

SENSITIVITY ANALYSIS FOR SPECIATED ISOTOPE DILUTION MASS SPECTROMETRY

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ABSTRACT. Speciated isotope dilution mass spectrometry is a technique that uses mass spectrometer readings to determine the species-specific isotopic profile of a chemical element in solution. The problem admits several approaches, each with its own computational quirks. In our view, matrix methods are attractive because they are conceptually clear, easy to implement, and amenable to formal analysis. The purpose of this note is to illustrate these virtues by using a matrix method to analyze the numerical sensitivity of a particular published example under both observation noise and changes in parameter space. The analysis blends numerical and theoretical techniques, and has implications both for experimental design and for practical uncertainty assessment.

1. INTRODUCTION

Speciated isotope dilution mass spectrometry is an analytical technique invented to quantify chemical species concentrations of an element in a sample. The basic data for the technique are obtained from mass spectrometer readings, whose ratios measure the relative abundances of different isotopes within each species. To pass from relative to absolute abundances, the sample is spiked with measured amounts of a solution whose species-isotope profile is known. Although the spikes and the ensuing sample treatment actually change the isotopic profile of the original sample, the technique can recover the original species concentrations as long as the number of measured isotopes exceeds the number of species. The recovery entails solving a system of equations whose exact form depends on the problem formulation: for a detailed comparative analysis of four of the most common approaches, see [1].

In [2], Meija et al. developed a matrix-based solution method in which the system of equations is decomposed into two sets of linear equations, each solvable via simple matrix inversion. Originally developed for the case of two species, this technique was later elegantly extended to the case of arbitrary numbers of species [3]. Since the mathematical demands of this method are limited to the elementary operations of linear algebra, and since there is an abundance of both open source and proprietary software that can execute these operations quickly, reliably, and transparently, the method lies within easy reach of the analytic chemistry community.

This paper considers a secondary advantage of the method, namely its amenability to sensitivity analysis. While the numerical sensitivity of isotope dilution methods has been addressed in ways that range from empirical

and numerical to formally analytic (see, e.g. [1], [4], [5], and [6]), our interest here is in the way matrix solutions uniquely open the problem to complementary theoretical and computational attacks. Among the advantages of using matrices are computational speed, coding transparency, and a well developed body of theory about errors under perturbations. Here we couple a Monte Carlo approach with matrix perturbation theory and a probability model to yield a technique for assessing error bounds on experimental results as well as a predictive technique for experimental design.

The structure of the paper is as follows: the next section reviews the basic framework for the matrix solution, establishing the notation and fundamental equations that will be referenced in the remainder. Section 3 contains the analysis proper, and is divided into two subsections, one on numerics and one on theory. The numerics subsection contains routine but paradigmatic experiments that set the stage for the probability model to follow, the goal of which is to show how matrix perturbation theory can be coupled with an analytic probability model to explain and quantify error trends. For clarity and consistency, this work focuses on a variant of the single, concrete example published in [2], and specifically focus on the issue of variations in the estimates under variations in spike levels. Our justification for this narrow focus is that these results are meant to be illustrative of a general approach, and in most cases can be applied with minimal modifications to other regimes of interest. The last section contains a discussion of both the strengths and limitations of this approach, and suggestions for further exploration.

The electronic supplement contains representative portions the code that was used to generate the analysis.

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The code is written in Matlab, an industry standard for matrix manipulations, and will run without changes in Octave [7], a freely downloadable open source alternative.

2. A MATRIX METHOD

The point of departure for any method is a data model which relates the parameters of interest to the observables. For the well-studied case of $m = 2$ species and $n \geq 3$ isotopes, the mass spectrometry reading for the i th isotope and j th species is related to the process parameters via

$$(1) \quad I_{ij} \approx (A_{i0}n_j + A_{ij}n_j^s)(1 - \alpha_{jk}) + (A_{i0}n_k + A_{ik}n_k^s)\alpha_{kj},$$

where $i = 1 \dots n$, $j = 1, 2$, $k \neq j$, the “ \approx ” symbol subsumes a scale factor (constant along species) and the symbols on the right hand side are:

knowns:

A_{i0}	natural abundance of i th isotope
A_{ij}	abundance of i th isotope in j th spike
n_j^s	moles of j th species in the j th spike

unknowns:

n_j	moles of j th species in sample.
α_{kj}	degree of conversion, species k to j

The goal is to use system (1) to solve for the unknowns n_j ; for a detailed development of this model, see [2], and for a nuanced discussion of the ambiguities latent in the α_{kj} 's, see [8].

Although notation is rather index heavy, it leads naturally into a matrix formulation of the problem: following [2], we write (1) as

$$\begin{bmatrix} I_{11} & I_{12} \\ \vdots & \vdots \\ I_{n1} & I_{n2} \end{bmatrix} = \begin{bmatrix} A_{10} & A_{11} & A_{12} \\ \vdots & \vdots & \vdots \\ A_{n0} & A_{n1} & A_{n2} \end{bmatrix} \begin{bmatrix} \frac{x_{01}}{x_{11}} & \frac{x_{02}}{x_{12}} \\ x_{21} & x_{22} \end{bmatrix},$$

i.e. as a matrix equation of the form $I = AX$, where the matrices I , A , and X have dimensions $n \times 2$, $n \times 3$, and 3×2 , respectively. (For notational consistency we symbolically partition these matrices to separate any row or column whose indices contain a 0 from the main body of the matrix.) Since A and I are known, X can be solved as

$$(2) \quad X = A^\dagger I,$$

where A^\dagger denotes the pseudo-inverse of A (which coincides with the inverse if A is square and non-singular.) From (1), the elements of X can be expressed algebraically as

$$(3) \quad \begin{array}{ll} x_{01} \approx n_1(1 - \alpha_{12}) + n_2\alpha_{21} & x_{02} \approx n_2(1 - \alpha_{21}) + n_1\alpha_{12} \\ x_{11} \approx n_1^s(1 - \alpha_{12}) & x_{12} \approx n_1^s\alpha_{12} \\ x_{21} \approx n_2^s\alpha_{21} & x_{22} \approx n_2^s(1 - \alpha_{21}). \end{array}$$

Using the numerical values from (2) to form quotients

$$(4) \quad r_{11} = \frac{x_{11}}{x_{21}}, \quad r_{12} = \frac{x_{12}}{x_{22}},$$

then substituting the algebraic expressions from (3) and simplifying leads to a matrix equation of the form

$$(5) \quad \begin{bmatrix} n_2^s r_{11} & n_1^s \\ n_2^s r_{12} & n_1^s \end{bmatrix} \begin{bmatrix} \alpha_{21} \\ \alpha_{12} \end{bmatrix} = \begin{bmatrix} n_1^s \\ n_2^s r_{12} \end{bmatrix}.$$

If the left hand side is non-singular, this equation can be solved for α_{12} and α_{21} . For the n_j 's, form quotients

$$(6) \quad r_{01} = \frac{x_{01}}{x_{11}}, \quad r_{02} = \frac{x_{02}}{x_{22}},$$

substitute (3) for the right hand sides, and again simplify to yield

$$(7) \quad \begin{bmatrix} 1 - \alpha_{12} & \alpha_{21} \\ \alpha_{12} & 1 - \alpha_{21} \end{bmatrix} \begin{bmatrix} n_1 \\ n_2 \end{bmatrix} = \begin{bmatrix} r_{01}(1 - \alpha_{12})n_1^s \\ r_{02}(1 - \alpha_{21})n_2^s \end{bmatrix}.$$

Once again, the system can be solved if the left hand matrix is non-singular.

In extending this approach to the general case of m species, the data model becomes

$$I_{ij} \approx (A_{i0}n_j + A_{ij}n_j^s) \left(1 - \sum_{k \neq j} \alpha_{jk} \right) + \sum_{k \neq j} (A_{i0}n_k + A_{ik}n_k^s) \alpha_{kj},$$

which can be written in matrix form as above. Matrix manipulations similar to those in (4)-(7) can then be used to solve first for the degrees of conversion α_{ij} and then the mass amounts n_j . In [3], the authors streamline this process by omitting the interconversion ratios and solving for the n_j directly from the x_{ij} via

$$\begin{pmatrix} x_{11} & \cdots & x_{m1} \\ \vdots & & \vdots \\ x_{1m} & \cdots & x_{mm} \end{pmatrix} \begin{pmatrix} n_1 \\ \vdots \\ n_m \end{pmatrix} = \begin{pmatrix} x_{01} \\ \vdots \\ x_{0m} \end{pmatrix}.$$

3. RESULTS AND DISCUSSION

The matrix method for speciated isotope dilution mass spectrometry is formal in the sense that given any set of intensity ratios and process parameters, it will find a unique solution for the mass profile of the analyte. The formal solution leaves untouched two important and related questions, however: first, how does uncertainty in the measurements I_{ij} translate into uncertainty in the estimates n_j ? and second, how should the configurable experimental parameters, the A_{ij} 's and the n_j^s 's, be chosen so as to minimize these uncertainties? The first question asks about the numerical stability of the problem for a fixed set of design parameters, while the second asks how to choose those parameters in such a way as to make the problem as stable as possible.

As mentioned, these fundamental questions have been addressed in a variety of different ways. In [1], the authors perform empirical comparisons, repeating a laboratory experiment in triplicate and re-solving for species amounts via a variety of methods. In [4], the authors use Monte-Carlo analysis to assess the effect of species interconversion on the uncertainty magnification factors of the mass estimates, and in [9], the author discusses the accuracy of the procedure in terms that relate to the underlying process mechanisms.

Our approach is slightly different in that it tries to take maximum theoretic and numeric advantage of an explicitly matrix-based formulation of the problem. In what follows we endeavor to illustrate the complementarity of these strategies by first presenting a sequence of routine but illuminating numerical experiments, and then showing how the results of these experiments can be explained analytically through perturbation theory and a sampling model.

3.1. Numerics. As a point of departure, we consider a set of results published in [2], where the authors calculate Cr(III) and Cr(VI) concentrations within a sample. Their experimental parameters are

$$A = \begin{pmatrix} 0.0435 & 0.9720 & 0.0004 \\ 0.8379 & 0.0258 & 0.0284 \\ 0.0950 & 0.0019 & 0.9698 \end{pmatrix} \quad n_j^s = \begin{pmatrix} 7.4175 \\ 13.6413 \end{pmatrix}$$

With these parameters, their (calibrated) raw data becomes, up to scale factors,

$$I = \begin{pmatrix} 0.6095 & 0.1941 \\ 0.5359 & 0.2011 \\ 0.5842 & 0.9601 \end{pmatrix},$$

and their solutions are

$$(8) \quad \alpha_{ij} = \begin{pmatrix} 0.22 \\ 0.38 \end{pmatrix} \quad n_j = \begin{pmatrix} 7.3657 \\ 0.1718 \end{pmatrix}.$$

Within a Monte Carlo framework, the stability of these results can be checked by first perturbing the observation matrix I with simulated noise, and then re-solving for both the α_{ij} and the n_j^0 . We did this with a Gaussian noise model, i.e. our perturbed observations \tilde{I}_{ij} satisfied $\tilde{I}_{ij} = I_{ij} + \epsilon$ where the ϵ were independent Gaussian random variables of mean zero and fixed standard deviation σ . Table 1 contains a statistical summary of the solution set under 1000 realizations of 1% noise.

	α_{12}	α_{21}	n_1	n_2
μ	0.38	0.22	7.36	0.18
σ	0.0078	0.0083	0.16	0.16
r	[0.36,0.40]	[0.21,0.24]	[7.01, 7.71]	[-0.20,0.51]

Table 1: Numerical mean, standard deviation, and range of estimate (8) under 1% observation noise.

The presence of negative values for n_2 in Table 1 raises the question of whether the estimates for the n_j might have been stabilized (i.e. have exhibited less variability) by strategically adjusting the spike levels. Since we do not know the true values of the parameters α_{ij} and n_j in the laboratory experiment, we cannot carry out this investigation directly, but we can consider a case which has comparable features, and use the generative data model (1) to carry out the experiments. To this end, we set

$$A = \begin{pmatrix} 0.0 & 1.0 & 0.0 \\ 1.0 & 0.0 & 0.0 \\ 0.0 & 0.0 & 1.0 \end{pmatrix}, \quad \alpha_{ij} = \begin{pmatrix} 0.2 \\ 0.4 \end{pmatrix}, \quad n_j = \begin{pmatrix} 10.0 \\ 0.10 \end{pmatrix},$$

and allowing the spike amounts n_1^s and n_2^s to vary independently, generating noisy pseudo-observations for each (n_1^s, n_2^s) pair and re-solving for n_1 and n_2 . The results are summarized in Figure 2, and suggest that the optimal spike ratio lies somewhere between 1 and the ratio of naturally occurring abundances. This is an interesting finding in light of classical error magnification factor calculations [10], [11], from which the traditional wisdom derives that spikes should be added in proportion to their naturally occurring analogues.

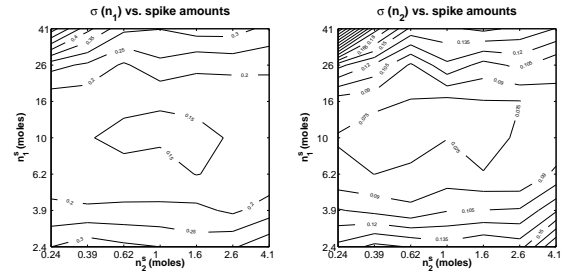


Figure 1: Sensitivity of the mass reconstruction as function of both spikes amounts. The plots were generated using a 1% Gaussian noise model.

To explain the location of the minima in Figure 1, we turned to the basic sensitivity theorem for square linear systems [12], which says that if v and \tilde{v} are the solutions to the matrix systems

$$Av = b, \quad \tilde{A}\tilde{v} = \tilde{b},$$

where \tilde{A} and \tilde{b} are perturbations from A and b , respectively, then

$$\frac{\|v - \tilde{v}\|}{\|v\|} \leq \epsilon(\delta A, \delta b)\kappa(A),$$

where $\|\cdot\|$ denotes the Euclidean vector norm, $\kappa(A)$ is the condition number of A and ϵ is a non-negative monotonically increasing function of the relative errors

$$(9) \quad \delta A = \|A - \tilde{A}\|/\|A\| \quad \text{and} \quad \delta b = \|b - \tilde{b}\|/\|b\|,$$

each defined in terms of, e.g., the Frobenius norm [12]. Since the parameter estimates emerge from solving matrix systems, we expect the largest range of estimation errors to coincide with spike levels that induce the largest range of matrix and vector errors.

To confirm this hypothesis, we looked at the matrix and vector quantities in (5), and recorded the average relative errors (9) under 1% noise and shifting levels of spike. For consistency, we considered the same problem parameters as before, and since the relative errors depend only on spike ratios, not total magnitudes, we fixed n_1^s at 10 moles and allowed n_2^s to vary. Figure 2 plots average relative errors against the spike level n_2^s , and show that the matrix error is minimized in the region $n_2^s \in [10, 20]$, while the vector error is minimized in the region $n_2^s \in [1, 20]$. Since the perturbation theorem guarantees small reconstruction error only if *both* matrix and vector errors are small, these findings suggest (but do not prove) that the value of n_2^s leading to the most stable reconstruction will be somewhere between 1 and 20 moles. This range is consistent with the observed minimizer in Figure 1.

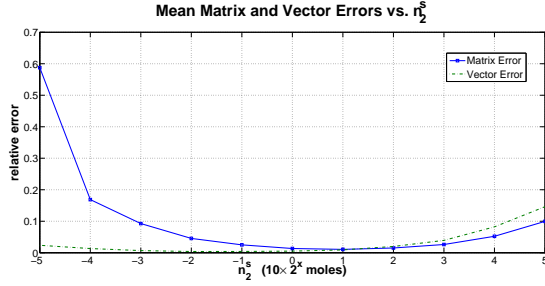


Figure 2: This plot shows how for the matrices and vectors that appear in (5), the expected relative errors (9) depend on the levels of the spikes.

3.2. A Probability Model. Figure 2 was generated numerically, but it begs the analytic question of what the relation might be between spike levels and matrix-vector errors. The answer can be understood as a consequence of our noise model. Two terms contribute to errors in matrix equation (5); the first is $n_2^s r_{11}$ and the second is $n_2^s r_{12}$. Using expressions (3) and (4), it is easy to see that in the absence of noise, these terms are given by

$$n_2^s r_{11} = \frac{n_1^s(1 - \alpha_{12})}{\alpha_{12}} \quad n_2^s r_{12} = \frac{n_1^s \alpha_{12}}{(1 - \alpha_{21})}.$$

In the presence of noise, these terms become $n_2^s \widehat{r}_{11}$ and $n_2^s \widehat{r}_{12}$, respectively given by

$$n_2^s \frac{s_1 n_1^s (1 - \alpha_{12}) + \eta_1}{s_1 n_2^s \alpha_{12} + \eta_2} \quad \text{and} \quad n_1^s \frac{s_2 n_1^s \alpha_{12} + \eta_3}{s_2 n_2^s (1 - \alpha_{21}) + \eta_4},$$

where each η_i represents a realization of a random noise term. If A is the identity, then under our noise model

these noise terms are independent Gaussians, each with a variance proportional to both the signal size and the noise-level,

$$\sigma(\eta_i) \approx \sigma \cdot \text{SIGNALSIZE}$$

where the signal is the vector of intensities given by (1). The relative errors become

$$(10) \quad \left| \frac{n_2^s r_{11} - n_2^s \widehat{r}_{11}}{n_2^s r_{11}} \right| = \left| 1 - \frac{\mu_1 + \eta'_1}{\mu_1 + \eta'_2} \right|$$

and

$$(11) \quad \left| \frac{n_2^s r_{12} - n_2^s \widehat{r}_{12}}{n_2^s r_{12}} \right| = \left| 1 - \frac{\mu_2 + \eta'_3}{\mu_2 + \eta'_4} \right|$$

where the numbers μ_1 and μ_2 are given by

$$(12) \quad \mu_1 = \alpha_{21}(1 - \alpha_{12})n_1^s n_2^s \quad \mu_2 = \alpha_{12}(1 - \alpha_{21})n_1^s n_2^s,$$

and the η_i are Gaussian random variables, each of mean zero, with standard deviations

$$(13) \quad \begin{aligned} \sigma(\eta'_1) &= \sigma \cdot \text{SIGNALSIZE} \cdot n_2^s \alpha_{21} \\ \sigma(\eta'_2) &= \sigma \cdot \text{SIGNALSIZE} \cdot n_1^s (1 - \alpha_{12}) \\ \sigma(\eta'_3) &= \sigma \cdot \text{SIGNALSIZE} \cdot n_2^s (1 - \alpha_{21}) \\ \sigma(\eta'_4) &= \sigma \cdot \text{SIGNALSIZE} \cdot n_1^s \alpha_{12}. \end{aligned}$$

Both error expressions are of the form $|1 - w|$, where w is a ratio of the form

$$w = \frac{\mu + \eta_a}{\mu + \eta_b},$$

with η_a and η_b zero-mean Gaussians. The exact distribution of such ratios is complicated, but analytic forms are known [9]. For our purposes, the important point is that this analytic form allows us to approximate the expected value of $|1 - w|$ for any choice of μ , η_a , and η_b .

Our first experiment was to calculate this expectation for expressions (10) and (11) above, where the associated μ 's, η_a 's, and η_b 's were those that obtained as n_2^s ranged over the values of the previous plots. Not surprisingly, the result was a plot that looked almost identical to Figure 3, where the blue line corresponded to the expected relative error of $n_2^s r_{11}$ and the green line corresponded to the expected relative error of $n_2^s r_{12}$. This similarity demonstrated the consistency of our stochastic model, and the accuracy of our formula for the density function of the quotient distribution.

Our second experiment was to turn the problem around, and ask which value of n_2^s yielded the 'best' quotient distribution, in the sense of one centered at 1 and with small variance. Intuitively, we would like to make the ratios $\mu/\sigma(\eta_a)$ and $\mu/\sigma(\eta_b)$ as large as possible, since in this case both the numerator and the denominator of w will cluster around the value of μ , and the value of the quotient will cluster near 1. On the other hand, if all we adjust is n_2^s , we have limited control over these ratios.

Figure 4 plots these ratios as a function of n_2^s . The left hand plot contains $\mu_1/\sigma(\eta'_1)$ and $\mu_1/\sigma(\eta'_2)$, while the

right hand plot contains $\mu_2/\sigma(\eta'_3)$ and $\mu_1/\sigma(\eta'_4)$. The ratios are calculated using (12) and (13), where the values of α_{12} , α_{21} , n_1 , n_2 , and n_1^s used in calculating SIGNAL-SIZE are exactly as in the previous plots. Note that the points where the lines cross correspond almost exactly to the minimizers of the matrix and vector curves in Figure 3, which in turn correspond the minimizers of the expected relative errors in the terms $n_2^s r_{11}$ and $n_2^s r_{12}$.

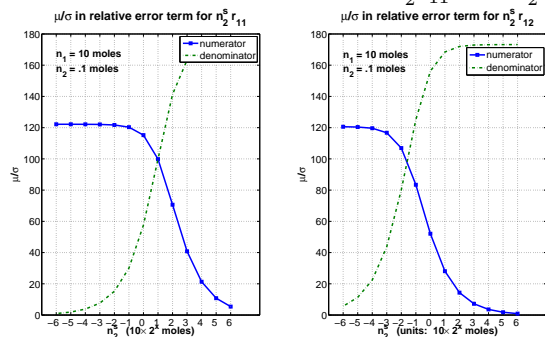


Figure 3: The points where these curves meet correspond almost exactly to the minima of the relative error curves in Figure 3, suggesting that the quotient is most stable when the standard deviations of the numerator and denominator are identical.

4. CONCLUSIONS

The thrust of this note has been to show how matrix methods facilitate the sensitivity analysis of speciated isotope dilution mass spectrometry. Although Monte Carlo analysis can be coupled with any solution method, matrix methods render the process efficient: the code to generate Table 1, for example, is a couple of lines long (see Supplement) and takes 0.7 seconds to execute using Matlab on a MacBook Pro laptop. On the other hand, a matrix approach was fundamental to our focus on linear perturbation theory and our subsequent development of the probability model.

The sensitivity analysis we have presented here, which is carried out in the spirit of *experimental mathematics* [13], should be considered representative but by no means the last word on the subject. While such purely formal approaches help illuminate the underlying numeric structure of the problem, they are no substitute for the laboratory, and are best coupled both with empirical evidence and a solid grounding in the underlying physical processes.

In this work we chose to focus on the particular experimental regime published in [2], looking mostly at shifts

in stability under shifts in spike amounts. Clearly, there are other regimes and other parameters of interest. Our choice to focus on spike amounts was motivated by two practical considerations. The first of these is that spike amounts are the easiest experimental parameters to adjust. The second is that spikes can be expensive, and thus determination of the optimal ratio between the spike and the sample concentrations can be an important budgetary consideration while maintaining accuracy.

On the level of design, even if the only choice facing the scientist is one of how much of each spike to add, the difficulty lies in the fact that the optimal choice depends on the composition of the sample, and this is by definition unknown. If this composition can be estimated a priori, the probability model of the last section can be used to guide the experimental design variables, but lacking such an estimate, the best use for this theory is probably as a means of generating error bounds on an existing estimate, or for repeating an experiment if the uncertainties on the first pass prove unacceptably high.

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