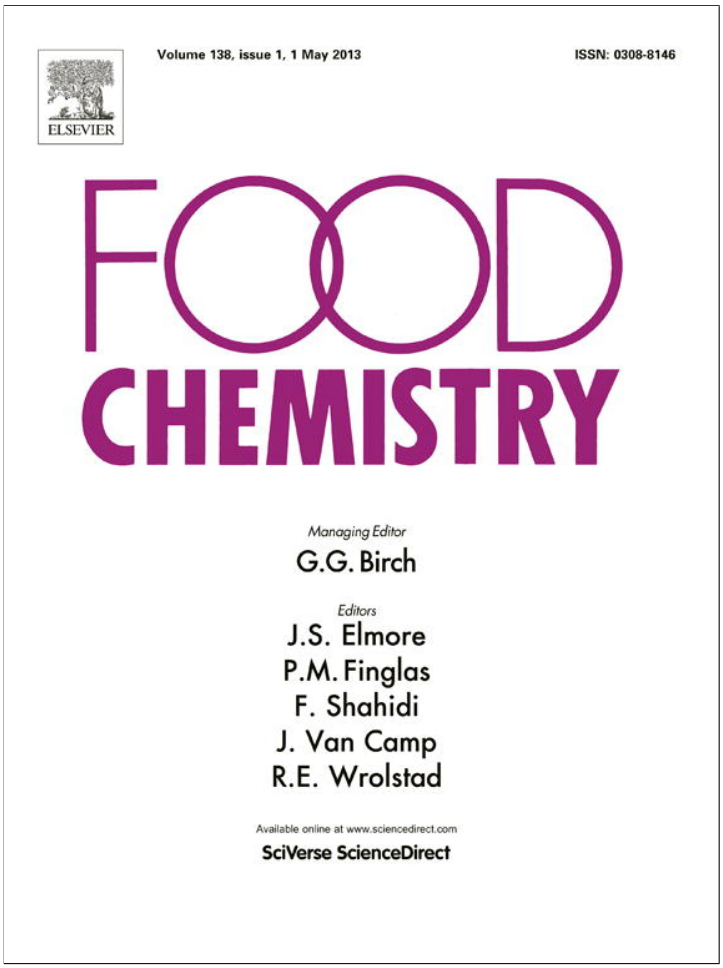

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Analytical Methods

Arsenic speciation analysis in mono-varietal wines by on-line ionic liquid-based dispersive liquid–liquid microextraction

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ABSTRACT

A highly efficient separation and pre-concentration method for arsenic species determination, based on ionic liquid (IL) dispersive microextraction technique implemented in a flow analysis system, is proposed. Highly selective separation of arsenite species [As(III)] was achieved by chelation with sodium diethyldithiocarbamate (DDTC) followed by dispersion with 40 mg of 1-octyl-3-methylimidazolium hexafluorophosphate ($[C_8mim][PF_6]$) IL. Analyte extraction, retention and separation of IL phase were achieved with a packed microcolumn and As(III) was determined in eluent solution by electrothermal atomic absorption spectrometry (ETAAS). Concentration of As(V) was deduced by the difference between total inorganic arsenic and As(III). Thus, determination of total arsenic was performed by previous degradation of organo-arsenic species, followed by a reduction. Under optimal conditions, As(III) extraction efficiency was 100% and a sensitivity enhancement factor of 46 was obtained with only 4.0 ml of sample The method was successfully applied for arsenic speciation studies in mono-varietal wines.

1. Introduction

Arsenic is a highly toxic metalloid that can be present in food, soil, water, air and living organisms (Cornelis, Caruso, Crews, & Heumann, 2003, 2005). It has been demonstrated in wine-related studies that arsenic is usually found in wines as a consequence of herbicides and insecticides used for grape production (Moreno, Cámara, Corns, Bryce, & Stockwell, 2000). International Office of Vine and Wine (OIV) has established the maximum contaminant level of arsenic in wines as 0.2 mg/l (2007). Despite marked differences in arsenic toxicology, there is no legislation for the maximum allowable concentration of specific arsenic species in wine. In order to obtain information about the bioavailability and toxicological effects of arsenic, it is necessary to obtain both qualitative and quantitative data regarding speciation.

Since arsenic concentrations in wine samples are usually very low, sensitive analytical techniques are required. Most of the works related to total arsenic determination in wines are based on hydride generation atomic absorption spectrometry (HG AAS) (Segura, Madrid, & Cámara, 1999; Tašev, Karadjova, & Stafilov, 2005) and inductively coupled plasma-mass spectrometry (ICP-MS) (Almeida, Vasconcelos, Barbaste, & Medina, 2002; Baxter, Crews, Dennis, Goodall, & Anderson, 1997). However, interference has been reported with ICP-MS in direct arsenic determination in wines because of the high ethanol content (Wangkarn & Pergantis, 1999). Different techniques have been used for arsenic speciation including gas chromatography (GC) (Campillo, Peñalver, Viñas, López-García, & Hernández-Córdoba, 2008) and high performance liquid chromatography (HPLC) (Šlejkovec, Van Elteren, & Byrne, 1997) because they offer advantages such as high sample number throughput and the potential for determining organo-arsenic species. These separation techniques are complex, and their instrumental and operation costs high for several routine analytical laboratories. For this reason, simple, sensitive and low cost non-chromatographic methods are needed for arsenic speciation studies in wine.

Generally, conventional microextraction techniques use volatile and toxic solvents for extraction (Munoz, Velez, & Montoro, 1999; Sounderajan, Udas, & Venkataramani, 2007). Ionic liquids (ILs) possess a number of unique properties such as negligible vapour pressure, thermal stability at high temperatures, and favourable viscosity and miscibility with water and organic solvents as well as specificity towards desirable ions (Liu, Jiang, & Jönsson, 2005). These properties make them attractive alternatives to replace

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those using environmentally unfriendly solvents that produce volatile organic compounds. The use of ILs in LLME has been implemented in different techniques such as single-drop microextraction (SDME) (Martinis, Berton, Altamirano, Hakala, & Wuilloud, 2010; Martinis & Wuilloud, 2010) and dispersive liquid–liquid microextraction (DLLME) (Berton & Wuilloud, 2010; Gharehbaghi, Shemirani, & Baghdadi, 2009). DLLME has been shown to be an efficient approach because of its simplicity, extraction efficiency and low consumption of solvents (Martinis, Berton, Monasterio, & Wuilloud, 2010). However, this technique has been used mostly in a batch mode, which is time consuming and associated with a high risk of contamination. For these reasons, ILs have been combined with flow injection (FI) techniques for automation and miniaturisation handling during sample preparation (Berton & Wuilloud, 2011) improving precision and enrichment whilst decreasing limits of detection (LODs).

Up to date, there are not analytical methods reported in the literature for pre-concentration and determination of arsenic species in wine samples. Therefore, the aim of this work was to develop a sensitive and selective on-line DLLME method for arsenic speciation studies in wine samples based on benign solvents. The proposed method was coupled to electrothermal atomic absorption spectrometry (ETAAS) for arsenic speciation in different monovarietal wines produced in the Mendoza province, Argentina. Moreover, this work is one of first focusing on this important matter.

2. Materials and methods

2.1. Instrumentation

The measurements were performed with a Perkin Elmer (Uberlingen, Germany) Model 5100ZL atomic absorption spectrometry equipped with a transversely heated graphite atomizer, an arsenic Electrodeless Discharge Lamp (EDL) and a Zeeman-effect background correction system. Instrumental conditions used for arsenic determination in IL-enriched phase are shown in Table 1. A centrifuge (Luguimac, Buenos Aires, Argentina) model LC-15 was used to accelerate the phase separation process. A vortex model Bio Vortex B1 (Boeco, Hamburg, Germany) was used for mixing the reagents. A Horiba F-51 pH metre (Kyoto, Japan) was used for pH determinations.

The flow injection system has been employed previously by our group (Berton, Martinis, & Wuilloud, 2010; Berton & Wuilloud, 2011). Gilson (Villiers Le-Bell, France) Minipuls 3 peristaltic pumps equipped with Tygon-type pump tubes (Gilson) were employed to propel the sample, reagent and eluent. The sample injection was achieved using six-way rotary valves from Upchurch Scientific (Oak Harbour, WA, USA). A microbore glass column (10 mm effective bed length; 2 mm internal diameter), filled with Florisil® and fitted with porous 25 μm glass frits was used for on-line retention of the dispersed IL phase.

Table 1
Instrumental and experimental conditions for arsenic species determination.

Instrumental conditions				
Wavelength				193.7 nm
Spectral band width				0.7 nm
EDL lamp current				300 mA
Matrix modifier				5 μg Pd [$Pd(NO_3)_2$] 3 μg Mg [$Mg(NO_3)_2$]
Step	Temperature (°C)	Ramp time (s)	Hold time (s)	Argon flow rate (ml/min)
Graphite furnace temperature program				
Drying 1	110	1	30	250
Drying 2	130	15	30	250
Pyrolysis	600	10	10	250
Pyrolysis	800	5	10	250
Atomization	2300	0	3	–
Cleaning	2400	1	2	250
Extraction conditions				
Sample volume				4 ml
DDTC concentration				7.5×10^{-4} mol/l
Working pH				4
Buffer concentration				2.5×10^{-2} mol/l
Triton X-114 concentration				0.05% (w/v)
$NaClO_4$ concentration				1.5% (w/v)
$[C_8mim][PF_6]$ IL amount				40 mg
Disperser solvent				Methanol (100 μl)
Shaking time with $[C_8mim][PF_6]$ IL				4 s
Eluent				Methanol (10% (v/v) HNO_3)
Eluent volume				100 μl
Loading flow rate				0.5 ml/min
Elution flow rate				0.25 ml/min

2.2. Reagents

Stock standard solutions of inorganic As(V) and As(III) species [1000 mg/l 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), respectively] were prepared with a final HNO_3 concentration of 0.05 mol/l. 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 arsenic/l) were prepared with ultrapure water and stored at 4 °C in amber-coloured HDPE bottles. Working solutions were prepared by diluting these stock solutions. A 1000 mg/l palladium nitrate solution [$Pd(NO_3)_2 \cdot 2H_2O$ (Fluka)] and 150 mg/l magnesium nitrate solution [$Mg(NO_3)_2$ (Merck, Darmstadt, Germany)] were prepared and used as chemical modifiers. These solutions were prepared in 0.1% (v/v) HNO_3 (Ultrex® II Mallinckrodt Baker, Phillipsburg, NJ, USA). A 4% (w/v) sodium diethyldithiocarbamate trihydrate (DDTC) > 99% (Fluka) solution was prepared in ultrapure water. A 2.0 mol/l acetic acid–acetate solution (Merck) adjusted to pH 4.0 by dissolution of sodium hydroxide (Merck) was employed as buffer solution. $[C_8mim][PF_6]$ IL was synthesized according to a method proposed by Huddleston et al. (2001). Methanol (Merck) was used as a dispersant. Solutions of potassium iodide (99.9%) (Ultrex® II Mallinckrodt Baker) and sodium thiosulfate (99%) (Sigma–Aldrich) were prepared for reducing As(V). Hydrochloric acid (37%) from Merck was used. A $NaClO_4 \cdot H_2O$ (Merck) solution 24% (w/v) was employed in order to adjust ionic strength. A surfactant solution containing 5% (w/v) Triton X-114 (Merck) was used to prevent the IL phase sticking to the Tygon tube walls. Synthetic magnesium silicate, Florisil® (100 Å pore size, 70–230 mesh particle size, Aldrich) was selected as filling material for the microcolumn. Ultrapure water (18 MΩ cm) was obtained from a Milli-Q water purification system (Millipore, Paris, France). All bottles destined for storing samples and standard solutions and the glassware were washed in 10% (v/v) nitric acid for 24 h and later rinsed with ultrapure water.

2.3. Sample collection and conditioning

Bottled wine samples were purchased at several local wine shops of Mendoza city (Argentina). Two typical varieties of wine in local consumption were studied. Malbec was chosen as the red variety and Sauvignon Blanc was selected as the white variety. All commercial products were 2009 vintage wine, with 6 months of ageing in oak barrels according to specifications given by manufacturers. Vintage year for wines was selected in order to analyse young wines, which are more accessible to common consumers. The wines were sampled by removing the cork, discarding approximately the first

100 ml of liquid and taking samples directly from the bottles. Red wine samples were ten-fold diluted before analysis to reduce organic matrix load in the extracts and potential increase of background signal during ETAAS determination. Samples of white variety were analysed without dilution.

2.4. Analytical procedures

2.4.1. On-line microextraction and pre-concentration procedure for As(III) species

An aliquot of 4 ml of wine sample was placed in a 10 ml graduated glass centrifuge tube with 300 μl of 10^{-2} mol/l DDTC solution, 50 μl of 2 mol/l (pH 4) acetate/acetic acid buffer, 250 μl of 24% (w/v) $NaClO_4$ and 40 μl of 5% (w/v) Triton X-114. [C_8mim][PF_6] (40 mg) was solubilized in 100 μl of methanol and injected into the sample. The resulting system was mixed using a vortex stirrer for ca. 4 s. Then, retention of the dispersed IL phase and analyte pre-concentration were performed using a flow injection system developed in previous works (Martinis et al., 2011). Basically, the mixture was propelled at a flow rate of 0.5 ml/min and the dispersed IL phase containing the As(III)–DDTC complex was retained by the filling material of the column. Finally, the retained IL rich phase was eluted with 100 μl of methanol acidified with 10% (v/v) nitric acid. The eluent was pumped with an air stream at a flow rate of 0.25 ml/min directly into the graphite tube of ETAAS for arsenic determination under the conditions shown in Table 1.

2.4.2. Total inorganic arsenic species

Selective reduction of As(V) to As(III) species was performed before pre-concentration. Volumes of 0.5 mol/l KI (1 ml) and 0.2 mol/l $Na_2S_2O_3$ (0.5 ml) solutions were added to each 4 ml of sample (placed in a 10 ml graduated glass centrifuge tube) before acidification with concentrated HCl (40 μl). The mixture was left to stand for 15 min, and total inorganic arsenic determined as described above.

2.4.3. Total arsenic

For determination of total arsenic, wine samples were placed in a digestion vessel. Subsequently, 500 μl of concentrated HNO_3 and 300 μl of 30% H_2O_2 were added. The mixture was heated to 200 °C until all organic matter was destroyed. As(V) released from organic fraction, along with the inorganic As(V) species originally present in the sample, were subsequently reduced to As(III) by KI and $Na_2S_2O_3$. Finally, total arsenic was determined with the same procedure as described earlier for As(III) species determination.

3. Results and discussion

3.1. Optimization of graphite furnace conditions for arsenic determination in IL matrix

Arsenic detection by ETAAS in an IL organic matrix can be affected by an increase in the background signal. Therefore, the graphite furnace program (pyrolysis and atomization temperatures) was optimised to obtain the highest absorbance-to-background signal ratio. In order to reduce potential matrix interference and increase accuracy, a chemical modifier or a modifier mixture is essential for ETAAS measurements. Thus, the effect of two different matrix modifiers [$Pd(NO_3)_2$, $Mg(NO_3)_2$] was studied in detail. When a mixture containing 5 μg Pd [$Pd(NO_3)_2$] and 3 μg Mg [$Mg(NO_3)_2$] was employed as a matrix modifier, the arsenic absorption signal was well shaped, i.e. narrow, sharp and symmetric peaks were observed, which is why this mixture in these quantities was selected for the analysis.

Arsenic could be partially volatilized during pyrolysis. To ensure sensitivity for arsenic determination, it was important to define an appropriate pyrolysis temperature that would remove the organic matter but minimize losses. An initial search was made for optimal temperatures during pyrolysis and atomization stages by injection of 50 μg/l arsenic solution (concentration) in presence of the IL organic matrix (40 mg of [C_8mim][PF_6], 5 μg of Pd and 3 μg of Mg). The influence of pyrolysis temperature on the absorption signal was studied in detail between 600 and 1200 °C. As can be seen in Fig. 1, the optimal pyrolysis temperature – for our system – was 800 °C with a hold time of 10 s. At temperatures above 900 °C led to significant loss of the analyte and hence decreased analytical signal.

Once the pyrolysis temperature had been selected, the effect of atomization temperature on absorbance was evaluated between 2000 and 2400 °C. The highest absorption signal was observed at 2300 °C (Fig. 1), with a hold time of 3 s. These conditions were used for all future analyses, and 2400 °C and 2 s hold used for cleaning. This final temperature was higher than atomization and no memory effects were observed during arsenic determination.

The pre-concentration procedure using DDTC, acetate/acetic acid buffer, $NaClO_4$, Triton X-114 and [C_8mim][PF_6] (methanol) was proved. A comparison using analysis of variance (ANOVA) at 95% confidence interval demonstrated that there is no significant difference amongst the achieved results using arsenic as a standard solution and following the pre-concentration method. Thus, accurate arsenic determination by ETAAS was demonstrated to be feasible even in the high organic content of IL of an enriched matrix.

3.2. Evaluation of column design and manufacturing

To be used as packing in pre-concentration columns, the filling material has to fulfill several requirements. Florisil® was used to separate analyte-containing IL phase because of its characteristics, namely its small particle size (100–200 mesh particle size) and corresponding ability to form a compact filtering media thus improving coalescence of the dispersed IL droplets and separation. Moreover, as a chemical homogeneous non-ionic structure, with a surface area of 289 m^2/g, Florisil® has been used in previous works by our research group for separation and pre-concentration of different analytes (Berton et al., 2010; Martinis, Olsina, Altamirano, & Wuilloud, 2009).

Since a more viscous, insoluble IL was used compared with previous reports, the effects of different variables during microcolumn design, such as internal diameter and length, were re-evaluated

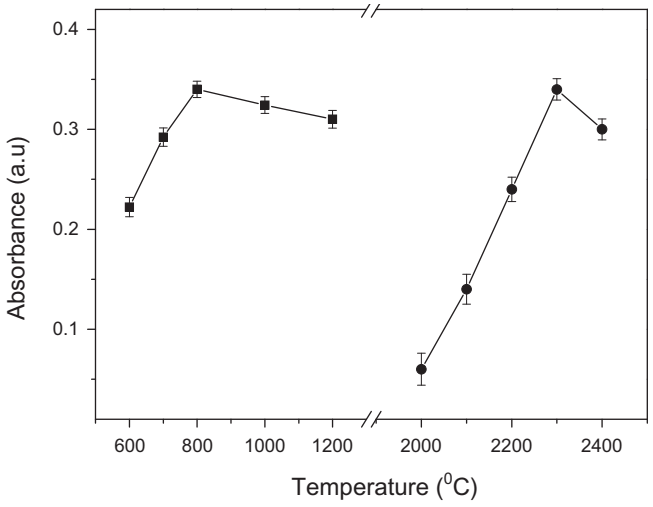

Fig. 1. Pyrolysis (■) and atomization (●) temperature curves for 50 μg/l arsenic solution mixed with 40 mg [C_8mim][PF_6] IL – methanol and using 5 μg of Pd and 3 μg of Mg as chemical modifiers. Other conditions were as mentioned in Table 1.

(Berton et al., 2010; Martinis, Olsina, et al., 2009). We observed a length of 10 mm was sufficient for complete retention of [C_8mim][PF_6] IL phase. Shorter columns were inefficient, as the filling material did not wholly retain IL phase. Increasing column length did not enhance arsenic recovery and larger volumes of eluent would have been necessary. Thus, a 10-mm column was selected.

Another variable considered in column design was inner diameter. Inner diameter is known to have an important effect on signal dispersion generated by the on-line pre-concentration system (Fang, 1993). Thus, the effect of different inner diameter of the microcolumn on analyte dispersion was considered, and a smaller diameter preferred to achieve low dispersion and allow analyte elution with a minimal volume of eluent. A 2-mm inner diameter was found to be effective for IL phase retention. The high back pressure built-up inside the FI system limited the use of microcolumns with smaller inner diameter (<2 mm).

3.3. Optimization of loading variables

Pre-concentration conditions were examined by modifying one variable at a time while keeping the others constant. Several variables were considered to optimise As(III)–DDTC complex formation and extraction as well as IL phase retention. It is a routine practise to add Na-DDTC to an aqueous phase to form the metal-DDTC chelate. It is also well known that DDTC behaves as a bidentate univalent anionic ligand and form complexes with more than 30 elements at above pH 4 (Cheng, Ueno, & Imamura, 1982). However, the reaction becomes more selective under acidic conditions (Cheng et al., 1982), and since the formation of a highly stable and hydrophobic chelate between As(III) species and DDTC is feasible (Sanllorente-Méndez, Dominguez-Renedo, & Arcos-Martínez, 2010), this reagent was used to improve affinity of arsenic for the IL phase. Thus, the effect of pH on As(III)–DDTC formation and extraction into [C_8mim][PF_6] IL phase was examined between pH 1 and 10. The results in Fig. 2(a) show the highest extraction efficiency was achieved within an interval between 3.5 and 5. This weakly acidic medium is also the best for quantitative formation of the complex under examination (Cheng et al., 1982). Hence, pH 4 was selected to adjust samples and standards before IL-DLLME, using an acetic acid/acetate buffer solution.

Furthermore, because reagent concentration is a critical variable in extraction methods based on a chelating agent such as DDTC, it was important to establish a minimal reagent concentration, which led to total complex formation whilst ensuring the highest possible extraction efficiency. As can be seen in Fig. 2(b), a concentration of 7.5×10^{-4} mol/l DDTC was the minimum concentration required to obtain the highest extraction efficiency.

Due to its notable hydrophobic character, [C_8mim][PF_6] IL is a good solvent to form biphasic systems. However, the amount of [C_8mim][PF_6] IL used in the pre-concentration phase is critical in obtaining high recovery of the analyte. The variation in arsenic recovery as a function of the amount of [C_8mim][PF_6] IL was investigated across the range 15–70 mg. Fig. 3 shows that extraction efficiency of the proposed system was affected significantly by IL amount; extraction efficiency increased with the amount of IL with the most arsenic recovered from 40 mg of [C_8mim][PF_6] IL. No significant changes were observed on the extraction efficiency by adding additional amounts of IL (95% confidence interval). Thus, 40 mg of IL was selected for further experiments.

A variety of disperser solvents including acetone, methanol and ethanol were assayed in this work. Since the highest analyte recovery was obtained with methanol, the influence of different volumes (100–500 μl) of this solvent on extraction efficiency was assayed. Thus, As(III) extraction efficiency reached 100% using 100–300 μl of methanol, while slightly decreased for higher volumes (95%).

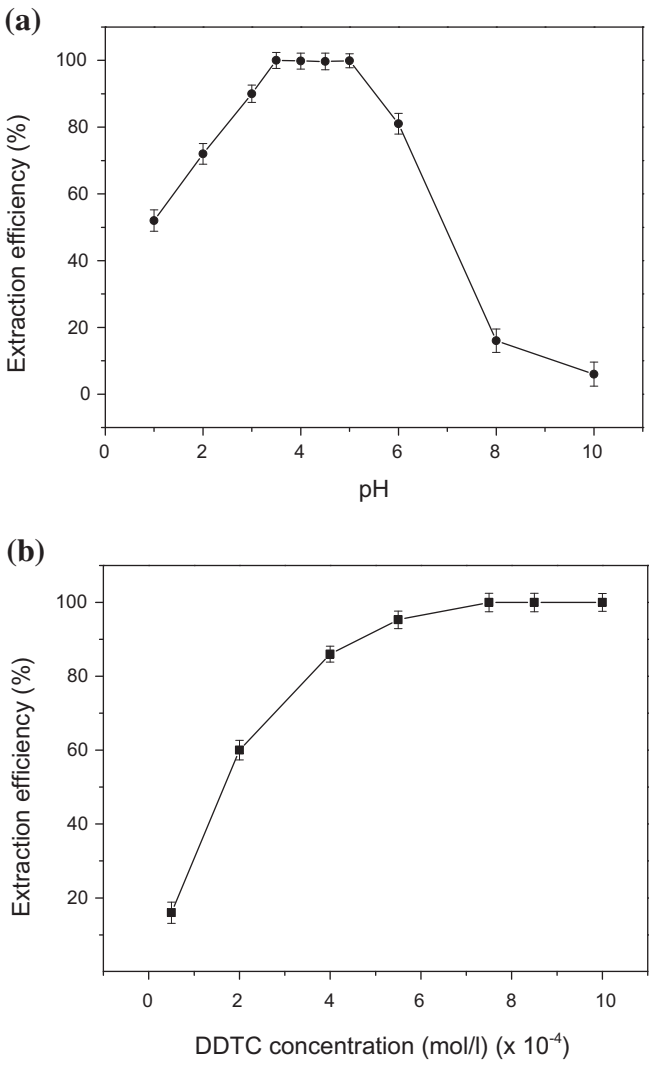

Fig. 2. (a) Influence of pH on As(III)–DDTC complex formation and extraction efficiency of As(III) by the on-line IL-DLLME system. (b) Effect of DDTC concentration on recovery of As(III). Other conditions are listed in Table 1.

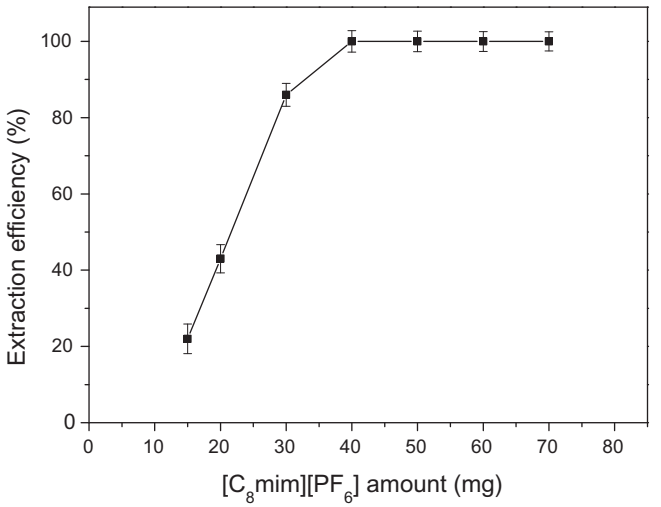

Fig. 3. Influence of [C_8mim][PF_6] IL amount on As(III) extraction efficiency obtained with the on-line IL-DLLME system. Other conditions are detailed in Table 1.

In order to minimize the consumption of volatile solvent, 100 μl of methanol was the volume used in all subsequent analyses. Moreover, methanol was useful in solubilizing the arsenic-enriched IL phase, making it easier and more reproducible for injection into the graphite furnace.

A common surfactant, Triton X-114, was added to the sample solution to prevent the IL adhering to the inner walls of the tubes, and thus improving the flow throughout of the FI system and promoting retention in the microcolumn. Triton X-114 surrounds ILs droplets reducing their interactions with the inner walls. However, large amounts of any surfactant could affect IL phase retention in the microcolumn. Thus, the impact of Triton X-114 on As(III)–DDTC extraction and IL phase retention in the microcolumn was examined across concentration range of 0.01–1% (w/v). It was observed that 0.05% (w/v) Triton X-114 yielded high extraction efficiency while allowing free running of the IL droplets in the lines. Higher surfactant concentrations led to insufficient retention in the column and hence poor results.

The effect of $NaClO_4$ concentration was studied in order to examine its impact onto the extraction efficiency. Concentrations between 0–3% (w/v) were evaluated. The extraction efficiency was moderately increased from 90% to more than 99% by increasing salt concentration up to 1%, remaining constant up to 3%. Thus, 1.5% (w/v) $NaClO_4$ concentration was chosen as optimal.

Sample flow rate is another important parameter since it is one of the variables controlling analysis time. Moreover, the effect of sample flow rate is critical to achieving retention of the IL phase. For these reasons, our study was developed with flow rates ranging between 0.25 and 2.0 ml/min. No major changes on the analytical response were observed up to 0.5 ml/min while it decreased at flow rates higher than 0.6 ml/min, and above 1.5 ml/min there was no retention of the IL phase. Thus, a flow rate of 0.5 ml/min was finally selected.

3.4. Study of elution conditions

An eluent should elute the analyte in a small volume without affecting the accurate determination of the analyte. To fully elute As(III), different types and amounts of organic eluents were assayed. Both acidified acetone and methanol were shown to be most effective for IL phase and As(III)–DDTC complex removal from the microcolumn. The best results were obtained when elution was counter-current, thus diminishing analyte dispersion in the elution volume (Hakim, Sabarudin, Oshita, Oshima, & Motomizu, 2008). Likewise, to reduce eluent volume further and minimize dispersion, air-segmentation, were air sandwiches the eluate, was implemented. For quantitative elution of the analyte in a small eluent volume, a low elution flow rate should be used, providing sufficient time for equilibrium between the phase containing the analyte and the eluent. Quantitative elution of the IL phase and As(III)–DDTC complex from the column were achieved with 100 μl of methanol acidified to 10% (v/v) nitric acid. A lower volume resulted in incomplete elution of the analyte and reduced sensitivity while larger volumes were limited by the capacity of the graphite furnace sample. Finally, a sample uptake rate in counter-current mode (0.25 ml/min) was chosen for the on-line system coupled to ETAAS.

3.5. Analysis of interferences

Several ions, including Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cl^-, PO_4^{3-}, citrate and tartrate are concomitant and regularly found in the samples under study. For this reason, their influence on arsenic extraction and determination was examined. Selectivity of the proposed method was assayed by evaluating the impact of possible concomitant ions at the levels usually found in wine samples. The experiments were performed by analysing a 1 μg/l As(III) standard solution containing concomitant ions, and applying the recommended extraction procedure. Concomitant ions were determined to interfere if this resulted in an analytical signal variation of ±5%. As shown in Table 2, none of the anions or cations examined produced significant interference effects, and they did not affect the extraction efficiency. The analytical signals of blank controls were also unchanged in the presence of concomitant ions.

3.6. Analytical performance

3.6.1. Calibration, detection limits and precision

The calibration graph was linear with a correlation coefficient of 0.9978 at levels near the detection limits (0.05 μg/l) and up to at least 6 μg/l As(III). The limit of detection (LOD), calculated based on three times the standard deviation of the background signal (Rickert et al., 2007) was 5 ng/l. Thus, the proposed methodology offers high sensitivity for determination of low concentrations of the analyte even below the maximum contaminant level (MCL) established by OIV. The relative standard deviation (RSD) for six replicate measurements at 0.2 μg/l of arsenic were 4.7%, 5.4% and 5.7% for As(III), As(V) and total organo-arsenic species, respectively.

3.6.2. Extraction efficiency and sensitivity enhancement factor

The extraction efficiency (ER%) was calculated using Eq. (1):

$$\mathrm{ER}\% = \frac{m_f}{m_0} = \frac{C_f \times V_f}{C_0 \times V_0} \times 100 \quad (1)$$

where m_f and m_0 are the mass of analyte in the final organic phase and the initial aqueous solution, respectively. Similarly, C_f and C_0 represents arsenic concentration, while V_f and V_0 are the volumes of the phases involved (Liu, Zhao, Zhu, Gao, & Zhou, 2009). An extraction efficiency of 100% was reached when the procedure was performed under optimal experimental conditions (Table 1).

The sensitivity enhancement factor (EF) was figured as the ratio of the slopes of the calibration curves for arsenic with and without the pre-concentration step (Martinis, Berton, Olsina, Altamirano, & Wuilloud, 2009). Thus, an enhancement (EF) factor of 46 was achieved with 4 ml of sample. Finally, the frequency of analysis was eight samples per hour.

3.6.3. Comparison with conventional methods

It is important to mention that there are not analytical methods reported in the literature for pre-concentration and determination of arsenic species in wine samples. A comparative study on analytical performance allows us to show the strengths of the proposed method with respect to other methods already reported in the literature (Table 3). Our method shows a LOD that is better than others developed for arsenic determination in wine samples by ETAAS. In contrast to other methods using ETAAS, this is a novel and effective method for speciation analysis of this toxic element. Although

Table 2
Effect of potential interfering ions on As(III) recovery.[a]

Ion	Added as	Concentration (mg/l)	Arsenic recovery (%)
Na^+	$NaNO_3$	125	100
K^+	KNO_3	2300	99.3
Ca^{2+}	$Ca(NO_3)_2$	150	96.9
Mg^{2+}	$Mg(NO_3)_2$	120	103
Fe^{3+}	$FeCl_3$	10	97.1
Cl^-	KCl	60	98.6
PO_4^{3-}	NaH_2PO_4	55	101
Citrate	Na-citrate	35	98.7
Tartrate	Na-tartrate	2500	102

[a] This study was performed using 4 ml of 1 μg/l arsenic standard solution.

Table 3
Analytical methods reported in the literature for arsenic determination in wine.

Method	Speciation analysis	LOD (ng L^{-1})	RSD (%)	Sample volume (mL)	Calibration range (μg L^{-1})	Refs.
ETAAS	No	5000	4.8	5	[a]–100	Ajtony et al. (2008)
ETAAS	No	6600	<10	8	[a]	Bruno, Campos, and Curtius (1994)
LC–ICP-MS	Yes	100	<10	5	0.1–10	Moreira et al. (2011)
HG-AFS	Yes	300	<8	0.1	1–10	Karadjova, Lampugnani, Onor, D'Ulivo, and Tsalev (2005)
IL-DLLME–ETAAS	Yes	5	4.7	4	0.05–6	This work

LC–ICP-MS: liquid chromatography–inductively coupled plasma-mass spectrometry.
HG-AFS: hydride generation-atomic fluorescence spectrometry.
[a] Non reported.

Table 4
Concentrations of As(III), As(V) and organo-arsenic species in wine samples (95% confidence interval; $n = 6$).

Sample		As(III)			As(V)			OrgAs		
		Added (μg/l)	Found (μg/l)	Recovery (%)[a]	Added (μg/l)	Found (μg/l)	Recovery (%)[a]	Added (μg/l)	Found (μg/l)	Recovery (%)[a]
White wine	1	0	1.08 ± 0.06	–	0	2.15 ± 0.14	–	0	nd[b]	–
		0.50	1.57 ± 0.08	98.2	0.50	2.66 ± 0.15	102	0.50	0.51 ± 0.05	102
	2	0	2.18 ± 0.09	–	0	4.97 ± 0. 18	–	0	0.08 ± 0.02	–
		0.50	2.68 ± 0.09	100	0.50	5.45 ± 0.20	96.3	0.50	0.59 ± 0.06	102
	3	0	3.20 ± 0.12	–	0	7.70 ± 0.25	–	0	0.25 ± 0.04	–
		0.50	3.69 ± 0.15	98.5	0.50	8.22 ± 0.24	104	0.50	0.74 ± 0.06	98.1
	4	0	5.60 ± 0.16	–	0	10.9 ± 0.30	–	0	0.83 ± 0.05	–
		0.50	6.09 ± 0.20	98.1	0.50	11.4 ± 0.28	100	0.50	1.31 ± 0.08	96.4
Red wine	1	0	1.56 ± 0.07	–	0	2.48 ± 0.15	–	0	nd[b]	–
		0.50	2.05 ± 0.08	98.3	0.50	2.99 ± 0.16	102	0.50	0.52 ± 0.05	102
	2	0	2.41 ± 0.08	–	0	3.35 ± 0.17	–	0	0.07 ± 0.03	–
		0.50	2.91 ± 0.09	100	0.50	3.83 ± 0.17	96.5	0.50	0.56 ± 0.05	98.2
	3	0	4.00 ± 0.18	–	0	6.40 ± 0.24	–	0	0.62 ± 0.05	–
		0.50	4.52 ± 0.19	104	0.50	6.92 ± 0.26	104	0.50	1.10 ± 0.06	96.0
	4	0	6.51 ± 0.17	–	0	11.2 ± 0.28	–	0	0.75 ± 0.06	–
		0.50	7.00 ± 0.22	98.2	0.50	11.7 ± 0.30	100	0.50	1.26 ± 0.08	102

[a] [(Found − Base)/Added] × 100.
[b] Not detected.

ICP-MS can be used for determination of As species because of its high sensitivity, selectivity and sample throughput, this instrumentation might not be always available in all routine analytical laboratories because of its high cost.

3.7. Determination of arsenic species in wine samples

The proposed analytical method was applied for the determination of As(III) and As(V) species, and total organo-arsenic fractions in selected wine samples. In the absence of a certified reference material for arsenic species in wines, a recovery study would be a valid alternative in elemental speciation studies (Cornelis et al., 2003). Recovery of known amounts of As(III) and As(V) added to wine samples was evaluated using the proposed IL-DLLME method. A similar procedure has been developed for organic arsenic species commonly found in wine samples (MMA + DMA) (Moreira et al., 2011) previously. The recovery values were between 98.1–104% for As(III), 96.3–104% for As(V) and 96–102% for organo-arsenic species (MMA + DMA) (Table 4). These results suggest the procedure is reliable for speciation analysis of arsenic in wine samples. Concentrations of arsenic species in white wine samples were in the range of 1.08–5.60 μg/l for As(III), 2.15–10.9 μg/l for As(V) and not detected–0.83 μg/l for organo-arsenic species. Arsenic concentrations for our selected red wines were between 1.56–6.51 μg/l for As(III), 2.48–11.2 μg/l for As(V) and 0.53–0.75 μg/l for organo-arsenic species. None of the wines contained total arsenic levels above the limits set by the International Office of Vine and Wine (OIV) (2007).

To sum up, As(III) and As(V) species were detected in all samples studied. Organic species (MMA + DMA) were present at very low concentrations levels, and in many cases they were not detected. It should be pointed out that concentrations of arsenic species found in the present work keep similarities from those reported by other authors in Argentinean wines (Moreira et al., 2011). In fact, they are not markedly different from those reported for wines produced in other well-recognised wine-producing regions around the word (Karadjova, Lampugnani, Onor, D'Ulivo & Tsalev, 2005).

4. Conclusions

We have demonstrated that As(III) species, as As(III)–DDTC complex, can be efficiently extracted using $[C_8mim][PF_6]$ IL. An extraction efficiency of 100% and an analytical sensitivity enhancement factor of 46 were obtained with only 4.0 ml of sample. The method developed exhibited advantages such as high sensitivity and low cost, and benefits from low detection limits. Moreover, the on-line retention and separation of IL-enriched phase increases the speed of the pre-concentration and analysis, in addition to reducing sample consumption and contamination risks generally present in batch procedures.

In summary, a simple non-chromatographic method with high selectivity and sensitivity has been developed in this work and its application successfully demonstrated for arsenic speciation at trace levels, with good accuracy and reproducibility, in local wine samples.

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