The determination of detection and quantitation limits in human biomonitoring

Biomonitoring Methods, Conceptual Topics – Translation of the German version from 2024

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Abstract

The working group "Analyses in Biological Materials" of the German Senate Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (MAK Commission) has evaluated six different procedures for the calculation or estimation of the detection and quantitation limits determined in the validation process of biomonitoring methods. Using the example of the biomonitoring parameter 1-hydroxypyrene in urine, the advantages and disadvantages of these approaches are considered, and all procedures are critically evaluated and discussed. In summary, the Commission does not recommend one preferred method for the calculation of detection and quantitation limits, but rather a careful consideration and fit-for-purpose check of the chosen procedure. A thorough documentation of the selected approach and its results is an essential part of a method description.

1   Introduction

The Commission has published a summary chapter on reliability criteria for analytical methods, including how these data can be verified (Bader et al. 2010). Among other aspects, this publication presents five different procedures for the determination of the limits of detection (LOD) and the limits of quantitation (LOQ) for biomonitoring methods. The critical discussion of these individual approaches is, however, briefly presented in this publication, as the focus was the general description of method validation. In the following sections, a detailed and critical comparison of the procedures for the determination of the limits of detection and the limits of quantitation described in the aforementioned publication as well as a comprehensive consideration of their advantages and disadvantages supplement the previous publication and substantiate these approaches using the biomonitoring parameter 1-hydroxypyrene in urine as an example.

2   General aspects

In chemical analysis–and thereby also in human biomonitoring–the challenge in the lower concentration range is to reliably distinguish the measurement signal of a sample from that of a blank as well as to be able to make a quantitative statement with sufficient precision. In order to complete this task, limits of detection and quantitation are determined as part of method validation.

In the field of chemical analysis, the limits of detection and quantitation are defined in DIN 32645 (DIN 2008). Both parameters are derived from the specification of the "critical value of a measured quantity", above which, based on a defined probability P for the type 1 error (significance level α), it is recognised that the quantity of an analyte in a sample is greater than that in a blank.

The detection limit is thereby defined as the concentration at which the β error (falsely negative result) reaches 50% and the α error (false positive result) of usually 1% or 5% has been established (Bader et al. 2010; DIN 2008).

For example, the measured value that is only exceeded by 1% of all blanks is selected as the upper limit for the blank (critical value). A measurement result which exceeds or falls short of this upper limit in repeated measurements at a rate of 50% each is referred to as the detection limit. This consideration explicitly means that, at the detection limit, the presence of an analyte is detected in half of the cases using the selected measurement method. In this respect, the detection limit presents the decision limit for the presence of an analyte in the sample matrix and can only be interpreted qualitatively (DIN 2008; Kromidas 2011).

In contrast, the quantitation limit is defined as the concentration at which the analyte can be quantified with a predetermined relative uncertainty of results, whereby a distinction is drawn between the lower and upper quantitation limit. According to most calculation and estimation procedures, the lower quantitation limit lies between three times (33.3% result uncertainty) and ten times (10% result uncertainty) the detection limit. Due to its defined result uncertainty, the lower quantitation limit enables a quantitative interpretation of measurement results and is a more meaningful measure for the actual performance of an analytical method than the detection limit. The upper quantitation limit is generally defined as the highest calibration standard for which a result uncertainty was determined during method validation (Bader et al. 2010). Unless otherwise specified, the term "quantitation limit" refers to the "lower quantitation limit".

As the quantitation limit is determined on the basis of a maximum allowable result uncertainty, its value depends on the specificity requirements (precision and accuracy) imposed on the analytical method. The quantitation limit should therefore be referenced for data evaluation and the communication of analytical results. The quantitation limit of a suitable analytical method, based on DIN EN 482 (DIN 2021) or ISO 20581 (ISO 2016), should be at least one-tenth of the decision-relevant concentration range (e.g. a biological limit value or biological reference value).

3 Determination procedures

There are a number of procedures for the determination of detection and quantitation limits. In the general chapter "Reliability criteria for analytical methods" (Bader et al. 2010) published by the Commission, five frequently used approaches were described. In summary, limits of detection and quantitation can be determined in an initial approach by visual estimation or via the signal-to-noise ratio. Furthermore, calculation can be performed using the blank-value or calibration-curve procedures described in DIN 32645 (DIN 2008).

Furthermore, detection and quantitation limits can be derived directly from precision data (Magnusson and Örnemark 2014).

3.1 Determination by visual estimation

For visual estimation, a blank sample (matrix blank) is spiked with increasing analyte concentrations and analysed. In the chromatograms obtained, the emergence of the analyte peak from the background noise of the baseline is visually assessed. The detection limit thereby corresponds with the lowest analyte concentration at which the analyte signal becomes clearly distinct from the background noise. Alternatively, the analyte concentration may be estimated by comparison with an adjacent measurement signal at a known concentration.

This procedure enables an initial estimation of the detection limit, whereby the area and height of the analyte signal depend both on the general condition of the analytical instrumentation as well as on the matrix. The value thus determined in a first approximation can serve as a starting point for a more precise determination using one of the following procedures. As a first approximation, the threefold detection limit can be assumed as the quantitation limit in this approach. In order to better assess the influence of the matrix, it is helpful to analyse various individual matrix blanks spiked with the analyte at the concentration visually determined as the detection limit.

3.2 Determination by the signal-to-noise ratio

In order to determine the detection limit from the signal-to-noise ratio, a matrix blank–or a sample which contains the analyte at a concentration near the detection limit–is measured. In the chromatogram obtained, the intensity of the background noise is determined in proximity to the peak to be analysed. The intensity of the background noise is thereby ascertained as the largest variation from the baseline (maximum-minimum difference) over at least ten outlier-free background-noise signals using, for example, a ruler or the trend-line function. The average noise intensity s_0 (positively or negatively from the baseline) is then multiplied by a factor of 3 to calculate the detection limit (LOD, 3-fold signal-to-noise ratio, Equation 1). The quantitation limit (LOQ) can be defined analogously as a signal-to-noise ratio of nine, for example (Equation 2).

$$LOD = 3 \times s_0 \tag{1}$$

$$LOQ = 9 \times s_0 \tag{2}$$

This approach represents a simple method for the estimation of the detection limit and can also easily be used in cases of non-linear calibration curves. Theoretically, an α error of 1% is pursued. Here, too, the background noise as well as the height and area of the analyte signal depend on the general condition of the analytical instrumentation and on the matrix. If the measurement was performed with pure, matrix-free standards, a transfer of the determined detection limit to real samples is correspondingly erroneous.

3.3   Blank-value method according to DIN 32645

DIN 32645 only applies to linear calibration functions, with normal distributions of the measured values, and with variance homogeneity (similar value distributions at the upper and lower ends of the calibration range). In order to ensure variance homogeneity, the calibration range selected for the determination of the LOD and LOQ should only extend to a maximum of about ten times the expected detection limit, and thereby usually lies considerably below the working range for subsequent routine analysis.

The determined concentration of a blank is subject to uncertainty which can be used for calculating the detection and quantitation limit: as long as there is no background from the analyte in the matrix, the detection limit can be determined using the blank-value method according to DIN 32645. In this "direct method", a matrix blank is analysed at least ten times. In addition, the slope of a calibration function is required to determine the analyte concentration; a calibration over the entire working range can be used for this purpose.

Using the standard deviation of the measured values of the blank as well as the slope of the calibration function, the detection limit can be calculated using Equation 3:

$$LOD = \frac{s_L}{b} \times t_{f;\alpha} \times \sqrt{\frac{1}{m} + \frac{1}{n}} \tag{3}$$

whereby s_L = standard deviation of the measured values of the blank; b = slope of the calibration curve; t = quantile of the t distribution in a one-tailed test for type 1 errors (Student factor) for f = n–1 degrees of freedom; m = number of measurements of the sample; n = number of blank-value measurements

In the blank-value method, the quantitation limit is calculated by rapid estimation (see Section 3.5); in routine analysis, the value of the calculated detection limit is often multiplied by a factor of 3.

By referring to the calibration function, the determination of the detection limit using the blank-value method according to DIN 32645 leads to a value resulting from the measurement method itself. However, this procedure can only be used insofar as blank samples are available. Due to background levels present in biomonitoring samples, this is not always possible, so that the indirect method (calibration-curve method, Section 3.4) may need to be used to determine the limits of detection and quantitation.

Due to the lack of matrix components, matrix-free blanks are only partially suitable for use and their application requires special testing.

3.4   Calibration-curve method according to DIN 32645

To determine the detection and quantitation limit using the calibration-curve method according to DIN 32645, the uncertainty of the blank is indirectly ascertained by extrapolating the regression data of a calibration. To this end, a calibration curve is generated by spiking the matrix with the analyte, whereby the lowest value should be close to the expected detection limit. The concentrations of the remaining calibration standards should span as equidistant points a concentration range up to about ten times the detection limit. The limits of detection and quantitation can be calculated from the calibration function thus generated as follows (Equation 4 and 5). The LOQ in the square root term of Equation 5 can be approximated by k × LOD.

$$LOD = s_{x0} \times t_{f;\alpha} \times \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{\bar{x}^2}{Q_x}} \tag{4}$$

$$LOQ = k \times s_{x0} \times t_{f;\frac{\alpha}{2}} \times \sqrt{\frac{1}{m} + \frac{1}{n} + \frac{(LOQ - \bar{x})^2}{Q_x}} \tag{5}$$

whereby s_{x0} = standard deviation of the procedure (in cases of variance homogeneity $s_{x0} \approx s_L/b$ applies with s_L = standard deviation of the measured values of the blank and b = slope of the calibration curve); t = quantile of the t distribution in a one-tailed test for type 1 errors (Student factor) for f = n–2 degrees of freedom; m = number of measurements of the analytical sample; n = number of calibration samples or total number of calibration measurements (with the same number of repeated measurements for each calibration point); $\bar{x}$ = arithmetic mean of the measured values of the calibration samples; Q_x = sum of deviation squares of x at calibration; 1/k = relative result uncertainty for the characterisation of the quantitation limit (e.g. 33.3% at k = 3).

Positive tests for variance homogeneity and linearity of the calibration function via F and t tests are a prerequisite for the applicability of the calibration-curve method. To ensure consistent variance homogeneity, the concentration range of the calibration curve may not exceed ten times the detection limit. When using this approach, it is likewise necessary to ensure that any background levels present in the sample matrix are subtracted from all measured values, although such a subtraction is not without its own problems (Magnusson and Örnemark 2014).

The calibration-curve procedure of DIN 32645 takes into consideration the natural scattering of measured values of a calibration, thereby enabling a high confidence for the detection and quantitation limits. The laboriousness of the procedure is, however, disadvantageous, as calibration within a concentration decade at the detection limit is rather unusual in practice. Furthermore, this approach is often subject to the fact that varying detection and quantitation limits are calculated depending on the selected calibration range. At higher concentrations, greater scattering is usually observed or greater deviation squares are to be expected in the linear regression, both of which, in turn, lead to higher calculated quantitation limits. The calibration curve used in practice, with analyte concentrations in the decision-relevant range, is therefore unsuitable to confirm the calculated detection and quantitation limits.

3.5 Rapid-estimation method according to DIN 32645

DIN 32645 notes the possibility of ascertaining the limits of detection and quantitation via rapid estimation. In this approach, the detection limit can be determined as a multiple of the standard deviation of the procedure, which can be ascertained by the blank-value or calibration-curve approach ($s_{x0} \approx s_L/b$), and Equations 6 and 7 are applied for individual measurements (m = 1).

$$LOD = \Phi_{n;\alpha} \times \frac{s_L}{b} \qquad \text{(blank-value method)} \tag{6}$$

$$LOD = 1.2 \times \Phi_{n;\alpha} \times s_{x0} \qquad \text{(calibration-curve method)} \tag{7}$$

For the quantitation limit, the following relationships result from Equations 8 and 9.

$$LOQ = k \times \Phi_{n;\frac{\alpha}{2}} \times \frac{s_L}{b} \qquad \text{(blank-value method)} \tag{8}$$

$$LOQ = 1.2 \times k \times \Phi_{n;\frac{\alpha}{2}} \times s_{x0} \qquad \text{(calibration-curve method)} \tag{9}$$

The factor Φ is given for various values of n and α in Table 2 of DIN 32645.

3.6 Standard-deviation method

Limits of detection and quantitation can also be derived directly from the precision data of the analytical procedure. To this end, samples with decreasing analyte concentrations are analysed multiple times in parallel, and the relative standard deviations of the repeated measurements are plotted against the corresponding analyte concentrations. The quantitation limit thereby corresponds to the concentration at which the relative standard deviation lies just below

the predetermined value (e.g. 10% or 33%). When using this method, it is important to ensure a sufficiently large number of analyte concentrations as well as a sufficient number of repeated measurements at each concentration level.

Eurachem (Magnusson and Örnemark 2014), for example, recommends using sixfold determinations at increasing degrees of dilution of an analyte in its matrix until the predetermined relative standard deviation is reached.

The standard-deviation method for the determination of the detection and quantitation limit takes all relevant parameters into consideration which may influence the detection and quantitation limit. The detection and quantitation limits determined with this approach are thereby robust and plausible values; the disadvantage of this procedure, however, is the significant amount of work involved.

4   Example

A concrete example is used to show that the calculation or estimation of the detection and quantitation limit applying the six procedures described above yields varying results. The determination of 1-hydroxypyrene is selected as an example from the field of human biomonitoring, whereby 1-hydroxypyrene was determined after hydrolysis in urine by gas chromatography with highresolution mass spectrometry (GC-HR-MS). 1-Hydroxypyrene-d_9 was used as internal standard (ISTD).

Figure 1 shows chromatograms of a blank, an unspiked urine sample, as well as a urine sample spiked with increasing concentrations of 1-hydroxypyrene. It can be seen that, with increasing concentrations, the 1-hydroxypyrene peak becomes increasingly more clear from the background noise of the baseline. The signal-to-noise ratio increases with increasing analyte concentrations. The **visually derived detection limit**, at which the analyte signal can be visually distinguished from the background noise, is 0.01 µg/l (Figure 1c). The detection limit determined from the **signal-to-noise ratio** lies between 0.01 and 0.02 µg/l.
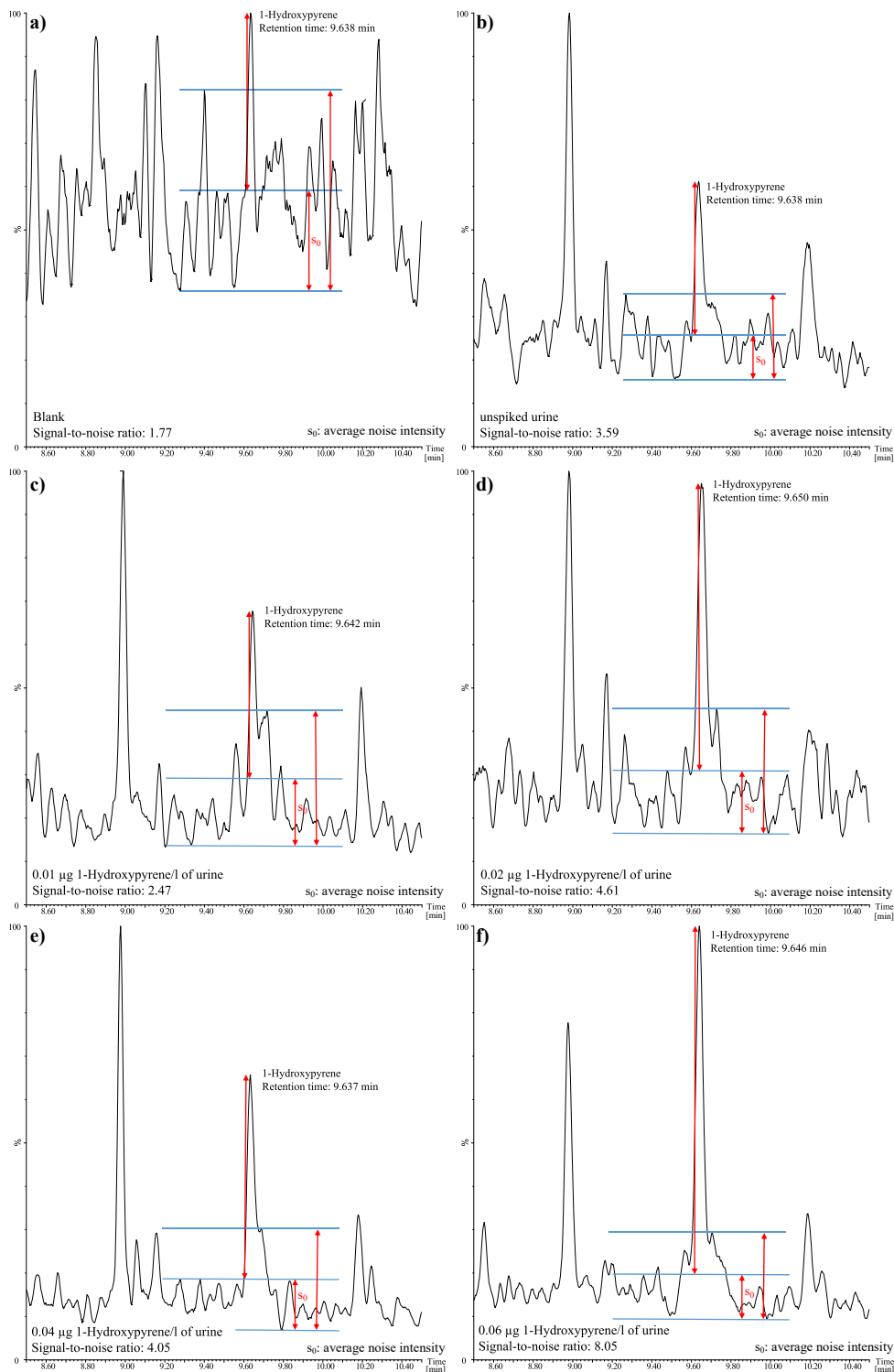
Fig. 1 Chromatograms of a) a blank, b) an unspiked urine sample, and c)–f) a urine sample spiked with increasing concentrations of 1-hydroxypyrene

To determine the detection limit using the **blank-value method according to DIN 32645**, a blank was processed and analysed ten times in parallel (Table 1), and a calibration function in a concentration range of 0.01 to 10 µg/l was

generated. Table 2 shows the data which were used to calculate the detection limit. A detection limit of 0.0492 μg/l was calculated using the blank-value method.

Tab. 1   Peak-area ratios of ten individual measurements of a blank

Individual measurement	y_L (peak-area ratio analyte/ISTD)
1	0.0054
2	0.0144
3	0.0108
4	0.0072
5	0.0108
6	0.0090
7	0.0126
8	0.0090
9	0.0144
10	0.0126

Tab. 2   Data which were used to calculate the detection and quantitation limits using the blank-value method according to DIN 32645

Number of blank measurements n	10	**t-Quantile for one-tailed confidence interval ($t_{9;0.01}$)**	2.821
Number of meas. of analytical samples m	1	**Mean $\bar{y}_L$**	0.0106
Significance level α	0.01	**Standard deviation s_L**	0.00299
Reciprocal rel. result uncertainty k	3	**LOD [μg/l]**	0.0492
Slope b [l/μg]	0.1795	**LOQ (per Equation 8) [μg/l]**	0.167

Using the **rapid-estimation method according to DIN 32645** (blank-value method), the detection limit is calculated as 0.0499 μg/l and the quantitation limit as 0.170 μg/l.

To determine the limits of detection and quantitation using the **calibration-curve method according to DIN 32645**, an equidistant ten-point calibration was generated in a concentration range of 0.01 to 0.1 μg/l (see Figure 2 and Table 3). In addition, a blank was processed and measured.
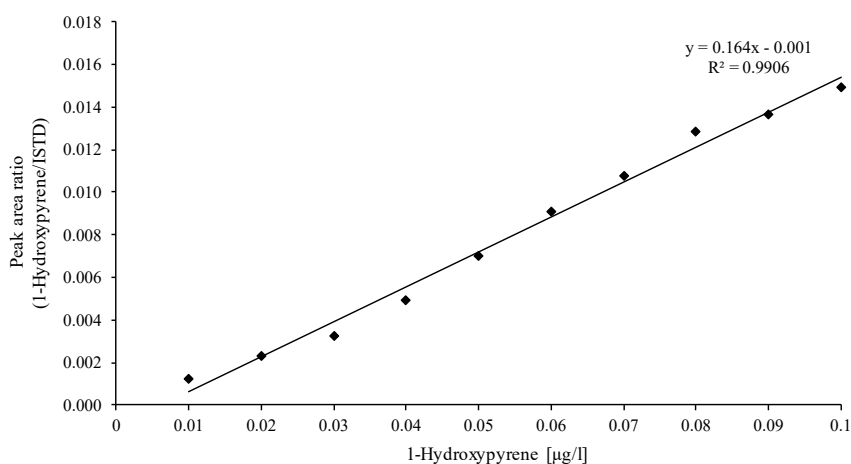

Fig. 2   Equidistant ten-point calibration in a concentration range from 0.01 to 0.1 μg 1-hydroxypyrene/l of urine for the determination of the limits of detection and quantitation using the calibration-curve method according to DIN 32645

Tab. 3 Data of the equidistant ten-point calibration in a concentration range of 0.01 to 0.1 µg 1-hydroxypyrene per litre of urine

1-Hydroxypyrene [µg/l]	Peak area 1-hydroxypyrene [counts per second, cps]	Peak area ISTD [counts per second, cps]	Peak-area ratio (1-hydroxypyrene/ISTD); background subtracted	Estimated value of the function	Bias [%]
0	532 209 700	250 872 000 000	0.000000	–	–
0.01	649 552 000	194 725 375 000	0.001214	0.000620	96.0
0.02	890 253 200	201 982 762 500	0.002286	0.002260	1.2
0.03	1 004 576 600	186 459 937 500	0.003266	0.003900	−16.3
0.04	1 310 319 900	185 881 475 000	0.004928	0.005540	−11.1
0.05	1 700 813 100	186 213 687 500	0.007012	0.007181	−2.3
0.06	2 313 397 100	206 608 612 500	0.009076	0.008821	2.9
0.07	2 299 595 100	178 268 562 500	0.010778	0.010461	3.0
0.08	2 841 040 200	189 599 912 500	0.012863	0.012102	6.3
0.09	3 311 612 100	210 045 625 000	0.013645	0.013742	−0.7
0.1	4 097 364 500	240 139 950 000	0.014941	0.015382	−2.9

The key data which are needed for the calculation of the detection and quantitation limits were calculated from this ten-point calibration and are presented in Table 4.

Tab. 4 Key data for the calculation of detection and quantitation limits using the calibration-curve method according to DIN 32645

Number of calibration samples n	10	**t-Quantile for one-tailed confidence interval ($t_{8;0.01}$)**	2.821
Number of meas. of analytical samples m	1	**Residual standard deviation of the measured values of the calibration $s_{y,x}$**	0.00051
Significance level α	0.01	**Standard deviation of the procedure s_{xo}**	0.00313
Reciprocal rel. result uncertainty k	3	**Sum of deviating squares from x for the calibration Q_x**	0.00825
Slope b ± standard deviation [l/µg]	0.16403 ± 0.00565	**LOD [µg/l]**	0.0107
Axis intercept ± standard deviation	−0.00102 ± 0.00035	**LOQ [µg/l]**	0.0339

From the calibration function obtained, detection and quantitation limits of 0.0107 µg/l and 0.0339 µg/l, respectively, were calculated using the **calibration-curve method according to DIN 32645**.

Using the **rapid-estimation method according to DIN 32645**, the procedural standard deviation of the calibration-curve method can be used to ascertain detection and quantitation limits of 0.0113 µg/l and 0.0383 µg/l, respectively.

Calibration curves in different concentration ranges lead to differing detection and quantitation limits (see Section 3.4). Calibration standards with higher analyte concentrations generally lead to higher calculated detection and quantitation limits. With respect to the data given in Table 5, it should be noted that the data were only calculated on the basis of DIN 32645, as only six calibration points were used for calculation (see Figure 3). Moreover, the concentration range of 0.01–10 µg/l exceeded the tenfold of the detection limit; however, the tests for variance homogeneity and linearity of the calibration function via F and t tests were positive, meaning that the calibration-curve method was applicable.
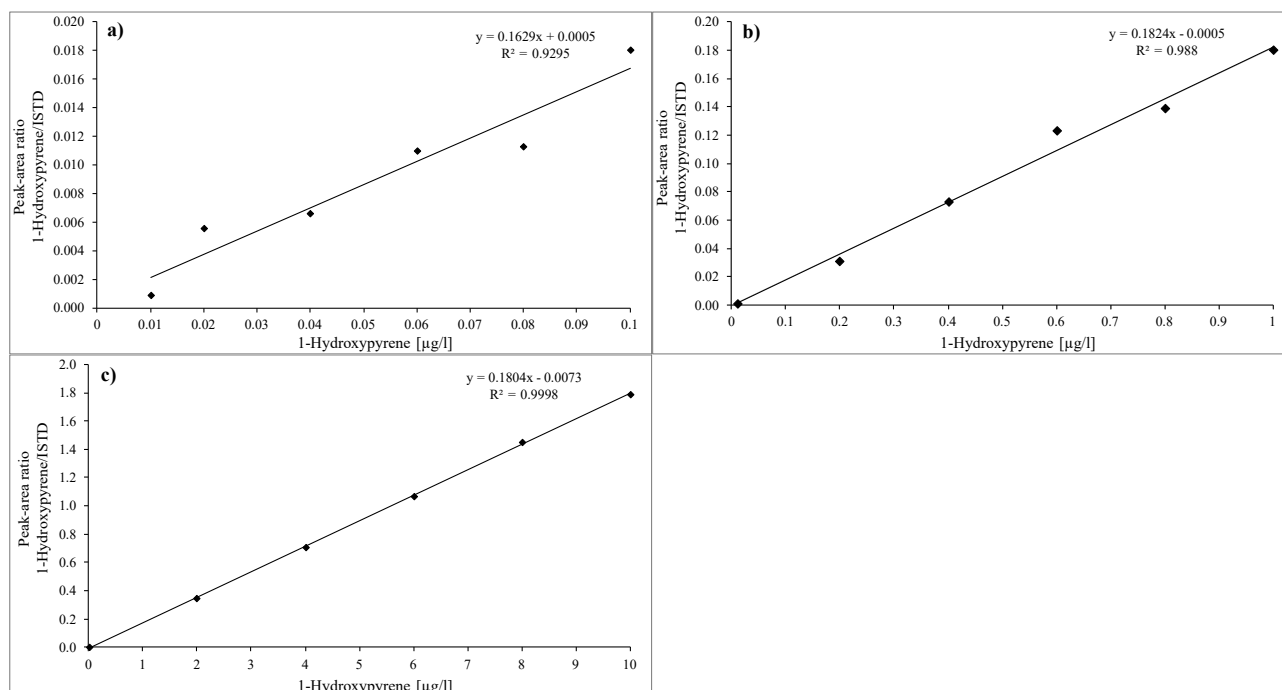
Fig. 3 Calibration curves for the determination of detection and quantitation limits based on the calibration-curve method according to DIN 32645; six calibration points in the following concentration ranges were used for calculation: a) 0.01–0.1 µg, b) 0.01–1 µg, and c) 0.01–10 µg 1-hydroxypyrene per litre of urine

Tab. 5 Determined limits of detection and quantitation using the calibration-curve method on the basis of DIN 32645; six calibration points in different concentration ranges were used for calculation

Calibration range [µg/l]	Detection limit [µg/l]	Quantitation limit [µg/l]
0.01–0.1	0.051	0.250
0.01–1	0.212	0.700
0.01–10	0.276	0.976

The detection limit calculated for the calibration range of 0.01–0.1 µg/l is considerably higher than the value calculated from the data in Table 3. This difference can be attributed to the fact that the data points are more scattered around the regression line (compare Figure 2 with Figure 3), but the lower number of calibration points is also reflected in the higher detection limit.

Finally, the quantitation limit for the quanti-fication of 1-hydroxypyrene in urine was determined using the **standard-deviation method according to DIN 32645**. To this end, urine samples were spiked with decreasing analyte concentrations, processed six times in parallel per concentration level, analysed, and the relative standard deviations were calculated. The previously defined precision of 20% was reached at a concentration level of 0.06 µg 1-hydroxypyrene/l urine (see Table 6).

Tab. 6   Determination of the quantitation limit using the standard-deviation method (n = 6)

Sample	Spiked concentration [µg/l]	Peak-area ratio (1-hydroxypyrene/ISTD)	Measured concentration [µg/l]
1		0.012	0.073
2		0.014	0.083
3		0.014	0.081
4	0.06	0.008	0.050
5		0.011	0.064
6		0.009	0.055
		Mean [µg/l]:	0.068
		Standard deviation [µg/l]:	0.0137
		Rel. standard deviation [%]:	20.2

The limits of detection and quantitation ascertained with the various procedures are summarised in Table 7. This comparison clearly demonstrates the problem that the different procedures lead to slightly different detection and quantitation limits, even with the exact same biomonitoring method. In the example given here, the ratio of the highest and lowest quantitation limit is about 5.

Tab. 7   Limits of detection and quantitation determined using different procedures

	Visual estimation	Signal-to-noise ratio	according to DIN 32645		Standard-deviation method
			Blank-value method	**Calibration-curve method**	
LOD	0.02	0.01−0.02	0.049	0.011	−
LOQ	0.06[a]	0.03−0.06	0.167	0.034	0.06

[a] was calculated approximately by multiplication by a factor of 3

5   Discussion

In principle, when developing or validating analytical methods, there is the possibility to choose from the variety of procedures for the determination of detection and quantitation limits. From the perspective of the Commission, all procedures possess certain strengths and weaknesses, depending on the objective, such that no uniform procedure can be established. In any case, it must be noted that the procedures described above are not given in a random order. In general, the determination of detection and quantitation limits using visual derivation, derivation from the signal-to-noise ratio, or using the blank-value method according to DIN 32645 is less robust than when using the calibration-curve method according to DIN 32645, and the standard-deviation method.

The lowest possible detection and quantitation limits should not be the decisive quality criteria for the evaluation of an analytical method, and should therefore neither be seen as the primary goal of method development. Rather, the robustness of the method must be considered and therefore only values which can be reliably determined under routine conditions should be given as quantitation limit. Beyond daily routine, when methods are only occasionally applied, detection and quantitation limits must be separately determined within each new analytical run.

The exemplary calculation of the detection and quantitation limits for the quantification of 1-hydroxypyrene in urine using the different determination procedures made it clear that the calculated values vary depending on the selected approach. In general, the detection and quantitation limits determined with varying approaches cannot be directly equated or interpreted with respect to the performance of an analytical method. When indicating detection and quantitation limits, therefore, the procedure used to determine these values must be given as well.

For the determination of the limits of detection and quantitation according to DIN 32645, further information to be provided may include: the method used, number of blank or calibration samples, number of measurements of the analytical sample(s), significance level α ("type 1 error"), significance level β ("type 2 error"), as well as the reciprocal relative result uncertainty k. In addition, relevant chromatograms and other relevant figures, if necessary, should be shown.

Even if the detection and quantitation limits are determined repeatedly with the same procedure, the values thus obtained may vary considerably. The results achieved ultimately reflect the current condition of the measurement system (analytical device, separation column, reagents, etc.). If the number of values used to calculate the confidence interval varies when using the same method, the quantitation limits thus obtained are not necessarily comparable. Moreover, the detection and quantitation limits calculated using the calibration-curve method depend on the selected concentration range, the correlation of the regression curves, and the matrix.

In the context of determining the detection limit, it is additionally necessary to differentiate between the detection limit of the analytical instrument and that of the analytical method. The detection limit of the analytical instrument is determined by measuring matrix-free analyte standards or even blank-value samples without further workup. In order to correctly determine the performance of an analytical method, however, analyte standards must be measured which have been prepared in matrix and processed analogously to any other samples according to the workup procedure described in the method. This especially applies to the visual estimation of the detection and quantitation limits as well as their determination from the signal-to-noise ratio.

The quantitation limit obtained should generally be verified using a suitable number of native samples with corresponding analyte concentrations. If necessary, also the robustness of the determined values must be verified by measuring various real-world samples with analyte levels close to the quantitation limit.

In conclusion, it can be stated that there is no single correct method for the calculation of the detection and quantitation limits, but rather that the selected approach must be carefully considered and tested in each case. Thorough documentation of the used determination approach is a necessary part of a method description. The quantitation limit as a performance characteristic for a validated measurement procedure should especially include information about the approach with which it was determined, the statistical reliability, and the relative uncertainty of results at the quantitation limit.

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

References

Bader M, Barr D, Göen T, Schaller KH, Scherer G, Angerer J (2010) Reliability criteria for analytical methods. Biomonitoring Method, 2010. In: Angerer J, Hartwig A, editors. The MAK-Collection for Occupational Health and Safety. Part IV: Biomonitoring Methods. Volume 12. Weinheim: Wiley-VCH. p. 55–101. Also available from https://doi.org/10.1002/3527600418.bireliabe0012

DIN (Deutsches Institut für Normung), editor (2008) DIN 32645:2008-11. Chemische Analytik – Nachweis-, Erfassungs- und Bestimmungsgrenze unter Wiederholbedingungen – Begriffe, Verfahren, Auswertung. Berlin: Beuth. https://doi.org/10.31030/1465413

DIN (Deutsches Institut für Normung), editor (2021) DIN EN 482:2021-05. Workplace exposure – procedures for the determination of the concentration of chemical agents – basic performance requirements. German version EN 482:2021. Berlin: Beuth. https://doi.org/10.31030/3211072

ISO (International Organization for Standardization), editor (2016) ISO 20581:2016. Workplace air – general requirements for the performance of procedures for the measurement of chemical agents. Berlin: Beuth

Kromidas S, editor (2011) Handbuch Validierung in der Analytik, 2nd ed. Weinheim: Wiley-VCH

Magnusson B, Örnemark U, editors (2014) Eurachem guide: the fitness for purpose of analytical methods – a laboratory guide to method validation and related topics, 2nd ed. Gembloux: Eurachem. https://www.eurachem.org/images/stories/Guides/pdf/MV_guide_2nd_ed_EN.pdf, accessed 14 Mar 2024