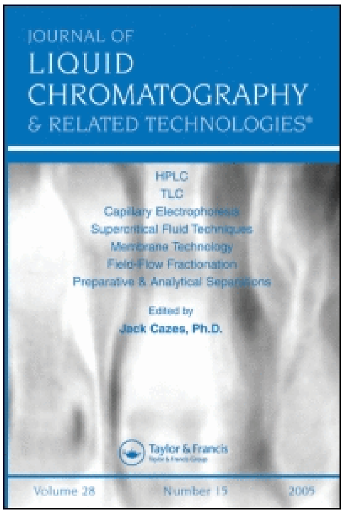

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Comparison of Countercurrent Chromatography with Flash Chromatography

W. D. Conway[a]; E. L. Bachert[a]; A. M. Sarlo[a]; C. W. Chan[a]
[a] Department of Pharmaceutics, School of Pharmacy State University of NY at Buffalo, Amherst, NY

To cite this Article Conway, W. D. , Bachert, E. L. , Sarlo, A. M. and Chan, C. W.(1998) 'Comparison of Countercurrent Chromatography with Flash Chromatography', Journal of Liquid Chromatography & Related Technologies, 21: 1, 53 — 63

To link to this Article: DOI: 10.1080/10826079808001935

URL: http://dx.doi.org/10.1080/10826079808001935

J. LIQ. CHROM. & REL. TECHNOL., 21(1&2), 53–63 (1998)

COMPARISON OF COUNTERCURRENT CHROMATOGRAPHY WITH FLASH CHROMATOGRAPHY

Walter D. Conway,* Elizabeth L. Bachert,†
Anthony M. Sarlo, Chung W. Chan

Department of Pharmaceutics
School of Pharmacy
State University of NY at Buffalo
Amherst, NY 14260

ABSTRACT

Studies of the separation of benzyl, phenylethyl and phenylpropyl alcohols by flash chromatography (FC) on 1.0 x 30 or 1.9 x 15 cm silica gel columns are compared with separation by countercurrent chromatography (CCC) on a 1.68 mm x 24 m, 56 mL, PTFE multilayer coil. Neither adjacent pair of alcohols could be separated by FC and separation of benzyl from phenylpropyl alcohol was poor. The small coil separation by CCC provided a resolution of 0.91 to 1.01 for benzyl and phenylethyl alcohols and better than baseline resolution of phenylpropyl alcohol from either benzyl or phenylethyl alcohol. Speed, sample capacity and selectivity of the two separation techniques are similar but resolution by CCC is enhanced by the characteristic symmetry of CCC peaks compared with the severe tailing observed in FC.

INTRODUCTION

The technique called *"Flash Chromatography"* introduced by Still et al.[1] is widely employed for the purification of crude products obtained in synthetic chemistry or isolated as extracts from natural products or fermentations. Columns of 40 μm silica gel, 10 or 20 mm diam. x 15 cm height are commonly used and developed with a mobile phase such as ethyl acetate in hexane driven by low pressure air.

Countercurrent chromatography (CCC) is a liquid-liquid chromatographic technique in which one liquid is retained in the column by centrifugal or gravitational force, while a second, immiscible liquid, passes through it. No supporting matrix is used.[2]

Our experience suggested that CCC, using a relatively short column in the Ito Multilayer Coil Planet Centrifuge,[3] should provide efficiency and separation times comparable to flash chromatography. This paper compares these two techniques for separation of benzyl alcohol, 2-phenylethanol and 3-phenylpropanol.

EXPERIMENTAL

Flash Chromatography (FC)

FC was carried out in 1.0 and 1.9 cm i.d. x 45 cm glass columns, the effluent ends of which were tapered and of low dead volume, adapted to flange-type low pressure fittings for connection to 0.8 mm i.d. PTFE tubing, which permitted the effluent to be monitored with a uv absorption HPLC detector. Columns were dry-packed as described by Still et al.[1] with Baker No. 7024-5 (40 μm) silica gel and developed with ethyl acetate in hexane. Column effluent was monitored at 254 nm using a 3-mm diam., cylindrical cell, vol. 60 μL, mounted for vertical flow in a Glenco 5480 monitor. Chromatograms were recorded at a chart speed of 1 cm/min. Solvent was delivered from a reservoir pressurized with air at 1 to 15 psi to obtain linear flow rates ranging from about 1.5 to 9 cm/min. The linear flow rate, as a function of pressure, was determined for each column by measurement of the effluent volume in a cylinder and, based on the column diameter, expressed in terms of the linear flow rate above the column bed. Mobile phase flow in the same column could be reproduced by adjustment of the reservoir pressure. After draining the mobile phase to the sand layer, samples in 1 mL of mobile phase, were introduced at the bed surface using a hypodermic syringe fitted with a long

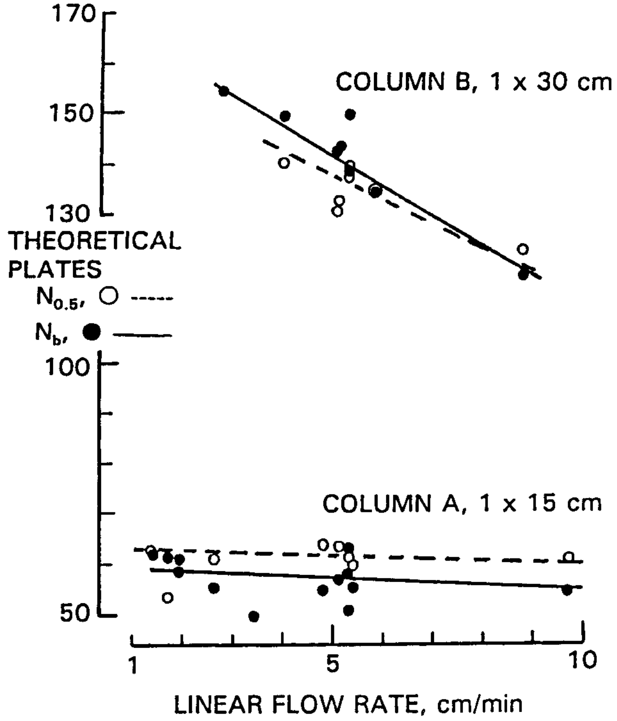

Figure 1. Effect of column length and flow rate on efficiency of 1-cm wide flash chromatography columns with 1-mL samples of 10 µL of benzyl alcohol in a mobile phase consisting of 20% ethyl acetate in hexane.

stainless steel needle bent at right angles at the end. The sample was drained into the bed and rinsed onto the column with two 1-mL aliquots of mobile phase prior to filling the column with mobile phase and initiating pressurized flow.

Countercurrent Chromatography (CCC)

CCC was carried out in a multilayer coil planet centrifuge[3] having an orbital radius of 10 cm and a multilayer coil of 24 m of 1.68 mm i.d. PTFE tubing, vol. 56 mL, ß range 0.5 to 0.85. Orbital frequency was 800 rpm. The solvent system was heptane/25% 2-propanol in water - 1:1 (v/v) using a mobile aqueous phase, pumped from a central column head to a peripheral tail at 4 mL/min. This solvent system was chosen by screening the partition coefficients

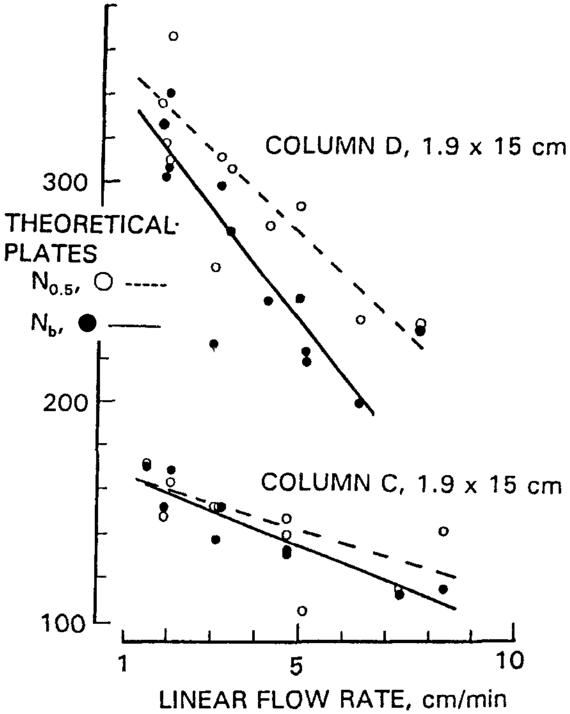

Figure 2. Effect of flow rate on efficiency for 1.9 cm wide flash chromatography columns with 1-mL samples of 10 µL of benzyl alcohol in a mobile phase consisting of 20% ethyl acetate in hexane.

of the test solutes in a series of heptane/2-PrOH/H_2O and heptane/MeOH/H_2O systems using an HPLC method to analyze the aqueous phase of the systems before and after equilibration with heptane. Column effluent was monitored at 254 nm using the same system described above for FC.

Standard Solutes

The test compounds, benzyl alcohol, 2-phenylethanol and 3-phenylpropanol, were commercial materials (Aldrich) used without additional purification. Injection solutions were prepared by diluting 10 to 20 µL volumes of the liquid test compounds to concentrations ranging from 10 to 20 µL/mL in mobile phase. Since the test compound densities vary from 1.01 to 1.04, 10 µL is essentially equal to 10 mg. Chromatographic solvents were HPLC grade.

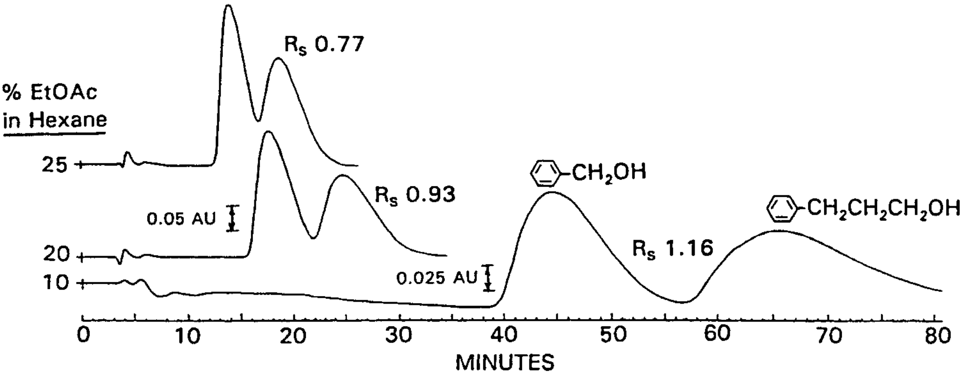

Figure 3. Effect of solvent strength on resolution by flash chromatography with column B. Sample is 10 µL of each compound in 1 mL of mobile phase. Mobile phases are 10, 20 and 25% ethyl acetate in hexane (v/v). Linear flow rate is 5.1 cm/min.

Evaluation of Chromatograms

Column efficiency was estimated using the conventional formulas, $N_b = 16\ (t/W_b)^2$ and $N_{0.5} = 5.55\ (t/W_{0.5})^2$, where t is the peak retention time and the subscripts b and 0.5, respectively, indicate the plate counts calculated from the 4σ base widths and the widths at half height, respectively. Resolution was calculated as $R_S = 2(t_2\text{-}t_1)/(W_1+W_2)$ where t_2 and t_1 are the retention times and W_1 and W_2 the 4σ base widths of the peaks. The separation factor, $\alpha = t_2'/t_1'$, where t' is the corrected retention time obtained by subtracting the retention time for a nonretained solute, t_0, from the solute retention time, $t' = t\text{-}t_0$.

RESULTS AND DISCUSSSION

Flash Chromatography Column Efficiency

A 10 µL sample of benzyl alcohol in 1 mL of mobile phase (20% ethyl acetate in hexane) was convenient for evaluating columns, since it provided peaks in a suitable absorbance range for monitoring. Measurements of theoretical plates, N, based on the peak width at half height were usually slightly higher than those determined from the 4σ base width.

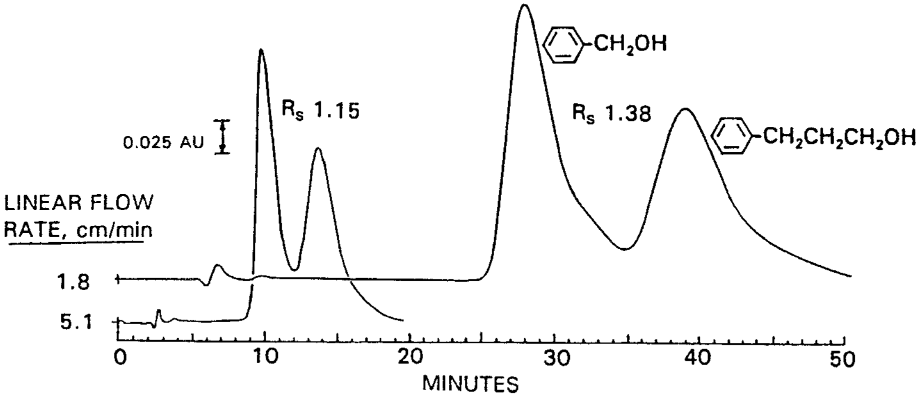

Figure 4. Effect of flow rate on resolution by flash chromatography with column D. Sample is 10 μL of each compound in 1 mL of mobile phase consisting of 20% ethyl acetate in hexane (v/v).

A 1 x 15 cm column provided about 60 theoretical plates which decreased only slightly with linear flow rate over the range from 1.5 to 9.7 cm/min (Fig. 1, Col. A). A column twice this length (Fig. 1, Col. B) provided a little more than double the plate number but the efficiency of the longer column decreased from about 150 plates at a flow of 3 cm/min to about 120 plates at 9 cm/min.

Two wider columns (1.9 x 15 cm), Fig. 2, showed quite different plate counts, about 140 and 260 plates at a linear flow rate of 5 cm/min, presumably as a result of variation in the uniformity of packing.

Resolution of benzyl alcohol and 3-phenylpropanol (Fig. 3) increased as solvent strength was decreased from 25% to 10% ethyl acetate in hexane. However, the elution time increased appreciably with an ethyl acetate content less that 20%. Therefore, FC performance using 20% ethyl acetate in hexane as eluent was used as a basis for comparison with CCC.

Still et al.[1] presented representative results for a single column 2 x 13 cm. At first glance it appears that their indices of column efficiency, based on the equations $R_b = t/W_b$ and $R_{0.5} = t/W_{0.5}$, could be used to calculate theoretical plate counts for comparison with our own work. However, such calculations yield unreasonably low plate counts, in the range of 3 to 9 for N_b and 6 to 18 for $N_{0.5}$. The reason for this is not clear but, perhaps their measurements of retention time and peak width were in different units. Nevertheless, they found that column efficiency decreased with increasing sample size. Their studies of the variation in efficiency with flow rate were done only with a 200 mg sample size. Based on those studies, they advocated a flowrate of 5 ± 0.25 cm/min, since the plate count dropped significantly at higher and lower flowrates. Our data using 10-µL samples of benzyl alcohol all show a steady decline in efficiency as flowrate is increased, with no indication of an optimum flowrate. We presume that this differs from Still's observation because of our lower sample size.

Flash Chromatography Resolution

Neither of the adjacent pairs in the homologous series of benzyl alcohol, 2-phenylethanol or 3-phenylpropanol, could be baseline separated by flash chromatography. Our best result was obtained with column D (1.9 x 15 cm), which provided a resolution of 1.38 for a mixture of 10 µL each of benzyl alcohol and 3-phenylpropanol (Fig. 4) at a flowrate of 1.8 cm/min.

Efficiency, N_b, for the benzyl alcohol peak is 326 plates. Elution time is about 50 minutes. Elution time was decreased to 13 min. at a flowrate of 5.1 cm/min but resolution fell to 1.15.

Resolution of the same pair was lower (R_S 0.91 to 0.96) on column C (1.9 x 15 cm) and was relatively unchanged at flowrates from 1.9 to 4.8 cm/min, but fell to 0.81 at 7.2 cm/min. Chromatography on the longer column B (1.0 x 30 cm) extended the elution time to 80 minutes, but resolution was only 1.16.

Countercurrent Chromatography

Based on measurements of the partition coefficients of the three alcohols in several heptane/methanol/water and heptane/2-propanol/water systems (Fig. 3 of ref 4), it was decided to employ the system heptane/25% 2-propanol-1:1 (v/v/) for CCC. This mixture, expressed here as a pseudo-2-component system,[4] corresponds to the system heptane/2-PrOH/H_2O-50:12.5:37.5 (v/v/v) which, on mixing, separates into two layers. Based on the ternary diagram, a table of phase compositions has been compiled which facilitates mixing the required volume of each phase for CCC.[4]

CCC was done in an apparatus essentially identical to that available commercially from P.C. Inc., Potomac MD, with the exception that the multilayer column employed was approximately one-fourth the size of the commercial column. It consisted of a 24 m length of 1.68 mm i.d. PTFE tubing

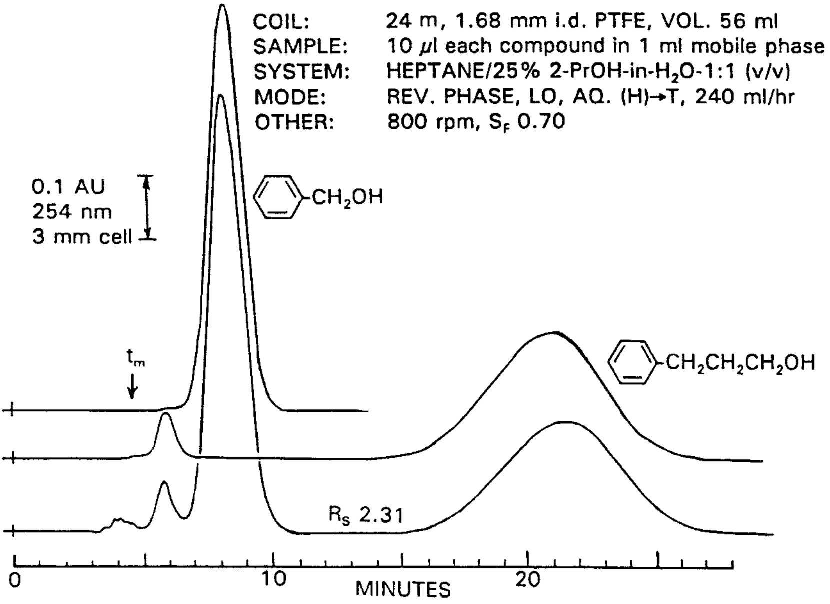

Figure 5. Resolution of benzyl alcohol and 3-phenylpropanol by CCC.

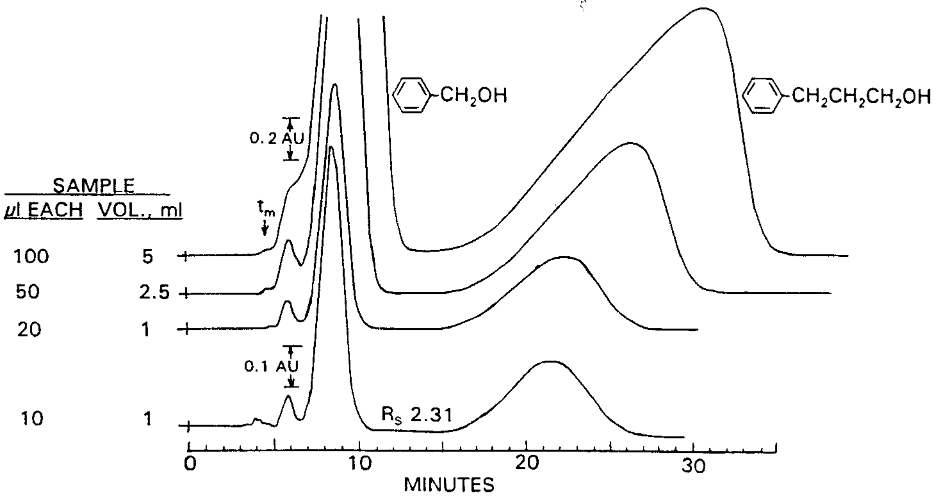

Figure 6. Effect of sample size on resolution of benzyl alcohol and 3-phenylethanol by CCC. Sample quantities and volumes are indicated in the figure. Other conditions as in Fig. 5.

Table 1

Comparison of CCC With FC

	CCC	**FC**
Column size	1.68 mm x 24 m, 56 mL	1 x 15 or 1 x 39 cm
Efficiency, N_b, benzyl alcohol	123	50-150
Sep'n factor, α, for benzyl and phenethyl alcohols		
This study	1.88	1.29
Crane et al.[5]	---	1.19, 1.23
Peak asymmetry, tail distance/front distance, at 10% height		
benzyl alcohol	1.05	2.32
Resolution, R_s		
benzyl and phenethyl	0.91-1.01	---
benzyl and phenylpropyl	2.09-2.23	1.38

and had a volume of 56 mL. The heptane, upper phase was employed as the stationary phase and the aqueous, lower phase was pumped through it in a central head to peripheral tail direction at 4 mL/min. Baseline resolution of benzyl alcohol and 3-phenylpropanol was easily achieved in less than 30 minutes (Fig. 5). To study the effect of sample size, first the concentration was doubled to inject 20 μL each of benzyl alcohol and 3-phenylpropanol in 1 mL of mobile phase. Then, the sample volume was progressively increased to 5 mL containing 100 μL of each compound (Fig. 6). This was the maximum size sample loop we had available, but it is apparent that baseline resolution could be obtained with a still larger sample. Elution time increased less than 10 minutes to about 35 minutes for the largest sample.

When the third alcohol, 2-phenylethanol, is added to the mixture (10 μL each compound), CCC resolution of the first eluted pair is about 1.01 while the second pair is still baseline resolved (Fig. 7).

SUMMARY

Several parameters comparing our separations by CCC with our FC results and FC separations by others[5] are listed in Table 1. In our experience, efficiency, run time, and column capacity on 56 mL CCC column is

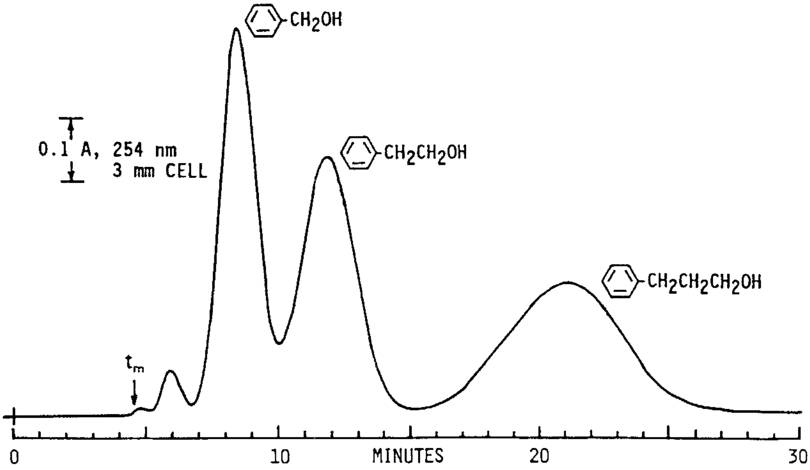

Figure 7. CCC separation of a mixture of 10 μL each of benzyl alcohol, 2-phenylethanol and 3-phenylpropanol by CCC using an injection volume of 1 mL. Other CCC conditions as noted in Fig. 5.

comparable to that obtained on a 1 x 30 cm or 2 x 15 cm silica gel flash chromatography column. However, the separation factor, α, for benzyl and phenylethyl alcohols is somewhat greater, 1.88, for CCC, than 1.29 for FC in our own study or in a study reported by Crane et al., 1.19 and 1.23.[6]

Measurements of resolution by FC are not reported by others but in this study, benzyl and phenylethyl alcohols were resolved by a factor of 0.91 to 1.01 by CCC but were not significantly separated by FC. Benzyl and phenylpropyl alcohols were more than baseline separated, R_s = 2.09 - 2.23 by CCC whereas we obtained a resolution of only 1.38 by FC.

The enhanced resolution obtained by CCC partly results from the greater α value obtained in the CCC system, but the minimal peak tailing in CCC as indicated by a peak asymmetry factor of 1.05 for benzyl alcohol compared to the significant tailing factor of 2.32 for FC on silica gel contributed strongly to resolution by CCC.

The resolution obtained using commercial CCC columns of 300 ml or more will be better than double that obtained with the experimental 56 ml column used in the present work.

ACKNOWLEDGMENTS

Presented, in part, at the Northeast Regional Meeting of the American Chemical Society, Rochester, NY, November 1987. This work was supported, in part, by a grant from the Northeast Regional Chromatography Discussion Group.

REFERENCES

[†] Present address: Pfizer Inc., Central Research Division, Eastern Point Road, Groton, CT 06340.

1. W. C. Still, M. Khan, A. Mitra, "Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution," J. Org. Chem., **42**, 2923 (1978).

2. W. D. Conway, **Countercurrent Chromatography**, VCH Publishers Inc., New York, 1990.

3. Y. Ito, "Efficient Preparative Countercurrent Chromatography with a Coil Planet Centrifuge," J. Chromatogr., **214**, 122 (1981).

4. W. D. Conway, "Pseudo-2-Component Notation for Heptane/Alcohol/Water Systems in Countercurrent Chromatography," J. Liq. Chromatog., **13**, 2409 (1990).

5. L. J. Crane, M. Zief, J. Horvath, "Low Pressure Preparative Liquid Chromatography," Amer. Lab. **13 (5)**, 128 (1981).

Received March 20, 1997
Accepted May 20, 1997
Manuscript 4416