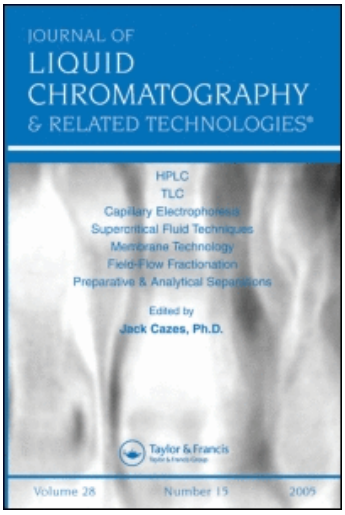

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Young Kwang Park[a]; Sung Taik Chung[a]; Kyung Ho Row[a]
[a] Department of Chemical Engineering, Inha University, Inchon, Korea

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PREPARATIVE CHROMATOGRAPHIC SEPARATION OF TAXOL FROM YEW TREES

Young Kwang Park, Sung Taik Chung, Kyung Ho Row

Department of Chemical Engineering
Inha University
253 Yonghyun-dong, Nam-ku
Inchon 402-751, Korea

ABSTRACT

Taxol shows promise for the treatment of a variety of human cancers, including leukemia and certain ovarian, breast, and lung tumors. In this work, the separation of taxol from Korean Yew tree was studied with solvent extraction and liquid chromatography. The content of taxol in the tree was analyzed to be 0.064 mg/g of dry powder. Methanol was used to extract taxol and the resulting extract was concentrated by the partition of water and methylene chloride. In the pretreatment step with open tubular chromatography, the first mobile phase was methylene chloride/acetone, 97/3 vol. % and the second mobile phase methylene chloride/acetone, 75/25 vol. %. The sample containing taxol was further purified by varying the composition of solvent, flow rate, and injection amount in analytical chromatography.

In a semi-preparative column (3.9×300 mm) with larger particle sizes the mobile phase composition was hexane/2-propanol/methanol, 90/4/6 vol.% in isocratic mode. Based on the mobile phase composition with 16 mL/min of mobile phase flow rate in a preparative column (22×250 mm), 2.41 mg of purified taxol was obtained in a single run.

INTRODUCTION

Taxol (Paclitaxel), an antitumor drug, isolated from the bark of the Pacific yew (*Taxus brevifolia*) has demonstrated clinical effectiveness in ovarian and breast carcinomas.[1] Discovered by Wall's group in 1967, taxol is a chemically and pharmacologically unique diterpene. The highly functionalized taxane diterpenes are known only from various species of Taxus and a few members of the Taxodiaceae.

Chromatographic methods have been developed to detect and isolate taxanes from the above sources on analytical and semi-preparative basis. A number of analytical HPLC methods exist, including the use of silica or C_{18} phase and reversed-phase separations on phenyl and cyano bonded silica.[2] These procedures suffer from insufficient selectivity among closely eluting taxanes. Additionally, the low solubility of taxanes in aqueous solutions often results in increased column back-pressure, caused by precipitation of products in the crude sample. In normal-phase, a preparative separation of taxol under the overload condition was conducted where high alcohol concentrations were used to increase sample solubility.[3]

Recently, the experimental work on the separation of taxol in an analytical scale has been performed at the High-Purity Separation Laboratory in Dept. of Chem. Eng., Inha Univ. In the commercial pentafluorophenyl (PFP) bonded silica column, the optimum mobile phases for binary and ternary system were water/acetonitrile, 60/40 (vol. %) and water/1-propanol/methanol, 96/2/2 (vol. %), respectively.[4] Also, taxol was separated by normal-phase Nova-Pak column with binary and ternary system in hexane/EtOH, 96/4 (vol. %) and hexane/1-propanol/methanol, 96/2/2 (vol. %), respectively.[5]

Based on the previous experimental results, we aim to find the condition of mobile-phase in preparative separation of taxol from the extracts of yew in normal-phase mode.

EXPERIMENTAL

The yew powder was provided by Forest Genetics Research Institute Suwon, Korea. Methanol (1L) was used to extract taxol from the dry powder (10 g). The concentrated methanolic extract (125 μL) was partitioned with chloroform ($CHCl_3$, 100 mL) and water (100 mL) for about 30 minutes and 10 hr. to allow for any emulsion to clear.[6,7] The solvent layer was drained off from the bottom into glass containers. The $CHCl_3$-extract was evaporated by a rotary evaporator (LABO-THERM SW 200, Resona Technics Co.), stripped of its solvent to a thick syrup. The syrup was dissolved into methanol and the concentration of 5,000 μg/mL was prepared for a subsequent step. The methanol solution of the extract was slowly passed through a open tubular

column (50×200 mm) packed with silica with the mobile phase of methylene chloride/acetone, 97/3 (vol. %). The eluant was changed to 30% acetone in methylene chloride and we obtained the band containing taxol.

The HPLC system consisted of a pump (Model 2326-26, TSP), Rheodyne 7125 injector (2 mL sample loop), pressure gauge (maximum pressure: 3000 psi, Span instrumant Co.), and Absorbance Detector (M720, Youngin Co.). The columns used were Nova-Pak Silica (60Å, 4 μm, 3.9×150 mm) and semi-prep column (3.9×300 mm) packed with Lichrospher Si 60 (15 μm). The dimensions of the preparative-scale column were 10 mm and 22 mm i.d. with 25 cm length. Effluents were monitored at 228 nm UV wavelength. The mobile phases were hexane, 2-propanol, 1-propanol, methanol, and ethanol in ACS grade purchased from J. T. Baker (Phillipsburg, NJ, U. S. A.).

The standards of cephalomannine, 10-deacetyltaxol, and taxol were cordially donated by NCI (the National Cancer Institute, Bethesda, MP, USA). In the analytical column, the injection volume and the flow rate of mobile phase were 5 μL and 1 mL/min, respectively. While in the preparative column, the experimental ranges were 25 μL~2 mL and 1.5~16 mL/min.[8] The columns were maintained at ambient temperature. The back-pressure problems were avoided by rinsing the column frequently with the same mobile phase.

RESULTS AND DISCUSSION

For preparative purification, the normal-phase mode has definite advantages over reversed-phase mode. Some of these are: use of solvents with low viscosity, high flow-rate (i.e., throughput), and simpler recovery of the products.[3,9,10,11] One of the difficulties in the currently employed isolation scheme is the separation of taxol and cephalomannine.

In 3 grams of Yew powder used in this experiment, 369.355 μg taxol was extracted by methanol. The methanol extract was further concentrated by the partition of water and methylene chloride. Impurities were removed by open tubular chromatography, where silica packings were packed. In the pretreatment the first mobile phase was methylene chloride/acetone, 97/3 (vol. %) and the second mobile phase methylene chloride/acetone, 75/25 (vol. %); and we collected the band containing taxol. The chromatogram of the effluent is shown in Fig. 1.

The experiments of separation were performed both in isocratic mode and gradient mode with 5 μL to 25 μL injection volumes and 1 ~ 1.5 mL/min mobile phase flow rates, respectively. The major mobile phase was hexane and the small amounts of ethanol, methanol, 1-propanol, and isopropanol were added to change the retention behavior.

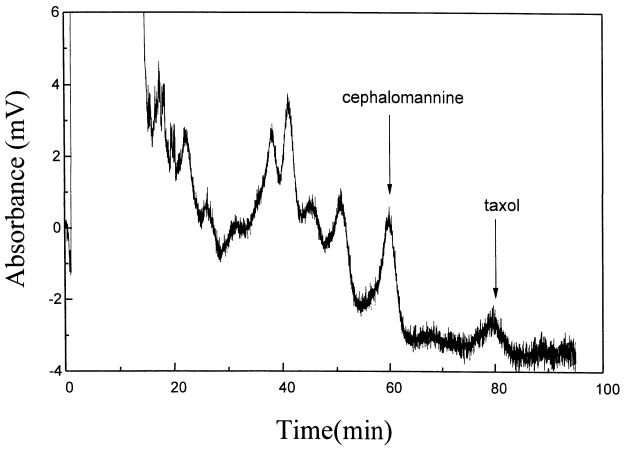

Figure 1. Elution profile of yew extract with methylene chloride/acetone, 75/25 (vol. %).

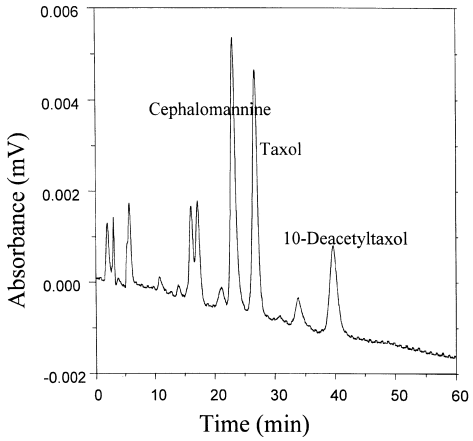

Figure 2. Analytical separation of taxol by Nova-Pak column (hexane/1-propanol/methanol, 96/2/2 (vol. %), 1 mL/min, 0.5μg).

Prior to a real sample, the artificial mixture of taxol, cephalomannine and 10-deacetyltaxol was separated. They were hard to be separated because of similar chemical structures. From the experimental results, in an analytical scale the ternary system was hexane/1-propanol/methanol, 96/2/2 (vol.%), and the chromatogram is shown in Fig. 2.

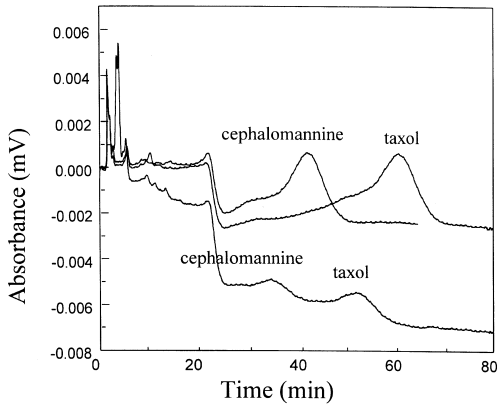

Figure 3. Separation of taxol in gradient mode (3.9×300 mm, 1st mobile phase : hexane/2-propanol/methanol, 86/4/10 (vol.%), for 17min, step change to the 2nd mobile phase: hexane/2-propanol/methanol, 92/2/6 (vol.%), 25 μg, 1.5 mL/min, 100 ppm).

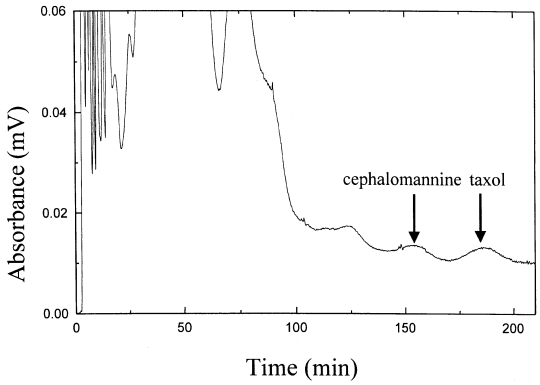

Figure 4. Separation of taxol in preparative-scale column (22×250 mm, 2 mL injection vol., flow rate : 16 mL/min, 2.41 mg).

The retention time of taxol was greatly influenced by 2-propanol and methanol in mobile phase, and the column efficiency was improved by methanol content. Even in the isocratic mode of hexane/2-propanol/methanol, 90/4/6 (vol. %), taxol was completely resolved from the real yew extracts. Fig. 3 shows the separation of taxol in gradient mode. The first mobile phase composition was hexane/2-propanol/methanol, 86/4/10 (vol. %) for 17 min and the second mobile phase was hexane/2-propanol/methanol, 92/2/6 (vol. %) in step-gradient mode.

The two larger columns of 10×250 mm and 22×250 mm were used to separate preparatively taxol from yew tree. In the smaller column (10×250 mm), the mobile phase composition was hexane/2-propanol/methanol, 88/4/8 (vol. %) and the effluent was collected from 35 min to 100 min. It was concentrated and injected into the column with the mobile phase in hexane/2-propanol/methanol, 90/4/6 (vol. %). In this procedure, pure taxol of 0.81 mg was obtained. In the larger column (22×250 mm), the mobile phase composition was hexane/2-propanol/methanol, 90/4/6 (vol. %) in isocratic mode (Fig. 4). Pure taxol of 2.41 mg was obtained.

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