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Speciation analysis of methylmercury via species specific isotope dilution GC-ICP-MS

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Goal

To demonstrate the utility of the Thermo Scientific™ Element™ HR-ICP-MS coupled with the Thermo Scientific™ Trace™ 1310 GC via the Thermo Scientific™ GCI 200™ Interface for speciation analysis of mercury.

1. Introduction

Mercury is known as a highly toxic element with ubiquitous occurrence due to its global cycle.[1,2] The toxicity, metabolism, and pathways depend on the mercury species.[2,3] When mercury is methylated by bacteria, its toxicity and environmental persistence are increased: methylmercury ($MeHg^+$) is bioaccumulated and biomagnified within the food chain and can have severe impacts on biota and human beings.[2] Hence, knowing the molecular species of mercury in the environment is crucial.

The determination of $MeHg^+$ with species-specific isotope dilution gas chromatography-inductively coupled plasma mass spectrometry (GC-ICP-MS) has evolved to be the gold standard in speciation analysis of mercury due to its accuracy, precision, and additional isotope information.[4,5] The strength of this technique is in the ability to account for non-quantitative recoveries and monitoring of Hg side products (methylation, demethylation) during sample preparation, which would not be recognized with GC-atomic fluorescence spectrometry (GC-AFS) where no isotope information is acquired.[4,6]

2. Method

2.1. Instrument setup

A Trace 1300 GC was coupled to the Element 2 HR-ICP-MS via the GCI 200 Interface (P/N BRE0008121).

The GC operational parameters and ICP-MS parameters were optimized and are listed in Table 1. The system was tuned with the following procedure:

- To create a stable Hg signal, a GC syringe was filled with 10 µL of a 10 mg/L methlypropylmercury (MeHgPr) solution in *n*-hexane and placed in the SSL injector without pressing the plunger down.
- The injector temperature and the oven were both set at 70 °C: these isothermal conditions are meant to create a steady stream of MeHgPr into the ICP.
- The Hg signal was monitored until it reached stable intensity over time.
- Source parameters were recursively changed via the Element 2 software to obtain the optimal Hg signal intensity.
- Alternatively, the system can be tuned on the Xe signal originating from the Xe content of the Ar gas used.

Table 1. Instrument configuration and operation parameters

GC parameters	Value
Oven program	Initial temperature of 40 °C for 1 min, ramp at 50 °C/min to 180 °C
Column	CP-Sil 5CB from Varian (15 m × 0.25 mm, 0.25 µm)
Injector volume	1 µL
Carrier gas	Argon
Injection mode	SSL, splitless
Injector temperature	250 °C
Flow rate	3 mL/min (Argon)
Transfer line	240 °C
ICP-MS parameters	**Value**
Interface	Pt tipped sampler Pt tipped skimmer
Resolution	Low
RF power	1150 W
Transfer line gas flow	0.6 L/min Argon
Monitored isotopes	^{200}Hg, ^{201}Hg, ^{202}Hg
Integration time	50 ms per isotope
Chromatogram duration	3.8 min

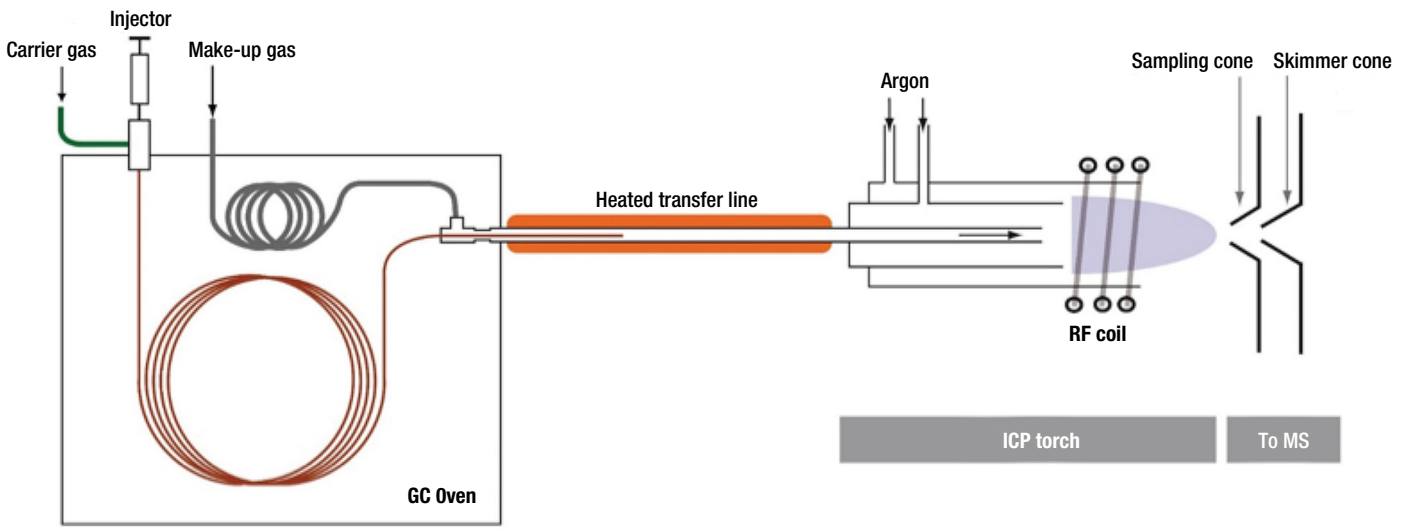

Figure 1. Scheme of the setup of the gas chromatograph (GC) coupled to a high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) via a heated transfer line

2.2. Sample preparation

2.2.1. Fish and biological material

One hundred micrograms of sample material (dried fish) were spiked with a $Me^{201}Hg^{+}$ enriched solution (ISC Science, Spain) targeting a ratio of $^{201}Hg/^{202}Hg$ of approximately 1. The spiked sample was digested in 5 mL of 25% m/v KOH in methanol at 60 °C. Concentrated hydrochloric acid was carefully added to bring the pH down to around 3.9, and 5 mL of a 0.5 M acetic acid/sodium acetate buffer at pH 3.9 was added to keep the solution at the optimal pH for propylation. One milliliter *n*-hexane was poured on top of the sample before adding 1 mL of the propylation reagent (1% (m/v) sodium tetrapropylborate in Milli-Q™ water). The sample was vigorously shaken for 10 min to extract the propylated mercury species into the *n*-hexane phase ($MeHg^{+}$ as MeHgPr and Hg^{2+} as $HgPr_2$; Pr=Propyl). The *n*-hexane phase was taken out and placed in a GC vial.

2.2.2. Water samples

Ninety-five milliliters of the aqueous sample was spiked with a $Me^{201}Hg^{+}$ enriched solution targeting a ratio of $^{201}Hg/^{202}Hg$ of approximately 1. The sample was then buffered to a pH of 3.9 by the addition of 5 mL 0.5 M acetic acid/sodium acetate buffer. The solution was left standing for 10 min. One milliliter of *n*-hexane was laid on top of aqueous solution and 1 mL of the propylating reagent was added. The mixture was shaken for 10 min. The *n*-hexane phase was then taken out and placed in a GC vial. If the concentration of $MeHg^{+}$ in the sample was very low, another preconcentration step was done by blowing an inert gas on top of the solution so that the *n*-hexane evaporated. In this process, the *n*-hexane evaporates easier than the propylated mercury species because its boiling point is lower.

2.2.3. Sediment samples

A selective extraction for organic mercury was done because $MeHg^{+}$ is typically less than 1% in most sediments. One hundred milligrams of sediment were placed in a vial and 5 mL of a solution containing 1 M sulfuric acid with 1.5 M KBr and 1 mL 1 M $CuSO_4$ were added. The sample was then spiked with a $Me^{201}Hg^{+}$ enriched solution targeting a ratio of $^{201}Hg/^{202}Hg$ of approximately 1 and shaken for 1 h. Two milliliters of toluene were added and the sample was shaken again for 30 min. The toluene phase was taken out after centrifugation and placed in a different vial with 5 mL 0.5 M acetic acid/sodium acetate buffer (pH 3.9). One milliliter of propylating reagent was added and the sample shaken for 10 min. The toluene phase was then taken out and placed in a GC vial.

2.3. Measurement

One microliter aliquots of the extracted sample were injected into the GC and the chromatogram was acquired over 3.8 min. The three mercury isotopes ^{200}Hg, ^{201}Hg, and ^{202}Hg were monitored. $MeHg^{+}$ elutes after 1.90 min and Hg^{2+} after 2.70 min (when the organic phase is *n*-hexane; see Figure 2).

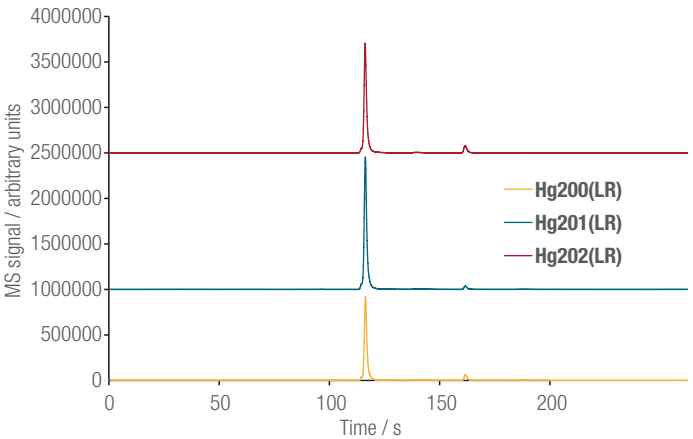

Figure 2. Chromatogram of mercury species in DORM-2. The sample was spiked with $Me^{201}Hg$ to get a ratio of 1 for $^{201}Hg/^{202}Hg$. The first peak is $MeHg^{+}$ as methylpropylmercury and the small peak is Hg^{2+} as dipropylmercury.

3. Data analysis

The $Me^{201}Hg^{+}$ and $Me^{202}Hg^{+}$ peaks were integrated from the obtained chromatograms and the isotopic ratio was calculated. The exact concentration of $MeHg^{+}$ was then calculated using the isotope dilution method, which combines known parameters like sample mass, spike volume, and spike concentration to calculate the concentration of $MeHg^{+}$.[7]

4. Results

For isotope dilution, it is crucial to accurately measure the isotope ratios in order to successfully quantify the species. ^{202}Hg has a natural abundance of 29.863%, ^{201}Hg of 13.181%, and ^{200}Hg of 23.096%. The enriched $Me^{201}Hg^+$ solution used for spiking contains 96.5% of ^{201}Hg and only 2.4% Hg^{202} and 0.9% Hg^{200} as $MeHg^+$. The different isotope ratios were calculated from those reference values and compared to the measured ratios (Table 2). The calculated isotope ratios compare very well to the measured isotope ratios. The deviation between the ratios is ≤1% except for the ratio $^{202}Hg/^{201}Hg$ from the enriched spike solution where the deviation is marginally higher (2.7%). The reason for this deviation is that the solution contains only a small amount of ^{202}Hg. A contribution of the mass bias to the result is therefore relatively small and can be neglected according to literature.[8] Therefore it can be concluded that this instrumentation determines the isotope ratios with sufficient accuracy.

The certified reference material DORM-2 (catfish muscle) was analyzed for $MeHg^+$. The CRM has a certified value of 4.47 ± 0.32 mg/kg of Hg in the form of $MeHg^+$. Three separate digestions of the material were performed and the *n*-hexane extracts for each sample were injected in triplicates. The instrument showed an exceptionally good stability with a relative standard deviation of ≤0.27% between three subsequent injections of the same extract. The method also showed to be reproducible with a relative standard deviation of 1% for the three separate extracts. Furthermore, a good accuracy was obtained highlighted by an average concentration of 4.43 mg/kg of $MeHg^+$ determined for DORM-2, which corresponds to a recovery greater than 98% (Table 3).

Distilled water was spiked with $MeHg^+$ of natural isotope constitution and the method tested on its limit of detection. A solution of 1, 10, and 100 ng/L MeHg was used for this purpose. Table 4 shows the results and Figure 3 a chromatogram of 1 ng/L $MeHg^+$. The 1 ng/L did give a concentration of 1.24 and 1.33 ng/L.

Table 2. Measured isotope ratios versus calculated reference values

	Ratio $^{202}Hg/^{200}Hg$	Ratio $^{202}Hg/^{201}Hg$	Ratio $^{202}Hg/^{200}Hg$	Ratio $^{202}Hg/^{201}Hg$
	Natural	Natural	Enriched $Me^{201}Hg^+$	Enriched $Me^{201}Hg^+$
Reference	1.2929	2.2656	2.6667	0.0249
Measured	1.3046	2.2889	2.6758	0.0242
Deviation / %	0.9	1.0	0.3	2.7

Table 3. Results for the analysis of three different digests of the certified reference material DORM-2. Each sample was injected 3 times.

Sample	c($MeHg^+$) / mg/kg	SD c($MeHg^+$) / Mg/kg (n=3)	RSD / % (n=3)	Recovery / %
DORM-2 a	4.38	0.010	0.22	98.0
DORM-2 b	4.47	0.008	0.18	100.0
DORM-2 c	4.43	0.012	0.27	99.1

Table 4. Results for the analysis of extracts from 1, 10, and 100 ng/L $MeHg^+$ (as Hg). The approximately peak area is listed in the last column.

Sample	c($MeHg^+$) / ng/L	SD c($MeHg^+$) / ng/L	App. Peak Area
1 ng/L $MeHg^+$	1.33	0.17	5000
1 ng/L $MeHg^+$ (baseline correction)	1.02	0.05	5000
1 ng/L $MeHg^+$ (evaporated)	0.97	0.04	70,000
10 ng/L $MeHg^+$	9.47	0.35	30,000
10 ng/L $MeHg^+$ (evaporated)	9.67	0.20	300,000
100 ng/L $MeHg^+$	95.97	1.59	300,000
100 ng/L $MeHg^+$ (evaporated)	98.04	0.17	3,000,000

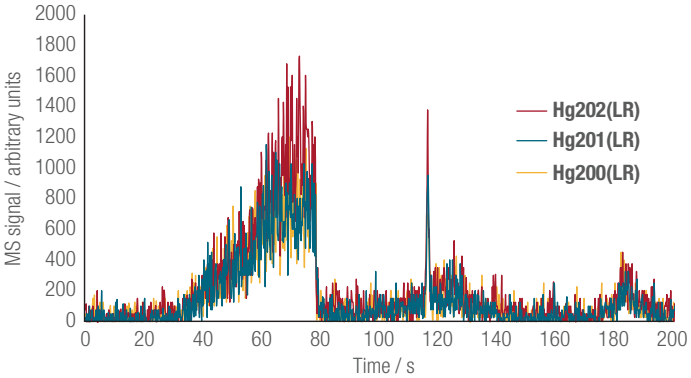

Figure 3. Chromatogram of Hg extracted from a 1 ng/L $MeHg^+$ solution. 1 pg $MeHg^+$ (as Hg) is injected. The $MeHg^+$ peak (as MeHgPr) is at 115 s.

Table 5. MeHg+ and T-Hg in filtered water from the Weser River (T-Hg was determined with CV-AFS)

Sample	c(T-Hg) / ng/L	c(MeHg) / ng/L	RSD c(MeHg) / %
WS	0.75 ± 0.05	0.076 ± 0.021	28
WF	0.76 ± 0.02	0.082 ± 0.016	19
K	0.80 ± 0.01	0.059 ± 0.014	24
UE a	0.74 ± 0.03	0.069 ± 0.005	8
UE b		0.060 ± 0.013	22
UE c		0.081 ± 0.021	26

The reason for a deviation of up to 30% from the real value is the small peak which is influenced by the noise in the background signal. One microliter of the extract from a 1 ng/L $MeHg^+$ solution contains an absolute mass of 1 pg of Hg, which gives a peak area of approximately 5000 and three subsequent injections of this solution can still have an RSD of 20%. It is therefore desirable to achieve a peak area of about 30,000 and more for getting accurate data. There are two ways to get the data more accurate.

When the peak area is corrected by the noise peak area, the concentration is accurately determined as 1.02 ng/L. Another way to overcome the peak area issue is a further pre-concentration step carried out by blowing an inert gas (here: argon) on top of the *n*-hexane phase. The *n*-hexane has a lower boiling point compared to the propylated mercury species and will therefore evaporate faster leaving a higher concentration of mercury species in the sample. The peak area for the 1 ng/L MeHg solution increased from 5000 to 70,000 and the concentration could be determined well (the measured value was 0.97 ng/L).

Four different water samples from the Weser River were collected at different points in Bremen and analyzed for their content of $MeHg^+$. The results are listed in Table 5. The samples were extremely low in MeHg+ (in the region of $MeHg^+$ in seawater) and had to be pre-concentrated by the described evaporation technique and the peak areas were then corrected by subtracting the baseline noise peak area. Because of the low concentrations, the RDSs for three subsequent injections of the same sample are relatively high. The preparation of sample UE as three individual extracts resulted in an RSD of 15%.

The method was tested further with an estuarine sediment, the certified reference material ERM-CC580. A selective extraction for organic mercury was done beforehand because $MeHg^+$ is typically less than 1% in most sediments. A value of 74.9 ± 0.75 µg/kg was achieved for a sediment, which is certified for a MeHg concentration of 75.5 ± 4 µg/kg as Hg. The method was further tested on sediments from the Weser River in Bremerhaven. The concentrations of $MeHg^+$ were low in the sediment and no visible $MeHg^+$ peak was detected from the extracts itself. The extracts were therefore treated further by blowing an inert gas (here argon) on top of the vial. The concentrated organic phase was then injected into the GC and a small $MeHg^+$ peak could now be detected with a delay in retention time of 144 s. Total mercury in the sediment was measured after aqua regia digestion on a CV-AFS and the $MeHg^+$ determined here comprises about 1% of the total mercury as expected beforehand (Table 6). Three subsequent injections of the same extract can give an RSD of up to 22% because the peaks are small and the background signal has here an effect, too. The concentration of MeHg is within the range of 0.64 to 2.31 µg/kg MeHg (as Hg) for 14 different sediment samples.

Table 6. Results for the analysis of sediments from the Weser River in Bremerhaven. Total mercury is listed next to the concentration of MeHg in each sediment.

Sample	c(T-Hg) / µg/kg	c(MeHg) / µg/kg	SD c(MeHg) / µg/kg	Ratio MeHg/T-Hg
1	244.2	0.88	0.15	0.36
2	228.6	0.64	0.34	0.28
3	240.3	0.85	0.07	0.35
4	234.9	1.63	0.08	0.69
5	260.1	1.35	0.13	0.52
6	263.8	2.31	0.15	0.88
7	288.9	1.09	0.33	0.38
8	200.7	1.50	0.02	0.75
9	262.5	1.11	0.34	0.42
10	240.3	1.23	0.06	0.51
11	243.6	1.24	0.22	0.51
12	249.9	1.06	0.09	0.42
13	244.3	1.63	0.04	0.67
14	250.0	1.51	0.24	0.60

Conclusion

The setup comprising a Trace 1310 GC coupled to the Element 2 HR-ICP-MS via the new GCI 200 Interface was applied successfully for the determination of methylmercury. A good analytical result depends on the accurate determination of the peak area, which is not given at very small peaks because the background noise has an influence on the peak area. The results for DORM-2 (fish muscle) and ERM-CC580 (estuarine sediment) showed good precision and accuracy in a short chromatography time of 3.8 min.

Provided a suitable preparation and extraction method, this technique can be applied for most environmental samples spanning from plant material to sediment samples and different water samples covering most applications for compound-specific Hg ultra-trace quantification.

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