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Journal of Applied Chemical Research, 15, 2, 19-30(2021)

Ultrasound-Assisted Emulsification Microextraction Followed by Gas Chromatography as an Efficient and Sensitive Technique for Determination of Olanzapine in Biological Samples

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(Received 08Aug. 2020; Final revised received 07Nov.2020)

Abstract

Ultrasound-assisted emulsification microextraction (USAEME) and gas chromatography – flame ionization detection (GC-FID) was presented for the extraction and determination of olanzapine in human urine and plasma samples. Chlorobenzene at microliter volume level as an extraction solvent without disperser solvent was used. The main advantages of this method are high speed, high recovery, good repeatability and extraction solvent volume at µL level. The effect of several variables such as type and volume of extraction solvent, ultrasonication time, centrifugation time, salt addition, etc. were evaluated, carefully. In the optimum conditions, the calibration curve was linear in the range of 70-2000 µg L^{-1} with the detection limit of 20 µg L^{-1}. The relative standard deviation (R.S.D.) for the five replicate measurements of olanzapine was 4.6 %. USAEME combined with GC-FID is a fast, simple and efficient method for the determination of olanzapine in human urine and plasma samples.

***Keywords*:** Ultrasound-assisted emulsification microextraction, Olanzapine, Human urine, Human plasma, Gas chromatography.

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Introduction

Olanzapine (OLN) is considered as one of the widely useful psychiatric drug for effective treatment of schizophrenia and related disorders. The structure of olanzapine was shown in the Figure 1. It is a very promising drug that is widely prescribed by physicians as an integral part of psychiatric treatment, due to its efficiency in controlling both positive and negative symptoms of schizophrenia, which is a general quality observed with most of the atypical antipsychotics as compared to typical ones such as Haloperidol [1-5].

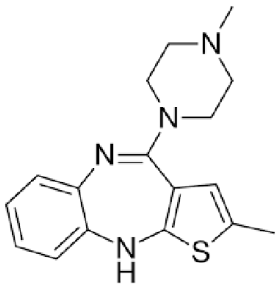

Figure 1. The structure of olanzapine.

Quantification of the urine or plasma concentration of olanzapine, is an important tool to ensure that a patient's drug dose is optimized. It can also be used to monitor drug adherence and reveal drug interactions. As the analysis is often requested among psychiatrists, a simple, rapid and robust analytical method, enabling high throughput, is a prerequisite to meet the needs of the clinicians.

There are several determination methods, HPLC-UV [6], HPLC-ECD [7], GC [8], liquid chromatography/tandem mass spectroscopy (LC-MS/MS) [9, 10] published for the measurement of olanzapine. Analytical methods, including liquid–liquid extraction (LLE) [11, 12] or solid-phase extraction (SPE) [13-16], have been for the extraction and determination of olanzapine from biological matrices. However, these pretreatment methods are time-consuming and laborious, and the large amounts of organic solvents used in the extraction procedures cause problems with regard to health and the environment.

Regueiro et al. developed a new technique called ultrasound-assisted emulsification–microextraction (USAEME) [17]. In this method, a small volume of extraction solvent is emulsified in the sample solution with the assistance of ultrasound energy. By formation of tiny droplets of an extraction solvent, the target analyte is extracted into the extraction solvent. Using ultrasound radiation causes the enlargement of the contact surface between two phases for the determination of trace amount of analyte with increment in extraction efficiency. The extraction solvent could be sedimented at the bottom of the centrifuge tube after centrifugation. There is no need to use a disperser solvent such as methanol or acetonitrile to disperse the extraction solvent into the sample solution which is the most important defect in dispersive liquid-liquid microextraction (DLLME).

Using a disperser solvent decreases the partition coefficient of analyte between the sample solution and extraction solvent which causes lower extraction efficiency. USAEME has been developed for the extraction and determination of different compounds [17, 18–22].

The present investigation has focused on the development of a reliable method for the determination of olanzapine in human urine and plasma samples. In this study, for the first time, we use the new pre-concentration method of USAEME, which is combined with GC for the determination of olanzapine in biological samples. The Influence of different extraction parameters on the performance of the proposed method is discussed in details.

Experimental

Chemicals and reagents

Olanzapine was obtained from EXIR Pharmaceutical Company (Borujerd, Lorestan). Carbon tetrachloride, chloroform, dichloromethane, chlorobenzene, acetone, acetonitrile, methanol, sodium chloride, and sodium hydroxide were obtained from Merck Company (Germany). Proper amounts of olanzapine were dissolved in methanol to obtain a stock solution of the analyte with a concentration of 100 mg L^{-1}. Working standard solutions were prepared by diluting the standard solution of the analyte with the deionized water to the required concentration. All the stock solutions were stored at 4°C.

Apparatus

Chromatographic analysis was performed using an Agilent Model 7890A GC equipped with a flame ionization detector. The GC was equipped with a HP-5, (5% phenyl, methylpolysiloxane), fused silica capillary column(30 m length, 0.25 mm i.d and 0.25μm film thickness) and split/splitless injection system.The chromatographic conditions were: helium was used as the carrier gas at 2 mL min^{-1}, inlet temperature 200 ºC,detector temperature 220 ºC, temperature program was 150 ºC, 2 min, followed by a 20 ºC min^{-1} ramp to 240 ºC, followed by 7 ºC min^{-1} ramp to 290 ºC,2 min. The detector gases were air and hydrogen, and their flow rates were regulated at 400 and 30 mL min^{-1}, respectively.

Ultrasonic cleaner model Elmasonic S 60 H was used (Gottlieb-Daimler, Germany). A centrifuge model ALC 4232 was used to perform the centrifuge process (USA). The pH- meter model 827 Metrohm (Herisau, Switzerland) was used for pH measurements. A 100 μL syringe was purchased from Hamilton (USA) for injection of extraction solvent into the sample solution and measuring the volume of the sedimented phase.

Ultrasound- assisted emulsification–microextraction procedure

A 5-mL sample solution was placed in a 15 mLscrew cap glass tube with a conical bottom. 50µL of chlorobenzene (extraction solvent) was added into the sample solution with the 100-µL syringe. The tube was then immersed in the ultrasonic water bath maintained at 25 ºC. Dispersion of very fine droplets of chlorobenzene in the sample solutioncaused high turbidity and a cloudy state in the aqueous phase. Centrifuging at 3000rpm was performed for 4 min and the extraction solvent was sedimented at the bottom of the conical tube. 1.0µL of the sediment phase was removed using a 1 µL syringe and injected into GC. The schematic of the extraction procedure is shown in the Figure 2.

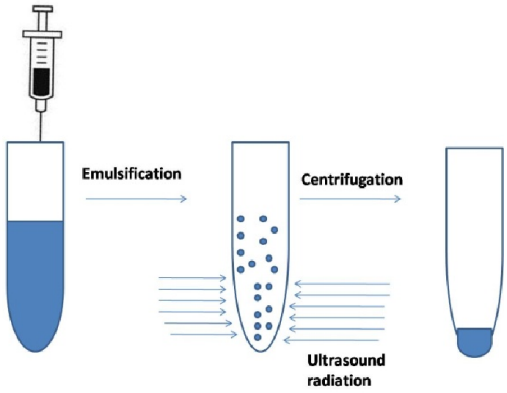

Figure 2. The schematic representation of the extraction procedure.

Results and discussion

In this research, USAEME combined with GC-FID was developed for the determination of olanzapine in biological samples. In order to obtain a high recovery, the effect of different parameters such as type and volume of extraction solvent, ultrasonication time, centrifugation time and finally, salt addition were examined and optimal conditions were selected.

Effect of pH

pH was the key parameter for the sample solution affecting the extraction efficiency. The sample solution must be adjusted to the desired pH where the analyte was uncharged, thus the uncharged molecular form of the analyte was extracted into the chlorobenzene droplets effectively. The pH of the samples was adjusted with 1 mol L^{-1} of NaOH to ensure that the neutral molecular form of the analyte is present prior to performing the microextraction step. The sample pH effect was tested in the pH range from 7 to 12. The results showed that, the extraction recoveries of the analyte were maximized at pH=11 and then slightly decreased. Thus, pH=11 was selected as the optimum value.

Selection of extraction solvent

Selection of the extraction solvent is one of the key parameters in the optimization of USAEME conditions in biological fluids. Four chlorinated solvents, carbon tetrachloride (CCl_4), chloroform ($CHCl_3$), chlorobenzene (C_6H_5Cl) and dichloromethane (CH_2Cl_2) which have densities above 1 g mL^{-1}, low water solubilities and different polarities were considered for the ultrasound-assisted emulsification microextraction of olanzapine. The effect of different types of extraction solvents on the extraction efficiency of olanzapine was shown in Figure3. The results showed that chlorobenzene has the highest extraction efficiency forolanzapine which could be due to the interaction between the benzenering of chlorobenzene and the benzene ring in the target analyte. Based on the above considerations, chlorobenzene was selected as a suitable solvent in the following experiment.

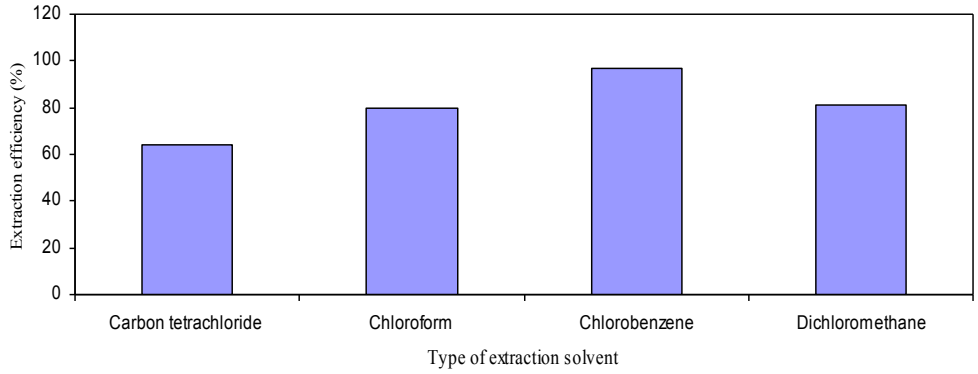

Figure 3. Effect of type of extraction solvent on the extraction efficiency.

Effect of extraction solvent volume

The effect of the volume of the extracting solvent on the extraction efficiency of olanzapine was also investigated at five levels in the range of 20-60 µL.Figure4 shows the extraction efficiency of olanzapine versus the volume of chlorobenzene. According to the results, as the volume of the extraction solvent increases to 50 µL, the extraction efficiency increases and then decreases, due to increasing the volume of the sedimented phase and dilution effect. In the following studies, 50.0 µL was selected as the optimal volume of the extraction solvent.

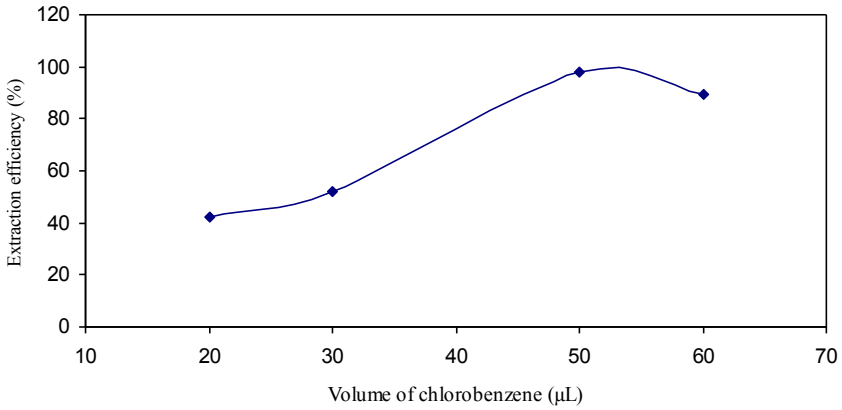

Figure 4. Effect of volume of extraction solvent on the extraction efficiency.

Effect of ultrasound time

The effect of ultrasound time on the extraction efficiency was examined in the range of 1-5minutes. As shown in Figure5, in theless than 3 min, extraction efficiency is low, because of the ultrasound time is not enough for the dispersion phenomenon and after 3 min, the extraction efficiency does not change significantly, because of equilibrium state was achieved. Therefore, 3 min was selected as the optimum value for further experiments.

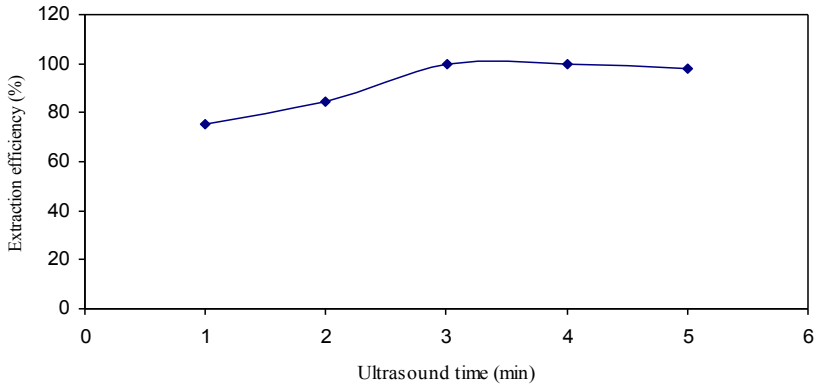

Figure 5.Effect of ultrasound time on the extraction efficiency.

Effect of centrifugation time

In the USAEME process, centrifugation is required to break down the emulsion and accelerate the phase-separation process. The effect of centrifugation times was investigated in the in the range of 1-5 min at 3000 rpm. Theoretically, a longer centrifuging time would result in more organic drops and higher extraction efficiency of the target compound because a fast separation of solvent extraction from the aqueous solutions would be difficult. Extraction solvent drops were very small

when the centrifuging time was too short, and excessing centrifuging time resulted in heat generation, dissolving of part of the extraction solvent, and losing sensitivity. Therefore, it is necessary to find a suitable centrifuging time. In the presented work, at higher centrifugation times (>4 min), the extraction efficiency decreased. Therefore, considering the extraction efficiency, 4 min was selected as the optimum centrifugation time.

Effect of extraction time

The extraction time is defined as; an interval time that started after dispersion and ended just before centrifugation. The results show that the extraction time has no significant effect on the extraction efficiency of the analyte. It is revealed that the surface area between the extraction solvent and the aqueous sample is infinitely large. Thereby, the transfer of the analyte from aqueous sample to the extraction solvent is fast. Subsequently, the equilibrium state is achieved quickly; as a result, the extraction time is very short. Therefore, in further experiments the centrifugation was carried out just after the dispersion process.

Salt addition

The influence of ionic strength was evaluated at 0-0.5% (*w/v*) NaCl levels while other parameters were kept constant. The experimental results (Figure 6) showed that salt addition had a negative effect on the extraction efficiency of the analyte and due to the increase in the volume of the sedimented organic phase and decrease in the dispersion efficiency, reduces the extraction efficiency. As mentioned above, by increasing the salt concentration, the volume of the sedimented organic phase increases, because of the decrease of solubility of the extraction solvent in the presence of salt. Therefore, all the following experiments were carried out without adding salt.

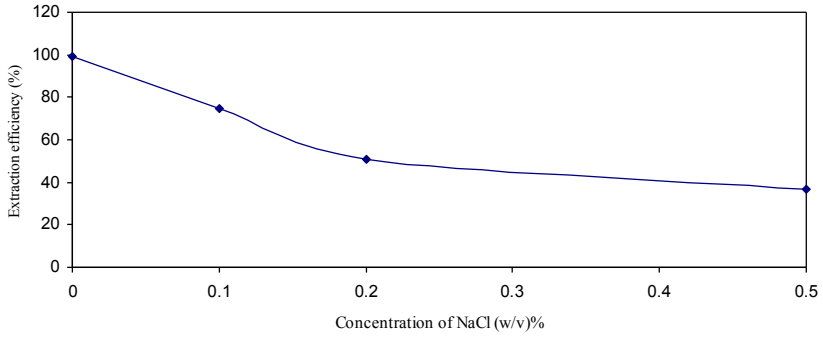

Figure 6. Effect of NaCl concentration on the extraction efficiency.

Method validation

Analytical performance

To evaluate the practical applicability of the USAEME method, analytical quality parameters such as linearity, repeatability, and LOD were investigated. The linear dynamic range 70 to 2000 μg L^{-1} was calculated. The relative standard deviation (RSD %) for the extraction and determination of the analyte was 4.6% based on 5 replicates. Good limit of detection (LOD) 20 μg L^{-1} was obtained, based on *S/N* = 3.

Table 1 compares the proposed method with the other extraction methods for the determination of the target analyte in water samples. The comparison of extraction time of the proposed method with liquid-liquid extraction (LLE) [24, 27] and solid-phase extraction (SPE) [25, 26] for the extraction of the target analyte indicates that this novel method has a very short equilibrium time comparing to the mentioned methods and the extraction time needed for the proposed method is a few seconds. Quantitative results of the proposed method without using a sensitive detector such as MS are better than SPE method with GC-electron ionization-mass spectrometry [26]. Quantitative results of the proposed method are comparable with LLE-LC-MS-MS [24, 27] and SPE-Ultra performance liquid chromatography-MS-MS [25] methods without using sensitive detector. Also, SPE and LLE methods are time-consuming and laborious, and the large amounts of organic solvents used in the extraction procedures cause problems with regard to health and the environment. Finally, consumption of disperser solvent in DLLME have led to some disadvantages such as decreasing of partition coefficients of the analyte into the extracting solvent and increasing of the cost as well as environmental pollution.There is no need to use a disperser solvent to disperse the extraction solvent in the proposed method.

Table 1. Comparison of the proposed method with other extraction methods for the determination of **o**lanzapine

Methods	R.S.D.%	Dynamic linear range (μg L^{-1})	Limit of detection (μg L^{-1})	Extraction time (min)	Ref.
LLE-LC-MS/MS	٣<	5.0-1000	5 (Limit of quantitation)	2.5	[24]
SPE-Ultra Performance Liquid Chromatography-MS-MS	١٤<	10-400	7.09	5	[25]
SPE-GC-Electron ionization-MS	٥<	300-50000	200	5	[26]
LLE-LC-MS-MS	٨<	0.1-50	٠.١	١٠	[27]
USAEME-GC-FID	٥<	70-2000	20	A few seconds	This work

Extraction of the olanzapine from biological samples

In order to study the suitability of the proposed USAEME method for the determination of analyte in the biological samples, the developed technique was applied for the extraction of the olanzapine from the human urine samples and human plasma samples. The urine from a healthy person was collected in disposable polyethylene containers and kept at 4 º C before analysis. A frozen human plasma sample was obtained from the Iranian Blood Transfusion Organization (Tehran, Iran). It was thawed and allowed to reach room temperature and then used. In order to reduce the matrix effect; the urine sample was diluted to 1:5, using deionized water. For doing the USAEME procedure on the plasma sample, some extra process is needed. At first, the human plasma was dissolved in a suitable amount of acetonitrile such as 1:1 (*V/V*) to reduce the matrix effect, and then the mixture was centrifuged. Secondly, it was filtered for getting a clear solution and removing the dirty solution at the bottom of the test tube. Finally, the clear solution was diluted to 1:10 for the USAEME procedure. Urine and plasma samples were spiked with the analyte standard to assess matrix effects. Ultimately, the extraction was performed at the optimized conditions using the proposed method, and the results are shown in Table 2. The results demonstrate that the urine and plasma matrices, in our present context, had little effect on the USAEME performance. The chromatograms of the urine and plasma sample (without spiked and with spiked) are shown in Figures 7 and 8, respectively.

Table 2. Determination of olanzapine in human plasma and urine by USAEME-GC-FID.

Sample	Spiked concentration ($mg\ L^{-1}$)	Recovery% ± SD (n=3)[a]
Human urine	0.5	95.0 ± 4.7
	1.0	97.0 ± 3.3
Human plasma	0.5	94.0 ± 5.6
	1.0	93.5 ± 4.5

[a]Standard deviation

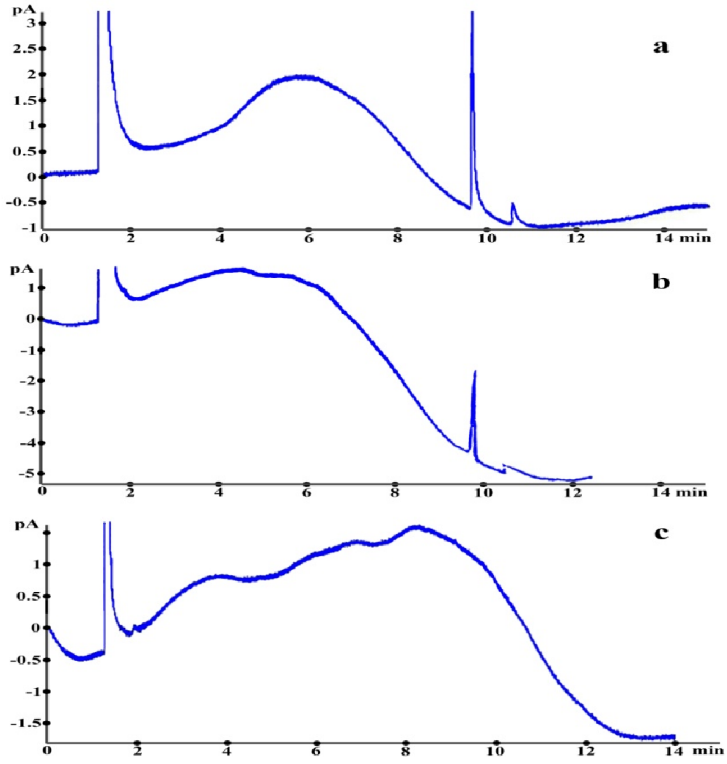

Figure 7. GC chromatograms, (c) before spiking with analyte in urine, (b) 0.5 mg L^{-1} and (a) 1.0 mg L^{-1} spiked of analyte in urine after extraction via proposed method at optimum conditions.

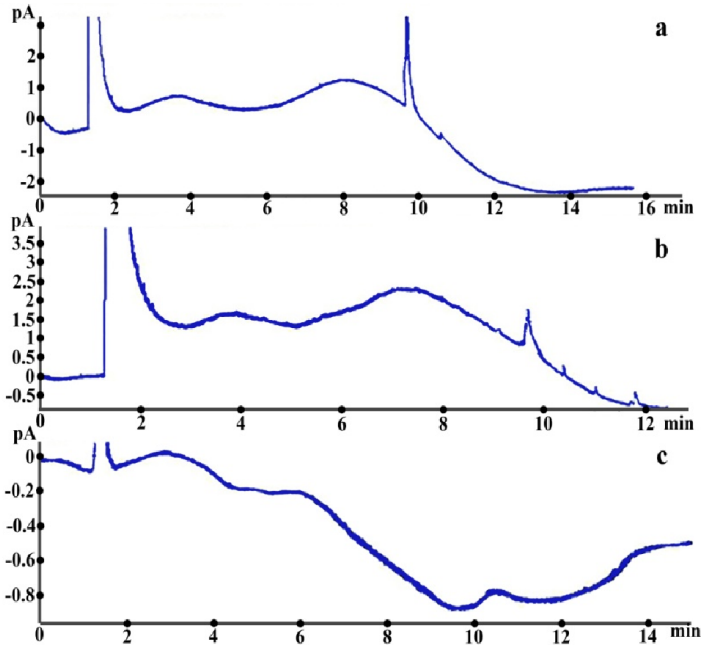

Figure 8. GC chromatograms, (c) before spiking with analyte in plasma, (b) 0.5 mg L^{-1} and (a) 1.0 mg L^{-1} spiked of analyte in plasma after extraction via proposed method at optimum conditions.

Conclusions

An ultrasound-assisted emulsification microextraction combined with GC-FID detection was presented for the concentration of olanzapine from human urine and plasma samples. The method is simple, rapid and inexpensive. In this method, sample preparation time as well as consumption of toxic organic solvents was minimized without affecting the sensitivity of the method. No matrix effect was observed when the proposed USAEME technique was applied to human urine and plasma samples spiked with the analyte. Finally, USAEME provides high extraction recovery and low LOD within very short time for olanzapine in human urine and plasma samples.

Acknowledgment

Financial support by Lahijan Branch, Islamic Azad University (Lahijan, Iran) during the period of this research is gratefully acknowledged.

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