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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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ISO 12787 was prepared by Technical Committee ISO/TC 217, *Cosmetics*.

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.

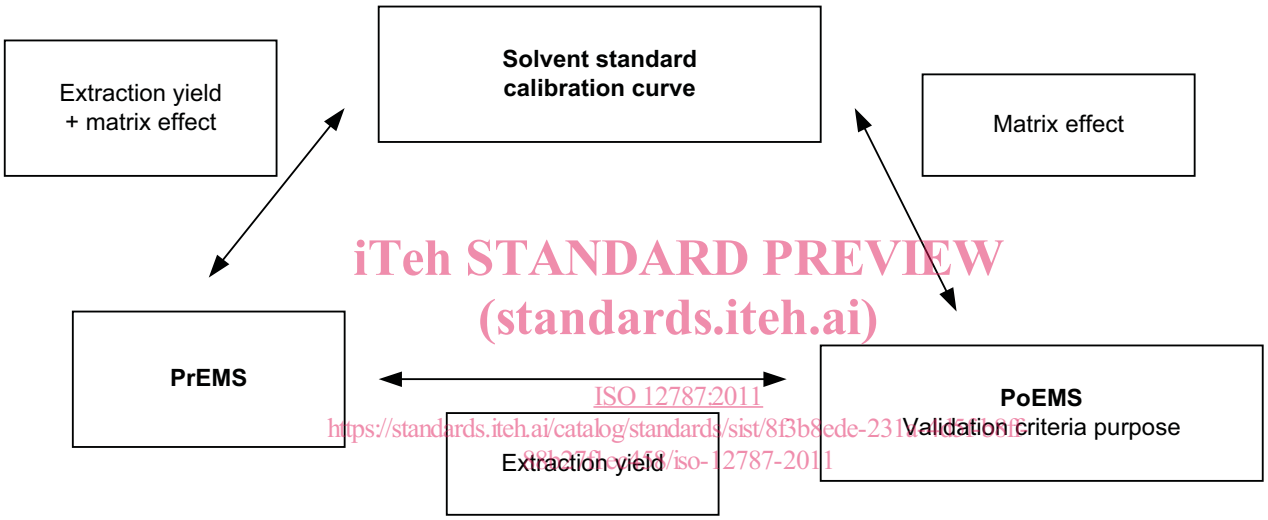

Figure 1 — Validation criteria for analytical results obtained using PrEMS, PoEMS and a solvent calibration curve

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.

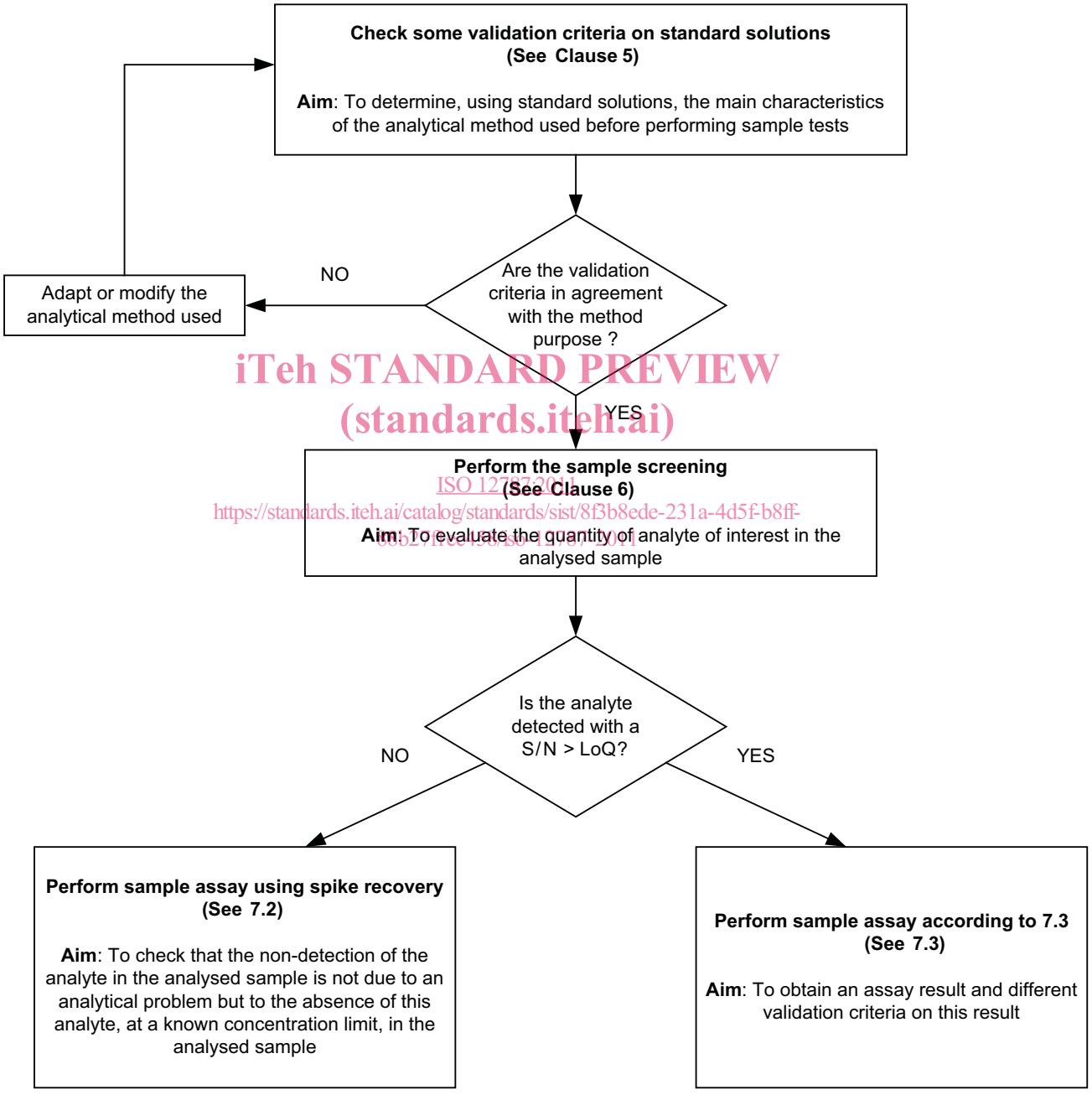

Figure 2 — Purpose of the approach and steps to be performed

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;