

Article

Parameter Optimization Considering the Variations Both from Materials and Process: A Case Study of *Scutellaria baicalensis* Extract

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Abstract: The Quality by Design (QbD) concept has been widely applied to the optimization of traditional Chinese medicine production processes recently. This work focused on optimizing the critical purification process of *Scutellaria baicalensis* extract used in the preparation of Zhushheyong Shuanghuanglian. Considering the impact of noise parameters and changes in herbal properties, an experimental design method was employed for optimization. Multiple batches of *Scutellaria baicalensis* decoction were prepared in this research, and quantitative models of *Scutellaria baicalensis* herbal properties, critical process parameters (CPPs), and process evaluation indicators were established. The R^2 of the quantitative models were all higher than 0.80. According to the model, the yield of baicalin was identified as a critical material property (CMA). The pH of first acid precipitation (X_1), first temperature holding time (X_2), pH of alkalization (X_3), ethanol amount (X_4), and end pH of ethanol washing (X_5) were CPPs. Considering the difficulty in controlling the end pH of the ethanol washing, it was considered to be a noise parameter. The Monte Carlo probability-based method was used to calculate the design space, determining the range of controllable parameters, which was successfully validated through experiments. Normal operation ranges for controllable parameters are recommended as follows: X_1 of 0.8–2.2, X_2 of 25–35 min, X_3 of 6.5–7.5, and X_4 of 0.8–1.2 g/g.

Keywords: Zhushheyong Shuanghuanglian; *Scutellaria baicalensis* extract; process parameter optimization; design space



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1. Introduction

Zhushheyong Shuanghuanglian is included in the 2020 edition of the *Pharmacopoeia of the People's Republic of China*. It is prepared from three herbs: *Scutellaria baicalensis*, *Lonicera japonica*, and *Forsythia suspensa* [1]. It has the effects of clearing heat and detoxifying, as well as dispelling wind and relieving exterior symptoms; it is commonly used for coughs, fever, pneumonia, etc., and also helps to enhance the body's immune function [2,3]. Among these, *Scutellaria baicalensis* (*Scutellaria baicalensis* Georgi) is widely used in traditional Chinese medicines, with its main active ingredients including various flavonoids such as baicalin, baicalein, and wogonoside [4–6]. These flavonoids have multiple pharmacological activities, including anti-tumor [7], antioxidant [8], antiviral activity [9], antibacterial [10], and immune-boosting [11].

Scutellaria baicalensis extract for Zhusheyong Shuanghuanglian is produced by processes such as water decoction, precipitation, ethanol washing, activated carbon treatment, and drying [1]. In a previous work, the preparation process of *Scutellaria baicalensis* extract was investigated [12] and determined that the critical processes are water decoction, acid precipitation, and ethanol washing based on mass transfer characterizations and similarity change. Additionally, it was found that controlling ethanol washing to a fixed pH value is challenging; therefore, this parameter is considered a noise parameter in the preparation process of *Scutellaria baicalensis* extract.

The steps for drug development are proposed in ICH Q8 (R2) [13], including identifying critical quality attributes (CQAs), determining critical process parameters (CPPs), developing design space, and continuous improvement. Before establishing the design space, it is usually necessary to build a mathematical model to describe the relationships between process inputs (material attributes and process parameters) and the CQAs of the drug [14]. The design space not only helps enhance the process's robustness but also improves production flexibility and reduces the regulatory burden [15]. For example, Wang et al. considered the quality differences between batches of herb materials and used definitive screening design (DSD) to optimize the preparation process of ginkgo leaf extract, establishing the design space based on risk levels [16]. When establishing the design space, parameters that are costly or difficult to control can be treated as noise parameters [17], as shown by Tai et al., who treated the refrigeration temperature as a noise parameter [18].

The differences between batches of medicinal materials were considered in this work, different properties of *Scutellaria baicalensis* decoction were prepared for purification, and a quantitative model of critical herb material attributes (CMAs), CPPs, and process evaluation indicators were established. By controlling the controllable parameters, the sensitivity to noise parameters, specifically the change of pH at the end of the ethanol washing, was reduced, enhancing the robustness of the preparation process of *Scutellaria baicalensis* extract. In this research, the Monte Carlo probability-based method was used to establish the design space, which was verified through experiments.

2. Materials and Methods

2.1. Reagents and Herb Materials

Hydrochloric acid (36–38%, analytical grade) and sodium hydroxide ($\geq 96.0\%$, analytical grade) were purchased from Sinopharm Chemical Reagents Co., Ltd. (Shanghai, China). Methanol ($\geq 99.9\%$, chromatographic purity) and acetonitrile ($\geq 99.9\%$, chromatographic purity) were both purchased from Merck (Darmstadt, Germany). Formic acid ($\geq 99.9\%$, chromatographic purity) was purchased from ROE Scientific Inc. (Newark, NJ, USA). The reference substances of wogonoside (lot:201702, 98.5% purity), baicalin (lot:202122, 94.2% purity), and baicalein (lot:201808, 97.9% purity) were all provided by the National Institutes for Food and Drug Control (Beijing, China). Information about the different batches of *Scutellaria baicalensis* herb materials is shown in Table 1.

Table 1. Number of different batches of herb materials.

Herb Identification Number	Batch of Herbs	Manufacturer
S1	B2008191-01	Hebei Chufeng Decoction Pieces Co., Ltd. (Hebei, China)
S2	210301	Anhui Daoyuantang Decoction Pieces Co., Ltd. (Anhui, China)
S3	168210701	Hebei Linyitang Pharmaceutical Co., Ltd. (Hebei, China)
S4	168200601	Hebei Linyitang Pharmaceutical Co., Ltd. (Hebei, China)
S5	201101	Anhui Daoyuantang Decoction Pieces Co., Ltd. (Anhui, China)
S6	211109	Bozhou Kangyiyin Biotechnology Co., Ltd. (Bozhou, China)
S7	211112	Luan Danbell Biological Technology Co., Ltd. (Luan, China)
S8	168211001	Hebei Linyitang Pharmaceutical Co., Ltd. (Hebei, China)
S9	168201702	Hebei Linyitang Pharmaceutical Co., Ltd. (Hebei, China)

2.2. Experimental Methods

According to previous research [12], the critical processes of *Scutellaria baicalensis* extract preparation are shown in Figure 1, including water decoction, acid precipitation, dissolution, and second acid precipitation. In the dissolution process, alkali and ethanol were both added.

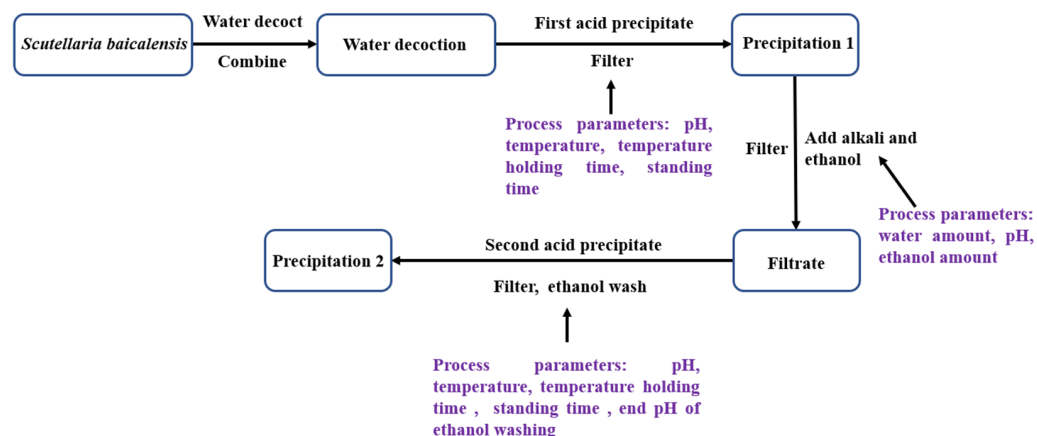


Figure 1. Extraction process of *Scutellaria baicalensis* products.

Preparation of the water decoction of *Scutellaria baicalensis*: *Scutellaria baicalensis* was extracted twice with water for 1 h each time. After filtration with filter paper under vacuum, the filtrates were combined.

Preparation of precipitate 1: a total of 2 mol/L of hydrochloric acid solution was added to adjust the pH to 1.0~2.0. The mixture was maintained at 80 °C for 30 min. Subsequently, it was left undisturbed for 12 h, filtered with filter paper under vacuum, and the precipitate was washed with water to remove residual acid on the surface. Finally, vacuum drying was carried out to obtain precipitate 1.

Preparation of the filtrate: eight times the volume of water was added to precipitate 1 under stirring, and the pH was adjusted to 7.0 using a 10% sodium hydroxide solution. An equal amount of ethanol was subsequently added, and under continuous stirring, the precipitate was dissolved. The solution was then filtered with filter paper under vacuum to obtain the filtrate.

Preparation of precipitate 2: the pH of the filtrate was adjusted to 2.0 with 2 mol/L hydrochloric acid solution, and the mixture was kept at 60 °C for 30 min before being allowed to stand for 12 h. It was then filtered with filter paper under vacuum, and the precipitate was washed with ethanol until its pH reached 4.0. Vacuum drying was finally applied to obtain precipitate 2.

2.2.1. Reference Solution Preparation

Appropriate amounts of the reference substances wogonoside, baicalein, and baicalin were vacuum-dried for 24 h and accurately weighed. They were then placed into volumetric flasks, dissolved by ultrasonication (LMTD15, Lumiere Tech Ltd., Beijing, China) with methanol, diluted to the mark, and shaken well to obtain single-reference solutions with mass concentrations of 0.594, 0.566, and 0.545 mg/mL, respectively. Accurate volumes of the individual reference solutions were then transferred into a common volumetric flask, diluted to the mark, and thoroughly mixed, yielding a mixed reference solution containing 0.119 mg/mL wogonoside, 0.113 mg/mL baicalein, and 0.109 mg/mL baicalin. Stepwise dilution of this mixed reference solution was performed to obtain a series of mixed reference solutions with varying concentrations. The resulting series of solutions were analyzed by high-performance liquid chromatography (Agilent 1100, Agilent Technologies Ltd., Santa

Clara, CA, USA) to obtain the corresponding peak areas. A standard curve was plotted using concentration as the x-axis and peak area as the y-axis, and the regression equation of peak area versus concentration was derived.

2.2.2. Preparation of Test Solution

An appropriate mass of the sample to be analyzed was accurately weighed and transferred into a volumetric flask of suitable size. The *Scutellaria baicalensis* decoction was diluted to the mark with ultrapure water, while precipitate 2 was diluted with a 10% sodium hydroxide solution. The solutions were thoroughly mixed and set aside for subsequent use. The solutions were filtered through a 0.22 μm membrane filter, and the filtrate was placed into sample vials for high-performance liquid chromatography (HPLC) analysis to obtain the corresponding peak areas. The flavonoid concentration in the sample was then calculated based on the standard curve equation established previously.

2.2.3. Analytic Procedure

The contents of baicalin, wogonoside, and baicalein, the three flavonoid components in the water decoction, and precipitate 2 of *Scutellaria baicalensis* were detected using chromatographic conditions from the literature [12]. The limits of detection (LOD) of baicalin, wogonoside, and baicalein are 0.00536 mg/mL, 0.00113 mg/mL, and 0.000369 mg/mL, respectively. The limits of quantification (LOQ) are 0.0162 mg/mL, 0.00342 mg/mL, and 0.00112 mg/mL, respectively. Due to the high content of baicalin in the water decoction, chromatographic analysis can easily become overloaded. Therefore, when detecting the content of baicalin, the sample was diluted with a larger volume of water. The analysis was performed on a Waters XBridge Shield RP18 column (250 mm $\times$ 4.6 μm , 5 μm), which was purchased from Waters (Massachusetts, USA). The mobile phase consisted of 0.1% formic acid aqueous solution (A) and acetonitrile (B), with gradient elution as follows: 0–10 min, 10–15% B; 10–20 min, 15–20% B; 20–30 min, 20–25% B; 30–60 min, 25–45% B; 60–70 min, 45–60% B; and 70–75 min, 60–10% B. The flow rate was set to 0.8 mL/min, the detection wavelength was 274 nm, the injection volume was 10 μL , and the column temperature was maintained at 28 $^{\circ}\text{C}$. The typical chromatogram obtained is shown in Figure 2.

2.2.4. Determination of Critical Process Evaluation Indicators

The main components of *Scutellaria baicalensis* are flavonoids. Currently, baicalin is the indicator component for quality control of *Scutellaria baicalensis* in the Chinese Pharmacopoeia. However, considering that traditional Chinese medicine integrates multiple components and targets for efficacy, three flavonoids were selected as indicator components: baicalin, wogonoside, and baicalein. In this research, the yields of the three active components and total solids were chosen as critical process evaluation indicators, namely, yield of baicalin (Y_1), yield of wogonoside (Y_2), yield of baicalein (Y_3), and yield of total solids (Y_4).

2.2.5. Research on Herb Properties

To research the impact of differences in *Scutellaria baicalensis* herbs, nine batches of *Scutellaria baicalensis* were selected for experiments. To quantitatively characterize the property differences among the nine batches, the following method of testing the properties of their decoctions was adopted. The fixed conditions for decocting the herbs were as follows: *Scutellaria baicalensis* herbs were decocted twice with 3 L of water, each time for 1 h, and the decoctions were combined. Then, the flavonoid contents and total solids of each decoction were measured, and the yields of flavonoids, total solids, and the purity of

each flavonoid were calculated as quantitative indicators of herb properties. The formula for calculating the purity of flavonoid is shown in Equation (1).

$$P_i = \frac{Y_i}{Y_s} \quad (1)$$

The purity of the i -th flavonoid is P_i , the yield of the i -th flavonoid is Y_i (mg/g), and the yield of the total solids is Y_s (mg/g).

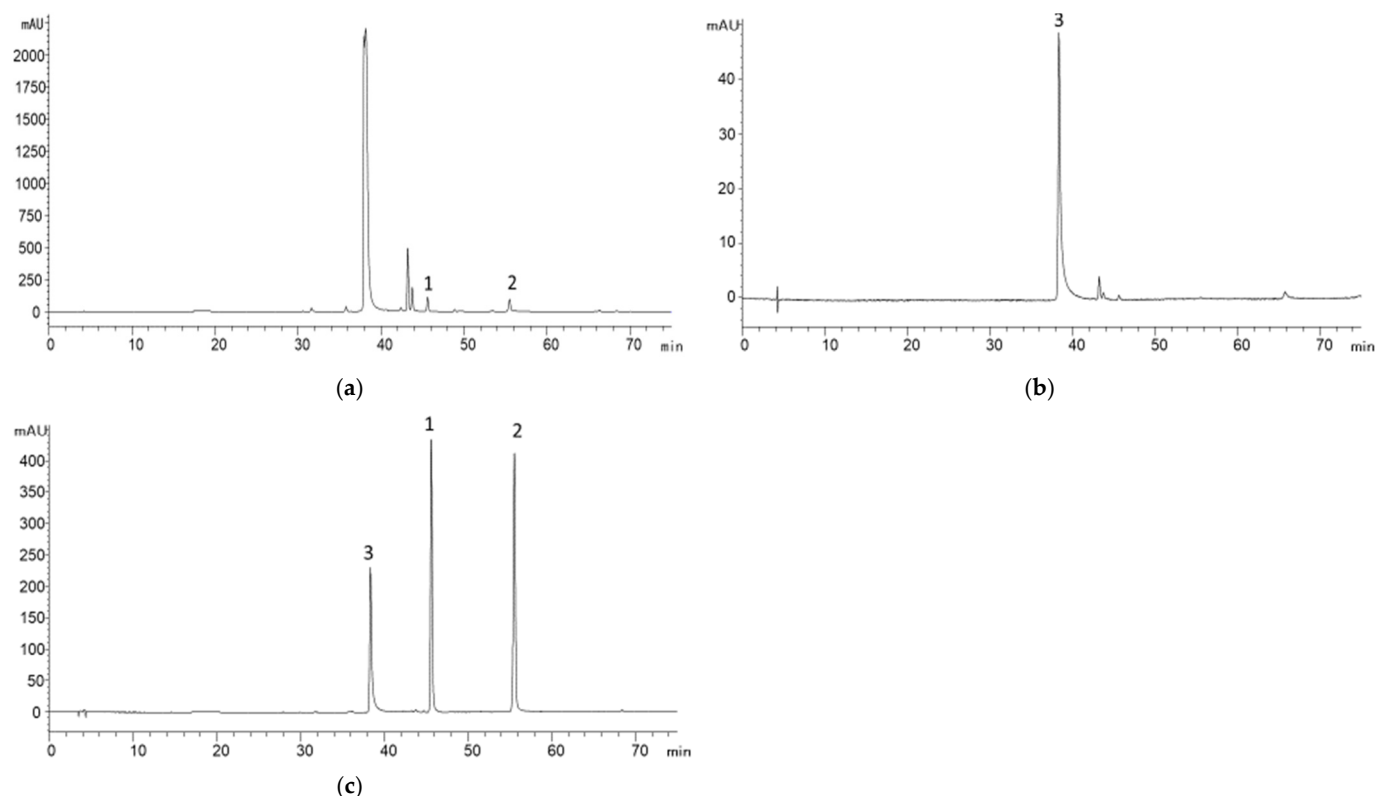


Figure 2. HPLC chromatogram of *Scutellaria baicalensis* extract. (a) The decoction was diluted 10 times. (b) The decoction was diluted 100 times. (c) Mixed reference solution. Peak 1 is wogonoside, Peak 2 is baicalein, and Peak 3 was baicalin.

2.2.6. Experiment Design

DSD was used to investigate the quantitative relationships among the CPPs, CMAs, and critical process evaluation indicators. Compared with other response surface designs, fewer experimental runs are required for DSD when researching the same number of parameters [19]. DSD cannot avoid mixing third-order and higher-order effects, but in this work, it is enough to establish a quantitative model containing second-order and first-order terms. Because the fluctuations in herbal properties have a greater impact on the composition of decoctions compared with changes in decoction process parameters [20], optimization of the decoction process's parameters was not conducted in this research.

After previous experiments and mechanism analysis [12], the selected CPPs are as follows: pH of first acid precipitation (X_1), first temperature holding time (X_2), pH of alkalization (X_3), ethanol amount (X_4), and end pH of ethanol washing (X_5). The ranges of CPPs were determined based on previous work [12]. The experimental design was carried out using Design Expert 12.0 software (Stat-Ease, Minneapolis, MN, USA), with the central points repeated five times for a total of 17 experiments. Due to the difficulties in controlling the pH values at the endpoint of the ethanol washing during the experiments, the measured values in the experiments were used. To research the impacts of herbal properties, different

decoctions were used in each experiment, and the experimental conditions are listed in Table 2.

Table 2. DSD parameter table.

Experiment Numbers	Number of Herb Material	pH of First Acid Precipitation X_1	First Temperature Holding Time (min) X_2	pH of Alkalization X_3	Ethanol Amount (g/g) X_4	End pH of Ethanol Washing X_5
1	S1	0.8	35	7.0	1.2	4.88
2	S2	2.2	35	7.5	0.8	4.35
3	S3	1.5	30	7.0	1.0	4.47
4	S1	2.2	30	6.5	1.2	4.34
5	S2	1.5	35	7.5	1.2	4.29
6	S4	0.8	25	6.5	1.2	4.75
7	S5	1.5	25	6.5	0.8	4.01
8	S4	0.8	30	7.5	0.8	4.18
9	S5	2.2	25	7.0	0.8	4.04
10	S6	0.8	35	6.5	0.8	4.10
11	S7	0.8	25	7.5	1.0	4.43
12	S6	2.2	35	6.5	1.0	3.96
13	S7	2.2	25	7.5	1.2	4.25
14	S3	1.5	30	7.0	1.0	4.19
15	S3	1.5	30	7.0	1.0	3.96
16	S8	1.5	30	7.0	1.0	4.29
17	S9	1.5	30	7.0	1.0	4.00

2.3. Data Processing

2.3.1. CMA Screening and Process Modeling

The quantitative model of the potential CMA, CPPs, and critical process evaluation indicators was established by Formula (2).

$$Y = b_0 + \sum_{i=1}^n b_i X_{c,i} + \sum_{i=1}^n b_{ii} X_{c,i}^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n b_{ij} X_{c,i} X_{c,j} + \sum_{k=1}^m c_k Z_{c,k} \quad (2)$$

The numbers of the CPPs and potential CMA are n and m , respectively; $X_{c,i}$ and $X_{c,j}$ are CPPs; $Z_{c,k}$ is the potential CMA; b_i , b_{ii} , b_{ij} and c_k are the partial regression coefficients for each term; and b_0 is the constant term. The backward stepwise regression method was used to screen the CMA, eliminating unimportant items to simplify the model, with the threshold set at 0.10. Data analysis was performed using Design Expert (Stat-Ease Inc., version 12.0, Minneapolis, MN, USA).

2.3.2. Design Space Calculation

MATLAB (R2018b, version 9.0, The Math Works Inc., Natick, MA, USA) was used for calculations, and the process was as follows [21–23]: It was assumed that the experimental results under different process conditions followed a normal distribution. The mean values and standard deviations of each response variable were obtained through center point experiments, and subsequently, Monte Carlo sampling was employed to simulate the values of each response under various process conditions. Modeling was carried out using stepwise regression according to Formula (2), with the models established from the Monte Carlo-simulated values used to compute predictive outcomes. Based on these model predictions, the probability of meeting all critical-process evaluation criteria was calculated. A minimum acceptable probability of 0.80 within the design space was set, with 500 simulation runs conducted. Parameter combinations satisfying the probability requirement were then summarized, followed by further statistical analysis of the controllable parameter

combinations that met the probability criterion under variations in noise parameters, from which the design space was determined.

3. Results

3.1. Properties of Decoction

In this work, nine batches of *Scutellaria baicalensis* herbs were selected, which were decocted as described in Section 2.2.5. The flavonoid contents and total solids of each batch of decoction were measured, as shown in Table 3. The total solids yield of each batch of the decoction ranged from 380 mg/g to 492 mg/g, indicating that the amount of solids transferred from per gram of herb varied among the different batches of *Scutellaria baicalensis* herbs. Among the three flavonoids, baicalin had the highest yield, reaching nearly 100 mg/g herb, making it one of the main components in *Scutellaria baicalensis* decoction. Wogonoside followed with the second-highest yield, while baicalein had a yield below 3 mg/g herb.

Table 3. Physicochemical properties of different batches of the *Scutellaria baicalensis* decoction.

Experiment Numbers	Number of Herb Material	The Yield of Baicalin (mg/g) Z ₁	The Yield of Wogonoside (mg/g) Z ₂	The Yield of Baicalein (mg/g) Z ₃	The Yield of Total Solids (mg/g) Z ₄	Purity of Baicalin (%) Z ₅	Purity of Wogonoside (%) Z ₆	Purity of Baicalein (%) Z ₇
1	S1	99.29	21.00	2.12	387.9	25.59	5.41	0.55
2	S2	90.78	19.11	1.97	458.7	19.79	4.17	0.43
3	S3	95.75	20.80	1.35	441.2	21.70	4.71	0.31
4	S1	99.29	21.00	2.12	387.9	25.59	5.41	0.55
5	S2	90.78	19.11	1.97	458.7	19.79	4.17	0.43
6	S4	82.74	20.17	1.75	491.2	16.84	4.11	0.36
7	S5	70.81	17.27	2.64	466.3	15.18	3.70	0.57
8	S4	82.74	20.17	1.75	491.2	16.84	4.11	0.36
9	S5	70.81	17.27	2.64	466.3	15.18	3.70	0.57
10	S6	93.78	20.62	1.91	440.4	21.29	4.68	0.43
11	S7	89.23	19.12	1.28	399.4	22.34	4.79	0.32
12	S6	93.78	20.62	1.91	440.4	21.29	4.68	0.43
13	S7	89.23	19.12	1.28	399.4	22.34	4.79	0.32
14	S3	95.75	20.80	1.35	441.2	21.70	4.71	0.31
15	S3	95.75	20.80	1.35	441.2	21.70	4.71	0.31
16	S8	86.21	21.18	0.910	453.9	18.99	4.67	0.20
17	S9	82.20	19.17	1.59	476.7	17.24	4.02	0.33

3.2. Potential CMAs Identified

The Pearson correlation analysis was conducted on the properties of different batches of the decoctions to preliminarily screen the attributes of the *Scutellaria baicalensis* herbs. The results are shown in Table 4. The correlation coefficients between the purity of baicalin (Z₅) and the yield of baicalin (Z₁) or the purity of wogonoside (Z₆) were all above 0.90. Therefore, the yield of baicalin (Z₁) can be used as an alternative to the purity of baicalin (Z₅) and the purity of wogonoside (Z₆) can be used as one of potential critical attributes of *Scutellaria baicalensis* herbs. The correlation coefficient between the purity of baicalein (Z₇) and the yield of baicalein (Z₃) was above 0.95, indicating a strong correlation. Thus, the yield of baicalein (Z₃) can be selected as a representative. Other potential CMAs include the yield of wogonoside (Z₂) and total solids (Z₄).

Table 4. Pearson correlation coefficient and the p value obtained from the correlation analysis of the properties of *Scutellaria baicalensis*.

	Yield of Baicalin (mg/g)	Yield of Wogonoside (mg/g)	Yield of Baicalein (mg/g)	Yield of Total Solids (mg/g)	Purity of Baicalin (%)	Purity of Wogonoside (%)
Yield of Wogonoside (mg/g)	Z ₂	0.808 (0.000)				
Yield of Baicalein (mg/g)	Z ₃	−0.413 (0.099)	−0.559 (0.020)			
Yield of Total Solids (mg/g)	Z ₄	−0.630 (0.007)	−0.297 (0.247)	0.138 (0.597)		
Purity of Baicalin (%)	Z ₅	0.914 (0.000)	0.640 (0.006)	−0.292 (0.255)	−0.887 (0.000)	
Purity of Wogonoside (%)	Z ₆	0.866 (0.000)	0.747 (0.001)	−0.366 (0.149)	−0.854 (0.000)	0.960 (0.000)
Purity of Baicalein (%)	Z ₇	−0.199 (0.444)	−0.424 (0.090)	0.954 (0.000)	−0.155 (0.552)	−0.012 (0.965)

(p value).

3.3. Influences of CMAs and CPPs

The results obtained from the DSD experiments are shown in Table 5. The yield of total solids was 2.8~49.33 mg/g. The yield of baicalin was mostly higher than 15 mg/g, and the yield of wogonoside was mostly higher than 0.1 mg/g.

Table 5. Experimental results of DSD.

Experiment Number	Number of Herb Materials	Yield of Baicalin (mg/g) Y ₁	Yield of Wogonoside (mg/g) Y ₂	Yield of Baicalein (mg/g) Y ₃	Yield of Total Solids (mg/g) Y ₄
1	S1	1.860	0.03220	0.01670	2.840
2	S2	17.64	0.2544	0.1662	24.87
3	S3	36.04	0.4843	0.2334	49.33
4	S1	17.45	0.1378	0.05260	21.96
5	S2	16.50	0.1568	0.05890	21.16
6	S4	5.630	0.0739	0.02130	8.590
7	S5	29.38	0.2767	0.1501	40.12
8	S4	24.50	0.3717	0.07680	32.70
9	S5	31.76	0.3138	0.1044	41.46
10	S6	26.66	0.3221	0.1861	35.27
11	S7	6.640	0.0981	0.01220	9.500
12	S6	30.29	0.2195	0.1066	40.62
13	S7	17.22	0.1622	0.05430	22.78
14	S3	22.51	0.3169	0.1213	30.39
15	S3	28.81	0.2877	0.2636	37.40
16	S8	24.92	0.3156	0.04880	32.94
17	S9	20.73	0.2179	0.1128	26.66

Quantitative mathematical models were established for each critical process evaluation indicator, with the potential CMAs and CPPs based on Formula (2). The regression coefficients and variance analysis results of each model are shown in Table 6. All p values were less than 0.05, indicating that the models were significant. The R^2 values for all models were greater than 0.80, with a small difference from R^2_{adj} , suggesting that these models can explain most variations and fit well. According to the model term screening results, Z₁ was the CMA, while X₁, X₂, X₃, X₄, and X₅ were all CPPs.

Table 6. Analysis of variance results for multiple regression models. Y_i is the yield of the i -th component (mg/g), and X_i is the i -th CPP.

Terms	Y_1		Y_2		Y_3		Y_4	
	Regression Coefficient	P	Regression Coefficient	P	Regression Coefficient	P	Regression Coefficient	P
constant	150.72	-	0.6993	-	5.77	-	187.96	-
Z_1					0.0045	<0.0001		
X_1								
X_2					−0.0039	0.0044		
X_3								
X_4					−0.2558	<0.0001	−18.14	0.0665
X_5	−30.65	<0.0001			−2.53	0.0007	−33.57	<0.0001
X_2^2			0.00002	0.0118				
X_5^2			−0.0077	0.0516	0.2751	0.0012		
X_1X_3					0.0018	0.0008		
X_2X_3					0.0002	0.0005		
X_4X_5			−0.0818	0.0005				
p value	<0.0001		<0.0001		<0.0001		<0.0001	
R^2	0.8223		0.8422		0.9593		0.8474	
R_{adj}^2	0.8104		0.8058		0.9276		0.8256	

Figures 3–6 show the contour plots of the effects of the CMA and CPPs on each critical-process evaluation indicator. The higher was X_5 , and the lower were Y_1 , Y_3 , and Y_4 . The CMA had little effect on the other three critical process evaluation indicators except baicalein yield.

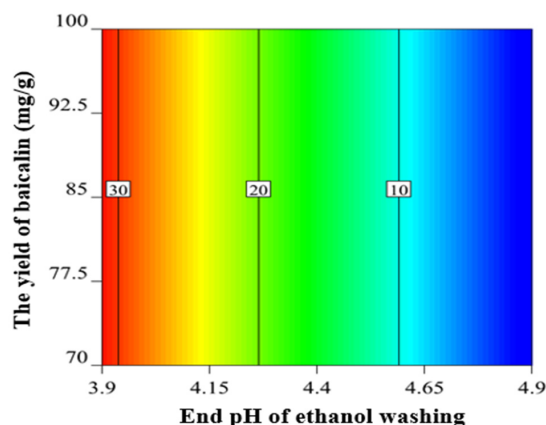


Figure 3. Contour plot of Y_1 . $X_1 = 1.5$; $X_2 = 30$ min; $X_3 = 7.0$; $X_4 = 1.0$ g/g.

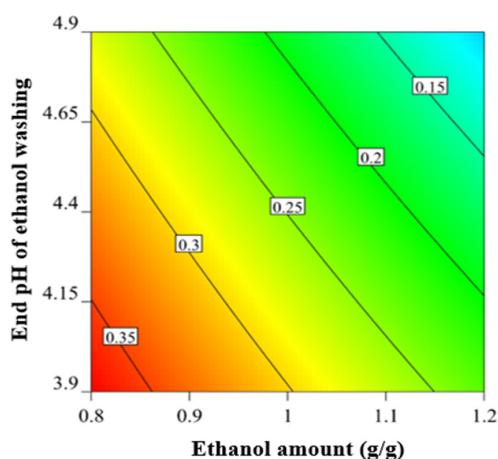


Figure 4. Contour plot of Y_2 . $X_1 = 1.5$; $X_2 = 30$ min; $X_3 = 7.0$.

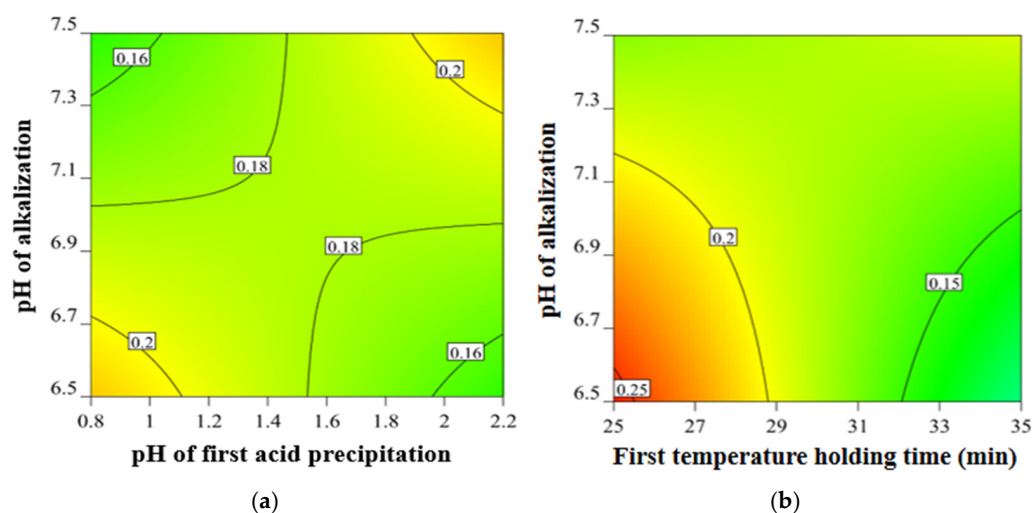


Figure 5. Contour plot of Y_3 . (a) $X_2 = 30$ min; $X_4 = 1.0$ g/g; and $X_5 = 4.2$. (b) $X_1 = 1.5$; $X_4 = 1.0$ g/g; and $X_5 = 4.2$.

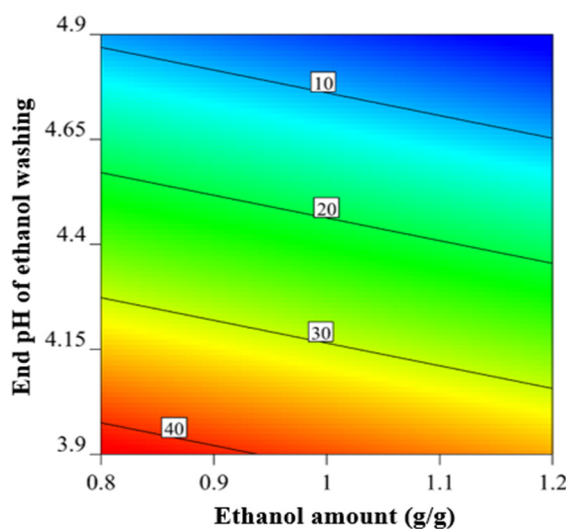


Figure 6. Contour plot of Y_4 . $X_1 = 1.5$; $X_2 = 30$ min; $X_3 = 7.0$.

3.4. Design Space Calculation and Verification

The lower limits and minimum probability of reaching the criteria for each critical process evaluation indicator are shown in Table 7. The design space obtained by the probability-based method, and verification points are shown in Figure 7.

Table 7. The lower limit of each critical process evaluation indicator and the probability requirements for meeting the criteria.

Critical Process Evaluation Indicators	Lower Limits	Minimum Probability of Reaching the Criteria
The yield of baicalin (mg/g)	13	$\geq 80\%$
The yield of wogonoside (mg/g)	0.1	
The yield of baicalein (mg/g)	0.03	
The yield of total solids (mg/g)	15	

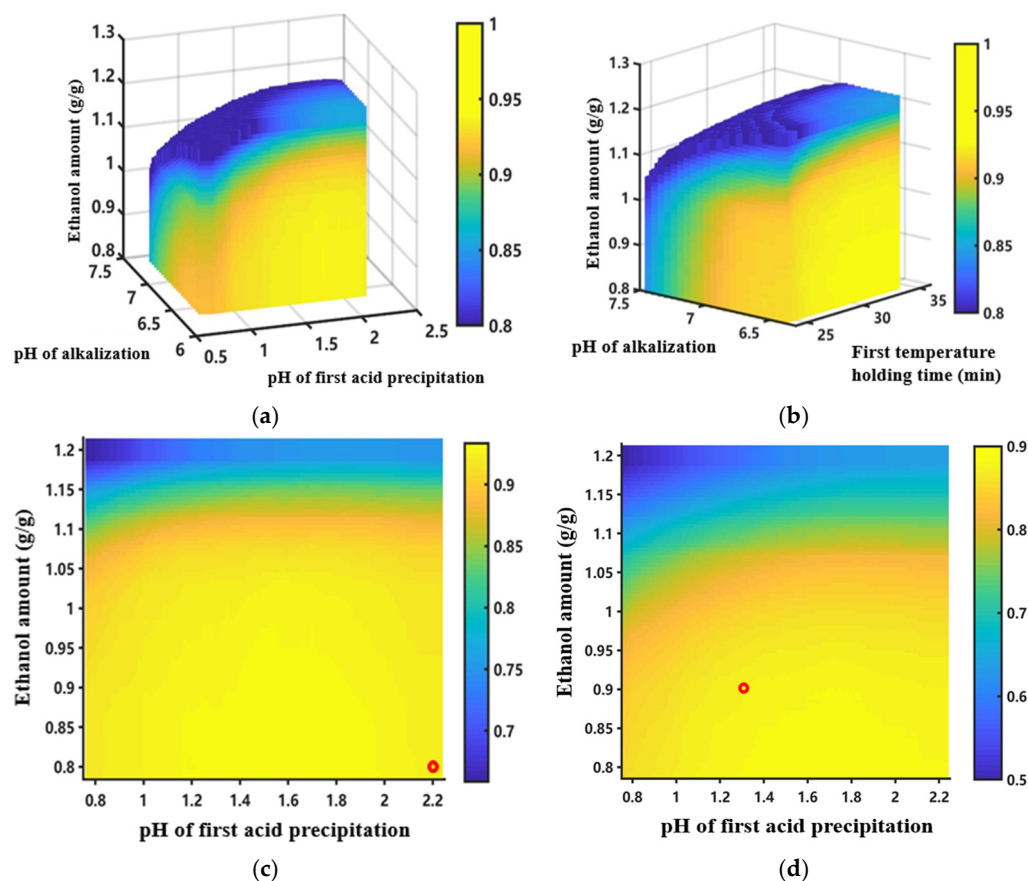


Figure 7. Design space diagram. (a) $Z_1 = 99.29$ mg/g; $X_2 = 35$ min; $X_5 = 4.0\sim 4.5$. (b) $Z_1 = 99.29$ mg/g; $X_1 = 2.2$; $X_5 = 4.0\sim 4.5$. (c) $Z_1 = 85.468$ mg/g; $X_2 = 35$ min; $X_3 = 6.5$; $X_5 = 4.0\sim 4.5$. (d) $Z_4 = 90.127$ mg/g; $X_2 = 30$ min; $X_3 = 7.5$; $X_5 = 4.0\sim 4.5$. The color bar indicates the probability of the critical process evaluation indicators reaching the criteria; ○ indicates the verification point.

To conduct validation experiments, two batches of new decoctions were prepared, and their CMAs were measured. The validation experiment conditions are listed in Figure 7c,d, with the results shown in Table 8. Most of the measured values from the validation experiments closely matched the predicted values, indicating the relatively good predictive performance of the model. However, there were still some validation experiments in which the measured values of Y_1 , Y_3 , and Y_4 differed significantly from the predicted values; this may be due to too few batches or unknown interference factors during modeling. The experimental results obtained at points within the design space met the set standards, suggesting that the constructed design space is relatively reliable.

Table 8. Verification of experimental point conditions and results ($n = 3$).

Experimental Conditions and Process Evaluation Indicators		Inside Design Space					
		V1	V2	V3	V4	V5	V6
Y_1	Z_1	85.468	85.468	85.468	90.127	90.127	90.127
	X_1	2.2	2.2	2.2	1.3	1.3	1.3
	X_2	35	35	35	30	30	30
	X_3	6.564	6.551	6.511	7.536	7.567	7.507
	X_4	0.8	0.8	0.8	0.9	0.9	0.9
	X_5	4.137	4.115	4.068	4.132	4.157	4.257
	Predicted values (mg/g)	23.91	24.58	26.02	24.06	23.29	20.23
	Measured values (mg/g)	34.97	37.25	38.57	25.31	19.97	26.89

Table 8. Cont.

Experimental Conditions and Process Evaluation Indicators		Inside Design Space					
		V1	V2	V3	V4	V5	V6
Y ₂	Predicted values (mg/g)	0.2871	0.2891	0.2931	0.3152	0.3127	0.3029
	Measured values (mg/g)	0.2782	0.3205	0.3225	0.3183	0.2710	0.3717
Y ₃	Predicted values (mg/g)	0.0468	0.0524	0.0633	0.1478	0.1389	0.1103
	Measured values (mg/g)	0.1321	0.2179	0.1186	0.0840	0.0758	0.1107
Y ₄	Predicted values (mg/g)	34.57	35.31	35.66	32.93	32.09	28.73
	Measured values (mg/g)	38.64	44.85	47.64	29.64	25.60	34.16

4. Conclusions

In this research, the critical processes of *Scutellaria baicalensis* extract preparation were optimized through design space methods based on the QbD concept. First, wogonoside yield, baicalin yield, baicalein yield, and total solids yield were selected as critical process evaluation indicators. Then, DSD experiments were performed to obtain data. After that, quantitative models were established using stepwise regression for the CMAs, CPPs, and critical process evaluation indicators. The model's R^2 was above 0.80, indicating that it can explain most variations of the data. According to the model, the yield of baicalin in the decoction was screened as the CMA, while the pH of first acid precipitation, the first temperature holding time, the pH of alkalization, the ethanol amount, and the end pH of ethanol washing were CPPs. The end pH of ethanol washing was regarded as a noise parameter. The Monte Carlo algorithm was used to calculate the design space of the controllable parameters within the process, which was then validated. The validation results indicate that operating within the design space can ensure that critical process evaluation indicators such as baicalin yield meet the standards and that the quality of the extracted product retains good batch-to-batch consistency. Normal operation ranges to attain the criteria of process evaluation indicators with a probability more than 80% are recommended as follows: X_1 of 0.8–2.2, X_2 of 25–35 min, X_3 of 6.5–7.5, and X_4 of 0.8–1.2 g/g.

In this work, there are still some shortcomings. Only nine batches of *Scutellaria baicalensis* were investigated, and the coverage of the differences in the sources and batches of herbal materials was limited. Therefore, in industrial production, it will be necessary to collect more data. If the sample size of the herbal materials is increased, the mathematical models and corresponding design spaces need to be further updated. Additionally, although this work selected three indicator components, there was no clear correlation between the standards and the therapeutic efficacy of the components. Correspondingly, the association between the CMAs of medicinal herbs and drug efficacy has not been established. This requires more pharmacological research and clinical data support.

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Abbreviations

The following abbreviations are used in this manuscript:

QbD	Quality by Design
CPP	critical process parameter
CMA	critical material attribute
CQA	critical quality attribute
LOD	limits of detection
LOQ	limits of quantification
DSD	Definitive Screening Design
HPLC	high-performance liquid chromatography

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