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Short communication

Preparative separation and purification of deoxyschisandrin and γ-schisandrin from *Schisandra chinensis (Turcz.) Baill* by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography (HSCCC) was successfully applied to the preparative separation and purification of deoxyschisandrin and γ-schisandrin from the crude petroleum ether extracts of *Schisandra chinensis (Turcz.) Baill*. The optimum solvent system composed of *n*-hexane–methanol–water (35:30:3, v/v) led to the successful preparation of deoxyschisandrin and γ-schisandrin. The analysis of HPLC for each peak fraction of preparative HSCCC showed that the purity of deoxyschisandrin (8 mg) was over 98% and γ-schisandrin (12 mg) was over 96% from 100 mg of the crude petroleum ether extracts in one-step separation.

Keywords: Counter-current chromatography; *Schisandra chinensis (Turcz.) Bail*; Deoxyschisandrin; γ-Schisandrin

1. Introduction

Schisandra chinensis (Turcz.) Baill has been used as a traditional Chinese medicine in China and Japan. The fruits of *Schisandra chinensis (Turcz.) Baill* are used as a tonic, a sedative, an antitussive and an anti-aging drug [1]. The major active compounds found in the fruit of *schisandra chinensis (Turcz.) Baill* are lignans, which have a dibenzocyclooctadiene skeleton, such as deoxyschisandrin and γ-schisandrin (Fig. 1). The effects of deoxyschisandrin on alanine transaminase, aspartate aminotransferase, albumin and total protein in serum show that deoxyschisandrin can afford protection against CCl_4 induced hepatic damage [2]. Modern medicine research suggests that these lignans have a protective effect on the liver and an immuno-modulating effect [3].

The separation and purification of deoxyschisandrin and γ-schisandrin using the conventional methods such as column chromatography requires several steps resulting in low recoveries of products. High-speed counter-current chromatography (HSCCC), being a support-free liquid–liquid partition chromatography, eliminates irreversible adsorption of sample onto the solid support. The method has been successfully applied to the separation and purification of various natural products, such as alkaloids [4,5], isoflavones [6,7]. However, no report has been published on the use of high high-speed counter-current chromatography for separation and purification of deoxyschisandrin and γ-schisandrin from *Schisandra chinensis (Turcz.) Baill*.

The present paper describes HSCCC separation and purification of deoxyschisandrin and γ-schisandrin from *Schisandra chinensis (Turcz.) Baill*. The optimized HSCCC condition thus obtained led to the successful preparation of deoxyschisandrin and γ-schisandrin.

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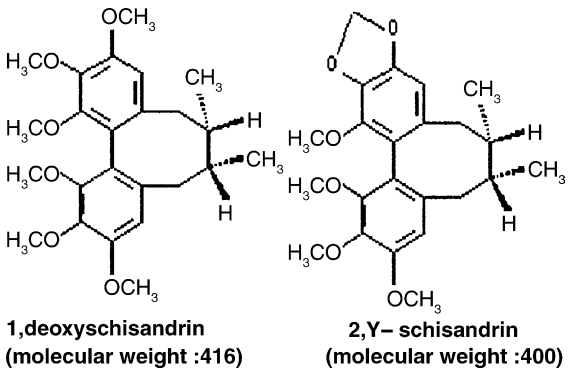

Fig. 1. Structural formulas of the deoxyschisandrin and γ-schisandrin.

2. Experimental

2.1. Apparatus

The preparative HSCCC instrument (Model TBE-300, Shanghai Tauto Biological Company, China) was equipped with three preparative coils connected in series (diameter of polytetrafluoroethylene (PTFE) tube, 2.6 mm; total volume, 119 ml) and a 10 ml sample loop. The revolution speed of the instrument could be regulated with a speed controller in the range between 0 and 1000 rpm, an optimum speed of 950 rpm was used in the experiment. The solvent was pumped into the column with a model NS-1007 constant-flow pump and continuous monitoring of the effluent was achieved with a model 8823A-AU monitor (Beijing Institute of New Technology Application, China); a portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqing, China) was used to draw the chromatogram.

The HPLC system used was waters 510 series with a UV–vis photodiode-array detector, an injection valve (model 7726) with a 20 μl loop, and two 510 pumps (Waters, USA). Microwave extractor (Model VIP 272, National Engineering Research Center for Chinese Traditional Medicine, Shanghai, China); ZX-4A vacuum pump and ZK 82J electrothermal vacuum desiccator (Shanghai Experimental Instrument Company, Shanghai, China).

2.2. Plant material and chemicals

The fruits of *Schisandra chinensis (Turcz.) Baill* were purchased from Shanghai Kangqiao Medicinal Materials Factory, and judged by Shanghai Chinese Traditional Medicine Research Institute and fitted for Chinese Pharmacopoeia.

Ethanol, *n*-hexane and petroleum ether were of analytical grade, methanol and water were of HPLC grade (Chinese Medication Group Shanghai Chemical Reagent Company, Shanghai, China). Deoxyschisandrin and γ-schisandrin standard samples were purchased from National Institute for the Control of Pharamaceutical and Biological Products, Ministry of Health, Beijing, China.

2.3. Solvent system for HSCCC

The present study utilized of two-phase solvent systems composed of *n*-hexane–methanol–water (35:30:3, v/v). Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

2.4. Sample preparation

The dried and powdered fruits of *Schisandra chinensis (Turcz.) Baill* were weighted and put into microwave extractor and extracted with the 810 W of microwave power, 90% of ethanol concentration, 1:12 of the proportion of raw material to solvent, and 5 min of radiation time. The extraction solution was vacuum filtrated and concentrated to dryness by rotatory evaporator. The ethanol extracts were dissolved in water in the separatory funnel. The petroleum ether for five times was added, shook and stratified, the five times petroleum ether extraction solutions were concentrated to dryness. The residues were stored in a refrigerator for the subsequent HSCCC separation. The sample solutions were prepared by dissolving proper residues in the mobile phase and stationary phase of solvent system for preparative separation.

2.5. HSCCC separation

The multiplayer coiled column was first entirely filled with the upper organic phase of solvent system as the stationary phase. Then, the apparatus was rotated at 950 rpm and the lower phase of solvent system was pumped through the column at a flow-rate of 1.0 ml/min. After the mobile phase emerged in the effluent and hydrodynamic equilibrium was established in the column, the sample solution was injected through the injection valve. The effluent was continuously monitored with UV detector at 254 nm and peak fractions were each collected according to the chromatogram. The retention of the stationary phase was calculated to the volume of the stationary phase to the total column capacity after the separation was completed.

2.6. HPLC analysis and EI-MS identification

The crude petroleum ether extracts and each purified peak fraction from the preparative HSCCC separation were analyzed by HPLC (Shim-Pack VP-ODS column, 3.5 μm, 150 × 4.6 mm). Mobile phase was performed with methanol–water (75:25). The flow rate was 1.0 ml/min. Detection wave was 254 nm. Temperature was 35 °C. The purified fractions of deoxyschisandrin and γ-schisandrin obtained from the preparative HSCCC separation were analyzed by electron impact mass spectroscopy (EI-MS) (GC-TOFMS, Micromass, UK).

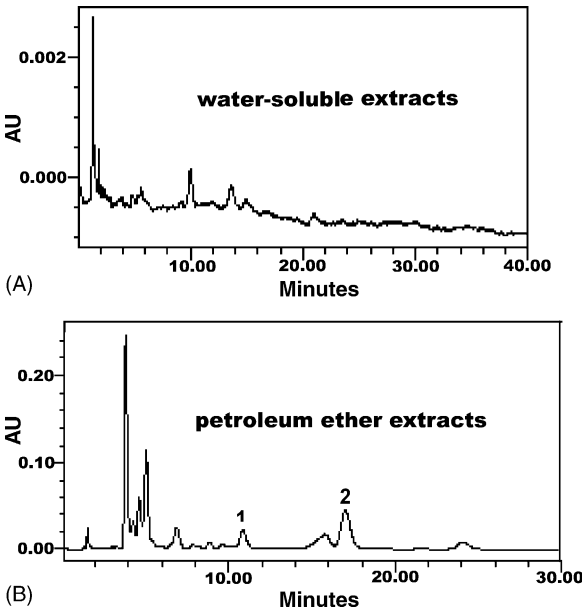

Fig. 2. Chromatogram of crude water-soluble extracts (A) and petroleum ether extracts (B) from *Schisandra chinensis (Turcz.) Baill* by HPLC analysis. Peak 1 = deoxyschisandrin and peak 2 = γ-schisandrin. Experimental condition, shim-pack VP-ODS column (3.5 μm, 150 × 4.6 mm); mobile phase, methanol–water (75:25); flow-rate, 1.0 ml/min; detection wave, 254 nm; temperature, 35 °C.

3. Results and discussion

The crude ethanol extracts of *Schisandra chinensis (Turcz.) Baill* containing deoxyschisandrin and γ-schisandrin were first separated into the petroleum ether and the water-soluble extracts by two-phase solvent extraction. As shown in Fig. 2, HPLC analysis of these extracts indicated that deoxyschisandrin and γ-schisandrin were concentrated in the petroleum ether extracts. Peak 1 corresponds to deoxyschisandrin and peak 2 corresponds to γ-schisandrin.

Because the sample solvent was extracted by petroleum ether, the target compounds mainly behave hydrophobic. Preliminary HSCCC studies were carried out with the two-phase solvent system composed of *n*-hexane–methanol–water (45:30:2, 35:30:1, 4:3:1, 3:2:1 and 6:5:5, v/v). Experiments showed that solvent system *n*-hexane–methanol–water at the volume ratio 4:3:1and 3:2:1 came out serious emulsification, solvent system *n*-hexane–methanol–water at the volume ratio 45:30:2 and 6:5:5 could not give effective separation, while solvent system *n*-hexane–methanol–water at the volume ratio 35:30:1 gave a good results and fit for separation demands on the whole. In subsequent studies, the experiments by changing the volume ratio of water in the solvent system of *n*-hexane–methanol–water (35:30:2, 35:30:3, 35:30:4 and 35:30:5,v/v) showed that the increase of water forwarded hydrophilic compounds and retarded hydrophobic compounds. Solvent system *n*-hexane–methanol–water at the volume ratio 35:30:3 achieved best separation. Fig. 3 shows the preparative

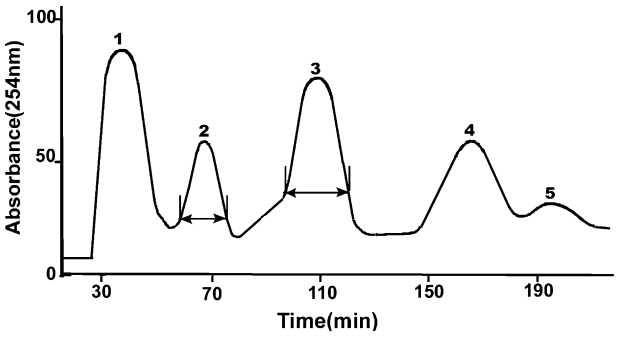

Fig. 3. Chromatogram of crude petroleum ether extracts from *Schisandra chinensis (Turcz.) Baill* by HSCCC. Experimental condition: mutilayer coil of 2.6 mm i.d. PTFE tube with a total capacity of 119 ml, rotary speed 950 rpm; solvent system, *n*-hexane–methanol–water (35:30:3, v/v); stationary phase, upper organic phase; mobile phase, lower aqueous phase; flow-rate, 1.0 ml/min; detection wave, 254 nm; sample size, 100 mg; retention of the stationary phase, 73%.

HSCCC separation of 100 mg of the petroleum ether extracts.

HPLC analysis of each peak fraction from this preparative HSCCC were compared with the deoxyschisandrin and γ-schisandrin standard samples and confirmed by their retention time and purity analysis. Routine sample calculations were made by comparison of the peak area with those of the standards. As shown in Fig. 4, the results revealed that peak 2 corresponds to deoxyschisandrin was 8 mg over 98%

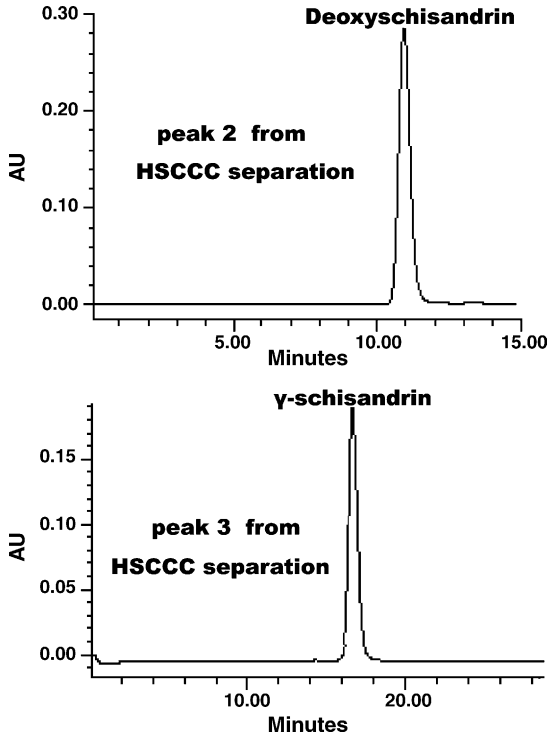

Fig. 4. HPLC chromatogram of each peak fraction from this preparative HSCCC. Peak 2 = deoxyschisandrin and peak 3 = γ-schisandrin. HPLC experimental conditions are shown in Fig. 2.

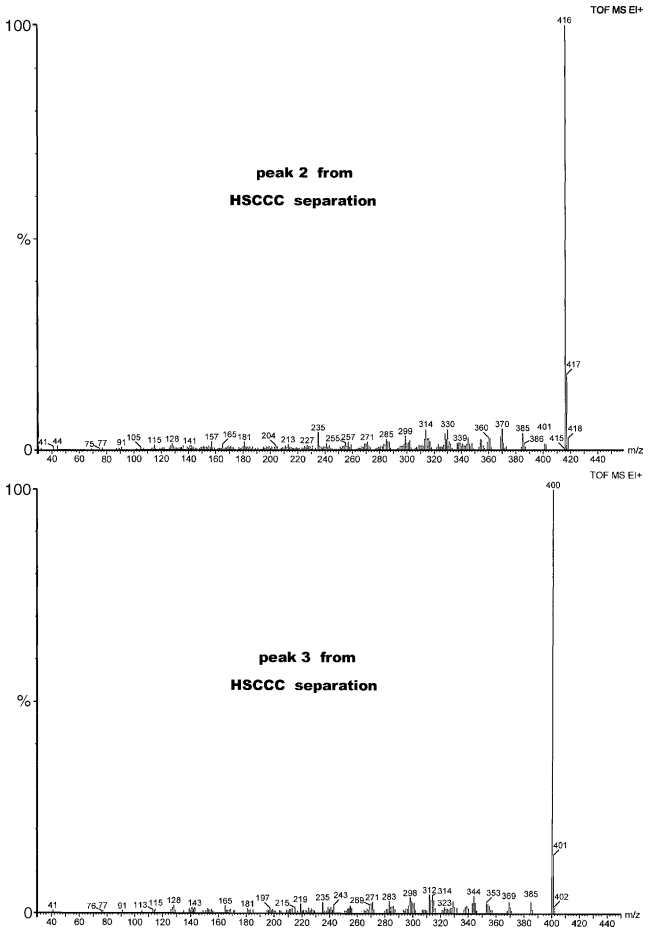

Fig. 5. EI-MS spectra of purified peaks 2 and 3 from this preparative HSCCC.

purity and peak 3 corresponds to γ-schisandrin was 12 mg over 96% purity.

Fig. 5 shows the electron impact-mass spectra of the purified peaks 2 and 3 from the preparative HSCCC. The structural identification of peak 2 and 3 was carried out by EI-MS as follows: the molecular ion at *m*/*z* 416 of peak 2, which is in agreement with the molecular formula $C_{24}H_{32}O_6$ of deoxyschisandrin, and the molecular ion at *m*/*z* 400 of peak 3, which is in agreement with the molecular formula $C_{23}H_{28}O_6$ of γ-schisandrin. Therefore, these results indicate that the compounds in peaks 2 and 3 are deoxyschisandrin and γ-schisandrin, respectively.

The overall results of our studies indicate that HSCCC is successfully used for separation and purification of deoxyschisandrin and γ-schisandrin from *Schisandra chinensis (Turcz.) Baill*. The present study indicates that HSCCC is a potential and powerful tool for separation and purification of biologically active substances from other traditional Chinese medicines.

Acknowledgements

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