Optimization of Tertiary Alkaloids Separation from *Corydalis yanhusuo* by Macroporous Resins

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Corydalis yanhusuo is used widely for the treatment of gastralgia, costalgia and dysmenorrhea in Chinese medicine. The alkaloid is the main active ingredient of *C. yanhusuo*. Response surface methodology was applied to optimize the separation and purification process for alkaloids by AB-8 resin-packed chromatogram column. The optimal conditions were found to be as follows: height-diameter ratio of AB-8 resin-packed chromatogram column, 10.50; concentration and pH of feed sample solution, 1.12 mg mL^{-1} and 7.16, respectively. The gradient elution program was 30 % ethanol for 2 BV (bed volume) followed by 80 % of ethanol for 5 BV at flow rate of 3 mL min^{-1}. After the AB-8 resin treatment, the contents of alkaloids and tetrahydropalmatine were increased respectively from 25.20 % and 2.12 % to 58.25 % and 6.58 %, the recovery of alkaloids and tetrahydropalmatine were 85.40 % and 65.21 %, respectively. The results indicated that the optimization of alkaloid separation from *C. yanhusuo* by macroporous resins is feasible and efficient.

Key words:
Macroporous resins, tertiary alkaloid, response surface methodology, *Corydalis yanhusuo*

Introduction

Corydalis yanhusuo W.T. Wang is a widely used traditional Chinese herbal medicine to promote blood circulation, reinforce vital energy, and alleviate pain such as headache and chest pain.[1–3] The dried and pulverized tubers of *C. yanhusuo* are often known as Rhizoma *C. yanhusuo*, whose major active components are tertiary alkaloids with very similar structures. One of these alkaloids is dl-tetrahydropalmatine, which possesses analgesic, sedative, hypnotic and antihypertensive effects.[4] The chemical composition of *C. yanhusuo* is complex, comprised of large numbers of starch, β-glucoside, small quantity of grume, naphtha besides alkaloid.[5]

A few methods have been developed to separate and purify the alkaloids from *C. yanhusuo,* such as microwave-assisted extraction,[6] supercritical fluid extraction,[7] HPLC,[8] and high-speed counter-current chromatography.[9,10] However, these methods are only suitable for initial separation or purification of small quantities for analytical purposes. Moreover, the aforementioned separation processes require multiple fractionation steps, expensive chromatographic matrices and frequent use of poisonous solvents resulting in lower recovery of the products, high costs of operation, and safety problems. Macroporous resins, as durable hydrophilic polymers, possess some attractive merits including high adsorption capacity to adsorbed molecules, relative low cost, and easy regeneration.[11] Macroporous resins have been successfully applied to industrial refining and purification of bioactive substances such as alkaloids, polyphenols, flavonoids and other components extracted from plant materials.[12–15] Although alkaloid with good physiological activity was previously reported to be separated from *C. yanhusuo* by macroporous resins,[16] the technical conditions and parameters were uncertain in that report.

Classical optimization studies use one-factor-at-a-time approach, in which only one factor is variable at a time while all others are kept constant. This approach is time consuming and expensive. In addition, possible interaction effects between variables cannot be evaluated, which may lead to drawing misleading conclusions. The response surface methodology (RSM) can overcome these limitations, since it allows accounting for possible interaction effects between variables.[17] Nowadays, RSM has become an effective method in helping to solve many practical problems in the course of production, such as reducing manufacturing costs, optimizing process operating conditions, and improving product quality. In practice, RSM has been used successfully to model and optimize biochemical processes in agriculture, biology, and chemistry.[18–20] Previously, researchers have adopted this method in analyzing the extraction process of phenolic compounds from *Inga* edulis,[21] oleanolic acid from

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Lantana camara roots,[22] and polysaccharides from boat-fruited sterculia seeds.[23] In this paper, the separation and purification process of alkaloid using macroporous resins by RSM was studied for the first time.

Materials and methods

C. yanhusuo was collected from Wenzhou County (Zhejiang, China). The herb was authenticated by the Huqingyutang Hospital of China.

Reference substance of tetrahydropalmatine was obtained from the National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, China). Macroporous resins AB-8, D101, D201, and NKA-2 were purchased from Chemical Factory of Nankai University. Methanol (chromatogram grade), ethanol (analytical grade), petroleum ether (30–60 °C, analytical grade), chloroform (analytical grade) and EtOAc (analytical grade) were obtained from local chemical suppliers.

The crude extract (180.4 g, content of alkaloid: 17.38 %) by ethanol was dissolved in 1 L water, with pH adjusted to 1~4 by 1 % HCl, and then defatted by petroleum ether. Following that, ammonia water was applied to adjust pH to 9, and the extract was with EtOAc. After recycling the solution, the alkaloid content of *C. yanhusuo* sample extracts (121.5 g) was 25.20 %, and the tetrahydropalmatine content was 2.12 %.

Detection of alkaloid contents

Acid-Dye Complexation Method was used to detect alkaloid contents.[24] From the 14.4 mg L^{-1} *C. yanhusuo* extract solution, 1, 2, 3, 4 and 5 mL was transferred to a series of separating funnels and 2 mL of pH-4 buffer was added into each funnel. Following that, 1 mL of 0.12 % w/v bromocresol green was mixed with the solution in each funnel and shaken well, followed by adding 10 mL of chloroform and shaking thoroughly. After keeping for a few minutes, the chloroform layer was separated and treated with anhydrous sodium sulphate, and the absorbance of the solution at 414 nm was measured against reagent blank. The final concentrations of analyzed solutions were determined in comparison with the standard curve prepared with tetrahydropalmatine, which ranged from 1.44 mg mL^{-1} to 11.52 mg mL^{-1}. The total alkaloid contents were expressed as mg tetrahydropalmatine per mL.

Content and recovery of alkaloids

On the basis of the alkaloids content of prepared samples measured by the standard curve, the content of alkaloids was calculated by eq. (1).

$$C_{A2}(\%) = \frac{c_{A2} \cdot V_2}{m_2} \cdot 100 \quad (1)$$

Recovery of alkaloids was determined by eq. (2),

$$R_A(\%) = \frac{C_{A2} \cdot m_2}{C_{A1} \cdot m_1} \cdot 100 \quad (2)$$

where R_A (%) represented the recovery of alkaloids, m_1 (mg) was the quantity of the sample extracts, C_{A1} (%) was the alkaloids content of the sample extracts, m_2 (mg) was the quantity of prepared sample by macroporous resins, C_{A2} (%) was the alkaloids content of the prepared sample, V_2 was the total volume of prepared sample (mL), and c_{A2} was the alkaloid concentration of the prepared sample (mg mL^{-1}).

Content of tetrahydropalmatine

Quantification of content of tetrahydropalmatine was performed by Agilent 1200. All separations were carried out on a ZORBAX Extend-C18 column (250 mm × 4.6 mm, 5 μm) from Agilent Technologies. The mobile phase consisted of methanol (solvent A) and 0.1 % phosphoric acid water solution with pH adjusted to 6.09 by triethylamine (solvent B) (55 : 45, v/v). The detection wavelength of tetrahydropalmatine was 280 nm. The flow rate of mobile phase was set at 1 mL min^{-1}, the injection volume was 10 μL, the column temperature was maintained at 25 °C, and the retention time of tetrahydropalmatine was 35.8 min. The working calibration curve based on tetrahydropalmatine standard solutions showed good linearity over the range of 0.1–2.3 mg mL^{-1}. Content of tetrahydropalmatine was calculated by the eq. (3)

$$P_T(\%) = \frac{c_T \cdot V_T}{m_T} \cdot 100 \quad (3)$$

where c_T was the tetrahydropalmatine concentration of the prepared sample (mg mL^{-1}), V_T was the volume of prepared sample (mL), and m_T was the quantity of prepared sample (mg).

Static adsorption and desorption tests for screening of resins

The static adsorption and desorption experiments were performed as follows: 10 mL pretreated resin (equal to 7 g dry resin) was introduced into a 100 mL conical flask. Then, 20 mL of sample extract with known alkaloids concentration was added to each flask. The flasks were maintained in the shaking incubator set at 180 rpm and 20 °C until reaching adsorption equilibrium. Desorption experiments were carried out as follows: the adsorbate-laden resins were first washed in deionized

Table 1 – *Content of alkaloids using different levels of purification variables for Plackett-Burman design*

	A	B	C	D	E	F	G	H	Content of alkaloids/%
1	+1(7.5)	–1(1.5)	–1(8)	–1(0.8)	+1(3.0)	–1	+1	+1	36.7
2	+1	+1(3.0)	–1(10)	+1(1.0)	–1(1.5)	–1	–1	+1	40.5
3	–1(6.5)	+1	+1	–1	–1	–1	+1	–1	44.7
4	+1	–1	–1	+1	–1	+1	+1	–1	39.8
5	+1	+1	+1	+1	+1	–1	–1	–1	46.9
6	+1	+1	+1	–1	–1	+1	+1	+1	35.2
7	–1	+1	–1	+1	+1	+1	+1	–1	53.5
8	–1	–1	+1	+1	+1	–1	+1	+1	55.0
9	–1	–1	+1	+1	–1	+1	–1	+1	51.1
10	+1	–1	+1	–1	+1	+1	–1	–1	44.3
11	–1	+1	–1	–1	+1	+1	–1	+1	47.8
12	–1	–1	–1	–1	–1	–1	–1	–1	49.5

A: pH of sample solution; B: Flow rate of sample solution (mL min^{-1}); C Height-diameter ratio; D: Concentration of sample solution (mg mL^{-1}); E: Flow rate of gradient elution (mL min^{-1})

water and then desorbed in 50 mL 90 % ethanol. Finally, the flasks were shake incubated at 180 rpm and 20 °C until desorption equilibrium.

Dynamic adsorption and desorption tests

Dynamic adsorption experiments were carried out in a glass column (12 mm × 500 mm) wet-packed with AB-8 adsorbents (equal to 35 g dry resin). The BV was 50 mL and the packing length of the resin bed was 12 cm. The sample solution was loaded into the column; the concentration of alkaloid in feed solution was 1.0 mg mL^{-1} and the leak flow rate was 3 mL min^{-1}. The adsorbate-laden column was washed with 2 BV of 30 % ethanol first, and then desorbed with 80 % ethanol at the flow rate of 3 mL min^{-1}. The elution of initial approximate 5 BV of 80 % ethanol was collected for alkaloid product.

Optimization of chromatogram column by RSM

RSM was applied in two stages. In the first stage, it was used to identify the significant parameters for the purification process of alkaloid using Plackett-Burman design criterion. In the second stage, the significant parameters resulting from Plackett-Burman design were optimized using a central composite design. The experimental design and statistical analysis of the data were done with Design Expert software package.

Plackett-Burman design

The Plackett-Burman factorial designs allow for the screening of main factors from a large number of process variables, and these designs are thus quite useful in preliminary studies in which the principal objective is to select variables that can be fixed or eliminated in further optimization processes.[25] In practice, the Plackett-Burman factorial designs can identify main factors for the desired response variables in a few runs. Five variables were analyzed with regard to their effects on the purification process of alkaloid using a Plackett-Burman design, and the design matrix selected for the screening of significant variables for content of alkaloid and its corresponding responses are shown in Table 1. The adequacy of the model was calculated, and the variables evidencing statistically significant effects were screened via *F*-test for ANOVA (Table 2). Factors evidencing probability (*P*) of less than 0.05 were considered to have significant effects on alkaloid purification process, and were therefore selected for further optimization studies.

Table 2 – *Results of regression analysis for purification variables of the Plackett-Burman design*[*]

Source	DF	*F*-value	Prob > *F*
A	1	39.04	0.0008[b]
B	1	0.70	0.4344[a]
C	1	6.31	0.0458[b]
D	1	9.43	0.0219[b]
E	1	1.02	0.3518[a]
Model	5	11.30	0.0052[b]

[*]Coefficient of determination (R^2) = 90.40 %
[a]Non-significant at $P < 0.05$;
[b]Significant positive effect.

Central composite design

RSM is the product of the combination of mathematics and statistics, which researches and optimizes the relationship between factors and response value. According to the results of Plackett-Burman design, three factors, pH of sample solution, concentration of sample solution, and height-diameter ratio, were selected for optimization. The responses functions (Y) measured were content and recovery of alkaloid, and contents of tetrahydropalmatine, respectively. The effects of pH of sample solution (X_1), concentration of sample solution (X_2), and height-diameter ratio (X_3) on the purification process of alkaloid are reported in Table 3, and expressed in the form of second-order polynomials below.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + \\ + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (4)$$

The coefficients of the polynomial were represented by b_0 (constant term), b_1, b_2, b_3 (linear effects), b_{11}, b_{22}, b_{33} (quadratic effects), b_{12}, b_{13}, and b_{23} (interaction effects). The ANOVA tables were generated, and the effect and regression coefficients of individual linear, quadratic and interaction terms were determined (Table 4). The significance of all terms in the polynomial were judged statistically by computing the F-value at a probability of 0.001, 0.01 or 0.05. The regression coefficients were then used to make statistical calculations to generate contour maps from the regression models.

Results and discussion

Adsorption/desorption capacities and desorption ratio of different resins

Four macroporous resins with different physical properties were employed for separation, and the results showed that the adsorption capacity of

Table 3 – *Central composite design criterion of purification parameters with corresponding experimental*

Serial number	Independent variables			Dependent variables		
	a	b	c	A	B	C
1	–1(6.5)	–1 (0.8)	–1 (8)	52.7	84.7	4.3
2	–1	–1	1 (12)	51.8	82.8	3.1
3	–1	1 (1.2)	–1	56.1	84.1	5.3
4	–1	1	1	54.5	82.5	5.6
5	1 (8.5)	–1	–1	43.2	85.0	2.6
6	1	–1	–1	52.7	84.6	7.1
7	1	1	–1	40.8	85.8	3.3
8	1	1	1	55.7	83.5	5.2
9	–1.68179 (5.8)	0 (1.0)	0 (10)	51.7	84.0	3.2
10	1.68179 (9.2)	0	0	47.1	83.7	4.9
11	0 (7.5)	–1.68179 (0.664)	0	52.8	83.8	5.0
12	0	1.68179 (1.336)	0	56.2	84.5	8.6
13	0	0	–1.68179 (6.64)	45.8	85.1	9.8
14	0	0	1.68179 (13.36)	50.9	81.5	7.7
15	0	0	0	58.5	85.8	8.9
16	0	0	0	58.7	85.8	6.8
17	0	0	0	57.9	86.1	6.2
18	0	0	0	58.3	84.8	7.1
19	0	0	0	58.3	85.3	6.0
20	0	0	0	58.0	85.5	6.7

a: pH of sample solution; b: Concentration of sample solution (mg mL^{-1}); c: Height –diameter ratio

A: Content of alkaloid (%); B: Recovery rate of alkaloid (%); C: Contents of tetrahydropalmatine (%)

Table 4 – *Regression analysis of central composite design data for purification of alkaloids from Corydalis yanhusuo*

Regression coefficient	Content of alkaloid/%	Recovery rate of alkaloid/%	Contents of tetrahydropalmatine/%
b_0	–129.41168	36.53015	
b_1	34.05322	7.91478	
b_2	87.42557	3.10891	
b_3	3.33947*	3.93839***	
b_{12}	–8.23154	2.34267**	
b_{13}	1.45474**	–0.13888	
b_{23}	2.60130	0.44441	
b_{11}	–2.80240**	–0.58564***	
b_{22}	–24.98191	–11.98938***	
b_{33}	–0.79341**	–0.19502***	
Lack of Fit *F*-value	0.84	0.50	1.64
R^2	0.8675	0.9466	0.5801
p or probability	0.0023**	< 0.0001***	0.2586

Subscripts: 1 = pH of sample solution, 2 = Concentration of sample solution, 3 = Height-diameter ratio.
*Significant at 0.05 level.
**Significant at 0.01 level.
***Significant at 0.001 level.

AB-8, D101, D201, and NKA-2 was 37.54 mg mL^{-1}, 37.66 mg mL^{-1}, 40.52 mg mL^{-1}, 45.32 mg mL^{-1}, and the desorption rate was 87.52 %, 80.21 %, 55.21 %, 44.21 %, respectively. In contrast the highest adsorption capacity, the desorption rate of alkaloid NKA-2 was only about half of that on AB-8 and D101. That can be explained by the difference in electrostatic interaction between the macroporous resin and alkaloid. NKA-2, as a polar resin, possesses greater affinity to alkaloid than low polar AB-8 and non-polar D101. Hence, it has a high adsorption capacity and low desorption capacity simultaneously. Considering adsorption and desorption properties, the AB-8 was chosen for further investigations.

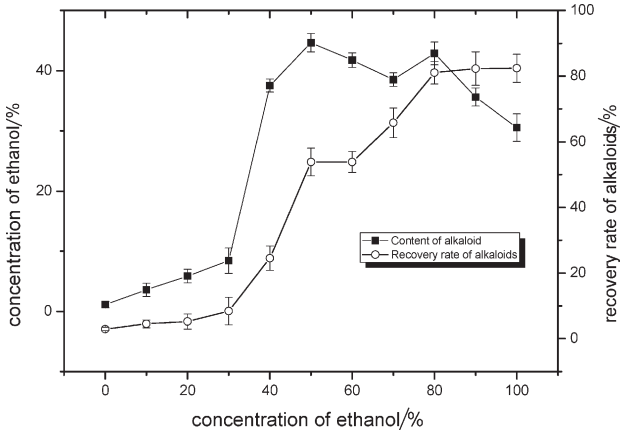


Fig. 1 – *Static desorption of alkaloids on AB-8 resin by contacting the different concentration of aqueous ethanol*

Optimization of ethanol concentration

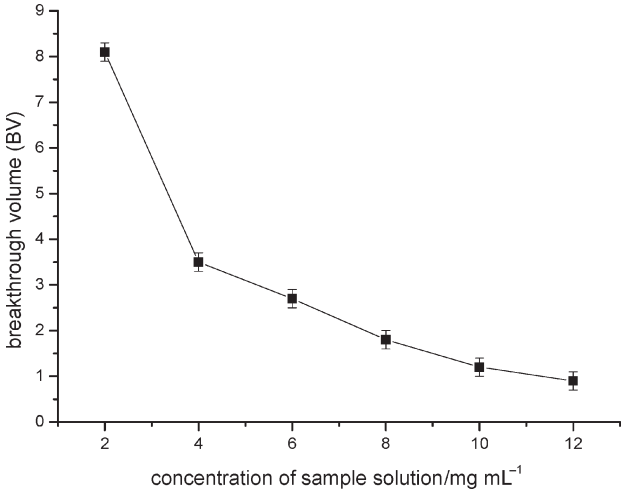
For optimizing the appropriate ethanol concentration in the separation of alkaloids, different concentrations of aqueous ethanol (2 BV), from 0 % to 100 % (v/v), were applied to perform desorption test after adsorption equilibrium. The result is shown in Fig. 1.

It was obvious that the adsorption ratios of alkaloids on AB-8 resin increased with ethanol concentration. Initially, the content of alkaloids was very low, demonstrating that the alkaloids were hardly desorbed on AB-8. The adsorption content of alkaloids increased sharply when ethanol concentration was over 30 %. The content of alkaloids acquired the peak value under the condition of ethanol concentration at ~50 %. Along with the rise of ethanol-water proportion to 60 : 40 (v/v), the content of alkaloids descended firstly, and then increased at the 70 % ethanol. The alkaloids of *C. yanhusuo* are mainly composed of quaternary amine alkaloids and tertiary amine alkaloids. The reason for the change of alkaloid content with ethanol concentration was that the quaternary amine alkaloids were very easy to wash down by 50 % ethanol while 70 % – 80 % ethanol is beneficial to the elution of tertiary amine alkaloids. Therefore, to retain alkaloids of *C. yanhusuo*, 30 % and 80 % aque-

ous ethanol was selected as the appropriate desorption solution in dynamic desorption experiments.

Breakthrough volume

The breakthrough volume is useful in determining the total sample capacity of the column for a particular solute. Breakthrough point was defined as the point at which the exit solute concentration reached 5 % of the inlet concentration. At the flow rate of 3 mL min^{-1}, the breakthrough volumes of alkaloids in different concentrations on AB-8 resin are shown in Fig. 2.



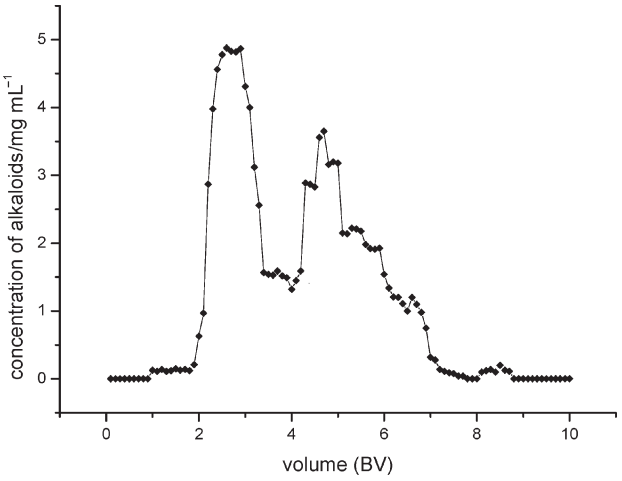
F i g . 2 – *Breakthrough volumes of different concentration alkaloids on AB-8 resin, at the flow rate of 3 mL min^{-1}*

Dynamic desorption curve on AB-8 resin

After adsorption equilibrium, the resin was flushed with 2 BV of 30 % ethanol firstly for removing the high polar components, such as polysaccharides and amino acids. Subsequently, the adsorbent was rinsed with 80 % ethanol. As the alkaloids could be desorbed completely using approximate 5 BV of 80 % aqueous ethanol, the elution solution of initial approximate 5 BV of 80 % ethanol was collected for alkaloids product. Fig. 3 shows the chromatograms of desorbed alkaloids solution.

The single-factor test of chromatogram column

Although tertiary alkaloids of molecular state are difficult to dissolve in water, the interaction between the low-polar macroporous resin and free tertiary alkaloids is stronger than that of ionized state tertiary alkaloids, different pH can change the state of tertiary alkaloids, so pHs were attempted in the adsorption procedure. In addition, adsorption and desorption procedures were also carried out at different concentration and flow rate of sample solution, flow rate of desorption solution, and height-diameter ratio. The effects of different operating parameters on the recovery and the content of alkaloids have been investigated by single factor analysis method. The results showed that the separation and the purification process of tertiary alkaloids on macroporous resins chromatogram column requires pH of sample solution 7.5, concentration of sample solution 1.0 mg mL^{-1}, height-diameter ratio 10, flow rate of sample solution 3.0 mg mL^{-1}, and flow rate of desorption solution 3.0 mg mL^{-1}. The operating parameters obtained by single factor analysis method were considered optimal in the present experiment.



F i g . 3 – *Dynamic desorption curves of alkaloids on AB-8 resin. The column packed with saturate AB-8 resin was gradually flushed with 30 % and 80 % aqueous ethanol (v/v) for respective different volumes at flow rate of 3 mL min^{-1}.*

The optimization of chromatogram column by Plackett-Burman and RSM

Plackett-Burman designs open to identify the active factors

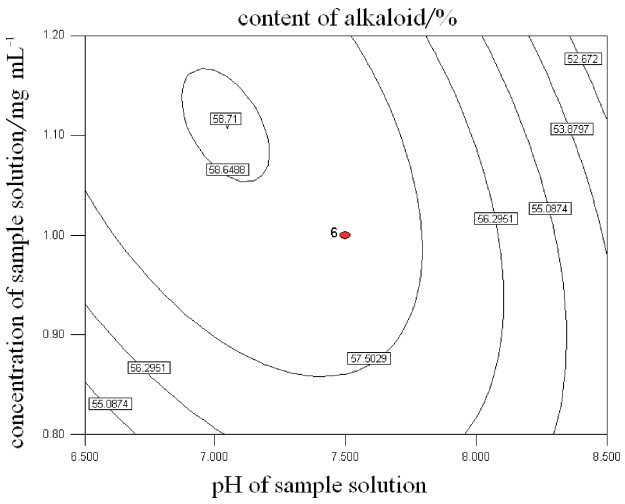
Five variables were analyzed with regard to their effects on content of alkaloids using a Plackett-Burman design (Table 1). The adequacy of the model was calculated, and the variables evidencing statistically significant effects were screened via regression analysis (Table 2). Among five extraction parameters studied, pH of sample solution, with a probability value of 0.0008, was identified to be the most significant factor, followed by concentration of sample solution (0.0219), and height-diameter ratio (0.0458). The lower probability values indicated the more significant factors on the content of alkaloids. Therefore, all other insignificant variables were neglected, and the optimum levels of the three variables, (pH of sample solution, concentration of sample solution, and height-diameter ratio) were further determined by an RSM design.

Optimization of chromatogram column by RSM

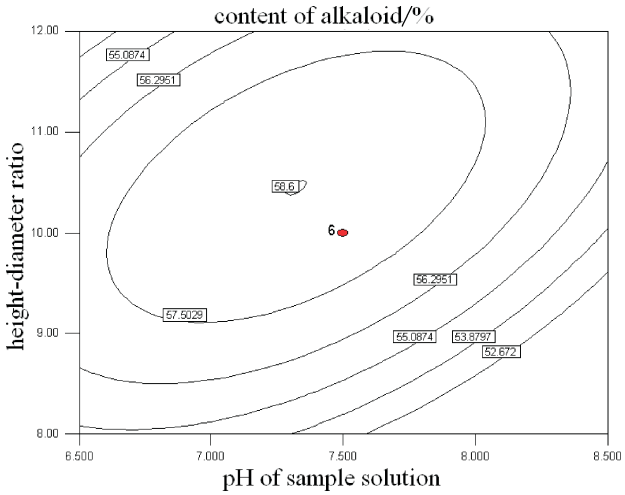
Twenty experiments were carried out according to the design and the experimental values are given in Table 3. All the experiments were implemented in duplicate and the mean value of content and recovery of alkaloids, as well as contents of tetrahydropalmatine were taken for statistical analysis. The statistical analysis indicated that the proposed model on the value of content and recovery of alkaloids was adequate, and the probability values of regression models on the value of content and recovery of alkaloids were 0.0023 and less than 0.0001, respectively, while the contents of tetrahydropalmatine had less relevance to the dependent variables in the model (p: 0.2586 > 0.05).

The effect of different parameters on the content and recovery of alkaloids were reported (Table 4) by the coefficient of the second order polynomials. As shown in Table 4, content of alkaloids was positively related to the linear effect of height-diameter ratio ($p < 0.05$), while the quadratic terms of height-diameter ratio ($p < 0.01$) and the quadratic terms of pH of sample solution ($p < 0.01$) had negative effect. Table 4 also indicated that the interaction between pH of sample solution and height-diameter ratio had significant influence on the purification process of alkaloids and all other interactive variables were insignificant. Recovery of alkaloids was negative related to the linear effect of height-diameter ratio ($p < 0.001$), the quadratic terms of pH of sample solution ($p < 0.001$), concentration of sample solution ($p < 0.001$), and height-diameter ratio ($p < 0.001$). Regression analysis of the experimental data (Table 4) showed that pH of sample solution and concentration of sample solution had significant positive linear effects on recovery of alkaloids while height-diameter ratio demonstrated negative linear effect on recovery of alkaloids. It was also found in Table 4 that the interaction between pH of sample solution and concentration of sample solution possessed significant effect on purification process of alkaloids and all other interactive variables are insignificant.

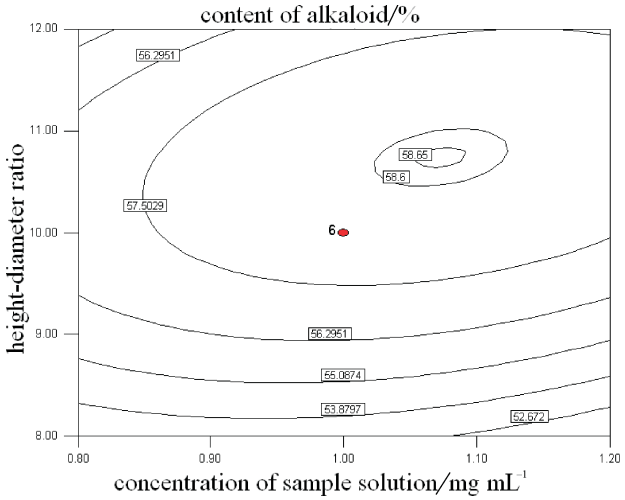
The closer the value of R^2 (multiple correlation coefficient) was to 1, the better the correlation between the observed and predicted values. The value of R^2 (0.8675 and 0.9466) indicated that the model can explain up to 86.75 % variation of the content of alkaloids and 94.66 % variation of recovery of alkaloids, respectively. The P value for models revealed that the experimental data obtained fitted well with the models which can describe the effect of pH of sample solution, concentration of sample solution, and height-diameter ratio on the content and recovery of alkaloids reliably. Figs. 4–5 showed the contour plots of purification process of



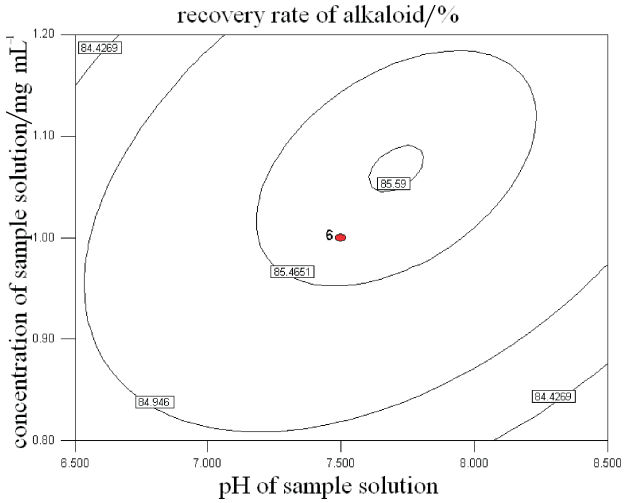
F i g . 4 a – *Contour plot for content of alkaloids as a function of pH of sample solution and concentration of sample solution (at height-diameter ratio 10)*



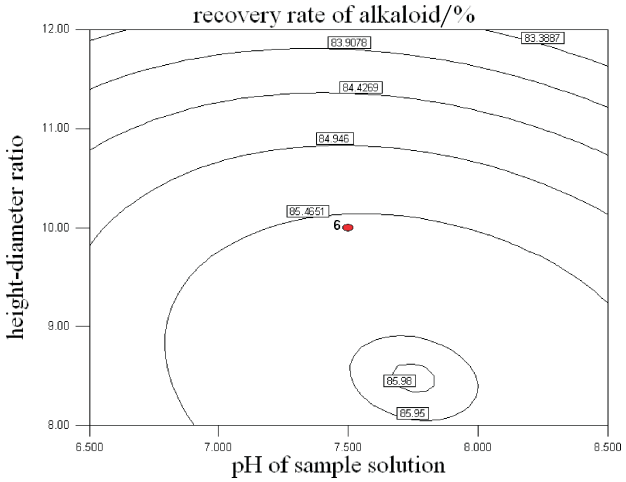
F i g . 4 b – *Contour plot for content of alkaloids as a function of height-diameter ratio and pH of sample solution (at concentration of sample solution 1.0 mg mL^{-1})*



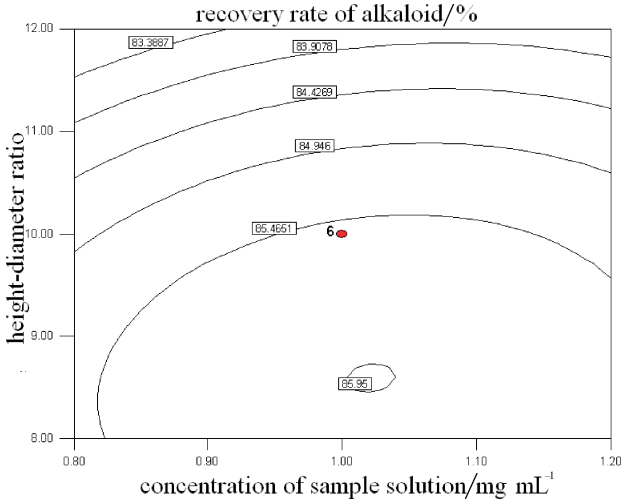
F i g . 4 c – *Contour plot for content of alkaloids as a function of height-diameter ratio and concentration of sample solution (at pH of sample solution 7.5)*



F i g . 5 a – *Contour plot for recovery of alkaloids as a function of pH of sample solution and concentration of sample solution (at height-diameter ratio 10)*



F i g . 5 b – *Contour plot for recovery of alkaloids as a function of height-diameter ratio and pH of sample solution (at concentration of sample solution 1.0 mg mL^{-1})*



F i g . 5 c – *Contour plot for recovery of alkaloids as a function of height-diameter ratio and concentration of sample solution (at pH of sample solution 7.5)*

alkaloids for each pair of purification parameters by keeping the rest of parameters constant at its middle level. The effects of pH and concentration of sample solution on the content of alkaloids are shown in Fig. 4a. Maximum content of alkaloids (58.71 %) was obtained at pH of sample solution (7.04) and concentration of sample solution (1.11 mg mL^{-1}). Further increase in pH or concentration led to the drop of content of alkaloids. Fig. 4b indicated that maximum content of alkaloids was 58.60 % when height-diameter ratio up was 10.45. Further augment in the mean height-diameter ratio induced the decrease in extraction of content of alkaloids. The contour plot of Fig. 4c shows that maximum content of alkaloids (58.66 %) occurred at the concentration of sample solution (1.07) and height-diameter ratio (10.74). The content of alkaloids ascended with height-diameter ratio up to 10.74 and further increase in height-diameter ratio leaded to decrease in the content of alkaloids. Fig. 5a indicates that maximum recovery of alkaloids was acquired at the pH of sample solution (7.71) and concentration of sample solution (1.07 mg mL^{-1}). Further increase in either of the two parameters led to the decline in recovery of alkaloids. As demonstrated in Fig. 5b, maximum recovery of alkaloids 85.98 % was obtained when height-diameter ratio was 8.48 and pH of sample solution (7.75). Fig. 5c contour plot showe that maximum recovery of alkaloids was acquired at the concentration of sample solution (1.02 mg mL^{-1}) and height-diameter ratio (8.59).

Validation of the model

During purification process of alkaloids by macroporous resins, content of alkaloids was an important index for evaluating the performance of this process. Therefore, the experimental data were fitted to the model and the optimum values were found to be: pH of sample solution (7.16), concentration of sample solution (1.12 mg mL^{-1}), and height-diameter ratio (10.50). At these optimum levels of purification parameters, the content and recovery of alkaloids were 58.25 % and 85.40 %, respectively, which was very close to their predicted values (58.86 % and 85.05 %). Meanwhile content of tetrahydropalmatine was 6.58 %, about three times that of the sample extracts, and the recovery of tetrahydropalmatine was 65.21 %.

Conclusion

In this study, a method was developed offering feasible and reliable recovery of alkaloids from *C. yanhusuo*. Currently, alkaloids of *C. yanhusuo, such as* tetrahydropalmatine, can be obtained by chromatography, but the pretreatment and enrich-

ment of samples are also important in order to decrease the interference of impurity and cost of chromatography. Although liquid–liquid extraction is capable of fulfilling the enrichment and preparative separation of alkaloids from *C. yanhusuo*, separation by macroporous resins was much more efficient. The separation of alkaloids on four widely used macroporous resins was compared with respect to their adsorption and desorption properties. Results showed that AB-8 possessed the best adsorption capacity and desorption capacity for alkaloids compared with other resins. The optimal adsorption and desorption conditions were: 2 BV of 30 % ethanol during the adsorption phase followed by 80 % ethanol at flow rate of 3 mg mL^{-1}during the desorption phase. The elution of initial approximate 5 BV of 80 % ethanol was collected for alkaloids product. Several important parameters for purification process using macroporous resins chromatogram column, such as flow rate and different pH of sample solution, flow rate of desorption solution, and height-diameter ratio, were optimized by RSM for realizing the most effective enrichment and preparative separation. The results of RSM showed pH of the sample solution, concentration of sample solution and height-diameter ratio had a remarkable influence on the process, and the sequence was pH of sample solution > concentration of sample solution > height-diameter ratio. These parameters can be correlated to the purification process conditions by second order polynomials. Optimized chromatogram purification process for obtaining tertiary alkaloids on macroporous resins was the following: pH of sample solution (7.16), concentration of sample solution (1.12 mg mL^{-1}), and height-diameter ratio (10.50). After the AB-8 resin treatment, the contents of alkaloids and tetrahydropalmatine were elevated from 25.20 % and 2.12 % to 58.25 % and 6.58 %, respectively. In addition, the recovery of alkaloids and tetrahydropalmatine was 85.40 % and 65.21 %, respectively. The experimental results showed that the purification process of tertiary alkaloids was efficient, with potential application in the future.

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List of symbols

R_A – recovery of alkaloids, %

m_1 – quantity of the sample extracts, mg

C_{A1} – alkaloids content of the sample extracts, %

m_2 – quantity of prepared sample by macroporous resins, mg

C_{A2} – alkaloids content of the prepared sample, %

V_2 – total volume of prepared sample, mL

c_{A2} – alkaloid concentration of the prepared sample, mg mL^{-1}

c_T – tetrahydropalmatine concentration of the prepared sample, mg mL^{-1}

V_T – volume of prepared tetrahydropalmatine sample, mL

m_T – quantity of prepared tetrahydropalmatine sample, mg

Y – response

X_1 – pH of sample solution

X_2 – concentration of sample solution

X_3 – height-diameter ratio

b_0 – constant term

b_i – linear effects

b_{ii} – quadratic effects

b_{ij} – interaction effects

P – probability

R^2 – multiple correlation coefficient

Abbreviations

BV – bed volume

RSM – response surface methodology

ANOVA – analysis of variance

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