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Coordinate swapping in standard addition graphs for analytical chemistry: a simplified path for uncertainty calculation in linear and nonlinear plots

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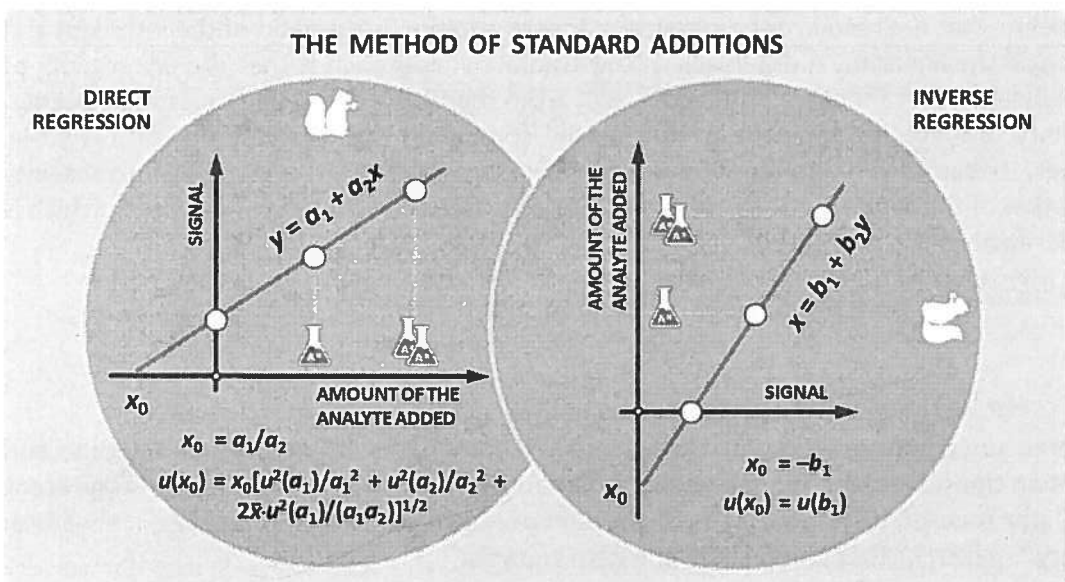
ABSTRACT

In order to simplify the data analysis from standard addition experiments, we propose *x*-*y* coordinate swapping followed by the conventional linear regression. The results of the standard addition experiment are obtained directly from the intercept of the inverse calibration approach. Consequently, the uncertainty evaluation becomes significantly simplified. The method is also applicable to nonlinear curves, such as the quadratic fit, without incurring any additional complexity.

GRAPHICAL ABSTRACT



Introduction

It is a common knowledge in analytical chemistry that the instrumental response can be affected by the sample matrix. This behaviour leads to biased results when the analytical signal produced from the sample is compared against the calibration graph prepared from matrix-free standard solutions. To overcome this problem, the method of standard additions was introduced in the 1930s.[1] To date, the method of standard addition remains a serious tool in quantitation and the best tool available when isotope dilution cannot be applied. Like any scientific tool, the method standard addition has witnessed new developments most notable of which is the concomitant use of the internal standard.[2]

To wit, if more than one addition of a standard is performed method of standard additions seeks the point with zero response (assuming all signals are corrected for blank contribution), i.e., the analytical result is

$$x_0 = -x_{y=0} \tag{1}$$

The calculation of x_0 is done by fitting of best line through the observed data $\{x, y\}$ where x is the amount of the added analyte, and y is the corresponding analytical response. For a commonly used straight line fit, $y = a_0 + a_1x$,

$$x_0 = a_0/a_1 \tag{2}$$

which means that the result of the classical standard addition is the ratio of the intercept and the slope of the linear fit. A drawback of this traditional approach is that the uncertainty of the analytical result cannot be obtained readily from the fitting parameters a_0 and a_1. Rather, uncertainty propagation must be performed by combining the uncertainties of a_0 and a_1. Moreover, because the intercept and the slope are correlated estimates, uncertainty propagation of their ratio must involve the covariance between these two estimates which is not trivial and is often neglected in practice:

$$\frac{u^2(x_0)}{x_0^2} = \frac{u^2(a_0)}{a_0^2} + \frac{u^2(a_1)}{a_1^2} - 2\frac{\mathrm{cov}(a_0, a_1)}{a_0 a_1} \tag{3}$$

where

$$\mathrm{cov}(a_0, a_1) = -\bar{x} \cdot u^2(a_1) \tag{4}$$

In general, uncertainty calculations of standard addition results are rather tedious; and treatises on the subject are riddled with mathematical equations.[3] In addition, several recent studies have focused on a more general problem of nonlinear calibration curves in analytical chemistry,[4,5] which further complicates the data analysis.

THEORY

Variable transformation for standard additions

To the annoyance of many chemists, traditional standard addition regression does not yield the analytical result directly. This problem has been noted before. Notably, Tellinghuisen has proposed an alternative approach whereby the variable-of-interest for standard additions, x_0, is cast as an explicit variable of the fitting model rather than calculated from the fitting parameters.[6,7] The method involves variable transformation from the classical $y = a_0 + a_1x$ (with $x_0 = a_0/a_1$) to $y = a_0(a_1 + x)$ which gives a direct estimate of the standard addition result, i.e., $x_0 = a_1$. Likewise, quadratic fitting of $y = a_0 + a_1x + a_2x^2$ can be done using a function $y = a_0(a_1 + x) + a_2(a_1 + x)^2$ which yields $x_0 = a_1$. Albeit simple in its concept, the variable

rearrangement from $y = a + bx$ to $y = a(b + x) = ab + ax$ leads to a nonlinear fitting problem because of the product of both variables, ab, in the model equation. As a result, no simple algebraic expressions can provide the results and iterative minimization methods must be employed, such as the Excel SOLVER macro.

Coordinate swapping

Consider a typical standard addition experiment where x is the vector of the masses of the added standard and y is the vector of the corresponding instrumental responses. Now, instead of fitting $y = a_0 + a_1x$, one performs fitting of $x = b_0 + b_1y$ which is known as the inverse calibration.[8] The advantage of the coordinate swapping becomes evident after calculation of $x_0 = -x_{y=0}$; it is equal the (negative) intercept of the inverse regression, i.e.,

$$x_0 = -b_0 \tag{5}$$

Consequently, the uncertainty of the standard addition result is the uncertainty of the intercept and no additional uncertainty propagation is necessary thus obviating the need to evaluate the covariance between parameters:

$$u(x_0) = u(b_0) \tag{6}$$

Nonlinear models

The difficulty of employing polynomial fitting in standard addition models is in large part due to the complexity of obtaining the analytical results. For example, the analytical result from a quadratic fit, $y = a_0 + a_1x^1 + a_2x^2$, is

$$x_0 = \frac{-a_1 \pm \sqrt{a_1^2 - 4a_0a_2}}{2a_0} \tag{7}$$

and the uncertainty calculation is prohibitively complex:

$$u^2(x_0) = \frac{u^2(a_0)}{z} + \frac{q^2u^2(a_1)}{4a_2^2z} + \frac{d^2u^2(a_2)}{4a_2^4z} - \frac{q\,\mathrm{cov}(a_0,a_1)}{a_2z} + \frac{d\,\mathrm{cov}(a_0,a_2)}{a_2^2z} - \frac{qd\,\mathrm{cov}(a_1,a_2)}{2a_2^3z} \tag{8}$$

where $z = a_1^2 - 4a_0a_2$, $q = a_1 \mp \sqrt{z}$, $d = a_1q - 2a_0a_2$, and the parameter covariances are calculated as follows:

$$\mathrm{cov}(a_0,a_1) = u^2(a_1)\frac{\sum x \sum x^4 - \sum x^2 \sum x^3}{\left(\sum x^2\right)^2 - n\sum x^4} \tag{9}$$

$$\mathrm{cov}(a_0,a_2) = u^2(a_2)\frac{\left(\sum x^2\right)^2 - \sum x \sum x^3}{\left(\sum x\right)^2 - n\sum x^2} \tag{10}$$

$$\mathrm{cov}(a_1,a_2) = u^2(a_2)\frac{n\sum x^3 - \sum x \sum x^2}{\left(\sum x\right)^2 - n\sum x^2} \tag{11}$$

In stark contrast to eqS. 8-11, the simplicity of the uncertainty calculation in the inverse calibration approach remains unchanged when polynomial models are employed:

$$u^2(x_0) = u^2(b_0) \tag{12}$$

This is a particularly attractive feature given the growing use of nonlinear calibration functions in analytical chemistry (see Table 1).[9]

Table 1. Comparison of the direct and inverse calibration approaches for standard additions

Direct calibration	**Result**
$y = a_0 + a_1x$	$x_0 = a_0/a_1$
$y = a_0 + a_1x^1 + a_2x^2$	$x_0 = (-a_1 \pm \sqrt{(a_1^2 - 4a_0a_2)})/(2a_0)$
$y = (a_0 + a_1x + a_2x^2)/(1 + a_3x)$	$x_0 = (-a_1 \pm \sqrt{(a_1^2 - 4a_0a_2)})/(2a_0)$
Inverse calibration	**Result**
$x = b_0 + b_1y$	$x_0 = -b_0$
$x = b_0 + b_1x^1 + b_2x^2$	$x_0 = -b_0$
$x = (b_0 + b_1y + b_2y^2)/(1 + b_3y)$	$x_0 = -b_0$

Regressions with Excel

Calculations using Excel spreadsheets have become the norm in science.[10][11] Yet, many fitting algorithms involve the SOLVER macro which performs minimization of a user-defined function against the user-defined argument. Although effective, the use of this macro requires active user input, and it does not provide the uncertainty estimates for the parameters. On the contrary, the LINEST function is a standard part of Excel and it performs linear and nonlinear fitting. Table 2 summarizes useful LINEST directives in order to perform fitting on linear or polynomial models, including the rational Padé functions.

Table 2. LINEST() fitting of functions commonly encountered in analytical chemistry[1]

Type	**Function**	**Excel directive**[2]
Direct	$y = a_0x$	=LINEST(y, x, FALSE, TRUE)
Linear	$y = a_0 + a_1x$	=LINEST(y, x, TRUE, TRUE)[3]
Quadratic	$y = a_0 + a_1x^1 + a_2x^2$	=LINEST(y, x^{0,1,2}, FALSE, TRUE)
Polynomial	$y = a_0 + a_1x^1 + \ldots + a_mx^m$	=LINEST(y, x^{0,…,m}, FALSE, TRUE)
Padé [1,1]	$y = (a_0 + a_1x)/(1 + a_2x)$	=LINEST(y, x^{0,1,0}*-y^{0,0,1}*x^{0,0,1}, FALSE, TRUE)
Padé [2,1]	$y = (a_0 + a_1x + a_2x^2)/(1 + a_3x)$	=LINEST(y, x^{0,1,2,0}*-y^{0,0,0,1}*x^{0,0,0,1}, FALSE, TRUE)

1 For inverse fitting $x = f(y)$, interchange a instances of x and y in the LINEST directive. For example, inverse linear fit becomes =LINEST(x, y, TRUE, TRUE).

2 Symbols x and y stand for the data input range. For example, if the values of x and y are in cells A1 to A5 and B1 to B5 respectively, then the linear fit is obtained by entering =LINEST(B1:B5, A1:A5, TRUE, TRUE). Also note that unlike many other functions which are entered in a singe cell by pressing enter, LINEST is an array function which is entered over several cells by pressing ctrl+shift+enter.

3 Alternatively, one can write =LINEST(y, x^{0,1}, FALSE, TRUE).

Experimental Part

Bromide was analyzed in certified groundwater reference material by using headspace gas chromatography mass spectrometry (GC/MS) after the aqueous derivatization with triethyloxonium tetrafluoroborate. Iodide ions naturally present in the groundwater were used as the internal standard for bromide. Experimental details are summarized in the Supplementary material and further details can be found elsewhere.[ref]

Results and discussion

To demonstrate the validity of the coordinate swapping in standard additions, and also to show the usefulness of this approach when dealing with nonlinear standard addition models, determination of bromide in groundwater certified reference material was undertaken using state-of-the-art GCMS method.[12] All relevant experimental data are given in Table 3. There, $m_{A(AA^*)}$ is the mass of the sample in the mixture of the sample (A) and standard (A*). Consequently, $m_{A^*(AA^*)}$ is the mass of the standard in the corresponding mixture. $m_{(AA^*)dil}$ is the mass of the AA* mixtures after addition of all reagents.

Table 3. Determination of bromide ions in groundwater (BCR-611): data[1]

$m_{A(AA^*)}$/g	$m_{A^*(AA^*)}$/g	$m_{(AA^*)dil}$/g	A_{EtBr}	A_{EtI}
2.005 99	0.000 00	2.353 82	14 610	8153
2.008 17	0.000 00	2.358 67	14 633	8176
2.180 35	0.000 00	2.355 67	13 422	7695
2.001 74	0.049 49	2.350 62	21 863	8190
2.004 35	0.050 05	2.353 49	22 161	8339
2.009 60	0.049 89	2.358 09	22 155	8199
1.999 61	0.099 24	2.348 73	29 786	8357
2.005 36	0.099 49	2.354 30	30 806	8652
2.009 01	0.099 91	2.358 00	30 427	8432
1.999 22	0.148 14	2.346 72	35 088	7983
2.002 76	0.148 72	2.351 28	37 794	8531
2.006 66	0.148 83	2.354 87	34 243	7731
1.997 26	0.197 03	2.344 29	42 221	7909
2.004 13	0.198 03	2.352 16	46 070	8682
2.006 93	0.200 21	2.357 14	38 640	7286

1 Mass fraction of Br^- in primary reference standard solution, $w_{A^*} = 1925(2)_{k=1}$ ng g^{-1}.

In our calculations we use a more elaborate formalism of standard additions which accounts for the (small) differences in the amount of sample in each aliquot, and accounts for the (small) differences in the dilution factors. We also employ an internal standard in order to attain highest precision. More details on this precision-formalism of the standard additions can be consulted elsewhere,[1314] and here we give only a brief summary of the variables which are involved in constructing the standard addition plots. When no internal standard is used, the following variables are considered:

$$y = A_{EtBr}\frac{m_{(AA^*)dil}}{m_{A(AA^*)}} \quad \text{and} \quad x = w_{A^*}\frac{m_{A^*(AA^*)}}{m_{A(AA^*)}} \tag{13}$$

When the internal standard is used, there is no need to account for the sample dilution factors, and the following variables are considered:

$$y = \frac{A_{EtBr}}{A_{EtI}} \quad \text{and} \quad x = w_{A^*}\frac{m_{A^*(AA^*)}}{m_{A(AA^*)}} \tag{14}$$

The incorporation of the mass fraction of the analyte in the primary standard, w_{A^*}, serves to simplify the calculations. Such practice is acceptable if the uncertainty of the w_{A^*} is negligible.

However, when the uncertainty arising from the w_{A*} needs to be explicitly accounted for in the combined uncertainty budget, one can remove the w_{A*} from eqs. 13 and 14. Then, the mass fraction of the analyte in the sample is $w_A = x_0 w_{A*}$, and the uncertainty is $(u(w_A)/w_A)^2 = (u(x_0)/x_0)^2 + (u(w_{A*})/w_{A*})^2$. This way one can ensure that the uncertainty associated with the primary standard is properly incorporated in the uncertainty of the result. For the sake of simplicity, this was not done in this work, also because the uncertainty in the primary reference standard, $u(w_{A*})$, is insignificant in comparison to the uncertainty of the fit for data presented in this work.

Using data from Table 3, standard addition calculations were performed with three models: linear, quadratic, and Padé[2,1] whose model equations are given in Table 1. Least squares fitting was performed using LINEST() function as detailed in Table 2. For direct calibration functions, the uncertainty of x_0 was obtained either from eqs. 3-4 (linear model) or eqs. 8-11 (quadratic and Padé[2,1] models). For all inverse calibration functions, the uncertainty of the result was obtained directly from eq. 6. The results are given in Table 4.

Table 4. Results of standard addition experiment.[1]

$y = A_{EtBr}/A_{EtI}$ $x = w_{A*} \cdot m_{A*(AA*)}/m_{A(AA*)}$	**Equations**	**Result, $w(Br^-)$/(ng/g)**	**Complexity, $N(x_0)$**	**Complexity, $N[u(x_0)]$**
Direct calibration				
$y = a_0 + a_1 x$	Eqs. 2, 3-4	96.45(1.14)	1	28
$y = a_0 + a_1 x + a_2 x^2$	Eqs. 7, 8-11	93.15(3.12)	8	170
$y = (a_0 + a_1 x + a_2 x^2)/(1 + a_3 x)$	Eqs. 7, 8-11	94.93(1.17)	8	170
Inverse calibration				
$x = b_0 + b_1 y$	Eqs. 5, 6	96.37(1.14)	0	0
$x = b_0 + b_1 x + b_2 x^2$	Eqs. 5, 6	93.09(3.34)	0	0
$x = (b_0 + b_1 y + b_2 y^2)/(1 + b_3 y)$	Eqs. 5, 6	94.94(1.12)	0	0

1 Mass fraction of bromide in BCR-611 as determined by the quadruple isotope dilution, $w(Br^-)$ = 96.28(42) ng/g (ref. 12). All uncertainties here are expressed with coverage factor $k = 1$.
2 Minimum number of elementary arithmetic functions (addition, multiplication, and exponentiation) needed to obtain the result starting from the results given by the LINEST() function (calculated for this dataset with $N = 15$).

In general, one can observe that the results of inverse calibration approach are no different than those obtained from the traditional direct fitting of analytical signal against the mass of the added analyte. The assignment of the x_0 values and their uncertainty estimates, however, is considerably simpler from the inverse fits. In addition, the marked simplicity of the inverse approach can spur the evaluation of the metrological performance of nonlinear standard addition models.

Conclusions

Although strictly speaking the classical and inverse linear fitting are not mathematically equivalent procedures, the distinction becomes relevant only for datasets that are very noisy. Since this is not a norm in analytical chemistry, the inverse fitting offers significant advantages to chemists. Given that the use inverse regression in standard addition experiments simplifies the data analysis significantly over the traditional fitting approach, the use of inverse regression should be embraced in the spirit of Occam's razor.

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