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# Simultaneous determination of mercury and selenium in fish by CVG AFS

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ABSTRACT

In this work, an analytical method was proposed for the simultaneous determination of mercury and selenium in fish samples using Atomic Fluorescence Spectrometry (AFS). Multivariate designs were performed to evaluate the variables and optimize the best condition of chemical vapor generation (CVG) and simultaneous determination of mercury and selenium by AFS. Fish samples were prepared via acid digestion in digester block with cold finger reflux system, which ensured that the elements were not lost by volatility. The proposed analytical method was validated, and excellent figures of merit have been achieved, such as detection limits of 0.33 and 9.18 ng $g^{-1}$ for mercury and selenium, respectively. The method was applied for simultaneous determination of mercury and selenium in canned sardines. Mercury concentrations ranged from 0.057 to 0.203 μg $g^{-1}$ and selenium concentration from 1.76 to 2.21 μg $g^{-1}$, providing a mean molar ratio (Se:Hg) equivalent to 36.

## 1. Introduction

Mercury is a toxic trace element that has the ability to bioaccumulate and biomagnify to higher trophic levels. Its organic compounds have a higher toxicity to humans (Bosch, O'Neill, Sigge, Kerwath, & Hoffman, 2016; Tuzen, Uluozlu, Karaman, & Soylak, 2009). Already selenium is an essential nutrient for the humans and animals health, but in high concentrations it is toxic. Selenates and selenides are its most toxic species (Looi, Aris, Haris, Yusoff, & Hashim, 2016). Fish is a widely consumed food and that provides many important nutrients for the human diet. They are organisms capable of absorbing elements that are contained in water or food, whether it be essential or non-essential, storing them in muscle tissue. Fishes are part of the group of foods that can accumulate in its structure large amounts of elements such as mercury and selenium (Berges-Tiznado, Marquez-Farias, Torres-Rojas, Galvan-Magana, & Paez-Osuna, 2015; Looi et al., 2016; Tuzen, Karaman, Citak, & Soylak, 2009). The presence of selenium in fish samples, as well as their determination, is of extreme importance to assess the risks associated with exposure to mercury via ingestion of contaminated food. It is suggested that the selenium has a protective effect against the toxic effects of Hg and thus, the determination of the Se:Hg molar ratio in samples allows a more representative investigation. It is estimated that, when the Se:Hg molar ratio is greater than 1 the protective effect of Se against Hg toxicity effects is achieved (Polak-Juszczak, 2015; Rayman, 2000).

Different techniques are employed in the individual or simultaneous determination of Hg and Se in various types of samples. For the simultaneous determination of these elements the most used techniques are the Inductively Coupled Plasma Mass Spectrometry (ICP-MS), whether for food analysis (Chevallier, Chekri, Zinck, Guerin, & Noel, 2015; Dubascoux, Nicolas, Rime, Payot, & Poitevin, 2015) or other sample types (Choe & Gajek, 2016), and the Inductively Coupled Plasma Optical Emission Spectrometry with Chemical Vapor Generation (CVG-ICP OES) (Baika, Dos Santos, Herrmann, & Grassi, 2016; Guerrero, Alonso, Pavon, Cordero, & de Torres, 2016). Atomic Fluorescence Spectrometry with Chemical Vapor Generation (CVG AFS) is a technique capable to carry out simultaneous determinations, having as main advantages: the high sensitivity, selectivity, easy operation, low relative cost and low gas consumption argon. Some works in the literature employed this approach in simultaneous determinations of As and Se in biological sample (Sun, Liu, Wu, Li, & Shi, 2005), As and Hg in human hair and saliva (Xu, Liu, Wei, Qiu, & Gao, 2013), Hg and Sb in lead-base alloys (Zhang & Duan, 2014), As, Bi, Te, and Se in tea leaves (Zhang, Fu, Fang, Feng, & Ke, 2011), As and Sb in water samples (Wu et al., 2011) and in clinical samples (Tu & He, 2015). However, it was not found papers involving simultaneous determination of Hg and Se by CVG AFS.

Determination of elements in food samples, such as fish, using spectroanalytic techniques requires a sample preparation step. Among the possible methods to transform the sample into a more proper form

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for analysis there is the acid digestion, which can be conducted in closed systems such as microwave oven and high pressure pumps or in open systems as block digesters (Korn et al., 2008). Acid digestion in a digester block is a simple and relatively low cost process that can be applied to a wide variety of samples, and that presents flexibility in the amount of mass to be employed. In addition, it allows the simultaneous digestion of a relatively large quantity of samples. However, they are not suitable processes when determining volatile elements. This problem can be overcome by employing the "cold finger" reflux system, increasing the applicability of this sample preparation procedure. The "cold finger" coupled to the top of the digesters tube allows the condensation and reflux of the acid used in the digestion and of the volatile species (Ferreira et al., 2013). It is an alternative and economical strategy that has been effectively applied to the determination of many volatile elements, as e.g. cadmium, lead, mercury, arsenic, antimony and selenium.

In order to achieve the best operating conditions and generate better analytical response for the simultaneous determinations are used chemometric tools. These tools are very effective in optimizing analytical methods. Two-level full factorial design allows us to understand the effects of factors and the interaction between them. Response Surface Methodologies (MSR) are used to establish the critical conditions of significant variables for the system under study (Bruns, Scarminio, & Neto, 2006; Ferreira et al., 2007). In simultaneous determinations there is more than one analytical response of interest. Thus, during the multivariate optimization of this analytical system it is necessary to establish a multiple response. The alternative strategy to the most used strategy (desirability function D) is the multiple response function (MR) proposed by a Brazilian research group, which has shown great simplicity and applicability (Ferreira et al., 2017; Novaes et al., 2016; Portugal, Ferreira, dos Santos, & Ferreira, 2007).

In this paper is propose an analytical method for the simultaneous determination of Hg and Se in fish using atomic fluorescence spectrometry with chemical vapor generation. This work seeks to develop a simple, practical and accurate method that could help the researches and the analysis laboratories in their works about nutritional parameters of the food and their potential risks to consumers. The method was optimized and developed employing multivariate design and using the acid digestion in digester block with "cold finger" reflux system as sample preparation procedure.

## 2. Experimental

### 2.1. Instrumentation

Mercury and selenium simultaneous determination was performed using an Atomic Fluorescence Spectrometer (Aurora Lumina 3300, Canada) coupled with a continuous flow chemical vapor generator. This spectrometer is fitted with a atomizer of quartz tube with diffusion flame (air-$H_2$) and automatic ignition, as well as two hollow cathode lamp (HCL) of high intensity, one of mercury (253.6 nm) and other of selenium (196.0 nm). Argon with a high purity (White Martins, Brazil) was used into the system for carrying the volatile species of the analytes to quartz cell and to act as a shield gas thereof. The work conditions for the simultaneous determination of Hg and Se were: 350 V for PMT voltage, 255 mL $min^{-1}$ for carrier gas flow (argon), 800 mL $min^{-1}$ for shield gas flow (argon) and 4.0 mL $min^{-1}$ for the flow of introduction of reagents. The lamp current was set to 20 mA for Hg and 120 mA for Se.

Fish samples were prepared employing acid digestion procedure in digester block (Tecnal, Brazil) with "cold finger" reflux system. For some tests, acid digestion of the sample were performed in microwave oven (Milestone, Italy). Pre-reduction of selenium was performed in thermal bath (Unique, Brazil) and using Erlenmeyer and "cold finger" system. A system of water purification Milli-Q Plus (Millipore, USA) was used to provide ultrapure water necessary for all experiments.

### 2.2. Reagents and solutions

Throughout the procedure were used analytical grade chemicals and ultrapure water. For the preparation of standard solutions of Hg and Se were used stock solution of 1000 mg $L^{-1}$ (Merck, Germany). Concentrated nitric acid (Merck, Germany) and 30% v/v hydrogen peroxide (Merck, Germany) were used in the acid digestion step. In the pre-reduction step was used concentrated hydrochloric acid (Merck, Germany). Dilute solutions of nitric and hydrochloric acid (6 mol $L^{-1}$), used to correct final acidity before the analyze, were prepared from the respective concentrated acids. Sodium tetrahydroborate solutions of 2.5% w/v (Merck, Germany) were prepared daily by dissolving the salt in sodium hydroxide (Merck, Germany) solution of 0.5% w/v.

### 2.3. Fish sample preparation procedure

The canned sardine samples were purchased in supermarkets in Salvador city (Bahia, Brazil). Samples of sardines, first, were separated from its preserves. Soon they were stored in bottles, which were subjected to freezing for subsequent lyophilization process. After freeze-drying, the samples were passed through a mesh (300 mesh), and then stored in the container. Finally, they were stored in a desiccators until the acid digestion procedure.

For acid digestion of sardine, 0.1 g of sample was weighed into a digestion tube. To each tube were added 3 mL of concentrated nitric acid. Then, the system was subjected to heating at 100 °C for 1 h in the block digester using "cold finger" reflux system attached to each tube to prevent loss of the analyte by volatilization. After this period, the tubes were removed from heating for 30 min to be cooled to room temperature and thus add 2 mL of $H_2O_2$. So, the tubes were reinserted in block at 100 °C for 1 h and 30 min, totalizing a digestion time of 3 h. Upon completion of the digestion procedure and with the system at room temperature, the contents of the tubes were carefully transferred to 125-mL Erlenmeyer (which allow coupling a cold finger reflux system) using ultrapure water to ensure complete transfer of the solution. For the pre-reduction of Se (VI) to Se (IV) were added 3 mL of concentrated hydrochloric acid and the system was heated in heat bath at 90 °C for 30 min. After this step and cooling, the tube content was transferred to 15 mL flasks using ultrapure water to ensure complete transfer of the solution to achieve a final volume of 15 mL. Finally, 1.7 mL of this solution were transferred to a volumetric flasks, to which were added 851 μL of 6 mol $L^{-1}$ $HNO_3$ to correct the final acid nitric concentration to the optimized value for Hg and Se determinations, and ultrapure water to reach a volume of 10 mL. The solution was conducted to reading in AFS. The same procedure was applied to the Oyster Tissue Certified Reference Material (NIST 1566b). For the Fish Liver Certificate Reference Material (DOLT 4) the general procedure was similar, but at the end of the experiment a larger dilution was performed on account of the relatively high concentration of Hg.

### 2.4. General procedure for the simultaneous determination of Hg and Se by CVG AFS

An Atomic Fluorescence Spectrometer coupled with a Chemical Vapor Generator (CVG AFS) was used for Hg and Se simultaneous determination. The volatiles species (cold vapor of mercury and selenium hydride) were generated by mixing the acidic solution of the analyte or acidified sample with a reducing solution of $NaBH_4$ (2.5% w/v) stabilized in NaOH 0.5% (w/v). Acidified sample and solution of $NaBH_4$ were transported by tygon tube with the aid of a peristaltic pump coupled to the instrument at a flow rate of 4.0 mL $min^{-1}$. Both solutions were transported and mixed in a reaction cell, which was also the first gas-liquid separator (GLS 1). The volatile species were formed and the gas-liquid separation occurred in this reaction cell. The chemical vapor formed was transported to a second gas-liquid separator (GLS 2) using argon as the carrier gas at a flow rate of 255 mL $min^{-1}$. The GLS 2