Journal of Pharmaceutical and Biomedical Analysis 56 (2011) 859–866

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Electrically enhanced microextraction for highly selective transport of three β-blocker drugs

Shahram Seidi, Yadollah Yamini*, Maryam Rezazadeh

Department of Chemistry, Tarbiat Modares University, P.O. Box 14115-175, Tehran, Iran

ARTICLE INFO

Article history:
Received 25 April 2011
Received in revised form 6 July 2011
Accepted 22 July 2011
Available online 29 July 2011

Keywords:
Electrical field
Hollow fiber
Microextraction
β-Blocker
Saliva

ABSTRACT

Facilitated transport of three β-blocker drugs including atenolol (ATE), betaxolol (BET) and propranolol (PRO) was investigated under electrical field across a supported liquid membrane (SLM) using phosphoric acid derivatives as selective ion carriers, dissolved in 2-nitro phenyl octyl ether (NPOE). In the presence of di-(2-ethylhexyl) phosphate (DEHP) and tris-(2-ethylhexyl) phosphate (TEHP) in the membrane phase, the three β-blockers showed completely different transport behaviors which enabled highly selective separation of the drugs. Each β-blocker migrated from 3 mL of sample solutions, through a thin layer of specific organic solvent 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. The influences of fundamental parameters affecting the transport of target drugs including type of ion carrier for selective separation of each drug and its concentration in the membrane phase, extraction voltage, time of transport, pH of donor and acceptor phases, stirring speed of donor phase and salt effect were studied and optimized. After microextraction process, the extracts were analyzed by high-performance liquid chromatography with ultraviolet detection. Under optimal conditions, ATE was selectively extracted from different saliva samples with recovery of 37%, which corresponded to preconcentration factor of 74. A good linearity was achieved for calibration curve with a coefficient of determination higher than 0.997. Limits of detection and intra-day precision ($n = 3$) were less than 2 μg L^{-1} and 8.8%, respectively.

1. Introduction

Hypertension is a growing medical concern in industrial countries. By annual increase in the number of persons suffering from hypertension, the use of antihypertensive medications has increased as well [1]. Adrenoceptor antagonists briefly called as beta-blockers are drugs mainly used for treatment of hypertension, angina pectoris, and cardiac dysrhythmias as well as in the follow-up treatment of myocardial infarctions [2]. β-Blockers can improve the athletes' abilities so that, Medical Commission of the International Olympic Committee (MCIOC) prohibits the use of these drugs and includes them in the list of forbidden substances [3]. Therefore, measurement of β-blockers in bio-fluids is important not only in controlling the therapy compliance of the patients or intoxication but also in the field of doping control.

Different techniques, including gas chromatography mass spectrometry (GC–MS) after derivatization [4], liquid chromatography–mass spectrometry (LC–MS) [5], capillary electrophoresis with UV detection (CE-UV) [6], thin-layer chromatography (TLC) [7], ultra-performance liquid chromatography-UV detection (UPLC-UV) [8] and UPLC–MS [9] have been applied for determination of different β-blockers. More recently, the use of GC/MS/MS [10], LC/MS/MS [11] and UPLC/MS/MS [12] has improved the selectivity and sensitivity of the method for screening β-blocker agents in the biological fluids. However, such expensive instruments are available only in a few laboratories. Current methods for toxicological purpose usually involve high performance liquid chromatography (HPLC) combined with electrochemical, fluorimetric and UV detection.

In general, sample preparation and concentration of the target analytes are often needed before analysis. Traditionally, liquid–liquid extraction (LLE) has been used for pre-treatment of biological samples, but LLE is laborious and requires environmentally toxic solvents. Due to several advantages, solid-phase extraction (SPE) has become more popular [13] compared to LLE, but it also requires an organic solvent for elution of analytes, as well as a solvent evaporation step prior to final analysis. In recent years, microscale extraction methods have been developed for extraction of different substances from small volumes of biological fluids; nevertheless, the problem of selectivity exists in these techniques. Recently, some attempts have been made to enable selectivity in microextraction techniques [14,15]. For example, Pawliszyn et al. have introduced an interesting method for creating selectivity in SPME technique using combination of molecular-imprinted

* Corresponding author. Tel.: +98 21 82883417; fax: +98 21 88006544.
E-mail address: yyamini@modares.ac.ir (Y. Yamini).

0731-7085/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2011.07.029

polymer (MIP) with in-tube SPME [14]. Furthermore, several works have reported the use of MIP-coated SPME fiber through the chemical bonding method [16]. These techniques need a synthesis step for selective separation of each analyte and have relatively high RSDs% among synthetic fibers.

Membrane technology has become a dignified separation technique over the past decade. One of the major applications of this type of technology is in the field of hollow fiber liquid-phase microextraction (HF-LPME) based on passive diffusion. The main advantages of this technique is high degree of clean-up especially in complex matrices such as biological fluids and also a good selectivity by selection of proper organic solvents. This technique has been used for extraction of many compounds so far [17]. HF-LPME procedure is time-consuming. Recently, Pedersen-Bjergaard et al. demonstrated the effect of applying an electrical potential on the transport of ionizable substances across a supported liquid membrane (SLM) [18]. In this technique, termed electromembrane extraction (EME), target analytes are extracted from an aqueous sample, into an immobilized organic solvent located in the pores of a porous hollow fiber, and then transported into an aqueous acceptor solution placed inside the lumen of the hollow fiber by applying an electrical potential across the SLM. Compared to passive diffusion, electrokinetic migration appears to be a much more efficient transport mechanism, providing high analyte recoveries in very short time. Nowadays, EME has been developed for extraction of different analytes [18–21].

The objective of this research was to develop a simple and inexpensive electrically enhanced microextraction for highly selective transport of atenolol (ATE) in the presence of other β-blocker drugs through a supported liquid membrane based on phosphoric acid derivatives as suitable ion carriers from saliva samples. The advantages of saliva drug testing are mainly twofold in comparison with urine and plasma [22].

2. Experimental

2.1. Equipment for electromembrane extraction (EME)

A 3 mL glass vial with internal diameter of 10 mm and 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 mm × 2 mm magnetic bar.

2.2. Chemicals and materials

Atenolol (ATE), betaxolol (BET) and propranolol (PRO) were kindly donated by the Department of Medical Sciences of Tehran University (Tehran, Iran). The chemical structures of the three main β-blockers including ATE, BET and PRO are shown in Table 1. 2-Nitrophenyl octyl ether (NPOE), tris-(2-ethylhexyl) phosphate (TEHP) and di-(2-ethylhexyl) phosphate (DEHP) were purchased from Fluka (Buchs, Switzerland). All of the chemicals used were of analytical reagent grades. The porous hollow fiber used for the SLM 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

Saliva samples were collected from four patients who were under treatment with ATE drug in Imam Khomeini Hospital (Tehran, Iran) and one person who had not consumed β-blocker at all (as match matrix for drawing the calibration curves). All samples were stored at −4 °C, thawed and shaken before extraction. A stock solution containing 1 mg mL^{-1} of ATE, BET and PRO was prepared in methanol and stored at −4 °C protected from light. Working standard solutions were prepared by dilution of these stock solutions in methanol.

2.4. HPLC conditions

Separation and detection of the target analytes were performed by a Varian HPLC (Walnut Creek, CA, USA) containing a 9012 HPLC pump, a six-port Cheminert HPLC valve from Valco Instruments (Houston, TX, USA) with a 15 μL sample loop and a Varian 9050 UV-Vis detector. Chromatographic data were recorded and analyzed using Chromana software (version 3.6.4). The separations were carried out on an ODS-3 column (250 mm × 4.0 mm, with 5 μm particle size) from TeknoKroma (Barcelona, Spain). An isocratic elution was performed at a flow rate of 1.0 mL min^{-1}. The mobile phase consisted of 10 mM phosphate buffer pH 4.0 and methanol (75:25, v/v). Total analysis time was 10 min. Quantification of all β-blockers was accomplished by measuring peak areas at wavelength of 224 nm. Calibration was run by injecting 15 μL of standards and samples.

2.5. Procedure for EME

Three milliliters of sample solution containing target analytes in 1 mM HCl was transferred into the sample vial. To impregnate the organic solution in the pores of hollow fiber wall, 6 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 air blowing using a 500 μL Hamilton 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 1250 rpm. The predetermined voltage was turned on and extraction was performed for 15 min. Under the applied voltage, the target analytes migrated from aqueous sample, into SLM, and then transported into acceptor phase (Fig. 1A). After the extraction was completed, the acceptor solution was collected by a microsyringe and injected into HPLC vial for further analysis.

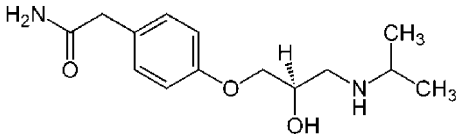
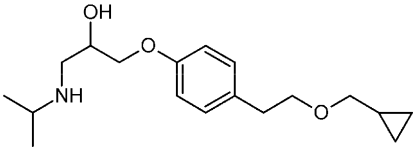
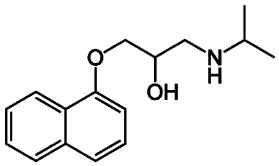
2.6. Calculation of preconcentration factor, extraction recovery and relative recovery

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

$$PF = \frac{C_{f,a}}{C_{i,s}} \quad (1)$$

where $C_{f,a}$ was calculated from a calibration graph obtained from direct injection of ATE standard solutions (1–100 mg L^{-1}) in

Table 1
Chemical structures, pK_a, log P and dose of atenolol, betaxolol and propranolol.

Chemical structure	Compound name	Abbreviation	pK_a[a]	Log P[a]	Dose[a] (mg/day)
	Atenolol	ATE	9.6	0.23	50–100
	Betaxolol	BET	9.4	2.81	10–20
	Propranolol	PRO	9.5	1.2[b]	30–400[c]

[a] Ref. [22].
[b] Log P (octanol/pH 7.4).
[c] This dose was reported for hydrochloride salt of propranolol.

100 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 ($n_{f,a}$).

$$ER\% = \frac{n_{f,a}}{n_{i,s}} \times 100 = \frac{C_{f,a} \times V_a}{C_{i,s} \times V_s} \times 100 \quad (2)$$

$$ER\% = \left(\frac{V_a}{V_s}\right) PF \times 100 \quad (3)$$

where V_a and V_s represent the volumes of acceptor phase and sample solution, respectively. Relative recovery (RR) was acquired from the following equation:

$$RR\% = \frac{C_{\text{found}} - C_{\text{real}}}{C_{\text{added}}} \times 100 \quad (4)$$

where C_{found}, C_{real}, and C_{added} are the concentrations 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.

2.7. Data analysis and statistical methods

Design generation and statistical analyses were performed by means of the software package Minitab Plus trial version 15 for Windows (State College, PA, USA).

3. Results and discussion

3.1. Variation in the SLM composition (organic solvent)

The chemical nature of the supported liquid membrane (SLM) is highly critical to succeed electrokinetic cross-membrane extraction. The flux of analyte is affected by analyte concentration gradient across the SLM; it is partially determined by the sample-to-SLM distribution ratio, and this in turn is controlled by the type of solvent used as the SLM. In addition, the type of solvent also affects the diffusion coefficient of the analyte [19] and it could also be tuned to increase the selectivity as well as to obtain good clean-up during extraction [20,21]. There are specific requirements for a solvent to be used as a SLM in EME. The organic phase should have a certain dipole moment or electrical conductivity to support a relatively low current flow in the system and it should have certain chemical properties to enable phase transfer and electrokinetic migration of the model analytes [21]. Furthermore, the solvent should be immiscible in water to avoid losses from the hollow fiber membrane wall pores and dissolution in the sample during stirring. Based on earlier finding, NPOE is an efficient organic solvent for electrokinetic migration of basic drugs through the SLM [16]. In a first experiment, electrokinetic transport was performed for the three β-blocker drugs with pure NPOE. The results are summarized in Fig. 2A. As can be seen, in the presence of NPOE, PRO and BET have comparable extraction efficiencies in comparison with ATE. The obtained results are in agreement with the reported log Ps for these drugs in Table 1. The non-polar drugs were readily transferred to the liquid membrane due to solvation. For the polar drugs, solvation was very slow for polarity reasons, and the polar drugs principally remained in the sample solution during the time frame of the experiment. It has been found that, addition of hydrophobic alkylated phosphate reagents to SLM could improve or decrease phase transfer and electrokinetic migration of basic analytes [20]. Based on these findings, a good selectivity can be reached for extraction of a specific analyte in the presence of other compounds. For this purpose, 10% of TEHP, DEHP and a mixture containing 5% of them were added to NPOE. Under these conditions, interesting results were obtained. Addition of TEHP increased the extraction efficiency of ATE whereas it led to moderate and vigorous reduction of PRO and BET extraction efficiencies, respectively. In the case of DEHP, a noticeable and intensive increase was obtained for ATE as well as a vigorous decrease for both of PRO and BET drugs; so in the latter case, detection of the compounds in the acceptor phase was not possible using HPLC-UV. NPOE containing 5% mixture of TEHP and DEHP yields the results similar to addition of 10% DEHP to NPOE. Generally, DEHP and TEHP interact with target analytes which accelerate analyte transport from sample solution into SLM [20]. At the liquid membrane/acceptor interface, the protons release the analytes by counter-ion exchange, and are co-transported through the liquid membrane and into the sample solution (Fig. 1B). Carrier-mediated transport, therefore, leads to a continuous loss of protons from the acceptor solution to the sample solution as the analytes are enriched in the acceptor solution. A sufficiently large excess of protons in the acceptor solution is

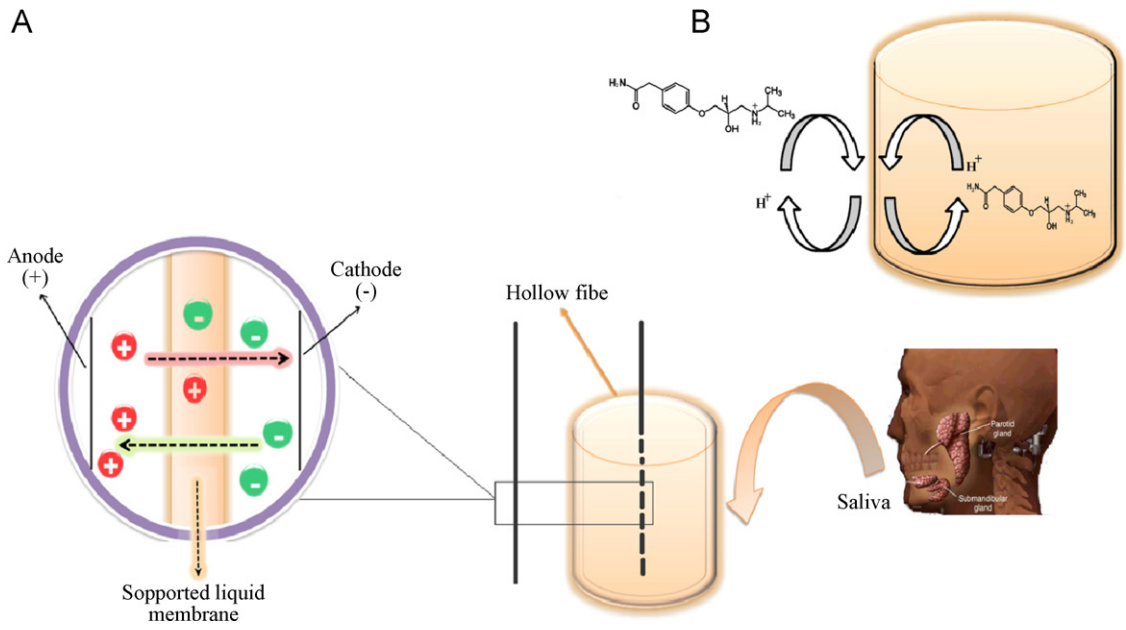


Fig. 1. Schematic illustration of EME for extraction of ATE from saliva samples (A), and co-transportation of ATE through the liquid membrane using ion-pair regents (B).

necessary both to provide extraction and to prevent back-extraction of analytes as ion-pairs with the carrier. In addition, the analytes are highly soluble in the acidic acceptor solution where they are ionized under acidic conditions. The reason for no transportation of BET and PRO in the presence of DEHP is not yet clear, but the strong interaction of the ion-pair complex of BET and PRO with the organic phase may be responsible for this behavior [20]. For the most hydrophobic substances, the analytes not detected in the acceptor phase can be distributed between the sample and the organic phase. To investigate this subject in more detail, subsequent analysis of the sample after the experiment was conducted. Very interestingly, in comparison with pure NPOE, more portions of hydrophobic substances were trapped within the organic phase in the presence of alkylated phosphates. Thus, the presence of these ion-pair reagents is highly beneficial to transfer the analytes into organic phase. However, these ion-pairs are highly hydrophobic and stable, and the strong interaction may partly prevent them from being released into the acceptor phase. This supported that mass transfer resistance most probably occurred at the interfaces at both sides of the organic phase. Based on the experiences yielded from DEHP and TEHP, a final experiment was conducted with a NPOE membrane containing different amounts of DEHP and TEHP

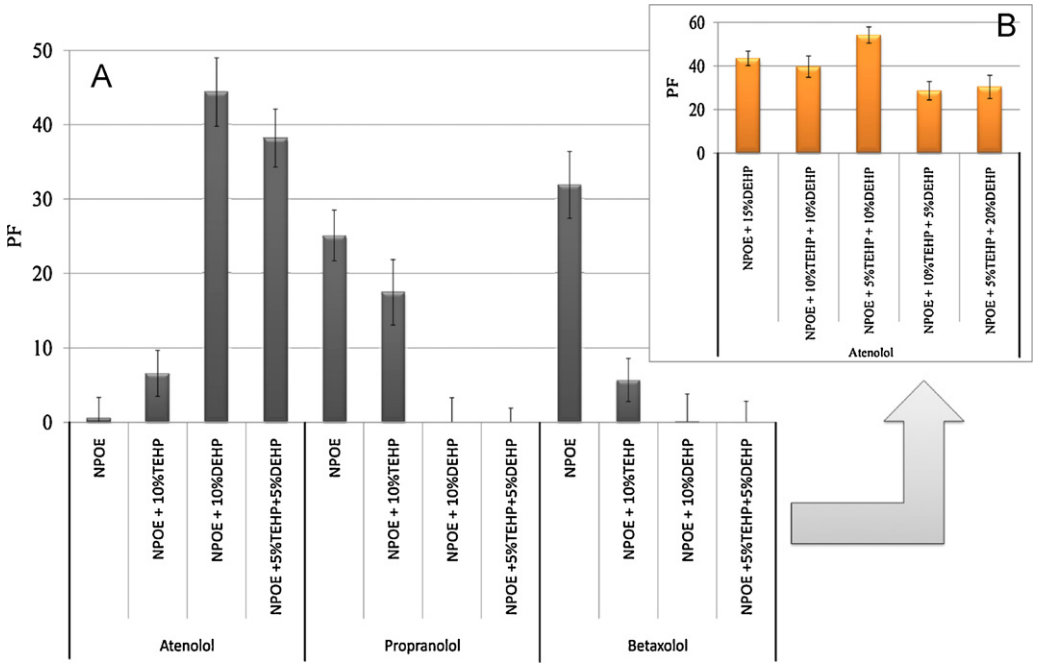


Fig. 2. Effect of SLM composition on extraction efficiency of ATE by EME; spiked concentration: 0.25 mg L^{-1}, voltage: 200 V, sample solution: 10 mM HCl, acceptor solution: 100 mM HCl, sample volume: 3 mL, extraction time: 15 min, and stirring rate: 1250 rpm.

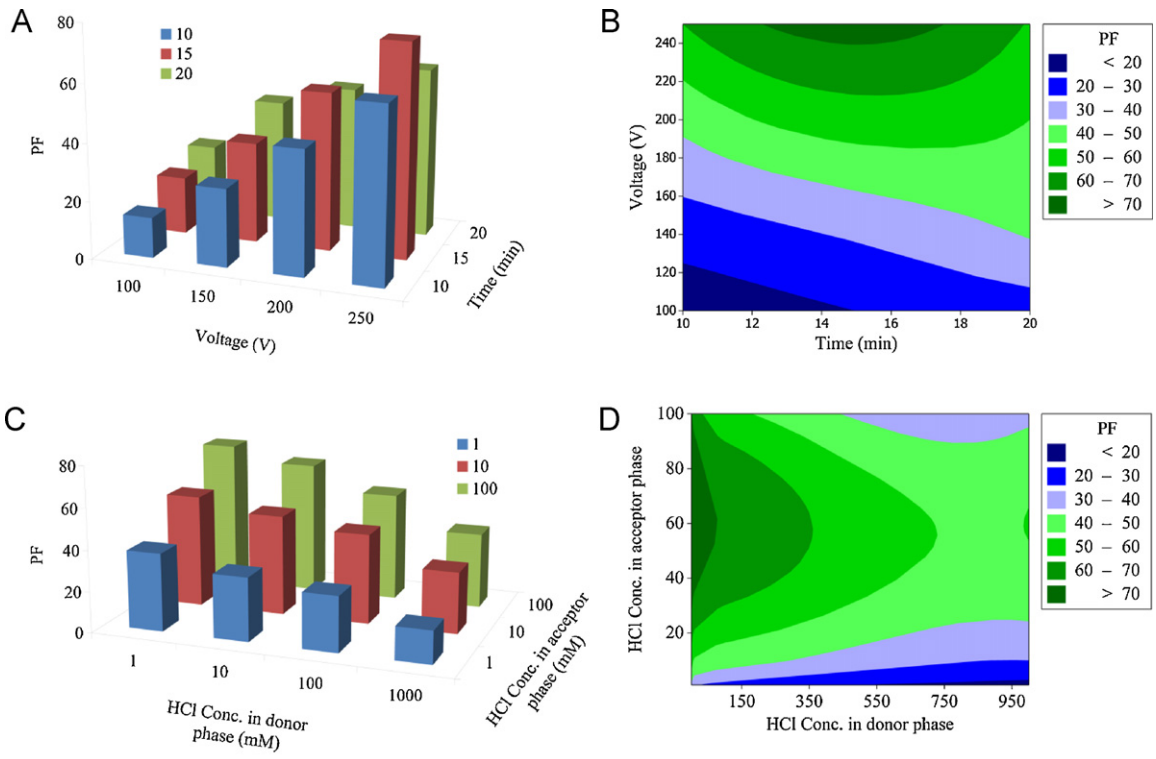


Fig. 3. Simultaneous investigation of time–voltage (A) as well as HCl concentration of donor $[HCl]_D$ and acceptor phases $[HCl]_A$ (C) on *PF* of ATE by EME. (B and D) are related to two-dimensional contour plots of time–voltage and $[HCl]_D$–$[HCl]_A$, respectively. Conditions for (A) are like Fig. 2 except variable time and voltage and for (B), are like Fig. 2 except voltage: 250 V, extraction time: 15 min and variable $[HCl]_{D\&A}$.

(Fig. 2B) in order to achieve higher extraction efficiency for ATE. As can be seen, NPOE containing 5% of TEHP and 10% of DEHP provides higher *PF* than other cases. In conclusion, DEHP and TEHP were found to effectively control the selectivity of the SLM, and the extractability of polar drugs can be improved at the expense of non-polar substances through suitable engineering.

3.2. Effect of extraction time and voltage

In EME, the electrokinetic migration of the analytes across the SLM into the acceptor solution is greatly dependent upon the applied voltage. In a recent paper, Kjelsen et al. reported that the flux of analytes (J_i) is affected by the magnitude of the applied voltage [19]:

$$J_i = \frac{-D_i}{h}\left(1+\frac{\upsilon}{\mathrm{Ln}\ \chi}\right)\left(\frac{\chi-1}{\chi-\exp(-\upsilon)}\right)(C_{ih}-C_{io}\exp(-\upsilon)) \quad (5)$$

where D_i denotes the diffusion coefficient for the analyte, h represents the thickness of the membrane, C_{ih} stands for the analyte concentration at the SLM/sample interface, and C_{io} is the analyte concentration at the acceptor/SLM interface. Also, υ is a function of electrical potential [19], and χ is the ratio of the total ionic concentration in the sample solution to that in the acceptor solution, and is defined as ion balance [19]. Time is another parameter which can affect the flux of analytes in EME. Both time and voltage directly increase the extraction recovery; but there is an antagonistic effect when they are simultaneously considered, thus an increase in extraction time limits the voltage and vice versa. For obtaining the best optimum extraction voltage and time, these parameters were considered at the same time. For this purpose, the extraction of ATE was studied in different EME durations and electrical potential differences ranging from 10 to 20 min and 100 to 250 V, respectively. Twelve experiments were designed by means of the software Minitab. The results are summarized in Fig. 3A. It also depicts two-dimensional contour plot (Fig. 3B) displaying the interaction between independent variables and assists in determining the optimum operating conditions for desirable responses. Based on the obtained results and contour plots in this figure, it can be observed that *PF* of ATE increases by increasing the voltage and extraction time to 250 V and 15 min, respectively. Further increase of voltage and extraction time from these levels leads to a decrease in response. Also, the obtained data were evaluated by analysis of variance (ANOVA). An effect whose *p*-value exceeds 0.05 may be considered significant. Results of ANOVA implied that, time is not significant whereas voltage is a significant parameter. It should be noted that EME is a non-exhaustive process. At the beginning of the process, recoveries increased rapidly by increasing the extraction time and voltage, but declined thereafter. This observation shows that EME reached the steady state at a time earlier than the investigated range. Therefore, time did not show meaningful effect on extraction recovery in the range of 10–20 min. The decreased peak area after the given time and voltage can most probably be attributed to saturation of the analyte in the acceptor phase and analyte back-extraction into the donor phase as pH increased slightly in the acceptor solution due to electrolysis. Electrolysis occurred at both electrodes according to the following reactions:

$$\text{Samplesolution}: H_2O \rightarrow 2H^+ + (1/2)O_2 + 2e^- \quad (6)$$

$$\text{Acceptorphase}: 2H^+ + 2e^- \rightarrow H_2 \quad (7)$$

Similar observation has previously been reported [19]. In addition, the gradual suppression of analyte net transfer resulted from heat generation at higher times and voltages can also decrease the extraction efficiency [21].

Table 2
Operating conditions for selective extraction of ATE in the presence of BET and PRO by EME.

Type of β-blocker	Operating conditions	Optimal amount
ATE	SLM	NPOE containing 5% TEHP and 10% DEHP
	Sample volume	3 mL
	Stirring speed	1250 rpm
	Voltage	250 V
	Extraction time	15 min
	Donor phase composition	1 mM HCl
	Acceptor phase composition	100 mM HCl
	Salt%	No salt

3.3. Effect of pH of donor and acceptor phases (ion balance effect)

In the following optimization process, the pHs of both acceptor and donor phases were considered. It was shown that the total ionic concentration of the donor phase to that of the acceptor phase, which is defined as the ion balance (χ), impresses the flux over the membrane [19]. The flux may be decreased as this ratio increased according to theoretical models [19]. To investigate the effect of this parameter, HCl concentration in the donor phase was changed from 1.0 to 1000 mM while it was changed in the range of 1–100 mM in the acceptor phase. The results are shown in Fig. 3C. Also, the related contour plot (Fig. 3D) is presented in this figure. As can be seen, the maximum response was obtained for the minimum value of χ (decreasing pH of acceptor phase and increasing pH of donor phase) as predicted by theoretical models [19]. By increasing pH of acceptor solution, χ increases and thus causes partial deprotonation of the analyte and accelerates its back-diffusion to the donor solution. On the other hand, at a low pH in the acceptor phase (below the pK_a of analyte), the analytes are easily released into the acceptor solution. Although extraction recoveries increased by increasing the HCl concentration in the acceptor phase, there are some limitations for this increasing. In EME, for neutralization in donor and acceptor phases, cations migrate toward cathode and anions migrate toward anode through the SLM (Fig. 1A). Increasing content of ions in each of donor and acceptor phases can increase number of ions migrate through SLM at a given moment, it increases the current level and thus Joule heating causes instability of SLM. Increasing of the current level between electrodes increase electrolysis reactions on the surfaces of electrodes and therefore bubble formation risk. Bubble formation increases uncertainties in obtained data by EME. Therefore, 100 mM HCl was chosen as the upper level of acceptor solution for this investigation. Results of ANOVA show significant effect for HCl concentration in both donor and acceptor phases. Increase of the proton ions' concentration in donor phase led to competition among proton and analytes ions to migrate toward cathode electrode. Therefore, extraction recovery decreased in comparison with sample solutions containing less proton ions. For the rest of this work, 1 mM HCl was utilized as the sample solution.

3.4. Effect of stirring rate

As is known, stirring speed plays an essential role in increasing the kinetics and efficiency of extraction by increasing the mass transfer and reducing the thickness of double layer around SLM. To study the effect of stirring rate in more detail, the effect of stirring speed on extraction efficiency of ATE was investigated. As shown in Fig. 4, the highest extraction efficiencies were obtained at the maximum stirring speed. Thus, a stirring rate of 1250 rpm was chosen in the subsequent experiments.

3.5. Salt effect

According to previous studies [19,21], 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. The effect of χ was investigated using solutions containing 2% and 5% NaCl. The obtained results are in full agreement with previous studies [19,21]. Thus, migration of the analytes would be more efficient in the absence of salt.

Table 3
Analytical performance for determination of ATE in a drug-free saliva sample, in the presence of BET and PRO by EME.

LOD (μg L^{-1})	2.0
Linearity[a] (μg L^{-1})	10–5000
Regression equation	Y = 4E+06 + 1274.8
R^2	0.997
RSD%[b]	6.4
PF[b]	74
ER%	37

[a] Linearity was investigated until concentration of 5000 μg L^{-1} of ATE.
[b] Preconcentration factors (PF) and RSD% were calculated for three-replicate measurements at concentration of 50 μg L^{-1}.

3.6. Method validation

To investigate the practical applicability of the proposed EME technique, figures of merit of the method were evaluated under the optimized extraction conditions (Table 2), whose results are summarized in Table 3. Drug-free saliva sample was used for validations. Under optimal conditions, ATE was effectively extracted with recovery of 37%, which corresponded to PF of 74, limits of detection of 2.0 μg L^{-1} and intra-day precision (n = 3) less than 8.8%. Comparison of the proposed method with different existing methods for extraction and determination of ATE is provided in Table 4. It is 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

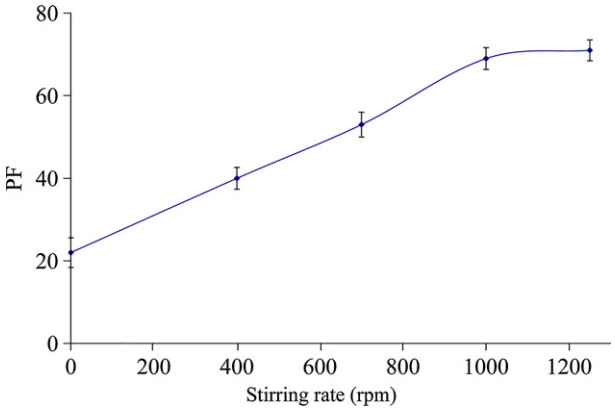


Fig. 4. Investigation of stirring rate on extraction efficiency of ATE by EME (a), conditions are like those used for investigation of $[HCl]_D$–$[HCl]_A$ except sample solution: 1 mM HCl, acceptor solution: 100 mM HCl, and variable stirring rates.

Table 4
Comparison of the proposed method with other analytical techniques for determination of ATE in different samples.

Analytical technique[a]	Sample	Extraction time (min)	Linear range ($\mu g\,L^{-1}$)	R^2	LOD ($\mu g\,L^{-1}$)	Sample volume (mL)	*ER*%	RSD% (intra-day)	Ref.
PC-HFME-CE	Water	50	0.025–5	0.996	7.0	20	–	4.0	[6]
SPE-UPLC/MS/MS	Water	–	0.0013–0.8	0.989	0.0013	500	22.2	15.2	[9]
LLE-LC/MS/MS	Eye tissue	–	10–2000	0.999	–	–	84–95.8	<9.9	[11]
HF-LPME-LC/MS	Plasma	60	25–1500	0.992	25[c]	0.1	11–17	<17.2	[23]
SPE-LC/MS/MS	Water, plant	–	10.6–6000	0.999	<0.049[c]	100–1000	81–108	<10.0	[24]
MIP-LC/MS/MS	Water	–	0.1–200	0.999	<0.0015[c]	25	43–110	<20.0	[25]
SPE-LC/MS/MS	Water	–	–	–	0.004	25	50–89	<12	[25]
SPE-LC/MS/MS	Water	–	0.001–18[b]	0.999	0.00107[c]	500	106	<7.77	[26]
SPE-LC/MS/MS	Urine	–	–	–	500[c]	1.0	55	<16	[27]
SPE-LC/MS/MS	Water	–	0.5–500	0.998	<0.0014	100–150	74–99	<8	[28]
EME-HPLC-UV	Saliva	15	10–5000	0.997	2.0	3.0	37	6.4	This work

[a] Hollow fiber (HF), liquid-phase microextraction (LPME), liquid–liquid extraction (LLE), liquid chromatography (LC), mass spectrometry (MS), solid-phase extraction (SPE), molecularly imprinted polymer (MIP), polymer-coated hollow fiber microextraction (PC-HFME), capillary electrophoresis (CE), ultra-performance liquid chromatography (UPLC), electromembrane extraction (EME).
[b] This linearity has been tested for concentration ranges that are normally measured in waste and surface waters.
[c] These data are related to limit of quantification (LOQ).

Table 5
Determination of ATE in saliva samples collected from one male and four females stricken to hypertension diseases.

Sample	Duration of drug consumption (year)		ATE
Saliva 1 (male, age 27)	Not at all	Initial concentration ($\mu g\,L^{-1}$)	n.d[b]
		RR%[a]	99.0
		RSD% ($n = 3$)	6.4
Saliva 2 (female, age 67)	6	Initial concentration ($\mu g\,L^{-1}$)	102.7
		RR%	102
		RSD% ($n = 3$)	7.8
Saliva 3 (female, age 32)	2	Initial concentration ($\mu g\,L^{-1}$)	20.0
		RR%	96.4
		RSD% ($n = 3$)	8.1
Saliva 4 (female, age 73)	10	Initial concentration ($\mu g\,L^{-1}$)	84.5
		RR%	94.8
		RSD% ($n = 3$)	8.8
Saliva 5 (female, age 70)	7	Initial concentration ($\mu g\,L^{-1}$)	41.2
		RR%	97.0
		RSD% ($n = 3$)	6.6

[a] 100 $\mu g\,L^{-1}$ of ATE was added to calculate relative recovery percent (RR%).
[b] n.d, not detected.

which is comparable with existing techniques. The consumption of organic solvents in this technique reaches the minimum amount. Selecting an appropriate organic solvent, EME can provide a high selectivity as well as high clean-up in complex matrices. These characteristics are keys of interest for laboratories doing routine trace analysis of ATE by EME.

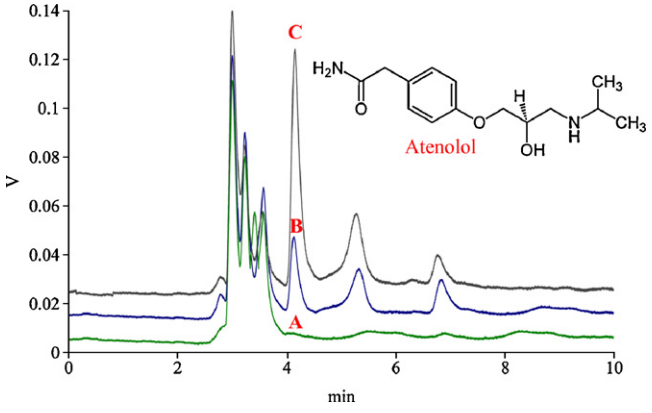


Fig. 5. Typical chromatograms of the ATE in a drug-free saliva sample (A) and a patient's saliva sample before (B) and after spiking at concentration of 100 $\mu g\,L^{-1}$ (C), respectively.

3.7. Analysis of real samples

To investigate matrix effects and applicability of EME technique, some experiments were carried out on different saliva samples. Firstly, 100 μL of HCl 100 mM was added to 5.0 mL of each saliva sample and centrifuged at 3000 rpm for 15 min. Afterward, samples were diluted 1:1 with ultrapure water and their pHs were adjusted at 3.0. Then, 3 mL of each solution was transferred into the sample vial and exposed under EME process. The obtained results are shown in Table 5. Relative standard deviations (RSDs%) based on three similar determinations and relative recoveries (RR%) were within the ranges of 6.4–8.8% and 94.8–102.7%, respectively. Proper RRs% indicate little effect of matrices on the extraction efficiency. Fig. 5 depicts the typical chromatograms of the ATE in a drug-free saliva sample (A) and a patient's saliva sample before (B) and after spiking (C).

4. Conclusions

In the present work, EME was applied for highly selective and efficient extraction of atenolol as an important β-blocker drug. A liquid membrane of pure NPOE was efficient for extraction of non-polar drugs, whereas addition of DEHP to the liquid membrane was required to extract polar substances. By a combination of NPOE, DEHP, and TEHP, high selectivity and high clean-up can be obtained. In other words, the electrokinetic transport across liquid

membranes may be altered dramatically by the composition of the liquid membrane, which may open future possibilities to effectively control selectivity in the aforementioned concept. Regarding short extraction time, satisfactory LODs and RSDs, EME may have a strong potential as a future sample preparation technique.

Acknowledgements

The authors gratefully acknowledge financial support from Tarbiat Modares University and gracious help of Miss Elham Ghaemi, M.Sc. student of analytical chemistry, Tarbiat Modares University (Tehran, Iran) and Mrs. Jamileh Pazhoom, nurse of Imam Khomeini Hospital (Tehran, Iran).

References

[1] R.D. Johnson, R.J. Lewis, Forensic Sci. Int. 156 (2006) 106–117.
[2] K. Parfitt (Ed.), Martindale: The Complete Drug Reference, 32nd ed., Pharmaceutical Press, London, 1990.
[3] D. Šatínský, H.S. Serralheiro, P. Solich, A.N. Araújo, M.C.B.S.M. Montenegro, Anal. Chim. Acta 600 (2007) 122–128.
[4] M.-J. Paik, D.T. Nguyen, K.-R. Kim, Chromatographia 64 (2006) 673–679.
[5] Y.Q. Xia, R. Bakhtiar, R.B. Franklin, J. Chromatogr. B 788 (2003) 317–329.
[6] C. Basheer, H. Wang, A. Jayaraman, S. Valiyaveettil, H.K. Lee, J. Chromatogr. A 1128 (2006) 267–272.
[7] R. Bhushan, S. Tanwar, J. Chromatogr. A 1217 (2010) 1395–1398.
[8] I. Baranowska, B. Kowalski, Water Air Soil Pollut. 211 (2010) 417–425.
[9] J. Magnér, M. Filipovic, T. Alsberg, Chemosphere 80 (2010) 1255–1260.
[10] L. Amendola, F. Molaioni, F. Botre, J. Pharm. Biomed. Anal. 23 (2000) 211–221.
[11] R.S. Kadam, U.B. Kompella, J. Chromatogr. B 877 (2009) 253–260.
[12] G.J. Murray, J.P. Danaceau, J. Chromatogr. B 877 (2009) 3857–3864.
[13] M. Josefsson, A. Sabanovic, J. Chromatogr. A 1120 (2006) 1–12.
[14] W.M. Mullett, P. Martin, J. Pawliszyn, Anal. Chem. 73 (2001) 2383–2389.
[15] C. Yao, T. Li, P. Twu, W.R. Pitner, J.L. Anderson, J. Chromatogr. A 1218 (2011) 1556–1566.
[16] X. Hu, J. Pan, Y. Hu, G. Li, J. Chromatogr. A 1216 (2009) 190–197.
[17] J. Lee, H.K. Lee, K.E. Rasmussen, S. Pedersen-Bjergaard, Anal. Chim. Acta 624 (2008) 253–268.
[18] S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1109 (2006) 183–190.
[19] T.M. Middelthon-Bruer, A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Sep. Sci. 31 (2008) 753–759.
[20] A.C. Moffat, M.D. Osselton, B. Widdop (Eds.), Clarke's Analysis of Drugs and Poisons, 3rd ed., Pharmaceutical Press, London, 2003.
[21] A. Gjelstad, K.E. Rasmussen, S. Pedersen-Bjergaard, J. Chromatogr. A 1124 (2006) 29–34.
[22] J. Lee, F. Khalilian, H. Bagheri, H.K. Lee, J. Chromatogr. A 1216 (2009) 7687–7693.
[23] T.S. Ho, J.L.E. Reubsaet, H.S. Anthonsen, S. Pedersen-Bjergaard, K.E. Rasmussen, J. Chromatogr. A 1072 (2005) 29–36.
[24] N.M. Vieno, T. Tuhkanen, L. Kronberg, J. Chromatogr. A 1134 (2006) 101–111.
[25] M. Gros, T.M. Pizzolato, M. Petrović, M.J.L. de Alda, D. Barceló, J. Chromatogr. A 1189 (2008) 374–384.
[26] S. Castiglioni, R. Bagnati, D. Calamari, R. Fanelli, E. Zuccato, J. Chromatogr. A 1092 (2005) 206–215.
[27] M. Kolmonen, A. Leinonen, A. Pelander, I. Ojanperä, Anal. Chim. Acta 585 (2007) 94–102.
[28] N.A. Al-Odaini, M.P. Zakaria, M.I. Yaziz, S. Surif, J. Chromatogr. A 1217 (2010) 6791–6806.