Chaoyang Ma[1]
Jian Tang[1]
Hongxin Wang[1]
Guangjun Tao[1]
Xiaohong Gu[1]
Liming Hu[2]

[1]State Key Laboratory of Food Science and Technology (Jiangnan University), School of Food Science and Technology, Wuxi, China
[2]Quartermaster Department, Air Force College, Xuzhou, China

Original Paper

Preparative purification of salidroside from *Rhodiola rosea* by two-step adsorption chromatography on resins

Salidroside is an effective adaptogenic drug extracted from *Rhodiola* species. In the present study, a simple and efficient method for preparative separation and purification of salidroside from the Chinese medicinal plant *Rhodiola rosesa* was developed by adsorption chromatography on macroporous resins. The static adsorption isotherms and kinetics of some resins have been determined and compared for preparative separation of salidroside. According to our results, HPD-200 resin is the most appropriate medium for the separation of salidroside and its adsorption data fit the Langmuir isotherm well. Dynamic adsorption and desorption were carried out in glass columns packed with HPD-200 to optimize the separation process. After two adsorption and desorption runs, a product with a salidroside content of 92.21% and an overall recovery of 48.82% was achieved. In addition, pure lamellar crystals of salidroside with a purity of 99.00% could be obtained from this product. Its molecular weight was determined by an ESI-MS method. The simple purification scheme avoids toxic organic solvents used in silica gel and high-speed counter-current chromatographic separation processes and thus increases the safety of the process and can be helpful for large-scale salidroside production from *Rhodiola rosea* or other plant extracts.

Keywords: Adsorption chromatography / Macroporous resins / Purification / *Rhodiola rosea* / Salidroside

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1 Introduction

Rhodiola rosea, which belongs to the family Crassulaceae and the genus *Rhodiola* [1], is one of the most popular Chinese traditional medicinal plant used to enhance the body's resistance to fatigue and to prolong life [2]. Research has shown that this herb has a number of bioactive effects such as antimicrobial [3, 4], anti-cancer [5 – 8], antidepressant, anti-inflammatory, and antidiabetic effects [9 – 11]. It has also been reported to enhance liver regeneration and memory [12, 13]. It has been defined as an adaptogen because of its ability to increase the resistance of an organism to environmental stress factors and avoid damage from such factors [14 – 16].

Salidroside ((4-hydroxyphenethyl)-β-D-glucopyranoside (Fig. 1)) has been identified as the most potent ingredient in *Rhodiola rosea*. Some studies have shown that it possesses various pharmacological functions including anti-aging, anticancer [17], sleep improving [18], anti-inflammatory [19], neuroprotective, hepatoprotective, and antioxidative effects [20 – 22]. Due to these beneficial effects, more attention has been focused on extraction and purification technologies for salidroside.

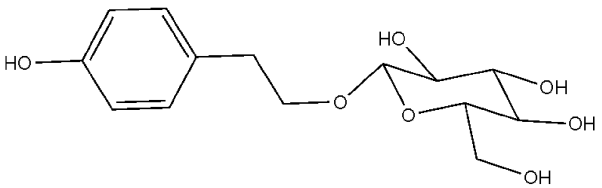

Figure 1. Molecular structure of salidroside.

Up to now, although some methods such as enzymatic synthesis [23] and plant cell culture [24] have been reported to produce salidroside, the most important source of salidroside is the natural plant extract. Numerous methods have meanwhile been developed for the industrial-scale extraction of salidroside. However, there are only few reports of its separation and purification. The conventional method for separation from crude

Correspondence: Dr. Hongxin Wang, State Key Laboratory of Food Science and Technology (Jiangnan University), School of Food Science and Technology, 1800 Lihu Road, 214122, Wuxi, China
E-mail: hxwang1964@yahoo.com.cn
Fax: +86-510-85329276

extracts of *R. rosea* is liquid–liquid extraction, followed by polyamide column and silica column chromatography [25, 26]. Conventional separation techniques create various problems such as excessive solvent wastage, high energy consumption, inefficiency of separation, residual solvent, and considerable labor intensity. Moreover, they are not suitable for industrial use. Recently, high-speed counter-current chromatography [27] has been applied to the preparation of salidroside in high purity; however, the method has the disadvantages of high cost and use of harmful organic solvents. On the other hand, the adsorption and desorption on macroporous resins is an efficient method with a moderate purification effect, high absorption capacity, low operating costs, low solvent consumption, and easy regeneration, and has been successfully applied to the separation and enrichment of effective components from many natural products [28–30]. There is increasing interest in the use of macroporous resins for separating bioactive compounds in industrial practice [31]. In a previous study, Fan *et al.* [32] also reported purification of salidroside on DA-201 resin; however, the purification efficiency was unsatisfactory with only 13.8% (w/w) content of salidroside in the resulting product.

In this study, we investigated the adsorption and desorption properties of salidroside on different macroporous resins. An efficient method was developed for preparative separation and purification of salidroside by adsorption chromatography on resins. After two-step adsorption and desorption and recrystallization, salidroside was obtained in high purity.

2 Experimental

2.1 Chemicals and reagents

Salidroside was isolated in our laboratories. Its structure was elucidated by comparison of spectral data (UV, IR, MS, ^{1}H-NMR, and ^{13}C-NMR) with previous literature data [33]. The purity was determined to be above 98% by HPLC analysis based on a peak area normalization method. HPLC-grade methanol was purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents including hydrochloric acid, sodium hydrate, ammonium acetate, ethanol, and methanol (Sinopharm Chemical Reagent Co., Ltd, Shanghai, China) were of analytical grade and deionized water was purified by a Milli-Q water-purification system from Millipore (Bedford, MA, USA).

2.2 Adsorbents

Five macroporous resins, *viz.* D101, HPD-200, AB-8, HPD-600, and ADS-17, were supplied by Bonchem Co., Ltd (Hebei, China) and Haiguang Chemical Ltd (Tianjin, China). The physical properties of the macroporous resins are summarized in Table 1. The macroporous resins were successively pre-treated with HCl (1 mol/L) and NaOH (1 mol/L) solutions to remove monomers and porogenic agents trapped inside the pores during synthesis process. Then they were dried to constant weight at 80°C under vacuum in a vacuum drying oven (HZF, Shanghai Hongyue Experimental Instrument Co., Ltd., China). Prior to the adsorption experiments, weighed amounts of resins were soaked in ethanol and then washed in a Büchner filter with deionized water and under vacuum until the ethanol concentration of eluate determined by an alcohol meter (Hangzhou Top instrument Co., Ltd, China) was 0%. The moisture content of these resins was determined as follows: three aliquots of each kind of macroporous resin were weighed and then dried in a drying oven (Cany Precision Instruments Co., Ltd., Shanghai, China) at 105°C until constant weight.

2.3 Preparation of sample solutions

The dried roots of *Rhodiola rosea* were provided by Shanghai Novanat Bioresource Co., Ltd. They were ground to powder and dried at 60°C to constant weight. The ground powder (500 g) was refluxed with 5000 mL of ethanol–water (60:40, v/v) solution in a water bath for 60 min. The extraction was repeated once. The extracted solutions were combined and centrifuged at 9000 rpm for 20 min (GL-25MS centrifuge, Shanghai Luxiangyi Centrifuge Instrument Co., Ltd., China). The supernatant extract was transferred to a rotary evaporator (R205B, Shanghai Senco Instrument, China) and concentrated under vacuum to dryness. The salidroside content in the dried extract was 5.55% (w/w); deionized water was added to obtain salidroside solutions of different concentrations.

2.4 HPLC analysis of salidroside

Sample analysis was performed by HPLC [34]. The HPLC system (Waters Corp., Milford, USA) consisted of a dual pump with an 996 diode array detector, a vacuum degasser, an autosampler, a column thermostat, and a Purospher STAR C18 column (250 × 4.6 mm id, 5.0 μm particle size) from Merck KGaA (Darmstadt, Germany). The column oven temperature was fixed at 30°C. A mobile phase consisting of methanol (A) and 20 mmol/L ammonium acetate buffer (pH 5.6) (B) was applied for the gradient elution: 10:90 to 100:0 in 25 min with the flow rate of 1.0 mL/min. Photodiode array detection was used to detect salidroside at 276 nm.

2.5 ESI-MS experiments

ESI-MS experiments were performed to analyze salidroside crystals in both positive and negative ionization

Table 1. Physical properties of five resins.

Resin series	Specific surface area (m^2/g)	Granular diameter (mm)	Pore radius (Å)	Moisture content (%)	Polarity
HPD-600	500 – 550	0.3 – 1.25	100 – 120	29.25	polar
D101	480 – 520	0.3 – 1.25	90 – 100	31.05	non-polar
AB-8	480 – 520	0.3 – 1.25	130 – 140	33.11	weakly polar
HPD-200	700 – 750	0.3 – 1.25	85 – 90	38.44	non-polar
ADS-17	250 – 300	0.3 – 1.25	90 – 150	38.57	polar

mode on a Waters Platform ZMD 4000 (Waters). Typical running parameters were as follows: the continuous infusion concentration of a solution of salidroside crystals dissolved in methanol was 50 ng/mL at a flow rate of 5 μL/min; capillary voltage, +3.87 kV for positive mode and −3.88 kV for negative mode; cone voltage, 25 V for positive mode and 24 V for negative mode; and source temperature, 120°C. Spectra were scanned over a mass range of *m/z* 100 – 1000.

2.6 Static adsorption experiments

2.6.1 Adsorption resins screening

The adsorption isotherms of salidroside on HPD-200 resin were investigated by mixing of 10 mL salidroside extracts at different initial concentrations with pretreated resin (0.5 g on dry basis) on a shaker for 12 h at 25°C. Each experiment was done in triplicate. The following equations were used to quantify the capacity of adsorption.

$$q_e = \frac{(C_o - C_e)V_i}{W} \quad (1)$$

where q_e is the adsorption capacity at adsorption equilibrium (mg/g resin), C_0 and C_e are the initial and equilibrium concentrations of salidroside in the solutions, respectively (mg/mL), W the weight of dry resin (g).

The equilibrium experimental data were fitted to the Langmuir and Freundlich isotherms to describe the interaction of adsorbates with adsorbent:

$$q_e = \frac{q_{max} k_L C_e}{1 + k_L C_e} \quad (2)$$

$$q_e = a C_e^{1/n} \quad (3)$$

where q_e and C_e represent the same parameters as in Eq. (1), q_{max} is the theoretically calculated maximum adsorption capacity (mg/g resin), k_L is the adsorption equilibrium constant related to the affinity between the adsorbent and adsorbate, a the Freundlich constant, an indicator of adsorption capacity, and $1/n$ an empirical constant related to the magnitude of the adsorption driving force.

2.6.2 Adsorption kinetics

The adsorption kinetics of HPD-200 and D101 resins was studied by adding 0.5 g of pretreated resin and 10 mL of 1.89 mg/mL salidroside extracts to each flask with a lid. The concentration of salidroside in the adsorption solution was determined by HPLC at different times until equilibration. The following pseudo-second-order kinetic equation was used to describe adsorption process.

$$q_t = \frac{k q_e^2 t}{1 + k q_e t} \quad (4)$$

where q_e and q_t is the amount adsorbed at equilibrium and at time t (mg/g), respectively, k is the rate constant for the adsorption process ($g \cdot mg^{-1} \cdot min^{-1}$).

2.7 Dynamic adsorption and desorption tests

Dynamic adsorption and desorption tests were performed in glass columns (16 mm × 320 mm, Shanghai Xiamei Biochemical Science Techne Development Ltd., Inc, China) which were wet-packed with HPD-200 resin (28.00 g on dry basis). The flow of samples in all cases was downward. The sample solution was pumped through the glass column at a prescribed flow rate by a peristaltic pump (DHL-A, Shanghai Qingpu-Huxi Instruments Factory, China) and the salidroside contents in the effluent were monitored by HPLC analysis of the eluted fractions collected at 30 mL intervals. The temperature was maintained at 25°C. The adsorbate-laden column was eluted by a certain solvent system and the salidroside contents in the desorption solution were analyzed by HPLC. In adsorption process, the effect of flow rate on adsorption was investigated. In the desorption process, the effect of different elution system on desorption was studied.

In addition, to make desorption more efficient, a two-run dynamic adsorption and desorption process was developed to purify salidroside by gradient elution. In the first-run process, adsorption was performed with 180 mL of a salidroside solution of concentration 1.89 mg/mL at a rate of 1 BV/h, desorption was conducted as follows: after adsorption equilibrium, desorption was successively performed with water and different ethanol aqueous solutions (5%, 10%, 60%). The elution volume was 1.5, 2.5, 2.5, and 3 bed volumes (BV), respectively. The

flow rate during desorption was 1 BV/h. In the second-run process, the eluates of 10% aqueous ethanol from the first-run process were combined and concentrated to dryness and the residue was dissolved in 30 mL of deionized water; then the solution was loaded onto the adsorbent column at the rate of 1 BV/h. After loading, the column was eluted at a rate of 1 BV/h with 2.5 bed volumes of 5% ethanol, then with 2.5 bed volumes of 10% ethanol, and finally with 3 bed volumes of 60% ethanol.

During adsorption and desorption, aliquots of the eluates were pooled at 30 mL intervals by an auto-fraction collector (BS-40A, Shanghai Qingpu-Huxi Instruments Factory, China). The concentration of salidroside in the effluent liquid was monitored using HPLC analysis and then concentrated to dryness under vacuum. The dynamic adsorption and desorption tests were done in triplicates under optimal conditions.

2.8 Recrystallization of salidroside

The fraction obtained with 10% ethanol elution in the second run was made into a saturated solution with ethanol. After being kept at 10°C for two days, the solution was centrifuged to obtain lamellar crystals of salidroside. The quantitative and qualitative analysis of crystals was performed by liquid chromatography/mass spectrometry.

3 Results and discussion

3.1 Adsorption resins screening

The static equilibrium adsorption isotherms for the five resins (HPD-600, D101, AB-8, HPD-200, and ADS-17) were obtained by mixing 10 mL of aqueous solution of salidroside extract at different initial concentrations with the pretreated resins in a shaker bath at 25°C. The initial concentration of salidroside in the solution was 0.20, 0.61, 1.26, 1.89, and 2.52 mg/mL, respectively. As shown in Fig. 2, the adsorption of salidroside onto each resin reached approximately the saturation plateau when the initial concentration of salidroside was 1.89 mg/mL. Thus, the concentration of salidroside in the feed solution was selected as 1.89 mg/mL.

The equilibrium adsorption data were fitted to the Langmuir and the Freundlich equation, respectively. Equilibrium parameters were obtained with Matlab 7.5 using Langmuir and Freundlich curve fitting. The parameter values obtained are listed in Table 2. Comparing two models, the correlation coefficients (R^2) indicated that the Langmuir equation fitted the experimental data better than the Freundlich equation in the studied concentration range. On the other hand, as can be seen from Table 2, non-polar (HPD-200 and D101) and weakly polar (AB-8) resins with greater Q_m values proved to be more

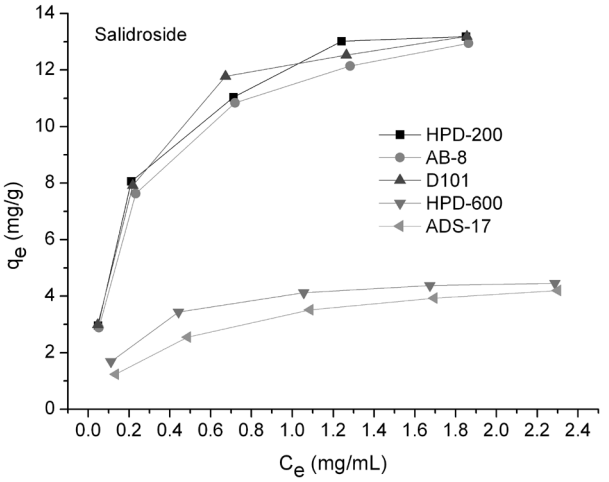

Figure 2. Static isotherm adsorption curves of salidroside on the five resins at 25°C (contact time 12 h).

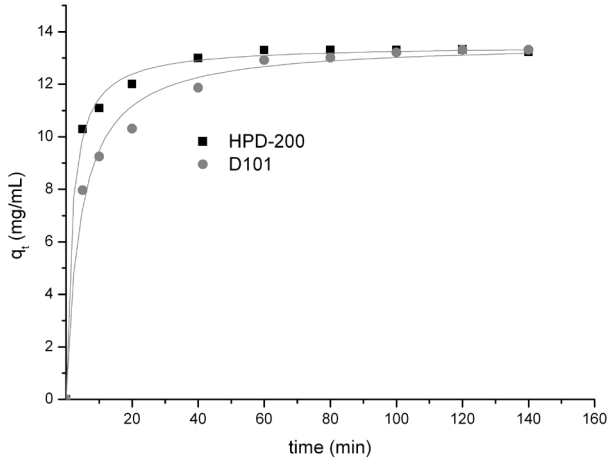

Figure 3. Adsorption kinetics curves of salidroside on the two selected resins at 25°C.

effective for the adsorption of salidroside than the other two polar resins. This behavior is expected, since the adsorption of salidroside molecules should occur in an aqueous environment mainly due to the van der Waals hydrophobic forces in both cases, which were stronger in the case of the non-polar and weakly polar resins. The HPD-200 and D101 resins, which had the largest adsorption capacities, were selected for the following experiments.

3.2 Adsorption kinetics

Adsorption dynamics of salidroside on the two selected resins was investigated at 25°C. Adsorption kinetics curves were obtained and are shown in Fig. 3. The static adsorption dynamics data were fitted to the pseudo-second-order kinetic equation. The kinetic parameters have

Table 2. Langmuir and Freundlich isotherm parameters and pseudo-second-order kinetic parameters of salidroside adsorption.

Resins	Langmuir equation			Freundlich equation			Pseudo-second-order constant		
	q_m (mg/g)	k_L	R^2	A	$1/n$	R^2	q_m (mg/g)	K (g · mg^{-1} · min^{-1})	R^2
HPD-600	4.92	4.75	0.996	3.79	0.271	0.904			
D101	14.48	5.67	0.998	11.62	0.308	0.917	13.59	0.017	0.989
AB-8	14.17	4.89	0.998		11.11	0.329	0.948		
HPD-200	14.49	5.54	0.993	11.63	0.319	0.936	13.48	0.0421	0.997
ADS-17	4.96	2.27	0.998	3.19	0.381	0.972			

been obtained by Matlab 7.5 using the non-linear curve fitting. The parameter values obtained are listed in Table 2. It could be observed from Fig. 3 that, in general, absorption increased rapidly in the first 20 min due to rapid attachment of salidroside to the surface of resin and thereafter rose slowly because of diffusion of salidroside into the micropores of the resin with high intraparticle mass transfer resistance, and reached equilibrium between adsorption and desorption at around 60 min, indicating that the two resins both belonged to the fast adsorption resin type. The good correlations showed that the model is adequate for describing the adsorption process. According to the equilibrium rate constant, k, the HPD-200 resin showed a comparatively faster adsorption rate and is more suitable for adsorption of salidroside than the D101 resin.

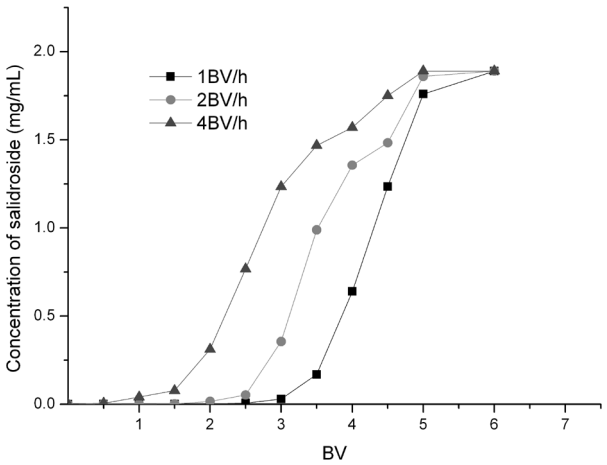

Figure 4. Dynamic breakthrough curves of salidroside on HPD-200 resin at different flow rates.

3.3 Dynamic breakthrough curves on HPD-200 resin

In dynamic adsorption chromatography, feed concentration, flow rate, and temperature have a significant effect on dynamic breakthrough curves. In this test, the initial concentration of salidroside was held constant at 1.89 mg/mL and the temperature at 25°C, and then the effect of flow rate on the adsorption of salidroside by HPD-200 was investigated. The breakthrough curves on HPD-200 were obtained for salidroside based on the elution volume and the concentration of solute therein (Fig. 4).

The flow rate can affect the residence time of solutes in a packed column and further influence adsorption. According to the results of kinetic experiments, the residence time of salidroside molecules on the packed column should be no less than 60 min in order to prevent the occurrence of early breakthrough. In general, a low flow rate was advantageous for adsorption of salidroside molecules because they would acquire sufficient time to interact with active sites at the surface of resins and *vice versa*. At the breakthrough point, the dynamic adsorption capacities of salidroside on HPD-200 were 12.15 mg/g, 10.12 mg/g, and 4.06 mg/g dry resin at flow rates of 1 BV/h, 2 BV/h, and 4 BV/h, respectively, showing that the best adsorption performance was obtained at a flow rate of 1 BV/h. A lower flow rate could decrease the productivity. Therefore, 1 BV/h was used in further experiments. Under these conditions the breakthrough volume was 3 BV (180 mL) with the capacity of 12.15 mg/g equivalent to 92.16% of the corresponding static saturation adsorption capacity.

3.4 Dynamic desorption of salidroside on HPD-200 resin

Dynamic desorption curves for the HPD-200 resin were obtained using different eluants. The results shown in Fig. 5 indicate that the best desorption was obtained when salidroside was eluted by 10% ethanol aqueous solution. 5% ethanol aqueous solution eluted only a little salidroside with the lowest peak; 20% ethanol aqueous solution gave a tailed peak; 10% ethanol aqueous solution gave the sharpest peak and the best desorption.

3.5 Gradient elution test

The results of the gradient elution tests are summarized in Table 3. As can be seen from this table, in the first-run

Table 3. Summary of results from the two-run adsorption and desorption process for the purification of salidroside in *Rhodiola* extract by HPD-200 resin ($n = 3$).

Purification process		*V* (mL)	*Ts* (g)	*Ws* (g)	*Ps* (%)	*Rs* (%)
First	Feed	180	6.1035	0.3402	5.57	100
	Flow-through	180	1.9373			
	Water	90	0.1962	0.0021		
	5% ethanol	150	0.6151	0.0311	5.06	9.14
	10% ethanol	150	0.5280	0.2752	52.12	80.89
	60% ethanol	180	2.8230	0.0215	1.48	6.32
Second	Feed	30	0.5498	0.2866		100
	5% ethanol	150	0.1980	0.0682	34.4	23.8
	10% ethanol	150	0.1821	0.1661	91.21	57.96
	60% ethanol	180	0.1691	0.0463	27.4	16.2

V = volume; *Ts* = total solids; *Ws* = weight of salidroside; *Ps* = purity of salisroside; *Rs* = recovery of salidroside.

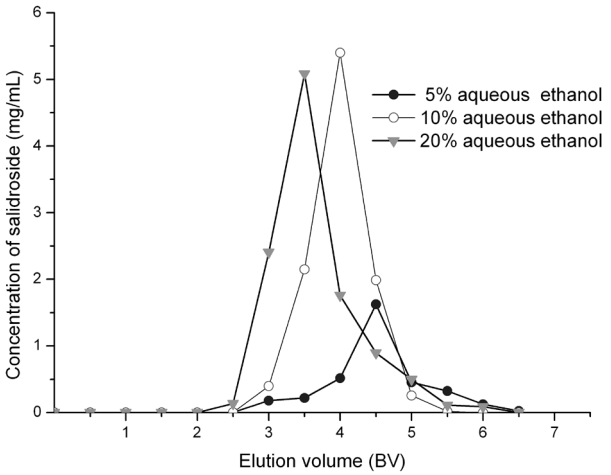

Figure 5. Desorption curves of different ethanol aqueous solutions for salidroside adsorbed on HPD-200 resin.

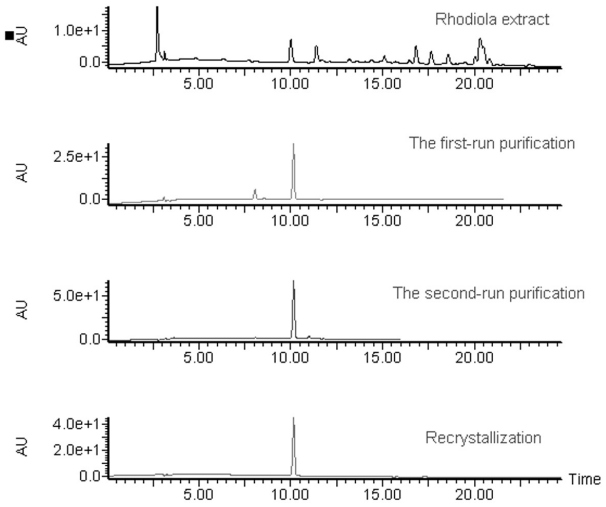

Figure 6. HPLC chromatograms of the extract and products from two-run purifications and recrystallization at a detection wavelength of 205 nm.

process some non-adsorbed components were washed out by elution with 90 mL of deionized water; some adsorbed impurities were also removed by elution with 180 mL of 60% aqueous ethanol. Elution with 150 mL of 10% aqueous ethanol gave the fraction best enriched in salidroside (purity 52.12%, recovery 80.89%). The HPLC chromatogram of the 10% ethanol eluate showed that most impurities present in the crude extract were absent and the relative peak area of salidroside increased significantly (Fig. 6).

According to Table 3, in the second-run process the 10% ethanol elution resulted in a product containing 91.21% salidroside according to HPLC analysis, with an overall yield of 48.82%. The chromatogram of the final product is depicted in Fig. 6.

3.6 Identification of salidroside crystals

The product containing 91.21% salidroside could be recrystallized to give lamellar crystals with a purity of 99.00% according to HPLC analysis. Figure 6 shows the typical HPLC chromatographic profiles for the crystals. Molecular validation of the crystals was performed by ESI-MS experiments. The ESI-MS of the crystals showed a pseudo negative molecular ion $[M-H]^-$ of *m/z* 299 and a pseudo positive molecular ion $[M+NH_4]^+$ of *m/z* 318, indicating that the molecular weight of the crystals is 300. This was in agreement with the value reported by Tolonen *et al.* [35].

4 Concluding remarks

In the present study, a method was successfully developed for preparative isolation of salidroside from extracts of *R. rosea* using adsorption chromatography on macroporous resins. Among five resins investigated, the nonpolar resin HPD-200 was the most appropriate for

separation of salidroside with the best separation behavior. Some process parameters, such as feed concentration and volume, flow rate, temperature and gradient, and volume of elution, were optimized for the most effective preparative separation. A purity of 91.21% and an overall yield of 48.82% for salidroside were achieved when crude extracts were purified by two runs of dynamic adsorption and desorption chromatography on the column packed with HPD-200 resin. Lamellar crystals of salidroside with a purity of 99.00% were obtained from the eluate of 10% ethanol in the second run. Compared to the conventional method, this method costs less, is less labor intensiveness, and gives a high separation efficiency. The results will be of help in the further development of *Rhodiola* resources and applications in pharmaceutical industry.

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