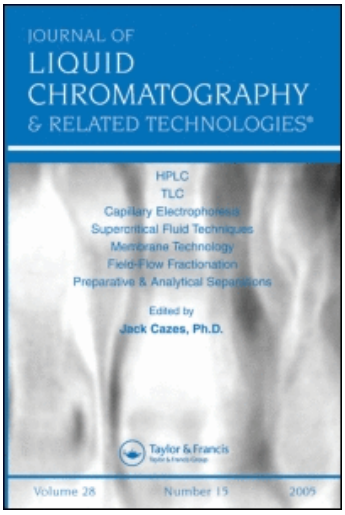

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Enantioseparation of Chlorpheniramine by High Speed Countercurrent Chromatography Using Carboxymethyl-β-cyclodextrin as Chiral Selector

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Abstract: High speed countercurrent chromatography was used for enantioseparation of chlorpheniramine. The separation was performed with a two-phase system composed of ethyl acetate : methanol : water (10 : 1 : 9) within 2 hours in a tail-to-head elution mode. The lower phase contained 20 mmol/L of carboxymethly-β-cyclodextrin as a chiral selector. The enantiomers separated were identified by chiral HPLC, which confirmed that this method was very useful for the chiral preparative separation.

Keywords: High speed countercurrent chromatography, Enantioseparation, Chlorpheniramine, Carboxymethyl-β-cyclodextrin

INTRODUCTION

The growth of analytical methodologies for the separation of enantiomers has been impressive. Attention is now turning to the preparative separation of enantiomers, particularly in the pharmaceutical industry. 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 preparative techniques for the separation of chiral molecules.[1]

High speed countercurrent chromatography (HSCCC) is a liquid-liquid partitioning chromatography method in which the stationary phase is

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immobilized by centrifugal force. It has a higher sample capacity because of the large volume of stationary phase involved in the separation process. With the advantage of eliminating the use of solid support matrix, the selectivity of enantioseparation can be adjusted by a chiral selector added to either phase. This method is very suitable for preparative separations of enantiomers.[2,3]

There are a few examples of chiral separation in countercurrent chromatography.[4] Nowadays, most of the chiral selectors focus on the N-dodecanoyl-L-proline-3,5-dimethylanilide,[5–7] sulfated β-cyclodextrin,[4] vancomycin,[8] bovine serum albumin,[9,10] and cinchona alkaloid derivatives,[11] and the enantiomers separated were amino acid derivatives.[3,4] To our knowledge, the literature of chiral separation published on high speed countercurrent chromatography was less than 10 papers.

Carboxymethyl-β-cyclodextrin, an ordinary chiral selector in CE and HPLC, can be easily synthesized. They form inclusion complexes with various organic compounds in aqueous solution. This is a first time report proving that carboxymethyl-β-cyclodextrin can be used as a chiral selector for the HSCCC.

Chlorpheniramine, an organic amine, is a basic anaphylactic medicine which is a classical H_1-receptor antagonist. There is a chiral carbon in its molecular structure (Figure 1). Each enantiomer possesses different medicinal properties.[12]

EXPERIMENTAL

Apparatus

The present investigations were performed with a multilayer coil planet centrifuge constructed at the Beijing Institute of New Technology Application, Beijing, China. The apparatus holds a pair of column holders, symmetrically, 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 separation column was prepared by winding a 1.6 mm i.d. polytetrafluoroethylene (PTFE) tube directly onto the

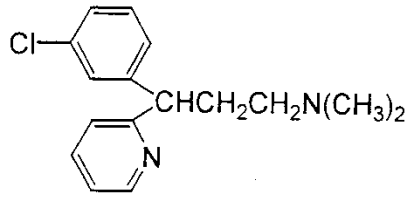

Figure 1. Structure of chlorpheniramine.

holder hub to form multiple coiled layers 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), a recorder, and a sample injection valve.

The HPLC analyses were performed on the cellulose tris(3,5-dimethylphenyl carbamate) chiral column (5 μm, 4.6 mm i.d. × 250 mm) or the ODS column (Hypersil C_{18}, 5 μm, 4.6 mm i.d. × 250 mm) using a Shimadzu LP-6A liquid delivery pump, a Shimadzu SPD-10AVP UV–Vis detector, and a CR-5A integrator (Shimadzu, Kyoto, Japan). Detection was examined at 254 nm.

Reagents

The racemate of chlorpheniramine was obtained from Sigma. The carboxymethyl-β-cyclodextrin was synthesized by our group. All organic solvents and other chemical reagents are analytical-reagent grade (Beijing Chemical Factory, China). Silica gel plates were from Qingdao Ocean Chemical Factory (China).

Preparation of Two-Phase Solvent System and Sample Solution

Each two-phase solvent mixture, composed of ethyl acetate : methanol : water (10 : 1 : 9, v/v/v), was thoroughly equilibrated in a separating funnel at room temperature and the two phases were separated before use. The carboxymethyl-β-cyclodextrin (20 mmol/L) was dissolved in the lower water phase and used as the stationary phase.

The sample solutions were prepared by dissolving 3 mg chlorpheniramine in 4 mL of above phase mixture consisting of equal volumes of each phase.

HSCCC Procedure

In each separation, the multiplayer, coiled column was entirely filled with the lower phase at a flow rate of 10 mL · min^{-1}. Then, the apparatus was rotated at 800 rpm while the upper phase was pumped into the inlet of the column at a flow of 2.0 mL · min^{-1} in the tail-to-head elution mode. After the front of mobile phase appeared at the outlet of the column, this was followed by an injection of a 4.0 mL sample solution containing 3 mg racemate of chlorpheniramine. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm. Fractions of peaks were collected according to the chromatograms.

RESULTS AND DISCUSSION

The solubility of carboxymethyl-β-cyclodextrin in water is good. The selection of a solvent system is the most important step in performing HSCCC. We have conducted a literature search for the suitable solvent systems previously used for the organic amine and considered the solubility of carboxymethyl-β-cyclodextrin in the solvent mixture. The two-phase solvent system composed of ethyl acetate : methanol : water (10 : 3 : 7, 10 : 2 : 8, and 10 : 1 : 9, v/v/v) were tested in the tail-to-head elution mode, but no enantioseparation was observed with 10 : 3 : 7 volume ratio. The best chiral resolution was obtained by using the solvent system 10 : 1 : 9.

Generally speaking, the most suitable range of partition coefficient value of solutes for HSCCC is 0.5–2 for C_U/C_L, where C_U indicates solute concentration in the upper phase and that of C_L in the lower phase. The partition coefficients of the chlorpheniramine were determined by a simple test tube procedure: 3 mg of the chlorpheniramine was thoroughly equilibrated with the two-phase solvent system, 2 mL of each phase, in a test tube. After the clear two layers were formed, each phase was separately analyzed on an ODS column (Hypersil C_{18}, 5 μm, 4.6 mm i.d. $\times$ 250 mm) using CH_3CN-H_2O (4 : 6, v/v) as an eluent, at a flow rate of 0.5 mL $\cdot$ min^{-1}. Detection was examined at 254 nm. From a pair of chromatograms obtained from each phase, the partition coefficient value was obtained by computing the ratio in the area between the corresponding peaks. When the concentration of carboxymethyl-β-cyclodextrin in the solvent system 10 : 1 : 9 was 0, 10 mmol/L, 20 mmol/L, and 30 mmol/L, the partition coefficients of the chlorpheniramine were 0.65, 0.55, 0.57, and 0.58, respectively. Therefore, the chiral selector introduced in the solvent system modified the partition behavior, but it only had a few different concentrations of chiral selectors ranging from 10 to 30 mmol/L.

The effects of the quantity of the chiral selectors were investigated by introducing various amounts of chiral selectors in the stationary phases. Figure 2 showed three enantioseparation chromatograms of chlorpheniramine (3 mg) with the two-phase solvent system composed of ethyl acetate : methanol : water (10 : 1 : 9, v/v/v) when the concentrations of carboxymethyl-β-cyclodextrin in the stationary phases were 10 mmol/L (Figure 2A), 20 mmol/L (Figure 2B), and 30 mmol/L (Figure 2C). Their retentions of stationary phase were close to 50%. The results showed that the resolutions of Figure 2A and Figure 2C were incomplete, good enantioseparation was achieved when the concentration of carboxymethyl-β-cyclodextrin was 20 mmol/L (Figure 2B). In order to keep the reversed pressure gradient constant in the tail-to-head elution, the flow rate of 2 mL $\cdot$ min^{-1} was used in all experiments. The chiral recognition mechanism may be due to carboxymethyl-β-cyclodextrin having chiral centers in its cavity.

The fractions of each enantiomer peak were collected and the solvents of the fraction were evaporated under vacuum at 50°C. The residue was

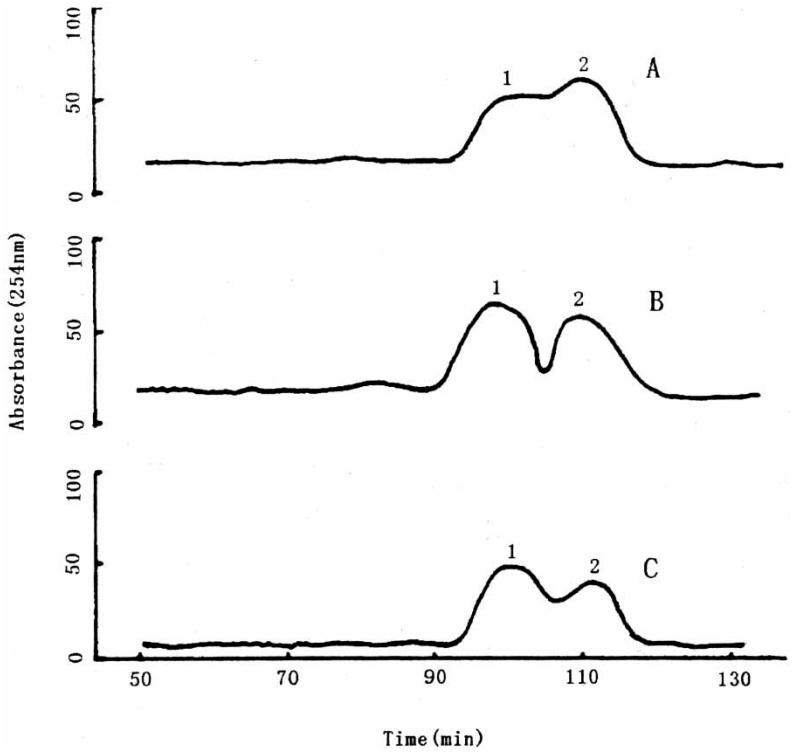

Figure 2. Enantioseparation chromatograms of chlorpheniramine obtained by HSCCC. Solvent system: ethyl acetate : methanol : water = 10 : 1 : 9, in which the lower phase contains carboxymethyl-β-cyclodextrin 10 mmol/L (A), 20 mmol/L (B), and 30 mmol/L (C); mobile phase: upper phase; sample: 3 mg of chlorpheniramine dissolved in 4 mL solvent.

washed with water to remove the carboxymethyl-β-cyclodextrin. The pure enantiomers were obtained. Before measurement with chiral HPLC, two enantiomers were spotted on silica gel plates and developed with chloroform : methanol (9 : 1, v/v). The visual detections were done by iodic vapor. Experimental results show that two spots on the TLC have same R_f (0.46) value.

Figure 3 showed the chiral HPLC chromatograms of HSCCC fractions from chlorpheiniramine in Figure 2B. The analysis was carried out on a chiral column of cellulose tris(3,5-dimethylphenyl carbamate) using hexane-2-propanol (90 : 10) as the eluent, at a flow rate of 0.5 mL $\cdot$ min^{-1}. Figure 3B was commercial chlorpheniramine, Figure 3A and Figure 3C were its enantiomers separated by HSCCC. Obviously, the complete resolutions were attained by HSCCC for the racemate of chlorpheniramine.

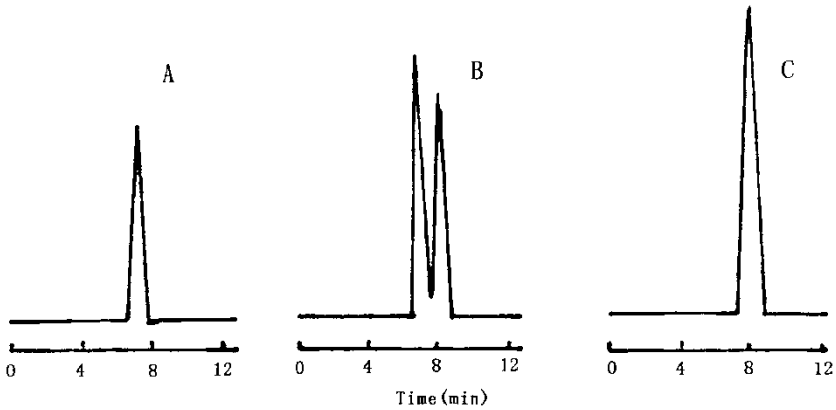

Figure 3. Chiral HPLC analysis of HSCCC fractions from chlorpheniramine in Figure 2B. (A) Peak 1; (B) racemate of chlorpheniramine; (C) peak 2. Experimental conditions: cellulose tris(3,5-dimethylphenyl carbamate) chiral column (5 μm, 4.6 mm i.d. × 250 mm); mobile phase: hexane-2-propanol (90 : 10, v/v); flow rate: 0.5 mL · min^{-1}; detection: 254 nm.

CONCLUSIONS

From the above comprehensive studies, we know that the chlorpheniramine can be separated into its two enantiomers by HSCCC using carboxymethyl-β-cyclodextrin as the chiral selector. The present method may be applied to enantioseparation of some other organic amine racemates if a new two-phase solvent system can be selected carefully.

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