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Limits of detection, identification and determination: a statistical approach for practitioners

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Abstract For the estimation of the limits of detection, identification and determination, considerations from analytical practice were applied to the statistics of the calibration line and its prediction interval. The detection limit was the concentration calculated from the maximum height of the prediction interval at zero spiking concentration. The identification limit was twice the detection limit and was the lowest concentration that could safely be detected. The determination limit was the lowest concentration fulfilling three criteria: 1. None of the signals resulting from determination limit concentration should interfere with any signal from detection limit concentration, thus providing an unambiguous separation between the two limits. 2. Recovery should be between 70% and 120%. 3. Lowest and highest predictable signal at determination limit concentration should not deviate more than ± 30% from the average. Practical analytical guidance and the necessary mathematical formulae are presented.

Key words Detection limit · Determination limit · Calibration line · Prediction interval · Validation

Introduction

Daily practice in a residue laboratory often makes analysts deal with concentration levels that are close to the bottom end of their clean-up procedures' and instruments' capacities. When analysing a sample in this low concentration range, the results will show a considerable variation. Controlling this variation and producing reliable results is one of the most challenging tasks for practitioners, for instance when "zero tolerance values" have effectively to be controlled (for an example, see [1]) or when the residue level of a food produce is decisive for its placing on the market [2].

The region of low concentration levels is governed by the "limit of detection" and "limit of determination". These expressions are well known and widely used and applied in daily routine, but there is no unanimous agreement on the best way to establish them. For instance, solutions of the pure substance in question may simply be injected into the gas chromatograph, and from the peak heights observed the limits may be estimated using practical experience. Other approaches require spiking experiments to be carried out, in order to imitate the usual sample clean-up, and their results are processed in quite complex mathematical-statistical calculations.

Different working procedures or mathematical-statistical approaches may however lead to limits of detection and determination that diverge by more than is reasonably acceptable, namely by up to a factor of 100 in different laboratories [3]. On the other hand, two re-

cognised concepts [4, 5] have been evaluated in a type of ring test, and standardisation recommendations have been given [6] which made the numerical values for the limits converge.

Yet these two concepts [4, 5] have certain drawbacks, some of them severe [7], which may hinder their acceptance by experienced practitioners. The approach presented here therefore combines the useful parts of these concepts, including the "identification limit", which cannot be left out of the discussion. In order to come closer to analytical practice and thus provide most realistic values for the limits, several new elements are added.

In this way, this approach is intended to provide convincing evidence that the usual laboratory habits in the area of very low concentrations may easily be placed on a sound mathematical-statistical basis. This should allow for more reliable results in everyday analytical practice.

Detection limit: analytical practice and statistical implementation

The experienced practitioner in residue analysis is accustomed to the signals, e.g., the peaks in a gas chromatogram, that come up in the analysis of a blank material. By definition, this material does not contain a residue, and the signal detected at the place where a compound of interest would normally occur stems from coextractives or other substances that could not be separated completely during clean-up.

From experience with a large number of blank analyses, the practitioner knows that the height of this signal may vary considerably, but that it would not go beyond a certain maximum height. He estimates this maximum height from the many chromatograms he has seen, and he includes in his estimate a "reserve" on top of the peak heights oberved, since he wants to be sure that no blank peak at any time in the future goes beyond that estimate. Should a signal now extend beyond this maximum height, a residue must be present. Thus the maximum height of the blank signal is the decisive criterion of whether a residue has been detected or not. To convert the maximum height into a concentration value, a calibration line is employed. The concentration value thus calculated is called "detection limit" (DTC).

In order to draw up the calibration line, several batches of blank material are spiked with increasing concentrations of the compound in question. Spiking should start at the concentration level of a provisionally estimated DTC and should go up to about ten times this level. Each spiking sample is taken through the whole analytical procedure. The signals obtained, e.g., their heights, are then mapped onto a 2-dimensional diagram in the direction of the y axis, whereas the corresponding spiking concentrations are noted on the x axis. A subsequent regression calculation provides the calibration line or, more precisely, the "calibration line of the complete analytical procedure".

For the mathematical-statistical implementation of this approach, the calibration line and its prediction intervals are considered (Fig. 1). The edges of the prediction intervals above and below the calibration line determine the maximum and minimum heights of a future signal at a selected concentration. Hence the upper prediction interval margin at zero concentration represents

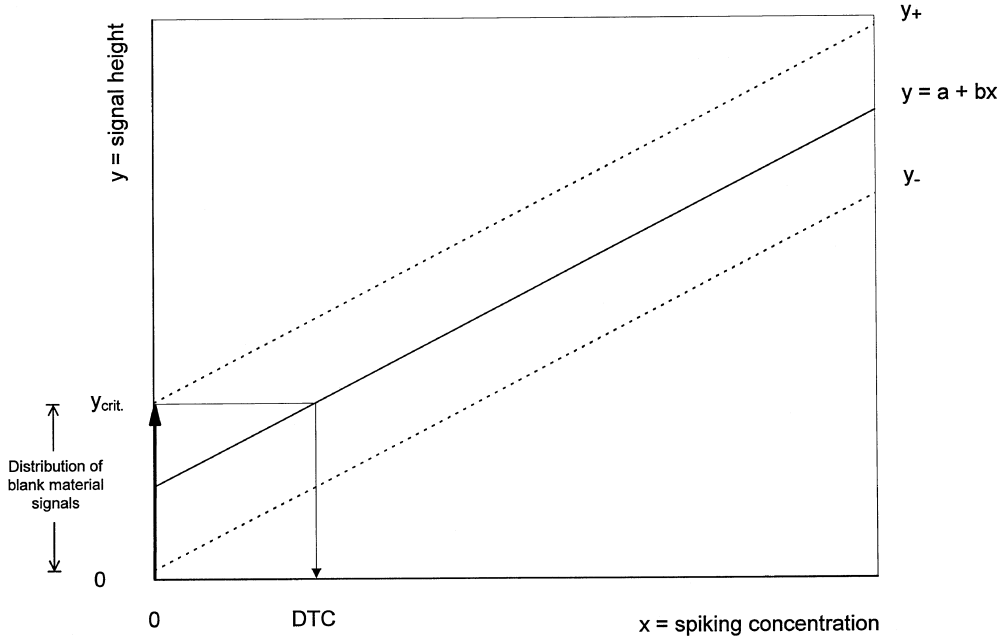

Fig. 1 Calibration line ($y = a + bx$) with prediction intervals ($y+$, $y-$) and graphical deduction of DTC. The arrow on the y axis reflects the maximum future signal height from a blank material

the maximum peak height that may be encountered from the analysis of a blank material (y_{crit} in Fig. 1). Transforming this signal height via the calibration line into a concentration provides DTC. In this way analytical practice is sufficiently well reflected, and this approach was taken as the basis of DIN 32645 [4].

In order to cover virtually every future peak from a blank material we calculate the upper prediction interval for a probability of 95% which is usually sufficient for analytical purposes [5]. Since only the maximum peak height is of interest, but not the maximum and minimum at the same time, we have a 1-sided statistical consideration. Consequently, the prediction interval is computed for 95%, for a 1-sided consideration and for a single future analysis.

The prediction intervals at both sides of the calibration line are limited by hyperbolic functions. In a "good" calibration these hyperbolae appear as straight lines parallel to the calibration line itself. This is also the case in the figures presented here, but it does not influence the mathematical-statistical considerations.

In analytical practice the lower prediction interval limit may well go to a negative signal value at zero spiking concentration. This corresponds to negative signals from the analysis of a blank material, which is very rare however. Thus we see here the limitations of the linear regression line model for the lower end of the calibration function, where signals are rather determined by coextractives than by the substance traces the analytical procedure intends to detect.

Analysis of a residue actually present: identification limit

When a residue is indeed present in a material, repeated analyses will result in a range of different concentration values because of the scatter of analytical results. The concentrations found will be above and below the actual residue concentration and will give a Gaussian distribution when a large number of analyses are carried out. Hence, with a residue concentration of DTC, analytical results will scatter around DTC. After a sufficiently large number of analyses, the distribution will show that 50% of the results are above DTC and 50% below. Thus, in 50% of the cases the residue will be declared "detected", whereas in the remaining 50% a "not detected" is reported (Fig. 2).

In daily laboratory routine, often large numbers of samples have to be inspected, and this allows only a single analysis to be performed on each sample. Consequently, when a sample contains an actual residue concentration of DTC, the analyst has only a 50% chance of detecting the residue. The probability of not detecting the residue is equally 50%, but this is an error. This false-negative error is called a 2nd order error or beta-error, and the probability for it is 50%.

The 50% error at DTC concentration has led to quite a few concepts (and to many more hours of heated discussions) that fix DTC at a higher concentration level than described here, in order to reduce the 2nd order error to an acceptable value of, say, 5%. The practitioner will however continue to employ DTC in the way he is used to, namely by saying that below DTC any residue is "not detected", whereas above DTC a residue is "detected". This is always a 50% 2nd order error for any DTC concentration level, no matter how high DTC is. Therefore setting DTC at a higher concentration level leads to contradictions in the statistical argumentation [7].

When an actual residue concentration is higher than DTC, the results from repeated analyses will scatter around this higher concentration, and fewer than 50% will fall below DTC. Therefore a single analysis has a higher chance than 50% of detecting the residue. When the concentration is sufficiently high, virtually all single analyses (e.g., 95% of them) will show that a residue is "detected", because virtually none of the analytical results lies below DTC. This concentration is the identifi-

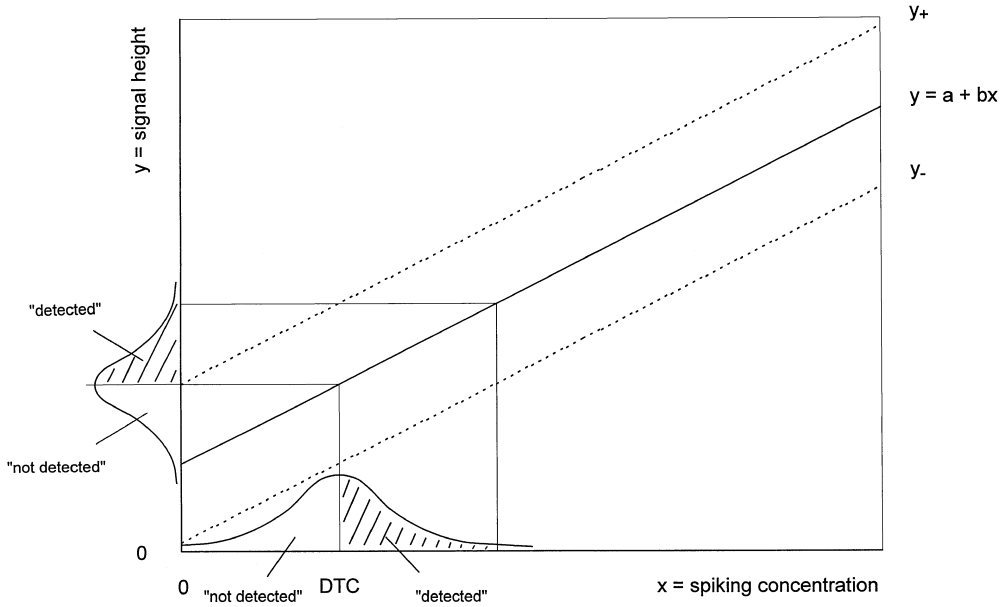

Fig. 2 Variability of analytical results at DTC concentration. The Gaussian distributions represent the abundance of the signals (y axis) and of the concentrations (x axis), respectively

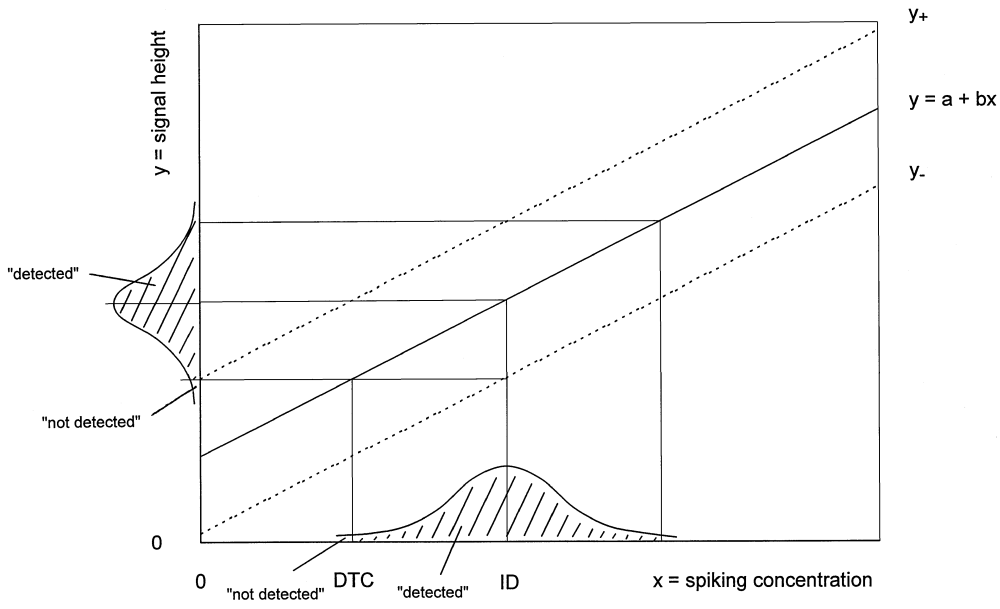

Fig. 3 Variability of analytical results at ID concentration. The Gaussian distributions represent the abundance of the signals (y axis) and of the concentrations (x axis), respectively

cation limit (ID). With the usual statistical assumptions, it is twice as high as DTC (Fig. 3) [4].

In practice this means that a horizontal line is drawn from the point where the upper prediction interval limit cuts the y axis to the right until it reaches the lower prediction interval limit, which is equally computed for 95% probability, for a 1-sided consideration and for a single future analysis. Going down to the x axis provides ID.

Both prediction interval limits have the same vertical distance from the calibration line at a given concentration. This can easily be recognized from the formulae [8]. Calculating ID as being 2 × DTC requires that they also be symmetrical in the horizontal direction. However, this did not seem to be the case with the computer programme which we had written in the course of this work [9] and which performed all calculations with a remarkable accuracy of 18 digits. In general, the lower prediction interval, when calculated via the height formula (= vertical height), was situated up to 2% of 2 × DTC to the left of 2 × DTC. Plotting the upper and lower prediction interval limits using either the formulae for the (vertical) height or for the (horizontal) width [8] gave two different lines for each limit. Therefore ID should be between 0% and 2% to the left of 2 × DTC. This minor difference can be taken account of by the computer programme employed. For the practitioner, an ID of 2 × DTC will probably be sufficient in most cases.

On the other hand, when going down from DTC to lower concentrations, the chance that a single analysis may detect an actual residue is always lower than 50%, because less than 50% of the results of repeated analyses would lie above DTC. This probability decreases with decreasing residue concentration; but also an actual residue below DTC can be detected with a single analysis.

Determination limit: analytical practice and statistical implementation

In the low concentration range, the variability of analytical signals is relatively high. This is due to the possible presence of coextractives or other components that are not separated during clean-up. With increasing concentration and, consequently, signal height, the relative scatter decreases and the results of analyses are increasingly precise. Thus, from a certain limit upwards, the result of an analysis may be reported as a reliable number. This limit is called “determination limit” (DTM).

Criterion 1: Sufficient distance between determination limit and detection limit

In analytical practice, a signal should be about three times as high as the largest possible signal from a blank analysis before a trustworthy figure may be given. Experience tells us that from this high point upwards the influence of coextractives on a signal is sufficiently small to allow for reporting a precise number. In this sense the region of high relative variability is below DTM concentration, in particular around DTC, and the region of low relative variability is at DTM concentration and above. Hence DTC and DTM must be sufficiently separated from each other. Since this is the first criterion that must be satisfied by DTM, the value resulting from this condition will be called DTM1.

If we take this into consideration in the statistical model, it means that the Gaussian curves produced by the concentrations DTC and DTM virtually do not overlap. Therefore, a horizontal line has to be drawn

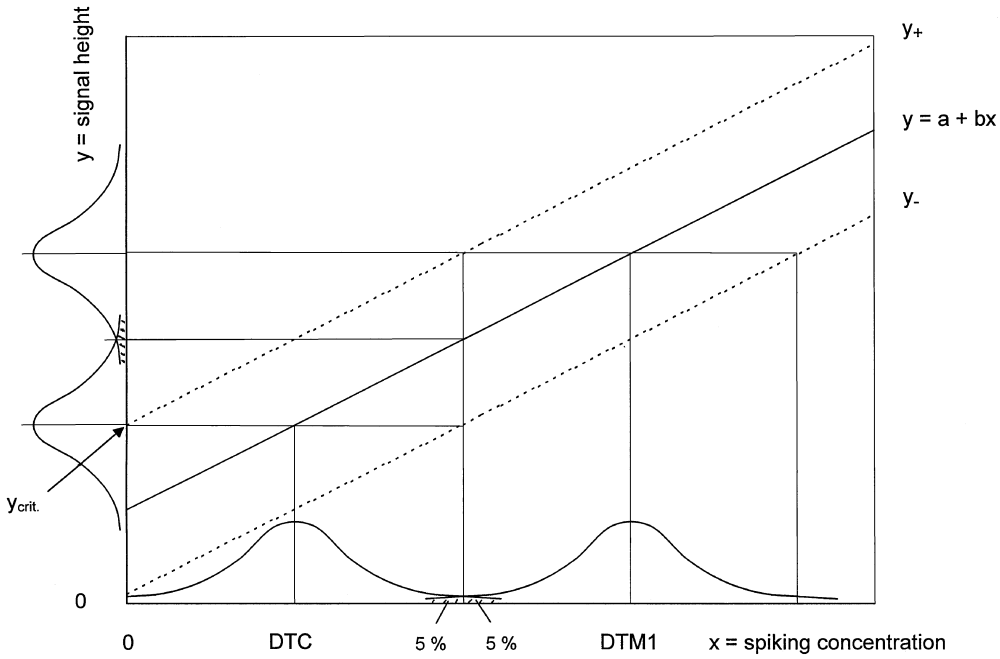

Fig. 4 Variability of analytical results at DTM1 concentration. The Gaussian distributions represent the abundance of the signals (y axis) and of the concentrations (x axis), respectively

from y_{crit} to the right towards higher concentrations (Fig. 4). The intersection of this line with the edge of the lower prediction interval is the upper end of DTC's Gaussian. At the same time this will be the lower edge of DTM's Gaussian. Hence a vertical line has to be drawn up to the borderline of the upper prediction interval. Going from this new point of intersection to the right until the lower prediction interval is reached gives the width of DTM's Gaussian. DTM itself is found when dropping down onto the x axis at the point where this horizontal line cuts the calibration line (Fig. 4). In this way, DTM1 only provides signals which are always larger than any signal from DTC concentration. Thus one can indeed say that DTC and DTM1 are sufficiently well apart from each other.

When taking the 1-sided prediction interval of 95% probability, the Gaussian curves of DTM1 and DTC overlap by only 5% each (Fig. 4). It seems plausible to apply the 1-sided interval also here, since for DTM1 only the variability of signals down to lower concentrations is of interest, whereas for DTC only the scatter upwards is considered.

Criterion 2: Complete recovery at determination limit and above

The second criterion for DTM is that recovery should be complete from this concentration level upwards [5]. In practical terms this means that recovery should neither be too low, nor should it go far beyond 100% (second criterion for DTM, giving DTM2). For residue analyses a widely accepted minimum is 70% [5], and 120% should be the upper mark for a "complete" recovery.

In order to check this criterion, the calibration line of the so-called "basic analytical procedure" has to be drawn up. Standard solutions containing only the compound of interest in a suitable solvent are injected into the analytical measurement system, thus providing signal heights resulting from the pure compound. The standards should have the same concentrations as the injected solutions from the "complete analytical procedure" when assuming a 100% recovery. From the signals obtained, the calibration line of the basic analytical procedure is calculated by linear regression.

Since the standards only contain the pure compound but no coextractives, signal variability should be rather small, much smaller than from the spiking assays. Hence it may be sufficient to inject just a few standards that cover the whole calibration concentration range. The minimum number for this is certainly 3, since this is necessary to compute a regression line in order to take account of the analytical variability. Taking 2 data points or even 1 point plus the origin of the coordinate system would be less adequate.

The calibration line of the basic analytical procedure serves to convert the signals from the spiking assays into the corresponding recovered concentrations. In a further diagram the recovered concentrations are then plotted (on the y axis) against the spiking concentrations (on the x axis). By a subsequent regression, the "recovery function" is obtained (Fig. 5).

The ideal recovery function is a straight line that starts at the origin of the coordinate system and has a slope of 1, which means a recovery of 100% (Fig. 5, line 1). With just 70% recovery the slope is only 0.7, and with 120% it is 1.2. Any recovery line through the origin and within the funnel limited by these two extreme

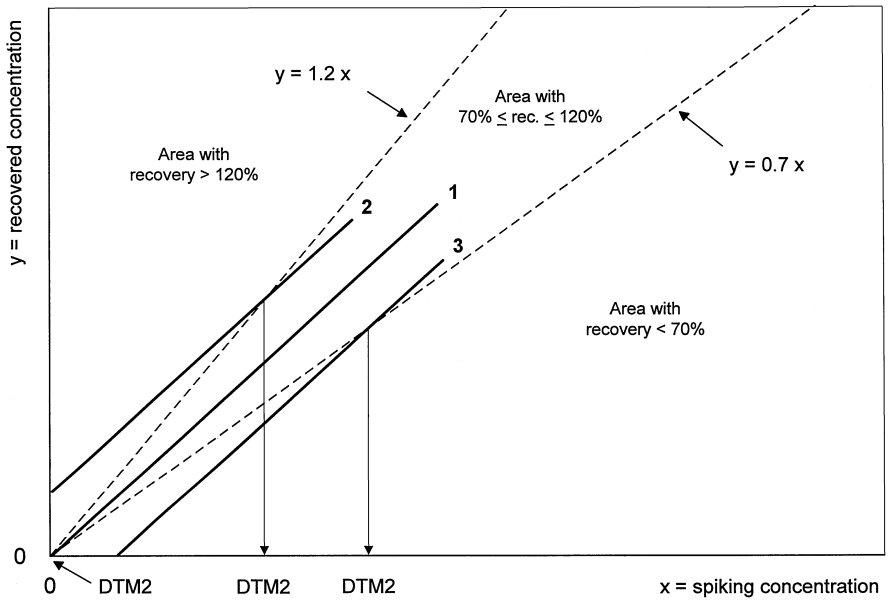

Fig. 5 "Recovery funnel". *1.* Ideal recovery function. *2.* Recovery function with a positive intercept. *3.* Recovery function with a negative intercept

recovery lines represents a recovery between 70% and 120%. Thus it satisfies the DTM2 criterion.

Should a recovery function have a positive intercept (Fig. 5, line 2), its slope must be below 1.2 in order to penetrate into the "recovery funnel". However, it must not be smaller than 0.7 because it should remain within this area. At the entrance point into the funnel a vertical line onto the x axis marks the concentration of DTM2 (Fig. 5).

In the case of a negative intercept (Fig. 5, line 3), the slope of the line must be steeper than 0.7 to reach the funnel area, but not larger than 1.2 as it must remain within it. This time the point of intersection with the lower edge of the funnel gives DTM2 on the x axis.

Even if a recovery line with a positive/negative intercept remains within the funnel, the recovery itself decreases/increases when going to higher concentrations. Should the recovery function not reach the funnel area within the calibration range, no DTM can be given, strictly speaking. However when a DTM must be reported, it should be accompanied by the actual recovery encountered at DTM concentration.

Criterion 3: Limited variability at determination limit and above

The third criterion for DTM is that variability should be sufficiently low at this concentration level (and above). In residue analysis the widely recognised quantitative criterion is a variation coefficient of ±0.2 or ±20% [5]. The variation coefficient is the standard deviation divided by the mean, and it describes the scatter of about 68% of the events that were measured in the past, provided that these events followed a Gaussian (= normal) distribution [10]. If we want to cover 68% of the signals that scatter around the calibration line, we draw two parallel lines above and below the calibration line itself at a distance of ± one residual standard deviation [11]. This band with a height of "± residual standard deviation" corresponds to the usual ± standard deviation around the mean. Dividing the (vertical) height of this band by the y value of the calibration line at a determined concentration gives the "relative residual standard deviation".

A more illustrative representation of the signal scatter around the calibration line is the prediction interval, e.g., the 2-sided prediction interval for a probability of 95%, which includes virtually all signals (more precisely: 95% of all signals) above and below the calibration line that may ever come up in the future. Dividing the (vertical) height of the band by the y value of the calibration line at a determined concentration gives the "relative prediction interval height" ($H_{\text{rel.}}$).

If we now try to establish a maximum variability criterion for DTM using the prediction interval height, we have to consider that this interval is based on the t distribution, which is wider than the normal distribution, and that it aims at future events, which enlarges variability even further. Therefore we have to go sufficiently beyond the ±20% criterion for the variation coefficient.

Inspections of more than 40 calibration lines of organochlorine compounds, sulfonamides, substances with hormonal action and heavy metals analysed by GC, HPLC, ELISA, RIA or atomic absorption showed that the concentrations where the relative residual standard deviation was ±20% provided a relative prediction interval height of ±47% to ±53% (Table 1). Thus, a relative prediction interval height of some ±50% would be about equivalent to the ±20% variation coefficient

Table 1 Relative prediction interval heights corresponding to a ±20% variation coefficient, and relative residual standard deviation corresponding to a ±30% relative prediction interval height

Substance class[a]	Relative prediction interval height, in ±% corresponding to a ±20% variation coefficient	Relative residual standard deviation, in ±% corresponding to a ±30% relative prediction interval
Organochlorine compounds	47–48	12–13
Sulfonamides	48–49	12–13
Substances with hormonal action	47–52	12–13
Heavy metals	49–51	12–13

[a] For details on compounds in each substance class see Table 3

criterion. Yet ±50% appeared to be too much for the DTM criterion that should assure a "limited" variability.

On the other hand, concentrations at a ±30% relative prediction interval height gave a relative residual standard deviation of ±12% to ±13% (Table 1). This was more conservative than the usual ±20% variation coefficient, but it seemed to be preferable from a practical point of view: A ±30% relative prediction interval height means that with a DTM peak of 3 cm in a chromatogram of 15 cm height, peaks from the analysis of a DTM material could scatter by about 1 cm at most, i.e., from 2 cm to 4 cm. This seems to be quite a lot, but, as explained before, it takes account of 95% of all peaks that may ever come up in a future analysis, and in addition it is more conservative than the usual ±20% variation coefficient.

The third criterion for DTM is thus that the relative vertical height of the 2-sided 95% prediction interval is ±30% at DTM concentration (Fig. 6). The resulting concentration will be called DTM3.

Final decision

Finally, the three provisional concentration values for DTM, namely DTM1, DTM2 and DTM3 are compared with each other (similar to [5]). The highest value is DTM, since it satisfies all three criteria.

Reporting analytical results

As a rule, the mathematical formulae for DTC, ID and DTM are designed for application to single analytical results only [4, 5], but not for instance to averages. This is quite well adapted to laboratory practice where large numbers of samples and limited resources in personnel and time often only allow a single analysis of each sample. In reporting analytical results in the concentration range of DTC, ID and DTM, the practitioner will not want to give the single result that he has found. He knows that variability is considerable around this concentration level, and consequently he may have found a result, e.g., above DTC, whereas the actual concentration in the sample is below. Therefore he has to formulate his finding accordingly (Table 2) [4].

Should the result be below DTC, a "not detected" should be reported. An actual concentration that may nevertheless be present will not be higher than ID, since ID is the lowest concentration that will virtually always be found. Therefore, in addition to the "not de-

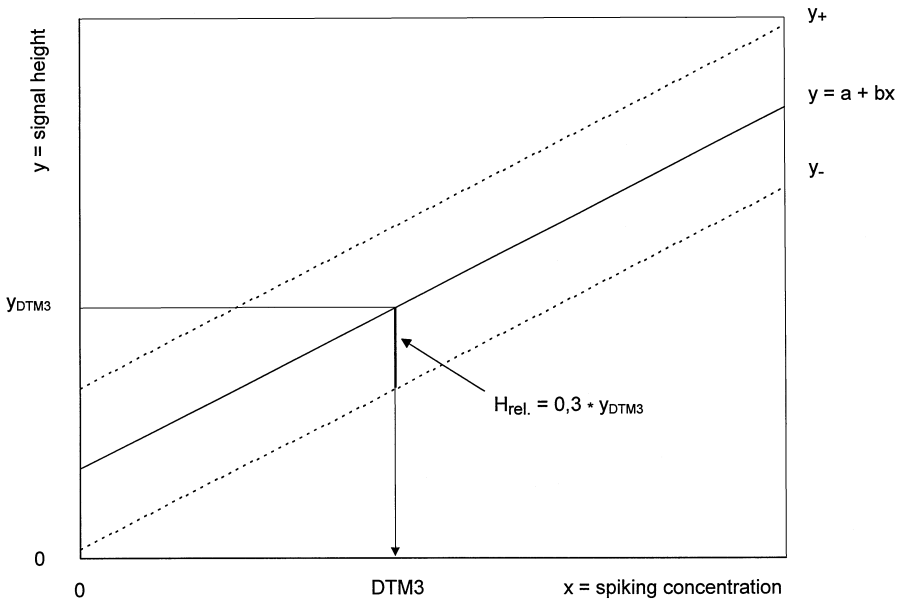

Fig. 6 Variability of analytical signals at DTM3 concentration. *H*rel. = relative prediction interval height

Table 2 Reporting analytical results

Result x	Report	Additional information
$x < \mathrm{DTC}$	Not detected	Maximum limit <ID>
$\mathrm{DTC} \leq x < \mathrm{DTM}$	Detected, not determinable	Determination limit <DTM>
$x \geq \mathrm{DTM}$	Concentration	Horizontal prediction interval width

tected", the "maximum limit [numerical ID value]" or "< [numerical ID value]" should be given. Reporting the numerical value of DTC, as is often done in analytical practice, provides less valuable information.

When the analysed compound is considered an "impurity", a "not detected" guarantees that the concentration is not above ID. In this sense ID is the "limit of guarantee of purity", an expression that was introduced in a very early concept on DTC and ID [12].

When the single analytical result lies between DTC and DTM it should be reported as "detected", adding eventually that it was "not determinable, determination limit [numerical value of DTM]". Since variability is still considerable in this concentration range, the actual concentration may even be slightly above DTM, but the numerical DTM value shows in which concentration area the result was obtained.

With an analytical result above DTM, the numerical value of the result should be reported. Even if the actual concentration may be slightly below DTM the variability is quite similar. Thus, the numerical value is sufficiently reliable. Further to the numerical value, the horizontal width of the 2-sided prediction interval at this concentration may be reported.

Application to analytical practice

Fortification experiments and computations

In order to illustrate the approach to DTC, ID and DTM described here, selected organochlorine compounds, sulfonamides, substances with hormonal action and heavy metals were added to blank samples of muscle, serum and urine samples. The spiking concentrations started at the level of the DTCs estimated from previous experience and raised to 8 or 10 times this level. Within this range, 3 fortification experiments were repeated at each of 4 evenly distributed, distinct concentration levels, thus giving 12 fortifications for each compound [7]. Details of sample clean-up and instrumental determination were described earlier [14, 15].

Regression lines and DTC, ID and DTM (Table 3) were calculated with a self-made computer programme [9], but may also be obtained from the formulae given below. It may be interesting to know that DTC, ID and DTM may as well be calculated from the recovery function of a substance.

Table 3 Parameters of the recovery function ($x_{\mathrm{fnd,fort}} = a_{\mathrm{rec}} + b_{\mathrm{rec}} x_{\mathrm{fort}}$) and $\mathrm{DTC_{C98}}$, $\mathrm{ID_{C98}}$ and $\mathrm{DTM_{C98}}$, calculated from the fortification experiments by applying the concept presented here. Results for pesticides and substances with hormonal action are given in ng/kg, those for sulfonamides and heavy metals in μg/kg

Substance	b_{rec} [%]	a_{rec}	Corr. coeff.[a]	$\mathrm{DTC_{C98}}$	$\mathrm{ID_{C98}}$	DTM1	DTM2	DTM3	$\mathrm{DTM_{C98}}$
DDE, pp	87.1	6	0.9949	30	61	90	18	**110**	110
HCB	85.9	−12	0.9944	32	63	94	77	**136**	136
HCH, α	83.7	− 3	0.9950	30	60	89	20	**119**	119
PCB 101	80.3	− 7	0.9925	37	73	108	64	**149**	149
Dexamethasone	108.0	128	0.9901	108	215	317	**1063**	293	1063
Diethyl-stilboestrol	119.9	−36	0.9960	34	68	101	71	**160**	160
Progesterone	85.1	76	0.9946	84	168	**248**	217	236	248
Salbutamol	83.3	308	0.9958	344	685	**1015**	839	967	1015
Sulfadimidine	97.2	− 9	0.9985	17	33	50	33	**75**	75
Sulfadoxine	83.3	4	0.9992	12	25	37	11	**44**	44
Sulfapyridine	90.0	− 5	0.9982	18	36	53	23	**76**	76
Sulfisoxazol	77.0	5	0.9984	18	35	52	13	**62**	62
Arsene	99.9	− 8	0.9990	5.2	10	16	28	**29**	29
Cadmium	118.0	− 2	0.9983	2.0	4.0	6.0	3.7	**9.4**	9.4
Mercury	104.9	11	0.9991	9.2	18	27	**75**	26	75
Selenium	97.7	− 2	0.9979	37	74	110	9.4	**147**	147

[a] correlation coefficient

A comparison with other approaches

In current analytical literature, DTC and DTM are often calculated from the blank average signal by adding a multiple of the blank signal variation. The basis for doing so can be found in a quite early concept [12] which calculated DTC from the "average blank signal + 3 standard deviations of the blank signal". In accordance with this, DTM was elsewhere computed from the "average blank signal + 10 standard deviations of the blank signal" [13]. In either case, however, the observed signal value (y axis) had to be mirrored at the calibration line of the complete analytical procedure in order to give DTC and DTM (on the x axis), respectively. In the following comparison we index the resulting DTCs with "3SD" and the DTMs with "10SD" (Table 4).

The results from a further approach, especially designed for residue analysis [5], will be marked "DFG" (Table 4), and an approach for chemical analysis in general [4] will be designated "DIN" (Table 4). As mentioned above, these two approaches served as the basis for the concept presented here. Finally the results computed from the present approach are labelled "C98" for "Concept '98" (Table 3).

Table 4 DTC, ID and DTM from other statistical approaches than that presented here, calculated from the fortication experiments (3SD from [12], 10SD from [13], DFG from [5], DIN from [4]). Results for pesticides and substances with hormonal action are given in ng/kg, those for sulfonamides and heavy metals in μg/kg

Substance	DTC_{3SD}	DTM_{10SD}	DTC_{DFG}	DTM_{DFG}	DTC_{DIN}	ID_{DIN}	DTM_{DIN}
DDE, pp	25	57	73	109	46	92	149
HCB	12	29	76	134	48	97	155
HCH, α	21	44	72	107	46	91	147
PCB 101	46	103	88	147	56	112	180
Dexamethasone	87	198	257	381	164	329	515
Diethyl-stilboestrol	72	172	82	158	52	104	166
Progesterone	75	159	202	300	129	259	411
Salbutamol	83	202	828	1232	529	1059	1684
Sulfadimidine	17	47	41	74	26	52	84
Sulfadoxine	7.2	20	30	45	19	38	62
Sulfapyridine	20	58	44	75	28	55	90
Sulfisoxazol	28	67	42	63	27	53	87
Arsene	0.3	0.8	13	19	8.0	16	26
Cadmium	0.5	1.5	4.9	7.3	3.1	6.2	10.1
Mercury	3.2	9	22	33	14	28	46
Selenium	18	48	89	133	56	113	183

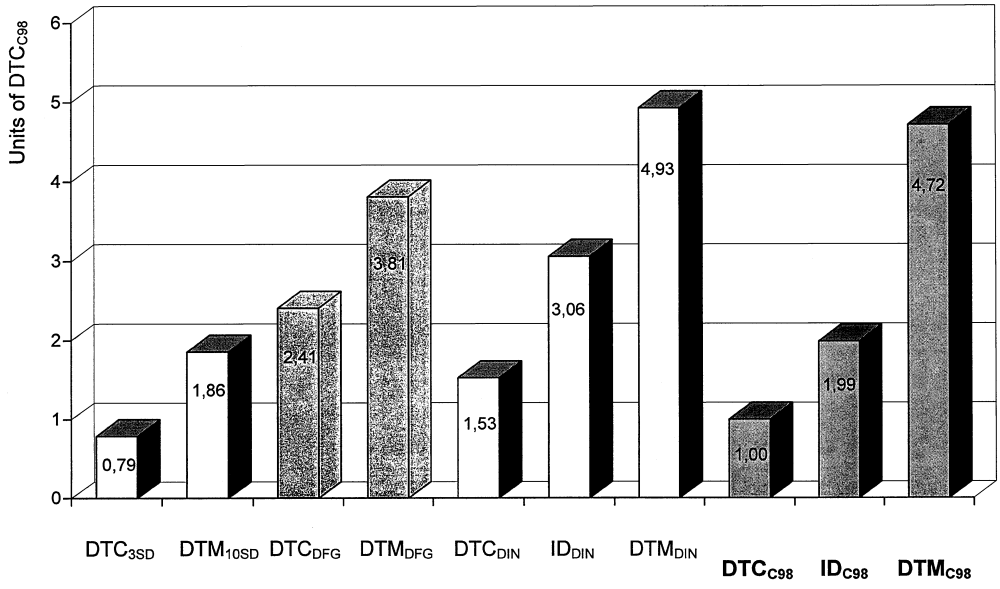

Fig. 7 Relative mean values of DTC, ID and DTM from different approaches, calculated from the fortification experiments, with DTC_{98} set to 1.00

When taking DTC_{C98} as the basis for comparison, DTC_{DIN} values were about 1.5 times higher (Fig. 7); the reason for this was that C98 employs the 95% prediction interval, whereas DIN requires 99%. DTC_{DFG} values were some 2.4 times above DTC_{C98}, because DFG has a different argumentation for deducing DTC (but see also [7]). DTC_{3SD} proved to be slightly lower than DTC_{C98}.

Comparing the ID showed that ID_{DIN} values were 1.5 times higher than ID_{C98} (Fig. 7); this again reflected the higher probability DIN uses for the prediction interval.

The calculated DTM_{C98} most often resulted from DTM3 (Table 3), i.e., the criterion for a limited variability was most often decisive. In comparison with DTM_{C98} the DTM_{10SD} values were only 0.39 times as high (Fig. 7). DTM_{DFG} values also were below DTM_{C98}, namely by a factor of 0.81, DTM_{DIN} values were somewhat above.

As explained above, during the deduction of DTM, the laboratory practitioner expects a sufficient distance between DTC and DTM. From experience a factor of about 3 would be the least to request.

The DTM_{C98} values calculated from the fortification experiments were about a factor of 4.7 higher than the DTC_{C98} (Fig. 7); this was somewhat more than what an analyst would look for, but reflects the "safety net" the three criteria for DTM_{C98} offer. In contrast to this, DTC_{3SD} and DTM_{10SD} only differed by a factor of 2.4, and between DTC_{DFG} and DTM_{DFG} there was only a factor of 1.6, which indeed proved to be too little. DTC_{DIN} and DTM_{DIN} provided a difference by a factor of 3.2.

Performance characteristics of the analytical procedure employed

When establishing DTC, ID and DTM as described here, the computations also provide a number of data that reflect the performance of the analytical procedure employed. These data include the slope of the calibration line (sensitivity of the procedure), the intercept (influence of coextractives), correlation coefficient (link of concentrations and signals), residual standard deviation (variability of signals around the regression line), standard deviation of the procedure (= residual standard deviation divided by the slope; reflects the procedure's absolute precision) and variation coefficient of the procedure (= standard deviation of the procedure divided by the mean of spiking concentrations; for comparison amongst different laboratories).

Taking advantage of modern instruments' capabilities

It is of the utmost importance that the obtained signal is converted into the analytical result via the calibration line of the complete analytical procedure. Only this calibration line sets the finding at the right place on the concentration axis (= x axis).

Modern analytical instruments in AAS, GC and GC-MS, HPLC, ELISA or RIA may offer the possibility to establish a calibration line by injecting several calibration solutions in the course of a series of samples. These calibration solutions should originate from spiking concentrations analysed in parallel with the samples, thus enabling the instrument to calculate the calibration line of the complete analytical procedure and, subsequently, the sample concentrations.

When the calibration solutions just contain the pure compound, only the basic analytical procedure is calibrated. This gives results that do not take account of the recovery, which may be extremely low, e.g., around 40% for polar pesticides in water, or which appear to be extremely high, e.g., about 180% for corticosteroids in urine. In the latter case, the measured signals would lead to much too high results and thus render concentrations "detectable" which indeed are not. The unpleasant fact is that recovery of such analytical procedures should be brought closer to 100%, even if this may be difficult for the practitioner working under unfavourable conditions.

Consequently, when calibrating only the basic analytical procedure in daily analyses, the resulting concentrations found by the instrument should be plotted onto the y axis of the recovery function and then be converted into actual concentrations on the x axis. In this way, the recovery established in the spiking assays is taken into account. The better way however is to always calibrate the complete analytical procedure.

Mathematical formulae

Calibration line of the complete analytical procedure

$$y = a + bx$$

with: y = signal value at concentration x
x = a substance's concentration in a sample
a = y intercept
b = slope

Slope b of the calibration line

$$b = \frac{\sum_{i=1}^{n} y_i x_i - \frac{1}{n} \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{\sum_{i=1}^{n} x_i^2 - \frac{1}{n} \left(\sum_{i=1}^{n} x_i \right)^2}$$

with: x_i = fortification concentration of sample i
y_i = signal value of sample$i$
n = total number of calibration analyses
i = index of calibration analyses

Intercept a of the calibration line with the y axis

$$a = \frac{1}{n}\sum_{i=1}^{n} y_i - \frac{b}{n}\sum_{i=1}^{n} x_i$$

A minor drawback of the linear regression calculation is that it requires constant variability over the entire calibration range. But it is well known from analytical experience that variability increases along with concentration [16]. This can already be observed when the lowest and highest concentration of the calibration differ by a factor of 10 as suggested in general in the concepts for DTC, ID and DTM. Nevertheless a weighted regression that takes account of the variability increase does not necessarily provide the significant changes in DTC, ID and DTM [17] that may be expected (e.g. [18]).

Residual standard deviation s_y

$$s_y = \sqrt{\frac{\sum_{i=1}^{n}(y_i - (a + bx_i))^2}{n-2}}$$

Critical signal height y_{crit}

$$y_{crit} = a + s_y\, t_{f;\alpha}\sqrt{1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^{n}(x - \bar{x})^2}}$$

with: $t_{f;\alpha}$ = quantil of t distribution (single-sided) for $f = n-2$ degrees of freedom and a probability of 95% (error probability $\alpha = 0{,}05$)
$\bar{x}$ = mean value of the fortification concentrations of all calibration analyses

Detection limit DTC

$$\mathrm{DTC} = \frac{(y_{crit} - a)}{b} = \frac{s_y}{b}\, t_{f;\alpha}\sqrt{1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}}$$

Identification limit ID

To calculate ID, we saw that the horizontal line has to cut the lower prediction interval at the height of y_{crit}:

$$y_{crit} = s_y\, t_{f;\alpha}\sqrt{1 + \frac{1}{n} + \frac{(\mathrm{ID} - \bar{x}^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}} \quad \text{or}$$

$$y_{crit} - s_y\, t_{f;\alpha}\sqrt{1 + \frac{1}{n} + \frac{(\mathrm{ID} - \bar{x}^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}} = 0$$

Since it would be rather complicated to derive a formula for ID from this expression (in the form if ID = ...), the computer programme we wrote during this work [9] approximated ID as being equal to 2 × DTC and calculated the above expression. Where the result was not zero, the programme diminished ID by 0.1% and calculated the expression again. with this iteration procedure it approached the ID value that would make the above expression equal to zero. To keep the necessary time short we stopped the iteration when the expression was smaller than 0.01. This resulted in ID values that were up to 2% smaller than 2 × DTC. Since this difference is quite small for the practitioner's purpose, we set:

$$\mathrm{ID} = 2 \times \mathrm{DTC}$$

Determination limit DTM

Calculation of DTM1

The lower edge of the Gaussian distribution around DTM1 is ID (Fig. 4). Using the formula for the branches $y_{\pm}$ of the single–sided prediction interval:

$$y_{\pm} = \bar{y} + b\,(x - \bar{x}) \pm s_y\, t_{f;\alpha}\sqrt{1 + \frac{1}{n} + \frac{(x - \bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}}$$

we obtain y_{DTM1} by replacing x by ID:

$$y_{DTM1} = \bar{y} + b\,(\mathrm{ID} - \bar{x}) + s_y\, t_{f;\alpha}\sqrt{1 + \frac{1}{n} + \frac{(\mathrm{ID} - \bar{x})^2}{\sum_{i=1}^{n}(x_i - \bar{x})^2}}$$

with: $t_{f;\alpha}$ = quantil of t distribution (single-sided) for $f = n-2$ degrees of freedom and a probability of 95% (error probability $\alpha = 0{,}05$)
$\bar{y}$ = mean value of the signal values of all calibration analyses

$$\mathrm{DTM1} = (y_{DTM1} - a)/b$$

Calculation of DTM2

Linear regression of the signal and concentration values obtained from the instrumental analysis of standard solutions gives the calibration line of the basic analytical procedure:

$$y = a_{std} + b_{std}\, x$$

with: x = concentration of standard solutions
y = signal values from the analysis of standard solutions
a_{std} = y intercept of the calibration line of the basic analytical procedure
b_{std} = slope of the calibration line of the basic analytical procedure

With this calibration line, the signal values obtained in the fortification experiments (*y*fort) are converted into "found concentrations" ($x_{\text{fnd, fort}}$):

$$x_{\text{fnd, fort}} = (y_{\text{fort}} - a_{\text{std}})/b_{\text{std}}$$

Linear regression of the $x_{\text{fnd, fort}}$ (on the *y* axis) and the fortification concentration leads to the recovery function:

$$x_{\text{fnd, fort}} = a_{\text{rec}} + b_{\text{rec}}\, x_{\text{fort}}$$

with: x_{fort} = fortification concentrations
$x_{\text{fnd, fort}}$ = found fortification concentrations
a_{rec} = *y* intercept of the recovery function
b_{rec} = slope of the recovery function

DTM2 is then calculated according to Table 5.

Calculation of DTM3

Auxiliary terms:

$$Q = \sum_{i=1}^{n} (x_i - \bar{x})^2;\; D = (0.3\; b)^2 - \frac{(s_y t_{f,\,\alpha})^2}{Q}$$

$$\text{DTM3} = \bar{x} - \frac{b\; 0.3^2}{D}\,\bar{y} + \frac{s_y t_{f,\,\alpha}}{D}\sqrt{\left(1 + \frac{1}{n}\right) D + \frac{0.3^2 \bar{y}^2}{Q}}$$

with: $t_{f;\,\alpha}$ = quantil of *t*–distribution (double-sided) for $f = n-2$ degrees of freedom and a probability of 95% (error probability $\alpha = 0{,}05$)

Table 5 Calculation of DTM2. $a_{\text{rec}} = y$ intercept of the recovery function, b_{rec} = slope of the recovery function

Case	a_{rec}	b_{rec}	Calculation of DTM2
1	0	$0.7 \le b_{\text{rec}} \le 1.2$	DTM2 = 0
2	>0	$0.7 \le b_{\text{rec}} < 1.2$	$\text{DTM2} = \frac{a_{\text{rec}}}{1.2 - b_{\text{rec}}}$
3	<0	$0.7 < b_{\text{rec}} \le 1.2$	$\text{DTM2} = \frac{a_{\text{rec}}}{0.7 - b_{\text{rec}}}$
4	>0 =0 <0	$b_{\text{rec}} < 0.7$ $b_{\text{rec}} > 1.2$	DTM cannot be calculated

Final decision for DTM

The largest numerical value from among DTM1, DTM2 und DTM3 is selected as DTM.

A comprehensive example

The numerical example in Table 6 enables the interested reader to inspect the necessary calculations in detail.

Table 6 Comprehensive example: calculation of DTC, ID and DTM

Description	Numerical values			
Signal values from 3 fortification experiments at each of 4 concentration levels	Fortification concentrations (µg/kg)		Signal values (are units)	
	x_1	20	y_1	5661
	x_2	20	y_2	6640
	x_3	20	y_3	7639
	x_4	80	y_4	20712
	x_5	80	y_5	21871
	x_6	80	y_6	23163
	x_7	140	y_7	35006
	x_8	140	y_8	36221
	x_9	140	y_9	37358
	x_{10}	200	y_{10}	50473
	x_{11}	200	y_{11}	51522
	x_{12}	200	y_{12}	52729
Number of signal values	n			12
Mean of all concentrations	$\bar{x}$			110.0000
Mean of all signal values	$\bar{y}$			29082.9160
Further values	$\bar{x}^2$			12100
	$\sum_{i=1}^{n} (x_i - \bar{x})^2$			54000
y intercept (*a*) and slope (*b*) of the calibration line of the complete analytical procedure	a			1754.57
	b			248.44
Residual standard deviation	s_y			1046.0726

Table 6 (Continued)

Description	Numerical values			
Quantil of the t distribution (single-sided) for $f=n-2$ degree of freedom at a probability of 95% ($\alpha=0.05$)	$t_{f,\alpha}$			1.8125
Critical signal value	y_{crit}			3922.50
Detection limit (μg/kg)	**DTC**			**8.7**
Identification limit (μg/kg) (The value found by iteration was: 17.2)	**ID**			**17.4**
Signal value of DTM1	y_{DTM1}			8202.76
DTM criterion 1	DTM1			25.72
Signal values from the analysis of standard solutions (basic analytical procedure)	Concentrations (μg/kg)		Signal values (area units)	
	x_{std1}	20	y_{std1}	4628
	x_{std2}	20	y_{std2}	5514
	x_{std3}	20	y_{std3}	6462
	x_{std4}	80	y_{std4}	20643
	x_{std5}	80	y_{std5}	21542
	x_{std6}	80	y_{std6}	22542
	x_{std7}	140	y_{std7}	37478
	x_{std8}	140	y_{std8}	38347
	x_{std9}	140	y_{std9}	39309
	x_{std10}	200	y_{std10}	53462
	x_{std11}	200	y_{std11}	54311
	x_{std12}	200	y_{std12}	55234
y intercept (a) and slope (b) of the calibration line of the basic analytical procedure	a_{std}			35.02
	b_{std}			272.01
Found concentrations ($x_{fnd,fort}$) after conversion of the signal values y (complete analytical procedure) by means of the calibration line of the basic analytical procedure	Fortification concentrations (μg/kg)		Found concentrations (μg/kg)	
	x_{fort1}	20	x_{fnd1}	20.68
	x_{fort2}	20	x_{fnd2}	24.28
	x_{fort3}	20	x_{fnd3}	27.95
	x_{fort4}	80	x_{fnd4}	76.02
	x_{fort5}	80	x_{fnd5}	80.28
	x_{fort6}	80	x_{fnd6}	85.03
	x_{fort7}	140	x_{fnd7}	128.57
	x_{fort8}	140	x_{fnd8}	133.03
	x_{fort9}	140	x_{fnd9}	137.21
	x_{fort10}	200	x_{fnd10}	185.43
	x_{fort11}	200	x_{fnd11}	189.28
	x_{fort12}	200	x_{fnd12}	193.72
y intercept (a) and slope (b) of the recovery function	a_{rec}			6.32
	b_{rec}			0.91
Intersection point ($x_{1,2}$) of the recovery function with the upper edge of the "recovery funnel" ($y=1.2\ x$; case 2 in Table 5)	$x_{1,2}$			22.05
DTM criterion 2	DTM2			22.05
Quantil of the t distribution (double-sided) for $f=n-2$ degrees of freedom with a probability of 95% ($\alpha=0.05$)	$t_{f,\alpha}$			2.2281
Auxiliary term Q	Q			54000
Auxiliary term D	D			5454.42
DTM criterion 3	DTM3			27.34
Determination limit (μg/kg)	**DTM**			**27.3**

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