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One-step separation of nine structural analogues from *Poria cocos* (Schw.) Wolf. *via* tandem high-speed counter-current chromatography

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ABSTRACT

A novel one-step separation strategy—tandem high-speed counter-current chromatography (HSCCC) was developed with a six-port valve serving as the switch interface. Nine structural analogues including three isomers were successfully isolated from *Poria cocos* (Schw.) Wolf. by one step. Compared with conventional HSCCC, peak resolution of target compounds was effectively improved in tandem one. Purities of isolated compounds were all over 90% as determined by HPLC. Their structures were then identified via UV, MS and ^{1}H NMR, and eventually assigned as poricoic acid B (**1**), poricoic acid A (**2**), 3β,16α-dihydroxylanosta-7, 9(11), 24-trien-21-oic acid (**3**), dehydrotumulosic acid (**4**), polyporenic acid C (**5**), 3-epi-dehydrotumulosic acid (**6**), 3-*o*-acetyl-16α-hydroxydehydrotrametenolic acid (**7**), dehydropachymic acid (**8**) and dehydrotrametenolic acid (**9**) respectively. The results indicated that tandem HSCCC can effectively improve peak resolution of target compounds, and can be a good candidate for HSCCC separation of structural analogues.

1. Introduction

Natural products (NPs) provide rich sources for biologically active compounds, which are highly significant to drug discovery and development [1]. Preparative separation is an indispensable procedure to obtain large quantities of monomers with high purities from NPs for the studies of structural information and biochemical properties. With advantages such as low risk of sample denaturation and irreversible adsorption, high sample recovery and large loading capacity [2], the continuous liquid–liquid partition chromatography-high-speed counter-current chromatography (HSCCC) has been widely employed in the separation of NPs for decades [3–8]. However, compared with high performance liquid chromatography (HPLC), the theoretical plates of HSCCC is relatively lower, and it usually leads to insufficient peak capacity and resolution. Thus, it is arduous to isolate all target compounds from complex extracts of NPs by conventional HSCCC with only one solvent system [9–14]. Since, it is common that structural analogues prevalently distributing in NPs [15], those compounds would be co-eluted in peaks due to their little differences of *K*-values [16].

According to the basic formula between length of column, theoretical plate number and theoretical plate height, the longer length of column, the higher theoretical plate number would give better peak resolution of target compounds. Recently, a twin-column based recycling HPLC (R-HPLC) strategy was developed in our own lab and peak resolution of target chiral compounds was effectively improved [17]. Analogously, extending length of CCC column is a feasible method to improve separation efficiency. Recycling HSCCC (R-HSCCC) equivalent to elongating CCC column has been found to possess abilities to isolate compounds with similar *K*-values, whereas limited peak capacity of HSCCC seriously hinder its application. For R-HSCCC separation is consistently performed in the same column, peak overlapping usually occur when it encounters complex samples demanded to prepurify samples before adopting recycling elution mode [18,19]. It is urgent to develop more facile and efficient methods to improve peak resolution of HSCCC separation. Connecting two HSCCC instruments in series would eliminate

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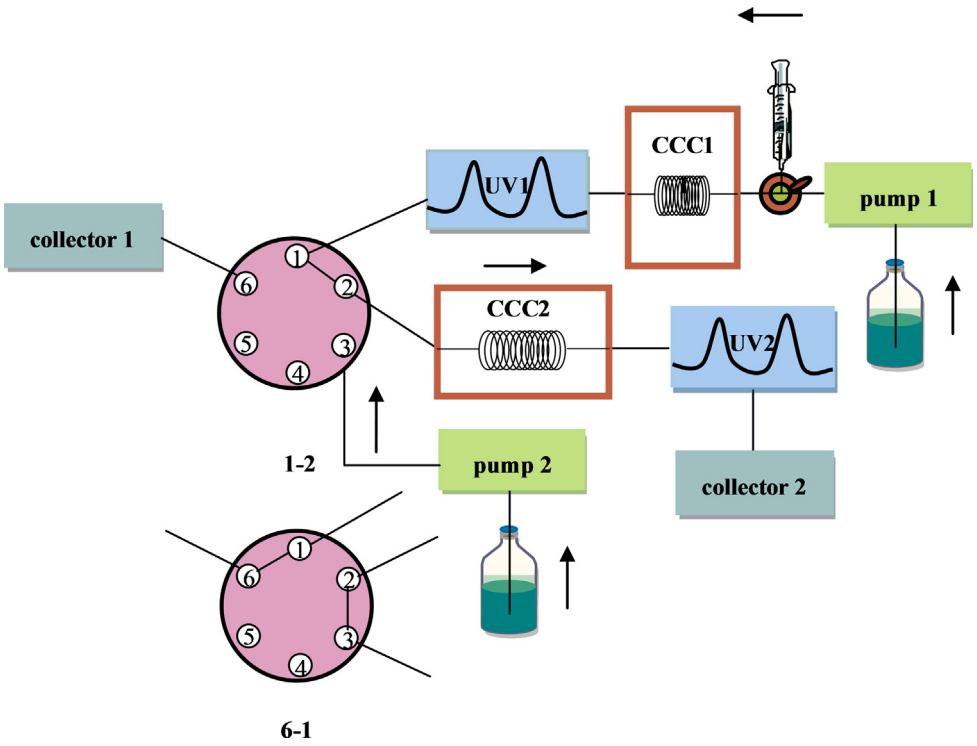

Fig. 1. Connection of T-HSCCC.

restriction of peak capacity of HSCCC, as the effluents of the first HSCCC would be introduced to another HSCCC column, rather than the same HSCCC instrument. Up to now, there is no literature report about tandem HSCCC separation. Thus, employing a six-port valve as the switch interface, we developed a novel separation system-tandem HSCCC (T-HSCCC). As shown in Fig. 1, a T-HSCCC separation can be achieved by switching the valve to two different positions. In this work, the developed T-HSCCC was evaluated by applying it to isolate principal compounds from petroleum ether fraction of *Poria cocos* (Schw.) Wolf. (*PC*), which is rich in biologically active structural analogues of lanostan triterpene and 3, 4-seco-lanostan triterpene [20,21].

2. Experimental

2.1. Apparatus

The HSCCC separation was performed using two HSCCC models TBE-300B (CCC1) HSCCC and TBE-300A (CCC2) HSCCC (Shanghai Tauto Biotechnique Co., Ltd., Shanghai, China). CCC1 is composed of an upright coil type-J planet centrifuge with three multilayered coils connected in series (diameter of tube, 1.6 mm, total capacity 260 mL) and a 20 mL manual sample loop. The rotation speed can be set from 0 to 1000 rpm. The CCC1 system was equipped with a TBP-1002 pump, a TBD-2000 UV detector, an HX1050 constant temperature regulator (Beijing Boyikang Lab Implement Co., Ltd., Beijing, China), a WH V4.0 workstation (Shanghai Wuhao Information Technology Co., Ltd., Shanghai, China) and a BSZ-100 automatic fraction collector (Shanghai Qingpu-Huxi Instruments Factory, Shanghai, China). CCC2 also has three multilayer coil separation columns (total volume 260 mL), a 20 mL sample loop and an HX1050 constant temperature regulator, but equipped with an ÄKTA prime system (Amersham Pharmacia Biotechnique Group, Sweden), and the data were collected by Sepu 3000 chromatography workstation (Hangzhou Puhui Science Apparatus Co., Ltd., Hangzhou, China).

HPLC apparatus (Dionex Ultimate 3000, USA) and a reversed-phase Waters Spherisorb ODS2 (250 mm × 4.6 mm i.d., 5 μm, Waters, Milford, MA, USA) column were employed for HPLC analysis. Dionex Ultimate 3000 system is comprised of an SRD-3600 6 degasser channels, a DGP-3600SD binary pump, a WPS-3000SL auto sampler, a TCC-3000SD thermo stated column compartment and a DAD-3000 multiple wavelength detector (Dionex, Sunnyvale, CA, USA). MS data were acquired in the negative ion mode from a Micromass® Quattro microTM API mass spectrometer (Waters Corp., Milford, MA, USA) with an ESI interface. NMR experiments were performed on a Bruker-400 (Bruker Corporation, Germany) NMR spectrometer.

2.2. Reagents

The dried *PC* was purchased from Tianjian Drugstore (Yuelu District, Changsha, Hunan, China) and identified by local pharmacist. Petroleum ether (60–90 °C), ethyl acetate and ethanol used for sample preparation and HSCCC separation were of analytical grade and obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Acetonitrile used for HPLC was of chromatographic grade (Merck, Darmstadt, Germany). Ultrapure water (18.2 MΩ resistivity) used in the present work was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.3. Preparation of crude sample

The dried and powdered (100 g) *PC* were extracted twice (2 h each) with 500 mL of 95% ethanol each. Then the extracts were combined, filtered and evaporated to dryness (8.03 g) by rotary evaporation at 30 °C under reduced pressure. The concentrated residue was dispersed in the 100 mL of ultrapure water and extracted with 100 mL petroleum ether for 3 times. The concentrated petroleum ether fraction was stored in a refrigerator (4 °C) and subjected for subsequent HPLC analysis and HSCCC separation.