Optimization of Preparation Technology and Development of Quality Standards for Chengyin Mixture

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Abstract [**Objectives**] To optimize the extraction and molding technology of Chengyin Mixture, an empirical prescription of Zhuang medicine, and to establish its quality control method. [**Methods**] An $L_9(3^4)$ orthogonal experimental design was adopted, with water addition amount, extraction time, and extraction times as investigative factors, and dry extract yield, transfer rate of forsythiaside, and transfer rate of chlorogenic acid as evaluation indices to optimize the process. Referring to the relevant methods specified in the *Chinese Pharmacopoeia* (2020 Edition), TLC identification and preparation inspections for Lonicerae Japonicae Flos, Forsythiae Fructus, and Glycyrrhizae Radix et Rhizoma were established. [**Results**] The optimal process was determined as follows: adding 10 times the amount of water, decocting twice, with one hour for each decoction. Validation tests showed that the mean dry extract yield was 24.24%, and the mean transfer rates of forsythiaside and chlorogenic acid were 71.86% and 69.48%, respectively, with all *RSD* values below 5%. TLC spots were clear with no negative interference. The properties, relative density, pH, filling volume, and microbial limits of three batches of pilot-scale samples all complied with the pharmacopoeia standards. [**Conclusions**] The optimized extraction process is stable and feasible, and the established quality standard is simple to operate and highly specific, which can be used for the quality control of Chengyin Mixture, providing a basis for its hospital preparation registration and standardized production.

Key words Chengyin Mixture, Orthogonal experiment, Extraction process, Quality standard, Thin layer chromatography (TLC)

1 Introduction

Chengyin Mixture is a clinical empirical prescription developed by Professor Qin Zujie, a distinguished TCM practitioner of Guangxi International Zhuang Medical Hospital, in response to clinical demands. The formula comprises four components: *Godoengzhoengz* (*Clerodendrum japonicum*), Forsythiae Fructus, Lonicerae Japonicae Flos, and Glycyrrhizae Radix et Rhizoma, integrating both Zhuang and traditional Chinese medicinal materials. According to its formula theory, the prescription exhibits actions of clearing heat, removing toxicity, reducing swelling, and relieving pain. It is primarily indicated for acute inflammatory throat conditions, including wind-heat type acute tonsillitis and acute pharyngitis[1].

At present, this prescription is still applied in the form of traditional decoction, which suffers from problems such as inconvenience in preparation, unstable quality, and poor patient compliance, seriously restricting its clinical application and promotion. In order to develop this empirical prescription into a stable hospital preparation, it is urgent to conduct systematic research on its preparation technology and quality standards. Following the principle of "traditional technology, modern evaluation", this study adopted orthogonal experimental design, using dry extract yield, transfer rates of forsythiaside and chlorogenic acid as evaluation indices to optimize the water decoction extraction process and select appropriate molding excipients, while maintaining the therapeutic efficacy of the original decoction. Meanwhile, referring to the methods specified in the *Chinese Pharmacopoeia* (2020 Edition), TLC identification methods and preparation inspection items for Lonicerae Japonicae Flos, Forsythiae Fructus, and Glycyrrhizae Radix et Rhizoma were established. The aim is to provide scientific evidence for the hospital preparation registration and standardized production of Chengyin Mixture, and to promote the modernization development of Zhuang medicinal preparations.

2 Materials

2.1 Instruments The following equipment and instruments were used in this study: a multi-function extraction tank (TQNS-1500), an extraction and concentration unit (TQNS-1500), a jacketed kettle (JCG), a trough mixer (CH-200), an oscillating granulator (YK-160), an oven (CT-C-1), a vibrating sifter (ZS-600), a three-dimensional mixer (SBH-600), a particle packaging machine (DXDK40II), an electronic platform scale (100 kg/10 kg), and a high-performance liquid chromatograph equipped with a UV detector.

2.2 Drugs and reagents *Clerodendrum japonicum* (batch No.: 20230801, conforming to the *Quality Standard of Zhuang Medicinal Materials of Guangxi Zhuang Autonomous Region* (*Volume II*), Lonicerae Japonicae Flos (batch No.: 20230915, conforming to the *Chinese Pharmacopoeia* (2020 Edition, Part I), Forsythiae Fructus (batch No.: 20230922, conforming to the

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Chinese Pharmacopoeia (2020 Edition, Part I), and Glycyrrhizae Radix et Rhizoma (batch No.: 20231005, conforming to the *Chinese Pharmacopoeia* (2020 Edition, Part I) were all purchased from Guangxi Xianzhu Traditional Chinese Medicine Technology Co., Ltd. The reference standards of forsythiaside (batch No. 110821-201912, purity ≥98.0%) and chlorogenic acid (batch No. 110753-202116, purity ≥98.0%) were both obtained from the National Institutes for Food and Drug Control (NIFDC), China.

3 Methods and results

3.1 Extraction process This prescription consists of four herbal components, with the main active constituents encompassing flavonoids, tannins, phenolic acids, organic acids, iridoid glycosides, saponins, polysaccharides, amino acids, and others, all of which possess favorable water solubility. In order to preserve the therapeutic effect of the original decoction while also considering manufacturing costs, the conventional decoction extraction method was employed for the process optimization study.

3.1.1 Optimization of extraction process parameters[2-3]. Based on the physicochemical properties of the extracts, the primary factors influencing the extraction efficiency include extraction time, extraction frequency, water-to-material ratio, and soaking time, among others. Taking into account the practical production conditions, water addition amount (A), extraction time (B), and extraction frequency (C) were selected as the independent variables for the orthogonal experimental design. The transfer rates of forsythiaside and chlorogenic acid, together with the dry extract yield, were employed as the evaluation indicators. An $L_9(3^4)$ orthogonal experiment design was applied to optimize the extraction process of Chengyin Mixture. The factors and their corresponding levels are shown in Table 1.

Table 1 Factors and levels for the $L_9(3^4)$ orthogonal experimental design

Level	Factor		
	Water addition amount (A)	Extraction time (B)//min	Extraction frequency (C)//times
1	8	30	1
2	10	60	2
3	12	90	3

As shown in Table 2, the order of factors affecting the dry extract yield was C (extraction frequency) > B (extraction time) > A (water addition amount), indicating that extraction frequency exerted the greatest influence, followed by extraction time and water-to-material ratio, with the optimal combination being $C_3B_3A_3$. Similarly, the order of factors affecting the forsythiaside transfer rate was C (extraction frequency) > B (extraction time) > A (water addition amount), with extraction frequency again showing the predominant effect, followed by extraction time and water-to-material ratio, and the optimal combination was $C_3B_3A_3$. For the chlorogenic acid transfer rate, the factor order was C (extraction frequency) > B (extraction time) > A (water addition amount), with extraction frequency as the most influential factor, followed by extraction time and water-to-material ratio, and the optimal combination was $C_2B_1A_3$. According to the ANOVA results (Tables 3-5), extraction frequency had a statistically significant effect on dry extract yield, forsythiaside transfer rate, and chlorogenic acid transfer rate ($P<0.05$), further confirming that extraction frequency was the most critical factor. In contrast, extraction time and water addition amount showed no statistically significant effects on any of the three response indices ($P>0.05$).

Table 2 $L_9(3^4)$ orthogonal experimental design array and corresponding results

Experiment No.		Factor				Evaluation indicator//%	
		A	B	C	D	Dry extract yield	Forsythiaside transfer rate	Chlorogenic acid transfer rate
1		1	1	1	1	13.82	34.81	56.75
2		1	2	2	2	24.18	66.35	68.81
3		1	3	3	3	28.25	84.52	65.63
4		2	1	2	3	24.03	63.80	78.22
5		2	2	3	1	28.98	89.53	73.81
6		2	3	1	2	16.92	50.36	50.64
7		3	1	3	2	28.78	83.12	80.37
8		3	2	1	3	15.90	52.87	51.34
9		3	3	2	1	25.76	88.69	76.68
Dry extract yield//%	K_1	22.08	22.21	15.55	22.85			
	K_2	23.31	23.02	24.66	23.29			
	K_3	23.48	23.64	28.67	22.73			
	R	1.40	1.43	13.12	0.57			
Forsythiaside transfer rate//%	K_1	61.89	60.58	46.01	71.01			
	K_2	67.90	69.58	72.95	66.61			
	K_3	74.89	74.52	85.72	67.06			
	R	13.00	13.95	39.71	4.40			
Chlorogenic acid transfer rate//%	K_1	63.73	71.78	52.91	69.08			
	K_2	67.56	64.65	74.57	66.61			
	K_3	69.46	64.32	73.27	65.06			
	R	5.73	7.46	21.66	4.02			

Table 3 Analysis of variance for dry extract yield

Variance source	Sum of squares of deviations	Degree of freedom	Variance	*F*	Significance
A	3.484 3	2	1.742 1	6.564 8	>0.05
B	3.099 1	2	1.549 5	5.839 0	>0.05
C	271.320 8	2	135.660 4	511.197 3	<0.01
Error	0.530 8	2	0.265 4		

Table 4 Analysis of variance for forsythiaside transfer rate

Variance source	Sum of squares of deviations	Degree of freedom	Variance	*F*	Significance
A	253.993 4	2	126.996 7	7.227 7	>0.05
B	300.033 2	2	150.016 6	8.537 8	>0.05
C	2 465.531 8	2	1 232.765 9	70.159 7	<0.05
Error	35.141 7	2	17.570 8		

Table 5 Analysis of variance for chlorogenic acid transfer rate

Variance source	Sum of squares of deviations	Degree of freedom	Variance	*F*	Significance
A	51.149 9	2	25.574 9	2.076 5	>0.05
B	106.604 1	2	53.302 0	4.327 7	>0.05
C	885.375 2	2	442.687 6	35.942 8	<0.05
Error	24.632 9	2	12.316 4		

Based on the above results, the order of factors affecting the extraction process of Chengyin Mixture was C (extraction frequency) > B (extraction time) > A (water addition amount), indicating that extraction frequency (C) was the primary influencing factor, while extraction time (B) and water addition amount (A) exhibited no significant effects. To conserve energy, accommodate large-scale industrial production, and maintain consistency of the material basis in alignment with the traditional decoction method, the selected extraction process was as follows: decocting twice, with 10 times the amount of water each time, and 1 h for each decoction.

3.1.2 Validation experiments. Three portions of the prescription amount of medicinal materials were weighed and decocted twice, each with 10 times the amount of water for one hour. The decoctions were filtered, and the filtrates were combined for HPLC determination of the active components. Meanwhile, 25 mL of the aqueous extract (in duplicate) was transferred into a constant-weight evaporating dish for the determination of dry extract yield.

As shown in Table 6, the transfer rates of the three batches of samples were stable, and the dry extract yields were generally consistent, indicating that the optimized process is stable and feasible.

Table 6 Validation experiment results %

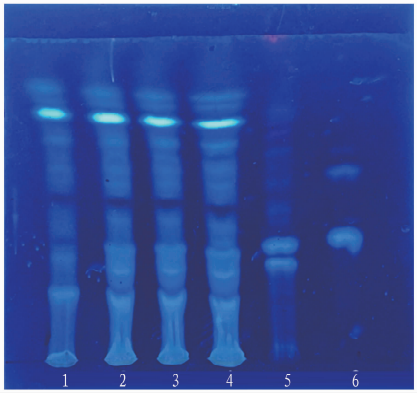
Experiment No.	Dry extract rate	Forsythiaside transfer rate	Chlorogenic acid transfer rate
1	24.35	68.91	71.59
2	24.68	75.02	69.48
3	23.76	71.65	67.36
Mean	24.24	71.86	69.48

3.2 Quality standard

3.2.1 Appearance. The three batches of pilot-scale samples (batch No.: 20240401, 20240402, and 20240403) were all brownish-black liquids with a slight amount of precipitate upon prolonged standing, possessing a slight aromatic odor and a slightly bitter taste. Accordingly, the appearance of this product is tentatively specified as a yellowish-brown to brownish-black liquid, with a small amount of precipitate upon prolonged standing, a slight aromatic odor, and a slightly bitter taste.

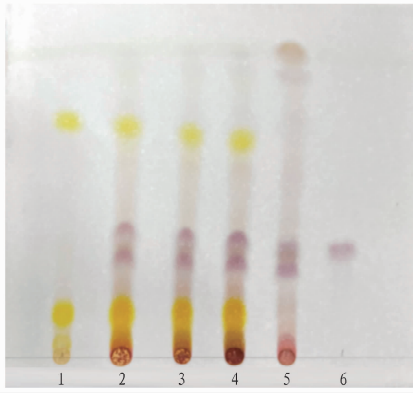
3.2.2 Thin-layer chromatography. (i) Lonicerae Japonicae Flos. A 10 mL portion of the sample was mixed with 5 mL of methanol, ultrasonicated for 10 min, cooled, and filtered. The subsequent filtrate was used as the test solution. A negative control solution was prepared in the same manner using 10 mL of the sample without Lonicerae Japonicae Flos. Separately, 0.2 g of the reference medicinal material powder of Lonicerae Japonicae Flos was added to 5 mL of methanol, ultrasonicated for 30 min, cooled, and filtered; the filtrate was used as the reference medicinal material solution. A reference solution was also prepared by dissolving chlorogenic acid reference standard in methanol to a concentration of 1 mg/mL. Following the TLC method described in the *Chinese Pharmacopoeia* (2020 Edition, General Chapter 0502), 3 μL of each of the above solutions (test, negative control, reference medicinal material, and reference standard) were spotted onto the same polyamide film. The plate was developed with the upper phase of a mixture of butyl acetate, formic acid, and water (7∶3∶2.5), then removed and air-dried. The spots were visualized under ultraviolet light at 365 nm. As shown in Fig. 1, the test solution exhibited fluorescent spots of the same color at positions corresponding to those in the reference medicinal material and reference standard chromatograms.

(ii) Forsythiae Fructus. A 10 mL portion of the sample was extracted twice with petroleum ether (60 – 90 ℃), 10 mL each time. The lower layer was retained, and the petroleum ether layer



NOTE 1. Negative control (without Lonicerae Japonicae Flos); 2 – 4. Test solutions (batches 20240401, 20240402, and 20240403); 5. Reference medicinal material solution of Lonicerae Japonicae Flos; 6. Chlorogenic acid reference standard solution.

Fig. 1 TLC chromatogram of Lonicerae Japonicae Flos

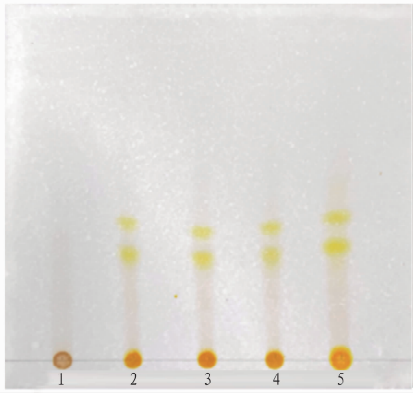


NOTE 1. Negative control (without Forsythiae Fructus); 2 – 4. Test solutions (batches 20240401, 20240402, and 20240403); 5. Reference medicinal material solution of Forsythiae Fructus; 6. Forsythiaside reference standard solution.

Fig. 2 Fig. 1 TLC chromatogram of Forsythiae Fructus

was discarded. The residue was evaporated to dryness to remove residual petroleum ether, then dissolved in 20 mL of methanol and ultrasonicated for 20 min. After cooling, the solution was filtered, and the filtrate was evaporated to dryness. The final residue was redissolved in 5 mL of methanol to obtain the test solution. A negative control solution was prepared in the same manner using 10 mL of the sample without Forsythiae Fructus. Separately, 1 g of the reference medicinal material powder of Forsythiae Fructus was placed in a stoppered flask with 25 mL of petroleum ether (60 – 90 ℃), ultrasonicated for 15 min, cooled, and filtered. The petroleum ether filtrate was discarded, and the residue was evaporated to remove residual petroleum ether. Methanol (20 mL) was then added, ultrasonicated for 20 min, cooled, and filtered. The filtrate was evaporated to dryness, and the residue was redissolved in 5 mL of methanol to serve as the reference medicinal material solution. A reference standard solution of forsythiaside was prepared in methanol at a concentration of 1 mg/mL. TLC was carried out on silica gel G plates according to the method described in the *Chinese Pharmacopoeia* (2020 Edition, General Chapter 0502). Aliquots of 2 μL each of the above four solutions (test, negative control, reference medicinal material, and reference standard) were spotted onto the same plate. The plate was developed with a mixture of chloroform and methanol (8 ∶ 1), removed, and air-dried. It was then sprayed with 10% sulfuric acid in ethanol and heated at 105 ℃ until the spots became clearly visible. In the chromatogram of the test solution, spots of the same color appeared at positions corresponding to those in the reference medicinal material and reference standard chromatograms (Fig. 2).

(iii) Glycyrrhizae Radix et Rhizoma. A 4 g portion of the sample was ground to a fine powder and refluxed with 50 mL of methanol for one hour After cooling, the mixture was filtered, and the filtrate was evaporated to dryness. The residue was dissolved in 40 mL of water, and the solution was extracted with three 20 mL portions of n-butanol. The n-butanol extracts were combined and washed with three 20 mL portions of water. The aqueous washings were discarded, and the n-butanol layer was evaporated to dryness. The residue was redissolved in 1 mL of methanol to obtain the test solution. A negative control solution was prepared in the same manner using 4 g of the sample without Glycyrrhizae Radix et Rhizoma. A 1 g portion of the reference medicinal material powder of Glycyrrhizae Radix et Rhizoma was refluxed with 40 mL of diethyl ether for 1 hour. After cooling, the mixture was filtered, and the ether filtrate was discarded. The residue was refluxed with 30 mL of methanol for 1 h, cooled, and filtered. The filtrate was evaporated to dryness, and the residue was dissolved in 40 mL of water. The solution was extracted with three 20 mL portions of n-butanol. The combined n-butanol extracts were washed with three 20 mL portions of water, and the aqueous washings were discarded. The n-butanol layer was evaporated to dryness, and the residue was redissolved in 1 mL of methanol to obtain the reference medicinal material solution. TLC was carried out on silica gel G plates according to the method described in the *Chinese Pharmaco poeia*



NOTE 1. Negative control (without Glycyrrhizae Radix et Rhizoma); 2 – 4. Test solutions (batches 20240401, 20240402, and 20240403); 5. Reference medicinal material solution of Glycyrrhizae Radix et Rhizoma.

Fig. 3 TLC chromatogram of Glycyrrhizae Radix et Rhizoma

(2020 Edition, General Chapter 0502). Aliquots of 2 μL each of the test solution, negative control, and reference medicinal material solution were spotted onto the same plate. The plate was developed with a mixture of cyclohexane, ethyl acetate, and formic acid (13∶6∶1), removed, and air-dried. It was then sprayed with 10% sulfuric acid in ethanol and heated at 105 ℃ until the spots became clearly visible. The plate was examined under ultraviolet light at 365 nm. As shown in Fig. 3, the test solution exhibited fluorescent spots of the same color at positions corresponding to those in the reference medicinal material chromatogram.

4 Discussion

4.1 Rationality of the extraction process The water decoction method was chosen for this study not only to maintain consistency with the traditional decoction, but also to meet the requirements of industrial production. The orthogonal experimental results showed that extraction frequency was the key factor affecting both the dry extract yield and the transfer rates of the marker components ($P < 0.05$ or $P < 0.01$), whereas water addition amount and extraction time had no significant effects. With consideration for energy conservation and production cycle reduction, two decoction cycles were selected instead of three, reflecting a process design principle that prioritizes therapeutic efficacy while taking efficiency into account. Validation experiments confirmed that the process was stable and feasible, with all *RSD* values within acceptable limits.

4.2 Characteristics of the quality standard The established TLC identification method covers the three main medicinal materials (Lonicerae Japonicae Flos, Forsythiae Fructus, and Glycyrrhizae Radix et Rhizoma) with strong specificity, no interference from negative controls, and simple operation. It can serve as a qualitative control indicator for the preparation. The results of all inspection items met the requirements for oral liquids (mixtures) specified in the *Chinese Pharmacopoeia* (2020 Edition), and the microbial limits were within the prescribed standards, indicating that the quality of this preparation is controllable.

4.3 Future research prospects As the principal herb in the formula, *Clerodendrum japonicum* currently lacks a well-established TLC identification method. Further efforts are needed to explore more favorable conditions for extraction, separation, and development, so as to establish a highly specific TLC or HPLC identification method for this herb. In addition, it is recommended that quantitative studies be carried out on forsythiaside and chlorogenic acid, and that a multi-component quality standard be developed to provide a more comprehensive basis for the quality control of the preparation.

5 Conclusions

The optimal extraction process for Chengyin Mixture established in this study was as follows: decocting twice with 10 times the amount of water, one hour for each decoction. Validation experiments confirmed that the process was stable and feasible, with a mean dry extract yield of 24.24%, and mean transfer rates of 71.86% for forsythiaside and 69.48% for chlorogenic acid, all with *RSD* values below 5%. The TLC identification methods developed were highly specific with no interference from negative controls. The appearance, relative density, pH, filling volume, and microbial limits of the preparation all met the requirements of the *Chinese Pharmacopoeia*. This process and the associated quality standards can serve as a basis for the hospital preparation registration and standardized production of Chengyin Mixture.

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