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Enzyme-assisted extraction and liquid chromatography-inductively coupled plasma mass spectrometry for the determination of arsenic species in fish

Fa Zhao[a,1], Yanming Liu[a,*], Xiqi Zhang[a], Rui Dong[a], Wenjiang Yu[a], Yanfang Liu[b], Zhimou Guo[b], Xinmiao Liang[b], Jianhua Zhu[a]

[a] Shandong Institute for Food and Drug Control, PR China
[b] Dalian Institute of Chemical Physics, Chinese Academy of Sciences, PR China

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ABSTRACT

A sensitive, simple and rapid method for the simultaneous determination of eleven arsenic species has been developed. A high performance liquid chromatography-inductively coupled plasma mass spectrometry (HPLC-ICP-MS) technique was used for the analysis of eleven arsenic species in less than 17 min. Different extraction solutions were explored and the recovery results using water and aqueous acidic solvents, aqueous organic solvents and enzymes showed that 40 mg protease with 0.75 mL 0.5% hydrochloric acid (*v/v*) as the extraction agent gave the best experimental results. Species separation with ammonium carbonate solution as the mobile phase was conducted on an anion-exchange chromatographic column using gradient elution. The column temperature was 20 °C and kinetic energy discrimination (KED) was employed to eliminate spectral interference. The use of KED mode effectively removed interference from ^{75}ArCl. The detection limit (L_D) of the method was in the range of 0.11–0.59 μg kg^{-1}. Repeatability values obtained for spiked real fish samples were in the range of 1.1%–7.6%. Accuracy was calculated based on the analysis of spiked real fish samples at five concentration levels. Obtained recoveries were 91%–106%. The validated method was used in a pilot study to analyze real samples of fish, the organic arsenic especially AsB was the major arsenic specie present in the analyzed samples, only trace amount of inorganic arsenic were detected. The analytical method should improve the assessment of human exposure associated with arsenic intake from fish.

1. Introduction

In recent decades, rapid urbanization and industrial expansion have led to increased levels of chemical pollution [1]. This has severely impacted the environment and could also be a disaster for the ecosystem [2]. Environmental pollution caused by arsenic, a heavy metal, has attracted the attention of geologists. Arsenic is present in nature as many different chemical species that can be classified into inorganic and organic arsenic. Inorganic arsenic species include As (III) and As (V), while the important organic arsenics include: arsenobetaine (AsB), dimethylarsinic acid (DMA), arsenocholine (AsC), monomethylarsonic acid (MMA), 4-nitrophenylarsonic acid (NIT), 4-hydroxy-3-nitrobenzenearsonic acid (ROX), 4-aminobenzenearsonic acid (ASA), carbarsone (CA) and p-hydroxyphenylarsonic acid (NAPP) [3–6].

The reasons for the multiple species of arsenic in the environment are various: pollutants resulting from a variety of industrial production processes, numerous household waste materials, animal drugs and fodder that are metabolized to multiple forms of arsenic [7]. Toxicity of the metal ion may differ depending on its oxidation state, with different species having various physicochemical properties and biological activities [8]. The toxicities of these arsenic species vary dramatically by several orders of magnitude.

Abbreviations: HPLC-ICP-MS, high performance liquid chromatography-inductively coupled plasma mass spectrometry; KED, kinetic energy discrimination; L_D, detection limit; AsB, arsenobetaine; DMA, dimethylarsinic acid; AsC, arsenocholine; MMA, monomethylarsonic acid; NIT, 4-nitrophenylarsonic acid; ROX, 4-hydroxy-3-nitrobenzenearsonic acid; ASA, 4-aminobenzenearsonic acid; CA, carbarsone; NAPP, P-hydroxyphenylarsonic acid; IARC, International Agency for Research on Cancer; FAO, Food and Agricultural Organization; EU, European Union; He, helium; L_Q, quantification limit.

* Corresponding author at: Shandong Institute for Food and Drug Control, 99 Tianluo Road, Jinan, 250100, PR China.
E-mail addresses: zhaofa1987@126.com (F. Zhao), msymliu@163.com (Y. Liu).
[1] Shandong Institute for Food and Drug Control, 99 Tianluo Road, Jinan, 250100, PR China.

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The inorganic arsenic species are highly toxic [9], with As (III) being the most toxic and a suspected human carcinogen [10,11]. While As (V) has relatively high toxicity [12], organic arsenics are less toxic and often considered hypotoxic or non-toxic [13]. Arsenic species can, however, be interconverted by metabolism in the human body, and organic arsenic can be converted into other arsenic species in animals [4]. For example, a variety of arsenic species can be detected in chickens receiving fodder containing ROX. ROX, generally used as an animal growth additive in fodder or as a drug, can accumulate and transform in the animal, leading to waste emissions to water that affect the ecosystem [14]. Documents issued by international organizations such as IARC (International Agency for Research on Cancer), FAO (Food and Agricultural Organization), WHO and EU (European Union) contain information on the toxicity and carcinogenicity of As species on living organisms. Despite this, there are no legal norms concerning concentration levels of every species of arsenic. Even if there are set levels, they only limit the total arsenic or inorganic arsenic concentrations. One of the major reasons for this is the lack of analytical procedures to rapidly obtain reliable results that are acceptable to the accrediting institutions.

In the last two decades, multi-element speciation studies, a successor of well-studied single element speciation, has gained more attention despite difficulties resulting from chromatographic separation. The following papers on the multi-element speciation in different matrices have been reported: As, Se, Cr, Sb, Te, Mo [15–21]. High performance liquid chromatography (HPLC) combined with inductively coupled plasma mass spectrometry (ICP-MS) has been used extensively for arsenic speciation analysis, taking advantage of efficient separation by HPLC and sensitive detection by ICP-MS [22–24]. Detection by ICP-MS is highly sensitive and element-specific, comparing favorably with other atomic spectrometric techniques, particularly when an aqueous mobile phase is used [25].

Fish is a nutritionally valuable food, including protein and micronutrients that are easily absorbed. All people in the world benefit from consumption of fish. However, arsenic in the environment can be enriched in fish through the food chain, causing harm to human health. Since different arsenic species have varying toxicity to humans, studies of fish should not only consider total arsenic content, but also the arsenic species present as the basis for food safety risk assessment. There are many studies on the morphology of arsenic, but because of the constraints of the chromatographic column and mobile phase, detection throughput is limited [26,27]. Only a few arsenic forms can be detected, and co-eluting compounds can easily lead to misidentification.

For fish samples, the arsenic species must be extracted into solution before HPLC analysis, The related pretreatment method must be highly efficient and keep the property of original arsenic species. Currently, extraction of arsenic species includes the use of water and aqueous acidic solvents, aqueous organic solvents, enzymes and other methods [3,28–34]. There are many organic compounds in fish tissue, however, that can be easily lost or transformed during extraction. Matrix interference during the chromatographic separation is also a problem. In general, a variety of mobile phases have been used, but the pH values need to be adjusted accurately [14,17,35–37]. The pH value is susceptible to the external environment, affecting the separation and the retention times of arsenic species. The linear range of the method is also narrow. For example, different separation conditions are required for the determination of solution concentrations above or below 10 ng mL^{-1} [38]. The operation is inconvenient and tolerance is poor. During ICP-MS detection, quantitative accuracy is also affected by ion interference [39,40].

The purpose of this study was to develop a high throughput method for determination of arsenic species in fish. Extraction methods and instrument operating conditions were explored to develop a simple operation having strong tolerance, strong anti-interference capability and fast separation. The analytical method should improve the assessment of human exposure associated with arsenic intake from fish.

Table 1
Optimization parameters for separation and determination of arsenic species using HPLC-ICP-MS.

	Parameter	Setting
HPLC	Instrument	Dionex UltiMate 3000 pump Dionex UltiMate 3000 autosampler Dionex UltiMate 3000 RS Column Compartment
	Column	AS7 Guard column(2 × 50 mm) AS7 Analytical column(2 × 50 mm)
	Elution	Multi-step gradient
	Mobile phase	Ammonium carbonate
	Mobile phase flow	0.4 mL min^{-1}
	Injection volume	5 μL
	Column temperature	20 °C
	Sampling depth	3mm
ICP-MS	Instrument	iCAP Q
	RF power	1500 W
	Nebulizer gas (Ar) flow rate	1.0 mL min^{-1}
	Auxiliary gas (Ar) flow rate	0.8 mL min^{-1}
	Plasma gas (Ar) flow rate	14 mL min^{-1}
	Sampler and skimmer cones	Pt
	Scan mode	Peak area
	Dwell time	0.2 s
	KED gas (He) flow rate	4.7 mL min^{-1}
	Atomic weight	75

2. Materials and methods

2.1. Instrumentation

The AG7 (Guard column, 2 × 50 mm, Thermo Fisher Scientific, Sunnyvale, USA) and AS7 (Analytical column, 2 × 250 mm, Thermo Fisher Scientific) anion exchange columns, installed in an UltiMate 3000 series HPLC system (Thermo Scientific, Germany), were used for separation of arsenic species. An iCAP Q ICP-MS system (Thermo Scientific, Germany) was used for detection. The instrument had a high efficiency sample introduction desolvating system equipped with a quartz cyclonic spray chamber and an additional mixing peristaltic pump. The quadrupole mass analyzer was operated in single ion monitoring mode (*m/z* 75) for detecting arsenic and in kinetic energy discrimination (KED) mode using helium (He) to eliminate interference. All of the measurements were performed under the operating conditions presented in Table 1. The separation conditions are shown in Table 2.

Table 2
Gradient elution conditions. A: 400 mmol L^{-1} ammonium carbonate, B: 5 mmol L^{-1} ammonium carbonate.

Time (min)	Flow (mL min^{-1})	A (%)	B (%)
0	0.4	0	100
2	0.4	0	100
2.1	0.4	10	90
5.5	0.4	10	90
5.6	0.4	25	75
8.6	0.4	25	75
8.7	0.4	100	0
17	0.4	100	0

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