Study on the Preparation Process of Shegan Qingwen Fuzheng Oral Liquid

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Abstract: Objective — To investigate the preparation process of Shegan Qingwen Fuzheng Oral Liquid (SQFOL) to fully extract active components, enhance the content and stability of pharmacologically active constituents in the formulation, and ensure clinical efficacy. Methods — The extraction process for volatile components, β-cyclodextrin inclusion process for distillate, and extraction process for distillation residue were investigated separately. Results — The optimal extraction process for volatile components was determined as follows: 8-fold water volume, 1-hour soaking time, and 4-hour distillation time. The optimal β-cyclodextrin inclusion process for distillate was a core-to-shell mass ratio of 1:4, inclusion temperature of 40°C, and inclusion time of 2 hours. The extraction process for distillation residue was adding 8 times the volume of 50% ethanol, performing two extractions: the first for 2 hours and the second for 1.5 hours. Conclusions — The extraction processes were validated to be stable and feasible.

***Keywords*:** Shegan Qingwen Fuzheng Oral Liquid; preparation process; volatile component extraction; inclusion process; reflux extraction.

1. Introduction

Traditional Chinese medicine (TCM) has seen its inheritance and innovative development elevated to a national strategic priority [1-4]. The Shegan Qingwen Fuzheng Formula is a distinctive prescription in TCM clinical practice. Its formulation adheres to the TCM therapeutic principle of “strengthening the body's defenses and expelling pathogens” [5], integrating multiple herbs that clear heat, detoxify, and fortify the body's constitution. This provides a novel solution for treating respiratory infections. Our research team developed the Shegan Qingwen Fuzheng Oral Liquid based on the Shegan Qingwen Fuzheng Formula and conducted process research. This work establishes key indicators for further process optimization, ensuring consistency in the formulation's material composition and achieving stable quality, reliable efficacy, and safe administration.

2. Instruments and Reagents

The chromatographic analysis employed an Agilent 1260 series HPLC system (Agilent Technologies, USA). The TU-1810 UV-Vis Spectrophotometer was purchased from Beijing Puxi General Instrument Co., Ltd. The crude drugs of Belamcandae rhizoma, Lonicerae Japonicae Flos, Isatidis Radix, Isatidis Folium, Taraxaci Herba, Eupatorii Herba and so on were purchased from Guoxiu Trading Center in Shijiazhuang High-Tech Zone and verified as authentic. Phosphoric acid of chromatography grade was procured from Chengdu Kelong Chemical Reagent Factory. Acetonitrile of HPLC grade was purchased from Thermo Fisher Scientific Inc. Methanol of HPLC grade and chlorogenic acid reference were purchased from Shanghai Aladdin Bio-Chemical Technology Co., Ltd. β-Cyclodextrin (purity ≥98%) was purchased from Shandong Tongwang Biotechnology Co., Ltd.

3. Methods and Results

3.1 Extraction Processes for Volatile Components

Take the prescribed amount of herbal materials, remove impurities, then rinse three times with purified water and drain thoroughly. Grind the herbs into coarse powder (passing through a 20-mesh sieve), mix thoroughly, and transfer into a multi-functional extraction vessel. To determine optimal process parameters for steam distillation extraction, three key factors of water addition volume, soaking time, and distillation duration were selected for evaluation based on preliminary experiments. Using essential oil yield (mL/g) as the primary metric, optimization was achieved through single-factor experiments.

3.1.1 Single-factor experiment

3.1.1.1 Water Addition Assessment

The fixed soaking time was 1 hour, and the distillation time was 4 hours. The effects of purified water at 6-, 8-, 10-, and 12-fold concentrations (w/v) on essential oil yield were examined. Results indicated that increasing the water volume from 6 to 8 times significantly enhanced the yield. This improvement stemmed from sufficient moisture facilitating uniform steam penetration through the herbal material to carry volatile components. Beyond an 8-fold water volume, yield gains became negligible while leading to excessively large distillate volumes, thereby increasing energy consumption and processing time. Consequently, an 8-fold water volume was determined to be the optimal level.

3.1.1.2 Soak Time Assessment

A fixed water-to-herb ratio of 8:1 and a distillation time of 4 hours were maintained, with soaking times of 0.5, 1, 1.5, and 2 hours evaluated. Results indicated that a 1-hour soaking time yielded the highest extraction rate. A duration shorter than 0.5 hours failed to sufficiently moisten the herbal materials, hindering component extraction and steam diffusion. Durations longer than 1.5 hours risked loss of water-soluble or hydrolyzable components without further improving yield. Thus, the optimal soaking time was determined to be 1 hour.

3.1.1.3 Distillation Time Assessment

A fixed water-to-material ratio of 8:1 was maintained, with a soaking time of 1 hour. Distillation times of 2, 3, 4, and 5 hours were examined. Experiments revealed that within the first 4 hours of distillation, distinct oil droplets were visible in the distillate, with yield increasing linearly over time. After 4 hours, no significant oil droplets remained in the distillate, and the yield stabilized. Considering both extraction efficiency and energy consumption, the optimal distillation time was determined to be 4 hours.

3.1.2 Process Validation

Under optimized conditions, add purified water at 8 times the volume (w/v) and soak for 1 hour to thoroughly moisten the herbal materials. Activate the steam heating system of the extraction vessel, maintaining steam pressure at 0.1–0.15 MPa to sustain an internal temperature of 95–100°C for steam distillation. During distillation, collect the distillate through the condenser tube, controlling the distillate flow rate at 200-300 mL/h. Continue distillation for 4 hours, stopping when no significant oil droplets appear in the distillate. Collect the distillate, which should be approximately 6-8 times the total weight of the herbal materials. Three batches of samples were prepared using this optimal process, yielding stable essential oil rates with an average of 0.48%.

3.2 β-Cyclodextrin Inclusion Process of Distillate

3.2.1 Evaluation Indicators

Transfer the distillate to a redistillation apparatus, add 0.5% (v/v) anhydrous sodium sulfate (for dehydration), stir thoroughly, then perform redistillation. Collect the first 80% fraction as the distillate. Immediately refrigerate the distillate (4°C) for storage and future use. To prepare distillate-β-cyclodextrin inclusion complexes with high inclusion efficiency and excellent stability, the inclusion temperature, inclusion time, and core-to-shell mass ratio (distillate:β-cyclodextrin) were selected as key factors based on preliminary experiments and single-factor experiments. Using the combined score of inclusion yield and inclusion efficiency as the primary evaluation indicator, the inclusion process was systematically optimized through orthogonal design experiments. Inclusion yield is used to reflect material loss during the process as Inclusion yield = (Actual mass of inclusions/Theoretical total mass of inclusions) × 100%. The inclusion rate reflects the actual encapsulation efficiency of β-cyclodextrin for volatile oils and serves as the core indicator for evaluating inclusion effectiveness. The content of volatile oil within the inclusion complex is determined via ultraviolet spectrophotometry using the following formula: Inclusion Rate = (Actual volatile oil content in inclusion complex / Theoretical volatile oil addition amount) × 100%. To comprehensively evaluate both yield and inclusion efficiency, a composite score is adopted as the final optimization indicator: Composite Score = Inclusion Rate × 0.7 + Yield × 0.3.

3.2.2 Orthogonal Design Optimization

After weighing β-cyclodextrin, dissolve β-cyclodextrin and distillate in purified water at 60°C according to a specific mass ratio, stirring until completely dissolved to prepare a β-cyclodextrin saturated solution (approximately 20% w/v). Cool to 40°C and set aside. Slowly add the chilled distillate to the β-cyclodextrin saturated solution while stirring continuously. After complete addition, continue stirring in a constant-temperature water bath to ensure the volatile oil components fully enter the β-cyclodextrin cavity structure. To investigate the interactions among core-to-shell mass ratio, inclusion temperature, and

inclusion time, and determine the optimal combination, a three-factor and three-level orthogonal experiment was designed based on single-factor trials. The factors selected were core-to-shell mass ratio (A), inclusion temperature (B), and inclusion time (C). Using a composite score of inclusion rate and yield as the evaluation indicator, range analysis and analysis of variance were conducted. The factor and level arrangements were detailed in Table 1. Experimental results were presented in Tables 2 and 3.

Table 1. Orthogonal design factors and levels for inclusion process

Levels	Factors		
	A:core-to-shell mass ratio	B: inclusion temperature (℃)	C: inclusion time (h)
1	1:3	30	1.5
2	1:4	40	2
3	1:5	50	2.5

Table 2. Statistical results for orthogonal experiment of inclusion process

No.	A	B	C	D	composite score
1	1	1	1	1	0.480
2	1	2	2	2	0.550
3	1	3	3	3	0.560
4	2	1	2	3	0.580
5	2	2	3	1	0.672
6	2	3	1	2	0.590
7	3	1	3	2	0.590
8	3	2	1	3	0.605
9	3	3	2	1	0.626
K_1	0.530	0.550	0.558	0.593	
K_2	0.614	0.609	0.585	0.577	
K_3	0.607	0.592	0.607	0.582	
R	0.084	0.059	0.049	0.016	

Table 3. Variance Analysis of Inclusion Process

Sources of variance	Sum of Squared Deviations	degree of freedom	F Value	Significance (P)
A	0.0130	2	32.42	P<0.05
B	0.0055	2	13.77	
C	0.0036	2	8.99	
D (Error)	0.0004	2		

The results of the analysis of variance indicate that the order of influence of various factors on the inclusion process is as follows: core-to-wall material mass ratio (A) > encapsulation temperature (B) > Encapsulation time (C), with factor A exhibiting a significant effect. Based on the range analysis results, the optimal process is $A_2B_2C_2$, corresponding to a core-to-wall material mass ratio of 1:4, an encapsulation temperature of 40℃, and an encapsulation time of 2 hours. Validation tests under these conditions demonstrated that both the inclusion rate and the comprehensive evaluation score exceeded those of any test group in the orthogonal array, confirming the process stability and feasibility.

3.3 Extraction Process of Distillation Residue

3.3.1 HPLC Fingerprint Analysis

3.3.1.1 Chromatographic Conditions

COSMOSIL 5C18-MS-II ODS column (5 μm, 4.6 mm × 250 mm) was purchased from Shanghai Puyu Kemao Co., Ltd. Mobile Phase was composed of aqueous phase A of 0.1% phosphoric acid-water solution and organic phase B of 0.1% phosphoric acid-acetonitrile solution. Gradient elution program was shown in Table 4. Flow rate was 1.0 mL/min. Detection wavelengths were set at 203 nm, 220 nm, 254 nm, 265 nm, and 327 nm. Column temperature was 35℃ and injection volume was 20 μL.

Table 4. Chromatographic Mobile Phase Elution Program

Gradient	0	3	9	22	30	45	60	80	90
A	100	100	95	88	84	82	70	40	40
B	0	0	5	12	16	18	30	60	60

3.3.1.2 Solution Preparation

Accurately weigh an appropriate amount of chlorogenic acid reference dried to constant weight. Dissolve in acetonitrile-water (95:5) to prepare a 400 μg/mL solution as the chlorogenic acid reference solution. Take the distilled residue, extract with solvent by reflux, filter, combine filtrates, and concentrate under reduced pressure to obtain the pre-concentrate. Obtain the concentrate by water precipitation or ultrafiltration membrane filtration. Accurately measure 10 mL of the concentrate, dilute 40-fold with mobile phase, filter through a 0.45 μm membrane, and take the filtrate to obtain the test solution.

3.3.2 Establishment and Evaluation of HPLC Fingerprint

Ten batches of self-prepared test solutions were injected separately, and chromatograms were recorded. Calculated based on 100% peak occurrence rate, the total number of fingerprint peaks at detection wavelengths of 203 nm, 220 nm, 254 nm, 265 nm, and 327 nm were 17, 17, 17, 17, and 13, respectively. The fusion fingerprint at these five wavelengths exhibited 17 peaks. Given chlorogenic acid's moderate retention time and good separation from adjacent peaks, it was selected as the reference peak (peak 6) for identifying common fingerprint peaks. A reference fingerprint (RFP) was generated using the average method, with the common peak labeling diagram shown in Figure 1.

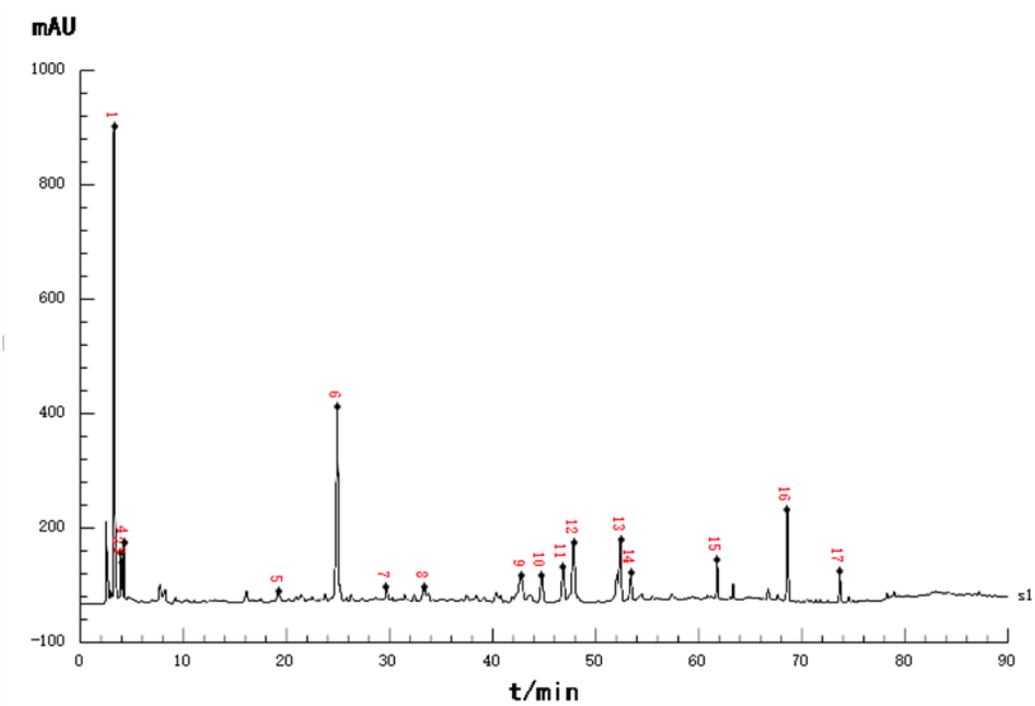

Figure 1. HPLC Fusion Fingerprint at five wavelengths

The resolution factor ***RF***, derived from chromatographic fingerprint [6], serves as an indicator for evaluating and optimizing the separation performance of samples. The ***RF*** reflects the number of effective signals in the chromatogram, resolution, and signal uniformity. Its calculation formula is as follows:

$$RF = \tau \left[\sum_{i=1}^{n-1} R_i \lg\left(\frac{A_i + A_{i+1}}{2}\right)_i + \frac{2\left(t_{R_n} - t_{R_1}\right)}{(n-1)(W_1 + W_n)} \lg\left(\frac{A_1 + A_n}{2}\right) \right] \quad (1)$$

Experimental parameters can be assessed using this indicator, where a higher RF value indicates more favorable experimental conditions[7].

3.3.3 Alcohol Extraction Process

Based on single-factor experiments, reflux extraction was conducted with ethanol concentration, solvent dosage, extraction time, and reflux cycles as variables[8]. Experiments were performed using an L9(34) orthogonal design, with factors and levels arranged as shown in Table 5. Evaluation was based on the separation index ***RF***. Experimental results were presented in Tables 6 and 7.

Table 5. Orthogonal Test Factors and Level Arrangement

Levels	Factors			
	A:Ethanol concentration (%)	B: Solvent Usage (Times)	C:Extraction time (h)	D: Number of reflows
1	40	6	1	1
2	50	8	1.5	2
3	60	10	2	3

Analysis of variance results indicated that the factors influencing the extraction process were: ethanol concentration (A) > number of extractions (D) > extraction time (C) > solvent volume (B), with factor A exhibiting a significant effect. Based on the range analysis results in Table 4, the optimal extraction process was A2B2C3D3, corresponding to an ethanol concentration of 50%, solvent volume of 8 times, extraction time of 2 hours, and extraction frequency of 3 times. Considering multiple factors including energy and time savings, the extraction process was finalized as follows: Add 8 times the volume of 50% ethanol, perform two extractions, with the first extraction lasting 2 hours and the second extraction lasting 1.5 hours. Using this process for three extractions yielded relatively stable ***RF*** values, with an average of 211.5, indicating that the selected process is reasonable.

Table 6. Orthogonal Test Results

No.	A	B	C	D	RF
1	1	1	1	1	141.0
2	1	2	2	2	162.0
3	1	3	3	3	171.0
4	2	1	2	3	197.0
5	2	2	3	1	199.5
6	2	3	1	2	204.0
7	3	1	3	2	178.0
8	3	2	1	3	177.0
9	3	3	2	1	155.0
K1	158.000	172.000	174.000	165.167	
K2	200.167	179.500	171.333	181.333	
K3	170.000	176.667	182.833	181.667	
R	42.167	7.500	8.833	16.500	

Table 7. Results of Orthogonal Test Analysis of Variance

Sources of variance	SS	degree of freedom	MS	F Value	Significance (P)
A	2832.056	2	1416.028	32.91	P<0.05
B	86.056	2	43.028	1.00	
C	217.389	2	108.694	2.53	
D	533.722	2	266.861	6.20	

4. Discussion

This study innovatively incorporated Astragali Radix and Ligustri Lucidi Fructus into the formulation of the antiviral injection based on Shegan Qingwen Fuzheng Formula, enhancing its tonifying and fortifying effects. This formula simultaneously achieves heat-clearing and detoxification alongside lung-nourishing and qi-tonifying actions. Utilizing modern extraction and inclusion complexation technologies, combined with multi-wavelength HPLC fingerprinting for comprehensive quality control, it ensures stable content of pharmacologically active compounds. resulting in a safe,

convenient oral liquid formulation suitable for preventing and treating respiratory viral infections.

Preliminary experiments on β-cyclodextrin inclusion complexation of distillate revealed that inclusion efficiency significantly increased with rising β-cyclodextrin dosage, peaking at a 1:4 ratio. This occurs because an optimal amount of β-cyclodextrin provides sufficient cavity space to accommodate volatile oil molecules. Beyond a 1:4 ratio, the inclusion rate showed no further significant increase, while inclusion complex yield decreased due to residual excess β-cyclodextrin, leading to lower overall scores. With a fixed core-to-wall mass ratio of 1:4 and an inclusion time of 2 hours, the effects of inclusion temperature (30°C, 40°C, 50°C, 60°C) were investigated. Results indicate that at excessively low temperatures (30°C), molecular thermal motion slows, diffusion rates decrease, and inclusion kinetics become insufficient. At excessively high temperatures (>50°C), β-cyclodextrin cavity structures may destabilize due to thermal agitation, while volatile oil components accelerate desorption and oxidation, both leading to reduced inclusion rates. With a fixed core-to-shell mass ratio of 1:4 and an inclusion temperature of 40°C, the effect of inclusion time (1 h, 1.5 h, 2 h, 2.5 h, 3 h) was investigated. Experiments revealed that the inclusion rate increased rapidly with time within the first 2 hours, then plateaued thereafter. This indicates that 2 hours is sufficient for the inclusion reaction to reach dynamic equilibrium. Prolonging stirring time not only fails to further enhance the inclusion rate but may also cause dissociation of formed inclusion complexes due to mechanical shear forces.

When evaluating solidification and drying conditions for the inclusion compounds, comparisons of different static solidification times (6 h, 12 h, 24 h) revealed that 12 hours at 4°C was sufficient for complete precipitation and solidification. Shorter times resulted in incomplete precipitation, while longer times did not significantly improve yield. Regarding drying methods, comparisons between atmospheric drying and vacuum drying (60°C) demonstrated that vacuum drying effectively prevents oxidation and loss of volatile oils during heating. The resulting inclusion complexes exhibited superior color and higher volatile oil retention rates.

In studying extraction processes for post-distillation residue, preliminary and single-factor experiments used water, 25% (v/v) ethanol, 50% (v/v) ethanol, 75% (v/v) ethanol, 95% (v/v) ethanol, anhydrous ethanol, and ethyl acetate as solvents for reflux extraction. Results indicate that medium-concentration ethanol yields chromatograms with more peaks when preparing samples. Given the affordability and availability of water and ethanol, coupled with the good solubility of the primary active components in the herbal materials used in the prescription in both water and ethanol, a specific concentration of ethanol was selected as the extraction solvent for subsequent orthogonal experiments. This choice ensures the thorough extraction of the active components.

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