



ORIGINAL ARTICLE

Pre-concentration and determination of amitriptyline residues in waste water by ionic liquid based immersed droplet microextraction and HPLC

M.T. Hamed Mosavian^{a,*}, Z. Es'haghi^b, N. Razavi^c, S. Banihashemi^c

^aChemical Engineering Department, Ferdowsi University of Mashhad, Mashhad, Iran

^bDepartment of Chemistry, Payame Noor University, 19395-4697 Tehran, Islamic Republic of Iran

^cChemistry Department, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran

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Abstract This paper describes a new approach for the determination of amitriptyline in waste-water by ionic liquid based immersed droplet microextraction (IL-IDME) prior to high-performance liquid chromatography with ultraviolet detection. 1-Hexyl-3-methylimidazolium hexafluorophosphate ($[C_6MIM][PF_6]$) was used as an ionic liquid. Various factors that affect extraction, such as volume of ionic liquid, stirring rate, extraction time, pH of the aqueous solution and salting effect, were optimized. The optimal conditions were as follows: microextraction time, 10 min; stirring rate, 720 rpm; pH, 11; ionic drop volume, 100 μ L; and no sodium chloride addition. In quantitative experiments the method showed linearity in a range from 0.01 to 10 μ g/mL, a limit of detection of 0.004 μ g/mL and an excellent pre-concentration factor (PF) of 1100. Finally, the method was successfully applied to the determination of amitriptyline in the hospital wastewater samples.

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1. Introduction

Amitriptyline hydrochloride is a tricyclic antidepressant drug. It is approved most commonly for the treatment of major depression [1]. This drug is chemically basic and is in the form of hydrochloride salt (pK_a 9.4) in the market [2]. The function of these drugs is to block the reuptake of the neurotransmitters, norepinephrine and serotonin in the central nervous system [3].

The analytical methods described in the literature to analyze antidepressants in biological fluids usually use conventional sample pretreatment techniques that are laborious, time

*Corresponding author. Tel.: +98 5118805040; fax: +98 5118816840.

E-mail addresses: hmosavian@gmail.com,
mosavian@um.ac.ir (M.T. Hamed Mosavian).

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consuming and require large amounts of organic solvents [4]. Recently, many important procedures have been reported for the sample pretreatment. Among them, liquid phase microextraction has been developed successfully and achieved much more attention due to its advantages [5–12].

Single drop microextraction (SDME) is a mode of liquid phase microextraction (LPME) that provides analyte extraction in a few microliters of an organic solvent. SDME avoids some problems of the solid phase microextraction (SPME) method such as sample carry-over and fiber degradation. It is also quick, inexpensive and uses very simple equipments. In the SDME technique, a microdrop of an organic solvent is immersed in a stirred aqueous sample solution [13–14].

Although organic solvents (i.e., octanol, cyclohexane, toluene, etc.) are useful as an extractant phase, recently, the use of ionic liquids (ILs) has been proposed in SDME [15–20].

ILs are organic salts in the form of liquid at room temperature and have high boiling points. They have various advantages over traditional organic solvents, such as low vapor pressure, high stability, large viscosity, moderate solubility of organic compounds, adjustable miscibility and polarity, good extractability for different organic and inorganic compounds, as well as the possibility of using longer sampling time and larger droplet volume [16–18]. Moreover, owing to their low volatility, flammability and toxicity, ILs have been proposed as a good alternative to organic solvents, and are known as green solvents for extraction [21–22].

In this study, the application of IL-IDME in combination with high performance liquid chromatography and UV detector for determination of amitriptyline in water was examined.

2. Experimental

2.1. Chemicals and reagents

The drug, amitriptyline hydrochloride was obtained from Darou Pakhsh Co. (Tehran, Iran).

1-Hexyl-3-methylimidazolium hexafluorophosphate ([C6MIM][PF6]) which was used as an ionic liquid, HPLC grade methanol, acetonitril, sodium hydroxide and sodium chloride were purchased from Merck (Darmstadt, Germany).

2.2. Preparation of standard solutions

Stock solution of amitriptyline (1000 µg/mL) was prepared by dissolving a calculated amount of drug in methanol. It was stored at 4 °C in the refrigerator and protected from light. Fresh working solutions were prepared daily by diluting the stock solution in double distilled water.

Ultra-pure water was prepared in the lab using a Water Purification System (HUMAN POWER 1, Korea).

All glassware used in the experiments were cleaned with pure water, then soaked in 6 M nitric acid for 24 h and then washed with purified water. 0.4 M of sodium hydroxide and concentrated hydrochloric acid were used for adjusting the pH value of the water samples.

2.3. Instrumentation

Chromatographic separations were carried out on a Cecil 1100 HPLC pump (Cecil, England) and injector valve equipped

with a 20 µL sample loop (RHEODYNE, USA) and consisted of a CE 1200, Height performance Variable Wavelength Monitor Cecil 1100 UV/Vis detector. Chromatographic data were recorded and analyzed using a Cecil instrument Data Control.

A reversed-phase HiCHROM LiChrosorb RP8-10 C18 column (250 mm × 4.6 mm I.D., particle size 5 µm) was used for separation at ambient temperature (25 ± 0.5 °C). The column was equilibrated with the mobile phase (flow rate 1.0 mL/min); methanol/water/acetonitril (25/65/10, v/v). The injection volume was 25 µL, and the detection wavelength was 240 nm.

2.4. Ionic liquid microextraction procedure

IL-IDME procedure was performed according to the following manner. The sample solution (5.0 mL aqueous donor phase, adjusted to pH 11 with 0.4 M NaOH) was added to the glass vial and a magnetic bar (7 mm × 3 mm) was placed into the vial. An ionic liquid droplet (100 µL) was immersed into the stirred aqueous solution by a microsyringe. Then the mixture was agitated for 10 min at 720 rpm.

Before injecting into HPLC column, a part of the ionic liquid which was collected after extraction at the bottom of the vial (50 µL) was withdrawn into a syringe, diluted with 1.0 mL methanol–acetonitril (50%, v/v) for easier injection of the viscous extract into the HPLC and 25 µL of the diluted solution was injected into the LC column for analysis.

3. Result and discussion

In this study, ionic liquid single drop microextraction combined with HPLC/UV detection was developed for the determination of amitriptyline. There are several factors that affect the extraction process such as sample pH, volume of ionic liquid, stirring rate, extraction time, and salting effect, which were optimized as follows.

3.1. Effect of sample pH

As mentioned in several researches, the pH of aqueous feed solution which contains acidic or basic drugs should be controlled in the extraction process [23].

Amitriptyline is a basic compound (pKa 9.4) [24]; therefore the pH of the aqueous feed solution should be higher than the pKa of the analytes. In this condition, analytes are largely neutral and it is obvious that the neutral form of an organic compound has a greater tendency to be extracted into the ionic liquid compared to the ionized form.

The pH of the sample solutions was changed in the range 7–12 with the addition of NaOH solution (4 M). Fig. 1 shows that with increase of pH, the peak area increases and after pH=11 decreases slowly. It may be explained that at the beginning, the analyte was in the form of amitriptyline hydrochloride, by the addition of NaOH to the solution, the ionic form of analyte could be converted to a molecular form. In the pHs higher than 11.0, addition of NaOH causes salting in effect due to simultaneous production of NaCl in the solution. This effect reduces the extraction efficiency. Therefore, pH 11 was selected as an optimal pH value.

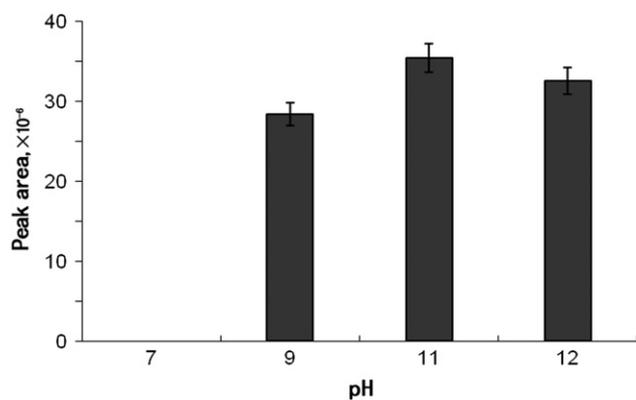


Figure 1 Effect of aqueous solution pH on the extraction of analyte into the drop. Experimental conditions are as follows: aqueous sample volume 5 mL concentration level at 1 $\mu\text{g}/\text{mL}$; 100 μL $[\text{C}_6\text{MIM}][\text{PF}_6]$; 480 rpm stirring rate; 5 min extraction time and 2.5 μL injection volume.

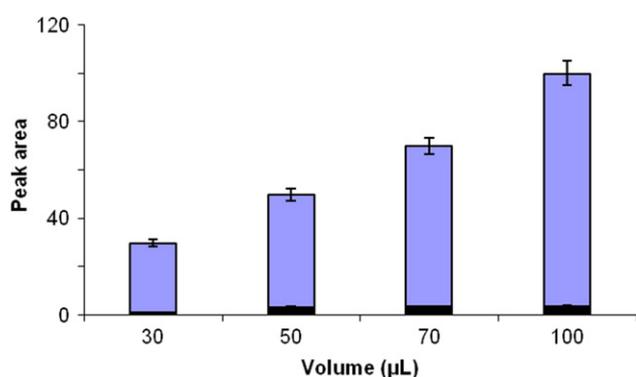


Figure 2 Effect of volume of ionic liquid on the extraction of amitriptyline. Experimental conditions are as follows: aqueous sample volume 5 mL concentration level at 1 $\mu\text{g}/\text{mL}$; sample pH 11; 480 rpm stirring rate; 5 min extraction time and 2.5 μL injection volume.

3.2. Effect of the volume of ionic liquid droplet

The volume of extraction solvent is a crucial parameter that seriously influences the extraction performance in the liquid phase microextraction. Theoretically, a larger volume of extraction solvent results in higher extraction efficiency.

In present study, the volume of extraction solvent was changed in the range 30–100 μL . The results are shown in Fig. 2. The results indicate that along with the increase of volume of ionic liquid, the peak area of amitriptyline increased and reached the largest one in 70–100 μL but collecting a part of a larger ionic liquid droplet was much easier. Hence 100 μL volume droplet was selected for use in the subsequent experiments.

3.3. Effect of stirring rate

Sample agitation is another important parameter having a great role for enhancing extraction efficiency. Fast agitation can increase the rate of mass-transfer of analyte to the ionic liquid. To evaluate the effect of stirring rate, sample solutions containing 1 $\mu\text{g}/\text{mL}$ amitriptyline, was extracted in triplicate with a 100 μL of ionic liquid with several stirring rates (480,

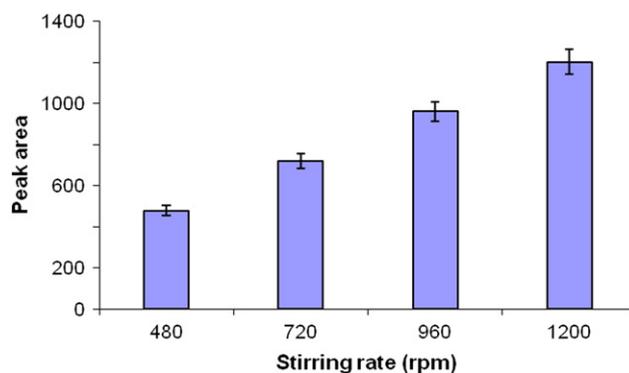


Figure 3 Effect of stirring rate on the extraction of amitriptyline. Experimental conditions are as follows: aqueous sample volume 5 mL concentration level at 1 $\mu\text{g}/\text{mL}$; sample pH 11; 100 μL $[\text{C}_6\text{MIM}][\text{PF}_6]$; 5 min extraction time and 2.5 μL injection volume.

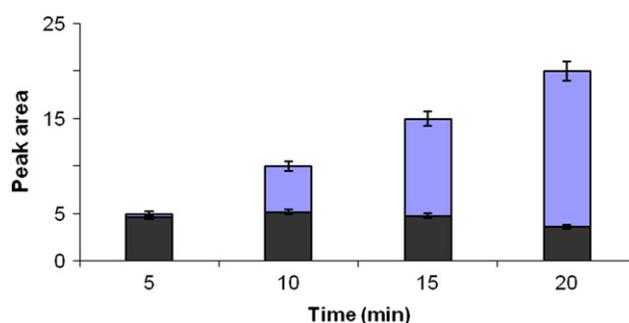


Figure 4 Effect of extraction time on the peak area of amitriptyline. Experimental conditions are as follows: aqueous sample volume 5 mL concentration level at 1 $\mu\text{g}/\text{mL}$; sample pH 11; 100 μL $[\text{C}_6\text{MIM}][\text{PF}_6]$; 720 rpm stirring rate and 2.5 μL injection volume.

720, 960 and 1200 rpm). Although high stirring rates increased the amount of analyte extracted considerably, the volume of re-collectable extracted droplet in the solution was decreased by increasing the ionic liquid solubility in the aqueous solution. The highest peak area was obtained at the stirring rate of 720 rpm. Therefore, the optimum stirring rate was selected at 720 rpm and used in all subsequent experiments. The results are shown in Fig. 3.

3.4. Effect of extraction time

Mass transfer between donor and acceptor phase is a time-dependent process. Different extraction times (5–20 min) were evaluated at room temperature with constant stirring speed (720 rpm). The results are displayed in Fig. 4. The amount of the analyte extracted increased with longer extraction time. But longer exposure time leads to ionic liquid dissolution. However, the optimal extraction time was needed for the ionic liquid to extract enough analyte. Therefore, an extraction time of 10 min was selected for this research.

3.5. Effect of ionic strength

In the extraction methods, the solubility of many analytes in aqueous solutions decreases with increasing ionic strength due to salting out effect [25].

The salt effect on extraction of amitriptyline was investigated by adding different amounts of sodium chloride in the range 0–10 %w/v. As it can be seen in Fig. 5, by the addition of salt, a reverse effect on extraction efficiency occurred. The results indicated that by increasing NaCl, the volume of ionic liquid decreased due to the increase in solubility of extraction solvent in the presence of salt (salting in effect). This would also confirm our results in Section 3.1.

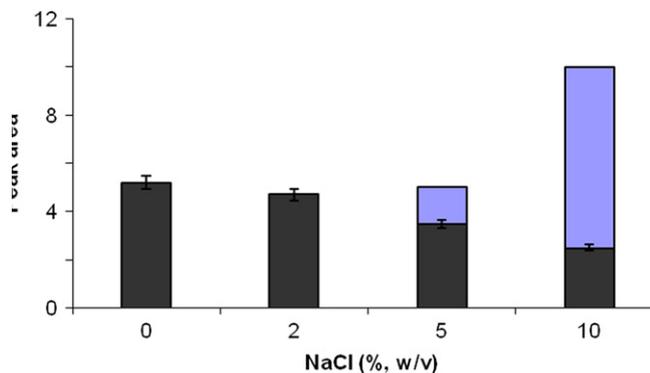


Figure 5 Effect of addition of NaCl on extraction efficiency. Experimental conditions are: aqueous sample volume 5 mL concentration level at 1 µg/mL; sample pH 11; 100 µL [C₆MIM][PF₆]; 720 rpm stirring rate; extraction time 10 min and 2.5 µL injection volume.

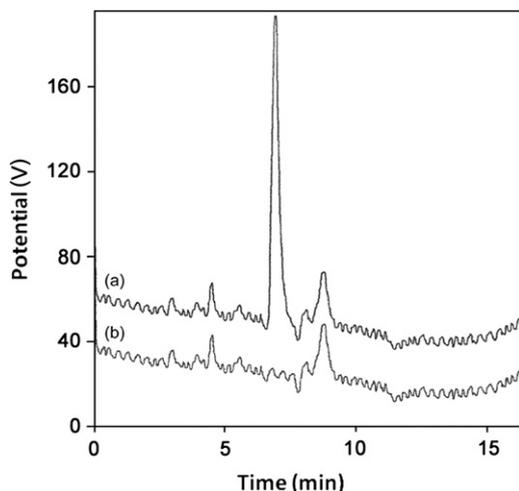


Figure 6 HPLC chromatograms of 0.05 µg/mL spiked waste water (a) and drug-free waste water (b).

3.6. Analytical performance

Calibration curve was drawn utilizing 10 spiking levels of drugs and was linear with correlation coefficient (r^2) 0.9987, in concentrations between 0.001 and 12 µg/mL in distilled water. For each level, three replicate extractions at optimal conditions were performed.

The limit of detection, (LOD) as the minimum concentration providing chromatographic signals minimum three times higher than background noise (S/N is 3) was 0.004 µg/mL ($n=6$). LOD was determined in distilled water [26]. Repeatability [relative standard deviation (RSD)] was evaluated on five replicate experiments at three concentration levels over the studied linearity interval (0.05, 1.00 and 5.00 mg/mL). The average of the three RSD readings (5.6%, 4.3% and 3.9%) was 4.6%.

For determination of pre-concentration factor (PF), peak area after extraction should be divided to peak area before extraction at the same condition (for example both of them should be injected with same syringe and same volume) and multiplied by dilution factor (1000/50). The experimental PF was found to be 1100.

3.7. Method application of wastewater

In order to study the suitability of the proposed method for the determination of the amitriptyline in the real sample, the developed technique was applied for the extraction of the drug from the hospital waste water. This real sample was examined and there were no observed signals related to the analyte. Thus, the hospital wastewater was spiked with drug and three replicate extractions were performed at optimized conditions using the proposed method. Chromatograms of drug-free water and a water sample containing 0.05 µg/mL amitriptyline are shown in Fig. 6. SDME is not an exhaustive extraction method, so the relative recovery was determined as the ratio of the concentration found in real samples and the distilled water sample, with both samples spiked at the same concentration level [23,27]. Under optimized conditions the relative recovery that was obtained for amitriptyline in water sample was 85.12%. RSD (0.05 µg/mL) in water sample was 2.25% and LOD was 0.006 µg/mL. The obtained results for the spiked sample indicated a good agreement with the original values.

3.8. Methods comparison

Ionic liquid based SDME has a short extraction time, higher pre-concentration factor, and non-organic solvent consumption. The main competing method (traditional liquid–liquid

Table 1 Method comparison for determination of amitriptyline.

Methods	Linear range (µg/mL)	LOD (µg/mL)	RSD (%)	E.F. ^a	Refs.
IL-SDME	0.01–10	0.004	4.3	1100	This study
DLLME ^b	0.005–16	0.005	5.6	740.04	[2]
HF-LPME ^c (HPLC)	0.005–0.5	0.0005	2–12	313	[3]

^aEnrichment factor.

^bDispersive liquid–liquid microextraction.

^cHollow fiber liquid-phase microextraction.

extraction) has a lower pre-concentration factor and organic solvent consumption. Also, dispersive liquid-liquid microextraction (DLLME) is possible to obtain high enrichment factor (EF) such as the presented technique, but it is more time-consuming. The review of some methods which were applied for the determination of amitriptyline in the environmental and biological samples is shown in Table 1.

4. Conclusion

In this method, the combination of immersed ionic liquid single drop microextraction and HPLC for determination of amitriptyline was demonstrated. The proposed method was proved to be very simple, selective, fast and environment friendly. It was successfully applied to monitor low concentration of amitriptyline in real water sample with good accuracy and precision. In addition, because of good selectivity and sensitivity of the method, its application may be extended to biological and geological samples.

The use of ionic liquids in this technique involves some advantages. First of all, the high affinity of the extractant to the target analyte produces an efficient pre-concentration of the analyte prior to analysis by HPLC-UV. Moreover, the low vapor pressure of the ionic liquid plays a key role in the whole process. On the other hand, it permits the use of more reproducible volumes in the SDME procedure since no-evaporation of the extractant takes place during the extraction. The most important advantage of ionic liquid is that it is environmental friendly that can be easily eliminated in the environment.

The proposed IL-SDME is an inexpensive and one-step microextraction technique that can be conveniently coupled with HPLC.

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