Definition of the limit of quantification in the presence of instrumental and non-instrumental errors. Comparison among various definitions applied to the calibration of zinc by inductively coupled plasma–mass spectrometry

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A B S T R A C T

The limit of quantification (LOQ) in the presence of instrumental and non-instrumental errors was proposed. It was theoretically defined combining the two-component variance regression and LOQ schemas already present in the literature and applied to the calibration of zinc by the ICP-MS technique. At low concentration levels, the two-component variance LOQ definition should be always used above all when a clean room is not available. Three LOQ definitions were accounted for. One of them in the concentration and two in the signal domain. The LOQ computed in the concentration domain, proposed by Currie, was completed by adding the third order terms in the Taylor expansion because they are of the same order of magnitude of the second ones so that they cannot be neglected. In this context, the error propagation was simplified by eliminating the correlation contributions by using independent random variables. Among the signal domain definitions, a particular attention was devoted to the recently proposed approach based on at least one significant digit in the measurement. The relative LOQ values resulted very large in preventing the quantitative analysis. It was found that the Currie schemas in the signal and concentration domains gave similar LOQ values but the former formulation is to be preferred as more easily computable.

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1. Introduction

The determination of the limit of quantification (LOQ) is a crucial step in the quantitative analysis as suggested by Currie [1]. Key aspects of the quantification concept, as well as of the detection one, were reviewed in relevant publications, as Gibbons [2], Gibbons et al. [3], Zorn et al. [4], Mocak et al. [5], Currie [6–8], Voigtman [9–12], Mermet et al. [13], and Carlson et al. [14] papers. In particular, Currie [1,6–8], following Adams et al. [15], defined the limit of quantification, L_Q, (actually called *determination limit* with L as the generic symbol for the quantity of interest, signal, or amount) from the maximum acceptable relative standard deviation for quantitative analysis:

$$L_Q = k_Q \sigma_{L_Q} \qquad (1)$$

where k_Q was the reciprocal of the requisite relative standard deviation. In the net signal domain, Eq. (1) becomes $Y_Q - \alpha = k_Q \sigma_{Y_Q - \alpha}$ (a default value $k_Q = 10$ was chosen), where α is the blank signal, whereas in the concentration domain, the LOQ is defined by $x_Q = k_Q \sigma_{x_Q}$. Zorn et al.[4]

generalized the limit of Currie to the case of non-constant variance by firstly modeling association between standard deviation and concentration, and then defining the quantification limit in the response domain as 10 times the standard deviation at the lowest detectable signal, *i.e.* the Currie's critical level Y_C, $Y_Q = 10 s_{Y_C} + \hat{\alpha}_w$, where s_{Y_C} is the standard deviation at the critical level and $\hat{\alpha}_w$ is the weighted intercept estimated via a weighted least-square regression. Currie [6] discussed open questions involving quantification capabilities in the signal and concentration domains. The proposed expression for the LOQ in the net signal domain was $Y_Q - \alpha = k_Q \sigma (1 + \frac{1}{n})^{1/2}$, where σ was the constant standard deviation of the measurements and the estimates of the signal and of the blank were based on 1 and n replicates, respectively. By using the ordinary least squares (OLS) intercept $\hat{\alpha}$ as an estimate of the blank, the LOQ was given by $Y_Q - \alpha = k_Q \sigma (1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum (x - \bar{x})^2})^{1/2}$. In this latter case, the calibration line $Y = \alpha + \beta x$ was estimated from a set of n calibration points $x_i (i = 1, 2, ..., n)$ with mean $\bar{x}$. If the measurement variance σ^2 was estimated by the sample standard error about regression $s_{y/x}$, the Currie experimental content domain LOQ was given by $x_Q = k_Q \frac{s_{y/x}}{\hat{\beta}} (1 + \frac{1}{n} + \frac{\bar{x}^2}{\sum (x - \bar{x})^2})^{1/2}$, where $\hat{\beta}$ is the OLS slope of the calibration

line. Further, Currie [6] directly estimated the concentration x_Q starting from the basic equation $\hat{x}_Q = k_Q \sigma_{\hat{x}_Q}$, where $\hat{x}_Q$ indicated the discriminated concentration obtained from the calibration function. The definition of the LOQ in the concentration domain is crucial because the estimate of a concentration value is always required in a quantitative analysis. The relationship between standard deviation and discriminated concentration, obtained using the Taylor expansion for $\hat{x}$ in first and second derivatives, gave rise to a quadratic equation from which the LOQ was calculated (see Eq. (26A) in ref. [6]). Moreover, Currie [7] offered another expression of the LOQ in the concentration domain in terms of the detection limit, x_D, equating the ratio x_Q/x_D to 3.04, when default values of false positive and false negative equal to 0.05 were employed, given normality and a measurement variance known and constant. These approaches appeared in the IUPAC Recommendations 1995 [7]. Recently, Carlson et al. [14] outlined that the decision level, not detection limit, is of primary utility for quantitation purposes. They also stressed a general lack of fundamental justification in the definitions of the LOQ appearing in the literature with the exception of the study of Coleman, Auses, and Grams in 1997 [16]. This study defined the LOQ as the lowest concentration that ensures at least one significant digit in the measurement, or, equivalently, the lowest concentration at which measurements have a relative measurement error (RME) satisfying the inequality RME ≤ 5%. Carlson et al. [14] assumed the absolute measurement error (AME) as the half-width of the 95% confidence interval (CI) in the net response domain. An important feature of this approach was the choice of defining LOQ basing on data quality (one significant digit in the measurement) and on a level of confidence ($CI_{95\%}$). The requirement of a confidence level for CI showed that LOQ could be statistically defined similarly to the Currie decision limit.

The major goal of this paper will be to define the quantification limit combining the above-mentioned approaches and the two-component variance regression, a recently developed regression procedure for measurement data affected by instrumental and non-instrumental errors [17]. In this context, the calibration line will be constructed with J concentration levels measured I times with an additive systematic error affecting the I replicates at the jth concentration. This systematic error will be assumed stochastic among the J concentration levels. The Currie schema in the concentration domain will be reviewed adding terms of the third order in the Taylor expansion since they were comparable to the second-order ones in the determination of the variance of the LOQ concentration and for this reason they cannot be neglected. Experiments performed by ICP-MS for calibrating Zinc will be made in order to compare the values of the LOQ obtainable with all the proposed definitions.

2. Theoretical

2.1. Statistical model of the two-component variance regression

In order to obtain the expression of the LOQ in the case of two-component variance regression, only the major features of the calibration will be considered, as it was dealt with in detail in previous publications [17,18]. The linear model:

$$y_{ij} = \alpha + \beta x_j + \eta_j + \varepsilon_{ij} \tag{2}$$

where $i = 1, 2, ..., I$ denotes the measurements at the levels x_j, is assumed to describe the relationship between the y measurement, the x concentration, and the analytical conditions at the spiked level j. The random variables ε_{ij} and η_j are assumed uncorrelated and Gaussian with zero mean and constant variance: $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$, $\eta_j \sim N(0, \sigma_\eta^2)$. The response y_{ij} is then normally distributed with mean $\alpha + \beta x_j$ and variance $\sigma_\eta^2 + \sigma_\varepsilon^2$: $y_{ij} \sim N(\alpha + \beta x_j, \sigma_\eta^2 + \sigma_\varepsilon^2)$. Estimates $\hat{\alpha}$ and $\hat{\beta}$ of the

calibration line parameters are determined by the OLS method applied to the mean responses at each concentration level, $\bar{y}_j = (1/I)\sum_{i=1}^{I} y_{ij} = \alpha + \beta x_j + \eta_j + \sum_i^I \varepsilon_{ij}/I$. The random variable $\bar{y}_j$ is normally distributed: $\bar{y}_j \sim N(\alpha + \beta x_j, \sigma_{\bar{y}_j}^2|$, with $\sigma_{\bar{y}_j}^2$ given by

$$\sigma_{\bar{y}_j}^2 = \sigma_\eta^2 + \frac{\sigma_\varepsilon^2}{I}. \tag{3}$$

The significant presence of the two variance components in Eq. (3) may be checked by an F-test computing the statistic

$$F = \frac{\hat{\sigma}_{\bar{y}_j}^2}{\frac{\hat{\sigma}_\varepsilon^2}{I}}, \tag{4}$$

where the OLS estimate

$$\hat{\sigma}_{\bar{y}_j}^2 = \frac{\sum_{j=1}^{J} \left(\bar{y}_j - \hat{y}_j\right)^2}{J-2} \tag{5}$$

with J-2 degrees of freedom, and the pooled variance

$$\hat{\sigma}_\varepsilon^2 = \frac{\sum_{j=1}^{J} \sum_{i=1}^{I} \left(y_{ij} - \bar{y}_j\right)^2}{J(I-1)} \tag{6}$$

with $J(I$-1) degrees of freedom may be used.

2.2. LOQs in the presence of instrumental and non-instrumental errors

In this section, the theory presented above will be combined with one definition of the LOQ in the concentration and two definitions in the net signal domains.

2.2.1. Content domain LOQ
The definition of the LOQ here considered is given by

$$x_Q = k_Q \sigma_{x_Q} \tag{7}$$

where k_Q equals the reciprocal of the requisite relative standard deviation, $\sigma_{x_Q} = \sigma_{\hat{x}}|_{x=x_Q}$, and $\hat{x}$ indicates a discriminated concentration obtained from the calibration function [6]. The variance of the estimated LOQ, $\hat{x}_Q$, is generated using the Taylor expansion of the function $\hat{x} = \bar{x} + (\bar{y}_M - \bar{y})/\hat{\beta}$ where $\bar{y} = \sum_j^J \bar{y}_j/J$, and $\bar{y}_M$ is the mean of M replicate responses at $\hat{x}$. The derivatives with respect to the three independent variables $\bar{y}_M, \bar{y}$, and $\hat{\beta}$ are evaluated at the expected values $E(\bar{y}_M), E(\bar{y})$, and β of the normally distributed variables: $\bar{y}_M \sim N(\alpha + \beta x_Q, \sigma_\eta^2 + \frac{\sigma_\varepsilon^2}{M}), \bar{y} \sim N(\alpha + \beta\bar{x}, \frac{\sigma_{\bar{y}_j}^2}{J})$, and $\hat{\beta} \sim N(\beta, \frac{\sigma_{\bar{y}_j}^2}{S_{xx}})$, where $\bar{x} = \sum_j^J x_j/J, S_{xx} = \sum_{j=1}^{J} (x_j - \bar{x})^2$. Using the expansion in first derivatives, the variance of $\hat{x}_Q$ is given by $\sigma_{x_Q}^2 = \frac{1}{\beta^2}[\sigma_\eta^2 + \frac{\sigma_\varepsilon^2}{M} + \frac{\sigma_{\bar{y}_j}^2}{J} + \frac{(x_Q - \bar{x})^2}{S_{xx}}\sigma_{\bar{y}_j}^2]$ or better, in view of Eq. (3), by

$$\sigma_{x_Q}^2 = \frac{1}{\beta^2}\left[\sigma_\varepsilon^2\left(\frac{1}{M} - \frac{1}{I}\right) + \sigma_{\bar{y}_j}^2\left(1 + \frac{1}{J} + \frac{(x_Q - \bar{x})^2}{S_{xx}}\right)\right] \tag{8a}$$