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Optimization of Reflux Extraction Process for Phenylethanol Glycoside from Cistanche Deserticola by Response Surface Methodology

Aili Duan, Hongyan Wang, Shengmei Yang

Wuwei Food Inspection and Testing Center, Wuwei, Gansu, 733000, China

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Abstract: This article takes the extraction rate of phenylethanol glycosides from Cistanche deserticola as an evaluation index to investigate the effects of factors such as ethanol temperature, time, volume fraction, solid-liquid ratio, and extraction times on the extraction effect. Response surface methodology is used to optimize the reflux extraction process conditions of phenylethanol glycosides from Cistanche deserticola, and the feasibility of the method is analyzed. The experimental results show that the solid-liquid ratio is 1; 10, the volume fraction of ethanol is 1:60, the extraction temperature is 80 ℃, and the average extraction rate of phenylethanol glycoside is 76.5880 mg/g. The relative error is 0.29%, with a small deviation. Based on the above results, the response surface methodology was applied to optimize the reflux extraction process of phenylethanol glycosides, laying a theoretical foundation for extracting active substances from Cistanche deserticola and industrial production[1].

1. Introduction

Cistanche deserticola is a precious traditional Chinese medicine in China. It has a sweet and salty taste, a mild nature, and has functions of moisturizing dryness and nourishing blood, invigorating the kidneys and strengthening yang, and promoting beauty and longevity. Its main effective ingredient is phenylethanoid glycoside, which can slow aging, improve sexual function, improve memory function, protect liver and calm nerves, etc. It has been widely used in food, cosmetics, traditional Chinese patent medicines and simple preparations and health care products. At present, the main extraction and separation methods of phenylethanol glycosides from Cistanche deserticola include alcohol extraction, multiple organic solvent extraction, macroporous resin adsorption, flocculation water extraction, biotransformation technology, nano emulsion separation and purification methods, microwave extraction, ultrasonic extraction, etc., and the purification are commonly performed by medium pressure column chromatography, reverse phase silica gel chromatography and gel chromatography. However, these methods for separating and purifying phenylethanol glycosides from Cistanche deserticola will increase carbon element consumption and carbon dioxide emissions during the production process, which is not conducive to promoting the achievement of China’s carbon peak and carbon neutrality strategic goals. Therefore, it is of great

significance to comprehensively screen out a fast and environmentally friendly separation and purification process for phenylethanol glycosides in the low-carbon and safe production of Cistanche deserticola[2].

This research analyzes and studies the reflux extraction process of phenylethanol glycosides from Cistanche deserticola using response surface methodology, laying a theoretical foundation for the development, utilization, and industrial production of phenylethanol glycosides in Cistanche deserticola.

2. Research Procedures

2.1 Material Preparation

Cistanche deserticola used in this research was purchased from Gansu Rongbao Biotechnology Co., Ltd. Echinacoside medicine (batch number: MUST-22110117, drug content: 99.6%); Methanol, ethanol, n-butanol, and ethyl acetate are all chromatographic grade, products from Thermo Fisher Scientific Company in the United States; UV visible spectrophotometer: BSA-24S, with its measurement accuracy 0.1mg; SZ-D (III) circulating water vacuum pump, DF-101S collector type constant temperature thermal magnetic stirrer; Rotary evaporator RE-52; Electric constant temperature blowing furnace DHG-9070A; 200 type crusher.

2.2 Research Methods

2.2.1 Extraction Process

Cistanche deserticola → drying (60 ℃, 12h) → crushing (40 mesh) → ethanol reflux extraction → filtration → absorption of night extraction → vacuum rotary distillation → extracting (dissolved in water) → water saturated ethyl acetate, n-butanol extraction → D101 macroporous adsorption resin → eluent → vacuum rotary distillation → powder of Cistanche deserticola phenylethanol glycosides.

2.2.2 Selection of Wavelength for the Determination of Echinacoside

Putting 8mg of Echinacoside into a 50mL volumetric flask, taking methanol as the solvent, bringing to volume to the mark and shaking fully, thereby the mother liquor is obtained. Taking 1.5mL of the mother liquor from a 10mL volumetric flask, bringing to volume with methanol to the mark and shaking[3-4]. In the 230-450nm wavelength range, a visible ultraviolet spectrophotometer was used to scan its ultraviolet spectrum, and it was found that its absorption peak was the highest in the 333nm wavelength range. Therefore, the 333nm wavelength range was chosen as its standard curve.

2.2.3 Drawing of Standard Curve for Echinacoside

Putting 0.5mL, 1.0mL, 1.5mL, 2.0mL, and 2.5 mL of mother liquor respectively into the 10mL volumetric bottles according to the requirements of “1.2.2”, and bringing to volume with methanol to the mark, then shaking fully. Measuring the absorbance value at 333nm using methanol as the blank control, and then performing three measurements. Drawing a standard line with the x-axis as the concentration of Echinacoside while the y-axis as the absorbance value (OD), and obtaining a regression equation with $y=0.0233x-0.0188$ and $r^2=0.9997$, where the content of Echinacoside is 8-40 μg/ml, there is a good linear correlation between the content of Echinacoside and its absorbance value.

2.2.4 Determination of Extraction Rate of Phenylethanol Glycosides

The extraction rate of phenylethanol glycosides is measured in units of (mg/g), and the extraction rate of phenylethanol glycosides is measured in units of mg/g as the total mass of the raw material (g). Determination of phenylethanol glycoside content in the extraction solution: Putting 0.2mL of the extraction solution into a 50mL volumetric bottle, diluting with methanol and shaking fully, then measuring the absorbance value at a wavelength of 333nm to calculate the phenylethanol glycoside content in the extraction solution.

2.2.5 Single Factor Test

Placing 1.0g of dried Cistanche deserticola powder in an eggplant shaped bottle and extract for 2 hours at 60℃, with a solid-liquid ratio of 1:10 and an ethanol volume fraction of 40-90%; Under constant temperature conditions of 50℃, 60℃, 70℃, 80℃, 90℃ and 100 ℃, using 60% ethanol volume fraction with a solid-liquid ratio of 1:10 as raw materials, constant temperature reflux extraction was performed at 50℃, 60℃, 70℃, 80℃, 90℃ and 100 ℃ for 2 hours. The results showed that the extraction time was 1.0,1.5,2.0,2.5,3.0 hours at a constant solid-liquid ratio, 60% ethanol volume fraction, and 80 ℃ temperature, respectively; At a 60% ethanol volume fraction and a temperature of 80 ℃, with a solid-liquid ratio of 1:6,1:8,1:10,1:12:12:14 and extracting for 2 hours; Under the conditions of constant solid-liquid ratio, alcohol content of 60%, and temperature of 80 ℃. This research conducted first, second, third, and fourth time extractions respectively, and detected the content of phenylethanol glycosides in different components[5].

2.2.6 Optimization of Extraction Process Using Response Surface Methodology

Under the premise of analyzing the single factor experimental results with ethanol volume fraction, extraction temperature and solid-liquid ratio as the main influencing factors, a 3-factor 3-level response surface analysis method was used. Based on the “Center Box Behnken” combination experiment, the extraction rate of phenylethanol glycoside Y was selected as the reaction parameter. This study used a response surface experiment with 3-factor and 3-level, with five central points as the research objects, consisting of 17 sets of experiments[6].

3. Results and Analysis

3.1 Single Factor Test Results and Analysis

3.1.1 Volume Fraction

When the ethanol volume fraction is 40%-60%, the extraction rate of phenylethanol glycosides increases in accordance with the increase of ethanol volume fraction. The extraction rate of phenylethanol glycoside ranges from 57.70-69.18 mg/g. Within this range, there has been a significant change in the extraction rate of phenylethanol glycoside, indicating that the ethanol volume fraction between 40% and 60% has a significant influence on the extraction rate of phenylethanol glycoside (show as Figure 1).

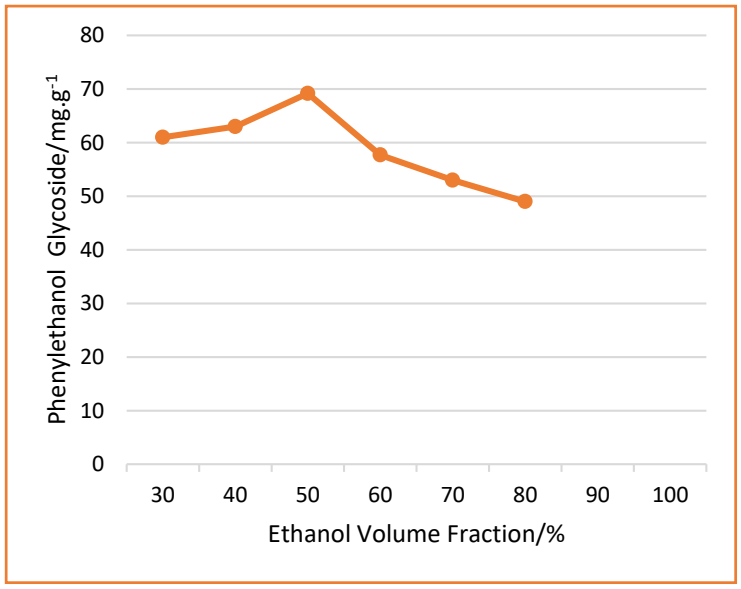

Figure 1: Effect of Ethanol Volume Fraction on the Extraction Rate of Phenylethanol Glycoside

The results showed that when the ethanol volume fraction was more than 60%, the content of phenylethanol glycosides in the extraction solution gradually decreased, and at 90% volume fraction, its content was only 47.61 mg/g, indicating that when the ethanol volume fraction was more than 60%, it had a significant influence on the extraction effect of phenylethanol glycosides[7]. Therefore, during extraction, the volume fraction of ethanol will have a significant influence on the extraction rate of phenylethanol glycosides, which will be selected as a factor for response surface analysis.

3.1.2 Temperature

Under temperature conditions of 50-100 ℃, the extraction rate of phenylethanol glycosides increases in accordance with the increase of temperature. However, when the temperature is higher than 80 ℃, its content decreases, indicating that the temperature high than 80 ℃ is unfavorable for its extraction. On this basis, 80 ℃ is proposed as the optimal extraction temperature, in order to avoid data bias, response surface analysis of the influencing factors of 80 ℃ is conducted to make it more accurate (show as Figure 2).

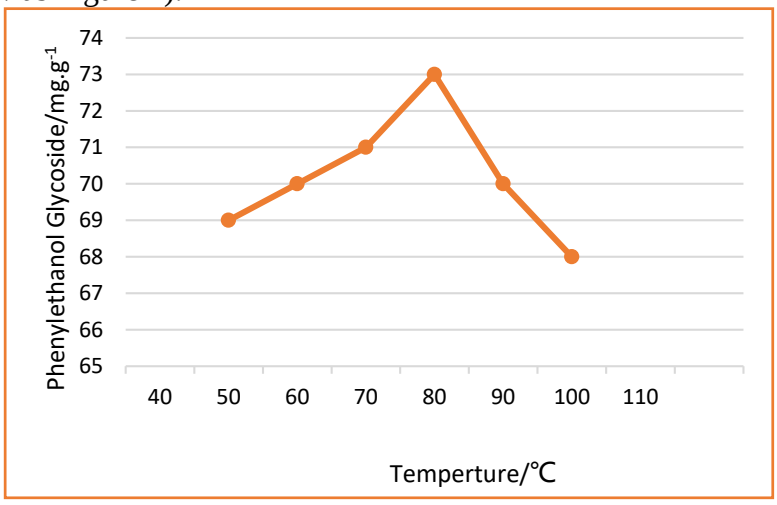

Figure 2: Effect of Temperature on the Extraction Rate of Phenylethanol Glycosides

3.1.3 Time

Within 1-3 hours, the content of phenylethanol glycosides increased with the time. Within 1-2 hours, the content of phenylethanol glycosides increased from 58.99-62.10 mg/g, with a variation of 3.11 mg/g; Within 2-3 hours, its content ranged from 62.10-63.28 mg/g, with a variation range of 1.18mg/g (show as Figure 3).

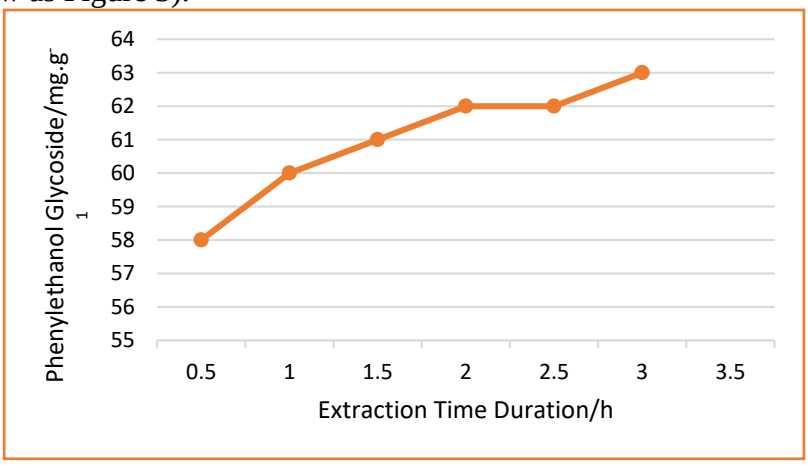

Figure 3: Effect of Time on the Extraction Rate of Phenylethanol Glycosides

The results showed that after 2 hours of extraction, the content of phenylethanol glycosides remained basically unchanged, indicating that the optimal extraction time is 2 hours, which can achieve the goal of energy conservation.

3.1.4 Solid-liquid Ratio

As the ratio of solid-liquid increases between 1:6 and 1:14, the content of phenylethanol glycoside gradually increases, and increases significantly from 63.99-72.93 mg/g between 1:6 and 1:10. After the solid-liquid ratio was greater than 1:10, the content of phenylethanol glycoside increased slowly. At the 1:14 ratio, the extraction rate was 75.08 mg/g, only increased by 2.15 mg/g. A thorough analysis was conducted on the response surface from the aspects of the amount of extraction solution and the cost of extraction solution (show as Figure 4)[8-10].

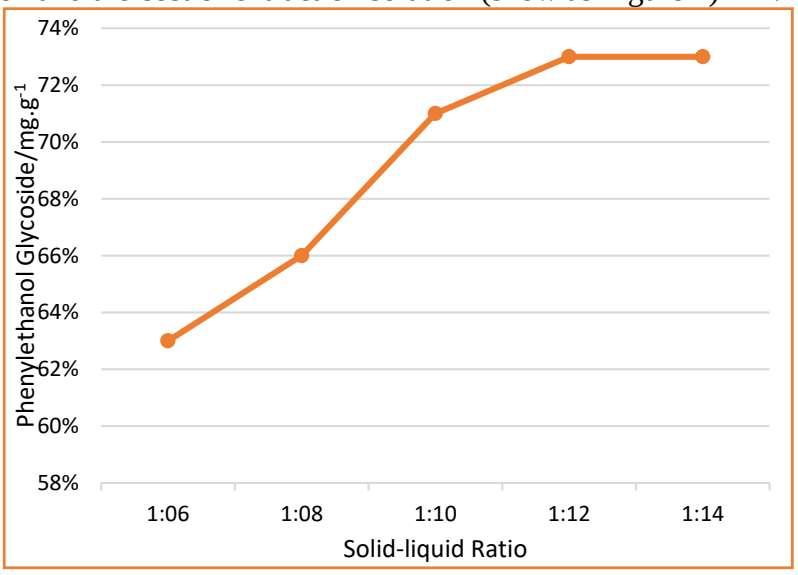

Figure 4: Effect of Solid-liquid Ratio on the Extraction Rate of Phenylethanol Glycosides

3.1.5 Extraction Times

As time goes by, the number of extraction times continues to increase, and the extraction rate of phenylethanol glycosides continues to improve. However, after two times, the extraction rate of phenylethanol glycosides is no longer significant. The multiple extraction process is both time-consuming and labor-intensive, and it also increases the complexity and energy consumption of the subsequent filtration process. Therefore, when considering various factors, the optimal extraction frequency is 2 times (show as Figure 5).

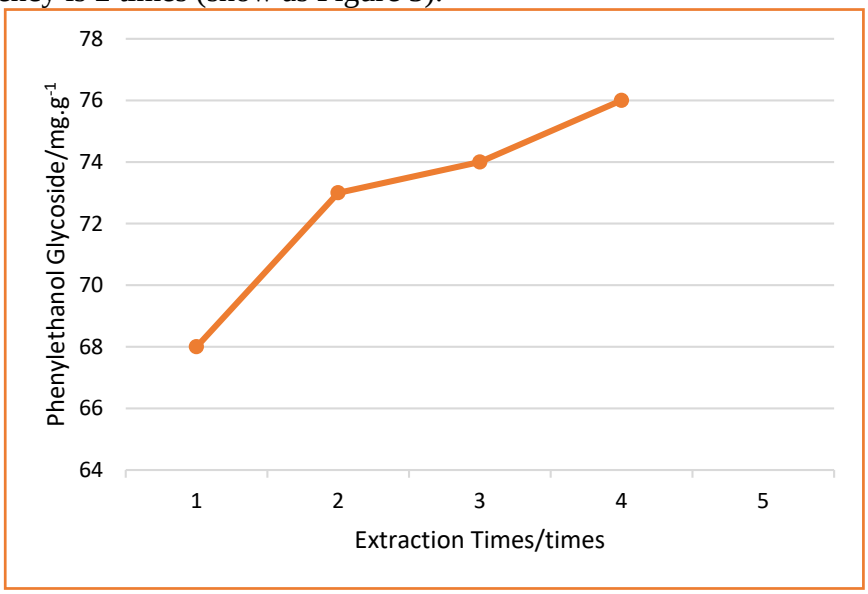

Figure 5: Effect of Extraction Times on the Extraction Rate of Phenylethanol Glycosides

3.2 Optimization of Response Surface Extraction Conditions

3.2.1 Selection of Factor Levels for Response Surface Analysis

This project is based on the combination design principle of Box Behnken center, with ethanol volume fraction A, extraction temperature B, and solid-liquid ratio C as the research objects. Lower values are represented by -1, moderate values are represented by 0, and higher values are represented by 1. After been extracted twice within 2 hours by the optimal experimental method of 3-factor and 3-surface, the design level and coding of the response surface experiment are listed in Table 1.

Table 1: Response Surface Test Factor Level

Factor	Level		
	-1	0	1
Ethanol volume fraction (%)	50	60	70
Extraction time(℃)	70	80	90
Solid-liquid ratio	8:1	10:1	12:1

3.2.2 Response Surface Test Design and Results

Taking the above factors as independent variables and the Phenylethanol glycoside ratio (Y) as the response value, a response surface was established. The experimental design and results are

shown in Table 2.

Table 2: Box Behnken Experimental Design and Results

Test order	A	B	C	Phenylethanol glycoside extraction rate
1	-1	-1	0	64.9591
2	1	-1	0	63.7790
3	-1	1	0	64.3164
4	1	1	0	63.0379
5	-1	0	-1	63.6035
6	1	0	-1	61.2206
7	-1	0	1	69.6733
8	1	0	1	68.1801
9	0	-1	-1	67.9924
10	0	1	-1	67.4138
11	0	-1	1	74.4026
12	0	1	1	73.3807
13	0	0	0	77.3796
14	0	0	0	78.3025
15	0	0	0	79.4507
16	0	0	0	77.2048
17	0	0	0	77.0036

Based on Table 2, regression analysis was conducted using Design-Expert 8.0.6.1 software. Through regression fitting of various factors, a quadratic multiple regression equation was obtained between the phenylethanol glycoside extraction rate (Y) and the influencing factors A, B, C:

$$Y = 76.87 - 0.79A - 0.37B + 3.18C - 0.025AB$$

$$+ 0.22AC - 0.11BC - 9.49A2 - 4.36B2 - 2.71C2$$

The square coefficient in the fitting formula of the response surface model is negative, indicating that its image shape is a downward parabola, and its maximum extremum point can be optimized. If the coefficients of other items are all positive, it indicates that there is a certain positive correlation between this factor and the response result. On the contrary, this factor is negatively correlated with the response value.

The results of variance analysis and significance testing conducted on the regression formula are listed in Table 3. The regression coefficient is significant ($P<0.0001$), while the mismatch term is not significant ($P=0.9556>0.1$), indicating that the difference between the mismatch term and pure error is not significant. On this basis, a quadratic regression model was established corresponding to factors such as ethanol volume fraction, extraction temperature, and solid-liquid ratio. The coefficient of determination r^2Adj=0.9837 indicates that the model has an explanatory rate of 98.37% for the value of the coefficient of determination. The correlation coefficient r^2 is 0.9929, indicating that the model fits well and the predicted values have a good correlation with the measured values. This experiment has only a slight error[9].

The results indicate that the established mathematical model can effectively reflect the content of phenylethanol glycosides in Cistanche deserticola. Analysis of variance shows that A and C have a significant influence on the response value of the first term of the equation, while A2, B2, and C2 of the second term of the equation have a significant impact on the response value, but the interaction term has no significant influence on the response value. Research has found that the effect of various experimental factors on response values is not a simple linear relationship. The magnitude

of each factor's influence is determined by the magnitude of the F-value, and a larger F-value indicates that each factor has a greater influence on the response value results. The statistical results of the variance table show that FA is 7.93, FB is 1.76, and FC is 12.49. The results showed that the influence order on the extraction rate of phenylethanol glycosides in Cistanche deserticola was: solid-liquid ratio>ethanol volume fraction>extraction temperature.

Table 3: Regression Model of Variance Analysis

Source of Variance	Sum of squares	Degree of freedom	Mean square	F Value	P_r>F	Significance
Model	617.96	9	78.65	108.32	<0.0001	3
A	6.02	1	6.12	7.93	0.0259	1
B	2.11	1	2.21	1.76	0.2264	0
C	90.69	1	90.79	12.49	<0.0001	3
AB	3.42E 一 003	1	3.52E-003	3.83E-003	0.9524	0
AC	1.2	1	1.3	0.31	0.5935	0
BC	1.049	1	1.149	0.078	0.7886	0
A2	478.92	1	478.93	598.75	<0.0001	3
B2	89.99	1	89.100	126.39	<0.0001	3
C2	40.97	1	40.98	48.94	0.0002	2
Residual	5.43	7	1.73			0
Misfitting term	1.31	3	1.2	0.1	0.9556	0
Pure error	5.12	4	2.13			0
Total dispersion	721.39	16				0
R^2=0.9929， R^2_{Adj}=0.9837						

3.2.3 Validation Test

The optimal extraction process of Phenylethanol Glycoside from Cistanche deserticola was analyzed using Design-Expert 12.0 software as follows: ethanol volume fraction is 1:62.91 (refer to the above table), solid-liquid ratio is 1:10.29 (refer to the above table), extraction temperature is 79.8 ℃ (refer to the above table), and the extraction rate of Phenylethanol Glycoside from Cistanche deserticola is 77.8%. Considering the operability of the process, the process conditions predicted by the model were adjusted to: ethanol volume fraction% is 1:60, solid-liquid ratio is 1;12, and extraction temperature is 80 ℃. Under these conditions, three parallel experiments were conducted to verify the results. The average extraction rate of phenylethanol glycosides from Cistanche deserticola was 77.8%, with a relative error of 0.29% and a small deviation. There was no statistically significant difference between the theoretical and actual values, indicating that the prediction conditions of the model were consistent with the actual situation. The model was successfully established, so the plan is feasible[11].

4. Conclusion

Based on response surface analysis, the optimal process conditions for reflux extraction of phenylethanol glycosides from Cistanche deserticola using ethanol as solvent were studied. The

correctness and feasibility of this method have been demonstrated through regression of the response surface equation. The reliability of the proposed method has been proven through the verification of the experimental results. The implementation of this project will lay the foundation for the development and industrialization of phenylethanol glycosides in Cistanche deserticola in China.

Acknowledgement

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