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Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics

Part 1:
Statistical evaluation of the linear calibration function

Qualité de l'eau — Étalonnage et évaluation des méthodes d'analyse et estimation des caractères de performance

Partie 1: Évaluation statistique de la fonction linéaire d'étalonnage

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Contents

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council. They are approved in accordance with ISO procedures requiring at least 75 % approval by the member bodies voting.

International Standard ISO 8466-1 was prepared by Technical Committee ISO/TC 147, *Water quality*.

ISO 8466 consists of the following parts, under the general title *Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics*:

— *Part 1: Statistical evaluation of the linear calibration function*

— *Part 2: Calibration strategy for non-linear calibration functions*

— *Part 3: Method of standard addition*

— *Part 4: Estimation of limit of detection and limit of determination of an analytical basis method*.

Annex A of this part ISO 8466 is for information only.

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Water quality — Calibration and evaluation of analytical methods and estimation of performance characteristics

Part 1:
Statistical evaluation of the linear calibration function

1 Scope

This part of ISO 8466 describes the steps to be taken in evaluating the statistical characteristics of the linear calibration function. It is applicable to methods requiring a calibration. Further parts of this International Standard will cover the determination of limit of detection and limit of determination, the effect of interferences and other performance characteristics.

It is intended especially for the evaluation of the pure analytical method and for the calculation of performance characteristics of the calibration function.

In order to derive comparable analytical results and as a basis for analytical quality control the calibration and evaluation of analytical methods have to be performed uniformly.

2 Definitions

For the purposes of this part of ISO 8466, the following definitions apply.

2.1 analytical method: An analytical method is composed of procedural, measuring, calibrating and evaluating instructions (see figure 1).

Whereas the procedural and measuring instructions depend on the method, and are therefore the object of standardization of the respective method, the calibrating and evaluating instructions are valid for any analytical method requiring calibration.

2.2 calibrating instruction: Describes the approach to determine the calibration function from information values, y_i, obtained by measuring given standard concentrations, x_i. The slope of the calibration function, b, as a measure of sensitivity of the analytical method and the standard deviation of the method, s_{xo}, are figures of merit and characteristics which result from the calibration experiment.

The standard deviation, s_{xo}, allows the comparison of independent analytical methods.

For the user of the method, these characteristics present criteria for the internal laboratory quality control.

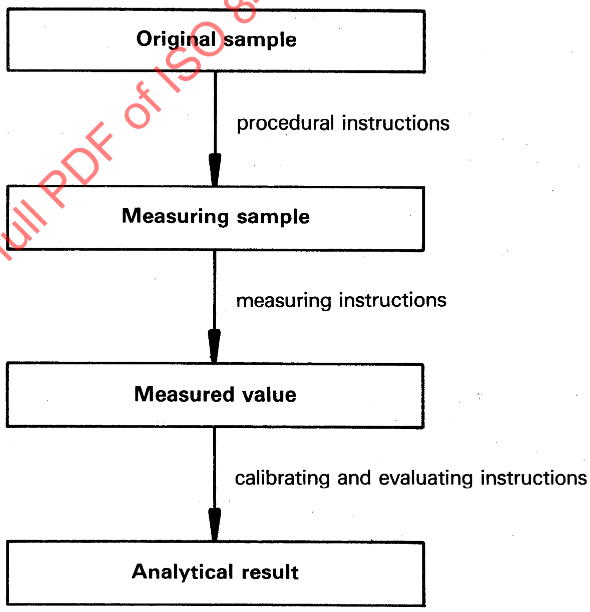

Figure 1 — The analytical method

2.3 evaluating instruction: A calculation guide for the computation of concentrations from the measured values by the use of the calibration function. Additionally, the confidence range permits an objective assessment of the imprecision of the analytical result[2].

2.4 measured values: The concentration-dependent initial values (e.g. extinction) of a measuring system.

NOTE — Information value and measured volume are synonymous.

2.5 residual standard deviation, s_y: The residual standard deviation describes the scatter of the information values about the calculated regression line. It is a figure of merit, describing the precision of the calibration.

For the purpose of this standard, the standard deviation of the method means the standard of deviation of the calibration procedure.

2.6 standard deviation of the method s_{xo}: The ratio of the residual standard deviation, s_y, to the sensitivity of the calibration function, b. It is a figure of merit for the performance of the analytical method, and is valid within the working range (see equation 13).

For the purpose of this standard, the standard deviation of the method means the standard of deviation of the calibration procedure.

2.7 coefficient of variation of the method, V_{xo}: The ratio of the standard deviation of the method s_{xo} to the appertaining mean, $\bar{x}$, which is the centre of the working range.

See also note to 2.5 and 2.6.

2.8 working range (of an analytical method): The interval, being experimentally established and statistically proved by the calibration of the method, between the lowest and highest quantity or mass concentration. The lowest possible limit of a working range is the limit of detection of an analytical method.

2.9 homogeneity of variances: Homogeneity of variances of pooled data, such as those resulting from replicate analyses at different levels, is confirmed if these variances are not significantly correlated to their appertaining concentrations.

2.10 sensitivity of the analytical method: The slope of the calibration function of the complete analytical method, inclusive of all procedural steps, within the working range in question.

2.11 measuring sample (reaction sample): A sample which can be directly submitted to the measurement of the determinand. A measuring sample is normally obtained by adding the required reagents to the analytical sample. Obviously, measuring and analytical sample are identical if no reagents have to be added to the analytical sample.

3 Symbols

x_i	Concentration of the i^{th} standard sample.
i	Subscript of the concentration levels, where $i = 1, 2, \ldots, N$.
N	Number of concentration levels (for this part of ISO 8466, $N = 10$).
x_1	Concentration of the standard sample at the lower level of the working range (1st standard sample).
x_{10}	Concentration of the standard sample at the upper level of the working range (10th standard sample).
$y_{i,j}$	j^{th} information value for the concentration x_i.
j	Subscript of the replicate j of level i, where $j = 1, 2, \ldots, n_i$.
n_i	Number of replicates per level x_i.
$\bar{y}_i$	Mean of the information values $y_{i,j}$ of standard samples, having the concentration x_i.
$\hat{y}_i$	Information value of the standard concentration x_i calculated from the calibration function.
s_i^2	Variance of the information values for the analyses of standard samples, having the concentration x_i.
f_i	Degrees of freedom for the calculation of the variance ($f_i = n_i - 1$).
a	Calculated blank (ordinate intercept of the calibration straight line).
b	Sensitivity of the method (slope of the calibration line; coefficient of regression).
$\bar{x}$	Mean of the standard concentrations x_i, resulting from the calibration experiment.
$\bar{y}$	Mean of the information values y_i, resulting from the calibration experiment.
s_y	Residual standard deviation.
s_{y1}	Residual standard deviation obtained by linear regression calculation.
s_{y2}	Residual standard deviation obtained by non-linear regression calculation.
DS^2	Difference of variances.
y	Information value of an analysed sample.
n	Number of replicates on the same analysed sample.
$\hat{\bar{y}}$	Mean of information values, resulting from n replicates.
$\hat{x}$	Concentration of the analytical sample, calculated from the information value y.
$\hat{\bar{x}}$	Concentration of the analytical sample, calculated from the mean of the information values $\hat{\bar{y}}$.
$t(f_1, 1-\alpha)$	Tabled value of the t-distribution with $f_1 = N - 2$ degrees of freedom and a confidence level of $(1 - \alpha)$ (t-factor of Student's distribution).
$F(f_1, f_2, 1-\alpha)$	Tabled value of the F-distribution (Fisher-Snedecor) with f_1 and f_2 degrees of freedom and a confidence level of $(1 - \alpha)$.
s_{xo}	Standard deviation of the method.
V_{xo}	Coefficient of variation of the method.
$\mathrm{VB}(\hat{x})$	Confidence interval for the concentration $\hat{x}$.
$\mathrm{VB}(\hat{\bar{x}})$	Confidence interval of the mean $\hat{\bar{x}}$ of the concentration.

4 Performance

4.1 Choice of working range

Each calibration experiment is started with the choice of a preliminary working range[3].

The working range depends on

a) the practice-related objective of the calibration.

The working range shall cover, as far as possible, the application range for water, waste water, and sludge analysis. The most frequently expected sample concentration should lie in the centre of the working range.

b) feasibilities of technical realizability.

The measured values obtained must be linearly correlated to the concentrations. This requires that the measured values obtained near the lower limit of the working range can be distinguished from the blanks of the method. The lower limit of the working range should therefore be equal to or greater than the limit of detection of the method. Dilution and concentrating steps should be feasible without the risk of bias.

c) the variance of the information values must be independent of the concentration.

The independence is verified by a statistical test on the linearity[6, 8].

4.1.1 Preparation of the calibration

After establishing the preliminary working range, measured values of at least five (recommended $N = 10$) standard samples are determined. The concentrations, x_i, of these standard samples shall be distributed equidistantly over the working range. In order to check for the homogeneity of the variances, ten replicates of each of the lowest and the highest concentrations (x_1 and x_{10}) of the working range are determined. Ten information values, $y_{i,j}$, result from these series of measurements (see table 1).

4.1.2 Test for homogeneity of the variances

Both data sets of the concentrations x_1 and x_{10} are used to calculate the variances s_1^2 and s_2^2, as given in equation (1):

$$s_i^2 = \frac{\sum\limits_{j=1}^{10} (y_{i,j} - \bar{y}_i)^2}{n_i - 1} \qquad \ldots \quad (1)$$

with the mean

$$\bar{y}_i = \frac{\sum\limits_{j=1}^{10} y_{i,j}}{n_i} \quad \text{for } i = 1 \text{ or } i = 10 \qquad \ldots \quad (2)$$

The variances are tested (F-test) for significant differences at the limits of the working range[5, 6].

The test value PG is determined for the F-test from equation (3).

$$\mathrm{PG} = \frac{s_{10}^2}{s_1^2} \quad \text{for } s_{10}^2 > s_1^2 \qquad \ldots \quad (3)$$

$$\mathrm{PG} = \frac{s_1^2}{s_{10}^2} \quad \text{for } s_1^2 > s_{10}^2$$

PG is compared with the tabled values of the F-distribution[5].

Decision:

a) If $\mathrm{PG} \leqslant F_{f_1;\, f_2;\, 0{,}99}$ the difference between the variances s_1^2 and s_2^2 is not significant.

b) If $\mathrm{PG} > F_{f_1;\, f_2;\, 0{,}99}$ the difference between the variances s_1^2 and s_2^2 is significant.

If the difference between the variances is significant, the preliminary working range should be made smaller until the difference between the variances is found to be random only.

Table 1 — Data sheet for the calibration

i	x_i	$y_{i,1}$	$y_{i,2}$	$y_{i,3}$	$y_{i,4}$	$y_{i,5}$	$y_{i,6}$	$y_{i,7}$	$y_{i,8}$	$y_{i,9}$	$y_{i,10}$
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											

4.1.3 Test for linearity[2, 6, 8]

The easiest test for the linearity is the graphical representation of the calibration data with the calculated regression line. Any unlinearity is evident (see figure 2).

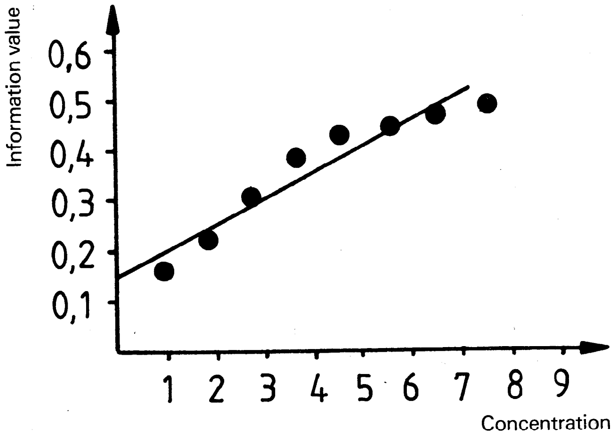

Figure 2 — Graphical linearity check

In the statistical linearity test the calibration data are used to calculate a linear calibration function as well as a non-linear calibration function, both with the residual standard deviation s_{y1} or s_{y2}.

The difference of the variances DS^2 is calculated from equation (4):

$$\mathrm{DS}^2 = (N-2)\,s^2_{y1} - (N-3)\,s^2_{y2} \qquad \ldots (4)$$

Degrees of freedom: $f = 1$.

DS^2 and the variance of the non-linear calibration function s_{y2} are submitted to a F-test in order to examine for significant differences.

The test value PG required for the F-test is calculated from equation (5)

$$\mathrm{PG} = \frac{\mathrm{DS}^2}{s^2_{y2}} \qquad \ldots (5)$$

Decision:

a) If PG $\leqslant F$: The non-linear calibration function does not lead to a significantly better adjustment, e.g. the calibration function is linear.

b) If PG $> F$: The working range should be reduced as far as possible to receive a linear calibration function; otherwise the information values of analyzed samples must be evaluated using the non-linear calibration function.

4.2 Calibration and characteristics of the method

After the final working range is established, ten standard samples are analyzed in accordance with all the steps of the analytical method in order to obtain ten ($N = 10$) measured values y_i (see table 2).

The measurement against a blank is not allowed, since thereby valuable information on the magnitude of the blank will be lost. The comparison medium for zeroing the instrument is always, if possible, a pure solvent (e.g. pure water).

Table 2 — Data sheet for simple linear regression

i	x_i	x_i^2	y_i	y_i^2	$x_i \cdot y_i$
1					
2					
3					
4					
5					
6					
7					
8					
9					
10 = N					
$\sum_{i=1}^{N}$					

The ten data sets, consisting of the values of x_i and y_i, are submitted to a linear regression analysis to obtain the coefficients a and b of the calibration function which describe the linear correlation between the concentration x as an independent variable, and the measured value y as a dependent variable.

The calibration function as well as the characteristics of the method should result from data obtained from a working range x_1 to x_{10}, as received from the measurement and not corrected for blanks. Generally, no blank value (concentration $x = 0$) is to be included in the calibration experiment and, consequently, in the least-squares fit of the regression.

The linear calibration function is given by equation (6)

$$y = a + bx \qquad \ldots (6)$$

The coefficients are obtained from equations (7) for sensitivity (slope of the calibration function) and (8) for the ordinate intercept (calculated blank)

$$b = \frac{\sum_{i=1}^{N} (x_i - \bar{x}) \cdot (y_i - \bar{y})}{\sum_{i=1}^{N} (x_i - \bar{x})^2} \qquad \ldots (7)$$

$$a = \bar{y} - b\bar{x} \qquad \ldots (8)$$

The coefficients provide an estimate of the true function, which is limited by the unavoidable procedural scatter. The precision of the estimate is quantified by the residual standard deviation,

s_y, which is a measure of the scatter of the information values about the calibration line and is given by equation (9).

$$s_y = \sqrt{\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)^2}{N - 2}} = \sqrt{\frac{\sum_{i=1}^{N} [y_i - (a + bx_i)]^2}{N - 2}} \qquad (9)$$

4.3 Assessment

The concentration of an analyzed sample is obtained

a) from the measured value y, to give $\hat{x}$

$$\hat{x} = \frac{y - a}{b} \qquad \ldots \quad (10)$$

or

b) from the mean of a series of replicates, $\bar{y}$, performed on the same original sample, to give $\hat{\bar{x}}$

$$\hat{\bar{x}} = \frac{\bar{y} - a}{b} \qquad \ldots \quad (11)$$

As to the uncertainty of an analytical result, keep in mind that the analytical error is a combination of the uncertainty of the determination of the measured value, and the uncertainty of the estimation of the regression coefficients[2].

From the law of error propagation it follows that, for each value x, a confidence interval for the true value y exists whose limiting points are on two hyperbolic paths bracketing the calibration line. Between these paths the true calibration function can be expected with a significance level of α ($f_1 : N - 2$, confidence level $= 1 - \alpha$), determined by Student's t-factor.

The confidence intervals for analytical results, calculated from the calibration function, are given by the intersections with the respective hyperbolic paths in figure 3. The estimation of the confidence intervals are given by equation (12) [7]

$$\hat{\bar{x}}_{1,2} = \hat{\bar{x}} \pm \text{VB}\,(\hat{\bar{x}})$$

$$\hat{\bar{x}}_{1,2} = \frac{\hat{y} - a}{b} \pm \left(\frac{s_y \cdot t}{b} \times \sqrt{\frac{1}{N} + \frac{1}{\hat{n}} + \frac{(\hat{\bar{y}} - \bar{y})^2}{b^2 \sum_{i=1}^{N} (x_i - \bar{x})^2}} \right) \qquad \ldots \quad (12)$$

NOTE — If $\hat{n} = 1$, $\hat{\bar{x}}_{1,2} = \hat{x}_{1,2}$.

Equation (12) indicates that the confidence interval VB($\hat{\bar{x}}$) brackets the true analytical value with a range governed by the statistical security of Student's distribution. The magnitude of VB($\hat{\bar{x}}$) is mainly determined by the number of replicates $\hat{n}$ and

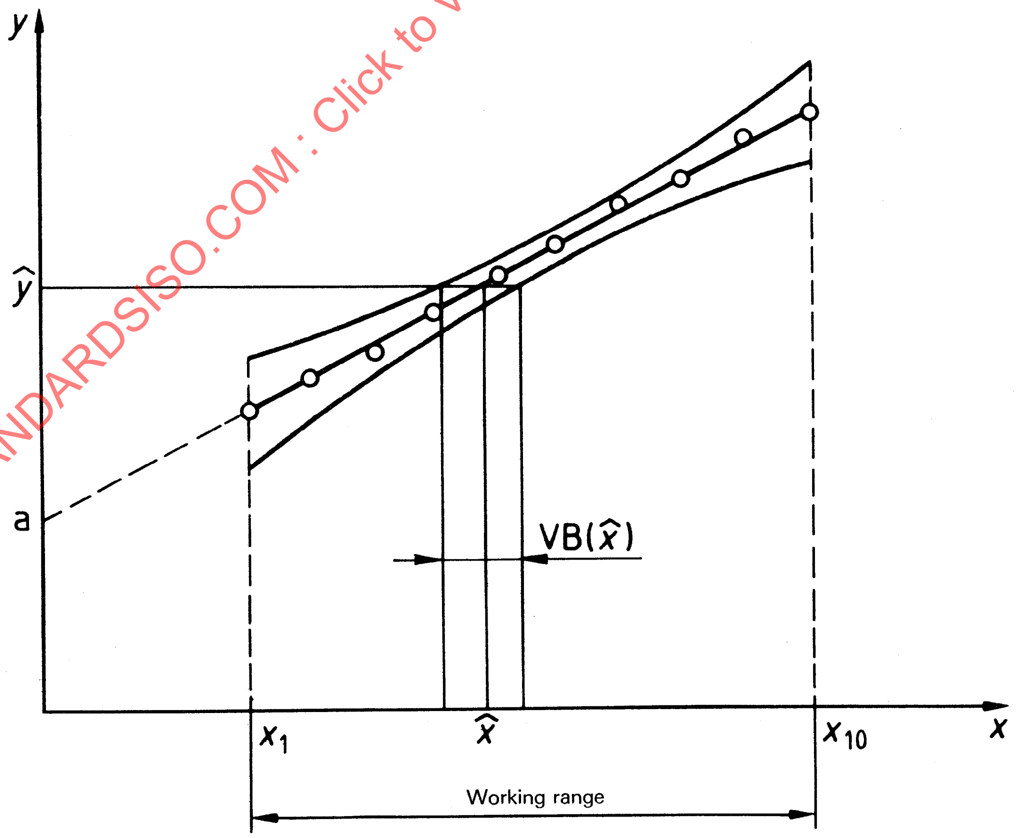

Figure 3 — Working range x_1 to x_{10}, calibration line with confidence band and a single analytical result with its appertaining confidence interval