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Determination of arsenic compounds in beverages by high-performance liquid chromatography-inductively coupled plasma mass spectrometry

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Abstract

Arsenic compounds including arsenous acid (As(III)), arsenic acid (As(V)), dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) were separated by high-performance liquid chromatography (HPLC) and detected by inductively coupled plasma mass spectrometry (ICP-MS). A Hamilton PRX-100 anionic-exchange column and a pH 8.5 K_2HPO_4/KH_2PO_4 5.0×10^{-3} mol L^{-1} mobile phase were used to achieve arsenic speciation. The separation of arsenic species provided peaks of As(III) at 2.75 min, DMA at 3.33 min, MMA at 5.17 min and As(V) at 12.5 min. The detection limits, defined as three times the standard deviation of the lowest standard measurements, were found to be 0.2, 0.2, 0.3 and 0.5 ng mL^{-1} for As(III), DMA, MMA and As(V), respectively. The relative standard deviation values for a solution containing 5.0 μg L^{-1} of As(III), DMA, MMA and As(V) were 1.2, 2.1, 2.5 and 3.0%, respectively. This analytical procedure was applied to the speciation of arsenic compounds in drinking (soft drink, beer, juice) samples. The validation of the procedure was achieved through the analysis of arsenic compounds in water and sediment certified reference materials.

Keywords: Arsenic speciation; HPLC-ICP-MS; Beverages

1. Introduction

The presence of arsenic in the atmosphere, aquatic systems, sediments and living organisms from both, natural and anthropogenic sources, causes its distribution in drinking water, foods and beverages, thus contributing to the daily intake of humans [1].

The European Union [2] and the World Health Organization [3] have established a guide limit of 10 μg L^{-1} of As in drinking water, but only some countries have specific legislation concerning the maximum concentration of As in beers. Spain fixed at 100 μg L^{-1} as the tolerable amount of As in beer [4]. Ireland and the United Kingdom recommended 500 μg L^{-1} and Bulgaria, Czech Republic, Hungary and Slovenia recommended 200 μg L^{-1} [5].

The physical, chemical and toxicological properties of As vary considerably as a function of its chemical forms. The inorganic compounds ((AsIII) and (As(V)) are more toxic than the methylated ones (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA)) [6]. So, it is important to develop speciation methods suitable to produce analytical data on natural samples concerning each one of the most common As chemical forms [7].

The quantitative determination of different chemical forms of As can be carried out with the use of a separation technique, such as high-performance liquid chromatography (HPLC) coupled with specific detectors, like inductively coupled plasma mass spectrometry (ICP-MS) and atomic fluorescence spectrometry (AFS) [8].

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The hyphenation of HPLC with atomic or mass spectrometry detectors produces high sensitivity and reproducibility [8–11] providing detection limits in the mg L^{-1} (ICP-OES) to μg L^{-1} (ICP-MS) range. The coupling of ICP-MS to HPLC combines the separation power of HPLC with the multi-element capability and the detection power of ICP-MS [8,12].

There are only few precedents on the determination of As in beverages due to the low concentration of this element in beverage samples. However, this kind of determination can be carried out by hydride generation atomic absorption spectrometry (HG-AAS) [13] or by electro thermal atomic absorption spectrometry (ETAAS) [14] the use, being necessary, of a dry-ashing sample treatment in order to reduce matrix effects and to improve the limits of detection through sample preconcentration.

Procedures by ICP-MS after microwave-assisted digestion have been developed for 23 trace element determination in Polish beers, including As also [15] and a direct hydride generation sample introduction ETAAS procedure has been applied for As, Sb, Se, Sn and Hg determination in beers using in situ preconcentration of the analytes onto Pd pre-treated furnaces [16].

Atomic fluorescence spectrometry has been applied to the direct determination of As in beer samples [17], a speciation methodology is also being developed to differentiate chemical forms of arsenic in beers [18].

There is a precedent about the determination of As(III), As(V), MMA and DMA in alcoholic and alcohol-free beer samples based on the use of HG-AAS after microwave assisted digestion for total As determination, and cationic-exchange followed by anionic-exchange sample treatment for speciation, being the species determined in the different elutes obtained after off line low pressure chromatographic separation by HG-AAS [19].

Coupling HPLC with ICP-MS has been applied to the determination of anionic arsenic species, DMA and MMA in kelp extracts and wine samples. Mild extracting conditions should be adopted in order to prevent any conversion of the arsenic species and to avoid interferences during the chromatographic separations [20].

The main objective of the present study was the development of a procedure for speciation of As in beverages after a simple and fast sample pre-treatment. Species of arsenic (As(III), DMA, MMA, As(V)) were separated by high-performance liquid chromatography using a strong anionic exchange column coupled with an ICP-MS system. The technique was applied to determine arsenic compounds in beverage samples (beer, juice) and reference material (certified for total As).

2. Experimental

2.1. Apparatus and reagents

A high-performance liquid chromatograph Hewlett-Packard model HP1050 (Waldbronn, Germany) equipped with a six-port injection valve Rheodyne was employed for As species separation. It was used with an anionic-exchange column Hamilton PRP-X10 of 250 mm length and 4.1 mm internal diameter (containing 10 μm particles of a styrene divinylbenzene copolymer with trimethylamonium exchange sites stable at pH values between 1 and 13 and with an exchange capacity of 0.19 meq g^{-1}). As a mobile phase was employed, a K_2HPO_4/KH_2PO_4 buffered solution of pH 8.5 with a phosphate concentration 5×10^{-3} mol L^{-1} and carrier flow of 1.0 mL min^{-1} was used for working in the isocratic mode.

An inductively coupled plasma mass spectrometer ELAN® 5000 (Perkin-Elmer, Thorn hill, Ont., Canada) equipped with a Ryton® Scott-type spray chamber and GemTip™ cross-flow nebulizer (Perkin-Elmer Instruments, Norwalk, CT, USA) was used as a chromatographic detector. The outlet of the HPLC column was connected directly to the nebulizer spray chamber using a Teflon (100 mm × 0.25 mm) tube. Before analysis, the ICP-MS was optimized using a solution containing Mg, Rh, and Pb at a concentration of 1.0 μg mL^{-1}. Mass 75 was monitored in the GRAPHICS Mode of the instrument. The Table 1 shows a summary of experimental details. The data was obtained by a XENIX Operating system.

All solutions were prepared from analytical reagent grade chemicals using ultra pure water, with 18 MΩ cm^{-1} resistivity, obtained from a Milli-Q water purification system of Millipore (Bedford, MA, USA). Standard stock solutions of 1000 μg mL^{-1} were prepared in water from $NaAsO_2$ PanReac (Barcelona, Spain) for As(III), from $Na_2HAsO_2{\cdot}7H_2O$ PanReac for As(V), from $C_2H_6AsNaO_2{\cdot}3H_2O$ Fluka (Buchs, Switzerland) for DMA and from $CH_3AsO(ONa)_2{\cdot}6H_2O$ Carlo Erba (Milano, Italy) for MMA. Working solutions with arsenic concentrations of 1.0–20.0 μg L^{-1} were prepared the day they were used.

Argon C-45, purity 99.995%, was used as auxiliary, nebulizer and plasma gas and obtained from Carburos Metalicos (Barcelona, Spain).

2.2. Methodology applied

Beer, soft drink and juice samples were degassed by filtration through a Whatman 42 filter paper. For chromatographic analysis, 2.0 mL of sample were passed through a 1.0 g C18 sep-pack and filtered using a 0.45 μm membrane and 100 μL were injected in the system. Data found for the peak height of the different As species were obtained and

Table 1
Instrumental conditions employed for determination of arsenic species by ICP-MS

Parameter	ICP-MS operating conditions
RF power (W)	1100
Auxiliary Ar flow (L min^{-1})	0.8
Nebulizer Ar flow (L min^{-1})	0.9
Plasma Ar flow (L min^{-1})	11.0

external calibration was used for quantification. For recovery procedures, samples were spiked at concentration levels from 5.0 to 10.0 μg L^{-1} of As(III), DMA, MMA and As(V) before the clean-up with C18.

3. Results and discussion

3.1. Previous studies on the chromatographic separation of As species

Based on the previously published literature [21] and on a systematic study of monoparametric optimization and multiparametric evaluation of the effect of pH, mobile phase concentration and carrier flow [22], it was selected the use of a K_2HPO_4/KH_2PO_4 5×10^{-3} mol L^{-1} at pH 8.5 with a flow of 1.5 mL min^{-1}.

In the aforementioned conditions, As(III), DMA, MMA and As(V) could be separated with retention times of 1.67, 2.08, 6.52 and 10.72 min, respectively, using an HPLC-HG-AFS system [18]. However, on coupling the HPLC with ICP-MS the retention times moved to 2.75, 3.33, 5.17 and 12.5 min for As(III), DMA, MMA and As(V), respectively, and selected conditions must be modified to K_2HPO_4/KH_2PO_4 5×10^{-3} mol L^{-1} at pH 8.5 with a flow of 1.0 mL min^{-1} (see the chromatogram in Fig. 1). The chromatographic resolution values obtained by the proposed procedure were 1.2, 3.9 and 9.5, respectively, for the separation of As species in the following order of elution: As(III), DMA, MMA and As(V).

3.2. Figures of merit of the developed procedure

Table 2 shows the results obtained using the proposed method. Calibration curves for the four arsenic compounds were examined under the best experimental conditions between 1.0 and 20.0 μg L^{-1} concentration of each compound. Peak height was used for quantification purpose. Linear regression curve of the individual compounds gave correlation coefficients of at least 0.9975. The reproducibility of the retention time and the relative standard deviations for the peak heights were determined by five successive injections of a standard solution containing 5.0 μg L^{-1} all four As compounds. Relative standard deviations for As(III), DMA, MMA and As(V) were 1.2, 2.1, 2.5 and 3.0%, respectively (see Table 2). Limits of detection were calculated as three times the standard deviation of the signal of the lowest standard solution divided by the sensitivity (slope of the calibration graph). They were found to be 0.2 μg As L^{-1} for As(III), 0.2 μg As L^{-1} for DMA, 0.3 μg As L^{-1} for MMA and 0.5 μg As L^{-1} for As(V).

3.3. Analysis of commercially available samples

Beverage samples were obtained from the Spanish market. The content of the arsenic species in the samples (soft drink, beer, juice) was evaluated using the procedure described previously. Samples were filtered, cleaned by passing through a C18 solid phase cartridge and injected in the chromatographic system. Table 3 shows the results obtained. As one can see, concentration levels of arsenic compounds of few μg L^{-1} were found for all analyzed samples. As(III) and As(V) were the only species detected and no organic arsenic species were found in the analyzed samples. For all samples, arsenic compounds were eluted with the same retention time that of standards, thus it not was observed any matrix effect during chromatographic elution step. To validate the method for the analyses of beverages, two samples were spiked with the four arsenic compounds to give a final concentration of 5.0 and 10.0 μg L^{-1} (Table 4). Recovery percentages in the range 90.8–116.0% were obtained, thus indicating that appropriate recoveries were found in all the cases.

Certified materials analyzed by the proposed methodology, after dilution, showed the presence of both inorganic

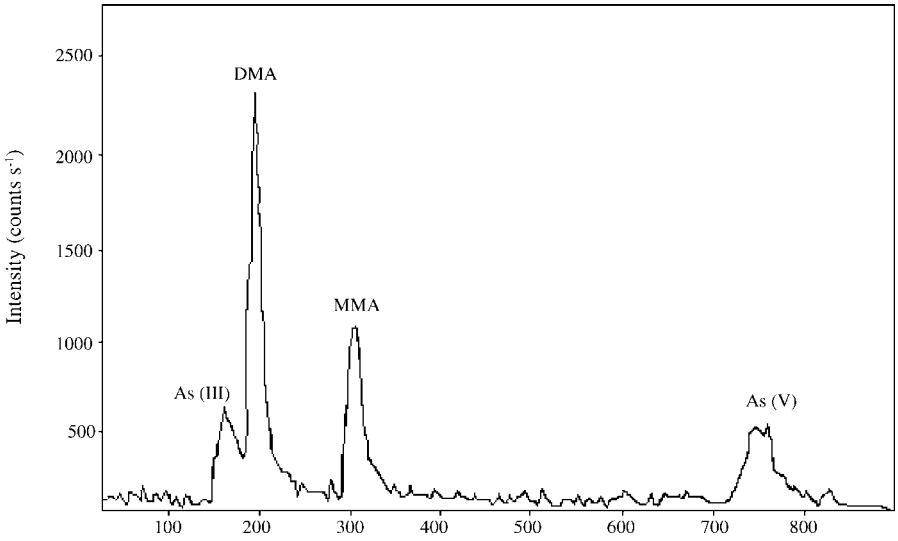

Fig. 1. Separation of As(III), DMA, MMA and As(V) using HPLC-ICP-MS. Standard solution: 10.0 μg As L^{-1} for each. Experimental conditions: see in text.

Table 2
Figures of merit of the proposed method

Parameter	Species			
	As(III)	DMA	MMA	As(V)
Calibration equation	$Y = 60.64X + 3.22$	$Y = 220.94X + 99.46$	$Y = 109.28X + 41.41$	$Y = 45.291X + 60.86$
R.S.D. (%)	1.2	2.1	2.5	3.0
LOD ($\mu g\,L^{-1}$)	0.2	0.2	0.3	0.5

R.S.D.: Relative standard deviation of five determinations of As species at concentrations levels of 5.0 $\mu g\,L^{-1}$. LOD: limit of detection obtained from three times the standard deviation of blank measurements divided by the slope of the calibration line. $Y =$ peak height and $X =$ As concentration.

Table 3
Determination of arsenic species in drinking samples by HPLC-ICP-MS, concentration values in $\mu g\,L^{-1}$

Sample	Arsenic species			
	As(III)	DMA	MMA	As(V)
Soft drink	1.19 ± 0.10	–	–	1.95 ± 0.20
Lemon juice	1.27 ± 0.10	–	–	0.43 ± 0.10
Beer	1.19 ± 0.10	–	–	1.37 ± 0.10

Table 4
Recovery experiments on As determination by HPLC-ICP-MS in the soft drink spiked with 5.0 and 10.0 $\mu g\,L^{-1}$ of As(III), DMA, MMA and As(V)

Sample	Arsenic species							
	As(III)		DMA		MMA		As(V)	
	Concentration	Recovery (%)	Concentration	Recovery (%)	Concentration	Recovery (%)	Concentration	Recovery (%)
Soft drink	1.19	–	–	–	–	–	1.37	–
Sample + 5.0 $\mu g\,L^{-1}$	5.24	104.8	5.22	104.4	5.33	110.7	4.54	90.8
Sample + 10.0 $\mu g\,L^{-1}$	11.26	112.6	10.80	108.0	11.6	116.0	9.3	93.0

Table 5
Determination of arsenic species in certified materials

Sample	Arsenic species					
	As(III)	DMA	MMA	As(V)	$\sum$ As	Certified[a]
APS-1075	49.3 ± 1.0[b]	–	–	31.0 ± 1.0[b]	80.3 ± 1.0[b]	80.0 ± 1.0[b]
APS-1066	–	–	–	0.61 ± 0.03[c]	0.61 ± 0.03[c]	0.60 ± 0.05[c]
APS-1071	103.5 ± 2.0[c]	–	–	–	103.5 ± 2.0[c]	100.0 ± 2.0[c]

APS-1075: Trace metals in drinking water; APS-1066: River sediment standard; APS-1071: primary drinking water metals.
[a] Certified value for total As.
[b] Values in $\mu g\,L^{-1}$.
[c] Values in $\mu g\,mL^{-1}$.

(As(III) and As(V)) species (Table 5). As(III) was the most abundant species in drinking water. However, only As(V) was detected in a river sediment extract. On comparing the data found for the four species, considered by HPLC-ICP-MS with the total As concentration established by direct ICP-MS measurement, a good coincidence can be observed at $\mu g\,L^{-1}$ concentration levels by results found comparable with the total As content at a probability level of 95%.

4. Conclusions

The methodology developed for HPLC-ICP-MS determination of As species in beverages provides a practically direct procedure, which involves a simple sample pre-treatment based on degassing and clean-up by C18 solid phase extraction. The HPLC-ICP-MS provides good recoveries of As(III), DMA, MMA and As(V) and total As concentrations comparable to those found by ICP-MS without chromatographic separation, thus being an interesting alternative for fast screening of the most toxic As species in beverages and environmental samples.

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