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Measurement of methyl mercury (I) and mercury (II) in fish tissues and sediments by HPLC-ICPMS and HPLC-HGAAS

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ABSTRACT

A procedure for the extraction and determination of methyl mercury and mercury (II) in fish muscle tissues and sediment samples is presented. The procedure involves extraction with 5% (v/v) 2-mercaptoethanol, separation and determination of mercury species by HPLC-ICPMS using a Perkin-Elmer 3 μm C8 (33 mm × 3 mm) column and a mobile phase 3 containing 0.5% (v/v) 2-mercaptoethanol and 5% (v/v) CH_3OH (pH 5.5) at a flow rate 1.5 ml min^{-1} and a temperature of 25 °C. Calibration curves for methyl mercury (I) and mercury (II) standards were linear in the range of 0–100 μg l^{-1} (r^2 = 0.9990 and r^2 = 0.9995 respectively). The lowest measurable mercury was 0.4 μg l^{-1} which corresponds to 0.01 μg g^{-1} in fish tissues and sediments. Methyl mercury concentrations measured in biological certified reference materials, NRCC DORM – 2 Dogfish muscle (4.4 ± 0.8 μg g^{-1}), NRCC Dolt – 3 Dogfish liver (1.55 ± 0.09 μg g^{-1}), NIST RM 50 Albacore Tuna (0.89 ± 0.08 μg g^{-1}) and IRMM IMEP-20 Tuna fish (3.6 ± 0.6 μg g^{-1}) were in agreement with the certified value (4.47 ± 0.32 μg g^{-1}, 1.59 ± 0.12 μg g^{-1}, 0.87 ± 0.03 μg g^{-1}, 4.24 ± 0.27 μg g^{-1} respectively). For the sediment reference material ERM CC 580, a methyl mercury concentration of 0.070 ± 0.002 μg g^{-1} was measured which corresponds to an extraction efficiency of 92 ± 3% of certified values (0.076 ± 0.04 μg g^{-1}) but within the range of published values (0.040–0.084 μg g^{-1}; mean ± s.d.: 0.073 ± 0.05 μg g^{-1}, n = 40) for this material. The extraction procedure for the fish tissues was also compared against an enzymatic extraction using Protease type XIV that has been previously published and similar results were obtained. The use of HPLC-HGAAS with a Phenomenox 5 μm Luna C18 (250 mm × 4.6 mm) column and a mobile phase containing 0.06 mol l^{-1} ammonium acetate (Merck Pty Limited, Australia) in 5% (v/v) methanol and 0.1% (w/v) L-cysteine at 25 °C was evaluated as a complementary alternative to HPLC-ICPMS for the measurement of mercury species in fish tissues. The lowest measurable mercury concentration was 2 μg l^{-1} and this corresponds to 0.1 μg g^{-1} in fish tissues. Analysis of enzymatic extracts analysed by HPLC-HGAAS and HPLC-ICPMS gave equivalent results.

1. Introduction

Mercury is considered to be a major environmental pollutant on a global scale [1]. Environmental and health risks for mercury are caused by methyl mercury that is formed by biomethylation of mercury (II) which is carried out by microorganisms in sediment and soil [1]. Methyl mercury concentrations, however, usually does not exceed 1.5% of the total mercury content in sediments [2]. Methyl mercury binds strongly with biological ligands containing sulfhydryl groups affecting the function of enzymes and interfering with protein synthesis [3]. It is a neurotoxin that readily crosses biological membranes and accumulates to harmful concentrations in organisms and biomagnifies in aquatic food webs to concentrations of toxicological concern [4].

Developing a sensitive, reliable and cost effective method to measure mercury species in environmental samples is important for monitoring mercury concentrations in biota and sediments to avoid ecotoxicological risks and understand the biogeochemical cycling of mercury species in the environment [5]. High Pressure Liquid Chromatography (HPLC) coupled to Inductively Coupled Plasma Spectrometry (ICPMS), Atomic Fluorescence Spectroscopy or Atomic Absorption Spectroscopy (AAS) with or without hydride generation can be used to separate and quantify mercury species [5–7].

One of the main problems in measuring methyl mercury concentrations in sediments is the extraction of this species from a

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Table 1
Operating conditions for ICP-MS.

Plasma conditions	
RF forward power	1200 W
Plasma argon flow rate	15 l min^{-1}
Auxiliary argon flow rate	1.2 l min^{-1}
Nebulizer gas flow	0.92 l min^{-1}
Mass spectrometer settings	
Acquisition mode	Peak hopping
Isotopes monitored	^{202}Hg, ^{201}Hg, ^{200}Hg, ^{198}Hg, ^{181}Ta, ^{184}W, ^{103}Rh, ^{115} In, ^{159}Tb, ^{165}Ho
Dwell time	50 ms
Sweep time	13.5 s
Sweeps per reading	15
Replicates	Total: 3
Integration time	750 ms per element

Table 2
Operating conditions for HPLC-ICP-MS.

Chromatography	
HPLC column	PE C8, 3 μm (33 mm × 3 mm)
Mobile phase	0.5% (v/v) 2-mercaptoethanol in 5% (v/v) CH_3OH, pH 5.3, flow rate, 1.5 ml min^{-1}; temp, 25 °C
Sample volume	100 μl
Plasma conditions	
RF forward power	1200 W
Plasma argon flow rate	15 l min^{-1}
Auxiliary argon flow rate	1.2 l min^{-1}
Nebulizer gas flow	0.84 l min^{-1}
Mass spectrometer settings	
Acquisition mode	Peak hopping
Isotopes monitored	^{202}Hg, ^{201}Hg, ^{200}Hg, ^{198}Hg, ^{181}Ta and ^{184}W
Dwell time	100 ms
Sweeps per reading	1
Replicates	1
Readings	500 (6.52 min)

Table 3
Operating conditions for HPLC-HG-AAS.

Chromatography	
HPLC column	Phenomenex Luna 5 μm C18 (250 mm × 4.6 mm) Mobile phase 5% (v/v) CH_3OH, 0.06 mol l^{-1} CH_3COONH_4 and 0.1% (w/v) L-cysteine, pH 6.8, flow rate, 1.0 ml min^{-1}; temp, 25 °C.
Sample volume	100 μl
Hydride generation	
HCl concentration	2% (v/v)
HCl flow rate	1 ml min^{-1}
$NaBH_4$	0.75% (w/v) in 0.05% (w/v) NaOH
$NaBH_4$ flow rate	1 ml min^{-1}
Gas flow rate	70 ml min^{-1}
Atomic absorption spectroscopy	
Wavelength	253.7 nm
Slit width	0.7 nm
Cell temperature	350 °C

complex matrix. The main requirement of an extraction method is complete separation of the analyte from the interfering matrix without analyte loss, no sample contamination or changes in speciation [2]. The most commonly used procedures for extraction of methyl mercury from sediment samples are the use of microwave heating [2,5–11], sonication [10], distillation [12–15] with acid [6,8,11,15–18], alkali [15] or thiol containing reagents such as 2-mercaptoethanol [19], thiourea [20], or cysteine [21] as extraction reagents. Most of these procedures, however, are incompatible with HPLC-ICPMS [10,14,15,18,22–25] as they use strong acids or bases that even after neutralisation result in poor chromatography of mercury species.

In our laboratory we routinely use HPLC-ICPMS to analyse mercury species in 100–200 fish samples per year and wished to develop a complementary alternative procedure using hydride generation-atomic absorption spectrometry (HGAAS) to reduce costs and free up our ICPMS. We have previously reported the successful use of Protease type XIV to extract mercury (II) and methyl mercury (I) from fish muscle tissues and the separation and quantitation of mercury species by HPLC-ICPMS [26].

This work reports the use of 2-mercaptoethanol for the extraction of mercury species from fish tissues and sediments and analysis by HPLC-ICPMS. The development of a complementary alternative HPLC-HGAAS system for the cold vapour measurement of mercury species in fish tissues is described. The 2-mercaptoethanol extraction procedure for the fish tissues was also compared with the enzymatic extraction using Protease type XIV.

2. Methods

2.1. Equipment

Total mercury concentrations in digests were determined using a Perkin Elmer SCIEX Elan DRC-e ICPMS (Table 1). Mercury species were determined using either a Perkin Elmer series 200 HPLC pump with a Perkin-Elmer 3 μm C8 (33 mm × 3 mm) column coupled to a Perkin Elmer Sciex Elan 6000 ICPMS (Table 2) or a GBC L1100 HPLC pump with a Phenomenox 5 μm Luna C18 (250 mm × 4.6 mm) column coupled to a Perkin Elmer 3100 atomic absorption spectrometer fitted with a Perkin Elmer electrically heated atomisation cell via a Perkin Elmer FIAS 400 flow injection analyser system (Table 3).

2.2. Standards

A stock solution (1000 mg l^{-1}) of mercury (II) was prepared by dissolving the appropriate amount of mercuric chloride (BDH Chemicals Ltd., England) in deionised water (Sartorius arium 611, Australia). The methyl mercury stock solution (1000 mg l^{-1}) was prepared by dissolving the appropriate amount of methyl mercury (I) chloride (Aldrich Chemical Company Inc., USA) in methanol and diluting to volume with deionised water. Stock solutions were stored in airtight bottles and refrigerated. Working standards (3, 6, 12, 25, 50,100 μg l^{-1}) were prepared daily from the stock solution by serial dilution using extracting solutions.

Table 4
Mercury measurements in the certified reference materials and fish tissues using 2-mercaptoethanol extraction and HPLC-ICPMS. Mean ± standard deviation, $n = 3$, where ± reported as 0.0 is <±0.005 μg g^{-1}.

Tissue	Total (μg g^{-1})	Extracted (μg g^{-1})	$MeHg^+$ (μg g^{-1})	Hg^{2+} (μg g^{-1})
Dogfish muscle – NRCC Dorm 2	4.64 ± 0.026	4.40 ± 0.00	4.4 ± 0.8	< 0.01
Dogfish liver – NRCC Dolt 3	3.37 ± 0.14	3.2 ± 0.3	1.55 ± 0.09	1.6 ± 0.2
Albacore Tuna – NIST RM 50	0.95 ± 0.03	1.1 ± 0.1	0.89 ± 0.08	0.18 ± 0.00
Tuna Fish – IRMM IMEP-20	4.32 ± 0.16	3.8 ± 0.6	3.6 ± 0.6	0.38 ± 0.00
Yellow fin Tuna 1 *Thunnus albacares*	1.39 ± 0.09	1.3 ± 0.3	1.14 ± 0.05	0.18 ± 0.00
Yellow fin Tuna 2 *Thunnus albacares*	1.3 ± 0.2	1.2 ± 0.1	0.97 ± 0.05	0.20 ± 0.00
Orange roughy *Hoplostethus atlanticus*	2.5 ± 0.2	2.4 ± 0.1	2.04 ± 0.05	0.34 ± 0.00