Journal of Pharmaceutical Analysis 2013;3(6):429–433

Contents lists available at ScienceDirect

Journal of Pharmaceutical Analysis

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ORIGINAL ARTICLE

# Preparative separation and purification of deoxyschizandrin from Schisandrae Sphenantherae Fructus by high-speed counter-current chromatography

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Received 4 February 2013; accepted 16 July 2013
Available online 24 July 2013

**KEYWORDS**

Schisandrae Sphenantherae Fructus;
High-speed counter-current chromatography (HSCCC);
Deoxyschizandrin

**Abstract** A high-speed counter-current chromatography (HSCCC) method was successfully developed for the preparative separation and purification of deoxyschizandrin from Schisandrae Sphenantherae Fructus in one step. The purity of deoxyschizandrin was 98.5%, and the structure was identified by MS, UV and NMR. This method was simple, fast, convenient and appropriate to prepare pure compound as reference substances for related research on Schisandrae Sphenantherae Fructus.



## 1. Introduction

Schisandrae Sphenantherae Fructus (Nan Wuweizi in Chinese), the dried ripe fruits of *Schisandra sphenanthera* Rehd. et Wils., is a famous traditional Chinese medicine. Various active effects including antihepatotoxic effect, antioxidant and detoxificant effect, and anticarcinogenic effect have been revealed [1,2]. More interestingly, it was indicated that Schisandrae Sphenantherae Fructus might be useful in the prevention and treatment of hyperproliferative and inflammatory skin diseases [3]. Lignans and volatile oils are the main components of Schisandrae Sphenantherae Fructus. It has been reported that the major active ingredients were lignans, including deoxyschizandrin and schisantherin A, B, C, D, E [4–8]. Therefore, large quantities of

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Peer review under responsibility of Xi'an Jiaotong University.

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http://dx.doi.org/10.1016/j.jpha.2013.07.002

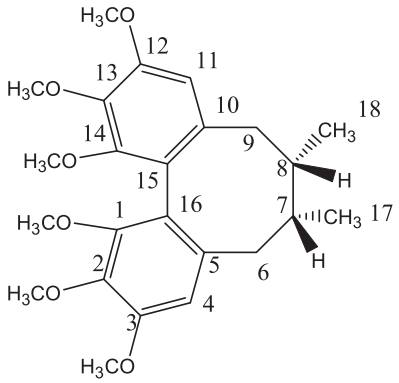


**Fig. 1** The chemical structure of deoxyschizandrin.

pure compounds are wanted as reference substances For related research based on this important traditional Chinese medicine.

Column chromatography is commonly adopted in separation and purification of lignans from Schisandrae Sphenantherae Fructus [9–11]. But these traditional methods are time consuming, have low efficiency and are complicated in operation. The high-speed counter-current chromatography (HSCCC) method, a newly developed technology of separation and purification that appeared in the 70s, has become one of the essential techniques in separation and purification. Isolation and separation of lignans from Schisandrae Chinensis Fructus (Bei Wuweizi in Chinese) by HSCCC has been reported [12]; however, no report has been published on the separation and purification of deoxyschizandrin from Schisandrae Sphenantherae Fructus (Nan Wuweizi in Chinese) using HSCCC. Therefore, this study focused on the establishment of an efficient method for the isolation and purification of deoxyschizandrin (Fig. 1) with high purity from Schisandrae Sphenantherae Fructus by HSCCC. Characterization of deoxyschizandrin was accomplished by MS, UV and NMR.

## 2. Materials and methods

### 2.1. Materials and reagents

Schisandrae Sphenantherae Fructus was purchased from Guangdong Guokang Pharmaceutical Ltd. (Batch no. 20111107). The sample was authenticated as the dried fruit of *Schisandra sphenanthera* Rehd. et Wils. by Doctor Xin-Jun Xu, School of Pharmaceutical Sciences, Sun Yat-Sen University. Methanol was HPLC grade (Honeywell B&J, SK Chemicals, Korea). Water was commercial ultrapure water. Other reagents were of analytical grade from Tianjin Damao Chemical Reagent Factory (Tianjin, China).

### 2.2. Apparatus

The HSCCC instrument was QuikPrep$^{TM}$ Chassis Mk5 high-speed counter-current chromatography. The unit had four identical coils of 3.2 mm o.d. and 2.16 mm i.d. Each coil had a volume of approximately 115 mL. Each coil in this configuration had a beta range of 0.85–0.62. The rotational speed of the apparatus could be regulated with a speed controller in the range of 0–860 rpm. Quattro CCC was manufactured by AECS-QuikPrep Ltd. (Bristol, UK), with a series II HPLC pump (SSI, USA). HPLC analysis was performed on a Lab Alliance HPLC (1500 pump, AS1000 autosampler, UV6000 detector, SSI, USA). A GX-10A 500 g multifunctional pulverizer (Shanghai Gaoxiang Food Machinery Factory), an ultrasonic machine (SB25-12DTD, Ningbo Scientz Bio-technology Co., Ltd., China), a KERN ABT 220-5DM electronic balance (0.1 mg, KERN, Germany) and a Yarong RE-300 rotational vacuum concentrator (Shanghai, China) were employed in preparing samples. Characterization was performed on a Finnigan LCQ DECA XP Liquid Chromatography Mass Spectrometer (Thermo, USA) and a Bruker Avance III 400 Nuclear Magnetic Resonance Spectrometer (Bruker, Germany).

### 2.3. Preparation of the crude extracts from Schisandrae Sphenantherae Fructus

Schisandrae Sphenantherae Fructus (about 100 g) was pulverized and extracted three times with 80% ethanol (1:4, w/v) for 30 min by ultrasound. The mixtures were filtered and the solution was evaporated to dryness by rotary vaporization under reduced pressure at 50 °C. 16.5 g of residue was obtained.

Then the residue was redissolved in water by ultrasound and extracted by petroleum ether. After evaporating the petroleum ether extracts to dryness by rotary vaporization under reduced pressure at 40 °C, 12.0 g of residue was obtained. The residue was preserved at 4 °C for subsequent separation and purification by HSCCC.

### 2.4. HPLC conditions

A Dikma-Diamonsil $C_{18}$ column (150 mm × 4.6 mm, 5 μm) with a $C_{18}$ guard column (4.6 mm × 10 mm, 5 μm) was used. The binary mobile phase consisted of methanol–water (75:25, v/v). The system was run with a gradient program at 1 mL/min. The effluent was monitored by a DAD detector at 220 nm. The sample injection volume was 10 μL and the column temperature was set at 30 °C.

### 2.5. Selection of two-phase solvent system

The two-phase solvent system was selected according to the partition coefficient ($K$) of the target compound. The $K$ values were determined as follows: two-phase solvent systems with different ratios of organic solvent and water were prepared. Upper and lower phases (2 mL each) were placed in test tubes and about 1 mg dry extract was added. The test tube was capped and shaken vigorously for several minutes to thoroughly equilibrate the sample between two phases. Then, equal volume (1 mL) of the upper and lower phases was separately evaporated to dryness. The residues were dissolved with 1 mL of 80% methanol and analyzed by HPLC to determine the $K$ values. The peak area of the upper phase was recorded as $A_U$ (area of upper phase) and that of the lower phase was recorded as $A_L$ (area of lower phase). The $K$ values were calculated according to the following equation: $K = A_U/A_L$.

### 2.6. Preparation of two-phase solvent system and sample solution

#### 2.6.1. Preparation of two-phase solvent system

The selected two-phase solvent system of *n*-hexane–ethanol–water was prepared by adding all the solvents into a separation funnel at the volume ratios of 6:5:4 (v/v/v) and thoroughly equilibrated by

shaking repeatedly. After stewing overnight, the two-phase solvent system was equilibrated and separated, and then each of the two phases was separated and filtered respectively for the subsequent HSCCC.

#### 2.6.2. *Preparation of sample solution*

The petroleum ether extract of Schisandrae Sphenantherae Fructus (160 mg) was dissolved in 10 mL of a mixture of upper and lower phases (1:1, v/v), and filtered by 0.45 μm millipore filters.

### 2.7. *HSCCC separation*

The stationary phase (the upper phase) of the solvent system of *n*-hexane–ethanol–water (6:5:4, v/v/v) was pumped into the HSCCC multilayer-coiled column at 8.0 mL/min firstly. When the column was fully filled with the stationary phase, the lower phase (the mobile phase) was pumped into the column from the head-to-tail at a flow rate of 2 mL/min while the apparatus was rotated at 860 rpm. After hydrodynamic equilibrium was reached, the sample prepared in Section 2.6 was loaded into the injection valve. The effluents from the column were continuously monitored at the wavelength of 220 nm and collected into test tubes with a fraction collector set at 3 min for each tube. The fractions were analyzed by HPLC–DAD and those containing the purified compounds were collected and dried separately.

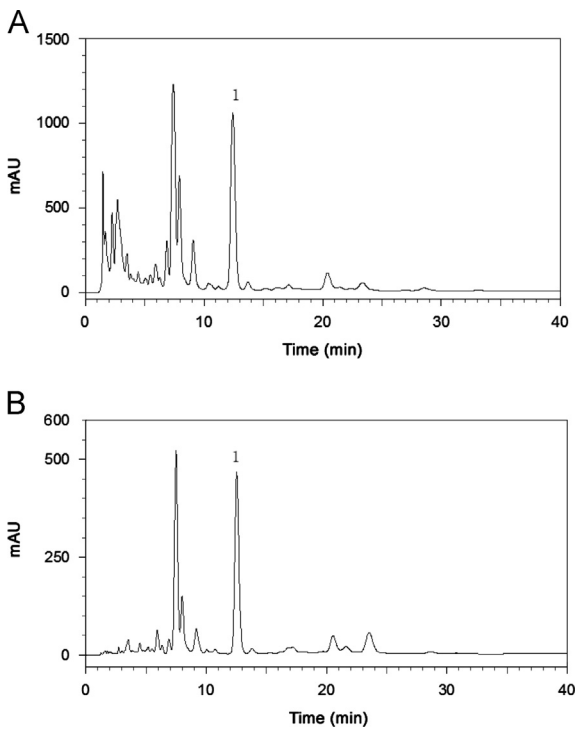


**Fig. 2** HPLC chromatograms of crude extract (A) and the petroleum ether extract (B) of Schisandrae Sphenantherae Fructus. Chromatographic conditions: column, Dikma-Diamonsil $C_{18}$ column (150 mm × 4.6 mm, 5 μm) with a $C_{18}$ guard column (4.6 mm × 10 mm, 5 μm); the binary mobile phase consisted of methanol–water (75:25, v/v); flow rate: 1 mL/min; DAD detector; injection volume: 10 μL; column temperature: 30 °C. Peak 1 corresponds to compound 1.

## 3. Results and discussions

### 3.1. *HPLC analysis of crude extract*

The crude extract and petroleum ether extract of Schisandrae Sphenantherae Fructus were analyzed by HPLC, and the chromatograms are shown in Fig. 2.

### 3.2. *Optimization of the solvent system*

The separation by HSCCC depends largely on a suitable two-phase solvent system that provides an ideal partition coefficient ($0.2 < K < 5$, $K$, solute concentration in the upper mobile phase divided by that in the lower stationary phase) for the target compound and a reasonably short settling time. In this experiment, according to the characters of the targeted compound and related reports [13,14], several solvent systems were tested, and the results are shown in Table 1. As indicated in Table 1, the $K$ value of the targeted compound in solvent system 1 was too small, which indicated that solvent system 1 was inappropriate. For solvent systems 2–5, the $K$ values were too big, which were inappropriate, too. The $K$ value (1.97) in solvent system 6 was moderate which

**Table 1** The $K$ values of the targeted fractions from Schisandrae Sphenantherae Fructus measured in different solvent systems.

| No. | Solution system (v/v/v or v/v/v/v) | $K^*$ values |
|---|---|---|
| 1 | *n*-Hexane–methanol–water (35:30:3) | 0.41 |
| 2 | *n*-Hexane–ethyl acetate–methanol–water (1:1:1:1) | 14.01 |
| 3 | *n*-Hexane–ethyl acetate–methanol–water (9:1:5:5) | 7.65 |
| 4 | *n*-hexane–methanol–water (2:1:1) | 7.15 |
| 5 | *n*-Hexane–ethanol–water (6:5:5) | 3.45 |
| 6 | *n*-Hexane–ethanol–water (6:5:4) | 1.97 |

*$K$ = solute concentration in the upper mobile phase divided by that in the lower stationary phase.

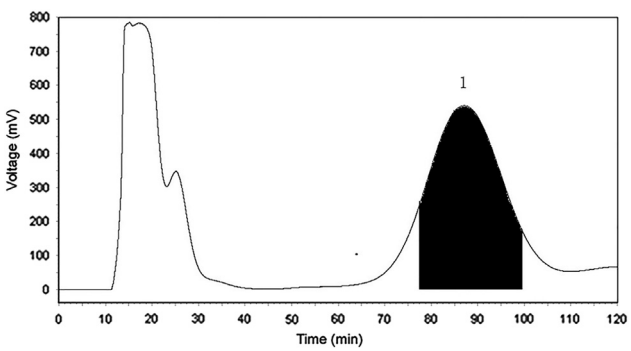


**Fig. 3** HSCCC chromatogram of the petroleum ether extract of Schisandrae Sphenantherae Fructus (the dark bar referred to the purity >98% for peak 1). Solvent system: *n*-hexane–ethanol–water (6:5:4, v/v/v); stationary phase: upper phase; mobile phase: lower phase; flow rate: 2.0 mL/min; revolution speed: 860 rpm; retention of stationary phase: 84.3%; sample size: 160 mg crude extract; detection at 220 nm. Peak 1 corresponds to compound 1.

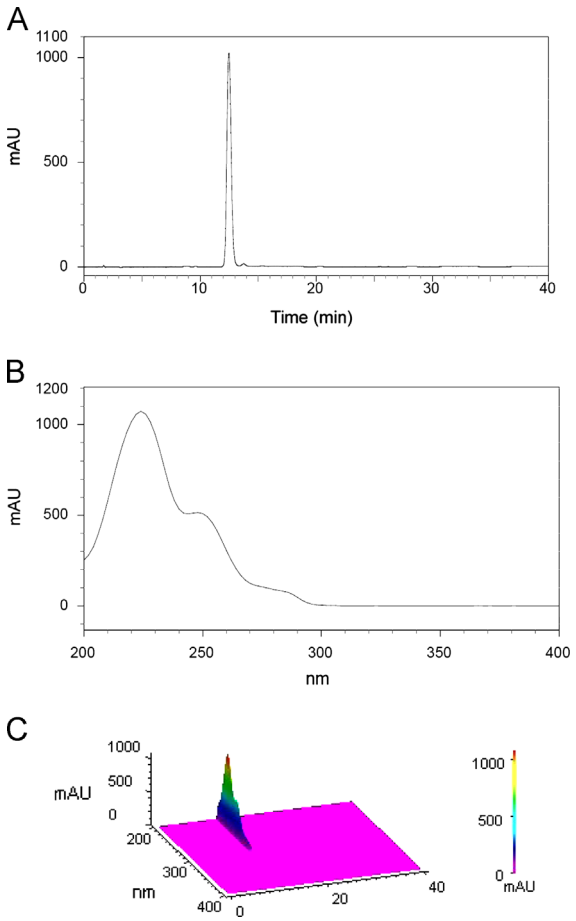


**Fig. 4** HPLC chromatograms of compound 1: (A) HPLC chromatogram; (B) UV spectrogram and (C) 3D graph.

achieved good resolution and acceptable separation time. Therefore, solvent system 6, *n*-hexane–ethanol–water (6:5:4, v/v/v), was selected as the solvent system for HSCCC separation. Under the optimized conditions, one fraction (corresponding to compound 1) was obtained in one-step elution (Fig. 3) and the retention of the stationary phase was 84.3%.

### *3.3. Purity detection of HSCCC peaks and structure confirmation*

#### *3.3.1. HPLC purity analysis*

The HSCCC separated collections were filtered and then analyzed by HPLC. The collections of the dark part in the HSCCC graph (Fig. 3) were combined and evaporated under reduced pressure at 50 °C. 27.1 mg of compound 1 was obtained, with purity of 98.5%, as determined by HPLC. The HPLC chromatogram of compound 1 is shown in Fig. 4.

#### *3.3.2. Structure confirmation of compound 1*

The compound was white powder. The structural data of the compound are listed as follows, which matched the data of deoxyschizandrin [15]. ESI-MS: *m/z*: $[M+N_a]^+$439.16. UV (MeOH) $\lambda_{max}$ nm: 220, 254, 282. $^1$H NMR and $^{13}$C NMR data are summarized in Table 2. The molecular formula of deoxyschizandrin was $C_{24}H_{32}O_6$.

**Table 2** $^1$H NMR (400 MHz) and $^{13}$C NMR (400 MHz) spectrum data of deoxyschizandrin (1) ($CDCl_3$, $\delta$ in ppm).

| No. | $^{13}$C NMR | $^1$H NMR |
|---|---|---|
| 1 | 151.7 | |
| 2 | 140.2 | |
| 3 | 153.0 | |
| 4 | 107.3 | 6.54 (1H, d, $J$=4.0) |
| 5 | 139.2 | |
| 6 | 35.7 | |
| 7 | 40.9 | 1.82 (1H, dd, $J$=1.6, 8.0) |
| 8 | 33.9 | 1.82 (1H, dd, $J$=1.6, 8.0) |
| 9 | 39.2 | 2.04 (1H, d, $J$=8.8), 2.27(1H, dd, $J$=1.6, 8.0) |
| 10 | 134.0 | |
| 11 | 110.6 | 6.54 (1H, d, $J$=4.0) |
| 12 | 151.6 | |
| 13 | 139.9 | |
| 14 | 151.5 | |
| 15 | 123.5 | |
| 16 | 122.4 | |
| 17 | 12.8 | 0.74 (3H, d, $J$=8.0) |
| 18 | 21.9 | 1.00 (3H, d, $J$=8.0) |
| $-OCH_3$ (–C1, 14) | 61.0 | 3.89 (6H, m) |
| $-OCH_3$ (–C2, 13) | 60.6 | 3.89 (6H, m) |
| $-OCH_3$ (–C3, 12) | 56.0 | 3.59 (6H, m) |

## 4. Conclusion

An HSCCC method was established for separation and purification of deoxyschizandrin from Schisandrae Sphenantherae Fructus in one step. The purity of deoxyschizandrin was 98.5% as determined by HPLC. The established method was simple, fast, effective and able to prepare pure compounds as reference substance from Schisandrae Sphenantherae Fructus for related research such as bioactivity, quality control, pharmacology and so on.

## Acknowledgments

This work was supported by the International Scientific and Technological Cooperation Program of China (No. 2009DFA31230) and the Industry–University–Research Cooperation Program from Science and Technology Department of Guangdong Province (No. 2010B090400533).

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