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Evaluation of drying conditions of fish tissues for inorganic mercury and methylmercury speciation analysis

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ABSTRACT

The influence of drying conditions on the behavior of Hg species (Hg^{2+} and CH_3Hg^+) present in fish tissues was evaluated. Drying conditions were evaluated for six fish species using air circulation drying oven in different temperatures (50 to 175 °C) and lyophilization (0.25 mm Hg, −2 °C). Evaluation of drying step was based on losses and conversions of original Hg species after each drying condition. The extraction efficiency was determined by comparing the concentration of total Hg in digested samples (wet digestion in closed system using HNO_3) with extracted Hg using L-cysteine solution. Chemical vapor generation-inductively coupled plasma mass spectrometry (CVG-ICP-MS) and liquid chromatography-chemical vapor generation-inductively coupled plasma mass spectrometry (LC-CVG-ICP-MS) were used for the determination of total Hg and Hg species, respectively. The accuracy was evaluated using certified reference materials and an agreement better than 97% with certified values was obtained for CH_3Hg^+ and total Hg. The relative standard deviation of the proposed method was below 5.5%. Limit of detection of 1.7 and 2.3 ng g^{-1} as Hg was obtained for Hg^{2+} and CH_3Hg^+, respectively. Results showed that with drying temperatures above 100 °C losses and conversions of CH_3Hg^+ to Hg^{2+} can occur for some fish species.

1. Introduction

One of the main problems related to chemical speciation analysis is to ensure the integrity of the species throughout the analytical procedure. However, several steps of analytical procedure may result in changes of the chemical species, which can lead to an erroneous interpretation of analytical results [1–3]. Considering the analysis of speciation, Hg could be pointed out as one of the most studied element, as well as As, Cr, Sb, Se, Sn, among others [4–10]. As observed for other elements, Hg presents some physical-chemical and toxicological characteristics that depends on the chemical form. Mercury can be found linked to organic groups, such as methyl, ethyl, and related compounds, where the related species are, in general, considered more toxic than elemental Hg and even its inorganic species [11–13].

The main exposure pathway of Hg to humans is through the consumption of fish where Hg is present mainly in the methylated form. Methylmercury (CH_3Hg^+) is efficiently adsorbed from the gastro-intestinal tract, and it passes the blood-brain and placenta barriers. Consequently, Hg compounds are associated to several diseases, mainly those related to central nervous system [14–17]. Hence, studies have been performed in order to check and control Hg levels and its chemical species in several kind of samples, mainly in fishes. In this sense, several sample preparation methods to speciation analysis were developed in recent decades [18,19].

Most of samples for Hg speciation analysis require a drying step, mainly biological samples. In general, dried samples are more suitable for homogenization, analyte extraction and sample conservation. Among the drying procedures commonly used in sample preparation, it can be highlighted the use of lyophilization and drying oven with forced air circulation [20].

Drying performed in ovens is frequently used as a pre-treatment for most of chemical analysis. It is considered practical and relatively cheaper than other drying procedures, such as lyophilization [21]. On this aspect, lyophilization is a drying process where the sample previously frozen is dried by sublimation using low temperature drying at reduced pressure. The key steps normally involved in this process are freezing, primary drying, and secondary drying [22]. Due to the low temperatures required in this process, many methods for speciation analysis have been employed using lyophilization in order to maintain the analytes integrity [23].

Currently, most of sample preparation methods related to Hg speciation analysis for biological samples employs some types of drying treatment [24–26]. However, little is known about the influence of drying on preservation and integrity of Hg species [27]. Furthermore, many studies were performed using dried certified reference materials (CRMs) which could not represent a "real sample", since these materials generally exhibit higher stability, [28–30] and therefore could be more resistant to variation such as temperature.

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In this sense, just a few systematic studies were performed taking into account the problems related to sample preparation step, especially when it is related to losses or conversion of Hg species during drying. Therefore, considering the volatile characteristics of some Hg species and the few studies related to the influence of drying step during the sample preparation, the aim of this work was to evaluate the influence of drying conditions on the possible interconversion of Hg species in fish samples during drying step. Six fish species were analyzed and mercury species were determined by liquid chromatography-chemical vapor generation-inductively coupled plasma mass spectrometry (LC-CVG-ICP-MS). Accuracy was evaluated by the use of certified reference materials of fish tissues. A comparison of two kind of drying techniques (air circulation oven and lyophilization) was performed and the conversion and losses of Hg species in fish samples were evaluated.

2. Experimental

2.1. Instrumentation

The evaluated drying processes were performed using a freeze dryer (Model LH 2000/3, Terroni Fauvel, Brazil) and an air-circulation drying oven (model 400/2ND, Nova Ética, Brazil). After drying, samples were ground in a cryogenic mill (model 6750, Spex Certiprep, USA).

Mercury species separation was carried out with a liquid chromatography (LC) system consisting of a quaternary pump (Model Series 200, PerkinElmer, USA) equipped with a Rheodyne six-port injector valve and a 200 μL sample loop. For separation of mercury species, a Si–C_{18} reversed-phase column (Discovery, 250×4 mm) with particle size of 5 μm was used. A guard column Si–C_{18} (Allchrom, 4×3 mm) packed with the same stationary phase was used. The mobile phase flow rate was kept at 1.0 mL min^{-1} and the elution was carried out in isocratic mode. The LC system was coupled to a chemical vapor generation system (CVG) that consists of a peristaltic pump (Ismatec, Switzerland) and a U-type gas-liquid separator. Tygon tubings of 1.14 mm i.d. were used for carrying 0.2% (m/v) $NaBH_4$ and 1.0 mol L^{-1} HCl solutions. These solutions were added to the column outlet solution using an X-type connector (0.8 mm i.d.). The mixture was pumped to the gas-liquid separator and mercury measurements were performed by ICP-MS. The CVG conditions were optimized and adapted from the procedure used by Pilz et al. [26]. The total Hg concentration in final digests was determined by CVG-ICP-MS using a homemade flow injection system, which was constructed as described in previous work [31].

Mercury determination was carried out with an inductively coupled plasma mass spectrometer (Model ELAN DRC II, PerkinElmer SCIEX, Canada). The operational conditions are shown in Table 1. Single ion monitoring at *m/z* 202 was used for data collection, which were obtained by integrating peak area, using Chromera software (PerkinElmer, version 1.2, 2006). The on-line LC-CVG-ICP-MS hyphenated system is shown in Fig. 1. Argon 99.996% (White Martins-Praxair, Brazil) was used for plasma generation and carrier gas.

In order to perform the total Hg determination, a microwave oven (Multiwave 3000, Anton Paar, Austria) equipped with eight high-pressure quartz vessels was used for sample digestion. The vessels capacity was 80 mL and the settings for maximum pressure and maximum temperature were 80 bar and 280 °C, respectively. This equipment was used for both digestion methods, microwave assisted digestion (MAD) and microwave induced combustion (MIC) [32,33].

A centrifuge (Model NT 810, Nova Técnica, Piracicaba, Brazil) was used for phase separation after analyte extraction procedures.

2.2. Samples, reagents and standards

Six frozen fish samples, bearded brotula (*Brotula barbata*), yellowfin tuna (*Thunnus albacares*), pirarucu (*Arapaima gigas*), salmon (*Salmo salar*), whitemouth croaker (*Micropogonias furnieri*) and mullet (*Mugil brasiliensis*) from different regions of Brazil were acquired in a local market (Santa Maria City, Rio Grande do Sul State, Brazil). According to information on the packaging of samples, Pirarucu was from Amazon region (rivers) and the other fish species were from Brazilian seacoast. Two CRMs, DORM-2 (Dogfish muscle) and DOLT-3 (Dogfish liver), from National Research Council of Canada (Ottawa, Canada) were used for accuracy evaluation.

Water was distilled, deionized and further purified using a Milli-Q system (Millipore Corp., USA). Analytical-grade nitric and hydrochloric acids (Merck, Germany) were purified in a sub-boiling system (Model duoPUR 2.01E, Milestone, Italy). Inorganic mercury (Hg^{2+}) standard stock solution (Titrisol, 1000 mg L^{-1}, Merck) and a 1000 mg L^{-1} Hg (as methylmercury, CH_3Hg^+) solution (prepared from CH_3HgCl dissolved in methanol, Aldrich, USA) were used. Solutions were stored in dark glass bottles at 4 °C. Analytical solutions ranging from 0.10 to 5 μg L^{-1} Hg (for Hg^{2+} and CH_3Hg^+) were prepared in 0.6% (m/v) L-cysteine solution (the same solution used for analyte extraction) by appropriate dilution of the stock solution. All Hg calibration solutions were prepared just before its use. Reagent-grade L-cysteine and sodium tetrahydroborate were obtained from Vetec (Brazil). Polypropylene vials, glass and quartz materials were soaked in 1.4 mol L^{-1} HNO_3 for 24 h and further washed with ultrapure water before use.

For Hg species separation by liquid chromatography, a mobile phase containing 0.1% (m/v) L-cysteine at pH 4.0 was used. These conditions were selected based on previous work [26].

Table 1
Operational conditions of LC-CVG-ICP-MS system.

Parameter	Conditions
LC	
Column	Si–C_{18} (250×4 mm, 5 μm)
Mobile phase (L-cysteine)	0.1% (m/v) (pH 4.0) (1.0 mL min^{-1})
Injected volume	200 μL
CVG	
Acid solution (HCl)	1.0 mol L^{-1} (2.0 mL min^{-1})
Reductant solution ($NaBH_4$)	0.2% (m/v) (2.0 mL min^{-1})
Carrier gas flow rate	1.12 L min^{-1}
ICP-MS	
RF Power	1300 W
Plasma gas flow rate	15 L min^{-1}
Auxiliar gas flow rate	1.2 L min^{-1}
Sampler and skimmer cones	Pt
Isotope monitored (*m/z*)	^{202}Hg
Dwell time	250 ms

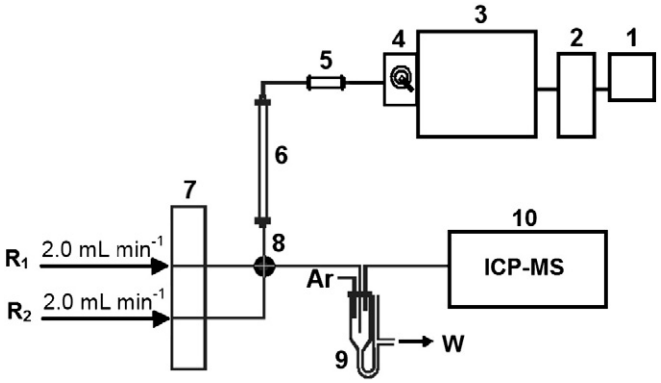

Fig. 1. Schematic diagram of the LC-CVG-ICP-MS system. R_1: HCl (1.0 mol L^{-1}); R_2: $NaBH_4$ (0.2% m/v); W: Waste; 1. Mobile phase (0.1%, L-cysteine); 2. Vacuum degasser; 3. LC pump (1.0 mL min^{-1}); 4. Injector (200 μL sample loop); 5. Si–C_{18} guard column (4×3 mm); 6. Si–C_{18} column (250 × 4.6 mm, 5 μm); 7. Peristaltic pump; 8. Confluence; 9. Gas-liquid separator; 10. Detector (ICP-MS).