Magnetic ionic liquids as non-conventional extraction solvents for the determination of polycyclic aromatic hydrocarbons

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HIGHLIGHTS

- MILs of hydrophobic nature and low water solubility have been used in microextraction.
- MILs are used for 1st time in an analytical method for hydrophobic analytes (PAHs).
- The magnet-based MILs in microextraction-HPLC-FD method was optimized and validated.
- ~20 μL of MILs were adequate for PAHs in waters & tea infusions with LODs ~ low ng L^{-1}.

GRAPHICAL ABSTRACT
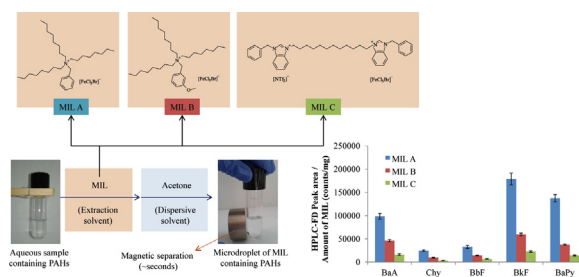
ABSTRACT

This work describes the applicability of magnetic ionic liquids (MILs) in the analytical determination of a group of heavy polycyclic aromatic hydrocarbons. Three different MILs, namely, benzyltrioctylammonium bromotrichloroferrate (III) (MIL A), methoxybenzyltrioctylammonium bromotrichloroferrate (III) (MIL B), and 1,12-di(3-benzylbenzimidazolium) dodecane bis[(trifluoromethyl) sulfonyl)]imide bromotrichloroferrate (III) (MIL C), were designed to exhibit hydrophobic properties, and their performance examined in a microextraction method for hydrophobic analytes. The magnet-assisted approach with these MILs was performed in combination with high performance liquid chromatography and fluorescence detection. The study of the extraction performance showed that MIL A was the most suitable solvent for the extraction of polycyclic aromatic hydrocarbons and under optimum conditions the fast extraction step required ~20 μL of MIL A for 10 mL of aqueous sample, 24 mmol L^{-1} NaOH, high ionic strength content of NaCl (25% (w/v)), 500 μL of acetone as dispersive solvent, and 5 min of vortex. The desorption step required the aid of an external magnetic field with a strong NdFeB magnet (the separation requires few seconds), two back-extraction steps for polycyclic aromatic hydrocarbons retained in the MIL droplet with n-hexane, evaporation and reconstitution with acetonitrile. The overall method presented limits of detection down to 5 ng L^{-1}, relative recoveries ranging from 91.5 to 119%, and inter-day reproducibility values (expressed as relative standard derivation) lower than 16.4% for a spiked

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level of 0.4 µg L^{-1} (n = 9). The method was also applied for the analysis of real samples, including tap water, wastewater, and tea infusion.

1. Introduction

Trends in sample preparation are focused on the development of non-conventional solvents as well as novel solid materials in extraction and preconcentration schemes to improve limitations and toxicity issues of current organic solvents and common sorbents [1–4]. Within non-conventional solvents, ionic liquids (ILs) have been widely studied in recent years due to their interesting structures and unusual properties [5]. These non-molecular solvents exhibit melting points below 100 °C, low to negligible vapor pressure at room temperature, high chemical stability as well as wide electrochemical windows. They have also been pointed out as green solvents because select classes of ILs do not generate volatile organic compounds and are hydrolytically stable [6,7]. Their structures can be easily modified by changing the nature of the cation/s and/or anion/s of the IL, or by incorporating different functional groups to the cationic/anionic moieties [8,9]. This simple structural versatility is accompanied by important modification of their properties, from low to high viscosity, moderate to high conductivity, and miscibility in water or in organic solvents, among others [10].

Several derivatives of ILs have been also designed with the purpose of combining some of their unique properties with those derived from other types of materials. Thereby, polymeric ionic liquids (PILs) combine the inherent properties of polymers as well as IL character [11,12], while IL-based surfactants offer the possibility to form a micellar media when dissolved in an aqueous media at a certain concentration [13,14]. Following this idea, magnetic ionic liquids (MILs) exhibit paramagnetic behavior under the application of an external magnetic field [15], and can be designed to incorporate paramagnetic metal anions or metal complexes. Tetrachloroferrate (III) ([FeCl$_4$]$^-$) and tetrabromoferrate (III) ([FeBr$_4$]$^-$)-based MILs are among those that are more commonly studied [15]. In recent years, other MILs containing anions based on Fe (III) [16,17], Co (II) [17], Mn (II) [17,18] and lanthanide [17,19] complexes have also been reported.

Magnet-based separations constitute a quite interesting and evolving advancement in sample preparation [20]. These methods utilize an extractant material with magnetic properties. Analytes are enriched in the material, which can be further separated from the remaining components of the sample with the aid of a strong magnet [21,22]. Analytes are finally eluted from the material and subjected to quantification. Thus, the overall method is quite simple because it does not require centrifugation or filtration to separate the magnetic material from the sample once extraction has been accomplished. ILs [23,24] and IL-based surfactants [25–27] have been described as extractants presenting a magnetic core (normally magnetite: α-Fe$_3$O$_4$) in a number of magnet-based extraction approaches. Bare Fe$_3$O$_4$ MNPs tend to aggregate, they easily oxidize in air, or they experience biodegradation, and thus coatings are required in an attempt to increase their stability [20]. Applications using Fe$_3$O$_4$@ILs (or @IL-based surfactants) require more than one synthetic step (that of the IL and that of the Fe$_3$O$_4$ magnetic nanoparticles, MNPs); and in some cases the whole extraction efficiency of the hybrid material is lower than that of the neat IL (or IL-based surfactant). In some approaches, dispersive solvents or agitation may be needed to disperse the extraction phase and improve the mass transfer of the analytes to the micro-amounts of material [20].

MILs have been utilized as extraction solvents for the preconcentration of DNA from a cell lysate [28]. They have also been shown to be highly compatible with the polymerase chain reaction thereby permitting high throughput DNA amplification from template DNA enriched in the MIL microdroplet [29]. MILs have been also used for the removal of certain compounds [17,30–34]. In these cases, there was no elution of the trapped analytes from the MIL prior to downstream analysis. In fact, there are few studies in the literature that exploit the use of neat MILs (paramagnetic MILs that do not combine ILs with α-Fe$_3$O$_4$) in analytical microextraction approaches involving further determination of analytes [35–37]. Two of these studies required the addition of carbonyl iron powder to the MIL because the paramagnetism of the tested MILs (1-hexyl-3-methylimidazolium [FeCl$_4$] [35] and 1-butyl-3-methylimidazolium-[FeCl$_4$] [36]) was not sufficient to enable the magnetic separation under the application of an external magnetic field. The remaining report was applied to the determination of polar analytes (phenols) using methyltrioctylammonium [FeCl$_4$] [37].

A main challenge encountered when working with MILs in microextraction approaches is the need to minimize water solubility of the MIL. This can largely be achieved by incorporating nonpolar moieties into the cation and/or using non-coordinating anions. The main aim of this work is the development of a MIL-based analytical microextraction approach devoted to the determination of hydrophobic analytes because so far the unique analytical determination study for small molecules with neat MILs has been devoted to the determination of polar compounds (phenols) [37]; and the majority of studies with MILs do not imply the further analytical determination of target compounds [17,30–34]. A group of five heavy polycyclic aromatic hydrocarbons (PAHs) was selected as test analytes, as model hydrophobic compounds widely determined using ILs or any other novel material [38–41]. The method was combined with high performance liquid chromatography and fluorescence detection (HPLC-FD) for the determination of these compounds within various aqueous samples.

2. Experimental

2.1. Chemicals, reagents, materials, and samples

Benzo(*a*)anthracene (BaA, ≥99.0%), chrysene (Chy, ≥99.0%), and benzo(*a*)pyrene (BaPy, ≥99.0%) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Benzo(*b*)fluoranthene (BbF, ≥99.0%) was purchased from Supelco (Bellefonte, PA, USA) whereas benzo(*k*)fluoranthene (BkF, ≥99.0%) was purchased from Fluka (St Louis, MO, USA). Ultrapure water (18.2 MΩ cm) was obtained from a Milli−Q water purification system (Millipore, Watford, UK).

All PAHs were individually dissolved in acetonitrile (≥99.9%), supplied by VWR International Eurolab S.L. (Barcelona, Spain) at a concentration of 1200 mg L^{-1} for BaA, 760 mg L^{-1} for Chy, and 1000 mg L^{-1} for the remaining PAHs. Three intermediate solutions of all PAHs at 1 mg L^{-1}, 0.5 mg L^{-1}, and 0.25 mg L^{-1}, in acetonitrile, were prepared by dilution of the individual standards. All experi-

ments were carried out by dilution of the intermediate solutions, or by spiking water with these intermediate solutions.

Calibration curves of the overall MIL-based microextraction-HPLC-FD method were carried out preparing standards of PAHs in ultrapure water, at concentrations between 0.05 and 5.0 $\mu g\ L^{-1}$.

The extraction method requires the use of NaOH ($\geq 98\%$), NaCl ($\geq 99.5\%$), acetone ($\geq 99.9\%$), n-hexane ($>97\%$), and acetonitrile ($\geq 99\%$), which were supplied by Sigma-Aldrich. Other reagents utilized during optimization of the method include chloroform ($\geq 99.5\%$), and dichloromethane (99.8%), obtained from Sigma-Aldrich, and the IL-based surfactants 1-hexadecyl-3-methylimidazolium bromide ([C_{16}MIm] [Br], with a critical micelle concentration (CMC) value of 0.7 mmol L^{-1} in pure water) and 1,3-didodecylimidazolium bromide ([$C_{12}C_{12}$Im] [Br], CMC = 0.1 mmol L^{-1} in pure water). Both ILs were synthesized and fully characterized as previously reported [42].

Three different MILs were utilized in the analytical micro-extraction approach. These include two monocationic MILs, benzyltrioctylammonium bromotrichloroferrate (III) ([$N_{8,8,8,B}$] [FeCl$_3$Br] denoted as MIL A) and methoxybenzyltrioctylammonium bromotrichloroferrate (III) ([$N_{8,8,8,MOB}$] [FeCl$_3$Br] denoted as MIL B), and a dicationic MIL, 1,12-di(3-benzylbenzimidazolium)dodecane bis[(trifluoromethyl)sulfonyl)]imide bromotrichloroferrate (III) ([(BBnIm)$_2$C$_{12}$] [NTf$_2$][FeCl$_3$Br] denoted as MIL C). These MILs were synthesized and characterized according to a recent report by Nacham et al. [16]. Table 1 shows their chemical structures together with some of their properties. Figure ESM-1 of the Electronic Supplementary Material (ESM) includes their ^{1}H NMR and ^{13}C NMR spectra.

0.22 μm polyvinylidene difluoride (PVDF) membrane filters (Whatman, Freiburg, Germany) were used before HPLC injection, while all aqueous samples were filtered through 0.45 μm PVDF Durapore membrane filters (Millipore, Darmstadt, Germany) before analysis.

Aqueous samples include tap water taken in the laboratory, wastewater collected from a wastewater treatment plant (WWTP) located in Tenerife (Spain), and a tea infusion of wild fruits tea bag bought in a local supermarket. Wastewater was subjected to 5 min of sonication and stored in an amber glass bottle at 4 °C until analysis. Tea infusion was prepared to mimic common preparation

of tea [43]: a tea bag was placed in a glass vessel containing 200 mL of previously boiled mineral water (acquired in a local supermarket) and kept at room temperature for 10 min.

2.2. Instrumentation

All MIL-based microextraction experiments were carried out in Pyrex® glass tubes of 25 mL (Staffordshire, United Kingdom). A cylindrical NdFeB magnet (5.08 cm diameter × 5.08 cm thickness; B = 0.9 T) was purchased from K&J Magnetics (Pipersville, PA, USA). Vortex was applied with a Reax-Control Heidolph GMBH (Schwabach, Germany). An ultrasonic bath KM (Shenzhen Codyson Electrical Co., Ltd., Shenzhen, China) was also employed. The evaporation step was performed in a Visiprep TM vacuum system of Supelco.

Separation and detection of the PAHs were carried out in a HPLC system equipped with a solvent delivery Varian ProStar 230 SDM module (Palo Alto, CA, USA) and a Rheodyne 7725i injection valve obtained from Supelco (Bellefonte, PA, USA) with a 5 μL loop. A fluorescence detection system (FD) Waters 474 (Waters, Milford, MA, USA) was used and connected through a Varian Star 800 module interface to the HPLC. The separation was achieved using a SUPELCOSIL™ LC-PAH column (25 cm × 2.1 mm I.D. × 5 μm particle size) supplied by Supelco and protected by a Pelliguard LC-18 guard column purchased from Supelco. The temperature of the column was controlled in the thermostat of a Varian ProStar 410 auto-sampler. LC workstation software (version 6.41) from Varian was used for data acquisition.
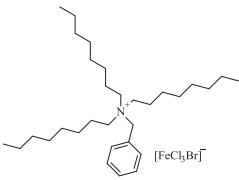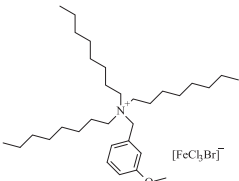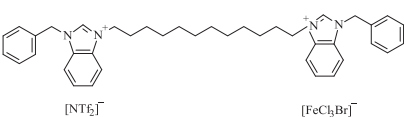
2.3. Procedures

2.3.1. HPLC-FD

The HPLC separation was achieved using a binary mobile phase of acetonitrile:water at a constant flow rate of 0.5 mL min^{-1}, and the following linear elution gradient: 3 min with 75% of acetonitrile (v/v), then up to 100% of acetonitrile (v/v) in 17 min, and finally kept for 5 min. The analytical column was maintained at a constant temperature of 30 °C.

Fluorescence detection required the use of excitation and emission splits of 18 nm and the following wavelength program:

Table 1
Chemical structures and properties of the three MILs studied [16].

MIL	Chemical formula	MW (g mol^{-1})	Thermal stability (° C)	μ_{eff}^a (μ_B)	Structure
MIL A	[$N_{8,8,8,B}$] [FeCl$_3$Br]	689.9	258	5.26	
MIL B	[$N_{8,8,8,MOB}$] [FeCl$_3$Br]	716.9	203	5.60	
MIL C	[(BBnIm)$_2$C$_{12}$] [NTf$_2$][FeCl$_3$Br]	1107.1	314	5.45	

[a] Effective magnetic moment, measured at 295 K.

initially, 248/370 (nm) (excitation wavelength/emission wavelength: $\lambda_{ex}/\lambda_{em}$) with a gain of 10, a change to 273/384 (nm) and to a gain of 100 at a chromatographic time of 6.5 min, following by a change to 254/451 (nm) at 9.9 min, then change to 288/406 (nm) at 12.3 min, and finally changed to 248/370 (nm) and to a gain of 10 at 16.3 min. Figure ESM-2(A) of the ESM shows a representative chromatogram of the PAHs (obtained without any preconcentration step, only HPLC-FD). PAHs eluted at the retention times reported in Table ESM-1 of the ESM, with RSD values lower than 1.2%.

2.3.2. MIL-based microextraction method

The MIL-based microextraction method under optimum conditions required 10 mL of the aqueous sample involving ultrapure water, tap water, wastewater, or tea infusion (with or without spiking PAHs, depending on the experiment). The NaCl content was adjusted to 25% (w/v), and 0.5 mL of NaOH 0.5 mol L^{-1} was added (final content in the extraction tube: 24 mmol L^{-1}). The optimum extraction step required ~20 μL of MIL A (as extraction solvent) and 500 μL of acetone (as dispersive solvent), which were sequentially added to the tube containing the aqueous sample, followed by 5 min of vortex to ensure proper dispersion of the MIL. Then, the cylindrical magnet was placed at one side of the extraction tube. The MIL was immediately attracted to the magnet and retained on the wall of the tube, allowing easy removal of the supernatant using a pipette. Once PAHs were enriched in the MIL droplet already separated from the remaining aqueous sample, the optimum back-extraction required 500 μL of n-hexane, 1 min of vortex, and separation of the eluent with a pipette and the aid of the magnet. This procedure was repeated twice. The total volume of eluent (~1 mL) was evaporated to dryness using a stream of air (~10 min). The residue was diluted up to 300 μL with acetonitrile, and then filtered through 0.2 μm PVDF membrane filters. Finally, a volume of 5 μL was injected in the HPLC-FD system. The overall procedure under optimum condition is summarized in Fig. 1.

All variables were properly studied in order to achieve the optimum conditions. Each optimization experiment was repeated in triplicate (n = 3).

3. Results and discussion

3.1. Use of MILs in the microextraction method for determining PAHs

3.1.1. Previous considerations of the MIL-based microextraction method

A group of three different MILs, described in Section 2.1, were examined as candidates for the magnet-based microextraction of PAHs. As previously discussed, the intrinsic hydrophobicity and high viscosity of the MILs studied exert an important influence in the method development.

Given the hydrophobicity of the MILs, it was challenging to homogeneously disperse the neat MIL into the aqueous solution by using stirring media (e.g., sonication, vortex, or manual stirring) or by incorporating any dispersive solvent. Furthermore, once dispersed, MILs easily stick on the walls of the tube making the recovery and back-extraction complicated. It was observed that the addition of sodium hydroxide before introduction of the MIL was beneficial for MIL dispersion (helping in the formation of multiple microdroplets) and subsequent recovery. A concentrated NaOH solution (spiked volume: 0.5 mL) was added to the aqueous sample. Two concentration levels of NaOH, 4.8 mmol L^{-1} and 24 mmol L^{-1}, were tested in the final aqueous solution. Figure ESM-3 of the ESM shows that similar extraction efficiencies (expressed as peak area of the PAHs) were obtained with both NaOH concentration values, even slightly higher for the lower NaOH content for BaA and BkF. However, the reproducibility was worse at lower values, and therefore the highest concentration of NaOH (24 mmol L^{-1}) was selected as the optimum condition and was used in the rest of the study.
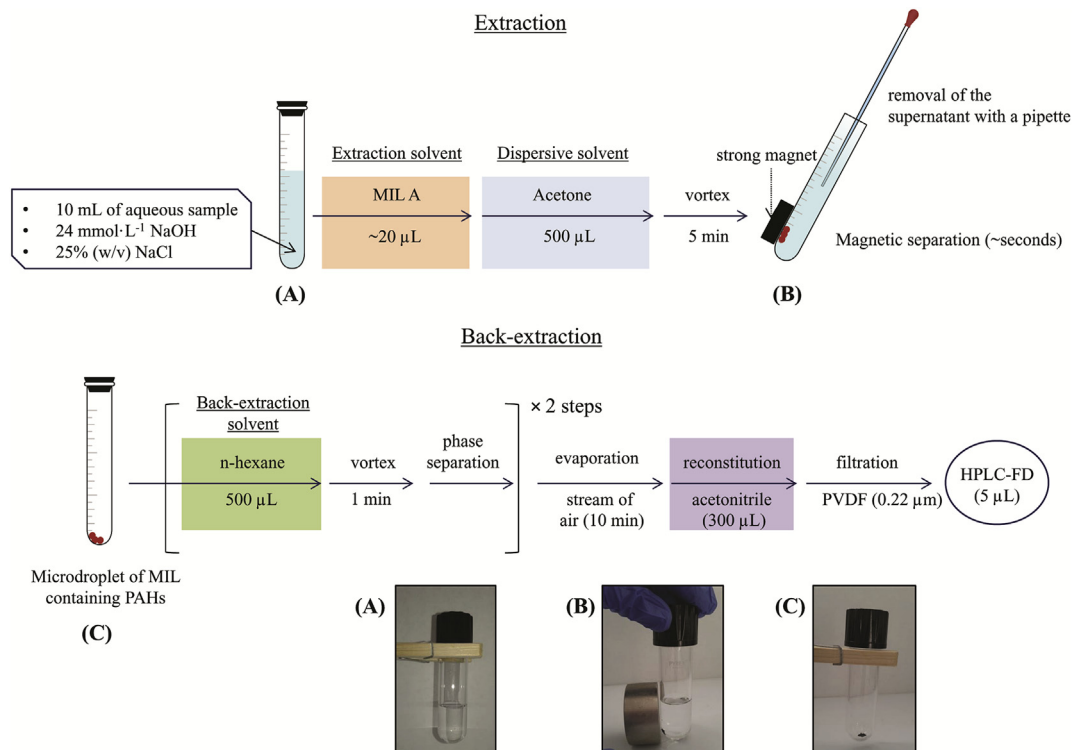

Fig. 1. Scheme of the overall MIL-based microextraction method-HPLC-FD performed under optimum conditions.

Finally, it is important to evaluate the influence of the ionic strength in the MIL-based microextraction approach. The use of high ionic strength is normally beneficial for exploiting the salting-out effect in the majority of liquid-phase microextraction methods [44]. In magnet-based extraction approaches using bare Fe_3O_4 as core of the magnetic material, the use of high ionic strength is normally not convenient when using ILs [45,46] or IL-based surfactants [25,27]. The use of high ionic strength can be accomplished only when Fe_3O_4 coated with silica ($Fe_3O_4@SiO_2$) is used, but requires an additional step in the preparation of the material [24,47]. Figure ESM-4 of the ESM shows the influence of the ionic strength (expressed as NaCl content), evaluated in the range of 0–25% (w/v) of NaCl. Clearly, an increase of the NaCl content increases the extraction efficiency for all PAHs. It is important to note that even when no NaCl is added, the net ionic strength of the sample is not zero due to the contribution of the NaOH (24 mmol L^{-1}). From these results, 25% (w/v) of NaCl was selected as optimum value for the further experiments. The ability to utilize high ionic strength in MIL-based extraction is advantageous compared to other magnet-based extraction methods in which the addition of salts has a negative effect in the extraction efficiency [25,27,45,46]. The MIL-based microextraction method is therefore clearly valid for aqueous samples with a high salt content.

3.1.2. Screening of MILs in the microextraction method for determining PAHs

The three hydrophobic MILs were tested for the determination of PAHs, using the fixed conditions described above in Section 3.2.1. The normalized peak area for each PAH was introduced as a tool to compensate for possible differences in weighing the MIL (as described in Section 3.2.1) when different MILs are compared. Thus, the normalized peak area is defined as the ratio between the chromatographic peak area and the amount of MIL weighed. The obtained results are shown in Fig. 2. It can be observed that better results are achieved with MIL A for all PAHs tested. MIL B showed adequate extraction efficiency (but lower than that obtained with MIL A), whereas MIL C poorly extracted the PAHs. It appears that MILs belonging to the ammonium family may be more suitable than those of the benzimidazolium family for the determination of PAHs. These results can be linked to the different viscosities of the MILs in which ammonium-based MILs (A and B) are less viscous
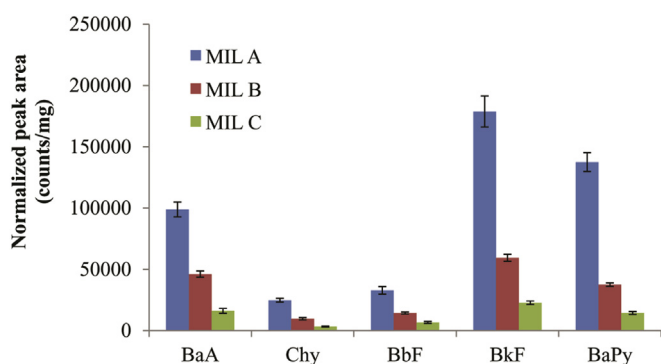

Fig. 2. Comparison of the extraction efficiency of the tested MILs in the microextraction-HPLC-FD method for PAHs determination. The normalized peak area is defined as the chromatographic peak area of each PAH divided by the amount of MIL weighed in each experiment. Fixed experimental conditions (n = 3): 10 mL of ultrapure water containing 5 µg L^{-1} of PAHs, spiked with 0.5 mL of NaOH solution of 0.5 mol L^{-1}; 25% (w/v) NaCl; 20 µL of MIL A, MIL B or MIL C (depending on the experiment); 500 µL of acetone as dispersive solvent; 5 min of vortex; magnetic separation (few seconds); two back-extraction steps using 500 µL of n-hexane and 1 min of vortex; evaporation; reconstitution in 500 µL of acetonitrile; filtration; and HPLC injection.

than MIL C and, consequently, they are more easily dispersed in the bulk of the sample, thus favoring the mass transfer of PAHs to the MIL microdroplets. Interestingly, MIL C was designed to possess higher hydrophobicity (which *a priori* could be beneficial for PAHs) but its higher viscosity hindered its performance within the magnet-based microextraction approach.

3.2. Optimization of the MIL-based microextraction-HPLC-FD method

The remaining parameters that have an influence in the MIL-based microextraction-HPLC-FD procedure were optimized using a simple factor by factor approach, but other mathematical models can be used in more complex optimizations [48]. The procedure was optimized with MIL A, which provided the best extraction efficiencies. The optimization study was carried out using a spiked level of 5 µg L^{-1} for the PAHs in ultrapure water, with the remaining conditions presented in Section 3.2 (NaOH 24 mmol L^{-1}, 25% (w/v) of NaCl, and 20 µL of MIL A).

The hydrophobic MILs used in this work possess high viscosity so their direct injection into HPLC is not recommended to avoid issues with high back pressure. Therefore, the MIL-based microextraction method was accomplished by designing a back-extraction step followed with reconstitution with acetonitrile. Regarding the back-extraction step, several parameters were fixed, including the volume of back-extraction solvent (500 µL, to ensure an adequate desorption volume to make contact with 20 µL of the MIL), together with the aid of vortex (1 min) to aid in the process. Once back-extracted, the time required to evaporate the solvent to dryness with the stream of air was dictated by the nature of the elution solvent. Finally, acetonitrile was selected to reconstitute the dry residue due to its HPLC compatibility.

The optimized parameters include the nature and volume of the dispersive solvent, the type and time of the stirring method during the extraction step, and the nature of the back-extraction solvent as well as the number of back-extraction steps.

3.2.1. Optimization of the extraction step

The dispersion of MIL A was studied using various commonly used organic dispersive solvents (e.g., acetonitrile, methanol, and acetone) as well as two different IL-based surfactants ([C_{16}MIm] [Br] and [$C_{12}C_{12}$Im] [Br], working in both cases at concentrations in the extraction tube that correspond with their CMC values). Acetonitrile and methanol caused the MIL to be soluble in the aqueous phase, precluding phase separation upon application of the external magnetic field. For the remaining solvents examined, the best results were achieved with acetone which produced peak areas around 27 times higher than [C_{16}MIm] [Br] and around 3 times higher than [$C_{12}C_{12}$Im] [Br], as can be observed in Fig. 3. Thus, acetone was selected for the subsequent experiments.

The volume of acetone was also studied in the range 0.25–1.5 mL (see Figure ESM-5 of the ESM). The use of acetone volumes equal to or higher than 1.0 mL caused the MIL to be soluble in the aqueous phase. The use of 500 µL of acetone provided best extraction efficiencies for PAHs (with the exception of BbF). A volume of 500 µL of acetone was selected as optimum value to avoid any partial solubility of the MIL in the aqueous phase.

Vortex and sonication were tested as dispersion modes to increase the mass transfer of PAHs to the MIL A microdroplet while also considering the stirring time. Results are included in Figure ESM-6 of the ESM. Vortex and sonication both gave higher extraction efficiency compared to when no stirring of any kind was applied. However, the application of sonication provoked partial solubility of MIL A in the aqueous solution causing lower extraction efficiency compared with vortex stirring. Therefore, the application
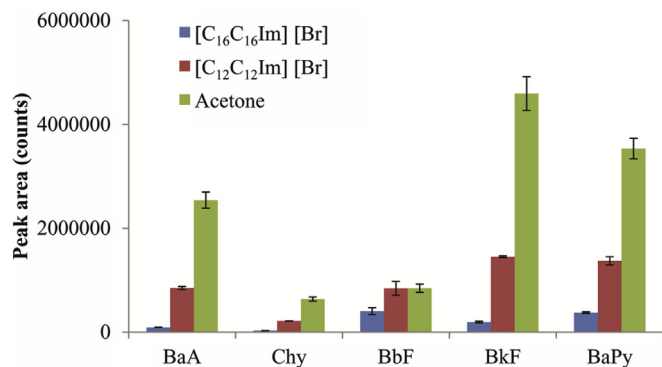
Fig. 3. Study of the effect of the nature of the dispersive solvent in the MIL-based microextraction-HPLC-FD method. Fixed experimental conditions (n = 3): 10 mL of ultrapure water containing 5 µg L^{-1} of PAHs, spiked with 0.5 mL of NaOH solution of 0.5 mol L^{-1}; 25% (w/v) NaCl; 20 µL of MIL A; 500 µL of 15 mmol L^{-1} [C$_{16}$MIm] [Br], 2.1 mmol L^{-1} of [C$_{12}$C$_{12}$Im] [Br], or acetone; 5 min of vortex; magnetic separation (few seconds); two back-extraction steps with 500 µL of n-hexane and 1 min of vortex; evaporation; reconstitution in 500 µL of acetonitrile; filtration; and HPLC injection.

of vortex was chosen to improve the efficiency of the method. The effect of the vortex time was then studied in the time range from 2.5 to 10 min. It was observed that a vortex of 10 min was adequate for the majority of PAHs (except for BbF). However, a vortexing time of 5 min was chosen with the goal of developing a fast extraction method but also considering that higher times are usually not recommended due to the health of laboratory personnel.

3.2.2. Optimization of the MIL-DLLME back-extraction step

The solvent used to accomplish the back-extraction step of PAHs from MIL A should be immiscible with the MIL and should be a good solubilizing medium for PAHs. Several solvents were tested, including dichloromethane, chloroform, and n-hexane. Of these solvents, only n-hexane satisfied the above conditions as the chlorinated solvents were partially miscible with MIL A. Thus, n-hexane was selected as the back-extraction solvent.

Regarding the number of back-extraction steps, successive back-extraction steps ranging from 1 to 4 steps using 500 µL of n-hexane and 1 min of vortex, followed by magnetic separation, were undertaken. The obtained results are shown in Figure ESM-7 of the ESM. The majority of PAHs were back-extracted with only 2 steps, with the exception of BbF (which required only one single step). Therefore, two successive back-extraction steps were chosen as optimum.

In order to increase the enrichment factor of the method, the final volume of reconstitution with acetonitrile was decreased from 500 µL to 300 µL without any loss of efficiency. A summary of the overall optimized MIL-based microextraction-HPLC-FD method is shown in Fig. 1.

3.3. Analytical performance of the MIL-based microextraction-HPLC-FD method

Calibration curves of the overall MIL-based microextraction-HPLC-FD method were obtained by applying the optimized method to standards of PAHs in ultrapure water. Figure ESM-2 (B) of the ESM shows a representative chromatogram obtained after the application of the entire method. Table 2 includes several quality analytical parameters of the method such as the linearity ranges, calibration levels, correlation coefficients, slopes and intercepts, and limits of detection and quantification.

The obtained calibration curves show a wide linearity range, from 0.05 to 5.0 µg L^{-1} for Chy and from 0.05 to 3.5 µg L^{-1} for the

remaining PAHs, with correlation coefficients (R) for the entire method ranging from 0.994 to 0.996.

Limits of detection (LODs) and limits of quantification (LOQs) of the entire method were estimated at three and ten times the signal-to-noise ratio, respectively. They were verified by preparing standards at those levels, which were then subjected to the whole microextraction-HPLC-FD method. Thus, LODs ranged from 0.005 µg L^{-1} for BbF to 0.02 µg L^{-1} for BaPy, while LOQs were always lower than 0.06 µg L^{-1}. The LODs of the overall method were ~13−54 times lower than those only associated with the HPLC-FD method (Table ESM-2 of the ESM). It is also interesting to mention that the PAHs are dissolved in acetonitrile for injection in the HPLC-FD after the MIL-based microextraction approach, and so this method is also suitable for LC-MS.

Table 2 also summarizes results regarding the reproducibility and extraction performance of the method. The reproducibility was evaluated as the relative standard deviation (RSD) using intra-day (n = 3) and inter-day (n = 9) experiments. Two spiked levels (0.4 and 1.5 µg L^{-1}) were selected for intra-day experiments. The obtained intra-day precision results ranged from 1.0% for Chy to 13% for BkF at the low spiked level, and from 7.5% for BbF to 13% for BkF for the intermediate spiked level (1.5 µg L^{-1}). The inter-day precision was evaluated at the low spiked level (0.4 µg L^{-1}) and during three non-consecutive days. RSD values ranged between 7.1% for BaA and 16% for BbF.

The extraction performance of the method was evaluated by means of the enrichment factor (E$_F$) and the extraction efficiency (E$_R$, in %) of each PAH (see Table 2). They were determined by performing experiments in ultrapure water at two spiked levels (0.4 and 1.5 µg L^{-1}, with n = 3).

The E$_F$ of the overall MIL-based microextraction-HPLC-FD method was calculated as the ratio between the predicted concentration obtained with chromatographic calibrations, without performing the MIL-based microextraction approach (see Table ESM-2 of the ESM) and the spiked concentration of each PAH. The obtained E$_F$ values ranged from 8.84 for BkF to 16.6 for BaPy at the low spiked level, and between 8.70 for BkF and 12.3 for Chy for the intermediate spiked level.

E$_R$ values were estimated as a percentage value by the ratio of the E$_F$ and the theoretical maximum preconcentration factor (E$_{F,max}$) of the overall method. The E$_{F,max}$ was calculated as the ratio of the initial volume in the extraction tube (10.5 mL) and the final volume after reconstitution with acetonitrile (0.3 mL). Following this consideration, the E$_{F,max}$ of the method is ~35.0. This value indicates the maximum preconcentration that can be achieved if all PAH are effectively extracted in the MIL and back-extracted by n-hexane in the method. Regarding E$_R$ values, they ranged from 25.3% to 47.3% at the low spiked level, and from 24.9% to 35.3% at the intermediate spiked level. This means that, on average, around 35.6% and 31.2% of the PAHs could be extracted at each spiked level, respectively. It is important to mention that E$_R$ values approaching ~100% are difficult to achieve in any microextraction procedure [49]. Thus, the obtained values are valid as long as the other analytical parameters of the method (mainly sensitivity and reproducibility) are sufficient for the intended analytical application. In any case, higher extraction efficiencies were obtained in this method using a hydrophobic MIL than those reported for the determination of phenols using a non-hydrophobic MIL, with calculated E$_R$ values ranging from 17.9 to 18.8% [37].

The accuracy of the method was evaluated by the determination of the relative recovery (RR, in %). RR was calculated as the ratio of the predicted concentration obtained in the calibration curves of the overall method (Table 2) and the initial spiked concentration. The obtained RR values were expressed as a percentage for the studied two spiked levels. RR values ranged from 91.5% to 119% at

Table 2
Analytical performance of the overall MIL-based microextraction-HPLC-FD method. The linearity range was 0.05—5.0 µg L^{-1} for Chy and 0.05—3.5 µg L^{-1} for the remaining PAHs, using 7 calibration levels.

PAH	(Slope ± SD[a])·10^{-6}	R[b]	LOD[c] (µg L^{-1})	LOQ[d] (µg L^{-1})	Low spiked level (0.4 µg L^{-1})				Intermediate spiked level (1.5 µg L^{-1})			
					E_F[e]	E_R[f] (%)	RR[g] (%)	RSD intra-day[h]/inter-day[i] (%)	E_F[e]	E_R[f] (%)	RR[g] (%)	RSD intra-day[h] (%)
BaA	1.5 ± 0.1	0.996	0.01	0.03	10.3	29.3	105	5.3/7.1	11.2	32.0	111	9.1
Chy	0.20 ± 0.01	0.995	0.01	0.05	13.9	39.6	98.9	1.0/10	12.3	35.3	107	9.6
BbF	0.43 ± 0.02	0.994	0.005	0.02	12.7	36.3	119	3.0/16	10.7	30.6	120	7.5
BkF	1.9 ± 0.1	0.994	0.01	0.04	8.84	25.3	91.5	13/16	8.70	24.9	99.0	13
BaPy	1.2 ± 0.1	0.994	0.02	0.05	16.6	47.3	107	6.2/12	11.6	33.2	89.8	9.2

[a] Standard deviation of slope.
[b] Correlation coefficient.
[c] Limits of detection, calculated as 3 times the signal to noise ratio.
[d] Limits of quantification, calculated as 10 times the signal to noise ratio.
[e] Enrichment factor.
[f] Extraction efficiency ($E_{F,max} = 35.0$).
[g] Relative recovery.
[h] Relative standard deviation (n = 3).
[i] Relative standard deviation (n = 9), calculated in 3 non-consecutive days.

the low spiked level. The obtained RR values in this study are comparable to those obtained in other studies with non-hydrophobic MILs [35—37].

3.4. Analysis of real aqueous samples

Once the overall MIL-based microextraction-HPLC-FD method was optimized and validated, it was applied for the analysis of real aqueous samples, including tap water, wastewater, and a tea infusion. The sampling and pretreatment of both samples prior to the analysis was performed as explained in Section 2.1. The studied PAHs were not detected in any of the samples analyzed. A representative chromatogram obtained with a non-spiked wastewater subjected to the whole method is included in Figure ESM-8 of the ESM.

The matrix effect of the method was evaluated with wastewater and tea infusion samples by the estimation of the reproducibility (as RSD) and the relative recovery. With this purpose, PAHs were spiked in these samples at 0.4 µg L^{-1} and experiments were repeated in triplicate (n = 3). A representative chromatogram obtained with a spiked wastewater subjected to the whole method is included in Figure ESM-8 of the ESM.

Regarding the reproducibility studies, low RSD values were obtained with both samples, with values ranging from 4.3% to 9.5%, and 3.5%—17% when wastewater and tea infusion were analyzed, respectively. RR values ranged from 85.5% to 135% when analyzing wastewater; and from 46.2% to 116% when tea infusion was analyzed. Clearly, matrix effects were present but the method could still be successfully performed in these complex samples. For these complex samples, the utilization of matrix-matched calibrations is advisable and, if possible, LC-MS.

This MIL-based microextraction approach provides similar extraction performance to other reported microextraction methods in the literature for determination of PAHs by HPLC-FD, in terms of extraction time, extraction solvent consumption, LODs, RR and reproducibility (see Table ESM-3 of the ESM) [39—41]. Similar conclusions can be derived from Table ESM-4 of the ESM, in which the proposed method is compared with other MIL-based approaches [35—37].

4. Conclusions

Magnetic ionic liquids have been successfully studied for first time in a magnet-based microextraction approach for the determination of a group of heavy hydrophobic PAHs. The extraction efficiency of three different hydrophobic and viscous MILs was compared and the results indicate that [N$_{8,8,8,B}$] [FeCl$_3$Br], denoted as MIL A, provided the best extraction performance for the group of PAHs.

The novel microextraction approach in combination with HPLC-FD was properly optimized and the method validated using MIL A. The proposed method is capable of performing adequate quantitation of heavy PAHs, in terms of high sensitivity (LODs down to 5 ng L^{-1}), adequate reproducibility and efficiency, while requiring low sample volumes, low amounts of MIL, low consumption of organic solvent, while being simple and fast.

Ongoing work is currently being conducted in our laboratories to prepare less viscous MILs while maintaining their hydrophobic nature. By doing this, a better dispersion of the MIL in the sample matrix can be obtained, thus decreasing sources of error and increasing the extraction efficiency and precision.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.aca.2016.06.014.

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