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Flow injection hydride generation electrothermal atomic absorption spectrometric determination of toxicologically relevant arsenic in urine

Panayot K. Petrov [a,*], Ivan Serafimovski [b], Trajce Stafilov [c], Dimiter L. Tsalev [a]

[a] *Faculty of Chemistry, University of Sofia, 1 James Bourchier Blvd., Sofia 1164, Bulgaria*
[b] *Food Institute, Faculty of Veterinary Medicine, Sts. Cyril and Methodius University, P.O. Box 95, MK-1000, Skopje, Macedonia*
[c] *Institute of Chemistry, Faculty of Science, Sts. Cyril and Methodius University, P.O. Box 162, MK-1000, Skopje, Macedonia*

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Abstract

Analytical procedure for the determination of toxicologically relevant arsenic (the sum of arsenite, arsenate, monomethylarsonate and dimethylarsinate) in urine by flow injection hydride generation and collection of generated inorganic and methylated hydrides on an integrated platform of a transverse-heated graphite atomizer for electrothermal atomic absorption spectrometric determination (ETAAS) is elaborated. Platforms are pre-treated with 2.7 μmol of zirconium and then with 0.10 μmol of iridium which serve both as an efficient hydride sequestration medium and permanent chemical modifier. Arsine, monomethylarsine and dimethylarsine are generated from diluted urine samples (10–25-fold) in the presence of 50 mmol L^{-1} hydrochloric acid and 70 mmol L^{-1} L-cysteine. Collection, pyrolysis and atomization temperatures are 450, 500, 2100 and 2150 °C, respectively. The characteristic mass, characteristic concentration and limit of detection (3σ) are 39 pg, 0.078 μg L^{-1} and 0.038 μg L^{-1} As, respectively. The limits of detection in urine are ca. 0.4 and 1 μg L^{-1} with 10- and 25-fold dilutions. The sample throughput rate is 25 h^{-1}. Applications to several urine CRMs are given.

Keywords: Electrothermal atomic absorption spectrometry; Flow injection hydride generation; In-situ trapping; Arsenic; Monomethylarsonate; Dimethylarsinate; Urine; Permanent modification; Zirconium and iridium treated graphite platform; Automation

1. Introduction

Arsenic is a ubiquitous toxic trace element, representing a major toxicological and environmental concern [1,2]. It has been permanently among the most popular analytes in recent decades and substantial progress in its quantification and speciation analysis has been reached (reviews [3–6]). Despite the remarkable methodological improvements, arsenic remains one of the most difficult analytes [3]; its concentration levels, oxidation and binding states, ionic and molecular forms and metabolic pathways vary strongly in different environmental compartments, food chains and ultimately in mammals.

Urine is a major route of excretion of arsenic from human organism, being an important and indispensable toxicokinetic test for monitoring of occupational, environmental, dietary, accidental and other exposure sources [1,5–7]. Four arsenic species represent main toxicological interest and are well recognized in human urine—in declining order of their toxicity: inorganic (i) arsenic(III) (i-As(III), arsenite), i-As(V) (arsenate), monomethylarsonate (MMA, $CH_3As(OH)_2O^-$) and dimethylarsinate (DMA, $(CH_3)_2As(OH)O^-$), the latter one being the prevailing yet the least toxic metabolite. Whereas these four species could nowadays be differentiated quantitatively by means of advanced separations such as liquid chromatography, ion exchange, selective hydride generation (HG), cryotrapping with subsequent fractional vaporization, etc. (reviews [4–6]), the determination of their sum, denoted as 'first order speciation' [8] or 'toxicologically-relevant arsenic' [9,10] is likely to gain popularity. Attractive features of this approach could be summarized: (i) its toxicological grounds and significance; (ii) this sum is essentially the 'hydride-reactive' fraction, since i-As(III), i-As(V), MMA and DMA are derivatized to their corresponding hydrides (AsH_3, AsH_3, CH_3AsH_2 and $(CH_3)_2AsH$, respectively, under well-defined conditions of HG [11,12]; (iii) very simple pre-treatment of urine samples is involved, e.g. dilution with appropriate reagent such

* Corresponding author. Tel.: +359 2 6256318; fax: +359 2 9625438.
E-mail address: pkpetrov@chem.uni-sofia.bg (P.K. Petrov).

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as L-cysteine [9–15]; (iv) lengthy decomposition procedures are avoided (MMA and DMA are rather resistant to wet mineralization treatments and could be seriously underestimated, even in microwave oven digests [3,12,16,17]); (v) differentiation from some dietary exposure sources with low toxicity and bioavailability such as seafood-derived arsenic (arsenobetaine, arsenocholine, tetramethylarsonium) can be achieved, hence reliability of occupational monitoring is improved; (vi) instrumental measurements could be automated and sampling frequencies greatly improved, especially in flow injection mode of operation [8–11]. Potential disadvantages of this approach are the narrow acidity interval for simultaneous hydride generation from arsenite, arsenate, MMA and DMA (compromise conditions), dependence of final pH on different acid/base buffering properties of individual urines at low dilution factors and individual foaming pattern of different urine samples. As long as other hydride forming species (e.g. phenylarsinic acid and derivatives [18,19]) are not metabolite products in arsenic detoxication and are hardly found in human body and body fluids, they are not in the scope of this study, moreover they could entrain the body only in case of specific compound poisoning. Their excretion is without conversion to above-mentioned four species [1].

The aim of this work was to elaborate a sensitive and robust atomic absorption spectrometric procedure for the determination of toxicologically relevant metabolite arsenic (sum of arsenite, arsenate, MMA and DMA) in human urine [1] by flow injection hydride generation and collection of generated inorganic and methylated hydrides on Zr–Ir treated platforms of graphite atomizer. To the authors' best knowledge, this approach has not been realized in analytical practice.

Expected advantages of the FI-HG–ETAAS coupling are: (i) better sensitivity owing to hydride trapping and electrothermal atomization, thus applying higher dilution factors and providing better tolerance to pH adjustment, foaming, kinetic effects on HG/stripping; (ii) elimination of atomization interferences; (iii) improved precision and long term stability; (iv) automated, high sample throughput measurements. These carbide–iridium modified platforms have already shown good performance for trapping and stabilization of hydride-forming elements (As, Sb, Se, Sn) and some organoelement species in hyphenated hydride-generation–ETAAS [13,14,20].

2. Experimental

2.1. Apparatus

A Perkin-Elmer AAnalyst 600 atomic absorption spectrometer (Perkin-Elmer, Norwalk, CT, USA) equipped with a Model 4100 ZL transverse-heated graphite atomizer with integrated, pyrolytic graphite coated platform (THGA®) and longitudinal Zeeman-effect background corrector [21–23] with an AS 800 Autosampler, a FIMS 100 Mercury Analysis System with an AS 93 Plus Autosampler, an electrodeless discharge lamp (EDL) powered by EDL System II were employed. 'End-capped' THGA® tubes (Part No. B300-0655) [24,25] were used for better sensitivity (ca. 1.3–1.4-fold versus normal tubes) and improved precision. Optimized instrumental parameters and temperature programs are given in Tables 1 and 2.

Table 1
Optimized instrumental parameters for HG–ETAAS measurements

Parameter	Setting
Wavelength (nm)	193.7
Bandpass (nm)	0.7 (low)
EDL System 2 power (mA)	380
Signal measurement	AA–BG (peak area)
Smoothing	5 points
Baseline offset correction (BOC) time (s)	2
Read delay (s)	0
Sample coil volume	500 μL, PTFE tubing, 1 mm i.d., 64 cm
Sample load conduit	Tygon tubing, "yellow/blue", 1.52 mm i.d., 7.0 mL min^{-1} at 80 rev min^{-1}
Carrier conduit	Tygon tubing, "yellow/blue", 1.52 mm i.d., 4.0 mL min^{-1} at 50 rev min^{-1}
Reductant conduit	Tygon tubing, "red/red", 1.14 mm i.d., 2.0 mL min^{-1} at 50 rev min^{-1}
Waste from GLS	Tygon tubing, "white/black", 3.18 mm i.d., 10.5 mL min^{-1} at 80 rev min^{-1}
Reaction coil	PTFE tubing, 1.3 mm i.d., 100 cm
Argon flow rate (mL min^{-1})	125

THGA® graphite tubes with integrated platform were pretreated with 2.7 μmol of Zr (250 μg) and then with 0.10 μmol of Ir (20 μg) by successive multiple injections of modifiers on platform and multi-stage thermal treatments as detailed elsewhere [20,26]. The amounts of permanent modifier components was increased in this work (versus 1.2 μmol of Zr and 10 nmol of Ir in original papers [13,14], thus providing better long-term stability of measurements and lifetimes of THGA® tubes up to 700–800 firings. Noteworthy, the *critical* values of atomization (2100 °C) and clean temperatures (2150 °C) may not be exceeded, in order to avoid vaporization and redistribution of permanent modifier. The quartz pipette tip was adjusted to deliver hydrides about 1.3 mm above the platform. All results are based on integrated absorbance (A_{int}) measurements.

The standard gas–liquid separator (GLS) made of polymethylpentene with internal volume of 2.15 cm^3 and PTFE membrane (Fig. 1(A)) was replaced in this work with a larger custom-made GLS (20 cm^3), which exhibited better tolerance to flooding and aerosol formation (Fig. 1(B)). This GLS is made of borosilicate glass tube (height 155 mm, i.d. 16 mm, o.d. 19 mm) and has one side-arm input (I) and two outlets: a central one for waste (W) and a side-one for the gas–vapor flow (O), all made of borosilicate glass tubes (i.d. 4 mm, o.d. 6 mm). The GLS is fed and drained by means of two PTFE capillaries (I, i.d. 1.0 mm and W, i.d. 1.3 mm) fitted into the side arm (I) and the central tube (W) of the GLS, respectively.