Validation of methodology for determining As, Pb and Cd in fish by using ICP-MS: Preliminary studies

Validação de método para análise de As, Pb e Cd em peixe por meio de ICP-MS: Estudos preliminares

RIALA6/1583

Lidiane Raquel Verola MATAVELI*, Luciana Juncioni de ARAUZ, Maria de Fátima Henriques CARVALHO, Paulo TIGLEA

*Endereço para correspondência: Núcleo de Contaminantes Inorgânicos, Centro de Contaminantes, Instituto Adolfo Lutz, Av. Dr Arnaldo, 355, Cerqueira César, São Paulo, SP, Brasil, CEP 01246-000. Tel.: (11) 3068-2923. E-mail: lmataveli@ial.sp.gov.br
Recebido: 28.08.2013 - Aceito para publicação: 25.11.2013

ABSTRACT
The progress of industry has led to the increased emission of pollutants into ecosystems**,** and fish consumption has been a remarkable source of human exposure to toxic metals. Arsenic, cadmium and lead constitute a potentially significant threat to human health because they are associated with several adverse health effects. Therefore, fish biomonitoring has been a crucial tool for assessing the environmental exposure to contaminants. This study aimed at determining arsenic, cadmium and lead contents in fish samples from a Proficiency Testing for Metals conducted by the Nuclear and Energy Research Institute (IPEN), in order to develop a routine analytical methodology using ICP-MS. The methodology was evaluated by using the certified reference oyster tissue material, which indicated good agreement between the certified and the determined concentrations for As, Cd and Pb. The report on the Proficiency Test performance evaluation**,** based on the *z-score* index, evidenced satisfactory results in both samples of the analyzed elements.
Keywords. fish, arsenic, cadmium, lead, ICP-MS

RESUMO
O progresso das indústrias tem causado aumento da emissão de poluentes em ecossistemas, e o consumo de peixe tem sido importante fonte de exposição dos homens aos metais tóxicos. Arsênio, cádmio e chumbo constituem uma potencial ameaça para a saúde humana, pois estão associados a efeitos adversos à saúde. Portanto, o biomonitoramento em peixe é fundamental ferramenta para realizar a avaliação da exposição aos contaminantes ambientais. O objetivo deste estudo foi de determinar a ocorrência de arsênio, cádmio, e chumbo em amostras de peixe, a fim de iniciar o desenvolvimento de um método analítico de rotina por meio de ICP-MS**.** Para conduzir este estudo, foram utilizadas as amostras provenientes do Teste de Proficiência em Metais organizado pelo Instituto de Pesquisas Energéticas e Nucleares (IPEN). A metodologia foi avaliada de acordo com os valores obtidos em material de referência certificado de tecido de ostra, que indicaram boa concordância entre as concentrações certificadas e aquelas determinadas para As, Cd e Pb. O relatório de avaliação de desempenho para o Teste de Proficiência, com base no índice *z-score*, mostrou resultados satisfatórios para as duas amostras dos elementos analisados.
Palavras-chave. peixe, arsênio, cádmio, chumbo, ICP-MS.

Elements such as arsenic, cadmium and lead, are natural trace components of the aquatic environment, but their levels have increased due to several human activities, such as industry, agriculture and mining.

When toxic elements accumulate to certain levels in the environment or in the human body, they can cause ecological damage and health problems to humans[1,2]. Even low metal concentrations may affect the health of aquatic and terrestrial organisms, including men, since it is known that the consumption of contaminated food can provide the transfer of these metals to human beings through the food chain[1].

That is why fish and other edible aquatic animals contamination caused by toxic metals has become a concern worldwide, not only because of the threat to these animals lives, but also due to health risks associated to the consumption of these animals. This problem already showed its effects in Brazilian ecosystems: Medeiros et al[3] observed arsenic levels of 0.002 and 11.8 $mg.kg^{-1}$ for two species of fish and, in another study[4], cadmium and lead contents were detected in different aquatic species with values ranging from 0.01 to 1.04 $mg.kg^{-1}$ and from 0.10 to 5.40 $mg.kg^{-1}$, respectively. It is important to notice that the highest concentration found in these studies are above (As, Pb) or in the limit (Cd) of the Brazilian legislation[5] maximum level. According to other recent studies carried out in Brazil, in which authors analyzed fish liver[6] and muscle[7], the results showed concentrations higher than the level allowed by legislation for these elements in a variety of species.

Thus the aim of this work was to start studying the validation of a method to analyze arsenic, lead and cadmium in aquatic organisms, and, as a public health laboratory, to provide methods that are able to analyze samples in concentrations that meet the Brazilian legislation.

The oyster tissue Certified Reference Material (CRM 1566b), used for the digestion procedure evaluation, was purchased from National Institute of Standards and Technology (NIST). Samples for the Proficiency Testing for Metals in Fish Tissue were sent to the inorganic contaminants laboratory of Instituto Adolfo Lutz by the Nuclear and Energy Research Institute (IPEN). According to IPEN information, these samples were acquired in the Amazon region, with Cd, As and Pb in concentration values within the Brazilian legislation.

Approximately 0.3 g from both CRM and samples for the analysis of Cd, Pb and As were weighed on an analytical balance (Ohaus Adventurer™). The CRM and samples, as well as blanks, were digested in a Marconi® digestion block at 90 °C for two hours, using 2.0 mL of Suprapur® HNO_3 (Merck) and 0.5 ml of H_2O_2 (30 % w/w, Merck). The digested sample had a final volume of 35 mL, completed with deionized water.

Table 1. Instrumental parameters of ICP-MS

RF power	1400 W
Plasma gas flow rate	18 $L.min^{-1}$
Auxiliary gas flow rate	1.0 $L.min^{-1}$
Nebulizer gas flow	0.99 $L.min^{-1}$
Integration time	750 ms (per element)*
Isotopes monitored	^{75}As, ^{114}Cd, ^{206}Pb**, ^{207}Pb**, ^{208}Pb**
Cell gas	NH_3, for As analysis

* For Pb, 750 ms per isotope ** Concentration values are expressed as the mean between the 3 isotopes

Analysis were performed using Inductively Coupled Plasma Mass Spectrometer (ICP-MS, ELAN DRC II, PerkinElmer), equipped with a glass Meinhard nebulizer and a cyclonic glass spray chamber. Daily performance of the equipment was checked using a multi-element standard solution (PerkinElmer). Relevant operating conditions of the equipment are shown in Table 1.

Metal concentration in the CRM and samples was calculated using analytical curves specific for each element, using 5 standard concentration ranging, with ranges from 1.0 to 40, 1.2 to 24 and 1.0 to 20 $\mu g.L^{-1}$ for As, Cd and Pb, respectively.

Main figures of merit were calculated as follows: instrumental limits of detection (LD) were calculated as 3 x standard deviation of ten repeated measures of blanks digested independently; instrumental limits of quantification (LQ) were calculated as 10x the standard deviation of ten repeated measures of blanks digested independently; precision was calculated as relative standard deviation (RSD), and accuracy was evaluated using the CRM 1566b subjected to the same digestion procedure as the samples.

Results for the measurement of As, Pb and Cd in two samples of fish and oyster certified reference material,

Table 2. Results of As, Pb and Cd in fish and oyster certified reference material using ICP-MS

	Certified Reference Material[c]					Samples	
	Obtained values			Certified values			
	LD[a] ($\mu g.L^{-1}$)	LQ[b] ($\mu g.L^{-1}$)	Concentration ($mg.kg^{-1}$)	Concentration ($mg.kg^{-1}$)	Recovery (%)	Sample 1 ($mg.kg^{-1}$)[d]	Sample 2 ($mg.kg^{-1}$)[d]
As	0.15	0.48	7.10 ± 0.19	7.65 ± 0.65	92.8	0.65 ± 0.09	1.09 ±0,09
Cd	0.15	0.51	2.48 ± 0.02	2.48 ± 0.08	100.2	0.48 ± 0.003	1.73 ± 0,04
Pb	0.30	0.99	0.33 ± 0.030	0.31 ± 0.009	108.3	1.03 ± 0.002	0.67 ± 0,02

[a] LD – Limits of detection for the analyzed elements
[b] LQ – Limits of quantification for the analyzed elements
[c] Results of NIST CRM 1566b analysis after oxidative digestion (average values, n=3 ± standard deviations)
[d] Results of sample 1 and 2 analysis after oxidative digestion (average values, n=9 ± standard deviations)

as well as limit of detection and limit of quantification using ICP-MS are shown in Table 02.

Accuracy of the sample preparation analysis was verified by analyzing CRM 1566b in the ICP-MS, and results are given in Table 02. Recoveries of measured elements were within 92.8-108.3 %, indicating a good agreement between certified and determined concentrations. Precision, as RSD, for analysis of 3 digested CRM samples varied from 0.8 to 9.7 %.

Two samples sent to the laboratory as part of the Proficiency Test for metals in fish tissue were analyzed, and the results are shown in Table 2. According to the report received, the performance evaluation of the analysis, based on the *z-score* index, showed satisfactory results for both samples regarding the analyzed elements. Precision as RSD for analysis of the digested samples varied from 0.9 to 12 %.

Lead absorption may constitute a serious risk to public health. It is well documented to cause neurotoxicity, nephrotoxicity, increase in blood pressure and cardiovascular diseases. In children, Pb can also cause slow cognitive development and impair intellectual performance[8]. The maximum Pb level established by the Brazilian legislation for fish is 0.3 $mg.kg^{-1}$ (raw weight)[5].Concentrations for the analyzed samples are higher (0.67 ± 0.02 $mg.kg^{-1}$ and 1.03 ± 0.002 $mg.kg^{-1}$) than the maximum concentration allowed by the legislation[9].

Cadmium absorption also constitutes a risk to humans, since it may induce kidney dysfunction, skeletal damage and reproductive disorders[8], and the maximum Cd level established by the Brazilian legislation[5] for fish range from 0.05 to 0.30 $mg.kg^{-1}$ (raw weight)[5] according to the species. Concentrations for both the analyzed samples (Table 02) are higher (0.48 ± 0.003 $mg.kg^{-1}$ and 1.73 ± 0.04 $mg.kg^{-1}$) than the maximum concentration allowed by the Brazilian legislation, demonstrating the capability of this method to meet its requirements.

The toxicity of arsenic differs mainly in terms of oxidation states and whether it is present in inorganic or organic forms. Studies[2] indicate that fish and other seafood account for 90 % of total As exposure, and the toxic species account for 1-3 % of the total As present[9]. This explains the lack of legislation regarding this element worldwide. For example, there is presently no legislation for arsenic in foodstuff in the European Union, and in the USA, only a regulatory limit for oral As reference dose is provided by EPA ($3x10^{-4}$ mg/kg/day)[9]. Brazilian legislation establishes the maximum As level for fish as 1.0 $mg\ kg^{-1}$ (raw weight)[5], and the method developed in this work is able to meet these values (0.65 ± 0.09 and 1.09 ± 0,09 $mg.kg^{-1}$).

According to the results, the concentration values found for the oyster CRM indicated a good agreement between certified and determined concentrations for As, Cd and Pb. In this preliminary study, the main figures of merit were established, and the results in the samples analysis (in this case, the Proficiency Testing samples) were promising. This provides data to develop a well established method capable of analyzing these elements, meeting the Brazilian legislation for As, Cd and Pb in fish.

ACKNOWLEDGMENT

To Luci Elaine Machado for all the help provided in the laboratory.

REFERENCES

1. Olmedo P, Pla A, Hernández AF, Barbier F, Ayouni L, Gil F. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environ Int. 2013;59:63-72.
2. Rahman MS, Molla AH, Saha N, Rahman A. Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. Food Chem. 2012;134:1847-54.

3. Medeiros RJ, Santos LMG dos, Freire AS, Santelli RE, Braga AMCB, Krauss TM, et al. Determination of inorganic trace elements in edible marine fish from Rio de Janeiro State, Brazil. Food Control. 2012; 23:535-41.

4. Santos LFP, Trigueiro INS, Lemos VA, Furtunato DMN, Cardoso RCV. Assessment of cadmium and lead in commercially important seafood from São Francisco do Conde, Bahia, Brazil. Food Control. 2013; 33:193-9.

5. Brasil. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução RDC nº 42, de 29 de agosto de 2013. Dispõe sobre o Regulamento Técnico MERCOSUL sobre Limites Máximos de Contaminantes Inorgânicos em Alimentos. Diário Oficial [da] República Federativa do Brasil. Brasília, DF, 30 ago. 2013. Seção 1, p. 33-35.

6. Meche A, Martins MC, Lofrano BESN, Hardway CJ, Verdade L. Determination of heavy metals by inductively coupled plasma-optical emission spectrometry in fish from the Piracicaba River in Southern Brazil. Microchem J. 2010; 94: 171-4.

7. Weber P, Behr ER, Knorr CL, Vendruscolo DS, Flores EMM, Dressler VL, et al. Metals in the water, sediment, and tissues of two fish species in a subtropical Brazilian river. Microchem J. 2013; 106: 61-6.

8. European Union. Summaries of EU Legislation. [acesso em 2013 Jul 10]. Disponível em: http://europa.eu/legislation_summaries/food_safety/contamination_environmental_factors/l21290_en.htm

9. Copat C, Arena G, Fiore M, Ledda C, Fallico R, Sciacca S, et al. Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. Food Chem Toxicol. 2013;53:33-7.