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Water quality — Calibration and evaluation of analytical methods —

Part 1:
Linear calibration function

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

Partie 1: Fonction linéaire d'étalonnage

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 147, *Water quality*, Subcommittee SC 2, *Physical, chemical and biochemical methods*.

This second edition cancels and replaces the first edition (ISO 8466-1:1990), which has been technically revised.

The main changes compared to the previous edition are as follows:

- the title has been modified;
- the scope of the document is the calibration for routine analysis;
- calculation of performance characteristics has been moved to the informative Annex A;
- the calibration range has been extended to several decade orders of magnitudes;
- the verification of the homogeneity of variances has been deleted;
- the linearity test has been modified;
- various calibration strategies have been described;
- the document has been editorially revised.

A list of all parts in the ISO 8466 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Calibration is a prerequisite for the quantification of analytes by means of physicochemical and chemical methods. In most cases, simple linear regression is applied because many measuring methods show a linear relationship between the indicated value and the sample content.

Since the publication of ISO 8466-1 in 1990, a huge progress has been made in the field of instrumental analysis, a consequence of which is that various calibration strategies have been developed in order to make best use of the equipment. The calibration range of many analytical methods was constrained to a maximum of one order of magnitude by the theoretical statistical requirement to only apply simple linear regression if homogeneity of variances exists across the selected working range. Due to the estimation of measurement uncertainty by calculation of the confidence interval in ISO 8466-1:1990, it had been necessary to conform to the required homogeneity of variances. Meanwhile, other methods for the estimation of measurement uncertainty that are independent of calibration have been established (e.g. ISO 11352).

Calibration is always done in two steps. The first step comprises the determination of the linear range, the second step is the calculation of the calibration function. The calibration strategies that are described in this document enable the analyst to individually define the calibration effort according to specified requirements. The method that is described in ISO 8466-1:1990 remains part of the informative annex since it can still be useful for specific purposes (e.g. method validation).

Water quality — Calibration and evaluation of analytical methods —

Part 1:
Linear calibration function

1 Scope

This document specifies various calibration strategies for physicochemical and chemical analytical methods and specifies the calculation of analytical results.

It defines the general context for linear calibration so that individual standards dealing with analytical methods for the examination of water quality can make reference to it.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at https://www.iso.org/obp
- IEC Electropedia: available at https://www.electropedia.org/

3.1
working range
interval, determined by calibration, between the highest and the lowest content, where the lowest possible limit of the working range is the limit of quantification of the analytical method

3.2
one-point calibration
calibration between the origin and the indicated value corresponding to the content in the *calibration sample* (3.8)

3.3
two-point calibration
calibration using two *calibration samples* (3.8) with different contents at the upper and at the lower working range limit

3.4
indicated value
y
quantity value provided by a measuring instrument or a measuring system

Note 1 to entry: In accordance with definition 4.1 “indication” of ISO/IEC Guide 99[7].

3.5

content

general term for the quantitative expression of the concentration or fraction of a substance in or of a substance mixture

Note 1 to entry: For example, it is the generic term for mass concentration, molar concentration, mass fraction.

3.6

total procedure

analytical method comprising all steps from sample pre-treatment to the report of results

3.7

calibration solution

standard solution without a matrix

3.8

calibration sample

standard solution with a matrix

3.9

measurement procedure

comprises all details of a measurement including all calculations for obtaining the measurement result

3.10

responsivity

R

input–output gain of a detector system, i.e. indicated value divided by the corresponding content in the *calibration sample* (3.8)

Note 1 to entry: For a system that responds linearly to its input, there is a unique responsivity. For nonlinear systems, the responsivity is the local slope.

4 Symbols

A_s	recovery rate for the sample
A_{Is}	recovery rate of the internal standard
a	intercept of the calibration line or the regression line for standard addition
b	slope of the calibration line or the regression line for standard addition or the coefficient of the linear term of the second-order calibration function
b_i	point-to-point slope i
b_{m}	median of the slopes b_i
c	regression coefficient of the quadratic term of the second-order calibration function
F	factor (taking into account sample preparation, e.g. enrichment factor)
f	conversion factor (e.g. 100 for expression in %)
f_1 or f_2	degree of freedom
i	index of the calibration solutions (1, 2, ..., N)
m	number of replicate measurements per spiking level for standard addition
N	number of calibration solutions

n	number of spiking levels for the method of standard addition (including unspiked sample)
P	confidence level
R	responsivity
R_0	responsivity threshold
R_{m}	responsivity median
V_{ssi}	volume of the spiking solution i
V_{M}	volume of the measurement solution
V_{s}	volume of the sample
V_{su}	volume of the sub-samples
x	analyte content
$\overline{x}$	mean value of the contents x_i
x_{e}	analyte content in the calibration solution
x_{eg}	analyte content in the spiked calibration sample
x_{g}	analyte content in the sample after calibration of the total procedure
x_{Ie}	content of the internal standard I in the calibration solution
x_{Ieg}	content of the internal standard I in the calibration sample
x_{Ig}	content of the internal standard I in the sample
x_{IM}	measured content of the internal standard I in the measurement solution
x_i	analyte content in the calibration solution i
x_{M}	analyte content in the measurement solution
x_{o}	analyte content in the original sample
x_{s}	analyte content in the sample
x_{sp}	content of the spiked sample
x_{ss}	content of the spiking solution
x_{z}	spiked content in the sample
x_{zi}	spiked content in the sub-samples i
y	indicated value
$\overline{y}$	mean value of the indicated values y_i
y_{e}	indicated value for external calibration
y_{eg}	indicated value for external calibration of the total procedure
y_{g}	indicated value of the analyte in the sample

y_{Ie}	indicated value of the internal standard I for calibration of the measuring method
y_{Ieg}	indicated value of the internal standard I for calibration of the total procedure
y_{Ig}	indicated value of the internal standard I in the sample
y_i	indicated value of the calibration solution i or indicated value of the sub-sample i for standard addition
y_{s}	indicated value of the (unspiked) sample
Δb_{i-m}	difference between the slope b_i and the median of the slopes b_{m}

5 Determination of the linear working range and establishment of the calibration range

5.1 General

Linearity of the analytical measuring method is tested within the practice-oriented working range in accordance with the following pattern:

a) First step: Establishment of the preliminary working range.

Prepare and analyse calibration solutions (with or without internal standards, analyte-dependent but matrix-independent) over one or more orders of magnitude.

b) Second step: Test linearity and establishment of the linear working range.

Calculate the linear calibration function.

5.2 Preliminary choice of working range

Each calibration starts with the selection of a preliminary working range. This is subject to:

- the objective with respect to the practical application;
- the technically feasible possibilities;
- the linearity of the functional relationship between the indicated values and the contents.

The working range should largely cover a wide application range for the purpose in hand.

The available measuring instruments often allow the choice of a wide linear working range (sometimes over several orders of magnitude). On the other hand, it is required that the indicated values obtained near the lower limit of the working range can be at least distinguished from the indicated values of the procedure blank. A lower limit of the working range is only reasonable if it is greater than or equal to the limit of quantification of this method. Moreover, dilution and concentration steps are required to be feasible and accurate.

5.3 Estimation of the linear working range

5.3.1 General

For testing the linearity of a working range covering one or more orders of magnitude, the following procedure which is based on the first-order calibration function has proved to be effective.

Prepare a minimum of five calibration solutions with different contents (x_i) and determine the corresponding indicated values (y_i). Subsequently, plot both the measuring points and the point-to-point slopes in a diagram and visually estimate the linear working range (see 5.3.2).

Here, the contents may be equidistant or be based on geometric series (e.g. 1; 2; 4; 8; ...) or, when testing linear working ranges that are expected to be large, such as for ICP-MS, be based on the power of ten.

Multiple measurements increase the reliability of the statement.

For many measuring methods showing a linear behaviour over two to five orders of magnitude, a series of contents (x_i) in the form of ... 0,1; 0,2; 0,5; 1; 2; 5; 10; 20; 50; 100; ... is suitable.

NOTE 1 For the recommended series of contents, the measuring points for the contents are nearly equidistant on a logarithmic scale. Instead of a series of contents of e.g. [... 0,2; 2; 20; ...], the series of contents of [... 0,25; 2,5; 25; ...] can also be applied.

NOTE 2 Multiple calibration solutions increase the reliability more than multiple determination.

5.3.2 Visual testing of measurement data — Testing using the x/y-diagram

The measuring points are plotted in a content/indicated value-diagram and the developing of the measuring points is visually assessed.

NOTE 1 When using a spreadsheet programme, the additional presentation of a linear regression function and a comparing second-order regression function (stating the correlation coefficient or the coefficient of determination) can be helpful.

NOTE 2 Analysis of residuals using a preliminary linear regression can give additional information.

5.3.3 Estimation of the linear range by calculating the point-to-point slope

For estimation of the linear working range calculate section-wise the slope b_i from the data sets of each two consecutive measuring points.

In case of a linear calibration function, a constant slope can be expected. Since there might be a non-linear sub-section, the median of the slope values should be used instead of the arithmetic mean value. In case of a linear working range, the section-wise calculated slopes should scatter around the median. A non-linear range at the beginning or at the end will be recognised by systematically increasing or decreasing deviations from the median of the slopes b_m.

For evaluation, plot the differences ($\Delta b_{i-\mathrm{m}} = b_i - b_\mathrm{m}$) between the slopes b_i and the median of the slopes b_m in a diagram.

If the procedure is linear across the selected working range, the differences will be normally distributed around zero (vary unsystematically around zero). Emerging trends indicate a non-linear relationship between content and indicated value.

If, in addition, a tolerance range with limiting lines above and below the zero-line is entered, then the working range that is accepted to be linear can be easily estimated. The tolerance range is defined in accordance with the accuracy requirements for analysis. The linear working range ends (or starts) at the measuring point (characterized as continuous index) that is just located within the accepted scatter around the zero-line or from which a systematic trend is recognized.

Annex B describes this procedure and gives examples.

NOTE 1 The tolerance range can affect measurement uncertainty.

NOTE 2 Especially in the case of measuring points not being equidistantly distributed, single points can visually lead to a false estimation of linearity. The limit of the linear working range can be specified more precisely by adding further measuring points.

As an alternative to the estimation of the linear range by determining the point-to-point slope, Annex C describes the testing of the linear working range by means of the empirical test of curvature. Both methods lead to the same result.

NOTE 3 An Excel spreadsheet for the testing of the linear working range can be downloaded from the website of the Water Chemistry Society[10]. It includes several sample data sets.

6 Calibration strategies

6.1 General

The calibration function that has been determined for a substance is only valid for the covered working range. Moreover, it depends on the operating condition of the measuring instrument and shall be tested within each series of measurements (see Clause 7). The calibration function is valid as long as the requirements for the measurement uncertainty are fulfilled.

The measurement uncertainty of analytical results comprises contributions from random and systematic deviations. The determination of the measurement uncertainty using quality assurance data is described in ISO 11352. Calibration itself contributes to the random and systematic deviations depending on the number of calibration levels applied and the number of parallel determinations, or to what extent a correction of matrix-related systematic deviations is done during calibration. Therefore, more or less complex calibration strategies can be selected according to the required measurement uncertainty for analysis. In this context, this document does not clearly specify the number of calibration levels, the number of standard additions or the number of parallel determinations.

For establishing the calibration functions, five modes of operation are described:

a) calibration of the measuring method using an external standard;

b) calibration of the measuring method using an internal standard;

c) calibration of the total procedure using an external standard;

d) calibration of the total procedure using an internal standard;

e) calibration according to the method of standard addition.

The following options for working ranges and for the number of calibration levels can be applied:

- for one-point calibration, selection of a content level at the upper working range limit. Blanks shall be negligible (below the limit of detection);
- if blanks are not negligible or are varying, measurement of at least one more content level near the lower working range limit and exclusion of the origin for the calculation of the calibration function;
- for multi-point calibration, distribution of content levels across the calibration range;
- for one-point and two-point calibration, a minimum of three measurements for each content level.

The lowest content level of the calibration range shall be greater than or equal to the limit of quantification. The determination of the limit detection and of the limit of quantification is carried out in accordance with documented procedures, for example according to ISO/TS 13530.

Table 1 summarizes the basic conditions and application ranges of various calibration strategies.

For large working ranges, some software packages offer the possibility of weighting by means of $1/x$ in order to improve trueness in the lower part of the working range, if necessary. This weighting procedure is presented in Annex D, including formulae for calculation.

Table 1 — Summary of various calibration strategies

Calibration method	Subclause	Characteristics/basic conditions	Preferred application range
a) calibration of the measuring method using an external standard, including determination of the recovery rate of the analyte	6.3	— matrix-free calibration solution; — abscissa: content values; — ordinate: indicated values; — regardless of the sample matrix, the content value of the sample is determined from the indicated value of the sample and the calibration function; — matrix effects or process-related effects are corrected by the recovery rate of spiked real or synthetic samples, if necessary	Analytical methods for which it is known due to experience from the standardization process or intralaboratory validation that the recovery rate is predominantly constant.
b) calibration of the measuring method using an internal standard, including determination of the recovery rate of the internal standard	6.4	— matrix-free calibration solution with internal standard; — abscissa: ratio of the analyte content in the calibration solution to the content of the internal standard in the calibration solution; — ordinate: ratio of the corresponding indicated value of the standard substance to the indicated value of the internal standard; — the content value of the sample is determined from the ratio of the indicated values of the analyte and the internal standard and the calibration function; — matrix effects are corrected by addition of the internal standard to the sample prior to sample preparation	Analytical methods for which the matrices influence the measurement results during preparation of different samples to an extent that is not negligible and not predictable
c) calibration of the total procedure using an external standard	6.5	— synthetic samples or analyte-free real samples are spiked with an analyte in order to prepare calibration samples and are subjected to the total procedure; — abscissa: content values; — ordinate: indicated values; — regardless of the sample matrix, the content value of the sample is determined from the indicated value of the sample and the calibration function; — the matrix effect is corrected by the recovery rate of spiked samples	Analytical methods for which it is known due to experience from the standardization process or intralaboratory validation that matrix-related interferences are present which, however, have a similar impact on the indicated values regarding amount and direction.

Table 1 *(continued)*

Calibration method	Subclause	Characteristics/basic conditions	Preferred application range
d) calibration of the total procedure using an internal standard	6.6	— for the preparation of calibration samples, synthetic or analyte-free real samples are spiked with an analyte and with an internal standard and finally subjected to the total procedure; — abscissa: ratio of the analyte content in the calibration sample to the content of the internal standard in the calibration sample; — ordinate: ratio of the corresponding indicated value of the standard substance to the indicated value of the internal standard; — matrix effects will be corrected due to the addition of the internal standard to the sample prior to sample preparation	Analytical methods for which the matrices influence the measurement results during preparation of different samples to an extent that is not negligible and not predictable. Analytical methods for which the analyte is converted during sample preparation to a different form that is not available as a standard substance.
e) calibration according to the method of standard addition	6.7	— the sample itself is used for the preparation of the calibration samples by spiking it with an analyte; — abscissa: spiked contents; — ordinate: corresponding indicated values; — the content value of the sample is determined by dividing the indicated value of the unspiked sample by the slope of the calibration function	Analytical methods for which it is known that each matrix significantly affects the indicated value in a non-predictable manner.

6.2 Calculation of the calibration function

Based on the data sets of calibration values (x_i and y_i), the coefficients a and b of the calibration line, which describes the linear relationship between the content x as an independent variable and the indicated value y as a dependent variable, are calculated in accordance with the rules of simple linear regression.

For the calculation of the simple linear regression, the uncertainty values of the contents are neglected.

The calibration function only results from those data which are based on a working range that has been determined from the standard contents (x_1 to x_N), i.e. no blank is subtracted from the individual indicated values.

The calibration function hence is as given in Formula (1):

$$y = a + b \cdot x \tag{1}$$

where

y is the indicated value;

a is the intercept of the calibration line;

b is the slope of the calibration line;

x is the analyte content.

The coefficients of the calibration function are the results from Formulae (2), (3) and (4):

Slope of the calibration function is given as Formula (2):

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

where

x_i is the analyte content of the calibration solution i;

$\bar{x}$ is the mean value of the contents x_i;

y_i is the indicated value of the calibration solution i;

$\bar{y}$ is the mean value of the indicated values y_i.

Ordinate intercept is given as Formula (3):

$$a = \bar{y} - b\cdot\bar{x} \tag{3}$$

This results in the general evaluation function which shall be adapted in accordance with the calibration strategies, if necessary, is given as Formula (4):

$$x = \frac{(y - a)}{b} \tag{4}$$

6.3 Calibration of the measuring method using an external standard, including determination of the recovery rate of the analyte

6.3.1 General

This type of calibration only concerns the measuring method, i.e. no steps of sample preparation such as extraction or digestion are carried out but only matrix-free standards (e.g. in pure solvent or high-purity water) are analysed. The influence due to the steps of sample preparation and of the matrix on the analytical result is taken into account by a separate determination of the recovery rate.

6.3.2 Establishing the calibration function

- For establishing the calibration function, measure the calibration solutions.
- Represent the calibration function graphically. Therefore, plot the indicated values y_e as the ordinate values and the corresponding contents x_e as the abscissa values.
- Determine the line of best fit by linear regression using the value pairs y_e and x_e in accordance with Formula (5):

$$y_e = b \cdot x_e + a \tag{5}$$

where

y_e is the indicated value of the external calibration;

x_e is the analyte content of the calibration solution.

6.3.3 Determination of the recovery rate

For the determination of the recovery rate, spike real samples with the analytes, e.g. by adding calibration solutions, and analyse these spiked samples and the original samples by the total procedure.

Spiking shall be such that the analyte contents in the spiked sample are at the upper working range limit.

The volume of the spike should be negligible compared to the volume of the sample, e.g. <1 %.

Determine the analyte contents of the spiked sample and of the original sample in accordance with Formula (6).

$$x = \frac{y - a}{b} \cdot F \tag{6}$$

where F is the factor (taking into account sample preparation, e.g. enrichment factor).

Calculate the recovery rate in accordance with Formula (7):

$$A_s = \frac{x_{sp} - x_0}{x_z} \cdot f \tag{7}$$

where

A_s is the recovery rate for the sample;

x_{sp} is the content of the spiked sample;

x_o is the content of the original sample;

x_z is the spiked content in the sample;

f is the conversion factor (e.g. 100 for expression in %).

If varying matrix effects exist with proportional systematic impacts, a correction by the sample-specific recovery rate (see Table 1 in 6.1), or the use of internal standards (6.6), or calibration according to the method of standard addition (6.7) is required.

NOTE Information on matrix effects can be obtained from recovery experiments.

6.3.4 Calculation of results

— Calculate the analyte content of the sample in accordance with Formula (8):

$$x_s = \frac{y - a}{b} \cdot \frac{F}{A_s} \cdot f \tag{8}$$

where x_s is the analyte content of the sample.

6.4 Calibration of the measuring method using an internal standard, including determination of the recovery rate of the internal standard

6.4.1 General

By using internal standards in the calibration solutions and samples, the variations of the measuring system are compensated and the recovery rates, including matrix effects, are determined and taken into account over the total analytical process.

The calibration solutions and each analytical sample are spiked with the same amount of the internal standard. The volume of the spike should be negligible compared to the volume of the sample, for example <1 %. The internal standard shall not be present in the samples to be examined. The internal standard and the substance to be determined should have a similar recovery rate and should show a similar behaviour during sample preparation and measurement. Depending on the spectrum of the analytes and the measuring method, it can be necessary to use several internal standards.

For the organic trace analysis, appropriate internal standards are, for example, homologous substances, and, especially for mass-spectrometric methods, the same analytes but composed of different isotopes, e.g. deuterated or ^{13}C labelled compounds. Analytes, for which no isotope-labelled compounds are available, may be analysed using other internal standards if it has been ascertained and documented that the recovery rate of the analyte, which has been determined by spiking, is in the same range for the examined sample types as the recovery rate of the selected internal standard.

6.4.2 Establishing the calibration function

Analyse the calibration solutions containing all substances to be determined and the internal standards; the contents of the internal standards shall be identical in all calibration solutions.

For the graphic representation of the calibration function, plot the ratios of the indicated values y_e/y_{Ie} as the ordinate values and the corresponding ratios of the contents x_e/x_{Ie} as the abscissa values.

Determine the line of best fit from the value pairs y_e/y_{Ie} and x_e/x_{Ie} by linear regression in accordance with Formula (9).

$$\frac{y_e}{y_{Ie}} = b \cdot \frac{x_e}{x_{Ie}} + a \tag{9}$$

where

y_{Ie} is the indicated value of the internal standard;

x_{Ie} is the content of the internal standard in the calibration solution.

6.4.3 Determination of the recovery rate

For the determination of the recovery rate, spike synthetic or real samples with the internal standard and analyse the spiked sample via the total procedure.

Spiking shall be such that the theoretical content in the measurement solution equals the content of the internal standard in the calibration solution.

Calculate the recovery rate of the internal standard A_{Is} in accordance with Formula (10):

$$A_{Is} = \frac{x_{IM}}{x_{Ie}} f \tag{10}$$

where

x_{IM} is the measured content of the internal standard I in the measurement solution;

f is the conversion factor (100 for expression in %).

NOTE x_{IM} is calculated by external calibration. This is usually a one-point calibration.

6.4.4 Calculation of results

Calculate the analyte content of the measurement solution in accordance with Formula (11):

$$x_M = \frac{\frac{y_e}{y_{Ie}} - a}{b} \cdot x_{Ie} \tag{11}$$

where x_M is the analyte content of the measurement solution.

Calculate the analyte content x_s in the water sample in accordance with Formula (12):

$$x_s = x_M \frac{x_{Ie}}{x_{IM}} \cdot \frac{V_M}{V_s} \tag{12}$$

where

x_M see Formula (11);

V_M is the volume of the measurement solution;

V_s is the volume of the sample.

6.5 Calibration of the total procedure using an external standard

6.5.1 General

This type of calibration concerns the total analytical procedure, including all steps of sample preparation. For calibration, synthetic samples[5] or representative analyte-free real samples are prepared by spiking them with analytes.

Calibration via the total procedure is necessary if the analyte is converted into another form during sample preparation, which is not available as a standard substance, e.g. by derivatization.

NOTE 1 The transferability onto real samples can be estimated by the indication of the application ranges in the subject-specific standards. They indicate details on possible problems or interferences.

Further matrix effects can be identified from real samples through the determination of the recovery rate.

If varying matrix effects exist with proportional systematic impacts, a correction by the sample-specific recovery rate (see Table 1 in 6.1), or the use of internal standards (6.6), or calibration according to the method of standard addition (6.7) is required.

NOTE 2 Information on matrix effects can be obtained from recovery experiments.

6.5.2 Establishing the calibration function

For establishing the calibration function, prepare and analyse the calibration samples in the same manner as real samples.

Represent the calibration function graphically. Therefore, plot the indicated values y_{eg} as the ordinate values and the corresponding contents x_{eg} in the spiked calibration samples as the abscissa values.

Determine the line of best fit by linear regression using the value pairs y_{eg} and x_{eg}:

$$y_{eg} = b \cdot x_{eg} + a \tag{13}$$

where

y_{eg} is the indicated value (dependent variable) for the external calibration of the total procedure;

x_{eg} is the analyte content (independent variable) of the spiked calibration sample.

6.5.3 Calculation of results

Calculate the content x_g in the sample in accordance with Formula (14):

$$x_g = \frac{(y_g - a)}{b} \tag{14}$$

where

x_g is the analyte content of the sample;

y_g is the indicated value of the analyte in the sample (prepared according to the same procedure as for calibration).

6.6 Calibration of the total procedure using an internal standard

6.6.1 General

This type of calibration concerns the total analytical procedure and corresponds to the method described in 6.4, except with the calibration sample being a synthetic sample[5] or an analyte-free real matrix. When adding an internal standard, the effects caused by the matrix and by sample preparation (e.g. incomplete digestion or extraction losses by clean-up) can be corrected.

Calibration of the total procedure is necessary if the analyte is converted to another form during sample preparation, which is not available as a standard substance, e.g. by derivatization.

The calibration samples and each analytical sample are spiked with the same amount of the internal standard. See 6.4.1 for the requirements for and the condition of the internal standards.

6.6.2 Establishing the calibration function

At the beginning of sample preparation, add the internal standard with a known concentration to the calibration samples. The content in the calibration samples and in the samples to be examined shall be identical.

Plot y_{eg}/y_{Ieg} as the ordinate values and x_{eg}/x_{Ieg} as the abscissa values.

Determine the calibration function by regression analysis as shown in Formula (15):

$$\frac{y_{eg}}{y_{Ieg}} = b \cdot \frac{x_{eg}}{x_{Ieg}} + a \tag{15}$$

where

y_{Ieg} is the indicated value of the internal standard I;

x_{Ieg} is the content of the internal standard I in the calibration sample.

6.6.3 Calculation of results

Calculate the analyte content of the sample in accordance with Formula (16):

$$x_{\text{g}} = \frac{\frac{y_{\text{g}}}{y_{\text{Ig}}} - a}{b} \cdot x_{\text{Ig}} \tag{16}$$

where

x_{g} is the analyte content of the sample;

x_{Ig} is the content of the internal standard I in the sample;

y_{g} is the indicated value of the analyte in the sample;

y_{Ig} is the indicated value of the internal standard I in the sample.

6.7 Standard addition

6.7.1 General

Calibration by means of standard addition permits the determination of an analyte in a sample in the presence of its individual matrix. Consequently, errors as a result of matrix-related proportional-systematic deviations are corrected and matrix-related effects on precision are quantified. The calibration solutions are therefore prepared by spiking sub-samples; the content in the unspoked samples directly results from the indicated values of the calibration measurements. The indicated value of the unspoked sample is therefore included in the calibration.

Here, a multi-point calibration with multiple spiking levels (n ≥2) adjusted by spiking, possibly equidistant, is carried out with at least one single measurement per content level (m ≥1).

n = number of spiking levels (including unspoked sample);

m = number of replicate measurements per spiking level.

No additive matrix effects that occur due to insufficient selectivity of the analytical method can be compensated by standard addition. The described method assumes that there is no blank or background signal. If corresponding effects are supposed, they shall be determined with a suitable method and the analytical result shall be corrected.

6.7.2 Procedure

Determine the indicated value of the unspiked sample (y_{S}).

Estimate the approximate content of the sample from the indicated value y_{s} by an existing calibration function.

Specify the number of spiking levels n.

Estimate the spiking content, which gives an indicated value for the highest spiked sample, which (a) does not exceed the indicated value of the upper working range limit and which (b) should be at least 2 y_s.

NOTE 1 In case of spiking, where the content is increased less than by a factor of two, it can be expected that the precision of the analytical result will be downgraded.

Prepare a spiking solution.

The analyte content of the spiking solution (x_{ss}) shall be selected such that the increase of the volume of the spiked sub-sample(s) is kept at a minimum compared to the volume of the unspiked sub-sample when adding the spiking solution.

NOTE 2 If the added volume of the spiking solution is more than 1 % of the volume of the sub-sample, then the dilution error can be corrected by adding a spiking blank solution to the sub-samples up to a constant end volume.

Take n sub-samples (i = 1, 2, ..., n) from the sample and add volumes of the spiking solution that are incremented in equidistant steps to n-1 sub-samples. Sub-sample i = 1 will remain unspiked.

Determine the indicated values of the sub-samples y_i.

Calculate the spiked contents in the sub-samples x_{zi} by using Formula (17):

$$x_{zi} = \frac{V_{ssi} \cdot x_{ss}}{V_{su}} \tag{17}$$

where

x_{zi} is the spiked content in the sub-samples i;

V_{ssi} is the volume of the spiking solution i;

x_{ss} is the content of the spiking solution;

V_{su} is the volume of the sub-samples.

6.7.3 Calculation of results

6.7.3.1 Graphical determination of the result

Plot the values of the spiked contents in the sub-samples x_{zi} on the abscissa and the indicated values y_i on the ordinate.

Plot the value pairs x_{zi} and y_i of the sub-samples i = 1 to n in the coordinate system.

Draw the line of best fit in the coordinate system and extrapolate the line to the left in excess of the ordinate until the line intersects with the abscissa;

Visually check linearity using the graphic representation of the indicated values y_i and the contents of the spiked sub-samples x_{zi}.

The intersection of the regression line with the abscissa represents the analyte content value of the sample x_s.

6.7.3.2 Mathematical determination of the result

Calculate the regression line from the value pairs x_{zi} and y_i. Include the indicated value of the unspiked sample (i = 1) in the calculation.

Calculate the analyte content of the sample in accordance with Formula (18):

$$x_s = \frac{a}{b} \tag{18}$$

where

x_s is the analyte content of the sample;

a is the intercept of the regression line;

b is the slope of the regression line.

NOTE An Excel spreadsheet for standard addition can be downloaded from the website of the Water Chemistry Society[10]. It includes several different fictional examples of standard addition.

7 Strategies for testing the validity of calibration

7.1 General

The validity of the existing calibration function shall be tested each working day prior to measurement regarding all parameters to be examined; in case of larger measurement series, it can be necessary to perform testing more frequently. The extent or effort of testing depends on the respective analytical method used, on the measuring instrument, on the size of the selected working range and, if applicable, on the matrix of samples to be examined, and shall be specified by the operator. The following strategies can be applied.

7.2 Testing by means of a control solution or control sample

Prior to commencement and at the end of each measurement series, an independent control solution/control sample with a known content is measured where the content shall be within the working range of the calibration line. The measurement result shall not exceed a specified tolerance range, otherwise a fresh control solution/control sample shall be prepared or recalibration needs to be performed.

For calibration according to 6.3 and 6.4, use an independent control solution, such as one that is prepared from a certified standard solution.

For calibration according to 6.5 and 6.6, use an independent control sample, such as a matrix-matched certified reference material or a stable real sample with a known content from collaborative studies.

The results of the control measurements should be documented on appropriate control charts.

7.3 Testing the slope of the calibration line

During or after each sample series measure a minimum of two control solutions or control samples with different contents at the upper (e.g. 80 %) and at the lower (e.g. 20 %) working range limit. If the slope of the line is within the specified tolerance range, the tested calibration may continue to be used. Otherwise, recalibration shall be done.

Annex A
(informative)

Goodness-of-fit test according to Mandel, standard deviation of the procedure, variation coefficient of the procedure and confidence interval

A.1 General

In ISO 8466-1:1990, homogeneity of variances had been a key requirement. Because of this, the working range was restricted in many cases to one order of magnitude. The goodness-of-fit test according to Mandel to statistically test whether the linear calibration function, compared to the quadratic calibration function, results in an adequate goodness of fit, works well for a working range over one order of magnitude and a nearly equidistant distribution of contents. In contrast, for calibrations over several orders of magnitude with contents that are represented nearly equidistant on a logarithmic scale, the goodness-of-fit test according to Mandel leads to a "non-linear" result and consequently to a restricted working range. For this reason, the linear range is estimated by identifying the point-to-point slope (see 5.3.3).

NOTE The coefficient of determination r^2 can also be used to support the decision-making process when assessing linearity and can be displayed when calculating the trend line using Excel.

In ISO 8466-1:1990, the confidence interval was calculated as a measure for the measurement uncertainty of an analytical method from the calibration data of the linear calibration function. Meanwhile, this approach has been replaced by other methods (e.g. ISO 11352). However, if no estimation of the measurement uncertainty is provided yet, the use of the confidence interval still is a practicable and reliable method. Moreover, some of the "performance characteristics" described in ISO 8466-1 have established in everyday laboratory practice, for example, in order to make quality-related statements when validating measuring methods. They include the standard deviation of the method (s_{xo}) and the coefficient of variation of the method (V_{xo}). These parameters can be used, for example, to compare the performance of different measuring methods or to evaluate the performance of a measuring method over time. In order to be still available to laboratories, these calculation methods are documented below in A.3.

A.2 Goodness-of-fit test in accordance with Mandel

Testing linearity by means of the goodness-of-fit test according to Mandel is carried out by comparison of the linear calibration function with the second-order calibration function (see Reference [8]). Therefore, the following functions and performance data are calculated from the calibration data sets:

a) the linear calibration function $y = a + b \cdot x$ (see 6.2);

b) the residual standard deviation s_{y1} of the linear calibration function (precision measure for the scatter of indicated values in y direction around the calibration function), see Formula (A.1):

$$s_{\mathrm{y1}} = \sqrt{\frac{\sum_{i=1}^{N}\left(y_i - \hat{y}_i\right)^2}{N-2}} = \sqrt{\frac{\sum_{\mathrm{i}=1}^{N}\left[y_i - \left(a + b \cdot x_i\right)\right]^2}{N-2}} \tag{A.1}$$

c) the quadratic calibration function, see Formula (A.2):

$$y = a + b \cdot x + c \cdot x^2 \tag{A.2}$$

NOTE For the calculation of the polynomial coefficients *a*, *b* and *c* see ISO 8466-2:2001, Clause 4.

d) the residual standard deviation s_{y2} of the quadratic calibration function, see Formula (A.3):

$$s_{y2} = \sqrt{\frac{\sum_{i=1}^{N}\left(y_i - \hat{y}_i\right)^2}{N-3}} = \sqrt{\frac{\sum_{i=1}^{N}\left[y_i - \left(a + b \cdot x_i + c \cdot x_i^2\right)\right]^2}{N-3}} \tag{A.3}$$

The difference DS^2 of the sums of squared deviations (with the degree of freedom $f = 1$) is calculated from the residual standard deviations s_{y1} and s_{y2}, see Formula (A.4):

$$DS^2 = (N-2) \cdot s_{y1}^2 - (N-3) \cdot s_{y2}^2 \tag{A.4}$$

The degree of freedom for DS^2 is: $(N\text{-}2)\text{-}(N\text{-}3) = 1$

The test value F_{calc} is determined for the *F*-test, see Formula (A.5):

$$F_{\text{calc}} = \frac{DS^2}{s_{y2}^2} \tag{A.5}$$

and is compared with the value from the *F*-table for the significance level $\alpha = 0{,}01$ (see ISO 8466-2:2001) for $f_1 = 1$ and $f_2 = N - 3$.

Decision:

- If $F_{\text{calc}} < F_{f_1;f_2;99\,\%}$, then the difference between DS^2 and the residual variance s_{y2}^2 is not significant. The calibration function can be assumed to be linear within the examined working range.
- If $F_{\text{calc}} \geq F_{f_1;f_2;99\,\%}$, then the difference between DS^2 and the residual variance s_{y2}^2 is significant. The calibration function is not linear within the examined working range and is rather described by the quadratic calibration function.

If the goodness-of-fit test according to Mandel yields “non-linear”, then one measuring point can be sorted out and the residual points can be tested again. This procedure can be repeated until the goodness-of-fit test according to Mandel leads to no other result than linearity.

A.3 Standard deviation of the method, variation coefficient of the method and confidence interval

A.3.1 Requirements

For the calculation of the performance characteristics, the standard deviation of the method and the variation coefficient of the method, the following requirements shall be conformed to:

- the working range comprises a maximum of one order of magnitude;
- the calibration function is determined from at least five calibration points;
- the contents are distributed equidistantly across the working range;
- the calibration data shall not comprise outliers.

NOTE Two different outlier tests are described in Reference [8].

For the calculation of the confidence interval, another requirement shall be conformed to:

- homogeneity of variances across the overall working range does exist (testing by means of the *F*-test[8]).

A.3.2 Standard deviation of the method

The standard deviation of the method (s_{xo}) is a measure for the performance of the analytical method and states that the performance increases with an increasing absolute value of the slope (b) of the calibration line and a decreasing residual standard deviation (s_{y}), see Formula (A.6).

$$s_{\mathrm{xo}} = \frac{s_{\mathrm{y}}}{b} \tag{A.6}$$

A.3.3 Coefficient of variation of the method

For the comparison of various analytical methods, the coefficient of variation of the method V_{xo} may be used, see Formula (A.7):

$$V_{\mathrm{xo}} = \frac{s_{\mathrm{xo}}}{\bar{x}} \times 100 \tag{A.7}$$

With this relative parameter, the standard deviation of the method s_{xo} is related to the centre of the working range.

A.3.4 Confidence interval

In accordance with the law of error propagation it follows that, for an analytical result x calculated from the calibration function, a confidence interval $VB(x)$ exists, within which the analytical result is assumed with a confidence level (P) that is given by the tabular value $t_{f1;\mathrm{P}}$, with $f_1 = N - 2$ *and* $P = 95\,\%$, of the t distribution.

The calculation of the confidence intervals $x_{1,2}$ (for individual measurements) is carried out in accordance with the following Formulae (A.8) and (A.9):

$$x_{1,2} = x \pm VB(x) \tag{A.8}$$

$$x_{1,2} = \frac{y-a}{b} \pm s_{\mathrm{xo}} \cdot t_{\mathrm{f}_1;\mathrm{P}} \cdot \sqrt{\frac{1}{N} + 1 + \frac{(y-\bar{y})^2}{b^2 \sum_{i=1}^{N} (x_i - \bar{x})^2}} \tag{A.9}$$

Details of the calculation are described in Reference [8].

NOTE A very comprehensive explanation of how to determine measurement uncertainties from linear calibration is given in ISO/TS 28037[6].

Annex B

(informative)

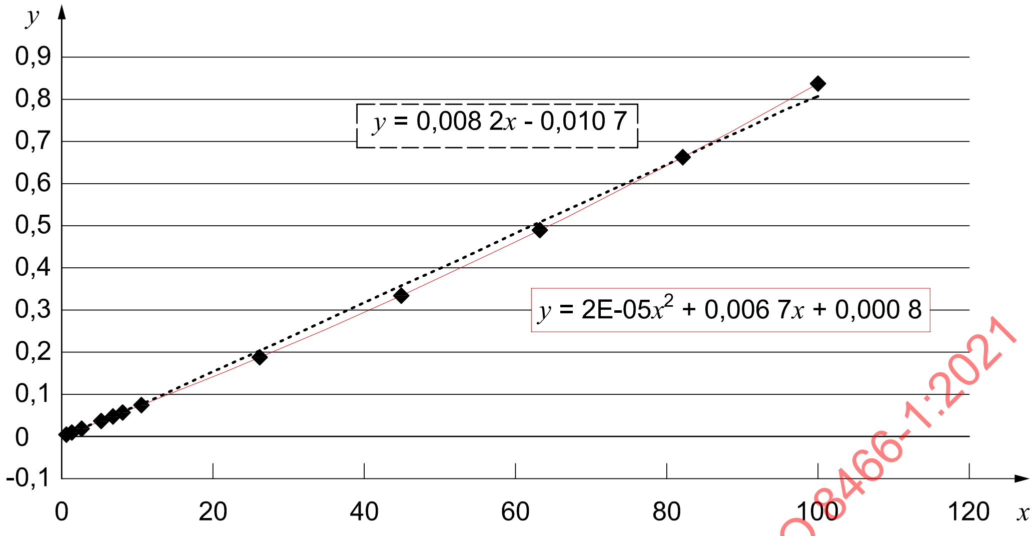
Examples of linearity testing

B.1 Examples 1a and 1b: Determination of nitrite in accordance with ISO 13395 and of ammonium in accordance with ISO 11732 using the continuous flow analysis (CFA)

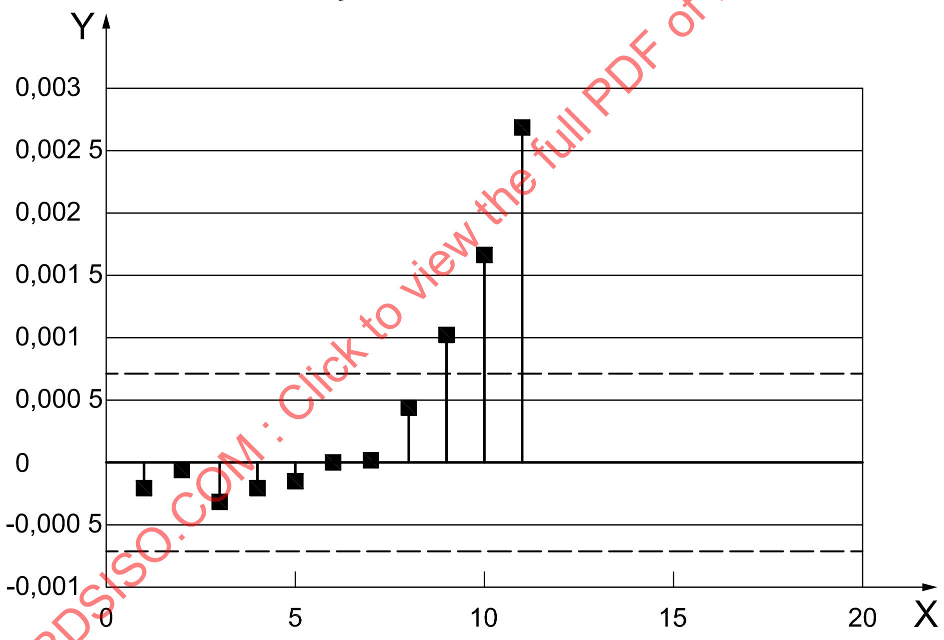
Table B.1 — Example data for nitrite [median 0,007 35 1/(µg/l)]

Reference no.	x values (concentration) µg/l	y values (absorbance)	b_i (point-to-point slope) $\frac{1}{\mu g/l}$	$\Delta b_{i-\mathrm{m}}$ (deviation from the median of the slopes) $\frac{1}{\mu g/l}$
1	0,66	0,003 7	0,007 12	−0,000 23
2	1,32	0,008 4	0,007 35	0,000 00
3	2,64	0,018 1	0,007 02	−0,000 33
4	5,26	0,036 5	0,007 12	−0,000 23
5	6,58	0,045 9	0,007 20	−0,000 15
6	7,9	0,055 4	0,007 33	0,000 02
7	10,6	0,075 2	0,007 36	0,000 01
8	26	0,188 5	0,007 77	0,000 42
9	44,71	0,333 9	0,008 36	0,001 01
10	63,19	0,488 4	0,009 01	0,001 66
11	82,18	0,659 5	0,010 03	0,002 69
12	100	0,838 3	—	—

NOTE Values in columns 4 and 5 are rounded.



a) Visual examination



b) Deviations of the point-to-point slopes from the median of the slopes

Key

x concentration, in micrograms per litre (µg/l)

y absorbance

X reference number as in Table B.1

Y $\Delta b_{i-\mathrm{m}}$ deviation from the median of the slopes, in 1/(µg/l)

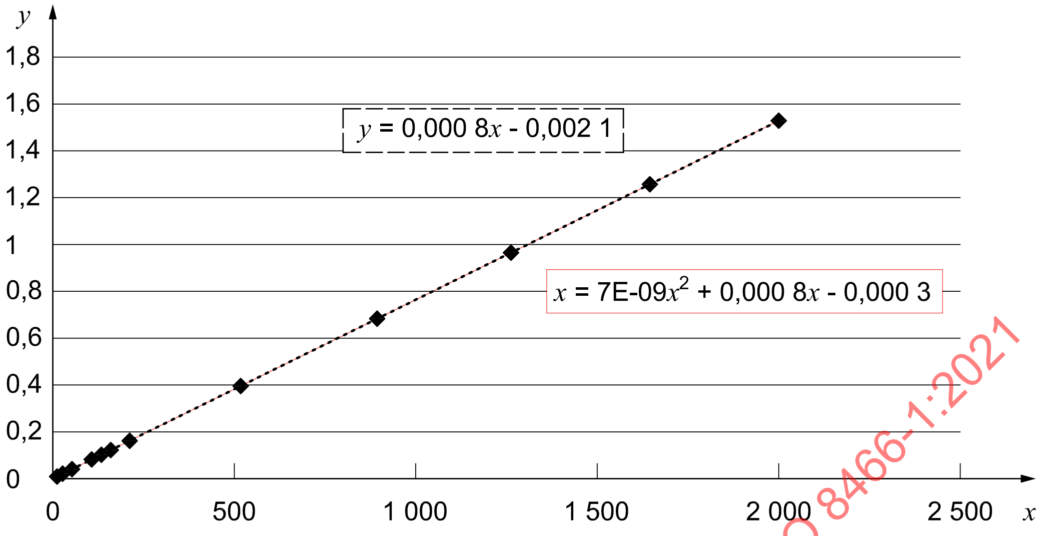
NOTE Deviation tolerance: 10 %.

Figure B.1 — Determination of nitrite using CFA (Example 1a)

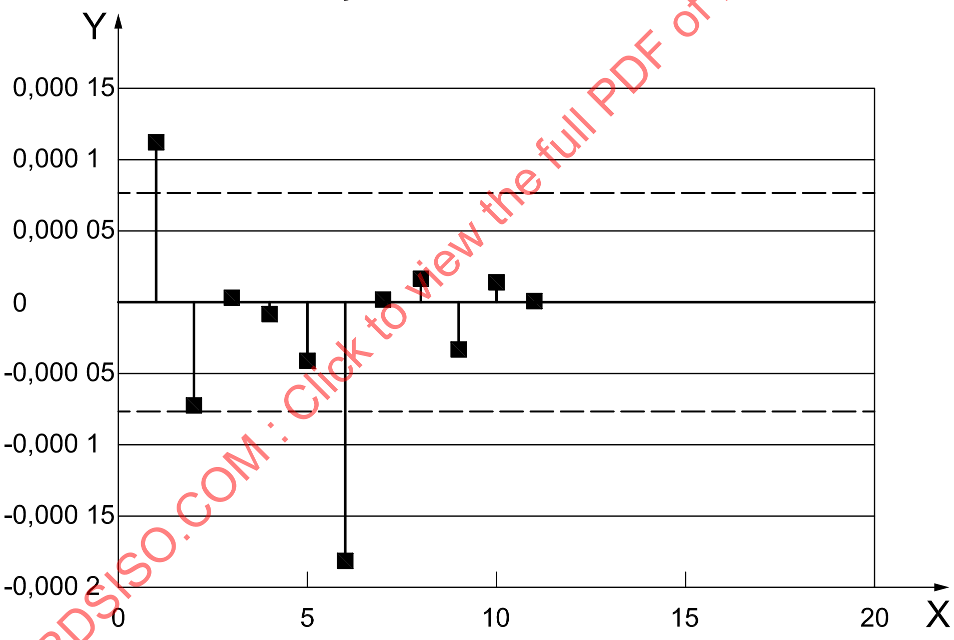
Table B.2 — Example data for ammonium [median 0,000 777 1/(µg/l)]

Reference no.	x values (concentration) µg/l	y values (absorbance)	b_i (point-to-point slope) $\frac{1}{\mu g/l}$	Δb_{i-m} (deviation from the median of the slopes) $\frac{1}{\mu g/l}$
1	13,21	0,009 9	0,000 888	0,000 110
2	26,39	0,021 6	0,000 701	−0,000 076
3	52,78	0,040 1	0,000 780	0,000 002
4	105,24	0,081 0	0,000 769	−0,000 008
5	131,63	0,101 3	0,000 736	−0,000 042
6	158	0,120 7	0,000 595	−0,000 182
7	212,08	0,152 9	0,000 778	0,000 001
8	520	0,392 5	0,000 793	0,000 016
9	894,14	0,689 3	0,000 743	−0,000 034
10	1 263,75	0,963 9	0,000 790	0,000 013
11	1 643,67	1,264 0	0,000 777	0,000 000
12	2 000	1,541 0	—	—

NOTE Values in columns 4 and 5 are rounded.



a) Visual examination



b) Deviations of the point-to-point slopes from the median of the slopes

Key

x	concentration, in micrograms per litre (μg/l)
y	absorbance
X	reference number as in Table B.2
Y	$\Delta b_{i-\mathrm{m}}$ deviation from the median of the slopes, in 1/(μg/l)

NOTE Deviation tolerance: 10 %.

Figure B.2 — Determination of ammonium using CFA (Example 1b)

Explanation:

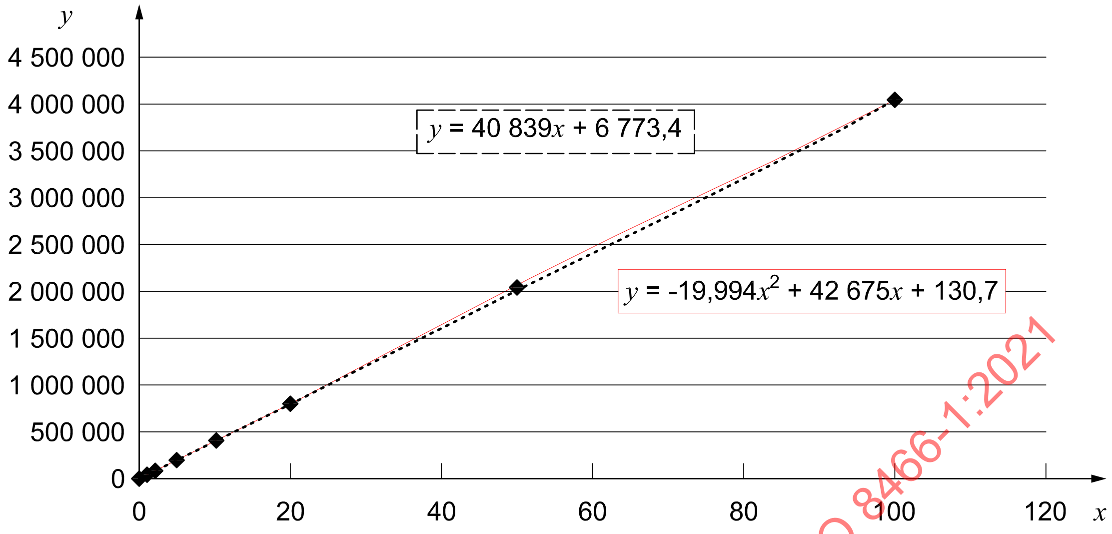
While the differences between the point-to-point slopes and the median of the slopes vary around zero for the determination of ammonium (Example 1b), a continuous increase can be noticed for the determination of nitrite (Example 1a) starting from the 8th measuring point.

Result: The linearity of nitrite determination consequently ends with 26 µg/l, while a linear dependence between absorbance and concentration can be assumed for the determination of ammonium in the tested range of 13 µg/l to 2 000 µg/l.

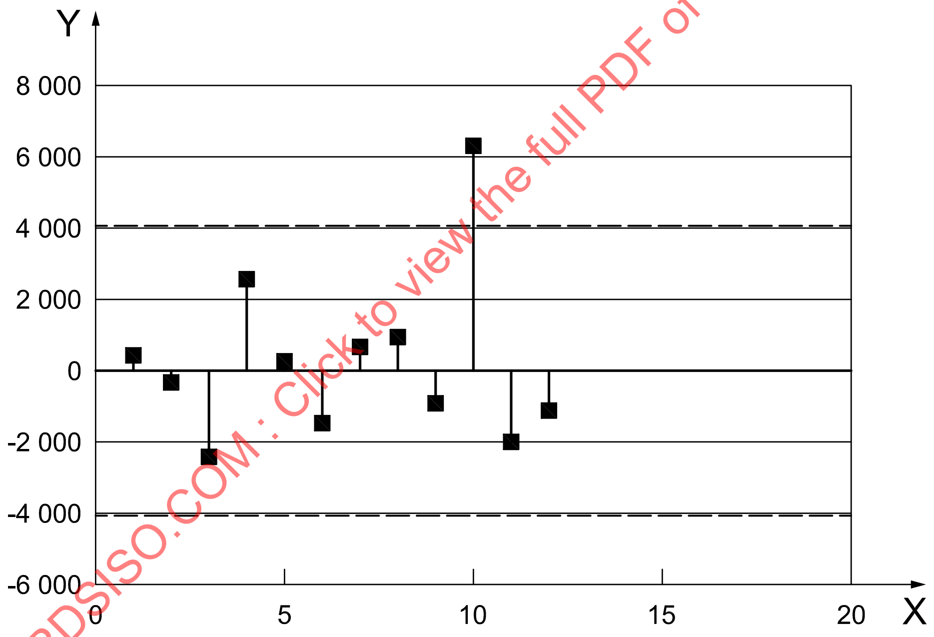
B.2 Examples 2a and 2b: Simultaneous determination of copper (327 nm) and lead (220 nm) using ICP-OES in accordance with ISO 11885

Table B.3 — Example data for copper [median 41 248,3 counts/(mg/l)]

Reference no.	*x* **values** (**concentration**) mg/l	*y* **values** (**indicated value**) counts	b_i (**point-to-point slope**) $\frac{\text{counts}}{\text{mg/l}}$	$\Delta b_{i-\text{m}}$ (**deviation from the median of the slopes**) $\frac{\text{counts}}{\text{mg/l}}$
1	0,01	413	41 700,0	451,7
2	0,02	830	40 966,7	−281,7
3	0,05	2 059	38 780,0	−2 468,3
4	0,1	3 998	43 870,0	2 621,7
5	0,2	8 385	41 530,0	281,7
6	0,5	20 844	39 774,0	−1 474,3
7	1	40 731	41 945,0	696,7
8	2	82 676	42 221,0	972,7
9	5	209 339	40 312,8	−935,5
10	10	410 903	47 546,5	6 298,2
11	20	886 368	39 250,5	−1 997,9
12	50	2 063 882	40 147,2	−1 101,1
13	100	4 071 243	—	—
NOTE Values in columns 4 and 5 are rounded.				



a) Visual examination



b) Deviations of the point-to-point slopes from the median of the slopes

Key

x	concentration, in milligrams per litre (mg/l)
y	indicated value, in counts
X	reference number as in Table B.3
Y	$\Delta b_{i-\mathrm{m}}$ deviation from the median of the slopes, in counts per mg/l

NOTE Deviation tolerance: 10 %.

Figure B.3 — Determination of copper using ICP-OES at 327 nm (Example 2a)

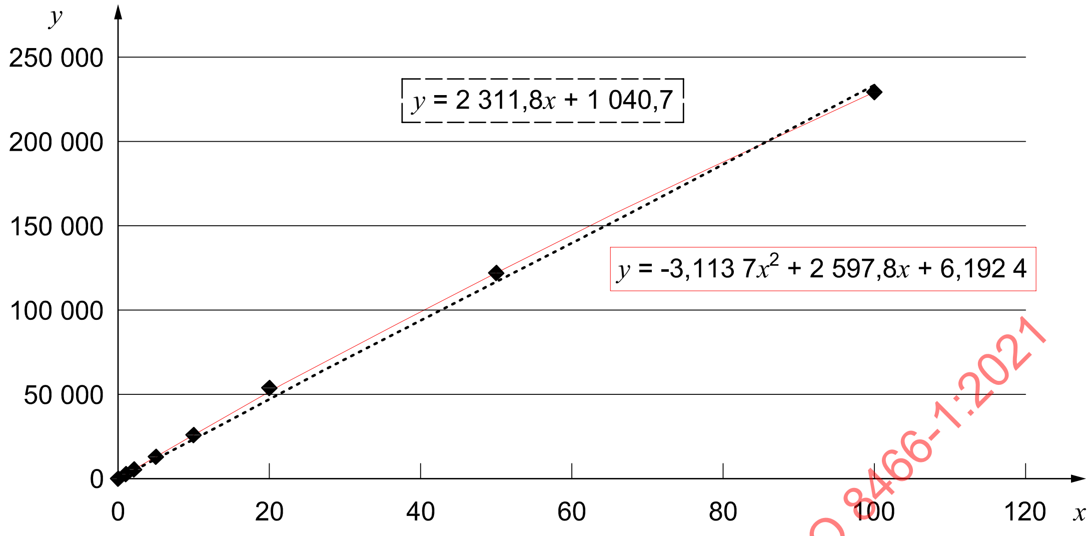
Examination of the point-to-point slopes, copper:

In Example 2a, the deviations of the point-to-point slopes from the median of the slopes vary unsystematically around the baseline. Therefore, measurement of copper at 327 nm within the concentration range of 0,01 mg/l to 100 mg/l can be assumed to be linear.

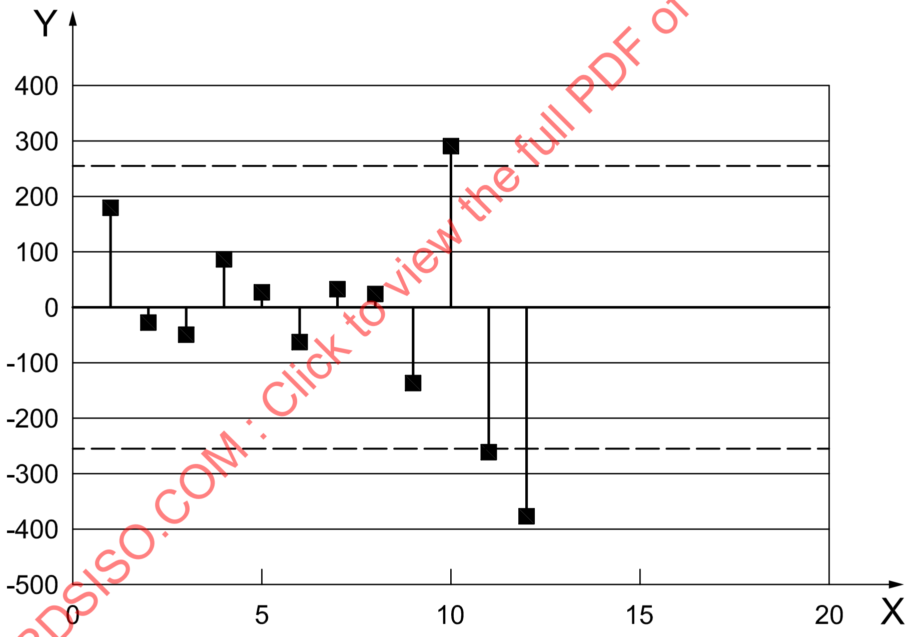
Table B.4 — Example data for lead [median 2 532,0 counts/(mg/l)]

Reference no.	*x* values (concentration) mg/l	*y* values (indicated value) counts	b_i (point-to-point slope) $\frac{\text{counts}}{\text{mg/l}}$	$\Delta b_{i-\mathrm{m}}$ (deviation from the median of the slopes) $\frac{\text{counts}}{\text{mg/l}}$
1	0,01	24,17	2 711,0	179,0
2	0,02	51,28	2 507,7	−24,3
3	0,05	126,51	2 484,0	−48,0
4	0,1	250,71	2 615,9	83,9
5	0,2	512,30	2 557,5	25,5
6	0,5	1 279,54	2 468,4	−63,5
7	1	2 513,75	2 564,6	32,7
8	2	5 078,39	2 556,3	24,3
9	5	12 747,17	2 395,4	−136,6
10	10	24 724,14	2 827,4	295,5
11	20	52 998,47	2 268,0	−263,9
12	50	121 039,76	2 156,0	−376,0
13	100	228 838,38	—	—

NOTE Values in columns 4 and 5 are rounded.



a) Visual examination



b) Deviations of the point-to-point slopes from the median of the slopes

Key

x	concentration, in milligrams per litre (mg/l)
y	indicated value, in counts
X	reference number as in Table B.4
Y	$\Delta b_{i\text{–m}}$ deviation from the median of the slopes, in counts per mg/l

NOTE Deviation tolerance: 10 %.

Figure B.4 — Determination of lead using ICP-OES at 220 nm (Example 2b)

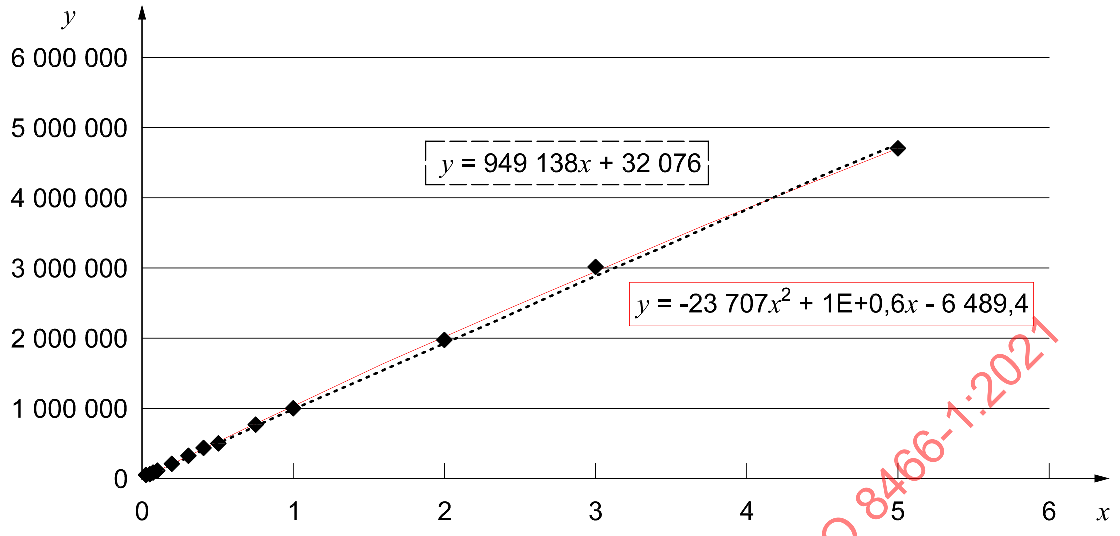
Examination of the point-to-point slopes, lead:

In Example 2b for the measurement of lead using ICP-OES at 220 nm, a deviation from the linear behaviour is probable from the 11th measuring point (20 mg/l).

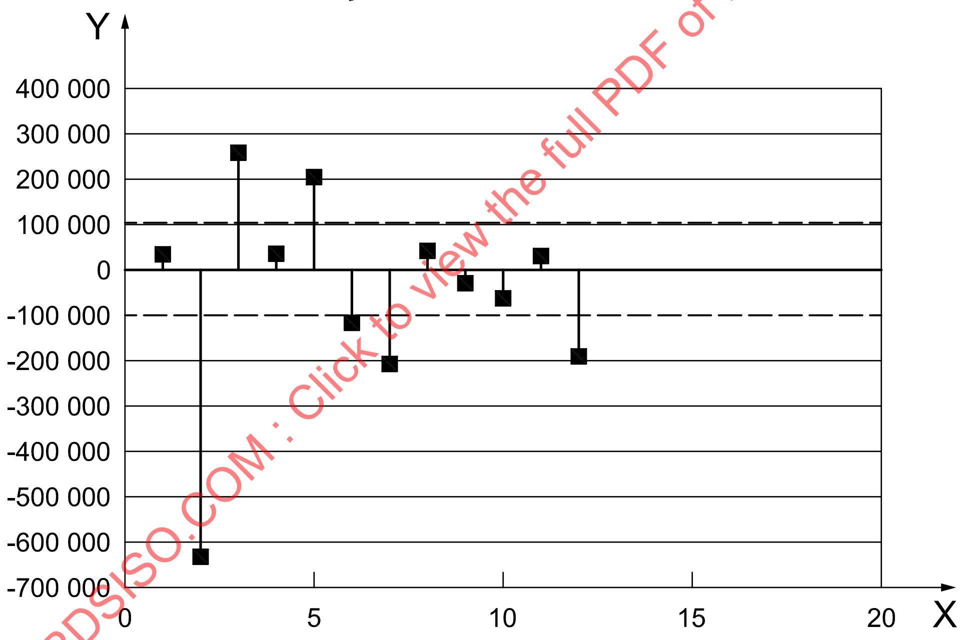
B.3 Examples 3a and 3b: Determination of desisopropylatrazine and carbamazepine using LC-MS/MS

Table B.5 — Example data for desisopropylatrazine [median 1 019 284 AU/(µg/l)]

Reference no.	x values (concentration) µg/l	y values (area) AU	b_i (point-to-point slope) $\frac{\text{AU}}{\text{µg/l}}$	$\Delta b_{i-\text{m}}$ (deviation from the median of the slopes) $\frac{\text{AU}}{\text{µg/l}}$
1	0,025	31 361	1 054 120	34 836
2	0,05	57 714	389 240	−630 044
3	0,075	67 445	1 279 600	260 316
4	0,1	99 435	1 053 560	34 276
5	0,2	204 791	1 222 260	202 976
6	0,3	327 017	904 240	−115 044
7	0,4	417 441	812 030	−207 254
8	0,5	498 644	1 060 052	40 768
9	0,75	763 657	988 832	−30 452
10	1	1 010 865	955 748	−63 536
11	2	1 966 613	1 049 736	30 452
12	3	3 016 349	828 876,5	−190 408
13	5	4 674 102	—	—



a) Visual examination



b) Deviations of the point-to-point slopes from the median of the slopes

Key

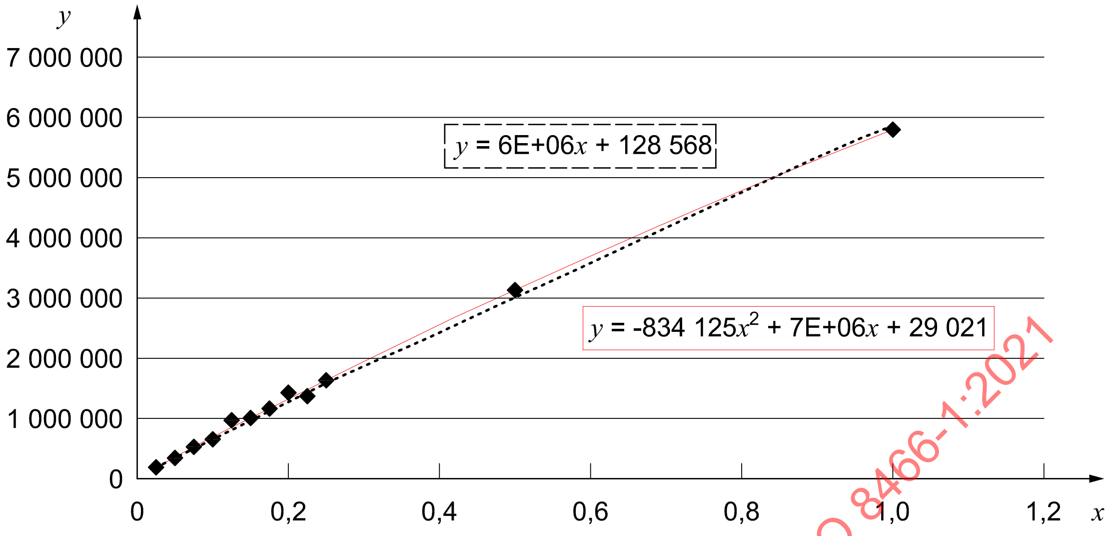
x	concentration, in micrograms per litre (µg/l)
y	area, in AU
X	reference number as in Table B.5
Y	$\Delta b_{i-\mathrm{m}}$ deviation from the median of the slopes, in AU per µg/l

NOTE Deviation tolerance: 10 %.

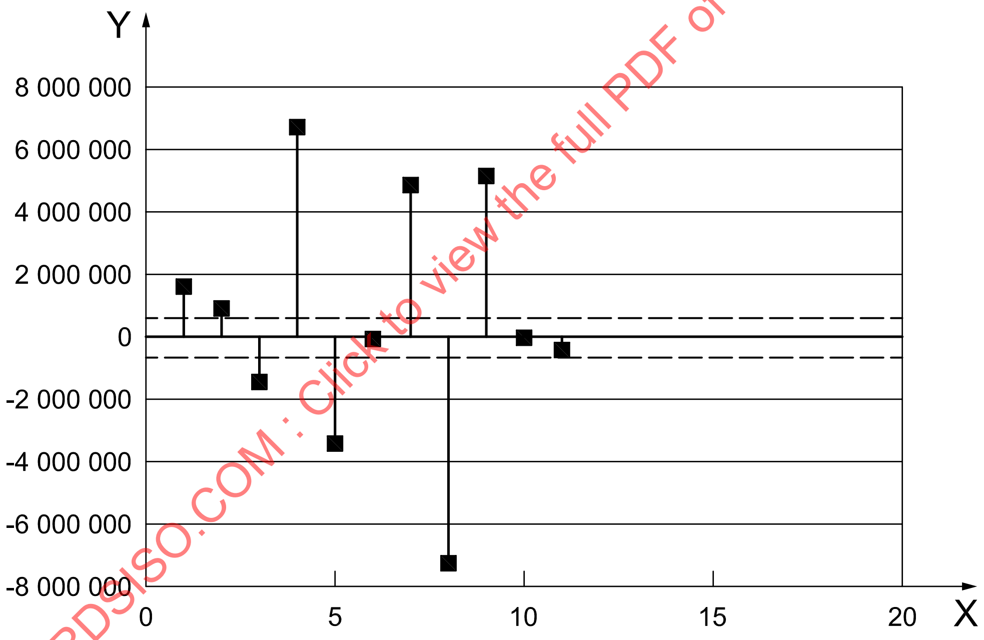
Figure B.5 — Determination of desisopropylatrazine using LC-MS/MS (Example 3a)

Table B.6 — Example data for carbamazepine [median 5 812 248 AU/(µg/l)]

Reference no.	*x* values (concentration) µg/l	*y* values (area) AU	b_i (point-to-point slope) $\frac{\text{AU}}{\mu\text{g/l}}$	$\Delta b_{i-\text{m}}$ (deviation from the median of the slopes) $\frac{\text{AU}}{\mu\text{g/l}}$
1	0,025	165 109	7 439 320	1 627 072
2	0,05	351 092	6 742 920	930 672
3	0,075	519 665	4 381 120	−1 431 128
4	0,1	629 193	12 529 840	6 717 592
5	0,125	942 439	2 421 680	−3 390 568
6	0,15	1 002 981	5 764 560	−47 688
7	0,175	1 147 095	10 692 000	4 879 752
8	0,2	1 414 395	−1 440 240	−7 252 488
9	0,225	1 378 389	10 958 680	5 146 432
10	0,25	1 652 356	5 812 248	0
11	0,5	3 105 418	5 418 260	−393 988
12	1	5 814 548	—	—



a) Visual examination



b) Deviations of the point-to-point slopes from the median of the slopes

Key

x concentration, in micrograms per litre (µg/l)

y area, in AU

X reference number as in Table B.6

Y $\Delta b_{i-\mathrm{m}}$ deviation from the median of the slopes, in AU per µg/l

NOTE Deviation tolerance: 10 %.

Figure B.6 — Determination of carbamazepine using LC-MS/MS (Example 3b)

Visual examination and testing of the point-to-point slopes:

While through visual examination of the measuring data exceedance of the linear working range can be assumed for the last two measuring points in both examples, examination of the point-to-point slope gives no clear evidence of an upper limit of the linear working range due to the scatter in both examples.

Annex C
(normative)

Examination of the linear working range using the empirical test of curvature

C.1 General

As an alternative to the estimation of the linear working range by determination of the point-to-point slope in accordance with 5.3.3, the empirical test of curvature can be applied. Therefore, a method is used that, for example, is also used for detectors to determine the linear range (see Reference [9]). In contrast to linearity testing, which is based on the calculation of the point-to-point slope, the slope of the line of origin through the measuring point is determined in the test of curvature. Both tests concentrate on the changes of the slope and provide comparable results. The deviation to be tolerated (in %) cannot be generally specified for both tests but shall be specified by the laboratory based on the test method used.

When applying the test of curvature, the measuring point from which a calibration function is assumed to be curved shall be determined. Therefore, responsivity y/x is calculated. This is illustrated in Figure C.1 with a simulated calibration example.