STATISTICAL ANALYSIS

Chemometric Protocol to Validate an Analytical Method in the Presence of Corrigible Constant and Proportional Systematic Errors

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A statistical methodology to verify the trueness of an analytical method in the presence of corrigible systematic errors is presented. This protocol enables detection of constant and proportional components of error. By using the data set obtained in the Youden calibration with different sample test portions, the constant component of the error (Youden blank) can be determined. An analysis of covariance was applied to 3 calibration curves established with standard solutions and with standard additions to 2 different sample test portions. The slopes were compared, and the presence of any matrix–analyte interaction was detected. A method for removing the numerical components of systematic errors is proposed: a calculation procedure to obtain a correct analytical result and a statistical test to verify the correctness of analyte contents obtained from different calibrations. For demonstration purposes, the protocol was applied to spectrofluorometric determination of oxalates in spinach leaves.

In analytical methodology, results are always affected by error, which has 2 components: systematic and random. Random error is ever present, and its determination, based on repetition of measurements and simple statistical treatment, enables a confidence interval to be established for the analytical result. Systematic error can be detected only when the result is different from the true value and lies outside the confidence interval determined by random error. Wilson (1) identified 4 types of error: (*1*) in the calibration process or the measurement system (for example, the use of standards that absorb humidity); (*2*) direct interference from the sample matrix, defined by Cardone (2) as any substance within the sample matrix that provokes a response that depends on the size of the sample and is greater or less than that due solely to the analyte, thus producing an unacceptably large systematic error; (*3*) constants, due to response variations that are not attributable to the analyte and are independent of sample size; and (*4*) proportional, due to response variations that depend on the matrix–analyte interaction and are proportional to the matrix–analyte relation present.

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The sources of errors (*1*) and (*2*) are clearly distinct from those of (*3*) and (*4*), because no statistical procedure is available to detect the former. It is also not possible to apply corrective techniques to eliminate errors (*1*) and (*2*) from the analytical result. On the contrary, the errors (*3*) and (*4*) may be detected by diagnostic statistical techniques and the resulting value may be used to correct analytical results.

To test the validity of an analytical method, it is necessary to determine its trueness by evaluating whether it is free of systematic errors and by checking that results are not significantly different from the true value. If suitable reference materials or methods are not available, it is not possible to test the validity of the method directly. It is then necessary to resort to other methodologies, such as the standard additions method (2–8).

The requirement for correct use of this methodology is the absence of systematic errors of types (*1*) and (*2*); that is, errors cannot be corrected.

In a previous study, we developed a statistical protocol to test the accuracy of a method by using standard additions (9). The use of this method was based on the absence of proportional systematic errors in the procedure; the presence of constant errors did not prevent its application. Three calibration curves were established: one with standard solutions; one with standard additions to a constant sample volume; and a Youden calibration, obtained from continuous sample variations. The absence of proportional systematic errors was confirmed by observing that no significant differences were observed in the slope of the calibration curve established with standard additions with respect to the slope of the calibration curve established with standard solutions. A *t*-test was applied to compare calibration slopes; the parallel lines showed the absence of matrix–analyte interaction.

Nevertheless, when this type of error is present, it is still possible to use standard additions methodology by measuring the saturation state of the interactive effect (7), that is, in a situation where a constant error level is obtained independently of the matrix–analyte relation, as described by Tyson (10). To test this saturation of the interactive effect, 2 standard additions curves, with different sample portions, are established and the slopes are examined to determine whether they are equal. By

this procedure, the matrix–analyte relation is modified and we observe whether the effect provoked is similar for the 2 cases (11).

This paper proposes a rigorous methodology to validate an analytical result by using the standard additions method in the presence of both constant and proportional systematic errors. The protocol describes a statistical method that detects both types of error and, when proportional errors exist, seeks a complete interval of sample portions for which saturation of the matrix–analyte interactive effect is obtained. It thus avoids the effects of this interference and arrives at a correct analytical result for the selected experimental region. The method, once validated, can be used in routine analysis with the standard solution additions method, provided that the sample concentration introduced into the calibration curve lies within the saturation interval of the matrix–analyte interactive effect.

Experimental

Three experiments are required to obtain the first data set necessary to check the presence of systematic errors. In each one, the same analytical procedure is applied:

(a) *Standard calibration (SC).*—Established from several replicates of different analyte standard solutions, including the blank as an additional value. From the data set obtained, the performance characteristics of the analytical method can be determined (12).

(b) *Youden calibration (YC).*—With the Youden method (13), a calibration curve is established by using increasingly larger portions from a treated sample solution. In this curve, the value corresponding to sample portion "zero" is not included. Replicates are not necessary.

This calibration must be performed before those established with standard additions. When there is no evidence of the content of the analyte in the sample, the curve of such a calibration, together with the analytical signal values corresponding to the sample quantity, provides an indication of which sample portions may be used for analysis without risk of insufficient reagents. Furthermore, with this calibration, it is possible to select 2 sample portions that are suitable for establishing the standard additions calibrations, taking into account that the total quantity of analyte in each addition (sample content plus added content) must not create an analytical signal greater than that corresponding to the highest concentration used to establish the standard solution calibration. Otherwise, there would be no certainty of working within the zone of linearity.

(c) *Standard additions calibrations (AC1 and AC2).*—Two calibrations can be obtained by the addition of continuous variations of the standard solution at 2 constant, different sample volumes and including the value of "zero" addition. It is not necessary to perform replicates for each addition. The sample portion and addition intervals should be as wide as possible to ensure the absence or the saturation of the interactive matrix–analyte effect.

By applying linear regression least-squares analysis, the slope, the intercept, and the regression standard deviation for each curve are calculated.

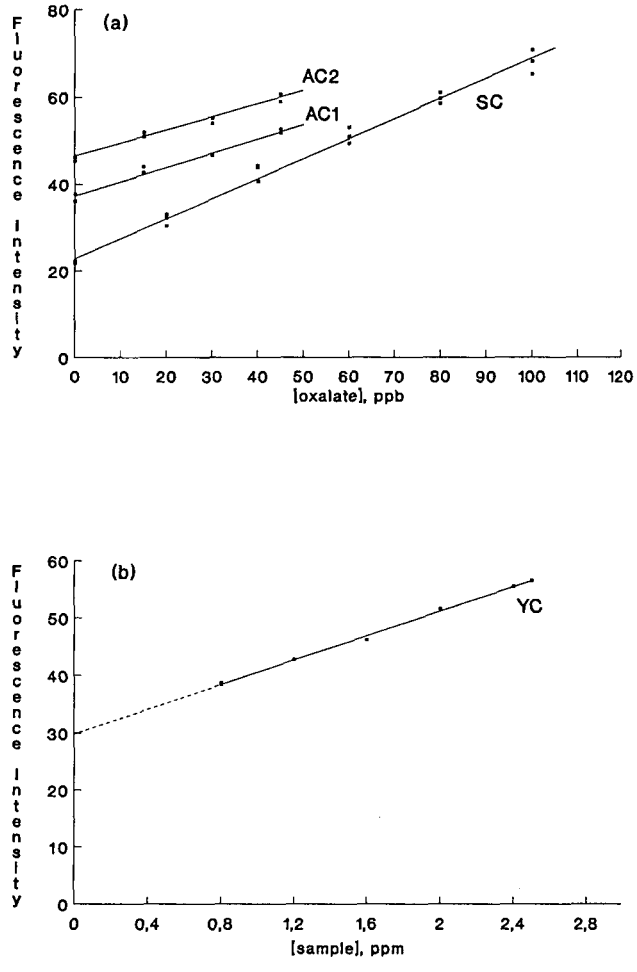

Figure 1. Different calibration curve plots: (a) SC, standard calibration; AC1 and AC2, standard additions calibrations with sample portions of 0.8 and 1.6 μg/mL; (b) YC, Youden calibration.

Statistical Procedure

The curves of the 3 different calibrations (standard calibration [SC], and standard additions calibrations with different sample portions [AC1, AC2]) can be represented by 4 spatial dispositions: (*1*) The 2 standard additions calibrations are displaced parallel to the standard calibration (i.e., there is a constant or translational bias in the selected sample portion interval); (*2*) one standard additions calibration is displaced parallel to the other, but the 2 curves have slopes that are different from that of the standard calibration (i.e., there is a proportional or rotational bias, but the matrix–analyte saturation effect is achieved); (*3*) only one standard additions calibration is displaced parallel to the standard calibration, whereas the other 2 curves have a different slope (there is a constant bias for the sample portion corresponding to the first calibration, and analytical results free from proportional error can be obtained only if this sample portion is used); and (*4*) the 3 curves have different slopes (i.e., there is a combination of both error types) (2).

To test the similarity between the slopes, a statistical technique known as analysis of covariance (ANCOVA) is used. This technique combines the aspects of variance and regression analysis, and among its many uses is the comparison of regression lines (14). Here we are exclusively interested in the joint comparison of the calibration slopes with standard solutions (SC) and standard additions calibrations (AC1 and AC2). The mathematics model used is based on the equation:

$$R_{ij} = \alpha_i + \beta_i c_{ij} + \varepsilon_{ij}$$

where $i = 1, 2, ..., r$ indicates each of the calibration curves; $j = 1, 2, ..., n_i$ is each of the experimental observations; α is the intercept; β is the slope; and ε is the normally distributed error of the model. The statistic test that should be done establishes a null hypothesis:

$$H_0 \equiv \beta_1 = \beta_2 = \ldots = \beta_r.$$

In this procedure, it is assumed that residual variances are homogeneous for all calibrations established and that the regression standard deviation depends only on the value of the analytical signal (15). The realization of this test implies a series of steps:

(a) *Initial calculations.*—When calibrations have been obtained, application of ANCOVA requires establishing a series of corrected sums of squares and products for each individual regression that represent deviations with respect to average values for concentrations (c) or analytical signals (R). The definition and brief expression of these, for purposes of calculation, may be represented, in simplified form, by the following equations:

$$(cc) = \sum(c_i - \bar{c})^2 = \sum c_i^2 - \frac{(\sum c_i)^2}{n} \quad (1)$$

$$(RR) = \sum(R_i - \bar{R})^2 = \sum R_i^2 - \frac{(\sum R_i)^2}{n} \quad (2)$$

$$(cR) = \sum(c_i - \bar{c})(Ri - \bar{R}) = \sum c_i R_i - \frac{\sum c_i \sum R_i}{n} \quad (3)$$

(b) *Regression deviations: sums of squares and means of squares of residuals.*—In each regression, the sum of the squares (SS) of the residuals represents the part of the total variability of the data set around the slope that is not explained by regression, depending instead on other variables and influences not considered in the linear model. The calculation is performed by using the following equation:

$$SS = (RR) - \frac{(cR)^2}{(cc)} \quad (4)$$

The means of squares (MS) of the residuals are obtained by dividing the sum of the squares by the degrees of freedom (DF) for each regression curve

$$MS = \frac{SS}{n-2} \quad (5)$$

where n is the total number of pairs of data for each calibration.

(c) *Full regression deviations: sum of squares and full mean of squares of residuals.*—Considering the 3 calibrations to be independent regressions, it is possible to obtain the full degrees of freedom as the sum of the degrees of freedom of each calibration and the full sum of squares, which is calculated by summing the sums of squares of each individual regression curve

$$\text{full DF} = (n_S - 2) + (n_{A1} - 2) + (n_{A2} - 2) = (n_S + n_{A1} + n_{A2} - 6) \quad (6)$$

$$\text{full SS} = (SS)_S + (SS)_{A1} + (SS)_{A2} \quad (7)$$

where n_s, n_{A1}, and n_{A2} represent the number of pairs of values for each calibration curve, respectively. The full mean of squares is obtained by dividing the full sum of squares by the full degrees of freedom

$$\text{full MS} = \frac{\text{full SS}}{\text{full DF}} \quad (8)$$

(d) *Reduced regression.*—By summing all the sums of squares and regression products, it is possible to establish the deviations of a model in which all the data of the different calibrations fit a single curve with slope, b_p, which is obtained by the following expression:

$$b_p = \frac{(cR)_S + (cR)_{A1} + (cR)_{A2}}{(cc)_s + (cc)_{A1} + (cc)_{A2}} \quad (9)$$

The reduced sum of squares is obtained from Eq. 4, in which each term has now been obtained from the sum of the corresponding terms of each individual regression, according to Eq. 10:

$$\text{reduced SS} = (RR)_S + (RR)_{A1} + (RR)_{A2} - \frac{[(cR)_S + (cR)_{A1} + (cR)_{A2}]^2}{(cc)_S + (cc)_{A1} + (cc)_{A2}} \quad (10)$$

and the reduced degrees of freedom, as a single pooled slope obtained for the 3 calibrations, are:

$$\text{reduced DF} = [(n_S - 1) + (n_{A1} - 1) + (n_{A2} - 1) - 1] = n_S + n_{A1} + n_{A2} - 4 \quad (11)$$

The reduced residual mean of squares, considering a model to fit a single slope, is obtained from the following expression:

$$\text{reduced MS} = \frac{\text{reduced SS}}{\text{reduced DF}} \quad (12)$$

(e) *Comparison of slopes.*—An F-test comparing 2 means of squares was performed to detect whether significant differences exist between the slopes of the 3 calibration curves. The

statistic used with ($k-1$, full DF) degrees of freedom was calculated according to the following expression:

$$F_{cal} = \frac{s_N^2}{s_D^2} \quad (13)$$

s_N^2 is the mean of squares calculated as the difference between the full sum of squares and the pooled sum of squares, with ($k-1$) degrees of freedom (k is the number of slopes to be compared). This variance measures the contribution of the difference observed between the slopes of the 3 calibration curves to the sum of squares of the deviations from the linear model. s_D^2 is the full mean of squares, obtained by Eq. 8 and with (full DF) degrees of freedom. This denominator measures the contribution of the deviations from the linear model, considering the calibrations to be independent (the variance within each curve). The calculation of the 2 is performed by using Eq. 14 and Eq. 15:

$$s_N^2 = \frac{(\text{reduced SS}) - (\text{full SS})}{k-1} \quad (14)$$

$$s_D^2 = (\text{full MS}) \quad (15)$$

(e.1) *Equal slopes (existence of a constant systematic error).*—The null hypothesis usually cannot be rejected for a significance level that is >5%. This means that the deviations introduced into the model by the differences existing between the slopes compared do not exceed the total random error of the calibration curves obtained independently (Eq. 15). It is thus concluded that there is no significant difference between the slopes of the 3 calibration curves. It may be deduced that there is no proportional systematic error component, at least if the method is applied within the interval of the sample portion under study (the maximum sample portion that may be used is given by the greatest sample concentration used in calibration by standard additions).

In this case, the pooled slope, b_p, obtained from Eq. 9, may be considered a representative value for the 3 calibration curves (with standard solutions and with standard additions). An estimated value of the analyte content of the sample can be obtained by dividing the corresponding slopes of the Youden calibration and the standard calibration curves (9).

From the value used for the slope, new intercepts of the addition curves A1 and A2 are calculated by using Eq. 16:

$$a'_A = \bar{R} - b_p \bar{c}_A \quad (16)$$

(e.1.1) *Estimation of constant error component: Youden blank.*—A difference between the intercepts of the curves SC and YC, a_S and a_Y, indicates the existence of a bias component due to the sample matrix effect. If a_Y is not included in the confidence interval value of a_S (9), the constant bias component, the total Youden blank, can be subtracted from the whole analytical signal to estimate the analyte content of the solution.

(e.1.2) *Check of trueness.*—This is performed by comparing the analyte content of the solution obtained from the standard calibration (SC) and that obtained from the standard additions calibration with the largest sample portion (AC2; to evaluate the effect of the maximum sample portion used). The analyte content in the solution, $c_{x,S}$, is calculated from the SC calibration, by applying the calibration equation with the intercept and slope values, as indicated in Eq. 17:

$$c_{x,s} = \frac{R_x - a_Y}{b_p} \quad (17)$$

where the analyte content calculated from AC2 calibration, $c_{x,A2}$, is obtained through Eq. 18:

$$c_{x,A2} = \frac{a'_{A2} - a_Y}{b_p} \quad (18)$$

Both values can be checked by using a statistic with $n_S + n_{A2} - 3$ degrees of freedom (there is an additional DF because the slope is the same for both curves), which is obtained by using the following expression derived from the Fieller theorem (16):

$$t(c) = \frac{\left|c_{x,s} - c_{x,A2}\right|}{\frac{s_p}{b_p}\sqrt{\frac{1}{n_S} + \frac{1}{n_{A2}} + \frac{(\bar{R}_s - \bar{R}_{A2})^2}{b_p^2[(cc)_S + (cc)_{A2}]}}} \quad (19)$$

where $\bar{R}_s$ and $\bar{R}_{A2}$ represent the means of the analytical signals for each data set of calibration curves SC and AC2, respectively, and s_p is the pooled standard deviation of regression of both calibrations, obtained from:

$$s_p = \sqrt{\frac{(n_S - 2)s_S^2 + (n_{A2} - 2)s_{A2}^2}{n_S + n_{A2} - 4}} \quad (20)$$

Using a simplified expression (Eq. 21), we obtain an approximate value that is always greater than that derived from the general expression (if the statistic is greater than the Student's t-value, it is necessary to use Eq. 19):

$$t(c) = \frac{\left|c_{x,s} - c_{x,A2}\right|}{\frac{s_p}{b_p}\sqrt{\frac{1}{n_S} + \frac{1}{n_{A2}}}} \quad (21)$$

If the null hypothesis is not rejected with a significance value of >5%, the 2 results are similar and the method is correct. This means that when the constant error component has been eliminated, the analyte content can be determined directly from the calibration curve established with standard solutions; this value is free from systematic errors, provided that the sample portion used in the analysis is equal to or less than the largest portion used in the calibration curve established with standard additions (AC2).

(e.2) *Different slopes (existence of a proportional systematic error). Comparisons by pairs.*—If the slopes are not similar, it is necessary to perform a test of comparisons by pairs to

Table 1. Analytical signals obtained to establish standard calibration (SC), standard additions calibrations (AC1 and AC2), and Youden calibration (YC)

Calibration	Concn of std analyte soln added, ng/mL	Concn of sample soln added, µg/mL	Analytical signal		
SC	0	0	22.1	21.6	21.7
	20		32.1	33.0	30.3
	40		43.7	44.2	40.5
	60		49.3	50.9	53.0
	80		58.4	59.6	60.9
	100		68.0	70.6	65.1
AC1	0	0.8		37.6	36.0
	15			43.9	42.6
	30			46.5	46.7
	45			51.6	52.5
AC2	0	1.6		46.1	45.3
	15			51.8	50.9
	30			53.9	55.0
	45			60.5	58.8
YC	0	0.8			38.6
		1.2			42.7
		1.6			46.1
		2.0			51.5
		2.4			55.4

identify which of them presents a significant variation from the others. For this purpose, the Bonferroni method (17) is used, in which all possible comparisons by pairs are made of the 3 slopes (b_S vs b_{A1}, b_S vs b_{A2}, b_{A1} vs b_{A2}). A Student's t-test is used; the expression of this for the first pair of slopes compared is:

$$t(b) = \frac{|b_s - b_{A1}|}{s_D \sqrt{\frac{1}{(cc)_S} + \frac{1}{(cc)_{A1}}}} \quad (22)$$

This expression is similar for other comparisons, because in every case the standard deviation of the denominator is that corresponding to the total mean of squares, s_D, which is a better estimator of the deviations from the linear model produced by all the calibrations. This method, with the aim of evaluating the pooled test error, uses a "penalized" Student's t-test; that is, it considers an error α divided among the q possible comparisons performed (error α/q). The null hypothesis (that the 2 slopes compared do not differ significantly) is not rejected for a level of significance of >1% (because the test with the normal value of 5% is too severe). This comparison may have 3 results:

(e.2.1) *b_s is different from b_{A1} and b_{A2}, but b_{A1} and b_{A2} are similar.*—In this case, there is an interval of sample portions for which a saturation of the matrix–analyte interactive effect has occurred. To avoid this effect, the analyte content in the measurement solutions is obtained from the 2 calibration curves established with standard additions (AC1 and AC2), as determined by Eq. 23 and Eq. 24:

$$c_{x,A1} = \frac{a'_{A1} - a_Y}{b_p} \quad (23)$$

$$c_{x,A2} = \frac{a'_{A2} - a_Y}{b_p} \quad (24)$$

In this case, a'_{A1} and a'_{A2} are calculated as in Eq. 16, except that now b_p is the pooled slope of calibrations AC1 and AC2 (*see* Eq. 9).

To obtain an acceptable estimate of the value of a_Y, using the Youden methodology (13), we can use the Youden calibration established for a particular range of sample portions, including those used to prepare calibration curves AC1 and AC2. Because replicates are not necessary, the linearity has been checked by residual analysis. In this way, it is possible to consider that this is the value of the Youden blank in the region of saturation of the interactive effect.

An approximate value for the analyte content in the sample may be obtained as in section **(e.1)**, by dividing the slope of the Youden calibration by the pooled slope of calibrations AC1 and AC2, b_p.

The analyte content of each sample portion used, $c_{x,A1}$ and $c_{x,A2}$, is then related to the final analyte concentration in the original sample, C_{A1} and C_{A2}. The intention is to test the trueness of the results, comparing the 2 values by using a t-test with $(n_{A1} + n_{A2} - 3)$ degrees of freedom, calculated according to the following equation:

$$t(c) = \frac{|C_{A1} - C_{A2}|}{\frac{s_p}{b_p}\sqrt{\frac{1}{n_{A1}} + \frac{1}{n_{A2}} + \frac{(\bar{R}_{A1} - \bar{R}_{A2})^2}{b_p^2[(cc)_{A1} + (cc)_{A2}]}}} \quad (25)$$

where s_p is the pooled standard deviation of regression of calibrations A1 and A2, obtained by:

$$s_p = \sqrt{\frac{(n_{A1} - 2)s_{A1}^2 f_1 + (n_{A2} - 2)s_{A2}^2 f_2}{n_{A1} + n_{A2} - 4}} \quad (26)$$

where f_1 and f_2 represent the respective dilution factors (numbers that multiply $c_{x,A1}$ and $c_{x,A2}$ to obtain C_{A1} and C_{A2}, the final analyte content of the original sample). The simplified expression for the $t(c)$ statistic (Eq. 27) is similar to Eq. 20, and the above-described considerations remain valid:

$$t(c) = \frac{|C_{A1} - C_{A2}|}{\frac{s_p}{b_p}\sqrt{\frac{1}{n_{A1}} + \frac{1}{n_{A2}}}} \quad (27)$$

Table 2. Parameters for SC, AC1, AC2, and YC calibrations

Parameter	AC1	AC2	SC	YC
n	8	8	18	4
a	37.31	46.05	22.81 ± 2.19	29.9
b	0.3273	0.2997	0.4604	10.60
s	0.9793	0.9164	1.7972	
b_p (SC, AC1, AC2)	0.4345			
SS	5.76	5.04	51.68	
MS	0.959	0.840	3.230	
		Initial data and F-test		
Full SS	62.48 (28 DF)			
Full MS	2.231			
Reduced SS	143.29 (30 DF)			
Reduced MS	4.776			
k	3			
s_N^2	40.40			
s_D^2	2.231			
F_{cal}	18.11[a] (P = 0.002%)			
		Bonferrani test for comparing slopes		
$t(b)$; b_S vs b_{A1}	4.017[b] (P = 0.12%)			
$t(b)$; b_S vs b_{A2}	4.849[b] (P = 0.01%)			
$t(b)$; b_{A1} vs b_{A2}	0.620[b] (P = 100%)			
		Pooled slope and Youden blank		
b_p (AC1, AC2)	0.3135			
a′	37.62	45.73		
YB				7.09

[a] Critical value, 3.340 (5%; 2, 28 DF).
[b] Critical value, 3.208 (1%; 28 DF); P, percentage of the Student's t or F distributions (P value).

The null hypothesis (the 2 results do not significantly differ) is not rejected with a level of significance that is >5%. This means the result is free from proportional systematic errors, provided the content is estimated from a calibration curve established with standard additions to a sample portion lying within the tested concentration interval. In this case, it is impossible to use the standard calibration curve (SC) to determine analyte content.

(e.2.2) *b_s is similar to b_{A1} and not to b_{A2}.*—In this situation, we may conclude that the matrix–analyte interactive effect does not exist, provided that the sample portion used in the analysis is equal to that used to establish the first calibration curve with standard additions to the smaller sample portion. In this range of concentrations, it is possible to affirm the absence of any proportional systematic error component. The analyte content may be obtained from the standard solution calibration (SC) or from the standard additions calibration (AC1); the verification of trueness is achieved by applying the expressions used in section **(e.1)**. In this case, if the analyte contents compared, $c_{x,S}$ and $c_{x,A1}$, do not differ significantly, it is possible to directly use the standard calibration (SC) for analyses, providing the sample portion is equal to that used for the AC1 calibration curve.

(e.2.3) *No pair of slopes compared presents similarity.*—This case may arise when there is a matrix–analyte interactive effect, but the experimental region in which this effect is saturated has not been localized. To find this saturation zone and to avoid a proportional systematic error, new calibrations must be performed with standard additions by using sample portions that are intermediate to those previously considered for the AC1 and AC2 calibrations. The object is to test whether, on varying the matrix–analyte relation, the saturation zone is bounded. When the new calibration has been established, the previously described statistical protocol is applied again.

Results and Discussion

To test the applicability of the statistical protocol established, it was applied to the determination of oxalates by the formation of a fluorescent ternary complex with Alizarin Red S and Zr(IV) (Aybar Muñoz et al., unpublished data). The standard calibration (SC) was obtained from the analytical sig-

Table 3. Accuracy of analyte content obtained from different calibrations

Parameter	AC1	AC2	YC
c_x, ng/mL	24.63	50.51	
f	1.250	0.625	
C, mg/g	30.79	31.57	33.81
s_p	0.9556		
t(c)[a]	0.512 (P = 61.7%)		

[a] Critical value, 2.160 (5%, 13 DF).

nal set for 3 replicates of oxalate standard solutions containing 0, 20, 40, 60, 80, and 100 ng/mL.

A solution of pretreated sample with a concentration of 0.4 mg/mL was prepared. To establish the Youden curve, sample solutions of 0.8, 1.2, 1.6, 2.0, and 2.4 μg/mL were prepared. Finally, the standard additions curves were obtained to add continuous volumes of analyte concentrations of 0, 15, 30, and 45 ng/mL to the same concentrations of sample solutions, 0.8 μg/mL (AC1) and 1.6 μg/mL (AC2). After the recommended procedure was applied, all the analytical signals were obtained. The results are presented in Table 1. Figure 1 shows the corresponding calibration curves established according to the above-mentioned procedure.

The parameters for 4 calibration curves are shown in Table 2. The constant error component is detected and must be corrected. ANCOVA shows that the 3 slopes jointly compared (b_S, b_{A1}, and b_{A2}) are significantly different, meaning that a proportional systematic error exists. The pair comparison test, applying the Bonferroni method, only concludes with the null hypothesis when b_{A1} and b_{A2} are compared, which confirms that using the selected sample portions produces a saturation of the matrix–analyte interactive effect. It is possible to determine the oxalate content in these samples from the standard additions calibrations.

Table 3 shows the analyte content of the solutions at concentrations of 0.8 and 1.6 μg/mL, obtained from the AC1 and AC2 calibrations, respectively. By using the corresponding factors, the final analyte contents in the sample are obtained, which are then compared by the t-test (using the approximated expression 27). This reveals that there is no significant difference between the 2 values and that the results obtained by using the 2 calibrations are free from systematic errors. From this study, it may be concluded that the oxalate content in this type of sample cannot be determined directly by using the calibration curve. However, if sample solutions are used with a sample concentration lying between 0.8 and 1.6 μg/mL, it is possible to determine the oxalate content by using the standard additions calibration, because there is no doubt that, within this interval, the matrix–analyte interactive effect is saturated. In every case, the Youden blank must be eliminated from all measurements.

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Glossary

AC1	Standard-additions calibration with test portion 1
AC2	Standard-additions calibration with test portion 2
a_{A1}	Intercept of AC1
a_{A2}	Intercept of AC2
a'_{A1}	Corrected intercept of AC1
a'_{A2}	Corrected intercept of AC2
a_S	Intercept of SC
a'_S	Corrected intercept of SC
a_Y	Intercept of YC
b_{A1}	Slope of AC1
b_{A2}	Slope of AC2
b_S	Slope of SC
b_Y	Slope of YC
b_p	Pooled slope
c	Concentration level
(cc)	Corrected sum of squares of concentrations
$c_{i,A1}$	Concentration of added-standard set used in AC1
$c_{i,A2}$	Concentration of added-standard set used in AC2
$\bar{c}_{A1}$	Average concentration of added standard set used in AC1
$\bar{c}_{A2}$	Average concentration of added standard set used in AC2
$c_{i,S}$	Concentration of standard set used in SC
$\bar{c}_S$	Average concentration of standard set used in SC
C_{A1}	Sample analyte concentration from A1
C_{A2}	Sample analyte concentration from A2
c_x	Solution analyte concentration
$c_{x,A1}$	Solution analyte concentration from AC1
$c_{x,A2}$	Solution analyte concentration from AC2
$c_{x,S}$	Solution analyte concentration from SC
(cR)	Corrected crossed product of sum of squares of concentrations and analytical signals
DF	Degrees of freedom
f_1	Multiplicative factor to obtain C_{A1} from $c_{x,A1}$
f_2	Multiplicative factor to obtain C_{A2} from $c_{x,A2}$
n_{A1}	Number of measurements used for AC1
n_{A2}	Number of measurements used for AC2
n_S	Number of measurements used for SC
n_Y	Number of measurements used for YC
R	Measured analytical signal
(RR)	Corrected sum of squares of analytical signals
$\bar{R}_S$	Average analytical signal for SC measurement set
$\bar{R}_{A1}$	Average analytical signal for AC1 measurement set
$\bar{R}_{A2}$	Average analytical signal for AC2 measurement set
R_i	Each analytical signal value of calibration measurement set
R_x	Sample analytical signal
SS	Sum of squares
MS	Mean of squares
SC	Standard calibration
s_{A1}	Regression standard deviation of AC1
s_{A2}	Regression standard deviation of AC2
s_S	Regression standard deviation of SC
s_Y	Regression standard deviation of YC
s_p	Pooled standard deviation
$t(b)$	Statistic for slope
$t(c)$	Statistic for concentration
YC	Youden calibration

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