Extraction Process of Wuteng Qufeng Zhitong Powder

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Abstract [**Objectives**] To establish the quality standard for Wuteng Qufeng Zhitong Powder. [**Methods**] The orthogonal design was used to optimize the water extraction process with the amount of water, extraction time and extraction times as the factors, and the content of protocatechuic acid and dry extract rate as the indicators. The content of protocatechuic acid of *Embelia parviflora* Wall in Wuteng Qufeng Zhitong Powder was determined by HPLC. [**Results**] The best water extraction process is: soaking for 0.5 h, adding water to decoct twice, adding 12 times of water for the first time, adding 10 times of water for the second time, decocting for 1 h each time. The average content of protocatechuic acid was 14.41 mg/g, and the average dry yield was 23.47%. [**Conclusions**] The preparation of Wuteng Qufeng Zhitong Powder by water extraction method has the characteristics of high efficiency and suitable for large-scale production. The quality control method is reliable, rapid and accurate, and can effectively control the quality of the lotion.

Key words Wuteng Qufeng Zhitong Powder, Extraction process, Content determination

1 Introduction

Wuteng Qufeng Zhitong Powder is ethnic medicine prescription of the Department of Rheumatology of Guangxi International Zhuang Medicine Hospital. It consists of 13 Chinese medicinal herbs, including *Parabarium micranthum*, *Embelia parviflora* Wall, *Tinospora sinensis*, *Sarcandra glabra*, *Piper kadsura*, *Phyllanthus reticulatus*, and *Semiliquidambar cathayensis*. It is suitable for rheumatism caused by wind-cold-dampness toxin, and has obvious curative effect. Traditional Chinese medicine therapy is an external treatment for rheumatism created by the Chinese people in their long-term medical practice[1]. From the Pre-Qin Dynasty to the Qing Dynasty, it has been documented and widely used in clinical practice[2]. According to the different skin symptoms, the appropriate drugs for treatment can directly act on the lesion site, and the effect of absorption through the skin is better. However, we should further study the mechanism and effective factors of TCM therapy, grasp the law of TCM treatment, and improve its efficacy.

By completing the relevant clinical data analysis, experimental research and writing of related materials of Wuteng Qufeng Zhitong Powder, and then submitting the materials to the Guangxi Zhuang Autonomous Region Drug Administration for the record, the national medicine preparation products are made into legal products, which are convenient for mass production and can improve the quality and competitiveness of the products of national medicine preparations and increase sales. Besides, it is expected that the project will be developed into a traditional Chinese medicine ethnic medicine preparation for the treatment of rheumatism with Guangxi characteristics in the future, which will promote the economic development of the region, build the industrial chain of ethnic medicine and strengthen the advantages of ethnic medicine. In order to further control the quality of the product and ensure its clinical efficacy, we studied its extraction process and quality standards.

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2 Materials

2.1 Instruments Agilent 1260 high performance liquid chromatograph; ME155DU electronic balance (Mettler); Simplicity ultrapure water system (Millipore China); SHB-III circulating water multi-purpose vacuum pump (Zhengzhou Changcheng Science, Industry and Trade Co., Ltd.); TGL-16G high-speed table centrifuge (Shanghai Anting Scientific Instrument Factory); HWS-26 electric thermostatic water bath (Shanghai Qixin Scientific Instrument Co., Ltd.).

2.2 Raw materials and reagents *P. micranthum*, *E. parviflora*, *T. sinensis*, *S. glabra*, *P. kadsura*, *Daemonorops margaritae*, *Spatholobus suberectus*, *L. japonicum*, *Rhododendron molle*, *Blumea balsamifera*, *Claoxylon indicum*, *P. reticulatus*, *S. cathayensis* were purchased from Guangxi Xianzhu Traditional Chinese Medicine Technology Co., Ltd.; Protocatechuic acid reference substance (batch No.: 110809-202207) was purchased from the China Institute for Food and Drug Control. Methanol (chromatographically pure, batch No.: 208502), acetonitrile (chromatographically pure, batch No.: 17006) was purchased from Thermo Fisher Technology Co., Ltd.

3 Methods and results

In this study, high performance liquid chromatography was used to

determine the content of protocatechuic acid in the extract of *E. parviflora Wall*, *and the detection wavelength was* 260 nm[3].

3.1 Preparation of reference substance solution Accurately weighed a proper amount of protocatechuic acid reference substance, added methanol to dissolve and fix the volume, and prepared a reference substance solution with a concentration of 0.962 5 mg/mL. Pipetted precisely 0.5 mL of the above reference substance solution into a 2 mL volumetric flask and added methanol up to the scale on the volumetric flask to bring the total volume of the solution to 2 mL, obtaining a concentration of 0.240 6 mg/mL.

3.2 Preparation of test solution Accurately weighed the required medicinal materials according to the formula, and extracting twice. The first time extraction: soaked for 0.5 h, added 10 times of water, and then extracted for 1 h. The second time extraction: added 8 times the amount of water and carried out the extraction for 1 h. Combined the decoctions obtained by the two extractions. Accurately measured the total volume (mL). Took out a proper amount of supernatant from the combined decoction, filtered the supernatant through a 0.45 μm microporous membrane, and collected filtrate, wherein the part of liquid is the test solution.

3.3 Chromatographic conditions Chromatographic column was Aglient C_{18} column (5 μm, 4.6 mm × 250 mm). The mobile phase consisted of acetonitrile (A)-0.1% phosphoric acid in water (B), and the gradient elution procedure is shown in Table 1. The flow rate was 1 mL/min, the column temperature was 30 ℃, the injection volume was 10 μL, and the detection wavelength of protocatechuic acid was set at 260 nm[4]. The number of theoretical plates should not be less than 3 000 to ensure good separation. Protocatechuic acid in the sample can be effectively separated from other components, and other components do not interfere with the determination result.

Table 1 Gradient elution procedure

Time//min	Mobile phase	
	Acetonitrile (A)//%	0.1% (*V*/*V*) Phosphoric acid aqueous solution (B)//%
0	5	95
8	15	85
20	35	65
30	64	36

3.4 Methodological investigation

3.4.1 Linear relationship investigation. Accurately pipetted a certain amount of the above reference substance solution (0.962 5 mg/mL), separately, put it into a 5 mL brown volumetric flask, and then diluted it to the scale with methanol to prepare 6 reference substance solutions with different concentrations. Separately injected the sample, the injection volume was 10 μL, the liquid chromatograph was used for detection, the peak area was determined, the concentration (mg/mL) was used as the abscissa, the peak area was used as the ordinate, and the standard curve was plotted. The regression equation was $Y = 14\,298X + 782.08$, $R^2 = 0.999\,6$. The calibration curve was linear in the range of 0.038 5 – 0.577 5 mg/mL.

3.4.2 Precision test. Accurately pipetted the same portion of protocatechuic acid reference substance solution for 6 consecutive injections. Determined the peak area of protocatechuic acid according to the liquid chromatography method in Section **3.3**[5-6]. The experimental results showed that the *RSD* of the peak area of protocatechuic acid was 0.79%, which was less than 3.00%, indicating that the instrument had good precision.

3.4.3 Stability test. Weighed 1 portion of Wuteng Qufeng Zhitong Powder medicinal materials according to the prescription proportion, and prepared the weighed medicinal materials into a test solution according to the method described in Section **3.2**. Then the test solution was injected for determination at 0, 2, 4, 8, 12 and 24 h, respectively. Determined the peak area of protocatechuic acid in the test sample according to the chromatographic condition method in Section **3.3** and made a record of it [5-6]. Finally, the *RSD* of the peak area of protocatechuic acid was calculated to be 0.87%, which was less than 3.0%, indicating that the test solution had good stability within 24 h.

3.4.4 Repeatability test. Weighed 6 portions of Wuteng Qufeng Zhitong Powder medicinal materials according to the proportion of the prescription. According to the method described in Section **3.2**, prepared each medicinal material into a test solution. Then, carried out detection and analysis according to the chromatographic conditions in Section **3.3**, determined the peak area of protocatechuic acid in each test solution, and calculated the average content of protocatechuic acid and its *RSD* value. The results showed that the average content of catechuic acid in Wuteng Qufeng Zhitong Powder was 12.82 mg/g, and the *RSD* was 1.61%, indicating that the experiment had good repeatability.

3.4.5 Sample recovery test. Precisely weighed 6 samples of known quantity of Wuteng Qufeng Zhitong Powder, each containing approximately 0.5 g, to a 10 mL volumetric flask. Accurately added appropriate amount of protocatechuic acid reference substance to each volumetric flask, and then added pure water to the scale mark. After shaking and mixing, took an appropriate amount of sample solution from each volumetric flask and centrifuged at 13 000 r/min for 10 min. Filtered the supernatant through a 0.45 μm microporous membrane. Recorded the peak area of protocatechuic acid and calculated the content of protocatechuic acid in the sample according to the chromatographic conditions under in Section **3.3**. The average recovery was 99.76% and *RSD* was 2.58% ($n = 6$), indicating that the method had good accuracy (Table 2).

3.5 Determination of dry yield The extraction conditions were studied, and the appropriate evaluation indicator, namely, dry yield, was selected according to the components of the medicinal materials. The experimental results were analyzed, and the dry yield was selected as one of the evaluation indexes of the extraction process to determine the extraction process parameters.

Table 2 Test results of recovery rate of Wuteng Qufeng Zhitong Powder ($n=6$)

No.	Sample weight//g	Sample weight//mg	Added amount//μg/mL	Measured amount//μg/mL	Recovery rate//%	Average sample recovery rate//%	*RSD*//%
1	0.500 2	6.41	6.42	13.00	102.65	99.76	2.58
2	0.500 6	6.42	6.42	12.69	97.75		
3	0.500 7	6.42	6.42	12.82	99.77		
4	0.500 6	6.42	6.42	13.03	102.93		
5	0.500 1	6.41	6.42	12.76	98.89		
6	0.500 3	6.41	6.42	12.62	96.60		

3.5.1 Establishment of determination method. After the extraction was completed, the total volume of the resulting liquid medicine was first measured. Then precisely pipetted 25 mL, transferred it to an evaporating dish pre-dried to constant weight, evaporated it in a water bath, and dried it in an electric heating constant temperature drying oven at 105 ℃ for 5 h. After drying, transferred the evaporating dish to a desiccator, cooled for 30 min, weighed accurately, dried at the above temperature for 1 h, took out, cooled and weighed again until the difference between two consecutive weighing results was not more than 5 mg. The dry extract yield was calculated according to the following formula:

Yield of dry extract = (Mass of dry extract/Sampling volume) × (Total volume of liquid medicine/Total mass of medicinal materials) × 100%

3.6 Orthogonal experiment The lotion was prepared by the traditional water extraction process. When the water decoction method was used to extract traditional Chinese medicine, the amount of added water, the extraction time, the soaking time of water added and the number of decoctions can all affect the extraction efficiency. Considering the actual mass production, work efficiency and economic cost, the amount of added water, the extraction time and the number of extractions have great influence on the extraction effect, which is more in line with the conditions of mass production. The orthogonal experiment was carried to optimize the extraction process by selecting the amount of water, decoction time and extraction times, and the content of protocatechuic acid and dry yield were used as evaluation indicators.

3.6.1 Factor-level design [7-8]. After considering the single factor experimental data and the actual production conditions, the key factors affecting the extraction efficiency of Wuteng Qufeng Zhitong Powder were determined as follows: the amount of added water (A), the extraction time (B) and the extraction times (C). In order to systematically evaluate and optimize the effect of these variables on the content of protocatechuic acid and the yield of dry extract, the $L_9(3^4)$ orthogonal array design method was used for experimental design. Specific experimental factor level settings are shown in Table 3.

Table 3 Factor levels of Wuteng Qufeng Zhitong Powder extraction process

Level	Factor		
	Added water//times (A)	Extraction time//h (B)	Extraction times (C)
1	8	0.5	1
2	10	1	2
3	12	1.5	3

3.6.2 Orthogonal design. According to the factors and levels of the orthogonal test design shown in Table 3, weighed the medicinal materials according to the proportion of the prescription, extracted, filtered and reserved. The results of the orthogonal experiment are shown in Table 4.

Table 4 Orthogonal experiment arrangement and results of Wuteng Qufeng Zhitong Powder extraction process

Level	Added water (A)	Extraction time (B)	Extraction times (C)	Blank (D)	Protocatechuic acid//mg/g	Dry yield//%
1	1	1	1	1	7.69	13.66
2	1	2	2	2	10.87	17.13
3	1	3	3	3	12.51	23.07
4	2	1	2	3	12.91	21.31
5	2	2	3	1	14.12	23.21
6	2	3	1	2	11.87	21.03
7	3	1	3	2	10.42	21.46
8	3	2	1	3	8.46	17.29
9	3	3	2	1	12.53	22.18
Protocatechuic acid	K_1	10.356	10.341	9.340	11.447	
	K_2	12.968	11.149	12.102	11.052	
	K_3	10.470	12.304	12.352	11.294	
	R	2.612	1.964	3.011	0.395	
Dry yield	K_1	17.953	18.810	17.327	19.683	
	K_2	21.850	19.210	20.207	19.873	
	K_3	20.310	22.093	22.580	20.557	
	R	3.897	3.283	27.833	0.873	

Table 5 Variance analysis of protocatechuic acid extraction

Source of variance	Sum of squares of deviations	Degrees of freedom	Variance	*F*	*P*
Amount of added water (A)	13.072 3	2.000 0	6.536 2	54.982 3	<0.05
Extraction time (B)	5.846 0	2.000 0	2.923 0	24.588 4	<0.05
Extraction times (C)	16.756 0	2.000 0	8.378 0	70.475 8	<0.05
Error (D)	0.237 8	2.000 0	0.118 9		

Table 6 Analysis of variance of dry yield extraction

Source of variance	Sum of squares of deviations	Degrees of freedom	Variance	*F*	*P*
Amount of added water (A)	23.109 5	2.000 0	11.554 7	18.257 5	>0.05
Extraction time (B)	19.253 9	2.000 0	9.626 9	15.211 4	>0.05
Extraction times (C)	41.524 6	2.000 0	20.762 3	32.806 2	<0.05
Error (D)	1.265 8	2.000 0	0.632 9		

The variance analysis of protocatechuic acid extraction (Table 5) showed that the factors of amount of added water (A), extraction time (B) and extraction times (C) had significant effects on the extraction process ($P < 0.05$). The primary and secondary factors were C > A > B, that is, the extraction times had the greatest effect, followed by the amount of added water and extraction time. The optimal process conditions were $A_2B_3C_3$.

The variance analysis of dry yield extraction (Table 6) showed that the factors of amount of added water (A) and extraction time (B) had no significant effect on the extraction process ($P > 0.05$), while the factor of extraction times (C) had significant effect on the extraction process ($P < 0.05$). The order of influencing factors is C > A > B, that is, the extraction times is the most important factor, followed by the amount of added water and extraction time, and the optimal process conditions are $A_2B_3C_3$.

Based on the results of variance analysis of protocatechuic acid and dry yield extraction, considering the factors of actual large-scale production and economic cost, in order to adapt to industrial large-scale production, and combining with traditional decoction methods, the extraction process of protocatechuic acid was determined as $A_2B_2C_2$, that is, after soaking in water for 0.5 h, extracting twice, adding 12 times of water in the first time (The water absorption rate of the medicinal materials was about 190%), extracting for 1 h, adding 10 times of water in the second time, and extracting for 1 h.

3.7 Extraction process validation Process validation was carried out according to the above extraction process optimization results. Weighed each medicinal material according to the prescription, soaked in water for 0.5 h, adding 12 times of water for the first time, adding 10 times of water for the second time, decocting twice, 1 h each time, and combined the decoctions. The test was repeated three times consecutively to determine the protocatechuic acid content and dry yield of the product, and the results are shown in Table 7.

Table 7 Validation test results of Wuteng Qufeng Zhitong Powder extraction process

Experiment No.	Protocatechuic acid//mg/g	Average value//mg/g	Dry yield//%	Average dry yield//%
1	14.19	14.41	23.41	23.47
2	14.68		23.76	
3	14.35		23.25	

The validation test results showed that the average content of protocatechuic acid was 14.41 mg/g and the average dry yield was 23.47% in the mixture prepared according to the optimized process, which indicated that the process was reasonable and feasible.

4 Conclusions

In order to control the quality of Wuteng Qufeng Zhitong Powder, the content of protocatechuic acid in the powder was determined by HPLC with the content of protocatechuic acid in the preparation as the indicator. Taking 13 Chinese medicinal herbs including *P. micranthum*, *E. parviflora* Wall, *T. sinensis*, *S. glabra*, *P. kadsura*, *P. reticulatus*, *S. cathayensis* as raw materials, the Wuteng Qufeng Zhitong Powder was prepared by water extraction and concentration process, and the quality standard of the preparation was preliminarily established to evaluate and control the quality of Wuteng Qufeng Zhitong Powder.

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