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Validation of Methods Employing Fast Alkaline Solubilization to Determine Cadmium in Fish Liver, Spleen, Gills and Muscle by Graphite Furnace Atomic Absorption Spectrometry

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ABSTRACT

The validations of analytical methods were performed to determine Cd in fish liver, spleen, gill, and muscle by graphite furnace atomic absorption spectrometry (GF AAS). The methods were based to alkaline solubilization of the samples with benzyltrimethylammonium hydroxide 40% v/v in water (Universol®), and when added to each sample, immediately solubilized the samples and formed an indefinitely stable and highly homogeneous solution, without a need for temperature, microwaves or any other apparatus. Ru was the best permanent modifier (500 μg) for all samples except for liver, while permanent Ir (500 μg) proved best for liver. The optimum pyrolysis temperatures were 450 °C for liver and spleen, 500 °C for gill, and 400 °C for muscle, while for atomization, the optimum temperatures were 1800, 1100, 1600, and 900 °C, respectively. No matrix effects were observed for liver and gill when external calibration was performed. For spleen and muscle, matrix effects were observed and matrix matching calibration was used. The limits of quantifications were 0.13, 1.20, 0.34, and 1.80 μg g^{-1} for liver, spleen, gill, and muscle, respectively. The absorption signals obtained for each sample were symmetrical and returned to baseline in less than 5 s. Recoveries levels (three levels of Cd for each matrix) ranged from 87.1 to 108.2. For the analysis of seven certified reference materials, the obtained values ($n = 5$ samples of each material) were not statistically different from the certified values (95% of confidence). This reagent (Universol®) promoted an immediate solubilization of the samples, thus forming an indefinitely stable suspension which can be an alternative for fast solubilization of any biological sample, such as food and other organic material.

1. Introduction

Cadmium, an IARC group 1 human carcinogen [1], is highly toxic to the renal, skeletal, nervous, respiratory and cardiovascular systems [2].

The contamination of aquatic environment occurs by numerous toxic agents that usually are produced by industrial, agricultural and domestic activities resultants from inappropriate use of water resource. This fact contributes to the degradation of the various environmental compartments (water, sediments, air and soil), as well as affects the health of individuals who lives in this area [3]. Heavy metals such as cadmium are pollutants highly dangerous to the ecosystem due to their persistence, bioaccumulation and genotoxicity, whose effects are achieved even in lower concentrations [4, 5].

The genotoxic effects of cadmium have been identified by interactive capabilities of this metal in inducing oxidative stress besides decreasing the cellular antioxidants [6–8].

Cadmium-induced oxidative stress is also associated to peroxidation of lipids. Their damage induce changes in permeability, fluidity, ion transport, inhibition of metabolic processes, modifications of transmembrane potential (depolarization), release of mitochondrial calcium, uncoupling and activation of caspase-3, generating fragmentation of DNA that could reach to apoptosis [8–10].

Tilapia (*Oreochromis niloticus*) stands as a good model in bioassays by presenting high prolificacy, resistance too many diseases [11], rapid growth and hardiness [12]. As an environmental bioindicator, tilapia presents considerable importance and, due to its easy adaptation to new environments, has a widespread distribution, responds well to chemicals and is used as protein resource for humans [13, 14].

In a work made by Pereira et al. [15], the best results obtained by cadmium in drinking water were with use of rhodium permanent modifier (500 μg) in a wall graphite tube with a characteristic mass of 1.0 pg (recommended by the manufacturer of 1.0 pg).

A method to test the feasibility of Ru as a permanent modifier for the determination of Cd in biological samples treated with tetramethylammonium hydroxide (TMAH) by ET AAS was investigated. In all certified reference samples the obtained results are in agreement with the certified. The use of TMAH needs 1 h at 60 °C in water bath to entirely solubilization of the samples [16].

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In a work presented by da Silva et al. [17] iridium (500 μg), rhodium (500 μg) and a mixture of both (250 μg of Ir plus 250 μg of Rh) were investigated for Ag, As, Bi, Cd and Sb in some certified reference materials (trace elements in water, 1643d and oyster tissue, SRM 1566a). For cadmium the best results in agreement with the certified and determined values for both certified samples were obtained with Ir or Ir + Rh permanent modifiers and for this metal. Poor results were obtained when using only the Rh permanent modifier.

Sobhanardakani et al. [18] proposed a method using anodic stripping voltammetry (ASV) to determine Cd (also Cu and Zn) in the muscle, gill, and liver tissues in five species of fish. The sample preparation was made using HNO_3 and $HClO_4$ in a hot plate. The concentrations of Cd(II), Zn(II), and Cu(II) were calculated in the sample solutions by using the standard addition method.

In another work [19], researchers studied the concentration of some heavy metals (including cadmium) in water from Lake Qarun, a private fish farm and "Sanhour River" (both in Egypt) and in four fish species by flame atomic absorption spectrometry (FAAS). Water was analyzed "in natura", together with fish organ samples, using the method of the Association of Official Analytical Chemists (AOAC) [20].

Reynders et al. [21], developed methods to determine total and dissolved cadmium (copper and zinc) in water and caged carp (*Cyprinus carpio*) and resident roach (*Rutilus rutilus*) at four locations along the Grote Nete River system (Belgium). The water was analyzed by using inductively coupled plasma mass spectrometry (ICP-MS). Tissue samples were digested using HNO_3 and microwave radiation [22]. Metal concentrations (Cd, Cu and Zn) in tissue samples were measured using ICP-AES (inductively coupled atomic emission spectrometry) or GF AAS.

Costa et al. [23] had validated an analytical method for the determination of cadmium in fish by GF AAS with Zeeman background correction. The decomposition of organic matter was performed with nitric acid in closed vessels and assisted by microwave radiation.

Sures et al. [24] had developed a method for the determination of cadmium and lead in fish by GF AAS. The sample solubilization was made with nitric acid in a microwave oven. The metal concentration in the GF AAS was obtained using standard addition calibration.

Direct solid sampling analysis [25] was proposed as a good method to determine cadmium and lead in fresh fish samples by ET AAS (DS-ET AAS, direct sampling electrothermal atomic absorption spectrometry). Aliquots between 2 and 8 mg, depending on the analyte concentration, were weighed directly onto the solid sampling platforms, and 10 mL of chemical modifier was added over the sample, and then the platform was transferred to the graphite tube and the determination of Cd and Pb was performed.

Ghanemi et al. [26], had developed a method for separation and preconcentration using sulfur-nano-nanoparticle-loaded alumina as adsorbent for the solid phase extraction (SPE) to determine cadmium (and Cu, Pb and Zn) in marine samples by FAAS after digestion in a furnace at 450 °C for 3 h [26]. Analysis of the certified reference material was in agreement with the certified value.

Yi-Ching and Shiuh-Jen [27] proposed a method to determine Cd (and Zn, Cu and Pb) in fish samples using slurry sampling and ETV-ICP-MS (electrothermal vaporization inductively coupled mass spectrometry) and ultrasonic nebulization and Na_4NO_3 as the chemical modifier. The applicability of the method to real samples was demonstrated by the analysis of reference materials. According to the authors the determined values were in agreement with the certified.

Aranha et al. [28] proposed an alternative procedure for sample calcination to determine Cd in meat, currently used by the Brazilian Federal Department of Agriculture (MAPA). The proposed method uses tetramethylammonium hydroxide (TMAH) which provides stable and homogeneous slurry at room temperature in 10 min.

In studies using other metals, Kruger et al. [29] studied alkaline solubilization with TMAH for Al distribution in human placental tissues by ET AAS.

Dogan and Sanin [30] used alkaline solubilization with NaOH and microwave irradiation (160 °C) to solubilize waste active sludge (WAS). In a third study, at pH 12, a significant improvement of the anaerobic digestibility of WAS and major reduction in the quantity of sludge were observed.

Silva et al. [31] used alkaline solubilization of biological materials for trace element analysis with methanolic solution of TMAH and maintained the samples overnight at room temperature or applied heat and/or ultrasonic agitation.

Soares and Nascentes [32] presented a simple method to determine Pb in lipstick using TMAH heating samples in a water bath for 60 min.

Matusiewicz and Golik [33] studied the determination of trace elements in biological materials by microwave ICP-OES (MIP-OES), adding TMAH in the samples and sonicated them (40 W) for 2–4 min.

In another study [34], the solubilization of biological materials for trace element determination by ET AAS was done using TMAH by heating at 60–70 °C for ca. 2 h.

Matusiewicz and Slachcinski [35] also used TMAH to solubilize biological samples. After the addition of the reagent to the samples, they were sonicated continuously for 3 min at 60 W. After 2 h, the samples were completed to the final 10 mL volume with water. The iodine content in the samples was determined by ultrasonic nebulizer, vapor generation and ICP-OES (US-VG-ICP-OES).

Tertiary amines (CFA-C) have also been used for alkaline solubilization. Krushevska and Barnes [36] determined low silicon concentrations in food and coral soil by ICP-OES solubilizing the samples with CFA-C using microwave radiation. Krushevska et al. [37] proposed a method for semiquantative ICP-MS protocol for biological materials analysis using CFA-C and microwave assisted procedure. Nóbrega et al. [38] used the solubilization of milk samples with CFA-C to determine trace and major elements using ICP-OES.

The objective of this study was to test the use of reagent Benzyltrimethylammonium hydroxide 40% m/v in water (international patent number – WIPO, WO2012045138) [39], to the immediate solubilization of liver, kidney and gills of fishes and fish tissues for Cd determination by GF AAS, employing permanent modifiers.

2. Experimental

2.1. Instrumentation

The integrated absorbances were obtained in an atomic absorption spectrometer, SpectrAA 220 from Varian (Victoria, Australia), equipped with a graphite furnace, an autosampler PSD 120, and the polarized Zeeman background correction, all from Varian. Hollow cathode lamp for Cd from Varian (part no. 56-101-008-00), operating at 10.0 mA, was used. Unless otherwise, the stated manufacturer recommended conditions were employed. Argon 99.999% from Air Liquid (Contagem, MG, Brazil) was used as the purge gas. Pyloric graphite coated tubes with Forked platform (Varian, part no. 63-1000-23-00 and 63-1000-24-00, respectively) were used. The samples and standard solutions, 20 μl, was introduced onto the platform at 40 °C to obtain the pyrolysis and atomization temperature curves. For the calibration curves (aqueous or matrix matching calibration) a volume of 20 μl was also used. The temperature program was optimized through pyrolysis and atomization temperature curves, and is shown in Table 1.

2.2. Reagents

All reagents employed were of analytical grade. Deionized water (18.2 MΩ cm) from a Milli-Q System (Millipore, Bedford, MA) was used to prepare all solutions. The new reagent Universol®, Benzyltrimethylammonium hydroxide 40% m/v in water was prepared in our laboratory from Sigma-Aldrich reagents (St. Louis, MO, USA, No. 100974415). The nitric acid used to stabilize the analytical solutions was from Merck (Darmstadt, Germany, No. 7587956). Cadmium