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Letter to Editor

A Critical Issue in Calibration Curve with Logarithmic Scale

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Dear Editor,

Developing a validated analytical method is critical in the quantification of chemical and biological compounds in various branch such as chemical, medical and pharmaceutical sciences. They were applied for the detection of an analyte in different matrices. Based on the types of analyte and matrix, a certain analytical method should be selected and a validation based on international guidelines must be used to confirm its accurate quantification. Various guidelines have been recommended which researchers applied them whenever developing an analytical method.[1] However, there are many reports which investigators did not completely observe these guidelines in the published articles.[2,3]

The main part of validation process of an analytical method is based on the calibration curve. A common type for calibration curve is a linear relation between concentration and response. However, other types such as multivariate calibration curve based on linear correlations or non-linear relations have been reported in the literature.[4,5] Recently, some concerns about type of chart[2] and logarithmic scale in calibration curve[6] have been published by scientific researchers. Calibration curve in logarithmic scale has been applied by researchers in high-quality journals[7-10] which some of them are highly cited.

Uran[6] requested avoid plotting analytical response against the logarithm of concentration because it is not appropriate for proper statistical evaluation. However, Kong et al[11] proved that transformation of data in the logarithm form will not affect the soundness of fit statistics. Hong et al[12] proposed paying more attention to operation signal transformation mechanism rather than data treatment by mathematical function e.g. logarithm in selecting linear and or non-linear correlation between concentrations and responses.

Transformation of data in the logarithmic form is a common method in modeling chemical and biological phenomena for data linearization e.g. in developing quantitative structure activity (or property) relationship (QSAR or QSPR).[13] However, against controversy between published perspectives in *Analytical Chemistry* journal, all of them approved that some issues should be considered in the plotting of calibration curve in logarithmic scale.[6,11,12] In this study, some points have been highlighted which should be considered in evaluation of calibration curve in logarithmic scale. The following example is represented to clarify the aim of this communication accurately:

Assume an analytical method composed of concentration = 10^3,10^4, 10^5, 10^6, 10^7 and 10^8 and the response = 0.2, 0.25, 0.30, 0.35, 0.42, 0.50 and 0.53. Figure 1 illustrated the calibration curve in the standard and logarithmic scales. In the first status, there is no appropriate linear correlation between concentration and response ($R^2 = 0.42$). However, the transformation of concentration to logarithmic scale improves the correlation and resulted in a large value for the correlation coefficient (R^2). Similar to modeling studies, R^2 is not enough to confirm the correlation between concentration and response.[3] Based on FDA guidelines,[14,15] computation of back-calculated concentration by the equation of calibration curve and comparison with nominal concentration of standard samples is necessary and some criteria were proposed in an earlier study. The back-calculated concentrations by the regression equation of calibration curve in logarithmic scale, in comparison with nominal concentration values shows that there is a significant difference, and applied calibration curve cannot give the concentration of unknown sample with appropriate accuracy (Table 1).

Developing analytical methods is critical in research and most of the conclusions in chemical, pharmaceutical and medical sciences result from the concentrations which obtained from calibration curves. Therefore, critical issues must be considered by researchers, reviewers and editors of the journals in the evaluation of these results. Logarithmic scale in calibration curve is possible, however, back-calculated concentration based on the regression equation of calibration curve is necessary and should be reported in research articles.

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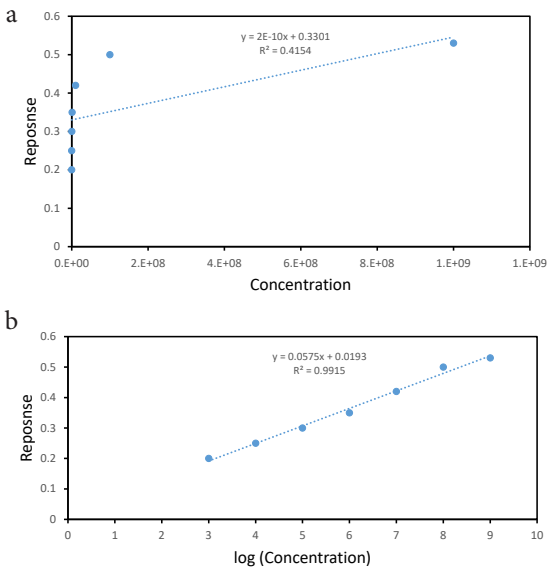

Figure 1. The calibration curve of a data set composed of concentration and response in standard and logarithmic scale

Table 1. Details of the calibration curve in logarithmic scale and back-calculated concentration and corresponding recovery based on the equation of calibration curve

Concentration	Log concentration	Response	Calculated concentration based equation of calibration curve	Recovery%
10^3	3	0.20	1.39×10^3	138.9
10^4	4	0.25	1.03×10^4	102.8
10^5	5	0.30	7.62×10^4	76.2
10^6	6	0.35	5.64×10^5	56.4
10^7	7	0.42	9.30×10^6	93.0
10^8	8	0.50	2.29×10^8	229.1
10^9	9	0.53	7.62×10^8	76.2

Conflict of Interest

The author have no conflicts of interest to declare.

Ethics Issues

Not Applicable

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References

1. Ozkan SA. Analytical method validation: the importance for pharmaceutical analysis. Pharm Sci. 2018;24(1):1-2. doi: 10.15171/ps.2018.01.
2. Shayanfar A. Beware of bar charts for plotting calibration curves for analytical method development. J AOAC Int. 2020;103(5):1424-5. doi: 10.1093/jaoacint/qsaa023.
3. Shayanfar A, Ershadi S. Developing new criteria for validity evaluation of analytical methods. J AOAC Int. 2019;102(6):1908-16. doi: 10.5740/jaoacint.19-0007.
4. Safi A, Campanella B, Grifoni E, Legnaioli S, Lorenzetti G, Pagnotta S, et al. Multivariate calibration in laser-induced breakdown spectroscopy quantitative analysis: the dangers of a 'black box' approach and how to avoid them. Spectrochim Acta Part B At Spectrosc. 2018;144:46-54. doi: 10.1016/j.sab.2018.03.007.
5. Chanda S, Hazarika AK, Choudhury N, Islam SA, Manna R, Sabhapondit S, et al. Support vector machine regression on selected wavelength regions for quantitative analysis of caffeine in tea leaves by near infrared spectroscopy. J Chemom. 2019;33(10):e3172. doi: 10.1002/cem.3172.
6. Urban PL. Please avoid plotting analytical response against logarithm of concentration. Anal Chem. 2020;92(15):10210-2. doi: 10.1021/acs.analchem.0c02096.
7. Hu LM, Luo K, Xia J, Xu GM, Wu CH, Han JJ, et al. Advantages of time-resolved fluorescent nanobeads compared with fluorescent submicrospheres, quantum dots, and colloidal gold as label in lateral flow assays for detection of ractopamine. Biosens Bioelectron. 2017;91:95-103. doi: 10.1016/j.bios.2016.12.030.
8. Wu Z, Xu E, Chughtai MFJ, Jin Z, Irudayaraj J. Highly sensitive fluorescence sensing of zearalenone using a novel aptasensor based on upconverting nanoparticles. Food Chem. 2017;230:673-80. doi: 10.1016/j.foodchem.2017.03.100.
9. Farshchi F, Saadati A, Kholafazad kordasht H, Hasanzadeh M. An innovative immunoanalysis strategy towards sensitive recognition of PSA biomarker in human plasma samples using flexible and portable paper based biosensor: a new platform towards POC detection of cancer biomarkers using integration of pen-on paper technology with immunoassays methods. ImmunoAnalysis. 2021;1(1):6. doi: 10.34172/ia.2021.06.
10. Mansouri M, Fathi F, Jalili R, Shoeibie S, Dastmalchi S, Khataee A, et al. SPR enhanced DNA biosensor for sensitive detection of donkey meat adulteration. Food Chem. 2020;331:127163. doi: 10.1016/j.foodchem.2020.127163.
11. Kong D, Zhao J, Tang S, Shen W, Lee HK. Logarithmic data processing can be used justifiably in the plotting of a calibration curve. Anal Chem. 2021;93(36):12156-61. doi: 10.1021/acs.analchem.1c02011.
12. Hong Q, Shen Y, Liu S, Zhang Y. Re-examination of plotting analytical response against different forms of concentration. Anal Chem. 2021;93(35):11910-4. doi: 10.1021/acs.analchem.1c02683.
13. Norouzi S, Farahani M, Nejad Ebrahimi S. The integration of pharmacophore-based 3D-QSAR modeling and virtual screening in identification of natural product inhibitors against SARS-CoV-2. Pharm Sci. 2021;27(Suppl 1):S94-S108. doi: 10.34172/ps.2020.98.
14. Analytical Procedures and Methods Validation for Drugs and Biologicshttps://www.fda.gov/files/drugs/published/Analytical-Procedures-and-Methods-Validation-for-Drugs-and-Biologics.pdf. Accessed November 2021.
15. Bansal S, DeStefano A. Key elements of bioanalytical method validation for small molecules. AAPS J. 2007;9(1):E109-14. doi: 10.1208/aapsj0901011.