Anal Bioanal Chem (2002) 374 : 513–519
DOI 10.1007/s00216-002-1492-7

ORIGINAL PAPER

Marta Segura · Juan Muñoz · Yolanda Madrid
Carmen Cámara

Stability study of As(III), As(V), MMA and DMA by anion exchange chromatography and HG-AFS in wastewater samples

Received: 24 April 2002 / Revised: 5 July 2002 / Accepted: 5 July 2002 / Published online: 10 September 2002

Abstract The stability of arsenic species {arsenate [As(V)], monomethylarsonate [MMA], dimethylarsinate [DMA] and arsenite [As(III)]} in two types of urban wastewater samples (raw and treated) was evaluated. Water samples containing a mixture of the different arsenic species were stored in the absence of light at three different temperatures: +4 °C, +20 °C and +40 °C. At regular time intervals, arsenic species were determined by high performance liquid chromatography (HPLC)-hydride generation (HG)-atomic fluorescence spectrometry (AFS). The experimental conditions for the separation of arsenic species by HPLC and their determination by AFS were directly optimised from wastewater samples. As(III), As(V), MMA and DMA were separated on an anion exchange column using phosphate buffer (pH 6.0) as the mobile phase. Under these conditions the four arsenic species were separated in less than 10 min. The detection limits were 0.6, 0.9, 0.9 and 1.8 μg L^{-1} for As(III), DMA, MMA and As(V), respectively. As(V), MMA and DMA were found stable in the two types of urban wastewater samples over the 4-month period at the three different temperatures tested, while the concentration of As(III) in raw wastewater sample decreased after 2 weeks of storage. A greater stability of As(III) was found in the treated urban wastewater sample. As(III) remained unaltered in this matrix at pH 7.27 over the period studied, while at lower pH (1.6) losses of As(III) were detected after 1 month of storage. The results show that the decrease in As(III) concentration with time was accompanied by an increase in As(V) concentration.

Keywords Arsenic · Speciation · Wastewater · Atomic fluorescence spectrometry · Stability

M. Segura · J. Muñoz · Y. Madrid · C. Cámara (✉)
Departamento de Química Analítica,
Facultad de Ciencias Químicas, Universidad Complutense,
28040 Madrid, Spain
e-mail: ccamara@quim.ucm.es

Introduction

Arsenic enters the aquatic environment from natural sources and by anthropogenic contamination as a result of human activities such as mining, smelting, glass making and pesticide manufacture. The maximum admissible concentration of total arsenic in wastewater samples is in the range 0.5–1 mg L^{-1} [1], depending on the type of industry, while in drinking water the concentration is about 10 μg L^{-1} [2].

Although there are no legislated values for arsenic species, it is established that the inorganic arsenic compounds are far more toxic than the organic ones. Consequently, it is important to have analytical methods and techniques for the identification and determination of arsenic species in different matrices. Most of them are hyphenated techniques which employ liquid chromatography separation coupled on-line with very sensitive, element specific detection methods such as inductively coupled plasma mass spectrometry (ICP-MS) [3, 4]; hydride generation (HG) atomic absorption spectrometry (HG-AAS) HG-ICP atomic emission spectrometry (HG-ICPAES) and HG atomic fluorescence spectrometry (HG-AFS) [4, 5, 6, 7, 8, 9], but in some cases, the sample is directly measured in the field using other techniques [10].

The achievement of reliable results in speciation studies requires not only sensitive techniques but also stability of the species. Few samples are analysed immediately upon receipt, and in many cases they have to be stored.

Several studies on arsenic species stability in water samples have been carried out, in some cases with contradictory conclusions concerning acidification, storage temperature, containers and type of matrix. Acidification prevents Fe from precipitating as the hydrous oxide, which would co-precipitate arsenic. Several acids have been used to reduce sample pH below 2: sulphuric acid [11], hydrochloric acid [12], nitric acid or ascorbic acid [13].

Storage temperature can play also an important role. Since the micro-organisms might metabolise and transform elemental species, a decrease in temperature is supposed to decrease biological activity and species transfor-

mation. Therefore Palacios et al. [14] recommended the freezing of aqueous samples containing arsenic species at –20 °C. However, Lidemann et al. [15] considered it an inconvenient storage method since there was a loss of almost 20% [of 20 μg L^{-1} As(III)] after the storage period of 30 days. A freezing process could induce chemical changes such as precipitation, which were not reversed in the melting process.

Contradictory information is available about the stability of arsenic species in different water matrices, too. Tallman and Shaikh [16] found that inorganic arsenic solutions (at a level of micrograms per litre) in distilled demineralised water, as well as in the presence of redox agents common in natural wastewaters (for example O_2, H_2S and Fe^{3+}), were stable for at least 3 weeks when stored in Pyrex glass with minimum precautions and without preservatives. Feldman reported that in unpreserved samples of As(III) at room temperature, 1–10 μg L^{-1} of As(III) oxidised completely within 4 days, 100 μg L^{-1} of As(III) oxidised completely within 7 days and 1 mg L^{-1} oxidised completely within 18 days without any losses owing to adsorption on the glass walls of the containers [17]. In a BCR experience, pure solutions of mixtures of As(III), As(V), MMA and DMA were shown to display degradation of some compounds after long periods of storage. Consequently, calibration stock solutions had to be stored as individual solutions of each compound in preference to mixtures [18]. The stability of the mixtures showed that, after 4 months of storage at +20 °C in the presence of light, As(III) was quantitatively transformed into As(V).

As(V) seems to be the predominant arsenic species in groundwaters [3], and in some wells and river waters As(III) was stabilised by its interaction with organic matter [19, 20]. In estuarine and oceanic environments the metabolisation of arsenates by phytoplankton and their transformation into soluble reduced species such as As(III), MMA or DMA have been documented [21]. It has been noted that the As(III) profile is depressed at the surface, probably due to more intense photochemical oxidation. Jean Yves Cabon et al. [22] also showed that As(III) was not stable in acidified seawater and the rate of conversion of As(III) to As(V) was dependent on the HCl concentration and the salinity. During the phytoplankton bloom all inorganic As(V) was converted to inorganic As(III) and afterwards, all As(III) was rapidly re-oxidised to inorganic As(V). MMA and DMA did not decrease after the phytoplankton bloom.

Little has been published on the speciation of wastewater samples. E. Russeva proposed a method of speciation of As(III) and As(V) though a column filled with inert support modified with the organotin reagent $(C_8H_{17})_2SnCl_2$, where only As(V) is retained and all the analysed samples were supposed to contain no organic arsenicals [23].

The aim of this work was to evaluate the stability of the arsenic species As(III), As(V), MMA and DMA in different wastewater samples under different temperatures and length of storage. Losses and species transformations were investigated. For this purpose the optimum conditions for the separation of arsenic species by HPLC and the HG conditions for their determination by AFS were established.

Materials and methods

Apparatus. The separation and determination of arsenic species was performed by HPLC-HG-AFS. The HPLC system consisted of a Milton Roy CM 4000 (Milton Roy, Ribera Beach, Fla., USA) equipped with an injection valve (Rheodyne 97251, Rheodyne, Calif., USA) and a 100 μL loop for sample introduction. The separation of the arsenic species was performed isocratically using an anion exchange column (250 mm×4.1 mm) (Hamilton, Reno, Nev., USA) PRP X-100 with 20 mmol L^{-1} phosphate buffer at pH 6.0 and a flow rate of 1.5 mL min^{-1} as the mobile phase.

The chromatograph was connected to the HG system by means of a PTFE tube (0.3 mm i.d.). HG of volatile compounds prior to the detection was performed by adding on-line solutions of HCl and $NaBH_4$ by means of a Gilson HP4 (Gilson, Villiers-le-Belle, France) peristaltic pump. The separation of the gaseous compounds from the liquid stream was performed in a gas-liquid separator (Philips, Eindhoven, Netherlands) using Ar (C-45) as a carrier gas. The argon stream was dried with a hygroscopic membrane dryer tube, Perma Pure (PS Analytical, Kent, UK) and close to the flame, a hydrogen flow was also added to support the hydrogen-argon diffusion flame of the detector.

AFS detection was achieved with a PSA Analytical Model 10.033 Excalibur detector (PS Analytical). The excitation source used in the instrument was an arsenic-boosted discharge hollow cathode lamp (Photron, Victoria, Australia) operated at primary and boost currents of 27.5 and 35.0 mA, respectively. A hydrogen-argon diffusion flame was used as the atom cell and an interference filter served to achieve wavelength isolation. The fluorescence signal was observed at 90° and was imaged by a lens onto a solar blind photomultiplier operating at 500 V. The signal was recorded on a BBC Goerz Metrawatt SE 120 recorder and the analytical peaks were evaluated as peak height.

Detection by ICP-MS was performed using a HP-4500 (Hewlett Packard, Tokyo, Japan). The system was optimised by nebulising 10 μg L^{-1} of a mixed standard solution containing Li, Co, Y, In and Ce. The optimisation was also checked using 10 μg L^{-1} arsenic standard solution, in order to improve further optimisation. Details of the operating conditions are given in Table 3.

Standards and reagents. All reagents were of the analytical-reagent grade of higher purity and de-ionised water obtained from Milli-Q system (Millipore, Bedford, Mass., USA) was used throughout. Stock solutions containing 1000 mg L^{-1} of As_2O_5 (Merck, Darmstadt, Germany) in 0.5 mol L^{-1} HNO_3 and 1000 mg L^{-1} of As_2O_3 in 4% HNO_3 (J.T. Baker, Phillipsburg, N.J., USA) were employed, together with 1000 mg L^{-1} of DMA and MMA prepared by dissolving in ???? appropriate amounts of $(CH_3)_2AsO_2Na.3H_2O$ (Sigma, St. Louis, Mo., USA) $(CH_3AsO(ONa)_2.6H_2O$ (Carlo Erba, Milan, Italy), respectively. Working arsenic solutions for analysis were prepared daily by dilution.

Borohydride solutions were prepared by dissolving $NaBH_4$ powder (Aldrich, Milwaukee, Wis., USA) in de-ionised Milli-Q water and stabilised with 0.3% w/v NaOH (Merck). Solutions were filtered before use to eliminate turbidity and HCl (Merck, 37%) was used for HG.

The phosphate mobile phase was prepared from H_3PO_4 (Carlo Erba) and $Na_3PO_4.12H_2O$ "Suprapur"(Merck) and was adjusted to different pHs.

Sampling and sample preparation. The urban wastewater samples were collected at an accessible site located in the proximity of the laboratory of a wastewater treatment plant (la China, Madrid) which deals with the wastewater coming from the centre of the city and whose influent is almost entirely of urban origin. The raw wastewater sample was collected with a magnetic pump without metal parts in contact with the solution, in an existing channel easily accessible and in which the effluent is considerably fast,

favouring sample homogenisation and avoiding large solid particles. The treated water was taken at the last stage of the wastewater treatment plant, before the tertiary filtration stage.

The samples were collected in high-density polyethylene containers (previously cleaned by leaching with 5% HNO_3 and rinsing with ultrapure water). Once received, the treated wastewater sample pH was adjusted to two pH values (1.60 and 7.27) and the raw wastewater sample pH was maintained at its natural value of 7.60. All wastewater samples were homogenised by stirring for a period of 16 h and pre-filtered through a 1.2 µm on-line prefilter cartridge (Millipore p.n KW1904NP3) and then filtered by means of 0.5 µm cartridges (Millipore p.n KWSC04NP3) [24]. Treated wastewater samples were spiked with 8, 15 and 30 µg L^{-1} of As(III), MMA and DMA, and the raw wastewater sample with 20, 30, 50 and 15 µg L^{-1} of As(III), MMA, DMA and As(V), respectively. The fortified samples were kept in 100 mL polyethylene containers filled almost to the top to avoid unnecessary contact with air, stored under various conditions until analysis: at +4 °C, +20 °C and +40 °C, and protected from light to avoid phototransformation of the species.

Procedure. Wastewater samples were loaded into a 100 µL loop and injected on to the anion-exchange column using buffering phosphate solution (20 mmol L^{-1}, pH 6.0) as eluent at 1.5 mL min^{-1}. The eluent from the column was merged at a T-piece with 1.0 mol L^{-1} HCl at 2.4 mL min^{-1}. The resulting acid solution was mixed with 1.0% w/v $NaBH_4$ in the reaction coil where the hydride formation took place. The hydrides were separated from the solution by an U-tube separator and swept by argon to a hygroscopic membrane dryer tube to remove moisture from the carrier gas stream. Afterwards hydrides were introduced into the atom-cell, which utilises an argon-diluted hydrogen diffusion flame. To improve the maintenance of the flame, an auxiliary stream of hydrogen of 144 mL min^{-1} was used. The As(III), As(V), DMA and MMA peaks were registered and evaluated as peak height. In all experiments the peak height was the average of the readings from three injections of each tested solution.

Results and discussion

Optimisation of experimental parameters of HPLC-HG-AFS for arsenic speciation

All the experimental parameters for arsenic speciation were directly optimised using wastewater samples.

Optimisation of chromatographic parameters. The isocratic chromatographic separation for the four arsenic species presented in the wastewater samples was carried out on an anion-exchange column (Hamilton PRP X-100) using phosphate buffer as the mobile phase. The effect of phosphate buffers at 10–20 mmol L^{-1}, pHs 4–8 and 1–2 mL min^{-1} flow rates were investigated. The results showed that low mobile phase concentration produced well-separated peaks but the last-eluting species, As(V), showed peak broadening due to the long retention time. Therefore 20 mmol L^{-1} phosphate was chosen for further experiments since it provides good separation of arsenic species in a reasonable time. Concerning the influence of pH, the retention of arsenic species decreased with increasing pH up to a value of 7.0, at which MMA and DMA peak overlapping occurred. The flow-rate of the mobile phase was optimised to 1.5 mL min^{-1}. Under these conditions the four arsenic species were separated in wastewater samples in less than 10 min. The retention times were 1.58, 2.33, 3.13 and 7.25 min for As(III), DMA, MMA and As(V), respectively.

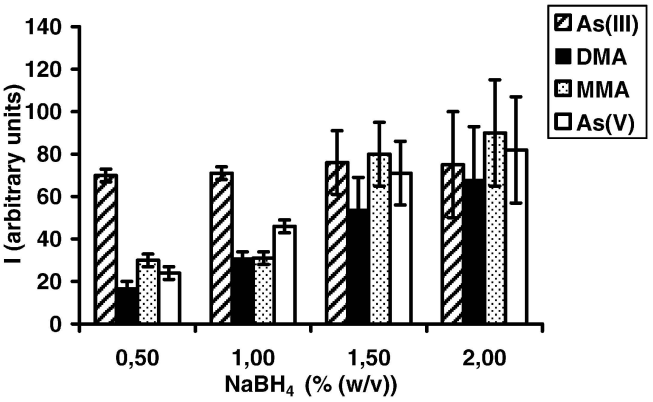


Fig. 1 Effect of $NaBH_4$ concentration on the hydride generation (HG) efficiency from urban raw wastewater containing 20 µg L^{-1} As(III), 50 µg L^{-1} dimethylarsinate (*DMA*), 30 µg L^{-1} monomethylarsonate (*MMA*), and 20 µg L^{-1} As(V). HCl concentration: 3 mol L^{-1}

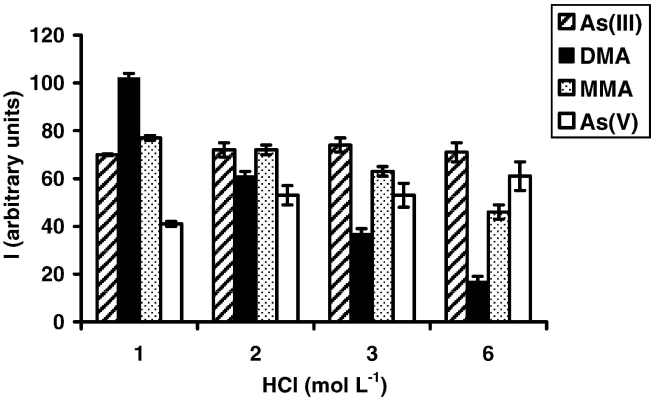


Fig. 2 Effect of HCl concentration on the HG efficiency from urban raw wastewater containing 20 µg L^{-1} As(III), 50 µg L^{-1} DMA, 30 µg L^{-1} MMA and 20 µg L^{-1} As(V). $NaBH_4$ concentration: 1% (m/v)

The optimum chromatographic parameters and the retention times remained unaltered even on varying the nature of the wastewater sample.

Optimisation of HG conditions. Once the optimum chromatographic conditions were fixed, the effect of various physical and chemical parameters were studied in order to identify those providing the maximum sensitivity in the HG step. It is well known that the yield of HG depends on the arsenic species: HG is less efficient for DMA and MMA than for inorganic species [25].

Wastewater samples (raw and treated) spiked with a mixture of the four arsenic species were injected into the system (under the optimal chromatographic conditions) to optimise the HG parameters. The influence of $NaBH_4$ concentration on the analytical response of the four arsenic species was investigated in the range of 0.5–2.0% (w/v) as shown in Fig. 1. The signals for the four arsenic

Table 1 Operating conditions for coupled HPLC-HG-AFS

Operating conditions		
Chromatography parameters (HPLC)	Anion exchange column	Hamilton PRP X-100 (250×4.1 mm i.d., 10 µm particle size)
	Mobile phase	20 mmol L^{-1} sodium phosphate (H_3PO_4, Na_3PO_4) adjusted to pH 6.0 at flow rate of 1.5 mL min^{-1}
	Injected volume	100 µL
Hydride generation	Acid	1.0 mol L^{-1} HCl at flow rate of 2.40 mL min^{-1}
	Reducing agent	1.0% (w/v) $NaBH_4$ in 0.3% (w/v) NaOH at flow rate of 1.78 mL min^{-1}
	Hydrogen flow rate	144 mL min^{-1}
	Carrier argon flow rate	300 mL min^{-1}
	Argon dryer flow rate	450 mL min^{-1}

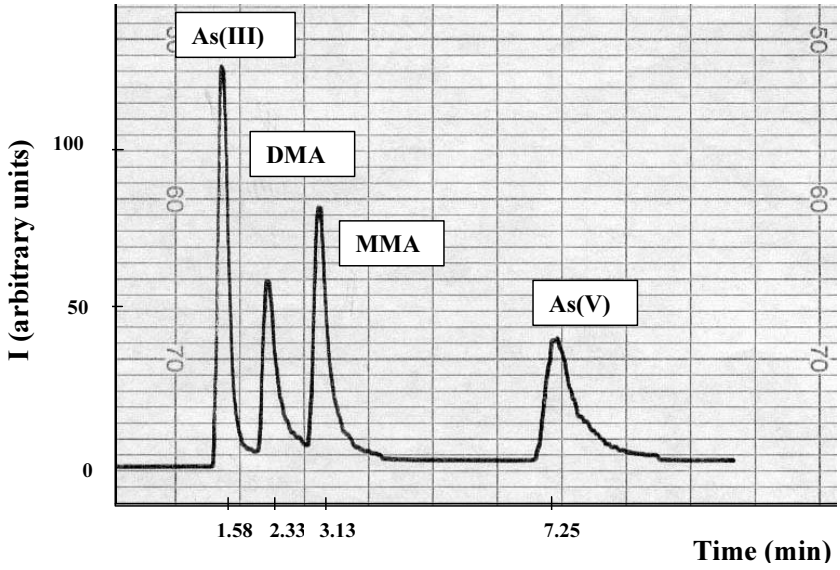


Fig. 3 Chromatogram of a sample containing 20 µg L^{-1} As(III), 30 µg L^{-1} DMA, 30 µg L^{-1} MMA and 20 µg L^{-1} As(V). Conditions are given in Table 1 for an injected volume of 100 µL (HPLC-HG-atomic fluorescence spectrometry coupling

species increased as the reductant concentration increased, this tendency being less pronounced for As(III). However, when working at a reductant concentration higher that 1.0% w/v a deterioration of the signal to noise ratio was observed, leading to a low reproducibility in the measurements. Hence a 1% (w/v) $NaBH_4$ concentration was chosen for further experiments.

The effect of acid concentration was studied for HCl over a range of 1–6 mol L^{-1} (Fig. 2). The best results for As(III), DMA and MMA were obtained with 1 mol L^{-1} HCl, whereas As(V) required higher concentrations to provide a higher response. A concentration of 1 mol L^{-1} HCl was chosen for continuing investigation since it provided the maximum response for the majority of the arsenic species tested. Due to the different yields of hydride generation provided by the different species, quantification was performed by standard addition of the four species.

Table 2 Analytical performance of the HPLC-HG-AFS. *LOD* Limit of detection

arsenicspecies	Retention time min	LOD µg L^{-1}	RSD %
As(III)	1.58	0.6	1.0
DMA	2.33	0.9	1.1
MMA	3.13	0.9	2.2
As(V)	7.25	1.8	9.0

[a]3×SD (n=10)

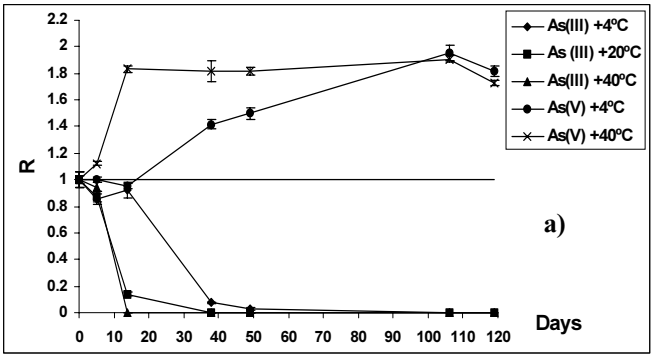
Table 3 Optimum inductively coupled plasma mass spectrometry (ICP-MS) operating conditions

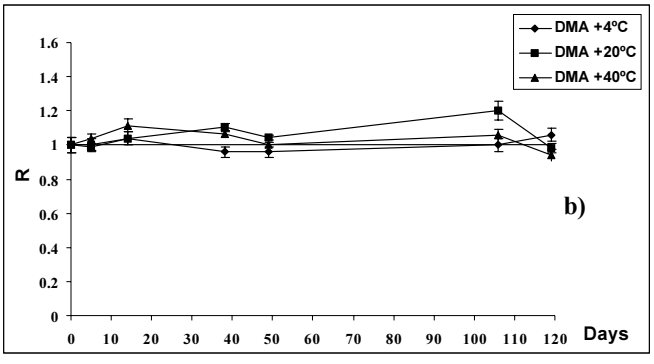
Operating conditions	
Rf power	1225 W
Plasma gas (Ar)	15 L min^{-1}
Auxiliary gas (Ar)	1.10 L min^{-1}
Nebuliser gas (Ar)	1.11 L min^{-1}
Spray chamber	Double pass (Scott type)
Nebuliser	Babington
Skimmer cone	Nickel, 0.4 mm orifice
Sampling cone	Nickel, 1.0 mm orifice
Sample uptake rate	1 mL min^{-1}
Data acquisition	^{7}Li, ^{59} Co, ^{75} As, ^{77}Se, ^{82} Se, ^{89} Y (internal standard), ^{115}In, ^{140}Ce
Integration time	0.1 s
Number of replicates	5

Table 4 Determination of arsenic species in wastewater samples. *n.d.* Not detected, *n*=3 determinations

Concentration μg L^{-1}		Species by HPLC-HG-AFS (added) found				HG-AFS As (total)	ICP-MS As (total)
		As(III)	DMA	MMA	As(V)		
Raw wastewater pH 7.6	Unspiked sample	n.d.	n.d.	n.d.	5±1	5.5±0.4	5.8±0.3
	Spiked sample	(20) 21.7±0.9	(50) 50±3	(30) 31±2	(15) 22±2	120±8	125±6
Treated wastewater pH 1.61	Unspiked sample	n.d.	n.d.	n.d.	5±1	5.4±0.6	4.8±0.1
	Spiked sample	(8) 8.2±0.6	(30) 31.4±0.6	(15) 15.0±0.5	6.6±0.5	60.3±0.8	60±1
Treated wastewater pH 7.27	Unspiked sample	n.d.	n.d.	n.d.	4±1	5.2±0.6	4.8±0.1
	Spiked sample	(8) 8.0±0.3	(30) 28.4±0.3	(15) 15.3±0.2	4.4±0.5	56±1	55±2

In the present method, $NaBH_4$ is used not only as reductant but also as hydrogen supply, which is necessary to sustain the argon-hydrogen flame. Under the optimised conditions, the flame generated using the hydrogen liberated from the reagents was not robust enough [26]. An additional external hydrogen flow of 144 mL min^{-1} was added to the system to overcome this limitation and it was optimum within the range 100–200 mL min^{-1} in terms of sensitivity and signal to noise ratio. Pure argon was used as carrier gas and its influence on the arsenic response was evaluated. Therefore a solution containing arsenite (10 μg L^{-1}) was analysed for the flow optimisation since its effect does not depend on the species. Argon flows from 250 to 400 mL min^{-1} were tested, and 300 mL min^{-1} was found to be optimum.





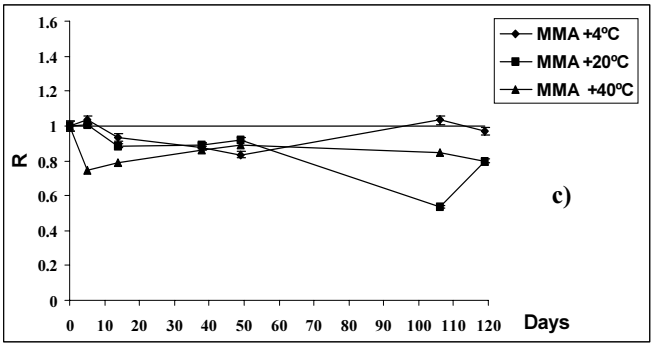


Fig. 4a–c Stability of arsenic species in raw urban wastewater at pH=7.60. **a** As(III), As(V). **b** DMA. **c** MMA

The optimum conditions for chromatographic separation and HG-AFS determination of As(III), DMA, MMA and As(V) are summarised in Table 1. A chromatogram obtained by applying the optimum conditions is shown in Fig. 3. The retention times, detection limits and relative standard deviations of each species are listed in Table 2.

Stability study of the arsenic species

The proposed method was applied to study the stability of arsenic species in urban wastewater samples taken from different stages of a wastewater treatment plant (raw and treated wastewater sample). Of all the investigated arsenic species only As(V) was detected in the wastewater samples. Prior to the stability study, the recovery of the method was evaluated by analysing spiked wastewater samples. To validate these results total arsenic was determined by ICP-MS as an alternative technique at the experimental conditions compiled in Table 3. The chloride contents found in the wastewater samples were 60±2 mg L^{-1} and 52±2 mg L^{-1} for raw and treated wastewater samples, respectively. At this level, possible formation of $^{40}Ar^{35}Cl^{+}$did not interfere in arsenic determination. Analyses were performed using an external calibration curve and Y was used as internal standard. The results, shown in Table 4, indicate that the proposed method is suitable and accurate for the simultaneous determination of the above-mentioned species at the 95% confidence interval.

For the stability study, treated wastewater samples were spiked with 8, 15 and 30 μg L^{-1} of As(III), MMA and DMA, respectively and the raw wastewater sample was spiked with 20, 30, 50 and 15 μg L^{-1} of As(III), MMA, DMA and As(V), respectively. Raw wastewater pH was maintained at its natural value (7.60) while treated wastewater pH was adjusted to two pH values: 1.60 and 7.27. All samples were stored at three different

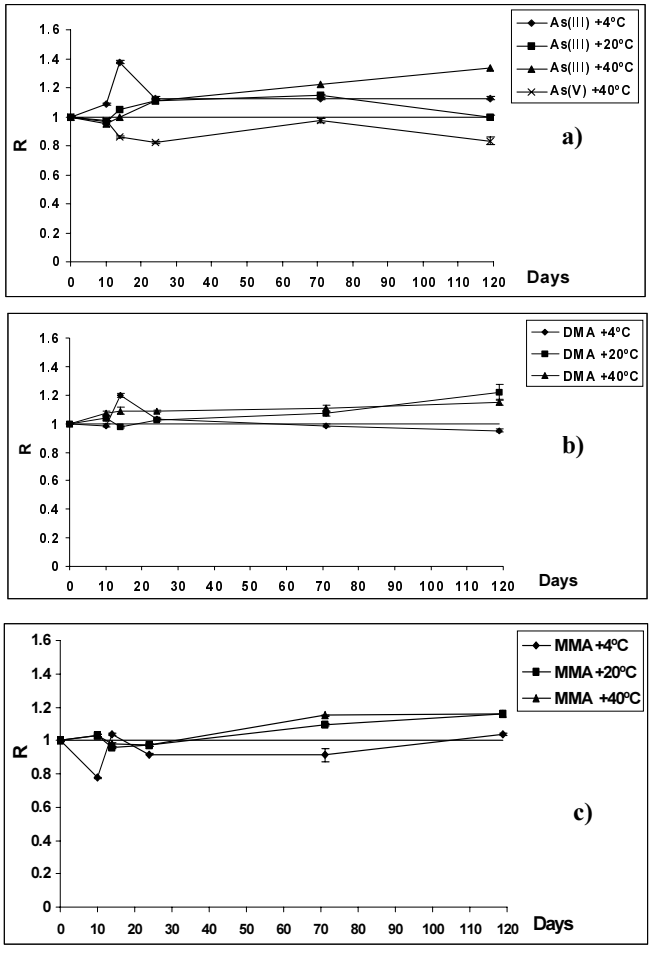


Fig. 5a–c Stability of arsenic species in treated wastewater at pH=7.27. **a** As(III), As(V). **b** DMA. **c** MMA

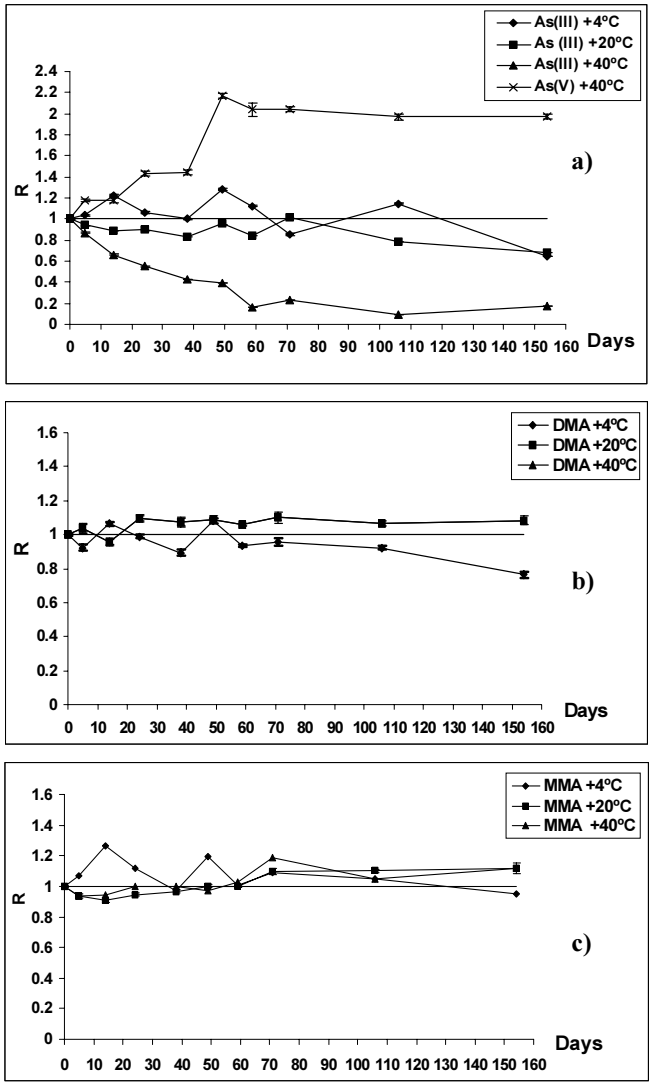


Fig. 6a–c Stability of arsenic species in treated wastewater at pH=1.61. **a** As(III), As(V). **b** DMA. **c** MMA

temperatures, +4 °C, +20 °C and +40 °C over a 4-month period.

The stability study at +4 °C, +20 °C and +40 °C was evaluated by comparing the results versus those of freshly spiked wastewater samples (t=0), and it was studied according to the relative variation observed as: $R_t=X_t/X_t=0$, where R_t is the ratio of the mean value X_t of three measurements made at different times and the mean value $X_{t=0}$ of the three measurements made at t=0.

The uncertainty U_t on the ratio R_t has been obtained from the coefficient of variation of each set of measurements: $U_t=(CV_t^2/n+CV_{t=0}^2/n)^{1/2}R_t$ where CV_t and $CV_{t=0}$ are coefficients of variation of X_t and $X_{t=0}$ respectively and n is the number of measurements made (n=3).

The results obtained are shown in Fig. 4, Fig. 5 and Fig. 6, in which R_t is plotted versus storage time for each arsenic species in the different type of samples.

The results indicate that MMA and DMA remained stable during the four-month period of storage at the three temperatures tested in both types of samples: raw wastewater (Fig. 4b, c) and treated wastewater at both pH values: 7.27 (Fig. 5b, c) and 1.61 (Fig. 6b, c). Different behaviour was observed for As(III), which was unstable in the raw wastewater sample at the temperatures tested, even at +4 °C (Fig. 4a). The As(III) losses were accompanied by a formation of As(V) whose concentration increased as the As(III) concentration decreased.

The As(III) stability is influenced by the sample matrix, as can be observed by comparing the results obtained in the study of raw and treated wastewater. Fig. 5a and Fig. 6a show the behaviour of As(III) in treated wastewater sample at two pH values (7.27 and 1.61). A better stabilisation of As(III) was obtained at pH 7.27, it being stable under this condition during the studied period of 4 months at all temperatures tested. However, a decrease in the pH of the sample provided negative effects for As(III) stability (Fig. 6a), it being stable for 70 days at +20 °C, and then decreasing its concentration and transforming slowly into As(V). At +40 °C the instability became even more critical and after 2 months almost the entire As(III) was transformed into As(V). No correlation was found between the organic matter present in the wastewater samples and the As(III) instability. The concentration val-

ues of 15 mg L^{-1} (BOD) and 67 mg L^{-1} (COD) in treated wastewater, and 285 mg L^{-1} (BOD) and 650 mg L^{-1} (COD) for raw wastewater could not explain why As(III) was more stable in the treated wastewater sample with a low content of the reductant organic matter.

The results obtained in this study indicate that MMA, DMA and As(V) remain stable in the wastewater samples during the storage time studied under all the conditions tested. As(III) is less stable and its stability depends on the nature of the matrix, pH and temperature. The effect of pH is critical at +40 °C when arsenic speciation is carried out in treated wastewater samples. Although acidification is recommended for total arsenic determination since it provides several advantages such as avoiding precipitation and colloidal formation, precautions have to be taken when species analysis is performed to avoid species interconversion. The acidification of the treated wastewater samples transformed As(III) into As(V) slowly at +4 °C and +20 °C, and the kinetics increased at +40 °C.

To conclude, the speciation and determination of the above-mentioned four arsenic species in wastewater samples was carried out by on-line HPLC-HG-AFS, providing detection limits in the range 0.6–1.8 μg L^{-1}. The long-term stability study showed that MMA, DMA and As(V) remain stable in these types of samples for long periods of time, even at room temperature. However transformation of As(III) into As(V) was noted in both water samples: raw and acidified treated wastewater samples. Although this does not present a problem for total arsenic determination, precautions during storage should be taken when arsenic speciation is carried out in these types of matrices. As a result, it is difficult to prepare a wastewater certified reference material in the arsenic speciation field to enable direct validation of the analytical methodology developed for species determination.

Acknowledgements. The authors thank the European Community for financial support under contracts SMT4-CT98–2226 and CICYT (PB98–0768).

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