We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

154
Countries delivered to

Our authors are among the

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Introductory Chapter: The Many Faces of Calibration and Validation in Analytical Methodology in the Present Day

Mark T. Stauffer

Additional information is available at the end of the chapter

1. The necessary processes of calibration and validation in chemical analysis

One of the most critical aspects of any analytical method, if not THE most critical aspect, is the *calibration* of the response of the particular equipment with respect to concentration of the sought-after analyte. This aspect of the quantitative determination of an interesting analyte is necessary to understanding the interesting, and sometimes complicated, relationship between the signal produced by the desired analyte present in an interesting sample and subsequently measured by the analyst, and the amount (e.g., mass, moles, concentration) of that analyte in the sample. Without such a relationship, let alone the methods and techniques to acquire it, analytical chemistry as we know it would be impossible, if not horrendously difficult.

In this introductory chapter, the author (Editor) wishes to provide some background information for the reader, toward appreciation and understanding of the relevance and necessity of the process of calibration in quantitative chemical analysis, and the equally relevant and necessary role of the process of validation, or verification, of the calibration process. The subsequent chapters of this book will deal with the (much) finer details of analytical calibration and validation in current applications to various analytical methodologies, and thus allow the reader to see the many "faces" of calibration and validation in the realm of chemical analysis.

1.1. What are *calibration* and *validation*, as applied to chemical analysis?

1.1.1. Calibration in chemical analysis

Calibration, in its broadest sense, may be defined as the process of bringing a task, method, procedure, or some operation in general, into conformity with a set of objectives and goals that are solidly established and highly reliable; i.e., based on information that is *precise* and *accurate* [1]. In analytical chemistry, calibration is defined as the process of assessment and refinement of the *accuracy* and *precision* of a method, and particularly the associated measuring equipment (i.e., an instrument), employed for the quantitative determination of a sought-after analyte [2]. The assessment of the analytical method and related instrumentation is based on analysis one or more *reference* samples that contain known, established quantities of the analyte(s) to be determined in the analysis. Usually, the number of reference samples, also known as *standards*, may be as few as two (i.e., a standard containing a known amount of analyte, and a *blank* or standard containing no known amount of analyte), or as many as, say, 10 (e.g., one blank and nine non-zero standards containing varying amounts of analyte). The scope of this process expands significantly when one considers the sample matrix in which the analyte(s) reside(s) and the processes needed to extract the desired analyte(s) from that matrix (if such processes are required), the instrumentation that produces and measures the analyte signal(s), and analysis of the calibration data [2].

1.1.2. Validation in chemical analysis

The term validation denotes, in general, verification of something; i.e., demonstrating by some means that an object, concept, etc. is accurate or valid [1]. In analytical chemistry, validation has the same meaning—in this case, though, the "something" to be verified is the analytical method used for analyte quantitation [3]. The calibration scheme employed in the analytical determination is particularly subject to verification, and must be, as the reliability of the analytical results produced by the determination is dependent on the reliability of the calibration expression that relates analyte signal to its concentration. Typical parameters used to validate a quantitative determination of an analyte include accuracy, precision, limits of detection and quantitation, limits of linearity of the calibration curve, dynamic range of calibration, robustness, sensitivity, and selectivity [3].

2. Rationale for calibration and validation in analytical chemistry

The rationale for performing a calibration of an analytical method may be stated as follows: *to obtain a valid relationship between the signal produced by the analyte and the quantity of analyte in two or more samples of known analyte concentration (standards), that can be described mathematically and used by the analyst to obtain quantitative information on the analyte in samples of unknown analyte concentration.* The calibration process for an analytical method involves measuring the signal produced by an analyte of interest in two or more standards (at least one blank and one non-zero standard) containing known quantities of the analyte. The measured signals from

all of the standards are total signals due to the contributions of the analyte plus other components in the standard matrix (e.g., an aqueous solution). The measured signals are then corrected for the blank signal, which is due to the other components in the standards. This blank correction of all standard signals yields a zero signal for the blank and non-zero signals for the non-zero standards, yielding in turn the signal due to the analyte only. The blank-corrected analyte signal, and the corresponding analyte concentration, are subjected to the appropriate mathematical and statistical treatment, usually linear least squares or other type of regression, to yield a mathematical equation for the best-fit line that describes the signal-concentration relationship [2, 3]. This mathematical expression may be used by the analyst to calculate the concentration of the sought-after analyte in samples of unknown analyte concentration.

The rationale for carrying out a validation study of the analytical calibration may be expressed as follows: *to verify the reliability of the calibration scheme, via assessment of the accuracy and precision of the calibration and the analytical results yielded by it.* For an analytical method to produce results that are both accurate and precise, the calibration setup employed must also be accurate and precise. The validation, or verification, process involves assessment of the calibration data, the outcome of the regression of those data, and the analytical results obtained. The assessment is accomplished by calculation of various statistics that address such parameters as the accuracy and precision of the analyte results, sensitivity of the method to the analyte, selectivity of the method for the analyte over other potentially interfering chemical species, and the lowest concentration of analyte that can be detected by the method as well as the lowest analyte concentration that can be detected with reasonable accuracy and precision [2, 3].

3. Calibration methodologies

One can say that the process of calibration has many 'faces". There is a myriad of possible approaches to the design and analysis of calibration schemes; all one has to do is peruse the published, peer-reviewed literature of analytical chemistry to get an idea of the breadth and depth of calibration methods that have been developed and subsequently implemented for a variety of quantitative analytical determinations over many years. Two aspects of the calibration process in chemical analysis, particularly, are critical to the development and implementation of calibration schemes for analytical methods. These aspects are:

- The design of the method, which would include such considerations as the number of analytes to be determined, the number of blanks and non-zero standards, the matrix of the blanks and standards, the concentration range of each analyte, and application of the method to univariate (one variable) or multivariate (more than one variable) data.

- The mathematical/statistical treatment of the calibration data (i.e., analyte signals and concentrations) that will yield a logical, workable relationship between signal and concentration.

Let us now discuss briefly the aforementioned items as they apply to calibration and validation of analytical methods.

3.1. Calibration methods: some established designs

A calibration scheme may consist of as few as two standards (a blank sample containing no known analyte plus a standard sample containing a known, non-zero quantity of analyte) to a series of standards (at least one blank sample and many standards containing known, and varying, amounts of the analyte) in which the analyte concentrations are arranged in order of increasing concentration. The resulting calibration method may be designed so that known quantities of the analyte are added to the sample matrix, or include a non-analyte chemical species that serves as an *internal* reference against which the analyte response may be ratioed to produce a relative response, or even prepare calibration standard solutions in, e.g., an aqueous medium, apart from the samples. The design of the method may be as simple as comparison of a standard sample containing a known concentration of the analyte to the unknown sample, or as complex as a series of calibration standards for one or more sought-after analytes. Let us now look briefly at some well known, widely used calibration methods employed for quantitation of interesting analytes.

3.1.1. External standard method

The external standard method is perhaps the best known and most widely used calibration method among analytical scientists. The external standard method employs a series of standards consisting of at least one blank that contains no known concentration of the sought-after analyte, and several non-zero standards containing known concentrations of the analyte and prepared in order of increasing analyte concentration. The calibration standards are prepared separate from (external to) the sample matrix, usually in a solvent, e.g., water, and containing the reagents used in sample preparation. The measured signals of the blank and non-zero standards are adjusted for the blank signal to yield a signal that reflects the signal due only to the analyte [3, 4]. The resulting blank-adjusted signals for the calibration standards are then regressed on the corresponding analyte concentrations to yield a calibration equation that is useful for determination of the concentration of the desired analyte in the unknown samples. If the analyte signal (I)-concentration (C) relationship is, e.g., first-order (i.e., "straight line") linear with a slope m, the resulting calibration function will be of the form given by Eq. (1) [3]:

$$I = mC + I_0 \tag{1}$$

The blank-corrected signals of the standards may also be plotted versus the corresponding analyte concentrations, as illustrated in **Figure 1**. The calibration equation for this plot is shown on the plot itself.

3.1.2. Standard addition method

This approach is employed mostly with samples that possess a component which yields a signal that interferes with the signal due to the analyte [3, 5, 6]. The method of standard addition involves direct addition (i.e., spiking) of known amounts of the analyte, usually as aliquots of a stock or working standard solution of the analyte, into equal-volume portions or aliquots of the sample itself. One of the sample aliquots is unspiked (i.e., no analyte added above what
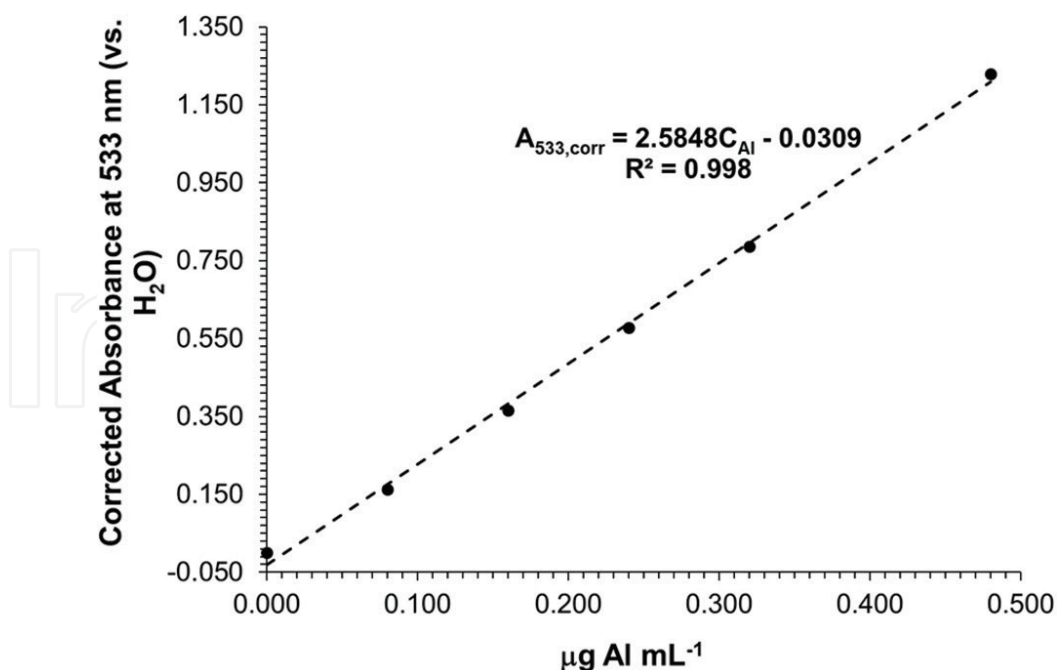
Figure 1. An example of a first-order linear calibration curve for determination of aluminum by the spectrophotometric Eriochrome Cyanine R method (courtesy of original research of the author, November 2015).

may already be in the sample), while the other aliquots are spiked with increasing amounts of the analyte, analogous to the scheme used for an external standard calibration. The effect of this addition of known amounts of the analyte to the aliquots of sample is to increase the signal due to the analyte in order to surmount the signal from the interfering species. The measured analyte signals of the unspiked and spiked sample aliquots are then regressed against the corresponding concentrations of spiked analyte to yield a calibration function that is utilized for determination of analyte concentration in the original sample by calculation of analyte concentration at zero signal. The standard addition method is used primarily to determine analytes in samples that contain chemical components which interfere with the signal produced by the analyte. An example of a standard addition plot is depicted in **Figure 2**.

3.1.3. Internal standard method

The internal standard method makes use of addition of a chemical species, different from the analyte, in a constant amount to calibration standards, blanks, and samples involved in the quantitative determination of the analyte [2, 3]. A ratio of the analyte signal ($I_{A,S}$) to the internal standard signal ($I_{IS,S}$) is calculated for the blank and each standard. Likewise, a ratio of the analyte concentration ($C_{A,S}$) to the internal standard concentration ($C_{IS,S}$) is calculated for each standard (including the blank) in the calibration set. The signal ratios ($I_{A,S}/I_{IS,S}$) are then plotted against the concentration ratios ($C_{A,S}/C_{IS,S}$) to produce a calibration curve and its calibration equation of the form given by Eq. (2):

$$\frac{I_{A,S}}{I_{IS,S}} = m \frac{C_{A,S}}{C_{IS,S}} \tag{2}$$
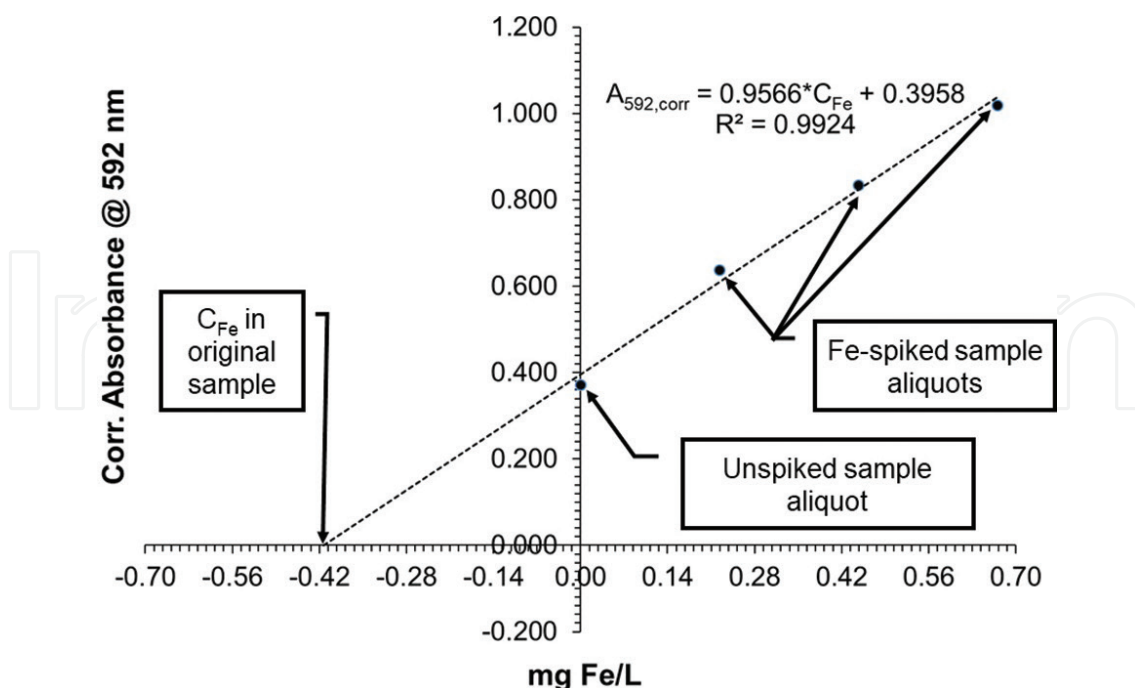
Figure 2. An example of a standard addition calibration curve for determination of iron in mine drainage by the colorimetric Ferene-S method (courtesy of original research of the author and former students, November 2010).

In Eq. (2), m is the slope of the calibration function. The internal standard method is used for irreproducible amounts of sample, varying signals from determination to determination, or losses of sample occurring during sample preparation.

3.2. Regression and analytical calibration

Regression is a statistical process in which the relationship between a dependent, or response, variable (e.g., "y") and an independent, or predictor, variable (e.g., "x") is determined and explained [7]. Through a collection of mathematical calculations, the equation that relates "y" to "x", and in essence explains it, is derived for its intended use. In a chemical analysis, the calibration process involves regression of the dependent variable (usually signal, but can be concentration regarding inverse calibration methods) on one or more independent variables; usually, concentration is taken as the independent variable, but signal is treated as the independent variable in inverse calibration methods [8–10]. The type of method employed for regression of calibration data in an analytical determination depends on how many analytes are to be determined as well as how many responses are to be measured; i.e., is the calibration model to be used *univariate* (one variable) or *multivariate* (more than one variable) in structure? This point is a good segue into the topic of univariate versus multivariate calibration, and some regression methods that are appropriate for either or both calibration models.

3.2.1. Univariate and multivariate calibration: regression methods

For sets of data/results, the term *univariate* refers to a one-variable set of data, e.g., a row or column of titration volumes. The term *multivariate* describes a set of data/results that contains

two or more variables, e.g., a group of sodium concentrations and the corresponding signal intensities measured by flame emission spectrometry—a two-variable system comprised of an independent variable and a dependent variable. In analytical calibration, however, the descriptor *univariate calibration* refers to one dependent variable (e.g., usually signal, but can be concentration) regressed on one or more independent variables (e.g., usually concentration, but can be signal), and the term *multivariate calibration* denotes two or more dependent variables (e.g., usually concentrations of two or more analytes) regressed on two or more independent variables (e.g., usually the corresponding signals) [9–13]. For each of these two broad categories of analytical calibration, there are regression methods that have been demonstrated to provide the sought-after relationship between an analyte signal and the corresponding analyte concentration [9–13]. **Table 1** provides a list of some of these well known, widely used regression methods for quantitative analytical calibration, and the type of regression. In the subsequent chapters of this book, some of the regression methods indicated in **Table 1** will be encountered, and their applications to calibration of various analytical methods illustrated.

Regression method	Univariate/multivariate mode
Ordinary least-squares (OLS)	Univariate (simple OLS, multiple OLS)
• Simple OLS (i.e., linear least-squares)	Multivariate (multivariate OLS)
• Multiple OLS (i.e., multiple linear regression (MLR))	
• Multivariate OLS (includes K-matrix and P-matrix methods)	
Stepwise	Univariate/multivariate
Weighted	Univariate/multivariate
Principal component (PCR)	Univariate/multivariate (usually, one dependent variable regressed on multiple independent variables)
Partial least-squares (PLS)	Univariate (PLS-1)
• PLS-1 (one dependent variable regressed on multiple independent variables)	Multivariate (PLS-2)
• PLS-2 (multiple dependent variables regressed on multiple independent variables)	
Canonical correlation analysis (CCA)	Multivariate
Ridge	Univariate
Lasso	Univariate
Regression trees	Univariate/multivariate
Artificial neural networks (ANN)	Univariate/multivariate

Table 1. Selected univariate and multivariate regression models applied to calibration data in quantitative analytical determinations [9–13].

4. Validation parameters for assessment of the reliability of calibration methods

Without the means to assess the reliability (i.e., accuracy and precision) of the calibration scheme used for quantitative determination of an interesting analyte, the calibration curve employed for quantitation of the analyte *and* the quantitative results for analyte concentration in the sample—in fact, the entire analytical method—become questionable and thereby unreliable. Thus, the need for *validation*, or assessment of the performance of the calibration for a quantitative analysis, becomes imperative for a successful analytical determination. Validation can also have more than one "face" as well.

4.1. Some examples of validation parameters

Various statistical parameters and methods have been developed over the years to accomplish the task of performing assessments of the reliability of calibration schemes used in quantitative analyses. These parameters examine such aspects of calibration schemes as the linearity of the resulting calibration curve, the goodness of fit of the regression model to the experimental calibration data, the precision of the calibration slope, and the standard errors of calibration (SEC) and prediction (SEP) [9–13], among other quantities. Such statistical parameters as the regression equation (i.e., slope (m) and y-intercept (b)), square of the Pearson correlation coefficient (R^2), and standard error of the regression (s_r) are among the assessors of calibration performance for a univariate case (i.e., an analyte signal dependent on a corresponding analyte concentration, or vice versa) [2–4, 8], and are parameters that are usually an undergraduate chemist's first exposure to calibration and validation in a quantitative analysis course. As those involved in data analysis know (all too well), there is much more to consider regarding calibration and validation methodologies. For both univariate and multivariate calibrations, parameters such as *total* and *explained residual variance* (TRV and ERV, respectively), *mean square error* (MSE), *root mean square error* (RMSE, an indicator of calibration *accuracy*), *standard error* (SE, or standard deviation of prediction errors, an indicator of calibration *precision*), *bias*, and the *coefficient of determination* (R^2, a.k.a. the square of the Pearson correlation coefficient) are widely and commonly used for assessment of calibration reliability. The formulas and descriptions for the aforementioned evaluation parameters may be found in any number of texts on chemometrics and statistics [9–13].

4.2. Validation methods: cross-validation and bootstrapping

Calibrations models are usually designed using two sets of response (dependent) variable and predictor (independent) variable data: a *training* set which, as the name suggests, "trains" or develops the model, and the *test* or *prediction*, or *validation*, set that "tests" the validity of the developed model. Assessment of the reliability of the developed calibration (training) model is made by application of that model to the validation (test) set via comparison of the predicted results to the known validation quantities; thus, it is the test set that acts as the *assessor* of the calibration model [11, 12]. Using a specific training and test set only once for development and testing of a calibration scheme may not always produce reliable results using the selected test data. Also, an insufficient amount of calibration data to yield a sufficiently large number of

predicted results can be problematical in evaluating the calibration scheme. Toward this end, two methods in particular, *cross-validation* and *bootstrapping*, have been developed to increase the number of predictions for a given calibration model.

4.2.1. Cross-validation

In cases for which there might be a paucity of data available to perform a thorough evaluation of the calibration model, a method known as *cross-validation*, that "resamples" both training and test data to produce a larger number of predictions, may be employed. Cross-validation works by splitting the total set of available calibration data into roughly equal-data segments, with one of the segments selected as the test set and the remaining segments serving as the training set. The calibration model is developed using the training set, and then tested on the test set. This process is continued until every segment has served as a test set. In this manner, the number of predictions for the model may be increased [10–13].

4.2.2. Bootstrapping

This method uses training sets with a set number of objects randomly selected from the available data set. A calibration model is developed from the training set and subsequently applied to the objects in the available data set that are not part of the training set. This process yields corresponding prediction values and their associated errors. The process is then repeated many (sometimes up to 1000) times. Two advantages of the bootstrap method are an uncomplicated approach and having the same number of objects in the training set; some disadvantages are labor-intensive calculations, the possibility of unequal consideration of all objects in the available data, and results that are sometimes overly optimistic [11].

4.3. Validation parameters for the analytical method itself

Let us not forget about other parameters that are useful for validation of the analytical method itself. These are the so-called "figures of merit" [2–4]—the accuracy (i.e., bias) and precision (i.e., standard deviation) of the analytical results, limits of detection (LOD), quantitation (LOQ), and linearity (LOL), the dynamic range (the range of concentration linear with signal from the LOQ to the LOL; i.e., LOL/LOQ), sensitivity, and selectivity [2–4]. All of these parameters for method validation are ultimately connected, and traceable, to the calibration scheme employed for quantitation of sought-after analytes using an analytical method or technique.

5. Some "faces" of calibration and validation to be found in this book

In this book on calibration and validation of analytical methods are a collection of research and review chapters on various applications and other aspects of calibration and validation in chemical analysis. In these highly interesting chapters, one can see the many and varied "faces" of calibration and validation revealing themselves to the reader, waiting to be studied and utilized by interested researchers. A quick glimpse of these "faces" should provide the reader with a preview of what is in store as one explores the content of this book:

- The impact of factorial design and machine learning strategies on pharmaceutical analysis

- Multivariate calibration methods applied to development of vibrational spectroscopic methods

- Approaches to method validation for pharmaceutical assessments, using high-performance thin-layer chromatography (HPTLC)

- A review of criteria for assessment of analytical method reliability

- Using internal standards for quantitation of proteins in biological matrices by LC-MS/MS

- Calibration methods for laser-induced breakdown spectroscopy (LIBS)

- Analytical method validation, presented in the context of laboratory competence and generation of reliable analytical results

I anticipate that the reader will find this assemblage of chapters dealing with analytical method calibration and validation useful as well as interesting, and possibly inspiring some ideas for future studies.

6. Summary

This introductory chapter to this book on calibration and validation of analytical methods was written to provide the reader with a general overview of a sort on the topics of calibration and validation as applied to problems in chemical analysis. This included a general explanation of calibration and validation, the importance of these topics in quantitative analysis, and a rationale for their use in analytical chemistry. Also presented were overviews on calibration and regression methods, and validation parameters and methods for calibration schemes and analytical results. Finally, a glimpse of the subsequent chapters in this text was given. This introductory chapter is meant to be general in scope; the reader will get much more detail in the following research and review chapters. Thus, I invite the reader to explore the following chapters to see the various "faces" of analytical method calibration and validation.

Acknowledgements

Author details

Mark T. Stauffer

Address all correspondence to: mtschem1@pitt.edu

Chemistry Department, Division of Natural Sciences, Mathematics and Engineering, University of Pittsburgh—Greensburg, Greensburg, Pennsylvania, USA

References

[1] BIPM. JCGM 200:2008. International vocabulary of metrology—Basic and general concepts and associated terms (VIM) [Internet]. Available from: https://www.bipm.org/utils/common/documents/jcgm/JCGM_200_2008.pdf [Accessed: January 31, 2018]

[2] Skoog DA, Holler FJ, Crouch SR. Principles of Instrumental Analysis. 6th ed. Pacific Grove: Brooks-Cole; 2007. ISBN-10: 0-495-01201-7

[3] Harris DC. Quantitative Chemical Analysis. 9th ed. New York: W. H. Freeman & Co.; 2016. ISBN-13: 978-1-4641-3538-5

[4] Danzer K, Currie LA. Guidelines for calibration in analytical chemistry. Pure and Applied Chemistry. 1998;**701**:993

[5] Bader M. A systematic approach to standard addition methods in instrumental analysis. Journal of Chemical Education. 1980;**57**:703

[6] Hyk W, Stojek Z. Quantifying uncertainty of determination by standard additions and serial dilutions methods taking into account standard uncertainties in both axes. Analytical Chemistry. 2013;**85**:5933

[7] Freedman DA. Statistical Models: Theory and Practice. New York: Cambridge University Press; 2009. ISBN-13: 978-0-521-74385-3

[8] Tellinghuisen J. Inverse vs. classical calibration for small data sets. Fresenius' Journal of Analytical Chemistry. 2000;**368**:585

[9] Kramer R. Chemometric Techniques for Quantitative Analysis. Boca Raton: CRC Press; 1998. ISBN-13: 978-0824701987

[10] Otto M. Chemometrics: Statistics and Computer Application in Analytical Chemistry. 3rd ed. Weinheim, Germany: Wiley-VCH; 2017. ISBN-13: 978-3527-34097-2

[11] Varmuza K, Filzmoser P. Introduction to Multivariate Statistical Analysis in Chemometrics. Boca Raton: CRC Press; 2009. ISBN-13: 978-1-4200-5947-2

[12] Pomerantsev AL. Chemometrics in Excel. Hoboken: John Wiley & Sons, Inc.; 2014. ISBN-13: 978-1-118-60535-6

[13] Brereton RG. Chemometrics: Data Analysis for the Laboratory and Chemical Plant. Chichester, West Sussex, England: John Wiley & Sons, Ltd.; 2003. ISBN-10: 0-471-48977-8