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Determination of total arsenic and toxicologically relevant arsenic species in fish by using electrothermal and hydride generation atomic absorption spectrometry

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Abstract

A scheme for the determination of total As by electrothermal atomic absorption spectrometry (ETAAS) and the sum of toxicologically relevant arsenic species (As(III), As(V), monomethylarsonate (MMA) and dimethylarsinate (DMA) using hydride generation AAS (HGAAS) in fish samples was developed. Simple and fast microwave assisted extraction in tetramethylammonium hydroxide (TMAH, 0.075% m/v) or in water–methanol mixture (80+20 v/v) for 20 min is proposed for quantitative leaching of arsenic species from fish tissue. Total As was measured by ETAAS directly in the TMAH extract under optimal instrumental parameters (pyrolysis temperature 1400 °C and atomization temperature 2000 °C) with Pd as modifier ensuring thermal stabilization and isoformation of all extracted arsenic species. The analytical features of the method are as follows: limit of detection (LOD) 0.45 μg g^{-1} (dry wt.), within-run and between-run precision in the range 4–8% and 5–12%, respectively, for arsenic contents 0.5–30 μg g^{-1} and recoveries 98–102%. The sum of toxicologically relevant arsenic species (As(III)+As(V)+MMA+DMA) was determined by flow injection HGAAS directly from the TMAH extract or water–methanol mixture and trapping of arsines onto Zr–Ir coated graphite tube followed by ETAAS measurement. L-cysteine is used as reagent for leveling off responses of different arsenic species in the presence of TMAH or water–methanol mixture. The LODs achieved are 0.0038 and 0.0031 μg g^{-1} (dry wt.), respectively, for fish extracts in TMAH and in water–methanol mixture. Within-batch and between-batch RSDs are in the range 3–5% and 4–7% for arsenic contents of 0.009–0.25 μg g^{-1} (dry wt.) for TMAH extracts and 2–4% and 3–6% for methanol water extracts, respectively. Selective reaction media for generation of respective hydrides from arsenic species were recommended for further speciation purposes in methanol–water extracts, viz. citrate buffer (pH 5.2) for the determination of As(III), 0.2 mol L^{-1} acetic acid for the determination of As(III)+DMA and 7 mol L^{-1} hydrochloric acid for the determination of inorganic As(III)+As(V). LODs are 0.0035, 0.0051 and 0.0046 μg g^{-1} (dry wt.) for As(III), DMA and As(V). The relative standard deviation is 4–8% for three arsenic species at As levels of 0.009–0.5 μg g^{-1} (dry wt.). The accuracy of the proposed speciation scheme is confirmed by the analysis of certified reference materials.

Keywords: Arsenic; Speciation; Fish; ETAAS; HGAAS

1. Introduction

The determination of total arsenic is not sufficient to assess the risk associated with consumption of arsenic containing foodstuff since the toxicity of As is highly dependent on its chemical form. Therefore much attention has been given to the elemental speciation of arsenic, especially in seafood products, known as a main source of As in human diet. Arsenobetaine considered as nontoxic has been identified to be the major arsenical in a variety of seafood including clams and many species of fish; other more toxic arsenic species such as As(III), As(V), MMA and DMA have been also determined [1,2]. Speciation procedure includes several steps: quantitative extraction with methanol, water or chloroform by using ultrasound or microwave-assisted procedures [3–7], separation of extracted species by high performance liquid chromatography or ion chromatography and instrumental measurement by highly sensitive method

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as ICP-MS, HGAAS or HGAFS [8–18]. Another option useful for routine laboratory practice is the so called nonchromatographic approach based on combination of different analytical techniques and procedures which ensure determination of the total As and toxicologically relevant As species [19–21]. As(III) is bound to the thiol groups in fish proteins, therefore organic solvents are not able to break these bonds, thus entailing underestimation of As(III) in fish samples. In the present study microwave assisted extraction in two solvents: tetramethylammonium hydroxide (TMAH, protein solubilizer) and water–methanol mixture (80+20 *v*/*v*) were compared for quantitative extraction of all arsenic species from lyophilized fish tissue. As a next step a scheme was proposed for total arsenic determination by ETAAS and toxicologically relevant As(III), As(V), MMA and DMA determination by HGAAS directly in fish extracts. TMAH is a known organic base used for solubilization of biological tissues which has been previously applied at relatively high concentration levels than applied in this study [22–25]. Preliminary experiments revealed strong depressive effect of TMAH on As signal in ETAAS measurements, therefore as small as possible amount of the organic base was used in our extraction procedure. Instrumental parameters for ETAAS determination of As were elaborated: (i) suitable modifier ensuring equal thermal behavior and sensitivity for different arsenic species in fish extracts; (ii) optimal pyrolysis and atomization temperatures; (iii) adequate calibration. According to the authors' best knowledge, arsines have not been generated directly from solutions containing TMAH, neither from methanol–water (80+20 *v*/*v*) mixtures. Careful optimization of all parameters for accurate and reliable determination of the sum of toxicologically relevant As species (As(III)+As(V)+MMA+DMA) has been performed in the presence of L-cysteine, a reagent supplemented for complexation, pre-reduction and leveling-off responses of different arsenic species in TMAH extracts or methanol–water mixtures. Different reaction media were proposed for selective determination of As(III), DMA and As(V)+MMA in methanol–water mixtures. The accuracy of the procedures developed for arsenic species determination in fish extracts was confirmed by analyses of certified reference materials (CRMs).

2. Experimental

2.1. Apparatus

ETAAS measurements were carried out with a PerkinElmer Model AAnalyst 600 atomic absorption spectrometer (PerkinElmer, Norwalk, CT) equipped with a transverse heated graphite atomizer (THGA®), longitudinal Zeeman effect background corrector and an AS-800 autosampler. THGA® graphite tubes with integrated platforms were used as atomizers. Sample aliquots of 20 and 5 μL modifier injections were performed successively. Instrumental parameters are presented in Table 1.

HGAAS measurements were performed by PerkinElmer FIMS® 100 Mercury Analysis System with an AS 93 Plus Autosampler. THGA® graphite tubes with integrated platform pre-treated with Zr (250 μg) and then with Ir (20 μg) by successive multiple injections of modifiers on platform and multi-stage thermal treatments were used [26,27]. An electrodeless discharge lamp for As (EDL System II, PerkinElmer) was used as radiation source. All measurements were in integrated absorbance mode (peak area, A_{int}). Custom made gas–liquid separator with better tolerance toward sample foaming was used [28]. Optimized instrumental parameters and temperature programs for FI-HGAAS are given in Tables 2 and 3.

A CEM Model MARSx closed-vessel microwave solvent extraction system with a HP500 rotor (CEM Corporation, Matthews, NC, USA), equipped with a pressure and temperature monitoring option was used for microwave assisted extraction of arsenic species from fish tissues.

2.2. Standards and reagents

Analytical grade reagents were used. Stock standard solutions for As were: 1000 μg mL^{-1} As(III) (AAS standard solution, No. 11082. Fluka); 1000 μg mL^{-1} As(V), As standard solution traceable to SRM from NIST, H_3AsO_4 in 0.5 mol L^{-1} HNO_3 (CertiPUR®, Merck, Darmstadt, Germany); 1000 μg mL^{-1} monomethylarsonate (MMA) prepared by dissolving of sodium methylarsonate (MMA), $CH_3AsO(ONa)_2{\cdot}6H_2O$ (Carlo Erba, Milan, Italy); 1000 μg mL^{-1} dimethylarsinate (DMA) prepared by dissolving sodium cacodylate, $C_2H_6O_2AsNa_3{\cdot}H_2O$ (Carlo Erba) in doubly distilled water; and BCR (Community Bureau of Reference, Geel, Belgium) CRM 626 BCR Arsenobetaine Calibrated Solution 1031 μg mL^{-1} as AsBet. The working standard solutions were prepared weekly and kept refrigerated at 4 °C. Stock standard solution of Pd 10000 μg mL^{-1} in 5% HCl, stock standard solution of Mg 10000 μg mL^{-1} in HNO_3 and stock standard solution of Ir 1000 μg mL^{-1} in 20% HCl were used for modifier solutions preparation. Tetramethylammonium hydroxide (TMAH, 25%, p.a., Merck) was used for alkaline solubilization of tissues. Solution of sodium tetrahydroborate, $NaBH_4$ (Fluka) (0.5% *m*/*v*) in NaOH (0.1% *m*/*v*) was prepared daily. Silicon antifoaming agent (Merck) (1 mL L^{-1}) was added to sodium tetrahydroborate solution. Aqueous solutions of L-cysteine, 0.7 or 0.9 mol L^{-1}, were prepared fresh daily from solid reagent (>99.5%, Fluka) and diluted as required. Doubly distilled water was used in all operations.

The following SRM and CRM were used for the validation purposes: NIST SRM 1566a Oyster Tissue from the National Institute of Standards and Technology (NIST, Gaithersburgh, MD, USA) and BCR CRM 627, Forms of As in Tuna Fish from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium).

Table 1
The optimized THGA program for ETAAS analysis of fish samples solubilized in TMAH

Step	Temperature, °C	Ramp time, s	Hold time, s	Ar flow rate, mL min^{-1}	Read
Drying 1	110	30	20	250	–
Drying 2	150	15	20	250	–
Pyrolysis	1400	25	30	250	–
Atomization	2000	0	5	0	On
Cleaning	2300	1	2	250	–