Process Optimization of Salidroside with Cellulase Auxiliary Ultrasonic-Assisted Extraction from *Rhodiola Cretinii* by Response Surface Methodology

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Abstract. The cellulase auxiliary ultrasonic-assisted extraction process of salidroside from *Rhodiola cretinii* was optimized by response surface methodology. The extraction content of salidroside was measured by HPLC. On the basis of single factor experiment, extraction time, quantity of cellulase, extraction temperature and ethanol concentrations were selected as independent variables, and the extraction content of salidroside was selected as response value. Box-Behnken design was adopted and the optimum extraction process was confirmed. The results showed that the optimum extraction conditions were as follows: 29 min, 0.18% cellulose, 43°C, 39% ethanol concentration, the extraction content of salidroside was 4.49 mg/g. Through the verified tests, the content of salidroside was 4.39 mg/g after cellulase was added, the content of salidroside without cellulase was 0.48 mg/g. The response surface analysis can predict the experimental results well, and this experiment can provide a more reasonable and effective method for extracting salidroside from *R. cretinii*.

Keywords: *Rhodiola Cretinii*, Salidroside, Ultrasonic-Assisted Extraction, Response Surface Methodology

1. Introduction

*Rhodiola cretinii* is mainly distributed in Sichuan, Jilin, Heilongjiang, Xinjiang in China [1]. Salidroside is the main active ingredient in *R. cretinii*. It has a wide range of pharmacological effects, such as eliminating fatigue, anti-aging, anti-hypoxia, anti-microwave radiation, anti-tumor and other functions [2-3]. According to the chemical research of *R. cretinii*, salidroside is the main active component, which has anti-inflammatory, anti-tumor and immunomodulatory effects. *R. cretinii* plays an important role in a variety of traditional Chinese medicine.

Compared with other techniques, UAE is driven by the driving force of acoustic cavitation, which is conducive to the rupture of cell wall and the improvement of extraction process [4]. Ultrasound has the advantages of simple operation and safety, because it is carried out under atmospheric pressure and ambient temperature, using a proper amount of solvent is repeatable, and requires relatively short
processing time [5].

The objective of this study were to seek the method of ultrasound-assisted extraction of as a approach applicable for the extraction of salidroside with cellulase auxiliary from R.cretini, to evaluate the influence and joint effect of main extraction independent variables, and to optimize the operational parameters to obtain the maximum possible content of salidroside in extracts with Response surface methodology (RSM).

2 Experimental

2.1 Materials

The roots of Rhodiola cretinii in Erdaobaihe of Jilin Province were studied. Plant samples were air dried in a cool place at room temperature and ground immediately before extraction. which was dried in 60 °C oven after 40 mesh sieve. The powder was kept in the dark in a dry, cool place until treated. The specimen was kept in the laboratory of pharmaceutical chemistry, Jilin Agricultural University of science and technology.

2.2 Design of Experiment

The technological parameters (dependent variables) of salidroside yield were studied by 3⁴ full-factor experimental designs. The range of independent variables (process parameters) was preliminarily investigated: extraction time (20, 30, 40 min, X₁), quantity of cellulose (0, 0.15%, 0.3%, X₂), extraction temperature (30, 40, 50 °C, X₃) and ethanol concentrations (30%, 40%, 50%, X₄). All experimental runs were carried out in three times.

2.2.1 RSM

The optimum combination of process parameters is assayed by RSM method on the tree structure level. The yield of salidroside (related parameter Y) was determined in triplicate., and the regression analysis is evaluated with average value. The experimental data were analyzed by design expert software to predict the optimal conditions, and fitted to the empirical second-order polynomial regression model [6]:

\[ Y = f(X₁, X₂, \ldots, X₄) + ε \]

Design-Expert 8.0.6 was used for experimental design, regression analysis and graphic analysis. The significance of independent parameters and their interactions, the adequacy of the established model and the statistical significance of regression coefficients were evaluated by Analysis of Variance (ANOVA).

2.3 Ultrasound-assisted Extraction

Ultrasound-assisted extraction was performed by a KQ-700V ultrasonic cleaner (Jining Tianhua Ultrasonic Electronic Instrument Co., Ltd). Ground plant material (1.00 g) adding cellulase (50 u/mg, “Shanghai yuanye Bio-Technology Co., Ltd.”) and pH 5.0 citric acid buffer solution, and different ethanol concentration (30%, 40%, 50%) of extraction temperature (30, 40, 50 °C) were put into a series of Erlenmayer flasks (50 mL). The extraction was performed at various extraction time (20, 30, 40 min). The filtrations of the two extractions were combined after the extraction cycle. The liquid extracts were condensed with a rotary evaporator at 45 °C. and transferred into a 50 mL Erlenmayer flask and fixed the volume to the scale with 60% ethanol. The extract solution was sonicated for 10 min and filtered through a 0.45 µm filter for High Performance Liquid Chromatograph [7].

2.4 Content Determination of Salidroside

2.4.1 Preparation of control solution

10.0 mg salidroside was weighed precisely, put it into a 20 mL volumetric flask, and fixed the volume to the scale with methanol, then shook well to obtain 0.5 mg/mL standard solution.

2.4.2 HPLC conditions

The chromatographic column:was ZORBAX SB-C18 column (4.6 × 150 mm, 5 µm), the mobile phase was made up of 0.5% glacial acetic acid - water solution (A) - methanol (B),
0~30 min 0 -55% A, 30~45 min 55-100% A. The injection volume was 1 µL, the detection wavelength was 270 nm, the column temperature was 30 ℃, and the flow rate was 1 mL/min[8].

2.4.3 Drawing of the standard curve 1, 2, 3, 4, 5 mL of standard solution was absorbed accurately and put them into 25 mL volumetric flasks respectively, and fixed the volume to the scale with methanol, shooked well. A series of volumetric flasks with water to the scale are obtained 20, 40, 60, 80, 100 µg/mL standard solution liquid. According to the above HPLC chromatographic conditions, 10 µL of sample was injected each time, and then the sample was analyzed by HPLC. The concentration (x) was the abscissa, and the peak area (y) was the vertical coordinate.

3 Results and Discussion

3.1 The Standard Curve

![Figure 1. High-performance liquid chromatography of salidroside](image)

The regression equation of salidroside was 

\[ y = 38.175x + 4.25, \quad R^2 = 0.9939. \]

3.2 RSM Modeling and Optimization

According to the test data in Table 1, Design-Expert 8.0.6 was used. Four factors and target peak area were obtained by software design analysis, 29 trial runs were carried out randomly in three times to study the effect of different variables on the production of salidroside. Table 1 listed the factors and their levels affecting salidroside yield, and indicated the predicted and experimental results.

| Run | X1  | X2  | X3  | X4  | extraction time (X1, min) | Quantity of cellulose (X2, %) | extraction temperature (X3, ℃) | ethanol concentrations (X4, %) | SY  | EV  | PV  |
|-----|-----|-----|-----|-----|---------------------------|-----------------------------|-----------------------------|-------------------------------|-----|-----|-----|
| 1   | -1  | 0   | 1   | 0   | 20.00                     | 0.15                        | 50.00                       | 40.00                         | 2.81| 2.11|     |
| 2   | -1  | 0   | -1  | 0   | 20.00                     | 0.15                        | 30.00                       | 40.00                         | 1.14| 0.83|     |
| 3   | 0   | 0   | 0   | 0   | 30.00                     | 0.15                        | 40.00                       | 40.00                         | 4.43| 4.49|     |
| 4   | 1   | 1   | 0   | 0   | 40.00                     | 0.30                        | 40.00                       | 40.00                         | 1.85| 1.76|     |
| 5   | 0   | 1   | 0   | -1 | 30.00                     | 0.30                        | 40.00                       | 30.00                         | 2.86| 2.61|     |
| 6   | 0   | -1  | -1  | 0   | 30.00                     | 0.00                        | 30.00                       | 40.00                         | 1.98| 2.36|     |
| 7   | 0   | 0   | 0   | 0   | 30.00                     | 0.15                        | 40.00                       | 40.00                         | 4.43| 4.49|     |
| 8   | 0   | 0   | 0   | 0   | 30.00                     | 0.15                        | 40.00                       | 40.00                         | 4.43| 4.49|     |
| 9   | 0   | 1   | 0   | 1   | 30.00                     | 0.30                        | 40.00                       | 50.00                         | 2.30| 2.07|     |
According to the experimental results, a second-order polynomial model was built to forecast the yield of salvodrose from *R. cretinii*:

\[
Y = -58.75887 + 0.9220X_1 + 18.82399X_2 + 1.28602X_3 + 1.08340X_4 - 0.47850X_1X_2 - 2.56320 \times 10^{-3}X_1X_3 - 1.92500 \times 10^{-3}X_2X_3 + 0.15541X_2X_4 - 0.07000X_3X_4 + 4.50 \times 10^{-3}X_1X_4 - 0.011483X_1^2 - 25.49379X_2^2 - 0.01490AX_3^2 + 0.013132X_4^2.
\]

**Table 2.** Analysis of Variance (ANOVA) of Box-Behnken Test

| Source | Sum of squares | df | Mean squares | F value | P value | 
|--------|----------------|----|--------------|---------|---------|
| Model  | 32.57          | 14 | 2.33         | 9.15    | < 0.0001 | Significant |
| $X_1$  | 0.34           | 1  | 0.34         | 1.34    | 0.2660  |
| $X_2$  | 0.012          | 1  | 0.012        | 0.049   | 0.8284  |
| $X_3$  | 3.84           | 1  | 3.84         | 15.11   | 0.0016  |
| $X_4$  | 0.36           | 1  | 0.36         | 1.43    | 0.2514  |
| $X_1X_2$ | 1.69         | 1  | 1.69         | 6.66    | 0.0218  |
| $X_1X_3$ | 0.29          | 1  | 0.29         | 1.13    | 0.3063  |
| $X_1X_4$ | 0.15           | 1  | 0.15         | 0.58    | 0.4579  |
| $X_2X_3$ | 0.19           | 1  | 0.19         | 0.73    | 0.4059  |

SY – salvodrose yield, EV – experimental values, PV – predicted values.
The F-value (9.15) and p-value (< 0.0001) indicated that the built model had statistically significant effects. $R^2$ and Adj. $R^2$ were 0.9015 and 0.8029 respectively, suggesting that the model was in good agreement with the experimental data. The effects of four independent variables (extraction time, quantity of cellulase, extraction temperature and ethanol concentrations) on the production of salidroside was reported through the significant (p<0.05) coefficient of the second-order polynomial model. The lower the values of F and P, the greater the impact on the response. From Table 2, extraction temperature ($X_3$) had a significant impact (p<0.05). In conclusion, the fitting degree of the regression model was good. The regression equation can be used to analyze and optimize the extraction conditions of salidroside in the samples.

Figure 2 showed that the production of salidroside added with the improvement of ethanol concentration from 50% to 90%, but with a further increase in the solid-liquid ratio, the production of salidroside reduced. The production of salidroside slightly added with the increase of extraction time from 2 to 4 times, while with the increase of solid-liquid ratio the production of salidroside increased evidently. Compared to the other analyzed factors, extraction concentration had the highest, while extraction times had the lowest affected on the production of salidroside.

**Figure 2.** 3D response surface plots for yield of salidroside

The optimum technological conditions for extraction of salidroside by cellulase-assisted ultrasound using were as follows: 28.98 min, 0.18% cellulose, 43.19°C, 38.69% ethanol concentration, and the
extraction content was 4.49mg/g. Combined with practical production applications, which was adjusted to 29 min, 0.18% cellulose, 43 °C, 39% ethanol concentration. The difference between the model and the predicted value was 0.025%, which was not much different from the predicted value of the model, the reliability of the model was fully verified.

3.3 Confirmatory Test
3.3.1 Test without cellulase
1.00 g R. cretinii was placed in Erlenmayer flasks, 10 mL PH 5.0 citric acid buffer solution was added and placed in 55 °C water bathing for 30 min, then adding 26 mL 39% ethanol solution. The samples were extracted under conditions of 43 °C, 100W, 29min, 3 times with Ultrasonic Cleaner. The filtrate was combined and concentrated, the solution was fixed in a 10 mL volumetric flask with 39% ethanol solution, shaken well. The content of salidroside was determined by "2.4.2". The extraction content was 0.48mg/g.

3.3.2 Test with cellulase
1.00 g R. cretinii was placed in Erlenmayer flasks, added 0.18% cellulase and 10 mL of citric acid buffer solution with pH 5.0, and placed in 55 °C water bathing for 30 min, then adding 26 mL 39% ethanol solution. The extraction conditions are the same as above “3.5.1”, The content of salidroside was determined by "2.4.2". The extraction content was 4.39 mg/g.

The results showed that the extraction rate of salidroside with cellulase was higher than that without cellulase.

4. Conclusion
The results of response surface method were pinpoint, effective and credible, and the model of interaction of different factors had a good fit within the experimental conditions. It can save reagents and raw materials, shorten the extraction time, improve efficiency, improve the bioavailability of salidroside from R. cretinii and provide a more effective method for the extraction of salidroside from R. cretinii.

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