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Cosmetics — Analytical methods — Validation criteria for analytical results using chromatographic techniques

Cosmétiques — Méthodes analytiques — Critères de validation pour les résultats analytiques utilisant des techniques chromatographiques

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Foreword

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Cosmetics — Analytical methods — Validation criteria for analytical results using chromatographic techniques

1 Scope

This International Standard defines validation criteria with which analytical results obtained from the analysis of cosmetic products should comply in order to give confidence in performance, reliability and quality of the final result. It sets out an analytical approach that can be used by a single laboratory to carry out chromatographic analyses on a given sample, or samples.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1 General

2.1.1
analyte
substance being subjected to analysis

2.1.2
bias
difference between the expectation of the test results and an accepted reference value

2.1.3
recovery
ratio between the quantity of analyte found by a particular analytical method compared to the quantity of analyte expected

2.1.4
post-extraction spiked matrix standards
PoEMS
samples taken through the entire extraction procedure and spiked with the analyte of interest at the end of the extraction immediately before, or very close to, detection

NOTE PoEMS are also called “Matrix-Matched Standards” or “Fortified Analytical Solutions (FAS)” and are used for determination of the bias.

2.1.5
pre-extraction spiked matrix standards
PrEMS
samples spiked with the analyte of interest at the beginning of the analytical procedure

NOTE PrEMS are also called “Spikes” or “Fortified Analytical Portions (FAP)” and are used for calibration and quantification of the target analytes in samples (extraction recovery).

2.1.6
matrix effect
combined effect of the presence of one or more components of a sample other than the analyte on the measured quantity of the analyte

NOTE The matrix effect could increase or decrease the chromatographic peak area for a same analyte concentration.

2.1.7
extraction yield
ratio between the quantity of analyte extracted during the extraction process from the sample matrix compared to the quantity of analyte present in the sample

2.1.8
solvent standard calibration curve
analyte calibration curve obtained from the analyses of at least five different standard calibration levels prepared in the solvent

2.1.9
control standard
independent standard solution used to verify the solvent standard calibration curve

2.2 Terms relating to validation criteria for analytical results

2.2.1
accuracy
closeness of agreement between a test result (the average value obtained from a large series of test results) and an accepted reference value

NOTE The accuracy is often expressed in terms of bias.

2.2.2
LoD
limit of detection
lowest amount of an analyte that can be reliably distinguished from zero with reasonable statistical certainty

2.2.3
LoQ
limit of quantification
lowest amount of an analyte that can be determined with an acceptable level of uncertainty under the stated conditions of test

2.2.4
linearity
ability of the method to obtain test results proportional to the concentration of the analyte

2.2.5
measurement uncertainty
MU
parameter, associated with the result of a measurement, that characterizes the dispersion of values that could be reasonably attributed to the measurand

2.2.6
precision
closeness of agreement between independent test results obtained under stipulated conditions

NOTE Precision depends only on the distribution of random errors and does not relate to the true value or the specified value.

2.2.7
working range
interval between the upper and lower concentration (amounts) of analyte in the sample for which it has been demonstrated that the analytical procedure has a suitable level of certainty

2.2.8
repeatability
precision under repeatability conditions where independent test results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time

2.2.9
intermediate precision
precision under conditions where independent test results are obtained with the same method on identical test items in the same laboratory by different operators using different equipment on different days

2.2.10
reproducibility
precision under reproducibility conditions, i.e. conditions where independent test results are obtained with the same method on identical test items from different laboratories at different times

2.2.11
selectivity
ability of a method to determine accurately and specifically the analyte of interest in the presence of other components in a sample matrix under the stated conditions of the test

2.2.12
sensitivity
change in the response of a measuring instrument divided by the corresponding change in the stimulus

2.2.13
specificity
ability of a method to measure only what is intended to be measured

2.2.14
target concentration
analyte concentration used as a reference for the determination of the analyte concentration in the sample

2.2.15
validation
confirmation of examination and provision of objective evidence that the particular requirements for a specified intended use are met

2.2.16
asymmetry
factor describing the shape of a chromatographic peak

NOTE The theory assumes a Gaussian shape and that peaks are symmetrical.

2.2.17
resolution
ability of a column to separate chromatographic peaks, usually expressed in terms of the separation of two peaks

3 Principle

The ingredients of cosmetic products are variable and complex, mainly due to the type of formulation. General analytical methods exist, or are to be developed, to assess the quality of cosmetics. These generalized methods, some of which might not be strictly certifiable, are intended to be widely usable, comprehensible and transferable.

The application of analytical methods to cosmetic products requires a specific validation approach in order to ensure the reliability of the results. For cosmetic products, the choice and use of a general method for analytical testing has to be supported by validation criteria specific to the sample matrix in order to ensure the reliability of the results. In this context, this International Standard aims to propose specific validation criteria to be evaluated for the use of a general method for testing cosmetic products. Validation criteria for analytical results to be evaluated include specificity, selectivity, recovery, confidence interval, limit of detection, limit of quantification, precision, accuracy and linearity.

Validation criteria shall be determined for each sample matrix. If a similar matrix is used, validation criteria need only be determined on the samples first analysed and extended to other samples in the same concentration range. Accordingly, this approach would not necessarily be applied in routine testing of cosmetic products if validation criteria were previously obtained. Careful consideration should be given to the sample matrix when determining if additional validation is required.

4 General information

4.1 Matrix effect

If the sample were submitted to an extraction process before injection (e.g. liquid-liquid extraction or solid-phase extraction), the recovery obtained on the PrEMS, using the solvent calibration curve, would include both the sample matrix effect and the extraction yield of the process.

From an analytical point of view, it would be interesting to distinguish the matrix effect from the extraction yield resulting from the sample preparation (extraction of the analyte from the cosmetic matrix). Use of a PoEMS would allow one to distinguish between the matrix effect and the extraction yield.

Figure 1 indicates the importance of preparing a PoEMS, in addition to a PrEMS and a standard calibration curve, in order to obtain different validation criteria on the analytical results, such as the extraction yield and/or the matrix effect.

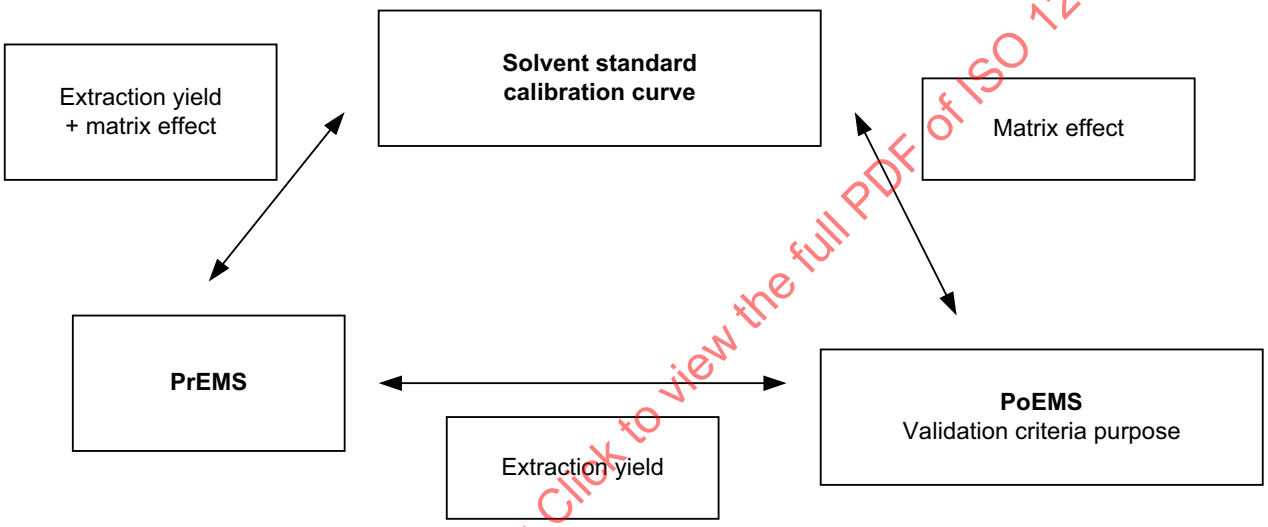

Figure 1 — Validation criteria for analytical results obtained using PrEMS, PoEMS and a solvent calibration curve

If an extraction process is performed, the matrix effect is given by the PoEMS recovery (using the solvent standard calibration curve). The difference between PoEMS and PrEMS recoveries gives the extraction yield of the sample process.

If no extraction process is performed, the extraction yield is equal to 100 %, and the matrix effect is given by the PrEMS (or PoEMS) recovery. If the recovery obtained on PrEMS, using the solvent standard calibration curve, is significantly different from the expected value, a matrix effect should be suspected. Under these circumstances, it is recommended that the method of standard addition be used.

PrEMS and PoEMS preparations should be carried out under the following conditions:

— use a solvent compatible with the sample preparation;

— use the minimum possible amount of solvent to introduce the analyte in the test solution;

— depending on the sample type, spiked samples (PrEMS) should be prepared by mixing the analyte solution with the sample, allowing dispersion into liquid samples and penetration/adsorption onto non-liquid or solid samples (this step should be adapted if the analyte is highly volatile);

— perform the PrEMS and the PoEMS at the estimated analyte concentration within the calibration range.

This analytical approach should only be used if the compound added to the cosmetic matrix behaves similarly to the compound present in the matrix. If not, certified or well-characterized standard samples could be proposed as an alternative. Careful consideration should be given to the use of spiked samples with solid cosmetic products.

4.2 Decision tree

The decision tree, represented in Figure 2, indicates the proposed approach and the different steps to be performed.

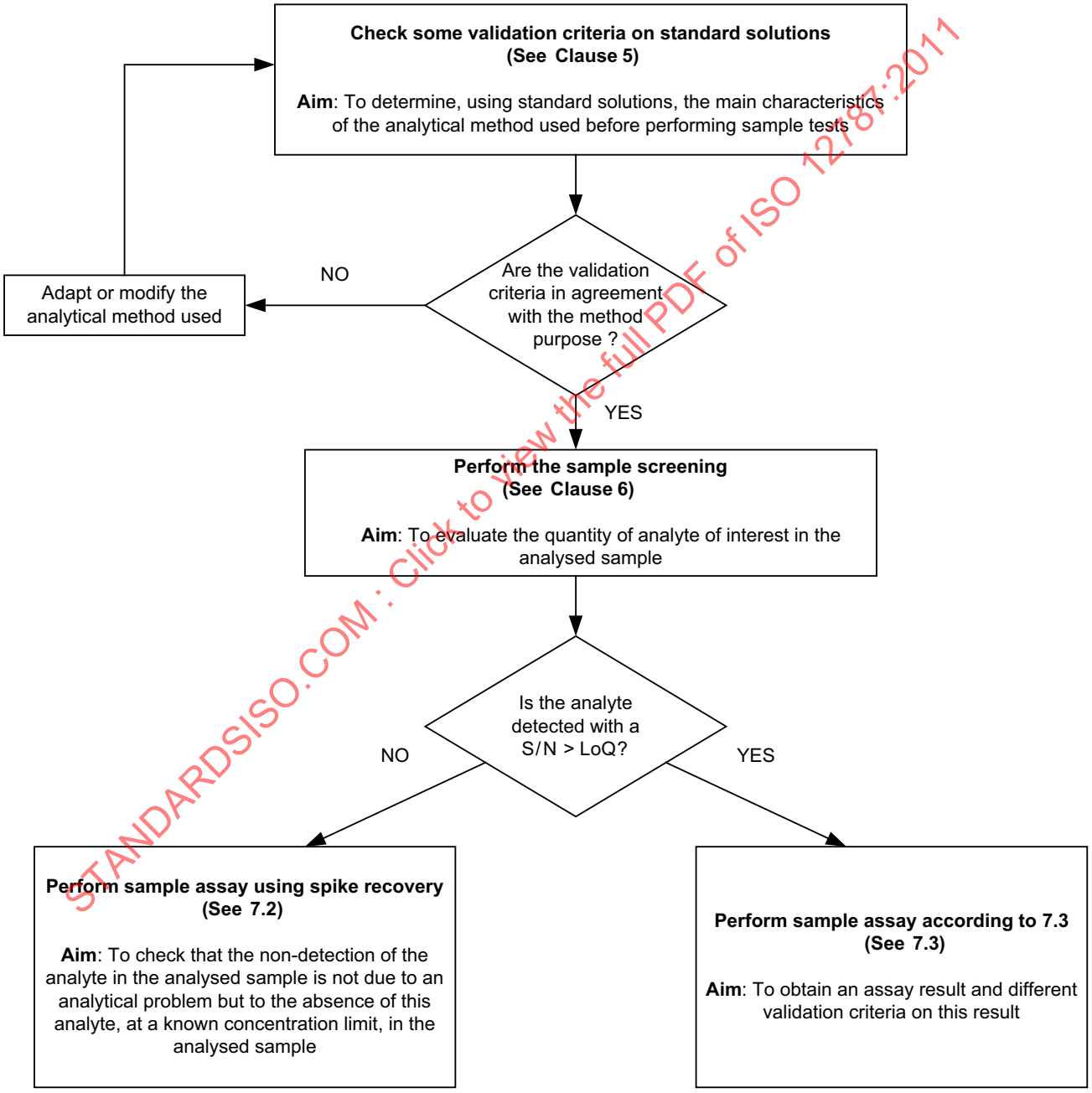

Figure 2 — Purpose of the approach and steps to be performed

5 First step — Minimum validation criteria on standard solutions

5.1 General

The aim of the first step is to determine, using standard solutions, the main characteristics of the analytical method before performing tests on samples.

Some general criteria should first be checked in order to determine assay conditions. For example, the apparatus conformity (injection repeatability, detector calibration, etc.) and the analyte stability in solution should be ascertained.

Validation criteria for analytical results to be considered are:

— analyte limit of quantification (LoQ) and limit of detection (LoD) using standard solutions;

— conformity of the chromatographic analysis, e.g. resolution factor, Rs, and asymmetry, As;

— linear range of the analyte signal;

— standard accuracy.

This first step is carried out once at the beginning of the analytical programme. This step should be performed again or adapted if any analytical parameter is changed (calibration solvent, injection volume, chromatographic column type, separation conditions, etc.) in order to check that the previous validation data still apply.

5.2 Estimation of detection and quantification limit in solvent (optional)

5.2.1 Assays

Inject in duplicate the dilution solvent to monitor any potential interference on the analyte and to estimate the LoD in solvent.

Inject low concentration standards to evaluate the analyte LoD and LoQ in standard solutions.

5.2.2 Results analysis

Using the dilution solvent, determine the LoD by measuring the noise level (standard deviation of the signal intensity) at the expected retention time of the analyte, in duplicate. The LoD is defined as three times the standard deviation (S/N ratio = 3).

Using a low concentration standard solution, calculate the standard deviation obtained for each injection. The LoQ is defined here as the concentration of analyte producing a signal ten times the standard deviation (S/N ratio = 10)[15][16][17].

NOTE 1 An estimate of LoD or LoQ could be obtained using the standard deviations of sample containing a small amount of analyte (typically a minimum of six replicates is required).

NOTE 2 For the LoD, an estimate could be obtained using the origin of the calibration curve[7].

NOTE 3 An estimate of both values (LoQ or LoD) could also be obtained using an analytical software calculation.

5.3 Analytical conformity

5.3.1 Assays

Prepare and inject a standard solution at a concentration level from the high end of the calibration curve expected. If an internal standard is used, add it to the standard preparation.

Inject the dilution solvent used.

5.3.2 Results analysis

Check the necessary conformity parameters as follows.

— Resolution factor (compulsory if more than one chromatographic peak is detected): the chromatographic separation between two peaks can be considered satisfactory if Rs is $> 1{,}5$.

— Asymmetry of the analyte peak: the asymmetry of the chromatographic peak can be considered satisfactory if $0{,}8 < As < 1{,}5$.

— Specificity of detection, if necessary.

Ensure the absence of interference peaks from the solvent at the retention times for the analyte and for the internal standard (if used).

5.4 Calibration: precision, linearity and accuracy

5.4.1 General

This subclause describes the recommended approach to estimating precision, linearity and accuracy.

5.4.2 Assays

Prepare three independent solvent calibration curves (containing a minimum of five concentration levels) by diluting three different standard stock solutions, then injecting them. The different calibration levels should be uniformly distributed along the calibration range and the same levels should be used for the three calibration curves.

NOTE For the determination of analytes in low concentration, the first calibration level should correspond to the quantification limit in solvent (two or three times the LoD in standard solutions). The upper end is usually signified by a change in instrument response.

5.4.3 Results analysis

The results analysis is performed as follows.

a) Determine the precision of the calibration curve using statistical analysis, e.g. as for variance homogeneity.

NOTE Assays performed on the same day by the same analyst indicate repeatability of the analytical method used on standard solutions. Assays carried out on different days and/or by different analysts indicate an estimation of intermediate precision.

b) Evaluate the linearity of the calibration curves using, for example, an analytical validation software package or by checking different regression factors on a plot of the data:

— determine the coefficient of determination, R^2 (a value of 0,990 or higher is recommended);

— determine the relative concentration deviation (bias) of each calibration level by examining the residuals in the linear regression analysis;

— determine the slope and the Y-intercept for the line produced from the linear regression analysis;

— determine the relative standard deviation (RSD) for the Y-intercept, which can be used to determine whether the Y-intercept is significantly different from zero.

NOTE If the regression model obtained is not linear even using a weighting factor, it is possible either to define a narrower concentration range or to choose a non-linear regression model. See Annex A for an example on how to select an appropriate regression model using a weighting factor.

The method accuracy on standard solution may be estimated at each calibration level, analysing in triplicate the bias obtained (three values for each level).

6 Second step — Sample screening

6.1 General

The aim of the second step is to evaluate the quantity of analyte in the sample.

6.2 Sample screening

6.2.1 Assays

Prepare and inject a calibration curve in the linearity concentration range determined in the first step.

Prepare and inject a control standard.

Prepare and inject the sample(s) with and without the internal standard (if used).

6.2.2 Results analysis

After checking the coefficient of determination, R^2, and accepting the result obtained for the control standard, check the chromatogram for any interference on the analytes, including the internal standard, if necessary. Evaluate the analyte amount in the sample using the standard calibration curve.

This result will present one of the following two cases:

— the sample contains no analyte, or contains the analyte at quantities less than the LoQ in the matrix ($S/N < 10$)(see 7.2);

— the sample contains the analyte at quantities higher than the LoQ in the matrix ($S/N > 10$)(see 7.3).

7 Third step — Assays

7.1 General

Validation criteria are determined for each sample matrix submitted for analysis. Validation data need only be determined for the first samples analysed and applied to all samples of a similar matrix. This approach should only be used for analyte concentration in the same range.

Once those validation criteria for analytical results are determined, other sample assay tests could be carried out using an external calibration curve for quantification, either after correcting the final results with the validation criteria obtained on the first samples analysed, or by expressing the result taking into account the uncertainty of the measurement.

7.2 Analytes not detected or detected at concentrations less than the LoQ

7.2.1 General

The aim of these assays is to ensure that the measured signal is not influenced by an interference compound or an analytical problem, e.g. a bad extraction yield.

The LoQ in the matrix can be evaluated as the spiked concentration that gives an S/N ratio in the sample that is equal to 10.

NOTE The LoQ in the matrix can also be estimated by checking the recovery obtained on spiked samples, after correction with the initial analyte concentration, using a solvent calibration curve. This estimation could be prevented by a possible matrix effect (suppression or enhancement of the quantifying signal due to the sample matrix).

7.2.2 Assays with spike recovery

7.2.2.1 Assays

Prepare and inject an unspiked sample.

Prepare and inject different spiked (PrEMS) samples, e.g. at 1 LoQ, 5 LoQ and 10 LoQ (the value of the LoQ was determined using standard solutions, as described in 5.2).

7.2.2.2 Results analysis

Using spiked and unspiked samples, check the specificity of the analyte detection in the sample matrix. The specificity criteria shall be checked before quantification in order to assess the identification of the analyte and the peak purity. Specificity can be verified using any relevant process and/or referential (see Reference [15]). If a doubt remains, the assay could be performed using another method or detection instrument. Evaluate the LoQ of the analyte in the matrix, checking the S/N ratio for each spiked and unspiked sample.

The final analyte estimation in the sample is given as follows:

Analyte concentration value $<$ LoQ matrix

NOTE If assays are performed using a target concentration value, the previous approach can be simplified as described in Annex B.

7.3 Analytes detected at a concentration greater than the LoQ

7.3.1 General

The aim of these trials is to determine the analyte concentration in the sample as well as several validation parameters, e.g. the matrix effect, the extraction yield, the accuracy, and the confidence interval. These are determined by performing statistical analyses on six preparations of the sample: three unspiked preparations, two PrEMS and one PoEMS.

Recoveries obtained from PoEMS and/or PrEMS lead to the determination of different validation criteria on the analytical results:

— the PoEMS recovery relative to calibration standards shows whether or not there is a matrix effect;

— the difference between the PoEMS and the PrEMS recoveries, relative to calibration standards, gives the extraction yield of the analytical process;

— the recovery obtained for the PrEMS relative to the PoEMS gives the accuracy of the analytical result;

— the RSD and confidence interval can be obtained by a statistical analysis of the replicates.

7.3.2 Assays

Make a standard calibration curve in solvent, covering all the estimated sample concentration values and their doubles in value (in order to correctly quantify the PoEMS or PrEMS). This calibration range shall be in the linear calibration range determined in 5.4.

Prepare and inject a control standard.

Prepare and inject one, two or three unspiked samples for the determination of the analyte amount. If an RSD or a confidence interval is to be determined on the final result, at least three unspiked samples should be assayed.

Prepare and inject a PoEMS by spiking the final sample extract, after all sample processing, at the estimated analyte concentration. The estimated analyte concentration was determined during the sample screening in Clause 6 (this step is optional if no extraction process is used).

NOTE 1 If possible, use one of the previous sample preparations (unspiked) to prepare this PoEMS.

Prepare and inject one or two spiked solutions (PrEMS) at the estimated analyte value. If an RSD or a confidence interval is to be determined on the final result, at least two spiked preparations should be assayed.

Prepare and inject a reagent blank preparation to ensure the specificity of the assay.

NOTE 2 If no extraction process is used (simple dilution), PrEMS preparations are similar to the matrix-matched standard (i.e. PoEMS).

NOTE 3 If spiked solutions are diluted to fall within the calibration range, corresponding unspiked preparations should also be diluted in the same way in order to preserve, if present, the same matrix effect.

7.3.3 Results analysis

7.3.3.1 General

Check the coefficient of determination, R^2, for the calibration curve and check the bias of the control standard relative to the calibration standard.

7.3.3.2 Matrix specificity

Using the PoEMS (i.e. PrEMS if no extraction process is used) and the corresponding unspiked sample, check all the specificity parameters:

— resolution factor (if necessary);

— asymmetry factor;

— analyte selectivity (detection).

NOTE Using another method or a detection instrument can help to confirm the selectivity of the assay.

7.3.3.3 Evaluation of the matrix effect

A matrix effect should be suspected if the recovery of the spiked amount from the PoEMS (or PrEMS if no extraction is used) relative to the calibration standards differs from the accuracy (bias) obtained for the corresponding calibration standard (determined during the first step in 5.4).

7.3.3.4 Estimation of the extraction yield

The extraction yield can be estimated, after correction for the initial analyte concentration, by the recovery of the spiked amount from spiked samples (PrEMS) relative to the PoEMS, or the solvent standard calibration curve if no matrix effect was observed.

NOTE This is not necessary if no extraction process is used.

7.3.3.5 Accuracy

Accuracy can be assessed by the recovery of the spiked amount (or the average recovery, if different spiked samples were analysed) from the PrEMS relative to calibration standards.

When a matrix effect has been observed, the accuracy can be assessed by comparing the recovery from the PrEMS relative to the PoEMS.

7.3.3.6 Analyte concentration

The final analyte concentration in the sample is determined in one of the following ways.

— If there is no matrix effect, the analyte concentration is determined relative to calibration standards as the average of unspiked sample values plus the percentage RSD, if the assay is carried out in triplicate.

NOTE 1 This result could be corrected for the extraction yield, if necessary.

— In the presence of a matrix effect, the analyte concentration is determined using the method of standard addition[42]. The single-point standard addition approach[15][43][44] might also be considered. If at least three unspiked samples and two spiked samples are analysed, the final result could be given using the mean square linear regression with a confidence interval.

NOTE 2 The method of standard addition can also be used in the absence of any matrix effect.

7.3.3.7 Confidence interval (optional)

The confidence interval can be determined using the method of standard addition. The confidence interval can be estimated using the “Fieller theorem” approach[45].

8 Summary

The following table shows the number of assays to be performed at each validation step.

Table 1 — Number of assays to be performed at each validation step

	Validation criteria using standard solution	Sample screening	Assays		
	1st step	2nd step	3rd step		
			Analyte not detected, or S/N < LoQ		Analyte detected, S/N > LoQ
			Assays with spike recovery	Assays using a target value	
Solvent standard calibration levels	5 (LoQ) + 15 (5×3) linearity	5			5
Unspiked samples		1	1	1	1 (3)[a]
Spiked samples (before treatment) i.e. PrEMS			3	1	1 (2)[a]
Spiked sample (after treatment) i.e. PoEMS					1
Check standard		1			1

[a] If an RSD or a confidence interval is to be determined on the final assay result.

Annex A
(informative)

Example of selection of a weighting factor

A.1 Mean concentrations corrected with different weighting factors at each calibration level

Table A.1 — Mean concentrations

Real calibration level concentration mg/kg	Measured concentration No weighting mg/kg	Measured concentration Weighting: $1/x$ mg/kg	Measured concentration Weighting: $1/y$ mg/kg	Measured concentration Weighting: $1/x^2$ mg/kg	Measured concentration Weighting: $1/y^2$ mg/kg
10,268 1	8,628	9,750	9,744	10,139	10,154
20,536 2	19,591	20,688	20,684	20,951	20,976
51,340 5	50,715	51,740	51,743	51,646	51,698
102,681	103,418	104,323	104,336	103,624	103,722
205,362	209,921	210,58	210,616	208,662	208,853
513,405	512,643	512,609	512,705	507,216	507,674
1 026,81	1 024,701	1 023,493	1 023,693	1 012,226	1 013,134
2 053,62	2 054,406	2 050,838	2 051,246	2 027,758	2 029,572

A.2 Relative errors corrected with different weighting factors at each calibration level

Table A.2 — Relative errors

Real calibration level concentration mg/kg	Relative absolute error No weighting %	Relative absolute error Weighting: $1/x$ %	Relative absolute error Weighting: $1/y$ %	Relative absolute error Weighting: $1/x^2$ %	Relative absolute error Weighting: $1/y^2$ %
10,268 1	15,970	5,043	5,107	1,260	1,116
20,536 2	4,601	0,739	0,720	2,021	2,139
51,340 5	1,219	0,779	0,783	0,596	0,696
102,681	0,718	1,599	1,611	0,919	1,014
205,362	2,220	2,541	2,559	1,607	1,700
513,405	0,148	0,155	0,136	1,205	1,116
1 026,81	0,205	0,323	0,304	1,420	1,332
2 053,62	0,038	0,135	0,116	1,259	1,171
Sum of errors	25,120	11,314	11,335	10,287	10,284

A.3 Interpretation of data and choice of regression model

The best regression model to use is the one giving the lowest sum of errors.

In the example, the sum of errors using no weighting factor is significantly higher than the sum of errors using any weighting factor. Since there is only a slight difference between sums of errors for different weighting factors, a weighting factor of $1/x$ could be used since it is the most simple.

Annex B
(normative)

Assays with a target value (simplified approach)

B.1 General

If assays are performed using a target concentration value, the general approach given in 7.2.2 can be simplified in the following way.

B.2 Assays

Prepare an unspiked sample.

Prepare the same sample after spiking it at the target concentration value (PrEMS).

B.3 Results analysis

Using spiked and unspiked samples, check the specificity of the analyte detection in the sample matrix and calculate the S/N ratio increase.

a) If the S/N ratio in the spiked sample (PrEMS) increases to 10 compared to the unspiked sample:

— for this sample, analyte can be quantified at the target concentration;

— result can be given as follows:

Analyte concentration value < Target concentration value

b) If S/N ratio in the spiked sample does not increase to 10 compared to the unspiked sample:

— for this sample, analyte cannot be quantified at the target concentration;

— it is necessary to determine the sample matrix LoQ.

As for the general approach, the interpretation of results when assays are performed using a target concentration value can be made by checking the recovery obtained on the spiked sample, after correction with the initial analyte value, using a solvent calibration curve. This determination could also be prevented by a possible matrix effect (suppression or enhancement of the quantifying signal due to the sample matrix).