Talanta 119 (2014) 178–180

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Strategy for determination of LOD and LOQ values – Some basic aspects

Jozef Uhrovčík [*]

Department of Ecochemistry and Radioecology, University of SS. Cyril and Methodius in Trnava, Nám. J. Herdu 2, SK-917 01 Trnava, Slovak Republic

ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form
24 October 2013
Accepted 30 October 2013
Available online 15 November 2013

Keywords:
Limit of detection
Limit of quantification
Electrothermal atomic absorption spectrometry
Lead

ABSTRACT

The paper is devoted to the evaluation of limit of detection (LOD) and limit of quantification (LOQ) values in concentration domain by using 4 different approaches; namely 3σ and 10σ approaches, ULA2 approach, PBA approach and MDL approach. Brief theoretical analyses of all above mentioned approaches are given together with directions for their practical use. Calculations and correct calibration design are exemplified by using of electrothermal atomic absorption spectrometry for determination of lead in drinking water sample. These validation parameters reached 1.6 $\mu g\,L^{-1}$ (LOD) and 5.4 $\mu g\,L^{-1}$ (LOQ) by using 3σ and 10σ approaches. For obtaining relevant values of analyte concentration the influence of calibration design and measurement methodology were examined. The most preferred technique has proven to be a method of preconcentration of the analyte on the surface of the graphite cuvette (boost cycle).

1. Introduction

Capability of detection and quantification are important performance characteristics of every measurement process. In chemistry, a representative characteristic of any analytical method is the smallest concentration or the mass of the analyte (the analysed sample component) that can be detected or quantified with a specified degree of certainty. The related quantity is the limit of detection (LOD), defined by International Union for Pure and Applied Chemistry [1]. Among them, the limit of quantification (LOQ), refers to the smallest analyte concentration or mass, which can be quantitatively analysed with a reasonable reliability by a given procedure [2]. These definitions represent the principle of 3σ and 10σ approaches which are nowadays normative approaches for establishment of LOD and LOQ values. Above mentioned approaches employ the mean blank signal value, as the reference point value for calculation of LOD and LOQ. Residual standard deviation or error of intercept of calibration line can be used in computation of LOD and LOQ values by 3σ and 10σ approaches instead of mean blank signal value due to the fact that they could be a more accurate estimate of mean blank value [3].

ULA2 approach [4] (abbr. *upper limit approach*) utilises the upper bound of the signal vs. concentration confidence band for obtaining concentration counterparts of the signal LOD and LOQ values. This model takes into account such requirements which can be achieved in real experiments.

MDL approach [5] (abbr. *method detection limit*) is widely used first of all in the USA and GB (recommendation of EPA). This approach is based on fulfilment of 4 simple assumptions, but their achievement expects advanced laboratory practice. This model utilises appropriate critical value of Student *t*-distribution that reflects limited number of observations for relevant concentration level.

The last implemented model was PBA approach [6] (abbr. *precision-based approach*) that employs dependence of relative standard deviation as a function of analyte concentration in suitable range. Inverse transformation from RSD domain ($\pm$33%) to concentration domain represents LOD value and ($\pm$10%) LOQ value. The aim of this article is to explain in a statistically correct way the calculation of the LOD and LOQ values in the region of low concentrations (trace analysis) and, in addition, to compare these limits each other. Moreover, it will be demonstrated the some aspects of correct strategy for transparent calibration design.

2. Material and methods

Procedure for determination of lead concentration in drinking water samples was adopted from related technical note [7]. Absorbance measurements were made on double beam atomic absorption spectrophotometer AA-7000 with graphite furnace (Shimadzu, Japan). For the preparation of calibration solutions and their sampling was used autosampler ASC-7000 equipped with intelligent dilution manager (Shimadzu, Japan). All analytical conditions were adjusted according to the manufacturer's recommendation. The analytical signal was measured at the wavelength 283.3 nm and the slit of monochromator was adjusted to 0.7 nm.

---

[*] Tel.: +421 33 55 65 321.
E-mail addresses: jozef.uhrovcik@ucm.sk, j.uhrovcik@gmail.com

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.10.061

The integrated absorbance for each point of calibration line was measured 5-times and average value was considered. Stock solution of Pb(II) with concentration 100 µg L^{-1} was prepared in deionised water (specific conductivity < 0.054 µS cm^{-1}) from standard solution (Merck, Germany) and stabilized with addition of concentrated solution of nitric acid (Merck, Germany). Magnesium matrix modifier for graphite furnace-AAS with concentration 1 g L^{-1} was prepared in deionised water from standard solution (Merck, Germany). Injection volume of calibration solution (sample) was 20 µL and matrix modifier 5 µL. Sample of drinking water marked as "*Florian*" was collected from artesian well.

3. Results and discussion

Validity of Beer's law was verified in concentration range from 0 to 15 µg L^{-1} Pb(II) (Fig. 1) with regard to supposed concentration of lead in drinking water sample and relevancy of homoskedasticity. Equation of calibration line can be expressed as $A = 0.0061 \pm 0.0002 \cdot c + 0.003 \pm 0.003$. Coefficient of determination reached value $R^2 = 0.9982$. Significance of intercept was checked by application of Student *t*-test and at the significance level $\alpha = 0.05$ was not revealed significant difference between obtained value of intercept and theoretical value 0. Homogeneity of variance was proved by application of Hartley's test [8] and linearity was proved by means of very powerful tool, namely QC parameter [9]. Characteristic mass reached value 20 pg. The precision of the method ($n = 7$) within the used range did not exceed ± 4% and trueness of the method was verified by application of spike-recovery procedure. Apparent recovery reached acceptable values 98–102%. Absence or presence of matrix effect was proved by application of the method of standard addition. Sample of drinking water was spiked with suitable addition of Pb(II) stock solution with concentration 100 µg L^{-1} and the final value of Pb(II) concentration was established in range 0–15 µg L^{-1} Pb(II). Due to the extrapolative character of determination of concentration by application of method of standard addition was necessary to remeasure every calibration point several times, namely 5-times and incorporate satisfactory number of calibration points. We have chosen 6 points with the linear range from 0 to 15 µg L^{-1} Pb(II). Obtained value of slope in case of application of method of standard addition reached 0.0059 µg^{-1} · L and in case of application of method of external calibration reached the value 0.0061 µg^{-1} L. Statistical comparison of these two values (*t*-test) was not revealed the statistically significant difference between them at the significance level $\alpha = 0.05$ and $\alpha = 0.01$. It may be conclude that response of model calibrations solutions is same as response for matrix in studied water sample. Thus, the presence of matrix effect has not been proved. The concentration of lead in analysed drinking water sample was established by means of three independent methods: method of external calibration (3.1 ± 0.6 µg L^{-1}), method of standard addition (3.0 ± 0.9 µg L^{-1}) and method of preconcentration of the analyte on the surface of the graphite cuvette (boost cycle) (3.8 ± 0.2 µg L^{-1}). The latter technique has been proved to be the most appropriate for determination of lead (and not only lead!). This technique provided orientation of analytical signal near to centroid of calibration line where is very favourable signal-to-noise ratio. Reason for this choice are conditional to the relative low level of Pb(II) in drinking water sample (< 10 µg L^{-1}) and sharp increase of relative standard deviation of determination in lower region of calibration range.

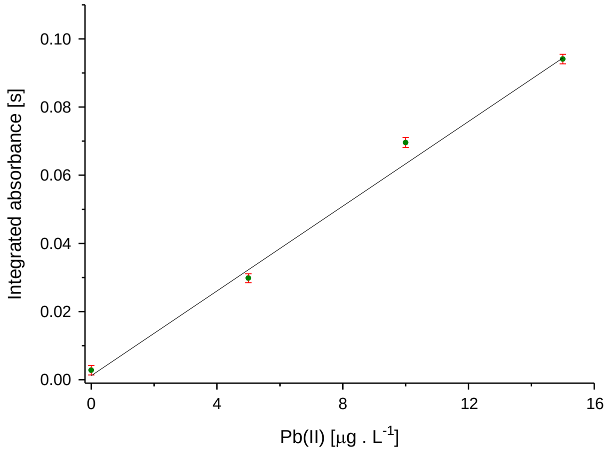

Fig. 1. Integrated absorbance as a function of lead concentration against a reagent blank.

Note: Data behind the sign ± represents estimation of standard deviation derived from related evaluation function.

The results for computation of LOD and LOQ values in concentration domain in model solutions by using 4 different statistical approaches are shown in Table 1.

Signal of blank was measured 20-times and average value was taken into account. Signal of Pb(II) near to estimated LOD and LOQ in case of PBA approach was measured 7-times for each concentration. The related dependence of application PBA approach is shown in Fig. 2. The value of LOD in case of application MDL approach reached 1.5 µg L^{-1}.

Results shown in Table 1 indicate relatively good agreement between values obtained by application of all above mentioned statistical approaches. Fact that these values do not differ in order is the most important conclusion and result of this work. 3σ and 10σ approaches are convenient first of all in case if the population characteristics of blank signal distribution are known ("well-known blank"). It is necessary to say that this model does not take into account the uncertainty of position of calibration line, in addition, due to the use of multiplication factor 3, the error of the second kind increases up to 50% and the error of the first kind not reached only 0.135%, but approximately 11% [10]. This model assumes the fact that slope and intercept of calibration line are error-free, but this assumption is never achieved in real experiments. Utilisation of residual standard deviation or error of intercept of calibration line in LOD and LOQ value, respectively can be better estimation of mean blank signal value. ULA2 approach represents statistically correct way of obtaining both LOD and LOQ values. This model is derived for limited set of observations and takes into account real conditions of experiments in analytical chemistry. The value of multiplication factors is not constant, but is dependent on calibration design and layout of calibration points. ULA2 approach utilises even the one-sided critical value of Student *t*-distribution instead of critical value of Gaussian (normal) distribution that is valid for infinite number of

Table 1
Computation of LOD and LOQ values by using four different approaches.

3 σ and 10 σ approach [µg·L^{-1}]			**ULA2 approach [µg·L^{-1}]**	
s_{blank}	LOD	1.6	LOD ($\alpha = 0.05$)	1.7
	LOQ	5.4	LOQ ($\alpha = 0.05$)	5.2
$s_{y/x}$ [a]	LOD	2.1	LOD ($\alpha = 0.01$)	2.9
	LOQ	7.1	LOQ ($\alpha = 0.01$)	8.7
s_{b0} [b]	LOD	1.5	**PBA approach [mg L^{-1}]**	
	LOQ	4.8	LOD	1.0
			LOQ	5.0

[a] Residual standard deviation.

[b] Error of intercept of calibration line.

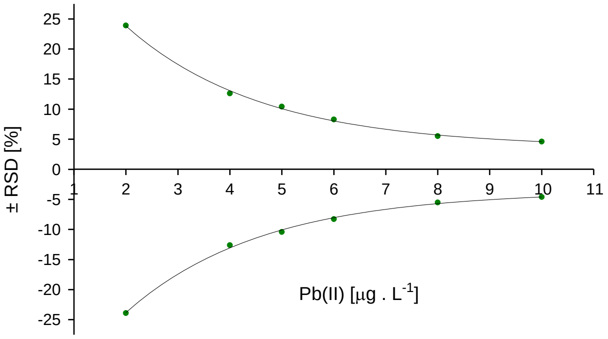

Fig. 2. Relative standard deviation as a function of lead concentration close to LOD and LOQ, respectively.

observations. 3σ and 10σ and ULA2 approaches, respectively can be used in some cases when method of standard addition is applied. MDL approach is very popular in GB and USA, but application of this model requires advanced experimental practise and it is time-consuming. Notation “MDL” is quite ambiguous, because value of this parameter is not only function of implemented method, but a function of instrument adjustment and type of samples, respectively. MDL approach makes use of one-sided critical value of Student *t*-distribution similarly as ULA2 approach and therefore takes into account real conditions of experiment. PBA approach is popular and extended in analytical chemistry and the computation of LOD and LOQ values is relative simple. Graphical presentation of relative standard deviation as a function of analyte concentration is analogy of Horwitz's trumpet, but the major source of error is choice of “proper” evaluation mathematical function. For the purpose of obtaining suitable type of mathematical function is needed to perform the regression analysis. Another feature of this approach is time consumption at the construction of experimental dependence between concentration of analyte and its relative standard deviation of determination. It is recommended to use at least 6 points for construction of this dependence and every point should be measured at least 7-times.

Finally, some fundamental assumptions of proper calibration design in regard to LOD and LOQ values establishment will be referred:

For a sufficiently wide calibration range, the assumption of constant population variance of the signal is not valid. However, in a narrow concentration range close to the LOD, this assumption is met. Therefore, for determination of the LOD and LOQ is recommended to use a concentration range of only 1.0–1.5 logarithmic units above the LOD value (i.e. 10–30 multiple of the LOD value).

Calibration design appropriate for the LOD and LOQ determination must be such that the region of calibration points is overlapping the determined LOD and LOQ values.

The inappropriate use of a calibration function as a convertor between the signal and concentration/mass domains is another source of error in LOD and LOQ calculations. It is strongly recommended to measure each point of calibration line as many times as sufficient and number of replicates should increase with decrease of analyte concentration.

It needs to be stressed that

- in practice the intercept of the calibration plot is not identical with the mean blank signal value even though this is assumed in both the IUPAC and ACS definitions.
- the calculated regression parameters are subject to errors.
- only limited number of observations are made and therefore obtained values of slope, intercept and related parameters are only estimations of “true” values.

4. Conclusions

It must be noted that limit of detection and limit of quantification represent typical example of non-rugged validation parameters and their values should be verified and defined at every construction and application of calibration line. The analysts have to bear in mind that above mentioned approaches are derived for straight line model and their implementation is not valid for non-linear calibration dependences. The usage of calibration line model is the most frequent case in analytical practice and majority of analytical techniques deal with this model. We chose the technique of electrothermal determination of lead in drinking water sample due to the fact that this method enables to detect and quantify very low concentrations of analyte and simultaneously deals with model of the straight line. In addition, the suitable calibration design for obtaining relevant values of limit of detection and limit of quantification in concentration domain is required. LOD parameter is critical quantity for comparison of different approaches, because the value of LOQ is set up by convention and by ability of reliable quantification of analyte in given matrix.

It can be argued that it does not exist universal model with satisfactory suitability for all cases of trace analysis and the proper choice of valid model for computation of LOD and LOQ values is dependent on requirements for reliable detection or quantification of analyte in different types of samples and such model can never exist, because for example spectrometric techniques are based on different principles in comparison with for example radiometric techniques and they are valid different assumptions about the validity of statistical distribution of observed values and data. Some important and significant articles focussed on verification and determination of LOD and LOQ values in concentration domain by means of selected spectroscopic techniques are listed in references [11–16].

References

[1] IUPAC, Spectrochim. Acta B 33 (1978) 241–245.
[2] ACS COMMITTEE, Anal. Chem. 52 (1980) 2242–2249.
[3] J.N. Miller, J.Ch. Miller, Statistics and Chemometrics for Analytical Chemistry, fifth ed., Ellison Horwood, Chichester, 2005.
[4] J. Mocák, A.M. Bond, S. Mitchell, G. Scollary, Pure Appl. Chem. 69 (1997) 297–328.
[5] J.A. Glaser, D.L. Foerst, G.D. McKee, S.A. Quave, W.L. Budde, Environ. Sci. Technol. 15 (1981) 1426–1435.
[6] L.R.P. Butler, Spectrochim. Acta B 38 (1983) 913–919.
[7] STN EN ISO 15586, 2004. Water quality. Determination of trace elements using atomic absorption spectrometry with graphite furnace.
[8] H.O. Hartley, Biometrika 37 (1950) 308–312.
[9] J. van Loco, M. Elskens, Ch. Croux, H. Beernaert, Accredit. Qual. Assur. 7 (2002) 281–285.
[10] G.L. Long, J.D. Winefordner, Anal. Chem. 44 (1983) 712A–724A.
[11] P.W.J.M. Boumans, Anal. Chem. 66 (1994) 459A–467A.
[12] N. Cressie, Chemometr. Intell. Lab. 22 (1994) 161–163.
[13] W. Huber, Accredit. Qual. Assur. 8 (2003) 213–217.
[14] I. Kuselman, A. Shenhar, Anal. Chim. Acta. 306 (1995) 301–305.
[15] J.M. Mermet, Spectrochim. Acta 63B (2008) 166–182.
[16] T.L. Rucker, J. Radioanal. Nucl. Chem. 192 (1995) 345–350.