Limit of Detection and Its Establishment in Analytical Chemistry

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ABSTRACT: Method validation is crucial for every procedure in analytical chemistry to ensure its reliability under defined conditions. As the fundamental element of method validation, limit of detection describes the smallest concentration or quantity of test substance that can be detected with a reasonable degree of certainty. In recent years, a number of documents have been published on the requirements of validation and the determination of limit of detection (LOD). A corresponding signal value measured in a procedure must reflect the true signal value as interfering factors can be contributed by the sample itself, instrument and the method used for analyte determination. Potential errors have to be evaluated during limit determination. The importance of this limit to distinguish the presence or absence of a test substance in a sample cannot be overlooked in an analytical analysis. This paper reviews various definition of LOD, factors affecting LOD and methods of LOD determination.

Keywords: limit of detection, method validation, analytical chemistry

Introduction

An analytical procedure is a series of steps from the receipt of sample until the production of final result. Through a given procedure, an analyst determines what he/she intend to; both describing the entity qualitatively and measuring the amount quantitatively. Additionally, the procedure should satisfy an agreed requirement and has to be validated to ensure the reliability of the procedure under the defined conditions. Therefore, a report on detection of the smallest concentration or amount of test substance with reasonable certainty becomes an important goal and essential part of quality assurance in analytical chemistry (CDER, 1994; ICH, 1997; EURACHEM, 1998; Walfish, 2006; NATA, 2009). Recent advancement in laboratory assays has led to development of more sensitive analytical procedures. These procedures are performed widely and allow detection down to the level of sub-part per trillion. Although with the best recognized analytical procedure, there may still consist of a certain degree of measurement uncertainty. The limit established today may also be superseded in coming future.

With increasingly lower level of measurement, several questions have to be answered in order to ensure the actual indication of the presence of test substance through a measurement or due to any error which is comparable to the reported value. First, if a test substance is not detected by an instrument (or analytical procedure), does it mean that there is an absence of test substance and no single molecule of that substance in the sample? Is the amount of test substance below the capability of the instrument? Is analytical procedure not appropriate to analyze the test substance? Or is there interference that quenches the signal or output?

In analytical chemistry, the capability of detection of any procedure is a typical performance characteristic with noteworthy impact on risk assessments or regularly decisions during laboratory analysis. It is necessary to characterize the performance of laboratory assay to understand its capability and limitations. It is also crucial to ensure that method "fits for purpose" for which the results are likely to be.

Definitions

The boundaries of analytical sciences are being challenged, thus, more precise, sensitive and accurate procedures shall be developed. Various analyses are performed with different instruments and different methods. The capability of an instrument and the method detecting limit are important in practices of chemical measurement and these have to be clearly specified with a fully defined measurement process (Currie, 1995). The importance of measurement limits and the associated problems have been highlighted with some common terms being used.

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Limit of detection (LOD) was the most widely used term for the description of the smallest concentration or quantity that can be reliably measured with a specified degree of certainty. M*inimum detectable value of the net state variable* (MDV) or *minimum detectable net concentration* was also used by International Standards Organization (ISO) (Mocak *et al.*, 2009) whereas the term *minimum detectable (true) value* was preferred by International Union of Pure and Applied Chemistry (IUPAC) (Currie, 1995).

The US Environmental Protection Agency (EPA) introduces *method detection limit* (MDL) in their published documents (U. S. EPA, 1992; U. S. EPA, 2010b; U. S. EPA, 2010a). In general, LOD was the term quoted by most chemists as a measure of the inherent detection capability and thus used throughout in this article. A number of international organizations had defined the definition summarised as follows:

The minimum concentration of an analyte that can be identified, measured and reported with 99 % confidence that the analyte concentration is greater than zero.

EPA (U. S. EPA, 1992; U. S. EPA, 2010b; U. S. EPA, 2010a)

Concentration or quantity derived from the smallest measure that can be detected with reasonable certainty for a given analytical procedure.

IUPAC (McNaught and Wilkinson, 1997)

The true net concentration or amount of the analyte in the material to be analysed which will lead with probability (1-β) to the conclusion that the concentration of the analyte in the analysed material is larger than that of the blank matrix.

ISO (EURACHEM, 1998)

Lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value.

International Conference of Harmonization (ICH) (ICH, 1995; ICH, 1997)

Lowest concentration of an analyte that the analytical process can reliably detect.

American Chemical Society (ACS) (ACS Committee on Environmental Improvement, 1980)

Lowest concentration of analyte in a sample that can be detected, but not necessarily quantitated, under the stated experimental conditions.

Center for Drug Evaluation and Research (CDER) (CDER, 1994)

The lowest concentration that can be determined to be statistically different from a blank with 99% confidence [NR 140.05 (12)].

and

The lowest concentration or amount of analyte that can be identified, measured and reported with confidence that the concentration is not a false positive value [NR 149.03 (41)].

Department of Natural Resources (DNR) (Ripp, 1996; DNR, 2008)

The smallest amount or concentration that can be readily distinguished from zero and be positively identified according to pre-determined criteria and/or level of confidence.

National Association of Testing Authorities (NATA) (NATA, 2009)

Capability of detection was described in two different components by U. S. EPA, namely *Instrument Detection Limit* (IDL) and MDL (U. S. EPA, 1992; Ripp, 1996; Johannes, 2003; U. S. EPA, 2010b; U. S. EPA, 2010a). IDL was expressed as the result of the development of various instruments and the limitations of each instrument on the amount of test substance that could be detected. It was defined as the "*concentration equivalent to the smallest signal, due to an analyte, that can be distinguished from the background noise by a particular instrument"* (U. S. EPA, 1992; Ripp, 1996). With varied analytical methods, MDL was applied for the determination of test substance within a given matrix that considered the whole analytical process and defined in previous section. In other words, MDL was a statistically determined value that reliably distinguished an analyte from the blank or background noise within a particular matrix by a specific protocol (U. S. EPA, 1992; Ripp, 1996; Johannes, 2003). Note that MDL must be distinguished from IDL as the latter involves only a single component of the entire analytical process (U. S. EPA, 2010b; U. S. EPA, 2010a) and therefore IDL is always lower than the value of MDL due to the presence of possible error.

Establishment of LOD in analytical chemistry

Practical determination of LOD was a complex process. It could be varied with the definition and the approach or concept chosen for analytical purposes (Johannes, 2003) with the possibility of errors arisen from method, matrix and analyte at certain degree of inaccuracy (ACS Committee on Environmental Improvement, 1980). These errors could be introduced at each step throughout the entire procedure (Ripp, 1996; Vanatta and Coleman, 2007). Therefore, an analyst had to evaluate any possible source of error and reduce the complexity of the procedure performed.

A number of approaches had been published for LOD determination that an analyst has to specify in an analytical procedure. Among them, visual determination, signal-to-noise ratio, and the standard deviation and slope determination were recommended (ICH, 1995; ICH, 1997; Walfish, 2006). An alternate approach with relative standard deviation against the analyte quantity is recommended in EURACHEM Guidelines (EURACHEM, 1998). Calculation of the limit through a series of spiking is used by U. S. EPA (U. S. EPA, 1992; Ripp, 1996; U. S. EPA, 2010a). Additionally, empirical and the statistical methods for limits determination were also reported (Armbruster *et al.*, 1994; Vial and Jardy, 1999; Armbruster and Pry, 2008).

LOD determination approaches

Both non-instrumental and instrumental methods could be used to determine the LOD through visual evaluation on the produced output. This involved only the analysis of analytes of known concentration at the minimum detectable level (ICH, 1997).

Apart from visual evaluation, analytical procedure with baseline noise was suggested using signal-to-noise ratio approach. Following this approach, comparison of measured signal with known concentration and blank sample are performed and its detection at the minimum level is established with certain degree of ratio. Generally, the signal-to-noise ratio of 2:1 or 3:1 is accepted, depending on the analytical procedure (ICH, 1997; Vial and Jardy, 1999).

LOD could be determined from a calibration curve drawn from a series of known concentration of analyte. From the slope and standard deviation, either from the blank, or the regression, or the y-intercept derived from the curve, detection limit could be expressed (ICH, 1997).

EURACHEM (1998) applied the determination of blank with non-zero standard deviation as the LOD calculation involved the value of blank plus three times the standard deviation (EURACHEM, 1998).

Additionally, LOD could also be determined when the relative standard deviation of analyses reached a pre-established level (EURACHEM, 1998; Vial *et al.*, 2003). A number of approaches by several agencies were stated by Johannes for limit determination and their comparison was performed (Johannes, 2003).

Factors affecting LOD

A corresponding signal value must reflect the value of true signal that is different significantly from blank signal value. However, this is not always the case for some analysis because the measurement of test substance could become relatively less precise with decrement in concentration (Mocak *et al.*, 1997). When defining analytical detection characteristics, influencing factors must be accounted for especially during a complex chemical measurement process. These factors include: matrix effects and interferences, the sensitivity of an instrument and its noise level, the variability in extraction process; efficiency of a method, and the performance of an analyst.

Matrix effects and interferences

In practice, test substance is present with other compounds that could cause matrix interferences. These compounds may present in large amount as opposed to the analyte of interest which could subsequently affect the analytical outcome. Interfering effect caused by these compounds could occur in several ways. The response of an analyte might also vary with sample matrices by different analytical methods (Johannes, 2003; NATA, 2009) and therefore the instrumental signal could be enhanced or suppressed as the result of the presence of matrix components. For instance, these compounds might absorb or emit in the same wavelength range as the analyte of interest in spectroscopic techniques. When chromatographic techniques are used, these matrix interferences might co-elute at the same time with the analyte of interets. Chemical modification or catalytic reaction could also occur with their presence and contribute to amount changes in the final product (Johannes, 2003).

Sensitivity of instrument

Newer instruments continue to improve their sensitivity in measurement. It is worth noting that each instrument has its performance limit at the low and high ends of its detection range. Different manufacturers and even the models of an instrument of the same manufacturer vary in sensitivity, especially the detector (CDER, 1994). Note also an automatic and digitalized measurement assay might give only the value of zero instead of a certain limit (Kaus, 1998). As mentioned earlier, IDL is highly dependent on this type of sensitivity (Ripp, 1996) as a much lower value is achieavable in latest instruments. On the other hand, ageing of existing instruments might potentially and gradually losing their sensitivities, restricting the decision precision at lower level. This is especially true with the long term usage of detector, ageing of chromatographic columns, and deterioration of elemental lamps (CDER, 1994).

Instrumental noise

Currie (1995) stated the instrumental background is the null signal without any presence of analyte or interference-derived signal. Two terms of blank were describe, i.e. (i) baseline, which is defined as the sum of above background and signals in the region of interest due to interfering species, and (ii) analyte blank, that arises from any contamination and sample preparation steps corresponding to the analyte (Currie, 1995).

Due to the effect of background signal, instrumental noises of various origins determine the IDL. It is also important to note that noise level of an instrument varies between detector manufacturers (CDER, 1994). As a result, the estimation of background level has to be specific for a particular instrument apart from the analytical procedure and the analyte of interest (Cox, 2005). Reduction in noisy results can decrease the LOD as seen in newly developed devices (Johannes, 2003).

Variability in extraction process

As stated previously, analyte of interest in a sample may come with a number of matrix interferences. Removal of these interfering compounds during the analysis is vital for analyte to be detected. Various extraction methods could be applied as a cleanup step but a complete elimination of interferences may not always be possible. Additionally, different extraction might vary in their efficiency (ACS Committee on Environmental Improvement, 1980; Ripp, 1996; Johannes, 2003). Therefore, the extraction procedure in a particular analysis has to be accounted for during the consideration of the limit.

Efficiency of method

A method determines the analytical outcome and therefore the right method shall be considered for a particular analyte. The efficiency of a method can be determined by the ratio of change in the response or signal to the change in analyte concentration (ACS Committee on Environmental Improvement, 1980). A complex chemical measurement process might include the sampling, extraction, analyte separation, purification before detection. There is every possibility to introduce uncertainty throughout the the entire procedure (Ripp, 1996) and therefore, the method for LOD determination must be reliable and avoid the involvement of long and tedious analytical procedures (Johannes, 2003).

Analyst's performance

Every analyst has different capabilities when performing an analytical procedure. Gaps in technical knowledge, either on sampling or measuring could be a problem. Sampling bias, undetected flaws or fault, and performance errors are instances leading to deviated results (ACS Committee on Environmental Improvement, 1980; Ripp, 1996). An analyst's experience and familiarity with the system operated affect the validity of LOD (Ripp, 1996).

LOD determination in method validation

Method validation ensures the reliability of an analytical procedure and the comprehensiveness of quality assurance system (CDER, 1994; ICH, 1997; EURACHEM, 1998; AOAC/FAO/IAEA/IUPAC, 1999; Walfish, 2006). Reliable analytical results are essential for compliance with international regulations or quality assurance. Revalidation of a method is therefore required in cases where there is modification or extension in analytical procedure, changes of sample matrix or sample nature (ICH, 1995; Ripp, 1996; AOAC/FAO/IAEA/IUPAC, 1999; NATA, 2009).

The presentation of raw data from an instrumental analysis could be a problem in most analytical laboratory. Absorbance unit in spectroscopic techniques and the peak areas (or peak heights) unit in chromatographic techniques required the transformation or interpretation process to a typical useful unit (Johannes, 2003; Vanatta and Coleman, 2007). To minimize the problem, instrumental parameters could be set for the standards, controls and samples with well defined threshold and width of peak on chromatogram (Johannes, 2003) whereas the reported value could be converted into the estimated concentration via a calibration curve (Cox, 2005). During interpretation, the baseline noise or extraneous peaks and any presence of undulation had to be discriminated (CDER, 1994; Johannes, 2003). Besides, the checking on detection range and the presence of any impurity with reference standard were suggested to avoid any bias (CDER, 1994).

Due to the matrix effects and interferences, the evaluation of such possible positive and negative effects was recommended to reveal the sensitivity and ensure the reliability of a method (ACS Committee on Environmental Improvement, 1980). Any presence of such effects must be taken into consideration during limits determination (Ripp, 1996; Johannes, 2003). ICH (1997) suggested the spiking of pure substances with some degree of impurities to ascertain their effect and determine the separation from other components in sample matrix (ICH, 1997). Also, precision of the procedure has to be determined with replicate measurement from samples processed through an entire analysis method (DNR, 2008) with the incorporation of number of measurement into the definition of the limits (Mocak

et al., 1997). Proper unit and matrix type must also be stated in an analysis report (Ripp, 1996).

The relationship between measured response and all relevant accompanying factors and influencing components could be accounted into calibration function (Danzer and Currie, 1998). Calibration curve was the choice to check on the possibility of the presence of interferences. Without the curve, incorporation of measurement error could be a risk with only the consideration of background noise of instrument. A plot of recovered concentration versus significant concentration could also be a choice to evaluate the recovery of an analysis, especially in chromatography (Vanatta and Coleman, 2007).

LOD is not a chemical concept but is statistically determined (Ripp, 1996). In order to statistically increase the confidence of estimation, a large replicated samples with more than one analysts and lot of reagents were suggested to increase the robustness of the procedure (Armbruster and Pry, 2008). Computers and software had greatly reduced the complexity and difficulties in limit determination and calculation (Johannes, 2003). Sample statistics are routinely used in chemistry (Mocak et al., 1997) with the selection of most appropriate calibration curve and the most suitable statistically estimation (Vanatta and Coleman, 2007). Unfortunately, no approach claims to meet all the requirements for every application. Thus, careful documentation of an analytical procedure is vital.

Conclusion

The importance of LOD for discrimination between the presence and absence of an analyte in a sample is vital in an analytical procedure. The analytical performance of laboratory assays has to be characterized to understand its capability and limitations. The analyst has to use the right procedure and the right instrument for a specific analyte in order to generate a meaningful LOD. More importantly, procedure for the determination of LOD must be clearly documented.

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