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Determination of methylmercury in fish tissue by gas chromatography with microwave-induced plasma atomic emission spectrometry after derivatization with sodium tetraphenylborate

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Abstract The detection of methylmercury species (MeHg) in fish tissue was investigated. Samples were digested with KOH-methanol and acidified prior to extraction with methylene chloride. MeHg was back-extracted from the organic phase into water. An aliquot of this aqueous solution (buffered to pH 5) was subjected to derivatization with sodium tetraphenylborate ($NaBPh_4$) and then extracted with toluene. The organic phase containing MePhHg was injected into a gas chromatograph (GC) which is on-line with a microwave-induced plasma atomic emission spectrometer (MIP-AED). The quantification limit was about 0.6 μg/g and 0.1 μg/g of MeHg (as Hg) for 0.08 g of freeze-dried fish powder and 0.5 g of fresh samples, respectively. Two certified reference materials, CRM 464 (tuna fish) from Community Bureau of Reference-BCR and DORM-2 (dogfish muscle) from National Research Council Canada-NRC were selected for checking the accuracy of the method. This methodology was applied to the determination of MeHg in some kinds of fish from the Carmo river with alluvial gold recovery activities ("garimpos") in Mariana, Minas Gerais, Brazil.

1 Introduction

Since the time Brazil was a colony, extensive damage has been caused to the environment by alluvial gold recovery activities in the Carmo river where metallic mercury is used to amalgamate gold. A lack of adequate technical procedures results in considerable occupational hazards and environmental contamination by mercury itself and its compounds.

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We worked on a project together with the state environmental regulatory agency (FEAM) of Minas Gerais in order to evaluate the mercury contamination by "garimpos" in Mariana region. Mercury is biomethylated and subsequently concentrated in the food chain. Information about the mercury species is relevant to understand the biogeochemical cycle of mercury and also as a prerequisite for toxicity investigations [1]. Because virtually all the mercury found in fish is in the toxic methylated form [2] fish must be monitored.

Mainly used methods for the determination of individual mercury species are based on gas chromatography (GC) separations with detection by electron capture (EC) [2, 3], atomic absorption spectrometry (AAS) and microwave induced plasma-atomic emission spectrometry (MIP-AED) [4–5]. Capillary GC with microwave-induced plasma atomic emission detection (GC-MIP-AED) was used in this work, due to the high separation efficiency of capillary GC, the element-specific nature of the detector, and its high sensitivity for certain elements such as Hg, Pb, halogens, etc.

Derivatization of the analytes is recommended for GC due to thermal instability of mercury species in the inlet system and/or in the GC column [6]. The most common derivatization procedures used are hydride generation with sodium borohydride ($NaBH_4$), aqueous-phase ethylation with tetraethylborate ($NaBEt_4$) and Grignard alkylation. Sodium tetraphenylborate ($NaBPh_4$) [7] was used in this study because the reaction product (MePhHg) is more suitable to liquid-liquid extraction. Besides that, $NaBPh_4$ is more stable than $NaBEt_4$ but after a long storage time (4–8 months) it looses its reactivity [8].

A simple solvent extraction technique [9] was used to isolate MeHg from the major matrix in order to eliminate interferences with the phenylation reaction. After the extraction MeHg was back-extracted into water, phenylated and the MePhHg species was extracted with toluene and injected into the GC-AED system.

2 Experimental

2.1 Reagents and standards. Standard solutions of Hg(II) were prepared by diluting mercury spectrometric standard (10.00 mg/mL in 10% HNO_3) from NIST in Milli-Q water (Millipore).

Methylmercury chloride (+ 99.8%) was obtained from Strem Chemicals. A stock standard solution was prepared by dissolution of methylmercury chloride in 1% HCl. This solution was kept in a Teflon bottle in the refrigerator. Working standards were prepared by serial dilution of the stock with Milli-Q water. Sodium tetraphenylborate (1% m/V) was freshly prepared a few minutes before its use.

A buffer solution of pH 5 was prepared using 0.2 M sodium acetate/acetic acid. Sodium acetate anhydrous (min. 98%), acetic acid (min. 99.8%), potassium hydroxide pellets (min. 85%), dichloromethane suprasolv, hydrochloric acid (37%) specific for trace mercury analyses, methanol uvasol (min. 99.9%), toluene uvasol (min. 99.9%), sodium tetraphenylborate (min. 99.5%) were purchased from Merck (Germany).

Oxygen (99.9995%) and hydrogen (99.997%) from White Martins (Brazil) were used as auxiliary gases. Helium was used as both the carrier and the plasma gas. Helium (99.995%) was purified by using a GC50 gas purifier (SAES Pure Gas, Inc.) to obtain He (99.9999%). N_2 (99.9995%) used for spectrometer purge was obtained by using a nitrogen generator (Peak Scientific, Inc.).

All glassware used was first soaked in 5% HNO_3 overnight, cleaned with 2% detergent solution (Dura Kleen), thoroughly rinsed with tap water, soaked in 10% HCl for 3 days and finally rinsed with deionized water.

2.2 Instrumentation and measurement. Gas chromatographic separation and detection were carried out with a 5890 Series II GC (Hewlett-Packard) with a 5921A atomic emission detector AED (Hewlett-Packard) tuned at 254 nm (for mercury) and 193 nm (for carbon). Data acquisition and reprocessing were carried out by means of a 3592A ChemStation System (Hewlett-Packard). Chromatographic and detection parameters were optimized. Operating conditions for the GC-MIP-AED are specified in Table 1.

Table 1 Optimum GC-AED parameters

GC parameters	
Column (HP-1)	25 m × 0.32 mm × 0.17 μm
Injection port	split-splitless
Injection port temperature	250 °C
Split mode	Splitless
Purge time: off/on	0–0.6 min/0.6–7 min
Septum purge	4 mL min^{-1}
Injection Volume	1.0 μL
Column head pressure	11.9 psi helium
Oven program	
Initial temperature	75 °C
Ramp rate	30 °C min^{-1}
Final temperature	250 °C (1 min)
AED parameters	
Wavelength	254 nm (Hg), 193 nm (C)
Helium make-up flow-rate	60 mL min^{-1}
Ferrule purge vent	20 mL min^{-1}
Scavenger gases:	
Hydrogen	70 psi
Oxygen	25 psi
Spectrometer purge	2 mL min^{-1} N_2
Solvent vent off-time	1–3 min
Cavity temperature	250 °C
Transfer line temperature	250 °C

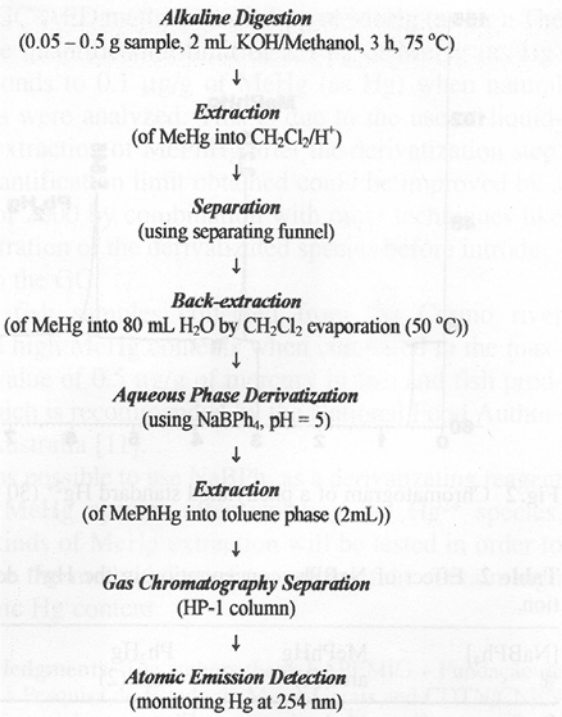

Fig. 1 Steps for MeHg determination in fish tissue

2.3 Procedure. The complete methodology for determination of MeHg in fish tissue by alkaline digestion, solvent extraction, aqueous phase phenylation, GC separation and AED detection are summarized in Fig. 1. The digestion and extraction of methylmercury from fish samples were based on the work described by Liang et al. [9]. The authors stated that MeHg is isolated from the matrix and that the technique has significant advantages for the analysis of samples containing large amounts of organic volatile compounds and for samples with high concentrations of Hg^{2+}, such as sediments.

The direct aqueous phase phenylation was based on the work of Minganti et al. [8] and Lückow and Rüssel [7].

3 Results and discussion

3.1 Derivatization

The result of the derivatization of Hg^{2+} standard solutions with $NaBPh_4$ is shown in Fig. 2. According to literature, a Hg^{2+} methylation is expected due to the quality of the derivatization reagent [10]. Then standard solutions of Hg^{2+} were derivatizated using $NaBPh_4$ in the range 0.01–1% to verify the effect of the reagent concentration over the Hg^{2+} methylation. It can be seen from Table 2 that the decrease of $NaBPh_4$ concentration did not inhibit the MeHg formation from Hg^{2+}. The relation between MePhHg and Ph_2Hg peak areas stayed almost constant with decreasing $NaBPh_4$ concentrations from 1 to 0.01%. On the other hand, the areas of MePhHg and Ph_2Hg peaks grew with the increasing concentration of $NaBPh_4$. This indicates that the use of an excess of derivatization reagent is important as recommended in the literature [7]. The formation of secondary species of Hg did not occur in the phenylation of MeHg standard solutions (Fig. 3). So it was possible to use $NaBPh_4$ as a derivatizating reagent to the

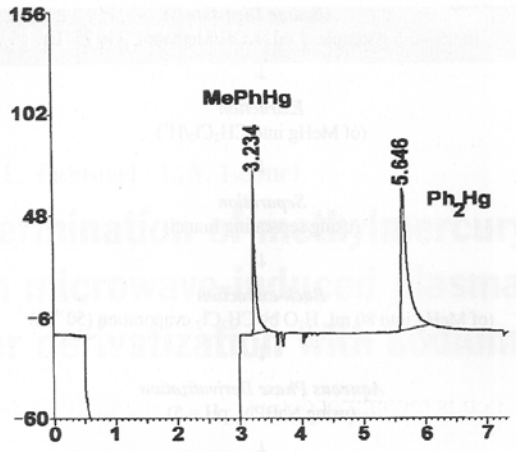

Fig. 2 Chromatogram of a phenylated standard Hg^{2+} (30 pg)

Table 2 Effect of $NaBPh_4$ concentration in the Hg^{2+} derivatization

[$NaBPh_4$] %	MePhHg area (1)	Ph_2Hg area (2)	(1)/(2) %
1	1983	5003	40
0.5	2058	5413	38
0.1	1958	4903	40
0.01	1780	4198	42

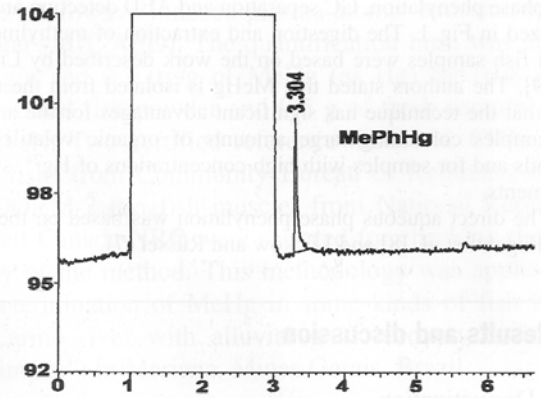

Fig. 3 Chromatogram of a phenylated standard MeHg (2.5 pg)

MeHg species. However, it is necessary to isolate the Hg^{2+} species before the MeHg derivatization.

3.2 Calibration

Calibration curves were prepared following two schemes: A) standard solutions of MeHg in the range 2.5–25 pg were submitted to the same treatment as the sample (Fig. 1); B) standard solutions with the same content of MeHg were directly phenylated. The calibration curves obtained by procedures A and B were linear ($r^2 \geq 0.99$) at the range (2.5–25 pg of MeHg as Hg) studied.

The MeHg content for CRM 464 obtained according to the two procedures was compared. Both procedures gave very similar results for CRM 464. Therefore procedure B was used for the subsequent determinations.

Table 3 Sample fish description

	Sample	Length (cm)	Wet weight (g)
Traíra (T1)	*Hoplias Malabaricus*	24.5	191.82
Traíra (T2)	*Hoplias Malabaricus*	18.0	84.66
Traíra (T2)	*Hoplias Malabaricus*	19.0	84.39
Traíra (T2)	*Hoplias Malabaricus*	19.5	99.97
Traíra (T2)	*Hoplias Malabaricus*	16.0	69.15
Traíra (T2)	*Hoplias Malabaricus*	15.5	55.03
Bagre (BG)	*Rhandia sp*	23.0	133.86
Bagre (BP)	*Rhandia sp*	16.0	39.76
Lambari (L)	*Asthyanax sp*	8.0	6.54
Lambari (L)	*Asthyanax sp*	8.5	7.39

Table 4 MeHg content in fish sample and in certified reference materials

Sample	MeHg obtained (μg/g)	MeHg certified (μg/g)
Traíra (T1)	0.62[a] ($n = 3$)	–
Traíra (T2)	0.39[a] ($n = 2$)	–
Bagre (BG)	0.46[a] ($n = 2$)	–
Bagre (BP)	0.35[a] ($n = 2$)	–
Lambari (L)	0.24[a] ($n = 2$)	–
Tilápia (T)	0.05[a] ($n = 2$)	
CRM 464	(5.54 ± 0.12)[b] ($n = 7$)	(5.50 ± 0.17)
DORM-2	(5.14 ± 0.17)[b] ($n = 9$)	(4.81 ± 0.32)

[a] wet weight
[b] corrected to dry mass
[n] number of determinations

3.3 Analytical application

Five fish samples collected from the Carmo river were analyzed using the methodology shown in Fig. 1. The fish samples were characterized according to the biological species, length and weight (Table 3). The MeHg content of these samples using the calibration curve B is shown in

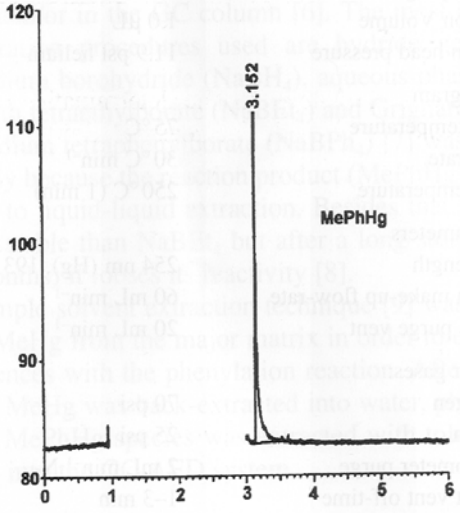

Fig. 4 Chromatogram of a fish sample (Traíra); MeHg concentration is 0.62 μg/g

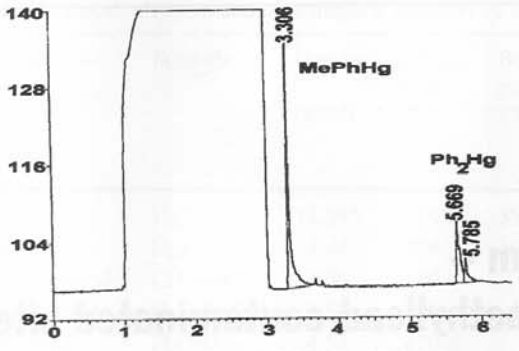

Fig. 5 Chromatogram of DORM-2 sample (dogfish muscle)

Table 4. As it could be expected, the concentration in the bigger fish (T1 and BG) was higher than in the smaller ones (T2 and BP). T was a fish sample *(Tilápia nigra)* from an uncontaminated area. In Fig. 4 a chromatogram of the phenylated sample T1 (Traíra) is shown.

3.4 Accuracy

The accuracy of the methodology employed was checked by analyzing about 0.075 g of the certified reference materials CRM 464 and DORM-2. The results obtained which were corrected to dry mass basis as recommended in the certificate of analysis are shown in Table 4. In the chromatogram shown in Fig. 5 the presence of a peak of Ph_2Hg can be observed. This indicates that the Hg^{2+} in DORM-2 was co-extracted. Then the higher result obtained for DORM-2 can be due to the methylation of the Hg^{2+} present increasing the MeHg concentration.

3.5 Detection limit of the GC-AED method

The detection limit (S/N 3:1) for the optimized GC-AED method was found to be 0.4 pg of MeHg as Hg.

4 Conclusions

A versatile methodology for the determination of MeHg in fish tissue is proposed. The detection limit for the optimized GC-AED method was 0.4 pg of MeHg (as Hg). The absolute quantification limit of 2.5 pg of MeHg (as Hg) corresponds to 0.1 µg/g of MeHg (as Hg) when natural samples were analyzed. This is due to the use of liquid-liquid extraction of MePhHg after the derivatization step. The quantification limit obtained could be improved by a factor of 2000 by combination with other techniques like concentration of the derivatizated species before introducing into the GC.

The fish samples collected from the Carmo river showed high MeHg contents when compared to the maximum value of 0.5 µg/g of mercury in fish and fish products which is recommended by the National Food Authority in Australia [11].

It was possible to use $NaBPh_4$ as a derivatizating reagent to the MeHg species after isolating the Hg^{2+} species. Other kinds of MeHg extraction will be tested in order to optimize the methodology for samples that contain high inorganic Hg content.

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