

DIN/ISO Compliant Calibrations

Essential performance characteristics
in instrumental analysis

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**"A standard is a document
established by consensus that
provides rules, guidelines or
characteristics for activities or
their results."**

(ISO/IEC Guide 2:2004)



Today Analytical Chemistry is embedded in a strictly regulated environment and it has to be ensured that verifiable and officially authorised methods are used. How can this be achieved in a reproducible and unequivocal way? Here we approach this question with respect to the problem of establishing and interpreting calibrations in Instrumental Analysis.

For a basic introduction to the problem let us go back to the days when we started our analytical education. Our first analytical experiments were qualitative ones, i.e. we tried to detect analytes by utilising reactions which for example generate specific colours. Let us assume that a student has the task of detecting Fe (III) in water with thiocyanate. For this purpose he adds a drop of a 1 molar NH_4SCN -solution to one drop of a weakly HCl-acidic Fe^{3+} -solution on a spotting plate. The production of a red colour caused by formation of $\text{Fe}(\text{SCN})_3$ indicates the presence of Fe(III), which means a “positive” test result. But students often tend to proclaim a positive test result even if the colour is only slightly orange and no iron at all is present. In such a case the test result is “false positive”. The left side of Fig. 1 shows the test results for 100 blank samples containing no iron. One of the tests is erroneously judged as positive by our student, i.e. the false positive rate is $\alpha=0.01$. The “specificity” [1] of this assay then is $1-\alpha=99\%$, i.e. 99 % of the results are “true negative”. The right side of Fig. 1 again shows 100 test results. But this time the sample drops contain 0.25 μg

Fe(III) which corresponds to the Limit of Detection (LOD) [2]. By judgement of the colour our student 95 times decides correctly that iron is present in the sample (“true positive” test results) and 5 times erroneously that the sample does not contain iron (“false negative” test results). Thus the false negative rate at the Limit of Detection (LOD) is $\beta_{\text{LOD}}=0.05$ and the “sensitivity” [1] of the assay is $1-\beta_{\text{LOD}}=0.95$, i.e. in 95 % of the cases our student is successful in detecting the above mentioned small amount of iron in the sample.

Focus on performance characteristics

Limit of detection

Checking the intensity of the red colour visually is a rather elementary approach. To enhance reproducibility we could substitute this by a photometric calibration [3]. But even then the first question to be posed refers to

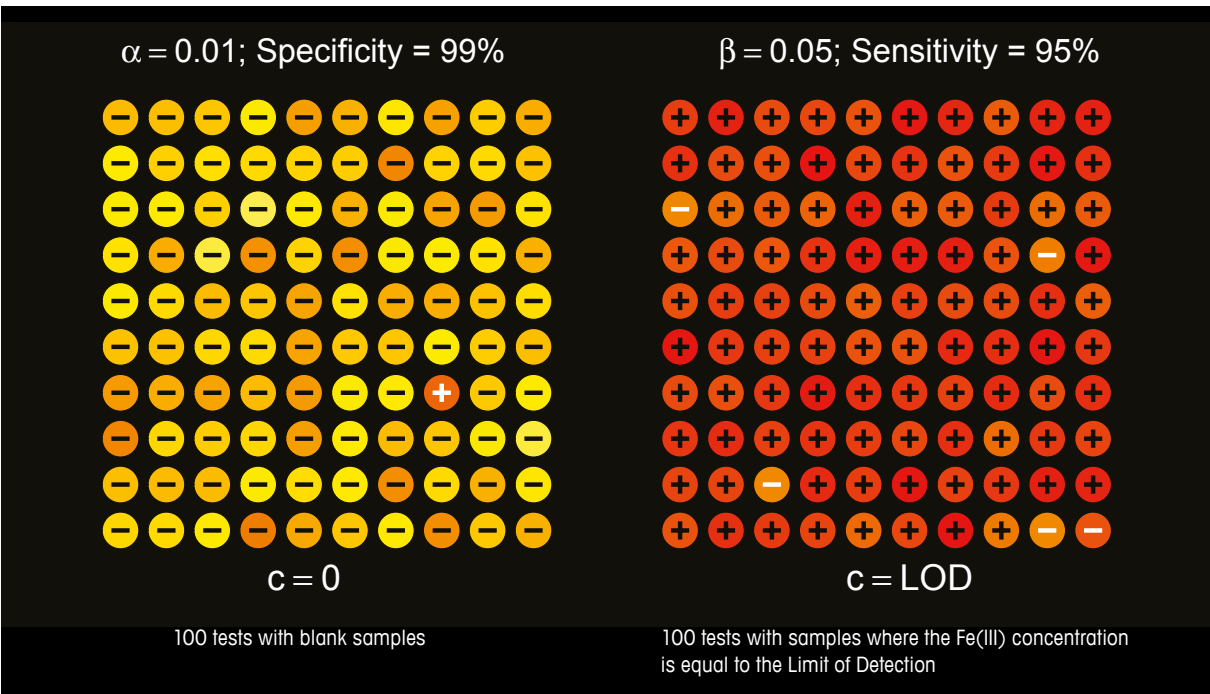


Fig. 1 Detection of Fe (III) with Thiocyanate on a spotting plate (Computer simulation)



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qualitative analysis: “What is the assay’s capability of detecting a certain analyte?” This can be answered unequivocally by determining the minimum detectable concentration, i.e. the “Limit of Detection” for a predefined specificity and sensitivity. The calculation of the LOD from a calibration is described in DIN ISO 11843-2:2006 (“Capability of detection – Part 2:Methodology in the linear calibration case”) and DIN 32645:2008 (“Decision limit, detection limit and determination limit under repeatability conditions - Terms, methods, evaluation”). Fig. 2 shows a simulated calibration example with 51 calibration standards [4] where x is the concentration and y the signal. The regression line can be established by common statistical software and the mathematical details of the corresponding calculations are described e.g. in ISO/TS 28037:2010 (“Determination and use of straight-line calibration functions”) and DIN ISO 11095:2008

(“Linear calibration using reference materials”). The red curve in Fig. 2 is the upper limit of the one-sided prediction intervals for $\alpha=0.05$. The intersection of this curve with $x=0$ determines the critical y -value and the corresponding critical value for x . The critical values are used as decision limits for deciding if the analyte has to be regarded as “detected” or “not detected”. Decision limits are applied a posteriori, i.e. if and only if they are surpassed by the measurement result of the sample, the analyte is assumed to be present in the sample. The green curve represents an additional safety interval taking into consideration the value chosen for β_{LOD} . This interval has to be added to the critical y -value and the corresponding x -value is the Limit of Detection. The LOD is a performance characteristic of the calibration, i.e. an a priori information which tells the user in advance the minimal concentration detectable by the assay in the long term.

Determination limit

After the question of the capability of detection of an assay with respect to qualitative analysis has been clarified, the next question immediately arises: “How far can the existing calibration be utilised for quantitative analysis?” ISO 8466-1:1990 (“Calibration and evaluation of analytical methods and estimation of performance characteristics - Part 1: Statistical evaluation of the linear calibration function”) describes how the standard deviation of a method can be calculated from the residual standard error of the regression. This ISO was derived from DIN 38402-51:1986. The capability of quantifying a certain analyte can be characterised further by its minimum quantifiable concentration, called Determination Limit or Limit of Quantification (LOQ). This depends on the maximum permissible value $1/k$ for the relative uncertainty of the result predicted by the calibration for the concentration x of the analyte. DIN 32645 suggests $k=3$, which in most cases is sufficient for trace analysis. In cases where a better precision is required the value of k should be chosen higher. The relative uncertainty of the result is given by $\Delta x/x$, where Δx is equal to half the two-sided prediction interval around x . Fig. 3 shows the

corresponding prediction band and demonstrates how the Limit of Quantification for $k=3$ can be derived from the calibration. It should be mentioned that an alternative for determining the Limits of Detection and Quantification is the evaluation of the standard deviation of blank samples (e.g. IUPAC [5]).

Here it must be pointed out that the term “sensitivity” has two different meanings. The first one, already discussed, is the sensitivity ($1-\beta_{LOD}$) of an assay with respect to qualitative analysis. The second one defines sensitivity as the slope of the calibration function. If the latter is a line this sensitivity is constant for all concentrations. However there are cases where non-linear calibration functions have to be applied. DIN ISO 8466-2:2004 (“Calibration strategy for non-linear second-order calibration functions”) gives a regression solution for a second-order polynomial. The question of the Limit of Detection in such non-linear cases is discussed generally in DIN ISO 11843-5:2010 (“Capability of detection – Part 5: Methodology in the linear and non-linear calibration cases”).

Recently we used non-linear calibration functions in the trace analysis of aqueous samples by Ion Mobility

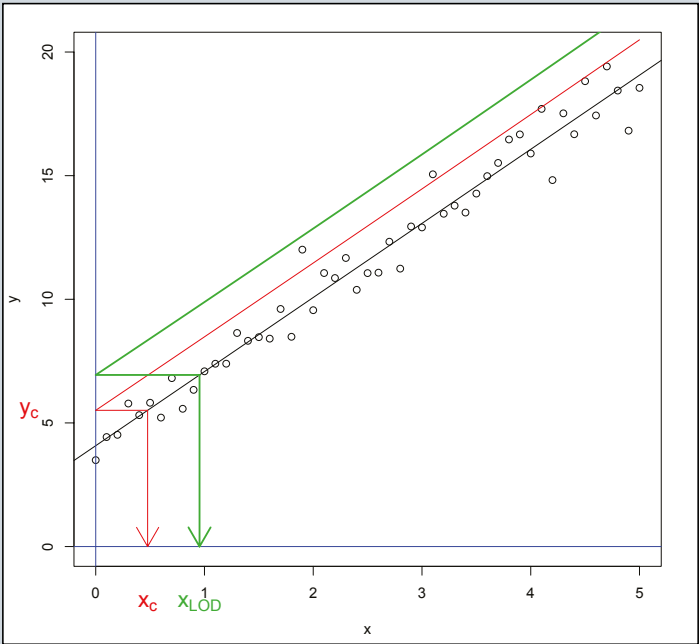


Fig. 2 Calibration with a linear function (Simulated example [4])
x = concentration; y = signal; Black points: Calibrations standards;
Black line: Calibration line; Red curve: One-sided prediction band ($\alpha=0.05$);
Green curve: Additional safety interval for guaranteeing a Sensitivity of $(1-\beta_{LOD})$
= 95%; y_c = Critical signal value; x_c = Critical concentration value; x_{LOD} = Limit
of Detection

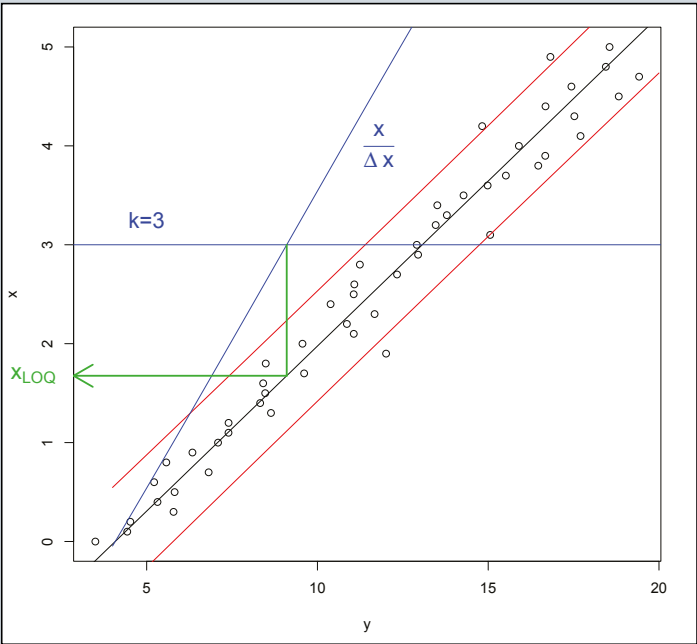


Fig. 3 Limit of Quantification (LOQ) for the calibration of Fig. 2
Red curves: Two-sided prediction band ($\alpha = 0.05$); $x/\Delta x$ = Inverse of the relative
uncertainty of the predicted x-values; Δx is equal to half the width of the
prediction band in x-direction; At the limit of quantification $x/\Delta x = k$ holds;
Due to DIN 32645 $k = 3$ was chosen; x_{LOQ} = Limit of Quantification

Spectrometry (IMS). In the corresponding paper [6] the calibration strategy of applying a quadratic calibration function is described explicitly. The calibration curve for lindane is shown in Fig. 4. The slope, i.e. the sensitivity is decreasing with increasing concentration and this at the same time means an increase of the relative uncertainty of the result. Therefore, for such a concave calibration curve, there exists not only a lower but also an upper Limit of Quantification, which is indicated by the red vertical lines in Fig. 4. Only in the range between these two lines is the relative uncertainty of the result less than 1/3.

Conclusion

In summary no analytical calibration should be established today without giving its performance characteristics. Besides the standard deviation of the method these are for predefined values of α , β_{LOD} and k the Limit of Detection and Quantification. What is the appropriate way to calculate these parameters? To some extent, commercial software is available. However, we prefer to use our own software. This is written in R, a free language and environment for statistical computing and graphics [7].

Our R-programmes are publicly available [8] on the BSCW (Basic Support for Communicative Work) Server of the University Duisburg-Essen. Some R programme libraries on this subject are also available from other authors [9,10]. Computer programmes should always be double checked with the numerical examples given in the corresponding DIN/ISO standards to ensure that the software delivers valid results.

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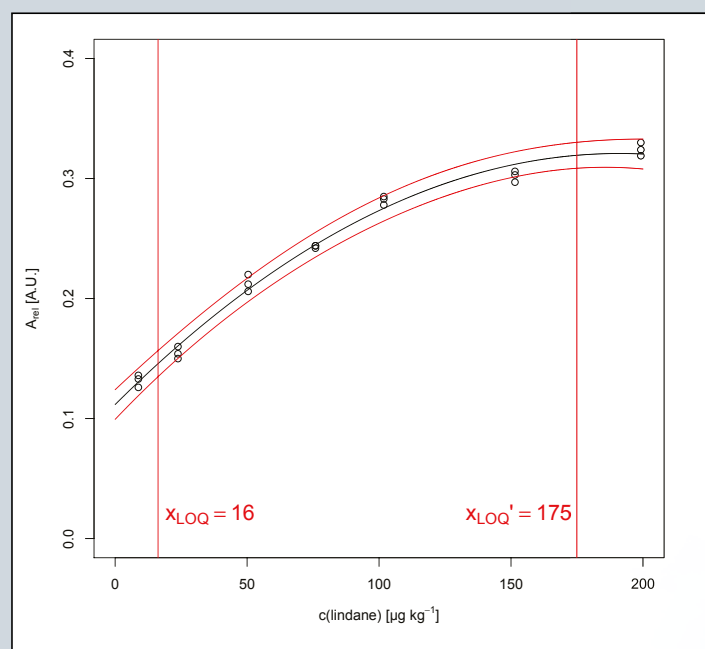


Fig. 4 Calibration curve for the IMS analysis of lindane in aqueous solutions [6]

Red curves: Two-sided prediction band ($\alpha = 0.05$, triple determination);

x_{LOQ} = Lower Limit of Quantification for $k=3$;

$x_{\text{LOQ}'}$ = Upper Limit of Quantification for $k=3$;

In the range $x_{\text{LOQ}} < c < x_{\text{LOQ}'}$ the relative uncertainty of the result is less than 1/3.