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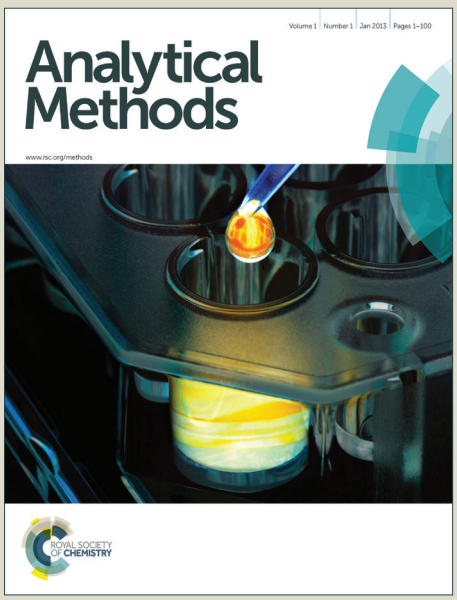

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TWO-PHASE ELECTRODRIVEN MEMBRANE EXTRACTION COMBINED WITH LIQUID CHROMATOGRAPHY FOR THE DETERMINATION OF TRICYCLIC ANTIDEPRESSANTS IN AQUEOUS MATRICES

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ABSTRACT

A two-phase low-voltage electrodriven membrane extraction (EME) combined with high performance liquid chromatography (HPLC) was developed for determination of tricyclic antidepressants (TCAs) in aqueous matrices. Three TCAs, namely imipramine (IMI), amitriptyline (AMI) and chlorpromazine (CHLO) were used as target analytes. The drugs

were extracted from aqueous sample solutions through a porous polypropylene membrane filter impregnated with 2-nitrophenyl octyl ether (NPOE) that served as a supported liquid membrane (SLM), and into acceptor phase with a potential difference of 10 V applied over the SLM. EME parameters such as type of organic solvent, pH of sample solution, extraction voltage, extraction time and stirring rate were evaluated and optimized. Optimal extractions were accomplished with NPOE as the organic solvent, pH of sample solution of 6, extraction time of 10 min, 10 V as the driving force with the whole assembly agitated at 1200 rpm. Under the optimized extraction conditions, the method demonstrated good linearity with coefficients of determination, $r^2 \geq 0.9987$ in the concentration range of 0.5-1000 μg L^{-1} for water, and 1.0-1000 μg L^{-1} for urine and good limits of detection in the range of 0.05-0.08 μg L^{-1} and 0.1-0.3 μg L^{-1} in water and urine samples, respectively. The method showed high enrichment factors in the range of 91-128 and high relative recoveries in the range of 98.4-103.1% and 86.7-107.4%, for water and urine samples, respectively with RSDs of < 9.0% (*n* = 3). The method was successfully applied to the determination of the drugs in water and human urine samples. The proposed method offered good features such as simple, easy handling, fast extraction time, low voltage and minimum organic solvent consumption which meet the green chemistry concept.

Keywords: Electrodriven membrane extraction, Supported liquid membrane, Polypropylene Membrane, Tricyclic antidepressants, High-performance liquid chromatography, Aqueous matrices.

1. Introduction

Hollow fiber liquid phase micro extraction (HF-LPME) is one of the most promising techniques in sample preparation [1-4]. This technique is based on the passive diffusion of analytes from a sample solution into the organic solvent inside the lumen of HF [5] and proved to be an effective method for the extraction of compounds from complicated matrices. This technique is very effective in discriminating compounds such as salts, acids, biological macromolecules and hydrophilic compounds which provide very clean acceptor solution [6]. However, HF-LPME has one major drawback of long extraction times. This is because HF-LPME allows passive diffusion that is a relatively slow process and thus the extraction procedures usually require extraction times in the range of 30-50 min [7-8].

There has been considerable interest to investigate the use of auxiliary energies such as electric field in order to reduce the extraction time. Pedersen-Bjergaard and Rasmussen [9] introduced electromembrane extraction (EME) as an alternative concept of LPME. EME is a liquid-phase microextraction technique based on the application of an electrical potential to migrate the charged analytes from an aqueous sample across a SLM [10]. In comparison with passive diffusion HF-LPME, EME was found to be more efficient than HF-LPME and was able to extract analytes in a short time [11]. Several works on EME have indicated high extraction recoveries and excellent clean-up in sample preparation and preconcentration of water and human biological samples [12-14]. Mostly, this method often employed SLM consisting of an organic solvent which impregnated in the walls of a hollow fiber [15-18]. However, the use of HF-SLM has a limited mechanical stability that can lead to a loss of the impregnated organic solvent during extraction due to the high electric fields and under sample agitation [19-20].

A novel extraction technique termed electric field-driven extraction using polymeric inclusion membrane (PIM) with three-phase system was developed to overcome the problem faced by EME [21]. PIM is self-supporting membrane that comprises a base polymer, a

plasticizer and a functional carrier. A high voltage of 700 V was used to extract the alkylsulfonate from spiked river water.

EME with combination of capillary electrophoresis (CE) has also been performed by group of Dominguez and co-workers to determine 29 different basic drugs model compounds under relatively low voltage [22]. The target analytes with log P $\geq$ 2.3 and with one basic group were all extracted by the SLM at low voltages less than 15 V. They concluded that mass transfer of protonated basic drug analytes across SLM under influence low applied voltage is highly structure dependent.

Tricyclic antidepressants (TCAs) are basic drugs commonly used to treat endogenous depression, panic attacks, neurophatic pain states, phobic states and pediatric enuresis [23]. The side effects of overdose of TCAs include dry mouth, urinary retention, dry nose, dry eyes and hyperthermia [24-25]. Therefore, the measurement of concentration of these drugs in biological fluids is becoming increasingly important to assess patient compliance especially for uncontrolled dosage and breakthrough seizures [26]. A number of conventional sample preparation methods have been employed in the analysis of TCAs in biological samples. These include liquid-liquid extraction (LLE) [27-29], solid phase extraction (SPE) [30-31] and solid-phase microextraction (SPME) [32].

A three-phase EME with gas chromatography flame ionization detector (GC-FID) was evaluated for determination of two TCAs (imipramine and clomipramine [33] from water, plasma and urine samples. These basic drugs analytes were extracted through a HF-SLM within 20 min of extraction with good linearity and acceptable LODs. Recently, two-phase EME was combined with gas chromatography mass spectrometry (GC-MS) with the utilization of polypropylene HF with SLM for the extraction and preconcentration of four basic drugs namely imipramine, citalopram, desipramine and sertraline [34]. The results suggested that two-phase EME method provided excellent clean-up and low detection limit.

In this work, a new design of low-voltage two-phase electrodriven membrane extraction using a polypropylene membrane filter is described for the first time. The polypropylene membrane was impregnated with NPOE which served as SLM for the migration of ions between a sample and acceptor phase solution. The robust design can eliminate the drawback of the poor mechanical stability of HF-SLM and the system was accomplished under two-phase mode system and low-voltage electrical potential. The developed system combined with high performance liquid chromatography with UV detector (HPLC-UV) was successfully applied to the analysis of selected TCAs in aqueous matrices. The extraction technique proved to be simple, provide faster extraction time, and compatible with other chromatographic and electrophoretic systems.

2. Experimental

2.1. Reagents and chemicals

Imipramine hydrochloride (IMI) and amitriptyline hydrochloride (AMIT) were purchased from Sigma-Aldrich (St. Louis, USA) while chlorpromazine hydrochloride (CHLO) was purchased from Clearsynth (Mumbai, India). . 2-nitrophenyl octyl ether (NPOE) was purchased from Sigma-Aldrich (St. Louis, USA). HPLC grade organic solvents (methanol, acetonitrile, toluene, heptanol, 1-Octanol) were obtained from JT Baker (Pennsylvania, USA). Sodium hydroxide (NaOH) and potassium dihydrogen phosphate (KH_2PO_4) were obtained from Merck (Darmstadt, Germany). Ultrapure water was obtained from a Millipore Milli-Q water purification system (Billerica, MA, USA).

2.2 *Chromatographic conditions*

All analyses were performed using high performance liquid chromatography (HPLC) (Agilent Technologies, California, USA) equipped with ultraviolet detection (Agilent Technologies) and a 20 µL sample loop. The chromatographic separation of TCAs was carried out on a Zorbax SB-C_{18} column (2.1 × 100 mm, 3.5 µm) from Agilent Technology (California, USA). Isocratic elution was used for chromatographic separation in which the mobile phase consisted of 25 mM phosphate buffer (pH 6), acetonitrile and methanol in a ratio of 30:55:15, (v/v). The flow rate and injection volume were set at 0.2 mL min^{-1} and 2 µL, respectively. Analytes were monitored at 240 nm and chromatographic data were recorded using Agilent Chemstation software.

2.3 *Preparation of standard solutions and samples*

Stock solutions of IMI, AMIT and CHLO (1000 µg mL^{-1}) were prepared separately in methanol. Urine was collected from a healthy volunteer with no recent history of drug-taking. Urine samples were prepared by dilution to 1:1 with water. All the standard and sample solutions were stored at 4°C and protected from light. The water and urine samples were spiked with TCAs mixture to give a final concentration of 1.0 µg mL^{-1} for each analyte. After dilution, the pH of the water and urine samples was adjusted to pH 6. All experiments involving human urine samples were performed in compliance with the institutional guidelines and approved by the Research Ethics Committee, Universiti Teknologi Malaysia. Informed consent was obtained for experimentations with human urine samples.

2.4 *Equipment for electrodriven membrane extraction (EME)*

The experimental setup used for the extraction procedure is shown in Fig. 1. A 12-mL glass sample vial (2.0 cm ID × 4.4 cm height) was used to hold the sample and the EME assembly. The EME assembly consisted of four glass tubes of different sizes prepared in our glass-blowing laboratory. These were tube A (40 mm × 2 mm ID, 1mm wall thickness), tube B (40 mm × 2 mm ID, 0.5 mm wall thickness), two identical tubes C and D (50 mm × 1.25 mm ID, 0.25 mm wall thickness). Three pieces of platinum wires (0.2 mm diameter) obtained from Mainland, China were used as electrodes with a three cable wires with a crocodile clip at each end were utilized. A Delta Elektronika DC power supply model ES 0300-0.45 (Zierikzee, Netherlands) with programmable voltage (0 - 300 V) and direct current (0 - 0.45 A) was used. A hot plate stirrer (Favorit, Malaysia) and a magnetic stir bar (12 mm × 4 mm) were used to stir the sample during extraction at stirring speeds in the range of 600-1300 rpm. A Whatman 47 mm diameter polypropylene membrane (membrane filters PTFE, polypropylene backed, 0.2 μm WTP-type) (Maidstone, England) was used in the extraction. It was cut into a certain size (ca. 2 cm × 2 cm) and attached at the end of a tube B (40 mm × 2 mm ID, 0.5 mm wall thickness) with epoxy adhesive (Windsor Chemical, Malaysia).

2.5 Procedure for electrodriven membrane extraction

Sample solution (10 mL) adjusted to pH 6.0 was pipetted into a 12-mL sample vial (Fig. 1). Tube A and Tube B were inserted through their respective septum and their tips were immersed into the sample solution. A polypropylene membrane filter sheet was attached at the end of a glass tube B to serve as a barrier during extraction. NPOE was used an organic solvent/acceptor phase. The pores of the membrane were impregnated by dipping the membrane in NPOE. A small amount of NPOE (25 μL) was introduced using a microsyringe into tube B and the tube was dipped into the sample solution.

The surface area of the membrane exposed to the sample solution was approximately 0.13 cm^2. Next, tiny glass tubes C and D were inserted into glass tube B. A platinum wire electrode was inserted into each tube A, C and D. The electrodes in tubes A and C were connected to the power supply voltage representing as anode and cathode. Meanwhile, the electrode in tube D was at virtual ground potential and connected to the input of a home-built current sensor using an I to V converter configuration to monitor the current that passed through the membrane during extraction. The power supply (10 V) was applied on the extraction was performed for a prescribed time (5 – 25 min). The sample was agitated at 1200 rpm using a magnetic stir bar. After the extraction (10 min), the power supply voltage was switched off and 2 µL of the acceptor phase was collected using a microsyringe and directly injected into the HPLC-UV instrument for analyte separation and quantification.

2.6 Validation of analytical method

The extraction method was assessed for limit of detection (LOD), linearity, repeatability and enrichment factor. The enrichment factor (EF) of the EME procedure was calculated according to the following equation:

$$EF = \frac{Ca,final}{Cs.initial} \quad (1)$$

3. Results and discussion

3.1. Optimization of EME process

Several parameters namely type of organic solvent/SLM, pH of sample solution, applied voltage, extraction time, and stirring speed were evaluated to obtain the optimum extraction efficiency. The optimization was carried out in triplicate using water samples

containing 1.0 μg mL^{-1} of each analyte. In this study, the salting out effect was not evaluated because of the high content of ionic compound that can cause a decrease in the flux of the analyte across the SLM [35]. Therefore, the migration of analyte would be more efficient without the salt addition and result in higher extraction efficiencies. Furthermore, the solubility and NPOE-water-partiton coefficients ($K_{o/w}$) of the less polar TCAs were not changed by the addition of salt into the sample solution [36]. In a two-phase system, the analytes can be extracted by a mixed-mode mechanism where the charged analytes of target molecules can be extracted by electrokinetic migration and the uncharged molecules can be extracted by passive diffusion [37, 8].

3.1.1 Selection of organic solvent

Organic solvent is one of the important parameters that affect the extraction efficiency in EME [5-9]. In this work, the organic solvent serves as the SLM and also as an acceptor phase. The extraction process involves the transfer of analytes from the sample solution across the pores of membrane impregnated with an organic solvent and then diffused into the acceptor phase assisted by electrical forces. Therefore, there were some considerations that should be taken into account in selecting a suitable organic solvent. Firstly, the organic solvent should be compatible with the membranes. The solvent should have a low boiling point or non-volatile to prevent solvent loss during the extraction due to the electrical potential generated [19, 34]. Secondly, the solvent must be immiscible with water and must have a good affinity for the target compounds [38]. Finally, the organic solvent must have sufficient electrical conductivity or dipole moment to allow a continuous electrical field being across in the midst of extraction system from the donor solution and the acceptor phase [39].

In this work, four organic solvents namely toluene, 1-heptanol, 1-octanol and NPOE were investigated. It was found that, NPOE gave the highest peak areas, followed by 1-

Heptanol, 1-octanol and toluene . NPOE also showed an efficient organic solvent for use to extract basic drugs through SLM [40], while 1-octanol was used to extract acidic drugs [41]. The lowest peak area was obtained with toluene. It can be explained by the relatively low boiling point of the solvent where the solvent is prone to lost during the extraction process due to the electrical potential applied [42, 19]. Therefore, NPOE was selected as the organic solvent and used in subsequent analysis.

3.1.2. Effect of sample pH

In order to determine the effect of the pH of the sample solution on the extraction efficiencies, experiments were carried out by varying the pH values from 3 to 7. Solutions of hydrogen chloride (HCl) with 0.1 M and sodium hydroxide (NaOH) with 0.1 M were used for pH adjustment. It was found that the peak area slightly increased with increasing pH from pH 3 to 6 (Fig. 2) and then dropped at pH 7 due to the ionization of the analytes that occurred at the neutral pH value [39]. The pK_a values of target TCAs analytes ranged from 9.3 to 9.5 and under the alkaline conditions, TCAs would exist in cationic form. Therefore, there was a competition between H^+ ion and cationic analytes that result in the decreased of the extraction efficiency [38]. Analytes with dissociation constants, pK_a of around 9.5 at low pH (1-6) was completely ionized. As the selected pH of the sample solution should be sufficiently low to maintain analytes in the ionized forms and prevent from back extracted into the organic phase [43, 18]. Therefore, pH 6 was selected as the best pH value and used in subsequent experiments.

3.1.3. Effect of applied voltage

In order to determine the effect of applied voltage on the performance of the method, experiments were carried out by varying the applied voltage from 5 to 60 V. The results are

summarized in Fig.3. It was found that highest peak areas were obtained at 10 V. This led to the deduction that the electrokinetic migration of protonated basic drug compounds across a membrane was influenced positively by relatively low electrical potential differences (< 15 V) [42]. The extraction slightly decreased when voltage applied was higher than 10 V. Therefore, 10 V was selected and applied in further analyses.

3.1.4 Effect of extraction time

Extraction times in the range of 5 - 25 min were studied. It was found that the peak area was rapidly increased from 5 to 10 min of extraction (Fig. 4). The highest extraction efficiency was obtained at extraction time of 10 min and beyond the peak areas decreased significantly. The latter phenomenon could be due to the back diffusion of analytes to the organic liquid membrane [43]. Therefore, 10 min was selected as the optimal extraction time and used in subsequent experiments.

3.1.5 Effect of stirring speed

In this work, the stirring rates from 600 to 1350 rpm were studied (Fig. 5). It was observed that, the peak area increased with stirring rate from 600 to 1350 rpm and reached maximum at 1200 rpm. Higher agitation rates facilitate the mass transfer of analytes into the acceptor phase [2, 33]. However, bubbles were observed at the surface of electrode at stirring rates of >1200 rpm at the surface of electrode and organic solvent could be potentially loss [44]. Therefore, stirring rate of 1200 rpm was chosen and used in subsequent experiments.

3.2 Theoretical considerations

In EME, the analytes can be extracted by a mixed-mode mechanism where the charged molecules of target analytes can be extracted by electrokinetic migration and the

uncharged molecules can be extracted by passive diffusion [34]. The flux of analytes (*Ji*) is affected by magnitude of the applied voltage; this was addressed by the group of Gjelstad [12].

$$J_i = -\frac{D_i}{H}\left(1+\frac{\upsilon}{ln\chi}\right)\left(\frac{\chi-1}{\chi-\exp(-\upsilon)}\right)(C_{ih} - C_{io}\exp(-\upsilon)) \quad (2)$$

where *Di* is the diffusion coefficient for the analyte, *h* is the thicknessof the membrane, *Cih* is the analyte concentration at the membrane/sample interface. Also, υ is a function of electrical potential while χ is defined as ion balance (the ratio of the total ionic concentration in the sample solution to the acceptor solution). Nevertheless, the above equation was not applicable in this work as the concentrations of the two-phase solutions were constant throughout the extraction process. Therefore, the only concentration gradient in this two-phase mode system was only the gradient across the membrane. Thus, the flux was greatly dependent on the stirring rate conditions [33].

In EME, there was also a potential that the mass transfer of basic drug substances across an SLM were under influenced a low applied voltage and was upon greatly depending on the structure of the compounds. Recently, work has demonstrated by Dominguez and co-workers [22] where a low applied voltage (0-15V) was applied to determine drug substances. This can also be expressed to by the Nernst equation for partitioning of charged compounds in two-phase systems whereby the partition coefficient of the ionized compound are dependent on the potential difference sustained over a liquid-liquid interface [45].

$$\Delta_o^W \emptyset = \emptyset^w - \emptyset^o = \Delta\frac{w}{o}\emptyset\frac{0}{i} + \frac{RT}{Z_iF}\ln\frac{{a_i}^o}{{a_i}^w} \quad (3)$$

where $\Delta_o^W \emptyset$ is the Galvani potential difference between the two phases, $\emptyset^o$ is the Galvani potential of the organic phase, $\emptyset^w$ is the Galvani potential of the aqueous phase, $\Delta \frac{w}{o} \emptyset \frac{0}{i}$ is termed the standard transfer potential, R is the ideal gas constant, T is the absolute temperature, Z_i is the charge of an ion i , F is the Faraday constant, a_i^o is the activity of an ion i in the aqueous phase.

3.3 Method validation and analytical performances of two phase EME-HPLC-UV

The two-phase EME-HPLC-UV method was validated by characterizing its analytical performance under optimal conditions as follows: NPOE as an organic solvent, pH of sample solution of 6, extraction time of 10 min, 10 V of extraction voltage and 1200 rpm of stirring speed. Under the optimum conditions, the proposed method was validated in terms of linearity, limit of detection (LOD), enrichment factor, inter-assay precision (RSD%) and relative recovery (RR%) in drug-free water and urine samples [46]. The result (Table 1) showed good linearity for all the compounds with coefficients of determination, $r^2 \geq 0.9987$. LODs were calculated at three times the signal noise-to-noise ratio (S/N × 3). The low LODs in the range of 0.05 - 0.3 μg L^{-1} was obtained with high enrichment factors in the range of 91 – 128. The relative recovery and inter-assay precision were determined at low and high concentrations (1 and 100 μg L^{-1}) with triplicate analyses using water and human urine samples. The results showed excellent relative recoveries in the range of 86.7 - 107.4% and good reproducibility with relative standard deviation (RSDs) of ≤ 9.5% (Table 2). Fig. 6 shows HPLC chromatograms of non-spiked and spiked water samples and human urine samples at concentration levels of 1.0 μg L^{-1} and 100 μg L^{-1}.

3.4 Comparison of proposed method with other reported methods

The performance of the proposed two-phase EME–HPLC-UV method was compared with other methods published previously for the extraction and determination of TCAs (Table 3. SBSE method is a very simple, solvent-free and fast technique; however, this method resulted in rather high limit of detection (40 μg L^{-1}) and suffered from a long extraction time (60 min) [47]. Although HF-LPME [48] and HF-LLLME-HPLC-DAD [49] methods resulted in good sensitivity, linearity and high enrichment factors; this method took a relatively longer extraction time (40 min) due to the passive diffusion of analytes. EME combined with solid phase microextraction (SPME) [39] utilized carbonaceous pencil lead as the electrode (cathode) which acted as a SPME sorbent. This method offered good detection limits and acceptable extraction efficiencies, but SPME suffers from sample carry over and fiber fragility [11]. EME combined with GC-FID [33], GC-MS [34] and DLLME with GC-FID [50] were demonstrated to enhance of the possibility to achieve more sensitivity of detection and high enrichment factor. Although, the results showed low LODs and high enrichment factors, direct injection of water in GC can eradicate the FID flame and most wide chemical compounds need to derivatization before analysis by GC. Furthermore, the combination EME with DLLME-GC-FID extraction was performed with relatively high voltage of 240 V.

As shown in comparison with other published methods, this study presented high enrichment factor ranged between 91 and 128 but the LODs and extraction time was comparable with HF-LPME-HPLC [48] and slightly better than three-phase EME-GC-FID [33] and two phase EME-GC-MS [34]. The proposed study demonstrated a direct interaction between sample solution and acceptor phase across a polypropylene membrane filter impregnated with NPOE that can overcome the problem often encountered in HF-SLM which had a limited mechanical stability that can lead to a loss impregnated organic solvent under

agitation process and high voltage. Furthermore, this method was performed under low voltage and low consumption of solvent. The overall result obtained indicate that two-phase of EME is a promising technique for analysis of TCAs from biological matrices and water and it was compatible with other chromatographic instrument and electrophoretic systems.

4. Conclusion

To the best of our knowledge, the present study demonstrates the first utilization of a two-phase low-voltage electrodriven membrane extraction (EME) in combination with HPLC-UV for the analysis of TCAs in water and human urine samples. In comparison with conventional EME, the developed method used a polypropylene membrane filter impregnated with NPOE to solve the problems encountered in HF-LPME including the poor mechanical stability, fragility and easy handling. Furthermore, this method performed under a two-phase system that makes it simple and faster extraction compared to three-phase system. More importantly, since the final phase in this extraction is an organic phase, this method becomes compatible with many types of instruments such as GC-FID/MS. In addition, the proposed method required a low voltage supply that can reduce the consumption of electrical energy sources. The application of the proposed two-phase EME-HPLC-UV method can expanded for wider applications such as environmental waste and food analysis. In short, the developed method provided a fast extraction time, simple step and minimum organic solvent consumption which meet the criteria for green analytical method.

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References

[1] M. Saaid, B. Saad, A. S. M. Ali, M. I. Saleh, C. Basheer and H. K. Lee, *J. Chromatogr. A*, 2009, **1216**, 5165-5170.

[2] J. Pawliszyn and S.P. Bjergaard, . *Chromatogr. A*, 2006, **44**, 291-307.

[3] E. Psillakis , D. Mantzavinos and N. Kalogerakis, *Anal. Chim. Acta*, 2004, **501**, 3-10.

[4] M. M. Sanagi, S. H. Loh, W. A. Wan Ibrahim and M. N. Hasan, *J. Chromatogr. A*, 2012, **1262**, 43-48.

[5] T. S. Ho, T. G. Halvorsen, S. Pedersen-Bjergaard and K.E. Rasmussen, *J. Chromatogr. A*, 2003, **998**, 61-72.

[6] S. Pedersen-Bjergaard and K.E. Rasmussen, *J. Chromatogr. A*, 2008, **1184**, 132–142.

[7] N. J. Petersen, S. T. Foss, H. Jensen, S. H. Hansen, C. Skonberg, D. Snakenborg, J. P. Kutter and S. Pedersen-Bjergaard, *Anal. Chem.,* 2011, **83**, 44-51.

[8] S. Seidi, Y. Yamini, M. Rezazadeh and A. Esrafili, *J. Chromatogr. A*, 2012, **1243**, 6-13.

[9] S. Pedersen-Bjergaard and K. E. Rasmussen, *J. Chromatogr. A*, 2006, **1109**, 183-190.

[10] M. Balchen, A. Gjelstad, K. E. Rasmussen and S. Pedersen-Bjergaard, *J. Chromatogr. A,* 2007, **1152**, 220–225.

[11] S. Seidi, Y. Yamini, A. Heydari, M. Moradi, A. Esrafili and M. Rezazadeh, *Anal. Chim. Acta*, 2011, **701**, 181-188.

[12] A. Gjelstad, T. M. Andersen, K. E. Rasmussen and S. Pedersen-Bjergaard, *J. Chromatogr. A*, 2007, **1157**, 38-45.

[13] M. Rezazadeh, Y. Yamini and S. Seidi, *J. Chromatogr. B*, 2011, **879**, 1143-1148.

[14] A. Gjelstad, and S. Pedersen-Bjergaard, *Anal. Methods*, 2013, **5**, 4549-4557.

[15] M. Balchen, L. Reubsaet and S. Pedersen-Bjergaard, *J. Chromatogr. A*, 2008, **1194**, 143-149.

[16] P. Kubáˇn, L. Strieglerova, P. Gebauer and P. Bocek, *Electrophoresis*, 2011, **32**, 1025-1032.

[17] S. Nojavan and A. R. Fakhari, *J. Sep. Sci*., 2010, **33**, 3231-3238.

[18] A. Gjelstad, K.E. Rasmussen and S. Pedersen-Bjergaard, *Anal and bioanalytical chemistry*, 2009, 393, 921-928.

[19] L. Xu, P. C, Hauser and H. K, Lee, *J. Chromatogr. A*, 2008, **1214**, 17-22.

[20] H. H. See, P.C. Hauser, M. M. Sanagi and W. A. Wan Ibrahim, *J. Chromatogr., A*, 2010, **1217**, 5832-5838.

[21] H. H. See and P.C. Hauser, *Anal. Chem*., 2011, **83**, 7507-7513.

[22] N. C. Dominguez, A. Gjelstad, A. M. Nadal, H. Jensen, N. J. Petersen, S. H. Hansen, K. E. Rasmussen and S. Pedersen-Bjergaard*, J. Chromatogr. A*, 2012, **1248**, 48-54.

[23] J. Wang, M. Bonakdar and C. Morgan, *Anal. Chem*., 1986, **58**, 1024-1028.

[24] V. F. Samanidou, M. K. Nika and I. N. Papadoyannis, *J. Sep. Sci*., 2007, **30**, 2391-2400.

[25] P. K. Gillman, *Br. J. Pharmacol*, 2007, **151**, 737-748.

[26] J. Lundmark, F. Bengtsson, C. Nordin, M. Reis and J. Walinder. *Acta Psychiatr Scand*., 2000, **101**, 354-359.

[27] M. P. Segatti, G. Nisi, F. Grossi, M. Mangiarotti and C. Lucarelli, *J. Chromatogr. A*, 1991, **536**, 319-325.

[28] Y. Chen, Z. Guo, X. Wang and C. Qiu, *J. Chromatogr. A.*, 2008, **1184**, 191-200.

[29] R. Theurillat and W. Thormann, *J. Pharm. Biomed. Anal.*, 1998, **18**, 751-760.

[30] L. Mercolini, M. Grillo, C. Bartoletti, G. Boncompagni and M. A. Raggi, *J. Anal. Bioanal. Chem*., 2007, **388**, 235-243.

[31] M. N. Uddin, V. F. Samanidou and I. N. Papadoyannis, *J. Sep. Sci*., 2008, **31**, 2358-2370.

[32] K. Jinno, M. Kawazoe and M. Hayashida*, Chromatographia*, 2000**, 52**, 309-313.

[33] S. S. H. Davarani, A. M Najarian, S. Nojavan and M. A. Tabatabaei, *Anal. Chim. Acta*, 2012, **725**, 51-56.

[34] S. S. H. Davarani, A. Morteza-Najarian, S. Nojavan, A. Pourahadi and M. B. Abbassi, *J. Sep. Sci*., 2013, **36**, 736-743.

[35] T.M. Middelthon-Bruer, A. Gjelstad, K.E. Rasmussen and S. Pedersen-Bjergaard, *J. Sep. Sci*., 2008, **31**, 753-759.

[36] J. Lee, F. Khalilian, H. Bagheri and H. K. Lee, *J. Chromatogr., A*, 1216 (2009) **1216**, 7687-7693

[37] K. S. Hasheminasab and A. R. Fakhari, *Anal. Chim. Acta*, 2013, 767, 75-80.

[38] H. Tabani, A. R. Fakhari and A. Shahsavani, *Electrophoresis*, 2013, **34**, 269-276.

[39] M. Rezazadeh, Y. Yamini, S. Seidi and B. Ebrahimpour, *J. Chromatogr. A*, 2013, **1280**, 16-22.

[40] A. Gjelstad, K. E. Rasmussen and S. Pedersen-Bjergaard, *J. Chromatogr. A*, 2006, **1124**, 29-34.

[41] M. R. Payán, M. A. B. López, R. F. Torres, M.V. Navarro, M. C. Mochón, *Talanta,* 2011, **85**, 394–399.

[42] C. Basheer, S. H. Tan and H. K. Lee, *J. Chromatogr. A*, 2008, **1213**, 14-18.

[43] L. Guo and H. K. Lee, *J. Chromatogr. A*, 2012, **1243**, 14-18.

[44] H. Tabani, A. R. Fakhari and E. Zand, *Anal. Methods*, 2013, **5**, 1548-1555.

[45] H. Jensen, V. Devaud, J. Josserand and H. H. Girault, *J. Electroanal. Chem.,* **537**, 77-84.

[46] N. Yahaya, M. M. Sanagi, H. Nur, W. A. Wan Ibrahim, S. Kamaruzaman and H. Y. Aboul-Enein, *Anal. Methods*, 2014, **6**, 3375-3381.

[47] A. R. Chaves, S. M. Silva, R. H. Costa-Queiroz, F. M. Lancas and M.E. Costa-Queiroz, *J. Chromatogr., B*, 2007, **850**, 295-302.

[48] A. Esrafili, Y. Yamini and S. Shariati, *Anal. Chim. Acta*, 2007, **604**, 127-133.

[49] M. Ghambarian, Y. Yamini and A. Esrafili, *J. Chromatogr. A*, 2012, **1222**, 5-12.

[50] S. Seidi, Y. Yamini and M. Rezazadeh, *J. Chromatogr. B*, 2013, **913**, 138-146.

Figure captions:

Figure 1. Schematic of two-phase electrodriven membrane extraction (EME).

Figure 2. Effect of pH of sample solution on the extraction efficiency: spiked concentration: 1.0 μg mL^{-1} of analyte, organic solvent: NPOE, pH of sample solution: 3, applied voltage: 50 V, extraction time: 15 min, and stirring rate: 1050 rpm.

Figure 3. Effect of applied voltage of sample solution on extraction efficiency; spiked concentration: 1.0 μg mL^{-1} of analyte, organic solvent: NPOE, pH of sample solution: 6, applied voltage: 50 V, extraction time: 15 min, and stirring rate: 1050 rpm.

Figure 4. Effect of extraction time of sample solution on extraction efficiency; spiked concentration: 1.0 μg mL^{-1} of analyte, organic solvent: NPOE, pH of sample solution: 6, applied voltage 10 V, extraction time: 15 min, and stirring rate: 1050 rpm.

Figure 5. Effect of stirring speed of sample solution on extraction efficiency; spiked concentration: 1.0 μg mL^{-1} of analyte, organic solvent: NPOE, pH of sample solution: pH 6, applied voltage: 10 V, extraction time: 10 min, and stirring rate: 1050 rpm.

Figure 6. HPLC Chromatograms of TCAs extracted using two-phase EME under optimized conditions from (A) water sample: (a) water sample spiked at concentration of 100 μg L^{-1} of each analyte (b) water sample spiked at 1.0 μg L^{-1} (c) non spiked water sample and (B) urine sample: (a) urine sample spiked at level of 100 μg L^{-1} of each analyte (b) urine sample spiked at level of 1.0 μg L^{-1} (c) non spiked urine sample on an Agilent Zorbax SB-C_{18} column (2.1 × 100 mm, 3.5 μm). HPLC conditions: isocratic mobile phase potassium dihydrogen phosphate buffer (25 mM, pH 6.0)-ACN-MeOH (30:55:15, v/v) at a flow rate of 0.2 mL min^{-1}, injection volume of 2 μL and detector wavelength at 240 nm. Peak identities: 1, NPOE; 2, imipramine (IMI); 3, amitriptyline (AMI); 4, chlorpromazine (CHLO).

Table 1

Quantitative results of two-phase EME-HPLC for tricyclic antidepressants (TCAs).

Sample	TCAs	Linear range (μg L^{-1})	Coefficient of determination (r^2)	LOD (μg L^{-1})	Enrichment factor	RSD % (n = 3)
Water	IMI	0.5-1000	0.9998	0.05	122	4.9
	AMI	0.5-1000	0.9995	0.08	103	6.3
	CHLO	0.5-1000	0.9987	0.07	128	2.4
Urine	IMI	1-1000	0.9994	0.1	116	5.5
	AMI	1-1000	0.9992	0.3	113	6.3
	CHLO	1-1000	0.9989	0.2	91	2.9

Table 2

Results of two-phase EME-HPLC of TCAs from water and human urine samples.

Analytes	Spiked concentration ($\mu g\ L^{-1}$)	Water		Urine	
		RR%[a]	RSD%[b] (n = 3)	RR%[a]	RSD%[b] (n = 3)
Imipramine (IMI)	1	100.5	4.3	86.7	4.8
	100	101.7	3.2	97.3	7.4
Amitriptyline (AMI)	1	101.6	3.2	102.3	6.6
	100	101.4	9.5	90.6	4.1
Chlorpromazine (CHLO)	1	103.1	5.1	95.2	3.4
	100	98.4	8.4	107.4	8.7

[a] Relative recovery
[b] Inter-assay precision

Table 3 Comparison of the proposed method with other published methods for the extraction and determination of TCAs.

Extraction technique [a]	LOD [b] ($\mu g\ L^{-1}$)	Linearity	Extraction Time	Extraction efficiency	Sample volume	Ref
SBSE-HPLC	10-40	10-1000	60 min	-	5 mL	[47]
HF-LPME-HPLC	0.5-0.7	5-500	40 min	298-315	11 mL	[48]
HF-LLLME-HPLC-DAD GC-MS	0.08-0.2 0.04	0.2-200	40 min	630-690	20 mL	[49]
Three-phase EME-GC-FID	0.35-0.8	5-1500	20 min	215-280	2.1 mL	[33]
Two-phase EME-GC-MS	0.1-0.25	1-500	15 min	140-175	1.2 mL	[34]
EME-SPME-GC-FID	0.5-5.0	2-500	20 min	-	24 mL	[39]
EME-DLLME-HPLC-UV	0.25-15	2-500	17 min	-	24 mL	[50]
Two-phase EME-HPLC-UV	0.05-0.3	0.5-1000	10 min	91-128	10 mL	Present work

[a] Stir bar sorptive extraction (SBSE), high-performance liquid chromatography (HPLC), hollow fiber (HF), liquid phase microextraction (LPME), liquid-liquid-liquid microextraction (LLLME), diode array detection (DAD), gas chromatography (GC), mass spectrometry (MS), electromembrane extraction (EME), flame ionization detector (FID), solid phase microextraction (SPME), dispersive liquid liquid microextraction (DLLME), ultraviolet (UV),
[b] Limit of detection.

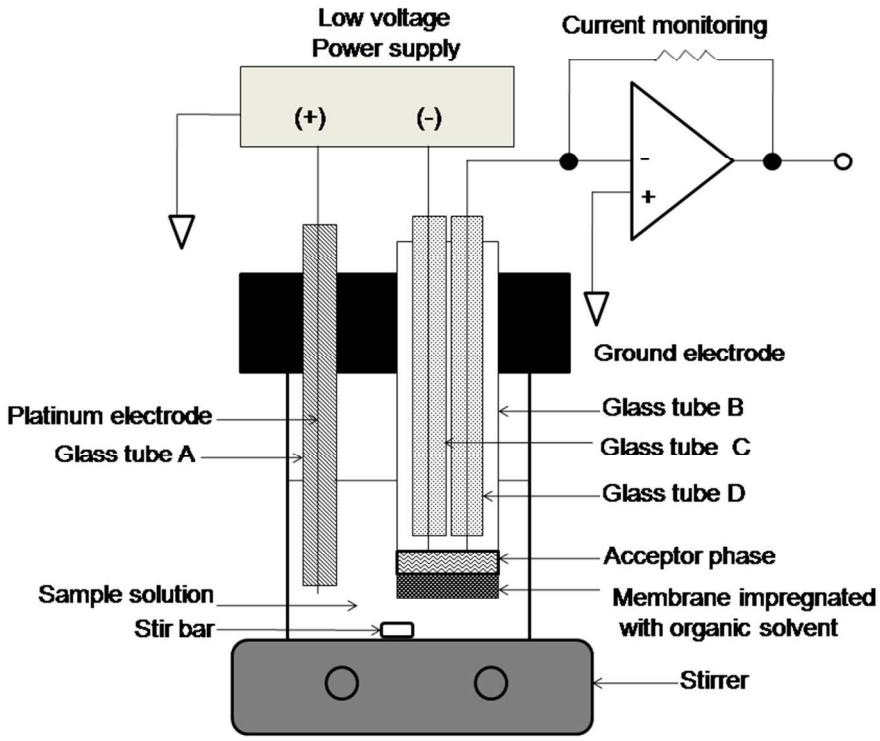


Figure 1. Schematic of two-phase electrodriven membrane extraction (EME).
254x200mm (96 x 96 DPI)

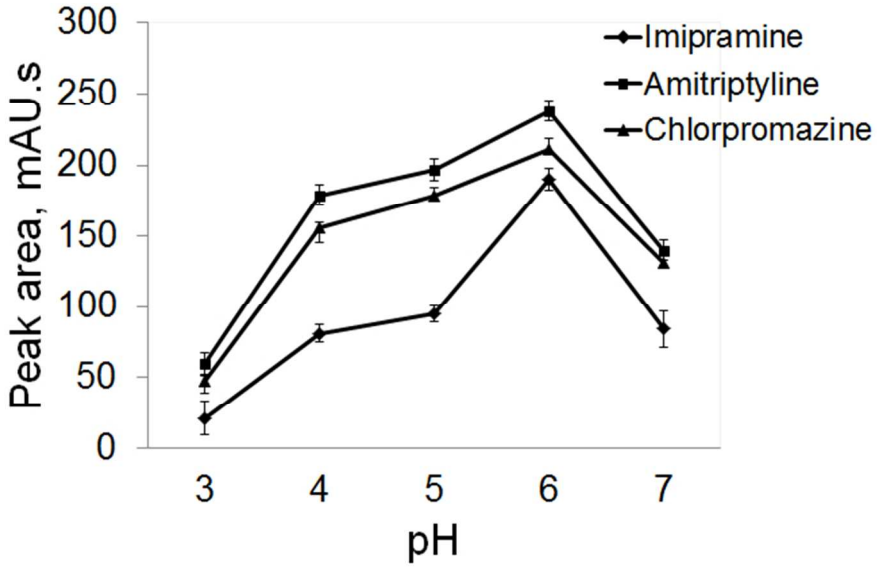


Figure 2. Effect of pH of sample solution on the extraction efficiency: spiked concentration: 1.0 μg mL−1 of analyte, organic solvent: NPOE, pH of sample solution: 3, applied voltage: 50 V, extraction time: 15 min, and stirring rate: 1050 rpm.
196x118mm (96 x 96 DPI)

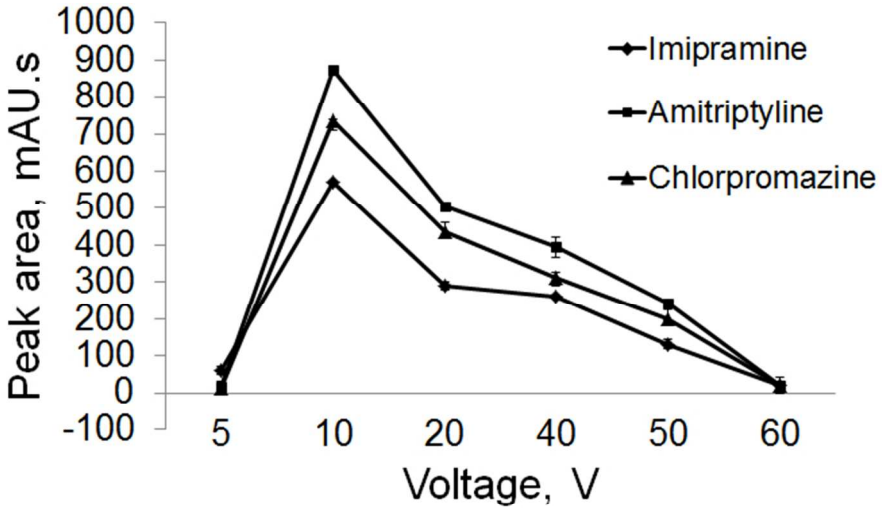


Figure 3. Effect of applied voltage of sample solution on extraction efficiency; spiked concentration: 1.0 µg mL−1 of analyte, organic solvent: NPOE, pH of sample solution: 6, applied voltage: 50 V, extraction time: 15 min, and stirring rate: 1050 rpm.
196x118mm (96 x 96 DPI)

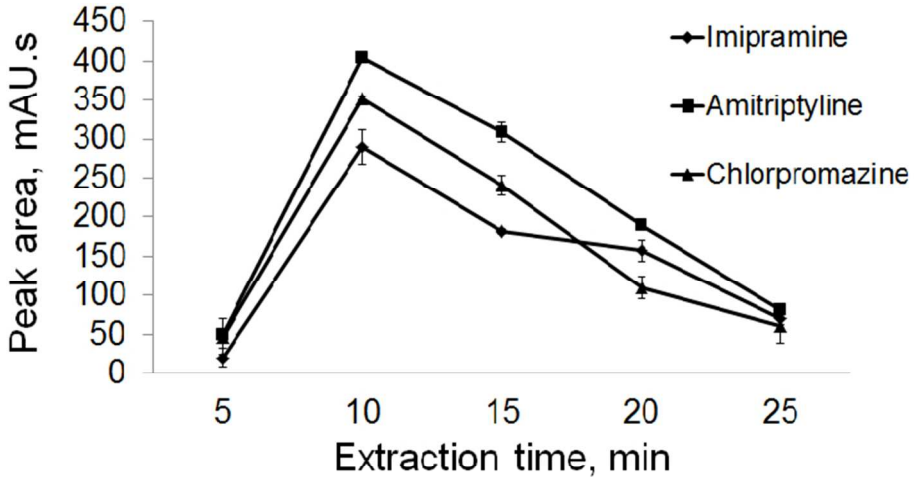


Figure 4. Effect of extraction time of sample solution on extraction efficiency; spiked concentration: 1.0 μg mL−1 of analyte, organic solvent: NPOE, pH of sample solution: 6, applied voltage 10 V, extraction time: 15 min, and stirring rate: 1050 rpm.
212x118mm (96 x 96 DPI)

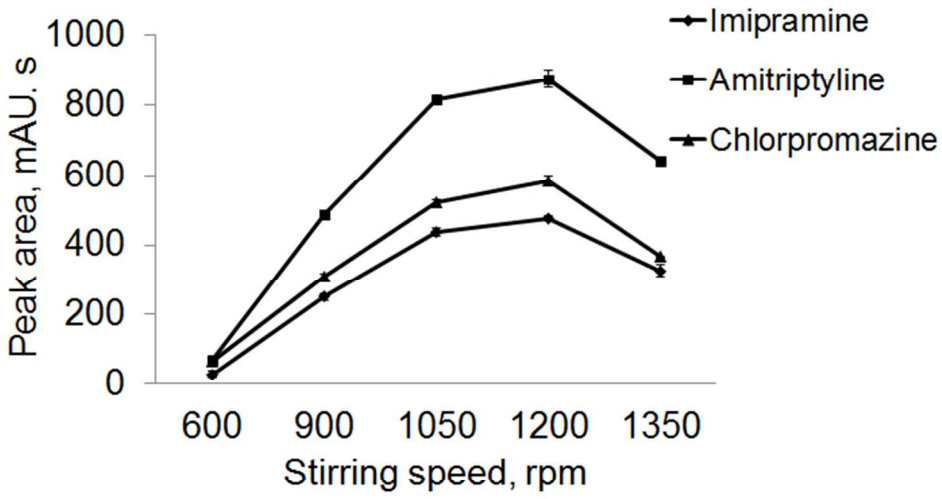
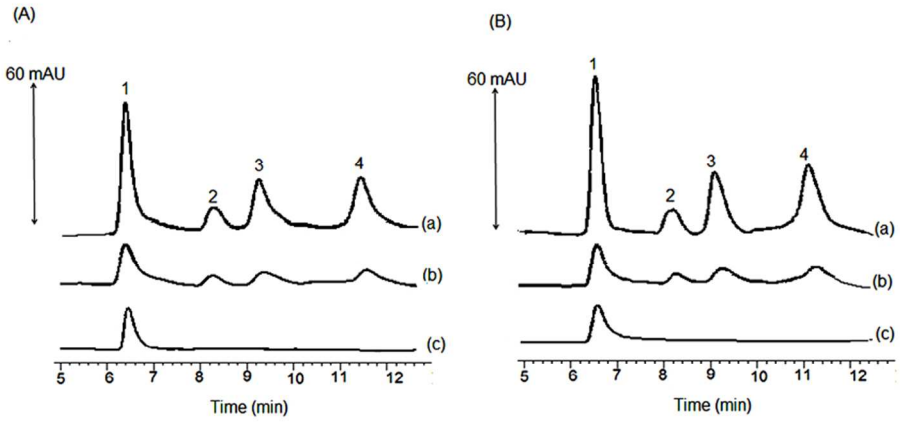


Figure 5. Effect of stirring speed of sample solution on extraction efficiency; spiked concentration: 1.0 µg mL−1 of analyte, organic solvent: NPOE, pH of sample solution: pH 6, applied voltage: 10 V, extraction time: 10 min, and stirring rate: 1050 rpm.
196x118mm (96 x 96 DPI)



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247x124mm (96 x 96 DPI)