Off-line Coupling of Ionic Liquid-Based Dispersive Liquid-Liquid Microextraction to HPLC-UV Method for the Drug Analysis in Pharmaceutical and Biological Samples

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In this study, an easy, fast, sensitive and accurate technique is described for extraction and quantitative analysis of fluoxetine and propafenone using off-line coupling of ionic liquid-based dispersive liquid-liquid micro-extraction with high performance liquid chromatography. The effective extraction variables including: the ionic liquid volume, the type and volume of dispersive solvent, the pH, the extraction and centrifugation time, and the volume of diluent solvent have been investigated and optimized. The optimum chromatographic conditions were also obtained for the drugs determination. Under optimum conditions, the analytical curves were linear ($r > 0.999$) within a wide concentration range (0.01-2.00 µg ml^{-1}). Relative standard deviations (precision) and detection limits for both drugs have been smaller than 5% and 0.005 µg ml^{-1}, respectively. The proposed method has been used successfully to detect and determine fluoxetine and propafenone in the capsule formulation and the spiked plasma samples; respectively, with the quantitative recovery results (94-97%).

INTRODUCTION

Fluoxetine hydrochloride, N-methyl-3-phenyl-3-[(4-(trifluoromethyl)phenoxy)]propan-1-amine hydrochloride is one of the anti-depressant drugs, which acts *via* inhibition of the serotonin uptake through neurons in brain. It is applied to treat depression, bulimia nervosa and obsessive-compulsive disorder. Fluoxetine would be consumed as capsule, tablet, or as oral solution with dosages of 20-60 mg per day [1-3]. Propafenone, 1-[2-[2-Hydroxy-3-(propylamino) propoxy]phenyl]-3-phenyl-1-propanone is a prominent antiarrhythmic drug for supra-ventricular and ventricular arrhythmias. It is a class Ic antiarrhythmic medicine acting on the Nav 1.5 and KCNH2 (hERG) ion channels, and possesses weak β-blocking impacts [4-6].

Drug manufacturing control and pharmacokinetic studies require to intensive analytical and chemical supports of each stage for ensuring the quality, safety and quantitative amount of drug. Several analytical techniques such as high performance liquid chromatography (HPLC) [7-9], gas chromatography (GC) [10-12] and spectrofluorimetry [13,14] have been reported for determination of fluoxetine in different samples. Also, there are some bio-analytical methods which describe the assessment of propafenone such as HPLC [15-17], liquid chromatography-mass spectrometry (LC–MS) [18], and spectrometry [19]. However, in order to decrease the waste of solvents and material, analysis time and costs, it is still necessitated introduction of developed sample preparation techniques in combination with the present methods.

Sample preparation is a major step in drug analysis. It depends on the sample matrix and levels of concentration of the target analytes. Nowadays, green sample preparation is one of the most challenging features in analytical chemistry.

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Dispersive liquid-liquid micro-extraction (DLLME) is one of the environmental-friendly extraction technique for preparing samples that was first introduced by Rezaee and co-workers in 2006 [20]. It is a ternary solvent system, being easy, rapid and inexpensive. DLLME begins with a fast injection of a mixture of a little amount of an extracting solvent and a dispersive solvent. It should be significantly miscible with the extracting solvent and the aqueous phase. DLLME has been used in various fields of chemistry [20-25].

Ionic liquids (ILs) are salts containing organic cations and various anions which have been introduced as green solvents. They are less toxic than other organic solvents and also due to their capacity to dissolve different compounds can be used in extraction techniques. Moreover, the capability to control the properties of ILs by changing their structure is a valuable advantage. Today, IL-based DLLME has been designed as an effective technique to extract various compounds [26-30].

In present research, an IL-DLLME process has been developed and optimized for extraction/pre-concentration of fluoxetine and propafenone from pharmaceutical and biological samples. ILs with the imidazolium cations and hexafluorophosphate [PF_6] anion are water-immiscible and useful in liquid-liquid extraction [31]. Herein, 1-hexyl-3-methylimidazolium [HMIM] cation with [PF_6] anion played the role of an extracting solvent in DLLME technique for extracting and pre-concentrating of the both drugs.

Accuracy, precision, detection limit, and linearity have been the variables employed for validating the technique. This research showed significant benefits such as: low extraction time, high enrichment factor, low volume of [HMIM][PF6] as IL (<80 µl), and fast determination by HPLC. On the other hand, the higher selectivity was also achieved due to combination of two stages of extraction and determination in the proposed method.

EXPERIMENTAL

Chemicals and Materials

Fluoxetine (99.5%) and propafenone (99%) were purchased from Aldrich and Fluka, respectively; and were used without further purification. Standard stock solutions (100 µg ml^{-1}) were procured *via* dissolution of appropriate amounts of drugs in mobile phases (methanol-water, 65:35 for fluoxetine; and acetonitrile-water, 60:40 for propafenone). The HPLC grade solvents were obtained from Merck and [HMIM][PF_6] was supplied from Aldrich. All of reagents were analytical quality and also the deionized water was used.

DLLME Procedure

A 10.0 ml of deionized water containing 1 µg ml^{-1} of drug (fluoxetine or propafenone) was transferred to a glass testing tube with conical bottom. The required pH was adjusted by adding sodium hydroxide solution (0.1 M). A mixture of acetone (disperser solvent) with [HMIM][PF_6] (extracting solvent) was injected quickly using a syringe into a sample solution. The obtained cloudy solution was allowed to stand for a few minutes (extraction time) and after centrifuging at 4000 rpm, IL–rich phase was deposited at the bottom of conical test tube. Then, the upper aqueous phase was drawn out with a syringe; the IL phase was separated and dissolved in methanol (diluent solvent) and was injected into HPLC system for drug determination. A schematic diagram of the extraction procedure employed is shown in Fig. 1.

Instrumentation and Chromatographic Conditions

In this project, for determination of fluoxetine; a HPLC system (Waters model 600E, U.S.A) equipped with a UV detector (2487 waters, setting at 254 nm for fluoxetine), helium degassing, autoinjector (Rheodyne model, 7125i U.S.A) with 10 µl loop, and a Novapak C18 column (Waters, Ireland) [150 × 3.9 mm id, 5.0 µm particle size] has been applied.

For quantification of propafenone, an Agilent 1200 series HPLC instrument (Agilent Technologies, Germany) composed of an online degasser, a quaternary pump, and a UV detector (setting at 280 nm) was used. All data were collected and analyzed using Agilent 1200 series HPLC Chemstation Software (Version B02.01).

The mobile phases consisted of methanol in water (65:35) for fluoxetine and acetonitrile in water (60:40) for propafenone were filtered using PTFE membrane filter, and Supor-450 (Model Waters Corporation, U.S.A) and was flowed at an isocratic mode. Sample solutions were injected *via* a syringe loading sample injector (model Millex-LCR,
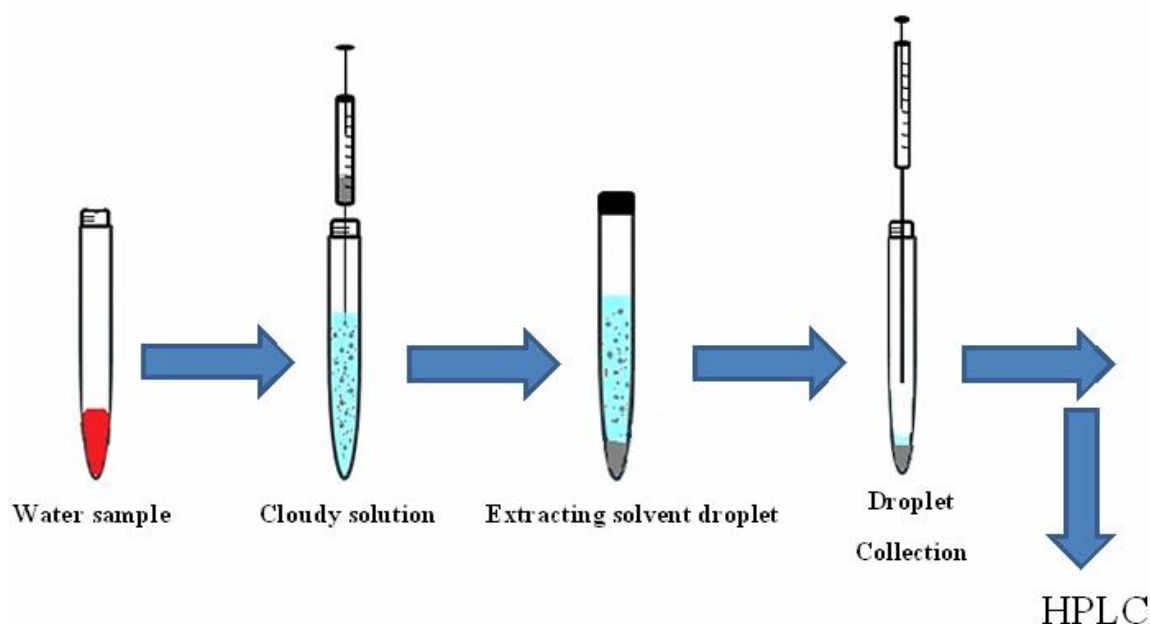
Fig. 1. A schematic diagram of the extraction procedure.

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Validity

International Conference on Harmonization Guidelines for validation of analytical procedure has been followed for the proposed method with considering the parameters: accuracy, precision linearity and limit of detection [32].

Determination of Fluoxetine and Propafenone in Real Samples

A total of 10 capsules of fluoxetine (pharmaceutical sample) were weighed and thoroughly grounded to a fine powder and an amount of it, equivalent to average weight of capsules, was transformed to a volumetric flask. The powder was dissolved in the mobile phase and shaked for a few minutes, and finally filtered.

10 ml of human plasma as a biological sample was obtained from a healthy person and then, various levels of propafenone were added to it. The pharmaceutical and biological sample solutions were exposed to analysis via the recommended method.

RESULTS AND DISCUSSION

In order to obtain high extraction efficiency, the effect of variables affecting extraction and chromatographic conditions were investigated and optimized. The extraction efficiency (Recovery) was calculated as:

$$\%R = \frac{C_{sed}V_{sed}}{C_0V_{aq}} \times 100$$

where, C_{sed}, C_0, V_{sed} and V_{aq} are the analyte concentration in the sediment, the initial analyte concentration in the aqueous samples, the volume of the sediment phase and the volume of the aqueous sample, respectively [20].

Optimum Chromatographic Conditions

In this study, an off-line coupling of IL-DLLME to HPLC has been developed and validated for determination of fluoxetine and propafenone in various samples. Chromatographic conditions should be optimized until retention and separation of analytes to be possible with a reverse phase column.

Based on the obtained results, a Nova-Pak C18 column showed a higher resolution and peak symmetry compared with other tested columns such as µBondapak C18, Symmetry C18, Finepak SIL C18, Reprosil C18 and Zorbax: Eclipse XDB. The isocratic mobile phases of methanol-water (65:35, v/v%) for fluoxetine and
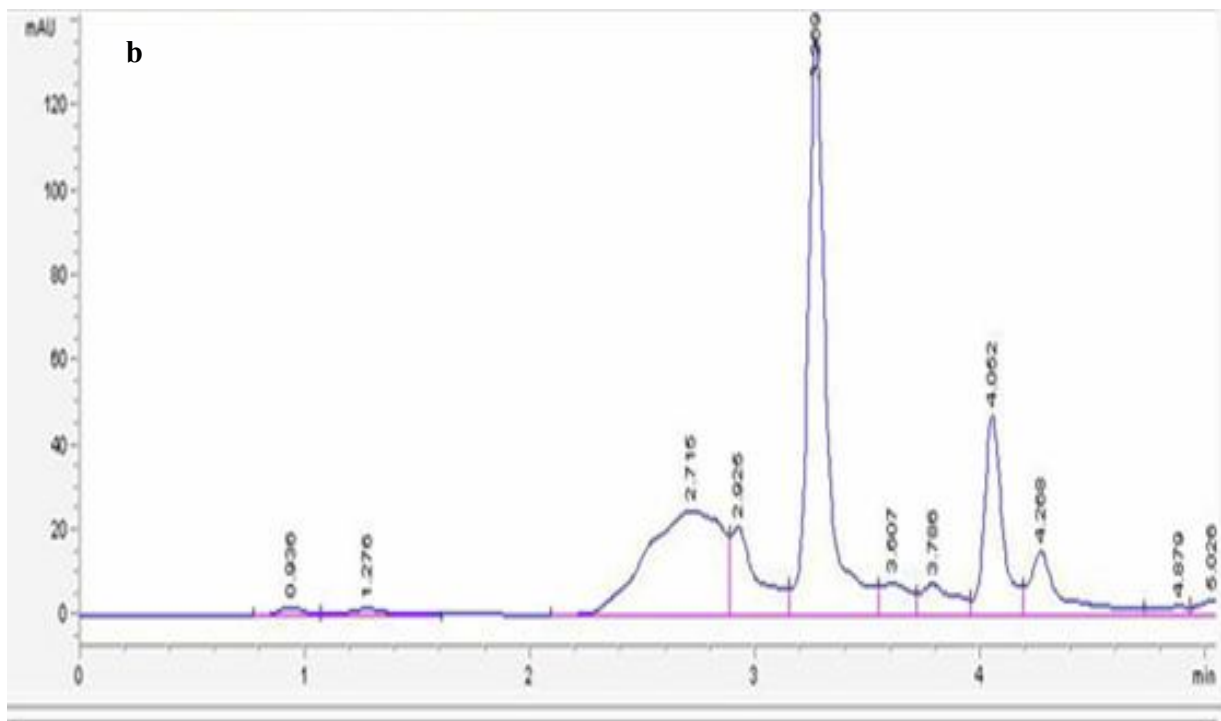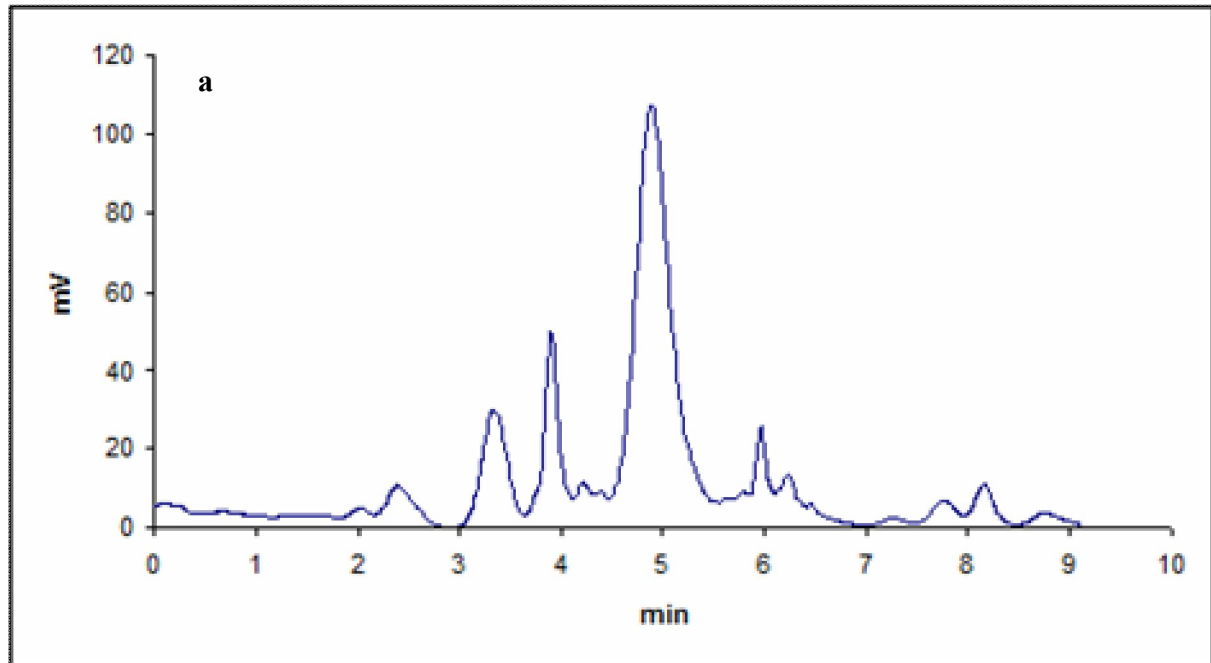
Fig. 2. Chromatogram of fluoxetine (a) and propafenone (b) in spiked samples.

acetonitrile-water (60:40, v/v%) for propafenone with a flow rate 1.0 ml min^{-1} were the suitable compositions for the analysis. The mobile phase buffer additives were also used which unsymmetrical peaks were obtained.

The chromatograms obtained under the mentioned optimum conditions for the spiked samples are shown in Fig. 2.

Optimization of IL-DLLME Procedure

Effect of dispersive solvent type and volume. Dispersive solvent must be miscible with both the aqueous and organic phases. In order to obtain the best solvent, acetonitrile, methanol, acetone, and ethanol were tested. Findings demonstrated that acetone gave better extraction efficiency than other solvents (data not shown). Acetone was also selected and used as a dispersive solvent in DLLME technique for extraction of other compounds [20, 29].

The effect of dispersive solvent volume on the analyte extraction was studied in the range of 0.5-2.5 ml. According to the curves of Fig. 3, 1.5 ml of acetone presented the best recoveries for the two drugs. Thus, this volume was selected for the following research.

Effect of [HMIM][PF$_6$] volume (extracting solvent). [HMIM][PF$_6$] has been applied as an extracting solvent in different separation methods. It provides advantages such as good performance, water-immiscibility, high hydrophobicity and viscosity for separation and preconcentration [28,33].

In this work, [HMIM][PF$_6$] was used as an extracting solvent in combination with acetone. The best combination of dispersive and extraction solvent has a major impact on DLLME. After choosing the appropriate combination of solvents, various volumes of [HMIM][PF$_6$] were investigated in the range of 20-100.0 μl. According to Fig. 4, by increasing the IL up to 80.0 to 100.0 μl, the extraction efficiency increased and then reduced. Therefore, this range of [HMIM][PF$_6$] was used in following experiments.

Effect of the sample pH. The optimum pH for IL-DLLME process depends on the chemical nature of pharmaceutical analytes. Since the studied drugs are basic compounds, the best recoveries have been predicted to be at basic pHs, at which they are non-ionized.

The effect of sample pH was studied (Fig. 5). As shown in Fig. 5, the pHs 8.5 and 8 offered the best recoveries for fluoxetine and propafenone, respectively. The low extraction efficiency was seen at pHs higher than optimum values. This can be probably due to the degradation of the formed micelle in higher pHs. Thus, pH 8.5 for fluoxetine and pH 8 for propafenone were found to be the optimum values.

Effect of duent solvent. One of the major criteria to select the diluent solvent is compatibility with mobile phase. In this study, methanol was used as diluent solvent. Under the optimized conditions, various volumes of methanol (0.1-0.5 ml) containing IL-rich of fluoxetine or propafenone were injected to HPLC system. According to Fig. 6, 0.3 and 0.4 ml of methanol were selected as optimum volumes for fluoxetine and propafenone, respectively.

Effect of the extraction and centrifugation time. For a complete and fast separation, the extraction and centrifugation (4000 rpm) time must be investigated and optimized. In DLLME, extraction time is defined as an interval time between injection of mixture of dispersive and extraction solvent and centrifugation process. The effect of these variables upon extraction efficiency were studied in the range of 1-9 min (data not shown). The recovery results increased up to 5 min, and then were constant. Therefore, 5 min was selected and used as the extraction and centrifugation time for the entire procedure.

Method Validation

The analytical curves for fluoxetine and propafenone standards were established via drawing the area under the curve of the primary peak vs. drug concentrations. Findings revealed that curves are linear between a wide concentration range (0.01-2.00 μg ml^{-1}) having a correlation coefficient (r) higher than 0.99. The straight-line equations achieved from experimental results are reported as below for fluoxetine (1) and propafenone (2):

$$Y = 10542X + 1150 \ (r = 0.9991) \qquad (1)$$

$$Y = 1356.6X + 72.8 \ (r = 0.9984) \qquad (2)$$

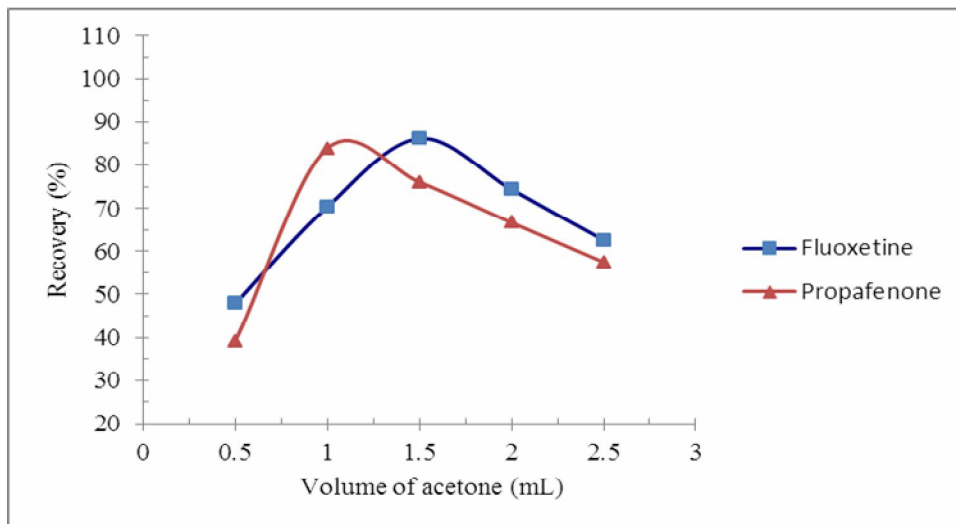Precision represents the variability in repeated examinations

Fig. 3. Effect of acetone volume (as dispersive solvent) on the HPLC peak area of fluoxetine and propafenone.
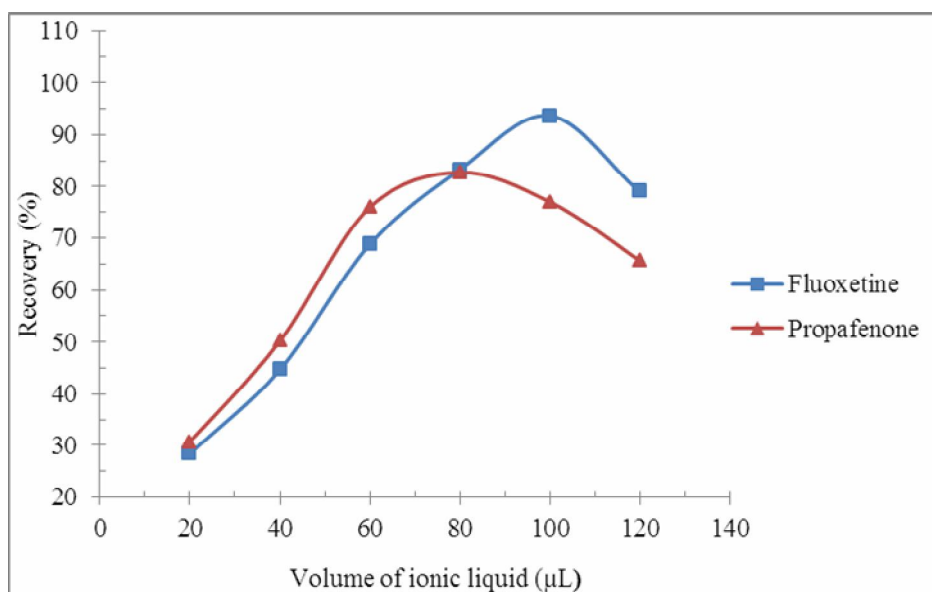

Fig. 4. Effect of [HMIM][PF6] volume (as extracting solvent) on the HPLC peak area of fluoxetine and propafenone.

of the samples under the same experimental conditions. It has been computed from an average of 10 determinations of homogeneous samples which relative standard deviations (RSDs), 2.1 and 4.6% obtained for fluoxetine and propafenone, respectively. The RSDs showed that the proposed method presented an acceptable precision.

Limit of detection (LOD) has been described as $3S_b/m$ where S_b represents standard deviation of the blank and m
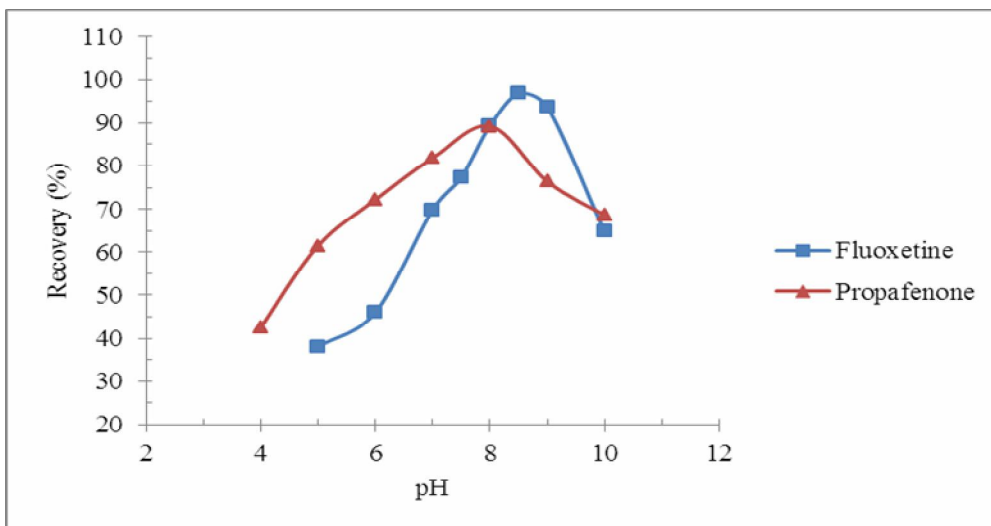
Fig. 5. Effect of pH on the HPLC peak area of fluoxetine and propafenone.
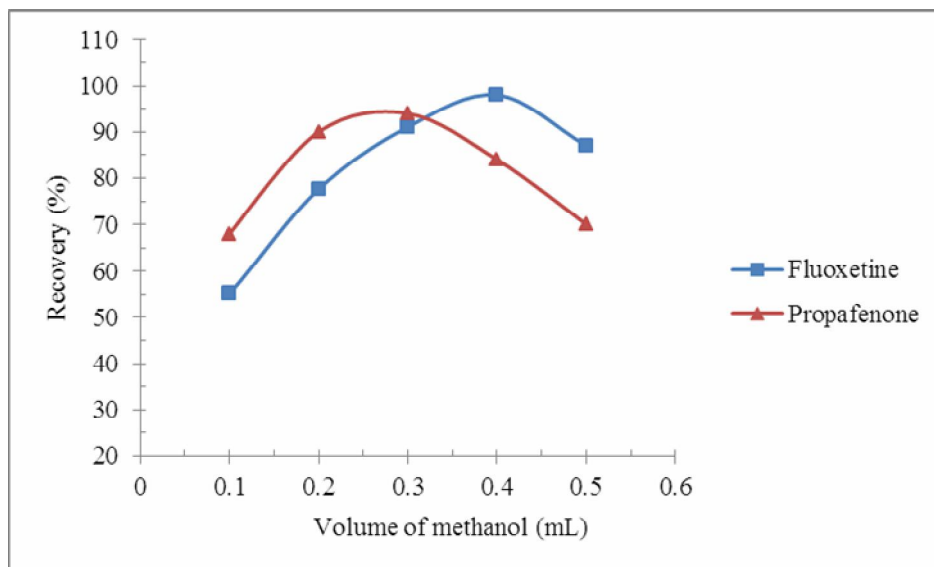

Fig. 6. Effect of methanol volume (as diluent solvent) on the HPLC peak area of fluoxetine and propafenone.

refers to the calibration curve slope. LOD has been calculated to be 0.005 $\mu g\ ml^{-1}$ for the both drugs.

Analysis of Real Samples

For evaluating usability of the recommended technique, it was employed to fluoxetine and propafenone determinations in pharmaceutical and biological samples.

Capsule of fluoxetine and the spiked plasma sample with propafenone were examined (Table 1).

According to the results reported in Table 1, mean recoveries ranged within 94.0-97% that these values are similar to those of IL-DLLME technique used for pre-concentration of other compounds [26-30]. The recovery results demonstrated accuracy and non-interference of the

Table 1. Analysis of Fluoxetine and Propafenone in Real Samples Using the Proposed Method (IL-DLLME-HPLC)

Sample	Amount labeled (mg)	Amount found[a] (mg)	Recovery (%)
Capsule (Fluoxetine)	10.00	9.70 ± 0.10	97
Plasma (Propafenone)	Added (μg ml^{-1})	Found (μg ml^{-1})[a]	Recovery (%)
	0.50	0.47	94
	1.00	0.96	96

[a]Mean ± standard deviation (n = 3).

Table 2. Comparison of Analytical Parameters of the Proposed IL-DLLME-HPLC Method with Various Methods for the Fluoxetine and Propafenone Determination

Method	LDR (μg ml^{-1})[a]	LOD (μg ml^{-1})	LOQ (μg ml^{-1})[b]	RSD (%)	Recovery (%)	Ref.
Fluoxetine						
SPE[c]-HPLC	0.003-1.20		0.003	2.12-12.9	95.5	8
GC-MS	0.060-3.0	0.012	0.041	1.5	99-102	10
LLE[d]-Spectrofluorimetry	0.040-1.0	0.016	-	1	96-105	14
IL-DLLME-HPLC	0.010-2.00	0.005	0.02	2.1	97	This work
Propafenone						
LLE-HPLC	0.010-0.750	-	10	7.8	90.5	17
SPME-LC-MS	0.01-0.15	0.0005	0.001	6.65	-	18
Spectrophotometry	0.17-14.0	1.0	-	0.5-4.65	95-106	19
IL-DLLME-HPLC	0.01-2.00	0.005	0.02	4.6	94-96	This work

[a]Linear dynamic range. [b]Limit of quantification. [c]Solid phase extraction. [d]Liquid-liquid extraction.

sample matrix in determination of the selected drugs.

The analytical parameters for fluoxetine and propafenone determination obtained by the proposed method were comparable with literature values (Table 2). Furthermore, simple procedure make the sample preparation easy and rapid, as only a few minutes were required before

HPLC analysis.

CONCLUSIONS

This study described the application of [HMIM][PF$_6$] (IL) in miniaturized liquid extraction technique as off-line coupling of IL-DLLME to HPLC method for extraction/pre-concentration and determination of fluoxetine and propafenone in pharmaceutical and biological samples. The recommended technique was fast and sensitive with satisfactory recoveries which introduced an analytical potential for determination of trace amounts of two drugs.

Furthermore, low volume of solvents, short time of analysis and low cost were the other advantages of the proposed method. The analytical parameters of developed method were acceptable and comparable to those of other methods for the determination of both drugs. Application of this method to real samples showed the ability and utility of IL-DLLME-HPLC for pharmacokinetic studies, since quantitative extractions were achieved.

ACKNOWLEDGEMENTS

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