Microchemical Journal 186 (2023) 108359

Contents lists available at ScienceDirect

Microchemical Journal

journal homepage: www.elsevier.com/locate/microc

Constructing a "Four in One" fingerprint quality evaluation system of *Cistanche Herba*

Xiang Li [a,*], Ting Yang [b], Hongzhou Bu [c], Huizhi Yang [b], Xinrong Liu [b], Jianhui Wang [d], Guoxiang Sun [b,*]

[a] Shenyang Medical College, Shenyang, Liaoning 110016, PR China
[b] School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, Liaoning 110016, PR China
[c] Drugs Control Centre of Qiqihaer, Qiqiihaer, Heilongjiang 161000, PR China
[d] Zhengzhou Food and Drug Inspection Institute, Zhengzhou, Henan 450000, PR China

ARTICLE INFO

Keywords:
Cistanche Herba
Quality evaluation system
UV
FTIR
DSC
Quantum fingerprint

ABSTRACT

Cistanche Herba, known as "Desert Ginseng", is widely sought after as a medicine and food homology. Owing to the one-sided quality control of several markers, a comprehensive "Four in One" fingerprint quality evaluation system based on HPLC, UV, FTIR and DSC was developed to ensure safety and improve quality. Except for HPLC quantitative analysis of echinacoside (1.41–60.23 mg/g) and acteoside (0.13–14.71 mg/g), UV and FTIR spectral analyses, 12 characteristic parameters of the DSC curve were first developed. Particularly, HPLC fingerprints with UV, FTIR and DSC quantum fingerprints established by merging point method were integrated to evaluate the sample quality with systematically quantified fingerprint method. 45 samples (*Cistanche deserticola*: 26 batches, *Cistanche tubulosa*: 19 batches) were evaluated separately to retain their fingerprint characteristics, whose significant inter batch differences and grade differentiation were manifested. This study can provide a comprehensive, strict and objective evaluation strategy for *Cistanche Herba* and even other samples.

1. Introduction

Cistanche Herba (Rou Cong-Rong in Chinese), bestowed with the honor of being named "Desert Ginseng", is derived from the dried succulent stems with scaly leaves of *Cistanche deserticola* Y. C. Ma (CD) or *Cistanche tubulosa* (Schenk) Wight (CT), belonging to *Cistanche* Hoffmg. et Link in the Orobanchaceae family [1–3]. It has been used for more than 3,000 years, as a superior tonic of medicine and food homology plant [4–6], *Cistanches Herba* is not only used to treat impotence, infertility, waist and knee crymodynia, and constipation induced by blood exhaustion, but also for tonifying the kidney and invigorating the *Yang*, second only to ginseng in rank [7,8]. It has been widely used in various food items and health products [4], such as Chinese JingJiu [9] or cooking *Cistanche Herba* mutton porridge. Due to the high medicinal value and nutritional effects, it is popularly used in traditional Chinese medicine and health care practices. This herb is being officially included in the Chinese Pharmacopoeia 2020 edition (ChP, 2020), and its main active ingredients, i.e. echinacoside (ECH) and acteoside (ACT), are described as "marker components" in the ChP for quality assessment [4]. However, in consideration of the quality of *Cistanche Herba* is affected by factors such as growth conditions, harvest season and processing methods [10], it must be admitted that it is insufficient to monitor the sample quality only through the low limit control of two ingredients, even within the same family [11]. Therefore, it is imperative to find a reasonable and comprehensive quality evaluation method.

For evaluating the quality, authenticity, superiority, and stability of plants and their related products with complex multi-components, fingerprint technology is convenient and has been internationally recognized [12–14]. HPLC, as the representative of chromatographic analysis method, has become the preferred and dominant technology for sample quality evaluation because of its high sensitivity, separation ability, reproducibility and adaptability [15]. It can be used to quantify multiple components as well as give an integral view of all components and display qualitative simplicity among various samples. With the

Abbreviations: ACT, acteoside; CD, *Cistanche deserticola* Y. C. Ma; CT, *Cistanche tubulosa* (Schenk) Wight; DAD, diode array detector; DSC, differential scanning calorimetry; ECH, echinacoside; HCA, hierarchical cluster analysis; ***LOD***, limit of detection; ***LOQ***, limit of quantification; QFP, quantum fingerprint; RFP, reference fingerprint; RPA, relative peak area; RRT, relative retention time; ***RSD***, relative standard deviation; SQFM, systematically quantified fingerprint method.

* Corresponding authors.
E-mail addresses: muxiang6199468@163.com (X. Li), gxswmwys@163.com (G. Sun).

https://doi.org/10.1016/j.microc.2022.108359
Received 10 October 2022; Received in revised form 7 December 2022; Accepted 23 December 2022
Available online 24 December 2022

prosperity and development of spectral technology, UV and IR are widely used in the quality evaluation of medicine and food homologous products, which stems from their advantages of integrity, fast, low loss and environmental protection, and can overcome the shortcomings of chromatographic technology [16,17]. Thus, apart from establishing HPLC quantitative fingerprints, the full wavelength (190–400 nm) UV spectra were collected by applying the flow injection analysis method [18] in this paper. Moreover, the IR spectra within 4000–400 cm^{-1} were scanned by FTIR technology to obtain a large amount of structural information including vibration of saturated bonds.

The widespread application of the above techniques has laid a solid foundation for the fingerprint of medicine and food homologous substances, and achieved the quality evaluation of different samples [11,19–24]. Moreover, the increasing rise of other technologies is playing a role that cannot be ignored, and differential scanning calorimetry (DSC) is emphasized here. This technology measures the difference of thermodynamic properties between the test sample and the reference sample at the programmed temperature [25], which is mainly used for the crystal study of pharmaceutical dispersion and the thermodynamic study of polymers and nanomaterials [26,27]. DSC curve with a large amount of information makes it have the characteristics of a fingerprint, which provides a strong premise for it to be used in sample identification and evaluation [28]. As the DSC fingerprint of *Cistanche Herba* has not been reported, this minimalist sample pretreatment technology was applied to this study to directly collect the characteristic thermograms of samples. Innovatively, 12 characteristic parameters related to the DSC curve were proposed for the first time and used for preliminary monitoring and analyzing the sample quality.

Generally, derivative processing and related partial least squares models for spectral data with continuous signals and redundant information are difficult to be used for overall qualitative and quantitative analysis. Especially for DSC curves with similar shapes but different data points, it is often expressed by a few one-sided enthalpy parameters. Actually, each thermal effect peak of DSC curve of complex sample is the comprehensive contribution of multiple components, which is difficult to belong to specific substances. The conventional fingerprint analysis method is not applicable, so that the comprehensiveness of the sample quality characterized by DSC curve is limited. The proposal of quantum fingerprint (QFP) [29–31] and its effective application and practical feasibility in UV, IR and electrochemical fingerprint data processing not only contribute a novel idea to the quality evaluation of traditional Chinese medicine but also reveal a new entry point for the characteristic data analysis and comprehensive quality evaluation of medicine and food homologous samples. This pretreating technology splits the data points into a defined number of consecutive peaks, which can perform sample analyses thoroughly the systematically quantified fingerprint method (SQFM) reported in the past [11,28].

On the premise of giving consideration to quantitative analysis, in the current study, the omnibearing research idea enabled us to focus on developing a comprehensive “Four in One” quality evaluation system of *Cistanche Herba*; a targeted quality evaluation strategy based on four kinds of analytical techniques was adopted to assess the quality of *Cistanche Herba*, which provided a basis for distinguishing and identifying its species as well as ensuring the quality and sustainable development of production and consumption. More importantly, this exploratory study with comprehensive quality assessment method can lay a solid foundation for the research of pharmacodynamic activity of *Cistanche Herba*.

2. Materials and methods

2.1. Reagents and chemicals

A total of 45 batches of *Cistanche Herba* samples (CD: S1-S26, CT: S27-S45) came from different regions were further authenticated by School of Traditional Chinese Medicine of Shenyang Pharmaceutical University. Echinacoside standard (purity 89.7%) was purchased from National Institutes for Food and Drug Control (Beijing, China), acteoside standard (purity 98.0%) was acquired from Chengdu Alfa Biotechnology Co., ltd. (Sichuan, China). Chromatographic grade acetonitrile and methanol were all obtained from Yuwang Industry Co., ltd. (Shandong, China), and phosphoric acid was supplied by Chengdu Kelong Chemical Reagent Factory (Sichuan, China). Sodium 1-heptane sulfonate was provided by Zhongmei Chromatographic Co., ltd. (Shandong, China). Spectrograde potassium bromide (KBr) was gained from Tianjin Nengpu Science and Technology Co., ltd. (Tianjin, China). Wahaha purified water was used throughout the experiments. All the other reagents and chemicals were of analytical grade.

2.2. Sample preparation

The sticky and soft *Cistanche Herba* samples were dried at 70 °C for 24 h to facilitate crushing into powder and analyzed by multiple techniques after passing through a 40-mesh sieve. Precisely weighed about 0.5 g of sample powder, accurately added 5 mL of methanol/water (50:50, v/v), sealed, weighed, and extracted in an ultrasonic bath (240 W, 40 kHz, JP-020, Shenzhen Jiemeng Cleaning Equipment Co., ltd., China) for 40 min after soaking for 30 min. Subsequently, the samples were cooled to room temperature, weighed again, made up for the weight loss with methanol/water (50:50, v/v), and shaken well. All the extracted sample solutions were filtered through 0.45 μm Millipore filters for HPLC or UV analysis. Appropriate amounts of ECH and ACT standards were weighed and dissolved with methanol/water (50:50, v/v) to make a mixed standard solution, and the concentration of them was about 500 and 100 μg/mL, respectively. All the solutions were stored at 4 °C until analysis.

In FTIR analysis, the sample powder was finely ground with previously dried KBr powder in the proportion of 1/40 (by weight), homogenized in an agate mortar, and then about 100 mg of the mixture was accurately weighed and compressed to a thin, almost transparent KBr wafer by a pressed powder machine (pressure: 15 MPa, time: 2 min). Similarly, the powder was also carefully ground in the agate mortar before collecting DSC curves.

2.3. Apparatus and conditions

2.3.1. HPLC chromatographic analysis

An Agilent Technology 1100 series HPLC instrument equipped with a degasser, a quaternary pump, an auto-sampler, a thermostatic column oven and a diode array detector (DAD) was used for the analysis of samples. The separation was carried out in an Agilent ZORBAX SB-C_{18} column (250 × 4.6 mm, 5.0 μm). The binary mobile phase consisted of water containing 0.2% (v/v) phosphoric acid and 5 mmol/L sodium 1-heptane sulfonate (solvent A) and acetonitrile and methanol (9:1, v/v, solvent B). The system was run 40 min with a gradient elution program: 0–7 min, 96% A to 90% A; 7–20 min, 90% A to 76% A; 20–27 min, 76% A to 70% A; 27–35 min, 70% A to 68% A; and 35–40 min, 68% A to 96% A. The flow rate and column temperature were kept constant at 1.0 mL/min and 35 °C, respectively. The sample injection volume and detection wavelength were set to 5 μL and 280 nm, respectively.

2.3.2. UV and FTIR spectroscopic analyses

In UV analysis, on the basis of HPLC conditions, the main change was the column component, that is, polyetheretherketone (PEEK) tube (5000 mm × 0.12 mm) was used to replace the C_{18} column. This popular flowing injection analysis method [18] is helpful to collect full-wavelength spectral information in less than 2 min to realize the rapid detection of a sample. The UV spectra were detected at 190–400 nm, and the step and slit width were 2 nm. The carrier was methanol/water (50:50, v/v), and 0.2 μL sample solution was injected into the PEEK tube at 35 °C with a flow rate of 0.3 mL/min.

An iCAN9 FTIR spectrometer (Tianjin Nengpu Science and

Technology Co., ltd., China) equipped with a deuterated triglycine sulfate detector was used for FTIR experiments. The KBr wafer was measured directly to record the relevant infrared spectrum in the optimized range from 4000 to 400 cm^{-1} at a spectral resolution of 32 cm^{-1} by accumulating 32 scans.

2.3.3. DSC thermal analysis

An intelligent DSC-500B instrument (Shanghai Yanjin Scientific Instrument Co., ltd., China) was used to collect characteristic DSC thermograms. The precisely weighed sample powder (about 8 mg) was evenly spread in the aluminum crucible (Φ 6.7 mm × 3 mm) and heated from 40 °C to 470 °C at a heating rate of 8 °C/min and maintained at 470 °C for 10 min to obtain a complete DSC curve. An empty aluminum crucible was used as a reference in the experiment.

2.4. Principle of SQFM

SQFM is a method with great potential for qualitative and quantitative evaluation of fingerprint, which is suitable for Chinese herbal medicine and its preparations and has been widely used and accepted. The quantity and distribution of chemical fingerprints were monitored based on the macro-qualitative similarity (S_m) listed in Eq. (1). Meanwhile, the total content of chemical fingerprints was monitored based on the macro-quantitative similarity (P_m) seen in Eq. (2). Under the monitoring of the two parameters, the quality of samples is divided into eight grades displayed in Supplementary Table S1, where grade 1 represents the highest quality. If the values of the two parameters of a sample are not at the same grade, the lower grade will be determined as its final evaluation grade.

$$S_m = \frac{1}{2}(S_F + S_F^{'}) = \frac{1}{2}\left[\frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2}\sqrt{\sum_{i=1}^{n} y_i^2}} + \frac{\sum_{i=1}^{n} \frac{x_i}{y_i}}{\sqrt{n\sum_{i=1}^{n}\left(\frac{x_i}{y_i}\right)^2}}\right] \quad (1)$$

$$P_m = \frac{1}{2}(C + P) = \frac{1}{2}\left[\frac{\sum_{i=1}^{n} x_i y_i}{\sum_{i=1}^{n} y_i^2} + \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} S_F\right] \times 100\% \quad (2)$$

3. Results and discussion

3.1. Optimization of experimental conditions

The slightly modified HPLC analysis conditions without optimization were obtained on the basis of our previous research [32]. The conditions of UV and FTIR analysis mentioned in Section 2.3.2 were obtained on the premise of quickly obtaining perfect and smooth spectra, which will not be described in detail here. Howbeit, to obtain a characteristic and complete DSC curve in an appropriate time, univariate experiments including the following factors were investigated one by one: heating rate, heating program (HP), and sample weight (*w*). Approximately 10 mg of sample (S12) powder was accurately weighed and tested at the heating rates of 8, 15 and 20 °C/min, with characteristic curves recorded for each. As can be seen in Fig. 1Aa, when the sample weight was constant (10 mg), the faster the heating rate, the higher the heat flow of each exothermic peak, and the sharper the peak, but the worse the integrity of the curve. Compared with others, 8 °C/min allowed the sample to be tested in an acceptable 60 min or so was undoubtedly the best option. Subsequently, on the basis of the rate of 8 °C/min, heating

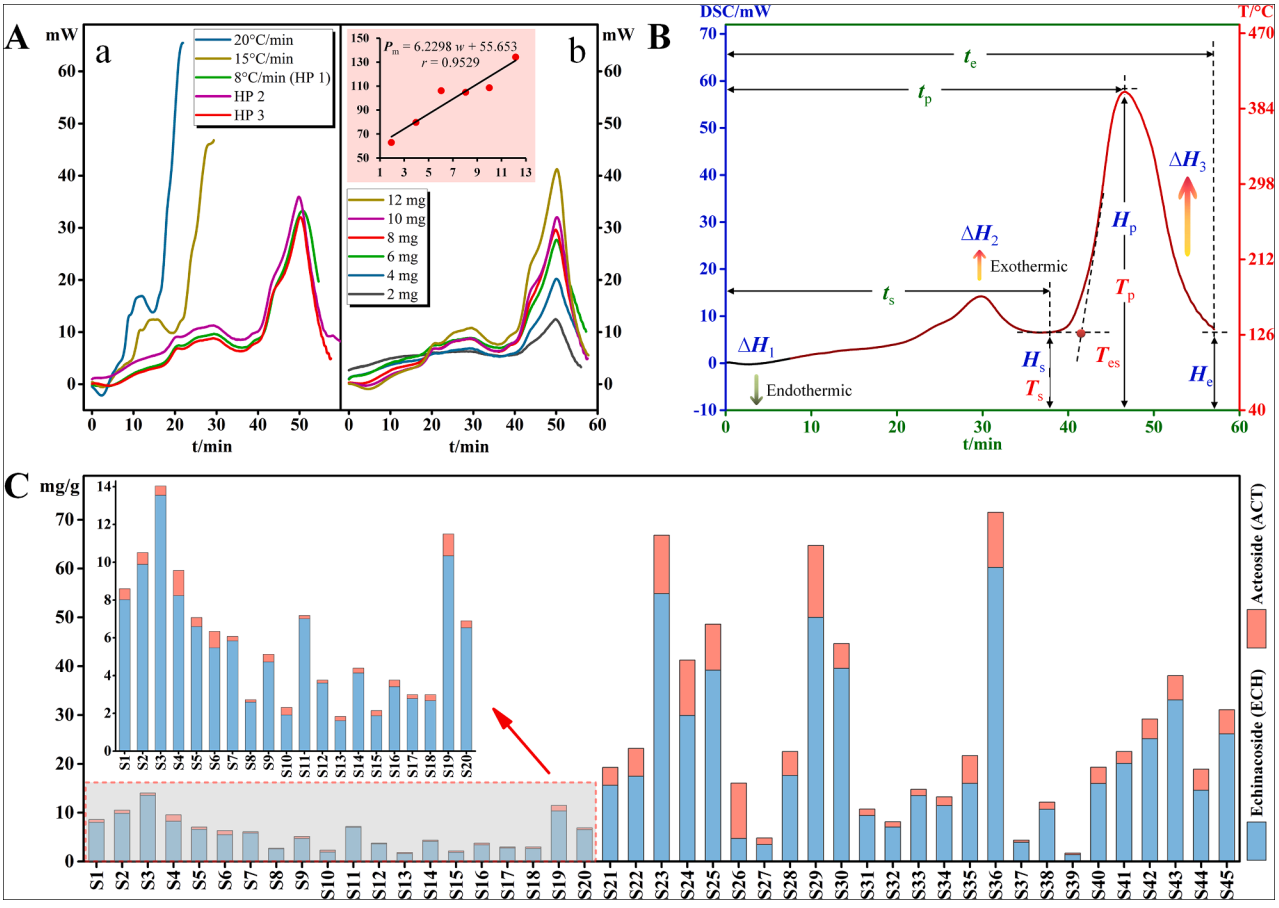

Fig. 1. Optimization of DSC experimental conditions (A): heating rate (8, 15 and 20 °C/min) and heating program (HP1, HP2, HP3) (a) and sample weight (2, 4, 6, 8, 10 and 12 mg) (b), the representative DSC curve (S29) and 12 characteristic parameters (B), the contents (mg/g) of ECH and ACT in 45 batches of samples (C).

program 1 (HP 1: 40 °C $\xrightarrow{8°C/min,\ 57.5\ min}$ 500 °C), heating program 2 (HP 2: 40 °C $\xrightarrow{8°C/min,\ 53.75\ min}$ 470 °C $\xrightarrow{0°C/min,\ 5\ min}$ 470 °C $\xrightarrow{8°C/min,\ 3.75\ min}$ 500 °C) and heating program 3 (HP 3: 40 °C $\xrightarrow{8°C/min,\ 53.75\ min}$ 470 °C $\xrightarrow{0°C/min,\ 10\ min}$ 470 °C) were set and investigated respectively to obtain a more complete DSC curve. As shown in Fig. 1Aa, the DSC curve of HP 1 was incomplete; the curve of HP 2 fluctuated obviously at the end when the temperature rose in the last section (470 °C $\xrightarrow{8°C/min,\ 3.75\ min}$ 500 °C). In contrast, HP 3 obtained a complete and beautiful curve; hence, it was finally selected for subsequent experiments. Besides, samples of different weights (2, 4, 6, 8, 10 and 12 mg) were tested to select the best one. As shown in Fig. 1Ab, the larger the sample weight, the more obvious the exothermic peak. The $\boldsymbol{P}_m$ values of the sample quantum fingerprint were linear with the weight in the range of 2–12 mg, and the regression equation was $\boldsymbol{P}_m = 6.2298\ w + 55.653$ ($r = 0.9529$). Ultimately, 8 mg of sample powder was tested under the condition of heating program 3 because of the suitable detection time and the perfect DSC curve profile.

3.2. Methodology validation

For the validation of the HPLC fingerprint method, S15 was randomly selected and used to investigate the precision, repeatability and stability within 24 h. The relative standard deviations (***RSD***s) were calculated based on the relative retention time (RRT) and relative peak area (RPA) of each common peak of the sample by selecting peak 11-ECH as the reference due to its appropriate peak position, moderate intensity, and good resolution with adjacent peaks. The results showed that the ***RSD***s of RRT and RPA of the above three validation items were less than 0.2% and 4.6% [Excluding peak 3 (6.2%), peak 7 (5.9%), and peak 8 (6.6%) in repeatability test; peak 3 (5.5%) and peak 6 (5.1%) in stability test], respectively. In the validation of two components quantitative analysis method, in addition to the above items, the accuracy determined by the standard addition method and expressed by the recovery, limit of detection (***LOD***, S/N = 3), limit of quantification (***LOQ***, S/N = 10), linearity and range were also verified. For precision, the ***RSD*** values of two marker peak areas were 0.54% (n = 6). For repeatability, the content ***RSD***s of two components in the samples were less than 3.7% (n = 6). The recoveries of ECH and ACT were in the range of 104.9%-108.5% (***RSD*** = 1.43%) and 96.6%-100.4% (***RSD*** = 1.39%), respectively. For stability within 24 h, the peak area ***RSD*** of the two markers was 0.35% and 0.43%, respectively. The mixed standard solution was diluted with methanol/water (50:50, v/v) to get a series of concentrations for establishing the calibration curves. The related regression equations and correlation coefficients were obtained by plotting the scatter diagram and adding the trend line (y, average peak area; x, the concentration of maker). The regression equation of ECH and ACT was $y = 4.0014x + 126.45$, $r = 0.9978$ and $y = 5.6656x - 2.2104$, $r = 1.000$, respectively. The range of linearity of them was 54.94–2197.65 μg/mL and 11.66–466.48 μg/mL. ***LOD***s and ***LOQ***s of ECH and ACT were 0.687 ng, 1.749 ng and 2.747 ng, 6.997 ng, respectively. The above results suggested that the established method is practicable and effective for evaluating the quality of samples qualitatively and quantitatively.

To validate the applicability of the other three fingerprint analysis methods (UV, FTIR and DSC), S12 was stochastically chosen for repeatability test. The ***RSD*** of the $\boldsymbol{P}_m$ values of sample quantum fingerprints was used to monitor whether the repeatability was qualified. The results showed that ***RSD*** were all less than 5.3% in the three methods. Furthermore, the 12 characteristic parameters of DSC curve (Fig. 1B) were extended and proposed for the first time. Taking S12 as an example, the ***RSD*** values of each parameter were calculated and analyzed to test the repeatability, which also laid a foundation for the later DSC fingerprint analysis of samples. As a result, the ***RSD*** values of 12 representative characteristic parameters [enthalpy change of the first endothermic peak (ΔH_1), enthalpy change of the first exothermic peak (ΔH_2), enthalpy change of the second exothermic peak (ΔH_3), and nine relevant parameters of the second exothermic peak including starting point heat flow (H_s), starting point temperature (T_s), epitaxial starting point temperature (T_{es}), starting point time (t_s), top point heat flow (H_p), top point temperature (T_p), top point time (t_p), end point heat flow (H_e), and end point time (t_e)] were 2.16%, 2.28%, 7.05%, 4.67%, 0.97%, 3.08%, 2.59%, 10.71%, 0.79%, 0.27%, 33.28%, and 0.41%, respectively. Accordingly, it is certain that the described fingerprint analysis methods have good repeatability and can be applied.

3.3. Quantitative analysis of samples

3.3.1. Sample analysis

The contents of ECH and ACT double markers in *Cistanche Herba* were assayed by the popular external standard method according to the established HPLC quantitative fingerprint method. With the help of the linear regression equations mentioned in Section 3.2, the contents of two markers of 45 batches of samples were calculated and manifested in Table 1. Except for S26 from Qinghai, the contents of ECH in other samples from Xinjiang were higher than that of ACT, so the origin is considered as the primary factor in this conspicuous difference. Specifically, a wide range fluctuation of the content, i.e. ECH, ACT and the total content of both was within the range of 1.41–60.23 mg/g, 0.13–14.71 mg/g and 1.71–71.53 mg/g, respectively, which exhibited obvious differences between batches and the dominant position of ECH in the total content. The stacked column chart of the two markers content was intuitively shown in Fig. 1C, where the contents of the two markers in most samples of CD were significantly lower than that of CT. Although the dominance of ECH seems to indicate that it can be used as a major marker to characterize the quality of *Cistanche Herba*, in fact, difference of marker content is a very common phenomenon. There are many factors affecting the components of medicine and food homology plants, such as growth environment, species and genera, fertilizer, sowing date, irrigation, plant growth regulating substances, harvesting, processing, storage and maintenance, etc. Although both of them belong to the *Cistanche Herba* genera of Orobanchaceae, the CD is parasitic on the roots of Haloxylon ammodendron, while CT is parasitic on the roots of Tamarix chinensis. Combined with the influence of many other factors, it finally leads to the significant difference of components represented by ECH and ACT. Therefore, in addition to a few markers, most unknown components also need to be monitored to ensure the safety and effectiveness of herbs containing complex components. It is very essential to analyze the characteristics of the fingerprint from a holistic and comprehensive perspective, which can provide more powerful support for the quality evaluation of samples.

3.3.2. Correlation analysis of P_{2C} with similarity parameters.

As verified in the reports [33,34], the strong correlation (Pearson correlation coefficient $r > 0.9$) between macro-quantitative similarity ($\boldsymbol{P}_m$) and the average percentage content of two analytes in each sample ($\boldsymbol{P}_{2C}$, Eq. (3)) as well as the weak correlation ($r < 0.9$) between macro-qualitative similarity ($\boldsymbol{S}_m$) and $\boldsymbol{P}_{2C}$ are the necessary conditions for SQFM to be used for quantitative and qualitative analysis. Thereupon, we also investigated the two relationships based on the evaluation results of HPLC-FP listed in Supplementary Table S2. The results (see Supplementary Table S3) showed that the Pearson correlation coefficient of $\boldsymbol{P}_m$ with $\boldsymbol{P}_{2C}$ was 0.914 with a two-tailed significance test of $p < 0.01$, indicating that $\boldsymbol{P}_m$ can represent the overall component information of samples for quantitative evaluation. Yet, r between $\boldsymbol{S}_m$ and $\boldsymbol{P}_{2C}$ was −0.001 meant there was no direct relationship between them. All these results not only further illustrate that the quality evaluation of complex samples needs to combine qualitative and quantitative analysis, but also suggest that SQFM is a very superior evaluation method that can be selected. In Eq. (3), C_{ECH}, C_{ACT} represents the content of ECH and ACT in each sample, respectively; $\overline{C}_{ECH}$, $\overline{C}_{ACT}$ represents the average content of ECH and ACT in 45 samples, respectively.

Table 1
The sample information and results of the content determination.

No.	Name	Origin	Collection time (MM/YYYY)	Content (mg/g) ECH	ACT	SUM[a]	No.	Name	Origin	Collection time (MM/YYYY)	Content (mg/g) ECH	ACT	SUM
S1	CD	Xinjiang	09/2016	8.02	0.58	8.60	S25	CD	Xinjiang	06/2019	39.18	9.43	48.61
S2	CD	Xinjiang	01/2017	9.89	0.63	10.51	**S26**	CD	Qinghai	05/2019	4.71	11.36	16.06
S3	CD	Xinjiang	04/2017	13.55	0.49	14.03	S27	CT	Xinjiang	12/2020	3.46	1.38	4.84
S4	CD	Xinjiang	02/2010	8.24	1.33	9.57	S28	CT	Xinjiang	09/2017	17.61	4.93	22.54
S5	CD	Xinjiang	02/2019	6.59	0.48	7.07	S29	CT	Xinjiang	05/2019	50.00	14.71	64.72
S6	CD	Xinjiang	09/2019	5.47	0.87	6.34	S30	CT	Xinjiang	08/2020	39.57	5.07	44.64
S7	CD	Xinjiang	09/2019	5.83	0.24	6.07	S31	CT	Xinjiang	04/2021	9.41	1.32	10.73
S8	CD	Xinjiang	12/2019	2.59	0.13	2.72	S32	CT	Xinjiang	01/2020	7.05	1.10	8.15
S9	CD	Xinjiang	11/2020	4.73	0.40	5.13	S33	CT	Xinjiang	05/2021	13.49	1.28	14.77
S10	CD	Xinjiang	04/2019	1.92	0.39	2.31	S34	CT	Xinjiang	04/2021	11.45	1.80	13.25
S11	Jiu CD[c]	Xinjiang	03/2021	7.01	0.17	7.18	S35	CT	Xinjiang	12/2019	16.01	5.68	21.69
S12	CD	Xinjiang	04/2019	3.61	0.15	3.76	S36	CT	Xinjiang	04/2019	60.23	11.31	71.53
S13	CD	Xinjiang	04/2019	1.62	0.22	1.84	S37	CT	Xinjiang	06/2020	3.93	0.46	4.40
S14	CD	Xinjiang	04/2019	4.14	0.26	4.40	S38	CT	Xinjiang	06/2019	10.72	1.45	12.17
S15	CD	Xinjiang	02/2017	1.87	0.28	2.15	S39	CT	Xinjiang	04/2020	1.41	0.30	1.71
S16	Jiu CD	Xinjiang	08/2018	3.42	0.34	3.76	S40	CT	Xinjiang	05/2021	15.97	3.34	19.31
S17	CD	Xinjiang	12/2018	2.78	0.21	2.99	S41	CT	Xinjiang	06/2021	20.10	2.42	22.51
S18	Jiu CD	Xinjiang	01/2019	2.67	0.32	2.99	S42	Jiu CT[c]	Xinjiang	03/2018	25.14	4.04	29.18
S19	Jiu CD	Xinjiang	01/2019	10.34	1.15	11.50	S43	CT	Xinjiang	10/2018	33.09	4.99	38.09
S20	Jiu CD	Xinjiang	02/2019	6.53	0.37	6.90	S44	CT	Xinjiang	10/2018	14.62	4.27	18.90
S21	CD	Xinjiang	04/2019	15.63	3.63	19.25	S45	CT	Xinjiang	06/2019	26.13	4.95	31.08
S22	CD	Xinjiang	08/2018	17.46	5.70	23.16	Min	–	–		1.41	0.13	1.71
S23	CD	Xinjiang	08/2018	54.86	11.99	66.85	Max	–	–		60.23	14.71	71.53
S24	CD	Xinjiang	06/2019	29.91	11.34	41.25	Mean	–	–		14.49	3.05	17.54
							RSD/%	–	–		101.52	127.28	102.98

[a] The total contents of two markers in each sample.
[b,c] Jiu CD/CT represent CD/CT were made with Chinese Baijiu.

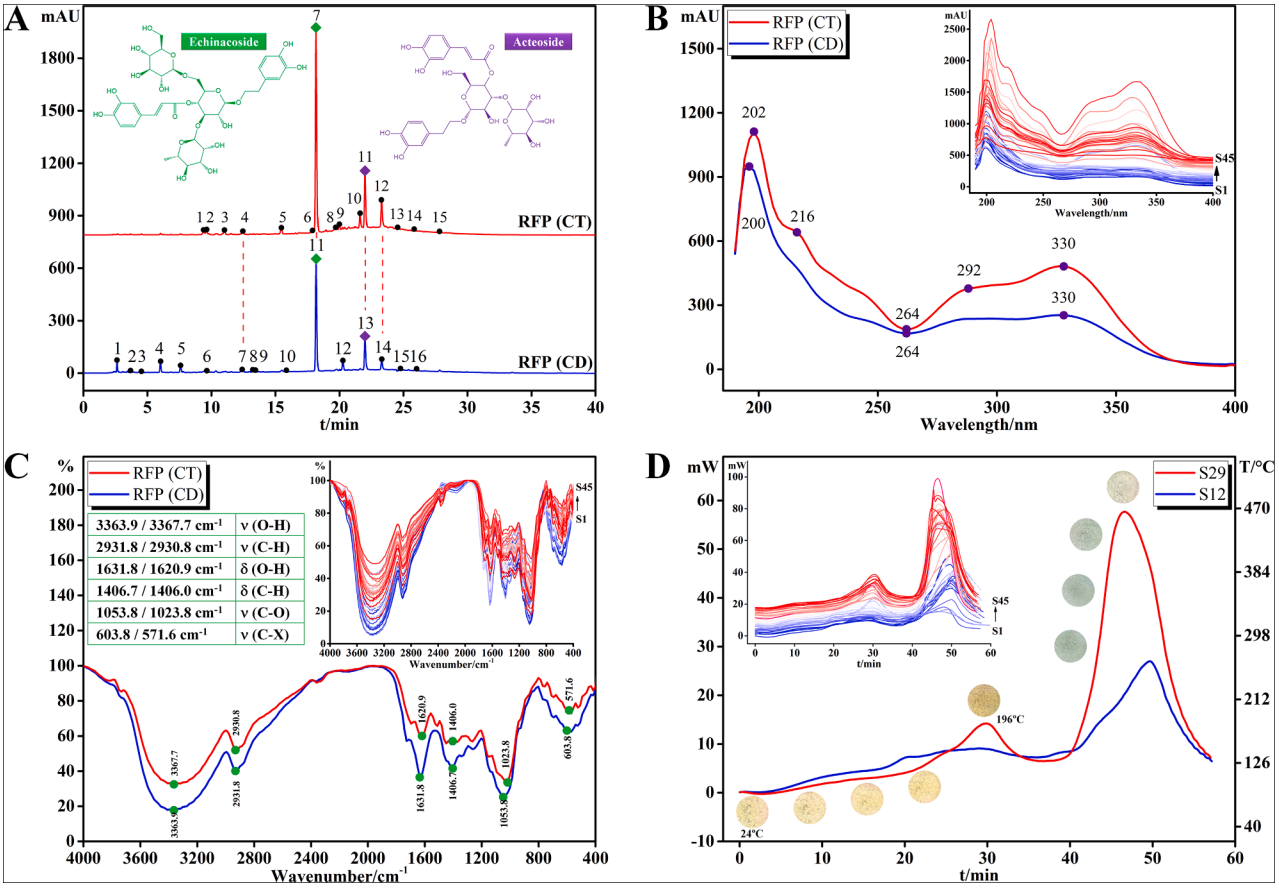

Fig. 2. RFPs of CD and CT and the structures of two markers (A), UV spectra of 45 batches of samples and RFPs of CD and CT (B), RFPs, the total FTIR spectra and peak assignments (C), DSC curves of all samples and representative curves, as well as the appearance changes (rising moon diagram) of S29 at different temperatures (D).

$$\boldsymbol{P}_{2C} = \frac{1}{2}(\boldsymbol{P}_{ECH} + \boldsymbol{P}_{ACT}) \times 100\% = \frac{1}{2}\left(\frac{C_{ECH}}{\overline{C}_{ECH}} + \frac{C_{ACT}}{\overline{C}_{ACT}}\right) \times 100\% \quad (3)$$

3.4. Fingerprint analysis

3.4.1. HPLC fingerprint analysis

CD and CT samples showed unique fingerprints (Supplementary Fig. S1), in which the former had 16 common peaks and the latter had 15, yet only four common peaks between their reference fingerprints (RFPs). As seen in Fig. 2A, RFP (CT) presented greater peak intensity than RFP (CD). To retain the corresponding characteristics, two types of fingerprints were evaluated by SQFM separately, with ECH peak as the reference peak (peak 11 in CD and peak 7 in CT). All the chromatographic integration files were imported into the independently designed professional data evaluation software (TCM Spectrum Quantum Profiling Consistency Digitized Evaluation System 4.0, software certificated NO. 7037415, China) to generate an RFP by the mean method, and then the RFP was used as the standard to calculate the $\boldsymbol{S}_m$ and $\boldsymbol{P}_m$ for evaluating the quality of the samples. The results (Supplementary Table S2) evaluated by SQFM showed that in CD, except S26 ($\boldsymbol{S}_m$ = 0.58), the $\boldsymbol{S}_m$ of other samples was more than 0.80, which indicated that the fingerprint had high similarity and similar chemical components. The particularity of S26 further verified the strong influence of origin on the sample from the perspective of the fingerprint. $\boldsymbol{P}_m$ presented a wide range of coverage, i.e. 24.5%-457.9%, which meant that there were great differences between batches in terms of quantification. In CT, the value of $\boldsymbol{S}_m$ was greater than 0.85, presenting that the qualitative similarities between batches were better than the CD. The narrow range of $\boldsymbol{P}_m$ fluctuation (9.8%-296.1%) also clearly showed the difference between batches. The wide range of $\boldsymbol{P}_m$ is consistent with the wide range of content determination results mentioned above. Concurrently, with the strong assistance of SQFM, the quantitative information of more components including ECH and ACT in the samples was reflected more comprehensively, strictly, truly and reliably. The characteristic $\boldsymbol{S}_m$ and $\boldsymbol{P}_m$ values eventually led to 45 samples covering all grades (1–8) of SQFM, of which only S19 (CD) and S41 (CT) were the best (grade 1) respectively.

3.4.2. UV spectroscopic fingerprint analysis

Consistent with the differences in HPLC fingerprints, the UV spectra of the two kinds of *Cistanche Herba* samples were also different (Fig. 2B). The RFP generated by the average method intuitively showed the difference between the two. RFP (CD) has a main absorption peak at 200 nm, which mainly comes from π-π* electronic transition of aromatic ring; the absorption peaks at 264 nm (valley) and 330 nm are not very obvious. In contrast, except for the similar highest peak (202 nm), RFP (CT) has stronger and obvious characteristic absorption, which is manifested in 216 nm (shoulder), 264 nm (valley), 292 nm and 330 nm. These absorption bands may be attributed to n–π* and π–π* electronic transitions of conjugated systems, such as aromatic rings and C═O in phenylpropanoids or phenylethanol glycosides. The apparent differences between the two UV spectra were inseparable from the result that the content of ECH and ACT in most of the CT samples determined by HPLC was higher than that in CD. Specifically, as the main components subordinate to phenylpropanoids and phenylethanol glycosides, respectively, they contributed more significantly to the UV spectrum than other substances with UV absorption. The high similarity of the UV spectra of the same *Cistanche Herba* is probably due to the comprehensive contribution of a variety of complex chemical components, which perfectly elucidates "seeking common ground while reserving differences".

3.4.3. FTIR spectroscopic fingerprint analysis

FTIR spectra present an overlap of absorption spectra of the complex system [35]. Fig. 2C showed the FTIR spectra of 45 batches of *Cistanche Herba* samples collected at 4000–400 cm^{-1} spectral window. It can be seen that there was no obvious difference in the position of characteristic peaks between the two kinds of *Cistanche Herba* samples except the intensity of transmittance (%), and there were six common characteristic absorption peaks between them. RFP (CD) and RFP (CT) were still selected as representative spectra to compare. Different from the weaker performance in HPLC and UV fingerprints, the transmittance of RFP (CD) is lower than RFP (CT), which indicates the total amount of infrared absorbing substances in the former is higher than that in the latter. As a species living in the desert, its water storage capacity is beyond doubt, which is manifested in the extremely obvious hydrogen-bonded O—H tensile vibration (~3300 cm^{-1}) from water, although all samples have been dried at the low temperature (to prevent excessive loss of components) described in Section 2.2. The second peak of transmittance (~1000 cm^{-1}) was attributed to the C—O stretching vibration of alcohols or phenols, which was well verified in the structures of ECH and ACT in Fig. 2A. The assignments of the other four characteristic spectral peaks were briefly listed in Fig. 2C.

3.4.4. DSC fingerprint analysis

As seen in Fig. 1B, the DSC thermogram of the *Cistanche Herba* contains a tiny endothermic peak and two obvious exothermic peaks. The DSC thermograms of all samples and representative samples (S12 and S29) were shown in Fig. 2D, which presented two kinds of DSC fingerprints visible to the naked eye. To explain the difference more specifically, 12 representative characteristic parameters of DSC fingerprint were displayed in Supplementary Table S4, in which end point heat flow (H_e), and end point time (t_e) presented the maximum and minimum ***RSD*** values respectively, indicating their largest and smallest fluctuations in all batches. In addition, the enthalpy changes (ΔH_1, ΔH_2, ΔH_3) of the three characteristic peaks, as the key parameters to be investigated, and the top point heat flow (H_p) of the second exothermic peak showed extremely significant fluctuations (***RSD*** greater than 39%) mentioned in Fig. 1B, which further manifested the characteristic differences of the samples in DSC thermal fingerprints. Those parameters with ***RSD*** values less than 5.0%, i.e. t_s, T_s, t_p, T_p, and T_{es}, seem to be more acceptable because of their relatively small fluctuations, which meant the little difference among samples. Based on the results of ***RSD*** values of each parameter in the repeatability test (see Section 3.2), nine parameters such as ΔH_1 are suggested to be used to evaluate the quality of samples. The smaller the difference from batch to batch, the better the consistency of DSC fingerprints. Relatively, the three parameters (ΔH_3, H_p and H_e) fluctuated greatly in the repeatability test, hence they are only used to assist in characterizing the DSC curve instead of quality evaluation.

Moreover, as we all know, high temperature will change the state and composition of many substances, especially the high temperature close to 500 °C in this study. To observe the appearance changes of samples at different temperatures, taking S29 as an example, the sample states in the microscope (10 × eyepiece, 2 × objective) at a specific temperature point were photographed and recorded, which were embedded and displayed in Fig. 2D like a "rising moon diagram" (from left to right were the sample states at 24, 52, 70, 97, 196, 291, 357, 435 and 470 °C). 196 °C was the first exothermic peak. Before that, the effect of temperature on the sample states was not obvious, mainly the loss of water; at 196 °C, the sample was accompanied by significant blackening in a large area. Then, the continuous temperature rise made the color of the sample change from black to gray-white, which was gradually ashed; after the second heat release at 470 °C, it was completely ashed. At this time, sugar and cellulose had been pyrolyzed, and organic and inorganic salts dominated these ashes. Not only the state and composition changed significantly, but also the sample quality decreased from the initial 8.19 mg (24 °C) to the final 0.21 mg (470 °C), losing about 97% of the mass.

3.4.5. Establishment and evaluation of UV, FTIR and DSC quantum fingerprints

UV, FTIR and DSC fingerprints have the characteristics of continuous, irregular curves, many data points and so on. Since it cannot be interpreted from a comprehensive perspective, as previously analyzed, reading a series of characteristic parameters seems to be the best analysis method, especially for DSC curves with unique data points for each curve. To retain all the information of each curve to evaluate the samples, based on the feasibility and practicability of the construction of QFPs, UV, FTIR and DSC fingerprints were preprocessed according to the quantization principle (fixed-point merging method) mentioned in our previous research [30]. It should be especially emphasized that the DSC fingerprint of *Cistanche Herba* was quantified for the first time, which is helpful to comprehensively and deeply analyze the thermal characteristics of samples. Considering that different original fingerprints contain different data points (UV/FTIR/DSC: 104/233/more than 13,000 points), for the sake of objectivity, 2% of the total data points were taken as the merging points this time. This meant that the number of the merging points of UV, FTIR and DSC were 2, 5 and 260 points respectively. As a result, the corresponding number of quantum peaks was 33, 37 and 50, as can be seen from Fig. 3A and Supplementary Fig. S2.

Compared with the original curve in Fig. 2B-D, the QFPs maintain the original profile but are more simplified and intuitive for analyzing easily. Happily, these obtained quantum peaks can achieve peak matching, which provides the possibility for the qualitative and quantitative analysis of spectral and thermal fingerprints of complex samples by SQFM. Following the idea of dividing evaluation into two types in HPLC, the evaluation results of the three QFPs of 45 samples can be seen in Supplementary Table S2. According to the comparative analysis, the macro-qualitative similarity $\boldsymbol{S}_{\mathrm{m}}$ values of the three QFPs of 45 batches of samples were in the range of 0.88–1.00, which implied that they had similar chemical composition and proportion respectively. However, in terms of quantification, great volatility was shown in a different and wide range of macro-quantitative similarity $\boldsymbol{P}_{\mathrm{m}}$ values. In 26 batches of CDs, the widest and narrowest $\boldsymbol{P}_{\mathrm{m}}$ ranges were UV (48.4%-258.7%) and FTIR (53.7%-143.0%) respectively, and in 19 batches of CTs, the widest and narrowest ranges were UV (26.1%-237.0%) and DSC (63.8%-111.5%) respectively. Such differences made the grades of all samples mainly dispersed in the range of 1–7 or 8, and only the DSC quantum fingerprints grade of CT samples was within grade 6.

Frankly speaking, the evaluation results of these three QFPs were not exactly similar, even different from those of HPLC, which made us fall into meditation. Common sense, different analytical techniques reflect the characteristics of samples from different angles, so the analytical results obtained from single technology are not the best, but better. Integrity, comprehensiveness and non-destructive are not only the pursuit of sample pretreatment in the analysis process but also the ideal direction of processing the obtained data. Therefore, it has to be said that constructing a variety of analytical techniques into a system to evaluate the quality of samples is the most objective and comprehensive strategy, which is worthy of our unremitting in-depth exploration.

3.4.6. Integrated evaluation by "Four in One" fingerprint system

Integrating the data obtained from various analytical techniques is an irresistible trend in the quality evaluation of food, traditional Chinese medicine and even medicine and food homologous samples, among which the equal weight method is the most common [11,31]. The powerful reason for this is data integration can overcome the shortcomings of a single technology and reflect the information of samples more objectively and comprehensively. Likewise, HPLC fingerprint, UV,

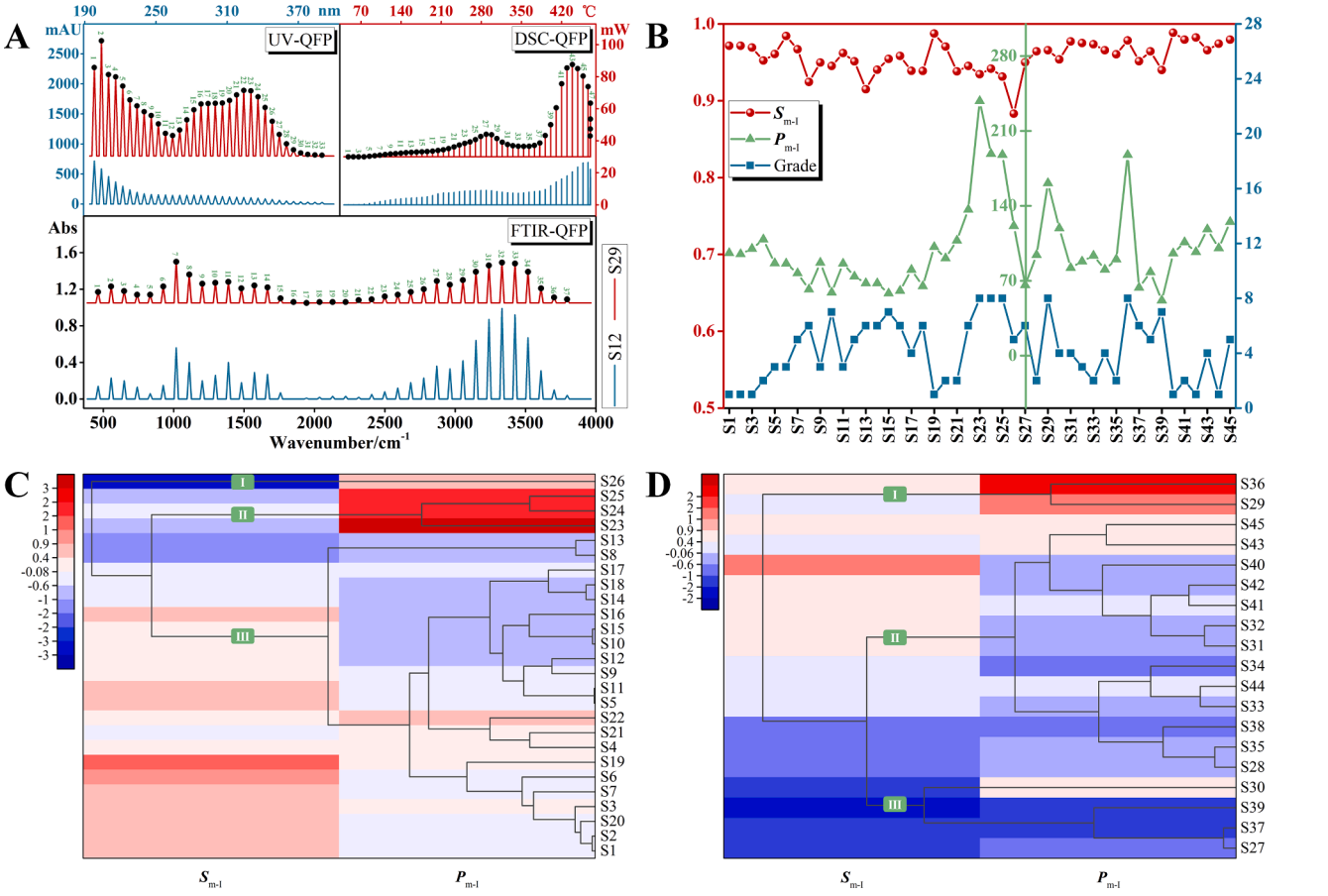

Fig. 3. Representative curves of three types of QFP (A), the integrated evaluation result of the "Four in One" fingerprint system (B), the heat map with dendrogram of CD and CT samples obtained by HCA, respectively (C, D).

FTIR and DSC quantum fingerprints were also integrated in an equal weight in this study, which is elegantly called the "Four in One" fingerprint system. Elaborately, SQFM was first introduced as an evaluation method to assess the quality of *Cistanche Herba* which belongs to medicine and food homology.

Two parameters characterizing the "Four in One" system, integrated qualitative similarity $\boldsymbol{S}_{\text{m-I}}$ and integrated quantitative similarity $\boldsymbol{P}_{\text{m-I}}$, were recorded by calculating the mean values of $\boldsymbol{S}_{\text{m}}$ and $\boldsymbol{P}_{\text{m}}$ corresponding to the above four fingerprints respectively and shown in Supplementary Table S2. The integrated grades of the system were judged according to the newly obtained $\boldsymbol{S}_{\text{m-I}}$ and $\boldsymbol{P}_{\text{m-I}}$ values and Supplementary Table S1. As illustrated in Supplementary Table S2, $\boldsymbol{S}_{\text{m-I}}$ values of 45 samples not less than 0.88 implied all samples had a good qualitative similarity, that is, the chemical composition and distribution proportion are similar between batches. The $\boldsymbol{P}_{\text{m-I}}$ values of CD and CT samples fluctuated in the range of 58.2%-238.0% and 51.7%-187.6% respectively, showing a significant difference in the total amount of compounds in the sample as a whole. To exhibit the evaluation results more intuitively, a multi-*y*-axis graph was plotted (Fig. 3B). Although the $\boldsymbol{P}_{\text{m-I}}$ value fluctuation of CT samples (S27-S45, $\Delta\boldsymbol{P}_{\text{m-I}} = 135.9\%$) is less than that of CD samples (S1-S26, $\Delta\boldsymbol{P}_{\text{m-I}} = 179.8\%$), actually, except for the significantly high $\boldsymbol{P}_{\text{m-I}}$ values of S22-S25 in CD samples and S29 and S36, the fluctuation scope of $\boldsymbol{P}_{\text{m-I}}$ value of other samples is relatively small and easy to accept. Owing to the significant fluctuation of $\boldsymbol{P}_{\text{m-I}}$ values, even if the four fingerprints have been integrated, the grade of the sample did not enter the narrower quality grade range than 1–8.

Considering the particularity of DSC thermal fingerprints in the previous article, here, S12 and S29 were selected as the representatives to compare and analyze the changes of $\boldsymbol{S}_{\text{m}}$, $\boldsymbol{P}_{\text{m}}$ and grade before and after integration. Both of them presented unique parameter values and grades in HPLC fingerprint, UV, FTIR and DSC quantum fingerprints. After integration, the three-parameter values of S12 and S29 were $\boldsymbol{S}_{\text{m-I}} = 0.95$, $\boldsymbol{P}_{\text{m-I}} = 74.0\%$, grade = 5 and $\boldsymbol{S}_{\text{m-I}} = 0.97$, $\boldsymbol{P}_{\text{m-I}} = 161.20\%$, grade = 8, respectively. Although the grade of S12 was evaluated as grade 8 in HPLC fingerprint, the integrated grade was 5. For S29, the grade of FTIR and DSC quantum fingerprints was all 2, but it was grade 8 after integration. These results indicate that the one-sidedness and limitation of single technology are common, and further prove the necessity of integration.

Hierarchical cluster analysis (HCA) has been widely used in the detection and quantitative classification of samples [36]. It can group variables or objects into a dendrogram based on potential similarity [37]. To verify the accuracy of "Four in One" fingerprint quality evaluation system, herein, the two parameter values ($\boldsymbol{S}_{\text{m-I}}$, $\boldsymbol{P}_{\text{m-I}}$) of 45 samples were normalized by Z Scores (standardize to N (0,1)), and the cluster dendrograms were depicted based on Euclidean distance with group average method for measuring the dissimilarity. The HCA results were shown in Fig. 3C, D. The 26 batches of CD samples could be divided into three main groups: S26 was clustered into group I, S23, S24, and S25 were clustered into group II, and other samples were group III. The 19 batches of CT samples could also be clustered into three main groups: S29 and S36 were group I, S27, S30, S37, and S39 were group III, and other samples were group II. The results of HCA were similar to those of SQFM, but not completely consistent. In Fig. 3C, the particularity of S26 was visually presented, and S23, S24, and S25 (grade = 8) was also distinguished. However, although S29 and S36 (grade = 8) were well distinguished, S30 (grade = 4) was divided into the same category as S27 (grade = 6), S37 (grade = 6), and S39 (grade = 7) (Fig. 3D). In contrast, SQFM, which divided samples into different grades, is more conducive to the quality evaluation of samples than HCA of simple grouping or clustering. Obviously, the results of the former were more intuitive and accurate. On balance, from the three perspectives of chromatography, spectroscopy and thermal analysis, the four fingerprints were integrated, which not only retains the popular equal weight fusion way but also innovatively starts with the strict quality evaluation method of traditional Chinese medicine and introduces SQFM. This will open a precedent for the systematic quality evaluation of medicine and food homologous samples.

4. Conclusion

In this study, taking comprehensive evaluation as the core and HPLC quantitative analysis as the basis, a solid "Four in One" fingerprint evaluation system was innovatively constructed to evaluate the quality of two kinds of *Cistanche Herba* samples. SQFM, as the chief evaluation method, was introduced into the quality evaluation of medicine and food homologous samples, which made 45 batches of samples well divided into different grades after sufficient qualitative and quantitative analysis. The "Four in One" fingerprint evaluation system proposed in this paper is a new overall layout, which includes not only HPLC fingerprints but also three emerging quantum fingerprints, namely UV-QFP, FTIR-QFP and DSC-QFP. The four complement each other and provide a systematic strategy for more holistic, objective and strict control of sample quality, which will undoubtedly contribute a more practical and reliable method to the quality analysis and comprehensive evaluation of other samples. The separate evaluation of CD and CT was the preliminary exploration of this study based on this system as well as the consideration of sample characteristics. In the future in-depth research, a more perfect full-scale evaluation system that tolerates sample characteristics and assesses the two kinds of *Cistanche Herba* samples simultaneously will be further developed, which is worthy of attention and expectation.

CRediT authorship contribution statement

Xiang Li: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Investigation, Project administration, Visualization, Writing – original draft. **Ting Yang:** Data curation, Formal analysis, Methodology, Investigation. **Hongzhou Bu:** Resources, Supervision. **Huizhi Yang:** Data curation, Investigation. **Xinrong Liu:** Data curation, Formal analysis. **Jianhui Wang:** Resources. **Guoxiang Sun:** Conceptualization, Funding acquisition, Project administration, Software.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (No. 81573586).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2022.108359.

References

[1] P.C.o.P.R. China, Pharmacopoeia of the People's Republic of China, China Medical Science and Technology Press, Beijing, 2020.
[2] Z. Li, H. Lin, L. Gu, J. Gao, C.M. Tzeng, Herba Cistanche (Rou Cong-Rong): One of the Best Pharmaceutical Gifts of Traditional Chinese Medicine, Front Pharmacol 7 (2016) 41, https://doi.org/10.3389/fphar.2016.00041.

[3] Y. Jiang, P. Tu, Analysis of chemical constituents in Cistanche species, J. Chromatogr. A 1216 (2009) 1970–1979, https://doi.org/10.1016/j.chroma.2008.07.031.
[4] L. Jiang, B. Zhou, X. Wang, Y. Bi, W. Guo, J. Wang, R. Yao, M. Li, The Quality Monitoring of Cistanches Herba (Cistanche deserticola Ma): A Value Chain Perspective, Front Pharmacol 12 (2021), 782962, https://doi.org/10.3389/fphar.2021.782962.
[5] X.Y. Wang, R. Xu, J. Chen, J.Y. Song, S.G. Newmaster, J.P. Han, Z. Zhang, S. L. Chen, Detection of Cistanches Herba (Rou Cong Rong) Medicinal Products Using Species-Specific Nucleotide Signatures, Front Plant Sci 9 (2018) 1643, https://doi.org/10.3389/fpls.2018.01643.
[6] L. Fan, Y. Peng, J. Wang, P. Ma, L. Zhao, X. Li, Total glycosides from stems of Cistanche tubulosa alleviate depression-like behaviors: bidirectional interaction of the phytochemicals and gut microbiota, Phytomedicine 83 (2021), 153471, https://doi.org/10.1016/j.phymed.2021.153471.
[7] L.-L. Wang, H. Ding, H.-S. Yu, L.-F. Han, Q.-H. Lai, L.-J. Zhang, X.-B. Song, Cistanches Herba: Chemical Constituents and Pharmacological Effects, Chinese Herbal Medicines 7 (2015) 135–142, https://doi.org/10.1016/s1674-6384(15)60017-x.
[8] Z. Fu, X. Fan, X. Wang, X. Gao, Cistanches Herba: An overview of its chemistry, pharmacology, and pharmacokinetics property, J Ethnopharmacol 219 (2018) 233–247, https://doi.org/10.1016/j.jep.2017.10.015.
[9] X. Sun, J. Du, Y. Xiong, Q. Cao, Z. Wang, H. Li, F. Zhang, Y. Chen, Y. Liu, Characterization of the key aroma compounds in Chinese JingJiu by quantitative measurements, aroma recombination, and omission experiment, Food Chem 352 (2021), 129450, https://doi.org/10.1016/j.foodchem.2021.129450.
[10] Z. Li, C. Zhang, G. Ren, M. Yang, S. Zhu, M. Li, Ecological modeling of Cistanche deserticola Y.C. Ma in Alxa, China, Sci Rep, 9 (2019) 13134, https://doi.org/10.1038/s41598-019-48397-6.
[11] F. Yang, T. Chu, Y. Zhang, X. Liu, G. Sun, Z. Chen, Quality assessment of licorice (Glycyrrhiza glabra L.) from different sources by multiple fingerprint profiles combined with quantitative analysis, antioxidant activity and chemometric methods, Food Chem., 324 (2020) 126854, https://doi.org/10.1016/j.foodchem.2020.126854.
[12] H. Li, X. Gong, Z. Wang, C. Pan, Y. Zhao, X. Gao, W. Liu, Multiple fingerprint profiles and chemometrics analysis of polysaccharides from Sarcandra glabra, Int J Biol Macromol 123 (2019) 957–967, https://doi.org/10.1016/j.ijbiomac.2018.11.103.
[13] M. Esteki, Z. Shahsavari, J. Simal-Gandara, Food identification by high performance liquid chromatography fingerprinting and mathematical processing, Food Res Int 122 (2019) 303–317, https://doi.org/10.1016/j.foodres.2019.04.025.
[14] Y. Xue, L. Zhu, T. Yi, Fingerprint analysis of Resina Draconis by ultra-performance liquid chromatography, Chem. Cent. J. 11 (2017) 67, https://doi.org/10.1186/s13065-017-0299-8.
[15] A. Sabir, M. Rafi, L.K. Darusman, Discrimination of red and white rice bran from Indonesia using HPLC fingerprint analysis combined with chemometrics, Food Chem 221 (2017) 1717–1722, https://doi.org/10.1016/j.foodchem.2016.10.114.
[16] M. Kharbach, R. Kamal, M. Bousrabat, M. Alaoui Mansouri, I. Barra, K. Alaoui, Y. Cherrah, Y. Vander Heyden, A. Bouklouze, Characterization and classification of PGI Moroccan Argan oils based on their FTIR fingerprints and chemical composition, Chemometrics and Intelligent Laboratory Systems, 162 (2017) 182-190, https://doi.org/10.1016/j.chemolab.2017.02.003.
[17] H.X. Huang, H.B. Qu, A comparative fingerprint study using high-performance liquid chromatography, ultraviolet, and near-infrared spectroscopy to evaluate the quality consistency of Danshen injections produced by different manufacturers, Analytical Methods 5 (2013) 474–482, https://doi.org/10.1039/c2ay25925g.
[18] E. Jaccoulet, C. Boughanem, L. Auduteau, P. Prognon, E. Caudron, UV spectroscopy and least square matching for high throughput discrimination of taxanes in commercial formulations and compounded bags, Eur J Pharm Sci 123 (2018) 143–152, https://doi.org/10.1016/j.ejps.2018.07.047.
[19] F. Zhang, X. Li, L. Lan, J. Wang, P. Guo, G. Sun, Simultaneous determination of eight components in Amomum villosum and its overall qualityconsistency evaluation by four-dimensional fingerprints assisted with antioxidant activity, J Chromatogr A 1674 (2022), 463135, https://doi.org/10.1016/j.chroma.2022.463135.
[20] M. Smolik, I. Ochmian, A. Bobrowska-Chwat, G. Chwat, L. Arus, P. Banaszczak, J. Bocianowski, P. Milczarski, K. Ostrowska, Fingerprinting, structure, and genetic relationships among selected accessions of blue honeysuckle (Lonicera caerulea L.) from European collections, Biotechnol Rep (Amst) 34 (2022) e00721.
[21] M. Liu, X. Li, T. Dai, Q. Li, Y. Huang, P. Guo, G. Sun, Multiple fingerprints and quantitative analysis for comprehensive quality evaluation of Citri reticulatae pericarpium within different storage years, New Journal of Chemistry (2022), https://doi.org/10.1039/d2nj02123d.
[22] T. Dai, G. Sun, The analysis of active compounds in Flos Chrysanthemi Indici by UHPLC Q exactive HF hybrid Quadrupole-Orbitrap MS and comprehensive quality assessment of its preparation, Food Funct 12 (2021) 1769–1782, https://doi.org/10.1039/d0fo03053h.
[23] B. Xu, M. Yang, Y. Du, S. Zhao, Y. Li, H. Pan, Fingerprint and multi-ingredient quantitative analyses for quality evaluation of hawthorn leaves and Guang hawthorn leaves by UPLC–MS, Revista Brasileira de Farmacognosia 28 (2018) 369–373, https://doi.org/10.1016/j.bjp.2018.03.005.
[24] H. Cheng, W. Wu, J. Chen, H. Pan, E. Xu, S. Chen, X. Ye, J. Chen, Establishment of anthocyanin fingerprint in black wolfberry fruit for quality and geographical origin identification, Lwt 157 (2022), https://doi.org/10.1016/j.lwt.2022.113080.
[25] M.R. Siddiqui, Z.A. AlOthman, N. Rahman, Analytical techniques in pharmaceutical analysis: A review, Arabian Journal of Chemistry, 10 (2017) S1409-S1421, https://doi.org/10.1016/j.arabjc.2013.04.016.
[26] A. Salerno, L. Verdolotti, M.G. Raucci, J. Saurina, C. Domingo, R. Lamanna, V. Iozzino, M. Lavorgna, Hybrid gelatin-based porous materials with a tunable multiscale morphology for tissue engineering and drug delivery, European Polymer Journal 99 (2018) 230–239, https://doi.org/10.1016/j.eurpolymj.2017.12.024.
[27] A.K. Attia, M.M. Abdel-Moety, S.G. Abdel-Hamid, Thermal analysis study of antihypertensive drug doxazosin mesilate, Arabian Journal of Chemistry, 10 (2017) S334-S338, https://doi.org/10.1016/j.arabjc.2012.08.006.
[28] L. Lan, Y. Zhang, M. Zhang, G. Sun, Evaluation of the quality of compound liquorice tablets by DSC and HPLC fingerprints assisted with dissolution, J Pharm Biomed Anal 175 (2019), 112715, https://doi.org/10.1016/j.jpba.2019.06.012.
[29] D. Gong, J. Chen, X. Li, G. Sun, W. Sun, A smart spectral analysis strategy-based UV and FT-IR spectroscopy fingerprint: Application to quality evaluation of compound liquorice tablets, J Pharm Biomed Anal 202 (2021), 114172, https://doi.org/10.1016/j.jpba.2021.114172.
[30] X. Li, F. Zhang, X. Wang, G. Sun, Evaluating the quality consistency of Rong'e Yishen oral liquid by UV+FTIR quantum profilings and HPLC fingerprints combined with 3-dimensional antioxidant profiles, Microchemical Journal 170 (2021), https://doi.org/10.1016/j.microc.2021.106715.
[31] X. Li, F. Zhang, Y. Shi, B. Bao, G. Sun, Assessing the quality consistency of Rong'e Yishen oral liquid by five-wavelength maximization profilings and electrochemical fingerprints combined with antioxidant activity analyses, Anal Chim Acta 1192 (2022), 339348, https://doi.org/10.1016/j.aca.2021.339348.
[32] X. Li, H. Yang, X. Pang, G. Sun, Entirely control the quality consistency of Rong'e Yishen oral liquid by both quantified profiling and quantitative analysis of multi-components by single marker method, J Pharm Biomed Anal 193 (2021), 113719, https://doi.org/10.1016/j.jpba.2020.113719.
[33] J. Chen, D. Gong, X. Liu, G. Sun, W. Sun, Quality and antioxidant activity evaluation of dandelion by HPLC with five-wavelength fusion fingerprint, New Journal of Chemistry 45 (2021) 9856–9863, https://doi.org/10.1039/d1nj01422f.
[34] D. Gong, J. Chen, Y. Sun, X. Liu, G. Sun, Multiple wavelengths maximization fusion fingerprint profiling for quality evaluation of compound liquorice tablets and related antioxidant activity analysis, Microchemical J. 160 (2021), https://doi.org/10.1016/j.microc.2020.105671.
[35] Y. Liu, G. Sun, J. Luan, J. Ling, J. Zhang, F. Yang, A comprehensive strategy to monitor quality consistency of Weibizhi tablets based on integrated MIR and UV spectroscopic fingerprints, a systematically quantified fingerprint method, antioxidant activities and UPLC-Q-TOF-MS chemical profiling, RSC Advances 6 (2016) 366–375, https://doi.org/10.1039/c5ra21468h.
[36] H. Guo, Z. Zhang, Y. Yao, J. Liu, R. Chang, Z. Liu, H. Hao, T. Huang, J. Wen, T. Zhou, A new strategy for statistical analysis-based fingerprint establishment: Application to quality assessment of Semen sojae praeparatum, Food Chem 258 (2018) 189–198, https://doi.org/10.1016/j.foodchem.2018.03.067.
[37] D. Gong, Z. Zheng, J. Chen, Y. Pang, G. Sun, Holistic quality evaluation of compound liquorice tablets using capillary electrophoresis fingerprinting combined with chemometric methods, New Journal of Chemistry (2021), https://doi.org/10.1039/d0nj05461e.