Best practices in establishing detection and quantification limits for pesticide residues in foods

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1 Introduction

Today, increased globalization has resulted in easy movement of agricultural products from one part of the world to another. The diversity in rules and regulations across the different nations has prompted the global harmonization of different regulatory agencies to establish joint tolerances or maximum allowable residue limits (MRLs), and establish best/uniform risk assessment guidelines. This has led to the necessity to adopt uniform methods to define the limitations of an analytical method. Without an appropriate definition, analyte concentrations reported by a method can be meaningless because calculated values of detection limits can vary over an order of magnitude depending on the method used to determine these values.[1,2]

The definitions of method detection and quantification limits should be reliable and applicable to a variety of extraction procedures and analytical methods. The issue is of particular importance to the US Environmental Protection Agency (EPA) and also pesticide regulatory and health agencies around the world in risk assessment. The critical question central to risk assessment is assessing the risk posed to a human being from the consumption of foods treated with pesticides, when the amount of the residue present in the food product is reported 'nondetect' (ND) or 'no detectable residues'.

If the analyte of interest is not detected by the instrument (or analytical procedure), does it mean that there is not a single molecule of that analyte present in the food product? Or does it mean that the analyte is present at a concentration that is just below the capability of the instrument? Or is it present at a concentration somewhere between these two extremes?

One major problem caused by Section 409 (c) (3) of the Federal Food, Drug and Cosmetic Act, commonly known as the 'Delaney Clause', which governed the registration of pesticides was the statement, 'No additive shall be deemed safe if it is found to induce cancer when ingested by man or animal, . . . '.[3] Dr Fred R. Shank, Director, Center for Food Safety and Applied Nutrition, US Food and Drug Administration, in

Handbook of Residue Analytical Methods for Agrochemicals.

his statement before the US House of Representatives' subcommittee on Health and the Environment, stated, 'The Delaney Clause read literally, requires absolute safety and would prevent the establishment of any tolerance for a residue in a processed food of any pesticide that is a human or animal carcinogen'.[4] He went on to add that at the time, 'the number of known or postulated carcinogens was fairly small, and the then state-of-the-art capability to detect a substance at a level of a few parts per million was considered ultra-sensitive.' At the time of passage of the Delaney Clause (1958), it was assumed that if residues were not detected then they were absent. As technology improved, more sensitive techniques capable of detecting residues in the sub-parts per trillion became available, thereby exposing the fallacy of this assumption. This clearly highlights the importance of defining the limitations of the analytical method when reporting the results of a test as ND.

Today, when a pesticide with no detectable residues is registered for use, a 'Tolerance' or 'maximum residue limit' (MRL) is established at the lowest concentration level at which the method was validated. However, for risk assessment purposes it would be wrong to use this number in calculating the risk posed to humans by exposure to the pesticide from the consumption of the food product. This would be assuming that the amount of the pesticide present in all food products treated with the pesticide and for which no detectable residues were found is just less than the lowest level of method validation (LLMV). The assumption is wrong, but there is no better way of performing a risk assessment calculation unless the limit of detection (LOD) and limit of quantification (LOQ) of the method were clearly defined in a uniformly acceptable manner.

In 1996, the US Senate and House of Representatives passed the Food Quality Protection Act (FQPA).[5] In order to make their exposure and risk assessments as accurate as possible, the US EPA Office of Pesticide Programs (OPP) has established guidelines to determine a value to assign for NDs.[6] These guidelines can be summarized as follows.

In the absence of any additional evidence implying lower residues:

1. for that percentage of the commodity that was not treated with the pesticide, a value of zero would be assigned;
2. for the remaining fraction, if a valid limit of detection exists, a value equal to half the LOD would be assigned;
3. if an LOD is not properly defined but a valid LOQ exists, a value equal to half the LOQ would be assigned;
4. if neither the LOD nor the LOQ are properly defined, then the value of the LLMV would be assigned;
5. if both the LOD and the LOQ are properly defined, and residues reported are between these two values, then a value equal to half the LOQ would be assigned.

This policy seems to be rational and would be of tremendous help to registrants in getting more uses registered for their products. This would be especially useful when new uses (worth millions of dollars to the registrant) were to be added to the label in cases where no detectable residues were found in the food product and the 'risk cup' (cumulative exposure risk) was nearly full. The following example highlights this point:

Risk cup $= 0.2\,\mathrm{mg\,kg^{-1}}$
LLMV $= 0.05\,\mathrm{mg\,kg^{-1}}$

If no detectable residues were present, the value used for risk assessment purposes would be $0.05\,\mathrm{mg\,kg^{-1}}$. This would allow only four uses with no detectable residues and even fewer uses where residues were present. If an appropriate LOD and LOQ had been calculated and reported as follows:

LOD $= 0.015\,\mathrm{mg\,kg^{-1}}$
LOQ $= 0.045\,\mathrm{mg\,kg^{-1}}$

As per US EPA guidelines, the value used for risk assessment purposes would now be $0.0075\,\mathrm{mg\,kg^{-1}}$, thereby permitting up to 26 uses for the product with no detectable residues.

There are several factors involved in defining the limitations of an analytical method. Selecting the right method for defining these limitations can be as important as the actual definitions. Factors that must be taken into consideration in defining detection and quantification limits are:

- instrumental noise
- matrix effects and interferences
- variability in extraction procedures, etc.

Several articles and books have been published dealing with this subject. In this article, some of the techniques which are relevant to methods for the analysis of foods for pesticide residues will be discussed.

1.1 Definitions

Several terms have been used to define LOD and LOQ. Before we proceed to develop a uniform definition, it would be useful to define each of these terms. The most commonly used terms are limit of detection (LOD) and limit of quantification (LOQ). The 1975 International Union of Pure and Applied Chemistry (IUPAC) definition for LOD can be stated as, '*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*'.[7] This term, although appearing to be straightforward, is overly simplified. It leaves several questions unanswered, such as, what does the term 'statistically different' mean, and what factors has the analyst considered in defining the 'blank'? Leaving these to the analyst's discretion may result in values varying between analysts to such an extent that the numbers would be meaningless for comparison purposes.

Later in 1995, IUPAC came up with additional recommendations for the definition of LOD.[8] Detection limit is defined as, '*The minimum detectable value of the net signal (or concentration) is that value for which the false negative error is β, given α. "α" is defined as the probability for a false positive ("analyte present" result when that is wrong) and "β" is defined as the probability of a false negative ("analyte absent" result when that is wrong)*'.[8] The values of α and β are defined by the analyst. This definition adds additional parameters to the definition of LOD, but does not solve

the fundamental issue of variability in the calculated value depending on the method used and parameters included in calculating LODs. Therefore, it is important that this term be defined in a manner such that it is easy to compare values reported by different analysts and laboratories. For most modern analytical methods, the LOD may be divided into two components, instrumental detection limit (IDL) and method detection limit (MDL).

In modern times, most analyses are performed on an analytical instrument for, e.g., gas chromatography (GC), high-performance liquid chromatography (HPLC), ultraviolet/visible (UV) or infrared (IR) spectrophotometry, atomic absorption spectrometry, inductively coupled plasma mass spectrometry (ICP-MS), mass spectrometry. Each of these instruments has a limitation on the amount of an analyte that they can detect. This limitation can be expressed as the IDL, which may be defined as the smallest amount of an analyte that can be reliably detected or differentiated from the background on an instrument.

The IDL is dependent on various factors such as sensitivity of the detector for the analyte of interest and electronic and detector (instrumental) noise of various origins, e.g., thermal noise, shot noise, flicker $(1/f)$ noise, environmental noise, etc.[8] Several books and articles have been published on the different types of instrumental noise, e.g., Skoog and Leary's 'Principles of Instrumental Analysis'.[9]

As the sensitivity increases, the IDL decreases, and as the instrumental noise decreases, so does the IDL. These aspects are key to selecting the correct instrument/detector system to perform the analysis.

Another factor of interest in defining the instrumental limitations is the instrumental quantification limit (IQL), which may be defined as the smallest amount of an analyte that can be reliably quantified by the instrument.

These two terms (IDL and IQL) define only the limitations of the instrument. When analyzing real-life samples such as plant or animal tissue or even soil and ground water samples, matrix interference must be taken into consideration in order to define detection limits. This is because these 'real-life matrices' are made up of hundreds (or even thousands) of compounds. These compounds may interfere in several ways in the detection and quantification of the analyte of interest.

In some cases, one or more of the matrix components may either elute at the same time as the analyte of interest in GC or HPLC or absorb or emit in the same wavelength range as the analyte. In other cases, the matrix components may either enhance or quench the analyte signal. In some cases, the matrix components catalyze reactions involving the analyte, for example, in GC or HPLC analysis involving pre- or post-column derivatization, matrix components may enhance or decrease the formation of the derivatized adduct. This may also be true of UV/VIS and fluorescence spectroscopy involving chemical modification of the analyte to enhance detection. As far as possible, the extraction method should be able to remove most of the interfering components. However, in complex matrices complete elimination of interfering components is impossible and therefore these effects must be taken into account when determining the LOD for an analyte–matrix combination. This leads us to a second set of terms that must be defined.

The method detection limit (MDL) is a term that should be applied to extraction and analysis methods developed for the analysis of specific analytes within a matrix. The MDL can be defined as the smallest amount of an analyte that can be reliably

detected or differentiated from the background for a particular matrix (by a specific method). All matrix interference must be taken into consideration when determining the MDL. Similarly, the method quantification limit (MQL) can be defined as the smallest amount of an analyte that can be reliably quantified with a certain degree of reliability within a particular matrix (by a specific method).

Finally, it is important to define the lowest level of method validation (LLMV). The LLMV is defined as the lowest concentration level expressed in terms of amount of analyte in the matrix, at which the method (extraction/analysis procedure) was validated or proven to be capable of reliably quantifying.

Depending on the method used to define the detection limit, the value can vary up to an order of magnitude, thereby rendering these numbers meaningless. This is why it is very important to have uniform definitions for each of these terms, in order to be able to compare values across matrices, methods and laboratories.[1]

2 Methods for defining LOD and LOQ

2.1 IUPAC method

In 1975, the IUPAC defined the LOD in terms of concentration (c_L) and the signal (x_L) generated by a solution of concentration c_L.[1] They defined the value of x_L in terms of the mean blank signal ($\bar{x}_B$) and the standard deviation (s_B) of these blank measurements as

$$x_L = \bar{x}_B + k \cdot s_B \tag{1}$$

where k is a numerical factor chosen in accordance with the confidence level desired .[1] Long and Winefordner[1] further link c_L to x_L as follows:

$$c_L = \frac{(x_L - \bar{x}_B)}{m} \tag{2}$$

where m is defined as 'analytical sensitivity' and expressed as the slope of the calibration curve line obtained from the linear regression analysis.[1] By substituting the value of x_L from equation (1) into equation (2), Long and Winefordner define c_L as

$$c_L = \frac{(k \cdot s_B)}{m} \tag{3}$$

Long and Winefordner along with several other authors agree on a value of $k = 3$, which allows a confidence level of 99.86% if the values of x_B follow a normal distribution, and 89% if the values of x_B do not follow a normal distribution.[1,10] A value of $k = 2$ has also been used by some workers, but this decreases the confidence level in c_L. The definition of LOD was later expanded on by IUPAC in 1995 to include the probabilities of false positives and negatives.

Both IUPAC and the American Chemical Society (ACS) have accepted the definition of c_L shown in equation (3). However, there are a few problems associated with

using this definition in today's automated chromatographic systems used for pesticide residue analysis in food/soil and also pesticide/drug analysis in animal fluids and tissues.

Modern chromatographic equipment is fitted with computers which gather data from the detector, process the data into a chromatogram (detector response vs time), integrate areas under peaks or measure peak heights and present the peak area/height in a report (printout or screen display). These integrators offer several modifiable parameters which must be set by the analyst in order to consistently integrate the peak area or measure peak height for standards, controls, fortified controls, and treated samples. Integration parameters cannot be changed within a batch or sequence.

Some of the parameters involved are as follows:

1. *Integrator on and off times*: Lets the computer know the time range of interest within the chromatogram [elution time range of the analyte(s) of interest].
2. *Minimum peak height/area*: Lets the computer know the minimum peak height or area below which the computer may disregard the peak (eliminates noise).
3. *Peak width definition (threshold)*: Lets the computer know how to distinguish between true peaks and detector spikes.
4. *Manual integration*: Permits the analyst to integrate the peak(s) of interest manually. In this case the analyst should be consistent in defining the baseline.

Figure 1 shows a chromatogram that may be obtained from the injection of a plant/animal tissue extract containing the analyte of interest (peak 11) after normal cleanup procedures. Figure 2 shows a chromatogram of the analyte standard in a pure solution and Figure 3 shows a chromatogram of an untreated control sample extract. If the integration parameters and ranges were improperly set, or set so as to integrate even the smallest 'blips', then all the peaks 1–12 and also the instrumental fluctuation observed along the baseline (peak 13) would be integrated. This would

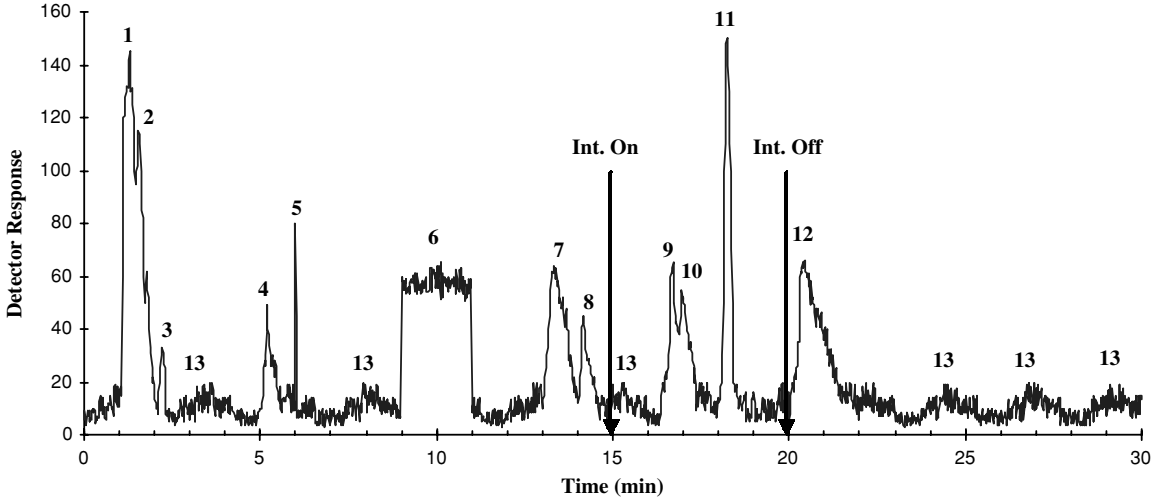

Figure 1 Example of a sample chromatogram with the analyte peak (11) eluting at 18.23 min, solvent peaks (1–3), matrix component peaks (4, 7–10, 12), and instrumental noise (5, 6, 13).

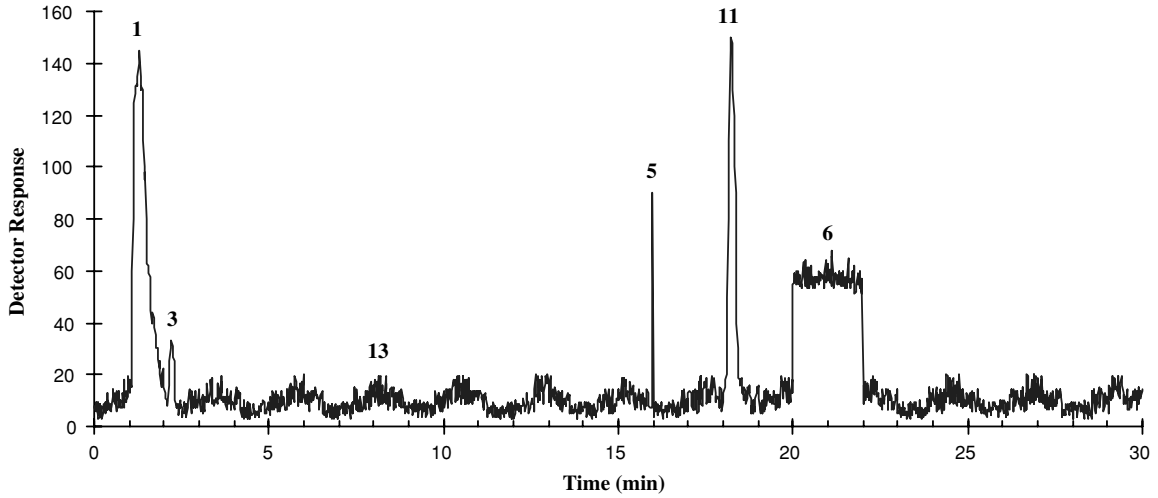

Figure 2 Example of a standard chromatogram (in pure solvent without matrix components) with the analyte peak (11) eluting at 18.23 min, solvent peaks (1, 3), and instrumental noise (5, 6, 13).

take a long time to generate and would overwhelm the analyst with a lot of useless information.

If the threshold and minimum peak heights/areas were appropriately defined, then only peaks 1–4 and 7–12 would be integrated, making the report much easier to read. Peaks 5, 6 and 13 are due to instrumental/detector noise. This would still provide a lot of extraneous information; however, the instrumental noise has been eliminated.

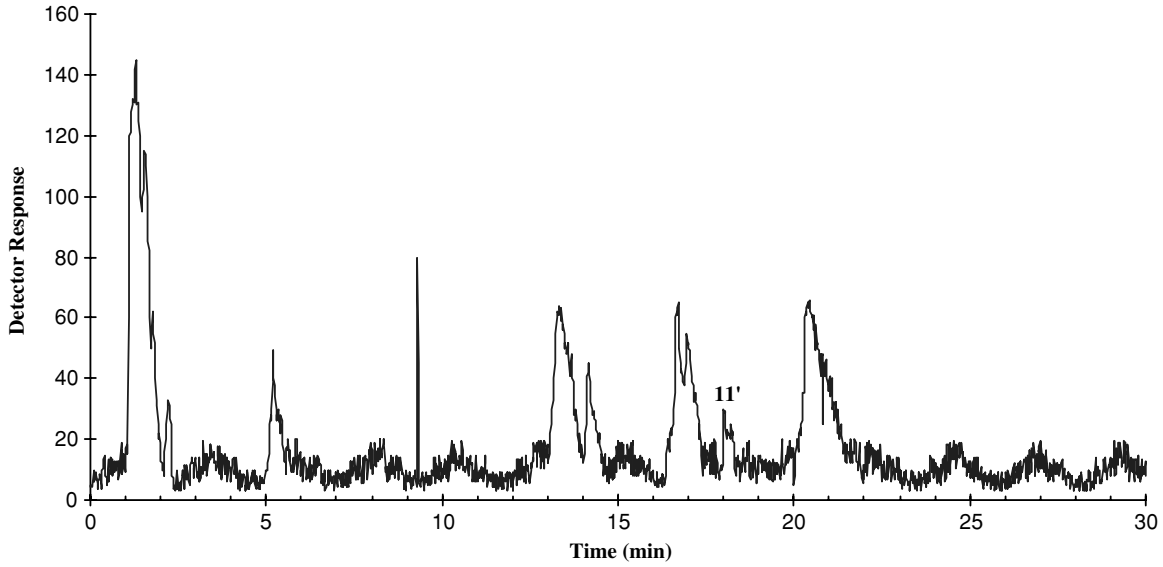

Figure 3 Example of an untreated control chromatogram with the interfering peak (11) eluting at 18 min, solvent peaks (1–3), matrix component peaks (4, 7–10, 12), and instrumental noise (5, 13).

Additionally, setting the correct 'integrator on and off' times would eliminate peaks 1–8 and 12, which are not of interest since the retention time of the analyte has been determined from the injection of a standard (Figure 2). The 'integrator on and off' times also need to be carefully determined.

Each of these parameters needs to be carefully set. Setting too large a range would provide the analyst with a lot of extraneous information, and setting too small a range would result in the possibility of incomplete integration due to slight shifts in analyte retention times. Also, setting the threshold or minimum peak height/area too high may result in the computer ignoring peaks of interest if they fall below the set minimum.

From the above discussion, it becomes apparent that if the standard deviation of the blank (s_B) had to be determined, the integration parameters would have to be set so as to integrate the background around the peak of interest. This would lead to a lot of useless information being generated and also improper integration of the analyte peak. The analyst would then be challenged to distinguish between the noise affecting the analyte peak and that which does not. Determining the value of s_B by integrating over a very narrow range (the width of the peak) may exclude matrix interferences (peaks eluting close to the analyte), which could result in under-calculating the value of s_B. On the other hand, integrating over too wide a range may result in noninterfering peaks contributing to the value of s_B. This makes the determination of the value of s_B very subjective, a major drawback in using the IUPAC method for calculating the MDL in dynamic systems such as chromatography. However, the IUPAC method provides a good estimate of MDL for techniques using static measurements such as spectrophotometers (UV/VIS, fluorimeters, AAS, ICP-MS, etc.).

2.2 Propagation of errors method

A variation of the IUPAC method called the 'propagation of errors' (PE) method has been discussed by Long and Winefordner.[1] In the PE method, the LOD is defined as

$$c_L = \frac{k \cdot \left\{ s_B^2 + s_i^2 + \left[\left(\frac{i}{m} \right)^2 \cdot s_m^2 \right] \right\}^{1/2}}{m} \tag{4}$$

where i is the intercept, s_i is the standard deviation of the intercept and s_m is the standard deviation of the slope m. If the value of s_m is small, then equation (4) reduces to

$$c_L = \frac{k \cdot \left(s_B^2 + s_i^2 \right)^{1/2}}{m} \tag{5}$$

If the value of s_i is much less than that of s_B, then equation (5) reduces to equation (3), the IUPAC definition.

It is important to note that the matrix effects, interferences, and variability in method efficiency are to be factored in when determining the MDL. If this was not done then only the background noise (see Figure 2, peak 13) would be considered in the definition of the MDL. In real-life samples there is a good possibility that matrix component peaks would either co-elute or elute at retention times close to

the analyte peak, thereby affecting the analyte signal (see Figure 3, peak 11). In order to factor these interferences into the determination of MDL, the calibration curves would have to be prepared by fortifying control samples with the analyte of interest at different concentrations around an estimated detection limit (within one order of magnitude). The fortified samples would then be extracted and analyzed and a calibration curve prepared in this manner. Furthermore, in order to measure s_i and s_m accurately, data from a minimum of five calibration curves would have to be evaluated. This would make the procedure tedious and time consuming for dynamic systems such as chromatography.

On the other hand, for static systems such as UV/VIS spectrophotometry, AAS, ICP-MS, etc., the PE method would be very easy to use since the values of s_B and s_i could be easily determined from analyzing multiple untreated control extracts. Furthermore, the PE method would be preferred over the IUPAC method because errors in analyte measurements can be incorporated into the MDL.[1]

2.3 Hubaux–Vos approach

A method for defining detection limits was discussed by Hubaux and Vos in a paper published in 1970.[11] This method involves the generation of multiple calibration curves and factors in the variability in the slope and intercept of the calibration curve. The Hubaux–Vos approach also factors analyst desired probabilities for false positives and negatives into the determination of the detection limit. Although these are important factors that must be factored in for certain types of analyses, the Hubaux–Vos approach is complicated and becomes very tedious and time consuming when matrix effects and interferences are to be factored in for determining the MDL. The same steps as outlined earlier, namely preparing the calibration curves from fortified matrix samples, would have to be applied when using this approach to determine the MDL. Additional information regarding the Hubaux–Vos approach can be found in the original paper.[11]

2.4 Two-step approach (proposed by the US EPA)

A method for determining the LOD and LOQ for water samples was proposed by the US EPA.[12] This method has also been discussed by Roy-Keith Smith in his book titled 'Handbook of Environmental Analysis'.[13] The method has also been proposed by the US EPA in their guidelines for 'Assigning Values to Non-detected/Non-quantified Pesticide Residues in Human Health Food Exposure Assessments'.[6]

This approach involves two steps for the determination of the MDL and MQL:

1. determining the instrumental limit of detection (IDL) and quantification (IQL) and using these values to estimate the MDL and MQL;
2. calculating the MDL and MQL for the extraction/analysis method for the crop/chemical combination.

For this approach, it is very important that both steps be followed in order that the calculated values of MDL and MQL are reliable. If only the first step is applied, then

the calculated value would not take matrix interferences and effects into consideration. On the other hand, if only the second step is used, the concentration of the fortified control samples used in calculating the standard deviation would bias the values of MDL and MQL. There are several ways in which the IDL and IQL could be determined. Any of the above-mentioned approaches can be used.

The simplest method for estimating the MDL and MQL would be to measure the peak-to-peak noise ($N_{p–p}$) around the analyte retention time and then estimate the concentration (of the analyte in the matrix) that would yield a signal equal to three times the $N_{p–p}$ (estimated MDL).

Alternatively, the following method would provide the analyst with a more reliable estimate of MDL:

1. analyze several (at least five) untreated control extracts;
2. measure the $N_{p–p}$ for each of the extracts;
3. calculate the average $N_{p–p}$ for the measurements;
4. calculate the concentration of a solution that would produce a signal three times the $N_{p–p}$ (taking into consideration expected losses of analyte during extraction/cleanup steps);
5. estimate the value of MDL by calculating the amount of analyte in the matrix that would yield the signal (using concentration/dilution factors).

2.5 RMSE method

Another method recommended by the US EPA[6] for estimating the LOD involves the generation of a calibration curve and calculating the root mean square error (RMSE). This method should be applied when a linear relationship exists between detector response and analyte concentration. The RMSE method involves the following steps:

1. Generate a 4–5-point calibration curve with standards of concentrations within an order of magnitude of the estimated detection limit. For this purpose, the detection limit may be estimated as a concentration that would yield a signal three times $N_{p–p}$. The calibration curve should be generated by plotting detector response (x) vs concentration (c).
2. Perform a regression analysis on the calibration curve and calculate the values of slope (m), intercept (i) and r^2 for a number of standards n.
3. The calibration curve can be defined by the following equation:

$$x = m \cdot c + i \tag{6}$$

4. Based on the values of slope m and intercept i, calculate the predicted response (x_p) for each of the standards.
5. Calculate the error (E) associated with each measurement $|x_p - x|$.
6. Calculate the square of the errors for each standard and then calculate the sum of the square of the errors ($\sum E^2$) for a number of points n.

7. The RMSE is then calculated as follows:

$$\text{RMSE} = \left[\frac{\sum_{j=1}^{n} E_j^2}{n-2} \right]^{1/2} \tag{7}$$

8. The predicted response at the IDL (x_L) is calculated as follows:

$$x_L = i + (3 \cdot \text{RMSE}) \tag{8}$$

9. Rearranging equation (6), the IDL (c_L) can be calculated as

$$c_L = \frac{(x_L - i)}{m} \tag{9}$$

Combining equations (8) and (9), we obtain

$$c_L = \frac{3 \cdot \text{RMSE}}{m} \tag{10}$$

The value of c_L determined here is measured in terms of concentration and solution. This value does not take matrix interferences into account since RMSE was determined from calibration standards. Therefore, this value should be reported as the IDL. This value provides a good starting point for the next step, which is calculating the MDL.

Comparing equations (10) and (5), the IUPAC definition for detection limit, the difference is that RMSE is used instead of s_B. For dynamic systems, such as chromatography with autointegration systems, RMSE is easier to measure and more reliable than s_B for reasons discussed earlier. Both are measures of variance and, although dissimilar, provide similar information. This is apparent in the equations used to calculate the values of s_B and RMSE:

$$s_B = \frac{\sum_{j=1}^{n} \left(x_{j_B} - \bar{x}_B \right)}{n-1} \tag{11}$$

and

$$\text{RMSE} = \left[\frac{\sum_{j=1}^{n} \left(x_j - x_{j_p} \right)^2}{n-2} \right]^{1/2} \tag{12}$$

The LOQ is regarded as the lower limit for precise quantitative measurements.[2] Several authors, such as Miller and Miller,[2] Skoog and Leary,[9] and Smith,[13] have suggested that the LOQ be defined as a concentration which would produce a signal 10–12 times s_B. At this point, the standard deviation is small enough (approximately 10–15%) so that the quantitated value can be deemed reliable.

Extending this principal to equation (10), the instrumental quantification limit (IQL, c_q) may be calculated using the equation

$$c_q = \frac{10 \cdot \text{RMSE}}{m} \tag{13}$$

2.6 *The $t_{99}s_{LLMV}$ method*

The second step in the two-step approach involves calculating the values of MDL and MQL. This method has been described previously by the US EPA[12] and by Smith[13] for the analysis of water samples. This method involves the following steps:

1. Weigh seven or more sub-samples (ground and homogenized) of an untreated control sample of the matrix of interest. The weight of each sub-sample should be the same as that proposed to be used during sample analysis.
2. Estimate the LOQ using any of the methods described earlier and equate the value in terms of amount in the matrix, factoring in any concentration and dilution factors from the extraction procedure.
3. Fortify each of these sub-samples with the analyte(s) of interest such that the concentration of the analyte(s) in the matrix equals the estimated limit of quantification (ELOQ).
4. Extract each of these fortified control samples using the extraction procedure used for the sample analysis.
5. Analyze each of the final extracts using the method used for sample analysis.
6. Determine the amount of residue found in each of the fortified samples.
7. Calculate the standard deviation of these measurements (s_{ELOQ}).
8. Determine the 'one-tailed t-statistic' for $n - 1$ observations at the 99% confidence level [$t_{99_{(n-1)}}$].
9. The MDL for the matrix/analyte(s) combination and the extraction/analysis procedure is defined as

$$\text{MDL} = t_{99_{(n-1)}} \cdot s_{\text{ELOQ}} \tag{14}$$

For seven replicates (six degrees of freedom) of the fortified control samples,

$$t_{99_{(n-1)}} = 3.143$$

As discussed earlier, a concentration that would produce a signal of approximately 10–12 times the standard deviation of the blank (or in this case s_{ELOQ}) is considered to be the limit of quantification. Therefore, if the LOQ was set at 10 times s_{ELOQ}, for 7 replicates (6 degrees of freedom) of the fortified control samples, $t_{99_{n-1}} = 3.143 - 10/t_{99_{n-1}} = 10/3.143 = 3.182$, which can be rounded off to 3 – then the method quantitation limit (MQL) for the matrix/analyte(s) combination and the extraction/analysis procedure is defined as

$$\text{MQL} = 3 \cdot \text{MDL} \tag{15}$$

This would provide a concentration limit above which fairly precise quantitative measurements can be reported.

Alternatively, during the course of method validation and sample analysis, control samples fortified at the ELOQ (determined by one of the methods described above) are extracted and analyzed. The standard deviation of these fortified control samples (s_{LLMV}) can also be used to calculate the MDL and the MQL for the method. In the latter case, s_{LLMV} would replace s_{ELOQ} in equation (14).

The value of MDL and MQL calculated by the two-step process described above takes into consideration several factors which affect the analyte signal, including:

- instrumental noise
- variability in instrument sensitivity
- variability in method efficiency
- matrix effects and interference

However, it is extremely important that the estimated LOD and LOQ be accurately determined. The fortification concentration greatly influences the final value of MDL and MQL determined by this method. If too high or too low a value of ELOQ is chosen for fortification for determining the MDL, then the calculated MDL and MQL may be different from the actual method capability.

The US EPA recommends that if the calculated values of LOQ (MQL) are significantly different from the estimated values, then steps 1–7 above should be repeated with the new 'estimates' of the LOD/LOQ and the MDL and MQL should be recalculated.[12] This should be done till the calculated values of LOD and LOQ are in the 'range' of the estimated values (LLMV = 2–5 times the MDL).

Although time consuming, this is important for determining accurate values of MDL and MQL. However, if the ELOQ/LLMV is properly determined by any of the methods described above, then an experienced chemist should be able to determine accurately the fortification levels (LLMV) for calculating MDL and MQL, thereby avoiding time-consuming repetitions. The two-step approach is a fairly accurate way for determining method limitations.

3 Confirmation

Once the MDL has been calculated, it is important to prove that the chosen analytical procedure is practically capable of detecting the analyte(s) at the MDL. To prove the practicality of the MDL, the analyst should spike triplicate sub-samples of an untreated control sample at the MDL, extract the fortified control samples and analyze them on the instrument. Well defined chromatographic peaks would prove the validity of the calculated MDL.

3.1 Representative data

The Interregional Research Project No. 4 (IR-4) was formed in the USA in 1963 to address the problem of lack of available pest control products for minor food crops (grown on 300 000 acres or less).[14] The problem of the lack of appropriate methods to

Table 1 Representative data: evaluating data obtained from various studies for calculating the LOD and LOQ values for the extraction/analysis procedure using the '3(RMSE)/slope' method to estimate the LOD/LOQ and the '$t_{99_{(n-1)}} s_{\mathrm{ELOQ}}$' method to calculate the MDL and MQL

Method[a]	Pesticide	Matrix	ELOQ (mg kg^{-1})	LLMV (mg kg^{-1})	Av. recovery ± standard deviation	Calc. MDL (mg kg^{-1})	Calc. MQL (mg kg^{-1})	Ref.
GC/ECD	Fipronil	Onion	0.002	0.005	0.0046 ± 0.0004	0.001	0.003	15
GC/ECD	Clopyralid	Canola	0.065	0.1	0.1124 ± 0.016	0.04	0.12	16
GC/ELCD	Sulfentrazone	Cabbage	0.03	0.05	0.0503 ± 0.005	0.014	0.042	17
GC/NPD	Zn_3P_2	Cucumber	0.05	0.05	0.0351 ± 0.0048	0.014	0.042	18
GC/FPD	Dimethoate	Grass	0.011	0.02	0.0196 ± 0.003	0.0086	0.026	19
GC/MS	Clopyralid	Canola	0.035	0.05	0.0593 ± 0.004	0.011	0.034	20
GC/MS	Quinoxyfen	Cantaloupe	0.005	0.01	0.0096 ± 0.00085	0.0027	0.008	21
LC/UV	Desmedipham	Spinach	0.024	0.05	0.0359 ± 0.002	0.0074	0.022	22
LC/MS	Fenhexamid	Pepper	0.031	0.02	0.0175 ± 0.0024	0.0065	0.0020	23

[a] GC/ECD, gas chromatography/electron capture detection; GC/ELCD, gas chromatography/electrochemical detection; GC/NPD, gas chromatography/nitrogen–phosphorus detection; GC/FPD, gas chromatography/flame photometric detection; GC/MS, gas chromatography/mass spectrometry; LC/UV, liquid chromatography/ultraviolet detection; LC/MS; liquid chromatography/mass spectrometry.

define method limitations has often plagued IR-4 analytical laboratories and chemists who have several years of experience in method development for pesticide residues in food. When the US FQPA was passed in 1996, the issue of calculating cumulative risks due to exposure from all sources has placed a very high burden on minor crops. The smaller market of minor crops has led some registrants to drop the use of pesticides in minor crops in order to retain the more profitable major crop uses such as corn, cotton, wheat and soybeans. For pesticide uses in minor crops where the use results in no detectable residues, IR-4 chemists have been challenged to develop methods with extremely low detection limits and prove the lack of detectable residue at these low limits, thereby increasing the number of minor uses by 'freeing up space in the use cup'.

Data from several laboratories within the Interregional Research Project No. 4 (IR-4) in the USA have been evaluated for determining the values of MDL and MQL. These data have been presented in Table 1. The two-step procedure described in the EPA guideline[6] was used to calculate the values of MDL and MQL. For the first step, the slope, intercept and RMSE values for the first three calibration curves of each study were separately calculated, then the IDL and IQL values calculated and the value of LOQ estimated for the method. These values were compared with the actual values of LLMV. The standard deviation of the spike recoveries at the LLMV (s_{LLMV}) was used to calculate the MDL and MQL. The values of LLMV were separately determined by the laboratory not using any of the methods described in this article.

Evaluating the data presented in Table 1 indicates that the values of ELOQ, LLMV and MQL are comparable, implying that the calculated values of MDL are fairly reliable. In a few cases (not reported here), the value of MQL was significantly (more than three times) different from the LLMV. In cases such as these, it would be advisable to repeat the procedure as discussed earlier.

Table 2 Comparison of methods for calculating detection and quantification limits for analytical methods used for food analysis

Method	Simple and easy to apply	Considers variability of calibration curve	Considers method efficiency and matrix effects	Variability between laboratories and analysts	Good for estimating LOD/LOQ	Comments
$N_{\text{p-p}}$	Yes	No	Yes	High	Yes	Very dependent on analyst interpretation
k_{S_B}/m	No	No	No	Moderate	No	Difficult to implement
PE	No	Yes	No	Low	Acceptable	Difficult to implement
Hubaux–Vos	No	Yes	No	Low	Very tedious and time consuming	Impractical for complex matrices
RMSE	Yes	Yes	No	Low	Yes	Good for IDL but not MDL
$t_{99}s_{\text{LLMV}}$	Yes	Yes	Yes	Low	No	Very dependent on value of LLMV chosen
Two-step approach using $t_{99}s_{\text{LLMV}}$ method	Yes	Yes	Yes	Low	Yes	Best for calculating MDL and MQL

4 Conclusions

Several methods have been discussed for the determination of method limitations when evaluating procedures for the determination of pesticides in food. A brief comparison of the methods discussed for the determination of the detection and quantification limits of methods used for the analysis of food products can be found in Table 2.

Although accepted by IUPAC and ACS, the '$k \cdot s_B/m$' definition is hard to implement and does not take either variability in method efficiency or matrix effects into consideration. This would be rectifiable if the calibration curves were prepared from control matrix samples fortified at different concentrations (within one order of magnitude of an estimated LOD).

A better alternative would be to use the 'propagation of errors' definition, which takes into consideration values of both s_B and s_i when calculating the MDL. This would involve generating at least five calibration curves in order to obtain an accurate measurement of s_i and s_m.

The $3N_{\text{p-p}}$ approach, although simple, leaves too much to the analyst's discretion, thereby rendering the values obtained hard to compare between analysts and laboratories. This method may be used in estimating the LOD and LOQ in the two-step approach.

The two-step approach involving the RMSE (or the $3 \cdot N_{\text{p-p}}$) method for estimating the LOQ and $t_{99_{(n-1)}} \cdot s_{\text{ELOQ}}$ is the most practical method for determining the MDL and MQL of the extraction/analysis procedure because it incorporates matrix effects and interferences and also variability of method efficiency in the final calculation.

The $t_{99_{(n-1)}} \cdot s_{\mathrm{ELOQ}}$ method can be applied to data generated during the course of a pesticide registration study. When data obtained during the course of a study are used, the standard deviation of the LLMV spike recoveries (s_{LLMV}) can be used instead of s_{ELOQ} provided that the LLMV was appropriately determined by one of the methods discussed in this article or any other statistically valid method.

Since several methods appear to be acceptable, it is important that when reporting values for MDL and MQL, the method used to define these values be clearly identified.

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Abstract: Limits of detection (LOD) and quantification (LOQ) are important parameters that define the limitations of an analytical method. The importance of having uniform working definitions has already been stressed upon by several authors including Long and Winefordner and Miller and Miller. This author reiterates the importance because, depending on the definition chosen, the value of LOD can vary over an order of magnitude, making the number meaningless for comparative purposes. It is important that the method chosen account for all factors affecting the analyte signal. It is equally important that the method for determining this value be reliable and easy to determine, and not involve long and tedious procedures. If these criteria are not met, it will result in chemists reverting to the old technique of 'Analyst's evaluation', normally done by 'eyeballing' a value with which he/she is comfortable, for reporting the values of LOD and LOQ. This would lead to a lot of variability between analysts and again render the reported value meaningless.

The reason behind chemists being reluctant to use statistics in evaluating analytical methods is the difficult and complex calculations involved. Today, modern computers and software packages have greatly reduced this task and therefore made the use of statistics more widespread in chemistry. Some of the methods being proposed here would not involve any additional work other than that done during method development, validation, and sample analysis.

Keywords: Limit of detection, limit of quantification, lowest level of method validation, method development, method validation, tolerance, maximum allowable residue limit, risk assessment, method detection limit, method quantification limit.