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Spectrometric Determination of Amitriptyline in Real Samples after Separation by Two Liquid-phase Microextraction Method

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ABSTRACT

The main aim of this research is using the preconcentration and sample preparation method, based on directly suspended drop microextraction method of amitriptyline prior to UV-vis spectrophotometer. Here, the target compound was extracted from an aqueous sample solution (donor phase) into an organic solvent drop (acceptor phase), which was suspended in the sample solution without using a microsyringe needle as a supporting device. Several important parameters which influence the extraction efficiency such as the kind of extracting solvent, extraction time, volumes of the sample solution and organic extraction solvent, stirring rate, pH of the donor phase and salt effect were investigated. Under the optimal conditions the enrichment factor was 25. By plotting absorbance versus concentration of the analyte in the standard solutions, calibration curve was obtained with correlation coefficient of 0.9931. The linearity of the method has been investigated between the ranges of 0.04-1 µg mL^{-1}. The Limit of Detection (LOD) of the method which calculated theoretically was 0.013 µg mL^{-1}. The precision or repeatability of the method is based on the average Relative Standard Deviation (RSD%) is 4.6, for three different concentrations of the analyte. Finally, the proposed method was applied for the determination of amitriptyline in urine and environmental water samples and the reasonable relative recoveries were obtained.

Keywords: Directly suspended drop microextraction (DSME), Amitriptyline, UV-vis spectrophotometer

INTRODUCTION

One of the most widespread and common mental disorders in the world is depression that is one of the serious global economic problems because the patients often lose the ability of working and also, it may ultimately result in suicide [1,2]. The depression treatment includes pharmacotherapy with medicines as well as various forms of psychotherapy. One of the largest groups of drugs for treatment of psychiatric disorders is the Tricyclic Antidepressants (TCAs) which are widely used for treatment of depression [3]. Amitriptyline is one of the TCAs has been used to treat endogenous depression, phobic states, panic attacks, neuropathic pain states, and pediatric enuresis.

Sample preparation is still the most important challenge for the analysis of different compounds from various complex matrices, especially real samples. Conventional Liquid–Liquid Extraction (LLE) and Solid Phase Extraction (SPE) have been usually applied as the useful sample preparation methods for determination of drug for many decades [4,5]. However, both methods have certain drawbacks. LLE is a time consuming and tedious procedure and needs very large amounts of the high-purity, expensive and also hazardous organic solvents. In SPE methods often the artifacts introduce into the sample solution; therefore, the limitation for pH ranges of this solution is very important factor. On the other hand, may require lengthy processing such as washing, conditioning, eluting and solvent evaporation [6]. During the last decade, new modern sample preparation methods with respect to simplification, miniaturization, and minimization of the organic solvent usage have been developed. Stir-Bar Sorptive Extraction (SBSE), Solid Phase Microextraction (SPME) and Liquid Phase Microextraction (LPME) are miniaturized techniques; introduced for these purposes [7-14]. SPME and SBSE are simple and solventless methods. However, the major disadvantages of SPME are SPME fibers are still comparatively expensive and the polymer coatings are fragile and have limited lifetime [12].

LPME can be divided into three broad categories: Single Drop Micro Extraction (SDME), Hollow Fiber Based Liquid Phase Microextraction (HF-LPME) and Dispersive Liquid-Liquid Microextraction (DLLME) [14-20]. Single drop microextraction is a

mode of LPME that provides analyte extraction in a few microliters of an organic solvent [14-18]. 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 [15-18]. In recent years, Lu and co-workers [21] developed Directly Suspended Droplet Microextraction Method (DSDME) as a new sampling method of SDME. In this method, a stirring bar is placed at the bottom of a vial containing an aqueous sample and rotated at a speed required to cause a gentle vortex. If a small volume of an immiscible organic solvent is added to the surface of the aqueous solution, the vortex results in the formation of a single droplet at or near the centre of rotation. The droplet itself may also rotate on the surface of the aqueous phase, increasing mass transfer. Compared with the other LPME techniques based on drop systems (e.g., SDME), it provides more flexibility in the choice of the operational parameters, especially the amount of the organic solvent and the stirring frequency. The possibility of applying larger volumes of organic solvents in this method also makes it a useful technique to match with HPLC and UV-vis spectrophotometer [13,21].

In the present work, we used DSDME method for extraction and preconcentration of the target compound (amitriptyline) in real biological and water samples (urine and groundwater) prior to UV-vis spectrophotometer.

MATERIALS AND METHODS

Chemicals and materials

Amitriptyline with purity of >99% was kindly supplied from Darou Pakhsh Co. (Tehran, Iran). Analytical reagents grade such as: 1-octanol, *n*-hexane, *n*-heptane and methanol (high purity) were purchased from Merck (Darmstadt, Germany). All the other used chemicals such as, acetone, NaCl, HCl and NaOH were purchased from Merck (Darmstadt, Germany) and used without further purification.

Preparation of samples

Stock solution of amitriptyline (1000 μg mL^{-1}) was prepared by dissolving calculated amount of the drug in methanol and stored protected from light in refrigerator at 4ºC. Fresh working solutions in various concentrations were prepared daily by diluting the appropriate amount of stock solution in distilled water and the solution pH was adjusted.

Apparatus

Spectrophotometric measurements were carried out using a UV-vis Unicode 2100 spectrophotometer (USA) equipped with a quartz microcell of appropriate path length with internal volume of 300 μL. The IKA heating magnetic stirrer (50-2500 rpm, Germany) was used for agitation of the sample solutions in the microextraction procedure. A 500 μL HPLC microsyringe with a flat needle was used for the sample injectors of the microextraction equipments.

Directly suspended droplet microextraction

The experimental setup of DSDME is illustrated in Figure 1. A cylindrical sample cell with a PTFE coated stirring bar was placed on a heating-magnetic stirrer. Then, a volume of 5 mL aqueous sample solution containing 1 μg mL^{-1} of amitriptyline was transferred into the vial as donor phase. The magnetic stirrer was turned on and adjusted to desired stirring speed. A trifle of *n*-hexane (180 μL, acceptor phase) was dripped on the centre of the aqueous sample surface carefully with a HPLC microsyringe and the mixture was agitated for 10 min at 1800 rpm. After this time, the acceptor phase was retracted into the microsyringe and transferred into the quartz microcell and introduced to the spectrophotometer for measuring the absorbance at 240 nm.

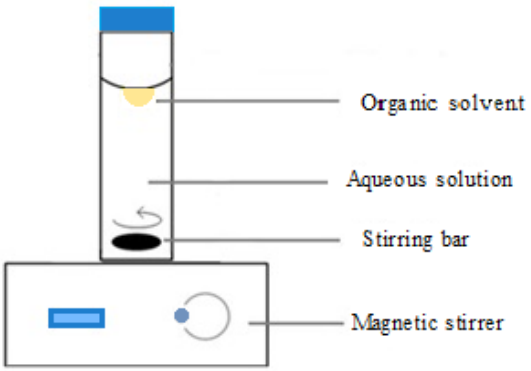

Figure 1: Schematic of the proposed microextraction method

RESULTS AND DISCUSSION

Optimization method

To obtain the optimal extraction conditions for the best efficiency, various parameters like the kind of organic solvent, extraction times, volume of the donor phase, volume of the acceptor phase, stirring speed, pH of the donor phase and salting effect were tested which can be discussed as follows.

Organic solvent selection

In two phases LPME, the type of the organic solvent is an essential factor for achieving the efficient analyte preconcentration. There are several requirements for obtaining the selected organic solvent. The appropriate organic solvents in this work should have lower density than the water to float on the top of the aqueous sample solution. They should be immiscible or have very low solubility with water for avoiding of dissolution in the water sample solution. On the other hand, the used organic extractants don't have any absorption in the maximum wavelength of the analyte. During this experiment, several organic solvents were tested to investigate their effect on the extraction efficiency. In this manner, three organic solvents such as, 1-octanol, *n*-hexane and *n*-heptane have been examined. Among of them, *n*-hexane was selected, because it has higher extraction efficiency (Figure 2).

Extraction time

Like the other techniques of microextraction, DSDME is a type of equilibrium extraction. Maximum efficiency is obtained at the equilibrium, and usually it takes too long and a further increase in extraction time does not affect the amount of the extracted analytes. Therefore, the extraction time is expected to be an important factor in the extraction efficiency of the process. The effect of the extraction time was examined by using the different times for stirring between 5 to 15 min. Experiments showed that the best extraction time was 10 min (Figure 3). With the shorter extraction times (5 min), the absorbance was lower due to the incomplete mass transfer of the target compound which was occurred at the equilibrium. And with the longer time (15 min), the absorbance decreased significantly; due to dissolve of the organic solvent in the aqueous sample solution. Therefore, in the following studies, the extraction time was set at 10 min.

Sample solution (donor phase) volume

In this experiment, the phase ratio of the donor and acceptor phases was changed by increasing the volume of the sample solution from 4-7 mL, whilst the volume of the organic drop (acceptor phase) was kept constant at 180 µL. As illustrated in Figure 4, the obtained results show the relationship between the absorbance (A) and the donor phase volume. For amitriptyline, the largest analytical response was obtained when 5 mL of the sample solution was extracted.

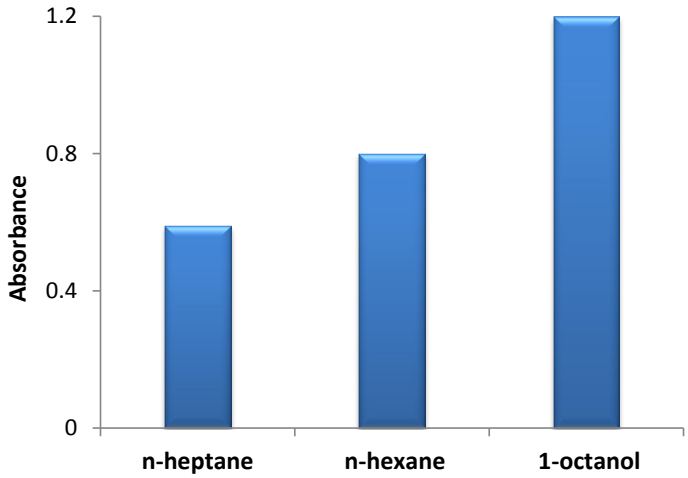

Figure 2: The effect of organic solvent on the extraction efficiency of amitriptyline

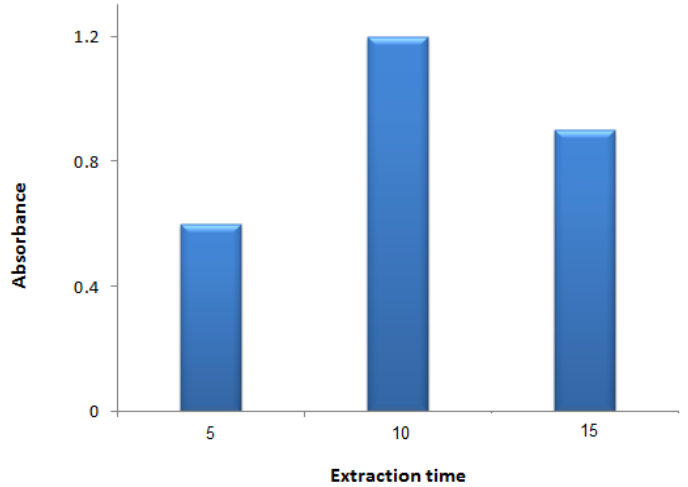

Figure 3: Effect of the extraction time on the absorbance of extracted amitriptyline in DSDME

Organic solvent (acceptor phase) volume

The organic solvent volume is almost the same as the micro extractant volume. It is a key parameter affecting the enrichment factor and the extraction kinetics. Additionally, the organic solvent volume should match with the typical injection volume of the analysis instrument. In DSDME a free drop was used as the extractant without using a microsyringe needle as the supporting device; therefore, a larger drop with a higher lifetime than conventional single drop microextraction methods can be used. So, DSDME can well match with UV–vis spectrophotometer directly. In order to optimize the organic phase volume, the range of drop volume between 160-200 µL was selected to carry out the DSDME procedure. The results indicate that the best volume of the organic solvent was found to be 180 µL. In lower volume of the organic drop (160 µL), the surface area for mass transfer of the target compound is smaller than the larger drop; so, the concentration of the extracted analyte was low and as a result, the measured absorbance was lower than the larger one. On the other hand, in higher volume of the organic drop (200 µL), the measured absorbance was lower too; because, the analyte concentration was decreased due to increase the volume of the organic drop (Figure 5). Consequently, a 180 µL volume of the organic drop was chosen for the subsequent extractions.

Stirring speed

As described before, increasing of the stirring speed is caused an increasing in the mass transfer and the extraction kinetics. In DSDME procedure, the stirring speed has a direct influence on both the shape of the droplet and the mass transfer characteristic in the aqueous sample. In this extraction method, the procedure adopts a symmetrical rotated flow field created by a stirring bar, placed at the bottom of the cylindrical sample cell and the organic single drop is delivered at the end of the aqueous sample solution vortex which was created by agitation. Thus, it forms a self-stable single microdrop system, easy to operate and control. Furthermore, the rotation of the microdrop around a symmetrical axis may cause an internal recycling and intensify the mass transfer process inside

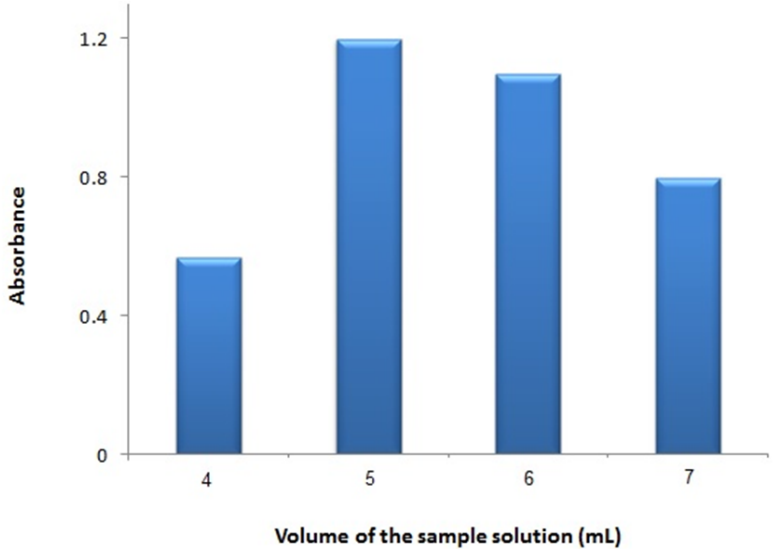

Figure 4: Effect of the aqueous sample solution volume on the analyte extraction efficiency

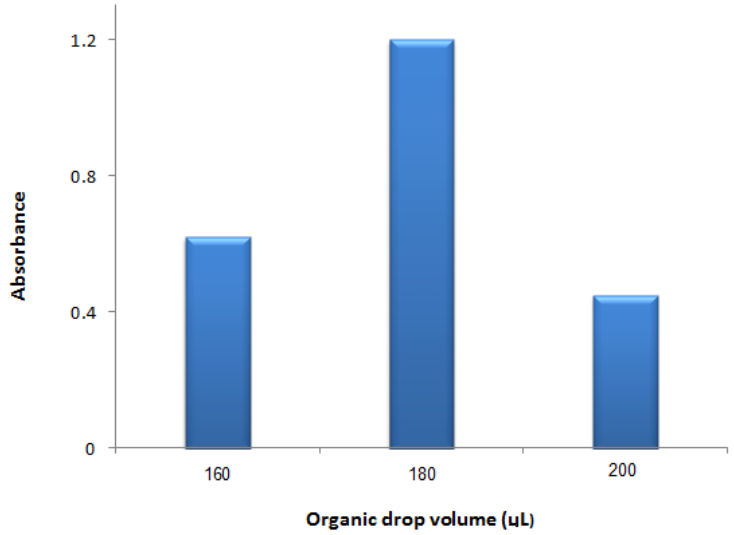

Figure 5: Effect of the organic drop (acceptor phase) volume on the analyte absorbance

the drop. Therefore, the stirring speed was also optimized for better extraction. As the stirring rate increases, the drop will collect towards the rotation axis and stretch along it. But exorbitant speed may make the drop break up and disperse into the aqueous phase. Besides, higher stirring speeds are often followed by a more unstable fluid field, which is unfavorable for operation. In general, a proper stirring speed should be convenient for operation and intensify mass transfer effectively. With these goals in mind, we investigated the extraction process at various stirring speeds and the results are shown in Figure 6.

Effect of pH

The sample pH usually plays an important role; because, the charges of the molecules depend on it. Since, the TCAs are weak basic

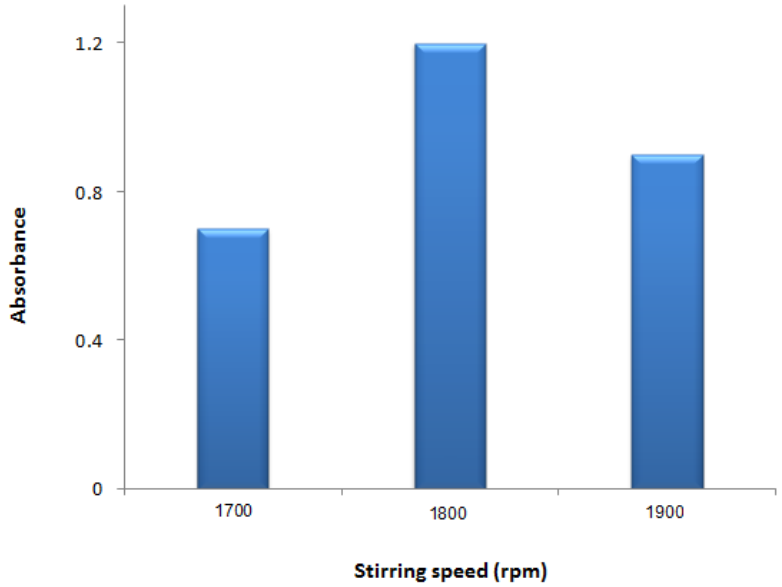

Figure 6: Effect of the stirring speeds on the absorbance of extracted amitriptyline in DSDME

Table 1: Analytical performance of the proposed extraction procedure

Target compound	RSD % [a], (n=5)	r[b]	LR[c]	LOD[d]	EF[e]
Amitriptyline	4.6	0.9931	1– 0.04	0.013	25

[a]Relative standard deviation; [b]Correlation coefficient; [c]Linear range (µg mL^{-1}); [d]Limit of detection (µg mL^{-1}); [e]Enrichment

Table 2: The relative recoveries of the target compound in real samples at 0.1 µg mL^{-1} spiking level (n=3)

Real samples	Amitriptyline
Urine sample	107 ± 6
underground water	98 ± 4

substances with low pK_a; they should be extracted in the alkaline medium. In this manner, the effect of pH on the analyte extraction efficiency was carried out in the different pH values by adding various amounts of NaOH into the aqueous sample solutions (0.0001-0.1 mol L^{-1} NaOH). According to the obtained results, the absorbance of the target compound increase when the pH of the aqueous sample solution (donor phase) increases up to pH 12 and after then decreases in pH 13. Therefore, the aqueous sample solution pH was selected 12 for future studies.

Effect of salt addition

In traditional liquid-liquid extraction, the addition of a salt often increases the extraction efficiency due to the salting out effect, whereby water molecules form hydration spheres around the ionic salt molecules. These hydration spheres reduce the amount of water available to dissolve analyte molecules in water; thus, it is expected that the target compounds will drive into the organic solvent. For this purpose, sodium chloride is normally used [22,23]. Wang et al. investigated the effect of salt on LPME method, and it was reported that the extraction efficiency decreased by increasing of the sodium chloride concentration (salting in effect) [24]. In the current work, NaCl was added into the donor sample solution in the range of 0-6% w/v. The results of the NaCl addition showed negative or salting in effect on the extraction efficiencies; so, NaCl was not added the sample solution for the subsequent extractions.

Quantitative consideration

The evaluation of the practical applicability of the proposed method, repeatability (RSD%), Linearity (LR), Limit of Detection

(LOD) and Enrichment Factor (EF) under the optimal extraction conditions were investigated by utilizing the standard solutions of amitriptilyne in water. The Calibration curve for the target compound was obtained by plotting absorbance vs. the various analyte concentrations for achieving the correlation coefficient (r). The analytical data are summarized in Table 1.

Real samples analysis

The real samples including urine and underground water samples were selected and extracted using directly suspended droplet microextraction method under the optimal conditions.

Real water sample was collected from Quchan (Iran). The urine sample was obtained from healthy volunteer and stored in appropriate polypropylene flask at −20°C until analysed. Before the extraction procedure, the sample was brought to the room temperature and centrifuged for 10 min at 2000 rpm. The supernatant was filtered through a 0.4 µm filter. After then, 3.5 mL of this was transferred into a clean glass volumetric balloon (50 mL) and adjusted to pH 12 with addition of NaOH solution and finally, subjected to the DSDME process. The results show that the contents of amitriptyline in two samples are under the detection limit. Accordingly, these samples were spiked with 0.1 µg mL^{-1} of amitriptyline and the extraction procedure was performed under the optimal conditions and the relative recoveries were subsequently calculated. The obtained results were compiled in Table 2.

CONCLUSION

The present work describes the possibility of using DSDME method in the extraction of amitriptyline from water samples prior to UV-vis spectrophotometer by utilizing a simple, rapid and cheap extraction device. In this method, contrary to the ordinary single drop liquid phase microextraction technique, an organic large drop is freely suspended without using a microsyring as supporting device. This large drop causes an increasing in mass transfer process and decreasing in equilibrium time. Compared to the most conventional extraction procedures, this extraction technique requires a very little aqueous sample solution and very little expensive and toxic organic extractants. On the other hand, this method is very fast, easy and simple. Using this technique, the analyte can be extracted from real biological and water samples quantitatively with a good linearity and reasonable relative recoveries.

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