Separation and Preparation of Gentiopicroside and Sweroside from the Extracts of *Swertia franchetiana* H. Smith by HSCCC

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Abstract [**Objective**] To separate and prepare gentiopicroside and sweroside from the extracts of *Swertia franchetiana* H. Smith by high speed counter-current chromatography (HSCCC). [**Method**] Chloroform:methanol:water = 4:4:2 (*V/V/V*) as solvent system, the upper one was stationary phase while the lower one was mobile phase; ultrasonic degassed for 20 min; the rotating velocity of 850 r/min; mobile phase flow rate of 2 ml/min; the detective wavelength of 254 nm; the purity of product was identified by polarimeter. [**Result**] 14 mg gentiopricroside and 27 mg swersoside were obtained from 100 mg crude extracts of *Swertia franchetiana* H. Smith, the specific rotation $[a]_D^t$ of which were −196.3° and −126.6° respectively, which suggested the products were of high purity. [**Conclusion**] The study would lay a feasible way for the large scale preparation of gentiopicroside and sweroside by HSCCC.

Key words *Swertia franchetiana* H. Smith, High speed counter-current chromatography, Gentiopicroside, Sweroside, China

Swertia franchetiana H. Smith, *Swertia* of Gentianaceae, perennial herbal plant[1], with the significant effects in clearing heat and nourishing gallbladder, dispelling damp, smoothing liver and promoting digestion, cardiac stimulation, detoxification, removing jaundice and keeping calm, has been recognized as common tibetan medicine in traditional popular prescription[2]. A large number of gentiopicroside and sweroside has been found in *Swertia franchetiana* H. Smith by Chen Hui-yun[3]. However, separating gentiopicroside and sweroside from *Swertia franchetiana* H. Smith by traditional chromatography was of long period and large consumption in reagents, plus with the irreversible adsorption of sample and difficulties in purifying, therefore establishing rapid and high efficient purification method is of great significance in separating high value added natural active ingredients.

As a new separating technology, the mechanism of high speed counter-current chromatography is to separate the solute from two immiscible liquid systems by different distribution coefficient without solid support. Compared with traditional chromatographic technology, high speed counter-current chromatography has the advantages of high efficiency, rapid, and simple, however without the adsorption phenomenon that are common to be seen in gas-liquid and solid-liquid chromatographic systems, therefore it has been extensive applied in separation and purification of active ingredients from natural medicines[4]. In this study, gentiopicroside and sweroside have been prepared from *Swertia franchetiana* H. Smith by high speed counter-current chromatography, the purity and structure of which were identified by polarimeter in order to lay foundation for the large preparation of gentiopicroside and sweroside.

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1 Materials and methods

1.1 Materials

1.1.1 Research object. *Swertia franchetiana* H. Smith, bought from medicine market and identified by researcher Lu Xue-feng, Northwest institute of plateau biology, Chinese academy of sciences.

1.1.2 Reagents. Gentiopicroside and sweroside reference substances, provided by Tibetan medicine research center of Northwest institute of plateau biology, Chinese academy of sciences; methanol, chromatogram class, other reagents were AR.

1.1.3 Instruments. TBE-300A high speed counter-current chromatography, Shanghai Tongtian biochemical technology Co., Ltd; TBD-23 UV Detector, HX-1050 constant temperature circulator, Beijing Boyikang experiment Co., Ltd; N2000 chromatographic working station, Zhejiang University institute of intelligence information engineering; 1200 HPLC, Agilent Corporation; FA2104 N electronic balance, Shanghai precision & scientific instruments Co., Ltd; FZ 102 micro-mill, Tianjing Taisite instruments Co., Ltd; RE-52 rotary evaporator, Shanghai Yarong biochemical instruments factory; XW-80 turbine mixer, Shanghai precision and science Co., Ltd.

1.2 Methods

1.2.1 Preparation of crude *Swertia franchetiana* H. Smith extract. 250 g crude drug was weighed precisely and smashed, ultrasonically extracted by 750 ml methanol for 40 min, repeated 3 times, then filtered. The filtrate was concentrated to 250 ml under reduced pressure then blended with ethyl acetate, the aqueous solution was concentrated under reduced pressure to 100 ml then passed activated carbon column, eluted with water:methanol (1:1 *V/V*), the elution was collected and evapo-

d to dry, then faint yellow powder was obtained.

.2 Selection of biphase solvent system[5-6]. Chloroform: hanol:water system was chosen, the ratio of chloroform and er was 4:2 (*V/V*) while the dosage of methanol was adjus- to the actual requirement, the mixed solvent system was ded well and kept overnight. Two shares of 1 ml solution e taken from the upper and lower phases respectively and into centrifuge tubes with 2 mg sample to be separated, centrifuged. The concentrations of tested sample in upper lower solution were determined by HPLC, the distribution ficient was figured out:

$K = C_{upper}/C_{lower} = A_{upper}/A_{lower}$

e equation, K was distribution coefficient; C_{upper} was the entration of tested sample in upper solution (mol/L); C_{lower} concentration of tested sample in lower solution (mol/L); was the peak area in upper solution (μV·s); A_{lower} was eak area in lower solution (μV·s).

3 HPLC conditions. Agilent TC-C18 column (250 mm × mm, 5 μm); mobile phase of methanol:water (2:8 *V/V*); rate of 1.0 ml/min; detective wavelength of 254 nm; tem- ture of 26 ℃, sampling volume of 10 μl.

4 Optimization of HPLC conditions. ①Chloroform-metha- ater (4:4:2, *V/V/V*) as biphase solvent system, flow of 2.0 ml/min, rotation speed of 750, 800, 850, 900, 950 , the influence of rotation speed was investigated by the tion rate of stationary phase. Retention rate = (column ne + channel soundness volume-pushed stationary phase ne)/(column volume- sampling circle volume) × 100%. lection of flow rate. Chloroform-methanol-water (4:4:2, /V) as biphase solvent system, rotation speed of 850 , flow rate of 1.0, 1.5, 2.0, 2.5 ml/min. The influence w rate was investigated by the retention rate of stationary e.

5 Separation and purification of crude *Swertia franchetiH.* Smith extract by HSCCC. Chloroform-methanol-water 2) was placed in 2 000 ml separating funnel, shaken well ept overnight. The upper level was stationary phase, the level was mobile phase, both of which were ultrasonic ally ssed for 20 min, the tested sample was dissolved in lower . Flow rate of 9.9 ml/min, the separating spiratron was with upper phase (stationary phase) then circulator d under preselected temperature, the lower phase (mo- hase) was pumped in with the speed of 2.0 ml/min. In eantime, the main engine and the detector were started he rotation rate of 850 r/min, the stationary phase and e phase were collected by graduate cylinder. The system ble once the mobile phase flew out and baseline was sta- he volume of stationary phase was recorded and the re- n rate of which was calculated. The air is extracted once mple solution was injected, recorded the volume, then ed the knob, the effluent was detected at 254 nm by ultra- detector and concentrated with rotary evaporator.

Determination of optical rotations of gentiopicroside and side. Gentiopicroside and sweroside are optical active nces because of the chiral molecule structure, as an important physical constant, the specific rotation of each substance is invariable, so the purity and the content of each optical active substance can be identified by determining the optical rotation and specific rotation. Let the sodium lamp stabilize by preheating the polarimeter, then the tube was filled with methanol for zero correction, rinsed with small amount of tested solution for 2 – 3 times then filled with tested solution, the indication was recorded. Repeated 2 – 3 times, the average was checked, then minus blank value, the actual optical rotation (a) was obtained. The specific rotation was calculated by the following equation:

$$[a]_D^t = a/C \times 1$$

In the equation, $[a]_D^t$ was specific rotation, a was optical rotation, C was the percentage concentration of tested sample.

2 Results and analysis

2.1 Selection result of biphase solvent system As can be known from Table 1, the distribution coefficient of gentiopicroside and sweroside was in the range of 0.5 – 1.0 when the solvent system was chloroform-methanol-water (4:4:2, *V/V/V*), under above condition, gentiopicroside and sweroside can be separated well. Sufficient time should be given to the mixed solvent system for the equilibrium of upper phase and lower phase, in addition the solubility of solute in single solvent is different from that in mixed solvent, so ultrasonic ally degassing should be carried.

Table 1 Distribution coefficient of gentiopicroside and sweroside in different solvent systems

Chloroform-methanol-water	Distribution coefficient	
	Gentiopicroside	Sweroside
4:1:2	0.15	0.20
4:2:2	0.36	0.38
4:3:2	0.42	0.47
4:4:2	0.57	0.78

2.2 Optimization result of high speed count-current chromatographic conditions

2.2.1 Effect of rotate speed on the separation activity. Generally speaking, the retention rate of stationary phase increased and better separating effect was achieved with higher rotate speed. As Fig. 1 showed, the retention rate of stationary phase was high when the rotate speed was over 900 r/min, while high rotate speed would result in emulsion and lead to the losing of stationary phase. Therefore 850 r/min was chosen as the separation speed for the consideration of instrumental wastage and energy-saving.

2.2.2 Effect of flow rate on the separation activity. High flow rate of mobile phase would reduce the retention rate of stationary phase, although the situation can be improved by lowering flow rate, the elution time would prolong and cause large consumption of mobile phase and trailing. As can be known from Fig. 2, the separating effect of high speed count-current chromatography was affected by the flow rate, the retention rate was decreased with the flow rate increased in the range of 1.0 – 2.5 ml/min.

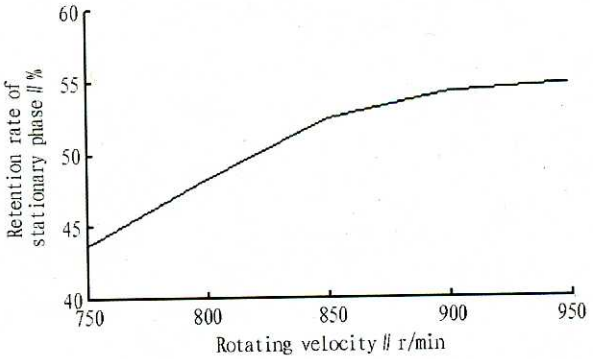


Fig. 1 Effect of rotate speed on the separation activity

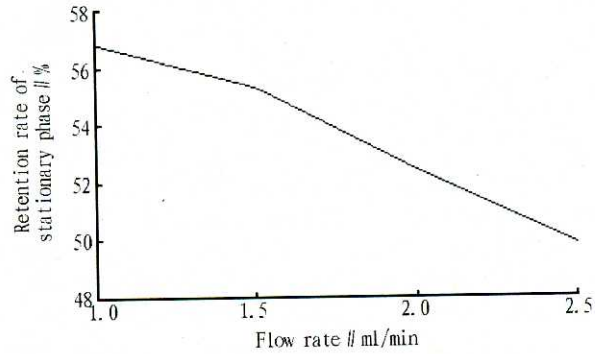
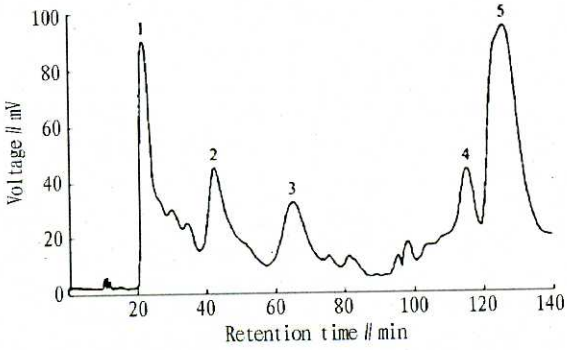


Fig. 2 Effect of flow rate on the separation activity

2.3 Purification result of crude *Swertia franchetiana* H. Smith extract The HSCCC chromatogram of the crude *Swertia franchetiana* H. Smith extract was shown in Fig. 3, 14 mg gentiopricroside and 27 mg swersoside were obtained from 100 mg crude extracts of *Swertia franchetiana* H. Smith by high speed count-current chromatography.



Note: 1 is gentiopicrosid; 3 refers to sweroside.

Fig. 3 HSCCC chromatogram of crude extract of *Swertia franchetiana* H. Smith

2.4 The specific rotation of gentiopricroside and swersoside The specific rotation $[a]_D^t$ of gentiopricroside and swersoside were $-196.3°$ and $-126.6°$ respectively, which was consistent with literature [7] and suggested the products were of high purity.

3 Conclusions and discussions

(1) Selection and optimization of solvent system are vital to the separation effect of high speed count-current chromatography, in which the two kinds of solvent are obviously stratified and the distribution coefficients of tested sample are suitable. Only if the retention rate of stationary phase is high, the effective separation result can be achieved in the range of 0.5-1.0. Suitable solvent separation system was selected in this study on the basis of referring to relative literatures and looking for similar examples, plus with the adjustments and experiments under specific circumstances, which has proved to be convenient and rapid.

(2) As a novel way to separate different substances, the sampling volume of high speed count-current chromatography is hundreds times than HPLC due to liquid as stationary phase; compared with common-pressure and low-pressure liquid chromatography, the renewal and balance of HSCCC system are more convenient and rapid, the separation effect is better with shorter separating time higher purity and completely recovery rate. Therefore, HSCCC will have an extensive application prospect in separation and purification of rare metals, natural drugs and protein.

(3) Creative in identifying, convenient and simple in application, high speed count-current chromatography can be applied in identifying the purity and structure of substances in large scale by utilizing the special physical natures of them[10].

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