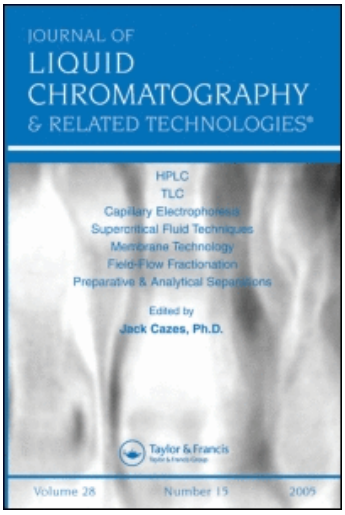

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Preparative Separation of Hydrocortisone and Its Stereoisomer by High-Speed Countercurrent Chromatography

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ABSTRACT

Using high-speed countercurrent chromatography (HSCCC), hydrocortisone, and its stereoisomer, epi-hydrocortisone was successfully separated from baseline with a chloroform–methanol–water (10 : 5 : 5, v/v/v) solvent system. After separation and purification, high-purity hydrocortisone and epi-hydrocortisone were obtained by HPLC and MS analyses. The purity of purified hydrocortisone was almost same with that of the

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commercial hydrocortisone standard (about 97% purity) and could meet the requirements for quantitative and qualitative analyses.

Key Words: High-speed countercurrent chromatography; Hydrocortisone; Epimer; Diastereomer; Epi-hydrocortisone; Separation.

INTRODUCTION

It has been known that enantiomers or diastereomers of an optically active substance are very similar to each other in their structures, while they usually show significant differences in their properties. In the past 20 years, the characteristics of these substances has attracted much attention in many fields, such as materials, fine chemicals, and especially pharmaceuticals, and so on, and demands for optical purity substances increased greatly.

Extensive efforts have been made in developing chiral separation methods, and chromatographic techniques[1] are often used for their high efficiency. Countercurrent chromatography (CCC) is a support-free liquid–liquid partition chromatography with less sample loss and contamination compared with other chromatography. Since 1982, researchers[2] have tried to separate optically active substances by CCC, and chiral resolution of some amino acids and their derivates had been achieved using chiral mobile phases or chiral stationary phases.

Hydrocortisone and epi-hydrocortisone are steroid compounds. They only differ with each other in the chiral center of carbon 11 in configuration, while their other chiral centers are of the same configurations and, therefore, they are epimers. Hydrocortisone could be used as anti-inflammatory drug, while epi-hydrocortisone has no therapeutic effectiveness and could only be used as an intermediate. Researchers[3] have shown that the epimers could be baseline separated unless cycle separation technology was applied with solid–liquid chromatography. In the paper by high-speed countercurrent chromatography (HSCCC), the epimers were separated completely with appropriate solvent systems.

EXPERIMENTAL

Equipment and Materials

HSCCC was performed using a multilayer coil planet centrifuge (Beijing Institute of New Technology, Beijing, China), and the total column (1.6 mm i.d.) volume was 234 mL. The solvents were delivered with pumps (Beijing Institute of New Technology). Column effluents were collected with a BSZ-100 fraction collector (Shanghai Huxi Instrument, Shanghai, China). A Shimadzu

series LC-10Avp HPLC apparatus (Kyoto, Japan) was used for quantitative analyses.

The hydrocortisone standard (about 97% purity) was obtained from Sigma Chemical Co. (St. Lousis, MO). Mixtures of crude hydrocortisone and epi-hydrocortisone were obtained from North China pharmaceutical Factory (Shengyang, China).

Methanol used for HPLC was HPLC grade. Other solvents were analytical grade.

HSCCC Separation Procedure

A solvent system containing chloroform–methanol–water (10 : 5 : 5, v/v/v) was thoroughly equilibrated in a separatory funnel, and the two phases were separated just before use. Descending mode was applied in the separation. The column was first filled with the stationary phase (upper phase), and then the samples, dissolved in equal volume of the upper and lower phase, were injected. Then, the column was rotated at 800 rpm while lower phase was delivered into the column with a flow-rate of 2 mL/min. The effluent was detected on line at 254 nm with a UV detector, and collected in test tubes after hydrodynamic equilibrium had been established.

HPLC Analyses

For the analyses of hydrocortisone and epi-hydrocortisone, a Diomond-ODS column (200 × 4.6 mm^2 i.d.) (China) was employed at 40°C. Methanol–water (65 : 35, v/v) was used as the mobile phase with a flow-rate of 1 mL/min. The epimers were detected at 254 nm.[4]

Measurement of Partition Coefficients

A mixture of crude hydrocortisone and epi-hydrocortisone was used to measure their partition coefficients with the classical "shake-flask" method.[5]

RESULTS AND DISCUSSION

Selection of Solvent Systems

The partition coefficients of hydrocortisone and epi-hydrocortisone were different to some extent in their solvents of alcohols–water, ketones–water,

ethers–water, esters–water, chlorohydrocarbons–water, aromatic hydrocarbons–water, and cyclohexane–water due to their polarity differences, and the epimers were more easily dissolved in esters, chlorohydrocarbons, and especially, in alcohols and ketones.[4] In the seven series solvents, considering boiling points of the organic solvents, separation factors, and solubility of the epimers, a chloroform–water system was more suitable for HSCCC separation. Methanol was added as the third solvent for further improving the solubility of the epimers to increase the sample loads, while as the volume percentage of methanol increased the separation factor decreased, which was shown in Fig. 1.

It has been known that when the separation factor of two components to be separated is about 1.5,[6] they might be completely separated by HSCCC. For ensuring the separation, we chose the solvent system containing chloroform–methanol–water (10 : 5 : 5, v/v/v) for the epimers HSCCC separation, and the separation factor should be larger than 1.8 in the solvent system estimated from Fig. 1.

Preparative Separation by HSCCC with Chloroform–Methanol–Water (10 : 5 : 5, v/v/v)

A mixture of hydrocortisone and epi-hydrocortisone (34.7 mg) was separated by HSCCC, and the chromatogram was shown in Fig. 2. As can be seen in Fig. 2, the epimers were completely separated and the first base peak was

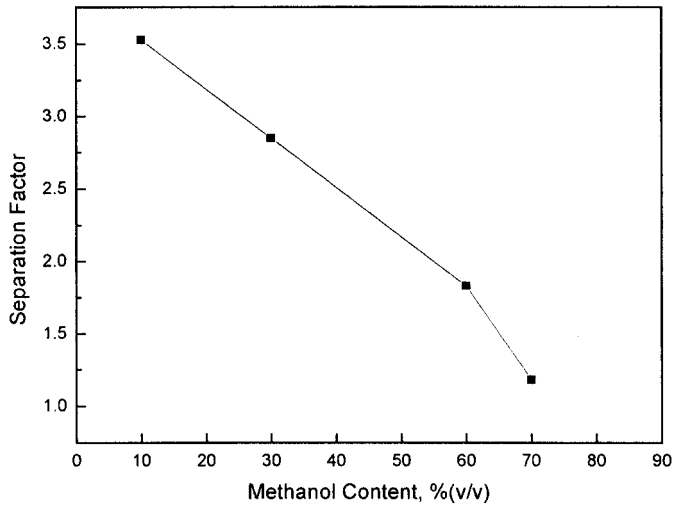

Figure 1. Separation factors in chloroform–methanol–water systems with different methanol contents.

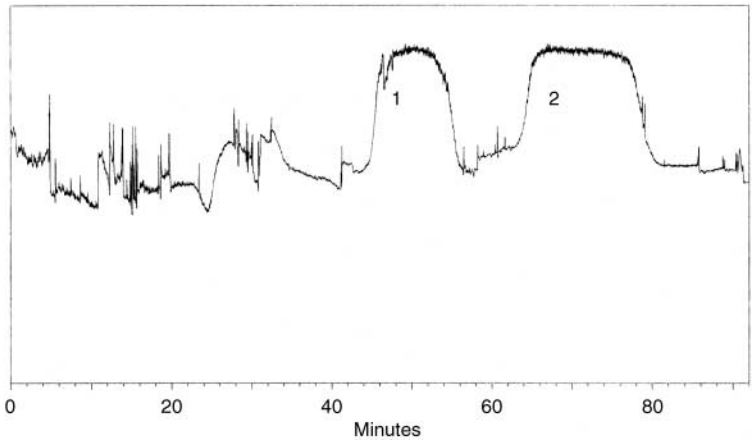

Figure 2. HSCCC chromatogram of hydrocortisone and epi-hydrocortisone.

hydrocortisone with chloroform being used as the mobile phase; baseline noise was noticeable. There are many reasons that cause noises, such as the properties of the solvents, the operation conditions, and so on. Researchers[7,8] found that heating the effluent before it goes into the detector, increased the pressure of the outlet, or applying other detectors such as an evaporative laser-light-scattering detector, could help to decrease the noise. In this paper, by adding methanol into the effluent as the complementary solvent, the noises were also eliminated effectively, as shown in Fig. 3.

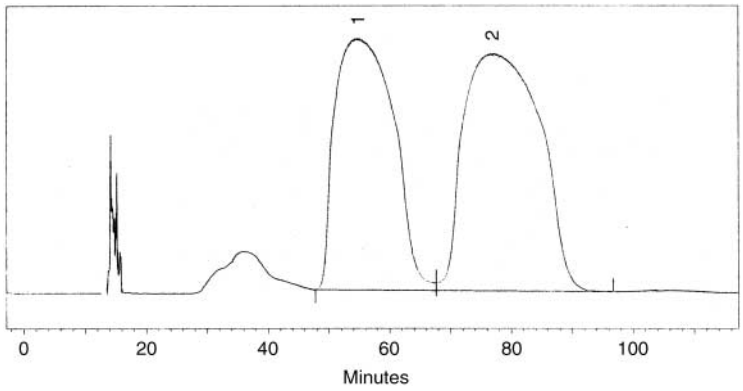

Figure 3. HSCCC chromatogram of hydrocortisone and epi-hydrocortisone (methanol as complementary solvent, 0.7 mL/min).

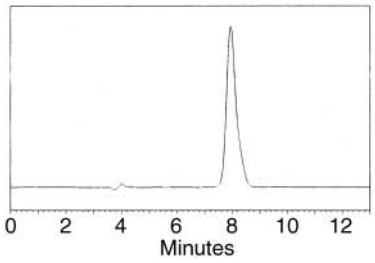

Figure 4. HPLC chromatogram of purified epi-hydrocortisone.

By collecting all the fractions of peaks 1 and 2, respectively, purified hydrocortisone and epi-hydrocortisone were obtained. Their purities were analyzed by HPLC and MS, as shown in Figs. 4–6. As can be seen from Fig. 4 and Fig. 5, impurities in the purified hydrocortisone and epi-hydrocortisone were not notable, and the purity of hydrocortisone was about 97% which was almost same with that of the respective standard by HPLC analyses. Figure 6 showed the results of MS for further detecting the impurities in purified hydrocortisone and epi-hydrocortisone. In Fig. 6, A, B, and C referred to the MS spectrum of hydrocortisone standard, purified hydrocortisone, and epi-hydrocortisone, respectively. In the three spectra, ions of m/z 363.3 and 385.2 were always observed as the base peaks with significant abundance, which were molecular ion peaks of hydrocortisone or epi-hydrocortisone in different forms, other peaks of impurities were negligible.

CONCLUSION

Complete separation of hydrocortisone and epi-hydrocortisone had been achieved using HSCCC. Purified hydrocortisone and epi-hydrocortisone

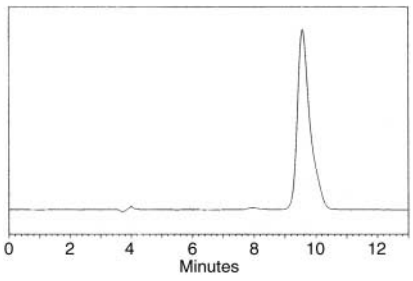

Figure 5. HPLC chromatogram of purified hydrocortisone.

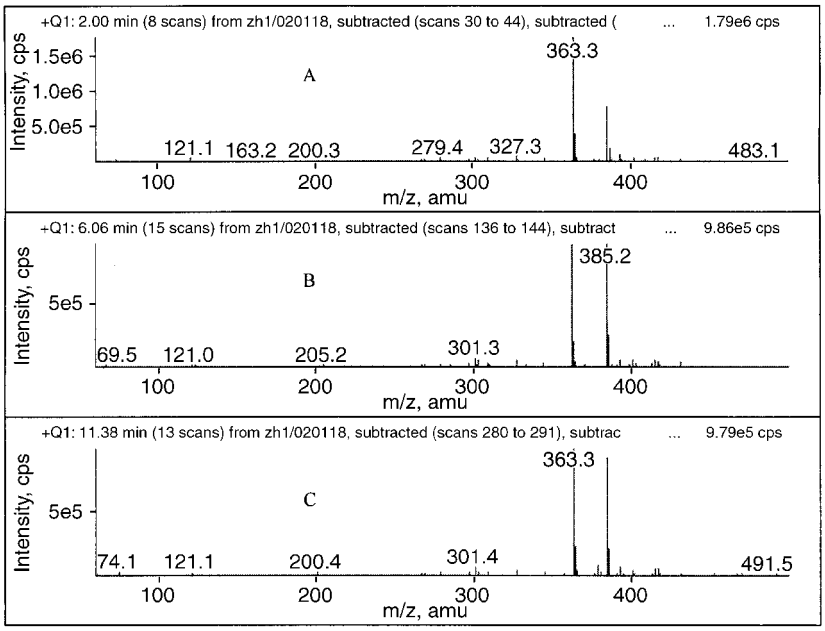

Figure 6. MS spectra of hydrocortisone and epi-hydrocortisone.

were obtained with high-purity analyzed by HPLC and MS. Chiral resolution is usually difficult in many cases, because it is not easy to find the suitable chiral selectors providing sufficient separation factors for the enantiomers to be separated. While, for epimers like hydrocortisone and epi-hydrocortisone with some difference in partition coefficients in some solvents, in this study HSCCC has been proven to be a better method of separation for high efficiency and easy operation. By calculating the solvent compositions[9,10] or referring to the phase diagram, the two liquid phase solvents could be solely prepared and, thus, more hydrocortisone and epi-hydrocortisone with high purity could be obtained easily with less solvent wastes.

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