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Publisher's version / Version de l'éditeur:

<https://doi.org/10.1007/s00216-013-6724-5>

Analytical and Bioanalytical Chemistry, pp. 1-9, 2013-03

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REDUCTION OF MEASUREMENT UNCERTAINTY BY EXPERIMENTAL DESIGN IN HIGH-ORDER (DOUBLE, TRIPLE, AND QUADRUPLE) ISOTOPE DILUTION MASS SPECTROMETRY: APPLICATION TO GC-MS MEASUREMENT OF BROMIDE

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ABSTRACT

Since its introduction a century ago, isotope dilution analysis has played a central role in developments of analytical chemistry. This method has witnessed many elaborations and developments over the years. To date we have single, double and even triple isotope dilution methods. In this manuscript we summarize the conceptual aspects of isotope dilution methods and introduce the quadruple dilution, and the concept of exact-matching triple and quadruple dilutions. The comparison of isotope dilution methods is performed by determination of bromide ions in groundwater using novel ethyl-derivatization chemistry in conjunction with GC/MS. We show that the benefits of higher-order isotope dilution methods are countered with a greater need for careful experimental design of the isotopic blends. Just as for ID²MS, ID³MS and ID⁴MS perform best when isotope ratio of one sample/spike blend is matched with that of a standard/spike blend (exact-matching).

KEYWORDS

Isotope dilution analysis, Double dilution, Triple dilution, Quadruple dilution, Exact-matching, Bromide, Triethyloxonium tetrafluoroborate, GC/MS

1. INTRODUCTION

Isotope dilution analysis is widely recognized as one of the most accurate tools of modern quantitative analysis [1–4]. In its original conception, the method of isotope dilution comprises the addition of isotopically-enriched substance to the sample. If the amount of added spike (n_B) is known, classical isotope dilution method yields the amount of substance in the sample (n_A), provided that the isotopic compositions of the analyte, of the spike, and of the resulting mixture are also known beforehand:

$$n_A = n_B \frac{R_B - R_{AB}}{R_{AB} - R_A} \cdot \frac{\Sigma R_A}{\Sigma R_B} \quad \text{or} \quad (1)$$

$$w_A = w_B \frac{R_B - R_{AB}}{R_{AB} - R_A} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \cdot \frac{\Sigma R_A}{\Sigma R_B} \cdot \frac{M_A}{M_B} \quad (2)$$

where w_A is the mass fraction of the analyte (A) in the sample, $m_{A(AB),aq}$ is the mass of the sample solution used to prepare the blend AB, etc. (see Table 1). Numerous practical challenges notwithstanding [3,4], it is inevitable that some of these variables, such as the amount of the added spike (n_B) or the isotopic composition of the spike (R_B) – are not known in practice. As a consequence, variations of the classical isotope dilution method have been sought to remedy the need for these requirements. This essay is an account for the possibilities of a successful isotope dilution analysis while obviating some of these variables.

Table 1. The list of variables

Symbol	Name
A	Analyte
A*	Primary reference standard of natural isotopic composition
B	Isotopically enriched standard
AB	Blend of A and B
A*B	Blend of A* and B
n_X	Amount of X (X = A, A*, or B)
M_X	Molar mass of X (X = A, A*, or B)
w_X	Mass fraction of X in the aqueous solution (X = A, A*, or B)
$m_{X(XY),aq}$	Mass of the aqueous solution of X used to prepare the blend of X and Y (X,Y =
R_X	Isotope amount ratio corresponding to material X
r_X	Isotope amount ratio in material X as measured by the mass spectrometer. All ratios correspond to $m/z = 108$ Da over 110 Da.
K	Mass-bias calibration factor, $K = R/r$
ΣR_X	Sum of all isotope amount ratios of the same denominator. For bromine, $\Sigma R_{Br} = R_{Br,79/81} + R_{Br,81/81} = R_{Br,79/81} + 1$.

In this work we perform the determination of bromide ions in a groundwater reference material, BCR-611, in order to support a critical discussion of the various isotope dilution quantitation approaches. For this purpose, triethyloxonium tetrafluoroborate, $\text{Et}_3\text{O}^+[\text{BF}_4]^-$, derivatization was used. This chemistry has only recently been introduced in analytical chemistry for the ethylation of various inorganic anions in aqueous media [5,6]. The ethyl-derivatives of many anions, such as bromoethane, are volatile, can be sampled in the headspace, and subsequently separated by gas chromatography, which offers high separation power in comparison to the traditional ion chromatography approach.

2. VARIATIONS

Because the results of mass spectrometry measurements are almost inevitably biased, determination of instrumental bias forms an integral part of any mass spectrometric measurement, including isotope dilution. For the majority of mass spectrometers, the isotope amount ratio response (r_i) is directly proportional to the actual (true) amount ratio (R_i), a feature commonly known as the linearity of response. Consequently, the measured isotope amount ratio can be linked to the actual amount ratio as follows:

$$R = K \cdot r \quad (3)$$

Here K is the mass-bias calibration factor. Note, however, that the above expression is not a definition. Rather, it is a statement which may or may not be valid for a particular measuring device (see for example [7]).

2.1. Single dilution. In the classical single dilution method the least number of variables is measured (r_{AB} , in fact) while leaving the maximum number of variables to be known in advance (w_B , R_B , R_A , K , ΣR_A , ΣR_B , M_A , and M_B):

$$w_A = w_B \frac{R_B - K \cdot r_{AB}}{K \cdot r_{AB} - R_A} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \cdot \frac{\Sigma R_A}{\Sigma R_B} \cdot \frac{M_A}{M_B} \quad (4)$$

This is a remarkably simple method for routine laboratories when all the necessary information regarding the concentration and the isotopic composition of the enriched spike is known beforehand.

As the first variation of Eq. 4, the isotopic composition of the spike and that of the analyte can be measured alongside the isotopic composition of the mixture. Assuming identical mass bias over the course of measuring ratios r_A , r_B , and r_{AB} , i.e., $R_A = Kr_A$, $R_B = Kr_B$, and $R_{AB} = Kr_{AB}$, the mass bias correction factor, K , cancels out and we obtain the following:

$$w_A = w_B \frac{r_B - r_{AB}}{r_{AB} - r_A} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \cdot \frac{\Sigma R_A}{\Sigma R_B} \cdot \frac{M_A}{M_B} \quad (5)$$

Equation 5 forms the basis of all isotope dilution methods. In addition to the isotope amount ratio in the blend of the analyte and the spike, r_{AB} , it contains many other variables – w_B , r_B , r_A , ΣR_A , ΣR_B , M_A , and M_B . Some of these variables are hard to establish in practice and they could be eliminated altogether if additional independent equations – just like the Eq. 5 – can be obtained. This can be achieved by producing and measuring additional blends AB or A*B. This gives rise to multiple dilution methods.

2.2. Double dilution. Isotopically-enriched materials are usually available in small quantities thus making the direct weighing a difficult task. In addition, the chemical purity of enriched materials is not always on par with primary standards of natural isotopic composition. For these reasons alone, the mass fraction of the spike in the primary spike solution (w_B) is the one variable whose elimination from the isotope dilution equation is the most sought-after variation of the traditional isotope dilution. This task is achieved by the coupling of two single isotope dilution measurements if the same solution of enriched spike (w_B) is used for both dilutions.

Consider the single dilution equation (Eq. 2). If the mass fraction of the enriched spike is measured in the same fashion, i.e., from a “reverse” isotope dilution of the enriched spike (B) with natural standard (A*), i.e.,

$$w_B = w_{A^*} \cdot \frac{R_{A^*} - R_{A^*B}}{R_{A^*B} - R_B} \cdot \frac{m_{A^*(A^*B),aq}}{m_{B(A^*B),aq}} \cdot \frac{\Sigma R_B}{\Sigma R_{A^*}} \cdot \frac{M_B}{M_{A^*}} \quad (6)$$

This expression, when combined with Eq. 2, yields the complete double dilution equation:

$$w_A = w_{A^*} \cdot \frac{R_{A^*} - R_{A^*B}}{R_{A^*B} - R_B} \cdot \frac{R_B - R_{AB}}{R_{AB} - R_A} \cdot \frac{m_{A^*(A^*B),aq}}{m_{B(A^*B),aq}} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \cdot \frac{\Sigma R_A}{\Sigma R_{A^*}} \cdot \frac{M_A}{M_{A^*}} \quad (7)$$

Evidently, the two variables concerning the enriched spike – i.e., ΣR_B and M_B – have been eliminated. This creates the first important advantage of the double spike method. In addition, if the natural standard (A*) and the analyte (A) are identical in their isotopic composition (i.e., $M_A = M_{A^*}$ and $R_A = R_{A^*}$), which is usually the case, the double isotope dilution equation simplifies to the following:

$$w_A = w_{A^*} \cdot \frac{R_A - R_{A^*B}}{R_{A^*B} - R_B} \cdot \frac{R_B - R_{AB}}{R_{AB} - R_A} \cdot \frac{m_{A^*(A^*B),aq}}{m_{B(A^*B),aq}} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \quad (8)$$

Although it is a common practice to assume that the isotopic composition of the analyte is identical to that of the natural standard, this might be not the case for elements such as lithium or lead. The isotope amount ratio $n(^{207}\text{Pb})/n(^{206}\text{Pb})$ in common materials, for example, varies in a 10% range. Therefore care must be taken when these elements are determined at high-levels of precision.

One can distinguish two strategies to apply Eq. 8 in practice. First, if R_A , R_B , R_{AB} and R_{A^*B} are all measured in the same sequence in order to attain identical mass bias, i.e. $R_i = K r_i$ ($i = A, B, AB, A^*B$) the mass bias factors cancel-out and the Eq. 8 becomes:

$$w_A = w_{A^*} \cdot \frac{r_A - r_{A^*B}}{r_{A^*B} - r_B} \cdot \frac{r_B - r_{AB}}{r_{AB} - r_A} \cdot \frac{m_{A^*(A^*B),aq}}{m_{B(A^*B),aq}} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \quad (9)$$

Alternatively, if the double dilution is designed so that $r_{AB} \approx r_{A^*B}$ by ensuring that

$$\frac{m_{A^*(A^*B),aq}}{m_{B(A^*B),aq}} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} = \left(\frac{m_{A^*(A^*B),aq}}{m_{A(AB),aq}} \cdot \frac{m_{B(AB),aq}}{m_{B(A^*B),aq}} \right) \approx 1 \quad (10)$$

then Eq. 8 becomes:

$$w_A \approx w_{A^*} \quad (11)$$

In practice, the condition given in Eq. 10 is attained by taking equal amounts of sample and primary standard, which is prepared to match the estimated mass fraction of the analyte in the sample, and followed by addition of equal amounts of enriched spike to both of these materials. This particular experimental design is called exact-matching double isotope dilution [8]. As it is evident from Eq. 11, the exact-matching creates a situation where mass spectrometer acts as a null-indicator. Therefore, all isotope ratio measurements become largely irrelevant to the resulting value of w_A when $r_{AB} \approx r_{A^*B}$. In particular, albeit r_B does enter in the double dilution equation (Eq. 9), its precise value does not need to be known.

2.3. Triple dilution. The robustification of double dilution into exact-matching double dilution obviates the need for the precise value of the isotopic composition of the enriched spike, r_B . An alternative means to avoid direct measurement of enriched spike is the triple isotope dilution method [9,10]. Here, double dilution is augmented with an additional blend of natural standard and enriched spike, A*B-2. Consider a set of two double dilution equations (Eq. 9):

$$w_A = w_{A^{*-1}} \cdot \frac{r_A - r_{A^{*B-1}}}{r_{A^{*B-1}} - r_B} \cdot \frac{r_B - r_{AB}}{r_{AB} - r_A} \cdot \frac{m_{A^{*}(A^{*B-1}),aq}}{m_{B(A^{*B-1}),aq}} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \quad \text{and} \quad (12)$$

$$w_A = w_{A^{*-2}} \cdot \frac{r_A - r_{A^{*B-2}}}{r_{A^{*B-2}} - r_B} \cdot \frac{r_B - r_{AB}}{r_{AB} - r_A} \cdot \frac{m_{A^{*}(A^{*B-2}),aq}}{m_{B(A^{*B-2}),aq}} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \quad (13)$$

From here it is possible to exclude r_B analytically:

$$w_A = \frac{m_{A^{*-2}}r_1 + m_{A^{*-1}}r_2}{r_3} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \quad (14)$$

where

$$m_{A^{*-i}} = w_{A^{*-i}} \frac{m_{A^{*}(A^{*B-i}),aq}}{m_{B(A^{*B-i}),aq}} \quad \text{and} \quad \begin{aligned} r_1 &= (r_{AB} - r_{A^{*B-1}})(r_A - r_{A^{*B-2}}), \\ r_2 &= (r_{AB} - r_{A^{*B-2}})(r_{A^{*B-1}} - r_A), \\ r_3 &= (r_{AB} - r_A)(r_{A^{*B-1}} - r_{A^{*B-2}}). \end{aligned}$$

We have obtained the model equation for the triple dilution method, which comprises a single blend of sample and spike (AB), and two blends of (natural) standard and spike (A*B). Note that triple dilution can also be performed using *two* blends of AB and *one* blend of A*B. In such case we obtain the following triple dilution equation:

$$w_A = \frac{r_{3^*}}{m_{A-2}r_{1^*} + m_{A-1}r_{2^*}} \cdot \frac{m_{A^{*}(A^{*B}),aq}}{m_{B(A^{*B}),aq}} \quad (15)$$

where

$$m_{A-i} = \frac{1}{w_{A^*}} \frac{m_{A(AB-i),aq}}{m_{B(AB-i),aq}} \quad \text{and} \quad \begin{aligned} r_{1^*} &= (r_{A^{*B}} - r_{AB-1})(r_A - r_{AB-2}), \\ r_{2^*} &= (r_{A^{*B}} - r_{AB-2})(r_{AB-1} - r_A), \\ r_{3^*} &= (r_{A^{*B}} - r_A)(r_{AB-1} - r_{AB-2}). \end{aligned}$$

For sake of brevity, we will not consider this variation of ID³MS in this manuscript.

Similar to the double dilution, triple dilution can be designed so that $r_{AB} \approx r_{A^{*B-1}}$. Akin to the exact-matching double dilution, Eq.14 simplifies to

$$w_A \approx w_{A^{*-1}} \quad (16)$$

Although the exact-matching triple dilution (Eq. 16) appears identical to the exact-matching double dilution (Eq. 11), the two approaches differ conceptually. The exact-matching double dilution eliminates quantity r_B numerically via the intelligent experimental design (robustification) thereby rendering the numerical value of r_B largely irrelevant. The exact-matching triple spike, however, builds from the exact-matching double dilution and, in addition, eliminates the variable r_B analytically via the expanse in the measurement complexity whereby r_B no longer appears as a variable.

2.4. Quadruple dilution. The isotopic composition of the natural standard (and the analyte) can further be eliminated from isotope dilution equations using quadruple dilution. Since the natural isotopic composition of elements is well-known, such a move might appear of no advantage in atomic spectrometry. However, mass-bias correction in isotope dilution methods can be obviated only when r_A , r_B and r_{AB} or r_{A*B} are *all* measured (as per Eq. 9 and alike). Direct measurement of r_A , however, is not necessarily a straightforward task, especially when minor isotopes are involved ($r_A > 50$), which is often the case in molecular spectrometry.

Quadruple dilution equation is obtained by combining three double dilution equations. This can be achieved in three different ways: (1) either by using two blends of AB and two blends of A*B, (2) by using three blends of AB and one blend of A*B or (3) by using one blend of AB and three blends of A*B. The latter approach yields the following quadruple dilution equation (assuming $R_A = R_{A*}$):

$$w_A = - \frac{m_{A*2}m_{A*3}r_4 + m_{A*1}m_{A*3}r_5 + m_{A*1}m_{A*2}r_6}{m_{A*1}r_4 + m_{A*2}r_5 + m_{A*3}r_6} \cdot \frac{m_{B(AB),aq}}{m_{A(AB),aq}} \quad (17)$$

where

$$m_{A*i} = w_{A*i} \frac{m_{A*(A*B-i),aq}}{m_{B(A*B-i),aq}}, \text{ and } \begin{aligned} r_4 &= (r_{AB} - r_{A*B-1})(r_{A*B-2} - r_{A*B-3}), \\ r_5 &= (r_{AB} - r_{A*B-2})(r_{A*B-3} - r_{A*B-1}), \\ r_6 &= (r_{AB} - r_{A*B-3})(r_{A*B-1} - r_{A*B-2}). \end{aligned}$$

As with the previous variations of isotope dilution, the situation $r_{AB} \approx r_{A*B-1}$ leads to the exact-matching quadruple dilution.

In a sense, the quadruple dilution is the epitome of the *indirect* measurements. Here, neither the sample, natural standard, nor the enriched spike is measured directly. Rather, only mixtures are measured. Such a strategy was also followed in the recent Avogadro project, where atomic weight of the highly-enriched silicon-28 was established without the direct measurement of the silicon-28 isotope [11-13].

3. EXPERIMENTAL

3.1. Reagents and materials. Standard solutions of bromide were prepared from a primary standard solution of bromide (NIST SRM-3184; Gaithersburg MA, USA). Triethyloxonium tetrafluoroborate was sourced from Fluka ($w > 0.97$ g/g) and an aqueous solution of ammonia (Tamapure AA-100; $w(\text{NH}_3) = 0.2$ g/g) was used for sample preparation. Groundwater reference

material certified for bromide content was obtained from the Institute for Reference Materials and Measurements (BCR-611, Individual identification No. 0049; Geel, Belgium). Isotopically labeled potassium bromide (K^{79}Br , $x(^{79}\text{Br}) \geq 0.94$ mol/mol) was sourced from Trace Sciences International (Richmond Hill ON, Canada). MilliQ water was used to prepare all solutions.

3.2. Safety considerations. Triethyloxonium tetrafluoroborate is a strong ethylating agent and must be handled in a fume-hood with appropriate personal protective equipment avoiding the exposure to fumes which arise from its decomposition (such as diethyl ether and hydrofluoric acid). Exposure to this reagent is minimized as it is a nonvolatile crystalline salt, which undergoes complete hydrolysis within 3 h of its dissolution in water. $\text{Et}_3\text{O}^+[\text{BF}_4]^-$ is highly hygroscopic so it is important to limit its exposure to moisture. It should be kept in a freezer.

3.3. Sample preparation. A 2 mL volume of the aqueous sample was introduced without pretreatment into a 4 mL vial and the K^{79}Br solution was then added. The resulting blend was alkalized with a 50 μL volume of $w(\text{NH}_3) = 0.2$ g/g solution, and then spiked with 100 μL of freshly prepared aqueous $\text{Et}_3\text{O}^+[\text{BF}_4]^-$ solution (x g of the solid salt dissolved in 1.5x g of water) in order to convert bromide ions into the ethyl derivative:

Sample vials were immediately sealed with a screw-cap containing an unpunctured poly-(tetrafluoroethylene)/silicone septum and were kept in the dark at room temperature for at least 3 h before the analysis. Alkaline conditions ($\text{pH} = 10$) were chosen in order to avoid any potential interference with bromate ions present as a result of the bromide/bromate disproportionation:

3.4. GC/MS. A Hewlett-Packard 6890 gas chromatograph equipped with a Hewlett-Packard 5973 mass selective detector was operated at constant flow of helium (0.9 mL /min) with a 60 m DB-624 capillary column (94% dimethyl polysiloxane with 6% cyanopropyl-phenyl groups). A narrow inlet liner designed for SPME (internal diameter of 0.75 mm) was used and a headspace volume of 250 μL was injected into the GC/MS. The GC was operated in pulsed split mode (5:1) with the pulse pressure of 172 kPa for 1 min. The inlet temperature was 100 °C and the oven was heated as follows: 30 °C for 7 min and then 20 °C/min to 200 °C. Transfer line temperature of 230 °C was chosen. Under these conditions the analyte elutes at 8.8 min (as bromoethane). Working

conditions of MS were standard (EI at 70 eV, ion source temperature at 250 °C, and quadrupole temperature at 150 °C). Detection of these two compounds was performed in single-ion monitoring mode at $m/z = 108$ and 110 Da ($\text{C}_2\text{H}_5\text{Br}^+$) with a 100 ms dwell time for each ion. Background-subtracted peak areas were used as analytical signals. All isotope ratios in this work are the ratios of $m/z = 108$ Da over 110 Da, which represent the amount ratio of $\text{C}_2\text{H}_5^{79}\text{Br}$ over $\text{C}_2\text{H}_5^{81}\text{Br}$. Instrumental limit of detection obtained by processing calibration solutions is $w(\text{Br}^-)_{\text{DL}} = 0.25 \text{ ng/g}$. This value is based on the signal-to-noise ratio calculated from the standard deviation of the baseline in the proximity of the analyte peak (i.e., detection limit corresponds to a signal-to-noise-ratio of three). Standard solution of $w(\text{Br}^-) = 2 \text{ ng/g}$ gives a signal-to-noise ratio of 26:1.

4. RESULTS AND DISCUSSION

To demonstrate the kinship between the variants of the isotope dilution methods, we have performed measurements of bromide ions in a certified groundwater reference material using a gamut of isotope dilution approaches: (1) double dilution, (2) exact-matching double dilution, (3) triple dilution, (4) exact-matching triple dilution, (5) quadruple dilution, and (6) exact-matching quadruple dilution. Figure 1 summarizes the assumptions and the input variables inherent to the each variant.

Figure 1. Variations of isotope dilution approaches from single to quadruple dilution.

Variables in white ellipses are determined experimentally (isotope amount ratios and mass-bias correction coefficient) whereas those in grey ellipses are known (such as the mass fraction of spike, or primary standard). Successive elimination of difficult-to-measure variables shows the kinship between all dilution methods: double dilution (ID^2MS) eliminates w_B , triple dilution (ID^3MS) further eliminates r_B , and quadruple dilution (ID^4MS), in turn, eliminates r_A . In all cases, the difficult-to-measure variables are replaced by easy-to-measure isotope amount ratios from additional standard/spike blends (r_{A^*B-i}). All dilutions become exact-matched when $r_{AB} = r_{A^*B-1}$.

In this work we performed 42 isotope amount ratio measurements – of standard (A^*) and enriched spike (B), both in sextuplicate, of three different sample/spike blends (AB), each in quintuplicate, and of fifteen sample/spike blends (A^*B). Figure 2 summarizes the isotopic composition of the blends used in this work and all experimental data pertinent to the preparation and measurement of all blends AB and A^*B are given in Table 2. In addition, the following average results were obtained from six replicate measurements over a three-day period: $r_{A^*} = 1.018(6)_{k=1}$ and $r_B = 15.81(10)_{k=1}$, where the numbers in parentheses are the combined standard uncertainties referred to the last applicable digits of the quoted value. Throughout this work we assume $R_A = R_{A^*}$ (and *ipso facto* $M_A = M_{A^*}$).

Figure 2. Isotopic composition of blends AB and A^*B prepared in this work for determination of bromide. Throughout this manuscript the individual blends are numbered for reference.

Table 2. Determination of bromide ions in groundwater sample (BCR-611) using double, triple and quadruple isotope dilution methods: experimental data^a

Preparation and measurement of blends AB			Preparation and measurement of blends A*B			
$m_{A(AB),aq}/g$	$m_{B(AB),aq}/g$	$r_{AB}/(V/V)$	$m_{A^*(A^*B),aq}/g$	$m_{B(A^*B),aq}/g$	$w_{A^*}/(ng/g)$	$r_{A^*B}/(V/V)$
2.007 58(3)	0.026 60(3)	2.642(1) _[1]	2.004 65(3)	0.059 93(3)	25.00(2)	8.681(4) _[28]
2.002 70(3)	0.026 65(3)	2.637(1) _[2]	2.001 68(3)	0.059 92(3)	49.97(4)	6.143(3) _[26]
2.000 57(3)	0.026 62(3)	2.645(1) _[3]	2.003 12(3)	0.060 13(3)	97.44(8)	4.125(2) _[23]
1.995 64(3)	0.026 66(3)	2.653(1) _[4]	2.001 14(3)	0.059 89(3)	199.9(2)	2.764(1) _[20]
2.000 00(3)	0.026 67(3)	2.646(1) _[5]	2.000 05(3)	0.059 89(3)	400.0(3)	1.945(1) _[18]
2.007 86(3)	0.059 52(3)	4.208(2) _[6]	2.007 30(3)	0.026 53(3)	25.00(2)	5.736(3) _[25]
2.011 24(3)	0.060 02(3)	4.230(2) _[7]	2.003 47(3)	0.026 70(3)	49.97(4)	3.849(2) _[22]
2.008 81(3)	0.059 90(3)	4.225(2) _[8]	1.999 54(3)	0.026 57(3)	97.44(8)	2.620(1) _[19]
2.008 11(3)	0.060 02(3)	4.226(2) _[9]	2.004 29(3)	0.026 54(3)	199.9(2)	1.849(1) _[17]
2.010 44(3)	0.060 15(3)	4.241(2) _[10]	1.998 10(3)	0.026 67(3)	400.0(3)	1.450(1) _[16]
2.005 03(3)	0.186 18(3)	7.819(4) _[11]	1.995 80(3)	0.185 06(3)	25.00(2)	12.354(6) _[30]
2.009 22(3)	0.186 24(3)	7.839(4) _[12]	1.997 15(3)	0.186 06(3)	49.97(4)	10.257(5) _[29]
2.008 41(3)	0.186 80(3)	7.825(4) _[13]	1.987 76(3)	0.185 78(3)	97.44(8)	7.827(4) _[27]
2.009 94(3)	0.186 12(3)	7.842(4) _[14]	1.992 06(3)	0.186 52(3)	199.9(2)	5.398(3) _[24]
2.007 27(3)	0.185 62(3)	7.803(4) _[15]	1.992 78(3)	0.185 13(3)	400.0(3)	3.555(2) _[21]

a. The subscripted numbers in the brackets refer to the numbering of r_{AB} and r_{A^*B} values (as per Fig. 2) while values in parentheses are uncertainties referring to the applicable digits (with coverage factor $k = 1$).

4.1. Multiplicity in triple and quadruple dilution. Given one blend AB and N_{A^*B} blends A*B, the number of distinct double isotope dilution results is N_{A^*B} . Triple dilution, however, considers a pair of A*B blends and there are $C(N_{A^*B}, 2) = N_{A^*B}(N_{A^*B} - 1)/2$ unique pairs in N_{A^*B} blends A*B. Likewise, quadruple dilution considers a trio of A*B blends and there are $C(N_{A^*B}, 3) = N_{A^*B}(N_{A^*B} - 1)(N_{A^*B} - 2)/6$ unique triplets in N_{A^*B} blends A*B. When $N_{A^*B} \geq 5$, the number of unique triple and quadruple dilution results exceeds the number of unique double dilution results – a situation that leads to combinatorial ‘explosion’ in the number of triple and quadruple dilution results as N_{A^*B} increases.

Given the multiplex nature of our experimental design, it is possible to establish a large number of distinct isotope dilution results. In this dataset of 15 blends AB and 15 blends A*B there are $15 \times C(15, 1) = 225$ double dilutions (such as A-1-16-B and A-11-30-B from Fig. 2), $15 \times C(15, 2) = 1575$ triple dilutions (such as A-6-19-30), and $15 \times C(15, 3) = 6825$ quadruple dilutions (such as 1-16-26-30). Note that we have only considered dilutions with one blend AB and multiple blends

A*B. Within these ‘dilutions’ we also distinguish the results of exact-matching dilution. In this work, we consider a situation as ‘exact-matching’ when $|r_{AB}/r_{A*B} - 1| < 3\%$. Unlike the traditional ‘one-analysis-one-result’ approach, the multiplex strategy provides not only with better understanding of the uncertainties of the results but also of the robustness of the model equations themselves.

Due to a large number of possible combinations, the uncertainty of the grand average for each isotope dilution method was obtained using Monte Carlo simulation [14]. All calculations were executed with *Mathematica* 8 (Wolfram Research; Champaign IL, USA).

Not all isotope ratio combinations are good choices. For example, the A-1-20-B combination for ID²MS yields a two times smaller uncertainty of w_A than the A-1-30-B combination owing to the fact that A-1-20-B design is exact-matched ($r_{AB[1]} = r_{A*B[20]}$) whereas A-1-30-B is not. For these reasons, combinations that lead to *extremely* large uncertainties were omitted. The results of bromide determination in BCR-611 are shown in Table 3. It is important to stress that the results quoted in Table 3 represent only the best performance in all isotope dilution methods; inspection of Figure 4 shows that many results of ID³MS and ID⁴MS have extreme uncertainties and this aspect has to be taken into consideration when IDMS methods are compared with one another.

Table 3. Determination of bromide ions in groundwater sample (BCR-611) using double, triple and quadruple isotope dilution methods: best results.

Method	$w_A/(\text{ng/g})^a$	Number of isotope ratio combinations ^b , N
ID ² MS	$96.28 \pm 0.81_{k=1}$	105 (half)
ID ³ MS	$96.25 \pm 0.76_{k=1}$	735 (half)
ID ⁴ MS	$96.21 \pm 0.76_{k=1}$	3193 (half)
ID ²⁼ MS	$96.25 \pm 0.53_{k=1}$	10 (most)
ID ³⁼ MS	$96.23 \pm 0.43_{k=1}$	94 (most)
ID ⁴⁼ MS	$96.28 \pm 0.42_{k=1}$	435 (most)
<i>Certified value</i>	$93(4)_{k=2}$	n/a

a. Uncertainty of the results is obtained from Monte Carlo simulations whereby uncertainty (i.e., normal distribution with a half-width equal to the uncertainty of the corresponding variable) was added to all appropriate variables, i.e., r_{AB} , r_{A*B} , r_A , r_B , w_B , w_A , $m_{A(AB),aq}$, $m_{B(AB),aq}$, $m_{A*(A*B),aq}$, and $m_{B(A*B),aq}$. Number of Monte Carlo simulations is $1000 \times N$ (ID²⁼MS) and $100 \times N$ (ID²MS, ID³⁼MS, ID⁴⁼MS), and $30 \times N$ (ID³MS, ID⁴MS) where N is the number of isotope ratio combinations used.

b. Number of unique combinations used to calculate the grand-average of w_A . For example, A-3-23-B (see Fig. 2) is one of the fifteen ID²⁼MS combinations. “Half” refers to all results with uncertainty below the median value (this corresponds to results displayed in the right-half of all plots of Fig. 4). “Most” refers to the majority of combinations with few isotope ratio combinations omitted due to large uncertainty.

4.2. Isotopic composition of spike. In 1994 Henrion introduced double-exact matching isotope dilution concluding that “for [this] method to work it is not necessary to exactly know the isotopic pattern or concentration of the spike” [8]. This statement is evident from Eq. 11. In practice, however, the matching of r_{AB} and r_{A^*B} can be achieved only to a certain degree. In line with the analysis of Milton and Wielgosz [15], Figure 3 shows the consequence of choosing various values for r_B in Eq. 9 when matching is not exact. This demonstrates the important role of exact-matching in eliminating the need for the precise value of r_B .

Figure 3. Unlike in double dilution (ID²MS, grey lines), it is not necessary to know the precise value of isotopic composition of spike in exact-matching double dilution (ID²⁼MS, black line). Note that the crossing of all three curves corresponds to the correct value of r_B . Here the open circles correspond to ID²MS with 10-15% mismatch between r_{AB} and r_{A^*B} ($r_{AB} = 4.208\text{--}4.230$, $r_{A^*B} = 3.555$ and $r_{AB} = 7.803\text{--}7.842$, $r_{A^*B} = 8.681$) whereas black diamonds are the average of all exact-matching combinations.

4.3. Robustness of the dilution methods. Consider a set of isotope amount ratios from a single mixture of sample and spike (AB) and two mixtures of standard and spike (A*B-1 and A*B-2). This yields a single result of triple dilution (provided that r_A is also measured), or two results of double dilution (given r_A and r_B):

$$\begin{aligned} (\text{ID}^2\text{MS}) \quad & r_A - r_{AB} - r_{A^*B-1} - r_B \text{ and } r_A - r_{AB} - r_{A^*B-2} - r_B \\ (\text{ID}^3\text{MS}) \quad & r_A - r_{AB} - r_{A^*B-1} - r_{A^*B-2} \end{aligned}$$

The question arises: is it better, in general, to subject the obtained dataset to a single ID³MS equation or two ID²MS equations? The same question can be formulated with quadruple dilution in play. To answer this question, all possible combinations of isotope amount ratios were selected

for double, triple and quadruple dilutions using the dataset in Table 2. Then, the mass fraction of bromide was calculated for each combination along with the corresponding uncertainty. Results are shown in Figure 4.

Figure 4. Robustness of isotope dilution methods.

Mass fraction of bromide in BCR-611 was calculated for every possible isotope amount ratio combination from dataset of Table 2. This means 210 combinations for ID²MS, 15 for ID²⁼MS, 2940 for ID³MS, and so forth. The resulting mass fractions of bromide (blue for ID²MS, ID³MS, ID⁴MS and black for ID²⁼MS, ID³⁼MS, ID⁴⁼MS) are ordered by their respective uncertainties, which were calculated using Monte Carlo simulations.

It is clear from these results that triple and quadruple dilutions can yield results with large uncertainty. On the contrary, double dilution is considerably more robust, and so are all methods that implement exact-matching. Such a conclusion is not surprising and can be explained.

For single isotope dilution to work, r_A and r_B need to differ from r_{AB} . In particular, the optimum conditions are reached when r_{AB} is the geometric average between r_A and r_B [15,16]. Same applies to r_{A*B} in double dilution. In triple dilution, however, an additional condition for the isotope amount ratio of blends arises: r_{A*B-1} and r_{A*B-2} must not coincide since Eq. 14 becomes indeterminate when $r_{A*B-1} = r_{A*B-2}$. Consequently, if $r_{A*B-1} \approx r_{A*B-2}$ Eq. 14 leads to large uncertainty in w_A . Likewise, quadruple dilution experiments must be designed so to avoid $r_{A*B-1} = r_{A*B-2} = r_{A*B-3}$. Although these examples are trivial, they illustrate the great importance of experimental design in triple and quadruple dilutions. A schematic atlas of possible experimental designs for various isotope dilution methods is given in Figure 5. As an alternative to exact-matching designs, lowest measurement uncertainty is achieved when the isotopic composition of the sample/spike blend (r_{AB}) is bracketed with the isotopic compositions of standard/spike blends (r_{A*B-i}) as shown in Fig. 5 for ID³MS and ID⁴MS.

Figure 5. Atlas of possible experimental designs for isotope dilution methods. For ID³MS to work $r_{A*B-1} \neq r_{A*B-2}$, and for ID⁴MS to work, $r_{A*B-1} \neq r_{A*B-2} \neq r_{A*B-3}$.

5. CONCLUSIONS

Variables that are most difficult to assess in isotope dilution practice are the mass fraction of enriched spike (w_B), isotopic composition of spike (r_B) and analyte (r_A). Variations of the classical single isotope dilution method have been sought to avoid direct measurements of these variables. ID²MS avoids direct measurement of w_B , ID³MS avoids direct measurement of w_B and r_B , and ID⁴MS furthers the elimination of w_B , r_B and r_A . However, the robustness of isotope dilution methods decreases significantly with the rise in the order of dilution, thus placing significant emphasis on suitable experimental design. Consequently, ID³MS or ID⁴MS methods can yield poor-precision results when sample/spike blends are not chosen properly whereas the exact-matching design has proven to yield robust and precise results in all variants of IDMS.

6. REFERENCES

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