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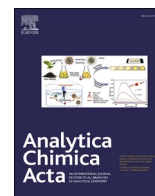
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Accurate measurements of lead using isotope dilution calibration curve method with the accounting for natural isotopic variations

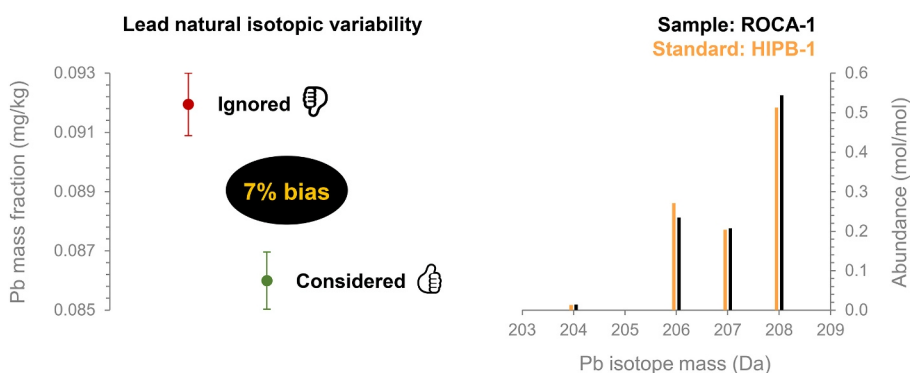
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HIGHLIGHTS

- A user-friendly, graphical isotope dilution method is proposed for accurate measurements of lead.
- The method accounts for natural variations of the isotopic composition of lead, essential to ensure accurate results.
- A software implementation of the method is provided.
- The method is well-suited for high-throughput analysis.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Isotope dilution mass spectrometry (IDMS) is a key method for high-precision measurements. Most implementations, however, implicitly assume that the isotopic compositions of the sample and the primary standard are identical, thereby neglecting the natural isotopic variations. Although this is a reasonable approximation for many chemical elements, ignoring the natural isotopic variations for elements such as lead, boron, or lithium can result in significant systematic errors.

Results: An IDMS calibration curve method capable of accounting for natural isotopic variations is developed and applied for the determination of lead in food samples. The fundamental equations describing the measurement model are derived from first principles and are supported by a suite of Excel-VBA functions that facilitate easy interpretation of the graphical calibration data and their uncertainty evaluation. Measurements of the NRC ROCA-1 cacao powder revealed that neglecting isotopic mismatch between lead in the cacao sample and the primary standard can introduce a 7% bias in the estimated mass fraction of lead. In general, biases of up to 20% are possible. The results obtained using the IDMS curve are compared with the traditional double isotope dilution (ID²MS). Both techniques yield highly accurate results, but the graphical method is easier to implement, more precise, and is better suited for high-throughput analyses.

Significance: In this study, a novel IDMS quantitation approach based on a multipoint calibration curve is proposed. The method effectively eliminates systematic errors resulting from significant differences in the isotopic compositions of lead between the sample and the primary standard. The graphical nature of this calibration

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approach marks a significant departure from current equation-based IDMS methods, aligning more closely with the practices commonly found in analytical laboratories.

Although the method is demonstrated on lead, a toxic element routinely measured in food commodities, this method can be extended to the accurate determination of other elements with significant natural isotopic variability, including technology critical elements such as lithium and boron.

1. Introduction

Isotope dilution mass spectrometry (IDMS) is a high-precision quantitation method that relies on the use of isotopically enriched internal standards [1–3] and is widely employed by National Metrology Institutes for Reference Material (RM) production [4–8]. The internal standard compensates for systematic effects that could otherwise bias the measurement results, such as the loss of analyte or variations in instrument response due to matrix effects. Like with all mass spectrometry-based methods, the accuracy of IDMS can be affected by the natural variations of the isotopic composition of the elements. In most cases, however, IDMS models overlook this effect and are prone to yield biased results. In order to explore the impact of this approximation, we revisit the IDMS method from the first principles.

The first step of IDMS quantification requires spiking the sample (A) with a known amount of an isotopically enriched internal standard (B). The chemical form of the analyte in the sample and the standard should be, ideally, the same, but the two materials must differ in their isotopic composition as much as possible. For a successful IDMS experiment, the blend of A and B (AB) must reach complete isotopic equilibration before analysis. Since isotopes of the same element exhibit near-identical chemical and physical behavior, the measured isotope ratio of the element in the blend AB (r_{AB}) remains largely unaffected by most systematic effects influencing the analytical response of each isotope. Thus, the ratio r_{AB} serves as a robust quantity for accurate quantitation. Typically, r_{AB} is measured by mass spectrometry by dividing the signal of the isotope most abundant in the sample by the signal of the isotope most abundant in the internal standard.

In the traditional IDMS formulation (i.e., single isotope dilution, ID¹MS), the mass fraction of the element in the sample is obtained by applying the following model equation [9]:

$$w_A = w_B \frac{m_{B(AB)} x_{a,B} - x_{b,B} K r_{AB} M_A}{m_{A(AB)} x_{b,A} K r_{AB} - x_{a,A} M_B} \quad (1)$$

where the mass fraction of analyte in the sample (w_A) is a function of the mass fraction of the internal standard (w_B), the gravimetric makeup of the blend AB ($m_{B(AB)}/m_{A(AB)}$), the measured isotope ratio (r_{AB}), isotopic abundances ($x_{i,E}$), and the molar masses (M_E) of the element in A and B (see Table 1).

The isotope ratios measured by mass spectrometry (r) are inevitably biased due to instrumental isotopic fractionation effects, and the isotope ratio correction factor (K) relates the observed isotope ratio (r) to the true isotope ratio (R) [10]:

$$R = K \cdot r \quad (2)$$

Assuming a linearity of response, as per Eq. (2) [11], Eq. (1) can be rewritten:

$$w_A = w_B \frac{m_{B(AB)} r_B - r_{AB} M_A x_{b,B}}{m_{A(AB)} r_{AB} - r_A M_B x_{b,A}} \quad (3)$$

Here, the direct measurements of r_A and r_B (the isotope ratios of the analyte in the pure sample and internal standard, respectively) result in the cancellation of K from the measurement model. However, this ID¹MS strategy has a major deficiency: the traceability of the measurement result rests with the mass fraction of the analyte in the internal standard (w_B). Isotopically enriched materials are usually obtained in small quantities, are difficult to weigh accurately, and are not characterized as

rigorously as calibration standards of natural isotopic composition. Double isotope dilution (ID²MS) overcomes this limitation, by introducing an additional blend – of a calibration standard having a natural isotopic composition (C) with the aforementioned internal isotopic standard (B) [12]. The measurement model is described with two equations of the same style as Eq. (1):

$$\begin{cases} w_A = w_B \frac{m_{B(AB)} x_{a,B} - x_{b,B} K r_{AB} M_A}{m_{A(AB)} x_{b,A} K r_{AB} - x_{a,A} M_B} \\ w_C = w_B \frac{m_{B(CB)} x_{a,B} - x_{b,B} K r_{CB} M_C}{m_{C(CB)} x_{b,C} K r_{CB} - x_{a,C} M_B} \end{cases} \quad (4)$$

The resulting ID²MS equation is:

$$w_A = w_C \frac{x_{a,B} - x_{b,B} K r_{AB} x_{b,C} K r_{CB} - x_{a,C} m_{B(AB)} m_{C(CB)} M_A}{x_{b,A} K r_{AB} - x_{a,A} x_{a,B} - x_{b,B} K r_{CB} m_{A(AB)} m_{B(CB)} M_C} \quad (5)$$

Eq. (5) is one of the most general formulations of ID²MS and accounts for potential differences in the isotopic composition of the analyte between the sample (A) and primary standard (C), and is applicable for accurate quantitation of lead. Eq. (5) is often simplified by assuming identical isotopic composition between A and C ($M_A = M_C$, $x_{a,A} = x_{a,C}$, and $x_{b,A} = x_{b,C}$), which is unsuitable for determination of lead and other elements that exhibit significant variability of their isotopic composition among terrestrial materials [13]. A key advantage of ID²MS is that the traceability of its results now rests with a well-characterized standard of natural isotopic composition (w_C).

To achieve best results, ID²MS is usually implemented in the *exact-matching* design whereby the isotopic composition of the sample and calibration blends are closely matched ($r_{AB} \cong r_{CB}$) [8,14–17]. While this strategy minimizes a variety of systematic errors [14], it does not compensate for isotopic mismatch between the materials A and C. In addition, although exact-matching ID²MS has been widely adopted in the metrological community, it is a single-level calibration approach tailored for a single sample at hand. Hence, this method does not allow to inspect the analytical response over a wider analytical range [18]. To address this issue, several multi-level IDMS methods have been reported

Table 1

Terms and symbols used in this study.

Symbol	Description
A	Sample material
B	Internal standard solution (isotopically enriched)
C	Calibration standard solution
AB	Sample blend (mixture of A and B)
CB	Calibration blend (mixture of C and B)
E	A generic material, can indicate either A, B, C, AB, or CB
a	Analytical (numerator) isotope, in this study $a = {}^{208}\text{Pb}$
b	Reference (denominator) isotope, in this study $b = {}^{207}\text{Pb}$
w_E	Mass fraction (mg/kg) of analyte in E
$m_{E(AB)}$	Mass (g) of E used to prepare blend AB
$m_{E(CB)}$	Mass (g) of E used to prepare blend CB
M_E	Molar mass (g/mol) of analyte in E
n_E	Amount (mol) of analyte in E, $n_E = (m_E \cdot w_E) / M_E$
$n_{i,E}$	Amount (mol) of isotope i ($i = a$ or b) in material E
$x_{i,E}$	Abundance (mol/mol) of isotope i ($i = a$ or b) in E, $x_{i,E} = n_{i,E} / n_E$
R_E	True isotope ratio of the analyte in E, $R_E = n_{a,E} / n_{b,E} = x_{a,E} / x_{b,E}$
r_E	Observed isotope ratio of the analyte in E, $r_E = K \cdot (n_{a,E} / n_{b,E}) = K \cdot (x_{a,E} / x_{b,E})$
K	Isotope ratio correction factor (mass bias), $K = R_E / r_E$
y	Dependent variable of the calibration curve (y-axis), $y = r_{CB}$
q	Independent variable of the calibration curve (x-axis), $q = w_C \cdot m_{C(CB)} / m_B$ (CB)

including triple (ID³MS) [19–21], and quadruple (ID⁴MS) isotope dilution [13]. Unfortunately, the mathematical framework of these methods becomes too complex to be of practical use whereas graphical, curve-based methods provide an attractive alternative [22].

When three or more calibration blends are available, a three-parameter calibration curve can be established [23,24]:

$$r_{CB} = \frac{a_0 + a_1q}{1 + a_2q} \quad (6)$$

$$q = w_C \frac{m_{C(CB)}}{m_{B(CB)}}$$

The isotope ratio of the calibration blend (r_{CB}) is reported on the y-axis and the gravimetric makeup of the blends (q) on the x-axis. The three fitting parameters (a_0 , a_1 , and a_2) are used to obtain the mass fraction of the analyte in the sample:

$$w_A = \frac{m_{B(AB)} r_{AB} - a_0}{m_{A(AB)} a_1 - a_2 r_{AB}} \quad (7)$$

The IDMS curve (Eqs. (6) and (7)) provides a considerably better alternative to the IDMS formulations such as those exemplified by Eq. (5). A key feature is its ability to accommodate any number of additional calibration points and, perhaps more importantly, to allow for visual inspection of the measurements associated with the calibration blends, facilitating the assessment of outliers [25]. However, the IDMS curve described by Eqs. (6) and (7) still assumes that the analyte element has the same isotopic composition in the sample and calibration standard.

In this study, we extended this method to account for potential mismatches in isotopic composition between the sample (A) and the calibration standards (C). The general theory of the method is presented with emphasis on the evaluation of the measurement uncertainty. Raw data and calculations are provided in the supplementary information, along with an Excel VBA-based tool developed for data reduction.

2. Theory and software

When lead quantitation is performed by monitoring individual ⁱPb isotopes, natural variations of its isotopic composition must not be ignored. The isotopic abundances of lead in both the sample and in the primary standard shall be determined first. These values are then used to adjust the isotope dilution calibration curve accordingly. In this section, the theory for accurate lead quantitation by isotope dilution calibration curves is described along with software solutions for data analysis.

2.1. Determination of lead isotopic abundances by mass spectrometry

The isotopic abundances of lead ($x_{i,E}$) in a given material (E) can be obtained from the mass bias-corrected isotope ratios (R_i):

$$x_{i,E} = \frac{R_i}{\sum_i R_i} \quad (8)$$

In practice, material E is analyzed directly by monitoring the ICP-MS signals of all stable isotopes ($i = 204, 206, 207$, and 208) and the isotope ratios are all expressed relative to the same denominator isotope.

In this study, mass bias correction was performed using Eq. (2) and the correction factors (K) were obtained by analyzing NRC HIPB-1 reference material certified for the isotopic composition of lead [26] with the isotope ²⁰⁷Pb chosen as the denominator isotope. Thus, $R_{204} = n(^{204}\text{Pb})/n(^{207}\text{Pb})$, $R_{206} = n(^{206}\text{Pb})/n(^{207}\text{Pb})$, $R_{207} = n(^{207}\text{Pb})/n(^{207}\text{Pb}) = 1$ by definition, and $R_{208} = n(^{208}\text{Pb})/n(^{207}\text{Pb})$.

Although the isotopic abundances $x_{i,E}$ can be calculated using Eq. (8), the uncertainty evaluation is more complex as both input (R_i) and output ($x_{i,E}$) quantities are correlated [27,28]. The methods described in JCGM 102:2011 provide a recipe for the evaluation of uncertainty associated with the isotopic abundances [29]. The variance-covariance matrix of the isotopic abundances (Σ_x) can be expressed as:

$$\Sigma_x = J_R \cdot \Sigma_R \cdot J_R^T \quad (9)$$

where Σ_R is the variance-covariance matrix of the isotope ratios, J_R is the Jacobian matrix of the output quantities with respect to the input quantities in Eq. (8), and J_R^T is its transpose. A detailed discussion of Eq. (9) is given in supplementary Paragraph S1, together with an example of calculation included in the accompanying Excel supplementary file where a VBA function named `xPb_COVAR` can be found. The function outputs both the isotopic abundances and their variance-covariance matrix. The following section describes how these quantities are used to adjust the isotope dilution calibration curves for lead.

2.2. Adjusting the lead isotope dilution calibration curve

Consider a multi-point isotope dilution in which three or more calibration blends are prepared by mixing known amounts of a primary lead standard of natural isotopic composition (C) with an isotopically enriched lead internal standard (B). For each blend CB, an ID¹MS equation of the same form as Eq. (1) can be written:

$$w_C = w_B \frac{m_{B(CB)} x_{a,B} - x_{b,B} \cdot K \cdot r_{CB} M_C}{m_{C(CB)} x_{b,C} \cdot K \cdot r_{CB} - x_{a,C} M_B} \quad (10)$$

This equation can be rearranged as a function of r_{CB} [23]:

$$r_{CB} = \frac{\left[K^{-1} \frac{x_{a,B}}{x_{b,B}} \right] + \left[K^{-1} \cdot w_B^{-1} \frac{M_B \cdot x_{a,C}}{x_{b,B} M_C} \right] \cdot \left(\frac{w_C \cdot m_{C(CB)}}{m_{B(CB)}} \right)}{1 + \left[w_B^{-1} \frac{M_B \cdot x_{b,C}}{x_{b,B} M_C} \right] \cdot \left(\frac{w_C \cdot m_{C(CB)}}{m_{B(CB)}} \right)} \quad (11)$$

In Eq. (11), the terms in square brackets are operational constants that are the fitting parameters indicated in Eqs. (6) and (7):

$$a_0 = K^{-1} \cdot \frac{x_{a,B}}{x_{b,B}}$$

$$a_1 = K^{-1} \cdot w_B^{-1} \cdot \frac{M_B}{x_{b,B}} \cdot \left(\frac{x_{a,C}}{M_C} \right) \quad (12)$$

$$a_2 = w_B^{-1} \cdot \frac{M_B}{x_{b,B}} \cdot \left(\frac{x_{b,C}}{M_C} \right)$$

The variable $w_C \cdot m_{C(CB)} / m_{B(CB)}$ is reported on x-axis and r_{CB} on y-axis of the calibration curve. Values of a_0 , a_1 , and a_2 can be obtained with a high accuracy by applying linear least squares fitting [23,30]. In Excel, this can be implemented using this line of code = `LINEST(Y, x^{1,1} * -y^{0,1}, TRUE, TRUE)`, where x is the column vector reporting the $(w_C \cdot m_{C(CB)} / m_{B(CB)})$ values, and y is the column vector with the corresponding isotope ratios r_{CB} .

These fitting parameters yield an accurate estimate of the mass fraction of lead in the sample (as per Eq. (7)) only when the isotopic composition of lead in this sample is identical to that of the primary standard (C). For accurate measurements of lead, the fitting parameters in Eq. (12) must be adjusted to eliminate their dependency on the isotopic composition of the primary standard. The adjusted parameters \tilde{a}_0 , \tilde{a}_1 and \tilde{a}_2 are given by the following formulas:

$$\tilde{a}_0 = a_0$$

$$\tilde{a}_1 = \frac{M_C \cdot x_{a,A}}{x_{a,C} M_A} a_1 \quad (13)$$

$$\tilde{a}_2 = \frac{M_C \cdot x_{b,A}}{x_{b,C} M_A} a_2$$

This adjustment depends on the isotopic abundances of lead in both the sample and in the primary standard as determined in the previous section (Fig. 1). The molar masses in Eq. (13), depend on $x_{i,E}$:

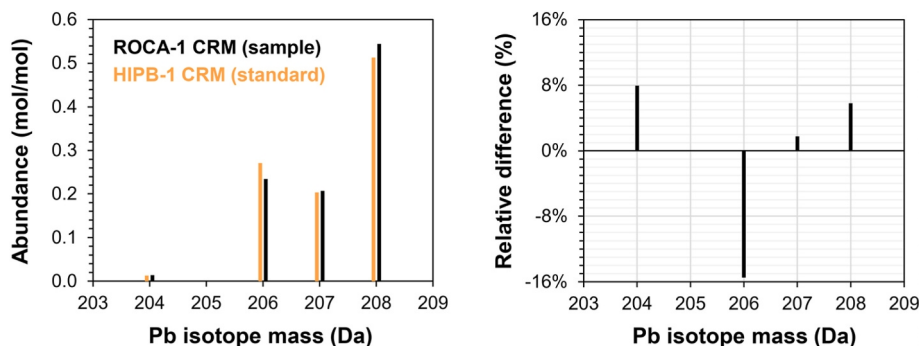


Fig. 1. Differences in the isotopic patterns of sample (NRC CRM ROCA-1) and calibration standard (NRC CRM HIPB-1) used for determination of lead. Overlooking such differences in isotopic composition would result in a 7% measurement bias when using m/z 208 as the analytical signal (numerator isotope) and m/z 207 as the internal standard signal (denominator isotope) in the isotope dilution quantitation of lead in ROCA-1.

$$M_A = \sum_i \left[M(^i\text{Pb}) \cdot x_{i,A} \right] \quad (14)$$

$$M_C = \sum_i \left[M(^i\text{Pb}) \cdot x_{i,C} \right]$$

where $M(^i\text{Pb})$ are the molar masses of the four lead stable isotopes. $M(^i\text{Pb})$ are related to the atomic masses: $M(^i\text{Pb}) = A_r(^i\text{Pb}) \cdot M_u$. In this study, the molar mass constant (M_u) was taken as 1 g/mol, and the values of $A_r(^i\text{Pb})$ were obtained from AME 2020 [31]. Uncertainties associated with M_u and $A_r(^i\text{Pb})$ were considered negligible.

The evaluation of uncertainty associated with \tilde{a}_0 , \tilde{a}_1 and \tilde{a}_2 requires a similar approach as reported in Eq. (9). A detailed discussion is given in supplementary Paragraph S2 with an example of calculation implemented in the Excel supplementary file where a VBA function named `FitIDMS_Pb` was developed. The `FitIDMS_Pb` function takes as input the calibration data, and lead isotope ratios for both the sample and the primary standard, and provides the adjusted fitting parameters along their uncertainties and their covariances.

2.3. Calculation of lead mass fraction in the sample

With the corrected fitting parameters \tilde{a}_0 , \tilde{a}_1 and \tilde{a}_2 , the mass fraction of Pb in the sample (w_A) can be obtained by applying an updated version of Eq. (7):

$$w_A = \frac{m_{B(AB)} \cdot r_{AB} - \tilde{a}_0}{m_{A(AB)} \cdot \tilde{a}_1 - \tilde{a}_2 r_{AB}} \quad (15)$$

For the uncertainty evaluation, the covariance of the corrected fitting parameters must be considered [32]. In the Excel supplementary file, a VBA function named `ResultIDMS_GS_Pb` is proposed to automate the calculation of w_A and its uncertainty.

3. Experimental session

3.1. Reagents and materials

Reagent-grade nitric acid (68-70% HNO_3), optima grade hydrogen peroxide (30-32% H_2O_2), and 8 mesh Drierite with indicator were acquired from Fisher Scientific (Ottawa, Canada). HNO_3 was further purified by sub-boiling distillation using a commercial system available from Milestone Inc. (Shelton, USA). Ultrapure water (18.2 $\text{M}\Omega$ cm) was produced in-house using a Milli-Q ion exchange system from MilliporeSigma (Oakville, Canada). All labware was cleaned by soaking in 0.8 M HNO_3 for several days and then rinsed with water prior to use. The NRC HIPB-1 CRM was used as primary standard [26]. A 3000 mg/kg stock solution of Pb was obtained by dissolving the HIPB-1 lead rod in 4.7 M HNO_3 . Working primary standard solutions were prepared by gravimetric dilution of the stock in 0.31 M HNO_3 . The isotopically

enriched lead internal standard ($w(^{207}\text{Pb}) > 0.92$ g/g) was acquired from Trace Sciences International (Richmond Hill, Canada) and used to prepare a 0.125 mg/kg ^{207}Pb solution in 0.31 M HNO_3 .

The sample analyzed in this study, named ROCA-1, is a cacao powder candidate Certified Reference Material (CRM) from NRC Canada. Additional CRMs were also analyzed as quality control samples including the baking chocolate SRM 2384 from NIST (Gaithersburg, USA), and the canola meal CAME-1 [33] from NRC (Ottawa, Canada).

3.2. Sample preparation

Sample preparation was carried out gravimetrically using a Mettler Toledo XPR 305D5 balance inside a class 10 cleanroom. The balance was equipped with an antistatic device to ensure stable readings and was calibrated daily using SI-traceable reference weights. A sample aliquot of 0.25 g was weighed into a pre-cleaned 50 mL Teflon digestion vessel and spiked with 0.25 g of ^{207}Pb isotopically enriched internal standard solution. Subsequently, 7.0 mL of high-purity nitric acid (68-70%) and 0.5 mL hydrogen peroxide (30-32%) were added.

The sample blends were then digested in a closed vessel microwave digestion system (Multiwave PRO, Anton Paar Canada, Montreal, QC, Canada) by ramping the power to 1400 W over 15 min, holding it for 30 min, and cooling to 55 °C before opening the vessels in a fumehood. The digests were transferred into pre-cleaned Teflon tubes, evaporated to near dryness (0.5 – 1 mL), and reconstituted with water to a final volume of 25 mL (corresponding to 0.3-0.6 M HNO_3). Five procedural blanks (spiked with 0.05 g of ^{207}Pb internal standard solution) were prepared similarly and used for blank subtraction. Calibration blends were prepared by mixing 0.25 g of primary standards (9 solutions were prepared to cover the 0.015-0.150 mg/kg analytical range) with 0.25 g of ^{207}Pb internal standard solution and diluting to 25 mL using 0.31 M HNO_3 . Blank solutions, calibration standards, and samples were analyzed by ICP-MS.

3.3. ICP-MS measurements

Measurements were performed using an Agilent 8900 ICP-MS/MS (Agilent Technologies, Santa Clara, USA) equipped with a standard sample introduction system consisting of a MicroMist glass concentric nebulizer, quartz spray chamber, and quartz torch with 2.5 mm ID injector. The interface was fitted with a nickel-plated copper sampling cone and a nickel skimmer cone. Since analytical sensitivity was not a limiting factor for this application, the instrument was operated in MS/MS mode using He collision gas at a flow rate of 5.0 mL/min to better control polyatomic interference during detection of ^{204}Pb , ^{206}Pb , ^{207}Pb and ^{208}Pb . For the ROCA-1 sample, the counts per second obtained were as follows: $1.8 \cdot 10^3$ for m/z 204, $2.9 \cdot 10^4$ for m/z 206, $2.7 \cdot 10^4$ for m/z 207, and $6.9 \cdot 10^4$ for m/z 208. The Agilent 8900 ICP-MS/MS acquisition software automatically performed deadtime correction. All test

solutions were introduced to the ICP-MS via a peristaltic pump at 0.32 mL/min. Daily optimization was performed according to manufacturer to ensure suitable sensitivity and stability.

3.4. Moisture content

All results are reported on a dry-weight basis. The dry-weight correction factor was obtained by mass loss of the sample after storage in a desiccator. A 0.5 g aliquot of sample was transferred in a glass weighing bottle and its mass (m_0) was measured using a Mettler Toledo XPR 305D5 balance. The sample was then stored open in a desiccator over Drierite, and reweighed periodically for 7 weeks until stable mass was reached (m_1). The dry-weight correction factor was m_1/m_0 .

4. Result and discussion

In this section, the performance of the IDMS curve method is compared to the exact-matching double isotope dilution (ID²MS) [34, 35] which, among higher-order IDMS formulations, is the only other method suitable for quantitation of lead (Eq. (5)). Although both IDMS models rely on the isotopic patterns of Pb to obtain unbiased results (Paragraph 2.1), their implementation differs in several important aspects, which are discussed below.

4.1. Adjustment of the IDMS calibration curve

A multi-point IDMS calibration curve for quantitation of lead was obtained by analysis of one blank and nine standard solutions prepared from HIPB-1 CRM [26]. Because the isotopic compositions of lead in HIPB-1 (primary standard, C) and ROCA-1 (sample, A) differ (Fig. 1), the calibration curve required a correction to account for this difference. Fig. 2 shows the magnitude of the adjustment necessary for accurate quantitation of Pb in ROCA-1. Neglecting the isotopic mismatch between the sample and the primary standard could result in a systematic bias of 7% in the reported mass fraction of lead.

The magnitude of this bias is proportional to the difference in isotopic composition between sample and standard, and, as shown in Fig. 2, also depends on the measured isotope ratio. Therefore, the adjustment required to obtain accurate results is sample-dependent. For example, determination of lead in other CRMs (*i.e.*, SRM 2384, and CAME-1) showed smaller bias, amounting to less than 2%.

Lead exhibits considerable isotopic variability within terrestrial materials which depends upon several factors, including geographical provenance and geological history [36]. As a result, ignoring this effect can potentially lead to biases greater than those observed in this work. For example, using the popular NIST SRM 981 primary standard to quantify Pb in certain igneous and sedimentary rock samples from India

could result in biases of 10-20% when the isotopic mismatch is not accounted for. In more extreme cases, such as for monazite samples composed almost exclusively of ²⁰⁸Pb, these biases could surpass 100%.

4.2. Inspection of the IDMS calibration curve

The graphical IDMS is based on a multi-point calibration curve which provides a visual representation of the analytical response. This is a departure from traditional ID²MS methods which only requires implementation of Eq. (5) for direct calculation of the results based on a single calibration level.

Since IDMS is an inherently nonlinear model that becomes asymptotic at high $m_{C(CB)}/m_{B(CB)}$ ratios [23,24], the ability to visualize calibration data is crucial for experimental design, method validation, and the detection of outlier data points [18].

Fig. 2 shows the nonlinearity encountered in the analysis of lead when the measured isotope ratio r_{CB} falls in the [0.1-0.6] interval. The r_{CB} upper limit of 0.6 was chosen to minimize the extent of nonlinear behavior to an acceptable level. Major departures from linearity, able to affect precision, could be observed when $r_{CB} \gg 0.6$. In this vein, the calibration curve could serve as a powerful diagnostic tool for identifying errors in experimental design, such as improper levels of isotope spiking. Furthermore, working with calibration curves instead of complex model equations like Eq. (5), may be more intuitive for analytical chemists familiar with the traditional quantitation approaches such as the external calibration.

4.3. Accuracy and precision

For validation purposes, the performance of the IDMS curve method was evaluated against the traditional exact-matching double isotope dilution (ID²MS, Eq. (5)). The experiment was designed to allow the simultaneous application of both models to the same dataset. As shown in Tables 2 and 3, the two techniques yielded comparable results, providing effective cross-validation. For the analysis of ROCA-1 (Table 2), the difference between the mass fraction of lead determined by the two methods is approximately 0.3%, well below the 1.1% standard uncertainty of the result. The small difference observed between the methods is due to the wider range of calibration blends used to define the IDMS curve. While ID²MS uses only the “exact-matching” calibration data (Fig. 2, red circles), the multipoint calibration curve approach can accommodate the use of all of them (Fig. 2, red circles and grey diamonds). Notably, the uncertainty obtained with the graphical approach is ~30% lower than that obtained using ID²MS. This finding is consistent with the early studies on triple isotope dilution (ID³MS) and quadruple isotope dilution (ID⁴MS), in which a 5-20% reduction in measurement uncertainty was observed when multi-point IDMS models

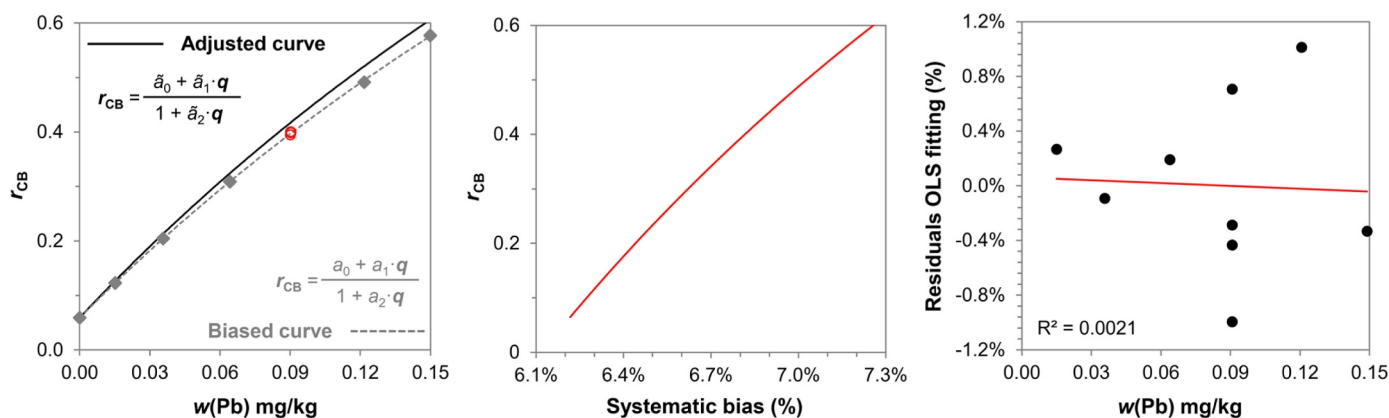


Fig. 2. Accounting for natural isotopic variation in the graphical IDMS method. Left: adjustment of the calibration curve for accurate quantitation of Pb in ROCA-1. Middle: magnitude of the systematic bias corrected by the method. Right: residual analysis.

Table 2

Mass fraction of lead in NRC ROCA-1 cacao powder as measured by ID²MS and by IDMS curve (mg/kg, dry-weight basis).

Unit	ID ² MS	IDMS curve	Difference
Identifier	w_A (mg/kg)	w_A (mg/kg)	(%)
ROCA096	0.0906 (14)	0.0908 (10)	-0.22%
ROCA649	0.0845 (13)	0.0847 (10)	-0.27%
ROCA384	0.0837 (13)	0.0840 (10)	-0.28%
ROCA653	0.0866 (13)	0.0868 (10)	-0.23%
ROCA456	0.0877 (13)	0.0879 (10)	-0.24%
ROCA408	0.0836 (13)	0.0838 (10)	-0.28%
ROCA503	0.0838 (13)	0.0840 (10)	-0.29%
ROCA191	0.0890 (13)	0.0892 (10)	-0.24%
ROCA072	0.0864 (13)	0.0866 (10)	-0.23%
ROCA480	0.0819 (12)	0.0821 (10)	-0.27%
ROCA655	0.0874 (13)	0.0876 (10)	-0.26%
ROCA024	0.0868 (13)	0.0870 (10)	-0.26%
ROCA263	0.0832 (13)	0.0834 (10)	-0.29%

Thirteen subsamples were measured. The differences between subsamples are due to the homogeneity of the CRM. Standard uncertainty is reported in the brackets and apply to the last two significant digits of the result.

Table 3

Mass fraction of lead in QC samples as measured by IDMS curve (mg/kg, dry-weight basis).

CRM	Certified value	IDMS curve
SRM 2384	0.0357 (23)	0.0368 (35)
CAME-1	0.0560 (40)	0.0521 (26)

Uncertainties reported in parenthesis are standard uncertainties ($k = 1$) and apply to the last two significant digits of the result. Three independent digestions of each QC sample were performed and the results are comparable to the certified values [37,38].

were applied instead of the single-point ID²MS [13,20]. Our results further reinforce the paradigm that increasing the number of calibration points improves measurement precision.

4.4. Analytical range

The traditional ID²MS serves as a reference method for the analysis of a single sample. Indeed, to effectively apply the exact-matching ID²MS method, the concentration of the analyte in the sample must be known beforehand so that a single calibration can be prepared to match the sample. The definition of ‘exact-matching’ remains ambiguous, and the extent of allowable mismatch is not clearly established. The literature includes both strict iterative methods [14,15] designed to achieve maximum matching, as well as more relaxed strategies that allow for ‘approximate matching’ [39,40], including arbitrary cutoffs such as a $\pm 25\%$ mismatch between the sample and standard [17]. For these reasons, ID²MS is not well suited for the analysis of multiple samples spanning over a wide range of concentration.

In contrast, the IDMS curve was developed to accommodate a wider range of samples using a single calibration set. In this case, the calibration curve was design to cover a Pb mass fraction range of 0.015–0.15 mg/kg, suitable for the analysis of all samples within a single sequence (Tables 2 and 3). The method upheld accuracy and precision as further demonstrated by the analysis of the residuals which are randomly distributed and exhibit the typical heteroscedastic trend associated with mass spectrometry response (Fig. 2). Given its wider calibration range and suitability for multi-sample analysis, the IDMS curve is well suited for implementation in high-throughput laboratories.

4.5. Mathematical complexity

The IDMS curve method enhances the mathematical efficiency of data analysis. The regression analysis required to construct the

calibration curve yields only three fitting parameters. Consequently, the model equation for the IDMS curve (Eq. (15)) involves only six variables, offering simplified calculations of results and uncertainty evaluation. In contrast, traditional ID²MS (Eq. (5)) does not utilize variable grouping, resulting in a fourteen-variable equation that is less intuitive and more cumbersome to use. As described in Section 2, a suite of Excel-VBA functions is developed to automate and further streamline data processing for the quantitation of lead using the IDMS curve.

5. Conclusions

The growing interest in isotope dilution methods based on calibration curves has underlined the need to extend this quantitation method to analytes which show significant natural isotopic variations. For elements like lead, lithium, and boron, isotopic differences between sample and primary standards can induce significant systematic biases, if left uncorrected. Therefore, the primary goal of the study was to provide the mathematical tools to address the issue. The foundational aspects of IDMS calibration curves were revisited from first principles, offering a transparent pathway for both value calculation and uncertainty evaluation. This IDMS method is based on a multipoint calibration curve that can be applied over a wide range of concentration. This approach offers several advantages over traditional IDMS methods. It provides a visual representation of the calibration curve, which helps in the identification of optimal experimental conditions to minimize nonlinearity. Additionally, the extended range covered by the calibration curve enables the analysis of multiple samples within a single sequence, allowing for high-throughput analysis. It is important to emphasize that the IDMS curve method preserves the accuracy and precision expected of an isotope dilution method while providing a simpler and more intuitive mathematical formulation compared to traditional approaches.

CRedit authorship contribution statement

Enea Pagliano: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Writing – original draft. **Kenny Nadeau:** Data curation, Investigation, Resources, Writing – review & editing. **Lu Yang:** Supervision, Writing – review & editing. **Patricia Grinberg:** Funding acquisition, Project administration, Supervision, Writing – review & editing. **Juris Meija:** Conceptualization, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aca.2026.345550>.

Data availability

All raw data, calculation and software codes used to produce the following results are provided in the supplementary Excel file.

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