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Optimization of Thin-film Microextraction using a Polyelectrolyte Multilayer Sorbent Combined with HPLC-UV for Separation and Determination of Tricyclic Antidepressant Residues in Aqueous Samples

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Thin-film polyelectrolyte multilayers (PEMs), namely poly(allylamine hydrochloride) (PAAH) and poly(styrene sulfonic acid) (PSS), were successfully prepared as a new extraction medium for drugs. Three kinds of tricyclic antidepressant drugs (TCAs), namely imipramine (IMI), amitriptyline (AMI), and chlorpromazine (CHLO) were selected as target model analytes. Thin-film microextraction (TFME) was performed by piercing 7 (approx. 8.4 mg) circular cellulose acetate-polyelectrolyte multilayers (CA-PEMs) (5 mm diameter) with a needle and directly dipping them into a sample solution which was agitated during the extraction process. The CA-PEMs were ultrasonicated for analyte desorption in 100 µL of organic solvent before analysis with high-performance liquid chromatography with ultraviolet detection (HPLC-UV). Several important parameters such as type of desorption solvent, the effect of sample pH, salting-out effect, extraction time, desorption time, the volume of desorption solvent, and stirring speed were evaluated and optimized. Under optimized extraction conditions, the technique demonstrated good linearity in the concentration range of 10-1000 µg L^{-1} for lake and river water samples, and 50-1000 µg L^{-1} for urine samples. The detection limits were in the range of 3.7 - 40.5 µg L^{-1}. The percentage recoveries were generally in the range of 99 - 111.18 %, with an RSD of 2.6 - 4.7 % (n=3). The facile synthesis conditions also indicate that the proposed method has good applicability in terms of simplicity, effectiveness, selectivity and accuracy. Thus, this method proved to be a suitable alternative green method for analysis of TCAs in different matrices.

Key words: Drug residues; HPLC-UV; thin-film microextraction; tricyclic antidepressant drugs

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Depressive disorder is the fourth leading cause of global disease burden and was predicted to be the second biggest health problem worldwide in 2020 and the largest disease burden in the world in 2030 [1]. Two derivatives of tricyclic antidepressants (TCAs), namely imipramine (IMI) and amitriptyline (AMI), are utilised intensively to treat psychiatric disorders [2]. The use of TCAs is expected to increase every year due to the growing number of patients with psychiatric disorders. Most TCAs have limits for therapeutic concentrations in the range of 80-300 ng mL^{-1}, with toxicity concentrations exceeding 500 ng mL^{-1} in the human body [2–4]. TCAs are not only present in the human body but also in aquatic systems through water reservoirs due to incomplete removal or degradation during sewage treatment [5]. These compounds can affect humans via the food chain or drinking water. Thus, monitoring the concentration of pharmaceutical residues in the aquatic environment is also an important issue to minimize their exposure to humans.

Sample preparation based on the micro-extraction technique has become an interesting subject for monitoring trace amounts of organic compounds. Sample preparation is an important part of the analysis of complex matrices, and involves extraction, concentration, and clean-up before instrumental detection b[6]. These steps aim to clean up the sample, enrich the analyte, and enhance the signal detected by the instrument. Sample preparation based on microextraction has recently gained attention in line with the green chemistry concept. The basic principle of microextraction is to extract analytes into micro-liters of organic solvent or adsorb the analytes onto small amounts (mg) of adsorbent [7]. Micro-extraction has advantages not only in terms of requiring less or

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no solvent but it also provides signal enhancement, as well as being a rapid and simple technique that is easily coupled with gas or liquid chromatography [8].

Thin-film microextraction (TFME) is one of the techniques developed for solid-phase microextraction (SPME). In TFME, a flat film with a high surface area to volume ratio is used as the extraction phase [9]. With this configuration, the extraction equilibrium is shortened due to a decrease in the thickness of the extraction phase, thereby accelerating the extraction rate. Polyelectrolyte-based sorbent is an alternative option for a thin-film sorbent. It can be prepared simply using a self-assembly technique [10].

In this work, a cellulose acetate-coated polyelectrolyte multilayer was proposed as an extraction phase in TFME. Three TCA drugs, imipramine (IMI), amitriptyline (AMI), and chlorpromazine (CHLO), were used as target analytes. The determination of pharmaceutical residues was performed using HPLC-UV. Optimization of extraction conditions was carried out to achieve the best extraction performance. Several parameters such as organic solvent selection, the pH of the sample solution, salt addition, extraction time, desorption time, desorption solvent volume, and stirring speed were studied. The optimum extracting conditions were applied to determine TCA residues in urine, as well as lake and river water samples.

MATERIALS AND METHODS

Materials

Chemicals and reagents for polyelectrolyte multilayer-coated cellulose acetate membranes (CA-PEM) were obtained from various sources. Cellulose acetate (CA) was purchased from R&M Chemicals, acetone for dissolved CA powder was obtained from Q-RëC, formamide as porogen and coating materials, poly (allylamine hydrochloride) (PAAH) (MW = 900,000), and poly(4-styrene sulfonic acid) (PSS) (MW = 75,000) were purchased from Sigma Aldrich. Deionized water of at least 18 MΩ was produced by a Nano ultra-pure water system. Imipramine (IMI) and amitriptyline (AMI) (pure analytical grade) were purchased from Sigma-Aldrich, while chlorpromazine (CHLO) was obtained from Clearsynth. HPLC grade organic solvents (acetonitrile (ACN), methanol (MeOH), ethanol (EtOH), and isopropanol (IPA)) were obtained from J.T. Baker and Merck. Potassium dihydrogen phosphate ($KH_2(PO_4)_3$) (analytical grade) was purchased from Merck. Nylon membrane filters (0.45 µm) from Gelman Sciences were used to filter buffer solutions and real samples. A Fisher Scientific ultrasonic water bath was used to sonicate the membrane during the desorption process. A hot plate stirrer and a magnetic stirrer bar (12 mm × 4 mm) were used to stir the sample solution during the TFME process. A Mitutoyo micrometer was used to measure membrane thickness.

Chromatographic Conditions

HPLC separations were performed on a Zorbax SB-C18 column (100 × 2.1 mm, 3.5 µm) from Agilent Technologies (California, USA) under isocratic conditions. A HPLC system with ultraviolet-visible (UV-Vis) detector and rheodyne (20 µL sample loop) from Agilent Technologies (California, USA) was used for determination of the analytes. The chromatographic data were recorded at a wavelength of 240 nm and processed using Agilent Chemstation software. The mobile phase consisted of acetonitrile-methanol-phosphate buffer (25 mM, pH= 6) in the ratio 55:15:30 (v/v). The injection volume and flow rate were set at 2 µL and 0.2 mL min^{-1}, respectively.

Preparation of CA-PEMs

A cellulose acetate (CA) flat sheet membrane was prepared by diluting 0.08% w/v of CA in acetone and casting the solution on a glass plate. The surface of CA was activated into becoming negatively charged by dipping the CA membrane into 0.5 M sodium hydroxide solution for 5 min. Then, the activated CA membrane was immersed in a 0.02 M PAAH solution (polycationic) at pH 2 for 5 min and then dried at room temperature. After the immersion process was complete, the membrane was rinsed with deionized water to remove excess polyelectrolyte, and dried at room temperature. The membrane was then dipped in 0.2 M PSS solution (polyanionic) at pH 2 for 5 min. This was repeated until 5 pairs of layers were formed. The coated film was then dried and rinsed with deionized water to remove the unreacted ions.

Thin Film Microextraction (TFME)

Thin-film microextraction (TFME) was performed by piercing 7 (ca. 8.4 mg) circular-shaped CA-PEM membranes (5 mm diameter) with a needle, directly dipping these into 10 mL of aqueous solution (500 µg L^{-1} of each IMI, AMI, and CHLO), and agitating the solution during the extraction process (Fig. 1). At the end of the extraction process, the CA-PEM membranes were ultrasonicated in 100 µL of organic solvent to desorb the analytes before HPLC-UV analysis. Parameters such as organic solvent selection, the pH of sample solution, salt addition, extraction time, desorption time, desorption solvent volume and stirring speed were optimized. The optimized extraction conditions were validated and applied to extract the selected TCAs (IMI, AMI, and CHLO) from the aqueous sample. The set-up of the technique is provided in Figure 1.

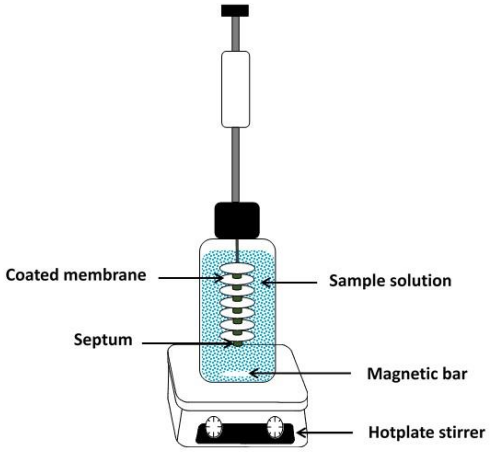

Figure 1. Set-up of thin-film microextraction (TFME) technique

Sample Analysis

Lake water and river water samples were obtained from Universiti Teknologi Malaysia Lake (Johor, Malaysia) and Skudai River (Johor, Malaysia) as the estuary of the university health centre's water channel. The urine sample was collected from a healthy volunteer with the inclusion criteria that the person had not consumed any drug in the two weeks prior. Lake water and river water samples (1 L each) were filtered separately using 0.45 µm Whatman filter paper and Buchner funnels to separate solid waste. The filtration process was repeated using 0.2 µm Whatman filter paper to remove smaller impurities. After filtration, the water sample was kept in the refrigerator at 4 ˚C before being used for analysis. Meanwhile, the urine sample was collected from the first urine in the morning and centrifuged to precipitate large molecules such as proteins and lipids. The sample was then filtered to separate the sediment from the supernatant. The aqueous solution collected from the filtered urine sample was then diluted with deionized water (1:1) and kept in a refrigerator at 4 ˚C before analysis. All these samples were extracted using CA-PEM-TFME and analyzed using HPLC-UV under optimized extraction conditions.

RESULTS AND DISCUSSION

1. Optimization of Organic Solvent Selection

In microextraction, the selection of an appropriate organic solvent is important to achieve the highest extraction efficiency. The stability of CA-PEM in different organic solvents was evaluated by dipping it into both semipolar (methanol, ethanol, and isopropanol) and non-polar (acetone, *n*-hexane, and toluene) solvents. The results showed that some organic solvents were incompatible with CA-PEM due to its miscibility in organic solvents. Among the six organic solvents tested, acetone, n-hexane and toluene showed incompatibility with CA-PEM. Meanwhile, methanol, ethanol, and isopropanol showed good compatibility and thus were selected for subsequent investigations (Figure 2). The best extraction efficiency was obtained using ethanol, followed by methanol and isopropanol.

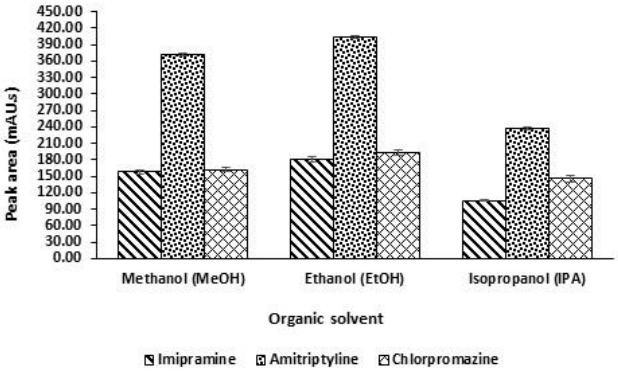

Figure 2. Optimization of organic solvent

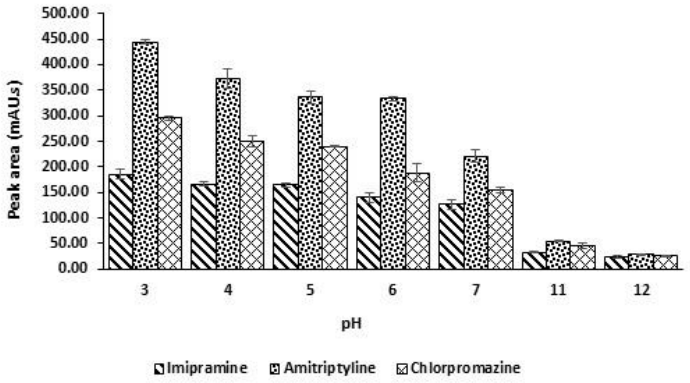

Figure 3. Optimization of sample solution pH

2. Optimization of Sample Solution pH

The pH value determines the form of an analyte in the aqueous sample solution based on its pKa value. TCAs are present in ionized form when the pH of the sample solution is lower than the pKa. Therefore, sample solutions which were acidic, neutral, and basic (pH 3-7 and 11-12) were tested. The results showed that the extraction efficiencies significantly decreased with an increase in pH from 3 to 12 (Figure 3). These results can be explained by the fact that that at lower pH, the sample solution is dominated with H^+, thus the amine groups in the backbones of IMI, AMI, and CHLO are in ionized form (positively charged). In ionized form, the analytes can easily migrate and adhere to the CA-PEM through electrostatic interactions and hydrogen bonding [10].

3. Optimization of Salt Addition

In microextraction methods, salt addition has an influential role in increasing or decreasing the solubility of polar analytes and enhancing their partitioning into the extraction solvent (organic phase) [11]. To evaluate the effect of salt in this study, sodium chloride (NaCl) was added to the sample solution in a concentration range of 0-2 % (w/v). It was evident that increasing the salt concentration was followed by a decrease in extraction efficiency for imipramine and amitriptyline, while the extraction efficiency for chlorpromazine (500 µg L^{-1}) increased when salt was added at a concentration of 0.3% (Figure 4). However, at higher salt concentrations, the extraction efficiency of chlorpromazine also decreased. This phenomenon indicates that the addition of salt can cause precipitation of analyte molecules and salt ions by electrostatic interaction, thus reducing mass transfer and extraction efficiency [12]. Also, because the chemical properties of the CA-PEM may cause it to change into charged molecules in water, the addition of salt may cause competition between salt and analyte to interact with CA-PEM, thus salt addition inhibits extraction efficiency [10]. Hence, no salt was added to the sample solution in subsequent experiments.

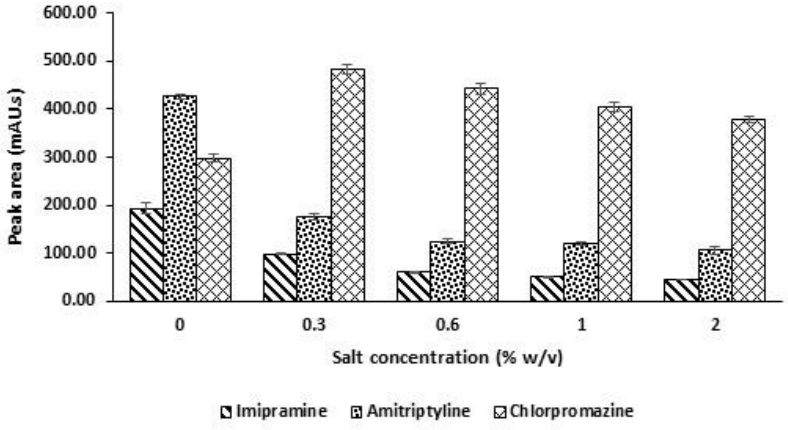

Figure 4. Optimization of salt addition

4. Optimization of Extraction Time

Extraction time is the required time for an analyte to migrate from the sample solution to the solid sorbent until an equilibrium condition is reached. Extraction efficiency increased with increasing extraction time up to 15 min, beyond which it remained almost stable until 30 min (Figure 5). A longer exposure time allows better equilibrium of mass transfer analyte from donor to acceptor phase. However, after equilibrium, the extracted analyte in the sorbent may be saturated and following a long period, it might be released back into the sample solution. Therefore, the equilibrium time of each TCA extracted using CA-PEM was determined to be 15 min. The equilibrium time was relatively fast probably because the low thickness of the extraction phase plays a vital role in the TFME process [Equation 1] [9]. It should be noted that the TFME technique generally provides a faster extraction time than other SPME procedures. The relationship is given by Equation 1 [9],

$$t_{95\%} = \frac{3\ x\ \delta Kes\ (b-a)}{D} \qquad (1)$$

where $t_{95\%}$ is the equilibrium time; b – a is the thickness of the extraction phase; Kes is expressed as the distribution coefficient; δ is the thickness of the boundary layer, and D is the diffusion coefficient. According to Equation 1, $t_{95\%}$ should decrease (faster equilibrium) with the decreasing thickness of the extraction phase.

5. Optimization of Desorption Time

Ultrasonic-assisted liquid desorption was used to accelerate the release of analytes from the CA-PEM thin film. The results show that extraction efficiency was optimum after the desorption time was increased up to 10 min (Figure 6). It can thus be concluded that in 10 min the analytes were completely extracted. However, when the desorption time was extended to 15 min, the extraction efficiency decreased. This was probably due to the longer sonication time, which produced heat that caused analyte degradation [11]. Prolonged ultrasonication can also produce a saturated organic solvent containing desorbed analytes, therefore the analytes might re-enter the porous sorbent. Hence, a desorption time of 10 min by ultrasonication was optimal, and this was used in subsequent experiments.

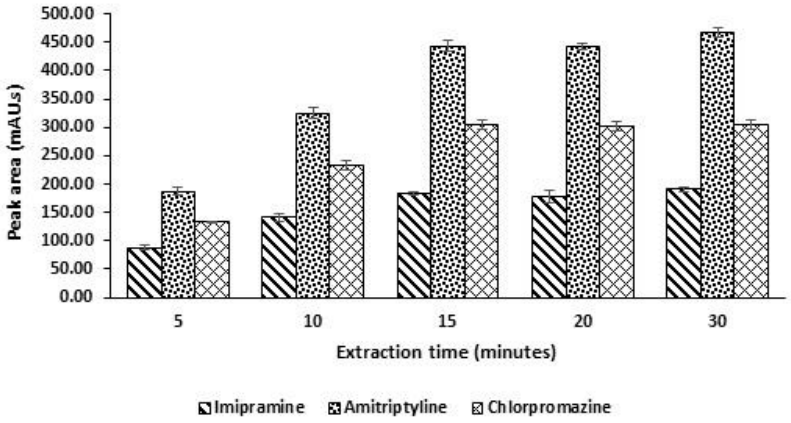

Figure 5. Optimization of extraction time

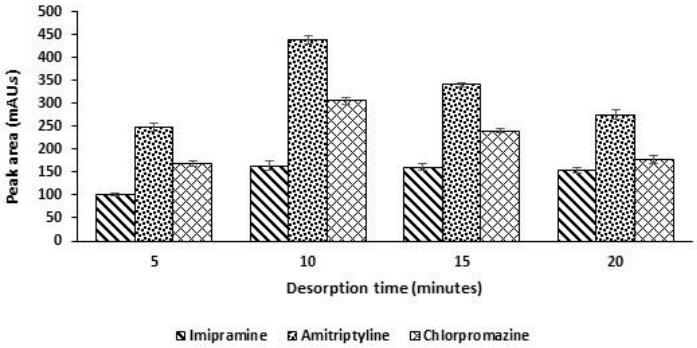

Figure 6. Optimization of desorption time

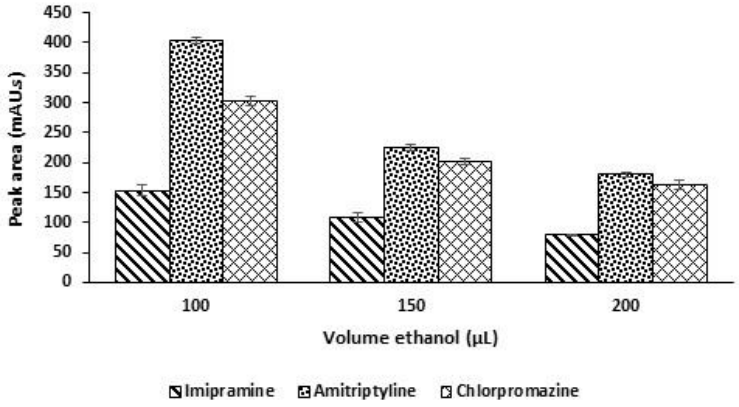

Figure 7. Optimization of desorption solvent volume

6. Optimization of Desorption Solvent Volume

In this experiment, three different volumes of desorption solvent (100 µL, 150 µL, and 200 µL) were investigated to determine the optimum volume. The results showed that extraction efficiency decreased with increasing desorption solvent volume (Figure 7). This condition occurred due to the dilution effect [13]. When a higher volume of desorption solvent was used, the extracted analytes also dissolved and became more dilute. However, a smaller volume of desorption solvent could not be used due to its inability to wet the entire sorbent. On the other hand, according to a previous study, a smaller volume (<100 µL) in µ-SPE can lead to higher peak areas, albeit with poor reproducibility [14]. Hence, 100 µL of organic solvent was selected as the optimum desorption solvent volume and applied in subsequent analyses. To investigate the carry-over effect, the desorbed CA-PEM membrane was desorbed again with a new desorption solvent for 10 min. If no analyte peaks were detected in the chromatogram after the second desorption, there was no carry-over effect.

7. Optimization of Stirring Speed

In the TFME process, stirring or agitation of the sample solution is a common method to increase the rate of mass transfer by raising the diffusion rate toward the increasing kinetic rate. A higher stirring speed also helps to reduce the extraction time needed to obtain thermodynamic equilibrium conditions due to the accelerating extraction kinetics [15]. The effect of stirring speed on extraction efficiency was investigated by continuous agitation at different speeds (600, 720, and 840 rpm). Peak areas increased with stirring speed (Figure 8). Higher stirring speeds were not used in this study as the stability of the sample bottle and TFME device could not be maintained during the extraction process. Fast agitation of the sample solution could enhance extraction efficiency as agitation permits the continuous exposure of the sample solution. However, the stability of the device also needs to be considered and thus, 840 rpm was chosen as optimum.

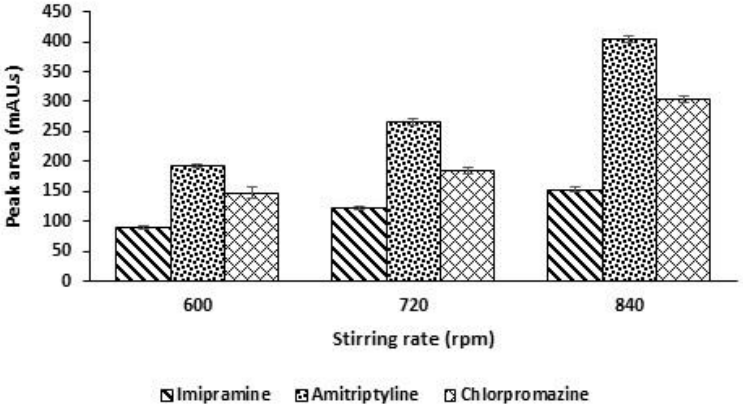

Figure 8. Optimization of stirring speed

Table 1. Method validation of CA-PEM-TFME in combination with HPLC-UV for analysis of TCAs

Analyte	Recovery (%)	Linearity range (μg L^{-1})	Correlation coefficient (r^2)	LOD (μg L^{-1})	LOQ (μg L^{-1})	RSD (%) (n = 3)
(a) *Lake water*						
Imipramine	104.4	10-1000	0.9998	7.6	25.1	2.6
Amitriptyline	102.54	10-1000	0.9997	3.7	12.2	3.7
Chlorpromazine	102.74	10-1000	0.9996	4.3	14.2	4.4
(b) *River water*						
Imipramine	104.6	10-1000	0.9971	9.3	30.7	3.3
Amitriptyline	99.00	10-1000	0.9968	5.4	17.8	4.0
Chlorpromazine	108.32	10-1000	0.9988	5.6	18.5	2.7
(c) Urine						
Imipramine	102.89	50-1000	0.9954	40.5	133.7	3.6
Amitriptyline	108.32	50-1000	0.9980	26.6	87.5	3.5
Chlorpromazine	111.18	50-1000	0.9974	19.7	65.0	4.7

8. Method Validation of CA-PEM-TFME

Three different sample-matched calibration curves were prepared. Calibration curves were prepared by plotting several spiked concentrations of analytes (IMI, AMI, and CHLO) in the blank of each real sample solution. The lake water and river water samples were spiked with target analytes (IMI, AMI, and CHLO) in the range of 10-1000 μg L^{-1}, respectively, while the urine sample was spiked with 50-1000 μg L^{-1} IMI, AMI, and CHLO to evaluate the accuracy of the extraction technique. At a concentration lower than 50 μg L^{-1}, the peak of TCAs were not visible due to impurities in the urine that blocked the porosity of the sorbent. The validation data are shown in Table 1. The results of the TFME procedure showed good linearity in the tested ranges, with R^2 values ranging from 0.9954 to 0.9998. Good detection limits in TFME were achieved in the range of 3.7-7.6 μg L^{-1}, 5.4-9.3 μg L^{-1} and 19.7-40.5 μg L^{-1} for lake water, river water, and urine, respectively. Meanwhile, the LOQs were in the range of 12.2-25.1 μg L^{-1}, 17.8-30.7 μg L^{-1} and 65.0-133.7 μg L^{-1} for lake water, river water, and urine, respectively. In this study, the urine sample had the lowest detection limits, probably due to its more complex matrix. Urine contains macromolecules like amino acids, lipids, as well as charged molecules like ammonia, uric acid, and urea. These compounds may enter the sorbent pores, and so inhibit its absorption performance. Higher interferences can decrease the LOD and LOQ significantly. Acceptable recoveries were obtained from 99.0 % to 111.18 %. For the repeatability test, the precision values (%RSD) obtained were in the range of 2.6 % to 4.7 %.

9. Application of CA-PEM-TFME on Real Samples

The optimized and validated CA-PEM-TFME technique was successfully used to analyze three kinds of aqueous samples, lake water, river water and urine. No TCA residues were detected in any of these samples. Therefore, spiked sample solutions with known concentrations of analytes were studied to investigate the analyte recoveries for the method. Spiking was performed in each sample matrix at different analyte concentration levels to determine recoveries by inter-day analysis. Spiked samples were extracted under optimal extraction conditions. All analyte extractions were carried out three times a day for three consecutive days. The spiked analyte concentrations for lake and river water samples were 500 μg L^{-1}, 50 μg L^{-1}, and 10 μg L^{-1} for IMI, AMI, and CHLO, respectively. The concentrations of the spiked analytes in urine were 50 μg L^{-1} and 500 μg L^{-1} for IMI, AMI, and CHLO, respectively. For the lake and river water samples, good percentage recoveries were obtained in the range of 93.5-112.4 % for amitriptyline, 90.3-110.1 % for amitriptyline, and 99.5%-107.8 % for chlorpromazine (Table 2). Meanwhile, the percentage recoveries for the urine sample were in the range of 95.5-101.0 %, 87.4-94.1 %, and 89.5-93.7 % for imipramine, amitriptyline, and chlorpromazine, respectively. These results are summarized in Table 2. Chromatograms of the selected TCAs are shown in Figure 9 (lake water), Figure 10 (river water), and Figure 11 (urine). The chromatograms of blank lake water, river water and urine showed no contamination with TCAs, while all the peaks that appeared in the chromatograms of extracted spiked samples showed good separation and resolution.

Table 2. Analyte recoveries for spiked lake water, river water and urine using the CA-PEM-TFME-HPLC-UV method

Sample	Spiked concentration (µg L^{-1})	Analyte recoveries (%)			RSD (n = 3)		
		IMI	AMI	CHLO	IMI	AMI	CHLO
Lake water	10	109.1	90.6	99.5	3.53	3.57	4.76
	50	112.4	108.6	101.8	4.96	3.44	1.17
	500	101.3	91.7	107.8	1.51	0.33	2.83
River water	10	104.9	96.7	105.1	4.17	2.63	2.56
	50	102.8	110.1	107.7	4.93	1.20	4.03
	500	93.5	90.3	99.7	3.25	0.71	2.56
Urine	50	95.5	94.1	93.7	6.93	5.52	3.09
	500	101.0	87.4	89.5	1.19	4.47	5.38

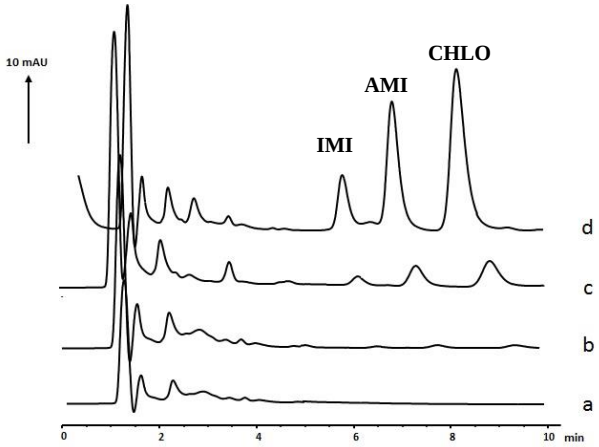

Figure 9. Chromatograms of TCAs in lake water, a. unspiked, b. spiked at 10 µg.L^{-1}, c. spiked at 50 µg.L^{-1}, d. spiked at 500 µg.L^{-1}

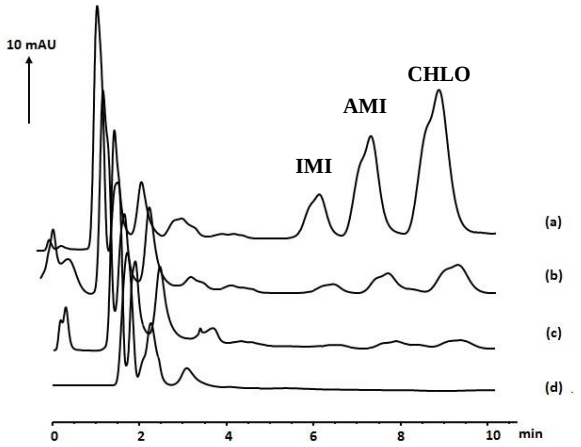

Figure 10. Chromatograms of TCAs in river water, a. unspiked, b. spiked at 10 µg.L^{-1}, c. spiked at 50 µg.L^{-1}, d. spiked at 500 µg.L^{-1}

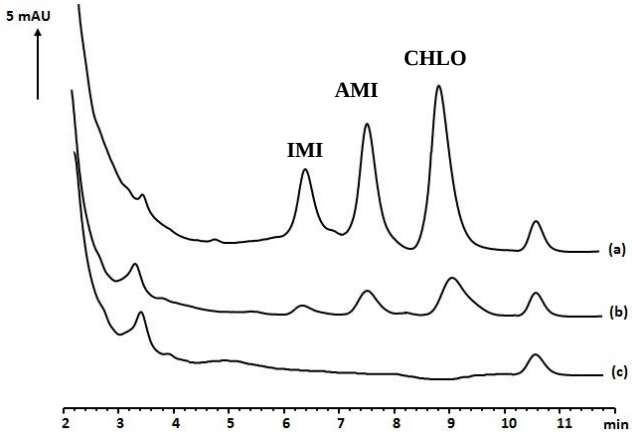

Figure 11. Chromatograms of TCAs in urine, a. unspiked, b. spiked at 50 µg.L^{-1}, c. spiked at 500 µg.L^{-1}

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

Extraction technique	LOQ (µg L^{-1})	Linearity range (µg L^{-1})	Extraction time (min)	References
HF-LPME-HPLC-UV	10-40	10-1000	60	[2]
SBSE-LC-UV	20-50	20-500	40	[3]
SPME-LC-UV	16-25	16-1200	60	[4]
SPME-HPLC-UV	10-17	10-400	16	[5]
Two-phase EME	0.33-0.99	0.5-1000	10	[6]
CA-PEM-TFME-HPLC-UV	12.2-133.7	10-1000	15	Present work

10. Comparison with other Reported Methods

The CA-PEM-TFME method was compared with other reported methods for the determination of TCAs (Table 3). Generally, each published method has both advantages and disadvantages. Stir bar sorptive extraction (SBSE) and SPME required longer extraction and desorption times due to the thickness of the extraction phase, but achieved good sensitivity [4, 15, 16]. The SBSE method has a similar principle with TFME in terms of the distribution of analytes. However, these techniques required a longer extraction time (60 min) than TFME due to the thickness of the PDMS layer extraction phase (0.5 mm thickness). The SBSE technique for the analysis of TCA developed by Melo and co-workers (2009) proposed a new extraction material for the SBSE layer, namely, PDMS/polypyrrole. This method also needed 40 min to reach equilibrium conditions due to the thickness of the extracting phase layer (0.5 mm). Furthermore, most of the SBSE methods required a higher volume of desorption solvent than TFME due to the larger surface area of the extracting phase that must be completely immersed. Electro membrane extraction (EME) had good sensitivity and a shorter extraction time due to the aid of electrical force, which supports analyte migration [4]. However, EME is a relatively high-cost technique due to the use of a platinum electrode.

CONCLUSION

In this study, CA-PEMs were successfully used as novel thin solid sorbents for the extraction of TCAs. This sorbent showed good extraction efficiencies for imipramine, amitriptyline, and chlorpromazine in aqueous samples. The parameters that influenced TFME were optimised as follows: methanol as desorption solvent, pH 3 sample solution, no salt addition in the sample solution, extraction time of 15 min, desorption time of 10 min, 100 µL of desorption solvent and a stirring rate of 840 rpm. Three different spiked concentrations of TCAs in lake water and river water (10 µg L^{-1}, 50 µg L^{-1}, and 500 µg L^{-1}) gave good percentage recoveries in the range of 93.5-112.41 % for imipramine, 90.31-110.05 % for amitriptyline, and 99.52-107.83 % for chlorpromazine. Meanwhile, the urine sample was spiked with two different concentrations (50 µg L^{-1} and 500 µg L^{-1}) and gave percentage recoveries in the range of 95.5-101.0 %, 87.4-94.1 %, and 89.5-93.7 % for imipramine, amitriptyline, and chlorpromazine, respectively. Thus, this CA-PEM-TFME method proved to be a suitable alternative green method for the analysis of TCAs in different matrices.

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