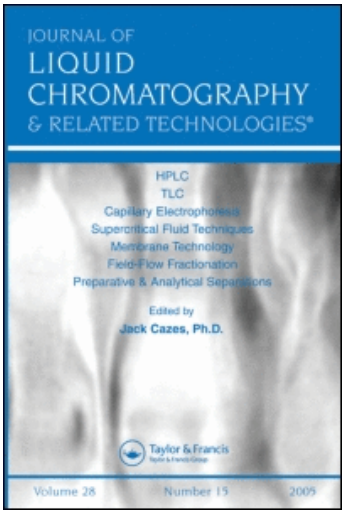

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PREPARATIVE ENANTIOSEPARATION OF OFLOXACIN BY HIGH SPEED COUNTERCURRENT CHROMATOGRAPHY USING L-(+)-TARTARIC ACID AS CHIRAL SELECTOR

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□ *High speed countercurrent chromatography was applied to the separation of racemic ofloxacin using L-(+)-tartaric acid as chiral selectors. The two-phase system composed of ethyl acetate/methanol/water = 10:1:9 was chosen. The upper phase contained 200 mmol/L of chiral selector. The maximum amount of sample in a one-time injection can be up to 20 mg. The enantiomers separated were identified by HPCE, which confirmed that this method was very useful for the chiral preparative separation.*

Keywords high speed countercurrent chromatography, L-tartaric acid, ofloxacin, preparative enantioseparation

INTRODUCTION

The rapidly increasing number of chiral drugs shows the essentiality in enantioseparation technologies. Countercurrent chromatography is a liquid-liquid partition chromatographic, which originates from the pioneering work of Yoichiro Ito et al. in the 1960s.[1] This method provides an advantage over the conventional column chromatography by eliminating the use of a solid support matrix to avoid the dangers of irreversible adsorption from the support.[2] Improvement of technique in subsequent years created high speed countercurrent chromatography (HSCCC), which was applied to enantioseparation.[3,4] HSCCC uses centrifugal force to fix a stationary phase in Ito's multilayered-coil column while the mobile phase is pumped through the column. An appropriate chiral selector is dissolved in the stationary phase, and a sample can be separated based on the

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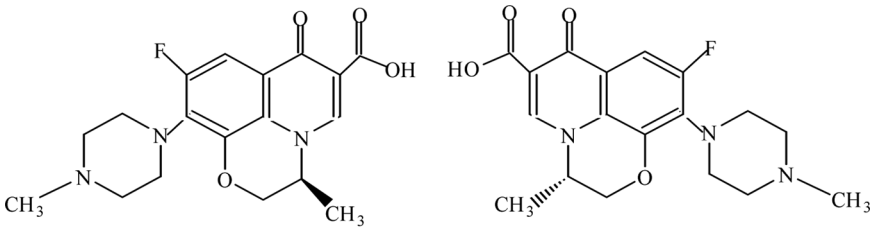

FIGURE 1 Molecular structure of ofloxacin.

difference of interaction force between the sample and the chiral selector in process. It is important that the chiral selector possesses a high selectivity and solubility in solvent system.

However, the examples of chiral separations in countercurrent chromatography are not numerous due to the difficulty of finding good chiral selectors.[5] Most chiral selectors use focus on the N-Dodecanoyl-L-proline-3,5-dimethylanilide,[6] Sulfated β-Cyclodextrin,[7] Bovine serum albumin,[8] Vancomycin,[9] and Cinchona Alkaloid Derivatives.[10] To our knowledge, the articles published on chiral separation that used HSCCC technology were less than 25 papers. Until now, there has been no report that L-(+)-tartaric acid can be used as a chiral selector for HSCCC except for a literature.[11]

Ofloxacin is an excellent third generation broad-spectrum fluoroquinolone antibacterial agent. Research has shown that the S-(-)-ofloxacin was 8 to 128 times more potent in inhibiting the multiplication of gram-positive and gram-negative bacteria than R-(+)-ofloxacin, and approximately two times more active than the racemate, (+/-)-ofloxacin.[12] There is a chiral carbon in its molecular structure, (Figure 1).

Although ofloxacin can be separated by classical crystallization of diastereomers, it is very difficult to simultaneously obtain two purified enantiomers. In this work, HSCCC was applied to the separation of racemic ofloxacin using L-(+)-tartaric acid as the chiral selectors; its enantiomers were identified and the pure enantiomers were obtained.

EXPERIMENTAL

Apparatus

The HSCCC experiment was performed with a multilayer coil planet centrifuge constructed at Beijing Institute of New Technology Application, China. The apparatus has a pair of column holders symmetrically placed on the rotary frame at a distance of 8 cm from the central axis of the centrifuge

($\beta = 0.5$–0.75). The multilayer coil was prepared by winding a 1.6 mm I.D. polytetrafluoroethylene (PTFE) tube directly onto the holder hub with a total capacity of 260 mL. 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), an injection valve, and a recorder.

A CL1020 CE system (Beijing Cailu Scientific Instrument Limited Company, China) equipped with a UV spectrophotometric detector (190–700 nm) and HW-2000 Chemstation software was used throughout. A 50 um i.d. and 375 um o.d. uncoated fused-silica capillary columns with extended light path (total and effective lengths of 50 cm and 41 cm, respectively) were used (Reafine chromatography Ltd., China). The capillary was stored overnight filled with water. Each day's operation was started by washing with 0.5 M sodium hydroxide solution followed by distilled water.

Reagents

R,S-oflxacin (98.6%) was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (China). Hydroxypropyl-β-cyclodextrin and L-(+)-tartaric acid were purchased from Sigma and Acros, respectively. L-tartaric acid n-decaester was synthesized by our group according to the literature.[13] All organic solvents and other chemical reagents are of analytical reagent grade (Beijing Chemical Factory, China). Silica gel G plates were obtained from Qingdao Ocean Chemical Factory (China).

Preparation of Two-Phase Solvent System and Sample Solution

Considering the good solubility of L-(+)-tartaric acid in the water, a two-phase solvent system composed of ethyl acetate:methanol: water (10:1:9, v/v/v) was selected. The solvent mixture was thoroughly equilibrated in a separation funnel at room temperature and the two-phase was separated shortly before use. A given amount of the L-(+)-tartaric acid (200 mmo/L) was added to the organic stationary phase.

The sample solutions were prepared by dissolving 20 mg of R,S-ofloxacin in 2.0 ml of the aforementioned phase mixture consisting of equal volumes of each phase.

HSCCC Procedure

The multilayer coiled column was first entirely filled with the upper phase at a flow-rate of 10.0 mL/min. The apparatus was rotated at

800 rpm while the mobile phase was pumped into the inlet of the column at a flow-rate of 2.0 mL/min in the head-to-tail elution mode. After the front of mobile phase appeared at the outlet of the column, a 4.0 ml of sample solution containing 20 mg R,S-ofloxacin was injected by an injected valve. The absorbance of the effluent was continuously monitored with a UV detector at 254 nm. The fractions of peaks were collected according to the chromatograms and the chirality of the fractions was assessed based on the HPCE.

HPCE Procedure

Phosphate buffers (70 mM) were prepared in distilled water and adjusted with sodium hydroxide to the required pH 2.16. HP-β-CD (40 mmol/L) was weighed directly into the background electrolyte (BGE). A stock solution of 4 mmol/L ofloxacin was dissolved by ultrasonic treatment in 10 mmol/L sodium hydroxide and stored at 4°C. The stock solutions were diluted with background electrolyte just before analysis. All solutions were filtrated with a 0.45 μm syringe-type Millipore membrane and sonicated prior to use. Between sample runs, the capillary was flushed with 0.2 mol/L NaOH, and then distilled water, followed by the carrier electrolyte, each for 3 min periods. Samples were press injected for 5 s and a voltage of 10 kV was applied for analysis. The UV spectrophotometric detector working at 254 nm was used for all measurements.[14]

RESULTS AND DISCUSSION

The selection of two-phase solvent system is very important for HSCCC, because selecting a solvent system means simultaneously choosing the column and eluent. We have conducted a literature search for suitable solvent systems previously used for ofloxacin and have determined the solvent systems can provide nearly equal volumes of the upper and lower phase solvent system with reasonable short settling times. On the basis of the aforementioned results, the two-phase system composed of ethyl acetate: methanol:water (10:3:7, 10:2:8, 10:1:9, v/v/v) was tested for enantioseparation of ofloxacin in the tail-to-head elution mode. As a result, the best solvent ratio to separate the ofloxacin was 10:1:9.

The influence of the quantity of chiral selector was studied by changing the amount of chiral selector in stationary phases. The concentration of L-(+)-tartaric acid was changed with 100, 150, 200, and 300 mmol/L. The results showed good enantioseparation was achieved when the concentration of L-(+)-tartaric acid was 200 mmol/L.

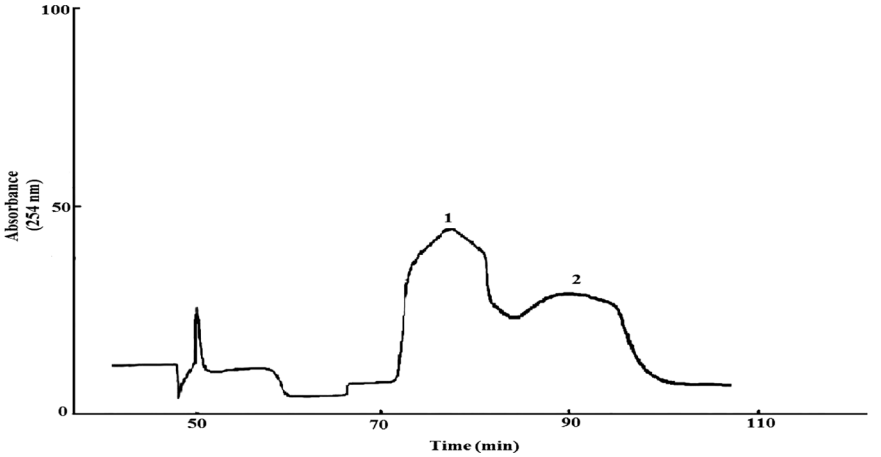

FIGURE 2 Enantioseparation chromatogram of R,S-oflocaxin obtained by HSCCC. Solvent system: ethyl acetate/methanol/water = 10:1:9 (v/v/v), in which the upper contains L-(+)-tartaric acid 200 mmol/L; mobile phase: lower; sample: 10 mg of R,S-ofloxacin dissolved in 4.0 mL solvent.

The maximum amount of sample in a one-time injection can be up to 20 mg. Figures 2 and 3 show HSCCC chromatograms of 10 mg and 20 mg of R,S-oflocaxin, respectively.

The fractions of each enantiomer peak were collected, and then they were evaporated in vacuum at 50°C. Purification of enantiomers was carried

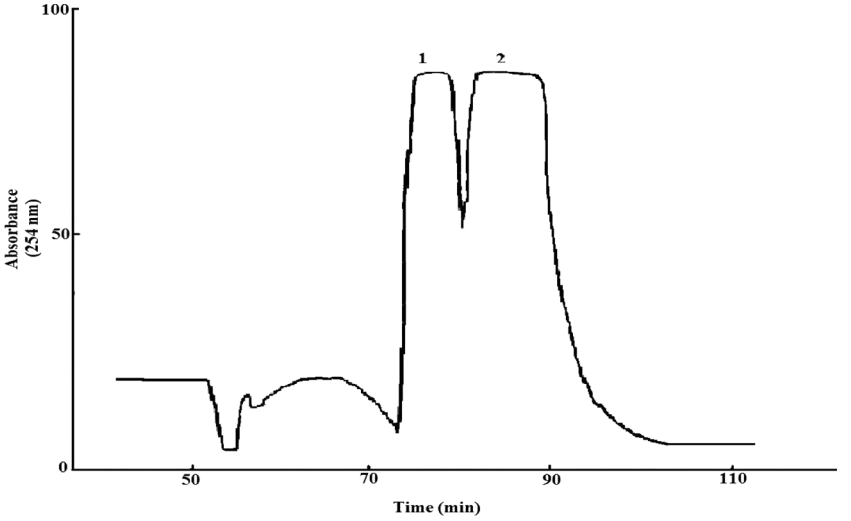

FIGURE 3 Enantioseparation chromatogram of R,S-oflocaxin obtained by HSCCC. Solvent system: ethyl acetate/methanol/water = 10:1:9 (v/v/v), in which the upper contains L-(+)-tartaric acid 200 mmol/L; mobile phase: lower; sample: 20 mg of R,S-ofloxacin dissolved in 4.0 mL solvent.

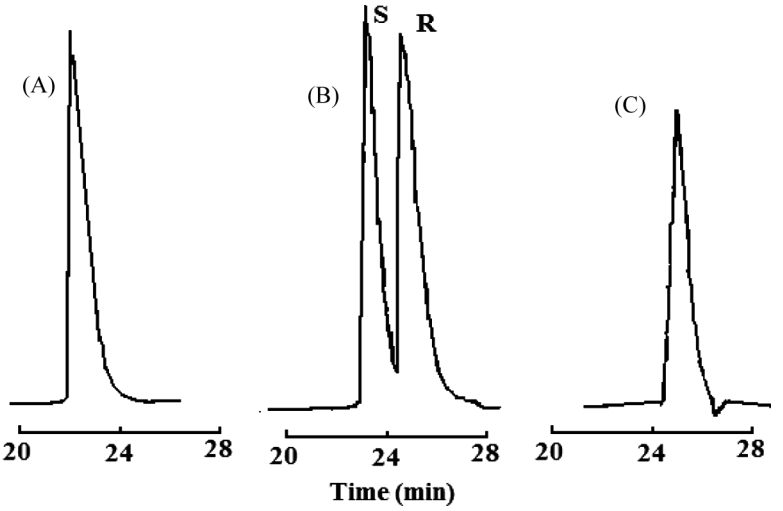

FIGURE 4 HPCE analysis of HSCCC fractions from ofloxacin in Figure 3. (A) peak 1; (B) racemate of ofloxacin; (C) peak 2. Experimental conditions: 70 mmol/L phosphate buffer (pH 2.16), containing 40 mmol/L HP-β-CD; applied voltage 10 kV, detection at 254 nm; temperature 25°C.

out by flash chromatography on silica gel (chloroform:methanol, 4:2, v/v) to remove L-(+)-tartaric acid. Before measuring with HPCE, each peak gave a single monochromatic spot on a silica gel G plate which was developed with chloroform:ethanol:toluene:dithylamine: water (10:10:5:2.5:1.5, v/v/v/v/v). The visual detections were done by iodic vapor. The three spots on TCL, which were peak 1, peak 2, and (R,S)-oflxacin, had the same R_f(0.53) value.

Figure 4 shows the enantioseparation chromatogram of oflxacin by HPCE using hydroxypropyl-β-cyclodextrin as the chiral selector. Figure 4B shows commercial R,S-ofloxacin, the first outflow is S-isomer and the latter out is the R-isomer; Figure 4A and 4C are its enantiomers separated by HSCCC in Figure 3. Obviously, complete resolutions were attained.

We tried to use L-(+)-tartaric acid n-decaester as a chiral selector to separate the ofloxacin with same two-phase solvent system in HSCCC, however, only part resolution was obtained for a 5 mg sample of ofloxacin.

CONCLUSION

From the aforementioned comprehensive studies, we know that the R,S-oflocaxin can be separated into its two enantiomers by HSCCC using L-(+)-tartaric acid as chiral selectors. The present method may be applied to enantioseparation of some other fluoroquinolones if a new two-phase solvent system can be carefully selected.

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REFERENCES

1. Ito, Y.; Weinstein, M.; Aoki, I.; Kimura, E.; Nunogaki, K. The Coil Planet Centrifuge. *Nature* 1966, 212, 985–987.
2. Ito, Y. Golden Rules and Pitfalls in Selecting Optimum Conditions for High-Speed Counter-Courrent Chromatography. *J. Chromatogr. A* 2005, 1065, 145–168.
3. Domon, B.; Hostettmann, K.; Kovacevic, K.; Prelog, V. Separation of the Enantiomers of (±)-norephedrine by Rotation Locular Countercurrent Chromatography. *J. Chromatogr.* 1982, 250, 149–151.
4. Ma, Y.; Ito, Y. Chiral Separation by High-Speed Countercurrent Chromatography. *J. Anal. Chem.* 1995, 67 (17), 3069–3074.
5. Foucault, A. P. Enantioseparations in Counter-Current Chromatography and Centrifugal Partition Chromatography. *J. Chromatogr. A* 2001, 906, 365–378.
6. Delgado, B.; Pérez, E.; Santano, M. C.; Minguillón, C. Enantiomer Separation by Counter-Current Chromatography: Optimization and Drawbacks in the Use of L-proline Derivatives as Chiral Selectors. *J. Chromatogr. A* 2005, 1092, 36–42.
7. Yuan, L. M.; Liu, J. C.; Yan, Z. H.; Ai, P.; Meng, X.; Xu, Z. G. Enantioseparation of Chlorpheniramine by High Speed Countercurrent Chromatography Using Carboxymethyl-B-Cyclodextrin As Chiral Selector. *J. Liq. Chromatogr. Related Technol.* 2005, 28 (19), 3057–3063.
8. Shinomiya, K.; Kabasawa, Y.; Ito, Y. Enantiomeric Separation of Commercial D,L-kynurenine with an Aqueous Two Phase Solvent System by Cross-Axis Coil Planet Centrifuge. *J. Liq. Chromatogr. Related Technol.* 1998, 21 (1–2), 135–141.
9. Duret, P.; Foucault, A.; Margraff, R. Vancomycin as a Chiral Selector in Centrifugal Partition Chromatography. *J. Liq. Chromatogr. Related Technol.* 2000, 23 (2), 295–312.
10. Gavioli, E.; Maier, N. M.; Minguillón, C.; Lindner, W. Preparative Enantiomer Separation of Dichlorprop with a Chinchona-Derived Chiral Selector Employing Centrifugal Partition Chromatography and High-Performance Liquid Chromatography: A Comparative Study. *J. Anal. Chem.* 2004, 76 (19), 5837–5848.
11. Cai, Y.; Yan, Z. H.; Zi, M.; Yuan, L. M. Preparative Enantioseparation of DL-α-methylbenzylamine by High-Speed Countercurrent Chromatography using L-(+)-tartaric Acid as Chiral Selector. *J. Liq. Chromatogr. Related Technol.* 2007, 30 (9), 1489–1495.
12. Hayakawa, I.; Atarashi, S.; Yokohama, S.; Imamura, M.; Sakano, K.; Furukawa, M. Synthesis and Antibacterial Activities of Optically Active Ofloxacin. *Antimicrob. Agents Chemother.* 1986, 29 (1), 163–164.
13. Heldin, E.; Lindner, K. J.; Pettersson, C.; Lindner, W.; Rao, R. Tartaric Acid Derivatives as Chiral Selectors in Liquid Chromatography. *J. Chromatogr.* 1991, 32 (9–10), 407–416.
14. Zhou, S. S.; Ouyang, J.; Baeyens, W. R. G.; Zhao, H. C.; Yang, Y. P. Chiral Separation of Four Fluoroquinolone Compounds Using Capillary Electrophoresis with Hydroxypropyl-β-cyclodextrin as Chiral Selector. *J. Chromatogr. A* 2006, 1130, 296–301.