Application Note: 40721

Determination of Methylmercury in Fish by GC Coupled with X Series ICP-MS

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Key Words

- GC
- ICP-MS
- Organo-Mercury
- Speciation
- Transient TRA

Introduction

Mercury is a well-known global pollutant of particular concern due to its toxicity. It's introduced in the environment via anthropogenic sources from its use in the agrochemical industry, in manufacturing processes and as a bactericide. The exploitation of natural sources such as mines and the combustion of fossil fuels and geochemical sources such as volcanic activity also contribute to the 3000 tonnes of mercury liberated into the environment each year. Mercury exists in three major forms; elemental Hg^0, inorganic Hg^{2+} and alkylated mercury.

Mono Methylmercury (MMHg) is to date, considered the most toxic form of mercury. The direct introduction of MMHg into the environment from use as a fungicide is now outlawed and the major source of MMHg is found from biotic or abiotic methylation of inorganic mercury. The methylation of mercury is of particular interest, as the transformation not only has an effect on the toxicity of the element but also on the bioavailability and mobility of the element in the environment. Thus, it is essential to investigate the speciation of mercury rather than total element concentration to fully understand the environmental consequences.

MMHg in the marine environment is of particular concern due to the phenomenon of bioaccumulation and biomagnification by marine biota. Accumulation occurs due to the high solubility of MMHg in the fat tissues of marine organisms and low excretion due to strong liaisons formed once it has entered the central nervous system and traversed the intracellular membrane. Some marine fish are found to be able to accumulate up to several ppm of MMHg in their tissues. Public health concerns have arisen out of the risk associated with eating too much fish or shellfish, which have accumulated MMHg. A broad spectrum of neurological disorders has been reported due to the effects of MMHg toxicity. As a result, regulatory bodies have issued guidelines for the amount of fish consumed, in particular for pregnant and breast-feeding mothers, where the effect on fetal and infant exposure is yet to be determined. For example, the United States Environmental Protection Agency (EPA) have recommended that pregnant women should limit their weekly intake to 6 ounces of marine fish and the Food and Drug Administration (FDA) have recommended that pregnant women should avoid eating shark and swordfish altogether due to the high levels of MMHg accumulated in those fish.

Albeit the MMHg accumulation levels pose a public health risk, concentrations representative of environmental samples are at levels that require sensitive methodology for their determination. The current EPA standard operating procedure for mercury speciation is based on AFS detection, which is unsuitable for the determination of ultra trace species in environmental matrices. Current analytical methodologies developed for the speciation of mercury generally encompass a coupled chromatographic separation technique with ICP-MS detection, which allows for rapid, specific and sensitive detection. This application note describes the use of the new GC-ICP-MS instrument package from Thermo Electron Corporation to determine trace and ultra trace concentrations of MMHg in various fish extracts. The analytical methodology is validated using a certified reference material (CRM) with a tuna fish matrix (BCR 464).

Instrument configuration

A FOCUS™ Gas Chromatograph and AS3000 autosampler were coupled to the X Series ICP-MS using the new Thermo GC-ICP-MS Coupling Pack (P/N 4600503) and FOCUS GC Wiring Harness (P/N 4600510.) This coupling pack includes all the required components to establish electrical and analytical connections between the GC and ICP-MS instrumentation and a schematic of the GC and ICP-MS coupling is illustrated in Figure 1.

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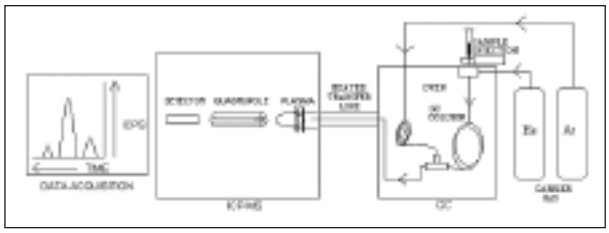

Figure 1: Schematic diagram for GC-ICP-MS coupling

Samples were injected onto the GC column in a flow of He carrier gas to enable separation of derivatized mercury species. The species were swept through the GC Transfer Line to the ICP-MS in a flow of Ar make-up gas. The GC Transfer Line was connected from the outlet of the GC column to the torch of the X Series ICP-MS via a 1/16-inch flexible transfer capillary located inside an insulated stainless steel capillary tube. The stainless steel capillary was also sheathed by a flow of heated Ar gas inside the steel capillary. These instrument features ensure a uniform heating profile for the Transfer Line to prevent the loss of species due to condensation and aid transmission of the separated species to the ICP-MS detector. The transfer line can be heated isothermally to temperatures in excess of 300°C and further technical details regarding the Thermo GC-ICP-MS Coupling Packs can be found in the Thermo note PS40674 (available to download from www.thermo.com/elemental).

The X Series ICP-MS was configured with HPI interface cones to enable enhanced instrument sensitivity and was also configured with the unique dual mode sample introduction system to facilitate simultaneous introduction of both liquid and gaseous samples. This dual sample introduction system allowed connection of the GC Transfer Line to the torch through the orifice used for standard mode sample introduction. A quartz concentric nebulizer and impact bead spray chamber arrangement was then mounted above the GC Transfer Line and connected to the third leg of the GC-ICP-MS torch as shown in Figure 2.

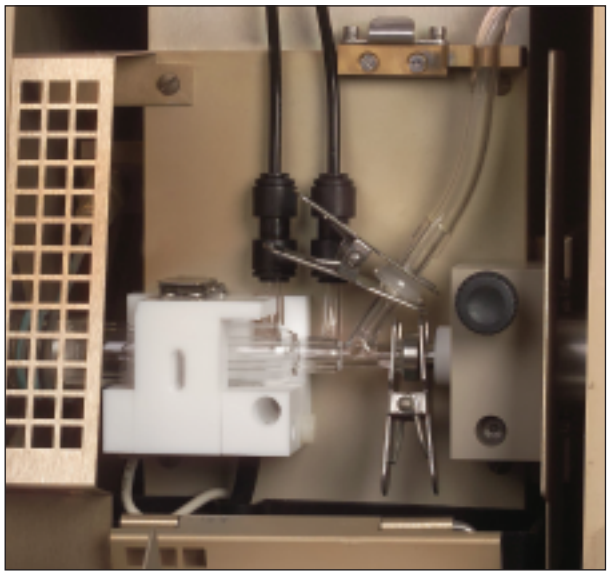

Figure 2: X Series dual mode sample introduction for GC-ICP-MS

Analytical Conditions for GC

The FOCUS GC and AS3000 autosampler were programmed from the X Series ICP-MS PC using ChromCard software to enable separation of the propylated mercury species and the analytical conditions used for the GC and GC Transfer Line are shown in Table 1.

Column	Restek MXT-1, 30 m, 0.53 mm i.d., PDMS 100 %, df 1 µm
Injection mode	Splitless
Injection port temperature	230°C
Injection volume	1 µL
Carrier gas flow	He @ 25 mL min^{-1}
Make up gas flow*	Ar @ 400 mL min^{-1}
Stop purge time	30 s
Transfer line temperature	250°C isothermal
Oven parameters	
Initial temperature	60 °C
Initial time	1 min
Ramp rate	50 °C min^{-1}
Final temperature	200 °C
Final time	1 min
Auto sampler	
Injection depth	Standard
Vial depth	Bottom
Syringe rinse cycles	3
Preinjection syringe sample rinses	7
Post injection syringe rinses	5

Table 1: GC, GC Transfer Line and Autosampler conditions
* delivered via the X Series Additional Mass Flow Controller

Analytical Conditions for ICP-MS

The X Series ICP-MS was performance tested, tuned and optimized as required for GC-ICP-MS analysis using the automated PlasmaLab Performance Test and Autotune facilities and analytical conditions for ICP-MS are shown in Table 2. An aqueous 1 µg L^{-1} Tl solution was aspirated continuously throughout the analysis using the unique dual mode X Series ICP-MS sample introduction system and ^{205}Tl was defined as a PlasmaLab Timeslice Internal Standard to allow correction for chromatographic baseline drift.

Forward Power	1350 W
Nebulizer Gas Flow	0.5 L min^{-1}
Auxilliary Gas Flow	0.8 L min^{-1}
Cool Gas Flow	13 L min^{-1}
Data Acquisition Mode	PlasmaLab Transient Time Resolved Analysis (TRA)
Isotopes and dwell times, ms	^{202}Hg (40 ms)
	^{205}Tl (10 ms)
Channels per AMU	1
Timeslice duration	128 ms
Timeslice Internal Standard pumped at 400 µL min-1	Aqueous Tl solution (1 µg L^{-1})
Transient acquisition time	210 s
Spray chamber	Conical with impact bead
Nebulizer	Standard, pneumatic concentric
Cones	HPI

Table 2: ICP-MS conditions

Preparation of MMHg Standard

A 1000 µg g^{-1} stock solution of MMHg was prepared by dissolving the appropriate quantity of the commercially available salt, MMHgCl in ultra-pure grade methanol. The stock was further diluted 10-fold with 1 % HCl in 18.2 MΩ water to produce working standard of 10 µg g^{-1} intended for the BCR 464 standard additions. A working solution of 10 ng g^{-1} MMHgCl was also prepared for standard additions with the commercial fish samples. Derivatization of external calibration standards was achieved by adding 0, 20, 100 and 200 µL of the 100 ng g^{-1} stock solution to 5 mL acetate/acetic acid buffer (0.1 M) at pH 3.9, adding 2 mL isooctane and 1 mL 1% Na BPr_4 (in 18.2 MΩ water) and agitating the mixture for approximately 5 minutes. The two liquid phases were allowed to separate in the vial and the derivatized standards were isolated for analysis by transferring a portion of the top, organic layer to a 2 mL, amber GC vial.

Preparation of CRM and Fish Samples

Sample preparation:

The commercially available fish samples were individually washed in 18.2 MΩ water, cut into small pieces with a scalpel and transferred to clean, dry vials. After freezing during 24 h the fish were subjected to freeze-drying during 48 h to remove all water from the tissues. The dried tissues were homogenized with a pestle and mortar and any dried fish unable to be reduced to powder was removed.

Sample extraction:

Prior to sub-sampling, the CRM BCR 464 was removed from storage at -20 °C, left to achieve ambient temperature for 1 h and homogenized by manual agitation for 5 min. Sub-samples of 250 ± 10 mg of sample were weighed accurately into 6 extraction vessels. Known amounts of MMHg were added to 3 of the extraction vessels to give standard additions of approximately twice, thrice and four times the certified concentration. For the commercially available fish samples, extractions were carried out in triplicate with approximately 250 mg of each freeze-dried fish tissue. 10 mL of tetra methyl ammonium hydroxide solution (TMAH, 25%) were added to each vessel and the vessels fitted with air-cooled condensers. After gentle stirring of this mixture, extraction was performed in an open focused microwave system (Prolabo, France) at 40W (20% power) for 2 min. After extraction, the sample was allowed to cool down and then transferred into a Teflon-capped vial. For each day of extraction 2 reagent blanks were prepared by the above procedure.

Derivatization of the extracts:

In clean Teflon®-capped vials, carefully measured aliquots of CRM BCR 464 extracts (0.05 mL) were buffered to pH 3.9 by adding 5 mL of acetic acid/Na acetate buffer (0.1 M). Depending on the estimated concentration of MMHg in the freeze-dried commercial fish, 4 aliquots (0.1 and 1 mL) of each extract were transferred to clean Teflon-capped vials and buffered to pH 3.9 by adding 5 mL of acetic acid/Na acetate buffer (0.1 M). pH was adjusted by adding small amounts of acetic acid (puriss). 1 or 2 mL of isooctane (depending on MMHg concentration in the respective fish) was added and propylation performed by adding 1 mL of aqueous 1% $NaBPr_4$ solution. The tube was capped immediately and shaken by hand for 5 min. Phase separation was accelerated by centrifuging for 10 min at 2000 rpm, and the upper, organic layer containing the derived MMHg was transferred into a 2 mL, amber GC vial. Derivatized samples were stored in the dark at –18 °C prior to analysis.

Fully Quantitative Data

Quantification was carried out by internal standard addition (prior to extraction) for the CRM to validate the pre-treatment methodology. External standard addition (after extraction) were then performed for the freeze-dried commercial fish samples. The chromatographic data is displayed automatically in the PlasmaLab software package following analysis and an example of the chromatographic separation of derived mercury species in freeze-dried fish samples is shown in Figure 3.

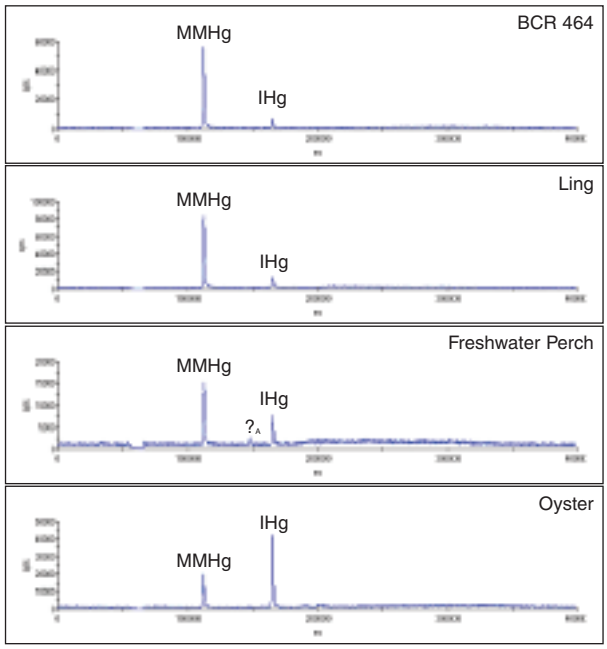

Figure 3: Chromatogram of derived mercury species in various freeze-dried fish extracts

The methodology allowed for the baseline separation of MMHg and inorganic Hg2+ (IHg) with retention times of 111 and 166 seconds respectively, and an unknown species at 146 seconds in the freshwater perch fish sample. Each extract was spiked with a MMHg standard solution in order to give standard additions of about twice, thrice and four times the concentration of the derived extract and the standard addition chromatography was integrated using

the PlasmaLab Transient TRA standard software tools. A method blank was prepared and analyzed to correct for systematic contamination. Examples of resultant standard addition curves are shown in Figure 4.

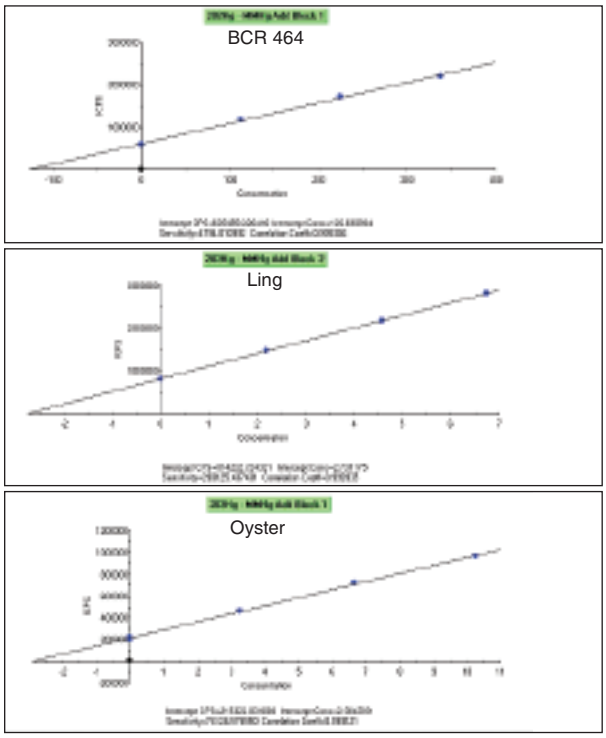

Figure 4: PlasmaLab Standard Addition curve for BCR 464, Ling and Oyster samples

CRM BCR 464 (certified for MMHg) was extracted, derivatized and analyzed to verify the GC-ICP-MS procedure. Two chromatographic peaks were observed in each of the samples and these peaks were identified from retention time correlations with standard calibrants. MMHg was quantified by standard addition. However, inorganic Hg was determined semi-quantitatively using the response curves derived from the MMHg standard addition curves (i.e. Compound Independent Calibration).

Hg Species (ng g-1)

SAMPLES MMHG BY STANDARD ADDITION					SEMI-QUANTITATIVE RESULTS	
CRM	**Source**	**MMHg**	**Certified**	**Recovery**	**IHg**	**?$_A$**
BCR 464	IRMM, Belgium	5199 ± 52	5500 ± 170	94.50%	513 ± 52	
Sample	**Source**	**MMHg**	**MMHg in wet fish**			
Red tuna	North-East Atlantic	1218 ± 132	335 ± 36		168 ± 33	
Canned tuna	Ecuador	328 ± 28	84 ± 7		203 ± 9	
Fillet of Trout	Farmed, France	168 ± 15	42 ± 4		143 ± 3	
Freshwater Perch	France	565 ± 25	107 ± 5		207 ± 11	58 ± 16
Black Pollock	North-East Atlantic	111 ±7	19 ± 1		87 ± 8	
Ling	North-East Atlantic	1159 ± 140	197 ± 24		177 ± 49	
Alaskan Hake fillets*	Pacific Ocean	46 ± 5	8 ± 1		152 ± 13	
Squid Heads*	Indian Ocean	58 ± 7	7 ± 1		108 ± 6	
Oyster$	French coast	106 ± 8			163 ± 5	

Table 3. Fully quantitative standard addition data for MMHg and semi-quantitative data for inorganic Hg and an unknown in various fish samples and BCR 464

* Frozen prior to pre-treatment

$ Moisture unknown – sample pre-prepared

?$_A$ - Uncharacterized Species

A third uncharacterized species was observed in the freshwater perch sample. However, the PlasmaLab software was used to generate semi-quantitive data for this species using the response curve derived from the MMHg calibrations. The concentration of mercury species in the CRM and freeze-dried fish tissues was calculated from the concentrations determined in the aliquots of derivatized extract by taking into account all dilution and weight factors. The CRM determinations were further corrected for moisture (determined as 5.2%). The estimated concentrations of MMHg in the commercial fish samples were calculated from the moisture removed in the freeze-drying process. All extraction procedures were carried out in triplicate and the mean and standard deviations determined are shown in Table 3.

The fully quantified standard addition of MMHg yielded a result of within 5.5% of the certified value in the CRM BCR 464. This analytical data demonstrates the suitability of the sample preparation for MMHg in fish and the accuracy of the GC-ICP-MS technique. Accordingly, the data generated for the commercial fish samples can be awarded a high level of confidence. Indeed, as expected, the tuna fish has the highest levels of MMHg along with the Ling (both carniverous fish susceptible to biomagnification of MMHg).

Detection limits for MMHg were determined in accordance with the 3σ model following fully quantitative analysis of the calibration blanks (n=5) and the absolute detection limit for MMHg was 35 fg.

Summary

The Thermo GC-ICP-MS speciation package provides a complete instrument solution for sensitive analyses of mercury species in fish tissues. The unique dual mode sample introduction system of the X Series ICP-MS provides a simple automated regime for GC-ICP-MS tuning, optimisation and performance testing and the intelligent productivity enhancing features of the X Series PlasmaLab software enable failsafe automated instrument operation.

Parts List

GC-ICP-MS Coupling Pack (P/N: 4600503)
Focus GC Wiring Harness (P/N: 4600510)
Trace GC Wiring Harness (P/N: 4600509)
Generic GC Wiring Harness (P/N: 4600494)

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