by Laura Reyes, H.M. Skip Kingston, Andrea Wille, Jürgen Knöll, and Alfred Steinbach

Mercury and Arsenic Speciation Analysis by IC-ICP-MS

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

The coupling of IC with multidimensional 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 light 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 a toxicological point of view, individual concentrations of element-containing species are far more significant than total element concentrations because different valence states of an element often have completely different properties. For example, while chromium(III) is an essential trace element for mammals since it is involved in glucose metabolization, all forms of hexavalent chromium are regarded as highly toxic and carcinogenic.

Arsenic

Arsenic is found ubiquitously 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 U.S. 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 properties. 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+}), and 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 U.S. FDA, the major exposure pathway to methylmercury in humans and wildlife is through consumption of contaminated fish. The U.S. 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]

*Figure 1 IC-ICP-MS system with 858 Professional Sample Processor, 850 Professional IC Anion—MCS (**Metrohm** CO_2 suppressor), and 7500 ICP-MS.*

Table 1 Instrumental operation conditions for the determination of the arsenic species via IC-ICP-MS

IC-ICP-MS conditions	
Column	Metrosep A Supp 15–150/4.0 (**Metrohm AG**)
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

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 U.S. EPA Method 6800,[2] each species is labeled 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 discusses 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.

Experimental

External calibration

Separation of mercury species was automated using the 858 Professional Sample Processor and the 850 Professional IC (both from **Metrohm AG**, Herisau, Switzerland) coupled to an ICP-MS model HP 4500 (**Agilent Technologies** [Palo Alto, CA] and **Yokogawa Analytical System Inc.** [Tokyo, Japan]). For the determination of the arsenic species, the IC system was coupled to an Agilent 7500 ICP-MS. The coupling of the IC system to ICP-MS is shown in *Figure 1*. The conditions used during the study are detailed in *Tables 1* and *2*. Each sample was analyzed 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 analyzed by ICP-MS.

SIDMS

To correct for mutual interconversion, Hg^{2+} and CH_3Hg^+ compounds were quantified by U.S. 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 were provided by **Applied Isotope Technologies (AIT**, Sunnyvale, CA). 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.

Table 2 Instrumental operation conditions for the determination of mercury species via SIDMS IC–ICP-MS

Operation conditions of ICP-MS	
RF power	1475 W
Plasma gas flow	Ar, 115 L min^{-1}
Auxiliary gas flow	Ar, 1 L min^{-1}
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 sec
Replicates	1
Total analysis time	300 sec
Separation conditions of ICP-MS	
Column	DVB-C18 column (**Metrohm, USA**, Riverview, FL), 150 × 4.6 mm, 2 µm
Injection volume	100 µL
Column temperature	Ambient
Eluent	50 mmol/L pyridine, 0.5% (w/v) cysteine, 5% (v/v) methanol, pH 3
Elution	Isocratic
Flow rate	1 mL/min

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); or 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 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, PA). Reagent-grade L-cysteine, L-cysteine hydrochloride hydrate, ammonium phosphate dibasic, pyridine, and protease XIV were obtained from **Sigma-Aldrich** (St. Louis, MO). 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 2* displays the peaks of a 10-µg/L standard solution containing monomethylarsenate, dimethylarsenate, arsenite, arsenate, and arsenobetaine (ASB).

Table 3 Concentrations of mercury species (in mg/kg Hg) determined in Tuna Fish Tissue Certified Reference Material (ERM-CE464) by external calibration and by U.S. EPA Method 6800 (SIDMS)*

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

*The values are means of ±95% confidence limits (n = 3). The percentage recoveries of total Hg and CH_3Hg^+ are indicated in parentheses.
[a]Inorganic mercury concentration was calculated as the difference between certified total mercury and methylmercury concentrations.
[b]Certified methylmercury and total mercury content in ERM-CE464 supplied by IRMM (Geel, Belgium).

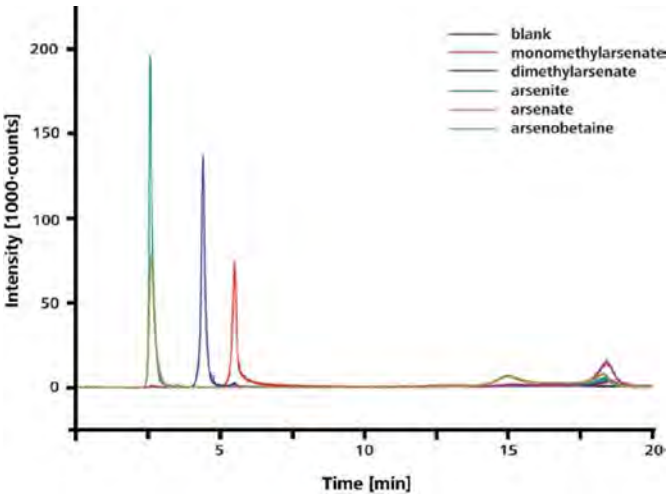

Figure 2 Separation and detection of arsenite, dimethylarsenate, monomethylarsenate, and arsenate.

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 3 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 5 min and the retention times were 1.87 ± 0.02 and 2.98 ± 0.03 min. Linear calibration curves for Hg^{2+} and CH_3Hg^+ were obtained in the range 1–20 µg/L. Detection limits were

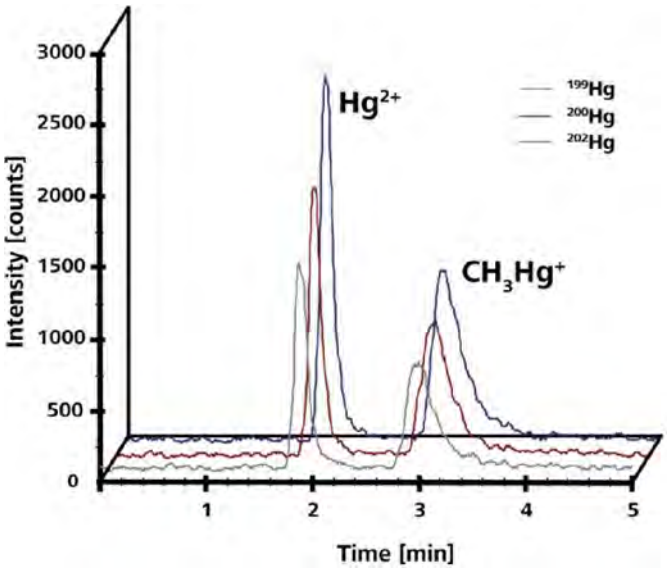

Figure 3 IC-ICP-MS chromatogram for 10 µg/L Hg^{2+} and CH_3Hg^+. Chromatograms obtained at different masses were shifted for clarity. Instrumental operation conditions are given in Table 2.

0.46 ± 0.02 and 0.78 ± 0.08 µg/L for Hg^{2+} and CH_3Hg^+, respectively.

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

Results obtained by external calibration

Based on 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 to 0.12 mg/kg Hg^{2+}—in the extracting reagent was observed.

Extraction procedures D, E, F, and H suffered from CH_3Hg^+ recoveries that were too low (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) were used. Due 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

Table 4 Estimated degree of mercury species transformation in ERM-CE464 during the eight extraction procedures evaluated by SIDMS IC-ICP-MS*

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

*Values are mean ±95% confidence level.

prevailing. Apparently, the elevated Hg^{2+} concentration of 0.27 mg/kg stems from the pronounced demethylation rate.

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

Conclusion

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 U.S. EPA Method 6800, is capable of tracing any interconversions that occur after spiking.

Because of the unique features and undisputed benefits of U.S. 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.

References

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Dr. Reyes is Associate Teacher in Chemical Sciences, University of Nuevo León, Mexico. Prof. Kingston is Professor of Analytical and Environmental Chemistry, Department of Chemistry and Biochemistry, Duquesne University, Pittsburgh, PA, U.S.A. Dr. Wille is Manager of the Competence Center Ion Chromatography, and Dr. Steinbach is a scientific writer, Marketing Department, ***Metrohm*** *International Headquarters, Oberdorfstrasse 68, CH-9101 Herisau, Switzerland; tel.: +41 71 353 86 10; fax: +41 71 353 89 01; e-mail: ast@metrohm.com. Mr. Knöll is a Ph.D. student, Faculty of Analytical Chemistry, Philipps University Marburg, Germany.*

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