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Method Development and Validation of Analytical Procedures

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1. Introduction

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. It is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Likewise the operator carrying out the studies must be competent in the analysis under study and have sufficient knowledge of the method/analysis to draw conclusions from the observations as the validation work proceeds. Quite often method validation evolves from method development and so the two activities are often closely tied, with the validation study employing the techniques and steps in the analysis as defined by the method development.
Analytical methods need to be validated or revalidated

- before their introduction into routine use;
- whenever the conditions change for which the method has been validated (e.g., an instrument with different characteristics or samples with a different matrix); and
- whenever the method is changed and the change is outside the original scope of the method.

Method validation has received considerable attention in the literature and from industrial committees and regulatory agencies.

- The U.S. FDA CGMP request in section 211.165 (e) methods to be validated: The accuracy, sensitivity, specificity, and reproducibility of test methods employed by the firm shall be established and documented. Such validation and documentation may be accomplished in accordance with Sec. 211.194(a). These requirements include a statement of each method used in testing the sample to meet proper standards of accuracy and reliability, as applied to the tested product. The U.S. FDA has also proposed industry guidance for Analytical Procedures and Methods Validation.
- ISO/IEC 17025 includes a chapter on the validation of methods with a list of nine validation parameters. The ICH has developed a consensus text on the validation of analytical procedures. The document includes definitions for eight validation characteristics. ICH also developed guidance with detailed methodology.

- The U.S. EPA prepared guidance for method's development and validation for the Resource Conservation and Recovery Act (RCRA). The AOAC, the EPA and other scientific organizations provide methods that are validated through multi-laboratory studies.

1.1 When should methods be validated?

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:

- Method just developed
- Revised method or established method adapted to a new problem;
- When a review of quality control indicates an established method is changing with time;
- When an established method is used in a different laboratory, with different analysts or with mdifferent equipment
- Demonstration of the equivalence between two methods, e.g. a new method and a standard. Certain areas of analytical practices, such as in clinical chemistry will specify validation requirements relevant to the method. This ensures that particular validation terminology together with the statistics used is interpreted in a manner consistent within the relevant sector. Official recognition of a method may require characterisation using a collaborative study.

1.2 Strategy for the validation of methods

The validity of a specific method should be demonstrated in laboratory experiments using samples or standards that are similar to unknown samples analyzed routinely. The preparation and execution should follow a validation protocol, preferably written in a step-by-step instruction format. This proposed procedure assumes that the instrument has been selected and the method has been developed. It meets criteria such as ease of use; ability to be automated and to be controlled by computer systems; costs per analysis; sample throughput; turnaround time; and environmental, health and safety requirements.

1. Develop a validation protocol, an operating procedure or a validation master plan for the validation
2. For a specific validation project define owners and responsibilities
3. Develop a validation project plan
4. Define the application, purpose and scope of the method
5. Define the performance parameters and acceptance criteria
6. Define validation experiments
7. Verify relevant performance characteristics of equipment
8. Qualify materials, e.g. standards and reagents for purity, accurate amounts and sufficient stability
9. Perform pre-validation experiments
10. Adjust method parameters or/and acceptance criteria if necessary
11. Perform full internal (and external) validation experiments
12. Develop SOPs for executing the method in the routine
13. Define criteria for revalidation
14. Define type and frequency of system suitability tests and/or analytical quality control (AQC) checks for the routine
15. Document validation experiments and results in the validation report

2. Steps in method validation

Successful acceptance of the validation parameters and performance criteria, by all parties involved, requires the cooperative efforts of several departments, including analytical development, QC, regulatory affairs and the individuals requiring the analytical data. The operating procedure or the Validation Master Plan (VMP) should clearly define the roles and responsibilities of each department involved in the validation of analytical methods.
The scope of the method and its validation criteria should be defined early in the process. These include the following questions:

- What analytes should be detected?
- What are the expected concentration levels?
- What are the sample matrices?
- Are there interfering substances expected, and, if so, should they be detected and quantified?
- Are there any specific legislative or regulatory requirements?
- Should information be qualitative or quantitative?
- What are the required detection and quantitation limits?
- What is the expected concentration range?
- What precision and accuracy is expected?
- How robust should the method be?
- Which type of equipment should be used? Is the method for one specific instrument, or should it be used by all instruments of the same type?
- Will the method be used in one specific laboratory or should it be applicable in all laboratories at one side or around the globe?
- What skills do the anticipated users of the method have?

The method's performance characteristics should be based on the intended use of the method. It is not always necessary to validate all analytical parameters that are available for a specific technique. For example, if the method is to be used for qualitative trace level analysis, there is no need to test and validate the method's limit of quantitation, or the linearity, over the full dynamic range of the equipment. Initial parameters should be chosen according to the analyst's experience and best judgment. Final parameters should be agreed between the lab or analytical chemist performing the validation and the lab or individual applying the method and users of the data to be generated by the method.

2.1 Quality control plan and implementation for routine

For any method that will be used for routine analysis, a QC plan should be developed. This plan should ensure that the method, together with the equipment, delivers consistently accurate results. The plan may include recommendations for the following:

1. Selection, handling and testing of QC standards
2. Type and frequency of equipment checks and calibrations (for example, should the wavelength accuracy and the baseline noise of an HPLC UV detector be checked after each sample analysis, or on a daily or weekly basis?)
3. Type and frequency of system suitability testing (for example, at which point during the sequence system should suitability standards be analyzed?)
4. Type and frequency of QC samples (for example, should a QC sample be analyzed after 1, 5, 20 or 50 unknown samples, and should there be single or duplicate QC sample analysis, or should this be run at one or several concentrations?)

5. Acceptance criteria for equipment checks, system suitability tests and QC sample analysis
6. Action plan in case criteria 2, 3 and/or 4 are not met.

In many cases, methods are developed and validated in service laboratories that are specialized in this task. When the method is transferred to the routine analytical laboratory, care should be taken that the method and its critical parameters are well understood by the workers in the departments who apply the method. A detailed validation protocol, a documented procedure for method implementation and good communication between the development and operation departments are equally important. If the method is used by a number of departments, it is recommended to verify method validation parameters and to test the applicability and usability of the method in a couple of these departments before it is distributed to other departments. In this way, problems can be identified and corrected before the method is distributed to a larger audience. If the method is intended to be used by just one or two departments, an analyst from the development department should assist the users of the method during initial operation. Users of the method should be encouraged to give constant feedback on the applicability and usability of the method to the development department. The latter should correct problems if any arise.

2.2 Transferring validated routine methods

Validated routine methods are transferred between laboratories at the same or different sites when contract laboratories offer services for routine analysis in different areas or when products are manufactured in different areas. When validated routine methods are transferred between laboratories and sites, their validated state should be maintained to ensure the same reliable results in the receiving laboratory. This means the competence of the receiving laboratory to use the method should be demonstrated through tests, for example, repeat critical method validation experiments and run samples in parallel in the transferring and receiving laboratories. The transfer should be controlled by a procedure, The recommended steps are:

- Designate a project owner
- Develop a transfer plan
- Define transfer tests and acceptance criteria (validation experiments, sample analysis: sample type, #replicates)
- Describe rational for tests
- Train receiving lab operators in transferring lab on equipment, method, critical parameters and troubleshooting
- Repeat 2 critical method validation tests in routine lab
- Analyze at least three samples in transferring and receiving lab
- Document transfer results

2.3 Revalidation

Most likely some method parameters have to be changed or adjusted during the life of the method if the method performance criteria fall outside their acceptance criteria. The question is whether such change requires revalidation. In order to clarify this question upfront, operating ranges should be defined for each method, either based on experience with similar methods or else investigated during method development. These ranges should be verified during method validation in robustness studies and should be part of the method characteristics. Availability of such operating ranges makes it easier to decide when

a method should be revalidated. A revalidation is necessary whenever a method is changed, and the new parameter lies outside the operating range. If, for example, the operating range of the column temperature has been specified to be between 30 and 40°C, the method should be revalidated if, for whatever reason, the new operating parameter is 41°C.
Revalidation is also required if the scope of the method has been changed or extended, for example, if the sample matrix changes or if operating conditions change. Furthermore, revalidation is necessary if the intention is to use instruments with different characteristics, and these new characteristics have not been covered by the initial validation. For example, an HPLC method may have been developed and validated on a pump with a delay volume of 5 mL, but the new pump has a delay volume of only 0.5 mL.

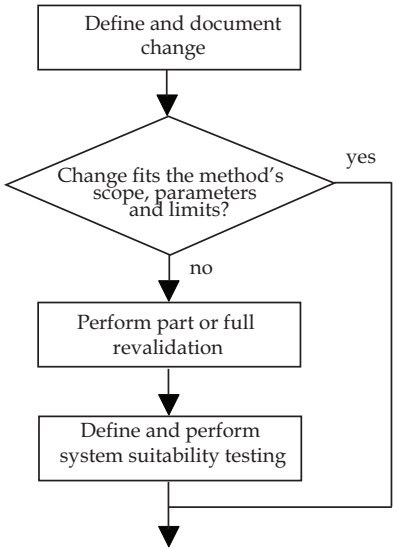

Fig. 1. Flow diagram for revalidation

Part or full revalidation may also be considered if system suitability tests, or the results of QC sample analysis, lie outside preset acceptance criteria and where the source of the error cannot be traced back to the instruments or any other cause.
Whenever there is a change that may require part or full revalidation, the change should follow a documented change control system. The change should be defined, authorized for implementation and documented. Possible changes may include

- new samples with new compounds or new matrices,
- new analysts with different skills,
- new instruments with different characteristics,
- new location with different environmental conditions,
- new chemicals and/or reference standards and
- modification of analytical parameters.

An evaluation should determine whether the change is within the scope of the method. If so, no revalidation is required. If the change lies outside the scope, the parameters for revalidation should be defined. After the validation experiments, the system suitability test parameters should be investigated and redefined, if necessary.

2.4 Bionalytical method development and validation

The process by which a specific bioanalytical method is developed, validated, and used in routine sample analysis can be divided into

1. Reference Standard preparation
2. Bioanalytical method develo`pment and establishment of assay procedure
3. Application of validated bioanalytical method to routine drug analysis and acceptance criteria for the analytical run and/or batch.

3. Parameters for method validation

The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature. Unfortunately, some of the definitions vary between the different organizations. An attempt at harmonization was made for pharmaceutical applications through the ICH where representatives from the industry and regulatory agencies from the United States, Europe and Japan defined parameters, requirements and, to some extent, methodology for analytical methods validation.

3.1 Selectivity/specificity

The terms selectivity and specificity are often used interchangeably, the term specific generally refers to a method that produces a response for a single analyte only, while the term selective refers to a method that provides responses for a number of chemical entities that may or may not be distinguished from each other. If the response is distinguished from all other responses, the method is said to be selective. Since there are very few methods that respond to only one analyte, the term selectivity is usually more appropriate Selectivity and specificity are measures of the reliability of measurements in the presence of interferences. Where the measurement stage is non-specific, method development should indicate which analytes do not interfere. 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 apply to both qualitative and quantitative analysis. 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 compared to other independent method/techniques. Another aspect of selectivity which must be considered is where an analyte may exist in the sample in more than one form such as: free or complexed; inorganic or organometallic; or the possibility of a component such as Chromium ion being present in different oxidation states such as Cr3+ or Cr6+.

3.2 Precision and reproducibility

Precision is method and concentration specific, which in prac tice 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 is reproducibility (this is the largest

measure of precision normally encountered). 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. The Purpose of carrying out a determination is to obtain a valid estimate of a 'true' value. When one considers the criteria according to which an analytical procedure is selected, precision and accuracy are usually the first time to come to mind. Precision and accuracy together determine the error of an individual determination. They are among the most important criteria for judging analytical procedures by their results. Precision refers to the reproducibility of measurement within a set, that is, to the scatter of dispersion of a set about its central values. The term 'set' is defined as referring to a number (n) of independent replicate measurements of some property. One of the most common statistical terms employed is the standard deviation of a population of observation. Standard deviation is the square root of the sum of squares of a deviations of individual results for the mean, divided by one less than the number of results in the set.

The standard deviation S, is given by

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

Standard deviation has the same units as the property being measured.

The square of standard deviation is called variance (s^2). Relative standard deviation is the standard deviation as a fraction of the mean, i.e. S/x. It is some times multiplied by 100 and expressed as a percent relative standard deviation. It becomes a more reliable expression of precision.

$$\% \text{ Relative Standard Deviation (RSD)} = S * 100/x$$

3.3 Accuracy and recovery

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. The true value for accuracy assessment can be obtained in several ways. One alternative is to compare the results of the method with results from an established reference method. This approach assumes that the uncertainty of the reference method is known. Secondly, accuracy can be assessed by analyzing a sample with known concentrations (e.g., a control sample or certified reference material) and comparing the measured value with the true value as supplied with the material. If certified reference materials or control samples are not available, a blank sample matrix of interest can be spiked with a known concentration by weight or volume. After extraction of the analyte from the matrix and injection into the analytical instrument, its recovery can be determined by comparing the response of the extract with the response of the reference material dissolved in a pure solvent. Because this accuracy assessment measures the effectiveness of sample preparation, care should be taken to mimic the actual sample

preparation as closely as possible. If validated correctly, the recovery factor determined for different concentrations can be used to correct the final results.

3.3.1 Calibration

Calibration is the most important step in bioactive compound analysis. A good Precision and accuracy can only be obtained when a good calibration procedure is adopted. In the Spectrophotometric methods, the concentration of a sample cannot be measured directly, but is determined using physical measuring quantity 'y' (absorbance of a solution). An unambiguous empirical or theoretical relationship can be shown between this quanity and the concentration of an analyte. The calibration between y = g(x) the calibration function can be obtained by fitting an adequate mathematical model through the experimental data. The most convenient calibration function is linear, goes through the origin and is applicable over a wide dynamic range. In practice however, many deviations from the ideal calibration line may occur. For the majority of analytical techniques uses the calibration equation.

$$Y = a + bX$$

In calibration, univarate regression is applied, when means that all observations are dependent upon a single variable X.

3.3.2 Standard deviation of slope (Sb)

The standard deviation of slope is proportional to standard error of estimate and inversely proportional to the range and square root of the number of data points.

3.3.3 Standard deviation of intercept, (Sa)

Intercept values of least squares fits of data are often to evaluate additive errors between or among different methods.

3.3.4 Correlation coefficient, (r)

The correlation coefficient r (x,y) is more useful to express the relationship of the chosen scales. To obtain a correlation coefficient the covariance is divided by the product of the standard deviation of x and y.

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

The absolute recovery of analytical method as the response of a processed spiked matrix expressed as a percentage of the response of pure standard. Which has not been subjected to sample pre-treatment and indicates whether the method provides a response for the entire amount of analyte that is present in the sample. It is best established by comparing the responses of extracted samples at low, medium and high concentrations in replicates at least 6 with those non- extracted standards, which represent 100% recovery.

$$\text{Absolute recovery} = \frac{\text{response of an spike into matrix (processed)}}{\text{response of an analyte of pure standard (unprocessed)}} \times 100$$

If an internal standard is used, its recovery should be determined independently at the concentration levels used in the method.

3.3.5 Linearity and sensitivity of the method

It may be demonstrated directly on the drug substance (by dilution of a standard stock solution) and/or separate weighings of synthetic mixtures of the drug product components, using the proposed procedure. The latter aspect can be studied during investigation of the range. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content.The correlation coefficient, y-intercept, slope of the regression line, and residual sum of squares should be submitted. A plot of the data should be included. According to the Beers Lambert Law, Absorbance is the ratio of logarithm of Intensity of incident light and Intensity of transmitted light, or $A = \varepsilon CT$. Knowledge of the sensitivity of the color is important and the following terms are commonly employed for expressing sensitivity. The absorbance (A) is proportional to the concentration (C) of the absorbing species, if absorptivity (ε) and thickness of the medium (t) are constant. When concentration is in moles per litre, the constant is called molar absorptivity. Beers Law limits and E_{max} values are expressed as µg/ml and moles/cm respectively. Sandell's Sensitivity refers to the number of µg of the drug to be determining, converted to the colored product, which in a column solution of cross section 1cm^2 shows an absorbance of 0.001(expressed as µg/cm).

3.4 Stability

The term system stability has been defined as the stability of the samples being analyzed in a sample solution. System stability should be determined by replicate analysis of the sample solution. System stability is considered appropriate when the RSD, calculated on the assay results obtained at different time intervals, does not exceed more than 20 percent of the corresponding value of the system precision. If, on plotting the assay results as a function of time, the value is higher, the maximum duration of the usability of the sample solution can be calculated.

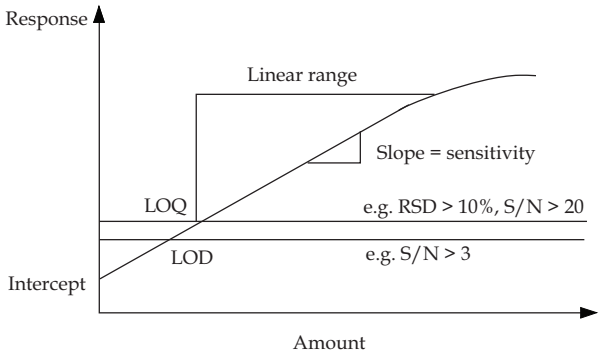

Fig. 2. Definitions for linearity, range, LOQ, LOD

3.5 Range

The range of an analytical method is the interval between the upper and lower levels (including these levels) that have been demonstrated to be determined with precision,

accuracy and linearity using the method as written. The range is normally expressed in the same units as the test results (e.g., percentage, parts per million) obtained by the analytical method. For assay tests, the ICH (5) requires the minimum specified range to be 80 to 120 percent of the test concentration, and for the determination of an impurity, the range to extend from the limit of quantitation, or from 50 percent of the specification of each impurity, whichever is greater, to 120 percent of the specification.

3.6 Limit of detection

The limit of detection is the point at which a measured value is larger than the uncertainty associated with it. It is the lowest concentration of analyte in a sample that can be detected but not necessarily quantified. The limit of detection is frequently confused with the sensitivity of the method. The sensitivity of an analytical method is the capability of the method to discriminate small differences in concentration or mass of the test analyte. In practical terms, sensitivity is the slope of the calibration curve that is obtained by plotting the response against the analyte concentration or mass.

In chromatography, the detection limit is the injected amount that results in a peak with a height at least two or three times as high as the baseline noise level. Besides this signal/noise method, the ICH (4) describes three more methods:

1. Visual inspection: The detection limit is determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.
2. Standard deviation of the response 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.
3. Standard deviation of the response based on the slope of the calibration curve: A specific calibration curve is studied using samples containing an analyte in the range of the limit of detection. 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.

3.7 Limit of quantitation

The limit of quantitation is the minimum injected amount that produces quantitative measurements in the target matrix with acceptable precision in chromatography, typically requiring peak heights 10 to 20 times higher than the baseline noise. If the required precision of the method at the limit of quantitation has been specified, the EURACHEM (22) approach can be used. A number of samples with decreasing amounts of the analyte are injected six times. The calculated RSD percent of the precision is plotted against the analyte amount. The amount that corresponds to the previously defined required precision is equal to the limit of quantitation. It is important to use not only pure standards for this test but also spiked matrices that closely represent the unknown samples. For the limit of detection, the ICH (5) recommends, in addition to the procedures as described above, the visual inspection and the standard deviation of the response and the slope of the calibration curve.

Any results of limits of detection and quantitation measurements must be verified by experimental tests with samples containing the analytes at levels across the two regions. It is equally important to assess other method validation parameters, such as precision, reproducibility and accuracy, close to the limits of detection and quantitation. Figure 6 illustrates the limit of quantitation (along with the limit of detection, range and linearity). Figure 7 illustrates both the limit of detection and the limit of quantitation.

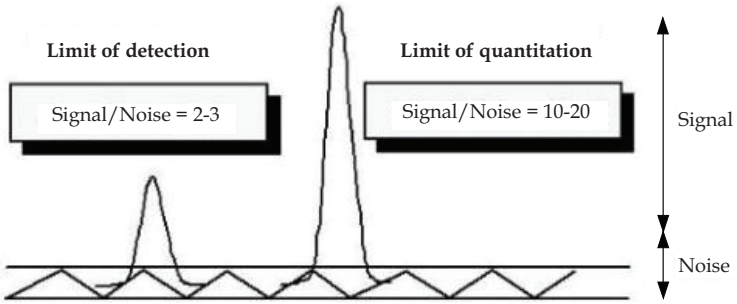

Fig. 3. Limit of detection and limit of quantitation via signal to noise

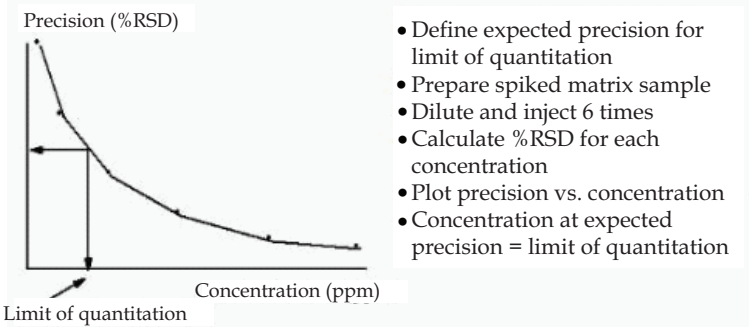

Fig. 4. Limit of quantitation with the EURACHEM (80) method

3.8 Repeatability

From the repeatability standard deviation or or sr 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.

3.9 Reproducibility

From the reproducibility standard deviation oR or sR it is useful to calculate the 'reproducibility limit 'R', 'which enables the analyst to decide wheth er the difference between duplicate analyses of a sample, determined under reproducibility conditions, is significant. These calculations can be performed directly with the built-in statistics function of the instrument, if available, or by using a pocket calculator or a PC (Personal Computer) with a suitable software pac kage (e.g. spreadsheet program).

3.10 Measurement uncertainty

Measurement uncertainty is a single parameter (usually a standard deviation with a coverage factor 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 is often termed an 'uncertainty budget' and 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.
- 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. 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. Note that, subject to additional consideration of effects outside the scope of a collaborative trial, the reproducibility standard deviation forms a working estimate of a 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 large than the published reproducibility standard deviation.

3.11 Sensitivity

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.

3.12 Ruggedness (or robustness)

Ruggedness is normally evaluated during method development, typically by the originating laboratory, before collaborating with other laboratories and is a measure how well a method 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 tests are normally applied to investigate the effect on either precision or accuracy.

4. The validation tools

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.

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 interference that would be encountered in the analysis of test samples.
3. 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 know 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.
4. Spiked material: These are material or solutions, which have been fortified with the analyte(s) of interest. 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 know. 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. The nature of the spike obviously needs to be identified.
5. (Measurement) Standards: 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 characterized to a sufficient extent it can be used for reference or calibration purposes.
6. Reference materials: frequently confused with *certified reference materials*. 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 materials does not need to have the high degree of characterisation, traceability and certifi cation more properly associated with certified reference materials.
7. 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.

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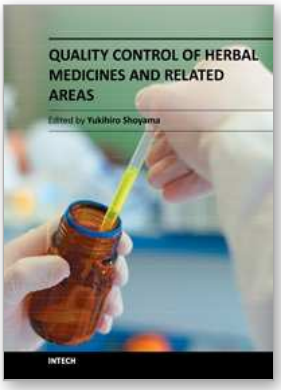

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The authors of this thematic issue provide a comprehensive summary of most recent knowledge and references on quality control in wide fields. Quality control is essential for natural products like natural medicine and related food products. In this issue fifteen chapters have been included, discussing in detail various aspects of quality control. It will certainly prove useful not only for phytochemical researchers, but also many scientists working in numerous fields. Much effort has been invested by the contributors to share current information. Without their efforts and input 'Quality Control of Herbal Medicine and Related Areas' could not exist.

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