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Analysis of Pharmaceutical Processes in Chinese Medicine Based on Quantity and Measure Value Transfer: A Case of *Salvia miltiorrhiza* Purified Extract Preparation

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Abstract: Quantity and measure value transfer is widely used to examine the correlation between the quality of Chinese herbs, Chinese herbal intermediates, and Chinese patent medicines. This study performed a quantity and measure value transfer analysis to assess the total solids yield, phenolic component yield, and phenolic component purity in the *Salvia miltiorrhiza* purified extract (SMPE) preparation process. The amount of extracted total solids was between 45–250 mg/g following the processes of reflux extraction, vacuum concentration, lime–sulfuric acid refining, first ethanol precipitation, second ethanol precipitation, first acidification, alkalization, thermal hydrolysis, and second acidification. Regarding yield and purity, Danshensu ranked first among all phenolic components. Additionally, a quantitative index defined as the total variation (TV) value was proposed to describe the consistency of the SMPE preparation process. The batch-to-batch variation in the SMPE came from the variable in herb quality and the preparation process, and the latter contributed more. The contribution of individual processes to the total variation (TVP) was proposed as an index to measure the impact of processes on batch-to-batch consistency. According to the TVP value, the lime–sulfuric acid refining process, the first ethanol precipitation process, and the second acidification process were all deemed crucial. When the similarity algorithms for the composition of the intermediates and SMPE were examined, the Euclidean distance outperformed the Pearson correlation coefficient, Spearman correlation coefficient, and cosine of the pinch angles. Based on the variation in the average Euclidean distance ($\Delta\overline{D_j}$) during the process, the second ethanol precipitation, alkalization, and thermal hydrolysis processes were determined to be critical. This study clarified the primary causes of extract quality fluctuation, identified the critical processes, and examined the pharmaceutical process of traditional Chinese medicine (TCM) from the standpoint of quantity and measure value transfer. The method can be used as a reference for the analysis of other TCM pharmaceutical processes.

Keywords: *Salvia miltiorrhiza* purified extract; critical processes; batch-to-batch consistency; sources of variation

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1. Background

Quantity and measure value transfer has recently been extensively used to examine the correlation between the quality of Chinese herbs, Chinese herbal slices, Chinese herbal intermediates, and Chinese patent medicines [1–4]. The findings could help to develop quality indicators and their control ranges for traditional Chinese medicines (TCMs). Chunhua Wang [5] et al. investigated the method of choosing "quality markers" [6] for Shengmai injection and chose six chemicals as the index components for quality control through

quantity and measure value transfer analysis during the production of Shengmai injection. Through an analysis of the quantity and measure value transfer between the Chinese herbal slices and the benchmark sample, the quality standard of the benchmark sample of the traditional recipe Houpuwenzhong Tang was clarified [7]. However, few studies have systematically reported pharmaceutical process characteristics based on quantity and measure value transfer.

Salvia miltiorrhiza (Danshen), belonging to the *Lamiaceae* family, is the dried root and rhizome of *Salvia miltiorrhiza* Bge. It has the effects of activating blood circulation, dispelling blood stasis, clearing and activating the channels and collaterals, clearing the heart and removing irritation, cooling the blood, relieving pain, etc. It is commonly used in clinical practice to treat coronary heart disease [8–10], angina pectoris [11–13], and ischemic stroke [14–16]. The phenolic components in *Salvia miltiorrhiza*, including salvianolic acid B, danshensu, protocatechuic aldehyde, and rosmarinic acid, are considered the main active ingredients, which are generally extracted by water decoction. The decoction can be further purified by ethanol precipitation, extraction, and column chromatography to obtain *Salvia miltiorrhiza* purified extract (SMPE). In industry, the purified extract is used to produce Danshen injection, compound Danshen spray, Senxiong glucose injection, and so on. However, a large dose of Danshen injection may cause peripheral vascular toxicity [17]. In addition, in practice, taking a large amount of oral preparations of Danshen can also stimulate the stomach.

Given the extensive clinical uses of *Salvia miltiorrhiza,* it is significant to look at the quantity and measure value transfer of active components in herbs, intermediates, and SMPE to raise the preparations' quality control standards. In this study, we chose the SMPE preparation process as the research object. The differences in the chemical composition of intermediates and purified extract that changed with the restorative materials' quality were investigated. Multiple indicators were used to measure the similarity of process intermediates and final product composition ratios. A novel indicator was represented for evaluating the contribution of processes on batch-to-batch consistency. The quality fluctuation sources of the SMPE were analyzed, and critical processes were identified based on the consistency change during the preparation process.

2. Materials and Methods

2.1. Reagents and Consumables

In this study, five analytical reagents (ARs) were utilized. The purity by volume of the ethanol solution purchased from Shanghai Lingfeng Chemical Reagent Co., Ltd. (Shanghai, China) and Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) was 95%. Sinopharm Chemical Reagent Co., Ltd. was the source of the other four ARs (sulfuric acid, calcium hydroxide, hydrochloric acid, and sodium hydroxide). Methanol and acetonitrile were chromatographically pure and were acquired from Merck (Darmstadt, Germany). Shanghai Winherb Medical Technology Co., Ltd. (Shanghai, China) supplied the Danshensu sodium, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B, all of which had a more than 99% purity. For more details regarding *Salvia miltiorrhiza* herbs, see Table 1.

2.2. Quality Characterization of Salvia Miltiorrhiza

The *Salvia miltiorrhiza* used in tests QC1-QC3 was from the same batch, Batch 180913. Process and detection were the main causes of purified extract quality variation in these three tests. Batches 180927, 180901, 181001, 190801, 210911, 211101, 210811T, and 211022T were utilized in tests QC4-Q11, respectively. In these eight tests, herbs, process, and detection were the primary contributors to quality variations.

Using The Technical Requirements for Quality Control and Standard Development of Chinese Medicine Formulated Granules [18] as a guide, this work prepared a *Salvia miltiorrhiza* standard decoction and assessed its solid yield, phenolic component yield, and purity to characterize the quality of *Salvia miltiorrhiza* herbs. The specific experimental

procedures were as follows: *Salvia miltiorrhiza* was reflux-extracted twice with 6 mL/g decoction water for 0.5 h after being steeped for 0.5 h. The two extracts were obtained by filtration and then combined.

Table 1. Information on *Salvia miltiorrhiza* slices.

Manufacturers	Places of Origin	Batch Number
Tonghua Tianshi Pharmaceutical Co.	Shandong Province, China	180913
Shandong Buchang Pharmaceutical Co.	Shandong Province, China	180927
Shandong Buchang Pharmaceutical Co.	Anhui Province, China	180901
Shandong Buchang Pharmaceutical Co.	Anhui Province, China	181001
Anhui Baicui Jinfang Pharmaceutical Co.	Shandong Province, China	190801
Anhui Hongentang Biotechnology Co.	Anhui Province, China	210911
Lu'an Danbeier Biotechnology Co.	Anhui Province, China	211101
Jiaxing Dongfang Chinese Traditional Medicine Tablet Co., Ltd.	Shandong Province, China	210811T
Jiaxing Dongfang Chinese Traditional Medicine Tablet Co., Ltd.	Shandong Province, China	211022T

2.3. Preparation of SMPE

The SMPE is used to prepare botanical injections. Tannins from *Salvia miltiorrhiza* herbs are considered the risk components that cause adverse reactions [19]. Tannins can bind to proteins in the tissue to form insoluble tannic proteins, causing pain, redness, swelling, and hardening at the injection site.

To assure the safety of the injection, many purification processes are used in industrial manufacturing. Many phenolic compounds will degrade in the lime–sulfuric acid refining process, and some other impurities will be removed as calcium salt. Some strong polarity impurities, such as salts or polysaccharides, can be precipitated in the ethanol precipitation process. The processes of alkalization and thermal hydrolysis help to decompose phenolic compounds. The components with good water solubility and stability will be retained, which reduces the risk of adverse reactions to botanical injection. Figure 1 shows the preparation process of the SMPE.

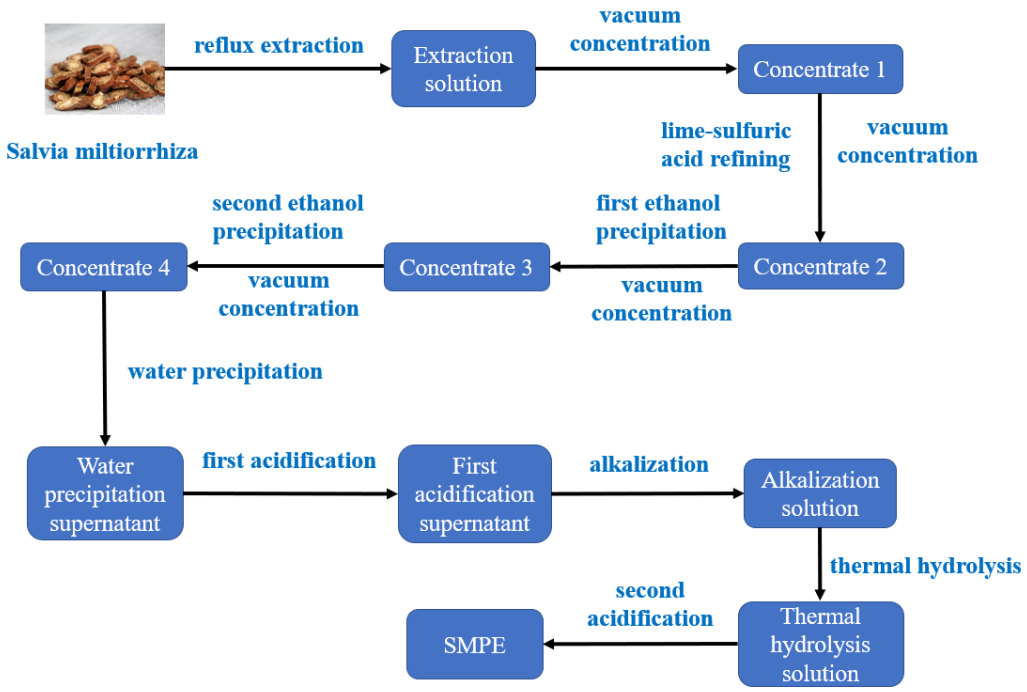

Figure 1. Process flow diagram for the preparation of the SMPE.

2.3.1. Preparation of *Salvia miltiorrhiza* Decoction

A total of 150 g *Salvia miltiorrhiza* herbs was placed in a round-bottom flask, combined with ten times as much water, and decocted twice for 1.5 h each using a heating jacket (ZNHW type, Hangzhou Mingyuan Instrument Co., Ltd., Hangzhou, China). The first decoction was timed from boiling after the herbs had been steeped for 0.5 h. The gauze was used to filter the decoction, and the two decoctions were mixed.

2.3.2. Preparation of Aqueous Extract Concentrate

The decoction was concentrated by a rotary evaporator (R-100, R-200, BUCHI, Switzerland), and the temperature was controlled not to exceed 70 °C. Aqueous extract concentrate (Concentrate 1) was obtained with a relative density of 1.03 g/cm^3.

2.3.3. Preparation of Lime–Sulfuric Acid Refining Solution

The Concentrate 1's pH was adjusted to 11.0 with 20% lime milk and allowed to stand for 2 h. After, 20% sulfuric acid solution was added to adjust the pH level to 5.0, and then the mixture was left for 24 h. Centrifugation was performed to separate and collect the supernatant. Subsequently, the supernatant was concentrated under vacuum to obtain a lime–sulfuric acid refining solution with a relative density of 1.20 g/cm^3 (Concentrate 2).

2.3.4. Preparation of the First Ethanol Precipitation Supernatant

First, 95% (v/v) ethanol solution was added to Concentrate 2 with a peristaltic pump (YZ15, Changzhou Visier Fluid Technology Co., Ltd., Changzhou, China) until the apparent ethanol content of the supernatant reached 60% (v/v). The mixture was stirred for 20 min and then placed in a low-temperature thermostat tank (THYD-1030 W, Ningbo Tianheng Instrument Factory, Ningbo, China). After standing for 24 h at 4 °C, the first ethanol precipitation supernatant was collected.

2.3.5. Preparation of Second Ethanol Precipitation Supernatant

The first ethanol precipitation supernatant was concentrated to a relative density of 1.15 g/cm^3 to obtain concentrate 3. Then, 95% (v/v) ethanol solution was added with a peristaltic pump until the ethanol content reached 70% (v/v). The mixture was stirred for 20 min and then allowed to stand at 4 °C. After 24 h, the second ethanol precipitation supernatant was gathered.

2.3.6. Preparation of First Acidification Supernatant

The second ethanol precipitation supernatant was evaporated to obtain concentrate 4 with a relative density of 1.05 g/cm^3. Ten times as much water was added, and after being agitated for 0.5 h, the mixture was chilled and let to stand for 24 h. The supernatant (supernatant from the water precipitation) was gathered. Then, the pH was adjusted to 3.0 with a 10% hydrochloric acid solution. After standing for 2 h, the first acidification supernatant was gathered by filtration under vacuum.

2.3.7. Preparation of Alkalization Solution

The first acidification supernatant was adjusted to pH 9.0 with a 10% sodium hydroxide solution to obtain the alkalization solution.

2.3.8. Preparation of the Thermal Hydrolysis Solution

The alkalization solution was heated and hydrolyzed at 100 °C for 1 h to obtain the thermal hydrolysis solution.

2.3.9. Preparation of the SMPE

The thermal hydrolysis solution was adjusted to pH 5.5 with a 10% hydrochloric acid solution to obtain the SMPE. This process is referred to as second acidification in this work.

2.4. Determination of Total Solids

A mass calculation was used to determine the total solids. After being accurately weighed and placed in a weighing bottle with constant weight, the required sample quantity was heated in an oven (XMTD-8222, Shanghai Jinghong Experimental Equipment Co., Ltd., Shanghai, China) at 105 °C for 3 h. The bottle was then moved to a desiccator until cooling to room temperature before being weighed. The total solid yield of the sample was calculated based on the difference in mass between before and after drying.

2.5. Determination of Phenolic Compounds

The chemical contents of the SMPE were determined by high-performance liquid chromatography (FL5090, Zhejiang Fuli Analytical Instruments Co., Ltd. (Taizhou, China); Agilent 1260, Agilent Technologies Ltd., Santa Clara, CA, USA). The method was adapted with some modifications from Cao [20] et al. Chromatographic separation was performed at 25 °C on an Extend- C18 column (4.6 × 250 mm, 5 μm). The mobile phases comprising pure acetonitrile (B) and 0.1% formic acid–water (A) had a flow rate of 1.0 mL/min. The gradient elution program was configured as follows: 0~10 min 7~17% B; 10~16 min 17~21% B; 16~32 min 21% B, 32~40 min 21~29% B; 40~44 min 29~35% B; 44~45 min 35~100% B. The sample volume injected was 2 μL.

The reference substances of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B were precisely weighed using an analytical balance (XS105DU, Mettler-Toledo Shanghai Co., Ltd., Shanghai, China), and then placed into the same 10 mL volumetric flask. The methanol solution was added to fully dissolve the substances and stabilize the volume. The reference solution was obtained after a thorough shake. The reference solution was gradually diluted with methanol to produce a series of reference solutions at various concentrations. The exact concentrations of each reference substance are listed in Appendix A Table A1.

An accurately appropriate amount of each step's intermediates and SMPE were placed into suitable volumetric flasks, and then the volume was fixed by diluting the mixture with methanol solution. After that, the solutions were filtered through a membrane with 0.22 μm pore size. High-performance liquid chromatography was used to collect the peak regions and determine the quantities of phenolic components using the standard curve from the following filtrate, which was put in a sample bottle. Figure 2 displays typical chromatograms of the reference solutions and samples.

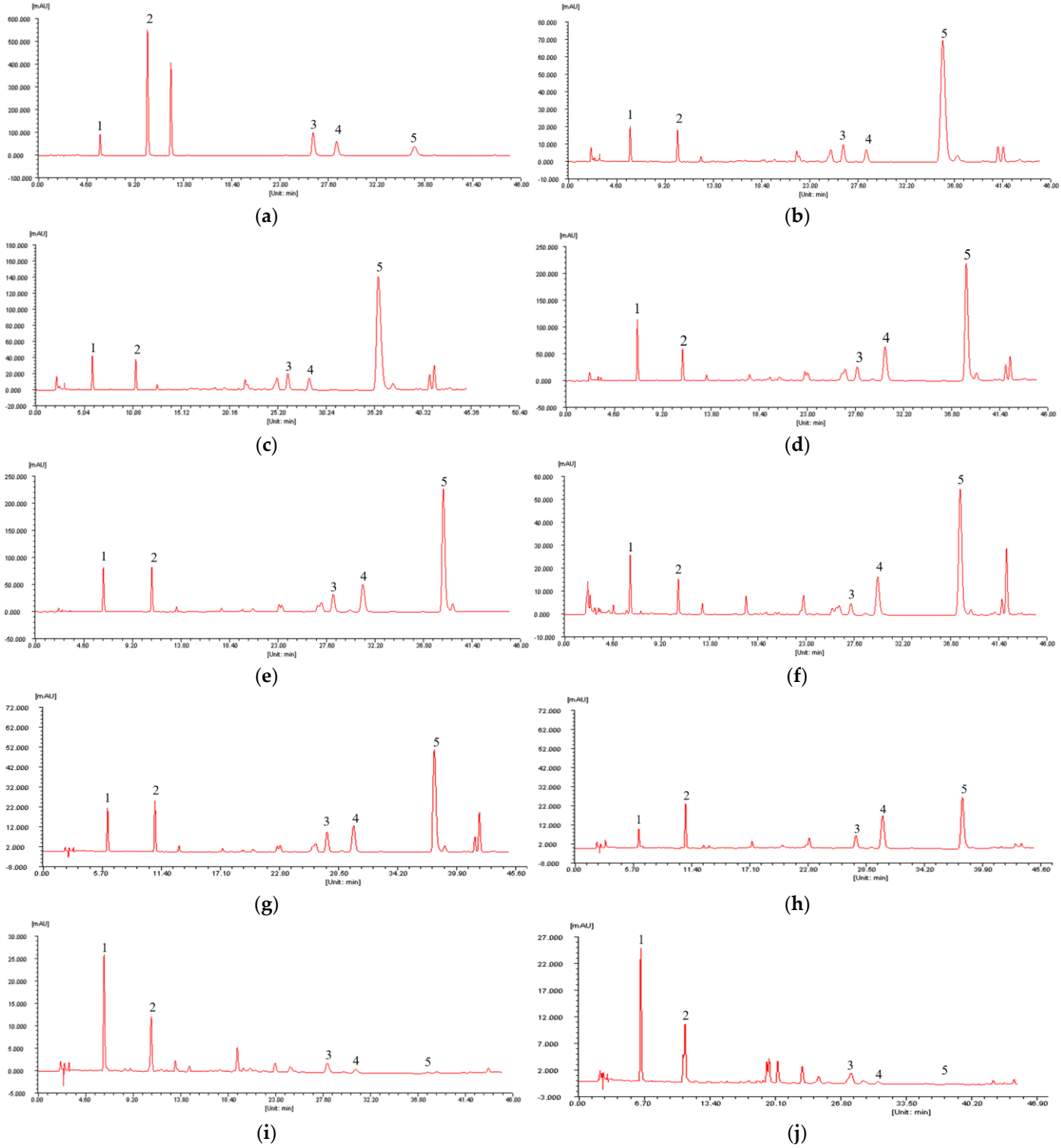

Figure 2. Typical chromatogram of the reference solution and samples: (**a**) reference solution, (**b**) salvia decoction, (**c**) aqueous extract concentrates, (**d**)lime-sulfuric acid refining solution, (**e**) first ethanol precipitation supernatant, (**f**) second ethanol precipitation supernatant, (**g**) first acidification supernatant, (**h**) alkalization solution, (**i**) thermal hydrolysis solution, and (**j**) SMPE. Peak 1 is danshensu, peak 2 is protocatechuic aldehyde, peak 3 is rosmarinic acid, peak 4 is lithospermic acid, and peak 5 is salvianolic acid B.

2.6. Calculation Method of Evaluation Indicators

Indicators for calculating the production process in this study included total solids, phenolic compound yield, and phenolic compound purity. The formula for estimating the yield of phenolic components is in Equation (1). The formulas for estimating the purity of phenolic components in Equation (2) and the total solid in Equation (3) are provided.

$$Phenolic\ component\ yield = \frac{Phenolic\ content\ of\ the\ intermediates \times Total\ mass\ of\ intermediates}{Salvia\ herb\ mass} \quad (1)$$

$$Purity\ of\ phenolic\ components = \frac{Content\ of\ intermediate\ phenolic\ components \times Total\ mass\ of\ intermediates}{Solid\ mass\ of\ intermediates} \quad (2)$$

$$Solids\ yield = \frac{Content\ of\ intermediate\ solid \times Total\ mass\ of\ intermediates}{Salvia\ herb\ mass} \quad (3)$$

The content of the undetected components in the solids ($x_{unknown}$) was included in calculating the similarity of the raw materials and each intermediate to the final product, which is given in Equation (4):

$$x_{unknown} = 1 - \sum_{i=1}^{n} p_{k,i} \quad (4)$$

where n is the number of detected components, which is 5 in this paper, and $p_{k,i}$ is the purity of the detected component i in the kth intermediate.

In this study, the Pearson correlation coefficient (r), Spearman correlation coefficient (r_s), cosine of the pinch angles (cos α), and Euclidean distance (D) were used to measure the similarity of process intermediates and final product composition ratios. The formula for calculating the Pearson correlation coefficient is given in Equation (5):

$$r = \frac{1}{n-1} \sum_{i=1}^{n} \left(\frac{x_{k,i} - \overline{x_k}}{S_k} \right) \left(\frac{x_{z,i} - \overline{x_z}}{S_z} \right) \quad (5)$$

where $x_{k,i}$ is the purity of component i in the kth intermediate, $\overline{x_k}$ is the mean value of the purity of all components in the kth intermediate, S_k is the standard deviation of the purity of all components in the kth intermediate, $x_{z,i}$ is the purity of component i in the final product, $\overline{x_z}$ is the mean value of the purity of all components in the final product, and S_z is the standard deviation of all components in the final product. The formula for calculating the Spearman correlation coefficient is given in Equation (6);

$$r_s = \frac{\sum_{i=1}^{n} (u_{k,i} - \overline{u_k})(v_i - \overline{v})}{\sqrt{\sum_{i=1}^{n} (u_{k,i} - \overline{u_k})^2} \sqrt{\sum_{i=1}^{n} (v_i - \overline{v})^2}} \quad (6)$$

where $u_{k,i}$ is the rank order of the purity of compound i in the kth intermediate, $\overline{u_k}$ is the mean of the rank order of the purity of all components in the kth intermediate, v_i is the rank order of the purity of compound i in the final product, and $\overline{v}$ is the mean of the rank order of the purity of all components in the final product. The formula for calculating the cosine of the pinch angles is given in Equation (7):

$$cos\alpha = \frac{\sum_{i=1}^{n} x_{k,i} x_{z,i}}{\sqrt{\sum_{i=1}^{n} x_{k,i}^2} \sqrt{\sum_{i=1}^{n} x_{z,i}^2}} \quad (7)$$

The formula for calculating the Euclidean distance is given in Equation (8):

$$D = \sqrt{\sum_{i=1}^{n} \left| x_{k,i} - x_{z,i} \right|^2} \quad (8)$$

3. Results

The raw materials, intermediates, and final products were labelled and are shown in Table 2.

Table 2. Experimental No. involved in the process.

Experimental Materials	Experimental No.
Salvia miltiorrhiza herbs	X1
Salvia miltiorrhiza decoction	X2
Aqueous extract concentrate	X3
Lime-sulfuric acid refining solution	X4
First ethanol precipitation supernatant	X5
Second ethanol precipitation supernatant	X6
First acidification supernatant	X7
Alkalization solution	X8
Thermal hydrolysis solution	X9
SMPE	X10

3.1. Total Solid

The total solids of raw materials are 1000 mg/g by default. As shown in Figure 3, the yield of total solid from raw materials to intermediates to purified extract decreased as the process proceeded. The total solid content of the final SMPE ranged from 45 to 250 mg/g.

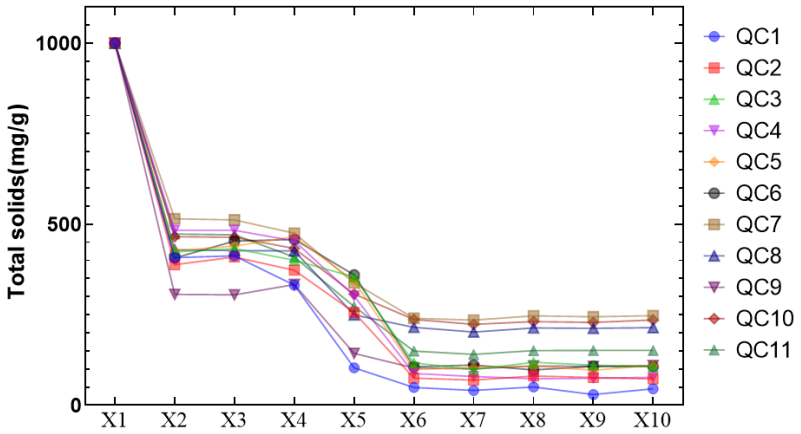

Figure 3. Variation in the total solid of raw materials, intermediates, and final products at each process stage.

The steps in which the yield of total solid significantly dropped are water decoction, first ethanol precipitation, and second ethanol precipitation. Approximately half of the solid was removed during decoction. From 306 to 516 mg/g of solid was dissolved in the aqueous decoction. Ethanol precipitation resulted in a further reduction in solid, with solid dissolved in the first ethanol precipitation supernatant ranging from 103 to 361 mg/g and in the second ethanol precipitation supernatant ranging from 49 to 241 mg/g. The decrease in total solids after the ethanol precipitation process can be explained by the fact that ethanol precipitation could remove polysaccharides, salts, proteins, and many other components [21].

3.2. Yield of Phenolic Components

Figure 4 demonstrates the variation in the yield of phenolic components. In Figure 4b,c, the protocatechuic aldehyde and rosmarinic acid yields remained relatively stable throughout the preparation. Figure 4a,d,e shows the yield variation of danshensu, lithospermic acid, and salvianolic acid B, respectively. After the lime-sulfuric acid refining process, the yield of danshensu and lithospermic acid showed significant growth, while the yield of

salvianolic acid B showed a dramatic decline. The main reason is that salvianolic acid B is easily hydrolyzed in an alkaline environment to transform into danshensu and lithospermic acid [22]. The yields of danshensu, lithospermic acid, and salvianolic acid B decreased after the first ethanol precipitation process, mainly due to the small solubility of these three phenolates in ethanol solution, which caused precipitation losses [23]. The thermal hydrolysis process decreased the yields of lithospermic acid and salvianolic acid B but increased the yield of danshensu. The reason is that heating in an alkaline environment can promote the hydrolysis of lithospermic acid and salvianolic acid B to produce danshensu [24]. The final yields of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B in the SMPE were 3.27–11.40 mg/g, 0.34–1.07 mg/g, 0.14–1.31 mg/g, 0.14–0.86 mg/g, and 0.02–0.55 mg/g, respectively.

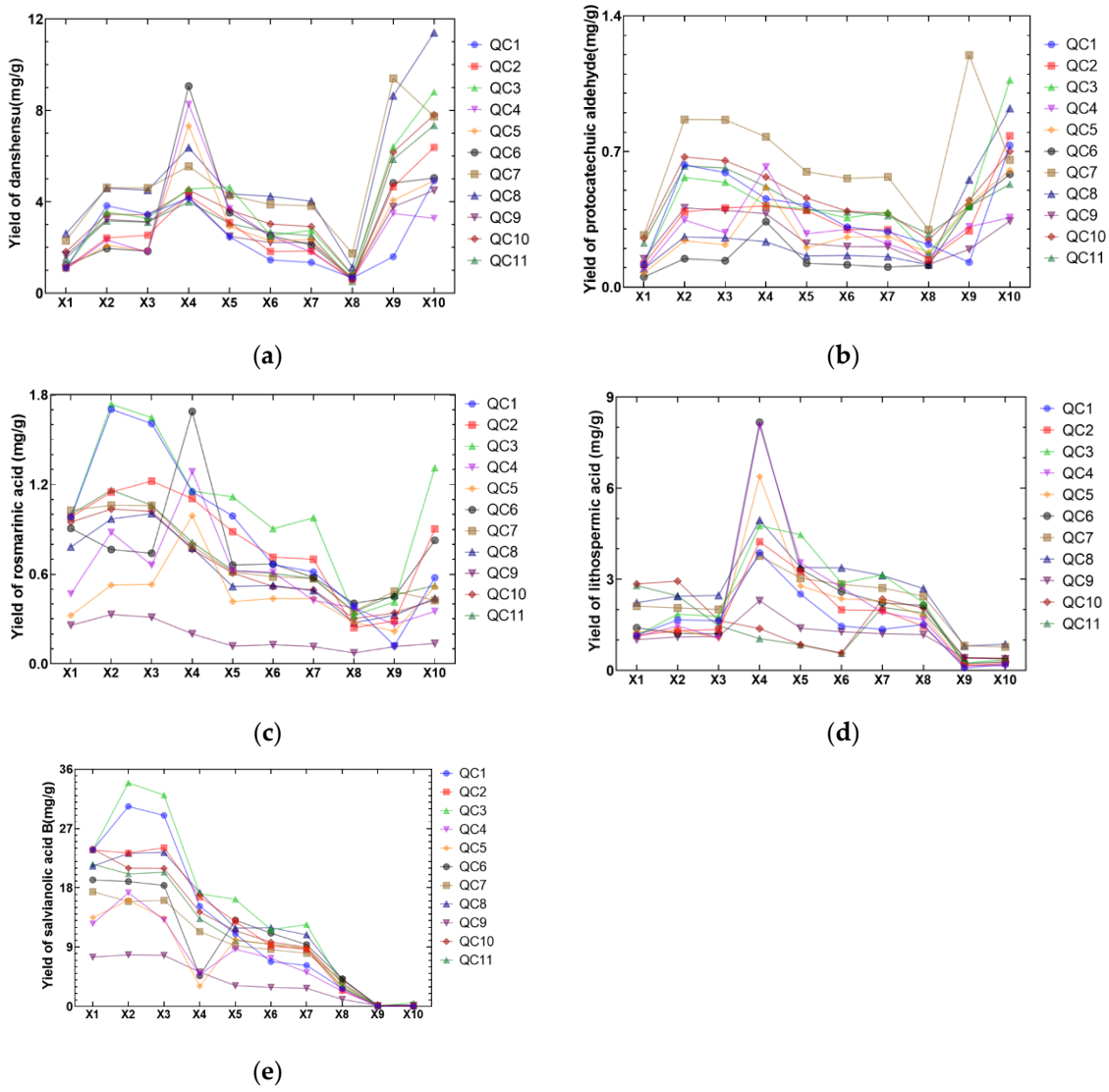

Figure 4. Variation in phenolic acid yield: (**a**) yield of danshensu; (**b**) yield of protocatechuic aldehyde; (**c**) yield of rosmarinic acid; (**d**) yield of lithospermic acid; (**e**) yield of salvianolic acid B.

3.3. Purity of Phenolic Components

The definition of purity can be seen in Equation (2). Figure 5 demonstrates the variation in the purity of the phenolic components. Water decoction and ethanol precipitation helped increase the purity of the phenolic components. After the second ethanol precipitation, the purity of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic

acid, and salvianolic acid B reached 1.27–2.95%, 0.07–0.64%, 0.12–1.36%, 0.23–3.12%, and 2.83–13.81%, respectively.

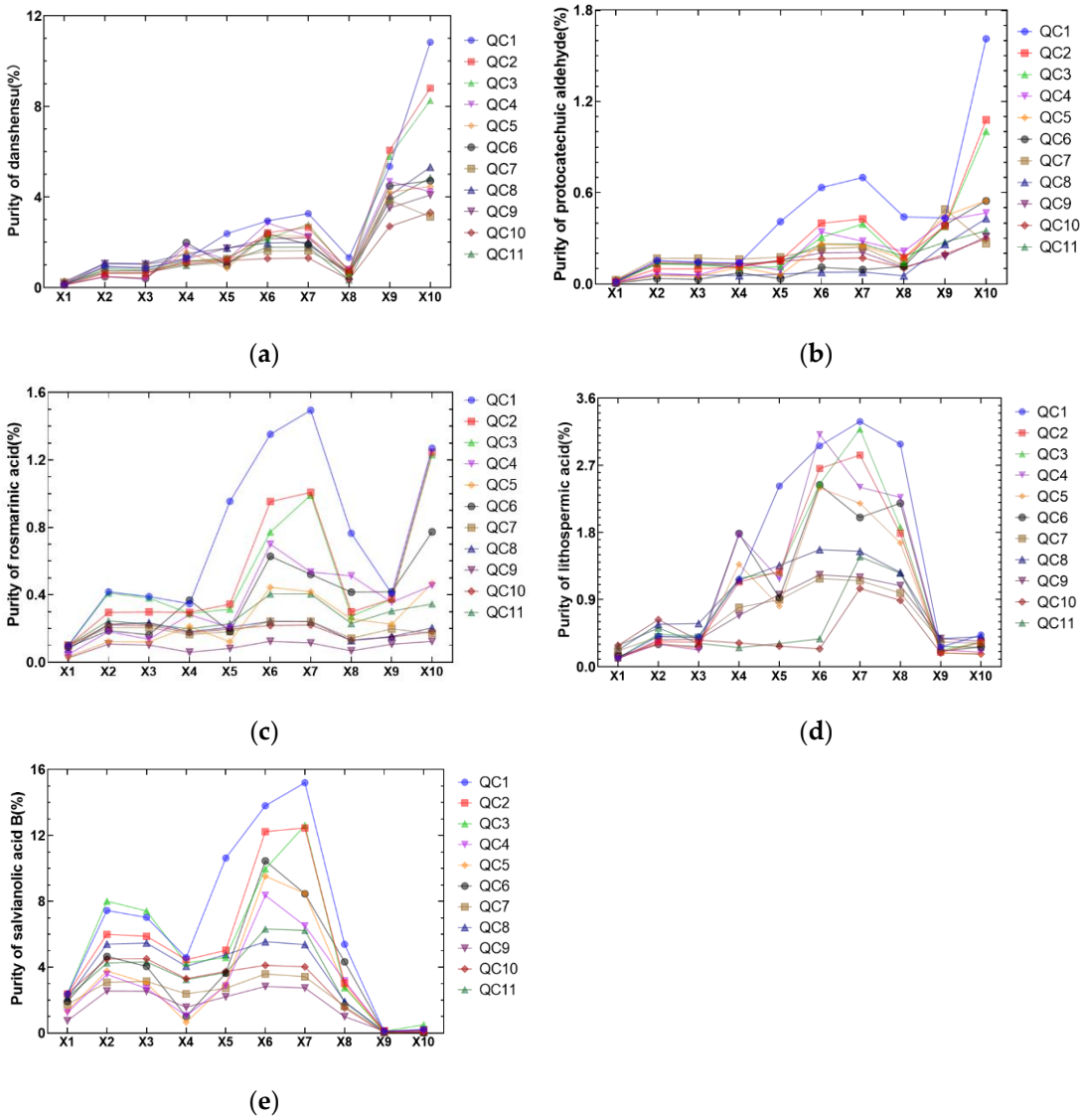

Figure 5. Variation in phenolic acid purity during the pharmaceutical process: (**a**) purity of danshensu; (**b**) purity of protocatechuic aldehyde; (**c**) purity of rosmarinic acid; (**d**) purity of lithospermic acid; (**e**) purity of salvianolic acid B.

The purity of phenolic components during the alkalization process is typically reduced due to their tendency to degrade easily. The thermal hydrolysis process significantly decreased the purity of lithospermic acid and salvianolic acid B. Nevertheless, there was an obvious increase in the purity of danshensu. The final purities of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B in the SMPE were 3.12–10.83%, 0.26–1.62%, 0.12–1.27%, 0.16–0.43%, and 0.02–0.52%, respectively.

4. Discussion

4.1. The Influence of Raw Materials and Processes on Inter-Batch Consistency

In this study, the relative standard deviation (RSD) of yield and purity was used to characterize the variation in consistency. Tests QC1, QC2, and QC3 all utilized the same batch of the herb from Batch 180913. RSD value fluctuation mostly represented the impact of the procedure and detection on consistency. Herbs from various batches, including batches 180927, 180901, 181001, 190801, 210911, 211101, 210811T, and 211022T, were utilized

in tests QC4-QC11, respectively. The RSD values were mostly affected by the quality of the herbs, pharmaceutical process, and detection.

4.1.1. Consistency of Total Solids

Figure 6 illustrates the variation in total solids during the pharmaceutical process. In the lime–sulfuric acid refining solution, the RSD of total solids yield was around 11%, but the ethanol precipitation process dramatically increased the RSD to approximately 45%. This means that attention should be paid to the ethanol precipitation process to control the overall amount of solid in each SMPE.

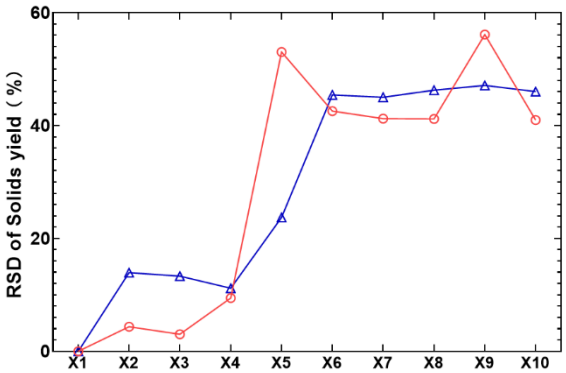

Figure 6. Variation in total solids yield. (○ represents data obtained with the same batch of *Salvia miltiorrhiza*; △ represents data obtained with the different batches of *Salvia miltiorrhiza*).

Total solids of SMPE obtained from the same batch and different batches had corresponding RSD values of 40.98% and 46.04%. The difference was negligible, proving that the pharmaceutical process rather than the quality of the herb was the primary cause of variance in the yield of total solids.

4.1.2. Yield of Phenolic Components

Figure 7 illustrates the variation in the yield of phenolic components during the pharmaceutical process. The RSDs of the yields of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B in the SMPE made from different batches of herbs were 37.21%, 31.94%, 47.19%, 61.25%, and 61.75%, respectively. While those obtained from the same batches of herbs were 29.28%, 21.10%, 39.57%, 30.87%, and 97.43%, respectively.

4.1.3. Purity of Phenolic Components

Figure 8 illustrates the variation in the purity of phenolic components during the pharmaceutical process. In the SMPE produced from different batches of herbs, the RSDs of the purity of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B were 37.74%, 59.81%, 82.79%, 31.59%, and 86.21%, respectively. As opposed to those obtained from the same batch of herbs, which were 14.58%, 26.93%, 1.57%, 16.13%, and 60.87%, respectively. The purity of the phenolic components of *Salvia miltiorrhiza* made from different batches of herbs was higher than that of the same batch. This suggests that the consistency of the purity of phenolic components in the SMPE is influenced by the fluctuation in herb quality.

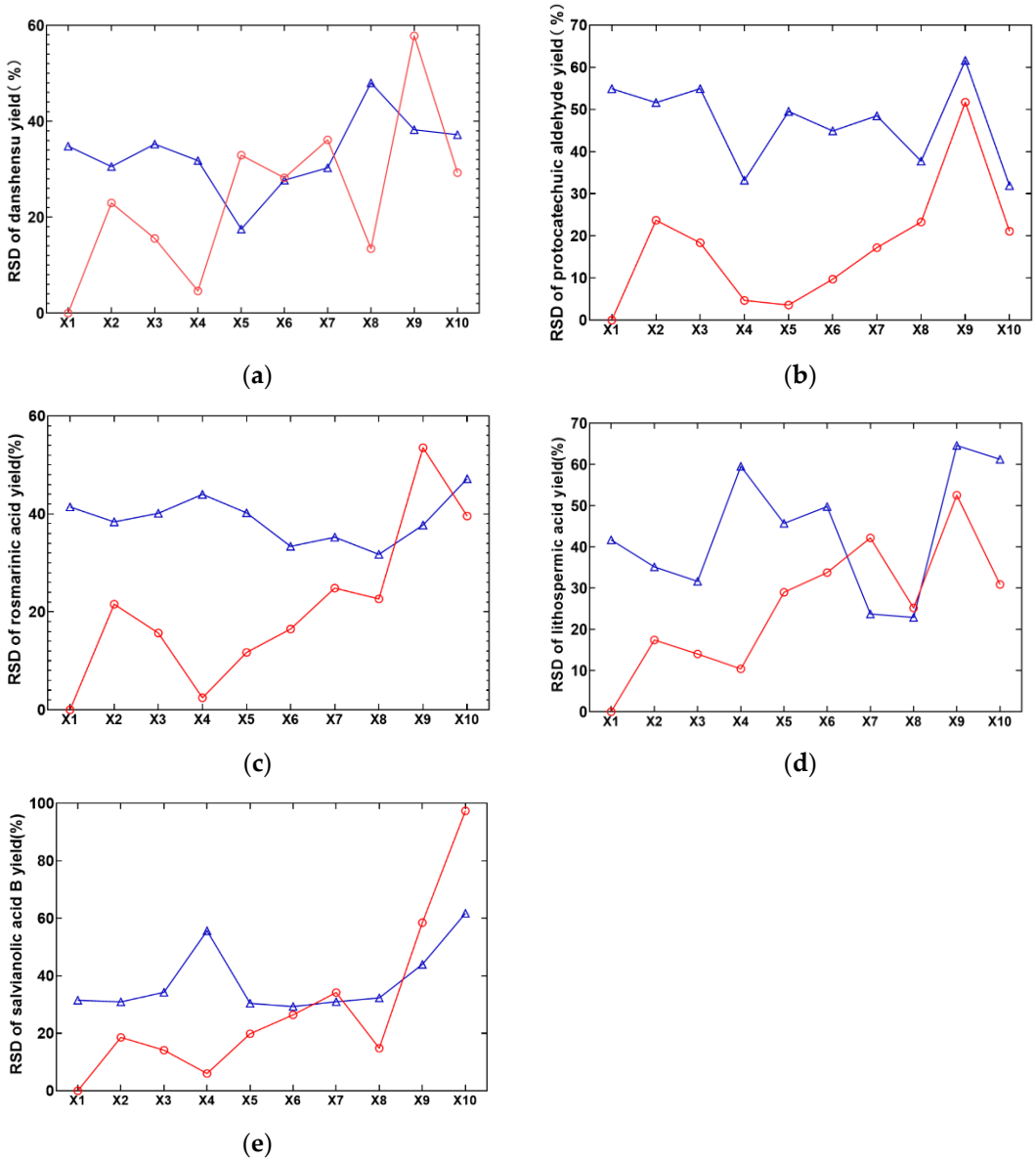

Figure 7. Variation in the yield of each phenolic compound: (**a**) RSD of danshensu yield; (**b**) RSD of protocatechuic aldehyde yield; (**c**) RSD of rosmarinic acid yield; (**d**) RSD of lithospermic acid yield; (**e**) RSD of salvianolic acid B yield. (○ represents data obtained with the same batch of *Salvia miltiorrhiza*; △ represents data obtained with the different batches of *Salvia miltiorrhiza*).

4.1.4. Quantitative Characterization of the Impact of Raw Materials and Pharmaceutical Processes on Batch-to-Batch Consistency

The RSD values of total solids, phenolic yield, and phenolic purity during the preparation of SMPE can all, to a certain extent, reflect the consistency of the overall process. In this study, the consistency of the SMPE preparation process was characterized by the amount of the total variation (TV) values, which were calculated in a weighted way. Equation (9) displays the calculation formula. The consistency becomes poorer as the TV value increases.

$$TV = w_1 \text{RSD of total solids} + w_2 \frac{\sum_{i=1}^{n} \text{RSD of each phenolic compound yield}}{n} + w_3 \frac{\sum_{i=1}^{n} \text{RSD of each phenolic compound purity}}{n} \quad (9)$$

where w_1, w_2 and w_3 are the weight coefficients, and they are taken as 0.2, 0.2, and 0.6 in this work, respectively.

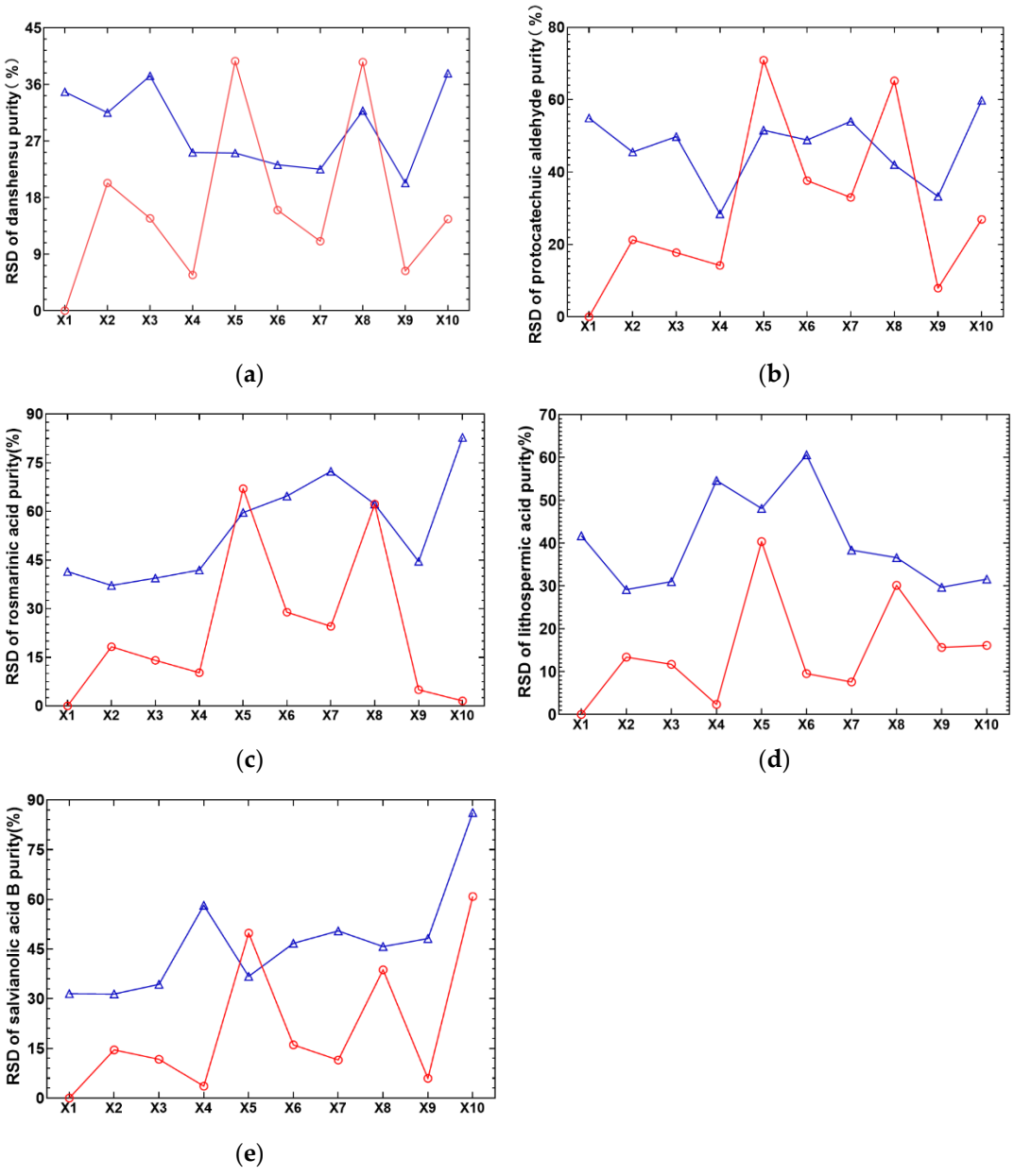

Figure 8. Variation in the purity of each phenolic compound: (**a**) RSD of danshensu purity; (**b**) RSD of protocatechuic aldehyde purity; (**c**) RSD of rosmarinic acid purity; (**d**) RSD of lithospermic acid purity; (**e**) RSD of salvianolic acid B purity. (○ represents data obtained with the same batch of *Salvia miltiorrhiza*; △ represents data obtained with the different batches of *Salvia miltiorrhiza*).

The total variation value was calculated to be 31.34% for the same batch of Salvia herbs after the preparation process of SMPE and 54.56% for different batches of Salvia herbs after the preparation process of SMPE. Assuming that the variation in data introduced by the analytical assay was 5%, the total variation effect of the preparation process on the SMPE was 26.34%, and the total variation effect of batch-to-batch variation in Salvia herbs on the SMPE was 18.22%. The contribution of the preparation process to the total variation was slightly greater than that of the batch-to-batch quality variation in the herbs.

The results of Zhong Wen et al. concluded that the contribution of batch-to-batch quality differences in herb quality to the total variation in herbal product quality was much greater than the effect of the process [25]. The results differ from this study mainly because of the presence of more chemical reactions and the purification processes in the process studied in this study, which have a greater impact on the chemical composition.

4.2. Identification of Critical Processes

4.2.1. Judgment of Critical Processes Based on Material Similarity

The results of the similarity calculation of the composition of raw materials, intermediates, and SMPE during the preparation process are shown in Figure 9. The closer the Pearson correlation coefficient, Spearman correlation coefficient, and cosine of the pinch angle were to 1.0, or the closer the Euclidean distance was to 0, the more similar the composition of each intermediate was to that of the SMPE.

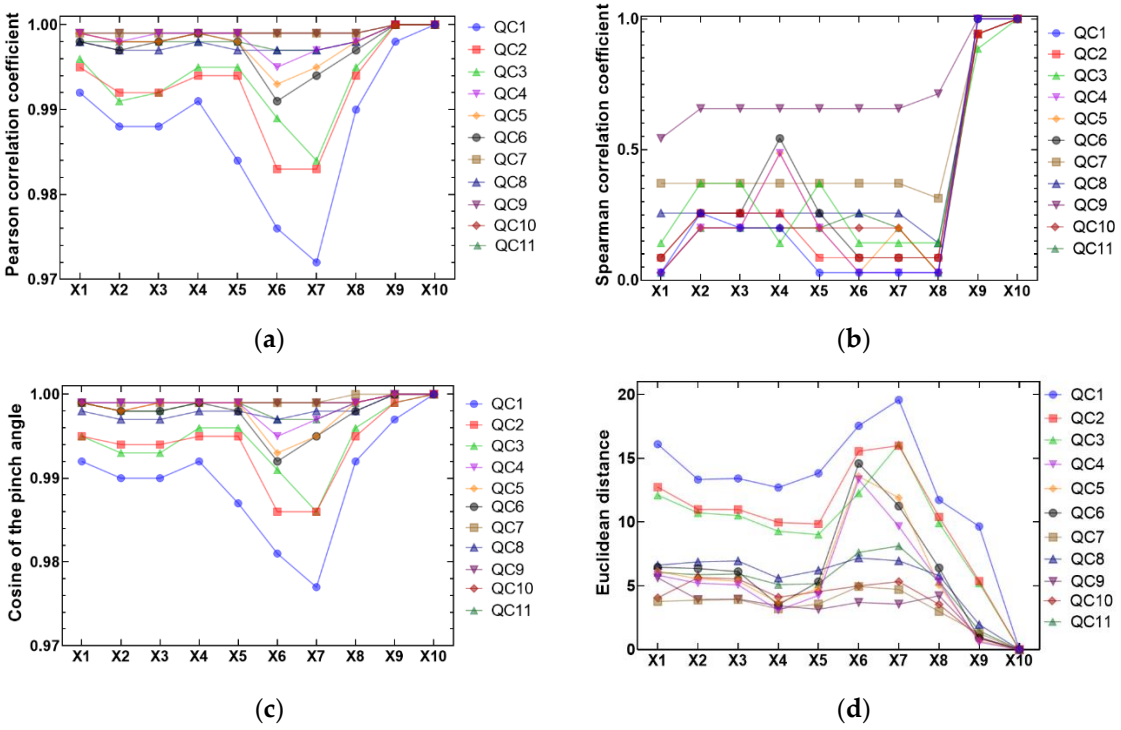

Figure 9. Changes in the similarity of raw materials, intermediates, and final products at each stage of the process: (**a**) Pearson correlation coefficient; (**b**) Spearman correlation coefficient; (**c**) cosine of the pinch angle; (**d**) Euclidean distance. (○ represents data obtained with the same batch of *Salvia miltiorrhiza*; △ represents data obtained with the different batches of *Salvia miltiorrhiza*).

In Figure 9a,c, the Pearson correlation coefficients and the cosine of the pinch angles in some batches do not reflect the differences between the pharmaceutical processes' materials, such as herbs and extracts, and the final products. In Figure 9b, the Spearman correlation coefficient is less sensitive and fails to reflect some preparation steps' effects because of the calculation's rank transformation step. In Figure 9d, the Euclidean distance characterized the similarity of the raw materials and each intermediate to the final product with high sensitivity.

The Euclidean distances of the intermediates obtained from different batches of herbs differed. After calculating their mean value $\overline{D_j}$, the change in the mean Euclidean distance before and after the process was further calculated as $\Delta\overline{D_j}$, and the equation is given in Equation (10):

$$\Delta\overline{D} = \left|\overline{D_j} \text{ before process} - \overline{D_j} \text{ after process}\right| \quad (10)$$

where the subscript j (j = 1 … … 9) in the formula represents the individual processes, which are reflux extraction, vacuum concentration, lime–sulfuric acid refining, first ethanol precipitation, second ethanol precipitation, first acidification, alkalization, thermal hydrolysis, and second acidification. The $\Delta\overline{D_j}$ calculation results are shown in Table 3. $\Delta\overline{D_j} \geq 3$ was considered the threshold value, and the second ethanol precipitation, alkalization, and thermal hydrolysis processes can be considered critical.

Table 3. Changes in the mean Euclidean distance in the process $\Delta\overline{D_j}$.

Process	$\Delta\overline{D_j}$
Reflux extraction	0.649
Vacuum concentration	0.054
Lime–sulfuric acid refining	1.282
First ethanol precipitation	0.549
Second ethanol precipitation	**4.143**
First acidification	0.194
Alkalization	**3.860**
Thermal hydrolysis	**3.788**
Second acidification	2.642

4.2.2. Judgment of Critical Processes Based on the Impact of the Process on Batch-to-Batch Consistency

To evaluate the effect of a specific process on consistency, this study calculated the difference in the RSD value of the total solids, the yield of each phenolic acid, and each phenolic acid purity before and after the process (ΔRSD).

$$\Delta\text{RSD} = \text{RSD before process} - \text{RSD after process} \tag{11}$$

The ΔRSD values for total solids, the yield of each phenolic compound, and the purity of each phenolic compound are listed in Appendix A Tables A2 and A3. A positive value of ΔRSD means the process improves quality consistency; a negative value means that it decreases consistency. The second ethanol precipitation process significantly increased the variation in total solids among the batches. First ethanol precipitation reduced the variation in salvianolic acid B yield, but lime–sulfuric acid refining and second acidification increased the variation in salvianolic acid B yield. The second acidification process reduced the consistency of the purity of most phenolic components.

The ΔRSD values were taken as absolute values and weighted to obtain the contribution of individual processes to the total variation (TVP) as an index to evaluate the influence of the process on consistency. The calculation formula is shown in Equation (12):

$$TVP_j = w_1 \text{total solids}|\Delta RSD| + w_2\frac{\sum_{i=1}^{n}\text{yield of each phenolic compfirstnt}|\Delta RSD|}{n} + w_3\frac{\sum_{i=1}^{n}\text{purity of each phenolic compfirstnt}|\Delta RSD|}{n} \tag{12}$$

The specific results are shown in Table 4. The critical processes were screened with TVP $\geq$ 10% as the threshold value. The critical processes were lime–sulfuric acid refining, first ethanol precipitation, and second acidification.

Table 4. Weighted contribution of individual processes to TVP.

Process	TVP_j (%)
Reflux extraction	7.06
Vacuum concentration	2.85
Lime–sulfuric acid refining	**13.59**
First ethanol precipitation	**13.73**
Second ethanol precipitation	9.29
First acidification	6.25
Alkalization	6.17
Thermal hydrolysis	9.56
Second acidification	**17.33**

4.2.3. Influencing Factors of Critical Process Judgment

Critical process identification can guide the optimization of pharmaceutical processes and production line automation of TCM. Many studies have been conducted to try to

identify the critical processes of pharmaceutical processes in TCM. Huali Chen [26] and Yao Li [27] used a qualitative risk assessment method to identify the critical processes for producing lyophilized powder for Panax ginseng and Danhong injection, respectively. This method is easy to implement but relies on subjective judgment. Jinjing Shen [28] proposed a method using the changes in the quantity, composition similarity, and purity of chemical components before and after the unit process as evaluative indexes to determine the critical processes for preparing honeysuckle extract. Pan et al. [29] identified the critical processes for preparing Panax notoginseng saponins considering the chemical composition, chemical consistency, and variation in biological activity.

Based on material similarity, the critical processes were considered second ethanol precipitation, alkalization, and thermal hydrolysis. According to the impact on batch-to-batch consistency, the critical processes were identified as lime–sulfuric acid refining, first ethanol precipitation, and second acidification. Therefore, the critical processes may vary depending on the perspective. The selection of the threshold value and the weight setting in this study can also affect the judgment of the critical process. Additionally, biological activity can indicate the general quality of the pharmaceutical solution. However, in this investigation, we did not assess the biological activity of the intermediates.

5. Conclusions

This paper studied the phenomenon of quantity and measure value transfer during the preparation of SMPE. After reflux extraction, vacuum concentration, lime–sulfuric acid refining, ethanol precipitation, acidification, alkalization, and thermal hydrolysis, the total solids ranged from 45–250 mg/g. The yields of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B were 3.27–11.40 mg/g, 0.34–1.07 mg/g, 0.14–1.31 mg/g, and 0.14–0.86 mg/g, respectively. The purities of danshensu, protocatechuic aldehyde, rosmarinic acid, lithospermic acid, and salvianolic acid B in the obtained SMPE were 3.12–10.83%, 0.26–1.62%, 0.12–1.27%, 0.16–0.43%, and 0.02–0.52%, respectively.

The differences between the SMPEs made from the same batch of herbs and those made from various batches of herbs were compared in this paper. The index TV was defined and calculated in a weighted manner. The magnitude of the TV values characterized the contribution to the consistency of the preparation process of SMPEs. The estimated TV values were 18.22%, contributed to by herb quality fluctuations, and 26.34%, contributed to by the preparation process. The contribution of herb batch-to-batch quality variation to the total variation was slightly smaller than that of the preparation process.

Furthermore, four similarity evaluation metrics, the Pearson correlation coefficient, Spearman correlation coefficient, cosine of the pinch angles, and Euclidean distance, were all compared. Euclidean distance was the most suitable for determining how comparable the intermediates and SMPEs were. The critical processes were judged to be the second ethanol precipitation process, the alkalization process, and the thermal hydrolysis process based on the change in the mean Euclidean distance ($\Delta\overline{D_j}$) before and after the process. The TVP value is also used as an index to evaluate how the process affects consistency. The first ethanol precipitation process, the second acidification process, and the lime–sulfuric acid refining process were found to be the critical processes based on the TVP value. In the manufacturing process of SMPEs, attention should be paid to the parameter control of these processes and related equipment. Priority should be given to these processes during industrial automation transformation.

This paper analyzed the pharmaceutical process of TCM from the perspective of quantity and measure value transfer, and the method used in this work can be extended to the pharmaceutical processes of other TCMs.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table A1. Exact concentrations of each reference substance.

Reference Substance	Concentration(mg/mL)	Peak Area (μAUs)
Danshensu	0.643	640.1
	0.3215	318.2
	0.0643	64.5
Protocatechuic aldehyde	0.567	4268.2
	0.2835	2145.1
	0.0567	437.1
Rosmarinic acid	0.589	1731
	0.2945	865.5
	0.0589	175.9
Lithospermic acid	0.489	854.5
	0.2445	415.5
	0.0489	84.1
Salvianolic acid B	0.602	1137
	0.301	542.5
	0.0602	105.8

Table A2. The ΔRSD values for total solids and the yield of each phenolic compound (%).

Process	Total Solids	Yield of Danshensu	Yield of Protocatechuic Aldehyde	Yield of Rosmarinic Acid	Yield of Lithospermic Acid	Yield of Salvianolic Acid B
Reflux extraction	−13.95	4.26	3.29	3.04	6.56	0.60
Vacuum concentration	0.62	−4.69	−3.30	−1.74	3.50	−3.35
Lime–sulfuric acid refining	2.15	3.43	21.74	−3.87	−27.91	−21.40
First ethanol precipitation	−12.55	14.28	−16.33	3.77	13.84	25.27
Second ethanol precipitation	−21.73	−10.20	4.61	6.84	−4.06	1.11
First acidification	0.43	−2.57	−3.57	−1.88	26.07	−1.67
Alkalization	−1.26	−17.72	10.74	3.50	0.84	−1.35
Thermal hydrolysis	−0.84	9.78	−23.88	−5.97	−41.72	−11.67
Second acidification	1.08	1.02	29.69	−9.45	3.32	−17.77

Table A3. The ΔRSD values for the purity of each phenolic compound.

Process	Purity of Danshensu	Purity of Protocatechuic Aldehyde	Purity of Rosmarinic Acid	Purity of Lithospermic Acid	Purity of Salvianolic Acid B
Reflux extraction	3.31	9.38	4.28	12.56	0.10
Vacuum concentration	−5.86	−4.27	−2.28	−1.84	−2.90
Lime–sulfuric acid refining	12.19	21.36	−2.48	−23.64	−23.88
First ethanol precipitation	0.09	−23.14	−17.72	6.55	21.48
Second ethanol precipitation	1.87	2.72	−5.10	−12.54	−10.00
First acidification	0.69	−5.14	−7.62	22.24	−3.75
Alkalization	−9.30	11.97	10.15	1.77	4.74
Thermal hydrolysis	11.50	8.78	17.65	6.91	−2.41
Second acidification	−17.44	−26.55	−38.23	−1.90	−38.06

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