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Purification of baicalin and wogonoside from *Scutellaria baicalensis* extracts by macroporous resin adsorption chromatography

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ABSTRACT

In this study, resin adsorption as a means to separate and purify baicalin and wogonoside from extracts of *Scutellaria baicalensis* was investigated. Among the ten tested resins, the non-polar resin HPD-100 offered the best adsorption and desorption properties. Langmuir and Freundlich isotherms were used to describe the interactions between solutes and resin at different temperatures, and the equilibrium experimental data were well fitted to the two isotherms. Column packed with HPD-100 resin was used to perform dynamic adsorption and desorption tests to optimize the separation process. After one round treatment with HPD-100 resin, the contents of baicalin and wogonoside were 3.6-fold and 12.0-fold increased with recovery yields of 85.7% and 65.6%, respectively. In addition, a laboratory preparative-scale separation was carried out under the final conditions. The results showed that the preparative separation of baicalin and wogonoside can be easily and efficiently achieved via adsorption and desorption on HPD-100 resin. The developed method is a promising basis for large-scale preparation of baicalin and wogonoside from *S. baicalensis* extracts.

1. Introduction

Baicalin and wogonoside (Fig. 1) are two major effective components in dried roots of *Scutellaria baicalensis* Georgi (*S. baicalensis*), which is widely used in traditional Chinese medicine to treat inflammatory, fever, hepatitis, jaundice, and hypertension [1,2]. The pharmacological efficacy and application potential of baicalin are mainly due to its antitumor [3], anti-inflammation [4], antioxidant [5], and antiviral effects on diseases such as AIDS caused by HIV [6], and it also has a chemoprevention effect [7]. Recently, it has been reported as a prolyl oligopeptidase inhibitor, which is a highly attractive basis from which to develop new treatments for schizophrenia [8]. More importantly, baicalin tablets have been used as an adjuvant drug in the clinical treatment of hepatitis [9]. Wogonoside, the second major flavonoid in *S. baicalensis*, has been reported to have similar pharmacological efficacy with that of baicalin such as anti-inflammatory, anti-allergic, antioxidant and hepatoprotective activities [5,10–12].

As so far, methods for purification of baicalin including solvent extraction [13], solvent sublation [14], supercritical fluid extraction [15], and high-speed counter-current chromatography (HSCCC) [16,17] were developed. For wogonoside, only the application of HSCCC was reported for its preparative separation and purification [17]. However, these established methods possess several disadvantages, such as bulk amount of solvent wastage, low capacity, time-consuming, low yields, or special instruments needed, thus are not suitable for large-scale industrial production. Presently both baicalin and wogonoside are only available in small quantities and at high prices. In view of the important pharmacological properties and the difficulties connected with existing purification methods, the application of low-cost technology to obtain baicalin and wogonoside from *S. baicalensis* is a rational strategy. Recently, macroporous resin chromatography has become increasingly used for separating bioactive components from crude extracts of herbal raw materials, attributing to its unique adsorption properties and advantages including high adsorption capacity, good stability, low operational cost, less solvent consumption and easy regeneration [18]. Resins have been widely used for the enrichment and separation of some secondary metabolites including flavonoids [19–23].

Consequently, this work aimed to investigate the adsorption and desorption properties of baicalin and wogonoside on various macroporous resins with different polarities, and to develop an efficient method for the preparative separation of baicalin and wogonoside from *S. baicalensis* crude extracts with the optimal resin. It is the first time that macroporous resins were systematically investigated for the simultaneous separation and purification of baicalin and wogonoside.

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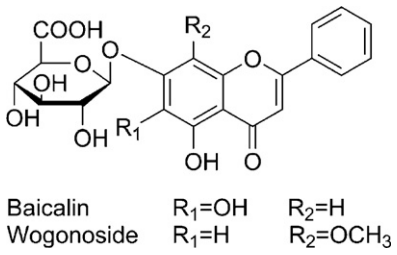

Fig. 1. Chemical structures of baicalin and wogonoside.

2. Materials and methods

2.1. Chemicals and reagents

Dry roots of *S. baicalensis* Georgi were purchased from Shenyang Chengdafangyuan Pharmacy Medicine Chain Co., Ltd. (Shenyang, China). Baicalin and wogonoside standards were prepared in our laboratories with the purities of 98.8% and 99.0% (HPLC analysis), respectively. Their structures were elucidated by comparison of the spectral data (MS, ^{1}H NMR and ^{13}C NMR) with previous literatures [17,24]. HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA, USA). Trifluoroacetic acid (TFA) was obtained from Dima Technology Inc. (Richmond Hill, USA). Ethanol and other reagents of analytical grade were obtained from Damao Chemical Reagent Factory (Tianjin, China). Deionized water obtained from distiller (Eyela Still Ace, Model SA-2100 E1, Tokyo, Japan) was used throughout this study.

2.2. Adsorbents

Ten macroporous resins including HPD-100, HPD-300, D-101, AB-8, HPD-450, ADS-17, HPD-750, DM-130, HPD-500, and HPD-600 were purchased from Cangzhou Bonchem Co., Ltd. (Hebei, China). The manufacturers' information regarding the characteristics of the resins is listed in Table S1. The resins were soaked in 95% (v/v) aqueous ethanol for 12 h, washed thoroughly with deionized water, and vacuum dried prior to use.

2.3. Preparation of S. baicalensis extracts sample solutions

The dry roots of *S. baicalensis* (1 kg) were extracted by 10 L of deionized water at 100 °C for 2 h, repeated twice. The extracts were combined and purified by membrane filtration and then vacuum concentrated by a rotary evaporator at 55 °C, and later lyophilized by a freeze dry system (Labconco, Model Freezone 6, MO, USA). The contents of baicalin and wogonoside in the extracts were 14.3% and 7.3%, respectively. Deionized water was added into crude extracts to get sample solutions. The initial concentrations of baicalin in the sample solutions were 0.215, 0.429, 0.634, 0.858 and 1.073 mg/mL, respectively. For wogonoside, the initial concentrations in the sample solutions were 0.109, 0.218, 0.328, 0.437 and 0.546 mg/mL, respectively.

2.4. HPLC analysis of baicalin and wogonoside

Quantification of baicalin and wogonoside was carried out on a Shimadzu HPLC system, consisting of LC-10ATVP binary pump, an SPD-10AVP detector, a CTO-10ASVP column oven, and an N3000 workstation. Analysis was accomplished on an Ultimate XB-C18 reverse phase column (250 mm × 4.6 mm i.d., 5 μm). The mobile phase consisted of 0.05% aqueous trifluoroacetic acid (A) and methanol (B). The elution system was as follows: 0–15 min, 30–40% of B, 15–35 min, 40–70% of B, 35–40 min, 70–30% of B. The flow rate was 1.0 mL/min and the injection volume was 20 μL and column temperature was maintained at 30 °C. The effluent was monitored at 277 nm. The retention time of baicalin and wogonoside was 16.2 min and 22.1 min, respectively. The regression lines for baicalin and wogonoside were $Y = 41{,}958X + 171{,}484$ ($R^2 = 0.9996$) and $Y = 21{,}524X + 42{,}404$ ($R^2 = 0.9995$), respectively, where Y is the peak area and X is the concentration (μg/mL).

2.5. Static adsorption and desorption tests

2.5.1. Resin selection

The adsorption tests were performed as follows: 1 g (dry weight) samples of hydrated test resins were put into flasks with a lid; 100 mL of sample solutions of obtained in Section 2.3 were added (the initial concentrations of baicalin and wogonoside were 0.858 and 0.437 mg/mL, respectively). The flasks were then shaken (120 rpm) for 12 h at 25 °C. After adsorption equilibrium was reached, the resins were first washed by deionized water and then desorbed with 100 mL ethanol–water (70:30, v/v) solution. The flasks were continually shaken (120 rpm) for 12 h.

The selectivity of resins was based on the capacities of adsorption (Q_e), capacities of desorption (Q_d), and ratio of desorption (D), which were quantified according to the following equations:

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

$$Q_d = \frac{C_d V_d}{W} \quad (2)$$

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

where Q_e (mg/g dry resin) is the concentration of solute per mass of adsorbent (solid phase), also known as adsorption capacity at equilibrium; Q_d is the desorption capacity after adsorption equilibrium (mg/g dry resin); C_0, C_e, and C_d are the initial, adsorption equilibrium and desorption concentrations of analyte in the solutions, respectively (mg/mL). V_i and V_d are the volume of the initial sample and desorption solution (mL), respectively. W is the dry weight of resin (g), and D is the desorption ratio (%).

The effect of pH on the adsorption capacities of baicalin and wogonoside was carried out by mixing 1 g (dry weight) of hydrated selected resin with sample solutions (100 mL each) in the pH range of 3.0–7.0. The sample pH was adjusted to the desired value with HCl or ammonia solution. Then, the flasks were shaken for 4 h at 25 °C.

2.5.2. Adsorption kinetics

The adsorption kinetics curves for baicalin and wogonoside on the selected HPD-100 resin were studied by mixing 200 mL sample solutions (the initial concentrations of baicalin and wogonoside were 0.858 and 0.437 mg/mL, respectively) with pre-weighed amounts of hydrated HPD-100 resin (equal to 1 g dry resin) in 250 mL flasks. The flasks were then shaken (120 rpm) in a constant temperature shaker at 25 °C for 7 h.

2.5.3. Adsorption isotherms

In order to investigate the effect of initial concentration and temperature on the target compounds adsorption, experiments of adsorption isotherm on HPD-100 resin were performed. Five aliquots of 200 mL sample solutions at different concentrations were contacted with pre-weighed amounts of hydrated resins (equal to 1 g dry resin) in a constant temperature shaker at 25, 35 and 45 °C for 4 h.

2.5.4. Adsorption isotherm models

Two popular theoretical models (the Langmuir and Freundlich models) were used to evaluate the experimental data. The

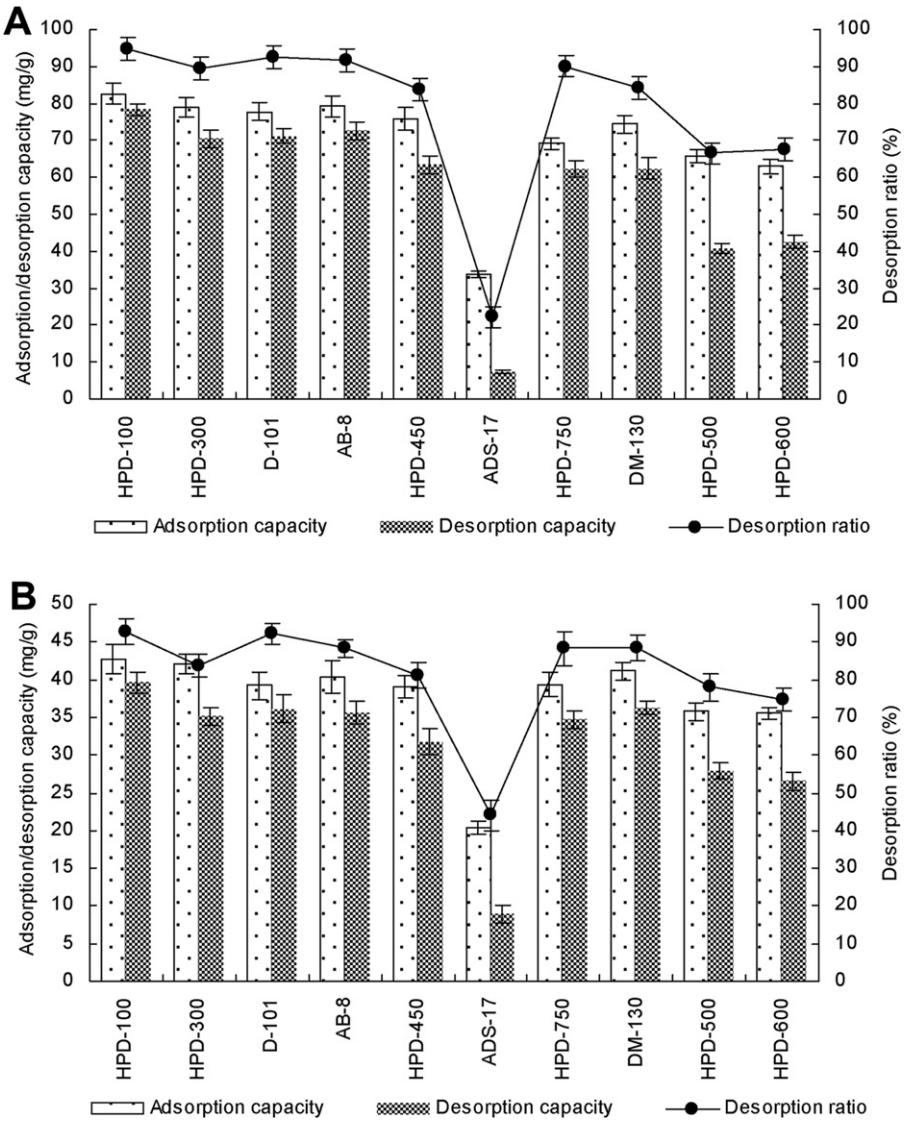

Fig. 2. Adsorption and desorption capacities and desorption ratio of baicalin (A) and wogonoside (B) on different resins.

Langmuir isotherm is the best known and the most frequently used isotherm for the adsorption of solutes from a solution [25]. The model of Langmuir can be expressed by the following mathematical formula:

$$Q_e = \frac{Q_m K_L C_e}{1 + K_L C_e} \tag{4}$$

where Q_e and C_e (mg/mL) are the same as those in Eq. (1); K_L (mg/mL) is the Langmuir constant; Q_m (mg/g resin) is the maximum adsorption capacity.

The Freundlich model is an empirical equation, used in the physical adsorption and chemical adsorption for nonideal adsorption systems [26]. The model of Freundlich can be expressed by the following mathematical formula:

$$Q_e = K_F C_e^{1/n} \tag{5}$$

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

2.6. Dynamic adsorption and desorption tests

Dynamic adsorption and desorption experiments were first carried out on glass columns (400 mm × 20 mm i.d.) wet-packed 18 g (dry weight) of HPD-100 resin with the bed volume (BV) of approximately 100 mL. The dynamic breakthrough tests were carried out first. Sample solution was loaded into the column at a predetermined flow rate (1.0, 2.0 and 4.0 BV/h). After adsorption equilibrium, the column was firstly washed with deionized water (5 BV), and then desorbed with 70% ethanol (5 BV) at the flow rate of 2 BV/h.

In order to separate the two target compounds, sample loading amount (10, 20 and 30 BV) was investigated on glass columns (500 mm × 35 mm i.d.) wet-packed 54 g (dry weight) of HPD-100 resin with the bed volume of approximately 300 mL. After adsorptive equilibrium, gradient elution using different concentrations of ethanol (0%, 10%, 20%, 30%, 40%, 50%, 60% and 70%) were used to desorb the target compounds successively at a flow rate of 2 BV/h. The elution volume of each concentration was kept constant at 5 BV. Baicalin and wogonoside in each eluent were detected by HPLC and then concentrated and lyophilized.

After adsorption equilibrium under the optimum condition, the column was washed with deionized water first, and then eluted

following the elution procedures determined in the tests above. The elution volume of each ethanol concentration was adjusted under the guidance of HPLC.

2.7. Laboratory preparative-scale separation

The dried extract of *S. baicalensis* (143.5 g) was dissolved in deionized water. The sample solution (pH 5.0) was applied to a glass column (100 cm × 7.5 cm i.d.) containing 1.0 kg of wet HPD-100 macroporous resin) with a bed volume of 2.4 L. Initially, the column was washed by 5 BV of deionized water, and then 5 BV of 10% ethanol was used to remove the high polar impurities. The column was then eluted with 6 BV of 30% ethanol to obtain the baicalin-rich fraction, and then the column was rinsed with 1 BV of 40% ethanol. Wogonoside was then flowed from the column using 6 BV of 50% ethanol. The flow rate of each gradient elution was set at about 80 mL/min (equals to 2 BV/h), and the eluate of 30% and 50% aqueous ethanol was collected, concentrated and freeze-dried.

3. Results and discussion

3.1. Screening of optimum resin

According to the "like dissolves like" principle, given baicalin and wogonoside both contain non-polar phenyl group (B-ring) and polar multi-hydroxy groups (glucuronyl), either non-polar resins or polar resins are applicable to adsorption of baicalin and wogonoside. Ten macroporous resins, ranging from non-polar to polar, were therefore employed for enrichment and separation of baicalin and wogonoside. As can be seen from Fig. 2, non-polar (HPD-100, HPD-300 and D-101) and weak polar (AB-8 and HPD-450) resins exhibited considerably higher adsorption capacities than those of other resins, and HPD-100 resin showed the best adsorption and desorption capacities. Non-polar and weak polar resins exhibited better adsorption capabilities than others not only because of their similar polarity with baicalin and wogonoside, but also because of their higher specific surface area. Obviously, the middle polar ADS-17 resin with low specific surface area and small average pore diameter possessed bad adsorption and desorption capabilities for both baicalin and wogonoside. In view of these results, non-polar resin HPD-100 was selected to perform the subsequent investigation.

3.2. Adsorption kinetics on HPD-100 resin

Adsorption kinetics was studied in order to choose the contact time to be used in the next steps of the optimization. As illustrated in Fig. 3, the adsorption capacities of baicalin and wogonoside increased rapidly with adsorption time before reaching the adsorption equilibrium. For baicalin, an asymptotic curve was reached after about 180 min of contact time, and an adsorption/desorption equilibrium was established, while for wogonoside, the equilibrium time was 150 min. Therefore, 180 min was sufficient to successfully reach adsorption equilibrium over the entire system, and the batch adsorption equilibrium tests were run for over 180 min.

3.3. Effect of pH value of sample solution

One of the most important parameters influencing the adsorption capacity is the initial pH of adsorption solution [19]. As shown in Table 1, the adsorption capacity increased for baicalin and wogonoside with the decrease of pH value. There was no obvious change on adsorption capacity of HPD-100 resin when pH value was between 3.0 and 5.0. This result suggested that hydrogen bonding might play an important role in the adsorption process of resins. At higher pH values, the adsorption of baicalin and wogonoside were probably caused by electrostatic interactions due to ionization of baicalin and wogonoside with the increase of pH. Therefore, the pH value of the solution was adjusted to 5.0 for all later experiments.

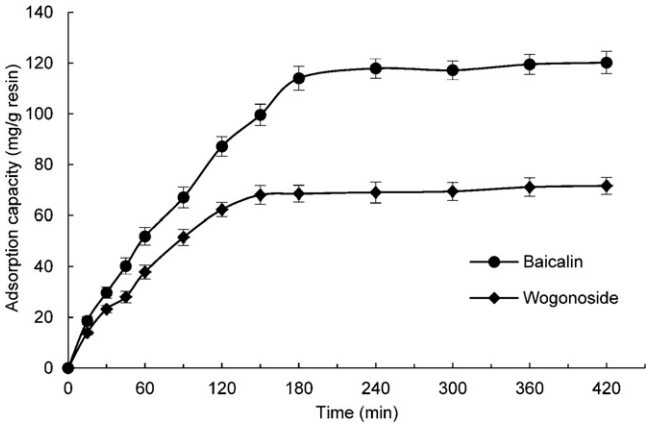

Fig. 3. Adsorption kinetics curves of baicalin and wogonoside on HPD-100 resin at 25 °C.

3.4. Adsorption isotherms

Equilibrium adsorption isotherms of baicalin and wogonoside on HPD-100 were investigated at the temperature of 25, 35 and 45 °C. The initial concentrations of baicalin were 0.215, 0.429, 0.634, 0.858 and 1.073 mg/mL, respectively. The initial concentrations

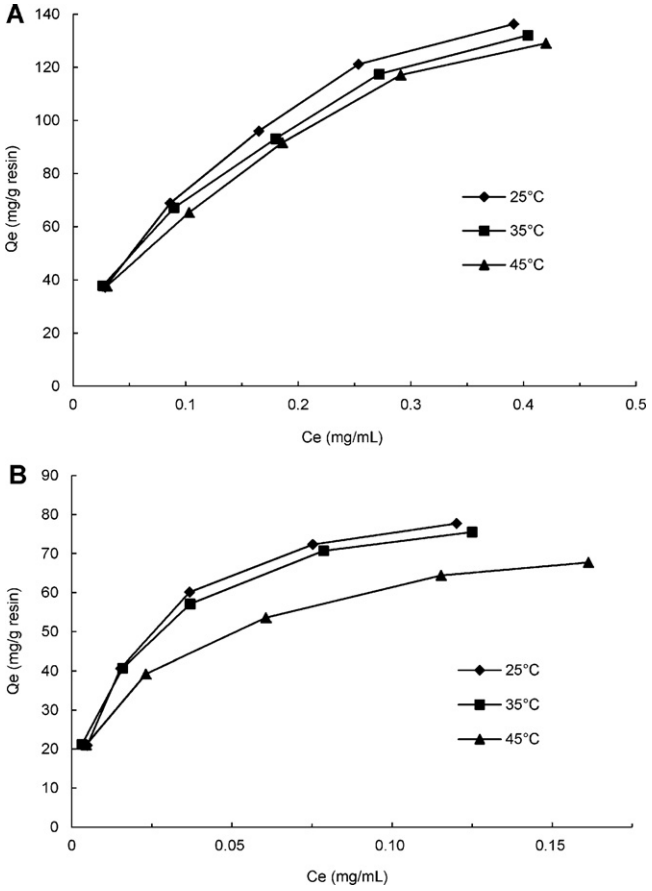

Fig. 4. Adsorption isotherms for baicalin (A) and wogonoside (B) on HPD-100 resin at 25, 35 and 45 °C.

Table 1
Effect of sample solution pH value and flow rate on the adsorption capacities of baicalin and wogonoside on HPD-100 resin.[a]

pH value	Adsorption capacity (mg/g resin)		Flow rate	Adsorption capacity (mg/g resin)	
	Baicalin	Wogonoside		Baicalin	Wogonoside
3	80.98 ± 2.14	41.15 ± 1.24	1.0 BV/h	117.32 ± 3.17	68.97 ± 2.79
5	81.57 ± 2.73	40.97 ± 1.05	2.0 BV/h	115.63 ± 3.36	67.84 ± 2.57
7	72.04 ± 2.39	36.57 ± 1.34	4.0 BV/h	84.58 ± 2.88	46.54 ± 2.45

[a] Results are means ± SD (n = 3).

Table 2
Langmuir and Freundlich parameters of baicalin and wogonoside on HPD-100 resin at different temperatures.

Compound	Temperature (°C)	Langmuir equation	R^2	Q_m (mg/g)	Freundlich equation	R^2	$1/n$
Baicalin	25	$C_e/Q_e = 0.0056\,C_e + 0.0007$	0.9907	178.57	$Q_e = 233.18\,C_e^{0.5074}$	0.9931	0.5074
	35	$C_e/Q_e = 0.0060\,C_e + 0.0007$	0.9808	166.67	$Q_e = 208.11\,C_e^{0.4684}$	0.9976	0.4684
	45	$C_e/Q_e = 0.0059\,C_e + 0.0008$	0.9768	169.49	$Q_e = 202.72\,C_e^{0.4821}$	0.9950	0.4821
Wogonoside	25	$C_e/Q_e = 0.0113\,C_e + 0.0002$	0.9997	88.50	$Q_e = 208.45\,C_e^{0.4124}$	0.9613	0.4124
	35	$C_e/Q_e = 0.0120\,C_e + 0.0002$	0.9962	83.33	$Q_e = 171.51\,C_e^{0.3559}$	0.9856	0.3559
	45	$C_e/Q_e = 0.0135\,C_e + 0.0002$	0.9949	74.07	$Q_e = 130.65\,C_e^{0.3311}$	0.9922	0.3311

Table 3
Purification results of baicalin and wogonoside on column packed with HPD-100 resin by final modified conditions.

Process	W_s (g)	P_b (%)	R_b (%)	P_w (%)	R_w (%)
Crude extract	143.5	14.3		7.3	
30% ethanol fraction	28.3	58.3	80.4		
50% ethanol fraction	8.0			83.3	63.4

W_s: weight of solids; P_b: purity of baicalin; R_b: recovery of baicalin; P_w: purity of wogonoside; R_w: recovery of wogonoside.

of wogonoside were 0.109, 0.218, 0.328, 0.437 and 0.546 mg/mL, respectively. As can be seen from Fig. 4, the adsorption capacities for both baicalin and wogonoside on HPD-100 resin increased with increasing equilibrium concentration, and reached saturation status at the turning point. The concentration at this point, which corresponds to the equilibrium concentration, was chosen as the proper initial concentration. Therefore, the initial concentrations of baicalin and wogonoside in sample solution for adsorption were selected as 0.858 and 0.437 mg/mL, respectively.

Table 2 lists the two isotherm equations at different temperatures and two important parameters: Q_m value (obtained from the Langmuir isotherm) and $1/n$ value (obtained from the Freundlich isotherm). The correlations (0.961–0.999) for baicalin and wogonoside on HPD-100 indicated that the two models were suitable for describing the tested adsorption system in the concentration ranges studied.

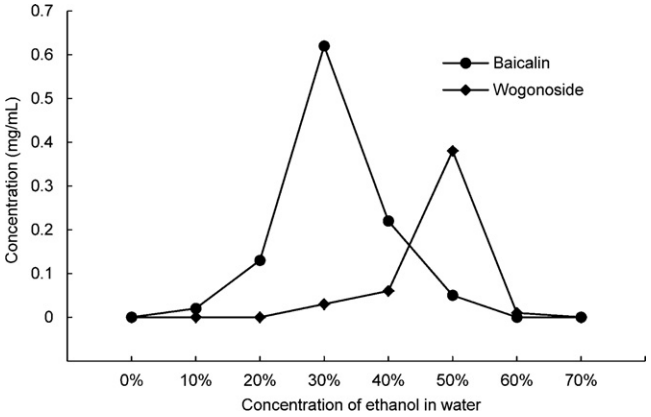

Fig. 6. Dynamic desorption curves of baicalin and wogonoside by different elution solvents with the same volume of 5 BV.

In general, in the Freundlich equation, the adsorption takes place easily when $1/n$ value is between 0.1 and 0.5, and it is not easy to happen if $1/n$ is above 1 [21]. In Table 2, the $1/n$ value were all between 0.1 and 0.5 except baicalin at 25 °C, demonstrated that the adsorption of baicalin and wogonoside on HPD-100 resin took-place easily and that HPD-100 resin was appropriate for the separation of both components.

In addition, it can be seen from Fig. 4, at the same initial concentration, the adsorption capacities decreased with temperature

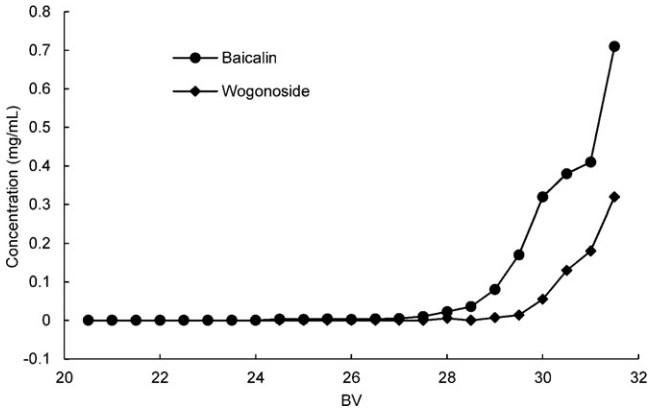

Fig. 5. Dynamic breakthrough curves of baicalin and wogonoside on HPD-100 resin at 2 BV/h.

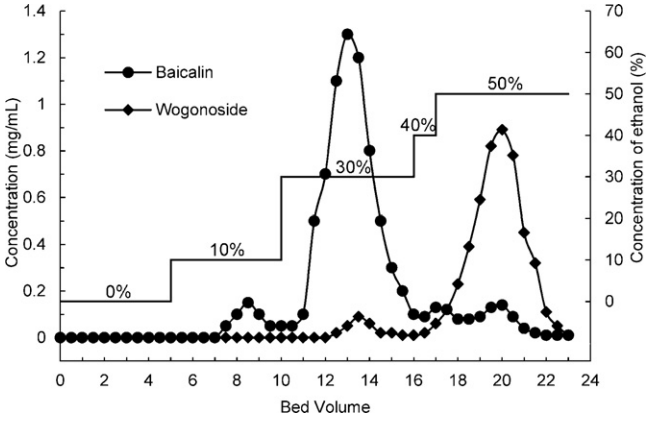

Fig. 7. Profile of desorption of baiclain and wogonoside on column packed with HPD-100 resin.

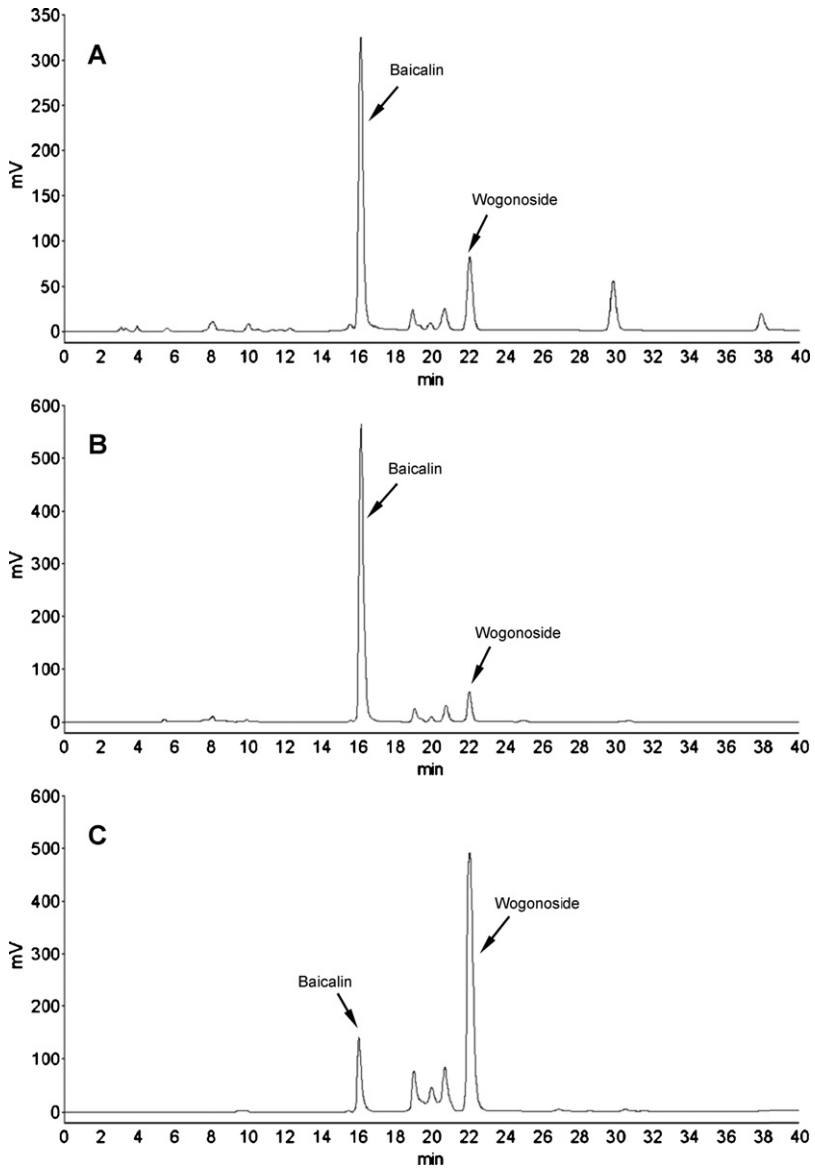

Fig. 8. HPLC chromatograms of samples before treatment (A) and fractions eluted by 30% ethanol (B) and 50% ethanol (C).

increase from 25 to 45 °C, implying that the adsorption process was an exothermic process. Similar results were obtained for the adsorption and desorption of flavone-*C*-glucosides using macroporous resin [27]. Therefore, 25 °C was selected as adsorption temperature.

3.5. Dynamic adsorption and desorption on HPD-100 resin

The effect of flow rate on dynamic adsorption capacities was carried out with constant loading volume 30 BV. As shown in Table 1, the adsorption capacities decreased a little as the flow rate increased from 1.0 to 2.0 BV/h, but decreased significantly as the flow rate increased from 2.0 to 4.0 BV/h, this is because that the interaction time of baicalin and wogonoside with active sites of the resin surface is deceased. Thus, taking the efficiency into account, the flow rate for loading sample was maintained constantly at 2.0 BV/h.

In general, the breakthrough point can be defined as 10% of the ratio of the exit solute concentration to the inlet solute concentration [21]. According to this standard, the corresponding breakthrough volumes of baicalin and wogonoside were 29.0 and 30.0 BV, respectively (Fig. 5). Accounting for both baicalin and wogonoside, 30 BV sample solution was determined as saturated adsorption volume for dynamic adsorption.

Different sample loading amounts (10, 20 and 30 BV) were investigated for the separation of the two target compounds. However, certain amount of wogonoside could be desorbed together with baicalin at the beginning of the elution by low concentration of ethanol water (20% or 30%) when the sample loading amount was 20 or 30 BV. Thus, 10 BV of sample solution was settled for dynamic adsorption. Fig. 6 shows the profile of desorption of baicalin and wogonoside by different elution solvents with the same volume of 5 BV when the sample loading amount was 10 BV. As shown in Fig. 6, at the 10% ethanol, baicalin and wogonoside was hardly desorbed. When the ethanol concentration was over 20%, the desorption of baicalin increased sharply and reached a peak value at 30% ethanol. Finally the elution of 20–40% aqueous ethanol gave the baicalin-rich fraction with a content of 51.0% on average and a total recovery yield of 85.7%. When the concentration of ethanol reached 30%, wogonoside started to be desorbed, but only a small amount of wogonoside was detected. Finally, 50% ethanol aqueous solution gave the wogonoside-rich fraction with a content of 87.1% and a

recovery yield of 65.6% (Table S2). Hence, a gradient elution procedure with 30%, 40% and 50% ethanol at a flow rate of 2 BV/h was applied for desorption of baicalin and wogonoside. In addition, the elution volume of each ethanol concentration was modified under the guidance of HPLC.

According to HPLC analysis, the elution scheme was modified as 5 BV 10% ethanol, 6 BV 30% ethanol, 1 BV of 40% ethanol, and 6 BV of 50% ethanol. As shown in Fig. 7, non-adsorption components were firstly washed out by 5 BV deionized water. Subsequently, some impurities were removed by elution of 5 BV 10% ethanol. The following 6 BV of 30% ethanol elution gave the product rich in baicalin. Then the column was washed by 1 BV of 40% ethanol, and finally 6 BV of 50% ethanol elution resulted in the product rich in wogonoside. Thus, the final separation and purification conditions for baicalin and wogonoside were determined as follows:

Adsorption: concentrations of baicalin and wogonoside in sample solution 0.858 and 0.437 mg/mL, respectively; pH 5.0; sample loading amount 10 BV; flow rate 2 BV/h; temperature 25 °C.
Desorption: deionized water and 10% ethanol each 5 BV, then 30% ethanol 6 BV, 40% ethanol 1 BV, 50% ethanol 6 BV, flow rate 2 BV/h.

3.6. Laboratory preparative-scale separation

Laboratory preparative-scale separation was carried out on a HPD-100 resin (1 kg, wet weight) column using the above determined conditions. As shown in Table 3, the elution of 6 BV 30% ethanol gave the baicalin-rich fraction with a content of 58.3% and a recovery yield of 80.4%, and the elution of 6 BV 50% ethanol gave the wogonoside-rich fraction with a content of 83.3% and a recovery yield of 63.4%. The HPLC chromatograms of samples before and after separation with HPD-100 resin were shown in Fig. 8. It can be seen that baicalin and wogonoside were successfully enriched in 30% ethanol eluate and 50% ethanol eluate, respectively.

4. Conclusion

An effective method for separation and purification of baicalin and wogonoside from *S. baicalensis* was developed in this study. Process parameters including sample concentration, sample loading amount, temperature, etc., were optimized for the separation procedure. The equilibrium experimental data of the adsorption of baicalin and wogonoside on HPD-100 resin at different temperatures were well-fitted to the Langmuir and Freundlich isotherms. HPD-100 resin was successfully applied to obtain products of baicalin and wogonoside with high contents. Conventional methods for purification of baicalin and wogonoside are solvent extraction and high speed counter-current chromatography. Compared with the former one, the present method is advantageous because of its high efficiency and less solvent wastage. Compared with the latter one, the developed method is simple, low cost and easy in scaling-up. Further studies considering the preparation of pure baicalin and wognoside from the high contents products are under investigation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2012.09.024.

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