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## Trace Determination of Duloxetine in Human Plasma by a Novel Ionic Liquid-Based Ultrasound-Assisted *In Situ* Solvent Formation Microextraction and High-Performance Liquid Chromatography

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For the first time, a novel and efficient ionic liquid-based ultrasound-assisted *in situ* solvent formation microextraction (IL-UA-ISFME) combined with high-performance liquid chromatography-ultraviolet detection (HPLC-UV) has been successfully developed for the determination of duloxetine (DLX) in human plasma. Herein, an environmentally-friendly hydrophobic ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate) was formed by addition of a hydrophilic ionic liquid (1-butyl-3-methylimidazolium tetrafluoroborate) to sample solution including NaPF<sub>6</sub> as an ion-pairing agent. The analyte was extracted into the ionic liquid although the microextraction solvent was dispersed among the sample solution using ultrasound radiation. The sample was then centrifuged and extracting phase injected into HPLC system. The developed sample enrichment method revealed a considerable robustness against the variations of sample ionic strength. Parameters controlling the performance of the microextraction were evaluated and optimized. The limit of detection was 0.8 μg l<sup>-1</sup> while a good linearity ( $r^2 = 0.996$ ) and a broad linear range (2.0-1500 μg l<sup>-1</sup>) were achieved. A reasonable relative recoveries (83.6-92.1%) and the appropriate intra-assay (4.0-5.1%,  $n = 5$ ) and inter-assay (4.3-7.6%,  $n = 9$ ) precisions along with an appropriate sample clean-up exhibited good performance of the analytical procedure. It was eventually validated for the screening purposes in human plasma after oral administration of the drug and some pharmacokinetic data were achieved. This green method is prompt, convenient, and reliable and offers satisfactory reproducibility as well as sufficient sensitivity.

**Keywords:** Ionic liquid-Based ultrasound-assisted *in situ* solvent formation microextraction, Human plasma, High-performance liquid chromatography, Duloxetine

### INTRODUCTION

The practical demands for analysis in the field of pharmaceutical, environmental, and life science are the driving forces for progressing sample preparation in analytical chemistry [1,2]. The foregoing attempts have focused on miniaturization, automation, high-throughput performance, on-line coupling with analytical instruments and low-priced operations through remarkably low or no solvent consumption [3-5]. Over the last decade, liquid-phase microextraction (LPME), a micro-scale

implementation of liquid-liquid extraction, has turned into a promising sample pretreatment technique, because it integrates extraction and enrichment into one step, and is low-priced, easy to operate and almost solvent-free procedure [6-8].

Ionic liquids (ILs) are categorized as liquid melted salts (at temperatures below 100 °C) in which they are usually included large and asymmetrical organic cations and organic or inorganic anions [9,10]. Besides the excellent physicochemical, nonflammability and thermal properties of ILs, they are frequently recognized by their worthy solvation ability for a wide range of compounds and materials. A positive characteristic of applying ILs as

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extracting solvent is their stability over a wide temperature range and extremely low vapor pressure at relatively high temperatures. Due to these properties, ionic liquids puts very few dangers to the environment and in comparison with toxic organic solvents they are classified as green solvents [11,12]. Recently, several studies have been reported addressing the successful application of ILs in different microextraction schemes [13,14].

In 2014, Zeeb *et al.* introduced a novel sample enrichment method as an efficient and practical preconcentration technique namely ionic liquid-based ultrasound-assisted *in situ* solvent formation microextraction (IL-UA-ISFME) [15]. It is set by dissolving of a hydrophilic ionic liquid in an aqueous medium containing analyte and an ion-pairing agent undergoing an in-situ metathesis reaction forming a hydrophobic ionic liquid. Therefore, target analyte is extracted and enriched once the hydrophobic ionic liquid is formed. There is no interface between the aqueous phase and the extraction solvent. So, the mass transfer process from aqueous media inside the ionic liquid, that is speeded up by ultrasound wave, has no meaningful influence on the method performance.

Duloxetine (DLX) is famous as a selective serotonin-norepinephrine reuptake inhibitor (SNRI) and antidepressant drug, and it is currently recommended for treatment of major depressive disorders [16,17]. The drug is approved by the United States Food and Drug Administration (U.S. FDA) for the cure of diabetic polyneuropathy and is recommended as a first line treatment for the purposes [18]. However, drugs like DLX have raised the chance of suicidal thoughts or actions in children and young adults. The risk may be greater in people who have had these thoughts or actions in the past and all people who take duloxetine need to be watched closely. So, the determination of DLX in biological fluids is of great importance for general practitioners for the next steps of a medical treatment process [19].

A literature survey revealed that many analytical methods have been reported for the analysis of DLX in pharmaceutical and biological media including ion-selective electrode [20], high performance thin layer chromatography [21], capillary electrophoresis [22] and chromatographic

methods [23,24]. Furthermore, the extraction and preconcentration techniques, which are generally employed for the drug monitoring, are liquid-liquid extraction (LLE) and solid-phase extraction (SPE). Nevertheless, the methods are considered high-priced, time-consuming and labor-intensive, which frequently result in high blank values [25]. Consequently, there is a growing need to develop new and practical microextraction protocols to provide higher efficiencies along with less usage of toxic organic solvents.

The goal of present study is to assess practical applicability of IL-UA-ISFME method for the determination of DLX in human plasma. The factors affecting microextraction efficiency are studied in details and optimum conditions are set. The analytical protocol in combination with HPLC-UV is finally validated for assessing the time profile of plasma concentrations of DLX while some pharmacokinetic parameters have been achieved.

## EXPERIMENTAL

### Apparatus

The chromatographic separations were performed on a HPLC system (Shimadzu, Kyoto, Japan) including a pump, an automatic injector equipped with 20  $\mu$ l sample loop and a UV detector (set at 230 nm). The analytical column was a RP-C<sub>18</sub> (LiChrospher, Merck Millipore, Darmstadt, Germany) with 5  $\mu$ m particle size and dimensions of 150 mm  $\times$  4.6 mm i.d., at temperature of 30  $\pm$  0.5  $^{\circ}$ C. Isocratic mobile phase consisted of ammonium formate (10 mM) and acetonitrile (40:60) with pH of 3.8 at a flow rate of 1.0 ml min<sup>-1</sup> was run through the column. A GS-6 centrifuge (Beckman, CA, USA) was applied to speed up the phase separation. The hydrophobic ionic liquid was dispersed into the aqueous sample using Sonorex ultrasonic baths (Bandelin, Berlin, Germany). The mobile phase was filtered *via* a 0.2  $\mu$ m membrane filter (Millipore, Bedford, MA, USA) and it was degassed constantly by an online degasser. The pH measurements were performed by a 827 pH lab instrument (Herisau, Switzerland).

### Reagents and Materials

Analytical reagent grade of all chemicals including 1-

butyl-3-methylimidazolium tetrafluoroborate [Bmim][BF<sub>4</sub>], sodium hexafluorophosphate (NaPF<sub>6</sub>), HCl, NaOH were purchased from Merck Company (Darmstadt, Germany). 1-Hexyl-3-methylimidazolium tetrafluoroborate [Hmim][BF<sub>4</sub>] was attained from Fluka Company (Steinheim, Switzerland). HPLC grade of acetonitrile and methanol were obtained from Merck Corporation (Darmstadt, Germany). DLX was acquired from Daroupakhsh Company (Tehran, Iran). Ultra-pure water (Millipore, Bedford, MA, USA) was used for the rest of the work. DLX tablets (60 mg) were bought from commercial resources.

### Preparation of Stock Solutions, Calibration Standards, and Quality Control Samples

The stock solution of DLX at concentration level of 100.0 mg l<sup>-1</sup> was prepared in methanol. The working standard solutions of DLX were prepared by dilution of the above stock solution with methanol to reach an appropriate concentration. The quality control (QC) samples at concentration levels of 5, 150, 500 and 1250 µg l<sup>-1</sup> were prepared by spiking an appropriate amount of working standard solutions into human plasma. A 1.0 g ml<sup>-1</sup> solution of [Bmim][BF<sub>4</sub>] acting as hydrophilic IL was made in methanol. A working solution of 160 mg ml<sup>-1</sup> of NaPF<sub>6</sub> as an ion-pairing agent was made by dissolving an appropriate amount of this salt in ultra-pure water. All the stock and working solutions were kept at -4 °C until analysis.

### Deproteinization of Plasma and Preparation of Calibration Standards

For preparing spiked samples, different concentration levels of DLX standard were added to 1.9 ml of human plasma [26]. Then, the spiked samples were deproteinized *via* 2.0 ml acetonitrile and vortexed for 4 min. Acetonitrile content of human plasma significantly increases the solubility of ionic liquid which has a negative effect on the extraction yield of target drug. Hence, the samples were centrifuged for 7 min at 5000 rpm and their acetonitrile contents were evaporated by nitrogen stream at 50 °C. About 2 ml of the remained phase was placed into a vial, diluted up to 6 ml with ultra-pure water and centrifuged again for 3 min at 5000 rpm. In the next step, the clear

upper phase was put into the new test tube and subjected to IL-UA-ISFM.

### IL-UA-ISFME Procedure

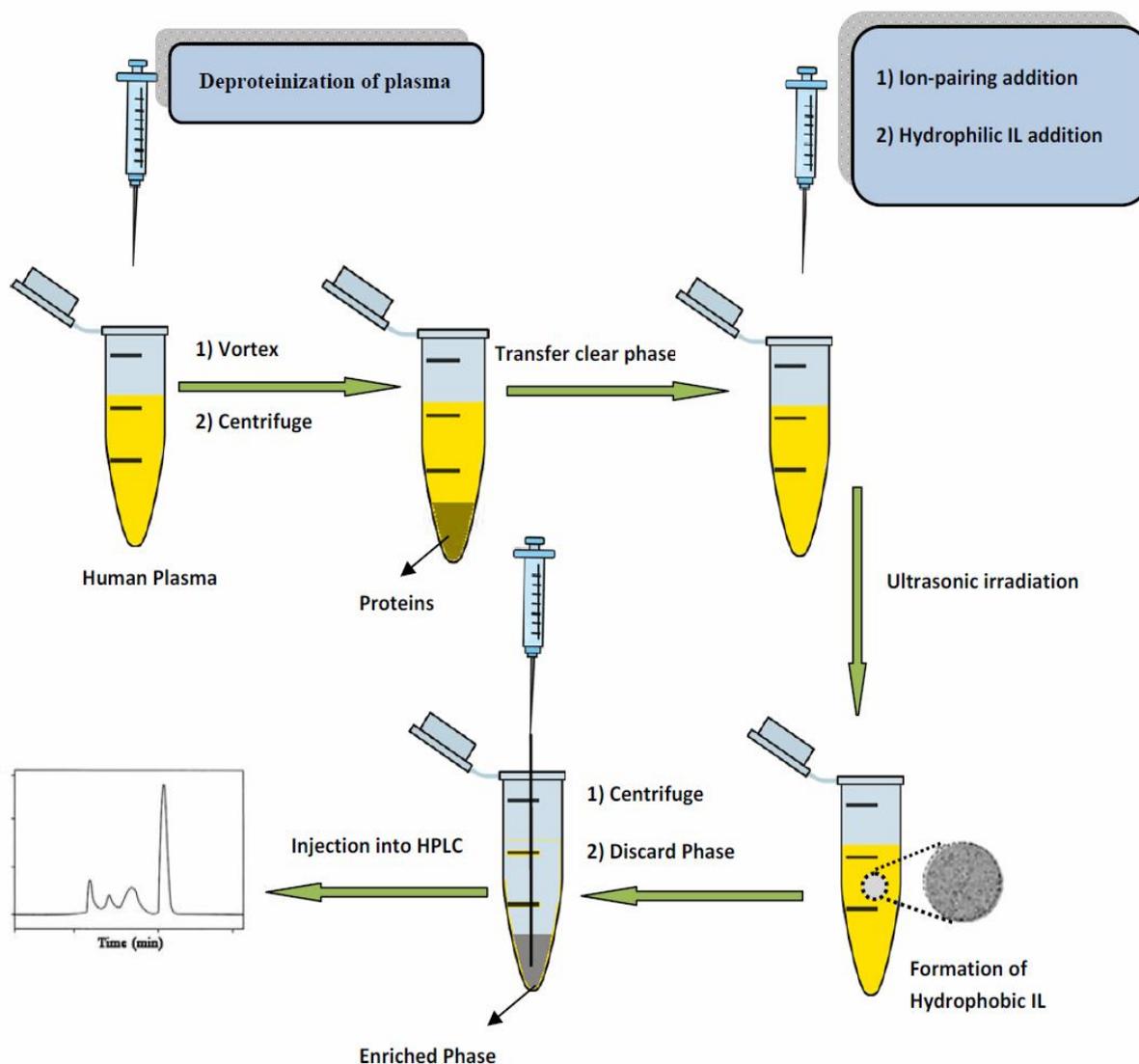
A 5.0 ml of the spiked human plasma was put into a centrifuge tube with a conical bottom while pH was adjusted at 3.0 using HCl 10<sup>-2</sup> M by a micropipette. Then, 0.5 ml of 180 mg ml<sup>-1</sup> NaPF<sub>6</sub> as an ion-exchange reagent was dissolved into the solution. In order to make water-immiscible [Bmim][PF<sub>6</sub>], 50 mg of water-miscible [Bmim][BF<sub>4</sub>] was added to the sample solution. For dispersing the hydrophobic IL into the sample, the test tube was moved within an ultrasonic bath and sonicated for 4.5 min. Following the mentioned conditions, the in-situ formed water-immiscible [Bmim][PF<sub>6</sub>] IL was completely dispersed through the aqueous phase and consequently, the analyte was quickly extracted into the fine drops of [Bmim][PF<sub>6</sub>]. For controlling the IL into the bottom of the tube and improve the phase separation, the sample solution was centrifuged for 5 min at 5000 rpm. In next step, the above phase was poured off and approximately 12 µl of enriched phase was diluted with acetonitrile to a final volume of 50 µl. The diluted phase was sonicated for 3 min and aftermost, 20 µl was injected into HPLC-UV. The developed procedure is presented in Fig. 1.

## RESULTS AND DISCUSSION

The quantifications in this study were based on the peak area of DLX from the average of three replicate measurements. In an effort to optimize the main parameters controlling the method performance, a one at a time approach was applied and a 500.0 ng ml<sup>-1</sup> concentration of DLX was used in optimization. Blanks were run periodically during the analysis to prove the absence of possible contaminants. In order to calculate enrichment factor (EF), the following equation was employed:

$$EF = C_{sed}/C_0$$

In this equation, C<sub>sed</sub> and C<sub>0</sub> denote the concentration of the target analyte in the enriched phase and its initial concentration in the aqueous phase, respectively. C<sub>sed</sub>, for



**Fig. 1.** Schematic diagram of IL-UA-ISFME-RP-HPLC in the determination of DLX.

extractor solvent, was measured using the calibration graph obtained from direct injection of DLX in enriched phase. For calculating extraction recovery (ER), the next equation was used:

$$ER = C_{sed} \times V_{sed} / C_0 \times V_{aq}$$

where  $V_{sed}$  and  $V_{aq}$  are the volume of the sediment phase and the volume of the aqueous sample, respectively.

### **Selection of Hydrophilic Ionic Liquid and Ion-pairing Agent**

Herein, a water-immiscible hydrophobic IL is made by addition of a water-miscible hydrophilic IL to sample including an ion-exchange reagent. In the interest of selection a desired water-miscible IL, the following criteria should be considered: (1) hydrophilic feature of IL (2) density of the in situ formed hydrophobic IL (3) extraction capability of target analyte and (4) chromatographic

compatibility. ILs containing  $\text{Cl}^-$ ,  $\text{BF}_4^-$  and  $\text{CF}_3\text{SO}_3^-$  provide hydrophilic property and ILs involving  $\text{PF}_6^-$  and  $(\text{CF}_3\text{SO}_2)_2\text{N}^-$  present hydrophobic manner. Ionic compounds consisting  $(\text{CF}_3\text{SO}_2)_2\text{N}^-$  are high-cost and others containing  $\text{BF}_4^-$  are pretty low-cost. 1-Butyl-3-methylimidazolium tetrafluoroborate  $[\text{Bmim}][\text{BF}_4]$  and 1-hexyl-3-methylimidazolium tetrafluoroborate  $[\text{Hmim}][\text{BF}_4]$  ILs, fulfilling the mentioned prerequisites, were preferred for optimization procedure. These ILs reveals satisfactory hydrophilic possessions and are well-designed for ISFME. However,  $[\text{Hmim}][\text{BF}_4]$  IL provided relatively low extraction recovery in comparison with  $[\text{Bmim}][\text{BF}_4]$  IL. So,  $[\text{Hmim}][\text{BF}_4]$  IL was removed from other experiments. Additionally, the density of the in-situ formed hydrophobic IL must be higher than that for water, in order to collect it at the bottom of the test tube. Lastly, to attain a balance between these facts, 1-butyl-3-methylimidazolium tetrafluoroborate  $[\text{Bmim}][\text{BF}_4]$  IL was chosen as an optimum hydrophilic IL. By dissolving this IL in aqueous phase containing  $\text{NaPF}_6$  (ion-pairing agent), it is likely to gain hydrophobic 1-butyl-3-methylimidazolium hexafluorophosphate  $[\text{Bmim}][\text{PF}_6]$  IL acting as the extraction solvent.

### Effect of Hydrophilic IL Amount

The influence of  $[\text{Bmim}][\text{BF}_4]$  IL dosage on the efficiency of the method was investigated. This factor was studied in the range of 30-110 mg. As shown in Fig. 2, stable and sensitive responses were gained by using 60 mg of  $[\text{Bmim}][\text{BF}_4]$  IL. It is clear that by rising the amount of  $[\text{Bmim}][\text{BF}_4]$  IL, the volume of the *in situ* formed hydrophobic IL significantly enlarged reasoning a major decrease in analytical signals. Hence, a value of 60 mg was used for all experiments.

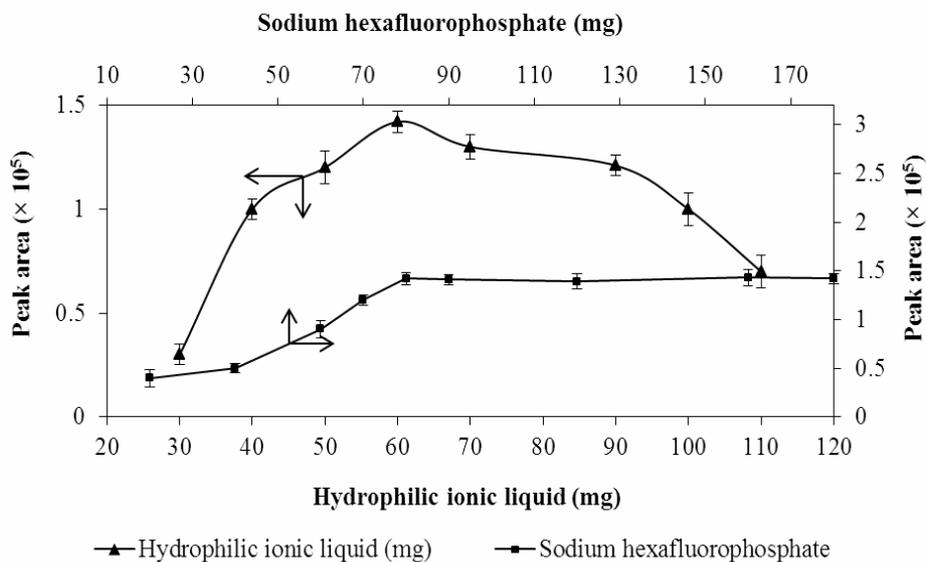
### Effect of $\text{NaPF}_6$ and Salt Addition

To produce water-immiscible  $[\text{Bmim}][\text{PF}_6]$  IL, extra amount of  $\text{NaPF}_6$  was dissolved in sample solution. The impact of  $\text{NaPF}_6$  quantity was evaluated in the range of 20-180 mg. As shown in Fig. 2, by increasing the amount of this reagent, the peak areas of DLX boosts up and after 80

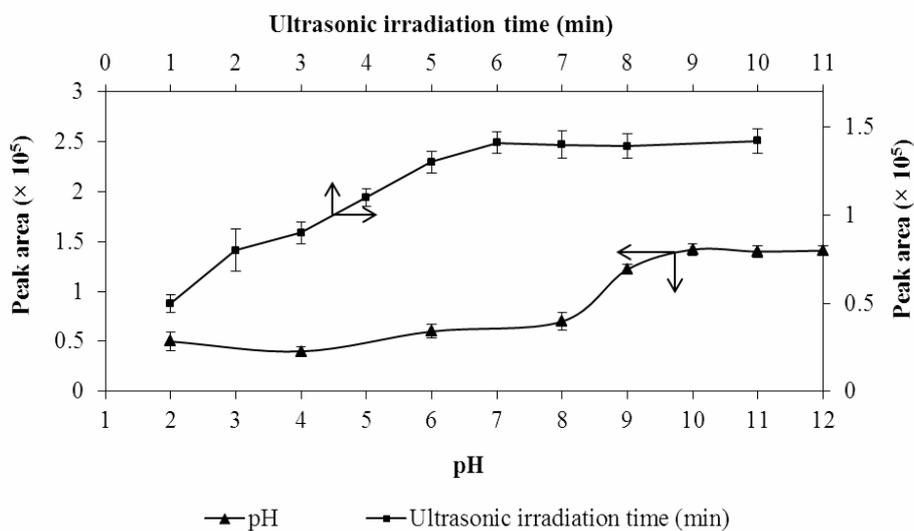
mg, it gains a steady form. In the end, in order to achieve stability between analytical sensitivity and repeatability, 80 mg of this agent was chosen as an optimum value. On the basis of literature survey, a small change in the salt content of the sample causes an extensive alter in the solubility of IL [27]. Thus, the presentation of sample pretreatment method is considerably affected by variation of ionic strength of sample media. Herein, to figure out this problem, a hydrophobic water-immiscible IL is formed by addition of a hydrophilic water-miscible IL to the sample solution containing an ion-exchange reagent. Accordingly, the analyte is extracted and enriched meanwhile the formation of tiny drops of hydrophobic IL. To this end, 1-butyl-3-methylimidazolium tetrafluoroborate  $[\text{Bmim}][\text{BF}_4]$  and sodium hexafluorophosphate ( $\text{NaPF}_6$ ) were selected as water-miscible IL and ion-exchange reagent, respectively. It should be noted that there is no indicative interface between the aqueous media and the extraction phase. Thereby, mass transfer from aqueous phase into extracting solvent has no meaningful influence on the efficiency of the microextraction method. Owing to the presence of excess amount of ion-exchange reagent in sample solution, which acts as a common ion, the solubility of IL plunges. Given this circumstance, the volume of the in situ formed IL is not affected by variations in the ionic strength [28]. The impact of salt concentration on the peak area of DLX was tested within the range of 0-20% (w/v) by  $\text{NaCl}$ . This factor has no significant impact on the analytical signals indicating the method robustness against high content of salt.

### Effect of Sample pH

It is well-known that pH has a reasonable effect on the extraction of ionizable compounds from aqueous media [29]. The extraction yield of ionic form of natural analyte is not satisfactory. In order to gain the best acquiesce of extraction, the uncharged form of DLX must be prevalent. The impact of sample pH was tested in the range of 2-12 using  $\text{HCl}$  and  $\text{NaOH}$  ( $10^{-2}$  M of each, by a micropipette). As shown in Fig. 3, stable and sensitive signals were achieved at pH 10, which is well-matched with equilibrium constant of DLX ( $\text{pK}_a = 9.7$ ) [30]. Hence, pH value of 10 was preferred for all experiments.



**Fig. 2.** Influence of [Bmim][BF<sub>4</sub>] and NaPF<sub>6</sub> dosage on extraction efficiency. Experimental conditions: DLX concentration: 500.0 μg l<sup>-1</sup>; pH: 10; ultrasonic irradiation time: 6 min; sample volume 5 ml.

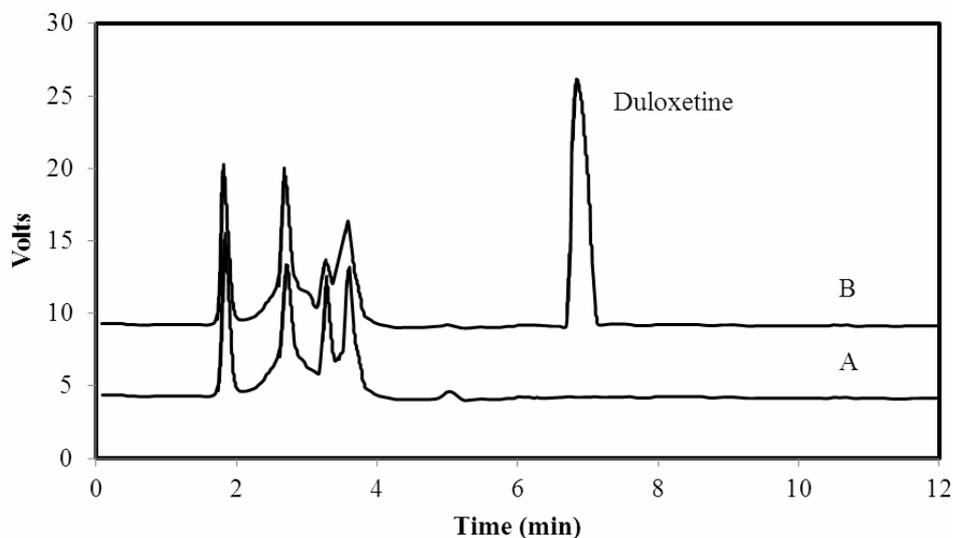


**Fig. 3.** Influence of pH and ultrasonic irradiation time on the extraction performance. Experimental conditions: DLX concentration 500.0 μg l<sup>-1</sup>; dosage of ion-pairing reagent 80 mg; dosage of [Bmim][BF<sub>4</sub>] IL 60 mg; sample volume 5 ml.

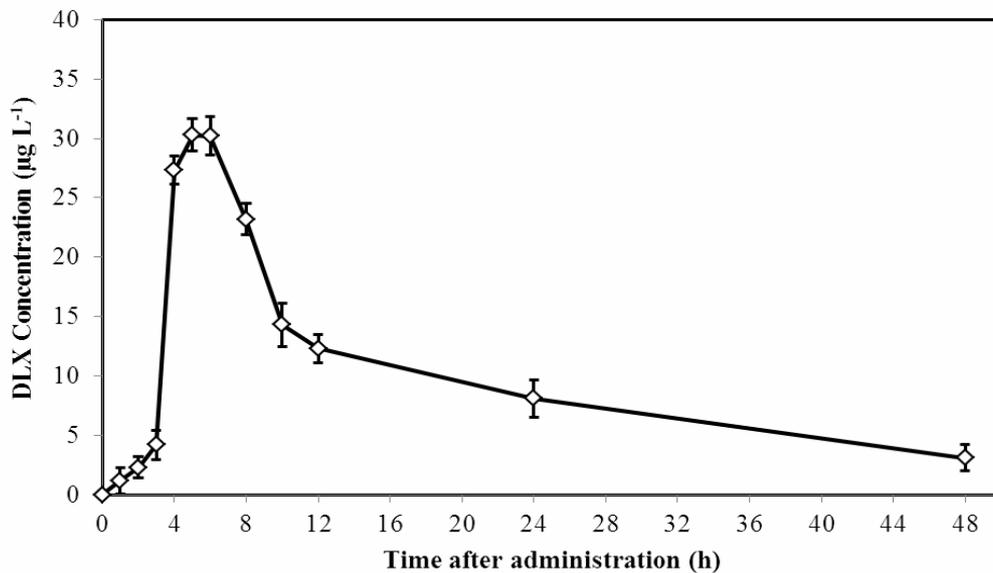
### Effect of Ultrasonic Time

Sufficient sonication time guarantees IL to disperse completely through the sample solution ensuring higher

extraction efficiency; it can improve mass transfer process. The mass transfer in IL-UA-ISFME is a process depending on equilibrium rather than exhaustive extraction, and to



**Fig. 4.** Typical chromatograms of DLX in human plasma; A: blank plasma; B: spiked plasma with 500.0  $\mu\text{g L}^{-1}$  of DLX.



**Fig. 5.** DLX concentration profile in plasma *versus* time after the single oral administration.

obtain acceptable repeatability, it is needful to choose a suitable extraction time [31]. In this context, ultrasound radiation was applied as a disperser agent, which

remarkably enhances the extraction yield and acceleration of the target analytes migration into the tiny droplet of [Bmim][PF<sub>6</sub>] IL. Herein, the sonication time profiles were

investigated in the range of 1-10 min at the power of 50 W. As shown in Fig. 3, by increasing the values up to 5 min, the extraction efficiencies rose rapidly and afterwards remained relatively constant. It is noteworthy that longer extraction time can result in the loss of the extracting solvent and contraction of the extraction yields. Consequently, the exposure time of 6 min was selected as an optimum value in the subsequent experiments.

### Analytical Performance

To assess the applicability of the method, calibration curves were plotted at the optimum conditions using spiked levels of the analytes. The limit of detection (LOD) based on the signal-to-noise ratio (S/N) of 3, the limit of quantitation (LOQ) based on the signal-to-noise ratio (S/N) of 10, the determination coefficients ( $r^2$ ), the linear range (LR) and the relative standard deviations (RSD) were calculated. LOD and LOQ were  $0.8 \mu\text{g l}^{-1}$  and  $2.0 \mu\text{g l}^{-1}$ , respectively, while linearity range varied in the range of  $2.0\text{-}1500 \mu\text{g l}^{-1}$  with correlation coefficient of 0.996. The typical chromatograms for blank and spiked human plasma shown in Fig. 4 indicate that there was no important interference in the whole analytical process. It is noteworthy that satisfactory compatibility of the proposed sample preparation method with the chromatographic analysis is observed in the chromatograms.

### Precision and Accuracy

The intra-day and inter-day precision at four concentration levels (5, 150, 500 and  $1250 \mu\text{g l}^{-1}$ ) for the QC samples are shown in Table 1. As illustrated, the intra-assay RSD determined at 5 runs in one day was 4.0-5.1%. Also, the inter-assay precision investigated on a three-day period with a total of 9 runs revealed RSD values in the range of 4.3-7.6%. As seen in Table 2, ER% and EF were in the range of 83.6-92.1% and 34.6-36.8, respectively.

### Application to Pharmacokinetic Study

For demonstrating the flexibility of the method in multipurpose analytical applications, the proposed approach was utilized to determine DLX in human plasma and achieving the main pharmacokinetic data. The presented

method under optimum conditions was employed to measure DLX concentration in human plasma. Oral administration of a 60 mg single dose was carried out to three healthy male volunteers. The blood samples of the volunteers were collected at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24 and 48 h after DLX tablet administration. The mean plasma concentration-time curve is shown in Fig. 5. The main pharmacokinetic parameters including  $T_{\text{max}}$ ,  $C_{\text{max}}$ ,  $\text{AUC}_{0\text{-}t}$ ,  $\text{AUC}_{0\text{-}\infty}$  and  $T_{1/2}$  are summarized in Table 3.

### Comparison with other Methods

A comparison of the method with formerly reported techniques for the analysis of DLX is brought in Table 4. It is clear that the LOQ of IL-UA-ISFME-HPLC-UV is better than the methods with the exception of protein precipitation-liquid chromatography-mass spectrometry (PP-LC-MS). As seen, the current protocol gives the best LR. Moreover, alongside the simplicity and low cost, an extra advantage of IL-UA-ISFME-HPLC is short extraction time. The data revealed a significant improvement in RSD excluding ultra-performance liquid chromatography (UPLC). A positive characteristic of applying ILs as extracting solvents is their stability over a wide temperature range and extremely low vapor pressure at relatively high temperatures; the advantages that stand out ILs from conventional organic solvents. These features are of great interest for the routine laboratories in the trace determination of DLX in plasma samples.

## CONCLUSIONS

The aim of the present study was to develop and validate a rapid, sensitive, robust and reliable method, IL-UA-ISFME combined with HPLC-UV for the quantitative determination of DLX in human plasma. The results obtained with the method described above indicated that it is a good alternative extraction technique for the drugs in plasma and offers highly interesting advantages from an analytical point of view; such as possibility of extracting and pre-concentrating of the analyte in the complex matrices. Because of the extremely low solubility of water in the hydrophobic IL, residual salinity of the real matrix is negligible. The mass transfer of the analyte also speeded up

**Table 1.** Intra-day and Inter-day Precision for the Determination of DLX in Spiked Human Plasma Samples

Compound	Intra-day, <i>n</i> = 5				Inter-day, <i>n</i> = 9		
	Concentration ( $\mu\text{g l}^{-1}$ )	Found value ( $\mu\text{g l}^{-1}$ ) <sup>a</sup>	RSD (%)	Accuracy (%)	Found value ( $\mu\text{g l}^{-1}$ ) <sup>a</sup>	RSD (%)	Accuracy (%)
DLX	5	4.6	5.0	8.0	4.5	4.7	6.0
	150	143.2	4.6	4.5	139.9	7.6	6.7
	500	514.0	5.1	2.8	470.5	3.9	5.9
	1250	1158.9	4.0	7.3	1153.1	4.3	7.7

<sup>a</sup>The average of three independent measurements.**Table 2.** Extraction Recovery and Enrichment Factor in the Proposed Method for the Determination of DLX in Human Plasma

DLX concentration ( $\mu\text{g l}^{-1}$ )	Plasma sample	
	ER <sup>a</sup> $\pm$ SD (%) <sup>b</sup>	EF <sup>c</sup>
5	83.6 $\pm$ 4.0	34.6
150	92.1 $\pm$ 4.1	36.8
500	90.7 $\pm$ 4.7	36.2
1250	91.2 $\pm$ 3.0	36.4

<sup>a</sup>Extraction recovery. <sup>b</sup>Standard deviation, *n* = 3. <sup>c</sup>Enrichment factor.**Table 3.** Pharmacokinetic Parameters of the Drug in Human Plasma after Oral Administration of 60 mg Dosage of DLX Tablet

Pharmacokinetic parameters	DLX (60 mg)	
	Mean	S.D.
T <sub>max</sub> (h)	5.1	0.7
C <sub>max</sub> ( $\mu\text{g l}^{-1}$ )	27.5	8.1
AUC <sub>0-48</sub> (ng h ml <sup>-1</sup> )	385.3	56.4
AUC <sub>0-∞</sub> (ng h ml <sup>-1</sup> )	412.9	65.8
T <sub>1/2</sub> (h)	10.9	1.6

T<sub>max</sub>: Time required for reaching maximum plasma concentration. C<sub>max</sub>: Maximum plasma concentration. AUC<sub>0-48</sub>: Area under curve. AUC<sub>0-∞</sub>: Area under curve at infinite time. T<sub>1/2</sub>: Time required for reaching to half Concentration.

**Table 4.** The Comparison of IL-UA-ISFME-RP-HPLC-UV with Previously Reported Methods for Quantification of DLX

Method	LOQ (ng ml <sup>-1</sup> )	LR (ng ml <sup>-1</sup> )	RSD (%)	Ref.
SPME-HPLC-UV <sup>a</sup>	16	16-2000	15.0	[33]
UPLC <sup>b</sup>	20	20-5000	1.5	[34]
PP-LC-MS <sup>c</sup>	0.8	0.8-100	5.4	[35]
DLLME-SFO-HPLC-FLD <sup>d</sup>	2.5	2.5-200	5.9	[36]
LLE-HPLC-UV <sup>e</sup>	5	5-2000	5.1	[37]
IL-UA-ISFME-RP-HPLC	2	2-2500	3.9	Represented work

<sup>a</sup>Solid phase microextraction-high performance liquid chromatography-ultraviolet detection. <sup>b</sup>Ultra performance liquid chromatography. <sup>c</sup>Protein precipitation-liquid chromatography-mass spectrometry. <sup>d</sup>Dispersive liquid-liquid microextraction solidification of floating organic solvent high-performance liquid chromatography-fluorescence detection. <sup>e</sup>Liquid-liquid extraction-high performance liquid chromatography ultraviolet detection.

through sonication, providing high recovery and enrichment factor while significantly shortage the entire analysis time. Good linearity and precisions were also obtained. Applicability of the method in a pharmacokinetic study was successfully demonstrated. Work is in progress to further enhancements.

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