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A comparative evaluation of different ionic liquids for arsenic species separation and determination in wine varietals by liquid chromatography – hydride generation atomic fluorescence spectrometry

Alexander Castro Grijalba[a,b], Emiliano F. Fiorentini[a], Luis D. Martinez[b,c], Rodolfo G. Wuilloud[a,b,*]

[a] Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, P.C 5500 Mendoza, Argentina
[b] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina
[c] INQUISAL-CONICET, Departamento de Química Analítica, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, San Luis, Argentina

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ABSTRACT

The application of different ionic liquids (ILs) as modifiers for chromatographic separation and determination of arsenite [As(III)], arsenate [As(V)], dimethylarsonic acid (DMA) and monomethylarsonic acid (MMA) species in wine samples, by reversed-phase high performance liquid chromatography coupled to hydride generation atomic fluorescence spectrometry detection (RP-HPLC-HG-AFS) was studied in this work. Several factors influencing the chromatographic separation of the As species, such as pH of the mobile phase, buffer solution concentration, buffer type, IL concentration and length of alkyl groups in ILs were evaluated. The complete separation of As species was achieved using a C_{18} column in isocratic mode with a mobile phase composed of 0.5% (v/v) 1-octyl-3-methylimidazolium chloride ([C_8mim]Cl) and 5% (v/v) methanol at pH 8.5. A multivariate methodology was used to optimize the variables involved in AFS detection of As species after they were separated by HPLC. The ILs showed remarkable performance for the separation of As species, which was obtained within 18 min with a resolution higher than 0.83. The limits of detection for As(III), As(V), MMA and DMA were 0.81, 0.89, 0.62 and 1.00 μg As L^{-1}. The proposed method was applied for As speciation analysis in white and red wine samples originated from different grape varieties.

1. Introduction

Arsenic (As) is a toxic metalloid that is present in the earth crust at levels as high as 3.4 mg kg^{-1} and it can be found in different concentration ranges in water, soil, air, food, plants and animals [1]. The toxicity of As strongly depends on its chemical association and speciation, with inorganic species being more toxic than organic ones [2]. In fact, total concentration of As does not provide full information about the real toxicological risks linked to this element. Consequently, the development of modern methodologies for As speciation analysis is mandatory, especially to assay the quality of foods typically consumed in human diet [3,4].

Arsenic can be present in widely consumed alcoholic beverages, such as wine, due to the use of contaminated herbicides or insecticides to vine plants. Other potential sources of As contamination for wines include, soil type, water quality used for watering, and the several procedures applied during winemaking, i.e. harvesting, crushing and pressing, fermentation, purification and storage conditions of the final product [5]. The International Office of Vine and Wine (OIV) regulates total As concentration in wines at a maximum residual level (MRL) of 200 μg L^{-1} [6]. This MRL raises some drawbacks for analytical chemists because highly sensitive analytical techniques are necessary for As determination. Moreover, this situation is more complex when speciation analysis has to be performed as concentration of As

* Corresponding author at: Laboratory of Analytical Chemistry for Research and Development (QUIANID), Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Padre J. Contreras 1300, 5500 Mendoza, Argentina.
E-mail addresses: rwuilloud@mendoza-conicet.gob.ar, rodolfowuilloud@gmail.com (R.G. Wuilloud).

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species is a fraction of total As. Different sensitive analytical methods have been reported for As speciation in wine using hyphenated techniques including, gas chromatography-atomic emission detector (GC-AED) [7] and high performance liquid chromatography-inductively coupled plasma-mass spectrometry (HPLC-ICP-MS) [8]. Likewise, non-chromatographic techniques such as ionic liquid-dispersion liquid liquid microextraction associated to electrothermal atomic absorption spectrometry (IL-DLLME-ETAAS) [9] or hydride generation-atomic fluorescence spectrometry (HG-AFS) [10] have been used. Normally, chromatography-based methods use expensive detectors such as ICP-MS, while non-chromatographic ones are time-consuming due to several experimental steps involved and have limitations to separate a high number of species. On the other hand, HPLC coupled to less expensive and highly sensitive detectors, such as AFS, is a valuable alternative for As speciation analysis, with a reasonable operating time that allows routine analytical laboratories to achieve an acceptable analytical frequency.

The main As species found in wines have been As(III), As(V), MMA and DMA [8,10]. Chromatographic hyphenated techniques such as HPLC-ICP-MS and HPLC-AFS have been useful for the determination of all these As species within a single chromatographic run [11]. However, the high concentration of ethanol occurring in complex beverage samples, such as wine, needs to be specially considered when AFS or ICP-MS detectors are used due to background signal increase and sensitivity loss effects caused by organic solvents [8,10]. Therefore, the most applied chromatographic technique for As speciation has been anionic exchange (AEC) because organic solvents are not necessary in the mobile phase. On the other hand, ion pairing reversed phase-HPLC (IP-RP-HPLC) has been successful for As species separation with minimal or none organic solvent added to the mobile phase [12]. Moreover, different ion pair reagents have been used as mobile phase additives in IP-RP-HPLC-HG-AFS to separate As species including, didocecyldimethylammonium bromide (DDAB), tetrabutylammonium hydroxide (TBA) or hexane-sulfonate [13].

Ionic liquids (IL) have unique properties such as negligible pressure vapour, good thermal stability and different solubility behaviours depending on the anions and cations that constitute them [14]. Since some ILs have the ability to form ion pairs with different species [15], this property has been exploited to develop ion pairing reverse phase liquid chromatography (IP-RP-HPLC) methodologies by employing them as additives in the mobile phase [16–18]. Thus, the role of ILs has been described as having a dual behaviour: (i) the anion or cation of ILs might interact with the stationary phase modifying its properties and (ii) they might interact with the analytes changing its distribution coefficient and retention on the stationary phase. However, the exact mechanisms by which ILs produce the separation of the analytes have not been fully explained and demonstrated. The advantages of using ILs in HPLC separations can be summarized as, the improvement of peak shape, better resolution, suppression of silanols and favourable changes in the retention behaviour of the analytes [16]. On the other hand, ILs have been scarcely explored as mobile phase modifiers in elemental speciation analysis by RP-HPLC-ICP-MS [18], but their application in RP-HPLC-AFS has not been studied.

In the present work, a complete study of the effects of several imidazolium and phosphonium type ILs on the separation and determination of different As species by RP-HPLC-HG-AFS was performed. The mechanisms involved during the separation of As(III), As(V), MMA and DMA were critically evaluated to understand how ILs improve the separation conditions. The effect of C_4-C_{16} methyl imidazolium ILs and tributyl(methyl)phosphonium methylsulphate used as mobile phase additives was studied. Likewise, hydride generation conditions were optimized by a multivariate methodology considering the possible effects of each IL.

Table 1
IL-HPLC-HG-AFS instrumental and chromatographic separation parameters.

HPLC	
Column	Kinetex C_{18} (4.6 mm i.d × 100 mm × 2.6 μm)
Guard column	HPLC Krudkatcher Ultra Column In-Line Filter 0.5 μm porosity × 0.01 in ID
Mobile phase	0.02 mol L^{-1} NH_4^+/NH_3 – 0.5% (v/v) $[C_8mim]^+Cl^-$ – 5% (v/v) methanol, pH 8.5
Mobile phase flow rate	0.5 mL min^{-1}
Injection volume	100 μL
Column temperature	25 °C
Chromatographic mode	Isocratic
HG-AFS	
Lamp and wavelength	As High intensity hollow cathode lamp, 197.3 nm
Main current	40 mA
Auxiliary current	0 mA
Photomultiplier detector voltage	−270 V
Reductant	1.4% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH
Carrier	8.5% (v/v) HCl
Reductant and carrier flow rates	Both at 6.0 mL min^{-1}
Carrier gas and flow rate	900 mL min^{-1} Ar
Atomization temperature	300 °C

The application of the methodology was assayed for the speciation analysis of As in different wine varietals.

2. Experimental

2.1. Instrumentation

The separation of As species was performed with a chromatographic system consisting of a YL9101 vacuum degasser, a YL9110 quaternary solvent delivery pump (YL Instrument Co., Ltd., Seoul, South Korea), a Rheodyne valve with a 100 μL loop injector (Cotati, CA, USA) and a Kinetex C_{18} column from Phenomenex (Torrance, CA, USA). Detection of As species was performed with a Rayleigh AF-640A atomic fluorescence spectrometer (Beijing Rayleigh analytical Instrument Corp., Beijing, China). Instrumental conditions are shown in Table 1. An ultrasound bath (40 kHz and 600 W) with temperature control (Test Lab, Buenos Aires, Argentina) was employed to degas the mobile phases and solvents before chromatography. A Horiba F-51 pH metre (Kyoto, Japan) was used for pH measurements in mobile phases, standard solutions and samples.

2.2. Reagents and solutions

Stock standard solutions of inorganic As(V) and As(III) species [1000 mg As L^{-1} as sodium arsenate dibasic heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$) (99.998%) (Sigma–Aldrich, Milwaukee, WI, USA) and sodium (meta)arsenite ($AsNaO_2$) (99%) (Fluka, Buchs, Switzerland)] were prepared at 0.1 mol L^{-1} HCl (Merck, Darmstadt, Germany). Disodium methylarsonate ($CH_3AsNa_2O_3{\cdot}6H_2O$) (MMA, 98%) (Fluka) and dimethylarsinic ($C_2H_7AsO_2$) (DMA, 98.6%) (Fluka) stock standard solutions (1000 mg As L^{-1}) were prepared with ultrapure water and stored at 4 °C in amber-coloured HDPE bottles. Working solutions were prepared by diluting these stock solutions.

Sodium borohydride (Fluka) (reducing agent) dissolved in 0.05% (w/v) NaOH (Aldrich) and hydrochloric acid (Merck) (carrier agent) were used for hydride generation. Tygon type tubes (Gilson, Villiers Le-Bell, France) were used to carry these reagents. All bottles used for storing samples, standard solutions and mobile phases along with glassware were washed in 10% (v/v) HNO_3 (Merck) for 24 h and later rinsed with ultrapure water (18 MΩ cm).

1-Butyl-3-methylimidazolium chloride ([C_4mim]Cl), 1-Hexyl-3-methylimidazolium chloride ([C_6mim]Cl), 1-Octyl-3-methylimidazolium chloride ([C_8mim]Cl), 1-Dodecyl-3-methylimidazolium bromide ([C_{12}mim]Br) and 1-Hexadecyl-3-methylimidazolium chloride ([C_{16}mim]Cl) were synthesized according to a method proposed by Baltazar et al. [19]. The identity of the synthesized ILs was confirmed by comparison of infrared spectra with available databases (Solvent Innovation GmbH, Köln, Germany). Tributyl(methyl)phosphonium methylsulphate ([$P_{4,4,4,1}$]CH_3SO_4) was from Sigma-Aldrich. Stock solutions of [C_4mim]Cl, [C_6mim]Cl and [C_8mim]Cl at 5% (v/v) and [C_{12}mim]Br, [C_{16}mim]Cl and [$P_{4,4,4,1}$]CH_3SO_4 at 5% (w/v) were prepared in ultrapure water and assayed as mobile phase additive. Citric acid (Sigma-Aldrich), acetic acid (Sigma-Aldrich), sodium dibasic phosphate (JT Baker) and 2-Amino-2-hydroxymethyl-propane-1,3-diol (JT Baker) were used as buffers for pH adjustment. Potassium iodide (JT Baker) and concentrated HNO_3 (Merck) were used for total As determinations.

2.3. Collection and wine sample pretreatments

Bottled wine samples were purchased at several local wine shops of Mendoza city (Argentina) and correspond to different wine varietals typically consumed in local and international markets. Wines were sampled by removing the cork, discarding approximately the first 100 mL of liquid and taking samples directly from the bottles. All wine samples were ten-fold diluted before analysis and the pH adjusted at 8.5 with ammonia/ammonium chloride buffer solution. Finally, all wine samples were filtered through 0.20 μm pore polyamide membrane filters and later injected into the HPLC column.

2.4. Analytical procedures

A volume of 2.0 mL of wine sample was mixed with 5 mL of 65% (w/v) HNO_3 in a beaker for digestion. The mixture was subjected to sequential heating steps (15 min at 50 °C, 30 min at 100 °C and 15 min at boiling temperature). Prior to total As determination, As(V) was reduced to As(III) by adding 2.5 mL of 37% (w/v) HCl and 2.5 mL of 10% (w/v) KI. Finally, the sample was diluted to 25 mL in a volumetric flask and the mixture left to stand for 1 h. Arsenic was determined by HG-AFS under the following conditions: 0.7% (w/v) $NaBH_4$, 5% (v/v) HCl, reductant and carrier flow rates at 12 mL min^{-1}, argon flow rate at 800 mL min^{-1} and atomizer temperature at 300 °C. Other conditions were as shown in Table 1.

A mobile phase composed of 0.5% (v/v) [C_8mim]Cl, 5% (v/v) MeOH and ammonia/ammonium buffer at 0.02 mol L^{-1} was prepared with ultrapure water. The samples (treated as mentioned in Section 2.3) were directly injected into the HPLC system after preconditioning of the C_{18} column with the mobile phase for 15 min at a flow rate of 0.2 mL min^{-1}. Separation of As species was performed under isocratic conditions at 0.5 mL min^{-1}. The different As species separated by HPLC were detected online by HG-AFS under the conditions detailed in Table 1. Peak area was employed for quantification. Other experimental conditions were as listed in Table 1.

3. Results and discussion

3.1. Influence of ILs on HG-AFS detection of As species

Initial studies were performed in order to evaluate a possible effect of the ILs proposed as mobile phase additives on the sensitivity of HG-AFS. It is known that volatile organic solvents as ethanol can have a profound influence on AFS signals for As and could affect its sensitivity [10]. However, there are no reports about the influence of ILs on As fluorescence signal and arsine generation. Previously, some authors have reported the enhancement of sensitivity caused by some ILs on the AFS signal, most probably, due to a possible improvement in the generation of Au, Cu, Ni and Ag vapors [20–22]. This effect was explained based on the property of ILs to inhibit the coalescence of nanoparticles possibly being formed during metal vapors generation, and hence, an increase number of volatile metal species reaching the atomizer. This mechanism cannot be applied to As since this metalloid does not form nanoparticles. Furthermore, ILs have a structure with high organic load, which raises some questions about a possible influence on arsine formation. Considering all the above-mentioned reasons, the effect of the different ILs used for the chromatography study on HG-AFS sensitivity was assayed in the present work.

For evaluating the influence of ILs on As fluorescence signal, each As species was measured in the presence of mobile phases with each IL at concentrations of 0%, 0.2% and 0.8% (v/v). For this study, the hydride generation conditions were adopted from a previous work done in our group [12]. Basically, 12.5% (v/v) HCl, 1.1% (w/v) $NaBH_4$ in 0.05% (w/v) NaOH were the concentration of reagents employed in this evaluation. The flow rate of these solutions was 5.6 mL min^{-1} and the argon flow rate was 600 mL min^{-1}. The pH was adjusted to 2.0 with a citric/citrate buffer system at 0.02 mol L^{-1}. Other conditions were as mentioned in Table 1. Mobile phases containing each IL at different concentrations were injected into the AFS detector by the HPLC pump at a flow rate of 1.0 mL min^{-1}.

It is important to mention that As(III) is the species with the most favorable oxidation state to generate arsine. Other As species form volatile compounds that can be atomized too, but they do it at lower yields [23]. The behavior of the fluorescence signal of the As species according the different ILs employed is shown in Fig. 1. The results obtained in this work demonstrated that the atomic fluorescence signal for As(III) was affected by all the ILs within the evaluated concentration range, thus decreasing in some cases up to 50% the peak area. Likewise, As(V), MMA and DMA species decreased their fluorescence signals 50%, 30% and 60%, respectively, with respect As(III) obtained in the absence of ILs. No significant differences were observed when ILs concentration was increased from 0.2% (v/v) (Fig. 1a) to 0.8% (v/v) (Fig. 1b). In the case of [C_{12}mim]Br at 0.2%, an increase of the signals for As(V), MMA and DMA was observed. Thus, this effect could be useful to enhance the sensitivity of some As species during AFS determinations. However, additional studies are required in order to fully understand this behavior. Also, it has to be mentioned that when [C_{12}mim]Br concentration was 0.8% (v/v) the formation of foam avoided its evaluation, which it was also observed when [C_{16}mim]Cl was assayed even at concentrations as low as 0.05% (v/v). The formation of foam during hydrides generation reaction is a common effect caused when some surfactants are used in the solutions. Based on these results, the effect of ILs on As species separation by HPLC was evaluated at concentrations of 0.2% and 0.5% (v/v), while 0.8% (v/v) was not included in this study for [C_{12}mim]Br only due to foaming effect.

3.2. Optimization of HPLC parameters for As species separation

The most common As species studied in speciation analysis are anionic and highly polar. Therefore, anion exchange chromatography has been a routinely applied technique for separation of As species. However, RP-HPLC has also been employed with good results, mainly by using several ion pairing reagents, which allows a myriad of possible separation approaches [13]. In this work, the application of different ILs and separation mechanism in RP-HPLC were studied with the aim of performing As speciation analysis in a complex sample such as wine.

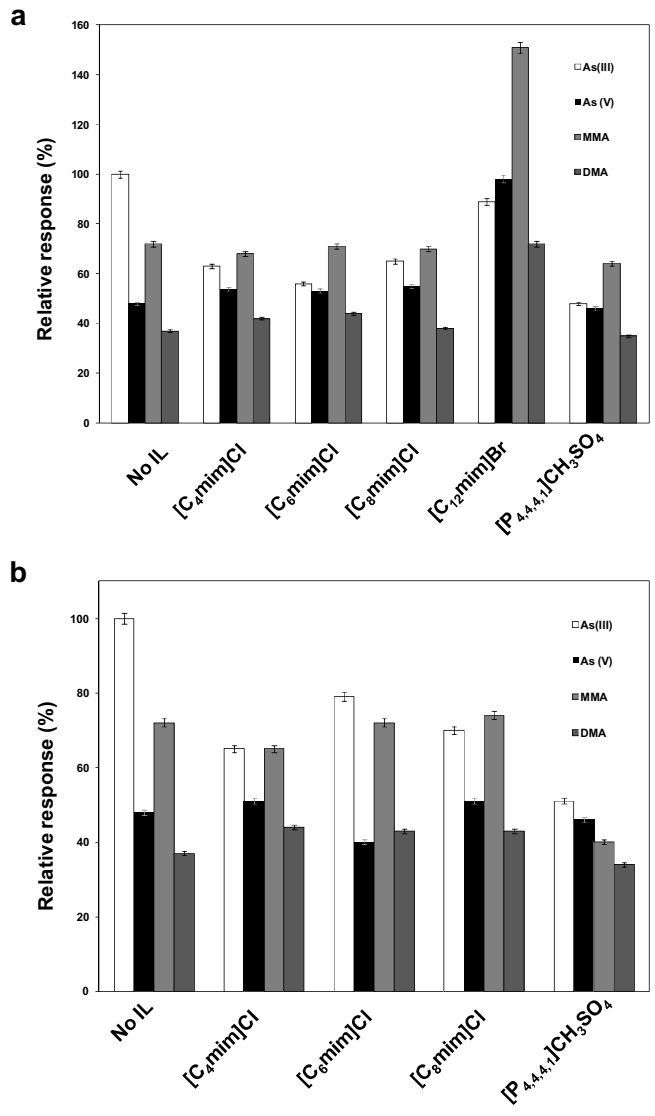


Fig. 1. Relative response of each arsenic species with respect to that of As(III) in presence of the different ILs studied this work. (a) 0.2% (v/v) IL b) 0.8% (v/v) IL. Concentration of each As species was 200 μg L^{-1}.

3.2.1. *Effect of pH on the separation of As species*

The dissociation equilibrium of As species depends strongly on the pH of solutions and pK_a values (see Table 2) [1]. Likewise, the formation of ion pairs is defined by pH because ion pair reagents are normally weak acids or bases [12]. Therefore, in a RP-HPLC separation method, the pH must be carefully evaluated to achieve the separation of As species. Since ILs are liquid salts, it can be assumed that ions are available for ion pair formation at all pH values [14]. Therefore, considering the pK_a values of As species studied in this work, the effect of pH was evaluated in the range of 2.0–9.0 with buffer systems at 0.015 mol L^{-1}. The IL [C_8mim]Cl was chosen for this study because it was considered to have a mid-length alkyl chain among all the imidazolium-type ILs included in this work. Other conditions were as mentioned in Table 1.

The differences in chromatographic resolution of the As species can be observed in Fig. 2. At pH 2.0, no separation was possible (Fig. 1a), which can be explained by the protonation of As species at this low pH and the difficulty to form ion pairs with the ILs. As pH increased, the dissociation of As species was higher, thus improving their separation until full resolution was obtained in the pH range of 8.0–9.0. Moreover, at these conditions, As(V) was highly retained

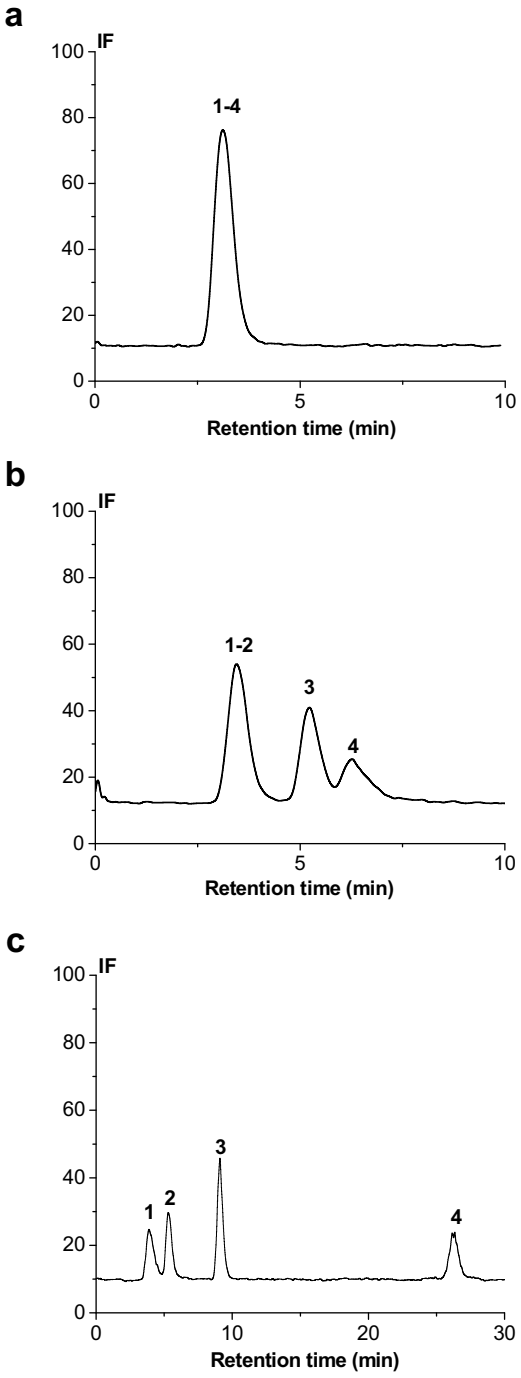
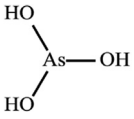
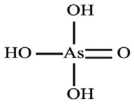
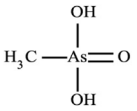
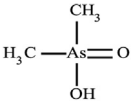


Fig. 2. Effect of pH on the separation of As species. (a) pH = 2.0, (b) pH = 5.0 and (c) pH = 8.5. IF: intensity of atomic fluorescence signal. (1) As(III), (2) DMA, (3) MMA, (4) As(V).

in the column and it was only eluted at retention times as high as 40 min. Finally, the pH for best chromatographic separation was 8.5 (Fig. 1c) and the total run was within 27 min with excellent resolution. In order to identify the retention times of each As species in the chromatogram, individual standards were injected, thus obtaining the following elution order: As(III) (3.88 min), DMA (5.30 min), MMA (9.09 min) and As(V) (26.33 min). According to Table 2, As(III) is not fully ionized until pH 9.23, therefore, this species did not show a significant interaction with the IL and hence, it was weakly retained on the C_{18} stationary phase at the pH selected in this work. The retention time of As(III) species was a bit higher than that (dead time) observed when a IL-free mobile phase was assayed. On the

Table 2
Chemical names and properties of As species included in this work.

Compound	Abbreviation	Formula	Structure	pK_a	Ref.
Arsenite	As(III)	$As(OH)_3$		pK_{a1} = 9.23 pK_{a2} = 12.1 pK_{a3} = 13.4	[5]
Arsenate	As(V)	$AsO(OH)_3$		pK_{a1} = 2.25 pK_{a2} = 7.25 pK_{a3} = 12.3	[5]
Monomethylarsonate	MMA	CH_5O_3As		pK_{a1} = 4.26 pK_{a2} = 8.25	[5]
Dimethylarsinate	DMA)	$C_2H_7O_2As$		pK_a = 6.25	[5]

other hand, since the other As species are ionized at pH 8.5, they were retained in the column.

Based on these observations, it can be assumed that the retention of As species in the column starts with the formation of ion pairs with the ILs, followed by the interaction of the ion pair formed with the stationary phase. Therefore, it can be hypothesized that the main separation mechanism was by ion-pairing formation. However, the elution order of As species coming out from the column was similar to that observed for anion exchange chromatography [24]. This observation raises the possibility that As species might also be retained following an anion exchange process with those IL molecules already retained on the C_{18} stationary. Therefore, the possible retention mechanism of As species in IL-RP-HPLC might include, indeed, a combination of different phenomena, i.e. ion-pairing, anion-exchange and hydrophobic partitioning [18].

3.2.2. *A comparative evaluation of different ILs on As species separation by RP-HPLC*

The ILs commonly used as mobile phases additives are miscible with water, have low viscosity and are used in concentrations of 0.1–1.5% (v/v) to avoid a significant increase of viscosity in the mobile phase. In aqueous mobile phases, both anion and cation of ILs can determine the chromatographic behaviour of the analytes [16]. Since ILs have been assayed as additives for HPLC mobile phases, the effect of several imidazolium-type ILs with different alkyl chain lengths were evaluated in this work for comparison. Likewise, a phosphonium-type IL, miscible with water was assayed. Fang et al. [17] have explored the effect of imidazolium ILs containing the anion $[BF_4]^-$ and alkyl groups with C_4, C_6 and C_8 lengths on the separation of As species, but longer alkyl chains were not evaluated. In fact, there is no other works reporting the effect of ILs containing a different anion than Cl^- and with longer alkyl chains than C_{12} and C_{16}.

The influence of IL concentration was evaluated considering the initial studies performed in this work on their effect AFS sensitivity. Consequently, the separation of As species by RP-HPLC was studied for all the ILs, i.e. [C_4mim]Cl, [C_6mim]Cl, [C_8mim]Cl, [C_{12}mim]Br and [$P_{4,4,4,1}$]CH_3SO_4 at 0.2 and 0.8% (v/v). [C_{16}mim]Cl was tested too, but significant foaming was observed inside the gas-liquid separator of the AFS detector and it was not possible its application. At 0.2% (v/v) of each IL, full resolution of the four As species was incomplete. The best chromatographic performance was obtained with [C_8mim]Cl with the first two peaks [As(III) and DMA] separated. On the other hand, for [C_4mim]Cl, [C_6mim]Cl and [$P_{4,4,4,1}$]CH_3SO_4 the first two peaks [As(III) and DMA] were not resolved, while in the case of [C_{12}mim]Br the first three peaks [As(III), DMA and MMA] are eluted together. The resultant chromatograms are shown in Fig. 3a–c.

The next evaluation was performed at 0.5% (v/v) of each IL. In case of [C_4mim]Cl, [C_6mim]Cl and [$P_{4,4,4,1}$]CH_3SO_4 the separation of the first two peaks [As(III) and DMA] was lower than the one observed at 0.2% (v/v). On the other hand, when [C_8mim]Cl was used, complete separation of the two first peaks [As(III) and DMA] was obtained. Finally, in the case of [C_{12}mim]Br better separation was achieved at concentration of 0.5% with respect to 0.2% (v/v), but full resolution of As(III), DMA and MMA was not feasible. Consequently, 0.5% (v/v) [C_8mim]Cl was chosen for optimal separation of As species and it was applied for further experiments.

It can be stated that the retention times and resolution are related with the interaction occurred between the paired-ion and the C_{18} stationary phase. Thus, with ILs containing short alkyl chains (C_4 and C_6) the interaction was not very strong and analytes were not much retained. On the other hand, C_8 offered an appropriate interaction and made feasible the complete resolution for all the As species. For the C_{12} alkyl chain, retention times were lower and the As species were not separated. In this case, it can be assumed that the interaction between the IL cation and the anionic As species is lower, leading to a decrease in ion pair formation. Consequently, retention times for As species were reduced compared to other ILs studied in this work containing shorter alkyl chains.

3.2.3. *Influence of type and concentration of buffer*

Buffers are needed in chromatography when the analyte is ionized at a specific pH value and its separation depends on its neutral or ionized form. Due to the importance of the buffer in RP-HPLC, concentration and type were studied in this work. Although several buffer systems could generate a similar pH value, the components of the buffer have different polarities and might affect the retention of analytes in the column [25]. Furthermore, the presence of buffers modifies the ionic strength of the mobile phase and affects the chromatographic separation of the analytes [26]. Regarding AFS detection, the type of buffer can affect the hydrolysis of $[BH_4]^-$ and the generation of As volatile species [23]. Therefore, the type of buffer and its concentration were deeply studied in this work.

For the evaluation of the buffer type three systems were selected to adjust the pH at 8.5: NH_3/NH_4^+, $H_2PO_4^-/HPO_4^{2-}$ and Tris. The

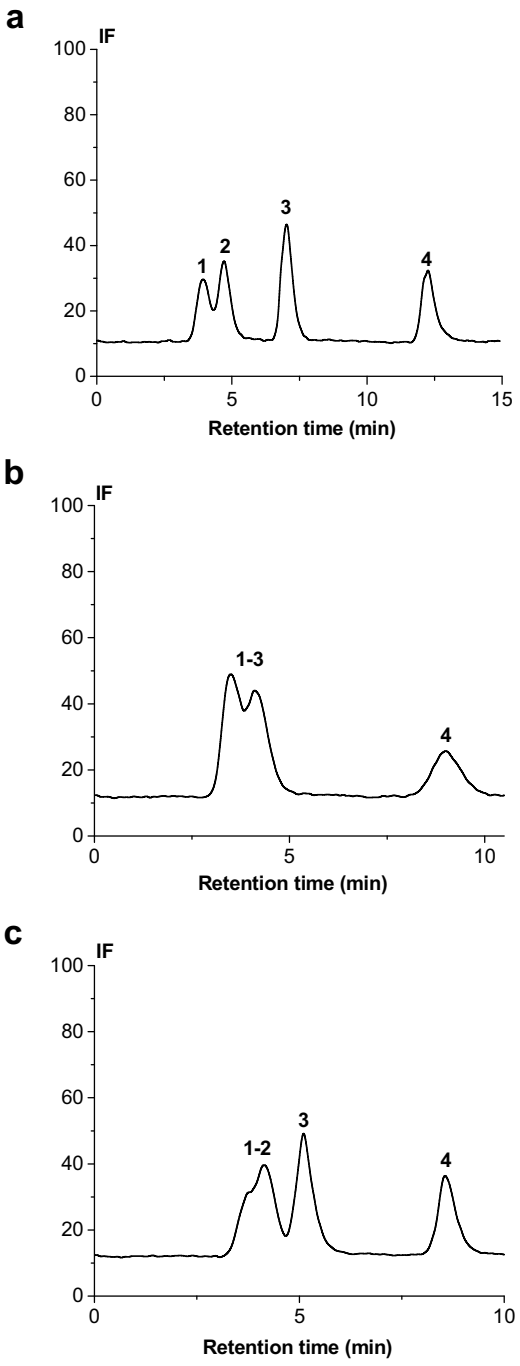


Fig. 3. Effect of type of IL on the separation of As species. a) $[C_6mim]Cl$, b) $[C_{12}mim]Br$, c) $[P_{4,4,4,1}]CH_3SO_4$. Aqueous standard mix of As species at 200 μg L^{-1}. (1) As(III), (2) DMA, (3) MMA, (4) As(V).

use of Tris resolved As(III) and DMA species even better than with NH_3/NH_4^+ buffer, however, As(V) species was not eluted. On the other hand, all As species were eluted within 8 min when phosphate buffer system was used, but resolution was loss for As(III), DMA and MMA, being eluted at the beginning of the chromatogram. Thus, complete separation of all As species under study was achieved only when NH_3/NH_4^+ buffer was used. Therefore, this last buffer system was chosen for the following experiments. Furthermore, it is well recognized that buffer systems do not generate the same ionic strength at a particular pH and concentration. Thus, among all buffers studied in this work, $H_2PO_4^-/HPO_4^{2-}$ system yielded the highest ionic strength and caused the elution of As species at the lowest retention times. In fact, this effect observed with phosphates buffers had been already confirmed when it was compared with acetic/acetate buffers for the separation of protonated bases by RP-HPLC [27]. On the contrary, Tris buffer system yielded the lowest ionic strength of all, leading to incomplete elution of As species even for retention times as long as 40 min. These findings are reinforcing our hypothesis commented earlier in this work, regarding an anion exchange process involved in the separation of As species by ILs-assisted RP-HPLC and not only pure ion-pairing phenomenon.

The effect of buffer concentration was evaluated at 0.01, 0.02 and 0.03 mol L^{-1}. It could be observed that when as NH_3/NH_4^+ concentration increased, retention times for all As species were significantly reduced. Likewise, the retention time of As(V) species, the last one eluted from the column, was shorten from 40 min to less than 15 min when buffer concentration was increased from 0.01 mol L^{-1} to 0.03 L^{-1}. However, the resolution was lost for As(III) and DMA at the highest concentration assayed. This behaviour could be related to the increase of ionic strength since the mobile phase becomes a stronger eluent. Therefore, the optimum buffer concentration was 0.02 mol L^{-1} and made feasible the separation of all As species in less than 18 min.

3.2.4. *Effect of methanol and mobile phase flow rate*

Organic solvents are common constituents of the mobile phases in RP-HPLC and can markedly modify the separation of species. In order to study the effect of methanol on the separation of As species, three different concentration were assayed: 5, 10 and 15% (v/v). Lower concentrations were not evaluated since 5% (v/v) was the lowest limit of organic solvent tolerated by the column used in this work. It was observed that when methanol concentration increased in the mobile phase, all the peaks were eluted at shorter retention times, causing that As(III) and DMA species were not resolved. This lost of chromatographic resolution is a normal effect observed in RP-HPLC due to the decrease in polarity of the mobile phase that elutes ion pairs more rapidly from the column.

Likewise, the effect of methanol on the AFS detection of As species was studied. The results showed that an increase of methanol concentration caused a significant loss of sensitivity (30%). Consequently, the optimum concentration of methanol in the mobile phase was 5% (v/v) [12]. It has be noted, that the effect of methanol on AFS sensitivity can be attributed to the formation of subproducts formed after the pyrolysis of organic solvents within an argon-hydrogen flame. Thus, carbon radicals and carbon particles might interfere during atomization of As volatile species [28].

The effect of mobile phase flow rate on the separation of As species was also studied in this work. In this case, experiments were performed at four flow rate values: 0.3, 0.4, 0.5 and 0.6 mL min^{-1}. The results showed that the optimum flow rate was 0.5 mL min^{-1}. Lower flow rates did not significantly change the separation of As(III) and DMA, while As(V) species was eluted at significantly higher retention times. On the other hand, when mobile phase flow rate was as high as 0.6 mL min^{-1}, the resolution for As(III) and DMA species was loss.

3.3. *Multivariate optimization of AFS detection conditions*

Most of the works developed on As speciation analysis using HPLC-AFS technique do not report a complete optimization that includes all the factors affecting the atomic fluorescence signal [29]. In the present work, a multivariate optimization of all variables involved in AFS detection of As species was performed. The mobile phase used for this evaluation was prepared according to the information detailed in Table 1. The variables involved in AFS detection were optimized to maximize the sensitivity and to obtain the lowest detection limits possible. A response surface methodology was employed for the optimization and a fractioned central

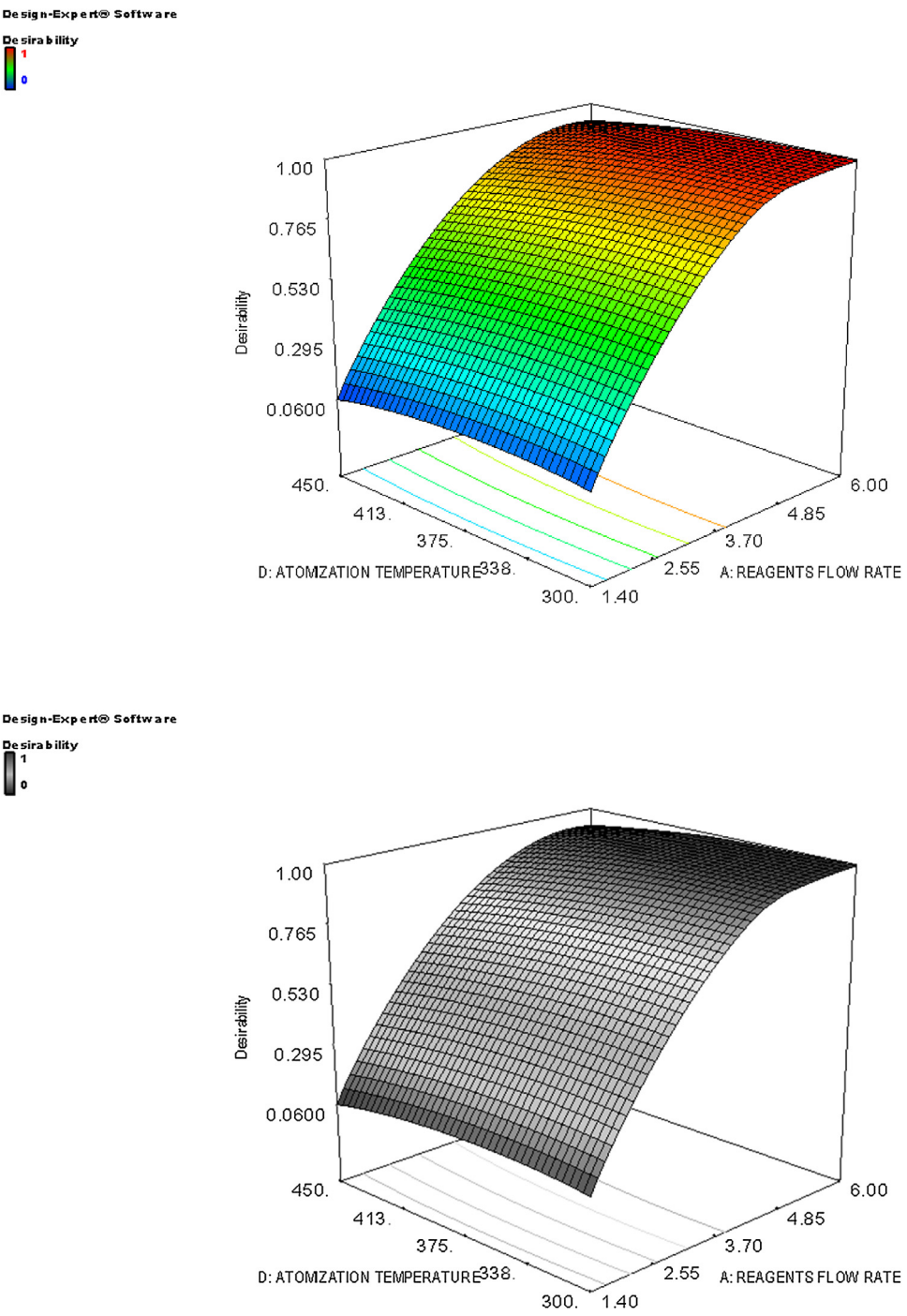


Fig. 4. Desirability function for the multivariate optimization of HG-AFS conditions for the detection of As species.

composite face centered design with six central points was applied. The evaluated factors were: $NaBH_4$ concentration, HCl concentration, flow rate of $NaBH_4$ and HCl, argon flow rate and atomization temperature. The low and high levels for these factors were as follows: 0.7–1.4% (w/v) $NaBH_4$, 5–15% (v/v) HCl, 1.4–6.0 mL min^{-1} of reagents flow rate, 700–900 mL min^{-1} argon and 300–450 °C atomization temperature. Other factors such as lamp current and photodetector voltage were fixed as constants. A total number of 34 experiments were needed for the central composite design. The response was peak area (PA) for each one of the evaluated species. Design Expert® 7.0 (Stat-Ease Inc., Minneapolis, USA) was used to process all the results obtained in this work. According to the results by this approach, several quadratic models with interactions were obtained as expressed by the following equations:

$$\begin{aligned}\mathrm{Log_{10}PA[As(III)]} &= 3.09 + 0.97A + 0.08B + 0.09C - 0.05D \\ &+ 0.05E + 0.07AC - 0.04AD - 0.03AE + 0.06BC - 0.01BD \\ &- 0.02BE + 0.01CD + 0.01CE - 0.07DE - 0.74A^2 - 0.11B^2 \\ &+ 0.07C^2 - 0.12E^2 \end{aligned} \quad (1)$$

$$\begin{aligned}\mathrm{Log_{10}PA[As(V)]} &= 2.83 + 0.92A + 0.10B + 0.12C - 0.04D \\ &+ 0.05E + 0.11AC - 0.04AD - 0.03AE + 0.06BC - 0.01BE \\ &- 0.07DE - 0.73A^2 - 0.05B^2 + 0.07C^2 - 0.07D^2 - 0.05E^2 \end{aligned} \quad (2)$$

Table 3
Chromatographic parameters and analytical performance for As species determination obtained with the proposed method.

Parameter	As(III)	DMA	MMA	As(V)
Lineal range ($\mu g\,L^{-1}$)	1.0–2000	1.0–2000	1.0–2000	1.0–2000
r^2	0.9954	0.9919	0.9996	0.9932
RSD (%)	1.98	1.84	1.56	2.36
LOD ($\mu g\,L^{-1}$)	0.81	1.00	0.62	0.89
R[a]	–	0.83	1.84	5.54
k'[b]	0.15	0.62	1.78	6.13
N[c]	64	136	248	1097
α[d]	–	4.13	2.87	3.44

[a] Resolution = $2\,(t_{R2} - t_{R1})/(w_2 + w_1)$.
[b] Capacity factor = $(t_R - t_0)/t_0$.
[c] Number of theoretical plates = $16\,(t_R/w)^2$.
[d] Selectivity = k_n/k_{n-1}.

$$[PA(MMA(V)]^{1/2} = 29.80 + 16.30A - 0.45B + 3.92C - 1.64D + 0.16E + 3.22AC - 1.05AD + 0.49AE - 0.51CE - 8.54A^2 + 2.62C^2 - 1.69D^2 - 1.50E^2 \quad (3)$$

$$[PA(DMA(V)]^{1/2} = 25.40 + 14.2A - 2.96B + 4.26C - 1.46D + 0.70E - 2.20AB + 4.03AC - 1.13AD + 1.28AE + 0.77BD - 8.31A^2 + 1.95C^2 - 1.44D^2 \quad (4)$$

where A = reagents flow rate, B = HCl concentration, C = $NaBH_4$ concentration, D = atomization temperature and E = argon flow rate. The model consisted of main and two-factor effects. R^2 was between 0.993 and 0.999 and adjusted R^2 was between 0.973 and 0.997. These values showed a good fitting quality with model equations. All the responses had to be transformed in order to obtain a lack of fit not significant and the appropriated model diagnostic plots as normal probability and residuals vs. predicted [30]. Therefore, the results were fitted to the obtained models. Observing the equations 1–4, it can be verified how the factors and their interactions affected to the responses. The coefficient of variation (CV) was between 0.94% and 5.12% indicating that the simulation can be considered as reproducible and that the precision and the experimental values were accurate. The ANOVA analysis showed that all models of the studied responses were highly significant ($p < 0.0001$).

A Derringersís desirability function (see Fig. 4) was employed to optimize the four responses simultaneously. All the factors were in range and the responses were maximum as optimization criteria. The adjusted optimum conditions given by the model were as follows: reagents flow rate of 6.0 mL min^{-1}, HCl concentration of 8.5% (v/v), $NaBH_4$ concentration of 1.4% (w/v), argon flow rate of 900 mL min^{-1} and atomization temperature of 300 °C. An experimental confirmation of these optimal conditions was performed. Peaks areas obtained for all responses were within an acceptable error range as depicted by the relative error (RE%). The RE values were between 0.84% and 2.06%. This confirmed that the model accurately predicted the response for all the As species. A chromatogram of a standard mixture containing the As species at the optimized conditions is shown in Fig. 5a. After multivariate optimization, a 3-fold enhancement in AFS sensitivity was achieved with respect to the initial measuring conditions.

3.4. Analytical performance and chromatographic parameters

The analytical figures of merit are reported in Table 3. The LODs, calculated based on the signal at the intercept and three times the standard deviation about regression of the calibration curve, obtained for As species were in the range of 0.62–1.00 $\mu g\,L^{-1}$.

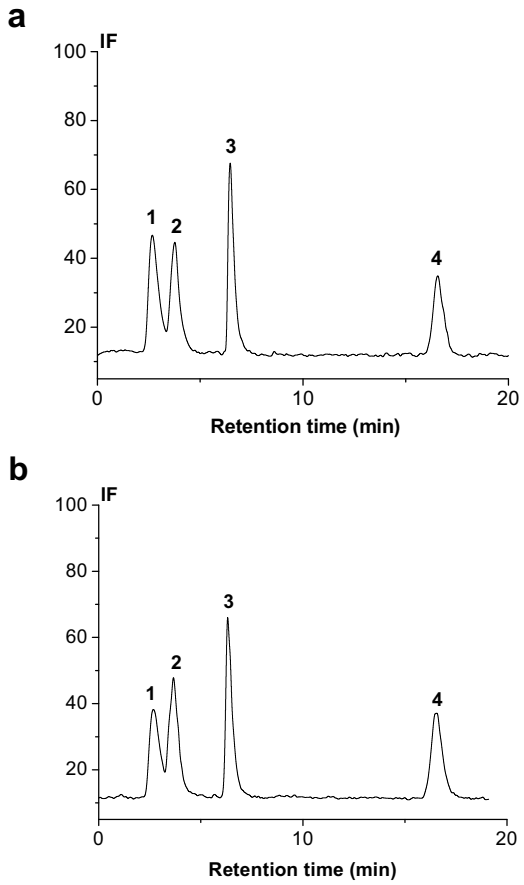


Fig. 5. IL-RP-HPLC-HG-AFS chromatograms of (a) aqueous standard mix of As species at 50 $\mu g\,L^{-1}$ as As and (b) a 10-fold diluted wine sample spiked at 50 $\mu g\,L^{-1}$ as As of each species. (1) As(III), (2) DMA, (3) MMA, (4) As(V).

The calibration curves showed a satisfactory linearity for each As species up to at least the upper limit assayed in this work, i.e. 2000 $\mu g\,L^{-1}$. The correlation coefficients were between 0.9919 and 0.9996. Table 3 also shows different parameters that characterize the chromatographic separation at the optimum conditions. Reproducible retention times were observed through 12 h. Relative standard deviations (RSD) were calculated for six replicate measurements of a standard mixture containing all As species studied in this work at 50 $\mu g\,L^{-1}$ each one. The results were in the range of 1.6% and 2.4%.

3.5. Application of ILs vs. common ion pairing reagents in RP-HPLC

Few methodologies have been developed for As speciation analysis using ion pairing reagents in HPLC-HG-AFS as compared to anion exchange. Most probably, because organic solvents needed for chromatographic separations can affect the sensitivity of AFS detector. Thus, anion exchange chromatography in the absence of volatile organic solvents has been heavily used for As speciation in HPLC-HG-AFS [31]. Among the several works using RP-HPLC-HG-AFS for As speciation analysis, two kind of methods have been developed, i.e. the first one using didodecyldimethyl-ammonium bromide (DDAB) [32] and the second one using tetrabutylammonium chloride (TBA) [33]. Likewise, sodium hexane-sulfonate has also been applied, but separation of As(III) and As(V) was impossible with this ion pairing agent [12]. The methodology using DDAB shows the disadvantage long time for conditioning the C_{18} column (500 mL of DDAB solution at 0.01 mol L^{-1}). On the other hand, the

Table 4
Comparison of methods for determination of As(III), As(V), MMA and DMA using RP-HPLC-HG-AFS based on different ion pairing agents.

Sample	Mobile phase	Elution Mode	Column Temperature (°C)	Elution time (min)	LOD ($\mu g\,L^{-1}$)	[Ref.]
Animal/plant[a] foodstuff	0.2% (v/v) $[C_6mim]BF_4$ + 0.4% (v/v) $[C_4mim]BF_4$ + 5% (v/v) methanol, pH = 6.0	Gradient	35	13	0.34–0.86	[17]
Urine	5.0×10^{-3} mol L^{-1} TBA + 3.0×10^{-3} mol L^{-1} malonic acid + 5% (v/v) methanol, pH = 5.9	Isocratic	50	6	0.50–2.00	[35]
Natural ground water	0.1% (v/v) DDAB + 0.5% (v/v) methanol + 0–50×10^{-3} mol L^{-1} sodium phosphate, pH = 6.0	Gradient[b]	N.R	6	0.30–1.00	[36]
Natural freshwater	5.0×10^{-3} mol L^{-1} TBA + 5% (v/v) methanol + 0.02 mol L^{-1} $(NH_4)_2CO_3$ + 0.02% (w/v) mercaptoethanol pH = 4.0	Isocratic	N.R	12	3.00–17.0	[37]
Wines	0.5% (v/v) (0.02 mol L^{-1}) $[C_8mim]Cl$ + 0.02 mol L^{-1} NH_4^+/NH_3 + 5% (v/v) methanol, pH = 8.5	Isocratic	25	18	0.62–1.00	This work

[a] ICP-MS detection.
[b] In this case, the gradient performed changing buffer concentration and not ion-pair reagent concentration. The column was conditioned previously with 500 mL of a DDAB solution at 1.0×10^{-2} mol L^{-1} with a flow rate for mobile phase of 1.0 mL min^{-1}.

methodology using TBA was applied to the analysis of water and urine samples with good detection limits and separation of all As species 12 min. However, a factor that might limit the widespread application of these methods could be attributed to the need for heating the mobile phase and column at temperatures that are significantly higher (50 °C) than room conditions.

In the method proposed in this work, the selection of conditions such as 0.5% (v/v) (0.02 mol L^{-1}) $[C_8mim]Cl$ in the mobile phase and an isocratic elution at room temperature, yielded good separation of all target As species within 18 min. A comparison of the above-mentioned methodologies using classical ion pairing agents with the proposed IL-RP-HPLC-HG-AFS method is shown in Table 4. It can be observed that the proposed method does not requires special operating conditions, performing the separation of As species at room temperature and under isocratic mode, which turns it into a simple and easy to operate method that might find extended utility in routine analytical laboratories. The detection limits (LOD) obtained in this work were comparable to those obtained by other methods, although it has been demonstrated the possibility of using the proposed method for As speciation analysis in highly complex matrix samples such as wine.

3.6. Determination of As species in different wine varietals

Total As concentrations in wine samples belonging to several grape varietals were evaluated by HG-AFS technique and the results are shown in Table 5. The highest total As concentration found was $26.4 \pm 0.9\ \mu g\,L^{-1}$, which is significantly lower than the MRL of $200\ \mu g\,L^{-1}$ established by the OIV [6]. Since there is no certified reference material for wines, accuracy study was performed by analyte recovery studies. Before the digestion procedure, additions of $20\ \mu g\,L^{-1}$ As standard solution were performed on wine samples. Recovery values were in the range of 94–105% showing a satisfactory accuracy for total As determination in wines.

Finally, the proposed methodology was applied for the determination of As species in several commercial wines produced in Argentina. Since a partial lack of chromatographic resolution was observed for As species when undiluted wine samples were injected into the HPLC column, 5-fold and 10-fold dilutions had to be assayed to overcome possible matrix effects. Thus, by diluting the wine samples 5 times with ultrapure water the separation of As species complete. However, a sensitivity loss was observed. On the other hand, a 10-fold dilution yielded symmetric peaks and optimum resolution, which was comparable to those obtained for As aqueous standards. Therefore, a 10-fold dilution was necessary for all wine samples prior to the injection into the HPLC instrument.

A typical chromatogram showing the separation of As(III), As(V), MMA and DMA species in wine samples is shown in Fig. 5b. It has to be mentioned that no matrix effects were observed when wine samples were injected into HPLC, evidenced by similar retention times and peak shapes obtained with respect to aqueous standards of As species (Fig. 5a). A recovery study was performed in the samples with additions at $50\ \mu g\,L^{-1}$ of each As species. Acceptable recovery values were obtained in the different wine varietals (95.4–108%). Concentration of As species in the wine samples were in the range of <LOD-$12.3 \pm 0.7\ \mu g\,L^{-1}$ for As(III) and of <LOD-$17.2 \pm 1.1\ \mu g\,L^{-1}$. The only As species found in the samples were As(III) and As(V), although MMA and DMA are probable As species to be found in wines [8]. Therefore, novel methods as the one proposed in this work are necessary to perform the accurate determination of both inorganic and organic As species in wine samples as part of food quality assurance.

3.7. Comparison of the proposed method with other anion exchange-based methods

Several reviews have been published in the literature discussing the current methods for As speciation analysis in food and water samples [11,13,34]. Thus, anion exchange chromatography has been the preferred technique due to the anionic nature of most As species and its simplicity for coupling to different detection systems. Furthermore, the pH of the mobile phases in these systems has been determined by columns properties, the composition of sample matrix and analytes under study. The method proposed in our work is simple and might not require a particular column, i.e. that a typical reversed phase column was demonstrated to be useful for efficient separation of several As species with the application of ILs as mobile phase modifiers, leading to achieve acceptable analytical figures of merits that were comparable with those obtained with classical anion exchange chromatography [34]. Therefore, the use of common columns and experimental conditions are remarkable benefits that allows the proposed method to be considered as an excellent choice by many routine analytical laboratories focused on As speciation analysis.

Table 5
Arsenic speciation analysis in different wine samples (95% confidence level, n = 3).

Sample	Total As ($\mu g L^{-1}$)	Recovery (%)[a]	As(III)			As(V)			MMA(V)			DMA(V)		
			Added ($\mu g L^{-1}$)	Found ($\mu g L^{-1}$)	Recovery (%)[a]	Added ($\mu g L^{-1}$)	Found ($\mu g L^{-1}$)	Recovery (%)[a]	Added ($\mu g L^{-1}$)	Found ($\mu g L^{-1}$)	Recovery (%)[a]	Added ($\mu g L^{-1}$)	Found ($\mu g L^{-1}$)	Recovery (%)[a]
Bonarda 1	25.8 ± 0.9	105	0	12.3 ± 0.7	–	0	13.0 ± 0.7	–	0	<LOD	–	0	<LOD	–
			50	59.4 ± 2.5	95.4	50	63.6 ± 3.7	101	50	49.8 ± 1.9	99.6	50	47.6 ± 1.24	95.2
Bonarda 2	19.8 ± 1.3	96	0	10.6 ± 0.7	–	0	13.2 ± 1.0	–	0	<LOD	–	0	<LOD	–
			50	62.1 ± 2.1	103	50	62.7 ± 2.1	99.0	50	49.8 ± 2.5	99.6	50	52.1 ± 1.5	104
Cabernet Franc 1	19.8 ± 0.9	94	0	<LOD	–	0	14.5± 0.5	–	0	<LOD	–	0	<LOD	–
			50	48.3± 1.7	96.6	50	63.1± 2.5	97.2	50	49.7 ± 1.8	99.4	50	50.4 ± 1.9	101
Cabernet Franc 2	<LOD	95	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	49.5 ± 1.7	99.0	50	48.7 ± 2.4	97.4	50	48.5 ± 1.2	97.0	50	51.6 ± 2.2	103
Cavernet Sauvignon	<LOD	105	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	51.4 ± 1.8	103	50	47.8 ± 1.4	95.6	50	52.6 ± 2.1	105	50	49.2 ± 1.2	98.4
Chardonnay	16.5 ± 0.7	96	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	50.8± 3.2	102	50	48.0± 2.8	96.0	50	52.3 ± 1.5	105	50	49.8 ± 2.5	99.6
Chenin	15.7 ± 1.5	102	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	50.5± 2.8	101	50	51.5± 1.8	103	50	49.8 ± 1.8	99.6	50	54.0 ± 1.4	108
Malbec 1	<LOD	103	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	51.4 ± 1.1	103	50	48.1 ± 1.2	96.2	50	51.8 ± 2.0	104	50	49.7 ± 0.9	99.4
Malbec 2	<LOD	93	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	48.3 ± 2.9	96.6	50	51.1 ± 1.9	102	50	52.1 ± 1.4	104	50	50.6 ± 1.9	101
Malbec 3	<LOD	98	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	49.6 ± 2.4	99.2	50	52.4 ± 2.7	105	50	48.7 ± 1.6	97.4	50	52.3 ± 2.1	105
Malbec 4	<LOD	99	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	49.0 ± 1.4	98.0	50	50.5 ± 2.1	101	50	51.4 ± 1.5	103	50	49.1 ± 1.9	98.2
Petit	14.7 ± 0.7	101	0	<LOD	–	0	12.8 ± 0.4	–	0	<LOD	–	0	<LOD	–
			50	51.4± 2.0	103	50	64.2± 1.7	103	50	49.5 ± 1.4	99.0	50	51.4 ± 1.8	103
Pinot 1	17.4 ± 1.3	96	0	<LOD	–	0	15.6± 0.9	–	0	<LOD	–	0	<LOD	–
			50	48.9± 1.7	97.8	50	63.3± 3.5	95.4	50	51.4 ± 2.7	103	50	48.6 ± 1.5	97.2
Pinot 2	<LOD	105	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	49.6 ± 1.4	99.2	50	50.1 ± 2.5	100	50	52.4 ± 2.0	105	50	49.5 ± 1.9	99.0
Red blend	26.4 ± 0.9	95	0	12.1± 0.5	–	0	16.8± 1.0	–	0	<LOD	–	0	<LOD	–
			50	61.1± 3.5	98.2	50	66.8± 3.1	100	50	49.8 ± 1.3	100	50	50.7 ± 1.9	101
Suavignon blanc	16.6 ± 0.8	101	0	<LOD	–	0	17.2 ± 1.1	–	0	<LOD	–	0	<LOD	–
			50	50.6 ± 1.9	101	50	64.2 ± 3.7	95.6	50	51.2 ± 3.1	102	50	52.1 ± 1.3	104
Syrah	12.4 ± 0.6	96	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	50.1 ± 3.0	100	50	48.7 ± 2.5	97.4	50	52.7.0 ± 1.4	105	50	48.8 ± 2.4	97.6
Tempranillo	<LOD	105	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	47.8 ± 1.4	95.6	50	51.3 ± 1.5	103	50	48.9 ± 2.2	97.8	50	51.4 ± 2.7	103
Torrontes	<LOD	94	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	48.6 ± 1.2	97.2	50	47.7 ± 1.6	95.4	50	52.3 ± 3.1	105	50	51.3 ± 2.0	103
Viognier	<LOD	97	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	48 ± 1.4	96.0	50	49.7 ± 2.1	99.4	50	48.9 ± 1.2	97.8	50	52.6 ± 2.1	105
White blend	14.7 ± 1.6	101	0	<LOD	–	0	<LOD	–	0	<LOD	–	0	<LOD	–
			50	49.5± 3.5	99.0	50	48.4 ± 2.1	96.8	50	52.0 ± 1.8	104	50	52.5 ± 2.2	105

[a] [(Found – base)]/added × 100.

4. Conclusions

A novel method for As speciation analysis in wine samples has been developed in this work. A comprehensive study of the effects of several ILs used as mobile phase additives for As species separation and determination by RP-HPLC-HG-AFS has been performed. The separation mechanisms involved when ILs are used in RP-HPLC could be attributed to different processes, i.e. anion exchange between anionic As species and IL molecules retained on the stationary phase, but also hydrophobic partitioning of the ion pairs formed between IL and As species with C_{18} stationary phase. Moreover, the choice of an appropriate type and concentration of IL was critical to obtain complete separation. In fact, different retention times for each As species and resolution of As species mix was observed depending on IL alkyl chain length of imidazolium-type ILs.

The method developed in this work showed detection limits that were comparable or even better than those reported in the literature using RP-HPLC-AFS. The separation of As species was performed without special operating conditions. Furthermore, a multivariate optimization study allowed a significant enhancement in AFS sensitivity for each As species, so it can be considered as an important allied during method development when hyphenated techniques are employed in elemental speciation analysis.

Finally, the present method was successfully applied to the analysis of samples with complex matrices, such as white and red wines, which demonstrates the high potential to use it for quality control of As speciation in this type of beverages consumed worldwide.

Acknowledgments

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