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# Evaluation of the quality consistency of powdered poppy capsule extractive by an averagely linear-quantified fingerprint method in combination with antioxidant activities and two compounds analyses

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A novel averagely linear-quantified fingerprint method was proposed and successfully applied to monitor the quality consistency of alkaloids in powdered poppy capsule extractive. Averagely linear-quantified fingerprint method provided accurate qualitative and quantitative similarities for chromatographic fingerprints of Chinese herbal medicines. The stability and operability of the averagely linear-quantified fingerprint method were verified by the parameter *r*. The average linear qualitative similarity $S_L$ (improved based on conventional qualitative "Similarity") was used as a qualitative criterion in the averagely linear-quantified fingerprint method, and the average linear quantitative similarity $P_L$ was introduced as a quantitative one. $P_L$ was able to identify the difference in the content of all the chemical components. In addition, $P_L$ was found to be highly correlated to the contents of two alkaloid compounds (morphine and codeine). A simple flow injection analysis was developed for the determination of antioxidant capacity in Chinese Herbal Medicines, which was based on the scavenging of 2,2-diphenyl-1-picrylhydrazyl radical by antioxidants. The fingerprint–efficacy relationship linking chromatographic fingerprints and antioxidant activities was investigated utilizing orthogonal projection to latent structures method, which provided important pharmacodynamic information for Chinese herbal medicines quality control. In summary, quantitative fingerprinting based on averagely linear-quantified fingerprint method can be applied for monitoring the quality consistency of Chinese herbal medicines, and the constructed orthogonal projection to latent structures model is particularly suitable for investigating the fingerprint–efficacy relationship.

**KEYWORDS**

antioxidant capacity, averagely linear-quantified fingerprint, multivariate statistical analyses, principle component analysis, traditional Chinese medicine

Abbreviations: ALQFM, averagely linear-quantified fingerprint method; ASAE, ascorbic acid equivalent; CHM, Chinese herbal medicines; CON, codeine; CV-ANOVA, analysis of variance of the cross-validated residuals; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FIA, flow injection analysis; IPC, ion-pair chromatography; MPE, morphine; OPLS, orthogonal projection to latent structures; PCA, principle component analysis; PPCE, powdered poppy capsule extractive; PTFE, polytetrafluoroethylene; RA, relative peak area; RFP, reference fingerprint; RT, relative retention time; SFP, sample fingerprint; VIP, variable influence on projection statistics

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## 1 | INTRODUCTION

Powdered poppy capsule extractive (PPCE) is partially dried latex obtained from *Papaver somniferum* L., known as Apian Fen in China. PPCE and their derivatives are the most effective analgesics available in clinical treatment, even though there are some side effects, such as addiction, drug resistance, and respiratory depression, and some of these compounds are also frequently abused as illicit drugs [1]. Modern research has shown that PPCE acts by modulating pain signaling pathways in the central nervous system. Varieties of pharmacological effects of PPCE have been found according to the previous studies, such as sedation, anti-tussive, and anti-diarrheal [2–4]. Among the chemical components of PPCE, isoquinoline alkaloids such as morphine (MPE) and codeine (CON) have been demonstrated to be the main and dominant active compounds [5,6]. Now PPCE has been documented in the present Chinese Pharmacopoeia (2015) as a chemical medicine, and the quantitative standard only involves MPE. Other reports concerning PPCE have been confined to the quantification of a limited number of bioactive components [7,8]. However, as a special chemical medicine characterized by complex components, the extraordinarily complex systems pose significant challenges in controlling their quality and finding out the mechanism of action [9,10]. Chromatographic fingerprint has been internationally acknowledged as a powerful and efficient method to determine the quality consistency of complex multicomponent medicine [11,12]. Therefore, chromatographic fingerprinting analysis of Chinese herbal medicines (CHM) represents a comprehensive approach for the purpose of the QC, and ensuring the consistency of CHM and their related products [13].

Current chromatography fingerprinting methods, especially HPLC, is preferred for QC due to its high sensitivity, reproducibility, and accessibility [14,15]. To investigate the interaction of two oppositely charged ions to form a neutral compound, the earliest ion-pair chromatography (IPC) was first introduced into LC by Schill et al. in 1960s [16]. For basic or amine compounds, various alkyl sulfonates are typically used as ion pair reagents. The separation of IPC is achieved by the difference in partition coefficients between the stationary phase and the mobile phase [17]. IPC also has an advantage over conventional LC, owing to the powerful separation capability for both charged and neutral or highly hydrophobic compounds [18,19].

The conventional chromatographic fingerprint analyses are mostly based on the qualitative similarity of the fingerprints, and often ignore the quantitative assessment [20–22]. For example, Li et al. adopted a qualitative hierarchial cluster analysis method to estimate the relative composition of steroidal alkaloids and analyze the fingerprints of Bulbus Fritillariae, and also monitored the content of alkaloids for the purpose of QC [23]. The capability of chromatographic fingerprinting in QC of CHM has been verified, nevertheless, multicomponent quantification is not credible in situations where some components are uncertain. But this is not the case currently. In the present work, the averagely linear-quantified fingerprint method (ALQFM) could achieve appraise the fingerprints from averagely linear qualitative and averagely quantitative similarities aspects, which was firstly developed and successfully applied to address the issue of qualitative and quantitative comparison of the reference standards and PPCE samples.

As is well known, antioxidants can decrease the risk of cardiovascular disease, cancer, and ageing, which are correlated with the damaging effects of free radicals [24–26]. Moreover, some reports present that isoquinoline alkaloids had the ability to scavenge free radicals in vitro [27,28], other reports suggest that antioxidants can alleviate pain and inflammation [29,30]. These information encourages us to investigate PPCE antioxidant activities and correlate them with chromatographic fingerprints. However, the batch 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay has disadvantages such as time consuming, tedious, high consumption, and strict adherence to reaction time. Flow-based methods, including injection analysis (FIA), sequential injection analysis, and multisyringe FIA, ideally suited for automated analysis, have been successfully applied for the purposes of fast screening of pesticide and foods [31,32]. According to the best of our investigation, other than the Chinese Pharmacopeia, there is almost no related publication describing the QC of PPCE. An attractive aspect is the use of orthogonal projection to latent structures (OPLS) [33] method to establish predictive model for the antioxidant activity, and the established model exhibited outstanding predictive performance.

## 2 | THEORY [34,35]

The sample fingerprint (SFP) and reference fingerprint (RFP) vectors can be expressed in $\vec{X} = (x_1, x_2, \cdots, x_n)$ and $\vec{Y} = (y_1, y_2, \cdots, y_n)$, where $x_i$ and $y_i$ are the $i$th peak area, respectively. For linear equation $\vec{x} = a + b\vec{y}$, the correlation coefficient $r$ is a basic qualitative similarity parameter as calculated by Eq. (1). To eliminate the dominance effect of large peak, the ratio SFP and ratio RFP vectors are expressed as $\vec{x}' = (\frac{x_1}{y_1}, \frac{x_2}{y_2}, \cdots \frac{x_n}{y_n})$ and $\vec{y}' = (1, 1, \cdots 1)$, respectively. The cosine of angle between $\vec{x}'$ and $\vec{y}'$ is calculated and defined as qualitative ratio similarity ($S'_r$), as shown in Eq. (2). Average linear qualitative similarity ($S_L$), as calculated by Eq. (3), is used as the final qualitative parameter to evaluate the number and distribution of fingerprint contents between SFP and RFP. The quantitative similarity ($P$, Eq. (4)) is the apparent content similarity (R%, Eq. (5)) corrected by the qualitative similarity

($S_F$, Eq. (6)), where R% is the ratio of overall contents and $S_F$ is the cosine of angle between $\vec{x}$ and $\vec{y}$. The slope of linear equation ($b$), as calculated by Eq. (7), can quantitatively compare $\vec{x}$ and $\vec{y}$ after weight-corrected by $m_S$ and $m_R$, where $m_S$ is the weight of each sample and the parameter $m_R$ is the average weight of 19 batches of samples. Similarly, $rb$ ($b$ corrected by $r$) also can be defined as a quantitative parameter. Therefore, average linear quantitative similarity ($P_L$), as calculated by Eq. (8), is defined as the final quantitative parameter to monitor the total content of all fingerprint components in the samples. The fingerprint variation coefficient (α, Eq. (9)), a statistical error, reflects the robustness of the linear model. $S_L$, $P_L$, and α are combined in the ALQFM. Eight grades (Supporting Information Table S1) are classified to assess the quality levels of medicines. The lower grade values, the better quality levels. Generally, it is the best approach to assess a complex fingerprint for the purpose of quality assurance by a combination of the qualitative and quantitative analyses.

$$r = \frac{\sum_{i=1}^{n}\left(x_i - \bar{x}_i\right)\left(y_i - \bar{y}_i\right)}{\sqrt{\sum_{i=1}^{n}\left(x_i - \bar{x}_i\right)^2}\sqrt{\sum_{i=1}^{n}\left(y_i - \bar{y}_i\right)^2}} \tag{1}$$

$$S'_F = \cos\theta' = \frac{\sum_{i=1}^{n}\frac{x_i}{y_i}}{\sqrt{n\sum_{i=1}^{n}\left(\frac{x_i}{y_i}\right)^2}} \tag{2}$$

$$\begin{aligned} S_L &= \frac{1}{2}\left(r + S'_F\right) \\ &= \frac{1}{2}\left(\frac{\sum_{i=1}^{n}\left(x_i - \bar{x}_i\right)\left(y_i - \bar{y}_i\right)}{\sqrt{\sum_{i=1}^{n}\left(x_i - \bar{x}_i\right)^2}\sqrt{\sum_{i=1}^{n}\left(y_i - \bar{y}_i\right)^2}} + \frac{\sum_{i=1}^{n}\frac{x_i}{y_i}}{\sqrt{n\sum_{i=1}^{n}\left(\frac{x_i}{y_i}\right)^2}}\right) \end{aligned} \tag{3}$$

$$P = \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} S_F \times 100\% \tag{4}$$

$$R\% = \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} \times \frac{m_R}{m_S} \times 100\% \tag{5}$$

$$S_F = \cos\theta = \frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2}\sqrt{\sum_{i=1}^{n} y_i^2}} \tag{6}$$

$$b = \frac{n\sum_{i=1}^{n} x_i y_i - \sum_{i=1}^{n} x_i \sum_{i=1}^{n} y_i}{n\sum_{i=1}^{n} y_i^2 - \sum_{i=1}^{n} y_i^2} \times \frac{m_R}{m_S} \times 100\% \tag{7}$$

$$P_L = \frac{1}{2}(rb + P) \tag{8}$$

$$\alpha = \left|\frac{R}{b} - 1\right| \tag{9}$$

## 3 | MATERIALS AND METHODS

### 3.1 | Chemicals and reagents

A total of 19 batches of PPCE samples (S1–S19) were supplied by Qinghai Pharmaceutical (Qinghai, China; Manufacturer A, including S5, S7, S8, S9, S12, S15) and China National Pharmaceutical (Hebei, China; Manufacturer B, including S1~S4, S6, S10, S11, S13, S14, S16~S19), respectively. CON and MPE were acquired from National Institute (Beijing, China) for the Control of Pharmaceutical and Biological Products. The structures of the two marker compounds are shown in Supporting Information Fig. S1. Sodium 1-heptanesulfonate was supplied by Zhongmei Chromatographic (Shandong, China). DPPH was provided by Sigma Chemical (St. Louis, MO, USA). Acetonitrile (HPLC grade), methanol (HPLC grade), and anhydrous ethyl alcohol (HPLC grade) were purchased from Yuwang Chemical Industry (Shandong, China). Phosphoric acid (HPLC grade) was obtained from Kermel Chemical Reagent (Tianjin, China). Deionized water and other reagents were of analytical grade.

### 3.2 | Preparation of sample and standard solutions

Approximately 0.12 g of the PPCE samples was weighed into a 50 mL volumetric flask and dissolved with methanol/water/phosphoric acid (160:40:1, v/v/v), and then ultrasonicated for 20 min at 45°C. CON and MPE were accurately weighed and dissolved in methanol. All solution were filtered through 0.45 μm Millipore filters and stored at 4°C before use. A series of standard ascorbic acid solutions (0.030–0.450 mM) were freshly prepared by the dilution of the ascorbic acid stock solution (4.500 mM). DPPH was dissolved in methanol (0.127 mM) before the experiments and protected from light.

### 3.3 | Instruments and HPLC chromatographic conditions

HPLC chromatographic analysis was performed on an Agilent 1100 HPLC series (Agilent Technology, USA), equipped with a UV-vis DAD, a low pressure mix quaternary pump, an auto sampler and an online degasser. Data acquisition and analysis were carried out using the Agilent ChemStation workstation (Agilent Technology, USA). The CAPCELL PAK C18 MG column (250 × 4.6 mm, 5.0 μm) was used for chromatographic separation. The mobile phase was composed of 5 mM sodium 1-heptanesulfonate and water containing 0.1% phosphoric acid (A) and acetonitrile/anhydrous ethyl alcohol/water containing 3% phosphoric acid (B; 82:10:8, v/v/v). The separation was affected using a linear gradient at 35°C with a flow of 1.0 mL/min as follows: 6–18% B at 0–10 min, 18–33% B at 10–20 min, 33–46% B at 20–32 min, 46–60% B at 32–45 min, 60–78% B at 45–60 min, 78–80% B at 60–65 min. The injection volume was set at 5 μL. Online UV spectra was obtained over a wavelength range of 190–600 nm. The detection wavelength was set at 220 nm.

The linearity was analyzed by a series of the two compounds solutions respectively at six concentration levels. The calibration curves were built by plotting the peak area ($y$) *versus* the concentration ($x$, mg/mL) of the analyzed components. The LOQ and LOD were determined by appropriately diluting the mixed standard solutions. The precision was determined by repeated loading S1 sample solution for six times, consecutively. The stability was assessed by analyzing S1 sample solution after prepared 0, 2, 4, 8, and 12 h. The repeatability was evaluated by analyzing six independently S1 sample solutions.

### 3.4 | Antioxidant activity conditions

The assays of DPPH radical scavenging activity were monitored by UV according to Mrazek et al. [36] with slight modification. Total antioxidant capacity was measured on an Agilent 1100 HPLC series (Agilent Technology, USA), equipped with a UV-vis DAD over the range 190–600 nm. The mobile phase A–B (50:50, v/v) was used as carrier, with FIA being adopted as the analytical principle as shown in Supporting Information Fig. S2. The parameters for separation were set as follows: flow rate 0.4 mL/min, PPCE sample injection volume 10 μL. The DPPH radical scavenging activity took place in a hollow polytetrafluoroethylene (PTFE) pipe (5000 mm × 0.18 mm id from Agilent) at a temperature of 35°C, where DPPH solution was delivered using a separate pump at a flow rate of 0.3 mL/min. Finally, the absorbance of the mixture was measured by a decrease at 517 nm with a UV-vis DAD after reacting in the PTFE pipe. All test samples were performed in triplicates, and the average value was used for the data analysis. Ascorbic acid was used as standard antioxidant. Thus, the antioxidant capacity was reported as ascorbic acid equivalent (ASAE). The linear range was 0.030–0.450 mM ($y = 10168x + 10.273$, $r = 0.9992$).

### 3.5 | Data analysis

Chromatographic fingerprints were assessed by laboratory-developed software (Digitized Evaluation System for Super-Information Characteristics of TCM Chromatographic Fingerprints 4.0; Software certificated NO. 0407573, China). SIMCA 13.0 was applied for data analysis.

## 4 | RESULTS AND DISCUSSION

### 4.1 | Optimization of chromatography conditions

To obtain as efficient separation as possible within a short-analysis time, the extraction solvents, detection wavelengths, the mobile phase and the linear gradient program for PPCE samples were investigated (Supporting Information Fig. S3). Chromatographic fingerprint resolution index *RF* [37] was used as an optimization objective function to optimize the chromatography conditions. The index *RF* reflects the effective fingerprint signal, the degree of resolution, and fingerprint peak spacing. The higher the *RF* value, the better the experimental condition. Supporting Information Fig. S3 shows that the optimized parameters were selected as follows: 80% MeOH (containing 0.5% phosphoric acid) was elected as sample extraction solvent, the optimum HPLC conditions were listed in Section 3.3, mobile phase 4 and linear gradient program 3 were used as mobile phase and linear gradient program for sample analysis, respectively. Particularly, the ion pair reagent could improve the retention of the analyte on the column and the separation between the analytes significantly. Then, combined with the maximum absorption wavelength of the analysts, the 3D-spectra, and the results of *RF* values, the detection wavelengths were determined at 220 nm.

### 4.2 | Determination of the two main compounds

#### 4.2.1 | Method validation of the determination

To support the quantitative analysis of the two compounds, the quantitative analysis method was validated for linearity, LOD, LOQ, repeatability, precision, stability, and accuracy, results were shown in Supporting Information Table S2. The correlation coefficients ($r$) were all above 0.9991, demonstrating that the linear correlations of the two compounds were excellent between the peak area and concentration over the linear ranges. According to the methodology validation experiments for fingerprint analysis, repeatability, precision, and stability

for quantitative analysis were calculated by the peak areas of the two main compounds, and the corresponding RSD values were found not to exceed 1.61, 1.16, and 1.55%, respectively. The accuracy of the quantitative analysis was evaluated by recovery using the standard addition method, and the mean recovery of the two main compounds was between 100.40 and 101.34%. The validation results demonstrated that the determination method was reasonable and acceptable to meet quantitative requirements, and was very suitable for the simultaneous quantitative analysis of the two main compounds in PPCE.

### 4.2.2 | Sample analysis

The content of MPE and CON in PPCE samples was determined using the established calibration curves (Supporting Information Table S2). From the contents (mg/g) of the two main compounds presented in Table 1, some variation in the content of MPE and CON was observed in all the PPCE samples in the ranges of 19.2197–33.1519 mg/g for MPE and 15.5974–23.1516 mg/g for CON, respectively. The possible explanation for the variation of the contents could be the difference quality of raw materials variability caused by complicated factors or the variability in manufacturing processes.

To evaluate the differentiating ability of the two compounds, the contents of the two marker compounds and the total amount (SUM) in Table 1 were adopted as the input data, and principle component analysis (PCA) was carried out to construct a 2D matrix (19 × 3) with 19 observations and three variables. A two-component PCA model was obtained with variance of 97.20 and 2.76% in PC1 and PC2, respectively, shown at the bottom of the loading scatter plot (Fig. 1A). Additionally, all three variables had the greatest correlativity with PC1, while CON had positively loading on PC2 significantly and MPE and SUM were negative correlated to PC2.

The PCA score scatter plot (Fig. 1B) shown that 19 samples were distinctly grouped into two clusters marked as Group 1 and Group 2, respectively. The samples in Group 1 (S5, S7, S8, S9, S12, and S15) all had negative correction on PC1, and the content of the two compounds (in the ranges of 19.2197–28.2731 mg/g for MPE, 15.5974–19.2267 mg/g for CON and 72.0–96.9 mg/g for SUM, respectively) were all lower than those in group 2. Similarly, the samples in Group 2 (S1∼S4, S6, S10, S11, S13, S14, and S16∼S19) exhibited positive values on PC1, and had relatively higher content of the two main compounds (in the ranges of 29.1657–33.1519 mg/g for MPE, 20.6558–23.1518 mg/g for CON and 105.1–115.1 mg/g for SUM, respectively). Consequently, the difference between the two groups was mainly attributed to the contents of the two compounds. Therefore, we could conclude that the established two-component PCA model shows good discriminating

**TABLE 1** Overview of the contents of two main compounds and the evaluation results assessed by ALQFM

| | Content (mg·g$^{-1}$) | | Percent content (%, m/m) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Sample | MPE | CON | MPE | CON | $P_{2C}$% | $S_L$ | $P_L$% | α | Grade | Quality |
| S1 | 31.4905 | 21.4496 | 111.2 | 104.8 | 108.0 | 0.96 | 112.7 | 0.03 | 3 | good |
| S2 | 31.4832 | 20.6558 | 111.2 | 100.9 | 106.1 | 0.97 | 106.4 | 0.05 | 2 | better |
| S3 | 30.6724 | 20.9049 | 108.3 | 102.2 | 105.2 | 0.93 | 105.0 | 0.05 | 2 | better |
| S4 | 31.2740 | 21.5202 | 110.4 | 105.2 | 107.8 | 0.95 | 105.7 | 0.04 | 2 | better |
| S5 | 19.2197 | 15.5974 | 67.9 | 76.2 | 72.0 | 0.89 | 71.7 | 0.14 | 5 | moderate |
| S6 | 30.3905 | 21.0758 | 107.3 | 103.0 | 105.1 | 0.93 | 111.7 | 0.02 | 3 | good |
| S7 | 28.2731 | 19.2267 | 99.8 | 94.0 | 96.9 | 0.90 | 97.1 | 0.09 | 3 | good |
| S8 | 23.2199 | 17.7961 | 82.0 | 87.0 | 84.5 | 0.93 | 86.8 | 0.12 | 3 | good |
| S9 | 22.3365 | 16.7900 | 78.9 | 82.1 | 80.5 | 0.96 | 83.4 | 0.09 | 4 | fine |
| S10 | 29.1657 | 23.0369 | 103.0 | 112.6 | 107.8 | 0.96 | 99.4 | 0.00 | 1 | best |
| S11 | 30.4969 | 21.9153 | 107.7 | 107.1 | 107.4 | 0.96 | 107.8 | 0.02 | 2 | better |
| S12 | 21.8861 | 16.8309 | 77.3 | 82.3 | 79.8 | 0.94 | 77.3 | 0.02 | 5 | moderate |
| S13 | 29.4156 | 22.9135 | 103.9 | 112.0 | 107.9 | 0.96 | 107.5 | 0.01 | 2 | better |
| S14 | 31.3142 | 21.8498 | 110.6 | 106.8 | 108.7 | 0.95 | 113.0 | 0.02 | 3 | good |
| S15 | 21.9428 | 16.3227 | 77.5 | 79.8 | 78.6 | 0.93 | 75.6 | 0.01 | 5 | moderate |
| S16 | 33.1519 | 23.1516 | 117.0 | 113.1 | 115.1 | 0.97 | 116.2 | 0.05 | 4 | fine |
| S17 | 30.8849 | 22.2967 | 109.0 | 109.0 | 109.0 | 0.95 | 102.4 | 0.04 | 2 | better |
| S18 | 30.4878 | 22.7534 | 107.6 | 111.2 | 109.4 | 0.94 | 110.5 | 0.01 | 3 | good |
| S19 | 30.9857 | 22.6949 | 109.4 | 110.9 | 110.2 | 0.96 | 105.2 | 0.05 | 2 | better |
| Mean | 28.3231 | 20.4622 | 100.0 | 100.0 | 100.0 | 0.94 | 99.76 | 0.05 | 2 | better |

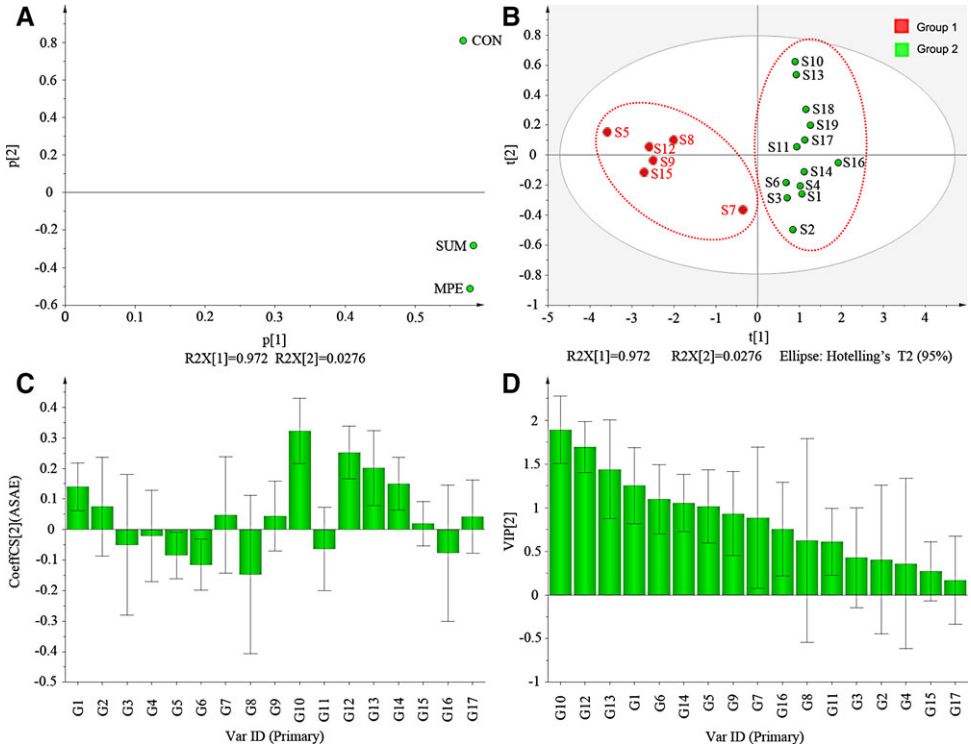


**FIGURE 1** The PCA model and OPLS model for all the PPCE samples. (A) PCA loading scatter plot; (B) PCA scores scatter plot; (C) OPLS coefficient plot; (D) OPLS VIP plot

ability among the 19 PPCE samples, and samples originated from manufacturer A (Group1) were obviously different from those from manufacturer B (Group 2).

## 4.3 | Chromatographic profiling quantifying

### 4.3.1 | Method validation of profiling quantifying

The peak of MPE was selected as the reference peak because of its strong signal absorption, appropriate retention time, as well as suitable separation with the adjacent peaks. Then the relative retention time (RT) and the relative peak area (RA) can be calculated. RSDs of RT and RA of all the samples were less than 1.5% for the precision, stability, and repeatability validation, indicating that the method satisfied the fingerprint analysis criteria for PPCE samples.

### 4.3.2 | Evaluation chromatographic fingerprints by ALQFM

The validated fingerprint method was successfully used to 19 PPCE samples, 17 common peaks were found in all samples at 220 nm, presented in Supporting Information Fig. S1. The sample fingerprints and the reference fingerprint (RFP, constructed as the authentically fingerprint by the mean method) were imported to the in-house software to calculate the evaluating results. As shown in Table 1, all the PPCE samples have average linear qualitative similarity ($S_L$) higher than 0.89 and error term $\alpha \leq 0.14$, indicating that they are similar in the number and distribution of chemical components, and there is little variability between the samples. In contrast, the average linear quantitative similarity ($P_L$) has a wider range (71.7–116.2%) and can exactly discriminate the PPCE samples from every content of fingerprints, but actually $S_L$ and α are disabled for this function. For example, S6 and S9 should be the grade 2 based on the parameters $S_L$ and α. However, they are the higher grades in combination with the average linear quantitative similarity ($P_L$). This result indicated that $P_L$ as a useful discriminating tool to differentiate the PPCE samples, should not be ignored, and it is a perfect combination with $S_L$ and α to evaluate the quality of medicines. Furthermore, $P_L$ as a quantitative method plays an important role in discriminating samples and it has a great potential to be associated with medicinal efficacy in clinics.

In terms of the criteria (shown in Supporting Information Table S1), the quality grade of S10 were best (Grade 1), those of S2, S3, S4, S11, S13, S17, and S19 were better (Grade 2), and those of S1, S6, S7, S8, S14, and S18 were good (Grade 3), and those of S9 and S16 were fine (Grade 4), except for that of S5, S12, and S15 as moderate (Grade 5) due to the much lower contents for the 17 components. Generally speaking, samples with the grade ≤5 were recommended as qualified ones. Therefore, in this study, the qualities of 19 samples were all judged as qualified; the quality consistency of the samples from manufacturer A (S5, S7, S8, S9, S12,

and S15) was worse than manufacturer B (S1∼S4, S6, S10, S11, S13, S14, and S16∼S19). Consequently, the quantitative fingerprint analysis can provide a more accurate, reliable, and feasible evaluation of CHM for the purpose of QC than the PCA method. From the ALQFM results, the products from the same manufacturer had relatively good consistency in quality, but exhibited large differentiation among different manufacturers.

## 4.4 | Correlation between average linear quantitative similarity and two components quantitative analysis

In this study, the two main compounds (MPE and CON) in the PPCE samples were accurately quantified. However, the reference standards, calibration, and more time were required for the quantitative analysis. Unfortunately, for most of CHM, some specific reference standards did not exist and the quantitation is not feasible. Consequently, fingerprint analysis will become a simple and fast method if it is consistent with the results of the quantitative analysis.

To explore the relationship between the average linear quantitative similarity ($P_L$) and the quantitative results of the two main compounds, data analysis processing was shown as follows. Linear regression was constructed using the $P_L$ and $\boldsymbol{P}_{\mathrm{MPE}}$, $\boldsymbol{P}_{\mathrm{CON}}$, the mean value of the content of the two marker compounds ($P_{2C}$) for each sample (Table 1), respectively. From Supporting Information Fig. S4, the correlation coefficients between $P_L$ and MPE, CON are 0.9695 and 0.9144, respectively, which were all above 0.9000 and, especially the correlation coefficient between $P_L$ and $P_{2C}$%, reached a more excellent value of 0.9620. This demonstrated that $P_L$ is basically consistent with the content of the two main compounds and it is very effective in quantitatively assessing the quality of CHM. Consequently, ALQFM, a simple and economic method, has the potential to replace the use of multicomponent quantitative analysis for QC.

## 4.5 | Correlation analysis between HPLC fingerprints and antioxidant activities

### 4.5.1 | Methodology validation of antioxidant activities

Instrumental precision was assessed by the absorbance of a single sample measured at 517 nm following consecutive injection. Repeatability experiment was tested by the absorbance of six independent samples from the same batch. The stability was evaluated by analyzing the PPCE solution stored at room temperature for 36 h. RSD of the precision, repeatability, and stability was 0.1, 0.3, and 0.8%, respectively, indicating the method of antioxidant activities assay was acceptable and reasonable.

### 4.5.2 | The antioxidant activities detection and the relationship with quantitative fingerprint

Under the conditions as described in Section "Antioxidant activity conditions," obtained at the fixed concentration of DPPH negative peak area was proportional to the concentration of DPPH free radical. This property is suitable for the determination of antioxidant activity. To explore the correlation between the HPLC fingerprint and the antioxidant activities, OPLS [38], a well-known multi- and megavariate data analysis method, was applied by taking the negative peak area as the response matrix Y, and relative peak area of the 17 copossessing fingerprints as the descriptor matrix X. The 19 PPCE samples were separated randomly into two groups (Table 2) of the calibration set (14 samples) and validation set (five samples) to established the OPLS model. The scatter plots of observed *versus* predicted values achieved calibration ($R^2$) and validation correlation coefficients ($Q^2$) of 0.9960 and 0.9390, a root mean square error of estimation and a root mean square error of cross-validation value of 0.00215 and 0.0067, respectively, indicating the present model was robust. To assess the performance of the obtained model, the remaining five samples not applied for calibration were used as a validation set. A satisfactory result with a root mean square error of prediction value of 0.0136 was obtained, indicating the predictive model was excellent. The relationships between the predicted *versus* measured antioxidant dates for both the validation and calibration models are shown in Table 2, no significant difference was found for all PPCE samples in calibration and prediction sets.

The standardized regression coefficients plot (Fig. 1C) reveal that many HPLC fingerprint components, such as peaks 1, 2, 7, 9, 10 (MPE), 12 (CON), 13, 14, 15, and 17 appeared to have a positive influence, and the rest peaks displaying negative correlation on the antioxidant activity. Variable influence on projection statistics (VIP) values reflected the overall contribution of each variable to the established model, the larger VIP values (VIP value > 0.5 usually used as a threshold value), the more relevant for variable classification. The identified variable responsible for class separation was the change in MPE (peak 10) and CON (peak 12) concentration, and the rest of the examined variables showed little influence on class separation (Fig. 1D).

### 4.5.3 | The validity testing of OPLS model [39]

To verify the reproducibility of the OPLS model and reduce the overfitting of the training set data, the response permutation testing was performed. The model was subjected to 50 cross-validation (i.e. the sample's dependent variable was randomly replaced by 50 times). When the $R^2$ and $Q^2$ acquired after the response permutation testing is less than obtained by the original data, or the intercept of $Q^2$'s regression line was less than 0, it indicated that the model was not overfilled.

**TABLE 2** **Table 2** The measured and predicted ASAE values of OPLS model

| Sample | S1[a] | S2[a] | S3[a] | S4[a] | S5[a] | S7[a] | S9[a] | S10[a] | S11[a] | S12[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| Measured ASAE (mM) | 0.1447 | 0.1388 | 0.1232 | 0.1347 | 0.0615 | 0.0941 | 0.0775 | 0.1208 | 0.1354 | 0.0803 |
| Predicted ASAE (mM) | 0.1469 | 0.1345 | 0.1235 | 0.1348 | 0.0605 | 0.0964 | 0.0770 | 0.1209 | 0.1363 | 0.0787 |
| RE[c] (%) | −1.53 | 3.20 | −0.25 | −0.06 | 1.63 | −2.34 | 0.64 | −0.02 | −0.68 | 2.02 |
| **Sample** | **S14[a]** | **S15[a]** | **S18[a]** | **S19[a]** | **S6[b]** | **S8[b]** | **S13[b]** | **S16[b]** | **S17[b]** | **Mean** |
| Measured ASAE (mM) | 0.1416 | 0.0762 | 0.1119 | 0.1064 | 0.1297 | 0.0825 | 0.1342 | 0.1367 | 0.1076 | 0.1125 |
| Predicted ASAE (mM) | 0.1415 | 0.0779 | 0.1123 | 0.1059 | 0.1210 | 0.0818 | 0.1227 | 0.1570 | 0.1249 | 0.1134 |
| RE[c] (%) | 0.05 | −2.25 | -0.31 | 0.47 | 7.18 | 0.83 | 9.34 | −12.92 | −13.87 | −0.47 |

[a]Used for the calibration model.
[b]Used for the prediction model.
[c]RE, relative error.

Figure 2 shows that the OPLS model is not overfilled and prove the effectiveness of the model. ANOVA of the cross-validated residuals (CV-ANOVA), applicable to single-Y cases, is a diagnostic tool to determine the reliability of OPLS models. CV-ANOVA result showed $p = 0.0003 < 0.05$, indicating that the model was highly significant.

## 5 | CONCLUSIONS

The present study established a multiprong approach, including quantitative fingerprint evaluation, antioxidant activity assay, and chemometric methods (OPLS) to evaluate the quality consistency of the PPCE samples. ALQFM for PPCE/CHM quality evaluation was firstly recommended. All PPCE samples showed similar average linear qualitative similarity ($S_L$) due to the same chemical composition, and distinctive average linear quantitative similarity ($P_L$) due to the variations in the overall content of the fingerprints. Thus, this assessment method can overcome the one-sidedness of a single qualitative criterion and reflect the accurate quality of CHM. $P_L$ was also shown to be highly correlated to the content of the two marker alkaloids (MPE and CON), indicating that quantitative analysis of multiple marker compounds may be substituted with ALQFM without any chemical standard for QC purpose. The quality of 19 PPCE samples from two manufacturers was well differentiated based on the ALQFM, which can be used to reliably assess the quality of CHM. In addition, FIA, a relatively rapid method and economic system for the determination of antioxidant capacity, was useful in antioxidants screening of large number of samples, and for the QC of antioxidants in CHM. Moreover, OPLS method was successfully applied to investigate the relationship between fingerprints and antioxidant activities. The established model had excellent predictive ability, providing important efficacy information for PPCE QC. Therefore, this multiprong approach is a good additional methodology and holistic analytical strategy to evaluate the quality consistency of PPCE samples.

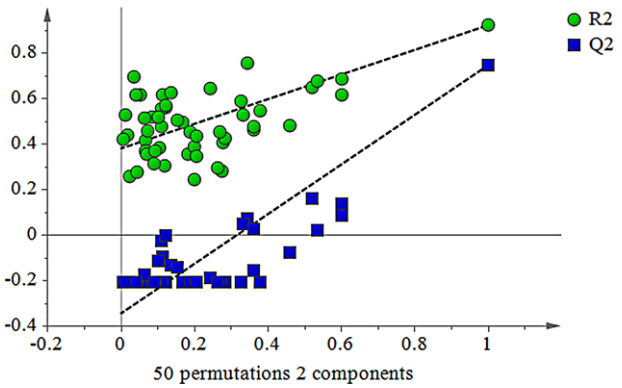


**FIGURE 2** Permutations plot for OPLS model (horizontal ordinate: the correlation coefficient of the dependent variable from original data and the dependent variable from permutation data; vertical coordinate: the values of $R^2$ and $Q^2$ corresponding to each model; the $R^2$ and $Q^2$ at top right corner belong to original data)

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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