Although the general concept of a detection limit as a lower practical operating limit for an analytical method is widely understood, the precise interpretation of results near the IUPAC limit of detection is rarely as well understood or implemented. It is important to distinguish between statements about the analytical result, which is known, and inferences about the true value, which are not known. It is also crucial to understand that there are at least two quite different limits involved and that the limit most commonly referred to as the ‘limit of detection’ is, surprisingly, not intended as the criterion for detection of an analyte. This Technical Brief describes the principal internationally recognised approach to decision and detection limits.

Introduction

Many analytes are prohibited or regulated at very low levels. For prohibited materials, the analyst’s problem is to establish whether the analyte is present or not. For materials regulated at low levels, we must establish whether our analytical method is capable of detecting the analyte at all at the regulated level.

In conformity with modern thought, these questions would best be answered by looking at the uncertainty associated with a result at low levels. Historically, however, the issue has been addressed by constructing limits for decisions and detectability. These have been defined differently over time, and are consequently often misused or misinterpreted. This Technical Brief describes the approach used by IUPAC and ISO for the construction and interpretation of decision and detection limits.

The IUPAC formulation

The present IUPAC formulation of limits related to detection of an analyte is based on theory presented in a seminal paper by Currie and described in an official IUPAC recommendation. The reasoning is based on statistical hypothesis testing and asks two fundamentally different questions:

(i) At what value is an observed analytical result significantly different from the result for a true ‘blank’ test material (that is, a material free from the analyte sought)?

(ii) At what true analyte concentration will the analytical result reliably exceed the level defined in (i)?

Question (i) defines the criterion for detection; any result above this value is to be declared ‘positive’. For that reason, together with its basis in hypothesis testing, IUPAC’s recommendation refers to this first level as a ‘critical value’, designated \( L_C \). Question (ii) is essentially asking the question ‘what is the lowest level that will reliably be detected in practice?’ Importantly, it is the second question that corresponds to the IUPAC definition of ‘limit of detection’ (LOD).

IUPAC designate this second value as \( L_D \).

Notice also why these questions are described as “fundamentally” different. The first asks about an observable signal – the analytical result leading to a declaration of presence. The second is asking about a true value – something we cannot see directly and can only make inferences about.

Calculating limits

Calculating the detection limit, \( L_D \), is a two-step process, starting with the critical value \( L_C \). \( L_C \) is constructed from

\[
L_C = \bar{x}_0 + s_0 t_{0.95,v_0}
\]

where \( \bar{x}_0 \) and \( s_0 \) are the estimated mean and standard deviation of results for a ‘blank’ material, and \( t_{0.95,v_0} \) is the one-tailed 95% quantile for Student’s \( t \) with \( v_0 \) degrees of freedom, which depends on the amount of data used to estimate \( s_0 \). Often, particularly for an estimate in method validation, \( t_{0.95,v_0} \) is replaced by its large-sample value of 1.645. \( \bar{x}_0 \) and \( s_0 \) can come from the simple replicated observation of a blank material; in some documentary standards, they are obtained as the intercept and residual standard deviation of a regression through calibration data. They can also be in either the ‘signal domain’ (for example, peak area, absorbance etc.) or in the ‘concentration domain’ after conversion to concentration. \( L_C \) itself of course appears in the same units as \( \bar{x}_0 \) and \( s_0 \) and can accordingly be expressed in either ‘domain’.
Interpretation of results near zero

Once we have a critical value \( L_C \) and a detection limit \( L_D \), we can use these to guide inference about the presence and absence of the analyte. Interpretation of new results follows Fig. 2, which divides the concentration range into three regions, A–C.

Table 1 overleaf summarises what can be said about the true concentration when the analytical result \( x \) falls in each of these ranges.

A result in region C can, of course, also be interpreted as evidence that the analyte is present with at least 95% confidence.

Notice that results in region B – between the critical value and the detection limit – are positive for the presence of analyte. It is the critical value that is the decision criterion for presence of analyte – not the LOD.

The quantitative inferences that can be made should also be noted. All observed results are valid estimates; it is, however, important to allow for the associated uncertainty in interpretation. Near zero, the relative uncertainty is usually large. For a single observation, the standard uncertainty cannot be less than the standard deviation used for calculating the detection limit; it is usually much larger because an estimate of uncertainty includes all of the factors that might affect the result.

Finally, results below \( L_D \) or even the critical value \( L_C \) are not meaningless; they just have a larger relative uncertainty than results above these limits. While it is clearly not harmful to report results as ‘less than’ \( L_D \) where this meets client requirements, it is important that laboratories remain able and willing to provide the raw results if requested.

Limit of quantitation (‘LOQ’) and other reporting limits

The concept of a ‘limit of quantitation’ is based on the idea that, as analyte levels reduce, the relative uncertainty – however that is described – increases to the point that the results no longer meet some requirement for acceptable uncertainty. While it can be useful to estimate a quantitation limit during method validation as an...
Room for confusion

While the general theory given here is now widely accepted, terminology remains confused and inconsistent. Historically, many writers referred to the critical value as the ‘detection limit’. ISO were obliged to define entirely new terms for critical value and LOD to avoid the confusion – but in doing so added yet more new terminology, necessitating a translation

Table 1 Interpretation of results near the detection limit

<table>
<thead>
<tr>
<th>Result in region</th>
<th>Description</th>
<th>Detection</th>
<th>Inference about the true value</th>
<th>Quantitative inference (all regions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>$x \leq L_C$</td>
<td>Not detected</td>
<td>Less than $L_D$ with at least 95% confidence</td>
<td>The best estimate of the true value is $x$. The standard uncertainty associated with this value is $s_x$ [see text]</td>
</tr>
<tr>
<td>B</td>
<td>$L_C &lt; x \leq L_D$</td>
<td>Detected</td>
<td>Greater than zero with at least 95% confidence</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>$x &gt; L_D$</td>
<td>Detected</td>
<td>Greater than $L_C$ with at least 95% confidence</td>
<td></td>
</tr>
</tbody>
</table>

Assuming $\alpha$ and $\beta$ are set at 5% for 95% confidence.

References

5. J. Mocak, A. M. Bond, S. Mitchell and G. Scollary, Statistical overview of standard (IUPAC and ACS) and new pesticides use CC and LOD for the IUPAC critical value and LOD respectively. And a later IUPAC publication may have confused matters even further by recommending that the IUPAC critical value be referred to as the detection limit. Further, while the idea of critical values and detection limits was intended to guide inference about the true analyte concentration, they are often used incorrectly as statements about the observed result. All of this results in considerable confusion.

The AMC recommends that if statements such as ‘less than LOD’ are made in relation to results, the basis for the calculation and interpretation should be made clear to the client, either explicitly in the report or by reference to a documented standard or procedure.

The AMC also recommends that raw data be made available to the client irrespective of whether it is above or below detection or reporting limits wherever it is important for the client’s needs – for example, for trend analysis, averaging or other summaries, or (for example) in proficiency testing. Where possible, information on the uncertainty of results should be given with any numerical values to prevent over-interpretation.

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