Implementation of isotope dilution mass spectrometry with one, two and three reverse steps

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Executive Summary

An extension to the established methods for analysing an unknown sample by isotope dilution mass spectrometry (IDMS) is proposed. The new method simplifies the presentation of established methods by introducing the "isotope dilution curve". This shows how a method can be developed that does not require the measurement of the isotope ratio of the highly enriched spike and therefore limits the range of isotope ratios that have to be measured accurately.

CONTENTS

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1. Introduction

Isotope dilution followed by isotope ratio measurement is used widely as an analytical method, usually known as IDMS. It has the major advantage that it is capable of measuring elemental and molecular amounts that are largely independent of the matrix. In its simplest form, it involves measuring the isotope ratios of the sample, a highly enriched spike and a blend of the two. Since these span a very wide range of isotope ratios, the method requires a mass spectrometer that has sufficient accuracy over the complete range of isotope ratios being measured, which is very complex and beyond the scope of routine laboratory analysis. Alternatively, standard reference materials with certified values of the isotope ratio can be used to calibrate the instrument.

Recent advances in the method include the development of the two-step IDMS method [1]. This extends the simple "direct" method by the introduction of a reverse step, in which the enriched spike is "assayed" against a pure material with the same isotope ratio as the sample. This eliminates the need to measure the concentration of the pure spike directly. However, it is still necessary to measure the isotope ratio of the highly enriched spike and memory effects, which arise when isotopes with vastly different ratios are measured, can limits the final accuracy of the measurement.

2. Measurement equation for direct IDMS

The basic principle of IDMS is that, an amount N_l of the sample is blended with an amount N_{sp} of enriched spike. The isotope ratio of the blend (R_{bl}) is then related to the isotope ratios of the spike (R_{sp}) and sample (R_s) by:

$$\frac{N_1}{} = \frac{R_{sp} - R_{b1}}{R_{b1} - R_s} \cdot \frac{\sum_i R_s}{\sum_i R_{sp}} \qquad 1)$$

where the summation is over all possible isotopes. This is the measurement equation for "direct" IDMS. In the following sections, this equation will be applied to each step in IDMS methods that use an unknown blend and up to three known blends.

3. IDMS with one reverse step

In "two-step" IDMS, a reverse step is introduced that "calibrates" the response of the spectrometer. The reverse step involves an additional blend with ratio R_{b2} made from an amount N_2 of pure material and an amount of enriched spike N_{sp2}. Applying (1) to the reverse step leads to:

$$\frac{N_2}{N_{sp2}} = \frac{R_{sp} - R_{b2}}{R_{b2} - R_p} \cdot \frac{\sum_i R_p}{\sum_i R_{sp}} \qquad (2)$$

The summations over all isotopes in the spike can be eliminated by dividing (1) by (2) and re-arranging to give:

$$\frac{N_1}{N_{sp1}} = G \cdot \frac{N_2}{N_{sp2}} \cdot \frac{R_{sp} - R_{b1}}{R_{b1} - R_s} \frac{R_{b2} - R_p}{R_{sp} - R_{b2}} \qquad (3)$$

where $G = \sum_i R_p \Big/ \sum_i R_s$ and the summations are over all isotopes (The measurement of G is discussed in Annex I). Equation (3) is the measurement equation for two-step IDMS. When the sample and pure reference have identical isotopic composition, (3) can be simplified to:

$$\frac{N_1}{N_{sp1}} = \frac{N_2}{N_{sp2}} \cdot \frac{R_{sp} - R_{b1}}{R_{b1} - R_s} \cdot \frac{R_{b2} - R_p}{R_{sp} - R_{b2}}$$

4. IDMS with two reverse steps

If a second reverse step is carried out, in which a second amount of pure material N_3 is blended with an amount of enriched material N_{sp3} to give another isotope ratio R_{b3}:

$$\frac{N_3}{N_{sp3}} = \frac{R_{sp} - R_{b3}}{R_{b3} - R_p} \cdot \frac{\sum_i R_p}{\sum_i R_{sp}}$$

Equations (2) and (4) can be combined to form the equality:

$$\frac{N_3}{N_{sp3}} \frac{R_{sp} - R_{b2}}{R_{b2} - R_p} = \frac{N_2}{N_{sp2}} \frac{R_{sp} - R_{b3}}{R_{b3} - R_p} \qquad (5)$$

which can be solved to give an explicit expression of R_{sp}:

$$R_s = \frac{\frac{N_2}{N_{sp2}} \cdot \left(R_{b2} - R_p\right) \cdot R_{b3} \quad \frac{N_3}{N_{sp3}} \left(R_{b3} - R_p\right) \cdot R_{b2}}{\frac{N_2}{N_{sp2}} \cdot \left(R_{b2} - R_p\right) \qquad \left(R_{b3} - R_p\right)} \tag{6}$$

Substituting (6) into (3), and re-arranging leads to

$$\frac{1}{G}\frac{N_1}{N_{sp1}} = \frac{N_2}{N_{sp2}} \cdot \frac{R_{b3} - R_{b1}}{R_{b1} - R_s} \cdot \frac{R_{b2} - R_p}{R_{b3} - R_{b2}} + \frac{N_3}{} \cdot \frac{R_{b2} - R_{b1}}{R_{b1} - R_s} \cdot \frac{R_{b3} - R_p}{R_{b2} - R_{b3}} \tag{7}$$

In the case where R_s=R_p, it follows that G=1 and equation (7) can be re-written as

$$\frac{N_1}{N_{sp1}} = \frac{N_2}{N_{sp2}} \cdot \frac{R_{b3} - R_{b1}}{R_{b1} - R_s} \cdot \frac{R_{b2} - R_p}{R_{b3} - R_{b2}} + \frac{N_3}{N_{sp3}} \cdot \frac{R_{b2} - R_{b1}}{R_{b1} - R_s} \cdot \frac{R_{b3} - R_p}{R_{b2} - R_{b3}}$$

Equations (7) have the important property that they are independent of R_{sp}. Hence a measurement that uses two reverse steps can be independent of the isotope ratio of the spike (R_{sp}). Alternatively, a similar equation could have been developed that involved R_{sp}, but was independent of R_p.

5. Simplified notation for the IDMS equations

Further manipulation of the IDMS equation is limited by the cumbersome notation used in previous sections. Therefore it is useful to re-formulate equation (1) by recognising that for any particular spike and sample, R_{sp} and R_s can be considered to be parameters while the variables are R_b and N_1/N_{sp1} or its equivalent, the mass ratio $x_1 = {}^{m_1}\!/_{m_{sp1}}$.

Hence (1) can be re-written as:

$$x_1 = \frac{a - y_1}{y_1 - b} \cdot Q$$

where $y_1 = R_{b1}$, $a = R_{sp}$, $b = R_s$, $Q = \frac{M_s}{M_{sp}} \sum_i R_s \Big/ \sum R_{sp}$ and M is the RMM.

This formulation of the equation highlights the fact that any IDMS method requires three unknown parameters to be determined and that x and y can be considered to be dependent and independent variables respectively. For example, in a "direct" IDMS measurement, a, b and Q are measured independently, which enables the unknown x to be calculated from a measurement of y.

When one reverse step is incorporated and the isotope ratio of the pure reference R_p is denoted as c, it yields a pair of measurements (x_1, y_1) through gravimetry and mass spectrometry. These enable Q to be eliminated and the IDMS equation to be re-written as:

$$x_1 = x_2 \cdot \frac{a - y_1}{y_1 - b} \cdot \frac{y_2 - c}{a - y}$$

where we have assumed that Q is the same for the unknown and the pure reference. The use of a second reverse step yields an additional pair of measurements (x_2, y_2) that enable both Q and a to be eliminated. This leads to:

$$x_1 = x_2 \cdot \frac{y_3 - y_1}{\text{[illegible]}} \cdot \frac{y_2 - c}{\text{[illegible]}} + x_3 \cdot \frac{y_2 - y_1}{\text{[illegible]}} \cdot \frac{y_3 - c}{\text{[illegible]}}$$

which corresponds to equation (7') in the new notation.

6. Graphical representation of IDMS

Further insight into the IDMS method can be gained by recognising that (8) is the basic measurement equation for IDMS, which can be applied to each step in an IDMS procedure. Hence every step must correspond to a point on a curve defined by (8). The form of this curve can be established by expanding and re-arranging (8):

$$(x + Q)(y - b) = Q(a - b) \qquad 1$$

Equation (11) is a rectangular hyperbola which is shown graphically for specific values of the parameters in Figures 1 and 2.

Figures 1 and 2 show the two cases of $R_{sp} > R_s$ and $R_{sp} < R_s$ respectively. When the three parameters a, b and Q are known, the curve is defined completely. Measurement of a value for y of an unknown blend then leads to a unique result for x (the mass ratio of sample to spike in the blend). Alternatively, since there are three unknowns in equation (11), the curve can be defined completely by knowledge of the coordinates (x,y) of any three points that lie on it. These might be the results, for example, of three reverse steps.

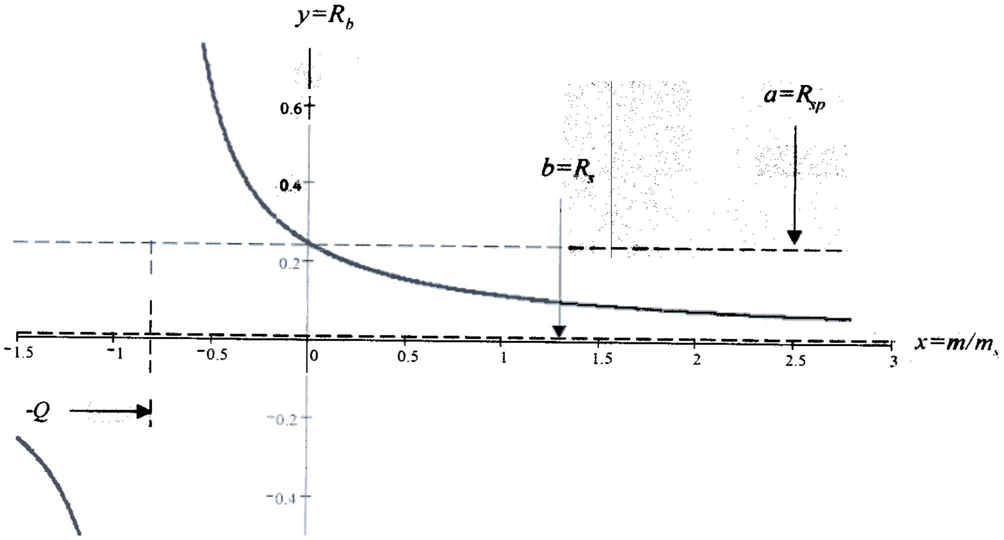

Figure 1: IDMS equation for the case of $R_{sp}>R_s$ (The values used are *$a=0.2435$, $b=0.01188$, $Q=0.8102$*).

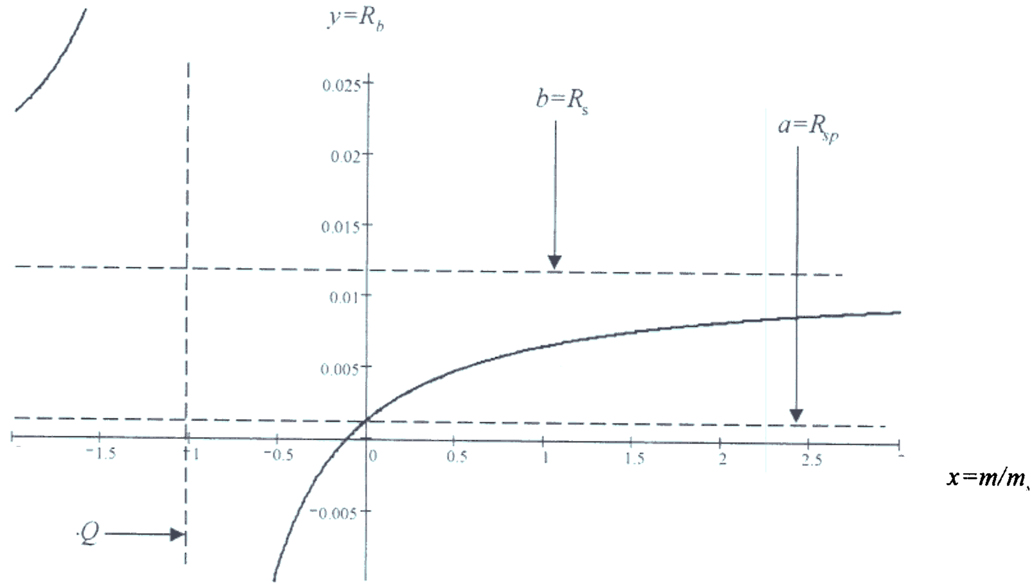

Figure 2: IDMS equation for the case of $R_{sp}<R_s$ (The values used are *$a=0.001302$, $b=0.01188$, $Q=1.011$*).

7. IDMS with one and two reverse steps expressed in δ-notation

Many mass spectrometers are configured to present results in terms of the quantity:

$$\delta = \frac{R}{R_{st}} \qquad (12)$$

where R is the measured isotope ratio and R_{st} is the isotope ratio of a "standard". In the examples developed here, R_p can be considered to play the role of a standard, and (11) can be re-written as:

$$(x+Q)\delta = Q\delta_{sp}$$

where $\delta = y/b-1$ and $\delta_{sp} = a/b-1$. If δ_s, δ_{b1}, δ_{b2}, and δ_{b3}, are used to denote the values of δ for the sample, the first, second and third blend respectively. It is now possible to re-express (10) as:

$$x_1 = Dx_2 + (1-D)x_3$$

where

$$D = \frac{\delta_{b2}(\delta_{b3} - \delta_{b1})}{(\delta_{b1} - \delta_s)(\delta_{b3} - \delta_{b2})}$$

or in the case where $R_s = R_p$

$$D = \frac{\delta_{b2}(\delta_{b3} - \delta_{b1})}{\delta_{b1}(\delta_{b3} - \delta_{b2})}$$

Equation (14) is the measurement equation for IDMS with two reverse steps and is equivalent to (7').

A graphical presentation of equation (13) is similar to Figures 1 and 2 except that the y-axis is displaced by b and re-labelled as δ. In equation (13), it is clear that, the unknowns a and Q can be determined through a measurement of the two pairs (δ_2, x_2) and (δ_3, x_3). The unknown x_1 can then be determined from a measurement of δ_1 as shown schematically in Figure 3:

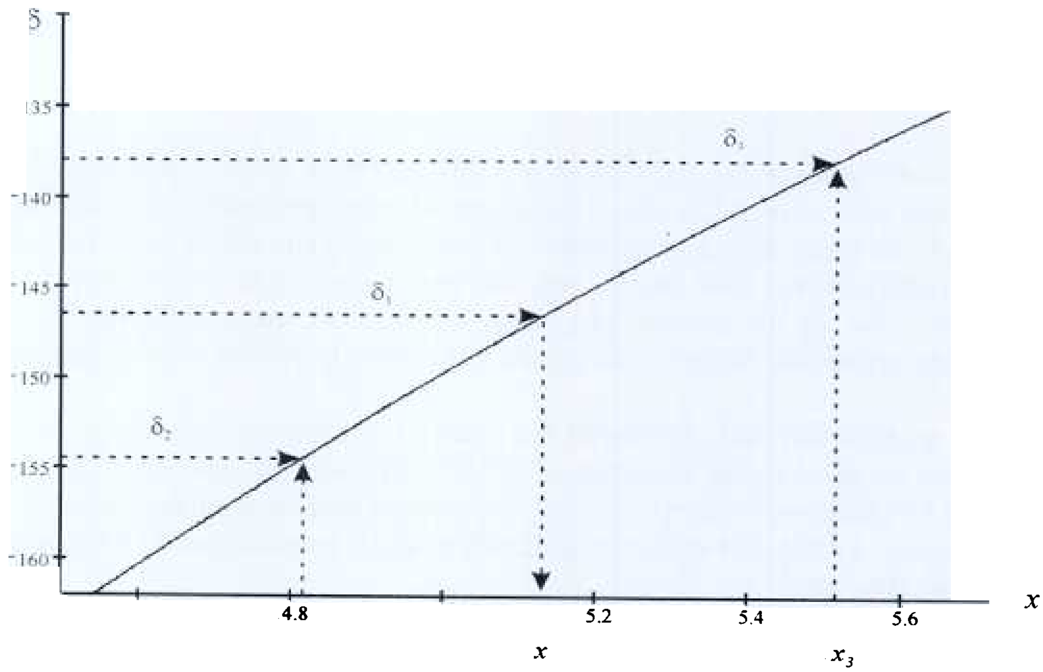

Figure 3: Graphical representation of equation (13) being used to calculate x from measured values of (δ_1, x_1), (δ_2, x_2) and δ (In this case b=0.01188 as for Figure 2).

8. Uncertainty analysis

In order to analyse the uncertainty of the different methods that use the isotope dilution curve, it is useful to write (13) in the form

$$x = Q\cdot(\frac{\delta_{sp}}{\delta} - 1)$$

If a series of values x_i and δ_i are used to define the isotope dilution curve, then their means are related by

$$x - \bar{x} = Q\cdot\delta_{sp}\cdot(\frac{1}{\delta_b} - \frac{1}{\bar{\delta}_b})$$

Applying the internationally-accepted method [2] for calculating measurement uncertainty to (16) leads to:

$$u^2(x) = u^2(\bar{x}) + (\frac{1}{\delta_b} - \frac{1}{\bar{\delta}_b})^2 \cdot u^2(Q\cdot\delta_{sp}) + (Q\cdot\delta_{sp})^2 \cdot u(\frac{1}{\delta_b} - \frac{1}{\bar{\delta}_b})$$

The term $u(Q\cdot\delta_{sp})$ would be determined from the uncertainty of the "best-fit" curve to the points (x_i, δ_i).

9. Summary

The measurement equations for IDMS with one, two and three reverse steps have been presented. It has been shown that the introduction of a second reverse step enables a measurement to be made that is independent of the isotope ratio of the enriched spike (R_{sp}). The introduction of a third reverse step also makes the measurement independent of the isotope ratio of the sample (R_s). Both of these developments help design experiments that reduce the range of isotope ratios that have to be measured accurately.

A simplified notation has been developed that leads to a graphical presentation which provides useful insight into the operation of IDMS. The use of δ notation enables the measurand to be expressed in terms of a set of quantities that are displayed directly by many instruments. Finally, the operation of IDMS methods is explained by reference to the "isotope dilution curve".

Annex I: The measurement of the *G* factor for carbon dioxide (CO_2)

When the sample and pure reference do not have identical isotope composition, the factor G in equation (3) must be evaluated, which is usually not possible by direct measurement. In the case of CO_2, G can be expressed as:

$$G = \frac{\sum_i R_s}{\sum_i R_p} = \frac{1 + R_s^{45} + R_s^{46} + \Delta R_s}{1 + R_p^{45} + R_p^{46} + \Delta R_p} \qquad \text{(A1.1}$$

where $\Delta R_s = R_s^{47} + R_s^{48} + R_s^{49}$ and $\Delta R_p = R_p^{47} + R_p^{48} + R_p^{49}$. Equation (A1.1) can be written in terms of two parameters that can be measured by comparing the isotope ratios of the sample and pure reference at the mass 45 and 46 plus other terms that can be determined either from other sources or through estimation when their values have no significant influence to the final results. For samples close to natural abundance, ΔR_p and ΔR_p will be very small compared with the other terms in the numerator and denominator and we can assume $\Delta R_s = \Delta R_p$. Hence:

$$G = \frac{+\left(\frac{R_s^{45}}{R_p^{45}}\right) \cdot R_p^{45} + \left(\frac{R_s^{46}}{R_p^{46}}\right) \cdot R_p^{46} + \Delta R_p}{+ R_p^{45} + R_p^{46} + \Delta R_p} \qquad \text{(A1.2)}$$

The isotope ratios of the pure CO_2 can be obtained through other sources such as those given in IUPAC [3]. The error caused by the assumption $\Delta R_s = \Delta R_p$ is very small since values of R^{47}, R^{48} and R^{49} are significantly small compared with R^{45} and R^{46}. The uncertainties resulting from the uncertainty in the values of R_p are very small due to their very small sensitivity coefficients and can be ignored.

Annex II: Analytical description of IDMS with three reverse steps

If a third reverse step is carried out, equation (6) can be extended to form the equality:

$$\frac{\dfrac{R_{sp}-R_B}{R_B-R_s}-\dfrac{R_{sp}-R_{B'}}{R_{B'}-R_s}}{\dfrac{N_s}{N_{sp}}-\dfrac{N_{p'}}{\;}}=\frac{\dfrac{R_{sp}-R_{B'}}{R_{B'}-R_s}-\dfrac{R_{sp}-R_{B''}}{R_{B''}-R_s}}{\dfrac{N_{p'}}{\;}\quad\dfrac{N_{p''}}{\;}}=\frac{\dfrac{R_{sp}-R_{B''}}{R_{B''}-R_s}-\dfrac{R_{sp}-R_{B'''}}{R_{B'''}-R_s}}{\dfrac{N_{p''}}{\;}\quad\dfrac{N_{p'''}}{\;}} \qquad \text{(A2.1)}$$

The numerator of each term in (A2.1) can now be re-arranged:

$$\frac{\dfrac{R_B-R_{B'}}{(R_B-R_s)\cdot(R_{B'}-R_s)}}{\left(\dfrac{N_s}{N_{sp}}-\dfrac{N_{p'}}{N_{sp'}}\right)}=\frac{\dfrac{R_{B'}-R_{B''}}{(R_{B'}-R_s)\cdot(R_{B''}-R_s)}}{\left(\dfrac{N_{p'}}{N_{sp'}}-\dfrac{N_{p''}}{N_{sp''}}\right)}=\frac{\dfrac{R_{B''}-R_{B'''}}{(R_{B''}-R_s)\cdot(R_{B'''}-R_s)}}{\left(\dfrac{N_{p''}}{N_{sp''}}-\dfrac{N_{p'''}}{N_{sp'''}}\right)} \qquad \text{(A2.2)}$$

Unfortunately, this expression does not have sufficient symmetry to proceed towards a simple elegant analytical solution. The matrix formulation described in the following Annex may then be used.

Annex III: IDMS with three reverse steps and their matrix representation

Measurement model

It is useful to consider the benefits of an IDMS measurement using three reverse steps. It is not feasible to extend the methods used above to provide a simple analytical solution for this case (as explained in Annex I). Hence, we express the fact that the three pairs (x_1, y_1), (x_2, y_2) and (x_3, y_3) are solutions of equation (11) in the form of a matrix:

$$\begin{bmatrix} 1 & -y & x_1 \\ 1 & -y_2 & x_2 \\ 1 & -y_3 & x_3 \end{bmatrix} \begin{bmatrix} C \\ Q \\ b \end{bmatrix} = \begin{bmatrix} x_1y_1 \\ x_2y_2 \\ x_3y_3 \end{bmatrix} \qquad \text{(A3.1)}$$

where $C=Qa$. Equation (A3.1) can be solved for the unknown C, Q and b:

$$\begin{bmatrix} C \\ Q \\ b \end{bmatrix} = \begin{bmatrix} 1 & -y & x_1 \\ 1 & -y_2 & x_2 \\ 1 & -y_3 & x_3 \end{bmatrix}^{-1} \begin{bmatrix} x_1y_1 \\ x_2y_2 \\ x_3y_3 \end{bmatrix} \qquad \text{(A3.2)}$$

When the parameters C, Q and b have been determined from the measured values of x_i and y_i $(i=1,2,3)$, equation (8) can be used to calculate the unknown x from a measurement of y:

$$x = \frac{C/Q - y}{y - b} \cdot Q \qquad \text{(A3.3)}$$

A significant advantage of using three steps in this way is that the sets of isotope ratios measured can be chosen to have values close to each other. This reduces the difficulties caused by memory effects, which arise when it is necessary to measure the vastly different isotope ratios of the sample and spike.

Uncertainty analysis

The uncertainty associated with these results can be calculated. Let e, f, δC, δQ and δb denote perturbations in x, y, C, Q and b, respectively, that satisfy equation (11)

$$(x+e)(y+f) - (b+\delta b)(x+e) + (Q+\delta Q)(y+f) = (C+\delta C) \qquad \text{(A3.4)}$$

After ignoring second order terms, it follows that,

$$ey + fx - be - x\delta b + Qf + y\delta Q = \delta C, \tag{A3.5}$$

or, in matrix notation,

$$\begin{bmatrix} 1 & -y & x \end{bmatrix} \begin{bmatrix} \delta C \\ \delta Q \\ \delta b \end{bmatrix} = \begin{bmatrix} y-b & x+Q \end{bmatrix} \begin{bmatrix} e \\ f \end{bmatrix}. \tag{A3.6}$$

Substituting for the measurement data, we obtain

$$\begin{matrix} -y_1 & x_1 \\ -y_2 & x_2 \\ -y_3 & x_3 \end{matrix} \begin{bmatrix} \delta C \\ \delta Q \\ \delta b \end{bmatrix} = \begin{matrix} y_1 - b & 0 & 0 & x_1 + Q & 0 & 0 \\ 0 & y_2 - b & 0 & 0 & x_2 + Q & 0 \\ 0 & 0 & y_3 - b & 0 & 0 & x_3 + Q \end{matrix} \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ f_1 \\ f_2 \\ f_3 \end{bmatrix}, \tag{A3.7}$$

With appropriate definitions this can be written as:

$$J_a \delta \mathbf{a} = J_e \mathbf{e}, \tag{A3.8}$$

or

$$\delta \mathrm{a} = J_a^{-1} J_e \mathbf{e}. \tag{A3.9}$$

where $\mathbf{a}=(C, Q, b)^T$, Then,

$$\delta \mathbf{a}(\delta \mathbf{a})^{\mathrm{T}} = J_a^{-1} J_e \mathbf{e}\mathbf{e}^{\mathrm{T}} J_e^{\mathrm{T}} J_a^{-\mathrm{T}}, \tag{A3.10}$$

and, taking expectations,

$$V_a = J_a^{-1} J_e V_e J_e^{\mathrm{T}} J_a^{-\mathrm{T}} \tag{A3.11}$$

Here, V_e is the covariance matrix for the model inputs (the measurement data) and V_a is the covariance matrix for the model outputs (the model parameters). The (6 × 6) matrix V_e contains the variances and covariances for the measurement data and requires *a priori* information about the data errors. The (3 × 3) matrix V_a contains the variances and covariances for the model parameters. From the conventional notations

$$u(x)^2 = E[(x - \mu_x)^2] \tag{A3.12}$$

and

$$u(x, y) = u(y, x) = E[(x - \mu_x) \cdot (y - \mu_y)] \tag{A3.13}$$

the full form of the covariance matrices V_e and V_a can be expressed as

$$V_a = \begin{bmatrix} u(C)^2 & u(C,Q) & u(C,b) \\ u(C,Q) & u(Q)^2 & u(C,b) \\ u(C,b) & u(Q,b) & u(b)^2 \end{bmatrix} \qquad \text{(A3.14)}$$

and

$$V_e = \begin{bmatrix} u(x_1)^2 & u(x_1,x_2) & u(x_1,x_3) & u(x_1,y_1) & u(x_1,y_2) & u(x_1,y_3) \\ u(x_1,x_2) & u(x_2)^2 & u(x_2,x_3) & u(x_2,y_1) & u(x_2,y_2) & u(x_2,y_3) \\ u(x_1,x_3) & u(x_2,x_3) & u(x_3)^2 & u(x_3,y_1) & u(x_3,y_2) & u(x_3,y_3) \\ u(x_1,y_1) & u(x_2,y_1) & u(x_3,y_1) & u(y_1)^2 & u(y_1,y_2) & u(y_1,y_3) \\ u(x_1,y_2) & u(x_2,y_2) & u(x_3,y_2) & u(y_1,y_2) & u(y_2)^2 & u(y_2,y_3) \\ u(x_1,y_3) & u(x_2,y_3) & u(x_3,y_3) & u(y_1,y_3) & u(y_2,y_3) & u(y_3)^2 \end{bmatrix} \qquad \text{(A3.15)}$$

Calculation

Given a new measured value y and its standard uncertainty $u(y)$, the corresponding value of x is calculated from equation (A3.3).

To a first order approximation, we have

$$x + \delta x = x + \begin{bmatrix} \frac{}{y-b} & \frac{-y}{y-b} & \frac{C-Qy}{(y-b)^2} \end{bmatrix} \delta \mathbf{a} - \frac{K(y-b)+(C-Qy)}{(y-b)^2}\delta y, \qquad \text{(A3.16)}$$

and, consequently, the standard uncertainty $u(x)$ of x is calculated from

$$u^2(x) = \begin{bmatrix} \frac{}{y-b} & \frac{-y}{y-b} & \frac{C-Qy}{(y-b)^2} \end{bmatrix} V_a \begin{bmatrix} \frac{1}{y-b} \\ \frac{-y}{y-b} \\ \frac{C-Qy}{(y-b)^2} \end{bmatrix} + \left(\frac{Q(y-b)+(C-Qy)}{(y-b)^2} \right)^2 u^2(y), \qquad \text{(A3.17)}$$

where we assume y and the parameters $\mathbf{a}$ are independent.

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