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Analysis of some anti-depressant drugs in aqueous samples using agarose film micro-electro driven membrane extraction

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ABSTRACT

Agarose film micro-electrodriven membrane extraction (AF-µ-EME) combined with liquid chromatography as a new microextractiontechnique was developed and applied for extraction of three selected anti-depressant drugs from tap water and human urine samples.Using 2-nitrophenyl octyl ether, NPOE as organic liquid membrane immobilized in the pores of a porous agarose film allowed the extraction of selective analytes from acidic sample solution into the acceptor phase. Different influencing parameters such selection of organic liquid membrane, sample pH, salt addition, extraction time and applied voltage were optimized.Under the optimal extractions conditions, the method demonstrated good linearity in the range of 0.5-500 µg L^{-1} withgood correlation of determination$r^2 \geq 0.9979$,low limits of detection (0.02- 0.20 µg L^{-1}), high enrichment factors (110-142), good relative recoveries in the range of (87-109.1%) and satisfactory precisions with RSDs of <8.9%(n = 3) were obtained. The validated method was successfully applied for the analysis of selected anti-depressantdrugs in tap water and human urine samples.

Keywords: Agarose film, micro-electrodriven membrane extraction, anti-depressant drugs, liquid chromatography, aqueous samples.

INTRODUCTION

Sample preparation based on the liquid phase microextractiontechnique has attracted and has been employed for the analysis of biological and water samples due to their favorable features [1]. The achievements of this extractionresulted with effective sample clean-up, low cost and excellent extraction performance, providing great promising and demanding in analytical sample preparation methods[2].Currently, electrical forces have been utilized in the sample preparation and received great considerable attentions by many researchers [3].Electromembrane extraction (EME) has been introduced by Pedersen-Bjergaard in 2006 to resolve problem long extraction time encountered by hollow fiber-liquid phase microextraction (HF-LPME) technique [4]. EME has been applied for the extraction of pharmaceutical compounds [5], wastewater [6] and heavy metals [7]. It has been received merits for simplicity, minimum organic solvent consumption, fast extraction time and powerful enrichment factors [8]. Most reported EMEs studies utilized conventional polypropylene supported liquid membrane (SLM) such as hollow fiber

(HF) for the extraction. Although, this membrane provided high pre-concentration and excellent efficiencies, it has several drawbacks such as inferior stability and possessed high pressure gradient especially for aqueous samples to pass through into membrane pores due to the capillary force in hydrophobic membranes [9]. Therefore, considerable attention has been focused on the use of hydrophilic biopolymer materials to allow more selectivity of analytes to pass through SLM for better performance of extraction efficiencies. In this work, two phase agarose film micro-electrodriven membrane(AF-µ-EME) extraction using hydrophilicagarose film as an interface was proposedand applied to the analysis of some anti-depressants drugs (imipramine, amitriptyline and chlorpromazine).Previously, these compounds were successfully extracted using two-phase EME with a polypropylene membrane as interface which has been explored by Sanagi and co-workers [10]. Thus, this work evaluated potentialofhydrophilic biodegradable agarose film served as interface in two phase of AF-µ-EME technique in order to achieve better performance extraction efficiencies. Therefore, agarose film micro-EME (AF-µ-EME)combinedhigh performance liquid chromatography-ultraviolet (HPLC-UV) was developed and validated for quantification of three selected antidepressant drugs intap water and human urine samples.

MATERIALS AND METHODS

Chemical and reagents

Imipramine hydrochloride (IMI) and amitriptyline hydrochloride (AMIT) were obtained from Sigma-Aldrich (St. Louis, USA). Chlorpromazine hydrochloride (CHLO) was obtained from Clearsynth (Mumbai, India). HPLC grade organic solvents (acetonitrile, methanol, 1-octanol) were obtained from J.T. Baker (Pennsylvania, USA). 2-nitrophenyl octyl ether (NPOE) was purchased from Sigma-Aldrich (St. Louis, USA). Reagentgrade sodium hydroxide (NaOH), hydrochloric acid (HCl) and potassium dihydrogen phosphate (KH_2PO_4) were obtained from Merck (Darmstadt, Germany).Agarose (molecular grade) was obtained from Promega (Madison, USA). Double-distilled water of at least 18 mΩ was purified by Nano utra-pure water system (Billerica, USA).

Materials

For this extraction, four glass tubes of different sizes made up in our glass-blowing laboratory was used. A voltmeter of a dc power supply 0-300V (Delta Elektronika BV model ES 0300-0.45) instrument (Zierikzee, Netherlands),providing a direct current in the range of (0-0.45 A) was used.Three cable wires, with a crocodile clip at each end and three platinum wires (Guangdong, China) with diameter of 0.2 mm were used as electrodes.The auto-ranging picoammeter 9103 (Oregon, United States) was used to monitor the current measurement ranges 2 nA to 2 mA.

Preparation of standard solutions, tap water and human urine samples

Standard solutions of IMI, AMIT and CHLO (1000 µg/mL) were prepared separately in methanol. All standard and sample solutions were stored at 4 °C and protected from light. Tap water was collected from Faculty of Science,UniversitiTeknologi Malaysia. The samples were pre-filtered with nylon filter due to the possibility particles. Human urine samples were collected from a healthy volunteer with no recent history of drug-taking. Urine samples were prepared by dilution to 1:1 with water. After dilution, the pH of the urine sample was adjusted to 6.0 with 0.1 M hydrochloric acid (HCL) and the solution was spiked with the drug mixture for further analyses. Human urine samples collection and experimental procedure were performed in compliance under guidelines and approved by the Research Ethics Committee, UniversitiTeknologi Malaysia.

HPLC-UV analysis

HPLC separations were carried out on a Zorbax Eclipse plus C_{18} column (2.1 × 100 mm, 3.5 µm) using an Agilent Technology HPLC system (California, USA) equipped with ultraviolet detector and a 20 µL sample loop. Analytes peaks were detected at 240 nm and chromatographic data were recorded using Agilent Chemstation software. The eluent was acetonitrile-methanol-phosphate buffer (pH 6.0, 25 mM) (55: 15:30) (v/v) and the flow rate was set at 0.2 mL/min.

Preparation of Agarose Film

Agarose film wasprepared by dissolving of 0.1 g of agarose in 10 mL deionized water and mixed thoroughly and stirred for 20 min. The warm solution of the aliquot was spread immediately into a 9 cm diameter glass petri-dish and it was allowed to cool and get at room temperature at least 30 min. The agarose film was cut into a certain size (ca. 2.5 cm x 2. 5 cm) and attached at the lower end of a glass tube.

Agarose film-microelectrodriven membrane extraction (AF-µ-EME) procedure

The equipment use forAF-µ-EME is illustrated in Fig.1. Asample solution (10-mL) pre-adjusted to pH 6.0 was introduced into a 12-mL sample vial.Agarose film was cut into (2.5 cm length and 2.5 cm width) and was attached at the lower end of glass tube and sealed with the parafilm. The surface area of the agarose film exposed to the sample

solution was approximately 0.13 cm^2. The pores of the membrane were impregnated by dipping in 2-nitrophenyl octyl ether (NPOE). The acceptor solution was introduced into the glass tube that attached with agarose film. The cathode and anode was subsequently coupled to the power supply. The power voltage supply was turned on and the extraction was performed for a prescribed time (5-15 min) with constant stirring using a magnetic stir bar at a rate of750 rpm. During extraction, the current flow was monitoredby the auto-ranging current measurement to make sure it was executed under low voltage system. At the end of the extraction, the power supply was turned off and 2 µL of the acceptor phase was withdrawn with a micro syringe and transferred to HPLC-UV system for separation and quantification.

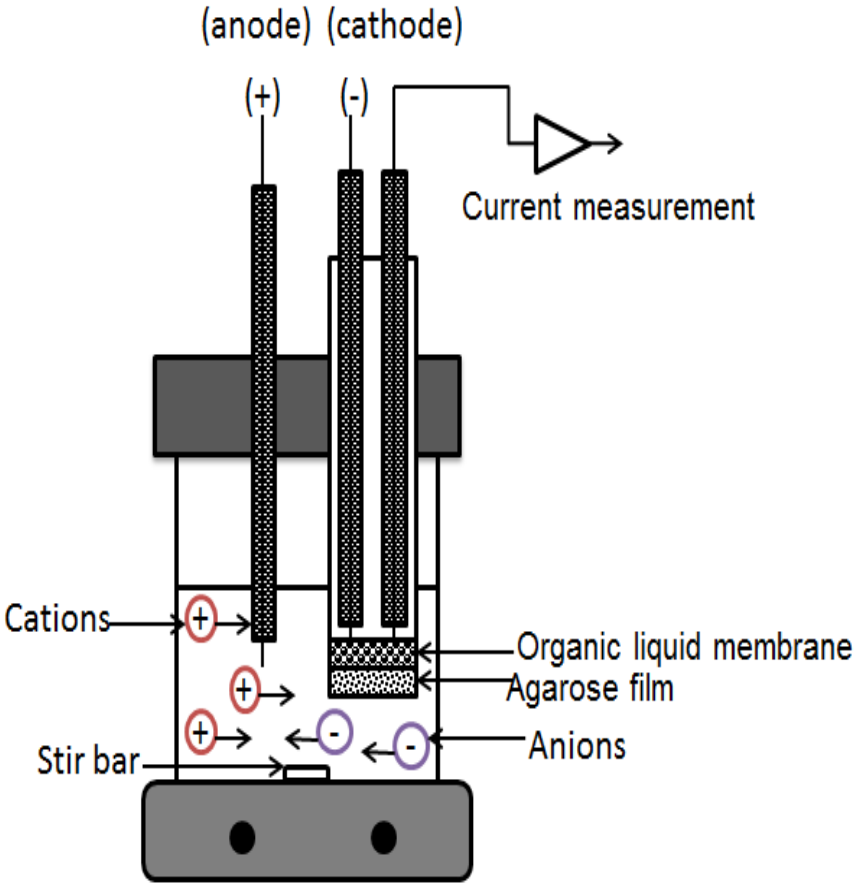

Fig. 1 Schematic representation ofAF-µ-EME system

RESULTS AND DISCUSSION

Optimization of AF-µ-EME procedure

Several important parameters namely (organic liquid membrane, sample pH, salt addition, extraction time and applied voltage) were studied and optimized. The optimization was evaluated by using deionized water samples spiked with each TCA at a concentration of 0.5 μgmL^{-1}. Each experiment was performed in triplicates (n = 3).

Selection of organic liquid membrane

Three types of organic solvents were evaluated namely 1-octanol, heptanol and 2-nitrophenyl octyl ether (NPOE) were investigated. The results revealed that NPOE gave the highest peak area compared to the other organic solvents (Fig. 2). This might probably due the compatibility of NPOE impregnated in the pores of the agarose film as SLM for the determination of basic drugs. NPOE is known as a very efficient organic solvents for electrokinetic migration of non-polar basic drugs through the SLM while, 1-octanol has been reported to be suitable for the extraction of acidic drugs[11]. Therefore, NPOE was chosen and used in subsequent experiments.

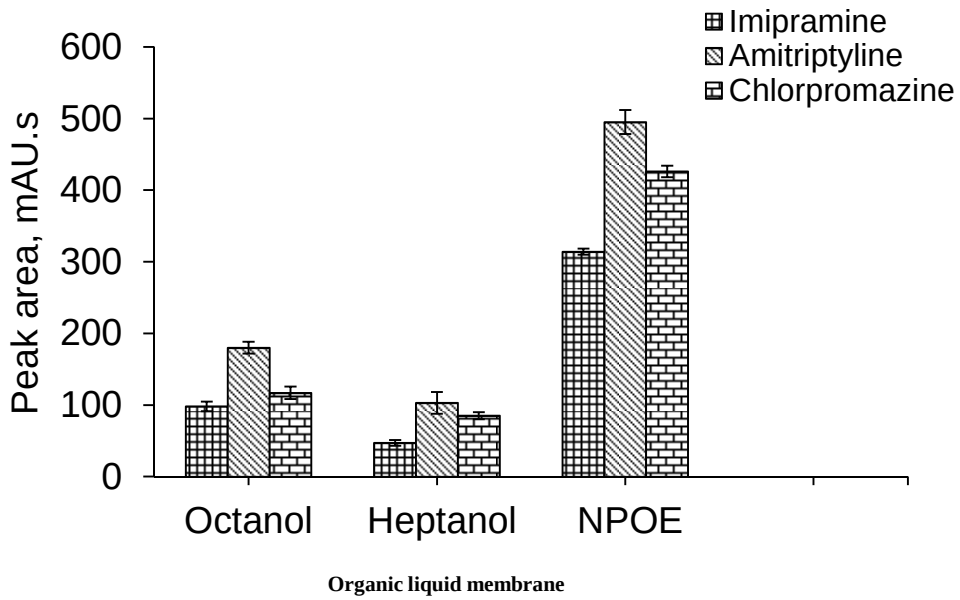

Fig. 2Effect of selection organic liquid membrane onAF-µ-EME of anti-depressant drugs (*n* =*3*). Error bars represent the standard deviations

Effect of sample pH

InAF-µ-EME procedure, the analytes must be possess and intended in ionized form due to the electrokinetic migration process.As the target compounds are basic drugs (pKa = 9.2-9.53), the pH of donor aqueous solutions was adjusted in the acidic range so as to deionize the target compounds.The basic analytes with the dissociation constant pKa ranging around 9.5 were completely ionized at low pH [12]. Therefore, the sample pH was evaluated by varying the pH in the range of 3.0-7.0 (Fig. 3). Therefore, when the pH of sample solution is more than 7, the ionization efficiency was decrease. Result showed, the highest peak area obtained at pH 6. Therefore, pH 6.0 was selected and used in further analyses.

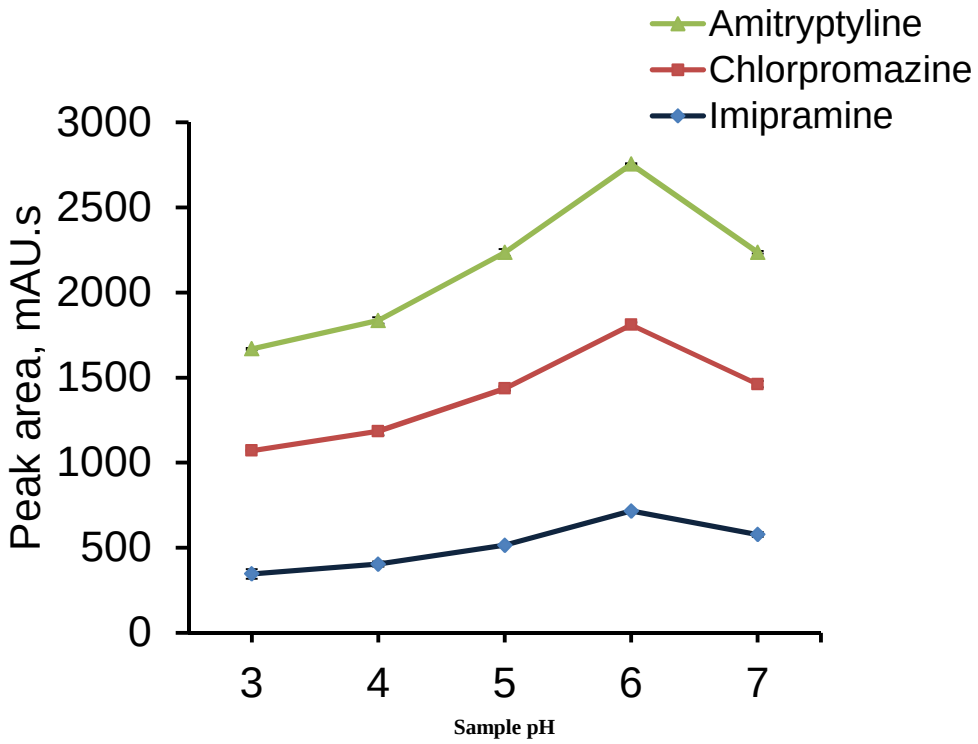

Fig. 3Effect of sample pH on AF-µ-EME of anti-depressant drugs (*n* =*3*). Error bars represent the standard deviations

Effect of salt addition, %(w/v)

The effect of sample solution ionic strength on AF-µ-EME efficiency was determined by adding different concentration of sodium chloride (0, 1, 2, 3 and 4 % w/v) in the sample solution.Results showed that addition of sodium chloride (NaCl) to the sample solution did not significantly increase the extraction efficiencies of all analytes (Fig. 4). The presence of salt in the sample solution will increase the value of the ion balance in the system, which will decrease the flux of analytes across the SLM [13]. Therefore, the presence of salt may decrease the extraction efficiencies.Thus, no addition of salt or 0% (w/v) to the sample solution was not performed in subsequent analysis.

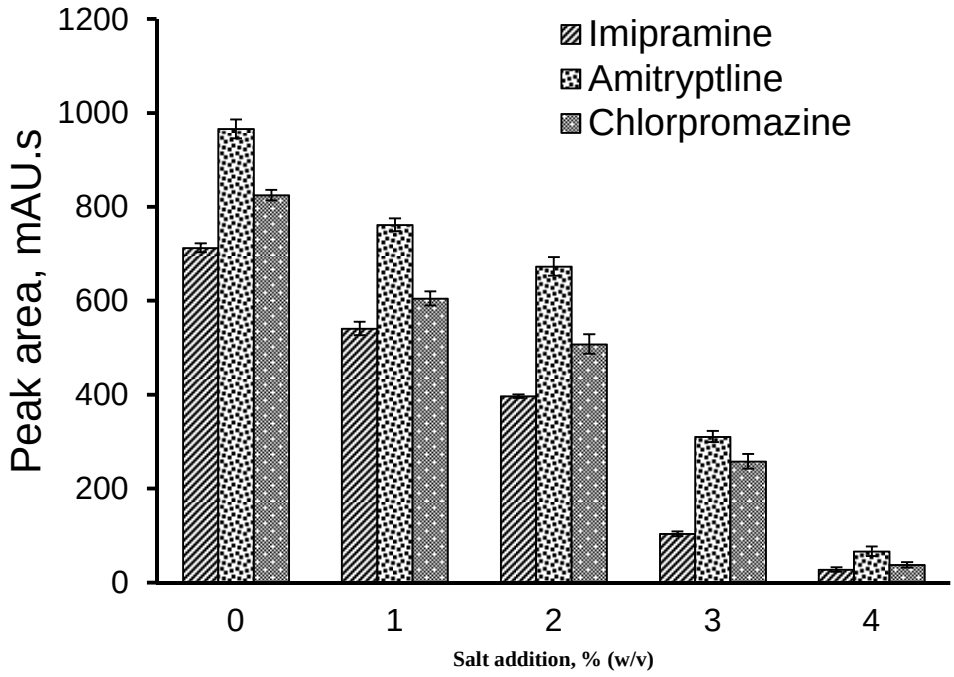

Fig. 4Effect of salt addition on AF-µ-EME of anti-depressant drugs (*n* =*3*). Error bars represent the standard deviations

Effect of extraction time

The effect of extraction times from 5 to 15 min were examined to determine the equilibrium time required for the analytes to partition into the acceptor phase. As can be seen (Fig. 5), the extraction efficiencies were slightly increased of response until 10 min extraction. However, extending the reaction time after 10 min, a slight decrease in peak areas observed for all three selected of TCAs analytes. It shows that AF-µ-EME is a non-exhaustive method in which it was a time-dependent system [14]. Hence, 10 min was adopted to be the most suitable extraction time.

Effect of applied voltage

AF-µ-EME is based on the driving force for electrokinetic migration of the analytes, applied voltage or electrical potential differences is one of the most important parameter that affect the performance of the extraction efficiency that should be optimized. In this study, the effect of applied voltage was examined in the range of 0 - 60 V. As illustrated in Fig. 6, there was sharp increase in peak area for TCAs analytes up to 10 V, but after that a slightly decreased response of peak area was observed. A high current may generate electrolysis at the both electrodes (anode and cathode) and this can lead a bubble formation due to the higher voltage [15]. The highest peak area for TCAs analytes was obtained at 10 V. Therefore, 10 V of applied voltage was chosen for the subsequent analyses.

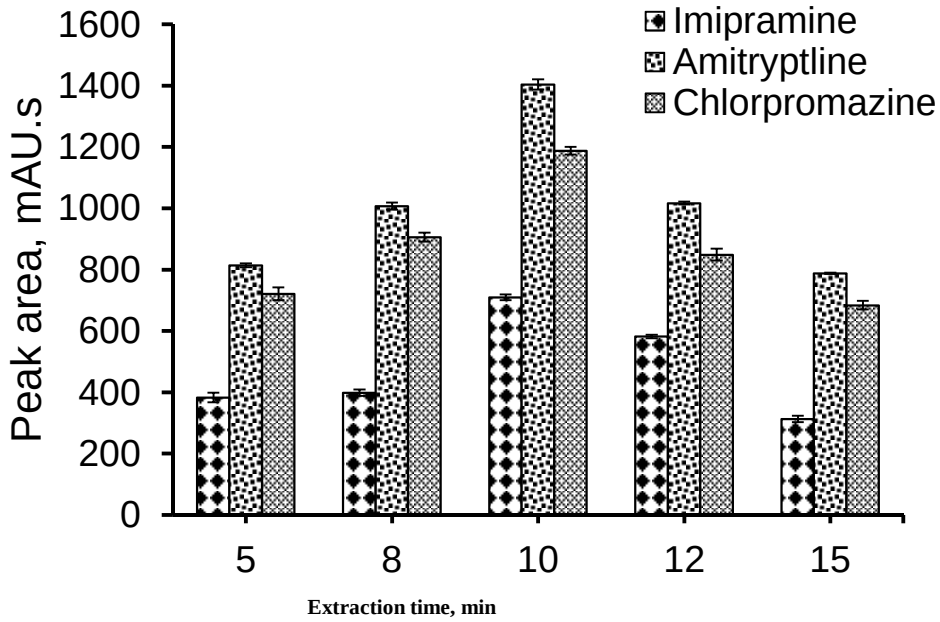

Fig. 5Effect of extraction time on AF-µ-EME of anti-depressant drugs (*n =3*). Error bars represent the standard deviations

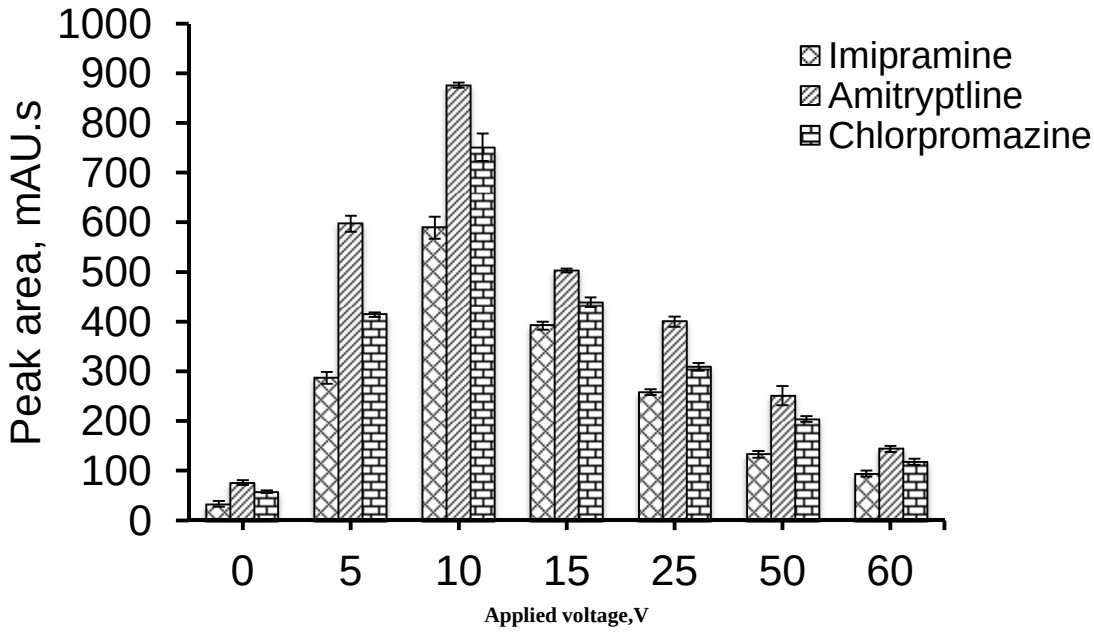

Fig. 6Effect of applied voltage on AF-µ-EME of anti-depressant drugs (*n =3*). Error bars represent the standard deviations

Table 1Summary of the optimum conditions ofAF-µ-EME extraction

Operating parameters	Optimum Condition
Organic liquid membrane	2-nitrophenyl octyl ether, NPOE
Salt addition	0 (%w/v), no salt addition
Sample pH	6
Extraction Time	10 min
Applied Voltage	10 V

Method Validation

The proposed of AF-EME method was evaluated under the most favorable extraction parameters determined earlier (organic liquid membrane, NPOE, pH of sample solution, 6, salt addition, 0% (w/v) or no salt addition, extraction

time, 10 min and applied voltage, 10 V). The results were tabulated in Table 1. Good linearity of response (peak area) for each analyte with coefficients of determination, r^2 of > 0.9979 over the concentration range of 0.5-500 µg L^{-1} (Table 2). The LODs were calculated based on the signal-to-noise ratio (S/N) of 3.The LODs obtained in (0.02-0.08) µg L^{-1} and(0.10-0.20)µg L^{-1} for tap water and human urine samples, respectively with relative standard deviations (RSDs) of <7.5% indicating good reproducibility.

The accuracy and the precisions of inter-day RSDs of the proposed method were measured with TCAs spike at two different concentrations (10 µg L^{-1} and 100 µg L^{-1}) in tap water and urine samples. The results are summarized in Table 3. The average recoveries were between87.0% -109.1 % and 86.1% -108.3 % for tap water and human urine samples respectively. The inter-day precisions of both anti-depressants were evaluated with the resulting RSDs less than 8.9 %. Figure 7 shows the typical HPLC/UV chromatograms of spiked tap water and human urine samples of three selected of TCAs at spiking with TCAs mixture standard solution at concentration (a) non-spiked sample (b) 10 µg $L^{-1}$and (c) 100 µg L^{-1}.The chromatogram revealed that all blank samples do not have contaminants of TCAs. Meanwhile, all peaks in spiked tap water and human urine were demonstrated well separated.

Table 2: Validation data of AF-µ-EME of TCAs from tap water and human urine samples

Sample	TCAs	Linear range (µg L^{-1})	Coefficient of determination, (r^2)	LOD (µg L^{-1})	EF	RSD % (n = 3)
Tap water	IMI	0.5-500	0.9993	0.08	134	6.6
	AMIT	0.5-500	0.9984	0.02	142	7.4
	CHLO	0.5-500	0.9985	0.06	114	4.9
Human urine	IMI	0.5-500	0.9991	0.18	128	5.4
	AMIT	0.5-500	0.9983	0.10	139	3.6
	CHLO	0.5-500	0.9979	0.20	110	6.5

EF=Enrichment factor

Table 3Extraction relative recovery (%) and method precision(RSD, %,n = 3) at two different concentrations of the anti-depressant drugs in tap water and human urine samples

Analytes	Spiked concentration (µg L^{-1})	Average relative recoveries, %(RSD, %)(n = 3)	
		Tap water	Human urine
Imipramine(IMI)	10	102.7 (5.1)	89.0 (5.3)
	100	105.8 (8.8)	108.3 (3.6)
Amitriptyline(AMIT)	10	87.0 (2.2)	86.1 (5.1)
	100	109.1 (7.8)	94.5 (2.8)
Chlorpromazine(CHLO)	10	99.2 (6.1)	95.1 (4.5)
	100	107.0 (2.8)	108.2 (6.3)

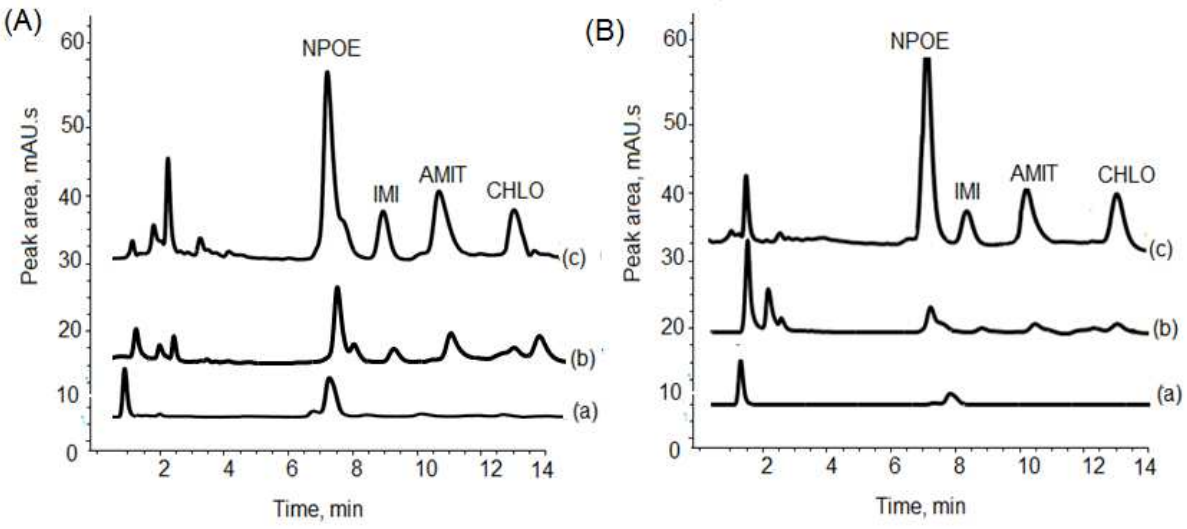

Fig.7 HPLC-UV chromatograms obtained after extraction of three TCAs drugs from (A) tap water and (B) human urine samples at concentration level of (a) non-spiked sample (b) 10 µg L^{-1} and(iii) 100 µg L^{-1}. HPLC conditions: separations were carried out on a ZORBAX Eclipse plus C_{18} column (2.1 × 100 mm, 3.5 µm thickness); detector: 240 nm

CONCLUSION

A new proposed method two phasemicroelectrodriven membrane extraction (AF-µ-EME) was successfully developed for the analysis and quantification of TCAs in tap water and human urinesamples. In this work, agarose

film has been prepared and utilized as interface in the extraction. The suitability of agarose film as an interface in EME extraction was successfully applied to the analysis of TCAs from tap water and human urine samples. This method exhibited provided a valuable value of the hydrophilic of agarose film (biodegradable polymer) as interface, fast time extraction, good limit of detection, high pre-concentration, acceptable repeatability and reproducibility, and minute of organic solvent consumption.

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