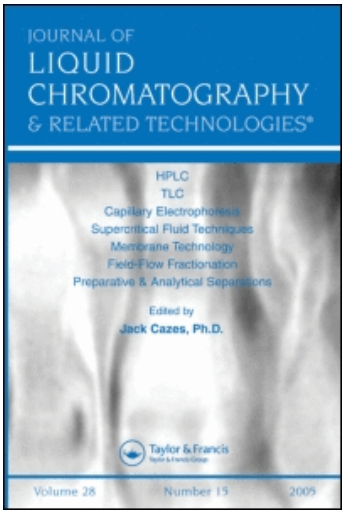

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RECYCLING HIGH-SPEED COUNTERCURRENT CHROMATOGRAPHY FOR SEPARATION OF TAXOL AND CEPHALOMANNINE

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ABSTRACT

A mixture of taxol and cephalomannine was subjected to recycling countercurrent chromatography (CCC) which allows the use of the same column for repetitive separation of the peak fractions to improve the peak resolution. When the total amount of 50 mg of the two components was recycled twice, peak resolution (R_s) increased from 0.7 to 1.27. The results also showed that R_s value increases according to the formula: $R_{s\text{-}n} = n^{1/2}R_{s\text{-}1}$ where n is the number of CCC runs, and $R_{s\text{-}1}$ and $R_{s\text{-}n}$, peak resolutions after the first and the nth run, respectively.

INTRODUCTION

Since the column capacity of the high-speed CCC instrument is constant, the peak resolution (R_s) of some components is limited and complete separation often requires the use of a longer separation column. In this case, the following two options are available: (1) Use multiple CCC instruments by connecting the columns in series;[1] and (2) using a single CCC instrument recycle the effluent through the same column. In this paper, we separated a mixture of taxol and cephalomannine by high-speed CCC using the above recycling method.

EXPERIMENTAL

Apparatus

High-speed CCC experiments were performed using a coil planet centrifuge equipped with a multilayer coil separation column mounted at a distance of 8 cm from the central axis of the apparatus. The apparatus was designed and fabricated at the Beijing Institute of New Technology Application, Beijing, China. The multilayer coil was prepared by winding a 1.6 mm ID PTFE (polytetrafluoroethylene) tube coaxially onto the column holder hub. The total column capacity measured 230 mL. The high-speed CCC centrifuge was rotated at 800 rpm to provide a force of about 56 x g on the axis of the holder. The system was equipped with an FMI pump (Zhejiang Instrument Factory, Hangzhou, China), an injection valve, a UV detector (UV-752, Shanghai, China), and a recorder.

Reagents

Hexane, ethyl acetate and methanol were analytical grade and purchased from Shanghai Chemical Factory, Shanghai, China. Taxol standard was purchased from Sigma Chemical Company, St Louis, MO, U.S.A. Cephalomannine standard was a gift from Zhejiang Medical Academy, China. A mixture of taxol and cephalomannine from *Taxus yunnansis* was purchased from Zhejiang Medical Academy. It consisted of 40% taxol and 51% cephalomannine.

Recycling High-Speed CCC Procedure

The high-speed CCC experiments were performed with a two-phase solvent system composed of hexane-ethyl acetate-methanol-water (6:4:5:5, v/v/v/v). The mixture was thoroughly equilibrated in a separatory funnel at room temperature and

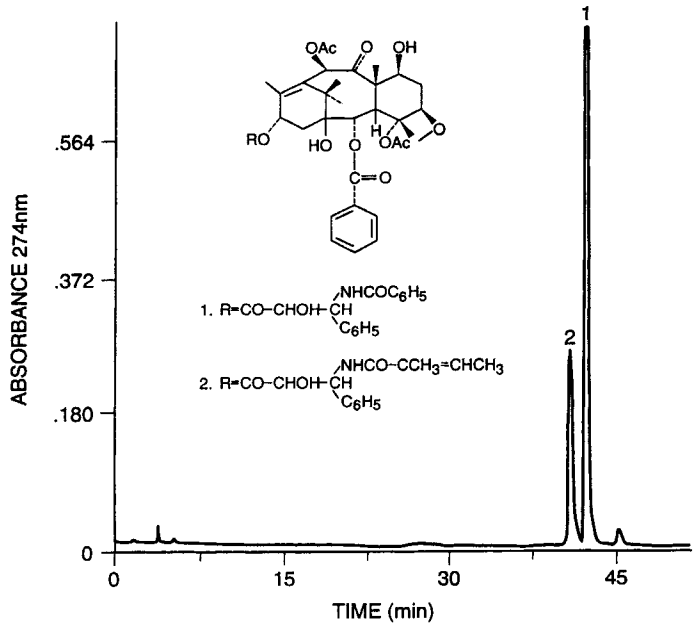

Figure 1. HPLC analysis of the original sample containing taxol 40% and cephalomannine 51%.

the two phases separated shortly before use. In each experiment, the multilayer coil separation column was first entirely filled with the organic stationary phase. This was followed by sample injection through the sample port. Then the aqueous mobile phase was eluted through the column while the apparatus was rotated at 800 rpm. Both sample injection and mobile phase elution was performed at a flow rate of 1.7 mL/min.

The effluent was continuously monitored with a UV monitor at 274 nm. When the target component began to elute, it was collected into a 1.6mm ID PTFE tube with a suitable capacity.

After all the components eluted from the column, the pump was stopped and the PTFE tube containing the peak fractions was inserted between the pump outlet and the inlet of the column to start the first recycling run. This recycling run was repeated until the two components were completely resolved (Fig. 2 B,C,D).

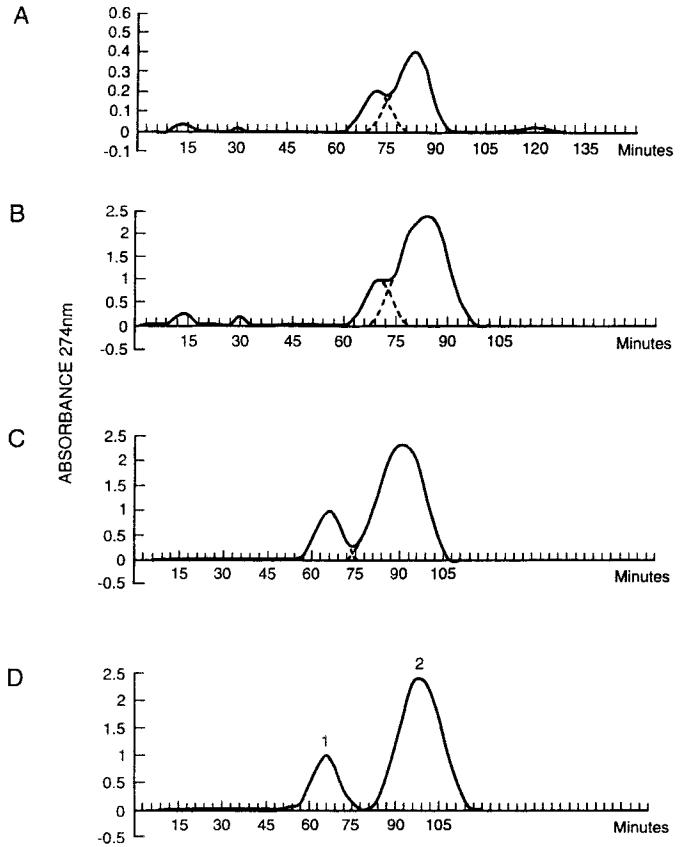

Figure 2. High-speed CCC separations of taxol and cephalomannine. A: 10 mg of the mixture dissolved in 3 mL of the mobile phase; B: 50 mg of the mixture in 3 mL of the mobile phase; C: the first recycling of the peak fractions obtained from B; D: the second recycling of the peak fractions obtained from C.

HPLC Analysis of Taxol and Cephalomannine

HPLC analysis of taxol and cephalomannine was performed with a Waters HPLC (Waters Chromatography Div., Millipore Co., Milford, MA, U.S.A.) consisting of a Model 510 pump, a Model 717 Auto-Injector, a Model 996 PDA detector and a Millennium 2010 data processor. HPLC separations were performed on a μ-Bondapak C_{18} column, 0.46 x 25 cm (Waters).

Table 1

Peak Broadening of Taxol and Cephalomannine During CCC Separation

	W (mg)	V_1 (mL)	V_2 (mL)	Peak Broadening (Times)	In Fig. 2
T	5.1	3	30	10	A
C	4.0	3	45	15	A
T	25.5	10	50	5	B
		50	55	1.1	C
		55	60	1.1	D
C	20.0	10	35	3.5	B
		35	37	1.1	C
		37	40	1.1	D

W: weight of component in the sample; V_1: injected volume; V_2: volume of the corresponding peak; Peak Broadening = V_2/V_1; T: taxol; C: cephalomannine.

The mobile phase, composed of solvent A (CH_3CN:H_2O, 20:80)(starting medium) and solvent B (CH_3CN), was eluted at 1 mL/min with a linear gradient from 0% to 90% B for 50 minutes while the effluent was monitored at 274 nm (Fig. 1).

RESULTS AND DISCUSSION

Fig. 2 A and B are chromatograms obtained by single runs from 10 mg and 50 mg of the sample, respectively. Fig. 2 C and D show the first and second recycled chromatograms using fractions obtained from 50 mg of the sample (Fig. 2 B). The fractions corresponding to peaks 1 and 2 in Fig. 2D were each pooled separately and lyophilized to yield 25 mg of taxol (peak 2) and 19.2 mg of cephalomannine (peak 1) as analyzed by HPLC.

As clearly shown from the diagrams (Fig. 2 B-D), the recycling of the peak fractions gives steady improvement of peak resolution. This recycling method is much more efficient than repeating the separation by pooling the collected fractions and mixing them before charging into the column for the next run.[2]

Based on the chromatograms shown in Fig. 2, one can compute the degree of peak broadening of taxol and cephalomannine during the separation. From Table 1, it is seen that the sample band width is not significantly affected by the original sample volume introduced into the column. More important, the broadening of each peak is almost negligible in the recycling runs.

From the chromatograms in Fig. 2, the peak resolution (R_s) of the two components is computed according to the following formula:

$$R_s = 2(R_2 - R_1)/(W_1 + W_2) \quad (1)$$

where W_1 and W_2 are peak widths and R_1 and R_2, the retention times of the respective peaks. Based on Fig. 2 B - D, in the first separation (B) the peak resolution R_s of the two peaks is 0.70 which is improved to 1.03 in the second run (C) and to 1.27 in the third run (D). The relationship between the number of CCC runs and peak resolution in the recycling operation may be expressed in the following formula:

$$R_{s\text{-}n} = n^{1/2}R_{s\text{-}1} \quad (2)$$

where n indicates the number of CCC runs, and $R_{s\text{-}1}$ & $R_{s\text{-}n}$, the peak resolution after the first and the nth runs, respectively. Thus, the desired R_s values can be obtained by increasing n and the elution time for the separation of closely related compounds.

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REFERENCES

1. Q.-Z. Du, Q.-K. Cheng, M.-J. Li, T.-Y. Zhang, Chinese J. Chromatogr., **12(2)**, 130-131 (1994).

2. C. Rao, X. Liu, P.-L. Zhang, W.-M. Chen, Q.-C. Fang, Chinese Acta Pharmaceutica Sinica, **26(7)**, 510-514 (1994).

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