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Is it really necessary to validate an analytical method or not? That is the question

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ABSTRACT

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 go about it. In addition, analysts' understanding of method validation is inhibited by the fact that many of the technical terms used in the processes for evaluating methods vary in different sectors of analytical measurement, both in terms of their meaning and the way they are determined. Validation applies to a defined protocol, for the determination of a specified analyte and range of concentrations in a particular type of test material, used for a specified purpose. In general, validation should check that the method performs adequately for the purpose throughout the range of analyte concentrations and test materials to which it is applied. It follows that these features, together with a statement of any fitness-for-purpose criteria, should be completely specified before any validation takes place.

1. Introduction

Reliable analytical methods are required for compliance with national and international regulations in all areas of the analysis. Accordingly, it is internationally recognised that a laboratory must take appropriate measures to ensure that it is capable of providing, and does provide, data of the required quality. Thus, method validation is an important requirement in the chemical analysis practice [1], and for this reason, it has received considerable attention in literature from industrial committees and regulatory agencies.

The final goal of the validation of an analytical method is to ensure that every future measurement in routine analysis will be close enough to the unknown true value for the content of the analyte in the sample [2]. Thus, the objectives of validation are not simply to obtain estimates of trueness or bias and precision but also to evaluate those risks that can be expressed by the measurement uncertainty associated with the result [3]. Method validation, together with uncertainty measurement or accuracy-profile estimation, can provide a way to check whether an analytical method is correctly fit for the purpose of meeting legal requirements [4]. Fitness for purpose is the extent to which the performance of a method matches the criteria that have been agreed between the analyst and the end-user of the data or the consumer and that describe their needs [1].

The purpose of validation is to test the suitability of methods, as well as the capacity of the staff and the laboratory. The validation is based on statistical parameters of the procedure. The procedures and scope of validation are not always the same and must be established individually.

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. A well-developed method should be easy to validate.

But, what is method validation? The word validation originates from the Latin validus meaning strong, and suggests that something has been proved to be true, useful and of an acceptable standard [5]. Method validation can be defined as the process of establishing the performance characteristics and limitations of a method, and of identifying the influences that may change these characteristics and to what extent [6]. Method validation is, therefore, an essential component of the measures that a laboratory should establish to be able to produce reliable analytical data. In general, validation should check that the method performs adequately for the purpose through the whole range of analyte concentrations to which it is applied. It therefore follows that these features, together with a statement of any fitness-for-purpose criteria, should be completely specified before any validation takes place. It is essential that validation studies are representative; that is, studies should, as far as possible, be conducted to provide a realistic survey of the number

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and range of effects operating during the normal use of the method, and to cover the concentration ranges and sample types within the method's scope. Several performance parameters should be studied, including specificity (the ability to measure a desired analyte in a complex mixture), accuracy (an agreement between the measured and the real value), linearity (the proportionality of the measured value to the concentration), precision (an agreement between series of measurements), range (a concentration interval where the method is precise, accurate and linear), detection limit (the lowest amount of analyte to be detected), limit of quantification (the lowest amount of analyte that can be measured), and robustness (reproducibility under normal but variable laboratory conditions). These concepts will be later explained in more detail.

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. 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.

In the last years, several manuscripts have already been published about method validation strategies [7–11], including measurement of uncertainty and accuracy profiles [12–15], guidance for robustness/ruggedness tests [16], quality assurance [17], focused in bioanalytical methods [18], and regulatory purposes in pharmaceutical and control of residues [19–23]. It should be taking into account that validation requirements are continually changing and vary widely.

Nowadays, there are several international renowned organizations offering guidelines on method validation and related topics [1,6,24–36]. Basic references are the Association of Official Analytical Chemists (AOAC), the American Society for Testing and Material (ASTM), the Codex Committee on Methods of Analysis and Sampling (CCMAS), the European Committee for Normalization (CEN), the Cooperation on International Traceability in Analytical Chemistry (CITAC), the European Cooperation for Accreditation (EA), the Food and Agricultural Organization (FAO), the United States Food and Drug Administration (FDA), the International Conference on Harmonization (ICH), the International Laboratory Accreditation Cooperation (ILAC), The World Health Organization (WHO), the International Organization for Standardization (ISO), the International Union of Pure and Applied Chemistry (IUPAC), the United States Pharmacopeia (USP), the analytical chemistry group EURACHEM, etc. The existence of many protocols and guidelines can confuse the analysts in their selection, and this can finally avoid the validation of a method.

In this manuscript we would like to highlight the necessity to validate analytical procedures in order to obtain reliable results. Some key aspects that should be considered when validating analytical methods will be discussed in order to derive useful information from experimental data and to draw robust conclusions about the validity of the method.

2. Why is method validation necessary?

Millions of analytical measurements are made every day in thousands of laboratories around the world. Virtually every aspect of society is supported in some way by analytical measurement. The cost of carrying out these measurements is high and additional costs arise from decisions made on the basis of the results. Thus, it is important to determine the correct result and be able to show that it is correct.

On the other hand, method validation enables chemists to demonstrate that a method is 'fit for purpose'. 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, method performance must be validated and the uncertainty on the result, at a given level of confidence, estimated.

In addition, method validation is required for the following reasons:

1. Assuring high quality.
2. Achieving acceptance of products by the international agencies.
3. Mandatory requirement purposes for accreditation as per ISO 17025 guidelines [37].
4. Mandatory requirement for registration of any pharmaceutical product or pesticide formulation.

3. How should methods be validated?

A generalised flowchart of the method validation process is detailed in Fig. 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. Usually national or international organizations, such AOAC International, ISO, etc., have undertaken the interlaboratory validation of the method in a method performance (collaborative) trial. The extent of laboratory internal validation and verification depends on the context in which the method is to be used.

If a method is being developed which will have wide-ranging use, then collaborative studies [38] 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, since those that might be interested could be competitors. 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.

The type of method and its intended use indicates which validation parameters need to be investigated, as can be seen in Table 1 according to the ICH criteria [27]. The laboratory has to decide which 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. 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. 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.

4. Method validation strategy

The necessity for laboratories to use a 'fully validated' method of analysis is now universally accepted or required within many sectors of analysis. Most method validation guides start with discussions on how criteria such as specificity, accuracy and precision of the method shall be established. The analytical problem, requirements of the customers and choices of analytical principles are seldom mentioned in this context. The first step in a 'full validation procedure' therefore should be to identify and document 'customer requirements' and the analytical problem, what is analytically and economically possible and other specific requirements on sampling, laboratory environment, external environment, etc.

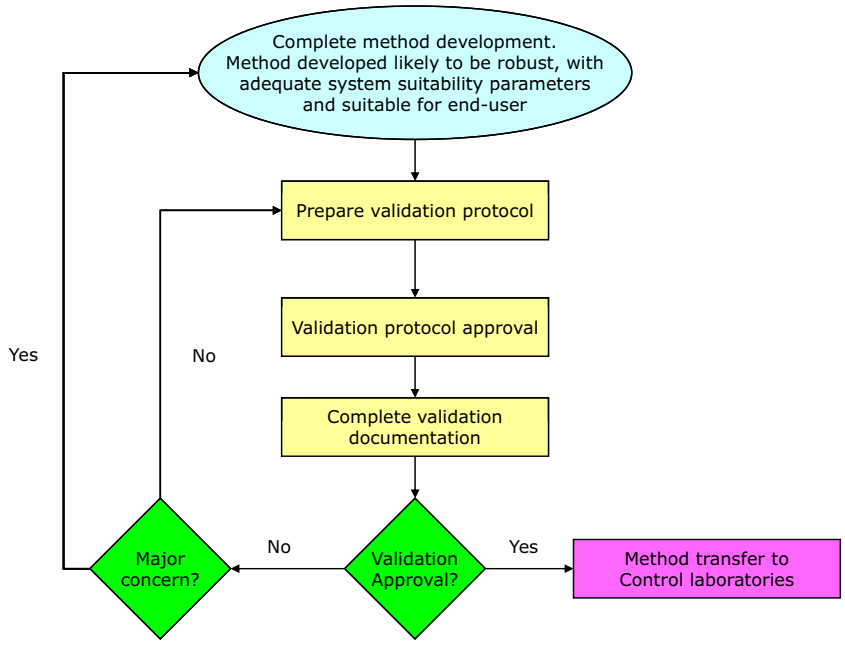

Fig. 1. Generalized method validation flowchart.

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A 'validation plan' should be written that indicates the method criteria needed and addresses questions such as:

- when is the method going to be used (e.g., official food control and in-house process control methods may have to fulfill different criteria on e.g., precision and accuracy),
- what type of answer is required (qualitative or quantitative), and
- in what state is the analyte.

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. Possible steps for a complete method validation are:

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 Standard Operating Procedures (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.

Table 1
Criteria to establish for different categories of methods of analysis.

Method-performance parameter	Type of assay			
	Identification test	Impurity test		Assay test – dissolution (measurement only) – content/potency
		Limit impurity test	Quantitative impurity test	
Specificity[a]	Yes	Yes	Yes	Yes
Accuracy	No	Yes	No	Yes
Precision	No	Yes	No	Yes
Repeatability	No	Yes	No	Yes
Intermediate precision	No	Yes[b]	No	Yes[b]
Reproducibility	No	Yes	No	Yes
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes
Limit of detection	No	No[c]	Yes	No
Limit of quantitation	No	Yes	No	No

[a] Lack of specificity of one analytical procedure could be compensated by other supporting analytical procedure(s).
[b] In cases where reproducibility has been performed, intermediate precision is not needed.
[c] May be needed in some cases.

Table 2
Parameters for method validation with reference to FDA, ICH, ISO 17025, IUPAC, and USP.

Parameter	Organization
Specificity	ICH, USP
Selectivity	FDA, ISO 17025, IUPAC
Accuracy	FDA, ICH, ISO 17025, USP
Precision	USP, ICH, FDA, IUPAC
Repeatability	ICH, ISO 17025
Intermediate precision	ICH
Reproducibility	ICH, defined as ruggedness in USP, ISO 17025, FDA
Trueness	IUPAC
Linearity	ICH, ISO 17025, IUPAC, USP
Range	ICH, USP
Limit of detection	FDA, ICH, ISO 17025, IUPAC, USP
Limit of quantitation	ICH, ISO 17025, IUPAC, USP
Robustness	FDA, included in ICH as method development activity, ISO, USP
Ruggedness	IUPAC, USP, defined as reproducibility in ICH
Sensitivity	FDA
Recovery	FDA, IUPAC
Applicability	IUPAC
Measurement uncertainty	IUPAC
Stability	FDA

15. Document validation experiments and results in the validation report.

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.

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 achieve to solve 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 Fig. 2, is method validation.

Here are some recommendations for the use of a single-laboratory method validation:

- Wherever possible and practical, a laboratory should use an analysis method whose performance characteristics have been evaluated through a collaborative trial that conforms to an international protocol.
- When such methods are not available, an in-house method must be validated before being used to generate analytical data.
- Single-laboratory validation requires the laboratory to select appropriate characteristics for evaluation (e.g., selectivity, calibration, accuracy, etc.).
- Evidence that these characteristics have been assessed must be made available.

During method validation, the parameters, acceptance limits and frequency of ongoing system suitability tests or quality control checks should be defined. Criteria should be defined to indicate when the method and system are beyond statistical control. The aim is to optimize these experiments so that, with a minimum number of control analyses, the method and the complete analytical system will provide long-term results to meet the objectives defined in the scope of the method. As shown in Fig. 3, there are required and fundamental controls that ensure the overall quality of the test data. The independent processes represented in Fig. 3 correlate to ensure the quality of the reported data [39].

5. 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. A revalidation is necessary whenever a method is changed, and the new parameter lies outside the operating range. 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.

6. Transferring validated routine methods

When validated methods are transferred between laboratories the receiving laboratory should demonstrate that it can successfully perform the method and 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. Typical instances when method transfer occurs are from the Research and Development (R&D) laboratory to the Quality Control (QC) laboratory. Currently, there is no official document available that can be used as a guide for performance demonstration of the receiving laboratory. However, the USP has published an article where the most common practices of method transfer are described [40]: comparative testing, co-validation between two laboratories or sites, complete or partial method validation or revalidation, and the omission of formal transfer, sometimes called the transfer waiver.

The transfer should be controlled by a procedure. The recommended steps are: (1) designate a project owner; (2) develop a transfer plan; (3) define transfer tests and acceptance criteria (validation experiments, sample analysis: sample type, replicates); (4) describe rational for tests; (5) train receiving laboratory operators in transferring laboratory on equipment, method, critical parameters and troubleshooting; (6) repeat 2 critical method validation tests in routine laboratory; (7) analyze at least three samples in transferring and receiving laboratory; and (8) document transfer results.

7. Validation method parameters

The parameters for method validation have been defined in different working groups of national and international committees and are widely 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 [27], 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. It should be noted that it is not the purpose of the manuscript to discuss the differences and similarities of the validation method parameters, nor even

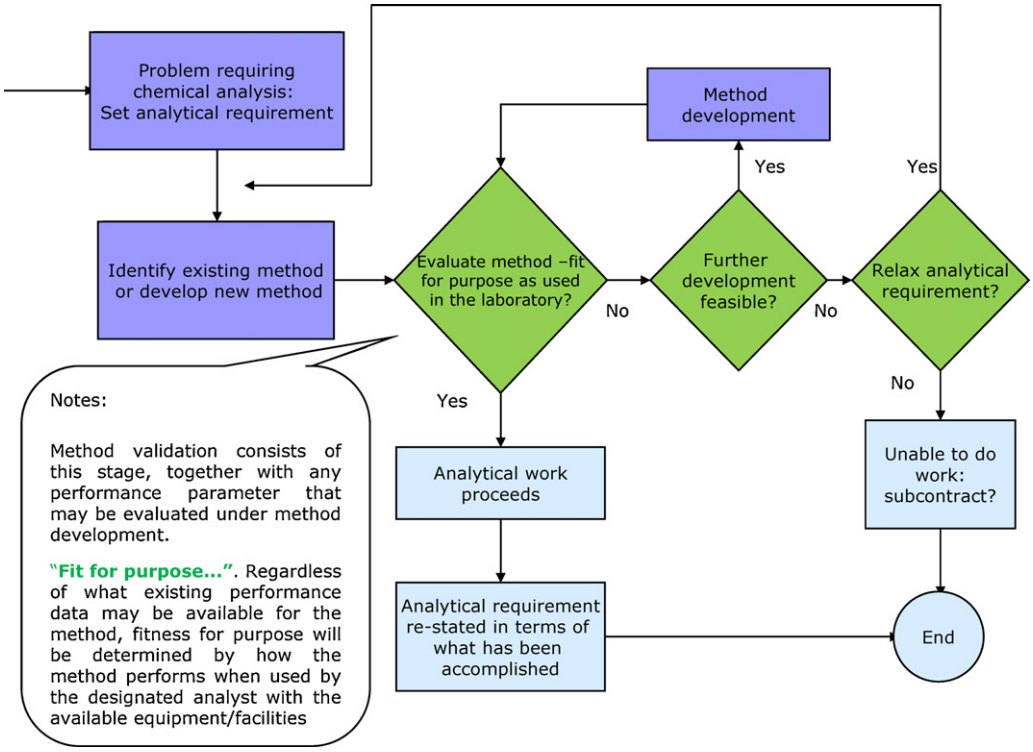

Fig. 2. Selection, development and method evaluation.

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how to measure them. Although Table 2 summarizes the parameters defined by the ICH and by other organizations, they will be next described briefly following only the ICH definition. It should be pointed out that ICH guidelines should be regarded as basis and philosophy of analytical validation, not as a checklist. "It is the responsibility of the applicant to choose the validation procedure and protocol most suitable for their product" [27]. Suitability is strongly connected with the requirements and the design of the given analytical procedure, which obviously varies and must, therefore, be reflected in the analytical validation. This includes the identification of the performance parameters relevant for the given procedure, the definition of appropriate acceptance criteria, and the appropriate design of the validation studies. In order to achieve this, the analyst must be aware of the fundamental meaning of these performance parameters, calculations, and tests and their relationship to his specific application. A lack of knowledge or (perhaps) a wrong understanding of "efficiency" will lead to validation results that address the real performance of the analytical

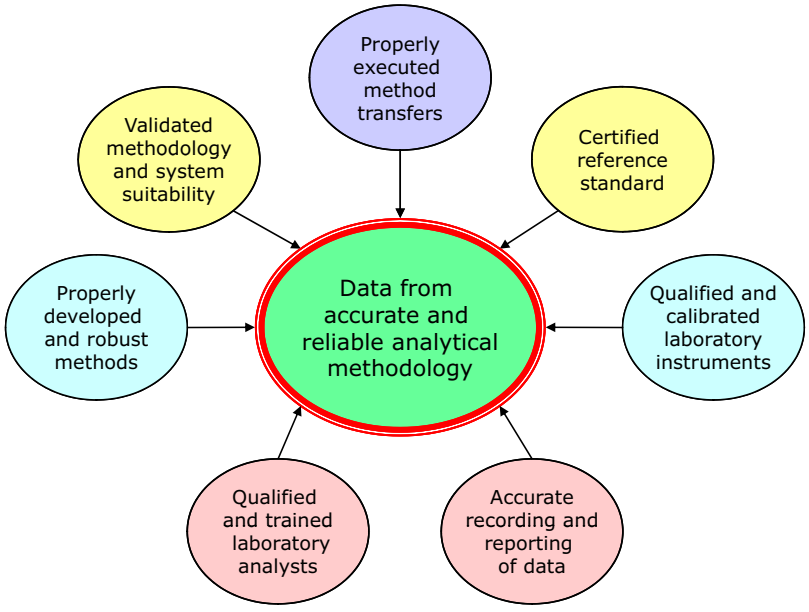

Fig. 3. Unified elements that ensure reliability of data from analytical methodology.

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procedure only partly or insufficiently. In the best case, it is a waste of resources because the results are meaningless.

There are no official guidelines on the correct sequence of validation experiments, and the optimal sequence may depend on the method itself. However, the more time-consuming experiments, such as accuracy and robustness, are included toward the end. Some of the parameters (such as, linearity, limit of quantitation, limit of detection, range, repeatability, intermediate precision and accuracy) can be measured in combined experiments. For example, in the validation of liquid chromatography methods, when the precision of peak areas is measured over the full concentration range, the data can be used to validate the linearity.

7.1. Selectivity and specificity

Specificity and selectivity both give an idea of the reliability of the analytical method. These terms have been the subject of intensive critical comments essentially focusing on the ways in which they are often defined by analysts working in method validation [41,42]. Selectivity refers to the extent to which a method can determine a particular analyte in a complex mixture without interference from other components in the mixture [43]. This definition is often wrongly used as equivalent to specificity, which is considered to be the ultimate in selectivity; it means that no interferences are supposed to occur [44]. By definition, the specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present [27]. Unfortunately, an inspection of the literature on method validation revealed that both terms are still used without distinction by some authors, even though by consulting the dictionary it is clear that these terms should not be used interchangeably. Selectivity should be connected with the word 'choose' while specificity with the word 'exact'. The term specificity refers always to 100% selectivity [45,46] or, conversely, 0% interferences.

7.2. Accuracy and recovery

The accuracy of an analytical procedure is the closeness of agreement between the conventional true value or an accepted reference value and the value found. This is sometimes termed trueness, which is stated quantitatively in terms of bias. Thus, since the determination of accuracy allows estimating the extent to which systematic errors affect a particular method, it has been highlighted that is the most crucial aspect that any analytical method should address [47].

The ICH document on validation methodology recommends accuracy to be assessed using a minimum of nine determinations over a minimum of three concentration levels covering the specified range (for example, three concentrations with three replicates each). Accuracy should be reported as percent recovery by the assay of known added amount of analyte in the sample or as the difference between the mean and the accepted true value, together with the confidence intervals. The expected recovery depends on the sample matrix, the sample processing procedure and the analyte concentration. The AOAC manual for the Peer-Verified Methods program [48] includes a table (Table 3) with estimated recovery data as a function analyte concentration.

7.3. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. It is usually expressed as the variance, standard deviation or coefficient of variation of a series of measurements. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility.

Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision.

Intermediate precision expresses variations within laboratories, such as different days, different analysts, different equipment, and so forth.

Reproducibility expresses the precision between laboratories (collaborative studies usually applied to standardization of methodology).

The objective of intermediate precision is to verify that in the same laboratory the method will provide the same results. However, the objective of reproducibility is to verify that the method will provide the same results in different laboratories. It should be pointed out that it is wrong to report a so-called "inter-day repeatability" term. Such a term should never be used in method validation. The term inter-day variation should be connected with intermediate precision or in some circumstances with reproducibility.

The ICH requires repeatability to be tested from at least six replications measured at 100 percent of the test target concentration or from at least nine replications covering the complete specified range. For example, the results can be obtained at three concentrations with three injections at each concentration.

Table 3 shows the estimated precision data as a function of analyte concentration recommended by the AOAC manual for the Peer-Verified Methods program.

7.4. Linearity, calibration curve and range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional (or by means of well-defined mathematical transformations) to the concentration (amount) of analyte in the sample.

Linearity may be demonstrated directly on the test substance (by dilution of a standard stock solution) or by separately weighing synthetic mixtures of the test product components. Linearity is determined by a series of five to six injections of five or more standards whose concentrations span 80–120 percent of the expected concentration range. A linear regression equation applied to the results should have an intercept not significantly different from zero. If a significant nonzero intercept is obtained, it should be demonstrated that this has no effect on the accuracy of the method [49]. It should be indicated that there are numerous analytical procedures that use non-linear calibration, but it is out of the scope of this manuscript to discuss them.

Range of an analytical procedure can be defined as the interval from the upper to the lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity.

7.5. Limit of detection and limit of quantitation

The limit of detection (LOD) of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value, whereas the limit of quantitation (LOQ) is the lowest amount of analyte in a sample that can be quantitatively determined with suitable precision and accuracy. LOD is the point at which a measured value is larger than the uncertainty associated with it, while LOQ is a parameter of quantitative assays for low levels of compounds in sample matrices, and is used particularly for the determination of impurities and/or degradation products.

For example, in chromatography, the detection limit is the injected amount that results in a peak with a height of at least two

Table 3
Acceptable recovery percentages and precision depending on the analyte level.

Analyte (%)	Analyte fraction	Unit	Recovery range (%)	RSD (%)
100	1	100%	98–102	1.3
10	10^{-1}	10%	98–102	2.8
1	10^{-2}	1%	97–103	2.7
0.1	10^{-3}	0.1%	95–105	3.7
0.01	10^{-4}	100 ppm	90–107	5.3
0.001	10^{-5}	10 ppm	80–110	7.3
0.0001	10^{-6}	1 ppm	80–110	11
0.00001	10^{-7}	100 ppb	80–110	15
0.000001	10^{-8}	10 ppb	60–115	21
0.0000001	10^{-9}	1 ppb	40–120	30

or three times as high as the baseline noise level. The LOQ is generally determined by the analysis of samples with known analyte concentrations and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. If the required precision of the method at the limit of quantitation has been specified, 5 or 6 samples with decreasing amounts of analyte are injected by replicate (e.g., 5–6 times). The calculated relative standard deviation (RSD) percent of the precision of these repetitive injections 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 unknown samples.

For the LOD and LOQ calculations, different approaches can be used: the visual inspection, the standard deviation (SD) of the response and the slope (S) of the calibration curve (3.3·SD/S for LOD and 10·SD/S for LOQ; the SD of the response can be determined based on the SD of the blank, on the residual SD of the regression line, or the SD of *y*-intercepts of regression lines), and the signal-to-noise ratio convention (usually 3:1 for LOD and 10:1 for LOQ), among others. The signal-to-noise ratio for LOQ is a good rule of thumb, but it should be remembered that its determination is a compromise between concentration and the required precision and accuracy. That is, as the LOQ concentration level decreases, precision increases.

Any results of limits of detection and quantitation measurements must be verified by experimental tests using samples containing analytes at levels across the two regions. It is equally important to assess other method validation parameters, such as precision, reproducibility and accuracy, which are close to the limits of detection and quantitation.

7.6. Stability

Another challenge encountered early in the development of methods intended to support stability studies is ensuring that the method is stability indicating. This process is typically achieved by conducting forced degradation studies. The design and execution of these studies requires thorough knowledge of the product being tested as well as a good understanding of the analysis technique.

Chemical compounds can decompose prior to chromatographic investigations, for example, during the preparation of the sample solutions, extraction, cleanup, phase transfer or storage of prepared vials (in refrigerators or in an automatic sampler). Under these circumstances, method development should investigate the stability of the analytes and standards. Stability testing is important for estimating the allowed time span between sample collection and sample analysis. The studies should evaluate the stability of the analytes during sample collection and handling after typical storage scenarios such as long-term storage (when frozen at intended storage temperatures), short-term storage (during a series of sample analyses at room temperature), and after freeze and thaw-cycles.

It is also important to evaluate an analytical method's ability to measure drug products in the presence of its degradation products. To force degradation, ICH also recommends conducting stress studies, in conditions such as elevated temperature, humidity or light.

7.7. Ruggedness and robustness

Ruggedness is not addressed in the ICH document [27]. This parameter evaluates the constancy of the results when external factors such as analyst, instruments, laboratories, reagents, days are varied deliberately. Its definition has been replaced by reproducibility, which has the same meaning. Ruggedness was defined by the USP until 2006 as the degree of reproducibility of results obtained under a variety of conditions, such as different laboratories, analysts, instruments, environmental conditions, operators and materials. Ruggedness cannot be erroneously used as a synonymous of robustness. However, it should be pointed out that from 2007, the United States Pharmacopoeia (USP 30-NF 25) [50] has revised chapter <1225> to harmonize more closely with ICH, using the term "intermediate precision", thus, deleting all references to ruggedness and introducing the concept of robustness.

Robustness evaluates the constancy of the results when internal factors (no external factors as in ruggedness) such as flow rate, column temperature, injection volume, mobile phase composition (in liquid chromatography) or any other variable inherent to the method of analysis (e.g., stability of analytical solutions, extraction time, etc.) are varied deliberately. ICH defines the robustness of an analytical procedure as 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. However, it is generally not considered in most validation guidelines.

Although robustness and ruggedness aim at testing the reproducibility of the test results regardless of internal or external influences respectively, the literature on method validation bears evidence that both terms are used interchangeably. The analyst performing a method validation should distinguish the similarities and differences between these validation parameters and avoid misconstruing ruggedness as robustness.

Robustness tests examine the effect that the operational parameters have on the analysis results. These method parameters are varied within a realistic range, and the quantitative influence of the variables is determined. If the influence of the parameter is within a previously specified tolerance, the parameter is said to be within the method's robustness range. Obtaining data on these effects helps to assess whether a method needs to be revalidated when one or more parameters are changed. In the ICH document, it is recommended to consider the evaluation of a method's robustness during the development phase, and any results that are critical for the method should be documented.

The most convenient way to determine the robustness of a method is by using chemometric experimental design procedures. The univariate approach is time-consuming but appropriate if the selection of experimental variables is adequate.

A common weakness in development and validation of methods is that the methods are not robust enough. If robustness is not built into methods early in development, then the result most likely will be loss of efficiency during routine quality control testing and a lengthy and complicated validation process as well.

8. Measurement uncertainty

Measurement uncertainty is a statistical parameter which describes the possible fluctuations of the result of a measurement. Uncertainty defined by the ISO Guide on Uncertainty of Measurement (GUM) [51] is "a parameter associated with the result of a measurement that characterises the dispersion of the values that could reasonably be attributed to the measurand". This parameter is usually a standard deviation, a given multiple of it, or the width of a confidence interval. The uncertainty expanded by a factor, 2 for e.g., is interpreted as an interval in which the true value of the result of a measurement resides with a defined probability. Measurement uncertainty defines thus a region around a routine result obtained from an incurred sample where it is highly probable to observe the real unknown true result. This parameter is estimated in order to judge the adequacy of a result for its intended purpose and to verify its consistency with other similar results. Recently, Meyer [52] revised the uncertainty measurement and indicated that some good working principles can help to obtain low measurement uncertainties. Any measurement uncertainty should be kept low but it is objectionable to state too low a value, e.g., by falsely reporting mere repeatability data instead of properly determined uncertainty data. However, it should be noted that measurement uncertainty is rarely used.

9. Is method validation always the best option?

After some decades insisting on validation and normalization of methods, the reliability of the results in Analytical Chemistry continues to be unsatisfactory. Also, the processes involved are too slow, with the effect that frequently, the most relevant results are obtained with methods not yet validated according to norms. Methods need to be developed or adapted in a short time, and chemical analysis often has an enormous impact. The present system contributes little to ensure the correctness of these results.

In addition, it seems that methods are perceived as machines: they are tested and after delivery they should work in every laboratory adequately respecting quality management requirements. A first problem originated from incomplete specifications in the methods, e.g., owing to differences in equipment. Hence, methods are not fully standardized and it remains up to the user to ensure that his system works properly. Most of the errors occurring in practice are failures rather than deviations somewhat exceeding the measurement uncertainty specified by the method.

To account for the instability of the performance, many methods require a kind of day-revalidation at the beginning and possibly at the end of a series of analyses. Day-revalidation shifts the focus from the initial method validation towards control within a series of analyses. Indeed, if the calibration provides the correct results for the check samples, it can be assumed that also the results of the samples to be analyzed will be correct.

However, sometimes verification of the results by tools built into the method provides better reliability than conventional validation of the method, since the performance of the critical steps or of the whole procedure is controlled for every sample. The design of a verification system starts from the analysis of the method: the potential sources of errors or deviations are listed and verification tools introduced to control them, possibly several being covered by the same tool. Then the method is overviewed to ensure that a maximum of critical points is covered. Points which cannot be covered by verification must be investigated within a classical validation program. The introduction of verification tools and the determination of the thresholds of acceptability, mean an extra effort in method development. However, if correct performance can be demonstrated for each sample, part of the method validation and day-revalidation are rendered unnecessary.

An important advantage of methods with a verification system is in their easy transfer to other laboratories. Reliability of the results can be ensured without time-consuming control of the method by other laboratories or normalization bodies: if the method does not work properly, the user will note it immediately. For this reason, methods with a far reaching verification are promising to increase the flexibility of analytical chemistry. It should be highlighted, however, that verification does not completely replace initial method validation. The procedure must be investigated, optimized and tested on quantitative performance, now even including the elaboration of the specifications for the verification tools.

10. Conclusions

Analytical methods are used for areas of far-reaching significance worldwide. To name a few examples, these could be areas of processing and quality control in the manufacture of all kinds of goods, food monitoring and application monitoring. Based on the precautionary principle and particularly in Europe, the consumer must be protected from hazards which could affect him through deficient products such as consumer goods, for example articles of daily use, cosmetics and tobacco, but particularly through the expansive and elementary area of food manufacturing and marketing. Nowadays, an analytical result is indispensible to take decisions. It has a significant impact on our society. For this reason, several standards and guides have been written and established for the sole purpose of ensuring the quality of results returned. The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories.

Validation is a constant, evolving process starting before an instrument is placed on-line and continues long after method development and transfer. A well-defined and well-documented validation process provides regulatory agencies with evidence that the system and method is suitable for its intended use. By approaching method development, optimization and validation in a logical, stepwise fashion, laboratory resources can be used in a more efficient and productive manner.

For analysts, method validation is the process of proving that an analytical method is acceptable for its intended purpose. In order to resolve this very important issue, analysts refer to regulatory or guidance documents which can differ in several points. Therefore, the validity of the analytical method is partially dependant on the chosen guidance, terminology and methodology. It is therefore highly essential to have clear definitions of the validation criteria used to assess this validity, to have methodologies in accordance with these definitions and consequently to use statistical methods which are relevant with these definitions, the objective of the validation and the objective of any analytical methods.

The objective of analytical method validation is to ensure that every future measurement in routine analysis will be close enough to the unknown true value for the content of the analyte in the sample. So, is it really necessary to validate an analytical method? No doubt, the answer is clearly yes. Even more, the analytical method validation should be a mandatory step to evaluate the ability of developed methods to provide accurate results for their routine application. Indeed, without results of adequate quality or reliability, the critical decisions that will be made during routine application of the method will be untrustworthy. It should be mentioned that the verification of the results could be an alternative to classical method validation, but taking into account that does not completely replace initial method validation.

The efficient development and validation of analytical methods are critical elements. Success in these areas can be attributed to several important factors, which in turn will contribute to regulatory compliance. Experience is one of these factors – both the experience level of the individual scientists and the collective experience level of the development and validation department. A strong mentoring and training programme is another important factor for ensuring successful methods development and validation.

New regulatory guidelines are being published governing the expectations of regulatory agencies throughout the world for method development and validation. Another challenge is that many laboratories must upgrade methods to meet current regulatory standards. From a simple method improvement to a complete redevelopment and subsequent cross-over to an older method, the upgrade of analytical methods can be a daunting task. For this reason, one must be alert to current trends in regulatory guidelines and to adopt a proactive approach to changes that may affect development and validation programmes. Finally, one of the key requirements for methods validation (which is also one of the key challenges) is that only well-characterised reference materials with well-documented purities should be used during methods validation activities. The challenge stems from the fact that, in some cases, the tools used to characterise reference standard materials are being developed and validated at the same time as the reference standard itself.

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