THE LIMIT OF DETECTION AND LIMIT OF QUANTIFICATION - THE BASIC STEPS IN A PROTOCOL FOR VALIDATION OF METHODS FOR THE ANALYSIS OF RESIDUES

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Abstract

The first experimental step in the validation of an analytical method is the set up for the detection limit (LOD). The modern methods of limit detection are values statistical determined, which define the degree for an analytical method can separate a specific chemical substance from the background noise. A common mistake is to considerate the method detection limit as the lowest concentration which can be measured. Actually, it is the concentration on which we can decide if an element is present or not (with an certain trust degree). The detection limits depends on the matrix, the equipment, the analyst and needs an analytical procedure well-done. Commission decision (2002/657/EC), of 12 august 2002, implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results.

Key words: (limit of detection, limit of quantification, residue analysis)

Establishing the limits of detection and quantification are the first steps in the experimental validation of analytical methods. The limits of detection and quantification of modern methods are statistically determined values which define the degree to which an analytical method can differentiate a certain chemical background noise.

The detection limit of the method is not the lowest concentration that can be measured but it is the concentration at which we decide whether an element is present or not (is minimum content measured from which it is possible to deduce the presence of the compound to determine with reasonable statistical certainty).

MATERIALS AND METHODS

Validation of methods is a process of obtaining a set of data to confirm the accuracy and fidelity of a method are corresponding to be applied to determine compound and sample type specified. These data are reunited in the „validation procedure".

In accordance with EURACHEM, Directive 2002/657/EC, ORD.ANSVSA 51/2005, method validation is defined as the process of establishing the following parameters related to analytical method:

- A set of common performance characteristics and validation model used and the factors that can change;
- Limitations of the analytical process of the proceedings dependent on a specific model.

The conclusion of a process of validation is that the analytical method is suitable purpose of testing and generates reliable results.

Validation shall demonstrate that the analytical method meets the criteria applicable to the relevant performance characteristics.

The limit of detection (LOD) and limit of quantification or limit the quantitative determination (LOQ), part of the validation of the method, are important parameters that require a separate approach. Those two parameters are the basic steps in the analysis of residues. Thus, with increasing interest connected with food safety will increase and efforts to associate a realistic estimate as to the limit of detection and quantification of measuring equipment increasingly complex.

A large number of effects become important at low concentrations, such as:

- presence of background noise;
- the presence of interference between the analytes which affect the analytical signal;
- influence of blank use;
- losses of active substance during calcination procedures, digestion, extraction, purification, etc..

Due to these effects, the relative uncertainty associated with the results tend to increase the decrease in concentrations, a substantial percentage of the initial result, reaching in a point where the confidence interval includes the value

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zero. This region basically is sometimes associated with a method detection limit. The residue analysis, the term of limit of detection is routinely used as a parameter that indicates the point from which one can deduce the presence of the analyte with reasonable statistical certainty.

The limit of quantification is the lowest analyte content that can be analyzed with the reasonable statistical certainty.

In ultra-trace analysis, many analytes are important at very low concentrations that it is inevitable that some measurements may be made and reported as such in this range (LOD - LOQ).

RESULTS AND DISCUSSION

One of the ways used to calculate LOD and LOQ of the analytical methods involving the use of samples fortified with the analyte of interest (an enhanced sample with a known amount of test substances analyte that are to be detected). Fortified samples can be considered as at best limit, but LOD - obtained from their use is often different from that obtained in naturally contaminated samples. The protocol for calculating the limit of determination must take into account all the variables that can lead to erroneous results obtained (the calibration of the equipment, the level of fortification, blank's contamination etc.) and include the following steps:

1. Verification of analytical performance

Crossing protocol usual equipment maintenance will ensure the correct functioning. LOD and LOQ study will be done only on equipment maintenance through all the checks specified in the analytical method.

2. Calibration of equipment

The correct calibration is essential in determining the limit of detection. For ultra-trace analysis is recommended that the standard calibration lowest concentration to be approximately equal to the quantification limit (or the limit of quantification estimated), and the remaining standards covering the whole concentration range normally encountered in the laboratory this analysis. You might not be able to obtain a low detection limit using calibrated equipment for analysis of samples with high levels of contamination. Routinely, the manufacturer of the equipment or test kits specific to the limit of quantification (equipment or kit) and this value is a good approximation of the limit of quantification of the laboratory, at least until the obtain sufficient data for a statistical calculation thereof.

Before starting the procedure of determining the limit of detection, will marking a new calibration curve (for some analytical methods it is obligatory before each set of samples - such as heavy metals).

When using calibration curves for quantification must be used at least 5 levels for marking the curve and to be described variation of work of the curve.

3. Choice of the level of fortification

One of the methods of determining the limit of detection is based on the standard deviation of the readings of a set of 10-20 blank samples.

This method is applicable to most determining residues. In the case of the ELISA method, this type of calculation leads to abnormally low detection limits, without a practical support.

Because of this fact, the calculation of the limit of detection is based on analysis of a number of fortified replicas at some level, and the chosen plays an important role in these calculations.

The LOD, is an estimate of the lowest level of the calibration curve, and the best level of fortification being equidistant steps from MRL's, as specified in Directive (EC) 657/2002, Reg (EC) 333/2007.

The LOD calculated to be less than one tenth of the level of fortification (or the MRL's (Reg EC 333/2007)). LOQ calculated to be less than a fifth of the level of fortification (or the MRL's (Reg. EC 333/2007)).

Thus, the next inequality can use for evaluating calculated detection limit (LOD):

LOD<fortification level<10 * LOD and LOQ>LOD

From the definition CCβ appears another condition for limit of detection and CCα for limit of quantification or decision namely error β for replicates analyzed at a particular level of fortification should be within the limits set by the operator. If all these conditions are met, the chosen level of fortification is corresponding.

4. Preparation of the fortified samples

The procedure requires analyzing of a number of minimum 20 replicates for a level of fortification. These must be prepared and processed exactly according working method and at the same time with the same equipment, reagents, operator, because LOD and LOQ depend great extent on the working method. In the case of deceleration of residues will be started from a

sufficiently large sample fortified order to be divided into 20 replicates.

They will be treated as individual samples and will go through all the steps provided by the working method and the results are quantified and reported in corresponding units of measurement. It is very important to check all matrices separately because it can not estimate the effect of matrix to calculate LOD and LOQ for a particular analyte.

Eliminating the effects of blank samples can lead to LOD and LOQ low artificially and increase the risk of false positive.

5. Calculation of the limit of detection and limit of quantification

In the calculation of LOD and LOQ take into consideration the following mathematical relationship:

$$xL = xsi - k * ssi$$

in which:

xL - detection limit of the method;

xsi - average readings fortified samples;

ssi - standard deviation of a set of measurements for samples fortified;

k - numerical factor chosen according to the chosen confidence level (Student factor).

$$LOQ = 6x(ssi)$$

LOD and LOQ calculated may be rounded, so a limit of 0.15 calculated value may be rounded to 0.2, if the results are reported with one decimal place. LOD and LOQ will not be rounded down, unless the operator demonstrates that consistently get rounded value.

LOD and LOQ of the method should be checked from time to time and whenever the analytical method changes or other variables that could affect the calculated limits.

In conclusion, there are three important things to be taken into account in calculating the LOD and LOQ, namely:

1) Standard deviation of the samples fortified;
2) Student factor corresponding;
3) Taking into account all significant digits.

CONCLUSIONS

Mentioned procedure is one of the modalities used to calculate LOD and LOQ.

Performance characteristics of different analytical methods should be comparable, so that if development and validation of the method expanded uncertainty of performance characteristics to be equal.

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