The ability to quantify a trace element or molecule in chemical and biological matrices using specific analytical methods is often viewed in terms of the limit of detection. This limit of detection is a number, expressed in units of concentration (or amount), that describes the lowest concentration level (or amount) of the element that an analyst can determine to be statistically different from an analytical blank (1). Although this definition seems rather straightforward, significant problems have been encountered in expressing these values because of the various approaches to the term “statistically different.” The calculated limit of detection for an element can easily vary an order of magnitude through the use of different statistical approaches (1–13). In spite of the fact that the International Union of Pure and Applied Chemistry (IUPAC) adopted a model for the limit of detection calculations in 1975 (1), and the ACS Subcommittee on Environmental Analytical Chemistry reaffirmed this standard in 1980 (2), acceptance of this model by the general analytical community has been slow. The result of this slow acceptance has led to a great deal of uncertainty when limits of detection are used as a basis for comparison between various analytical procedures, methods, or analytical instruments. Unless the limits of detection are calculated in a consistent manner, the comparison may be meaningless.

It is the purpose of this REPORT to examine the statistical meaning of limit of detection values in a format consistent with the IUPAC definition. It is not intended to be a comprehensive review of the various methods of calculating limit of detection values. For a more complete review, the reader is referred to the excellent articles by Kaiser (3–6), Boumans (7–9), Currie (10), Glaser et al. (11), as well as available textbooks (12, 13). Rather, this REPORT is intended to be a simple and general discussion on methods for calculating limits of detection; it is geared to the analyst who does not have a rigorous knowledge of statistics. In this examination, the significance of the limit of detection values will be emphasized, and the possible problems encountered when using
The limit of detection is the lowest concentration level that can be determined to be statistically different from an analytical blank. Significant problems have been encountered in expressing these values because of the various approaches to the term "statistically different".

Definition

The IUPAC definition, adopted in 1975, states that "the limit of detection is the lowest concentration of an analyte that can be reliably detected" (1). This concept is further clarified by the ACS definition, which states "the limit of detection is the lowest concentration of an analyte that can be reliably detected" (2). To understand what a reasonably certain measure or a reliable detection is, the method of measurement as well as the errors (including noises) associated with the measurement must be well understood.

Measurements

Generally, most analytical methods require the construction of analytical calibration curves for the determination of unknowns. These curves are generally plots of signal, x, vs. analyte concentration, c, and are represented as

\[ x = mc + i \]  

where m is the slope or analytical sensitivity and i is the intercept. When an unknown sample containing the analyte is subjected to the analytical procedure, a value, \( x_U \), can be measured. This value may then be inserted into Equation 1 to determine the concentration of the unknown, \( c_U \). However, the ability to solve accurately for \( c_U \) is dependent upon how well the m and i

\[ x_U = mc_U + i \]
values are known. As long as the calibration curve is obtained in the linear response region of the method, the more points obtained in the construction of the calibration curve the better defined the m value will be. Also, if points are sampled near the origin, the i value may be better defined. However, if the m and i values are not well-defined because of nonlinearity in the calibration curve or a poor choice of calibration curve ranges, the result of the unknown determination, C0, may be subject to considerable error.

**Statistics**

The amount of error associated with a measurement of x can be statistically estimated. Most measurements are subject to error that follows a normal distribution. If a sufficiently large number of observations is made, plotting the measured responses would produce a curve similar to that shown in Figure 2. The mean value of the responses, μ, occurs at the center of the curve. The curve is symmetric around μ and extends outward in units of standard deviation, σ. Since this curve includes all x values that could be obtained from the procedure for the sample, the area under the curve can be expressed in terms of probability, P (i.e., there is a 100% chance, P = 1, that a measured x value would fall somewhere under the curve).

The relationship between area and probability can be measured to estimate the chance that a newly measured x value, xB, would be a certain number of standard deviation units away from the mean response, μ. In Figure 2, xB is shown to the right of μ and can be measured to be kσ away from μ. By dissecting the curve with a line drawn at this x value, the area to the right of the line, α, is the probability that xB > (μ + kσ). This chance, which is represented by the pink shaded area, can be determined from:

\[
\text{area} = \frac{1}{2} \int_k^\infty \exp \left( -\frac{k^2}{2} \right) \, dk
\]

where the xB value is kσ away from μ, (i.e., (xB - μ)/σ = k).

This illustration can also be used to aid in the explanation of the smallest detectable signal, xL, in the IUPAC definition. When the determination of a limit of detection is performed, blank measurements, xB, are normally taken. The question, however, is how well are these xB values known? A mean value of the blank responses, x̄B, can be calculated as:

\[
x̄B = \frac{\sum_{i=1}^{n_B} x_{Bi}}{n_B}
\]

and the standard deviation as:

\[
\sigma_B = \sqrt{\frac{\sum_{i=1}^{n_B} (x_{Bi} - x̄B)^2}{n_B - 1}}
\]

for nB observations. Because a finite small number of blank readings is normally taken, e.g., nB = 20 or greater, x̄B must be used instead of σB. If a sample of this size is used, xL can be considered to be a reasonable indicator of σB. If the random error follows a normal distribution, a plot of these responses (frequency of occurrence vs. xB values) would resemble Figure 2. The probability that the smallest discernible analytical signal, xL, can be measured and not be a random fluctuation of the blank is dependent upon how many standard deviation units xL is from x̄B. If xL is 3σB away from x̄B, the area to the right of xL is no less than 0.0013. Thus, there is a 0.13% chance that a signal measured at xL or greater would be the result of a random fluctuation of the blank signal. This small chance of error can then fulfill the requirement of a reasonably certain signal.

In defining C1, IUPAC states that:

\[
x_L = x̄B + kSB
\]

where k is a numerical factor chosen in accordance with the confidence level desired. The C1 is a function of xL and therefore

\[
C1 = \left( \frac{x_L - x̄B}{m} \right)
\]

where m is the analytical sensitivity. Because the mean blank reading, x̄B, is not always 0, the signal must be background corrected. By substituting Equation 5 into Equation 6, Equation 7 is obtained

\[
C1 = \frac{kSB}{m}
\]

This definition of C1 can be illustrated as shown in Figure 3. The limit of detection is found by relating k SB to a concentration value by dividing by the slope of the calibration curve line obtained from the linear regression analysis. However, the C1 value obtained can only be a true reflection of the limit of detection when m is well-defined and i is essentially 0.

The use of k = 3 allows a confidence level of 99.86% that xL > (x̄B + 3σB) for a measurement based on the error of the blank signal following a normal distribution. It must be emphasized that if xB does not follow a normal distribution, then the probability that xL > (x̄B + 3σB) would be 100(1 - 1/k²), or 98% according to Tschebyscheff’s inequality (5). Hence, values of k < 3 should not be used for limit of detection calculations.

**Other Approaches**

The majority of the other approaches to calculating C1 values are similar to the IUPAC model in that σB and k factors are involved. However, it is because of these terms that trouble may be encountered when C1 values are used as a basis for the comparison between procedures, methods, or instruments. The most widely debated of the two factors has been the choice of a value for k. Kaiser was perhaps the first to stress the use of k = 3 for C1 values (3, 4). This value has also been agreed upon by other authors (9, 13), by IUPAC (17, 14), and by the ACS (2). A value of 2 for k had been initially suggested (8) but this value corresponds to a 97.7% confidence level for normal distribution and 75% for a non-normal distribution of measurement error.

Although the use of k = 3 instead of k = 2 slightly increases the C1 value, it is clear that C1 values must differ by a factor of three for the values to be significantly different. Nevertheless, factors of less than three have been commonly used for comparison purposes. In order to minimize confusion, IUPAC suggested that xL values be reported in all literature with their k value, xL(kSB). It would be extremely useful to go one step further and include the k value when C1 values are reported, C1(kSB). This change would be beneficial because C1 values are more commonly reported than xL values.

A problem encountered in the comparative use of C1 values is the use of the standard deviation of the mean, σB (17), the pooled standard deviation, σp (7, 12), or the relative standard deviation (RSD) (7, 9). Although each of these standard deviation expressions is important and has its place in analytical chemistry, the use or misuse of these expressions in C1 calculations may result in significant deviation.
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In many ICP determinations, the RSD has been measured to be 0.01 (as determined by emission flicker noise). Unfortunately, many ICP values in papers and current manufacturers’ bulletins have been calculated with \( k = 2 \). Inserting these values into Equation 13 yields

\[
\text{cL} = \frac{k \left( \frac{\bar{s}_B}{\bar{x}_B} \right)}{m} = k \text{(RSD)} \frac{\bar{s}_B}{m} \tag{13}
\]

A problem that may be encountered with this approach is that the RSD for some spectrometric detection systems may be >0.01. Before using this method, an analyst should reconfirm the validity of RSD < 0.01. Failure to measure and correct for high RSD values could result in erroneous \( c_L \) values. An attempt to compare an ICP \( c_L \) value obtained from this method and an IUPAC model value for the same analysis procedure would be meaningless.

A well-based but seldom used concept in the calculation of detection limits is the limit of identification, \( c_I \), as described by Boumans (7) or the limit of guarantee for purity, \( C_G \), described by Kaiser (3). These concepts are essentially the same and are based on the idea that the lowest statistically discernible signal should be

\[
\text{x}_I = \text{x}_L + 3\text{s}_I \tag{15}
\]

where \( s_I \) is the error associated with the measurements at the \( x_I \) level, or

\[
\text{x}_I = \text{x}_L + 3s_B \tag{16}
\]

if \( s_I = s_B \). This concept is illustrated in

![Figure 4. Normal distribution curves for the blank observation, \( x_B \) (pink line), the limit of detection, \( x_L \) (blue line), and the limit of identification, \( x_I \) (purple line) Here all standard deviations follow a normal distribution and are equal](image-url)

from the IUPAC model.

The standard deviation of the mean (or the standard error), \( s_e \), is expressed by

\[
s_e = \frac{s_B}{\sqrt{n_B}} \tag{8}
\]

This value is calculated by dividing the standard deviation of the blank measures, \( s_B \), by the square root of the number of blank observations, \( n_B \). Usually when \( s_B \) values are used, the \( k \) factor is replaced by a \( t \) distribution value. Even though the \( t \) values reflect the problem of defining a standard deviation for a finite number of observations, \( s_B > t_n s_B/(n_B) \) for \( \alpha = 0.005 \) and five observations (four degrees of freedom). This inequality greatly increases as \( n_B \) increases. If a large number of observations are taken on the blank, say 30, the use of the \( t \) and \( s_B \) will reduce the value of \( c_L \) by a factor of six from the IUPAC model.

The use of the pooled standard deviation includes the number of blank measurements as well as sample measurements. Generally, the pooled standard deviation, \( s_p \), is expressed as

\[
s_p = \frac{s_B^2 + s_B^2}{n_B} \tag{9}
\]

where \( s_B \) is the standard deviation of a sample measurement and \( n_B \) is the number of sample observations. If \( s_B = s_B \), then Equation 9 reduces to

\[
s_p = \frac{s_B}{\sqrt{n_B}} \tag{10}
\]

For \( n_B = 1 \), \( s_p \) is essentially the same as \( s_B \). However, if \( n_B \) and \( n_S \) are both large, \( s_p < s_B \). Again, the use of a large number of observations as mentioned above would cause a significant reduction in the \( c_L \) value as compared to the IUPAC model.

Another common practice involves the use of the RSD, which is defined as

\[
\text{RSD} = \frac{s_B}{\bar{x}_B} \tag{11}
\]

This relationship is useful in expressing the “concentrational limit of determination,” \( c_D \). Here, the measure \( x_D \) corresponding to \( c_D \) is defined as

\[
x_D = k_D s_B \tag{12}
\]

where \( s_B \) is the standard deviation of the blank (not the RSD), and \( k_D \) is a confidence factor that is defined by the reciprocal of the magnitude of the RSD value. For a maximum allowed RSD of 5%, \( k_D = 20 \) and for a RSD of 10%, \( k_D = 10 \).

Another limiting expression involving RSD values involves the rearrangement of Equation 7 (9). If the background signal is included in the numerator and denominator of Equation 7, then

\[
s_B = \frac{\sum_{i=1}^{n} c_i x_i}{\sqrt{\sum_{i=1}^{n} c_i}} \tag{14}
\]

\[
\text{Table I. Statistical Expressions for the Slope and Intercept}
\]

\[
\begin{align*}
\text{m} &= \left( \frac{n \sum_{i=1}^{n} c_i x_i}{\sqrt{\sum_{i=1}^{n} c_i^2}} \right) - \left( \frac{n \sum_{i=1}^{n} c_i}{\sqrt{\sum_{i=1}^{n} c_i^2}} \right) \\
\text{i} &= \frac{\left( \sum_{i=1}^{n} x_i \right)^2 - \left( \sum_{i=1}^{n} c_i \right)^2}{n} \\
s_m &= \frac{s}{\left( \bar{s}_{cc} \right)^{1/2}} \\
s_x &= \frac{s_{xx}}{\left( \bar{s}_{cc} \right)^{1/2}} \\
s_{sx} &= \frac{s_{sx}}{\left( \bar{s}_{cc} \right)^{1/2}} \\
s_c &= \frac{\sum_{i=1}^{n} c_i x_i}{\left( \bar{s}_{cc} \right)^{1/2}} \\
s_{cc} &= \frac{\sum_{i=1}^{n} c_i}{\left( \bar{s}_{cc} \right)^{1/2}} \\
s_{xx} &= \frac{\sum_{i=1}^{n} x_i^2}{\left( \bar{s}_{cc} \right)^{1/2}} \\
s_{sx} &= \frac{\sum_{i=1}^{n} c_i x_i}{\left( \bar{s}_{cc} \right)^{1/2}} \\
\end{align*}
\]
Figure 4. When measurements are made for a sample, the x values obtained should follow normal distribution around a mean value. If a sample were measured to have a mean at the xL value, the distribution of these x values around xL would resemble the high probability, the limit of xI is set as the IUPAC definition. To avoid this situation around a mean value. If a sample obtained should follow normal distribution below the xL limit, the area of the x distribution curve below xL, β, is no less than 0.0013. Thus, there is a 0.13% chance that an x value measured at xL would fall below the xL limit and not be considered as a true signal.

This idea of further statistically separating the blank measurements distributions and true signal distributions has been proposed by the ACS Subcommittee on Environmental Analytical Chemistry and has been termed the limit of quantitation (LOQ). Since the numerical significance of the analyte concentration increases as the analyte signal increases above xL, a minimum criterion, representing the ability to quantify the sample, can be established reasonably far away from xB. This criterion, called the limit of quantification (LOQ), is 10σ away from xB. For limit of detection work, σ = 3σB. Samples that are measured as having a signal, x, where x > 10σB are termed to be in the region of quantitation while samples where 3σB ≤ x ≤ 10σB are termed to be in the region of detection.

By setting the quantitation level as 10σB or the identification limit as 6σB, a much higher probability is afforded that the sample signal is not just a random fluctuation of the blank. However, when making comparisons using LOQ or xL to IUPAC xL values, the analyst must bear in mind the difference in the k factors for each limit.

Methods Involving Analytical Sensitivity Error

The previous models for calculating detection limits consider the error in the blank measurements. These models also consider the analytical sensitivity, m, as a well-defined value. In practice, however, m may have significant error due to nonlinearity in the calibration curve, or measurement errors.

The following proposed detection limit approaches include errors associated with measurements of the analytical sensitivity. The first method, a graphical approach, includes the standard deviation of the slope, σm, in the cL expression. The second method, a propagation of errors approach, considers the standard deviation of the concentration, σc. This value is calculated by including the standard deviations of the blank, slope, and intercept in the equation. The statistical expressions for these values are listed in Table I.

Although these models require additional calculations, most linear regression analyses are performed using calculators or microcomputers. With additional programming, these calculations can be easily performed, allowing more accurate determinations of cL values to be made. These values may also be used for a truer comparative look at the ability of an analytical method or instrument to quantify trace elements (or compounds) in a sample.

Graphical Approach

To obtain a more reliable cL value, the m value should be expressed as a confidence interval m ± tσm, where σm is the standard deviation of the slope and t is a t distribution value chosen for the desired confidence level, α, and the degrees of freedom, v. The insertion of this interval into Equation 7 produces

\[
\text{cL} = \frac{k\sigma_B}{m \pm t v \sigma_m} \quad (17)
\]

The effect of the inclusion of the confidence interval can best be seen by referring to Figure 5. The error bars (confidence interval) generated around the regression line are indicated as white dashed lines. Because of error in the slope, three concentration...
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values are found for a given \( x_1 \) value. When \( t_{s_m} = 0 \), (as in Equation 7), the value of \( c_l \) is obtained. However, the reduced analytical sensitivity yields a value, \( c_r \), to the right of \( c_l \). If \( m \gg t_{s_m} \), then the \( c_r \) will not be statistically different from \( c_l \). For the situation when \( t_{s_m} \) is not sufficiently small as compared to \( m \), a \( c_r \) may be substantially larger than \( c_l \). Therefore, only the larger value should be used in reporting a limit of detection.

An important consideration in the use of this model is the choice of a \( t \) value. The recommended \( k \) value of 3 involves a 99.87% confidence level. Therefore, the \( t \) should reflect a similar level. An \( \alpha = 0.0005 \) results in a confidence level of 99.9% for a two-tailed \( t \) distribution. The degrees of freedom, \( v \), are \( n - 2 \) for a linear regression model. The \( n \) value used should be the number of points used to prepare the calibration curve, i.e., each point consists of a mean measure and a concentration value.

### Propagation of Errors Approach

In the second approach used to evaluate \( c_l \), the error in the intercept term, \( i \), as well as the error in \( m \) are considered. To include these errors, Equation 1 must be rearranged to

\[
c = \frac{x - i}{m} \tag{18}
\]

From Equation 18, any value of \( x \) can be related to a concentration using the \( m \) and \( i \) values obtained from the linear regression model. The \( i \) term is usually neglected in most cases and assumed to be 0 because the analytical measures are background subtracted. But in most linear regression analyses, \( i \neq 0 \). If a true reflection of the error in the determined concentration is sought, the error in \( m \) and \( i \) must be included in Equation 7.

The contribution of each term to the total error may be found by taking the first derivative of \( c \) with respect to each term:

\[
s_c^2 = \left( \frac{\partial c}{\partial x} \right)^2 s_x^2 + \left( \frac{\partial c}{\partial i} \right)^2 s_i^2 + \left( \frac{\partial c}{\partial m} \right)^2 s_m^2 \tag{19}
\]

Taking the designated derivatives and the square root gives

\[
s_c = \left[ \left( \frac{1}{m} \right) s_x^2 + \left( \frac{1}{m} \right) s_i^2 \right]^{1/2} + \left( \frac{1}{m^2} \right) s_m^2 \right]^{1/2} \tag{20}
\]

and finally combining like terms yields

\[
s_c = \left[ s_x^2 + s_i^2 + \left( \frac{1 - x^2}{m} \right) s_m^2 \right]^{1/2} \tag{21}
\]
Equation 21 allows the determination of standard deviation in a c value calculated from any x value. In the case of limits of detection, the EL value is actually a confidence value expression of how well the blank is known. If Equation 7 is reconsidered, it can be written as

\[ CL = k_se + (m - i)se + \frac{1}{2} \left( \frac{1}{m} \right) s_m^2 \]  

(22)

where the \( s_p/m \) term describes the error in terms of c if \( c \sim c_i \). Equation 21 could now be used to evaluate where \( x \) is the blank signal, \( x_B \), and \( s_B \) is substituted for \( s_e \). By measuring \( x_B \), \( s_B \), and calculating \( m \), \( s_m \), and \( i \), the value of \( s_e \) can be determined.

In most determinations, the data are background corrected, that is, \( x_B = 0 \). Substituting the above measured and calculated values in Equations 21 and 22, the expression for \( CL \) is further simplified to

\[ CL = \frac{k(se^2 + s_i^2)^{1/2}}{m} \]  

(23)

In the event that no significant error occurred in the slope, Equation 21 reduces to

\[ CL = \frac{k(se^2 + s_i^2)^{1/2}}{m} \]  

(24)

If the error in the intercept, \( s_i \), is sufficiently small, Equation 23 reduces to Equation 7, which is the IUPAC definition of the limit of detection.

**Evaluation of Approaches**

The IUPAC, graphical, and propagation of errors models will be applied to four different sets of experimental data to show the effect of certain experimental conditions on the estimation of \( CL \) values (Table II). The data in Table II have been taken from a recent paper on ICP-excited ICP fluorescence detection limits (15). The four sets of typical experimental conditions are: A, Ca(II) fluorescence data which have well-defined \( m \) and \( i \) values; B, Ca(II) fluorescence data where \( SB = 0 \), the calibration curve data are taken far away from CL resulting in a poorly defined \( i \) value and a well-defined \( m \) value; C, Cu(I) fluorescence data where there is nonlinearity in the calibration curve resulting in ill-defined \( m \) and \( i \) values; and D, Co(II) fluorescence data where extreme nonlinearity in the calibration curve results in severe errors in both \( m \) and \( i \) values. The limit of detection values, \( CL_{(k=3)} \), for the three methods are tabulated in Table III. Here the \( CL_{(k=3)} \) values are reported only to one significant figure, as all \( CL \) values should be.

The values obtained in case B emphasize the problem of an ill-defined, nonzero intercept. This problem is the direct result of constructing calibration curves for detection limits when the lowest point of the calibration curve data is considerably removed from the \( CL(k=3) \) value. Only the propagation of errors model accounts for this error, while the other two methods indicate an erroneously low \( CL(k=3) \) value. Although there are no set guidelines for constructing calibration curves, this approach clearly illustrates the problem of sampling too far away from the limit of detection.

In some instances, calibration curves may not be linear. Although the linear regression procedure will fit a line through the data, the resulting \( m \) value is by no means a “true” representation of the analytical sensitivity at all concentrations. Nonlinear calibration curves generally produce significant \( s_m, s_i \), and \( i \) values. Case C represents such conditions. The IUPAC model results in a \( CL_{(k=3)} \) of 0.03 ppm. Repeating the \( CL \) calculation using the graphical approach results in a value of 0.3 ppm. Finally allowing \( m \) and \( i \) to be included results in a \( CL_{(k=3)} \) of 5 ppm for the propagation of errors method. The propagation of errors \( CL \) value is 170 times the IUPAC \( CL_{(k=3)} \) value. The problem of nonlinearity can be further emphasized by considering case D. Here, the errors associated with \( m \) and \( i \) are greater; however, the large value of \( s_m \) results in a special problem with the graphical approach. If \( t_se > m \), then the concentration value for the limit of detection can even be negative. Such negative values are the direct result of the graphical model not being statistically valid. Although the graphical model is easier to
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use than the propagation of errors approach, the former may give erroneous results as seen in Case D. Thus, the graphical method could be used only for approximating $c_i$ values. The detection limit using the propagation of errors approach is calculated to be 6 ppm. Correspondingly, the detection limit for the IUPAC model is 0.5 ppm, differing by a factor of 12 between the two methods.

Conclusions

Based on the above considerations, the graphical approach to $c_i$ should not be used. The IUPAC approach is valid only if the major source of error is in the blank, i.e., $s_0^2 \gg s_i^2$ or $s_m^2$. Therefore, the IUPAC approach in most cases gives artificially low values of $c_i$. The propagation of errors approach is certainly the most liberal approach and will give values of $c_i$ consistent with the reliability of the blank measurements and the signal measures of the standards.

We recommend that analysts report limits of detection using the IUPAC approach with $k = 3 (c_{IUPAC} - 3)$. The use of the propagation of errors approach is also recommended because errors in the analyte measurements can be incorporated into the $c_i$ value. By adopting these approaches, meaningful comparisons of analytical methods and instruments based on $c_i$ values can be made.

References

(1) "Nomenclature, symbols, units and their usage in spectrochemical analysis". IUPAC, Pure Appl. Chem. 1972, 44, 15.

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