Study on HPLC Fingerprint and Chemical Pattern Recognition of Guizhi Shaoyao Zhimu Granules

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Keywords: Guizhi Shaoyao Zhimu Granules, HPLC Fingerprint, Chemical Pattern Recognition, Similarity Evaluation.

Abstract: Objective: To establish the fingerprint of Guizhi Shaoyao Zhimu Granules, and evaluate its quality in combination with the chemical pattern recognition method, so as to provide a reference for the quality control of the preparation. Method: Use Waters XSelect HSS T3 (4.6 mm ×250 mm, 5 μm) chromatographic column; mobile phase is acetonitrile-0.1% phosphoric acid aqueous solution; gradient elution; flow rate is 1.0 min·m L^{-1}; column temperature is 35℃; the detection wavelength was 210 nm; the injection volume was 10 μL. Ten batches of Guizhi Shaoyao Zhimu Granules fingerprints were established, and similarity evaluation, cluster analysis, principal component analysis and orthogonal partial least squares discriminant analysis were performed. Results: The established HPLC fingerprint of Guizhi Shaoyao Zhimu Granules identified 16 common peaks, and identified ephedrine hydrochloride, D-pseudu-ephedrine hydrochloride, mangiferin, paeoniflorin, liquiritin, and 5-O-methylvisammioside , cinnamic acid, glycyrrhizic acid, 6-ginger phenol; 9 batches of Guizhi Shaoyao Zhimu Granules fingerprint pattern and control pattern similarity are all> 0.990, cluster analysis and principal component analysis results are basically the same, the orthogonality is the smallest. The two-fold discriminant analysis method screened out 9 quality difference markers. Conclusion: The method is simple and reliable, and can provide a reference for the quality control of Guizhi Shaoyao Zhimu Granules.

1 INTRODUCTION

Guizhi Shaoyao Zhimu Granules is a traditional Chinese medicine compound preparation made from 9 medicinal materials of Cinnamomum cassia, Paeonialactiflora, Glycyrrhiza uralensis, Ephedra sinica, Zingiber officinale Roscoe, Atractylodes macrocephala, Anemarrhena asphodeloides, Saposhnikovia divaricata, and Aconitum carmichaelii. It has the effects of expelling wind and dampness, warming menstruation and dispelling cold, the main clinical treatment of rheumatoid arthritis, gouty arthritis and knee osteoarthritis and other diseases. The preparation is composed of 9 medicinal materials with complex ingredients, and only controlling the content of one or several of them cannot reflect the quality level of the preparation as a whole. The fingerprint of traditional Chinese medicine is a comprehensive and quantifiable identification method, which can comprehensively evaluate the quality and stability of traditional Chinese medicine and its preparations as a whole, and provide effective means for its quality control. However, fingerprints have shortcomings such as large amount of information, fuzzy data, and difficult analysis, so it is necessary to use chemometric method to reduce the dimension of the data. Chemical pattern recognition technology is a comprehensive technology that analyzes information with the help of computers, which can quantify the entire fingerprint information, so as to more objectively reflect the differences in the quality of Chinese medicine, and achieve the purpose of

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comprehensive control of the quality of Chinese medicine. Therefore, in this study, the HPLC fingerprint of Guizhi Shaoyao Zhimu Granules was established to characterize the types of chemical components at the overall level. At the same time, cluster analysis, principal component analysis and orthogonal partial least squares discriminant analysis were combined to analyze each sample. The main markers for the quality difference of Guizhi Shaoyao Zhimu Granules of different batches are screened to provide a scientific basis for its quality control.

2 INSTRUMENTS AND MATERIALS

2.1 Instrument

Shimadzu LC-20AT system (SPD-M20A PDA detector, SIL-20A autosampler): Japan Shimadzu Technology Co., Ltd.; MS105DU electronic balance: METTLER TOLEDO Instrument Co., Ltd.; TGL16M high-speed centrifuge: Hunan Kaida Scientific Instrument Co., Ltd.; Ultrasonic Cleaner: Tianjin Autosines Instrument Co., Ltd.

2.2 Material

Liquiritin, glycyrrhizic acid, paeoniflorin, 6-ginger phenol, mangiferin, cinnamic acid, 5-O-methylvisammioside reference substance (Chengdu Desite Biotechnology Co., Ltd., batch numbers are DST200412-009, DSTDG000601, DSTDS007001, DST190716-027, DST190305-031, DST190413-045, DST190213-006, all with a purity of 98.0%); Ephedrine hydrochloride and D-pseudu-ephedrine hydrochloride reference substances (China Institute for Food and Drug Control, batch numbers are 171241-201809 and 171237-201809, respectively, with a purity of 100.0%). Anemarrhena asphodeloides, Glycyrrhiza uralensis and Aconitum carmichaelii were purchased from Hebei Renxin Pharmaceutical Co., Ltd.; Cinnamomum cassia, Paeonialactiflora, Atractylodes macrocephala and Saposhnikovia divaricata were purchased from Anguo Anxing Chinese Medicine Decoction Pieces Co., Ltd.; Ephedra sinica was purchased from Inner Mongolia Pukang Pharmaceutical Co., Ltd.; Zingiber officinale Roscoe was purchased From Jilin Hongjian Pharmacy. Guizhi Shaoyao Zhimu Granules, batch numbers are 200913, 200924, 201107, 201113, 201118, 201125, 210319, 201012, 210402, 201206, serial number S1~S10, laboratory self-made. Acetonitrile is chromatographically pure, water is pure water, and other reagents are analytically pure.

3 METHODS AND RESULTS

3.1 Chromatographic Conditions

Waters XSelect HSS T3 column (4.6 mm × 250 mm, 5 μm); mobile phase: acetonitrile (A)-0.1% phosphoric acid water (B), gradient washout (0-5 min, 95.5% B; 5-8 min, 95.5% B → 91% B; 8-13 min, 91% B → 82% B; 13-28 min, 82% B → 80% B; 28-35 min, 80% B → 77% B; 35-43 min, 77% B → 72% B; 43-51 min, 72% B → 57% B; 51-59 min, 57% B → 30% B; 59-64 min, 30% B→ 5% B; 64-70 min, 5% B; 70-73 min, 5% B→95.5% B; 73-80 min, 95.5% B). Flow rate: 1.0 min·m L^{-1}; column temperature: 35℃; injection volume: 10 μL; detection wavelength: 210 nm.

3.2 Solution Preparation

3.2.1 Mixed Reference Solution

Accurately weigh the appropriate amount of ephedrine hydrochloride, D-pseudu-ephedrine hydrochloride, mangiferin, paeoniflorin, liquiritin, 5-O-methylvisammioside, cinnamic acid, glycyrrhizic acid, and 6-ginger phenol reference substance. Dissolve with methanol to prepare a single reference solution containing the above-mentioned control quality concentrations of 1.008, 1.012, 1.024, 1.007, 1.013, 1.008, 1.000, 1.001, 1.015 mg·m L^{-1}; take an appropriate amount of each of the above single reference solution and dilute with methanol to prepare a mixed reference solution with mass concentrations of 10.08, 10.12, 10.24, 10.07, 10.13, 10.08, 10.00, 10.01, and 10.15 μg·m L^{-1}.

3.2.2 Test Solution

Take an appropriate amount of Guizhi Shaoyao Zhimu Granules, grind it into small pieces, take 0.8 g, accurately weigh it, place it in a stoppered erlenmeyer flask, accurately add 5 ml of methanol, ultrasonically treat (220 V, 50 Hz) for 20 min, 10,000 rpm, centrifuge for 10 min, take the supernatant, and pass through a 0.22 μm microporous membrane to get it.

3.2.3 Single Medicinal Solution

Weigh about 50 g of Guizhi Shaoyao Zhimu Granules prescription medicinal materials Anemarrhena asphodeloides, Glycyrrhiza uralensis, Aconitum carmichaelii, Cinnamomum cassia, Paeonialactiflora, Atractylodes macrocephala, Saposhnikovia divaricata, Ephedra sinica, and Zingiber officinale Roscoe respectively, and prepare each single medicinal material sample according to the prescription process of the preparation. Prepare a single medicinal solution according to the method under "3.2.2", and get it.

3.3 Methodological Review

3.3.1 Precision Test

Precisely draw the same test solution, inject 6 times continuously, and record the chromatogram. With liquiritin as the reference peak, the relative retention time and relative peak area of each shared peak were calculated. The RSD of the relative retention time of each chromatographic peak was less than 0.74% (n=6), and the RSD of the relative peak area was less than 2.90% (n=6), indicating that the precision of the instrument was good.

3.3.2 Stability Test

Precisely draw the same test solution, inject samples at 0, 4, 8, 12, 18 and 24 h, and record the chromatogram. With liquiritin as the reference peak, the relative retention time and relative peak area of each shared peak were calculated. The relative retention time RSD of each common peak was less than 0.69% (n=6), and the RSD of the relative peak area was less than 2.80% (n=6), indicating that the test product was stable within 24 hours.

3.3.3 Repeatability Test

Take the same batch of Guizhi Shaoyao Zhimu Granules, prepare 6 test solution solutions according to the method under "3.2.2", inject 6 samples for determination. With liquiritin as the reference peak, the relative retention time and relative peak area of each shared peak were calculated. The relative retention time RSD of each common peak was less than 0.27% (n=6), and the RSD of the relative peak area was less than 2.68% (n=6), indicating that the method has good repeatability.

3.4 Establishment of Fingerprint Atlas and Evaluation of Similarity

3.4.1 Establishment of Fingerprint Atlas

Take 10 batches of Guizhi Shaoyao Zhimu Granules and inject them for determination under the chromatographic conditions under "3.1". Analyzed by "Chinese Medicine Chromatographic Fingerprint Similarity Evaluation System (2012 Edition)", with S1 sample chromatogram as the reference map, the time width is set to 0.1 min, and the multi-point calibration is set to automatically match, and the median method is used to generate the overlay map and the control map, as shown in Figure 1. A total of 16 common peaks were calibrated, of which peak No. 10 (liquiritin) had a good separation effect and was in the middle position, so it was taken as the reference peak.

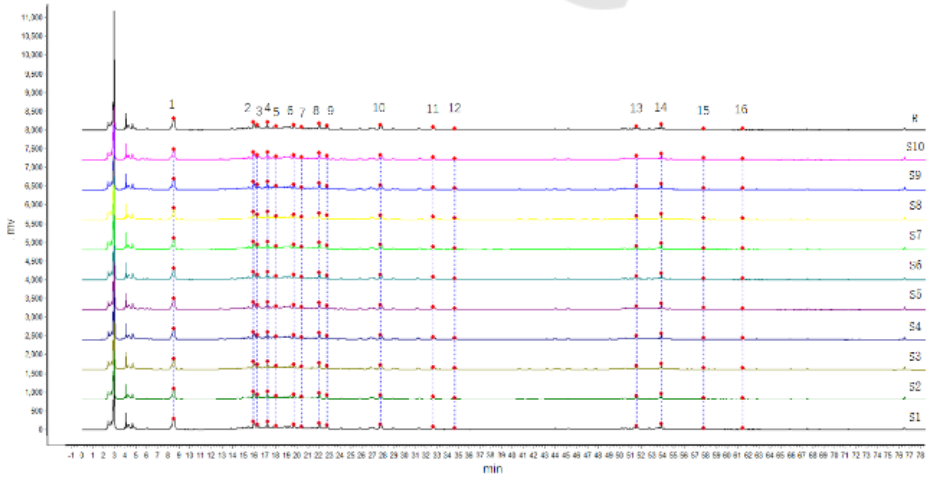
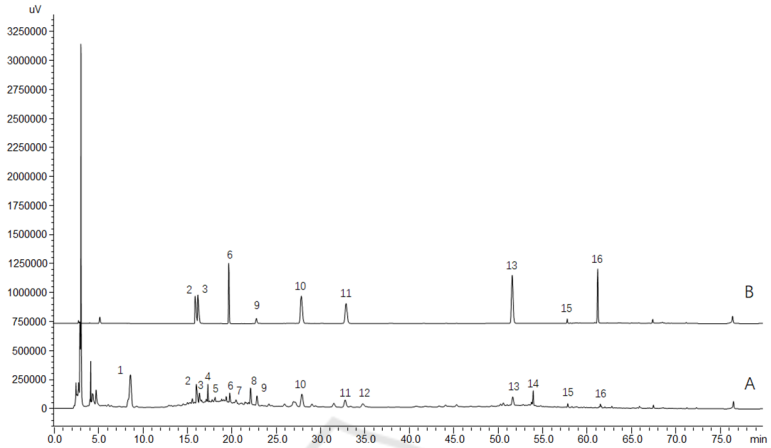


Figure 1: HPLC Superimposed Chromatogram (S1-S10) and Control Chromatogram (R) of 10 batches of Guizhi Shaoyao Zhimu Granules.

3.4.2 Identification of Common Peaks

Measure the test solution and the mixed reference solution under the chromatographic conditions under "3.1", and compare the retention time of each peak to identify 9 components, namely 2 (ephedrine hydrochloride), 3 (D-pseudu-ephedrine hydrochloride), 6 (mangiferin), 9 (paeoniflorin), 10 (liquiritin), 11 (5-O-methylvisammioside), 13 (cinnamic acid), 15 (glycyrrhizic acid), 16 (6-ginger phenol), see Figure 2.

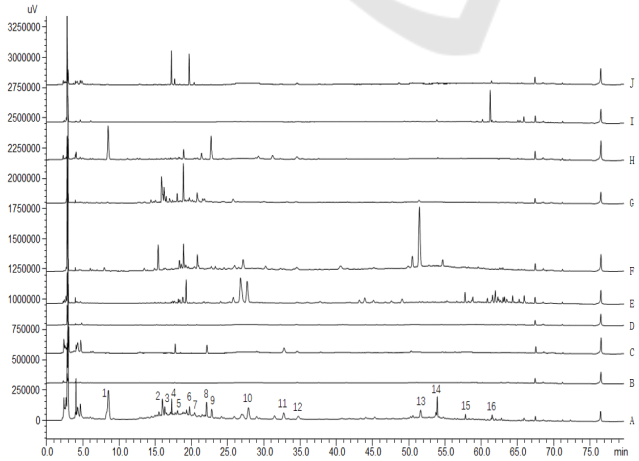


2.ephedrine hydrochloride 3. D-pseudu-ephedrine hydrochloride 6.mangiferin 9.paeoniflorin 10.liquiritin 11.5-O-methylvisammioside 13.cinnamic acid 15.glycyrrhizic acid 16.6-ginger phenol

Figure 2: HPLC chromatograms of test solution (A) and mixed reference solution (B).

3.4.3 Attribution of Shared Peaks

By comparing the HPLC chromatograms of the single medicinal solution and the test solution, the common peaks of the fingerprints are assigned to the medicinal materials, as shown in Figure 3.Peaks 1, 9 are from Paeonialactiflora; peaks 2, 3, and 5 are from Ephedra sinica; peaks 4, 6, and 7 are from Anemarrhena asphodeloides; peaks 8, 11 are from Saposhnikovia divaricata; peaks 10 and 15 are from Glycyrrhiza uralensis; peak 12 is shared by Saposhnikovia divaricata, Glycyrrhiza uralensis, Paeonialactiflora, and Ephedra sinica; peak 13 is derived from Cinnamomum cassia; peaks 14 and 16 are derived from Zingiber officinale Roscoe. The results show that the preparation has a good correlation with the single medicinal material.



A. test solution B. Atractylodes macrocephala C. Saposhnikovia divaricata D. Aconitum carmichaelii E. Glycyrrhiza uralensis F. Cinnamomum cassia G. Ephedra sinica H. Paeonialactiflora I. Zingiber officinale J. Anemarrhena asphodeloides

Figure 3: HPLC chromatograms of test solution and single medicinal materials.

3.4.4 Fingerprint Similarity Evaluation

The similarity evaluation system of fingerprints of traditional Chinese medicine (2012 edition) was used to calculate the similarity of fingerprints of 10 batches of Guizhi Shaoyao Zhimu Granules, as shown in Table 1. The similarities of 10 batches of preparations are all greater than 0.990, indicating that the similarity between batches of the preparations is good and the quality is relatively stable.

Table 1: Table of similarity evaluation results of 10 batches of Guizhi Shaoyao Zhimu Granules.

Peak number	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	R
S1	1.000	1.000	0.996	0.996	0.996	0.997	0.999	0.993	0.999	0.996	0.999
S2	1.000	1.000	0.996	0.996	0.996	0.998	0.999	0.994	0.999	0.996	0.999
S3	0.996	0.996	1.000	1.000	0.999	0.998	0.995	0.990	0.995	0.992	0.998
S4	0.996	0.996	1.000	1.000	0.999	0.998	0.995	0.991	0.995	0.991	0.998
S5	0.996	0.996	0.999	0.999	1.000	0.998	0.995	0.992	0.996	0.990	0.998
S6	0.997	0.998	0.998	0.998	0.998	1.000	0.997	0.994	0.997	0.993	0.999
S7	0.999	0.999	0.995	0.995	0.995	0.997	1.000	0.996	1.000	0.996	0.999
S8	0.993	0.994	0.990	0.991	0.992	0.994	0.996	1.000	0.996	0.992	0.996
S9	0.999	0.999	0.995	0.995	0.996	0.997	1.000	0.996	1.000	0.995	0.999
S10	0.996	0.996	0.992	0.991	0.990	0.993	0.996	0.992	0.995	1.000	0.996
R	0.999	0.999	0.998	0.998	0.998	0.999	0.999	0.996	0.999	0.996	1.000

3.5 Chemical Pattern Recognition

3.5.1 Cluster Analysis (HCA)

Using SPSS 20.0 software, taking the peak areas of 16 common peaks in 10 batches of Guizhi Shaoyao Zhimu Granules as variables, the original data matrix of 10×16 order was obtained, and the Ward method combined with mean euclidean distance was used as the metric to perform cluster analysis, see Figure 4. When the mean euclidean distance is 10, 10 batches of samples can be grouped into two types: samples S1, S2, S7, S8, and S9 are grouped into one type; samples S3, S4, S5, S6, and S10 are grouped into one type. When the squared Euclidean distance is 5, 10 batches of samples are aggregated into three categories: samples S1, S2, and S8 are aggregated into the first category; samples S7 and S9 aggregated into the second category; samples S3, S4, S5, S6, and S10 aggregate it is the third category.

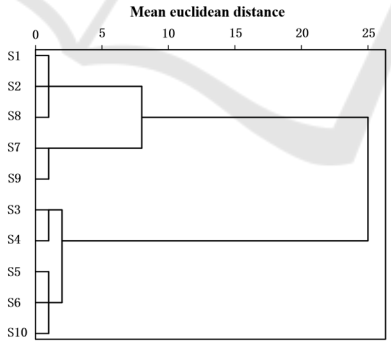


Figure 4: Cluster analysis tree of 10 batches of Guizhi Shaoyao Zhimu Granules.

3.5.2 Principal Component Analysis (PCA)

Using the common peak area as a variable, SPSS 20.0 software was used to perform principal component analysis on 10 batches of Guizhi Shaoyao Zhimu Granules, and 4 principal components were extracted (eigenvalues> 1), and the cumulative variance contribution rate was 88.609%, which is good Represents most of the information in the fingerprint, see Table 2. Use SIMCA 13.0 software to draw a principal component score chart, as shown in Figure 5. The results show that 10 batches of samples can be roughly divided into 3 categories, which are basically consistent with the cluster analysis results, and further verify the classification results of the cluster analysis.

Table 2: Principal component eigenvalues and variance.

Element	Eigenvalues	Variance contribution rate /%	Cumulative variance contribution rate /%
1	6.688	41.801	41.801
2	4.748	29.674	71.475
3	1.466	9.163	80.638
4	1.275	7.971	88.609

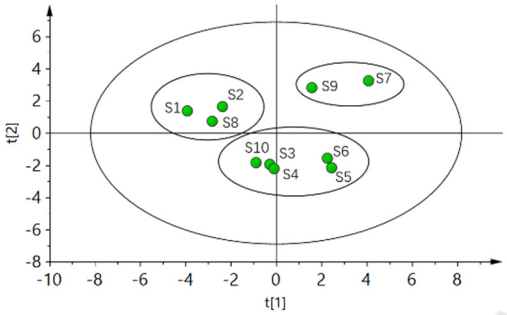


Figure 5: Principal component analysis score chart of 10 batches of Guizhi Shaoyao Zhimu Granules.

3.5.3 Discriminant Analysis of Orthogonal Partial Least Squares (OPLS-DA)

OPLS-DA analysis of common peak area of 10 batches of Guizhi Shaoyao Zhimu Granules using SIMCA 13.0 software. Under this model, RX2=0.794, RY2=0.974, and model prediction parameters Q2=0.905, all greater than 0.5. It shows that the OPLS-DA model established in this research is stable and has strong predictive ability. The 10 batches of samples are divided into 3 categories (Figure 6), which is consistent with the results of cluster analysis and principal component analysis. In order to further screen out the components that cause differences in 10 batches of samples, the variable importance projection (VIP) method was used for analysis, and the VIP values of 16 common peaks in the OPLS-DA model were extracted. The compounds with VIP values greater than 1 summarize the sample classification. The rate is greater than 50%, which is a marker of difference. Therefore, the differential markers of 10 batches of Guizhi Shaoyao Zhimu Granules have chromatographic peaks 5, 14, 6 (mangiferin), 10 (liquiritin), 13 (cinnamic acid), 7, 3 (D-pseudu-ephedrine hydrochloride), 1, 8, see Figure 7.

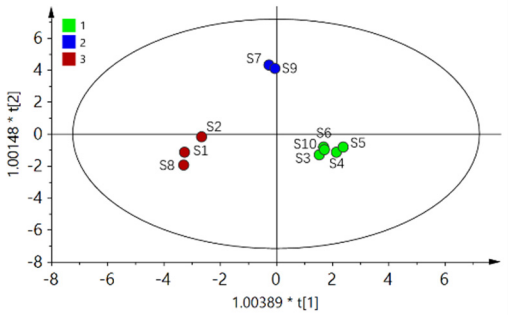


Figure 6: OPLS-DA score diagram of 10 batches of Guizhi Shaoyao Zhimu Granules.

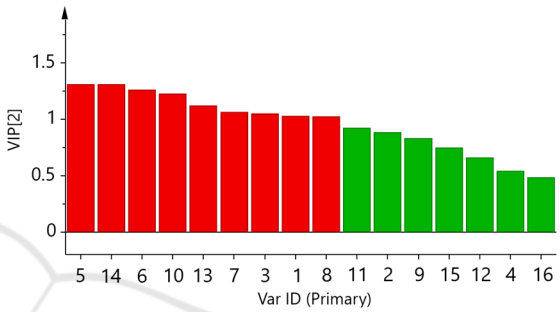


Figure 7: VIP value of each chromatographic peak in OPLS-DA model.

4 CONCLUSIONS

The similarity of 10 batches of Guizhi Shaoyao Zhimu Granules is above 0.990, indicating that the correlation between the batches is good, but the results of cluster analysis and principal component analysis show that there are certain differences between the sample groups, which may be different from the original formulation used. The origin of medicinal materials is related to the quality difference between batches. The chromatographic peaks 5, 14, 6 (mangiferin), 10 (liquiritin), 13 (cinnamic acid), 7, 3 (D-pseudu-ephedrine hydrochloride), and 1, 8 were selected by orthogonal partial least square discriminant analysis. In the quality control of Guizhi Shaoyao Zhimu Granules, the main marker ingredients that cause differences between the preparations should focus on the quality changes of these ingredients. In summary, the HPLC fingerprint of Guizhi Shaoyao Zhimu Granules established in this study combined with the chemical pattern recognition method can provide a reference for its overall quality evaluation.

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