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# The reversed-axis method to estimate precision in standard additions analysis

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ABSTRACT

The standard additions (SA) method is one of the most important calibration strategies in quantitative chemical analysis. It is a powerful tool to minimize matrix effects and enable precise and accurate determinations. On the other hand, the SA method is time-consuming and cumbersome because it requires the preparation of a calibration curve for each individual sample. Considering the statistical treatment required, the estimation of precision in SA determinations can be as laborious and cumbersome as the experimental procedure itself. In this work, we describe a simple method to quickly estimate standard deviations in SA analyses using the determination of Na and K in biodiesel by flame atomic emission spectrometry (FAES) as a model. By taking analyte concentration as the dependent variable ($y$-axis), and instrument response as the independent variable ($x$-axis), the standard deviation of the analyte concentration in the sample is equal to the error in the $y$-axis intercept of the SA calibration curve. This value can be easily calculated using a simple equation or the regression feature in MS Excel. Standard deviation values calculated using MS Excel and this reversed-axis method are compared to results from the traditional statistical methods of extrapolation and error propagation. In all determinations, the results from the extrapolation, error propagation with covariance, and reversed-axis methods are identical, which demonstrates their equivalence.



## 1. Introduction

The first description of the standard additions (SA) calibration method was published in a 1937 book on polarography, *Chemische Analysen mit dem Polarographen*, by Hans Hohn (1906–1978). However, the method only became more widely used starting in 1953, when it was applied in X-ray fluorescence and atomic spectrometry [1]. Since then, the SA method has become one of the most important developments in quantitative chemical analysis, especially because it provides a powerful correction of matrix effects [1,2]. The SA method is routinely used in the most diverse fields and it is present in almost all analytical chemistry textbooks [3–6]. Although it cannot correct for translational effects (*i.e.*, interference signal directly produced by concomitant species), SA is very efficient at minimizing the most prominent rotational effects (*i.e.*, variations in the analytical signal caused by concomitant species) [7]. In its most common version, increasing volumes of a standard reference solution are added to 4–5 different volumetric flasks containing a fixed volume of sample. The mixture is then diluted to the mark with solvent before analysis. No standard reference solution is added to the first flask, and the analyte concentration in the sample corresponds to the $x$-axis intercept of the least-squares linear regression produced from the calibration curve points [1–7]. Fig. 1 shows an example of SA application to determine Na in biodiesel by flame atomic emission spectrometry (FAES).

Despite its efficiency at minimizing matrix effects, the SA method is laborious because it requires the preparation of a calibration curve for each individual sample. Considering the statistical treatment required, the estimation of precision in SA determinations can be as laborious and cumbersome as the experimental procedure itself. The main approaches to estimating precision in SA determinations are based on extrapolation or error propagation, as described below.

### 1.1. Extrapolation method

The extrapolation method is described in different textbooks and literature sources [3,4,8]. The standard deviation of the SA-determined analyte concentration, $s_c$, is calculated using Eq. (1). Statistical parameters required to apply the extrapolation method are represented by Eqs. (2)–(7).

$$s_c = \frac{s_{Er}}{m}\sqrt{\frac{1}{N}+\frac{\bar{y}^2}{m^2\,S_{xx}}} \tag{1}$$

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$$s_{Er} = \sqrt{\frac{S_{yy} - m^2 S_{xx}}{N-2}} \tag{2}$$

$$\overline{y} = \frac{\sum y_i}{N} \tag{3}$$

$$m = \frac{S_{xy}}{S_{xx}} \tag{4}$$

$$S_{xx} = \sum (x_i - \overline{x})^2 \tag{5}$$

$$S_{yy} = \sum (y_i - \overline{y})^2 \tag{6}$$

$$S_{xy} = \sum (x_i - \overline{x})\,(y_i - \overline{y}) \tag{7}$$

where $s_{Er}$ = standard deviation about regression; $m$ = calibration curve slope; $N$ = number of points in the calibration curve; $\overline{y}$ = instrumental response mean; $x_i$ = individual concentration values; and $y_i$ = individual instrumental response values.

### 1.2. Error propagation method

As previously discussed, the analytical concentration in the sample ($C$) is calculated in SA by extrapolating the calibration curve line and determining the $x$-axis intercept (Fig. 1). Therefore, for a calibration curve equation, $IR = mC + b$ (where $IR$ is the instrumental response), $C$ can be determined for $IR = 0$. In this case, $C = -b / m$, where $b$ is the $y$-axis intercept and $m$ is the slope of the calibration curve line. The error propagation method, also known as algebraic method [8], assumes that the relative variance of the analyte concentration, $s_c^2$, is the sum of the relative variances of $b$ and $m$ ($s_b^2$ and $s_m^2$, respectively). To be properly employed, the error propagation method also requires the introduction of a covariance term, $s_{bm}$, into the derivation to prevent underestimation of concentration variances [8,9]. Equation deductions with and without the covariance term are demonstrated below.

If $IR = mC + b$, and $IR = 0$, then $C = \frac{-b}{m}$. By applying the error propagation strategy using partial derivatives:

$$s_c^2 = \left(\frac{\partial C}{\partial b}\right)^2 s_b^2 + \left(\frac{\partial C}{\partial m}\right)^2 s_m^2 = \left(\frac{-1}{m}\right)^2 s_b^2 + \left(\frac{b}{m^2}\right)^2 s_m^2. \tag{8a}$$

Then, multiplying the first term by $\frac{b^2}{b^2}$, isolating the common term, and taking the square root of both sides:

$$s_c^2 = \left(\frac{b}{m}\right)^2 \left[\left(\frac{s_b}{b}\right)^2 + \left(\frac{s_m}{m}\right)^2\right], \tag{8b}$$

$$s_C = \frac{b}{m}\sqrt{\left(\frac{s_b}{b}\right)^2 + \left(\frac{s_m}{m}\right)^2}. \tag{8c}$$

To introduce the covariance term:

$$\begin{aligned} s_c^2 &= \left(\frac{\partial C}{\partial b}\right)^2 s_b^2 + \left(\frac{\partial C}{\partial m}\right)^2 s_m^2 - \left(\frac{\partial C}{\partial b}\right)\left(\frac{\partial C}{\partial m}\right) s_{bm} \\ &= \left(\frac{-1}{m}\right)^2 s_b^2 + \left(\frac{b}{m^2}\right)^2 s_m^2 - 2\left(\frac{-1}{m}\right)\left(\frac{b}{m^2}\right), \end{aligned} \tag{9a}$$

$$s_c^2 = \left(\frac{b}{m}\right)^2 \left[\left(\frac{s_b}{b}\right)^2 + \left(\frac{s_m}{m}\right)^2 + \frac{2s_{bm}}{bm}\right], \tag{9b}$$

$$s_C = \frac{b}{m}\sqrt{\left(\frac{s_b}{b}\right)^2 + \left(\frac{s_m}{m}\right)^2 + \frac{2s_{bm}}{bm}}. \tag{9c}$$

### 1.3. Reversed-axis method

By taking analyte concentration (C) as the dependent variable ($y$-axis), and instrument response (IR) as the independent variable ($x$-axis):

$$IR = mC + b, \tag{10a}$$

$$C = \frac{IR}{m} - \frac{b}{m}, \tag{10b}$$

$$C = m'IR + b'. \tag{10c}$$

If $IR = 0$, then C = b′, and $s_c = s_{b'}$. Therefore, the analyte concentration, C, and its standard deviation, $s_c$, in the sample can be found by determining the $y$-axis intercept of the calibration curve, $b'$, and its corresponding standard deviation, $s_{b'}$. In MS Excel, this can be easily accomplished by using the "*Regression*" feature, under "*Data Analysis*", when the "*Analysis Toolpak*" add-in is enabled (Procedures S1 and S2).

In the present work, we use Na and K determination in biodiesel by FAES as a model to demonstrate the efficiency of the reversed-axis

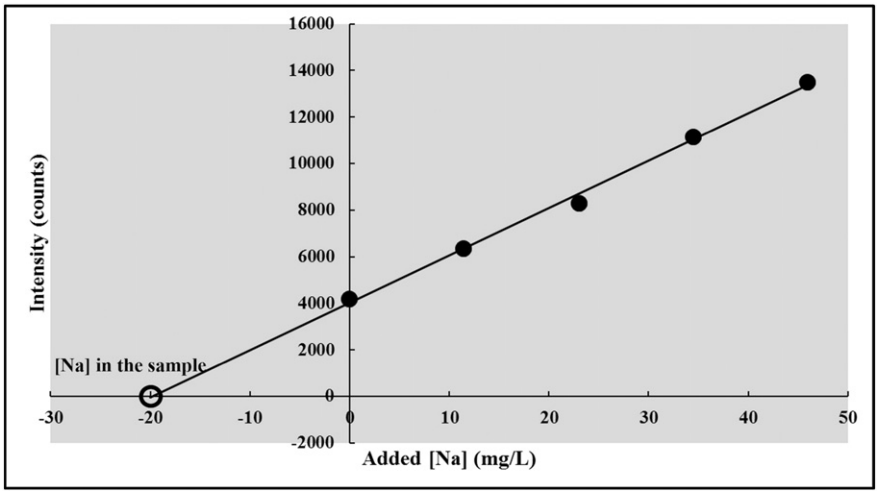


**Fig. 1.** Extrapolation of a standard additions calibration curve line to determine the Na concentration in a biodiesel sample by FAES.

**Table 1**
Added concentrations and the corresponding atomic emission net intensities used to determine Na and K concentrations and calculate the respective standard deviations.

| Concentration added (μg/g) | Atomic emission net intensity (counts) | | |
|---|---|---|---|
| | Na at 589.0 nm | K at 766.5 nm | K at 769.9 nm |
| 0.00 | 4231.80 | 3649.56 | 1394.99 |
| 0.00 | 4115.37 | 3568.56 | 1346.55 |
| 0.00 | 4190.64 | 3658.87 | 1380.34 |
| 0.00 | 4194.73 | 3569.58 | 1347.37 |
| 0.00 | 4177.53 | 3632.66 | 1371.13 |
| 11.4 | 6396.81 | 5419.41 | 2029.65 |
| 11.4 | 6470.74 | 5465.69 | 2120.38 |
| 11.4 | 6406.23 | 5498.67 | 2094.37 |
| 11.4 | 6265.74 | 5358.07 | 2045.93 |
| 11.4 | 6242.39 | 5219.12 | 1991.56 |
| 23.0 | 8317.40 | 6901.22 | 2665.65 |
| 23.0 | 8333.78 | 6869.68 | 2616.50 |
| 23.0 | 8295.48 | 6789.19 | 2592.33 |
| 23.0 | 8235.78 | 6921.49 | 2642.51 |
| 23.0 | 8298.66 | 6998.91 | 2653.77 |
| 34.5 | 11,143.28 | 8797.94 | 3435.38 |
| 34.5 | 11,049.90 | 8684.38 | 3319.77 |
| 34.5 | 11,085.84 | 8781.56 | 3411.42 |
| 34.5 | 11,345.73 | 8968.23 | 3489.34 |
| 34.5 | 11,098.02 | 8785.25 | 3370.05 |
| 45.9 | 13,448.89 | 10,598.21 | 4177.46 |
| 45.9 | 13,432.50 | 10,489.77 | 4036.87 |
| 45.9 | 13,437.32 | 10,502.67 | 4111.00 |
| 45.9 | 13,556.92 | 10,512.20 | 4099.02 |
| 45.9 | 13,582.93 | 10,542.51 | 4150.63 |

method. Standard deviation values calculated using MS Excel and the reversed-axis method are compared to results from the traditional methods of extrapolation [3,4,8] and error propagation [8,9].

## 2. Experimental

Biodiesel is a notoriously complex matrix that frequently requires the application of the SA method to ensure accurate and precise results [10]. The data used in this work were obtained from Na and K determination in biodiesel by FAES using SA. Approximately 0.1 g of a standard reference material of biodiesel (Metals in Biodiesel, VHG Labs, Manchester, NH, USA) was accurately weighted in polypropylene tubes using an electronic balance (Mettler AE 100, Hightstown, NJ, USA). Adequate volumes of trace metal-grade nitric acid (Fisher, Pittsburgh, PA, USA) and standard reference solutions of Na and K (SPEX CertPrep, Metuchen, NJ, USA) were added to the samples for final concentrations of 1% *v*/*v* $HNO_3$, and 0.00, 11.4, 23.0, 34.5 and 45.9 μg of added Na and K per gram of biodiesel sample. HPLC-grade methanol (Fisher) was then used to make up the volume of the calibration curve standards to 10.00 mL (Table S1). The calibration solutions were mixed for 2 min with a vortex mixer (Fisher) before Na and K determination by FAES. Analytical blanks (1% *v*/*v* $HNO_3$ in methanol) were run prior to the reference solutions to calculate the emission net intensities presented in Table 1.

A modular FAES system composed of a handheld spectrometer (USB4000, Ocean Optics, Dunedin, FL, USA), a 25 mm diameter, 75 mm focal length fused silica lens, and a 5.3-cm-slot burner head was used in all determinations. A stoichiometric mixture of air/acetylene was used in all flame measurements. The spectrometer operational conditions were: 10 ms integration time, 2 box-car smoothing, 5 replicates per run, and 2 spectra averaged for each replicate.

**Table 3**
Parameters used to calculate Na and K concentrations and the corresponding error estimates using the extrapolation and the error propagation methods.

| Parameter | Na at 589.0 nm | K at 766.5 nm | K at 769.9 nm |
|---|---|---|---|
| $s_{Er}$ | 228.74 | 112.19 | 62.34 |
| $N$ | 25 | 25 | 25 |
| $\bar{y}$ | 8694.18 | 7047.34 | 2715.76 |
| $m$ | 203.71 | 150.02 | 59.55 |
| $b$ | 415.99 | 3602.27 | 1348.23 |
| $s_m$ | 2.82 | 1.38 | 0.77 |
| $s_b$ | 79.20 | 38.85 | 21.59 |
| $S_{xx}$ | 6601.33 | 6601.33 | 6601.33 |
| $s_{bm}$ | 182.02 | 43.79 | 13.52 |

## 3. Results and discussion

Table 1 shows the added concentrations and corresponding atomic emission net intensities used to calculate Na and K concentrations and the corresponding standard deviation estimates. The analyte concentrations were calculated based on approximately 0.1 g samples and a 1.00 mg/L working reference solution prepared in methanol (Table S1). Table 2 presents the results for the different error estimate methods, and Table 3 shows the parameters used in the extrapolation and the error propagation calculations. As expected, the error propagation results for calculations ignoring the slope–intercept covariance (Eq. (8c)) are underestimated [8]. On the other hand, all the other methods provided the same error estimate, which indicates their equivalence. Fig. 2 shows the MS Excel output statistics for an example of Na determination in biodiesel by FAES using SA and the reversed-axis method.

To check the accuracy of the reversed-axis method, one can verify the relationship $s_c = s_{b'}$ (from Eq. (10c)) by recalculating the standard deviation values using Eq. (11) [3,8]. In this case, the alternative parameters for the 589.0, 766.5 and 769.9 nm analytical lines are: N = 25; $s_{Er'} = 1.12$, 0.75 and 1.04; $\sum x_i'^2 = 2.16 \times 10^9$, $1.39 \times 10^9$ and $2.08 \times 10^8$; and $S_{xx} = 2.75 \times 10^8$, $1.49 \times 10^8$ and $2.35 \times 10^7$. The respective $s_{b'}$ results from Eq. (11) are 0.63, 0.46 and 0.62 μg/g, which are identical to the ones calculated with the reversed-axis method and MS Excel (Table 2).

$$s_{b'} = s_{Er'} \sqrt{\frac{\sum x_i'^2}{S_{xx'} N}} \tag{11}$$

## 4. Conclusions

The extrapolation, error propagation with covariance, and reversed-axis methods provide the same precision estimate values. However, the reversed-axis strategy is much simpler and more straightforward. It can

**Table 2**
Sodium and potassium concentrations in biodiesel determined by SA and FAES. The standard deviations were calculated by the extrapolation, error propagation and reversed-axis methods. Values are the mean ± 1 standard deviation in μg/g (n = 5).

| Analyte | Wavelength (nm) | Reference[a] (μg/g) | Precision estimate method | | | |
|---|---|---|---|---|---|---|
| | | | Extrapolation | Error propagation | Error propagation (no covariance) | Reversed-axis |
| Na | 589.0 | 20.00 | 19.71 ± 0.63 | 19.71 ± 0.63 | 19.71 ± 0.47 | 19.53 ± 0.63 |
| K | 766.5 | 20.00 | 24.01 ± 0.46 | 24.01 ± 0.46 | 24.01 ± 0.34 | 23.92 ± 0.46 |
| K | 769.9 | 20.00 | 22.64 ± 0.62 | 22.64 ± 0.62 | 22.64 ± 0.47 | 22.47 ± 0.62 |

[a] Metals in Biodiesel, VHG Labs.

SUMMARY OUTPUT

| *Regression Statistics* | |
|---|---|
| Multiple R | 0.997810721 |
| R Square | 0.995626235 |
| Adjusted R Square | 0.995436071 |
| Standard Error | 1.120416447 |
| Observations | 25 |

ANOVA

| | *df* | *SS* | *MS* | *F* | *Significance F* |
|---|---|---|---|---|---|
| Regression | 1 | 6572.456036 | 6572.456036 | 5235.627487 | 1.222E-28 |
| Residual | 23 | 28.87265933 | 1.255333014 | | |
| Total | 24 | 6601.328695 | | | |

| | *Coefficients* | *Standard Error* | *t Stat* | *P-value* | *Lower 95%* | *Upper 95%* | *Lower 95.0%* | *Upper 95.0%* |
|---|---|---|---|---|---|---|---|---|
| Intercept | **-19.52747157** | **0.628554004** | -31.06729326 | 2.75632E-20 | -20.82773459 | -18.22720854 | -20.82773459 | -18.22720854 |
| X Variable 1 | 0.004887442 | 6.75456E-05 | 72.357636 | 1.222E-28 | 0.004747713 | 0.005027171 | 0.004747713 | 0.005027171 |

**Fig. 2.** MS Excel output statistics for the determination of Na in biodiesel by FAES using SA and the reversed-axis method. The analyte concentration in the sample and the corresponding standard deviation are highlighted.

easily be employed with MS Excel, and the results may be checked by mathematically proving the relationship $s_c = s_{b'}$ using statistical parameters.

The reversed-axis method is obviously not restricted to biodiesel analysis by FAES. It can be used in any instance where a complex sample matrix requires the application of the standard additions method. Therefore, similar to the traditional methods, the reversed-axis method has wide applications in many fields.

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## Appendix. A Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.microc.2015.08.006. The supplementary material is composed of two MS Excel procedures (Procedures S1 and S2): installing the Analysis Toolpak and applying the reversed-axis method; and a solution preparation table for standard additions (Table S1).

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