APPLICATION NOTE

GC/ICP - MS

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Mercury Speciation in Biological Tissue and Sediments by GC/ICP-MS Using the NexION 300D/350D

Introduction

The chemical determination of mercury (Hg) species in the environment is gaining increasing interest both for improved understanding of their reactional pathways and also to meet regulation limits in both Europe and the U.S. Mercury species play an important role in environmental pollution because they can result from anthropogenic activities, as well as natural biomethylation processes.[1]

Inorganic mercury (Hg^{2+}) is the main form present in water and sediment samples, while methylmercury (MMHg) compounds are considered more toxic than inorganic mercury and can be accumulated in biological tissues. Fish tend to concentrate MMHg by a factor of 10^5-10^7; hence, fish consumption is the major contributor to Hg risk in humans and wildlife.[2] The European Union (EU) added Hg and its compounds in the list of priority pollutants (Decision 2455/2001/EC amending the Water Framework Directive 2000/60/EC).[3] In addition, the EU established 0.5 µg/g (wet weight) as maximum level of Hg in different foodstuffs (Commission Regulation EC-78/2005 amending regulation CE-466/2001).[3] The U.S. Food and Drug Administration (FDA) set an advisory standard of 1 µg/g wet weight in fish flesh.[2] In 2003, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a Provisional Tolerable Weekly Intake (PTWI) of 1.6 µg MMHg/kg body weight (bw) and 5 µg THg/kg bw.[4] In 2010, the PTWI for total Hg was withdrawn by the Committee and replaced by a PTWI for inorganic mercury (Hg^{2+}) of 4 µg/kg bw.[4]

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Gas chromatography coupled with inductively coupled plasma mass spectrometry (GC/ICP-MS) is the method of choice for trace analysis because of its high sensitivity, selectivity and multielemental and multiisotopic detection capabilities.[1] Mercury speciation can be performed in all environmental matrices on a routine basis using well-established extraction and derivatization techniques. The main extraction method employed is open focused microwave extraction, due to its speed and efficiency. Once extracted, the various Hg species must be converted into volatile forms for GC separation. This conversion is accomplished with derivatization techniques using alkylation reagents, such as sodium tetrapropylborate and sodium tetraethylborate. The use of derivatization in situ allows reduction in the occurrence of possible interferences during the analytical steps and detection.[5]

The focus of this work is to examine the instrumental conditions and parameters necessary for the coupling of a Clarus® GC to a NexION® ICP-MS system with a GC transfer line and its application towards the speciation of mercury in biological tissues and sediments.

Experimental

Reagents and chemicals

Mercury (II) chloride (Hg^{2+}) and methylmercury chloride (MMHg) were obtained from Sigma-Aldrich®, and stock solutions were prepared in ultrapure water and methanol (Optima grade, ThermoFisher Scientific®), respectively. Sodium tetrapropylborate ($NaBPr_4$) and sodium tetraethylborate ($NaBEt_4$) (Merseburger Spezialchemikalien, Germany) were used as derivatization agents. Buffer solution (0.1 M, pH 3.9) was prepared by dissolving sodium acetate (Sigma-Aldrich®) and glacial acetic acid (HAc) (GFS Chemicals®) in MQ water. Analytical reagent-grade isooctane (HPLC Grade, ThermoFisher Scientific®) and high-purity ammonium hydroxide (NH_4OH), nitric acid (HNO_3), hydrochloric acid (HCl) and tetramethylammonium hydroxide (TMAH; 25%) were obtained from GFS Chemicals®. The reference materials BCR 710 Oyster Tissue and CE-464 Tuna Fish were obtained from the Institute for Reference Materials and Measurements (IRMM) Geel, Belgium, and the IAEA 405 Estuarine sediment was obtained from the International Atomic Energy Agency (IAEA) Vienna, Austria.

Instrumentation

A Clarus 580 GC was coupled to a NexION 300D ICP-MS (both PerkinElmer Inc., Shelton, CT, USA) via a GC transfer line (Part Nos. N0777440 for 110V; N0777361 for 220V), which works under dry plasma conditions, meaning that the spray chamber is removed, and the transfer line is inserted into the central channel of quartz torch and injector.[6]

The GC transfer line, equipped with an inner deactivated capillary column, was coupled to the base of the injector support. Optimization of the operational parameters, such as the distance of the inner deactivated capillary column from the injector tip, the argon (Ar) makeup gas flow rate, and the oxygen flow rate are evaluated and presented in the Results and Discussion section. The extraction of the mercury species from the solid matrices was carried out using an SP-D Discover open focus microwave digestion system (CEM Corporation®, Matthews, NC, U.S.). Operating conditions and instrumentation parameters are summarized in Table 1. Data collection and analysis were accomplished with NexION and Chromera® speciation software.

Figure 1. Clarus 580 GC (right) connected to NexION 300D ICP-MS (left) via GC transfer line.

Table 1. **GC/ICP-MS operating conditions.**

GC Parameters (Clarus)	**Conditions**
Column:	Elite-5MS (5% Diphenyl-dimethylpolysiloxane) (30 m, i.d. 0.25 mm, d.f. 0.25 µm)
Injection port:	Splitless
Injection port temperature:	250 °C
Injection volume:	1.0 µL
He carrier gas flow (mL/min):	2.0
Transfer line temperature:	300 °C
Transfer line capillary:	Elite – Siltek deactivated fused-silica (i.d. 0.25 mm)
Oven program:	50 °C → ramp 10 °C/min → 100 °C → ramp 45 °C/min → 290 °C (3 min)
ICP-MS Parameters (NexION)	**Conditions**
RF power (W):	1600 W
Nebulizer flow rate (L/min):	0.98
Auxiliary gas flow rate (L/min):	1.2
Plasma gas flow rate (L/min):	15
Oxygen gas flow rate (L/min):	0.025
Injector diameter:	1.2 mm i.d.
Isotope/dwell times:	Hg: 199, 201, 202; N: 15 (30 ms)

Sample preparation

The sample preparation, microwave extraction and derivatization procedures have been presented previously in the literature.[2,3] Stock solutions of 1000 µg/mL Hg^{2+} and MMHg were prepared in H_2O and MeOH, respectively. Working standard solutions were prepared fresh daily in 1% HCl by appropriate dilution of the stock solutions and stored in the refrigerator. Calibration curves were generated by triplicate injections of the standards solutions. An acetate buffer solution (0.1 M, pH 3.9) was prepared by weighing approximately 1.0 g of sodium acetate and 2.4 mL of HAc and dissolving with H_2O to a final volume of 500 mL. Also, 1% and 2.5% $NaBPr_4$ solutions of the derivatizing agent were prepared in H_2O.

For the microwave extraction of Hg^{2+} and MMHg from the oyster tissue, tuna fish and estuarine sediment, 0.2 g, 0.1 g and 0.3 g of dry sample were added to separate extraction vials, respectively. Next, 4 mL of 25% TMAH was added to the biological tissue and 4 mL of 6N HNO_3 was added to the sediment. A magnetic stir bar was added to each vial, which was then placed in the microwave oven, operating under the conditions shown in Table 2. After extraction, the samples were centrifuged (5 minutes at 2500 rpm), transferred to clean vials, and stored in the refrigerator until analysis.

Table 2. **Optimized microwave extraction conditions.**

CEM Microwave (SP-D Discover)	Conditions
Power	35 W for sediments 75 W for biological tissue
Ramp time (min)	1.0
Temperature	70 °C
Hold time (min)	4.0

For derivatization, 5 mL of the buffer solution was used. Appropriate concentrations of mercury standards were added to make the desired calibration curves. Likewise, appropriate amounts of sample extracts were used. In this work, 20 (tuna fish), 200 (oyster tissue), and 1000 µL (estuarine sediment) of the sample extracts were added, to account for the mercury concentrations in each sample. Next, 1 mL isooctane and 250 µL of 1% $NaBPr_4$ (for standard solutions) and 500 µL of 2.5% $NaBPr_4$ (for sample extracts) were added to each vial. The vials were capped and manually shaken for five (5) minutes. The organic phase was transferred to the GC vial for analysis. Figure 2 shows a summarized flow chart of the sample preparation procedure employed throughout this work. The same experiments were also carried out with $NaBEt_4$ as the derivatization agent.

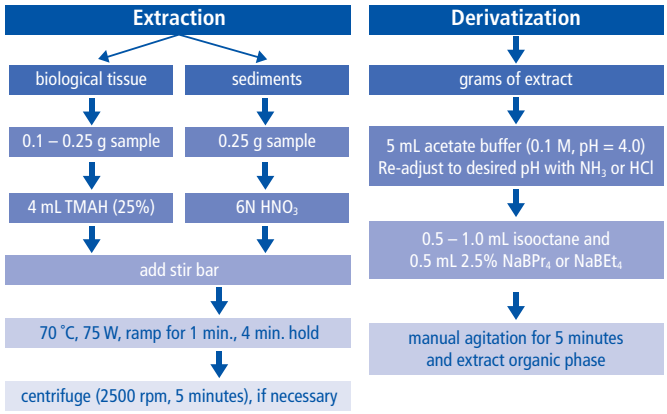

Figure 2. Flow chart of the extraction and derivatization procedure.

It is very important to remember to work under clean conditions for successful analysis at low concentrations. The containers were washed with detergent, followed by successive HNO_3 and HCl baths, and rinsed with MQ water between steps. Once the cleaning procedure was finished, the vials were dried under a laminar flow hood and stored until use. Also, remember that Hg compounds are highly toxic and must be handled with appropriate personal protection.

Results and Discussion

Optimization of the GC/ICP-MS Interface

The alignment of the torch and optimization of the operational parameters, such as the distance of the inner capillary column and the Ar makeup gas flow rate (i.e. the nebulizer gas), were evaluated using the nitrogen impurity ($^{15}N^+$) from the He carrier gas to allow the efficient introduction of the analytes under the dry plasma configuration. The addition of oxygen gas (O_2) was necessary to prevent carbon deposition on the sampler cone.[6] The optimization of the operating conditions can also be performed with the introduction of a xenon/argon (Xe/Ar) gas mixture (2.6 mL/min). Nitrogen or xenon provides a continuous signal which allows for an easy optimization of the operational parameters.[8]

The GC transfer line has been designed to be used with either the Clarus or the AutoSystem GC (PerkinElmer, Inc.) and can be coupled to the NexION, as well as ELAN® families of ICP-MS instruments (PerkinElmer, Inc.). The temperature of the GC transfer line, which is composed of a Silcosteel® tube surrounding an inert silica capillary, is directly heated by the GC oven to maintain the analytes in the gas phase, as well as for the prevention of cold spots in the transfer line.[6] The Ar makeup gas flow rate, controlled from the ICP-MS nebulizer control and necessary to achieve sufficient flow to sweep the analytes into the plasma, is heated once it enters the transfer line.

The O_2 gas flow was controlled using a mass flow controller mounted in the Clarus GC and added prior to the transfer line via a T-connection. After setup was complete and the plasma lit, the $^{15}N^+$ response was measured and torch alignment performed. Afterwards, the Ar makeup gas flow (with the O_2 flow set to 0.025 L/min) was optimized by varying the nebulizer gas between 0.9-1.1 L/min until a maximum signal for $^{15}N^+$ was obtained. Once the optimum condition for the Ar makeup gas was determined, injection of isooctane was performed to confirm that the analytes reaches the plasma. Figure 3 shows the typical response of the $^{15}N^+$ isotope, where the drop in signal indicates the solvent front.

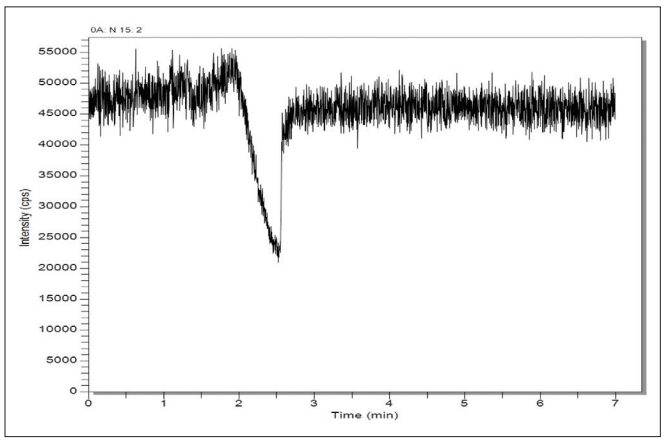

Figure 3. Typical $^{15}N^+$ isotope signal for an isooctane solvent injection.

Finally, the position of the inner deactivated capillary column in the transfer line was optimized with respect to the tip of the transfer line with injections of a 1 ng/mL solution composed of a mixture of MMHg and Hg^{2+}. The capillary position was varied by moving the column between 0-10 cm back from the tip of the Silcosteel® tube in the transfer line. The optimum response for the $^{202}Hg^+$ signal was found to be at approximately 7 cm. The zone between the end of the capillary and the plasma serves as a mixing area for the Ar and O_2 gases with the He carrier gas and analytes from the GC. Table 1 shows the optimum conditions for the GC-ICP-MS system.

GC Conditions

The parameters for the GC separation are optimized to obtain symmetrical peaks and good baseline resolution. The chromatographic conditions are chosen in a way that elution of the species is away from the zone disturbed by the solvent elution (in this case the isooctane).[1] The temperature program listed in Table 1 provided good separation for the mercury compounds in less than 8 minutes with peak widths of 2-3 s. Figure 4 shows the chromatogram obtained under the optimum conditions for a 1.0 ng/mL standard solution containing propylated MMHg (1.2 ng/mL) and Hg^{2+} (1.7 ng/mL) at mass 202. Multiple Hg isotopes (199, 201, 202) were monitored during method development, but only results for $^{202}Hg^+$ are presented for simplicity. The peaks at 4.6 and 7.0 minutes corresponds to the propylated MMHg and Hg^{2+}, respectively. In addition, the Hg peak at 4.8 min is probably due to $NaBEt_4$ impurities in the $NaBPr_4$ reagent employed during the derivatization procedure. Further work needs to be performed to corroborate the identity of the peak.

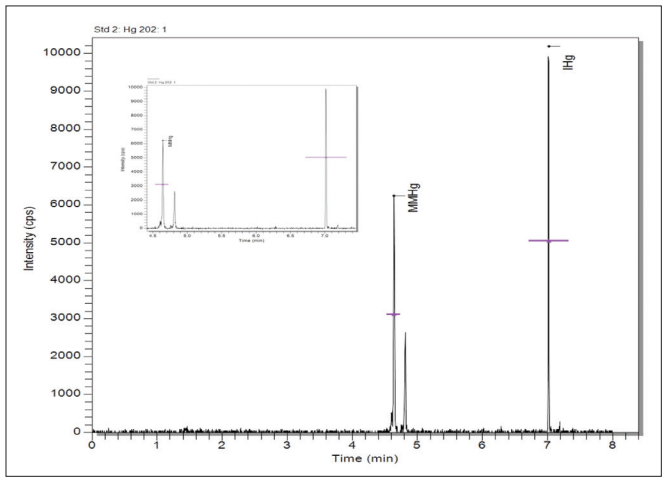

Figure 4. GC/ICP-MS chromatogram for $^{202}Hg^+$ for 1 ng/mL standard solution of MMHg (1.2 ng/mL) and Hg^{2+} (1.7 ng/mL).

Analytical Performance

Once the optimization of the operational parameters for the GC transfer line was achieved, the analytical response characteristics were determined for MMHg and Hg^{2+} using standard solutions. The calibration curves were generated for the $^{202}Hg^+$ peak areas for MMHg and Hg^{2+} through triplicate 1 µL injections across a concentration range from 0 (i.e. analytical blank) to 2 ng/mL. Good linearity and satisfactory coefficients of correlation (R^2 values) were observed for the $^{202}Hg^+$ response functions (Figure 5). The limits of detections (LODs = $3\sigma_{blank}/n$, where "n" is the number of readings) were determined from each calibration response function, and absolute LODs of 4 and 5 fg were obtained for MMHg and Hg^{2+} as $^{202}Hg^+$, respectively.

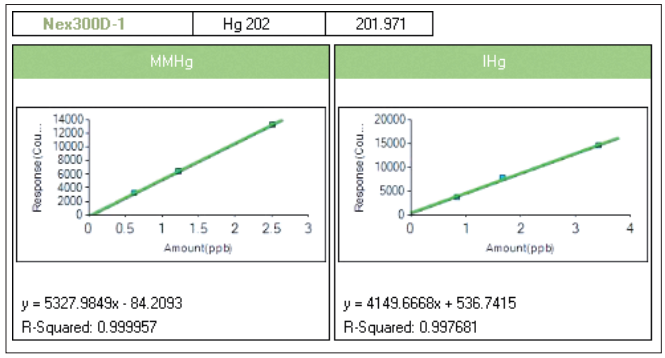

Figure 5. Calibration curves for $^{202}Hg^+$.

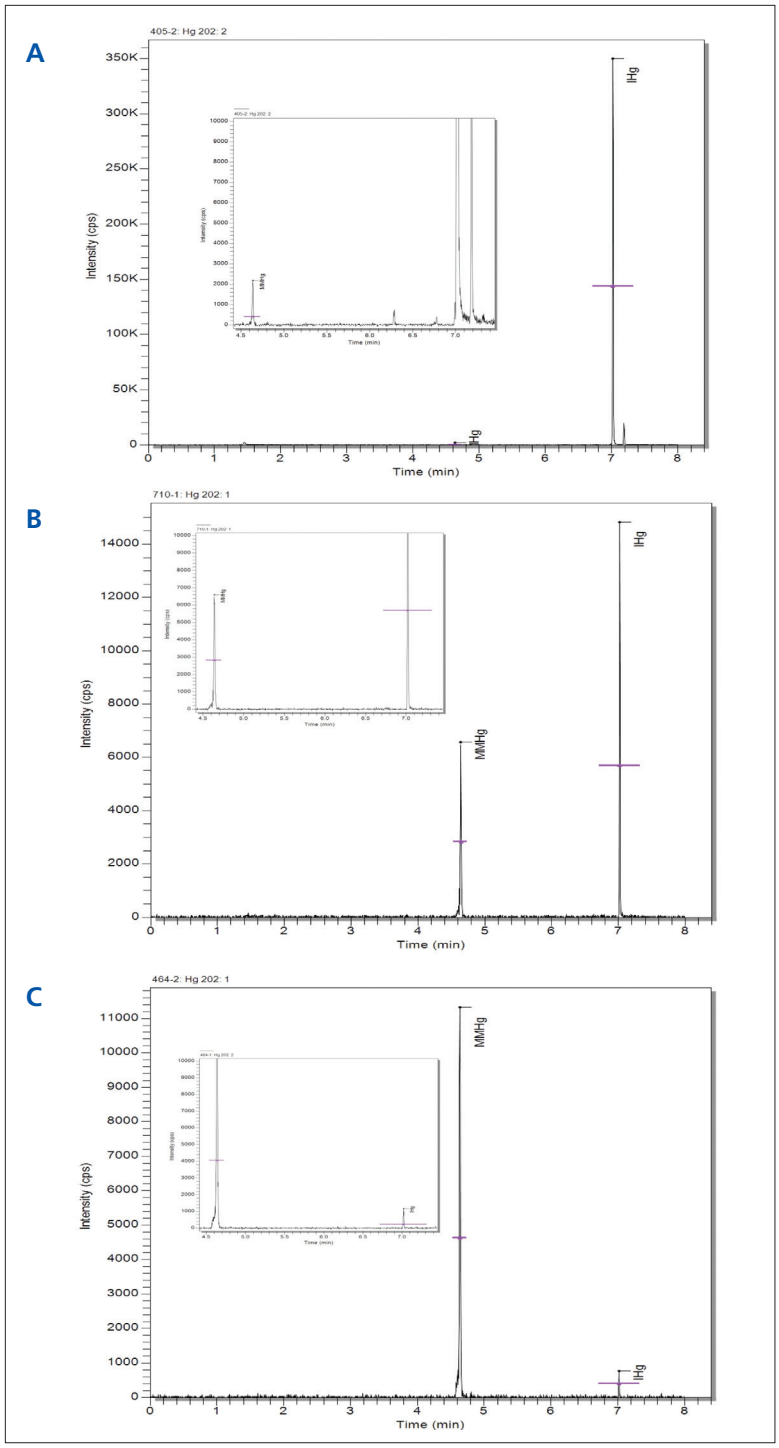

Figure 6. GC/ICP-MS chromatogram for $^{202}Hg^{+}$ for: (A) BCR 710-oyster tissue; (B) IAEA 405-estuarine sediment; (C) BCR 464-tuna fish extracts.

Table 3. Concentration results for MMHg (expressed ng/g as ^{202}Hg) obtained in the analysis of various reference materials (n=3).

Reference Materials	Certified Values	Experimental Values	%RSD	Recovery (%)
BCR 464 (tuna fish)	5500 ±170	4505 ±98	2.2	82
BCR (oyster tissue)	107 ±17	104 ±7	6.7	97
IAEA 405 (estuarine sediment)	5.49 ±0.53	5.41 ±0.34	6.3	99

Application to Reference Materials

The GC chromatographic separation and ICP-MS detection conditions for mercury speciation are presented in Table 1. It is important to remember that the GC conditions are chosen in such a way that elution of the species is away from the zone disturbed by solvent elution (i.e. after 2.5 minutes, as shown in Figure 3 for isooctane).[1] Figures 6A-C show the chromatographic separation for the propylated Hg species ($^{202}Hg^{+}$ isotope) in oyster tissue, estuarine sediment and tuna fish reference materials, respectively. Quantification results by external calibration were obtained for MMHg in the biological tissues and sediment reference materials, with percent recoveries of greater than 82% (Table 3). The low recovery value for tuna fish (with high mercury levels) is most likely the result of incomplete extraction of all the MMHg. The extraction procedure used was optimized for low MMHg concentrations and may need further refinement for use when higher MMHg concentrations are expected.

Conclusions

The work presented here shows the successful coupling the Clarus 580 GC and the NexION 300D ICP-MS through the GC transfer line. Once the optimum conditions were achieved, response functions with satisfactory linearity were generated and absolute LODs in the single femtogram level were determined. The GC chromatographic separation of mercury species was applied to the analysis of reference materials composed of biological tissues and sediment matrices. Quantification and validation of MMHg was performed by external calibration with recoveries of ≥ 82% and RSDs of ≤ 7%.

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