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Ionic liquids for improving the extraction of NSAIDs in water samples using dispersive liquid–liquid microextraction by high performance liquid chromatography-diode array–fluorescence detection

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ABSTRACT

A rapid, sensitive and efficient analytical method based on the use of ionic liquids for determination of non-steroidal anti-inflammatory drugs (NSAIDs) in water samples was developed. High-performance liquid chromatography equipped with a diode array and fluorescence detector was used for quantification of ketoprofen, ibuprofen and diclofenac in tap and river water samples. This new method relies on the use of two ionic liquids with multiple functionalities: one functions as an extraction solvent (1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), and the other changes the polarity in the aqueous medium (1-butyl-3-methylimidazolium tetrafluoroborate, ([BMIM][BF_4]). Factors such as the type and volume of the ILs and dispersive solvent, sample volume, and centrifugation time were investigated and optimized. The optimized method exhibited good precision, with relative standard deviation values between 2% and 3%, for the three NSAIDs. Limits of detection achieved for all of the analytes were between 17 and 95 ng mL^{-1}, and the recoveries ranged from 89% to 103%. Furthermore, the enrichment factors ranged from 49 to 57. The proposed method was successfully applied to the analysis of NSAIDs in tap and river water samples.

1. Introduction

Among the classical analytical extraction techniques, liquid–liquid extraction is widely used for the extraction of analytes from liquid matrices. However, one of the major drawbacks of liquid–liquid extractions is that they are carried out with relatively large volumes of toxic organic solvents and the extract often must be subjected to cleaning and subsequent evaporation (preconcentration) to improve the limit of detection. The general consensus in the research community is that this procedure is time consuming and tedious and requires too many steps [1]. Analytical strategies for improving the methods used to extract analytes from liquid samples have focused primarily on reducing the volume of organic solvent used. In this regard, dispersive liquid–liquid microextraction (DLLME) [2–18] which was development by Assadi et al., represents a success [2]. In DLLME, a mixture of a small amount (on the order of microliters) of extraction solvent and dispersive solvent is rapidly injected into the aqueous sample. The rapid injection produces a strong turbulence that causes the formation of micro-droplets distributed throughout the aqueous phase, leading to the extraction of the analyte. Finally, the extraction solvent is separated from the aqueous phase by centrifugation. This extraction method provides several advantages compared to traditional liquid–liquid extraction, such as simplicity, rapidity, ease of operation, low cost, high recovery and enrichment factor [2].

In recent years, ionic liquids (ILs) have begun to gain popularity as an environmentally friendly alternative to traditional organic solvents. Unlike common molten salts, ILs are salts with a melting point below 100 °C. Room-temperature ionic liquids (RTILs) describe the subset of ionic liquids that are liquid at room temperature (25 °C). Several advantages of ILs are their high viscosity, high thermal stability and low vapor pressure. They are also highly reusable and are therefore considered to be efficient compared to volatile organic solvents [19–22]. ILs are generally composed of an organic cation such as tetraalkylammonium, tetraalkylphosphonium or *N*-alkyl- or *N*,*N*-dialkylimidazolium, and a polyatomic anion, such as hexafluoroborate, hexafluorophosphate, tetrafluoroacetate, triflate or triflimide [23]. Because of their unique polarities, ILs have been commonly used as solvents in conventional liquid–liquid extraction (LLE), ultrasound-assisted extraction (UAE), and dispersive liquid–liquid microextraction (DLLME), and other methods [24]. In this context, the use of IL-DLLME has reduced both the amount of time and organic solvent required [25–35]. The IL-DLLME technique was introduced by Zhou et al. [36] and after further development, four methods have been established currently: conventional IL-DDLME [28], temperature-controlled IL-DLLME [36],

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additional-energy (ultrasound assisted extraction, vortex-assisted extraction, microwaves assisted extraction) IL-DLLME [25,29,30,37], and in situ IL-DLLME [23,27].

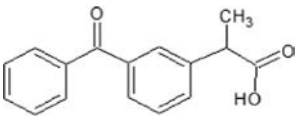
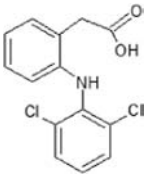
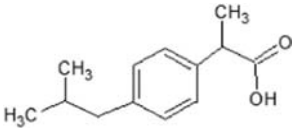
Polarity is one of the most widely applied solvent concepts, and its study for IL has been a major theme in their development. However, it is still unclear the real functionalities of ILs in solutions. Studies of reaction rates and spectra of some solute species have indicated that ILs behave similarly to polar organic solvents. However, for the same ILs, spectroscopic results have shown that have low static dielectric constants as, similar to non-polar organic solvents. The differences between these results prevent a proper understanding of the interactions that dominate ILs solutions [38]. Moreover, it has been shown that as the mole fraction of water increases, ILs exhibit structural transitions from a continuous phase, to domains, to ion-pairs and finally into individual ions at high water concentrations. However, such behavior will depend on the identity of the IL. In a very dilute solution, it has been assumed that the IL ions exist as isolated ions and their hydration is complete. But, this has not been demonstrated completely [39]. In this regard, Mathew et al. [38] worked with a mixture of ILs. They concluded that in organic solvents, ionic species can exist as contact ion pairs, solvent-separated ion pairs or solvated free ions, but in each case the solute cation and anion require each other's proximity in order to preserve charge neutrality. ILs, conversely, solvate individual solute ions completely as the IL itself is capable of preserving charge neutrality. On the other hand, Yee et al. [39] concluded that the dissociation decreases with increasing alkyl chain length on the cation when the identity of the anion remains the same. Similarly, for the same cation, the tendency for dissociation increases when the anionic nature of the IL is more hydrophilic [39]. As can be seen, many parameters influence the behavior of the ILs in solution. Therefore, in the case of IL-DLLME and ILs mixtures, it is difficult to anticipate any behavioral tendency.

Our study incorporates two ILs in DLLME. One IL serves as the extraction medium and a second IL is used to change the solubility of the analytes in the aqueous medium, resulting in a more efficient extraction. This additional IL replaces the NaCl typically added to the aqueous sample.

Emerging contaminants in water have attracted increasing attention from both the general public and government agencies [28]. In recent years, among the emerging contaminants, drugs have generated the greatest concern and have been the most widely studied. Of particular interest are non-steroidal anti-inflammatory drugs (NSAIDs), which are the most used group of analgesics and anti-inflammatory drugs worldwide [40]. They are widely used to treat symptoms related to pain, such as arthritis and other rheumatic diseases [41]. NSAIDs can enter the water supply from domestic or industrial wastewater discharges, commercial food treatment procedures, and the ground application of manure. According to reports, the most suitable analytical technique for the analysis of these pharmacologic compounds is LC-MS/MS. This is a highly reliable technique for the identification of compounds. However, the matrix effect and selection of suitable internal standards should be adequately addressed. The concentrations of NSAIDs detected in surface water or ground water are typically in the range of ng L^{-1}–mg L^{-1}, whereas they persist for longer periods of time in soils and sediments, reaching concentrations on the order of g kg^{-1} [42]. Therefore, a challenge remains in reaching these levels of detection using a method that is inexpensive and simple and does not require highly skilled personnel.

In this study, we propose a rapid and efficient IL-DLLME method for the determination of trace levels of three NSAIDs using HPLC with diode array (DAD) and fluorescence (FD) detection. The two ionic liquids employed were [BMIM][PF_6] for extraction and the moderately polar [BMIM][BF_4]. Ketoprofen, diclofenac and ibuprofen were selected as analytes because of their extensive use in therapeutics (Table 1). Several factors affecting their recovery were evaluated, such as the sample volume, centrifugation time, salting-out effect and type and volume of extraction solvent and dispersive solvent. Finally, under the established optimal conditions, this method was successfully applied to determine trace levels of the three drugs in tap and river water samples.

Table 1
Investigated NSAIDs and some of their physical properties.

Compounds	Chemical structure	Log P	pK_a
Ketoprofen		3.12	4.45
Diclofenac		4.51	4.15
Ibuprofen		3.97	4.91

2. Experimental section

2.1. Reagents

All reagents used were of analytical grade or better. The three NSAIDs drugs studied were obtained from Sigma-Aldrich (St. Louis, USA). Standard solutions of the drugs were prepared at 25 μg mL^{-1} using ultrapure water ($\rho = 18\ M\Omega\ cm$) from a Millipore Milli-Q system (MQ water). For the mobile phase, formic acid (98–100%), methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). 1-Butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]), 1-butyl-3-methylimidazolium hexafluorophosphate ([BMIM][PF_6]), ethyl-dimethyl-propylammonium bis(trifluoromethylsulfonyl) imide ([NEMMP][NTF]) and ethyl-dimethyl-(2-methoxyethyl)ammonium tris(pentafluoroethyl)trifluorophosphate ([MOEDEA][FAP]) were all purchased from Sigma-Aldrich (St. Louis, USA). Whatman™ filter paper (125 mm) was used to filter the drinking and river water samples.

2.2. Apparatus

A Hettich EBA 20 centrifuge (Hettich Lab. Technology, Tuttlingen, Germany) was used to accelerate the phase separation in 15 mL conical centrifuge tubes. A Radwag AS 60 analytical balance (RADWAG Wagi Elektroniczne, Radom, Poland) was used to weigh the standard drugs. A Thermolyne Maxi-Mix II Vortex Mixer (Thermoscientific, Waltham, MA, USA) was used for extraction of the analytes in DLLME.

2.3. IL-DLLME procedure

The working solution (500 ng mL^{-1} of each analyte) was first adjusted to pH 2.5 using H_3PO_4. Then, 5 mL of the working solution was placed in a 15 mL screw-capped conical-bottom graduated glass centrifuge tube. Two hundred microliters of [BMIM][BF_4] was injected into the sample solution to change the polarity of the sample, which was then stirred manually to promote mixing. DLLME was performed by rapidly injecting a 300 μL mixture of [BMIM][PF_6] (90 μL) and methanol (210 μL) into the water sample using a syringe. The rapid

and strong injection of the extraction mixture produced a cloudy sample solution, which was subsequently vortexed for 30 s and centrifuged for 5 min at 4000 rpm. The upper aqueous phase was removed with a syringe, and the sedimented phase (90 μL) was withdrawn using a 100 μL microsyringe. Finally, 20 μL of sediment IL was injected into the HPLC system. All measurements were performed in triplicate, and the syringe was rinsed with methanol to remove residual analytes and ILs.

For experiments with NaCl, a solution of 200 g L^{-1} NaCl was previously prepared. Corresponding aliquots were added to the aqueous sample to achieve a final concentration between 0 and 8 g L^{-1}. These aliquots were injected into the water sample before IL-DLLME. For the specifically case with [BMIM][BF_4], aliquots of NaCl solution were added first.

2.4. Chromatographic conditions

The HPLC analyses were carried out on a Jasco LC Net II system equipped with a quaternary gradient pump (PU-2089 U plus), a DAD (MD-2018), a fluorescence detector (FP-2020), and a column thermostat (CO-2060) (Easton, MD, USA). Separations of the analytes were performed on a Kinetex-Phenomenex reversed-phase (Torrance, CA, USA) C-18 column. The mobile phase consisted of methanol, formic acid (0.2% v/v) and acetonitrile (10/80/10) and was introduced at a flow rate of 1 mL min^{-1} at 40 °C. The injection volume was 20 μL. The detection wavelength was set at 256 nm for ketoprofen and 275 nm for diclofenac. Fluorescence detection with excitation and emission wavelengths of 220 and 290 nm, respectively, was used for ibuprofen.

2.5. Real sample preparation

The samples were pretreated before the microextraction process. In the case of river water, the samples were pre-filtered with Whatman filter due to the possibility to co-extracting impurity particles. In the case of tap water, the samples were boiled due to high level of Cl^- added in the industrial potability process, that interfere with chromatographic sign in first step. After boiling process, the samples were filtered.

3. Results and discussion

3.1. Optimization of microextraction procedure

To determine the optimal performance of this IL-DLLME procedure, various parameters were investigated, including the selection of the extraction and dispersive solvents, the volume of the extraction and dispersive solvents, the volume of the hydrophilic IL, the sample volume, the centrifugation time and the salting-out effect. Five-milliliter samples of water containing 500 ng mL^{-1} of each analyte were used for the optimization experiments. In a liquid–liquid extraction, the pH of the aqueous phase determines the effectiveness of the extraction of analytes containing ionizable groups. For this reason, the aqueous solution and standard solution were adjusted to pH 2.5 to ensure extraction of the non-ionized compound, taking into account that the pK_a values of each analyte are greater than 4 (Table 1). The pH was also chosen on the basis of previously reported DLLME studies involving these analytes [17,28].

3.1.1. Selection of the extraction and dispersive solvent

The selection of an extraction IL is determined by several requirements: it should be immiscible with the aqueous matrix solution, be able to extract the analytes, and be compatible with chromatographic detection method (HPLC in this case). In this study, 100–μL of different ILs ([BMIM][PF_6], [NEMMP][NTF] and [MOEDEA][FAP]) were investigated as potential extractants. These ILs were dispersed in methanol. [BMIM][BF_4] was previously added to the water sample before DLLME to change the polarity of the aqueous system and achieve better extraction of the analytes. Subsequently, DLLME was performed according to the procedure described in Section 2.3 and the experimental conditions shown in the captions of Fig. 1. The results (Fig. 1A) indicate that [BMIM][PF_6] gave the highest recoveries; it was therefore considered to be the most appropriate extractant for subsequent experiments. This IL has been used previously for the extraction of other types of NSAIDs, including ketoprofen [18], whereas this work involves NSAIDs, including ibuprofen and diclofenac, of a different structural class that have not yet been studied. In contrast, we observed that the use of [NEMMP][NTF] is unsuitable because of the high standard deviations obtained for all of the analytes in comparison with those obtained using other ILs (see Fig. 1A). Three organic solvents methanol, acetonitrile, and acetone were evaluated as potential solvents to disperse the [BMIM][PF_6] in the aqueous phase. These solvents must be miscible with both the aqueous phase and the IL. The effect of these solvents on the extraction of the analytes was quantitatively

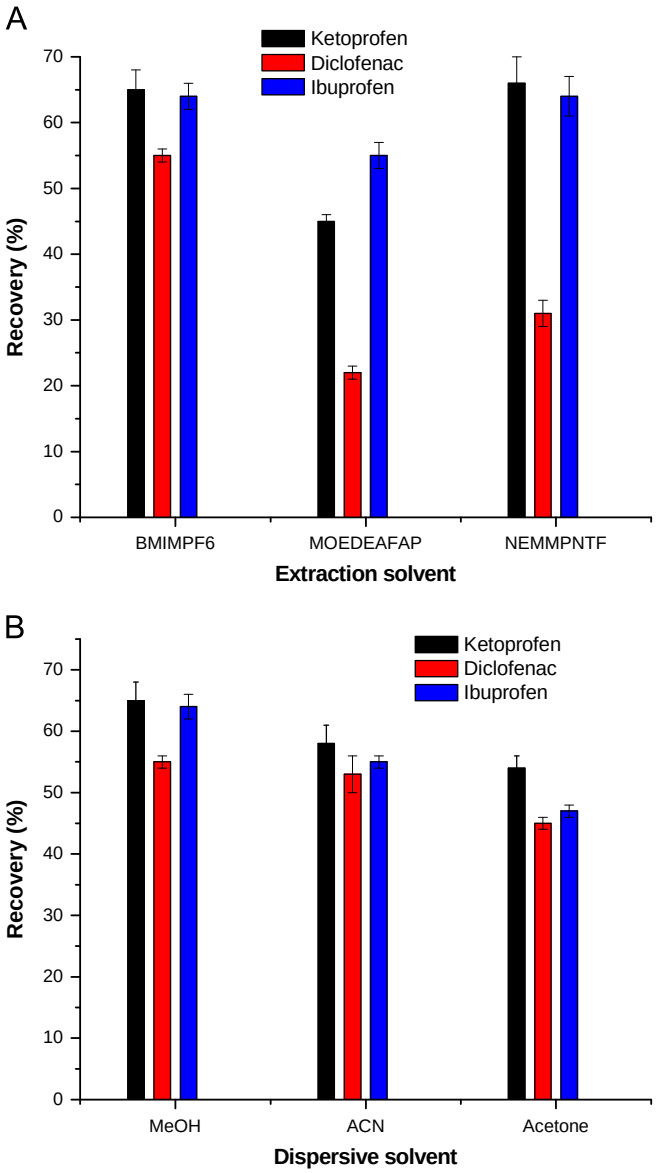


Fig. 1. (A) Effect of the three ILs as extraction solvents on the NSAID recoveries. (B) Effect of the dispersive solvent on NSAID recovery (5 mL total sample volume, 100 μL of IL, 500 μL [BMIM][BF_4], 30 s vortexing, 5 min centrifugation at 4000 rpm).

evaluated using the experimental conditions described in Fig. 1B. The results show similar tendencies for all of the analytes; however, methanol gave slightly better extraction efficiencies in all instances. This result differs from those reported by Cruz-Vera et al. [18], who observed that acetonitrile was the best dispersive solvent for ketoprofen extraction using [BMIM][PF_6] as the extraction solvent. This discrepancy leads us to believe that, for a given IL extractant, the choice of the other phases is essential for obtaining good recoveries. Therefore, the use of an additional, moderately polar IL, such as [BMIM][BF_4], plays an essential role in this type of microextraction.

3.1.2. *Effect of the [BMIM][PF_6] volume*

The amount of IL used in the DLLME is an important factor for obtaining a high extraction performance. Therefore, we studied the effect of varying the [BMIM][PF_6]-methanol volume on the analyte recovery to ensure the best extraction. Different volumes of [BMIM][PF_6] ranging from 30 to 100 μL were tested under the previously described extraction conditions. A total [BMIM][PF_6]-MeOH volume of 300 μL was injected for DLLME. Fig. 2 shows that the addition of 30 μL of [BMIM][PF_6] is a very small volume so that the analytes can hardly be separated from the aqueous phase during the centrifugation step, thereby preventing recoveries greater than 15%. In contrast, the addition of 50 μL [BMIM][PF_6] significantly increases the recovery for all three analytes. The maximal recoveries were obtained at 70 μL of [BMIM][PF_6] for diclofenac and at 90 μL of [BMIM][PF_6] for ibuprofen and ketoprofen. Beyond 90 μL, the recoveries of all three analytes decrease because the disperser volume, is not sufficient to disperse efficiently the [BMIM][PF_6] and therefore the extraction is reduced. On the basis of these values, a volume of 90 μL of [BMIM][PF_6] was chosen for the subsequent optimization experiments.

3.1.3. *Effect of [BMIM][BF_4] volume*

The addition of a water-miscible IL, [BMIM][BF_4], was carried out to facilitate the extraction of the analytes with [BMIM][PF_6]. To the best of our knowledge, this is the first report of using a water-miscible IL to improve extraction in IL-DLLME. Its use has been mainly linked to the in situ-DLLME as one of the extraction solvent [28] but not as a salt, which decreases the solubility of the analytes in the aqueous sample. Therefore, we carefully studied the effect of adding increasing volumes of [BMIM][BF_4] using a range of 0–500 μL. The results are shown in Fig. 3. The addition of 100 μL of [BMIM][BF_4] considerably increases the recovery of analytes, which reaches 75% in the case of diclofenac, and the addition of 200 μL [BMIM][BF_4] enables 100% recovery for ketoprofen and ibuprofen. However, [BMIM][BF_4] volumes greater than 300 μL decrease the recovery of the three analytes because they promote premature precipitation of [BMIM][PF_6] before vortexing, destabilizing the dispersion effect. Therefore, the optimal volume of [BMIM][BF_4] was determined to be 200 μL.

3.1.4. *Effect of the dispersive solvent volume*

The dispersive solvent can affect the formation of the emulsion and the dispersity of the IL. Using a fixed volume of 90 μL of [BMIM][PF_6], we performed NSAID extractions with different volumes of methanol in the range of 110–710 μL. The results are shown in Fig. 4. The optimal recovery occurs with 300 μL of ([BMIM][PF_6])-MeOH. With larger volumes of ([BMIM][PF_6])-MeOH, the extraction efficiency decreases. In particular, methanol volumes in excess of 500 μL result in a sharp decrease in analyte recoveries, which is explained by the fact that a higher volume of methanol increases the solubility of [BMIM][PF_6] in the aqueous phase, resulting in a significant decrease

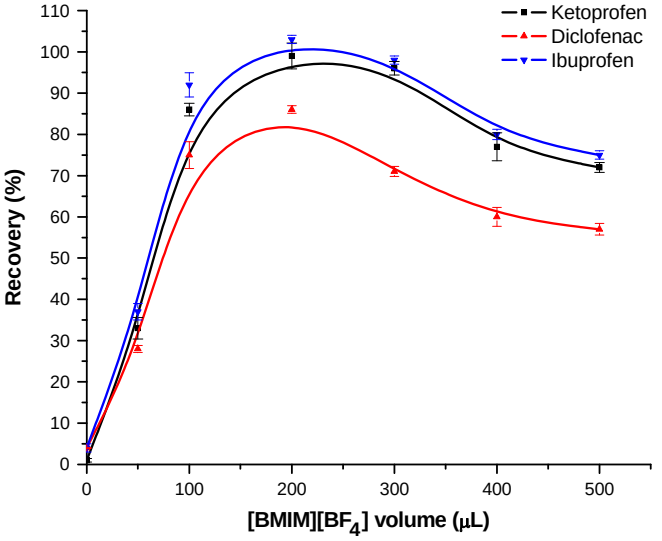


Fig. 3. Effect of the [BMIM][BF_4] volume on the NSAID recoveries (5 mL total sample volume; 90 μL of [BMIM][PF_6]; 30 s shaking vortex time; 5 min centrifugation at 4000 rpm).

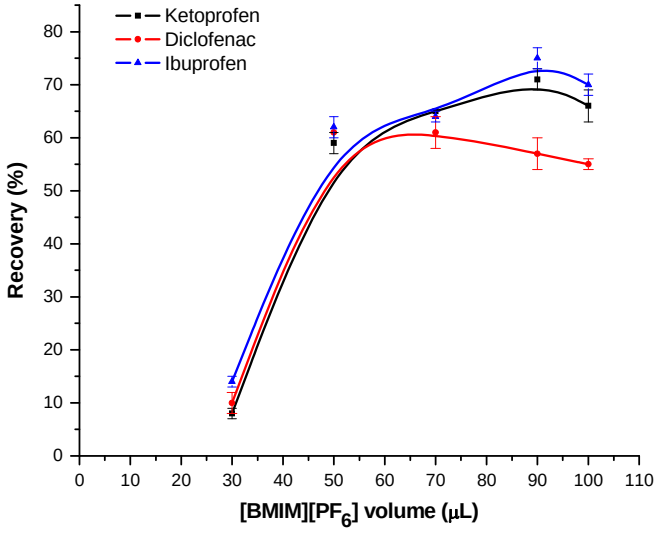


Fig. 2. Effect of the [BMIM][PF_6] volume on the NSAID recoveries (5 mL total sample volume; 500 μL of [BMIMBF_4]; 30 s shaking vortex time; 5 min centrifugation time at 4000 rpm).

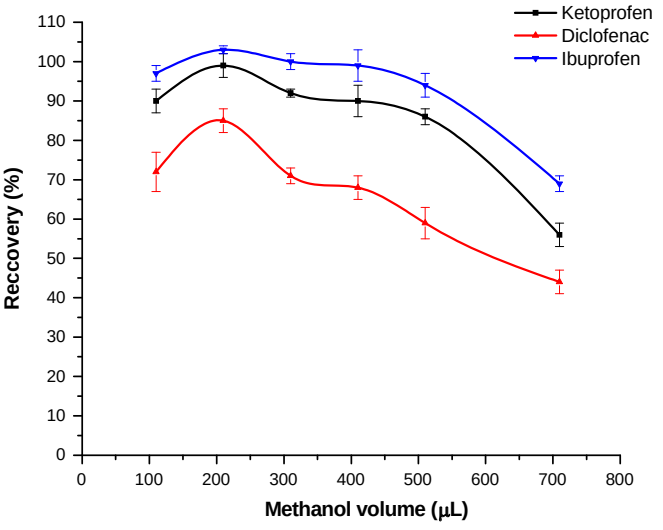


Fig. 4. Effect of the dispersive solvent (methanol) volume on the NSAID recoveries (5 mL total sample volume; 90 μL extraction volume; 200 μL of [BMIM][BF_4]; 30 s shaking vortex time; 5 min centrifugation at 4000 rpm).

in the volume of the sedimented phase. This effect hinders makes subsequent injections of the IL into the chromatographic system.

3.1.5. Sample volume effect

We assessed the effect of the sample volume on the extraction of the NSAIDs by extracting different volumes of the same sample (500 ng mL^{-1}) while maintaining a constant volume of 90 μL of [BMIM][PF_6] and 210 μL of methanol. Fig. 5 shows that recovery of all the analytes was unchanged up to a volume of 6 mL of sample. However, further increases of the sample volume resulted decreasing in the recoveries, mainly due to the saturation of the IL and the large increase in the amount of analyte to be extracted. Based on these results, 5 mL was selected as the optimal sample volume.

3.1.6. Ionic-strength effect

The addition of salt improves the efficiency of DLLME in most cases. In general, the addition of salt reduces the solubility of the analytes in the aqueous sample and enhances their distribution [27]. However, some research has indicated that the opposite effect can occur when an IL is used as the extractant solvent [29,43]. In this work, the effect of ionic strength on the IL-DLLME extraction efficiency was evaluated by increasing the concentration of NaCl in the aqueous matrix from 0 to 8 g L^{-1}. We observed that the recovery of all three NSAIDs decreased with increasing amounts of NaCl added (Fig. 6A). We also investigated a possible negative effect of [BMIM][BF_4] on NaCl. With the addition of only NaCl to the aqueous sample, an optimal value of 2 g L^{-1} was determined (Fig. 6B), however this optimal value is insufficient to extract 100% of the NSAIDs (recovery range: 60–97%). In contrast, when [BMIM][BF_4] was used alone, recoveries ranging from 86 to 103% were obtained (0 g L^{-1} NaCl in Fig. 6A). From these results, we concluded that the use of an additional IL that switches the polarity of the aqueous system is a more effective method for increasing the recovery of analytes than increasing the ionic strength with the use of a salt. The simultaneous use of [BMIM][BF_4] and NaCl (Fig. 6A) decreases the extraction power of [BMIM][PF_6], which may be primarily due to the increased miscibility of the IL in the water supernatant. This increased miscibility prevents further separation of the two phases during centrifugation, which decreases the efficiency of the IL-DLLME extraction method. Other researchers have previously reported this effect [17,27,29,43]. In our case, the use of NaCl and NaCl-[BMIM]

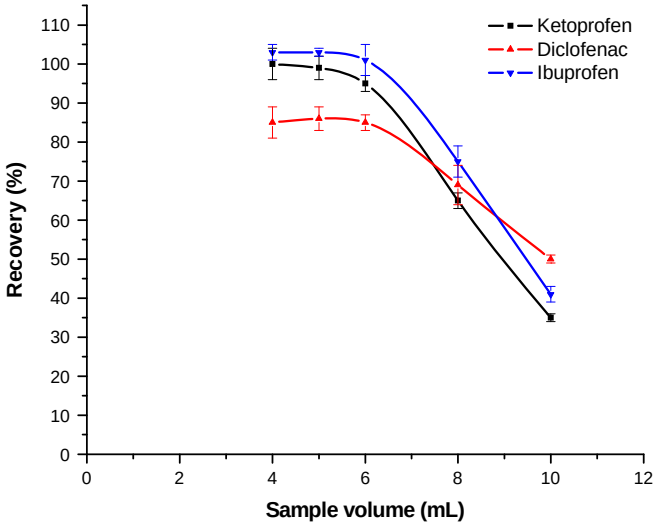


Fig. 5. Effect of sample volume on the NSAID recoveries (90 μL of [BMIM][PF_6]; 200 μL of [BMIM][BF_4]; 30 s shaking vortex time; 5 min centrifugation at 4000 rpm).

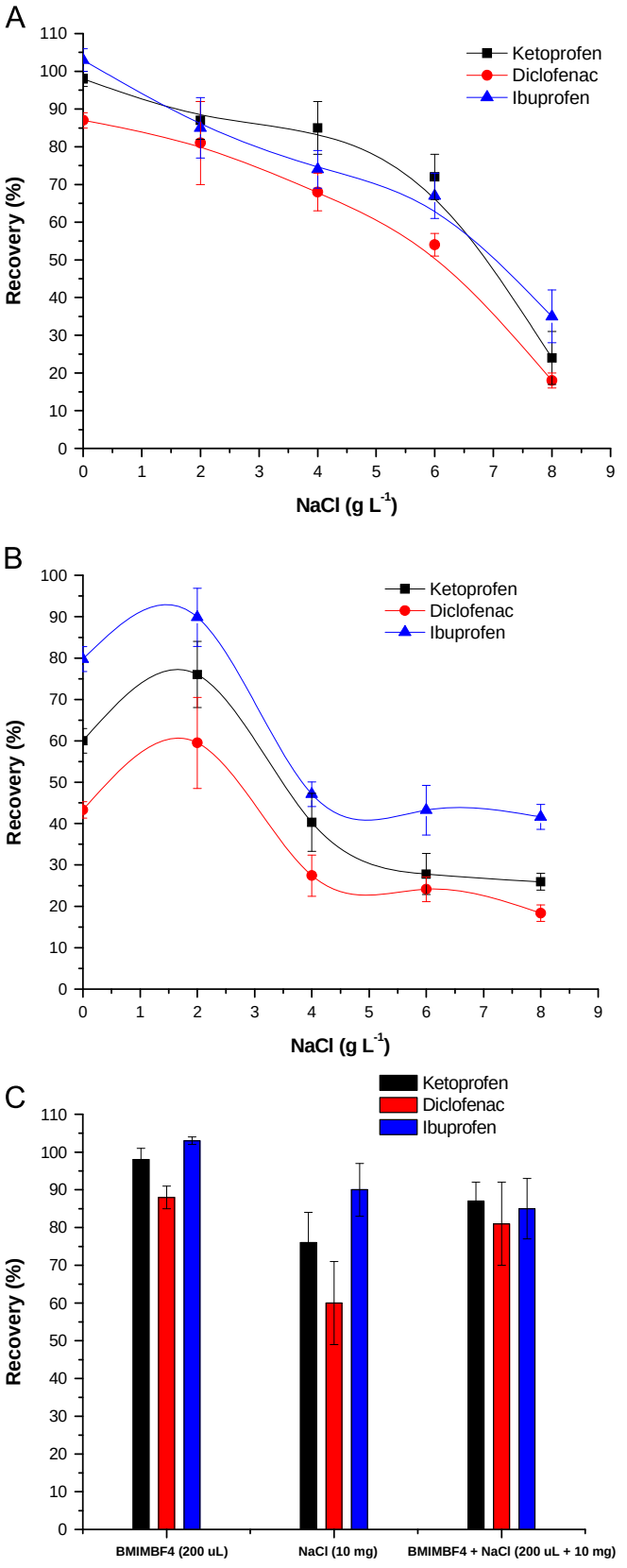


Fig. 6. Effect of using (A) NaCl plus 200 μL [BMIM][BF_4] and (B) NaCl on the NSAID recoveries. (C) Comparative maximum responses for three methods on the NSAID recoveries. 5 mL total sample volume; 90 μL of [BMIM][PF_6]; 30 s shaking vortex time; 5 min centrifugation at 4000 rpm.

[BF_4] also increases the error associated with the measurements (3–11%), whereas the measurements performed using exclusively [BMIM][BF_4] exhibit small deviations not exceeding 3% (Fig. 6C). Therefore, the addition of NaCl is not necessary in this method.

3.1.7. Centrifugation time

To optimize the extraction efficiency, centrifugation times were examined in the range of 2–8 min at a centrifugation speed of 4000 rpm. At 4 min of centrifugation, recoveries of the analytes began to level off. However, beyond 6 min of centrifugation, removal of the extraction phase with a syringe became difficult because of the high viscosity of the IL, and introduction of an additional dilution step of the IL was necessary. Therefore, the optimal centrifugation time was determined to be 5 min.

3.2. Analytical features and enrichment factors

The optimized IL-DLLME method was evaluated by characterizing its analytical performance in terms of linearity, precision, recoveries, enrichment factor, limit of detection (LOD), and limit of quantification (LOQ). Calibration plots of each analyte, prepared at five concentration levels in the range of 100–50000 ng mL^{-1} were observed to be linear, with correlation coefficients (r) ranging between 0.9995 and 0.9996. For LOD and LOQ determination, signal-to-noise (S/N) ratios of 3 and 10, respectively, were employed. The repeatability, described as the percent relative standard deviations (RSDs) of the results from six replicate experiments using 500 ng mL^{-1} NSAIDs were in the range of 2–3%. The results are shown in Table 2. The recovery values were also investigated for six replicate experiments performed under the determined optimal conditions. Additionally, the enrichment factors were calculated according to the formula:

$$\text{E.F.} = \frac{C\,(IL)}{C\,(aqueous)},$$

where $C\,(IL)$ is the final concentration of the IL microdroplet obtained by IL-DLLME (obtained by interpolation in the calibration plots) and $C\,(aqueous)$ is the initial concentration in the aqueous sample before IL-DLLME (also obtained by interpolation in the calibration plots) [44]. We observed that the optimized extraction process was highly efficient, with good recoveries and enrichment factors ranging from 89% to 103% and from 49 to 57, respectively (Table 2).

3.3. Application of IL-DLLME to real water samples

Real tap water and river water samples were examined to validate the applicability of the developed IL-DLLME method and to evaluate matrix effects for the extraction of NSAIDs.

An initial pretreatment for both matrices was performed due to the high amount of Cl^- present in the drinking water of the city of Santiago (Chilean norm of 400 mg L^{-1}) [45]. Table 3 shows the concentration and recovery of the three studied analytes spiked into the real water samples. No analytes were detected in the blank extraction of the water samples (Fig. 7). The recoveries ranged from 91 to 103% and from 90 to 102% for tap water and river water, respectively. These results indicate that the recovery of the analytes exhibits almost no matrix effect compared with spiked nanopure water (Tables 2 and 3). Finally, if the proposed method is compared with other previously reported for the extraction of NSAIDs in aqueous samples (Table 4), the new method has several improvements particularly in the simplicity of the extraction process. Ultrasound assisted extraction coupled with subsequent cooling is not used, thereby shortening the extraction process and minimizing the energy requirements [43]. Also shows an improvement in the repeatability (standard deviation decreases) and half of sample is used without impairing the enrichment factor.

4. Conclusions

A new analytical method that uses IL-DLLME in combination with HPLC for the determination of ketoprofen, ibuprofen and diclofenac in water samples has been developed. The new method does not require additional steps, as has been the case in some previous IL-DLLME reports for NSAIDs, where the use of ultrasound-assisted extraction and subsequent cooling of the sample increased the time of the extraction process. The high affinity of [BMIM][PF_6] to the analytes allows for the extraction and preconcentration of NSAIDs in one step, requiring less energy and resulting in significant time savings compared to similar techniques. Moreover, the addition of NaCl was observed to reduce the extractive power of the IL; thus, the use of salt in this type of microextraction is unnecessary. Our results also indicated that using [BMIM][BF_4] as an additional semipolar IL can further increase the efficiency of the extraction process, through changing the polarity of the extraction system and decreasing the miscibility of the analytes in the aqueous phase. The newly developed IL-DLLME method exhibited a large linear range and good repeatability, precision, and accuracy for the three studied drugs. It also provided several other advantages, such as a good enrichment factor, simplified and fast operation, and very low consumption of organic solvent. Finally, the method was applied to the determination of the studied drugs in drinking and river water samples. No serious matrix effect was observed, and good recoveries (over 90%) were obtained at two different NSAID concentrations for spiked

Table 3
Application of the new method to spiked real samples.

Analytes	Spiked concentration (ng mL^{-1})	Recovery (%)	
		Tap water	River Water
Ketoprofen	150	102.9 ± 5	100.6 ± 5
	500	97.3 ± 4	95.3 ± 3
Diclofenac	500	92.9 ± 3	90.5 ± 4
	800	91.1 ± 4	89.8 ± 4
Ibuprofen	250	103.0 ± 3	102.2 ± 3
	500	99.0 ± 3	101.5 ± 4

Table 2
Analytical features of NSAID extraction by IL-DLLME.

Analyte	Retention time (min)	Linear range (ng mL^{-1})	r	LOD (ng mL^{-1})	LOQ (ng mL^{-1})	R^a (%)	RSD[b] (%)	E.F.
Ketoprofen	7.4	100–50000	0.9995	17	57	100	3	56
Diclofenac	8.6	400–30000	0.9995	95	316	89	3	49
Ibuprofen	8.9	200–30000	0.9996	41	137	103	2	57

[a] Spiked recovery to 500 ng mL^{-1}.
[b] Repeatability with $n=6$.

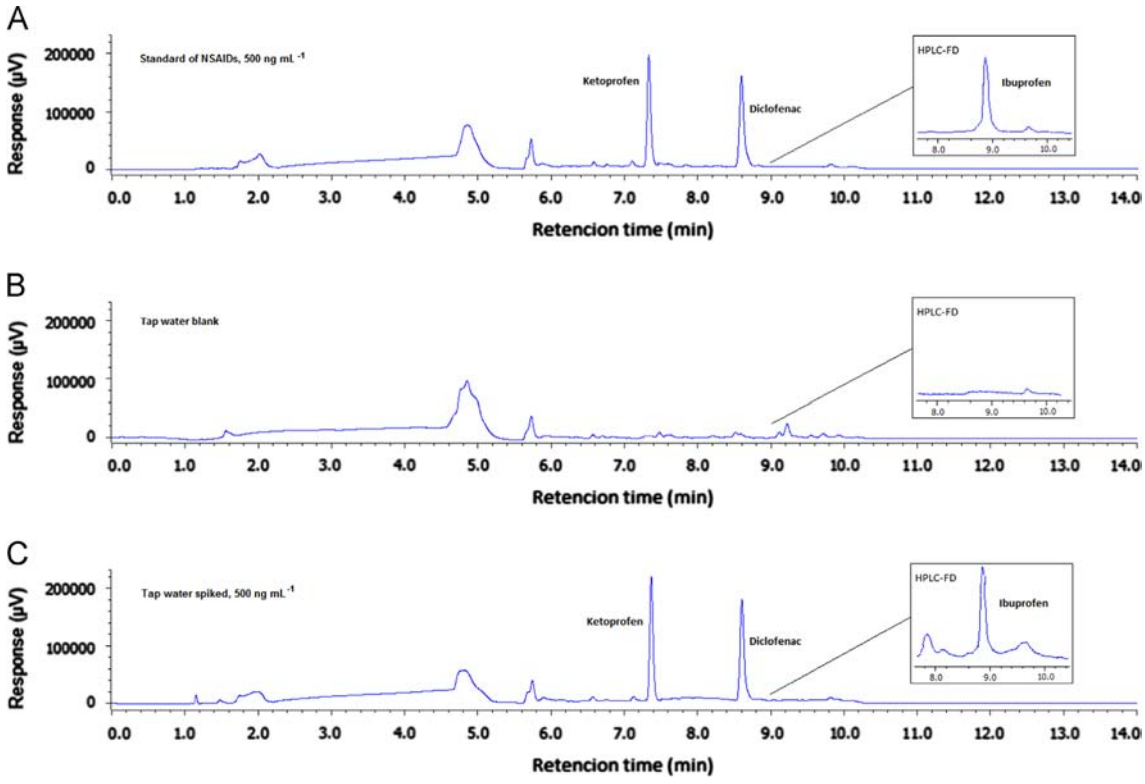


Fig. 7. Chromatogram of (A) solution of analyte standards at 500 ng mL^{-1}, (B) tap water blank and (C) tap water spiked with 500 ng mL^{-1} analytes using IL-DLLME.

Table 4
Comparative IL-DLLME methods for NSAIDs in real samples.

Method	Matrix	Analytes	Chromatographic technique	Sample preparation time (min)	Extraction/ dispersive solvent	Sample volume (mL)	LOD ($\mu g\ L^{-1}$)	Recovery (%)	RSD (%)	E.F	Ref.
In Syringe IL-DLLME	Urine	Ketoprofen Naproxen Flurbiprofen Indomethacin	HPLC-UV	< 5	[BMIM][PF_6]/ACN	10	32 9.2 16.3 8.3	100–107	3–9	36	[18]
In situ IL-DLLME	Tap and creek water	Naproxen Ibuprofen	HPLC-UV	5.5	([BMIM][Cl]+ $LiNTF_2$)/ [BMIM][Cl]	10	0.5 2.0	98–102	1–6	833	[28]
Vortex-IL-DLLME	Tap and creek water	Naproxen Ibuprofen	HPLC-UV	5.5	[HNH_2MPL][FAP]/ MeOH	10	0.5 55	96–102	1–6	333	[28]
US-IL-DLLME	wastewaters	Ketoprofen Naproxen Ibuprofen	LC-MS/MS	16	[C_8MIM][PF_6]/ACN	10	0.004 0.06 0.002	88–107	2–9	20	[43]
Vortex-IL-DLLME	Tap and river water	Ketoprofen Ibuprofen Diclofenac	HPLC-UV/FP	5.5	[BMIM][PF_6]/MeOH	5	17 95 41	90–103	2–3	56	This work

real samples compared with distilled nanopure water (differences on recovery lower 5% than for a 500 ng L^{-1} NSAIDs were obtained. Therefore, we conclude that our IL-DLLME method, in conjunction with HPLC, is a rapid and efficient analytical method.

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