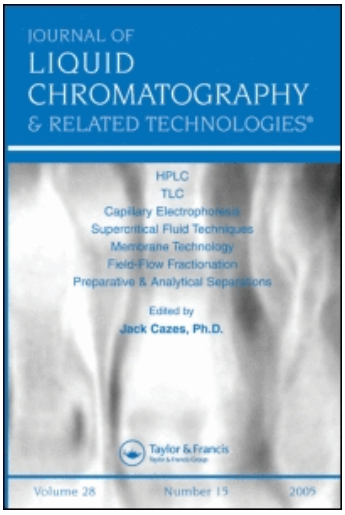

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Fu-Ying Chiou[a]; Pei Kan[a]; I-Ming Chu[a]; Chau-Jen Lee[a]
[a] Department of Chemical, Engineering National Tsing Hua University Hsinchu, Taiwan

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SEPARATION OF TAXOL AND CEPHALOMANNINE BY COUNTERCURRENT CHROMATOGRAPHY

Fu-Ying Chiou, Pei Kan, I-Ming Chu,* Chau-Jen Lee

Department of Chemical Engineering
National Tsing Hua University
Hsinchu, 300, Taiwan

ABSTRACT

Countercurrent chromatography was used to separate taxol from cephalomannine, on a preparative scale for the final purification of taxol. A solvent system consisting of hexane:ethyl acetate:methanol: ethanol:water in 5:7:5:1:6.5 was found to be suitable in achieving near base-line resolution of the two components. It was found that the amount of methanol in the solvent system was very critical to a successful separation operation.

INTRODUCTION

Taxol[1,2] has been found to be active toward various kinds of cancers and is a major focus of research in chemotherapeutic agents during recent years. Typically, barks, needles or twigs from trees of *Taxus* genus were ground and extracted by a series of polar or non-polar solvents, such as methylene chloride, methanol and, in some cases, supercritical carbon dioxide, to get rid of most

contaminants and recover taxol.[3,4,5] However, to separate cephalomanine, a contaminant that is most difficult to separate taxol from, which is present in most plant sources in about half as much as taxol, higher resolution methods are required. Column chromatography using silica gel with normal phase elution is widely used.[6]

In preparation of pure taxol in gram quantities for laboratory uses, countercurrent chromatocraphy (CCC) provides a convenient altenative.[7] This technique uses no solid particles and can adjust to the variable conditions of crude extract by adjusting solvent composition to achieve good results. Search for suitable solvent systems is the first step of CCC separation. A successful system was found and reported below.

MATERIALS AND METHODS

Taxol was purchased from suppliers of Yunnan, China in partially purified form. The off-white powder contains about 75 wt % of taxol and 25 wt % cephalomannine. Hexane, ethyl acetate, anhydrous ethanol and methanol were of HPLC grade. The countercurrent chromatography device used was Model CCC-1000 from PharmaTech, Baltimore, USA. Three consecutive coaxial multilayer coil columns were used with a total volume of 320 mL.

An appropriate amount of solvents were mixed and two phases were equilibrated. The top phase was chosen to be the stationary phase and filled the columns first and then the columns were rotated at 1000 rpm. Mobile phase was then pumped in via an HPLC pump at a rate of 1.5 mL/min. After the mobile phase fluid emerged at the exit of the columns, 1.0 mL of sample was injected. The sample contained 6.1 mg of the partially purified taxol, dissolved in 50:50 stationary and mobile phase. Fractions were collected in a size of 6.0 mL, and analyzed by HPLC. A C_{18} reverse phase column (LiChroCART 100, 5μm, Merck) was used, and taxol was detected at 229 mn. The mobile phase used for HPLC analysis was composed of water/methanol (60: 40) at a flow rate of 0.5 mL/min.

Partition coefficients measurement was made for taxol and cephalomannine in liquid-liquid two-phase systems. Partially purified taxol was dissolved in equilibrated two-phase solution. Samples were drawn from two phases and analyzed for taxol and cephalomannine. Samples from the top phase were first vacuumed to dryness and redissolved in the methanol before the HPLC analysis.

Table 1

Partition Coefficients of Taxol and Cephalomannine in the Solvent Systems*

Systems	Hexane	E.A.	MEOH	EtOH	Water	Pt	Pc
1	5	7	4	1	6.5	5.67	4.31
2	5	7	5	1	6.5	1.80	1.42
3	5	7	6	1	6.5	1.09	1.09
4	5	7	7	1	6.5	0.20	0.19
5	5	7	8	1	6.5	0.29	0.27

E.A.: ethyl acetate
Pt: partition coefficient of taxol
Pc: partition coefficient of cephalomannine
* expressed in volume ratio of their constituents

RESULTS

A basic formulation of the solvent system was first chosen, considering past experiences in CCC separation and taxol's general properties, such as its hydrophobicity. The hexane-ethyl acetate-methanol (or ethanol)-water system was commonly used in CCC for the separation of slightly hydrophobic components. The top phase was rich in hexane and the bottom phase was rich in water. Preliminary tests revealed that, in the hexane-ethyl acetate-ethanol-water system (5: 7: 4: 6.5), the elution times for both taxol and cephalomannine were very long compared to the mobile phase elution time. Thus a typical run would require up to 12 h for taxol or cephalomannine to exit the columns. This is caused by the high partition coefficients in this system, i.e. high affinity for the upper phase (stationary phase) by taxol and cephalomannine. Thus, this was not a practical system for the separation. The system using methanol instead of ethanol, e.g. hexane-ethyl acetate-methanol--water (6: 4: 6.5: 3.5) suffered from the opposite problem, i.e., very low affinity for the upper phase by the two components; thus, insufficient resolution. The two components were not separated at the exit, and emerged in one peak.

It seemed reasonable to try using both ethanol and methanol in the solvent system, as their opposite effects on affinity to the upper phase by these two solvents may cancel out. Partition coefficients of taxol and cephalomannine in a series of solvent systems were measured. The results are shown in Table 1.

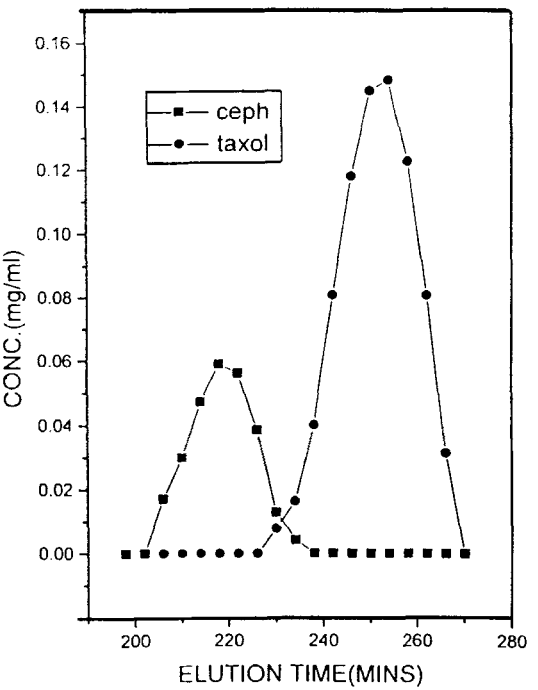

Figure 1. Elution profiles for taxol and cephalomannine. ●: taxol, ■: cephalomannine.

It was somewhat surprising to find that the amount of methanol in this solvent system had a major impact on the partition behavior of taxol and cephalomannine. The reason for this particular phenomenon was not determined.

The solvent system chosen for the CCC separation system was hexane-ethyl acetate-methanol-ethanol-water in 5: 7: 5: 1: 6.5 (system 2 in Table 1), since this was the only one that would result in suitable elution time and separation factor. Rotational speed of the CCC was set at 1000 rpm, the normal operating speed for this model. Mobile phase flow rate was 1.5 mL/min. The stationary phase retention was 264 mL in 320 mL total volume. Thus the mobile phase volume was 56 mL, the elution time for the mobile phase was thus 38 min. As shown in Fig. 1, cephalomannine was ahead of taxol, and near base-line resolution was achieved. The number of theoretical

plates was calculated to be 560 and the resolution was 0.98. The results were consistent with the partition coefficients (taxol: 1.80, cephalomannine: 1.42). Recovery of taxol was 90% with purity above 98%, when the collected fractions were dried under vacuum. These results can be helpful in scaling up the process in larger countercurrent chromatography units, in order to process gram levels of taxol in various kinds of extracts.

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