

**NPL REPORT
DQL-AS 014**

**Analytical methodologies
with very low blank levels:
implications for practical
and empirical evaluations
of the limit of detection**

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NOT RESTRICTED

January 2005

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ISSN 1744-0602

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We gratefully acknowledge the financial support of the UK Department of Trade and Industry (National Measurement System Policy Unit)

Approved on behalf of Managing Director, NPL
By S Windsor, Business Leader, Division of Quality of Life

Executive Summary

Limit of detection criteria in analytical chemistry are regularly specified in terms of the distribution of the measured blank response. The consequences of an analytical blank response with a negligible distribution of measured values on the calculation and significance of limit of detection criteria receive scarce attention. In this report a consideration of a hypothetical analytical methodology where the blank response is zero and the distribution of blank values is negligible is described. The impact of this situation on the traditional limit of detection criteria and on calibration relationships is discussed in detail. A simple, empirical method of estimating indicative method detection limits based on whole-method repeatability is proffered. This model has been validated with experimental data from NPL and LNE (France).

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Introduction

The limit of detection [1,2] is an important performance characteristic of an analytical method [3,4]. However, despite being a straightforward concept with readily understood application, it is difficult to define clearly and unambiguously [5]. Detection criteria are not boundaries in a binary state indicating the presence or absence of an analyte but are indications of when a specified probability for the presence or absence of an analyte has been attained [6].

The IUPAC compendium of Chemical Terminology [7] defines the detection limit (in analysis) as, “The minimum single result which, with stated probability, can be distinguished from a suitable blank value. The limit defines the point at which the analysis becomes possible and this may be different from the lower limit of the determinable analytical range.” In contrast, the VIM [8] (the international dictionary of metrology) contains no definition relating to limit of detection.

The problem of defining the limit of detection is usually formulated in terms of the critical detection limit (L_C) defined as, “the level at which it is possible to decide whether or not the result of an analysis indicates detection”, and the detection limit (L_D) defined as, “the level at which a given analytical procedure may be relied up on to yield detection”. L_D is a measure of the inherent detection capability, which is a performance characteristic of the analytical procedure, requiring knowledge of the acceptable false negative and false positive rates and of the probability distribution function of the blank and sample measurement. Consequently in the absence of a well-defined measurement process the detection limit may be without meaning.

The relationship between the blank-subtracted zero response (0_{corr}) and L_C , L_D , α and β is shown in Figure 1.

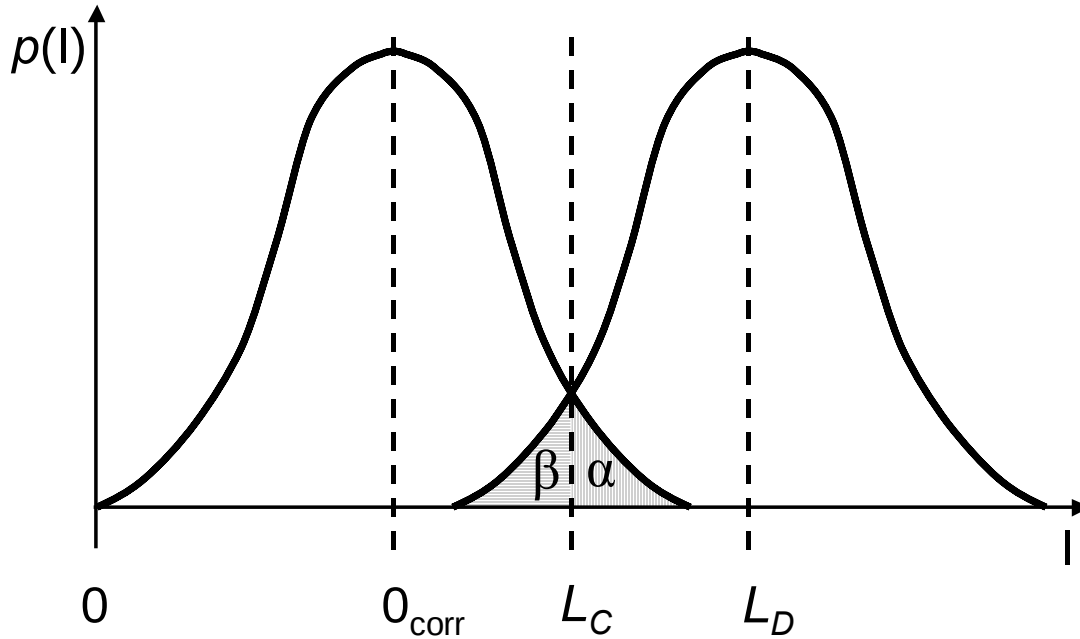


Figure 1. The probability density $p(I)$ displayed as a function of signal intensity (I) for blank (left) and sample (right) signal distributions under circumstances where the blank value is finite and the distribution of blank values is non-negligible. The relationship between the blank-subtracted zero response (0_{corr}) and L_C , L_D , α and β , is shown.

Critical decision limits L_C are, by usual definition, based on the distribution of the ‘blank’ signal response, σ_B , and the acceptable false positive rate, α . When L_C has been established, an *a priori* detection limit, L_D , may be defined by additional knowledge of the acceptable false negative rate, β , and the distribution of the sample signal, σ_S , at a level L_D [9]. An *a priori* limit of quantification, L_Q , may then also be defined as the minimum signal level at which the relative standard deviation of values attributable to the signal is $\leq 10\%$ of L_Q , the level at which a given procedure will be sufficiently precise to yield a satisfactory quantitative estimate [6]. For most routine uses of these quantities homoscedastic behaviour is assumed at low signal levels and

the assumption that $\sigma_S = \sigma_B$ is made. At low signal levels the distribution of these values is usually considered to be normal and the detection criteria, including detection and quantification limits that strictly rely additionally on the distribution of sample values, are simply defined in terms of multiples of the standard deviation of the (assumed) normal distribution of the blank values alone. However little consideration is given to situations where 'blank' signals are immeasurable or zero and exhibit a vanishingly small distribution of values. It is often impractical to use blank signal levels to establish detection criteria under these circumstances. It is not obvious how quantities relating to detection criteria are then established when the basis for traditional formulation is removed.

Discussion

To appreciate the implications of methodologies yielding very low blank levels on detection criteria it is first necessary to understand the importance of the role of the blank in analytical measurement [10]. Blank determinations are usually intrinsic to the accuracy of analytical measurements and the calculation of detection limits for analytical processes. Blank levels must be repeatedly analysed in order to achieve a realistic estimation of the analyte level within the blank, with a well-defined statistical distribution. Only then can blank corrections be implemented properly, detection criteria established and the robustness of analytical results retained. Three types of blank may be described in the context of analytical measurement:

- 1) The instrumental background; the signal response produced from the analytical instrument when no analyte is present.
- 2) The baseline; the instrumental background plus the signal produced by interfering species in the region of interest.
- 3) The analyte blank; the signal that arises due to the target analyte from all sources apart from the sample itself.

It is a type 3) blank which is usually used to assess detection criteria. This represents the situation where the largest signal response due to target analyte contamination would be expected, without actually introducing any sample. It is the only blank measurement expected actually to contain any of the target analyte. It is necessarily this blank level from which detection criteria are established and upon which discrimination of analyte present in the sample to that present in the blank is made (often L_C is defined as three times the standard deviation of the blank measurement [1]). However, circumstances occur practically where type 3) blanks are no larger than type 1) blanks. In fact all the blank levels may be vanishingly small with negligible distributions. The traditional procedures for establishing detection criteria must then be reconsidered.

Analytical techniques with very exacting and specific identification and detection criteria can lead to very low blank levels and often very narrow distributions of the

blank value. For example, during the sequential gas chromatography-mass spectrometry (GC-MS) identification of specific polyaromatic hydrocarbons (PAH) on ambient air-sampled filters, in order to be identified and detected, the target analyte species must both elute at the correct time from the GC and have the correct mass signature on the MS analyser. In effect this means that only the specific PAH requiring measurement will provoke ‘a non-zero’ response from the analytical instrument. The ultra-low levels of these specific analytes in the filters used for sampling and the solvents used for extraction, result in most PAH levels in the analyte blanks (or filter blanks in this application) being effectively zero with a negligible distribution of possible values. This situation is quite different to, for example, the analysis of total trace metals in ambient air by ICP-MS where the analytical methodology and analyte identification is not molecularly specific but instead measures a sum total of all the compounds containing the target elemental analyte regardless of specific speciation characteristics. It is unsurprising then that blank levels for the ICP-MS procedure and the associated distribution of the blank measurements are significantly larger than for the GC-MS methodology. The former situation, where blank levels are realisable and finite, is routinely considered when limit of detection theory is developed and discussed. However, if it is not practical or possible to use blank signal levels for the production of detection criteria, as in the GC-MS analysis of PAHs, other considerations may be necessary.

Consider that the blank response for the hypothetical methodology under consideration is zero. That is to say that the measurement of a blank cannot be distinguished from a situation where no sample is introduced into the machine. Consider also that the distribution of values around this zero response, effectively the noise of the detection system in its resting state, is negligible. In such a situation the detection decision limit, L_C , and analytical method-based detection levels, L_D and L_Q , will depend only on the distribution of values of the sample measurement and will not rely on the distribution or variability of the blank measurement. Under these conditions the critical detection level, L_C is zero and all positive signals result in an affirmative detection decision. The negligible distribution of blank results also necessarily confers a zero false positive rate on analytical measurements. The relationship between the absolute zero response, the blank-subtracted zero response, L_C , L_D , α and β under these circumstances is shown in Figure 2. (In traditional

considerations of detection theory, where the distribution of blank values is finite, a critical detection level of zero necessarily yields a 50% false positive rate [6]).

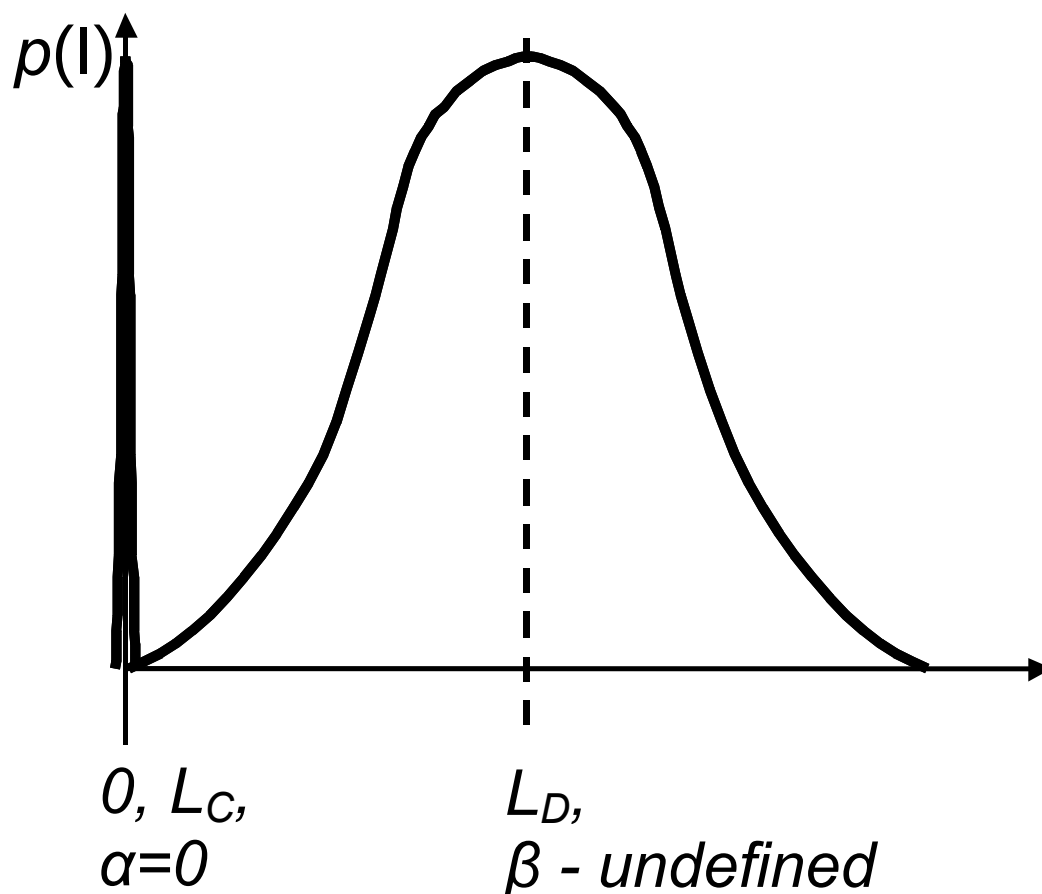


Figure 2. The probability density $p(I)$ displayed as a function of signal intensity (I) for blank (left, expanded for illustrative clarity) and sample (right) signal distributions under circumstances where the blank value is zero and the distribution of blank values is negligible. The relationship between the absolute zero response, 0 , L_C , L_D , α and β , is shown.

The critical detection criteria may then be directly related to the sensitivity of the machine, the instrumental detection limit. The critical detection limit practically becomes the minimum amount of target analyte species required to cause a change in the intensity response of the instrument, analogous to the definition of sensitivity:

$$L_C = \frac{\Delta x_i}{\Delta I} \quad (1)$$

Where Δx_i is the minimum change in mass fraction of the target analyte i required to cause an incremental change ΔI in the observed signal intensity.

In this situation, calculation of the limit of detection in terms of multiples of the standard deviation of the blank is impracticable and leads to unrealistically low, or zero, detection and quantitation limits. These limits will be defined purely by the distribution of sample measurements and the allowable false negative rate, β . The actual meaning of β under these circumstances is discussed later.

Appreciation of the relationship of the detection criteria to relative signal levels is simply the first stage in defining whole method detection limits. It is necessary to appreciate that the limit of detection is a property of a method, with defined parameters, related to a situation where an amount of a target analyte becomes detectable, and not a property solely of signal intensity. Similarly to other performance characteristics, detection limits may not be rigorously established in the absence of a fully defined measurement process, measurement equation, measurand and robust uncertainty statement. Treatments of detection limit criteria involving multiples of measured signal levels are relatively straightforward. However complications often occur when these quantities are translated from the signal domain to the analyte amount content domain. It is in the signal domain, though, where these quantities become realistically useful in judging compliance with limit values and upholding legislative limits. Whilst the concentration domain is limited at the low end by zero, under usual circumstances, where the finite blank response and its distribution is finite, and the response domain is in principle unbounded. There is no inherent requirement for the calibration function to pass through the origin. This situation is shown diagrammatically in Figure 3.

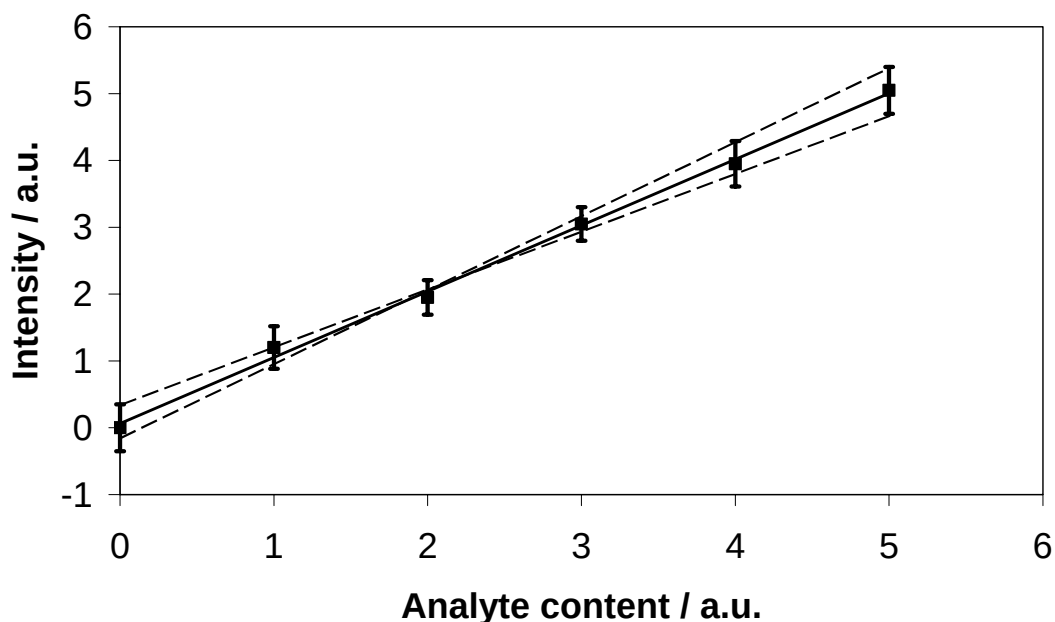


Figure 3. A generic calibration relationship of analyte content against signal intensity where the (corrected-for) blank response exhibits a non-negligible distribution of values and the calibration relationship is not constrained to pass through the origin. The calibration relationship of best-fit (solid) and the relationships the lowest and highest feasible calibration gradients (dashed) are shown. Uncertainty in the calibration relationship is significant at low signal levels and absolute precision is highest for values near the centroid of the calibration relationship.

As expected for this non-zero intercept model, the best absolute precision occurs at values towards the centroid of the calibration relationship [11]. Often, uncertainty in the gradient of the calibration relationship which defines the relationship between the signal and concentration domains will confer a range of ‘detection limit amount contents’ [12], ‘non-normal distributions of detection limits’ [13] or ‘random variable detection limits’ depending on the origin and distribution characteristics of the input signal distribution [14,15]. This is a particular problem at low signal levels. IUPAC has suggested dealing with this problem by always selecting the median of the distribution of such ‘detection limits’ [16].

However, for ultra-low, or zero blank levels and negligible blank value distributions this situation is actually simplified somewhat. For the determination of calibration curves of intensity against analyte concentration, one assumes that the calibration curve must be constrained to pass exactly through the origin, because the blank response is zero with a negligible uncertainty, *i.e.* the distribution of possible blank values at zero signal level is nil. In this way one does not expect to obtain negative analyte values from such a calibration curve. This is a result of constraining the calibration curve exactly through the origin with negligible uncertainty and because, by definition, negative (blank corrected) intensity values may not be realised [3,17]. (An analytical methodology with a finite blank response to which a blank correction has been applied would require a finite uncertainty in the signal domain to recognise this variability of the blank response and would then not necessarily be constrained to pass exactly through the origin as in Figure 3). As a result of the constraint to pass directly through the origin the uncertainty of the gradient at low analyte levels will be very low and problems that can be caused to the meaningfulness of detection limits transferred from the signal to concentration domains would largely disappear. This situation is shown diagrammatically in Figure 4. In contrast to Figure 3 the best absolute precision for this zero intercept model occurs when the analyte content is zero [11].

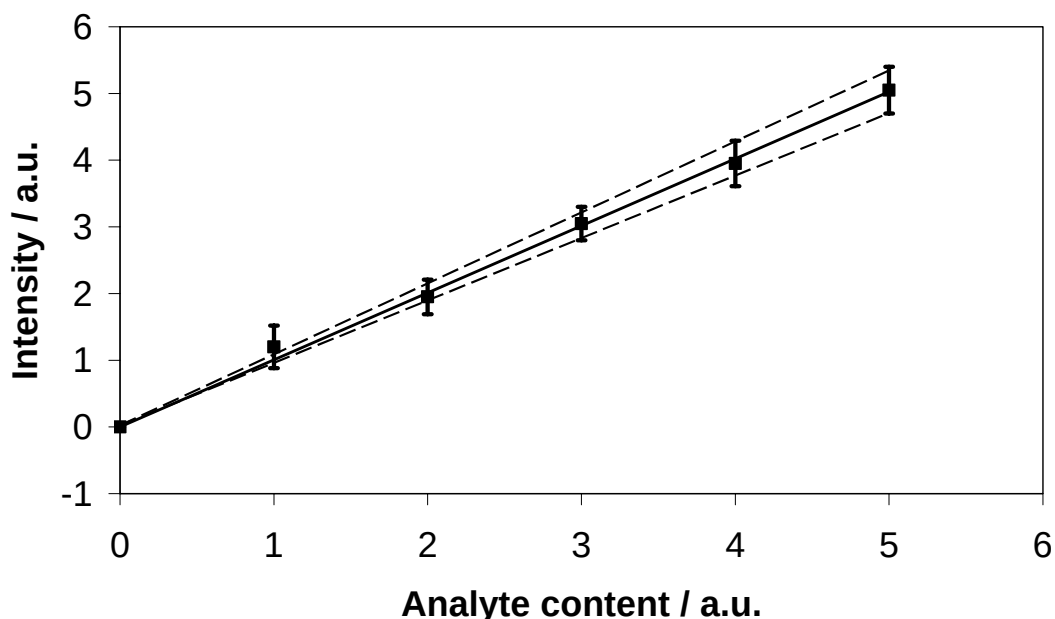


Figure 4. A generic calibration relationship of analyte content against signal intensity where the blank response is zero and exhibits a negligible distribution of values. The calibration relationship is constrained to pass directly through the origin. The calibration relationship of best-fit (solid) and the relationships the lowest and highest feasible calibration gradients (dashed) are shown. Absolute precision in the calibration relationship is highest at low signal levels.

Another interesting and philosophical result of the very low uncertainty of the calibration curve at low levels and the prohibition of negative signal and concentration levels is that probability distribution functions at very low analyte levels would be expected to be largely asymmetrical. The asymmetry would be expected to decrease as the analyte concentration moves away from zero. In situations where the calibration curve is not constrained through the origin obtaining negative analytical values is possible and entirely reasonable [3].

The limit of detection in hypothetical methodology under consideration should be determined by multiples of the standard deviation of the distribution of sample measurement with a false negative probability to be defined by the requirements

imposed on the analytical method. However, in this situation the concept of a false negative has little meaning. A false negative corresponds to the proportion of the sample distribution curve that falls below the critical detection decision limit, L_C . Here this would correspond to a negative signal intensity value, which is forbidden in this consideration. Hence the limit of detection may then best be defined as the minimum value of the target analyte for which the expanded uncertainty, at the z % confidence level, of the entire analytical measurement represents z % of the measured value of the analyte. No part of this distribution of sample values should be expected to fall in the negative signal domain. (This limit of detection could analogously be considered to be the minimum analyte concentration value at which analyte concentration distribution could reasonably be expected to be normal).

The output of this approach is that it results is a special case where both limits of detection and quantification may be determined before a rigorous understanding of the analytical methodology to be employed is established; and before repeated measurements of blank, sample or calibration relationships have been made. This is possible by consideration of empirical relationships that relate relative standard deviations (RSD) of analytical measurements to analyte mass fractions for the entire analytical procedure. Such an empirical relationship has been developed by Horwitz [18,19]:

$$RSD(\%) = 2^{(1-0.5 \log_{10} x)} \quad (2)$$

Where $RSD(\%)$ is the relative standard deviation of the analytical process expressed as a percentage, and x is the mass fraction of the target analyte in the sample under test. Alternatively, and more accurately, the relationship between whole-method repeatability and analyte content could be determined separately for the individual methodology under consideration yielding a more general relationship, $RSD(\%) = f(x)$. The detection limit of an analyte at the z % confidence level may be empirically expressed using equation 2 thus:

$$\frac{U(\%)_{P=z}}{t_{n-1, P=z}} = 2^{(1-0.5 \log L_D)} \quad (3)$$

Where $U(\%)_{p=z}$ is the expanded measurement uncertainty, expressed as a percentage of the measurand value, at the z % confidence level, $t_{p=z}$ is student t factor yielding a probability of z for the n measurements and L_D is the empirical limit of detection. Equation 3 pre-supposes that the analyte limit of detection at the 95% confidence interval would be reached at approximately a mass-fraction of 10^{-9} .

The limit of quantification is generally accepted to be the lowest concentration of analyte for which the standard deviation of the method reaches an acceptable level for quantification. This level is usually taken to be 10%. Again *a priori* estimation of this limit is possible using the modified Horwitz relationship in equation 3. This pre-supposes that the analyte limit of quantification would be reached at approximately a mass-fraction of 10^{-6} .

In this modified approach the limit of detection has assumed a similar definition to the limit of quantification in traditional approaches. Both are defined by a predetermined level of whole-method repeatability and not by the distributions of blank measurements. The critical decision limits still remains zero.

It may be worth additional consideration that the RSD of the system may not rise quite so fast as the Horwitz equation predicts as analyte concentration approaches zero as normally the blank response would contribute an increasing proportion of the RSD as analyte concentration decreases, but in this case the blank is extremely small and would not have this additive effect.

These results represent acceptable estimates for the performance of most methods but since the relationship is based on the empirical spread of inter-laboratory results for many different analytes in a variety of matrices it should be confirmed by repeated experimentation.

Validation

In the following applications, we used equation (2) instead of equation (3) to evaluate the detection limit. This simplification is acceptable only if there is no significant systematic errors, such as interferences or non-linearity, in the method. Equation 2 may be re-written thus:

$$RSD(\%) = a^{(1-b \log_{10} x)} \quad (4)$$

where a and b are constants relating to a particular measurement method. These constants may be determined by using data obtained for the RSD of repeat measurements, using this method, at a variety of analyte mass fractions. This has been done for the measurement of polycyclic aromatic hydrocarbons (PAHs) with GC-MS at NPL. The data are displayed in Figure 5.

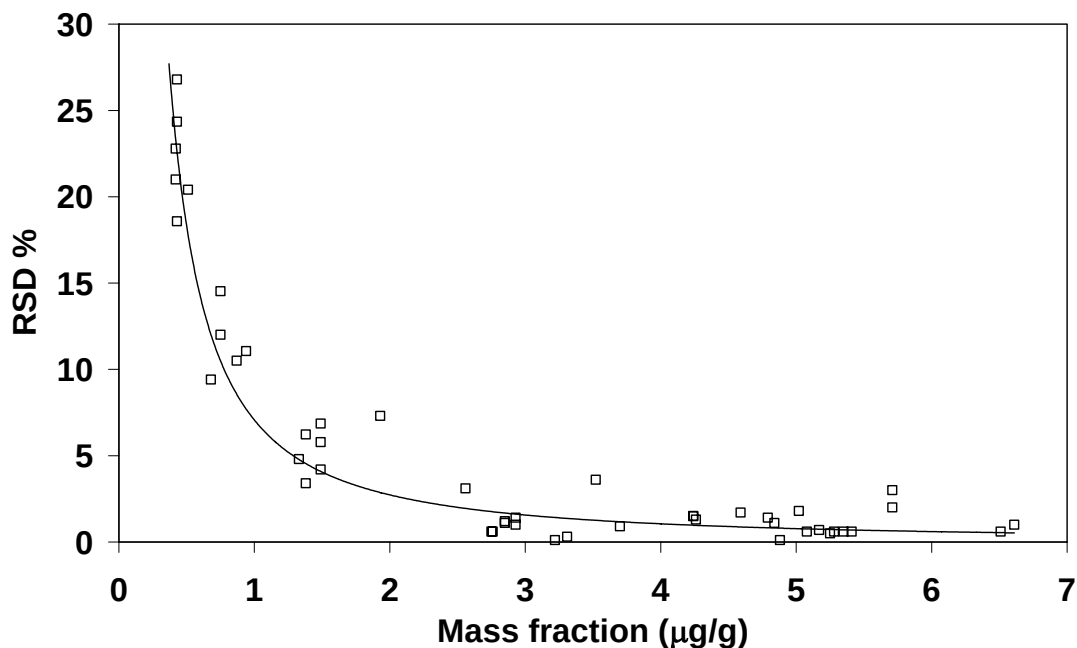


Figure 5. The relative standard deviation of measurements of polycyclic aromatic hydrocarbons (PAHs) at a variety of mass fractions. When the observed relationship is fitted to equation 4, and assuming the detection limit is reached when the RSD is 50%, the detection limit (L_D) determined is approximately 0.005 µg/g (5 ppb).

Assuming the detection limit is reached when the RSD of the method is 50% this yields a detection limit of 5 ppb. This value is of the same order of magnitude as the commonly accepted detection capability for this analyte using GC-MS at NPL.

The same approach was used for the analysis of copper by ICP-OES at LNE, where a and b were determined to be 1159 and 0.47 respectively (with x expressed as $\mu\text{g/l}$). This yielded a detection limit of 10 $\mu\text{g/l}$, which is in excellent agreement with previously accepted estimate of the detection limit. These results seem to confirm that this approach can additionally be used for techniques giving blank responses different from zero.

Conclusion

The routine determination of detection limit criteria in analytical chemistry relies on the measurement of the distribution of the analytical blank response. A hypothetical situation where the measurement blank response is zero and the distribution of the blank response is negligible has been considered. The impact of this situation on traditional limit of detection criteria has been discussed. The critical decision limit is then defined only by the sensitivity of the analytical instrument and the false positive rate of the critical detection decision is necessarily zero. Under these circumstances the limit of detection and limit of quantification levels have been shown to be determined solely by the distribution of measured sample values. The false negative rate has an undefined meaning in this consideration as the hypothetical analytical methodology does not allow for values occurring in the negative part of the signal domain. It has been proposed that the limit of detection and quantification at the required confidence level may then be practically estimated from a consideration of the whole method repeatability of the analytical procedure in using an adaptation of the Horwitz relationship. Although not as robust a methodology as repeated measurements of the blank and sample response in order to establish detection criteria, the approach provides a useful route to the estimation of the potential method capabilities of any analytical technique before it is employed and will prove a valuable indication for method development, and routine analytical laboratories and practitioners of analytical methodologies.

It must be appreciated that to be of any use detection limits should really be quoted with the rigorously defined parameters that define them. Moreover it should be realised that even then detection limits only represent an indication of the performance of an analytical method. For the purposes of reporting results it is always preferable to quote the measurement results obtained with the associated measurement uncertainty at a stated confidence interval.

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