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Harmonization of strategies for the validation of quantitative analytical procedures A SFSTP proposal – Part II

Ph. Hubert[a,*], J.-J. Nguyen-Huu[b], B. Boulanger[c], E. Chapuzet[d], P. Chiap[e], N. Cohen[f], P.-A. Compagnon[g], W. Dewé[h], M. Feinberg[i], M. Lallier[j], M. Laurentie[k], N. Mercier[d], G. Muzard[l], C. Nivet[m], L. Valat[n], E. Rozet[a]

[a] *Laboratory of Analytical Chemistry, Bioanalytical Chemistry Research Unit, Institute of Pharmacy, University of Liège, CHU, B36, B-4000 Liège, Belgium*
[b] *Sanofi-Aventis, quai Jules Guesde, B.P. 14, F-94403 Vitry sur Seine, France*
[c] *UCB Pharma SA, Chemin du Foriest, B-1420 Braine-L'alleud, Belgium*
[d] *Qualilab, rue de la Bergeresse, F-45160 Olivet (Orléans), France*
[e] *Advanced Technology Corporation (A.T.C.), Institute of Pathology B23, University Hospital Center of Liège, B-4000 Liège 1, Belgium*
[f] *Expanscience, rue des quatre Filles B.P. 25034, F-28231 Epernon, France*
[g] *French Agency for Health Products Safety (AFSSAPS), Boulevard Anatole France, Les Portes de Pleyel, F-93285 St. Denis, France*
[h] *GlaxoSmithKline Biologicals, 89, Rue de l'Institut, B-1330 Rixensart, Belgium*
[i] *National Institute for Agricultural Research (INRA), rue Claude Bernard, F-75231 Paris, France*
[j] *Innothera, Avenue Paul Vaillant Couturier, F-94110 Arcueil, France*
[k] *LERMDV, French Food Safety Agency (AFSSA), B.P. 90203, F-35032 Fougères, France*
[l] *Merck-Theramex, Avenue Prince Héréditaire Albert, F-98007 Monaco, France*
[m] *CERB, Chemin de Montifault, F-18800 Baugy, France*
[n] *Viatris-Manufacturing, Avenue J.F. Kennedy, B.P. 100, F-33701 Mérignac, France*

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Abstract

As reported in a previous paper [1], the main objective of the new commission of the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) was the harmonisation of approaches for the validation of quantitative analytical procedures. In a series of meetings, members of this Commission have first tried to review the objectives of analytical methods and the objectives of validation methods and to recommend the use of two-sided β-expectation tolerance intervals for total error of validation samples (accuracy profile) in the acceptance/rejection of analytical method in validation phase.

In the context of the harmonization, the other objectives were: (i) to propose a consensus on the norms usually recognized, while widely incorporating the ISO terminology; (ii) to recommend to validate the analytical procedure accordingly to the way it will be used in routine; (iii) to elaborate a rational, practical and statistically reliable strategy to assure the quality of the analytical results generated. This strategy has been formalised in a guide and the three latter objectives made by the Commission are summarised in the present paper which is the second part of summary report of the SFSTP commission.

The SFSTP guide has been produced to help analysts to validate their analytical methods. It is the result of a consensus between professionals having expertise in analytical and/or statistical fields. The suggestions presented in this paper should therefore help the analyst to design and perform the minimum number validation experiments needed to obtain all the required information to establish and demonstrate the reliability of its analytical procedure.

Keywords: Analytical procedure; Validation; Harmonization; Quantitative analysis; Accuracy profile; Total error; Experimental protocol

* Corresponding author. Tel.: +32 4 366 43 16; fax: +32 4 366 43 17.
E-mail address: ph.Hubert@ulg.ac.be (Ph. Hubert).

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1. Introduction

Irrespective of the sector of activity (chemistry, pharmacy, bio-pharmacy, food processing, environment, cosmetology, etc.), the goal of validation is to establish that the analytical method is suitable for its intended use, i.e. to prove the reliability of method results within well defined limits. An approach that is used currently is to define acceptance criteria based only on point estimates of assay parameters without an assessment of uncertainty. With this approach, the risk of accepting an unsuitable assay and rejecting a suitable assay are unknown and uncontrolled [2,3]. An alternative approach that controls these risks is to use accuracy profiles based on two-sided β-expectation tolerance intervals for total measurement error (including both bias and precision) of validation standards [4,5]. Such an approach reflects more directly the performance of individual assays and will result in fewer rejected in-study runs than the current procedure that compares point estimates of observed bias and precision with the target acceptance criteria, e.g. 2% (bulk drug), 5% (dosage form) or 15% (bioanalysis) [6]. It is why this approach has been adopted by the new SFSTP commission on the harmonisation of approaches for the validation of quantitative analytical procedures [1,7]. The accuracy profile constitutes for the analyst a visual tool allowing him to evaluate the capability of its method. In the context of harmonization, it is also needed to make the difference between the statistical tools that allow taking a decision (accuracy profiles) and the statistical tests that have a diagnostic purpose (estimation of trueness and precision). Indeed, as mentioned in the first part of the SFSTP guide [7], every analytical method is characterized by a "true bias" (systematic error) and a "true variance" (random error). These parameters are inherent in each method and they are also always unknown. In fact, an estimation of the method bias and variance can be obtained from the experiments carried out during method validation. These estimates will be more reliable if the experimental design and the number of experiments performed in the method validation are appropriate [8,9]. On the basis of these estimates for method bias and variance, the acceptance limits for the performance of the method make it possible to define the concept of "good analytical method" for a given field (e.g. bioanalysis) [4]. It is in this context that the statistical analysis of the validation results can find its real dimension and that the new commission proposed to review the bases of the analytical validation for developing harmonized approach, by distinguishing notably the diagnosis rules and the decision rules as reported in the first part of the SFSTP summary report [1,7].

On the other hand, considering official documents on validation of analytical methods [6,10–17], similarities (e.g. determination of accuracy, use of confidence intervals) and discrepancies (e.g. determination of linearity, interpretation of accuracy) can be found. It is why the new SFSTP guide also aims to propose in the present paper (part II of the summary report of SFSTP Commission [7]) a consensus on the norms usually recognized, while widely incorporating the ISO terminology. It also emphasizes the necessity to validate the analytical method in the same way it will be used in routine. However, as can be seen from the scientific literature, even if the validation criteria are defined, validation methodology together with practical experimental protocols are highly discussed [see for example 18–34] Thus, the new SFSTP guide finally presents an experimental strategy for the validation of the dosage procedures, regardless of the industrial sector, to optimally use experiments performed, to extract a maximum of information from the results and to minimize in routine the risks to re-analyze samples. The overall SFSTP approach [1,7] will therefore minimize considerably the risk to accept a procedure that would not provide sufficiently accurate results or, to the opposite, to reject a procedure that would be capable [35,36].

Table 1
illustration of the interpretation of the concept of accuracy

	Accuracy vs. trueness
Statistics	Total error = systematic error + random error = bias + standard deviation
ISO [15,16]	Total error = trueness + precision = accuracy
ICH [10]	Total error = ? accuracy (Q2R1, Part I) [10] ? = accuracy (Q2R1, Part II) [10] + precision accuracy (Q2R1, Part II) [10] = trueness ISO [15]

2. Terminology

The following generally accepted validation criteria [6–17] are listed in the SFSTP guide:

Specificity – selectivity	Trueness
Response function (calibration curve)	Accuracy
Linearity	Limit of detection (LOD)
Precision (repeatability and intermediate precision)	Limit of quantitation (LOQ)
	Assay range

In addition, according to the domains concerned, other specific criteria can be required, for example the following ones: (i) analyte stability; (ii) recovery; (iii) effect of the dilution, etc.

It must be underlined that the validation criteria mentioned above must be evaluated, as much as possible, in the same matrix as the one of the samples intended to be analysed. Nevertheless, the definition of a matrix depends on analyst responsibility and some matrix regrouping, generally admitted by the profession for an application domain given, can be performed. Moreover, each modification of a previously validated method automatically involves a re-validation, the extent of which depends on the modifications made and their possible influence on specific validation criteria [7,12,37].

On the other hand, it is important to specify that there is not yet a global consensus between the various regulatory documents (ISO, ICH, AFNOR, SANCO, FDA, . . .) for the definition of the criteria to be tested during the validation step [6–17]. For example, the linearity criterion can appear or not and its interpretation can be different from one document to another [6,10–12,37–39]. It is the same for the trueness that can be confused with the accuracy according to the referential used [10–17] as illustrated in Table 1. The definitions of the validation criteria selected by the SFSTP Commission are most often those given in the ICH text Q2R1 [10] excepted for the four criteria, described below, for

which the ISO norm was in particular selected as referential in order to obtain harmonized definitions.

2.1. Response function (calibration curve)

The response function of an analytical procedure is, within the range, the existing relationship between the response (signal) and the concentration (quantity) of the analyte in the sample. The calibration curve is the simplest monotonous response function [6,12,37,40].

The response function can be linear (straight line), but non linear models, sometimes induced by the detection method or by a wide concentration range, can also be observed [37,39,40]. The response function must however be monotonous, i.e. strictly increasing or decreasing. It must be noticed that the estimation of such a function using common fitting methods (e.g. least squares method) assumes that whatever the concentrations the response variance is a constant (homoscedasticity). However, this hypothesis is rarely met when the range is wider. The function meeting these requests and fitting the response is the calibration curve, which is then used to calculate the concentrations, i.e. the results.

2.2. Linearity (of the results)

The linearity of an analytical procedure is its ability within a definite range to obtain results directly proportional to the concentrations (amount) of the analyte in the sample [10].

The linearity criteria must only be applied to the results [calculated concentration = f (introduced concentration)], not to the responses [signal = f (introduced concentration)]. The linearity is required for the evaluation of the trueness, but a linear relationship between calculated concentrations and introduced ones does not guarantee the trueness of the analytical procedure (e.g. when bias is present) [37,40].

2.3. Trueness (bias)

The trueness of an analytical procedure expresses the closeness of agreement between the mean value obtained from a series of measurements and the value which is accepted either as a conventional true value or an accepted reference value (international standard, standard from a pharmacopoeia) [15].

The measure of trueness is generally expressed in terms of recovery and of absolute or relative bias (systematic error). It must be again notified that the trueness was also called "accuracy" or "accuracy of the mean". Nevertheless, this use is not recommended [15,40].

2.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value found and the value which is accepted either as a conventional true value or an accepted reference value [10,15,40].

The closeness of agreement observed is the resultant of the sum of the systematic and random errors, in other terms, the total error linked to the result. Consequently, the accuracy is the expression of the sum of the trueness and precision.

In ISO definitions of 1994 [15], accuracy is very close to the trueness. However, the note shows that it is a mixture between the trueness (a bias) and precision (a standard deviation). This is why the accuracy term is always accompanied by trueness and precision in the title of the ISO 5725 norm. In fact, one cannot measure in only one parameter, difference compared to a reference value and a dispersion of the results. This is why, the preference is now given to the terms, on the one hand, of uncertainty which is characterized by a composite standard deviation (of which one of the components is the random component of the bias of trueness), on the other hand, of trueness. It is still to note that the only interest of uncertainty compared to precision is to be close to the traditional vocabulary of metrology.

3. Validation protocols

3.1. Life cycle

As illustrated in Fig. 1, the implementation of a dosage procedure can be decomposed essentially in four successive phases: (1) a selection phase that allows to define the objectives and the initial operational conditions; (2) a development phase, with or without optimization by means of experimental designs (robust optimization); (3) a phase of validation that could be preceded by a pre-validation phase; (4) an application phase in routine, including most often a validation in routine and sometimes a partial validation.

The validation of a method has to be considered as an element intervening after the development of a new analytical procedure and so its performances will evolve during the course of the different phases of his life cycle (cf. Fig. 1). Confidence in the analytical results will considerably evolve during the course of the two first phases to go towards an increased confidence of which the validity is assessed in validation phase. However, the objective of the analytical procedure is not its validity but its routine use. Consequently, this usage in routine necessarily must be coupled with the set up of a quality control (QC) of which the objectives are on one hand the validity of the obtained results on

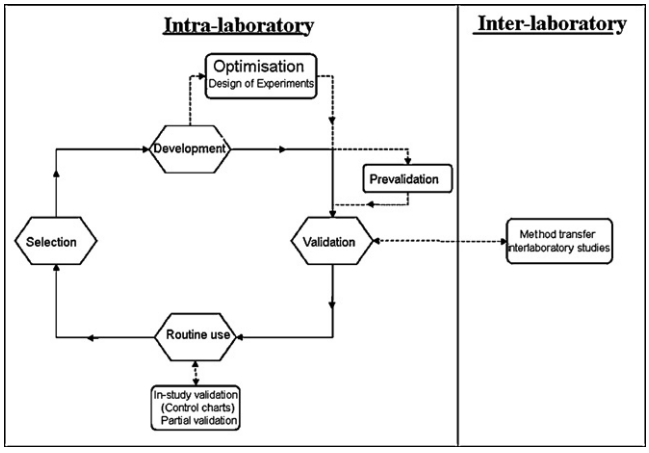

Fig. 1. Life cycle of an analytical procedure.

the unknown samples, and on the other hand the assessment of the continuity of the performances of the analytical procedure at the time of its use in routine.

Consequently, before beginning the validation, basic information on the performances of the analytical procedure are desirable, and even essential in some cases. These prerequisites to the validation concern in particular the relevance of the regression model retained to be the response function, the knowledge of the variability of the results, the limit(s) of quantification and the assay range. Two cases are frequent: either this information was obtained during the development, because of the use of an experimental design for example, or this information is unknown at the end of the development phase. In such a case, a pre-validation phase completes the development of the analytical procedure. Nevertheless, in the case of simple procedures, it is conceivable that the analyst begins directly with the phase of validation. For that reason, the validation will be described in the present paper before the pre-validation protocol.

3.2. Protocols in validation phase

The calibration standards (CSs), which are samples of known concentrations, with or without matrix, that allow to draw the calibration curve, must be prepared according to the protocol that will be applied in routine, i.e. the same operational mode, the same number of concentration levels (calibration point) and the same number of repetition by level. The validation standards (VSs), which are samples reconstituted in the matrix or in any other reference material with true values set by consensus and used to validate the analytical procedure, have to be independent samples (within-series variability) if it is applicable. Indeed, they actually represent, in validation phase, the futures samples that the analytical procedure will have to quantify. Each VS has to be prepared and treated independently as a future sample. The independence is essential for a good estimation of the between-series variance. In practice, the effects of "day", and "operator" are most often considered. It has to be noticed that, following the analytical constraints, the day effect can be replaced by the series effect, for example while realizing two series on a same day, as far as the analytical procedure is recommenced in its entirety between the series (sample preparation, different solutions, reactive, calibration, etc.). Moreover, in order to estimate the intermediate precision, i.e. the precision within the laboratory of interest under different operational conditions, alternative experiments have to be envisaged. Indeed, the analytical procedure is not developed to quantify in routine with the same operator and on the same equipment a single unknown sample during a day but a very large number of samples through time and so, implying often several operators and several equipments. The independent character of the calibration standards is less important as far as the protocol used in validation phase would be the same as the one that will be employed in routine. The calibration method is actually part of the definition of the analytical procedure. Finally, it appears for us important to insist on the fact that the validation phase is the ultimate stage, before the exploitation of the analytical procedure, allowing us to reasonably estimate its performances in the expected operational conditions and also to check its capability to quantify each unknown sample that it will have to measure. The Fig. 2 presents a decision tree to help the analyst to select an experimental pro-

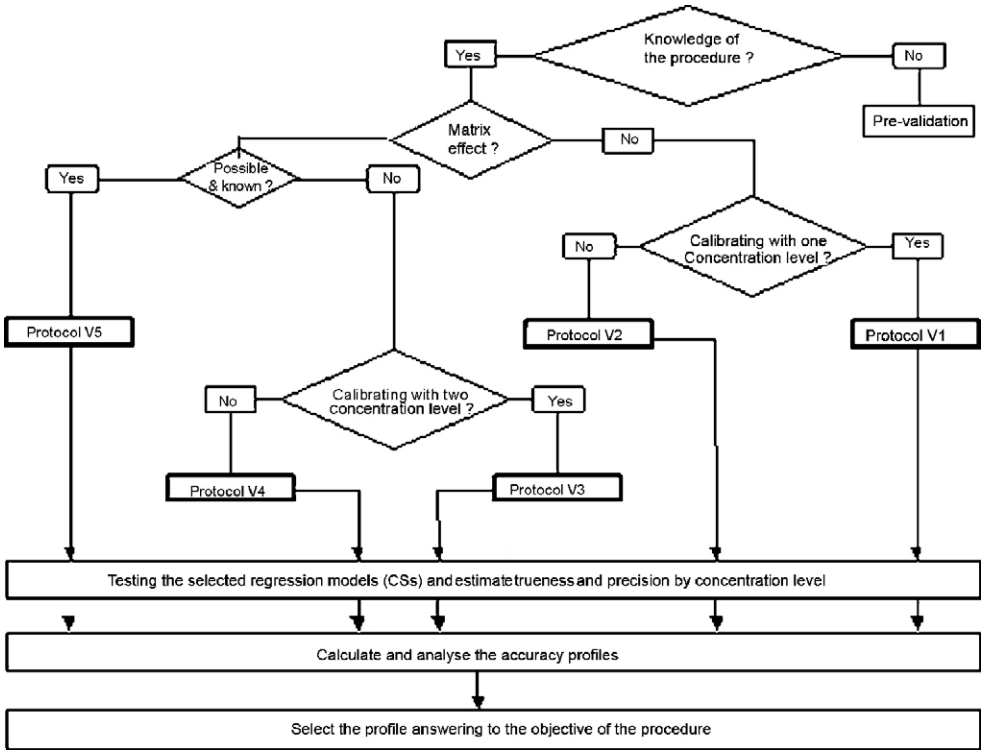

Fig. 2. Algorithm to select a validation protocol; CSs: calibration standards; VSs: validation standards.

Table 2
Choice of number of calibration standards and validation standards depending on the selected protocol

Standards	Concentration levels	PROTOCOL				
		V1	V2	V3	V4	V5
CSs without matrix	Low		2		2	
	Mid	2	(2)[a]	2	(2)[a]	
	High	(2)[b]	2	(2)[b]	2	
CSs within matrix	Low				2	2
	Mid			2	(2)[a]	(2)[a]
	High			(2)[b]	2	2
	Additional					(2)[c]
VSs within matrix	Low	3	3	3	3	3
	Mid	3	3	3	3	3
	High	3	3	3	3	3
Minimum number of series		3	3	3	3	3
Total number of experiments (minimum)		33	45	39	63	45

[a] Considering the regression model selected (ex.: simple regression line), the possible suppression of the mid range concentration level depending on the regression model considered to express the response function (for example: model as the simple regression line). In this case, there are 39 experiments for the protocols V2 (without matrix) and V5 (within matrix). There are 51 experiments for the protocol V4.
[b] Selection of a concentration level higher than the target concentration in order to calibrate (for example: 120% of target concentration).
[c] Addition of a concentration level for a more complex response function (for example: 4-parameter logistic regression).

tocol of validation according to the constraints or specificities linked to the dosage procedure of interest.

As can be shown from Fig. 2, without relevant information obtained during the development or special knowledge of the analyst on the performances of the procedure, a pre-validation phase is recommended. However, if these information are available, it is first of all recommended questioning on the presence or the absence of a matrix effect. In case of absence, the following question is about the concentration levels that will be used in routine for calibration. Depending on the answers, the protocols V1 (only one concentration level) or V2 are recommended. In case of evidence of matrix effect, the protocol V5 has to be considered. Finally, in case of doubt, the protocols V3 and V4 are proposed according to the wanted calibration levels.

These different protocols are summarized in the Table 2, also presenting the types of standards (CSs and VSs) as well as the concentration levels to use accordingly to the selected validation protocol. For example, the protocol V1 recommends a calibration out of matrix using two CSs at the same concentration level (at the target concentration or at a slightly higher concentration) and a minimum of nine independent VSs at three different concentration levels. The VSs have, as much as possible, to be prepared in the matrix and be independent. They must simulate the best the future samples that the analytical procedure will have to quantify.

According to the selected protocol, Table 2 shows the total number of experiments to be performed. It has to be noticed that they are the lightest protocols built according to regulatory constraints. Consequently, the total number of experiments can sometimes be reduced but also be developed depending on the objective of the analytical procedure. So the minimal number required of VSs can be reduced to a minimum of two or the

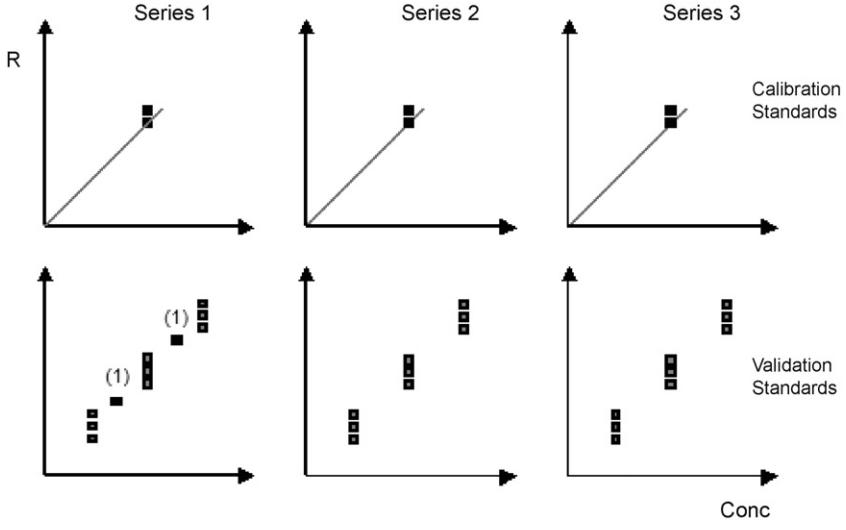

Fig. 3. Description of protocol V1. (1) Additional validation standards (linearity ICH).

Table 3
Examples of possible concentration levels by type of procedure

	Procedure					
	1	2	3	4	5	6
Calibration standards						
Low		LQ	(80%)[a] LA	LQ/LA[a]	C_{min}%	LQ
Mid	100%[b]	(1/2C_{max})[a]	100% LA	(50%)[a]	(50%)[a]	1/2C_{max}
High	(120%)[c]	(C_{max})[a]	(120%)[a] LA	120%	120%	C_{max}
Additionnal(s)						(x)[d]
Validation standards						
Low	80%	LQ	80% LA	LQ/LA	C_{min}%	LQ
Mid	100%	1/2C_{max}	100% LA	50%	50%	1/2C_{max}
High	120%	C_{max}	120% LA	120%	120%	C_{max}

LOQ: limit of quantification; LA: admitted limit; C_{max}: maximum concentration.
[a] Possible suppression of a concentration level for calibrating (for example: model as the simple regression line).
[b] Using only one concentration level implies that the zero would be included in the calibration system (cf. Fig. 3).
[c] Selection of a concentration level higher than the target concentration in order to calibrate (for example: 120% of target concentration).
[d] Addition of a concentration level for a more complex response function (for example: 4-parameter logistic regression).

number of concentration levels for these VSs can be increased from 3 to 5 in order to be compliant to the ICH requirements (Fig. 3).

It should be noticed that in the case of so-called "absolute" procedures, such as a potentiometric titration, the validation protocol is only limited to the use of standards of validation.

As an example, Table 3 illustrates some concentration levels that can be used for the following comparative procedures (by ex: HPLC):

1. determination of a single chemical substance for which the reference is available or determination of a chemical substance (active ingredient, preservative) in a pharmaceutical specialty (matrix);
2. determination of an available synthesis impurity of a chemical substance or of an available degradation products in this same substance or a pharmaceutical specialty (matrix) at concentration levels higher than the limit of quantification;
3. determination of an available synthesis impurity of a chemical substance or of one of its available degradation products in this same substance or a pharmaceutical specialty (matrix), around the impurity limit (impurity limit > limit of quantification);
4. simultaneous determination of a chemical substance and one of its non-available or not identified impurities or degradation products in this same substance or a pharmaceutical specialty (case of the use of the chemical substance concerned to the allowed maximum concentration as tracer of the impurity or the degradation product);
5. determination of a chemical substance (active ingredient) within the framework of dissolution kinetics for a dry dosage form (matrix);
6. determination of a chemical substance in a complex matrix (ex: active ingredient and its metabolites in plasma (drugs), drug residues or other contaminants in food . . .).

Fig. 3 illustrates protocol V1 in which using only one concentration level implies that the zero is included in the calibration system. This protocol can be applied to the procedures 1, 2 and 3 previously described as well as, in some cases, to the procedures 4 and 5. It has to be noticed that two validation standards have been added to one of the series in comparison with the protocol V1 presented in Table 2. Even if the reason of their presence is questionable, they are proposed in order to answer to the methodological specifications about linearity recommended by ICH (five concentration levels) but they are not maintained in the following series because of the weakness of information on the capability of the method.

Different versions of protocol V1 are presented in Fig. 4: protocols V2 and V3, respectively. In protocol V2 (Fig. 4A), calibration is always realized without matrix but contains two or three concentration levels (for a simple regression model) instead of one. The protocol V3 (Fig. 4B) is characterized by a single concentration level realized alternately within and without matrix. The objective of this double calibration system is to assess the effect of the matrix and especially to evaluate its impact on the accuracy profile.

In order to obtain a good estimate of the intermediate precision, it is important to perform the p series of experiments in conditions as representative as possible of the routine practice (day, operator, equipment, . . .). For example, if two operators will be involved with the method in routine use, a simple experimental design, such as illustrated in Table 4 can be applied.

At the end of the validation experiments performed following one of the mentioned protocols (Fig. 2 and Table 2), it is recommended to identify first, using the calibration standards, the relationship between the response Y and the concentration X

Table 4
Example of experimental conditions for estimating the intermediate precision

	Series					
	1	2	3	4	5	6
Day	1	1	2	2	3	3
Operator	A	B	A	B	A	B

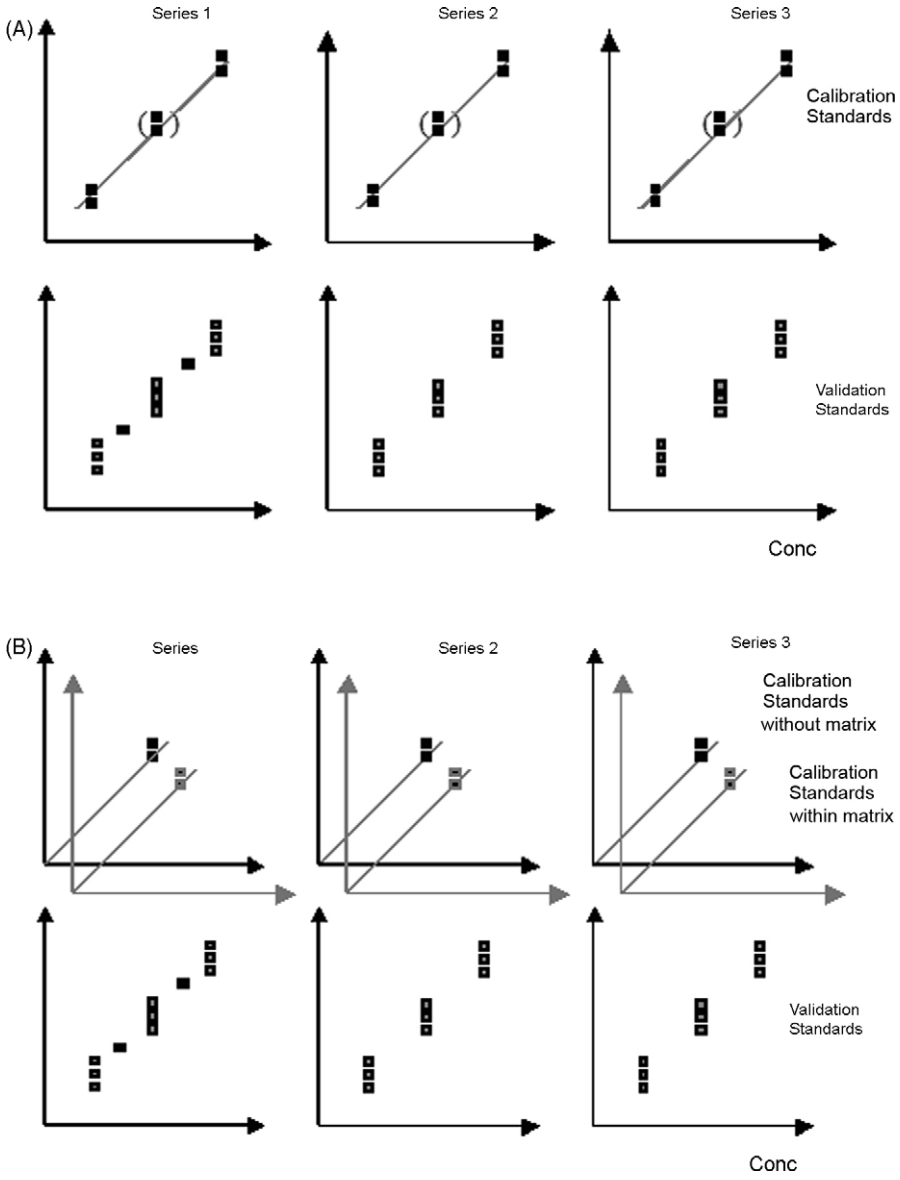

Fig. 4. Description of protocols V2 (A) and V3 (B) with within-matrix validation standards.

(response function). So, several mathematical regression models have to be fitted, their accuracy profile calculated and one of them selected in order to take a decision about the validity of the procedure of interest. The choice depends on one hand on procedure type (pharmaceutical method, bio-analytical method, immuno-assay, ...) and on the other hand on objectives fixed by the analyst. A linear regression (through 0 or not) will be envisaged for most of the pharmaceutical and bio-analytical methods. Mathematical transformations applied to the concentrations X and to the responses Y can be also used: the neperian logarithm, the square root, ... (Fig. 5). The quadratic regression can be useful in some cases while for the immuno-assays, the preference will be a 4 parameter logistic regression (4PL).

Then the concentrations of the VSs will be back-calculated by series using the different equations of calibration curves (functions of selected responses). However, before back-calculating the concentrations, it is essential, if it is not the case, to align all the introduced concentrations by concentration level.

Finally, for each concentration level, the trueness and the precision of the procedure will be estimated and then limits representing the accuracy of the results will be calculated. These limits should include a large (depending on the objective) proportion of results. For each fitted model, these limits are used to build the accuracy profile (Fig. 5). These profiles will constitute for the analyst a visual decision tool allowing him to evaluate the capability of the procedure. If none of the accuracy profiles is within pre-fixed acceptance limits, the analyst can, either restrict the dosing range by determining new limits of quantification or extend acceptance limits (which is not always possible). In this case, diagnosis tools like residual plots, lack-of-fit testing, ... will be available to identify problem(s).

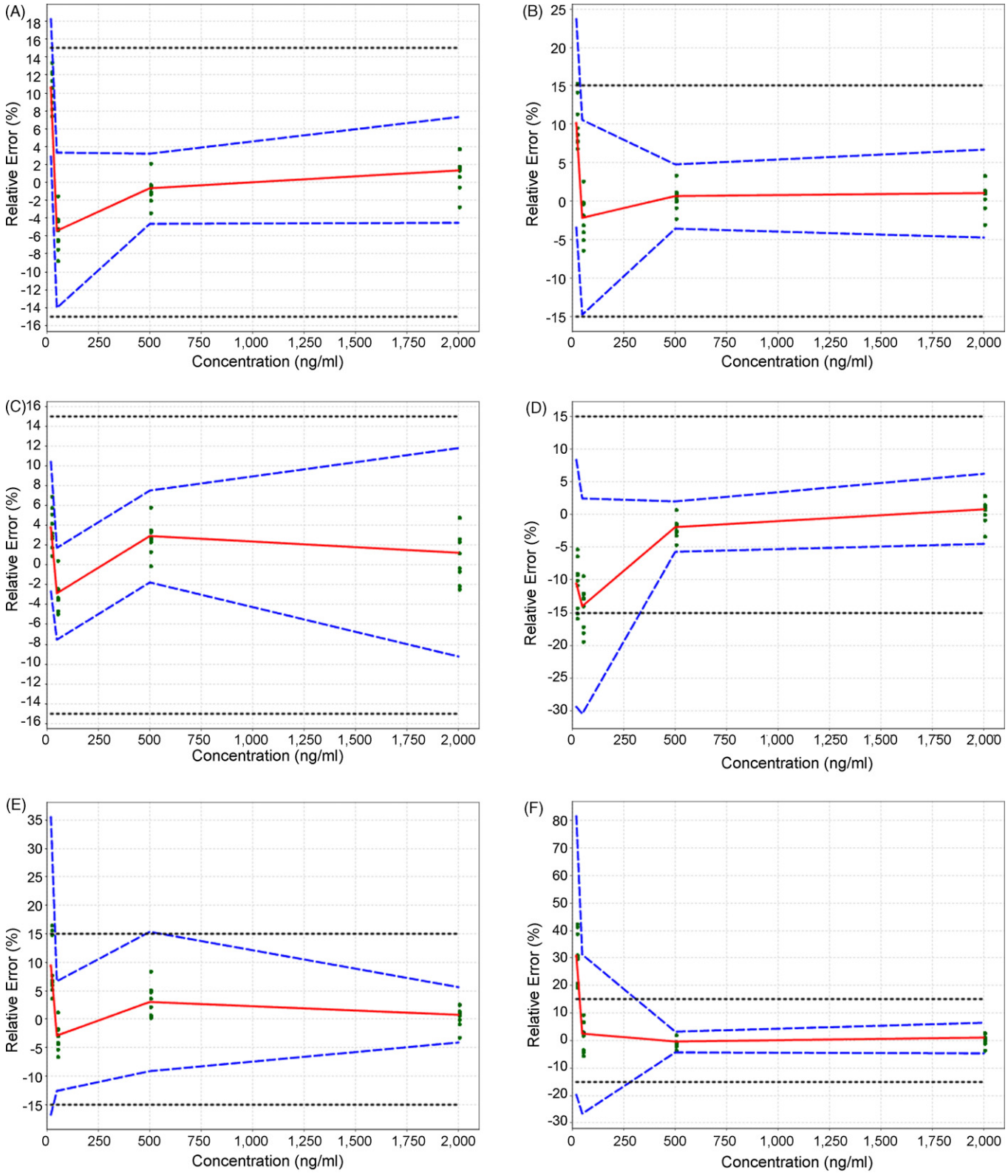

Fig. 5. Examples of accuracy profiles obtained with the same validation protocol (relative error vs. concentration). Dosing range: 20–2000 ng/ml. (A) Weighted linear regression (weighted factor: 1/X); (B) linear regression after square-root transformed data; (C) linear regression after log transformed data; (D) linear regression through 0; (E) quadratic regression; (F) linear regression. The continuous lines are the relative bias, the dotted lines are the ±15% acceptance limits and the dashed lines are the upper and lower relative 95%-expectation tolerance limits. The dots represent the relative back-calculated concentrations of the validation standards.

The accuracy profiles obtained for the validation of a dosing procedure of a chemical substance in a biological matrix are illustrated in Fig. 5. In this case, the protocol V5 was applied and some concentration levels were added. These levels were essentially low in order to have a good estimate of the lower limit of quantification. This choice was based on one hand on the expertise of the analyst and on the other hand, on the previous experiments performed during the optimisation of the analytical procedure. As described in Fig. 5, only one over six response functions (linear regression after log transformed data) answered the objective, i.e. being within the acceptance limits (±15%). Therefore, the accuracy profile allows us to decide about the capability of the procedure. In this example, the dosing range in which the procedure is able to quantify with a known accuracy (trueness + precision) was estimated to be between 20 and 2000 ng/ml.

Using such a practical decision tool, the analyst can choose the response function the most appropriate or the simplest that answers the objectives of the analytical procedure. In a way, this confirms the fitness for purpose of the response function. In the meantime, he has validated the dosing range. It is also possible to obtain an estimation of the overall accuracy of the results produced with the procedure by checking the linearity of the relationship between the estimated concentration and the introduced concentration.

In order to demonstrate the specificity of the method, experiments have to be added to the experimental design. These are generally performed at the beginning of the validation phase.

3.3. Protocols in prevalidation phase

The aim of the prevalidation phase is to prepare all the elements needed for the formal validation of the procedure, such as the precise configuration of the equipment to be used, the preparation of the stock and diluted solutions for the calibration standards and the preparation of the validation standards [41]. All these information should be included ideally in a protocol before starting the validation phase.

Moreover, the main objectives of the prevalidation phase are to:

- identify the response function (linear, non-linear, mathematical transformation, weighting) that will be used for calibration during validation;
- define the limit of detection (if it is necessary);
- estimate the limit(s) of quantitation (according to the procedures);
- evaluate the range and the number of calibration points;
- determine the extraction efficiency (if an extraction step is involved in the procedure);
- check the specificity before starting the validation phase.

All these results must be included in a documented report and their analysis should guide ideally to a detailed validation protocol describing the experimental procedure, the compounds to be assayed, the validation criteria and their acceptance limits.

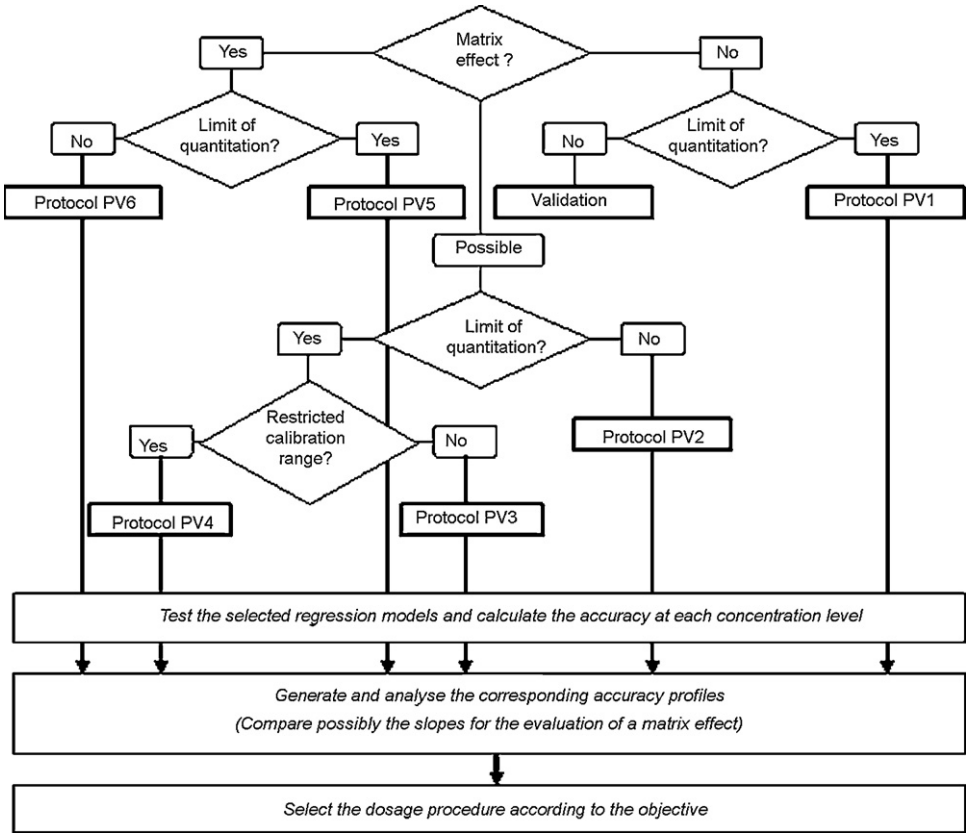

Fig. 6. Algorithm for the selection of the prevalidation protocol.

The algorithm presented in Fig. 6 illustrates the approach to select the experimental protocol in prevalidation according to the constraints or the features related to the dosage procedure of interest. Nevertheless, it is important to underline that this prevalidation step is not essential and is related especially to more complex procedures including more particularly the selection of a response function, the estimation of the limit(s) of quantitation prior to the validation phase. The prevalidation step can be replaced by an optimisation phase. It is also needed to remind that the experiments are performed only from the calibration standards in prevalidation. Therefore, for example the response function will have to be confirmed during the formal validation phase [42,43].

As shown in Fig. 6, it is first recommended to check whether there is or not a matrix effect. Then, irrespective of the answer, the limit(s) of quantitation will be or not estimated keeping in mind that one of the first aims in prevalidation is to define the most appropriate response function, i.e. the best relationship between response and concentration. The different protocols are summarised in Table 5 for which different concentration levels are proposed as examples. Each sample must be replicated at least two times and a minimum of two series must be performed. It is left to the analyst to form an opinion on the feature of independence for the replicates and especially for the series on which the power of the precision estimate will depend.

In addition to the establishment of appropriate response functions, the protocols proposed in Table 5 can be applied more particularly to:

- PV1: the determination of the limit of quantitation for an available synthesis impurity of a chemical substance or for one of its available degradation products;
- PV2: the evaluation of a matrix effect for the assay of a chemical substance (active ingredient, preservative) in a pharmaceutical form;
- PV3: the determination of the limit of quantitation and the evaluation of a matrix effect for the simultaneous assay of a chemical substance and one of its impurities or degradation products not available or not identified in a pharmaceutical form (case of the use of the chemical substance concerned at the allowed maximum concentration like a tracer of the impurity or the degradation product);
- PV4: the determination of the limit of quantitation and the evaluation of a matrix effect for the assay of an available synthesis impurity or for one of its available degradation products in a pharmaceutical form;
- PV5: the determination of the limit(s) of quantitation (lower and upper) for the assay of a chemical substance in a complex matrix (ex.: drug residues or other food contaminants, ...). It has to be noticed that if only the LLOQ is determined, the point corresponding to 0.85 C_{max} can be removed. In this case, the total number of experiments is equal to 24;
- PV 6: the definition of the range for the determination of a chemical substance in a complex matrix (ex.: active ingredient with a known matrix effect, determination of an endogenous component or a physiological parameter, ...).

If some intermediate (or additional) concentrations are needed, they can be determined as follows:

$$X_{i(j-1)k} = \frac{X_{i2k} + X_{ijk}}{2}$$

i.e. by selecting halfway concentrations between the nearest concentration level previously estimated and x_{i2k} (see Fig. 7).

Table 5
Non-exhaustive examples for the choice of the concentrations levels of calibration standards according to the kind of prevalidation protocol selected

Calibration standards	PROTOCOL					
	PV1	PV2	PV3	PV4	PV5	PV6
Calibration without matrix						
	LOD LOQ 3 LOQ x LOQ C_{max}	Low (80%) Medium (100%) High (120%)	LOD LOQ 3 LOQ Medium High (C_{max})	LOD LOQ 3 LOQ x LOQ		
Calibration within matrix						
		Low (80%) Medium (100%) High (120%)	LOD LOQ 3 LOQ Medium High (C_{max})	LOD LOQ 3 LOQ x LOQ	LOD LOQ 3 LOQ x LOQ Medium 0.85 C_{max} C_{max}	Low Medium High (*)
Minimum number of series						
	2	2	2	2	2	2
Total number of experiments (minimum)						
	20	24	40	32	28	24

LOD: limit of detection; LOQ: foreseen limit of quantitation, since it is not validated yet; C_{max}: the maximum concentration of the range defined a priori.
* Some additional calibration points may be required in the case of a wide range tested or according to the foreseen regression model.

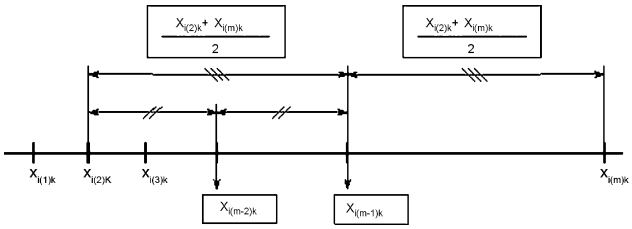

Fig. 7. Selection of the intermediate concentrations.

3.4. Optimal number of experiments to perform in validation

It is possible to determine the optimal number (replicates and series) of the validation standards for the validation phase. This optimal number is based on the precision results obtained in prevalidation or during optimisation. Indeed, performing too few experiments could lead to the rejection of the analytical procedure even though it is actually valid. On the other hand, too many experiments will make the validation phase longer than necessary. Between these two extremes, an optimal number of experiments exists as shown in Table 6. This table has been obtained by simulation methods at the 5% level of significance, assuming that the expected bias at the concentration level considered is not greater than 2% of the theoretical concentration.

For coefficient of variation values smaller than those presented in Table 6, a minimum of three series and four replicates per series must be envisaged. For the combination of values where no number of experiments is proposed in the table, it is recommended to continue the development of the analytical procedure, at the risk of never being able to validate it. If the expected bias at the concentration level j is close to 0%, one replicate per series less than proposed in Table 6 can be envisaged, which corresponds to the validation protocol with three series and three replicates (see Table 2).

Table 6
Recommended number of series and replicates per series for the validation standards as a function of the coefficients of variation for repeatability and between-series obtained from the calibration standards in prevalidation

CVj(g)	Nb. series	CV(r)				
		4%	5%	6%	7%	8%
		Nb replicates				
4%	3	4	4	5	6	
	4	4	4	4	5	9
	5	4	4	4	5	5
5%	3	4	4	4	5	
	4	4	4	4	6	
	5	4	4	4	5	8
	6	4	4	4	4	5
6%	3	4	4	6	10	
	4	4	4	6	7	
	5	4	4	5	7	
	6	4	4	5	5	6
7%	3	6	8			
	4	4	4	6		
	5	4	4	5	7	
	6	4	4	5	7	9
8%	4	9				
	5	6	8			
	6	4	5	8		

$CV_j(r)$: repeatability coefficient of variation. $CV_j(g)$: between-series coefficient of variation.

4. Conclusion

The objective of the SFSTP commission on analytical method validation was to provide analysts with a harmonized and practical guide to perform such validations since the regulatory documents of the domain do not achieve this.

The present paper is the second part of the summary report of a new SFSTP commission on the validation of quantitative analytical procedures which reviews some validation criteria and proposes harmonized protocols by in particular distinguishing the diagnosis rules and the decision rules. These latter are based on the use of the accuracy profile using the concept of total error (bias + standard deviation). At the same time, this approach permits to simplify the validation approach of an analytical procedure and to control the risk associated with its use.

The document is a compromise and did appear to be reasonable and acceptable to all members of the group with respect to the validation of an analytical procedure. Throughout commission reflections, the common concern was to rationalize the decision-making to go in the direction of an improvement of the coherence and documentation of the choices carried out and thus, in the long term, of quality.

So that this step is applicable to the laboratory level, the commission tried to take into account the practical constraint of the experimental approach suggested. The protocols propose a sufficient but realistic number of experiments. The gain in quality is not obtained by increasing the total cost of the validation process.

The statistical aspects as well as the examples of applications will be the subject of a later publication.

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