Study on the digitized and quantified evaluating method for the super information cluster of traditional Chinese medicine ultraviolet spectral fingerprints

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Abstract: The theories of ultraviolet spectral fingerprint (UVFP) index, information index, fluctuation index, information fluctuation index combined with the quantified UV fingerprint method (QUFM) had been established and put into practice in the Ginkgo Tablets (GT) quality evaluation. The flowing injection analysis (FIA) coupled with a diode array detector was applied as a novel method to obtain the UVFP in the region of 190-400 nm at which the absorption can reflect all the information of the chemical constituents contained $\pi \to \pi^*$, $n \to \pi^*$ and $n \to \sigma^*$ transition. The result showed that all batches were qualified (Grade ≤3) except S8 for its too high contents. It was proved that this method made the expression of superposed information in UVFP of traditional Chinese medicine (TCM) digitized and simple. What's more, an approach which can test the total chemical content with the chromophoric characteristics in the complex system of TCM rapidly, simply and accurately was achieved by the application of QUFM. In one word, it made the exploration of the general characteristic information of the molecular absorption complex TCM in the ultraviolet regions feasible and possible.

Key Words: Ginkgo tablet; ultraviolet spectral fingerprint (UVFR) index; information index; fluctuation index; information fluctuation index; quantified UV fingerprint method (QUFM)

Introduction

Nowadays, the quality testing about traditional Chinese medicine (TCM) is always by High Performance Liquid Chromatography (HPLC) method with ultraviolet detector. Although the ultraviolet spectrum analysis technology has an important application in quantitative determination, the features of only single information contained and ordinary characteristics results in its poor practical usage in the study of TCM fingerprint information. Meanwhile the extracts of TCM or their preparation certainly contain dozens or even hundreds of components, so the qualitative and quantitative information acquired at single or several wavelengths is obviously not comprehensive and not overall (1,2). Consequently, ultraviolet absorption spectra in the region

190-400 nm in a dilute solution can cover the superposed information of chemical composition, that is to say, ultraviolet fingerprint (UVFP) can be served as a simple and accurate quantitative technology for TCM identification (3,4). In addition, the information of unsaturated chemical bonds and conjugate system of the total contents in TCM extract can be detected or determined by UV spectrum analysis. It is just this fact that provides the basis for testing the overall chemical contents in TCM accurately by UVFP. In this paper, the evaluating method of the Super Information Cluster of Ultraviolet Spectrum Fingerprints was further developed according to the theories of UVFP Index, Information Index, Fluctuation Index, Information Fluctuation Index and quantified Ultraviolet fingerprint

method (QUFM). GTs, the most popular herbal drug (HD) used extensively in at least 130 countries with $5 billion worldwide sales per year (5), can increase peripheral and cerebral blood flow, treat dementia and decrease mental vitality at old age, tinnitus, Alzheimer's disease and depression (6-8). Numerous studies had reported to detect the contents of flavone glycosides and terpene lactones which were much inadequate to represent its real therapeutic effect (9-11). Furthermore, increasing interest in chemical fingerprint especially HPLC fingerprint (HPLC-FP) analysis can be observed, however, complex data processing, longer analysis time, and pollution of organic solvent limited its utility in manufacturer's accompanying inspection.

Therefore, in this study, we developed the UVFP as a fast, accurate, and effective method to reveal the complex system of GT based on the change of the multidimensional super information cluster obtained from the 211 UV data points from 190-400 nm. An effective, practical, simple and accurate method came into being to display the overall quantitative characteristics of the TCM with the feature of complex giant system by using UVFP.

Theory for digitized super information of UVFP (12,13)

UVFP index theory

Fingerprint point n

Considering each peak point in UVFP as fingerprint point, each fingerprint point represents the total absorbency of various chemical components (only thoroughly saturated chemical component cannot be effectively detected). Using all peak points of UVFP to quantitatively evaluate the overall quality of TCM possesses the advantages of abundantly, accurately and briefly quantitative information that was a very important innovation for TCM quality control.

Fingerprint separation ratio (β)

Fingerprint separation ratio was defined to reflect the peak point numbers and purity of monochromatic light seen in Eq. [1], where $\Delta\lambda$, d (nm), n, λ_1 and λ_2 is the adjacent wavelength interval of a continuous spectrum, slit width, fingerprint points, the starting and ending wavelength of scanning, respectively. The bigger fingerprint point number and the much purer of monochromatic light are, the larger β, which is usually one.

Fingerprint frequency ρ

Fingerprint frequency ρ is termed as the number of peak points obtained in unit wavelength, seen in Eq. [2], which usually is 1 or 2.

Fingerprint total signal intensity (LR)

Fingerprint total signal intensity (LR) is defined as the total sum of each fingerprint point absorbance, seen in Eq. [3], which represents the total signal overlap intensity of all fingerprint points, where A_i (T_i) is the absorbance or transmittance under the ith wavelength.

Fingerprint AUC

Fingerprint AUC is the integral area under curve of an ultraviolet spectrum that is performed by Simpson's integral method, so it is another index to reveal the total contents of TCM chemicals. When ρ is equal to one, AUC is equal to LR.

Leveling coefficient of peak signal γ

Leveling coefficient of peak signals γ is termed as the intersection angle cosine of $\vec{a}=(1,1,1,…,1)$ and $\vec{A}=(A_1, A_2, A_3, …, A_n)$ to reveal how uniform are the fingerprint signals, seen in Eq. [4]. The closer to one for γ, the poorer characteristics of a profile. Likewise, the geometric arithmetic ratio δ is defined as the ratio of the geometric mean of absorbances to the arithmetic mean of absorbances, respectively, seen Eq. [7], where $\overline{A}$ and A_0 are the arithmetic mean of absorbances and geometric mean of absorbances, separately, seen Eq. [5,6] that can also reveal how uniform are the peak signals.

Fingerprint space occupying ratio η

Fingerprint space occupying ratio η is defined as the percentage of AUC accounted for the largest spectrum area that is the wavelength range multiples the biggest value of absorbances, seen Eq. [8].

Apparent injection mass Q (mg)

Q is an apparent mass of raw materials or their preparations to be prepared sample solution, of which extracts have been loaded to be analyzed during UVFP process, where its unit is 1 mg.

Apparent absorption coefficient E

Apparent absorption coefficient E is defined as the absorbance of extractum of 1 mg raw materials or preparations at a certain wavelength, where the maximum of E_{max} is usually selected to express the UVFP characteristics, seen Eq. [9].

$$\beta = \frac{\Delta\lambda}{d} = \frac{\lambda_2 - \lambda_1}{nd} \quad [1]$$

$$\rho = \frac{1}{\Delta\lambda} = \frac{n}{\lambda_2 - \lambda_1 + 1} \quad [2]$$

$$LR = \sum_{i=1}^{n} A_i = -\sum_{i=1}^{n} \lg T_i \quad [3]$$

$$\gamma = \frac{\overline{A} \cdot \overline{a}}{\|\overline{A}\|\|\overline{a}\|} = \frac{\sum_{i=1}^{n} A_i}{\sqrt{n\sum_{i=1}^{n} A_i^2}} = \frac{\overline{A}}{\sqrt{\frac{1}{n}\sum_{i=1}^{n} A_i^2}} = [\frac{1}{n}\sum_{i=1}^{n}(\frac{A_i}{\overline{A}})^2]^{-\frac{1}{2}} \quad [4]$$

$$A_0 = \sqrt[n]{\prod_{i=1}^{n} A_i} \quad [5]$$

$$\overline{A} = \frac{1}{n}\sum_{i=1}^{n} A_i \quad [6]$$

$$\delta = \frac{A_0}{\overline{A}} \quad [7]$$

$$\eta = \frac{AUC}{(\lambda_2 - \lambda_1 + 1)A_{max}} \times 100\% \quad [8]$$

$$E = \frac{A}{Q} \quad [9]$$

UVFP index F
Taking the signal response, leveling coefficient, separation ratio and effective information into consideration, UVFP index F is defined to directly indicate both the fingerprint information amount and the signal intensity and also the uniformity, seen Eq. [10].

UVFP standard index $F_{r(q)}$
Considering the influence of different injection mass, UVFP standard index $F_{r(q)}$ is F corrected to Q=1 mg for further expressing the mass information of a sample, seen Eq. [11].

Fixed wavelength range index $F_{r(\lambda)}$
Besides the fingerprint information amount and the signal

intensity and uniformity, $F_{r(\lambda)}$ is termed as Eq. [12] that can reveal the spectrum efficiency of the whole profile at a fixed wavelength range.

The relative index F_r
F_r is defined as a comprehensive relative index that shows the relative information acquired at the condition of a fixed wavelength range and per unit injection mass, seen Eq. [13]. If the number of fingerprint points is 1,000, and A_i is equal to 1.0, meanwhile γ, ρ, and β is 1, respectively, then F_r is less than 100.

$$F = \eta\rho\beta\gamma S \ln LR = \eta\gamma\, d^{-1} S \ln LR = \eta\gamma\, d^{-1} S \ln(\sum_{i=1}^{n} A_i) \quad [10]$$

$$F_{r(q)} = \eta\gamma\, d^{-1} S \ln(\frac{1}{Q}\sum_{i=1}^{n} A_i) = \eta\gamma\, d^{-1} S \ln(\frac{LR}{Q})$$
$$= \eta\gamma\, d^{-1} S \ln(\sum_{i=1}^{n} E_i) \quad [11]$$

$$F_{r(\lambda)} = \frac{200F}{\lambda_2 - \lambda_1 + 1} \quad [12]$$

$$F_r = \frac{200F_{r(q)}}{\lambda_2 - \lambda_1 + 1} \quad [13]$$

Information amount theory

Peak point entropy S_i
According to Shannon entropy, the fingerprint peak point entropy S_i may be defined as Eq. [14,15], where p_i is the normalization value (A_T) of each fingerprint point absorbance (A_i).

Total fingerprint entropy S
Total fingerprint entropy S is the sum of each peak points entropy S_i, seen Eq. [16]. The greater the S the more information reflected by it.

Fingerprint information amount index I
I is defined as the total sum of the natural logarithm of absorbance peak A_i multiplied by the entropy S_i, leveling coefficient of peak signal γ and the effective entropy, seen Eq. [17]. So it can comprehensively show how high the signal responses are, leveling coefficient of peak signals and the information amount of the fingerprint profiles.

Standard information amount index $I_{r(q)}$

$I_{r(q)}$ is the corrected I by the apparent loading mass, accounting for 1 mg of the crude drug or preparation denoted as Eq. [18]. Beside the signal responses, leveling coefficient of the peak signals and the information amount of the profiles, it can also represent the apparent loading mass of the raw materials or their preparations that will be extracted for sample solution.

Fixed wavelength range information amount index $I_{r(\lambda)}$

In order to express the spectrum efficiency, I is corrected by the fixed range of wavelength to get $I_{r(\lambda)}$ that is shown in Eq. [19], which refers to I multiplied by 200 and divided by the difference of the beginning scan wavelength λ_1 and the ending one λ_2 to depict the information contained whether more or less than that acquired at 200 nm wavelength.

Relative information amount index I_r

Summing the above two factors, i.e., the apparent loading mass and the spectral efficiency, I_r is defined to display how about the relative information of chemical constituents in samples, seen Eq. [20]. Not only the signal responses, leveling coefficient of the peak signals, and the information amount, but also the loading mass of the raw materials or their preparations and the spectral efficiency of the profile can be thoroughly described by this parameter.

Inverse ratio of two indexes ω

In order to compare the difference between F and I, the inverse ratio of the two indexes ω was shown in Eq. [21], for which is often less than 1.0. The uniformity gets better as the ω value gets bigger.

$$S_i = -p_i \ln|p_i| \tag{14}$$

$$p_i = \frac{A_i}{A_T} \tag{15}$$

$$S = -\sum_{i=1}^{n} p_i \ln|p_i| = -\sum_{i=1}^{n} \ln\left|\frac{A_i}{A_T}\right| \tag{16}$$

$$I = \eta\gamma S \sum_{i=1}^{n} S_i \ln A_i \tag{17}$$

$$I_{r(q)} = \eta\gamma S \sum_{i=1}^{n} S_i \ln\frac{A_i}{Q} \tag{18}$$

$$I_{r(\lambda)} = \frac{200I}{\lambda_2 - \lambda_1 + 1} \tag{19}$$

$$I_r = \frac{200I_{r(q)}}{\lambda_2 - \lambda_1 + 1} \tag{20}$$

$$\omega = \frac{I}{F} \tag{21}$$

The number of arrest points and the central tendency theory

In order to further explore a UVFP fluctuation, the number of arrest points N_{sp}, the fingerprint range R and the fingerprint range ratio R_{std} have been proposed.

The number of arrest points N_{sp}

N_{sp} is termed as the number of the first derivatives being zero that are namely the extreme points on the curve, which obviously reveals the fluctuation of a fingerprint profile. The bigger the number is, the heavier the fluctuation. However, smoothing a profile can cause the number down.

Fingerprint range R

R is the difference between maximum and minimum point on a UV curve, which can characterize the variation of the fingerprint distribution and that of the dispersion degree.

Fingerprint range ratio R_{std}

R_{std} is defined as the ratio of maximum absorbance and fingerprint range R, seen Eq. [22]. It is another parameter to characterize the variation of the fingerprint distribution and that of the dispersion degree as well as R.

Median ratio m

What's more, the median ratio m is defined as the ratio of the average absorbance to the maximum absorbance, which depicts how closer the fingerprint points are to the central tendency. When m does exceed 0.5 to denote the number of the higher absorbance points is bigger, neither the case nor the number of those is big. If m is equal to 1, in which the uniformity is the best, but the characteristic of UVFP is very poor, seen Eq. [23].

$$R_{std} = \frac{A_{max}}{R} \tag{22}$$

$$m = \frac{\overline{A}}{A_{max}} \tag{23}$$

Fluctuation amount index theory

Fluctuation amount index *AF*

AF is termed as such an index that takes the fingerprint leveling coefficient, the number of arrest points, the fingerprint range ratio, the effective information entropy and the total responses all into consideration, described as Eq. [24]. It can tell us how about the total signal responses, the effective amount of information, the uniformity and the fluctuation of a UV curve. Actually **AF** is the sum of each absorbance common logarithm when γ=1, N_{sp}=0, *m*=1 and R_{std}=∞. The better leveling coefficient, the higher responses and the larger information amount will contribute to a bigger value of **AF**.

Standard fluctuation amount index $AF_{r(q)}$

$AF_{r(q)}$ is the corrected AF_r after testing extract from 1 mg of the raw materials or their preparation denoted as Eq. [25]. A lot of information including the total signal response, the effective amount of information, the uniformity, the fluctuation of a UV curve and the apparent loading mass can be comprehensively expressed by this index.

Fixed wavelength range fluctuation amount index $AF_{r(\lambda)}$

$AF_{r(\lambda)}$ is termed as the corrected AF_r by the fixed range of 200 nm, seen Eq. [26]. The greater fluctuation positively correlates the smaller range of wavelength. $AF_{r(\lambda)}$ synthesizes the spectral wavelength efficiency besides the information of AF_r.

Relative fluctuation amount index AF_r

The above two parameters are simultaneously taken into calculation: the AF_r is defined as Eq. [27]. It is such an index that manages to disclose not only the leveling coefficient, the number of arrest points, the fingerprint range ratio, the effective information entropy and the total response but also the apparent loading amount and the spectral efficiency.

$$AF = \eta\gamma^{-1}SR_{std}^{N_{sp}}\ln LR$$
$$= \eta\gamma^{-1}SR_{std}^{N_{sp}}\ln\left(\sum_{i=1}^{n}A_i\right) \tag{24}$$

$$AF_{r(q)} = \frac{\eta S}{\gamma}R_{std}^{N_{sp}}\ln\left(\frac{LR}{Q}\right) = \frac{\eta S}{\gamma}R_{std}^{N_{sp}}\ln\left(\sum_{i=1}^{n}\frac{A_i}{Q}\right) \tag{25}$$

$$AF_{r(\lambda)} = \frac{200AF}{\lambda_2 - \lambda_1 + 1} \tag{26}$$

$$AF_r = \frac{200AF_{r(q)}}{\lambda_2 - \lambda_1 + 1} \tag{27}$$

Information fluctuation amount index theory of UVFP

Information fluctuation index *AI*

In order to establish an index combined the total amount of information with the fluctuation degree, **AI** is put forth as Eq. [28]. It is a comprehensive index that can simultaneously indicate how about the fingerprint leveling coefficient, the number of arrest points, the fingerprint range ratio, the effective space information entropy and the total response. If the ideal conditions are met γ=1, N_{sp}=0 and R_{std}→∞, then $N_{sp}^{R_{std}}$=1. A bigger value of **AF** usually means the larger volatility of the effective spatial information.

Standard information fluctuation index $AI_{r(q)}$

Assumed the most adequate 1 mg of **Q**, **AI** is corrected by the apparent loading amount to be called the standard information fluctuation index $AI_{r(q)}$ denoted as Eq. [29], which refers to the practical **AI** as **Q** is corrected as equal to 1.0 mg.

Fixed wavelength range information fluctuation amount index $AI_{r(\lambda)}$

Considering the spectral efficiency, $AI_{r(\lambda)}$ is termed as the corrected index of **AI** seen Eq. [30], which refers to **AI** multiplied by 200 and divided by the difference of the beginning scan wavelength λ_1 and the ending one λ_2 to depict information contained whether more or less than that acquired in 200 nm.

Relative information fluctuation amount index AI_r

AI_r defined as Eq. [31] is introduced for correcting the apparent loading mass and the spectral efficiency for the same purpose as AF_r. It is such an index that manages to comprehensively disclose how high are the leveling coefficient, the number of arrest points, the fingerprint range ratio, the effective space information entropy and the total signal response as well as the apparent loading amount and the spectral efficiency of the profile.

Inverse ratio of fluctuation indexes *aω*

The inverse ratio of fluctuation **aω** is applied to compare the difference between **AF** and **AI** just as **ω**, seen Eq. [32]. The uniformity is positively correlated with the value of **aω**.

$$AI = \eta\gamma^{-1}\omega R_{std}^{N_{sp}}\sum_{i=1}^{n}S_i\ln A_i \tag{28}$$

Table 1 The quality level rule assessed by the quantified UVFP method

Para.	1	2	3	4	5	6	7	8
$S_m \geq$	0.95	0.90	0.85	0.80	0.70	0.60	0.50	$S_m < 0.5$
P_m /%	95-105	90-110	80-120	75-125	70-130	60-140	50~150	0-∞
$\alpha \leq$	0.05	0.10	0.15	0.20	0.30	0.40	0.50	>0.50
Quality	best	better	good	fine	moderate	common	defective	inferior

$$AI_{r(q)} = \frac{\eta\omega}{\gamma} R_{std}^{N_{sp}} \sum_{i=1}^{n} S_i \ln\frac{A_i}{Q} \qquad [29]$$

$$AI_{r(\lambda)} = \frac{200\,AI}{\lambda_2 - \lambda_1 + 1} \qquad [30]$$

$$AI_r = \frac{200\,AI_{r(q)}}{\lambda_2 - \lambda_1 + 1} \qquad [31]$$

$$a\omega = \frac{AI}{AF} \qquad [32]$$

$$P_m = \frac{1}{2}(C+P)\frac{m_{RFP}}{m_i} = \frac{1}{2}\left(\frac{\sum_{i=1}^{n} x_i y_i}{\sum_{i=1}^{n} y_i^2} + \frac{\sum_{i=1}^{n} x_i}{\sum_{i=1}^{n} y_i} S_F\right)\frac{m_{RFP}}{m_i} \times 100\% \qquad [34]$$

$$\alpha = \left|1 - \frac{\gamma_x}{\gamma_y}\right| = \left|1 - \frac{P}{C}\right| \qquad [35]$$

Principle for the development of UVFP

Flowing injection analysis (FIA) method

Ultraviolet spectrum possesses the fingerprinting characteristics of the different chemical composition system for its specially recognizing the chemical information of $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ and $n \rightarrow \sigma^*$. Therefore, this feature makes the UVFP much possible to perform the quality control of TCM as well as its preparation. To avoid the shortcomings of traditional ultraviolet analysis, FIA method shown in *Figure 1* was employed in high performance liquid chromatography (HPLC) system. FIA method, which used a packless column instead of the reversed phase column and detected the ultraviolet signal by DAD, had the advantages of high speed, stability and accurate reproduction.

Quantified UV fingerprint method (QUFM)

Although the digitized parameter criteria of the supper information characteristics for HD or TCM-UVFP has many abundant information, that cannot directly provide the detailed quality control information. Thereof the accurately qualitative and quantitative measurements of all chemicals have not been effectively performed. Based on the complexity science principle, we proposed a powerful method called quantified UV fingerprint method (QUFM) to effectively assess the quality level of GT. QUFM, taking each spectral point as the calculating unit, is a method combining the macro qualitative similarity S_m in Eq. [33] with the macro quantitative similarity P_m in Eq. [34] and the relative deviation coefficient α in Eq. [35] to simultaneously monitor or identify TCM and HD authentic quality level, in which x_i and y_i are the peak point absorbance of a sample and the reference UVFP, respectively, meanwhile m_{RFP} and m_i are the loading mass. The method combined S_m with P_m and α to determine or identify TCM quality level is called QUFM, in which the TCM and HD quality is divided into 8 grades in terms of QUFM criteria, listed in *Table 1*.

$$S_m = \frac{1}{2}(S_F + S_F') = \frac{1}{2}\left(\frac{\sum_{i=1}^{n} x_i y_i}{\sqrt{\sum_{i=1}^{n} x_i^2}\sqrt{\sum_{i=1}^{n} y_i^2}} + \frac{\sum_{i=1}^{n} \frac{x_i}{y_i}}{\sqrt{n\sum_{i=1}^{n} (\frac{x_i}{y_i})^2}}\right) \qquad [33]$$

Establishment of reference UVFP

For fingerprinting similarity analysis, one of the most common and easiest applying tools is to establish the reference fingerprint that is a crucial step. As for establishing the reference of UVFP, two following approaches can be employed. One way is calculating the whole ultraviolet spectral fingerprint points of more than 10 batches of the representative TCM raw materials or the extract solution of patent medicines by the averaging method. Another one is determining the famous-region drug or standard preparations by continuously for 6 times to develop the mean mode of them. Obviously, the mean or median fingerprint of the data
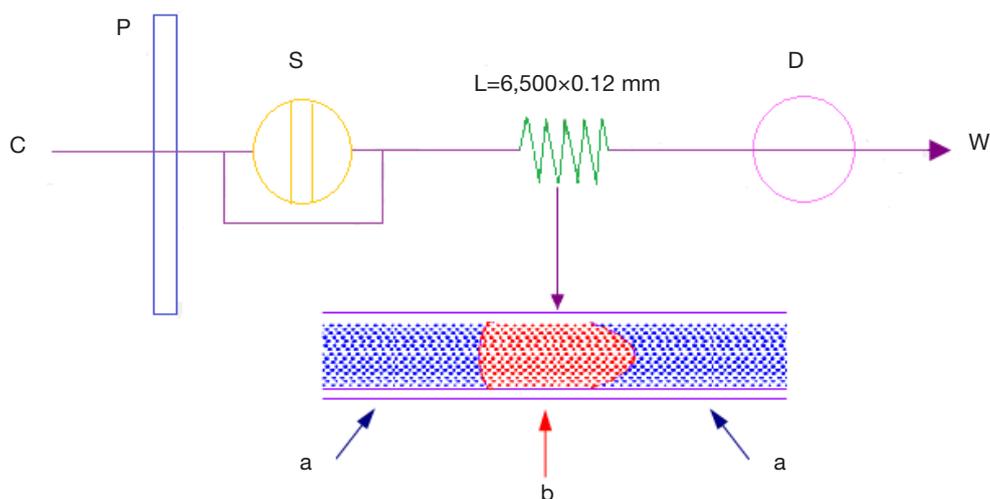
Figure 1 Diagram of determining TCM UVFP by the single channel FIA. C is carrier; P is a pump; S is the sample injector; L is the hollow pipe; D is a DAD detector; W is the disposal bottle; a is the carrier solution; b is the sample solution

set is usually taken for it is more feasible and more likely to achieve the accompanying quantitative analysis. Generally speaking, the reference UVFP can be used as the qualitative and quantitative standard after eliminating the system errors from the different instruments.

Types and characteristics of UVFP

UVFP for the extract approaches that directly exert an effect upon the stability and reproducibility essentially into four categories: UVFP of water-soluble ingredients, UVFP of liposoluble components, UVFP of whole components and UVFP of effective and characteristic groups. As one of the modern analytical means for the fingerprint testing, the UVFP has the following advantages: (I) rapid analysis (analysis time less than 1 min); (II) high stability and repeatability; (III) abundant quantitative information (detect in a wide range of wavelength at less 190-400 nm); (IV) accurately quantitative analysis; (V) significant digital characteristics. Therefore, even despite the single qualitative assay, the UVFP is more comprehensive and more precise than HPLC-UV in reflection of the whole information of TCM chemical ingredients.

Experimental

Apparatus and reagents

The analysis was performed on an Agilent 1100 HPLC series (Hewlett Packard, CA), equipped with a DAD detector, low pressure quaternary pumps, an online degasser and an autosampler. A Sarturius-BS110S analytic scale (Saiduolisi scale company limited, Beijing, China) was used during the analysis.

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

The 14 batches of GTs were bought from shenyang drugstores to specify as following. S1 (091103) and S12 (110602) were from the pharmaceutical factory A; S2 (20100901) and S11 (20110601) were from factory B; The others , S3 (20110101), S4 (110101), S5 (110202), S6 (110303), S7 (110406), S8 (1104218), S9 (1105015), S10 (1105251), S13 (110809) and S14 (20110901) were all produced in different factories, respectively. The names of manufacturers had been removed to maintain confidentiality.

Preparation of the sample solution

Ten tablets of GTs were accurately weighed to get the average weight for each one. A quantity equivalent to two tablets in powdered states was weighed and extracted with 20 mL methanol in an ultrasonic water bath for 20 min. The extracted solution was filtered and then diluted to 25 mL in a flask with methanol. The solution was centrifuged at 3,500 rpm for 10 min, and then the supernatant obtained was filtered through a 0.45 μm Millipore filter before analysis.
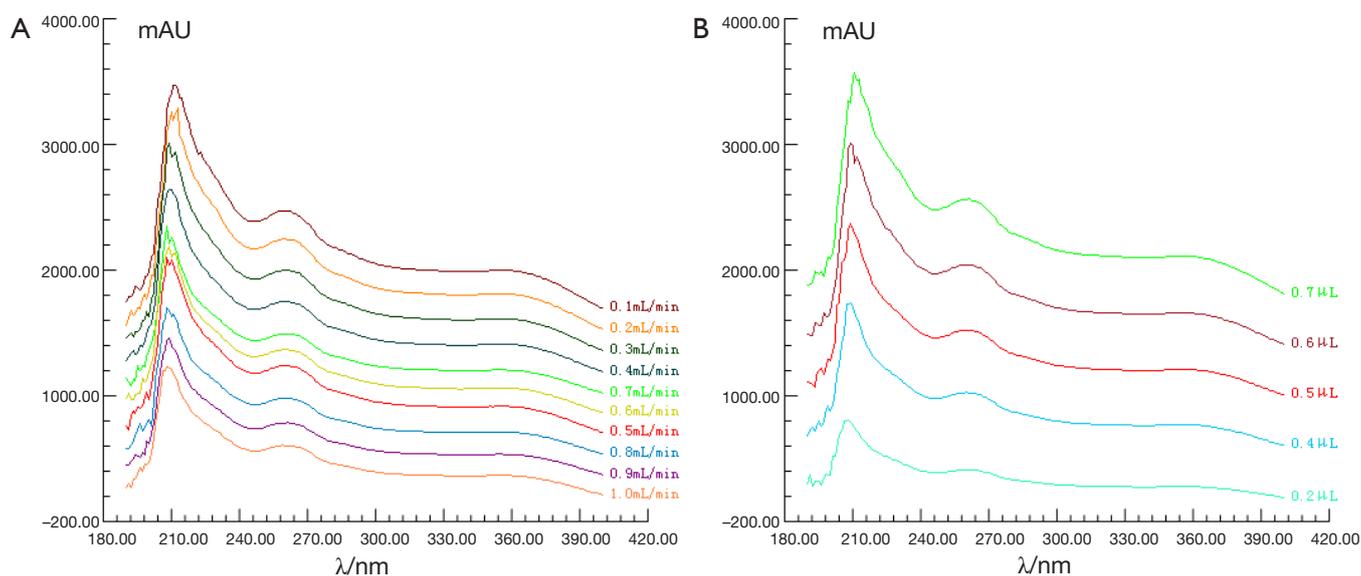
Figure 2 The UV spectra of GT under the different flows (A) and the different loads (B)

Experimental conditions

The unseparated chromatograms in UVFP were gained on an Agilent polytetrafluoro-ethylene (PTFE) tube (6,500 mm × 0.12 mm) with the column temperature of 30.00 (±0.15) ℃. The mobile phase was methanol at a flow rate of 0.5 mL/min and with an injection volume of 0.5 μL. Data acquisition was at the wavelength of 190-400 nm (DAD), with interval of 1 nm and slit width of 1 nm. Therefore 211 data points were gained in every UV spectrum.

Data analysis

All data acquired were processed by a ChemStation workstation (Agilent technology Inc). Similarity analysis of UV based on QUFM was performed on software 3.0 of Digitized Evaluation System for Super Information Characteristics of TCM UV Fingerprints (software Certificate No: 0462756 China) invented by Prof. Guo-Xiang Sun, etc.

Results and discussion

Experiment condition selection and method validation

Selection of flow rate

In this study, the flow rate (*Fc*) of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mL/min were investigated as loading 0.50 μL sample solution and the ultraviolet spectra at 190-400 nm were recorded. It suggested that a lower *Fc*

resulted in a higher **AUC** with the regression equation of $y=151942-7642.5x$, $r=-0.9589$. Finally, 0.5 mL/min was selected because there was no great difference between the ultraviolet spectra in the range of 0.5 to 1.0 mL/min shown in *Figure 2A* and the maximum absorbance of UVFP was rightly appropriate.

Selection of injection amount

The injection volume shown in *Figure 2B* of 0.20, 0.40, 0.50, 0.60 and 0.70 μL were loading into the column with the flow rate of 0.5 mL/min and the ultraviolet spectra at 190-400 nm were recorded. Obviously a linear correlation ($y=11616x+7429.6$, $r=1.00$) can be observed between **AUC** and the apparent injection amount in the range of 3.41 to 11.93 μg. Collecting spectrum at the peak point after deducting the nearest baseline based on its 3D spectrum, loading 0.50 μL had an advisable absorbance in the range of 600-800 mAU, in which 0.50 μL was eventually selected.

System suitability test

0.5 μL of sample solution was loaded and the contour map of absorbance of sample in UV spectral region from 190-400 nm was recorded as *Figure 3*. The unseparated chromatograms at 228, 246, 265, 286 and 326 nm selected on the basis of the contour map were recorded and compared shown in *Figure 4*. Theoretical plate number of this system was no less than 54 as the analysis time for 1 min.
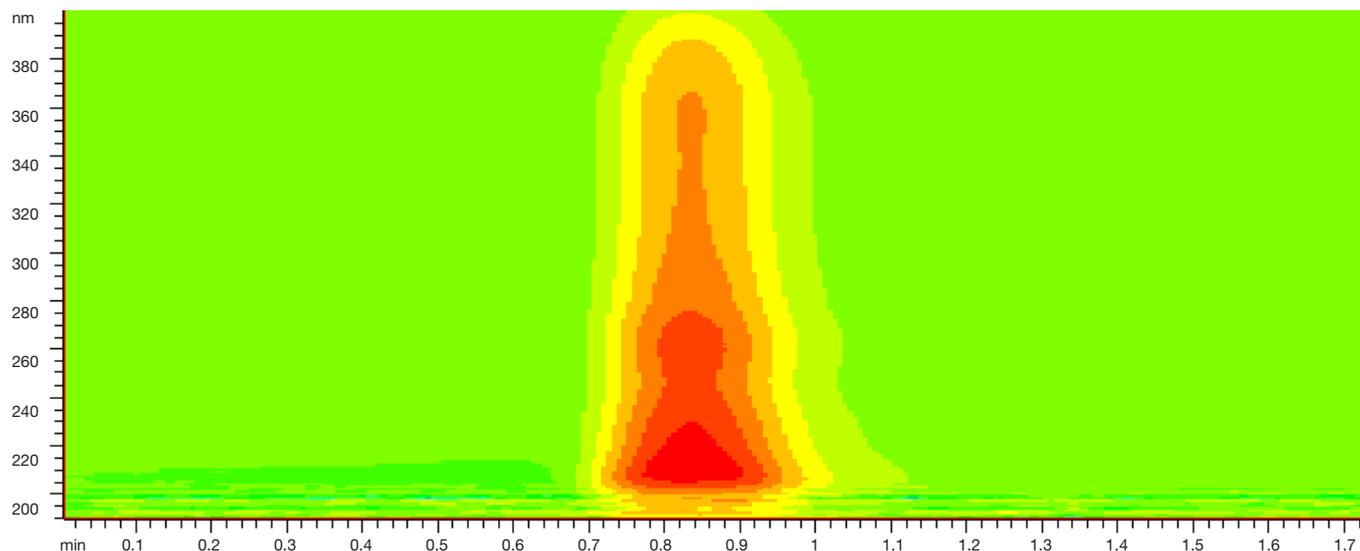
Figure 3 The contour map of absorbance of sample in UV spectral region from 190 to 400 nm
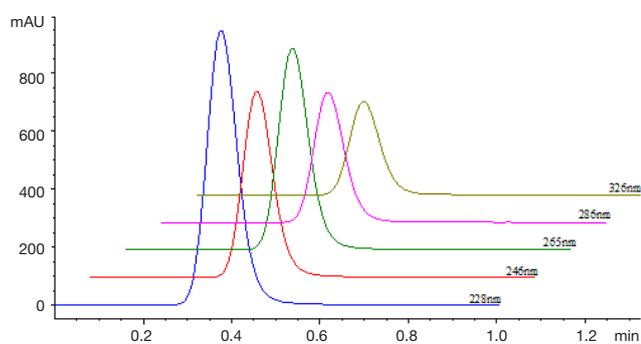
Figure 4 The unseparated chromatograms at five wavelengths

Figure 5 Ultraviolet spectral fingerprints of the 14 batches of GTs and their UV-RFP

Methodology validation

All tests below were carried out on the sample solution prepared as described in Section 4.2. The injection precision was determined by replicate HPLC injections of the same sample solution 6 times per day. Precision of sample stability was determined with measurements from a single sample solution stored at room temperature for 0, 1, 2, 4, 6 and 8 h. The retention time (**RT**) and peak area (**PA**) were recorded for estimating the precision and stability and the results were as following—precision: the relative standard deviations (RSD) of **RT** and **PA** were found not to exceed 0.40% and 1.70%, respectively; sample stability—below 0.40% and 1.40%, respectively. Thus, all results indicated that the quality of the studied samples and the FIA coupled with HPLC-DAD measurements were relatively stable and under control.

Development of the UVFP and analysis (14-16)

Under the present approach, the UV fingerprint of each sample was recorded. The UV absorption spectral fingerprints of 14 batches of GT samples from 190 to 400 nm were revealed in *Figure 5*. The variation between the 14 samples was significant especially from 205 to 280 nm. Two obvious broad peaks at 265 and 360 nm were the spectral feature flavonoids or their glycosides components in GTs possibly, such as kaempferol, quercetin and rutin. While majority

Table 2 The results of 14 batches of GTs assessed by UVFP according to the QUFM

Types	Para.	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	Mean	RSD%
	S_m	0.997	0.999	0.999	0.999	0.999	0.995	0.999	0.997	0.998	0.999	0.999	0.998	0.999	0.999	0.998	0.12
	P_m	82.3	87.6	109.7	92.5	91.5	112.7	108.4	208.7	94.6	94.0	87.3	95.9	94.0	110.2	105.0	29.86
UV	α	0.013	0.002	0.006	0.004	0.004	0.024	0.007	0.017	0.004	0.003	0.008	0.011	0.006	0.013	0.009	69.38
	Grade	3	3	2	2	2	3	2	8	1	2	3	1	2	3	3	56.40
	Quality	good	good	better	better	better	good	better	infer	best	better	good	best	better	good	-	-
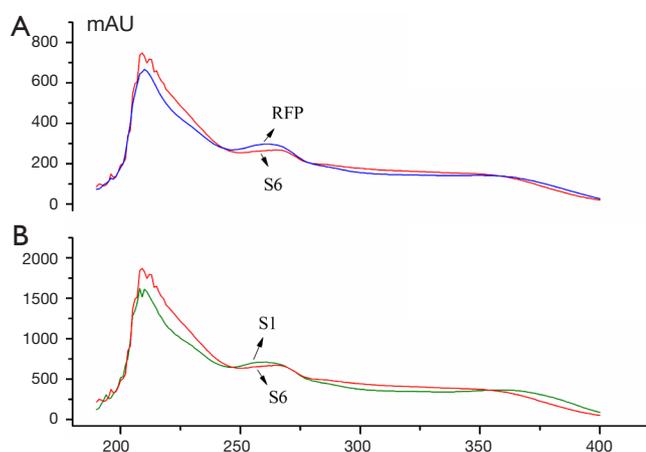
Figure 6 Detailed spectrum contrast for similarity and uniformity: A. S6 and RFP; B. S1 and S6

of components with chemical bonds $\pi \rightarrow \pi^*$, $n \rightarrow \pi^*$ in structure may contribute to the strong absorption at 210 nm, like terpene lactones components in GTs.

The UV spectra of 14 batch of GT samples and their reference fingerprint (RFP) obtained by the averaging method were converted into the file layout of CSV respectively and imported into the above software 3.0. Similarity parameters S_m, P_m and α and final quality grades were presented in *Table 2*, which were all based on each point absorbance in every wavelength within 190-400 nm. It was investigated through all samples that were almost similar with a low relative standard deviation (RSD) of 0.12%, the qualitative similarity S_m of S6 is the lowest which was mainly due to the variance of absorbance in the region of 240-400 nm shown in *Figure 6A*. Obviously, the absorbance of S6 was lower than the RFP in the range of 240-275 and 350-400 nm, however it was higer in the range of 275-350 nm. Even qualitative similarity S_m was not characteristic for the comparison of UVFP, it was proved that subtle difference can be distinguished. For the quantitative similarity P_m, the great difference can be

observed with high RSD of 29.86% from batch to batch which makes a obvious distinction between samples directly. As the results shown in *Table 2*, sample S2 and S11 from the manufactory B were proved to be accordant in quality, while sample S1 and S12 from the same source got the inconsistent results. It was conceivable that being active at keeping relatively stable raw material or technology in one company does well to the batch-to-batch consistency of the final products. Generally speaking, all batches were qualified (Grade ≤3) except S8 for its too higher contents which also necessarily might harm the patient's health and safety or lead to a series of problems to the therapeutic effect caused by overdosage.

Evaluate GTs by the super information cluster of ultraviolet spectum fingerprints

The spectral data of 14 batches of GT samples was imported into the above software 3.0. Enormous information of the UVFP contained potentially was presented in *Table 3*. Part of results were described and analyzed as following: (I) fingerprint separation ratio (β) and fingerprint frequency (ρ) are all one which meet the requirement of fingerprints points numbers and purity degree of monochromatic light; (II) fingerprint total signal intensity LR=100,122-126,720, fingerprint AUC=99,991-122,749, arithmetic mean absorbance $\overline{A}$=475-601, and geometric mean absorbance A_0=392-497 all above values indicated that the UV spectrum had higher response signals. Among all batches of samples, the response signal of S13 was the poorest; (III) γ and δ were in the range of 0.798 to 0.848, 0.820 to 0.852 revealed the uniformity of signal better. But relatively speaking, the signal uniformity for S6 (γ=0.798, δ=0.848) was lowest instead of sample S1 (γ=0.820, δ=0.852), which can be also observed in *Figure 6B* that the amplitude of fluctuation for S6 was more evident in the whole spectrum; (IV) arrest point number N_{sp} within 11-33 and fingerprint range R within 1,451-1,816 illustrated

Table 3 The digital results of super information characteristics of GT-UVFPs

No.	Para	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
1	λ_1	190	190	190	190	190	190	190	190	190	190	190	190	190	190
2	λ_2	400	400	400	400	400	400	400	400	400	400	400	400	400	400
3	Δ	1	1	1	1	1	1	1	1	1	1	1	1	1	1
4	d	1	1	1	1	1	1	1	1	1	1	1	1	1	1
5	n	211	211	211	211	211	211	211	211	211	211	211	211	211	211
6	β	1	1	1	1	1	1	1	1	1	1	1	1	1	1
7	ρ	1	1	1	1	1	1	1	1	1	1	1	1	1	1
8	LR	111,911	120,806	114,982	107,228	105,010	119,137	122,738	113,605	122,782	112,505	120,041	126,720	100,122	101,876
9	A_0	450	471	451	421	412	451	480	446	480	441	469	497	392	397
10	$\overline{A}$	530	573	545	508	498	565	582	538	582	533	569	601	475	483
11	δ	0.848	0.822	0.827	0.828	0.828	0.798	0.825	0.828	0.825	0.826	0.825	0.828	0.827	0.822
12	γ	0.852	0.842	0.845	0.844	0.837	0.820	0.835	0.826	0.844	0.843	0.847	0.850	0.835	0.829
13	AUC	111,730	120,585	114,831	107,191	104,930	119,029	122,585	113,517	122,749	112,327	119,938	126,531	99,991	101,612
14	η	32.7	32.5	31.4	32.8	31.4	30.2	31.0	29.6	33.4	32.9	34.0	32.3	31.3	30.8
15	Q	0.01	0.01	0.008	0.009	0.009	0.008	0.008	0.004	0.01	0.009	0.01	0.009	0.008	0.007
16	F	16.8	16.6	16	16.6	15.7	14.9	15.6	14.7	17.1	16.7	17.4	16.7	15.5	15.2
17	F_r	22.3	22.0	21.6	22.3	21.1	20.0	21.0	20.6	22.7	22.3	23.1	22.2	21.0	20.7
18	$F_{r(\lambda)}$	16.0	15.8	15.2	15.8	14.9	14.2	14.9	14.0	16.3	15.9	16.6	15.9	14.8	14.5
19	$F_{r(q)}$	23.4	23.1	22.6	23.4	22.2	21.0	22.0	21.6	23.9	23.4	24.3	23.3	22.1	21.8
20	S	5.2	5.2	5.2	5.2	5.2	5.1	5.2	5.2	5.2	5.2	5.2	5.2	5.2	5.2
21	I	47.7	47.3	45.5	47.0	44.4	42.3	44.7	41.7	48.9	47.4	49.8	47.9	43.8	42.8
22	I_r	78.3	77.0	76.2	78.4	74.1	70.4	73.9	73.9	79.9	78.5	81.1	78.1	73.8	73.0
23	$I_{r(\lambda)}$	45.5	45.1	43.3	44.8	42.2	40.3	42.5	39.7	46.5	45.1	47.4	45.6	41.7	40.8
24	$I_{r(q)}$	82.2	80.8	80.0	82.3	77.8	74.0	77.6	77.6	83.9	82.4	85.2	82	77.5	76.7
25	ω	2.84	2.86	2.85	2.83	2.83	2.85	2.86	2.84	2.86	2.84	2.86	2.87	2.82	2.82
26	A_1	1621	1756	1736	1548	1585	1870	1876	1816	1740	1618	1670	1856	1514	1563
27	E_1	162,059	171,976	223,948	180,572	186,057	236,380	223,093	447,332	181,425	182,779	164,538	190,762	191,457	2,264,753
28	N_{sp}	15	26	22	24	14	19	28	11	33	32	20	18	31	27
29	R	1,532	1,683	1,670	1,482	1,513	1,816	1,795	1,737	1,663	1,550	1,601	1,783	1,451	1,501
30	R_{std}	1.057	1.043	1.039	1.044	1.047	1.029	1.045	1.046	1.046	1.044	1.043	1.041	1.044	1.041
31	m	0.327	0.326	0.314	0.328	0.314	0.302	0.310	0.296	0.334	0.330	0.341	0.324	0.313	0.309
32	AF	38.8	50.0	37.2	46.8	30.0	25.8	53.6	24.0	76.4	65.1	40.8	34.5	59.0	45.4
33	AF_r	51.6	66.3	50.3	62.9	40.4	34.7	71.9	33.7	101.7	87.2	54.1	45.8	79.9	61.9
34	$AF_{r(\lambda)}$	37.0	47.7	35.5	44.6	28.6	24.6	51.1	22.9	72.8	62.0	38.9	32.9	56.2	43.2
35	$AF_{r(q)}$	54.2	69.6	52.8	66.0	42.4	36.5	75.5	35.4	106.7	91.6	56.8	48.1	83.9	65.0
36	AI	60.5	79.0	58.4	72.6	46.4	40.6	84.8	37.5	120.9	101.8	64.4	54.8	90.7	69.9
37	AI_r	57.7	75.2	55.6	69.2	44.2	38.7	80.7	35.7	115.1	96.9	61.3	52.2	86.4	66.6
38	$AI_{r(\lambda)}$	99.3	128.5	97.7	121.0	77.5	67.6	140.2	66.5	197.7	168.6	104.9	89.4	152.9	119.2
39	$AI_{r(q)}$	104.3	135	102.6	127.1	81.4	71.0	147.2	69.8	207.6	177.0	110.1	93.8	160.6	125.1
40	$A\omega$	1.56	1.58	1.57	1.55	1.55	1.58	1.58	1.56	1.58	1.56	1.58	1.59	1.54	1.54
41	f_{wi}	0.83	0.81	1.07	0.96	0.97	1.05	0.98	2.04	0.86	0.93	0.81	0.85	1.05	1.20

bigger fluctuation of spectrum. Moreover, it was observed that smaller absorbance point is more as the median ratio m (0.296-0.341) was less than 0.5; (V) according to the values of F (14.7-17.4), S (5.1-5.2), I (41.7-49.8), AF (24.0-76.4), and AI (37.5-120.9), high single response, good leveling coefficient and rich effective information can be displayed. After the calibration, F_r (20.0-22.7), I_r (70.4-81.1), AF_r (34.7-101.7), and AI_r (15.2-37.9) were all higher than uncorrected values which were caused by high spectrum efficiency or rich information of chemical ingredients in the extraction. However, lower than the uncorrected values $F_{r(\lambda)}$, $I_{r(\lambda)}$, $AF_{r(\lambda)}$ and $AI_{r(\lambda)}$ denote to the low spectrum efficiency therefore, higher F_r, I_r, AF_r and AI_r were mainly depend on the last factor which can be further illustrated by higer $F_{r(q)}$, $I_{r(q)}$, $AF_{r(q)}$ and $AI_{r(q)}$.

Conclusions

An accessible and feasible digitized and quantified evaluating method for super information cluster of traditional Chinese medicine UVFP was successfully established and applied to explore the potential information of the UVFP. In this method, 46 parameters were together to explore the information of ultraviolet spectra which can scarcely been observed by the visual examination. The UVFP analysis achieved its fast testing by the FIA method with high stability, precision and accessiblity in practice. Based on the above theory, 14 batches of GTs were finally evaluated as the different quality grades. It was suggested that all batches were qualified (Grade ≤3) except S8 whose quality grade was inferior for its too high contents which also necessarily can lead to a series of influences to the therapeutic effect. Our results have demonstrated that employing multi-dimensional digital and quantitative UVFP identification to reveal the characteristics of whole chemical contents is a practical, powerful, reliable and feasible method which can also be comprehensively performed on the quality control of the diverse TCM concerning the accompanying monitoring in manufacturers.

Acknowledgements

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