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Short communication

GFAAS determination of mercury in muscle samples of fish from Amazon, Brazil

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ABSTRACT

In the present study, a simple, rapid and sensitive method was developed for the determination of mercury concentrations in the muscle tissue of fish from the Brazilian Amazon using graphite furnace atomic absorption spectrometry (GFAAS) following acid mineralization of the samples in an ultrasonic cold water bath. Using copper nitrate as a chemical modifier in solution and sodium tungstate as permanent modifier, we were able to attain thermal stabilization of the mercury up to the atomisation temperature of 1600 °C in the GFAAS assay. The calculated limits of detection (LOD) and quantification (LOQ) were 0.014 and 0.047 mg kg^{-1}, respectively.

1. Introduction

Chemical and biological pollution in the Madeira river basin is one of the primary social and environmental problems in the Amazon. Mercury contamination, which scarred the Amazon from the 1980s through the mid-1990s, is once again a topic of discussion due to the environmental hazards associated with gold mining activities and the danger of contamination that these activities pose to society. The construction and use of the Jirau hydroelectric plant in the Madeira River basin may, because of environmental changes, modify the chemical species of mercury, resulting in high levels of soluble forms. Mercury species, including methylmercury, are present in aqueous medium, can be absorbed by aquatic biota and accumulate in organisms. As a result, the level of mercury is magnified at high levels of the food chain (Pfeiffer, Lacerda, Salomons, & Malm, 1993).

In the Amazon, fish is the main source of food for coastal communities. Several studies performed over the last two decades indicate high concentrations of mercury in Amazonian fish. In general, all of these studies suggest that bioaccumulation throughout the food chain is the reason behind the high mercury levels in Amazonian fish. Mercury from natural and/or anthropogenic sources, upon entering aquatic ecosystems, participates in biogeochemical cycles mediated by microorganisms. In these cycles, mercury is chemically transformed, bioaccumulated and biomagnified in the food chain. Thus, predatory fish, including the gilthead bream and peacock bass, accumulate high levels of mercury and can act as a vehicle of this toxin to their consumers, such as reptiles, birds and humans. Thus, the consumption of fish may pose a risk to human health (Bidone, Castilhos, Santos, Souza, & Lacerda, 1997).

The determination of the concentrations of mercury species in biological samples is challenging due to low concentrations of the mercury species, and the evaluation of toxicity is difficult because the toxicity depends on the specific mercury species. Several studies in the literature describe methods for the determination of the concentrations of mercury species using atomic spectrometry techniques. Examples of these techniques include flow injection analysis (FIA), coupled with cold vapour atomic absorption spectrometry (CVAAS) and assisted by sample mineralization in a microwave oven for total mercury determination (Gallignani et al., 1998); solid-phase methylmercury preconcentration and concomitant determination by CVAAS (Mondal & Das, 2003); acid extraction and slurry sampling procedures for the determination

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of methylmercury and total mercury concentrations by GFAA (Bermejo-Barrera, Verdura-Constenla, Moreda-Piñeiro, & Bermejo-Barrera, 1999); and the determination of total mercury concentrations in biological samples using extraction with quaternary ammonium salts and subsequent quantification by CVAAS (Torres, Vieira, Ribeiro, & Curtius, 2005). The major challenge in the determination of mercury concentrations using GFAAS is preventing the loss of mercury by volatilization during the drying and pyrolysis of the sample. This analyte loss can be prevented with the use of chemical modifiers that stabilize mercury prior to atomization. Several types of chemical modifiers for the determination of mercury concentrations have been reported in the literature. Palladium, magnesium–palladium mixtures and gold are the most commonly used chemical modifiers (Bermejo-Barrera, Moreda-Piñeiro, Moreda-Piñeiro, & Bermejo-Barrera, 1994).

In this study, we developed a GFAA-based method for the determination of mercury concentrations in the muscle tissue of dourada (*Brachyplatystoma rousseauxii*), pacu (*Mylossoma* sp., *Myleus* sp.) and jaraqui (*Semaprochilodus* sp.), fish from the Brazilian Amazon, using copper nitrate and sodium tungstate as chemical modifiers to stabilize the mercury after the acid mineralization of samples in an ultrasonic water bath.

2. Materials and methods

2.1. Reagents, standard solutions and samples

High purity deionised water (18.2 MΩ cm^{-1}) produced using an ELGA LabWater PURELAB Ultra purification system (Saint Maurice, France), high purity sulfuric acid (Merck) and hydrogen peroxide (Merck) were used throughout the study. The working solutions containing copper and/or tungsten were prepared by diluting 1 g L^{-1} copper nitrate and/or sodium tungstate (Merck) stock solutions with ultrapure water.

All vials for the storage of samples and standard solutions, all glassware and all autosampler cups of the atomic absorption spectrometer were washed with 10% v/v nitric acid for 24 h, rinsed with ultrapure water and dried by shaking before use.

2.2. Equipment

The mercury levels were determined using a SHIMADZU AA 6800 atomic absorption spectrometer with an ASC-6100 automatic sampler and an electrothermal atomization system in a GFA-EX7 graphite furnace. A UNIQUE ULTRASONIC CLEANER, model USC1800A, was used in the mineralization of the samples of fish muscle tissue.

2.3. Mineralization of samples

The muscle tissue samples of each fish species studied were mineralized using a procedure previously described in the literature (Neves et al., 2009; Silva, Padilha, Pezzato, Barros, & Padilha, 2006; Silva et al., 2007) with several modifications, as described below: approximately 250 mg (weighed in triplicate using an analytical scale, accurate to hundredths of a milligram) of the fish muscle tissue was transferred to a 25 mL digestion flask. Concentrated sulfuric acid (3 mL) and 30% (w/w) hydrogen peroxide (1 mL) were transferred to each digestion flask. The set of digestion flasks was placed in the UNIQUE ultrasonic water bath at a temperature of 40 °C and sonicated at 135 W until the mineralization of the samples was complete (transparent extract). Then, the acid extracts were transferred to 10 mL volumetric flasks, and the volume was brought up to 10 mL with ultrapure water. The Fish Protein DORM-4 NRC certified standard containing 0.410 ± 0.055 mg kg^{-1} of total mercury was used to validate the mineralization method. The volume of the acid extract obtained for this standard was adjusted to 5 mL with ultrapure water.

2.4. Determination of mercury concentrations by GFAAS

A SHIMADZU model AA-6800 atomic absorption spectrometer was used for the determination of the mercury concentrations. This spectrometer was equipped with a background absorption corrector with a deuterium lamp and a self-reverse system (SR), a pyrolytic graphite tube with an integrated platform and an ASC-6100 automatic sampler. A Shimadzu hollow cathode mercury lamp was used and operated at a minimum current of 12 mA and a maximum current of 400 mA (current used in background correction – BG). The wavelength was 253.7 nm, and the spectral resolution was 0.5 nm. Argon was used as the inert gas, and a constant flow of 1 L min^{-1} was maintained during the entire heating program except for the atomization stage, during which the gas flow was stopped. The absorbance signals were measured based on the peak area. The inner walls of the pyrolytic graphite tubes with integrated platforms used in the mercury analyses were coated with tungsten. For this purpose, 25 μL aliquots of 1000 mg L^{-1} sodium tungstate modifier solution were injected into the atomizer, which was then subjected to the program described by Silva et al. (2007). Tungsten ions were deposited on the graphite tube platform with heating up to 500 °C, forming a tungsten carbide layer that acts as chemical modifier. We were able to use the graphite tube in 483 firings of the samples after this treatment.

2.4.1. Preparation of the analytical curve

The analytical curve was prepared using a stock standard solution of mercury (Merck). The mercury standards were prepared using the autosampler. Aliquots of 2, 4, 8, 12 and 16 μL of a 2.50 μg L^{-1} mercury standard solution were combined with 4 μL of 100 mg L^{-1} of Cu(II) nitrate solution and sufficient ultrapure water to bring the final volume of the solutions to 20 μL. The concentrations of the mercury standard solutions ranged between 0.25 and 2.00 μg L^{-1}. These solutions were then injected into the spectrometer's graphite tube coated with tungsten carbide using the micropipette autosampler. The absorbance measurements were performed in triplicate, and the graphite tube's heating program, optimized for mercury analysis, is described in Table 1.

2.4.2. Analytical procedures

After the sample preparation stage (preparation of acid extracts), 20 μL aliquots, obtained by mixing 10 μL of acid extract with 4 μL of 100 mg L^{-1} Cu(II) nitrate solution and 6 μL of ultrapure water, were injected into the graphite tube coated with tungsten carbide using the micropipette autosampler. The measurements were performed in triplicate, and the graphite tube's heating program, optimized for mercury analysis, is described in Table 1.

Table 1
Graphite tube heating program optimised for mercury determination in acid extracts obtained from mineralised samples of fish muscle.

Steps	Temperature (°C)	Stages		Argon flow (L min^{-1})
		Ramp (s)	Hold (s)	
Drying	90	5	0	1
Drying	120	5	5	1
Pyrolysis	250	5	5	1
Pyrolysis	800	5	10	1
Atomization	1600	2	5	0
Cleanup	1800	5	0	1

3. Results and discussions

3.1. Optimization of the instrumental conditions for the determination of mercury concentrations by GFAAS

In the determination of the concentrations of metals and/or metalloids by GFAAS, obtaining accurate and reproducible analytical results depends on the optimization of the pyrolysis and atomization temperatures. Thus, pyrolysis and atomization curves were constructed to determine the optimal temperatures of mercury pyrolysis and atomisation using the standard solutions and the acid extracts of muscle tissue samples. The effects of the pyrolysis and atomization temperatures on the absorbance signal obtained for mercury in a 1.00 μg L^{-1} standard solution and in the acid extracts of muscle tissue samples, both treated with copper(II) nitrate as a chemical modifier and analyzed using a pyrolytic graphite tube with an integrated platform coated with tungsten carbide, are shown in Figs. 1 and 2.

The pyrolysis temperature of 800 °C was chosen because, as shown in Fig. 1, the mercury absorbance signal remained constant starting at 400 °C and exhibited a rapid decrease starting at 900 °C. All remaining experiments were conducted at 800 °C for the pyrolysis stage because the mercury species were thermally stable up to this temperature.

Regarding the atomization temperature (Fig. 2), the mercury absorbance signals were constant from 1300 °C to 1700 °C. The atomization temperature of 1600 °C was selected for all remaining experiments because a higher linearity of the standard curve was attained at this temperature, in addition to better reproducibility and repeatability of the measurements. The good thermal stability of mercury observed in these experiments demonstrates the efficacy of the Cu(II) chemical modifier when co-injected with the sample and of the tungsten carbide, which acted as a permanent chemical modifier. It is possible that with the gradual temperature increase (1000–1700 °C), Hg(II) ions formed metal bonds with the copper during the process of reduction to the elemental state and, subsequently, with the carbide tungsten film adhered to the graphite tube platform, causing a significant increase in the mercury volatilization temperature and, consequently, in the temperature of atomization (Silva et al. (2006), (2007)).

3.2. Obtainment of the analytical curve

Using the optimized parameters (pyrolysis and atomization temperature), an analytical curve for mercury was constructed in the concentration range of 0.25–2.00 μg L^{-1}. The analytical curve for mercury corresponded to the equation:

$$C_{(Hg)} = \text{Ab.} - 0.0016/0.048 \quad (1)$$

where $C_{(Hg)}$ is the concentration of mercury in μg L^{-1} and Ab. is the absorbance signal. The analytical curve enabled the calculation of the characteristic mass (m_0), the limit of detection (LOD) and the limit of quantification (LOQ); these analytical parameters were crucial for the validation of the proposed mercury quantification process (Currie, 1999). The characteristic mass calculated for the 1.00 μg L^{-1} mercury standard was 1.70 pg, and the limits of detection (LOD) and quantification (LOQ), calculated based on the standard deviation of 10 readings of the standard solution blank and on the slope of the analytical curve (LOD = 3σ/slope and LOQ = 10σ/slope), were 27 and 89 ng L^{-1} mercury, respectively. The graphite tube's lifetime was equivalent to 483 firings, confirming that the sample preparation method using an ultrasonic water bath effectively mineralized the organic matter and preserved the mercury in the obtained aqueous extract.

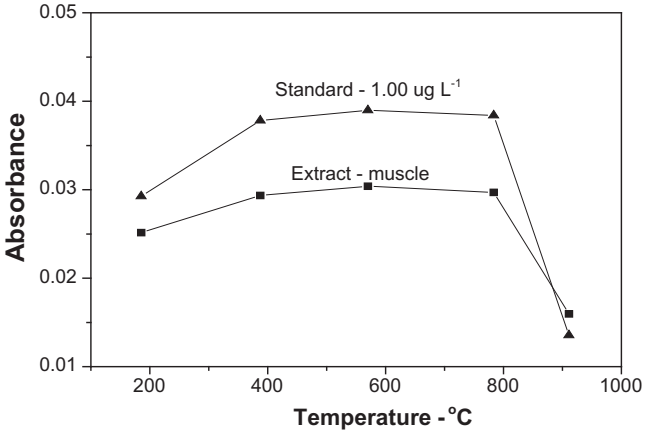

Fig. 1. Pyrolysis temperature curves of the acid extracts from dourada muscle samples and of the standard containing 1.00 μg L^{-1} of mercury.

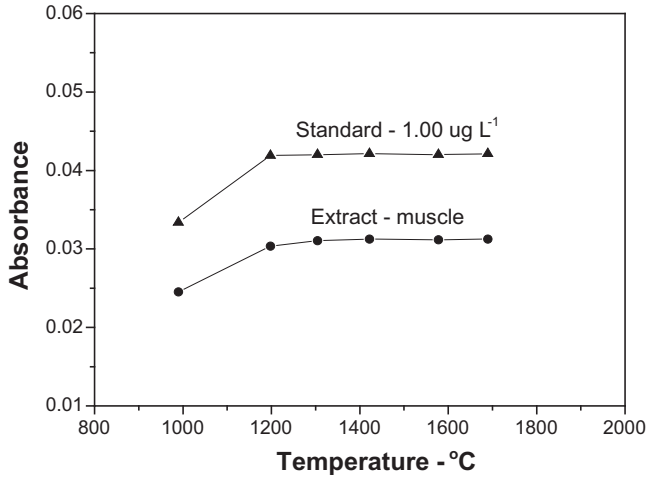

Fig. 2. Atomization temperature curves of the acid extracts from dourada muscle samples and of the standard containing 1.00 μg L^{-1} of mercury.

3.3. Suitability of the proposed method for the determination of mercury concentrations

After the optimization of the parameters of the extraction process and the quantification by GFAAS, the proposed method was used to determine the mercury concentrations of muscle tissue samples from dourada, pacu and jaraqui, fish from the Brazilian Amazon. The analytical results obtained in these determinations are listed in Table 2. The mercury concentrations determined for these three species fall below the maximum of 500 μg kg^{-1} permitted by the World Health Organization (WHO). The reliability of the proposed method for total mercury determination in fish samples was tested with recovery experiments by spiking mercury standard solution into the mineralization solutions. The analytical results and recoveries are also listed in Table 2. It can be observed that the analytical recovery percentages obtained in the three fish digest solution present in the range of 98.90–99.50%, which can be considered good analytical recovery. The accuracy of the method was assessed using the Fish Protein DORM-4 NRC certified standard. The mercury concentration determined for the DORM-4 NRC was 0.403 ± 0.012 mg kg^{-1} (n = 6). The concentration value determined is close to the certified value (DORM-4 NRC – 0.410 ± 0.055 mg kg^{-1}). The precision of method (repeatability and reproducibility) also was evaluated by the relative standard

Table 2
Analytical results and of recoveries for total mercury determination in fish samples.

Samples	Determined Concentration in samples[a] (mg kg^{-1})	Determined Concentration in spikes[a,b] (mg kg^{-1})	Recovery (%)
Dourada	0.331 ± 0.007	0.429 ± 0.01	99.50 ± 2.30
Pacu	0,081 ± 0.002	0.179 ± 0.005	98.90 ± 2.7
Jaraqui	0.132 ± 0.004	0.230 ± 0.006	99.10 ± 2,60

[a] Average value ± standard deviation (n = 6).
[b] Determination after adding 0.100 mg kg^{-1}.

deviation obtained in the mercury concentration determined for the DORM-4 NRC. In this calculus was used the HORRAT value (Mcclure & Lee, 2003), which was calculated dividing the RSD_R obtained of collaboratively (RDS_R = 3.92%) by $PRSD_R$ calculated from the HORWITZ equation ($PRSD_R$ = 2.30), (Horwitz & Richard, 1997). The precision of the method determined using these calculus conditions was 1.70%. The (LOD) and (LOQ) determined in relation to certified standard, using 0.01 g of Fish DORM-4 NRC and the method of analysis proposed were 0.014 and 0.047 mg kg^{-1}, respectively. It can be observed that the mercury values determined for samples of fish were all higher than the method's LOQ. Moreover, the digestion method proposed has the advantage of allowing the complete mineralization of the muscle tissue samples without the use of high-temperature heating and the time of determination of mercury by GFAAS is lower than the other methods described in the literature (Bermejo-Barrera et al., 1999; Gallignani et al., 1998; Moreda-Piñeiro, López-Mahía, Muniategui-Lorenzo, Fernández-Fernández, & Prada-Rodríguez, 2002; Zhang & Adeloju, 2012: Bermejo-Barrera et al., 1994;). These advantages strengthen the use of GFAAS in the determination of mercury in biological samples.

4. Conclusions

The proposed acid digestion procedure using an ultrasonic water bath enabled the complete mineralization of the fish muscle tissue samples for the determination of the total mercury concentration by GFAAS. The combination of copper nitrate as a chemical modifier and tungsten carbide as a permanent modifier thermally stabilized the mercury up to the atomization temperature of 1600 °C, enabling the determination of mercury concentrations in ppb. Therefore, the proposed method can be used to monitor the levels of mercury in fish muscle tissue samples.

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