



**ACCREDITATION SCHEME FOR LABORATORIES**

**Guidance Notes C&B and ENV 001  
Elements of Method Validation for  
Chemical Testing**

Guidance Notes C&B and ENV 001, 8 Feb 2019  
The SAC Accreditation Programme is managed by Enterprise Singapore

© All rights reserved

## **0 Introduction**

This document presents a discussion of the characteristics that should be considered during the validation of analytical procedures extracted from EURACHEM [1], International Conference on Harmonization (ICH) Guidelines [2,3], and the AOAC Guidelines [4]. Its purpose is to provide some guidance and recommendations on how to consider the various validation characteristics for each analytical procedure. In some cases (for example, demonstration of specificity/selectivity), the overall capabilities of a number of analytical procedures in combination may be investigated in order to ensure the quality of the products.

Approaches other than those set forth in this guideline may be applicable and acceptable. It is the responsibility of the laboratory to ensure that the validation procedure and protocol chosen is most suitable for the product. However it is important to remember that the main objective of validation of an analytical procedure is to demonstrate that the procedure is suitable for its intended purpose. Due to their complex nature, analytical procedures for biological and biotechnological products in some cases may be approached differently than in this document.

Well-characterized reference materials, with documented purity, should be used throughout the validation study. The degree of purity necessary depends on the intended use.

In practice, it is usually possible to design the experimental work such that the appropriate validation characteristics can be considered simultaneously to provide a sound, overall knowledge of the capabilities of the analytical procedure, for instance: specificity/selectivity, linearity and range, accuracy and precision.

## **1 Selectivity**

Selectivity is the degree to which the method can quantify the target analyte in the presence of other analytes, matrices, or other potentially interfering materials.

An investigation of selectivity should be conducted during the validation of a test method. The procedures used to demonstrate selectivity will depend on the intended objective of the analytical procedure.

It is not always possible to demonstrate that an analytical procedure is specific for a particular analyte. In this case, a combination of two or more analytical procedures is recommended to achieve the necessary level of selectivity.

<b>Selectivity</b>		
<b>Procedure</b>	<b>Calculation/Determination</b>	<b>Additional Remarks</b>
1. Analyze samples, <i>reference materials</i> by test method and/or other independent methods.	Use the results from the qualitative confirmatory techniques to assess the ability of the method to confirm the analyte identity and its ability to measure the analyte in isolation from other interference such as constituents in its matrix .	Decide how much supporting evidence is reasonably required to give sufficient reliability.
2. Analyze samples containing various suspected interference in the presence of the analyte of interest.	Examine effect of interference - does the presence of the interference enhance or inhibit detection or quantification of the measurands.	If detection or quantified is inhibited by the interference, further method development will be required, such as selective solvent extraction, chromatographic or other phase separation techniques.

*Note (1): Reference Materials are defined as 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.*

Suitable qualitative tests should be able to differentiate between compounds of closely related structures which are likely to be present. The selectivity of a procedure may be confirmed by obtaining positive results (perhaps by comparison with a known reference material) from samples containing the analyte, coupled with negative results from samples which do not contain the analyte. In addition, the identification test may be applied to materials structurally similar to closely related to the analyte to confirm that a positive response is not obtained. The choice of such potentially interfering materials should be based on sound scientific judgement with a consideration of the interferences that could occur.

In cases where a non-specific measurement is used, other supporting analytical procedure should be used to demonstrate overall selectivity. It is also possible to infer that certain analytes do not interfere if checks have been performed in the first place. 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.

## **2 Linearity and Range**

A linear relationship should be evaluated across the range of the analytical procedure. 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 quantification. At the upper end of the concentration range limitations will be imposed by various effects depending on the instrument response system.

Linearity should be evaluated by inspection of a plot of signals as a function of analyte standard concentration or diluted reference material solution, covering a reasonable range of signal response from the instrument. A linear response is desirable as it simplifies the subsequent data analysis. Under a linear relationship, test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. In some cases, to obtain linearity between assays and sample concentrations, the test data may need to be subjected to a mathematical transformation, such as logarithmic linearization prior to the regression analysis. Data from the regression line itself may be helpful to provide mathematical estimates of the degree of linearity.

The regression coefficient, y-intercept, slope of the regression line and residual sum of squares should be submitted. A plot of this data should also be included. In addition, an analysis of the deviation of the actual data points (residuals) from the regression line may also be helpful for evaluating linearity.

A high regression coefficient  $R^2$  (e.g.  $> 0.99$ ) is often recommended as evidence of goodness of fit in analytical chemistry. Six to eight points, approximately equally spaced over the concentration range of interest, performed in duplicate but measured at random is a suitable calibration pattern. Other approaches should be justified.

<b>Working and Linear Range</b>		
<b>Procedure</b>	<b>Calculation/Determination</b>	<b>Additional Remarks</b>
1. External standard method - Analyze blank and reference materials or spiked sample blanks at various concentrations to determine the linear range.	Plot measurement response ( $y$ axis) against measurand concentration ( $x$ axis)  Identify approximate linear range and upper and lower boundaries of the working range.  Proceed to analyze reference materials ( step 2)	Ideally the different concentrations should be prepared independently, and not from aliquots of the same master solution.  This will confirm whether or not the working range is linear. This stage is necessary to test a working range, thought to be linear and where it is intended to use single point calibration.
2. Analyze Reference materials or spiked sample blanks with at least 6 different concentrations	Plot measurement response ( $y$ axis) against measurand concentration ( $x$ axis). Examine for outliers which	It is unsafe to remove outliers without first checking using further determinations at nearby concentrations.

<p>within the linear range.</p> <p>Run at least duplicates for each concentration.</p>	<p>may not be reflected in the regression.</p> <p>Calculate appropriate regression coefficient, <math>R^2</math>. Calculate and plot residual values (difference between actual <math>y</math> value and the <math>y</math> value predicted by the straight line for each <math>x</math> value). Random distribution about the straight line confirms linearity. Systematic trends indicate non-linearity.</p> <p>Proceed to determine the Quantitation Limit. (step 3)</p>	<p>If variance of replicates is proportional to concentration then use a weighted regression calculation rather than a non-weighted regression.</p> <p>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.</p>
<p>3. Analyze spiked aliquots of a sample blank at various analyte concentrations close to the LOD.</p> <p>Measure, once each, at least 7 independent replicates at each concentration level [5].</p>	<p>Calculate the standard deviation (<math>s</math>) of the analyte value at each concentration. Plot <math>s</math> against concentration and assign a value to the Quantitation Limit by inspection.</p> <p>Express Quantitation Limit as the lowest analyte concentration, which can be determined with an acceptable level of uncertainty.</p>	<p>Work successively lower concentrations until the accuracy and precision becomes unacceptable.</p>
<p>4. Internal standard method – addition of a known amount of a compound that is easily distinguished from the analyte but which exhibits similar chemical properties.</p>	<p>Add a known amount of internal standard similar to that expected for the analyte at any early stage of the method.</p>	<p>Due to its similar properties, the internal standard chosen behaves similarly with the analyte in the analysis process and compensates any loss during the process.</p> <p>This method is common in gas and liquid chromatographic analyses.</p>
<p>5. Standard addition method – useful when the matrix effect on an analyte is unknown or variable</p>	<p>Make measurements on the isolated analyte solution and add a series of known amounts of the standard analyte at the same level, at twice and three (or known</p>	<p>Note that the main assumption of this method is that the response is linear in the working region.</p>

	<p>fractions) times the original level.</p> <p>Plot the signal against the concentration with the initial unknown concentration set at zero.</p> <p>Extrapolate the line connecting the measured responses back to zero response and read the concentration value off the (negative) <i>x</i>-axis.</p>	<p>It is frequently used with emission spectroscopy, HPLC, electrochemistry, and radiolabeled isotopes in mass spectrometric methods.</p>
--	---	---

### 3 Accuracy

Accuracy is a measure of the closeness of a result to the “true” or accepted value and should be established across the specified range of the analytical procedure. The difference of the reported value from the true value, whether it is an individual value, an average of a set of values, or the average of a number of averages, or an assigned value, is known as the bias under the reported conditions. It may be noted that the frequently used term for bias or “accuracy” when the average of a set of values is reported is called “trueness”.

Method validation seeks to quantify the likely accuracy of results by assessing both systematic and random effects on results.

<b>Accuracy</b>			
<b>Procedure</b>	<b>Minimum No. of Times</b>	<b>Calculation/Determination</b>	<b>Additional Remarks</b>
1. Reagent blank and reference materials using in-house method	7	<p>Mean blank value subtracted from mean analyte value for reference material.</p> <p>Compare with true or accepted values for the reference material by calculating the % recovery.</p>	<p>Subject to the uncertainty of the blank being a true blank, characterization of the reference material.</p>
2. Reagent blank and reference/test material using in-house method and independent (preferably	7	<p>Gives a measure of the method’s bias. Mean blank value for reference/test material.</p> <p>Compare results with similar measurements made using independent/primary method. Conduct a one-way analysis of variance (ANOVA) to test for</p>	<p>Independent method may have biases of its own, hence not an absolute measure of accuracy.</p> <p>Primary method ideally has no biases so is a better</p>

primary) method.		any significant difference between two sets of data.  Gives a measure of the method's bias relative to the independent/primary method.	measure of accuracy.
------------------	--	--	----------------------

Acceptable recovery is a function of the concentration and the purpose of the analysis. The AOAC guidelines[3] state some acceptable recovery requirements for individual assays as follows:

Concentration	Recovery limits
100 %	98 – 101%
10 %	95 – 102%
1 %	92 – 105%
0.1 %	90 – 108%
0.01 %	85 – 110%
10 µg/g(ppm)	80 – 115%
1 µg/g	75 – 120%
10 µg/kg (ppb)	70 – 125%

However, these limits may be modified as needed in view of the variability of individual results or which set of regulatory requirements are referenced.

#### 4 Precision

Precision is of paramount importance in all analytical works. It 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.

Robustness refers to the precision sensitivity of an analytical method in the presence of minor deviations of some experimental factors of the method *within* the laboratory; see Section (7). Ruggedness tests on the other hand check the degree of reproducibility of the results obtained under a more severe variety of conditions, such as the same test method being run in a different laboratory with different analysts and reagents in an inter-laboratory comparison study, and are normally applied to study the effect on either precision or accuracy. See Section (8) below. The significance of any variation in method robustness and ruggedness can be confirmed by the ANOVA techniques.

##### Repeatability Precision, $s_r$

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.

##### Intermediate Precision (or intermediate reproducibility), $s_{R'}$

The precision determined from replicate determinations conducted within a single laboratory not simultaneously, i.e. on different days, with different analysts, with

different calibration curves, with different analysts and instruments, etc. is called intermediate precision.

Reproducibility,  $s_R$

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 (2): Intermediate precision lies between the within- (repeatability) and among- (reproducibility) laboratories precision, depending on the conditions that are varied.*

*Note (3): Normally in the precision mode, the term “repeatability” which applies to parameter calculated from simultaneous replicates is used. It also represents the minimum variability equated to the “within-laboratory” parameter. However, if an “intermediate” within-laboratory precision is used, a statement of the experimental conditions shall be given.*

<b>Repeatability Precision and Reproducibility Precision</b>			
<b>Procedure</b>	<b>Minimum No. of Repeats (independent)</b>	<b>Calculation/ Determination</b>	<b>Additional Remarks</b>
Standards, reference materials or spiked samples blanks at various concentrations across the working range.			
a. Same analyst, equipment, laboratory, short timescale	7	Determine the standard deviation ( $s$ ) at each concentration	This determines repeatability standard deviation, $s_r$ at each concentration range.
b. Different analysts, equipment, same laboratory, extended timescale	7	Determine standard deviation ( $s$ ) at each concentration.	This determines intra-laboratory or intermediate reproducibility standard deviation, $s_R$ at each concentration range
<b>OR</b> c. Different analysts, equipment, different laboratories, extended timescale	7	Determines standard deviation ( $s$ ) at each concentration.	This determines inter-laboratory or reproducibility standard deviation $s_R$ at each concentration range



## 5 Limit of Detection

The concept of Limit of Detection (LOD) has been, and still is, one of the most controversial in analytical chemistry, mainly due to multiple definitions and calculation methods proposed by international learned organizations.

The LOD is usually defined as the lowest quantity or concentration of a component that can be reliably detected with a given analytical method.

Several approaches for determining the LOD are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

### 5.1 Based on Signal-to-Noise

This approach can only be applied to analytical procedures which exhibit electronic baseline noise in, for example, chromatographic and spectrometric techniques.

Determination of the signal-to-noise ratio is performed by comparing measured signals from samples with a series of decreasing low concentrations of analyte (ideally spiked samples) with those of blank samples and establishing the minimum concentration at which the analyte can be reliably detected.

In the chromatographic context, it means the measurement of standard solutions with decreasing concentrations until a peak is found whose height is three times taller than the maximum height of the baseline (measured at both sides of the chromatographic peak). Normally, a signal-to-noise S/N ratio between 3:1 or 2:1 is generally considered acceptable for estimating the LOD.

### 5.2 Based on the Standard Deviation of the Response and Slope

The LOD may be expressed as:

$$LOD = \frac{3.3\sigma}{S}$$

where  $\sigma$  = the standard deviation of the response  
 $S$  = the slope of the calibration curve

The slope  $s$  may be estimated from the calibration curve of the analyte. The estimate of  $\sigma$  may be carried in a variety of ways, for example:

#### *5.2.1 Based on the Standard Deviation of the Blank*

Measurement of the magnitude of analytical background response is performed by analyzing an appropriate number of blank samples and calculating the standard deviation of these responses.

#### *5.2.2 Based on the Calibration Curve*

A specific calibration curve should be studied using samples containing an analyte in the range of LOD. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

### 5.3 Documentation of LOD

The LOD and the method used for determining the LOD should be presented/ documented. If LOD is determined based on signal to noise ratio, the presentation of the relevant chromatograms is considered acceptable for justification.

In cases where an estimated value for the LOD is obtained by calculation or extrapolation, this estimate may be subsequently be validated by the independent analysis of a suitable number of samples known to be near or prepared at the LOD.

<b>LOD</b>	
<b>Procedure</b>	<b>Calculation/Determination</b>
<p>a. Analyse at least 7 independent sample blanks measured once each.</p> <p><b>OR</b></p> <p>b. At least 7 independent sample blanks spiked at lowest acceptable concentration measured once each.</p>	<p>Sample standard deviation (<math>s</math>) of a) sample blank values, or b) spiked sample blank values.</p> <p>Express LOD as the analyte concentration corresponding to a) mean sample blank value + <math>3s</math> or b) <math>0 + 3s</math></p>
<p>This approach assumes that a signal more than <math>3s</math> 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.</p>	
<p>c. At least 7 independent sample blanks spiked at lowest acceptable concentration, measured once each.</p>	<p>Sample standard deviation (<math>s</math>) of the spiked sample blank values.</p> <p>Express LOD as the analyte concentration corresponding to sample blank value + <math>4.65s</math> (derived from hypothesis testing).</p>
<p>The 'lowest acceptable concentration' is taken to be the lowest concentration for which an acceptable degree of uncertainty can be achieved.</p> <p>Assumes a normal practice of evaluating sample and blank separating and correcting for the blank by subtracting the analyte concentration corresponding to the blank signal from the concentration to the sample signal.</p> <p>If measurements are made under repeatability conditions, this also gives a measure of the repeatability precision.</p>	

*Note (4): A distinction shall be made between LOD and method detection limit (MDL). In the case of MDL estimation, the standard solutions of different low concentrations are to go through the whole analytical process including sample preparation stage before subject to instrumental measurements.*

## 6 Limit of Quantitation (LOQ)

Several approaches for determining the limit of quantitation (LOQ) are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

### 6.1 Based on Visual Evaluation

Visual evaluation may be used for non-instrumental methods but may also be used with instrumental methods.

This quantitation limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision.

### 6.2 Based on Signal-to-Noise Approach

This approach can only be applied to analytical procedures that exhibit baseline noise.

Determination of the signal-to-noise is performed by comparing measured signals from samples with known low concentrations of analyte with those of blank samples and by establishing the minimum concentration at which the analyte can be reliably quantified. A typical signal -to- noise ratio is 5:1 or 10:1.

### 6.3 Based on the Standard Deviation of the Response and the Slope

The limit of quantitation (LOQ) may be expressed as:

$$LOQ = \frac{5\sigma}{S} \quad \text{or} \quad LOQ = \frac{10\sigma}{S}$$

where  $\sigma$  = the standard deviation of the response ,  $S$  = the slope of the calibration curve

The slope  $S$  may be estimated from the calibration curve of the analyte. The estimate of  $\sigma$  may be carried out in a variety of ways, for example:

#### 6.3.1 *Based on Standard Deviation of the Blank*

Measurement of the magnitude of analytical background response is performed by analyzing at least 7 blank samples and calculating the standard deviation of these responses.

#### 6.3.2 *Based on the Calibration Curve*

A specific calibration curve should be studied using samples, containing an analyte in the range of LOQ. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

### 6.4 Documentation of Limit of Quantitation (LOQ)

The quantitation limit and the method used for determining the quantitation limit should be presented/ documented.

The limit should be subsequently validated by the analysis of a suitable number of samples known to be near or prepared at the quantitation limit.

<b>Quantitation Limit</b>		
<b>Procedure</b>	<b>Calculation/Determination</b>	<b>Additional Remarks</b>
1. Analyse at least 7 independent sample blanks measured once each.	Sample standard deviation ( $s$ ) of sample blank value.  Express quantitation limit as the analyte concentration corresponding to the sample blank value plus either $10s$ .	
2. Fortify aliquots of a sample blank at various analyte concentrations close to the quantitation limit.  Measure, once each, at least 7 independent replicates at each concentration level.	Calculate the standard deviation ( $s$ ) of the analyte value at each concentration.  Plot ( $s$ ) against concentration and assign a value to the quantitation limit by inspection.  Express quantitation limit as the lowest analyte concentration which can be determined with an acceptable level of uncertainty.	Normally quantitation limit forms part of the study to determine the working range. It should 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.

## 7 Robustness

The evaluation of robustness should only be considered during the development phase and depends on the type of procedure under study. It should show the reliability of an analysis with respect to deliberate variations in method parameters.

If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g. resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used.

System Suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be

evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Examples of typical variations are:

- stability of analytical solutions
- small change of pH value in the experiment
- a change of extraction solvent (e.g. from benzene to toluene)
- extraction time and/or temperature

In the case of liquid chromatography, examples of typical variations are

- influence of variations of pH in a mobile phase,
- influence of variations in mobile phase composition
- different conditions (different lots and/or suppliers),
- temperature,
- flow rate.

In the case of gas chromatography, examples of typical variations are

- different columns (different lots and/or suppliers)
- change of oven temperature and its programming
- carrier gas flow rate.

<b>Robustness</b>			
<b>Procedure</b>	<b>No. of times</b>	<b>Calculation/ Determination</b>	<b>Additional Remarks</b>
Identify variables that could have a significant effect on method performance. Set up experiments (analyzing 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 by one-way or two-way ANOVA, or factorial experimental technique.  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.

## **8 Ruggedness**

Ruggedness is the degree of reproducibility of the results obtained under a more severe variety of conditions (such as being run in different analytical laboratories with different analysts, equipment and reagents as a collaborative study), expressed as variance. It means that the quality of test data is independent of the operating variations of the procedure, and the results can be reproducible by other laboratories.

The analytical method is considered to be 'rugged' if the variances are comparable under whatever experimental conditions considered, by the one-way ANOVA. When the variations of results from a group of participating laboratories are studied, there are 2 possible sources of variations:

- *Random error* in measurement
- *Controlled or fixed-effect factor* (i.e. performance of the participating laboratories).

It is important to note that in order to be meaningful in the statistical analysis of the data given by the different laboratories, one must look at a total of 18 sets of data, meaning to get 9 labs for 2 sets of data or 6 labs for 3 sets of data.

## 9 Measurement Uncertainty

The importance of estimation of measurement uncertainty in chemical analysis needs no further emphasis. It gives the uncertainty interval for a test result given in the form of ' $\pm$ ' range, covering the true or target value of the sample with 95% confidence. In other words, a report value with an estimated measurement uncertainty must be accompanied by a uncertainty interval to be meaningful, for example,  $0.150 \pm 0.006\%$ .

All analytical methods involve a number of steps and each step is characterized by certain uncertainty. According to the ISO TAG 4 '*Guide to the Expression of Uncertainty in Measurement*' (GUM) and the EURACHEM/CITAC Guide '*Quantifying Uncertainty in Analytical Measurement*', the overall measurement uncertainty is a function of all the uncertainties in each step of the analytical process.

*Note (5): As the analytical method under study has not been put into routine practice, the alternative holistic top-down method performance approaches using the precision, accuracy and trueness under stable QA/QC protocols may not be adopted for evaluating the measurement uncertainty of the method under validation or verification. The GUM (bottom-up) approach is preferred.*

*Example:*

In weighing a certain amount of sample into a beaker during sample preparation, we have to consider the uncertainties in the:

- repeatability of weighing
- calibration of analytical balance
- sensitivity of analytical balance

and,

when we are to dissolve the sample in a volumetric flask, we have then to consider the uncertainties involved in:

- repeatability of the volume measured in the volumetric flask
- manufacturer's uncertainty of the volume claimed

- expansion of volume due to glass expansion at a temperature different from the calibration temperature

Furthermore, when a calibration curve is concerned, we then need to know the standard deviation of the gradient of the straight line curve and also the uncertainty of the value of say, concentration  $x$  value based on the observed, say absorbance  $y$  value.

In general under unbiased environmental conditions, when the individual independent steps are considered in the form of standard deviations, the overall standard uncertainty  $u$  is given by:

$$s_{total} = \sqrt{s_1^2 + s_2^2 + s_3^2 + \dots + s_n^2}$$

On the other hand, in certain instrumental analysis such as GC or HPLC, we are involved with calculation of multiplication and divisions, a standard uncertainty  $u$  in this case will have to be based on comparing their individual relative standard deviations (*RSDs*). For example in the following calculation:

$$A = \frac{B \times C}{D}$$

then, we shall calculate the standard uncertainty result expressed as standard uncertainty of  $A$  as:

$$\frac{s_A}{\bar{A}} = \sqrt{\left(\frac{s_B}{\bar{B}}\right)^2 + \left(\frac{s_C}{\bar{C}}\right)^2 + \left(\frac{s_D}{\bar{D}}\right)^2}$$

The expanded uncertainty,  $U$  is then calculated by multiplying the combined uncertainty with a  $k$  coverage factor of 2 or 3, depending on 95% or 99% confidence level required.

The test result shall be reported as  $X \text{ (unit)} \pm U \text{ (unit)}$ .

Uncertainty must also be estimated when a calibration curve is established. One has to provide:

- standard deviations of the replicate measurements obtained from instrumentation;
- error bars on the data points in the linear calibration curve;
- measurement uncertainty on the  $x$ -value (e.g. concentration) when a experimental  $y$ -value (e.g. absorbance, peak area, etc.) is read from the calibration curve or from the linear calibration equation.

## 10 Qualitative Methods

The validation of qualitative tests differs from the quantitative tests principally because there are no numerical results but binary results such as positive/negative, absent/present results. These tests are particularly common in foods/feeds, and

clinical/medical laboratories. A decision point or “cut-off” concentration is required on an ordinal scale which is linked to the “pure” qualitative test result against certain reference material where only a binary condition is known.

The Eurachem Guide [1] has described some principles for chemical analysis that are also relevant for qualitative methods in the determination of the presence of one or more analytes, e.g. the concepts of selectivity and limit of detection (LOD).

## 11 References

- [1] Eurachem Guide, The Fitness for Purpose of Analytical Methods, First Internet Version, December 1998
- [2] International Conference on Harmonization (ICH) Guidelines, *Validation of Analytical Procedures: Methodology*, 6 November 1996
- [3] International Conference on Harmonization (ICH) Guidelines, Text on Validation of Analytical Procedures, 27 October 1994
- [4] AOAC Guidelines for Single Laboratory Validation of Chemical Methods for Dietary Supplements and Botanicals, 19 December 2002
- [5] AOAC Guidelines for Standard Method Performance Requirements, AOAC Official Methods of Analysis (2016).