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Research Article

Development of a dispersive liquid–liquid microextraction method using a lighter-than-water ionic liquid for the analysis of polycyclic aromatic hydrocarbons in water

A dispersive liquid–liquid microextraction method using a lighter-than-water phosphonium-based ionic liquid for the extraction of 16 polycyclic aromatic hydrocarbons from water samples has been developed. The extracted compounds were analyzed by liquid chromatography coupled to fluorescence/diode array detectors. The effects of several experimental parameters on the extraction efficiency, such as type and volume of ionic liquid and disperser solvent, type and concentration of salt in the aqueous phase and extraction time, were investigated and optimized. Three phosphonium-based ionic liquids were assayed, obtaining larger extraction efficiencies when trihexyl-(tetradecyl)phosphonium bromide was used. The optimized methodology requires a few microliters of a lighter-than-water phosphonium-based ionic liquid, which allows an easy separation of the extraction solvent phase. The obtained limits of detection were between 0.02 and 0.56 $\mu\text{g/L}$, enrichment factors between 109 and 228, recoveries between 60 and 108%, trueness between 0.4 and 9.9% and reproducibility values between 3 and 12% were obtained. These figures of merit combined with the simplicity, rapidity and low cost of the analytical methodology indicate that this is a viable and convenient alternative to the methods reported in the literature. The developed method was used to analyze polycyclic aromatic hydrocarbons in river water samples.

Keywords: Dispersive liquid-liquid microextraction / High-performance liquid chromatography / Ionic liquids / Polycyclic aromatic hydrocarbons / Water samples

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1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous compounds present in the environment and produced by natural or anthropogenic sources. Sixteen of these compounds have been classified as priority pollutants by the United States Environmental Protection Agency (US-EPA) due to

their mutagenic or carcinogenic activity [1–4]. This institution as well as the World Health Organization (WHO) recommend the routine monitoring of benzo[*a*]pyrene (B[*a*]P), one of the most carcinogenic compounds [4, 5], in drinking waters. Its maximum allowed concentration should not exceed 0.20 $\mu\text{g/L}$ according to the US-EPA, and 0.70 $\mu\text{g/L}$ for the WHO [4, 5].

Most PAHs are released into the environment due to leaks or spills during extraction, fuel combustion, and emissions of oil refineries, among other sources [6]. Since PAHs have poor or very poor solubility in water, they are usually present in aqueous matrices at trace levels. Thus, efficient preconcentration methods are mandatory before detection. SPE, SPME, stir bar sorptive extraction (SBSE), and liquid–liquid microextraction (LLME), are the most popular ones used to determine PAHs in water samples [7–10].

The dispersive liquid–liquid microextraction technique (DLLME) has been applied to the determination of several compounds in different matrices [11, 12]. The method is based on a ternary solvent system in which an extraction solvent

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Abbreviations: EF, enrichment factor; FLD, fluorescence detector; IL-DLLME, ionic-liquid-based dispersive liquid–liquid microextraction; OVAT, one-variable-at-a-time; PB-RTIL, phosphonium-based room temperature ionic liquid; PAH, polycyclic aromatic hydrocarbon; SAM, standard addition method; US-EPA, United States Environmental Protection Agency

(e.g. dichloromethane, hexane, toluene) is firstly dissolved in a disperser solvent (e.g. isopropanol, acetone) and this mixture is rapidly injected into the aqueous sample by using a syringe. The disperser solvent must be miscible with the aqueous phase. Thus, during the dispersion process, the disperser solvent remains solubilized in the aqueous phase and, simultaneously, very small droplets of the extraction solvent are formed, increasing the mass transfer due to the high surface contact area. In classical DLLME, droplets collapse at the bottom of a conical tube to form the organic phase, insoluble in the aqueous phase. If the molecular interactions between the extracting solvent and the analytes are strong enough, the analytes will remain solubilized in the very small volume of the organic solvent drop, obtaining high enrichment factors. Different variations to the classical technique, which include vortex-, ultrasound-, or surfactant-assisted DLLME, as well as DLLME with in situ formation of the extraction solvent or with the solidification of a floating extraction solvent drop and several other alternatives, have been developed [13].

Usually, the extraction solvent in DLLME is heavier than water, which makes the collection of the organic drop some tedious and slow, since the aqueous phase has to be discarded carefully. However, several works using lighter than water organic solvents such as chloroform, isooctane, and long chain alcohols, among others, have been reported [10, 14, 15]. In this case, to facilitate the collection of the organic phase, specially designed glass tubes with a narrow neck at the top have been used [16, 17].

Room-temperature ionic liquids (RTILs) are a form of melting salts composed of organic cations and organic or inorganic anions. They emerged as possible environmentally friendly solvents ("green" solvents) [18] and have a wide application field in separation sciences [19–22] among other research areas, due to their unique properties such as low volatility, chemical, and thermal stability, and good solubility for both organic and inorganic molecules. RTILs are slowly replacing the classical organic solvents in sample preparation.

The use of RTILs in DLLME (IL-DLLME, ionic-liquid-based dispersive liquid–liquid microextraction) has spread quickly [23] since they offer important advantages as compared to the organic solvents usually employed in LLE such as chloroform, carbon tetrachloride, or diethyl ether. These specific solvents have very low miscibility with the usual reversed HPLC mobile phases [23] and, therefore, solvent change (e.g. by evaporation) is required before injection, which can result in a significant loss of some analytes [24]. On the contrary, RTILs can be directly injected into the chromatographic column avoiding the evaporation step, which can affect the method reproducibility. A disadvantage of some RTILs as compared with typical organic solvents is its cost, as occurs with the nitrogen-based RTILs (NB-RTILs).

Phosphonium-based room temperature ionic liquids (PB-RTILs), known for a long time, have been almost ignored in the literature, particularly as extraction solvents in LLE, as compared to their imidazolium- or pyrrolidinium-based

counterparts. PB-RTILs have higher thermal and chemical stability, usually more favorable solvation properties [25–27] and they are much economic than the NB-RTILs. These RTILs are made of tetra-alkylphosphonium cations with different organic or inorganic anions. At present, there are about 20 different types of PB-RTILs commercially available [25]. Recently, a flow injection system for online IL-DLLME using the lighter than water IL (tetradecyl)phosphonium bromide for cobalt preconcentration was reported [27].

In this work, we have developed a dispersive liquid–liquid microextraction method using a phosphonium-based ionic liquid as the extraction solvent, combined with HPLC–FL-DAD for the determination of the 16 US-EPA priority PAHs in water samples. Three PB-RTILs were tested and the trihexyl-(tetradecyl)phosphonium bromide, $[(C_6)_3C_{14}P][Br]$ was selected as the extraction solvent. The goal was to explore the extraction ability of this family of ionic liquids toward PAHs present in aqueous samples because of two main reasons: (i) they are lighter than water, which facilitates its collection in specially designed glass tubes (e.g. with a narrow neck at the top), and (ii) from the economical point of view, they are more convenient than the typical NB-RTILs used in IL-DLLME. The effects of experimental parameters on the extraction efficiency (type and volume of the IL and the disperser solvent, type, and salt concentration and extraction and centrifugation times) were investigated and optimized. From the obtained figures of merit, it can be concluded that the developed methodology allows to obtain very low LODs and LOQs, high enrichment factors (EFs) and *R*% values and very good reproducibility. To the best of our knowledge, the IL-DLLME method using a lighter than water PB-RTIL for the extraction of PAHs from water samples has not been used yet. The applicability of the proposed procedure was tested by the determination of PAHs present in two water samples collected from two docks contiguous to a petrol refinery in Berisso city and next to the Rio de la Plata river (Argentina).

2 Materials and methods

2.1 Chemicals and materials

The PAH standards naphthalene (Nap), acenaphthylene (Acp), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Flt), pyrene (Pyr), chrysene (Cry), benz[*a*]anthracene (B[*a*]A), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), indeno[1,2,3-*cd*]pyrene (InP), dibenz[*a,h*]anthracene (DBA), and benzo[*g,h,i*]perylene (BPe), were bought from AccuStandard as a reagent kit containing all 16 priority PAHs listed by the US-EPA.

Trihexyl(tetradecyl)phosphonium chloride, $[(C_6)_3C_{14}P][Cl]$ (CYPHOS[®] IL 101), trihexyl(tetradecyl)phosphonium bromide, $[(C_6)_3C_{14}P][Br]$ (CYPHOS[®] IL 102), trihexyl(tetradecyl)phosphonium dicyanamide, $[(C_6)_3C_{14}P][N(CN)_2]$ (CYPHOS[®] IL 105) were provided by Cytec Industries (New Jersey, USA).

Reagents were of analytical grade or better: potassium chloride, sodium chloride, sodium phosphate dibasic anhydrous, and sodium bicarbonate (Anedra, Industria Argentina), potassium phosphate (Matheson, Coleman & Bell, Norwood, Ohio, USA), acetone (Merck, Industria Argentina), acetonitrile, and methanol HPLC grade (J. T. Baker, Estado de Mexico, Mexico). Solutions were prepared in MilliQ® water (Milli-Q system, Millipore).

100 and 50 μL microsyringes supplied by Hamilton (Reno, USA) and Agilent Technologies (Mulgrave, Australia), respectively, were used.

For the DLLME technique using a lighter than water ionic liquid, homemade narrow neck glass tubes were used. The glass tubes were 9.5 x 12 mm id with necks of 6 mm id. The total volume was 5 mL and the volumes of the collected IL phase, measured by a calibrated microsyringe, were between 20 and 25 μL ($\pm 1 \mu\text{L}$).

2.2 Instrumentation and chromatographic conditions

Chromatographic analysis was carried out using an HP 1100 liquid chromatograph equipped with binary pump, degasser, thermostat-controlled column compartment containing a Zorbax Eclipse XDB-C18 column (250 x 4.6 mm id; 5 μm) connected to a guard column. A DAD and a fluorescence detector (FLD), connected to a HP workstation was used. Peak identities were confirmed by comparing the DAD spectra with those stored in the spectral library.

A gradient consisting of: 70–100% acetonitrile in 43 min was used. For fluorescence detection, an optimum excitation wavelength set at 260 nm for all PAHs was used, while the optimum emission wavelengths were 340 nm for Nap, Ace, Flu, and Phe, 400 nm for Ant, Pyr, Cry, BaA, and DBA, 420 nm for BbF, BkF, BaP, and BPe, and 500 nm for Flt and InP. The absorbance of Acp, a nonfluorescent PAH, was recorded at 230 nm.

2.3 Preparation of stock and standard solutions

A stock solution of PAHs was prepared by dissolving the compounds (5 mg/L) with acetonitrile and stored in the refrigerator for up to one month. The state of conservation of this solution was checked weekly by comparing the chromatographic peak areas with those obtained immediately after the solution was prepared.

2.4 Analysis of real samples

The river water samples were collected in amber glass bottles from the surface of the river, stored in the refrigerator at 4°C and prefiltered through 0.45 μm nylon membranes before use. A 6.03% w/v KCl solutions were prepared using the two river water samples. The solutions for the standard addition method (SAM) were prepared as follows: 4.145 mL of the river water samples containing KCl and different volumes of

ACN (between 705 and 855 μL) were put into 5 mL narrow-neck glass tubes and spiked with different volumes (between 0 and 150 μL) of the stock solution (5 mg/L) containing the 16 PAHs and then homogenized. The final concentrations of the solutions for the calibration curve were 0 to 0.15 mg/L for PAHs containing 17.1%v/v of ACN and 5%w/v of KCl.

2.5 Extraction procedure

The optimized IL-DLLME procedure was performed as follows: 90 μL of a mixture containing 33% v/v of $[(\text{C}_6)_3\text{C}_{14}][\text{Br}]$ in methanol was rapidly expelled from a 100 μL syringe and a turbid dispersion was obtained. After 10 min, centrifugation at 4000 rpm during 20 min was performed. An average volume of 23.6 μL (± 0.2) of the IL phase, floating in the neck of the tube was immediately obtained (Fig. 1). Finally, using a syringe, 10 μL of this phase was taken, and directly injected



Figure 1. Photograph of the separated RTIL-water phases after centrifugation within the narrow-neck glass tube.

into the chromatographic column. All experiments were performed in triplicate.

3 Results and discussion

3.1 Optimization of the developed methodology

In the experimental procedure, a one-variable-at-a-time (OVAT) optimization scheme was used. In this experiments, five PAHs were selected as probe molecules: Nap (43 $\mu\text{g/L}$), Ant (41 $\mu\text{g/L}$), Pyr (57 $\mu\text{g/L}$), Cry (53 $\mu\text{g/L}$), and B[k]F (38 $\mu\text{g/L}$).

Some important parameters that would affect the extraction performance including the type and volume of extraction and disperser solvent, the extraction and centrifugation time, and salt addition (salting-out effect) were studied and optimized. A constant volume (5.00 mL) of the standard solution was used in all the optimization experiments. Thus, in Figs. 2–4 the extraction yields were directly expressed as “area ratios,” A_r , i.e. the area of the corresponding PAH in the IL phase after extraction divided by the area in the aqueous

phase before extraction. All assays were carried out in triplicate.

3.1.1 Selection of ionic liquid

When an IL is going to be used as extraction solvent in DLLME previous to an HPLC separation, several properties have to be fulfilled: (i) the ionic liquid must be immiscible (or have very low mutual solubility) in water; (ii) it should have strong extraction capability toward the compounds of interest, so that low amounts of the RTIL are needed and, thus, high enrichment factors can be achieved; (iii) it should have good chromatographic behavior avoiding baseline disturbance, ghost peaks, or UV-absorption; (iv) although a liquid–liquid microextraction technique requires very small amounts of the extraction solvent phase (around 20 to 100 μL), inexpensive RTILs are preferable, and (v) it should have low viscosity if direct injection into the HPLC column is desired to avoid decreasing the enrichment factor. Otherwise, the RTIL drop must be diluted in some fluid solvent (e.g. acetonitrile) to obtain reproducible injections. Using high viscous RTILs, the aqueous phase can be discarded by just inverting the test tube,

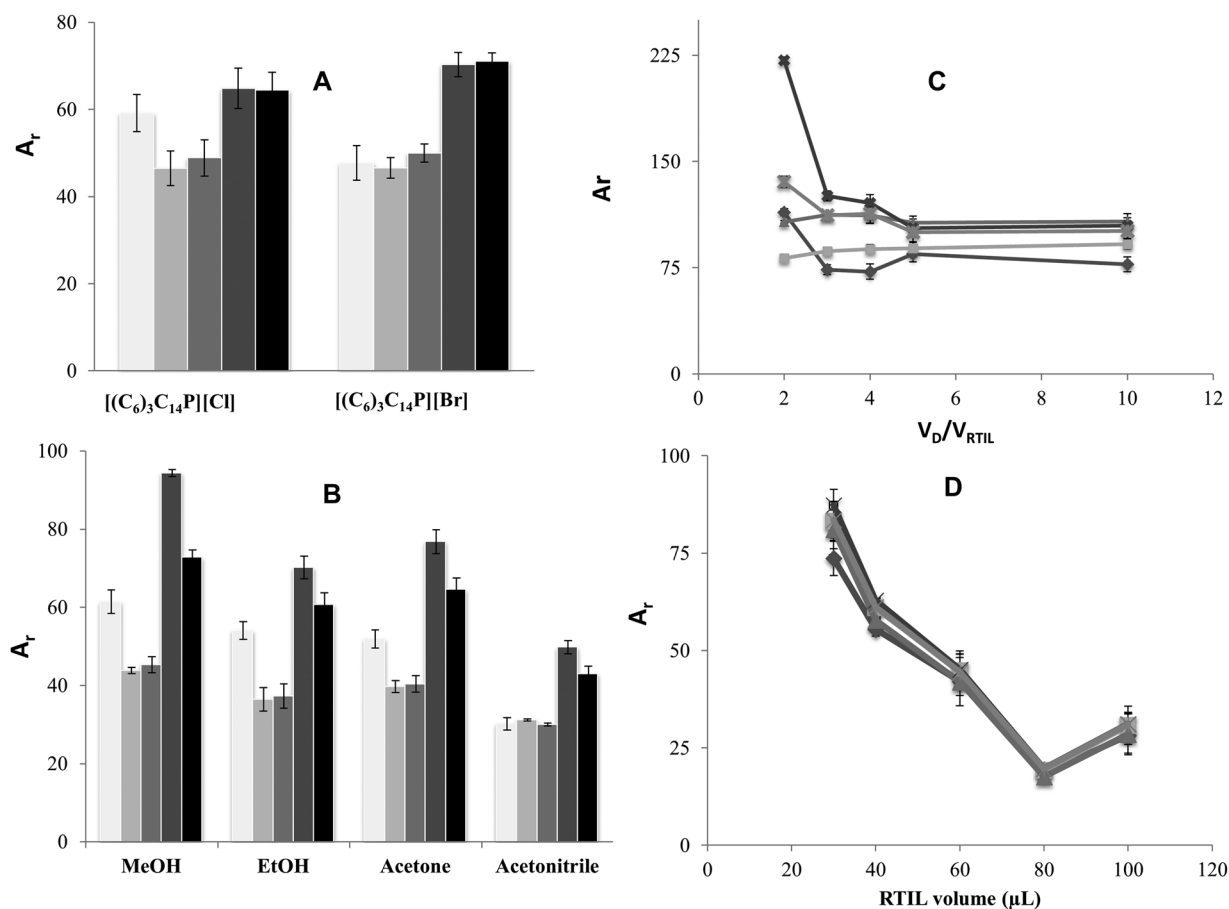


Figure 2. (A) Effect of the ionic liquid type and, (B) effect of the disperser, on the extraction yield, A_r . (■) Nap, (■) Ant, (■) Pyr, (■) Cry, (■) B[k]F; (C) Effect of the disperser volume, V_D , and (D), effect of the RTIL volume on extraction yield. V_{RTIL} is the fix volume of ionic liquid. (◆) Nap, (■) Ant, (▲) Pyr, (×) Cry, (※) B[k]F.

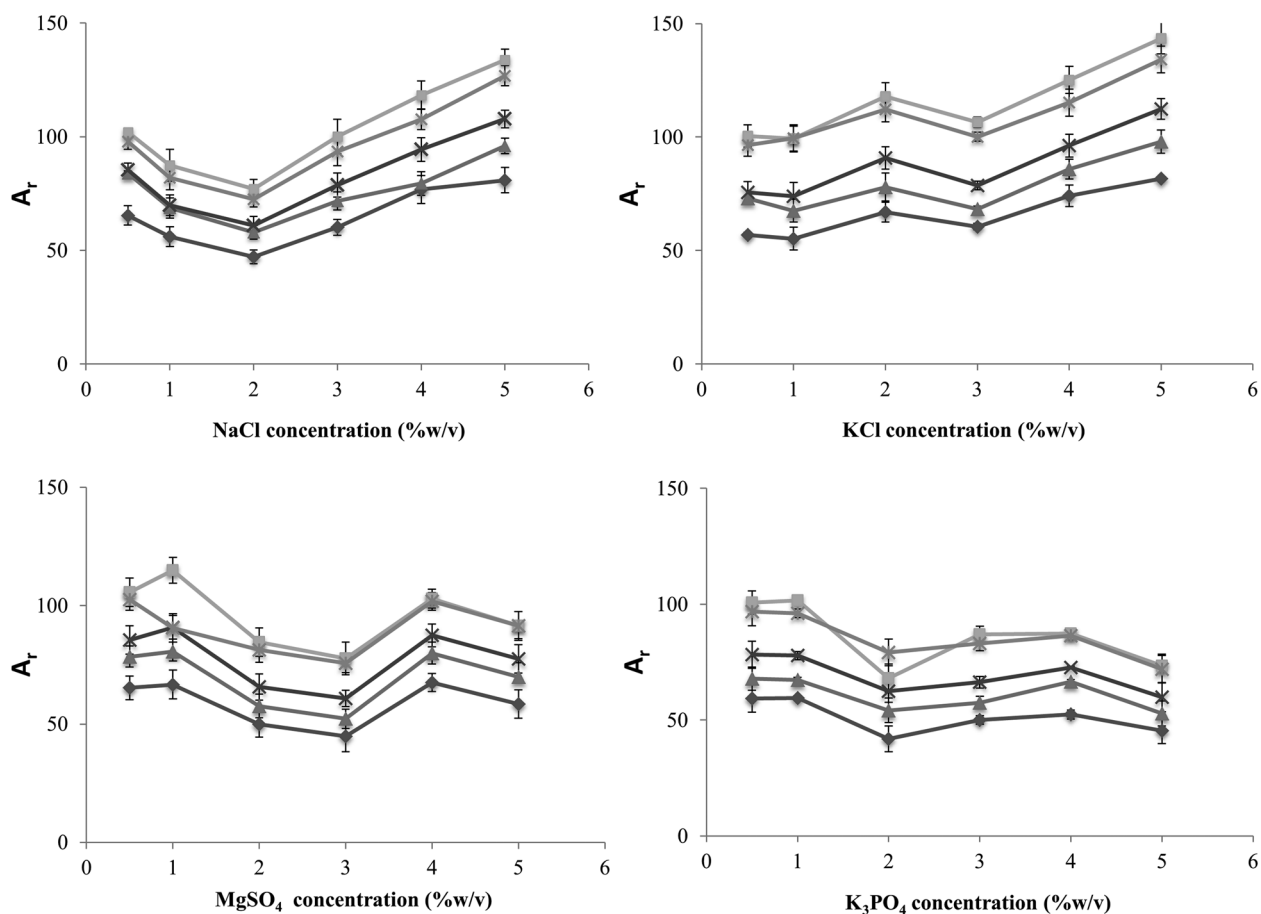


Figure 3. Effect of the type and concentration of different salts on extraction yield, A_r (♦) Nap, (■) Ant, (▲) Pyr, (×) Cry, (*) B[k]F.

since the ionic liquid remains usually stuck at the bottom of the tube. However, with RTILs lighter than water the drop extraction is carried out directly from the top of the tube.

In this work, three PB-RTILs lighter than water were assayed: $[(C_6)_3C_{14}P][Cl]$, $[(C_6)_3C_{14}P][Br]$, and $[(C_6)_3C_{14}P][N(CN)_2]$. These RTILs fulfill almost all the aforementioned requirements desirable for a DLLME methodology. However, although the three ILs were properly purified as described in [20], the chromatograms obtained with $[(C_6)_3C_{14}P][N(CN)_2]$ were not acceptable (ghost peaks and peak tailing prevented its use). In Fig. 2A, the extraction yields expressed as area ratios, A_r , for $[(C_6)_3C_{14}P][Cl]$ and $[(C_6)_3C_{14}P][Br]$ are shown. It is observed that results are very similar, although for the more hydrophobic analytes Cry and B[k]F the A_r values are higher with $[(C_6)_3C_{14}P][Br]$. Thus, this specific IL was selected for the extraction of PAHs from water samples.

In the subsequent experiments, the following amounts of the different experimental parameters were selected: 80 μ L of IL and two times this volume of methanol, used as disperser solvent. The chosen salt used in the aqueous phase was NaCl at 3% w/v. The values of extraction time and centrifugation time were 10 and 20 min, respectively. The different parameters (type and amount of disperser solvent, amount of RTIL,

type and amount of salt, extraction, and centrifugation times) were changed one at a time, while the other parameters were fixed.

3.1.2 Selection of the disperser solvent

As it was explained before, the disperser solvent must be miscible with both the RTIL and the aqueous sample. Acetone, ethanol, acetonitrile and methanol were selected and compared as disperser solvents. Different solutions containing 80 μ L of each solvent and 40 μ L of $[(C_6)_3C_{14}P][Br]$ were studied. The results shown in Fig. 2B indicate that the A_r values are higher when methanol was used. Consequently, this solvent was selected as the disperser solvent for the subsequent experiments.

3.1.3 Amount of disperser solvent

The solubility of the IL in the aqueous solution is affected by the volume of the disperser solvent and, thus, this parameter will affect the final volume of the extraction solvent phase. Extraction experiments were conducted to obtain the optimal volume of the disperser solvent; thus, different methanol to IL volume ratios (2, 3, 4, 5, and 10 times the IL volume, which

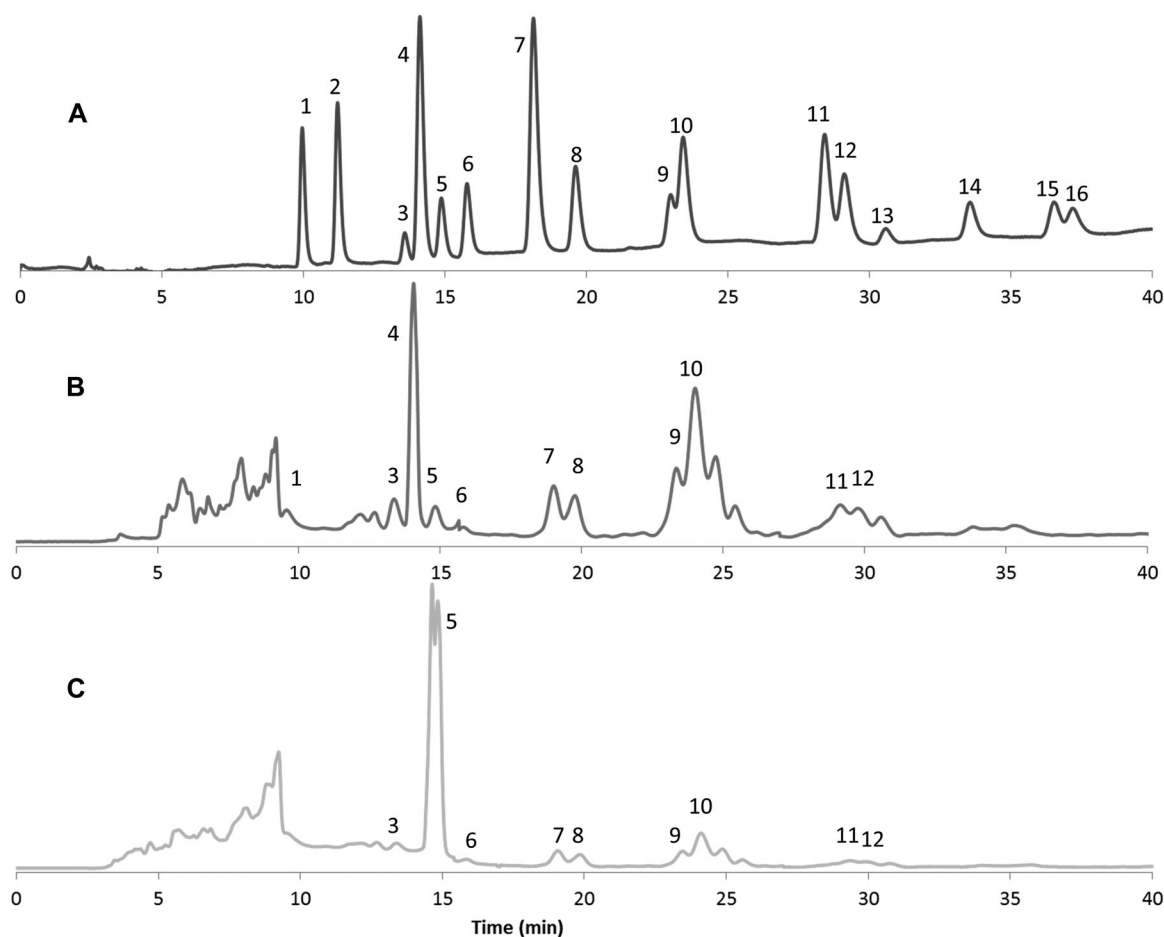


Figure 4. Chromatograms corresponding to: (A) standard mixture of the 16 PAHs (DAD signal to show the nonfluorescent Acp); (B) and (C) the two river water samples after the developed IL-DLLME methodology for “dock 1” and “dock 2,” respectively (FLD signals). 1: Nap, 2: Acp, 3: Flu, 4: Ace, 5: Phe, 6: Ant, 7: Flt, 8: Pyr, 9: Cry, 10: B[a]A, 11: B[b]F, 12: B[k]F, 13: B[a]P, 14: InP, 15: DBA, 16: BPe.

was 40 μL) were studied. Figure 2C shows that the extraction performance is better (or remains almost constant) for the minimum amount of methanol (two times the IL volume). As the amount of the disperser solvent increases, solubility of PAHs in the aqueous phase increases. Consequently, the optimum volume of methanol was two times the volume of IL.

3.1.4 Amount of ionic liquid

To determine the optimum amount of $[(\text{C}_6)_3\text{C}_{14}\text{P}][\text{Br}]$, different extraction experiments using different IL volumes (30, 40, 60, 80, and 100 μL) but maintaining the volume of the disperser solvent (methanol) at two times the IL volume were performed. It is clear from Fig. 2D that the A_r values decrease when the amount of IL increases. This is because all the PAH probes were completely extracted with the minimum amount of IL, and higher amounts only dilutes the analytes in this phase. Thus, since real aqueous samples contain PAHs in ppb or ppt levels, 30 μL is a sufficient amount of IL to extract all the compounds solubilized in 5 mL of the aqueous sample.

3.1.5 Salt effects

The use of salts in the aqueous phase to improve the extraction of organic compounds (“salting-out” effect) in LLE experiments is a common practice. However, when the extraction solvent is an IL, the addition of salts may have a counterproductive effect since the electrostatic interactions between the salt ions and the IL ions could enhance its solubility in the aqueous phase and, thus, decrease the extraction yield [28, 29].

The effect of salt addition on extraction yield was studied by using NaCl, KCl, MgSO_4 , and K_3PO_4 at concentrations between 0.5 and 5% w/v. The absence of salt in the aqueous phase avoids a clear formation of the RTIL/water interface. The results in Fig. 3 indicate that extraction performance depends on the salt (type and concentration) present in the aqueous phase. A_r values decrease as salt concentration increases for MgSO_4 , K_3PO_4 , and for the initial additions of NaCl. From this result would be inferred that IL solubility in the aqueous phase increases in presence of these salts, decreasing the effective volume of the extraction solvent phase and, thus, the extraction efficiency. On the contrary, when KCl

or larger amounts of NaCl are used, the A_r values increase due to the well-known salting-out effect. It is observed in Fig. 3 that the amount of extracted analytes is somewhat higher when KCl is used, as compared to NaCl, and the maximum is reached at 5.00% w/v. Thus, this concentration of KCl was used in further experiments.

3.1.6 Extraction and centrifugation times

The extraction time was defined as the interval between the instant when the IL was added to the sample solution until the time when the sample is centrifuged. The extraction times were varied from 5 to 30 min. The extraction efficiency increases up to 10.0 min and then decreases (Supporting Information Fig. S1). As a consequence, this value was selected as the optimum one.

The centrifugation time was defined as the time that the tube remains inside the centrifuge. Centrifugation helps to separate the IL phase from the aqueous phase particularly when viscous ILs sticks to the tube walls, as occurs when these RTILs are used. Centrifugation times were varied from 5.0 to 30.0 min at 4000 rpm, the maximum speed of the centrifuge. The highest A_r values were observed at 20 min for some compounds but at 30 min for some others, although the increments are not very significant decreases (Supporting Information Fig. S1). Since the total analysis time should be as short as possible, 20 min was selected as the optimum time.

3.2 Analytical performance of the proposed methodology

To perform an internal validation of the optimized methodology, the following figures of merit were evaluated: LODs, LOQ, linear range (LR), EF, recovery (%R), trueness (δ), and reproducibility (%RSD).

The LODs and LOQs were calculated at $S/N = 3.0$ and 10, respectively. The results gathered in Table 1 show that the LODs are in the range of 0.02–0.49 $\mu\text{g/L}$ for the fluorescent PAHs and it is 0.56 $\mu\text{g/L}$ for the nonfluorescent AcP, while the LOQs are in the range of 0.08–1.65 $\mu\text{g/L}$, while it is 1.85 $\mu\text{g/L}$ for AcP. Since no curvature of the calibration curve before of the fluorescence signal saturation was observed, the upper limit of the linear range (LR) was established at the corresponding limit concentrations.

The EF, %R and %RSD and δ values for each PAH were determined in water spiked (by quintuplicates) with the target species at three concentration levels: 0.025, 0.040, and 0.060 mg/L, within the linear zone of the calibration curve. The δ values were calculated according to Eq. (3).

$$\delta = 100 \frac{v - V}{V} \quad (3)$$

Here, V is the spiked concentration value and v is the average value of the determined concentrations.

Table 1. LODs, LOQs, and concentration of PAHs found in the water samples of docks "1" and "2"

Compound	LOD ($\mu\text{g/L}$)	LOQ ($\mu\text{g/L}$)	Concentration ($\mu\text{g/L}$)	
			"dock 1"	"dock 2"
Nap	0.28	0.95	1.6 \pm 1.1	ND*
AcP	0.56	1.85	ND	ND
Flu	0.06	0.20	<LOQ**	1.2 \pm 1.8
Ace	0.05	0.17	9.7 \pm 3.1	ND
Phe	0.24	0.82	<LOQ	ND
Ant	0.04	0.12	1.6 \pm 1.8	3.0 \pm 2.3
Flt	0.25	0.85	<LOQ	<LOQ
Pyr	0.05	0.18	0.48 \pm 0.8	0.4 \pm 1.9
Cry	0.06	0.19	0.6 \pm 1.1	2.0 \pm 1.2
B[a]A	0.03	0.08	3.0 \pm 1.5	3.2 \pm 2.1
B[b]F	0.08	0.25	<LOQ	2.6 \pm 1.4
B[k]F	0.03	0.09	1.6 \pm 1.2	2.3 \pm 1.5
B[a]P	0.02	0.08	ND	ND
DBA	0.23	0.77	ND	ND
InP	0.49	1.65	ND	ND
Bpe	0.12	0.39	ND	ND

*ND, detectable not quantifiable.

**Below the LOQ.

The EF values were obtained using the PAHs concentration in water, C_{aq} , and determining the final concentration extracted into the RTIL phase, C_{IL} , according to Eq. (4).

$$\text{EF} = \frac{C_{\text{IL}}}{C_{\text{aq}}} \quad (4)$$

R% values were determined by using Eq. (5) and calculating the phase ratio, ϕ (with V_{aq} as the initial volume of water sample and V_{IL} as the final volume of the RTIL phase) and using the corresponding previously obtained EF values.

$$R\% = 100 \frac{C_{\text{IL}} V_{\text{IL}}}{C_{\text{aq}} V_{\text{aq}}} = 100 \cdot \text{EF} \cdot \phi \quad (5)$$

The obtained EF, R%, the intraday reproducibility values (%RSD) and trueness (δ) are gathered in Table 2. EF values between 109 and 228, recovery values between 60 and 108%, %RSD between 3 and 12% and trueness (δ) values between 0.4 and 9.9% were obtained, depending on the analyte and spiking level. The presence of acetonitrile in the standard solutions, necessary to solubilize the PAHs and, also to avoid adsorption of the molecules on the glass surface, decreased the obtained R% values. However, if lower amounts of acetonitrile in the aqueous sample are used, the obtained volume of the RTIL phase obtained at the end of the extraction is very small, which makes the collection of the RTIL drop very difficult.

From the obtained figures of merit, it can be concluded that the developed IL-DLLME-HPLC-FLD-DAD methodology allows obtaining very low LODs and LOQs, high EFs and R% values and very good reproducibility and accuracy.

Table 2. Enrichment factors, EF, recovery, R%, reproducibility, RSD%, and trueness, δ , for the developed IL-DLLME technique

Analyte	Spiked amount (mg/L)											
	0.025				0.040				0.060			
	EF	R%	RSD%	δ	EF	R%	RSD%	δ	EF	R%	RSD%	δ
Nap	128	64	6.2	1.1	109	62	4.6	4.7	116	63	6.2	6.8
Acp	137	65	3.4	8.6	130	61.2	10.2	4.7	125	62	8.8	4.3
Flu	126	60	5.5	6.7	139	66	7.0	8.9	126	64	11.9	1.9
Ace	187	88	8.2	2.6	156	74	6.7	8.4	119	71	10.2	8.1
Phe	130	80.8	4.1	2.2	188	89	6.6	9.8	153	72	10.8	9.4
Ant	133	63	6.2	4.5	145	68	7.3	8.0	138	65	11.4	7.2
Flt	109	61	5.6	3.3	120	64	6.9	6.3	118	65	10.5	6.3
Pyr	137	65	5.6	2.9	145	68	7.3	6.2	138	65	10.9	3.5
Cry	124	62	10.0	6.4	123	62	7.4	6.6	134	65	7.6	9.4
B[a]A	152	76	9.5	5.5	164	82	8.1	9.9	134	67	7.6	5.2
B[b]F	139	66	7.3	8.2	154	73	6.7	9.9	138	65	11.2	7.4
B[k]F	144	68	8.3	4.9	138	65	7.9	8.2	143	67	11.5	4.8
B[a]P	146	69	7.7	5.6	155	73	7.5	7.9	140	66	11.9	4.8
DBA	221	105	7.5	9.8	192	91	5.5	4.6	196	92	10.1	3.4
InP	228	108	8.1	2.6	226	107	7.2	5.7	187	95	11.2	0.6
Bpe	148	70	7.6	0.4	137	64	6.9	7.7	150	99	11.5	0.8

3.3 Determination of polycyclic aromatic hydrocarbons in river water samples

The optimized method was applied to the determination of PAHs in river water samples collected from the two docks located on the east and west sides (labeled as “dock 1” and “dock 2,” respectively) of the oil refinery located in Berisso City (Buenos Aires, Argentina). Both docks are next to the Rio de La Plata river. To avoid matrix interferences usually present in such complex samples, the SAM was applied. For this purpose, calibration curves were constructed with seven to nine concentration levels depending on the PAH probe. Each concentration level was prepared in triplicate and then, extraction was carried out using the optimized IL-DLLME methodology. Finally, the RTIL drop was easily collected from the neck of the glass tube and directly injected into the RPLC column. Linear regressions of peak areas versus spiked concentrations for each PAH were made. Calibration parameters for water samples of “dock 1” and “dock 2,” together with the calibration ranges (or spiking intervals) used for each compound, are shown in Supporting Information Tables S1 and S2. The calibration plots were linear within those calibration ranges.

Figure 4 depicts the chromatograms (FLD signals) for the nonspiked water samples of both docks treated according to the optimized IL-DLLME methodology together with a chromatogram (DAD signal to show the nonfluorescent Acp) for a standard solution containing the 16 PAHs. From Table 1 it can be observed that 11 PAHs could be identified in the “Dock 1” sample and eight in the “Dock 2” sample, although some of them are present below the obtained LOQ. Neither Acp nor B[a]P, one of the most carcinogenic PAHs, were detected in any sample.

3.4 Comparison of the proposed methodology with similar studies from the literature

The developed analytical methodology was compared with other DLLME methods recently published in the literature for the determination of PAHs in water samples using HPLC.

Fatemi et al. [14], have used an “alcohol-assisted dispersive liquid–liquid microextraction” (AA-DLLME) method to determine six PAHs in water samples. The extraction solvents were lighter than water alcohols and the dispersive solvents were methanol, ethanol or 1-propanol. Enrichment factors of PAHs were in the range of 310–325 with limits of detection of 0.002–0.8 $\mu\text{g/L}$. The intra- and interday RSD were in the range of 1.7–7.0 and 5.6–7.3, respectively.

Fernández et al. [29] have determined 15 PAHs in tap water, rain waters, and river surface waters samples by an automated DLLME-FLD–HPLC procedure using a system of multisyringe FIA. They have used trichloroethylene as the extraction solvent. The enrichment factors of PAHs were in the range of 86–95 with LODs of 0.02–0.6 $\mu\text{g L}^{-1}$. The RSD for intra- and interday of extraction of PAHs were in the range of 1.6–4.7 and 2.1–5.3, respectively.

In both of the previous works, organic solvents instead of green solvents such as RTILs have been used. Cela et al. [30] as well as Zhou and Gao [31] have used a heavier-than-water NB-RTIL (1-octyl-3-methylimidazolium hexafluorophosphate) for the DLLME. In the first-mentioned work, the sample containing the RTIL has been cooled in an ice bath after the dispersion was generated, which took several minutes. Each PAH was excited and detected at different wavelengths to specifically increase sensitivity.

In the Zhou and Gao’s work [31], a temperature-controlled DLLME, in which the sample containing the RTIL

was heated up to 90°C and then cooled into an ice bath (total extraction time of 30 min) was used. On the other hand, Yao and Anderson [32] have used an in situ formation (“in situ IL-DLLME”) of the ionic liquid 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide. The imidazolium salt was supercooled previously to the metathesis reaction with the bis[(trifluoromethyl)sulfonyl]imide salt. Although the amounts of ionic liquid used in each experiment are small, it is important to remark that the NB-RTILs used in all the aforementioned works are much more expensive than the PB-RTIL used in the present study.

With respect to the LODs, the reported values of [30] (fluorescence detection), [31] and [32] (both UV detection) were 0.03–2 ng/L, 0.0005–0.88 µg/L and 0.02–0.3 µg/L, respectively, in comparison with 0.02 to 0.49 µg/L (FLD detection) obtained in the present work. Similar LODs are obtained if the RTIL phase is heated and then cooled in an ice bath after the DLLME method [31] or if it is obtained by using an in situ formation processes [32]. On the other hand, much lower LODs are obtained by cooling the RTIL phase combined with fluorescence detection [30]. However, addition of heating or cooling steps to the classical DLLME method increases the total analysis time. In the present work, no additional heating or cooling steps were added. Unfortunately, although fluorescence detection was used, higher LODs were obtained as compared with those in [30]. The main reason for this result could be attributed mainly to the used ionic liquid type (imidazolium- versus phosphonium-based RTIL). Thus, although strong dispersive interactions between PAHs and the long alkyl chains of the PB-RTILs could be established, additional π – π interactions with imidazolium-based RTILs could increase even more the extraction yields, obtaining in this case lower LODs.

In summary, it can be concluded that the figures of merit such as enrichment factors, LODs, and reproducibility obtained in the present work using a green and very cheap RTIL are similar to those obtained with other reported procedures using toxic organic solvents or expensive NB-RTILs. The only exception is the much better LOD obtained in [30] with the ionic liquid 1-octyl-3-methylimidazolium hexafluorophosphate, together with an additional cooling step was used. However, this type of heavier-than-water NB-RTIL makes the collection of the extracting drop more difficult.

4 Concluding remarks

A dispersive liquid–liquid microextraction technique coupled to LC with fluorescence/diode array detection to determine the 16 US-EPA priority polycyclic aromatic hydrocarbons in water samples has been developed. For the first time, a phosphonium-based room temperature ionic liquid, namely the [(C₆)₃C₁₄P][Br], was used as extraction solvent. Very small amounts of this green solvent, which is more economic than the typical imidazolium-based ILs commonly used in IL-DLLME, were required. The used ionic liquid is lighter than water, which makes the procedure easier to manipulate as

compared with heavier-than-water extraction solvents. By using an OVAT procedure, the experimental conditions for the extraction of these analytes were investigated and optimized. The developed methodology has good reproducibility and accuracy, very low detection and quantification limits, and high recovery values. In summary, the proposed dispersive liquid–liquid microextraction method is more convenient in terms of costs, speed, and simplicity than other similar procedures reported in the literature to analyze polycyclic aromatic hydrocarbons in water samples. Those procedures usually involve the use of toxic organic solvents (usually in large amounts) or more expensive nitrogen-based ionic liquids for extraction, additional evaporation of the organic solvent or additional steps such as heating or cooling the extraction solvents, among other factors, before the quantification step.

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