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Short communication

Purification of taxol by industrial preparative liquid chromatography

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Abstract

A method has been developed for the preparative purification of taxol from a crude chloroform extract of *Taxus yunnanensis* using an industrial preparative liquid chromatography (IPLC) system based on polymeric stationary phase (D956 resin). Using single-system apparatus, about 5 kg of the crude extract could be loaded on a column initially, and finally, through three chromatographic runs, about 50-g quantities of taxol (>99% pure) could be obtained within 155 h without any organic solvent waste. The recovery of taxol was greater than 80%. The D956 resin has shown greater selectivity and capacity of taxol than silica gel and C_{18} materials. With the system, industrial-scale production of taxol could be realized.

Keywords: Preparative chromatography; Taxol

1. Introduction

Taxol is a widely-known diterpene amide that was initially isolated from the bark of the *Taxus brevifolia* [1] and showed unique antitumor and antileukemic activities. Although the total synthesis and semi-synthesis as well as the plant tissue culture of taxol have been reported, the yew bark and leaves are still the richest sources of taxol and related compounds. Hence, chromatographic methods have been developed to detect and isolate taxol from the above sources on analytical and semi-preparative basis [2–10]. However, the isolation procedures are complicated not only by the small amount of taxol present in these sources but also by the presence of many interfering compounds, especially cephalomannine and 7-epi-taxol that coelute with taxol. Hence, these procedures are mainly tied to small laboratory-scale chromatography and suffer from low selectivity, recovery and throughput of taxol. In this paper, the above problems were solved successfully by industrial preparative liquid chromatography (IPLC) based on polymeric stationary phase (D956 resin). The system could separate kilograms of the crude extract at a pressure of around 11 bar with a flow-rate range of 79~226 ml/min. Some experiments were carried out for comparison of D956 with silica gel and C_{18} phases. The result indicated that D956 phase could provide effective purification, high recovery, sustained usability and economy.

2. Materials and apparatus

The crude chloroform extracts prepared from the bark of *T. yunnanensis* were purchased from Yunnan province (China) which were determined to consist of 1.2% (w/w) taxol by analytical high-performance

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liquid chromatography (HPLC). The solvents used were of reagent grade except acetonitrile which was of chromatographic grade. The taxol standard was purchased from Sigma (USA). The analytical HPLC system purchased from TSP (USA) consisted of a SpectraSystem P2000 pump, a LINEAR 206PHD UV detector, a Spherisorb C_6H_5 column (250 mm×4.6 mm I.D., 5 μm), PC1000 2.6 chromatography software as well as a 20-μl sample injector. A stainless steel ''C-I'' column (250 mm×40 mm I.D.) was purchased from Tianjing Scientific Instrument (China) and was packed with C_{18} (55 μm). A glass ''S-I'' column (250 mm×26 mm I.D.) packed with silica gel (60 μm) was also used. An IPLC system was devised by us which consisted of a metering pump with a pre-pump filter, a stainless steel ''D-I'' column (4000 mm×200 mm I.D.) packed with D956 resin, a fraction collector and some auxiliary equipment. The D956 resin consisted of macroporous methacrylate–divinylbenzene copolymers synthesized by us. Wet density, particle size, specific surface, porosity and hole diameter were 1.21 g/ml, 60 μm, 350 m^2/g, 7.45 ml/g, 80 Å, respectively.

3. Results and discussion

A sample of the crude chloroform extract (4800 g) was dissolved in acetone (extract–acetone, 1:4, w/w) and filtered through 300 mesh stainless steel gauze filter. The filtrate was mixed with D956 resin (extract–resin, 1:5, w/w). The mixture was evaporated under vacuum at 30–40°C and the resulting powder was distributed on the top of the ''D-I'' column (4000 mm×200 mm I.D.) and then chromatographed with heart-cutting and batch-type recycling of three runs. A metering pump was used to propel the mobile phase. In the primary run, the column was eluted with 1 bed volume (BV) of acetone–water (40:60, v/v) followed by 2 BVs of acetone–water (50:50, v/v) and then 2 BVs of acetone–water (58:42, v/v) at a flow-rate of 226 ml/min. In the second and third runs, the taxol-rich fractions from the former run were combined and diluted in water. The resulting liquid sample was pumped into the regenerated ''D-I'' column and then eluted as above but the flow-rate was 79 ml/min. In each run, the fractions eluted with acetone–water (58:42, v/v) afforded taxol, purity of which was evaluated by analytical HPLC (>50% pure in primary run, >70% pure in second run and >98% pure in third run). The preparative chromatogram of the third run is shown in Fig. 1a. Afterwards, the taxol-rich fractions from third run were cooled and recrystallized at 4°C yielding 49.7 g of super-pure taxol products. The HPLC chromatogram of the products (Fig. 1b) indicated that the purity was greater than 99%.

Practically speaking, among the taxol-related compounds, cephalomannine and 7-epi-taxol are the most difficult to separate from taxol. However, the IPLC system was capable of separating taxol from these compounds completely with 0.32 g/h throughput of super-pure taxol.

A series of samples of super pure taxol, 0.75 g, 0.85 g, 1.0 g, 2.0 g, 4.0 g mixed with the same amounts of cephalomannine, respectively, were loaded on a ''D-I'' column (1000 mm×26 mm I.D.) and then were eluted with acetone–water (50:50, v/v) at a flow-rate of 1.4 ml/min, respectively. The results (Fig. 2) showed that the column efficiency (N) steeply decreased with the mass-load of taxol. However, when the mass-load of taxol increased from 1.0 g to 4.0 g, the resolution (R_s) between cephalomannine and taxol did not steeply decrease. This result coincided with the displacement-effect theory [11]. Hence, the rate of production of taxol could be enhanced significantly under mass-overloading conditions. According to Fig. 2, it was appropriate to load 2.0 g, i.e., 3.8 mg/ml BV, of the sample on the column.

Fig. 1a showed that taxol could be free of other related compounds except small amounts of cephalomannine and 7-epi-taxol before third chromatographic run, and furthermore, taxol of greater than 98% purity could be obtained in third run with a recovery of greater than 80%.

Up to now, most of reported separations of taxol have been based on silica and C_{18} columns. Because cephalomannine cannot be removed completely on silica columns, silica runs are generally used initially to achieve partially purified taxol and are then followed by C_{18} runs for improving further purity. Although super-pure taxol can be finally obtained, the selectivity, capacity, concentration of taxol in fractions are poor and the processes are not economical. To compare the D956 stationary phase with

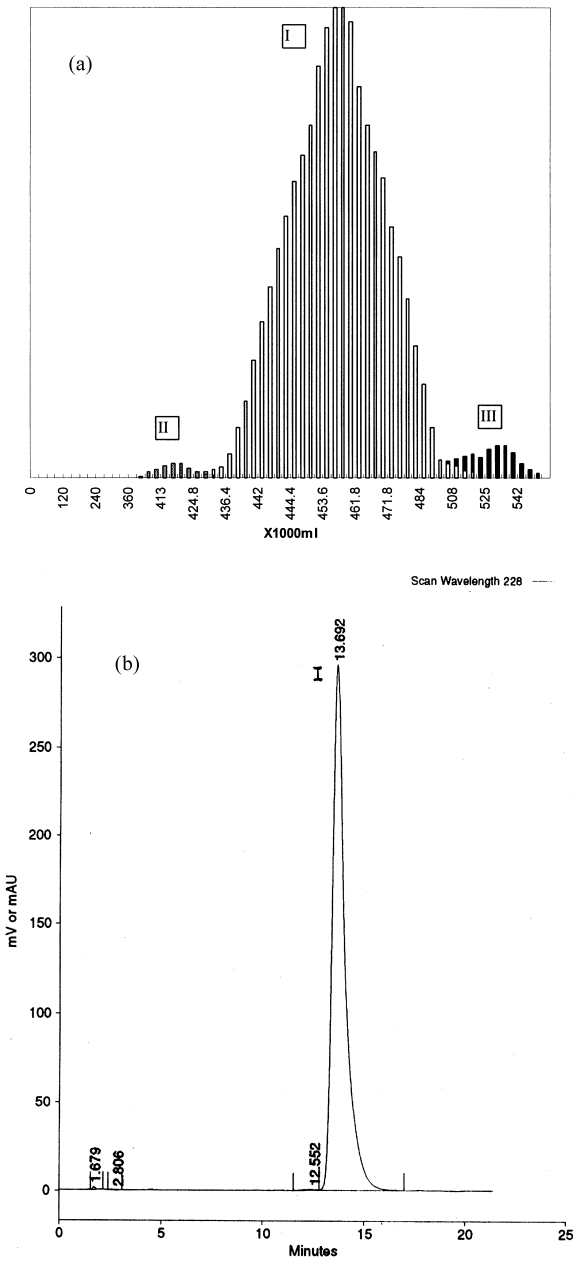

Fig. 1. (a) IPLC preparative chromatogram of third run. Conditions were as in Section 3. I=Taxol; II=cephalomannine; III=7-epi-taxol. (b) HPLC analysis chromatogram of super-pure taxol. Analytical conditions: column, Spherisorb C_6H_5 (250 mm×4.6 mm I.D.); mobile phase, MeOH–water–MeCN (20:42:38); flow-rate, 0.9 ml/min; wavelength: 228 nm; I=taxol.

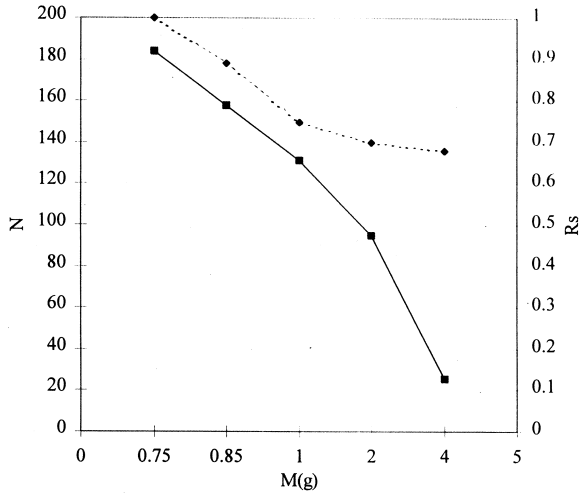

Fig. 2. Influence of mass-load of taxol on resolution (R_s) and column efficiency (N) in IPLC system. M=Mass-load of taxol; —=M~N curve; - - -=M~R_s curve.

silica gel and C_{18} phases, we obtained the following experimental results:

In the primary run, 3 g of the crude extract was loaded on the ''S-I'' column (200 mm×26 mm I.D.) and then chromatographed using linear gradient elution starting with 5% and ending with 80% of acetone in hexane at a flow-rate of 1.4 ml/min. The taxol-rich fractions were determined by analytical HPLC and the analytical chromatogram is shown in Fig. 3. In the third run, 5 ml of liquid sample which contained 10 mg taxol and a small amount of cephalomannine was injected onto the top of the ''C-I'' column (250 mm×40 mm I.D.) and then eluted with MeOH–water (70:30, v/v) at a flow-rate of 3.0 ml/min. The effluent was monitored by UV detection at 228 nm and the result shown in Fig. 4.

Comparison of Fig. 1a,b and Fig. 2 with Figs. 3 and 4 clearly indicates that the D956 stationary phase provided higher selectivity and capacity of taxol than silica gel and C_{18} phases. In addition, the IPLC system can be used repeatedly for up to as many as 50 times without degradation of recovery and only 3.6 l of acetone in the mobile phase was consumed for 1 g of super-pure taxol. D956 resin could be applied to an industrial-scale column at a middle pressure due to its better permeability than that of silica gel or C_{18} phase. Although low flow-rates were employed in the system, the throughput of taxol was

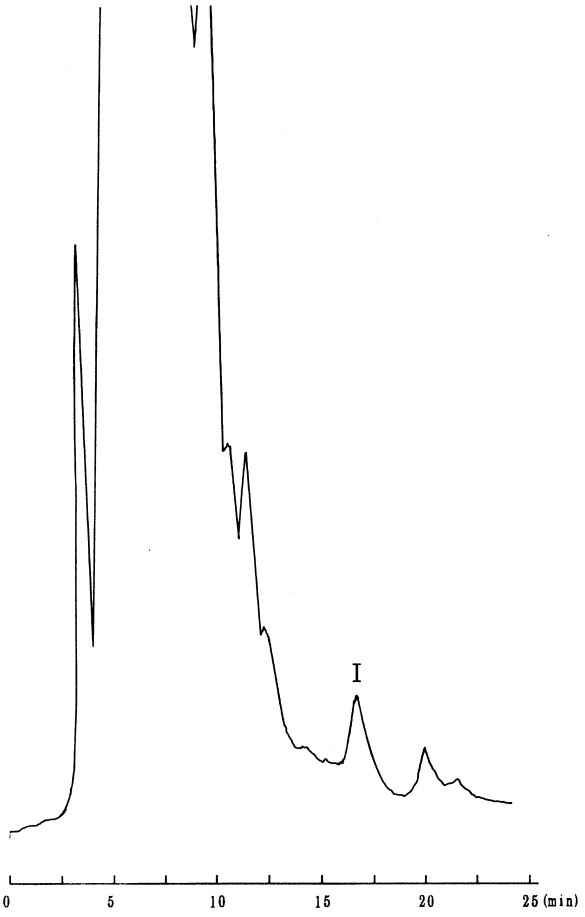

Fig. 3. HPLC analysis chromatogram of taxol-rich fraction eluted from the 'S-I' column. Analytical conditions: column, Spherisorb C_6H_5 (250 mm×4.6 mm I.D.); mobile phase, MeOH–water–MeCN (20:42:38); flow-rate, 0.8 ml/min; wavelength: 228 nm; I=taxol.

greater due to mass-overloading and good selectivity was obtained. Unlike the silica gel phase, the D956 phase did not adsorb taxol irreversibly and higher taxol recovery could be achieved.

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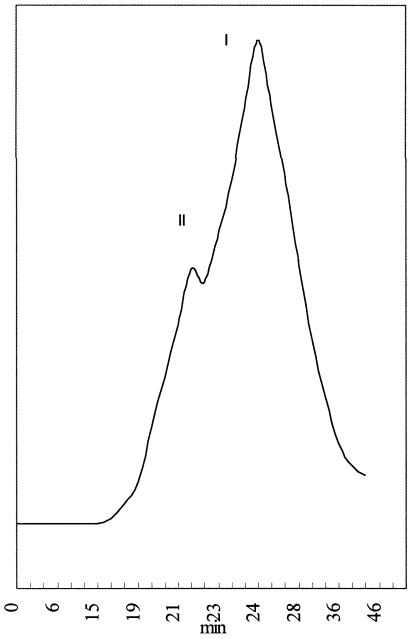

Fig. 4. Preparative chromatogram on the 'C-I' column loaded with a sample of mixture of taxol and cephalomannine. Conditions were as in Section 3. I=Taxol; II=cephalomannine.

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