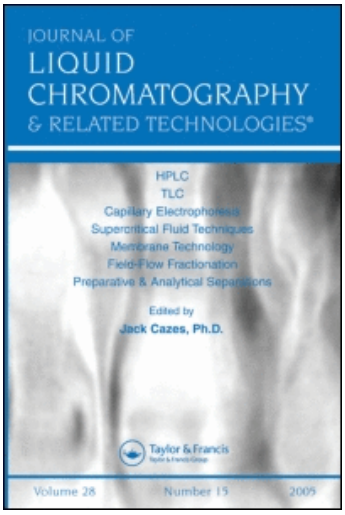

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Preparative Separation and Purification of Schisandrin and Schisantherin from *Schisandra Chinensis* (*Turcz*) *Baill* by High Speed Countercurrent Chromatography

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Abstract: High speed countercurrent chromatography (HSCCC) was successfully applied to the preparative separation and purification of schisandrin and schisantherin from *Schisandra chinensis* (*Turcz*) *Baill* by microwave-assisted extraction. The petroleum ether extract was separated with a two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (22:8:20:20, v/v). The analysis of HPLC for each fraction from preparative HSCCC showed that the purity of schisandrin (16 mg) was over 98% and schisantherin (6 mg) was over 96% from the 100 mg of petroleum ether extract in a one-step separation.

Keywords: Countercurrent chromatography, *Schisandra chinensis* (*Turcz*) *Baill*, Schisandrin, Schisantherin, Microwave-assisted extraction

INTRODUCTION

Schisandra chinensis (*Turcz*) *Baill* has been used as a traditional Chinese medicine in China for thousands of years. The fruits of *Schisandra*

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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 schisandrin and schisantherin. The effects of schisandrin on transaminase, aspartate, aminotransferase, albumin, and total protein in serum show that schisandrin 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 schisandrin and schisantherin using the conventional method, such as column chromatography requires several steps resulting in low recoveries of the target products. High speed countercurrent chromatography (HSCCC), being a support-free liquid-liquid partition chromatography, eliminates irreversible adsorption of the sample onto the solid support. The method has been successfully applied to the separation and purification of some natural products.[4–7] However, no report has been published on the use of high speed countercurrent chromatography for separation and purification of schisandrin and schisantherin from *Schisandra chinensis* (*Turcz*) *Baill*. The present paper describes HSCCC separation and purification of schisandrin and schisantherin from *Schisandra chinensis* (*Turcz.*) *Baill*. The optimized HSCCC condition thus obtained led to the successful preparation of schisandrin and schisantherin.

EXPERIMENTAL

Apparatus

The preparative HSCCC instrument (Model TBE-300, Shanghai Tauto Biotechnology Company, Shanghai, 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, Beijing, 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 with a 20 μL loop, and two 510 pumps (Waters, USA), Electron impact-mass spectrometry (EI-MS) (GC-TOFMS, Micromass, England); VIP 272 microwave-assisted extractor (National Engineering Research Center for Traditional Medicine, Shanghai, China); ZX-4A vacuum pump, and ZK 82J electrothermal vacuum desiccator (Shanghai Experimental Instrument Company, Shanghai, China).

Plant Material and Chemicals

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

Ethanol, n-hexane, ethyl acetate, and petroleum ether were of analytical grade; methanol and water were of HPLC grade (Shanghai Chemical Reagent Company Chinese Medicine Group, Shanghai, China). Schisandrin and schisantherin standard samples were purchased from National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China.

Solvent System for HSCCC

The present study utilized a two-phase solvent system composed of n-hexane-ethyl acetate-methanol-water (22:8:20:20, v/v). Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature, and the two phases were separated shortly before use (Figure 1).

Sample Preparation

The dried and powdered fruits of *Schisandra chinensis* (*Turcz.*) *Baill* were weighed and put into a microwave-assisted extractor and extracted with 90% of ethanol concentration (1:12 of the proportion of raw material to solvent), and 5 min of radiation time under 850 W of microwave power. The extraction solutions were vacuum filtrated and concentrated to dryness under reduced pressure. The ethanol extract was dissolved in water in the separatory

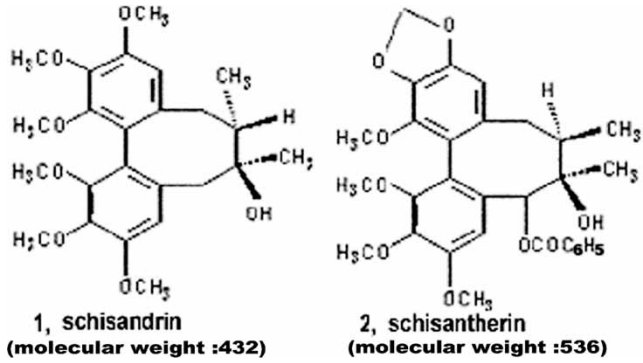

Figure 1. Structural formulas of schisandrin and schisantherin.

funnel. The petroleum ether was added, shook, and stratified five times and the extraction solutions were concentrated to dryness. The residues were stored in a refrigerator for the subsequent HSCCC separation. The sample solution was prepared by dissolving proper residues in the mobile phase and stationary phase of the solvent system for preparative separation.

HSCCC Separation

First, the multiplayer coiled column was 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 1.5 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 each fraction was collected according to the chromatogram. The retention of the stationary phase was calculated by the volume of the stationary phase to the total column capacity after the separation was completed.

HPLC Analysis and EI-MS Identifcation

The petroleum ether extract and the purified fractions from the preparative HSCCC separation were analyzed by HPLC (Shim-pack VP-ODS column, 3.5 μm, 150 × 4.6 mm), respectively. 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 schisandrin and schisantherin obtained from the preparative HSCCC separation were analyzed by electron impact-mass spectrometry (EI-MS).

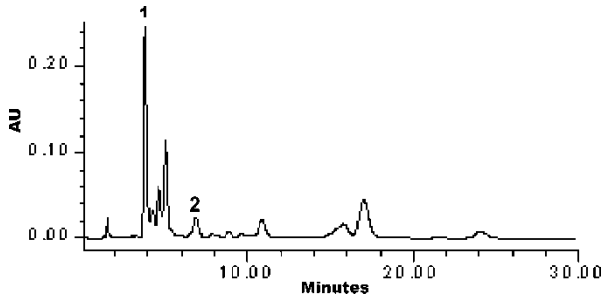

Figure 2. Chromatogram of petroleum ether extract from *Schisandra chinensis* (*Turcz.*) *Baill* by HPLC analysis. Peaks 1 = schisandrin and peak 2 = schisantherin. Conditions: shim-pack VP-ODS column (3.5 μm, 150 × 4.6 mm); mobile phase: methanol-water (75:25); flow-rate: 1.0 mL/min; detection wave: 254 nm; temperature: 35°C.

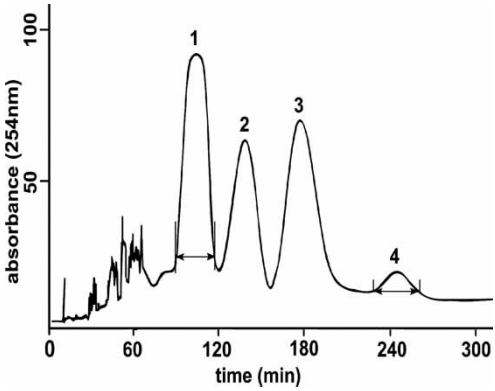

Figure 3. Chromatogram of petroleum ether extract from *Schisandra chinensis* (*Turcz.*) *Baill* by HSCCC separation. Conditions: mutilayer coil of 2.6 mm I.D. PTFE tube with a total capacity of 119 mL, rotary speed 950 rpm, solvent system: n-hexane-ethyl acetate-methanol-water (22:8:20:20, v/v); stationary phase: upper organic phase; mobile phase: lower aqueous phase; flow-rate: 1.5 mL/min, detection wave: 254 nm; sample size: 100 mg; retention of the stationary phase: 72%.

RESULTS AND DISCUSSION

Figure 2 shows HPLC analysis of the petroleum ether extract of *Schisandra chinensis* (*Turcz.*) *Baill*. Peak 1 corresponds to schisandrin and peak 2 corresponds to schisantherin.

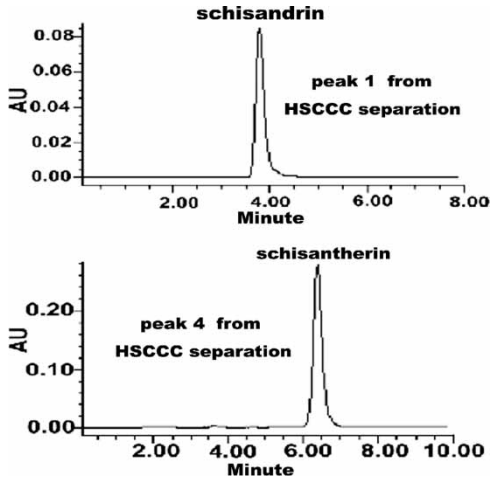

Figure 4. HPLC Chromatogram of each fraction from preparative HSCCC. Peak 1 = schisandrin and peak 4 = schisantherin. Conditions are shown in Figure 2.

Preliminary HSCCC studies were carried out with the two-phase solvent systems composed of chloroform-methanol-water (10:5:5, 7:3:5, v/v) and n-hexane-methanol-water (45:30:2, 6:5:5, v/v). The results showed that the solvent system chloroform-methanol-water at the volume ratio 10:5:5 and

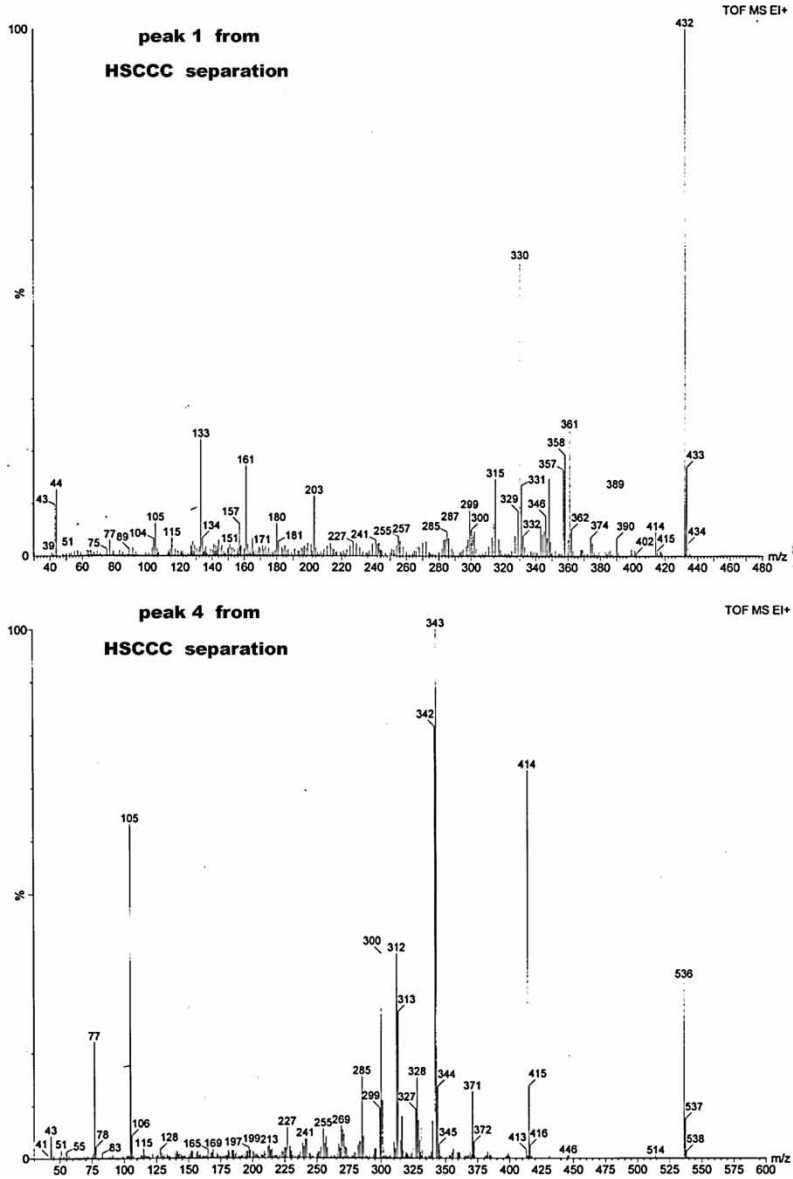

Figure 5. EI-MS spectra of purified peak 1 and peak 4 from preparative HSCCC separation.

7:3:5 came out seriously emulsified; solvent system n-hexane-methanol-water at the volume ratio 45:30:2 and 6:5:5 could not achieve effective separation. In the subsequent studies, two solvent systems composed of n-hexane-ethyl acetate-methanol-water (24:4:20:20, 24:40:20:12, v/v) were evaluated in terms of peak resolution. The results showed these solvent systems could improve the separation slightly. In order to separate schisandrin and schisantherin, the above solvent system should be further changed. A solvent system composed of n-hexane-ethyl acetate-methanol-water (22:8:20:20, v/v) could achieve effective separation, as shown in Figure 3.

Figure 4 shows the HPLC chromatogram of the fractions from the preparative HSCCC separation. HPLC analysis of each fraction from the preparative HSCCC were compared with the schisandrin and schisantherin 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. The results revealed that peaks 1 corresponding to schisandrin was 16 mg with purity over 98% and peak 4 corresponding to schisantherin was 6 mg with purity over 96% from 100 mg of petroleum ether extract.

Figure 5 shows the electron impact-mass spectra of the purified peak 1 and peak 4 of the preparative HSCCC. The molecular ion at m/z 432 of peak 1 is in agreement with the molecular formula $C_{24}H_{32}O_7$ of schisandrin, and the molecular ion at m/z 536 of peak 4 is in agreement with the molecular formula $C_{30}H_{32}O_9$ of schisantherin. These results indicated that the compounds in peak 1 and peak 4 are schisandrin and schisantherin, respectively.

The results of our studies indicate that HSCCC can be successfully used for separation and purification of schisandrin and schisantherin from *Schisandra chinensis* (*Turcz*) *Baill*, which implies that HSCCC is a potential and powerful tool for separation and purification of biologically active substances from other traditional Chinese medicine.

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