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Preparative separation of taxol in normal- and reversed-phase operations

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Abstract

A method is described that separates taxane compounds from crude extracts under either reversed- or normal-phase conditions using a Zorbax-based bonded material, "SW-Taxane". A crude plant extract of *Taxus canadensis* and five standard taxane compounds were separated. The method provides high selectivity and recoveries of compounds from a taxol extract in the isocratic mode. Under normal-phase conditions, the packing material has shown significantly greater selectivity of taxol-related compounds than bare silica and most other commercially available silica-based bonded materials. Reversed-phase separations on the column, are also superior compared to typical C_8 separations. In addition, a loading study demonstrates that this phase has a high taxol saturation capacity due to its large surface area.

1. Introduction

Taxol has gained increased significance as a chemotherapeutic drug in the fight against cancer and is in great demand. Taxol may be isolated from plant extracts such as bark and regenerable parts of yews such as *Taxus brevifolia* [1,2] or *Taxus canadensis*. Plant tissue cultures [3,4], and partial or complete synthesis [5], are also being explored as alternative sources of these potential drugs with increased yields.

Chromatographic methods have been developed to detect and isolate taxanes from the above sources on analytical and semi-preparative basis [6–11]. Frequently, they use reversed-phase C_{18} and other bonded phases in gradient elution modes. 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 order to purify a large quantity of various compounds, preparative high-performance liquid chromatography (HPLC) is usually operated under the mass-overload condition. A considerable amount of literature on preparative HPLC in elution has been published by Knox and Pyper [12], Snyder and co-workers [13–18], as well as Guiochon and co-workers [19–25] in the past years. These studies have suggested that the rate of production of a purified product (i.e. throughput) in preparative HPLC can be significantly enhanced under the overload condition. Maximization of throughput can be achieved by optimizing separation conditions [26], column variables [27] and sample sizes [19,28]. It is also shown that the saturation capacities of solutes

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and their isotherms have an important influence on throughput in the preparative HPLC [29,30].

In this paper, we report a method for preparative purification of taxol using either normal- or reversed-phase systems in isocratic elution. In normal phase, a preparative separation of taxol under the overload condition was conducted where high alcohol concentrations were used to increase sample solubility. Loading studies were conducted to determine the saturation capacity of taxol using this method and column.

2. Experimental

2.1. Materials

Columns (25 × 0.46 cm) packed with Impaq 1010C8 (100 Å, 10 μm, C_8), 1010Si (100 Å, 10 μm, bare silica), and Zorbax "SW-Taxane" (60 Å, 10-μm silica particles, bonded with a proprietary phase) were from BTR Separations (Wilmington, DE, USA). Alkyl phenyl (60 Å, 10 μm) and pentafluorophenyl (PFP, 60 Å, 10 μm) columns (25 × 0.46 cm) were purchased from ES Industries (Berlin, NJ, USA). Taxol was obtained from Sigma (St. Louis, MO, USA). 10-Deacetylbaccatin III (10DAB), baccatin III (BIII), and 7-*epi*-taxol were from Hauser Chemical Research (Boulder, CO, USA). Hexane, ethanol, isopropanol and methanol were from EM Science (Gibbstown, NJ, USA). Heptane was from Fisher Scientific (Malvern, PA, USA). The series 410 LC pump, LC-95 UV–Vis detector, and Nelson 2700 chromatography software were purchased from Perkin-Elmer (Cupertino, CA, USA). A 7125 injector from Rheodyne (Cotati, CA, USA) was used for sample injection. We thank Biolyse (Port Daniel, Canada) for *T. canadensis* crude plant extract, which was prepared by methanol extraction and further by dichloromethane–water extraction as described in [31].

2.2. Methods

The dimensions of all the columns were 25 × 0.46 cm; no guard columns were used in this work. Unless stated otherwise, column effluents were monitored at a 227 nm wavelength; mobile phase flow-rate was 1 ml/min, and the columns were maintained at ambient temperature.

2.3. Comparison between SW-Taxane and other bonded phases

A 2–3-μl sample of 0.2–0.3 mg/ml per compound was injected into each column for comparison experiments. Separations of 10DAB, BIII, cephalomannine, taxol and 7-*epi*-taxol on Zorbax SW-Taxane, alkyl phenyl and PFP columns were conducted in normal phase at a flow-rate of 0.5 ml/min. For the Zorbax SW-Taxane, alkyl phenyl and PFP columns, the mobile phases were heptane–ethanol (50:50), heptane–ethanol (96:4) and heptane–ethanol (93:7), respectively. Separation of these five taxane standards on this medium was also carried out in the reversed-phase mode utilizing a methanol–water (60:40) mobile phase.

Separations of cephalomannine and taxol on Zorbax SW-Taxane were compared with C_8 in reversed phase, and with bare silica in normal phase. In reversed phase, the mobile phase was methanol–water (65:35). In normal phase, the mobile phase was heptane–isopropanol (60:40) for the Zorbax SW-Taxane column, and hexane–ethanol (90:10) for the bare silica column.

2.4. Purification of a crude plant extract of T. canadensis

Separations of crude plant extract on Zorbax SW-Taxane were carried out in both reversed and normal phases. In reversed phase, a 2-μl sample of 10 mg/ml (20 μg) was injected onto the column and the mobile phase was methanol–water (60:40). In normal phase, 12.5 mg of crude plant extract dissolved in 1 ml of mobile phase was loaded onto the column. In this preparative separation, the mobile phase was heptane–ethanol (75:25), with a flow-rate of 0.7 ml/min. The effluent was monitored at a wavelength of 260 nm. The fractions were collected every 30 s during the time interval 32–40.5 min. These

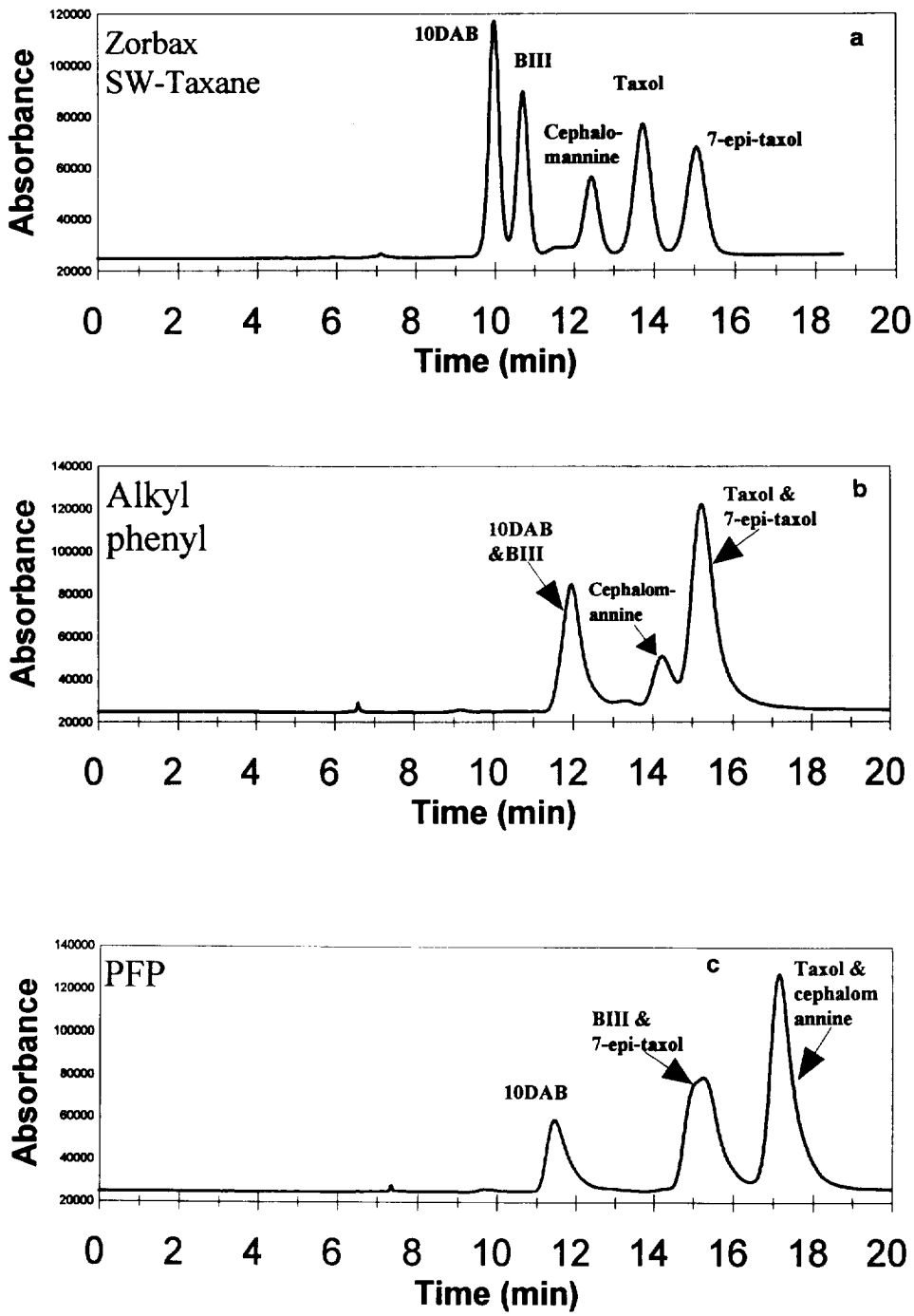

Fig. 1. Separation of a mixture of five standard taxanes containing 10DAB, BIII, cephalomannine, taxol and 7-*epi*-taxol in normal phase using (a) Zorbax SW- Taxane, mobile phase heptane–ethanol (50:50); (b) alkyl phenyl, mobile phase heptane–ethanol (96:4); (c) PFP, mobile phase heptane–ethanol (93:7). Column dimensions: 25 × 0.46 cm; sample concentration: 0.2–0.3 mg/ml per compound; sample volume: 2–3 μl; flow-rate: 0.5 ml/min; wavelength: 227 nm.

fractions were analyzed by re-injection into the column under reversed-phase conditions.

2.5. Column capacity study

Solutions of taxol and BIII were prepared at two concentrations (0.5 and 25 mg/ml for each compound). The mixtures were injected onto a column packed with Zorbax SW-Taxane at sample loadings of each compound from 2.5 μg to 1 mg and monitored at a wavelength of 270 nm. In normal phase, the mobile phase was heptane–ethanol (50:50). In reversed phase the mobile phase was methanol–water (70:30). The saturation capacity of taxol was calculated by [32,33]:

$$W_s = (3/8) \cdot [N_0 N/(N_0 - N)] \cdot [k_0'/(1 + k_0')]^2 W_x$$

where W_x, W_s, N_0, N and k_0' are sample load, saturation capacity, column efficiency at analytical loading, column efficiency at the sample load, and capacity factor at analytical loading, respectively.

3. Results and discussion

3.1. Comparison between SW-Taxane and other bonded phases

Fig. 1 presents separations of five standard taxanes on SW-Taxane, alkyl phenyl and PFP columns in normal phase. The method using the SW-Taxane column demonstrates a baseline resolution at high alcohol (i.e., ethanol) concentrations. In preparative taxol purification, high alcohol concentration is necessary to increase the solubility of the taxol components and to enhance the throughput of the process. Additionally, use of high alcohol concentrations decreases the likelihood of precipitation which often results in increased back-pressures and shortened column lifetime. All three columns performed equally well under reversed-phase conditions. Results of separations on Zorbax SW-Taxane phase are shown in Fig. 2. In addition, separation of cephalomannine and taxol on SW-Taxane was compared with that on bare silica and C_8. The Zorbax SW-Taxane results in a better

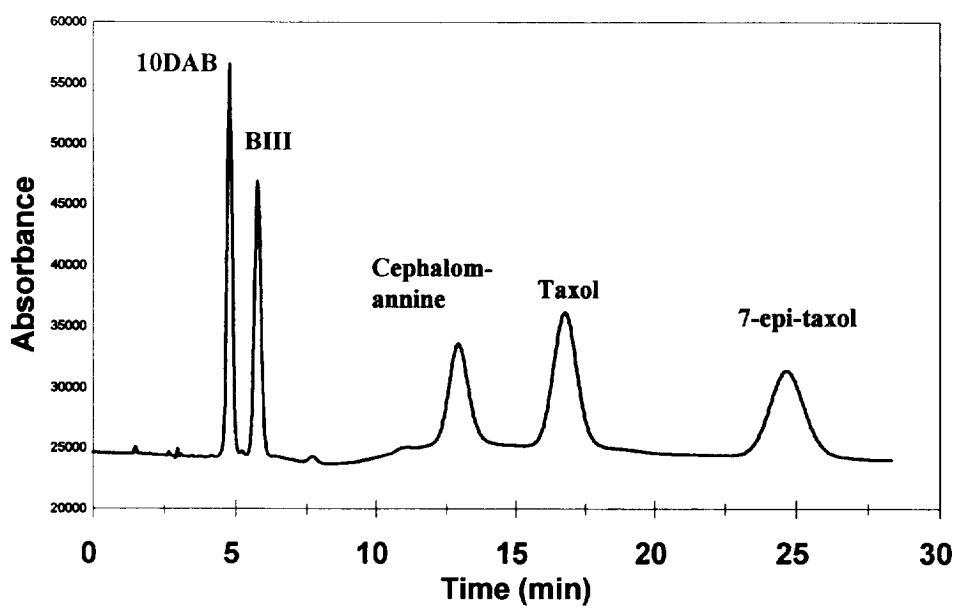

Fig. 2. Separation of a mixture of five standard taxanes containing 10DAB, BIII, cephalomannine, taxol and 7-*epi*-taxol in reversed phase using Zorbax SW-Taxane. Mobile phase: methanol–water (60:40); flow-rate: 1 ml/min. Other conditions as in Fig. 1.

resolution between cephalomannine and taxol than bare silica in normal phase (Fig. 3), and C_8 in reversed phase (figure not shown).

3.2. Purification of a crude plant extract of T. canadensis

Separation of taxol from crude extract of *T. canadensis* in reversed phase is especially useful for those interested in analytical studies (Fig. 4). However, for preparative purification, the normal-phase mode has definite advantages over reversed phase. Some of these are: use of solvents with low viscosity, high flow-rate (i.e., throughput), and simpler recovery of the products. Fig. 5 shows a normal-phase, preparative taxol purification loading 12.5 mg of crude extract of *T. canadensis* on a SW-Taxane column.

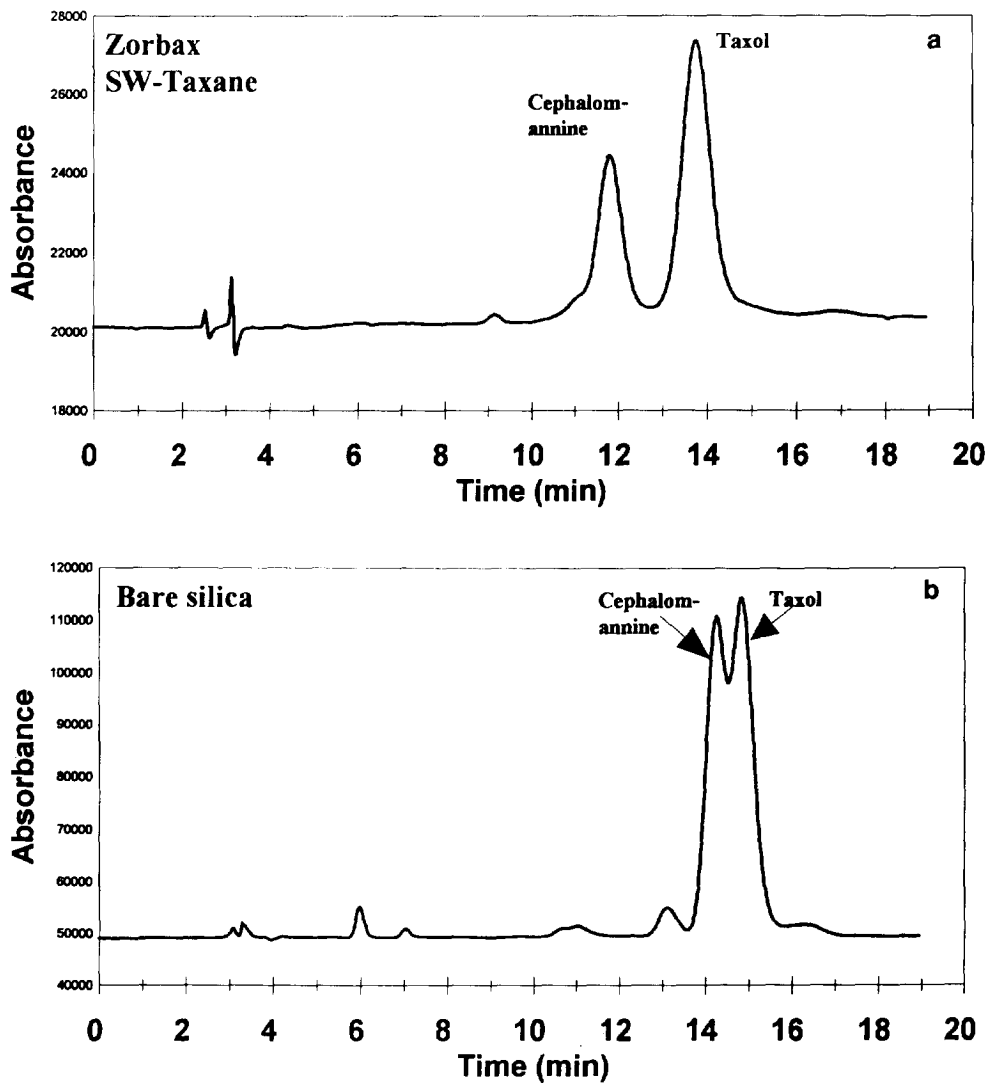

Fig. 3. Comparison of separation of cephalomannine and taxol in normal phase using (a) Zorbax SW-Taxane (mobile phase heptane–isopropanol, 60:40), and (b) bare silica (mobile phase hexane–ethanol, 90:10). Flow-rate: 1 ml/min. Other conditions as in Fig. 1.

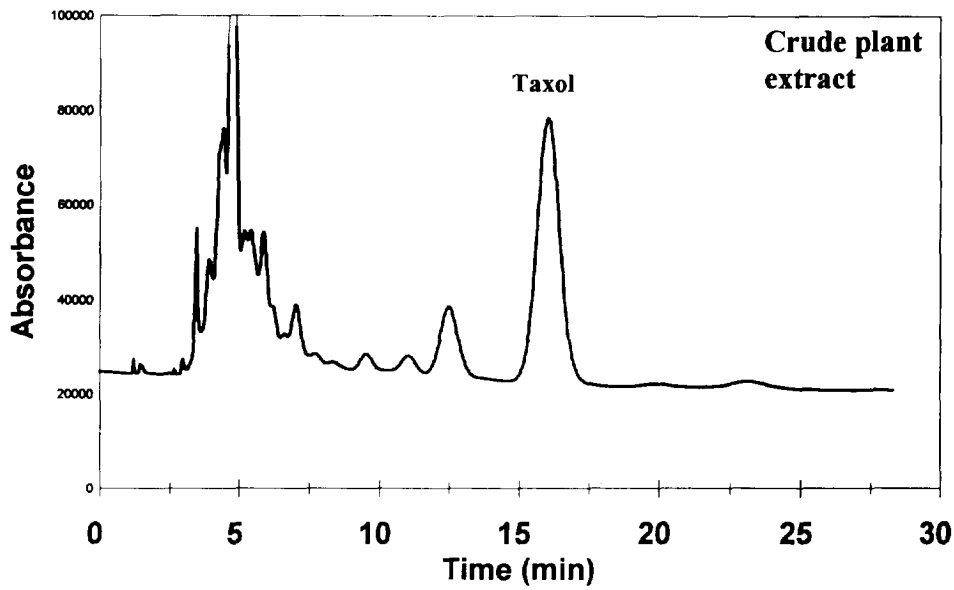

Fig. 4. Separation of taxol from crude plant extract of *T. canadensis* in reversed phase using Zorbax SW-Taxane. Mobile phase: methanol–water (60:40); flow-rate: 1 ml/min; sample concentration: 10 mg/ml. Other conditions given in the Experimental section.

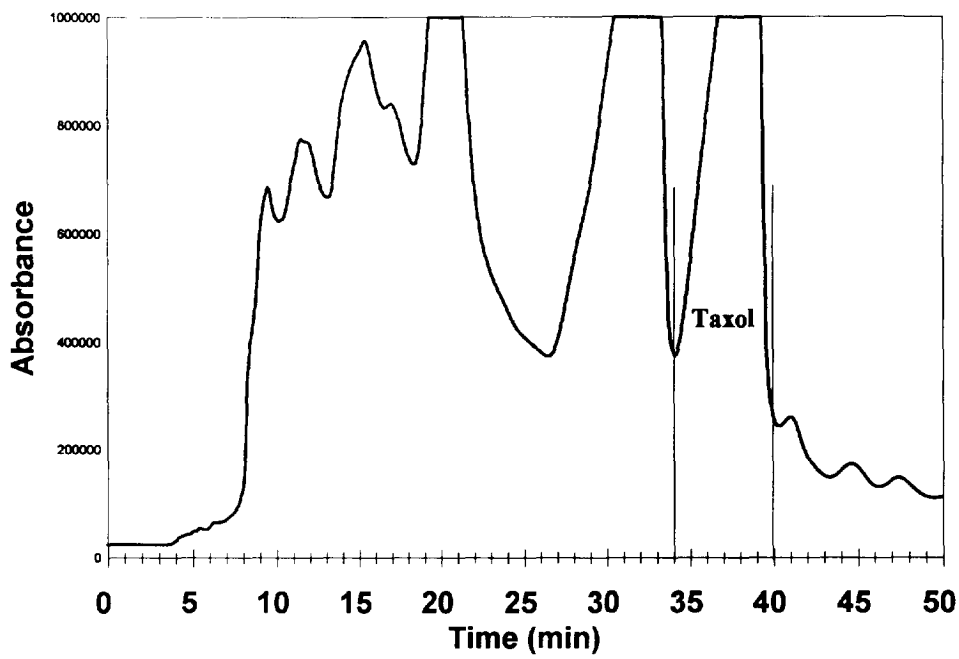

Fig. 5. Preparative separation of taxol from crude plant extract of *T. canadensis* in normal phase using Zorbax SW-Taxane. Mobile phase: heptane–ethanol (75:25); sample concentration: 12.5 mg/ml; sample volume: 1 ml; flow-rate: 0.7 ml/min; wavelength: 260 nm. Other conditions given in the Experimental section.

The rear part of the taxol peak is steeper than the front part, which indicates taxol follows non-Langmuir isotherms (e.g., S-shape). The taxol fractions were collected and analyzed (Fig. 6). These pooled taxol fractions (from 34 to 40.5 min) having a purity of about 98% (due to trace amounts of 7-*epi*-taxol), are free of cephalomannine. The recovery of taxol is greater than 90%. Practically speaking, a two-step process may be more practical for purification of crude extracts: partial purification by C_8 or bare silica, and then final polishing step using the methods outlined above.

3.3. Column capacity study

Saturation capacity (W_s) is the maximum mass of solute that can be taken up by the stationary phase when all the active sites of the absorbent surface are covered by a monolayer of solute molecules (Langmuir adsorption). The value of W_s, increasing with the higher surface area of the stationary phase, can be obtained either from isotherm data or from elution profiles under overload conditions [32,33]. Actual values of W_s per unit surface area can vary widely from 0.02 to 0.6 mg/m^2 for small molecules [29-30,34]. For adsorption in a flat configuration, the value of W_s per unit surface area is estimated to be 0.3 to 0.4 mg/m^2 [29,30]. Fig. 7 shows separation of taxol and BIII in normal phase at sample loadings ranging from 2.5 μg to 1 mg of each compound. The capacity factors (k') and plate counts for analytical and overload conditions are calculated. Based on the formula in the experimental section, the saturation capacities of taxol and BIII are 323 and 350 mg, respectively. With the total surface area of 977 m^2 in the column (2.9 g of packing material with 336 m^2/g), saturation capacities of taxol and BIII per unit surface area are 0.33 and 0.36 mg/m^2, respectively, which are in good agreement with the literature data cited above. The high level of saturation capacity achievable, is due to the large surface area of Zorbax 60 Å. This characteristic is desired in preparative purification in order to obtain a high throughput. Taxol saturation capacity in the reversed phase is calculated to be 295 mg (following the same procedure as in the normal phase), corresponding to 0.3 mg/m^2.

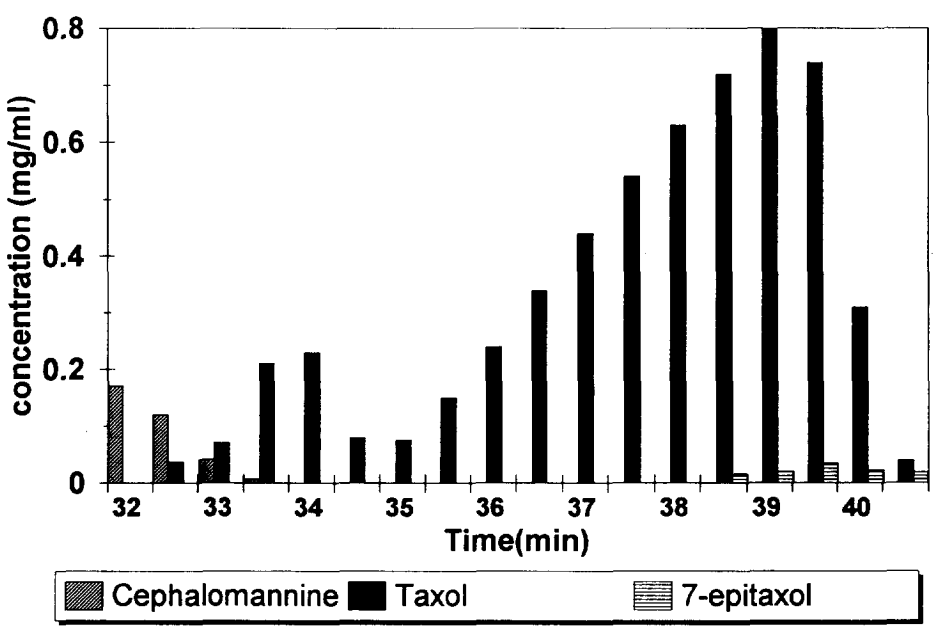

Fig. 6. Analysis of chromatogram of the taxol fractions collected from run represented in Fig. 5.

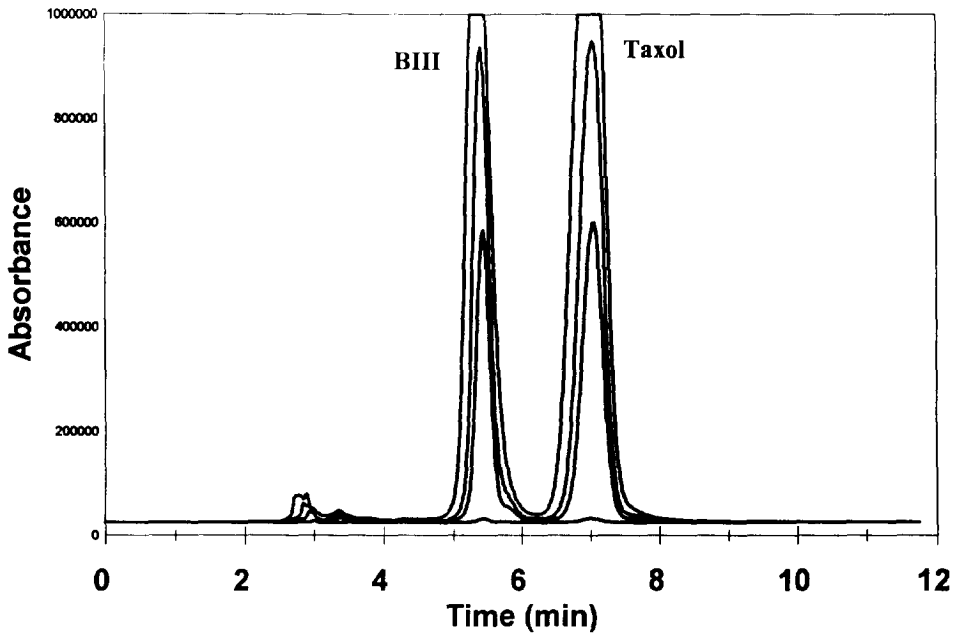

Fig. 7. Elution of a mixture of BIII and taxol at sample loadings of 2.5 μg to 1 mg of each compound in normal phase using Zorbax SW-Taxane. Mobile phase: heptane–ethanol (50:50); flow-rate: 1 ml/min; wavelength: 270 nm. Other conditions given in the Experimental section.

4. Conclusions

A method has been developed for preparative separation and purification of taxane compounds from crude extract using the Zorbax SW-Taxane in isocratic elution. Rapid separation with high selectivity is assured in either reversed or normal phase. The process works well at high alcohol concentrations. The increased solubility of the taxane compounds at high alcohol concentrations leads to a greater throughput. Zorbax SW-Taxane offers a large surface area and therefore a high taxol saturation capacity.

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