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# Separation of matrine and oxymatrine from *Sophora flavescens* extract through cation exchange resin coupled with macroporous absorption resin

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A simple method for separation of matrine and oxymatrine from Sophora flavescens was developed with cation exchange resin coupled with macroporous resin. Based on the adsorption characteristics of matrine and oxymatrine, 001×732 cation exchange resin was used to absorb target alkaloids for removing most of the foreign matter, while BS-65 macroporous resin was chosen to purify these alkaloids. The result showed that the equilibrium adsorption data of matrine and oxymatrine on 001×732 resin and BS-65 resin at 30°C was fitted to Langmuir isotherm and Freundlich isotherm, respectively. The contents of matrine and oxymatrine were increased from 0.73% and 2.2% in the crude extract of the root of Sophora flavescens to 67.2% and 66.8% in the final eluent products with the recoveries of 90.3% and 86.9%, respectively.

**Keywords:** *Sophora flavescens*, BS-65 macroporous resin, 001×732 cation exchange resin, separation, alkaloids.

## INTRODUCTION

*Sophora flavescens* (Fabaceae), a well-known traditional Chinese medicine, is widely planted in China. The root of *Sophora flavescens* has a long history in traditional Chinese medicine with the function of relieving heat, depriving the evil wetness, clear internal heat for detoxification and killing parasitic to relieve itching *ect.*, which has been used extensively as a creditable drug for the treatment of diarrhea, gastrointestinal hemorrhage, jaundice and eczema[1]. Matrine and oxymatrine are the main two of bioactive alkaloids rich in *Sophora flavescens* (Fig.1A). Modern pharmacological studies demonstrated that matrine and oxymatrine exert a variety of pharmacological effects such as anti-inflammatory, antitumor, antivirus, anti-fibrosis, immunoregulatory[2–6], and even against several pathophysiological states such as viral hepatitis, cardiac arrhythmia and asthma[7]. Therefore, it is necessary and valuable to optimize the separation method of matrine and oxymatrine for its pharmacological research and clinical application.

A few separation methods have been developed for matrine and oxymatrine from *Sophora flavescens*, such as liquid-liquid extraction, silica gel column chromatography[8], aluminum oxide column chromatography[9], ion exchange resins[10] and macroporous resins[11] *etc*. Although all these methods could achieve separation, the process of silica gel column and aluminum oxide column chromatography separations are time-consumption, labor intensiveness and more environmental pollution, while compared to the above separation methods, the ion exchange resins and macroporous resins are possessing some advantages including high adsorption capacity, easy elution, low operation expense, less solvent consumption and easy regeneration[12–15], both of which are all durable hydrophilic polymers. Recently, ion exchange resins[16] and macroporous resins have been extensively applied to separate the bioactive constituents from various herbs[10, 17–19]. Based on electrostatic force between cations, cation exchange resin can be usually used to absorb and isolate cationic compound from complicated sample solutions, while macroporous resins are more and more attention to purify the bioactive compounds in chemical and pharmaceutical fields[12, 20], depending on its affinities in different molecules since the differences of molecule polarity, shape and weight.

In our previous experiment, cation exchange resin and macroporous resin were respectively used to separate matrine and oxymatrine from crude extract, but the purity is too low to satisfy the quality of the product. Matrine and oxymatrine are belong to quinolizidine alkaloids, which could changed into ionic matters, and can be easily adsorbed on the cation exchange resin due to the ion bond force, while the other foreign matters in the crude extract will be removed in non-ionic forms. Subsequently, matrine and oxymatrine desorbed in non-ionic forms, and flowed through a macroporous resin column, further isolated from the other similarities based on the different affinities among non-ionic molecules on the macroporous resin. Thus, according to the chemical properties described above, these two alkaloids can be reasonably separated by cation exchange resin coupled with macroporous resin.

This paper reports a method that gives an acceptable cation exchange resin coupled with macroporous resin column chromatogram for separation of matrine and oxymatrine. In this study, the adsorption and desorption properties of these two alkaloids on cation exchange resins and macroporous resins were investigated. The type of resin, eluant, eluant pH values and eluant volume *etc*. were analyzed for identifying the best separation conditions. This separation method was designed to not only removing impurities but also improving purities of matrine and oxymatrine, which was expected to be an efficient method for the separation and purification of matrine and oxymatrine for their practical use.

## EXPERIMENTAL

### Apparatus

Microwave oven (PJ21C-AN, GD Midea Holding Co., Ltd., China); Thermostatic waterbath (HH-6, Jintan Jingda apparatus factory, China); Rotary evaporator device

RE52CS-1 (Shanghai Yali-Rong Biochemical Instrument Co., Ltd., China); Centrifuge (CR3i multifunction, Thermo Co., Ltd., Germany); Vacuum oven (DZF-6050, Shanghai Boxun Industry & Commerce Co., Ltd., China); Shaker (SHZ-82, Taicang Experiment Equipment Co., Jiangsu, China); Acidometer (pHs-25, Shanghai Hong Yi Instrument Co., Ltd., China); LC-10 High performance liquid chromatography with UV detection and SCL-10A controller (Shimadzu Co., Ltd., Japan).

### Sample, chemicals and reagents

The dried roots of *Sophora flavescens* were purchased from Choi-chi Lin, Guangzhou Pharmaceutical Co., Ltd. (Guangzhou, China).

Standards of matrine and oxymatrine were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); The matrine and oxymatrine sample were supplied by Xi'an Xuhuang Bio-tech Co., Ltd. (Xi'an, China) with a purity ≥ 98.0%, the two alkaloids were mixed and dissolved at different concentrations, which were used as composition solutions for the studies of separation performances on 001×732 resin and macroporous resins; HPLC-grade methanol and acetonitrile were from Merck (Darmstadt, Germany); All the other reagents including ethanol, diethyl ether, acetic acid, triethylamine, hydrochloric acid and sodium hydroxide (Guangzhou Chemical Reagent Factory, Guangzhou, China) were of analytical grade.

### Adsorbents

001×732 strong-acid cation exchange resin (-$SO_3H$ as the functional group) with 0.3–1.25 mm granular diameter and more than 4.5 mmol/g exchange capacity was provided by Liaoyuan New Material Co., Ltd. (Anhui, China). Five macroporous resins (D101, BS-30, BS-65, HPD-400 and CAD-45) were obtained from Liaoyuan New Material Co., Ltd. (Anhui, China) and Bonchem Co., Ltd. (Hebei, China). The physical and chemical properties of the tested macroporous resins are summarized in Table 1.

### Pretreatment of resins

001×732 strong-acid cation exchange resin was soaked in deionized water, then rinsed in 2 mol/L HCl solution and washed by deionized water; Subsequently soaked in 2 mol/L NaOH solution, and washed by deionized water; kept it in 2 mol/L HCl solution again for 24 h, finally the resin was washed by deionized water repeatedly before using[21]. The macroporous resins were soaked in ethanol for 24 h to swell completely, and then thoroughly removed the ethanol with deionized water for application[22].

### Preparation of the sample solution

Take 50 g *Sophora flavescens* root powder, soaked with water in a beaker, and irradiated by microwave in the condition of 60 s power on and 15 s power off for 12 min, then extracted with 500 ml 0.3% HCl solution at 90°C in water bath for 1 h. The extracts were filtrated with straining cloth and centrifuged. The supernatant were condensed and then dried in the vacuum oven at 50°C until constant weight. Dissolve the crude extract in deionized water to prepare the sample solution, which containing matrine and oxymatrine at the concentration of 0.31 and 0.92 mg/ml, respectively.

### HPLC analysis of matrine and oxymatrine

The sample solutions were analyzed on a Shimadzu LC-10 liquid chromatographic system, which with a Phenomenex Gemini C18 column (250×4.5 mm, 5.0 $\mu$m) by isocratic elution at 30°C with acetonitrile/0.1% acetic acid solution (4:96 v/v, pH 5.60 controlled by triethylamine) as the mobile phase. The flow rate was 1.0 ml/min and the ultraviolet detector wavelength was 220 nm.

### Static adsorption and desorption test on cation exchange resin

Place 1.0 g wet 001×732 cation exchange resin into the glass vial with a lid, add 50 ml composition solution. The vial was sealed tightly and shaken at 30°C for 12 h in a shaker under pH 2, the shaking index was 100 rpm. The supernatant was analyzed by HPLC to make sure the adsorption finished, remove the residual solutions and wash the resin with 50 ml deionized water. Furthermore, add 50 ml 1 mol/L NaCl solution to desorb the alkaloids, shake it at 30°C for 12 h and the desorption solution was analyzed by HPLC.

The adsorption isotherms of matrine and oxymatrine were conducted on 001×732 resin as follows, contact 10 ml different concentrations of the composition solution with 0.1 g wet resin under pH 2 in the shaker at 30°C respectively, then measure adsorption quantities of alkaloids and did the plot curves of Langmuir equations and Freundlich equations for matrine and oxymatrine. The fitness ranges of matrine and oxymatrine to Langmuir equations and Freundlich equations were evaluated.

The kinetic study was carried out on 1.0 g wet 001×732 resin with 50 ml composition solution under pH 2. The respective concentration of matrine and oxymatrine were monitored by HPLC at certain intervals until adsorption equilibrium.

### Static adsorption test on macroporous resins

As presented in Table 1, the static adsorption of the composition solution under pH 10 was carried out on macroporous resins. Place 0.5 g wet macroporous resin with 25 ml composition solutions into the glass vials with a lid. The vials were sealed tightly and shaken in a shaker at 30°C for 12 h with the shaking index at 100 rpm. The resins were selected by their adsorption capacities, which were analyzed by HPLC after final adsorption. In

**Table 1.** Physical and chemical properties of the test resins

| Trade name | Structure | Specific surface area [$m^2$/g] | Granular diameter [mm] | Average pore diameter [nm] | Polarity |
|---|---|---|---|---|---|
| BS-65 | Polystyrene | 580–600 | 0.25–0.83 | 7 | Nonpolar |
| D101 | Styrene diving-benzene | 480–520 | 0.25–0.83 | 9–10 | Nonpolar |
| BS-30 | Styrene acrylate | 450–460 | 0.25–0.83 | 8 | Weak polar |
| HPD-400 | Diviny-benzene | 500–550 | 0.30–1.25 | 7.5–8 | Semipolarity |
| CAD-45 | Diviny-benzene | 450–500 | 0.25–0.83 | 5–6 | Semipolarity |

this study, BS-65 resin was selected as the absorbent for further research due to its good performance.

Similarly, the adsorption isotherms of matrine and oxymatrine on BS-65 resin were conducted by contacting 10 ml composition solution under pH 10 at the different concentrations with 0.1 g wet resin in the shaker bath at 30°C. The fitness ranges of matrine and oxymatrine to Langmuir equations and Freundlich equations were evaluated.

In addition, keep 0.5 g wet BS-65 resin in 25 ml composition solution under pH 10 for kinetic study. The respective concentration of matrine and oxymatrine were monitored at certain intervals by HPLC until adsorption equilibrium.

**Procedure for dynamic adsorption and desorption tests**

The dynamic adsorption and desorption tests for matrine and oxymatrine were conducted in two glass columns (60 cm × 2.0 cm i.d.), with 10.0 g (wet weight) 001 × 732 resin and 45.0 g (wet weight) BS-65 resin, respectively. The bed volumes (BV) of 001 × 732 resin and BS-65 resin were respectively about 19 ml and 85 ml. The sample solutions flowed through 001 × 732 resin first at the flow rate of 2 ml/min, then flushed by water and 1 mol/L NaCl solution at 2 ml/min flow rate after adsorption equilibrium. Subsequently, the eluents which was adjusted to pH 10 by 1 mol/L NaOH solution, flowed through BS-65 resin at 2 ml/min flow rate, then flushed with water after adsorption equilibrium. Finally, use diethyl ether to flush matrine and 50% ethanol solution to flush oxymatrine respectively with 2 ml/min flow rate. For breakthrough volume and desorption experiment, the matrine and oxymatrine in the eluents were also analyzed by HPLC.

## RESULTS AND DISCUSSION

**HPLC analysis**

HPLC conditions such as mobile phases, flow rate and column temperatures were achieved as follows: the mobile phase was acetonitrile/0.1% acetic acid solution (4:96, v/v) with pH 5.6 (controlled by trimethylamine). Flow rate, column temperature and UV detection wavelength were obtained at 1.0 ml/min, 30°C and 220 nm, respectively. The chromatograms of the mixed standards and the sample solutions from *Sophora flavescens* extracts are shown in Figs. 1B, 1C. The regression equation and linear ranges of matrine and oxymatrine were performed in the above HPLC method. The correlation coefficient ($R^2$>0.9992) values were found to be good linearity between concentrations of investigated alkaloids and their peak areas within the tested ranges (Table 2). Based on the method established, the contents of matrine and oxymatrine in the dried crude extract were found to be 0.73% and 2.2%, respectively.

**Adsorption capability of macroporous resins**

Adsorption capability is one of the most important parameters for a suitable resin selecting, which is quantified according to the following equations.

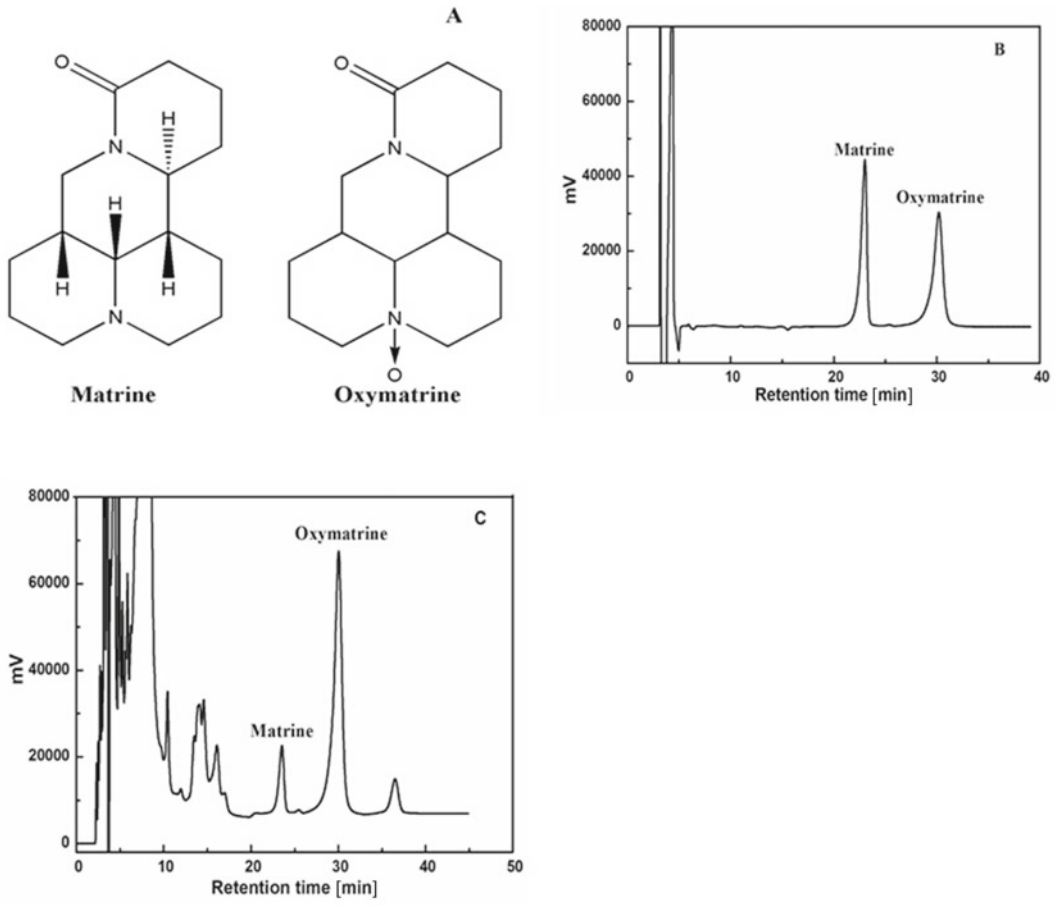


**Figure 1.** Structure of matrine and oxymatrine (A), HPLC chromatograms of mixed standards (B) and sample extracts from *Sophora flavescens* (C)

**Table 2.** Calibration curves for matrine and oxymatrine; a y: peak area; x: concentration [$\mu$g/ml]

| Analytes | Retention time [min] | Calibration curve[a] | $R^2$ | Test range [μg/ml] |
|---|---|---|---|---|
| Matrine | 23.4 | Y = 9825.1x – 64922 | 0.9993 | 53–848 |
| Oxymatrine | 30.1 | Y = 10773x – 73972 | 0.9992 | 53.5–856 |

[a]y: peak area; x: concentration [μg/ml].

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

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

Here, $Q_e$ (mg/g wet resin) is the adsorption capacity at adsorption equilibrium; $E(\%)$ is the adsorption ratio; $C_0$ and $C_e$ (mg/ml) are the initial and equilibrium concentration of analyte; $V_i$ (ml) and $W$ (g) are the volume of the initial feed and the weight of wet resin, respectively[13].

As shown in Table 3, the adsorption capacities and adsorption ratios of the five tested macroporous resins for matrine and oxymatrine were ranked as: BS-65 > HPD-400 > D101, CAD-5 > BS-30, which reflect that different resins shows different adsorption performances for matrine and oxymatrine. From this result, BS-65 showed the best adsorption efficiency than the other resins. Therefore, BS-65 microporous resin was selected as further to use.

**Effect of pH value on adsorption**

Initial pH values of the sample solution is an important influence factor for the adsorption capacities on cation exchange resin. pH values will change the exist molecular forms of the alkaloids, and affect the interaction with the adsorptive sites on cation exchange resin. As shown in Figure 2A, the adsorption capacities of matrine and oxymatrine on the cation exchange resin decreased with the pH value increase in the composition solution. pH decrease or $H^+$ concentration increase will makes matrine and oxymatrine exist mainly in cationic forms in solution, which will increase the exchange efficiency between $H^+$ and cationic alkaloids, and simultaneously increased the adsorption capacity. However, in this experiment, we found that the adsorption capacity would not increase any more when pH ≤ 2. Thus, here we selected pH 2 as the optimal conditons for further research.

In the same way, pH values also influence the adsorption capacity of BS-65 microporous resin for matrine and oxymatrine. As shown in Figure 2B, the adsorption capacities for matrine and oxymatrine on BS-65 resin increased with the pH increase in sample solution, and there was no obvious change when pH ≥ 10. Higher pH values will promte matrine and oxymatrine surrounded with the surface of BS-65 resin in non-ionic forms, which could improve the affinity interactions of these two alkaloids and the surface functional groups of BS-65 resin. Therefore, adjusting the solution to pH 10 is helpful to adsorb and recover alkaloids for further investigation.

**Adsorption isotherms**

Generally, the Langmuir equation can be used to describe a monolayer adsorption, whereas the Freundlich equation can be used to describe a monolayer adsorption as well as a multilayer adsorption. These two models are very popular, and frequently used to evaluate the experimental data of adsorption isotherms since its simplicity and accuracy[23].

Langmuir equations[24]:

$$Q_e/Q_m = K_L C_e/(1 + K_L C_e) \quad \text{(nonlinear form)} \quad (3)$$

$$1/Q_e = 1/(K_L Q_m C_e) + 1/Q_m \quad \text{(linear form)} \quad (4)$$

$K_L$ (ml/mg) is the adsorption equilibrium constant, $Q_m$ (mg/g-resin) is the theoretical maximum adsorption capacity, $Q_e$ (mg/g) is the equilibrium adsorption capacity and $C_e$ is the equilibrium concentration in liquid phase.

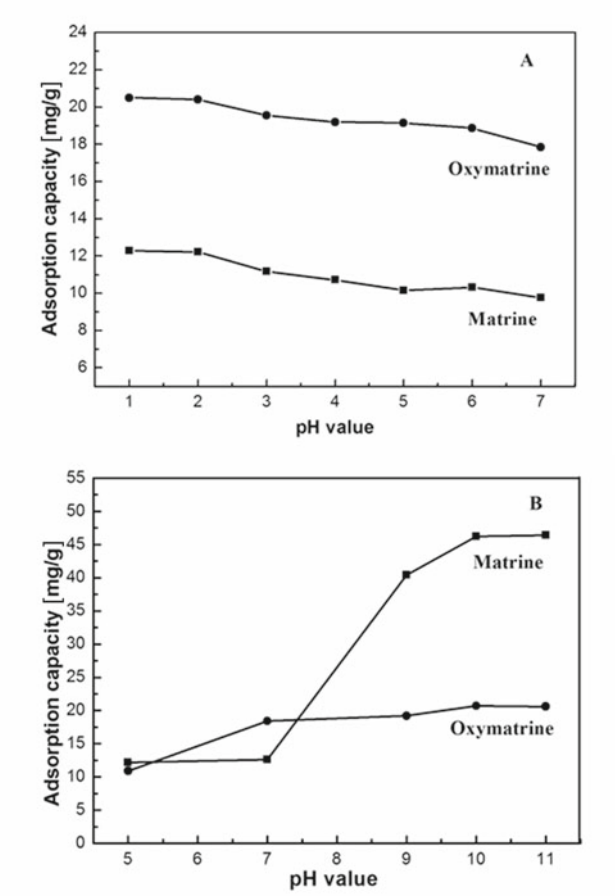


**Figure 2.** Effect of pH on the adsorption capacity of matrine and oxymatrine on 001×732 resin (A) and BS-65 resin (B)

**Table 3.** Adsorption capacities and adsorption ratios of matrine and oxymatrine on the different macroporous resins

| Name | Adsorption capacity [mg/g wet resin] | | Adsorption ratio [%] | |
|---|---|---|---|---|
| | matrine | oxymatrine | matrine | oxymatrine |
| BS-65 | 45.4 | 18.4 | 86.6 | 36.7 |
| D101 | 38.5 | 12.6 | 73.6 | 25.7 |
| BS-30 | 33.5 | 11.7 | 65.3 | 23.4 |
| HPD-400 | 42.4 | 14.5 | 80.6 | 29.1 |
| CAD-45 | 38.3 | 13.2 | 70.0 | 26.9 |

Freundlich equations[25]:

$$Q_e = K_F C_e^{1/n} \quad \text{(nonlinear form) (5)}$$

$$\ln Q_e = \frac{1}{n} \ln C_e + \ln K_F \quad \text{(linear form) (6)}$$

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

The equilibrium adsorption isotherms of matrine and oxymatrine on 001×732 and BS-65 resins constructed at 30°C, and the initial concentrations ($C_0$) of matrine and oxymatrine in the composition solution were 0.31, 0.62, 1.23, 1.87, 2.48 mg/ml and 0.92, 1.83, 3.67, 5.51,7.34 mg/ml, respectively. As shown in Figures 3A and 3B, the adsorption capacity of 001×732 and BS-65 resins increased with the initial concentration increase, the parameters and correlation factor ($R^2$) in two models were listed in Table 4.

The theoretical maximum adsorption capacity $Q_m$ on two resins determined from the Langmuir equation was ≥ 101.0 (mg/g resin) for matrine and oxymatrine. $K_L$ is a stability indicator for the combination of adsorbate and adsorbent, and the constants $K_L$ showed between 0.1535 and 1.9414 ml/mg by Langmuir equation. In Freundlich equation, the adsorption takes place easily when the 1/n value is between 0.1 and 1[18, 26]. As shown in Table 4, the 1/n values were between 0.38 and 0.87, which indicated that the tested resins are favorable for the separation of matrine and oxymatrine.

### Adsorption kinetics of matrine and oxymatrine on 001×732 and BS-65 resins

Adsorption kinetics of matrine and oxymatrine on 001×732 and BS-65 resins were investigated at 30°C in a thermostatic shaker. The adsorption kinetic curves were obtained and showed in Figure 4. The adsorption capacities of 001×732 resin for matrine and oxymatrine become increases with the adsorption time increased until reaching equilibrium at about 60 min (Fig. 4A), while the adsorption capacities on BS-65 resin increased rapidly in the initial 30 min (Fig. 4B), which indicated

**Table 4.** Langmuir and Freundlich adsorption isotherm parameters of matrine and oxymatrine on 001×732 and BS-65 resins at 30°C; [a] x: $1/C_e$, y: $1/Q_e$; [b] x: $1/Q_e$, y: ln $C_e$

| Name | Content | Langmuir equation | | | | Freundlich equation | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | linear equation[a] | $K_L$[ml/mg] | $Q_m$[mg/g] | $R^2$ | linear equation[b] | $K_F$[mg/g] | 1/n | $R^2$ |
| 001×732 | matrine | y = 0.0066x + 0.0031 | 0.4697 | 322.6 | 0.9843 | y = 0.8385x + 4.8021 | 121.8 | 0.8385 | 0.9815 |
| | oxymatrine | y = 0.0058x + 0.0080 | 1.3793 | 125.0 | 0.9968 | y = 0.7820x + 4.9705 | 144.1 | 0.7820 | 0.9923 |
| BS-65 | matrine | y = 0.0051x + 0.0099 | 1.9414 | 101.0 | 0.9866 | y = 0.3811x + 4.5841 | 97.92 | 0.3811 | 0.9826 |
| | oxymatrine | y = 0.0241x + 0.0037 | 0.1535 | 270.3 | 0.9954 | y = 0.8681x + 3.5214 | 33.83 | 0.8681 | 0.9940 |

[a] x: 1/*eC*, y: 1/*eQ*; [b] x: 1/*eQ*, y: ln*eC*

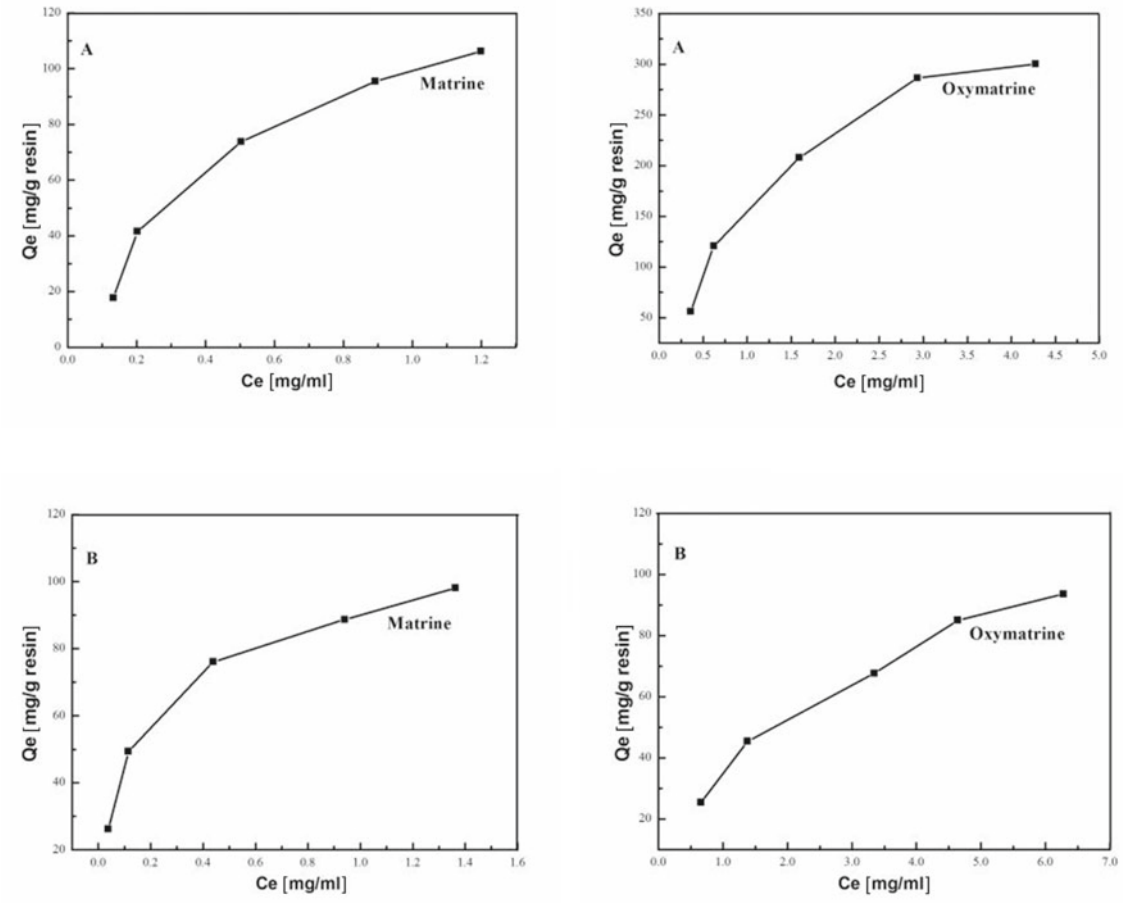


**Figure 3.** Adsorption isotherm at the temperature of 30°C for matrine and oxymatrine on 001×732 resin (A) and BS-65 resin (B)

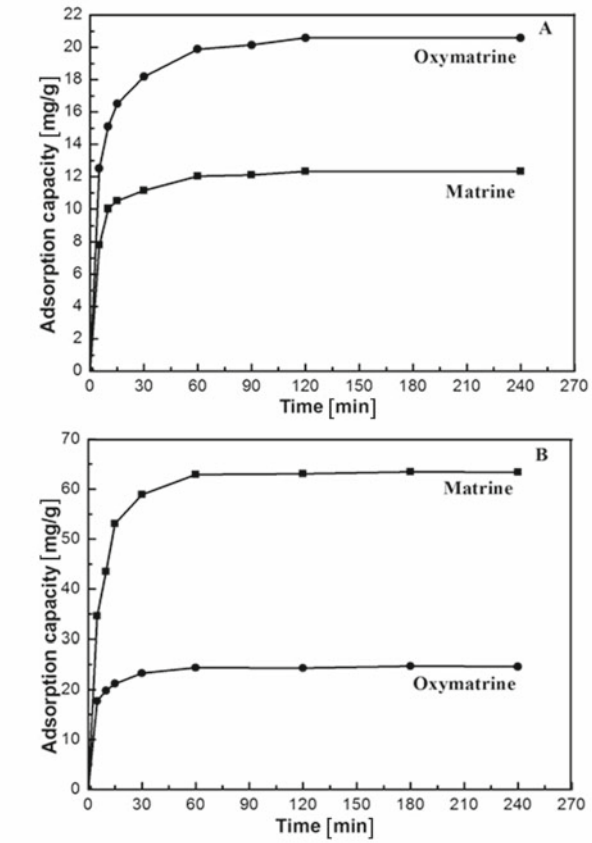


**Figure 4.** Adsorption kinetic curves of matrine and oxymatrine on 001×732 resin (A) and BS-65 resin (B)

that the selected resins possessed a good adsorptive rate for matrine and oxymatrine. Therefore, the duration of adsorption was set respectively to more than 60 min and 30 min for 001×732 resin and BS-65 resin in the following research.

**Static desorption of matrine and oxymatrine on 001×732 resin**

The desorption ratio of matrine and oxymatrine is quantified in the following equation.

$$D = \frac{C_d V_d}{(C_0 - C_e)V_i} \times 100\% \quad (7)$$

Where $C_d$ is the concentration of analyte in the desorption solution (mg/ml); $V_d$ is the volume of the desorption solution (ml); $D$ is the desorption ratio (%); $C_0$, $C_e$ and $V_i$ are the same as described above.

As shown in Figure 5A, the desorption ratio of the selected eluents NaCl, HCl and NaOH solutions on 001×732 resin for matrine and oxymatrine, ranked as: NaCl > HCl > NaOH, so NaCl solution was chosen as the eluent. Furthermore, the result of the appropriate concentration test showed that the desorption ratio of matrine and oxymatrine increased with the NaCl concentration increase (Fig. 5B), and there was no obvious change when the NaCl concentration over 1 mol/L. So, 1 mol/L NaCl solution was efficient to elute matrine and oxymatrine in the later experiments.

**Dynamic breakthrough curve and desorption curve on 001×732 resin**

– Dynamic breakthrough curve

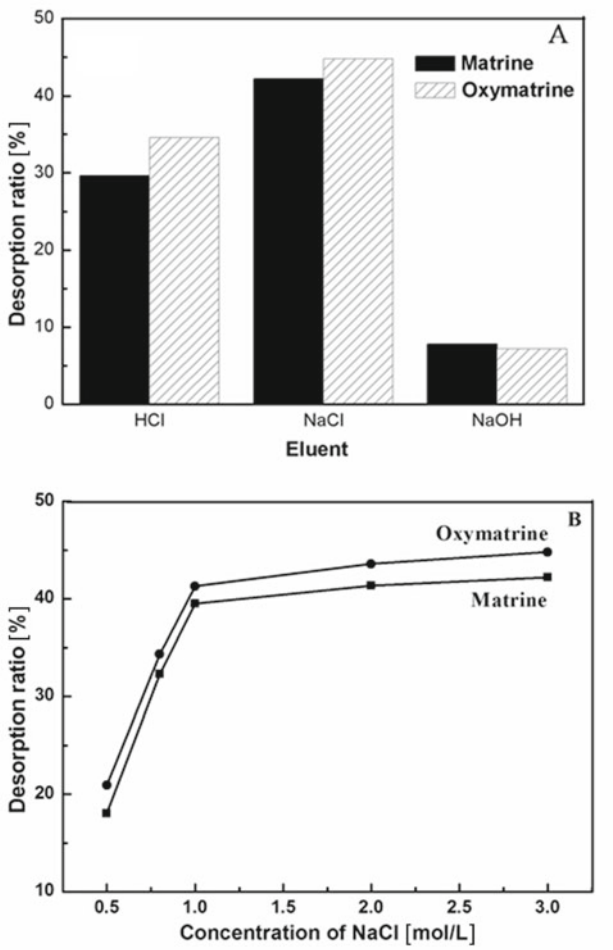


**Figure 5.** Static desorption ratio of matrine and oxymatrine on 001×732 resin at 30°C with three eluents (A); the effect of NaCl concentration on desorption ratio of matrine and oxymatrine (B)

Breakthrough volume is important in solid phase extraction because it represents the sample volume that can be pre-concentrated without loss of analytes during the loading of sample. In this test, the initial concentration of matrine and oxymatrine was respectively 0.31 mg/ml and 0.92 mg/ml at the flow rate of 2 ml/min. As shown in Figure 6, the breakthrough volume of matrine and oxymatrine on 001×732 resin was approximately 100 ml (5BV).

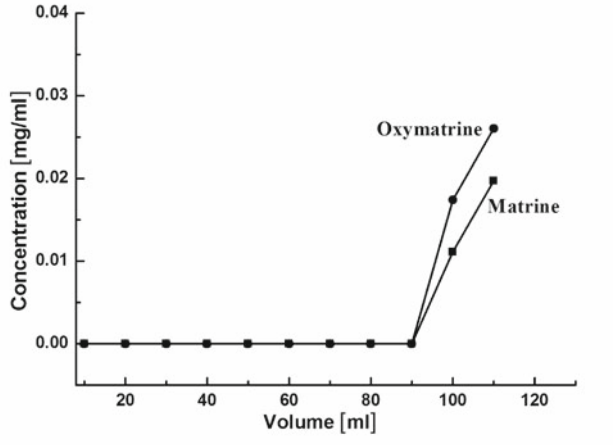


**Figure 6.** Dynamic leakage curves of matrine and oxymatrine from *sophora flavescens* extract on 001×732 resin

– Dynamic desorption curve

Based on the breakthrough volume determined above, 100 ml sample solution was fed on the column, which packed with 10.0 g (wet weight) 001×732 resin. After adsorption equilibrium, the resin was flushed with 100 ml deionized water for removing the non-cationic components. Subsequently, elute matrine and oxymatrine with 1 mol/L NaCl solution at the flow rate of 2 ml/min. As shown in Figure 7A, oxymatrine was totally desorbed in 460 ml (24BV), while matrine was totally desorbed in 280 ml (15BV).

**Separation of matrine and oxymatrine on BS-65 resin**

The eluent from 001×732 resin was adjusted to pH 10 with 1 mol/L NaOH solution and then fed onto the column packed with 15.0, 30.0 and 45.0 g (wet weight) BS-65 resin respectively. The resins were flushed with 100 ml deionized water when adsorption equilibrium. Subsequently, use diethyl ether and 50% ethanol to flush matrine and oxymatrine, respectively. As results, a part of oxymatrine could be found in diethyl ether eluent with 15.0 g and 30.0 g BS-65 resin, while 45.0 g BS-65 adsorbent could completely separated the matrine and oxymatrine. Moreover, the eluent volumes were investigated as Figure 7B. Matrine could be desorbed completely using approximate 200 ml (2.5 BV) diethyl ether, and oxymatrine was easily desorbed by about 120 ml (1.5 BV) 50% ethanol solution.

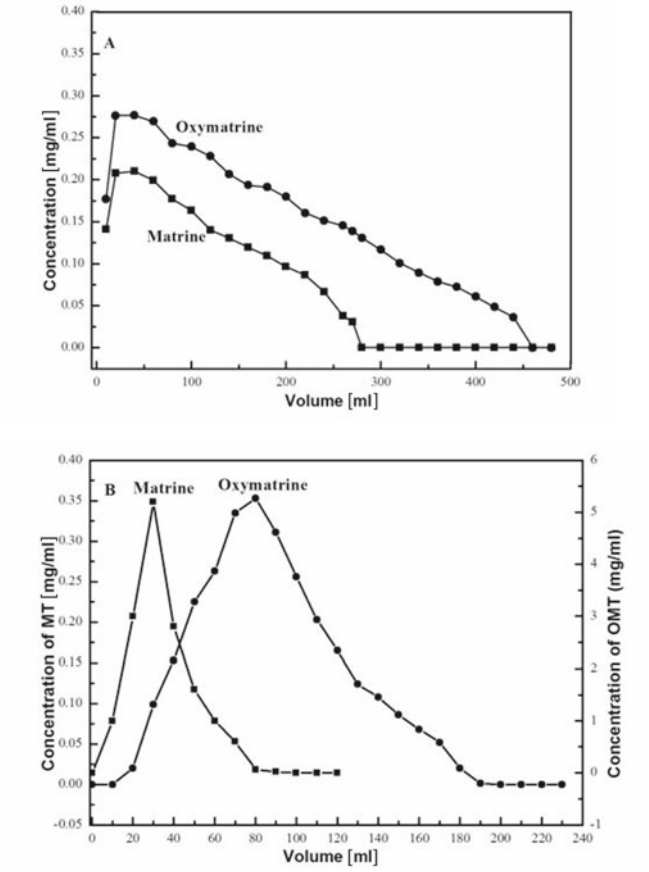


**Figure 7.** Dynamic desorption curves of matrine and oxymatrine from *Sophora flavescens* extract on 001×732 resin (A); The dynamic desorption curves of matrine and oxymatrine in sample solution from *Sophora flavescens* on BS-65 resin (B)

As shown in Figure 8, the results of separate efficiency of matrine and oxymatrine were revealed by chromatograms using 001×732 resin and 001×732 resin combined with BS-65 resin. The contains of matrine and oxymatrine in feeding solutions were about 31.0 mg and 92.0 mg respectively. 28.0 mg matrine and 79.9 mg oxymatrine were obtained from 001×732 resin coupled with BS-65 resin separation, and the recovery of matrine and oxymatrine was reached to 90.3% and 86.9%, respectively. Subsequently, the eluent product was collected through evaporating and drying, the content of matrine and oxymatrine have reached to 67.2% and 66.8% respectively in the drying products through HPLC analysis. In contrast, the content of matrine and oxymatrine was only 0.73% and 2.2% respectively in the drying crude extract before separation. While the same test on BS-65 resin lonely showed that the content of matrine and oxymatrine were only 7.7% and 12.4% respectively in the drying eluent products.

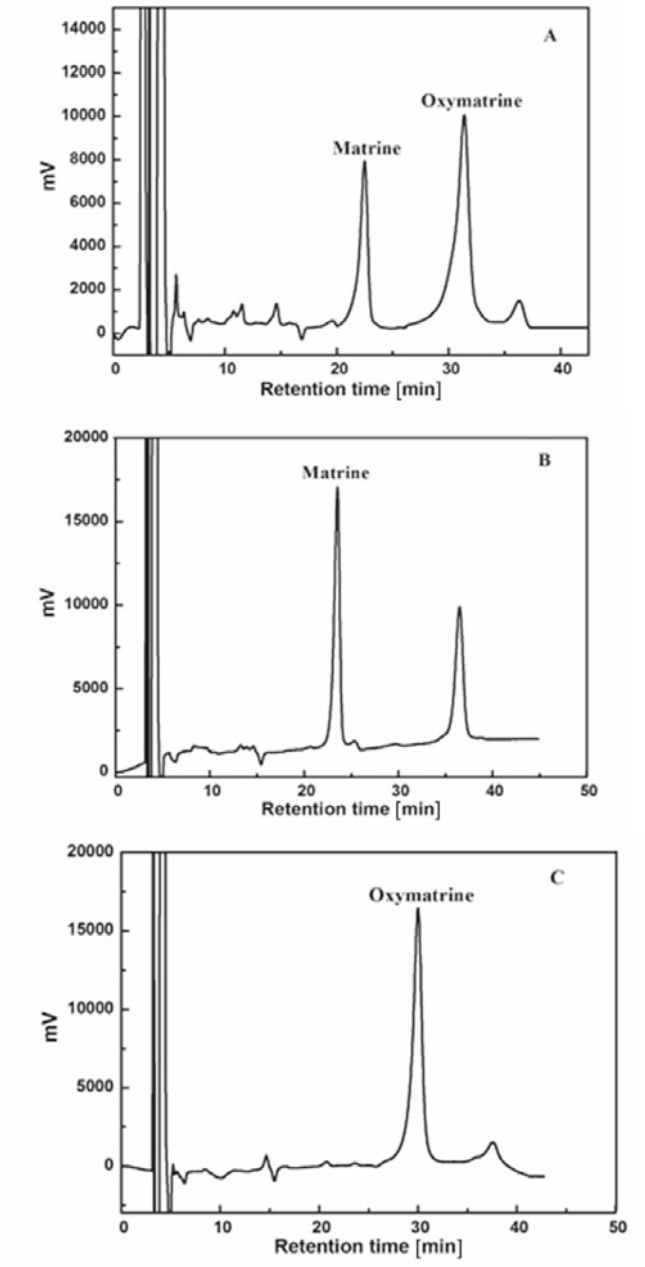


**Figure 8.** HPLC chromatograms of the products desorbed on 001×732 resin with 1 mol/L NaCl solution (A), desorbed on BS-65 resin with diethyl ether (B) and 50% ethanol solution (C)

## CONCLUSIONS

The separation of matrine and oxymatrine extracted from the root of *Sophora flavescens* was successfully developed on 001×732 cation exchange resin coupled with BS-65 macroporous resin. As cation exchange resin could remove the non-ionic impurities, and macroporous resin could retain the desired alkaloids in molecular forms, so combined these two type of resins is the best way for the separation of matrine and oxymatrine. In the five tested macroporous resins, BS-65 resin offered the best separation performances for matrine and oxymatrine because of its high surface area and appropriate surface functional polarity. The equilibrium adsorption experiment at 30°C on 001×732 resin and BS-65 resin was fitted to Langmuir isotherm and Freundlich isotherm. The optimized experimental conditions in this study showed that matrine and oxymatrine from extracts of the root of *Sophora flavescens* could be better separated under pH 2 on 001×732 resin and under pH 10 on BS-65 resin with 1 mol/L NaCl eluent solution. Based on the difference of elution ability, the matrine and oxymatrine adsorbed on BS-65 resin were successfully eluted with diethyl ether and 50% ethanol solution, respectively. Consequently, purities of matrine and oxymatrine were significantly improved from 0.73% and 2.2% in the crude extract to 67.2% and 66.8% in the final eluent product, respectively. The separation method of matrine and oxymatrine from *Sophora flavescens* could also be successfully applied to the same genus herbs, such as *Sophora tonkinensis* Gagnep. and *Sophora alopecuroides* L., and which will be useful for many applications of medical and pharmaceutical areas for the relative primary products supply.

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