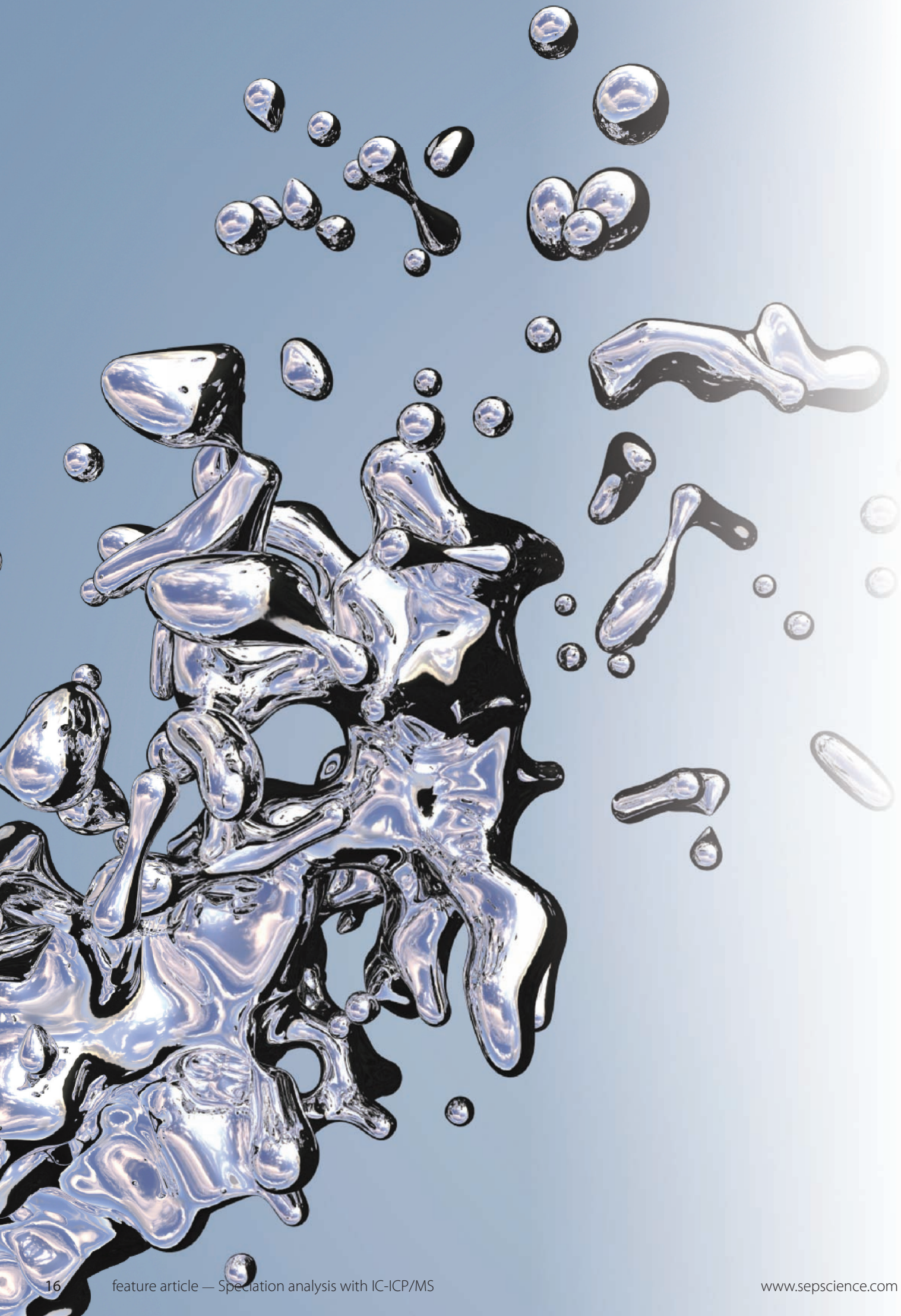

Mercury and arsenic speciation analysis by IC-ICP/MS

Laura Reyes,[1] H.M. Skip Kingston,[1] Andrea Wille,[2] Jürgen Knöll[3] and Alfred Steinbach[2]

[1]Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, Pennsylvania, USA
[2]Metrohm International Headquarters, Herisau, Switzerland
[3]Philipps Universität Marburg, Germany

By means of IC-ICP/MS, different valence states of arsenic and mercury in the form of inorganic and organic species can be sensitively and unambiguously identified in one single run. Owing to the absence of interconversions during sample preparation, the determination of arsenic species in biological and environmental matrices is straightforward and can be performed by traditional speciation analysis down to the sub-ppb level. In contrast, species transformations of mercury during sample preparation require the use of specific isotope dilution mass spectrometry (SIDMS). EPA Method 6800 was applied to evaluate and efficiently compensate for potential errors during measurement.

Introduction

Ion chromatography (IC) with conductivity detection has been successfully used to determine anionic and cationic substances as well as polar compounds such as amines or organic acids. However, in environmental samples a higher sensitivity and selectivity are required to test for potentially toxic substances with low maximum contaminant levels (MCL).

The coupling of IC with multi-dimensional detectors such as electrospray ionization mass spectrometers (ESI-MS) or inductively coupled plasma mass spectrometers (ICP/MS) solves even complex separation problems, simultaneously achieving high sensitivity and selectivity. Additionally, these hyphenated techniques allow unambiguous peak identification and are less prone to matrix interferences than conductivity detection.

Especially in view of the *zero tolerance* policy concerning chromium, arsenic and selenium compounds in drinking water and mercury in food samples, ICP/MS detectors have gained increasing importance. IC-ICP/MS can distinguish between different oxidation states and chemical forms of a given element. This approach is called speciation analysis. From the toxicological point of view, individual concentrations of element-containing species are far more significant than total element concentrations as different valence states of an element often have completely different properties. For example, while chromium(III) is an essential trace element for mammals as it is involved in glucose metabolization, all forms of hexavalent chromium are regarded as highly toxic and carcinogenic.

Arsenic

Arsenic is ubiquitously found in a high number of minerals and its use as a weed killer and rat poison illustrates its high toxicity. Inorganic arsenical derivatives are considered to be carcinogenic and possibly teratogenic. Therefore, the EPA proposes a maximum allowable drinking water concentration of 10 ppb. In environmental and biological samples, more than 20 arsenic species have been identified. Depending on their binding characteristics, they have different toxicities and chemical behaviours. Based on structural data, IC-ICP/MS allows separation and unambiguous identification of different arsenic species in inorganic and organic forms.

Mercury

Mercury is found in several forms, particularly as elemental (Hg^0),

inorganic (Hg^{2+}), or alkylated mercury (CH_3Hg^+). Of the most common mercury species found in the environment, methylmercury is considered the most toxic species. It is classified as a neurotoxin that rapidly bioaccumulates and can cause major health problems or death, even in small quantities. According to the US Food and Drug Administration (FDA), the major exposure pathway to methylmercury in humans and wildlife is through consumption of contaminated fish. The US Environmental Protection Agency (EPA) stipulates a reference dose for methylmercury (R_f) of 0.1 µg/kg of body weight per day, while the World Health Organization (WHO) has set a tolerable dose of 1.6 µg/kg of body weight per week [1].

However, mercury species are prone to interconversion. Mercury shows a pronounced transformation from inorganic mercury (Hg^{2+}) to the biologically active and highly toxic methylmercury (methylation) and vice versa (demethylation). Similarly, the extraction techniques used for separation and preconcentration tend to alter the original distribution of mercury species, which affects the legal defensibility of the data.

Speciated isotope dilution mass spectrometry (SIDMS) has been developed to correct for species conversions. According to US EPA Method 6800 [2] each species is labelled with a different isotope-enriched spike in the corresponding form. By measuring the isotope ratio of both the unspiked and spiked sample and knowing the isotopic ratio of the addition, interconversions between the species become traceable and can be corrected.

This article deals with the determination of organic and inorganic arsenic and mercury compounds by means of IC-ICP/MS. Arsenic species (monoisotopic) are not prone to interconversion and are thus determined by traditional speciation analysis. Several established extraction techniques used for mercury speciation in biological samples are evaluated by applying both internal SIDMS and external calibration.

Table 1

Instrumental operation conditions for the determination of the arsenic species via IC-ICP/MS	
Column	Metrosep A Supp 15 – 150
Injection volume	10 µL
Column temperature	Ambient
Eluent	8 mmol/L NH_4NO_3, pH = 8.3
Elution	Isocratic
Flow rate	0.7 mL/min
ICP/MS	Without reaction or collision mode
m/z	75

Table 2

Instrumental operation conditions for the determination of mercury species via SIDMS IC–ICP/MS.	
RF power	1475 W
Plasma gas flow	Ar, 115 L/min
Auxiliary gas flow	Ar, 1 L/min
Nebulizer	Quartz, concentric
Spray chamber	Quartz
Sample and skimmer cones	Ni, 1.1 and 0.8 mm, respectively
Measurement parameters of ICP/MS	
Monitoring isotopes	^{199}Hg, ^{200}Hg, ^{201}Hg and ^{202}Hg
Acquisition mode	Time-resolved analysis
Integration time per mass	0.20 s
Replicates	1
Total analysis time	300 s
Separation conditions	
Column	DVB-C18 column, 150 × 4.6 mm, 2 µm
Injection volume	100 µL
Column temperature	Ambient
Eluent	50 mmol/L pyridine, 5% (v/v) methanol, pH 3, 0.5% (w/v) cysteine,
Elution	Isocratic
Flow rate	1 mL/min

Experimental

External calibration: Separation of mercury species was automated using the 858 Professional Sample Processor and the 850 Professional IC (both Metrohm AG, Herisau, Switzerland) coupled to an ICP/MS model HP 4500 (Agilent Technologies, Palo Alto, California, USA and Yokogawa Analytical System Inc., Tokyo, Japan). For the determination of arsenic species, the IC system was coupled to an Agilent 7500ce ICP/MS. The conditions used during the study are detailed in Tables 1 and 2. Each sample was analysed three times. Quantitation was based on peak areas by external calibration using the arsenic isotope *m/z* 75 and the most abundant mercury isotope *m/z* 202. Quantitation using the mercury isotopes *m/z* 199, 200 and 201 yielded similar results. For the determination of total mercury concentrations, the digested and extracted solutions were analysed by ICP/MS.

SIDMS: To correct for mutual interconversion, Hg^{2+} and CH_3Hg^+ compounds were quantified by EPA 6800 protocol specifications, spiking the sample before the extraction with $^{199}Hg^{2+}$ and $CH_3{}^{200}Hg^+$ and application of SIDMS equations [2]. The isotopic species reagents and calculation software in a SPC-M Mercury Speciation Kit was provided by Applied Isotope Technologies (AIT, Sunnyvale, California, USA). This double-spike approach allowed tracking of any artifact stemming from methylation/demethylation reactions that might have occurred during the sample preparation and/or analysis procedure.

Extraction methods: The extraction methods to be evaluated were based on literature-referenced methods such as alkaline extraction with potassium hydroxide (KOH) or tetramethyl ammonium hydroxide (TMAH) solution; acid leaching with hydrochloric acid (HCl), nitric acid (HNO_3) or glacial acetic acid (CH_3COOH); extraction with L-cysteine hydrochloride and enzymatic digestion with protease XIV. The methods are summarized in Table 3 [3]. The reference material used for the comparison of sample preparation methods was Tuna Fish Tissue Certified Reference Material (ERM-CE464) supplied by IRMM (Geel, Belgium), which is certified for total mercury and methylmercury content.

Reagents, standard solutions and eluents: All reagents used in this

Figure 1

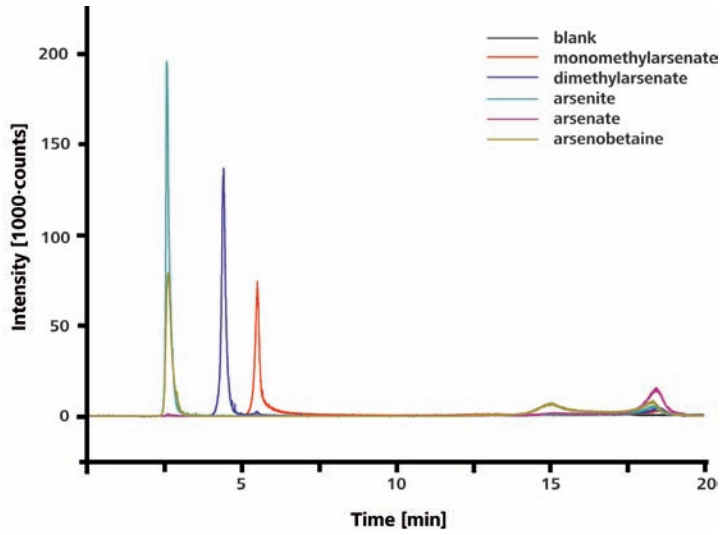

Figure 1: Separation and detection of arsenite, dimethylarsenate, monomethylarsenate and arsenate.

Figure 2

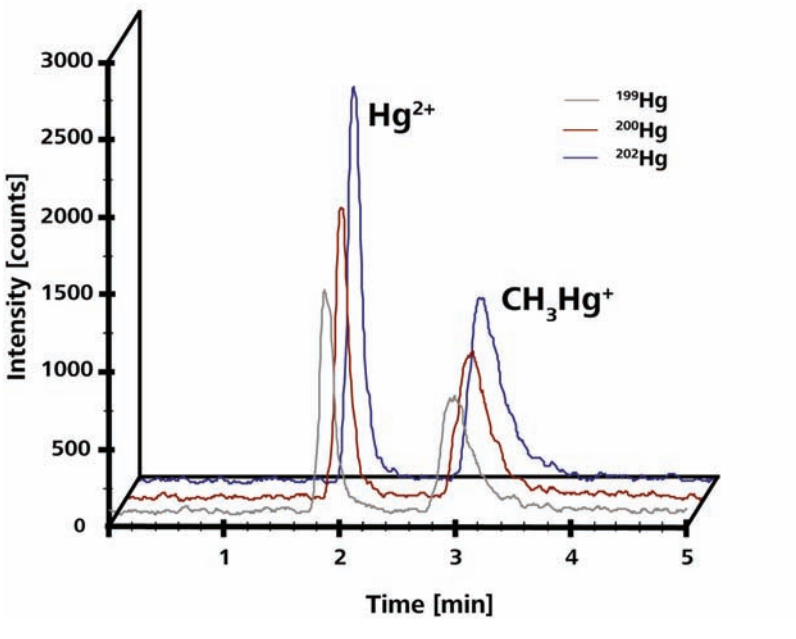

Figure 2: IC-ICP/MS chromatogram for 10 µg/L Hg^{2+} and CH_3Hg^+. Chromatograms obtained at different masses were shifted for clarity. Instrumental operation conditions are given in Table 2.

work were of the highest purity grade (puriss. p.a.). Analytical reagent grade HNO_3, HCl, TMAH, potassium hydroxide, optima-grade methanol and HPLC-grade glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, Pennsylvania, USA). Reagent grade L-cysteine, L-cysteine hydrochloride hydrate, ammonium phosphate dibasic, pyridine and protease XIV were obtained from Sigma Aldrich (St. Louis, Missouri, USA). The arsenic standard solutions were purchased from Fluka (Sigma Aldrich, Buchs, Switzerland). All solutions were prepared with deionized water with a specific resistance higher than 18 MΩ•cm.

Results and Discussion

Arsenic: IC-ICP/MS allows the separation and unambiguous identification of different arsenic species in inorganic and organic forms. Figure 1 displays the peaks of a 10 µg/L standard solution containing monomethylarsenate, dimethylarsenate, arsenite, arsenate and arsenobetaine (ASB). Under the given conditions (Table 1), ASB is not separated from the trivalent arsenic species. However, ASB interference can be overcome by changing the chromatographic parameters.

Mercury: Figure 2 shows the chromatogram of the separation of the divalent mercury ion from methylmercury [3] on a polymer-based C18 reversed-phase column. Separation was achieved in less than five minutes and the retention times were 1.87 ± 0.02 and 2.98 ± 0.03 minutes. Linear calibration curves for Hg^{2+} and CH_3Hg^+ were obtained in the range from 1–20 µg/L. Detection limits were 0.46 ± 0.02 and 0.78 ± 0.08 µg/L for Hg^{2+} and CH_3Hg^+,

Table 3

Concentrations of mercury species (in mg/kg Hg) determined in Tuna Fish Tissue Reference Material (ERM-CE464) by external calibration and by EPA Method 6800 (SIDMS). The values are means of ± 95% confidence limits (n = 3). The percentage recoveries of total Hg and CH_3Hg^+ are indicated in parentheses.

Extraction procedure				External calibration			EPA Method 6800 (SIDMS)		
				Hg^{2+}	CH_3Hg^+	Sum of species	Hg^{2+}	CH_3Hg^+	Sum of species
				0.12[b]	5.12 ± 0.16[a]	5.24 ± 0.10[a]	0.12[b]	5.12 ± 0.16[a]	5.24 ± 0.10[a]
	Extraction technique, extraction reagent	**Temp. [°C]**	**Time [min]**	**mg/kg Hg**			**mg/kg Hg**		
A	Sonication/water bath, 25% (w/v) KOH in methanol	70	180	0.06 ± 0.02[b]	5.05 ± 0.13 (99 ± 3)	5.11 ± 0.13 (98 ± 3)	0.07 ± 0.02[b]	5.22 ± 0.31 (102 ± 6)	5.29 ± 0.31 (101± 6)
B	Sonication/water bath, 25% (w/v) TMAH in methanol	70	180	0.12 ± 0.03[b]	5.05 ± 0.18 (99 ± 4)	5.17 ± 0.18 (99 ± 3)	0.07 ± 0.03[b]	5.20 ± 0.18 (102 ± 4)	5.27 ±0.18 (101 ± 6)
C	Microwave, 5% (w/v) TMAH in methanol	180	20	0.18 ± 0.05[b]	4.88 ± 0.17 (95 ± 3)	5.06 ± 0.18 (97 ± 3)	0.30 ± 0.07[b]	5.18 ± 0.13 (101 ± 3)	5.48 ± 0.15 (105 ± 3)
D	Sonication bath, 5 mol/L HCl	25	5	0.07 ± 0.02[b]	4.29 ± 0.39 (84 ± 8)	4.36 ± 0.39 (83 ± 7)	0.13 ± 0.05[b]	5.11 ± 0.38 (100 ± 7	5.24 ± 0.38 (100 ± 7)
E	Microwave, 4 mol/L HNO_3 (EPA 3200)	180	20	0.06 ± 0.04[b]	3.94 ± 0.12 (77 ± 2)	4.00 ± 0.13 (76 ± 2)	0.11 ± 0.07[b]	5.60 ± 0.33 (109 ± 6)	5.71 ± 0.34 (109 ± 6)
F	Microwave, glacial CH_3COOH	165	10	0.35 ± 0.08[b]	3.29 ± 0.14 (64 ± 3)	3.64 ± 0.16 (69 ± 3)	0.27 ± 0.12[b]	5.12 ± 0.19 (100 ± 4)	5.39 ± 0.22 (103 ± 4)
G	Water bath, 1% L-cysteine hydrochloride	60	120	0.45 ± 0.10[b]	4.87 ± 0.20 (95 ± 4)	5.32 ± 0.22 (102 ± 4)	1.05 ± 0.14[b]	5.08 ± 0.25 (99 ± 5)	6.13 v 0.29 (117 ± 5)
H	Hybdridization oven, Enzymatic digestion with protease XIV	37	120	0.16 ± 0.07[b]	4.42 ± 0.14 (86 ± 3)	4.58 ± 0.16 (87 ± 3)	0.07 ± 0.02[b]	5.09 ± 0.24 (99 ± 5)	5.29 ± 0.31 (100 ± 5)

[a]Certified methyl mercury and total mercury content in Tuna Fish Tissue Certified Reference Material (ERM-CE464) supplied by IRMM (Geel, Belgium) [b]inorganic mercury concentration was calculated as the difference between certified total mercury and methylmercury concentrations.

respectively.

Table 3 shows the accuracy of the extraction procedures tested by both external calibration analysis and SIDMS using the Tuna Fish Tissue Certified Reference Material (ERM-CE464). For seven of the eight extraction procedures evaluated, the CH_3Hg^+ values calculated by using SIDMS were in good agreement with the certified reference value. Only for extraction procedure E, a too high methylmercury content was found. Hence, transformations and losses of Hg^{2+} and CH_3Hg^+ can be directly linked to pretreatment steps.

Results obtained by external calibration: Based upon the data shown in Tables 3 and 4, the concentrations found for methylmercury in the alkaline extraction procedures using either the ultrasonic-assisted system (procedures A and B) or the microwave device (procedure C) were in good agreement with the certified values at the 95% confidence level. These procedures yielded similar methylation and demethylation rates (~ 6%). Although procedure G was suitable for CH_3Hg^+ determination, inorganic mercury contamination – 0.45 compared with 0.12 mg/kg Hg^{2+} – in the extracting reagent was observed.

Extraction procedures D, E, F and H suffered from too low CH_3Hg^+ recoveries (64–86%). Despite frequent use of acid leaching for the extraction of mercury species from tuna fish samples, the lowest concentration of methylmercury on the ERM-CE464 and the highest mercury species transformation occurred when microwave-assisted extraction with glacial acetic acid (procedure F) and extraction with 4 mol/L HNO_3 (procedure E) was used. Owing to the relatively low ratio of Hg^{2+} to CH_3Hg^+, the high methylation rate of 18% in procedure E did not cause a significant error regarding CH_3Hg^+ concentration. In contrast, a pronounced demethylation rate has a considerable effect if, as in the case of procedure F, high CH_3Hg^+ to Hg^{2+} ratios are prevailing. Apparently, the elevated Hg^{2+} concentration of 0.27 mg/kg stems from the pronounced demethylation rate.

Table 4

Extraction procedure	Mean degree of transformation [%]	
	Methylation	**Demethylation**
A	5 ± 3	6 ± 1
B	6 ± 2	4 ± 1
C	3 ± 2	6 ± 2
D	5 ± 3	3 ± 1
E	18 ± 4	0.8 ± 0.6
F	4 ± 2	27 ± 5
G	4 ± 2	4 ± 1
H	4 ± 2	1.4 ± 0.5

The SIDMS protocol is an invaluable tool for overcoming nonquantitative recoveries and species transformations observed during the evaluation of extraction procedures.

Conclusions

Ion chromatography coupled to an ICP/MS is a powerful tool to determine different organic and inorganic species unambiguously in one single run. In the absence of interconversions, traditional speciation analysis provides accurate results down to the sub-ppb level. Species interconversions, however, require correction. SIDMS according to EPA Method 6800 is capable of tracing any interconversions that occur after spiking.

Because of the unique features and undisputed benefits of EPA Method 6800, it is expected that utilization of SIDMS will increase and that this valuable tool for optimizing and validating sample preparation procedures for trace-metal speciation, involving extraction, separation and detection, will gain much wider acceptance by analytical chemists.

Acknowledgments

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