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Semiautomatic method for the ultra-trace arsenic speciation in environmental and biological samples via magnetic solid phase extraction prior to HPLC-ICP-MS determination

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ABSTRACT

A novel magnetic functionalized material based on graphene oxide and magnetic nanoparticles (MGO) was used to develop a magnetic solid phase extraction method (MSPE) to enrich both, inorganic and organic arsenic species in environmental waters and biological samples. An automatic flow injection (FI) system was used to preconcentrate the arsenic species simultaneously, while the ultra-trace separation and determination of arsenobetaine (AsBet), cacodylate, As^{III} and As^{V} species were achieved by high performance liquid chromatography combined with inductively coupled plasma mass spectrometry (HPLC-ICP-MS). The sample was introduced in the FI system where the MSPE was performed, then 1 mL of eluent was collected in a chromatographic vial, which was introduced in the autosampler of HPLC-ICP-MS. Therefore, preconcentration and separation/determination processes were automatic and conducted separately. To the best of our knowledge, this is the first method combining an automatic MSPE with HPLC-ICP-MS for arsenic speciation, using a magnetic nanomaterial based on MGO for automatic MSPE. Under the optimized conditions, the LODs for the arsenic species were 3.8 ng L^{-1} AsBet, 0.5 ng L^{-1} cacodylate, 1.1 ng L^{-1} As^{III} and 0.2 ng L^{-1} As^{V} with RSDs <5%. The developed method was validated by analyzing Certified Reference Materials for total As concentration (fortified lake water TMDA 64.3 and seawater CASS-6 NRC) and also by recovery analysis of the arsenic species in urine, well-water and seawater samples collected in Málaga. The developed method has shown promise for routine monitoring of arsenic species in environmental waters and biological fluids.

1. Introduction

Arsenic is considered a metalloid, which arises from natural and anthropogenic sources [1]. Its presence in water, soil and air over the recommended limits causes environmental pollution and public health problems, being established a daily intake maximum of 10 μg L^{-1} by the World Health Organization (WHO) [2]. As is absorbed by the lungs and the gastrointestinal tract and is excreted in the urine. The exposure caused by this element can be evaluated by analyzing urinary As levels, being considered an excellent biomarker of health condition. Moreover, there are more than thirty species of organic and inorganic arsenic, presenting different properties and toxicities [3]. Inorganic arsenic species (As^{III} and As^{V}) are considered carcinogenic agents in humans by the International Agency for Research on Cancer (IARC) [4], being As^{III} 60 times more toxic than As^{V} [5,6], while organic arsenic species, such as cacodylate and arsenobetaine (AsB), are considerably less toxic or nontoxic and usually found in biological samples [4]. Therefore, the speciation methods for the arsenic determination in environmental samples are crucial to evaluate the pollution level adequately, while the analysis of biological samples like urine allows the study of As exposure.

Usually, in order to develop a speciation method for As, the coupling of a separation system and an atomic spectrometer is widely used. In this work, high performance liquid chromatography (HPLC) and inductively coupled plasma mass spectrometry (ICP-MS) are combined with a common interface to achieve the separation and the determination of the arsenic species. HPLC-ICP-MS system has demonstrated a wide applicability to develop speciation methods for different elements and its compounds [7–10], offering a high sensitivity and large linear ranges. Furthermore, this technique has been already used for the separation and determination of arsenic species such as arsenosugars [11], arsenolipids [12] and phenylarsenicals [13]. Among the main species for the monitoring of arsenic, As^{III}, As^{V}, cacodylate and AsBet are commonly

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analyzed [14–16].

Despite the HPLC-ICP-MS advantages, an additional dilution takes place when this system is used due to the mixture of the sample and the mobile phase during the HPLC separation. Moreover, As concentration in environmental and biological samples is extremely low [17]. For these reasons, a preconcentration pre-treatment is needed in order to ensure an adequate analysis. Solid phase extraction (SPE) is used as an extraction/preconcentration technique, presenting the following advantages: no emulsions, rapidity, easy to use, automation, limited consumption of organic solvents and high enrichment factors (EFs) can be achieved, improving the selectivity, sensitivity and precision of the method [18–20]. Nowadays, nanomaterials are widely used for this purpose due to their large surface area and highly active surface sites, being graphene based nanomaterials one of the most recently studied adsorbents. Graphene oxide (GO) is a graphite monolayer and it has been exploited as a solid-phase material due to its large specific surface area, numerous oxygen-containing functional groups (epoxy, carboxylic acid, carbonyl and hydroxyl groups) and aromatic sp^2 domains which enables interaction with analytes through π-π, hydrogen bonding and electrostatic interactions [21]. The adsorption on GO is good, but some difficulties limit its operation: tedious to use, slow separation from the matrix and high sample volumes are needed. Iron oxide (Fe_3O_4) magnetic nanoparticles (MNPs) have also been exploited as solid-phase materials due to their biocompatibility, degradability, physiological and chemical stability, low toxicity and high magnetic response [22,23]. Magnetic materials allow a variation of the classical SPE, denominated magnetic solid-phase extraction (MSPE). The MNPs–GO coupling seeks compensation for the disadvantages of both MNPs and GO. Scattering the MNPs over GO layers avoids the MNPs agglomeration [24], which would decrease the specific surface area and facilitate the separation of the adsorbent from the matrix, turning it fast and easy with MGO. For this reason, GO–MNPs tandem (MGO) has recently been recognized as an excellent magnetic adsorbent material to develop a MSPE pre-treatment method [24,25]. A material based on functionalized MNPs, previously synthesized by P. Montoro-Leal et al., was proven to be effective for inorganic arsenic speciation [18]. Besides, graphene based nanomaterials have been previously applied in As speciation [26,27], being suitable for the adsorption of both inorganic and organic species, due to the high variety of interactions mentioned above. Therefore, MGO can be considered as a potential adsorbent to develop an As speciation method.

In this study, a magnetic nanomaterial functionalized with [1,5-bis (2-pyridyl) 3-sulfophenylmethylene] thiocarbonohydrazide (MGO-PSTH), previously synthesized by us [28], was used to develop an automatic preconcentration system by using a knotted reactor. This method has been proposed for the enrichment of inorganic and organic arsenic species in aqueous environmental and biological samples prior to HPLC-ICP-MS determination. The strategy of this work was focused on the compatibility between the MSPE eluent and the mobile phase of HPLC system, resulting in an efficient and reliable semiautomatic preconcentration and detection of inorganic and organic arsenic speciation. The developed method was validated by analyzing Certified Reference Materials for total As concentration (fortified lake water TMDA 64.3 and seawater CASS-6 NRC) and also by recovery analysis of the arsenic species in urine, well-water and seawater samples collected in Málaga. The developed method has shown promise for routine monitoring of arsenic species in environmental waters and biological samples.

2. Experimental

2.1. Instrumentation

A NexION 2000 inductively coupled plasma mass spectrometer directly connected to Flexar HPLC, both controlled by Empower software (PerkinElmer SCIEX Instruments, Waltham, MA, USA) were used throughout. The ICP-MS instrument, with standard nickel sampler and skimmer cones, was optimized daily and operated as recommended by the manufacturer. The nebulizer gas flow rate was adjusted so that Ce^{++} (70)/Ce (140) ratio and CeO (156)/Ce (140) were less or equal than 0.03 and 0.025, respectively. For HPLC separation of arsenic species an IonPac AS9-HC column 4 × 250 mm and IonPac AG9-HC 4 × 50 mm precolumn, with 9 μm particle size (Dionex Corporation, Sunnyvale, CA, USA), were used. The Flexar HPLC presents a 100 positions autosampler tray for the automation of the injection of the chromatographic vial content. The outlet of the HPLC column and the ICP-MS nebulizer were connected through an 80 cm 0.8 mm i.d. Capillary tube. The optimum operation conditions of the HPLC-ICP-MS system are summarized in Table 1. The adsorption mechanisms was studied by X-Ray photoelectron spectroscopy (XPS) with ESCA 5701 instrument (Physical Electronics, Chanhassen, MN, USA).

A PerkinElmer FIAS-400AS system, which consists of two peristaltic pumps and a five-port way rotary valve was used as the flow injection (FI) accessory. The magnetic knotted reactor (MKR) containing the MGO-PSTH was made with PTFE tube (500 mm, 0.5 mm i.d.) packed with 50 mg of MGO-PSTH and knotted round a circular Nd/Fe/B magnet (Outer diameter: 40 mm; Inner diameter: 23 mm; Height: 5 mm; Holding strength: 81.4 N) and sandwiched between other two identical circular magnets; at both ends of the PTFE tubes, polyethylene filters (Omnifit, Cambridge, UK) were fixed to prevent material loss. The MKR was placed in the sample loop of the five-port rotary valve. Magnet knotted reactors (MKR) have been previously used by us [29] as the best solution to pack magnetic materials. FIAS-400AS system was not connected directly to NexION 2000, the outlet of the eluent tube was collected into a chromatographic vial. The FI system was controlled by a second independent computer using Syngistix software (PerkinElmer, Waltham, MA, USA). A vortex mixer VX-2500 Multi-Tube (VWR international, Radnor, PA, USA) was used for the studies of the adsorption capacities of the species.

2.2. Reagents and solutions

High purity reagents were used in all experiments. Doubly de-ionized water (18 MΩ cm) obtained from a Milli-Q water system (Millipore, Bedford, MA, USA) was used throughout. All plastic and glassware were cleaned with 10 % w/w nitric acid and stored soaked with this acid. They were rinsed several times with doubly de-ionized water immediately before use.

Table 1
HPLC-ICP-MS conditions.

ICP-MS	
Analyte	As^{75}
Replicates	3
Radiofrequency power/W	1600
Waste flow rate/mL·min^{-1}	1.4
Distance from HPLC to ICP-MS/cm	80
Nebulizer type	Cyclonic chamber
Gas flows/L·min^{-1} (Plasma, auxiliary, nebulizer)	15/1.2/0.96
Torch alignment/mm (Horizontal, vertical, depth)	−0.07/0.84/0.00
HPLC	
Injection volume/μL	100
Column temperature/ C	30 ± 1
Mobile phase flow rate/mL·min^{-1}	0.9
Column	IonPac AS9-HC 4 × 250 mm
Mobile phase A	$NaHCO_3$ 0.005 M
Mobile phase B	Na_2CO_3 0.1 M
Mobile phase C	NaOH pH 12.0
Gradient program	12.0%A, 4.0%B, 84.0%C 3.2 min (stabilization) 0.0%A, 71.4%B, 28.6%C 1.8 min (ramp) 0.0%A, 71.4%B, 28.6%C 6.0 min

As^{III} and As^{V} stock standard solutions 1000 mg L^{-1}, ≤98% Sodium cacodylate trihydrate and ≤95% Arsenobetaine (Merck, Darmstadt, Germany) were used for standards preparation. The structures of these arsenic species are shown in supplementary material (Fig. S1). For the preconcentration system, standards and samples were adjusted to pH 1.0 by using HNO_3, and 0.1% m/v thiourea +0.1% m/v L-cysteine solution in NaOH pH 12.0 was used as eluent. For the mobile phase, a NaOH pH 12.0, 0.1 M sodium carbonate and 0.005 M sodium hydrogen carbonate solution was prepared. All reagents were also purchased from Merck.

The certified reference materials (CRMs) analyzed to determine the accuracy of the proposed procedure were obtained from National Research Council of Canada (NRCC): Fortified Lake Water TMDA 64.3 and Nearshore Seawater CASS-6. In order to study the applicability of the method, seawater, well-water and urine samples were collected in polypropylene bottles. Samples were immediately filtered by using a membrane of 0.45 μm pore size cellulose nitrate filters from Millipore. After that, the samples were acidified to 0.1% v/v by the addition of concentrated HNO_3 and were stored in low density polypropylene bottles at 4 °C as recommended by Method 3010B from the Environmental Protection Agency (USA), for less than 3 days until analysis.

2.3. Preparation of MGO-PSTH

MGO-PSTH has been previously synthesized and characterized by P. Montoro-Leal et al. [28]. A comparative study was conducted in that work, and this material was selected as the most proper extractant, presenting excellent properties such as suitable yields of functionalization and high adsorbent capacity for inorganic arsenic species. This new material was protected by applying a National Patent, P202030050 on 01/22/2020 with a favourable inform of the Spanish Office of Patents and Marks (OEPM) from the day June 10, 2020. Furthermore, the right for international patent was requested on 01/21/2021, with code PCT/ES2021/070039. This is the first time MGO-PSTH is used to develop an analytical method. A brief description of the synthesis and structure of this nanomaterial is reported in the supplementary material (Fig. S2). Besides, XPS spectra were registered of MGO-PSTH and MGO-PSTH exposed to each individual arsenic species to study the adsorption mechanisms.

2.4. Study of adsorption capacity towards arsenic species

In order to evaluate the adsorption capacities of the MGO-PSTH towards arsenic species, an in-batch MSPE procedure was performed. For that, four portions of 5 mg of MGO-PSTH were weighted and placed in four 50 mL beakers with 25 mL of different solutions containing As^{III}, As^{V}, cacodylate and AsBet 2 mg L^{-1} at pH 1. The mixtures were stirred by vortex for 10 min at room temperature and after magnetically separated. The supernatants were analyzed for the determination of unextracted ions. The adsorption capacities towards each species were calculated by the difference between the added and found concentration.

2.5. Automatic preconcentration and elution procedure

The FI system used for automatic preconcentration and elution of the arsenic species is shown in Fig. 1 and it was operated as follows: during the 180s sample loading period, valve in position 1, a 4.0 mL min^{-1} flow rate of standard or sample at pH 1 was pumped via P1 through the reactor, which was located replacing the sample-loop of the injection valve. The target analytes were retained on the MGO-PSTH, while the sample matrix components were directed to waste. At the end of 180 s loading period, the valve position was changed and the pump P1 stopped. In position 2, the eluent was pumped via P2 through the reactor and the retained analytes were eluted at a flow rate of 1.2 mL min^{-1}. A chromatographic vial was used to collect 1 mL of the eluent. This vial was introduced in the HPLC autosampler tray for the measurement. It was estimated that 10 s were necessary for the sample to reach the vial when the MKR was eluted. So, the first 10 s were always rejected in order to ensure the maximum preconcentration factor as possible.

2.6. HPLC-ICP-MS measurements

100 μL of the vial content were injected and the arsenic species (As^{III}, As^{V}, Cacodylate and AsBet) were separated in a single run. The optimized chromatographic gradient program is summarized in Table 1 and three different solutions were involved (sodium hydrogen carbonate as phase A, sodium carbonate as phase B and NaOH pH 12.0 as Phase C).

2.7. Optimization strategy

There were numerous parameters to optimize, which can be divided in two groups: 1) MSPE parameters and 2) HPLC separation/ICP-MS determination parameters. Two optimization strategies were used for both: univariate, changing one parameter and keeping the others constant, and multivariate, by means surface designs with multiple responses. The statistical software Statgraphics Centurion 18-X64 (Statgraphics Technologies, Inc., The Plains, VA, USA) was used to process the results of the experiments. The significance of the effects was checked by analysis of the variance (ANOVA).

Five MSPE parameters were identified to be optimized (eluent composition: concentration of thiourea, L-cysteine and NaOH pH; sample flow rate and elution flow rate). Three of them were optimized using a multiple response experiment design [30] of 9 experiments, which were randomly performed (2^3 + 1 central point). The chosen response functions were the signal to noise ratio for the four arsenic species. The variables to be optimized in this design were those related to the eluent

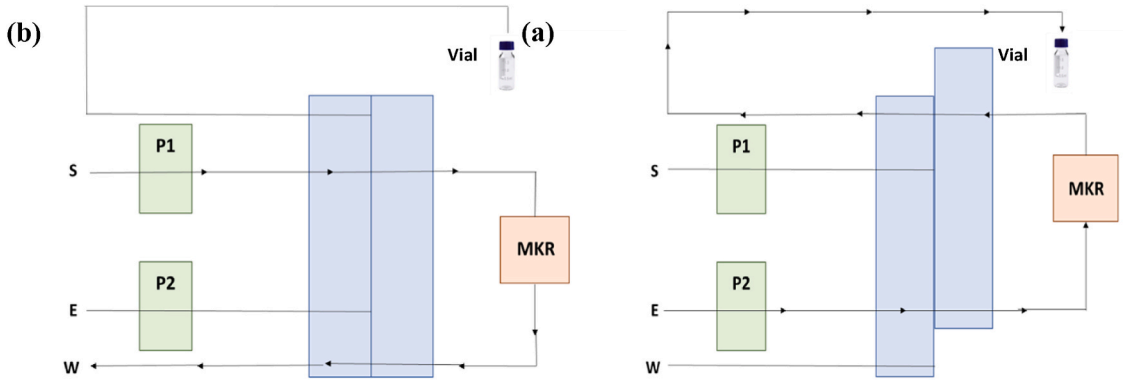

Fig. 1. FI system schematic diagram for preconcentration (a) and elution (b) steps. P1 and P2, peristaltic pumps; MKR, magnetic knotted reactor; W, waste; S, sample; and E, eluent.

composition (concentration of thiourea, L-Cysteine and NaOH pH). The ranges studied for each parameter were 0.1–2%, 0.1–0.25% and 9.0–12.0, respectively. After this optimization, a univariate study for the rest of parameters was performed. Sample and elution flow rates were varied by changing the speed of the peristaltic pumps (P1 and P2) and the inner diameter of the pump tubes. The studied ranges for these parameters were 1.8–5.9 mL min^{-1} for sample flow rate, and 0.6–1.6 mL min^{-1} for eluent flow rate. The response functions to be maximized were the signals for the four species. MSPE parameters were optimized by using the FI-ICP-MS coupling (HPLC system was not connected), and each analyte was performed separately in order to simplify the instrumentation during the performance of the experiments.

Five HPLC-ICP-MS parameters were identified (mixture percentage of phase B, ramp time, stabilization time, ICP-MS waste flow rate and ramp profile), and an optimization strategy similar to the above was followed. Three parameters were selected to be optimized by multiple response experiment design. For the design, 16 experiments were required and randomly performed (2^3 + 2·3 + 2 central points). The chosen response functions were the resolutions of the closest peaks in the chromatogram, AsBet and Cacodylate peaks resolution (R1), Cacodylate and As^{III} peaks resolution (R2); the As^{III} signal and the total time required to obtain the chromatogram (Fig. 2). The variables to be optimized in design were the mixture percentage of phase B, ramp time and stabilization time. The ranges studied for each factor were 30–100%, 0.3–4.0 min and 1.0–5.0 min, respectively. As^{III} signal was selected due to the toxicity of this inorganic form of arsenic, favouring its determination. Moreover, it should be noted that R1, R2 and As^{III} signal, were maximized, while the total time was minimized in order to increase the sample throughput of the method. After this optimization, a univariate study for the waste flow rate and ramp profile was performed, studying the ranges of 1.2–2.0 mL min^{-1} and changing the slope and the form of the ramp from 1 to 5 (Fig. 3). In this case, the response functions selected were the signal for the four species and the same response functions as the HPLC/ICP-MS design (R1, R2, total time and As^{III} signal), respectively.

2.8. Sample preparation

For the analysis of the samples, aliquots were placed in volumetric flasks of 50 mL, the pH was adjusted to 1.0 by using 5% HNO_3 and then made up to the mark with doubly de-ionized water. In all cases, external calibration with aqueous standards was used.

3. Results and discussion

3.1. Study of sample pH

It is well known that pH affects the complexation of the analytes when a chelating agent is used. Therefore, pH must be studied in order to find the best conditions to retain the arsenic species. Standard solutions of 5 μg L^{-1} of As^{III}, 5 μg L^{-1} of As^{V}, 5 μg L^{-1} of Cacodylate and 5 μg L^{-1} of AsBet were prepared in the pH range between 1.0 and 11.0 and were introduced separately in FI-ICP-MS system (HPLC was not connected). pH from 1.0 to 2.0 was adjusted using HNO_3; from 3.0 to 5.0 using sodium acetate-acetic acid buffer; and from 7.0 to 11.0 using borax-boric acid buffer. The results can be seen in Fig. 4, mostly showing complex curve profiles. This indicates that there are several adsorption mechanisms implicated, between them, the complexes formation has been demonstrated by the XPS studies described below (section 3.2.). At basic pH, both, arsenic species and MGO-PSTH, are deprotonated ($pK_{1(HSO3)}$ = 3.2, $pK_{1(As^{III})}$ = 9.2, $pK_{1(As^{V})}$ = 2.3, $pK_{1(Cacodylate)}$ = 6.3, $pK_{1(AsBet)}$ = 2.9). Therefore, under basic conditions, analytes are electron rich molecules and the adsorbent presents a negatively charged surface, producing electronic repulsions between them. At acidic pH, all the arsenic species were protonated, becoming neutral (except for AsBet) and favouring adsorption on MGO-PSTH. A pH of 1.0 was chosen for the retention of all the species. The structure of functional groups can be seen in the supplementary material (Fig. S2(B)).

Once the pH was optimized, the adsorption capacity of MGO-PSTH towards each arsenic species at the optimum pH was determined as described in section 2.4. The results obtained were 1.6, 5.0, 3.2 and 1.1 mg g^{-1}, for As^{III}, As^{V}, cacodylate and AsBet, respectively. As can be seen, As^{V} was the most adsorbed species while AsBet was the least adsorbed.

3.2. XPS results. Adsorption mechanisms

XPS spectra of (a) MGO-PSTH (b) deconvoluted sulfur spectrum of MGO-PSTH (c) deconvoluted nitrogen spectrum of MGO-PSTH, and (d) deconvoluted XPS nitrogen spectrum of MGO-PSTH with adsorpted As^{V} on the surface were shown in Fig. 5. The general view XPS confirmed the double coupling of MNPs–GO (Fig. 5a). In sulfur spectrum (Fig. 5b), three oxidation states for sulfur were identified, being assigned to the tautomers of C═S (167.8 eV), C-SH bond (164.0 eV) and the presence of a sulfonic group (168.5 eV) in the structure (Fig. S2). In Fig. 5c two peaks of nitrogen can be observed, being assigned to amine and pyridine groups (398.7 eV) and amide (400.8 eV) bonds. Amide bonds resulted from the condensation reaction between aminopropyl groups of MNPs

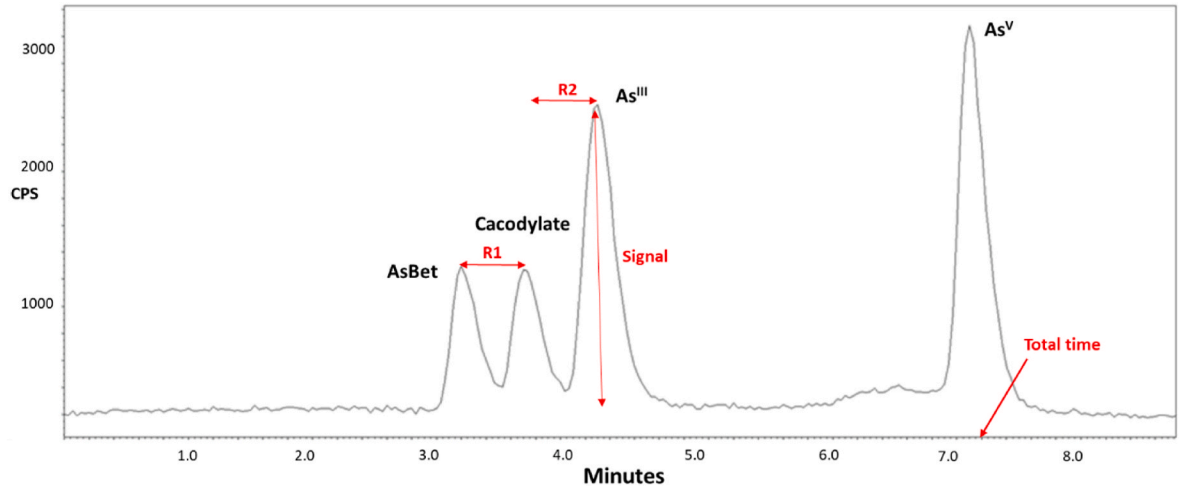

Fig. 2. Chromatogram of the arsenic species. Text in red are the response functions selected for the optimization of HPLC parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

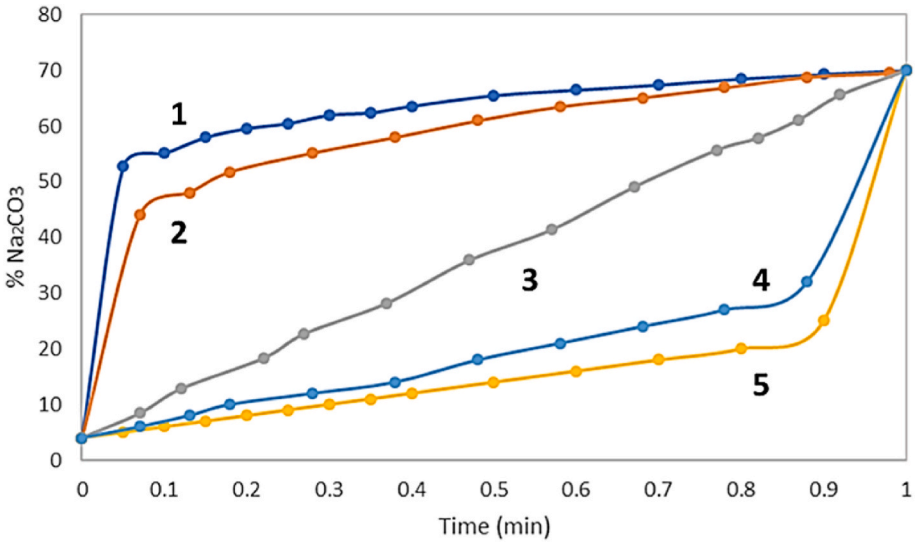

Fig. 3. Studied ramp profiles for the optimization of the HPLC running program. Labels 1–5 indicate the ramp profiles studied.

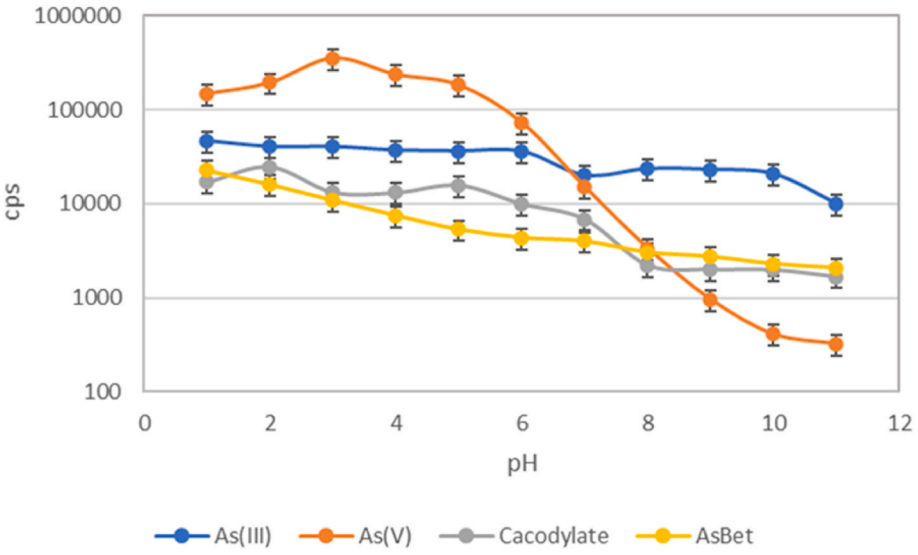

Fig. 4. Effect of pH on the adsorption of the arsenic species. Axis "y" is expressed in logarithmic scale. Concentration for all species: 5 μg L^{-1}.

and –COOH functional groups on GO [28].

As can be seen in Fig. 5d, an additional peak appeared at higher binding energy (406.9 eV) which was assigned to the formation of coordinated complex As–N. This interaction appeared when MGO-PSTH was exposed to the four studied arsenic species (As^{III}, As^{V}, Cacodylate and AsBet), demonstrating that the adsorption process was based on the formation of coordinated species of arsenic and the functional group. The position of the additional peaks of As^{V}, Cacodylate, As^{III} and AsBet were 406.9, 406.7, 406.5 and 406.5 eV, respectively. The changes of binding energy indicate the existence of distinct charge transfer phenomena in ligand molecules after the ligands forming As–N complexes [31]. This displacement is related to the bond energy of the resulting As–N species, being stronger when the peak is more displaced. According to this, As^{V} and Cacodylate presented the highest displacements while As^{III} and AsBet showed the lowest binding energy, agreeing with adsorption capacities calculated.

After being confirmed the complexes formation by XPS, the differences between adsorption capacities towards each arsenic specie, could be explained by several facts. First, PSTH-MNPs from PSTH-MGO better adsorbed As^{III} between pH 4–9, and As^{V} more readily in an acidic pH 2–7 [18], thus at pH 1, As^{III} is less retained than As^{V}. Second, the formation of hydrogen bonds between the protonated PSTH sulfonic group, hydroxylic groups on GO, and protonated forms of the arsenic species are favoured at acid pH. It is well known that hydrogen bonding can play a reinforced role in adsorption capacity of the materials when GO is used as sorbent [32]. The differences between the adsorption capacities of As^{III} and AsBet can be explained due to their capacity to form hydrogen bonds, being remarkable in the case of As^{III} because of the presence of several –OH groups in the structure (Fig. S1). Moreover, AsBet presents an organic chain in the structure, which could produce steric hindrance to interact with the functional groups. In fact, all species were adsorbed more efficiently at acid pH range, except As^{III}, which adsorption is less dependent of the pH by the competition between two mechanism, chelation and hydrogen bonds. Third, it is well known that GO due its π electron cloud better adsorbs molecules with resonant π electrons [33, 34], the only arsenic species without π electrons is As^{III}.

Summarizing, three mechanisms were implicated: chelation, hydrogen bonds and electronic π interactions. As^{V} adsorption is the most favoured by the three mechanisms at acid pH, followed of cacodylate with less capacity to form hydrogen bonds; the complex formation was

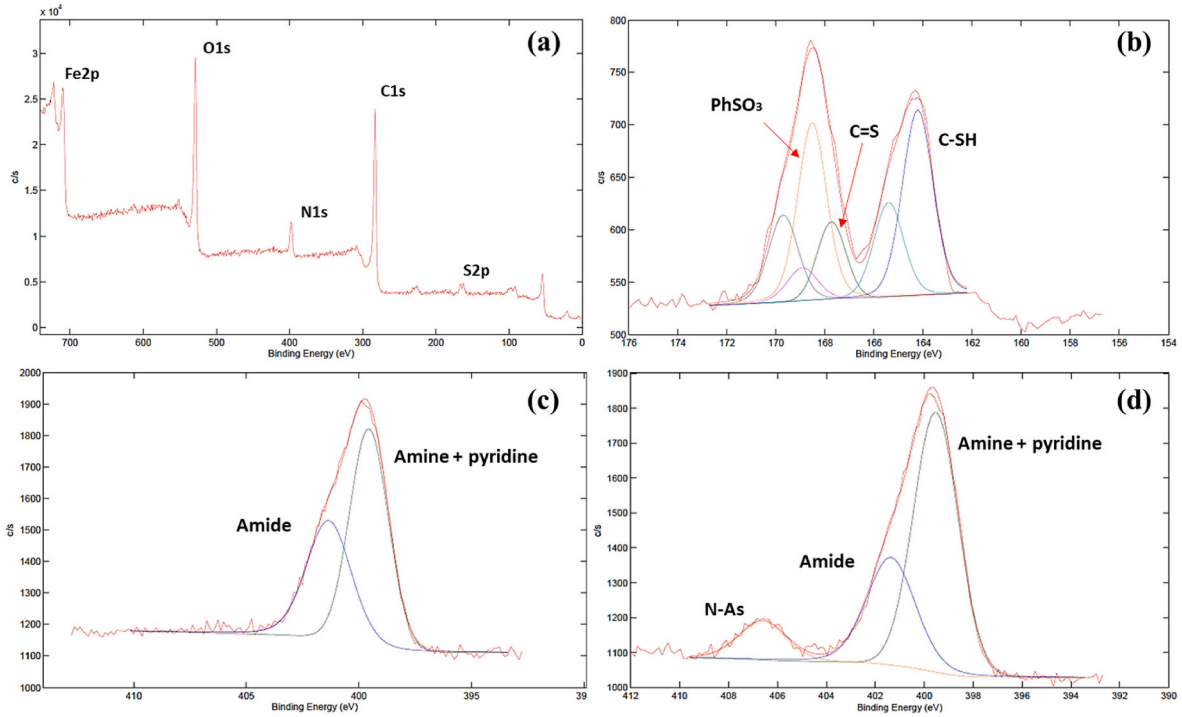

Fig. 5. XPS spectra of (a) MGO-PSTH (b) deconvoluted sulfur spectrum of MGO-PSTH (c) deconvoluted nitrogen spectrum of MGO-PSTH, and (d) deconvoluted XPS nitrogen spectrum of MGO-PSTH with adsorpted As on the surface.

less favoured at acid pH for As^{III} and for AsBet by steric hindrance, in addition, AsBet has less ability to form hydrogen bonds, being the specie less retained.

3.3. Selection of the eluent/mobile phase

The selection of the eluent/mobile phase is crucial, because phases with similar matrix solutions are necessary to ensure the compatibility of the preconcentration and separation systems. First, several mobile phase solutions, found in bibliography, were tried as eluents, such as 3 mM $(NH_4)_2HPO_4$ pH 8.7 [35] and HNO_3 solutions (phase A 1.0 mM pH 2.9, 1% MeOH and phase B 80 mM HNO_3 pH 1.3, 1% MeOH) [36]. In general, acid media has previously been proved as a suitable arsenic eluent [18], however, the results obtained were unsatisfactory due to low signal-to-noise ratios and lack of reproducibility.

According to the pH study shown in Fig. 4, arsenic species were less retained by MGO-PSTH at basic pH. For this reason, a chromatographic method with basic mobile phase for the separation of arsenic species was tried [37]. In that method, a gradient program was used for the mixture of sodium hydrogen and carbonate as phase A, sodium carbonate as phase B and NaOH pH 12.0 as Phase C. Therefore, NaOH was selected as a possible eluent, showing a suitable sensitivity and reproducibility. Then, several experiments were performed to test if the addition of L-cysteine and thiourea in the eluent solution could improve the elution

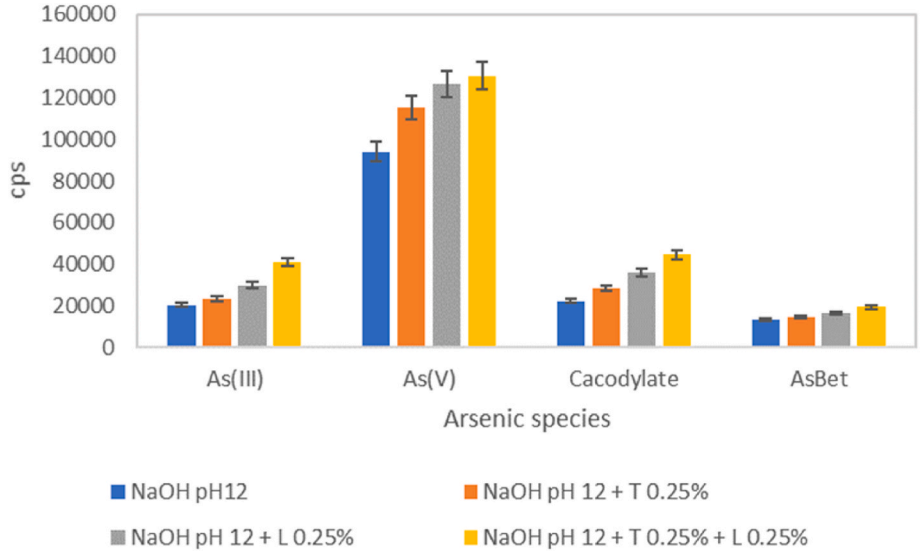

Fig. 6. Performed experiments for the improvement of elution efficiency by adding L-Cysteine (L) and Thiourea (T). Concentration: 5 μg L^{-1} arsenic species, 0.25 % T and 0.25 % L.

efficiency. Despite both substances present reductant properties, they have been previously used by us for elution purposes without modifying the arsenic species distribution in the sample [18]. Standard solutions of 5 μg L^{-1} As^{III}, As^{V}, Cacodylate and AsBet at pH 1 were prepared and introduced separately in the FIAS-ICP-MS system using 2 min of loading time. As can be observed in Fig. 6, L-cysteine and thiourea increased the elution efficiency.

Thus, L-cysteine and thiourea concentrations besides NaOH pH were included in a multiple response surface experiment design to be optimized, as mentioned in section 2.7. In order to know the upper values of the ranges to be studied, several previous experiments were necessary. For that, standard solutions mixing 5 μg L^{-1} As^{III}, As^{V}, Cacodylate, AsBet and different concentrations of L-cysteine and thiourea diluted with NaOH pH 12, were prepared and introduced in the HPLC-ICP-MS system (FI system was not connected). The range studied for both reagents was 0–2%. The chromatograms were obtained, and the results showed that the system presented the maximum tolerance for thiourea (2%), while negative effects such as peak overlaps were observed when L-cysteine exceeded 0.25%. Therefore, 2% and 0.25% were selected as the upper values to be studied in the surface design, respectively. The NaOH was studied between 9.0 and 12.0.

3.4. Optimization results

As has been said before, two multiple response experiment designs were conducted in order to optimize MSPE and chromatographic separation/ICP-MS determination parameters. The ANOVA analysis to evaluate the significance of the variables produced graphs showing the standardized effects on the response function for both designs. In the case of MSPE parameters, none of the variables or their interactions were considered statistically significant at the 95% confidence level ($p < 0.05$). However, the pareto graphs for chromatographic separation/ICP-MS determination parameters showed several statistically significant variables at the same confidence level (Fig. 7). Obviously, the mobile phase composition (A) was the most influential parameter, the sodium carbonate percentage showed a negative influence on the resolution between cacodylate and As(III) (Fig. 7b), decreasing the total time of the chromatogram and the As^{III} signal (Fig. 7c and d, respectively); the stabilization time (C) had also a negative influence on the As^{III} signal (Fig. 7d); the ramp time (B) increased the total time of the chromatogram (Fig. 7c) and decreased the As(III) signal (Fig. 7d); the stabilization time (C) decreased the As^{III} signal (Fig. 7d); and the interaction between the ramp (B) and stabilization time (C) improved the resolution between cacodylate and As^{III} (Fig. 7b). Finally, the obtained response surfaces are shown in Fig. 8. In these figures can be observed the values of the experimental parameters to obtain the maximum desirability for the response functions, Fig. 8a for MSPE parameters and Fig. 8b for chromatographic separation/ICP-MS. These optimal conditions were summarized in Table 2.

A univariate strategy was followed for the optimization of sample flow rate, eluent flow rate and waste flow rate. As a result, 4 mL min^{-1}, 1.2 mL min^{-1} and 1.4 mL min^{-1} were selected as the optimum values (Fig. 9), respectively. For sample flow rate optimization (Fig. 9A), the signal increased when the flow rate was higher, 4 mL min^{-1} was finally considered as the most suitable flow rate in order to avoid overpressure problems. The ramp profile was also optimized by this strategy, being selected the profile 5 (Fig. 3) as the optimum.

3.5. Reuse of the column

The regeneration capacity of the adsorbent material was evaluated as the MKR was subjected to successive adsorption/elution cycles connected to FI-ICP-MS. 12 mL of the mixed arsenic species were preconcentrated (5 μg L^{-1} As^{III}, As^{V}, Cacodylate and 5 μg L^{-1} AsBet at pH 1), monitoring the results in order to know the number of cycles allowed without obvious signal loss. The experiments showed high long-term stability and regeneration capacity of the MKR, which could be reused in 200 adsorption-elution cycles. Once this limit is reached, the MKR needs to be refilled again.

3.6. Analytical features

After optimization, analytical features were determined and summarized in Table 3. Calibration curves were prepared in the indicated range on the table for LOD and LOQ determination. LODs and LOQs were defined as $3.3S_a/b$ and $10S_a/b$, respectively. If the calibration

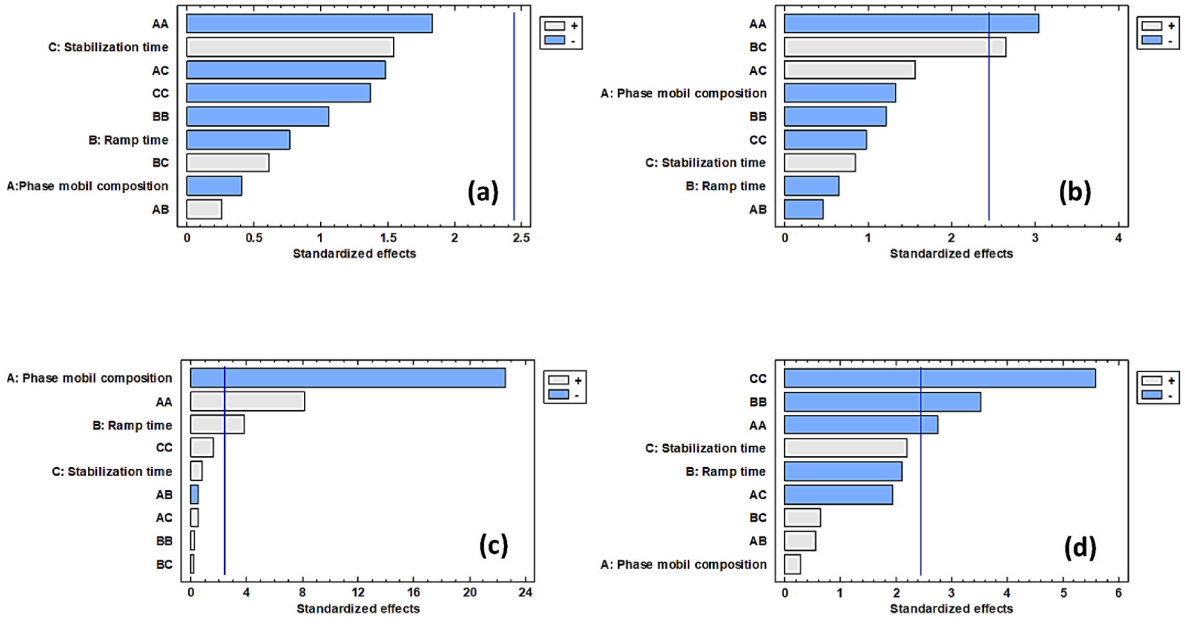

Fig. 7. Pareto graphs resulting from ANOVA for the study of separation/determination parameters (a) R1; (b) R2; (c) Total time and (d) As^{III} signal.

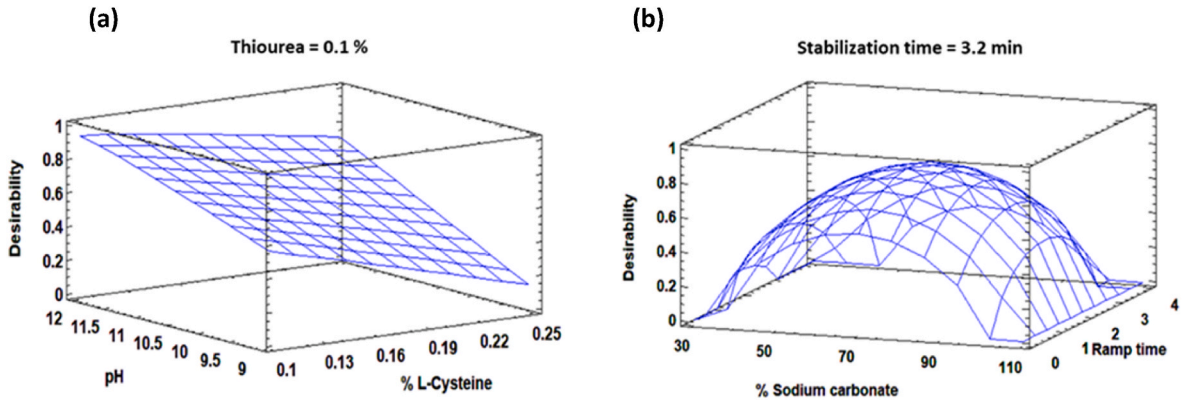

Fig. 8. Response surfaces for (a) online MSPE parameters and (b) chromatographic separation/ICP-MS determination parameters.

Table 2
Optimal conditions for preconcentration and separation/determination systems optimized by experiment design.

Variables		Optimized results
Preconcentration	Thiourea (%)	0.1
	L-Cysteine (%)	0.1
	NaOH pH	12
Separation/determination	Sodium carbonate (%)	71.4
	Ramp time (min)	1.8
	Stabilization time (min)	3.2

curve is defined as $y = bx + a$, the parameter "b" is the slope of the calibration curves and S_a is the intercept uncertainty (considered as standard deviation of the blank). For precision determination, a standard solution containing 0.05 μg L^{-1} of As^V and Cacodylate, 0.1 μg L^{-1} As^{III} and 0.5 μg L^{-1} AsBet was prepared and analyzed seven times, then the percentage of relative standard deviation (% RSD) was calculated. The EFs were studied by using the FI-ICP-MS system (HPLC was not connected) and calculated as the ratio of the slopes of the calibration curves obtained with and without preconcentration (changing the MKR by another unfilled). For this purpose, standard solutions of As^{III}, As^V, Cacodylate and AsBet in the range of 1–10 μg L^1 were prepared. Although the calculated EFs seem low for some species, they are adequate for an automatic FI method.

The comparative study shown in Table 4 proves that the additional dilution when HPLC is coupled has been adequately compensated, as this work has presented lower LODs than HPLC-ICP-MS methods without preconcentration pre-treatment [16,38]. Moreover, the MSPE-HPLC-ICP-MS method with MGO-PSTH has presented an excellent analytical performance, being the most sensitive method (with the best LODs and low sample consumption, only 12 mL). As can be seen, although one of the compared analytical procedures showed higher enrichment factors [39], higher sample volumes were needed. Obviously, increasing the sample loading time, a higher sample volume can be loaded, and the enrichment factors would be improved; however, the time of the total analysis would be also increased. Therefore, the proposed method presents the best analytical characteristics between the compared methods in Table 4.

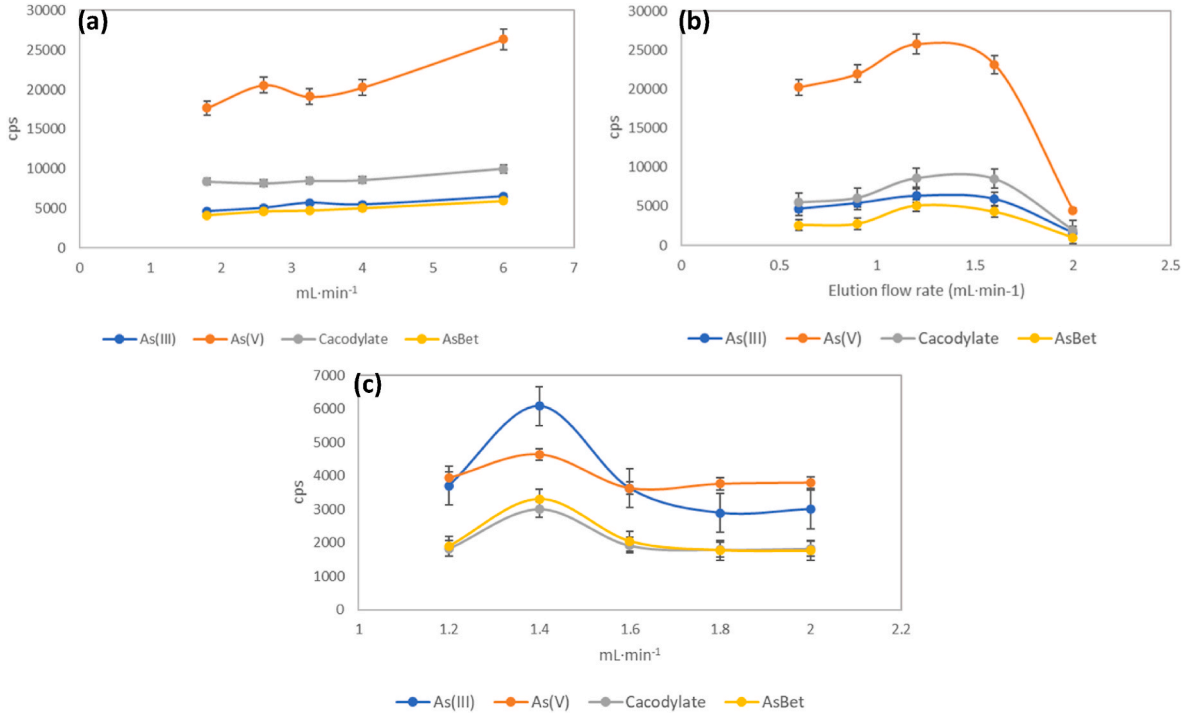

Fig. 9. Univariant optimization of (a)Sample flow rate (ml min^{-1}), (b) eluent flow rate (ml min^{-1}) and (c) waste peristaltic pump speed (ml min^{-1}).

Table 3
Analytical characteristics of the online MSPE coupled with HPLC-ICP-MS.

Parameters	Analytical Features			
	As^{III}	As^{V}	Cacodylate	AsBet
Studied linear range (μg·L^{-1})	0.005–10	0.005–10	0.005–10	0.02–10
Correlation coefficients	0.9904	0.9990	0.9981	0.9996
Slope	2143.5	2355.8	1066.6	11.2
Intercept (a)	5322.7	11758.6	4294.3	390.0
Intercept uncertainty (S_a)	721.3	155.2	161.4	12.8
Limit of detection (ng·L^{-1})	1.1	0.2	0.5	3.8
Limit of quantification (ng·L^{-1})	3.4	0.7	1.5	11.4
Precision, RSD (%)	4.5	3.0	4.7	3.8
Enrichment Factors	6	19	12	4

3.7. Samples analysis

For validation purposes, the optimized method was applied to the analysis of real samples (urine, well-water and Malaga seawater), and certified samples (Nearshore Seawater Certified Reference Material CASS-6 and Fortified Lake Water TMDA 64.3). All the analyses were made in triplicate. Samples with certified concentrations of the different arsenic species are scarce and expensive, so the accuracy of the proposed method was studied using spike tests in real samples and by comparison of total As certified values with total As found. Results were shown in Tables 5 and 6, respectively. As can be observed, the accuracy of the method has been satisfactory, the *t*-test showed that there were no significant differences between the certified and found values, with calculated t values of 3,90 and 0,87 less than the tabulated t value for 95 % of confidence, 4,30. Also, high recoveries (90–115%) were obtained for all the spiked samples, this fact confirms that the distribution of arsenic species was not modified during the MSPE method. The As content in drinking well water was in good agreement with WHO recommendations (<10 μg L^{-1}). As expected, a remarkable concentration of organic arsenic species was found in urine samples, while inorganic species were the predominant forms of arsenic in environmental waters. The samples were analyzed by external calibration. These certified and real samples had included trace elements such as transition metals, besides high salinity content with high concentration in chloride, thus, it can be concluded that this method does not present any interference from these kinds of matrices.

It is well known the interference $Ar^{40}Cl^{35}$ when As^{75} is monitored. Malaga seawater was analyzed to study this interference, an additional peak appeared at t_R = 5.5 min. So, $Ar^{40}Cl^{35}$ was adequately separated and no peak overlap was observed. This effect had been previously studied by P. Zbinden et al. [37], proving that a mobile phase based on NaOH, $NaHCO_3$ and Na_2CO_3 is able to separate this interference and the arsenic species. The non-appearance of interferences is due to both the selective extraction process and an adequate chromatographic program optimization. However, the elimination of this additional peak was studied by us using He flow rate (collision cell) during the analysis in the range of 0–3 mL min^{-1}. Finally, 3 mL min^{-1} was estimated as an adequate value to considerably decrease the presence of the $Ar^{40}Cl^{35}$ peak if necessary. In all analyzed samples, no additional peaks appeared in the chromatogram indicating that other arsenic species were not detected.

4. Conclusions

The proposed speciation method based on the compatibility of a FI MSPE method and HPLC-ICP-MS analysis has proved to be a promising monitoring routine of inorganic and organic As in environmental waters and biological samples, including complex matrix samples as seawater and urine. Moreover, the MSPE-HPLC-ICP-MS method has allowed the sequential determination by external calibration of four arsenic species (two inorganic species, As^{III} and As^{V}, and two organic species, Cacodylate and AsBet) without the requirement of collision or reaction cells,

Table 4
Comparative study of analytical performance with ICP-MS speciation methods found in bibliography.

Technique / Material	Sample (mL)	LOD (ng·L^{-1})				Enrichment Factor				Ref.
		AsIII	AsV	Cacodylate	AsBet	As^{III}	As^{V}	Cacodylate	AsBet	
HPLC	–	190	180	130	70	–	–	–	–	[16]
HPLC	–	60	220	40	–	–	–	–	–	[38]
Offline SPE Functionalized Silica	4	25	12.5	–	–	20	20	–	–	[40]
Online SPE-HPLC Polymer microspheres	25	1.2	0.91	0.96	–	28	30	28	–	[39]
Online MSPE Functionalized MNPs[a]	13	2.7	3.2[a]	–	–	2	2[a]	–	–	[18]
Online MSPE-HPLC Functionalized MGO	12	1.1	0.2	0.4	3.8	6	19	12	4	This work

[a] Analytical performance of total inorganic arsenic. As^{V} is not directly measured and it is calculated subtracting total inorganic As and As^{III}.

Table 5
Analytical applications.

Sample	Added (μg·L^{-1})				Found (μg·L^{-1})				Recovery (%)			
	As^{III}	As^{V}	Cacodylate	AsBet	As^{III}	As^{V}	Cacodylate	AsBet	As^{III}	As^{V}	Cacodylate	AsBet
Urine 1	–	–	–	–	1.8 ± 0.3	5.5 ± 0.9	94 ± 12	164 ± 6	–	–	–	–
	2	2	20	20	3.7 ± 0.4	7.3 ± 0.9	117 ± 15	185 ± 6	95	90	115	105
	4	4	60	60	6.0 ± 0.4	9.8 ± 0.9	153 ± 15	224 ± 13	105	108	98	100
Urine 2	–	–	–	–	3.9 ± 0.5	13.8 ± 1.1	7.4 ± 0.5	27 ± 2	–	–	–	–
	2	2	20	20	6.0 ± 0.6	16 ± 2	27.5 ± 0.8	50 ± 4	105	110	101	115
	4	4	60	60	7.7 ± 0.5	17.5 ± 1.4	67 ± 3	87 ± 4	95	93	99	100
Well water	–	–	–	–	2.1 ± 0.2	0.65 ± 0.06	0.052 ± 0.005	<0.0038	–	–	–	–
	0.2	0.2	0.2	0.2	2.28 ± 0.04	0.87 ± 0.06	0.25 ± 0.02	0.21 ± 0.03	90	110	99	105
	0.4	0.4	0.4	0.4	2.51 ± 0.12	1.05 ± 0.12	0.450 ± 0.009	0.395 ± 0.003	103	100	100	99
Sea water	–	–	–	–	2.38 ± 0.09	13 ± 3	<0.0005	<0.0038	–	–	–	–
	10	10	10	10	12.4 ± 0.4	24 ± 3	9.8 ± 0.3	9.9 ± 0.6	100	110	98	99
	30	30	30	30	32.5 ± 1.4	42 ± 3	30.4 ± 0.3	30.2 ± 1.0	100	97	101	101

Table 6
Certificated samples analysis.

Samples	Found ($\mu g \cdot L^{-1}$)				Total As ($\mu g \cdot L^{-1}$)	Certificate value ($\mu g \cdot L^{-1}$)	t value
	As^{III}	As^{V}	As(Cacodylate)*	As(AsBet)*			
TMDA 64.3	72 ± 2	80 ± 3	0.44 ± 0.06	<0.0114	154 ± 4	163 ± 13	3.90
CASS-6	0.60 ± 0.03	0.163 ± 0.014	<0.0015	0.28 ± 0.03	1.04 ± 0.04	1.02 ± 0.10	0.87

● Values expressed as As concentration. This conversion is necessary in order to sum the results and compare with the certificate values (also expressed as As concentration).

washing steps or salinity adjustments. This method is not susceptible to interferences thanks to the preconcentration/separation step and an adequate chromatographic program. Ten parameters (five parameters related to MSPE, and five parameters related to HPLC-ICP-MS separation/determination) have been optimized in order to maximize the signals and the resolution of the peaks, and minimize the analysis time, resulting in a sample throughput of 5 h^{-1}. The comparative study conducted with other ICP-MS speciation methods described in the bibliography has demonstrated that the proposed method presents good sensitivity and precision, with the best detection limits of 1.1, 0.2, 0.5 and 3.8 ng L^{-1} for As^{III}, As^{V}, Cacodylate and AsBet, and RSDs of 4.5, 3.0, 4.7 and 3.8%, respectively. The proposed method is semiautomatic, selective, consumes low sample volumes (12 mL) and, although it presents some instrumental complexity, the implementation of automatic MSPE and HPLC autosampler simplifies the methodology. The nanomaterial MGO-PSTH had previously been characterized and designed by us and has proved to be stable and resistant (the MKR filled with the nanomaterial was useful for 200 adsorption-elution cycles). For all the experiments described in this work, the MKR had to be refilled only three times. This method presents one of the first automatic MSPE preconcentration systems based on MGO (the first one was published in 2019 by the research group [29]), and, to the best of our knowledge, there is no previous report of a method based on automatic FI MSPE based on MGO compatible with HPLC-ICP-MS for arsenic speciation analysis.

Credit author statement

E.I. Vereda Alonso: Conceptualization, Methodology, Formal analysis, Writing - review & editing, Data curation, Supervision, Resources, Funding acquisition. **A. García de Torres**: Conceptualization, Methodology, Formal analysis, Writing - review & editing, Data curation, Supervision, Resources. **P. Montoro Leal**: Investigation, Methodology, Writing - original draft, Writing - review & editing, Validation, Formal analysis, Data curation. **J.C. García-Mesa**: Investigation, Methodology, Validation, Formal analysis, Writing - review & editing, Data curation. **I. Morales-Benítez:** Writing – review & editing.

Declaration of competing interest

The author(s) declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.talanta.2021.122769.

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