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The ICP-MS validated for measuring arsenic levels in muscle tissues of freshwater fishes in Ho Chi Minh City

Nguyen Quoc Thang[1*], Le Van Tan[1], Nguyen Thi Kim Phuong[2]

[1]*Chemical Engineering faculty - Industrial University of Ho Chi Minh City*

[2]*Institute of Chemical Technology, Vietnam Academy of Science and Technology*

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Abstract

The present study was carried out to investigate contamination of arsenic in 10 freshwater fish species (*Oreochromis sp, Ophiocephalus maculatus, Anabas testudineus, Oreochromis niloticus, Trichogaster pectoralis, Clarias macrocephalus, Pangasius hypophthalmus, Pangasius bocourti, Cyprinus carpio, Macrognathus siamensis*) that are most commonly consumed by the habitant of Ho Chi Minh City. The arsenic concentration in fish muscle was analyzed using ICP-MS. Linearity was evaluated by repeated three times using different concentrations in the range from 0.5 to 20 μg/L with a determination coefficient (r^2) of 0.9998. The limit of detection (LOD) and limit of quantification (LOQ) of the method were found to be 0.076 μg/L and 0.253 μg/L, respectively. Intra-assay precision levels were between 0.41 and 2.69 %. Inter-assay precision levels were between 2.11 and 4.17 %. Recovery of arsenic from fish muscle was found to be from 105 to 114 %. The resulted showed that the mean arsenic concentration in muscle of fish ranged from 0.38±0.06 to 6.28±0.20 ng/g. The overall results indicated that arsenic concentrations in fish muscle were lower than the permitted level set by Ministry of Health Vietnam and Food and Drug agent. Therefore, the consumption of the fish species might not pose a risk of arsenic toxicity.

Keywords. Fish, Arsenic, ICP-MS.

1. INTRODUCTION

Elemental arsenic is found naturally in the Earth's crust at concentration of 2-5 ppm [1]. Arsenic is released into the environment through both natural sources (i.e., soil erosion, volcanoes) as well as anthropogenic sources (e.g. release from metal mining and smelting, pesticide application, coal combustion waste incineration). Most arsenic release into the environment is inorganic and accumulates by binding to organic matter in the environment [2].

Arsenic is classified as chemical hazards. Arsenic pollution in aquatic environment has become a serious problem and also an important factor in the decline of water sediment and fish quality. Water with high arsenic concentrations can yield fish with exceedingly elevated arsenic levels [3]. Therefore, fish is the highest dietary source of arsenic [4]. Arsenic concentrations for fish and seafood average 4-5 ppm [5], significantly higher than concentrations found in grain and cereals, with an average of 0.02 ppm [6]. Chronic exposure to arsenic does occur from dietary sources. Chronic arsenic toxicity may cause peripheral neuropathies, paresthesia, ataxia, cognitive deficits, fatigue, and muscular weakness [3]. Fish are widely consumed in many parts of the world by humans and polluted fish may endanger human health [7].

The aim of the study was to set up and validate the quality criteria of ICP-MS for analyzing arsenic levels in arsenic-spiked fish muscle samples. The application of this method was then tested by analyzing arsenic levels in 10 freshwater fish species (*Oreochromis* sp., *Ophiocephalus maculatus, Anabas testudineus, Oreochromis niloticus,…*) that are commonly in Vietnam to determine the levels of arsenic in the muscle tissue in order to assess fish quality and the health risk for humans.

2. MATERIALS AND METHODS

2.1. Chemicals

Analytical grade nitric acid (65 %), hydrogen peroxide (30 %) and arsenic stock standard solution (1000 mg/L) were acquired from Merck (Darmstadt, Germany). The working standard solutions were freshly prepared by diluting an appropriate aliquot of the standard stock solutions. All glassware was

treated with 20 % (v/v) HNO_3 for 24 h and then rinsed three times with deionizer water before use.

2.2. Sample collection

Ten kinds of fish which are the most commonly consumed by the habitant of Ho Chi Minh City. Fresh fish were collected and randomly bought during January to March, 2016 from the local markets in Ho Chi Minh City. Table 1 shows the name, number of samples to be tested.

Table 1: Scientific name, number of samples and number of individuals for each sample

Scientific Name	No. of analysis sample	No. of individual per one sample
Oreochromis sp	5	10
Ophiocephalus maculatus	5	10
Anabas testudineus	5	10
Oreochromis niloticus	5	10
Trichogaster pectoralis	5	10
Clarias macrocephalus	5	10
Pangasius hypophthalmus	5	10
Pangasius bocourti	5	10
Cyprinus carpio	5	10
Macrognathus siamensis	5	10

2.2. Sample preparation

As soon as arrived in the laboratory, fish samples were treated with removing inedible parts, then washed and frozen. All samples were kept at -75ºC without any prior treatment. Before analysis, composite sample of each species was prepared by mixing and ground homogenously. All composite samples were packed into polyethylene covered cup, stored in freezer at -20 ºC and analyzed within a week. Before digestion process, samples were dried for 72 h at 60-70ºC using air oven and grinded using mortar.

One gram dry fish muscle sample was weighed out in the reaction vessel. Four milliliters of 65% HNO_3 were then added to each vessel; two milliliters of 30% H_2O_2 were then added. The vessel was heated to 85ºC over 30 min. After digestion, the sample was allowed to cool. Then, elute was transferred to a volumetric flask and made up to 25 mL with 2% HNO_3 (sample solution). The solution was filtered through a 0.45 µm Millipore filter.

2.3. Fish muscle arsenic analyses

The quantification of As was performed with Inductively Couple Plasma-Mass Spectrophotometry (ICP-MS 7700, Agilent, USA). The assay characteristics, including intra- and inter-assay variability, were assessed using an internal standard as described below.

Defining assay characteristics: Assay linearity was assessed by directly injecting the standard solutions in the range 0.5 µg/L and 20 µg/L. The lower limit of detection (LOD) and lower limit of quantification (LOQ) were calculated:

$$LOD = \frac{3.\ S_\gamma}{B} \quad \text{and} \quad LOQ = \frac{10.\ S_\gamma}{B}$$

Where S_γ: standard error of estimate

B: coefficient B of linear regression (Y = A + B.X)

Intra-assay imprecision and inaccuracy were assessed by analyzing two quality controls (0.5 and 5 µg/L of arsenic) as six replicates during a single day. The mean, standard deviation, and coefficient of variation values were calculated for each quality control. The inaccuracy of the estimates for each quality control was determined as the difference between the mean measured concentration and the nominal concentration as a percentage of the nominal concentration. Inter-assay imprecision was evaluated in six assays run on separate days with two quality controls containing arsenic concentrations within the working range. This was again expressed as the coefficient of variation. The assay recovery rate was determined by adding 0.5 and 5.0 µg/L of arsenic to *Oreochromis* sp. muscle samples. Each sample was analyzed six times, and the recovery rates were calculated. The recovery was calculated:

$$H\% = \frac{C_{standard+sample} - C_{sample}}{C_{standard}} \text{ x } 100$$

The ICP-MS conditions were shown in table 2.

Table 2: ICP-MS parameters

Parameters	Optimal condition
Mass	75
Acid	HNO_3 2%
Analysis mode	Helium gas used in collision mode
RF Power	1350 W
Nebulizer gas flow	0.85 L/min
Carier gas flow	1.09 L/min
Plasma flow	15 L/min
Nebulizer pump	0.1 rps
Sample depth	8.00 mm

3. RESULTS AND DISCUSSION

3.1. Quality control criteria for the method

Linearity was evaluated by repeated three times using different concentrations in the range from 0.5 to 20 µg/L with linear regression is $y = 930.25x - 26.73$ and a determination coefficient (r^2) of 0.9998. The LOD and the LOQ were 0.076 µg/L and 0.253 µg/L, respectively. This was well below the working range for arsenic (i.e., 0.5-20 µg/L).

Validation results of assay precision, accuracy and recovery are shown in tables 3 and 4. Intra-assay and inter-assay precision levels were assessed by analyzing the quality control samples. The RSD precision within-day was between 0.41 and 2.69 %. The accuracy of the method within-day was between 98.0 and 98.6 %. The accuracy of the day-to-day data of this study was from 96.0 to 97.8 %. The stability of the samples was found to be at least 6 days. Arsenic recovery was measured based on recovery from *Oreochromis* sp muscle matrix samples containing known concentrations of arsenic. Recovery of arsenic from *Oreochromis* sp muscle was found to be from 105 to 114 %, and CV precision levels ranged from 2.56 to 7.04 %.

Table 3: Precision and accuracy data

Certified value (µg/L)	Intra-assay (n = 6, single day)			Inter-assay (n = 6, six days)		
	Value found (µg/L)*	RSD (%) precision	CV (%) Accuracy	Value found (µg/L)*	RSD (%) Precision	CV (%) accuracy
0.50	0.49±0.01	2.69	98.0	0.48±0.01	2.11	96.0
5.00	4.93±0.02	0.41	98.6	4.89±0.20	4.17	97.8

* Mean ± SD (n = 6).

Table 4: Recovery of arsenic in *Oreochromis* sp. muscle

Oreochromis sp. muscle (µg/L)	Added concentration (µg/L)	Observed concentration* (µg/L)	Calculated concentration* (µg/L)	CV (%) precision	Recovery (%)
0.33±0.03	0.5	0.92±0.07	0.57±0.04	7.04	114
	5.0	5.58±0.12	5.25±0.13	2.56	105

* Mean ± SD (n = 5).

3.2. Arsenic level in freshwater fishes

The observed arsenic concentration in muscle tissues of different fish species collected from local market in Ho Chi Minh city during January to March, 2016 were presented in Table 5. In this study, the arsenic concentrations in muscle tissues varied significantly among the ten fish species. The differences in arsenic concentration in each sample were depending on species, sex biological cycle and the portion of sample being analyzed [8]. Furthermore, ecological factors such as season, place of development, nutrient availability, temperature and salinity of the water may also contribute to the inconsistency of metals concentration in fish tissue [8,9]. Moreover, some aquatic organisms have the ability to concentrate heavy metals in their tissue in several orders of magnitude higher than those in water and sediment [10]. Arsenic is toxic for living organisms because of their accumulation properties. Therefore, at the top of the trophic chain, human beings are especially sensitive to this contaminants due to bioaccumulation.

Table 5: Concentration of arsenic (mean ± SD) (n=5) in muscle tissues of fish

Fish species	As level (µg/kg)
Oreochromis sp	3.70±0.14
Ophiocephalus maculatus	4.55±0.25
Anabas testudineus	0.83±0.04
Oreochromis niloticus	0.75±0.04
Trichogaster pectoralis	0.38±0.06
Clarias macrocephalus	6.07±0.30
Pangasius hypophthalmus	3.20±0.08
Pangasius bocourti	3.97±0.23
Cyprinus carpio	4.10±0.28
Macrognathus siamensis	6.28±0.20
Health criteria level (µg/g)*	2

*Ministry of Health Vietnam recommended health criteria concentration [12].

The highest concentration of As accumulation was observed in *Macrognathus siamensis* (6.28 μgkg^{-1}), followed by *Clarias macrocephalus (*6.07 μgkg^{-1}), *Ophiocephalus maculatus (4.55* μgkg^{-1}), *Cyprinus carpio* (4.1 μgkg^{-1}), *Pangasius bocourti* (3.97 $\mu g\ kg^{-1}$), *Oreochromis* sp (3.70 $\mu g\ kg^{-1}$), *Pangasius hypophthalmus* (3.2 μgkg^{-1}), *Anabas testudineus (*0.83 μgkg^{-1}), *Oreochromis niloticus (*0.75 μgkg^{-1}) and *Trichogaster pectoralis (*0.38 μgkg^{-1}) The significant different concentration of arsenic in fish species could be due to the habitat of fish species. *Macrognathus siamensis, Clarias macrocephalus, Ophiocephalus maculatus* habitated in the middle strata and bottom strata, nearly to the sediment where various kinds of hazardous and toxic substances are accumulated [11] while *Oreochromis* sp, *Oreochromis niloticus, Pangasius hypophthalmus, Trichogaster pectoralis* and *Anabas testudineus* habitated in the upper strata. Arsenic present in all of samples but the values obtained were well below safe limits set by the Ministry of Health Vietnam (2007) [12].

4. CONCLUSION

The ICP-MS validated for measuring arsenic levels in muscle tissues of fish species is shown to be sensitive (LOD = 0.076 µg/L and LOQ = 0.253 µg/L). This study was taken to provide information on trace arsenic concentration in fish species which are the most commonly consumed by the habitant of Ho Chi Minh city. The results of this study showed that the mean arsenic concentration in muscle of freshwater fish species ranged from 0.38±0.06 to 6.28±0.20 ng/g.

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Corresponding author: **Nguyen Quoc Thang**
Chemical Engineering Faculty
Industrial University of Ho Chi Minh City
12, Nguyen Van Bao street, Ward 4, Go Vap district, Ho Chi Minh City
E-mail: thangdhcntphcm@gmail.com.