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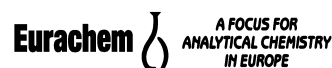
Quantifying Uncertainty in Analytical Measurement

First Edition 1995

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Quantifying Uncertainty in Analytical Measurement

Eurachem A FOCUS FOR ANALYTICAL CHEMISTRY IN EUROPE

English Edition

First Edition 1995

Contents

Foreword

Many important decisions are based on the **results [B.10]** 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 chemical analysis results, 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 results obtained outside the users own organisation is a prerequisite to meeting this objective. As a consequence of this, 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, irrespective of the methods used. An extremely useful measure is **measurement uncertainty [B.11]**

The "Guide to the Expression of Uncertainty in Measurement" **[G.1]** published in 1993 by ISO in collaboration with BIPM, IEC, IFCC, IUPAC, IUPAP and OIML, establishes general rules for evaluating and expressing uncertainty in measurement across a broad spectrum of measurements. This document shows how the concepts in the Guide may be applied in chemical measurement. The document 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. The process is illustrated by worked examples in Appendix A.

The evaluation of uncertainty requires the analyst to look closely at all the possible sources of uncertainty. A detailed study of this kind may require a considerable effort. However, the effort expended should not be disproportionate. In practice, a preliminary study will quickly identify the most significant sources of uncertainty, and as the examples show, the value obtained for the total uncertainty is almost entirely controlled by the major contributions. It follows that a good estimate can be made by concentrating effort on the largest contributions. Further, once evaluated for a given method applied in a particular laboratory, the uncertainty estimate obtained can be 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.

NOTE   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.2]**, the Guide **[G.1]** and ISO 3534 (Statistics - Vocabulary and symbols) **[G.3].** Appendix C shows, in general terms, the overall structure of a chemical analysis leading to a measurement result. Appendix D describes the calculations used to combine uncertainty components, 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 by Appendix G.

1. Scope

1.1. This protocol establishes general rules 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. Some common areas in which chemical measurements are needed and in which the principles of this protocol may be applied are:

- Quality control and quality assurance in manufacturing industries.

- Testing for regulatory compliance.

- Calibration of standards and equipment.

- Development and certification of reference materials.

- Research and development.

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 protocol, 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.2]** is "A parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the **measurand [B.6]**."

Note 1   The parameter may be, for example, a standard deviation **[B.22]** (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 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. Error and Uncertainty

2.2.1."Error" **[B.18]** 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. In principle, the systematic part of an error can be corrected for if all the sources of error are known, though the random part of an error is (by definition) variable from one determination to the next.

> NOTE   The term **true value** is an idealised concept. In this protocol, the terms *value of a* measurand (or of a quality) and *true value of a measurand* (or of a quality) are viewed as equivalent.

2.2.2. 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.

2.2.3. 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 the result is to the value.

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

2.3. Uncertainty Components

2.3.1. In practice the uncertainty on the result may arise from many possible sources, including examples such as:

- Incomplete definition of the measurand (for example, failing to specify the exact form of the analyte being determined).

- Sampling - the sample measured may not represent the defined measurand, for example a sub-sample may not be representative of the bulk, or the sample tested may have degraded with time since sampling.

- Incomplete extraction and/or pre-concentration of the measurand.

- Matrix effects and interferences.

- Contamination during sampling or sample preparation.

- Inadequate knowledge of the effects of environmental conditions on the measurement procedure or imperfect measurement of environmental conditions.

- Personal bias in reading analogue instruments.

- Uncertainty of weights and volumetric equipment.

- Instrument resolution or discrimination threshold.

- Values assigned to measurement standards and reference materials.

- Values of constants and other parameters obtained from external sources and used in the data reduction algorithm.

- Approximations and assumptions incorporated in the measurement method and procedure.

- Random variation.

> NOTE   These sources are not necessarily independent.

2.3.2. 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 may be referred to as an Uncertainty Component. When expressed as a standard deviation, an uncertainty component is known as a **standard uncertainty [B.12]** and is denoted **u(y).**   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.

2.3.3. For a measurement result **y**, the total uncertainty, termed **combined standard uncertainty [B.13]** 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 variance and covariance components, however evaluated, using the law of propagation of uncertainty (see Appendix D).

2.3.4. For most purposes in analytical chemistry, an **expanded uncertainty [B.14]** **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.15]** 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.

3. Basic Concepts

3.1. Measurement and Analysis

3.1.1.A quantitative analysis is a particular type of **measurement [B.7]**. The objective of measurement is to determine the value of the measurand, that is, the value of the specific quantity to be measured.   In chemistry, the quantity of interest is most commonly a concentration, mass or mass fraction. However, because the result of a procedure depends on the conditions and on exactly what is being measured, a measurement begins with an appropriate specification of the measurand, the **method of measurement [B.9]** and the **measurement procedure [B.8]**.

3.1.2. In associating an estimate of uncertainty with a procedure, it is essential that the procedure be adequately defined and in statistical control. For an analytical procedure, the method must be fully documented and operating within prescribed quality control limits.

3.2. Specification

3.2.1. In practice, the specification or definition of the measurand depends on the required accuracy of the measurement. The measurand should be defined with sufficient exactness relative to the required accuracy so that for its intended purposes its value is unique.   Incomplete definition of the measurand can itself give rise to an uncertainty sufficiently large that it must be included in the overall estimation of uncertainty.

EXAMPLE:
If a concentration (volume in a liquid) is required to four significant figures, its specification should include the temperature at which the concentration is defined. Thus the concentration should be specified as, for example, the concentration at 25°C. If the concentration is to be determined to only two significant figures, its specification would not normally require a precise defining temperature or a value for any other defining parameter. However, the lack of definition of temperature leads to an element of uncertainty which should be included in the estimated uncertainty of the two-figure result.

3.2.2. In specifying the procedure for which the uncertainty is to be estimated, it is essential to state which operations are included. For example, estimates of uncertainty will often be very different for a complete procedure which includes sampling and analysis and for the same analytical procedure without prior sampling.

3.2.3. In some cases, the measurand is defined in terms of a measurement procedure only. For example, 'loss on drying' (under specified conditions) is a procedure description which does not specify the nature of the material lost. Such methods are sometimes referred to as 'empirical methods' and their use may be agreed on for trade or regulatory purposes. When such methods are used then this should be clearly stated when reporting the results.

3.3. Determining the Measurand

3.3.1. Every analytical result is a combination of intermediate measurement results, like weighings, volumes and instrument readings, with other parameters like molecular weights. The relationship between these parameters and the value of the measurand can be simple or complex. An example of a simple relationship is that for the determination of the concentration (c) from the mass (m) present and the volume (v) at STP. *i.e.* c=m/v. It is customary to break complex relationships down into a combination of simpler relationships where possible (see Appendix D).

4. Errors and corrections

4.1. General

4.1.1. In general, an analytical procedure has imperfections that give rise to an error in the result. For example the value of the result can be affected by quantities that are not included in the definition of the measurand. These are called **influence quantities [B.5]** and their effects and that of other imperfections in the analytical procedure have to be evaluated.

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

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

4.2. Random error

4.2.1. Random error [B.19] typically arises from unpredictable variations of influence quantities. The effects of such variations, hereafter referred to as 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.21]** 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.

NOTE 2   Great care should be taken to distinguish between the terms error and uncertainty; they are not synonyms but represent completely different concepts. They should not be confused with one another or misused.

4.3. Systematic error

4.3.1.A **systematic error [B.20]** 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.

4.3.2. 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.

4.3.3. 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.

4.3.4. A gradual increase in the temperature of a set of samples during a chemical analysis can lead to progressive changes in the result. Sensors and probes that exhibit ageing effects over the time-scale of an experiment can also introduce non constant systematic errors.

4.3.5. 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.

4.4. Spurious Errors

4.4.1.A further type of error, which may be considered an extreme case of random error, is a spurious error. Errors of this type invalidate a measurement and typically arise through human failure or instrument malfunction. Transposing digits in a number while recording data or an air bubble lodged in a spectrophotometer flow-through cell are common examples of this type of error.

4.4.2. 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.

4.4.3. 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.

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

5. The Uncertainty Estimation Process

5.1. General

5.1.1. Uncertainty estimation is simple in principle. The following steps summarise the tasks that need to be performed in order to obtain an estimate of the uncertainty associated with a measurement.

- **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.

- **Identify Uncertainty Sources**
  For each parameter in this relationship, list the possible sources of uncertainty. These must include chemical assumptions.

- **Quantify Uncertainty Components**
  Measure or estimate the size of the uncertainty associated with each potential source of uncertainty identified.

- **Calculate Total Uncertainty**
  Combine the quantified uncertainty components expressed as standard deviations, according to the appropriate rules, to give a combined standard uncertainty, apply the appropriate coverage factor to give an expanded combined uncertainty.

Figure 5.1 (p18) shows the process schematically.

5.1.2. In practice it may not be necessary to carry out an evaluation of all the components identified in steps 2 and 3, since in certain circumstances it may be possible to obtain the uncertainty associated with the combined effect of several components. This document provides 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 components.

5.1.3. In addition check standards and control charts should be used to ensure that the measurement process is under statistical control.

5.2. Step 1 - Specification

5.2.1. Most chemical measurement results are obtained at the end of a procedure, which yields a numerical value for the measurand that is dependent upon a number of intermediate or input quantities. These may be other measurands or constants (constants also have uncertainties).

5.2.2. The measurand has a relationship to these other quantities which, in principle can be expressed algebraically as $\mathbf{y}=f(p,q,r, ...)$. Such a formal approach is useful for a theoretical discussion and can help in certain circumstances. However in practice, except in the simplest cases, it is rare to utilise this approach. It is more usual to break down the measurement procedure into a number of blocks. The results of the uncertainty evaluations on these simple blocks can then be used to obtain the combined uncertainty.

5.2.3. For the purpose of uncertainty calculations, it is also advisable to break down the relationship between the measurand

and the input quantities into simple expressions that conform to one of the standard forms given in Appendix D. Alternatively, Appendix D gives details of a numerical method, suggested by Kragten, which makes effective use of standard spreadsheet software **[G.5]**.

5.3. Step 2 - Identifying Uncertainty Sources

5.3.1. For each block of the measurement procedure a list of the possible sources of uncertainty associated with the input quantities that contribute to the value of the measurand has to 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 the next step, the best way of treating each source will be considered. Additional information is given in Appendix C (Structure of Analytical Procedures). This gives a list of stages followed during typical analytical procedures. 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.

- Instrument bias

  Limits of accuracy on the calibration of an analytical balance.

  A temperature controller that may maintain a mean temperature which differs 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

  Volumetric glassware may be used at a temperature different from that at which it was calibrated.

  The use of materials 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

  The uncritical use of computer software can introduce errors into the reported results. Both commercially produced and in-house programs are subject to conceptual errors such as coding population instead of sample standard deviation.

  Selection of an inappropriate calibration model, *e.g.* using a straight line calibration on a curved response.

  Early truncation and round off can lead to inaccuracies in the final result.

- Contamination

  Cross contamination between samples and contamination from the laboratory environment as a result of poor working practice is an ever present risk which should be minimised whenever possible. In trace analysis work it is particularly important to be aware of this possibility.

- Operator bias

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

  Possibility of slight misinterpretation 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.

5.3.2. It may not be necessary to write down all possible sources of uncertainty. For example, rather than write down temperature, pressure, and calibration error as sources of uncertainty for every determination of weight by difference, it may be more sensible to regard all three as parts of a single "weighing uncertainty" which can be evaluated directly.

5.4. Step 3 - Quantifying Uncertainty

5.4.1. The uncertainties identified must now be quantified. However, 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. The first step in the quantification is to make a preliminary estimate of the contribution of each component to the combined uncertainty and to eliminate those that are not significant. It is also useful to review the lists obtained in Step 2 with a view to simplification. The aim is to identify

groups of uncertainty components which can be evaluated as a single uncertainty component.

5.4.2. Calibration enables the combined uncertainty associated with the blocks of the measurement process included in the "calibration loop" to be obtained without a detailed evaluation of each component within the blocks.

5.4.3. Where uncertainty sources are grouped in this way, the groups should be identified and the uncertainty sources included should be checked against the list generated in step 2. This provides an auditable record of which contributions to uncertainty have been included.

5.4.4. 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 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.

5.4.5. There are four basic ways in which the individual components can be estimated

- by experimental work by the laboratory carrying out the analysis

- use of measurements on reference materials

- by utilisation of the data and results from previous work carried out in house or elsewhere

- using the judgement of the analyst based on experience.

Experimental Quantification

5.4.6. 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.

5.4.7. 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.

5.4.8. 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.

Use of Reference Materials

5.4.9. 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. 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.

5.4.10. 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.

Estimation based on previous results/data

5.4.11. Where experiment is impractical, some of the standard uncertainties have to be evaluated by using whatever relevant information is available about the uncertainty on the quantity concerned. Fortunately, such information is very often already available. The following paragraphs suggests some sources of information.

5.4.12. 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.

5.4.13. Inter-laboratory studies.
A collaborative study carried out, for example according to AOAC/IUPAC or ISO 5725 standards, to validate a published method, is an excellent source of data to support an uncertainty estimate. How useful the data is depends on the factors taken into account during the study. It is unlikely that any study will have covered all possible sources of uncertainty, particularly where systematic effects are likely, and the results of the study and prior validation experiments need to be examined carefully to identify any

sources of uncertainty outside the scope of the inter-laboratory study. In particular, sources which may need further consideration include:

- Sampling. Studies rarely include a sampling step.

- Pre-treatment (in most studies, samples are homogenised before distribution)

- Method bias. Although often examined by comparison with reference methods or materials, the uncertainty on the estimate of bias is seldom stated directly.

- 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.

- 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.

These and other factors preclude use of reproducibility figures (e.g. s_R) as unqualified estimates of uncertainty. Nonetheless, such figures, and the data from which they are derived, can substantially reduce the effort required to estimate uncertainty. An example of uncertainty estimation using collaborative trial data is given as Appendix A.4.

5.4.14. Proficiency Testing schemes are also of value. A laboratory's results can be used as a check on the evaluated uncertainty, since they 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 5.4.13) must also be taken into account.

5.4.15. Quality Assurance (QA) data. As noted previously (sec. **1.**) 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 samples are used in QA checks, section 1. shows how the data can be used to evaluate uncertainty. In other cases, QA data forms a continuing check on the value quoted for the uncertainty. Clearly, the combined uncertainty arising from random effects cannot be less than the repeatability of the QA measurements.

Estimation based on judgement

5.4.16. 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.

5.4.17. 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 functional values of all distributions are directly proportional to the frequency of observations in each particular range along the measurement scale. Such distributions can be said to have a frequentistic interpretation that is also reflected in the understanding of the standard deviation.

Depending on the exact form of a distribution a certain percentage of all observations lies within the interval of ±1 standard deviation. The quantification of these distributions and their associated standard deviations is done through repeated measurements.

5.4.18. 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.

5.4.19. 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.

5.4.20. 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.

5.4.21. 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.

5.4.22. 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.

5.4.23. 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.

5.4.24. 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.

Standard Uncertainties

5.4.25. All uncertainty contributions must be expressed as standard uncertainties, that is, as standard deviations, before combination. 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.

5.4.26. Where the uncertainty component was evaluated experimentally then it can readily be expressed as a standard deviation

5.4.27. 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 ±*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 $(0.2/1.96) \approx 0.1$.

5.4.28. If limits of ±*x* are given without a confidence level then it is normally appropriate to assume a rectangular distribution, with a standard deviation of $x/\sqrt{3}$ (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.

5.4.29. 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 value which could reasonably occur in practice (excluding simple mistakes). If a smaller value is considered

substantially more likely, this estimate should be treated as *a* in (2) above. If there are no grounds for believing that a small error is more likely than a large error, the estimate should be treated as a rectangular distribution.

5.4.30. Conversion factors for the two most commonly used distribution functions, the normal distribution and the rectangular distribution, are given in Appendix E**.**

5.5. Step 4 - Calculating the Combined Uncertainty

5.5.1. The next stage is to combine the standard uncertainties using one of the procedures described in detail in Appendix D. If the relationship between the measurand and the input quantities cannot be expressed in terms of the simplified standard forms then the general procedure, requiring the generation of partial differentials, must be employed.

5.5.2. Computer software forms an alternative to algebraic manipulation, and its use is recommended for any but the simplest cases. A particularly convenient method, suggested by Kragten **[G.5]**, is the use of standard spreadsheet software. This method is described in Appendix E.

5.5.3. 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.

5.5.4. 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.

For most purposes it is recommended that k is set to 2 in accordance with section 6. However, the *Guide* **[G.1]** gives additional guidance on choosing k, especially where a very small number of measurements is used to estimate large random effects.

Figure 5.1. The Uncertainty Estimation Process

Specification	Write down a clear statement of what is being measured and the relationship between it and the parameters on which it depends (Section 5.2)
Identify Uncertainty Sources	List sources of uncertainty for each part of the process or each parameter (Section 5.3)
Quantify Uncertainty Components	Estimate the size of each uncertainty. At this stage, approximate values suffice; significant values can be refined in subsequent stages (Section 5.4)
Convert to Standard Deviations	Express each component as a standard deviation (Section 5.4.20)
Calculate the Combined Uncertainty	Combine the uncertainty components, either using a spreadsheet method (Appendix E) or algebraically. Identify significant components. (Section 5.5)

Re-evaluate the Significant Components

Do the Significant components need re-evaluating?

YES   **NO**   **END**

6. Reporting uncertainty

6.1. General

6.1.1. 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.

6.1.2. 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.

6.2. Information required

6.2.1.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

6.2.2. 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.

6.2.3. 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.1**]).

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.

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

6.3. Reporting expanded uncertainty

6.3.1. Unless otherwise required, the result **x** should be stated together with the expanded uncertainty **U** calculated using a coverage factor k=2. 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.

EXAMPLE:

> Total nitrogen:3.52 $\pm$ 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%.

6.4. Reporting standard uncertainty

6.4.1. When uncertainty is expressed as the combined standard uncertainty $\mathbf{u_c}$ (that is, as a single standard deviation), the following form is recommended:

"(Result): **x** (units) [with a] standard uncertainty of $\mathbf{u_c}$ (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 $\pm$ 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.

6.5. Numerical expression of results

6.5.1. 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.

6.6. Compliance against limits

6.6.1. 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.

6.6.2. 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.

6.6.3. 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.
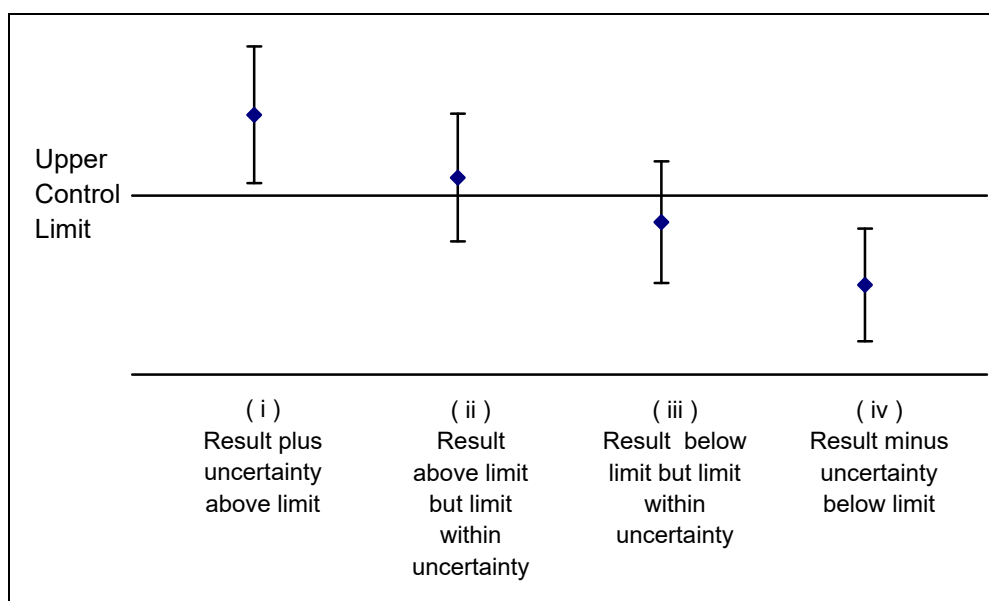
Figure 6.1: Uncertainty and compliance limits

Appendix A - Examples

Introduction

A0.1 Introduction

The following examples show detailed calculations of uncertainty in analytical measurement for several representative cases. The section aims to give

- Examples of the general form of uncertainty estimation procedures in analytical measurement

- A wide range of detailed examples of the evaluation of individual components of uncertainty.

Since the intention is to provide a range of detailed example calculations, all the individual components of uncertainty are considered in detail. This will not normally be the case in practice, as such detailed consideration need only be given to the most significant components. For minor components of uncertainty, it will normally be sufficient to use a preliminary estimate.

A0.2. Description of examples

Example 1 - An acid/base Titration
Titration provides examples of uncertainties in weighing and volumetric operations, use of certified reference materials and calculations involving molecular weights. Details are given at each stage of the experiment. An alternative description, combining all components in a single calculation, is also given, together with a summary of the main sources of uncertainty in titrimetry.

Example 2 - Determination of Cadmium release from ceramic ware by Atomic Absorption Spectrophotometry.
Example 2 gives an example of an uncertainty arising from a chemical process - in this case, acid leaching - and shows how environmental and chemical effects may be included in the calculation of a result and its associated uncertainty. The example also includes a rigorous treatment of uncertainties in a relatively complex expression. Note that Appendix E gives an alternative calculation based on a simple spreadsheet approach.

Example 3 - Determination of organophosphorus pesticides in bread
Trace organic analysis is typically subject to large uncertainties associated with extraction processes, and example 3 includes a consideration of the problem. Example 3 also considers an uncertainty arising from a sampling step, and an additional section discusses one method of modelling the effects of inhomogeneity.

Example 4 - Determination of meat content
Example 4 covers the important case of an uncertainty estimation where substantial prior information is available from collaborative trials and other method development studies. The example shows an alternative approach to the detailed examination of individual operations.

Appendix A - Examples

Example 1 - An acid/base Titration

A1.1 Introduction

This example discusses an experiment to determine the concentration of a solution of hydrochloric acid (HCl). The HCl is to be titrated against a freshly prepared solution of sodium hydroxide (NaOH) standardised with potassium hydrogen phthalate (KHP). It is assumed that the HCl concentration is known to be of the order of 0.1 mol.l^{-1}

The experiment involves a series of steps in which the calculated output value of one step forms an input value to a succeeding step. Each step consists of a set of logically grouped operations and has a simple expression for its measurement goal. This illustrates an important point, *viz.,* that although the expression for a measurand may be quite complex, the uncertainty of the measurement result can be easily obtained by decomposing the expression into a series of simpler stages and determining the uncertainties of the intermediate results represented by each stage.

A1.2 Method

The method can be broken down into the steps in the table (right) and the complete set of calculations is:

$$C_{KHP} = \frac{1000 \times m_{KHP} \times P_{KHP}}{V_f \times F_{KHP}} \quad (1)$$

where

C_{KHP} = concentration of KHP (mol.l^{-1})
m_{KHP} = weight of KHP taken (g)
F_{KHP} = formula weight of KHP (g.mol^{-1})
P_{KHP} = purity of KHP (mass fraction)
V_f = volume of KHP flask (ml)

$$C_{NaOH} = \frac{C_{KHP} \times V_{KHP}}{V_{N1}} \quad (2)$$

where

C_{NaOH} = concentration of NaOH solution (mol.l^{-1})
V_{N1} = volume of NaOH solution titrated at step 5 (ml).
V_{KHP} = titration volume of KHP (ml).

$$C_{HCl} = \frac{C_{NaOH} \times V_{N2}}{V_{HCl}} \quad (3)$$

where

C_{HCl} = concentration of HCl (mol.l^{-1})
C_{NaOH} = concentration of NaOH (mol.l^{-1})
V_{N2} = volume of NaOH titrated at step 8 (ml).
V_{HCl} = titration volume of HCl solution(ml).

Step	Description	Symbol
1	Weighing of KHP primary standard.	m_{KHP}
2	Dissolution of KHP and bulking to final volume.	V_f
3	Calculate KHP concentration.	C_{KHP}
4	Preparation of NaOH solution	-
5	Transfer of aliquot of NaOH to titration vessel.	V_{N1}
6	Titration of NaOH against KHP standard.	V_{KHP}
7	Calculate concentration of NaOH solution.	C_{NaOH}
8	Transfer of fixed volume of NaOH to volumetric flask.	V_{N2}
9	Titration of NaOH with HCl.	V_{HCl}
10	Calculate concentration of HCl solution.	C_{HCl}

A1.3 Evaluating Uncertainty Components

Step 1: Weighing of KHP primary standard (m_{KHP})

The first stage involves the preparation of a standard solution of KHP. A 250 ml quantity of an 0.1 mol.l^{-1} solution of KHP is to be prepared. This entails weighing out an amount as close as possible to

$$\frac{204.2236 \times 0.1 \times 250}{1000} = 5.10559g$$

The relevant weighings are:

container and KHP   36.1284g (observed)

container less KHP   31.0234g (observed)

KHP 5.1050g (calculated)

The final weight is a weight by difference. The uncertainties are therefore due to the variability of weights by difference in the appropriate region, and the uncertainty associated with the calibration of the balance. The quality control log shows a standard deviation of 0.07mg for check weighings of weights up to 50g. The calibration certificate establishes that a weight obtained by difference within the same range is within ±0.1mg of the displayed value with 95% confidence. This quantity therefore needs to be divided by 1.96 to give the component of uncertainty as a standard deviation, at 0.1/1.96=0.052mg.

The two components are then combined by taking the square root of the sum of their squares (see Appendix D) to give an uncertainty $\mathbf{u}(m_{KHP})$:

$$\mathbf{u}(m_{KHP}) = \sqrt{(0.052^2 + 0.07^2)} = 0.087mg$$

Step 2: Dissolution of KHP and bulking to final volume V_f

The volume of solution contained in the volumetric flask is subject to three main sources of uncertainty:

1. The uncertainty in the stated internal volume of the flask.

2. Variation in filling the flask to the mark.

3. The flask and solution temperatures differing from the calibrated temperature.

The first is indicated by the manufacturer as a $\pm$ figure. For a 250 ml volumetric flask this figure is typically ±0.15 ml. Because the figure is given without a confidence level, a rectangular distribution is assumed, and the appropriate standard deviation is therefore $0.15 / \sqrt{3} = 0.087\,\text{ml}$.

The uncertainty due to variations in filling can be estimated from a repeatability experiment on the flask in question. A series of ten fill and weigh experiments on a typical 250ml flask gave a standard deviation of 0.012 ml. The standard deviation can be used directly.

Finally, the effect of temperature difference from the flask calibration temperature can be calculated from an estimate of the temperature range and the coefficients of volume expansion. Since the volume expansion of the liquid is considerably greater than that of the flask, only the former need be considered. Taking a possible temperature variation of ±3K (with 95% confidence), and taking the coefficient of volume expansion for water as 2.1×10^{-4} $°C^{-1}$ gives a 95% confidence interval for a volume V of $\pm$V x 3 x 2.1×10^{-4}. For the target value of 250ml, this gives a 95% confidence interval of 250 x 3 x 2.1×10^{-4} = 0.158ml. Dividing this by 1.96 to obtain the standard deviation gives a value of ±0.08ml for the uncertainty due to incomplete temperature control.

Combining the three contributions to the uncertainty $u(V_f)$ of the volume V_f gives a value of

$$u(V_f) = \sqrt{(0.08^2 + 0.012^2 + 0.087^2)}$$
$$\Rightarrow u(V_f) = 0.12 \text{ml}$$

Step 3: Calculation of concentration C_{KHP} of KHP solution

The concentration C_{KHP} of this solution is calculated from

$$C_{KHP} = \frac{1000 \times m_{KHP} \times P_{KHP}}{V_f \times F_{KHP}} \quad (1)$$

where

C_{KHP} = concentration of KHP (mol.l^{-1})
m_{KHP} = weight of KHP taken (g)
F_{KHP} = formula weight of KHP (g.mol^{-1})
P_{KHP} = purity of KHP
V_f = volume of KHP flask (ml)

V_f and m_{KHP}, together with their uncertainties, have been determined in steps 1 and 2. The expression for C_{KHP} includes two additional parameters, P_{KHP} and F_{KHP}, for which values and uncertainties are needed.

Formula weight F_{KHP}

Potassium hydrogen phthalate has the empirical formula

$$C_8H_5O_4K$$
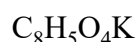
The uncertainty in the formula weight of a compound can be derived by combining the uncertainties in the atomic weights of its constituent elements. A table of atomic weights including uncertainty estimates is published biennially by IUPAC in the Journal of Pure and Applied Chemistry.

From the latest IUPAC table, the atomic weights and listed uncertainties for the constituent elements of KHP are:

Element	Atomic weight	Quoted Uncertainty	Standard Uncertainty
C	12.011	± 0.001	0.00058
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 these values by $\sqrt{3}$.

The separate element contributions to the formula weight, together with the uncertainty contributions for each, are:

	Calculation	Result	Uncertainty
C_8	8×12.011	96.088	0.0046
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

$$F_{KHP} = 96.088 + 5.0397 + 63.9976 + 39.0983$$
$$= 204.2236 \text{ g.mol}^{-1}$$

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

$$u(F_{KHP}) = \sqrt{\begin{array}{l} 0.0046^2 + 0.0002^2 + \\ 0.00068^2 + 0.000058^2 \end{array}}$$

$$\Rightarrow u(F_{KHP}) = 0.0047 \text{ g.mol}^{-1}$$

NOTE: Since the element contributions to F_{KHP} are simply the sum of the single atom

contributions, it might be expected from the general rule for combining uncertainty contributions that the uncertainty for each element contribution would be calculated from the sum of squares of the single atom contributions, that is, for example for carbon,

$$u(C) = \sqrt{(8x0.00058^2)} = \pm 0.0016$$

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 contribution is obtained by multiplying the value from a single determination by 8, the uncertainty is also derived by multiplying the single value by 8. Notice that the contributions from different elements are independent, and will therefore combine in the usual way.

Purity P_{KHP} of KHP

The purity of KHP is quoted in the supplier's catalogue as 99.9 ±0.1%. P_{KHP} is therefore 0.999 ±0.001. The quoted uncertainty is taken as a rectangular distribution, so the standard uncertainty $u(P_{KHP})$ is $0.001/\sqrt{3} = 0.00058$.

Concentration C_{KHP}

C_{KHP} is given by

$$C_{KHP} = \frac{1000 \times m_{KHP} \times P_{KHP}}{V_f \times F_{KHP}} \quad (1)$$

The intermediate values and their uncertainties are given in the table below, together with the uncertainties expressed as **relative standard deviations** (RSD) **[B.25]**.

	Value V	Uncertainty **u**	Uncertainty as RSD (U/V)
m_{KHP}	5.105	0.000087	1.7×10^{-5}
P_{KHP}	0.999	0.00058	5.8×10^{-4}
V_f	250	0.12	4.8×10^{-4}
F_{KHP}	204.2236	0.0047	2.3×10^{-5}

Using the values obtained above:

$$C_{KHP} = \frac{1000 \times 5.105 \times 0.999}{250 \times 204.2236}$$
$$= 0.0999 \text{ mol l}^{-1}$$

In order to combine the uncertainties associated with each component of a multiplicative expression (as above), the relative standard deviation must be used. The uncertainty $u(C_{KHP})$ in the concentration of KHP is therefore given by

$$\frac{u(C_{KHP})}{C_{KHP}} = \sqrt{\begin{pmatrix} (1.7 \times 10^{-5})^2 + (5.8 \times 10^{-4})^2 \\ +(4.8 \times 10^{-4})^2 + (2.3 \times 10^{-5})^2 \end{pmatrix}}$$

$$\Rightarrow \frac{u(C_{KHP})}{C_{KHP}} = 7.5 \times 10^{-4}$$

The standard uncertainty $u(C_{KHP})$ in the concentration of KHP is

$$u(C_{KHP}) = 0.0999 \times 7.5 \times 10^{-4} \approx 8 \times 10^{-5} \text{ mol.l}^{-1}$$

The KHP concentration is therefore 0.0999 mol l^{-1} with a standard uncertainty of 8×10^{-5} mol l^{-1}.

Step 4: Preparation of sodium hydroxide solution

A 0.1 mol.l^{-1} solution of sodium hydroxide is to be prepared. This material has a molecular weight of 39.9971 g.mol^{-1} and, as with the KHP, there will be an uncertainty associated with this value and an additional uncertainty associated with the purity of the material.

However, since the concentration of the NaOH is to be determined by assay against the reference KHP solution, and not by direct calculation, no information on the uncertainties connected with the molecular weight or the weight taken is required.

In order to prepare 250 ml of solution, it is necessary to weigh out an amount as close as possible to 0.9989g. For this example, the weight of NaOH actually taken was 1.0078g. Again, the weighing uncertainty is irrelevant since no subsequent calculations depend upon values connected with this stage.

Step 5: Transfer of aliquot V_{N1} of NaOH to titration vessel.

25 ml of the NaOH solution is to be transferred by means of a pipette. As in the case of a flask, the uncertainty in the value of 25 ml arises from three components:

1. The uncertainty in the stated internal volume of the pipette.

2. Variation in filling the pipette to the mark.

3. The pipette and solution temperatures differing from the calibrated temperature.

The uncertainty in the stated internal volume of the pipette is given by the manufacturer as ±0.03 ml. This can be approximated to a rectangular distribution with a standard uncertainty of $0.03/\sqrt{3} = 0.017$.

As before, a replicate weighing exercise is used to establish the uncertainty due to variability in filling, giving a sample standard deviation of 0.0092 ml. This can be used directly as the standard uncertainty for this operation.

NOTE: It is assumed throughout this example that only a single titration is performed. If several titrations were performed, each using a different aliquot V_{N1} and averaging the results, the uncertainty in the average value arising from random variation in aliquot removal and later titrations would be correspondingly smaller; for n replicates, the standard deviation found for the replicate weighings above would be divided by $\sqrt{n}$ to obtain the relevant contribution. The contribution of temperature and calibration uncertainty would not be affected, as neither would change significantly between replicates, assuming the same pipette were used to withdraw the aliquots.

The uncertainty due to the lack of temperature control is calculated as for step 1, again taking a possible temperature variation of ±3°C (with 95% confidence) and using the coefficient of volume expansion for water as 2.1×10^{-4} °C^{-1}. This gives a value of ±0.008ml for the uncertainty due to incomplete temperature control.

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.

Combining the three different sources of uncertainty in the volume V_{N1} gives an uncertainty $u(V_{N1})$ of

$$u(V_{N1}) = \sqrt{0.017^2 + 0.0092^2 + 0.008^2}$$
$$= 0.021 \text{ml}$$

<u>Step 6: Titration of NaOH against KHP standard (V_{KHP})</u>

Titration is accomplished using a 50ml burette. The same three main sources of uncertainty are present as for previous volumetric operations: repeatability, specification range and temperature effects. Additionally, the use of a burette, unlike that of a bulb pipette, does not in general involve the complete discharge of its contents. When estimating the variability of delivery it will be necessary to quote the graduation marks between which discharge occurs. For a given burette, several such sets of delivery limits should be investigated and recorded; *e.g.* 0-25, 25-50, 20-45 ml *etc.* Similarly, it may be necessary to investigate the repeatability of different volumes delivered, such as 5, 10, 15ml etc.

In this example, knowing the anticipated delivery will be close to 25ml, a series of deliveries of 25ml volumes were checked, giving a sample standard deviation of 0.013ml, used directly as a standard uncertainty.

The two other uncertainty contributions are derived as before. The manufacturer specifies a calibration accuracy of ±0.05ml, which is converted to a standard deviation of $0.05/\sqrt{3} = 0.029$. As an aqueous solution of volume near to 25ml, the uncertainty contribution from temperature effects (taking ±3°C as before) is again 0.008ml.
In this instance, V_{KHP} is found to be 24.85ml. Using the figures above, the uncertainty $u(V_{KHP})$ is calculated as

$$\mathbf{u}(V_{KHP}) = \sqrt{0.013^2 + 0.029^2 + 0.008^2}$$
$$= 0.033\text{ml}$$

<u>Step 7: Calculation of concentration C_{NaOH} of NaOH solution</u>

The concentration C_{NaOH} is calculated from

$$C_{NaOH} = \frac{C_{KHP} \times V_{KHP}}{V_{N1}}$$

where
  C_{NaOH} = concentration of NaOH (mol.l^{-1})
  V_{N1} = volume of NaOH titrated at step 5 (ml).
  V_{KHP} = volume of KHP delivered from burette (ml).
  C_{KHP} = concentration of KHP solution (mol.l^{-1}).

From steps 4 to 6 above, the values and their uncertainties are:

	Value	Uncertainty	Uncertainty as RSD
C_{KHP}	0.0999	0.00008	8×10^{-4}
V_{KHP}	24.85	0.033	1.3×10^{-3}
V_{N1}	25.0	0.021	8.4×10^{-4}

The concentration of the NaOH solution is

C_{NaOH}=(0.0999×24.85)/25.0
$\qquad\qquad$ = 0.0993 mol l^{-1}

In order to combine the uncertainties associated with each component of a multiplicative expression such as (2) above, the relative standard uncertainties must be used. This gives:

$$\frac{\mathbf{u}(C_{NaOH})}{C_{NaOH}} = \sqrt{\begin{array}{c}(8 \times 10^{-4})^2 + (1.3 \times 10^{-3})^2 \\ + (8.4 \times 10^{-4})^2\end{array}}$$

$$= 0.0017$$

The standard uncertainty $\mathbf{u}(C_{NaOH})$ in the concentration of the NaOH is

$$\mathbf{u}(C_{NaOH}) = 0.0017 \times 0.0993$$
$$= 0.00017 \text{ mol l}^{-1}$$

The concentration of the NaOH solution is therefore 0.0993 mol l^{-1} with a standard uncertainty of 0.00017 mol l^{-1}

Step 8: Transfer of fixed volume V_{N2} of NaOH to volumetric flask

This step is the first in the titration of the HCl against the standardised NaOH solution. An 25ml aliquot of the NaOH solution is required. As before, a 25ml pipette is used. This is exactly the same as step 5. V_{N2} is therefore 25ml, with a standard uncertainty $\mathbf{u}(V_{N2})$ of 0.021ml.

Step 9: Titration of NaOH with HCl (V_{HCl})

A 50ml graduated burette is used, giving a volume V_{HCl} of 25.45ml. As similar equipment is used to that for the titration in step 6, and the assumptions on temperature effects will be the same, the combined standard uncertainty $\mathbf{u}(V_{HCl})$ is 0.033 ml.

Step 10: Calculate concentration C_{HCl} of HCl

C_{HCl} is calculated from

$$C_{HCl} = \frac{C_{NaOH} \times V_{N2}}{V_{HCl}} \qquad (3)$$

where
    C_{HCl} = concentration of HCl solution (mol.l^{-1}).
    C_{NaOH} = concentration of NaOH solution (mol.l^{-1}).
    V_{N2} = Aliquot of NaOH titrated at step 8 (ml).
    V_{HCl} = volume of HCl from burette (ml).

The values and their uncertainties are as follows:

	Value	Uncertainty	Uncertainty as RSD
C_{NaOH}	0.0993	0.00017	1.7×10^{-3}
V_{N2}	25.0	0.021	8.4×10^{-4}
V_{HCl}	25.45	0.033	1.3×10^{-3}

From (3), the concentration C_{HCl} of the HCl is

$$C_{HCl} = (0.0993 \times 25.0) / 25.45$$
$$= 0.0975 \text{ mol l}^{-1}$$

Again, in order to combine the uncertainties associated with each component of a multiplicative expression such as (3) above, the relative standard uncertainties must be used, giving

$$\frac{\mathbf{u}(C_{HCl})}{C_{HCl}} = \sqrt{\begin{array}{c}(1.7 \times 10^{-3})^2 + (8.4 \times 10^{-4})^2 \\ + (1.3 \times 10^{-3})^2\end{array}}$$

$$= 2.3 \times 10^{-3}$$

$$\mathbf{u}(C_{HCl}) = 2.3 \times 10^{-3} \times 0.0975$$
$$= 0.00022 \text{ mol l}^{-1}$$

A1.4 Calculation of expanded uncertainty

The expanded uncertainty $\mathbf{U}(C_{HCl})$ is obtained by multiplying the standard combined uncertainty by a coverage factor of 2.

$$\mathbf{U}(C_{HCl}) = 0.00022 \times 2 = 0.00044 \text{ mol l}^{-1}$$

Thus the concentration of the HCl solution is 0.0975 ± 0.00044 mol l^{-1}

A1.5 Alternative calculation.

The stepwise calculations above are straightforward and simple, but do not make clear the relative sizes of all the components. An alternative form, in which the complete expression is written and the uncertainties of all the values included explicitly, follows.

From equations (1) to (3)

$$C_{KHP} = \frac{1000 \times m_{KHP} \times P_{KHP}}{V_f \times F_{KHP}} \quad (1)$$

$$C_{NaOH} = \frac{C_{KHP} \times V_{KHP}}{V_{N1}} \quad (2)$$

$$C_{HCl} = \frac{C_{NaOH} \times V_{N2}}{V_{HCl}} \quad (3)$$

it follows that

$$C_{HCl} = \frac{1000 \times m_{KHP} \times P_{KHP} \times V_{KHP} \times V_{N2}}{V_f \times F_{KHP} \times V_{N1} \times V_{HCl}}$$

As before, for multiplicative expressions, the individual contributions are combined as relative standard deviations. The necessary values are:

	Description	Value	Uncertainty as RSD
m_{KHP}	Weight of KHP	5.1050	1.7×10^{-5}
P_{KHP}	Purity of KHP	0.999	5.8×10^{-4}
V_{KHP}	Volume of NaOH	24.85	1.3×10^{-3}
V_{N2}	NaOH aliquot for KHP titration	25.0	8.4×10^{-4}
V_f	Stock volume of KHP	250	4.8×10^{-4}
F_{KHP}	Formula wt of KHP	204.2236	2.3×10^{-5}
V_{N1}	NaOH aliquot for HCl titration	25.0	8.4×10^{-4}
V_{HCl}	Volume of HCl	25.45	1.3×10^{-3}
C_{HCl}	Concentration of HCl	0.0975	2.3×10^{-3}

This gives:

$$C_{HCl} = \frac{1000 \times 5.105 \times 0.999 \times 24.85 \times 25.0}{250 \times 204.2236 \times 25.0 \times 25.45}$$
$$= 0.0975 \text{ mol l}^{-1}$$

and

$$\frac{u(C_{HCl})}{C_{HCl}} = \sqrt{ \begin{array}{l} (1.7 \times 10^{-5})^2 + (5.8 \times 10^{-4})^2 + (1.3 \times 10^{-3})^2 \\ (8.4 \times 10^{-4})^2 + (4.8 \times 10^{-4})^2 + (2.3 \times 10^{-5})^2 \\ + (8.4 \times 10^{-4})^2 + (1.3 \times 10^{-3})^2 \end{array} }$$

$$= 2.3 \times 10^{-3}$$

$$u(C_{HCl}) = 0.0975 \times 2.3 \times 10^{-3}$$

$$= 0.00022 \text{ mol l}^{-1}$$

It is instructive to examine the complete table (left), comparing the relative contributions of different steps. For example, the two volumes V_{KHP} and V_{HCl} each contribute 1.3×10^{-3} to the total uncertainty. If these two values are combined, the contribution is 1.8×10^{-3} - 80% of the estimated uncertainty. By contrast, the uncertainties m_{KHP} and F_{KHP} contribute under 2% of the overall uncertainty. Clearly, any improvements to the accuracy of the experiment would start with the burette titrations. Further examination shows that significant improvements here could be made by replication of the titrations, as a substantial fraction of the burette uncertainties arises from repeatability variation.

A1.6 Sources of Uncertainty in Titrimetry

This example has not attempted to address all of the possible sources of error that could affect the final result. A list of possible sources of error is given below, including those taken into account in this example. In particular cases some of the additional sources may be significant and would have to be taken into account.

1. Weighing - balance calibration and repeatability*.

2. Weighing - buoyancy effect of air.

3. Temperature effects*

4. Purity of weighed material.*

5. Formula weight of material*.

6. Nature of impurities *e.g.* other bases in a base.

7. Systematic errors in volumetric glassware*.

8. Endpoint detection.

9. Competing reactions *e.g.* adsorption of CO_2 from the air.

*Included in example

Appendix A - Examples

Example 2 - Determination of Cadmium release from ceramic ware by Atomic Absorption Spectrometry

A2.1 Introduction

This example considers the uncertainty in the determination of lead or cadmium by atomic absorption spectrometry. The method employed is that described in BS6748 "Limits of metal release from ceramic ware, glassware, glass ceramic ware and vitreous enamel ware" **[G.6]**. An extract from BS 6748 is given at the end of this example (Section A2.7). This forms the 'specification' of the measurand. This test is used to determine the amount of lead or cadmium leached from the surface of ceramic ware by a 4%(v/v) aqueous solution of acetic acid.

A2.2 Method

The concentration of lead or cadmium in the acetic acid is determined by atomic absorption spectrometry. For vessels that can be filled completely with the leaching solution, the amount of metal leached is expressed as the concentration C_0 of metal in milligrams per litre of leaching solution. For vessels that cannot be filled completely the standard calls for the result to be expressed as mass, **r**, of Pb or Cd leached per unit area. **r** is given by:-

$$r = \frac{C_0 \cdot V_L}{a_V} \qquad (1)$$

where
    C_0 is the concentration found (see (2) below)
    V_L is the volume of leachate
    a_V is the surface area of the vessel

The concentration is calculated by means of the bracketing technique described in section A.6 of BS6748. In this technique two standards are employed; one having a metal concentration below that expected, the other having a metal concentration higher expected of the determinand.

The expression given in the standard for the concentration C_0 is:

$$C_0 = \left[\left(\frac{A_0 - A_1}{A_2 - A_1} \right) \cdot (C_2 - C_1) + C_1 \right] \cdot d \qquad (2)$$

where

A_0 = absorbance of the metal in the sample extract.

A_1 = absorbance of the metal in the lower bracketing solution.

A_2 = absorbance of the metal in the upper bracketing solution.

C_0 = metal content of the sample extract (in mg l^{-1}).

C_1 = metal content in the lower bracketing solution (in mg l^{-1}).

C_2 = metal content in the upper bracketing solution (in mg l^{-1}).

d is the factor by which the sample was diluted.

The following experimental steps are involved:

Step	Description	Quantities (eq 2, 3)
1	Prepare 4% Acetic acid	-
2	Fill ceramic ware	V_L
3	Allow to stand	f_{time}, f_{temp}, f_{acid}
4	Prepare upper reference	C_2
5	Prepare lower reference	C_1
6	Determine absorbances	A_0, A_1, A_2
7	Calculate concentration	C_0
8	Calculate metal/area	a_V, **r**

A2.3 Uncertainty Components

Though equation (2) is exactly that given in the standard, it is not entirely sufficient for estimating uncertainty. The standard allows latitude in temperature, and there will be additional uncertainties in the acid concentration and the leaching time which need to be explicitly considered, as they could reasonably be expected to affect the amount of metal leached. These factors can be included in equation (2) by writing

$$C_0 = \left[\left(\frac{A_0 - A_1}{A_2 - A_1} \right) \cdot \left(C_2 - C_1 \right) + C_1 \right] \cdot d \cdot f_{acid} \cdot f_{time} \cdot f_{temp}$$

(3)

where f_{acid}, f_{time} and f_{temp} are correction factors for each parameter differing from that specified in the standard. Equation (2) implicitly assumes these factors are identically equal to 1 (i.e. no variation in the parameters). Including the factors as in equation (3) shows how the respective uncertainty contributions will contribute to the uncertainty in C_0.

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

A2.4 Evaluating Uncertainty Components

The individual components are evaluated against the experimental steps as follows.

Step 1. Prepare 4% v/v aqueous acetic acid

The acetic acid solution is prepared volumetrically, using a 1l volumetric flask and dispensing the acid in two aliquots from a 20ml pipette, with both operations nominally at ambient temperature (estimated to lie within 3°C of the calibration temperatures). The three significant contributions to uncertainty arise from the repeatability of the operation, the specification limits for the glassware and any temperature effects.

Repeated fill and weigh operations on a similar flask gave a standard deviation of 0.14ml, and the manufacturer quotes a calibration accuracy of ±0.2ml which, converted to a standard deviation, gives a calibration uncertainty of 0.12ml. Combining these values gives an uncertainty in the flask volume of $\sqrt{0.14^2 + 0.12^2} = 0.18$ml in 1000ml, or a relative standard deviation of 0.00018. For the pipette, the calibration specification is ± 0.015ml, which, since the same pipette is used twice, is doubled to ±0.03ml in the 40ml required giving a calibration uncertainty of 0.017ml as standard deviation. Repeatability experiments establish a standard deviation for pipettes in this volume range of 0.016ml. For two different deliveries from the same pipette, this standard deviation is multiplied by √2 to give the uncertainty in the double delivery due to repeatability variation as 0.022ml. Combining the calibration and repeatability uncertainty components for the two 20ml deliveries gives an uncertainty of $\sqrt{0.017^2 + 0.022^2} = 0.028$ml in 40ml, that is, a relative standard deviation of 0.0007.

Since the percentage of acetic acid in the solution is given by the ratio of the two volumes, the two glassware uncertainties are combined as relative standard deviations, giving a value of $\sqrt{0.0007^2 + 0.00018^2} = 0.00072$ for the uncertainty in the acid concentration expressed as a relative standard deviation. For the nominal value of 4% v/v, the uncertainty as a standard deviation is 0.00072×4 %v/v = 0.0029%v/v.

As the temperature is for practical purposes constant for both operations, the ratio of acetic acid to total volume at STP is affected only by the differences in the temperature coefficients of expansion between acetic acid and the bulk solution. (This contrasts with example 1, where the main effect is due to anticipated differences in temperature). For two liquids of volume coefficients of expansion α_1 and α_2, the volume ratio $(V_1/V_2)_T$ at temperature T is given by

$$\left(\frac{V_1}{V_2}\right)_T = \left(\frac{V_1}{V_2}\right)_0 \cdot \left(\frac{1 + \alpha_1 \cdot \delta T}{1 + \alpha_2 \cdot \delta T}\right)$$

where $(V_1/V_2)_0$ is the volume ratio at a reference temperature T_0 and δT is the temperature change from this temperature. The <u>change</u> is obtained by difference. Taking the coefficients of expansion for acetic acid and the aqueous solution as 1.07×10^{-3} and 2.1×10^{-4} respectively gives the effect on a nominal 4% solution of preparation up to 3° C away from the calibration temperature as $4 \times (1 + 3 \times 0.00107)/(1 + 3 \times 0.00021) - 4 = 0.01$. Converting to a standard deviation from this rectangular distribution gives an uncertainty component of $0.01/\sqrt{3} = 0.0058$.

Combining this value with the glassware uncertainty of 0.0029% v/v gives an uncertainty in the nominal 4% concentration of acetic acid of $\sqrt{0.0058^2 + 0.0029^2}$ =0.0064 %v/v.

NOTE: It will be seen that this level of uncertainty in the acid concentration represents a very small contribution. Where this is known to be the case, an approximate value could have been estimated from experience of similar calculations. For example, for class A glassware over about 20ml capacity, the manufacturers' estimates and the repeatability standard deviations typically combine to give uncertainties of approximately 0.05% of the nominal value. Similarly, for aqueous solutions, temperature effects contribute approximately 0.02% per degree C, while for organic liquids, the temperature effect on volume is approximately 0.1% per degree C. These values would have prompted a working estimate of about 0.1% of the nominal value in this case, or about 0.004% v/v.

Step 2. Fill ceramic ware.

The standard requires the vessel to be filled 'to within 1mm from the brim'. For a typical drinking or kitchen utensil, 1mm will represent about 1% of the height of the vessel. The vessel will therefore be 99.5± 0.5% filled (ie V_L will be approximately 0.995±0.005 of the vessel's volume). The volume V_L used is to be recorded to within 2%; in practice, use of a measuring cylinder allows an accuracy of about 1% (ie 0.01 x V_L). Both of the above figures represent rectangular distributions and must be converted to standard deviations as usual, giving uncertainty contributions to the volume V_L of $0.01 \times V_L/\sqrt{3} = 0.006 V_L$ and $0.005 \times V_L/\sqrt{3} = 0.003 V_L$. Combining the two components as usual gives an uncertainty on the volume V_L of $V_L \cdot \sqrt{0.006^2 + 0.003^2} = 0.007 V_L$ as standard deviation, or 0.007 expressed as a relative standard deviation. For this example, the volume used is 332ml, with an uncertainty of $0.007 \times 332 = 2.3$ml.

Step 3. Allow to stand.
The standard specifies the standing time and temperature as 24±0.5h and 22±2°C respectively. During this period, the time, temperature and acid concentration will determine the amount of metal leached, and there is some possibility of changes in V_L due to evaporative loss (which must be minimised). Equation (3) shows that the uncertainties associated with the first three of these effects appear as uncertainties on the correction factors f_{time}, f_{temp} and f_{acid}. Information on these effects is available from published studies of metal release from ceramics[1-5]. Evaporative loss will increase the uncertainty of V_L.

3.1 Effect of leaching time f_{time}.
For a relatively slow process such as leaching, the amount leached will be approximately proportional to time for small changes in the time. Krinitz and Franco[1] found a mean change in concentration over the last 6 hours of leaching was approximately 1.8mg.l^{-1} in 86, that is, about 0.3%/hr. 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; as a standard deviation the uncertainty $u(f_{time})$ is $0.0015/\sqrt{3} ≈ 0.001$. Since $f_{time}=1$, the relative standard deviation is also 0.001.

3.2 Effect of temperature f_{temp}
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 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 standard, this leads to a factor f_{temp} of 1±0.1; converting this to a standard deviation gives an uncertainty of $0.1/\sqrt{3}=0.06$ on f_{temp}.

3.3 Effect of acid concentration f_{acid}.
One study[1] of the effect of acid concentration on lead release showed that changing concentration from 4 to 5% v/v increased the lead released from a particular ceramic batch from 92.9 to 101.9 mg.l^{-1}, ie 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; taking the uncertainty of 0. 0064%v/v on the acid concentration (calculated above) suggests an uncertainty for f_{acid} of 0. 0064×0.1=0.00064. As the uncertainty on the acid concentration is already expressed as a standard deviation, 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; see the note at section 1 above for additional comment.

Steps 4, 5. Prepare calibration solutions.

The bracketing method requires two solutions of known concentration. These are prepared from a stock solution of cadmium acetate obtained from a commercial supplier. The concentration of cadmium was stated to be 1000 ±2 mg of cadmium in 1 litre of 4% v/v acetic acid.

From past experience, the expected level of cadmium in the solution presented to the AA analyser was approximately 0.2 mg l^{-1}. This suggested that a pair of bracketing solutions have concentrations of 0.1 and 0.5 mg l^{-1} respectively. The sample dilution factor, d, was 1.

In order to prepare the bracketing solutions a dilution scheme must be designed. The one used in this example is illustrated schematically below, using the 1000 mg l^{-1} stock solution of cadmium acetate as a starting point.
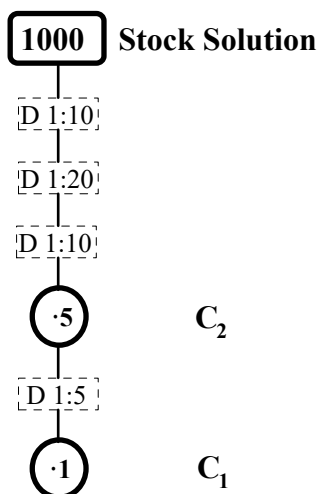
To calculate the uncertainty associated with each of the concentrations C_1 and C_2, it is necessary to determine the uncertainty in the stock solution concentration, estimate the uncertainties in the dilution chain, and combine the two sources of uncertainty.

As with previous volumetric measurements on liquids, the three main sources of uncertainty in the dilution chain are temperature, repeatability and the specification limits of the glassware.

During the dilutions, the temperature will be essentially constant, so temperature effects cancel at each dilution stage (the dilution factor will be unchanged by temperature, as aliquot and diluent are dilute aqueous solutions or pure water respectively). However, the final concentration, expressed as mg/l at the glassware calibration temperature, will be affected by temperature; this contribution to the uncertainty will be considered below.

The 1:10 dilutions are to be performed using 10 ml pipettes and 100 ml volumetric flasks. The 1:20 dilution was performed using a 5 ml pipette and a 100 ml flask. The 1:5

dilution was performed using a 20 ml pipette and a 100 ml flask. Contributions due to imperfect repeatability and variation within specification limits must be determined and combined for each type of glassware used.

As before, the uncertainty arising from imperfect repeatability is estimated from the standard deviation of a number of fill and weigh operations, while the uncertainty arising from variation within specification limits is obtained by dividing the manufacturer's specification by $\sqrt{3}$ to convert from rectangular limits to a standard deviation. The table below summarises the data so obtained for the types of glassware in use:

	Description	std dev	Spec. /$\sqrt{3}$
V_5	5ml pipette	0.0048	0.0087
V_{10}	10ml pipette	0.012	0.012
V_{20}	20ml pipette	0.0067	0.017
V_{100}	100ml flask	0.024	0.046

The resulting combined uncertainties, shown both as standard deviations $\mathbf{u}(V_i)$ and as relative standard deviations (RSD) are :

	Calculation	$u(V_i)$ (ml)	u as RSD $u(V_i)/V_i$
V_5	$\sqrt{0.0048^2 + 0.0087^2}$	0.010	0.0020
V_{10}	$\sqrt{0.012^2 + 0.012^2}$	0.016	0.0016
V_{20}	$\sqrt{0.0067^2 + 0.017^2}$	0.019	0.00095
V_{100}	$\sqrt{0.024^2 + 0.046^2}$	0.052	0.00052

To obtain the uncertainties for each dilution step, it is simplest to treat each step as applying a dilution factor, made up from the volumes of the two items of glassware used at each step. Three dilution factors are employed in the dilution chain and arise from 1:20, 1:10 and 1:5 dilutions. Since, in each dilution step, there is an uncertainty connected with the initial and final volumes taken, the dilution factor for that step also has an uncertainty associated with it. The same dilution factor uncertainty may be applied to each dilution of the same kind

even though, in practice, different pipettes and flasks (of the same nominal capacity) will be used.

Each dilution factor is calculated from

$$f = \frac{V_f}{V_i}$$

where

f = dilution factor
V_f = final volume
V_i = initial volume

For a 1:20 dilution, the relevant dilution factor f_{20} is:

$$f_{20} = \frac{V_{100}}{V_5} = 20$$

Since the factor is calculated by division, the associated uncertainty is given by

$$\frac{u(f_{20})}{f_{20}} = \sqrt{\left(\left(\frac{u(V_{100})}{100}\right)^2 + \left(\frac{u(V_5)}{5}\right)^2\right)}$$

that is, the uncertainty in f_{20} as a relative standard deviation is calculated from the sum of squares of the uncertainties in the volumes expressed as relative standard deviations. From the Table above, this gives

$$\frac{u(f_{20})}{f_{20}} = \sqrt{0.00052^2 + 0.002^2}$$

$$= 0.0021$$

$$\Rightarrow \mathbf{u}(f_{20}) = 0.0021 \times 20 = 0.042$$

For a the 1:10 dilution, the dilution factor is

$$f_{10} = \frac{V_{100}}{V_{10}} = 10$$

The uncertainty is given by

$$\frac{u(f_{10})}{f_{10}} = \sqrt{\left(\left(\frac{u(V_{100})}{100}\right)^2 + \left(\frac{u(V_{10})}{10}\right)^2\right)}$$

$$= \sqrt{0.0052^2 + 0.0016^2}$$

$$= 0.0017$$

$$\Rightarrow \mathbf{u}(f_{10}) = 0.0017 \times 10 = 0.017$$

and finally, for the 1:5 dilution using the 20ml pipette and 100ml flask (V_{20} and V_{100} respectively), the uncertainty $\mathbf{u}(f_5)$ is calculated from

$$\frac{u(f_5)}{f_5} = \sqrt{0.00052^2 + 0.00095^2}$$

$$= 0.0011$$

$$\Rightarrow \mathbf{u}(f_5) = 0.0011 \times 5 = 0.0055$$

The stock solution concentration C_{stock} is, as noted above, $1000 \pm 2 mg.l^{-1}$. As usual this is treated as a rectangular distribution and divided by $\sqrt{3}$ to obtain the value of $2/\sqrt{3} = 1.2 mg.l^{-1}$, or 0.0012 as a relative standard deviation.

The values involved in the dilution, with their standard uncertainties and uncertainties expressed as relative standard deviations (RSD), are therefore

	Value	Uncertainty $\mathbf{u}$	$\mathbf{u}$/Value (RSD)
C_{stock}	1000	1.2	0.0012
f_5	5	0.0055	0.0011
f_{10}	10	0.017	0.0017
f_{20}	20	0.042	0.0021

The dilution chain for the standards requires four dilutions to be made. For the upper standard (C_2) of 0.5 mg.l^{-1}, the dilutions are 1:10, 1:20 and 1:10 whilst to obtain the low standard $(C_1$, 0.1 mg.l$^{-1})$, the high standard is diluted further by 1:5. This gives

$$C_2 = C_{stock} / (f_{10} \times f_{20} \times f_{10})$$

$$C_1 = C_{stock} / (f_{10} \times f_{20} \times f_{10} \times f_5)$$

Both expressions only involve multiplication and division, so in each case the uncertainties are combined as relative standard deviations, that is, for C_2:

$$\frac{u(C_2)}{C_2} = \sqrt{\left(\frac{u(C_{stock})}{C_{stock}}\right)^2 + \left(\frac{u(f_{10})}{f_{10}}\right)^2 + \left(\frac{u(f_{20})}{f_{20}}\right)^2 + \left(\frac{u(f_{10})}{f_{10}}\right)^2}$$

Using the relative standard deviations in the table above,

$$\frac{u(C_2)}{C_2} = \sqrt{0.0012^2 + 0.0017^2 + 0.0021^2 + 0.0017^2}$$

$$= 0.0034$$

$$\Rightarrow u(C_2) = 0.5 \times 0.0034 = 0.0017 \text{ mg.l-1}$$

The lower standard (C_1) is obtained from the upper standard (C_2) by dilution; the uncertainty is therefore best considered by making the substitution

$$C_1 = C_2 / f_5$$

in equation (3) to give

$$C_0 = \left[\left(\frac{A_0 - A_1}{A_2 - A_1}\right) \cdot \left(C_2 - \frac{C_2}{f_5}\right) + \frac{C_2}{f_5}\right] \cdot d \cdot f_{acid} \cdot f_{time} \cdot f_{temp}$$

$$(4)$$

and adding in the uncertainty for the dilution factor f_5 (see below).

So far, the uncertainty arising from unknown constant temperature offset during the dilution has not been considered. The effect can readily be calculated from the known coefficients of expansion (2.1×10^{-4}) and the possible ambient temperature range $(\pm 3°C)$ to give a factor of 6.3×10^{-4}, or, as a relative standard deviation about C_i, 3.6×10^{-4}. However, it is important to be certain how this value will apply. Any constant temperature offset during the dilution process will affect C_1 and C_2 by the same factor. If this factor were incorporated in equation 3 as a correction factor f_{dil} on the concentrations C_1 and C_2, it can be seen as a factor which applies directly to C_0. From equation 4 (neglecting other corrections for clarity):

$$C_0 = \left[\left(\frac{A_0 - A_1}{A_2 - A_1}\right) \cdot \left(f_{dil} \cdot C_2 - f_{dil} \cdot \frac{C_2}{f_5}\right) + f_{dil} \cdot \frac{C_2}{f_5}\right] \cdot d$$

$$= f_{dil} \cdot \left[\left(\frac{A_0 - A_1}{A_2 - A_1}\right) \cdot \left(C_2 - \frac{C_2}{f_5}\right) + \cdot \frac{C_2}{f_5}\right] \cdot d$$

This contribution $(u(f_{dil}))$ to the uncertainty in the concentrations C_1 and C_2 can therefore be associated directly with C_0 rather than making separate assessments. In this instance, this avoids some difficulties in dealing with the problems of a correlated temperature effect on separate parameters in equation 4.

Step 6. Determine absorbances A_0, A_1, A_2.

Each of the absorbance measurands A_0, A_1, and A_2 have uncertainties connected with them and these uncertainties were estimated by making replicate measurements. Up to ten replicates were obtained for each measurand. The sample obtained from the ceramic ware was used undiluted, that is d in equation 3 is exactly 1, and no uncertainty allowance is required.

The results are summarised in the table below. The value SD/√n is the standard

deviation of the mean of the n observations. This value can be used directly as the estimate of the standard uncertainty associated with the mean values above.

	Absorbance		
Value:	A_0	A_1	A_2
	55	23	101
	54	24	101
	53	21	102
	54	22	102
	50	22	101
	52	22	101
	53	22	100
	-	22	102
	-	20	102
	-	20	102
Mean	53.0	21.8	101.4
Std dev (SD)	1.63	1.23	0.70
n	7	10	10
SD/√n	0.62	0.39	0.22

NOTE: In principle, an allowance needs to be made for any non-linearity of response in the instrument. This type of error would affect the calculation of C_0 directly, as it violates the assumption of linearity on which the bracketing method depends. In this example, the instrument non-linearity over the range is small compared to the absorbance uncertainties above and no further allowance is made.

Step 7. Calculate concentration C_0.

The concentration C_0 is calculated from:

$$C_0 = \left[\left(\frac{A_0 - A_1}{A_2 - A_1}\right)\cdot\left(C_2 - \frac{C_2}{f_5}\right) + \frac{C_2}{f_5}\right]\cdot d \cdot f_{acid} \cdot f_{time} \cdot f_{temp}$$

(4)

Carrying out some simplification gives:-

$$C_0 = \frac{C_2}{(A_2 - A_1)}\cdot[(A_0 - A_1) - (A_0 - A_2)/f_5]$$

(5)

The values and their uncertainties have been calculated previously, and are summarised below.

	Value	Uncertainty u	u/Value (RSD)
A_0	53.0	0.62	0.012
A_1	21.8	0.39	0.018
A_2	101.4	0.22	0.002
f_5	5	0.0055	0.0011
C_2	0.5	0.0017	0.0034
d	1	0	0
f_{acid}	1	0.0064	0.0064
f_{time}	1	0.001	0.001
f_{temp}	1	0.06	0.06
f_{dil}*	1	0.00036	0.00036

* f_{dil} is the correction factor for the reference solution dilution temperature described in step 5.

From equation (5) (ignoring the factors nominally equal to 1 for clarity);

$$C_0 = \left[\left(\frac{53.0 - 21.8}{101.4 - 21.8}\right)\cdot(0.5 - 0.1) + 0.1\right]$$

$$= 0.26 \text{ mg.l}^{-1}$$

A2.5 Combined Uncertainty

Unfortunately it is not possible to express the formula for C_0 in terms of the standard expressions since all of the terms are not independent. A_1 appears in both the denominator and the numerator and in addition C_1 is a function of C_2 via $C_1 = C_2/f_5$. The uncertainty contributions from the absorbance and concentration terms therefore have to be calculated using the formula given in Appendix D, that is:

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

(6)

where y(p,q,...) is a function (such as eqn 3 above) of several variables p,q,..., and $\partial y/\partial p$ is the partial differential of y with respect to p. Each variable's contribution is just the square of the relative standard deviation

multiplied by the square of the relevant partial differential. For this example, the formal differentials will be derived.

NOTE: While formal differentiation is given here, Appendix E gives a simpler alternative using standard spreadsheet software.

Differentiating equation 5, and substituting the values from the table above, gives:

$$\frac{\partial C_0}{\partial A_0} = \frac{C_2}{(A_2 - A_1)} \cdot (1 - 1/f_5) = 0.005$$

$$\frac{\partial C_0}{\partial A_1} = \frac{C_2(A_0 - A_2)}{(A_2 - A_1)^2} \cdot (1 - 1/f_5) = -0.0032$$

$$\frac{\partial C_0}{\partial A_2} = \frac{C_2 \cdot (A_1 - A_0)}{(A_2 - A_1)^2} \cdot (1 - 1/f_5) = -0.0020$$

$$\frac{\partial C_0}{\partial C_2} = \frac{(A_0 - A_1) - (A_0 - A_2)/f_5}{(A_2 - A_1)} = 0.51$$

$$\frac{\partial C_0}{\partial f_5} = \frac{C_2 \cdot (A_0 - A_2)}{(A_2 - A_1) \cdot f_5^2} = -0.012$$

Applying equation (6) to the individual relative standard deviations from the table and partial differentials calculated above gives the combined contribution **u(a,c)** of the uncertainties in absorbance and concentration values as

$$\sqrt{\begin{array}{l} 0.005^2 \times 0.062^2 + (-0.0032)^2 \times 0.39^2 \\ +(-0.002)^2 \times 0.22^2 + 0.51^2 \times 0.0017^2 \\ +(-0.012)^2 \times 0.0055^2 \end{array}}$$

$$\Rightarrow \mathbf{u(a,c)} = 0.0035 \text{ mg.l}^{-1},$$

or, as a relative standard deviation, **u(a,c)**/C_0 = 0.0035/0.257=0.014 .

This value needs to be combined with the remainder of the uncertainty components, ie those in the correction factors f_{time} etc. As these parameters are simple multipliers in the concentration expression, the uncertainties are combined as for normal multiplicative

expressions, that is, as the root sum of squares of the relative standard deviations.

From the table, this gives

$$\frac{u(C_0)}{C_0} = \sqrt{\begin{array}{l} 0.014^2 + 0.001^2 + 0.06^2 \\ 0.0064^2 + 0.00036^2 \end{array}}$$

$$= 0.062$$

(ie 6.2%) and

$$\mathbf{u(C_0)} = 0.062 \times 0.26 = 0.016 \text{ mg.l}^{-1}$$

For articles complying with categories 2 and 3 in the standard, the concentration C_0 is the value required. The uncertainty should be quoted after multiplication by a suitable coverage factor, usually 2. This leads to a reported uncertainty of 0.032 mg.l^{-1}.

The concentration is therefore reported as

Cadmium release measured according to BS6748: 0.26 ± 0.032 mg.l^{-1}, where the reported uncertainty is calculated using a coverage factor of 2.

<u>8. Calculate amount leached per unit area **r**</u>

The amount of metal leached per unit area is calculated from

$$\mathbf{r} = \frac{C_0 \cdot V_L}{a_V} \qquad (1)$$

For this example, the total surface area a_V of the sample was calculated, from measured dimensions, to be 2.37 dm². Since the items are approximately cylindrical but not perfectly regular, measurements are estimated to be within 2mm at 95% confidence. Typical dimensions are about 10cm leading to an estimated dimensional measurement uncertainty of 0.01 as relative standard deviation (after dividing the 95% figure by 1.96). Area measurements typically require two length measurements, either height and width or two independent

diameter measurements, which results in a relative uncertainty in the area of approximately $0.01 \times \sqrt{2} = 0.014$. Since the items are not perfect geometric shapes, there is also an uncertainty in any area calculation; in this example, this is estimated to contribute an additional 5% at 95% confidence. Converting to standard deviation and combining these two values for the purpose gives a relative standard deviation for the area uncertainty of $\sqrt{0.014^2 + 0.025^2}$ =0.029. For the estimate of $2.37 dm^2$ this gives an uncertainty of $0.069 dm^2$.

The volume V_L of acetic acid used was 332 ml; the uncertainty as RSD has already been estimated (step 1 above) as 0.007, giving a volume uncertainty of 2.3 ml.

The relevant values are:

	Value	Uncertainty	as RSD
C_0	0.26 mg.l^{-1}	0.016	0.06
V_L	0.332 l	0.0023	0.007
a_V	2.37 dm^2	0.069	0.029

Inserting these values in the above expression yields

$$r = \frac{0.26 \times 0.332}{2.37} = 0.036 \text{ mg.dm}^{-2}$$

The uncertainty as RSD is obtained by combining the individual RSD values:

$$\frac{u_c(r)}{r} = \sqrt{0.06^2 + 0.007^2 + 0.029^2} = 0.067$$

$$\Rightarrow u_c(r) = 0.067 \times 0.036 = 0.0024 \text{ mg.dm}^{-2}$$

An expanded uncertainty for r is obtained by applying a coverage factor of 2 which gives a value of 0.0048 mg.dm^{-2}

The result can therefore be reported as

Cadmium release measured according to BS6748:1986:
$$0.036 \pm 0.005 \text{ mg.dm}^{-2}$$
where the reported uncertainty is calculated using a coverage factor of 2.

A2.6 References.

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. Steele, *J. AOAC* **66**, 610-619 (1983)
4. T. D. Seth, 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)

A2.7 Summary of procedure
From BS 6748:1986, "Limits of metal release from ceramic ware, glassware, glass ceramic ware and vitreous enamel ware"

A2.7.1 Reagents

Water, complying with the requirements of BS3978.

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 litre of 4% v/v acetic acid

- 500 ±0.5 mg Cd in 1 L of 4% v/v acetic acid.

A2.7.2 Apparatus

Atomic absorption spectrophotometer, with a detection limit of at least 0.2 mg l^{-1} Pb (in 4% v/v acetic acid) and 0.02 mg l^{-1} 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.

A2.7.3 Preparation of samples

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

A2.7.4 Procedure

- 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 acetic acid solution (**A.1.4**) 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 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 evaporative loss.

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

A2.7.5 Analysis

- The AA instrument is set up according to the manufacturer's instructions 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 and of the 4% v/v acetic acid solution indicate minimal drift, the results may be calculated by the bracketing technique (below), or from a manually prepared calibration curve or by using the calibration facilities of the instrument.

A2.7.6 Calculation of results by the bracketing technique

The lead or cadmium content, C_0 expressed in mg l^{-1} of the extraction solution, is given by the equation:

$$C_0 = \left[\left\{ \left(\frac{A_0 - A_1}{A_2 - A_1} \right) (C_2 - C_1) \right\} + C_1 \right] d$$

where

A_0 is the absorbance of the lead or cadmium in the sample extract.

A_1 is the absorbance of the lead or cadmium in the lower bracketing solution.

A_2 is the absorbance of the lead or cadmium in the upper bracketing solution.

C_1 is the lead or cadmium content of the lower bracketing solution (in mg l^{-1}).

C_1 is the lead or cadmium content of the upper bracketing solution (in mg l^{-1}).

d is the factor by which the sample was diluted.

NOTE The lower and upper bracketing solutions should be chosen to have absorbance values close to that of the sample extract or diluted sample extract.

A2.7.7 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(ies) of extracting solution(s) 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 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 High Road, London, W4 4AL, England. ☎0181 996 7000.

Appendix A - Examples

Example 3 - Determination of organophosphorus pesticides in bread

A3.1 Introduction

The aim of the measurement is to determine the amount of an organophosphorus pesticide residue in bread. The method is applicable to a small range of chemically similar pesticides at levels between 0.01 and 2 mg.kg^{-1}.

A3.2 Method

The method is illustrated schematically below.
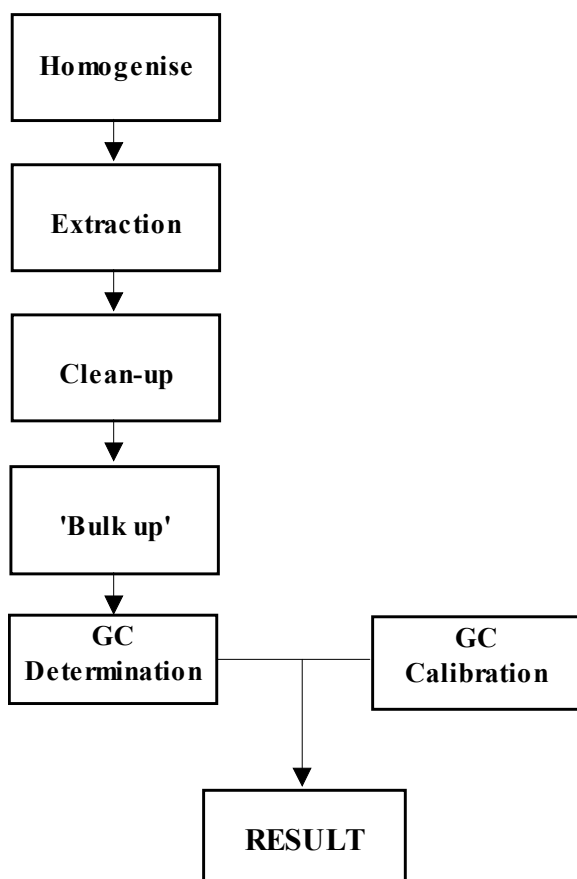
Figure: Organophosphorus pesticide analysis

The separate stages are:

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

ii) Weighing of sub-sample for analysis (gives mass M_{sample}).

iii) Extraction. Quantitative extraction of the analyte with organic solvent, decanting and drying through a sodium sulphate column, 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 blow-down of extract to near dryness.

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

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

viii) GC calibration via preparation of an approximately 5μgml^{-1} standard (actual mass concentration C_{ref}) and injection and GC measurement of 5μl of standard to give a reference peak intensity I_{ref}.

Calculation

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

$$C_{op} = C_{ref} \times \frac{I_{op}}{I_{ref}}$$

and the estimate $\hat{P}_{op}$ of the level of pesticide P_{op} in the bulk sample (in $\mu g.g^{-1}$) is given by

$$\hat{P}_{op} = \frac{C_{op} \times V_{op}}{M_{sample}} \times 10^6$$

A3.3 Uncertainty components

From the expressions above, it appears that the uncertainty in the final value $\hat{P}_{op}$ can be derived directly from the uncertainties in the individual values C_{ref}, I_{ref}, I_{op}, M_{sample} and so on. However, it is clear from examination of the experimental stages that the unreliability of extraction and clean-up and the effect of inhomogeneity will contribute appreciably to the uncertainty in $\hat{P}_{op}$ as a value for the bulk sample.

The basic calculation does not make this sufficiently explicit, especially since it is not immediately clear how these factors affect the individual measurements. To show the effects of uncertainties arising from these operations clearly, it is useful to write

$$\hat{P}_{op} = F_{ext} \times F_{hom} \times F_{con} \times \frac{C_{op} \times V_{op}}{M_{sample}} \times 10^6$$

where F_{ext} and so on are correction factors assumed to be unity in the original calculation.

This makes it clear that the uncertainties in these correction factors must be included in the estimation of the overall uncertainty. The final expression also shows how the uncertainties 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 a "correction factor" which is normally assumed to be unity. For example, the uncertainty in C_{op} can be expressed as a standard deviation for C_{op}, or as the standard deviation which represents the uncertainty in a "correction factor". In the latter case, the value is incidentally the uncertainty for C_{op} expressed as a relative standard deviation.

A3.4 Evaluating Uncertainty Components

Table A3.7 lists the individual steps in the method, and shows the best estimate of the "correction factor" associated with each step and the uncertainty in each. The following paragraphs show how the components of uncertainty were evaluated.

i)   Homogeneity

No literature data were available on the distribution of trace organic components in bread products, despite an extensive literature survey (at first sight this is surprising, but most food analysts attempt homogenisation rather than evaluate inhomogeneity separately). Nor was it practical to measure homogeneity directly. The contribution of inhomogeneity therefore had to be estimated on the basis of the sampling method used.

To aid estimation, a number of possible pesticide residue distribution scenarios were considered, and simple binomial statistical distributions used to calculate standard deviations for the total included in the analysed sample (see section A3.6). The scenarios, and their calculated relative standard deviation in the amount of pesticide in the final sample, were:

a) Residue distributed on the top surface only. RSD 0.58.

b) Residue distributed evenly over the surface only. RSD 0.20.

c) Residue distributed evenly through the sample, but reduced in concentration by evaporative loss or decomposition close to the surface. RSD 0.05-0.10 depending on "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 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.

ii) Weighing

The method calls for 10g material to be taken for extraction. Table A3.1 gives weighing data for the two-figure balance in use in the laboratory. The data has a standard deviation of 0.03g for repeatability experiments, and a very similar figure for long term variability. The standard deviation of 0.03 g was taken as the contribution to the uncertainty of random run to run variation. The long term data show no significant error or drift; nonetheless, the limited amount of data makes it uncertain that the balance error is identically zero. The size of this uncertainty can be estimated using the **standard deviation of the mean [B.24]** of the long term data. This gives a value of 0.008g. The two values are combined to give the value of $\sqrt{0.03^2 + 0.008^2} = 0.031g$.

iii) Extraction

The contribution of the extraction step to the overall uncertainty is estimated using analysis of spiked samples and replicate analysis of samples containing the pesticides of interest.

The main points to consider are the value and uncertainty of the recovery estimate F_{ext}, and the run to run reproducibility. The best available data in this laboratory are given in tables A3.2 and A3.3. Experiments are direct recovery estimates from spiking, extraction and concentration.

Table A3.2 shows the available results of long term studies of spiked samples of various types. The relevant line is the "Bread" entry line which shows a mean recovery for forty-two samples of 90%, with a relative standard deviation of 0.31 (of 90%). This leads to an estimated value for F_{ext} of 1.1 ±0.048 calculated as the standard deviation of the mean $(0.31/\sqrt{42})$. Note that since this factor is built in to the calculation, no amount of replication on real samples will reduce the value.

The additional effect of variation from extraction to extraction still needs to be considered. Table A3.3 gives the available duplicate test data for typical organophosphorus pesticides found in a number of bread samples. The normalised difference data (the difference divided by the mean value) provides a measure of the variability. To obtain the estimated relative standard deviation 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 deviation for the single values. This gives a value for the uncertainty due to run to run variation in the extraction process of 0.27 (expressed as RSD).

Combining these two values gives a relative standard deviation of $\sqrt{0.27^2 + 0.048^2} = 0.27$, that is, $F_{ext} = 1.1 \pm 0.30$.

The extract is reduced from an initial 100-150ml volume to 10ml by evaporation at ambient pressure. There is some risk of loss both by evaporation and decomposition. However, the extraction experiments (above) include the concentration step; it follows that no further allowance need be made.

NOTE   The extraction experiments also include the later stages of the experiment, and in principle no further allowance appears necessary. However, it is unlikely that the extraction experiments covered the full range of variability observed in later stages in practice; for example, operator and other effects would not be covered effectively. Additional allowance will, therefore, be necessary; the following paragraphs give the values used.

iv) Liquid-liquid extraction and drying

No data was available for liquid-liquid partition and drying; the analyst's best estimate was a value of 0.03 (as RSD) for the additional uncertainty for the two operations. No separate random variability was identified. *i.e.* $F_{LL} = 1.0 \pm 0.03$. This value is taken as the estimate of additional uncertainty arising from this step.

v) Concentration of washed extract

Following liquid partition, the liquid is reduced to a small volume by gas "blow down" at ambient temperature.

The experimental data on organochlorine pesticides in table A3.2 shows a mean recovery of 94%, with RSD of 0.045 and standard deviation of the mean of 0.006. This gives the value for F_{con} as 1.064 ± 0.006. The main cause of loss is expected to be direct evaporation, and this is expected to be controlled largely by molecular weight in both organophosphorus and organochlorine pesticides, hence the organochlorine data is adopted without modification as a working estimate.

In principle, an estimate is also required of that run-to-run variation of concentration which is not included in the extraction repeatability noted above. However, examination of the independent GC repeatability (below) suggests that the majority of the variation found in the concentration experiments arises from GC variability; accordingly, no further allowance is considered appropriate here.

The relative standard deviation expressing the uncertainty for F_{con} is therefore $0.006/1.064 = 0.0056$.

vi) Dilution to standard volume

The reduced solution is made up to 2ml in a graduated tube. A series of fill/weigh experiments using water gave the data in table A3.4.

This data shows a pooled standard deviation of 0.08ml, which, for a target volume of 2ml, becomes 0.04 RSD. In this instance, the data also show a bias of 0.09ml for the 2ml volume. From experience, this is a typical error magnitude for the type of measurement, and is therefore used directly as an estimate of the uncertainty in assumptions about the dilution step. In terms of the correction factor F_{dil}, $F_{dil} = 1.0 \pm 0.045$. This step, too, is included in the extraction repeatability experiments; no additional uncertainty allowance is therefore made for the imperfect repeatability of the dilution step.

vii) GC measurement

Available data on a series of GC experiments, repeated by several analysts over a period of time, are summarised in table A3.6. The RSD for several pesticides was determined; the best and worst for each analyst/instrument combination are available. The mean over all the available RSD values is 0.066.

In principle, the GC variability is included in the extraction repeatability trials. However, GC variability also occurs in the course of the dilution trials, which show an RSD of only 0.045. Clearly, the wide ranging study across different GC instruments, operators and times provides evidence that only part of the possible GC variability is included in the prior experiments. An additional allowance is therefore appropriate. Following basic statistical principles applicable to

combination of variances, an additional variance of approximately $0.066^2 - 0.045^2 = 0.0023$ is required to account for the additional GC variability found. This leads to an estimate of $\sqrt{0.0023} = 0.048$ as an estimate of the additional allowance required.

viii) Calibration of the GC response

a) Preparation of standard

The standard is prepared by weighing out 50mg of the organophosphorus pesticide reference material, dissolving it in 100ml solvent, and diluting twice by a factor of ten, using fresh 10ml pipettes and 100ml volumetric flasks.

There are three different sources of uncertainty; the purity of the standard, the weighing uncertainty and the volumetric uncertainty. The purity of the certified standard is given as 99.53% ±0.06%. For a four figure balance, the weighing uncertainty is estimated from other studies as ±0.3mg (RSD 0.006).

The 100ml flask has a manufacturer's tolerance of ±0.08 ml (RSD 0.0008), while the pipette has a tolerance of 0.01ml in 10ml (RSD 0.001). Each dilution is therefore subject to an uncertainty of

$$\sqrt{\left(0.0014^2 + 0.0005^2\right)} = 0.0015$$

For the complete dissolution/dilution process, therefore, the overall volumetric uncertainty is

$$\sqrt{\left(0.0005^2 + 0.0015^2 + 0.0015^2\right)} = 0.002$$

as an RSD (the first term is for the initial 100ml dissolution).

In addition, given that the operations are carried out at ambient temperature, an allowance is necessary for unknown absolute temperature offset.

NOTE   Since the aliquot and diluent can reasonably be considered as being at the same temperature for the complete dilution process, the uncertainty arising from constant, unknown temperature offset will appear directly in the final concentration, not once for each dilution.

Taking the temperature variability as ±5° C (RSD) and assuming hexane as solvent gives an uncertainty in volume at STP of ±0.005 (as RSD). Combining this value with the volumetric and weighing uncertainties gives a figure of

$$\sqrt{\left(0.003^2 + 0.002^2 + 0.005^2\right)} = 0.006$$

This represents the uncertainty in C_{ref}.

b) GC determination of standard

The response for the standard is determined once, using the same GC equipment as the sample. From the work above, the GC determination variability in addition to that allowed for in prior stages is characterised by an RSD of 0.048.

This represents the uncertainty of I_{ref}.

NOTE   This estimate ignores most systematic errors in the GC determination. This is possible here only because the value obtained is compared directly with the intensity value for the sample; any such systematic errors therefore largely cancel in the final result.

A3.5 Combined Uncertainty

The expression for the value P_{op} shows that all the terms combine multiplicatively. Using the rules in Appendix F, the combined standard uncertainty in this situation is the root of the sum of squares of the relative standard deviations used to express the individual components.

Using Table A3.7, the following figures are obtained:

Combined correction: 1.17
Combined Uncertainty: 0.35

This is the uncertainty for the determination expressed as a relative standard deviation.

Normally, the expanded uncertainty should be reported; in the absence of other indications the value of k=2 is used, giving

Expanded uncertainty:0.70 as RSD (70% as CV).

A3.6 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 standard deviation s of the amount of material in n equal portions selected randomly after separation.

These values are given by

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

$$\mu = np_1.(l_1 - l_2) + n.l_2 \qquad [1]$$

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

where l_1 and l_2 are the amounts of substance in portions from regions in the sample containing total fractions 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 $12\times12\times24$ cm, using a portion size of $2\times2\times2$ 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; 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

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

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

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

$$\Rightarrow RSD = \sigma/\mu = 0.58$$

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

$$X = 432/15 \times 2\cdot5 \times l_1 = 72 \times \frac{X}{72} = X$$

This result is typical of random sampling; the expectation value of the mean is exactly the mean value of the population For random sampling, there is thus no contribution to overall uncertainty other than 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

$$P_1 = \frac{((12x12x24) - (8x8x20))}{(12x12x24)} = 0.63$$

i.e. p_1 is that fraction of the sample in the "outer" 2cm.

Using the same assumptions then

$$l_1 = \frac{X}{272}$$

This gives:

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

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

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

$$\Rightarrow \text{RSD} = \sigma/\mu = 0.2$$

Scenario (c)

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

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

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

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

$$\Rightarrow \text{RSD} = \sigma/\mu = 0.33$$

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

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

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

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

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

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

NOTE   In this case, the reduction in uncertainty arises because the inhomogeneity is on a smaller scale than the portions 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 large 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.

A3.7 Tables

Table A3.1 **Weighing data**

1. Repeat weighings, replicate readings, five second interval.

Reading Weighing	1	2	3	4
1	10.53	10.51	10.47	10.50
2	10.48	10.46	10.44	10.49
3	10.52	10.47	10.48	10.46
4	10.46	10.44	10.47	10.47
5	10.50	10.49	10.46	10.48
6	10.45	10.45	10.46	10.45
7	10.50	10.43	10.42	10.42
8	10.45	10.44	10.43	10.44
9	10.40	10.43	10.43	10.42
10	10.44	10.46	10.49	10.45
11	10.42	10.41	10.44	10.43
12	10.45	10.43	10.43	10.44

Mean: 10.46
Standard deviation **s**: 0.030
Relative standard deviation: 0.003

2. Calibration data: Stated weight calibration Uncertainty

Nominal wt	Uncertainty	Validity
100	0.00005g	Jun 93
50	0.00006g	Nov 92
20	0.00005g	Nov 92
10	0.00004g	Nov 92
5	0.000035g	Nov 92

3. Monthly Check weights (10g) (Log book data).

Date	Reading	Error
3 Apr 92	9.99	0.01
1 May 92	10.00	0.00
2 Jun 92	10.01	-0.01
1 Jul 92	9.99	0.01
31 Jul 92	10.01	-0.01
2 Sep 92	10.00	0.00
30 Sep 92	9.93	0.07
1 Dec 92	10.02	-0.02
4 Jan 93	9.99	0.01
1 Feb 93	10.02	-0.02
1 Mar 93	9.97	0.03

Mean: 9.994
Standard deviation **s** 0.026

Standard deviation
of the mean

$$\frac{0 \cdot 026}{\sqrt{11}} = 0 \cdot 008$$

Table A3.2 **Pesticide recovery data from spiked samples**

Substrate	Residue Type	Conc mgkg^{-1}	N[1]	Mean[2] %	s[2] %	RSD
Waste Oil	PCB	10.0	8	84	9	0.11
Butter	OC	0.65	33	109	12	0.11
Compound Animal Feeds I	OC	0.325	100	90	9	0.10
Animal and Vegetable Fats I	OC	0.33	34	102	24	0.24
Brassicas 1987	OC	0.32	32	104	18	0.17
Bread	OP	0.13	42	90	28	0.31
Rusks	OP	0.13	30	84	27	0.32
Meat & Bone Feeds	OC	0.325	8	95	12	0.13
Maize Gluten Feeds	OC	0.325	9	92	9	0.10
Rape Feed I	OC	0.325	11	89	13	0.15
Wheat Feed I	OC	0.325	25	88	9	0.10
Soya Feed I	OC	0.325	13	85	19	0.22
Barley Feed I	OC	0.325	9	84	22	0.26

The "Recovery Data" heading spans the N, Mean, s, and RSD columns.

(1) The number of experiments carried out.
(2) The mean and sample standard deviation **s** are given as percentage recoveries.

Table A3.3 **Results of duplicate analyses on bread samples**

Residue	D1 mgkg^{-1}	D2 mgkg^{-1}	Mean mgkg^{-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
Pirimphos 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	0.075	-0.01	-0.133
Chlorpyrifos Methyl	0.01	0.01	0.00	0.00	0.000
Pirimiphos Methyl	0.06	0.03	0.045	0.03	0.667
				standard deviation s	**0.382**

Table A3.4 **Glassware volume repeatability.**

Nominal volume	Actual Volume (weight water / density)		
1 ml	0.82	0.82	1.01
	0.80	1.03	0.87
	0.94	0.93	0.92
	0.92	0.70	0.81
2 ml	1.84	1.95	1.93
	1.79	2.00	1.96
	1.96	1.86	2.00
	1.82	1.81	1.96
5 ml	4.88	4.89	5.05
	4.99	4.91	4.95
	4.97	4.94	4.86
	4.79	4.99	4.99

The data are obtained from a series of replicate fill/weigh operations.

Table A3.5 **Gas blow-down loss data**

Recovery of organochlorine pesticide standard evaporated from 10ml of standard solution to the specified volume and bulked back to 10ml. Values are the ratio of GC intensity after evaporation and bulking up to that before evaporation. DEGS and FS16 refer to different GC columns used for the determinations.

Summary for minimal loss procedure (Evaporate to $\geq$ 0.25ml, tables 1-3)

	DEGS	**FS16**
Mean recovery	0.934	0.946
Number of determinations	27	27
Standard deviation s	0.046	0.044
Pooled recovery	0.940	
Pooled standard deviation	0.045	
Standard deviation of the mean	0.006	

The following tables show the data from which the summary table is drawn.

1.   **Evaporated to 1ml**

Component	DEGS column	FS16 column
HCB	0.889	0.988
A-HCH	0.935	0.990
G-HCH	0.950	1.045
B-HCH	0.944	1.037
PP-DDE	0.981	0.989
Dieldrin	0.975	0.940
OP-DDT	0.963	0.914
PP-TDE	0.971	0.955
PP-DDT	0.981	0.958
Mean	0.954	0.980
s	0.030	0.043

2.   **Evaporated to 0.5ml**

Component	DEGS column	FS16 column
HCB	0.814	0.870
A-HCH	0.905	0.869
G-HCH	0.922	0.880
B-HCH	0.936	0.913
PP-DDE	0.959	0.945
Dieldrin	0.961	0.939
OP-DDT	0.964	0.936
PP-TDE	0.964	0.975
PP-DDT	0.986	0.973
Mean	0.935	0.922
s	0.052	0.042

3.   **Evaporated to 0.25ml**

Component	DEGS column	FS16 column
HCB	0.797	0.943
A-HCH	0.881	0.934
G-HCH	0.902	0.941
B-HCH	0.927	0.954
PP-DDE	0.929	0.955
Dieldrin	0.962	0.964
OP-DDT	0.947	0.956
PP-TDE	0.945	0.883
PP-DDT	0.937	0.896
Mean	0.914	0.936
s	0.050	0.028

Additional Data:

The following data is provided for information only; it was not used to construct the summary table above.

4.   Evaporated to just dry and then bulked to 10ml.

Component	DEGS column	FS16 column
HCB	0.514	0.581
A-HCH	0.649	0.644
G-HCH	0.736	0.733
B-HCH	0.885	0.901
PP-DDE	0.918	0.937
Dieldrin	0.935	0.966
OP-DDT	0.940	0.994
PP-TDE	0.956	0.944
PP-DDT	0.959	0.973
Mean	0.832	0.853
s	0.161	0.157

5.   Evaporated to dryness (15min) and then bulked to 10ml.

Component	DEGS column	FS16 column
HCB	0.000	0.000
A-HCH	0.029	0.018
G-HCH	0.098	0.089
B-HCH	0.591	0.597
PP-DDE	0.685	0.667
Dieldrin	0.608	0.581
OP-DDT	0.760	0.735
PP-TDE	0.893	0.795
PP-DDT	0.895	0.805
Mean	0.507	0.476
s	0.365	0.340

Table A3.6 **Precision of GC injections**

Analyst ID	Instrument ID	n[1]	Worst[2] RSD	Best[2] RSD
HK	1	10	0.083	0.038
HK	2	10	0.047	0.044
KP	1	10	0.037	0.022
CL	1	4	0.045	0.024
DG	2	9	0.082	0.021
DG	1	5	0.100	0.061
RT	1	8	0.159	0.040
RL	3	6	0.180	0.120
RL	5	6	0.100	0.100
RL	4	7	0.161	0.101
SV	1	8	0.115	0.078
MR	5	8	0.040	0.040
TL	1	10	0.081	0.058
TL	2	10	0.035	0.022
MA	1	10	0.029	0.016
MA	1	10	0.110	0.061
SB	1	10	0.020	0.019
SB	2	10	0.020	0.019
AR	1	10	0.020	0.020
IP	1	10	0.056	0.056
IP	2	10	0.147	0.131
Means	-	-	0.079	0.052

(1)   Number of separate GC runs.
(2)   RSD was determined for each of eight to ten peaks for each analyst/instrument combination. The best and worst are given.

Table A3.7 **Uncertainty components in determination of organophosphorus pesticides in bread**

	Component	Value	u (SD)	u/f (RSD)	Comment
i)	**Homogenisation F_{hom}**	1.0	0.2	0.2	Estimated by modelling
ii)	**Weighing M_{sample}**	10	0.031	0.0031	QA data
iii)	**Extraction and concentration F_{ext}**	1.1	0.30	0.27	Spiked sample recovery
iv)	**Liquid-liquid extraction F_{LL}**	1.0	0.03	0.03	Estimated
v)	**Concentration F_{con}**	1.06	0.006	0.0056	Experimental data
vi)	**Dilution to standard volume F_{dil}**	1.0	0.045	0.045	Experiment and estimation
vii)	**GC determination (Iop)**	1.0	0.047	0.047	Experiment
viii)	**Calibration Cref**	1.0	0.008	0.008	Specification and calculated
	Iref	1.0	0.047	0.047	Experiment

$$\text{Combined Correction} = 1{\cdot}0 \times 1{\cdot}0 \times 1{\cdot}1 \times 1{\cdot}0 \times 1{\cdot}06 \times 1{\cdot}0 \times 1{\cdot}0 \times 1{\cdot}0 \times 1{\cdot}0 = 1{\cdot}17$$

$$\text{Combined Uncertaint y} = \sqrt{\begin{array}{l} 0.2^2 + 0.0031^2 + 0.27^2 + 0.03^2 + 0.0056^2 \\ + 0.045^2 + 0.047^2 + 0.008^2 + 0.047^2 \end{array}} = 0.35$$

Appendix A - Examples

Example 4 - Determination of meat content

A4.1 Introduction

Meat products are regulated to ensure that the meat content is accurately declared and that only permitted additives are present. In consequence, there is a substantial range of information on particular analytical methods in the open literature. This example shows how this information can be used to provide estimates of uncertainty for total meat content both for meat without nitrogen-containing additives and for a meat product containing soya protein.

A4.2 Method

Total meat content M_{tot} is given by

$$M_{tot} = P_{meat} + F_{tot} \qquad [1]$$

where

P_{meat} = total meat protein (%w/w)
F_{tot} = total fat content (%w/w).

Meat protein P_{meat} (in %) is calculated from

$$P_{meat} = 100 * N_{meat} / NF \qquad [2]$$

where NF is a nitrogen factor specific to the material, and N_{meat}, the total meat nitrogen content. In this instance N_{meat} is identical to the total nitrogen content N_{tot} determined by Kjeldahl analysis.

The table (above right) shows the experimental steps involved.

Step	Description	Quantities (eq 1,2)
1	Determine fat content	F_{tot}
2	Determine nitrogen content by Kjeldahl	N_{meat}
3	Calculate 'defatted meat' content (eq 2)	P_{meat}, NF
4	Calculate total meat content (eq. 1)	M_{tot}

A4.3 Uncertainty Components

The components of uncertainty to consider are those associated with each of the parameters in the table above. The most significant relate to P_{meat}, which constitutes some 90% of M_{tot}. The largest uncertainties in P_{meat} arise from

i) uncertainty in the factor NF owing to incomplete knowledge of the material
ii) the reproducibility of the method, which is subject to variations both from run to run and in detailed execution in the long term and
iii) the uncertainty associated with method bias.

Note: These uncertainties are associated with the sample, the laboratory and the method respectively. It is often convenient to consider each of these three factors when identifying gross uncertainties as well as any necessary consideration of the individual steps in the procedure.

In addition, the uncertainty in fat content F_{tot} needs to be considered.

A4.4 Evaluating Uncertainty Components

i) Uncertainty in NF

The uncertainty in NF can be estimated from a published range of values. Reference 2 gives the results of an extensive study of nitrogen factors in beef, which show a clear variation between different sources and cuts of meat; the extremes are 3.57 and 3.73 (for defatted meat). The mean value is 3.65 (also the centre point of the range). The observed range is 0.16; if taken as a rectangular distribution, this gives a standard deviation of $0.16/2\sqrt{3}=0.046$ (0.013 as relative standard deviation). Reference 2 also permits calculation of the observed standard deviation over a large range of samples, giving a value of 0.052 (0.014 as relative standard deviation). The latter figure is most appropriate as it represents an observed standard deviation.

ii) Uncertainty in N_{tot}

Information in two collaborative trials[1,6] allows an estimate of the uncertainty arising from imperfect reproducibility or execution of the method. Close examination of the trial conditions shows first, that each was conducted over a broad range of sample types and with a good, representative range of competent laboratories, and second, that the reproducibility standard deviation s_R correlates well with level of nitrogen. For both trials, the best fit line is given by

$$s_R = 0.021 \ N_{tot}$$

Thus the figure of 0.021, which is actually a relative standard deviation, is a good estimate of the uncertainty in N_{tot} arising from reasonable variations in execution of the method.

Some consideration also needs to be given to uncertainty in N_{tot} arising from unknown bias within the method. In the absence of reliable reference materials, comparison with alternative methods operating on substantially different principles is an established means of estimating bias. Reference 6 reports the results of a recent comparison of Kjeldahl and combustion methods for total nitrogen. The study, which covered a range of 30 samples of different types across a number of laboratories, found a small but statistically significant difference in results, averaging 0.01 of the value observed. This leads to an estimated correction of $+0.005.N_{tot}$ to the Kjeldahl result (based on the mean of the two methods). Since only two methods are compared, however, the case for applying this correction is relatively weak. The result does suggest, however, that a bias of $0.005N_{tot}$ is quite possible. On this basis, a judgement of the uncertainty can be made; the figure of 0.005 is decided on as representing the relative standard deviation. While this is a comparatively crude estimate, it will be seen to give a sufficient indication of the uncertainty involved.

The overall uncertainty on N_{tot} can now be estimated from the two components above as $\sqrt{0.021^2 + 0.005^2} = 0.022$ (as RSD). Note that the figure of 0.005 makes very little contribution; there is thus no need to further refine the estimate of the uncertainty associated with method bias.

iii) Uncertainty in F_{tot}

From experience and knowledge of additional collaborative trial data, analysts estimate that the uncertainty in fat content will be adequately represented by a relative standard deviation of 0.02 (that is, a standard deviation of $0.02F_{tot}$).

A4.5. Combined uncertainty (unadulterated meat)

The table below shows the individual values and the uncertainties calculated using the above figures.

Parameter	Value V	u	u/V
Fat content F_{tot} (%)	5.5	0.11	0.02
Nitrogen content N_{meat}	3.29	0.072	0.022
Nitrogen factor NF	3.65	0.051	0.014
Meat protein P_{meat} (%)	90.1	90.1 x 0.026= **2.5**	$\sqrt{0.022^2 + 0.014^2}$ =0.026
Meat content M_{tot} (%)	95.6	$\sqrt{2.5^2 + 0.11^2}$ =**2.5**	

A level of confidence of approximately 95% is required. This is provided by multiplying the total uncertainty by a coverage factor k of 2, giving an estimated uncertainty U on the meat content of U = 5%; that is, M_{tot} = 95.6±5%

A4.6 Uncertainty in meat content with soya protein present

The case involving soya protein involves correction of the total nitrogen for soya-derived nitrogen to give the meat-derived nitrogen content. The expression is therefore:

$$M_{tot} \ (\%) = 100 * (N_{tot} - N_{soya}) / NF_{beef} \qquad [3]$$

where
N_{tot} = total nitrogen found
N_{soya} = total soya-derived nitrogen
NF_{beef} = the nitrogen factor for beef.

The uncertainties on all but N_{soya} have already been estimated (above).

N_{soya} is calculated from:

$$N_{soya} = \frac{P_{soya}}{NF_{soya}}$$

where
P_{soya} = soya protein content P_{soya} measured by ELISA
NF_{soya} = nitrogen factor for soya protein, estimated as 5.71

Both these values have uncertainties.

ELISA determination of soya protein P_{soya}

The former can be estimated from existing collaborative trial data (refs 3, 4) which give a value of 0.2 for the reproducibility (as relative standard deviation) of ELISA analysis of meat containing soya grits (flour or similar). For the figure of P_{soya}=4.4g/100g this gives an uncertainty of 0.88g/100g.

Nitrogen factor for Soya protein NF_{soya}

The conversion factor is based on the known (exact) value of nitrogen content for glycinin, the dominant protein in soya; the uncertainty arises primarily from the unknown contribution of the secondary protein, legumellin. Ref. 5 quotes nitrogen contents of 17.5 and 16.1 wt % respectively for these proteins, leading to figures of 5.71 and 6.21 for the nitrogen factor. Taking glycinin as over 50% of the total leaves a limiting range of values between 5.71 and 5.95 (calculated from the % nitrogen figures). This leads to an uncertainty estimate of (5.95-5.71)/2$\sqrt{3}$=0.07 or 0.012 as relative standard deviation. Note that this applies strictly only to the mean nitrogen factor estimate, 5.8: in this instance, it is used as an estimate based on the accepted value of 5.71. In practice, the overall estimate of soya content will be somewhat high if legumellin is present in quantity.

Combining these two figures (as relative standard deviations, since the result is calculated by division) leads to an uncertainty in N_{soya} of $\sqrt{(0.012^2+0.2^2)}=0.2$ as relative standard deviation. As expected, the value is dominated by the large ELISA uncertainty.

A4.7 Combined uncertainty in meat content with soya protein

The table below summarises the figures obtained, together with their calculated uncertainties and the relevant calculations. The standard uncertainty $\mathbf{u}(M_{tot})$ is calculated as 4.4 g/100g.

For approximately 95% confidence, the combined uncertainty in the defatted meat content is multiplied by 2, giving a total meat content of 48.8±8.8g/100g.

Parameter	Value	Uncertainty $\mathbf{u}$
N_{tot}	2.55	0.022 * 2.55 = 0.056
N_{soya}	0.77	0.2 * 0.77 = 0.15
$N_{meat} =$ $N_{tot}-N_{soya}$	1.78	$\sqrt{((0.056^2+0.15^2)}$ = 0.16
NF_{beef}	3.65	0.014 (as RSD)

Defatted meat M_{tot} (g/100g)	48.8	$\sqrt{(.014^2+(0.16/1.78)^2)}$
		    =0.09 (as RSD)
		    = 4.4 g/100g

A4.8 References

1. B. Shure, P. A. Corrao, A. Glover, A. J. Malinowski: *J. AOAC* **65** 1339 (1982)

2. Analytical Methods Committee: *Analyst* **118** 1217 (1993)

3. C. Hall, C. H. S. Hitchcock, R. Wood: J. APA **25** 1 (1987)

4. MAFF collaboratively tested non-statutory methods, No. V.25, April 1992

5. D. Breese Jones: US Department of Agriculture Circular no. 183, August 1931

6. M. King-Brink, J. G. Sebranek: *J. AOAC Int*. **76**, 787 (1993)

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.2,** 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.2,** 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.2,** 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.2,** 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.2,** 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.2,** 2.1].

B.8 Measurement Procedure

Set of operations, described specifically, used in the performance of measurements according to a given method [**G.2,** 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.2,** 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.2,** 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.2,** 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 Standard uncertainty

$u(x_i)$ uncertainty of the result of a measurement expressed as a standard deviation. [**G.1**, 2.3.1]

B.13 Combined standard uncertainty

$u_c(y)$ standard uncertainty of the result 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.1**, 2.3.4].

B.14 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.1**, 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.15 Coverage factor

k   numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty [**G.1**, 2.3.6].

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

B.16 Type A evaluation (of uncertainty)

Method of evaluation of uncertainty by the statistical analysis of series of observations [**G.1**, 2.3.2].

B.17 Type B evaluation (of uncertainty)

Method of evaluation of uncertainty by means other than the statistical analysis of series of observations [**G.1**, 2.3.3]

Error

B.18 Error (of measurement)

The result of a measurement minus a true value of the measurand [**G.2**, 3.10].

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

B.19 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.2**, 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.20 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.2,** 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.21 Arithmetic mean

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

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

B.22 Sample Standard Deviation

s   an estimate of the **population standard deviation** σ [**B.21**] from a sample of n results.

$$s = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}}$$

B.23 Population Standard Deviation

σ the standard deviation of a population using all data in that population.

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n}}$$

B.24 Standard deviation of the mean

$s_{\bar{x}}$ The standard deviation $s_{\bar{x}}$ of the mean $\bar{x}$ of n samples taken from a population is given by

$$s_{\bar{x}} = \frac{\sigma}{\sqrt{n}}$$

The terms "standard error" and "standard error of the mean" have also been used to describe the same quantity.

B.25 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.

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

Appendix C - Structure of Analytical Procedure

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, indicates the factors which should be considered.

1. **Sampling**

   Homogeneity estimate.
   Need several samples from different parts of bulk?
   Is bulk medium static or flowing?
   Physical state of bulk (solid, liquid, gas?)
   Temperature, pressure effects.
   Does sampling process affect composition? *e.g.* differential adsorption in sampling system.

2. **Sample preparation**

   Procedures for ensuring representative sub-sample.
   Dissolution.
   Extraction.

   Contamination.
   Derivatisation (chemical effects)
   Dilution errors.
   Concentration.
   Control of speciation effects.

3. **Presentation of Certified Reference Materials to the measuring system**

   Carry-over in auto analysers.
   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

5. **Analysis**

   Operator effects, *e.g.* colour blindness, parallax, other systematic errors.
   Avoidance of contamination and cross contamination.
   Reagent purity.
   Instrument parameter settings, *e.g.* myriad parameters on integrators.

6. **Data Processing**

   Averaging.
   Control of rounding and truncating.
   Statistics.
   Processing algorithms (model fitting, *e.g.* 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 - Calculating Combined Uncertainty

D.1 Following the estimation of individual components of uncertainty, the next stage is to combine the standard uncertainties using one of the procedures described below. If the mathematical model does not correspond to one of the simplified standard forms then the general procedure, requiring the generation of partial differentials, must be employed.

D.2 The final stage is to multiply the combined standard uncertainty by the chosen coverage factor in order to assign a confidence level to the result.

Combined Standard Uncertainty

D.3 The general relationship between the uncertainty $\mathbf{u(y)}$ of a value $\mathbf{y}$ and the uncertainty of the independent parameters p, q, ... on which it depends is

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

where y(p,q,...) is a function of several parameters p,q,..., $\partial y/\partial p$ is the partial differential of y with respect to p and so on. Each variable's contribution is just the square of the associated uncertainty expressed as a standard deviation multiplied by the square of the relevant partial differential.

D.4 Where variables are not independent, the relationship is more complex:

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

where $\mathbf{s}(x,ik)$ is the covariance between x_i and x_k. In practice, the covariance is often related to the correlation coefficient $\mathbf{r}_{ik}$ using

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

where $-1 \leq \mathbf{r}_{ik} \leq 1$.

D.5 For many purposes, these expressions 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.* $\mathbf{y}=k(p+q+r+...)$ where k is a constant, the combined standard uncertainty $\mathbf{u}_c(y)$ is given by

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

Rule 2
For models involving only a product or quotient, *e.g* $\mathbf{y}=k(pqr...)$, where k is a constant, the combined standard uncertainty $\mathbf{u}_c(y)$ is given by

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

where $(\mathbf{u}(p)/\overline{p})$ 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.

D.6 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.

D.7 The following examples illustrate the use of the above rules:

Example 1

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

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

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

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.

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

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

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

Combined Expanded Uncertainty

D.8   Once a value for the combined standard uncertainty **u**$_c$(y) has been calculated, a final stage is required in order to produce the expanded uncertainty value, which provides an interval expected to include a large fraction of the distribution of values reasonably attributable to the measurand. This is achieved by multiplying the combined standard uncertainty by the chosen coverage factor k.

D.9   The choice of coverage factor will depend on knowledge of the use to which the result is put, experience and the degree of confidence required. The value of k will normally be in the range 2 to 3. For most purposes, the value of 2 is recommended.

D.10 Where the distributions concerned are normal, a coverage factor of 2 gives an interval containing approximately 95% of the distribution of values.

D.11 Continuing with the previous examples gives:

Example 1

For most purposes, k is chosen as 2.

$U = 2 \times 0.260 = \pm 0.52$

$\Rightarrow \mathbf{y} = 7.61 \ \pm 0.52$

Example 2

For a higher level of confidence, k is chosen as 3.

$U = 3 \times 0.024 = 0.072$

$\Rightarrow \mathbf{y} = 0.56 \pm 0.072$

Where the distribution about **y** is known to be normal, a coverage factor of 3 gives an interval including over 99.6% of the distribution.

D.12 It is not recommended that a value of k=2 is taken to imply a 95% confidence interval without a knowledge of the distribution concerned.
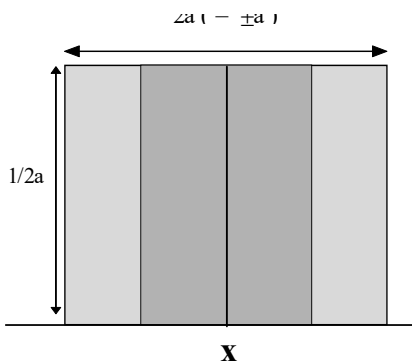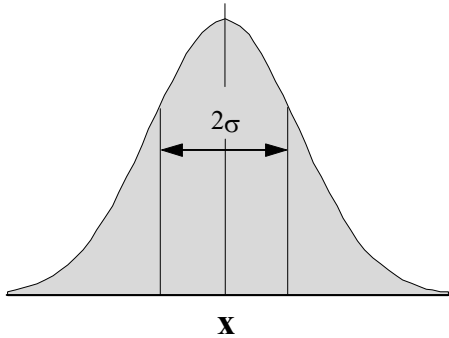
Appendix E - Useful Statistical Procedures

E.1 Choosing 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 (eg 25ml ± 0.05ml) • An estimate is made in the form of a maximum range (±a) with no knowledge of the shape of the distribution.	$u(x)=\dfrac{a}{\sqrt{3}}$

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 or σ, a relative standard deviation $s/\overline{x}$, 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.	$u(x) = s$ $u(x) = s$ $u(x)=x.(s/\overline{x})$ $u(x)=\dfrac{CV}{100}\cdot x$ $u(x) = \mathbf{I}/2$ (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 Appendix D. 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.5]**.

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

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

provided that either $\mathbf{y}(x_1, x_2...x_n)$ is linear in x_i or $\mathbf{u}(x_i)$ is small compared to x_i, the partial differentials $(\partial y/\partial x_i)$ can be approximated by:-

$$\frac{\partial y}{\partial x_i} \approx \frac{y(x_i + \mathbf{u}(x_i)) - y(x_i)}{\mathbf{u}(x_i)}$$

Multiplying by $\mathbf{u}(x_i)$ to obtain the uncertainty $\mathbf{u}(y,x_i)$ in y due to the uncertainty in x_i gives

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

Thus $u(y,x_i)$ is just the difference between the values of y calculated for $[x_i + \mathbf{u}(x_i)]$ and x_i respectively.

E.2.3 The assumption of linearity or small values of $\mathbf{u}(x_i)/x_i$ 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 $\mathbf{u}(x_i)$. Reference G.5 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 ..) 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 $2 \times r(p,q) \times u(y,p) \times u(y,q)$ is added to the calculated sum before taking the square root. Correlation can therefore easily be included by adding suitable extra terms to the spreadsheet.

EXAMPLE:

Applying this procedure to the calculation of the uncertainty on C_0 in example 2 in Appendix A gives the spreadsheet shown in Figure E2.4 The uncertainty on C_0, given in cell B19 is 0.016 in agreement with the result calculated algebraically. Examination of row 17 shows that the largest component comes from f_{temp} and the only other significant component comes from A_0.

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					

Figure E2.4
Example: Numerical calculation for example A.2

	A	B	C	D	E	F	G	H	I	J	K	L
1			A.0	A.1	A.2	f.5	C.2	d	f.acid	f.time	f.temp	f.dil
2		Value:	53	21.8	101.4	5	0.5	1	1	1	1	1
3		Uncertainty	0.62	0.39	0.22	0.0055	0.0017	0	0.0064	0.003	0.06	0.00036
4												
5	A.0	53	53.62	53	53	53	53	53	53	53	53	53
6	A.1	21.8	21.8	22.19	21.8	21.8	21.8017	21.8	21.8	21.8	21.8	21.8
7	A.2	101.4	101.4	101.4	101.62	101.4	101.4	101.4	101.4	101.4	101.4	101.4
8	f.5	5	5	5	5	5.0055	5	5	5	5	5	5
9	C.2	0.5	0.5	0.5	0.5	0.5	0.5017	0.5	0.5	0.5	0.5	0.5
10	d	1	1	1	1	1	1	1	1	1	1	1
11	f.acid	1	1	1	1	1	1	1	1.0064	1	1	1
12	f.time	1	1	1	1	1	1	1	1	1.003	1	1
13	f.temp	1	1	1	1	1	1	1	1	1	1.06	1
14	f.dil	1	1	1	1	1	1	1	1	1	1	1.00036
15	C.0	0.257	0.260	0.256	0.256	0.257	0.258	0.257	0.258	0.258	0.272	0.257
16			0.00312	-0.00120	-0.00043	-0.00007	0.00087	0.00	0.00085	0.00077	0.01541	0.00009
17		2.51E-04	9.71E-06	1.43E-06	1.87E-07	4.46E-09	7.53E-07	0.00	7.18E-07	5.93E-07	2.37E-04	8.55E-09
18												
19	u(C.0)	**0.016**										
20												

The concentration C_0, given by

$$C_0 = \left[\left(\frac{A_0 - A_1}{A_2 - A_1} \right) \cdot \left(C_2 - \frac{C_2}{f_5} \right) + \frac{C_2}{f_5} \right] \cdot d \cdot f_{acid} \cdot f_{time} \cdot f_{temp}$$

This calculation is inserted at row 15, using the values in the column immediately above. Row 2 and Column B contain the experimental values.

The table (right) shows the remaining calculations.

NOTE: Column C is an example. For column D, cell D**6** contains the value D2+D3, with the remainder of D5:D14 set to D2 to L2 respectively

Cell	Formula
C5	C2+C3 (Note 1)
C6...C14	D2...L2
C16	C15-B15
C17	C16∗C16
B17	SUM(C17:L17)
B19	SQRT(B17)

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.:

$$y = mx + c$$

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

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

It is usual to determine the constants m and c 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 m and c, their variances var(m), var(c) and their covariance, covar(m,c), are determined by the method of least squares, the variance on x, var(x), obtained using the formula in Appendix D and differentiating, is given by

$$var(x) = \frac{var(y) + x^2 \cdot var(m) + 2 \cdot covar(c,m)}{m^2}$$

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

From the RMS error or the variance of residuals S.
var(x) is approximately equal to S^2/m^2, where S^2 is the variance of the y values about the fitted line:

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

and $(y_i - \hat{y}_i)$ is the residual for the ith point. S can also be calculated from the RMS error using

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

it follows that S is given by

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

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

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

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

$$u(x_{obs}, y) =$$

$$\sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{m^2 \cdot (n-2)} \cdot \left(1 + \frac{1}{n} + \frac{(y_{obs} - \bar{y})^2}{m^2 \cdot (\sum(x_i^2) - (\sum x_i)^2/n)}\right)}$$

where $(y_i - \hat{y}_i)$ is the residual for the ith point, n is the number of data points in the calibration, m 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 :-

$$var(x) = [\, s(y_c)\, /m\,]^2$$

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

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

which 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 $u(y,const)$ and $u(x,const)$, then the uncertainty on the interpolated value x_{obs} is given by:-

$$u(x_{obs})^2 =$$
$$u(x,const)^2 + (u(y,const)/m)^2 + var(x)$$

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 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, taken from the body of the document

- A reference to the body of the document where possible.

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
				Example	Value	
Mass (absolute)	Balance calibration uncertainty	Limited accuracy in calibration	Stated on calibration certificate, converted to standard deviation	4-figure balance	$0.5/\sqrt{3} = 0.3$ mg	Example 3 table OPT1.1
	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.	Example 3 table OPT1.3
	Run to run variation	Various	Standard deviation of successive sample or check weighings		ca. 0.5x last significant digit.	Example 3 table OPT1.1
	TOTAL specification uncertainty	Combination of above	Combine above as standard deviations	4-figure balance	0.5 mg	Example 3.ii)
	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
				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	Example 3 table OPT1.1; Example 3.ii)
	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	$\sqrt{0.07^2 + \left(\frac{0.1}{1.96}\right)}$ $=0.087$mg	Example 1, step 1

Determination	Uncertainty Components	Cause	Method of determination	Typical values		Example
				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)	$0.02 / \sqrt{3} = 0.01$ ml	Example 1, section 2
	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 2×10^{-4} K^{-1} for water and 1×10^{-3} K^{-1} for organic liquids.	100 ml water	0.03 ml for operating within 3°C of the stated operating temperature	Example 1, section 2;
	Run to run variation	Various	Standard deviation of successive check deliveries (found by weighing)	25 ml pipette	Replicate fill/weigh: $s = 0.0092$ ml	Example 1, section 2 (Steps 5 & 6); Example 3, part vi) and table A3.4

Determination	Uncertainty Components	Cause	Method of determination	Typical values		Example
				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/√3 = 0.06%	Example 1, sec 2, Purity P_a
	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/√3 = 1.2 mg.l^{-1} (0.0012 as RSD)	Example 2, sec 4,5.
	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	Example 2, sec 4,5

Determination	Uncertainty Components	Cause	Method of determination	Typical values		Example
				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	$1.63/\sqrt{7} = 0.62$	Example 2, sec 6.
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 contaminat-ed and 360 uncontam-inated bulk portions: RSD = 0.58	Example 3, sec A3.6

Determination	Uncertainty Components	Cause	Method of determination	Typical values		Example
				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/√42= 4.3% (0.048 as RSD)	Example 3, sec iii)
	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.	Example 3, sec iii)

Appendix G - Bibliography

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Index