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Electrokinetic extraction on artificial liquid membranes of amphetamine-type stimulants from urine samples followed by high performance liquid chromatography analysis

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ABSTRACT

Electromembrane extraction (EME) coupled with high performance liquid chromatography and ultraviolet detection was developed for determination of amphetamine-type stimulants in human urine samples. Amphetamines migrated from 3 mL of different human urine matrices, through a thin layer of 2-nitrophenyl octyl ether (NPOE) containing 15% tris-(2-ethylhexyl) phosphate (TEHP) immobilized in the pores of a porous hollow fiber, and into a 15 μL acidic aqueous acceptor solution present inside the lumen of the fiber. Equilibrium extraction conditions were obtained after 7 min of operation. Experimental design and response surface methodology (RSM) were used for optimization of EME parameters. Under optimal conditions, amphetamines were effectively extracted with recoveries in the range of 54–70%, which corresponded to preconcentration factors in the range of 108–140. The calibration curves were investigated in the range of 0–7 μg mL^{-1} and good linearity was achieved with a coefficient of estimation better than 0.991. Detection limits and inter-day precision ($n = 3$) were less than 0.01 μg mL^{-1} and 11.2%, respectively.

1. Introduction

Amphetamines are a major class of central nervous system stimulants and are often abused by drug addicts and recreational users. Amphetamine has been firstly used as a nasal decongestant, an appetite suppressant, or to combat fatigue. Nevertheless, its medical uses are now limited. These stimulants are known by different names such as “crystal” and “ecstasy” [1]. These drugs increase self-confidence, wakefulness, alertness, competitiveness, and aggression [2]. They are also associated with psychosis, paranoia, violence and increased stroke risk [2]. Some deaths have been reported due to consumption of amphetamines [3]. Unfortunately, amphetamines usage rate dramatically increases worldwide especially among young population and constitutes serious social problems. Therefore, their determination is a main issue in clinical and forensic laboratories. Their abuse is generally verified by the analysis of biological samples, such as urine, blood, saliva, hair, etc. Among them, urine is a biological fluid that has been used more than the others for testing drug abuse since large volume of sample is available, and its collection is easy and non-invasive [4]. In addition, urine testing provides a relatively long detection window for drugs due to stability of drugs in its medium [5]. To date, many methods have been reported to assess amphetamines in human urine samples [6–15]. In 2006, Pedersen-Bjergaard et al. introduced an interesting analytical extraction technique, termed electromembrane extraction (EME). This technique has been demonstrated for extraction of different basic or acidic compounds [16–25]. In the present work, EME was applied for extraction of some amphetamine-type stimulants from urine samples. Different variables on EME were optimized with response surface methodology (RSM) and experimental design. After EME, the target analytes in the extract were separated and determined by HPLC and diode array detection (DAD). The present study was recommended by Research Center of Antinarcotic Police of Iran to develop a simple and easy method for discrimination of amphetamine stimulants in urine samples.

2. Experimental

2.1. Equipment for electromembrane extraction (EME)

The equipment used for the extraction procedure is shown in Fig. 1. A three milliliter vial with internal diameter of 10 mm and

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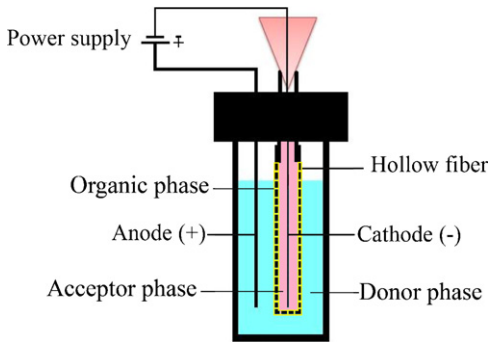


Fig. 1. Schematic illustration of the equipment for electromembrane extraction (EME). Different parts of this set-up are shown in the figure.

height of 8 cm was used. The electrodes used in this work were platinum wires with diameters of 0.2 mm and 0.5 mm for cathode and anode, respectively, and were obtained from Pars Pelatine (Tehran, Iran). The electrodes were coupled to a power supply model 8760T3 with a programmable voltage in the range of 0–600 V and with a current output in the range of 0–500 mA from Paya Pajoohesh Pars (Tehran, Iran). During the extraction, the EME unit was stirred with a stirring speed in the range of 0–1250 rpm by a heater-magnetic stirrer model 301 from Heidolph (Kelheim, Germany) using a 5.0 mm × 2.0 mm magnetic bar.

2.2. Chemicals and materials

Amphetamines (Table 1) were kindly donated by the Research Center of Antinarcotic Police (Tehran, Iran). 2-Nitrophenyl octyl ether (NPOE), tris-(2-ethylhexyl) phosphate (TEHP), and di-(2-ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs, Switzerland). All chemicals used were of analytical-reagent grades. The porous hollow fiber used for the SLM and for housing the acceptor solution was a PPQ3/2 polypropylene hollow fiber from Membrana (Wuppertal, Germany) with inner diameter of 0.6 mm, wall thickness of 200 μm, and pore size of 0.2 μm. Ultrapure water was obtained from a Milli-Q water purification system from Millipore (Madrid, Spain).

2.3. Biological matrices and standard solutions

Urine samples were collected from six young persons who were suspicious to consumption of amphetamines and one person who had not consumed amphetamines at all (as match matrix for drawing the calibration curves). The samples were stored at −4 °C, thawed and shaken prior to extraction. A stock solution containing 1 mg mL^{-1} of target analytes was prepared in methanol and stored at −4 °C protected from light. Sample solutions were prepared by dilution of the stock solutions.

2.4. HPLC conditions

Chromatographic separations were performed with a HPLC system from Waters (Milford, MA, USA) consisted of a 1525 binary pump, a 717 plus automatic injector, a 1500 series column heater, and a 2998 photodiode-array detector. The separations were carried out on ODS-3 column (250 mm × 4.0 mm, with 5 μm particle size) from Waters. It was thermostated at 27.0 ± 0.5 °C. Chromatographic data were recorded and analyzed using Empower™ software. An isocratic elution was performed at a flow rate of 1.0 mL min^{-1}. Eluent *A* was 1% (v/v) orthophosphoric acid in water containing 4 mL *n*-hexyl amine whose pH was adjusted at 3.0 by dropwise addition of 4 M NaOH and/or orthophosphoric acid 1 M and eluent *B* was acetonitrile (70:30). Total analysis time was 15 min. Quantification of all amphetamines was accomplished by measuring peak areas at 210 nm. Calibration was run by injecting 10 μL of standards and samples.

2.5. Determination of amphetamines distribution ratios

Three hundred microliters of amphetamines standard solutions (50 mg L^{-1} in 10 mM HCl) was mixed with 300 μL of NPOE in a 1.5 mL conical microtube. The microtube was vigorously shaken for 15 min by a Vortex (IKA® MS 3 basic, USA) at 3000 rpm. Subsequently, the microtube was centrifuged at 3000 rpm for 15 min, and then 10 μL of the aqueous phase was removed and analyzed by HPLC-DAD. Distribution ratio (D) was determined by the following equation:

$$D = \frac{C_{org}}{C_{HCl}} \quad (1)$$

where C_{org} is the concentration of the analyte in the NPOE and C_{HCl} is the concentration of it in the aqueous phase (10 mM HCl). C_{HCl} was determined by direct analysis by HPLC, whereas C_{org} was calculated based on C_{HCl} and a total mass balance of the system. The results are provided in Table 1.

2.6. Procedure for EME

Three milliliters of sample solution containing the analyte in 1 mM HCl was transferred into the sample vial. To impregnate the organic solution in the pores of hollow fiber wall, 7 cm piece of hollow fiber was cut out and dipped in the solution for 5 s and then the excess of organic solution was gently wiped away by blowing air with a medical syringe. The upper end of hollow fiber was connected to a medical needle tip as a guiding tube which was inserted through the rubber cap of the vial. Fifteen microliters of 100 mM HCl (acceptor solution) was introduced into the lumen of the hollow fiber by a microsyringe and the lower end of hollow fiber was sealed with a small piece of aluminum foil. One of the electrodes, the cathode, was introduced into the lumen of the fiber. The fiber containing the cathode, SLM and the acceptor solution was afterward directed into the sample solution. The other electrode, the anode, was led directly into the sample solution. The electrodes were subsequently coupled to the power supply and the extraction unit was placed on a stirrer with stirring speed of 1000 rpm. The power supply was turned on and extraction was performed for 7 min. After the extraction was completed, the acceptor solution was collected by a microsyringe and transferred into HPLC vial for further analysis.

3. Calculation of preconcentration factor, extraction recovery and relative recovery

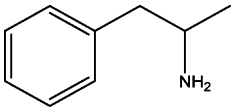
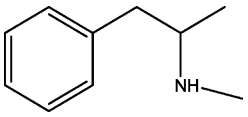
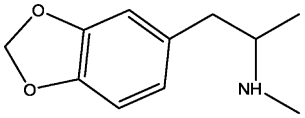
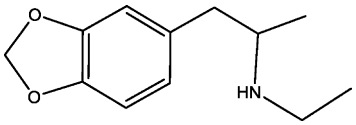
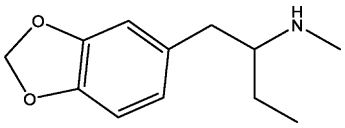
The preconcentration factor (*PF*) was defined as the ratio between the final analyte concentration in the acceptor phase ($C_{f,a}$) and the initial concentration of analyte ($C_{i,s}$) in the sample solution:

$$PF = \frac{C_{f,a}}{C_{i,s}} \quad (2)$$

where $C_{f,a}$ was calculated from a calibration graph obtained from direct injection of amphetamines standard solutions (5–100 mg L^{-1}) in 10 mM HCl. The extraction recovery (*ER*) was defined as the percentage of the number of moles of analyte originally present in the sample ($n_{i,s}$) which was extracted to the acceptor phase ($n_{f,a}$).

$$ER = \frac{n_{f,a}}{n_{i,s}} \times 100 = \frac{C_{f,a} \times V_{f,a}}{C_{i,s} \times V_{i,s}} \times 100 \quad (3)$$

Table 1
Structures, pK_a, log P and log D for some of the amphetamine-type stimulants.

Chemical structure	Compound name	Abbreviation	pK_a[a]	log P[b]	$-\log D \pm$ SD[c]
	Amphetamine	AM	10.1	1.76	1.04 ± 0.12 (1.3)[d]
	Methamphetamine	MAM	9.87	2.07	1.12 ± 0.15 (1.6)[d]
	3,4-Methylenedioxymethamphetamine	MDMA	10.32	1.81	1.39 ± 0.21
	3,4-Methylenedioxyethamphetamine	MDEA	10.34	2.34	1.47 ± 0.17
	Methylbenzodioxolylbutanamine	MBDB	10.46	2.33	1.84 ± 0.23

[a] Ref. [26].
[b] Ref. [26].
[c] Standard deviation ($n = 3$).
[d] Ref. [27].

$$ER = \left(\frac{V_{f,a}}{V_{i,s}}\right) PF \times 100 \quad (4)$$

where $V_{f,a}$ and $V_{i,s}$ are the volumes of acceptor phase and sample solution, respectively. Relative recovery (RR) was acquired from the following equation:

$$RR = \frac{C_{found} - C_{real}}{C_{added}} \times 100 \quad (5)$$

where C_{found}, C_{real}, and C_{added} are the concentration of analyte after addition of known amount of standard into the real sample, the concentration of analyte in real sample, and the concentration of known amount of standard which was spiked into the real sample, respectively.

4. Data analysis and statistical methods

A two-step optimization strategy, including a full-factorial experimental design and a face-centered central composite design (response surface methodology), was employed to optimize the extraction of amphetamines by EME. In all cases, design generation and statistical analyses were performed by means of the software package Statgraphics Plus version 5.1 for Windows (Rockville, MD, USA).

5. Results and discussion

5.1. Optimization of organic solvent (SLM composition)

Based on earlier findings for basic drugs, EME was employed for amphetamines with NPOE as SLM in a first experiment [16,21–25]. As seen from Fig. 2, amphetamines were poorly extracted in the initial electromembrane system based on NPOE as the SLM solvent. This can be due to amphetamines' polar nature which limited their distribution into the SLM (Table 1). In a new experiment, di-(2-ethylhexyl) phosphate (DEHP) and tris-(2-ethylhexyl) phosphate (TEHP) were gradually added to NPOE to reach 5, 15 and 30% (w/w) for TEHP and 15% (w/w) for DEHP, respectively. The results are demonstrated in Fig. 2. As seen, addition of both TEHP and DEHP to NPOE facilitated extraction of the amphetamines. It should be noted that, compared to DEHP, TEHP exhibited more effect on increasing the extraction recovery of target analytes [23,27]. Extraction of amphetamines increased up to 15% (w/w) of TEHP, whereas further addition of TEHP decreased the extractability of the amphetamines. This may be attributed to decrease in electrical resistance of SLM and increase in the current level and bubble formation. Redox processes at the surface of electrodes increase the pH of acceptor solution and decrease the pH of donor solution. Raising the pH in the acceptor solution decreases efficiency of proton/analyte exchange

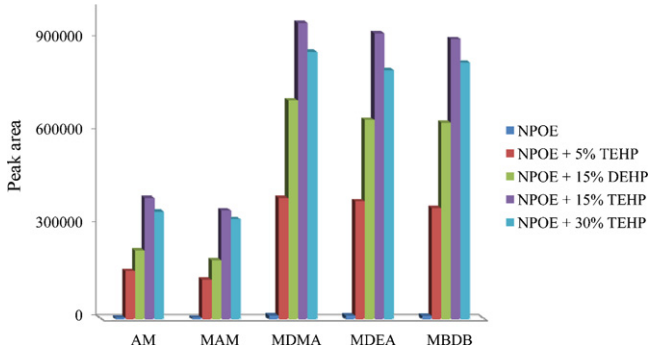


Fig. 2. Effect of SLM composition on EME extraction; spiked concentration: 0.5 mg L^{-1}, voltage: 150 V, sample solution: 10 mM HCl, acceptor solution: 100 mM HCl, sample volume: 3 mL, extraction time: 5 min, and stirring rate: 1000 rpm.

at the SLM/acceptor phase interface, which in turn reduces analyte release and extraction efficiency. Also, increasing the pH of acceptor phase can increase back diffusion of analyte.

5.2. Salt effect

According to previous studies [20,27], the presence of high content of ionic substances causes an increase in the value of the ion balance (χ) in the solution, which in turn decreases the flux of analytes across the SLM. In this study, the effect of χ was investigated using solutions containing 5% NaCl. The obtained results are in full agreement with previous studies [20,27]. Thus, migration of the analytes would be more efficient in the absence of salt and all of the subsequent experiments in EME were performed in the absence of salt.

5.3. Selection and screening of factors using two level full factorial design (2^n)

Different variables can affect the extraction efficiency in the EME procedure, including type of organic solvent (SLM), volume of sample solution, pH of donor and acceptor phases, stirring rate, salt%, temperature, extraction time and voltage. Two level factorial designs are very useful for preliminary studies or in the initial steps of an optimization due to their simplicity and relatively low cost. With factor 9, number of required runs is 2^9 (512) whereas one of the main aims of experimental methodologies is to obtain the best operating conditions with least runs. Therefore, a strategy should be selected for reducing the number of experiments like Placket–Burman design (P–B) or fractional factorial design. But, the interactions among the factors are considered negligible in P–B and also some information on higher-order interactions among factors can be lost in fractional factorial design compared to a full factorial design. Among the ways that can use full factorial design with least experiments is logical reduction of variables. For example, investigation of SLM in factorial design experiments not only increases the number of runs but also requires some complicated designs with more number of experiments to be accomplished. Study of this parameter separately can give optimum SLM as well as simplicity of experimental design method and reduction of number of runs. Therefore, this parameter was separately optimized at the first and thus NPOE + 15% TEHP was chosen as the best SLM for extraction of amphetamines by EME. As is known, stirring speed plays an essential role in increasing the kinetic and efficiency of extraction by increasing the mass transfer and reducing the thickness of double layer around SLM [21,22,28]. Therefore, investigation of stirring speed is not necessary and it can be removed from variables by selection of a proper speed (a speed which has maximum amount without bubble formation around SLM). In this study, 1000 rpm was chosen for all experiments. Also, an amount of 3 mL of sample solution was used for further experiments. According to previous reports in EME, mass transfer is accomplished by electrokinetic migration and convection. Since the strength of electrical field is stronger in a much smaller sample compartment due to shortened migration distance of analytes towards the membrane [20,22], thus an increase in recovery was expected. As mentioned before, migration of the analytes would be more efficient in the absence of salt. Therefore, all of the subsequent experiments in EME were performed in the absence of salt. Temperature is another factor which can affect the flux of ions through SLM. Theoretically, increasing the temperature decreases electrical driving force in EME whereas it increases the diffusion coefficient of ions into SLM [29]. But according to previous studies and our experience, increasing the temperature caused the punctuation of SLM, as well as increase in bubble formation and arc phenomenon, probably due to increasing the solubility of membrane organic solvent especially for long extraction time. Therefore, studying the effect of temperature can be removed from experiments. In the following, pH of donor and acceptor phases was combined as the ion balance (χ), i.e. the ratio of the total ionic concentration in the sample solution to that in the acceptor solution [29], to further reduce the number of runs. For this purpose, in all experiments the acceptor solution was kept constant at 100 mM HCl and the donor phase was varied between 1 and 100 mM HCl for making χ in the range of 1–0.01 for both full factorial and face-centered central composite design experiments. By this strategy for factor selection and using a two level full-factorial design, 8 permutations of conditions were obtained by varying each of the investigated variables at the lower and upper levels (designated −1 and +1 in Table 2, respectively). Normalized peak area for each run was selected as response objective for the study [30]. To normalize the peak areas of target analytes, all of the experiments were first performed based on Table 2. Afterwards, the peak area of each amphetamine was divided by its smallest peak area which was obtained in all of the experiments. Normalized peak area for different amphetamines were subsequently added for each run and used in calculation of total normalized peak area. The obtained data were evaluated by analysis of variance (ANOVA) and the main effects were visualized by use of a Pareto chart (Fig. 3a). This chart implied that all of the three factors and the interaction between some of them displayed statistically significant effects at the $P < 0.05$ level. Therefore, they were considered in the following optimization step.

Table 2
Experimental factors and levels of the full-factorial design for determination of amphetamines using EME.

Factors	Symbol	Levels	
		Low (−1)	High (+1)
Extraction time (min)	t	2	5
Voltage (V)	V	100	300
Ion balance[a]	χ	0.01	1

Effective factors, levels and matrix of the face-centered central composite design (FCCCD)

		Low (−1)	Center (0)	High (+1)
Extraction time (min)	t	2	5	8
Voltage (V)	V	100	200	300
Ion balance	χ	0.01	0.505[a]	1

Trial No.	t	V	χ	Normalized peak area
1	−1	0	0	16.25
2	1	−1	1	11.75
3	1	1	1	33.49
4	−1	1	1	12.97
5	0	0	−1	104.10
6	−1	−1	−1	5.00
7	−1	1	−1	24.35
8	0	0	1	23.81
9	−1	−1	1	11.75
10	0	−1	0	35.38
11	1	1	−1	59.46
12	0	1	0	36.73
13	1	0	0	50.48
14	1	−1	−1	61.31
15[b]	0	0	0	45.12
16[b]	0	0	0	42.73
17[b]	0	0	0	39.74

[a] For generation of χ = 0.01, 0.5 and 1.0, concentration of HCl was selected to be 100 mM in acceptor phase and 1, 50, and 100 mM for donor phase, respectively.
[b] Replications in center point.

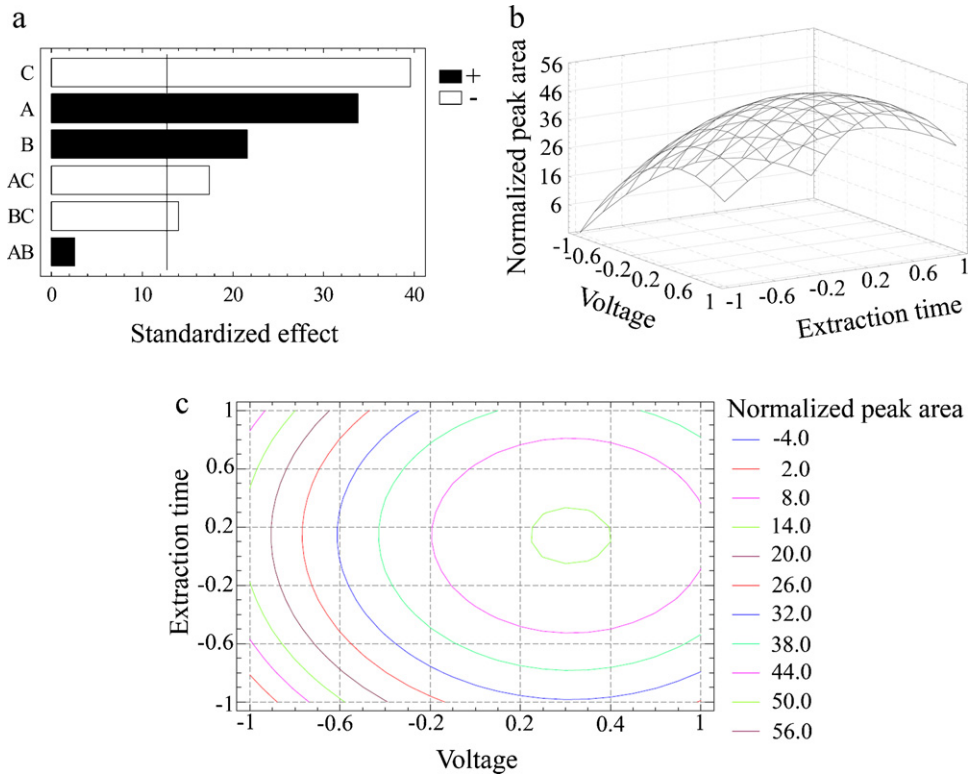


Fig. 3. (a) Pareto chart of the main effects obtained from a full factorial design (2^3) for amphetamines. (A) Voltage, (B) extraction time, (C) ion balance; (b) response surface of amphetamines using FCCCD which illustrates the relationship between the extraction time, voltage and experimental responses in a three-dimensional representation; (c) Two-dimensional contour plot of extraction time *vs.* voltage which displays the interaction between independent variables and assists in determining the optimum operating conditions for the desirable response.

5.4. Results for the central composite design

This step is concerned with optimizing the values of significant variables in order to obtain the best response. In statistics, central composite design (CCD) is one of the most frequently used response surface designs. CCD is a second-order model takes the following form for three independent variables [31]:

$$y = a_0 + \sum_{i=1}^{3} a_i x_i + \sum_{i=1}^{3}\sum_{j=1}^{3} a_{ij} x_i x_j + \sum_{i=1}^{3} a_{ii} x_i^2 \quad (6)$$

where y is the dependent variable (normalized peak area); x_i is the independent variable; the a_i terms represent the regression coefficient of the model and a_0 is the deviation between the observed and predicted responses in the design point. In order to optimize the parameters that simultaneously influence the determination of amphetamines, face-centered central composite design (FCCCD), which is considered to be 1 in α (star point), was carried out in this study. The three significant factors: time of extraction (t), voltage (V), and ion balance (χ) were considered in order to maximize the experimental response (normalized peak area) based on full factorial design experiments. The total number of design points needed (N) is determined by the following equation:

$$N = 2^f + 2f + N_o \quad (7)$$

where f is the number of variables and N_o is the number of center points [32]. Therefore, with 3-factor and 3-center points totally 17 experiments had to be run for the FCCCD (Table 2). By using multiple regression analysis, the experimental responses shown in Table 2 were correlated with the three significant factors. The results are listed in Table 3. The goodness of fit of the quadratic polynomials is expressed by the coefficient of determination, R^2.

Table 3
Coefficients of the regression equation for simultaneous determination of some amphetamines.

	Coefficients of the regression (a)
Constant (a_0)	47.2116
x_1 (voltage)	14.6167
x_2 (extraction time)	4.18053
x_3 (ion balance)	−16.0347
x_1^2	−17.3519
x_2^2	−14.6615
x_3^2	13.1927
x_1x_2	−0.0868667
x_1x_3	−8.86274
x_2x_3	0.680647
R^2%	80.3346

According to Joglekar and May [33], R^2 should be at least 0.80 for a good fit of a model. As is observed, the coefficient of determination, R^2, was more than 0.80 which means that the obtained equation is adequate for correlating the experimental results. Also, ANOVA was performed and showed that the model was significant and the "lack of fit" was not significant (P = 0.05), which implied that the model was fitted. Statistical significance was evaluated on the basis of the magnitudes of coefficients in the regression equation (Table 3). As can be seen, the quadratic terms of the voltage (x_1) and linear term of ion balance (x_3) have the largest influence on the response. The next most significant factors were quadratic terms of extraction time (x_2) and ion balance and the linear term of voltage. The linear term of the extraction time and interactions between x_1 and x_3 also showed significant effects on the response. RSM (Fig. 3b) and two-

Table 4
Operating conditions for simultaneous extraction of some amphetamines by EME.

SLM	NPOE + 15% TEHP
Sample volume	3 mL
Stirring speed	1000 rpm
Voltage	250 V
Extraction time	7 min
Donor phase solution	1 mM HCl
Acceptor phase solution	100 mM HCl
Salt %	No salt
Temperature	Ambient

Table 5
Figures of merit of EME in an amphetamines-free urine sample.

Analyte	LOD (mg L^{-1})	Linearity[a] (mg L^{-1})	R^2	RSD%[b]	PF	ER%
AM	0.01	0.1–7	0.994	8.4	114	57
MA	0.01	0.1–7	0.997	10.2	108	54
MDMA	0.005	0.05–7	0.995	8.3	140	70
MDEA	0.005	0.05–7	0.998	6.7	132	66
MBDB	0.005	0.05–7	0.991	5.6	126	63

[a] Linearity was investigated until concentration of 7 mg L^{-1} of each analyte.
[b] Percent relative standard deviations for three replicate measurements of the elements with the concentration of 100 μg L^{-1} in the urine samples.

dimensional contour plot (Fig. 3c) were applied to analyze the effect of independent variables on the response. Based on the analysis and plots present in this figure, it can be observed that the normalized peak area of amphetamines increases in a quadratic manner with decreased ion balance and increased voltage and extraction time to given levels. More increase of voltage and extraction time from these levels leads to a decrease in response. Application of high voltage reduces deprotonation opportunity of basic drugs which have relatively high tendency to deprotonate in the artificial liquid membrane so as to promote their efficient migration through SLM. This resulted in higher extraction recovery. As can be seen in Fig. 3b and c, maximum response was yielded at 250 V. Beyond that, a decrease in the EME performance was observed. This phenomenon most probably was caused by analyte back-extraction into the SLM and sample solution as pH increased slightly in the acceptor solution due to electrolysis as well as the gradual suppression of net transfer of the analyte due to heat generation at higher voltages [21]. It should be noted that EME is an equilibrium distribution process [28]. As can be seen, the normalized peak area decreased after 7 min that can most probably be attributed to saturation of the analyte in the acceptor phase, increase in pH of the acceptor solution after these times and therefore, back-diffusion of the analyte to the sample solution; similar observation has previously been reported [22]. Also, the flux of analyte can be improved by lowering the ion balance (χ) over the SLM [29]. Table 4 shows all of the obtained optimum conditions used for rest of this work.

5.5. Analytical performance

To evaluate the practical applicability of the proposed EME technique, under optimized extraction conditions (Table 4) the figures of merit of the method were investigated in an amphetamines-free human urine sample (Table 5). Comparison of the proposed method with different existing methods for extracting and determining the amphetamines is provided in Table 6. It was shown that along with its simplicity, this technique demonstrated wide linearity range, high sensitivity, and an acceptable reproducibility with an important emphasis on the extraction time which seems to be short and no need to sample preparation step.

5.6. Analysis of real samples

To investigate matrix effects and applicability of the technique to biological samples, final experiments were carried out on six urine samples. In this case, pH of urine samples was adjusted by addition of proper amount (μL) of HCl 100 mM such that the final concentration of HCl reached 1 mM. With the current direction of the polarity, only basic compounds can enter the SLM. Also, DEHP and TEHP were found to effectively control the selectivity of the SLM [23]. Regarding these points, as shown in Fig. 4, chromatograms with noticeable clean-up were obtained. The obtained results are given in Table 7. As is obvious, RSDs% and the relative recoveries for the spiked samples are in acceptable range (95–103%).

Table 6
Comparison of the proposed method with other analytical techniques for determination of amphetamines in urine samples.

Analytical technique[a]	Analytes[b]	LODs (μg L^{-1})	ER%	Extraction time (min)	Reference
HF-LPME-GC-FID	AM, MAM, MDA, MDMA	8–82	4.2–22.7	20	[6]
In-tube SPME-HPLC-UV	AM, MAM, MDA, MDMA	1.4–4	–	10	[9]
SPE-GC–MS	AM, MAM	0.08–0.1	78.2–82.9	8	[15]
SPE-GC–MS	AM, MAM, MDA, MDMA	2–4	73–104.6	8[c]	[13]
SPME-HPLC-FLD	AM, MAM, MDMA	100–1000	0.17–0.63[d]	>20	[8]
SPME-HPLC-FLD	AM, MDA	250	50–104	>40	[34]
HS-SPME-GC-FID	AM, MAM	3–9	–	22	[35]
HS-SPME-GC–MS	AM, MAM, MDA, MDMA, MDEA	0.016–0.193	16.9–19.6	30	[7]
HS-SPME-GC-FID	MAM	0.6	–	35	[36]
Three-phase SDME-HPLC-UV	AM, MAM	0.5	43.66–58.91	80	[10]
HS-HF-LPME-GC–MS	AM, MDA	0.25–1.0[e]	5.2–19.6	30	[11]
SPE-HPLC-DAD	AM, MAM, MDA, MDMA	100	–	5	[12]
Three-phase HF-LPME-CE-UV	AM, MAM	–	40–75	45	[37]
LE- HPLC-FLD	MDA, MDMA, MDEA	10–20	85–102	>20	[38]
LPME-FIA-APCI-MS-MS	AM, MAM, MDA, MDMA, MDEA, MBDB	2–100	24–68	15	[39]
EME-HPLC-DAD	AM, MAM, MDMA, MDEA, MBDB	5–10	54–68	7	This work

[a] Hollow fiber (HF), liquid phase microextraction (LPME), flame ionization detector (FID), solid phase microextraction (SPME), solid phase extraction (SPE), mass spectrometry (MS), fluorescence detector (FLD), headspace (HS), single drop microextraction (SDME), capillary electrophoresis (CE), flow injection analysis (FIA), atmospheric pressure chemical ionization (APCI), diode array detector (DAD).
[b] Amphetamine (AM), methamphetamine (MAM), 3,4-methylenedioxyamphetamine (MDA), 3,4-methylendioxymethamphetamine (MDMA), 3,4-methylendioxyethamphetamine (MDEA), methylbenzodioxolylbutanamine (MBDB).
[c] Twenty min was needed for evaporation.
[d] Percentage of extracted analyte with derivatization in solution.
[e] LOQ.

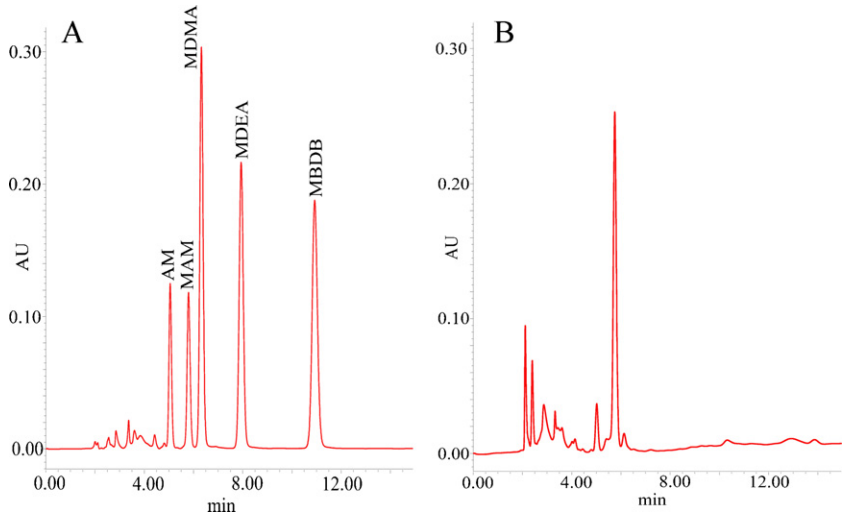


Fig. 4. Typical chromatograms from (A) a spiked healthy urine sample at 1 mg L^{-1} concentration of each amphetamine; (B) urine sample 6 related to a person suspicious of amphetamines consumption, obtained under optimal extraction conditions.

Table 7
Determination of some amphetamines in different urine samples.

Sample		AM	MAM	MDMA	MDEA	MBDB
Urine 1	Initial concentration (mg L^{-1})	<LOD	<LOD	<LOD	<LOD	<0.05
	RR%[a]	97	96	102	98	95
	RSD% ($n=3$)	8.4	7.5	9.3	6.9	8.2
Urine 2	Initial concentration (mg L^{-1})	0.15	0.30	<LOD	<LOD	<LOD
	RR%	95	97	98	96	94
	RSD% ($n=3$)	5.8	8.4	10.0	7.3	6.5
Urine 3	Initial concentration (mg L^{-1})	0.16	1.66	<LOD	<LOD	<LOD
	RR%	95	103	99	97	95
	RSD% ($n=3$)	6.7	7.7	8.1	5.9	9.9
Urine 4	Initial concentration (mg L^{-1})	0.15	1.66	<LOD	<LOD	<LOD
	RR%	98	101	103	97	97
	RSD% ($n=3$)	8.4	11.2	9.5	8.6	7.3
Urine 5	Initial concentration (mg L^{-1})	2.02	6.25	0.17	<LOD	<LOD
	RR%	98	100	96	101	95
	RSD% ($n=3$)	5.6	6.9	7.4	6.8	9.1
Urine 6	Initial concentration (mg L−1)	0.32	2.16	<0.05	<LOD	<LOD
	RR%	102	98	101	102	99
	RSD% ($n=3$)	8.9	9.8	9.4	6.2	7.4

[a] 0.1 mg L^{-1} of each amphetamine was added to calculate relative recovery percent (RR%).

6. Conclusions

In the present work, EME was conducted for determination of amphetamine-type stimulants directly from urine samples. For the first time, a central composite design and full fractional factorial design were carried out for optimization of variables of interest in EME. Concerning the excellent recoveries in a short time and in a single step with highly efficient sample clean-up, as well as good accuracy and reproducibility, EME may be a very powerful and innovative future sample preparation technique in drug analysis from different and complex biological matrices.

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