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Fast and accurate separation of the paclitaxel from yew extracum by a pseudo simulated moving bed with solvent gradient

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ABSTRACT

A pseudo simulated moving bed (SMB) with solvent gradient was used to trap and separate paclitaxel from yew extracum. This SMB process consisted of three steps: feeding, purification and recovery. In comparison with methanol/water as an eluent, acetonitrile/water could give a better separation but had a poor dissolubility of the yew extracum, and therefore methanol/water was used in the feeding followed by acetonitrile/water in the purification. In the first two steps, water was deliberately added into zone III to modulate the eluotropic strength of the liquid entering zone III, so as to make paclitaxel separation from impurities be more efficient. Once most of impurities discarded, the columns were in turn eluted to recover the trapped paclitaxel of 98% yield with a purity of 78% from the yew extracum containing 1.5% paclitaxel. Afterward, an additional operation of crystallization improved the purity further to 97.8% with the yield of 95%.

1. Introduction

Paclitaxel is a well known drug for the treatment of several cancers including ovarian cancer, breast cancer and Kaposi's sarcoma. The demand for paclitaxel has been increasing in the past two decades. There are three ways to produce paclitaxel: extraction and purification of paclitaxel from dried plant materials [1,2], semi-synthesis from 10-deacetybaccatin III [3,4], and plant cell culture process [5,6]. Among these methods, extraction and purification from the leaves of yew trees was first developed to provide the natural product, and hence more attractive.

Because paclitaxel at very low content coexists with many interfering taxane compounds in yew extracum, the separation and purification of paclitaxel from the crude extracts becomes difficult. Normally, the purification of paclitaxel requires a multi-step procedure involving liquid-liquid partition, normal-phase chromatography, reverse-phase chromatography, and re-crystallization, etc [7–16]. After the tedious steps of operation, the yield of products is not high. For example, a multi-step combination of adsorbent treatment, first precipitation, secondary precipitation and ODS-HPLC purified paclitaxel from 6.3% to 95% with the total recovery of 63.3% only [15]. In addition, the operation cycle is time-consuming such as three chromatographic operations gave paclitaxel (>99%) within 155 h [9]. Obviously, developing a more effective process for paclitaxel separation and purification becomes increasingly important and urgent.

As an effective continuous chromatographic process, simulated moving bed (SMB) has been increasingly used in liquid phase separations [17,18]. The SMB consists of several chromatographic columns connected in series. The ports of feed, desorbent, raffinate and extract are switched periodically in the liquid flowing direction to simulate the counter movement of the solid against the liquid, making the SMB significantly enhance the efficiency of separation. For the separation of paclitaxel from yew extracum containing a lot of ingredients, however, the SMB was merely used in the final step to separate a finite number of key components including cephalomannine, 10-deacetylpaclitaxel and paclitaxel [19–23], because it was uneconomical and inefficient for full separation of a complex mixture system.

Wei et al. herebefore developed a pseudo SMB with solvent gradient for ternary and quaternary separations [24–26]. Unlike the conventional SMB, the target components could move forward in zone II but backward in zone III to be selectively trapped in the two zones due to the solvent gradient, and consequently, the desired component could be isolated from both pre-impurities and post-impurities. In this paper, such a pseudo SMB will be introduced into the one-shot separation and purification of the paclitaxel from yew extracum. The pseudo SMB should be attractive and profitable as the operation procedure could be simplified to increase the yield and productivity accordingly.

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2. Experimental

2.1. Materials and instruments

The yew extracum at about paclitaxel content of 1.5% was obtained from Chongqing Sansheng Industrial Co., Ltd., Chongqing, China. The extracum had been treated by the adsorption of macropore resin to discard the impurities with very strong retention, and hence was used as the raw material without additional pretreatment. 100 g of the extracum was dissolved in 1 L solution of methanol/water (65/35, v/v), and the insoluble impurities were removed by filtration to give a supernatant liquid as the feed of the SMB process.

The solvents, methanol and acetonitrile, were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai, China, and the two-time distilled water was prepared in the laboratory at the location of this study. Eight semi-preparative columns (length 100 mm, diameter 10 mm) were packed with ODS silica gel (particle diameter 15 μm, pore diameter 10 nm, Fuji, Japan) following a slurry-packing technique at a pressure of 20 MPa.

The SMB unit was set up in our own laboratory and had been described in detail [24]. There were six (8 + 1)-port multi-position valves (EMT-6CSD8UW, Vici-valco, Switzerland) connected to 8 columns to control the positions of two desorbents, extract, feed, modifier and raffinate ports. A check valve was installed between every two columns to prevent the liquid from backflushing. In this work, the SMB would be operated in a short-cut mode as illustrated in the later section.

2.2. Analysis

Samples from SMB operation were analyzed with a HPLC system (P1201 pump, 1201 UV detector, Elite, Dalian, China) at 227 nm using an ODS column (Kinetex® XB-C18, 5 μm, 150 × 4.6 mm, Phenomenex, USA). The mobile phase at the flow rate of 1.0 mL/min was acetonitrile /water (38/62, v/v) during 0–35 min and then switched to be acetonitrile after 35 min. The chromatogram of yew extracum is presented in Fig. 1, in which peak 5 at about 30 min was the elution peak of paclitaxel, and peaks 1, 3 and 4 were not identified. The peak of cephalomannine was overlapped with the tail of peak 1 and labeled as peak 2.

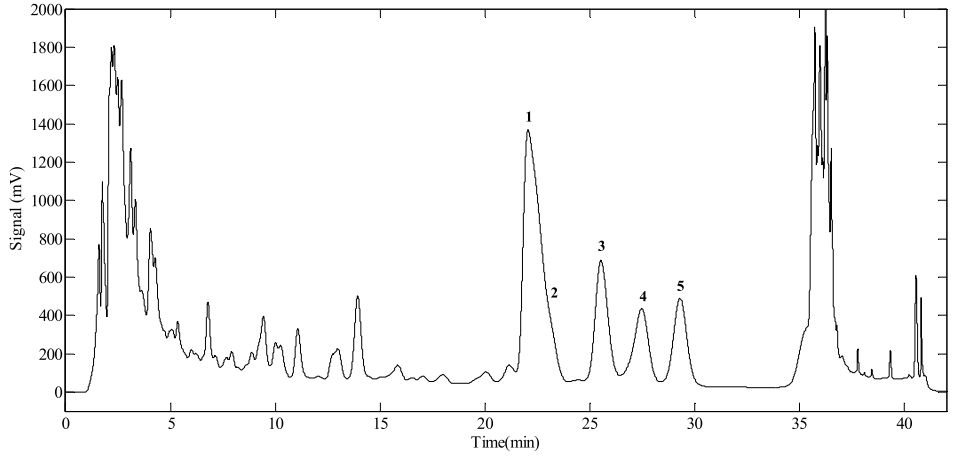

Fig. 1. Chromatogram of the yew extracum (peak 2-cephalomannine, peak 5-paclitaxel).

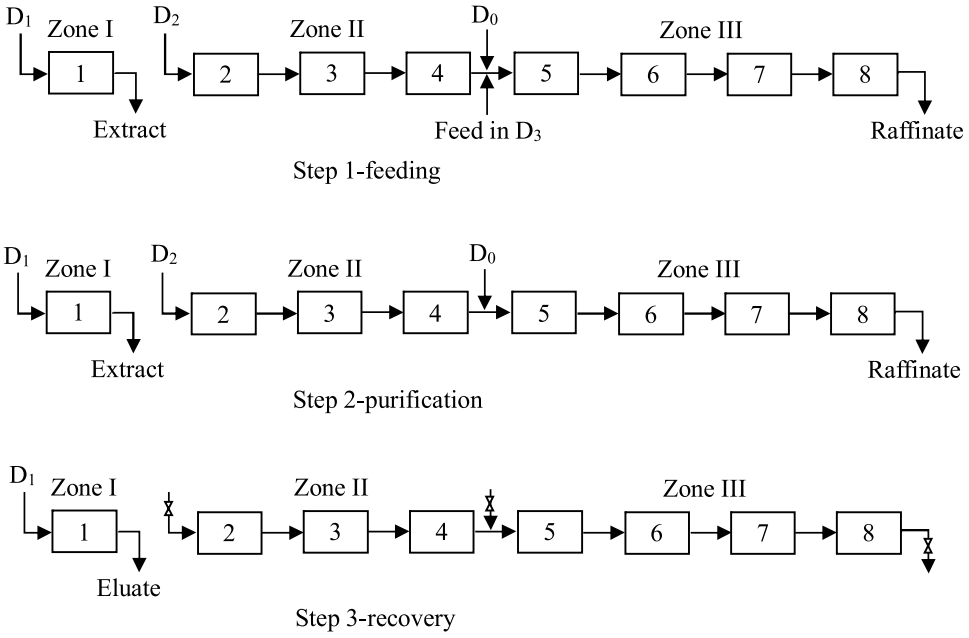

Fig. 2. Scheme of the pseudo SMB.