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Short communication

High-performance liquid chromatography–hydride generation–atomic fluorescence spectroscopic determination of arsenic species in water

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Abstract

A method was developed for the determination of arsenite (As^{III}), monomethylarsonate (MMAs), dimethylarsinate (DMAs), arsenate (As^{V}) from waters by using ion-pair chromatography hydride generation and atomic fluorescence spectrometry. A C_{18} bonded silica column modified by didoctyldimethylammonium bromide (DDAB) was used for separation. The effect of phosphate and methanol content of the eluent on the separation was investigated. The influence of five metals on hydride generation efficiency was tested. The optimal hydrogen chloride and sodium borohydride quantity for hydride generation was determined. The detection limit of the developed method, by using a 250-mm^3 loop, was 0.4 ng/cm^3 for As^{III}, 0.8 ng/cm^3 for DMAs, 0.6 ng/cm^3 for MMAs and 1.2 ng/cm^3 for As^{V}. The responses for all species tested were linear in 10–3000 ng/cm^3 range.

Keywords: Hydride generation–atomic fluorescence spectroscopy; Interfaces; Arsenic compounds; Metals; Organoarsenic compounds

1. Introduction

Many previous publications dealt with the speciation of arsenic [1–3]. The different species of arsenic exibit a wide range of toxicity, for example As^{III} and As^{V} are prominently toxic, whereas arsenobetaine and arsenocholine are virtually non-toxic. Moreover, arsenic is on the way to being recognised as essential for human health.

Deep and well waters in some areas of Hungary are contaminated by arsenic for hydrogeological reasons. These waters are the main sources of drinking water in some parts of Hungary. The measurement of arsenic, particularly the analysis of the specific chemical forms is of primary importance in identifying toxic levels of the element and gaining precise knowledge of its biochemical cycling [4].

High-performance liquid chromatography (HPLC) is the most powerful technique for the separation of arsenic species. Ion-exchange chromatography has been the most popular method to separate the different species [5,6]. However, the low matrix tolerance, the short lifetime and the high cost of ion-exchange HPLC columns exhibit a general problem. A recent paper by Liu et al. [7] presented the separation of As^{III}, MMAs, DMAs, As^{V}, the four arsenic species bearing reasonably high toxicity. This method was efficient and fairly robust, therefore it

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was used (chosen to be) as the starting point for the development of this new system [8].

Arsenic species absorb in the ultraviolet region, therefore following HPLC separation the use of UV detection proves to be adequate [4]. For the detection of inorganic ions electrochemical detection methods are similarly used [9]. The advantages of UV and electrochemical detectors are: they are widely used and it is simple to couple them to HPLC systems. Since these detectors are not selective for arsenic compounds, the matrix effect has to be considered. The high detection limit of the above mentioned detectors exclude the possibility of the direct analysis of arsenic species from water samples.

The direction for further steps on the field of trace element speciation was assured by coupling element-specific (spectroscopic) detection methods with HPLC separation techniques [10]. Today, such combinations are called "hyphenated techniques". HPLC with atomic absorption spectroscopy (AAS) [11], inductively coupled plasma atomic emission spectroscopy (ICP-AES) [12], inductively coupled plasma mass spectroscopy (ICP-MS) [13] are the most frequently used "hyphenated techniques" for arsenic speciation. Boosted-discharge hollow cathode lamps, which have recently become commercially available, rendered the use of atomic fluorescence spectroscopy (AFS) possible. The consequence of the measurement principle of AFS detectors is an outstandingly good selectivity [14].

The main technical problem of spectroscopic detection is the connection of the HPLC systems to the atomic source (flame or plasma) of the detectors without losing the separation characteristics of the HPLC system. Therefore the "interfacing appliance" used should be able to decrease the outlet volume of the HPLC system, transform the outlet to gas or gas-dispersion and transfer it to the atomic source. Most commonly the interfacing appliance is a nebuliser. Using an HPLC–ultrasonic nebulizer–AFS system, relatively good sensitivity and selectivity had been achieved in previous experiments [8].

Provided that the "interfacing appliance" is selective (by which only the metal species to be tested are transferred) the signal-to-noise ratio of the detector spectacularly improves. One of the selective "interfacing appliances" is the hydride generation technique. Based on the experience obtained in the above described way, our system constructed for arsenic speciation is built up of ion-pair chromatographic separation, hydride generation interface and AFS detection.

2. Experimental

2.1. Instrumentation

A Shimadzu Model LC-7A HPLC pump was attached to a sample injection valve (six-port Rheodyne system, LMIM, Hungary). A 250-μl sample loop was used for sample introduction. The analytical column was a Bio Separation Technologies (BST) C_{18} Rutin column (25×4.6 mm I.D., 10 μm particle size). A four-channel peristaltic pump (Ismatec MS-CA 4, Switzerland), a 100 cm long mixing coil functioned as continuous hydride generator. A laboratory-made liquid–gas separator was used in the system. The nebulizer was further connected to an AFS system (PSA Excalibur, PS Analytical, Sevenoaks, Kent, UK) that utilises an arsenic boosted discharge hollow cathode lamp (Superlamp, Photron, Vic., Australia) as an excitation source. Measurements were carried out around the resonance wavelength of arsenic (193.7 nm) using a multi-reflectance filter having a spectral bandpass between 20 and 40 nm. Argon functioned as a carrier gas and mixed with the hydrogen, supported the diffusion flame. The constant gas flows were maintained by Cole-Palmer rotameters (Niles, IL, USA). Data collection and evaluation was totally automated by a Borwin Chromatographic software (JMBS, Grenoble, France). All peaks were evaluated by their peak height, as it was found easier to carry out than the peak area method and no difference was found between the results of the two methods. A schematic diagram of the system is shown in Fig. 1.

2.2. Reagents

Arsenite stock solution was prepared dissolving 1.320 g of As_2O_3 in 25 cm^3 of 0.5 mol/dm^3 NaOH solution and then diluting the solution to 1 dm^3 with 0.6 mol/dm^3 HCl. As^V stock solution was obtained from Merck (Darmstadt, Germany). MMAs stock solution was prepared from strychrotonin solution

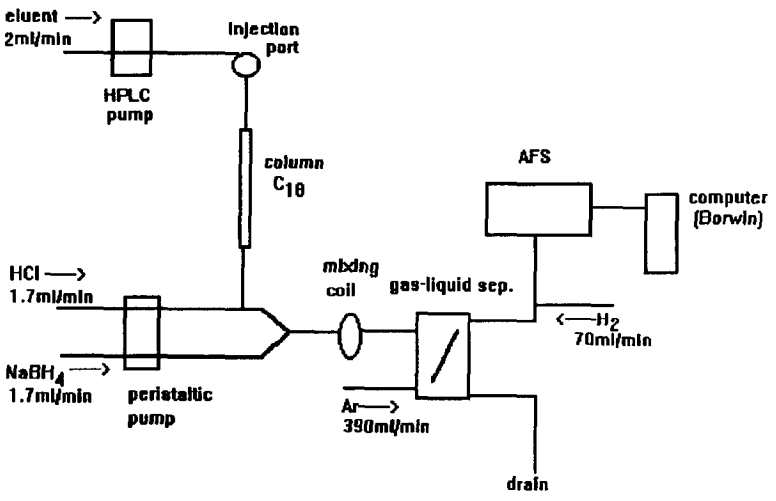

Fig. 1. Schematic diagram of the HPLC–hydride generation–AFS system used for the arsenic speciation.

(Chinoin, Budapest), the DMAs was obtained from Fluka. The 1000 mg/dm^3 stock solutions of MMAs, DMAs, As^{III} and As^{V} were further diluted in deionized water daily.

The DDAB solution (0.01 mol/ dm^3) was prepared by adding 0.5% (v/v) methanol and 0.1% (v/v) of 0.01 mol/dm^3 DDAB solution to the Na_2HPO_4 buffer solution (20 mmol/dm^3). The pH was set to 6.0 by the addition of NaH_2PO_4 solution containing the same amount of phosphate, methanol and DDAB as the eluent.

Chemicals for hydride generation: the (2%, w/v) sodium tetrahydroborate solution was prepared daily by dissolving $NaBH_4$ powder (Aldrich) in 0.5% (w/v) NaOH solution, the HCl was obtained from Reanal (Hungary). A Reanal potassium iodide and sodium thiosulfate was used for preparing the 0.1%, 1% and 10% solutions for reducing As^{V} to As^{III}.

2.3. Procedures

2.3.1. Column modification

The C_{18} bonded silica column was modified by passing through 500 cm^3 of a DDAB solution (1×10^{-2} mol/dm^3) in methanol–water (50:50) at a flow-rate of 1 cm^3/min. Then de-ionised water was passed through the column. The modified column was kept in de-ionised water when not in use and more than 400 measurements (mainly standard solutions and water samples) were made before renewing.

2.3.2. Arsenic speciation

A 250-mm^3 volume of the working standard solutions of a mixture of arsenic species are injected directly onto the HPLC column through the injection port (Fig. 1). After separation, first HCl than $NaBH_4$ solutions are introduced into the stream of the column effluent. Hydride generation takes place in a mixing coil, the generated gas phase is being separated from the liquid phase continuously in the gas–liquid sepator. A continuous stream of argon transfers the generated hydrides and hydrogen from the separator to the AFS detector. The hydrogen–argon diffusion flame is maintained by externally introduced hydrogen where the hydrogen gas enters the system via an Y-shape connection tube about 25 cm from the flame. (Tube lengths were reduced as much as possible to minimise dead volumes.) All chromatographic peaks were evaluated by their peak heights.

2.3.3. Optimization of HCl and $NaBH_4$ quantity for hydride generation

In order to determine the optimal quantity of HCl and $NaBH_4$, their concentration was changed between the following values:

$NaBH_4$ % (w/v)	0.25	0.5	1.5	3

Table 1
Operating conditions for the HPLC–hydride generation–AFS system

HPLC system	
Column	BST C_{18} Rutin column (25×4.6 mm I.D., 10 μm particle size)
Column temperature	24°C
Sample loop size	250 mm^3
Mobile phase	
0–2 min	0 mmol/dm^3 Na_2HPO_4–NaH_2PO_4 buffer
2–4 min	0⇒50 mmol/dm^3 Na_2HPO_4–NaH_2PO_4 buffer
4–8 min	50 mmol/dm^3 Na_2HPO_4–NaH_2PO_4 buffer +1·10^{-5}mol/dm^3 DDAB+0.5% (v/v) methanol (pH 6.0)
Pump flow-rate	2 ml/min
Hydride generation	
(a)	HCl 1.5 *M*
(b)	$NaBH_4$ 2% (w/v)
Pump flow-rate	1.7 ml/min
Atomic fluorescence detector	
Primary current	27.5 mA
Boost current	35 mA
Detection wavelength range 190–210 nm	
Gas flow-rates	
Argon	390 cm^3/min
Hydrogen	70 cm^3/min

HCl (mol/dm^3)	0.75	1.5	3.0	4

The optimal values proved to be 2% (w/v) in the case of $NaBH_4$ and 1.5 mol/dm^3 in the case of HCl. The details of the optimization are shown elsewhere [15].

The optimal parameters for the separation and the detection of the four arsenic species are presented in Table 1.

3. Results and discussion

3.1. Determination of optimal eluent composition

In ion-exchange chromatographic systems an increase of the buffer concentration of the eluent results in a decrease in retention time. According to our experiments, the most adequate way of influencing retention times is to change the phosphate concentration of the buffer.

The effect of the phosphate buffer concentration on retention time was studied by changing the amount from 10 to 50 m*M* systematically, as shown in Fig. 2. As expected, the most spectacular decrease in retention time was observed in the case of As^V (the highest charge of all the solutes tested). Based on the results previously obtained, we developed a gradient elution method by which the first three eluted components (As^{III}, DMAs, MMAs) are separated more efficiently than by isocratic methods. The parameters of the gradient methods are shown in Table 1. With the gradient elution method, the long retention time of As^V was reduced to 6.4 min. This way the total HPLC analysis time was decreased by 40%. According to the results of earlier experiments, changing the methanol content of the eluent has basically no effect on the system.

3.2. Effect of matrix on separation, hydride generation and detection

The effect of some elements (Na, Ca, Fe, Cu, Se) was investigated on the chromatographic method, hydride generation and detection. These metals occur most frequently in water samples. The reasons for choosing these metals for the experiment, besides

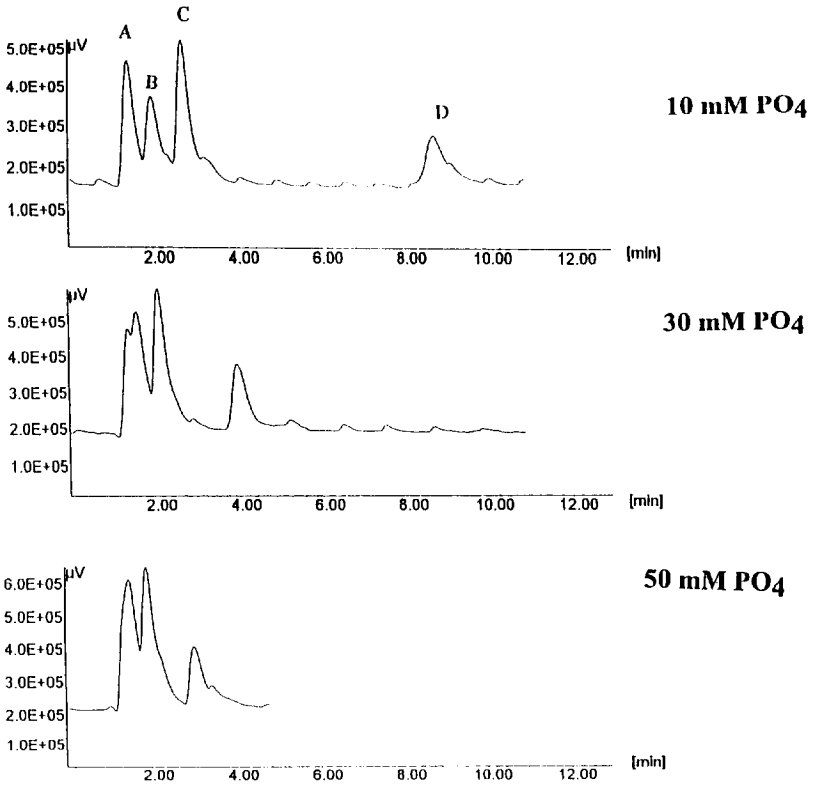

Fig. 2. Effect of phosphate buffer concentraion on retention time (A, As^{III}; B, DMAs; C, MMAs; D, As^{V}).

these being the most frequently occurring ones in water samples, are the following:

- Na and Ca may cause spectral interference during detection, because of their relatively low ionisation potential
- Fe, Cu and Se may reduce the effectiveness of hydride generation
- All of the five metals may deviate the redox equilibriums which determine the conditions of separation.

To a standard solution containing 100 ng/cm^3 of all of the four arsenic species the five tested metals were added in 0.1, 1, 10 mg/cm^3 concentration.

The recovery of arsenic species from the standard solution containing the five metals was practically 100%. In all the cases deviation remained within the relative standard deviation (R.S.D.) of the chromatographic method. The retention time of all the arsenic species remained uneffected. Therefore we may conclude that the conditions of separation, hydride generation and detection were not changed by the presence of the metals investigated above in the standard As solution. The obtained results prove the stability and robustness of the separation technique and thus the whole measuring system. A typical chromatogram of the four arsenic species is shown on Fig. 3.

3.3. Characteristics of the method

The retention time, the detection limit ($3r$ value) and the R.S.D. (on the basis of nine replicate measurements) of our system for the four arsenic species are shown in Table 2. In order to be able to compare our system with other systems, detection limits of various other systems are presented in Table 3. On the basis of Table 3, we can conclude that only the significantly more expensive LC–ICP-MS system

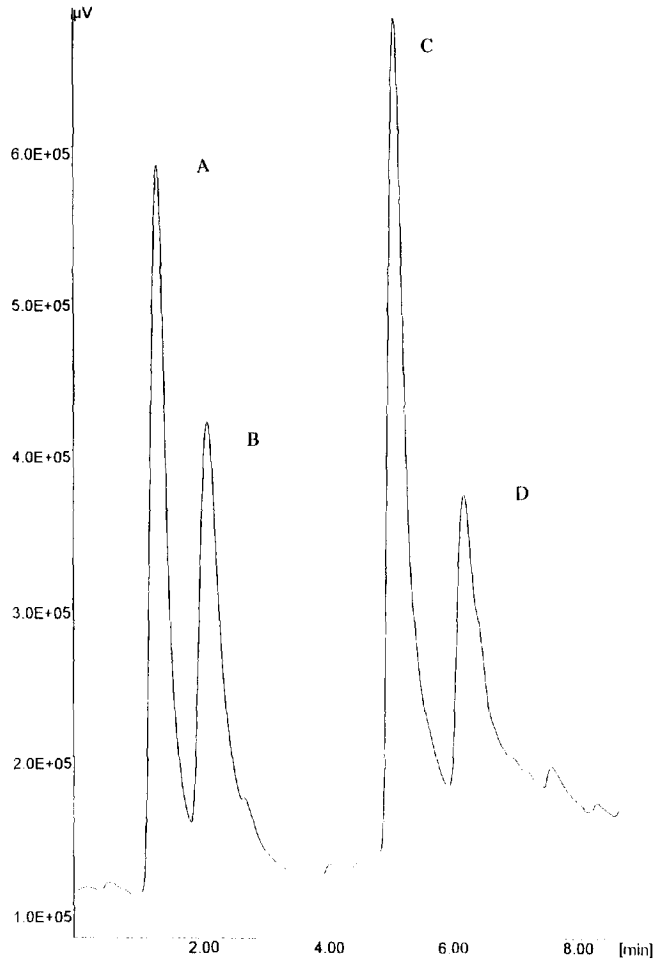

Fig. 3. As speciation from standard solution by gradient elution (A, As^{III}; B, DMAs; C, MMAs; D, As^{V}; concentration of all arsenic species was 40 ng/cm^3).

Table 2
Retention, detection limit and R.S.D. of the four As species

As species	t_R (min)	DL[a] (ng)	R.S.D.[b] (%)
As^{III}	1.35	0.4	3.2
DMAs	2.15	0.8	2.9
MMAs	5.13	0.6	4.3
As^{V}	6.27	1.2	4.5

[a] Detection limit.
[b] Relative standard deviation at 100 ng/ml (n=9).

resulted in better detection limits than our relatively simple and inexpensive method.

Responses for all the four arsenic species tested was linear in 10–3000 ng/cm^3 range, based on five replicate measurements of each solutions of four species at eight different concentrations. The detection limit range required by the samples is well satisfied with this range of linearity.

The calibration curves used for water sample analysis range from 10 to 130 ng/cm^3. The calibration curve parameters for the four arsenic species are found in Table 4. The good correlation coefficient means the linearity of the calibration curves in

Table 3
Detection limits of the four As species measured by different systems

As species	LC–HG–ICP-AES [7]	LC–ICP-MS [11]	LC–FAAS [11]	LC–ultrasonic nebulizer–AFS [8]	LC–UV detection [4]
As^{III}	0.5	0.7	110	35	10
DMAs	1	0.3	70	20	–
MMAs	0.6	0.3	140	20	–
As^{V}	1.2	0.3	140	50	25

Table 4
Calibration parameters of the four As species

Species	A	B	Coeff. of correlation
As^{III}	10.22	0	0.999
DMAs	6.16	0	0.999
MMAs	9.96	1.4	0.996
As^{V}	5.05	1.5	0.963

$y = Ax + B$.

the above mentioned concentration range and the higher sensitivity of As^{III} and MMAs is the effect of the relatively high A values.

3.4. Arsenic content of waters

Averages of five replicate determinations of arsenic content of water samples taken from drilled wells are seen in Table 5. In the first row the NIST 1643c fresh water standard is seen. The total arsenic content of the standard is certified. The origin of the two species measured from the NIST CRM needs more investigation.

The reference values for all the other water samples were determined in laboratories elsewhere, by ICP-AES and graphite furnace-AAS technique. Knowing the detection limits of the above-mentioned techniques regarding arsenic determination, the values provided by us are acceptable since recovery was practically 100% of the NIST standard. The big deviation at sample N.5 can be explained by the detection limits of the graphite furnace-AAS technique. The relative standard deviation of five replicates was in range of 5–15%.

4. Conclusion

The main advantage of our newly developed chromatographic system compared to the previously applied methods is its remarkably low detection limit which makes the direct analysis of different water samples possible. It is proved by a series of experiments that the efficiency and robustness of the separation guarantees precise direct As species analysis of different fluid samples (wine, beer, tea etc.).

The system is very simple and stable. Regarding analytical parameters, our system is qualified to enter into competition with the more complicated and more expensive ICP-AES, ICP-MS and AAS systems.

Table 5
Results of As determination from different samples

Sample	Certified (ng/ml)	Measured (ng/ml)				Total As (ng/ml)	Recovery (%)
		As^{III}	DMAs	MMAs	As^{V}		
NIST 1643c	82±1.2	43.0	–	39.7	–	82.7	101
Water N.1	56[a]	–	–	–	52.1	52.1	93
Water N.2	56[a]	39.0	–	–	23.0	62.0	111
Water N.3	55[b]	–	–	–	63.0	63.0	115
Water N.4	58[b]	10	–	–	50.5	60.5	103
Water N.5	10[b]	–	–	–	16.5	16.5	165
Water N.6	63[b]	–	–	–	70.8	70.8	111

[a] ICP-AES.
[b] Graphite furnace-AAS.

Acknowledgments

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