Isotope pattern deconvolution for internal mass bias correction in the characterisation of isotopically enriched spikes†

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Enriched spikes of ^{53}Cr, ^{57}Fe, ^{67}Zn, ^{95}Mo, ^{111}Cd, ^{143}Nd, ^{150}Sm, ^{199}Hg and ^{207}Pb were characterised on a multi-collector ICP-MS instrument both in terms of isotopic composition and concentration. The isotopic composition of the spikes was determined using either a natural abundance or certified standard for mass bias correction, except for lead where thallium internal correction was used. It was observed that the use of weighted regression lines for mass bias correction provided similar or, in most cases, lower uncertainties in the isotopic composition of the spikes in comparison with unweighted regression lines or single ratio measurements. For the establishment of the spike concentration by reverse isotope dilution analysis, isotope pattern deconvolution was evaluated and mass bias could be corrected internally in each blend by minimising the variance of the multiple linear regression model. It was also observed that isotope pattern deconvolution with internal mass bias correction provided lower uncertainties in the concentration of the spikes in comparison with the usual procedure based on external mass bias correction.

1. Introduction

The characterisation of isotopically enriched spikes, both in terms of isotope abundance and concentration, is the first link in the traceability chain of any analytical process based on isotope dilution (ID) analysis. In many cases this first link is the weakest one, as the uncertainties in the concentration of the spikes could be a major source of the overall analytical uncertainty, particularly when using multi-collector ICP-MS instruments.[1,2] Several approaches, such as double IDMS or exact matching double IDMS, have been followed to minimise the effect of spike concentration uncertainty in the overall analytical results.[1,3,4] However, these procedures, which are useful for the preparation of reference materials, require a very good prior estimation of the unknown concentration of the element and they would not be easily implemented in the routine application of ID in testing laboratories except, perhaps, in the case of on-line ID.[5] Ideally, the final uncertainty of the concentration of the isotopically enriched spike should be as close as possible to the uncertainty of the concentration of the natural abundance standard used for certification. However, other terms, such as mass bias correction uncertainty,[6] natural abundance uncertainty[7] and/or the precision of the

isotope ratio measurements in the blend,[4] contribute significantly to the total combined uncertainty.

As is well known, mass bias in ICP-MS causes deviation of the measured isotope ratios with respect to the theoretical values because of a mass dependent transmission efficiency in the mass spectrometer.[8,9] Several algorithms for mass bias correction have been published,[9,10] including the linear, power law and exponential model.[9] The Russell equation[11] is another exponential model taken from TIMS isotope ratio measurements which are also applied nowadays in multi-collector ICP-MS instruments.[12,13] As in any analytical procedure where a systematic error has to be taken into account, the correction for mass bias increases the uncertainty of the measurements. Hence, it is necessary to use mass bias correction procedures, which minimise the propagated uncertainty. In most applications of ID analysis, except for the time-consuming exact matching double IDMS, mass bias needs to be corrected either externally or internally. External mass bias correction uses an isotopically certified standard measured before and/or after the measurement of the samples.[12] For the certification of the isotopic composition of enriched spikes external mass bias correction is usually applied.[14,15] In many instances the mass bias factor is calculated from a single ratio measurement[16] or from the slope of the linear plot between the natural logarithm of the ratio between the measured and certified isotope ratio *versus* the nominal mass difference between the isotopes used to calculate the ratio.[15,17] Under those circumstances the uncertainties in the natural or certified isotope abundances are the largest part of the uncertainty budget in the calculation of the mass bias factor but no statistical weight is usually given to the experimental points. In theory, the use of weighted

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† Electronic supplementary information (ESI) available: uncertainty propagation for the determination of the molybdenum content in the ^{95}Mo spike by isotope pattern deconvolution, isotope pattern deconvolution for samarium—internal correction of the mass bias factor and experimental isotope ratios. See DOI: 10.1039/b710595a

regression lines for mass bias correction should provide lower uncertainties in the isotopic composition of the spikes, as most statistical weight is given to the isotope ratios measured with lower uncertainties.

On the other hand, for internal mass bias correction another element is added to the sample in order to calculate the mass bias factor, e.g., thallium for lead isotope ratio measurements,[6] usually applying the exponential model. It is not completely accepted that another element close in mass to the element of interest will suffer the same mass bias in ICP-MS. For lead isotope ratio measurements, thallium has proved to be a good internal mass bias standard[18] but examples of disagreement, such as the use of strontium for the correction of selenium isotope ratios,[19] have been reported in the literature. On the other hand, this mode of correction is ideal when there is mass bias drift or matrix effects on the mass bias factor as the correction is performed in the sample and at the instant of the measurement.[20] Unfortunately, internal correction has been described only for a few isotope systems (Zn–Ga, Pb–Tl, Cu–Zn, Si–S) so, at the moment, there is no universal procedure for internal mass bias correction which could be applied to any isotope system.

Another important factor affecting the uncertainty of the spike concentration is the precision of the measured isotope ratio in the blend.[1,2] Traditionally, isotope dilution analysis is a single ratio procedure and the relative uncertainty in the measured isotope ratio propagates to the measured concentration by a multiplicative factor higher than 1. Multiple isotope ratio procedures, such as those needed to calculate isotope abundances, may reduce the contribution of this factor to the overall standard uncertainty.

Isotope pattern deconvolution is a mathematical technique which can calculate the contribution of individual mass spectra in mixtures of different "isotopic patterns".[21–23] In this paper this mathematical procedure has been applied to the characterisation of enriched spikes by isotope dilution analysis. In this case, the isotope pattern observed in the blend can be assumed to be a linear combination of two isotope abundance patterns: the isotope pattern in the sample (usually natural) and the isotope pattern in the spike. The calculation of the individual contributions (molar fractions) to the overall isotope composition in the blend is performed by multiple least squares.[24] The characterisation of enriched isotope spikes by multi-collector ICP-MS is an ideal test for this methodology as several isotope ratios can be measured simultaneously with low standard uncertainties. We have observed that, by

applying this procedure, mass bias correction can be performed internally by minimising the variance of the multiple linear regression. In addition, different procedures for the evaluation of mass discrimination and their associated uncertainties are compared.

2. Experimental

2.1. Reagents and materials

All reagents used were of analytical grade. Ultra-pure water was obtained from a Milli-Q system (Millipore Co., Bedford, MA, USA). Individual certified standard solutions (1000 mg L^{-1}) of Cr, Fe, Zn, Mo, Cd, Nd, Sm, Hg, Pb and Tl were purchased from Merck (Darmstadt, Germany). Hydrochloric and nitric acids (analytical grade, Merck) were purified by sub-boiling distillation prior their use for spikes digestions and further solutions.

Enriched ^{53}Cr, ^{67}Zn, ^{95}Mo, ^{111}Cd, ^{199}Hg and ^{207}Pb spikes were prepared from solid materials (metal or oxide) supplied by Cambridge Isotope Laboratories (Andover, MA, USA). Solid ^{57}Fe, ^{143}Nd and ^{150}Sm were obtained from Isoflex (San Francisco, CA, USA). The nominal enrichment of the most abundant isotope in the spike was given by the manufacturer, but no abundance values were given for the other isotopes nor any uncertainty data on their isotopic composition. Solid spikes were dissolved in nitric acid or in hydrochloric acid and made up to volume with Milli-Q water.

2.2. Instrumentation

The characterization of the spikes was performed using a MC-ICP-MS instrument (Neptune, Thermo Fisher Scientific, Germany) located at the University of Vigo (Spain). This instrument incorporates a double focusing sector field mass spectrometer with variable mass dispersion capable of operating in low, medium and high resolution modes. It is equipped with eight adjustable Faraday cups and one fixed centre cup. In order to increase the sensitivity, the instrument is also equipped with a guard electrode that improves the ion transmission efficiency and prevents formation of secondary discharges. The low and medium resolution modes were used throughout this study, with low resolution being nemployed for all elements except Cr and Fe, which were measured at medium resolution. The cup configuration employed for each element is given in Table 1 including, in some cases, other elements to correct for isobars. Instrument tuning and

Table 1 Resolution mode and Faraday cup configuration for the different isotopically enriched spikes certified by MC-ICP-MS

Element	Resolution	Cup configuration								
		L4	L3	L2	L1	C	H1	H2	H3	H4
Cr	Medium			^{50}Cr		51.93	^{53}Cr	^{54}Cr		
Fe	Medium		^{53}Cr		^{54}Fe	55.90	^{57}Fe	^{58}Fe		^{60}Ni
Zn	Low			^{64}Zn		^{66}Zn	^{67}Zn	^{68}Zn		^{70}Zn
Mo	Low		^{92}Mo	^{94}Mo	^{95}Mo	^{96}Mo	^{97}Mo	^{98}Mo		^{100}Mo
Cd	Low		^{108}Cd	^{110}Cd	^{111}Cd	^{112}Cd	^{113}Cd	^{114}Cd	^{116}Cd	
Nd	Low	^{142}Nd	^{143}Nd	^{144}Nd	^{145}Nd	^{146}Nd	^{147}Sm	^{148}Nd	^{150}Nd	
Sm	Low		^{144}Sm	^{147}Sm	^{148}Sm	^{149}Sm	^{150}Sm	^{152}Sm	^{154}Sm	
Hg	Low	^{194}Pt	^{196}Hg	^{198}Hg	^{199}Hg	^{200}Hg	^{201}Hg	^{202}Hg	^{204}Hg	^{206}Pb
Pb	Low		^{202}Hg	^{203}Tl	^{204}Pb	^{205}Tl	^{206}Pb	^{207}Pb	^{208}Pb	

optimization of signal intensity were performed before each measurement session. The analyses were conducted in the static mode. Samples and standards were diluted with sub-boiled 1% (w/w) HNO_3 and introduced into the plasma through a microconcentric PFA nebulizer (50 μL min^{-1}) operating by free aspiration and a quartz spray chamber arrangement (cyclone + Scott double pass).

2.3. Procedures

External mass bias correction. The determination of the isotopic composition of the enriched spikes was carried out by external mass bias correction using the natural abundance element (Merck standard) as reference, except for lead where thallium internal correction was used.

The measured intensities of the isotopes of a given element, within the different cups of the multi-collector, were transformed to isotope ratios with reference to the most abundant isotope free of potential isobaric interferences, and then the mass bias factor, K, was computed as the slope of the regression line given by the equation:

$$\ln \frac{R_{\exp}}{R_{\text{theo}}} = K\Delta M \qquad (1)$$

where $R_{\exp}$ is the measured isotope ratio for the natural abundance standard, R_{theo} is the theoretical value calculated from the IUPAC isotope abundances,[25] and ΔM is the nominal mass difference between the isotopes considered.

For the linear regression a weighted line was calculated taking in consideration the theoretical and experimental uncertainties in the isotope ratios. It should be emphasized here that the theoretical uncertainties in the isotope ratios were calculated based on the uncertainties in the natural isotope abundances taken from the IUPAC tables,[25] which included possible natural variations. Variations in the natural isotopic composition of analytical standards larger than the stated IUPAC uncertainties have been observed previously.[20] For the purpose of this work, isotope abundances in analytical standards slightly different from those tabulated by IUPAC will only increase the uncertainty of the mass bias correction factor, which will be then propagate to the uncertainty in the concentration.

It can be demonstrated that the uncertainty for the expression $\ln \frac{R_{\exp}}{R_{\text{theo}}}$ can be calculated using:

$$u\left(\ln \frac{R_{\exp}}{R_{\text{theo}}}\right) = \sqrt{\left(\frac{s_{R_{\exp}}}{R_{\exp}}\right)^2 + \left(\frac{s_{R_{\text{theo}}}}{R_{\text{theo}}}\right)^2} \qquad (2)$$

So, eqn (2) allows us to calculate the weight factors, w, which need to be applied to every point in the regression line.[26]

Isotope pattern deconvolution. In the application proposed here a natural abundance element, of known isotopic composition and stated isotope abundance uncertainties, or better, an isotopically certified standard, is mixed with the same element isotopically enriched in one of its isotopes. Thus, in the spiked sample or blend we will have two different isotope patterns. The amount (mol) of element in a given blend, b, can be distributed between the two sources by:

$$N_{\text{b}} = N_{\text{nat}} + N_{\text{enr}} \qquad (3)$$

In eqn (3), N_{b} is the total amount of element found in the blend b, N_{nat} is the amount coming from the element with natural isotope abundances, and N_{enr} is the amount of the enriched element in the blend coming from the spike added. Similar mass balances can be also obtained for all isotopes of the element considered, as shown by eqn (4), illustrated for a theoretical isotope i as an example:

$$N_{\text{b}}^{\text{i}} = N_{\text{nat}}^{\text{i}} + N_{\text{enr}}^{\text{i}} \qquad (4)$$

Eqn (4) can be expressed as a linear combination of the total amounts and the isotope abundances of the natural abundance element and the enriched isotope present in the blend:

$$N_{\text{b}} \cdot A_{\text{b}}^{\text{i}} = N_{\text{nat}} \cdot A_{\text{nat}}^{\text{i}} + N_{\text{enr}} \cdot A_{\text{enr}}^{\text{i}} \qquad (5)$$

where A_{b}^{i} is the isotope abundance of the isotope i of the element in the blend and $A_{\text{nat}}^{\text{i}}$ is the natural isotope abundance of the same isotope, whereas, $A_{\text{enr}}^{\text{i}}$ is the isotope abundance of isotope i in the isotopically enriched element. When we divide eqn (5) by eqn (3), the following expression is obtained:

$$A_{\text{b}}^{\text{i}} = x_{\text{nat}} \cdot A_{\text{nat}}^{\text{i}} + x_{\text{enr}} \cdot A_{\text{enr}}^{\text{i}} \qquad (6)$$

where

$$x_{\text{nat}} = \frac{N_{\text{nat}}}{N_{\text{nat}} + N_{\text{enr}}} \qquad (7)$$

and

$$x_{\text{enr}} = \frac{N_{\text{enr}}}{N_{\text{nat}} + N_{\text{enr}}} \qquad (8)$$

In expressions (7) and (8), the variables x_{nat} and x_{enr} indicate the molar fractions of the element in the isotopically altered sample arising from the two different sources of the element (natural or isotopically enriched). If we have an element containing n isotopes, equations for all the isotopes of the element can be obtained in a similar way to eqn (6), and they can be expressed in matrix notation as:

$$\begin{bmatrix} A_{\text{b}}^1 \\ A_{\text{b}}^2 \\ A_{\text{b}}^3 \\ \dots \\ A_{\text{b}}^{n-1} \\ A_{\text{b}}^n \end{bmatrix} = \begin{bmatrix} A_{\text{nat}}^1 & A_{\text{enr}}^1 \\ A_{\text{nat}}^2 & A_{\text{enr}}^2 \\ A_{\text{nat}}^3 & A_{\text{enr}}^3 \\ \dots & \dots \\ A_{\text{nat}}^{n-1} & A_{\text{enr}}^{n-1} \\ A_{\text{nat}}^n & A_{\text{enr}}^n \end{bmatrix} \cdot \begin{bmatrix} x_{\text{nat}} \\ x_{\text{enr}} \end{bmatrix} + \begin{bmatrix} e^1 \\ e^2 \\ e^3 \\ \dots \\ e^{n-1} \\ e^n \end{bmatrix} \qquad (9)$$

As we have more parameters (isotopes abundances) than unknowns (molar fractions) an error vector is included in eqn (9). The best values of x_{nat} and x_{enr} are found by least square minimisation of the error vector "e". If we name the vector of the isotope abundances in the blend as "y", the matrix of the isotope abundances of the pure components as "A" and the vector of the unknown molar fractions as "x", the least square solution of this over-determined system of equations can be calculated as:[21,24]

$$x = (A' \times A)^{-1} \times (A' \times y) \qquad (10)$$

where A' indicates the transpose of "A" and superscript -1 the inverse. When we have more parameters than unknowns (a minimum of three isotopes) we can also determine the standard uncertainties for those parameters using the variance-

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covariance matrix, $V(x)$. The diagonal elements of this matrix are the variances of the variables x_{nat} and x_{enr}. From the multivariate linear regression this matrix can be calculated as:[24]

$$V(x) = (A' \times A)^{-1} \times s_e^2 \qquad (11)$$

where s_e^2 is the variance of the regression model (the sum of squares of errors divided by the degrees of freedom, $n - 2$). Once the molar fractions of the element and their uncertainties are calculated by the linear regression, the amount of natural abundance element can be easily calculated since the amount of spike added is known or *vice versa*.

Internal mass bias correction. We have used the exponential model for mass bias correction.[6] The intensities obtained in each Faraday cup in the multi-collector instrument for the blend are divided by the intensity observed in the reference isotope (the spike isotope). Then, the isotope ratios are corrected using the exponential model by the equation:

$$R_{cor} = \frac{R_{exp}}{e^{K \cdot \Delta M}} \qquad (12)$$

where R_{cor} is the mass bias corrected isotope ratio, R_{exp} is the measured ratio, K is the mass bias factor ($K = 0$ before iteration), and ΔM is the nominal mass difference between the considered isotopes. Then, the corrected isotope abundances in the blend are calculated using the equation:

$$A_b^i = \frac{R_{cor}^i}{\sum\limits_{i=1}^{n} R_{cor}^i} \qquad (13)$$

These corrected abundances are then introduced into the isotope pattern deconvolution procedure, and the internal mass bias correction factor, K, is calculated by minimising the variance of the regression, s_e^2. For iteration, the excel SOLVER application was used (changing K until s_e^2 is a minimum). An example Excel file for samarium is given in the Electronic Supplementary Information†.

3. Results and discussion

Enriched spikes of ^{53}Cr, ^{57}Fe, ^{67}Zn, ^{95}Mo, ^{111}Cd, ^{143}Nd, ^{150}Sm, ^{199}Hg and ^{207}Pb were characterised on a multi-collector ICP-MS instrument, both in terms of isotopic composition and concentration. These spikes cover a large proportion of the element mass scale and contain at least 4 stable isotopes for simultaneous multiple isotope ratio measurements. First, the

spikes were characterised in isotopic composition by means of external mass bias correction using a weighted regression line, and then their concentration was determined by reverse isotope dilution analysis. Isotope pattern deconvolution was applied, which allowed internal mass bias correction. Finally, full uncertainty calculations were carried out, both for the isotopic composition and concentration.

3.1. Determination of the isotope composition of the spikes and their uncertainty

The isotope composition of the nine spikes under study was measured using a bracketing mode in which the natural abundance element was measured before and after the enriched spike for external mass bias correction. No statistical differences in the mass bias factor were found for the standards measured before or after the spike, so we can assume that no mass bias drift was present during the measurements. The mass bias factor, K, was calculated using the exponential model[6,10] using a weighted regression line in which the declared uncertainties in the natural isotopic composition of the elements[25] were taken into account in the linear regression. The uncertainty in the mass bias factor, $u(K)$, was calculated from the uncertainty in the slope of the weighted regression line.[26] It was observed that this exponential model provided better adjustment of the experimental points than using the Russell equation,[11] particularly for large mass differences between the measured isotopes.

Table 2 shows the parameters used for the determination of the mass bias factor, K, with natural abundance samarium using the weighted regression line. As can be observed, the uncertainty for each experimental point is different and, particularly for the ratios 144/147 and 154/147, larger than for other measured ratios, which justifies the selection of the weighted least squares procedure. Similar results were observed for the rest of measured elements. The weighting factors used were the inverse of the variances of each experimental point normalised to the total number of points in the line.[24] As can be also observed in Table 2, most of the uncertainty of each point in the regression line arose from the uncertainty in the natural isotope abundances and very little contribution came from the experimentally measured ratios. This trend was also observed for all the other isotope systems measured in the multi-collector instrument. Please note that we have included the ratio 147/147 in the calculations. The experimental isotope ratio uncertainty for these measurements is 0, but the theoretical uncertainty can be calculated as for the rest of isotope ratios.

Table 2 Parameters employed in the determination of mass bias using a weighted regression line and their standard uncertainties for samarium

Ratio	Parameters						
	R_{exp}	$s_{R_{exp}}$	R_{theo}	$s_{R_{theo}}$	$\ln(R_{exp}/R_{theo})$	$u[\ln(R_{exp}/R_{theo})]$	w
^{144}Sm/^{147}Sm	0.19787	0.00005	0.2048	0.0030	−0.0344	0.0149	0.34
^{147}Sm/^{147}Sm	1.00000	0.00000	1.0000	0.0098	0.0000	0.0098	0.78
^{148}Sm/^{147}Sm	0.75882	0.00006	0.7498	0.0065	0.0119	0.0086	1.01
^{149}Sm/^{147}Sm	0.94396	0.00007	0.9219	0.0069	0.0236	0.0075	1.33
^{150}Sm/^{147}Sm	0.51015	0.00005	0.4923	0.0034	0.0356	0.0070	1.55
^{152}Sm/^{147}Sm	1.89184	0.00017	1.7845	0.0138	0.0584	0.0077	1.25
^{154}Sm/^{147}Sm	1.64712	0.00019	1.5177	0.0153	0.0818	0.0101	0.74

Table 3 Measured mass bias factors and their relative uncertainties for all isotope systems studied

Element	Reference isotope	Weighted regression line		Unweighted regression line		Ratio	Single ratio	
		K	$u(K)/K$ (%)	K	$u(K)/K$ (%)		K	$u(K)/K$ (%)
Cr	52	−0.034 49	0.4	−0.034 47	1.7	^{53}Cr/^{52}Cr	−0.033 93	3.1
Fe	56	−0.033 21	0.8	−0.035 51	6.6	^{57}Fe/^{56}Fe	−0.031 66	8.7
Zn	66	−0.020 44	0.6	−0.020 20	1.1	^{67}Zn/^{66}Zn	−0.022 93	14.8
Mo	98	−0.020 32	0.6	−0.020 28	1.5	^{95}Mo/^{98}Mo	−0.018 99	12.3
Cd	114	−0.015 38	0.7	−0.015 46	0.7	^{111}Cd/^{114}Cd	−0.014 92	22.4
Nd	146	−0.013 92	0.4	−0.013 91	0.4	^{143}Nd/^{146}Nd	−0.014 30	32.2
Sm	147	−0.011 65	0.2	−0.011 64	0.2	^{150}Sm/^{147}Sm	−0.011 85	19.6
Hg	202	−0.008 19	7.1	−0.008 94	5.6	^{199}Hg/^{202}Hg	−0.007 70	39.2
Pb	—	—	—	—	—	^{203}Tl/^{205}Tl	−0.006 35	3.7

The measured mass bias factors and their uncertainties for all measured isotope systems studied in this work are given in Table 3. In this table, we compare the mass bias factor obtained, both using the weighted and unweighted regression lines, and the traditional single ratio procedure used for lead and other isotope systems. As can be observed, similar or lower uncertainties in the mass bias factor were always observed using the weighted regression line, except for the case of mercury, where the uncertainties in the natural isotope abundances were extremely high in comparison with the rest of the elements. The use of a single isotope ratio to calculate the mass bias factor was the only option applied for lead (using thallium internal correction), and for the rest of the elements it provided mass bias uncertainties which were between 10 and 90 times higher that those obtained from the weighted regression line. Therefore, it is clear that the use of weighted regression lines is advantageous and it provides a more realistic account of the mass bias correction procedure, since those isotope ratios for which the natural abundances have higher uncertainties were weighted lower in the linear regression.

The isotopic compositions of the enriched spikes were calculated using eqns (12) and (13), and their relative uncertainties[2,6,27] calculated using equations:

$$\left(\frac{s_{R_{\mathrm{cor}}}}{R_{\mathrm{cor}}}\right)^2 = \left(\frac{s_{R_{\mathrm{exp}}}}{R_{\mathrm{exp}}}\right)^2 + (K\Delta M)^2\left(\frac{s_K}{K}\right)^2 \qquad (14)$$

and

$$\left(\frac{s_{A_i}}{A_i}\right)^2 = \sum_{i=1}^{n}\left[\left(\frac{R_i}{\sum R_i}\right)^2\left(\frac{s_{R_i}}{R_i}\right)^2\right] + \left(1 - \frac{2R_i}{\sum R_i}\right)\left(\frac{s_{R_i}}{R_i}\right)^2 \qquad (15)$$

As can be observed, the uncertainties were calculated taking into account the uncertainties in the mass bias factors and the experimental uncertainties in the measured isotope ratios where all R_i in eqn (15) are corrected ratios. The final isotope composition of the enriched spikes and their standard uncertainties calculated, as shown above, are given in Table 4 for all measured isotope systems. Please note that in Table 4, each isotope can be identified by referring to Table 1 with regards to cup configuration. As can be observed, in all cases the standard uncertainty in the isotopic composition of the most abundant isotope in the spike solutions is lower than 0.01%. This is due to the very low uncertainties in both the experimental isotope ratios measured in the multi-collector instrument and the mass bias factor (eqn (14)) calculated using the weighted regression line in the multi-collector instrument. For these final calculations, the uncertainties in the natural abundance standards need not to be taken into account as they are included in the mass bias factor.

3.2. Determination of the concentration of the spikes

For the determination of the concentration of the spikes, three blends were prepared containing different amounts of spike and natural abundance element. The idea behind this procedure was to evaluate the presence of systematic errors that would not be detected if all blends had been prepared aiming at the same isotope ratio in the mixture. Also, this procedure would give a more realistic account of the uncertainty in the concentration of the spike by taking into account the effect of the measured isotope ratio in the blend.

All three blends were measured and the data processed, as explained in the procedures for isotope pattern deconvolution and internal mass bias correction. No corrections for blanks or

Table 4 Isotope composition of the isotopically enriched spikes and their standard uncertainties (in brackets for the last digit)

| Element	L4	L3	L2	L1	C	H1	H2	H3	H4
Cr			0.077 (1)		1.915 (1)	**97.865 (2)**	0.143(1)		
Fe				0.0066 (1)	2.717 (5)	**95.361 (5)**	1.915 (1)		
Zn			0.8219 (3)		1.5754 (2)	**94.0127 (6)**	3.5124 (4)		0.077 69 (3)
Mo		0.3741 (7)	0.7555 (7)	**93.807 (2)**	2.3193 (8)	1.6736 (8)	0.8275 (7)		0.2433 (6)
Cd		0.010 11 (3)	0.6027 (1)	**96.4830 (3)**	1.7910 (2)	0.4230 (1)	0.5905 (2)	0.0997 (1)	
Nd	2.5378 (1)	**78.9286 (7)**	15.3524 (7)	2.1870 (2)	0.8335 (1)		0.094 26 (3)	0.066 41 (3)	
Sm		0.065 45 (1)	0.416 37 (4)	0.471 64 (3)	1.143 33 (3)	**94.8180 (2)**	2.3891 (1)	0.696 12 (8)	
Hg		0.0829 (1)	1.717 (1)	**90.426 (4)**	5.229 (3)	0.926 (1)	1.373 (2)	0.2482 (7)	
Pb				0.0129 (2)	2.153 (1)		**92.323 (2)**	5.512 (1)	
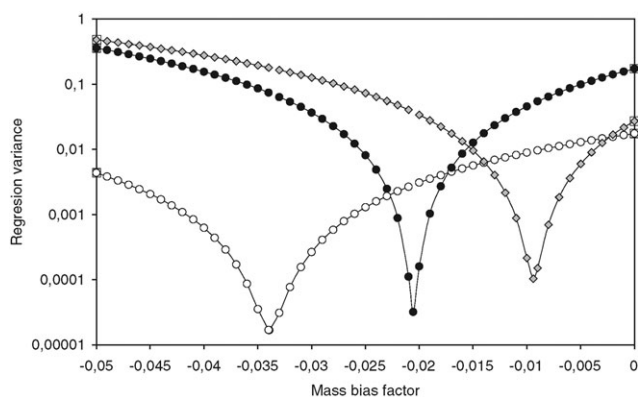
Fig. 1 Determination of the mass bias factor, K, by minimising the regression variance of each blend for chromium ($\bigcirc$), molybdenum ($\bullet$) and mercury ($\diamond$).

interfering isotopes were needed as pure solutions were measured, thus simplifying the data treatment and uncertainty calculations. From the measured isotope ratios we applied eqns (12) and (13) to calculate the isotopic composition of the blend. Then, isotope pattern deconvolution was applied, taking into account the natural and enriched isotope abundances. Finally, the Excel SOLVER application was used to minimise the regression variance by changing the mass bias factor applied for correction in eqn (12). When this was finished (a few ms), the molar fractions, x_{nat} and x_{enr}, and their uncertainties, were used to calculate the concentration of the spike since the amount of natural element added to the blend is known.

Fig. 1 shows the optimisation process in the calculation of the mass bias factor for one of the blends prepared for the certification of ^{53}Cr, ^{95}Mo and ^{199}Hg. As can be observed, in all three cases a minimum in the regression variance is obtained for a certain value of the mass bias factor. All three blends prepared for each element provided very similar optimisation values for the mass bias factor. Obviously, as can be observed in Fig. 1, the mass bias factor changed depending on the mass range measured, as was shown in Table 3. At the same time, a natural abundance standard was measured and

Table 5 Comparison of the mass bias factor calculated by internal and external mass bias correction for the different isotope systems

Element	Internal mass bias correction			External mass bias correction weighted model Natural
	Blend 1	Blend 2	Blend 3	
Cr	−0.0339	−0.0339	−0.0340	−0.0345
Fe	−0.0329	−0.0327	0.0328	−0.0332
Zn	−0.0208	−0.0207	−0.0207	−0.0204
Mo	−0.0206	−0.0206	−0.0204	−0.0203
Cd	−0.0153	−0.0153	−0.0153	−0.0154
Nd	−0.0139	−0.0139	−0.0138	−0.0139
Sm	−0.0113	−0.0117	−0.0116	−0.0117
Hg	−0.0094	−0.0094	−0.0094	−0.0082
Pb[a]	−0.0065	−0.0064	−0.0064	−0.0064[b]

[a] The isotopic composition of the natural abundance lead standard was calculated previously using Tl internal mass bias correction. [b] Thallium internal mass bias correction.

the mass bias factor was calculated using the external procedure shown before for the isotopic composition of the spikes. The comparison of the internal and external mass bias factor calculated for all the spike solutions is given in Table 5. As can be observed, the values obtained by internal mass bias correction for the three blends agree quite well between them. Also, the agreement with those values determined using the natural abundance standard is remarkable, with the clear exception of mercury, indicating that this procedure is adequate for internal mass bias correction in mixtures of natural abundance and isotopically enriched spikes. Please note finally that, for lead, the isotopic composition of the "natural abundance" Merck standard had to be measured previously, also using Tl internal correction. Therefore it is not so remarkable that the results shown in Table 5 agree well for this element. If we could have used a certified natural abundance lead standard (certified standards for lead natural abundance, both in isotopic composition and mass fraction, are not commercially available) the data treatment would have been analogous to that for the rest of the elements.

As can be deduced from the model, the procedure proposed here for internal mass bias correction can be considered as universal and it could be applied to any isotope system containing at least three isotopes. In theory, this procedure will eliminate the need for the measurement of a standard solution of a certified isotope abundance element when performing any isotope dilution determination, simplifying still further the analytical procedure and making it more suitable for routine application. For two isotope systems (*e.g.*, Tl, Cu or Sb) this procedure cannot be applied and external mass bias correction will still be needed.

3.3. Uncertainty propagation in the concentration of the spikes

The uncertainty in the mass fraction of the spikes was calculated by propagating the uncertainties obtained in the molar fractions x_{enr} and x_{nat}, calculated from eqn (11), using the variance–covariance matrix of the multiple linear regression. An example of uncertainty propagation for molybdenum is given in the ESI†. For this element it was observed that the final relative standard uncertainty for each blend (*ca.* 0.32%) was only slightly higher than the relative uncertainty of the concentration of the natural molybdenum standard used for certification (0.29%), indicating that the main source of uncertainty in the concentration of the spike was the stated uncertainty in the mass fraction of the natural abundance element. For the remainder of the measured elements we also calculated the fraction of the standard uncertainty of each blend measurement coming from the uncertainty in the mass fraction of the natural abundance element used for the reverse ID experiment. For elements such as Cr, Fe, Zn, Mo, Cd, Nd and Sm more than 90% of the uncertainty in the spike concentration arose from the uncertainty in the mass fraction of the natural abundance element, indicating that other sources of uncertainty, such as the measurement of isotope ratios or mass bias correction, were negligible. Notable exceptions were mercury and lead. For mercury, only 39% of the uncertainty was explained by the uncertainty in the concentration of the natural standard. For this element the uncertainty

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of the isotope pattern deconvolution regression was high due to the large uncertainties in the isotope abundances of natural mercury. In the case of lead, 69% of the uncertainty was due to the uncertainty in the mass fraction of the natural abundance standard. In this case, the determination of the isotopic composition of the natural abundance element (Merck standard) using thallium for mass bias correction was an important additional source of uncertainty.

Conclusions

We have developed a procedure for isotope dilution analysis based on isotope pattern deconvolution which allows internal mass bias correction for isotope systems containing three or more isotopes with lower uncertainties in comparison with standard measurement procedures based on single ratio measurements. This procedure could be useful in the routine application of isotope dilution analysis as it is not necessary to measure a certified isotope standard for mass bias correction. Of course, it is necessary for the isotopic composition of the spike and that of the natural element for quantitation to be known beforehand. When applying this procedure in a routine testing laboratory blanks should be treated as samples and spiked accordingly. Then, the concentration found in the blank should be subtracted from that in the sample.

Additionally, we have applied a weighted regression line for the calculation of the mass bias factor in the determination of the isotopic composition of the enriched spikes used. We have observed that this procedure provided the lowest uncertainties between alternative procedures used in the literature (single ratio or unweighted linear regression).

We consider that the combination of both measurement procedures, external mass bias correction with weighted regression lines and isotope pattern deconvolution with internal mass bias correction, will provide enriched spikes which could be certified for isotopic composition and concentration with very low uncertainties for the routine application of isotope dilution analysis.

Acknowledgements

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