General equation for multiple spiking isotope dilution mass spectrometry

Ouerdane, Laurent; Mester, Zoltán; Meija, Juris

Publisher's version / Version de l'éditeur:
https://doi.org/10.1021/ac900205b
Analytical Chemistry, 81, 12, pp. 5075-5079, 2009-06-15

Anal. Chem. **2009,** *81,* 5075–5079

General Equation for Multiple Spiking Isotope Dilution Mass Spectrometry

Laurent Ouerdane,* Zoltán Mester, and Juris Meija

Institute for National Measurement Standards, National Research Council Canada 1200 Montreal Rd., Ottawa ON K1A 0R6, Canada

Isotope dilution is a well-known primary ratio method of quantitative analysis that yields good-quality metrological results. Many equations have been proposed to calculate the amount of substance from the isotope ratio measurements, and these have been used successfully for more than a half-century. Decades ago, isotope dilution equations were extended to correct for analyte formation during analysis, which is especially apparent in the analysis of methylmercury or chromium(VI). Considering only methods for the determination of these two analytes, many variables that are involved must be considered (for example, the extent of analyte formation, the number of isotopes monitored for each analyte, the number of substances, or the nature of mass spectra (elemental versus molecular)). To date, no master equation that can adequately address all of these aspects of the problem has been proposed. In this manuscript, we propose a general equation for isotope dilution.

Quantitation in analytical chemistry is usually achieved using external calibration. However, in the presence of matrix interferences, a standard additions method[1] or an internal standard method[2] is used to reduce or eliminate various sources of error. The standard additions method relies on signal intensity measurements, which are prone to instrumental drift and variations in analyte recovery during extraction or separation. To reduce the measurement uncertainty that is due to these effects, ratio methods are used where all signals are normalized to the internal standard. Isotope dilution, which uses a known amount of an isotopically labeled internal standard, is a combination of these two methods.[3,4] A recent trend in analytical chemistry is to clarify and generalize traditional methods, such as linear calibration,[5] standard additions,[1] and internal standards.[2] Because no generalized mathematical representation of isotope dilution exists, here, our objective is to provide one.

Multiple Spiking Isotope Dilution. Biologists and sociologists often must estimate the size of a population that is known to exist but is impossible to sample entirely. In addition, it is rather challenging to account for the changes in population size during the analysis. In biology, this occurs in the form of the birth or death of animals; in chemistry, this occurs in the form of the loss or formation of the analyte during the sample analysis. The addition of not just one but multiple spikes of known amounts of the substances involved efficiently solves the problem of quantifying interconverting analytes.[6,7] In essence, when substances B and C, for example, are known to produce analyte A after the addition of isotopically enriched A to the sample, an accurate initial amount of substance A can be obtained only when known amounts of enriched substances B and C are also added (hence, multiple-spiking isotope dilution) and all three substances (A, B, and C) then can be measured. The measurand in isotope dilution is the amount of substance (at the time of spiking) and the measured quantity is the isotope pattern of the analyte(s) (more specifically, isotope ratios). Isotope dilution was initially practiced using radioactive isotopes of lead as spikes (tracers).[8] Here, we present a comprehensive approach for isotope dilution analysis using partial or complete isotope patterns of analyte(s), enriched spike(s), and their mixtures. Using this approach, isotope dilution is treated mathematically as the superimposition of the natural isotope pattern of the analyte on the isotopically altered (enriched) isotope pattern, as illustrated in Scheme 1.[9,10]

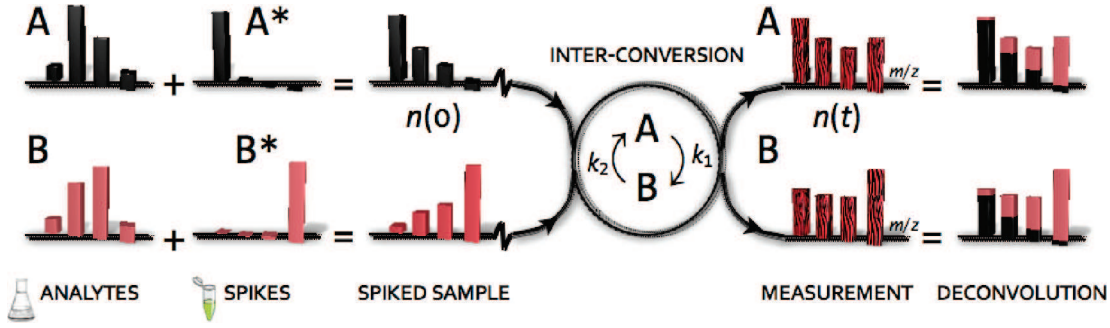
Multiple spiking isotope dilution methods are common in analytical chemistry; yet, the implementation of this advanced calibration approach is slow, because of the complexity of the mathematical equations. In the literature, there are many examples of equations for two- or three-component systems that fill entire pages; still, the reader is left without explicit expressions for the estimate of the measurand.[11−16] Such complexity is unwarranted and, in our opinion, may impede development of innovative

* To whom correspondence should be addressed. E-mail: laurent.ouerdane@univ-pau.fr.

(1) Ellison, S. L. R.; Thompson, M. *Analyst* **2008**, *133*, 992–997.
(2) Burke, D. G.; Mackay, L. G. *Anal. Chem.* **2008**, *80*, 5071–5078.
(3) Rittenberg, D.; Foster, G. L. *J. Biol. Chem.* **1940**, *133*, 737–744.
(4) Heumann, K. G. *Mass Spectrom. Rev.* **1992**, *11*, 41–67.
(5) Hibbert, D. B. *Analyst* **2006**, *131*, 1273–1278.
(6) Kingston, H. M. U.S. Patent 5,414,259, 1995.
(7) Kingston, H. M.; Huo, D.; Lu, Y.; Chalk, S. *Spectrochim. Acta B* **1998**, *53*, 299–309.
(8) von Hevesy, G.; Paneth, F. *Z. Anorg. Chem.* **1913**, *82*, 323–328.
(9) Meija, J.; Caruso, J. A. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 654–658.
(10) Meija, J.; Yang, L.; Caruso, J. A.; Mester, Z. *J. Anal. Atom. Spectrom.* **2006**, *21*, 1294–1297.
(11) Ruiz Encinar, J.; Rodríguez-González, P.; García-Alonso, J. I.; Sanz-Medel, A. *Anal. Chem.* **2002**, *74*, 270–281.
(12) Point, D.; Davis, W. C.; García-Alonso, J. I.; Monperrus, M.; Christopher, S. J.; Donard, O. F. X.; Becker, P. R.; Wise, S. A. *Anal. Bioanal. Chem.* **2007**, *389*, 787–798.
(13) Monperrus, M.; Rodríguez-González, P.; Amouroux, D.; García-Alonso, J. I.; Donard, O. F. X. *Anal. Bioanal. Chem.* **2008**, *390*, 655–666.
(14) Van, D. N.; Bui, T. T. X.; Tesfalidet, S. *Anal. Bioanal. Chem.* **2008**, *392* (4), 737–747.
(15) Rodríguez-González, P.; Ruiz Encinar, J.; García-Alonso, J. I.; Sanz-Medel, A. *J. Anal. Atom. Spectrom.* **2004**, *19*, 685–691.
(16) Tirez, K.; Brusten, W.; Cluyts, A.; Patyn, J.; De Brucker, N. *J. Anal. Atom. Spectrom.* **2003**, *18*, 922–932.

10.1021/ac900205b CCC: $40.75 Published 2009 by the American Chemical Society
Published on Web 05/20/2009

Scheme 1. Principle of Multiple Spiking Isotope Dilution for Interconverting Substances



applications of isotope dilution for the study of complex biological and environmental systems. It is not only the complexity of the equations that threatens the independent development of applications; no general framework has been proposed for multiple spiking isotope dilution, and the state of the art rests entirely with specific validated equations. While some of these solutions can be downsized, none of them can be extended and generalized to arbitrary systems. As a result, "new" equations are frequently published for each particular application. In this work, we outline a comprehensive approach for the interpretation of multiple spiking isotope dilution results. This method also facilitates the use of isotope tracers to provide information not only about the degradation-corrected amount of substances but also about the reaction rate constants and the extent or degree of interconversion reactions, as presented recently for a two-component system.[17]

For isotope dilution to provide estimates of the initial concentration of the analytes, the system must be closed and isotope patterns for all analytes before spiking must be known. The addition of the enriched spikes must be designed so that each compound is defined by at least one unique isotope pattern (in its natural or enriched form) and at least $m + 1$ of these isotope patterns are different. To improve the precision of the isotope dilution results, it is advantageous to use enriched spikes with isotope patterns that are as different as possible from each other (i.e., to ensure the orthogonality of isotope patterns). One of the limitations of multiple spiking isotope dilution is usually the complexity of the chemical systems studied. Factors such as the presence of multiple reaction pools, open reaction systems, and sampling or analysis constraints, restrict the quality and accuracy of the information that can be accessed. Currently, several isotope dilution approaches exist to estimate the amounts of substance for two-component systems (with three or more isotopes monitored)[7,17] and three-component systems (with only four isotopes measured),[15] using isotope dilution mass spectrometry.

Although traditional measurement equations for isotope dilution have been successfully applied for a long period,[17−19] no unifying general framework exists to reconcile the various experimental strategies in isotope dilution (i.e., the varying number of monitored isotopes and correction for species interconversion). In this manuscript, we show that the isotope pattern deconvolution approach, which has been recently developed for two-component systems,[10] can be generalized for an arbitrary number of components and isotopes, and it can be applied to interpret both organic and inorganic mass spectra.

Isotope Pattern Deconvolution. Consider a system of m interconverting analytes with p isotopes measured for each of the m substances ($p \geq m + q$, where q is the number of unique natural isotope patterns among the m substances and $1 \leq q \leq m$). In routine elemental speciation analysis, all analytes usually have indistinguishable isotope patterns ($q = 1$). Such situations are encountered in elemental speciation using low-resolution quadrupole inductively coupled plasma−mass spectrometry (ICP-MS). Similarly, when high-precision mass spectrometers are employed, such as the multicollector ICP-MS, natural fractionation of isotopes becomes evident and species of the same element show different isotope patterns.[20] Moreover, when reverse isotope dilution is performed (i.e., to estimate the concentration of the isotopically enriched substance using known amounts of natural isotopic composition standard), initial patterns of analytes are usually rather different, because of idiosyncratic isotopic enrichment procedures for each substance, whereas the spikes, which represent the natural isotopic composition, might have identical isotope patterns.

All m compounds of interest are determined simultaneously using isotope dilution, which is comprised of the addition of the isotopically enriched internal standards (spikes), followed by chromatographic separation and mass spectrometric detection.[21,22] Let the known amounts of isotopically enriched analytes M_1*, ..., M_m* added to the analyzed sample be represented as $n(M_i^*) = n_{0,i}^*$. After isotopic equilibration, the resulting isotopic patterns of all analytes are measured using mass spectrometry.

When elemental mass spectra are used, the observed spectra can be processed directly for isotope dilution equations; however, molecular mass spectra of the interconverting analytes first must be deconvoluted into pseudo-elemental spectra (i.e., isotopomer composition), so that the isotopic signatures can be directly compared between the interconverting substances. Several methods exist to extract isotope patterns of elements from the molecular ions, starting from the pioneering work of Biemann.[23−25]

After the elemental spectra of all m interconverting species are obtained, the observed isotope patterns of all analytes (**I**) can be expressed as a linear combination of the pure component

(17) Meija, J.; Ouerdane, L.; Mester, Z. *Analyst* **2009**, *134*, 466–471.
(18) Kipphardt, H.; De Bièvre, P.; Taylor, P. D. P. *Anal. Bioanal. Chem.* **2004**, *378*, 330–341.
(19) Rodríguez-González, P.; Monperrus, M.; García Alonso, J. I.; Amouroux, D.; Donard, O. F. X. *J. Anal. Atom. Spectrom.* **2007**, *11*, 1373–1382.
(20) Yang, L.; Sturgeon, R. E. *Anal. Bioanal. Chem.* **2009**, *393* (1), 377–385.
(21) Meija, J.; Mester, Z. *Anal. Chim. Acta* **2008**, *607*, 115–125.
(22) Rodríguez-González, P.; Marchante-Gayón, J. M.; García-Alonso, J. I.; Sanz-Medel, A. *Spectrochim. Acta B* **2005**, *60*, 151–207.
(23) Biemann, K. *Mass Spectrometry: Organic Chemical Applications*; McGraw−Hill: New York, 1962.
(24) Brauman, J. I. *Anal. Chem.* **1966**, *38*, 607–610.
(25) Jennings, M. E. I.; Matthews, D. E. *Anal. Chem.* **2005**, *77*, 6435–6444.

spectra (**X**) and the pure component amount in the resulting (observed) patterns (**A**), i.e., $\mathbf{I} = \mathbf{X}^{\bullet}\mathbf{A}$.[9] The same can be done with the observed isotope abundances or isotope ratios, instead of intensities. Nevertheless, the use of isotope ratios is ubiquitous in isotope dilution. Consequently, we have the expression $\mathbf{R} = \mathbf{X}^{\bullet}\mathbf{A}'$, or

$$R_{i,k} = \sum_{j=1}^{m+q} a_{j,k} x_{i,j} \tag{1}$$

$$\begin{pmatrix} R_{1,1} & \cdots & R_{1,m} \\ R_{2,1} & \cdots & R_{2,m} \\ \vdots & \vdots & \vdots \\ R_{p,1} & \cdots & R_{p,m} \end{pmatrix} = \begin{pmatrix} x^{*}_{1,1} & \cdots & x^{*}_{1,m} & x^{\mathrm{nat}}_{1,m+1} & \cdots & x^{\mathrm{nat}}_{1,m+q} \\ x^{*}_{2,1} & \cdots & x^{*}_{2,m} & x^{\mathrm{nat}}_{2,m+1} & \cdots & x^{\mathrm{nat}}_{2,m+q} \\ \vdots & \vdots & \vdots & \vdots & \vdots & \vdots \\ x^{*}_{p,1} & \cdots & x^{*}_{p,m} & x^{\mathrm{nat}}_{p,m+1} & \cdots & x^{\mathrm{nat}}_{p,m+q} \end{pmatrix} \cdot \begin{pmatrix} a_{1,1} & \cdots & a_{1,m} \\ a_{2,1} & \cdots & a_{2,m} \\ \vdots & \vdots & \vdots \\ a_{m+q,1} & \cdots & a_{m+q,m} \end{pmatrix} \tag{2}$$

Here, $R_{i,j}$ denotes the measured peak area ratios for the ith isotope of compound M_j ($^{i}\mathrm{M}_j$), where $R_{i,j} = I(^{i}\mathrm{M}_j)/I(^{\mathrm{ref}}\mathrm{M}_j)$, and $x_{i,j}$ are the isotopic abundances of all m pure spikes, $x^{*}_{i,j} = x(^{i}\mathrm{M}_j{}^{*})$, and natural isotopic abundances of all analytes, $x^{\mathrm{nat}}_{i,m+q}$ ($1 \le q \le m$). Clearly, all of these quantities must be corrected for systematic instrument biases, such as mass bias, uneven signal suppression (encountered in molecular mass spectrometry), spectral interferences, and detector deadtime.

Coefficients $a_{j,k}$ are the link between the observed mass-bias-corrected isotope ratios and pure component spectra and, most importantly, $a_{j,k}$ can be used to calculate the amount of the involved substances. It is important that the isotopic abundances that are used in eq 2 be fractions of all the atoms of a particular element, rather than be only normalized abundances of the measured isotopes. Similarly, the abundances cannot be scaled to relative abundances, e.g., where the maximum abundance is set to 100%. This also applies to the deconvolution of molecular mass spectra into pseudo-elemental spectra.

To obtain the amounts of m interconverting substances, at least $m + q$ isotopic abundances must be measured for each compound. In the simplest case, when $p = m + q$, the contribution coefficient matrix **A** (or **A′**) is determined via matrix inversion: $\mathbf{A}' = \mathbf{X}^{-1}\mathbf{R}$. For $p > m + q$, on the other hand, this can be achieved by obtaining the least-squares solution to eq 2, using the Moore−Penrose pseudo-inverse ($\mathbf{A}' = (\mathbf{X}^{\mathrm{T}}\mathbf{X})^{-1}\mathbf{X}^{\mathrm{T}}\mathbf{R}$), among other methods.[26] The least-squares solution can also be obtained using the LINEST function in Microsoft Excel. Note that the LINEST function is equipped with built-in statistical features that can greatly simplify the uncertainty analysis of the obtained results or the internal mass-bias correction that operates by minimizing the squared sum of isotope pattern residuals.[27] Ultimately, the unknown variables of interest are the amounts of substance M_1, ..., M_m in the sample at the time of spiking, $n^{0}(\mathrm{M}_i) = n_{0,i}$.

Amount of Substance. Realizing that the rows of the coefficient matrices **A** or **A′** are linearly dependent, because they represent the relative contribution of individual isotopic sources to the observed signal, the following identity can be established ($j = 1, ..., m$):

$$\sum_{z=1}^{q} a_{m+z,j} = \sum_{i=1}^{m} a_{i,j}\left(\frac{n_{0,i}}{n^{*}_{0,i}}\right) \tag{3}$$

From these m equations, the m unknowns ($n_{0,i}$) can be determined by combining eqs 2 and 3. This leads to a general equation for the amount of all analytes in the sample at the time of spiking ($t = 0$):

$$n_{0,i} = n^{*}_{0,i}\left(\frac{|\mathbf{A}_i|}{|\mathbf{A}_{*}|}\right)(-1)^{m+i} \tag{4}$$

Here, $|\mathbf{A}_{*}|$ is the determinant of the $m \times m$ truncated coefficient matrix $\mathbf{A}_{*}$ that contains only the contributions from the enriched spikes, i.e., $a_{1,1}$ to $a_{m,m}$, whereas $|\mathbf{A}_i|$ is the determinant of the $m \times m$ truncated matrix $\mathbf{A}_i$ obtained by deleting the ith row in **A** (or **A′**). To the best of our knowledge, this is the most general equation of isotope dilution and applies to the simultaneous quantitation of m interconverting compounds with multiple spiking isotope dilution mass spectrometry. In a particular case of two interconverting substances, such as chromium(III)/chromium(VI), eq 4 reduces to the recently published expression[10] ($m = 2$, $q = 1$, $p = 3$):

$$n_{0,\mathrm{M}_1} = n^{*}_{0,\mathrm{M}_1}\left(\frac{a_{2,2}a_{3,1} - a_{2,1}a_{3,2}}{a_{1,1}a_{2,2} - a_{1,2}a_{2,1}}\right) \tag{5}$$

$$n_{0,\mathrm{M}_2} = n^{*}_{0,\mathrm{M}_2}\left(\frac{a_{1,1}a_{3,2} - a_{1,2}a_{3,1}}{a_{1,1}a_{2,2} - a_{1,2}a_{2,1}}\right) \tag{6}$$

When the amount of a single substance is determined by isotope dilution and two isotopes are monitored, eq 2 reduces to the following:

$$\begin{pmatrix} R_{1,1} \\ R_{2,1} \end{pmatrix} = \begin{pmatrix} x^{*}_{1} & x^{\mathrm{nat}}_{1} \\ x^{*}_{2} & x^{\mathrm{nat}}_{2} \end{pmatrix} \cdot \begin{pmatrix} a_{1,1} \\ a_{2,1} \end{pmatrix} \tag{7}$$

Therefore, $\mathbf{A} = \mathbf{X}^{-1}\mathbf{R}$ and

$$\begin{pmatrix} a_{1,1} \\ a_{2,1} \end{pmatrix} = \frac{1}{|\mathbf{X}|}\begin{pmatrix} x^{\mathrm{nat}}_{2}R_{1,1} - x^{\mathrm{nat}}_{1}R_{2,1} \\ -x^{*}_{2}R_{1,1} + x^{*}_{1}R_{2,1} \end{pmatrix} \tag{8}$$

Recognizing that either $R_{1,1}$ or $R_{2,1} = 1$, the above expression yields the familiar basic isotope dilution equation:[4,22,28]

$$n_{0,1} = n^{*}_{0,1}\left(\frac{a_{2,1}}{a_{1,1}}\right)(-1)^{1+1} \tag{9}$$

$$n_{0,1} = n^{*}_{0,1}\left[\frac{(x^{*}_{2}/x^{*}_{1}) - R_{2,1}}{R_{2,1} - (x^{\mathrm{nat}}_{2}/x^{\mathrm{nat}}_{1})}\right] \cdot \left(\frac{x^{*}_{1}}{x^{\mathrm{nat}}_{1}}\right) \tag{10}$$

The amount of substance obtained from this equation is traceable to the *Système International d'Unités*;[29] hence, the continuity of the aforementioned proposed general equation is certain. [Note that, in the aforementioned equations, n_i refers to the amount of the natural analytes, not the total amount of the substances M_i (natural and enriched spikes).]

(26) Lawson, C. L.; Hanson, R. J. *Solving Least Squares Problems*; Prentice Hall: Englewood Cliffs, NJ, 1974.

(27) Rodríguez-Castrillón, J. Á.; Moldovan, M.; Ruiz Encinar, J.; García Alonso, J. I. *J. Anal. Atom. Spectrom.* **2008**, *23*, 318–324.

(28) De Bièvre, P.; Peiser, H. S. *Fresenius J. Anal. Chem.* **1997**, *359*, 523–525.

(29) Milton, M. J. T.; Wielgosz, R. I. *Metrologia* **2000**, *37*, 199–206.

With further regard to the general expression for the amount of analyte, general equations can be written for the amount of correction factors, which are commonly used in multiple spiking isotope dilution calculations:[15,17,22]

$$F_{i\rightarrow j} = F_{i,j} = \left(\frac{a_{i,j}}{a_{j,j}}\right) \cdot \left(\frac{n^*_{0,j}}{n^*_{0,i}}\right) \tag{11}$$

An alternative form of eq 4 can be obtained by combining eqs 3 and 11: $\mathbf{n} = (\mathbf{F}^{-1})^{\mathrm{T}}\mathbf{n}^{\dagger}$, where $\mathbf{F}$ is the correction factor matrix and $\mathbf{n}$, $\mathbf{n}^{\dagger}$ are the vectors of analyte amounts, corrected and uncorrected, for species interconversion, respectively. The hypothetical interconversion uncorrected analyte amount is given as follows:

$$n^{\dagger}_i = n^*_{0,i} \sum_{z=1}^{q} \frac{a_{m+z,i}}{a_{i,i}} \tag{12}$$

Similarly, the degree of conversion is a quantity that is often used to describe the interconversion of analytes.[17] In a closed system of m interconverting compounds, the degree of conversion ($\alpha_{i,j}$) corresponds to the fractional amount of compound M_i that is present in the form of M_j after the interconversions. The relationship between the degrees of conversion and the correctional amount factors has been established previously for a two-component system,[17] and its generalization for m components is given as follows:

$$F_{i,j} = \frac{\alpha_{i,j}}{1 - \sum_{z\neq j}^{m} \alpha_{j,z}} \tag{13}$$

This equation can be expressed and solved for $\alpha_{i,j}$ in matrix form:

$$\alpha_{i,j} = F_{i,j}\left(\frac{|\mathbf{F}_j|}{|\mathbf{F}|}\right) \tag{14}$$

where $|\mathbf{F}|$ is the determinant of the $m \times m$ correction coefficient matrix $\mathbf{F}$ and $|\mathbf{F}_j|$ is the determinant of matrix $\mathbf{F}$ with the entries in the jth column replaced by ones. In the case of two interconverting compounds, eq 14 reduces to the following:

$$\alpha_{1,2} = F_{1,2}\frac{\begin{vmatrix} 1 & 1 \\ F_{2,1} & 1 \end{vmatrix}}{\begin{vmatrix} 1 & F_{1,2} \\ F_{2,1} & 1 \end{vmatrix}} = F_{1,2}\left(\frac{1 - F_{2,1}}{1 - F_{1,2}F_{2,1}}\right) \tag{15}$$

In summary, the amount of substance can be calculated from any multiple spiking isotope dilution experiment results by solving two general equations (i.e., eqs 2 and 4). The single-component case refers to traditional isotope dilution experiments such as post-column isotope dilution analysis of sulfur[30] or iron[31] for protein quantification, whereas the two-component case can be applied to interconverting systems such as chromium(III)/chromium(VI),[10] CH_3Hg^+/Hg^{2+},[13] lead(II)/lead(IV), Br^-/BrO_3^-, iron(II)/iron(III),[32] L/D-racemization, or cis/trans-isomerization. Among the most common three-component systems that are encountered in current analytical practice are $Ph_3Sn^+/Ph_2Sn^+/PhSn^+$,[14] $Bu_3Sn^+/Bu_2Sn^+/BuSn^+$,[15] and $Hg^0/Hg^{2+}/CH_3Hg^+$.[13] Four-component systems are also encountered in analytical chemistry (for example, when two compounds are distributed between two phases (solid/liquid)). A particular case is the determination of chromium(III)/chromium(VI) from solid matrices, arguably a key application in the industrial sector.

Example of Calculation. Consider a closed system of four interconverting compounds B_1, B_2, B_3, and B_4 with identical natural isotope patterns and their isotopically enriched analogues (five isotopes, $p = 5$):

$$\mathbf{X} = \begin{array}{c} \begin{matrix} B_1^* & B_2^* & B_3^* & B_4^* & B_{i=4} \end{matrix} \\ \begin{pmatrix} 0.875 & 0.002 & 0.001 & 0.001 & 0.129 \\ 0.082 & 0.021 & 0.001 & 0.002 & 0.478 \\ 0.021 & 0.941 & 0.005 & 0.005 & 0.110 \\ 0.002 & 0.015 & 0.961 & 0.032 & 0.132 \\ 0.001 & 0.005 & 0.022 & 0.948 & 0.054 \end{pmatrix} \end{array}$$

One gram of sample that contains unknown amounts of these four compounds is spiked with known amounts (1.0 mol) of isotopically enriched spikes, each with a distinct isotope pattern. After 3 h, traditional chemical analysis occurs, involving the extraction, derivatization, and separation of all analytes. The following isotope ratios of all four compounds are obtained (with respect to the first isotope):

$$\mathbf{R}(B_i) = \begin{array}{c} \begin{matrix} i=1 & 2 & 3 & 4 \end{matrix} \\ \begin{pmatrix} 1.000 & 1.000 & 1.000 & 1.000 \\ 1.220 & 2.024 & 1.511 & 1.559 \\ 0.663 & 1.901 & 0.608 & 0.925 \\ 1.097 & 1.122 & 1.669 & 0.857 \\ 0.361 & 1.055 & 0.575 & 1.149 \end{pmatrix} \end{array}$$

Isotope dilution calculations are now applied to obtain the amount of all analytes in the sample at the time of spiking. The corresponding coefficient matrix $\mathbf{A}'$, which satisfies the equation $\mathbf{R} = \mathbf{X}\cdot\mathbf{A}'$, is given as follows:

$$\mathbf{A}' = \begin{array}{c} \\ \begin{pmatrix} 0.787 & 0.538 & 0.694 & 0.681 \\ 0.401 & 1.525 & 0.268 & 0.596 \\ 0.797 & 0.555 & 1.302 & 0.419 \\ 0.223 & 0.860 & 0.402 & 1.021 \\ 2.397 & 4.070 & 3.026 & 3.113 \end{pmatrix} \end{array} \begin{array}{l} \\ \left.\begin{matrix} \\ \\ \\ \\ \end{matrix}\right\} \mathbf{A}'_* \\ \left.\begin{matrix} \end{matrix}\right\} \mathbf{A}'_1 \end{array}$$

Using eq 4, the following amount of all analytes were obtained: $n(B_1) = 0.80$ mol, $n(B_2) = 1.20$ mol, $n(B_3) = 1.25$ mol, and $n(B_4) = 1.30$ mol. In particular, the amount of B_1 is calculated as follows:

$$n(\mathbf{B}_1) = n(\mathbf{B}_1^*)\left(\frac{|\mathbf{A}'_1|}{|\mathbf{A}'_*|}\right)(-1)^{4+1} = 1.00 \times \frac{0.380}{0.474} = 0.80\ \mathrm{mol} \tag{16}$$

Many analytical chemists have a general aversion to matrix algebra; therefore, it is worth noting that all matrix computations, such as transposition, inversion, multiplication, or finding a determinant, can be calculated in an instant using standard Excel functions. More specifically, $\mathbf{A}'$ can be obtained using the formula MMULT(MINVERSE(**X**), **R**) which corresponds to the equation $\mathbf{A}' =$

(30) Zinn, N.; Krüger, R.; Leonhard, P.; Bettmer, J. *Anal. Bioanal. Chem.* **2008**, *391*, 537–543.

(31) Del Castillo Busto, M. E.; Montes-Bayón, M.; Bettmer, J.; Sanz-Medel, A. *Analyst* **2008**, *133*, 379–384.

(32) Welch, S. A.; Beard, B. L.; Johnson, C. M.; Braterman, P. S. *Geochim. Cosmochim. Acta* **2003**, *67*, 4231–4250.

$\mathbf{X}^{-1}\mathbf{R}$. The matrix determinants in eq 16—0.380 and 0.474—can be calculated using the formula MDETERM(**A**), where **A** represents the input range of the corresponding matrix.

Uncertainties of all output variables, i.e., n_0 or F_{ij}, can be evaluated using Monte Carlo simulations,[33] which, in essence, are comprised of the addition of random noise (for example, 1%) to the measured isotope ratios of each compound. Alternatively, uncertainties of the output variables can be evaluated using the Kragten method.[34] Here, each input variable (measured isotope ratio) is perturbed with noise separately and the resulting changes in output variables are then summed in quadrature. However, in these simulations, correlation between the isotope ratios cannot be dismissed.[35]

(33) Patterson, K. Y.; Veillon, C.; O'Haver, T. C. *Anal. Chem.* **1994**, *66*, 2829–2834.
(34) Kragten, J. *Analyst* **1994**, *119*, 2161–2165.
(35) Meija, J.; Mester, Z. *Metrologia* **2008**, *45*, 53–62.

CONCLUSIONS

Currently, data analysis remains a major obstacle for the development of innovative multiple spiking isotope dilution methods that are capable of incorporating an arbitrary number of isotopes monitored or correcting for the formation and loss of the analyte during sample preparation and/or analysis. The general formulation of the isotope dilution equation, which is also applicable to molecular mass spectrometry, offers an intuitive expansion for the future development of quantitative methods for labile analytes and provides tools to extract kinetic information from the analyzed systems, as was recently described for the two-component system.[17]

Received for review January 27, 2009. Accepted April 8, 2009.

AC900205B