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Živila - Smernice za kalibracijo in kvantitativno določanje ostankov pesticidov in organskih kontaminantov (onesnaževal) z uporabo kromatografske metode

Foodstuffs - Guidelines for the calibration and quantitative determination of pesticide residues and organic contaminants using chromatographic methods

Lebensmittel - Leitfaden für die Kalibrierung und die Auswertung von Analysenergebnissen bei der Anwendung chromatographischer Methoden für die quantitative Bestimmung von Pflanzenschutzmittelrückständen und organischen Kontaminanten

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Foodstuffs - Guidelines for the calibration and quantitative determination of pesticide residues and organic contaminants using chromatographic methods

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This draft Technical Specification is submitted to CEN members for Vote. It has been drawn up by the Technical Committee CEN/TC 275.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (FprCEN/TS 17061:2017) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the vote on TS.

1 Scope

This Technical Specification describes the execution of calibration and quantitative evaluation of chromatographic procedures for the determination of pesticides and organic contaminants in residue analysis. In addition, the essential requirements for calibration are outlined.

The calibration of analytical procedures and the evaluation of analytical results need to be conducted according to uniform principles in order to allow for a comparison of analytical results (even from different analytical procedures). They constitute the basis of any method validation and of the quality assurance within laboratories [1], [2], [3].

This Technical Specification does not consider issues of identification/qualification and extraction efficiency.

2 Principle

This document describes the approach for the calibration of chromatographic procedures. The following types of calibration are discussed in more detail:

— external calibration with linear calibration function;

— external calibration with quadratic calibration function;

— calibration with internal standard and linear calibration function;

— calibration with internal standard and quadratic calibration function;

— calibration with standards labelled with stable isotopes (isotopic dilution analysis);

— standard addition to final extract;

— standard addition to sample.

For this purpose, the calibration function and the selection criteria are illustrated on the basis of examples. The calculation formulae refer to the final extract ready for analysis (“test solution”).

The description is rounded off by essential items of quality assurance, e.g. the qualification of chromatographic systems or the quality control chart.

3 General

Calibration of a system is understood as the determination of a functional relationship between a measurable quantity and a concentration to be determined. The chosen type of calibration depends on the various analytical problems/tasks. It is performed in connection with the respective series of measurements.

Basic calibration is regarded as the determination of the functional relationship when an analyte is to be determined for the first time by means of a particular measurement system.

Depending on the problem and on the type of reference solution used, it is distinguished between:

— *calibration with external standard*;

— *calibration with internal standard*;

— *calibration with standard addition*;

— *calibration of the entire procedure*.

In case of the calibration with external standard, the calibration solutions can be prepared either with a pure solvent or with sample extracts which evidently do not contain the analyte(s) (matrix-matched standards).

The application of a simple linear-regression calculation requires a linear relationship between the content of substance and the measured value. The linearity test can be performed visually and/or mathematically. A mathematical check is performed, e.g. by means of the goodness-of-fit test according to Mandel or by means of residual analysis. The residuals are the deviations of the measurement values from the values predicted by the regression line (see Example 1 in 7.1).

4 Execution and calculation of calibrations

4.1 General/specifications

4.1.1 Working range

The range of measurement represented by the lowest and the highest calibration point constitutes the range of concentrations for which the determined calibration function applies (working range). Only within this range, the measured values are reliable and, therefore, can be used for the calculation of analyte contents. At the upper and lower end, the prediction interval becomes wider i.e. the measurement error increases progressively. The highest precision is found in the middle of the working range [4].

The detector response from the analytes in the sample extract has to lie within the working range. Extracts containing residues above the calibrated range shall be diluted. If the calibration solutions are matrix-matched the matrix concentration in the calibration standards should also be diluted, see [5].

The calibration range shall be adjusted to the respective residue concentrations in the test solution (real-sample concentrations which often occur in practice) and should cover a maximum of two orders of magnitudes. Where appropriate, several calibration functions shall be established by means of calibration solutions.

The lower limit of the practical working range usually represents the lowest calibration level, see [5]. It shall be equal to or lower than the Reporting Limit (RL). The RL may not be lower than the Limit of Quantification (LOQ).

4.1.2 Number of calibration points

4.1.2.1 General

For the working range of calibration functions, calibration solutions with different concentrations of pesticides or contaminants are prepared (depending on the requirement, three to five calibration points), the concentrations of which are as equidistantly distributed over the working range as possible. The concentrations shall start at the lower limit of the practical working range. If the working range has to cover one order of magnitude, three calibration points are necessary, while five calibration points are necessary for two orders of magnitude (depending on the covered concentration range, e.g. 1, 3, 10, 30 and 100 times the lowest calibrated concentration).

4.1.2.2 Acceptability of single-point calibrations

A single-point calibration is sufficient if the linearity of the calibration function has been checked over a longer period of time and has been evaluated as stable and if the blank values as well as the intercept are negligibly small. The concentration level should be in the upper fraction of the working range. The analyte concentrations in the calibration and test solutions should be within the range proposed DG-SANTE, (at present ± 30 %) if the test solution is compared to one calibration solution only (see [5] for more details). However, a check of the basic calibration shall be made every working day, and the measurement of a minimum number of representative analytes is indispensable, see [5].

4.1.3 Permissible quantities for determination of response (peak areas or peak heights and peak ratio, respectively)

The analyte signal, thus the peak, produced by the detector can be quantitatively evaluated through determination of the peak height or peak area. By principle, the height as well as the area of the peak depends on the analyte concentration and mass, respectively. The peak height indicates the distance from the baseline to the maximum of the peak. In case of well resolved peaks, the peak height is proportional to the analyte concentration. The evaluation by means of peak height should only be performed in case of reproducible peak shape and constant width at half-height (half-width). It leads to difficulties if there are two peaks with a poorer resolution than approximately 1,25.

A resolution R can be defined by means of the distance ΔE and the width $4 \times s$ of the peaks, see Figure 1 and Formula 1. The width $4 \times s$ is determined through the intercept of both inflexional tangents on the baseline or calculated from the standard deviation s.

$$R = \frac{\Delta E}{4 \times s} \tag{1}$$

In case of well resolved peaks, the peak area is proportional to the analyte concentration. In contrast to the peak height, the peak area usually provides accurate results, even for asymmetric peaks. The prerequisite for peak-area determinations is always the precise definition of the baseline.

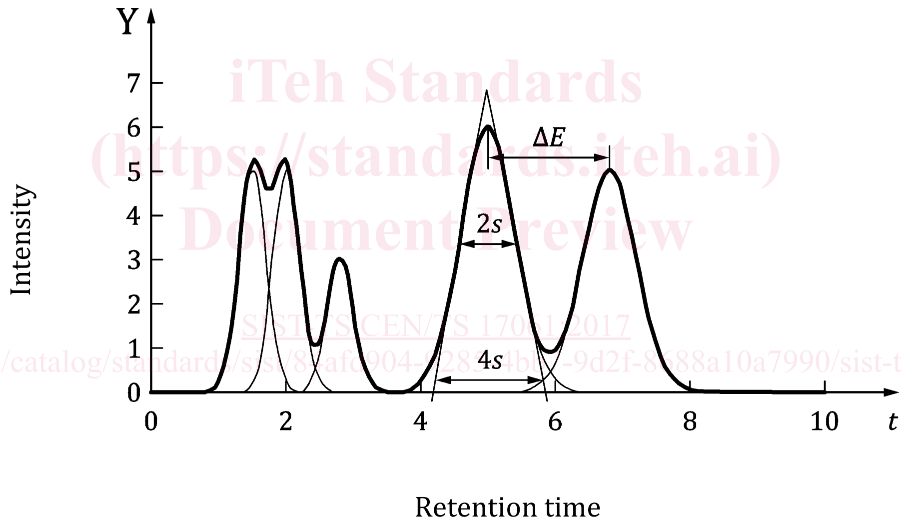

Figure 1 — Definition of the resolution of peaks

The calculation by means of the intensity ratios of peaks (peak ratio) is used for calibrations with internal standard (ISTD), e.g. for EN 15662 (QuEChERS) and for calibrations with internal standard labelled with stable isotopes (stable-isotope labelled standards). This procedure of calibration and calculation requires that the amounts of internal standard added to the analysis samples and calibration standards are known.

4.1.4 Stability of calibration functions

The calibration standards should be injected at least at the start and end of a sample sequence (bracketing calibration). Bracketed samples containing pesticide residues or organic contaminants should be re-analysed if the drift between the two bracketing injections exceeds the limit given by DG-

SANTE. In general such bracketed samples have to be re-analysed if the calibration level corresponding to the RL was not measurable throughout the batch, see [5] for more details.

4.2 Calibration functions

4.2.1 Selection of appropriate calibration function

Calibration functions can be linear, logarithmic, exponential as well as polynomial of 2nd order.

Whenever possible, the simplest acceptable calibration function should be used. The use of linear weighted regression (e.g. $1/x$ or $1/x^2$ weighting) is recommended.

A calibration function is a unique plotting of the set of all *x*-values (concentration or mass) against the set of all *y*-values (peak area or height), i.e. exactly one *y*-value is assigned to each *x*-value.

Before determining the established type of calibration function and testing the linearity of a calibration function, the homogeneity of variances should be checked first as it represents a basic prerequisite for the applicability of statistical methods.

For this purpose, calibration solutions are prepared where between three and ten concentration levels with two to six measurements per concentration are recommended or ten standard samples of the lowest and the highest working-range concentration are separately analysed at a time. The variation (scatter) of the measured values at the limits of the working range is tested for significant differences by means of a simple F-test for variance inhomogeneity.

In case that the F-test indicates a significant difference of variances, there are three opportunities to proceed:

— selection of a narrower working range;

— application of weighted regression;

— application of multiple curve fitting.

The non-consideration of a present inhomogeneity of variances results in a wider prediction interval so that an analytical result which has been determined accordingly shows a higher uncertainty of measurement.

The linearity of the calibration function can be mathematically checked by means of a linearity test. For this purpose, the goodness-of-fit test according to Mandel or the residual analysis is appropriate. In every case, a graphical representation (plot) of the calibration data are recommended (see Example 2 in 7.2).

4.2.2 Visual linearity test

In the simplest case, the determination of the type of calibration function is performed by means of graphical representation of the calibration data including the calibration line and a subjective assessment. If this indicates an obvious nonlinearity of the measured values (see Figure 2), a separate statistic linearity test can be omitted. In cases of doubt, however, the linearity should be checked mathematically [5].

Calibration curves (graphs) shall not be forced through the zero point (origin). Further examples can be found in Clause 7 on Examples.

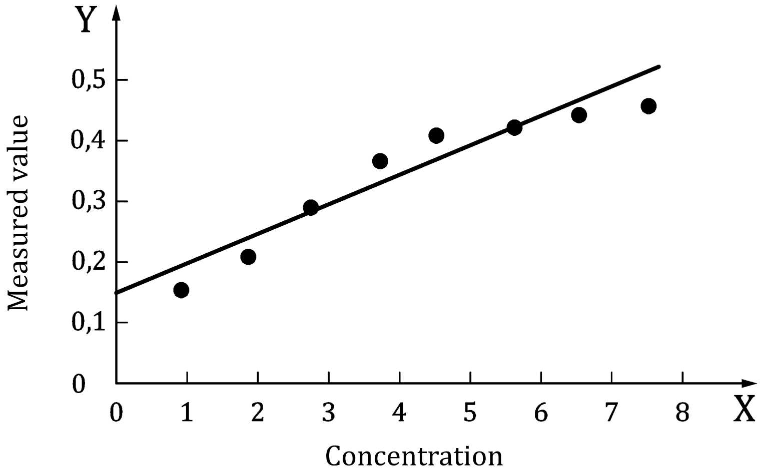

Figure 2 — Graphical representation of calibration data

4.2.3 Mathematical check of linearity

Within the laboratory, the mathematical check of linearity is of secondary importance. Regarding the execution of linearity tests refer to the Examples 1 and 2 and to [6].

As an important criterion of linearity, the coefficient of determination, R^2, is well accepted. However, the coefficient of determination does not allow a sufficient statement regarding the statistical significance of the linear relationship. Calculation of the residuals is recommended to avoid overreliance on coefficients of determination. If individual residuals exceed an acceptable level as defined by DG-SANTE, an alternative calibration function shall be used according to [5].

4.2.4 Calibration with interpolation functions

Several methods of measurement show a basically nonlinear relationship between the measured signals and the concentration and the amount of analyte, respectively. In cases where the linear regression is inappropriate, a polynomial of 2nd order (quadratic calibration function) is usually fitted to the calibration data.

Like the linear calibration function, the polynomial of 2nd order has a prediction interval. Nevertheless, the functional equation has a higher degree of complexity which is why this procedure needs computer support for evaluation.

In case of the nonlinear calibration function, the number of necessary calibration points depends on the desired accuracy and on the reasonable effort. For a global interpolation function, a single mathematical equation describes the entire calibration. However, this is only possible in exceptional cases. In most cases, such a function does not show the expected properties between the calibration points (e.g. polynomial "oscillation"). Therefore, local interpolation functions are employed where each interval between two interpolation nodes is characterized by a separate interpolation function. The application of the calibration function requires the selection of the corresponding local interpolation function for each signal value.

In case of interpolation with polynomials calculated from calibration points in proximity, the curvature of the calibration graph between the interpolation nodes can also be taken into account. The computational effort increases especially when determining the inverse function. Therefore, the calibration function is often calculated once the x-values and y-values have been interchanged.

The calibration function piecewise defined by polynomials will show break (knee) points between the interpolation nodes. With further increased computational effort, this can be prevented by means of