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Preparative isolation and purification of salidroside from the Chinese medicinal plant *Rhodiola sachalinensis* by high-speed counter-current chromatography

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Abstract

High-speed counter-current chromatography was applied to the isolation and purification of salidroside from the Chinese medicinal plant *Rhodiola sachalinensis* A. Bor. The crude salidroside was obtained by extraction with methanol from *Rhodiola sachalinensis* A. Bor. Preparative high-speed counter-current chromatography with a two-phase solvent system composed of *n*-butanol–ethyl acetate–water (2:3:5, v/v) was successfully performed yielding salidroside (32 mg) at 98% purity from 250 mg of the crude extract in a one-step separation.

Keywords: *Rhodiola sachalinensis*; Counter-current chromatography; Salidroside

1. Introduction

Rhodiola sachalinensis A. Bor. (Hongjingtian in Chinese) is one of the most popular traditional Chinese medicines. This herb has been shown to possess medical functions such as resisting anoxia, microwave radiation and fatigue [1,2]. As a drug of ''source of adaptation to environment'' in Chinese traditional medicine, it has been used in such special posts as diver, astronaut, pilot and mountaineer to enhance the body's ability to survive in adverse environments [3–5]. Furthermore, its effect on extending human life was also found [6]. The major active constituent of this medicinal herb is salidroside.

High-speed counter-current chromatography (HSCCC) is a unique liquid–liquid partition chromatography that uses no solid support matrix. Therefore, it eliminates irreversible adsorptive loss of samples onto the solid support matrix used in the conventional chromatographic column. The method has been successfully applied to the analysis and separation of several natural products [7–15]. However, no report has been published on the use of HSCCC for the isolation and purification of salidroside from plants. The purpose of this study, therefore, was to develop a method for the isolation and purification of salidroside from the Chinese medicinal plant *Rhodiola sachalinensis* A. Bor. by HSCCC.

2. Experimental

2.1. Apparatus

Preparative HSCCC was carried out with a Model

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CCC-1000 high-speed counter-current chromatograph (Pharma-Tech Research, Baltimore, MD, USA). The apparatus consisted of three preparative coils, connected in series (diameter of tube, 2.6 mm; total volume, 325 ml). The revolution radius or the distance between the holder axis and central axis of the centrifuge (R) was 7.5 cm, and the β value varied from 0.47 at the internal terminal to 0.73 at the external terminal ($\beta = r/R$ where r is the distance from the coil to the holder shaft). The HSCCC system was equipped with a Model Series II HPLC pump (Pharma-Tech Research, USA), a Model SPD-10Avp UV–Vis detector (Shimadzu, Japan), a Model L 120 E flat-bed recorder (Linseis, Germany), and a sample injection valve with a 10-ml sample loop.

2.2. Reagents

All solutions were prepared with analytical grade compounds. Reverse osmosis Milli-Q water (18 MΩ) (Millipore, USA) was used for all solutions and dilutions. Ethyl acetate, *n*-butanol, *n*-hexane, methanol and ethanol were obtained from BDH (Poole, UK). The standard salidroside was purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Ministry of Health, Beijing, China. The salidroside stock solution was 1.00 mg ml^{-1} which was prepared by dissolving 10.0 mg of salidroside in 10.00 ml methanol and then stored in a refrigerator. The working solutions were prepared by suitable dilution of the stock solutions with methanol. All other solutions were prepared by dissolving appropriate amounts of commercially available chemicals in water.

The dried root of *Rhodiola sachalinensis* A. Bor. was purchased from a local drug store.

2.3. Preparation of crude salidroside from the *Rhodiola sachalinensis A. Bor.*

Preparation of crude salidroside was carried out according to the literature [16]. In brief, the dried roots of *Rhodiola sachalinensis* A. Bor. were ground into powder. The powder (100 g) was extracted by reflux with 400 ml of methanol for 2 h. The mixture was filtered, and the filtrate was collected. The extract was then evaporated to dryness by rotary vaporization at 50°C, and the residue (5.286 g) was stored in a refrigerator for the subsequent HSCCC separation.

2.4. Preparation of two-phase solvent system and sample solution

In the present study, we selected a two-phase solvent system composed of *n*-butanol–ethyl acetate–water at various volume ratios. Each solvent mixture was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use.

The sample solution was prepared by dissolving the crude sample in the solvent mixture of lower phase and upper phase (1:1, v/v) of the solvent system used for separation.

2.5. HSCCC separation procedure

In each separation, the coiled column was first entirely filled with the upper phase (stationary phase), and then the apparatus was rotated at 1000 rpm, while the lower phase (mobile phase) was pumped into the column at a flow-rate of 2.0 ml min^{-1}. After the mobile phase front emerged and hydrodynamic equilibrium was established in the column, about 10 ml of the sample solution containing 250 mg of the crude salidroside was injected through the injection value. The effluent of the column was continuously monitored with UV–Vis detection at 276 nm. Peak fractions were collected according to the elution profile.

2.6. HPLC analysis

The crude sample and each peak fraction obtained by HSCCC were analyzed by high-performance liquid chromatography (HPLC) according to the literature [16]. The HPLC system used throughout this study consisted of a Waters 510 pump (Waters, Milford, MA, USA), a sample injector (Rheodyne,

Cotati, CA, USA) with a 20 μl loop, and a Waters 996 photodiode array detector. Evaluation and quantification were made on a Millenium chromatography data system (Waters). The column used was a reversed-phase μBondapak C_{18} column (300×3.9 mm I.D., 10 μm, Waters). The mobile phase was methanol–water (15:85, v/v) and the flow-rate was 1.0 ml min^{-1}. The effluent was monitored at 276 nm.

3. Results and discussion

Fig. 1 shows HPLC analysis of the crude salidroside from *Rhodiola sachalinensis* A. Bor. as well as the chemical structure of salidroside. Peak A corresponds to salidroside.

Preliminary HSCCC studies were carried out with the two-phase solvent system composed of *n*-hexane–ethanol–water at various volume ratios (10:5:5, 10:2:8 and 10:7:3). It was very difficult to separate salidroside from other substances (data not shown). As shown in Table 1, the *K* (partition coefficient) values of salidroside in the two-phase solvent system composed of *n*-hexane–ethanol–water were too small. Thus, the two-phase solvent system composed of *n*-hexane–ethanol–water was not suitable for the separation of salidroside from plant, although this two-phase solvent system was satisfactory for the separation of lutein from algae by HSCCC [7]. Consequently, another two-phase solvent system was then tested.

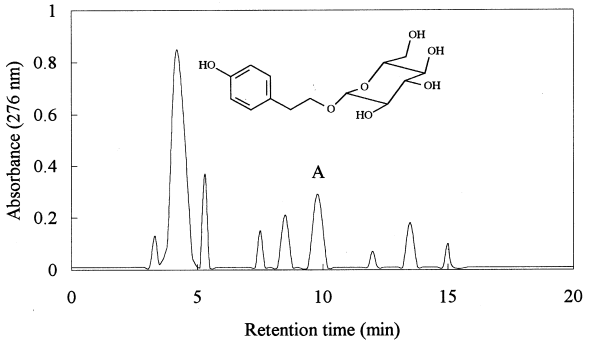

Fig. 1. Chromatogram of crude salidroside from *Rhodiola sachalinensis* A. Bor. by HPLC analysis as well as the chemical structure of salidroside, A=salidroside. Conditions: column: reversed-phase μBondapak C_{18} column (300×3.9 mm I.D., 10 μm); mobile phase: methanol–water (15:85, v/v); flow-rate: 1.0 ml min^{-1}; detection: 276 nm.

Table 1
The *K* (partition coefficient) values of salidroside in several solvent systems

Solvent system	*K* value
n-Hexane–ethanol–water (10:5:5)	0.011
n-Hexane–ethanol–water (10:2:8)	0.010
n-Hexane–ethanol–water (10:7:3)	0.014
n-Hexane–ethyl acetate–ethanol–water (5:5:5:5)	0.135
n-Hexane–ethyl acetate–ethanol–water (5:5:2:8)	0.124
n-Hexane–ethyl acetate–ethanol–water (5:5:7:3)	0.142
n-Butanol–ethyl acetate–water (4:1:5)	0.845
n-Butanol–ethyl acetate–water (3:2:5)	0.751
n-Butanol–ethyl acetate–water (2.5:2.5:5)	0.706
n-Butanol–ethyl acetate–water (2:3:5)	0.524
n-Butanol–ethyl acetate–water (1:4:5)	0.339

It was also difficult to separate salidroside from other substances with the two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water at various volume ratios (5:5:5:5, 5:5:2:8 and 5:5:7:3). As shown in Table 1, the *K* values of salidroside in the two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water were also small. Thus, the two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water was not suitable for the separation of salidroside from plant, although this two-phase solvent system was satisfactory for the separation of astaxanthin from algae by HSCCC [17]. In subsequent studies, another two-phase solvent system was thus tested.

Performance of the two-phase solvent system composed of *n*-butanol–ethyl acetate–water at various volume ratios (4:1:5, 3:2:5, 2.5:2.5:5, 2:3:5 and 1:4:5) was evaluated in terms of peak resolution. The retention of the stationary phase was small (about 20%) with the two-phase solvent system at ratio 4:1:5, and it was very difficult to separate salidroside from other substances. When the two-phase solvent system at ratio 3:2:5 was used, the retention of the stationary phase was enhanced (about 30%), but, the peak resolution was still not satisfactory (data not shown). When the two-phase solvent system at ratio 2.5:2.5:5 was used, the peak resolution and the

retention of the stationary phase (about 35%) were both improved. When the two-phase solvent system at ratio 2:3:5 was used, the peak resolution and the retention time were even better. When, the two-phase solvent system at ratio 1:4:5 was also tested, the retention of the stationary phase was good (about 50%), but the peak resolution was poorer. Fig. 2 shows the preparative HSCCC separation of 250 mg of the crude sample using the solvent system composed of *n*-butanol–ethyl acetate–water (2:3:5, v/v). After salidroside was eluted, in order to save solvents and time, the remaining compounds in the column were removed by pumping out the stationary phase instead of eluting them with the mobile phase because the stationary phase was not to be reused. HPLC analysis of each peak fraction of this preparative HSCCC revealed that salidroside corresponding to peak A was over 98% pure. The yield of salidroside was 32 mg. The HPLC chromatogram of salidroside as purified from the preparative HSCCC is shown in Fig. 3.

The *K* values of salidroside in several solvent systems were measured according to the literature [18], and are given in Table 1.

In conclusion, HSCCC was successfully used for the isolation and purification of salidroside from *Rhodiola sachalinensis* A. Bor., and yielded 32 mg salidroside at 98% purity from 250 mg of the crude extract in a one-step separation.

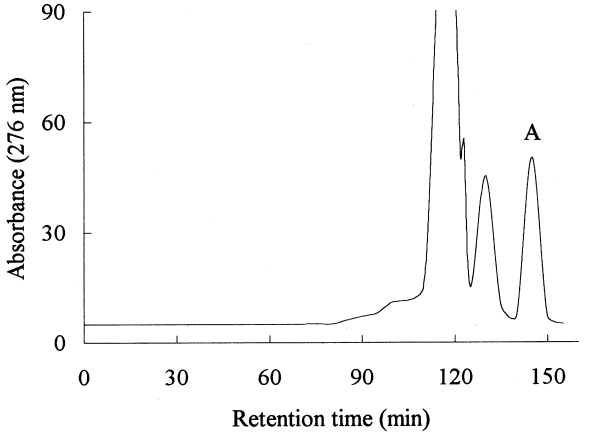

Fig. 2. Chromatogram of crude salidroside from *Rhodiola sachalinensis* A. Bor. by HSCCC separation, A=salidroside. Conditions: column: multilayer coil of 2.6 mm I.D. PTFE tube with a total capacity of 325 ml: rotary speed: 1000 rpm; solvent system: *n*-butanol–ethyl acetate–water (2:3:5, v/v); mobile phase: lower phase (water); flow-rate: 2 ml min^{-1}; detection: 276 nm; sample size: 250 mg; retention of the stationary phase: 42%.

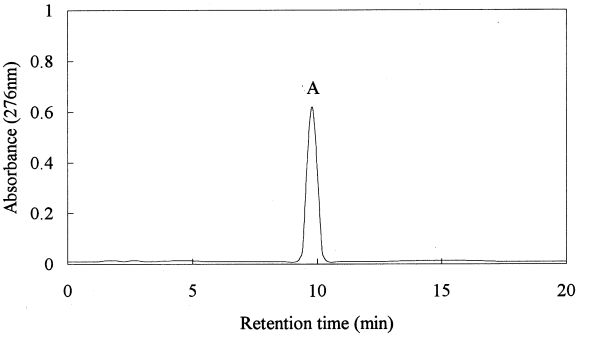

Fig. 3. HPLC chromatogram of salidroside purified from *Rhodiola sachalinensis* A. Bor. Conditions: column: reversed-phase μBondapak C_{18} column (300×3.9 mm I.D., 10 μm); mobile phase: methanol–water (15:85, v/v); flow-rate: 1.0 ml min^{-1}; detection: 276 nm.

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