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Preparative Separation of Glabridin from *Glycyrrhiza glabra* L. Extracts with Macroporous Resins

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Abstract: In the present study, the performance and adsorption characteristics of five macroporous resins for the separation of glabridin from *Glycyrrhiza glabra* L. have been evaluated. The adsorption and desorption properties of glabridin on macroporous resins including HPD100, HPD300, HPD800, NKA and H103 were compared. HPD100 resin offered the best adsorption and desorption capacities based on the research results. Both Langmuir and Freundlich isotherms were used to describe the interactions between the solutes and resins at different initial concentrations. Dynamic adsorption and desorption experiments on HPD100 resin packed column were conducted to optimize the separation process of glabridin from licorice extracts. After the treatment with stepwise elution on HPD100 resin, the content of glabridin in the product increased from 0.21% to 32.2% which is 153-fold higher than it in *G. glabra* L. roots and the recovery yield was 79.7%. The results indicated the good ability of HPD100 resin for separation glabridin and the study may provide scientific references for the large-scale glabridin production from *G. glabra* L. or other plants extracts.

Keywords: Glabridin, *Glycyrrhiza glabra* L., macroporous resins, separation

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INTRODUCTION

The root of *Glycyrrhiza glabra* L. (licorice) has been used medically for more than 5000 years (1). It was usually used as a single herbal preparation, or combined with other herbs to treat various diseases in respiratory, digestive, endocrine, cardiovascular, and central nervous system as traditional Chinese medicine (2–3). Licorice was also widely used as antiallergic and anti-inflammatory agents for the treatments of health problems in western countries (4). Glabridin is a polyphenolic flavonoid and a main constituent in the hydrophobic fraction of licorice extract. It has been revealed that glabridin has a wide variety of pharmacological activities, such as cytotoxic activity (5), antimicrobial activity against both *Helicobacter pylori* and *Mycobacterium tuberculosis* (6–7), estrogenic and anti-proliferative activity against human breast cancer cells (8). Significantly glabridin has also exhibited inhibitory effect on melanogenesis, inflammation (9) and activities of human cytochrome P450s 3A4, 2B6, and 2C9 (10), anti-oxidantive effect on LDL oxidant (11) and protection of liver mitochondria (12).

The conventional method for separation of glabridin was performed by means of solid-liquid extraction from natural resources, followed by gel chromatography and then solid-liquid extraction is conducted by using different solvents (13), this method to obtain glabridin is inefficient regarding reagents, energy consumption and labor intensiveness. Alternatively, the adsorption-desorption process on macroporous resins is an efficient separation method with a moderate purification effect and can be used economically for recovery and concentration targeted phytochemicals in industrial practices. Macroporous resins have a large specific surface area, a small size and a hollow and layered structure. They are durable polar, non-polar or slightly hydrophilic polymers having high adsorption capacity with possible recovery of the adsorbed molecules, relative low cost, and easy regeneration (14). Therefore macroporous resin adsorption technology has been applied in the field of separation flavonoids and polyphenols from many plants, for example, macroporous resins have been used for adsorption of luteolin from pigeonpea leaves extracts (15), flavonoids and glycyrrhizic acid from licorice extracts (16), polyphenols from *Inga edulis* leaves extracts (17), etc. However, to date, few studies have been involved in the separation of glabridin from *G. glabra* L. by macroporous resins and a low purity of glabridin from crude extracts was achieved after treatment by macroporous resins (18). Consequently, an efficient method with detailed process for preparative separation and purification of glabridin from licorice roots is needed.

In this paper, the development of macorporous resins method for separation of glabridin from the crude ethyl acetate extracts is described.

An efficient method for the preparative separation of glabridin with the optimal resin is developed after investigating the adsorption and desorption properties of glabridin on different macroporous resins. The results in this study are of significances for the preparative separation of *G. glabra* L. extracts or other herbal materials in general.

EQUILIBRIUM MODEL

The adsorption process is the distribution of the solute molecules between the adsorbent and the liquid phase. The equilibrium experimental data were fitted to the Langmuir and Freundlich equations to describe the interaction of solutes with the resin.

The Langmuir isotherm is the best known and the most often used isotherm for the adsorption of solutes from a solution. This model assumes monomolecular layer adsorption with a homogeneous distribution of adsorption energies and without mutual interaction between adsorbed molecules. Langmuir equation (1) is based on a theoretical model where the maximum adsorption capacity corresponds to a monolayer saturated with adsorbate molecules on the adsorbent surface, which is energetically homogeneous.

$$Q_e = \frac{aC_e}{1 + bC_e}, a = Q_{\max}k_l, b = k_l \tag{1}$$

Where Q_e (mg/g) is the adsorption capacity at adsorption equilibrium point, a is the solute adsorptivity (ml/g), $Q_{\max}$ is a constant related to the adsorptive capacity (mg/g), k_l is the parameter which relates to the adsorption energy (ml/mg), C_e is the equilibrium concentrations of glabridin in solutions (mg/ml).

A linearized form of Eq. (1) can be written as:

$$\frac{C_e}{Q_e} = \frac{1}{k_l Q_{\max}} + \frac{Ce}{Q_{\max}} \tag{2}$$

The Langmuir equation was converted to the linearized form with C_e and C_e/Q_e as independent variable, the experimental data were statistically analyzed and R^2 was obtained.

The Freundlich model is used extensively in the physical adsorption and chemical adsorption, and can be used to describe the adsorption behavior of monomolecular layer as well as that of the multi-molecular layer. It assumes a heterogeneous distribution among the adsorption sites at different energies. It is a two-parameter model widely employed for

many different adsorbate/adsorbent system for liquid and gas phase adsorption.

The experimental data were fitted to the Freundlich Eq. (3), describing the interaction of solutes with the resins:

$$Q_e = k_f C_e^{\frac{1}{n}} \tag{3}$$

Where k_f and n are both the Freundlich constants, k_f reflects the adsorption capacity of the adsorbent and n reflects the adsorption affinity of the adsorbent to the adsorbate.

A linearized form of Eq. (3) can be written as:

$$\ln Q_e = \ln k_f + 1/n \ln C_e \tag{4}$$

The k_f and n values can be obtained from the intercept and slope, respectively, and the linear regression line from a plot of ln Q_e versus ln C_e.

EXPERIMENTAL

Chemicals and Reagents

The roots of *G. glabra* L. were collected from Kazakhstan, provided by China National Group Corporation of Traditional & Herbal Medicine (Beijing, China) and identified by Doctor Zhimei Zhang (China Agricultural University, Beijing, China). Glabridin standard was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethanol and ethyl acetate, used for sample preparation and separation were of analytical grade and purchased from Beijing Chemical Factory (Beijing, China). Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from Dima Technology Inc. (VA, USA). All solutions prepared for HPLC were subject to filtration through 0.45 μm nylon membranes.

Adsorbents

Macroporous resins including HPD100, HPD300 and HPD800 were provided by Bon Adsorber Technology Company (Hebei, China), and NKA, H103 from the Chemical Plant of NanKai University (Tianjin, China). Macroporous and their physical properties are listed in Table 1. 100 g (wet weight) macroporous resins were pre-treated at 25°C with 200 ml 1 N HCl and NaOH solutions successively for 1 h to remove the

Table 1. Physical properties of the test macroporous resins

Type	Surface area (m^2/g)	Average pore radius (Å)	Particle diameter (mm)	Polarity	Dipolar moment
HPD100	650–700	90–95	0.3–1.25	Non-polar	0.3
HPD300	800–870	50–55	0.3–1.25	Non-polar	0.3
HPD800	700–750	90	0.3–1.25	Moderate-polarity	1.8
NKA	250–290	85–95	0.3–1.25	Non-polar	0.3
H103	1000–1100	85–95	0.3–1.25	Non-polar	0.3

monomers and porogenic agents trapped inside the pores during the synthesis process, and then were subsequently washed by 400 ml deionized water for 2 h. Pre-treated adsorbents dried at 60°C under vacuum were soaked with 200 ml 95% ethanol for 12 h at 25°C. Subsequently the ethanol was thoroughly replaced with 400 ml deionized water by twice filtration.

HPLC Analysis of Glabridin

Quantification of the glabridin concentration was carried out on a Shimadzu LC-20AVP system with two LC-20AT solvent delivery units, an SPD-20A UV/VIS detector, a CTO-10ASVP column oven (Shimadzu, Kyoto, Japan), T2000P workstation (Beijing, China) and a reversed phase C18 column (250 mm × 4.6 mm, 5 μm, Diamodsil^{TM}). The solvent system of HPLC analysis was consisted of acetonitrile and 0.02% (v/v) TFA in water. The eluation started of 60% acetonitrile and lasted for 20 min. Then acetonitrile was raised to 85% during 2 min and maintained for 15 min to purge the column. The flow rate was 1.0 ml/min. An aliquot of 10 μl sample was injected into the column and detected by UV at 283 nm.

Preparation of Crude Licorice Extracts

Licorice sample, weighing 200 g, dried at 50°C, was minced and grounded into powder of 180 μm by a disintegrator and extracted with 1500 ml ethyl acetate. By sonication in an ultrasonic bath for 30 min, the insoluble residue was extracted again under the same condition. The combined extraction solution was purified by membrane filtration and then concentrated to dryness by removing the ethyl acetate solvent under reduced pressure in the rotary evaporator at 40°C. The content of glabridin was

determined by HPLC as 0.21% in the licorice and 5.25% in the extracts, respectively. The solution of ethanol and water (80:20, v/v) was added to obtain sample solutions containing glabridin in the concentration range of 0.1–0.7 mg/ml.

Static Adsorption and Desorption Tests

The static adsorption tests of glabridin extracts on all macroporous resins were performed as follows: pre-weighed amount of hydrated resins, equaling to 0.5 g dry resins, was put into an air-tight Erlenmeyer flask. Then 80 ml of 20% ethanol aqueous solution of glabridin extracts at different concentrations was added into each flask, respectively. These flasks were shaken on a constant temperature water-bath shaker (120 rpm) at 25°C for 24 h to reach adsorption equilibrium. Their extents of fitness to Freundlich and Langmuir equations were evaluated.

The desorption process was conducted as follows. After adsorption equilibrium was reached, the resin was first washed by 150 ml deionized water and then desorbed with 80 ml ethanol-water (80:20, v/v) solution. The flask was shaken (180 rpm) for 4 h at a constant temperature of 25°C. The desorption solution was analyzed by HPLC.

The adsorption kinetic curves of glabridin on the selected resin were studied as follows: 0.5 g (dry weight) resins were kept in contact with 80 ml ethanol-water (20:80, v/v) solution with 0.45 mg/ml extract. The respective concentration of glabridin in the liquid phase was monitored at certain intervals till adsorption equilibrium by HPLC analysis.

Dynamic Adsorption and Desorption

Dynamic adsorption and desorption experiments for glabridin were carried out in a glass columns (12 mm × 180 mm) wet-packed with the selected resin. The bed volume (BV) and length was 10 ml and 14 cm, respectively. The flow of samples in all cases was downward. Regeneration of the resin was also carried out in a downflow fashion. The sample solution flowed through the glass column at different flow rates and the concentration of glabridin in the effluent liquid was monitored by HPLC analysis.

After reaching adsorptive saturation, the adsorbate-laden column was first washed with 2BV deionized water and then eluted by ethanol-water solution at a certain flow rate. The test for the content of glabridin in the desorption solution was carried out by HPLC. The effect of the sample flow rate and desorption flow rate on the capability of adsorption and desorption were studied.

The stepwise elution tests were carried out as follows: While adsorptive equilibration, the adsorbate-laden column was washed first with 2 BV deionized water, and then eluted with ethanol-water (30:70, 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, v/v) solutions successively, and the volume of each eluent was 2 BV. Each part of the desorption solutions was analyzed by HPLC and then concentrated to dryness under vacuum.

Calculation of Adsorption Capacity, Ratios of Adsorption and Desorption

The following equations are used to quantify the sorption and desorption capacities:

Sorption evaluation

$$Q_e = \frac{(C_0 - C_e) \times V}{W} \tag{5}$$

$$E = \left(\frac{C_0 - C_e}{C_0}\right) \times 100\% \tag{6}$$

Where C_0 is the initial concentrations of glabridin in solutions (mg/ml), V is the volume of solutions (ml), W is the weight of the dry adsorbent (g), E is the adsorption ratio (%) which is the percent of the quantity adsorbed to the initial quantity under equilibrium, and C_e is the same as described above.

Desorption evaluation:

$$D = \left(\frac{C_d \times V_d}{(C_0 - C_e) \times V}\right) \times 100\% \tag{7}$$

Where D is the desorption ratio (%), C_d is the concentration of glabridin in the desorption solution (mg/ml), V_d is the volume of the desorption solution (ml).

RESULTS AND DISCUSSION

Adsorption Isotherms

Equilibrium adsorption isotherms were obtained for all the resins by contacting 40 ml of aqueous solution of licorice extract at different concentrations with the resins in a shaker bath controlled at 25°C.

Table 2. Langmuir and Freundlich adsorption parameters of glabridin on five resins at 25°C

Adsorbent	Langmuir equation	R^2	Freundlich equation	R^2
HPD100	$C_e/Q_e = 0.0236C_e + 0.0004$	0.9972	$Q_e = 49.112C_e^{0.1697}$	0.9715
HPD300	$C_e/Q_e = 0.0263C_e + 0.0014$	0.9919	$Q_e = 45.654C_e^{0.3021}$	0.9584
HPD800	$C_e/Q_e = 0.0266C_e + 0.0049$	0.9799	$Q_e = 46.280C_e^{0.5724}$	0.9188
NKA	$C_e/Q_e = 0.0248C_e + 0.0011$	0.9950	$Q_e = 51.537C_e^{0.3122}$	0.9454
H103	$C_e/Q_e = 0.0391C_e + 0.0036$	0.9893	$Q_e = 29.696C_e^{0.3727}$	0.9429

The C_0 of glabridin in the solutions were 0.099, 0.201, 0.300, 0.402, and 0.701 mg/ml, respectively. The Langmuir and Freundlich parameters were summarized in Table 2. As shown in Table 2, both the Langmuir and the Freundlich equations may be considered suitable for describing the tested adsorption system in the studied concentration range and for comparing the adsorption capacity of the different resins at 25°C. Compared with other resins, the correlation coefficients of both equations and the k_f of glabridin on HPD100 were higher, indicating its better performance for separation of glabridin. The correlation coefficient of the Langmuir equation is closer to 1.0, suggesting that the Langmuir isotherm model was slightly better for describing the biosorption equilibrium than Freundlich model.

The amount of glabridin adsorbed at different C_e was shown in Fig. 1. As can be seen from Fig. 1, the adsorption of HPD100 was highest and it would be preferred for the particular application. The adsorption of HPD100 increased slightly at the C_e larger than 0.169 mg/ml, and the corresponding C_0 was 0.402 mg/ml. Thus, the C_0 of glabridin in the sample solution for adsorption were selected as near 0.4 mg/ml.

Adsorption Capacity, Ratios of Adsorption and Desorption

Five kinds of macroporous adsorption resins with different physical properties were employed for separation of glabridin. The initial concentration of glabridin was 0.30 mg/ml. The results of static adsorption and desorption tests for screening among these macroporous resins are shown in Table 3.

The selection of proper resins should be in accordance with their polarities, as well as their average pore radii, surface areas, etc., and the features of the adsorbates. The nonpolar resin HPD100 exhibited that the best adsorption and desorption capabilities may be partly due to the matrix with the strong affinity for glabridin, its larger specific surface areas, or suitable average pore radii.

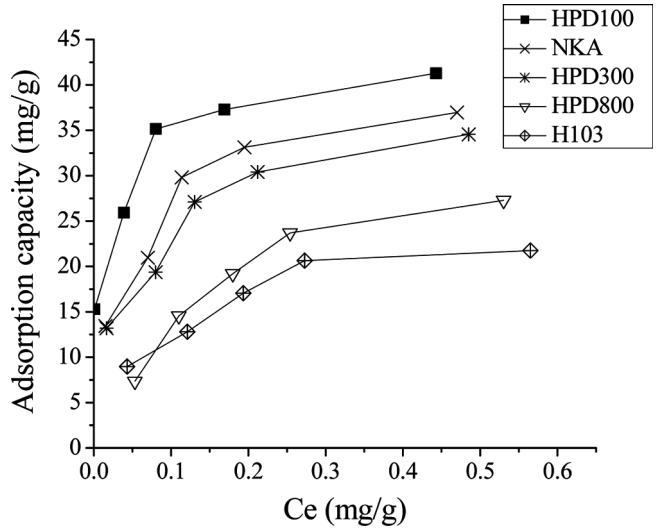

Figure 1. Adsorption isotherm of glabridin on five resins. Initial concentrations of glabridin in adsorption solutions were 0.099, 0.201, 0.300, 0.402, and 0.701 mg/ml. Temperature: 25°C; shake speed: 120 rpm.

In the comprehensive consideration of the fitness to the Langmuir and Freundlich equations, the adsorption capacity and the desorption ratio, HPD100 resin was selected as the most suitable resin for the separation of glabridin and was used in the following test.

Adsorption Kinetics on HPD100

Adsorption kinetics curves were obtained for glabridin on HPD100. As can be seen from Fig. 2, the adsorption capacity towards glabridin

Table 3. The adsorption capacities (mg/mg resin), adsorption and desorption (%) of glabridin on different resins at 25°C. (Initial concentration, 0.30 mg/ml)

Adsorbent	Adsorption capacity (mg/g resin)	Adsorption (%)	Desorption (%)
HPD100	35.13	73.2	92.6
HPD300	27.10	56.5	89.7
HPD800	18.61	38.8	84.9
NKA	29.80	62.1	90.4
H103	17.06	35.5	71.8

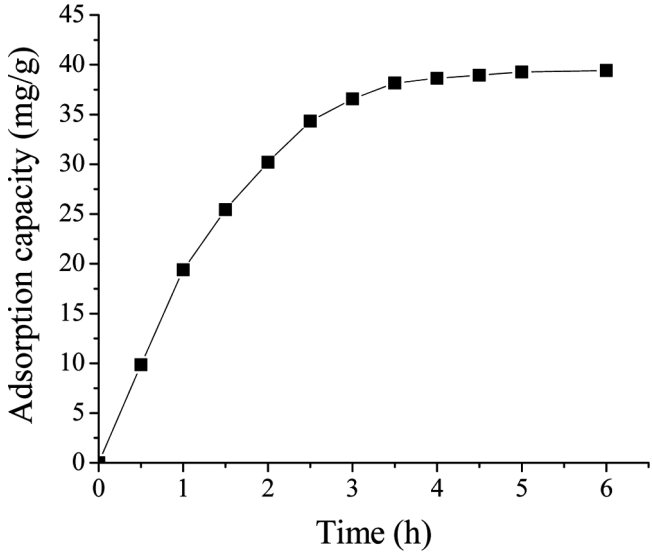

Figure 2. Adsorption kinetics curve for glabridin on HPD100. Initial concentration of glabridin in adsorption solution: 0.45 mg/ml; adsorption temperature: 25°C; shake speed: 120 rpm.

increases rapidly in the first 2 h, and then they increased slowly and reached equilibrium in about 4.0 h.

The adsorption behavior is consistent with the Langmuir monomolecular layer adsorption theory. The fast initial step is likely due to the occurrence of adsorption in the easily accessible mesopores of the particles, proceeding with low mass transfer in the bulk solution. The later slower uptake, on the other hand, is indicative of processes with high intraparticle mass transfer resistance within the macroporous resin.

Static Desorption on HPD100 Resin

The adsorption process was conducted by the procedure described in Static adsorption and desorption tests Section, initial concentration of glabridin in the adsorption solution was 0.45 mg/ml. After the adsorption equilibrium was reached, the adsorbates were desorbed for 6 h in shakers at 25°C using 80 ml of different concentrations of ethanol-water solutions (30:70, 40:60, 50:50, 60:40, 70:30, 80:20, and 90:10, v/v) to choose proper desporption solution, respectively. As shown in Fig. 3, the desorption ratios of glabridin increased rapidly at the range of ethanol concentration from 30 to 50%, and slowly from 60 to 90%.

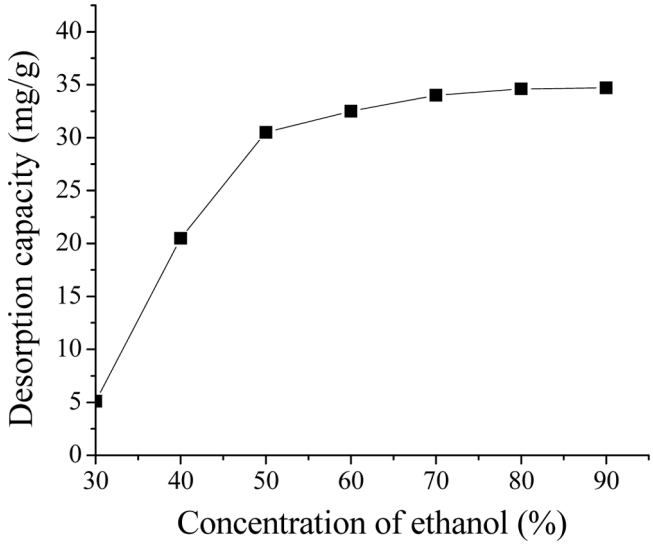

Figure 3. Effect of ethanol concentration on the desorption ratio on HPD100. Initial concentration of glabridin in adsorption solution: 0.45 mg/ml; adsorption and desorption temperature: 25°C; shake speed: 120 rpm.

Thus for the principle of efficiency and economy, ethanol-water (50:50, v/v) solution was selected as the appropriate desorption solution and used in the dynamic desorption experiment.

Effects of Feed Concentration and Flow Rate on Dynamic Adsorption Breakthrough Point on HPD100 Resin

The adsorption effect of the macroporous resin was determined by surface adsorption and sieve classification, surface electrical property, hydrogen bonding interactions, etc. When the adsorption reaches the breakthrough point, the adsorption effect decreases and even disappears. So it is important to set up the breakthrough point in order to calculate the proper sample feed concentration, the flow rate, and the processing volume of the sample solution. Thus, in order to obtain optimum experimental conditions, the effects of the feed concentration and flow rate on the adsorption capacity were studied and the results are presented in Table 4. When investigating one parameter, another parameter was kept constant. As seen in Table 4, as the feed concentration increased, the volume of adsorption solutions at the 5% breakthrough point increased, the adsorption capacity increased at first, then decreased. It reached its

Table 4. The effects of feed concentration and flow rate on adsorption capacity. (a) Flow rate of sample solution is 3 BV/h, (b) Feed concentration of sample solution is 0.50 mg/ml

Feed concentration (mg/mL)	Volume of adsorption solutions (mL)	Adsorbed amount at 5% break-through point (mg/g)
(a)		
0.40	445	34.5
0.45	440	39.1
0.50	420	41.6
0.55	380	40.3
Flow rate (BV/h)	Volume of adsorption solutions (mL)	Adsorbed amount at 5% break-through point (mg/g)
(b)		
2	426	41.8
3	420	41.6
4	390	38.6
5	370	36.4

peak value at a feed concentration of 0.50 mg/ml. As the flow rate increased, both the volume of adsorption solutions at the 5% break-through point and the adsorption capacity decreased. Thus, the optimum flow rate was set as 3 BV/h considering the time consumption. Consequently, in order to make the adsorption effectively, the appropriate feed concentration and flow rate should be controlled at 0.50 mg/ml and 3 BV/h, respectively. The processing volume of the sample solution on HPD100 resin was approximately 42 BV, the absorption capacity of glabridin was 41.6 mg/g.

Dynamic Desorption Curve on HPD100 Resin

The dynamic desorption curves on HPD100 resin were obtained based on the volume of desorption solution and the concentration of glabridin in the desorption solution. Figure 4 shows the dynamic desorption curves of glabridin on column packed with HPD100 resin at different desorption flow rates. The ethanol-water (50:50, v/v) solution was used to elute glabridin. The flow rate investigated in this test was 5, 6, and 7 BV/h. The desorption capacity and product purity at different desporption flow rates were summarized in Table 5. As can be seen in Table 5, the effort

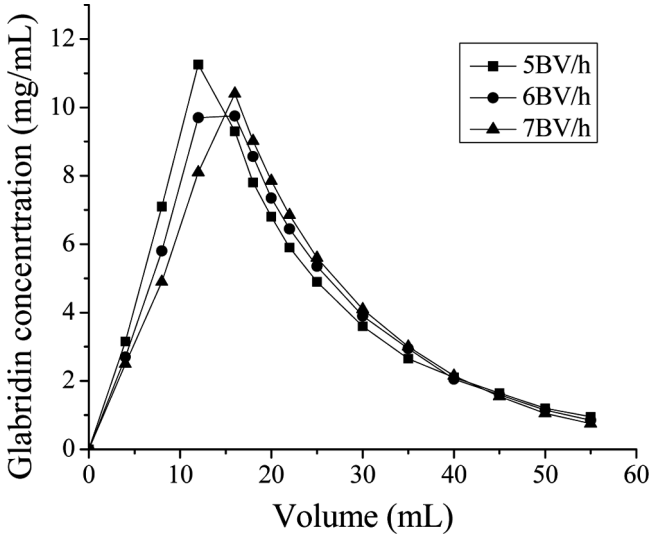

Figure 4. Dynamic desorption curves of glabridin on column packed with HPD100 resin at different desorption flow rates. The eluent was ethanol–water (50:50, v/v) solution. Temperature: 25°C.

of the desorption flow rate on the purity and desorption rate of glabridin is not apparent. Therefore 7 BV/h was chosen as the optimal desorption flow rate in consideration of the lower time and volume consumption. Approximately 5 BV of the desorption solution could completely desorb glabridin from the HPD100 resin at a flow rate of 7 BV/h.

Stepwise Elution Test

In order to decrease the consumption of reagents and make desorption effectively, stepwise elution tests were carried out under the following

Table 5. The desorption capacity of glabridin on column packed with HPD100 resin at different desorption flow rates at 25°C (The eluent was ethanol–water (50:50, v/v) solution)

Flow rate (BV/h)	Adsorption capacity (mg/g)	Purity (%)
5	39.51	27.5
6	38.82	27.6
7	38.48	27.9

conditions: the adsorption and desorption process was conducted as in the Dynamic adsorption and desorption section. The adsorption process was the concentration of glabridin in sample solution is 0.5 mg/ml, the volume of desorption solution is 5 BV, the flow rate of the sample solution and eluted solution is 3 BV/h and 7 BV/h respectively. The results were summarized in Table 6. The following equation was used to quantify the recovery yield of glabridin:

$$Y = \frac{C_d V_d}{(C_0 - C_a) V_p} \times 100\% \quad (7)$$

Where Y is the recovery yield of glabridin (%); C_a the concentration of glabridin in the effluent liquid (mg/ml); V the processing volume of the sample solution (ml); C_0, C_d, and V_d are the same as defined in the Calculation of adsorption capacity, ratios of adsorption and desorption Section.

As shown in Table 6, most glabridin adsorbed on HPD100 resin was eluted by 2 BV ethanol-water (40:60, v/v) solution followed by 2 BV ethanol-water (50:50, v/v) solution and the content of glabridin increased from 0.21% to 32.2% and the recovery yield of glabridin was 79.7%. The chromatograms of the test samples before and after treatment with HPD100 resin were shown in Fig. 5. By comparison, it can be seen that many hydrophilic impurities with strong polarity were removed and the relative peak area of glabridin increased obviously after the separation on HPD100 resin. However, the hydrophobic components maintained almost the same concentration. It may due to the correlations with the matrix of HPD100 and the chemical features of the adsorbed substance. Usually, no-polar resins exhibited stronger adsorption ability towards non-polar substances because of the strong affinity of the matrix with non-polar hydrophobic compounds (19). Therefore no-polar resins such as XAD-4 are commonly used for purification of hydrophobic flavonoids from products and byproducts of food processing plants (20–21).

Table 6. Results of stepwise elution of glabridin on packed with HPD100 resin

Concentration of ethanol	30%	40%	50%	60%	70%	80%	90%
Mass of dried residue (mg)	56.95	231.23	284.46	148.63	76.12	41.33	0.61
Mass of glabridin (mg)	4.89	64.07	101.78	20.96	6.34	0.13	0.03
Content of glabridin (%)	8.59	27.71	35.78	14.11	8.33	0.32	0.05

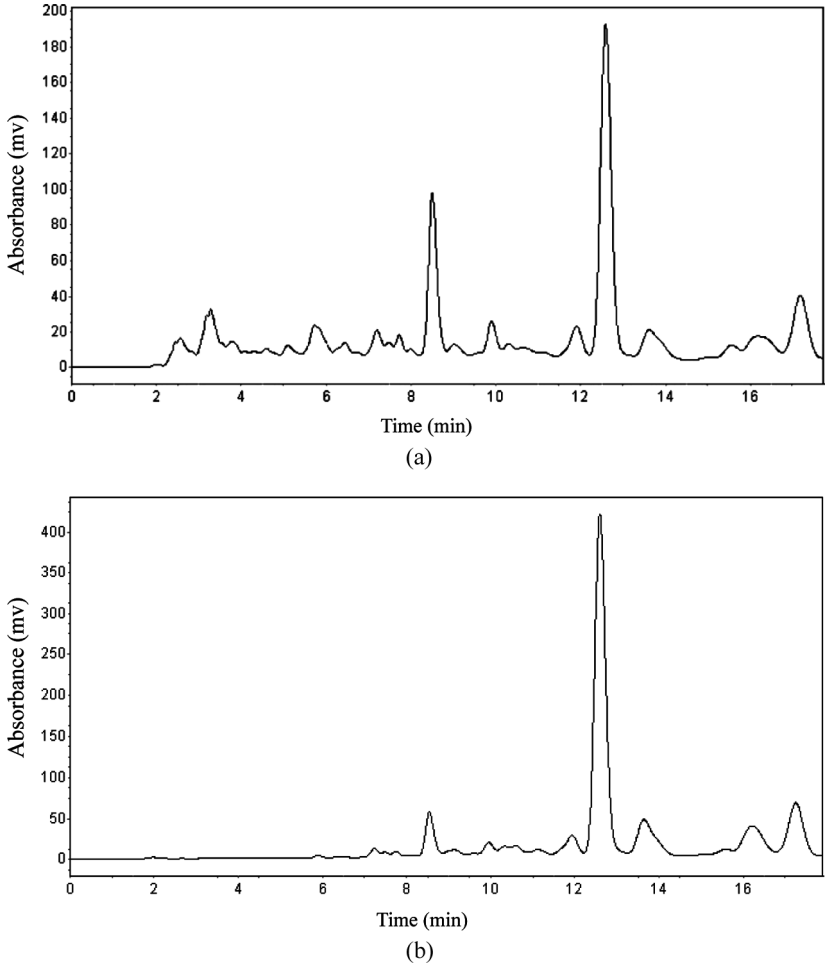

Figure 5. The chromatograms of sample solution before (a) and after (b) separation on a column packed with HPD100 resin. HPLC column: (250 mm × 4.6 mm, 5 μm, Diamodsil™. HPLC condition of glabridin was as follows: the solvent system consisted of acetonitrile and 0.02% (v/v) TFA in water was 60:40 for 20 min, then the percentage of acetonitrile was raised to 85% over 2 min and run isocratically for 15 min to purge the column., flow rate was 1.0 ml/min, 10 μl samples were injected into the column and detected by UV at 283 nm in 30°C.

Regeneration of HPD100 Resin

The regeneration ability is an important property of macroporous resins, which is directly related to the cost of production.

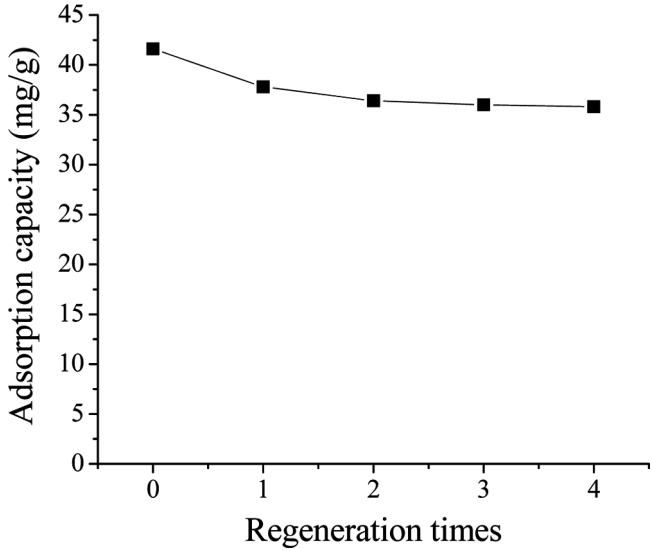

Figure 6. Adsorption capability of the new HPD 100 resin in the best condition after different regeneration times. Temperature: 25°C.

HPD100 resin was regenerated by 2BV 1 N NaOH solution, 4BV deionized water, 4BV 95% ethanol, and 4BV deionized water successively. Figure 6 shows that the adsorption capacity of the new HPD100 resin in the best condition changed with regeneration time. It can be seen from Fig. 6 that the adsorption capacity of HPD100 resin decreased slightly after four times of regeneration, which showed the good reproducibility of the process and regeneration of the HPD100 resin.

CONCLUSIONS

In this study, the performance and separation characteristics of five widely used macroporous resins for preparation of glabridin have been critically evaluated. Among the five resins investigated, HPD100 resin provides the best separation power for glabrdin in licorice extracts and its equilibrium adsorption data fits best to the Langmuir isotherm. Several important parameters in the separation process, such as the concentration and the flow rate of the feeding sample, the concentration and the volume of the eluent, were optimized for the most effective enrichment and preparative separation. Using HPD100 resin at optimal conditions, the glabridin content in the product was increased from 0.21% to 32.2% which is 153-fold higher than it is in *G. glabra* L. roots with a recovery yield of 79.7%. The

adsorption capacities under different regeneration times indicated that the HPD100 resin was durable and easily regenerated. Compared to the conventional method, the adsorption-desorption method on HPD100 macroporous resin possesses lower costs, labor intensiveness, high separation efficiency, and procedural simplicity. The present study showed that HPD100 resin may provide a strongly predictive value in the adsorption of flavonoids, a similar result was already reported on the study of XAD and EXA macroporous resins with detailed analysis on equilibrium (20,22–23). This kind of procedure can be referenced for large-scale production of a specific compound from plant extracts. The economic value for industry is considerable because of the low cost of HPD100 resin and the expensive price of glabridin product.

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