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Simultaneous determination of mercury and arsenic species in natural freshwater by liquid chromatography with on-line UV irradiation, generation of hydrides and cold vapor and tandem atomic fluorescence detection

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Abstract

An approach based on the coupling HPLC–UV–CV/HG–mAFS–AFS has been developed for the simultaneous determination of mercury and arsenic species. A home modified AFS detector has been used for the analysis of mercury coupled in series with another similar detector for arsenic determination. The determined species were Hg^{2+}, methylmercury ($MeHg^{+}$), As(III), As(V) and monomethylarsinate (MMA). A critical aspect is the chromatographic separation, which was carefully optimized for the separation of all the species. The detection limits for these species are 11, 8, 17, 17 and 3 ng ml^{-1}, respectively. Linear curves for MMA were obtained between 10 and 200 ng ml^{-1}. The linear dynamic range for all the other species was comprised between the detection limit and 500 ng ml^{-1}. The influence of cations and anions at the concentration usually present in natural freshwater was studied. The procedure was validated by application to spiked natural freshwater samples from the south-west Spain, and it can be considered for routine analysis of polluted sites.

Keywords: Speciation analysis; Atomic fluorescence detection; Water analysis; Multielemental speciation analysis; Mercury; Arsenic

1. Introduction

Arsenic and mercury are two elements of both environmental and health concern due to their high toxicity at trace levels [1]. Arsenic is probably the best known trace element for its toxic properties [2] and has been used as a poison for centuries [3]. Mercury is one of the most prevalent and toxic contaminants in the environment [4]. It is well known that the toxicity of most elements depends on the chemical form in which they are present in the environment. Thus, in the case of arsenic, inorganic salts are more toxic than methylated species, and larger molecules (such as arsenobetaine or arsenosugars) become even innocuous for human consumption [5,6]. The case of mercury is the opposite, being the organic species the most toxic ones [7].

Arsenic is related with dermal injuries (including change of pigmentation and keratosis) [8] blackfoot disease (a peripheral vascular disorder) [9] and skin cancer. It has also been demonstrated that arsenic binds to proteins [10] and affects to gene expression [11].

Mercury causes neurological diseases, and in extreme cases, it produces severe congenital effects (infants born to mothers with high mercury levels may suffer cerebral palsy, blindness and mental retardation) [12].

Since both mercury and arsenic are highly toxic elements, several international organisms have established maximum recommended levels for their consumption. Thus, the WHO sets a maximum tolerable weekly intake of 5 μg kg^{-1} of body weight of total mercury, of which no more than 3.3 μg kg^{-1} should be present as methylmercury. The guideline level for total mercury in drinking water is established at 0.001 mg l^{-1} [13].

The average intake for arsenic inorganic forms from water is estimated to be similar to that from food [14]. The European Union and the World Health Organization set the guideline for arsenic in water at 10 μg l^{-1} [14,15].

The most common procedures for the determination of mercury and arsenic are based on the reduction of these ele-

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ments into volatile forms to improve sensitivity of the detectors, namely hydride generation (HG) for arsenic, and cold vapor (CV) technique for mercury. The detection is normally carried out using atomic spectroscopy or mass spectrometry techniques, being the most commonly used AAS [3,16], AFS [17,18] and ICP–MS [17,19]. Speciation is required in order to determine the potential toxicity of mercury and arsenic. Therefore, HPLC is commonly used for arsenic speciation, whilst most procedures for the determination of mercury are based on gas chromatography [18–20]. However, mercury speciation can also be performed by HPLC [21,22].

Speciation analysis is traditionally carried out with sensitive and selective techniques, which involves the determination of only one element each time; although multielemental detectors like ICP–MS, ICP–OES or MIP–AED allow the simultaneous speciation of several elements. More recently, a commercial multichannel AFS detector has been developed by Beihing Haiguang Instrument Company (China), which enables simultaneous multielemental analysis [23,24]. At the present a coupling based on the join of two AFS in series has been developed in our laboratory. The system includes a modification in the first fluorescence detector (mAFS) consisting on the introduction of a quartz flow cell, which allows the detection of methylmercury in a confined area [25]. Once the carrier gas has crossed the flow cell and mercury is determined, it is driven to the second AFS, were arsenic can be detected.

In this work, the coupling HPLC–UV–CV/HG–mAFS–AFS has been performed and optimized. Mercury and arsenic species are separated by liquid chromatography (HPLC) and subsequently on-line oxidized under an ultraviolet lamp (UV). Then, a reducer reagent is introduced online in order to generate cold vapor and hydrides (CV and HG), which provides suitable species for the final detection of mercury and arsenic with a home modified atomic fluorescence detector (mAFS) connected to a standard AFS system. The approach has been applied for the determination of Hg^{2+}, $MeHg^{+}$, As(III), monomethylarsinate and As(V) in spiked freshwater samples.

2. Experimental

2.1. Chemicals and standards

The 1000 mg l^{-1} stock solutions of arsenic species (As) were prepared from arsenic trioxide (Panreac, Barcelona, Spain), sodium arsenate (Merck, Darmstadt, Germany) and sodium monomethylarsinate (Carlo Erba, Milan, Italy) by diluting with Milli-Q water (Millipore, Watford, UK). The 1000 mg l^{-1} stock solution of Hg^{2+} was purchased (Merck). A solution of CH_3HgCl (Merck) with similar concentration was prepared by dilution in methanol. All the stocks were stored at 4 °C in the dark. Calibrants were prepared daily by appropriate dilution of stock solutions with water.

2-Mercaptoethanol and potassium persulfate were purchased from Sigma–Aldrich. *tetra-n*-Butylammonium bromide, ammonium acetate, hydrochloric acid, sodium tetrahydroborate and sodium hydroxide (Merck) were of analytical grade. Methanol of gradient quality (Romil LTD, Cambridge, UK) was also used. Sodium acetate, potassium bromide, magnesium oxide, calcium hydroxide, iron chloride, copper chloride, ammonium acetate and ammonium sulfate (Merck) of analytical grade were used for the study of interferences.

2.2. Instrumentation

The HPLC system consisted of an Agilent 1100 quaternary pump and degasser (Agilent Technologies, Waldbronn, Germany), equipped with a Rheodine 7125i injector and a 200 μl loop for sample introduction. The separation was car-

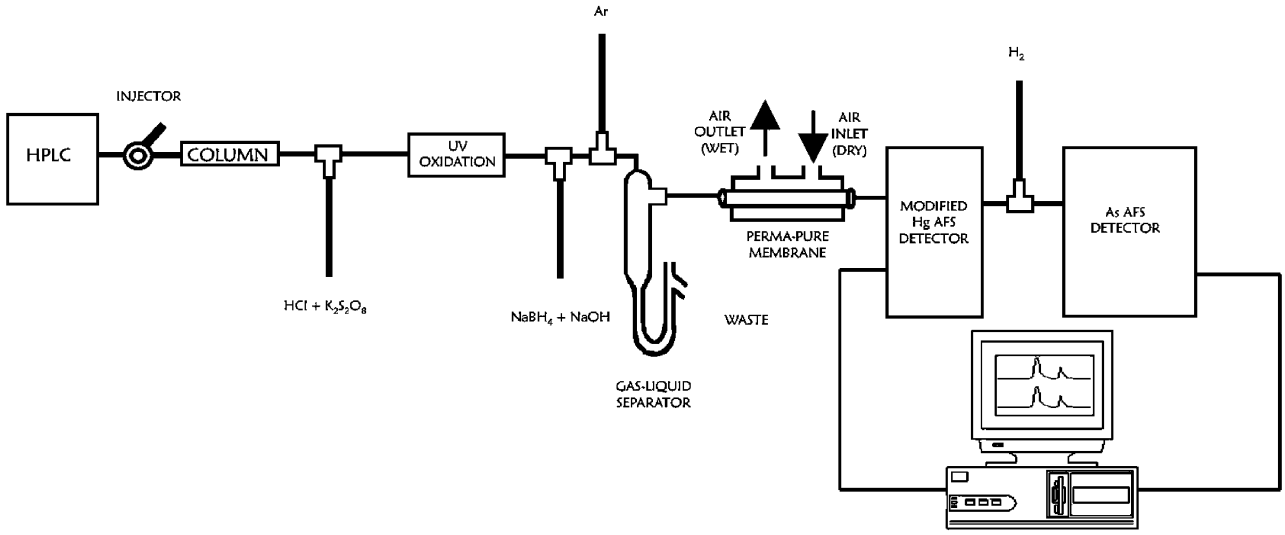

Fig. 1. Scheme of the coupling HPLC–CV/HG–mAFS–AFS.

ried out in a 25 cm × 4.6 mm × 5 μm Prodigy ODS(3) column (Phenomenex, Aschaffenburg, Germany). Photooxidation was carried out in a 8 m long Teflon tube (i.d.: 0.5 mm) wrapped around a low pressure Hg lamp (TNN 15/32, Heraeus, South Plainfield, NJ, USA), after addition online of a potassium persulfate solution in hydrochloric acid medium. Separation of volatile compounds (arsines, elemental mercury) was performed in a gas–liquid separator "B" type (PS Analytical Ltd., Orpington, Kent, UK). A peristaltic pump (Gilson Minipuls 3, Villiers le Bel, France) was used for the addition of all the reagents.

The detection of mercury was carried out with a home modified [25] Merlin Mercury Atomic Fluorescence Detector model 10.023 (PS Analytical Ltd.), and arsenic was determined with a Excalibur mod. 10.044S atomic fluorescence detector (PS Analytical Ltd.). Both detectors are connected in series. A scheme of the coupling is shown in Fig. 1.

Briefly, the modification of the mercury AFS detector was based on the inclusion of a closed quartz flow cell (QFC) in the sample compartment. The inlet of the QFC is connected with Teflon tubes to the gas liquid separator, and the outlet is coupled to the second AFS detector previous online addition of hydrogen for feeding the flame of the system.

2.3. Samples and spiking procedure

The analytical procedure proposed in this work was validated with spiked samples of natural freshwater since no reference materials with both mercury and arsenic were available. An amount of 50 ml of water were spiked with 0.25 ml of standard solutions (10 mg l^{-1}) of each mercury and arsenic species. The final concentration in the samples was around 50 ng ml^{-1}. The samples were filtered prior to the analysis.

3. Results and discussion

3.1. Optimization of the coupling variables

The HPLC–CV/HG–mAFS–AFS device was optimized for arsenic and mercury simultaneous determination. The optimization criterion was to achieve the maximum sensitivities for each one these elements, although a compromise has to be adopted for the simultaneous determination of both. The parameters under optimization were reagents concentration, and flow rates of both reagents and gases. For the chromatographic separation, the mobile phase composition was also assayed. A mixture of 100 ng ml^{-1} of Hg^{2+}, $MeHg^{+}$, As(III), MMA and As(V) (as the elements) was prepared in Milli-Q water, and employed throughout the optimization.

3.1.1. Hydrochloric acid

The concentration of this acid was optimized in the range of 0.1–2 mol l^{-1} (Fig. 2a). As can be observed, the best signals for mercury were obtained with the minimum HCl concentration, possibly due to the formation of $HgCl_2$, highly stable, whilst the highest areas for arsenic are reached with larger concentrations of HCl. A compromise was achieved at 1 mol l^{-1}, since sensitivity for both analytes was in the same order of magnitude.

3.1.2. Potassium persulfate

The concentration of potassium persulfate in the hydrochloric acid solution was investigated in the range of 0–2% (w/v). Methylarsinate and methylmercury standards were used for this purpose. Low differences were observed for arsenic, but higher areas were obtained for mercury at 1% (w/v), that was selected for further experiences.

3.1.3. Sodium tetrahydroborate

The concentration of the reductant was ranged from 0.1 to 1.5% (w/v). In all cases, the solutions were pre-

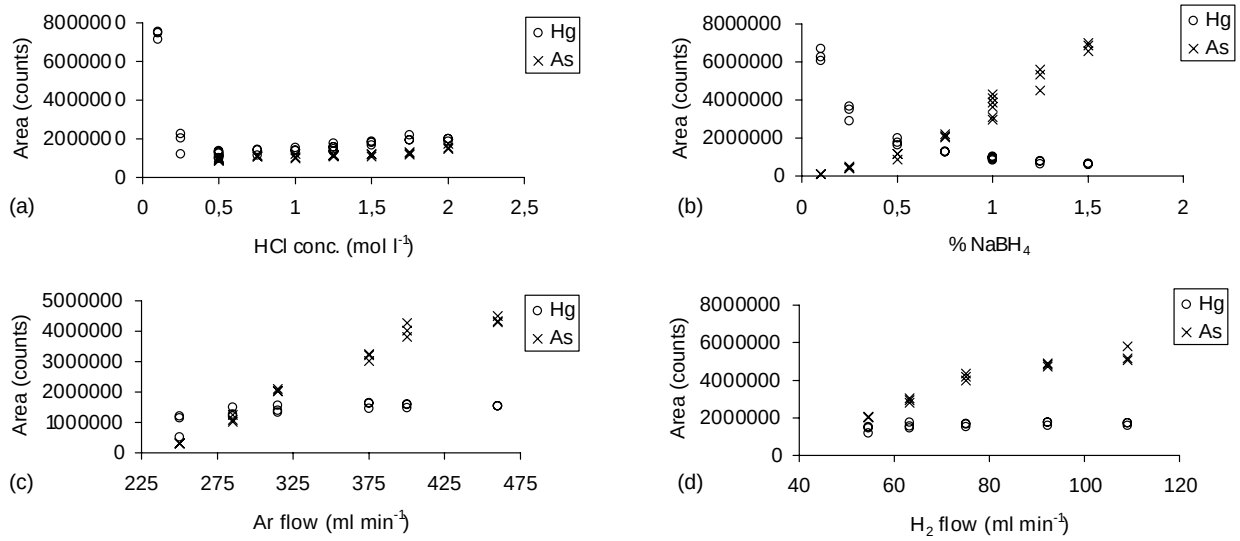

Fig. 2. Optimization of the operating condition.

pared in basic media (NaOH 1% (m/v)), in order to stabilize this reagent. Mercury is reduced to its elemental form, and arsenic reacts to generate arsine. These reactions occur under acidic conditions, previously stated by the addition of HCl together with potassium persulfate. In acidic medium, sodium tetrahydroborate is decomposed and generates hydrogen, which can produce quenching effect during the determination of mercury. As can be observed (Fig. 2b), mercury and arsenic exhibit opposite responses respect to the concentration of this reagent. Peak area of mercury decreases when sodium tetrahydroborate concentration increases; with arsenic the results are just the opposite. Finally, it was chosen the reductant concentration in the crossing point of both graphs, 0.5% of $NaBH_4$.

3.1.4. Carrier gas flow (Ar)

The separation of the arsines and elemental mercury generated after the reduction step is assisted by the introduction of an argon stream into the transference line. Latterly, the mixture of gas and aqueous fluxes was separated in a gas–liquid separator device. The argon flow rate was also optimized, by varying from 250 to 450 ml min^{-1} as shown in Fig. 2c. Mercury signal is poorly affected by this parameter, but the signal for arsenic rapidly increases with higher flows of argon. Thus, the maximum flow rate compatible with the system was used.

3.1.5. Hydrogen flow rate

The flow of this gas was studied from 55 to 110 ml min^{-1}, with no effects on mercury signals. However, arsenic response enhanced with higher flows of hydrogen (Fig. 2d). Thus, an optimum flow rate was set at 110 ml min^{-1}.

3.2. Optimization of the mobile phase

A complex mobile phase had to be considered in order to separate both mercury and arsenic species due to their very different physico-chemical properties. Mercury species are positively charged, while arsenic species are anionic. For this reason, the selection of reverse phase chromatography was necessary. The selection of the mobile phase is also critical. Different reagents have to be included in the mobile phase for a suitable separation of all the species. 2-Mercaptoethanol contributes to the retention of mercury species due to its complexing capability that increase the interaction of positive charged mercury species and the non polar stationary phase. Otherwise, an ion pairing reagent, such as tetrabutyl ammonium bromide (TBA), is necessary to balance the strong polar character of arsenic species caused by their negative charges. Other mobile phase modifiers such as methanol as well as the influence of pH value can also contribute to the chromatographic resolution. To improve the separation performance the use of a mobile phase gradient also has to be considered, as mentioned below.

3.2.1. Mobile phase 1

The following components were considered:

(i) *tetra-n-Butylammonium bromide*, ion pairing agent, which concentration does not affect to mercury species retention time, but can change those corresponding to arsenic species. The concentration of this reagent was studied between 5×10^{-3} and 30×10^{-3} mol l^{-1}. High concentrations of TBA increase the time of the chromatographic run. Thus, when 30 mM of TBA was used, the elution time for As(V) increased over 30 min. The optimum separation of all the species of arsenic was achieved when the concentration was fixed at 5 mM.

(ii) *2-Mercaptoethanol* for mercury complexation, which concentration was studied from 0.001 to 0.5%, and no differences in retention time were observed for Hg^{2+} and $MeHg^{+}$. However, higher areas were obtained for concentrations of 0.02%.

(iii) *Methanol* that prevents the irreversible retention of both inorganic mercury and methylmercury in the column. However, this solvent has a quenching effect on the arsenic signal in the second AFS detector. Therefore, a compromise between arsenic detection and the chomatographic run length (<1 h is advisable) has to be achieved. The concentration of methanol in the mobile phase was investigated in the range of 1–25% (v/v). When 5% methanol was used, the chromatographic run was reduced to 1 h. Higher amounts of this solvent produced slightly reduced retention times for mercury species, but sensitivity for arsenic was lower. Therefore, the concentration of methanol was fixed to 5% (v/v).

The pH of Mobile phase 1 was studied from 4.0 to 8.0. The buffer concentration used in all the cases was 20×10^{-3} mol l^{-1}. An appropriate buffer was selected for each pH value (NH_4CH_3COO for pH 4.0–6.0, $KHPO_4$ in the range 6.0–8.0). This parameter only affected the retention times for arsenic species. A suitable separation of the three arsenic species was achieved when pH 4.0 was used.

3.2.2. Mobile phase 2

3.2.2.1. 100% methanol. As explained above, methanol is adequate for the elution of mercury species, but it produced quenching effect on arsenic signal. Therefore, this mobile phase should be used to accelerate the elution of mercury species, but not during the determination of arsenic. Since arsenic species are rapidly eluted from the column, and mercury species are retained for a longer time, methanol is used once arsenic species have been eluted.

3.2.2.2. Gradient program. Good separation was achieved with Mobile phase 1 alone, but the time of the chromatographic run was too long (about 1 h). Methanol concentra-

Table 1
Optimized operating conditions for the simultaneous determination of mercury and arsenic species

HPLC	
Column	ODS-3 column, 250 × 4.6 mm i.d., 5 μm
Mobile phase A	5% CH_3OH:0.02% (w/v) 2-mercaptoethanol:5m mol l^{-1} TBA:20 m mol l^{-1} NH_3CH_3COO; pH 4.0
Mobile phase B	100% CH_3OH
Gradient	Initial: 100% A; 12 min, 30% B; 15 min, 100% A
Sample injection	200 μl
On-line digestion	
Type of oxidation	UV (15 W)
Digestion coil	0.5 mm i.d., 8 m length
Oxidant solution	$K_2S_2O_8$ 1% (w/v) in HCl 1 mol l^{-1}; 1 ml min^{-1}
Cold vapor/hydride generation	
Reducing solution	$NaBH_4$ 0.5% (w/v) in 1% NaOH (w/v); 1 ml min^{-1}
Air flow rate	450 ml min^{-1}
Detection	
Hg mAFS gain	10 × 10
H_2 flow rate	110 ml min^{-1}
As AFS gain	10 × 4

Table 2
Mean recovery experiments of As and Hg species in water in presence of interferent ions

	Hg^{2+} (%)	$MeHg^+$ (%)	As(III) (%)	MMA (%)	As(V) (%)
Na^+	104	67	81	95	65
K^+	89	86	90	99	107
Mg^{2+}	104	92	72	96	65
Ca^{2+}	101	82	88	99	109
Cl^-	86	84	81	97	95
$SO_4{}^{2+}$	81	44	91	106	103
Fe^{3+}	104	109	96	101	103
Cu^{2+}	109	110	90	101	109

tion was fixed at a low value (5%) until the elution of arsenic species, and latterly increased up to 30% for the fast mercury elution. Finally, the system was returned to the initial conditions until its stabilization for the next injection.

The optimized operational conditions are summarized in Table 1.

3.3. Method performance

The relative standard deviation (R.S.D.) of 10 sequential injections of a 50 ng ml^{-1} Hg^{2+}/$MeHg^+$/As(III)/As(V)/MMA mixed standard solution in water was 4.2, 7.3, 5.4, 5.6 and 5.0%, respectively. Linear calibration lines were obtained between 25 and 500 ng ml^{-1} (10–200 ng ml^{-1} in the case of MMA). The detection limits achieved for arsenic and mercury species (calculated as 3σ) were 11 and 8 ng ml^{-1} for Hg^{2+} and $MeHg^+$, respectively. The values found for As(III), As(V) and MMA were 17, 17 and 3 ng ml^{-1}.

3.4. Method validation for natural freshwater analysis

To our knowledge, no reference materials are available for neither mercury nor arsenic speciation in water. Moreover, there are not water reference materials containing both certified mercury and arsenic values. For this reason, validation had to be achieved with spiked natural water. A preliminary interference study was carried out on natural freshwater spiked with the most common ions present in them [26]. The tests were performed at two concentration levels: Na^+ (10 and 20 mg l^{-1}), K^+ (2 and 4 mg l^{-1}), Ca^{2+} (20 and 40 mg l^{-1}), Mg^{2+} (10 and 20 mg l^{-1}), Cl^- (10 and 20 mg l^{-1}) and $SO_4{}^{2-}$ (15 and 30 mg l^{-1}). In addition, potential interferences by Fe^{3+} (20 and 40 ng ml^{-1}) and Cu^{2+} (2 and 4 mg l^{-1}) were also investigated. A mixed standard solution containing 100 ng ml^{-1} of all the species of mercury and arsenic was used throughout. The results obtained are summarized in Table 2. As can be seen, the chromatographic method is rather robust and only a slight influence from sodium (for $MeHg^+$ and As(V)), but especially from sulfate (for $MeHg^+$) can be remarked. Double concentration of interferents does not produce a significant change in recoveries except in the case of sulfate.

The procedure has been applied to different natural freshwater samples from non-polluted rivers in the south-west Spain. Blanks performed with filtered Milli-Q water were also analyzed together with the samples. The concentration of arsenic and mercury species were always under the detection limits in all the cases. Therefore, spike experiments were performed on these samples in order to test the ap-

Table 3
Analysis of mercury and arsenic species in south-west Spain non-polluted rivers ($n = 3$)

	Hg^{2+}		$MeHg^+$		As(III)		MMA		As(V)	
	$\overline{X} \pm \sigma$	Recovery (%)	$\overline{X} \pm \sigma$	Recovery (%)	$\overline{X} \pm \sigma$	Recovery (%)	$\overline{X} \pm \sigma$	Recovery (%)	$\overline{X} \pm \sigma$	Recovery (%)
Carreras river	53.9 ± 2.4	108	52.0 ± 4.0	104	44.5 ± 3.1	89	49.1 ± 2.5	98	55.0 ± 3.2	110
Piedras river	54.2 ± 2.6	108	49.5 ± 3.9	99	42.5 ± 2.6	85	47.8 ± 2.7	96	53.8 ± 2.9	108
San Pedro river	51.5 ± 2.5	103	50.4 ± 4.1	101	43.8 ± 2.8	88	50.2 ± 3.0	100	54.2 ± 3.6	108
Guadalete river	48.3 ± 2.3	97	51.2 ± 3.7	102	42.9 ± 3.0	86	48.6 ± 2.9	97	52.9 ± 4.3	106

Samples were spiked with 50 ng ml^{-1} each species.

plicability of the proposed method (Table 3). Results show good recoveries, in the range of 85–110%, with precisions (R.S.D.) from 4.5 to 8.1%.

4. Conclusions

A methodology for simultaneous mercury and arsenic speciation based on the approach HPLC–UV–CV/HG–mAFS–AFS has been developed. A compromise in the experimental conditions has been reached to get similar sensitivities for both mercury and arsenic species. The approach can be used for the simultaneous speciation of mercury and arsenic in natural water, although the detection limits make it especially suitable for heavily polluted water assessment.

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