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Determination of inorganic arsenic species As(III) and As(V) by high performance liquid chromatography with hydride generation atomic absorption spectrometry detection

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Abstract: The paper presents the principles and advantages of a technique combining high performance liquid chromatography and hydride generation atomic absorption spectrometry (HPLC-HGAAS) applied to speciation analysis of inorganic species of arsenic As(III) and As(V) in ground water samples. With separation of the arsenic species on an ion-exchange column in the chromatographic system and their detection by the hydride generation atomic absorption spectrometry, the separation of the analytical signals of the arsenic species was excellent at the limits of determination of 1.5 ng/ml As(III) and 2.2 ng/ml As(V) and RSD of 4.3% and 7.8% for the concentration of 25 ng/ml. The hyphenated technique has been applied for determination of arsenic in polluted ground water in the course of the study on migration of micropollutants. For total arsenic concentration two independent methods: HGICP-OES and HGAAS were used for comparison of results of real samples analysis.

Keywords: speciation, hyphenated techniques, arsenic, hydride generation absorption atomic spectrometry, high performance liquid chromatography

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1 Introduction

The toxic effect of arsenic is directly dependent on the particular species and its occurrence. Arsenates (III) are ten times more toxic than arsenates (V), which are still more toxic than the organic species monomethylarsenicacid (MMAA) and dimethylarsenicacid (DMAA), while arsenobetaine AsB is non toxic [1]. The content of inorganic species of arsenic in ground water provides important information on migration of pollutants and conditions of circulation of the ground water, in the recharge area of ground water and on intensifying anthropogenic pressure. Therefore, the interest in determination of arsenic species, especially inorganic As(III) and As(V) [2] has recently increased.

A number of analytical methods have been used for the purpose AAS, ICP-MS, ICP-AES, in combination with the technique of hydride generation HG [3]. The use of hydride generation as a technique for introduction of the analyte in spectrometric methods permits isolation of the element under determination from the interfering environmental matrix and determination of particular species on the basis of the differences in the kinetics of hydride generation by As(III) and As(V) [4], [5]. The use of spectrometric methods in combination with the hydride generation atomic absorption spectrometry as a detector in gas or liquid chromatography has considerably extended the possibilities of speciation determinations. The hyphenated systems HPLC-HGICP-MS [6], HPLC-HGAFS [1], [7], [8–9] or HPLC-HGAAS [10–13] permit determinations of the content of inorganic arsenic species As(III) and As(V) [6–13] and organic ones MMAA, DMAA [6–8, 10–13], AsB [1], [6] or others [11], [12] and compete with the systems combined with direct detection HPLC-ICP-MS [14], [15]. The possibility of determination of organic species of arsenic has been achieved by applying on-line mineralisation of organic compounds realised by the thermal method [1], with microwave heating [12], UV-radiation assisted UV [8], [11] or sometimes with preliminary reduction of arsenic species to As(III) [10]. Particularly important, also because of the cost of analysis, are the methods HGAAS and HGAFS used as detection systems in hyphenated systems.

This paper reports results of determinations of the inorganic species of arsenic As(III) and As(V) in samples of polluted ground water performed by the hyphenated technique HPLC-HGAAS. The inorganic arsenic species were separated in a chromatographic system with an ion-exchange column, using an on-line atomic absorption spectrometer with hydride generation as a detector. The analytical solution proposed was characterised and its application in determination of arsenic species in environmental samples was exemplified. The use of constructed analytical HPLC-HGAAS set, the technique that was already discussed in the literature but is hardly ever applied to speciation analysis, allowed to determine the content of inorganic arsenic species in ground water. The results obtained will be used to study the migration of microelements in ground water in the porous hydrogeological medium.

2 Experimental

2.1 Instruments

The hyphenated system constructed is schematically shown in Figure 1. It comprises a liquid chromatograph Shimadzu LC-10A equipped with a pump with a degassing unit, a 250 mm anion-exchange column Supelco LC-SAX1 and a guard column. The tubings were made of PEEK. The eluant was directed to the hydride generation chamber of an atomic absorption spectrometer. The detector was an atomic absorption spectrometer Varian SpectrAA 220FS equipped with a hydride generation unit VGA 77 (flow-through system) with an electrothermally heated quartz cell (ETC-60) as an atomiser and a lamp with hollow cathode (HCL) for arsenic determination (Varian). Argon was used as the carrier gas. The conditions of chromatographic separation, concentrations of reagents used in the system of hydride generation and the conditions of the AAS determinations are specified in Table 1. HGICP-OES system consisted of the spectrometer Jobin-Yvon Model 170 Ultrace (Jobin-Yvon, Longjumeau, France) and hydride generator (Labtech Brno). The hydride generator was equipped with a glass mixing coil and glass gas-liquid separator. The separator was connected directly to injector of the lateral ICP torch. The instrumental parameters were: power 950 W, plasma gas (Ar) flow rate 12 l min^{-1}, sheat gas (Ar) flow rate 0,4 l min^{-1}, carrier gas (Ar) flow rate 0,6 l min^{-1}, sample solution flow rate 2,5 ml min^{-1} and NaBH$_4$ solution flow rate 1 ml min^{-1}.

HPLC	
Column	Supelco LC-SAX1
Mobile phase	2 mmol/l Na$_2$HPO$_4$20 mmol/l KH$_2$PO$_4$
Flow rate	2 ml/min
Injection volume	200 μl
Hydride generation	
HCL concentration / flow	1 mol/l / 1 ml/min
NaBH$_4$concentration / flow	1% / 1 ml/min
AAS	
Atomizer temperature	900^{o}C
Lamp current	10 mA
Wavelength / Slit	193.7 nm/ 0.5 nm

Table 1 Experimental condition for arsenic speciation analysis by HPLC-HGAAS.

2.2 Reagents

All chemical reagents used were analytically pure and the water was redistilled and subjected to ion-exchange process in a Milli-Q unit (Milipore). The basic solutions of the standards at a different degree of oxidation (As(III) and (V)) of the concentration

1 mg/ml were made by dissolution of appropriate weighted portions of sodium arsenate (III) $NaAsO_2$ and sodium hydroarsenate (V) $Na_2HAsO_4^{\cdot}7H_2O$ (Sigma–Aldrich). The standard solutions of lower concentrations were obtained by dilution. For determinations in the hyphenated system the standard solutions contained both arsenic species under determination. The solution of sodium borohydride was made on the day of the analysis by dissolving $NaBH_4$ in a 1% (w/v) solution of sodium hydroxide. The solution of hydrochloric acid was made from a 10 mol/l Suprapur solution (Merck). The phosphate buffer of pH 6.0 was made from a solution of disodium hydrophosphate and a solution of potassium dihydrophosphate (Merck). The solution contained 0.4 mmol/l $Na_2HPO_4$and 4 mmol/l KH_2PO_4. The buffer was filtered and then degassed at the ultrasonic bath. For determinations in the HGICP-OES system 2,5 ml of concentrated HCl and 0,5 ml of 20% KI solution were added to 10 ml of sample. After 15 min 0,5 ml of 10 % ascorbic acid solution was added to the resulting mixture.

2.3 Samples

The samples of ground water were collected from piezometres localised over the dumping ground of a chemical plant, with the help of a pump for collection of ground water samples. After collection, the samples were transported and stored in polyethylene vessels for trace analyses (Nalgene). The samples were stored in the laboratory for no longer than a few days at a temperature close to -30 ^{o}C.

3 Results and discussion

3.1 Hyphenated analytical system HPLC-HGAAS

The inorganic species of arsenic arsenates (III) and arsenates (V) were separated on an anion-exchange column Supelco LC-SAX1. The mobile phase was phosphate buffer (0.4 mmol/l $Na_2HPO_4$and 4 mmol/l KH_2PO_4 of pH = 6.0 ensuring the presence of both arsenic species in the dissociated form. The chromatographic separation was made in isocratic conditions at a constant rate of flow of the mobile phase (2 ml / min). The eluant from the column was supplied to the chamber of hydride generation playing the role of an interface between the chromatographic system and the AA spectrometer. The flow rates used enabled a direct introduction of the eluant into the reaction system of hydride generation. AsH_3 formed was directed in the stream of neutral gas (argon) to the atomiser of the AA spectrometer, which was an electrothermally heated quartz cell. The analytical signal obtained revealed peaks corresponding to particular species. A very good separation of the peaks assigned to As(III) and As(V) was obtained and the corresponding retention times of ∼130 s and 225 s, at no interferences (Figure 2).

3.2 Characterisation of the analytical method

The content of arsenic species was proportional to the area and the height of the peak obtained. However, because of the asymmetry and variation of the peak shape in the quantitative determinations only area of the peak was taken into regard. A number of validation parameters characterising the analytical method were determined (Table 2).

Arsenic species	Linear range of calibration curve ng/ml	Correlation coefficient	Detection limit (6σ)	RSD at 25 ng/ml
As(III)	DL* - 200	0.9903	1.5	4.3
As(V)	DL* - 200	0.9948	2.2	7.8

* Detection Limit

Table 2 Analytical characteristic obtained for arsenic species (n=10) for HPLC-HGAAS determination.

Because of the impossibility of checking the traceability due to the lack of certified reference materials for determination of inorganic arsenic species, the recovery of each species was determined at the standard addition to the environmental sample (Figure 2). The recovery (Table 3) of each form is high. The results indicate that the proposed analytical technique HPLC-HGAAS allows determinations of the inorganic arsenic species As(III) and As(V).

		As (III)		As (V)	
Sample	added ng/ml	found ng/ml	recovery %	found ng/ml	recovery %
1	100	104.1 ± 4.1	104 ± 4	103.7 ± 5.3	104 ± 5
	25	22.6 ± 2.8	90 ± 11	21.8 ± 3.5	87 ± 14
2	100	97.9 ± 4.5	98 ± 4	92.9 ± 5.2	93 ± 5
	25	27.1 ± 3.2	108 ± 13	26.5 ± 3.1	106 ± 12

Table 3 Recovery of arsenic at the addition of standards (n=5).

3.3 Determination of As(III) and As(V) in environmental samples

The proposed method of inorganic arsenic species determination was applied in analysis of ground water samples from piezometres over the area of a dumping ground of a chemical plant. The results obtained in hyphenated system HPLC-HGAAS by the method of the standard addition were in good (below 20% of RSD) agreement with those obtained by the methods of HGICP-OES and HGAAS (Table 4).

The content of arsenic in the samples studied was a hundred times greater than its average level in ground water [16], which testifies to a strong effect of dumped substances on the underground waters. The dominance of the species As(V) suggests a hypothetical

Sample	HGICP-OES Total ng/ml	HGAAS Total ng/ml	HPLC-HGAAS As(III) ng/ml	 As(V) ng/ml
H(I)	1,04 ± 0,05	1,15 ± 0,07	-*	-*
J(I)	32,8 ± 0,1	38,6 ± 0,4	-*	36,3 ± 3,2
L(I)	43,7 ± 0,2	42,3 ± 0,4	-*	39,9 ± 4,0
L1(I)	47,7 ± 0,4	55,7 ± 0,6	-*	59,0 ± 5,2

* Below detection limit

Table 4 Results of determination forms of arsenic (n=5).

character of migration of arsenic to the underground waters in the process of vertical migration through the aeration zone [2].

4 Conclusions

The hyphenated technique HPLC-HGAAS ensures effective determination of inorganic arsenic species As(III) and As(V). Relatively low limit of determination and a wide dynamic range for determinations of both species of inorganic arsenic at a good precision make this technique a unique analytical tool competitive to the other methods of speciation analysis. The hyphenated HPLC-HGAAS technique proposed is expected to become a very important tool in analytical study of not only environmental samples.

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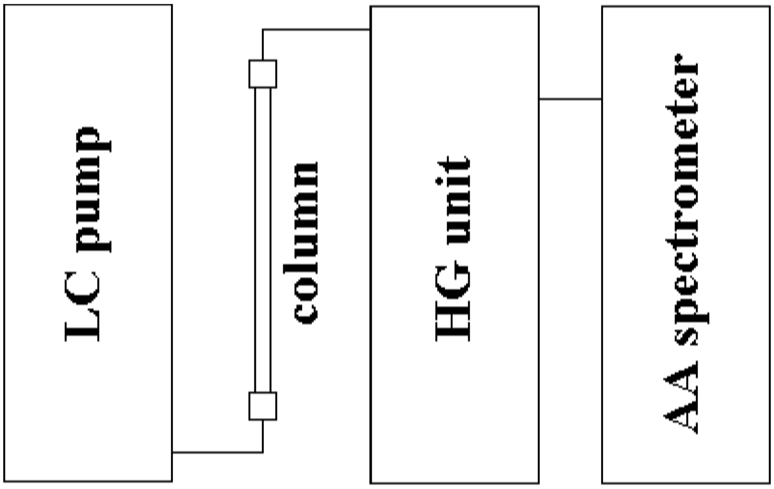
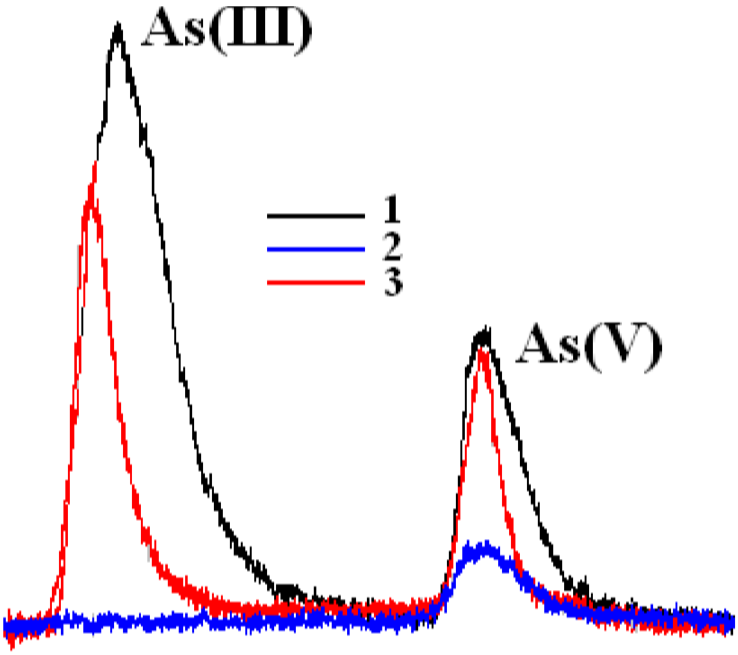


Fig. 1 Schematic diagram of the HPLC-HGAAS-instrumentation.



1 – standard (200 ng/ml each form),
2 – sample 1,
3 – standard addition to sample 1 (100 ng/ml each form)

Fig. 2 Chromatograms of two arsenic forms As(III) and As(V) (corresponding with Table 3).