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Separation of scutellarin from crude extracts of *Erigeron breviscapus* (vant.) Hand. Mazz. by macroporous resins

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Abstract

Scutellarin, a flavone glycoside, popularly used in the treatment of heart disease, has been efficiently separated using macroporous resins from crude extracts of Chinese medicinal plant *Erigeron breviscapus* (vant.) Hand. Mazz. HPD-800 resin offered the best adsorption and desorption capacity for scutellarin among the eight macroporous resins tested, and its adsorption data at 25 °C fit best to the Langmuir isotherm. The dynamic adsorption and desorption experiments have been carried out on a HPD-800 resin packed column to optimize the separation process of scutellarin from the crude extracts of *E. breviscapus*. After one run treatment with HPD-800 resin, the scutellarin content in the product was increased 15.69-fold from 2.61% to 40.96% with a recovery yield of 95.01%. The preparative separation process via adsorption–desorption method developed in this study provides a new approach for scale-up separation and purification of scutellarin for its wide pharmaceutical use.

Keywords: Adsorption; Desorption; *Erigeron breviscapus* (vant.) Hand. Mazz.; Macroporous resins; Scutellarin

1. Introduction

Erigeron breviscapus (vant.) Hand. Mazz. is one of the two most important Chinese traditional medicinal plants for heart disease [1], and is also used for expelling the cold, relieving exterior syndrome, dispelling the wind and dampness, removing stagnancy of indigested food and relieving pain [2–4]. Scutellarin (Fig. 1), the major bioactive component in *E. breviscapus*, is drawing particular interest because it significantly dilates blood vessel, improves microcirculation, increases cerebral blood flow and inhibits platelet aggregation activity [5,6].

The conventional method for separation of scutellarin from the crude extracts of *E. breviscapus* was performed by solid–liquid extraction or liquid–liquid extraction, followed by polyamide chromatography and gel chromatography [7,8]. The traditional separation process is not effective regarding reagents, energy consumption and labor intensiveness. Recently, the preparative high-speed counter-current chromatography (HSCCC) has been developed to obtain scutellarin with high purity [9]. However, the HSCCC method is expensive to obtain a large amount of scutellarin from the crude extracts for commercial use. Alternatively, the adsorption–desorption process of macroporous resins is an efficient method for moderate purification, and can be used economically for recovery and concentration of targeted phytochemicals in industrial practices. Macroporous resins can be used to selectively adsorb constituents from aqueous solution as well as non-aqueous systems through electrostatic force, hydrogen bonding interaction, complexation, size sieving action, etc., and they are durable non-polar (polystyrene), middle polar (ester group) and polar (amide, amidocyanogen, acylamino polystyrene) macroporous polymers having a high adsorption capacity. There has been a growing interest in employing macroporous resins to separate bioactive compounds from crude extracts of herbal raw materials because of their unique adsorption properties including ideal pore structure and various surface functional groups avail-

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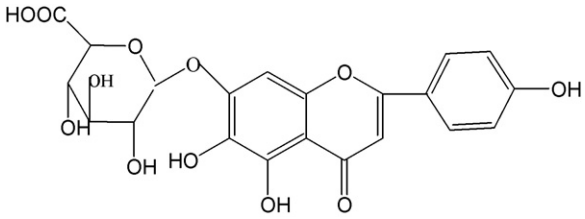

Fig. 1. Chemical structure of scutellarin.

able, low operation expense, less solvent consumption and easy regeneration [10–12].

The objective of the current study was to assess the adsorption and desorption properties of scutellarin on different macroporous resins in order to achieve an efficient method for preparative separation of scutellarin from crude extracts of *E. breviscapus* by a suitable macroporous resin. The information is of significance in the selection of adsorption resins for the preparative separation of scutellarin extracts or other herbal materials in general.

2. Experimental

2.1. Chemicals and reagents

Analytical-grade methanol, *n*-butanol, acetic acid, ethyl acetate, acetonitrile and *n*-hexane were purchased from Atoz Fine Chemicals Co. Ltd. (Tianjin, China). HPLC-grade methanol was obtained from Concord Tech Co. Ltd. (Tianjin, China). All aqueous solutions were prepared with pure water produced by Milli-Q system (Bedford, MA, USA).

The standard sample of scutellarin was supplied by the State Food and Drug Administration of China. Stock solution of scutellarin (1.0 mg/ml) was prepared in methanol. A series of standard working solutions with concentrations in the range of 10.0–200 μg/ml for scutellarin were obtained by further dilution of the stock solution with methanol.

Crude extracts of *E. breviscapus* with 2.61% purity were supplied by Wanfang Company (Yunnan, China). The sample solution was prepared by dissolving 2 g of the crude extracts in 50 ml of pure water.

2.2. Adsorbents

Macroporous resins including HPD-100, HPD-300, HPD-400, HPD-450, HPD-500, HPD-600, HPD-700 and HPD-800 were provided by Bonherb Technology Company (Hebei, China), and their physical properties are listed in Table 1. These macroporous resins were pre-treated with 1N HCl and NaOH solutions successively to remove the monomers and porogenic agents trapped inside the pores during the synthesis process, and then were subsequently washed by pure water. Pre-weighed amounts of pre-treated adsorbents dried at 60 °C under vacuum were soaked with 95% ethanol for 12 h, and subsequently the ethanol was thoroughly replaced with pure water.

Table 1
Physical properties of macroporous resins

Name	Surface area (m^2/g)	Average pore diameter (Å)	Polarity
HPD-300	800–870	50–55	Non-polar
HPD-700	650–700	85–90	Non-polar
HPD-100	650–700	90–100	Non-polar
HPD-400	500–550	75–80	Moderate-polarity
HPD-450	500–550	90–110	Moderate-polarity
HPD-800	700–750	90–110	Moderate-polarity
HPD-500	500–550	100–120	Polar
HPD-600	500–550	100–120	Polar

2.3. HPLC analysis of scutellarin

The HPLC analysis was carried out on an Agilent 1100 system which is composed of a quaternary pump with a degasser, a thermostatted column compartment, a variable wavelength detector, an auto-sampler with a 20 μl injection loop and 1100 ChemStation software [13]. Sample analyses were performed on a Shimadzu C_{18} column (250 mm × 4.6 mm i.d., 5 μm) with a liner elution using 0.1% TFA:methanol (60:40) in 20 min. The flow rate was 0.9 ml/min and the effluent was monitored at 335 nm by UV detector.

2.4. Static adsorption and desorption

The static adsorption tests of scutellarin extracts on macroporous resins were performed as follows: pre-weighed amount of hydrated resins (equal to 400 mg dry resin) and 10 ml of aqueous solution of scutellarin extracts were added into Erlenmeyer flask. The flasks were shaken on a shaker (100 rpm) at 25 °C for 2 h. The solution after adsorption was analyzed by HPLC. After the adsorption equilibrium was reached, the resins were desorbed with 10 ml 95% ethanol solution. The desorption solution was analyzed by HPLC. The preliminary choice of these resins was evaluated by their adsorption capacities, and the ratios of adsorption and desorption.

The adsorption kinetics of the selected macroporous resins was studied by adding pre-weighed amount of hydrated adsorbent (equal to 400 mg dry resin) and 10 ml of sample solution of scutellarin extracts into each flask. These flasks were shaken on an incubation shaker (100 rpm) at 25 °C, the concentration of scutellarin in the adsorption solution was analyzed at different time by HPLC. The adsorption isotherm of scutellarin extracts on the selected resins was investigated by contacting 10 ml of scutellarin extracts at different concentrations with pre-weighed amount of hydrated resins (equal to 400 mg dry resin) on an incubation shaker (100 rpm) at 25 °C, and their degrees of fitness to Freundlich and Langmuir equations were evaluated.

2.5. Dynamic adsorption and desorption

Dynamic adsorption and desorption experiments were carried out in a glass column (16 mm × 50 mm) wet-packed with the selected HPD-800 resin. The bed volume (BV) of the resin was 10 ml, and the feed flow rate was 1 ml/min. The concentration

of scutellarin in the effluent liquid was monitored by HPLC analysis of the eluted aliquots collected at 5 ml intervals by a BSZ-100 auto-fractional collector (Shanghai, China). When the dynamic adsorption reached equilibrium, the adsorbate-laden column was washed first by pure water, and then eluted with ethanol–water (70:30, v/v) solution at a flow rate of 1 ml/min. The concentration of scutellarin in each part of the collected desorption solutions was determined by HPLC.

2.6. Calculation of adsorption capacity, ratios of adsorption and desorption

The following equations were used to quantify the capacity of adsorption as well as the ratios of adsorption and desorption.

- Adsorption capacity:

$$Q_e = \frac{V_0(C_0 - C_e)}{W} \quad (1)$$

- Adsorption ratio:

$$E(\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

where Q_e is the adsorption capacity, which represents the mass of adsorbate adsorbed on 1 g dry resin at adsorption equilibrium; E the adsorption ratio, which means percentage of total adsorbate being adsorbed at adsorption equilibrium; C_0 and C_e the initial and equilibrium concentration of scutellarin in the solutions, respectively; V_0 the initial solution added into the flask; W is the weight of the dry resin.

- Desorption ratio:

$$D(\%) = \frac{C_d V_d}{(C_0 - C_e)V_0} \times 100 \quad (3)$$

where D is the desorption ratio (%); C_d the concentration of the solutes in the desorption solutions (mg/ml); V_d the volume of the desorption solution; C_0, C_e and V_0 are the same as those defined above.

3. Results and discussion

3.1. Adsorption capacity, ratios of adsorption and desorption

Eight macroporous resins with different physical properties were employed for separation of scutellarin, and the results were listed in Table 2. The adsorption and desorption ratios of scutellarin on HPD-100, HPD-400, HPD-450 and HPD-800 resins were considerably higher than those of other resins, which correlates with the capacities of the resins and the chemical features of the adsorbed substance. Resins with moderate-polarity showed stronger adsorption ability to moderate-polar substance scutellarin composed by non-polar flavone and polar glycoside. The non-polar resin HPD-100 also showed better adsorption and desorption capacities to scutellarin because HPD-100 resin has a relative bigger average pore diameter and surface area. Similar results were also observed on luteolin separation from pigeon pea leaves by macroporous resins [12]. The selection of resins should be in accordance with the resins polarities, as well as their average pore diameters, surface areas and so on. The moderate-polar resin HPD-800 showed the best adsorption and desorption capacities because of its larger specific surface areas and average pore diameters. Therefore, HPD-800 was selected for the further study of adsorption process of scutellarin.

Table 2
Results of adsorption capacity, adsorption and desorption ratios of different resins

Resin	Adsorption capacity (mg/g)	Adsorption ratio (%)	Desorption ratio (%)
HPD-300	8.92	62.17	96.13
HPD-700	8.85	61.67	42.80
HPD-100	10.65	74.22	88.50
HPD-400	9.15	63.76	92.79
HPD-450	10.5	73.17	69.15
HPD-800	13.72	95.61	98.69
HPD-500	6.15	42.86	64.02
HPD-600	6.45	44.95	60.12

3.2. Adsorption kinetics

Adsorption kinetics curve was obtained for scutellarin on HPD-800 resin. As shown in Fig. 2, the adsorption capacity of HPD-800 resin increased rapidly in the first 30 min, and then increased slowly and reached equilibrium at around 90 min. The fast adsorption process of HPD-800 resin in 30 min was due to high diffusivity of scutellarin into micropores of the resin in the bulk solution, and the slow adsorption process of HPD-800 resin after 30 min was due to the high intraparticle mass transfer resistance within the macroporous resin.

3.3. Adsorption isotherms

Equilibrium adsorption isotherms were obtained by contacting 10 ml of aqueous solution of scutellarin crude extracts at different concentrations with the HPD-800 resin on an incubation shaker at 25 °C. As shown in Fig. 3, the adsorption capacity

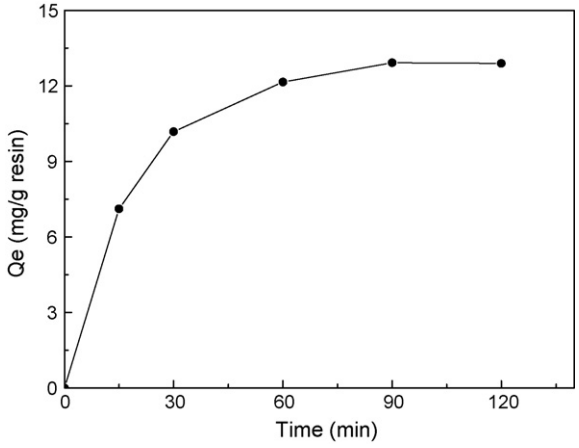

Fig. 2. Adsorption kinetics of scutellarin onto HPD-800 at 25 °C.

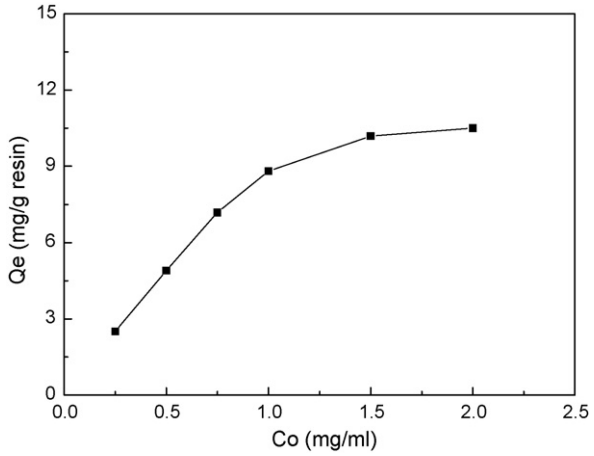

Fig. 3. Adsorption isotherm of scutellarin on HPD-800 at 25 °C.

increased with the initial concentration and reached the saturation plateau when the initial concentration of scutellarin was 1.5 mg/ml. The Langmuir and Freundlich equations are the most popular ones frequently used in description of the experimental data of adsorption isotherms because of their relative simplicity and reasonable accuracy [14]. The Langmuir equation can be used to describe a monolayer adsorption, whereas the Freundlich equation can be used to describe a monolayer adsorption as well as a multilayer adsorption.

Freundlich equation:

$$Q_e = K_F C_e^{1/n} \quad (4)$$

where K_F is the Freundlich constant that indicates the adsorption capacity, and $1/n$ is an empirical constant related to the magnitude of the adsorption driving force.

Langmuir equation:

$$Q_e = \frac{Q_0 K_L C_e}{1 + K_L C_e} \quad (5)$$

where K_L is the adsorption equilibrium constant and Q_0 is an empirical constant.

The Langmuir and Freundlich parameters were summarized in Table 3. Langmuir equation described the better adsorption behavior of scutellarin on HPD-800 because the correlation coefficient (0.995) of Langmuir equation was higher than that (0.973) of Freundlich equation. In the Freundlich equation, the adsorption is very difficult to occur if $1/n$ value is above 1 [15]. In Table 3, the $1/n$ value is 0.81, which indicates that the HPD-800 resin is appreciated for separating scutellarin.

Table 3
Freundlich and Langmuir adsorption isotherm parameters for scutellarin on HPD-800 at 25 °C

Langmuir	
Linear equation	$1/Q_e = 0.02352 + 0.09294/C_e$
R^2	0.995
Q_0 (mg/g)	42.517
K_L (l/mg)	3.951
Freundlich	
Linear equation	$\ln Q_e = 2.113 + 0.810 \ln C_e$
R^2	0.973
K_F (mg/g)	8.273
$1/n$	0.810

Table 4
Breakthrough volume and mass of scutellarin on HPD-800 resin at different feed concentrations under dynamic adsorption conditions

C_0 (mg/ml)	Breakthrough volume (ml)	Mass of scutellarin absorbed (mg)
0.522	50	26.1
0.783	50	39.2
1.044	50	52.2
1.305	31	40.4
1.566	24	37.6

3.4. Dynamic adsorption and desorption

The dynamic adsorption results on HPD-800 were summarized in Table 4. The highest adsorption capacity was observed when the initial concentration of scutellarin was 1.044 mg/ml. When the feed concentration was low, the adsorbate relative to active sites was low, and adsorption increased with increasing concentration of the scutellarin. However, with further increase of feed concentration, the amount of impurities in the crude extracts also increased and the active site-to-adsorbate ratio reduced.

The dynamic desorption curve on HPD-800 was obtained based on the volume of desorption solution and the concentration of solute herein. Most of the scutellarin adsorbed on HPD-800 resin was eluted by 30% ethanol–water solution (data not shown), and the content of scutellarin in the product was increased 15.69-fold from 2.61% to 40.96% with a recovery yield of 95.01%. The chromatograms of the tested samples before and after treatment with HPD-800 resin were shown in Fig. 4. By comparison, it can be seen that some impurities in the crude extracts were removed and the relative peak area of scutellarin increased significantly after the separation on HPD-800 resin.

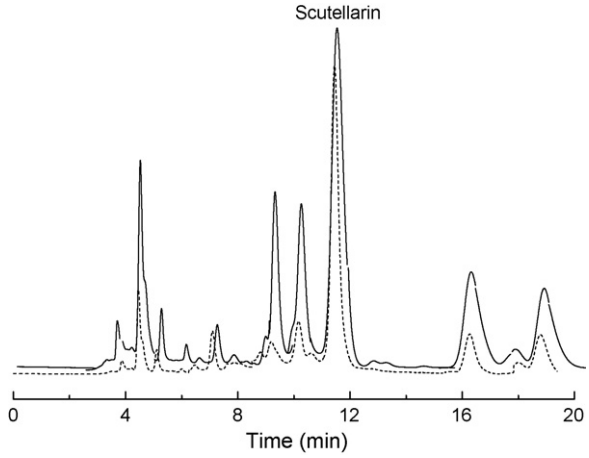

Fig. 4. Chromatograms of sample solution before (solid line) and after (dot line) separation on a column packed with HPD-800 resin.

4. Conclusion

The preparative separation process of scutellarin with macroporous resin has been successfully developed in this study. Among the eight macroporous resins tested, HPD-800 gave the best separation efficiency for scutellarin because of its high surface area, optimum average pore diameter and appropriate surface functional residues, and its adsorption data at 25 °C fit better to the Langmuir isotherm. Using the HPD-800 resin at optimal conditions, the scutellarin content in the product was increased 15.69-fold from 2.61% to 40.96% with a recovery yield of 95.01%. Compared to the conventional method, the adsorption–desorption method is advantageous because of its procedural simplicity, low cost, high efficiency, and ease in scaling-up.

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