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Application of regression methods to solve general isotope dilution measurement equations

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Abstract. Isotope dilution is among the most accurate quantitation approaches in chemical analysis. This calibration method is often employed using a plurality of mathematical formulations. While most analysts find the calibration curve approach most appealing, there is a lack of rigorous general procedures involving calibration curves in isotope dilution and analysts resort to empirical polynomial calibration functions. In this contribution we discuss the adoption of regression analysis, commonly known as least squares methods, to solve isotope dilution equations of varied complexity. This manuscript introduces general regression-based methods to isotope dilution applicable to all known variants of classical isotope dilution known to date, including the fusion of the isotope dilution and standard additions methods.

Keywords: isotope dilution, least squares methods, graphical approach

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1. Introduction

Isotope dilution is often the method of choice in high-quality quantitation of chemical substances [1, 2]. This method has a rich history with beginnings in the form of the mark-and-recapture method to count fish at the turn of the 20th century. Early applications of this method in chemistry involved the use of isotopes as tracers to study chemical processes, as epitomized with the 1943 Nobel Prize in chemistry to de Hevesy.

In its simplest form, quantitation of substances using isotope dilution proceeds by taking an aliquot of the sample (A) which is then mixed with a known amount of isotopic standard (B). After an appropriate equilibration period, the isotopic composition (isotope ratio, R_{AB}) of the resulting blend (AB) is measured. From here, the mass fraction of the analyte in the sample can be obtained if the isotopic compositions of A and B (R_{A}, R_{B}) are known beforehand:

$$w_{\mathrm{A}} = w_{\mathrm{B}} \frac{m_{\mathrm{B}}}{m_{\mathrm{A}}} \frac{R_{\mathrm{B}} - R_{\mathrm{AB}}}{R_{\mathrm{AB}} - R_{\mathrm{A}}} g({}^{k}\mathrm{A}, {}^{k}\mathrm{B}) \qquad (1)$$

Here, $g({}^{k}\mathrm{A}, {}^{k}\mathrm{B}) = (x_{k,\mathrm{B}} M_{\mathrm{B}}^{-1})/(x_{k,\mathrm{A}} M_{\mathrm{A}}^{-1})$ and k refers to the denominator (reference) isotope in measured ratios R_{A}, R_{B}, R_{AB}. Standard symbols are used for quantities as explained in Table 1.

The above expression contains three variables whose values are typically unknown to the analyst: R_{A}, R_{B}, and w_{A}. The Eq. 1 can be solved for the isotope ratio of the blend, R_{AB}:

$$R_{\mathrm{AB}} = \frac{(R_{\mathrm{B}} w_{\mathrm{B}} g_{\mathrm{A,B}}) \cdot m_{\mathrm{B}} + (R_{\mathrm{A}} w_{\mathrm{A}}) \cdot m_{\mathrm{A}}}{(w_{\mathrm{B}} g_{\mathrm{A,B}}) \cdot m_{\mathrm{B}} + (w_{\mathrm{A}}) \cdot m_{\mathrm{A}}} \qquad (2)$$

The Eq. 2 can be recast in the form of a three-parameter hyperbolic relationship between the mass ratios of A and B by dividing its numerator and denominator with the factor $w_{\mathrm{B}} g_{\mathrm{A,B}} m_{\mathrm{B}}$:

$$R_{\mathrm{AB}} = \frac{c_1 + c_2 \cdot m}{1 + c_3 \cdot m} \qquad (3)$$

where $m = m_{\mathrm{A}}/m_{\mathrm{B}}$. This expression can be used to build the calibration function by measuring the isotopic composition of mixtures of the natural and isotopic standards [3, 4]. In practice, isotopic standards are significantly different from the analyte in terms of their isotopic patterns which leads to $c_3 \approx 0$ and a linear relationship between the mass ratio and the resulting isotope ratio in their mixture (see Fig. 1 for A+C).

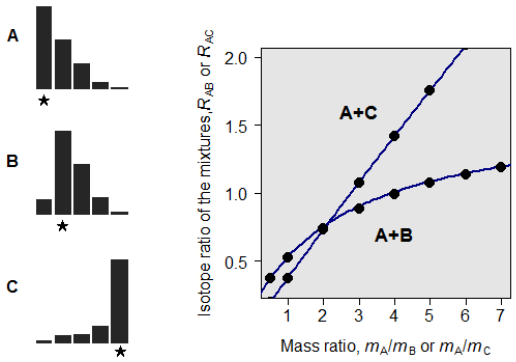

Figure 1. Relationship between the mass ratio of substances and the isotopic composition of their mixtures. Consider substance A and its isotopic analogues B and C. Overlap between the isotope patterns generally leads to curved relationship (as in A+B) whereas larger difference between the isotopic patterns of the analyte and spike (as in A+C) exhibit nearly linear relationship. Note that the shape of this curve depends also on the relative amounts of the substances with some parts being more linear than others.

This contribution explores the mathematical framework to enable accurate isotope dilution analysis using regression-based methods. In particular, this work builds on the concepts introduced in our previous study [5] by extending the regression models to three-component mixtures which enable isotope-based measurements with matrix matching.

2. Theory

Consider the sample (A), natural standard (A∗), and the isotopic standard (B). Analyzing the isotopic composition of the various mixtures of these three materials forms the conceptual landscape for the isotope-based methods of quantitation. In general, we have the following equation for the balance of the chemical amount of isotope i of the analyte, $n({}^{i}\mathrm{A}) + n({}^{i}\mathrm{A}*) + n({}^{i}\mathrm{B})$, in any mixture of these materials:

$$n_{i,\mathrm{AA*B}} = \sum_{\mathrm{E} \in \mathrm{A,A*,B}} x_{i,\mathrm{E}} w_{\mathrm{E}} m_{\mathrm{E}} M_{\mathrm{E}}^{-1} \qquad (4)$$

Although most isotope-based quantitation approaches are confined to the analyses of binary mixtures, the analysis of trinary mixtures affords experimental designs where all measurements are performed

Table 1. Notation of materials and quantities.[a]

Symbol	Description
Materials	
A	Sample
A∗	Natural standard
B	Isotopic standard (enriched spike)
E	A, A∗, or B
AB	Binary mixture of materials A and B
AA∗B	Trinary mixture of materials A, A∗, and B
Quantities	
w_{E}	Mass fraction of the analyte in the solution of E
m_{E}	Mass of solution of E having mass fraction w_{E}
M_{E}	Molar mass of the analyte in the material E
n_{E}	Total amount of E, $n_{\mathrm{E}} = m_{\mathrm{E}} w_{\mathrm{E}} / M_{\mathrm{E}}$
$n_{i,\mathrm{E}}$	Amount of isotope i in E
$x_{i,\mathrm{E}}$	Abundance of isotope i in E, $x_{i,\mathrm{E}} = n_{i,\mathrm{E}} / n_{\mathrm{E}}$
R_{E}	Isotope amount ratio in E, $R_{\mathrm{E}} = n(^{i}\mathrm{E}) / n(^{k}\mathrm{E})$

[a] We use symbol ID^{N}MS to distinguish various isotope dilution strategies where N denotes the number of binary blends (of A or A∗ mixed with B) measured and SA-ID^{N}MS to denote standard additions strategies involving trinary blends (of A and A∗ mixed with B).

in the presence of identical amounts of the sample in order to minimize the effects of the sample matrix on the measurements.

Given that all isotope ratios are expressed relative to the same denominator isotope (^{k}E), the isotopic abundances $x_{i,\mathrm{E}}$ and $x_{k,\mathrm{E}}$ are related: $R_{\mathrm{E}} = x_{i,\mathrm{E}} / x_{k,\mathrm{E}}$. With this in mind, Eq. (4) provides the isotope amount ratio, $R_{\mathrm{AA*B}}$, in any ternary mixture of A, A∗, and B:

$$R_{\mathrm{AA*B}} = \frac{\sum\limits_{\mathrm{E}\in \mathrm{A},\mathrm{A*},\mathrm{B}} x_{i,\mathrm{E}} w_{\mathrm{E}} m_{\mathrm{E}} M_{\mathrm{E}}^{-1}}{\sum\limits_{\mathrm{E}\in \mathrm{A},\mathrm{A*},\mathrm{B}} x_{k,\mathrm{E}} w_{\mathrm{E}} m_{\mathrm{E}} M_{\mathrm{E}}^{-1}} \qquad (5)$$

The Eq. (5) forms the basis for the generalized isotope dilution equations which rely upon the analysis of multiple binary or ternary mixtures of the sample (A), natural standard (A∗), or isotopic standard (B). Analytical solutions of this equation with respect to w_{A} are available for various isotope dilution models. These include ID^{N}MS where $N = 1 \ldots 4$ [6, 7] and SA-ID^{N}MS where $N = 1 \ldots 3$ [8]. Although such solutions are rigorous and provide an explicit measurement models, they have drawbacks. For one, analysts must choose the mathematical equation which is tailored to the particular experimental design. Thus, variations in the experimental design require new mathematical measurement models, such as the double isotope dilution (ID^{2}MS) or triple isotope dilution (ID^{3}MS) among others. In addition, explicit mathematical measurement models cannot easily accommodate situations where there are more measurements than necessary (overdetermined systems).

The above issues notwithstanding, the most notable drawback of the analytical solutions of IDMS equations is the rapid rise in the algebraic complexity with the number of mixtures analysed. The simplest of all isotope dilution methods is ID^{1}MS (which relies on measuring the isotopic composition of a single binary mixture). When fully expanded, this equation (Eq. 1) contains 46 indivisible subexpressions, known as the leaf count. The algebraic complexity of higher-order ID^{N}MS equations rises rapidly with the number of mixtures measured as shown in Table 2. Such algebraic complexity is prohibitive for adoption of higher-order IDMS methods and the purpose of this article is to describe affordable numerical alternatives to solve IDMS equations of arbitrary complexity. Special cases where analyte is formed during the analysis are not considered here but have been treated at length elsewhere [9].

Table 2. Algebraic complexity of IDMS model equations.[a]

Binary models	Complexity	Trinary models	Complexity
ID^{1}MS	46	SA-ID^{1}MS	85
ID^{2}MS	129	SA-ID^{2}MS	431
ID^{3}MS	295	SA-ID^{3}MS	1065
ID^{4}MS	1221	SA-ID^{4}MS	8917

[a] The complexity is measured as the leaf count (*Mathematica*) and assuming identical isotopic composition of A and A∗.

2.1. Regression method

Numerical approaches to solve Eq. 5 for w_{A} can be devised and may be preferable over the analytical solutions for a variety of reasons. We have discussed the application of the least squares method to provide solutions of standard isotope dilution models which rely on binary mixtures [5]. Indeed, it appears that many analysts do prefer numerical methods over analytical expressions which has led to the adoption of various empirical models to construct the calibration curves, including the quadratic fits [10, 11, 12]. Empirical fitting functions are approximations to the underlying theoretical measurement models and it is inevitable that such approximations lead to biases. Here we propose the adoption of non-empirical fitting models over the polynomial functions.

Consider a set of mixtures made by combining A, A∗, and B in various proportions. Eq. 5, governs the resulting isotope ratios of such mixtures as a function of masses of the constituent components and the mass fractions of the substances in these solutions. Thus, the isotopic composition of any such mixture can be expressed in a form of a rational function as follows:

$$R = \frac{k_{\mathrm{A}} m_{\mathrm{A}} + k_{\mathrm{A*}} m_{\mathrm{A*}} + k_{\mathrm{B}} m_{\mathrm{B}}}{h_{\mathrm{A}} m_{\mathrm{A}} + h_{\mathrm{A*}} m_{\mathrm{A*}} + h_{\mathrm{B}} m_{\mathrm{B}}} \qquad (6)$$

Here, k_{E} and h_{E} represent a grouping of variables whose values depend solely on the characteristics of the material E and are therefore unchanged during the experiment. As an example, $k_{\mathrm{A}} = x_{i,\mathrm{A}} w_{\mathrm{A}} M_{\mathrm{A}}^{-1}$. Consequently, we can consider these six variables ($k_{\mathrm{A},\mathrm{A}*,\mathrm{B}}$ and $h_{\mathrm{A},\mathrm{A}*,\mathrm{B}}$) as nuisance parameters and Eq. 6 can be rewritten in the linear rational form by dividing both the numerator and denominator with h_{B}:

$$R = \frac{a_1 m_{\mathrm{A}} + a_2 m_{\mathrm{A}*} + a_3 m_{\mathrm{B}}}{a_4 m_{\mathrm{A}} + a_5 m_{\mathrm{A}*} + 1 \cdot m_{\mathrm{B}}} \tag{7}$$

In the following sections we shall demonstrate the utility of this expression and how the coefficients a relate to the mass fraction of analyte, w_{A}.

2.2. Measurement models

The coefficients $a_1 - a_5$ in Eq. 7 provide a mathematical link between the mass fractions of the analyte in sample, natural standard or isotopic standard. As an example, consider the ratio a_1/a_2:

$$\frac{a_1}{a_2} = \frac{k_{\mathrm{A}}}{k_{\mathrm{A}*}} = \frac{x_{i,\mathrm{A}} w_{\mathrm{A}} M_{\mathrm{A}}^{-1}}{x_{i,\mathrm{A}*} w_{\mathrm{A}*} M_{\mathrm{A}*}^{-1}} \tag{8}$$

Thus, when the quantitation of substance A is traceable through a standard of natural isotopic composition (A$*$), the mass fraction of the analyte in the sample, w_{A}, is related to the regression coefficients as dictated from Eq. 8 and alike:

$$w_{\mathrm{A}} = w_{\mathrm{A}*} \frac{a_1}{a_2} g({}^{i}\mathrm{A}, {}^{i}\mathrm{A}*) \tag{9}$$

$$w_{\mathrm{A}} = w_{\mathrm{A}*} \frac{a_4}{a_5} g({}^{k}\mathrm{A}, {}^{k}\mathrm{A}*) \tag{10}$$

where the parameter $g_{\mathrm{A},\mathrm{A}*}$ accounts for the possible discrepancy between the molar masses and isotopic composition of A and A$*$:

$$g({}^{j}\mathrm{A}, {}^{z}\mathrm{A}*) = \frac{x_{\mathrm{A}*,z} M_{\mathrm{A}*}^{-1}}{x_{\mathrm{A},j} M_{\mathrm{A}}^{-1}} \tag{11}$$

Typically, the isotopic compositions of A and A$*$ are nearly identical. As a result, the numerical value of $g({}^{j}\mathrm{A}, {}^{z}\mathrm{A}*)$ is close to 1 when $j = z$ and this factor therefore plays a minor role in practice.

Note that Eq. 7 affords several estimates for the mass fraction w_{A} depending on the choice of the regression coefficients. In the absence of measurement errors, these estimates will be identical. In practice, however, the solution with the smallest uncertainty ought to be selected and typically will involve the numerator coefficients a_1, a_2, or a_3 (as in Eq. 9).

In cases when the mass fraction of B (w_{B}) in the isotopic standard is known, it can be used to obtain the mass fraction of A:

$$w_{\mathrm{A}} = w_{\mathrm{B}} \frac{a_1}{a_3} g({}^{i}\mathrm{A}, {}^{i}\mathrm{B}) \tag{12}$$

$$w_{\mathrm{A}} = w_{\mathrm{B}} a_4 \cdot g({}^{k}\mathrm{A}, {}^{k}\mathrm{B}) \tag{13}$$

Here, $g_{\mathrm{A,B}}$ accounts for the discrepancy between the molar masses and isotopic composition of A and B:

$$g({}^{j}\mathrm{A}, {}^{z}\mathrm{B}) = \frac{x_{\mathrm{B},z} M_{\mathrm{B}}^{-1}}{x_{\mathrm{A},j} M_{\mathrm{A}}^{-1}} \tag{14}$$

Given that the isotopic compositions of A and B are markedly different by design, the value of $g_{\mathrm{A,B}(j)}$ will not be close to 1 and this factor therefore cannot not be overlooked. In the case of near-zero overlap between the isotope patterns of A and B, $x_{\mathrm{B},k} \approx x_{\mathrm{A},i}$ and the following model is useful:

$$w_{\mathrm{A}} = w_{\mathrm{B}} a_1 \cdot g({}^{i}\mathrm{A}, {}^{k}\mathrm{B}) \approx w_{\mathrm{B}} a_1 \cdot M_{\mathrm{A}}/M_{\mathrm{B}} \tag{15}$$

2.3. Linear least squares fitting

Eq. 7 provides regression-based path to obtain the mass fraction of analyte, w_{A}, using the method of isotope dilution. This nonlinear five-parameter model can be fitted to the data using a variety of computational methods. More conveniently, linear regression can also be used to obtain the coefficients of Eq. 7 [5, 13, 14]. For this, it can be rewritten as follows:

$$\begin{aligned} R m_{\mathrm{B}} = a_1 \cdot m_{\mathrm{A}} + a_2 \cdot m_{\mathrm{A}*} + a_3 \cdot m_{\mathrm{B}} \\ - a_4 \cdot R m_{\mathrm{A}} - a_5 \cdot R m_{\mathrm{A}*} \end{aligned} \tag{16}$$

The above parametrization is not unique but it eschews mass ratios to avoid potential division by zero. If only the mixtures of A and B are employed, as in ID^{1}MS, the Eq. 16 becomes (with regression coefficients renumbered to maintain unbroken sequence):

$$R m_{\mathrm{B}} = a_1 \cdot m_{\mathrm{A}} + a_2 \cdot m_{\mathrm{B}} - a_3 \cdot R m_{\mathrm{A}} \tag{17}$$

The ordinary least squares estimates of coefficients a are given as

$$\mathbf{a} = (\mathbf{X}^T \mathbf{X})^{-1} \mathbf{X}^T \mathbf{Y} \tag{18}$$

where $\mathbf{Y}$ is a column vector of $R m_{\mathrm{B}}$ values and $\mathbf{X}$ is the experimental design matrix:

$$\mathbf{X} = \{m_{\mathrm{A}}, m_{\mathrm{A}*}, m_{\mathrm{B}}, -R m_{\mathrm{A}}, -R m_{\mathrm{A}*}\} \tag{19}$$

or, in the case of measurements involving mixtures of A and B only,

$$\mathbf{X} = \{m_{\mathrm{A}}, m_{\mathrm{B}}, -R m_{\mathrm{A}}\} \tag{20}$$

Regression coefficients can be readily obtained by solving Eq. 18 using common spreadsheet software capable of performing basic matrix manipulations (see Supplementary Information).

The linearized version of the rational measurement model allows us to employ linear least squares. However, strictly speaking, ordinary least squares fit might not always be an appropriate method. First, the stimulus variables cannot be regarded as exact. In addition, not all variables are independent of each other: there are correlations among the stimulus variables as between m_{A} and Rm_{A} in Eq. 16, for example. Furthermore, there are correlations between some of the stimulus variables and the response variable (for example, between m_{B} and Rm_{B}). When considering the various fitting algorithms, the impact of these correlations has to be evaluated, for example, by using the generalized distance linear regression which can take into account the covariance structure. One can also consider Bayesian methods and fully model the relationships between the fitting variables. The uncertainty propagation of isotope dilution results can be performed using the Monte Carlo method [15] with tutorial examples of such evaluations readily available [16].

2.4. Simplified cases

The simplest case, and perhaps one that is most encountered in laboratory practice, corresponds to a situation of negligible isotopic overlap between the analyte and the isotopic standard. This scenario leads to $h_{\mathrm{A}} = 0, h_{\mathrm{A}*} = 0, k_{\mathrm{B}} = 0$ and, consequently, Eq. 6 simplifies to

$$Rm_{\mathrm{B}} = a_1 m_{\mathrm{A}} + a_2 m_{\mathrm{A}*} \tag{21}$$

where $a_1 = k_{\mathrm{A}}/h_{\mathrm{B}}$ and $a_2 = k_{\mathrm{A}*}/h_{\mathrm{B}}$. In Table 3 we provide a summary of regression models applicable for isotope dilution. Note that such simplification might not be justified in practice when affordable spikes are poorly enriched.

2.5. Validation

Regression-based (graphical) solutions of the various isotope dilution designs are derived from first principles and these expressions can be further validated by comparing them with the corresponding analytical solutions. To evaluate the generic five-parameter regression model (M1, Table 3), we have developed an explicit analytical measurement model tailored for five isotope ratio measurements from trinary mixtures, similar to the models which we have developed previously for less complex experimental designs [8]. The compact form of this equation fills an entire page and is given in the Supplementary Information. Both measurement models ('analytical' and 'graphical') provide identical results on synthetic data (see Supplementary Information).

3. Tutorial examples

To add further familiarity with the regression-based isotope dilution methods we provide several practical examples.

3.1. Example 1: Simple measurement of meloxicam

In 2018 the NRC released a Certified Reference Material of veterinary drug residues in bovine muscle, BOTS-1 [17]. In short, the method relies on taking beef sample aliquots which were spiked with either known amounts of natural or isotopic standard (or both). Samples were then subjected to extraction followed by liquid chromatography (LC) and the eluting substances were detected using mass spectrometry (MS). Among the simplest ways to perform isotope-based quantitation is to use isotopic standard with the measurement results shown in Table 4. To obtain the mass fraction of meloxicam in BOTS-1 sample, w_{A}, we use the linearized two-parameter measurement model (Table 3, M4) which gives $a_1 = 0.228$ from ordinary least squares fitting. Assuming $g_{\mathrm{A,B}} = M_{\mathrm{A}}/M_{\mathrm{B}}$ and $w_{\mathrm{B}} = 13.3$ ng/g, we obtain the following result:

$$w_{\mathrm{A}} = w_{\mathrm{B}} \cdot a_1 \cdot \frac{M_{\mathrm{A}}}{M_{\mathrm{B}}} = 13.3 \cdot 0.228 \cdot \frac{351}{354} = 3.0 \text{ ng/g} \tag{22}$$

3.2. Example 2: Elaborate measurement of meloxicam

The above example relies on isotopic standard and uses linearized measurement model. A set of more elaborate measurements are summarized in Table 5.

The mass fraction of meloxicam in BOTS-1 can be calculated using the conventional ID^{2}MS measurement model (assuming $g_{\mathrm{A,A}*} = 1$) which does not require the mass fraction of meloxicam-d_3 standard to be known:

$$w_{\mathrm{A}} = w_{\mathrm{A}*} \frac{m_{\mathrm{A}*,5} m_{\mathrm{B},4} (R_3 - R_4)(R_2 - R_5)}{m_{\mathrm{A},4} m_{\mathrm{B},5} (R_4 - R_1)(R_5 - R_3)} \tag{23}$$

Using measurements Nr. 1-5 from Table 5, we obtain $w_{\mathrm{A}} = 3.12$ ng/g for meloxicam in BOTS-1. Identical result can be obtained using regression method when same input data are used. For this, we use the full 5-parameter measurement model (Table 3, M1). The regression coefficients a_1, a_2 or a_4, a_5 provide the mass fraction of the analyte:

$$w_{\mathrm{A}} = w_{\mathrm{A}*} \frac{a_1}{a_2} = 13.03 \frac{0.2332}{0.9743} = 3.12 \text{ ng/g} \tag{24}$$

$$w_{\mathrm{A}} = w_{\mathrm{A}*} \frac{a_4}{a_5} = 13.03 \frac{0.001\,50}{0.006\,29} = 3.12 \text{ ng/g} \tag{25}$$

Table 3. Summary of regression models applicable for isotope dilution.

Model	Components	Full regression model	Measurement model for w_A
M1	A, A∗, B	$Rm_B = a_1 \cdot m_A + a_2 \cdot m_{A*} + a_3 \cdot m_B - a_4 \cdot Rm_A - a_5 \cdot Rm_{A*}$	$w_A = w_{A*} \cdot (a_1/a_2) \cdot g({}^iA, {}^iA*)$ $w_A = w_{A*} \cdot (a_4/a_5) \cdot g({}^kA, {}^kA*)$
M2	A, B	$Rm_B = a_1 \cdot m_A + a_2 \cdot m_B - a_3 \cdot Rm_A$	$w_A = w_B \cdot (a_1/a_2) \cdot g({}^iA, {}^iB)$ $w_A = w_B \cdot (a_3) \cdot g({}^kA, {}^kB)$
Model	Components	Simplified regression model (linear approximation)	Measurement model for w_A
M3	A, A∗, B	$Rm_B = a_1 \cdot m_A + a_2 \cdot m_{A*}$	$w_A = w_{A*} \cdot (a_1/a_2) \cdot g({}^iA, {}^iA*)$
M4	A, B	$Rm_B = a_1 \cdot m_A$	$w_A = w_B \cdot (a_1) \cdot g({}^iA, {}^kB)$

Table 4. Determination of meloxicam in bovine muscle Certified Reference Material BOTS-1 using LC-MS.

Nr.	ID	m_A/g	m_B/g	R
1	AB	0.5073	0.0924	1.270
2	AB	0.5022	0.0926	1.246
3	AB	0.5029	0.0914	1.245
4	AB	0.5044	0.0945	1.206
5	AB	0.5006	0.0929	1.216

The mass fraction of meloxicam-d_3 (B) in the standard solution is $w_B = 13.3$ ng/g. Molar mass of meloxicam is $M_A = 351$ g/mol and $M_B = 354$ g/mol. Isotope ratio, R, is the average measured ratio from MS-MS transitions of m/z 352 → 115 and m/z 355 → 115 from three replicate measurements.

Table 5. Determination of meloxicam in bovine muscle Certified Reference Material BOTS-1 using LC-MS.

Nr.	ID	m_A/g	m_{A*}/g	m_B/g	R
1	A	0.4956	0.0000	0.0000	155
2	A∗	0.0000	0.1845	0.0000	155
3	B	0.0000	0.0000	0.1827	0
4	AB	0.5073	0.0000	0.0924	1.270
5	A∗B	0.0000	0.1852	0.1845	0.972
6	AA∗B	0.4967	0.1839	0.2786	1.052

The mass fraction of meloxicam in the standard solution is $w_{A*} = 13.03$ ng/g.

An advantage of using regression methods over custom-tailored IDMS equations is that one can readily accommodate additional measurements which would be otherwise incompatible with the IDMS equations at hand. As an example, if one wishes to incorporate measurement Nr. 6 from Table 5, new measurement model equation needs to be developed specifically for this case as it contains a mixture of *three* components. Such equations are unwieldy and incorporating all six measurements shown in Table 5 leads to expressions that are far too complex for them to be useful. Thus, ignoring measurement Nr. 5 leads to measurement model equation

$$w_A = w_{A*} \frac{\begin{array}{r} -m_{B,4} m_{A*,6} (R_3 - R_4)(R_2 - R_6) \\ + m_{A,4} m_{B,6} (R_4 - R_1)(R_3 - R_6) \\ + m_{B,4} m_{A,6} (R_3 - R_4)(R_1 - R_6) \end{array}}{\begin{array}{r} + m_{A*,4} m_{B,5} m_{B,6} (R_5 - R_6)(R_4 - R_2) \\ - m_{A*,5} m_{B,4} m_{B,6} (R_4 - R_6)(R_5 - R_2) \\ + m_{A*,6} m_{B,4} m_{B,5} (R_4 - R_5)(R_6 - R_2) \\ - m_{A,4} m_{B,5} m_{B,6} (R_5 - R_6)(R_4 - R_1) \\ + m_{A,5} m_{B,4} m_{B,6} (R_4 - R_6)(R_5 - R_1) \\ - m_{A,6} m_{B,4} m_{B,5} (R_4 - R_5)(R_6 - R_1) \end{array}} \quad (26)$$

whereas omitting result Nr. 3 leads to a significantly more complex measurement model equation

$$w_A = w_{A*} \frac{\begin{array}{r} + m_{A*,4} m_{B,5} m_{B,6} (R_5 - R_6)(R_4 - R_2) \\ - m_{A*,5} m_{B,4} m_{B,6} (R_4 - R_6)(R_5 - R_2) \\ + m_{A*,6} m_{B,4} m_{B,5} (R_4 - R_5)(R_6 - R_2) \\ - m_{A,4} m_{B,5} m_{B,6} (R_5 - R_6)(R_4 - R_1) \\ + m_{A,5} m_{B,4} m_{B,6} (R_4 - R_6)(R_5 - R_1) \\ - m_{A,6} m_{B,4} m_{B,5} (R_4 - R_5)(R_6 - R_1) \end{array}}{\begin{array}{r} + m_{A*,4} m_{B,5} m_{B,6} (R_5 - R_6)(R_4 - R_2) \\ - m_{A*,5} m_{B,4} m_{B,6} (R_4 - R_6)(R_5 - R_2) \\ + m_{A*,6} m_{B,4} m_{B,5} (R_4 - R_5)(R_6 - R_2) \\ - m_{A,4} m_{B,5} m_{B,6} (R_5 - R_6)(R_4 - R_1) \\ + m_{A,5} m_{B,4} m_{B,6} (R_4 - R_6)(R_5 - R_1) \\ - m_{A,6} m_{B,4} m_{B,5} (R_4 - R_5)(R_6 - R_1) \end{array}} \quad (27)$$

In both cases we assume $g_{A,A*} = 1$. The former measurement model provides $w_A = 3.12$ ng/g whereas the latter gives $w_A = 3.14$ ng/g. While analytical expressions are far too complex to accommodate all results shown in Table 5, the regression method has little difficulty to do so, giving $w_A = 3.12$ ng/g from either the coefficients a_1, a_2 or a_4, a_5 in the model M4 (Table 3).

In summary, data presented in Table 5 can be evaluated using several IDMS equations depending on which subset of the measurements is employed. In contrast, regression-based methods can accommodate a variety of data with a single general equation that can be applied to all three scenarios. Moreover, the same regression model can be used to obtain results from overdetermined system of measurements involving measurements Nr. 1-6.

3.3. Example 3: Elaborate measurement of nitrate

We have performed determination of nitrate using triethyloxonium derivatization and gas chromatography mass spectrometry (GC-MS) [18]. Measurements were done on a standard solution with known mass fraction of nitrate and results are shown in Table. 6. While the experiment shown here is not necessarily a 'good' design for isotope dilution, it aims to illustrate the flexibility of the underlying mathematical technique.

Applying all measurements from Table 6, the ordinary least squares fit of the full measurement model (M1) yields $a_1 = 0.2711$ and $a_2 = 0.5722$ which, in turn, leads to the following mass fraction of nitrate in

Table 6. Determination of nitrate in standard solution with known mass fraction, $w_A = 50.6(1)$ mg/kg, using GC-MS.

Nr.	ID	m_A/g	m_{A*}/g	m_B/g	R
1	AA∗B	0.4995	0.4971	0.6947	0.5888
2	AA∗B	0.6989	0.7962	0.5972	1.0478
3	AA∗B	0.2999	1.6884	1.1911	0.8547
4	AA∗B	0.4987	1.9895	0.4959	2.4670
5	AA∗B	0.6966	2.2896	0.3972	3.5663
6	AA∗B	0.6981	3.7811	0.7947	2.8261
7	AA∗B	0.4976	4.4727	0.4962	5.0209

The mass fraction of nitrate in the primary standard solution is $w_{A*} = 107.3(2)$ mg/kg and the isotope ratio, R, is the ratio of fragment ions m/z 46 ($^{14}NO_2^+$) and 47 ($^{15}NO_2^+$).

the sample standard solution:

$$w_A = w_{A*}\frac{a_1}{a_2} = 50.8 \text{ mg/kg} \tag{28}$$

When the experiment provides more data points than regression parameters, as in Table 6, the uncertainty of the result can be estimated by propagating the uncertainty of the regression coefficients. Thus, when all seven measurements are taken into consideration, the ordinary least squares fit yields coefficients with standard uncertainties $u(a_1) = 0.0067$ mg/kg and $u(a_2) = 0.0026$ mg/kg, and correlation coefficient $r(a_1, a_2) = 0.358$. These uncertainties are then propagated in accordance with the GUM (as implemented in the *NIST Uncertainty Machine* at uncertainty.nist.gov) to yield a value $w_A = 50.8$ mg/kg with standard uncertainty $u(w_A) = 1.2$ mg/kg which is in agreement with the known mass fraction of nitrate in this sample, $w_A = 50.6(1)$ mg/kg.

4. Conclusion

In this manuscript we present a general framework in support of regression-based approach for isotope dilution. This method can be implemented in *Excel* as it is based on multiple linear regression and, more importantly, it avoids the use of polynomial functions which can lead to percent-level errors. Overall, the proposed regression method offers minimal algebraic complexity compared to analytical solutions, has the ability to deal with overdetermined systems, and can accommodate a variety of experimental designs under a single underlying mathematical expression. Furthermore, a key advantage of the regression-based method is that the mathematical complexity does not rise with the amount of data considered.

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