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Different Quantification Approaches for the Analysis of Biological and Environmental Samples Using Inductively Coupled Plasma Mass Spectrometry

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For the analysis of biological and environmental materials by inductively coupled plasma mass spectrometry (ICP-MS), several quantification procedures can be used depending on the precision and accuracy required. Semi-quantitative methods based on the molar response curve were compared with conventional external calibration and standard additions for the analysis of waters and sewage sludges. For the analysis of biological materials, where higher quality data were required, isotope dilution analysis using enriched isotopes was applied. It was observed that the molar sensitivity for different elements in ICP-MS was a simple function of the mass of the isotopes measured after normalization for ionization efficiency which could be fitted to a third-order polynomial equation. Element ionization adjustments for the third-order polynomial, using the Saha equation, allowed the calculation of the plasma ionization temperature and electron density. For the determination of trace metals in waters and sewage sludges, the samples were spiked with different internal standards, ionization corrections were performed and the results obtained agreed with those obtained by external calibration and standard additions within a factor of 2 but, on average, the agreement was within 20%. The determination of molybdenum in biological reference materials was performed by isotope dilution analysis taking into account possible sources of error in the measurements by ICP-MS such as mass discrimination, detector dead time, isobaric interferences and random error propagation. © **1997 by John Wiley & Sons, Ltd.**

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INTRODUCTION

The determination of trace metals in biological and environmental samples is generally performed using atomic spectrometric techniques.[1] Methods based on graphite furnace atomic absorption spectrometry (AAS), cold vapour or hydride generation AAS and inductively coupled plasma atomic emission spectrometry (ICP-AES) are the most popular and are widely used owing to their high sensitivity and selectivity.[2] However, these techniques require the use of calibration graphs, using the element(s) to be determined, or standard addition procedures. These can be time consuming when a large number of samples and/or elements have to be measured for screening purposes. Also, AAS-based methods are essentially monoelemental and do not allow internal standardization. For ICP-AES there is a limited choice of suitable internal standards, which is more problematic for difficult samples.

In the last few years, the use of ICP-MS for the analysis of biological and environmental samples has increased dramatically.[3] Quantification procedures used in ICP-MS derive from those applied before by AAS and ICP-AES (calibration graphs and standard additions). However, the special characteristics of the technique allow the use of more elaborate quantification procedures which were not available with optical-based techniques. It was observed[4,5] that the analytical response of ICP-MS to different elements depends on the masses of the elements and on their ionization potentials.[6] This allows the application of simple semi-quantitative procedures for multielemental trace analysis using ICP-MS.[7] However, previous work in this field did not take into account more than four or five elements in order to build the response curve and ionization corrections were done based on response tables prepared by the manufacturers (semiquantitative software). The use of response curves based on more than ten elements, with large isotope distributions and different ionization potentials, could allow a better definition of the response function and plasma parameters, and high-quality semiquantitative results could be achieved. Also, the use of internal standards added to

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the sample would help in reducing matrix effects by building the response curve directly for the sample and not from standard solutions.

Isotope dilution analysis (IDA) has been considered as a definitive analytical method under certain conditions.[8] For ICP-MS determinations using IDA, the changes in response for different isotopes of the same element observed in the semiquantitative measurements[9] have to be taken into account for accurate results to be obtained.[10] Mass bias functions vary for different instruments from purely linear functions[9,10] to exponential and power functions for multicollector magnetic sector ICP-MS instruments.[11] The detector dead time and isobaric interferences also have to be considered for accurate IDA measurements.[10,12] Finally, random error propagation from the measured isotope ratios to the final concentration values has to be optimized.[10,13]

In this work, alternative quantification procedures to those classically used for trace elemental determinations were evaluated and results are reported for some biological and environmental samples.

EXPERIMENTAL

Instrumentation

The inductively coupled plasma mass spectrometer used in this work was a Hewlett-Packard Model HP4500 fitted with a Meinhard concentric nebulizer. The operating conditions were optimized both for the semiquantitative measurements and for molybdenum isotope dilution analysis. The optimum conditions used are summarized in Table 1. A Milestone Model MLS1200 microwave digestor with an EM-45/A extractor module and an AC-100 open/close module with medium-pressure PTFE vessels were employed for digestions of samples. The digestion programs both for sewage sludges and biological materials are given in Table 2.

Reagents and reference materials

Natural element standard solutions containing $\sim$1000 $\mu g\ g^{-1}$ of Mo, Ni, Cr, Zn, In, Cs, Tb, Pr, Sr, Mg, Sb, Cu, Pd, Sn, Nd, Tl, Pb, Bi and Se from Merck (Darmstadt, Germany) and Th, Co, Y, Mn, V, U and Sc from J. T. Baker (Phillipsburg, NJ, USA) were used. The response curve was prepared using Mg, Al, Sc, V, Mn, Co, Cu, Zn, Se, Sr, Y, Mo, Pd, Sn, In, Sb, Cs, Pr, Nd, Tb, Tl, Pb, Bi, Th and U diluted by mass from the stock solution to prepare a $\sim$10 $\mu g\ g^{-1}$ multielemental solution. Further dilution of this solution to 10 ng g^{-1} was performed daily.

For IDA, a 1.2904 ± 0.0130 $\mu g\ g^{-1}$ stock solution of natural Mo was prepared by diluting the Mo standard solution (Merck) with ultrapure water. Enriched ^{95}Mo spike was obtained from Cambridge Isotope Laboratories (Woburn, MA, USA) as metal and was dissolved in high-purity nitric and hydrochloric acids to prepare an 886 ± 7 $\mu g\ g^{-1}$ stock solution which was standardised by reverse IDA. A 1.1097 ± 0.0091 $\mu g\ g^{-1}$ substock solution of enriched ^{95}Mo spike was prepared by diluting the stock solution with ultrapure water.

All dilutions were performed with filtered (0.22 mm) 18 MΩ deionized water (ultrapure water) or 1% (v/v) nitric acid. This water was obtained from a Milli-Q system (Millipore, Eschborn, Germany). Nitric acid (Suprapur, Merck) was used throughout.

Environmental samples analysed included acidified natural waters and sewage sludges from an urban waste water treatment plant. Lyophilized biological tissues analyzed in this work were NIST SRM 1577 Bovine Liver and SRM 1572 Citrus Leaves from the National Institute of Standards and Technology (Gaithersburg, MD, USA) and IAEA H-8 Horse Kidney and IAEA V-10 Hay from the International Atomic Energy Agency (Vienna, Austria). Solid and digested samples were conserved at 4 °C in the dark.

Table 1. Optimum operating conditions

R.f. power	1200 W,[a] 1350 W[b]
Sampling depth	5.5 mm,[a] 6.2 mm[b]
Carrier gas flow rate	1.3 l min^{-1},[a] 1.28 l min^{-1} [b]
Intermediate gas flow rate	1.0 l min^{-1}
Outer gas flow rate	15.0 l min^{-1}
Sample uptake flow rate	0.3 ml min^{-1}
Nebulizer type	Meinhard (concentric)
Spray chamber	Double pass/Peltier cooler (2 °C)
Sampler and skimmer cones	Nickel, 1 and 0.4 mm orifice, respectively

[a] Isotope dilution analysis.
[b] Semiquantitative analysis, external calibration and standard additions.

Table 2. Microwave oven heating program for biological and environmental samples dissolution when six vessels plus a pressure reference vessel are placed in the sample carousel[a]

Biological samples

Step	Time (min)	Power (W)
1	1	120
2	2	360
3	3	600

First digestion: 0.5 g of sample + 5.0 ml of 65% (w/v) HNO_3 + 0.1 ml of 48% (w/v) HF (optional)
Second digestion: + 2.0 ml of 30% (w/v) H_2O_2

Sewage sludges

Step	Time (min)	Power (W)
1	5	120
2	5	250
3	3	0
4	3	500

Digestion: 0.5 g of sample + 3.0 ml of 65% (w/v) HNO_3

[a] If the number of vessels is below five the power and time must be decreased by 25%.

Procedures

Digestion of sewage sludges. A portion of 0.500 g of wet sample was transferred into a PTFE digestion vessel

and 3 ml of concentrated nitric acid were added. The sample was digested in a microwave oven using the heating program given in Table 2. This resulted in a clear digest. After cooling, the samples were diluted by mass to 50 g with ultrapure water. A second dilution (1:10) was then performed and the elements were Sc, Co, Y, In, Tb, Tl and Th added as internal standards (10 ng g^{-1} final concentration).

Digestion of biological materials. Wet sample amounts ranging from 0.05 to 0.5 g were weighed directly into a PTFE vessel and 5.0 ml of HNO_3 (65%, w/v) were added. In the case of vegetable tissue samples, the addition of 0.1 ml of HF (48%, w/v) was necessary. For IDA, appropriate amounts of Mo spike were added to the samples directly in the PTFE vessels at this point. After tightly capping the vessels, the sample carousel was placed in the microwave oven and the heating program given in Table 2 was started. After cooling, 2.0 ml of H_2O_2 (30%, w/v) were added to the samples and the digestion heating program was repeated. Finally, the acid sample solutions were transferred into polypropylene containers and diluted to 50 g with ultrapure water.

Semiquantitative analysis. A multielemental standard solution containing Mg, Al, Sc, V, Mn, Co, Cu, Zn, Se, Sr, Y, Mo, Pd, Sn, In, Sb, Cs, Pr, Nd, Tb, Tl, Pb, Bi, Th and U each at ~10 ng g^{-1} was measured to evaluate the ICP-MS molar response factor (*MRF*) using the following equation:

$$MRF_{(i,e)} = \frac{\text{counts}_{(i)} \times \text{atomic mass}_{(e)}\ (\text{ng nmol}^{-1}) \times 100}{\text{isotope abundance}_{(i,e)}\ (\text{mol\%}) \times \text{concentration}_{(e)}\ (\text{ng g}^{-1})}$$

where the subscripts i and e refer to the ith isotope of the element e. *MRF* has the units of counts per $\text{nmol}_{(i,e)}$ g^{-1}. Ionization corrections were performed using the Saha equation[14] taking into account the temperature-dependent atomic and ionic partition functions.[15] The method of least squares was applied for the calculation of the ionization temperature and electron density in the plasma which best fitted the molar response curve to a third-order polynomial. Samples were spiked with Sc, Co, Y, In, Tb, Tl and Th as internal standards, which cover a mass range from 45 (Sc) to 232 (Th), at 10 ng g^{-1} levels and the molar response curve in the sample was evaluated from the internal standard elements. Finally, ionization corrections based on the previously measured ionization temperature and electron density were applied to the test elements and their concentrations were evaluated from their measured counts in the sample.

Calibration graph and standard additions procedures. The concentrations of Al, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Sb, Hg, Pb and U in natural waters and Cd, Cr, Cu, Ni, Hg, Pb and Zn in sewage sludges were determined in parallel by calibration graph and standard addition methods using Sc, Co, Y, In, Tb, Tl and Th as internal standards in both procedures.

Isotope dilution analysis. The isotope dilution equation has been expressed in various forms. For this work, the equation used was[10]

$$C_s = C_{sp} \frac{W_{sp}}{W_s} \frac{A_{W_n}}{A_{W_{sp}}} \frac{A_{sp}^{95}}{A_n^{98}} \frac{R_m - R_{sp}}{1 - R_m R_n}$$

where C_s is the unknown concentration of Mo in the sample (s), C_{sp} is the concentration of Mo in the spike (sp), W_s and W_{sp} are the masses taken from sample and spike, respectively, A_{W_n} and $A_{W_{sp}}$ are the Mo atomic masses in the sample (natural atomic mass) and spike, respectively, A_{sp}^{95} is the isotope 95 abundance (at%) in the spike and A_n^{98} is the isotope 98 abundance (at%) in the sample (natural abundance), R_m and R_{sp} are the isotope ratios ($^{98}Mo/^{95}Mo$) in the mixture and the spike, respectively, and R_n is the natural isotope ratio ($^{95}Mo/^{98}Mo$).

RESULTS AND DISCUSSION

Molar response curve

The response curve was measured using 10 ng g^{-1} of Mg, Al, Sc, V, Mn, Co, Cu, Zn, Se, Sr, Y, Mo, Pd, Sn, In, Sb, Cs, Pr, Nd, Tb, Tl, Pb, Bi, Th and U. Table 3 shows the raw counts measured, masses, ionization potentials, isotope abundances and atomic masses used for the establishment of the response curve. The data for Pr, Nd, Tb, Th and U are not included in the table as partition functions were not available. We have assumed a 100% ionization degree for these elements. Elements with different ionization potentials were measured from mass 24 (Mg) to 238 (U). Also, most isotopes for each element were measured. The molar response curve obtained directly is shown in Fig. 1. As can be observed, elements such as Zn, Se, Pd, Sb and Bi show clearly a low ionization degree in the plasma. Elements such as Mg, Al, Sc, Sr, Y, In, Cs, Pr, Nd, Tb, Tl, Pb, Th and U are almost completely ionized in the plasma. Figure 2 shows the same results after ionization correction using an ionization temperature (T_{ion}) of 6980 K and an electron density (n_e) of 1.33×10^{15} e cm^{-3}, which was the best fit obtained for this particular experiment. The values of the ionization temperature and electron density were obtained by fitting a third-order polynomial to the response curve using the Saha equation. The ionization degree obtained for the different elements is also indicated in Table 3. It was observed that the presence of different matrix elements changed only slightly the values of the ionization degrees found for the different elements.[16] As an example, Table 4 shows the results obtained (best fit) for T_{ion} and n_e with increasing concentrations of Ca and the calculated ionization degrees for Se, Sb and Pb, elements which show different ionization degrees in the plasma. As can be observed, the mathematical fitting procedure produced a range of results for T_{ion} and n_e but the calculated ionization degrees changed only slightly with increasing Ca concentration up to 200 μg g^{-1}, allowing the use of those values found for the standard solution in the quantification procedure. Also, the

Table 3. Parameters used to elaborate the molar response curve (elements present at ~10 ng g^{-1})

Element	Raw counts	Mass	Ionization potential (eV)	Isotope abundance (at%)	Atomic mass	Ionization degree[a] (%)
Mg	96 828	24	7.6	79	24.3	92.1
Mg	13 395	25	7.6	10	24.3	92.1
Mg	17 239	26	7.6	11	24.3	92.1
Al	129 211	27	5.98	100	26.98	94.5
Sc	144 204	45	6.54	100	44.96	98.5
V	129 858	51	6.74	99.8	52	96.2
Mn	137 337	55	7.43	100	54.94	86.8
Co	120 586	59	7.86	100	58.93	79.6
Cu	67 333	63	7.72	69.2	63.55	69.8
Zn	28 095	64	9.39	48.6	65.39	41.2
Cu	31 553	65	7.72	30.8	63.55	69.8
Zn	16 776	66	9.39	27.6	65.39	41.2
Zn	11 771	68	9.39	18.8	65.39	41.2
Se	1 386	77	9.75	7.6	78.96	9.8
Se	5 722	78	9.75	23.8	78.96	9.8
Se	1 678	82	9.75	8.7	78.96	9.8
Sr	18 609	86	5.69	9.9	44.96	99.5
Sr	13 226	87	5.69	7.0	44.96	99.5
Sr	158 125	88	5.69	82.6	44.96	99.5
Y	191 416	89	6.38	100	88.9	94.4
Mo	24 941	92	7.10	14.8	95.94	93.1
Mo	16 036	94	7.10	9.3	95.94	93.1
Mo	27 597	95	7.10	15.9	95.94	93.1
Mo	29 198	96	7.10	16.7	95.94	93.1
Mo	16 997	97	7.10	9.6	95.94	93.1
Mo	42 982	98	7.10	24.8	95.94	93.1
Mo	17 458	100	7.10	9.6	95.94	93.1
Pd	15 445	104	8.33	11.1	106.4	77.7
Pd	31 131	105	8.33	22.3	106.4	77.7
Pd	38 068	106	8.33	27.3	106.4	77.7
Pd	37 023	108	8.33	26.5	106.4	77.7
Pd	16 646	110	8.33	11.7	106.4	77.7
In	6 079	113	5.78	4.3	114.8	96.9
In	135 370	115	5.78	95.7	114.8	96.9
Sn	22 512	116	7.34	14.5	118.7	97.7
Sn	11 025	117	7.34	7.7	118.7	97.7
Sn	34 691	118	7.34	24.2	118.7	97.7
Sn	13 221	119	7.34	8.6	118.7	97.7
Sn	47 034	120	7.34	32.6	118.7	97.7
Sb	36 779	121	8.64	57.4	121.8	37
Sn	6 810	122	7.34	4.6	118.7	97.7
Sb	27 832	123	8.64	42.7	121.8	37
Sn	8547	124	7.34	5.8	118.7	97.7
Cs	130 910	133	3.9	100	132.9	99.9
Tl	52 738	203	6.1	29.5	204.4	96.7
Tl	125 206	205	6.1	70.5	204.4	96.7
Pb	43 397	206	7.4	24.1	207.2	90.4
Pb	41 431	207	7.4	22.1	207.2	90.4
Pb	87 323	208	7.4	52.4	207.2	90.4
Bi	173 009	209	8.1	100	209	77

[a] Calculated from the Saha equation for T_{ion} = 6980 K and n_e = 1.33 × 10^{15} e cm^{-3}.

ionization degrees shown in Table 4 are similar to those in Table 3 for Se, Sb and Pb for a different set of electron density and ionization temperature values.

Analysis of natural waters

Acidified natural waters were spiked with a multielemental solution containing Sc, Co, Y, In, Tb, Tl and Th to a final concentration of 10 ng g^{-1}. The response curve, corrected for ionization efficiency, was calculated using only the internal standard elements. From these data and the values of electron density and ionization temperature, the concentrations of the test elements in the sample were evaluated. Table 5 shows the results obtained for Al, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Sb, Hg, Pb and U in one of those samples. The results can be compared with those obtained based on a calibration graph and a standard additions procedure. As can be observed, the agreement between the different quantification approaches is, in general, satisfactory except for Al (the low value found for Al is due to the extrapolation of the response curve to masses lower than ^{45}Sc) and As. In general, the agreement is within a factor of 2, but on average the agreement was within 20%.

Table 4. Effect of increasing amounts of Ca on the T_{ion} and n_e obtained using the response curve method and their effect on the calculated ionization degrees for Se, Sb and Pb

Ca concentration (μg g^{-1})	T_{ion} (K)	n_e (e cm^{-3})	α_{Se}	α_{Sb}	α_{Pb}
0[a]	5740	3.13 × 10^{13}	0.093	0.422	0.961
0[a]	5856	4.38 × 10^{13}	0.101	0.436	0.960
50	6154	1.26 × 10^{14}	0.097	0.407	0.946
100	6788	8.22 × 10^{14}	0.096	0.374	0.913
200	6458	3.49 × 10^{14}	0.090	0.372	0.926
400	6394	3.41 × 10^{14}	0.077	0.337	0.918

[a] Measured on different days.

Analysis of sewage sludges

A similar quantification procedure was applied for sewage sludge samples. Figure 3 shows the molar response curve obtained for one of these samples spiked with the seven internal standard elements. As can be

Table 5. Results obtained for a natural water sample comparing different quantitation procedures (concentrations in ng g^{-1})

Element	Calibration	Standard additions	Response curve
Al	21	18	8.8
Cr	0.56	0.47	0.42
Mn	150	187	165
Ni	2.2	2.9	1.9
Cu	2.0	2.2	2.5
Zn	5.0	7.0	5.7
As	0.20	0.23	0.14
Se	0.95	1.3	1.3
Cd	ND[a]	0.04	0.04
Sb	0.24	0.25	0.27
Hg	0.16	0.27	0.18
Pb	0.03	0.10	0.12
U	0.86	0.81	0.86

[a] Not detected.

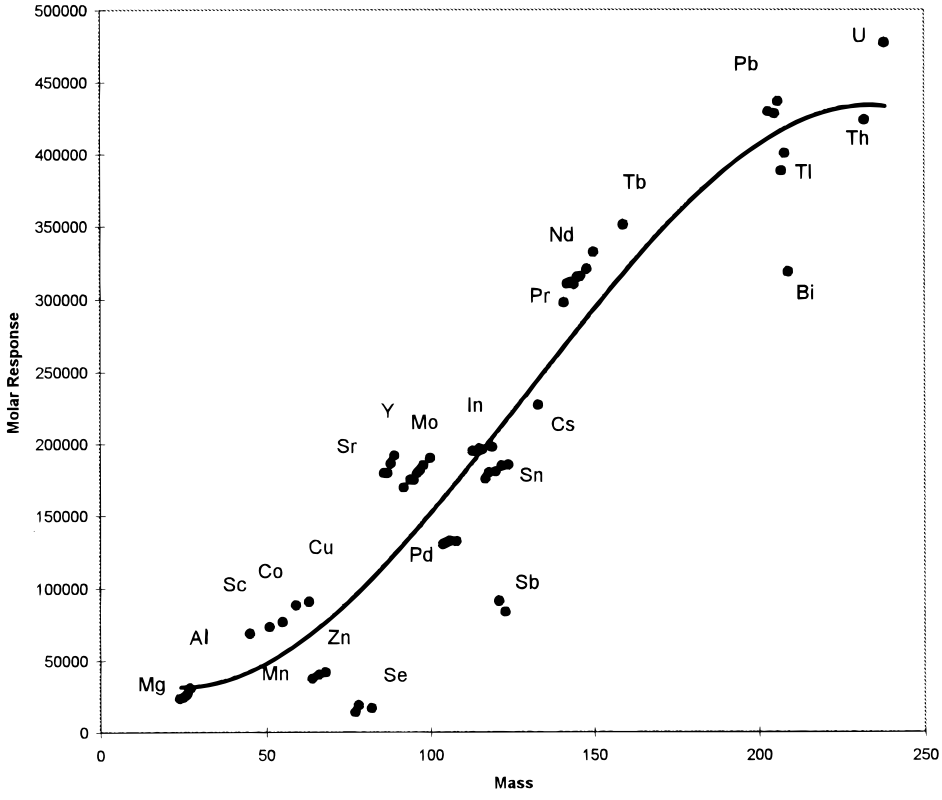

Figure 1. Molar response curve obtained for the HP4500 instrument using a multielement standard solution containing Mg, Al, Sc, V, Mn, Co, Cu, Zn, Se, Sr, Y, Mo, Pd, In, Sn, Sb, Cs, Pr, Nd, Tb, Pb, Tl, Bi, Th and U.

observed, the shape of the curve is similar to that shown in Fig. 2. From this response curve, the concentrations of Cd, Cr, Cu, Ni, Hg, Pb and Zn were determined. The results obtained were compared with those found by external calibration and standard additions and are given in Table 6. As can be observed, the agreement is in all cases within 30%, the agreement between standard additions and the response curve generally being

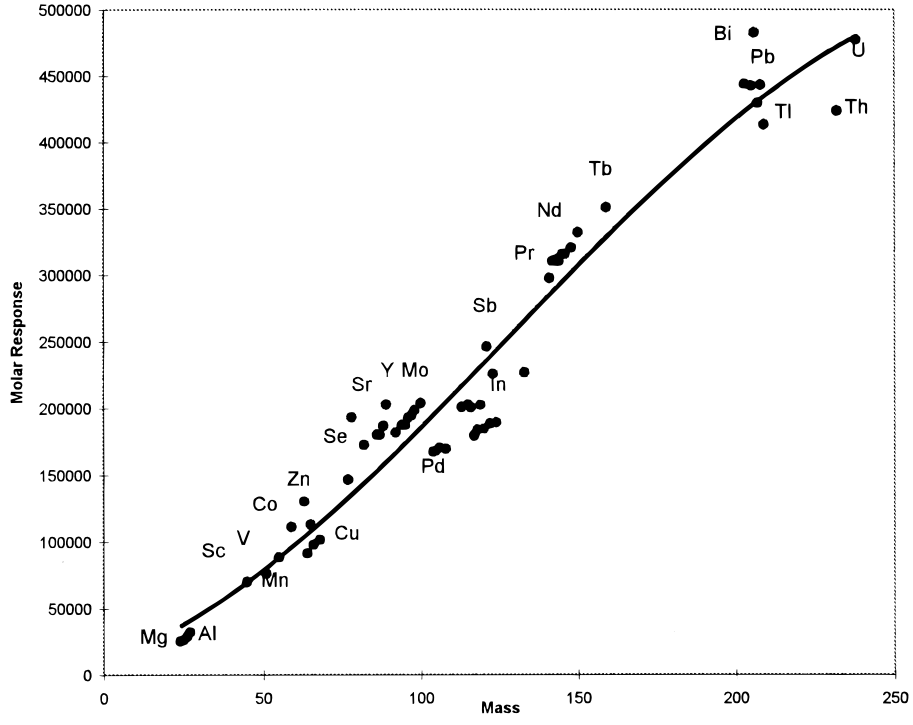

Figure 2. Molar response curve after correction for $T_{ion} = 6980$ K and $n_e = 1.33 \times 10^{15}$ cm^{-3}. Elements as in Fig. 1.

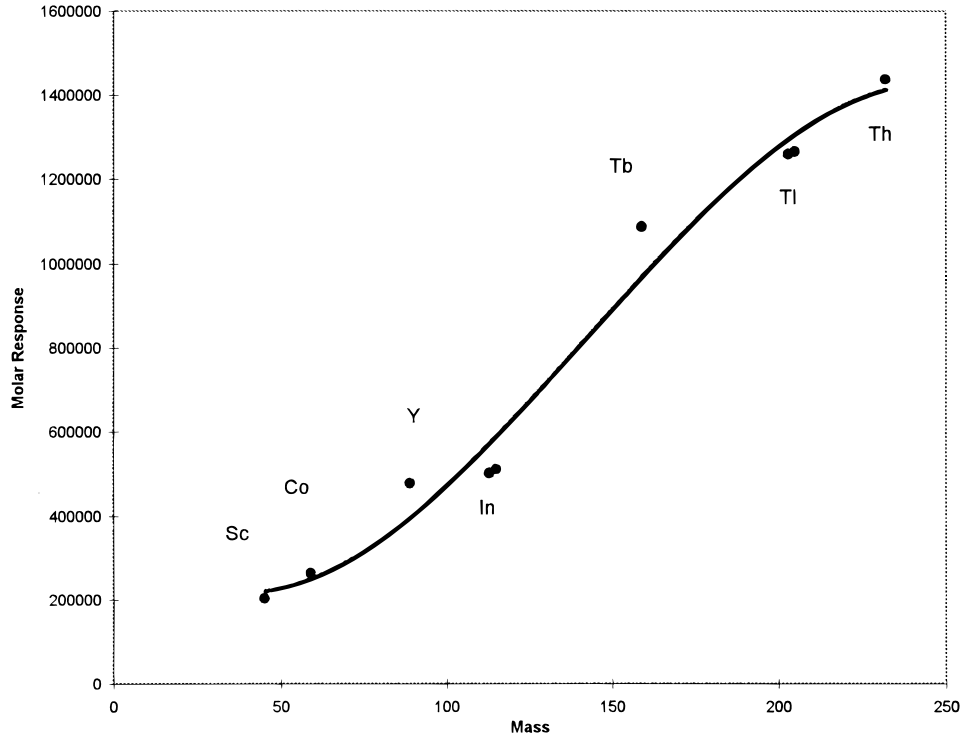

Figure 3. Molar response curve obtained for the sewage sludge sample using the internal standard elements shown.

better than that between the calibration graph and standard additions, which seems to indicate that the response curve method compensates for matrix effects which could be present, and not corrected for, by external calibration. The addition of the elements Sc, Co, Y, In, Tb, Tl and Th to the sample for building the response curve in the actual sample seems to be responsible for this correction of matrix effects.

Optimization of isotope dilution analysis

Factors affecting the precision and accuracy of IDA have to be considered first. The precision of the isotopic ratio measurement is one of the major determinants of the overall precision of IDA due to random error propagation.[10] In order to obtain optimum conditions for the precise measurement of Mo isotopic ratios, the effect of integration time was examined using a 50 ng g^{-1} solution of natural Mo. The dependence of the RSD ($n = 5$) of the $^{95}Mo/^{98}Mo$ ratio measurement on the integration time per unit mass showed that the measured RSD decreased from 1% at a 0.1 s integration time per unit mass to 0.3% at a 0.5 s integration time per unit mass and it became constant for longer integration times. However, for lower abundance isotopes longer integration times had to be used. Table 7 shows the selected integration times for Mo isotope ratio measurement together with the natural Mo isotopic composition[17] and the measured isotopic composition of the ^{95}Mo spike (not certified for isotopic composition).

Table 6. Results obtained for a sewage sludge sample comparing different quantitation procedures (concentrations in mg kg^{-1} dry sample)

Element	Calibration	Standard additions	Response curve
Cd	1.5	1.8	1.8
Cr	76	80	79
Cu	74	111	101
Ni	75	92	89
Hg	5.3	4.7	7.1
Pb	46	39	37
Zn	1622	—	1852

It has been shown previously[10] that mass discrimination and detector dead time losses could be a serious source of systematic errors in isotope dilution analysis with ICP-MS. From Figs 1–3 it is clear that the response for different isotopes of the same element will vary which will, in turn, produce biased isotope ratio measurements (the so-called mass bias factor). For a limited mass range, the mass discrimination factor, K, could be considered constant and the relative error proportional to the mass difference between the measured isotopes.[10] This can be expressed as

$$K\Delta M = \frac{R_{exp} - R_{theo}}{R_{theo}}$$

where $(R_{exp} - R_{theo})/R_{theo}$ is the relative error and ΔM the mass difference.

In order to determine simultaneously the value of the mass bias factor and the detector dead time, τ, a series of increasing concentrations of natural Mo were measured in the peak hop mode (one point per peak, 10 ms

Table 7. Integration times for Mo isotope ratio measurements and isotopic abundances of natural molybdenum[17] and the ^{95}Mo spike (at%)

Type of solution	Integration time (s) Isotope 98	Isotope 95	Other 5 isotopes	Uptake time (s)	Rinse time (s)	Total time (s)
Natural standard	1	2	2	30	180	275
Mixtures (samples)	1	1	—	30	180	220
spike	10	1	10	30	180	515

Mass	Natural[17]	Spike[a]
92	14.84 ± 0.04	0.38 ± 0.01
94	9.25 ± 0.03	0.77 ± 0.01
95	15.92 ± 0.05	93.67 ± 0.08
96	16.68 ± 0.05	2.39 ± 0.03
97	9.55 ± 0.03	1.69 ± 0.03
98	24.13 ± 0.07	0.85 ± 0.02
100	9.63 ± 0.03	0.25 ± 0.01

[a] Determined after mass discrimination correction using natural molybdenum.

dwell time). The experimental isotopic ratios, R_{exp}, were calculated with respect to the main ^{98}Mo isotope. Five measurements were performed for each concentration level and the mean isotopic ratio was used in the calculations. The mean intensity was used in the dead time correction procedure. The mass bias factor and the detector dead time were calculated using the equation[10,12]

$$R_{corr} = \frac{R_{exp}(1 - \tau I^2_{meas})}{1 - \tau I^1_{meas}} \over 1 + K\Delta M$$

were R_{exp} are the measured isotopic ratios, I^2_{meas} and I^1_{meas} the count rates for isotopes 1 and 2, respectively, ΔM the mass difference between the measured isotopes and R_{corr} the corrected isotope ratio. First, tentative values for the mass bias factor and detector dead time were introduced and the R_{corr} values for all isotope ratios measured were determined. The R_{corr} values were then compared with the theoretical ratios,[17] R_{theo}, and the square relative error was evaluated. An iteration procedure was then initiated which changed the values of K ant τ until the sum of errors was a minimum. The best fit corresponded to a dead time of 11 ns and a mass bias factor of -0.0112 per mass unit. It has been shown previously[12] that this procedure provides more precise data on detector dead time and mass bias factor than the determination of both parameters independently.

The dead time value obtained was very low and, taking into account that concentrations higher than 50 ng g^{-1} were not measured, did not need to be taken into account for the measurement of isotopic ratios. In consequence, only the mass bias factor needed to be evaluated in order to correct the experimental isotope ratios measured with the instrument. This factor was constant within any particular working day (RSD $< 0.5\%$) but it fluctuated from day to day. Figure 4 shows the plot of R_{exp}/R_{theo} *vs.* the mass difference between the measured isotopes, ΔM, using weighted linear regression calculations. The value of K, the mass bias factor, was derived from the slope of the weighted regression line. Once K had been calculated, the corrected isotope ratios, R_{corr}, were calculated. As can be observed in Fig. 4, the corrected isotope ratios are now very close to the theoretical values, $R_{exp}/R_{theo} = 1$. The data in Table 7 for the spike isotope composition were also corrected for mass bias.

The ^{95}Mo isotope suffers from spectral interferences from potassium as a matrix element (concentration in the digested animal tissues, 100 μg g^{-1} and, in the digested vegetable tissues 200 μg g^{-1}). This interference can be attributed to the formation of the polyatomic ion ^{40}Ar^{39}K^{16}O$^+$. An apparent Mo concentration of 0.2 ng g^{-1} was found at m/z 95 for a K concentration of 200 μg g^{-1}, which represented ~12% of the Mo concentration in the digested citrus leaves sample. This could explain the higher ^{95}Mo/^{98}Mo isotopic ratio observed with the digested citrus leaves sample (K/Mo ratio = 10^5:1) and is in agreement with the results published by other workers.[18] The K/Mo ratio in the other samples studied was much lower and no spectral interferences were expected.

Determination of Mo in biological materials by isotope dilution analysis

In order to test the precision of the proposed method, three different sample to spike mass ratios were assayed. In the case of animal tissue samples, which contain higher Mo concentrations, 0.5 g of spike solution, with a Mo concentration of ~1 μg g^{-1}, was added to 0.05, 0.1 and 0.5 g of solid undigested sample. In the case of vegetable tissue samples, which contained much lower Mo concentrations, 0.75, 0.25 and 0.1 g of spike solution were added to 0.5 g of solid undigested sample. The Mo concentrations found by the proposed procedure after mass discrimination correction are compared with the certified values in Table 8. As can be seen, very good agreement with the reference values was generally obtained except for the citrus leaves sample. The precision of each measurement ($n = 5$) is also shown in Table 8. As can be observed, for bovine liver and horse kidney, which contain higher Mo concentrations, the

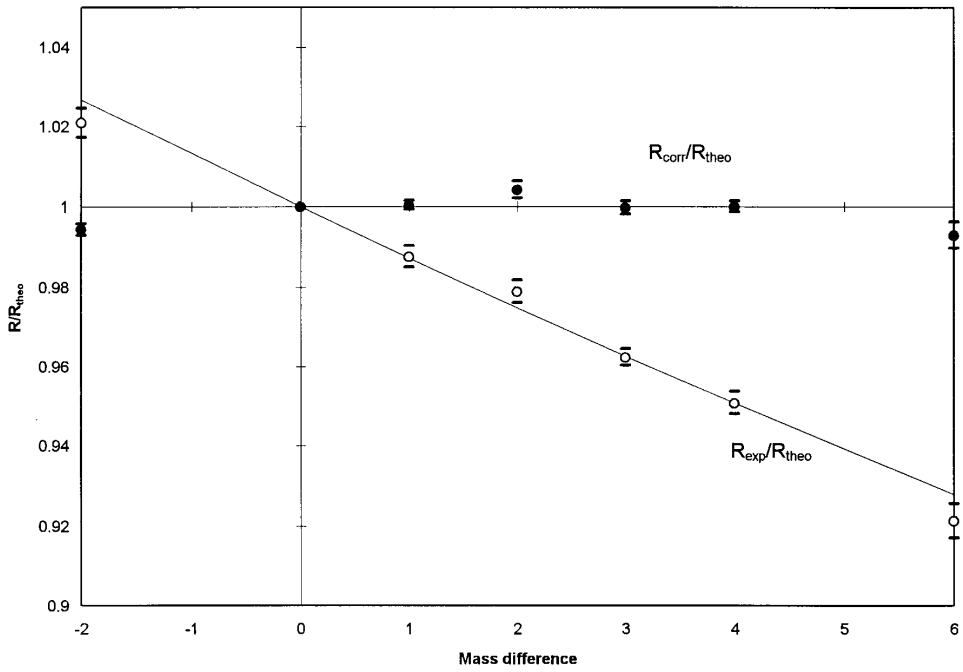

Figure 4. Accuracy of the measured isotopic ratios *vs*. the mass difference between the measured isotopes. (○) Experimental data; (●) data corrected for mass bias using $K = -0.0112$.

precision seemed to be limited by the digestion procedure or sample heterogeneity. For the hay sample, similar precisions were obtained for the measurement and for the digestion, but this could be due to the lower Mo content in this sample.

CONCLUSIONS

The use of ICP-MS for the analysis of biological and environmental samples offers distinct advantages over classical AAS and ICP-AES techniques. Fast screening analysis can be performed with adequate accuracy for environmental samples, such as natural waters and sludges, using semiquantitative approaches. The determination of target elements in such samples at levels well below those required by current legislation can be performed with a fast and straightforward procedure taking into account the molar response curve of the instrument and the ionization characteristics of the test elements.

Trace element determinations in biological materials for nutritional, toxicological and biomedical studies require the best precision and accuracy possible. In this context, IDA with ICP-MS can provide, under certain conditions, highly precise and accurate results at very low concentration levels which could not be matched by current AAS and AES techniques.

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Table 8. Molybdenum concentrations in reference materials determined by isotope dilution analysis (concentrations in μg g^{-1}, standard deviations based on $n = 5$)

Sample (g)	Spike 1 ppm (g)	NIST SRM 1577 Bovine Liver	IAEA H-8 Horse Kidney	IAEA V-10 Hay	NIST SRM 1572 Citrus Leaves
0.50	0.75	—	—	0.78 ± 0.01 (1.3%)	0.12 ± 0.01 (8.3%)
0.50	0.50	3.66 ± 0.01 (0.3%)	2.23 ± 0.01 (0.5%)	—	—
0.50	0.25	—	—	0.78 ± 0.01 (1.3%)	0.12 ± 0.01 (8.3%)
0.50	0.10	—	—	0.78 ± 0.01 (1.3%)	0.19 ± 0.02 (11%)
0.10	0.50	3.42 ± 0.01 (0.3%)	2.17 ± 0.02 (0.9%)	—	—
0.10	0.50	3.67 ± 0.01 (0.3%)	2.25 ± 0.01 (0.4%)	—	—
0.10	0.50	3.82 ± 0.01 (0.3%)	2.16 ± 0.02 (0.9%)	—	—
0.05	0.50	3.51 ± 0.01 (0.3%)	2.18 ± 0.01 (0.5%)	—	—
Mean value		3.62 ± 0.16 (4.4%)	2.20 ± 0.04 (1.8%)	0.78 ± 0.01 (1.3%)	0.14 ± 0.04 (29%)
Reference value		3.5 ± 0.5 (14%)	2.21 ± 0.3 (14%)	0.9 ± 0.3 (33%)	0.17 ± 0.09 (53%)

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