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REVIEW

Klaus G. Heumann

Isotope-dilution ICP–MS for trace element determination and speciation: from a reference method to a routine method?

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Abstract This critical review discusses the conditions under which inductively coupled plasma–isotope dilution mass spectrometry (ICP–IDMS) is suitable as a routine method for trace element and element-speciation analysis. It can, in general, be concluded that ICP–IDMS has high potential for routine analysis of trace elements if the accuracy of results is of predominant analytical importance. Hyphenated techniques with ICP–IDMS suffer both from lack of commercially available isotope-labeled spike compounds for species-specific isotope dilution and from the more complicated system set-up required for species-unspecific ICP–IDMS analysis. Coupling of gas or liquid chromatography with species-specific ICP–IDMS, however, enables validation of analytical methods involving species transformations which cannot easily be performed by other methods. The potential and limitations of ICP–IDMS are demonstrated by recently published results and by some unpublished investigations by our group. It has been shown that possible loss of silicon as volatile SiF_4 during decomposition of a sample by use of hydrofluoric acid has no effect on trace silicon determination if the isotope-dilution step occurs during digestion in a closed system. For powder samples, laser ablation ICP–IDMS can be applied with an accuracy comparable with that only available from matrix-matched standardization, whereas the accuracy of electrothermal vaporization ICP–IDMS was strongly dependent on the element determined. The significance of easy synthesis of isotope-labeled spike compounds for species-specific ICP–IDMS is demonstrated for monomethylmercury and Cr(VI). Isotope-exchange reactions between different element species can prevent the successful application of ICP–IDMS, as is shown for iodinated hydrocarbons. It is also shown for monomethylmercury that species transformations during sample-pretreatment steps can be followed by species-specific ICP–IDMS without loss of accuracy. A relatively simple and time-efficient procedure for determination of monomethylmercury in environmental and biological samples is discussed. The method, which entails a rapid microwave-assisted isotope dilution step and in-situ extraction of the derivatized species, has good potential for routine application in the future.

Keywords Inductively coupled plasma isotope dilution mass spectrometry · Trace elements · Element species · Species-specific and species-unspecific spiking · Routine method · Validation

K. G. Heumann (✉)
Institute of Inorganic Chemistry and Analytical Chemistry,
Johannes Gutenberg-University Mainz, 55099 Mainz, Germany
e-mail: heumann@mail.uni-mainz.de

Introduction

Before 1990 determination of trace elements and, less frequently, element species by isotope-dilution mass spectrometry (IDMS) was almost exclusively performed by use of thermal ionization (TI–IDMS) [1, 2]. Until this time determination of trace elements by inductively coupled plasma mass spectrometry (ICP–MS) was usually performed by external calibration with the corresponding standard solutions or by internal calibration by the standard addition method. Since 1990 an increasing number of such ICP–IDMS analyses have been published (e.g. Refs. [3, 4]) and in 1994 the first reports of element speciation coupling HPLC with ICP–IDMS appeared in the literature [5, 6].

Nuclear technology and geochemistry have been the major fields of application of trace-element determination by TI–IDMS [7, 8]. In the past TI–IDMS was also often used for certification of reference materials [9]. Introduction of the isotope-dilution technique to ICP–MS also enabled use of this technique, internationally well known for its highly accurate analytical results, by many analytical laboratories not specialized in TI–MS measurements. Even if the precision of isotope-ratio measurements by TI–MS is better than that for single-collector ICP–MS instruments, ICP–IDMS is usually not strongly affected by this difference, because other factors such as sample inhomogeneity and sample preparation have a much greater

effect on IDMS results. Corrections can be made for mass bias in ICP–MS. Although in recent years ICP–IDMS has most often been applied during certification campaigns or in connection with analytical quality assurance [10, 11], an increasing number of routine analytical problems, such as the determination of lanthanides and actinides in fission products or of heavy metals in plastic materials, have also been solved by ICP–IDMS [12, 13]. The multi-element capability, the usually simpler sample preparation compared with TI–IDMS, and the resulting higher sample throughput are the major reasons why ICP–MS is now the preferred analytical method for trace-element determination by IDMS.

Online coupling of ICP–IDMS with chromatographic techniques is possible; this is a powerful tool for accurate element speciation analysis. With TI–MS only offline coupling is possible; this is more time-consuming and less practical. It must, however, be taken into account that ICP–IDMS analysis, in contrast with the more selective thermal ionization technique with its different analyte-isolation steps (usually used in this connection), implies a high risk of spectrometric interferences and, therefore, a higher probability that the accuracy of results can be affected. Because an isotope ratio of the isotope-diluted sample is always measured in IDMS determinations, two isotopes must be free from interference.

The important question to be discussed in this critical review is whether or not the worldwide distribution of many ICP–MS instruments and the mentioned advantages of ICP–IDMS are sufficient to enable it compete as a routine method in comparison with other analytical methods? Essential presuppositions must be fulfilled for an analytical procedure to become a routine method. Important topics are a simple sample-pretreatment procedure, an easy to handle and matrix-unaffected calibration method, good time- and cost-efficiency, sufficient robustness, and the requirement that accuracy and precision fit the purpose of the analysis. With regard to the known high accuracy of IDMS analysis, it must be assumed that ICP–IDMS is especially suitable as a routine method in all cases where this criterion is important to the analytical results. The major aim of this paper is to critically review the potential of ICP–IDMS as a routine method for trace element and element speciation analyses.

Is sample preparation for ICP–IDMS suitable for routine analysis?

The fundamental principles of IDMS are described in different textbooks [1, 14, 15]. In IDMS analysis Eq. (1) is the basic formula for calculating the number of analyte atoms in a sample:

$$N_S = N_{Sp} \cdot \left(h^2_{Sp} - R \cdot h^1_{Sp}\right) / \left(R \cdot h^1_S - h^2_S\right) \quad (1)$$

where R is the isotope ratio of the isotope-diluted sample, N_S and N_{Sp} are number of analyte atoms in the sample and the number of spike atoms in the added isotope-enriched spike solution, respectively, and h^1 and h^2 are the isotope abundances of the reference isotope (usually most naturally abundant) and spike isotope, respectively.

The only value to be measured for IDMS analyses is the isotope ratio R of the isotope-diluted sample. This means that after equilibration of the sample (analyte isotopes) with the spike isotopes, possible loss of the isotope-diluted sample has no effect on the result. This is because loss of substance does not change the isotope ratio in the remaining isotope-diluted analyte. This is one important advantage of IDMS, because loss of substance during sample preparation, e.g. by separation or evaporation steps, is a substantial problem in trace and ultra-trace analysis. When other analytical methods are applied it must either be guaranteed that the total amount of the analyte is isolated for detection or that the recovery of the corresponding analytical step can be determined precisely; this is not always an easy task.

In principle, TI–IDMS is also not affected by loss of the isotope-diluted analyte. However, for TI–MS measurements more complicated and, therefore, more time-consuming sample pretreatment steps must usually be performed. This is especially obvious for analyses in which TI–IDMS and ICP–IDMS have been applied to identical samples, as has been described in the literature, for example, for determination of heavy metals in polyolefins and of traces of iridium in photographic emulsions [13, 16].

A good example of the advantage of ICP–IDMS compared with other analytical methods is the determination of platinum-group elements (PGE) in environmental and geological samples. Because of many serious spectrometric interferences with the different PGE isotopes by matrix elements, even if a sector-field mass spectrometer at high mass resolution is applied, the platinum-group elements must be separated from interfering elements after sample digestion. This can be achieved by simple chromatographic separation on a strongly basic anion-exchange column only 8 cm long. After introduction of the isotope-diluted sample to the top of the column, all interfering elements, for example Hf, Cu, and Zn, are eluted with 2 mol L^{-1} HNO_3 and the PGE are then collected in a small fraction by use of concentrated HNO_3 [17]. Recoveries of the different PGE differ substantially and the reproducibility of this analytical step varies strongly from one separation to the next (Pt=11–22%, Pd=19–35%, Ru=87–92%, Ir=4–8%). Nevertheless, accurate results have been obtained, even when a quadrupole instrument was used, as has been demonstrated by analysis of certified geological reference materials and by participation in an interlaboratory study for the determination of PGE in road dust samples [17]. The interlaboratory study results for Pd, in particular, which ranged from about 3 ng g^{-1} to approximately 1000 ng g^{-1} for an accepted value of <5 ng g^{-1} clearly showed that many other methods cannot be used as routine methods for accurate determination of PGE in environmental samples.

That loss of analyte has no effect on the result after the isotope-dilution step has taken place is not only advantageous for analytical methods with non-quantitative separation techniques, but also for those with digestion proce-

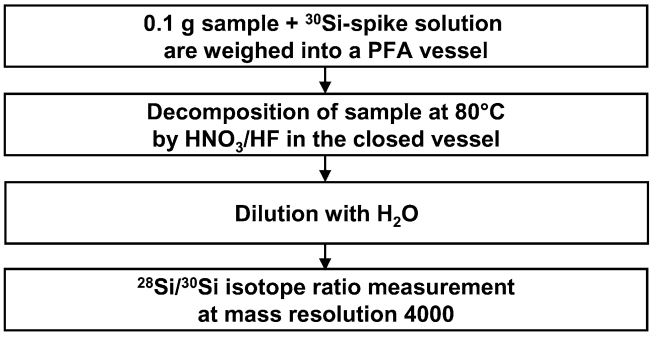

Fig. 1 Sample preparation for determination of traces of silicon in GaAs wafers by sector-field ICP–IDMS [18]

dures in which the analyte exists in form of a volatile compound after acid treatment. An impressive example of the solution of such a problem is the determination of traces of silicon in gallium arsenide (GaAs) semiconductor materials. For complete and rapid digestion of GaAs use of hydrofluoric acid is necessary; this produces volatile SiF_4 from the target analyte element. The formation of this volatile compound implies a high risk of analyte loss after the vessel is opened, even if decomposition of the sample has taken place in a closed system. Figure 1 depicts the sample-treatment scheme for determination of traces of silicon in GaAs by ICP–IDMS with use of a sector-field instrument for mass separation of interferences from the silicon isotopes [18]. During decomposition of the sample in the closed system complete equilibration between sample and spike silicon takes place. Afterwards, loss of isotope-diluted silicon as SiF_4 has no effect on the analytical result, assuming enough substance remains for measurement of the silicon isotope ratio *R*. Compared with optical atom spectrometric methods ICP–MS detection of silicon is more sensitive, so ICP–IDMS has exclusive advantages as a routine method for trace silicon determination, especially when HF digestion techniques are applied. A similar situation also occurs for other elements forming volatile compounds during sample digestion, e.g. determination of boron or osmium where volatile BF_3 and OsO_4 are formed during digestion with HF and with oxidizing acids, respectively.

In Table 1 results from determination of trace silicon in three different GaAs samples by ICP–IDMS [18] are listed and compared with those obtained by spark-source mass spectrometry (SSMS) [19]. Results from ICP–IDMS are much more precise than those from SSMS; this was expected, because possible sample inhomogeneities are better compensated by the wet-chemical IDMS method. The mean silicon content determined by both methods differs significantly, however, because of lack of GaAs calibration standards for SSMS. On the other hand, multi-element information without chemical sample treatment was obtained by SSMS in the same analytical run. Thus, ICP–IDMS should be the preferred method if precision and accuracy of single-element analysis are the main requirements of a routine method, but for rapid semi-quantitative multi-element information the use of SSMS or laser ablation (LA)–ICP–MS is more convenient.

Table 1 Determination of traces of silicon in GaAs wafers by ICP–IDMS and SSMS

Sample	Silicon content (μg g^{-1})	
	ICP-IDMS [18]	SSMS [19]
GaAS-1	12.4±1.2	16±3
GaAS-2	89.3±1.5	60±12
GaAS-3	132.9±2.9	84±20

Does internal calibration by use of an isotope-enriched spike meet the needs of routine analysis?

A homogeneous mixture of sample and spike is one essential precondition for application of IDMS. This is best achieved by use of solutions in which the analyte and the spike occur in the same ionic form. This alone guarantees total equilibration between analyte and spike isotopes and is the reason IDMS analyses have been performed almost exclusively with wet-chemical sample-preparation procedures and by applying a spike solution. Such an isotope-diluted solution is highly compatible with the ICP–MS equipment usually used, in which solutions are normally introduced into the plasma.

Internal calibration by means of an isotope-enriched spike compensates for possible matrix effects by enabling measurement of the ratio of two isotopes identically affected by the matrix. Figure 2 shows the difference between analytical results obtained by ICP–IDMS and ICP–MS using external non-matrix-matched calibration for analysis of a 10 ng mL^{-1} molybdenum standard solution containing increasing concentrations of dissolved organic carbon (DOC) [20]. The results for external calibration differ by up to approximately 8.5% from the true value for DOC concentrations of 100 mg L^{-1}, whereas ICP–IDMS results were not affected by matrix concentration. The accuracy of ma-

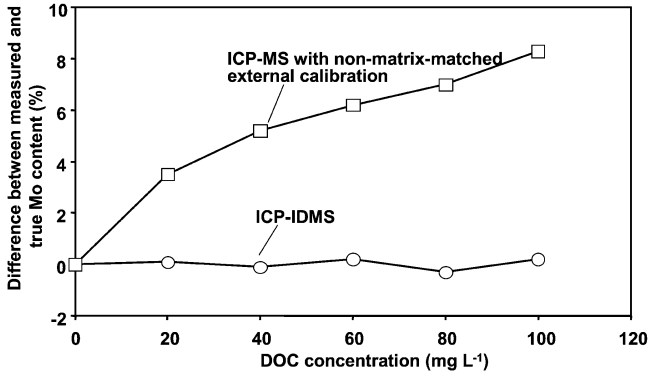

Fig. 2 Comparison of molybdenum determinations (10 ng mL^{-1}) by ICP–MS with external non-matrix-matched calibration and by ICP–IDMS in solution with increasing DOC content [20]

trix-independent results is, therefore, a substantial advantage of IDMS.

For IDMS analyses the isotope ratio R of only one (isotope-diluted) solution of the sample must be measured, so this technique is a one-point internal-calibration method. When the commonly used standard addition method is used for internal calibration, at least two sample aliquots (one without and another with standard addition) must be analyzed; this is less time-efficient than ICP–IDMS. For both methods the approximate order of magnitude of the analyte concentration must be known (or determined by any kind of pre-analysis) to enable optimization of the spike and standard addition, respectively. Highly accurate IDMS results for measurement of R also require mass-bias correction; this can be achieved by analyzing isotope standards under identical experimental conditions. In addition, for a few elements for which variation of the natural isotopic composition is possible (this is especially relevant for ICP–IDMS of Li, B, S, Sr, and Pb) one must determine the corresponding isotope abundances in the unspiked sample also.

Although isotope-enriched substances are now commercially available for almost all elements, most of the necessary spike solutions must be prepared, and their isotopic composition and spike concentration must be characterized. This means that routine analyses by ICP–IDMS are acceptable only if the same spike solution can be applied for a relatively large number of analyses. Otherwise the advantage of the one-point calibration method is lost. Spike standard solutions for twelve elements (B, Mg, Cr, Fe, Ni, Cu, Zn, Ag, Cd, Ba, Tl, and Pb) are available commercially [21]; this enables easy application of ICP–IDMS for these elements. Dahmen et al. were among the first to introduce ICP–IDMS for analytical control of an industrial process by analyzing high-purity chemicals used for processing of semiconductors [22].

IDMS is often judged as a costly analytical method, because of the relatively high price of isotopically enriched substances. This is totally incorrect for trace and ultra-trace analysis by IDMS and only becomes relevant for determinations at concentrations $>100\,\mu g\,g^{-1}$. To minimize the error multiplication factor of the isotope ratio measurement R, the ratio of analyte atoms to spike atoms must be optimized; this is usually achieved by using ratios in the range 0.1–10 [1]. From this it follows that an amount of spike of less than 1 µg is normally sufficient for one trace analysis by IDMS. Depending on the element, the cost of 1 mg of an isotopically enriched element varies in the range of approximately 1–100 Euro. Thus, one IDMS trace analysis costs less than 0.1–10 Cents, which can be neglected compared with the other costs of the analysis. Only for determinations at higher concentrations does the isotope spike contribute significantly to the total cost of the analysis. For special purposes when inaccurate analytical results can become extremely costly it also pays to apply ICP–IDMS at relatively high concentration levels. For example, control of platinum and palladium concentrations in automotive catalysts requires accuracy better than 1% relative standard deviation, because of the high cost of these noble metals. This is why, a few years ago, a leading company producing automotive catalysts evaluated ICP–IDMS for routine control [23]. One drawback of final establishment of ICP–IDMS for production control was the need to separate elements interfering with palladium measurement; a simple solution such as that described above was not available at the time.

Is ICP–IDMS possible in connection with direct solid sampling?

In principle, application of IDMS is also possible for direct analysis of solid samples if homogeneous mixing of the sample and spike can be achieved in the solid phase. In addition, if analyte and spike exist in different chemical forms the subsequent evaporation and ionization processes must guarantee their total equilibration, to eliminate possible discrimination between the corresponding ICP–MS signals. For compact solid samples this can be guaranteed only by chemical digestion of the sample with subsequent addition of the spike. Re-conversion of the corresponding isotope-diluted solution into a solid sample is necessary. This type of solid-sample IDMS analysis has been used to obtain accurate analytical data by spark-source mass spectrometry [24]. However, this procedure eliminates one of the major advantages of a direct solid-sampling technique – analysis without large-scale chemical manipulation. Pulverized substances are, therefore, the only samples which can be successfully and easily handled for possible routine analysis by direct sampling ICP–IDMS.

In principle, electrothermal vaporization (ETV) and laser ablation (LA) can be used to introduce a powder sample, first mixed with a corresponding spike in solution or in the pulverized form, into the plasma source of an ICP–MS. For ETV–ICP–IDMS the isotope-diluted powder can be used directly whereas for LA–ICP–IDMS a pellet must be pressed. For example, ETV–ICP–IDMS has been used to determine heavy metals in polyolefins by introducing the powder sample and 10 µL spike solution into the graphite furnace. An argon gas flow and three-step temperature program (drying at 120 °C, ashing at 600 °C, evaporation of heavy metals at 2000 °C) transported the isotope-diluted analytes into the plasma source. In Fig. 3 the results for three heavy metals are compared with the corresponding results from ICP–IDMS and TI–IDMS, using sample digestion for both [25]. Excellent agreement was obtained between the ETV–ICP–IDMS result for lead and that obtained from wet-chemical IDMS methods. For copper the ETV–ICP–IDMS data agree with the ICP–IDMS results within the relatively high standard deviation, but for cadmium no agreement was observed between ETV–ICP–IDMS and the other two methods. The worst cadmium result obtained by use of ETV–ICP–IDMS can be explained by the high volatility of this element and the different probabilities of evaporation for sample and spike cadmium. On the other hand, different volatilities of mercury compounds were recently used by Gelaude et al. to develop a successful ETV–ICP–IDMS method for mercury specia-

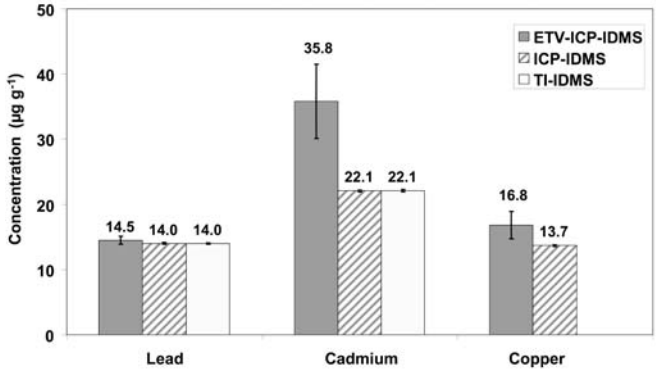

Fig. 3 Direct determination of heavy metal traces in polyolefins by ETV–ICP–IDMS and comparison with results obtained by wet-chemical ICP–IDMS and TI–IDMS [25]

tion in biological samples using a permeation tube with ^{200}Hg-enriched elemental mercury for the isotope-dilution step [26]. The examples of ETV–ICP–IDMS analyses discussed show that this method may be useful in special cases but is not, in general, applicable to routine analysis. Reliable results are strongly dependent on suitable ETV conditions for a single element and the special type of sample.

Tibi and Heumann recently demonstrated that LA–ICP–IDMS can be used for successful multi-element trace determinations of powder samples [27, 28]. Homogenization of the isotope-diluted sample was achieved by suspending the sample powder in the spike solution, followed by evaporation of the suspension to dryness and subsequent formation of a pellet by means of a laboratory press. During the applied laser-ablation process, with a special laser system (LINA-Spark-Atomizer), by focusing of the laser approximately 15 mm behind the sample surface and use of pulse energies of up to 350 mJ the isotope-diluted sample was first molten and then evaporated, which equilibrated the analyte with the spike. A large variety of different samples were analyzed by LA–ICP–IDMS, including alkaline earth fluorides, sediments, and biological samples.

Lack of a reliable calibration method is one of the major problems preventing LA–ICP–MS from becoming a widely adopted routine method for solid samples. Matrix-matched calibration was found to be the best calibration method for LA–ICP–MS, as has been demonstrated by many publications, e.g. Ref. [29]. Comparison of results from determination of trace elements in alkaline earth fluoride samples by LA–ICP–MS, using matrix-matched reference standards, with those obtained by LA–ICP–IDMS showed accuracy, precision, and detection limits were similar [27]. However, lack of suitable reference materials often prevents application of a matrix-matched calibration. In such circumstances non-matrix-matched calibration with other reference materials, in combination with internal standardization by means of an element of known concentration in both the reference material and the sample, can be applied [30]. Non-matrix-matched calibration

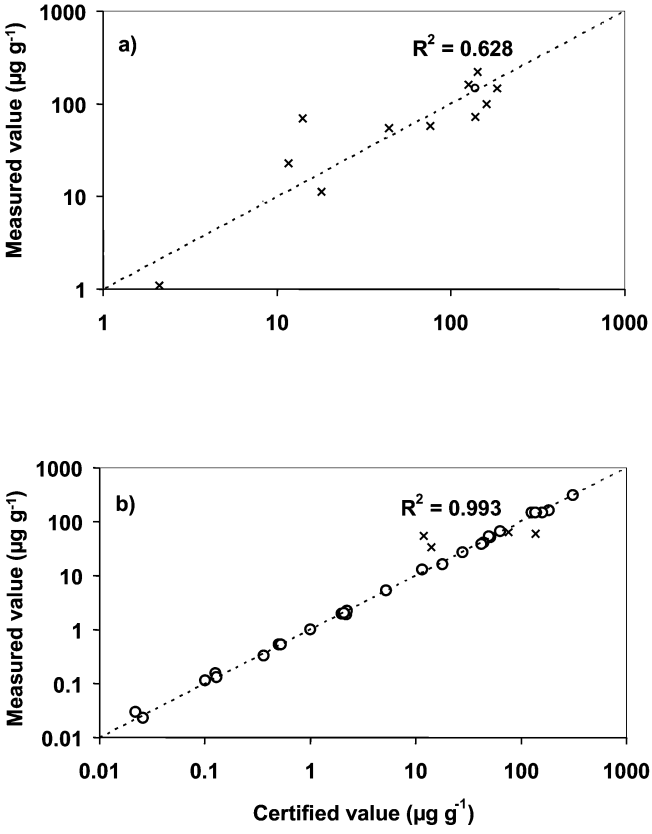

Fig. 4 Correlation of measured and certified trace metal concentrations in different reference materials by using: (**a**) LA–ICP–MS with non-matrix-matched calibration and an internal standard, and (**b**) LA–ICP–IDMS [32, 33] (*circle*, measured value within the uncertainty of the certified value; *crosses*, measured value outside the uncertainty of the certified value)

often does not produce accurate analytical results, as was demonstrated, for example, by a recent publication by Rodushkin et al. [31] and also by the results represented in Fig. 4a. An important alternative to calibration by use of matrix-matched reference materials is, therefore, LA–ICP–IDMS, for which external calibration standards are not needed.

The accuracy of LA–ICP–IDMS was demonstrated by analyzing up to seven trace elements (Cr, Fe, Cu, Zn, Sr, Cd, Pb) in seven different certified reference materials (BCR 60 aquatic plant, BCR 150 spiked skim milk powder – lower level, BCR 151 spiked skim milk powder – higher level, SRM 1567a wheat flour, SRM 1577b bovine liver, CRM 320 river sediment, and SRM 1646 estuarine sediment). Twenty-eight of a total of thirty-two trace element concentrations determined by LA–ICP–IDMS were in agreement, within the corresponding standard deviations, with the certified values and their given uncertainties [28] (Fig. 4b). LA–ICP–IDMS analyses result in more accurate data than non-matrix-matched calibration with an internal reference element, as can be seen by comparison of the results shown in Figs. 4a and 4b. In Fig. 4a the corresponding Cr, Fe, Cu, and Zn data, determined in four of the seven reference materials (CRM 320, SRM 1646,

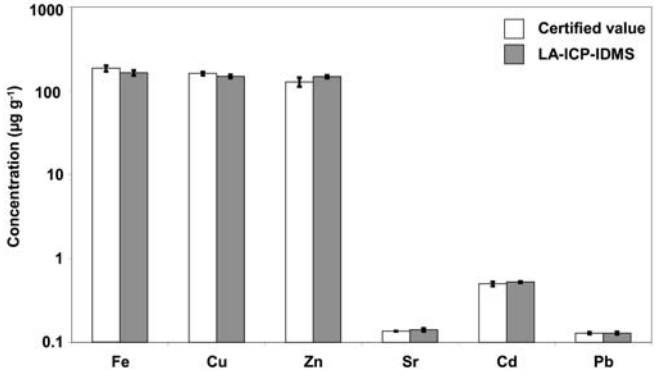

Fig. 5 Comparison with the certified values of trace metal concentrations obtained by LA–ICP–IDMS in the standard reference material SRM 1577b (bovine liver) [33]

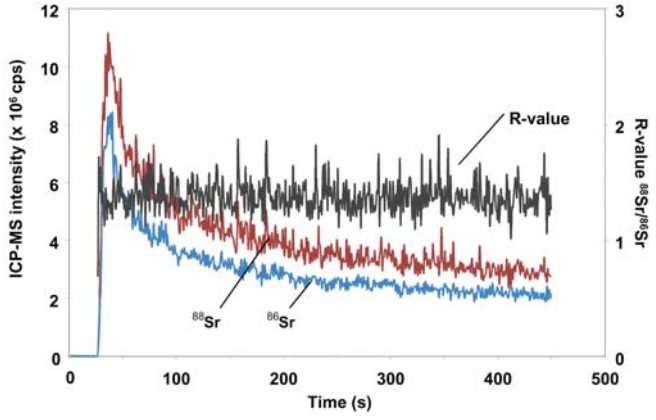

Fig. 6 Time-dependent signals of ^{86}Sr and ^{88}Sr and of the corresponding isotope ratio $^{88}Sr/^{86}Sr$ during LA–ICP–IDMS analysis of an ^{86}Sr-spiked alkaline earth fluoride sample [33]

SRM 1567a, SRM 1577b) by non-matrix-matched calibration, are also correlated with the certified values. For non-matrix-matched calibration an alkaline earth fluoride standard containing known concentration of the elements to be determined, and Rh for internal standardization, was used. The slope of the curve for the LA–ICP–IDMS data (Fig. 4b) is 0.984, with an excellent correlation coefficient (R^2=0.993), whereas the slope of the non-matrix-matched calibration curve (Fig. 4a) is only 0.80 (R^2=0.628) [32].

A more detailed example of one of these LA–ICP–IDMS analyses is represented in Fig. 5, in which the results for the standard reference material SRM 1577b (bovine liver) are shown. The results for six metals determined by LA–ICP–IDMS, covering more than three orders of magnitude in trace element concentration, agree well within the uncertainties given for the certified values for the standard reference material. One important reason accurate data are obtained by use of LA–ICP–IDMS is that ICP–MS signals of isotopes produced by laser ablation, even if they are unstable with time, always result in a time-independent isotope ratio, R, of the isotope-diluted sample. This is demonstrated in Fig. 6 for an alkaline earth fluoride sample spiked with ^{86}Sr for strontium analysis [33].

The results presented in Figs. 4, 5, and 6 show that the unique advantages of the isotope dilution technique, well-known from solution analysis, have been successfully transferred to the direct analysis of powder samples. Simple sample pretreatment only – suspension of the sample in the spike solution with subsequent drying – is necessary for the isotope dilution step. An alternatively easy procedure is the application of a solid-spike where the spike isotopes are absorbed on nano-particles. Preliminary results demonstrated recently the usefullness of such a solid-spiking technique for LA–ICP–IDMS. LA–ICP–IDMS is, therefore, highly suitable for routine analysis of pulverized products in the future, especially when reference materials are not available for matrix-matched calibration.

Is ICP–IDMS suitable as a routine method for element speciation?

Element speciation has become one of the most important topics in trace element analysis in recent years, because scientists learned it is often not sufficient to know the total amount of an element only, because of the different behavior of element species with regard to, for example, bioavailability, toxicity, and mobility in the environment [34]. Nevertheless, international legislation or guidelines relating to trace elements in food, occupational health, or the environment are usually based on total element concentrations – few regulations or guidelines pay attention to element species [35]. Currently, therefore, there is no great pressure to develop routine methods for element speciation. There might, however, be a change in the near future when the huge importance of element speciation, especially in the environment, in medicine, and in occupational health, is better recognized by legislative bodies. For example, in the European Union butyltin compounds must be determined on a routine basis in fresh waters and industrial effluents. The allocation of a US patent to a *method of speciated isotope dilution mass spectrometry* in 1995 [36] reflects the expectation that ICP–IDMS of element species will become necessary for routine analyses also, even if the license for this patent totally ignored the fact that element speciation by IDMS was first conducted long before this patent appeared, e.g. by TI–IDMS in 1990 [2] or by ICP–IDMS in 1994 [5, 6]. Also, the approval of a European Virtual Institute for Speciation Analysis (EVISA) at the beginning of 2003 by the European Union [37], both for members of research institutes and those in industry, demonstrates the increasing recognition by political bodies of the importance of element speciation.

These facts show that element speciation is, currently, not usually performed routinely. ICP–IDMS must, therefore, not be evaluated solely on the basis of whether it can substitute other analytical procedures as a routine method, but also whether it is suitable for possible routine analyses in the future. One of the most powerful hyphenated techniques used for element speciation is coupling of ICP–MS

with separation methods such as capillary gas chromatography (CGC), high-performance liquid chromatography (HPLC), and capillary electrophoresis (CE) [34, 38]. Coupling of ICP–IDMS with these separation techniques has been applied, e.g., for determination of mercury species by CGC–ICP–IDMS [39, 40], analysis of trimethyllead, using a species-specific spike, by HPLC–ICP–IDMS [5], and analysis of heavy metal complexes of humic substances using a species-unspecific spike and HPLC–ICP–IDMS [20]. CE–ICP–IDMS also was recently used for the first time to characterize and quantify metallothionein isoforms [41].

Coupling of ICP–MS with CGC or HPLC is especially easy because the gas and liquid flow, respectively, of these two separation methods can be introduced directly into the ICP–MS without splitting or dilution. The low gas flow of CGC does not usually disturb plasma stability and, as an additional advantage, 100% of the analyte is introduced into the plasma torch. The eluent flow from HPLC, on the other hand, fits exactly that normally applied for nebulizer systems of ICP–MS instruments.

Two different modes of spiking can be used in element speciation by ICP–IDMS – use of species-specific or species-unspecific spiking solutions [42]. For species-specific spiking the composition and structure of the element species must be known and the spike must be available or synthesized in an isotopically labeled form of the element species to be determined. The sample should then be spiked before separation of the different species to make total use of one of the major advantages of IDMS – that loss of substance after the isotope-dilution step has no effect on the analytical result. For determination of volatile element species or those which have been converted into volatile species by derivatization, CGC–ICP–IDMS with species-specific spiking is, currently, the procedure usually used. Examples include determination of methylmercury and alkyltin species [43, 44, 45].

The main problem preventing more frequent use of species-specific ICP–IDMS is the lack of commercially available isotope-labeled spike compounds. The isotope-labeled spike must, therefore, normally, be synthesized if species-specific ICP–IDMS is to be applied; this is not usually too complicated for inorganic species, for example Cr(VI), as can be seen from Eqs. (2) and (3). Commercially available ^{53}Cr-enriched chromium metal is dissolved in hydrochloric acid and the Cr(III) formed can easily be converted into Cr(VI) by oxidation with hydrogen peroxide in ammonia solution [46]:

$$2\ {}^{53}\mathrm{Cr} + 6\ \mathrm{HCl} \rightarrow 2\ {}^{53}\mathrm{CrCl_3} + 3\ \mathrm{H_2} \qquad (2)$$

$$2\ {}^{53}\mathrm{CrCl_3} + 3\ \mathrm{H_2O_2} + 10\ \mathrm{NH_3} + 2\ \mathrm{H_2O} \rightarrow 2\ (\mathrm{NH_4})_2\ {}^{53}\mathrm{CrO_4} + 6\ \mathrm{NH_4Cl} \qquad (3)$$

The synthesis of organometallic compounds is usually more complicated than the synthesis of inorganic isotope-labeled substances, because many of these reactions must be conducted in water-free solutions because of water-sensitive intermediates or reactants, for example Grignard reagents. Alternative methods have also been described, however, for example, the relatively simple reaction of mercuric chloride with methylcobalamin (Me-[Co]) in aqueous solution to synthesize an isotope-labeled monomethylmercury spike [43, 47]. Commercially available ^{201}Hg-enriched mercuric oxide can first be converted into mercuric chloride by treatment with concentrated HCl and, in a second step, mercuric chloride can react with methylcobalamin in aqueous solution:

$$^{201}\mathrm{HgO} + 2\ \mathrm{HCl} \rightarrow \quad ^{201}\mathrm{HgCl_2} + \mathrm{H_2O} \qquad (4)$$

$$^{201}\mathrm{HgCl_2} + \mathrm{Me\text{-}[Co]} \rightarrow \quad ^{201}\mathrm{HgMeCl} + \mathrm{Cl\text{-}[Co]} \qquad (5)$$

Species-specific spiking can also used successfully in HPLC–ICP–IDMS, as was first shown in 1994 for determination of trimethyllead [5] and for iodide and iodate [6]. A schematic diagram of an HPLC–ICP–IDMS system for species-specific and species-unspecific spiking modes is represented in Fig. 7 [20, 42]. The UV flow-through cell of the system provides additional information about unknown species, usually on the organic ligands of an element. Transient signals from both the spike and the reference isotope must be measured in the separated fractions for all the element species. Even if the isotope ratio for an element species, isotope-diluted by a species-specific spike, should, theoretically, be constant over the total peak, evaluation of the corresponding peak areas usually results in more precise data.

Whereas for total element analysis by ICP–IDMS equilibration of the spike with all species of the element to be determined is required, in element speciation by the species-specific spiking mode it must be guaranteed that no isotope exchange occurs between the different species until they are completely separated from each other. This is not always fulfilled, as the results presented in Fig. 8 show – different iodinated hydrocarbons of natural isotopic composition in water were mixed with a ^{129}I-labeled 1- and 2-propyl iodide spike solution [48]. As can be seen from the CGC–ICP–IDMS chromatogram of the iodine isotopes, not only did the two isomers of propyl iodide con-

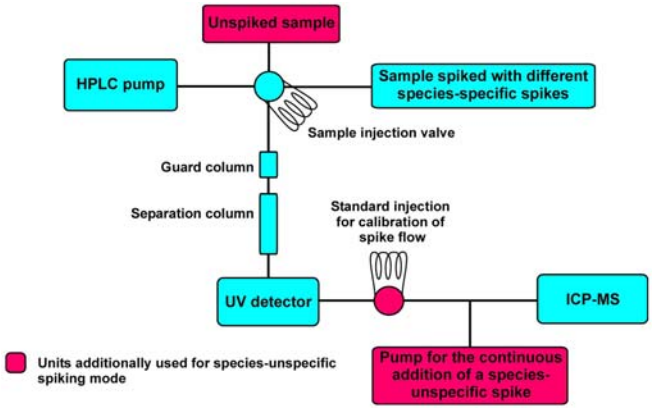

Fig. 7 Schematic figure of a HPLC–ICP–MS system for determination of element species by species-specific and species-unspecific IDMS [20, 42]

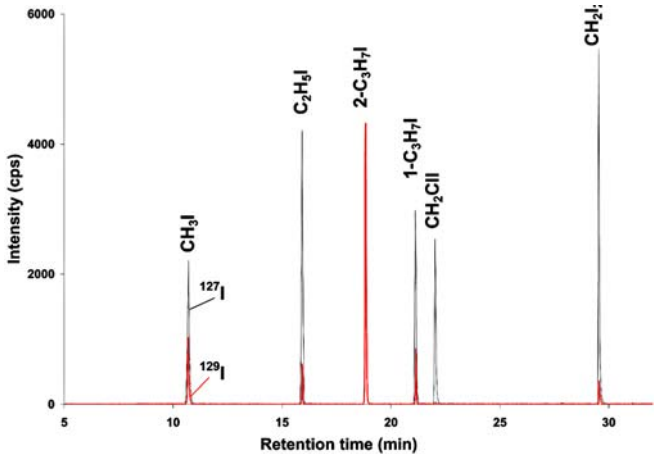

Fig. 8 CGC–ICP–MS chromatogram of the iodine isotopes ^{127}I and ^{129}I after spiking of a solution of iodinated hydrocarbons of natural isotopic composition with ^{129}I-enriched 1- and 2-propyl iodide [48]

tain substantial amounts of ^{129}I but also the other iodinated hydrocarbons all afford more or less significant intensities of ^{129}I not occurring in natural iodine. This is because of nucleophilic isotope exchange of ^{129}I in the spike compound with all other iodinated hydrocarbons, as represented by methyl iodide in Eq. (6). Possible isotope exchange between different element species must, therefore, always be checked in species-specific ICP–IDMS, to ensure such exchange does not affect the accuracy of results.

$$CH_3{}^{127}I + C_3H_7{}^{129}I \rightarrow CH_3{}^{129}I + C_3H_7{}^{127}I \quad (6)$$

Use of species-unspecific spiking, in which the spike can occur in any chemical form, eliminates possible problems caused by isotope exchange between different element species, because spike and analyte are mixed after complete separation of the element species. Up to this step, loss of substance is not allowed, so one of the usual advantages of IDMS is not valid for the species-unspecific isotope-dilution mode. However, use of a completely closed system, as shown in Fig. 7, does not involve high risk of loss of analyte during separation. Total equilibration between the separated element species and the spike in the plasma of the ICP–MS is highly desirable for this type of spiking. The species-unspecific spiking mode must always be used when the exact composition and structure of the element species is not known or when the corresponding labeled compounds cannot be synthesized. This is true, for example, for metal complexes with humic substances and for most metal–protein complexes. A schematic illustration of species-unspecific HPLC–ICP–IDMS is given in Fig. 7. Fractions of separated element species are mixed with a continuous spike flow just before entering the ICP–MS. In contrast with species-specific spiking mode the isotope ratio R varies over the total transient signal, so R-values at each point of the corresponding isotope-diluted fraction represent the corresponding time-dependent amount of the analyte given by Eq. (1). Transformation of such an

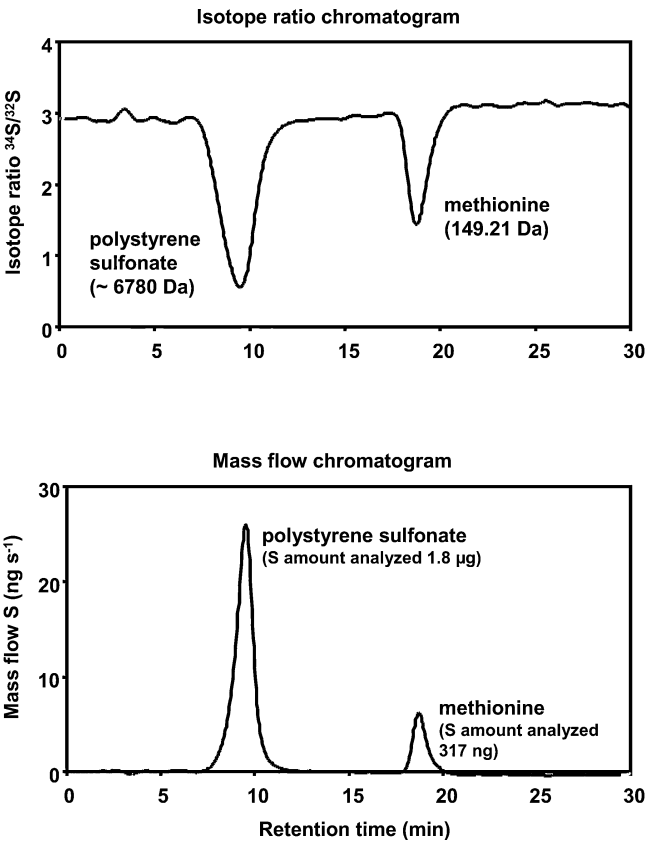

Fig. 9 $^{34}S/^{32}S$ isotope ratio and corresponding sulfur mass-flow chromatogram of a standard solution containing polystyrene sulfonate (approx. 1.6 µg sulfur by weight) and methionine (exactly 322 ng sulfur) obtained by SEC–ICP–IDMS using a sector-field mass spectrometer at mass resolution 4000 and species-unspecific spiking with ^{34}S-enriched sulfate [49]

isotope-ratio chromatogram into a mass flow chromatogram and, thereby, into the corresponding concentrations is discussed in detail elsewhere [20]. ICP–IDMS is currently the only possible means of determining "real-time" concentrations of element species in separated fractions by hyphenated techniques. For example, Fig. 9 depicts results obtained from the determination of sulfur in a standard solution of two sulfur-containing compounds by coupling of size-exclusion chromatography (SEC) with ICP–IDMS [49]. The applied spike was ^{34}S-enriched sulfate solution; a sector-field mass spectrometer was operated at mass resolution 4000.

For species-unspecific spiking of gaseous species, a continuous gas flow of a volatile spike compound must be mixed with the separated fractions. A permeation tube, filled with an isotopically labeled spike, can be used for this purpose, as in the analysis of monomethylmercury and inorganic mercury in biological samples by use of ETV–ICP–IDMS [26]. The analytical unit in which this isotope-dilution step occurred is shown schematically in Fig. 10.

When the species-unspecific spiking mode is used it must be shown whether this causes calibration problems as a result of discrimination between the element species to be determined and the spike compound. For nebulizer

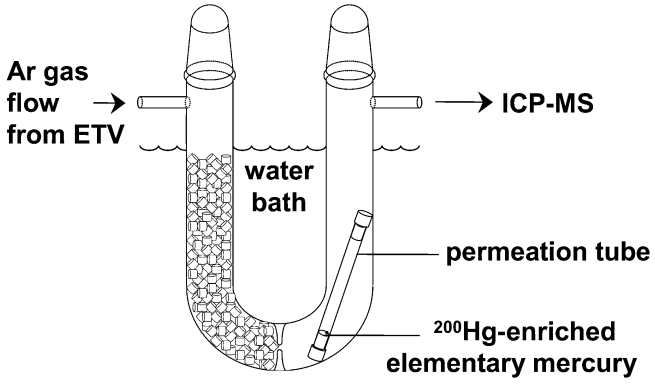

Fig. 10 Species-unspecific spiking for determination of mercury species after selected evaporation by an ETV system using a permeation tube filled with ^{200}Hg-enriched elemental mercury [26]

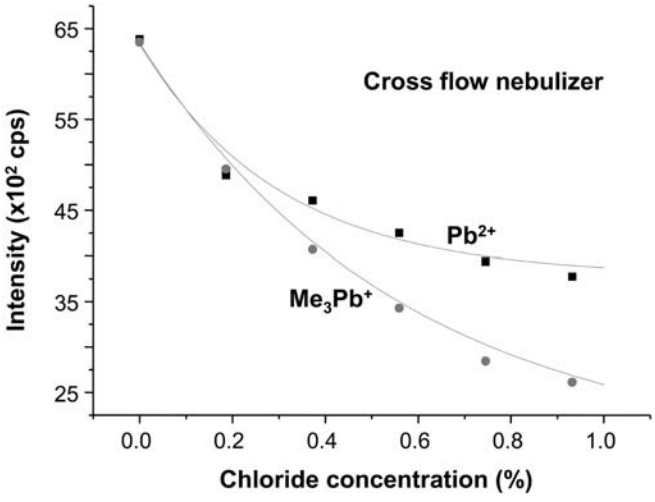

Fig. 11 Dependence on the chloride concentration of a NaCl solution of lead species discrimination for inorganic Pb^{2+} and Me_3Pb^+ (Pb concentration of both solutions is 12 ng Pb mL^{-1}) [50]

systems commonly applied in ICP–MS, e.g. the cross-flow nebulizer, no differences between signal intensities from different element species were found under the conditions usually used. For some special cases, however, for example use of an ultrasonic nebulizer with a membrane desolvator or at high chloride concentrations, significant differences were measured when comparing inorganic lead and trimethyllead (Me_3Pb^+) solutions with an identical lead content of 12 ng mL^{-1} [50]. Figure 11 shows that in a 0.9% (*w*/*w*) sodium chloride solution the ICP–MS response for Me_3Pb^+ is reduced by about 60% compared with that for a matrix-free solution, and that the difference between the signal intensity and that for inorganic lead is approximately 15% at this high chloride concentration. At high matrix concentrations Me_3Pb^+ possibly forms a chloride compound which is enriched in the larger droplets of the nebulization process and, therefore, it is less abundant in the plasma. Even if element-species discrimination in ICP–MS introduction systems does not occur very often, it should always be checked that species-unspecific spiking mode does not cause calibration problems.

The lack of commercially available isotope-labeled element species, on the one hand, and the more complicated instrumentation and measurement technique for application of the species-unspecific spiking mode, on the other hand, are major reasons why hyphenated ICP–IDMS techniques are currently a long way from being used as routine methods in element speciation. There is, in fact, a general lack of analytical methods suitable for application as routine methods for determination of element species.

Validation of analytical methods by species-specific ICP–IDMS

Even if hyphenated ICP–IDMS techniques cannot be described as routine methods, they are the only convenient procedures for validation of other analytical methods or single sample-pretreatment steps. Transformation of element species during sample pretreatment is a great problem in element speciation and often affects the accuracy of results. If the isotope-dilution step occurs before possible species transformation, subsequent loss of parts of the isotope-diluted element species has no effect either on the measured isotope ratio *R* or, therefore, on the analytical result. In addition, transformation of an element species can be followed by means of the isotope-labeled spike compound. For example, there were doubts about the accuracy in monomethylmercury ($MeHg^+$) speciation when using ethylation by sodium tetraethylborate to convert $MeHg^+$ into a volatile compound for determination by CGC–ICP–MS [51]. Demuth and Heumann found that transformation of $MeHg^+$ into elemental Hg was highly dependent on chloride concentration during the ethylation process (Fig. 12); this particularly affected the accuracy of $MeHg^+$ results obtained from ocean water samples or those from HCl extraction procedures [43, 52].

It was found that propylation, in contrast with ethylation, did not convert $MeHg^+$ into Hg, as depicted in Fig. 13 for analysis of this mercury species in a river water sample [52]. However, $MeHg^+$ results obtained by use of CGC–

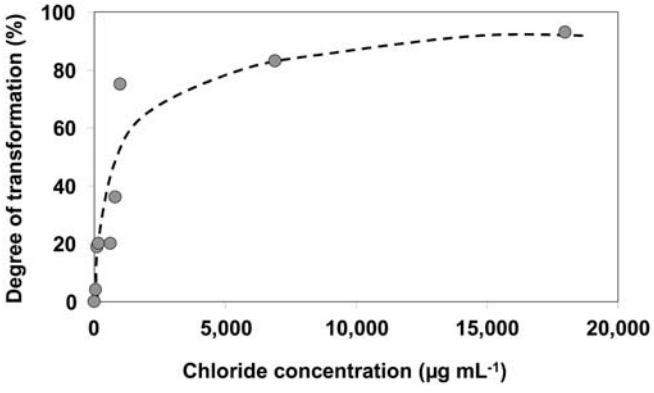

Fig. 12 Dependence on the concentration of chloride in different aquatic systems of the degree of transformation of $MeHg^+$ into elemental mercury during ethylation with $NaBEt_4$ (for reference, 18,000 µg mL^{-1} is the average concentration of chloride in ocean water) [43]

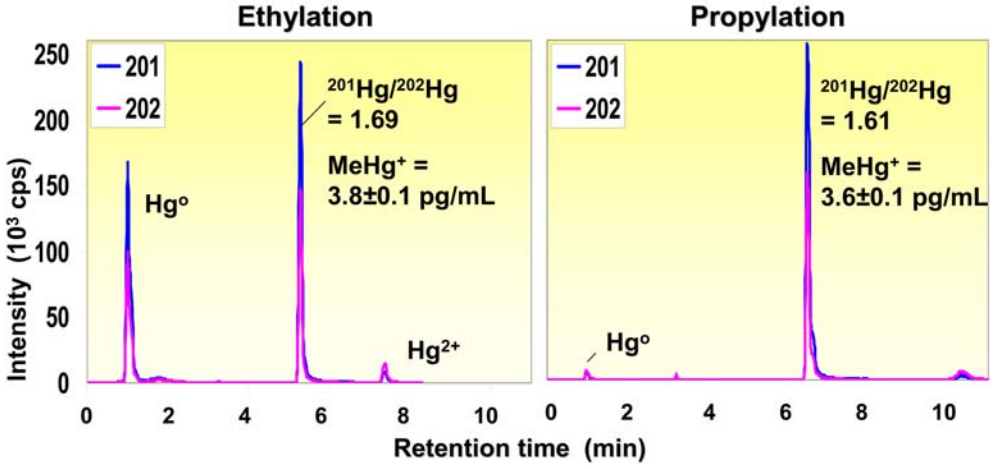

Fig. 13 Comparison of $MeHg^+$ results from analysis of a river water sample by CGC–ICP–IDMS after derivatization with $NaBEt_4$ and $NaBPr_4$ [52]

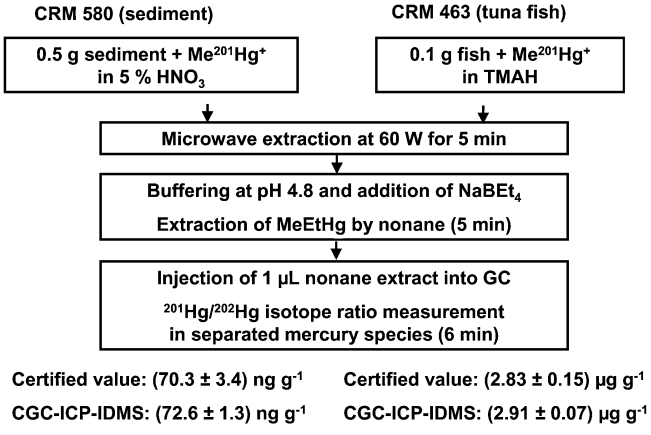

Fig. 14 Method for rapid determination of $MeHg^+$ in environmental and biological samples by species-specific CGC–ICP–IDMS [52]

ICP–IDMS were identical within the given standard deviations for both derivatization processes (3.8±0.1 pg mL^{-1} and 3.6±0.1 pg mL^{-1}, respectively, for three parallel analyses each). This means that the described CGC–ICP–IDMS method is independent of possible species transformations, because the isotope-dilution step occurred before species conversion during derivatization.

A relatively easy to handle and time-efficient CGC–ICP–IDMS method has been developed for determination of $MeHg^+$ in environmental samples such as sediments and biological materials (Fig. 14) [52]. When a sediment and a tuna fish reference material were analyzed by this method the results agreed well with the certified values. Because total mixing of the sample with the spike mercury species occurs during extraction, the isotope-dilution step is also complete after only 5 min of this sample-treatment step, so the long equilibration times (up to 14 h) recommended in the literature [53], are not needed. Extraction of monomethylmercury from environmental samples has also been performed by HCl extraction [54]; this leads to a high risk of monomethylmercury transformation by subsequent derivatization with sodium tetraethylborate. It was, however, found that in-situ extraction of the MeEtHg compound with nonane reduces this species transformation; this indicates that a kinetic effect obviously controls conversion of $MeHg^+$ into elemental Hg by chloride ions in the aqueous phase [52].

A modern trend in the validation of element species analyses is the use of a variety of species of a single element labeled with different isotopes. Such a multi-isotope labeling experiment enables identification of reciprocal conversions of one species into another and simultaneous detection of transformations of different element species. Examples have been published of the correction of species transformation in the analysis of Cr(VI) [55] and, recently, of evaluation of extraction techniques for determination of butyltin compounds in sediments [56]. Although this multi-isotope spike technique is extremely elegant and can be used to identify several species transformations simultaneously, the complex system of mathematical equations needed for evaluation and the lack of availability of isotope-labeled spikes will not enable routine application of this method in analytical quality assurance in the near future.

Summary

Although the question whether or not ICP–IDMS is suitable for routine analysis is answered in the points listed below, a general answer can never be presented, because the specific demands of each routine analysis must always be taken into account.

1. It must first be stated that, with regard to the special features of the isotope dilution technique, ICP–IDMS should, preferably, be taken into consideration if (one of) the most important aspects of a routine analysis is the accuracy of results.
2. Sample preparation for ICP–IDMS is much simpler than for TI–IDMS and is often also simpler than for other atom-spectrometric methods. Sample treatment for ICP–IDMS is not, however, automatically an absolutely simple procedure without possible sources of error.

3. One of the most important advantages of IDMS is that loss of the analyte has no effect on the analytical result after the isotope dilution step has occurred. Because total or reproducible recovery is often a great problem in sample pretreatment procedures for trace analysis, this special advantage alone might quite often qualify ICP–IDMS as the *best* routine method.
4. The isotope-dilution step is easy to perform for all dissolved samples. This, in principle, qualifies ICP–IDMS as a routine method for solutions for which matrix-independent results are requested. Compared with the standard addition method, which also guarantees results not affected by the matrix, ICP–IDMS is a one-point calibration method, whereas for the standard addition technique, at least, two aliquots of the sample (one with and another one without a standard addition) must be measured.
5. ICP–IDMS is suitable for routine analysis of a small number of samples only if the corresponding isotope-enriched spike solutions are available; preparation and characterization of spike solutions is, on the other hand, justified only if a sufficiently large number of similar analyses must be performed. In trace analysis the cost of the isotope-enriched spike can be neglected.
6. The robustness of ICP–IDMS analysis depends primarily on the quality of the instrument. When quadrupole instruments or sector-field instruments are used in the low-resolution mode results are usually more robust, because of the stable measurement conditions, than those from sector-field measurements in high-resolution mode, e.g. as a result of possible problems in mass calibration. The robustness of IDMS determinations suffers from the specific problem of spectrometric interference in ICP–MS measurements. Because an isotope ratio is determined in IDMS analysis, two isotopes must always be free from interference; this might be a special problem for some routine analyses in which only incomplete separation of interfering elements is possible.
7. Direct solid sampling is possible for powder samples by application of LA–ICP–IDMS; this especially qualifies this method for accurate routine analysis if reference materials are not available for matrix-matched calibration.
8. In principle, IDMS is only applicable for polyisotopic elements and for those for which a synthetic long-lived radioactive nuclide is available as spike isotope. This precondition is fulfilled for most elements. The multi-element capability of ICP–MS is, however, usually reduced by applying the isotope dilution technique. ICP–IDMS is therefore a better choice for oligo- or single-element analysis than for multi-element routine analysis.
9. Hyphenated techniques such as CGC–ICP–MS and HPLC–ICP–MS are often used for characterization of element species. These coupling systems can, in general, also be used in conjunction with the isotope-dilution technique, even lack of commercially available isotope-labeled spike compounds limits their use as routine methods. The increasing importance of analytical quality assurance might, however, also increase the routine application of these hyphenated IDMS techniques to quality control of element speciation. Species-specific IDMS is a useful method for clearly identifying possible species transformations during sample-pretreatment steps.

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