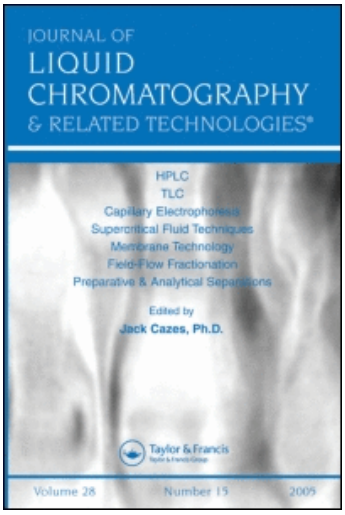

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Y. Cai[a]; Z. H. Yan[a]; M. Zi[a]; L. M. Yuan[a]
[a] Department of Chemistry, Yunnan Normal University, Kunming, P. R. China

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Preparative Enantioseparation of DL-α-Methylbenzylamine by High-Speed Countercurrent Chromatography using L-(+)-Tartaric Acid as Chiral Selector

Y. Cai, Z. H. Yan, M. Zi, and L. M. Yuan
Department of Chemistry, Yunnan Normal University,
Kunming, P. R. China

Abstract: Enantiomers of DL-alpha-methylbenzylamine were separated by high-speed countercurrent chromatography using L-(+)-tartaric acid as a chiral selector. The two phases system composed of chloroform:methanol:water = 4:3:1 was chosen, which contained 278 mmol/L of chiral selector in the upper phase. The maximum of sample in a one time injection can be up to 120 mg. The enantiomers separated were identified by TLC and chiral HPLC, which confirmed that the method was very feasible for chiral preparative separation. This is the first report that L-(+)-tartaric acid was used as a chiral selector in the HSCCC.

Keywords: High-speed countercurrent chromatography, Preparative enantioseparation, DL-Alpha-methylbenzylamine, L-(+)-Tartaric acid

INTRODUCTION

The separation of enantiomers is an important analytical operation in many fields of academic, industrial, and pharmaceutical research.[1] With the increasing demand of enantiomerical pure compounds, efficient strategies for analytical and preparative separation of enantiomers are required.

The high-speed countercurrent chromatography (HSCCC) is a liquid-liquid portioning chromatography method, and its stationary phase is immobilized by centrifugal force.[2] The enantioseparation by HSCCC has two advantages: (A) it has no solid support matrix and can eliminate irreversible

Address correspondence to L. M. Yuan, Department of Chemistry, Yunnan Normal University, Kunming 650092, P. R. China. E-mail: yuan_limingpd@yahoo.com.cn

adsorption, which often happens in gas chromatography and liquid chromatography, so high-speed countercurrent chromatography is suitable to separate large polar chiral compounds and biological active molecules; (B) it has a higher sample capacity in preparative enantioseparation because of its particular mechanism of separation.[3] However, the examples of chiral separations in countercurrent chromatography are not numerous due to the difficulty of finding good chiral selectors, which have a high selectivity and solubility in the solvent system. The literature of chiral separation published using high-speed countercurrent chromatography are fewer than 15 papers.

Chiral selectors play an important role for enantioseparations in countercurrent chromatography.[2] Now, most chiral selectors use focus on the sulfated β-cyclodextrin,[4] N-dodecanoyl-L-proline-3,5-dimethylanilide,[5–7] vancomycin,[8] bovine serum albumin,[9,10] and cinchona alkaloid derivatives.[11] In our lab, carboxymethly-β-cyclodextrin has been used as a chiral selector.[12]

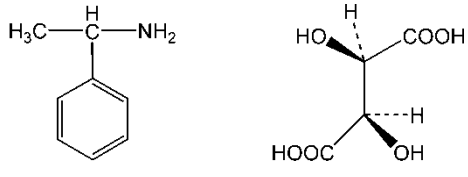
L-(+)-Tartaric acid, an ordinary chiral selector for chemical resolution, is inexpensive and can be easily bought from the reagent company. Until now there has been no report that L-(+)-tartaric acid can be used as a chiral selector for the HSCCC.

DL-alpha-methylbenzylamine is an important substance. Also, DL-alpha-methylbenzylamine is often used as a raw material, active intermediate, reagent, etc., in the chemical and other fields. Although it can be separated by classical crystallization of diastereomers, it's very difficult to simultaneously obtain its two purified enantiomers. In this work, the preparative separation of the racemate of DL-alpha-methylbenzylamine was performed, using L-(+)-Tartaric acid as the chiral selector, by HSCCC, its enantiomers were identified and the pure enantiomers were obtained. Figure 1 shows the molecular structures of alpha-methylbenzylamine and L-(+)-tartaric acid.

EXPERIMENTAL

Apparatus

The HSCCC experiments were performed using a multilayer coil planet centrifuge constructed at the Beijing Institute of New Technology Application,



alpha-methylbenzylamine L-(+)-tartaric acid

Figure 1. Molecular structures of alpha-methylbenzylamine and L-(+)-tartaric acid.

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), a recorder, and an injection valve.

The chiral HPLC analyses were performed with a cellulose tris(3,5-dimethylphenylcarbamine) chiral column (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, Japan). Detection was carried out at 254 nm.

Reagents

DL-Alpha-methylbenzylamine (99%) and L-(+)-tartaric acid were purchased from Acros Organics. All organic solvents and other chemical reagents are of analytical reagent grade (Beijing Chemical Factory, China). Silica gel G plates were from Qingdao Ocean Chemical Factory, China.

Preparation of Two Phase Solvent System and Sample Solution

The two phase solvent system composed of chloroform:methanol:water (4:3:1, v/v/v) was used. At first, the solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and then the two phases were clearly separated. Lastly, the chiral selector L-(+)-tartaric acid (278 mmol/L) was dissolved in the upper phase.

The sample solutions were prepared by dissolving 120 mg of DL-alpha-methylbenzylamine in 4.0 mL of the above 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 4.0 mL/min, the lower phase was pumped into the inlet of the column at a flow of 2.0 mL/min in the head-to-tail elution mode and the apparatus was rotated at 800 rpm. When the front of the mobile phase elutes from the outlet of the column, a 4.0 mL sample solution containing 120 mg of DL-alpha-methylbenzylamine was introduced into the column through an injection valve. 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 selection of a solvent system is important for HSCCC, because selecting a solvent system means simultaneously choosing the column and the eluent. In the present study, first we conducted a literature to search for the suitable solvent systems previously used for organic amines and considered the solvent systems providing nearly equal volumes of the upper and lower phase with reasonably short settling times. Secondly, as L-(+)-tartaric acid is soluble in water, the solubility of L-(+)-tartaric acid in the water phase of the solvent system was checked. Lastly, the partition of DL-alpha-methylbenzylamine between the two phases of solvent system were estimated by silica gel TLC, so that the range of the optimum distribution coefficient of DL-alpha-methylbenzylamine was about 0.2–2.

On the basis of the above results, the two phase solvent system composed of chloroform:methanol:water (4:1:2, 4:2:2, and 4:3:1, v/v/v) was tested using the head-to-tail elution mode. As a result, the best solvent ratio to separate DL-alpha-methylbenzylamine was 4:3:1.

The effect of L-(+)-tartaric acid concentration was examined. The concentration of L-(+)-tartaric acid was changed with 111, 167, 278, or 389 mmol/L in the stationary phase, the best separation was attained when L-(+)-tartaric acid was at 278 mmol/L.

We also attempted to separate the racemate as well as possible, and the sample size was 120 mg in a single injection. Figures 2 and 3 show HSCCC chromatograms of 60 mg and 120 mg of D,L-alpha-methylbenzylamine, respectively.

The fraction for each enantiomer peak was collected and the solvents were evaporated under vacuum at 50°C. All residues were combined for peak 1 and peak 2, respectively. Purification of the mixtures was carried out by flash chromatography on silica gel (chloroform:methanol, 6:1, *v*/*v*) to remove L-(+)-tartaric acid. Before measuring by chiral HPLC, the two

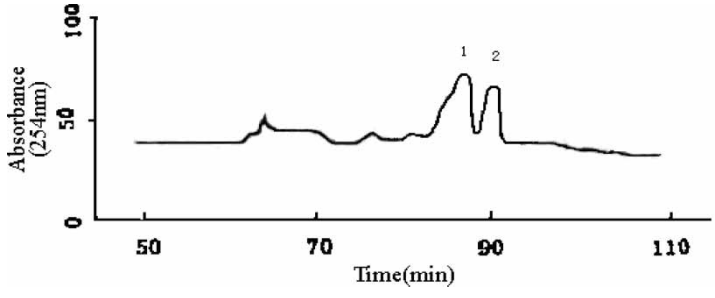


Figure 2. HSCCC chromatogram of D,L-alpha-methylbenzylamine. Solvent system: chloroform:methanol:water = 4:3:1, in which the upper phase contains L-(+)-tartaric acid 278 mmol/L; Mobile phase: lower phase; Sample: 60 mg of D,L-alpha-methylbenzylamine dissolved in 4.0 mL solvent.

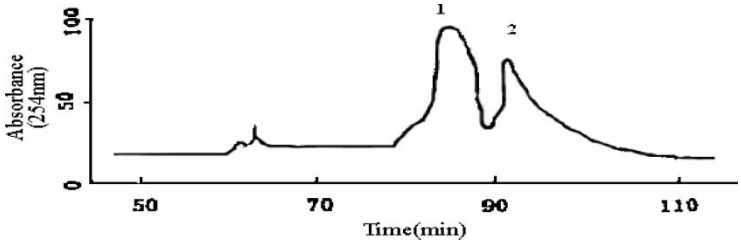


Figure 3. HSCCC chromatogram of D,L-alpha-methylbenzylamine. Solvent system: chloroform:methanol:water = 4:3:1, in which the upper phase contains L-(+)-tartaric acid 278 mmol/L; Mobile phase: lower phase; Sample: 120 mg of D,L-alpha-methylbenzylamine dissolved in 4.0 mL solvent.

enantiomers were spotted onto silica gel G plates and developed with chloroform:methanol (6:1, v/v). The visual detections were done by ninhydrin. Figure 4 shows TLC chromatograms of HSCCC fractions from Figure 3. The three spots on the TLC, which were DL-alpha-methylbenzylamine, fraction 1, and fraction 2, had the same R_f (0.61) values.

Figure 5 shows the enantioseparation chromatograms of DL-alpha-methylbenzylamine on cellulose tris-(3,5-dimethylphenylcarbamine) chiral column using hexane-propanol(90:10,v/v) as eluent. Figure 5(B) was commercial DL-alpha-methylbenzylamine; Figure 5(A) and Figure 5(C) were eanantiomers separated by HSCCC in Figure 3. Obviously, complete resolutions was attained.

CONCLUSION

From the above comprehensive studies, we know that DL-alpha-methylbenzylamine can be separated into its two enantiomers by HSCCC using the

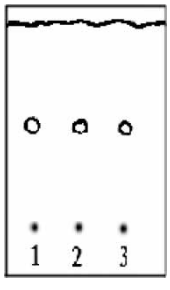


Figure 4. TLC chromatograms of HSCCC fractions in Figure 3. (1) DL-alpha-methyl-benzylamine; (2) fraction 1; (3) fraction 2. The analyses were made on a silica gel G TLC plate developed with chloroform:methanol (6:1, v/v). The visual detections were done by ninhydrin.

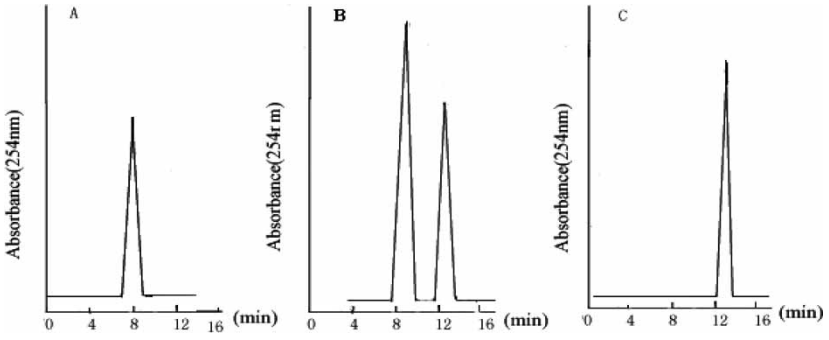


Figure 5. Chiral HPLC analysis of HSCCC fractions from alpha-methylbenzylamine in Figure 3. (A) Peak 1; (B) racemate of alpha-methylbenzylamine; (C) peak 2. Experimental conditions: cellulose tris(3,5-dimethylphenyl carbamate) chiral column; mobile phase: hexane:2-propanol (90:10); flow rate: 0.5 mL/min; detection: 254 nm.

two phase system of chloroform:methanol:water = 4:3:1. The present method may be applied to enantioseparation of some other organic amine racemates if a new two phase solvent system can be selected properly. This is a first time report of L-(+)-tartaric acid being used as the chiral selector in HSCCC.

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REFERENCES

1. Maier, N.M.; Franco, P.; Lindner, W. Separation of enantiomers: needs, challenges, perspectives. J. Chromatorgr. A **2001**, *906*, 3–33.
2. Yuan, L.M. *Preparative Chromatography Technology and Application*; Chemical Industry Press: Beijing, 2005; 107–135.
3. Foucault, A.P.; Chevolot, L. Countercurrent chromatography: instrumentation, solvent selection and some recent applications to natural product purification. J. Chromatogr A **1998**, *808*, 3–22.
4. Foucault, A.P. Enantioseparations in countercurrent chromatography and centrifugal partition chromatography. J. Chromatogr. A **2001**, *906*, 365–378.
5. Ma, Y.; Ito, Y.; Foucault, A. Resolution of gram quantities of racemates by high-speed countercurrent chromatography. J. Chromatogr. A **1995**, *704*, 75–81.
6. Ma, Y.; Ito, Y. Chiral separation by high-speed counter-current chromatography. Anal. Chem. **1995**, *67* (17), 3069–3074.

7. Ma, Y.; Ito, Y.; Berthod, A. A chromatographic method for measuring K_F of enantiomer-chiral selector complexes. J. Liq. Chromatogr. & Rel. Technol. **1999**, *22* (19), 2945–2955.
8. Duret, P.; Foucault, A.; Margraff, R. Vancomycin as a chiral selector in centrifugal partition chromatography. J. Liq. Chromatogr. & Rel. Technol. **2000**, *23* (2), 295–312.
9. Armstrong, D.W.; Menges, R.; Wainer, I.W. Use of centrifugal partition chromatography and proteins in the preparative separation of amino acid enantiomers. J. Liq. Chromatogr. & Rel. Technol. **1990**, *13* (18), 3571–3581.
10. 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. & Rel. Technol. **1998**, *21* (1–2), 135–141.
11. Franco, P.; Blanc, J.; Oberleitner, W.R.; Maier, N.M.; Lindner, W.; Minguillon, C. Enantiomer separation by countercurrent chromatography using cinchona alkaloid derivatives as chiral selectors. Anal. Chem. **2002**, *74* (16), 4175–4183.
12. Yuan, L.M.; Liu, J.C.; Yan, Z.H.; Meng, X.; Xu, Z.G. J. Liq. Chromatogr. & Rel. Technol. **2005**, *28*, 3057–3063.

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