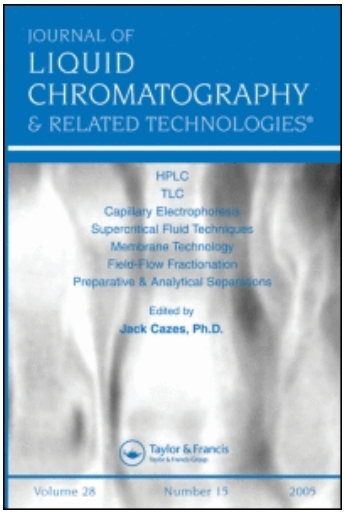

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ABSTRACT

Countercurrent chromatographic separation of hippuric acid (HA) and related compounds was performed using the cross-axis

coil planet centrifuge equipped with a pair of eccentric coil assemblies mounted in an off-center position. Partition coefficients of HA and a set of related compounds were determined on a polar two-phase solvent system composed of methyl t-butyl ether, 1-butanol, acetonitrile and aqueous 0.1% trifluoroacetic acid at various volume ratios. The optimal volume ratio of 1 : 0 : 0 : 1 was successfully used to resolve p-amino HA, HA, p-methyl HA, and (±)-mandelic acid by the lower aqueous phase mobile, and benzoic acid, p-methyl HA and HA by the upper organic phase mobile.

INTRODUCTION

Countercurrent chromatography (CCC) is a form of liquid-liquid partition chromatography in which the stationary phase is retained in the open column space free of a solid support matrix. Consequently, the system eliminates various complications arising from the use of the solid support. Among many existing CCC instruments, type-J multilayer coil planet centrifuge and cross-axis coil planet centrifuge (cross-axis CPC) have proven most useful for separation of various natural and synthetic products.[1-3]

The cross-axis CPC produces a unique planetary motion such that the column holder rotates about its horizontal axis while revolving around the vertical axis of the centrifuge.[4,5] This motion produces satisfactory retention of the stationary phase for viscous low-interfacial tension two-phase solvent systems such as aqueous-aqueous polymer phase systems.

An improved model of the cross-axis CPC was constructed in our laboratory for performing CCC with aqueous two-phase solvent systems.[6-11] Our previous studies demonstrated that the cross-axis CPC equipped with a pair of multilayer coils or eccentric coil assemblies in the off-center position was useful for the separation of proteins with polyethylene glycol-potassium phosphate solvent systems.[6-8] The apparatus is also useful for the separation of highly polar compounds such as sugars which requires the use of polar two-phase solvent systems.[12]

In the present paper, we report the separation of hippuric acid (HA) and related compounds by CCC using the cross-axis CPC.

EXPERIMENTAL

CCC Apparatus

The cross-axis CPC employed in the present studies was constructed at the Machining Technology Center of Nihon University, Chiba, Japan. The design of the apparatus was previously described in detail.[6–8]

Preparation of Separation Columns

A pair of eccentric coil assemblies was used in the present study. Each assembly was prepared by winding a 1 mm-ID PTFE (polytetrafluoroethylene) tubing (Flon Kogyo, Tokyo, Japan) onto 7.6 cm long, 5 mm-OD nylon pipes forming a series of tight left-handed coils. A set of these coil units was symmetrically arranged around the holder hub of 7.6 cm diameter in such a way that the axis of each coil unit is parallel to the axis of the holder. Two sets of coil assemblies were mounted on the rotary frame, one on each side, and serially connected with the flow tube. The total column capacity is 26.5 mL.

Reagents

Hippuric acid (HA), p-amino HA, o-, m- and p-methyl HAs, (±)-, (+)- and (-)-mandelic acids, benzoic acid, 3-indoleacetic acid, 2-naphthoic acid and phenaceturic acid were purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents were of reagent grade.

Preparation of Two-Phase Solvent Systems and Sample Solutions

A set of two-phase solvent systems was prepared from methyl t-butyl ether (MBE), 1-butanol, acetonitrile (AcN) and aqueous 0.1% trifluoroacetic acid (TFA) at various volume ratios. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases separated after two clear layers were formed. Standard sample solutions for CCC separation were prepared by dissolving each sample mixture in 0.5 mL of each phase of the two-phase solvent system used for separation.

Measurement of Partition Coefficients of HA and Related Compounds

Successful CCC separation highly depends on the choice of the two-phase solvent system which provides suitable partition coefficient values for a set of analytes. In the present study, partition coefficient (K) of each standard sam-

ple was determined spectrophotometrically using a simple test tube experiment described by Oka et al.[13] as follows: Two milliliters of each phase of an equilibrated solvent system were delivered into a test tube to which about 1 mg of the sample was added. The contents were thoroughly mixed and allowed to settle at room temperature. After the two clear layers were formed, a 1 mL aliquot of each phase was diluted with 2 mL of methanol. The absorbance was measured at 254 nm using a spectrophotometer (Model UV-1600, Shimadzu Corporation, Kyoto, Japan). The K value was obtained by dividing the absorbance value of the upper organic phase by that of the lower aqueous phase.

Separation Procedure

Each separation was initiated by completely filling the column with the stationary phase followed by injection of the sample solution into the column inlet. Then, the mobile phase was pumped into the column using a reciprocating pump (Model KHU-W-52H, Kyowa Seimitsu Co., Tokyo, Japan), while the column was rotated at 800 rpm in a counterclockwise direction. The effluent from the outlet of the column was collected in test tubes (0.8 mL/tube) using a fraction collector (Model SF-200, Advantec Co., Tokyo, Japan).

Analysis of CCC Fractions

Each collected fraction was diluted with 2 mL of methanol and the absorbance was measured at 254 nm using a spectrophotometer (Model UV-1600, Shimadzu Corporation, Kyoto, Japan).

RESULTS AND DISCUSSION

HA and related compounds are relatively hydrophilic while containing a hydrophobic aryl group in each molecule (Figure 1). In the present studies, the polar two-phase solvent systems composed of MBE/1-butanol/AcN/aqueous 0.1% TFA at various volume ratios were examined for suitable K values on a set of standard samples. TFA was added to protonate the carboxyl group to increase hydrophobicity of the molecule.

Figure 2 illustrates the K values of HA and a set of related compounds in the above solvent systems. K values of glycine conjugates (HA, o-, m-, p-methyl HAs and phenaceturic acid) increased by decreasing the hydrophobicity of the solvent system, while the original aromatic compounds such as benzoic acid, 3-indoleacetic acid, and 2-naphthoic acid were almost unilaterally partitioned in the organic phase regardless of the phase composition. p-Amino HA was found to be most hydrophilic among all compounds examined. From the

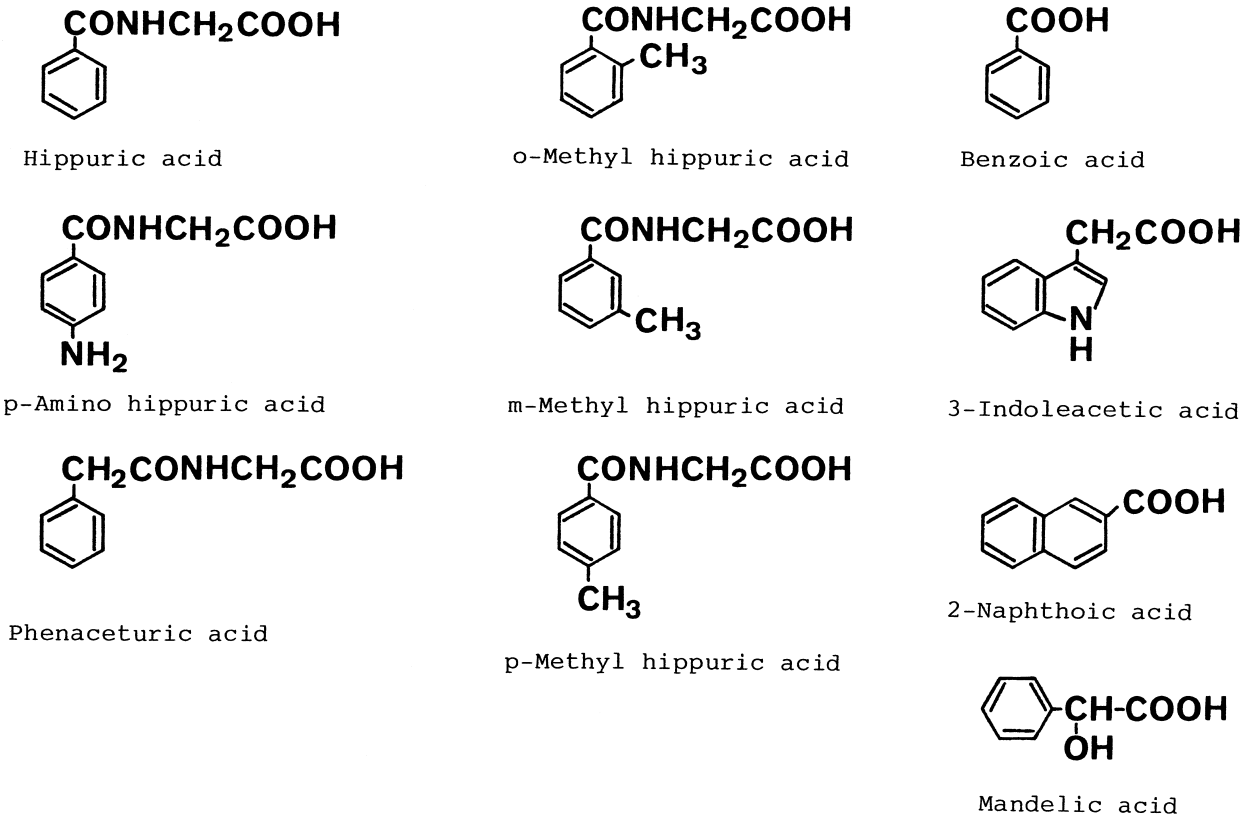

Figure 1. Chemical structures of hippuric acid and related compounds.

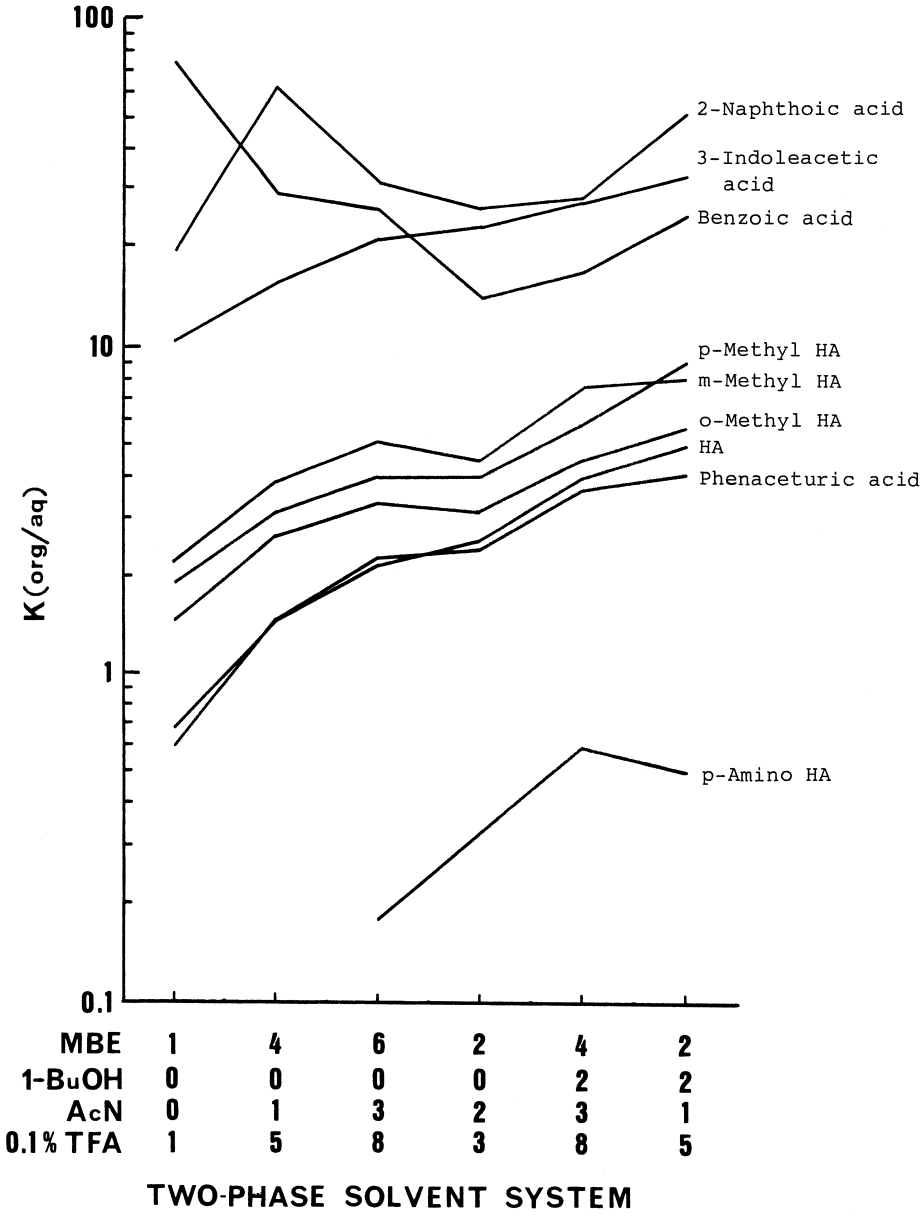

Figure 2. Partition coefficients (K = Corg/Caq) of hippuric acid and related compounds in methyl t-butyl ether/1-butanol/acetonitrile/aqueous 0.1% trifluoroacetic acid system. MBE = methyl t-butyl ether; 1-BuOH = 1-butanol; AcN = acetonitrile; TFA = trifluoroacetic acid.

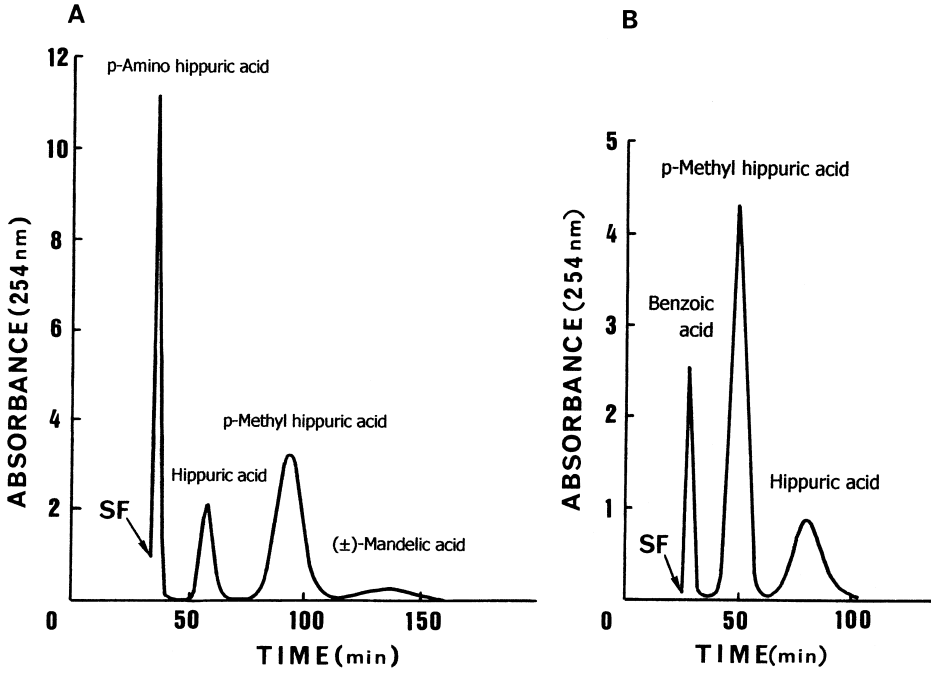

Figure 3. CCC separation of hippuric acid and related compounds by cross-axis CPC. Experimental conditions: apparatus: cross-axis CPC equipped with a pair of eccentric coil assemblies, 1mm ID and 26.5 mL total column capacity; sample: (A) p-amino hippuric acid (2.5 mg), hippuric acid (1.5 mg), p-methyl hippuric acid (2.5 mg) and (±)-mandelic acid (10 mg); (B) benzoic acid (2.5 mg), p-methyl hippuric acid (2.5 mg) and hippuric acid (2.5 mg); solvent system: methyl t-butyl ether/aqueous 0.1% trifluoroacetic acid (1:1); mobile phase: (A) lower phase; (B) upper phase; flow rate: 0.4 mL/min; revolution: 800 rpm. SF = solvent front.

above results, a two-phase volume ratio of 1 : 0 : 0 : 1 (left of phase compositions in Figure 2) was selected for CCC separation, since K values of HA and phenaceturic acid are relatively well separated from those of methyl HA groups in an ideal range of around K = 1.

Figure 3 illustrates the CCC chromatograms of HA and related compounds obtained with the above solvent system composed of MBE/aqueous 0.1% TFA (1:1). In Figure 3A, p-amino HA, HA, p-methyl HA, and (±)-mandelic acid were separated using the lower phase as a mobile phase. All components were well resolved and eluted 2.5 h. In Figure 3B, benzoic acid, p-methyl HA, and

HA were separated using the upper organic phase as a mobile phase under otherwise identical experimental conditions.

The overall results of the present study indicates that a variety of urinary metabolites such as HA, methyl HAs, and mandelic acid can be resolved by cross-axis CPC using a pair of eccentric coil assemblies mounted in the off-center position.

REFERENCES

1. **Countercurrent Chromatography: Theory and Practice**, N. B. Mandava, Y. Ito, eds., Marcel Dekker, New York, 1988.

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

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

4. Y. Ito, Sep. Sci. & Technol., **22**, 1971 (1987).

5. Y. Ito, Sep. Sci. & Technol., **22**, 1989 (1987).

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

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

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

9. K. Shinomiya, Y. Kabasawa, Y. Ito, J. Liq. Chrom. & Rel. Technol., **21**, 111 (1998).

10. K. Shinomiya, Y. Kabasawa, Y. Ito, J. Liq. Chrom. & Rel. Technol., **21**, 1727 (1998).

11. K. Shinomiya, Y. Kabasawa, Y. Ito, Prep. Biochem. & Biotechnol., **29**, 139 (1999).

12. K. Shinomiya, Y. Kabasawa, Y. Ito, J. Liq. Chrom. & Rel. Technol., **22**, 579 (1999).

13. F. Oka, H. Oka, Y. Ito, J. Chromatogr., **538**, 99 (1991).

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