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Evaluation of the linear calibration in terms of uncertainties

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ABSTRACT

A method is presented for the assessment of the calibration line in analytical chemistry. It is based on a combination of a chemometric data interpretation and a calculation of the minimum standard deviation (STDEV) of experiments. The more favourable concentration range of linear calibration could be obtained by iteration. This condition links the lower limit of analysis (LLA) to an upper limit of analysis (ULA), and thus completes the statistically appropriate extension of the calibration line. In addition, a minimum STDEV of measurement was expressed in terms of STDEV's on slope (S_a) and on intercept (S_b) and the calculation was performed by using a modified version of the law of propagation of errors (LPE). The method was validated on experiments of gas chromatography (GC), liquid chromatography (LC), electrochemistry, flow-injection analysis (FIA), atomic emission spectrometry (AES), flame atomic absorption spectrometry (FAAS) and inductively-coupled-plasma mass-spectrometry (ICP-MS). The comparison of theory with experiments suggests that an appropriate concentration range of calibration was obtained by iteration when the intercept decreased to a value below a factor of two of the corresponding standard deviation. The proposed method is uncomplicated and facilitates the determination of the concentration range of calibration and the LLA in one working operation. © 2008 Trade Science Inc. - INDIA

KEYWORDS

Quality assurance;
Measurement uncertainty;
Limit of detection;
Atomic absorption;
Calibration.

INTRODUCTION

Although non-linear methods are available^[1-2], calibration of analytical instruments is most frequently performed, as to establish a concentration range of linear response^[3-6]. One of the reasons for the preference to use a straight line is related to the calculation of uncertainties, which amplifies in complexity if non-linear equations were applied^[1]. However, non-linear methods and the laborious calculations of uncertainties may be facilitated by the use of appropriate spreadsheets^[2] that are accessible in any analytical laboratory. In experiments

where the results need correction for the presence of interferences, it is important to experimentally establish the linear range of calibration for every single component of the matrix. Otherwise, the calculations of adjustments to concentrations of unknowns for the presence of interferences become mathematically complex.

Several methods of linear calibrations have been promoted by international organisations such as IUPAC^[7], ISO^[8], BIPM^[9] and EURACHEM/CITAC^[10]. The methods were formulated by the equation of a straight line added to an error function that could be determined experimentally^[7,11,12]. The calculations were

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performed on the basis of a straight line, and the predicted uncertainties of concentrations were evaluated in terms of the LPE^[3,13,14]. In order to isolate the concentration value in the equation of a straight line, the resulting equation was earlier denoted as the inverse equation^[9,15-16]. By applying the LPE to the inverse equation, it was shown that an equation could be obtained^[3], which complies with the equation of uncertainties of the Guide to Uncertainties in Measurements (GUM)^[17]. The parameters of the straight line and the STDEV thus obtained on responses of blanks(s) were required for a calculation of the LOD^[5]. However, the standard deviation on the slope of the calibration line (S_{α}) and the standard deviation on the intercept (S_{β}) were not included in the calculation of the LOD but relied entirely on the average value of blanks, A_0 ^[5]:

$$\text{LOD}(3s) = \frac{A_0 + 3 \cdot s_{A_0}}{\alpha} \quad (1)$$

The problem of omitting the standard deviation on slope and intercept in the calculation of the LOD is illustrated in figure 1. The two lines of figure 1 are apparently different because both slopes and intercepts appear to be different? However, by taking into account the uncertainties on slopes and intercepts, the lines are equal within error. The calibration by analytes^[5], may display working curves of high-precision(\times), figure 1) but a larger spread of data(\circ), figure 1) is most frequently encountered by analysing analytes in a matrix or by using, e.g., an injection accessory attached to the detection unit. In the tentative experiment of figure

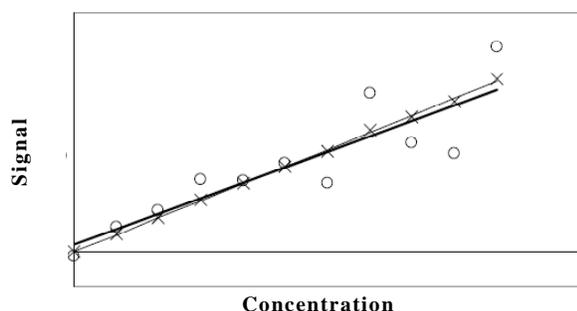


Figure 1 : Two tentative experiments that exhibited the same limits of detection(LOD(3s)), according to the classical definition. The spread of data may be introduced by matrix effects or by sample-injection accessories. By taking into account the general level of uncertainty, the two calibration lines were equal.

1, the noise level of the instrument remains approx. unaltered if an accessory of any type were applied. According to the definition(eq. 1), the LOD of the two experiments(figure 1) would also be almost the same, which is not reasonable a priori because the low precision results exhibit such a large spread of data. Since the LOD signifies a lower limit, it is rarely accessible to analysis, particularly in the case where a large spread of data prevails(\circ), figure 1). A more practical limit could therefore be considered, which takes into account all accessible standard deviations thus providing a practical lower concentration that could always be monitored at acceptable precision. This problem was addressed in the ISO11843-2 standard^[8] where weighted regressions was considered and by Huber^[18] who suggested that the limit of determination is an unnecessary concept, when the spread of data were included in the assessment of the LOD. In most contemporary works of analytical chemistry, a calibration range of linear response was reported but no reference was made to the method of determination, as a rule. This observation indicates that there is a demand for an uncomplicated method of data analysis. The concentration of maximum linear response and the LOD(eq. 1) are important, and both concepts need consideration because the full range of calibration is required for an estimate of the concentration range of minimum uncertainty. The calibration may be improved by including correlation terms, which slightly reduces the uncertainty but the procedure is not straightforward^[19,20].

A method was therefore developed, which enables the user to determine the full range of calibration, from a low concentration(LLA) and up to a limit(ULA), where the deviation of the experimental values deviate significantly from the position of the calibration line. The LLA and the ULA are determined by iteration. The concentrations were thus estimated by using the inverse relation^[9,15,16] and the corresponding standard deviations were calculated by the LPE^[3], excluding correlation terms^[19,20]. It was therefore suggested that the response of the blank subtracted from all measurements resulted in an intercept that ought to be close to zero or lower than the concentration of the corresponding LLA. The method simplified data analysis and it was based entirely on the standard deviations of measurements, and it was verified on experiments of a wide range of ex-

perimental technologies^[21-25].

EXPERIMENTAL

Chemicals

The standards were prepared by dissolution of Sr(NO₃)₂ (Merck p.a.), Ba(NO₃)₂ (Merck p.a.) and Fe₂(SO₄)₃ (Merck p.a.) in Millipore water (Resistivity 18 MΩ).

Apparatus

The measurements of Sr²⁺, Ba²⁺ and Fe³⁺ were performed by using three different modes of an atomic absorption spectrophotometer (Perkin Elmer M2100). Strontium was measured in the absorbance mode at a wavelength of 460.7 nm, slit width 0.2, air flow of 8.0 mL/min and an acetylene flow of 2.8 mL/min. Barium was monitored in the emission mode using a flame of nitrous oxide and acetylene and a modifier of 0.2% KCl where the element was detected at 553.6 nm.

Iron was measured in the electrothermal AAS (ETAAS) mode at 248.3 nm using a hollow cathode lamp (S. & J. Juniper, UK) operated at 30 mA and slit 0.2 nm. Aliquots of 20 μL were applied to the graphite furnace and the temperature programme of measurement included steps of preheating at 70°C for 10 seconds, drying at 130°C for 10 seconds and 180°C for 20 seconds, pyrolysis at 1400°C for 20 seconds cooling to 20°C for 15 seconds and atomisation at 2400°C for 5 seconds and, finally, cleaning the tube at 2600°C for 3 seconds. The flow rate of argon was maintained at 300 mL/min, and a modifier of 0.05 M Mg(NO₃)₂ was applied. Great care was exerted, as to ensure homogeneity and repeatability of all measurements by using the same settings throughout.

Strontium was also determined by ICP-MS (Perkin-Elmer, Sciex, ELAN 5000) equipped with a cross-flow nebulizer. The samples were aspirated at a flow rate of 1.1 mL/min and strontium was analysed by using the more abundant isotope of ⁸⁸Sr. The argon flow rate was 0.8 L/min and the auxiliary argon gas was supplied at a rate of 10 L/min.

RESULTS AND DISCUSSION

Measurement uncertainty

In the uncertainty budget, the standard deviation of an unknown may be estimated by adding contributions of standard deviations of chemicals, glassware, temperature etc., which may be determined separately before measurement^[17]. The calibration line may also be used to estimate the standard deviation before analysis and, thus, the prediction of the calibration line belongs to the uncertainty budget. Accordingly, it becomes possible to predict the standard deviation on a single determination of an unknown^[17].

If the average concentration of an unknown (\bar{C}) were determined by a number of repetitions (N) that were calculated by the working curve, then the experimental standard deviation (S'_c) is found by:

$$s'_c = \sqrt{\frac{\sum_i (c_i - \bar{c})^2}{N-1}} \quad (2)$$

The result may be compared to the predictions of the uncertainty budget^[10,13,14]. However, the uncertainty on a single measurement of an unknown cannot be determined by eq. 2 but may be predicted from the uncertainty budget that is treated in further detail in the following. The corresponding standard deviation (S_A) on the average-response values of the unknown (\bar{A}) belongs to the uncertainty budget:

$$s_A = \sqrt{\frac{\sum_i (A_i - \bar{A})^2}{N-1}} \quad (3)$$

The response of a straight line (A) and concentration (C) may be characterised by the linear equation of slope (α) and intercept (β), which provides the concentration through the inverse relation^[9,5-16]:

$$c = \frac{A - \beta}{\alpha} \quad (4)$$

The uncertainty (S_c) on the concentration of an unknown may thus be estimated by applying to eq. 4 the LPE^[3,10,17,19,20]. However, the STDEV of the unknown is generally overestimated by adding up all the contributions of the LPE of a straight line. It is unlikely that all the uncertainties involved in the LPE simultaneously add to the total uncertainty. It is more likely that some contributions add to the total uncertainty and other contributions diminish the total uncertainty. In analogy to the

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definitions of eqs. 2 and 3, the STDEV of the unknown may therefore be calculated by an average of the collected contributions. It is therefore proposed that the uncertainty of chemical experiments is calculated by the LPE averaged by the number of terms that appear in the final expression with the subtraction of unity, that is, in the case of a straight line; $(N_{\text{terms}} - 1) = 2$:

$$s_c = \sqrt{\frac{\left(\frac{\partial c}{\partial A} s_A\right)^2 + \left(\frac{\partial c}{\partial \beta} s_\beta\right)^2 + \left(\frac{\partial c}{\partial \alpha} s_\alpha\right)^2}{2}} \quad (5)$$

In the conventional version of the LPE, the uncertainty becomes overestimated because all contributions add up, which only represent one out of eight possible combinations. Since the LPE contains the uncertainty contributions squared, the only viable method, as to correct for the possible combinations of positive and negative contributions to the total uncertainty, is by averaging. Equation 5 may be evaluated and simplified, which provides an expression of the absolute-standard deviation of an unknown, and it depends on concentration within the linear range of responses^[3,10]:

$$s_c = \frac{1}{\alpha} \cdot \sqrt{\frac{s_A^2 + s_\beta^2 + (\bar{c} \cdot s_\alpha)^2}{2}} \quad (6)$$

The equation (eq. 6) shows, contrary to eq. 2, that a STDEV is defined for a single measurement of the unknown, i.e., when $S_A = 0$. The calculation of S_c is more conveniently calculated by eq. 6, which contains information on the standard deviations of the working curve. In earlier publications, the significance of S_A (eq. 6) was not discussed in greater detail^[3,10]. It does depend on concentration however, and the precise relation may differ between analytical technologies. Division of eq. 6 by concentration provides the RSD:

$$\frac{s_c}{c} = \frac{1}{\alpha \cdot c} \cdot \sqrt{\frac{s_A^2 + s_\beta^2 + (\bar{c} \cdot s_\alpha)^2}{2}} \quad (7)$$

In eqs. 5-7 the number of measurements of the calibration line and the number of repetitions enter the equations by the calculation of S_A , S_β , S_α . Similarly, the standard deviation on the signal, S_A , contains the number of repetitions according to eq. 3. Equation 7 was derived, by definition, on the assumption of zero uncertainty on standards; this condition was most frequently fulfilled

because standards were prepared by dilution of high-purity stock solutions, with RSD's of dilution less than 0.5%. However, if many standards and less accurate means of dilution were applied to the calibration, the STDEV on concentration of standards might add considerably to the overall STDEV of measurement (eq. 6).

Limits of analysis

Since the STDEV depends on concentration^[11,17,26], it is worthwhile to consider the value at the extremes. The STDEV of eqs. 6,7 may be compared to the measurement error of the IUPAC convention^[7] with the exception, however, that the STDEV needs to be converted to the corresponding confidence levels, which relates the distribution of STDEV's to the normal distribution. In eqs. 6,7, it may be noted that the RSD approaches the RSD on the slope, at very high concentrations, that is, it becomes independent of the STDEV on the intercept, S_β :

$$\frac{s_c}{c} \approx \frac{s_\alpha}{\alpha \cdot \sqrt{2}} \text{ when } \text{ULA} > c \gg \frac{\sqrt{s_{A_0}^2 + s_\beta^2}}{s_\alpha} \quad (8)$$

which expresses that at very high concentrations, the RSD becomes constant, and thus independent of the concentration (c), which occurs when the concentration is close to the ULA. The RSD at very low concentrations approaches the rational function (eq. 9):

$$\frac{s_c}{c} \rightarrow \frac{1}{\alpha \cdot c} \cdot \sqrt{\frac{s_A^2 + s_\beta^2}{2}} \rightarrow \infty \text{ when } c \rightarrow 0 \quad (9)$$

Thus, the influence of the standard deviation on the slope, S_α , diminishes at low concentrations, which seems reasonable because the slope is not properly defined within the noise level and below the LLA of the measurement. Further, according to eq. 9, the RSD on a measurement of an unknown approaches infinity, as the concentration approaches zero. The determination of A_0 (eq. 1) may result in small negative values, which leads to a lower value of the LLA, as compared to the value obtained by only considering the term that contains the standard deviation ($3 \cdot S_c$). This is another feature of the conventional LOD of eq. 1, which is inconvenient. It may thus be proposed that an LLA based entirely on standard deviations would be the more comprehensible approach.

Minimum accessible concentration

In reality, all calibration lines are approximations to a curve that is not linear within the full range of concentrations^[27]. This non-linear behaviour is observed in most analytical methods such as electrochemistry, atomic absorption spectrometry, mass spectrometry and UV-VIS spectrometry. Therefore, the samples must be diluted, as to enter the linear range of responses that provides the minimum intercept. These considerations about the intercept lead to the determination of an appropriate value of the LLA.

At zero concentration, the signal reaches a constant value that according to eq. 6 leads to a minimum STDEV on concentration:

$$s_{c_0} = \frac{1}{\alpha} \cdot \sqrt{\frac{s_{A_0}^2 + s_{\beta}^2}{2}} \quad \text{at } c = 0 \quad (10)$$

This equation (eq. 10) corresponds to eq. 1 with the difference; however, that eq. 10 contains information on the actual appearance of the calibration results (figure 1) including both information on blanks (S_{A_0}) and on the spread of data by the parameter S_{β} . Thus, eq. 10 represents a minimum accessible concentration; whereas the conventional LOD of eq. 1 corresponds to a limit that is defined by the instrument noise level and by the slope. In contrary to the conventional LOD (eq. 1), the RSD on the concentration (eq. 8) cannot reach values below the RSD on the slope. The values of S_{α} and S_{β} may be calculated by using a spreadsheet and the value of S_{A_0} is calculated by using eq. 3. At zero concentration, the values of S_{A_0} and S_{β} are not equal because S_{A_0} is determined by a number of blanks while S_{β} originates from the distribution of data around the calibration line. Accordingly, the LLA of the present method always exceeds the value of the conventional LOD.

Correspondence with empirical relation

Experiments have earlier shown that standard deviations of unknowns follow a general trend. This trend corresponds to the empirical relation of the Eurachem Guide^[10], where the absolute standard deviation obeys the equation:

$$s_c \approx \sqrt{\kappa_1^2 + \kappa_2^2 \cdot c^2} \quad (11)$$

which is approximately linear at small concentrations (κ_1 and κ_2 are constants). In addition, it was noted that the

empirical relation is identical to the results of the present method (eq. 7) if the dependence on S_A were constant:

$$\kappa_1^2 = \frac{s_A^2 + s_{\beta}^2}{2 \cdot \alpha^2} \quad \text{and} \quad \kappa_2^2 = \frac{s_{\alpha}^2}{2 \cdot \alpha^2} \quad (12)$$

The results of eq. 12 thus strongly indicate that correlations were not usually identified, that is, a tentative correlation term of eq. 12 was less significant and therefore not recognised in the empirical relation of eq. 11^[10]. Accordingly, the LPE (eq. 5) seems to provide a straightforward explanation of the expression given by the empirical equation (eq. 11). However, the results of eq. 12 are only valid if the value of S_A is very small, if it depends weakly on concentration or if it were proportional to the concentration squared. The details of this potential dependence thus need further attention but much data of several methods is required for a reliable estimate.

Validation

In order to demonstrate the versatility of the method, it was tested on a number of different calibrations and on data published earlier^[21-25]. Before the data were subjected to the analysis, four steps of calibration were observed:

The calibration line extends from the concentration of the LLA (eq. 10) and up to concentrations (ULA) where the data differ significantly from the straight line.

If the blank of the calibration standards were displaying a signal, it constituted a background value that should subsequently be subtracted from all responses measured. Some instruments, e.g. UV-VIS spectrometers, were zeroed before measurement. Other instruments, such as potentiometers and mass-spectrometers, provide an electrochemical potential difference of zero concentration or a count number of zero concentration, respectively, that may be subtracted from all standards and samples. Subtraction of a background value from the standards may accidentally lead to negative values but this frequently indicates that the concentration of the analyte falls below the LLA^[17]. Thus, if the instrument were operated with standards below the LLA of the method, then both negative and positive values of the responses were expected to occur.

The STDEV on the intercept of the calibration line is a key parameter of the present method. The calibra-

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tion line was determined satisfactorily when the intercept, as obtained by a stepwise elimination of high concentration values, declined below the STDEV on the intercept multiplied by a factor of x , that is:

$$\beta < x \cdot s_{\beta} \quad (13)$$

at the optimum straight line (x : whole number). The choice of ' x ' controls the extension of the calibration line. An x -value of unity results in many data being eliminated from the data set and a narrow concentration range of calibration but yields low standard deviations. Conversely, x -values larger than 1 provide a wide calibration range and higher standard deviations.

No data outside the calibration range can be discarded during the measurements. All data must be retained before entering the statistical evaluation.

In the theoretical section (above) was only considered the example of STDEV's of a straight line. Any trend line other than the linear relation would result in a magnitude of the deviation that differed from the value given by eq. 6. Thus, in a first approximation, the magnitude of the STDEV of experimental data that belongs to a straight line must follow the prediction of eq. 6. The distribution of data around the straight line should, for a given concentration, follow a normal distribution, and if some systematic unusual tendency were observed in the distribution of data, then these data could be identified in a depiction of the theoretical STDEV (eqs. 6 and 7) vs. observed deviations. In figure 2 is shown an ideal distribution of data according to such a depiction, which represents a 2D-normal distribution of data where the majority of data was located within a circle of radius of one STDEV. Unless a high number of data were applied to the analysis, the distribution of data did not follow the distribution of figure 2, and it would be difficult to identify a high density of data around the centre of the chart. Deviations that exhibit larger values than those of eqs. 6 and 7 may be identified by the formation of additional groupings in depictions of this type (figure 2). These data were subsequently removed from the data set and a re-calculation of a new straight line with a corresponding distribution of deviations followed. This iterative approach was repeated until the characteristic circular pattern of figure 2 was obtained. However, it may be difficult to estimate by a visual inspection if the data should be retained or removed from

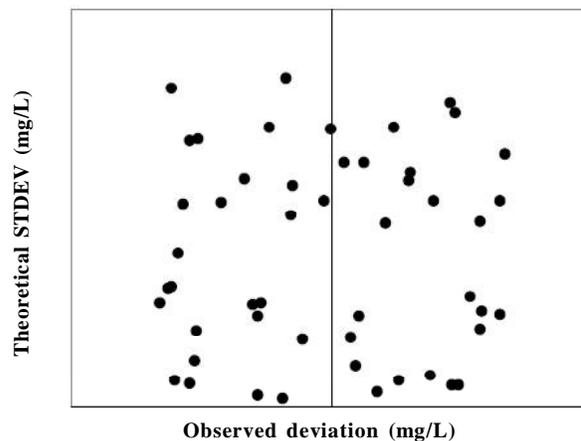


Figure 2 : A depiction of the STDEV of a straight line (eqs. 6 and 7) as a function of the observed deviations from the straight line results in a circular pattern of deviations without groupings. Any deviation from such a grouping signifies that the corresponding data do not belong to the straight line. A homogenous circular distribution of data means that the predicted and observed deviations are uncorrelated.

the data set for the subsequent iteration. Therefore, the additional request of eq. 13 was introduced. The intercept of the optimum straight line must be less than the corresponding value of the STDEV multiplied by a factor of ' x ' on the intercept ($x \cdot S_{\beta}$) (TABLE 1).

1. Analysis of barium by AES

In figure 3a is demonstrated the principle of straight-line analysis where barium was analysed by AES. The calibration line of this technique frequently produces a response that is non-linear. A planar distribution of deviations was obtained by calculating the standard deviation of a straight line (eqs. 6 and 7) and depicting it vs. the observed deviation of the data from the same straight line, as shown in figure 3a (right-hand side). If the data were displaying a pattern or a grouping, then some of data might not belong to the model. In figure 3a however, the deviations are evenly distributed around a centre value and some of the points that deviate from the centre belong to points within the calibration range of figure 3a. Only data outside the calibration range can be discarded. This implication must be added to the condition of the ratio between the intercept and slope being lower than $x \cdot S_{\beta}$ (eq. 13). This condition ensures that the intercept equals zero within experimental error. The theoretical STDEV's correspond well to the ob-

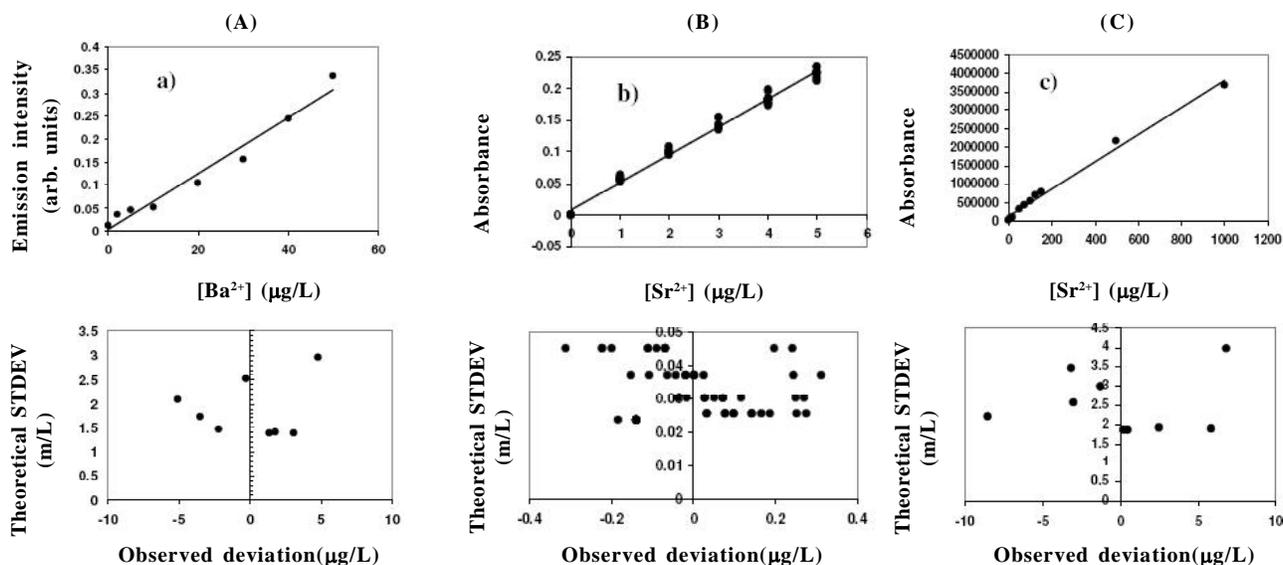


Figure 3 : Calibration results of analysis by three methods of analysis. Initial state before elimination of data with the corresponding 2D-plot of deviations depicted to the right-hand side. (a) Intensity of emission of Ba depicted as a function of concentration. An approximate linear response was observed. No data eliminated (TABLE 1) (b) Determination of the calibration results of strontium measurements obtained by FAAS. Data above 2mg/L eliminated, according to the condition of eq. 13 with $x=2$. (c) Calibration results of strontium measurements that were performed by ICP-MS. The weak curvature of the calibration line was not significant, and all data were retained (TABLE 1).

served deviations (figure 3a, eq. 6) within the calibration range. In TABLE 1 is shown that the ratio between intercept and slope was lower than the corresponding $1 \cdot S_{\beta} / \alpha$, which is a confirmation of the correctness of the calibration line of figure 3a without eliminating any data. Thus, in this particular series of experiment, the intercept was smaller than the STDEV within $1 \cdot S_{\beta}$. The calibration range could extend even further than indicated by the results of figure 3a and of TABLE 1 but these data were not reported in this particular example. Thus, the concentrations of calibration extends from 2 $\mu\text{g/L}$ (at the LLA) and up to 50 $\mu\text{g/L}$ (at the ULA), as shown in TABLE 1. The minimum RSD was calculated as 11% (TABLE 1). Thus, under no circumstances, an RSD less than 11% on a measurement of an unknown could be obtained by this particular experiment.

2. Analysis of strontium by FAAS

In a second experiment, the concentration of strontium ions were determined by FAAS, and the distribution was investigated of spread of data around the calibration line. As opposed to the barium calibrations of figure 3a, repetitions were included, as shown in figure 3b. The general trend of the response vs. concentration is linear and the distance of data from the working curve

increases as a function of concentration. Since a large number of measurements were performed in this particular experiment, the distribution of deviations (figure 3b) follows the expected almost circular pattern (figure 2). The distribution of deviations shows that all data belong to the same group and the plot implies that no data should be removed from the data set. However, the application of the condition given by eq. 13 results in the removal of data above 2mg/L, as shown in TABLE 1. The data were removed iteratively by first eliminating the data of 5mg/L and subsequently re-calculating a new calibration line, and continuing until the condition of eq. 13 was fulfilled. Thus, the linear range of figure 3b was reduced from the initial 5mg/L to only 2 mg/L with $x=2$, which is a result of a slight curvature of the original data and the spread of data around the line. If a value of $x=1$ was chosen as the condition for stop of iteration, the calibration range narrowed to 1 mg/L and the LLA would be lower (TABLE 1).

3. Analysis of strontium by ICP-MS

In a third series of experiments, calibration data of strontium was obtained by ICP-MS where repetitions were omitted, however (figure 3c, TABLE 1) a significant curvature of the data was observed. The pattern

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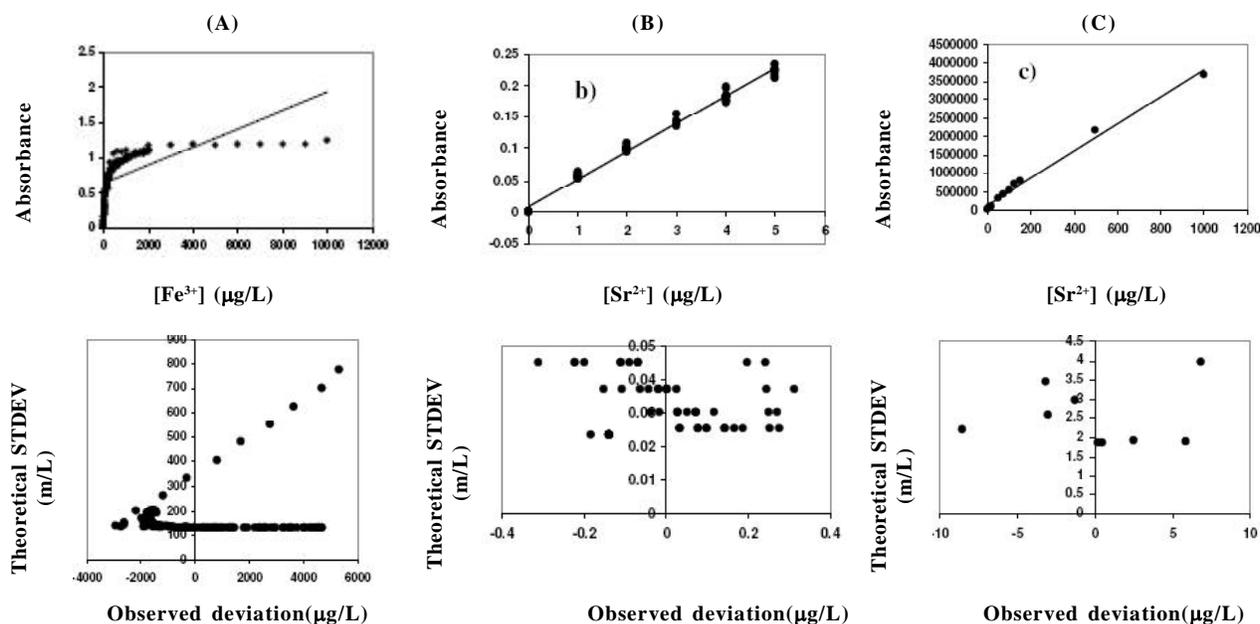


Figure 4 : Determination of the calibration line in experiments of determination of iron by ETAAS. A large number of measurements were performed under equal conditions and no outliers were eliminated. (a) The first iteration shows a lack of compliance to stipulated conditions and experiments deviate significantly from linearity. (b) The second iteration also exhibits a poor correspondence with linearity and a grouping of data is seen in the lower part of the plot of STDDEV's. (c) The third iteration provides the final calibration line of experiments with an almost circular distribution of STDDEV's and all conditions fulfilled (TABLE 1).

of STDEV's in figure 3c correspondingly exhibits a random distribution, half circular, where no outliers were detected. Most likely, the calibration line of Sr-determination by ICP-MS does extend further than the calibration range of 28-500 µg/L (TABLE 1) with the condition of $3 \cdot S_{\beta}$ but in the present series of experiments no data were available that could prove such an expectation. In this example, the condition of $1 \cdot S_{\beta}$ or $3 \cdot S_{\beta}$, i.e. $x=1$ or $x=3$ (eq. 13), were the only options for determination of the calibration range, owing to the limited number of data available to the analysis. The present method merely comments on the data obtained by an actual experiment and it does not impose any model on the data other than a linear relation. According to this particular calibration, the apparent curvature was insignificant for linear calibrations, as evidenced by the results of figure 3 and of TABLE 1.

4. Analysis of iron by ETAAS

In a fourth series of experiments, iron species were determined by ETAAS over a wide range of concentrations and applying many repetitions within the range of concentrations where a linear response was ex-

pected. In order to demonstrate the lack of dependence on concentration at concentrations above the detectors threshold value, additional data were measured at high concentrations (figure 4a). In the first iteration of fitting a straight line to the data of figure 4a, a rather poor correspondence was obtained, as identified by a high value of the LLA (TABLE 1) and the obvious grouping of data observed in the STDEV plot of figure 4a. In addition, the value of S_{β} is smaller than the value of β (TABLE 1). By discarding the significant outliers that belong to the upper-half area of the STDEV plot of figure 4a, the second iteration provides a new plot with yet another grouping of data, as shown in figure 4b. After this second iteration, the data still do not fit to a linear response, as identified by the parameters of TABLE 1 and the grouping of data in figure 4b. According to the plot of STDEV's (figure 4b), the more natural choice is to eliminate the data in the upper-half area at standard deviations higher than 20 µg/L. The following step finalises the iterations and the resulting calibration line fulfils all the required conditions, as shown in figure 4c and in TABLE 1. Thus, this particular example demonstrates that, although the calibration con-

tained a large number of data, only a very limited number of iterations were required, as to obtain the statistically correct calibration range of concentrations. The present method was not applicable to the identification of outliers within the calibration range because all data were maintained within the linear range of calibration. The method merely identified the concentration where the data started to deviate significantly from linearity at high concentrations.

5. Analysis of literature investigations

A detailed analysis of calibration results in novel procedures of analytical chemistry are rarely printed in contemporary literature because they are considered to be of minor interest to the reader. However, when the condition for determination of the calibration range is not copied in the text, it may, in some cases, seem established simply from a matter of judgement not anchored in statistics. Janos et al.^[25] comments on these problems and attempts several approaches to the cali-

bration problem. Their results were based on calibrations of a low number of measurements, which was also true for a number of other publications where calibration results were carefully reproduced and discussed^[21-25]. By using the data of the literature^[21-25], the method outlined above could be validated on various technologies including, gas chromatography(GC), liquid chromatography(LC), electrochemistry, flow-injection analysis (FIA) and atomic absorption spectrometry (AAS). Similar to the results of figure 1 and 3, the number of measurements was low but the method also applied to these results. Only in a few instances, the ULA was reported, as shown in the column with heading $3 \cdot S_{\beta}$ in TABLE 1. The LOD was more frequently reported, and it was determined according to the conventional procedure(eq. 1). In TABLE 1 all columns are indicated by $1 \cdot S_{\beta}$, $2 \cdot S_{\beta}$ and $3 \cdot S_{\beta}$, which indicates the respective condition applied to the acceptance or rejection of the calibration line during iterations. A condition of $3 \cdot S_{\beta}$ imposed as a condition to the determination of

TABLE 1: Results of the determination of linear range of calibration that was defined by the LLA(eq. 10) and by the ULA. The most favourable value of α (eq. 13) that provided the wider range of calibration was estimated by applying the method to working curves of the literature and to results of the present work(see text)

Reference	Measureand	Method	Units	N	LLA			β/α			ULA			RSD at ULA(%)		
					$1 \cdot S_{\beta}$	$2 \cdot S_{\beta}$	$3 \cdot S_{\beta}$	$1 \cdot S_{\beta}$	$2 \cdot S_{\beta}$	$3 \cdot S_{\beta}$	$1 \cdot S_{\beta}$	$2 \cdot S_{\beta}$	$3 \cdot S_{\beta}$	$1 \cdot S_{\beta}$	$2 \cdot S_{\beta}$	$3 \cdot S_{\beta}$
21/reported	Tetraethyllead	Gas Chromatography	$\mu\text{g/L}$	8	-	-	1	-	-	-	-	-	1000	-	-	-
21/present method	Tetraethyllead	Gas chromatography	$\mu\text{g/L}$	8	0.64	1.4	-	0.6	2.1	-	20	100	-	3	3	-
22/reported	Leukotrienes	Liquid chromatography	ng/mL	6	-	-	0.2	-	-	-	-	-	1	-	-	-
22/present method	Leukotrienes	Liquid chromatography	ng/mL	6	0.071	0.085	0.25	0.024	0.15	0.66	0.5	2	5	13	6	10
23/reported	Glucose	Amperometry	mM	14	-	-	0.2	-	-	-	-	-	12	-	-	-
23/present method	Glucose	Amperometry	mM	14	0.066	0.29	0.49	0.0048	0.66	1.4	12	18	21	0.7	4	5
24/reported	Iron	FIA	μM	13*	-	-	-	-	-	-	-	-	-	-	-	-
24/present method	Iron	FIA	μM	13*	14	28	61	6.3	79	230	1200	1500	2000	2	3	6
25/reported	Europium	Differential pulse polarography	mmol/L	8	-	-	0.014	-	-	-	-	-	-	-	-	-
25/present method	Europium	Differential pulse polarography	mmol/L	8	0.0045	0.0057	0.0071	0.0035	0.011	0.022	0.075	0.125	0.175	8	7	6
Figure 1	Ba	AES	$\mu\text{g/L}$	8	1.4	-	-	0.6	-	-	50	-	-	8	-	-
Figure 2	Sr	FAAS	mg/l	73	0.014	0.021	-	0.003	0.03	-	1	2	-	2	2	-
Figure 3	Sr	ICP-MS	$\mu\text{g/L}$	11	1.8	-	8.5	0.2	-	28	150	-	500	2	-	3
Figure 4	Fe	GFAAS	$\mu\text{g/L}$	169	3.8	5.0	5.2	0.2	5.0	6.0	60	100	125	5	6	5

*Average of three repetitions

Full Paper

the intercept thus signifies that a relatively large spread of data was allowed to the acceptance of the calibration line. Accordingly, a wide calibration range was accepted, which leads to a low sensitivity and a high value for the LLA. Conversely, a condition of $1 \cdot S_\beta$ leads to a narrow calibration range, and it leads to a high sensitivity and a low value for the LLA. In most cases for the results presented in TABLE 1, the condition of $1 \cdot S_\beta$ provides an impractical narrow calibration range that requires careful dilution of samples. This drawback was eliminated by applying the condition of $2 \cdot S_\beta$ to the choice of calibration line, which provided an acceptable range of calibrations and an acceptable level of standard deviation at ULA (TABLE 1). Thus, a general value of $x=2$ in eq. 13 provides acceptable working ranges and LLA's. The results of TABLE 1 were obtained by eliminating data one by one starting from the higher concentrations but when a very large number of data was applied to the calibration (TABLE 1, figure 2 and 4); more data could be identified for removal by the depiction of standard deviations (figures 1-4). Although some calibration data exhibited a large spread around the calibration line, the correct calibration line was obtained by the first iteration, as seen for the analysis of tetraethyllead by GC and for the analysis of Sr by FAAS (TABLE 1). The overall impression of the analysis of the results presented in TABLE 1 is the more favourable calibration range, the low LLA's and the acceptable RSD at ULA, which were obtained by applying the condition of $2 \cdot S_\beta$ to the limit of iterations. Since the condition of $2 \cdot S_\beta$ unambiguously was linked to the determination of the intercept (eq. 13), the ratio of intercept and slope could be considered, as to be a measure of the LLA (TABLE 1). In fact, the definition of eq. 6 provides values that are approximately equal to the value of β/α , which implies that this latter quantity could be applied as a useful approximation of the LLA. It may be supported by assuming that eq. 13 ($x=1$) inserted into eq. 10 with $S_{A_0} = S_\beta$, which corresponds to a similar magnitude of STDEV's of blanks and of the STDEV of the intercept (TABLE 1). This disclosure further implies that the LLA may be determined without performing measurements of blanks, which is an attractive property of the procedure. The iterative elimination of data that originates from the non-linear range of concentrations results in a slope of the working curve, which

further increases after continuous iterations. The iteration halts when the condition of eq. 13 is fulfilled, which occurs when the intercept decreases to a value close to the magnitude of $2 \cdot S$. Thus, the correspondence between LLA and β/α is a result of the curvature of the data and the value of β/α always becomes a small positive number close to the LLA. The conventional definition of the LOD (eq. 1) considers only the noise level of the detector and the slope of the calibration line while the LLA of the present method includes the general level of noise that results from variations caused by the sample introduction system and variations in chemical reactions induced by, e.g., a sample-introduction accessory. Thus, in general the LLA provides higher LLA's and more realistic limits of detection, as compared to the values given by eq. 1. This observation is a result of the instrument accessories low influence on the magnitude of the noise level on the blanks. In summary, the present method shows that the minimum RSD was obtained at the ULA, and that all the analytical protocols exhibited an average RSD of 4% at the ULA by applying the condition of $x=2(2 \cdot S_\beta)$ (TABLE 1). Similarly, the RSD of the LLA (eq. 6) was on average 40%.

3.6 Distribution of data

According to eq. 6, the calculation of S_c allows the determination of the distribution function ($F(c)$) of data scattering around the calibration line, as represented by:

$$f(c) = \frac{\alpha}{\sqrt{\pi \left(s_A(\bar{c})^2 + s_\beta^2 + (\bar{c} \cdot s_\alpha)^2 \right)}} \times \exp \left(- \frac{\alpha^2 \cdot (\bar{c} - \bar{c})^2}{\left(s_A(\bar{c})^2 + s_\beta^2 + (\bar{c} \cdot s_\alpha)^2 \right)} \right) \quad (14)$$

At a given absorbance, the average concentration (\bar{c}) is situated at the calibration line and it is calculated by eq. 4. The frequency of data at the calibration line is at its maximum at zero concentration and decreases as a function of concentration (figure 5a). Furthermore, the width of the data distribution increases as a function of concentration, which was confirmed experimentally by the experiments of figures 3,4. The overall influence on

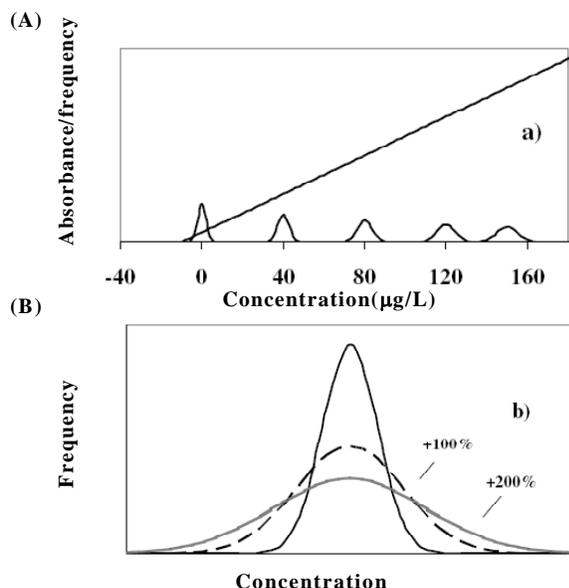


Figure 5 : Evolution of the scattering of data(exemplified by data of ETAAS) around the calibration line, as represented by the distribution of eq. 14. The width of the distribution increases as a function of concentration and the probability of the data at the calibration line decreases. (a) At zero concentration, the width of the data distribution represents the LLA(eq. 10). (b) Increments of the scattering of data(eq. 14) results in higher values of S_A , S_β , S_α . The value of S_A was estimated by eq. 3. This results in a wide distribution and low occurrence at the position of the calibration line, as shown by increments of the parameters by 100% and by 200%(eq. 14)

the distribution function is a widening of the function associated with decreasing frequencies(figure 5b). In order to make an acceptable determination of eq. 14, it is worthwhile to perform a thorough investigation of the calibration line, including one hundred data or more, which ensures that the law-of-great numbers is obeyed. It may therefore be suggested that the determination of the distribution function of eq. 14 ought to be an intrinsic property of any calibration procedure of chemical analysis. Similar to the results of eq. 12, the distribution function(eq. 14) requires an investigation of the dependence on concentration of S_A .

CONCLUSION

A method of data analysis is presented that enables determination of the full concentration range of calibration by a statistical data interpretation. A minimum value of the expected standard deviation of the straight line

was thus established and compared to experiments. By inspection of a depiction of the STDEV as a function of observed deviations, identification was facilitated of data groups belonging to the straight line. Data outside the prevailing grouping of data were subsequently removed by an iterative procedure of fitting straight lines that proceeded until the twice the value of the STDEV, $x=2$ in eq. 13, on the intercept ($2 \cdot S_\beta$) exceeded the value of the intercept(β). The method thus links the LLA to the upper limit of analysis(ULA), where calibration is feasible. A low number of iterations was required, as to fulfil the conditions of the method both in calibration experiments of small data sets and in calibration experiments of multiple data. Thus, by examining four different experiments of calibration, it is suggested that the method is generally applicable to a multitude of analytical technologies. It is proposed that calibration should be performed experimentally by measuring all responses from zero concentration and up to concentrations where the signal of the detector saturates. A subsequent data treatment by the present method then delivers the statistically correct range of calibrations and a higher value of the LLA, as compared to the conventional LOD. The method provides both the LLA and the ULA, which narrowed the range of calibration but alleviated the effort of defining the limits. In addition, an absolute minimum value of the standard deviation was assessed, which stipulates the terms of precision. The validation of the method showed that measurements performed without including the STDEV of an unknown, resulted in RSD's of approx. 4%. Thus, the demand for a prediction of the uncertainty on a single measurement, according to the BIPM philosophy^[17], may be provided by an evaluation of the standard solutions of a given calibration. In summary, the following schedule is proposed for the experiments of calibration:

1. Prepare standard solutions of the analyte from concentrations close to the expected LOD(eq. 1) and up to concentrations where the signal of the detector starts to saturate.
2. Perform the measurements and prepare the calibration line in a spreadsheet(e.g. Excel/tools/data analysis/regression) that also provides values of S_α and S_β (eq. 6).
3. Calculate STDEV's of a straight line(eq. 6) and depict experimental data as a function of corre-

Full Paper

sponding observed deviations from the straight line.

4. Delete data from the data set, which exhibit large deviations from a grouping (figure 2) and belong to the group of high concentrations.
5. Calculate a new calibration by the remaining data of the data set and continue deleting data of high concentrations and re-calculation of the straight-line parameters until the condition is fulfilled, $\beta < 2 \cdot S_{\beta}$ (eq. 13).
6. The LLA of the method is given by eq. 10:

$$7. \quad LLA = \frac{1}{\alpha} \cdot \sqrt{\frac{s_{A_0}^2 + s_{\beta}^2}{2}}$$

8. The distribution of data scattered around the calibration line as a function of concentration is estimated by eq. 14.

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