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Short Communication

An improved concept of minimum detectable limit in pesticide residue analysis

R. RAY AND S. F. HAMEED
Department of Entomology, Rajendra Agricultural University, Pusa 848 125, Samastipur, Bihar.

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Abstract

A simple method for working out minimum detectable limit of the conventional pesticide residue assays (colorimetry and microbioassay) has been proposed as sum total of lower measuring limit of an analytical instrument and sensitivity of a method. A concept of lowering the detectable limit of a method has been put forward. The concept of $'t'_{sen}$ (time to dissipate to the level of sensitivity) has been replaced by more logical $'t'_{MDL}$ (time to dissipate to the level of minimum detectable limit).

Key words: Pesticides residue, minimum detectable limit, colorimetry, microbioassay.

1. Introduction

The detection of a small amount of a given pesticide by an analytical technique is known as the residue analytical limit of detectability. The decay curve, a simplified graphic representation of the disappearance of a pesticide, wherein concentration is plotted against time, could be extrapolated to an infinitely low concentration, like the radioactivity decay curve. Thus, theoretically, no chemically treated substrate can become free of residues and it is seemingly impossible ever to employ the term no residue while reporting data.

In the microanalysis of pesticide residue, the words BDL, *i.e.*, below detectable limit or ND, *i.e.*, non-detectable are often used while referring to the quantity which an analytical method fails to detect. From BDL it is sometimes concluded that cent per cent dissipation of residues has occurred. But it is misleading to conclude that quantity not detected is the quantity not present in the substrate sample. Quantity not detected by one method may very well be measured by a more efficient method as the minimum level of detection would vary with different analytical methods with reference to pesticides. Hence, it is important to work out the 'minimum detectable limit' (MDL) of a method and should always be mentioned when the word BDL or ND is used.

2. Proposition

Sensitivity is often confused with the minimum detectable limit of a method[1,2]. Sensitivity gives the value for minimum noticeable deviation caused by the smallest change in the quantity, whereas the minimum detectable limit gives a measure of the minimum quantity to be detected reliably by the method as such. Therefore, sensitivity and the minimum detectable limit of a method are not one and the same thing, rather both in combination indicate the overall efficiency of the analytical method.

For any analysis it is required to work out the linearity range of the chemical by a linear standard curve. The linearity range of a method is given with reference to the chemical and the analytical tool used. Each experimenter may have his own working limits within the prescribed linearity range and thus his working range would have a lower and higher measuring limits. Extrapolating the line back to zero often leads to erroneous results. Therefore, the + ve value of sensitivity of a method, when added to the lower measuring limit, *i.e.*, the lowest quantity in the standard curve, would give reliability to the minimum measurement and may be termed as the 'minimum detectable limit', *i.e.*, MDL = lower measuring limit $\pm$ sen.

Let us examine the case with colorimetric assay as well as microbioassay with reference to residue analysis.

2.1. *Colorimetry*

Sensitivity and minimum detectable limit of a method are worked out using one and the same formula of Bates[3]. Here, sensitivity $= \pm 2S/\sqrt{n}$ where S is the estimate of the standard deviation of the readings of the blank samples and n the replication per sample estimate. The value of S is obtained in the absorbance scale.

Here, MDL = ODL + sen, where ODL = lowest quantity in the standard curve and sen = + ve value of sensitivity of the method. Again, MDL = (ODL + sen)/$R \times V$ μg g^{-1} where R is the extraction ratio and V the maximum aliquot size contained in the reaction tube. If ODL + sen = MDQ, then MDL = MDQ/$R \times V$ μg g^{-1}, where MDQ is the minimum quantity measured by the instrument.

As the value of MDQ, the minimum quantity being measured by the method/instrument is a constant, the MDL $\propto R^{-1} \cdot V^{-1}$. Again, when V, *i.e.*, the maximum aliquot size is also a constant, MDL $\propto R^{-1}$, *i.e.*, the minimum detectable limit is inversely proportional to the extraction ratio, where others are constant. Based on the above proposition, MDL may be calculated from the following data[4]. Sensitivity $= \pm 0.03$ in the absorbance scale where $S = 0.02125$ and $n = 2$. This is equivalent to $\pm$ 3.07 μg corresponding to the standard curve, and hence the + ve value of sensitivity = + 3.07 μg. Therefore, MDL = (10.0 + 3.07)/20·R μg g^{-1}, where ODL = 10 μg, V = 20 ml or MDL = 0.65 R^{-1} μg^{-1} g., or MDL $\propto$ R^{-1} μg g^{-1}, where 0.65 is a constant.

MDL in colorimetric assay varies due to different values of R (Table I).

Table I
Minimum detectable limit of endosulfan in various substrates by colorimetric and microbioassay methods[a]

Sl no.	Substrate	R(g/ml)	Colorimetric assay			Microbioassay					
			V (ml)	MDQ (μg)	MDL ($\mu g\,g^{-1}$)	V (ml)	F*	LD_{16} (μg)	LD_{50} (μg)	Sen ($\mu g\,g^{-1}$)	MDL ($\mu g\,g^{-1}$)
1	Green plant material	50/50 = 1	20	13.07	0.65	2	0.10	0.2754	0.5466	0.0273	0.1650
2	Dried plant material	50/50 = 1	20	13.07	0.65	2	0.11	0.2163	0.4382	0.0241	0.1322
3	Whole grain	150/50 = 3	20	13.07	0.22	2	0.13	0.3388	0.5867	0.0127	0.0691
4	Dehusked rice	150/50 = 3	20	13.07	0.22	2	0.12	0.4027	0.6492	0.0130	0.0801
5	Rice husk	50/50 = 1	20	13.07	0.65	2	0.13	0.3803	0.5801	0.0377	0.2279
6	Field water	1000/50 = 20	20	13.07	0.03	2	0.15	0.2884	0.4636	0.0017	0.0089
7	Soil	100/50 = 2	20	13.07	0.33	2	0.10	0.2917	0.4505	0.0113	0.0842

*F, the factor of accuracy, has been calculated after Ray *et al*[8]

2.2. *Microbioassay*

Sensitivity of microbioassay method is worked out as $\pm D \times F/R \times V\,\mu g\,g^{-1}$ where D is the LD_{50} value, F the factor of accuracy, R the extraction ratio, and V the volume of extract added to each test jar[5]. Let the measuring range of the standard curve be LD_{16} to LD_{84}, where LD_{16}, the lower measuring limit, is the dose/concentration of toxicant bringing about 16% mortality in the test population, and LD_{84} refers to 84% mortality, the higher measuring limit.

Here, again MDL is the lower measuring limit + sensitivity, *i.e.*, $MDL = (LD_{16} + (LD_{50} \times F))/R \times V\,\mu g\,g^{-1}$. If $R \times V$ factor is not considered, the rest gives the minimum quantity detected by the method, which is constant for a particular set of experiments and may be given by MDQ.

Therefore,

$$MDL = MDQ/R \times V\,\mu g\,g^{-1}$$

or

$$MDL \propto 1/R \times V\,\mu g\,g^{-1}.$$

The MDL of an insecticide analysed by microbioassay method would vary due to different values of R, V, F, LD_{16} and LD_{50} obtained from different substrates (Table I). The prescribed tolerance limit of endosulfan for paddy is 0.1 μg/g. To make the analysis meaningful, the minimum detectable limit of the method was suitably lowered by increasing the extraction ratio accommodating greater sample size.

3. Discussion and conclusion

Clear distinction between the sensitivity and the MDL of an analytical method is not made because sensitivity is confused as MDL[1,2,6]. In microbioassay, the sensitivity may vary with the substrates, the test organism remaining the same, because of different LD_{50} and F values, but the situation is not similar with colorimetric analysis. Here, the clean up is so rigorous that the estimate of standard deviation of readings of the blank samples of different sets seldom differs significantly. Thus, sensitivity of colorimetric method is considered with reference to an analytical instrument and the product.

Minimum detectable limit would consequently vary with substrates because of varying sensitivity in microbioassay. But there would be no such variation with colorimetric assays (Table I).

Problem arises when sensitivity is mistaken as the minimum detectable limit. Bates[3] calculated the sensitivity as well as MDL from the same formula but some problems surfaced when Verma and Pant[1] used this model to work out the sensitivity/MDL of a sample size of 30 g treated with endosulfan as 0.054 ppm. However, when the sample analysis was replicated (at least twice), the instrument should have detected minimum amount, *i.e.*, $0.054 \times 15 = 0.81\ \mu g$. Even if the entire sample (30 g) were to be used, the minimum amount estimated by the instrument would have been 1.62 μg. But the method of Maitlen *et al*[7], by which endosulfan was estimated, clearly defined the linearity range from 5 to 100 μg. Bates[3] formula of sensitivity for calculating MDL can be used only when the lower measuring limit of the standard reference curve is zero. But seldom in practice it is so. Again, in the regression line, $y = a + bx$ representing standard curve, the value of a may be close, but not equal, to zero. For this reason, it is better not to extrapolate the standard curve back to zero. Thus, the lowest quantity of toxicant above zero within the linearity range taken for working out the standard curve should be regarded as the lower measuring limit, unless specified otherwise by the author of the method.

The term 't_{sen}' (time required for the residues to dissipate to the level of sensitivity) is sometimes used in residue data. In colorimetry, if the lower measuring limit is taken as zero, then only it is possible to measure a quantity as small as sensitivity. In microbioassay, the measure of LD_{50} is the most reliable one but when it is multiplied with the factor of accuracy ranging from 0.2 to 0.1 to give the sensitivity it becomes 5 to 10 times smaller than LD_{50} value and hence cannot be measured reliably. Therefore, it seems logical to change the concept of 't_{sen}' with that of 't_{MDL}', *i.e.*, the time required for the residues to dissipate to the level of minimum detectable limit. The MDL of a method should be below the accepted tolerance limit of the substrate concerned. Sometimes, it is observed that the prescribed tolerance limit of a substrate is so low that it is even lower than the minimum detectable limit of the method. This problem can be overcome by increasing the sample size resulting in increased extraction ratio (R) which, on the other hand, is inversely proportional to MDL. Increasing the aliquot size (V) is not always possible because of experimental limitations.

The minimum detectable limit of a method must be stated along with the sensitivity while reporting the residue data. The concept of MDL is of utmost importance in designing the

residue analysis trial right from the sampling stage. It helps in optimising the sample size enabling detection to a level below the tolerance limit.

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