Controversies on Limit of Detection

The limit of detection (LOD) is an important characteristic of a test method involving trace analysis but its concept has been, and still is, one of the most controversial in analytical chemistry.

Its general definition is the minimum concentration of the analyte that can be reliably detected from zero with a specified level of confidence or significance for a given method. It is normally referred to the smallest concentration of the analyte giving a significant response of the instrument which can be distinguished as being present to above the blank or background response.

However, many learned international professional organizations have come up with their own definitions and calculation methods. This has created confusions amongst the analytical chemists. Efforts have been put forward by ISO (e.g., ISO 11843 "*Capability of Detection*" series) and IUPAC to harmonize this situation by reaching a consensus in their definitions and issuing guidelines on the LOD estimation method. But, it still seems to have a long way to go to achieve a satisfactory outcome.

Therefore, when quoting LOD values, it is proper for the laboratory to state the approach taken.

What are the common evaluation methods that we can use?

1. The LOD can be evaluated by obtaining the standard deviation of results obtained from 6-8 replicate analysis of a blank sample (containing none of the analyte of interest), or a sample containing only a very small amount of the analyte, taken through the whole analytical process. This LOD is also known as method detection limit.

   The limit is calculated by multiplying the standard deviation with a suitable factor, normally by a value of 3, based on statistical reasoning with a consideration to keep the risk of false positive (wrongly declaring

the presence of analyte when it is actually absent) and false negative (wrongly declaring the absence of analyte when it is present) at an acceptable level (say, a 5% probability for both types of error).

By this manner, the LOD of the instrument response is therefore $y_B + 3s_B$, where subscript B refers to a blank determination. The corresponding concentration is then calculated from the calibration equation $y = a + bx$ covering a series of sample matrix with very low concentrations of the analyte, by assuming that the equation is valid down to that concentration:

$$\hat{x}_{DL} = \frac{y_B + 3s_B - a}{b} \qquad [1]$$

2. In certain instrumental analysis, it may be not possible to make a measurement in the absence of the analyte. In this case, we shall substitute the intercept of the calibration equation for the blank response (i.e., when the x-axis for the concentration is zero), and the standard error of the regression, $s_{y/x}$, for the standard deviation of the blank. That means in equation [1], therefore, $y_B = a$ and $s_B = s_{y/x}$, which gives:

$$\hat{x}_{DL} = \frac{a + 3s_{y/x} - a}{b} = \frac{3s_{y/x}}{b} \qquad [2]$$

where $s_{y/x} = \sqrt{\frac{\sum(y_{i,obs} - y_{i,cal})^2}{n-2}}$; n = number of data points; $y_{i,obs}$ = the experimental response value for concentration x_i; $y_{i,cal}$ = the calculated response value from the equation $y = a + bx$ for concentration x_i.

3. ISO 11843-2 "*Capability of detection — Part 2: Methodology in the linear calibration case*" estimates the critical value and detection limit directly from a calibration curve. It gives a more statistically defensible equation from calibration data as shown below:

$$\hat{x}_{DL} = \frac{2.t_{0.05,n-2}.s_{y/x}}{b} \sqrt{\frac{1}{R} + \frac{1}{I.J} + \frac{\bar{x}^2}{J\sum(x_i - \bar{x})^2}} \qquad [3]$$

Here, a calibration is performed with I independent calibration materials (including a blank if possible and a calibrator having a value near the expected detection limit) each measured J times. R is the number of replicate measurements that will be done on each test solution to give an average response.

For large n (number of dataset), the t-statistic limits get closer to the normalized z-value of 1.645 at $\alpha = 0.05$ level.

Therefore, the multiplication factor of equation [3] of 2 x $t_{0.05,n-2} \cong 3.3$ with $R = 1$ will give somewhat greater detection limits than equation [2].

4. There is another approach for LOD estimation, based on a signal-to-noise (S/N) ratio of three. This approach can only be applied to analytical procedures which exhibit baseline noise, such as in chromatographic analysis.
   In this case, working at the minimal attenuation of the chromatographic signal, the measurement of standard solutions (normally spiked samples) with stepwise decreasing concentrations until a peak is found whose height is three times taller than the maximum height of the baseline (measured at both sides of the chromatographic peak). The concentration corresponding to that peak is then taken to be the LOD.

   The *European Pharmacopoeia* defines the signal-to-noise ratio (S/N) as

$$S/N = \frac{2H}{h} \qquad [4]$$

   where:

   H is the height of the peak corresponding to the analyte concerned in the chromatogram with the low concentration sample. It is measured from the maximum of the peak to the extrapolated baseline of the signal observed over a distance equal to 20 times the width at half height;

h, is the range of maximum amplitude of the background noise obtained after injecting a blank solution and observed over the interval mentioned above, situated around the time where the peak would be found.

Limit of quantitation

The limit of quantitation (LOQ) is the smallest concentration of analyte that can be determined with an acceptable level of uncertainty. We may use a factor of 5 or 10 of the standard deviation of results from replicate measurements of a blank or low-concentration sample. It may be noted that in this case, there is no statistical basis to the convention used for estimating LOQ. The requirements of the analysis, precision in particular, are to be taken into account.

The aim of reporting LOQ is to identify the concentration below which the measurement uncertainty becomes unacceptable. It has been shown that the factor of 10 provides a reasonable estimate for many test methods although this proposal has not found great favor.