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REVIEW

The Importance of Teaching Metrology for Chemistry Undergraduate Courses

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PITFALLS THAT SHOULD BE ADDRESSED IN CHEMISTRY METROLOGY COURSE SYLLABUS

Are the variances always homogeneous?

Should every analytical calibration curve (ACC) have 1st degree polynomial (straight-line) adjustment?

Where is the most precise region of an analytical calibration curve (ACC)?

Must the measurement uncertainty arising from sampling in chemical analyses be neglected?

Is the laboratory precision the measurement uncertainty of the analytical result?

The Chemistry undergraduate courses, in general, aim to train professionals for research, teaching and activities in industry. In all these areas, the statistical treatment of data supported by metrology disciplines has been a decisive boost to consolidate conclusions in Analytical Chemistry. The main objective of this work was to stimulate the teaching of the metrology in the chemistry undergraduate courses in Brazil and Portugal. In this work we present some concepts and practical cases of metrology and statistics, which together guarantee the suitability of analytical measurement results for specific purposes. Knowledge of these concepts is essential for the chemist to act effectively and responsibly, avoiding some pitfalls when processing data in Analytical Chemistry. A supplementary material with Excel spreadsheets is available, containing the statistical treatment of some examples and complementing the discussions in this work. A survey is presented among the most important universities in Brazil and Portugal, from which we can see the lack of teaching

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these subjects in undergraduate chemistry courses. For this reason, this work recommends a syllabus for creating chemical metrology disciplines in undergraduate chemistry courses. To subsidize this syllabus, an extensive, but not exhaustive, bibliographical references is indicated.

Keywords: homogeneity of variances, analytical calibration curve fit, sampling uncertainty, metrology course syllabus, compliance assessment, chemical metrology

INTRODUCTION

"Metrology", the science of measurement and its applications,[1] develops the knowledge to guarantee that the results of a measurement enable decisions that are as assertive as possible in relation to its intended use; this is what is called the "fitness for purpose" or "fitness to intended use"[1-4] of the "measurement results".[1] In the recent past, analytical chemists were aware of mainly the "measurement trueness"[1] related to the "systematic error"[1] and its "bias"[1] estimation to enable the analytical result "correction".[1] More recently, the awareness with the "measurement precision"[1] as "repeatability"[1] or "reproducibility"[1] or "intermediate precision"[1] and especially with the "measurement uncertainty"[1] as well as with the "metrological traceability",[1] "metrological comparability"[1] and "metrological compatibility"[1] entered within the horizon chemist's interest. All these metrological concepts combined with a solid knowledge of statistics are extremely important for the chemist's professional training and can be a differentiator in employability. In this work, we will show that some statistical concepts are necessary to evaluate some of the above metrological concepts and propose some contents that should be taught in undergraduate chemistry courses and related courses. Also, we will review the availability of Brazilian publications and of the undergraduate and graduate disciplines on metrology. Finally, to help the persons interested in these subjects' relations of bibliographic references and internet sites are presented.

Based on the authors' academic and industrial experience, several times, analytical chemists think that the results from chemical analyses always belong to an ideal universe; that is, they are homoscedastic, the analytical curve is rectilinear (linear on the analyte concentration, the measurand, as in the example of the analytical calibration curve for Cd determination discussed below),[5] and there is no significant influence of the sampling step (not considering the uncertainty due to sampling).[6] All these issues have contributed to the measurement uncertainty. The violation of these assumptions can cause problems or risks in decision-making using the analytical chemistry results.

Often, analytical instrumental responses variability does not have constant variance; that is, it does not present homoscedastic behavior. Thus, the parameters of the analytical calibration curves (ACC) obtained using an unweighted, simple linear regression by ordinary least-squares (OLS),[7-9] when all points have the same influence on the fitting procedure, are not the most adequate, leading to biased fitted parameters. Due to this data heteroscedasticity, these curves are conceptually correctly fitted by a simple linear regression by weighted least-squares (WLS);[10-17] that is, the coefficients of the analytical curve are estimated to give higher weights to the points where the variability (uncertainty) is lower.

Unfortunately, analytical calibration curves (ACC) do not always behave as a straight line (polynomial of degree one) over the entire working range. It is not uncommon that polynomials of a higher degree or non-linear on the fitted parameters fits (e. g., $y = ax^b$, exponential functions $y = ae^{bx}$, $y = e^{a+bx}$, etc) are better suited in instrumental analysis. Thus, analytical chemists must know how to check correctly and decide whether to stick with the rectilinear adjustment or fit the analytical calibration curve (ACC) with a polynomial of higher order on the analyte concentration or a function non-linear on the fitted parameters.

Due to the degree of heterogeneity (i.e., inhomogeneity) on sampling targets, the uncertainty arising from sampling, besides the analytical uncertainty, must be evaluated. Ratifying this statement, item 7.6.1 of ISO/IEC 17025:2017 says:[18-19] "… all contributions that are of significance, including those arising from sampling, shall be taken into account using appropriate methods of analysis." If sampling uncertainty is neglected and is not statistically negligible, the uncertainty information used in the compliance assessment may lead to erroneous conclusions regarding the sampling target and relates only to the laboratory sample.

Misunderstanding related to these very important issues can occur because there is a gap between the analytical chemistry and metrology concepts.[20] Thus, this review aims to shed light on the importance and lack of connection between metrology in teaching and research in Analytical Chemistry in Brazil and Portugal and, finally, propose a syllabus for the metrology course in Chemistry and some related undergraduate courses.

PROBLEMS OR RISKS IN DECISION-MAKING WHEN METROLOGICAL AND STATISTICAL CONCEPTS ARE NOT CORRECTLY APPLIED

As some authors are or were professors in undergraduate and postgraduate Chemistry courses, unfortunately, it was noticed that the disciplines of Analytical Chemistry and Instrumental Analysis do not closely connect with metrological concepts. In these situations, analytical chemists should remember that one of the final steps of the analytical process is the data treatment. Any mistake in this important stage can compromise the results and conclusions.

Are the variances always homogeneous? The case of sulfur determination in mineral oil by X-ray fluorescence

Table I presents the results of concentrations and instrumental responses (IR) for an ACC of the sulfur mass fraction of certified reference materials in the mineral oil matrix.[21] The experiment was carried out on the XOS SINDIE GEN 3 equipment, based on the ASTM D7039-15a (2020) standard method,[22] whose analytical technique is monochromatic wavelength dispersive x-ray fluorescence.

Table I. Analytical calibration curve (ACC) from 0 to 50 mg kg^{-1} for sulfur in mineral oil determination by X-ray fluorescence

Mass fraction, mg kg^{-1}	IR = Counts					
0.00	177	182	172	182	164	199
5.04	922	1085	927	987	991	919
10.29	1842	1841	1791	1962	1843	1897
15.16	2562	2606	2566	2650	2720	2687
25.18	4023	4246	4387	4168	4016	4206
50.48	**8100**	8408	8404	8419	8400	**8306**

Data extracted from the Technical Note[21] titled “Avaliação Metrológica da Curva Analítica para Determinação do Teor de Enxofre em Baixíssimas Concentrações Em Óleo Diesel S-10”. By de Farias, S. I.; da Costa, L. G.; Calili, R. F.; Rios, R. M.; de Oliveira, E. C. *Quim. Nova* **2015**, *38*, (6), 852-858. http://dx.doi.org/10.5935/0100-4042.20150071 with permission granted by Quím. Nova, PubliSBQ, on March 14, 2024.

Although the values in bold were considered outliers according to the Grubbs’ pair test and the residues show independent behavior, according to the Durbin-Watson test, these values weren’t excluded from the ACC statistical treatment since no technical event was reported by the analyst during the analytical work. Also, the standard deviations of the IR smoothly increase with the analyte calibrator concentration, and by removing these two values, the standard deviation of the last calibrated level becomes even lower than that of the first calibrated level.

The ordinary (unweighted) regression ANOVA indicates that the straight-line OLS fit is adequate; however, the Cochran test assesses that the behaviour is heteroscedastic; therefore, WLS linear regression is more suitable.

For this study, the prediction "combined standard measurement uncertainty",[1,23,24] $u(c_{pred})$, were calculated by Equation 1, considering the regression as unweighted (OLS) and weighted (WLS) for two CRMs of concentrations of 10.29 mg kg^{-1} and 25.18 mg kg^{-1} with IR of 1860.67 and 4174.33, respectively, around the centroid (17.7, 3026.58) of the ACC, and for a hypothetical sample solution with IR 9000 using the values available in Table II. Equation 1 (Equation E3.3 of section E.4.3 at reference 24, Equation 6 without the first term at reference 25) is obtained by applying the law of propagation of uncertainty (LPU) on the "measurement function"[1] for the analyte concentration predicted on the ACC as a straight-line,[25] which can be used for all the OLS, WLS and bivariate regressions. Note that the widely used Equation E3.5 at reference 24 is a particular case of Equation 1 and can be deduced from it, but it can be used only when an OLS regression is performed on a homoscedastic ACC.

$$u(c_{\mathrm{pred}}) = u(x^*) = u(x_o) =$$

$$\sqrt{\left\{u^2(y_o)/K + \begin{bmatrix}1 & x_o\end{bmatrix}\begin{bmatrix}u^2(a) & \mathrm{cov}(b,a)\\ \mathrm{cov}(a,b) & u^2(b)\end{bmatrix}\begin{bmatrix}1\\ x_o\end{bmatrix}\right\}b^{-2}} = \qquad \text{Equation 1}$$

$$\sqrt{\frac{s^2(y_o)/K + \left\{s^2(a) + (c_{\mathrm{pred}})^2 s^2(b) + 2c_{\mathrm{pred}}\,\mathrm{cov}(a,b)\right\}}{b^2}}$$

Table II. Parameter fitted by OLS and WLS and their standard-deviations and their covariances; predicted concentrations (c_{pred}) on the fitted ACC, $c_{pred} = (y - a)/b$, for sulfur determination in mineral oil and their standard uncertainties ($u(c_{pred})$). The measured concentrations for two CRMs and a sample solution with their prediction standard uncertainties.

Fitted Parameter	OLS	WLS	c_{CRM} / mg kg^{-1}	$c_{pred} \pm u(c_{pred})$ by OLS / mg kg^{-1}	$c_{pred} \pm u(c_{pred})$ by WLS / mg kg^{-1}
Intercept *a*	171.33 ± 21.65	179.26 ± 4.70	10.29	10.48 ± 0.56	(10.43 ± 0.39)
Slope *b* / kg mg^{-1}	161.39 ± 0.89	161.46 ± 0.79	25.18	24.80 ± 0.56	(24.74 ± 0.58)
cov(*a*,*b*) / kg mg^{-1}	–14.0393	–9440	IR = 9000	54.70 ± 1.21	54.63 ± 2.03

It is observed that the confidence intervals for one standard uncertainty with confidence probability of nearly 68%, for both the OLS and WLS regressions, contain the CRMs values and that the prediction standard uncertainty for the unweighted OLS regression increases symmetrically but very slightly around the centroid, at the concentration 17.6917 mg kg^{-1}, being practically constant (Figures 1(A) and 1(C)) throughout the calibrated range, while for weighted WLS regression the confidence interval increases with concentration (Figures 1(B) and 1(D)).

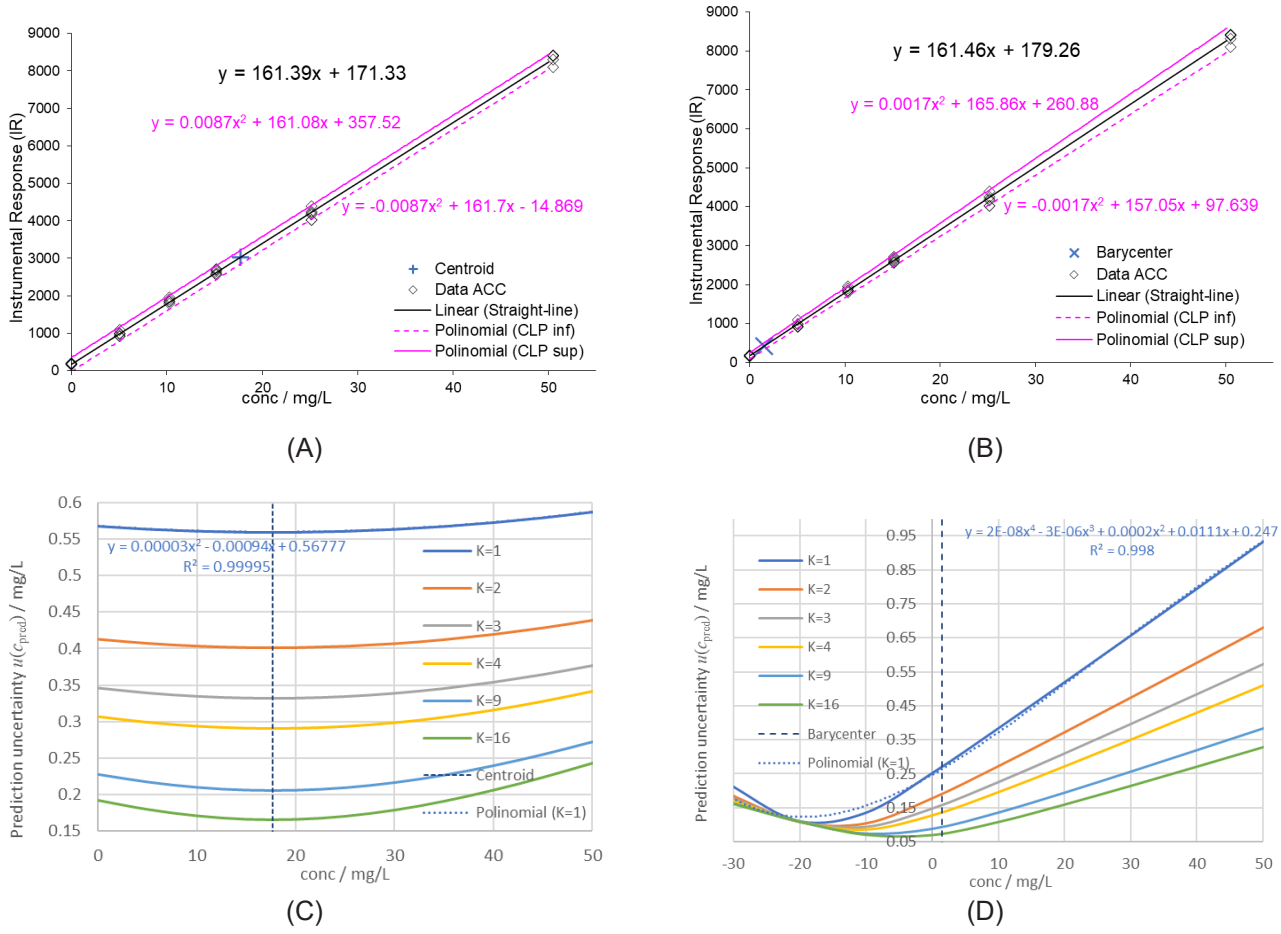


Figure 1. The sulfur analytical calibration curve (ACC) from Table I.

The "calibration diagram"[1] shows the calibration function and the prediction confidence band around the calibration function for a 95% confidence level for (A) OLS and (B) WLS fit, with the upper (CLP sup) and lower (CLP inf) prediction confidence limit curves.

The curves for the variation of the prediction uncertainty, according to Equation 1, against the predicted concentration and the number K of replications of measurement of the IR for the sample solution for (C) OLS and (D) WLS fits. These curves, as well as the CLP sup and CLP inf curves, are hyperbolas, but can be approximated by a polynomial of even degree, as represented for K = 1.

Should every analytical calibration curve (ACC) have 1st degree polynomial (straight-line) adjustment? The case of cadmium determination in water by AAS

The data for the ACC for the determination of Cd in water by atomic absorption spectroscopy (AAS) adapted from Example A5 from QUAM,[24] Table III, must be conceptually fitted by the weighted least squares (WLS) method, as the variances of the calibrated levels IR are heteroscedastic as depicted in the last column in Table III and in Figure 2(A), where a rectilinear line well fit the increasing IR repeatability standard deviations. This heteroscedastic IR of the analytical instruments, based on electromagnetic, but also ionization, radiation detection is naturally expected when consider the intrinsic behavior of this detector noise (see the section "Effect of the instrumental noise on the spectrometric analysis" at reference 26). For teaching purposes, aiming to emphasize the effect of different regression models and techniques on the measurement result (value and uncertainty), this study considers the first (straight-line) and second (parabola, quadratic) degree polynomials models, first using the OLS fitting and after the WLS fitting. Figure 2(B) shows the not conceptually correct use of the OLS to fit a straight-line (dashed line) on the ACC, as realized at reference 24, and to fit a parabola (continuous line), which presents a best coefficient of

multiple determination R^2 = 0.9991. The fitted OLS values for the intercept a and slope b and their standard uncertainty were obtained using the command “Regression” on Excel, a = 0.0097, $u(a)$ = 0.002847 and b = 0.23766667, $u(b)$ = 0.004956. These same values are obtained by the OLS fit (model 1b) using the CCC Software[27] and by the spreadsheet in the tab “Cd Expl A5 QUAM OLS” in the supplementary material. The CCC Software and the spreadsheet present the value of the covariances between these fitted parameters, $\text{cov}(a,b) = -1.228205\times10^{-05}$, not presented by the Excel command “Regression” at "Data", "Data Analysis", or by the function PROJ.LIN. Note that these fitted parameters are very near, within one standard deviation of the values of the uncorrected data fitted at the original publication.[24]

Table III. Data of the analytical calibration curve (ACC) for Cd determination in water by atomic absorption spectroscopy (AAS) (adapted from Example A5 from reference 25)

Concentration (mg L^{-1})	Absorbance (A)			Standard-deviation s_A
0.1	0.028	0.029	0.029	0.000577
0.3	0.084	0.083	0.081	0.001528
0.5	0.135	0.131	0.133	0.002000
0.7	0.180	0.181	0.183	0.001528
0.9	0.215	0.220*	0.216	0.002646

* This value replaces 0.230 in the original publication.[24] Without replacing (correction) the repeatability standard-deviation s_A of the last level becomes 0.008386, larger than threefold the expected from the linear behavior of the first four levels, Figure 2(A). This pathologic behavior is probably due to a typo.

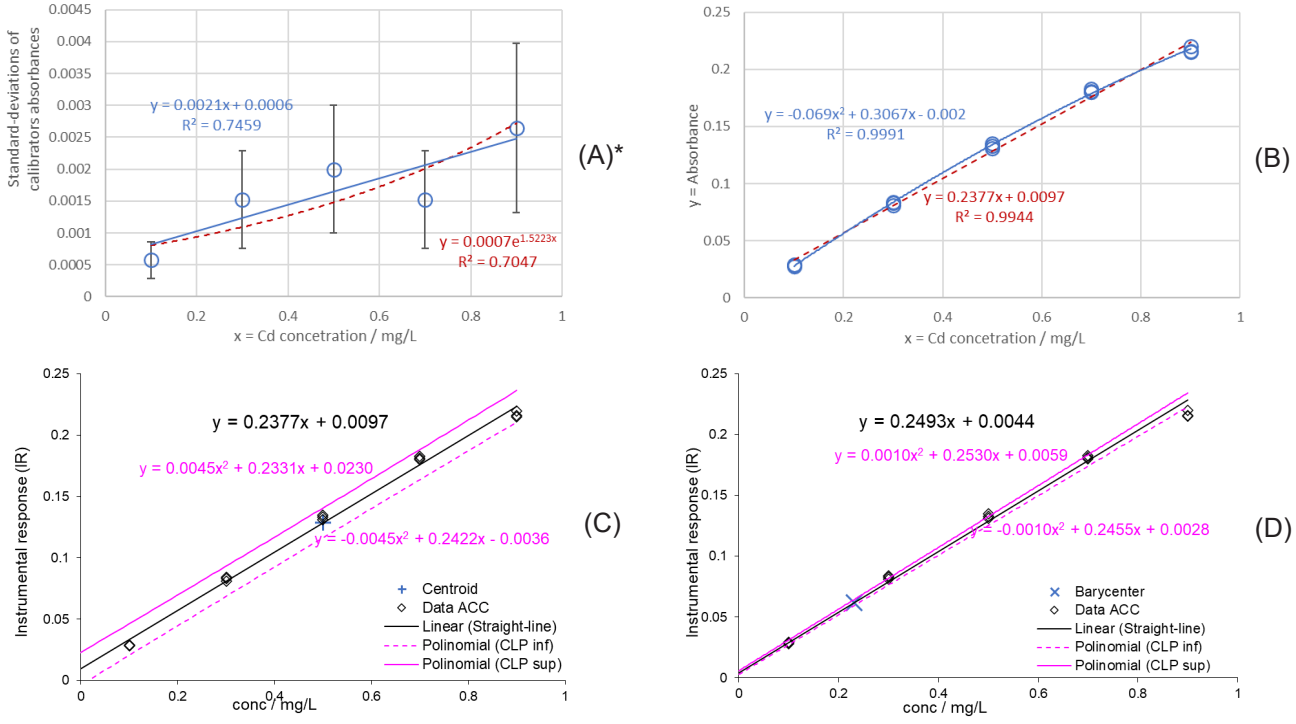
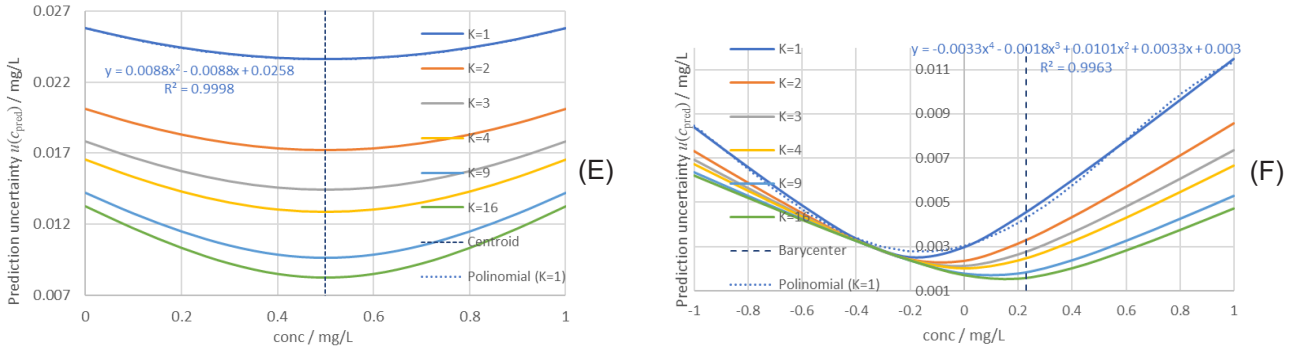


Figure 2. The Cd analytical calibration curve (ACC) from Table III. (continues on the next page)



* The first four levels are well fitted by the straight-line s_A = 0.0017x + 0.0007 with R^2 = 0.5161. Without the replacement of the original 0.23 value by 0.22, the five points of s_A vs. concentration are best fitted by an exponential function: $S_A = 0.0005^{2.6759x}$ with R^2 = 0.8082. The error bars are 50%, as nearly expected by equation E.7 in section E.4.3 of the GUM[23] for three IR replications for each calibration level.

Figure 2. The Cd analytical calibration curve (ACC) from Table III. (continuation)

(A) The OLS fits of the Standard deviations of the instrumental responses (IR) at each calibration level. (B) The Cd ACC is fitted by the OLS as a straight-line and a parabola. The "calibration diagrams"[1] shows the calibration functions, and the prediction confidence bands around the calibration functions for a 95% confidence level for (C) OLS and (D) WLS fits, with the upper (CLP sup) and lower (CLP inf) prediction confidence limit curves.
The curves for the variation of the prediction uncertainty, according to Equation 1, against the predicted concentration and the number K of replications of measurement of the IR for the sample solution for (E) OLS and (F) WLS fits. These curves, and CLP sup and CLP inf, are hyperboles but can well be approximated by a polynomial of even degree, as depicted for *K*=1.

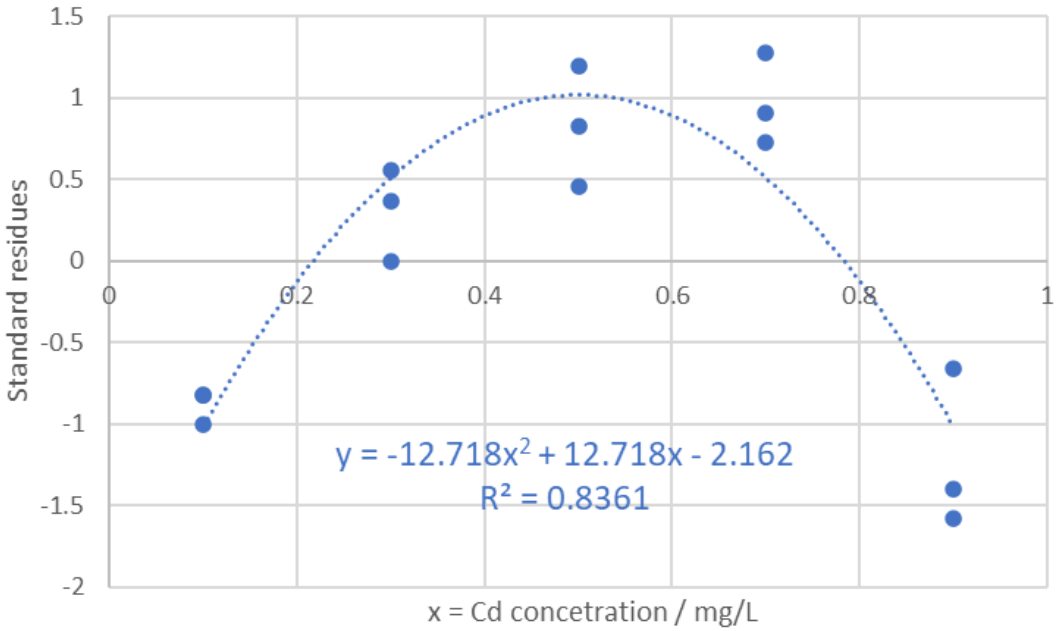


Figure 3. Standard residues, the residues divided by the residual standard deviation, of the OLS rectilinear fit of the analytical calibration curve (ACC) for cadmium determination (Table III).

The rectilinear (first-degree polynomial) OLS fit results show a coefficient of multiple determination $R^2 = 0.9944$ or $r = 0.9972$ (for the rectilinear fit $R^2 = r^2(x,y)$, the square of the coefficient of linear correlation or Pearson correlation coefficient between x and y, see sections 8.3 and 10.3.1.2 at reference 28), with a residual standard-deviation of $s_{res} = 0.005429$. Note that this residual standard-deviation is even larger than the largest IR standard-deviation at the last calibration level! Visually, the rectilinear fit looks like an

excellent model (rejection of the null hypothesis $b = 0$), but it lacks fit (rejection of the null hypothesis $MS_{LOF} = MS_{EE}$), as indicated by Figure 3, and confirmed by the unweighted ANOVA, Table IV. The concave pattern of the plot of the standard residues in Figure 3 also indicates that this ACC is not adequately fitted by a straight-line. This pattern is yet more pronounced from the rectilinear WLS fit of this ACC (see the Figure "Plot of the standard residues" in the tab "CCC WLS rectilinear" in the supplementary material) because the OLS residual standard-deviation is inflated due to the lack of fit.

Table IV. Ordinary (unweighted) Analysis of Variance for first-degree polynomial (straight-line) OLS fit of the Cd analytical calibration curve (ACC) at Table III and Figure 2(B)

Y = 0.0097	**+**	**(0.237666667) X**
Correlation Coefficient	**=**	**0.99719**
% Explained Variation	**=**	**99.44**

SOURCE OF VARIATION	SUM OF SQUARES		DEGREES OF FREEDOM	MEAN SQUARE (MS)
Regression (Reg)	0.067782533		1	0.067782533
Residual (Res)	0.0003832		13	2.94769E-05
Lack of fit (LOF)	0.0004		3	0.000117067
Experimental Error (EE)	0.0000		10	3.2E-06
Total	0.068165733		14	
MS_{Reg}/MS_{Res}	2299.512	>	$F_{CRITICAL}$	4.667
		The model is suitable		
MS_{LOF}/MS_{EE}	36.583	>	$F_{CRITICAL}$	3.708
		The model fit is not suitable		

These results show that even on heteroscedastic data, the OLS fit can be used for an initial exploratory study of the ACC, but not for its definitive fit. The OLS fit can be used to model the plot of the standard-deviations of the IR of the calibration levels against the analyte concentration of the calibration standard solutions. This is because in routine analysis, the number of replications of the measurement of these calibrators is, in general very low, and the corresponding standard-deviations are very uncertain, Figure 2(A).

To try to eliminate the lack of fit, a quadratic term was added, and the OLS fitting of a second-degree polynomial (parabola) was realized, Figure 2(B), resulting in a coefficient of multiple determination $R^2 = 0.9991$ and a residual standard deviation of $s_{res} = 0.002288$, which is half of this one for the rectilinear fit and around the values of s_A in Table III.

Table V. Ordinary (unweighted) Analysis of Variance for second-degree (quadratic) polynomial OLS fit of the Cd analytical calibration curve (ACC) at Table III and Figure 2(B)

$Y = \beta_0 + \beta_1 X + \beta_2 X^2$		
β_0	=	-0.00204
β_1	=	0.30671
β_2	=	-0.06905
r	=	0.99954
% Explained Variation	=	99.91

SOURCE OF VARIATION	SUM OF SQUARES		DEGREES OF FREEDOM	MEAN SQUARE (MS)
Regression (Reg)	0.068102914		2	0.034051457
Residual (Res)	6.2819E-05		12	5.23492E-06
Lack of fit (LOF)	0.0000		2	1.54095E-05
Experimental Error (EE)	0.0000		10	3.2E-06
Total	0.068165733		14	
MS_{Reg}/MS_{Res}	6504.675	>	$F_{CRITICAL}$	3.885
		The model is suitable		
MS_{LOF}/MS_{EE}	4.815	>	$F_{CRITICAL}$	4.103
		The model fit is not suitable		

The predicted standard uncertainty, $u(c_{pred}) = u(x^*)$, for the linear and quadratic OLS models varies from 0.0235 mg L^{-1} to 0.0250 mg L^{-1} (see the Figure 10 in the tab “Cd Expl A5 QUAM OLS” in the supplementary material) and from 0.019 mg L^{-1} to 0.031 mg L^{-1} (see the Figure 12 in the tab “Cd Expl A5 QUAM OLS” in the supplementary material), respectively, what can justify the quadratic fit, leading lower prediction uncertainties at low concentrations. Also, the quadratic OLS fit standard residues are now randomly distributed around the zero, Figure 4, as expected from a good fit, although the lack of fit indicated by the unweighted ANOVA in Table V; however, the heteroscedasticity of the ACC requires the use of the WLS fit.

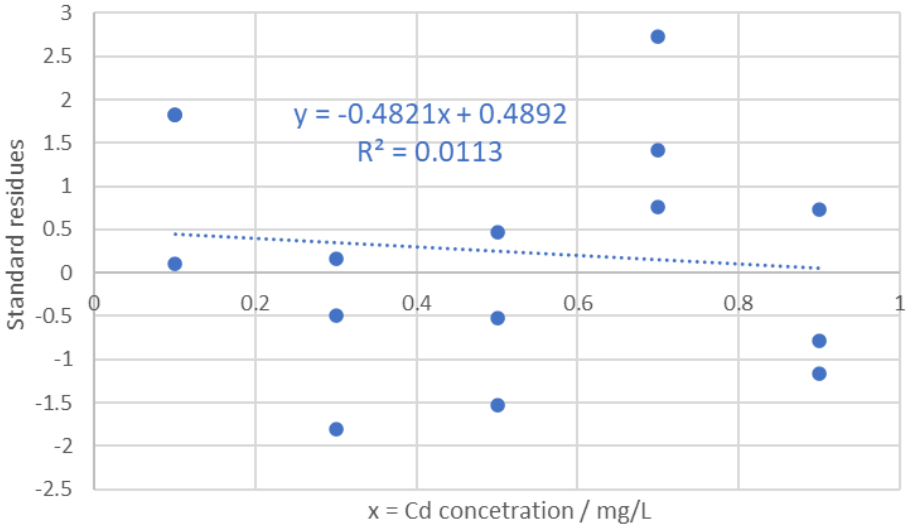


Figure 4. Standard residues of the OLS parabola (quadratic or second-degree polynomial) fit of the analytical calibration curve (ACC) for Cd determination.

The above treatment of the ACC demonstrates that its correct fit should be realized using the WLS. The quadratic WLS fit was performed using the CCC Software[27] using the option "model 2b" which estimates the IR variances (squared standard-deviation) from the replications of each calibrated level. The fitted values, their standard uncertainties, and the covariance matrix among the three fitted parameters are presented in Table VI and the plot of the fitted parabola is in Figure 5(A). The standard residues of this fit are depicted in Figure 5(B) (see also Figure 11 in the tab "Cd Expl A5 QUAM WLS" in the supplementary material). The prediction standard uncertainty ($u(c_{pred})$) calculation for the fit of a parabola is a little cumbersome and can be realized using a generalized matrixial form of the Equation 1. Using the covariance matrix associated with fitted (calculated) values y (y_{fit}) provided by the CCC Software, we calculated the prediction standard uncertainty, only for K = 1, at the five calibrated levels. It was used the LPU as in Equation 2 (see cells U412:U440 in the tab "Cd Expl A5 QUAM WLS" in the supplementary material), where $u^2(y_{fit})$ are the diagonal elements of the y_{fit} covariance matrix from the CCC Software and $s^2(y_o)$ was assumed to be equal the repeatability standard deviation of a calibrator with the same concentration of c_{pred}. This last hypothesis is especially valid for ACC prepared from a blank sample or matrix-matched ACC.

$$u(c_{pred}) = u(x^*) = u(x_o) = \sqrt{\frac{s^2(y_o)/K + u^2(y_{fit})}{\left(\partial y / \partial x\right)^2}} \qquad \text{Equation 2}$$

The behavior of this prediction uncertainty with the analyte concentration is complex, as depicted by the continuous line in Figure 6, but grossly increases within the calibrated region (see Figure 12 in the tab "Cd Expl A5 QUAM WLS" in the supplementary material), as also happen for the case of the WLS straight-line fit, and the lowest prediction uncertainty is below the calibrated range (the minimum of the dashed parabola in Figure 6 is at -4.5 mg/L = -8.0138E-03 / 2 / 8.9039E-04). The pathological local minima at the continuous line, Figure 6, is due to the low IR repeatability standard deviation at a concentration of 0.7 mg/L, below the expected value for this calibration level, as inferred from the straight-line in Figure 2(A). The dashed line parabola in Figure 6 shows the prediction uncertainty $u(c_{pred})$ if the IR repeatability standard deviations of the calibrators and sample test solutions $s^2(y_o)$ obey the straight-line in Figure 2(A).

Table VI. Results of the quadratic (parabola) WLS fit $y = a + bx + cx^2$ carried out by the CCC Software[27] on the analytical calibration curve (ACC) data for Cd determination by AAS in Table III

	Fitted value	Standard uncertainty	Covariance matrix		
a	-0.000918166	0.000689964	+4.760506e-07	-3.472725e-06	+3.767259e-06
b	0.30110662	0.00567432	-3.472725e-06	+3.219796e-05	-3.691866e-05
c	-0.06224945	0.00665795	+3.767259e-06	-3.691866e-05	+4.43283e-05

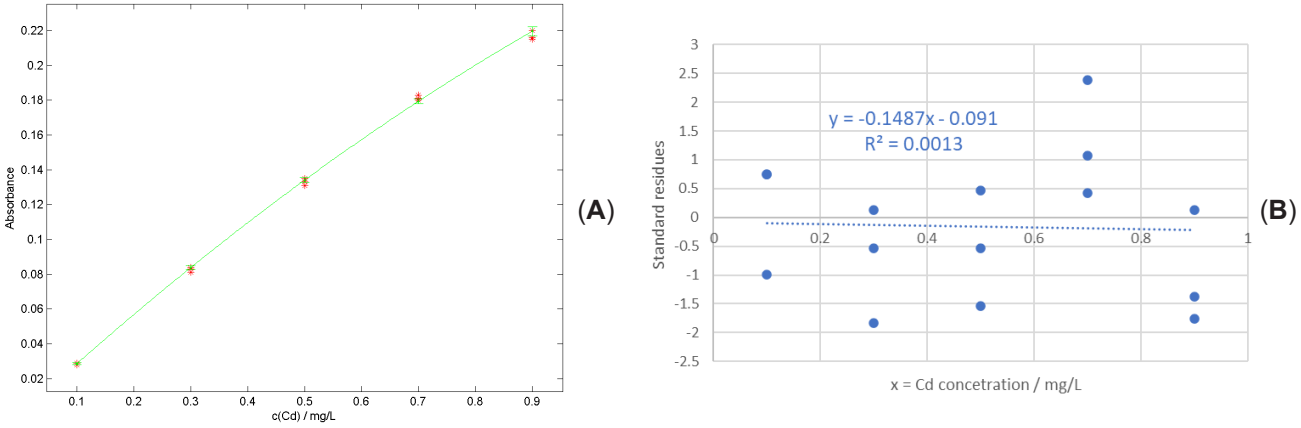


Figure 5. The analytical calibration curve (ACC) for cadmium determination (Table III) and the WLS fitted parabola (quadratic fit). (A) The "calibration diagram"[1] showing the calibration function and the prediction confidence band, as error (uncertainty) bars, around the calibration function for a 95% confidence level as depicted by the CCC Software.[27] The prediction error bars are shown on the fitted curve at the five calibrated levels. (B) Standard residues of the WLS parabola (quadratic) fit. Note that, as expected for a good fit, all the estimated parameters for the straight-line in this figure are nearest from zero than those for the OLS parabola fit in Figure 4.

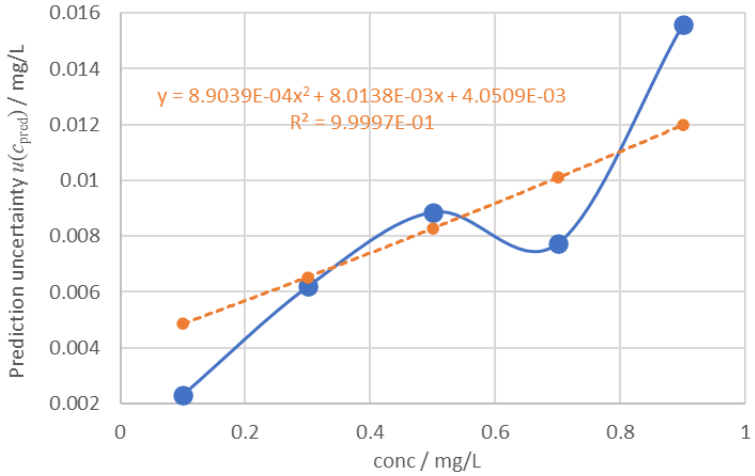


Figure 6. The curve for the variation of the prediction uncertainty, $u(c_{pred})$, according to Equation 2, against the predicted concentration for the WLS parabola fitted on the cadmium analytical calibration curve (ACC) for one unique sample test solution measurement, *K*=1. The dashed line parabola well fits the expected behavior of $u(c_{pred})$ if the IR repeatability standard deviations of the sample test are given by the straight-line in Figure 2(A).

Where is the most precise region of an analytical calibration curve (ACC)?

We learned in our first courses in instrumental analytical chemistry that an ACC should be designed to have the sample test solution in the middle of the calibrated range, which is the more precise region of the ACC. Note that the middle of the ACC is not necessarily equal to its centroid (see the symbol + in Figure 1(A) and Figure 2(C)), the mean values of the calibrators concentrations and their IR. However, this is true only for a very particular and rare case: a homoscedastic ACC with statistically the same IR standard deviation in all calibrated levels, calibrated levels equally spaced, and finally, with the same number of replications of the IR in all calibration levels. This is **not** the case for ACCs in Table I and Figure 1 for sulfur determination, neither in Table III nor Figure 2 for Cd determination.

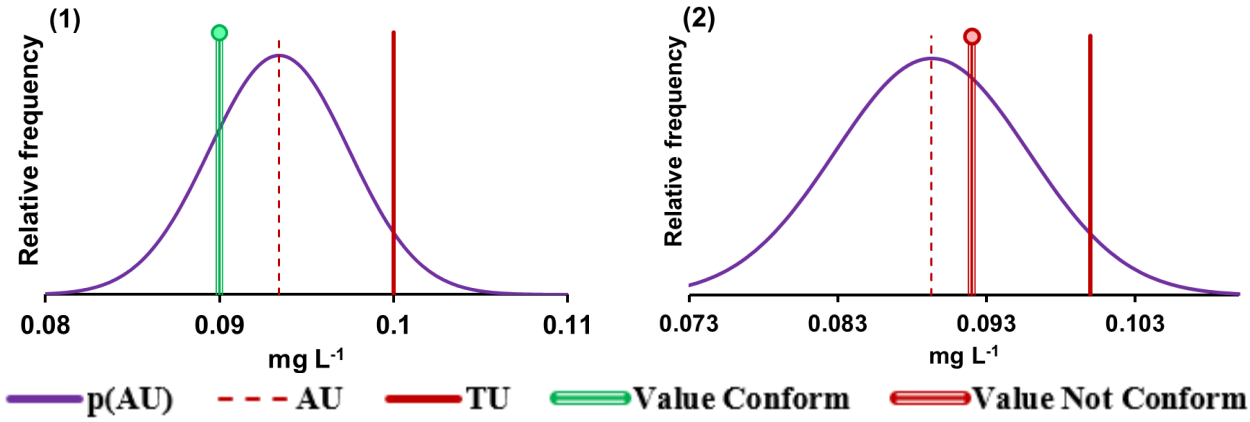
The highly precise region for the OLS regression is at the centroid, at a concentration 17.6917 mg kg^{-1} (Figures 1(A) and 1(C)) for sulfur determination, and at 0.5 mg L^{-1} (Figures 2(C) and 2(E)) for cadmium determination, however none of these ACCs are homoscedastic, but heteroscedastic, the IR standard deviations for each calibration level are different among them. Also, in the case of the sulfur determination, the calibrator's concentrations design of the ACC is not equally spaced, making the centroid not the middle of the calibrated range. Both the heteroscedasticity and the not equally spaced calibrators design dislocate the more precise region of the ACC from its middle point towards the region with a higher density of measured calibrators and with lower IR repeatability uncertainties (standard deviations), which appears to be obvious.

The highest precise region for the WLS regression is below the barycenter, which is generally near the lowest concentrations' levels, at concentration 1.5239 mg kg^{-1} (Figures 1(B) and 1(D)) for sulfur determination and at 0.23 mg L^{-1} (Figures 2(D) and 2(F)) for cadmium determination, when the IR are increasingly heteroscedastic. This behavior becomes clearer when looking for the dependence of the prediction standard uncertainty, $u(c_{\text{pred}})$, against the predicted concentration for both the OLS (Figures 1(C) and 2(E)) and WLS (Figures 1(D) and 2(F)) regression.

To design an ACC owing to a fit for purpose prediction uncertainty, it is necessary to consider not only the expected analyte concentration in the sample test but also prior information concerning the precision (uncertainties) of the IR, $u(\text{IR}_{\text{std}i})$ at the different calibrator concentrations. Finally, if the calibrator concentrations, $u(c_{\text{std}i})$, uncertainties are not negligible, at least $3b$ x $u(c_{\text{std}i}) \leq u(\text{IR}_{\text{std}i})$ for all the calibration levels for a rectilinear ACC (see the equation 23 at the bibliographical reference: Magalhães, W. F. "Cálculo de Incerteza de Grandezas Obtidas por Regressão pelos Métodos de Mínimos Quadrados de Modelos Físico-Químicos Linearizados, uma Abordagem Estatística e Metrológica". *Revista Virtual de Química* **2020**, *12* (5) 1507. Available at: https://rvq.sbq.org.br/), the univariate OLS or WLS treated here cannot be used, but a bivariate least squares (BLS) regression, as performed by the CCC Software.[27] In this case, also the behavior of the calibrator's concentrations uncertainties will contribute to determining the highest precise region for the bivariate regression of the ACC.

Must the measurement uncertainty arising from sampling in chemical analyses be neglected?

Let us think about the determination of the manganese molar concentration in freshwater. Based on an elemental analysis technique ICP-MS, a test result of 0.090 mg L^{-1} complies with the Brazilian specification limit of 0.100 mg L^{-1}?[29] Considering two scenarios. (1): the analytical expanded uncertainty with a coverage factor k = 2 for nearly 95% of coverage probability is 0.008 mg L^{-1}, and (2) the analytical expanded uncertainty, including the sampling uncertainty, is 0.013 mg L^{-1}. Figure 7 shows the upper acceptance limits for the two cases.



p(AU) – probaility density function centered at the upper acceptance limit; AU – Upper acceptance limit; TU – Upper tolerance limit.

(1) Based only on the analytical uncertainty: AU = 0.1 – 0.008/2 x 1.64 = 0.09344 > 0.090 the test result (conform).

(2) Based on the analytical uncertainty plus the sampling uncertainty: AU = 0.1 − 0.013/2 x 1.64 = 0.08934 <0.090 the test result (non-conform).

Where 1.64 is the one-tail normal z-score for a "specific consumer's risk"[30] or a level of significance of 5%.

Figure 7. Risk assessment in manganese molar concentration in freshwaters.

Here, the uncertainty information in compliance assessment was used, considering the guard band concept to be a "specific consumer's risk" of 5%.[30] This measurand was considered compliant based on scenario (1), but not compliant based on scenario (2).

Is the laboratory precision the measurement uncertainty of the analytical result?

A requisite of the laboratory quality systems, as ISO/IEC 17025:2017[18] is the determination during the analytical procedure validation of the "intermediate precision",[1] often called "laboratory reproducibility", "internal reproducibility", "in-house reproducibility", etc. Chemists generally consider this validation performance characteristic as the analytical result measurement uncertainty. Although it is true that the intermediate precision is one of the highest uncertainty sources, it competes with the sampling uncertainty and the recovering uncertainty for this position. Finally, to guarantee metrological traceability, it is important to combine these three previous sources of uncertainties with those ones due to mass, volume measurements and that one due to analytical instrument calibration. This uncertainty combination can be performed using the LPU according to the well-known BIPM[23] or Eurachem/CITAC[24] guides or using a numerical approach like the Monte-Carlo[31] or Kragten[24] methods.

BRAZILIAN JOURNALS

A search in the SCOPUS database was carried out using the terms "metrology", and more specifically "uncertainty", in the article title, abstract and keywords during the years 2001 to 2022, considering the Brazilian journals: Brazilian Journal of Analytical Chemistry (BrJAC), Eclética Química (Ecletica), Journal of the Brazilian Chemical Society (JBCS), Química Nova (QN) and Revista Virtual de Química (RVQ). Few works were found, Figure 8. The authors supposed that this could indicate that the Brazilian chemists are not very familiarized with these knowledges, what justifies its teaching in the university's courses related with chemical analysis.

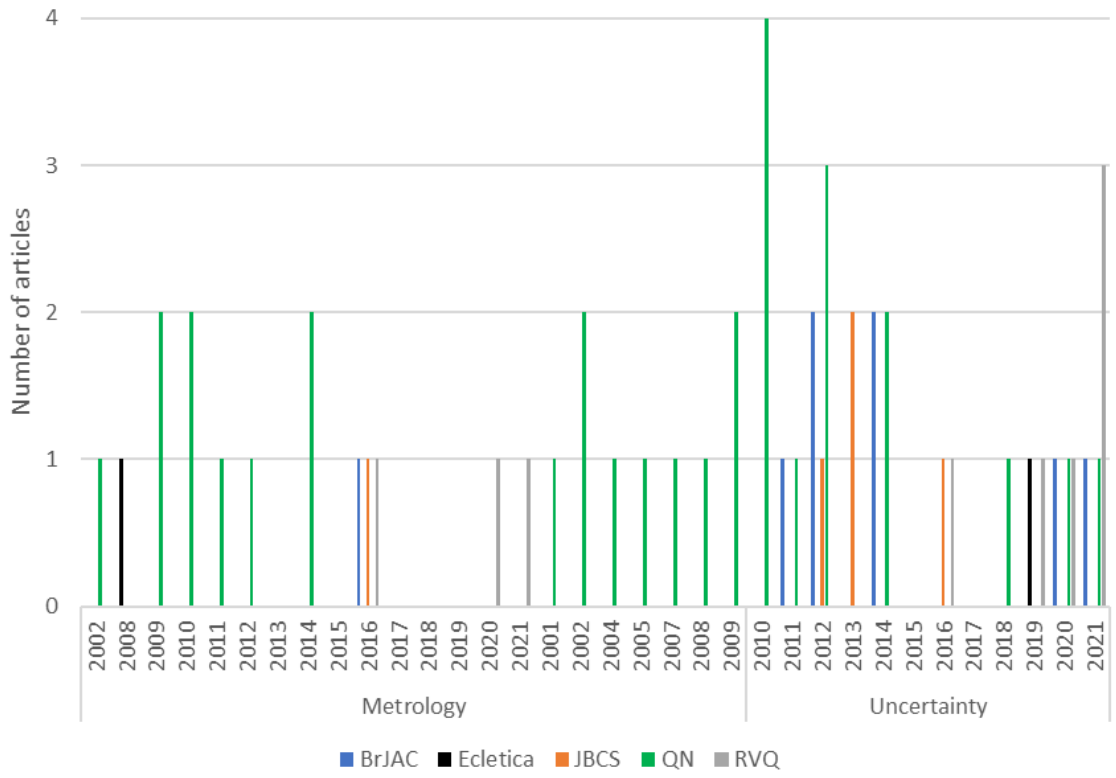


Figure 8. Profile of metrological academic production in Brazilian chemistry journals.

UNDERGRADUATE COURSES

Brazil

Looking at the syllabus design of the first hundred Brazilian Chemistry undergraduate courses according to the "Folha de São Paulo" University Ranking of 2019,[32] only nineteen courses offered in the last years a discipline where some aspects of chemical metrology, as measurement uncertainty, analytical measurement procedure validation, quality control, quality guaranty, quality management or ISO 17025 are treated in their syllabuses our courses programs or bibliographies, as disponible in their course's sites (Table VII). There are only two pos-graduation programs on metrology in Brazil: (i) from the National Metrology Institute (INMETRO),[33] and (ii) from the Pontifical University Catholic of Rio de Janeiro – PUC-Rio,[34] both at the Rio de Janeiro city, RJ, Brazil.

Table VII. Brazilian chemistry undergraduate courses that offered disciplines concerning metrology concepts in the last years

i	RUF Rank	University	University URL	Further information
1	1	USP	www.usp.br	https://uspdigital.usp.br/jupiterweb/obterDisciplina?sgldis=7500077&codcur=75014&codhab=200 https://uspdigital.usp.br/jupiterweb/obterDisciplina?sgldis=7500062&codcur=75014&codhab=200 https://uspdigital.usp.br/jupiterweb/obterDisciplina?sgldis=7500078&codcur=75014&codhab=500
2	2	Unicamp	www.unicamp.br	https://www.iqm.unicamp.br/arquivos/QA851%20-%20Valida%C3%A7%C3%A3o%20de%20M%C3%A9todos%20Anal%C3%ADticos.pdf

(continues on the next page)

Table VII. Brazilian chemistry undergraduate courses that offered disciplines concerning metrology concepts in the last years (continuation)

i	RUF Rank	University	University URL	Further information
3	3	UFRJ	www.ufrj.br	https://siga.ufrj.br/sira/repositorio-curriculo/ListaCursos.html?_ga=2.221801368.1809578526.1658765001-55904377.1658765001&_gl=1*wx8kso*_ga*NTU5MDQzNzcuMTY1ODc2NTAwMQ..*_ga_S9CWPVF04S*MTY1ODc2NTAwMC4xLjEuMTY1ODc2NTA3Ni4w
4	4	UFMG	www.ufmg.br	https://www2.ufmg.br/quimica https://www2.ufmg.br/quimica/quimica/Home/Cursos/Bacharelado https://www2.ufmg.br/quimica/quimica/Home/Cursos/Bacharelado
5	6	UFRGS	www.ufrgs.br	http://www.ufrgs.br/ufrgs/ensino/graduacao/cursos/exibeCurso?cod_curso=343
6	7	UNESP	www.unesp.br	https://www.iq.unesp.br/#!/graduacao1260/cursos/bacharelado-em-quimica/ https://www.iq.unesp.br/#!/graduacao1260/programas-de-ensino/ https://www.fc.unesp.br/Home/Departamentos/quimica201/gradescurriculares/bacharelado-em-quimica-tecnologica.pdf https://www.ibilce.unesp.br/Home/Graduacao450/quimica/quibqa2019.pdf
7	10	UFSC	www.ufsc.br	https://quimica.paginas.ufsc.br/files/2014/03/PPC_QMC_Bacharelado_2021.1-1.pdf https://quimica.paginas.ufsc.br/files/2014/03/PPC_QMC_Tecnol%C3%B3gica_2021.1-1.pdf
8	11	UFF	www.uff.br	https://www.uff.br/?q=curso/quimica/312700/bacharelado/niteroi https://app.uff.br/iduff/consultaMatrizCurricular.uff
9	12	UFPE	www.ufpe.br	gradquimica@ufpe.br 55 81 2126.844
10	14	UFSM	www.ufsm.br	https://www.ufsm.br/cursos/graduacao/santa-maria/quimica/
11	25	UNEB	www.uneb.br	https://dcv1.uneb.br/wp-content/uploads/2021/06/ESTRUTURA-CURRICULAR-farmacia.pdf
12	33	UFPB	www.ufpb.br	http://www.ufpb.br/graduacao/quimicacca
13	37	UNIFESP	www.unifesp.br	
14	39	UFES	www.ufes.br	https://quimica.vitoria.ufes.br/pt-br/grade-curricular
15	45	IFRJ	http://www.ifrj.edu.br/	https://portal.ifrj.edu.br/cursos-graduacao/licenciatura-quimica-nilopolis https://portal.ifrj.edu.br/cursos-graduacao/licenciatura-quimica-duque-caxias
16	50	UFAM	http://www.ufam.edu.br/	
17	52	PUC-Campinas	http://www.puc-campinas.edu.br/	https://www.puc-campinas.edu.br/graduacao/quimica/
18	53	UEPB	http://www.uepb.edu.br/	

(continues on the next page)

Table VII. Brazilian chemistry undergraduate courses that offered disciplines concerning metrology concepts in the last years (continuation)

i	RUF Rank	University	University URL	Further information
19	82	IFMA	http://www.ifma.edu.br/ https://portal.ifma.edu.br/inicio/	https://acailandia.ifma.edu.br/cursosofertados/ https://caxias.ifma.edu.br/cursosofertados/quimica/ https://montecastelo.ifma.edu.br/licenciatura-em-quimica/ https://zedoca.ifma.edu.br/cursosoferecidos/licenciatura-em-quimica/

Portugal

The research was based on websites of the Portuguese society of chemistry, physics, biology, for possible courses in metrology, at universities, and found only five universities that offer metrology in undergraduate courses related to the technological area, Table VIII.

Table VIII. Undergraduate courses in Portugal with metrology disciplines

University	Course	Curricular unit	Six-monthly workload
Universidade Nova de Lisboa	Engineering and Industrial Management	Metrology and Measurement System	38 hours
Universidade de Aveiro	Engineering Sciences	Metrology	60 hours
Universidade de Coimbra	Applied Physics	Metrology	Not available
Universidade da Beira Interior	Engineering and Industrial Management	Instrumentation, Automation and Control	30 hours
Universidade de Évora	Instrumentation Engineering and Metrology		

PROPOSED SYLLABUS FOR A METROLOGY COURSE

Motivation: To propose a knowledge base to implement a metrology discipline, including uncertainty calculation in the curriculum of undergraduate courses in which analytical chemistry is applied;

Name suggestions: Chemical Metrology, Metrology applied to Analytical Chemistry;

Target audience: Undergraduate students in the areas of chemistry, chemical engineering, food engineering, pharmacy, and related areas;

Half-year working hours: 60;

Main objectives: To know the basic concepts of metrology, probability and statistics. Know the main references of the JCGM, International Metrology Vocabulary (VIM) and International Guide to the Expression of Measurement Uncertainty (GUM). Build measurement models. Estimate the result of a measurand with measurement uncertainty. Apply uncertainty as a criterion in decision-making. Use and choose certified reference materials (CRM). Validate analytical measurement procedures. Critically evaluate all previous steps.

Complete Program

1. Objectives of Metrology and Basic Industrial Technology: Standardization and Conformity Assessment, intellectual and industrial property, and management techniques. Purpose of the TIB. TIB as a tool for competitiveness and innovation. Risk management in commercial relationships. Intellectual property (trademarks and patents).
2. International Vocabulary of Metrology – Basic and. General Concepts and Associated Terms (VIM) concepts and definitions: Quantities and their properties. Quantity. Unit of measurement. Unit system. International System of Units. Value of quantity. Measurement. Metrology. Measuring. Measuring principle. Measurement method. Measurement procedure. Measurement result. Metrological traceability. True value of quantity. Measurement accuracy. Measurement accuracy. Measuring accuracy. Measurement error. Systematic error. Measurement bias. Random error. Measurement repeatability condition. Intermediate precision condition. Reproducibility condition. Measurement uncertainty. Type A assessment of measurement uncertainty. Type B assessment of measurement uncertainty. Standard uncertainty. Combined standard uncertainty. Relative standard uncertainty. Expanded measurement uncertainty. Coverage probability. Scope factor. Scope range. Calibration. Validation. Measurement model. Measuring function. Input quantity in a measurement model. Output quantity in a measurement model. Correction.
3. Random variable: Descriptive measures and graphical representations. Continuous random variable. Position and dispersion parameters. Probability distributions. Interval estimation.
4. Least Squares Linear Regression: Assumptions of the Least Squares Method. Cochran test. Estimation of regression parameters. Analysis of the quality of fit. Confidence curves and forecast limits.
5. Linear regression for a second-degree polynomial: Estimation of parameters and matrix of variances and covariances. Estimation of the predicted value and its uncertainty.
6. Special Topics: Analytical calibration curve (ACC) with significant uncertainty in standards. Law of propagation in matrix form. Evaluation of interlaboratory programs. Monte Carlo simulation and Bayesian approach.
7. Measurement model: Measurement function. Expectation and variance properties of a function of random variables. First-order Taylor series approximation of the variance of a function of random variables (law of propagation of uncertainties – LPU).
8. Input quantities: Estimation of position and dispersion of an input quantity. Treatment of outliers (Dixon, Grubbs and Chauvenet tests). Estimation of the standard uncertainty of an input quantity (Type A and Type B). Degree of freedom of an input quantity.
9. Sensitivity coefficients and uncertainty contributions: partial derivatives of input quantities. Uncertainty contributions to variance. Covariance uncertainty contributions.
10. Estimation of the combined standard uncertainty: Application of the Law of Propagation of Uncertainties to the measurement model. Estimation of input quantities. Estimation of the uncertainty of the output quantity by both strategies “Bottom-up” and “Top-down” uncertainty evaluations.
11. Estimate of expanded uncertainty: Scope factor. Coverage probability. t-Student distribution. Equation of the effective degree of freedom. Statement of result with expanded uncertainty.
12. Calibration and Metrological Traceability: Concepts of calibration and metrological traceability of the measurement result. Calibration measurement templates. Applying calibration correction and recovery correction to the measurement result.
13. Reference material and analytical validation: Reference material and certified reference material. Homogeneity and stability tests. Purpose of analytical validation. Concepts of accuracy and veracity in analytical validation. Analytical quality control.

14. Analytical validation parameters: Concepts and applications of accuracy, veracity, precision, selectivity, specificity, working range and quantification limits, decision, and detection limits. Validation planning. Declaration of results.
15. Decision Limit and Decision Rule: Normative limit and reference values. Calculation of the Decision Limit under the assumption of normality. Construction of the Decision Rule. Detection limit.
16. Interlaboratory Trials or Programs: Proficiency Trials (PT) and Collaborative Trials.

Resource Suggestion

Hours distributed in theoretical classes and practical classes in a computer lab with programming in spreadsheet and R software.

Internet sites: (links between <>)

- Agência Nacional de Vigilância Sanitária - Anvisa: <https://www.gov.br/anvisa/pt-br>
- Anvisa, Validação, RDC Nº 166, DE 24 DE JULHO DE 2017 validação de métodos analíticos: <http://antigo.anvisa.gov.br/documents/10181/2721567/RDC_166_2017_COMP.pdf/d5fb92b3-6c6b-4130-8670-4e3263763401>
- Bureau Internacional de Pesos e Medidas – BIPM: <https://www.bipm.org/en/home>
- Carta de nuclídeos, isótopos, isótonos, radionuclídeos, decaimentos radiotativos, etc: <https://www.nndc.bnl.gov/nudat3/>
- Constants, Units and Uncertainty: <https://physics.nist.gov/cuu/Constants/index.html>
- Documentos para acreditação no INMETRO: <http://www.inmetro.gov.br/credenciamento/organismos/doc_organismos.asp?tOrganismo=CalibEnsaios>
- Eurachem: <https://eurachem.org/>
- Euramet: <https://euramet.org/>
- Fundamental Physical Constants: <https://www.nist.gov/pml/fundamental-physical-constants>
- Guias da Eurachem sobre validação, incerteza, qualidade, acreditação, teste de proficiência, rastreabilidade e materiasi de referência: <https://eurachem.org/index.php/publications/guides>
- INMETRO: <https://www.gov.br/inmetro/pt-br>
- IUPAC: International Union of Pure And Applied Chemistry <https://iupac.org/>.
- JCGM Publications: Guides in Metrology: <https://www.bipm.org/en/committees/jc/jcgm/publications>
- Ministério da Agricultura, Pecuária e Abastecimento – MAPA: <https://www.gov.br/agricultura/pt-br/>
- NIST Livro de Química na Web: <https://webbook.nist.gov/chemistry/>
- National Institute of Standards and Technology – NIST: <https://www.nist.gov/>
- Physical Reference Data: <https://www.nist.gov/pml/productsservices/physical-reference-data>
- Redes metrológicas no Brasil: São Paulo, REMESP: <https://www.remesp.org.br/>
  Minas Gerais, RMMG: <https://www.rmmg.com.br/>
  Rio grande do Sul, <https://redemetrologica.com.br/>
- Sociedade Brasileira de Metrologia – SBM: <https://metrologia.org.br/wpsite/>

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CONCLUSIONS

As demonstrated through this work, the knowledge of metrological and statistical concepts, as well as their correct use, is paramount to leads for analytical results that could enable risk-based decision-making. It is shown that the incorrect use of the regression techniques has little effect on the analyte sample test predicted concentration; however, its measurement uncertainty can be completely different according to the used regression techniques. Contrarily, the common sense of the presented examples of analytical calibration curves shows that most of the analytical instruments based on electromagnetic and ionization radiation detection lead to heteroscedastic analytical instruments responses, and its calibration curve data should be fitted using the weighted least squares regression or the bivariate regression, as performed by the free CCC Software. To increase students' interest in statistics subjects, it is strongly recommended that examples of applications from routine chemical work be used. As future work, a critical assessment of the financial impact on the economy resulting from the correct use of data processing in Analytical Chemistry vis-à-vis the pitfalls discussed here is recommended.

Supplementary Material

The tabs "Data", "Var_x" and Var_y" in the Excel file are to be used to perform regressions using the CCC Software as explained by its "User manual (for Release 1.3)" available at the INRIM site.[27] The tabs "Cd Expl A5 QUAM OLS" and "Cd Expl A5 QUAM WLS" are spreadsheets with the OLS and WLS regressions to fit a straight line on the calibration curve data for Cd determination. These spreadsheets can be used to fit straight lines on other calibration curves. The following four tabs with names stating "CCC" have the results from CCC Software V. 1.3 for OLS or WLS rectilinear or parabole fit and some calculations in grey cells for the adapted Example A5 QUAM for Cd determination. The final four tabs have data to be pasted on the first three tabs of the file to use the CCC Software to fit the calibration curves of Cd or sulfur determinations.

This supplementary material can be accessed at the following doi: 10.30744/brjac.2179-3425.RV-98-2023-supp-material.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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