The Fitness for Purpose of Analytical Methods

A Laboratory Guide to Method Validation and Related Topics

This document has been developed by a EURACHEM Working Group from a draft originally produced by LGC. The membership of the EURACHEM group is

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THE FITNESS FOR PURPOSE OF ANALYTICAL METHODS

A Laboratory Guide to Method Validation and Related Topics

1. Preface

An initiative in the UK to promote good practice in analytical measurement has identified six principles of analytical practice which, taken together, are considered to constitute best practice. The six principles which are described in more detail in a separate guide [1] are:

1. “Analytical measurements should be made to satisfy an agreed requirement.” (i.e. to a defined objective)

2. “Analytical measurements should be made using methods and equipment which have been tested to ensure they are fit for purpose.”

3. “Staff making analytical measurements should be both qualified and competent to undertake the task.” (and demonstrate that they can perform the analysis properly).

4. “There should be a regular independent assessment of the technical performance of a laboratory”

5. “Analytical measurements made in one location should be consistent with those made elsewhere.”

6. “Organisations making analytical measurements should have well defined quality control and quality assurance procedures”

These principles are equally relevant to laboratories whether they are working in isolation or producing results which need to be compared with those from other laboratories.

This document is principally intended to assist laboratories in implementing Principle 2, by giving guidance on the evaluation of testing methods to show that they are fit for purpose.

2. Introduction

2.1 Method validation is an important requirement in the practice of chemical analysis. However, awareness of its importance, why it should be done and when, and exactly what needs to be done, seems to be poor amongst analytical chemists. Much advice related to method validation already exists in the literature, especially related to particular methods, but more often than not is underused. Some analysts see method validation as something that can only be done by collaborating with other laboratories and therefore do not do it.

2.2 The purpose of this guide is to discuss the issues related to method validation and increase readers' understanding of what is involved, why it is important, and give some idea of how it can be achieved.

2.3 The guide is expected to be of most use to a) laboratory managers who are responsible for ensuring the methods within their responsibility are adequately validated and b) the analysts responsible for carrying out studies on methods for validation purposes. Other staff may find the guidance of use as a source of background information - senior staff from a management point of view and junior staff from a technical point of view.

2.4 The guide is aimed at laboratories needing to validate methods but working in isolation, with no immediate possibility of participation in collaborative trials. It aims to direct the reader towards established protocols where these exist and where they do not, give a simple introduction to the processes involved in validation and provide some basic ideas to enable the reader to design their own validation strategies. It includes references to further material on particular technical aspects of validation.

2.5 The guide avoids a large emphasis on the use of statistics although undoubtedly those with a working knowledge of simple statistics will find the method validation process easier to understand and implement. Where appropriate, formulae have been included with the definitions in Annex A.

2.6 The analyst's understanding of method validation is inhibited by the fact that many of the technical terms used in processes for evaluating methods vary in different sectors of analytical measurement, both in terms of their meaning and also the way they are determined. This guide cannot say where a term is used correctly or incorrectly although it is intended to provide some clarification. The best advice when using a term that may be subject to misinterpretation is to state which convention has been used, so that any confusion to others using the data is avoided.

2.7 **Notes on the use of this guide:**

2.7.1 The most important terms used in the guide are defined in Annex A. ISO and IUPAC definitions have been provided wherever possible. Users should note that there is no universal agreement on the definition of some of the terms used in method validation.

2.7.2 In chapter 6, the shaded boxes provides quick reference advice for determining each method performance parameter. However, it is recognised that in many cases laboratories will not have the time and resources to carry out experiments in such detail. Carrying out the operations described in the boxes, using less replication than suggested, will still yield useful information and is certainly better than no work at all. However, the information provided will be less reliable than if full replication had been utilised.

3. What is method validation?

3.1 The ISO definition of ***validation*** is given in Annex A. One can interpret this for ***method validation*** as being the process of defining an analytical requirement, and confirming that the method under consideration has performance capabilities consistent with what the application requires. Implicit in this is that it will be necessary to evaluate the method's performance capabilities. This is consistent with the interpretation of the ISO definition by Morkowski [2]. The judgement of method suitability is important; in the past method validation has tended to concentrate on the process of evaluating the performance parameters.

3.2 It is implicit in the method validation process that the studies to determine method performance parameters are carried out using equipment that is within specification, working correctly, and adequately calibrated. Likewise the operator carrying out the studies must be competent in the field of work under study and have sufficient knowledge related to the work to be able to make appropriate decisions from the observations made as the study progresses.

3.3 Method validation is usually considered to be very closely tied to method development, indeed it is often not possible to determine exactly where method development finishes and validation begins. Many of the method performance parameters that are associated with method validation are in fact usually evaluated, at least approximately, as part of method development.

4. Why is method validation necessary?

(a). Importance of Analytical Measurement

4.1 Millions of analytical measurements are made every day in thousands of laboratories around the world. There are innumerable reasons for making these measurements, for example: as a way of valuing goods for trade purposes; supporting healthcare; checking the quality of drinking water; analysing the elemental composition of an alloy to confirm its suitability for use in aircraft construction; forensic analysis of body fluids in criminal investigations. Virtually every aspect of society is supported in some way by analytical measurement.

4.2 The cost of carrying out these measurements is high and additional costs arise from decisions made on the basis of the results. For example, tests showing food to be unfit for consumption may result in compensation claims; tests confirming the presence of banned drugs could result in fines, imprisonment or even, in some countries, execution. Clearly it is important to determine the correct result and be able to show that it is correct.

(b) The Professional Duty of the Analytical Chemist

4.3 If the result of a test cannot be trusted then it has little value and the test might as well have not been carried out. When a “customer” commissions analytical work from a laboratory, it is assumed that the laboratory has a degree of expert knowledge that the customer does not have themselves. The customer expects to be able to trust results reported and usually only challenges them when a dispute arises. Thus the laboratory and its staff have a clear responsibility to justify the customer’s trust by providing the right answer to the analytical part of the problem, in other words results that have demonstrable ‘fitness for purpose’. Implicit in this is that the tests carried out are appropriate for the analytical part of the problem that the customer wishes solved, and that the final report presents the analytical data in such a way that the customer can readily understand it and draw appropriate conclusions. Method validation enables chemists to demonstrate that a method is ‘fit for purpose’.

4.4 For an analytical result to be fit for its intended purpose it must be sufficiently reliable that any decision based on it can be taken with confidence. Thus the method performance must be validated and the uncertainty on the result, at a given level of confidence, estimated. Uncertainty should be evaluated and quoted in a way that is widely recognised, internally consistent and easy to interpret. Most of the information required to evaluate uncertainty can be obtained during validation of the method. This topic is dealt with briefly in section 6; further guidance is listed in the bibliography.

4.5 Regardless of how good a method is and how skilfully it is used, an analytical problem can be solved by the analysis of samples only if those samples are appropriate to the problem. Taking appropriate samples is a skilled job, requiring an understanding of the problem and its related chemistry. A laboratory, as part of its customer care, should, wherever possible, offer advice to the customer over the taking of samples. Clearly there will be occasions when the laboratory cannot themselves take or influence the taking of the samples. On these occasions results of analysis will need to be reported on the basis of the samples as received, and the report should make this distinction clear.

5. When should methods be validated?

5.1 A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem. For example:

- new method developed for particular problem;
- established method revised to incorporate improvements or extended to a new problem;
- when quality control indicates an established method is changing with time;
- established method used in a different laboratory, or with different analysts or different instrumentation;
- to demonstrate the equivalence between two methods, e.g. a new method and a standard.

5.2 The extent of validation or revalidation required will depend on the nature of the changes made in reapplying a method to different laboratories, instrumentation, operators, and the circumstances in which the method is going to be used. Some degree of validation is always appropriate even when using apparently well-characterised standard or published methods This issue is dealt with in more detail in Section 7.

6. How should methods be validated?

(a) Who carries out method validation?

6.1 The laboratory using a method is responsible for ensuring that it is adequately validated, and if necessary for carrying out further work to supplement existing data. For example, where a method has been validated by a standards approving organisation, such as AOAC International, the user will normally need only to establish performance data for their own use of the method.

6.2 Much has been published in the literature concerning method validation by collaborative study. There are a number of protocols relating to this type of validation [3-7]. If a method is being developed which will have wide-ranging use, perhaps as a published standard procedure, then collaborative study involving a group of laboratories is probably the preferred way of carrying out the validation. However, it is not always a suitable option for industrial laboratories. The application for which the method is required may be esoteric to the extent that no other laboratories would be interested in collaboration. Those that might be interested could be competitors. Where it is inconvenient or impossible for a laboratory to enter into collaborative study, a number of questions are raised:

- Can laboratories validate methods on their own, and if so, how?
- Will methods validated in this way be recognised by other laboratories?
- What sort of recognition can be expected for in-house methods used in a regulatory environment?

6.3 Working in isolation inevitably reduces the amount of validation data that can be gathered for a method. Principally it restricts the type of information on inter-laboratory comparability. This information is not always required so this may not be a problem. If necessary, it may be feasible to get some idea of the comparability of measurement results of any given method with others obtained elsewhere by measuring certified reference materials or by comparing the method against one for which the validation has been carried out.

6.4 Whether or not methods validated in a single laboratory will be acceptable for regulatory purposes depends on any guidelines covering the area of measurement concerned. It should normally be possible to get a clear statement of policy from the appropriate regulatory body. The following example is taken from UK Drinking Water Inspectorate guidelines [8].

> "A laboratory using an analytical method which is not referenced to a fully validated authoritative method will be expected to demonstrate that the method has been fully documented and tested to the standard currently expected of an authoritative reference method. It should demonstrate that the following have been established:
>
> a. the required tolerances of all measurements undertaken within the method (volume, temperatures, masses etc.);
> b. the forms of the determinand measured, including speciation;
> c. the effect of interferences has been widely investigated and quantified;
> d. significant sources of error have been identified and adequate means of controlling them have been identified."

6.5 The Association of Official Analytical Chemists (AOAC International) has always been a strong supporter of interlaboratory trial as the preferred way of validating methods. More recently it has introduced its "Peer Verified Method Program" [9] for the validation of methods by laboratories working with only one or two others.

(b) Deciding what degree of validation is required

6.6 The laboratory has to decide which method performance parameters need to be characterised in order to validate the method. Characterisation of method performance is an expensive process and inevitably it may be constrained by time and cost considerations. Starting with a carefully considered analytical specification provides a good base on which to plan the validation process, but it is recognised that in practice this is not always possible. The laboratory should do the best it can within the constraints imposed, taking into account customer requirements, existing experience of the method, and the need for compatibility with other similar methods already in use within the laboratory or used by other laboratories. Some of the parameters may have been determined approximately during the method development stage. Often a particular set of experiments will yield information on several parameters, so with careful planning the effort required to get the necessary information can be minimised.

6.7 The implications of the constraints discussed above are particularly critical where the method is not going to be used on a routine basis. Validation of methods which are going to be used on a routine basis is a comparatively straightforward process. However, should the same validation processes be applied to ad-hoc analysis? Clearly the same principles apply as for routine testing. It is necessary to be able to have an adequate level of confidence in the results produced, otherwise the work is not worth doing. Striking the balance between time and costs constraints and the need to validate the method is difficult and in some circumstances it may be more appropriate to subcontract the work to another laboratory where it can be performed on a routine basis.

6.8 Validation requirements may be specified in guidelines within a particular sector of measurement relevant to the method and it is recommended that where these are available they are followed. For example validation of a method for food analysis should be consistent with the validation strategy used by AOAC International. This will ensure that particular validation terminology together with the statistics used are interpreted in a manner consistent within the relevant sector. Official recognition of a method may require characterisation using collaborative study. Regulatory requirements may require a particular method to be followed to the letter even though the laboratory considers it to be unsound or inaccurate. Additional validation will be needed to confirm the satisfactory performance of the analyst.

(c) The Analytical Requirement

6.9 Faced with a particular analytical problem, ideally the laboratory should firstly agree with the customer an analytical requirement which defines the performance requirements that a method

must have to be suitable for solving the analytical problem. In response to this requirement, the laboratory can evaluate existing methods for suitability and if necessary develop a new method. This iterative process of development and evaluation continues until the method is deemed capable of meeting the requirement; further development is unnecessary and the analytical work can proceed. This process of evaluation of performance criteria and confirming that the method is suitable, illustrated in Figure 1, is method validation. Table 1 shows the sort of questions which might be posed in formalising an analytical requirement (column 1) and the corresponding performance parameters of the method which may need to be characterised (column 2).

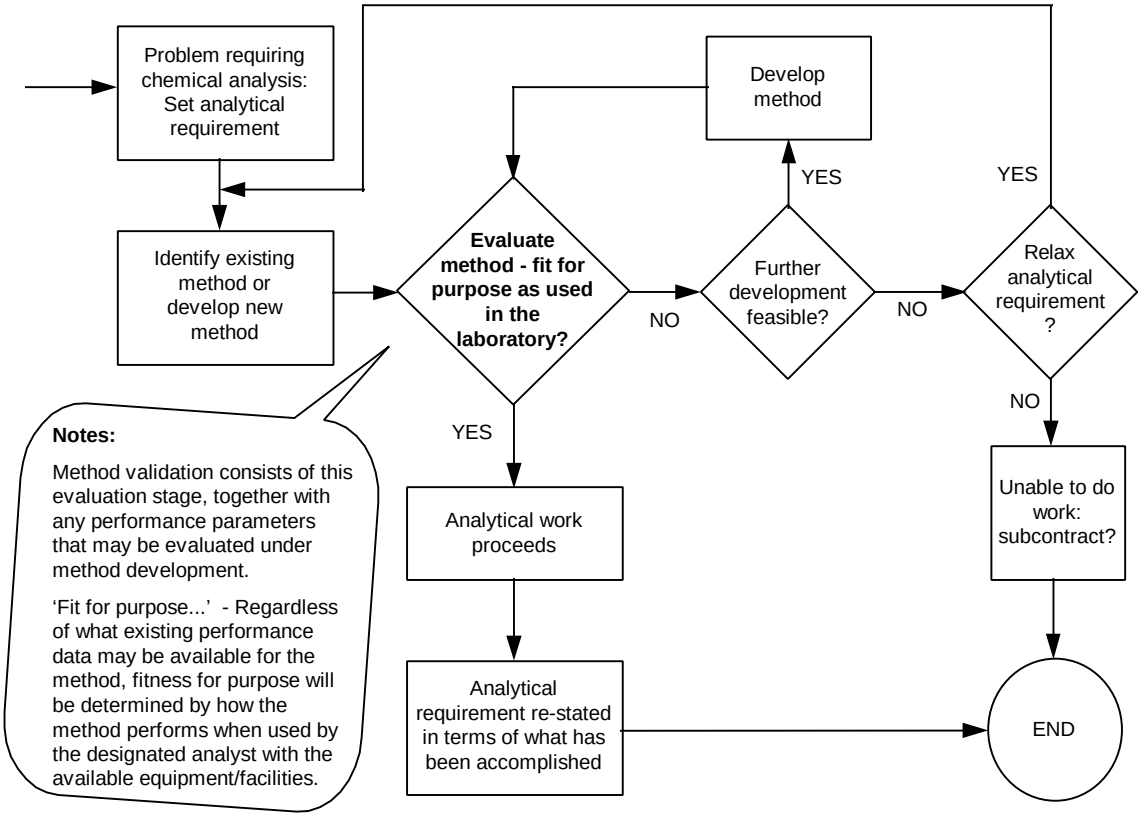


Figure 1: Choosing, developing and evaluating methods

6.10 In reality an analytical requirement is rarely agreed beforehand in such a formal way. More often, if it is stated at all, it will be done retrospectively. Customers usually define their requirements in terms of cost and/or time and rarely know how well methods need to perform, although performance requirements for methods may be specified where the methods support a regulatory requirement or compliance with a specification. It will usually be left to the analyst's discretion to decide what performance is required from the method and very often this will mean setting an analytical requirement in line with the method's known capability. Financial

constraints may dictate that development of a method that satisfies a particular analytical requirement is not economically feasible, in which case the decision must be taken whether to relax the requirement to a more achievable level or rethink the justification for the analysis.

Table 1: Analytical requirement elements and related performance characteristics

Analytical requirement elements	Related performance characteristics..	..dealt with in section	Notes
What sort of answer is required - qualitative or quantitative?	Confirmation of identity, selectivity/specificity Limit of detection Limit of quantification	6.13-6.19 6.20-6.23 6.24-6.25	
Is the analyte dispersed or localised?			a)
Is the analyte present in more than one form, are you interested in extractable, free or total analyte?	Confirmation of identity Recovery	6.13-6.19 6.46-6.47	
What are the analyte(s) of interest and the likely levels present (%, $\mu g g^{-1}$, $n g g^{-1}$, etc.)?	Confirmation of identity Limit of detection Limit of quantification Working & linear ranges	6.13-6.19 6.20-6.23 6.24-6.25 6.26-6.29	
How accurate and precise must the answer be? / what degree of uncertainty is allowed and how is it to be expressed?	Recovery Accuracy / trueness Repeatability precision Reproducibility precision	6.45-6.46 6.30-6.36 6.37-6.39 6.37-6.38, 6.40	b)
What is the chemical, biological and physical nature of the matrix?			a)
What are the likely interferences to the analyte(s)?	Selectivity/specificity	6.13-6.19	
Is sampling and subsampling required (and will this be done within the laboratory)?			a)
What if any are the restrictions on sample size/availability?			a)
Do resource constraints apply and how - people, time, money, equipment & reagents, laboratory facilities?			a)
Do results need to be compared with results from other laboratories?	Ruggedness/robustness Reproducibility precision	6.45 6.37-6.38, 6.40	
Do results need to be compared with external specifications?	Accuracy Reproducibility precision	6.30-6.36 6.37-6.38, 6.40	

Notes:

a) Not all of the elements of the analytical requirement will link directly to method validation requirements. Some of them will dictate more generally as to whether particular techniques are applicable. For example, different techniques will be applicable according to whether the analyte is dispersed through the sample or isolated on the surface.

b) One essential element of the analytical requirement is that it should be possible to judge whether or not a method is suitable for its intended purpose and thus must include the required uncertainty expressed either as a standard uncertainty or an expanded uncertainty.

(d) Method Development

6.11 Method development can take a number of forms. At one extreme, it may involve adapting an existing method, making minor changes so that it is suitable for a new application. For example, a method required to determine toluene in water might be adapted from an established method for benzene in water. The matrix is the same, and the two analytes have broadly similar properties. It is likely that the same principles of isolation, identification, and quantitation that are applied to the benzene can also be applied to the toluene. If, on the other hand, a method is required to determine benzene in soil, adaptation of the benzene in water method may not be the best option. Adaptation of some other method determining organics in soil may be a better starting point.

6.12 At the other extreme the analytical chemist may start out with a few sketchy ideas and apply expertise and experience to devise a suitable method. It may involve significant innovation based on novel exploitation of known properties of the analyte or measurand. This clearly involves a great deal more work, and initially at least a degree of doubt as to whether the final method will be successful. It is not infrequent for method development to involve working on a number of different ideas simultaneously and eventually choosing one winner.

(e) The different performance parameters of a method and what they show

Confirmation of identity and selectivity/specificity *(see Annex A for definitions)*

6.13 In general analytical methods can be said to consist of a measurement stage which may or may not be preceded by an isolation stage. It is necessary to establish that the signal produced at the measurement stage, or other measured property, which has been attributed to the analyte, is only due to the analyte and not from the presence of something chemically or physically similar or arising as a coincidence. This is confirmation of identity. Whether or not other compounds interfere with the measurement of the analyte will depend on the effectiveness of the isolation stage and the selectivity/specificity of the measurement stage. Selectivity and specificity are measures which assess the reliability of measurements in the presence of interferences. Specificity is generally considered to be 100% selectivity but agreement is not universal. Where the measurement stage is non-specific, it is possible to state that certain analytes do not interfere, having first checked that this is the case. It is far more difficult to state that nothing interferes since there is always the possibility of encountering some hitherto unrecognised interference. There will be cases where chemical interferences can be identified for a particular method but the chances of encountering them in real life may be improbable. The analyst has to decide at what point it is reasonable to stop looking for interferences. These parameters are applicable to both qualitative and quantitative analysis.

6.14 If interferences are present which either cannot be separated from the analyte of interest, or if interferences which the analyst is not aware of are present, then those interferences will have a number of effects. Depending on how the identity of the analyte is established interferences may inhibit confirmation, for example by distorting the signal arising from the analyte. They may also have the effect of apparently enhancing the concentration of the analyte by contributing to the signal attributed to the analyte, (or conversely suppressing the concentration of the analyte if they contribute a negative signal). Interferences will usually affect the slope of the calibration curve differently than will the analyte of interest, so the slope of the calibration curve in the method of additions may affect the linearity of the curve. This effect has the potential to indicate the possible presence of a hidden interference, but it is not helpful if the recovery curve is inherently non-linear.

6.15 The selectivity of a method is usually investigated by studying its ability to measure the analyte of interest in test portions to which specific interferences have been deliberately introduced (those thought likely to be present in samples). Where it is unclear whether or not interferences are already present, the selectivity of the method can be investigated by studying its ability to measure the analyte compared to other independent methods/techniques.

6.16 **Examples:**

6.16.1 A peak in a chromatographic trace may be identified as being due to the analyte of interest on the basis that a reference material containing the analyte generates a signal at the same point on the chromatogram. But, is the signal due to the analyte or to something else which coincidentally co-elutes? It could be either or both. Identification of the analyte by this means only is unreliable and some form of supporting evidence is necessary. For example, the chromatography could be repeated using a column of different polarity, to see whether the signal and the signal generated by the reference materials still appear at the same time. Where a peak is due to more than one compound, a different polarity column may be a good way of separating the compounds. If available, gas chromatography with mass spectrometric detection could be used to confirm the identity of the analyte or show a mixture to be present. Some detectors can monitor peak purity.

6.16.2 In infra-red spectrometry, identification of unknown compounds may be made by matching absorbances (i.e ‘peaks’) in the analyte spectrum with those of reference spectra stored in a spectral library. Once it is believed the correct identification has been made a spectrum of a reference material of the analyte should be recorded under exactly the same conditions as for the test portion. The more exact the match is required to be (i.e. the more absorbances which are required to coincide between analyte and reference material) the better the confidence which can be placed on the

identification being correct. To be confident of a good match analyte and reference material spectra would need to be recorded under similar conditions and it would also be worthwhile examining how dependant the shape of the spectrum was with respect to how carefully the analyte was isolated and prepared for I.R. analysis. For example, if the spectrum was recorded as a salt disc, the particle size distribution of the test portion in the disc might influence the shape of the spectrum.

6.17 Use of confirmatory techniques can be useful as a means of verifying identities and amounts of analyte. The more evidence one can gather, the better. Inevitably there is a trade-off between costs and time taken for analyte identification and the confidence with which one can decide the identification has been made correctly.

6.18 Some validation protocols confuse confirmation of identity with repeatability. Whereas evaluation of repeatability requires the measurement to be performed several times by one technique, confirmation (i.e. of analyte identity) requires the measurement to be performed by several, preferably independent techniques. Confirmation increases confidence in the technique under examination and is especially useful when the confirmatory techniques operate on significantly different principles. In some applications, for example, the analysis of unknown organics by gas chromatography, the use of confirmatory techniques is essential. When the technique under evaluation is specific, the use of other confirmatory techniques may not be necessary.

6.19 Another aspect of selectivity which must be considered is where an analyte may exist in the sample in more than one form such as: bound or unbound; inorganic or organometallic; or different oxidation states.

Confirmation of identity and selectivity/specificity - Quick Reference

What you do..	..how many times	Calculate / determine	Comments
Analyse samples, and reference materials by candidate and other independent methods	1	Use the results from the confirmatory techniques to assess the ability of the method to confirm analyte identity and its ability to measure the analyte in isolation from other interferences	Decide how much supporting evidence is reasonably required to give sufficient reliability
Analyse samples containing various suspected interferences in the presence of the	1	Examine effect of interferences - does the presence of the interferent enhance or inhibit detection or quantification of the measurands	If detection or quantitation is inhibited by the interferences, further method development will be required.

analytes of interest

Limit of Detection *(see Annex A for definitions)*

6.20 Where measurements are made at low analyte or property levels, e.g. in trace analysis, it is important to know what is the lowest concentration of the analyte or property value that can be confidently detected by the method. The importance in determining this, and the problems associated with it, arise from the fact that the probability of detection does not suddenly change from zero to unity as some threshold is crossed. The problems have been investigated statistically in some detail and a range of decision criteria proposed. Additional confusion arises because there is currently no universal agreement on the terminology applicable. The terms 'limit of detection' or 'detection limit' are not generally accepted although they are used in some sectoral documents[9,10]. ISO uses as a general term 'minimum detectable value of the net state variable' which for chemistry translates as 'minimum detectable net concentration'[11]. IUPAC is cautious in the use of 'detection limit', preferring 'minimum detectable (true) value' [12].

6.21 For validation purposes it is normally sufficient to provide an indication of the level at which detection becomes problematic. For this purpose the "blank + 3s" approach will usually suffice. Where the work is in support of regulatory or specification compliance, a more exact approach such as that described by IUPAC [12] and various others [13-14] is likely to be appropriate. It is recommended that users quote whichever convention they have used when stating a detection limit.

Limit of Detection (LoD) Quick Reference	
What to analyse	**What to calculate from the data**
a) 10 independent sample blanks measured once each or	*Sample standard deviation* '*s*' of a) sample blank values, or b) fortified sample blank values
b) 10 independent sample blanks fortified at lowest acceptable concentration measured once each	Express LoD as the analyte concentration corresponding to a) mean sample blank value + 3*s* or b) 0 + 3*s*
This approach assumes that a signal more than 3s above the sample blank value could only have arisen from the blank much less than 1% of the time, and therefore is likely to have arisen from something else, such as the measurand. Approach a) is only useful where the sample blank gives a non-zero standard deviation. Getting a true sample blank can be difficult.	
c) 10 independent sample blanks fortified at lowest acceptable concentration, measured once each	*Sample standard deviation 's'* of the fortified sample blank values

	Express LoD as the analyte concentration corresponding to sample blank value +4.65*s* (derives from hypothesis testing)
The 'lowest acceptable concentration' is taken to be the lowest concentration for which an acceptable degree of uncertainty can be achieved. Assumes a normal practice of evaluating sample and blank separately and correcting for the blank by subtracting the analyte concentration corresponding to the blank signal from the concentration corresponding to the sample signal. If measurements are made under repeatability conditions, this also gives a measure of the repeatability precision (Annex A, A20)	

6.22 Note that both the mean and the standard deviation of the sample blank are likely to be dependant on the matrix of the sample blank. Limit of detection will therefore be matrix dependent. Similarly, where such criteria are used for critical decisions, the relevant precision values will need to be re-determined regularly in line with actual operating performance.

6.23 For qualitative measurements, there is likely to be a concentration threshold below which specificity becomes unreliable. The threshold may vary if the experiment is repeated at another time with different reagents, fortification, spiking materials, etc. In the example shown in Table 2, positive identification of the analyte has ceased to be 100% reliable below 100 $\mu g.g^{-1}$.

Limit of Detection (LoD) - Qualitative Measurements - Quick Reference

What to analyse	What to calculate from the data
Sample blanks spiked with the analyte at a range of concentration levels. At each concentration level, it will be necessary to measure approximately 10 independent replicates Measurement of the replicates at the various levels should be randomised	A response curve of % positive (or negative) results versus concentration should be constructed, from which it will be possible to determine, by inspection, the threshold concentration at which the test becomes unreliable.

Table 2: Qualitative Analysis - Illustration of how cut-off (i.e. threshold) concentration is determined

Concentration/ $\mu g g^{-1}$	No. of replicates	Positive/negative results
200	10	10/0

100	10	10/0
75	10	5/5
50	10	1/9
25	10	0/10

Limit of Quantitation *(see Annex A for definitions)*

6.24 The 'limit of quantitation' (LoQ) is strictly the lowest concentration of analyte that can be determined with an acceptable level of repeatability precision and trueness. It is also defined by various conventions to be the analyte concentration corresponding to the sample blank value plus 5, 6 or 10 standard deviations of the blank mean. It is also sometimes known as 'limit of determination'. LoQ is an indicative value and should not normally be used in decision making.

6.25 Note that neither LoD nor LoQ represent levels at which quantitation is impossible. It is simply that the size of the associated uncertainties approach comparability with the actual result in the region of the LoD.

Limit of Quantitation (LoQ) - Quick Reference

What to analyse	**What to calculate from the data**
a) 10 independent sample blanks measured once each	*Sample standard deviation 's'* of sample blank value Express LoQ as the analyte concentration corresponding to the sample blank value plus either: i) 5*s*, ii) 6*s*, iii) 10*s*
Getting a true sample blank can be difficult.	
b) Fortify aliquots of a sample blank at various analyte concentrations close to the LoD. Measure, once each, 10 independent replicates at each concentration level.	Calculate the standard deviation '*s*' of the analyte value at each concentration. Plot *s* against concentration and put assign a value to the LoQ by inspection. Express LoQ as the lowest analyte concentration which can be determined with an acceptable level of uncertainty.
Normally LoQ forms part of the study to determine working range. It should not be determined by extrapolation below the lowest concentration fortified blank. If measurements are made under repeatability conditions, a measure of the repeatability precision at this concentration is also obtained.	

Working & Linear Ranges *(see Annex A for definitions)*

6.26 For any quantitative method, it is necessary to determine the range of analyte concentrations or property values over which the method may be applied. Note this refers to the range of concentrations or property values in the solutions actually measured rather than in the original samples. At the lower end of the concentration range the limiting factors are the values of the limits of detection and/or quantitation. At the upper end of the concentration range limitations will be imposed by various effects depending on the instrument response system.

6.27 Within the working range there may exist a linear response range. Within the linear range signal response will have a linear relationship to analyte concentration or property value. The extent of this range may be established during the evaluation of the working range. Note that regression calculations on their own are insufficient to establish linearity. To do this a visual inspection of the line and residuals may be sufficient; objective tests, such as 'goodness-of-fit' tests, are better still [15-17]. In general linearity checks require points at at least 10 different concentrations/ property values.

6.28 Evaluation of the working and linear ranges will also be useful for planning what degree of calibration is required when using the method on a day-to-day basis. It is advisable to investigate the variance across the working range Within the linear range, one calibration point may be sufficient, to establish the slope of the calibration line. Elsewhere in the working range, multi-point (preferably 6+) calibration will be necessary. The relationship of instrument response to concentration does not have to be perfectly linear for a method to be effective but the curve should be repeatable from day to day. Note that the working and linear range may be different for different matrices according to the effect of interferences arising from the matrix.

6.29 Where the method uses some sort of instrumentation it may be useful to consider 'sensitivity', 'discrimination', 'discrimination threshold', and 'response time' (see Annex A).

Working and Linear Range - Quick Reference

Analyse	Repeats	What to calculate from the data	Comments
1. Blank plus reference materials or fortified sample blanks at various concentrations Need at least 6 concentrations plus blank	1	Plot measurement response (y axis) against measurand concentration (x axis) Visually examine to identify approximate linear range and upper and lower boundaries of the working range then go to 2.	Ideally the different concentrations should be prepared independently, and not from aliquots of the same master solution. This will give visual confirmation of whether or not the working range is linear
2., reference materials or fortified sample blanks at at least 6 different concentrations within the linear range	3	Plot measurement response (y axis) against measurand concentration (x axis) Visually examine for outliers which may not be reflected in the regression Calculate appropriate regression coefficient. Calculate and plot residual values (difference between actual y value and the y value predicted by the straight line, for each x value). Random distribution about the straight line confirms linearity. Systematic trends indicate non-linearity then go to 3.	This stage is necessary to test a working range, thought to be linear and where it is intended to use single point calibration. It is unsafe to remove outliers without first checking using further determinations at nearby concentrations. If variance of replicates is proportional to concentration then use a weighted regression calculation rather than a non-weighted regression. In certain circumstances it may be better to try to fit a non-linear curve to the data. Functions higher than quadratic are generally not advised

3. As for LoQ (b)	As for LoQ LoQ effectively forms the lower end of the working range	Work with successively lower concentrations until the accuracy and precision becomes unacceptable

Accuracy *(see Annex A for definitions)*

6.30 'Accuracy' expresses the closeness of a result to a true value (The ISO 3534-1 definition has been assumed in this guidance). Method validation seeks to quantify the likely accuracy of results by assessing both systematic and random effects on results. Accuracy is, therefore, normally studied as two components: 'trueness' and 'precision'. The 'trueness' (of a method) is an expression of how close the mean of a set of results (produced by the method) is to the true value. Trueness is normally expressed in terms of bias. 'Precision' is a measure of how close results are to one another, and is usually expressed by measures such as standard deviation, which describe the spread of results. In addition, an increasingly common expression of accuracy is 'measurement uncertainty', which provides a single figure expression of accuracy. These three different parameters will be discussed in turn.

Trueness

6.31 Practical assessment of trueness relies on comparison of mean results from a method with known values, that is, trueness is assessed against a reference value (*i.e*. true value or conventional true value). Two basic techniques are available: checking against reference values for a characterised material or from another characterised method. Reference values are ideally traceable to international standards. Certified reference materials are generally accepted as providing traceable values; the reference value is then the certified value of the CRM. Note that reference values, certified or otherwise, may be absolute (traceable to the SI) or conventional, that is, generally agreed upon for a particular purpose.

6.32 To check trueness using a reference material, determine the mean and standard deviation of a series of replicate tests, and compare with the characterised value for the reference material. The ideal reference material is a certified, natural matrix reference material, closely similar to the samples of interest. Clearly, the availability of such materials is limited. Reference materials for validation may accordingly be:

- prepared by spiking typical materials with pure certified reference materials or other materials of suitable purity and stability;
- typical, well-characterised materials checked in-house for stability and retained for in-house QC.

6.33 Validation needs to fit the purpose, so the choice of reference material may be affected by the end use. The reference material must be *appropriate to the use.* For regulatory work, a relevant certified material should be used, ideally matrix matched. For methods used for long-term in-house work, a stable in-house material or certified reference material should be used. For short-term or non-critical work, a prepared standard or spike is often sufficient.

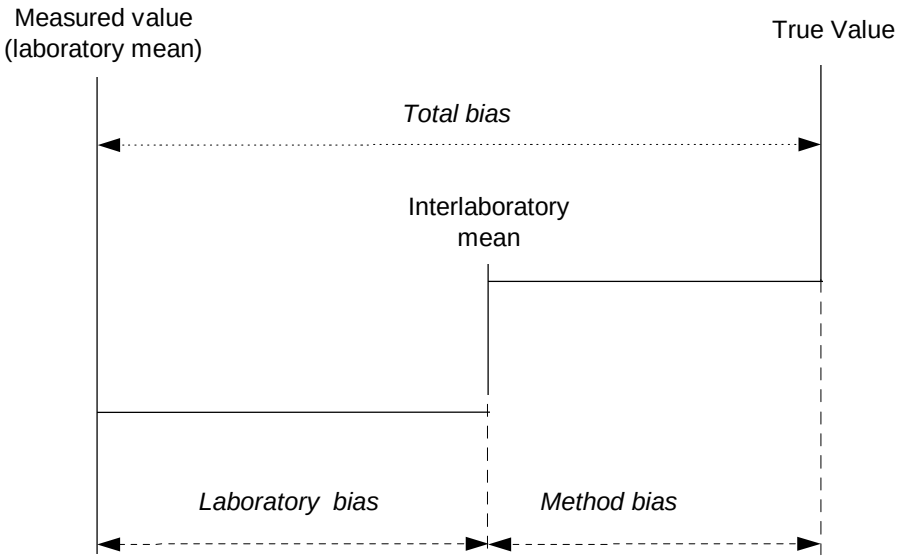
6.34 To check against an alternative method, compare results from the two methods for the same sample or samples. The sample(s) may be CRMs, in-house standards, or simply typical samples. There are advantages to using CRMs, since these have known stability and homogeneity, and additionally give an indication of bias with respect to international standards. On the other hand, CRMs are costly and may not be representative of typical samples.

Note: it may be necessary to repeat a trueness check where materials are encountered that have radically different matrices or analyte concentration levels from those originally checked.

Interpreting bias measurements

6.35 Figure 2 shows two components of bias, referred to here as method and laboratory components of bias. The method bias arises from systematic errors inherent to the method whichever laboratory uses it. Laboratory bias arises from additional systematic errors peculiar to the laboratory and its interpretation of the method. In isolation, a laboratory can only estimate the combined bias. However, in checking bias, it is important to be aware of the conventions in force for the purpose at hand. For example in much Food regulation, regulatory limits are set in terms of the results obtained by the standard method. Bias arising solely from the particular method (see Figure 2) is therefore compensated for, and comparability with other laboratories using the same method is the main concern. The overall bias determined by a particular laboratory during validation should then be compared with any reported bias for the regulatory method.

6.36 For most purposes, however, acceptability of bias should be decided on the basis of overall bias measured against appropriate materials or reference methods, taking into account the precision of the method, any uncertainties in reference material values, and the accuracy required by the end use. Statistical significance tests are recommended.



Note: Laboratory and method biases are shown here acting in the same direction. In reality this is not always the case.

Figure 2: Types of bias

Accuracy and Trueness - Quick Reference

Analyse	No of times	Calculate	Comments
a) Reagent blank and reference material using candidate method	10	Mean blank value subtracted from mean analyte value for reference material. Compare with true or accepted true values for the reference material Gives a measure of the method's bias	Subject to the uncertainty of the blank being a true blank, characterisation of the reference material
b) Reagent blank and reference/test material using candidate method and independent (preferably primary) method	10	Mean blank value subtracted from mean analyte value for reference/test material. Compare with similar measurements made using independent/primary method Gives a measure of the method's bias relative to independent/primary method	Independent method may have biases of its own hence not an absolute measure of accuracy Primary method ideally has no biases so is a better measure of accuracy

Precision *(see Annex A for definitions)*

6.37 'Precision' is normally determined for specific circumstances which in practice can be very varied. The two most common precision measures are 'repeatability' and 'reproducibility'. They represent the two extreme measures of precision which can be obtained. Repeatability (the smallest expected precision) will give an idea of the sort of variability to be expected when a method is performed by a single analyst on one piece of equipment over a short timescale, i.e. the sort of variability to be expected between results when a sample is analysed in duplicate. If a sample is to be analysed by a number of laboratories for comparative purposes then a more meaningful precision measure to use is reproducibility (this is the largest measure of precision normally encountered, although it does formally exclude variation with respect to time). It may be that some in-between measure is the most useful in particular cases; for example precision measured between different analysts, over extended timescales, within a single laboratory. This is sometimes known as 'intermediate precision', but the exact conditions should be stated. Precision is usually stated in terms of standard deviation or relative standard deviation. Both repeatability and reproducibility are generally dependent on analyte concentration, and so should be determined at a number of concentrations and if relevant, the relationship between precision and analyte concentration should be established. Relative standard deviation may be more useful in this case because concentration has been factored out and so it is largely constant over the range of interest provided this is not too great.

6.38 Note these statements of precision relate to quantitative analysis. Qualitative analysis can be treated in a slightly different way. Qualitative analysis is effectively a yes/no measurement at a given threshold of analyte concentration. For qualitative methods precision cannot be expressed as a standard deviation or relative standard deviation, but may be expressed as true and false positive (and negative) rates. These rates should be determined at a number of concentrations, below, at and above the threshold level. Data from a confirmatory method comparison should be used if such an appropriate method is available. If such a method is not available fortified and unfortified blank samples can be analysed instead.

% false positives = false positives X 100/total known negatives
% false negatives = false negatives X 100/total known positives

Note that biological analytical chemists and microbiologists treat false positives and false negatives slightly differently, using the terms selectivity and specificity in a way that conflicts with chemical usage.

Repeatability Precision and Reproducibility Precision - Quick Reference

Analyse	Repeats (indepen -dent)	What to calculate from the data	Comments
Standards, reference materials or fortified sample blanks at various concentrations across working range			
a) Same analyst, equipment, laboratory, short timescale	10	Determine standard deviation *(s)* at each concentration	Determines repeatability standard deviation at each concentration
b) Different analysts, equipment, same laboratory, extended timescale	10	Determine standard deviation *(s)* at each concentration	Determines intra-laboratory reproducibility standard deviation at each concentration
c) Different analysts, equipment, laboratories, extended timescale	10	Determine standard deviation *(s)* at each concentration	Determines inter-laboratory reproducibility standard deviation at each concentration Requires collaborative study

Repeatability *(see Annex A for definitions)*

6.39 From the repeatability standard deviation σ_r or s_r it is useful to calculate the 'repeatability limit '*r*'', which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under repeatability conditions, is significant.

Reproducibility *(see Annex A for definitions)*

6.40 From the reproducibility standard deviation σ_R or s_R, it is useful to calculate the 'reproducibility limit '*R*', 'which enables the analyst to decide whether the difference between duplicate analyses of a sample, determined under reproducibility conditions, is significant.

Measurement uncertainty *(see Annex A for definitions)*

6.41 A full discussion of measurement uncertainty is beyond the scope of this guide; detailed expositions will be found elsewhere [18-20]. Measurement uncertainty is a single parameter (usually a standard deviation or confidence interval) expressing the range of values possible on the basis of the measurement result. A measurement uncertainty estimate takes account of *all recognised effects* operating on the result; the uncertainties associated with each effect are combined according to well-established procedures.

An uncertainty estimate for analytical chemistry should take into account:

- the overall, long-term precision of the method;
- bias and its uncertainty, including the statistical uncertainty involved in the bias measurements, and the reference material or method uncertainty. It may be necessary to increase the estimate where a significant bias is detected but left uncorrected [20];
- calibration uncertainties. As most equipment calibration uncertainties will be negligibly small by comparison with overall precision and uncertainty in the bias; this needs only to be verified;
- any significant effects operating in addition to the above. For example, temperature or time ranges permitted by the method may not be fully exercised in validation studies, and their effect may need to be added. Such effects can be usefully quantified by robustness studies (see 'Ruggedness' below) or related studies which establish the size of a given effect on the result.

6.42 Where the contribution of individual effects is important, for example in calibration laboratories, it will be necessary to consider the individual contributions from all individual effects separately.

6.43 Note that, subject to additional consideration of effects outside the scope of a collaborative trial, the reproducibility standard deviation forms a working estimate of measurement uncertainty provided that the laboratory's bias, measured on relevant materials, is small with respect to the reproducibility standard deviation, the in-house repeatability precision is comparable to the standard method repeatability, and the laboratory's intermediate precision is not larger than the published reproducibility standard deviation.

Sensitivity *(see Annex A for definitions)*

6.44 This is effectively the gradient of the response curve, i.e. the change in instrument response which corresponds to a change in analyte concentration. Where the response has been established as linear with respect to concentration, i.e. within the linear range of the method, and the intercept of the response curve has been determined, sensitivity is a useful parameter to calculate and use in formulae for quantitation. Sensitivity is sometimes used to refer to limit of detection but this use is not generally approved.

Ruggedness (or Robustness) *(see Annex A for definitions)*

6.45 A measure of an effective analytical method is how well its performance stands up to less than perfect implementation. In any method there will be certain stages which, if not carried out sufficiently carefully, will have a severe effect on method performance and may even result in the method not working at all. These stages should be identified, usually as part of method development, and if possible, their influence on method performance evaluated using 'ruggedness tests', sometimes also called 'robustness tests'. This involves making deliberate variations to the method, and investigating the subsequent effect on performance. It is then possible to identify the variables in the method which have the most significant effect and ensure that, when using the method, they are closely controlled. Where there is a need to improve the method further, improvements can probably be made by concentrating on those parts of the method known to be critical. Ruggedness is normally evaluated during method development, typically by the originating laboratory, before collaborating with other laboratories. An established technique for ruggedness testing is described by the AOAC [21]. Ruggedness tests are normally applied to investigate the effect on either precision or accuracy.

Ruggedness Testing - Quick Reference

Analyse	No of times	Calculate	Comments
Identify variables which could have a significant effect on method performance Set up experiments (analysing reference materials, samples of known composition or certified reference materials) to monitor the effect on accuracy and precision of systematically changing the variables	Analyse each set of experimental conditions once	Determine the effect of each change of condition on the mean. Rank the variables in order of the greatest effect on method performance	Design quality control in order to control the critical variables Concentrate on these variables for method improvement

Recovery *(see Annex A for definitions)*

6.46 Analytical methods do not always measure all of the analyte of interest present in the sample. Analytes may be present in a variety of forms in samples not all of interest to the analyst. The method may thus be deliberately designed to determine only a particular form of the analyte. However a failure to determine all of the analyte present may reflect an inherent problem in the method. Either way, it is necessary to assess the efficiency of the method in detecting all of the analyte present.

6.47 Because it is not usually known how much of a particular analyte is present in a test portion it is difficult to be certain how successful the method has been at extracting it from the matrix. One way to determine the efficiency of extraction is to spike test portions with the analyte at various concentrations, then extract the fortified test portions and measure the analyte concentration. The inherent problem with this is that analyte introduced in such a way will probably not be held as strongly as that which is naturally present in the test portion matrix and so the technique will give an unrealistically high impression of the extraction efficiency. It is however the most common way of determining recovery efficiency, and it is recognised as an acceptable way of doing so. However the drawback of the technique should be borne in mind. Alternatively it may be possible to carry out recovery studies on reference materials, if suitable materials are available. Provided these have been produced by characterisation of natural materials rather than by characterisation of synthetic materials into which the analyte has been spiked, then the recovery study should accurately represent the extraction of real test portions.

Recoveries - Quick Reference

Analyse	Repeats	What to calculate from the data	Comments
Matrix blanks or samples unfortified and fortified with the analyte of interest at a range of concentrations	6	Determine recovery of analyte at the various concentration Recovery (%) = (C1-C2)/C3 X 100 where C1 = concentration determined in fortified sample C2 = concentration determined in unfortified sample C3 = concentration of fortification	Fortified samples should be compared with the same sample unfortified to assess the net recovery of the fortification Recoveries from fortified samples or matrix blanks will usually be better than real samples in which the analyte is more closely bound
Certified reference materials (CRM)		Determine recovery of analyte relative to the certified value	Depending on how the CRM was produced and characterised, it may be possible to get >100% recovery.

(f) The tools of validation

6.48 **Blanks**: Use of various types of blanks enable assessment of how much of the measured signal is attributable to the analyte and how much to other causes. Various types of blank are available to the user:

6.48.1 **Reagent blanks**: Reagents used during the analytical process (including solvents used for extraction or dissolution) are analysed in isolation in order to see whether they contribute to the measurement signal. The measurement signal arising from the analyte can then be corrected accordingly.

6.48.2 **Sample blanks**. These are essentially matrices with no analyte. They are difficult to obtain but such materials are necessary to give a realistic estimate of interferences that would be encountered in the analysis of test samples.

6.49 **Samples / test materials:** Test materials taken from real samples are useful because of the information they yield on interferences *etc*. which could be realistically encountered in day-to-day work. If the true analyte content of a test material is accurately known it can be used as a way of assessing the accuracy of the method. However the true analyte content is usually difficult to determine unless there is the possibility of using other methods which are known to show negligible bias.

6.50 **Fortified materials / solutions:** These are materials or solutions which have been fortified with the analyte(s) of interest. The fortification is usually made by spiking. These materials or solutions may already contain the analyte of interest so care is needed lest fortification inadvertently leads to levels outside of the range of applicability of the method. Fortification with a known amount of analyte enables the increase in response to the analyte to be measured and calculated in terms of the amount added (assuming 100% recovery), even though the absolute amounts of analyte present before and after the fortification are not known. Note that most methods of fortification add the analyte in such a way that it will not be as closely bound to the sample matrix as it would be if it was present naturally. Therefore, recovery determinations obtained by fortification can be expected to be over-optimistic.

6.51 **Spiked materials:** These are similar to fortified materials, indeed to some extent the terms are interchangeable. Spiking does not necessary have to be restricted to the analyte of interest. It could include anything added to the sample in order to gauge the effect of the addition. for example the sample could be spiked with varying amounts of a particular interference in order to

judge at what concentration of the interferent, determination of the analyte was adversely affected. The nature of the spike obviously needs to be identified.

6.52 **Incurred materials:** These are materials in which the analyte of interest may be essentially alien, but has been introduced to the bulk at some point prior to the material being sampled. The analyte is thus more closely bound in the matrix than it would be had it been added by spiking. The analyte value will depend on the amounts of analyte in contact with the material, the rates of take-up and loss by the matrix and any other losses through metabolism. The value of incurred sample for calibration purposes depends on how well the analyte value can be characterised. The following are examples of incurred materials:

1. Herbicides in flour from cereal sprayed with herbicides during its growth.
2. Growth promoters in meat derived from beast fed with feeds containing the promoters
3. Active ingredients in pharmaceutical formulations added at the formulation stage.

6.53 **Independently characterised materials:**. It is difficult to determine the bias of a method without knowing the true analyte content of the test material. If a material has been characterised by other means, for example, by a method which is known to have negligible bias, then it can be used as a reference material, a comparison can be made and the bias of the method under examination assessed.

6.54 **(Measurement) Standards:** Care must be taken when referring to standards as the term is also commonly used to refer to written standards, such as ISO standards. Where the term is used to refer to substances used for calibration or identification purposes it is convenient to refer to them as measurement standards or calibrants. These are traditionally thought of as solutions of single substances but in practice can be anything in which a particular parameter or property has been characterised to the extent it can be used for reference or calibration purposes. The term standard includes items in which a range of physical parameters may be calibrated (*e.g*. a calibrated thermometer). Strictly, these are physical standards.

6.55 **Reference materials:** It is commonplace to confuse ***reference materials*** with ***certified reference materials*** [22] (and see Annex A). *Reference materials* can be virtually any material used as a basis for reference, and could include laboratory reagents of known purity, industrial chemicals, or other artefacts. The property or analyte of interest needs to be stable and homogenous but the material does not need to have the high degree of characterisation, traceability and certification more properly associated with certified reference materials.

6.56 **Certified reference materials:** The characterisation of the parameter of interest in a ***certified reference material*** is generally more strictly controlled than for a ***reference material,*** and in

addition the characterised value is certified with a stated uncertainty by a recognised institution. Characterisation is normally done using several different methods, so that as far as possible, any bias in the characterisation is reduced or even eliminated.

6.57 **Statistics:** This is useful for analysing the variability inherent in analytical measurements. Analysts should familiarise themselves with at least the more basic elements of statistical theory particularly as an aid to evaluation of accuracy, precision, linear range, limits of detection and quantification and measurement uncertainty. A number of useful books introducing statistics for analytical chemistry are listed in the bibliography.

6.57.1 **Replication:** Properly used, this gives the analyst more information on the underlying statistics behind a particular measurement. Experiments involving replicate analysis should be designed to take into account all of the variations in operational conditions which can be expected during routine use of the method. The aim should be to determine typical variability and not minimum variability.

7. Using validated methods

7.1 When using someone else's method, whether it is a method developed elsewhere within the laboratory, a published method, or even a standard or regulatory method, there are two issues which need to be considered. Firstly is the existing validation data adequate for the required purpose or is further validation necessary? Secondly, if the existing validation data is adequate, is the laboratory able to achieve the level of performance claimed possible in the method? In other words is the analyst sufficiently competent? Are the available equipment and facilities adequate? If the method has been validated by extensive testing under all extremes of operating conditions, then a new competent analyst will probably operate satisfactorily within the existing performance data, although actually it is more relevant to check the analyst's performance against what is required by the analytical specification, rather than against the existing published data. However, this should always at least be checked. It is the level of performance the analyst can achieve with the method that is important, not what other analysts have achieved in the past.

7.2 Usually, standard methods are generally produced by some form of collaborative study and the standardisation bodies which produce them frequently have statistical experts to help ensure that validation studies are correctly designed, performed and evaluated. The level of validation of methods is improving but it is dangerous to assume that just because a method is standard that you can take for granted that its published validation will be adequate.

7.3 Similarly, it is often assumed that standard methods can be used straight off the shelf and the published performance data achieved straight away by whoever uses the method. This is not a safe assumption. Even those who are familiar or expert in the particular field of chemistry covered by the method will need to practice before becoming fully proficient. This is covered more fully below.

7.4 When using validated methods (or for that matter any methods) the following rules are recommended to ensure that acceptable performance is achieved.

7.4.1 Firstly, the analyst should make themselves completely familiar with a new method before using it for the first time. Ideally the method will first be demonstrated to the analyst by someone already expert in its use. The analyst should then use it under initially close supervision, working with reference materials or practice samples. The level of supervision will be stepped down until the analyst is deemed sufficiently competent to "go solo". For example competence might be established in terms of the analyst's ability to achieve the levels of performance stated in the method, such as repeatability, limit of detection, etc. This is typical of the way someone might be trained to use a new method and laboratory training procedures will frequently be designed in this way with objective measures in place to test competence at intervals during the training. In any case, the analyst should have read through the method and familiarised themselves with the theory behind the measurement, mentally rehearsing the various stages, identifying points where breaks can be taken, and parts of the process where the analyst is committed to continuous work. Where reagents need to be prepared, how stable are they once prepared? Do they need to be prepared in advance? A classic pitfall is to spend several hours preparing a number of samples and then finding the preparation of the reagent needed for the next stage of the work involves a complicated synthesis. Meanwhile the samples themselves are degrading....

7.4.2 Secondly, an assessment needs to be made of how many samples can be conveniently handled at a time. It is better to analyse a few samples well than to try to analyse a large number and have to repeat most of them.

7.4.3 Finally, make sure every thing needed for the method is available before work is started. This involves gathering together the right sort of equipment, reagents and standards (with any attendant preparation), perhaps reserving space in fume-cupboards, etc.

7.5 If it is necessary to adapt or change someone else's validated method then appropriate revalidation will be necessary. Depending on their nature, the changes may well render the original validation data irrelevant.

8. Using validation data to design QC

8.1 'Quality control' and 'quality assurance' [23] are terms whose meanings are often varied according to the context. In practical terms quality assurance relates to the overall measures taken by the laboratory to ensure and regulate quality, whereas quality control describes the individual measures which relate to the monitoring and control of particular analytical operations.

8.2 Method validation gives an idea of a method's performance capabilities and limitations which may be experienced in routine use while the method is in control. In routine use, specific controls need to be applied to the method to verify that it remains in control, *i.e.* is performing in the way expected. During the validation stage the method was largely applied to samples of known content. Once the method is in routine use it is used for samples of unknown content. Suitable control can be applied by continuing to measure samples of known content, thus allowing the analyst to decide whether the variety of answers obtained truly reflects the diversity of samples analysed or whether unexpected and unwanted changes are occurring in the method performance. In practice these known samples should be measured with every batch of samples as part of the quality control process.

8.3 The sort of checks made will depend on the nature, criticality and frequency of the analysis, batch size, degree of automation, and test difficulty and also on the lessons learnt during development and validation processes. Quality control can take a variety of forms, both inside the laboratory (internal) and between the laboratory and other laboratories (external).

8.4 ***Internal QC:*** This includes the use of: blanks; chemical calibrants; spiked samples; blind samples; replicate analyses and QC samples [21]. The use of control charts is recommended, particularly for monitoring results from QC control samples.

8.5 The sorts of QC adopted must be demonstrably sufficient to ensure the validity of the results. Different sorts of quality control may be used to monitor different types of variation within the process. QC samples, analysed at intervals in the analytical batch will indicate drift in the system; use of various types of blank will indicate what are the contributions to the instrument signal besides those from the analyte; duplicate analyses give a check of repeatability.

8.6 QC samples are typical samples which over a given period of time are sufficiently stable and homogeneous to give the same result (subject to random variation in the performance of the analytical method) and available in sufficient quantities as to be available for repetitive analysis. Over this period the random variation in performance of the analytical method can be monitored by monitoring the analysed value of the QC sample, usually by plotting it on a control chart [25-

[26]. Limits are set for the values on the chart (conventionally 'warning limits' are set at ±2σ (±2*s*) about the mean value, and 'action limits' are set at ±3σ (±3*s*) about the mean value. See Annex A, A28 for information on σ and *s*). Provided the plotted QC values conform to certain rules pertaining to the set limits, the QC is deemed to be satisfactory. As long as the QC sample value is acceptable it is likely that results from samples in the same batch as the QC sample can be taken as reliable. The acceptability of the value obtained with the QC sample should be verified as early as practicable in the analytical process so that in the event of a problem as little effort as possible has been wasted on unreliable analysis of the samples themselves.

8.7 In order to set realistic limits on the control chart, the initial calculations of mean and standard deviation must reflect the way the method is actually intended to be used on a day-to-day basis. Thus readings should mimic all possible variations in operating conditions: different analysts; variations in laboratory temperature *etc*.. If this is not done, then the standard deviation will be unrealistically small, resulting in limits being set on the chart , which cannot possibly be complied with in normal use.

8.8 The use of various types of blanks enable the analyst to ensure that calculations made for the analyte can be suitably corrected to remove any contributions to the response which are not attributable to the analyte.

8.9 Replicate analysis provides a means of checking for changes in precision in an analytical process, which could adversely affect the result. Replicates can be adjacent in a batch (to check repeatability) or placed randomly (to check for drift).

8.10 Blind analysis is effectively a form of repeat analysis and provides a means of checking precision. It consists of replicated test portions placed in the analytical batch, possibly by the laboratory supervisor, and is so-called because the analyst is not normally aware of the identity of the test portions or that they are replicates. Thus the analyst has no preconceived ideas that the particular results should be related.

8.11 Standards and chemical calibrants placed at intervals in an analytical batch enable checks to be made that the response of the analytical process to the analyte is stable.

8.12 It is the responsibility of the laboratory management to set and justify an appropriate level of quality control, based on risk assessment, taking into account the reliability of the method, the criticality of the work, and the feasibility of repeating the analysis if it doesn't work correctly first time. It is widely accepted that for routine analysis, a level of internal QC of 5% has been identified as reasonable, *i.e.*, 1 in every 20 samples analysed should be a QC sample. However, for robust, routine methods with high sample throughput, a lower level of QC may be

reasonable. For more complex procedures, a level of 20% is not unusual and on occasions even 50% may be required. For analyses performed infrequently, a full system validation should be performed on each occasion. This may typically involve the use of a reference material containing a certified or known concentration of analyte, followed by replicate analyses of the sample and spiked sample (a sample to which a known amount of the analyte has been deliberately added). Those analyses undertaken more frequently should be subject to systematic QC procedures incorporating the use of control charts and check samples.

8.13 ***External QC:*** for example proficiency testing (also known as external quality assessment). A recognised way for a laboratory to monitor its performance against both its own requirements and the norm of peer laboratories is through regular participation in proficiency testing schemes. Proficiency testing helps to highlight reproducibility performance between laboratories and systematic errors, *i.e*. bias. It can also be used to determine repeatability but this can also be checked more cost effectively using internal controls. Proficiency testing and other types of intercomparison are accepted as being an important means of monitoring traceability at national and international levels. Accreditation bodies recognise the benefit of these schemes and strongly encourage laboratories to participate in proficiency testing as an integral part of their quality assurance protocols. It is important to monitor proficiency testing results as a means of checking quality assurance and take action as necessary. In certain instances, accreditation bodies may specify participation in a particular proficiency testing scheme as a requirement of accreditation. The value of proficiency testing is of course only as good as the schemes themselves. Very often there may not be scheme available which is relevant to the types of analysis that the laboratory wishes to check, especially if it is working in isolation.

9. Documentation of validated methods

9.1 Once the validation process is complete it is important to document the procedures so that the method can be clearly and unambiguously implemented. There are a number of reasons for this. The various assessments of the method made during the validation process assume that, in use, the method will be used in the same way each time. If it is not, then the actual performance of the method will not correspond to the performance predicted by the validation data. Thus the documentation must limit the scope for introducing accidental variation to the method. In addition, proper documentation is necessary for auditing and evaluation purposes and may also be required for contractual or regulatory purposes.

9.2 Appropriate documentation of the method will help to ensure that application of the method from one occasion to the next is consistent.

9.3 Since the quality of documentation of a method has a direct effect on how consistently it can be applied, it therefore has an influence on its reproducibility and the likely estimate of uncertainty associated with it. In fact, the uncertainty contribution associated with inadequately documented methods could be so large that it effectively makes the method useless. Any anomalies in the documentation must be resolved before a sensible estimate of the uncertainty can be obtained.

9.4 It is not easy to document a method properly. Information should appear in roughly the order that the user will be expected to need it. A common trap is to assume that everyone will understand the mechanics of the method to the same extent as the person who has developed and documented the method. This assumed knowledge can be dangerous. A useful way to test the documentation is for a competent colleague to work through the documentation exactly in the way described. If this corresponds to what was intended then the documented method should stand up well to use by a variety of analysts and deliver consistent results. If not then redrafting is necessary to describe the procedures in more detail and reduce ambiguity.

9.5 A number of standards provide guidance on what sort of information should be included when documenting a method. From the chemists' point of view probably the most useful are the ISO 78 series, which describe the documentation of a number of different types of chemical analysis methods (Standardisation bodies produce, validate and of course document a large number of methods each year, and need as consistent an approach as possible and produce these standards principally for the benefit of their own technical committees). ISO 78/2 [27] advises on method documentation for general chemical methods. A layout based around this standard is included in Annex B. The standards indicate a logical order for material with recommended headings and advice on the sort of information which should appear under each heading. When using these standards the reader should note the need to balance flexibility of approach against consistency. Whilst it is desirable that all methods should have the same document format, it should also be recognised that not all methods warrant the same degree of detail and frequently it will be appropriate to omit some of the recommended sections from the documentation.

9.6 A laboratory documenting its own methods may well benefit from developing a "house style". As well as presenting relevant information in a logical easy-to-use way, it also enables the burden of method writing to be spread across a number of authors. Draft generated by a number of authors can be checked for consistency using a single checking authority.

9.7 Documented methods form an important part of a laboratory's quality system and should be subject to an appropriate degree of document control. The purpose of this is to ensure that only methods and procedures which have been authorised as fit for use are actually used. Therefore as part of the documentation process methods should carry information which enables the user to judge whether the method has been authorised for use and whether it is complete. Other

information should be available regarding the version number and date of the method; the author; how many copies of the method exist; and any copying restrictions.

9.8 From time to time methods may require updating. The technology underpinning the procedure may for example have been improved, necessitating amendments to the documentation. Document control enables the smooth withdrawal of obsolete methods and issue of revised methods. These days the process of document control is greatly simplified using word-processing. Changes should be made only by those so authorised. This may be controlled in word processing where the relevant files may have widespread 'read-only' access and very limited 'write' access.

10. Implications of validation data for calculating results and reporting

10.1 It is important that the analyst is able to translate the data, generated during analysis of samples using the validated method, into answers which directly relate to solving the customer's problem. The performance characteristics established during the validation process help to do this. Precision data for repeatability and reproducibility can be used to establish whether differences found when analysing samples are significant. Quality controls based on the validation data can be used to confirm that the method is in control and producing meaningful results. Estimation of the measurement uncertainty, associated with the method performance, enables expression of the result as a range of values in which the true value for the measurement can be said to lie with an accepted level of confidence.

10.2 It is important that the analyst has access to validation data which can be used to support the validity of the results. Whether or not such information is passed to the customer is another matter. Very often the customer will not have the technical skills to appreciate the significance of the data. In such circumstances it is perhaps safer to make the data available on request.

10.3 Issues such as method validation, variability and measurement uncertainty need to be treated carefully in certain circumstances, such as for example, in legal or forensic contexts. It may be better to be open about the existence of uncertainty attached to measurements and be prepared to justify decisions made in the light of knowing that uncertainty.

10.4 Care need to be taken when trying to use an analytical result with its accompanying uncertainty to try to decide whether or not the original consignment from which the sample has been taken complies with a specification or limit. Such a decision may not be the responsibility of the analyst however the analyst may be required to provide technical advice to assist in the decision making process.

10.5 When reporting results, the analyst must decide whether to correct for any biases which may have been detected or to report results uncorrected but acknowledge the existence of the bias.

10.6 Care should be taken when reporting results as 'not detected'. On its own this statement is uninformative and should be accompanied by an explanation of what is the limit of detection in that instance. Sometimes it is appropriate to report a numerical value even though this may be below the apparent limit of detection.

10.7 As has been explained above much of the information required to evaluate the uncertainty is obtainable from the validation process. It is assumed that before the method is used on unknown samples the laboratory will have demonstrated that it can achieve the performance parameters set out in the method and that it has satisfactory QC data and satisfactory results from measurements on reference materials. In which case the extra factors that need to be taken into account in evaluating the uncertainty on the result of a measurement on an unknown sample are any differences between the sample matrix composition and the sample used in the method validation. This would include differences that would affect analyte recovery or produce interferences that would affect the measured result.

10.8 Where a statement of uncertainty is required with results it may be appropriate to quote an expanded uncertainty by applying a suitable coverage factor, e.g. a coverage factor of 2, approximates to 95% confidence.

11. References and Bibliography

(a) References cited in the text

1. The Manager's Guide to VAM, UK Department of Trade and Industry, Valid Analytical Measurement Programme

2. Characterisation and Validation of Test Methods, J.S. Morkowski, paper presented at the EUROLAB Symposium, Berlin, 5-7 June 1996.

3. Guidelines for Collaborative Study - Procedures to Validate Characteristics of a Method of analysis, AOAC International, revised May 1994, originally published *J. Assoc. Off. Anal. Chem.* 72, 694-704 (1989).

   Note 3, 4, & 6 are effectively the same text but differ slightly editorially.

4. Harmonised Protocols for the Adoption of Standardised Analytical Methods and for the presentation of their Performance Characteristics, (IUPAC), *Pure & Appl. Chem*., 1990, **62(1)**, pp. 149-162.

5. Pocklington, W.D., "Guidelines for the development of standard methods by collaborative trial", LGC, 5th Edition, 1990. ISBN 0 948926 03 1.

6. Protocol for the Design, Conduct and Interpretation of Method-Performance Studies, (IUPAC), *Pure & Appl. Chem*., 1995, **67(2)**, pp. 331-343.

7. ASTM E 1060 - 85: Standard Practice for Interlaboratory Testing of Spectrochemical Methods of Analysis.

8. UK Drinking Water Inspectorate (DWI) Information Letter 8/93.

9. AOAC Peer Verified Methods Program - Manual on policies and procedures, AOAC International, Arlington VA 22201-3301, USA

10. International Conference on Harmonisation*, Draft Guideline on Validation of Analytical Procedures: Definitions and Terminology*, Federal Register, Volume 60, pp.11260, March 1 1995.

11. ISO 11843-1:1997. Capability of detection - Part 1 Terms and definitions.

12. Nomenclature in evaluation of analytical methods, including detection and quantification capabilities (IUPAC Recommendations 1995). *Pure & appl. Chem*., 1995, **67**, 1699 - 1723.

13. Detection in analytical Chemistry - Importance, Theory, and Practice. L. A. Currie, ACS Symposium Series 361, American Chemical Society, Washington, DC 1988. Various chapters are recommended, particularly Ch4 (Kirchmer C.J.) and Ch 16 (Kurtz D.A. *et al*.).

14. Analytical Methods Committee, "Recommendations for the Definition, Estimation and Use of the Detection Limit", *The Analyst*, 1987, **112**, 199-204.

15. Sahai, H., Singh, R.P., "The Use of R^2 as a Measure of Goodness of Fit: An Overview", *Virginia Journal of Science,* 1989, **40(1)**, 5-9.

16. Analytical Methods Committee, "Uses (Proper and Improper) of Correlation Coefficients", *The Analyst*, 1988, **113**, 1469-1471.

17. Miller, J.N., “Basic Statistical Methods for Analytical Chemistry Part 2. Calibration and Regression Methods, A Review”, *The Analyst*, 1991, **116**, 3-14.

18. ISO/IEC (with BIPM, IFCC, IUPAC, IUPAP, OIML): “Guide to the expression of uncertainty in measurement”, 1993. ISBN 92 67 10188 9.

19. Quantifying Uncertainty in Analytical Measurement, 1st Ed, 1995, EURACHEM

20. Ellison, S.L.R., Williams, A., “Measurement Uncertainty: the Key to the Use of Recovery Factors?”, from “The Use of Recovery Factors in Trace Analysis”, edited by Parkany, M., pub. Royal Society of Chemistry, 1996. ISBN:0-85404-736-0.

21. Youden, W. J., and Steiner, E. H., "Statistical Manual of the AOAC", Association of Official Analytical Chemists, 1975.

22 ISO/IEC Guide 30:1992. Terms and definitions used in conjunction with reference materials.

23. ISO 8402:1994. Quality - Vocabulary .

24. Harmonised guidelines for internal quality control in analytical chemistry laboratories, (IUPAC), *Pure & Appl. Chem*., 1995, **67(4)**, pp.649-666,

25. ISO 7870:1993. Control charts - General guide and introduction.

26. British Standard BS 5700:1984, + AMD 5480:1987. "Guide to process control using quality control chart methods and cusum techniques". (complements BS 5701:1980 and BS 5703:1980).

27. ISO 78-2:1982. Layouts for standards - Part 2: Standard for chemical analysis (under revision).

(b) Bibliography related to method development and validation in specific sectors

	Sector
A Manual on Analytical Quality Control for the Water Industry, NS30, Water Research Centre, UK, 1989, ISBN 0 902156 85 3	Environmental - water analysis
AOAC International - AOAC Peer-verified Methods Program.	Food analysis
ASTM D 2777 - 86: Standard Practice for Determination of Precision and Bias of Applicable Methods of Committee D-19 on Water.	Environmental analysis, precision, bias
Codex Alimentarius Commission on Methods of Analysis and Sampling (CX/MAS 95/3; 1995); Criteria for evaluating acceptable methods of analysis for Codex purposes.	Food analysis
Green, J.M., “A Practical guide to Analytical Method Validation”, *Analytical Chemistry News & Features*, 1/5/96, pp305A - 309A.	Pharmaceuticals
Guideline for Submitting Samples and Analytical Data for Methods Validation, Food and Drug Administration, 1987.	Pharmaceuticals

Reviewer Guidance, Validation of Chromatographic Methods, Center for Drug Evaluation and Research, Food and Drug Administration, 1994.	Pharmaceuticals
ICH Topic Q2 Validation of Analytical Methods, The European Agency for the Evaluation of Medicinal Products: ICH Topic Q 2 A - Definitions and Terminology (CPMP/ICH/381/95) ICH Topic Q 2 B - Methodology (CPMP/ICH/281/95)	Pharmaceuticals
USA - Environment Protection Agency - Report on Minimum Criteria to Assure Data Quality, July 1989.	General QA Environmental analysis
Validation of methods for the analysis of soils from potential contaminated land. Recommendations of the Working group on Soil Analysis, UK Department of the Environment	Environmental analysis

(c) Other references and bibliography

	Relevance
Analytical Methods Committee, "Uncertainty of measurement: Implications of its use in analytical science, *The Analyst*, 1995, **120**, 2303-2308.	Measurement uncertainty
Association of Public Analysts, "A Protocol for Analytical Quality Assurance in Public Analysts' Laboratories", APA, London, 1986.	General QA
ASTM C-26 Proposal P 207. "Proposed Terminology Relating to Precision and Bias Statements in Test Method Standards".	Terminology, precision, bias
ASTM C 1009 - 83: "Standard guide for Establishing a Quality Assurance Program for Analytical Chemistry Laboratories Within the Nuclear Industry".	General QA
ASTM D 4210 - 89: "Standard Practice for Intra laboratory Quality Control Procedures and a Discussion on reporting Low-level Data".	QC
ASTM D 4621 - 92b: "Standard Guide for Accountability and Quality Control in the Coal Analysis Laboratory".	QA, QC
ASTM D 4697 - 87: "Standard Guide for Maintaining Test Methods in the User's Laboratory".	QA, QC
ASTM E 1212 - 92: "Standard Practice for Establishment and Maintenance of Quality Control Systems for non-destructive Testing Agencies".	General QA, QC
ASTM E 882 - 87: "Standard Guide for Accountability and Quality Control in the Chemical Analysis Laboratory".	QC
BS 4778:Part 2:1991. "Quality vocabulary, part 2 - Quality concepts and related definitions".	Terminology
Caulcutt, R., Boddy, R., "Statistics for Analytical Chemists", Chapman and Hall, London, 1991.	Statistics

Compendium of Chemical Technology (IUPAC Recommendations), Blackwell Scientific, 1987, ISBN 0-632-01767-8.	Terminology
Dell, D., "Views on method validation", *Methodol. Surv. Biochem. Anal.,* 1990, Vol. 20, pp. 9-22.	Method validation
Dietrich, C. F., "Uncertainty, calibration and probability", 2nd edition 1991, Adam Hilger, London.	Measurement uncertainty
Dux, J. P., "Handbook of Quality Assurance for the Analytical Chemistry Laboratory", Van Nostrand Reinhold, New York, 1986.	General QA
	Relevance
EAL-G4 - "Accreditation for Chemical Laboratories - Guidance on the interpretation of the EN 45000 series of Standards and ISO/IEC Guide 25", edition 1, 4/93 (originally EURACHEM/ WELAC GD1/WGD2).	General QA
"Estimating uncertainties in testing - A guide to estimating and reporting uncertainties of measurement in testing" - 1st edition, October 1994 - British Measurement and Testing Association (BMTA).	Measurement uncertainty
Garfield, F. M., "Quality Assurance Principles for Analytical Laboratories", Association of Official Analytical Chemists", Arlington VA, 1992.	General QA
"General Guidelines for use with a Protocol for Quality Assurance of Trace Analysis" (2nd draft), UK DTI VAM Programme.	General QA
Günzler, H., "Accreditation and Quality Assurance in Analytical Chemistry", Pub. Springer Verlag, 1994. ISBN 3-540-60103-1.	General QA including chapter on method validation
ISO "VIM (International vocabulary of basic and general terms in metrology)", 2nd edition, 1993.	Terminology
ISO 3534-1:1993. "Statistics - Vocabulary and symbols - Part 1: Probability and general statistical terms".	Statistics, terminology
ISO 3534-2:1993. "Statistics - Vocabulary and symbols - Part 2: Statistical quality control".	Statistics, terminology
ISO 3534-3:1985. "Statistics - Vocabulary and symbols - Part 3: Design of experiments".	Statistics, terminology
ISO 5725-1:1994. "Accuracy (trueness and precision) of measurement methods and results - Part 1. General principles and definitions".	Terminology, accuracy
ISO 5725-2:1994. "Accuracy (trueness and precision) of measurement methods and results - Part 2. Basic methods for the determination of repeatability and reproducibility of a standard measurement method".	Accuracy
ISO 5725-3:1994. "Accuracy (trueness and precision) of measurement methods and results - Part 3. Intermediate measures of the precision of a standard measurement method".	Accuracy

ISO 5725-4:1994. "Accuracy (trueness and precision) of measurement methods and results - Part 4. Basic methods for the determination of the trueness of a standard measurement method".	Accuracy
ISO 5725-6:1994. "Accuracy (trueness and precision) of measurement methods and results - Use in practice of accuracy values".	Accuracy
ISO 7873:1993. "Control charts for arithmetic average with warning limits".	Statistics, QC
ISO 7966:1993. "Acceptance control charts".	Statistics, QC
ISO 8258:1991. "Shewhart control charts".	Statistics, QC
ISO/CD 5725-5. "Accuracy (trueness and precision) of measurement methods and results - Part 5. Alternative methods for the determination of the precision of a standard measurement method".	Accuracy
	Relevance
ISO/DIS 11095 (April 1993). "Linear calibration using reference materials".	Calibration, reference materials
ISO/IEC Guide 2:1991. "General terms and their definitions concerning standardisation and related activities".	Terminology
ISO/IEC Guide 32 (in preparation). "Calibration of chemical analyses and use of certified reference materials".	Calibration, reference materials
ISO/IEC Guide 33:1989. "Uses of certified reference materials".	Reference materials
ISO/TAG4/WG3:1993. "Guide to the Expression of Uncertainty in Measurement".	Measurement uncertainty
Kateman, G., Buydens, L., "Quality Control in Analytical Chemistry", J Wiley & Sons, 1991.	General QA, QC
Mesley, R. J., Pocklington, W. D., Walker R. F., "Analytical Quality Assurance - A Review", *Analyst*, 1991, **116 (10)**, 975-1092.	General QA
Miller, J. C., and Miller, J. N., "Statistics for Analytical Chemistry", Ellis Horwood, Chichester, 1988.	Statistics
Murdoch, J., "Control Charts", Macmillan 1979.	Statistics, QC
NATA - Technical Note #17 - "Requirements for the Format and Content of Test Methods and Recommended Procedures for the Validation of Test Methods".	Documentation of methods, general method validation
"Nomenclature for the Presentation of Results of chemical analysis (IUPAC Recommendations 1994)", *Pure & Appl. Chem*., 1994, **66(3)**, pp. 595-608,.	Terminology, statistics
Parkany, M., "Quality Assurance for Analytical Laboratories." Special publication 130, Royal Society of Chemistry, UK. ISBN 0-85186-705-7	General QA
Prichard, E., "Quality in the Analytical Chemistry Laboratory", ACOL,	General QA

Wiley.

Taylor, B. N., Kuyatt, C. E., “Guidelines for evaluating and expressing uncertainty in NIST measurement results”, NIST technical note 1297, 1994, National Institute of Standards and Technology.	Measurement uncertainty
Taylor, J. K., "Quality Assurance of Chemical Measurements", Lewis Publishers, Michigan, 1987. ISBN 0-87371-097-5	General QA
“Validation of test methods - General principles and consepts (sic)”, Committee paper EAL/GA(96)58, European Accreditation of Laboratories, Oct. 1996	Method validation
WECC Document 19 1990.	Measurement uncertainty

Annex A - Definitions

Contents

A1 **Accuracy:**

'The closeness of agreement between a test result and the accepted reference value.

Note: *The term accuracy, when applied to a set of test results, involves a combination of random components and a common systematic error or bias component.*'

[ISO 3534-1]

'A quantity referring to the differences between the mean of a set of results or an individual result and the value which is accepted as true or correct value for the quantity measured.'

[IUPAC Compendium of Chemical Technology, 1985]

A1.1 **Accuracy (of a Measuring Instrument):**

'Ability of a measuring instrument to give responses close to a true value.

Note: *In this context accuracy is a qualitative concept.*'

[IUPAC 'Orange' Book]

A2 **Bias:**

'The difference between the expectation of the test results and an accepted reference value.

Note: *Bias is the total systematic error as contrasted to random error. There may be one or more systematic error components contributing to the bias. A larger systematic difference from the accepted reference value is reflected by a larger bias value.*'

[ISO 3534-1]

'Characterises the systematic error in a given analytical procedure and is the (positive or negative) deviation of the mean analytical result from the (known or assumed) true value.'

[IUPAC Compendium of Chemical Technology, 1985]

'The difference between the limiting mean (μ) and the true value (τ); i.e., $\Delta = \mu - \tau$.'

[IUPAC 'Orange' Book]

A3 **Calibration Curve:**

'Graphical representation of measuring signal as a function of quantity of analyte.'

[AOAC - PVMC]

A4 **Cross Reactivity:**

'Response (of method) to analogues, metabolites, or other non-target components that may be present in the matrix(es).'

[AOAC - PVMC]

A5 **Discrimination**

'The ability of a measuring instrument to respond to small changes in the value of the stimulus.'

[VIM 1984]

A5.1 **Discrimination Threshold**:

'The smallest change in a stimulus which produces a perceptible change in the response of a measuring instrument.

Note: *The discrimination threshold may depend on, for example, noise (internal or external), friction, damping, inertia, quantization.*'

[VIM 1984]

A6 **Error (of Measurement):**

'The result of a measurement minus the true value of the measurand.'

Note: *Since a true value cannot be determined, in practice a conventional true value is used.*

[VIM 1993]

'The value of a result minus the true value.'

[IUPAC Compendium of Chemical Technology, 1985]

A6.1 **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.

Note: *Random error is equal to error minus systematic error. Because only a finite number of measurements can be made, it is possible to determine only an estimate of random error.'*

[VIM 1993]

'The difference between an observed value (x_i) and the limiting mean (μ); i.e. $\delta = x_i - \mu$.'
[IUPAC 'Orange' Book]

A6.2 **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.'

Note: *Systematic error is equal to error minus random error. Like true value, systematic error and its causes cannot be known.*

[VIM 1993]

A7 **False Negatives / Positives:**

'For qualitative methods the false positives/negatives rate may be determined. Data from a confirmatory method comparison should be provided if such method(s) is applicable to the same matrix(es) and concentration range(s). In the absence of a method comparison, populations of negative and positive fortified samples must be analysed. False positives / negatives may be determined as follows:

False positive rate (%) = false positives X 100/total known negatives

False negative rate (%) = false negatives X 100/total known positives
[AOAC Research Institute - Performance tested Methods Programme, Procedure]

A8 **Fitness for Purpose:**

'Degree to which data produced by a measurement process enables a user to make technically and administratively correct decisions for a stated purpose.'

[IUPAC 'Orange' Book]

A9 **Limiting Mean:**

'The asymptotic value or population mean of the distribution that characterises the measured quantity; the value that is approached as the number of observations approaches infinity.'
[IUPAC 'Orange' Book]

A10 **Limit of Detection:**

'The lowest content that can be measured with reasonable statistical certainty.'

[AOAC - PVMC]

'The lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated under the stated conditions of the test'.

[NATA Tech Note#13]

'The limit of detection, expressed as the concentration c_L, or the quantity q_L, is derived from the smallest measure x_L, that can be detected with reasonable certainty for a given analytical procedure. The value of x_L is given by the equation:

$x_L = x_{bl} + ks_{bl}$

where x_{bl} is the mean of the blank measures and s_{bl} the standard deviation of the blank measures, and *k* is a numerical factor chosen according to the confidence level desired.'

[IUPAC Compendium of Chemical Technology, 1985]

It may also be known as **Minimum detectable net concentration**, or **Limit of Determination /Limit of Decision,** which are respectively defined as:

'The true net concentration or amount of the analyte in the material to be analysed which will lead with probability (1-β), to the conclusion that the concentration of the analyte in the analysed material is larger than that of the blank matrix.'

[ISO/DIS 11843-1]

and

'The lowest analyte content, if actually present, that will be detected and can be identified.'

[AOAC - PVMC]

This whole subject is dealt with in great detail by IUPAC [12].

A11 **Limit of Quantitation**:

'(The content) equal to or greater than the lowest concentration point on the calibration curve.'

[AOAC - PVMC]

It is also known as **Limit of Reporting:**

'The lowest concentration of an analyte that can be determined with acceptable precision (repeatability) and accuracy under the stated conditions of the test.'

[NATA Tech Note #13]

It is also known as **Quantification Limit:**

'Quantification limits are performance characteristics that mark the ability of a chemical measurement process to adequately 'quantify' an analyte.

Note: *The ability to quantify is generally expressed in terms of the signal or analyte (true) value that will produce estimates having a specified relative standard deviation (RSD), commonly 10%.*

Thus: $L_Q = k_Q\, s_Q$

Where L_Q is the Quantification Limit, s_Q is the standard deviation at that point, and k_Q is the multiplier whose reciprocal equals the selected quantifying RSD. The IUPAC default value for k_Q is 10.'

[IUPAC 'Orange' Book]

A12 **Linearity:**

'Defines the ability of the method to obtain test results proportional to the concentration of analyte.

Note: *The Linear Range is by inference the range of analyte concentrations over which the method gives test results proportional to the concentration of the analyte.* '

[AOAC - PVMC]

A13 **Measurand:**

'Particular quantity subject to measurement.

Note: *Specification of a measurand may require statements about quantities such as time, temperature and pressure.* ’

[VIM 1993]

A14 **Measurement:**

'Set of operations having the object of determining a value of a quantity.'

[VIM 1993]

A14.1 **Measurement Procedure:**

'Set of operations, described specifically, used in the performance of measurements according to a given method.

Note: *A measurement procedure is normally recorded in a document that is sometimes itself a measurement procedure or measurement method and is usually in sufficient detail to enable the operator to carry out a measurement without additional information.* '

[VIM 1993]

A14.2 **Method of Measurement:**

'A logical sequence of operations, described generically, used in the performance of measurements.'

[VIM 1993]

A15.1 **Precision:**

'The closeness of agreement between independent test results obtained under stipulated conditions.'

Note: *Precision depends only on the distribution of random errors and does not relate to the true value or specified value. The measure of precision is usually expressed in terms of imprecision and computed as a standard deviation of the test results. "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* ***Reproducibilit****y are particular sets of extreme conditions.*

[ISO 3534-1]

'A measure for the reproducibility of measurements within a set, that is, of the scatter or dispersion of a set about its central value.'

[IUPAC Compendium of Chemical Technology, 1985]

A15.2 **Intermediate Precision:**

'Intermediate precision expresses within laboratories variation: different days, different analysts, different equipment, etc.'

[ICH Q2A, CPMP/ICH/381/95]

A16 **Proficiency Testing:**

'A periodic assessment of the performance of individual laboratories and groups of laboratories that is achieved by the distribution by an independent testing body of typical materials for unsupervised analysis by the participants.'

[IUPAC 'Orange' Book]

A17 **Quality:**

'The totality of features and characteristics of a product or service that bear on its ability to satisfy stated or implied needs.'

[ISO 8402:1994]

A17.1 **Quality Assurance**

'All those planned and systematic activities implemented within the quality system , and demonstrated as needed, to provide adequate confidence that an entity will fulfil requirements for quality.'

[ISO 8402:1994]

A17.2 **Quality Control:**

'The operational techniques and activities that are used to fulfil requirements of quality.'

[ISO 8402:1994]

A17.3 **Internal Quality Control:**

'Set of procedures undertaken by laboratory staff for the continuous monitoring of operations and the results of measurements in order to decide whether results are reliable enough to be released.'

[IUPAC 'Orange' Book]

A18 **Range (Measuring - Working):**

'Set of values of measurands for which the error of a measuring instrument is intended to lie within specified limits.'

[IUPAC 'Orange' Book]

A19 **Recovery:**

'The fraction of analyte added to a test sample (fortified or spiked sample) prior to analysis, the unfortified and fortified samples, percentage recovery (%R) is calculated as follows:

$$\%R = [(CF-CU)/CA] \times 100$$

Where CF is the concentration of analyte measured in the fortified sample; CU is the concentration of analyte measured in the unfortified sample; CA is the concentration of analyte added (measured value, not determined by method) in fortified sample.'

[AOAC-PVMC]

A20 **Reference Material (RM):**

'Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.'

Note: *The term reference material describes materials which are often also called measurement standards, e.g. chemical substances used for calibration or identification purposes Care is necessary when using the term 'standard' as it is commonly used in two different contexts. The term may refer to 'measurement standards' in the reference material sense, or it may refer to written standards, such as standard methods. It is important to ensure the distinction is always clear.*

[ISO/IEC Guide 30 - 1992, 2.1]

A20.1 **Certified Reference Material (CRM):**

'Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure, which establishes its traceability to an accurate realisation of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence.'

[ISO/IEC Guide 30 - 1992, 2.2]

A21 **Repeatability:**

'Precision under repeatability conditions, i.e. conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.'

[ISO 3534-1]

A21.1 **Repeatability (of results of measurements):**

Closeness of the agreement between the results of successive measurement of the same measurand carried out in the same conditions of measurement.'

[IUPAC 'Orange' Book]

A21.2 **Repeatability (of a measuring instrument):**

'Ability of a measuring instrument to provide closely similar indications for repeated applications of the same measurand under the same conditions of measurement.'

[IUPAC 'Orange' Book]

A21.3 **Repeatability Standard Deviation:**

'The standard deviation of test results obtained under repeatability conditions.

Note: *This is a measure of dispersion of the distribution of test results under repeatability conditions. Similarly "repeatability variance" and "repeatability coefficient of variation" could be defined and used as measures of the dispersion of test results under repeatability conditions.* '

[ISO 3534-1]

A21.4 **Repeatability Limit "*r*":**

'The value less than or equal to which the absolute difference between two test results obtained under repeatability conditions may be expected to be with a probability of 95%.'

Repeatability (limit) is given by the formula:

$$r = t_{\infty} \times \sqrt{2} \times s_r$$

where t_{∞} is the Student's two tailed value for $\nu = \infty$ for a given confidence (normal confidence level state is 95% where the value is 1.96), and s_r is the standard deviation measured under repeatability conditions (see A20.3).

[ISO 3534-1]

A22 **Reproducibility:**

'Precision under reproducibility conditions, i.e. conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

Note: *A valid statement of reproducibility requires specification of the conditions changed. Reproducibility may be expressed quantitatively in terms of the dispersion of the results.*'

[ISO 3534-1]

A22.1 **Reproducibility Standard Deviation:**

'The standard deviation of test results obtained under reproducibility conditions.

Note: *This is a measure of dispersion of the distribution of test results under reproducibility conditions. Similarly "reproducibility variance" and "reproducibility coefficient of variation" could be defined and used as measures of the dispersion of test results under reproducibility conditions.* '

[ISO 3534-1]

A22.2 **Reproducibility Limit "R":**

'The value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions may be expected to be with a probability of 95%.

Reproducibility (limit) is given by the formula:

$$R = t_{\infty} \times \sqrt{2} \times s_R$$

where t_∞ is the Student's two tailed value for $\nu = \infty$ for a given confidence (normal confidence level state is 95% where the value is 1.96) and s_R is the standard deviation measured under reproducibility conditions (see A21). '

[ISO 353 4-1]

A23 **Response Time**:

'The time interval between the instant when a stimulus is subjected to specified abrupt change and the instant when the response reaches and remains within specified limits of its final steady value.'

[VIM 1984]

A24 **Result of a Measurement:**

'Value attributed to a measurand, obtained by measurement.

Note: *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. A complete statement of the result of a measurement includes information about the uncertainty of measurement.* '

[VIM 1993]

A25.1 **Ruggedness Test:**

'Intra-laboratory study to study the behaviour of an analytical process when small changes in the environmental and/or operating conditions are made, akin to those likely to arise in different test environments. Ruggedness testing allows information to be obtained on effects of minor changes in a quick and systematic manner.'

[AOAC - PVMC]

A25.2 **(Robustness):**

'The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.'

[ICH Q2A, CPMP/ICH/381/95]

A26 **Selectivity (or Specificity):**

'The ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test.'

[NATA Tech Note #13]

A26.1 **Selectivity (in analysis):**

'Qualitative - the extent to which other substances interfere with the determination of a substance according to a given procedure.'

[IUPAC Compendium of Chemical Terminology, 1987]

'Quantitative - A term used in conjunction with another substantive (e.g. constant, coefficient, index, factor, number) for the quantitative characterisation of interferences.'

[IUPAC Compendium of Chemical Terminology, 1987]

A27 **Sensitivity**:

'The change in the response of a *measuring instrument* divided by the corresponding change in the stimulus.

Note: *Stimulus may for example be the amount of the measurand present*. *Sensitivity may depend on the value of the stimulus. Although this definition is clearly applied to a*

measuring instrument, it can also be applied to the analytical method as a whole, taking into account other factors such as the effect of concentration steps. '

[VIM 1984 and IUPAC 'Orange' Book]

A28 **Specificity:**

'The ability of a method to measure only what it is intended to measure.'

[AOAC - PVMC].

'Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.'

[ICH Q2A, CPMP/ICH/381/95]

A29 **Standard Deviation:**

This is a measure of how values are dispersed about a mean in a distribution of values:

The standard deviation σ for the whole population of *n* values is given by:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{n}\left(x_i - m\right)^2}{n}}$$

In practice we usually analyse a sample and not the whole population The standard deviation *s* for the sample is given by:

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

A30 **Traceability:**

'Property of the result of a measurement or the value of a standard whereby it can be related with a stated uncertainty, to stated references, usually national or international standards (i.e. through an unbroken chain of comparisons.'

Note: *The standards referred to here are measurement standards rather than written standards.*

[ISO/IEC Guide 30 - 1992, 3.8]

A31 **Trueness:**

'The closeness of agreement between the average value obtained from a large set of test results and an accepted reference value.

Note: *The measure of trueness is normally expressed in terms of bias. The reference to trueness as "accuracy of the mean" is not generally recommended.* '

[ISO 3534-1]

A32 **Uncertainty (of Measurement) i.e. Measurement Uncertainty:**

'Parameter associated with the result of a measurement, that characterises the dispersion of the values that could reasonably be attributed to the measurand.

Note: *The parameter may be, for example, a standard deviation (or a given multiple of it), or the width of a confidence interval. 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. 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. '

[VIM 1993]

A32.1 **Standard Uncertainty**

'**$u(x_i)$** - uncertainty of the result of a measurement expressed as a standard deviation.'

[ISO GUM]

A32.2 **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 co-variances of these other quantities weighted according to how the measurement result varies with these quantities.'

[ISO GUM]

A32.3 **Expanded Uncertainty**

'**U** - quantity defining an interval about a 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.

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:

$U = k \times u_c$ '

[ISO GUM]

A32.4 **Coverage factor**

'**k** - numerical factor used as a multiplier of the combined standard uncertainty in order to obtain an expanded uncertainty.

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

[ISO GUM]

A33.1 **Validation:**

'Confirmation by examination and provision of objective evidence that the particular requirements for a specified intended use are fulfilled.'

[ISO 8402:1994]

A33.2 **Method Validation:**

1. The process of establishing the performance characteristics and limitations of a method and the identification of the influences which may change these characteristics and to what extent.

Which analytes can it determine in which matrices in the presence of which interferences? Within these conditions what levels of precision and accuracy can be achieved?

2. The process of verifying that a method is fit for purpose, i.e. for use for solving a particular analytical problem.

Note: *1. is applicable where a method is developed without any particular problem in mind. 2 is applicable where a method is being developed for a specific purpose. In analytical chemistry the other commonly encountered use of the term validation is in the context of instrumentation.* ***Instrument validation*** *is used to describe the process of establishing that an instrument at any given moment is able to perform according to its design specification This process might be achieved for example by means of calibration or performance checks.*

A34 **Value:**

A34.1 **Accepted Reference Value:**

'A value that serves as an agreed-upon reference for comparison and which is derived as:

a) a theoretical or established value, based on scientific principles;
b) an assigned or certified value, based on experimental work of some national or international organisation;
c) a consensus or certified value , based on collaborative experimental work under the auspices of a scientific or engineering group;
d) when a), b), and c) are not available, the experimentation of the (measurable) quantity, i.e. the mean of a specified population of measurements.'

[ISO 3534-1]

A34.2 **True Value:**

'Value consistent with the definition of a given particular quantity.

Note: *This is a value that would be obtained by a perfect measurement. True values are by nature indeterminate. 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 particular quantity.* '

[VIM 1993]

A34.3 **Conventional True Value:**

'Value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose.

Note: *"Conventional true value" is sometimes called "assigned value", "best estimate" of the value, "conventional value" or "reference value". Frequently a number of results of measurements of a quantity is used to establish a conventional true value.* '

[VIM 1993]

'Conventional true value (of a quantity) is the value attributed to a particular quantity and accepted, sometimes by convention, as having an uncertainty appropriate for a given purpose.

Note: *Should not be confused with reference value*'

[IUPAC 'Orange' Book]

A35 **Verification:**

'Confirmation by examination and provision of objective evidence that specified requirements have been fulfilled.'

[ISO 8402:1994]

Annex B - Method Documentation Protocol

The adequate documentation of methods is discussed in chapter 9 of the guide. The following format is included for reference as a suitable layout. It is based loosely on ISO 78-2 [(27)] , but contains additional advice on calibration and quality control, and document control.

B0 UPDATE & REVIEW SUMMARY

This section has a twofold purpose. Firstly, it is intended to enable minor changes to be made to the text of the method without the need for a full revision and reprint of the method. Secondly, it is recommended that every method should be reviewed for fitness-for-purpose periodically and the summary serves as a record that this has been done. The summary typically would be located at the front of the method, just inside the front cover.

B0.1 *Updates*

Any hand written changes to the text of the method would be accepted provided the changes were also recorded in the table below (hand-written entries acceptable) and appropriately authorised. It would be implicit that the authorisation endorsed the fact that the effects of the changes on the method validation had been investigated and caused no problems, and that the changes had been made to all copies of the method.

#	Section	Nature of Amendment	Date	Authorisation
1 (e.g.)	3.4	Change flow rate to 1.2 ml.min-1	8/2/96	DGH

B0.2 *Review*

At any given time it would be expected that the date at which a method was seen to be in use would be between the *review* and *next review* dates, as shown in the table. Two years has been suggested as a suitable interval.

Review Date	Outcome of Review	Next Review Date	Authorisation

B1 TITLE

Preferred format:

Determination of A{*analyte or measurand*} (in the presence of B{*interference*}) in C {*matrix*} using D {*principle*}.

B2 SCOPE

This section enables a potential user to see quickly whether the method is likely to be appropriate for the desired application. The following details should be covered:

- the analyte(s) which can be determined by the method;
- the form in which analyte(s) are determined - speciation, total/available *etc*.;
- the sample matrix(es) within which those analyte(s) may be determined;
- the concentration range of analyte(s) over which the method may be used;
- known interferences which prevent or limit the working of the method;
- the technique used by the method;
- the minimum sample size.

B3 WARNING & SAFETY PRECAUTIONS

Detailed precautions may be given in the relevant sections, but notice must be drawn to the existence of hazards and need for precautions here. Include nil returns.

Provide suitable warnings of any hazards involved with:

- handling the samples;
- handling or preparing solvents, reagents, standards, or other materials;
- operation of equipment;
- requirements for special handling environments e.g. fume cupboards;
- consequences of scaling up experiment (explosion limits).

B4 DEFINITIONS

Define any unusual terms, use ISO definitions wherever possible. Quote sources. Analytical structures can be included here if relevant.

B5 PRINCIPLE

Outline the principle by which the analytical technique operates. A flow-chart may help. This section should be written so as to allow an at-a-glance summary of how the method works. Include an explanation on the principle of the calculation. Where appropriate to clarifying the working of the method or calculations, include details of any relevant chemical reactions (for example, this may be relevant where derivatisation is involved, or titrimetry).

e.g.: 'The concentration is derived from a 6 point calibration curve by reading off the concentration, corresponding to the sample absorbance, corrected for the blank value, and multiplying it by the concentration factor.'

B6 REAGENTS & MATERIALS

List all of the reagents materials, blanks, QC samples and standards and certified reference materials required for the analytical process, numbered for later reference. List:

- details of any associated hazards including instructions for disposal;
- analytical grade;
- need for calibration and QC materials to come from independent batches;
- details of preparation, including need to prepare in advance;
- containment and storage requirements;
- shelf life of raw material and prepared reagent;
- required concentration, noting whether w/v, w/w or v/v;
- labelling requirements;
- disposal hazards.

B7 APPARATUS & EQUIPMENT

Describe individual equipment and how they are connected in sufficient detail to enable unambiguous set-up. List minimum performance requirements and verification requirements, cross-referenced to the calibration section and any relevant instrument manuals. Number for later reference. For glassware include grade where applicable (bear in mind that use of a particular grade may require justification and that proof of compliance may be required). Include environmental requirements (fume cupboards *etc*.).

Diagrams and flowcharts may assist clarity.

B8 SAMPLING & SAMPLES

This section is not intended to include sample selection, which will probably feature in a separate sample plan.

Include sufficient detail to describe how the test portion is arrived at starting with the sample as received by the laboratory. Include storage, conditioning and disposal details.

If this stage is particularly complicated, a separate extraction method may be justified.

B9 **CALIBRATION**

Identify the critical parts of the analytical process. These will have to be controlled by careful operation and calibration. Cross-reference to the relevant sections above.

Include calibration of equipment - what needs to be calibrated, how, with what, and how often? Consider appropriate traceability of calibrants.

B10 **QUALITY CONTROL**

Explain what form the quality control takes, frequency of quality control checks during batch analysis, pass/fail criteria, action to take in the event of a failure. Cross-reference to the relevant sections above.

B11 **PROCEDURE**

Describe the analytical procedure, cross-referencing previous sections as appropriate including numbered reagents, apparatus and instrumentation. Where parameters are expressed (time, temperature) which are critical to the procedure, cross-reference to the relevant part of the calibration section. Indicate at which point in the analytical procedure the quality control, and calibration procedures should be performed.

B12 **CALCULATION**

Lay out the formulae for calculating the results ensuring all terms are clearly defined and derived. Indicate requirements for checking, cross-reference to QC requirements.

B13 **REPORTING PROCEDURES INCLUDING EXPRESSION OF RESULTS**

Indicate how results should be reported, including: rounding of numbers; final units: ± uncertainty; confidence interval.

B14 **NORMATIVE REFERENCES**

Any references which give fundamental background to the method. Use CHEMABS format.

B15 **(Appendix on -) METHOD VALIDATION**

Depending on the volume of data in support of the validation, it may be appropriate to list it here or provide reference to a separate file. *E.g.*: For linear range as stated in Section 3 “Scope”

above there should be data, either in this appendix or in a separate file that show how the range was arrived at.

B16 **(Appendix on -) MEASUREMENT UNCERTAINTY**

The major sources of uncertainty, relating to the method should be identified and the assigned values listed. Those contributions not used in the final calculation, because they are considered insignificant, should be mentioned. The overall uncertainty should be listed together with an explanation of how it was derived. A more detailed treatment may be in a cross referenced file.

Annex C - EURACHEM Working Group

Development of this guide was undertaken by a group working by correspondence. The following were involved in this group:

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