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# A Comprehensive Strategy to Quantify the Complex System by Ultraviolet and Infrared Spectra Analyses Coupled with Combustion Heat for Recognizing the Quality Consistency of San-Huang Tablets

**Yan Wang[1,2], Guoxiang Sun[1*], Yang Jin[3], Jing Zhang[1], Jiayue Gao[1], Eboka Majolene Benedicta Sabi-Mouka[4]**

[1]College of Pharmacy, Shenyang Pharmaceutical University, Shenyang, China
[2]College of Pharmacy and Chemistry, Dali University, Dali, China
[3]Department of Pharmacy, Traditional Chinese Medicine Hospital of Dali, Dali, China
[4]Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing, China
Email: *gxswmwys@163.com

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## Abstract

Spectral quantitative fingerprinting including ultraviolet (UV) and Fourier transform infrared (FT-IR) coupled with combustion heat (CH) analytical techniques was employed and compared for rapid screening quality grade and discriminating San-Huang Tablets (SHT) of different commercial brands. The systematic quantified fingerprint method (SQFM) was applied to evaluate, qualitatively and quantitatively, the quality consistency of the herbal preparation. It was possible to deduce that the quantitative similarity analysis by SQFM was enabled to make a good discrimination of the tested samples. It was a particularly useful method for the overall quality evaluation of herbal medicine and their preparations.

## Keywords

Combustion Heat, San-Huang Tablets, Spectral Fingerprint, Systematic Quantified Fingerprint Method

## 1. Introduction

Traditional Chinese medicine (TCM) and herbal drugs (HD) are very popular in different systems of medicines such as Chinese medicine, naturopathy and homeopathy. They play increasingly important roles in healthcare of the majority

of the population worldwide [1]. Traditionally, one or two markers' determination is performed on TCM identification [2], and such approach might be unreliable and far from satisfactory. As the therapeutic effects of TCM and HD are based on the complex interaction of numerous ingredients, they are different from those of synthetic drugs [3].

Up to now, chemical fingerprint techniques have already been internationally acknowledged and employed by existing regulations and guidelines such as the World Health Organization, the American Food and Drug Administration and The Chinese State Food and Drug Administration. Meanwhile, multiple kinds of techniques such as high performance liquid chromatography in combination with different detectors, gas chromatography, nuclear magnetic resonance [4] [5] [6] have been successfully applied to establish fingerprints for quality control of TCM and HD. Though the reported results evidenced the feasibility of these methodologies, it should be pointed out that there are also some disadvantages with respect to them. For instance, source identification requires complicated peak matching techniques, which lead to more challenges in data processing and fingerprint comparison. Meanwhile, other rests of the proposed strategies need expensive equipment and laborious sample preparation prior to analysis. Therefore, spectroscopy techniques such as ultraviolet (UV), infrared (IR), near infrared (NIR) [7] [8] [9] [10] with the characteristics of fast analysis, non-destructiveness, low equipment cost have become powerful analytical tools.

In addition, most published reports evaluated the similarity between sample fingerprint (SFP) and reference fingerprint (RFP) only qualitatively [11] [12], but quantitative fingerprint evaluation can provide a more comprehensive view of the fingerprints not only based on the similarity of peak distribution, but also the quantitative content of the peaks.

The Chinese Pharmacopoeia lists the monograph of San-Huang Tablets (SHT), and there are also some literature reports regarding the qualitative and quantitative study of SHT [13] [14], but studies with respect to the fast quality analysis can rarely be found. Therefore, in this experiment, SHT, the most popular herbal formulation for the treatment of gingival bleeding, intense heat in the body, reddish urine and constipation in clinical practice [15], was taken as an example to propose a comprehensive strategy for its quality consistency monitoring. The aim of the study was to establish visible spectral quantitative fingerprints combined UV with IR. Moreover, the combustion heat (CH) frequently used in physical chemistry was employed for the first time to reflect the whole chemical information of SHT. Finally, the integrated assessment method involving UV and IR quantitative fingerprints coupled with CH based on systematic quantified fingerprint method (SQFM) was applied and analyzed the quality consistency of samples from batch to batch and manufactory to manufactory.

## 2. Materials and Methods

### 2.1. Chemicals and Materials

A total of thirty batches of SHT samples (labeled S1-S30) from nineteen manu-

facturers were listed in Table 1. Methanol (purchased from Yuwang Industry Limited Company, Shandong, China) was HPLC grade. The other reagents were all analytical grade.

## 2.2. Sample Preparation

Ten tablets of SHT were accurately weighed to get the average weight for each one. A quantity equivalent to two tablets in powdered states was weighed and extracted with 20 mL methanol in an ultrasonic water bath for 20 min. The

Table 1. The source of 30 San-Huang Tablets samples in this study.

| | Source | |
|---|---|---|
| Sample no. | Batch no. | Manufacturer |
| S1 | D95015 | Baishang Tangwei Pharmaceutical Co., Ltd. |
| S2 | D95019 | Baishang Tangwei Pharmaceutical Co., Ltd. |
| S3 | 110301 | Anhui Renhe Pharmaceutical Co., Ltd. |
| S4 | 120506 | Guangxi Banyutianlong Pharmaceutical Co., Ltd. |
| S5 | 111195 | Handan Moluodan Pharmaceutical Co., Ltd. |
| S6 | D15045 | Hebei Shijitangwei Pharmaceutical Co., Ltd. |
| S7 | 11104 | Henan Fusen Pharmaceutical Co., Ltd. |
| S8 | 120401 | Henan Huaiqing Pharmaceutical Co., Ltd. |
| S9 | 20120401 | Henan Kangqi Pharmaceutical Co., Ltd. |
| S10 | 20120301 | Henan Jishi Pharmaceutical Co., Ltd. |
| S11 | KL100939 | Jindu Nianantang Pharmaceutical factory |
| S12 | 110303 | Luoyan Junsan Pharmaceutical Co., Ltd. |
| S13 | 120101 | Luoyan Junsan Pharmaceutical Co., Ltd |
| S14 | 120501 | Luoyan Junsan Pharmaceutical Co., Ltd. |
| S15 | 120401 | Luoyan Junsan Pharmaceutical Co., Ltd. |
| S16 | 110601 | Guangxi Banyutianlong Pharmaceutical Co., Ltd. |
| S17 | 110901 | Shandong Jianming Pharmaceutical Co., Ltd. |
| S18 | 20111105 | Sanxi Hetai Pharmaceutical Co., Ltd. |
| S19 | 110304 | Sanxi Hetai Pharmaceutical Co., Ltd. |
| S20 | 20120404 | Sanxi Hetai Pharmaceutical Co., Ltd. |
| S21 | 120502 | Shanxi Lijun Pharmaceutical Co., Ltd. |
| S22 | 100805 | Xiangfan Longzhong Pharmaceutical Co., Ltd. |
| S23 | 131201 | Guangxi Banyutianlong Pharmaceutical Co., Ltd. |
| S24 | 20120401 | Xinxiang Zuojinming Pharmaceutical Co., Ltd. |
| S25 | 110321 | Yabao Pharmaceutical Co., Ltd. |
| S26 | 111122 | Yabao Pharmaceutical Co., Ltd. |
| S27 | 120212 | Yabao Pharmaceutical Co., Ltd. |
| S28 | 20100902 | Zhengzhou Yumi Pharmaceutical Co., Ltd. |
| S29 | 20120502 | Xinxiang Zuojinming Pharmaceutical Co., Ltd. |
| S30 | 110801 | Hubei Wudang Pharmaceutical Co., Ltd. |

extracted solution was filtered and then diluted to 25 mL in a volumetric flask with methanol. The solution was centrifuged at 3500 rpm for 10 min, and the supernatant obtained was filtered through a 0.45 μm Millipore filter for UV spectroscopic analysis.

1 mg of the powdered and dried SHT was weighed, then 100 mg of KBr powder was added. After thorough mixing with an agate mortar, they were pressed into a KBr crystal tablet for IR spectroscopic analysis.

## 2.3. Analysis Conditions

Flow injection analysis (FIA) was employed, and the unseparated chromatograms of UV spectra were recorded on an Agilent 1100 HPLC series (Hewlett Packard, CA), coupled with an Agilent polytetrafluoroethylene tube (6500 mm × 0.12 mm) and a DAD detector (190 - 400 nm, acquired at 1 nm intervals). The column temperature was set at 30.00 (±0.15)˚C. The mobile phase was 100% (v/v) methanol at a flow rate of 0.8 mL/min with an injection volume of 0.2 μL. FIA method (specified in Figure 1), which used a packless column instead of the reversed phase column and detected the UV signal by DAD.

The Fourier transformed infrared (FT-IR) scans were performed on a Bruker IFS-55 type Fourier infrared spectral instrument equipped with a deuterated triglycine sulfate (DTGS) detector (BRUGG GROUP). Spectral scans were recorded between 4000 and 400 $cm^{-1}$ at 8 $cm^{-1}$ resolution. The spectra were converted to ASCII format and transferred to an Excel file for statistical analysis.

1 g of the dried SHT was weighed and performed on a ZDHW6000 computer automatic calorimeter (Hebi ying hua instruments limited company, China) for CH analysis.

## 2.4. Data Analysis

All original data acquired were processed by a ChemStation workstation (Agilent technology Inc.). Similarity analysis of UV and IR fingerprints based on

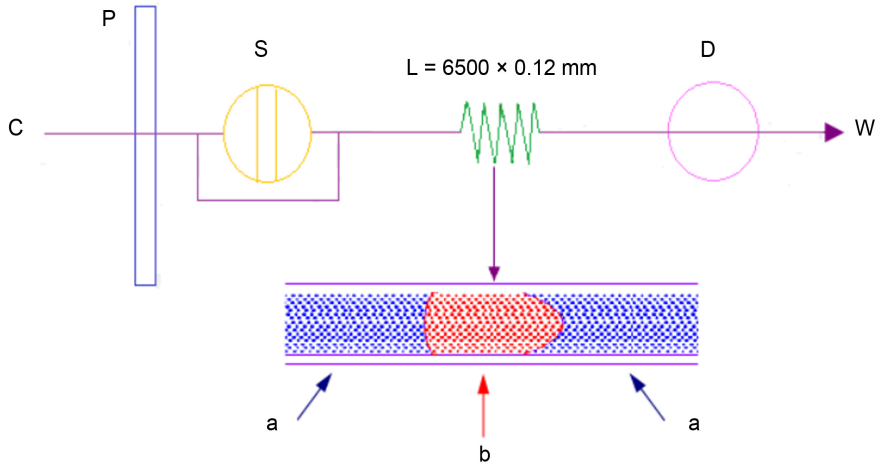


Figure 1. Diagram of FIA method. C: carrier; P: pump; S: sample injector; L: hollow pipe; D: diode array detector; W: disposal bottle; a: carrier solution; b: sample solution.

SQFM was performed on software 3.0 of Digitized Evaluation System for Super-Information Characteristics of UV Fingerprints (software Certificate No: 0462756, China) and software 4.0 of Digitized Evaluation System for Super-Information Characteristics of IR Fingerprints (software Certificate No: 0474079, China), respectively.

## 2.5. Theory

The qualitative similarity $S$, describes in Equation (1) can monitor the number of chemical fingerprint and the distribution proportion between SFP and RFP. The $S$ ranges between 0 and 1, with 0 meaning no similarity between the fingerprints and 1 identical fingerprints. The quantitative similarity $P$ describes in Equation (2) can monitor the overall content of chemical fingerprints of the system. Relative deviation $\alpha$ (in Equation (3) defines as the relative deviation of leveling coefficient is proposed to discover the similarity between SFP and RFP. From Equation (1) to Equation (3), $n$ represents the fingerprint peak number, $x_i$ and $y_i$ is the peak area of the $i$th common constituent existing in sample fingerprint vector $\boldsymbol{x}=(x_1,x_2,\cdots,x_n)$ and reference fingerprint vector $\boldsymbol{y}=(y_1,y_2,\cdots,y_n)$, respectively. SQFM, is a method combining $S$ with $P$ and $\alpha$ to simultaneously determine or identify the quality level of TCM, and the quality is divided into 8 grades in terms of SQFM criterion (summarized in Table 2).

$$S=\frac{1}{2}\left(S_{\mathrm{F}}+S_{\mathrm{F}}'\right)=\frac{1}{2}\left(\frac{\sum_{i=1}^{n}x_iy_i}{\sqrt{\sum_{i=1}^{n}x_i^2}\sqrt{\sum_{i=1}^{n}y_i^2}}+\frac{\sum_{i=1}^{n}\frac{x_i}{y_i}}{\sqrt{n\sum_{i=1}^{n}\left(\frac{x_i}{y_i}\right)^2}}\right) \tag{1}$$

$$P=\frac{1}{2}(C+P)=\frac{1}{2}\left(\frac{\sum_{i=1}^{n}x_iy_i}{\sum_{i=1}^{n}y_i^2}+\frac{\sum_{i=1}^{n}x_i}{\sum_{i=1}^{n}y_i}S_{\mathrm{F}}\right)\times 100\% \tag{2}$$

$$\alpha=\left|1-\frac{\gamma_x}{\gamma_y}\right|=\left|1-\frac{P}{C}\right| \tag{3}$$

Table 2. The quality grade identified by SQFM.

| Para. | I | II | III | IV | V | VI | VII | VIII |
|---|---|---|---|---|---|---|---|---|
| $S\geq$ | 0.95 - 1.00 | 0.90 - 0.95 | 0.85 - 0.90 | 0.80 - 0.85 | 0.70 - 0.80 | 0.60 - 0.70 | 0.50 - 0.60 | <0.5 |
| $P$/% | 95 - 105 | 90 - 110 | 80 - 120 | 75 - 125 | 70 - 130 | 60 - 140 | 50 - 150 | 0-∞ |
| $\alpha<$ | 0.05 | 0.10 | 0.15 | 0.20 | 0.30 | 0.40 | 0.50 | >0.50 |
| Quality | best | better | good | fine | moderate | common | defective | inferior |

## 3. Results and Discussions

### 3.1. UV Fingerprint

#### 3.1.1. Methodology Validation

Unseparated chromatograms at 254 nm and UV spectra in the region of 190 - 400 nm were recorded, the retention time (RT) and peak area (PA) of unseparated chromatograms of samples were used to estimate the repeatability, precision and stability. The repeatability test was determined by analyzing six replicates of the same batch of sample, and the relative standard deviation (RSD) of RT and PA were less than 1.0% and 5.0%, respectively. Instrument precision was determined by injecting the same sample solution six times consecutively, and the RSD of RT and PA were found to be 0.4% and 3.3%, respectively. The solution stability was measured by testing a single sample solution stored at room temperature for 0, 0.25, 0.5, 1, 2 and 4 hours, and the RSD of RT and PA did not exceed 1.0% and 3.0%, respectively.

#### 3.1.2. Spectrum Analysis

All the thirty samples were extracted under the optimized conditions and analyzed using the above-established method. The UV absorption spectra of the tested samples from 190 to 400 nm were revealed in **Figure 2(a)**. The variation of the thirty samples was significant especially from 210 to 280 nm, and the majority of components with chemical bonds $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ in structure may contribute to the strong absorption at around 215 nm, 275 nm and 355 nm (**Figure 2(c)**), such as flavonoids and anthraquinones components in SHT.

#### 3.1.3. Fingerprint Analysis in Terms of SQFM

The UV spectra of the collected samples and the RFP obtained by average method were converted into the file layout of CSV, and then imported into the in-house software. Similarity parameters ($S_{UV}$, $P_{UV}$, $\alpha_{UV}$) and final quality grades were calculated in **Table 3**, and it was indicated that all samples were almost similar to the RFP with qualitative similarity $S_{UV} \geq 0.93$. However, a great difference was observed in quantitative similarity $P_{UV}$, which made an obvious distinction of all samples. The results in **Table 3** shown that most of the samples were qualified (grade ≤ 5) [13] except S8, S12, S18, S28, S30 for their higher contents in the range of 145.5% - 253.8%, and S17, S21, S22, S25, S27 for their lower contents in the range of 46.3% - 67.5%. Moreover, S1 and S2 from the same manufactory were proved to be accordant in quality of grade 2 and 3, respectively. However, S23 and S24 from the same vendors, got the inconsistent results of grade 1 and 5, respectively.

### 3.2. IR Fingerprint

#### 3.2.1. Methodology Validation

The repeatability test was determined by analyzing six replicates of the same batch of sample. The qualitative similarity $S_{IR}$ and RSD were calculated, and the results showed that $S_{IR} \geq 0.99$ with RSD ≤ 3.0%. Instrument precision was

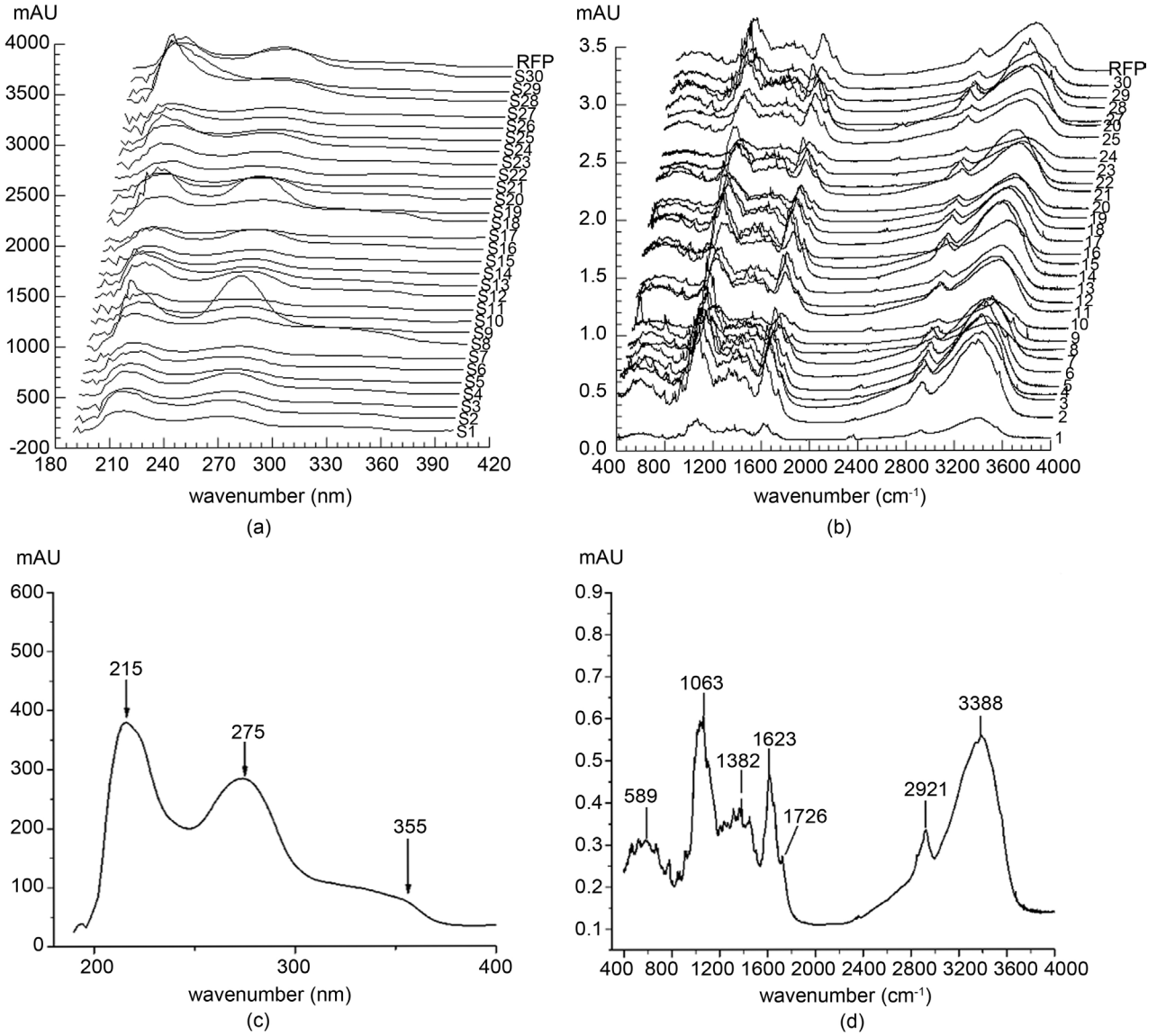


**Figure 2.** The spectral fingerprints of 30 batches of SHT and their reference fingerprints. (a): UV fingerprints; (b): IR fingerprints; (c): UV reference fingerprint; (d): IR reference fingerprint.

determined by analyzing the same sample solution six times consecutively. The sample solution stability was evaluated by analyzing a single sample stored in the dryer at room temperature for 0, 0.25, 0.5, 0.75 and 1.0 hour, and the results showed that $S_{IR} \geq 0.99$ with RSD ≤ 1.0%.

### 3.2.2. Spectrum Analysis

Nine-points smoothing technique was performed for original spectral data in order to remove unwanted variations and increase spectral resolution. The IR spectra fingerprints of thirty SHT samples from 4000 to 400 $cm^{-1}$ and the RFP generated by averaging all of the spectra were shown in **Figure 2(b)**. The variation of the collected samples was significant especially from 1500 to 400 $cm^{-1}$ (mainly fingerprinting region) and several fingerprint characteristics were

**Table 3.** The similarity analysis results in terms of UV, IR and CH by SQFM.

| | Para. | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UV | $S_{UV}$ | 0.99 | 0.99 | 0.98 | 0.98 | 0.97 | 0.99 | 0.97 | 0.95 | 0.97 | 0.99 | 0.96 | 0.99 | 0.98 | 0.99 | 0.97 | 0.98 | 0.98 |
| | $P_{UV}$ (%) | 92.2 | 114.0 | 85.8 | 109.2 | 83.6 | 86.2 | 71.0 | 253.8 | 103.6 | 90.4 | 78.4 | 147.7 | 115.5 | 82.7 | 70.5 | 113.8 | 46.3 |
| | $\alpha_{UV}$ | 0.02 | 0.00 | 0.01 | 0.01 | 0.01 | 0.01 | 0.00 | 0.00 | 0.05 | 0.02 | 0.02 | 0.01 | 0.03 | 0.02 | 0.00 | 0.02 | 0.03 |
| | Grade | 2 | 3 | 3 | 2 | 3 | 3 | 5 | 8 | 2 | 2 | 4 | 7 | 3 | 3 | 5 | 3 | 8 |
| | Para. | S18 | S19 | S20 | S21 | S22 | S23 | S24 | S25 | S26 | S27 | S28 | S29 | S30 | RFP | Average | | RSD (%) |
| | $S_{UV}$ | 0.98 | 0.98 | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 | 0.99 | 0.97 | 0.94 | 0.96 | 0.93 | 0.99 | 1.00 | 0.98 | | 1.56 |
| | $P_{UV}$ (%) | 227.3 | 78.2 | 145.5 | 66.3 | 67.5 | 96.6 | 127.3 | 66.5 | 76.8 | 54.1 | 176.2 | 102.4 | 171.3 | 100 | 106.69 | | 45.33 |
| | $\alpha_{UV}$ | 0.02 | 0.02 | 0.02 | 0.01 | 0.00 | 0.01 | 0.03 | 0.01 | 0.05 | 0.02 | 0.06 | 0.14 | 0.01 | 0.00 | 0.02 | | 121.20 |
| | Grade | 8 | 4 | 7 | 6 | 6 | 1 | 5 | 6 | 4 | 7 | 8 | 3 | 8 | 1 | —— | | —— |
| | Para. | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 |
| IR | $S_{IR}$ | 0.96 | 1.00 | 0.99 | 1.00 | 0.99 | 1.00 | 1.00 | 0.99 | 0.94 | 0.99 | 0.99 | 1.00 | 0.99 | 1.00 | 1.00 | 0.99 | 0.99 |
| | $P_{IR}$ (%) | 148.7 | 140.6 | 168.8 | 124.2 | 118.3 | 115.7 | 120.4 | 77.7 | 76.2 | 65.6 | 118.2 | 84.7 | 87.6 | 129.3 | 116.3 | 73.5 | 103.9 |
| | $\alpha_{IR}$ | 0.07 | 0.02 | 0.01 | 0.01 | 0.04 | 0.03 | 0.01 | 0.03 | 0.06 | 0.03 | 0.00 | 0.00 | 0.00 | 0.03 | 0.02 | 0.02 | 0.02 |
| | Grade | 7 | 7 | 8 | 4 | 3 | 3 | 4 | 4 | 4 | 6 | 3 | 3 | 3 | 5 | 3 | 5 | 1 |
| | Para. | S18 | S19 | S20 | S21 | S22 | S23 | S24 | S25 | S26 | S27 | S28 | S29 | S30 | RFP | Average | | RSD (%) |
| | $S_{IR}$ | 1.00 | 1.00 | 1.00 | 1.00 | 0.99 | 1.00 | 0.99 | 0.99 | 0.99 | 1.00 | 0.99 | 0.99 | 0.99 | 1.00 | 0.99 | | 1.16 |
| | $P_{IR}$ (%) | 87.4 | 83.0 | 67.8 | 113.2 | 72.2 | 68.7 | 67.1 | 111.0 | 140.1 | 76.4 | 102.4 | 74.7 | 74.7 | 100 | 100.28 | | 28.21 |
| | $\alpha_{IR}$ | 0.00 | 0.01 | 0.01 | 0.02 | 0.02 | 0.01 | 0.05 | 0.04 | 0.01 | 0.02 | 0.04 | 0.01 | 0.01 | 0.00 | 0.02 | | 79.15 |
| | Grade | 3 | 3 | 6 | 3 | 5 | 6 | 6 | 3 | 7 | 4 | 1 | 5 | 5 | 1 | —— | | —— |
| | Para. | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 |
| CH | $Q_v$ (kJ/g) | 16.73 | 16.51 | 15.78 | 16.03 | 16.68 | 17.30 | 16.57 | 17.07 | 16.10 | 16.88 | 16.58 | 16.92 | 16.53 | 16.68 | 16.13 | 15.73 | 15.34 |
| | $S_q$ | 0.97 | 0.98 | 0.97 | 0.99 | 0.97 | 0.93 | 0.98 | 0.95 | 0.99 | 0.96 | 0.98 | 0.95 | 0.98 | 0.97 | 1.00 | 0.97 | 0.95 |
| | $P_q$ (%) | 103.3 | 101.9 | 97.4 | 99.0 | 103.0 | 106.8 | 102.3 | 105.4 | 99.4 | 104.2 | 102.4 | 104.5 | 102.1 | 103.0 | 99.6 | 97.1 | 94.7 |
| | $\alpha_q$ | 0.03 | 0.02 | 0.03 | 0.01 | 0.03 | 0.07 | 0.02 | 0.05 | 0.01 | 0.04 | 0.02 | 0.05 | 0.02 | 0.03 | 0.00 | 0.03 | 0.05 |
| | Grade | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 |
| | Para. | S18 | S19 | S20 | S21 | S22 | S23 | S24 | S25 | S26 | S27 | S28 | S29 | S30 | Average | | | RSD (%) |
| | $Q_v$ (kJ/g) | 16.07 | 16.05 | 16.07 | 15.68 | 16.01 | 15.87 | 15.73 | 15.20 | 16.19 | 16.45 | 16.49 | 15.16 | 15.31 | 16.20 | | | 3.46 |
| | $S_q$ | 0.99 | 0.99 | 0.99 | 0.97 | 0.99 | 0.98 | 0.97 | 0.94 | 1.00 | 0.98 | 0.98 | 0.94 | 0.95 | 0.97 | | | 1.97 |
| | $P_q$ (%) | 99.2 | 99.1 | 99.2 | 96.8 | 98.9 | 98.0 | 97.1 | 93.8 | 100.0 | 101.6 | 101.8 | 93.6 | 94.6 | 100 | | | 3.46 |
| | $\alpha_q$ | 0.01 | 0.01 | 0.01 | 0.03 | 0.01 | 0.02 | 0.03 | 0.06 | 0.00 | 0.02 | 0.02 | 0.06 | 0.05 | 0.03 | | | 67.80 |
| | Grade | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 2 | —— | | | —— |
| | Para. | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 |
| Integrated | $S_I$ | 0.97 | 0.99 | 0.98 | 0.99 | 0.98 | 0.97 | 0.98 | 0.96 | 0.97 | 0.98 | 0.98 | 0.98 | 0.98 | 0.99 | 0.99 | 0.98 | 0.97 |
| | $P_I$ | 114.7 | 118.8 | 117.3 | 110.8 | 101.6 | 102.9 | 97.9 | 145.6 | 93.1 | 86.7 | 99.7 | 112.3 | 101.7 | 105.0 | 95.5 | 94.8 | 81.6 |
| | $\alpha_I$ | 0.04 | 0.01 | 0.02 | 0.01 | 0.03 | 0.04 | 0.01 | 0.03 | 0.04 | 0.03 | 0.01 | 0.02 | 0.02 | 0.03 | 0.01 | 0.02 | 0.03 |
| | Grade | 3 | 3 | 3 | 3 | 1 | 1 | 1 | 7 | 2 | 3 | 1 | 3 | 1 | 1 | 1 | 2 | 3 |
| | Para. | S18 | S19 | S20 | S21 | S22 | S23 | S24 | S25 | S26 | S27 | S28 | S29 | S30 | Average | | | RSD (%) |
| | $S_I$ | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.99 | 0.98 | 0.97 | 0.99 | 0.97 | 0.98 | 0.95 | 0.98 | 0.98 | | | 0.94 |
| | $P_I$ | 138.0 | 86.8 | 104.2 | 92.1 | 79.5 | 87.8 | 97.2 | 90.4 | 105.6 | 77.4 | 126.8 | 90.2 | 113.5 | 102.32 | | | 15.84 |
| | $\alpha_I$ | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.04 | 0.04 | 0.02 | 0.02 | 0.04 | 0.07 | 0.02 | 0.02 | | | 56.78 |
| | Grade | 6 | 3 | 1 | 2 | 4 | 3 | 1 | 2 | 2 | 4 | 5 | 2 | 3 | —— | | | —— |

RFP: Reference fingerprint; RSD: Relative standard deviation.

revealed in **Figure 2(d)**, the strongest peak at 3388 $cm^{-1}$ indicated the stretching vibration of O-H groups associated with phenolic hydroxyl of flavonoids and phenoliacids. The peak at 2921 $cm^{-1}$ and 1382 $cm^{-1}$ belonged to the stretching vibration of $-CH_2$ and $-CH_3$ groups, respectively, and the stronger peaks in the range of 1200 - 1000 $cm^{-1}$ mainly attributed to the stretching vibration of C-O, which might be the characteristic absorptions of glycosides.

#### 3.2.3. Fingerprint Analysis in Terms of SQFM

The IR spectra of all SHT samples and the RFP were imported into the in-house software to calculate similarity parameters ($S_{IR}$, $P_{IR}$, $\alpha_{IR}$) and final quality. It was demonstrated from **Table 3** that although qualitative similarity $S_{IR}$ was able to slightly discriminate samples from batch to batch, a wide range of quantitative similarity $P_{IR}$ led to final various quality grades. Most of the samples are qualified (grade ≤ 5) except for S1-S3, S10, S20, S23, S24 and S26.

What's more, compared with the UV fingerprint, enormous difference has been found in some samples. A case in point was S8, instead of inferior (grade 8) in UV analysis, S8 was recognized as qualified (grade 4) with IR analysis. To our knowledge, it is mainly attributed to the different principles between IR and UV. UV fingerprint reveals the features of $\pi \to \pi^*$, $n \to \pi^*$ and $n \to \sigma^*$ chemical bonds of compounds while IR fingerprint chiefly reflects the vibration and rotation of chemical bonds (especially saturated bonds). It reminds us that multi-analysis methods based on diverse principles is necessary to comprehensively control the quality of TCM or HD.

### 3.3. CH Analysis

CH as a traditional parameter according to the First Law of Thermodynamics can disclose the total chemical energy in single compounds or mixture. CH can reflect not only the total chemical contents but also the therapeutic effect of TCM or HD to some extent [16] [17].

Consequently, it was introduced as an important approach in this study to analyze the quality of herbal medicines. The CH of thirty samples was determined using the calorimeter after instrument adjustment and precision validation. Qualitative similarity $S_q$, quantitative similarity $P_q$ and variation coefficient $\alpha_q$ were defined as Equations (4)-(6), of which $Q_v$ was the CH value of each sample and $\overline{Q}_v$ served as the reference value calculated by the average method.

$$S_q = 1 - \left| 1 - \frac{Q_v}{\overline{Q}_v} \right| \tag{4}$$

$$P_q = \frac{Q_v}{\overline{Q}_v} \times 100\% \tag{5}$$

$$\alpha_q = 1 - S_q \tag{6}$$

It was noticed that all samples had similar quality (grade 1 - 2) with qualitative

similarity $S_q \geq 0.93$ and quantitative parameter $P_q \geq 93.6\%$ shown in Table 3.

### 3.4. Integrated Evaluation Involving UV and IR Fingerprinting as Well as CH Analysis

In order to avoid bias detection at a single method, an integrated evaluation was carried out by means of equal weights. The integrated $S_I$, $P_I$ and $\alpha_I$ values were calculated according to Equations (7)-(9).

$$S_I = \frac{1}{3}\left(S_{UV} + S_{IR} + S_q\right) \tag{7}$$

$$P_I = \frac{1}{3}\left(M_{UV} + M_{IR} + P_q\right) \tag{8}$$

$$\alpha_I = \frac{1}{3}\left(\alpha_{UV} + \alpha_{IR} + \alpha_q\right) \tag{9}$$

Our results illustrated that S8 and S18 were found to be outliers with the quality of grade 7 and 6, respectively, the remaining twenty-eight samples had qualified grade in the range of 1 - 5 (specified in Figure 3).

### 3.5. Comparative Analysis of Results Obtained from Multiple Methods

Table 3 displays the discrepant quantitative similarity values of 30 batches of sample, a possible explanation of the result might be due to the variability in the raw material associated with a wide range of factors (e.g., climate, geographical location, harvest time, etc.) and variability in the manufacturing processes. As shown in Figure 4, the quantitative similarity of many samples exhibited large diversity, which reminded us that it was essential to adopt multi-approaches integration to disclose the quality information of TCM and HD.

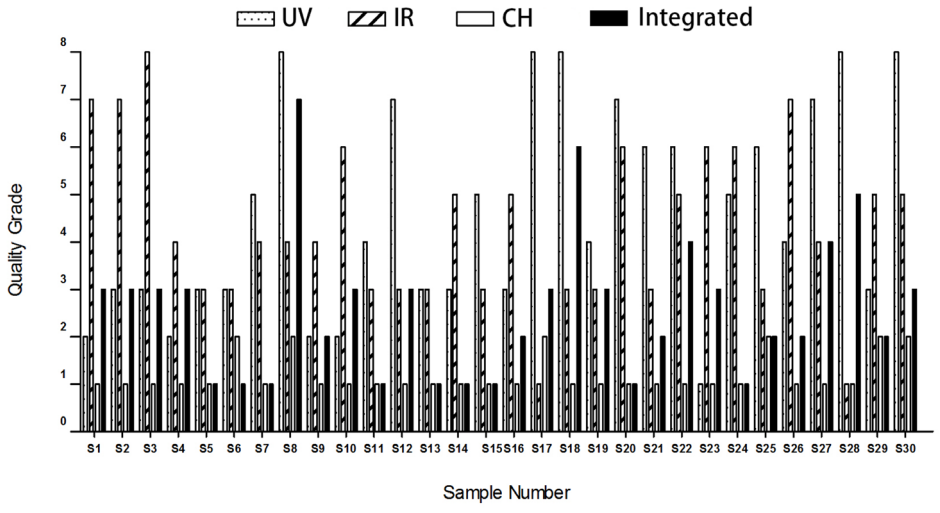


**Figure 3.** The quality grade of 30 samples identified by different methods.

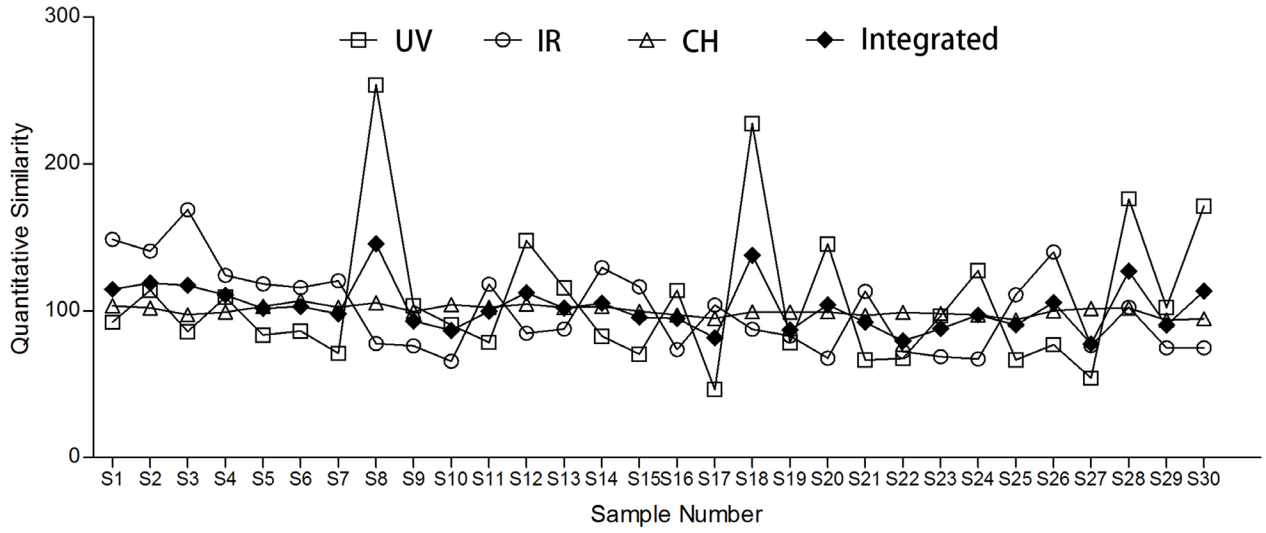


**Figure 4.** The comparison of quantitative similarity analysis for 30 batches of SHT in different analytical approaches calculated by SQFM.

## 4. Conclusion

Three approaches, UV and IR fingerprinting coupled with CH analysis, developed in the present study, represent fast and alternative way to traditional chromatographic analyses for the differentiation of SHT samples. They are all capable of acquiring data allowing discrimination of the quality consistency of herbal medicines from different sources. The promising results obtained from combination of multiple analytical methods encourage the application of the proposed strategy for the comprehensive quality assessment of raw herbal medicine and their preparations.

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