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Short communication

Enantioseparation of lomefloxacin hydrochloride by high-speed counter-current chromatography using sulfated-β-cyclodextrin as a chiral selector

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ABSTRACT

Enantiomers of lomefloxacin hydrochloride were separated by high-speed counter-current chromatography (HSCCC) using sulfated-β-cyclodextrin as a chiral selector (CS). The separation was performed with a two-phase solvent system composed of ethyl acetate–methanol–water (10:1:10, v/v) containing CS at 0–60 mmol/l in a head-to-tail elution mode, while obtained fractions were identified by polarimeter and spectropolarimeter. The results show that the concentration of the CS in the system strongly affects the peak resolution (Rs). As the concentration of CS increases, the Rs first increases reaching the maximum at 50 mmol/l and then decreases. When the CS concentration is kept constant in the solvent systems, the Rs decreases as the concentration of the lomefloxacin hydrochloride increases. The overall results of our studies indicated that sulfated-β-cyclodextrin is very useful for the chiral separation by HSCCC.

1. Introduction

The rapidly increasing number of new chiral drugs creates a high demand for improving enantioseparation technologies [1]. The growth of analytical methodologies for the separation of enantiomers has been impressive. This interest is due to the different pharmacokinetic characteristics and pharmacological activities of each enantiomer in a racemic drug. There has been a great demand for the development of analytical and preparative techniques for the chiral separation [2].

High-speed counter-current chromatography (HSCCC) is a liquid–liquid partitioning chromatography method in which the stationary phase is retained in the column by a centrifugal force field [3,4]. The method eliminates various complications such as sample loss due to irreversible adsorption onto the solid support. In addition, it has a large sample loading capacity because of a large volume of the liquid stationary phase involved in the separation process. In chiral separation one can add a chiral selector to either phase. Therefore, this method is very suitable for preparative separations of enantiomers [5,6]. Another advantage of HSCCC is the possibility to apply a variety of elution modes, which increase the potential of technique in operation mode and scale [7,8]. Some of these modes have been applied with the aim of improving enantioseparation. In addition, the HSCCC technique is very efficient in chiral separation because the method permits repetitive use of the same column for a variety of chiral separations [9]. Nevertheless, there are few examples of chiral separation in counter-current chromatography [10]. In the past, most of the enantioseparation in HSCCC were focused on amino acid derivatives using *N*-dodecanoyl-L-proline-3,5-dimethylanilide, vancomycin and cinchona alkaloid derivatives as chiral selectors [11–16]. Cyclodextrins (CDs) and their derivatives are the most widely employed chiral additives because of their high resolution capability towards racemic compounds belonging to different classes. In the aspect of separation time frame, resolving power and solubilities, derivatized CDs have been demonstrated to be better than the parent CDs. The modifications influence the overall hydrophobic characters of the CDs, resulting in changes in the shape and size of their cavities and their hydrogen bonding ability [17]. Sulfated β-cyclodextrin is usually employed, since it has higher solubility in aqueous solutions than native CDs [18]. Furthermore, compared with other β-cyclodextrin derivatives such as methyl-β-cyclodextrin, synthesis process of sulfated β-cyclodextrin was relatively simple. Therefore, in this paper sulfated β-cyclodextrin was employed for the first time as a chiral selector in chiral separation by counter-current chromatography, which can be used in the aqueous phase of the polar CCC solvent systems.

Lomefloxacin hydrochloride containing a chiral carbon in its molecular structure is an antibiotic quinolones drug used for a wide variety of bacterial infections. It has a wide range of antimicrobial spectrum with a high ability of inhibiting bacterial DNA synthesis and replication. When administered, it distributes widely in the body with strong penetrability, long half-time, little side effect and drug resistance [19]. Each enantiomer possesses

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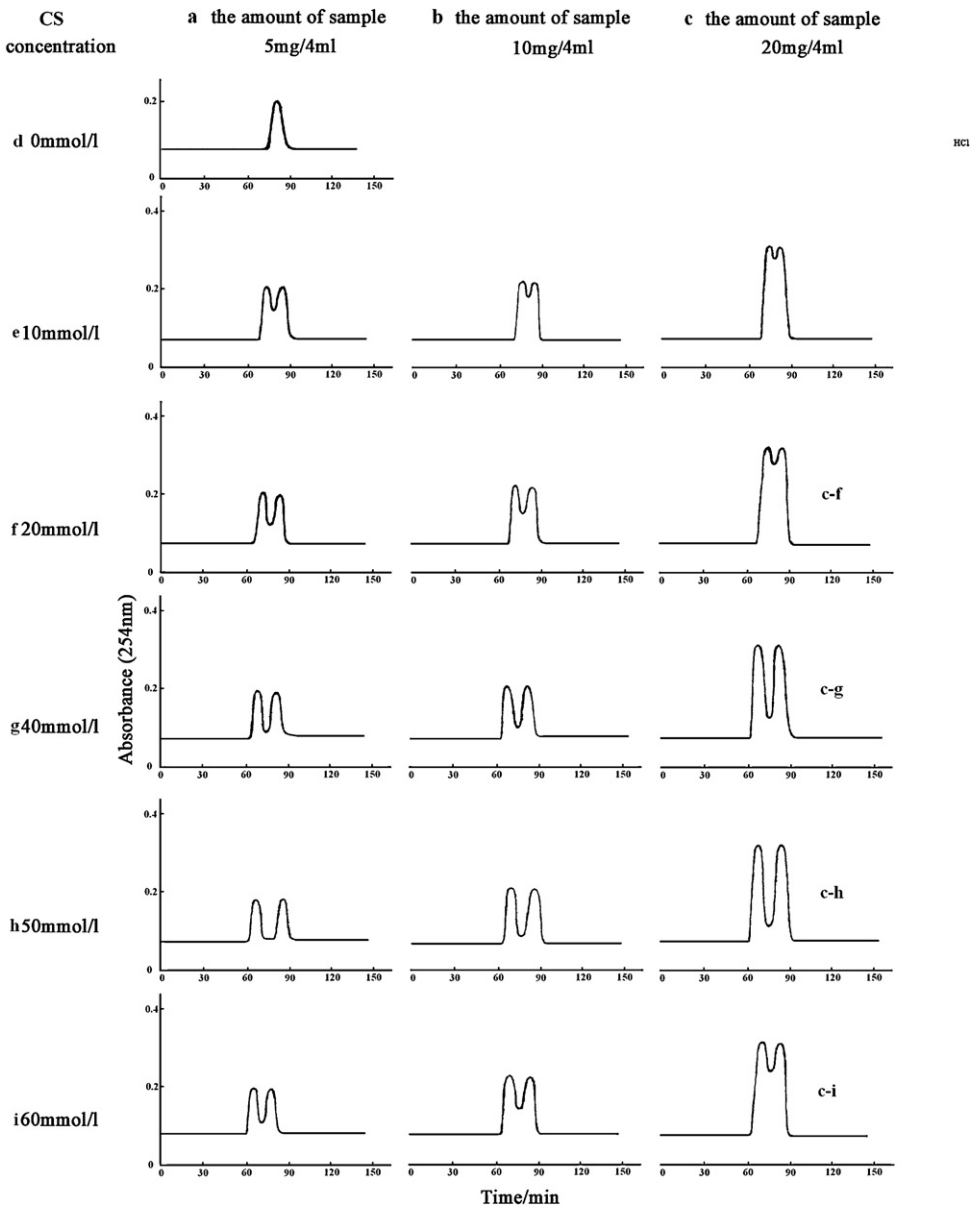

Fig. 1. Effects of the concentration of CS and the amount of sample on the separation of racemic lomefloxacin hydrochloride. Apparatus: HSCCC with multilayer coil consisting of 1.6 mm I.D. PTFE tubing with a total capacity of 150 ml; sample: racemic lomefloxacin hydrochloride 5–20 mg dissolved in 4 ml of solvent (2 ml of upper phase and 2 ml of lower phase); solvent system: ethyl acetate–methanol–water (10:1:10, v/v), different CS concentrations (0, 10, 20, 40, 50 and 60 mmol/l) in this solvent systems; stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow rate: 1 ml/min; revolution speed: 800 rpm. In all resolved chromatograms, the first peak represents (−)-enantiomers and the second peak, (+)-enantiomers.

different medicinal properties [20]. In this paper, enantiomers of lomefloxacin hydrochloride were separated by HSCCC using sulfated-β-cyclodextrin as a chiral selector consisting in a two-phase solvent system composed of ethyl acetate–methanol–water (10:1:10, v/v).

2. Experimental

2.1. Apparatus

Two HSCCC equipments have been used in this study. The first preparative HSCCC instrument is a Model GS10 multilayer coil planet centrifuge equipped with a polytetrafluoroethylene (PTFE) multilayer coil of 71 m × 1.6 mm I.D. with a total capacity of 150 ml. It was designed and constructed in Beijing Emilion Science & Technology Co., Ltd (Beijing, China). The second preparative HSCCC instrument with a Model GS10 multilayer coil planet centrifuge was constructed in the Beijing Institute of New Technology Application (Beijing, China). In this instrument the multilayer coil separation column was prepared by winding a 1.6 mm I.D. PTFE tube directly onto the holder hub to form multiple coiled layers with a total capacity of 250 ml, β values ranging from 0.5 at the internal terminal and 0.75 at the external terminal. The system was equipped with a metering pump (Model NS-1007, Beijing Institute of New Technology Application, China), a UV detector (Model 8823A-UV, Beijing Institute of New Technology Application, China), a recorder, and a sample injection valve.

The WZZ-2S automatic digital polarimeter was constructed at Pudong Shanghai Physical Optics Instrument Factory (Shanghai, China).

The model J-810 spectropolarimeter was constructed at JASCO Corporation (Japan).

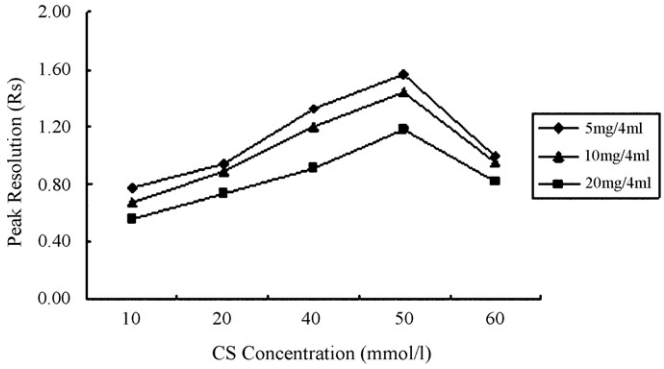

Fig. 2. Effects of the CS concentration on peak resolution.

The high-performance liquid chromatography (HPLC) equipment used was a Shimadzu LC-20A system including two LC-20A solvent delivery units, an SPD-M20A UV–VIS photodiode array detector (DAD), a Model 7725 injection valve with a 20 μl loop, an SCL-20A system controller, and a Class-VP-LC workstation (Shimadzu, Kyoto, Japan).

2.2. Reagents

The racemate of lomefloxacin hydrochloride was obtained from Hangzhou Jonquil Pharmaceutical Co., Ltd (Hangzhou, China). β-Cyclodextrin was purchased from Shanghai Chemical Reagent Company (Shanghai, China). The sulfated-β-cyclodextrin was synthesized in our laboratory. All analytical grade organic solvents and other chemical reagents were purchased from Beijing Chemical Factory (Beijing, China).

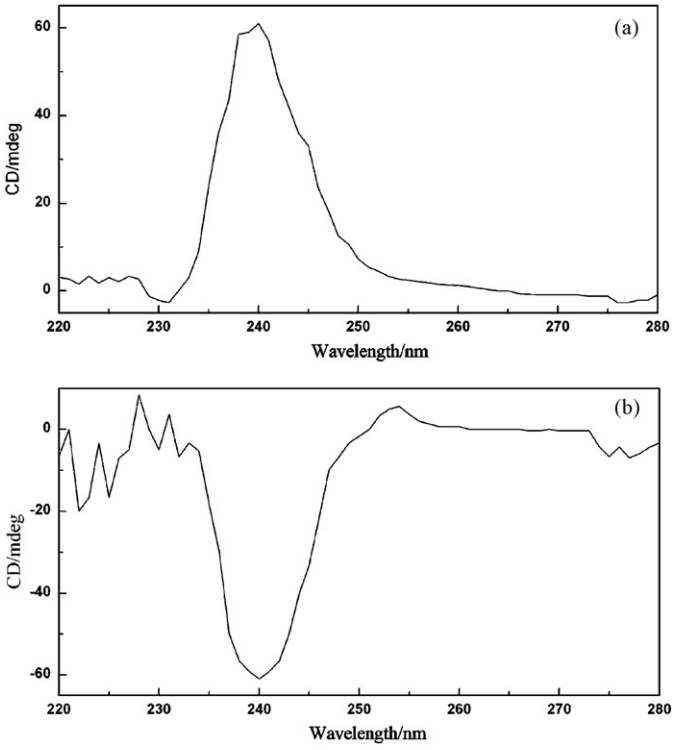

Fig. 4. Circular dichroism spectra of HSCCC fractions. (a) The second peak (+)-enantiomer; and (b) the first peak (−)-enantiomer.

2.3. Synthesis of sulfated-β-cyclodextrin

A 10 g amount of β-cyclodextrin and 30 ml of 80% (mass fraction) sulfuric acid were mixed in a 100 ml round-bottom flask. The

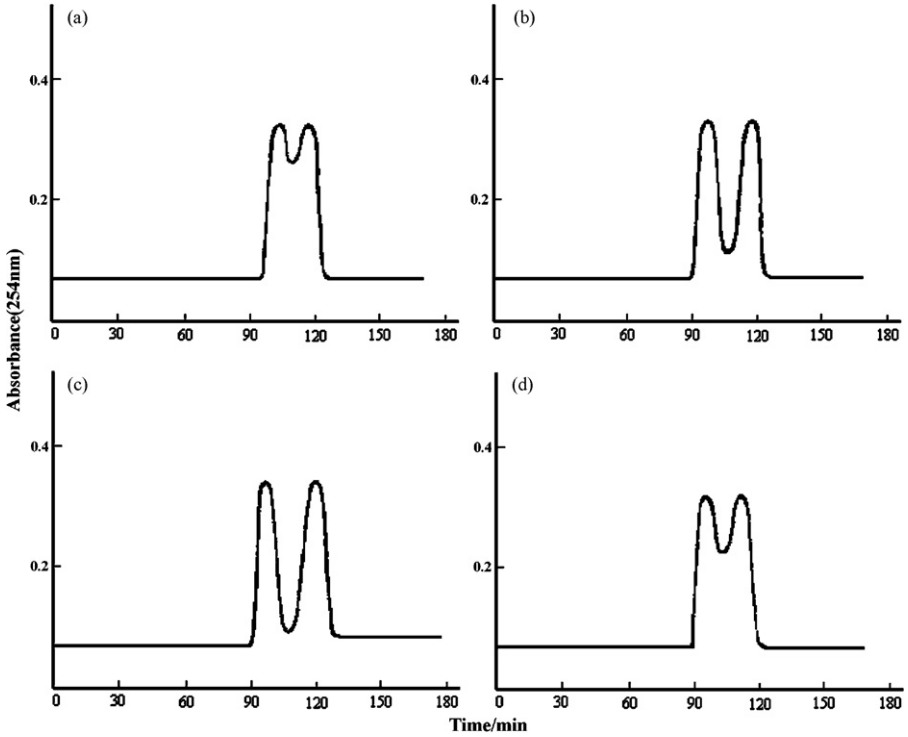

Fig. 3. Effects of total capacity of HSCCC on the separation of racemic lomefloxacin hydrochloride Apparatus: HSCCC with multilayer coil consisting of 1.6 mm I.D. PTFE tubing with a total capacity of 250 ml; sample: racemic lomefloxacin hydrochloride 20 mg dissolved in 4 ml of solvent (2 ml of each phase). Other experimental conditions are the same as those in Fig. 2. (a) CS concentration 20 mmol/l; (b) CS concentration 40 mmol/l; (c) CS concentration 50 mmol/l; and (d) CS concentration 60 mmol/l.

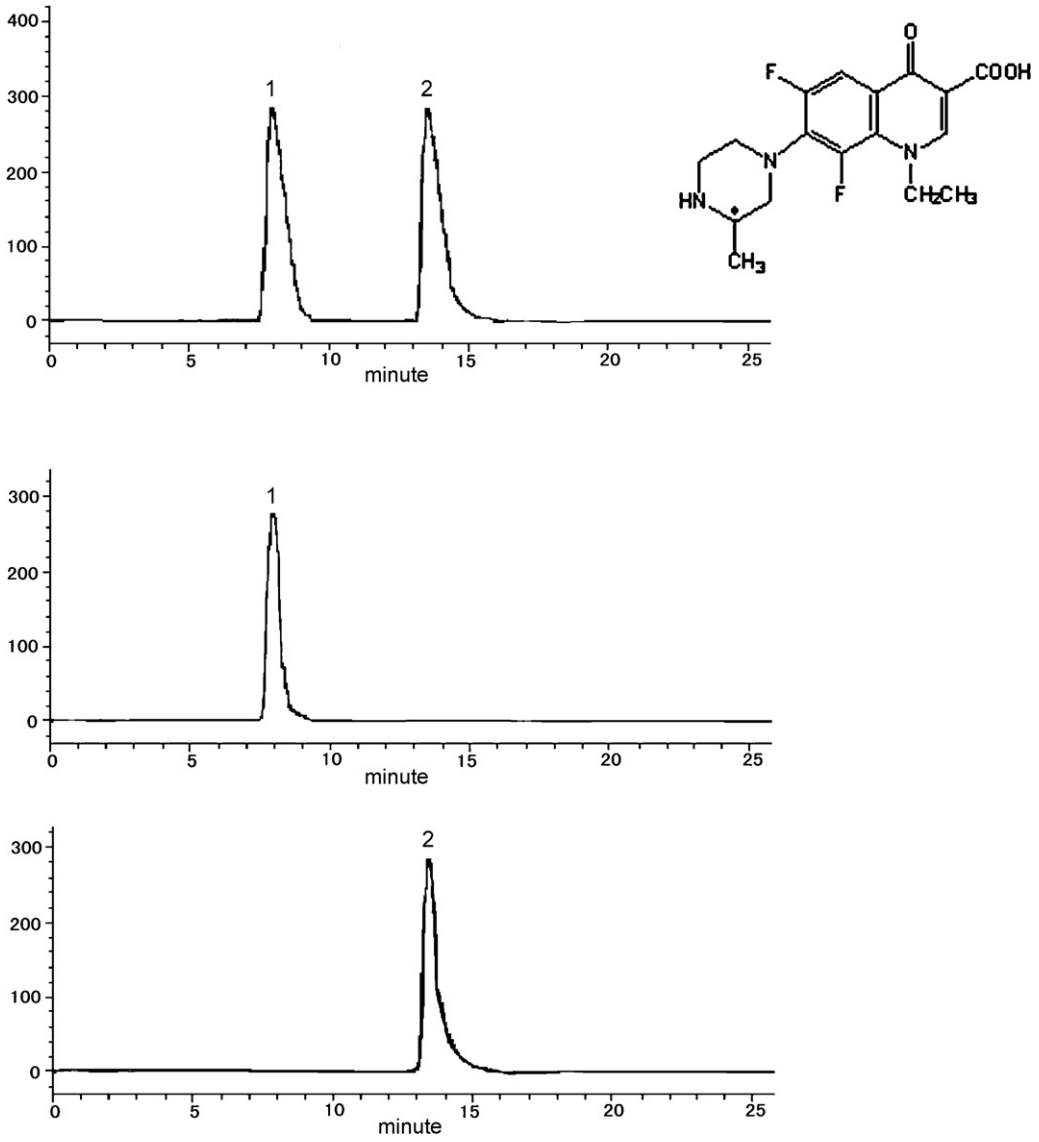

Fig. 5. HPLC analyses of racemic lomefloxacin and fractions from HSCCC. HPLC conditions: a chiral column made in our laboratory (150 mm × 4.6 mm I.D.). Mobile phase: hexane–isopropanol (90:10, v/v), flow rate: 0.5 ml/min, monitored at 254 nm by a DAD detector. Peak 1: (−)-enantiomer, Peak 2: (+)-enantiomer.

mixture was stirred for 1 h at 0–5 °C. The product was poured into a beaker with 500 ml water, followed by adding 55 g of calcium carbonate. A large amount of precipitates generated was eliminated by filtration. Then 100 ml of 95% (volume percentage) ethanol was added in the filtrate. The mixture was kept for 12 h at 0–5 °C. Then the precipitates were removed by filtration. The pH of filtrate was adjusted to 10.5 with sodium carbonate. The solution was again filtered. After adjusting the pH at 7.0 with acetic acid, the filtrate was concentrated and mixed with 500 ml ethanol, resulting in formation of a large amount of white precipitates. The precipitates were washed with ethanol, acetone, and diethyl ether in turn and dried under vacuum at 50 °C for 2 h [21] yielding about 7.2 g of sulfated-β-cyclodextrin.

2.4. Preparation of two-phase solvent systems and sample solutions

The sulfated-β-cyclodextrin was dissolved in the water at concentrations of 0, 10, 20, 40, 50, and 60 mmol/l. The two-phase solvent system composed of ethyl acetate–methanol–CS aqueous solution (10:1:10, v/v) was thoroughly equilibrated in a separatory funnel at room temperature and separated before use.

The sample solutions were prepared by dissolving 5, 10 and 20 mg of lomefloxacin hydrochloride in 4 ml of equal volumes of each phase.

2.5. HSCCC procedure

In each separation, the multiplayer coiled column was first entirely filled with the upper phase at a flow rate of 10 ml/min. Then, the lower phase was pumped into the head end of the column at a flow rate of 1.0 ml/min, while the apparatus was run at a revolution speed of 800 rpm. After hydrodynamic equilibrium was established, as indicated by a clear mobile phase eluting at the tail outlet, the sample solution was injected through the sample port. The effluent from the tail end of the column was continuously monitored with a UV detector at 254 nm. Each peak fraction was collected according to the chromatogram. The retention of the stationary phase was calculated from the volume of the stationary phase collected from the column after the separation was completed.

2.6. Effects of various parameters on peak resolution

The effect of the chiral selector (CS) concentration on the separation resolution was investigated using a two-phase solvent system composed of ethyl acetate–methanol–water (10:1:10, v/v). The lower phase of solvent system contained CS at various concentrations (0, 10, 20, 40, 50 and 60 mmol/l). A 5 mg amount of racemic lomefloxacin hydrochloride was injected in each run.

The effect of the sample size on the peak resolution was investigated by injecting 10 or 20 mg of racemic lomefloxacin hydrochloride in the two-phase solvent system composed of ethyl acetate–methanol–water (10:1:10, v/v).

The preparative capacity of the HSCCC column on the chiral separation was also examined by successively injecting 20 mg of racemic lomefloxacin hydrochloride at each CS concentration (20, 40, 50 and 60 mmol/l) into the separation column with a total capacity of 250 ml.

2.7. Analysis of racemic lomefloxacin and fractions by HPLC

The racemic lomefloxacin and HSCCC peak fractions were all analyzed by HPLC. HPLC conditions: a chiral column made in our laboratory (150 mm × 4.6 mm I.D.). Mobile phase: Hexane–isopropanol (90:10, v/v), flow rate: 0.5 ml/min, monitored at 254 nm by a DAD detector.

3. Results and discussion

Fig. 1 showed a set of chromatograms of racemic lomefloxacin hydrochloride obtained by different concentrations of CS in the two-phase solvent systems composed of ethyl acetate–methanol–water (10:1:10, v/v). These chromatograms were arranged from a to c according to the amount of sample ranging from 5 mg/4 ml to 20 mg/4 ml, and from b to i according to the amount of CS in solvent systems ranging from 0 to 60 mg/l. In the CS-free separation (0 mg/l), the racemic lomefloxacin hydrochloride only gave a single peak as expected. As the concentration of CS increased, the racemate was resolved into their isomers where (−)-enantiomer was eluted earlier than (+)-enantiomer. Increasing the concentration of CS in the solvent systems tends to decrease the retention time of the first peak. This may be due to the fact that CS is more strongly combined with (−)-enantiomers than (+)-enantiomers.

Fig. 2 indicated that the peak resolution was improved by increasing the concentration of CS in the solvent system up to 50 mmol/l. Further increase of CS concentration, however, decreased the peak resolution. Keeping the CS concentration constant in the solvent system, peak resolution decreased as the amount of the lomefloxacin hydrochloride increased.

Fig. 3 illustrates a set of preparative chromatograms of racemic lomefloxacin hydrochloride obtained by HSCCC with a total capacity of 250 ml. As expected, the peak resolutions were increased with a larger volume column. The flow rate of 1 ml/min was used in all experiments in the head-to-tail elution. The fractions of each enantiomer peak were collected and dried under vacuum at 42 °C. The residues were washed with water to remove the sulfated-β-cyclodextrin. The specific rotation of the first fraction is $[\alpha]_D^{25} = -74.1$ ($c = 0.334$, methanol), and the specific rotation of the second fraction is $[\alpha]_D^{25} = +73.7$ ($c = 0.334$, methanol).

Fig. 4 is circular dichroism spectra of HSCCC fractions from racemic lomefloxacin hydrochloride. Fig. 4a is (+)-enantiomer and Fig. 4b is (−)-enantiomer separated by HSCCC indicating that racemic lomefloxacin hydrochloride is well resolved.

In order to determine the purity, the racemic lomefloxacin and fractions from HSCCC were analyzed by HPLC method. Seen from Fig. 5, the Peak 1 is (−)-enantiomer, the Peak 2 is (+)-enantiomer. These results indicate that racemic lomefloxacin hydrochloride is well resolved by HSCCC. HPLC is an efficient technique to further demonstrate the quality of chiral separation by HSCCC.

4. Conclusions

The lomefloxacin hydrochloride racemate was resolved into its two enantiomers by HSCCC using sulfated-β-cyclodextrin as a chiral selector added to the two-phase solvent system composed of ethyl acetate:methanol:water (10:1:10, v/v). It is suggested the present method may be applied to enantioseparation of some other organic amine racemates by selecting the suitable two-phase solvent system.

The overall results of the present studies indicated that the peak resolution of the racemates is increased with higher CS concentration up to 50 mmol/l and further improved by the use of large volume column.

Acknowledgements

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