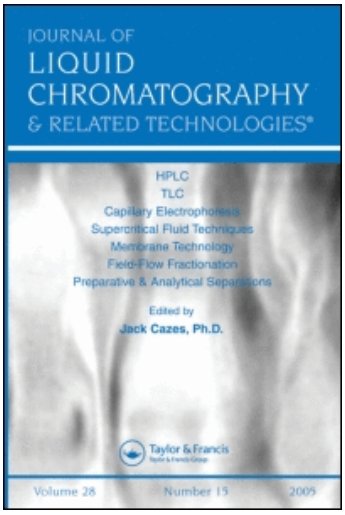

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Enantiomeric Separation of Commercial D,L-Kynurenine with an Aqueous Two-Phase Solvent System by Cross-Axis Coil Planet Centrifuge

K. Shinomiya[a]; Y. Kabasawa[a]; Y. Ito[b]
[a] College of Pharmacy Nihon University, Chiba, Japan [b] Laboratory of Biophysical Chemistry, National Heart, Lung, and Blood Institute, Bethesda, MD, USA

To cite this Article Shinomiya, K. , Kabasawa, Y. and Ito, Y.(1998) 'Enantiomeric Separation of Commercial D,L-Kynurenine with an Aqueous Two-Phase Solvent System by Cross-Axis Coil Planet Centrifuge', Journal of Liquid Chromatography & Related Technologies, 21: 1, 135 — 141
To link to this Article: DOI: 10.1080/10826079808001942
URL: http://dx.doi.org/10.1080/10826079808001942

J. LIQ. CHROM. & REL. TECHNOL., 21(1&2), 135–141 (1998)

ENANTIOMERIC SEPARATION OF COMMERCIAL D,L-KYNURENINE WITH AN AQUEOUS TWO-PHASE SOLVENT SYSTEM BY CROSS-AXIS COIL PLANET CENTRIFUGE

Kazufusa Shinomiya,[1,]* Yozo Kabasawa,[1] Yoichiro Ito[2]

[1] College of Pharmacy
Nihon University
7-7-1, Narashinodai, Funabashi-shi
Chiba 274, Japan

[2] Laboratory of Biophysical Chemistry
National Heart, Lung, and Blood Institute
National Institutes of Health
Building 10, Room 7N-322
Bethesda, MD 20892, USA

ABSTRACT

Commercial D,L-kynurenine was resolved by high-speed countercurrent chromatography using a cross-axis coil planet centrifuge. The separation was performed with an aqueous-aqueous polymer phase system composed of 10% (w/w) polyethylene glycol 8000 and 5% (w/w) dibasic sodium phosphate containing 6% (w/w) bovine serum albumin as a chiral selector. The lower phosphate-rich mobile phase eluted the L-enantiomer first followed by the D-enantiomer. The peak resolution was 0.94 for 2.5 mg of the sample. The separation was completed within 3.5 h.

INTRODUCTION

Countercurrent chromatography (CCC) performs liquid-liquid partitioning using no solid support.[1,2] Elimination of the solid support provides various advantages over the conventional chromatographic methods such as lack of sample loss to adsorption, easy adjustment of either phase, high sample capacity, easy prediction of retention times, etc. Since the 1980's high-speed CCC has been widely used for separation of natural and synthetic products.[3]

Recently, an improved model of the cross-axis coil planet centrifuge (X-axis CPC) was constructed in our laboratory for performing CCC with aqueous-aqueous polymer phase systems.[4-6] The apparatus undergoes a synchronous planetary motion in such a way that the column holder rotates about its horizontal axis and simultaneously revolves around the vertical axis of the centrifuge, both at the same angular velocity. The centrifugal force field produced by this planetary motion ensures a good retention of the stationary phase, even for viscous aqueous-aqueous polymer phase systems with extremely low interfacial tension.[7] Our previous studies demonstrated that the apparatus was useful for the separation of proteins with polymer phase systems.[4-6]

In the past, Albertsson et al. conducted a series of experiments to resolve racemates such as D,L-tryptophan[8] and D,L-kynurenine[9] using aqueous-aqueous polymer phase systems containing bovine serum albumin (BSA) as a chiral selector. However, these experiments were not successful, mainly due to lack of an adequate partition technique. Recently, we have used rotation locular CCC (RLCC) for the resolution of D,L-kynurenine with a similar polymer phase system.[10] Although the method achieved a partial peak resolution, it required a long separation time of over 60 hr.

This paper describes the CCC resolution of D,L-kynurenine by the use of the X-axis CPC which provides much higher efficiency in terms of both peak resolution and separation time.

EXPERIMENTAL

Apparatus

The X-axis CPC used in the present study was constructed at the machine shop of Nihon University (Chiba, Japan). The basic features of the apparatus were reported earlier.[4-6]

Preparation of the Column

The columns used in the present study were a pair of eccentric coil assemblies. Each assembly was prepared by winding a single piece of 1 mm I.D. polytetrafluoroethylene (PTFE) tubing (Flon Kogyo, Tokyo, Japan) onto 7.6 cm long, 5 mm O.D. nylon pipes, forming 20 units of serially connected left-handed coils. These coil units are symmetrically arranged around the holder with their axes parallel to the holder axis. A pair of identical coil assemblies was connected in series to obtain a total column capacity of 28 mL.

Reagents

D,L-kynurenine, BSA and dibasic sodium phosphate were purchased from Wako Pure Chemicals (Osaka, Japan). Polyethylene glycol (PEG) 8000 was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents were of reagent grade.

Preparation of the Two-Phase Solvent System and Sample Solution

The enantiomeric separation was performed using 6% (w/w) BSA as a chiral selector in the aqueous two-phase solvent system composed of 10% (w/w) PEG 8000 and 5% (w/w) dibasic sodium phosphate. The solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated after clear layers were formed.

The sample solution was prepared by dissolving 2.5 mg of commercial kynurenine in 5 mL of the solvent mixture consisting of equal volumes of each phase.

Separation of D,L-Kynurenine

In each separation, the coiled column was completely filled with the PEG-rich upper stationary phase and the sample solution was charged into the column through the sample port. Then, the phosphate-rich lower mobile phase was pumped into the column at 0.2 mL/min while the column was rotated at 800 rpm in a counterclockwise direction. The effluent was collected in test tubes (0.4 mL/tube) using a fraction collector (Model SF-200, Advantec Co., Tokyo, Japan).

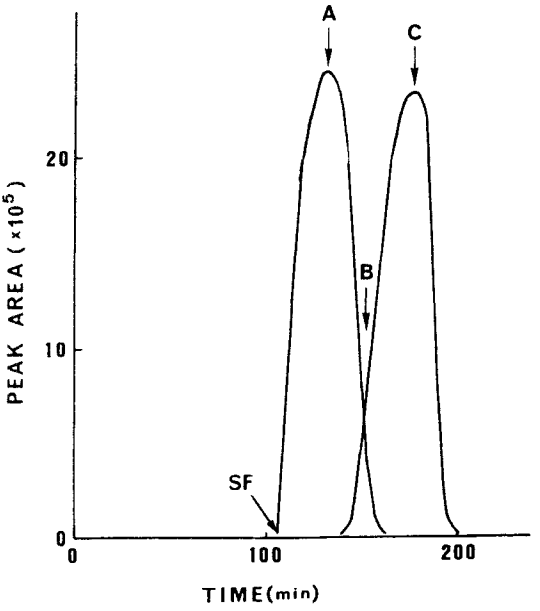

Figure 1. Enantiomeric separation of commercial D,L-kynurenine by X-axis CPC. The vertical axis indicates the peak area of each enantiomer in HPLC analysis of CCC fractions. Experimental conditions; solvent system: 10% (w/w) PEG 8000 - 5% (w/w) dibasic sodium phosphate containing 6% (w/w) BSA; mobile phase: lower phase; flow rate: 0.2 mL/min; sample size: 2.5 mg; revolution: 800 rpm. SF = solvent front

Analysis of CCC Fractions

An aliquot of each fraction was submitted to analysis by high-performance liquid chromatography (HPLC). The HPLC equipment included a reciprocating pump (Type 880-PU Nihon Bunko Co., Tokyo, Japan), a sample injector (Type 7125, Reodyne Inc., Berkeley, CA, USA), a UV detector (Type UVIDEC-100, Nihon Bunko Co., Tokyo, Japan) and a variable input recorder (Type C-R5A, Shimadzu Seisakusho, Kyoto, Japan).

Analytical conditions were as follows: column, TSKgel ODS-120T (4.6 mm ID x 150 mm, Tosoh Co., Tokyo, Japan) coated by C_{12}-hydroxyproline according to the method by Takeuchi et al.;[11] eluent, 0.05 M acetic acid containing 5 mM copper (II) acetate; flow rate, 1.0 mL/min; column temperature, room temperature; detection, 350 nm and injection volume, 10 μL.

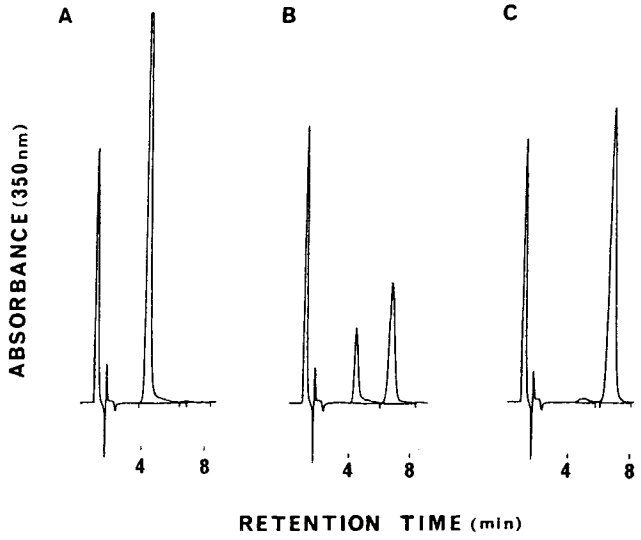

Figure 2. HPLC analysis of CCC fractions obtained by X-axis CPC. (A) the summit of the first peak, (B) between the first and second peaks and (C) the summit of the second peak. Experimental conditions: column: ODS-silica coated by N-n-dodecyl-L-hydroxyproline (4.6 mm ID x 150 mm L); mobile phase: 0.05 M acetic acid containing 5 mM copper (II) acetate; flow rate: 1.0 mL/min; detection: 350 nm; sample size: 10 μL.

RESULTS AND DISCUSSION

The separation of D,L-kynurenine was first reported by Sellergren et al. using an aqueous two-phase solvent system composed of 8% (w/w) dextran, 7% (w/w) PEG 8000 and 6% (w/w) BSA in 0.05 M sodium phosphate buffer (pH 8.5) containing 0.1 M sodium chloride.[9] We found, however, that this solvent system was inadequate for the enantiomeric separation by either RLCC[10] or X-axis CPC, because of its high viscosity caused by the presence of a large amount of dextran. In the RLCC separation, this solvent system required a long settling time in the locule, while in the X-axis CPC technique, the lower dextran-rich mobile phase totally displaced the upper PEG-rich stationary phase, resulting in loss of stationary phase.

In our previous studies with RLCC,[10] this problem was greatly alleviated by using a solvent system composed of 10% (w/w) PEG 8000, 5% (w/w) dibasic sodium phosphate and 6% (w/w) BSA. Hence, this solvent system was employed in the present study.

Fig. 1 shows the CCC separation of 2.5 mg of commercial D,L-kynurenine by the X-axis CPC with the above solvent system. Using the lower phosphate-rich phase as the mobile phase, L-kynurenine was eluted first followed by D-kynurenine, as confirmed by the following HPLC analysis.

The three HPLC chromatograms in Fig. 2 were obtained from the summit of the first peak (A), between the peaks (B) and from the summit of the second peak (C). In all chromatograms, BSA present in the effluent was eluted at the solvent front, followed by the main kynurenine peak(s). Using standards, the first peak was identified as L-kynurenine and the second peak, D-kynurenine.

The results indicate that peak resolution (R_s) between the two enantiomers is 0.94 and the total separation time was about 3.5 h compared with 60 hours required for the even less efficient separation by RLCC.[10] The successful chiral separation in the present study was attributed to the interaction between BSA and D,L-kynurenine in the column where the BSA is almost entirely partitioned into the mobile phase, indicating that earlier eluting L-kynurenine has a greater affinity for BSA than does D-kynurenine.

The overall results of the present studies indicate that the X-axis CPC is useful for enantiomeric separations of certain amino acids using an aqueous polymer phase system containing BSA as a chiral selector.

ACKNOWLEDGMENT

The authors thank Ms. Tamaki Yoshikura and Kaori Takacho for their technical assistance. The authors are also indebted Dr. Henry M. Fales of National Institutes of Health for editing the manuscript.

REFERENCES

1. Y. Ito in **Countercurrent Chromatography: Theory and Practice**, N. B. Mandava, Y. Ito, eds., Marcel Dekker, New York, 1988 Chapter 3, pp. 79-442.

2. W. D. Conway, **Countercurrent Chromatography: Apparatus, Theory and Applications**, VCH, New York, 1990, p. 195.

3. Y. Ito, W. D. Conway, eds., **High-Speed Countercurrent Chromatography**, Wiley-Interscience, New York, 1996.

4. K. Shinomiya, J.-M. Menet, H. M. Fales, Y. Ito, J. Chromatogr., **644**, 215 (1993).

5. K. Shinomiya, N. Inokuchi, J. N. Gnabre, M. Muto, Y. Kabasawa, H. M. Fales, Y. Ito, J. Chromatogr., **724**, 179 (1996).

6. K. Shinomiya, M. Muto, Y. Kabasawa, H. M. Fales, Y. Ito, J. Liq. Chromatogr. & Rel. Technol., **19**, 415 (1996).

7. P. A. Albertsson, **Partition of Cell Particles and Macromolecules**, Wiley-Interscience, New York, 1986.

8. B. Ekberg, B. Sellergren, P. A. Albertsson, J. Chromatogr., **333**, 211 (1985).

9. B. Sellergren, B. Ekberg, P. A. Albertsson, K. Mosbach, J. Chromatogr., **450**, 277 (1988).

10. Y. Sato, K. Shinomiya, Y. Kabasawa, J. Chem. Soc. Jpn, Chemistry and Industrial Chemistry, 1994 (12), 1067.

11. T. Takeuchi, R. Horikawa, T. Tanimura, Anal. Chem., **56**, 1152 (1984).

Received March 20, 1997
Accepted May 23, 1997
Manuscript 4424