Research Article

Analysis of the HPLC Fingerprint and QAMS for Sanhuang Gypsum Soup

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A valid and encyclopaedic evaluation method for assessing the quality of Sanhuang Gypsum Soup (SGS) has been set up based on analysis of high-performance liquid chromatography (HPLC) fingerprint combined with the quantitative analysis of multi-components by single marker (QAMS) method, hierarchical cluster analysis (HCA), and similarity analysis. 20 peaks of the common model were obtained and used for the similarity analysis and HCA analysis. Berberine was selected as an internal reference, and the relative correction factors of mangiferin, geniposide, liquiritin, epiberberine, coptisine, baicalin, palmatine, harpagosid, wogonoside, cinnamic acid, cinnamic aldehyde, baicalein, glycyrrhizic acid, and wogonin were established. The accuracy of quantitative analysis of multicomponents by the single-marker method was verified by comparing the contents of the fourteen components calculated by the external standard method with those of the quantitative analysis of multicomponents by the single-marker method. No significant difference was found in the quantitative results of the established quantitative analysis of multicomponents by a single-marker method and an external standard method. In summary, these methods were applied to evaluate the quality of SGS successfully. As a result, these evaluation methods have great potential to be widely used in the quality control of traditional Chinese medicines (TCM).

1. Introduction

Perspiration is a considerable physiological phenomenon to maintain and control body temperature. Excessive sweat secretion can cause armpit moisture, resulting in unpleasant body odour, embarrassment, and inconvenience [1, 2]. Hyperhidrosis is an excessive sweating disease which can bring severe psychological burden and affect the quality of life of patients negatively. There are a variety of medical treatments and surgery for treating primary hyperhidrosis. However, the side effects of these drugs include thirst, dry eyes, dizziness, drowsiness, constipation, and urinary retention, which limit the scope of their use. Surgical treatment is mainly applied to patients who are not suitable for the abovementioned methods. Surgery is more traumatized and risky than other treatments. Therefore, surgical operation should be as a second or third option [3]. There is still a paucity of effective nonsurgical therapies. With the development of modern society, the elimination of body odour is given more and more people’s attention; this article is about introducing Sanhuang gypsum soup (SGS) which is a significant antiperspirant. Through the use of classical Chinese medicine SGS to regulate the internal environment of the human body, it achieves a good antiperspirant effect with small side and remarkable effects, which make up for deficiency of some medicine and surgery. Every year, more than ten thousands of patients have benefited from SGS by reducing excessive perspiration symptoms. SGS is a hospital preparation of Jiangsu Provincial Hospital of Traditional Chinese Medicine, which consists of coptidis rhizoma, phellodendri chinensis cortex, scutellariae radix, scrophulariae radix, anemarrhenae rhizome, gardeniae fructus, cinnamomi cortex, glycyrrhizae radix et rhizoma preparata cum melle, and gypsum fibrosum. These herbs can be used for treating and could exhibit action on excessive perspiration through anti-inflammatory and antipyretic properties. Studies have shown that SGS has many chemical constituents such as mangiferin, geniposide, coptisine,
wogonin, wogonoside, baicalein, baicalin, and cinnamic aldehyde [4–13] in accordance with herbs and preparations known to be beneficial for the treatment of excessive per-
spiration through anti-inflammatory properties and so on.

Although the SGS is prepared as a prescription with the combination of these herbs in well-defined formulae, no standard quality control method for this product has been reported up to now. Since the effect of SGS might result from the synergy of multiple components, a reliable, sensitive, and uncomplicated quantitative method based on the diverse constituents is need to be developed.

Our findings have established an HPLC method to evaluate the quality of SGS comprehensively. Due to the variety of components of traditional Chinese medicine preparations, any one of the active ingredients cannot reflect the overall curative effect of traditional Chinese medicine. Therefore, a comprehensive macroscopic analysis will become an inevitable trend. Chromatographic fingerprint analysis with integrated, macroscopic, and “fuzzy” nonlinear characteristics is more adapted to the traditional Chinese medicine theory needs. Under the premise of efficacy, toxicology, and clinical trials which have confirmed safety and efficacy of preparation, we can not only verify the authenticity of the preparation but also determine whether the stability of the quality exists or not along with a practical fingerprint. Unlike the content determination, the fingerprint can provide more informative and useful message than the determination of any single component. The US Food and Drug Administration (FDA) allows applicants to provide product chromatographic fingerprinting information in the phytomedical guidance (Draft for Comment). British Herbal Codex, Ayurvedic Codex, the Canadian Society of Medicinal and Aromatic Plants [14], and the German Society of Medicinal Plants [15] also accept chromatographic fingerprint. One of the first measures that China’s State Drug Administration has taken to strengthen the supervision of traditional Chinese medicine injections requires the research on the fingerprint of injections, which has taken into account its necessity and feasibility. It is accepted that preparation of acceptable quality can be exerted on its drug efficacy, what really matters is establishing an accurate and easy method.

Previously, our laboratory has researched on the fingerprints of the existing preparations which have been applied in the control of preparation quality. Also, to make up for the limitations of fingerprint that cannot be quantified accurately, a QAMS method using berberine as the standard was developed and validated for the simultaneous quantitative of 14 components [16]. This strategy can not only reduce the cost of the experiment and time of detection but also be independent of the availability of all the target ingredients [15]. To our knowledge, quality control of herb extracts and botanical ingredient by QAMS have been included both in USP 33-NF and in Ch.P.2010 edition (volume I). Our results showed that no significant difference was found in the results between our established QAMS method and the external standard method. No one has yet studied the fingerprints of SGS; this article first established the SGS fingerprinting method and also used the QAMS method to measure the preparation of 14 kinds of pharmacodynamics components. This method could potentially be applied for the identification of qualitative and quantitative quality of SGS.

This HPLC fingerprint method, therefore, provides a comprehensive platform for quality evaluation of SGS with more chemical information. The combination of HCA and similarity analysis presents the differences and similarities of the HPLC fingerprints. In the meantime, QAMS method was adopted to quantify the main active components by comparing with the external standard method (ESM) in all the SGS samples. Our findings offer a new routine for assessing the quality of TCM.

2. Materials and Methods

2.1. Chemicals and Reagents. Analysis was applied on three different HPLC systems, including (a) Agilent 1100 series with vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1316A), and a ChemStation Workstation with VWD detector; (b) Agilent 1260 series with DAD detector and Agilent ChemStation Workstation; and (c) Waters 2695-2996 series with 2998PDA detector and empower workstation. The chromatographic separation was performed on an AmethyC18 (4.6 mm × 250 mm, 5 μm) column, Agilent C18 (4.6 mm × 250 mm, 5 μm) column, and HedaR1 (4.6 mm × 250 mm, 5 μm) column. The SPSS software (Edition 2.0) was used for conducting cluster analysis.

BP-211D electronic analytical balance (Germany Sar-torius Company) was used to weigh the drugs. Sonicator (SK6200H, Shanghai Branch guided ultrasound instrument Co., Ltd.) was used to help dissolve the sample.

2.2. Materials. The batch numbers and origins of eight qualified Chinese herbal pieces of decoction are shown in Table 1. All pieces were purchased from Anhui Concord Pharmaceutical Pieces Co., Ltd. and identified by Professor Zhihui Liu of Nanjing University of Traditional Chinese Medicine.

Fifteen batches of Sanhuang Gypsum Soup were provided by the Department of Pharmacy of Jiangsu Provincial Hospital. Their batch numbers were S1 (1707010), S2 (1704006), S3 (1712019), S4 (1711016), S5 (1704005), S6 (1703004), S7 (1711013), S8 (1711017), S9 (1705007), S10 (1704003), S11 (1704002), S12 (1704001), and S13 (1702015). Each single piece preparations and its negative preparations are made by our laboratory as per the preparation standard process.

2.3. Chemical Reagents and Standards. Mangiferin, geni-
poside, liquiritin, epiberberine, coptisine, baicalin, palma-
tine, berberine, harpagosid, wogonoside, cinnamic acid, cinnamic aldehyde, baicalein, glycyrrhizic acid, and wogonin were all supplied by Chengdu Mansi Biotechnology Co., Ltd. The purity of each ingredient was greater than 98% as determined by HPLC. Acetonitrile of HPLC grade and formic acid of analytical grade were purchased from Merck (Darmstadt, Germany) and Roe Scientific Inc. (USA).
A Milli-Q water (Millipore, Inc., USA) purification system was applied to purify water for the HPLC analysis.

2.4. Preparation of the Sample Solution. The sample solution of SGS was precisely absorbed (5 ml) and immersed in 25 ml volumetric flask with methanol. Additional methanol was added to compensate the weight loss after ultrasonic extraction for 30 min. All solutions were filtered through 0.45 μm filter membranes before being injected into the HPLC system precisely.

2.5. Reference Solution Preparation. A mixed stock solution containing reference standards was prepared by dissolving weighed samples of each compound in methanol accurately. Then, the stock solutions were diluted to establish the calibration curves based on six appropriate concentrations with the ranges of 2.80–88.70 μg·ml⁻¹ for mangiferin, 14.80–472.90 μg·ml⁻¹ for geniposide, 3.20–101.00 μg·ml⁻¹ for liquiritin, 1.40–44.30 μg·ml⁻¹ for epiberberine, 6.40–204.10 μg·ml⁻¹ for coptisine, 19.80–632.50 μg·ml⁻¹ for baicalin, 1.80–56.80 μg·ml⁻¹ for palmatine, 15.30–488.40 μg·ml⁻¹ for berberine, 2.60–82.60 μg·ml⁻¹ for harpagosid, 4.60–145.82 μg·ml⁻¹ for wogonoside, 0.30–9.51 μg·ml⁻¹ for cinnamic acid, 0.20–6.34 μg·ml⁻¹ for cinnamic aldehyde, 0.50–15.85 μg·ml⁻¹ for baicalein, 1.10–34.00 μg·ml⁻¹ for glycyrrhizic acid, and 0.30–9.51 μg·ml⁻¹ for wogonin.

2.6. Chromatographic Procedures. Analytes were separated on a reverse phase C₁₈ column (Amethyl-ODS-2 C₁₈ column, 250 mm × 4.6 mm × 5 μm).

Mobile phase consists of 0.1% phosphoric acid (A)-acetonitrile (B), gradient elution program was as follows: 0–2 min, 12% B; 2–7 min, 12%–20% B; 7–17 min, 20%–25% B; 17–25 min, 25%–32% B; 25–32 min, 32%–35% B; 32–45 min, 35%–44% B; 45–50 min, 44%–45% B; 50–55 min, 45%–50% B; 55–56 min, 50% B; 56–61 min, 12% B. Flow rate: 0.8 ml·min⁻¹; column temperature: 35°C; injection volume: 10 μl; UV detection wavelength: 250 nm. On the basis of chromatographic conditions, all the components had good resolution.

2.7. Data Analysis. The data were analyzed and evaluated by Similarity Evaluation System for chromatographic fingerprint of TCM (Version 2004 A) which was recommended by the SFDA of China for evaluating similarities of chromatographic profiles of TCM. The similarity among different chromatograms was determined by calculating the correlation coefficient or cosine value of the vectorial angle [17–19]. HCA was carried out by calculating Squared Euclidean distance to distinguish preparation of different batches using SPSS. At the same time, we used the external standard method (ESM) and QAMS to calculate the 15 active components in 13 batches of SGS, respectively, to verify the feasibility of QAMS.

3. Results and Discussion

3.1. Chromatograph Optimization. At present, there are no single liquid phase conditions that can divide 15 components of SGS with good resolution. As the ingredients of SGS are very intricate, it is critical to establish a favorable mobile phase system, gradient elution system, and detection wavelength to obtain efficient separation of the numerous target components. The suitable ingredient of the HPLC method was investigated by checking peak resolution and the peak purity of SGS. In this case, some different mobile phases were tested which were acetonitrile-water, methanol-water, methanol-water containing phosphoric acid or formic acid at different concentrations, acetonitrile-water with acetic acid, formic acid, and phosphoric acid at different concentrations. Experimental results show that acetonitrile-water containing 0.2% phosphoric acid system produced...
Figure 1: Continued.
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Figure 1: (a) Mixed standard solution, (b) negative sample without *coptidis* rhizoma, (c) negative sample without *scrophulariae* radix, (d) negative sample without *anemarrhenae* rhizome, (e) negative sample without *gardeniae* fructus, (f) negative sample without *coptidis* rhizoma and *phellodendri* chinensis cortex, (g) negative sample without *glycyrrhizae* radix et rhizoma preparata cum melle, (h) negative sample without *cinnamomi* cortex, (i) negative sample without *phellodendri chinensis* cortex, (j) negative sample without *scutellaria* radix, and (k) SGS sample. 1, mangiferin; 2, geniposide; 3, liquiritin; 4, epiberberine; 5, coptisine; 6, baicalin; 7, palmatine; 8, berberine; 9, harpagosid; 10, wogonoside; 10, cinnamic acid; 11, cinnamic aldehyde; 12, baicalein; 13, glycyrrhizic acid; and 14, wogonin.

Table 2: Standard curves of fifteen kinds of reference components.

| Compounds       | Regression equations | Linear ranges (μg·mL⁻¹) | R²   |
|-----------------|----------------------|--------------------------|------|
| Mangiferin      | y = 29.897x + 30.359 | 2.80–88.70               | 0.9998|
| Geniposide      | y = 11.306x – 40.123  | 14.80–472.90             | 0.9977|
| Liquiritin      | y = 6.9865x – 5.2633  | 3.20–101.00              | 1.0000|
| Epiberberine    | y = 23.477x – 12.998  | 1.40–44.30               | 0.9988|
| Coptisine       | y = 11.677x – 31.945  | 6.40–204.10              | 0.9988|
| Baicalin        | y = 17.312x – 189.17  | 19.80–632.50             | 0.9999|
| Palmatine       | y = 39.570x – 21.105  | 1.80–56.80               | 0.9999|
| Berberine       | y = 38.357x + 108.6   | 15.30–488.40             | 0.9999|
| Harpagosid      | y = 9.5715x + 8.4144  | 2.60–82.60               | 0.9998|
| Wogonoside      | y = 16.462x + 11.105  | 4.60–145.82              | 0.9999|
| Cinnamic acid   | y = 32.7x + 0.4639    | 0.30–9.51                | 0.9998|
| Cinnamic aldehyde| y = 15.523x – 0.1567 | 0.20–6.34                | 0.9998|
| Baicalein       | y = 25.996x + 3.789   | 0.50–15.85               | 0.9998|
| Glycyrrhizic acid| y = 7.5138x + 1.333  | 1.10–34.00               | 0.9999|
| Wogonin         | y = 21.652x + 0.8509  | 0.30–9.51                | 1.0000|

Table 3: The results of recovery of fifteen components in samples (n = 6).

| Compound       | Original (mg) | Added amount (mg) | Detected amount (mg) | Recovery (%) | RSD (%) |
|----------------|---------------|-------------------|----------------------|--------------|---------|
| Mangiferin     | 0.0241        | 0.0241            | 0.0478               | 93.96        | 1.18    |
| Geniposide     | 0.1036        | 0.1036            | 0.2032               | 99.58        | 2.60    |
| Liquiritin     | 0.0198        | 0.0198            | 0.0380               | 96.11        | 3.42    |
| Epiberberine   | 0.0073        | 0.0073            | 0.0142               | 103.46       | 3.61    |
| Coptisine      | 0.0223        | 0.0223            | 0.0438               | 110.19       | 3.16    |
| Baicalin       | 0.1226        | 0.1226            | 0.2139               | 81.82        | 2.61    |
| Palmatine      | 0.0104        | 0.0104            | 0.0200               | 97.21        | 2.28    |
| Berberine      | 0.0748        | 0.0748            | 0.1539               | 101.87       | 5.31    |
| Harpagosid     | 0.0212        | 0.0212            | 0.0433               | 100.01       | 3.56    |
| Wogonoside     | 0.0307        | 0.0307            | 0.0620               | 99.81        | 3.33    |
| Cinnamic acid  | 0.0023        | 0.0023            | 0.0048               | 111.45       | 2.38    |
| Cinnamic aldehyde | 0.0023    | 0.0023            | 0.0040               | 70.08        | 5.00    |
| Baicalein      | 0.0048        | 0.0048            | 0.0095               | 79.84        | 3.73    |
| Glycyrrhizic acid | 0.0079    | 0.0079            | 0.0155               | 94.08        | 2.52    |
| Wogonin        | 0.0028        | 0.0028            | 0.0056               | 98.34        | 4.19    |
Table 4: Similarities of 13 batches SGS.

|    | S1   | S2   | S3   | S4   | S5   | S6   | S7   | S8   | S9   | S10  | S11  | S12  | S13  | R    |
|----|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| S1 | 1    | 0.984| 0.984| 0.982| 0.989| 0.981| 0.991| 0.99  | 0.978| 0.984| 0.982| 0.987| 0.978| 0.994|
| S2 | 0.984| 1    | 0.975| 0.978| 0.985| 0.986| 0.985| 0.995 | 0.997 | 0.987 | 0.979 | 0.973 | 0.994 | 0.994|
| S3 | 0.984| 0.975| 1    | 0.991| 0.987| 0.978| 0.974| 0.974 | 0.964 | 0.983 | 0.995 | 0.995 | 0.959 | 0.991|
| S4 | 0.982| 0.978| 0.991| 1    | 0.991| 0.99  | 0.98  | 0.975 | 0.972 | 0.99  | 0.987 | 0.988 | 0.96  | 0.992|
| S5 | 0.989| 0.985| 0.987| 0.991| 1    | 0.985| 0.992| 0.989 | 0.98  | 0.985 | 0.988 | 0.988 | 0.975 | 0.996|
| S6 | 0.981| 0.986| 0.978| 0.99  | 0.985| 1    | 0.983| 0.982 | 0.985 | 0.993 | 0.972 | 0.972 | 0.974 | 0.992|
| S7 | 0.991| 0.985| 0.974| 0.98  | 0.992| 0.983| 1    | 0.992 | 0.983 | 0.98  | 0.975 | 0.979 | 0.983 | 0.992|
| S8 | 0.99 | 0.995 | 0.974| 0.975 | 0.989 | 0.982| 0.992| 1    | 0.994 | 0.982 | 0.977 | 0.975 | 0.993 | 0.994|
| S9 | 0.978| 0.997 | 0.964| 0.972 | 0.98  | 0.985| 0.983| 0.994 | 1    | 0.985 | 0.969 | 0.963 | 0.994 | 0.989|
| S10 | 0.984| 0.987 | 0.983| 0.99  | 0.985| 0.993| 0.98  | 0.982 | 0.985 | 1    | 0.977 | 0.977 | 0.973 | 0.993|
| S11 | 0.982| 0.979 | 0.995| 0.987 | 0.988 | 0.972 | 0.975 | 0.977 | 0.969 | 0.977 | 1    | 0.994 | 0.966 | 0.99|
| S12 | 0.987| 0.973 | 0.995| 0.988 | 0.988 | 0.972 | 0.975 | 0.975 | 0.963 | 0.977 | 0.994 | 1    | 0.962 | 0.99|
| S13 | 0.978| 0.994 | 0.959| 0.96  | 0.975 | 0.974 | 0.983 | 0.993 | 0.994 | 0.973 | 0.966 | 0.962 | 1    | 0.985|
| R  | 0.994| 0.994 | 0.991| 0.992 | 0.996 | 0.992 | 0.992 | 0.994 | 0.989 | 0.993 | 0.99  | 0.99  | 0.985 | 1    |
sharp and symmetrical chromatographic peak shapes, good separation, and prevented the peak tailing. Chromatogram with the maximum number of peaks also relies on best conditions for preparation of sample solution. On the basis of the investigation of different solvent and ultrasonic time, it can be concluded that samples are dissolved in methanol and ultrasound 30 minutes; we can get better resolution and reproducibility of fingerprint chromatograms under the conditions of Section 2.6. Under the above chromatographic conditions, all the components were well separated (Figure 1).

3.2. Method Validation

3.2.1. Linearity. A mixed solution containing all the reference substances were prepared and diluted in series with methanol to obtain six different concentrations. The different concentration of the mixed solution was used for constructing the reference curve. As shown in Table 2, good calibration curves of 15 compounds were obtained, and high correlation coefficient values ($R^2 > 0.999$) were shown with good linearity at a wide range relatively. In response to sample concentration, the peak area of the analyte is determined by least squares linear regression to obtain a linear equation.

3.2.2. Precision, Stability, Repeatability, and Recovery. The same mixed standard solution of 10 μl was injected for six consecutive times under chromatographic conditions, and their RSDs were calculated. The RSD of mangiferin, geniposide, liquiritin, epiberberine, coptisine, baicalin,
Table 5: RCFs of berberine to mangiferin, geniposide, liquiritin, epiberberine, coptisine, baicalin, palmatine, harpagosid, wogonoside, cinnamic acid, cinnamic aldehyde, baicalein, glycyrrhizic acid, and wogonin on different instruments and different columns.

| Instrument Column | Mangiferin | Geniposide | Liquiritin | Epiberberine | Coptisine | Baicalin | Palmatine | Harpagosid | Wogonoside | Cinnamic acid | Cinnamic aldehyde | Baicalein | Glycyrrhizic acid | Wogonin |
|------------------|------------|------------|------------|--------------|-----------|---------|----------|-----------|-----------|--------------|-----------------|-----------|----------------|---------|
| Waters2695-2998  | Amethy     | 0.7632     | 0.3112     | 0.1888       | 0.6033    | 0.3144  | 0.4421   | 1.0451    | 0.2561    | 0.4321       | 0.8611          | 0.4043    | 0.6811         | 0.1993  |
| Hedra            | 0.7794     | 0.3008     | 0.1791     | 0.6215       | 0.3211    | 0.4351  | 1.0733   | 0.2671    | 0.4411    | 0.8835       | 0.4156          | 0.6632    | 0.1911         | 0.5783  |
| Agilent          | 0.7794     | 0.2948     | 0.1821     | 0.6121       | 0.3044    | 0.4513  | 1.0316   | 0.2495    | 0.4292    | 0.8525       | 0.4047          | 0.6777    | 0.1959         | 0.5645  |
| Amethy           | 0.7553     | 0.3138     | 0.1813     | 0.5988       | 0.3198    | 0.4579  | 1.0651   | 0.2355    | 0.4351    | 0.8421       | 0.4255          | 0.6843    | 0.2021         | 0.5547  |
| Hedra            | 0.7421     | 0.3097     | 0.1923     | 0.5899       | 0.3176    | 0.4621  | 1.0688   | 0.2466    | 0.4284    | 0.8311       | 0.4322          | 0.6731    | 0.2124         | 0.5832  |
| Agilent          | 0.7342     | 0.3201     | 0.1799     | 0.6031       | 0.3021    | 0.4633  | 1.0803   | 0.2611    | 0.4511    | 0.8941       | 0.4167          | 0.6864    | 0.2145         | 0.5401  |
| Mean             | 0.7589     | 0.3084     | 0.1839     | 0.6048       | 0.3132    | 0.4520  | 1.0607   | 0.2527    | 0.4362    | 0.8607       | 0.4165          | 0.6776    | 0.2025         | 0.5623  |
| RSD (%)          | 2.48       | 2.97       | 2.91       | 1.80         | 2.58      | 2.52    | 1.75     | 4.45      | 1.98      | 2.81         | 2.67            | 1.26      | 4.56           | 2.90    |

Table 6: Retention time (min) of berberine to mangiferin, geniposide, liquiritin, epiberberine, coptisine, baicalin, palmatine, harpagosid, wogonoside, cinnamic acid, cinnamic aldehyde, baicalein, glycyrrhizic acid, and wogonin on different instruments and different columns.

| Instrument Column | Mangiferin | Geniposide | Liquiritin | Epiberberine | Coptisine | Baicalin | Palmatine | Harpagosid | Wogonoside | Cinnamic acid | Cinnamic aldehyde | Baicalein | Glycyrrhizic acid | Wogonin |
|------------------|------------|------------|------------|--------------|-----------|---------|----------|-----------|-----------|--------------|-----------------|-----------|----------------|---------|
| Waters2695-2998  | Amethy     | 0.38       | 0.40       | 0.58         | 0.80      | 0.83    | 0.92     | 0.97      | 1.04      | 1.14         | 1.17            | 1.34      | 1.41           | 1.60    |
| Hedra            | 0.41       | 0.43       | 0.62       | 0.82         | 0.84    | 0.96    | 0.97     | 1.06      | 1.11      | 1.18         | 1.28            | 1.54      | 1.59           | 2.02    |
| Agilent          | 0.36       | 0.39       | 0.57       | 0.75         | 0.81    | 0.90    | 0.95     | 1.02      | 1.13      | 1.16         | 1.30            | 1.48      | 1.60           | 1.91    |
| Amethy           | 0.37       | 0.41       | 0.58       | 0.77         | 0.83    | 0.92    | 0.96     | 1.05      | 1.12      | 1.16         | 1.33            | 1.40      | 1.58           | 1.79    |
| Hedra            | 0.40       | 0.42       | 0.60       | 0.82         | 0.85    | 0.94    | 0.99     | 1.06      | 1.16      | 1.19         | 1.36            | 1.43      | 1.62           | 1.83    |
| Agilent          | 0.40       | 0.42       | 0.60       | 0.82         | 0.85    | 0.94    | 0.99     | 1.06      | 1.16      | 1.19         | 1.36            | 1.43      | 1.62           | 1.83    |
| Mean             | 0.39       | 0.41       | 0.59       | 0.79         | 0.83    | 0.93    | 0.97     | 1.05      | 1.13      | 1.17         | 1.32            | 1.44      | 1.60           | 1.86    |
| RSD (%)          | 5.00       | 3.03       | 3.38       | 4.03         | 2.76    | 2.66    | 1.90     | 1.85      | 2.32      | 1.77         | 2.95            | 4.33      | 1.95           | 5.00    |
Palmitate, berberine, harpagosid, wogonoside, cinnamic acid, cinnamic aldehyde, baicalein, glycyrrhizic acid, and wogonin was 1.94%, 0.72%, 0.88%, 0.54%, 0.62%, 0.97%, 0.93%, 0.98%, 1.33%, 1.40%, 0.98%, 1.33%, 0.67%, 1.40%, 1.08%, 0.96%, and 1.49% which indicated that the developed method had a good precision.

The stability of the sample solutions was analyzed at 0, 2, 4, 8, 12, and 24 h at room temperature. It was found that the sample solutions were stable within 24 h (RSD ≤ 5.0%). To confirm the repeatability of the method, six independently prepared solutions from the same batch were analyzed. The RSD values of the peak are 0.37%, 0.31%, 1.52%, 0.72%, 1.00%, 0.20%, 0.71%, 0.18%, 0.88%, 0.50%, 3.02%, 3.65%, 2.30%, 1.68%, and 3.23%, respectively. The results indicated the method is reproducible.

The recovery was performed by adding a known amount of individual standards into a certain amount of the SGS sample. The mixture was extracted and analyzed by using the method mentioned above. The average recoveries of 6 samples are shown in Table 3. The results show that the method is accurate. The recoveries of the 15 compounds which are shown in Table 3 ranged from 70.08% to 111.45% with RSDs ≤ 5.0%.

### 3.3. HPLC Fingerprint and Similarity Analysis

Thirteen batches of samples were prepared according to Section 2.5, and 10 μL was injected into the HPLC system according to the chromatographic conditions given under Section 2.6, and then the chromatograms were recorded and entered into the similarity analysis software. We selected S (1) as the reference chromatogram, the utilization of the average correlation coefficient method of 13 batches of samples for multipoint correction, time window width is set to 0.5, while the establishment of a common model is to generate a control fingerprinting SGS, the antithesis fingerprint chromatogram was shown in Figure 2. Fingerprint chromatograms of 13 batches of SGS can be seen in Figure 3. As compared with the reference fingerprint chromatograms, the similarities of 13 batches of samples shown in Table 4, and the results are all above 0.95. On the basis of these results, we concluded that SGS between different batches are of good consistency and in line with the relevant requirements of the fingerprints. Palmitate is the main active ingredient of coptidis rhizoma; the corresponding peaks have favorable resolution, and the retention time is stable and moderate. Therefore, we selected palmitate (no. 11 peak) as the reference peak and calculated the relative retention time of the other common peaks. We can see that the retention time of the common peak is stable. According to the retention time of each fingerprint, a total of 20 common peaks were identified while 14 of them were determined. However, it should be pointed out that the chemical property of cinnamic aldehyde is very unstable due to its alkene structure of the molecule which has poor stability when exposed to light and oxygen, so it is not within the category of the common peaks [20]. This can be in accordance with S11 without the peak of cinnamic aldehyde.

To gain better understanding of ascription of common peaks, reference standards and single TCM pieces were used. The peaks 2, 3, 6, 8, 9, 10, 11, 12, 13, 16, 17, 18, 19, and 20 were identified as mangiferin, geniposide, liquiritin, epiberberine, coptisine, baicalin, palmatine, berberine, harpagosid, wogonoside, cinnamic acid, cinnamic aldehyde, baicalein, glycyrrhizic acid, and wogonin, respectively (Figure 4). The peak 1 belongs to phellodendri chinensis cortex. The peak 4 belongs to both phellodendri chinensis cortex and coptidis rhizoma. The peaks 5, 14, and 15 belong to scutellariae radix. The peak 7 belongs to scrophulariae radix.

### Figure 6: The contents of 15 active components in 13 batches of SGS.

3.4. Hierarchical Cluster Analysis. The 13 * 20 matrices were obtained from 20 common peak areas of fingerprints of 13 batches of SGS. The cluster analysis was performed by using ssps 2.0 software. The Euclidean distance was chosen as the measure of the distance between groups. The results are shown in Figure 5. S3, S4, S5, and S12 batches of samples are divided into a category; the remaining batches are divided into another classification, which indicates that there are differences in the content of the components in the samples prepared from different raw material TCM pieces. And it suggested that HCA was a valid method for the identification of the source of TCM pieces.
| Batch number | Berberine | Mangiferin | Geniposide | Liquiritin | Epiberberine | Coptisine | Baicalin | Palmatine | Harpagoside | Wogonoside | Cinnamic acid | Cinnamic aldehyde | Baicalein | Glycyrrhizic acid | Wogonin | SMD |
|--------------|-----------|------------|------------|------------|--------------|-----------|---------|-----------|------------|------------|----------------|-----------------|------------|-----------------|-----------|-----|
| 1            | 109.5341  | 40.2024    | 40.2261    | 181.4930   | 189.7332     | 23.0219   | 24.3703 | 6.3113    | 7.0281     | 46.8326    | 50.7789        | 134.5759        | 148.9816   | 11.4799         | 12.3100   | 4.84%|
| 2            | 89.2249   | 37.5741    | 37.7510    | 179.5150   | 188.7602     | 15.1217   | 16.3549 | 5.8212    | 6.5595     | 40.4232    | 44.4416        | 140.3703        | 150.3753   | 10.8018         | 11.7349   | 5.00%|
| 3            | 129.1133  | 35.8398    | 35.6103    | 157.0895   | 164.0831     | 15.6869   | 16.7843 | 7.5026    | 8.2207     | 54.0515    | 57.9725        | 163.0149        | 177.5168   | 13.4391         | 14.1080   | 5.00%|
| 4            | 124.5509  | 39.7362    | 39.6241    | 177.6354   | 185.2223     | 16.2344   | 17.3568 | 8.0797    | 8.8171     | 57.6934    | 61.7406        | 178.8141        | 193.4197   | 13.8791         | 14.7108   | 5.00%|
| 5            | 131.7465  | 42.8953    | 42.8017    | 215.0841   | 223.2552     | 13.4517   | 14.4941 | 8.1313    | 8.8596     | 58.7696    | 62.7683        | 177.6743        | 192.4197   | 13.8791         | 14.7108   | 5.00%|
| 6            | 91.8581   | 33.9407    | 33.9713    | 167.7467   | 176.4659     | 13.4688   | 14.5137 | 8.0163    | 8.8171     | 41.8712    | 45.8975        | 161.3843        | 177.2857   | 13.0916         | 14.0284   | 5.00%|
| 7            | 93.6830   | 33.7007    | 33.7037    | 184.5859   | 193.7133     | 12.6430   | 13.7785 | 5.6230    | 6.3466     | 40.0688    | 44.0134        | 121.2770        | 135.8693   | 10.4254         | 11.2738   | 5.00%|
| 8            | 83.4633   | 28.7922    | 28.7534    | 161.0843   | 170.0976     | 11.7671   | 12.9197 | 4.9025    | 5.6224     | 37.9355    | 41.9581        | 118.3847        | 133.3277   | 9.5326          | 10.3893   | 4.84%|
| 9            | 87.8692   | 35.7417    | 35.8779    | 191.4261   | 201.1430     | 15.2532   | 16.4990 | 5.7200    | 6.5601     | 44.2423    | 48.6466        | 171.3295        | 187.5168   | 11.7349         | 12.7388   | 5.00%|
| 10           | 96.2826   | 34.0947    | 34.0971    | 161.2528   | 171.6896     | 10.7070   | 11.9177 | 4.4526    | 5.1965     | 32.0308    | 36.1347        | 95.5600         | 110.5690   | 7.9011          | 8.7719    | 4.84%|
| 11           | 127.4969  | 35.4701    | 35.2424    | 160.0752   | 167.1788     | 18.2031   | 19.3607 | 6.7088    | 7.4114     | 56.9690    | 60.9699        | 139.2935        | 153.3139   | 11.8422         | 12.6385   | 5.00%|
| 12           | 66.2826   | 26.8813    | 27.0141    | 161.2528   | 171.6896     | 10.7070   | 11.9177 | 4.4526    | 5.1965     | 32.0308    | 36.1347        | 95.5600         | 110.5690   | 7.9011          | 8.7719    | 4.84%|

| p-value      | 1.0000    | 0.9990    | 1.0000     | 1.0000     | 1.0000      | 1.0000     | 1.0000  | 1.0000    | 0.9990     | 1.0000     | 1.0000         | 1.0000         | 1.0000    | 1.0000          | 1.0000    |

| SMD (%)      | 0.14%     | 4.84%     | 5.00%      | 4.40%      | 3.88%       | 4.64%      | 5.00%  | 4.00%     | 4.84%      | 4.40%      | 3.88%          | 4.64%          | 5.00%     | 4.00%           | 4.84%     |

Table 7: Comparison of the results from the ESM and QAMS (μg·ml⁻¹).
3.5. Quantitative Analysis of Multicomponents by a Single Marker (QAMS). It is well established that many variations of experimental conditions, such as concentrations of standard, detector, and peak measurement parameters, would extremely influence the RCFs. Accordingly, the accuracy of RCFs may affect the final analysis results. Nevertheless, RCFs, which was calculated by linear-regression equation in the experiment, was considered to be accurate and stable [21, 22]. The RCFs were calculated using the calibration curves as follows:

\[ F_{K,S} = \frac{a_k}{a_s} \]  

(1)

The content of the measured component was calculated as follows:

\[ C_k = \frac{A_k}{(A_s \times F_{K,S})} \]  

(2)

\( a_s \) is the ratio of the slope of internal standard reference calibration equations; \( a_k \) is the ratio of the slope of measured component calibration equations; \( A_k \) is the peak area of the measured component; \( A_s \) is the peak area of the internal standard reference.

We investigated the influence of different instruments and different columns on the RCF values, and results are shown in Table 5 which illustrated RCF values had good repeatability on different chromatographic systems and different columns.

In this paper, we selected cheap, readily available, and chemically stable berberine as an internal reference standard for the quantitative determination of other active components. In addition to that, berberine is the main active ingredient of phellodendri chinensis cortex and coptidis rhizoma, so this study eventually takes it as main active ingredient of phellodendri chinensis cortex active components. In addition to that, berberine is the standard for the quantitative determination of other chemically stable berberine as an internal reference standard.

Different columns.

We measured the multicomponent content of 13 batches SGS (Figure 6); the results showed that there were significant differences in some contents of 15 ingredients, such as cinnamic aldehyde and baicalein, which indicated that only a few ingredients of the standard determination of content could not control the quality of SGS effectively. It is necessary to use multiple active ingredients as index components to control the quality of TCM preparations more comprehensively.

To validate the difference between ESM and QAMS method using RCFs, 13 SGS samples were analyzed for their active ingredients. The calculated results are shown in Table 7. Standard method difference (SMD) is calculated according to the following equation:

\[ \text{SMD} = \left( \frac{C_{ES} - C_{QAMS}}{C_{ES}} \right) \times 100\% \]  

(4)

where \( C_{ES} \) and \( C_{QAMS} \) represent the concentrations of an analyte assayed by the external standard method and QAMS method, respectively [23]. All the values of standard deviation (SMD < 0.05) revealed that there were no significant differences between ESM and QAMS methods of all SGS samples.

4. Conclusion

On the basis of these results, we concluded that HPLC fingerprint method based on chemical constituents profiling was an effective and stable tool, and QAMS method was feasible to quantify the active compounds by RCFs for evaluating the quality of SGS. Along with similarity analysis and HCA of synthesis, the quality of SGS would be evaluated and better identified comprehensively. This method could potentially be applied in the quality control of TCM.

Data Availability

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request. All data generated or analyzed during this study are included in this submitted manuscript.

Disclosure

Minghui Dong is the co-first author.

Conflicts of Interest

The authors declare no conflicts of interest.

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