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大孔树脂纯化黄藤中生物碱的工艺优化

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摘　要:本文以巴马汀和药根碱纯度为检测指标,优化大孔树脂纯化黄藤活性部位提取液的工艺。首先考察4种大孔树脂对黄藤中生物碱的静态吸附及解吸附性能,筛选出最优大孔树脂ADS-3。其次,选取六个变量对ADS-3树脂的动态吸附及解吸附性能进行优化。结果如下:在吸附过程中,树脂与药材用量比4∶1(V∶M),吸附流速2.0 BV/h且3.0 BV的水洗脱除杂(柱体积为30 mL);解吸附过程中,使用4.0 BV的40%乙醇溶液洗脱,洗脱流速为2.0 BV/h。经纯化后,巴马汀和药根碱的回收率分别达到95.93%及96.03%。因此,ADS-3型大孔树脂纯化工艺稳定,值得在工业上推广应用。

关键词:黄藤;巴马汀;药根碱;大孔树脂;单因素试验;

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Optimization of Purification Process of Alkaloids from *Fibraurea recisa* Pierre. with Macroporous Resin

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Abstract: The *Fibraurea recisa* Pierre. has been applied to a diverse range of diseases as a heat-clearing, detoxicating, anti-inflammatory and laxative drug. This study is aimed to optimize the technology of purifying major alkaloids (palmatine and jatrorrhizine) from *Fibraurea recisa* Pierre. by macroporous resin using purity as index. Firstly, the static adsorption and desorption properties of four different macroporous resins were compared to select the appropriate resin. Furthermore, the dynamic adsorption and desorption experiments were carried out on selected ADS-3 resin to optimize the separation process of alkaloids with six variable conditions. The results showed the optimized conditions as below: In the adsorption process, 1. the mass ratio of resin to materials was 4∶1; 2. sample feeding rate is 2.0 BV/h; 3. The volume of water eluent was 3.0 BV (30ml of Bed Volume). During the optimal desorption process, 4. 40% ethanol was used as elution solution; 5. The volume of elution is 4.0 BV; 6. The desorption rate is 2.0 BV/h. Results reveal that after separation, the desorption rate of palmatine and jatrorrhizine were achieved at 95.93% and 96.03% respectively, indicating ADS-3 can be successfully applied to enrich and purify alkaloids in FRP.

Key words: *Fibraurea recisa* Pierre.; palmatine; jatrorrhizine; macroporous; single-factor experiment

Fibraurea recisa Pierre. (FRP), belonging to Menispermaceae, is found mainly in the tropical and semitropical zones of Asia and Africa[1]. The stem bark of *F. recisa*, known as 'Huangteng' in Chinese traditional medicines, was recorded in Chinese Pharmacopoeia as a heat-clearing, detoxicating, anti-inflammatory and laxative drug[2].

The major alkaline components in *F. recisa* extracts are palmatine and jatrorrhizine. Palmatine is proved to exhibit the same biological activities as 'Huangteng'[3] and other activities including anti-fungal[4], WNV NS2B-NS3 pro-tease inhibition[5] as well as cognitive dysfunction improvement in streptozotocin-induced diabetic rats[6]. Jatrorrhizine can prominently inhibit the cell proliferation in human colon cancer cells. Therefore, it can be a good candidate potential to be further developed into a chemotherapeutic drug[7]. It also displays synergetic cholesterol-lowering efficacy as a complex with four other alkaloids. In addition, jatrorrhizine is also regarded as a potential drug for hypercholesterolemia[8]. Thus, it is of significance to purify two alka-

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loids in FRP simultaneously.

Conventional extraction of alkaloids from FRP including hot maceration[9,10], cold maceration method[9], ultrasonic method[10] and salting out method[11,12] are not effective to obtain these compounds concerning regents, labor, energy consumption and environmental protection. Now great focus has been put into employing macroporous resins to enrich and purify bio-active constituents from traditional Chinese herbs[13]. Macroporous resins can selectively adsorb targeted phytochemicals due to unique characteristics including ideal pore structure, various surface functional groups. Besides, macroporous has lower operation expense, less solvent consumption, and easier regeneration[14]. Chen HP[3] used D101 macroporpus resin to separate and purify palmatine from FRP. He managed to get extract of palmatine and jatrorrhizine. However, research is still scarce on screening the species of resin that affect properties of palmatine as well as jatrorrhizine. Moreover, the extraction ratio of palmatine and jatrorrhizine were only 54.6% and 21.6%, respectively. Consequently, these methods are not suitable for industrialization.

In this paper, four macroporous resins with different chemical and physical properties were screened to investigate the adsorption and desorption process. ADS-3 macroporous resin was selected for further optimization by six parameters including resin-materials ratio, feeding rate, eluenting water volume, eluent fraction, eluent rate and eluent volume to develop a convenient and efficient method for enrichment and separation of alkaloids from FRP.

Materials and Methods

Materials

Plant materials

FRP were collected in December 2014 in Cambodia and authenticated by Prof. Jiang Lin of School of Pharmaceutical Science, Sun Yat-sen University. A voucher specimen (accession number: CAFR201412) has been deposited at the School of Pharmaceutical Science, Sun Yat-sen University.

Main reagents

Standard samples of palmatine and jatrorrhizine were purchased from the National Institute for food and drug control, Ministry of Health, Beijing, China. Methanol (HPLC grade), acetonitrile (HPLC grade), Sodium hydroxide, hydrochloric acid, phosphoric acid, diethyl amine, alcohol (all AC grade) were purchased from Tianjin Zhi Yuan chemical reagent. (Tianjin, China). De-ionized water was prepared by a Milli-Q water purification system (Millipore, Billerica, MA, USA). The macroporous resins including ADS-3、D101、HP20、AB-8 were purchased from Tianjin chemical plant Nankai University. (Tianjin, China). 0.45 μm membranes (Chromatography Science and Technology Co., Tianjin, China) were used for filtration.

Instruments

HPLC analysis was performed using LC-20AB pump, SIL-20A automatic injector, SPD-M20A diode array detector (Shimadzu Corporation from Japan); SB25-12DTD ultrasonic cleaning machine (Ningbo xinzhi biological technology Co.), CA-1111 cooling device and N-1001 Rotary evaporator (AiLang instrument Co., Shanghai), SHZ-D (Ⅲ) circulating water pump (Yu Hua instrument Co., Gongyi, China), DZG-6020 vacuum drying oven (Sen Xin experimental equipment Co., Shanghai), governor oscillator SHA-BAB (Jintan Ronghua Instrument Manufacturing Co., Ltd.) were use during the experimental process.

Methods

Extraction of total alkaloids

FRP was placed in the drying oven for 5.0 h, smashed and filtered through a 60-mesh sieve. 75.0 g of powder was accurately weighed and placed in a round-bottom flask, added with 70% ethanol, placed in an ultrasonic cleaning machine for extraction for 0.5 h, the extraction was repeated twice. Extracts were collected for centrifugal (5000 r/min) and concentration. Subsequently, the extracts were diluted to appropriate concentrations according to the experimental requirements.

Analytical Methods

An Ultimate AQ-C_{18} (4.6 mm × 250 mm, 5 μm) was employed for the detection of samples. H_2O-CH_3CN-

H_3PO_4-$C_4H_{11}N$ (70 : 30 : 0.4 : 0.4) was adopted as mobile phase. The column temperature was fixed at 25 ℃. Flow rate was 1ml · min^{-1}. The detector was set to 345 nm and injection volume was 20 μL. All solutions were prepared in 1% hydrochloride methanol and filtered through 0.45 μm membranes before HPLC analysis. Alkaloids concentration was calculated using the standard sample of palmatine and jatrorrhizine as the calibration standard. Good linear relationship of palmatine and jatrorrhizine were obtained over the range of 1.482 ~ 23.712 mg/mL and 0.417 ~ 6.672 mg/mL, respectively. And the regression were y = 3526.2x + 4000000 (R^2 = 0.9993) and y = 4197.7x + 419228 (R^2 = 0.9998), where y was the absorbance at 345nm, x was the concentration of alkaloids (ug/ml) and R was the regression coefficient.

Pretreatment of tested resins

The tested resins were pretreated by soaking in ethanol for 24 h and then washed with ethanol until there was no turbidity when a five-fold volume of water was added into the eluent[15]. The resins were subsequently washed with de-ionized water until the ethanol was thoroughly replaced. The resins were further successively washed with 5% HCl, de-ionized water, 2% NaOH, and de-ionized water till its neutral before use[16].

Static adsorption capacity and desorption ratio

Selection of macroporous adsorption resin

The static adsorption test for screening the optimal resins was performed as follows: Suitable amount of each resin (equivalent to 2.0 g dry resin) was mixed in a vial with 20 mL diluted solution of crude extract. And the vials were shaken in an oscillator at a frequency of 100 times per min at 25 ℃ for 24 h to establish the adsorption equilibrium. Then the supernatant was sampled (1 mL), filtrated, and measured by HPLC. Each experiment was repeated in triplicate. After reaching the adsorption equilibrium, the supernatant was discarded by filtration and the saturated resin was washed with 5 mL distilled water. A volume of 40 mL 95% ethanol was added in the vial, followed by shaking at a frequency of 100 times per min at 25 ℃ for 24 h to estimate desorption ratio of two main alkaloids from the resin. The adsorption and desorption ratio were calculated with the following formula:

$$\alpha(\%) = \frac{C_0 - C_1}{C_0} \times 100\%$$

$$\beta(\%) = \frac{C_2}{C_0 - C_1} \times 100\%$$

where (α%) is the adsorption ratio of equilibrium, (β%) is the desorption ratio. C_0 is the initial concentration of crude extraction (mg/mL), C_1 is the equilibrium concentration (mg/mL). C_2 is the concentration of two main alkaloids in the desorption solution.

ADS-3 resin adsorption process optimization

Determination of specific volume

0.8 g of dry ADS-3 macroporous resin was accurately weighed and soaked. Then it was placed in a graduated cylinder to record volume.

Static adsorption ratio of ADS-3

Suitable amount of ADS-3 resin (equivalent to 2.0 g dry resin) was mixed in a vial with 20 mL diluted solution of crude extract. Adsorption time and the pH of crude extraction were set as the single variables. And the vials were shaken in an oscillator at a frequency of 100 times per min at 25 ℃ for 24 h to establish the adsorption equilibrium. Then the supernatant was sampled (1 mL), filtrated, and measured by HPLC. Each experiment was repeated in triplicate. Static adsorption curves were drew according to the adsorption ratio of different variables (T, pH), respectively.

Static desorption ratio of ADS-3

2.0 g ADS-3 resin was weighed accurately and mixed in a vial with solution of extract solution. The adsorption process was performed according to 1.2.4.2.2. After static adsorption, the resin was filtered, dried and added with 40 mL of 95% ethanol. The erlenmeyer flask was placed in an oscillator at a frequency of 100 times per min for 24 h and filtered after desorption. Desorption time and the pH of ethanol were set as the single variables. The concentration of alkaloids in the filtrate was detected to calculate the desorption rate of different variables (T, pH) and to draw static desorption curves, respectively.

Dynamic adsorption and desorption ratio optimization

Dynamic adsorption experiments were carried out in

glass columns wet-packed with selected resin (ADS-3). The bed volume (BV) of the resin was 30 mL. The origin conditions were as follows: resin-materials ratio = 4∶1 (pH = 11.0), feeding rate 2.0 BV /h, elution rate 2.0 BV /h, 4.0 BV of 50% aqueous ethanol (pH = 5.0). Then the specific condition was changed according to six factors. The feed solution's pH was all set at 11.0 and eluent ethanol's pH was set at 5.0. All the dynamic experiments were performed at room temperature. Apart from that, all the equilibrium adsorption time and desorption time were set at 2.0 hrs and 3.0 hrs, respectively. The adsorbate-laden column was washed with de-ionized water, and then desorbed with certain concentration of ethanol. The elutriant was concentrated in the rotary evaporation apparatus and dried under vacuum before further analysis. The adsorption rates of resin-material ratio, feed rate, volume of de-ionized water were calculated with the formula used in 1.2.4.1α(%). The desorption rates of 3 factors (eluent concentration, rate, volume) were calculated with the formula used in 1.2.4.1β (%).

Results and Discussion

Static adsorption rate and desorption rate of the four types of resin

It is known that differences in adsorption and desorption capacity can be found in different species of resins due to many factors including polarity, surface area, particle size, aperture, adsorptive polarity, molecular size. Generally, better adsorption capacity will be achieved when the adsorptive and the resin possess the same or similar polarity. Based on such knowledge, we chose 4 types of resins, whose physical properties are listed in Table 1 to perform static adsorption desorption screening.

As can be seen from Fig. 1 (1A, 1B), ADS-3 has the highest adsorption rate and desorption rate among the four types of resins. Hence, ADS-3 resin was selected for dynamic adsorption and desorption experiments to explore the optimal technological conditions.

Table 1 Physical properties of the 4 kinds of resins

Model resin	Exterior	Polarity	The specific surface area (m^2/g)	The average pore size(nm)	Porosity (%)	Pore (m^2/g)	Size range (mm)
AB-8	White spherical particles	moderate polar	480 ~ 520	130 ~ 140	42 ~ 48	0.73 ~ 0.77	0.315 ~ 1.25
ADS-3	White spherical particles	non-polar	480 ~ 650	130 ~ 150	30 ~ 52	0.74 ~ 0.78	0.32 ~ 1.45
HP-20	White spherical particles	non-polar	550 ~ 650	120 ~ 140	56 ~ 78	1.14 ~ 1.34	0.35 ~ 1.05
D101	White spherical particles	non-polar	500 ~ 550	90 ~ 100	65 ~ 75	1.18 ~ 1.24	0.3 ~ 1.25

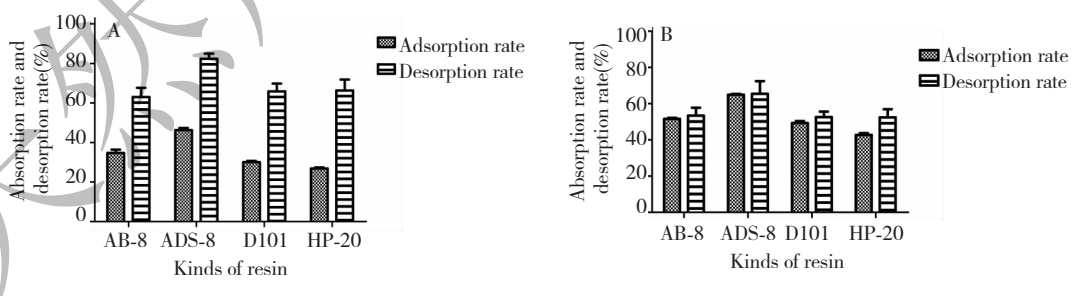

Fig. 1 Effect of four macroporous resins on adsorption and desorption rates of jatrorrhizine (A) and palmatine (B) from FRP

Note: Compared to other resins, ADS-3 has significance difference at $P < 0.05$

Static adsorption and desorption on ADS-3

Determination of specific volume

Firstly, we carried out resin specific volume determination experiments and identified the specific volume of ADS-3 resin is 6.784 mL/g (RSD: 0.76%).

Static adsorption ratio of ADS-3

Determination of adsorption time

As seen from Fig. 2 (A), the adsorption capacity in-

creases with adsorption time and reaches equilibrium in about 4.0 h. The initial rate was likely due to the occurrence of adsorption in the easily accessible mesoporous of the particles. The slower uptake later, on the other hand, is the indication of process with high mass transfer resistance inside the particle. The adsorption rate achieved equilibrium in 78.85% at 4.0 h. However, the difference between the rate of 2.0 h and 4.0 h is merely 10%. Thus, 2.0 h is chosen as adsorption time considering efficiency cause.

Determination of feeding extract's pH

The column were fed by crude extract (pH was adjusted at 5.0, 7.0, 9.0, 11.0, 13.0) of FRP. As shown in Fig. 2(B), the resin achieves optimal adsorption result at pH 11. Two alkaloids of FRP have nitrogen heterocyclic structures and show weak basicity. Thus, components generally exist as molecules under alkaline conditions and react with resin by the Van der Waals' force, which leads to good adsorption results. However, when the condition is over basic, the alkaloids would generate complex salt and lead to poor adsorption results. Therefore, the pH of feeding solution is set at 11.0 in subsequent trials.

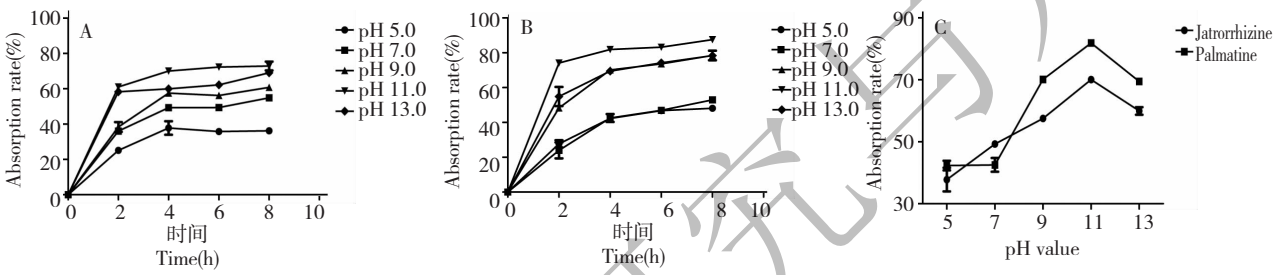

Fig. 2 Effect of time: Jatrorrhizine (A) and Palmatine (B); pH (C) on adsorption rate of two main alkaloids from *F. recisa*

Static desorption ratio of ADS-3

Determination of desorption time

As shown in Fig. 3 (A), the desorption rate of jatrorrhizine and palmatine are 84.86%, 81.56% while equilibrium time is 3.0 h. Then, the desorption rate remains stable. For economical consideration, the 3.0 h is finally chosen as desorption time.

Determination of ethanol's pH

The columns were eluted by 40mL of 95% ethanol and pH was set at 5.0, 7.0, 9.0, 11.0, 13.0, respectively. As obtained from Fig. 3 (B), the desorption rate of two alkaloids decreases with the increasing of eluent's pH value. For ethanol weakens the adhesion between alkaloids and resin. Besides, the ionization of acid increases the polarity of ethanol which make it easier for alkaloids to be washed out. Therefore, pH 5.0 ethanol (95%) is chosen to be adapted.

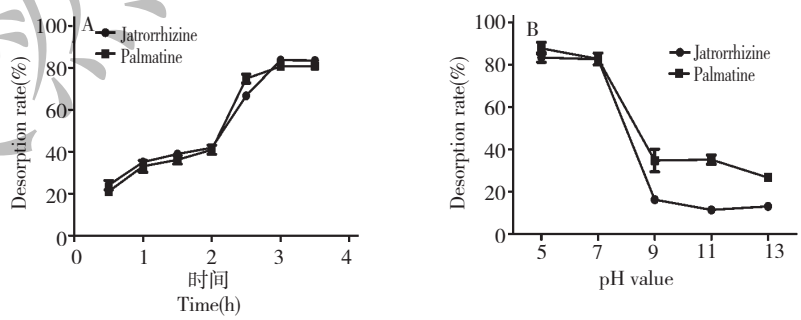

Fig. 3 Effect of time (A), pH (B) on desorption rate of two main alkaloids from FRP.

Dynamic adsorption and desorption on ADS-3

Determination of resin-material ratio

The purification efficacy is influenced by the amount of macroporous resin and crude drugs. If there are excessive crude drugs, macroporous resin will be overloaded which indicates that components could not be effective-

ly adsorbed. On the contrary, resin redundant will cause waste of resin which leads to production costs. Therefore, appropriate proportion is significantly needed.

Sample solution with resin-materials ratio of 2.0, 3.0, 4.0, 5.0, 6.0 were respectively prepared, and the pH was adjusted to 11.0. As depicted in Fig. 4 (A), the adsorption rate achieves the maximum while the resin-materials ratio is 4.0 and 6.0; however, the amount of extraction feeding into column can not meet with industrial manufacture when the ratio is 6.0. The adsorption rate is reduced from ratio 4.0 to 2.0, which due to the early leak of overload sample concentration. It indicates that the ratio 4.0 is the most appropriate for subsequent experiments.

Determination of feeding rate

The rate of feeding solution were 0.5, 1.0, 2.0, 3.0, 4.0, respectively. The result in Fig. 4 (B) indicates that low feeding rate can be profitable for the adsorption process. However, when velocity is less than 2.0 BV/h, adsorption rate barely increases with the velocity decreases. The increase is not statistically significant. Besides, substantial time is wasted because of slow feeding rate. Above all, feeding rate of 2.0 BV/h is chosen.

Determination of water volume

After the completion of the adsorption, eluting with de-ionized water can remove proteins, amylose and other impurities from crude extract. The volume of de-ionized water is 1.0, 2.0, 3.0, 4.0, 5.0 BV (bed volume of column). Fig. 4 (C) demonstrates that the adsorption rate of main alkaloids is maximum with 3.0 bed volume (30mL/BV) of water while gradually declines with the increase of water volume. To sum it up, 3.0 BV water can remove impurities as well as retain the two main alkaloids of FRP.

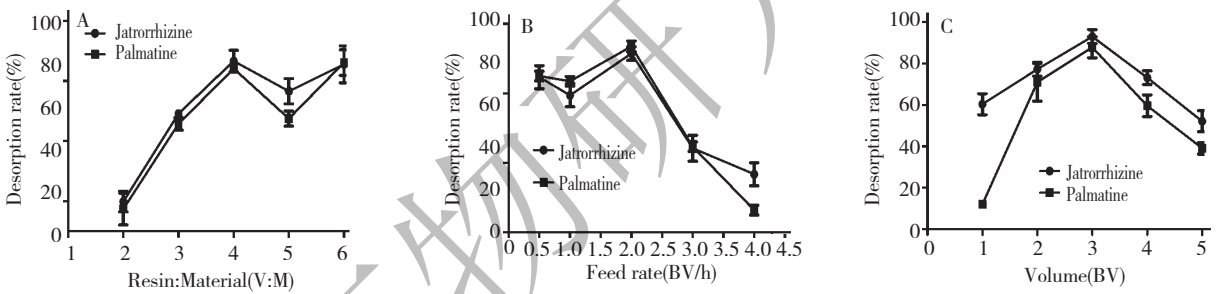

Fig. 4 Effect of resin-materials ratio (A), sampling flow rate (B), washing consumption (C) on dynamic absorption rate of two main alkaloids from FRP

Determination of the eluent volume fraction

The column were eluted by 20%, 30%, 40%, 50%, 60% concentrations of ethanol. The elution curves is shown in Fig. 5 (A). Ethanol solution with volume fraction of 40% shows optimal ability for eluting two alkaloids. The desorption rate decreases after 40% extract contains due to aqueous alkaloids existing in the extraction. Comprehensively considering the cost factors, ethanol solution with volume fraction of 40% is selected as the optimizes eluent.

Determination of eluent rate

The columns were eluted by 40% ethanol at the rate of 0.5, 1.0, 2.0, 3.0, 4.0, respectively. As evinced in Fig. 5 (B), eluent volume decreases with the increase of eluent rate. The extensive rate causes same concentration elunt leaking from column before the alkaloids are fully eluted. On the contrary, extreme slow rate will prolong the production period. Therefore, elution rate is selected at 2.0BV/h.

Determination of the eluent volume

The solution from 1.0 to 6.0 bed volume eluted by 40% ethanol was collected, concentrated, dried. As observed in Fig. 5 (C), the maximum content of alkaloids is around 1.0 to 4.0 BV. The peak is sharp and wide, the linear on the left side of the peak is increasing which indicates rapid elution. The highest contents of alkaloids is obtained at 1.5 BV with color brown eluate. The lowest is observed at 4.0 BV with fading color, indicating most alkaloids has already been desorbed away. Therefore, 4.0 BV is defined as suitable eluent

volume.

Table 2 demonstrates that the recovery rate of Palmatine and Jatrorrhizine are 95.93% and 96.03%, respectively. Compared to the results from Chen HP[3], the rate increase 40.94% and 75.27%.

Table 2 Desorption rate of the Palmatine and Jatrorrhizine

Eluent volume	Desorption rate of Palmatine(%)			Desorption rate of Jatrorrhizine(%)		
1.0 BV	22.67	22.63	22.34	12.18	11.42	11.45
1.5 BV	41.48	42.51	42.62	41.21	41.29	41.39
2.0 BV	19.24	19.43	19.53	22.43	23.52	23.26
2.5 BV	7.62	7.55	7.71	12.09	11.72	11.88
3.0 BV	2.30	2.27	2.34	5.61	5.71	5.65
4.0 BV	1.95	1.86	1.75	2.45	2.42	2.40
Recoverry rate	95.26	96.25	96.29	95.97	96.08	96.03
$\bar{X}$		95.93			96.03	

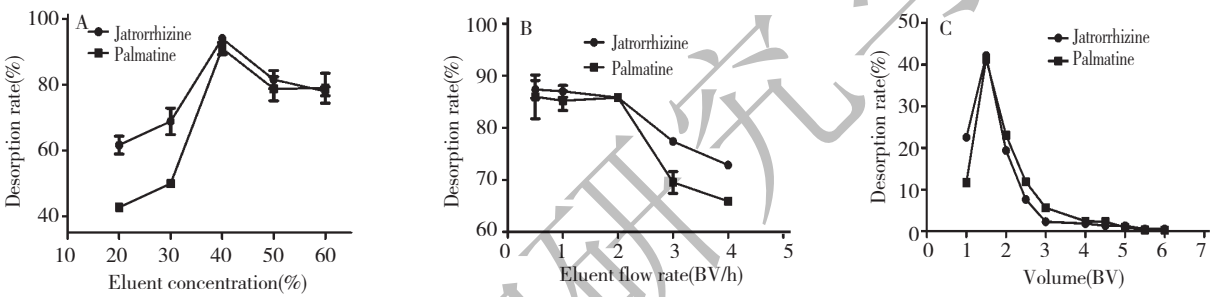

Fig. 5 Effect of eluent concentration (A), eluent velocity (B), eluent volume (C) on dynamic desorption rate of two main alkaloids from FR

Conclusion

In this study, the adsorption characteristics of four widely used macroporous resins were critically evaluated for the enrichment and recovery of palmatine and jatrorrhizine from FRP. Among them, ADS-3 was selected for its performance in static adsorption and desorption tests. Then, the effects of several factors were investigated to optimize the adsorption and desorption conditions using ADS-3 resin. The optimal technological conditions were selected: in the adsorption process, the mass ratio of resin to materials was 4∶1; the pH of sample solution was 11.0; the equilibrium adsorption time was 2.0 h, with a sample feeding rate of 2.0 BV/h; the volume of water eluent was 3.0 BV. During the optimal desorption process, the pH of sample solution was 5.0; 40% ethanol was used as elution solution, with a volume of 4.0 BV; the equilibrium desorption time was 3.0 h, with a solution rate of 2.0 BV/h.

The process is unprecedented and significant. To the best of our knowledge, Chen HP[3] use single index palmatine which is lacking of accuracy in the enriching and purifying process of macroporous. Our study shows that palmatine and jatrorrhizine's content chosen as index can give a full and objective evaluation on optimal technical conditions.

Secondly, we select 4 potantial resins for further optimization instead of chosing D101 macroporous resin according to Chen HP[3]'s research. Thirdly, static absorption and desorption on ADS-3 optimize the proper pH and reaction time which both increase the recovery of two compounds significantly. Last but not the least, the transfer rate of palmatine is 95.93% and 40% higher than the former research demonstrated by Chen[3] (about 54.6%). In addition, jatrorrhizine was separated and purified from FRP with yield 4 times

more than former study[3] (about 21.6%).

In conclusion, the results of our study suggested that ADS-3 resin is a feasible purification procedure for alkaloids from FRP. Further investigations are required regarded to the potential to produce palmatine and jatrorrhizine by resin adsorption at manufacturing scale.

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