The MAK Collection for Occupational Health and Safety

Calibration

Air Monitoring Methods, Conceptual Topics – Translation of the German version from 2016

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Calibration

1 Introduction

The main task of applied analytical chemistry is the determination of the type and concentration of the substances contained in solids, liquids or gases. Therefore, the precision and accuracy of the result, which are sometimes predefined, must be suitable for the subject under investigation. This is generally accomplished by direct comparison with known references – frequently along a chain of comparisons.

Thus measurement results can be traced back to so-called standards. In this context, traceable basic standards are e.g. substance quantity, length, mass, time, current strength and temperature.

When calibrating a measuring device, a functional correlation between a known concentration and a measurement signal is established (calibration function). The calibration of measurement devices plays a key role when carrying out quantitative determinations and ensures the quality of the analytical results.

In order to achieve sufficient reliability, repetition of the measurements with several known concentrations of preferably traceable standards is required. Unknown concentrations are determined by converting the calibration function into an analysis function (inverse function), with the help of which a concentration is calculated from the measurement signal. Conclusions on the uncertainty can be drawn from the calibration experiments and from further information (e.g. round robin tests) gleaned from the specifically applied analytical methods (see “Preliminary Remarks“, Vol. 13, chapter “Quality assurance at workplace measurements“ 2012). The calibration thus essentially determines the reliability of the entire analytical method.

Note:

In the past the terms calibration and gauging were often used synonymously. The differences are described below, in order to show that official gauging does not play a significant role in the context of workplace measurements. A definition of the term official gauging can be found in the German 'Measurement and Official Gauging Law' (*Mess- und Eichgesetz* [1]). *Here it is stated that: “Gauging is each official or officially required testing, assessment and labelling of a measurement device, in connection with the permission to continue using the measurement device for its intended purpose and under the appropriate conditions of use for a further period until renewed official gauging is required.” Thus official gauging establishes whether a measurement device or a measurement aid (e.g. so-called test or calibration weights) stays within predefined error limits and whether it can be assumed that this will remain the case throughout the entire period until renewed official gauging is required. Thus it becomes clear that an official gauging, e.g. of an atomic absorption spectrometer, is practically impossible when this definition is applied.*

Calibrations are carried out before the background of different investigations. As part of the development of a measurement method, for example, a calibration of the analytical basic procedure is carried out. In this case the concentration range, in which there is a linear relationship between the concentration of the analyte and the respective measurement signal, can be determined. The calculation of the scatter of the measurement values around the calibration curve offers the possibility of calculating analytical parameters such as the limit of detection (LOD) and the limit of quantification (LOQ) [2].

Further aspects have to be considered in the case of a calibration that is to be carried out for the purpose of evaluating samples, which have been taken as part of a validated measurement method. The composition of the calibration solutions should then correspond to that of the respective samples as closely as possible. The concentration range, for which the calibration is carried out, depends on the concentration range, over which the measurement method is expected to deliver unambiguous results. Due to the fundamental importance of the calibration of measurement devices for the determination of concentrations of hazardous substances at workplaces a special chapter is dedicated to this subject. As a general rule measuring institutions that perform workplace measurements must carry out their own various calibrations and take any errors arising from them into consideration.

Note:

The employer, who carries out or commissioned workplace measurements, must ensure or check the competency of the contracted measuring institution. A measuring institution can establish its competency through the national accreditation body e.g. by accreditation as stipulated in standard EN ISO/IEC 17025 [3]. An accreditation gives rise to the presumption of the accuracy of the results obtained from workplace measurements, according to § 7 Section 10 of the German Hazardous Substance Ordinance (Gefahrstoffverordnung) [4]. However, accreditation is not mandatory. Competency can also be proven by other means such as by a quality management system or by participation in round robin tests.

2 Traceability and reference materials

2.1 Traceability

Traceability is the property of a measurement result that can be traced back to national or international measurement standards via an uninterrupted chain of calibrations [3]. This means that all links of the chain of calibration – from the current test object to the legally defined basic reference quantity for the measurement parameter – have been documented in a traceable manner. The unbroken chain of comparison measurements is also known as a traceability chain. In this manner traceability serves the purpose of determining or ensuring the accuracy of measurements. Measurements mainly consist of comparisons of an unknown quantity, whose value is being sought, with a standard, whose value is regarded as being known. Therefore, for instance the results from routine methods should be traceable to the results of reference methods, and these in turn should be verifiably traceable to the results of definitive methods. Traceability is usually ascertained by the use of certified reference materials.

Hence traceability is primarily a process, by which information can be retraced to its source. It includes information required for result confirmation, such as detailed specifications for the measurement method and the reference materials to be used as well as all the various pertinent uncertainties. It should be noted that traceability in quantitative chemical analysis (chemical metrology) is more difficult to achieve than in physical metrology due to the complexity of the entire analytical process

[5]. Chemical analyses require the comparison of important characteristics of the sample with known characteristics of reference materials. This comparison can either be carried out directly or indirectly with the help of devices that have been calibrated using one or several accepted reference values.

Therefore, for instance, in the chemical analysis of a sample consisting of several components several reference materials may be required if reference values are required at different concentrations for each component. On the other hand, the matrix that contains the analytes can cause severe interference to the analytical results. This shows that exact requirements and procedures (standard operating procedures), which are necessary to ensure the traceability, can be very problematic. Strictly speaking, perfect reproduction of accuracy in chemical analysis can only be achieved with the help of very detailed standard operating procedures. Each procedural instruction should be regarded as a fundamental component of the respective analytical procedure and therefore become an integral part of an internationally standardised method.

2.2 Reference materials

A reference material is a substance or an object, for which one or several properties have been sufficiently well determined to enable validation of a measurement system. Its composition should closely match that of the sample under investigation.

There are various types of reference materials, such as e.g. [6]:

- Certified reference material that is supplied and certified by a recognised organisation (in Germany e.g. certified reference materials are available from the *Physikalisch-Technische Bundesanstalt* (PTB) [The National Metrology Institute of Germany]).
- External reference material that is provided from someone other than the user.
- Internal (in-house) reference material that has been produced by the users for their own purposes.

Reference materials can be solid, liquid or gaseous substances. Their chemical and physical properties are important for the accuracy and comparability of analytical results. Likewise composition, homogeneity and stability of the reference materials are of great relevance in chemical analyses. Reference materials are essential for carrying out chemical analyses, as these are mainly based on comparative measurements (relative procedure). In such procedures the measurement system generates a signal, which must then be referenced to a known value (calibration). Reference materials, whose measurement signals are equivalent to exact contents or concentrations, are necessary for this comparison.

Reference materials must be characterised in the following manner:

- Exact specification of the content of the analyte, including the confidence interval.

- Specification of the validated measurement procedure for conducting certification.
- Information for the purpose of metrological traceability.

The manufacturer supplies a certificate that contains information on tested properties, their variations and on the certification procedure with each certified reference material [5, 7, 8, 9]. Reference standards (e.g. gauged weights) [6] or reference materials [3] are preferably used for the validation of test procedures.

Reference materials for workplace air samples are not available in practice. Only reference materials for individual substances or mineral substance mixtures with certified metal contents are commercially available. In the case of the latter it is essential to know which analytical procedure was used to obtain the measurement values. Often, for example, the digestion procedures are not comparable with those recommended for workplace measurements (see “Preliminary Remarks“, Vol. 9, chapter “Sampling and determining aerosols and their chemical components“ 2005). For this reason, the use of reference materials in workplace measurements has only played a minor role to date.

3 Requirements for calibration standards

3.1 Quality of the chemicals for the preparation of calibration solutions

Chemicals to be used as starting materials for the preparation of calibration solutions must fulfil the following requirements:

- Highest possible purity.
- Content information with associated uncertainty.
- Information on type and quantity of contaminants.

The quality (purity, concentration) of the substances used must be taken into consideration when preparing calibration solutions. The following procedure is recommended in this case.

The content information can be written as e.g. ≥ 99%. In this case the user must decide whether the content that deviates from 100% should be taken into consideration when calculating the concentration of the calibration solution prepared from this chemical. The manufacturers guarantee that for contents such as this the proportion of the chemical exceeds 99% in each case. The error in the concentration of the calibration solutions is so small that no correction is necessary. However, a correction of the concentration should be carried out when the content is less than 99%. The procedure must always be described in the documentation of the method. In the case of chemicals, whose content differs markedly from 100% according to the specification, an analysis certificate for the respective batch should be obtained from the manufacturer so that the concentration can be calculated using the actual content. The uncertainty associated with content information is generally not stated by the manufacturer.

Note:

Analysis certificates can be accessed online from some manufacturers when the batch number is provided.

A calibration should always be checked by testing a control sample prepared separately from the calibration solution. If a certified material is used in this investigation then the accuracy of the calibration can also be checked. As certified materials are not available for all analytical investigations, only a limited check of the accuracy can be carried out. In order to at least carry out a plausibility check, a calibration can be checked with an independently prepared reference standard, which is sourced from another preparation if possible.

3.2 Preparation of calibration standards

It should be ensured that the individual solutions and devices have reached the appropriate temperatures, for which the devices used have been calibrated. Deviations can lead to errors in volume measurements. Furthermore, calibration solutions should contain the chemicals used in the preparation of the sample solution in the same concentration or a concentration similar to that of the analytical sample. Preparation of the solutions must avoid contamination.

Calibration standards must be prepared from materials with known analyte content. Such materials can be e.g.:

- Pure substances (e.g. metals, solvents, derivatives).
- Solutions and substance mixtures.

Calibration stock solutions are initially prepared, from which the calibration standards are obtained by dilution. The calibration standards have to be checked for stability under defined storage conditions.

3.3 Labelling and storage of calibration standards

Calibration standards must be stored in suitable containers taking the manufacturer/supplier’s instructions or other specifications into consideration. Calibration stock solutions and calibration standards have to be labelled unambiguously and stored separately from the samples. The label contents must provide information on the type of analyte and its concentration as well as the date of preparation and the period of use. The labelling of standards must also conform to TRGS 201 [10]. Exact instructions for handling calibration stock solutions and calibration standards must be included in the standard operating procedures.

The stability of calibration standards and calibration functions can be determined with control standards that have been independently (separately) prepared. In this case deviations must be within defined limits that have been derived from the calibration curve, control charts or justified by empirical values. It is advisable to also carry out a trend test [11] when checking the storage stability.

3.4 Preparation of dilutions

Calibration solutions that cover the working range of the analytical method are required for quantitative determinations. These are generally prepared by dilution of a stock solution that contains the analyte in a known concentration. In the case of dilution series a differentiation must be made between the stepwise dilution, for which each diluted solution is prepared by dilution of the previous step dilution (serial dilution), and those in which all the dilution steps are prepared directly from the starting (stock) solution (parallel dilution). Vessels and working aids of known accuracy, which may need to be calibrated if necessary, must be used for all working steps.

It is important to minimise errors when preparing stock and calibration solutions. Therefore e.g. balances of sufficient accuracy (0.01 mg) and volumetric vessels of the highest quality (class A) should be used. The pipettes used must be regularly checked [12], whereby the permissible tolerances are stated in ISO 8655 [13] in the case of piston pipettes. Pipettes that do not meet the minimum requirements listed in Table 1 should not be used. The specific data for a pipette can be found in the manufacturer's certificate. Requirements for tolerances for pipettes are stated in ISO 648 [14] and those for volumetric flasks in ISO 1042 [15].

Table 1 Permitted tolerances for pipettes with frequently used volumes [13][1]

Nominal volume	Error limits (±)			
	Accuracy		Precision	
[µL]	[%]	[µL]	[%]	[µL]
2	4	0.08	2	0.04
10	1.2	0.12	0.8	0.08
200	0.8	1.6	0.3	0.6
1000	0.8	8	0.3	3

[1] The acceptable error limits with respect to the nominal volume apply to the entire usable volume of the pipette. This means that e.g. the systematic measurement deviation for a piston pipette with a usable range of 100 to1000 µL for each measured volume is not more than ± 8 µL.

Serial dilution. If calibration solutions are prepared by serial dilution from the stock solution, then a procedure with as little variation as possible and with the greatest accuracy possible should be chosen when planning the dilution steps. The dilution error increases with the number of dilutions as well as with the magnitude of the dilution step. Dilution factors of more than 1:100 per dilution step should not be exceeded. In the case of very large dilutions from the stock solution it must be checked whether the error is less if diluting in one or several steps. Even though the total error of serial dilution is added according to the law of error propagation, this can nevertheless be smaller than in a one-step dilution.

Parallel dilution. Parallel dilution with dilution factors much greater than 1:100 should only be carried out when e.g. interfering reactions due to the substances used or evaporation can be anticipated. In cases such as these, the error in a one-step dilution can be less than the errors caused by such possible interferences.

4 Calibration functions

4.1 Determination of the calibration functions

Prior to the quantitative determination of the content of analytical samples the relationship between the content x (concentration) and the corresponding measurement signal y must be determined. This is carried out using so-called calibration functions $y = f(x)$. For this purpose a number of calibration standards are prepared, which undergo multiple analysis.

When determining calibration functions, the following conditions should be complied with [16]:

- The calibration points should be distributed at equidistant intervals over the working range for the content x.
- The error for the content x must be small in comparison to the error of the measurement signals y.

If the functional relationship is determined in the form of $x = f(y)$, then this is the so-called analytical function, which is inverse to the calibration function. This analytical function is implemented in numerous analytical systems by the evaluation algorithm. A distinction is made between first-, second-, third-degree etc. polynomials in the calibration function, depending on the respective form of the relationship $y = f(x)$. A calibration function (Equation (1)) of the type:

$$y = a + b \cdot x \qquad (1)$$

is described as linear, i.e. a first-degree polynomial. However, if the type of the function (Equation (2)) is:

$$y = a + b \cdot x + c \cdot x^2 + \ldots \qquad (2)$$

then it is described as a higher degree polynomial for the calibration function. Here a, b, c, etc. are the polynomial coefficients. The coefficient a is conventionally also called the absolute term or intercept. In the case of a first-degree polynomial, b is known as the slope (gradient).

The quality of a calibration function can be assessed by the residual variance. In this case the residual variance reduces with the increasing degree of the polynomial, i.e. a greater accuracy of the degree of fit of the calibration function to the calibration points x,y on which it is based is produced. However, it should be pointed out that it is not the highest possible, but only the required degree of the corresponding polynomial that should be used for the determination of a calibration function. The quality of the fit (residual variance) should be equivalent to the quality of the measurement signals (reproducibility variance). Therefore experience has shown that a first- or second-degree polynomial is completely adequate as a calibration function.

Calibration functions can often also be represented by a logarithmic function. This particularly applies, if measurement ranges are very large. In contrast to the calibration functions obtained with Equations (1) and (2), where the absolute error of the measurement signals are largely constant, a constant relative error is found in the measurement range. The calibration function is calculated as follows according to Equation (3):

$$\log y = \log a + b \cdot \log x \tag{3}$$

After taking the corresponding logarithm of the pairs of values (x, y), such a calibration function can also be calculated by means of linear regression. It should be noted that exponentiation can lead asymmetrical confidence intervals arising from the results.

A further significant point to be considered when determining the calibration function is the so-called origin ($x = 0$, $y = 0$), i.e. the coordinate origin. The assumption that the calibration function runs through this point ($0,0$) is only justified, if it can be proven by an appropriate statistical test that the absolute term is not significantly different from 0. For this purpose the quotient from the absolute term and its standard deviation are calculated and compared with a previously stipulated test parameter of the t-distribution [17]. If the determined quotient is less than the test value, then a calibration function according to Equation (4), can be used as follows:

$$y = b \cdot x \tag{4}$$

4.2 Verification of the calibration by means of control charts

Analytical systems can change their response characteristics over the course of time, e.g. through matrix effects, contamination of the working materials and ageing of the separation columns. This leads to systematic deviations or even to a change in the fluctuation range of the analytical signal. In order to recognise the influence of these changes on the analytical results, measurements with reference samples are carried out and recorded on quality control charts. The results of the screening are presented in tabular as well as graphic form. Graphic presentation enables early recognition of significant as well as slowly and continuously developing quality changes. An example of a graphic presentation is the Shewhart control chart shown in Figure 1.

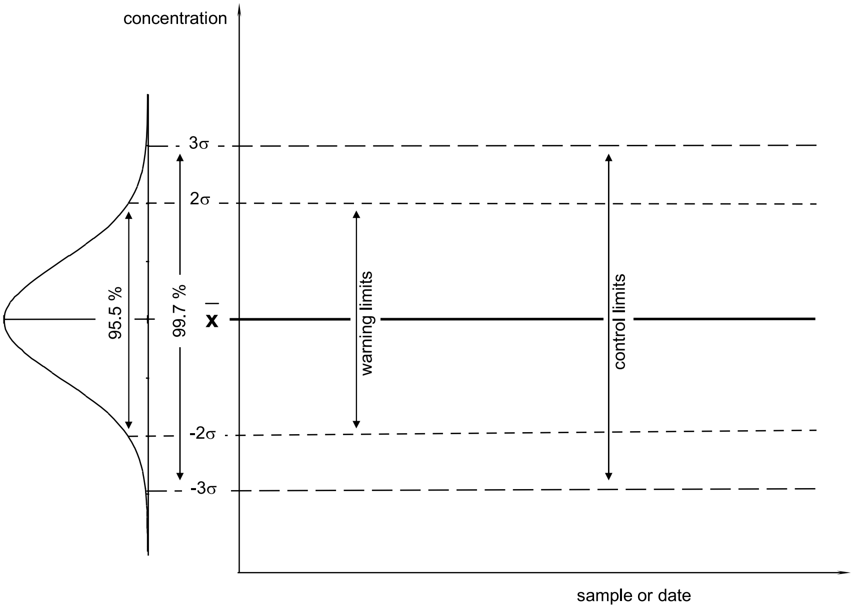

Figure 1 Shewhart chart (see Section 9.3.4 of the 'General Introduction', Chapter 9 'Quality assurance' 2000) with $\bar{x}$ as the average and σ as the standard deviation of the measured values

The use of the Shewhart control chart is based on the assumption that the results of the screening are normally distributed. In order to determine the limits the standard deviation and mean value of the pre-analytical period are calculated. The alert limit, which is allowed to be exceeded once, has a distance of twice the standard deviation from the mean value. The control limit, which when exceeded requires immediate measures to be taken, has a distance of three times the standard deviation from the mean value.

The following 'out-of-control situations', which require immediate action, must be considered:

- A value outside the control limit.
- Two out of three consecutively recorded values outside the action limit.
- Seven consecutively recorded values to one side of the mean value line.

- Seven consecutively recorded values with an ascending trend.
- Seven consecutively recorded values with a descending trend.

If several data points are close to the action limit, then action is required.

The cause for the ‘out-of-control situation’ must be investigated and rectified prior to processing further samples. Only when control samples that fall within alert limits have been recorded can further processing of real samples continue. Further details on establishing, maintaining and evaluating control charts are described in Section 9.3 of the ‘General Introduction’, Chapter 9 ‘Quality assurance’ 2000 of this method collection.

The calibration should be checked prior to each sample series by analysing a control sample. The control sample, which is independently prepared from the calibration solution, contains the substances to be determined using the analytical method or at least individual substances, which are representative for the substance groups to be determined in the method in each case. The concentrations of the weighed-in substances should lie within the working range prescribed by the valid limit or empirical values. If an 'out-of-control situation’ occurs, then the measurement system must be stopped and the cause for the deviation must be searched and eliminated. Renewed release can only occur after the requirements of the control chart have been fulfilled. The scatter of the results of these investigations of the measurement system is included in the determination of the uncertainty.

Maintaining a control chart for the analytical device does not make much sense for rarely performed investigations, as on the one hand the data pool is too small to compile the distribution function and on the other the calibration is often not consistent over the long intervening intervals. In this case the analytical device must be calibrated anew in each case and the calibration must be checked with an independently prepared control sample.

4.3 Blank value correction

The blank value corresponds to the measurement signal (measurement value) of a sample without the analyte to be investigated being present in the sample. It represents the background of a measurement and in most cases contains several components.

Blank value of the analytical device. In many cases an analytical device produces a signal even when no sample is present. This usually very small signal is known as the device background or the blank value of the analytical device.

Method blank value. The measurement value of a blank sample that is subjected to the complete measurement procedure, excluding sampling during the collection phase, and that contains all the reagents described in the method – except the analyte to be determined – is known as a method blank value.

Typical examples of methods influenced by blank values are the determination of diesel engine emissions, aldehydes, inorganic acids, alkaline and alkaline earth hydroxides, cooling lubricants as well as phthalates. The blank values occurring in these cases can arise from a variety of sources, e.g. from the collection medium as well as from reagents, or due to transport or storage conditions. Blank values cause systematic errors and must be subtracted from the sample measurement value before calculating the final result. This is known as blank value correction. The blank value is preferably calculated from multiple determinations.

4.4 Calibration in the presence of matrix effects

The entirety of the concomitant substances in a sample solution is known as the sample matrix, and the influences on the measurement signal arising from this are called the matrix effect. Matrix effects are caused by the presence of associated material in the analytical sample, which become more or less strongly enriched in the sample during the measurement procedure – from sampling to sample preparation, right up to analytical measurement – or can alter the physical properties of the sample. Matrix effects can be of a chemical or physical nature.

Matrix effects can be compensated for by an appropriate course of action during calibration. This includes e.g. the internal standard method and the standard addition method (see Section 6). In this manner e.g. the internal standard method can be used. However, this method works only in the case of linear response characteristics. In this instance the internal standard is a substance, whose behaviour in analytical devices is similar to that of the analyte to be determined.

The intensity ratio between the measurement signal of the analyte and that of the added internal standard is consistently measured. If, for example, ionisation occurs during determination by atomic spectrometry, the internal standard is influenced to a similar extent, so that this results in compensation. Furthermore, different atomisation yields are compensated in the spectral source.

If different matrix effects cannot be compensated from one sample to the next, even through the use of internal standards, then the standard addition method should be applied [18].

4.5 Validity of calibrations

The validity of calibrations can be checked most simply by maintaining mean value control charts (see Section 4.2). However, this procedure is only suitable for analytical systems, which show a signal that is stable over time. This is the case with e.g. photometers and gas chromatographs with flame ionisation detectors. In the case of gas chromatographs with mass spectrometric detection calibration is generally less stable over time than in gas chromatographs with flame ionisation detectors. Therefore, these devices must be calibrated more frequently. Calibration each working day is necessary e.g. for atomic absorption and atomic emission spectrometry.

In order to facilitate decisions on measures to be taken and time intervals that shall apply Document 71 SD 4 027 "*Leitlinien und Beispiele für Überwachungsfristen von Prüf-/Messmitteln für Laboratorien in den Bereichen Gesundheitlicher Verbraucherschutz, Agrarsektor, Chemie und Umwelt sowie Veterinärmedizin und Arzneimittel*" (Guidelines and examples for monitoring test intervals and measurement aids for laboratories in the fields of health consumer protection, agriculture, chemistry and the environment as well as veterinary medicine and pharmaceuticals) issued by the National Accreditation Body for the Federal Republic of Germany [Deutschen Akkreditierungsstelle] [19] can be consulted.

5 Calibration of measuring devices

All measuring devices, including devices for measuring ambient conditions that have a significant influence on the measurement results must be calibrated before being put into operation as stipulated in the requirements for the traceability of the measurement results according to EN 17025 [3]. Measurement results are traced back to the SI units by means of the calibration. The measuring devices include e.g. balances, syringes, piston pipettes and their tips, burettes, dispensers as well as the measurement systems used for checking relative humidity, temperature and atmospheric pressure.

Information on the measuring devices, i.e. on the type of device, manufacturer, serial and inventory number, the certificates and specifications of the manufacturer, the dates of receipt and release as well as an internal identification, with which the respective device is permanently labelled, should be recorded at a central location – e.g. in a database. Measuring devices of the same type are organised in device groups, for which clear rules for carrying out the respective tests are specified. After the initial testing upon receipt, during which compliance with the required specifications is checked, a control chart can be established if necessary. Relevant instructions must be taken into consideration for this purpose [19]. The alert or control limits can be adopted from empirical values, such as the control charts of similar measurement systems. The deviation and scattering determined during the investigations can be used for calculation of the uncertainty of the analytical result.

Balances must be calibrated in an accredited test laboratory at least every two years. The calibration certificate, issued after testing is complete, states the uncertainty at different test loads and the time period until the next test. Furthermore, the balances have to be checked every working day with certified calibration weights and the results recorded in the respective control chart. The calibration weights are checked and certified at an accredited test laboratory at maximum intervals of four years [19].

The conformity of volumetric measuring devices with pistons, such as piston pipettes, direct displacement pipettes, piston burettes, dilutors or dispensers can be gravimetrically traced back to the mass [12]. The standard provides information and specifications for carrying out the test, such as the duration and number of the test cycles, the test liquid, the test volume, the necessary resolution of the balance as well as the metrological conditions during the measurement. In the case of small volumes this test can also be carried out photometrically [20].

Pressure, temperature or relative humidity measuring devices – if they have a significant influence on the measurement result – must be calibrated at regular intervals at an accredited test laboratory. The checked measurement range and the uncertainty are recorded on the calibration certificate issued following the check.

6 Calibration of analytical methods

Adjustments of the sampling systems (pumps) and calibrations of the determination procedures must be carried out for the measurement procedures used for workplace measurements.

The calibration of the measurement procedure has to be adapted to the respective task. Suitable calibration ranges must be specified, taking the sensitivity of the analytical system and the available sampling system as well as normative requirements with regard to the concentration ranges to be monitored into consideration.

6.1 Pump adjustment

In the case of personal sampling the sampling pumps must fulfil the requirements of standard ISO 13137 [21] for flow rates of up to 20 L/min. It is advisable to utilise pumps as described in appendix C of this standard.

- The pump must be regularly checked, at least annually by recording the characteristic curve of the pump.
- Pumps show a run-in behaviour. Therefore it is advisable to let pumps run for a certain time (up to 30 min) until their volume output is constant before use.
- The pumps must be adjusted to the desired flow rate prior to each sampling. The flow rate should be set according to the sampling system used – consisting of the pump, sampling head and collection medium. It must be ensured that the collection medium used for calibration originates from the same manufacturer's batch as the collection media used in the workplace measurements.
- Immediately before sampling, the flow rate of the sampling system used must be determined.
- Immediately after sampling, the flow rate must be checked. Deviations greater than ± 5% with respect to the set flow rate are not acceptable. In this case it is advisable to check the flow rate with the loaded sampling system.
- It is not permissible to use the pumps adjusted for one collection medium for another collection medium under the assumption that the flow rate will be the same. Adjustment must always be performed anew.

The following measurement devices with external calibration can be used for the adjustment of pumps:

- Thermal flow meters (mass flow meters)
- Rotameter flow meters
- Soap bubble flow meters
- Dry-piston flow meters
- Gas meters

It is advisable to calibrate these devices annually.

6.2 Calibration of measurement procedures

Analytical procedures require calibrations for:

- specification of the working range (concentration range) and for
- determination of the characteristics of the method.

The characteristics of the method must be determined according to the normative requirements. In this case the important standards are EN 482 [23], EN 1076 [24], EN 838 [25], EN 13890 [26], EN 13936 [27] as well as DIN 32645 [2].

6.2.1 Determination of the working range

The required minimum measurement range of a measurement method according to EN 482 [23], in which the method should produce unambiguous results, is between a tenth and twice the limit value.

In order to determine the calibration range, the minimum measurement range as well as the sensitivity of the analytical system must be taken into consideration. Taking the sampling time, the flow rate during sampling and the sample preparation steps into account, it is possible to calculate which minimum concentration in the measurement solution must be determined with certainty. The concentration of the lowest calibration standard may not exceed this minimum concentration. The calibration range established in this manner will be henceforth referred to as the working calibration.

Note:

Experience has shown that the calibration range should not be greater than one to two orders of magnitude and consist of at least five calibration points.

6.2.2 Limit of quantification

The limits of detection (LODs) and limits of quantification (LOQs) are important characteristics for the evaluation of an analytical method. The scattering of the measurement values of a calibration experiment can be used to determine them (calibration line method) [2]. The blank value method, which is also described in DIN 32645 [2], using the scattering of the measurement values of a measurement sample of very low concentration, which is analysed under repeatability conditions, is not considered more closely here.

Starting at the lowest calibration concentration, calibration is performed over one order of magnitude, whereby the calibration concentrations are distributed at regular intervals from the lowest up to the highest concentration (equidistant). A total of n = 10 calibration solutions are tested – with duplicate determinations if possible. The evaluation of the calibration data pair concentration *vs.* the measurement signal is carried out according to Section 4.1. The calibration curve used must be linear and free of outliers. Important test methods can be found e.g. in [28, 29]. The LOD is approximately a third of the LOQ.

6.2.3 Characteristics of the method from the working calibration

The following characteristics of the method are determined from the working calibration as specified in Section 6.2.1:

- The standard deviation or coefficient of variation of the method.
- The confidence interval for the analytical results.

Calculation of the standard deviation or coefficient of variation of the method. The measure for the scattering of the measurement values around the line of best fit is the residual standard deviation (see Equations (5) and (6)).

$$s_y = \sqrt{\frac{\sum (y_i - \hat{y}_i)^2}{N - 2}} \tag{5}$$

with

$$\hat{y}_i = a + bx_i \tag{6}$$

where

s_y is the residual standard deviation
y_i are the measurement values of the calibration samples $i = 1 \dots N$
$\hat{y}_i$ is a value calculated from the calibration function for the standard concentration x_i
N is the number of calibration concentrations
a, b are the function coefficients
x_i is the concentration of the calibration samples $i = 1 \dots N$

The standard deviation of the method s_{x0} is calculated from the residual standard deviation as a measure for the scattering of the concentrations around the line of best fit using Equation (7) as follows:

$$s_{x0} = \frac{s_y}{b} \tag{7}$$

Finally, the coefficient of variation of the method V_{x0}, which is a relative measure of the scattering based on the median value of the calibrated concentration range (see Equation (8)), can be calculated. It is a value that is well suited for comparing precisions of different analytical methods with one another.

$$V_{x0} = \frac{s_{x0}}{\bar{x}} \times 100 \tag{8}$$

where

$\bar{x}$ is the mean value of the calibration concentrations

If the distribution of the calibration points is not equidistant when determining the characteristics of the method, then determination of the characteristics of the method is not possible.

6.2.4 Determination of the confidence interval for the analytical results

On the basis of the calibration data the range can be calculated, in which the analytical result $\hat{x}$ lies for a measurement signal $\hat{y}$ with a given statistical certainty. This range is known as a confidence interval.

According to Equations (9) and (10) the result of an investigation is then:

$$\hat{x} \pm VB(\hat{x}) \tag{9}$$

with

$$VB(\hat{x}) = s_{x0} \times t_{f,P} \times \sqrt{\frac{1}{M} + \frac{1}{N} + \frac{(\hat{x} - \bar{x})^2}{\Sigma(x_i - \bar{x})^2}} \tag{10}$$

where

$\hat{x}$	is the concentration of a sample obtained from the analytical function
$VB(\hat{x})$	is the confidence interval for the concentration $\hat{x}$
s_{x0}	is the standard deviation of the method
$t_{f,P}$	is the value in the table of the t-distribution with f = N – 2 and P = 95% (two-sided)
M	is the number of the repeat measurements of a sample

6.3 Calibration procedures

6.3.1 Standard addition

The standard addition procedure is aimed at minimising instrumental errors and matrix effects – above all in the low concentration range [18]. This method is also described as the spike addition method or spiking. This calibration procedure consists of adding a defined amount of the analyte (standard) in pure form to the sample to be investigated and measuring the increase in the analytical signal (at least three different concentrations).

This type of calibration is preferably used, in particular for unknown, complex or frequently changing sample matrices. The prerequisites for using the standard addition method are a linear relationship between the concentration and the measurement signal as well as a homogeneously divisible analytical sample. The sample is divided into aliquots in the standard addition method. One aliquot is measured without addition; different amounts of the analyte (standard) are added to the other aliquots and these are also measured.

Figure 2 shows the graphic evaluation according to the standard addition method. The measurement points of a regression curve are calculated according to Section 4.1. The intersection of this curve with the abscissa is equivalent to the concentration of the non-spiked sample. This intersection is equivalent to the starting concentration of the analyte in the sample (as an amount). The advantage of the standard addition method, i.e. that matrix effects can be corrected, is counterbalanced by an increase in the amount of work required and possible problems in exceeding the linear range.

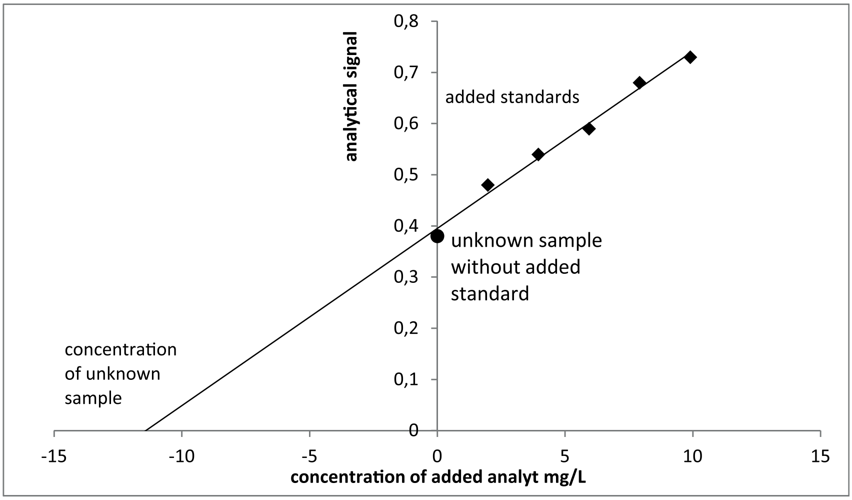

Figure 2 Example of a standard addition with five spiked quantities

Note:

In order to establish whether a matrix effect is present, a single spiking of one aliquot of the sample to be investigated can be carried out. The original sample and the spiked sample are evaluated using an external calibration curve. A deviation between recovered (actual value) and calculated spiking (theoretical value) is an indication of the occurrence of a matrix effect. The standard addition method should be used in the case of a proven matrix effect.

6.3.2 Calibration with external standards

Calibration using the external standard method is frequently used in similar analyses with similar or uniform samples. The reason for the popularity of this calibration procedure lies in its simplicity and, in some steps, its automatic applicability. The composition of the solutions used for calibration should be equivalent to that of the analytical solutions.

As two principally completely independent analytical steps are brought together in the analysis, some requirements must be imposed on the method.

- All the analytical steps must be reproducible.
- The recovery must be known, taken into consideration and may not be subject to any matrix effects.

Note:

In the case of gas chromatographic measurement procedures the use of solvents with low boiling points should be avoided in this calibration type, as errors in volume can occur due to evaporation.

If these conditions are satisfied, the calibration can be carried out by creation of the calibration function as described in Section 4.1. At least five calibration solutions are prepared for this purpose in concentration steps as equidistant as possible over the designated working range of the method and measured in the same manner as the sample solutions. The calibration function is obtained using the method of least squares. The inversion of this function can be used as an analytical function for the calculation of the substance concentrations in the sample solution.

When applying the external standard method, standard solutions must be regularly analysed alongside the sample solutions, in order to control the stability of the measurement system. Control charts have been successfully used in these cases (see Section 4.2).

6.3.3 Calibration with internal standards

The calibration and evaluation according to the internal standard method is used in order to avoid not only systematic errors, such as sample loss during preparation or errors in volume dosing, but also random errors, such as measurement errors through fluctuating device parameters. For this purpose the internal standard must ideally behave like the analyte for the entire analytical run, i.e. it should have properties chemically and physically similar to the analyte. Equation (11) below is proposed for a linear analytical function to enable calibration and evaluation. The signal ratios of the analyte to the internal standard are plotted versus the concentration ratios of the analyte to the internal standard.

$$\frac{S_A}{S_{IS}} = a + b \cdot \frac{c_A}{c_{IS}} \tag{11}$$

where

S_A is the measurement signal of the analyte
S_{IS} is the measurement signal of the internal standard
c_A is the concentration of the analyte
c_{IS} is the concentration of the internal standard

When selecting the internal standard, it must be ruled out that the internal standard is already present as a blank value or becomes enriched – in the same manner as the analyte – during sampling. This can almost always be achieved with isotope-labelled analytes, which are used as internal standards. They are ideal internal standards with regard to the chemical behaviour or the physico-chemical properties of the substance. As the signals of the analytes and of the internal standard are registered simultaneously in the analytical determination, they do not influence one another. The use of isotope-labelled analytes as internal standards is recommended in analytical determination using mass spectrometric detection.

The internal standard should be added as early as possible to the calibration or sample solution. It is advisable to ensure that the concentration of the internal standard in the calibration and sample solutions is the same. The addition of the internal standard when preparing the desorption solution has proved successful. The calibration solution and the control solution are prepared alongside the desorption solution for the maintenance of the control chart. The signal strength of the internal standard should also be recorded in the control chart. This allows timely detection of changes in the analytical system as well as changes in concentration of the internal standard in the desorption solution (e.g. through evaporation of the solution). The concentration of the internal standard should be chosen, so that the anticipated analytical signal is of approximately the same magnitude as that of the internal standard.

In the case of thermal desorption the internal standard is added after conditioning of the adsorption tubes in the laboratory before sampling. For this purpose, e.g. in the case of Tenax TA as an adsorption material, a few microlitres of a methanolic solution of the internal standard are dosed onto the glass wool plug above the sorbent and then nitrogen is passed through the tube for 10 minutes at a flow rate of approx. 50 mL/min to evaporate the methanol. Collection tubes prepared in this manner are used for sampling or calibration. For the purpose of calibration, for example, the solution of the calibration mixture (a few µL) is applied to a prepared tube.

The collection tubes can also be loaded with a test gas.

References

1 Gesetz über das Inverkehrbringen und die Bereitstellung auf dem Markt, ihre Verwendung und Eichung sowie über Fertigpackungen (Mess- und Eichgesetz – MessEG), BGBl. 2013 Part I No. 43, pp. 2723 – 2744), http://www.gesetze-im-internet.de/bundesrecht/messeg/gesamt.pdf

2 DIN 32645 (2008) Chemical analysis – Decision limit, detection limit and determination limit under repeatability conditions. Beuth Verlag, Berlin, Germany

3 ISO/IEC 17025 (2005) General requirements for the competence of testing and calibration laboratories. Beuth Verlag, Berlin, Germany

4 Verordnung zum Schutz vor Gefahrstoffen (Gefahrstoffverordnung – GefStoffV) dated 26th November 2010 (BGBl. I p. 1643) revised by article 2 of the law dated 28th July 2011 (BGBl. I p. 1622) by article 2 of the ordinance dated 24th April 2013 (BGBl. I p. 944), article 2 of the ordinance dated 15th July 2013 (BGBl. I p. 2514) and article 2 of the ordinance dated 3rd February 2015 (BGBl. I p. 49), http://www.baua.de/de/Themen-von-A-Z/Gefahrstoffe/Rechtstexte/pdf/Gefahrstoffverordnung.pdf?__blob=publicationFile&v=14

5 ISO/WD Guide 35 (2006) Reference materials – General and statistical principles for certification. Beuth Verlag, Berlin, Germany

6 ISO 11095 (2008) Linear calibration using reference materials. Beuth Verlag, Berlin

7 ISO/CD Guide 31 (2000) Reference materials – Contents of certificates and accompanying documentation. Beuth Verlag, Berlin, Germany

8 ISO Guide 32 (1997) Calibration in analytical chemistry and use of certified reference materials. Beuth Verlag, Berlin, Germany

9 ISO Guide 33 (2000) Use of certified reference materials. Beuth Verlag, Berlin, Germany

10 TRGS 201 (2010) Einstufung und Kennzeichnung bei Tätigkeiten mit Gefahrstoffen: GMBl 2011 No. 42/43, p 855 (24th November 2011) http://www.baua.de/de/Themen-von-A-Z/Gefahrstoffe/TRGS/pdf/TRGS-201.pdf?__blob=publicationFile&v=4

11 Gottschalk G (1980) Auswertung quantitativer Analysenergebnisse. In ANALYTIKER Taschenbuch Vol. 1 Kienitz H, Bock R, Fresenius W, Huber W, Tölg G (eds.) Springer-Verlag Berlin, Heidelberg, New York, pp 63–99

12 ISO 8655-6 (2002) Piston-operated volumetric apparatus – Part 6: Gravimetric methods for the determination of measurement error. Beuth Verlag, Berlin, Germany

13 ISO 8655-2 (2002) Piston-operated volumetric apparatus – Part 2: Piston pipettes. Beuth Verlag, Berlin, Germany

14 ISO 648 (2009) Laboratory glassware – Single-volume pipettes. Beuth Verlag, Berlin, Germany

15 ISO 1042 (1999) Laboratory glassware – One-mark volumetric flasks. Beuth Verlag, Berlin, Germany

16 Doerffel K, Eckschlager K (1981) Optimale Strategien in der Analytik. Deutscher Verlag für Grundstoffindustrie, Leipzig, Germany

17 Youden WJ (1947) Technique for Testing the Accuracy of Analytical Data. *Anal. Chem.* 19 (12), pp 946-950

18 DIN 32633 (2013) Chemical analysis – Methods of Standard addition – Procedure, evaluation with CD-ROM. Beuth Verlag, Berlin, Germany

19 Deutsche Akkreditierungsstelle (DAkkS) Leitlinien und Beispiele für Überwachungsfristen von Prüf-/Messmitteln für Laboratorien in den Bereichen Gesundheitlicher Verbraucherschutz, Agrarsektor, Chemie und Umwelt sowie Veterinärmedizin und Arzneimittel, Document 71 SD 4 027,
www.dakks.de

20 ISO 8655-7 (2005) Piston-operated volumetric apparatus – Part 7: Non-gravimetric methods for the assessment of equipment performance. Beuth Verlag, Berlin, Germany

21 EN 13137 (2014) Workplace atmospheres – Pumps for personal sampling of chemical and biological agents – Requirements and test methods. Beuth Verlag, Berlin, Germany

22 Physikalisch Technische Bundesanstalt (2007) Das internationale Einheitensystem. PTB-Mitteilungen 117 Part 2, Braunschweig, Germany

23 EN 482 (2012) Workplace atmospheres – General requirements for the performance of procedures for the measurement of chemical agents. Beuth Verlag, Berlin, Germany

24 EN 1076 (2010) Workplace exposure – Procedures for measuring gases and vapours using pumped samplers – Requirements and test methods. Beuth Verlag, Berlin, Germany

25 EN 838 (2010) Workplace exposure – Procedures for measuring gases and vapours using diffusive samplers – Requirements and test methods. Beuth Verlag, Berlin, Germany

26 EN 13890 (2010) Workplace exposure – Procedures for measuring metals and metalloids in airborne particles – Requirements and test methods. Beuth Verlag, Berlin, Germany

27 EN 13936 (2014) Workplace exposure – Procedures for measuring a chemical agent present as a mixture of airborne particles and vapour – Requirements and test methods. Beuth Verlag, Berlin, Germany

28 Funk W, Dammann V, Donnevert G (2005) Qualitätssicherung in der Analytischen Chemie. Wiley-VCH, Weinheim, Germany

29 Doerffel K (1984) Statistik in der analytischen Chemie. Verlag Chemie, Weinheim, Germany; Deerfield Beach, USA; Basel, Switzerland

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