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A green and efficient protocol for industrial-scale preparation of dioscin from *Dioscorea nipponica* Makino by two-step macroporous resin column chromatography

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ABSTRACT

A green and efficient method for the large-scale preparation of dioscin from *Dioscorea nipponica* Makino, a well known traditional Chinese medicinal herb, was developed. The preparation procedure consisted of two separation steps. In the first step, several types of macroporous resins including NKA-II, DA201, DM-301, AB-8, D1400, D4020, X-5, HPD-100 and D101 were investigated in order to choose the best resin. D101 resin was selected according to its adsorption and desorption properties. Other parameters during static and dynamic procedures were also investigated. In order to obtain a pure target, a second step was established, which involved the screening of various types of macroporous resins (D900, D318, D315, D280 and D301). The results demonstrated that D900 resin was the most suitable resin for use in this study. Under these conditions, the large-scale preparation of dioscin was carried out. The purity of dioscin after the first step was increased 9.16-fold from 9.35% to 85.64% with a recovery of 81.47%. The purity of dioscin then increased to 96.55% with a recovery of 89.64% after the second purification. A total of 1.486 kg of pure dioscin was produced from 100.0 kg raw material by the developed two-step macroporous resin column chromatography method using only aqueous ethanol as the solvent. Our study provides a green and efficient protocol for industrial-scale preparation of dioscin for pharmaceutical use.

1. Introduction

Dioscin (diosgenyl 2, 4-di-*O*-a-L-rhamnopyranosyl-p-D-glucopyranoside) is one of the most important saponins in the Chinese medical herb *Dioscorea nipponica* Makino, whose chemical structure is given in Fig. 1. It has anti-tumor and anti-fungal effects [1–3], which have been shown in pharmacology studies. The proliferation of HL-60 and HeLa cancer cells was significantly inhibited by dioscin *in vitro* [4,5]. Furthermore, it can be hydrolyzed to diosgenin directly, which can be used to synthesize many useful steroid hormone and contraceptive drugs [6,7]. As a result of these beneficial effects, further scientific research and the clinical application of dioscin require large amounts of high purity dioscin. Thus, the establishment of an efficient method for the large-scale preparation of pure dioscin from medicinal plants is necessary.

Many methods for the preparative separation and purification of dioscin from medicinal plants have previously been reported, including silica gel and polyamide column chromatography, high-speed counter-current chromatography (HSCCC) and preparative high-performance liquid chromatography (p-HPLC) [8–12]. Pure dioscin can be separated by these methods, however, these techniques have some shortcomings, such as they are small-scale, consume large amounts of organic solvents and are complicated procedures. Thus, they are not suitable for simple and rapid industrialization production, as they are not environmentally friendly and consume numerous resources. Thus, an industrial-scale, simple, green and effective method is urgently needed for dioscin separation.

Macroporous resin, a type of adsorbent, has distinguishing features including a large adsorption capacity, it is suitable for the enrichment of a wide range of chemicals, has easy operation, low cost, and can be used more than one thousand times [13–15]. Importantly, only aqueous ethanol has been used to desorb target compounds, and the use of macroporous resins is a green method. In principle, substances are purified according to their molecular weight, polarity, and shape. The effective adsorption of macroporous resin is related to its surface adsorption, electrical property,

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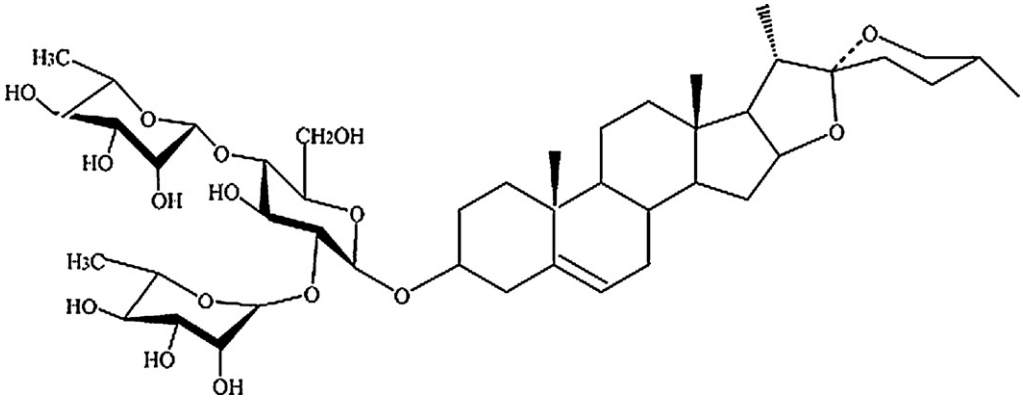

Fig. 1. The chemical structure of dioscin.

sieve classification, and hydrogen bond interactions. Therefore, different resin adsorbents can be selected for the preparation of different compounds with special characteristics.

The aim of the present study, therefore, was to establish a green and efficient method for the large-scale preparation of pure dioscin from *D. nipponica* Makino by macroporous resin column chromatography. D101 and D900 resins were chosen and used in the developed two-step protocol, respectively. A total of 1.486 kg dioscin with a purity of 96.55% and recovery of 72.99% was obtained from 100.0 kg raw material. As far as we know, this is the first time that a new approach has been reported for the industrial-scale preparation of dioscin for pharmaceutical use.

2. Materials and methods

2.1. Reagents and materials

Acetonitrile was of HPLC grade (TEDIA, USA). Ethanol was of analytical grade and purchased from ShenLian Chemical Factory (Dalian, China). Reverse osmosis Milli-Q water (18 MΩ) (Millipore, USA) was used for the preparation of deionized water. The dioscin standard was isolated in our laboratory with a purity >98%, and the structure was identified by UV, MS, NMR and confirmed by reported literature [16].

D. nipponica Makino was purchased from a local drug store (Dalian, China) and authenticated by Dr. Yunpeng Diao (Dalian Medical University, Dalian, China).

2.2. Absorbents

Macroporous resins (NKA-II, DA201, DM-301, AB-8, D1400, D4020, X-5, HPD-100, D101, D900, D318, D315, D280 and D301) were purchased from Tianjin Haiguang Chemical Ltd. (Tianjin, China). The physical and chemical properties of the resins are summarized in Table 1. The macroporous resins were soaked in 95% aqueous ethanol and shaken for 24 h, and were then washed thoroughly with deionized water. The moisture content of each resin was determined by drying the resins at 110 °C until the mass did not change [17,18]. The moisture content of each resin was then calculated (Table 1).

2.3. Preparation of standard solutions and the extract from D. nipponica Makino

Standard stock solutions of dioscin were prepared by dissolving dioscin in methanol, which were then diluted to seven different concentrations for the calibration plots and verification of the analysis method.

Dry powder of *D. nipponica* Makino (100.0 kg) with a dioscin content of 2.44% was extracted three times with 60% aqueous ethanol (solvent and sample ratio = 8:1, v/w) under reflux for 2 h each time [19]. The extract was then collected and evaporated under reduced pressure in a rotary evaporator at 60 °C. The powdered extract (20.95 kg) was obtained for subsequent separation and purification, and the content of dioscin was determined.

2.4. Static adsorption and desorption tests

In order to choose the most suitable macroporous resin to separate dioscin from the crude extract, the adsorption and desorption properties of the different resins were characterized, and the static adsorption and desorption tests were performed using the following procedure:

Table 1
Physical property of the tested macroporous resins.

Trade name	Type of skeletons	Surface area (m^2/g)	Average pore diameter (nm)	Particle diameter (mm)	Polarity	Water content
DA-201	Styrene copolymer	≥150	140–170	0.315–1.25 mm ≥ 95% (60–16 mesh)	Polar	60.75%
AB-8	Styrene copolymer	480–520	130–140	0.315–1.25 mm ≥ 90% (60–16 mesh)	Weak polar	57.50%
DM-301	Styrene copolymer	≥480	155–165	0.315–1.25 mm ≥ 90% (60–16 mesh)	Middle polar	61.12%
D101	Styrene copolymer	≥400	100–110	0.315–1.25 mm≥90%(60–16 mesh)	Non-polar	53.60%
HPD-100	Styrene copolymer	650–700	85–90	0.315–1.25 mm≥90%(60–16 mesh)	Non-polar	63.72%
D1400	Styrene copolymer	≥550	120–130	0.315–1.25 mm ≥ 95%(60–16 mesh)	Non-polar	57.08%
X-5	Styrene copolymer	500–600	290–300	0.315–1.25 mm ≥ 95%(60–16 mesh)	Non-polar	52.79%
NKA-II	Styrene copolymer	160–200	145–155	0.315–1.25 mm ≥ 95%(60–16 mesh)	Polar	57.48%
D4020	Styrene copolymer	540–580	100–105	0.315–1.25 mm ≥ 95%(60–16 mesh)	Non-polar	59.09%
D900	Styrene copolymer	300–380	115–120	0.315–1.25 mm≥90%(60–16 mesh)	Alkalescence	57.43%
D318	Polyacrylic acid	540–620	95–105	0.315–1.25 mm ≥ 95%(60–16 mesh)	Alkalescence	54.94%
D315	Polyacrylic acid	410–490	120–140	0.315–1.25mm≥95%(60–16 mesh)	Alkalescence	62.45%
D280	Polystyrene	350–480	140–150	0.315–1.25 mm≥95%(60–16 mesh)	Alkalescence	59.36%
D301	Polystyrene	300	170–180	0.315–1.25 mm ≥ 95%(60–16 mesh)	Middle polar	50.21%

In the first step, the macroporous resins (NKA-II, DA201, DM-301, AB-8, D1400, D4020, X-5, HPD-100 and D101) (equal to 1.0 g dry weight) were placed into flasks with a lid, and 50 mL sample solution (dioscin concentration 0.812 mg/mL) was added. The flasks were then shaken (160 rpm) for 6 h at 25 °C. The supernatant after adsorption was analyzed by HPLC. After reaching the adsorption equilibrium, the solutions in the flasks were removed, and the resins were washed three times with deionized water. The desorption processes were then carried out. The resins were desorbed with 50 mL 80% (v/v) aqueous ethanol. The flasks were shaken (160 rpm) for 6 h at 25 °C, and the desorption solutions were analyzed. The adsorption capacity, desorption capacity and desorption ratio for dioscin were calculated on each resin.

In the second step, five macroporous resins, D900, D318, D315, D280 and D301, were tested. The sample solution used was the product of the first step process, which was then re-dissolved in water to afford a dioscin concentration of 1.405 mg/mL. The resins (equal to 1.0 g dry resin) were placed into flasks with a lid, and 50 mL sample solutions were added. The static adsorption and desorption tests were then carried out.

2.5. Dynamic adsorption and desorption tests

The selected resins, D101, AB-8, D900 and D301, were studied to choose the best resin, and the adsorption equilibration time and the initial concentration of dioscin in contact with each resin were optimized according to the adsorption kinetic curves. Dynamic adsorption and desorption experiments were carried out using a glass column (20 mm × 100 mm) wet packed with 5.0 g (dry weight) with 10 mL of the bed volume (BV). Sample solutions flowed through the glass column at 0.5 mL/min, and the concentration of dioscin was analyzed. When the adsorption equilibrium was reached, i.e. when the concentration of the sample solution did not change, the adsorbate-laden column containing D101 and D900 resins was first washed with deionized water, and then successively eluted with different concentrations of aqueous ethanol,. The volume of each fraction was 5 BV, and all parts of the desorption solutions were analyzed.

2.6. Adsorption isotherms of dioscin on D101 and D900 resins

The tests for equilibrium adsorption isotherms on D101 and D900 resins were conducted by adding 50 mL sample solutions at eight different concentrations of the extract from *D. nipponica* Makino. The flasks were shaken (160 rpm) for 6 h at 25, 30 and 35 °C, respectively. The initial and equilibrium concentrations of dioscin were determined, and the temperature for dioscin separation was optimized according to the equilibrium data.

2.7. HPLC analysis

The analysis of dioscin was carried out by HPLC on an Agilent 1200 system (Agilent, Germany) equipped with an online vacuum degasser, a quaternary gradient pump, an autosampler and a 2000ES evaporation light scanning detector (ELSD) (Alltech, USA). Analysis was performed on a Lichrosorb C_{18} column (150 mm × 4.6 mm, 5 μm) (Zhonghuida, Dalian, China) at room temperature. The flow-rate was 0.8 mL/min, and the effluent was monitored by ELSD (nebulizer gas flow: 2.5 L/min, drift tube temperature: 100 °C) according to the literature [20,21]. The peak of the HPLC profile was identified by comparing the retention time with the standard substance. All the samples were filtered through a 0.45 μm syringe filter before injection into the HPLC system.

2.8. Large-scale purification

The crude extract from *D. nipponica* Makino (20.95 kg) was redissolved in water and chromatographed on a stainless steel column (60.0 cm × 180.0 cm, containing 100.0 kg D101 macroporous resin) with 100 L of BV. Initially, water was used to wash the elution solution until almost no color was noted, then 8 BV of 30% ethanol was used to remove the high polar components, and the adsorbent was finally rinsed with 10 BV 80% ethanol. The flow rate of each gradient was 450 mL/min, and the elution of 80% ethanol was collected, concentrated and dried. The second step was carried out on a stainless steel column (40.0 cm × 120.0 cm, containing 50.0 kg D900 macroporous resin), and the resin column was decolorized by 12 BV of 20% ethanol. Dioscin then flowed from the column using 60% ethanol in about 15 BV. The flow rate of each gradient elution was set at 200 mL/min, and the elution of 60% aqueous ethanol was collected, concentrated and dried.

3. Results and discussion

3.1. Adsorption and desorption capacities, desorption ratio of the macroporous resins

In order to calculate the adsorption and desorption capacities and the desorption ratio of dioscin on macroporous resins, a method for the measurement of dioscin using HPLC coupled with an ELSD was established. A calibration curve of dioscin was constructed using a series of concentrations of the standard solutions, and the regression equation for concentration and corresponding peak area was calculated in the form of $Y = aX + b$. A good calibration curve of dioscin was obtained ($Y = 7520.8X + 476.35$ ($R^2 = 0.9992$)). The inter-day and intra-day precisions for dioscin expressed as relative standard deviation (RSD) were lower than 3.0% ($n = 5$).

The adsorption capacity, desorption capacity and desorption ratio of the resins for the target were calculated according to the following Eqs. (1)–(3), respectively.

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

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

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

where q_e is the adsorption capacity at adsorption equilibrium (mg/g dry resin); C_0 and C_e are the initial and equilibrium concentrations of dioscin in the solutions (mg/mL); V_i is the volume of the initial sample solution (mL) and W is the weight of the dry resin (g); Q_d is the desorption capacity; D is the desorption ratio (%); C_d represents the concentration of dioscin in the desorption solution (mg/mL), and V_d is the volume of the desorption solution (mL).

In the present paper, the adsorption capacities, desorption capacities and desorption ratios of dioscin on different macroporous resins were distinct. The selection of suitable resins was mainly based on the polarities of the chemicals and the resin polarities, as well as the average pore diameters and surface areas, listed in Table 1. Nine adsorption resins with different polarities were investigated to select the best resin for the first step separation process. Among these resins, DA-201 and NKA-II showed polarity; AB-8 had low polarity; DM-301 had middle polarity; D101, HPD-100, D1400, X-5 and D4020 were non-polar. For dioscin with a relatively low polarity, the resins with low polarity possessed a better adsorption capacity. As shown in Fig. 2A, the adsorption and desorption capacities, and the desorption ratios of D101 resin were 35.52 mg/g, 28.92 mg/g and 85.6%, respectively, and these parameters in AB-8

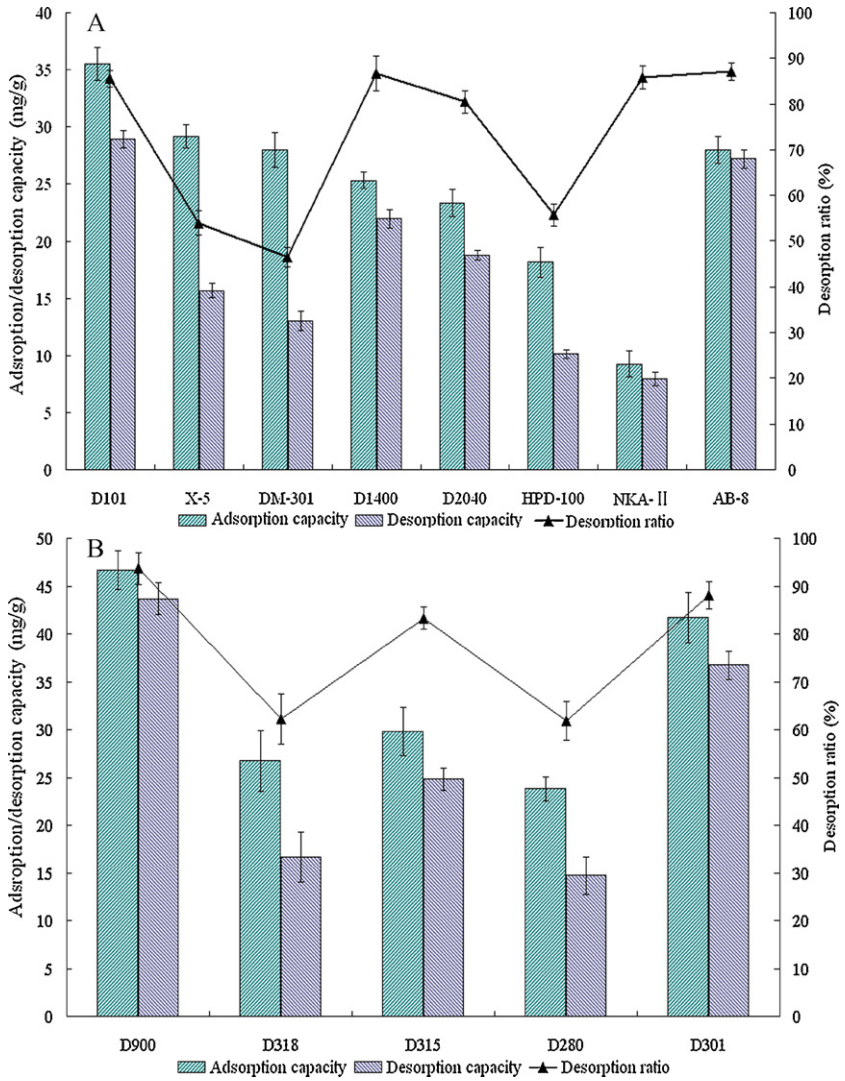

Fig. 2. Adsorption and desorption capacities, and desorption ratio of dioscin on macroporous resins. (A) The resins for the first step; (B) the resins for the second step.

resin were 28.01 mg/g, 27.20 mg/g and 87.08%, respectively. These were considerably higher than the other adsorbing resins. However, the adsorption and desorption capacities of dioscin on D101 resin was higher than on AB-8, but AB-8 had a better desorption ratio. Therefore, further investigation on the adsorption kinetics of AB-8 and D101 resins were carried out.

In the resin screening test for the second step separation of dioscin, adsorption and desorption capacities, and desorption ratios of another five types of decolorizing macroporous resins were obtained. The adsorption capacities and desorption ratio of D900 resin were 46.64 mg/g and 93.65%, and those of D301 resin were 41.72 mg/g and 88.06%, respectively. These data are shown in Fig. 2B. The absorption and desorption capacities of D900 and D301 resins were close to each other, thus an adsorption kinetics experiment to choose the best resin was needed.

3.2. Adsorption kinetics of dioscin on AB-8, D101, D900 and D301 resins

The adsorption capacity was increased transiently with contact time before reaching the adsorption equilibrium. Fig. 3A shows the uptake of dioscin on AB-8 and D101 resins over the experimental time. The equilibrium time for dioscin was 180 min on D101 resin, and was about 300 min on AB-8 resin. Therefore, 360 min was sufficient to reach adsorption equilibrium. Comparing the two resins, D101 resin showed more advantages in the adsorption of dioscin than AB-8 resin. Thus, D101 resin was selected for dioscin separation.

Adsorption kinetic curves were also obtained for dioscin on D900 and D301 resins. As can be seen from Fig. 3B, the adsorption capacities of the two resins increased with adsorption time. Equilibrium was reached at approximately 2 h. In the first 1 h, the adsorption capacities increased rapidly. After 1.5 h, the slopes at different times varied little. In the comprehensive consideration of the adsorption capacity and the desorption ratio of the two resins, these parameters were higher on the D900 resin than on the D301 resin. Hence, the D900 resin was selected as the most suitable resin for dioscin separation.

3.3. Adsorption isotherms

In the present study, D101 and D900 resins were selected to further characterize the adsorption behavior of dioscin. Equilibrium adsorption isotherms for dioscin on D101 and D900 resins with eight concentrations of dioscin at the temperature of 25 °C, 30 °C and 35 °C were constructed. The initial concentrations of dioscin in the solution were 2.853, 2.533, 2.130, 1.836, 1.190, 0.775, 0.525 and 0.253 mg/mL, respectively. As seen in Fig. 4A, the adsorption

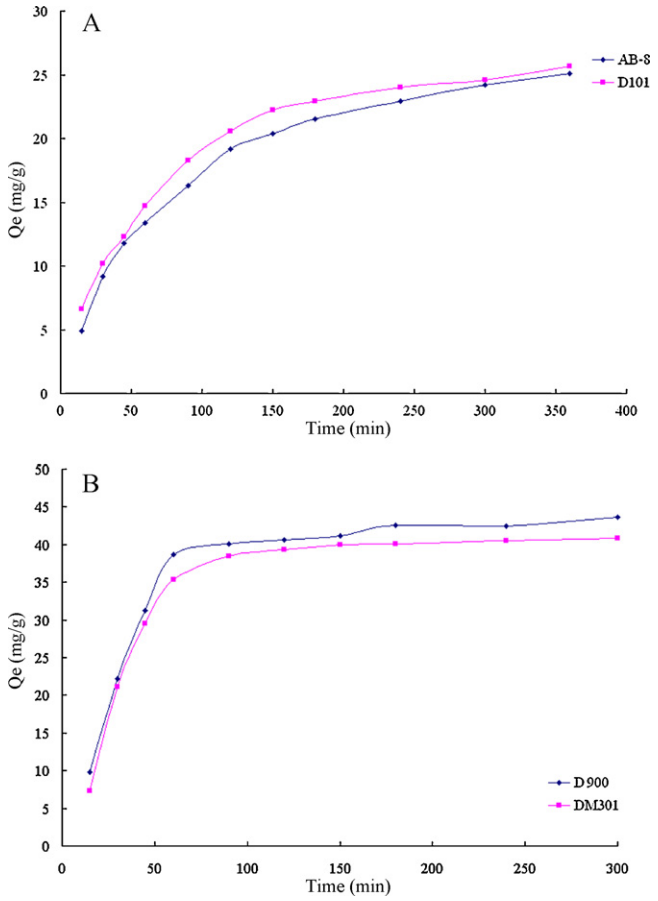

Fig. 3. Adsorption kinetics curves for dioscin on (A) D101 and AB-8 resins; (B) D900 and D301 resins.

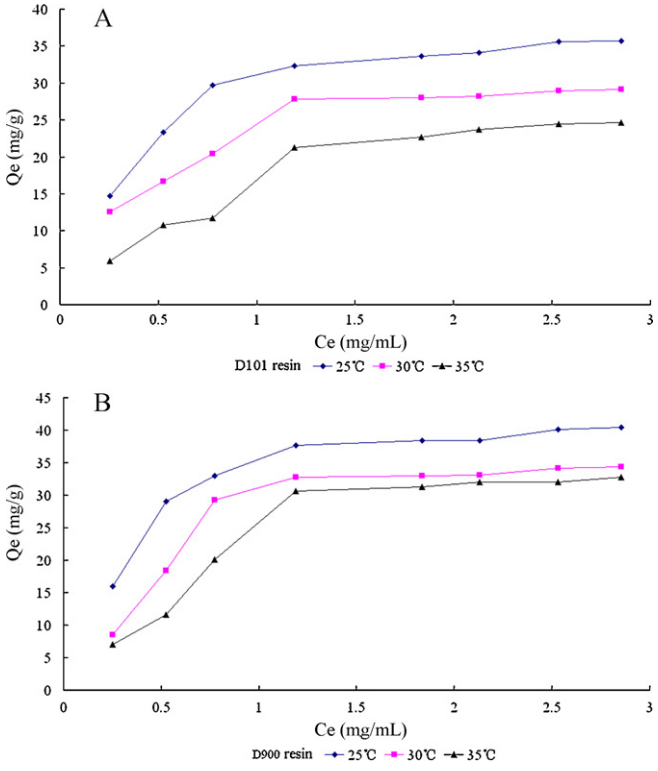

Fig. 4. The adsorption isotherms of dioscin on selected resins. (A) The D101 resin at 25, 30 and 35 °C; (B) the D900 resin at 25, 30 and 35 °C.

capacity for dioscin on D101 resin increased when the initial concentration of dioscin increased, and reached saturation status when the initial concentration was 1.190 mg/mL. Thus, the initial concentration of dioscin in the sample solution for adsorption should be higher than 1.190 mg/mL. In Fig. 4B, the adsorption capacity of D900 resin for dioscin under the same conditions is also shown and the initial concentration of dioscin needed was also higher than 1.190 mg/mL.

The initial and equilibrium data demonstrate the affinity between solutes and adsorbent. The relationship between them can be described by the Langmuir isotherm and the Freundlich equation. The two equations are used to show linearity and to describe how the components interact with the resins. Application of the Langmuir equation indicates that each site of the adsorbent can adsorb only one particle. Hence, the Langmuir equation describes the adsorption behavior of a monomolecular layer. The Langmuir isotherm is used mainly due to its simplicity. This model can be converted to a linear line with C_e and C_e/q_e, and is usually given as:

$$\frac{C_e}{q_e} = \frac{C_e}{q_0} + \frac{1}{Kq_0} \quad (4)$$

where K is the adsorption equilibrium constant, which is related to the maximum adsorption capacity; q_0 is the empirical constant, which is related to the adsorption energy of the resin.

The Freundlich equation is used extensively in physical and chemical adsorptions. It can be used to describe the adsorption behavior of a monomolecular layer as well as that of a multimolecular layer. It is a two-parameter model widely used for many different liquid and gas adsorption systems where the following formulae (5) and (6) are used:

$$q_e = KC_e^n \quad (5)$$

$$\log q_e = \log K + \left(\frac{1}{n}\right) \log C_e \quad (6)$$

where K is a Freundlich constant that is taken as an indicator of adsorption capacity; the $1/n$ value is obtained from the slope in the linear regression result, which is correlated with the adsorption intensity of the resin.

The adsorption capacities of D101 and D900 resins for dioscin were compared using the two standard theoretical models. The linearity of each model for each resin is shown in Fig. 5, and the parameters obtained frpm the regression equations at 25 °C, 30 °C and 35 °C are listed in Table 2. The R^2 values of the Langmuir isotherm model for each resin at each temperature were all above 0.98, but were best at 25 °C. Adsorption can take place easily when the n value of the Freundlich model is between 0.1 and 0.5, and there was only one regression equation at 30 °C which was beyond this range. Thus, dioscin adsorption on D101 and D900 resins can be described by the Langmuir and Freundlich models, and the adsorption and desorption of dioscin takes place much easier at 25 °C.

3.4. Dynamic desorption curves on macroporous resins

In order to decrease the consumption of reagents and make desorption more efficient, optimization of ethanol concentrations to elute dioscin from D101 and D900 resins was carried out. Different concentrations of aqueous ethanol (from 10% to 80%) were used in desorption tests after adsorption equilibrium, and the dynamic desorption curves are shown in Fig. 6A. These concentrations of aqueous ethanol were used to remove high polarity compounds and elute dioscin from the resins.

With increasing ethanol concentration, the desorption ratio of dioscin on D101 was obviously increased. At 30% aqueous ethanol,

Table 2
Langmuir and Freundlich equation parameters of dioscin on D101 and D900 resins at 25 °C, 30 °C and 35 °C.

Resin	Temperature (°C)	Langmuir equation	R^2	Freundlich equation	R^2
D101	25	$Q_e = 0.3036C_e + 0.0502$	0.9948	$Q_e = 2.923e^{0.3398}$	0.8699
	30	$Q_e = 0.3238C_e + 0.0391$	0.9835	$Q_e = 2.079e^{0.5356}$	0.9225
	35	$Q_e = 0.2529C_e + 0.0429$	0.9960	$Q_e = 3.414e^{0.3147}$	0.8320
D900	25	$Q_e = 0.0739C_e + 0.019$	0.9989	$Q_e = 9.582e^{0.3580}$	0.8989
	30	$Q_e = 0.0843C_e + 0.0276$	0.9967	$Q_e = 7.799e^{0.4431}$	0.8476
	35	$Q_e = 0.086C_e + 0.0414$	0.9887	$Q_e = 6.937e^{0.4698}$	0.9278

little dioscin was desorbed, however, when the ethanol concentration was over 40%, the desorption ratio increased sharply and reached a peak value at 80% aqueous ethanol. Thus, 30% and 80% aqueous ethanol were selected as the cleaning solution and desorption solution, respectively. Gradient elution was also carried out on D900 resin. At 20% ethanol, little dioscin was desorbed. The desorption capacity increased when the concentration of ethanol solution increased until it reached 60%. Hence, 20% and 60% aqueous ethanol were selected as the cleaning solution and desorption solution, respectively.

When adsorption saturation was reached, the glass column was eluted to choose suitable volumes of the cleaning solution and desorption solution to remove impurities and elute dioscin. The elution curves for the cleaning solution and desorption solution selected for desorption of dioscin on D101 and D900 resins were obtained. As shown in Fig. 6B and C, the D101 resin was flushed with 8 BV 30% aqueous ethanol to remove high polar components in the extract. The adsorbent was then rinsed with 10 BV 80% aqueous ethanol to collect dioscin. In the second step, the D900 resin was decolorized with 12 BV 20% aqueous ethanol, and dioscin was allowed to flow from the column using 15 BV 60% aqueous ethanol.

In summary, a two-step separation method was established for the purification of dioscin from *D. nipponica* Makino. In the first step, a D101 resin column was used, and 8 BV of 30% aqueous ethanol and 10 BV 80% aqueous ethanol were used to wash the column. In the second step, 12 BV of 20% aqueous ethanol and 15 BV 60% aqueous ethanol were used to wash the column packed with D900 resin.

Table 3
Concentration and recovery of dioscin in products after the two procedures.

Process	Amount (kg)	Purity of dioscin (%)[c]	Recovery (%)[d]
Raw material	100.0	2.44	–
Crude extract	20.95	9.35	80.52
First-step[a]	1.869	85.64	81.47
Second-step[b]	1.486	96.55	89.64
Total[e]	1.486	96.55	72.99

[a] The process was carried out on D101 resin.
[b] The process was carried out on D900 resin.
[c] P_x (%) = $C_x V_x / W_x$
[d] R_x (%) = $W_x P_x / W_i P_i$
[e] $R_{total} = R_{step1} \times R_{step2}$

where P_x is the purity of dioscin after the separation; C_x is the concentration of dioscin in the desorption solution collected; V_x is the volume of the desorption solution collected; W_x is the weight of the product obtained after the separation. R_x is the recovery of bioscin; P_i is the purity of dioscin in the sample before separation; W_i is the weight of the sample used for the separation; R_{step1} is the recovery of dioscin of the first step, and R_{step2} is the recovery of dioscin of the second step.

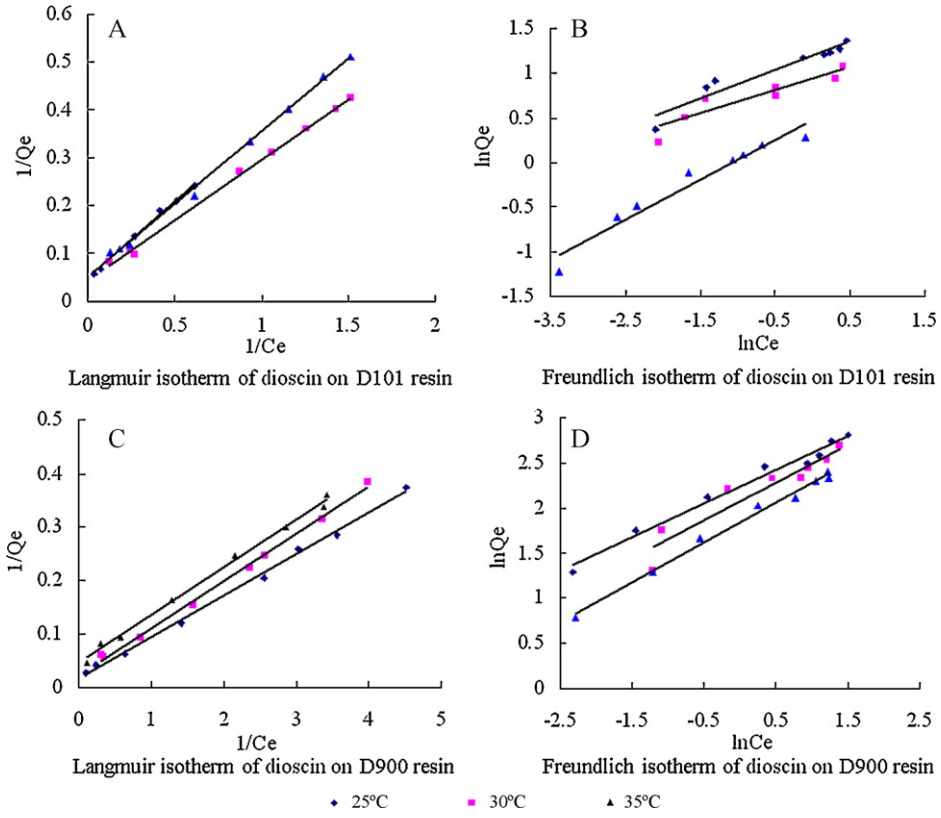

Fig. 5. The linear correlations of dioscin on D101 and D900 resins at 25, 30 and 35 °C on bases of Langmuir and Freundlich models.

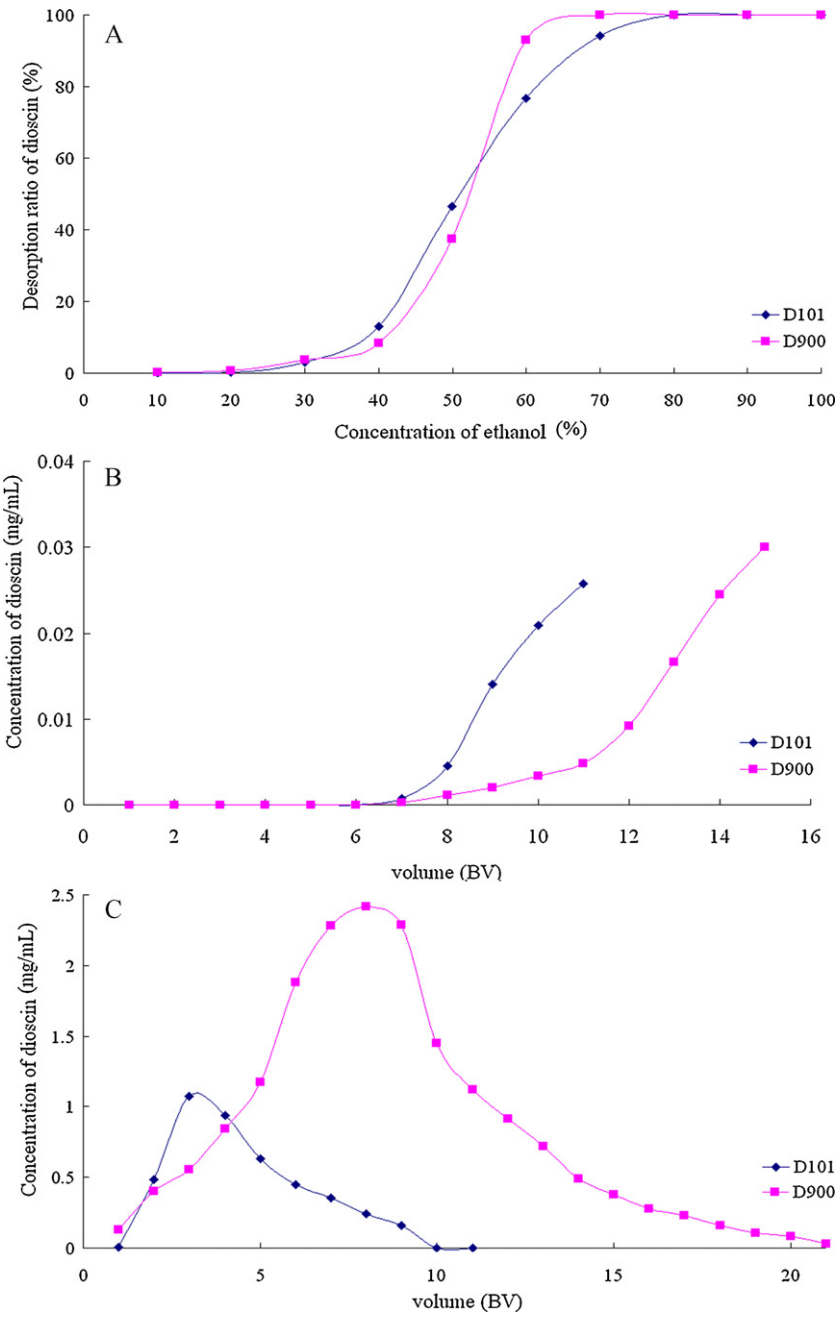

Fig. 6. Dynamic desorption tests of dioscin on D101 and D900 resins. (A) Dynamic desorption curves of dioscin using different concentrations of aqueous ethanol; (B) the volumes of 30% aqueous ethanol on D101 resin and 20% aqueous ethanol on D900 to remove the impurities; (C) the volumes of 80% aqueous ethanol on D101 resin and 60% aqueous ethanol on D900 to elute dioscin.

3.5. Industrial-scale preparation

All the methods used for separating dioscin from *D. nipponica* Makino were investigated in a small-scale preparation. This was followed by a large-scale preparation of pure dioscin using the above-mentioned conditions. A D101 resin (10 kg) column was used with a BV of 10 L. The dry brown powdered extract (20.95 kg) from 100.0 kg plant material containing 9.93% dioscin (Fig. 7A) was separated. 8 BV of 30% aqueous ethanol and 10 BV 80% aqueous ethanol were used to wash the column. A product of 1.869 kg with pallide-flavens containing 85.64% dioscin was obtained (Fig. 7B), and a recovery of 81.47% was reached after the first step. In the second step, 12 BV of 20% aqueous ethanol and 15 BV 60% aqueous ethanol were used to wash the column packed with D900 resin, and a product of 1.486 kg consisting of a white powder containing 96.55% dioscin was produced (Fig. 7C), and a recovery of 89.64% was reached. Thus, the total recovery was 72.99%. The purity of dioscin in the two steps was calculated according to formula (7) and the recoveries of dioscin were calculated based on formula (8). The total recovery (R_{total}) was then determined according to formula (9). Production data are shown in Table 3.

$$P_x\,(\%) = \frac{C_x V_x}{W_x} \tag{7}$$

where P_x is the purity of dioscin after separation; C_x is the concentration of dioscin in the desorption solution collected; V_x is the volume of the desorption solution collected; W_x is the weight of

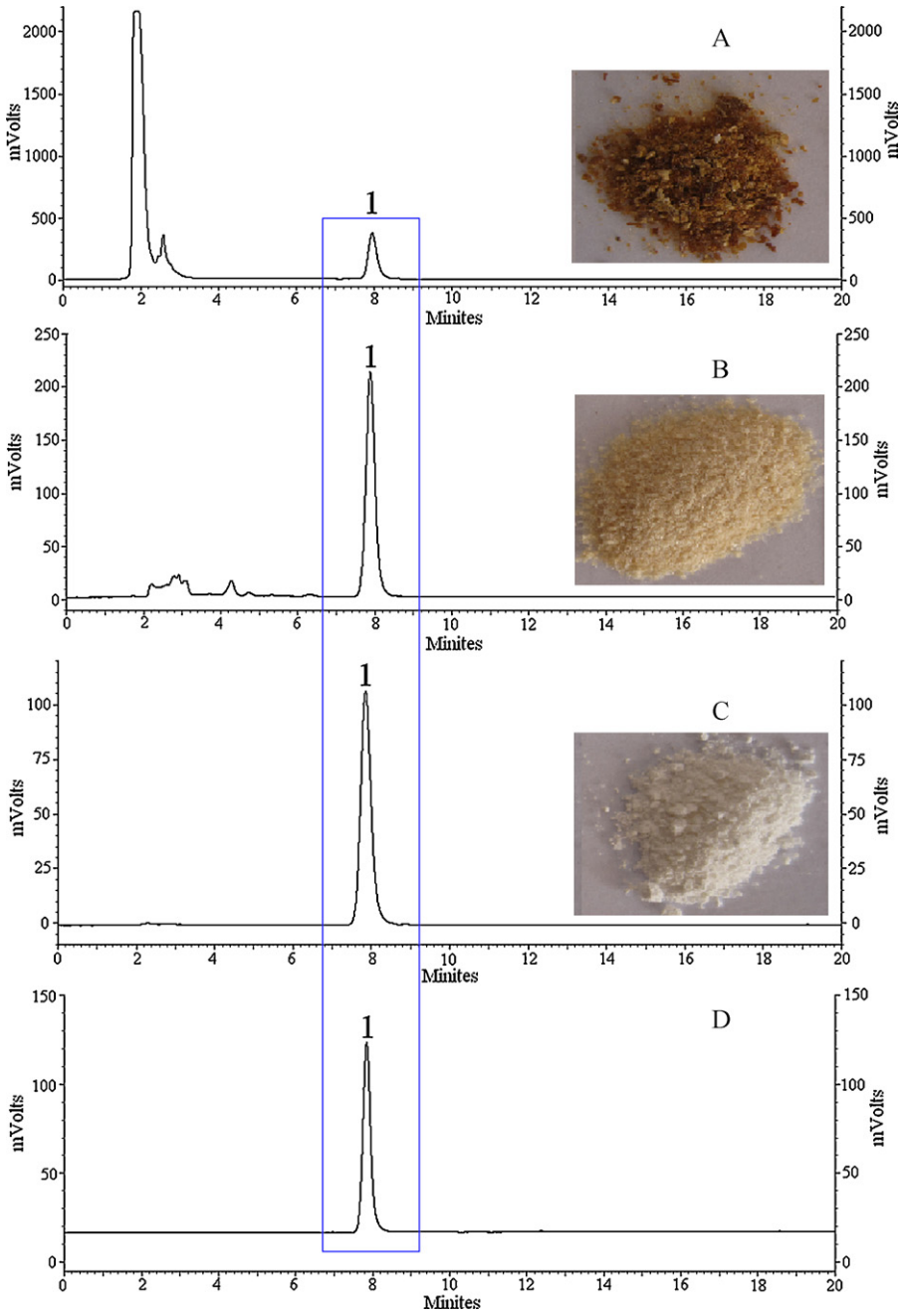

Fig. 7. Chromatograms and the pictures of the sample and products. (A) Before the first-step; (B) after the D101 resin process; (C) after the second procedure; (D) the standard of dioscin.

the product obtained after separation.

$$R_x\,(\%) = \frac{W_x P_x}{W_i P_i} \tag{8}$$

$$R_{total} = R_{step1} \times R_{step2} \tag{9}$$

In the equations, R_x is the recovery of dioscin; P_i is the purity of dioscin in the sample before separation; W_i is the weight of the sample used for the separation; R_{step1} is the recovery of dioscin in the first step, and R_{step2} is the recovery of dioscin in the second step.

4. Conclusions

In the present study, the performance and characteristics of several macroporous resins for the preparation of dioscin were investigated. Among them, the D101 and D900 resins were most suitable, and best fitted the Langmuir isotherm at 25 °C. Using optimized parameters, an industrial-scale process was carried out, and the target purity of the extract of *D. nipponica* Makino was increased 10.33-fold from 9.35% to 96.55% with a recovery of 72.99%. Therefore, this method has high throughput, is green and effective, and is very suitable for the large-scale separation of dioscin from medicinal plants for pharmaceutical use.

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