Pharmacogn. Mag.
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ORIGINAL ARTICLE

Novel Mathematical Model for the Assessment of Similarity of Chromatographic Fingerprints of Volatile Oil from *Houttuynia cordata*

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Submitted: 22-May-2020 Revised: 06-Jul-2020 Accepted: 15-Dec-2020 Published: 15-Apr-2021

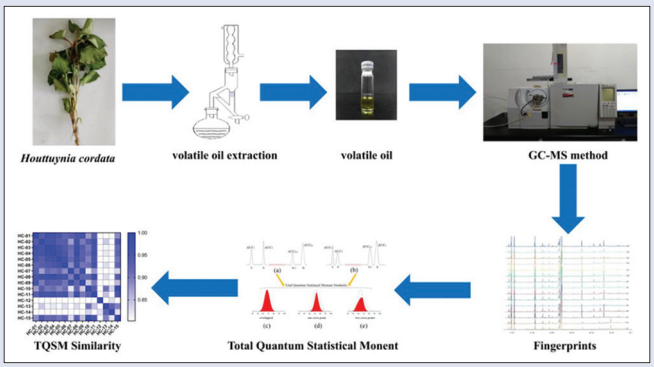
ABSTRACT

Background: The analysis of similarities among fingerprints of Chinese herbal medicines is an important quality control tool to determine the authenticity of the herbal medicines. **Objectives:** In this study, we aimed to develop a novel mathematical model to analyze the similarity of the chromatographic fingerprints of *Houttuynia cordata* (HC). **Materials and Methods:** Total quantum statistical moment similarity (*TQSMS*) expressions were deduced to evaluate the similarities between two chromatographic fingerprints. The volatile oil samples of HC were analyzed with gas chromatography-mass spectrometry, and the fingerprints were constructed by the area under the peak of the chromatograms. **Results:** There were nine peaks in common, and a total of 733 chemical constituents observed among 15 batches of samples. The number of peaks in the chromatographic fingerprints of the 15 batches of HC was 49–137, with a relative standard deviation (RSD) of 30.13%. The sum of area under the peak was 1.159×10^7–3.437×10^8 μv × s, with an RSD 174.56%; $MCRT_T$ was 9.410–18.602 min, with an RSD of 20.79%; and $VCRT_T$ was 37.549–81.504, with an RSD of 23.27%. The volatile oil composition and content of HC showed strong fluctuation. Therefore, its quality control from the variety and content of the components is impractical. Since *TQSMS* method can characterize the sample similarity, we can quantitate the correct probability of positive and negative conclusions regardless of the population origin of the samples. **Conclusion:** Our results show that *TQSMS* can be an additional method that can be used to assess the similarity of two chromatographic fingerprints.

Key words: Chromatographic fingerprints, gas chromatography-mass spectrometry, herbal medicine, *Houttuynia cordata,* total quantum statistical moment similarity, volatile oil

SUMMARY

- The volatile oil composition and content of *Houttuynia cordata* showed strong fluctuation
- The total quantum statistical moment similarity can characterize the sample similarity, and we can quantitate the correct probability of positive and negative conclusions regardless of the origin of the samples.



Abbreviations used: *TQSMS*: Total quantum statistical moment similarity; *TQSM*: Total quantum statistical moment; GC-MS: Gas chromatography-mass spectrometry; AUC_T: Area under the curve of total quantum; $MCRT_T$: Mean chromatographic retention time of total quantum; $VCRT_T$: Variance of mean chromatographic retention time of total quantum; *D*: Deviation; *pV*: Variable probability; 1 – β: Confidence of probability S_T: Similarity of total quantum statistical moment; α: Confidence coefficient; HC: *Houttuynia cordata*; RSD: Relative standard deviation.

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DOI: 10.4103/pm.pm_192_20

Access this article online
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INTRODUCTION

Chinese herbal medicines have been successfully used to treat various diseases since thousands of years, yet there is a lack of adequate evidence to substantiate the quality of herbal medicines. Recently, chromatographic fingerprints have been proposed to identify the authenticity of herbal medicines, which provide quality control measures for complex systems with multiple components.[1-3] Currently, many mathematical models have been applied to assess the similarities or differences between various herbal medicines, such as angle cosine, correlation coefficient, fuzzy cusp T distribution, and Euclidean distance.[4] The methods used in the fingerprint similarity analysis usually are divided into the fingerprint characteristic peak response value into discrete data information, adopted to a corresponding characteristic peak of a multidimensional vector method, and calculated to find the similarity, often causing an unstable result.[5]

Cite this article as: Zhou J, Fan Q, Zhang Y, Castillo R, Xiao M, Liu H, *et al*. Novel mathematical model for the assessment of similarity of chromatographic fingerprints of volatile oil from *Houttuynia cordata*. Phcog Mag 2021;17:154-62.

Although great progress has been made in the field of fingerprint analysis, some problems still need attention.[6,7] First, under the same conditions of analysis, researchers have obtained variations in peak retention times and heights of a given sample. However, it is noteworthy that the peak areas of a given concentration were relatively stable. Second, the differences among the chromatographic fingerprints are influenced by factors such as analytical technics, geographical factors, and harvest time.[8-12] In other words, for one sample of herbal medicine, any change in conditions, analytical or environmental, will lead to differences in their chromatographic fingerprints. Composition and characteristics of herbal medicines vary with their collection time. Unfortunately, existing methods usually do not compare samples with an objective statistic to evaluate the similarities or differences between two fingerprints. Thus, an objective method is needed to evaluate the similarities among fingerprints for quality control and routine authenticity of herbal medicines.

Houttuynia cordata (HC, Yuxingcao in Chinese), the dried aerial part of HC Thunb. (*Saururaceae*),[13] is one of the best-known natural herbs used since thousands of years in the history of traditional Chinese medicine. Modern pharmacological studies have shown that it shows pharmacological activities, such as antimutagenic,[14] anti-inflammatory,[15] antiviral,[16] antibacterial,[17] antiallergic,[18] antidiabetic,[19] antioxidant,[14] and antiobesity.[20] The primary active components of HC include volatile oils, flavonoids, alkaloids, organic acids, and trace elements. The volatile oils are considered as the major functional components in HC.[21] As is known, the composition of HC changed greatly in the variety and contents with planting environment and harvesting time.[22] However, these existing methods of similarity analysis, including the angle cosine method and correlation coefficient method, cannot accurately reflect the reality of multidimensional vector deviation degree and are more sensitive to the change in peaks with a larger response and are less sensitive to small peak.

To address this issue, we established a novel qualitative and quantitative mathematical model for chromatographic fingerprint analysis, namely, the total quantum statistical moment similarity (*TQSMS*). *TQSMS* can be used to characterize the characteristic information of chromatographic fingerprints. Specifically, the area under the curve of total quantum (AUC_T) can be used in quantitative analysis, whereas the mean retention time of total quantum ($MCRT_T$) and variance of mean retention time of total quantum ($VCRT_T$) can be used in qualitative analysis. According to the properties of the normal distribution probability density function and the total quantum statistical moment, the *TQSMS* can then be established and elucidated. To test the model, we analyzed gas chromatographic fingerprints of the volatile oil of HC.

MATERIALS AND METHODS

Total quantum statistical moment similarity established for chromatographic fingerprint

Statistical moment methods and theory are powerful techniques for characterizing the chromatographic peaks of fingerprints.[23] In detail, the area of a peak is defined as the zeroth moment, the retention time is defined as the first moment, the variance in retention time is defined as the second moment, whereas the higher moments are associated with the peak shape.[24] However, for a more in-depth analysis, further elaboration of zeroth, first, and second moment of a chromatographic fingerprint is essential.

Zeroth moment of total quanta (AUC_T)

Each chromatographic peak in a fingerprint can be considered as a Gaussian curve. Therefore, a complete chromatographic fingerprint can be regarded as the superposition of *m* characteristic peak response curves. The area under curve (AUC) of the chromatographic fingerprint spectrum (AUC_T) was defined as the zeroth moment of total quanta, i.e., all chromatographic peak areas integrated versus time from zero to infinity, and can be calculated by Equation (1).

$$AUC_T = \sum_{i=1}^{n} AUC_i \tag{1}$$

First moment of total quanta ($MCRT_T$/t_T)

The first moment of total quanta is defined as the mean chromatographic retention time ($MCRT_T$). It can be calculated by Equation (2).

$$MCRT_T = \frac{\sum_{i=1}^{n}(t_i \times AUC_i)}{\sum_{i=1}^{n} AUC_i} \tag{2}$$

Second moment of total quanta ($VCRT_T$/$\overline{\sigma}_T^2$)

The second moment of total quanta was defined as the variance of the chromatographic retention time ($VCRT_T$), a degree of residence as Equation (3).

$$VCRT_T = \frac{\sum_{i=1}^{n}((\sigma_i^2 + t_i^2)\times AUC_i)}{\sum_{i=1}^{n} AUC_i} - MCRT_T^2 \tag{3}$$

Total quantum statistical moment similarity

The two *TQSM* parameters, first moment ($MCRT_T$), i.e., mean, and second moment ($VCRT_T$), i.e., variance, illustrate the mean chromatographic retention time center and discrete degree for the chromatographic fingerprints [Figure 1]. The two parameters can be converted into a normal distribution probability density function,[25,26] as shown in the following Equation (4).

$$F(t) = \int_{-\infty}^{+\infty} \frac{1}{\sqrt{2\pi}\sigma_T} \exp\left(-\frac{(t-t_T)^2}{2\sigma_T^2}\right) d\lambda \quad (-\infty < t < +\infty) \tag{4}$$

Where t means the chromatographic retention time, t_T is the $MCRT_T$, and σ_T^2 is the $VCRT_T$. Assume that the first moments for two chromatographic fingerprints are t_a and t_b and second moments are σ_a^2 and σ_b^2. The intersection point of two normal curves of distribution represents t_1 and t_2, yielding Equation (5).

$$\frac{1}{\sqrt{2\pi}\sigma_a} e^{-\frac{(t-t_a)^2}{2\sigma_a^2}} = \frac{1}{\sqrt{2\pi}\sigma_b} e^{-\frac{(t-t_b)^2}{2\sigma_b^2}} \quad (-\infty < t < +\infty) \tag{5}$$

Then, Equation (5) was regulated, and Equation (6) was obtained.

$$(\sigma_b^2 - \sigma_a^2)t^2 - 2(t_a\sigma_b^2 - t_b\sigma_a^2)t + (\sigma_a^2\sigma_b^2 ln\frac{\sigma_a^2}{\sigma_b^2} + \sigma_b^2 t_a^2 - \sigma_a^2 t_b^2) = 0 \tag{6}$$

Finally, two solutions following Equation (7) are given.[27,28]

$$t_{1(2)} = \frac{(t_b\sigma_a^2 - t_a\sigma_b^2) \pm \sqrt{(t_b\sigma_a^2 - t_a\sigma_b^2)^2 - (\sigma_a^2 - \sigma_b^2)(\sigma_b^2\sigma_a^2 ln\frac{\sigma_b^2}{\sigma_a^2} + \sigma_a^2 t_b^2 - \sigma_b^2 t_a^2)}}{\sigma_a^2 - \sigma_b^2} \tag{7}$$

The *TQSMS* for two chromatographic fingerprints can then be defined as the overlapping area for two probability density functions depicted in Figure 1, as in Equation (8).

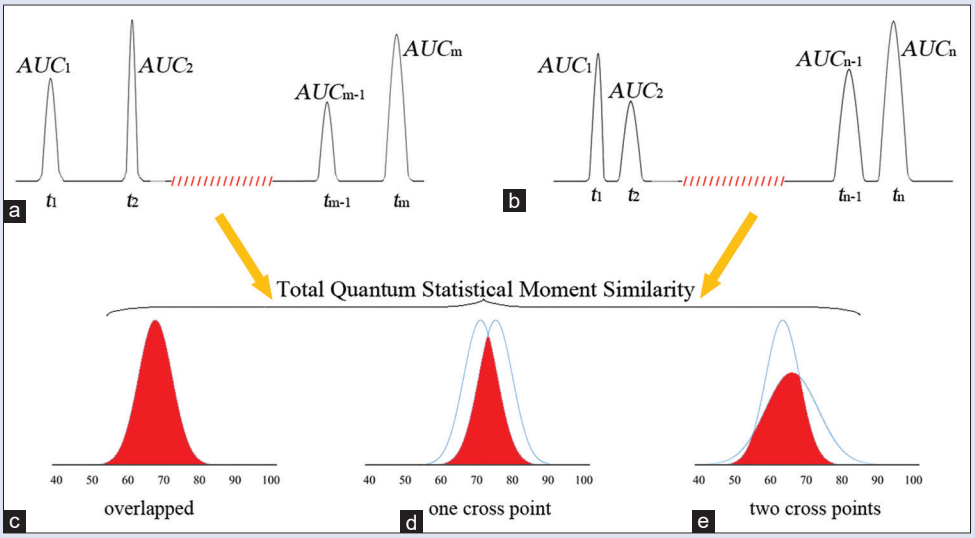

Figure 1: Total quantum statistical moment similarity modeling process for chromatographic fingerprint. (a and b) Chromatographic fingerprints of two samples. (c-e) Three scenarios of total quantum statistical moment similarity model: (c) two chromatographic fingerprints were overlapped completely and the similarity was 1; (d) only one cross-point exists; (e) two cross-point exists

$$TQSMS = 1 - \left| \int_{t_1}^{t_2} \frac{1}{\sqrt{2\pi}\sigma_a} \exp\left(-\frac{(t_a - t)^2}{2\sigma_a^2} \right) dt - \int_{t_1}^{t_2} \frac{1}{\sqrt{2\pi}\sigma_b} exp\left(-\frac{(t_b - t)^2}{2\sigma_b^2} \right) dt \right| \quad (-\infty < t < +\infty) \tag{8}$$

There were three scenarios as follows:

1. When $\sigma_a = \sigma_b$, $t_a > t_b$ as shown in Figure 1b in Figure 1c, two chromatographic fingerprints were overlapped completely and the similarity S_T was 1
2. When $\sigma_a = \sigma_b$, $t_a > t_b$ as shown in Figure 1a in Figure 1d, only one cross-point t_1 exists as in Equation (9)

$$t_1 = \frac{t_a + t_b}{2} \tag{9}$$

Then, *TQSMS* can be calculated by Equation (10).

$$TQSMS = 1 - \left| \int_{-\infty}^{t_1} \frac{1}{\sqrt{2\pi}\sigma_a} \exp\left(-\frac{(t_a - t)^2}{2\sigma_a^2} \right) dt - \int_{t_1}^{+\infty} \frac{1}{\sqrt{2\pi}\sigma_b} exp\left(-\frac{(t_b - t)^2}{2\sigma_b^2} \right) dt \right| \quad (-\infty < t < +\infty) \tag{10}$$

3. When $\sigma_a \neq \sigma_b$, $t_a \neq t_b$, $\sigma_a > \sigma_b$ as shown in Figure 1c in Figure 1e, two cross-points both t_1 and t_2 exist in two probability density functions where similarity S_T is calculated by Equation (8).

Standard total quantum statistical moment similarity, deviation, variable probability, positive or negative judgment, and critical values for standard normal distribution

Similar to our previous study on *TQSMS* of pharmacokinetics,[27] the parameters of the statistical test for *TQSMS* of chromatographic fingerprints can be obtained. Under test size u_α, the parameters, standard *TQSMS* (*TQSMSu*), deviation (*D*), variable probability (*p*V), and the confidence of probability (1 − β), can be calculated and shown in Supplementary Table 1 for negative judgment and Supplementary Table 2 for positive judgment, and finally, their critical values can also be ascertained. With this, it is convenient to make judgment about similarity or differences in the chromatographic fingerprints.

When *D* = 1.96, *TQSMSu* would be 0.05, and the *p*V is 95%, whereas the confidence of probability (*1* − β) varied with the confidence coefficient α. If a negative judgment was made that the two chromatographic fingerprints were different, then Supplementary Table 1 should be adopted. When α value is 0.05, *1* − β is 0.5, i.e., there is 50% confidence of probability to make a judgment on 95% of chromatographic fingerprints being different. When 1 - β value is more than 0.75 and *TQSMSu* is less than 0.008 (α = 0.05, significance level) or 0.001 (α = 0.01), it can be considered as the critical value of negative conclusion that two chromatographic fingerprints are from different populations. If a positive judgment was made that the two chromatographic fingerprints were similar, then Supplementary Table 2 should be adopted. When *1* − α is valued 0.95, β = 0.95, *TQSMSu* = 0.803, i.e., there is 95% confidence of probability to make a judgment on 95% of two fingerprints are similar, and then it can be considered as a critical value to a positive conclusion that the two samples were from a same population; as the routine requirement that *1* − α value was less than 0.95, β value *is* more than 0.900, and *TQSMSu* is more than 0.803, then there is 90% confidence of probability to make a judgment on 95% of two samples are similar. In other words, the critical value of *TQSMSu* to determine whether the two fingerprints are similar is 0.8030, which is an important and objective statistical parameter to judge the similarity between two samples. Within 25%–75% confidence of probability, a precise judgment would be made that the two chromatographic fingerprints are from the same population, while the risk of these conclusions can also be estimated. The availability of statistical test parameters is an important feature of the *TQSMS* model superior to other similarity methods.

Chemicals and materials

Standards 2-undecanone (Lot. 110834-200502, purity ≥ 99.8%), α-pinene (Lot. 897-2000001, purity ≥ 98.0%), and internal standard *n*-pentadecane (Lot. 11677-200401, purity ≥ 100.0%) were obtained from the China National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Ethyl acetate (Lot. 013092701, purity ≥ 99.5%) was purchased from Chengdu Chron Chemicals (Chengdu, China), *n*-hexane (Lot. K46764991 524, purity ≥ 99.9%) from Merck Ltd. (Darmstadt, German), and ethanol (Lot. 0907360, purity ≥ 99.7%) from Anhui Ante Biochemistry Co. Ltd. (Suzhou, China). All samples of fresh HC were collected from

Changsha Hunan from April 2017 to August 2017, and Prof. Ji-Lian Shi (Professor from Hunan University of Chinese Medicine, Changsha, China) authenticated the plants. *n*-Pentadecane (0.1358 g) was dissolved in 10 mL of *n*-hexane to and diluted to obtain a final concentration of 0.679 mg/mL. Briefly, 2-undecanone (0.0726 g) and α-pinene (0.1393 g) were dissolved in *n*-pentadecane internal standard solution (0.679 mg/mL) to a final volume of 10 mL and final concentrations of 7.29 and 13.93 mg/mL. All these solutions were kept at 4°C until for gas chromatography-mass spectrometry (GC-MS) analysis.

Apparatus and chromatographic conditions

The procedure for the extraction of HC volatile oil and GC-MS analysis is presented in our previous study.[29] Volatile oil of HC was extracted by an extractor apparatus purchased from Sichuan Shubo (Group) Co., Ltd. (Chongzhou, China). GC-MS-QP2010 (SHIMADZU, Japan) was used for GC-MS analysis, and a quartz capillary column SE-30 (the stationary phase: AT SE-30, 0.25 mm × 30 m × 0.25 μm, Dalian Physiochemical Institute, China) was used for chromatographic separation. The injection volume of 2.0 μL was used for analysis. The injection port temperature was held at 250°C. The oven temperature was kept at 60°C for 3 min and then increased up to 140°C at 2°C/min, held at 140°C for 5 min and then increased up to 200°C at 10°C/min, and finally held at 200°C for 5 min. The total flow rate was kept at 37.1 mL/min, the column pressure was maintained at 65.2 kPa, and the temperature of transfer line and source was maintained at 230°C, with split mode (ratio 30:1). The temperature of ESI ion source was held at 230°C, the interface at 280°C, and the quadruple temperature at 150°C; the electron energy was maintained at 70 eV. SCAN mode was used for detection. The solvent cutoff time was 3.5 min. Constituents were identified by contrasting their mass spectra of chromatographic peak from NIST08.

Sample preparation

A total of 15 batches of fresh HC were weighed and chopped into 2–3 cm pieces. The HC volatile oil was extracted five times by steam distillation with water.[13] The distillate was collected (100%) (2.0 mL) and sequentially labeled as HC-01–HC-15 and stored at −20°C until GC-MS analysis. To perform GC-MS analysis, 0.5 μL of the sample was diluted to 1.0 mL volume with ethyl acetate.

From previously reported systems,[27] integral conditions for the chromatographic fingerprint of HC volatile oil were obtained as follows: peak height of 200 μv, peak area of 4000 μv × s, and drift value of 15 μv.

Method validation

Standard solution was determined for precision by five continuous times. The RSD of the peak retention time was <2.56% and the RSD of the peak area was <2.83%, indicating that the precision of the method meets standard requirements.

Stability of the sample solutions was tested by comparing sample solutions that were kept at room temperature with standard solutions in 1, 2, 4, 6, 12, and 24 h. We found that the sample solutions were stable within 24 h (the RSD of peak retention time <2.45%, the RSD of peak area <4.62%).

Five independent samples were prepared and analyzed to determine the repeatability. The RSD of peak retention time was <0.44% and the RSD of peak area was <4.10%, indicating that the repeatability of the method met standard requirements.

Data analysis

The chromatographic fingerprints obtained were analyzed with *TQSMS* method established in this study according to Equations (1)–(10). Supplementary Tables 1 and 2 are applied for the statistical tests. Furthermore, the included angle cosine similarities and correlation coefficient were also used to compare with the *TQSMS* method.[30]

RESULTS AND DISCUSSION

Identification of volatile compounds in *Houttuynia cordata*

The volatile oils from the 15 batches of HC samples were analyzed by GC-MS. Figure 2 shows the representative chromatograms. The peak area of each component was chosen as the analytical signal for the relative content, and these identified components from the 15 batches of HC are listed in Supplementary Tables 3-17. The composition and relative content of volatile oil obtained by steam distillation of 15 batches of HC were all different. There were nine peaks in common, and a total of 733 chemical constituents were observed among 15 batches of samples. Figure 3 shows the mass scan spectra and chemical structures of the nine common compounds [Figure 2]. Table 1 shows the retention time, peak area, and relative content of common chemical compounds of essential oils from 15 batches of HC. The RSD of retention time of nine common chemical compounds ranged from 0.47% to 1.90%, indicating that the precision of the method met the standard requirements. The RSD of peak area and relative content of nine common chemical compounds ranged from 33.79% to 182.04% and from 37.45% to 101.04%, respectively. As outlined in Table 2 and Figure 4, the common components are represented from 20.74% to 74.13% of the extracted volatile oils. The identified volatile constituents consisted of aromatic, aliphatic, and terpenoid compounds. The most abundant component of the HC volatile oils was 2-undecanone (4.24–29.88%). Furthermore, the volatile oils also contained β-myrcene (5.41%–27.00%), β-pinene (0.83–17.90%), α-pinene (0.37%–10.46%); 2-tridecanone (0.76%–8.80%); bicyclo[3.1.0] hexane, 4-methylene-1-(1-methylethyl)- (0.02%–8.09%); *n*-decanoic acid (0.53%–6.62%), camphene (0.13%–1.86%), and *n*-hexadecanoic acid (0.26%–1.65%). However, the peak area percentages of other volatile constituents ranged from 79.26% to 25.87%. These results show that the composition of volatile oil and content of HC have a strong fluctuation, hinting that its quality control cannot be considered solely from the variety and content of the components. In situations like these, *TQSMS* method was a better tool to assess the similarities of these chromatographic fingerprints.

Total quantum statistical moment similarity analysis of gas chromatography-mass spectrometry fingerprint of volatile oil from *Houttuynia cordata*

Chromatographic fingerprints of volatile oil from HC were evaluated by the *TQSMS* method established in this study. The *TQSMS* parameters of the chromatographic fingerprints of the 15 batches of HC volatile oil are presented in Table 3 and Figure 5. The peak numbers of chromatographic fingerprints of 15 batches of HC volatile oil were 49–137, with an RSD value of 30.13%; the sums of peak area were 1.159×10^7–3.437×10^8 μv × s, with an RSD value of 174.56%; $MCRT_T$ was 9.410–18.602 min, with an RSD value of 20.79%; $VCRT_T$ was 37.549–81.504, with an RSD value of 23.27%. The *TQSMS* of the chromatographic fingerprints of the 15 batches of HC volatile oil was also obtained [Table 4]. *TQSMS* close to 1 suggests a high similarity between the two chromatographic fingerprints. As outlined in Table 4, the *TQSMS* was ranging from 0.4973 (HC-7 and HC-12) to 0.9905 (HC-4 and HC-8). According to the statistical test for *TQSMS* and our previous work,[27] α error (Type-I error) = 0.05, β error (Type-II error) = 0.95, and S_T = 0.8030, *TQSMS* of chromatographic fingerprints for HC volatile oil was shown as significant deviation among the batches of HC volatile oil. The

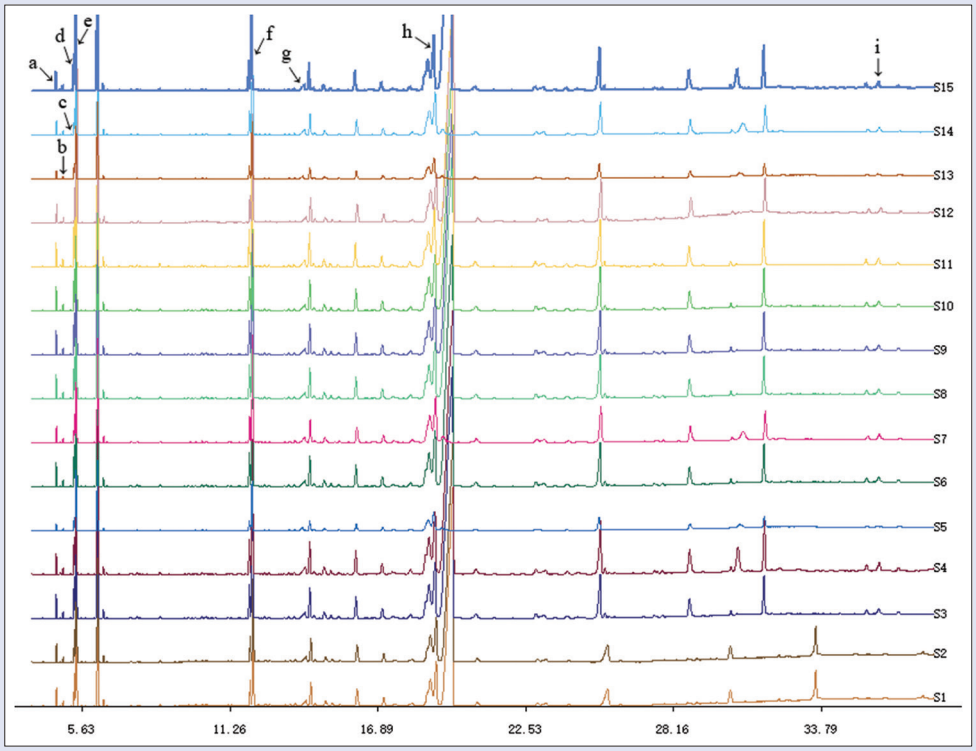

Figure 2: Gas chromatography fingerprint of *Houttuynia cordata* volatile oil samples. S1–S15, respectively, represent the gas chromatography fingerprint of batch 1 to batch 15 *Houttuynia cordata* volatile oil samples. (a) .alpha.-pinene; (b) camphene; (c) bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-; (d) .beta.-pinene; (e) .beta.-myrcene; (f) 2-undecanone; (g) n-decanoic acid; (h) 2-tridecanone; (i) n-hexadecanoic acid

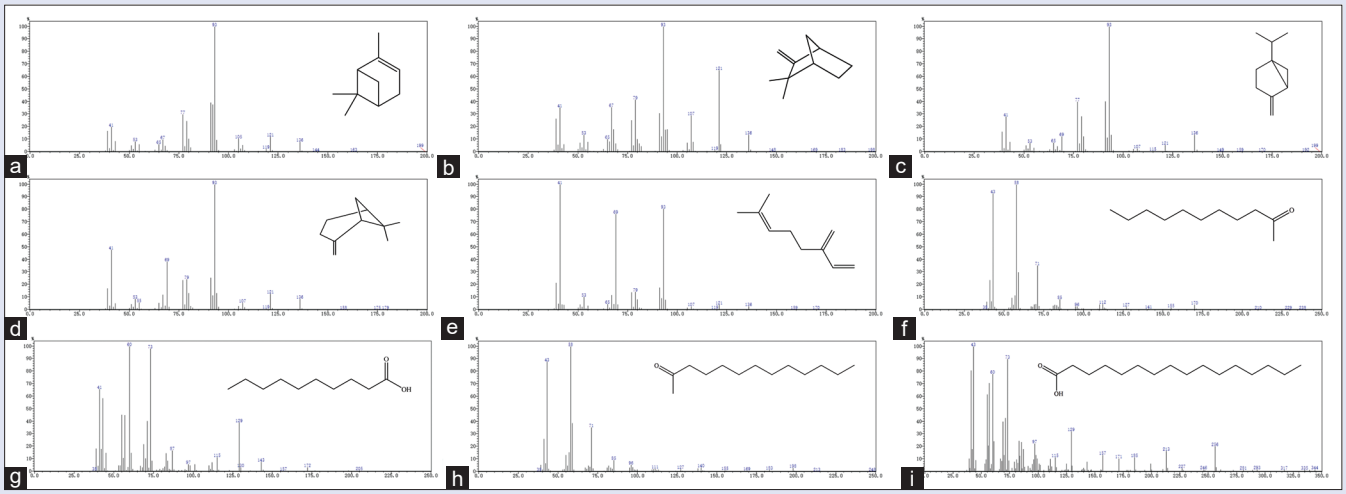

Figure 3: Ion fragmentation patterns and chemical structures of (a) .alpha.-pinene; (b) camphene; (c) bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-; (d) .beta.-pinene; (e) .beta.-myrcene; (f) 2-undecanone; (g) n-decanoic acid; (h) 2-tridecanone; (i) n-hexadecanoic acid

reason for the occurrence of these differences is that the biosynthesis of secondary metabolites during the growth of the plant is closely related to soil, temperature, water quality, ecological environment, and other factors. Based on the values in Table 4, samples from HC-01 to HC-09 were to have similarity (range from 0.8752 to 0.9905), whereas samples from HC-10 is similar to HC-15 (*TQSMS* = 0.9893), HC-11 is similar to HC-02 (*TQSMS* = 0.9705), HC-12 is similar to HC-14 (*TQSMS* = 0.8282), HC-13 is similar to HC-14 (*TQSMS* = 0.9264), HC-14 is similar to HC-13 (*TQSMS* = 0.9264), and HC-15 is similar to HC-10 (*TQSMS* = 0.9893). Otherwise, based on the mean *TQSMS* of each HC sample to other 14 samples, the order from largest to smallest is HC-08 (*TQSMS* = 0.9036), HC-11 (*TQSMS* = 0.9025), HC-04 (*TQSMS* = 0.9020), HC-03 (*TQSMS* = 0.8982), HC-02 (*TQSMS* = 0.8972), HC-05 (*TQSMS* = 0.8913), HC-06 (*TQSMS* = 0.8887), HC-15 (*TQSMS* = 0.8878), HC-01 (*TQSMS* = 0.8832), HC-10 (*TQSMS* = 0.8827), HC-09 (*TQSMS* = 0.8591), HC-07 (*TQSMS* = 0.8383), HC-13 (*TQSMS* = 0.8249), HC-14 (*TQSMS* = 0.7703), and HC-12 (*TQSMS* = 0.6510). In other words, samples HC-08, HC-11, and HC-04 have high similarities to other 14 samples. However, samples

Table 1: Retention time, peak area, and relative content of common chemical compounds of volatile oils from 15 batches *Houttuynia cordata*

Common compounds	Retention time (min)	Peak area (μv × s)	Relative content (%)
.alpha.-Pinene	4.48±0.08	1119663.33±378311.68	5.95±3.59
Camphene	4.72±0.09	229216.93±78631.16	1.08±0.57
Bicyclo [3.1.0] hexane, 4-methylene-1-(1-methylethyl)-	5.02±0.09	623831.00±441557.95	4.02±3.17
.beta.-Pinene	5.14±0.09	2125719.73±736129.13	10.93±6.48
.beta.-Myrcene	5.21±0.10	6887956.27±11069992.24	11.46±8.37
2-Undecanone	11.91±0.11	5535069.47±3497279.81	21.22±7.95
n-Decanoic acid	14.17±0.10	704160.20±521870.84	2.86±2.14
2-Tridecanone	18.41±0.34	2442806.20±4446977.96	3.22±3.26
n-Hexadecanoic acid	35.67±0.17	367685.00±643569.86	0.73±0.39

Table 2: Relative content (%) of common chemical compounds of volatile oils from *Houttuynia cordata* of different samples

Common compounds	HC-01	HC-02	HC-03	HC-04	HC-05	HC-06	HC-07	HC-08	HC-09	HC-10	HC-11	HC-12	HC-13	HC-14	HC-15
.alpha.-Pinene	10.46	8.76	8.69	7.55	7.88	7.65	7.03	7.53	9.64	2.04	7.96	0.37	0.89	0.74	2.08
Camphene	1.86	1.44	1.44	1.35	1.41	1.39	1.17	1.25	1.81	0.56	1.32	0.13	0.27	0.24	0.58
Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-	7.99	8.09	7.21	5.49	6.79	2.36	5.78	5.96	3.96	0.32	5.93	0.02	0.06	0.05	0.32
.beta.-Pinene	17.90	15.25	15.38	14.75	14.25	14.04	13.57	14.98	17.85	3.48	14.37	0.83	1.90	1.63	3.53
.beta.-Myrcene	7.30	5.84	5.87	6.01	5.55	7.13	5.61	6.12	8.73	24.91	5.41	9.96	27.00	21.20	25.24
2-Undecanone	23.94	27.09	26.46	26.94	26.21	25.04	29.88	26.93	23.15	16.48	26.33	4.24	9.51	9.57	16.61
n-Decanoic acid	2.27	5.04	5.85	0.57	4.56	1.25	6.62	2.82	0.71	2.63	5.54	0.53	0.75	1.18	2.65
2-Tridecanone	0.76	1.15	0.93	1.34	1.12	1.44	1.12	1.56	1.00	8.18	0.79	3.98	7.91	8.80	8.27
n-Hexadecanoic acid	1.65	0.48	0.27	0.56	0.77	1.35	0.36	0.78	0.26	0.83	0.56	0.68	0.43	1.06	0.85
Total content	74.13	73.14	72.1	64.56	68.54	61.65	71.14	67.93	67.11	59.43	68.21	20.74	48.72	44.47	60.13

Table 3: Total quantum statistical moment parameters of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

Batch	Peak number	AUC_T (μv × s)	$MCRT_T$ (min)	$VCRT_T$ (min^2)
HC-01	49	1.159×10^7	10.395	41.854
HC-02	68	1.267×10^7	11.298	48.181
HC-03	81	1.315×10^7	10.644	45.916
HC-04	112	1.701×10^7	10.671	48.056
HC-05	134	1.530×10^7	10.18	50.711
HC-06	137	1.716×10^7	10.322	57.294
HC-07	86	2.069×10^7	9.410	37.549
HC-08	78	1.476×10^7	10.746	49.822
HC-09	130	2.015×10^7	9.778	39.321
HC-10	125	2.284×10^7	12.038	60.659
HC-11	73	1.491×10^7	10.986	53.460
HC-12	85	3.437×10^8	18.602	57.459
HC-13	77	7.053×10^7	13.665	77.568
HC-14	65	1.350×10^8	15.297	81.504
HC-15	115	2.253×10^7	11.855	59.177
Mean	94.33	5.013×10^7	11.726	53.902
RSD (%)	30.13	174.56	20.79	23.27

AUC_T: Area Under the Curve of Total Quantum; $MCRT_T$: Mean Retention Time of Total Quantum; $VCRT_T$: Variance of Mean Retention Time of Total Quantum

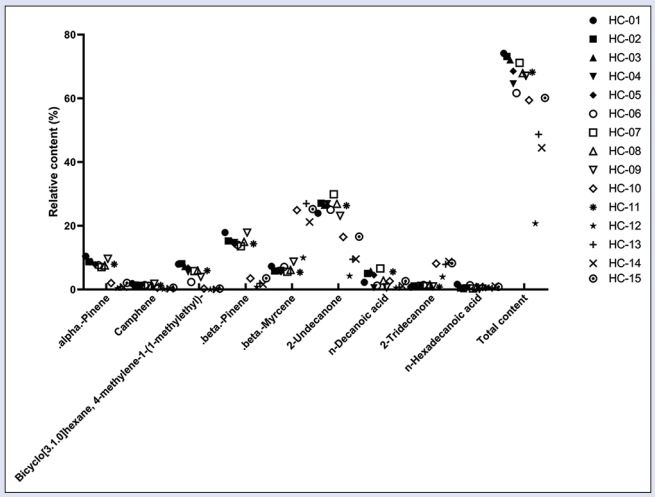

Figure 4: Score plot of relative content of common chemical compositions of volatile oils from 15 batches of *Houttuynia cordata*

HC-12 and HC-14 showed significant differences from that of other 14 samples. These results indicate that the volatile oils in HC varied slightly. The *TQSMS* of samples HC-12 and 14 is different from other samples, probably due to some environmental factors.[31,32] Therefore, the specific reasons for these differences and whether these differences are related to their efficacy need further research.

The included angle cosine and correlation coefficient of these fingerprints are, respectively, listed in Tables 5 and 6, and the corresponding heatmaps are shown in Figures 6 and 7. As outlined in Table 5, the correlation coefficient ranged between 0.3172 (HC-05 to HC-13) and 0.9987 (HC-02 to HC-05). Meanwhile, as outlined in Table 6, the correlation coefficient ranged between 0.1183 (HC-11 to HC-12) and 0.9988 (HC-10 to HC-15). According to the principle of each similarity calculation method, the included angle cosine method and correlation coefficient method cannot accurately reflect the reality of multidimensional vector deviation degree. The two methods are more sensitive to the change of the peaks with a larger response and less sensitive to small peak. Therefore, the aforementioned two methods for fingerprints of herbal medicine with different batches, less characteristic peak, and more fingerprint peak are less effective. The composition and content of herbal medicine are complicated, which will be affected by a series of factors such as variety, origin, and processing conditions. Therefore, the similarity analysis of fingerprint is more important to the analysis of the whole spectrum for quality control of herbal medicine. The *TQSMS* method significantly reduces the requirements on the test method so that its similarity mainly reflects

Table 4: Total quantum statistical moment similarity of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

Batches	HC-01	HC-02	HC-03	HC-04	HC-05	HC-06	HC-07	HC-08	HC-09	HC-10	HC-11	HC-12	HC-13	HC-14	HC-15
HC-01	1.0000	0.9393	0.9740	0.9637	0.9523	0.9241	0.9342	0.9542	0.9595	0.8783	0.9337	0.5556	0.7874	0.7203	0.8887
HC-02	0.9393	1.0000	0.9608	0.9639	0.9358	0.9354	0.8752	0.9679	0.9001	0.9341	0.9705	0.6136	0.8430	0.7769	0.9437
HC-03	0.9740	0.9608	1.0000	0.9889	0.9659	0.9443	0.9137	0.9796	0.9383	0.9035	0.9595	0.5774	0.8119	0.7434	0.9140
HC-04	0.9637	0.9639	0.9889	1.0000	0.9702	0.9543	0.9076	0.9905	0.9311	0.9117	0.9699	0.5835	0.8202	0.7505	0.9223
HC-05	0.9523	0.9358	0.9659	0.9702	1.0000	0.9697	0.9169	0.9680	0.9351	0.8949	0.9543	0.5662	0.8079	0.7358	0.9051
HC-06	0.9241	0.9354	0.9443	0.9543	0.9697	1.0000	0.8882	0.9606	0.9053	0.9103	0.9619	0.5847	0.8280	0.7551	0.9197
HC-07	0.9342	0.8752	0.9137	0.9076	0.9169	0.8882	1.0000	0.8997	0.9747	0.8209	0.8801	0.4973	0.7326	0.6644	0.8313
HC-08	0.9542	0.9679	0.9796	0.9905	0.9680	0.9606	0.8997	1.0000	0.9226	0.9199	0.9793	0.5907	0.8285	0.7582	0.9305
HC-09	0.9595	0.9001	0.9383	0.9311	0.9351	0.9053	0.9747	0.9226	1.0000	0.8437	0.9024	0.5196	0.7542	0.6861	0.8541
HC-10	0.8783	0.9341	0.9035	0.9117	0.8949	0.9103	0.8209	0.9199	0.8437	1.0000	0.9391	0.6691	0.9068	0.8361	0.9893
HC-11	0.9337	0.9705	0.9595	0.9699	0.9543	0.9619	0.8801	0.9793	0.9024	0.9391	1.0000	0.6088	0.8482	0.7769	0.9497
HC-12	0.5556	0.6136	0.5774	0.5835	0.5662	0.5847	0.4973	0.5907	0.5196	0.6691	0.6088	1.0000	0.7564	0.8282	0.6586
HC-13	0.7874	0.8430	0.8119	0.8202	0.8079	0.8280	0.7326	0.8285	0.7542	0.9068	0.8482	0.7564	1.0000	0.9264	0.8964
HC-14	0.7203	0.7769	0.7434	0.7505	0.7358	0.7551	0.6644	0.7582	0.6861	0.8361	0.7769	0.8282	0.9264	1.0000	0.8256
HC-15	0.8887	0.9437	0.9140	0.9223	0.9051	0.9197	0.8313	0.9305	0.8541	0.9893	0.9497	0.6586	0.8964	0.8256	1.0000

Table 5: Correlation coefficient of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

Batches	HC -01	HC-02	HC-03	HC-04	HC-05	HC-06	HC-07	HC-08	HC-09	HC-10	HC-11	HC-12	HC-13	HC-14	HC-15
HC-01	1.0000	0.9816	0.9802	0.9716	0.9789	0.9617	0.9503	0.9761	0.9792	0.5414	0.9732	0.3599	0.3351	0.3498	0.5384
HC-02	0.9816	1.0000	0.9984	0.9808	0.9987	0.9647	0.9896	0.9911	0.9554	0.5549	0.9969	0.3634	0.3291	0.3505	0.5445
HC-03	0.9802	0.9984	1.0000	0.9775	0.9983	0.9679	0.9909	0.9882	0.9582	0.5569	0.9985	0.3642	0.3348	0.3579	0.5436
HC-04	0.9716	0.9808	0.9775	1.0000	0.9840	0.9836	0.9739	0.9951	0.9689	0.5702	0.9811	0.3873	0.3502	0.3842	0.5672
HC-05	0.9789	0.9987	0.9983	0.9840	1.0000	0.9748	0.9931	0.9934	0.9607	0.5685	0.9982	0.3513	0.3172	0.3685	0.5692
HC-06	0.9617	0.9647	0.9679	0.9836	0.9748	1.0000	0.9655	0.9816	0.9832	0.6358	0.9730	0.4558	0.4198	0.4599	0.6309
HC-07	0.9503	0.9896	0.9909	0.9739	0.9931	0.9655	1.0000	0.9852	0.9315	0.5764	0.9954	0.3775	0.3397	0.3800	0.5726
HC-08	0.9761	0.9911	0.9882	0.9951	0.9934	0.9816	0.9852	1.0000	0.9676	0.5712	0.9911	0.3982	0.3531	0.3863	0.5770
HC-09	0.9792	0.9554	0.9582	0.9689	0.9607	0.9832	0.9315	0.9676	1.0000	0.6187	0.9543	0.4667	0.4316	0.4572	0.6179
HC-10	0.5414	0.5549	0.5569	0.5702	0.5685	0.6358	0.5764	0.5712	0.6187	1.0000	0.5503	0.9662	0.9605	0.9732	0.9998
HC-11	0.9732	0.9969	0.9985	0.9811	0.9982	0.9730	0.9954	0.9911	0.9543	0.5503	1.0000	0.3709	0.3356	0.3707	0.5491
HC-12	0.3599	0.3634	0.3642	0.3873	0.3513	0.4558	0.3775	0.3982	0.4667	0.9662	0.3709	1.0000	0.9827	0.9981	0.9685
HC-13	0.3351	0.3291	0.3348	0.3502	0.3172	0.4198	0.3397	0.3531	0.4316	0.9605	0.3356	0.9827	1.0000	0.9832	0.9606
HC-14	0.3498	0.3505	0.3579	0.3842	0.3685	0.4599	0.3800	0.3863	0.4572	0.9732	0.3707	0.9981	0.9832	1.0000	0.9739
HC-15	0.5384	0.5445	0.5436	0.5672	0.5692	0.6309	0.5726	0.5770	0.6179	0.9998	0.5491	0.9685	0.9606	0.9739	1.0000

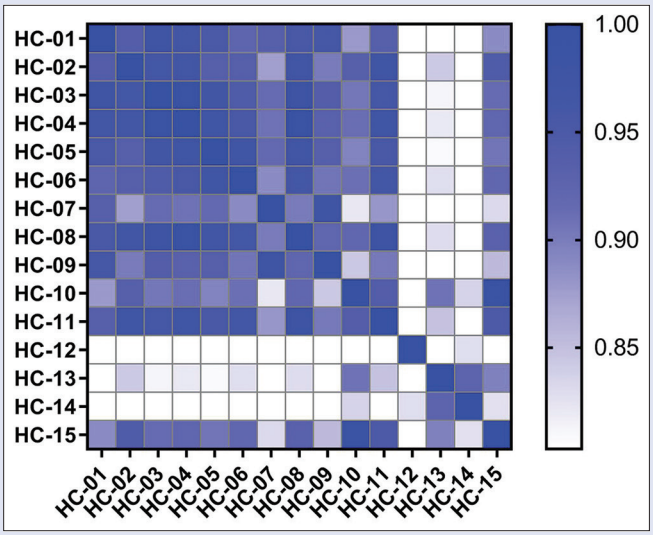

Figure 5: Heatmap of total quantum statistical moment similarity of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

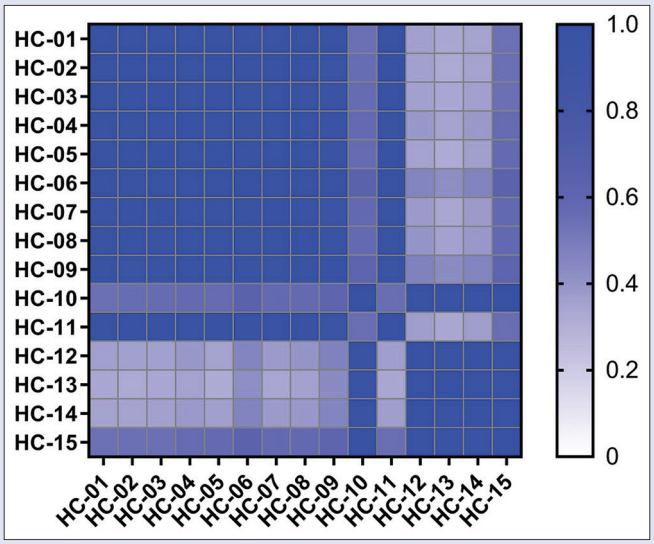

Figure 6: Heatmap of correlation coefficient of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

the similarity degree of the components, which can be used to analyze the fingerprint with no obvious characteristic peak. To sum up, *TQSMS* is an effective method to analyze the chromatographic fingerprints with some major outstanding characteristics, such as reducing the requirement of test method and having a coupling ability to couple with multiple varies to form a multidimensional functional curve and calculate the multi-dimensional *TQSMS* parameters.[27] *TQSMS* model has been elucidated and established according to the *TQSM* parameters

Table 6: Angle cosine of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

Batches	HC-01	HC-02	HC-03	HC-04	HC-05	HC-06	HC-07	HC-08	HC-09	HC-10	HC-11	HC-12	HC-13	HC-14	HC-15
HC-01	1.0000	0.9838	0.9822	0.9765	0.9730	0.9692	0.9502	0.9811	0.9783	0.5990	0.9618	0.1226	0.4060	0.4207	0.6005
HC-02	0.9838	1.0000	0.9966	0.9826	0.9890	0.9688	0.9812	0.9902	0.9598	0.6022	0.9796	0.1201	0.3913	0.4145	0.6033
HC-03	0.9822	0.9966	1.0000	0.9804	0.9879	0.9690	0.9806	0.9882	0.9595	0.6010	0.9797	0.1195	0.3907	0.4131	0.6025
HC-04	0.9765	0.9826	0.9804	1.0000	0.9757	0.9816	0.9662	0.9942	0.9662	0.6091	0.9665	0.1231	0.4011	0.4228	0.6105
HC-05	0.9730	0.9890	0.9879	0.9757	1.0000	0.9657	0.9813	0.9831	0.9595	0.6051	0.9788	0.1206	0.3933	0.4172	0.6046
HC-06	0.9692	0.9688	0.9690	0.9816	0.9657	1.0000	0.9595	0.9823	0.9785	0.6496	0.9572	0.1319	0.4373	0.4558	0.6513
HC-07	0.9502	0.9812	0.9806	0.9662	0.9813	0.9595	1.0000	0.9761	0.9435	0.6148	0.9865	0.1209	0.3924	0.4196	0.6117
HC-08	0.9811	0.9902	0.9882	0.9942	0.9831	0.9823	0.9761	1.0000	0.9689	0.6175	0.9748	0.1248	0.4058	0.4290	0.6185
HC-09	0.9783	0.9598	0.9595	0.9662	0.9595	0.9785	0.9435	0.9689	1.0000	0.6391	0.9538	0.1315	0.4429	0.4524	0.6379
HC-10	0.5990	0.6022	0.6010	0.6091	0.6051	0.6496	0.6148	0.6175	0.6391	1.0000	0.6004	0.2602	0.9149	0.8841	0.9988
HC-11	0.9618	0.9796	0.9797	0.9665	0.9788	0.9572	0.9865	0.9748	0.9538	0.6004	1.0000	0.1183	0.3909	0.4246	0.5973
HC-12	0.1226	0.1201	0.1195	0.1231	0.1206	0.1319	0.1209	0.1248	0.1315	0.2602	0.1183	1.0000	0.2902	0.2919	0.2602
HC-13	0.4060	0.3913	0.3907	0.4011	0.3933	0.4373	0.3924	0.4058	0.4429	0.9149	0.3909	0.2902	1.0000	0.9809	0.9155
HC-14	0.4207	0.4145	0.4131	0.4228	0.4172	0.4558	0.4196	0.4290	0.4524	0.8841	0.4246	0.2919	0.9809	1.0000	0.8844
HC-15	0.6005	0.6033	0.6025	0.6105	0.6046	0.6513	0.6117	0.6185	0.6379	0.9988	0.5973	0.2602	0.9155	0.8844	1.0000

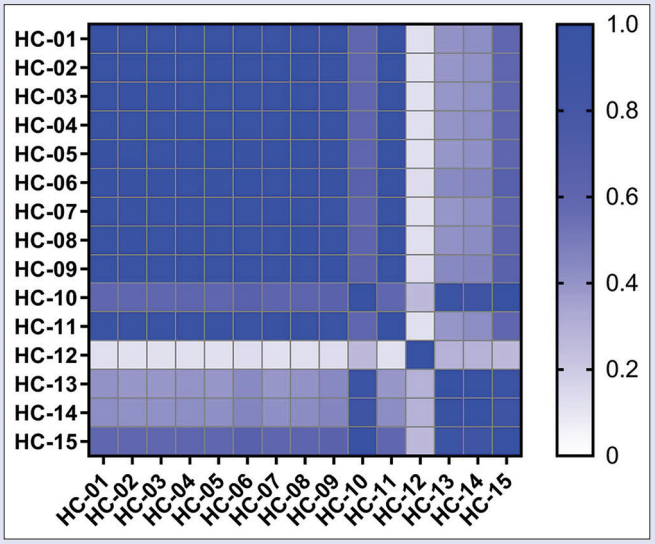

Figure 7: Heatmap of included angle cosine of gas chromatography fingerprints of 15 batches of *Houttuynia cordata* volatile oil

and normal distribution probability density function properties. Thus, we can quantitative analysis the correct probability to make positive and negative conclusions regardless of the origin of the samples with any confident coefficient α.

CONCLUSION

Using statistical moment theories, the *TQSMS* was applied to analyze chromatographic fingerprints in this study. The volatile oil composition and content of HC have strong fluctuations. As a result, its quality control cannot be considered solely from the variety and content of its components. The *TQSMS* of chromatographic fingerprints of the 15 batches of HC volatile oil ranged from 0.4973 to 0.9905. Except for samples HC-12 to HC-14, the 13 other samples were found to be highly similar to each other, whereas samples HC-12 and 14 were found to be significantly different from others samples. Due to the simplicity of the *TQSMS* similarity method, it can be easily adopted and applied. Thus, *TQSMS* can be an additional method applied in the assessment of the similarity of two chromatographic fingerprints of herbal medicine or other complex systems with multiple components.

Acknowledgements

The authors are thankful to the help from colleagues of Hunan Key Laboratory of Druggability and Preparation Modification for Traditional Chinese Medicine, Yunfeng Lu research group of University of California Los Angeles, and China Scholarship.

Financial support and sponsorship

National Natural Science Foundation of China (Grant No. 81903759, 81874507, 81703824, 81573691, 81803729), the Natural Science Foundation of Hunan Province (Grant No. 2017JJ3236), the Youth Foundation of Hunan Province Department of Education (Grant No. 17B200), The First-Class Discipline of Pharmaceutical Science of Hunan (Grant No. 2018XY09), Changsha Science and Technology Bureau project (Grant No. kq2004059), the Open Fund of Hunan Key Laboratory of Druggability and Preparation Modification for Traditional Chinese Medicine supported the study.

Conflicts of interest

There are no conflicts of interest.

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