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Extraction and preconcentration of β-blockers in human urine for analysis with high performance liquid chromatography by means of carrier-mediated liquid phase microextraction

Li Zhang, Xiaoli Su, Chenggong Zhang, Li Ouyang, Qingji Xie, Ming Ma*, Shouzhuo Yao

Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education of China), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, PR China

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A B S T R A C T

A novel method was developed for the analysis of four β-blockers, namely sotalol, carteolol, bisoprolol, and propranolol, in human urine by coupling carrier-mediated liquid phase microextraction (CM-LPME) to high performance liquid chromatography (HPLC). By adding an appropriate carrier in organic phase, simultaneous extraction and enrichment of hydrophilic (sotalol, carteolol, and bisoprolol) and hydrophobic (propranolol) drugs were achieved. High enrichment factors were obtained by optimizing the compositions of the organic phase, the acceptor solution, the donor solution, the stirring rate, and the extraction time. The linear ranges were from 0.05 to 10.0 $mg L^{-1}$ for sotalol and carteolol, and from 0.05 to 8.0 $mg L^{-1}$ for bisoprolol and propranolol. The limits of detection (S/N = 3) were 0.01 $mg L^{-1}$ for sotalol, carteolol, and bisoprolol, and 0.005 $mg L^{-1}$ for propranolol. The relative standard deviations were lower than 6%. The developed method exhibited high analyte preconcentration and excellent sample clean-up effects with little solvent consumption and was found to be sensitive and suitable for simultaneous determination of the above four drugs spiked in human urine. Furthermore, the successful analysis of propranolol in real urine specimens revealed that the determination of β-blockers in human urine is feasible using the present method.

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1. Introduction

β-Blockers, e.g., sotalol, carteolol, bisoprolol, and propranolol (structures shown in Fig. 1) are widely used as standard therapies in the treatment of high blood pressure, arrhythmias, and angina pectoris. Besides, β-blockers can also improve the heart's ability to relax and exhibit calming neurological effects decreasing anxiety, nervousness and stabilizing motor performance. The improved psychomotor performance may be beneficial in sports requiring coordination, steady hands, precision, and accuracy such as shooting, archery, golf, billiards, and gymnastics [1,2]. Therefore, sotalol, carteolol, bisoprolol, and propranolol are listed as the prohibited substances in athletic competitions by the Medical Commission of the International Olympic Committee (MCIOC) [3]. After administration, small amounts of sotalol, carteolol, bisoprolol, and propranolol are excreted unmetabolized in the urine. Usually, due to the low concentration of drugs and a high number of interferents existing in urine, sample preconcentration and cleanup must be carried out before the drugs can be determined. Liquid–liquid extraction (LLE) [1,4], solid phase extraction (SPE) [2,5,6], and solid phase microextraction (SPME) [7,8] combined with HPLC, CE, HPLC-MS, or GC–MS were used successfully to determine β-blockers in human fluids. However, the LLE and SPE techniques are time consuming and involve large organic solvent consumption. In SPME, the fiber needs to be treated to avoid carry-over effects after sample desorption every time and the SPME–HPLC interface requires a desorption chamber as part of a switching valve, and thus it is not convenient to use [9].

Liquid phase microextraction (LPME), emerged in the mid-to-late 1990s [10,11], was greatly developed as an efficient method for sample preparation [12–26]. In LPME, the extraction efficiency is governed simultaneously by the partition behavior of analytes between the donor solution and the organic phase and between the organic phase and the acceptor solution. Therefore, it is usually difficult to obtain a high extraction efficiency for polar analytes [27]. Ho et al. [28,29] and Wu and Lee [30,31] added an ion-pair reagent to the donor solution of LPME to form a hydrophobic ion-pair with an ionized analyte and significantly increased the extraction efficiency of the analyte. Yazdi and Es'haghi [32–35] enhanced the efficiency of LPME for the determination of basic drugs of abuse in hair by adding a surfactant in the donor solution and for aromatic amines by adding a crown ether to the acceptor solution.

* Corresponding author. Tel.: +86 731 88872681; fax: +86 731 88872681.
E-mail address: mingma@hunnu.edu.cn (M. Ma).

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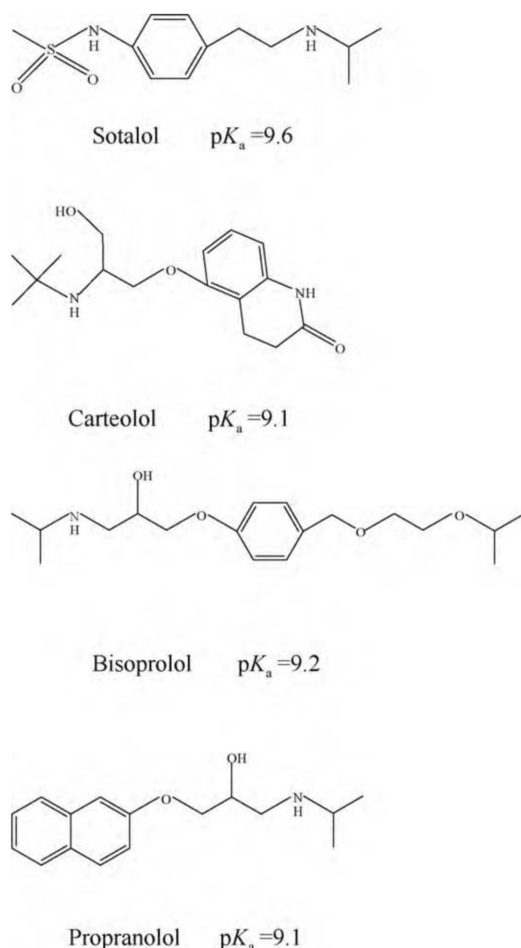

Fig. 1. Structures and pK_a values of sotalol, carteolol, bisoprolol, and propranolol.

Recently, Yamini et al. [36,37], Ma and co-workers [19], and Jiang and co-workers [38] demonstrated efficient extraction and preconcentration of drugs and aromatic amines using LPME with organic phase containing an anionic carrier. Based on the carrier-mediated transport, high enrichment factors for the hydrophilic drugs were obtained.

In the present paper, we report a new carrier-mediated liquid phase microextraction (CM-LPME) method for simultaneous extraction and enrichment of hydrophilic and hydrophobic drugs including sotalol, carteolol, bisoprolol, and propranolol, which have an octanol–water partition coefficient (log P) of 0.24, 1.42, 1.87, and 3.48 [1], and a dissociation constant (pK_a) of 9.6, 9.1, 9.2, and 9.1 [6], respectively. High precision, good accuracy, and high sensitivity were achieved by coupling the CM-LPME with HPLC. The new CM-LPME system is different from our previous study [19] in the compositions of the organic phase (particularly the carrier), the donor solution, and the acceptor solution besides the target compounds.

2. Experimental

2.1. Reagents and apparatus

Sotalol hydrochloride, carteolol hydrochloride, bisoprolol fumarate, and propranolol hydrochloride were reference substances (purity > 98%) purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The above four drugs were dissolved in water to contain 100 mg L^{-1} of the analytes and stored at 4 °C as stock solutions. Standard working solutions of these four drugs were prepared by suitable dilutions of the stock solutions in water and also stored at 4 °C. The pH of the standard working solution was adjusted to a desired value with 5.6 mol/L sodium hydroxide solution as the donor solution just before the extraction experiment.

HPLC-grade acetonitrile was obtained from Tedia (Fairfield, OH, USA). Benzene, toluene, *o*-xylene, *n*-hexane, *n*-octanol, and isoamyl alcohol were all of analytical grade and purchased from Hunan Chemical Reagent Ltd. (Changsha, Hunan, China). The carriers used, trioctylmethylammonium chloride (Aliquat 336) and tetraoctyl ammonium bromide (TOAB) purchased from Fluka (Buchs, Switzerland) and Xiamen Pioneer Technology Inc. (Xiamen, China), respectively, were of chemical grade and directly used without further purification. The water used was purified with a Milli-Q system from Millipore (Bedford, MA, USA).

Spiked urine samples containing sotalol, carteolol, bisoprolol, and propranolol were prepared by diluting the stock solutions in the urine obtained from a healthy female volunteer and were stored at 4 °C and tested within 24 h. Real urine specimen for determination of propranolol was collected from a female patient, who took two tablets of 20 mg propranolol hydrochloride prescribed by a physician, at 4.0 h post-administration with her written consent.

An HJ-2 magnetic stirrer, purchased from Hengfeng Instrument Factory (Jintan, Jiangsu, China), was used in the LPME setup.

2.2. HPLC system

The Agilent 1100 HPLC system from Agilent Technologies (Santa Clara, USA) consisted of two G1312A pumps, a G1316A thermostat, and a G1315B DAD detector. A Hypersil C18 analytical column (5 μm, 4.6 mm × 250 mm) purchased from Dalian Johnsson Separation Science & Technology Corporation (Dalian, Liaoning, China) was used for the chromatographic separation. The mobile phase consisted of (A) a mixture of 10 mM triethylamine and 20 mM potassium dihydrogen phosphate solution (adjusted to pH 3 with phosphoric acid) and (B) acetonitrile. The gradient elution was programmed as follows: 0–5 min, 85% A; 5–10 min, 85–70% A; 10–19 min, 70–55% A. The flow rate was 1 mL min^{-1} throughout and the UV detection wavelength was set at 222 nm.

2.3. Extraction procedures

The CM-LPME device is illustrated in Fig. 2. Briefly, 2.1 mL of the donor solution was transferred into a home-made sample vial with magnetic stirring to facilitate the mass transfer process. 190 μL of an organic phase was carefully pipetted along the inner wall of the sample vial to form a solvent thin layer above the donor solution. To prevent the evaporation of the organic solvent, the sample vial was covered with a PTFE lid, which had a hole in the center for inserting a 10-μL HPLC microsyringe. For an extraction, the microsyringe was rinsed with pure water, methanol, and acceptor solution (each for three times), respectively, to ensure that no air bubble and other impurities were left in the barrel and the needle. Then, 1.4 μL of the acceptor solution was drawn into the syringe, which was held by a clamp, and the needle tip of the syringe was dipped into the organic phase. The plunger of the syringe was depressed completely to suspend a microdrop of the acceptor solution at the needle tip and to expose the drop to the organic phase. Then, the aqueous donor solution began to be stirred to promote mass transport. After extraction, the drop was carefully withdrawn into the microsyringe and the plunger was depressed to 1.0 μL. The tip was wiped carefully. Finally, 1.0-μL of the acceptor solution was directly injected into the HPLC system for analysis. All LPME experiments were conducted at 30 ± 0.5 °C.

Conventional liquid–liquid extraction experiments were also carried out at 30 ± 0.5 °C. The donor solution was 5 mg L^{-1} of drugs

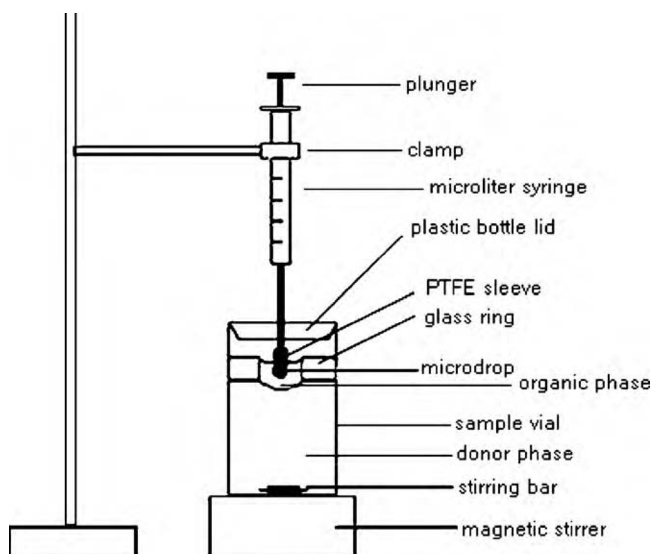

Fig. 2. Schematic illustration of the CM-LPME system. Donor phase: 2.1 mL sample solution, pH 12.0; organic phase: 190 μL 0.005 M TOAB-toluene; microdrop: 1.4 μL 0.1 M HCl (acceptor solution). After 30 min of CM-LPME pretreatment, 1.0 μL of the acceptor solution was analyzed by HPLC.

standard working solutions (pH 11) and the organic phase contained 0.005 M carrier. 1 mL of the organic phase and 2 mL of the aqueous donor solution or the acceptor solution (0.1 M HCl solution) were added into a centrifugal tube and mixed on a vibrator for 30 min. After phase separation, the concentration of the analyte in the donor solution or in the acceptor solution was determined by means of HPLC, and the concentration of the analyte trapped in the organic phase was calculated from the material balance.

2.4. Related calculation formulas

Enrichment factor (E_f) was calculated as the following equation:

$$E_f = \frac{c_{a,fin}}{c_{d,ini}} \quad (1)$$

where $c_{a,fin}$ and $c_{d,ini}$ represent the final concentration of the analyte in the acceptor solution after CM-LPME and the initial concentration of the analyte in the donor solution, respectively. The method relative recovery (R) was calculated as the following formula:

$$R = \frac{c_{d,det}}{c_{d,ini}} \quad (2)$$

where $c_{d,det}$ is the determined concentration of the analyte in the donor solution with the present method. In liquid–liquid extraction experiments, the extraction efficiency (E) and the back-extraction efficiency (E_R) are defined as follows:

$$E = \frac{n_{d,ini} - n_{d,fin}}{n_{d,ini}} \quad (3)$$

$$E_R = \frac{n_{a,fin}}{n_{d,ini} - n_{d,fin}} \quad (4)$$

where $n_{d,ini}$, $n_{d,fin}$ and $n_{a,fin}$ are the initial molar quantity of the analyte in the donor solution before extraction, the final molar quantity of the analyte in the donor solution and in the acceptor solution after extraction, respectively.

3. Results and discussion

The CM-LPME procedure used includes two consecutive extraction steps: the donor solution–organic phase extraction and the organic phase–acceptor solution back-extraction. Sotalol, carteolol, bisoprolol, and propranolol are all basic compounds. In the acidic and neutral solution, the compounds are protonated and, in the basic solution, they are unprotonated. Therefore, prior to CM-LPME, it is necessary to adjust the pH of the donor solution according to the properties of the analytes and the carrier so that the analytes can easily penetrate into the organic phase. Owing to low pH of the acceptor solution (HCl solution), the analytes react easily with H^+ in the acceptor solution at the interface of organic phase–acceptor solution and are then back-extracted to the acceptor solution. In the following experiments, operating parameters of CM-LPME were first optimized and then the optimal CM-LPME procedure was coupled with HPLC for determination of the four drugs in urine samples.

3.1. Selection of the organic solvent and the carrier in the organic phase

In order to achieve satisfactory analyte preconcentration, the selection of appropriate organic solvent is crucial in LPME [37,38]. Six organic solvents with different polarity (Table 1 [39]), namely benzene, toluene, *o*-xylene, *n*-hexane, *n*-octanol, and isoamyl alcohol, were investigated for their effects on the extraction efficiency with liquid–liquid extraction experiments. The results are shown in Fig. 3. Fig. 3a indicates that all of six organic solvents exhibited relatively high extraction efficiencies (>83.8%, except 36.4% of extraction efficiency for bisoprolol with *n*-hexane as the extraction solvent) for bisoprolol and propranolol and relatively low extraction efficiencies (<13.9%) for sotalol. However, relatively high extraction efficiencies of carteolol (>96.3%) were only obtained in *n*-octanol and isoamyl alcohol. Moreover, Fig. 3b shows that *n*-octanol and isoamyl alcohol gave obviously lower back-extraction efficiencies for the analytes compared to other extraction solvents. This may be because *n*-octanol and isoamyl alcohol own higher polarity, i.e., relative larger dielectric constant and dipole moment, which is favorable for enhancing E and reducing E_R of the polar analytes. Relatively high back-extraction efficiencies (>52.1%) for all of analytes were obtained only in toluene. This implies that a high enrichment factor is expected to be obtained only with toluene as the organic solvent in LPME. Nevertheless, with any of the six reagents as the extraction solvent, very low extraction efficiencies were observed for sotalol, due to its high hydrophilicity. Therefore, toluene was selected as the extraction solvent and attempts were made to promote the extraction and realize the preconcentration of sotalol by adding an appropriate carrier into the organic phase.

According to our previous study [19], the anionic carrier, Aliquat 336, can mediate the transport of unprotonated basic analytes under a base condition (pH 11.5). Yang et al. [40] also observed that

Table 1
Characteristics of the investigated organic solvents [39].

Solvents	Dielectric constant (20 °C)	Dipole moment (10^{-30} C m)	Solubility in water (g/100 g)	Viscosity (mPa s, 25 °C)
Benzene	2.283	0 (20–60 °C)	Insoluble	0.6010
Toluene	2.24	1.23	Insoluble	0.5866 (20 °C)
o-Xylene	2.266	1.47	Insoluble	0.754
n-Octanol	10.34	5.60	0.01–0.05	8.93 (20 °C)
Isoamyl alcohol	14.7 (25 °C)	6.07	Slightly soluble	4.2 (20 °C)
n-Hexane	1.890	0.27	Insoluble	0.307

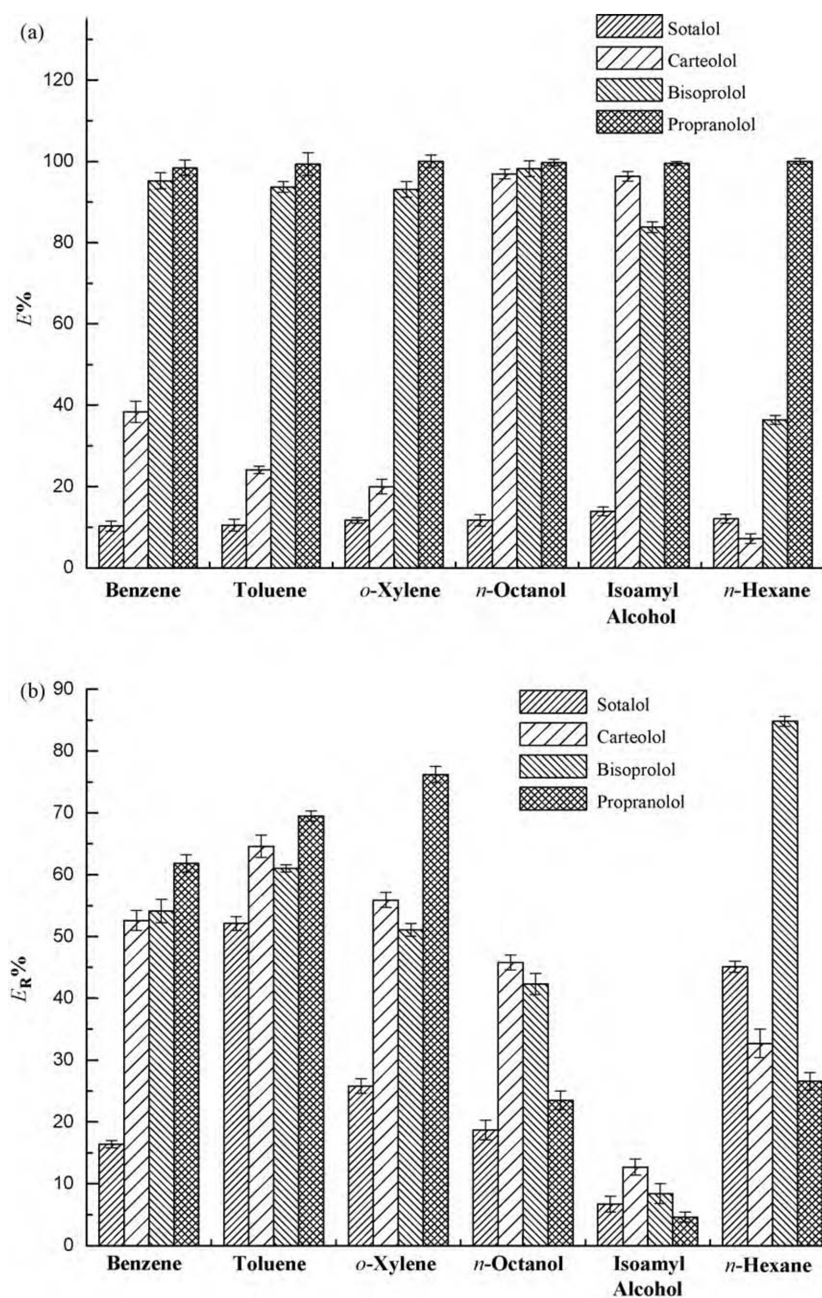

Fig. 3. Effect of the organic solvent on the extraction efficiency and the back-extraction efficiency.

Aliquat 336 could extract both dissociated and undissociated forms of carboxylic acids. Therefore, two anionic carriers, Aliquat 336 and TOAB, were investigated as a carrier under a weak base condition (pH 11). The results are summarized in Table 2. Compared to the extraction efficiencies shown in Fig. 3a and the enrichment factors in toluene alone (0.0545, 0.155, 0.571, and 0.690 for sotalol, carteolol, bisoprolol, and propranolol, respectively), the addition of TOAB or Aliquat 336 obviously increased the extraction efficiencies and enrichment factors for sotalol, carteolol, and bisoprolol. These enhancing effects were probably due to the increase of the polarity of the organic phase with addition of a polar carrier and the association of the unprotonated polar analyte with the carrier through non-ionic forces [19,40]. However, it should be noted that the enrichment factor for propranolol decreased when the carrier TOAB or Aliquat 336 was added into organic phase. This may be because propranolol is a strong hydrophobic analyte and thus its extraction efficiency decreased with the increase of the polarity of the organic phase due to the addition of the carrier. Moreover, the stability of the acceptor solution droplet in Aliquat 336-toluene was worse than that in the TOAB-toluene. Therefore, TOAB-toluene was selected as the organic phase although Aliquat 336 gave higher enrichment factors.

Table 2
Effect of the carriers on the extraction behavior of analytes.

Analyte	0.005 M TOAB			0.005 M Aliquat 336		
	E	E_R	E_f	E	E_R	E_f
Sotalol	0.983	0.761	0.748	1.00	0.923	0.923
Carteolol	0.768	0.822	0.631	0.593	0.965	0.572
Bisoprolol	1.00	0.662	0.662	1.00	0.792	0.792
Propranolol	1.00	0.383	0.383	1.00	0.528	0.528

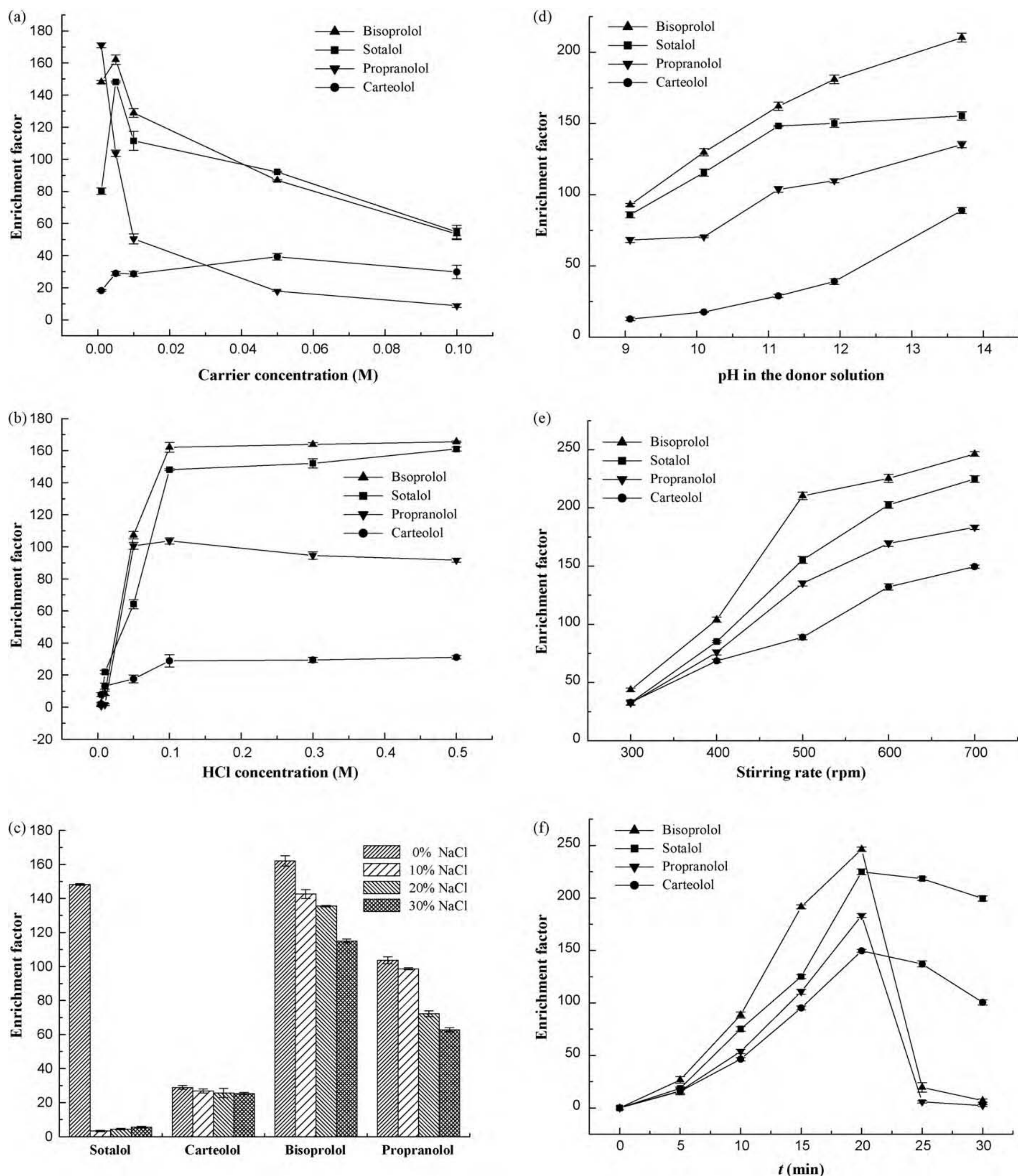

Fig. 4. Effects of related parameters on the enrichment factor: (a) effect of TOAB concentration on the enrichment factor (acceptor solution: 0.1 M HCl; stirring rate: 500 rpm; extraction time: 20 min; pH in the donor solution: 11); (b) effect of HCl concentration in the acceptor solution on the enrichment factor (carrier: 0.005 M TOAB; stirring rate: 500 rpm; extraction time: 20 min; pH in the donor solution: 11); (c) effect of the concentration of NaCl in the donor solution on the enrichment factor (carrier: 0.005 M TOAB; acceptor solution: 0.1 M HCl; stirring rate: 500 rpm; extraction time: 20 min; pH in the donor solution: 11); (d) effect of pH in the donor solution on the enrichment factor (carrier: 0.005 M TOAB; acceptor solution: 0.1 M HCl; stirring rate: 500 rpm; extraction time: 20 min); (e) effect of the stirring rate on the enrichment factor (carrier: 0.005 M TOAB; acceptor solution: 0.1 M HCl; extraction time: 20 min; pH in the donor solution: 13.7); (f) effect of the extraction time on the enrichment factor (carrier: 0.005 M TOAB; acceptor solution: 0.1 M HCl; stirring rate: 700 rpm; pH in the donor solution: 13.7).

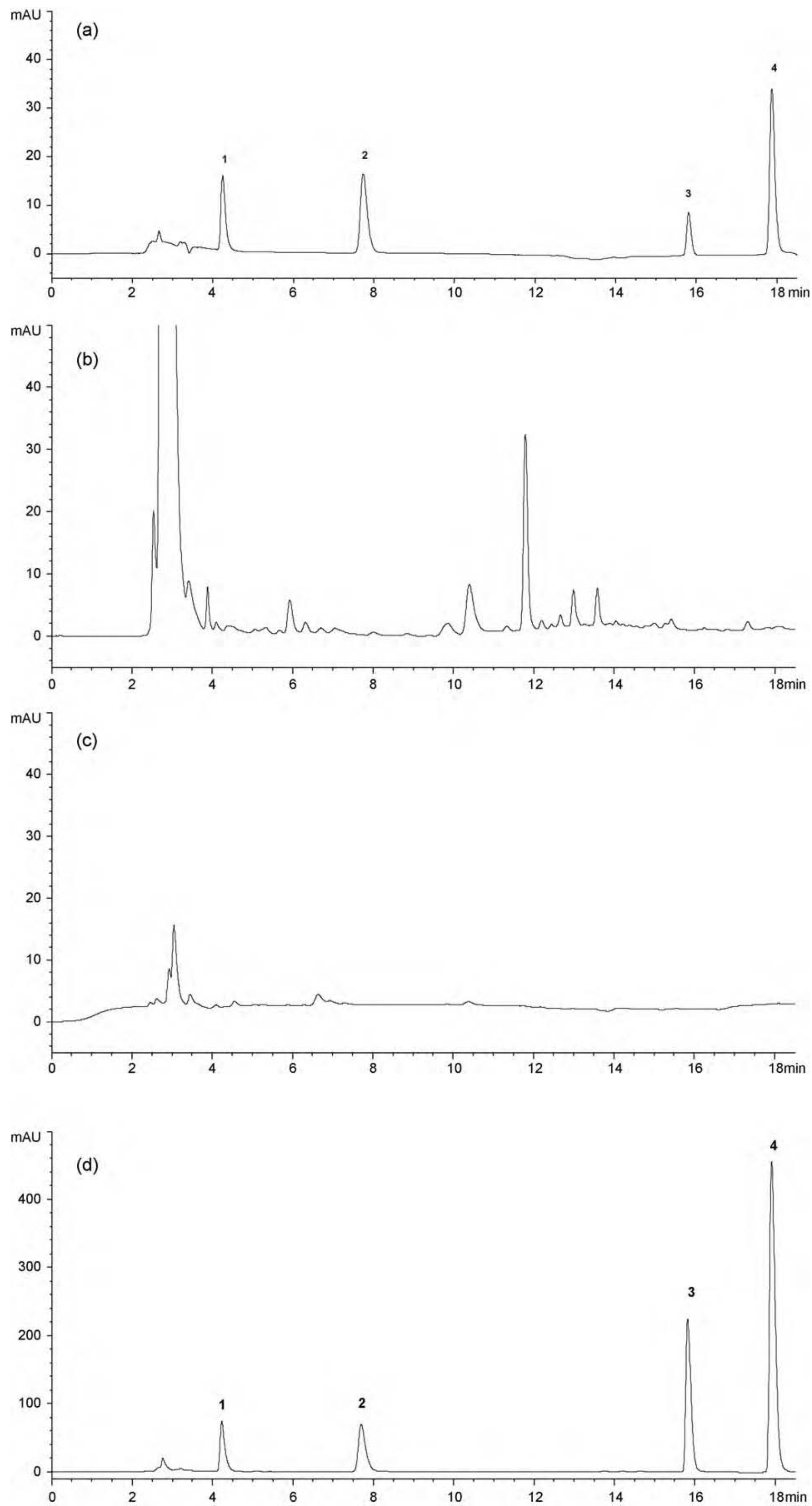

Fig. 5. Chromatograms of (a) 10 μL of the standard solution containing the four drugs, (b) 10 μL of the blank urine, (c) 1.0 μL of the CM-LPME pretreated blank urine sample, and (d) 1.0 μL of the CM-LPME pretreated urine sample spiked with the four drugs. In both (a) and (d), the concentration of each analyte is 5 mg L^{-1}. (1) Sotalol; (2) carteolol; (3) bisoprolol; (4) propranolol.

Table 3
Effect of the composition of the acceptor phase on enrichment factor.

Analyte	Enrichment factor			
	0.1 M HCl	0.1 M CH_3COOH	0.1 M H_3PO_4	0.1 M HCOOH
Sotalol	148.2	45.6	43.6	52.4
Carteolol	28.9	19.8	5.3	12.7
Bisoprolol	162.1	145.6	143.1	103.6
Propranolol	103.7	152.4	251	138

Stirring rate: 500 rpm; extraction time: 20 min; pH in the donor solution: 11.

The effect of TOAB concentration in the organic phase on the enrichment factors was investigated in the range of 0.001–0.1 M. As shown in Fig. 4a, 0.005 M TOAB in toluene gave the maximum enrichment factors for all of the studied analytes. Higher concentration of TOAB increased the viscosity of the organic phase and hence blocked the transport of analytes, resulting in decreased enrichment factors.

Therefore, pH 11 and 0.005 M TOAB-toluene were chosen for the donor solution and the organic phase, respectively, for subsequent experiments.

3.2. Effects of other factors on the enrichment factor

In addition to the optimization of the above two factors, effects of other important parameters including the composition and the concentration of the acceptor phase, salt concentration, pH in the donor phase, the stirring rate, and the extraction time on the efficiency of CM-LPME were also investigated.

Based on the mechanics for the analytes to transport through the organic phase to the acceptor solution, the acceptor should be an acidic solution. Four common acids, CH_3COOH, HCl, HCOOH, and H_3PO_4 were investigated under the same conditions and the results were summarized in Table 3. According to Table 3, HCl solution was selected as the acceptor phase because it resulted in obviously higher enrichment factors for sotalol, carteolol, and bisoprolol compared to other acids. Then, the effect of HCl concentration was investigated in the range of 0.001–0.5 M. As indicated in Fig. 4b, the enrichment factors increased markedly with increasing HCl concentration in the range of 0.001–0.1 M and reached a plateau thereafter. Hence, 0.1 M HCl is enough to strip the analytes from the organic phase, and this was selected as the acceptor solution.

The effect of adding salt to the donor solution on the extraction usually has two aspects [41]. In one side, the solubility of analyte in the donor solution decreases due to the addition of a certain amount of salt in the donor solution, and this enhances the partition ratio of analyte in the organic phase. On the other hand, the physical properties of the Nernst diffusion film is changed by adding salt to the donor solution and this restricts the extraction of analytes. The effect of NaCl concentration (0–30%, w/v) on enrichment factor was shown in Fig. 4c. It is evident that the highest enrichment factor was obtained in the absence of NaCl. The enrichment factor decreased gradually with the increase of NaCl concentration, and the decrease in enrichment factor was marked especially for sotalol. Accordingly, no salt was added into the donor solution in later experiments.

To extract the basic analytes from the donor phase into the organic phase with a high efficiency, the analytes should be unprotonated. According to the pK_a values of the studied analytes, the effect of pH was investigated in the range of 9–13.7. Fig. 4d demonstrates that enrichment factors increased slowly with the increase of pH due to the increase in ratio of unprotonated forms of the analytes. The pH of the donor phase was adjusted to 13.7 in subsequent experiments.

Stirring can decrease the diffusion layer thickness and enhance the convective-diffusive mass transfer rate; the stirring rate is thus expected to be an important factor affecting the extraction efficiency. Fig. 4e illustrates the effect of the stirring rate in the range of 300–700 rpm. As can be seen, for all the analytes, the enrichment factors increased with increasing stirring rate as expected. However, when the stirring rate was increased to 750 rpm, the acceptor droplet became unstable and the organic phase became emulsified. Considering the stability of the droplet and organic phase, the stirring rate was kept constant at 700 rpm during CM-LPME.

CM-LPME is a preconcentration process and its efficiency is expected to increase with extraction time until reaching the extraction equilibrium. The effect of the extraction time is depicted in Fig. 4f. The enrichment factors for all the four analytes increased with extraction time in the range of 0–20 min. However, contrary to our expectations, the enrichment factors decreased for all analytes when the extraction time was longer than 20 min, particularly for bisoprolol and propranolol. For exploring the reason leading to the decrease of the enrichment factors, the stability of the analytes in 0.1 M HCl solution and the change of pH of the acceptor droplet after extraction were investigated. The stability investigation indicated that the analytes were stable in 0.1 M HCl solution in 3 h studied, whereas the pH of the acceptor droplet was increased from the initial value of 1 to near 3 with an extraction time of 25 min. This implies that some OH^- in the donor solution with a pH of 13.7 could be transported into the acceptor solution through the organic solvent with the mediation of the quaternary ammonium salt carrier. It was also possible that a part of analytes extracted in the acceptor solution could be re-migrated to the organic phase due to the increase in the deprotonation of the basic analytes in the acceptor solution when the pH rose. A blank extraction experiment (i.e., without any analyte) shows that with an extraction of 25 min, the pH in the acceptor droplet was kept changeless. This result indicates the transport of OH^- in the donor solution (pH 13.7) is company with the transport of analytes. The larger decreases in the enrichment factors of bisoprolol and propranolol with the extraction time of >20 min coincide with their higher hydrophobicity compared to sotalol and carteolol. The competition transport of high concentration of anions coexisting with analytes in the donor solution through the liquid membrane containing quaternary ammonium salt as the carrier was also observed in the previous reports [42–44].

According to the above results, an extremely high pH such as 13.7 was inappropriate for the donor solution in this work. Therefore, a pH of 12 was chosen for the donor phase in subsequent experiments. Under this condition, with an extraction time of 20, 25, and 30 min, the enrichment factors were 76.3, 114, and 152 for carteolol, 113, 147, and 182 for propranolol, 181, 216, and 236 for sotalol, and 191, 226, and 254 for bisoprolol, respectively. More prolonged extraction time resulted in the acceptor droplet becoming unstable. Finally, an extraction time of 30 min and a pH of 12 for the donor phase were selected.

Table 4
Analytical characteristics of the proposed CM-LPME-HPLC method.

Analyte	Linear range (mg L^{-1})	r	RSD (%) (n = 3) 8.0 mg L^{-1}	RSD (%) (n = 3) 1.0 mg L^{-1}	LOD (mg L^{-1})
Sotalol	0.05–10.0	0.9995	5.0	0.6	0.01
Carteolol	0.05–10.0	0.9996	1.4	2.1	0.01
Bisoprolol	0.05–8.0	0.9999	5.0	1.4	0.01
Propranolol	0.05–8.0	0.9998	5.9	2.1	0.005

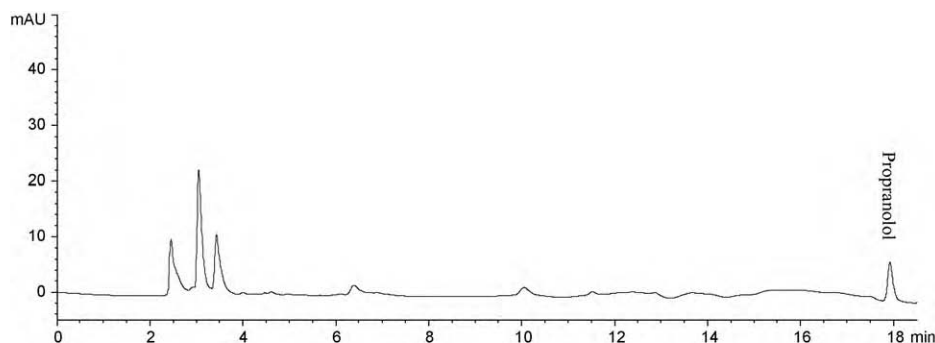

Fig. 6. Chromatogram of the real urine sample collected from a female patient at 4.0 h post-oral administration of 20 mg of propranolol.

3.3. Analytical characteristics and sample analyses

For investigating the effect of biological fluid, the HPLC method was applied to analyze a standard solution containing the four drugs, each at 5 mg L^{-1} (Fig. 5a), a blank urine sample (Fig. 5b), an CM-LPME pretreated blank urine sample (Fig. 5c), and an CM-LPME pretreated urine sample spiked with the four drugs, also each at 5 mg L^{-1} (Fig. 5d). As can be seen in Fig. 5a, the four drugs were separated completely under the given HPLC condition; however, in the absence of CM-LPME, their peak heights were relatively small compared to Fig. 5d. Fig. 5b demonstrates that some potential interferents existed in the blank urine sample. However, as illustrated in Fig. 5c and d, these interfering components were eliminated effectively through CM-LPME. The peak areas of carteolol, sotalol, propranolol, and bisoprolol obtained in Fig. 5d are 4.4, 5.1, 13.5, and 27.2 times those obtained in Fig. 5a, respectively, although the sample injection volume used in Fig. 5d was only 1/10 of that used in Fig. 5a. Hence, the concentrations of the analytes in the test solution were increased by 44- to 272-fold through the CM-LPME pretreatment. The result also shows that the enrichment factors of sotalol, carteolol, and propranolol obtained in spiked urine were obviously lower and the order of the enrichment factors for sotalol and propranolol was different when compared to those obtained in the standard solution under the same conditions (see Section 3.2). This was likely due to the matrix effects existing in the urine samples including the salt effect, which decreased the enrichment factor for sotalol most obviously (Fig. 4c).

For evaluating the practical applicability of the proposed CM-LPME technique, the linearity, precision, and limit of detection were investigated with the urine samples spiked with the four drugs in the range of 0.05–10.0 mg L^{-1} under the optimal extraction conditions. Calibration curves were obtained by plotting peak areas vs. concentrations of the analytes in the spiked urine samples. The linear range, correlation coefficient (r), relative standard deviation (RSD), and the limit of detection (LOD) at the signal-to-noise ratio of 3 of the studied four β-blockers were presented in Table 4. The linear ranges were from 0.05 to 10.0 mg L^{-1} for sotalol and carteolol, and from 0.05 to 8.0 mg L^{-1} for bisoprolol and propranolol. The RSDs at 8.0 and 1.0 mg L^{-1} were lower than 6% and 3%, respectively. The LODs were 0.01 mg L^{-1} for sotalol, carteolol, and bisoprolol, and 0.005 mg L^{-1} for propranolol, respectively.

Table 5
The relative recovery of the method.

Analyte	Relative recovery (%)	
	1.0 mg L^{-1}	5.0 mg L^{-1}
Sotalol	104.7	91.1
Carteolol	104.2	109.4
Bisoprolol	100.8	100.3
Propranolol	99.3	97.9

The above results are superior to those obtained with GC–MS [45] (LOD: 0.1 mg L^{-1} for sotalol, 0.01 mg L^{-1} for propranolol) and comparable to those obtained with HPLC-MS [2] (LOD: 0.007 mg L^{-1} for sotalol, 0.003 mg L^{-1} for bisoprolol, and 0.018 mg L^{-1} for carteolol and propranolol); however, the MS detector, although characterized by its inherent high sensitivity, is sophisticated and very expensive. While in the proposed method, a conventional DAD detector was used and the urine samples were subjected to CM-LPME without any other pretreatment.

To evaluate the accuracy of the present method, the relative recoveries of the four β-blockers were investigated in spiked urine samples at two analyte concentrations, 1.0 and 5.0 mg L^{-1}, and the results were shown in Table 5. It shows that the relative recoveries were in the ranges of 91.1% to 109.4%, indicating that the present method is a feasible method for analysis of sotalol, carteolol, bisoprolol, and propranolol in human urine.

The efficiency of the proposed method was further evaluated by determining the concentration of propranolol in a real urine specimen collected from a patient taking propranolol hydrochloride. Fig. 6 gives the CM-LPME chromatogram of the urine sample collected at 4.0 h post-administration. According to the linear regression equation of propranolol, the concentration of propranolol in the urine sample was determined to be 0.07 mg L^{-1}.

4. Conclusion

The present work demonstrates that CM-LPME is a powerful and useful sample pretreatment technique for HPLC as it can provide simultaneous extraction of hydrophilic and hydrophobic drugs with high enrichment factors and excellent sample cleanup. With aid of the carrier-mediated transport, the proposed CM-LPME method has expanded the application of LPME to extract hydrophilic substances from a complex biological matrix, which is very challenging for the conventional LPME. The results indicate that the proposed CM-LPME-HPLC method is a convenient and feasible technique for quantitative determination of β-blockers such as sotalol, carteolol, bisoprolol, and propranolol in human urine samples.

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