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An approach to develop two-dimensional fingerprint for the quality control of *Qingkailing* injection by high-performance liquid chromatography with diode array detection

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Abstract

An approach was proposed to develop two-dimensional fingerprint (2D fingerprint) by means of principal component analysis (PCA) of high-performance liquid chromatography with diode array detection (HPLC/DAD) data. The approach was applied to establish 2D fingerprints of various *Qingkailing* injections which were produced by different manufacturers and procedures. In comparison with common one-dimensional fingerprint (1D fingerprint) at fixed wavelength, 2D fingerprint compiled additional spectral data and was hence more informative. Principal component analysis of the 2D fingerprint data was performed in this study, and it led to an accurate classification of various samples on their manufacturers and procedures. The quality of *Qingkailing* samples was further evaluated by similarity measures and the same results were achieved. For comparison, four conventional 1D fingerprints were also applied to the quality assessment for the same samples. Finally, we demonstrated that 2D fingerprint was a more powerful tool to characterize the quality of samples, and could be used to comprehensively conduct the quality control of traditional Chinese medicines.

Keywords: Two-dimensional fingerprint; Quality control; High-performance liquid chromatography with diode array detection; Traditional Chinese medicines; *Qingkailing* injection

1. Introduction

In many Asian countries, especially in China, traditional Chinese medicine (TCM) has a long therapeutic history over thousands of years, and currently it is still attracting ever-increasing attention worldwide [1–3]. It is well known that TCM, either presenting as single herb or as a collection of herbs in composite formulae, is a complex mixture containing hundreds of chemically different constituents which are usually responsible for the therapeutic effects [4]. Therefore, quality control of TCM, unlike that of a chemically synthetic drug with much purity, is beyond the ability of conventional analysis which concentrates basically on single or few marker components, whereas the fingerprint technique emphasizes on the systemic characterization of compositions of samples and appears to offer a more logical approach for quality control [5–8]. So far, fingerprint of TCM, especially the chromatographic fingerprint, has gained more and more attention and been internationally accepted as a feasible means for the quality control of TCM [9–11].

Several chromatographic techniques such as high-performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC), capillary electrophoresis (CE), can be applied for fingerprinting [12]. Fingerprint developed by these approaches contains only chromatographic information, and thus could be regarded as one-dimensional fingerprint (1D fingerprint). In general, one could use 1D fingerprint to obtain a relatively complete picture of common TCM for

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quality control purpose. However, in some cases, the limited information provided by 1D fingerprint might be not enough to comprehensively reveal the quality characteristics of some extremely complex TCM products, such as composite formulae samples prepared from a large number of herbs. Under these circumstances, additional and/or complementary information is often required. In 2000, Luo et al. proposed a solution of multi-dimensional and multi-informational fingerprint by hyphenated techniques such as HPLC/DAD, CE/DAD, GC/MS, HPLC/MS, HPLC/DAD/MS/MS, CE/MS, CE/DAD/MS/MS and HPLC/NMR [13]. Compared with conventional 1D fingerprint, the proposed novel fingerprint has several advantages in terms of on-line structural information, measurement precision, selectivity, chromatographic separation abilities, elimination of instrumental interferences, and so on [7,14]. Moreover, with additional spectral information contained, it gives a much more complete profile of TCM for quality control purpose.

Among all the detection techniques of hyphenated instruments, tandem mass spectrometry is generally considered as one of the best tools for TCM analysis due to its excellent selectivity, specificity and powerful ability of structural qualification [15–17], and hence HPLC/MS/MS tend to be widely employed to control the quality of TCM in the future. However, at present, HPLC/DAD is by far the most common hyphenated instrument, and for the most TCM manufacturers, it is practical and meaningful to efficiently perform quality control by such a common and simple instrument. It is true that DAD has its own limitations such as poor selectivity and specificity, but just as a lot of literatures reported [18–21], by means of fingerprint technique, HPLC/DAD can be successfully applied to reveal the quality characteristics of TCM products in an 'integral' and 'fuzzy' way. Therefore, to develop novel fingerprint based on HPLC/DAD is meaningful for the quality control of TCM.

To the best of our known, DAD is widely applied to simultaneous determination [22], peak purity checking [23], chromatographic discrepancy correction [24,25], identification of chromatogram peaks and so on, but it has not been applied to generate multi-dimensional fingerprint yet. It was recently reported that multi-wavelength chromatographic fingerprints of *Fructus Gardenise* could be generated from chromatograms at three different wavelengths [26], but only very limited DAD data was utilized and it could not comprehensively reveal the quality characteristics of samples. In this study, using HPLC/DAD, we proposed an approach to develop novel fingerprint (2D fingerprint) containing two-dimensional information of chromatogram and UV spectrum. Compared with conventional 1D fingerprint, the proposed 2D fingerprint has several advantages. First, with additional UV spectral information, it is possible to perform qualitative analysis of peaks. Secondly, with additional chromatographic information at various wavelengths compiled, 2D fingerprint contains more peaks than 1D fingerprint, because it is inevitable that some components might have no peaks at a fixed wavelength in 1D fingerprint but have UV responses at other UV wavelengths; thirdly, under different monitoring wavelengths, 1D fingerprints might give different results in quality assessment, whereas 2D fingerprint technique is more objective and credible.

As one of the most commonly used TCM, *Qingkailing* injection has excellent efficacy on circulation system disease, phlogistic disease, virosis and some inexplicable fever [27]. It is prepared from eight medicinal materials or their extracts, including *Radix Isatidis*, *Flos Lonicerae*, *Fructus Gardenise*, *Cornu Bubal*, *Concha Margaritifera*, *Baicalinum*, *Acidum Cholicum*, and *Acidum Hyodesoxy-cholicum*. Due to the extreme complexity of the components, the quality control of *Qingkailing* injection is a rather difficult work. From the literature reported [28,29], HPLC coupled with UV detector or DAD is widely adopted as an efficient tool for qualitative and quantitative analysis to control the quality of *Qingkailing* injection, however, so far there are still quite a few reports on its side effects [30]. Therefore, efficient novel approaches are urgently required to ensure the safety, efficacy and batch-to-batch conformity of *Qingkailing* injection. In this study, based on hyphenated instrument of HPLC/DAD, a meaningful solution of 2D fingerprinting was proposed. We obtained 2D fingerprint of *Qingkailing* injection by PCA of HPLC/DAD data, and applied it to evaluate the quality of various samples in comparison with the approaches of conventional 1D fingerprint. As a result, 2D fingerprint is more likely to be accepted as an effective tool in the quality control of *Qingkailing* injection.

2. Theory and methods

2.1. 2D information extraction from DAD data based on PCA

PCA [31,32] is a well-known chemometric method for the decomposition of two-dimensional matrices. Suppose $\mathbf{X}$ ($n \times m$) is a two-dimensional data matrix measured by DAD with n observations and m variables. If the retention time is set as observations (rows) and the wavelength as variables (columns), $\mathbf{X}$ can be described as m one-dimensional chromatographic fingerprints at various wavelengths, and each fingerprint is measured at n regular time intervals, as shown in the following,

$$\mathbf{X} = [\mathbf{x}_1, \mathbf{x}_2, \ldots, \mathbf{x}_m]$$

$$\mathbf{x}_i = [x_{i1}, x_{i2}, \ldots, x_{in}]^{\mathrm{T}}, \quad i = 1, 2, \ldots, m \tag{1}$$

here the vector $\mathbf{x}_i$ ($n \times 1$) denotes the chromatographic fingerprint measured at the ith wavelength. By PCA, $\mathbf{X}$ can be decomposed into three matrix, say the score matrix $\mathbf{T}$ ($n \times m$), the loading matrix $\mathbf{V}$ ($m \times m$), and the residual matrix $\mathbf{E}$ ($n \times m$) as follows

$$\mathbf{X} = \mathbf{T}\mathbf{V}^{\mathrm{T}} + \mathbf{E} \tag{2}$$

with the restrictions that $\mathbf{T}^{\mathrm{T}}\mathbf{T}$ is diagonal and $\mathbf{V}^{\mathrm{T}}\mathbf{V}$ equals **I** (identity). The score matrix **T** captures the systematic variation related to the observations. The loading matrix **V** captures systematic information related to the variables, and **E** consists of unmodeled variation. Then, with the residual matrix omitted, the score matrix can be estimated as

$$\mathbf{T} = \mathbf{XV} \tag{3}$$

In the quality control, an authentic sample (AUS) or a standard sample is often required for reference [33,34]. Assume that $\mathbf{X}^0$ is the DAD matrix of AUS, and we can calculate the *i*th principal component (PC) of the score matrix, $\mathbf{t}_i^0$, as follows

$$\mathbf{t}_i^0 = \mathbf{X}^0\mathbf{v}_i^0 \tag{4}$$

where $\mathbf{v}_i^0$ is the *i*th column of loading matrix. Considering that the first principle component (PC1) represents the maximal variation of the data matrix, we simply use PC1 to describe the systematic information,

$$\mathbf{t}_1^0 = \mathbf{X}^0\mathbf{v}_1^0 = v_{11}\mathbf{x}_1^0 + v_{21}\mathbf{x}_2^0 + \cdots + v_{m1}\mathbf{x}_m^0 \tag{5}$$

where vector of $\mathbf{v}_1^0$ is the first column of loading matrix, and $\mathbf{x}_1^0 \sim \mathbf{x}_m^0$ are chromatographic fingerprints of AUS at various wavelengths. In Eq. (5), $\mathbf{t}_1^0$ is a linear combination of 1D chromatographic fingerprints at fixed wavelengths, and the weights of each fingerprint are expressed by the loading vector of $\mathbf{v}_1^0$. Obviously, both the information of chromatogram and spectrum are extracted and compiled into $\mathbf{t}_1^0$.

2.2. 2D fingerprint generating

By the method described in Section 2.1, the PC1 of the score matrix can be considered as 2D fingerprint of the sample. In Eq. (5), the weights, i.e. the loading vector $\mathbf{v}_1^0$, is referred to a one-dimensional axis, through which the data of DAD are projected onto to form a model to extract 2D information. Generally, the loading vector of $\mathbf{v}_1^0$ derived from AUS describes the common spectral characteristics for the same species of samples. Then in the practice of quality control, we can conveniently generate 2D fingerprint of a new sample just from its DAD matrix $\mathbf{X}^{\mathrm{new}}$ and the weight vector of $\mathbf{v}_1^0$ as the following,

$$\mathbf{t}_1^{\mathrm{new}} = \mathbf{X}^{\mathrm{new}}\mathbf{v}_1^0 \tag{6}$$

AUS is generated from the samples which are well recognized to have good quality and then perform HPLC/DAD analysis. Fig. 1 shows the contour map of the DAD data. At the wavelengths less than 240 nm, there are strong background absorptions and baseline drifts, while above 450 nm most components have no or only very weak responses. Thus in this study, we utilize the DAD data at the wavelengths of 240–450 nm to generate 2D fingerprint.

By the method of Section 2.1, vectors of $\mathbf{t}_1^0$ and $\mathbf{v}_1^0$ are obtained, which are plotted in Figs. 2 and 3, respectively. In Fig. 2, $\mathbf{t}_1^0$ explains over 99.5% variance of the data matrix,

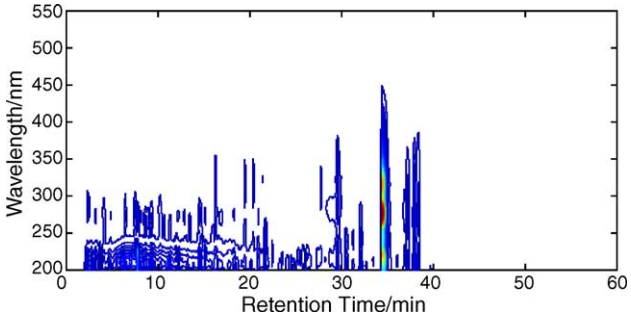

Fig. 1. Contour map of HPLC/DAD chromatogram of AUS.

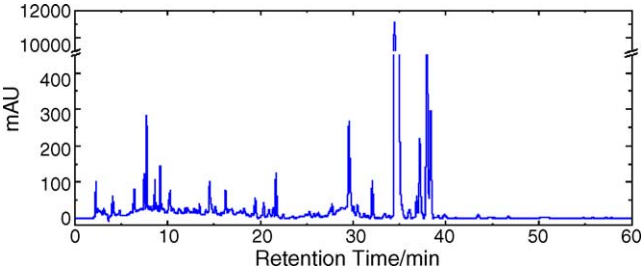

Fig. 2. Plot of the score vector $\mathbf{t}_1^0$ of AUS vs. retention time PC1 explains over 99.5% variance.

which indicates that the most information of DAD data is successfully extracted.

Fig. 3 shows the plot of $\mathbf{v}_1^0$ (in solid line), which represents the weights to generate 2D fingerprint. It reveals that all the chromatograms at various wavelengths contribute to 2D fingerprint, while at 285, 330 nm and their adjacent wavelengths have the largest contribution. Further investigation suggests that the loading plot is quite similar to the UV spectrum of the components of baicalin (shown in Fig. 3 with dashed line). That is, with such a weight vector, the 2D fingerprint contains the most chromatographic information of baicalin. It is logical because baicalin is of the highest amount in *Qingkailing* injection, and moreover, it is one of the most effectual components with high pharmacological activity.

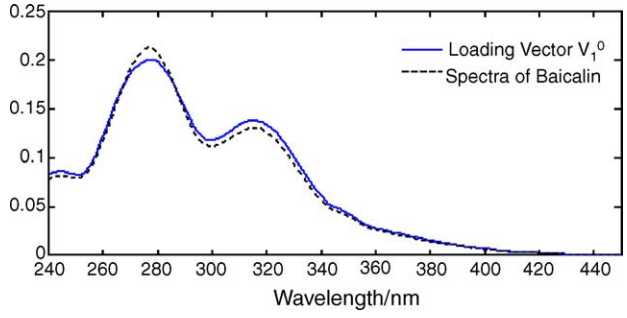

Fig. 3. Plot of loading vector $\mathbf{v}_1^0$ of AUS vs. wavelengths and the spectrum of the component of Baicalin.

2.3. Quality control by 2D fingerprint

In this study, we use the data of all the available peaks in 2D fingerprint for quality control. However, the data matrix of peak areas cannot directly reveal the quality characteristics of products, so the methods of PCA and similarity evaluation are employed.

PCA describes the variation in data with minimum latent variables. The score values for the first two PCs, say PC1 and PC2, are often used to represent the characteristics of the samples. Therefore, grouping or classification of the fingerprints is more easily discovered, and outliers (abnormal fingerprints) can be diagnosed or detected.

Similarity measurements between a tested sample and a reference sample (AUS) can be used to quantitatively conduct quality assessment. The most commonly used standard is congruence coefficient [7], as expressed by the following formula:

$$r = \frac{\sum_{i=1}^{n} x_i x_i^0}{\sqrt{\sum_{i=1}^{n} x_i^2 \sum_{i=1}^{n} x_i^{0^2}}}$$

where r is the value of similarity value between fingerprint $\mathbf{x}$ and that of AUS, and x_i, x_i^0 represent the ith elements of the ith peak of each fingerprint, which can be the response value of signal, area or height of the peak, etc. The value r of the congruence coefficient is in the range $0 < r \leq 1$. The larger the value of r is, and the higher the quality of target samples is. When r equals 1, they are identical.

3. Experimental

3.1. Instrumentation and materials

The HPLC/DAD analysis was performed using a Dionex P580 liquid chromatograph (Dionex Inc.) equipped with a diode-array UV detector (USD340S), an intelligent quaternary pump, a column oven and a manual injection system with a 20 μL loop.

Standard chemicals, baicalin, geniposide, adenosine, uridine, caffeic acid and chlorogenic acid were purchased from the National Institute of China for the Control of Pharmaceutical and Biological Products. Methanol and acetonitrile were of HPLC grade (Sigma Inc., USA). Ultrapure water (18.2 MΩ) was prepared with Milli-Q water purification system (Millipore, France).

Totally 14 *Qingkailing* injection samples (marked as 1–14) were collected from two Chinese medicine manufacturers. Samples 1–5 and 6–10 were from the same manufacturer (manufacture A), while produced in two different procedures. Samples 11–14 were from manufacturer B. The names of manufacturers had been removed in order to preserve confidentiality.

3.2. Sample preparation

AUS was prepared by blending samples 11–14 in equal proportions. All the 15 samples (include AUS) were diluted to 1/5 (v/v) with ultrapure water, and then filtered through a 0.45 μm membrane filter for HPLC analysis.

3.3. HPLC/DAD analysis

The column of C_{18} RP-ODS (250 mm × 4.6 mm, 5 μm, Phenomenex Luna, USA) and another C_{18} guard column (7.5 mm × 4.6 mm, 5 μm, Alltech, IL) were used. The mobile phases were composed of water/formic acid (100/0.1, A) and methanol/acetonitrile (4/1, B). Gradient elution was performed using the following linear gradient: 0 min-100% A; 33 min-34% A; 60 min-12% A. The column compartment was kept at the temperature of 35 °C, and DAD detection was performed in the range of 200–594 nm at 1 nm/step.

3.4. Data analysis

Data analysis was performed on a Pentium IV 1.7G processor. Origin7.0 (OriginLab Co.) and similarity evaluation system for chromatographic fingerprint of TCM (Chinese Pharmacopoeia Committee, 2004A) were used to process 2D fingerprint. PCA and other involved programs were coded in MATLAB 5.3 (Mathworks Inc.).

4. Results and discussion

4.1. Comparison of 2D fingerprint and conventional 1D chromatographic fingerprint

By the presented approach, 2D fingerprint of each sample were generated. The conventional 1D chromatographic fingerprints of *Qingkailing* injection were also obtained at wavelengths of 240, 254, 280 or 330 nm (shown in Fig. 4), which were maximal UV absorption wavelengths of the most important four species of active components respectively (i.e. nucleoside, iridoid glucoside, organic acid and flavone glycoside).

Compared with 1D chromatographic fingerprint, 2D fingerprint contains more peaks. For instance, as shown in Fig. 4, 1D chromatographic fingerprints of 280, 254 and 240 nm have less or rather weak peaks in the retention time of 16–21 min, and that of 330 nm even has no signals in the first 16 min. We also studied the DAD chromatogram of six standard chemicals which were known as the important effectual components in *Qingkailing* injection. From the retention times and UV spectra, we determined their corresponding peaks in each fingerprint (marked as peak 1–6, in Fig. 4). It can be seen that each 1D chromatographic fingerprint lacks at least one peak, while 2D fingerprint contains all the six peaks. It is well known that, many of the constituents of

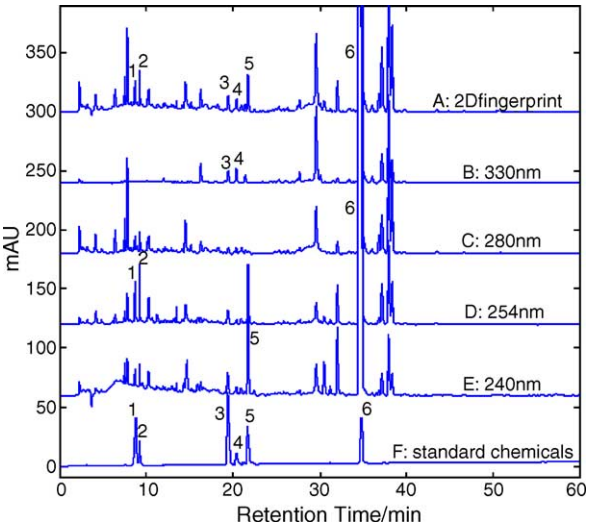

Fig. 4. Comparison of 1D chromatographic fingerprints and 2D fingerprint: (A) 2D fingerprint; (B)–(E) 1D chromatographic fingerprint (at 330, 280, 254, 240 nm, respectively); (F) chromatogram of standard chemicals (at 254 nm, 1. uridine 2. adenosine 3. chlorogenic acid 4. caffeic acid 5. geniposide 6. baicalin).

TCM, whether active or inactive, exert synergistic activity and hence the fingerprint should ideally include the most of the possible constituents of TCM. In the sense, the developed 2D fingerprint shows superiority than conventional 1D fingerprint in the quality control.

4.2. Validation of 2D fingerprinting

To ensure the validity of this newly developed 2D fingerprinting method, validation tests of precision, repeatability and stability were performed. Origin 7.0 was used to acquire peak area and retention time, while too many peaks integrated, it was impossible to test each peak, so peaks 1–6 (Fig. 4) in 2D fingerprint were selected as the representatives of the constituents in *Qingkailing* for validation. All the validations were measured by calculating their relative standard deviations (RSD) of the relative areas and retention times of each target peak. The method precision of 2D fingerprinting was determined by performing five reduplicate HPLC/DAD analysis of the same working solution and then their 2D fingerprints were generated. To confirm the repeatability, five different working solutions produced from a same sample were analyzed. The stability test was performed with sample solutions over a period of 2, 4, 8, 12 and 24 h.

The data of precision, repeatability and stability tests of the proposed method are listed in Tables 1 and 2. Most RSDs are less than 5%, which indicate that the method is precise and repeatable, and the sample is stable to be analyzed in 24 h. The proposed 2D fingerprinting, therefore, is acceptable.

4.3. Quality control for Qingkailing injections using 2D fingerprint

In the old quality criterion [35], only the quantification of marker substances of baicalin, geniposide and the total

Table 1
Precision data of the proposed method

Peak no.	Compounds	Observations					Mean	RSD (%)
		1	2	3	4	5		
1	Uridine							
	Area[a]	5.43	5.09	5.3	4.99	5.57	5.23	4.51
	R.T.[b]	8.84	8.75	8.78	8.88	8.91	8.83	0.767
2	Adenosine							
	Area	8.49	9.04	8.57	8.83	9.25	8.84	3.59
	R.T.	9.58	9.45	9.38	9.46	9.45	9.46	0.764
3	Chlorogenic acid							
	Area	3.88	3.95	4.06	3.89	4.05	3.97	2.16
	R.T.	19.49	19.58	19.61	19.55	19.51	19.55	0.254
4	Caffeic acid							
	Area	2.62	2.35	2.41	2.45	2.48	2.46	4.10
	R.T.	20.87	20.89	20.98	21.01	20.96	20.94	0.285
5	Geniposide							
	Area	12.68	13.00	12.83	13.21	12.98	12.94	1.53
	R.T.	21.98	22.03	21.93	21.91	22.10	21.99	0.351
6	Baicalin							
	Area	2377.88	2473.23	2410.25	2337.49	2280.17	2375.80	3.07
	R.T.	35.23	35.67	35.7	35.53	35.58	35.54	0.527

[a] Area: peak area (mAu min).
[b] R.T.: retention time (min).

Table 2
Repeatability and stability data of the proposed method

Peak no.	Compounds	Repeatability ($n = 5$)				Stability ($n = 5$)			
		Peak area (mAu min)		R.T. (min)		Peak area (mAu min)		R.T. (min)	
		Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
1	Uridine	5.35	5.85	8.97	1.28	5.65	6.03	9.02	1.04
2	Adenosine	9.02	5.02	9.53	0.98	8.76	5.80	9.60	0.80
3	Chlorogenic acid	3.98	5.33	19.92	0.91	4.32	1.38	19.65	0.20
4	Caffeic acid	2.56	5.60	21.06	1.11	3.11	4.20	20.92	0.26
5	Geniposide	13.21	4.60	22.14	0.65	13.18	2.44	21.89	0.40
6	Baicalin	2392.32	2.38	35.40	0.53	2436.77	3.48	34.93	0.17

amount of nitrogen are required, which could hardly ensure the quality effectively. In this study, 2D fingerprint was applied for quality control of *Qingkailing* injection.

We integrated all the available peaks of 2D fingerprint for each sample (including AUS). The corresponding peaks of various fingerprints were matched by the software of similarity evaluation system for chromatographic fingerprint of TCM. Data normalization transformation was also performed using $x'_{ij} = x_{ij} - \min(x_j)/\max(x_j) - \min(x_j)$, here min ($x_j$) and max ($x_j$) were respectively the minimal and maximal value of vector x_j. Then, we ran quality assessment on these corrected objects.

Just from the figure of 2D fingerprints, it is difficult to tell the differences from various samples. But if we simply perform PCA upon the data of 2D fingerprints, the discrepancies of the samples are shown clearly in the scores plot of PC1 versus PC2 (in Fig. 5). From the scatter points, the samples can be classified into three groups, which are marked as groups I–III, respectively. Samples of groups I and II, which are all from manufacturer A, are far apart from AUS, while samples of group III (from manufacturer B) are projected together with AUS, which suggests that the products from manufacturer B are superior to those from manufacturer A. Although samples of groups I and II are produced by the same manufacturer, they differ clearly in quality due to their different preparation procedures.

Sample 1 and sample 6 were picked out as representatives of the samples produced in two different procedures. We compared their 2D fingerprints with that of AUS (shown in Fig. 6) to study the effect of preparation procedures on the product quality. In the fingerprint of sample 1, compared with that of AUS, the peak around the retention time of 22 min (geniposide) is much higher, and moreover, many extra peaks appear in the fingerprint of sample 1, especially at the retention time of 11–14 min (marked by a rectangle frame), while some other peaks around 15 min and 20 min are almost disappear. The fingerprint of sample 6 seems much more similar to that of AUS, only with the exception of an additional peak at the retention times of 21 min, and lack of two peaks at 27 min and 32 min.

Further investigation indicates that the preparation procedures of the samples in group I are different from the conventional procedures issued by the Department of Health of the Republic of China, especially the extraction of *Fructus Gardenise*. In order to take full utilization of herbal material and to acquire more geniposide to meet the old quality criteria, *Fructus Gardenise* is extracted with the solvent of alcohol, while water is used in the traditional procedure. As a result, the yield of geniposide increases, while unex-

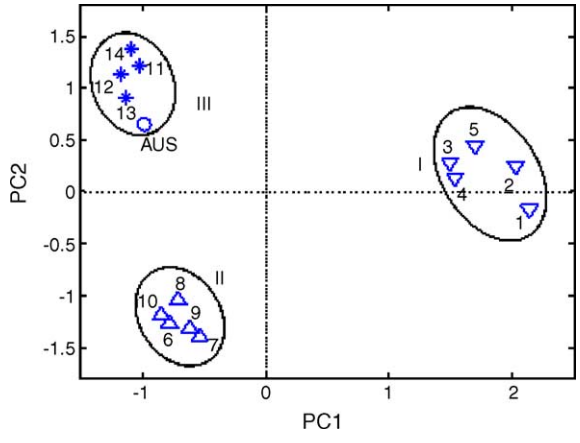

Fig. 5. Representation of 2D fingerprints of various *Qingkailing* injections and AUS on PC1 and PC2 (83.4% variance explained): (▽) samples of manufacturer A with initial preparation procedures; (△) samples of manufacturer A with adjusted preparation procedures; (*) samples of manufacturer B; (◯) AUS.

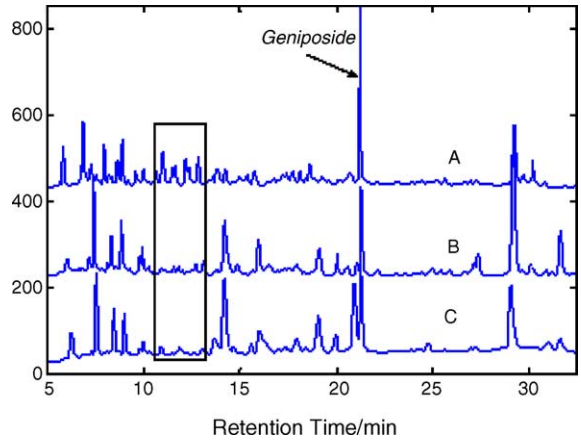

Fig. 6. An amplified segments of 2D fingerprints of *Qingkailing* injections and AUS: (A) sample 1 (B) AUS (C) sample 6.

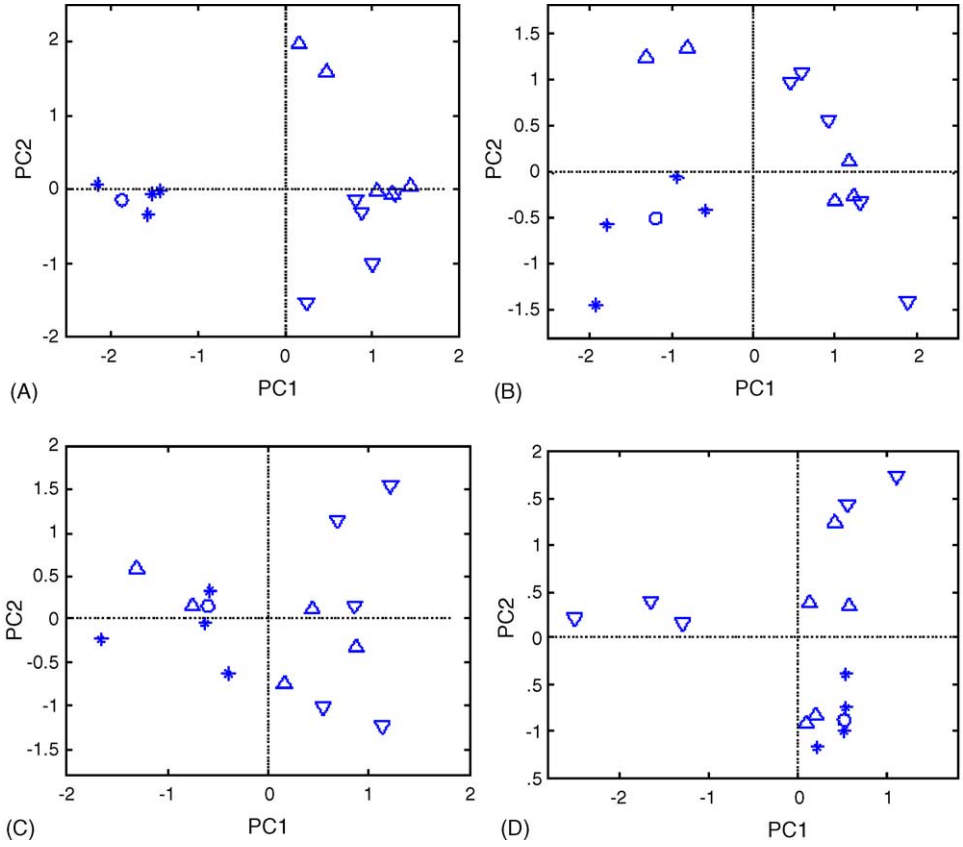

Fig. 7. Representation of 1D fingerprints of *Qingkailing* injections and AUS on PC1 and PC2: (A) fingerprint at 240 nm (65.0% variance explained); (B) fingerprint at 254 nm (83.7% variance explained); (C) fingerprint at 280 nm (65.0% variance explained); (D) fingerprint at 330 nm (73.0% variance explained); (▽) samples of manufacturer A with initial preparation procedures; (△) samples of manufacturer A with adjusted preparation procedures; (*) samples of manufacturer B; (◯) AUS.

pected components are simultaneously extracted and some other components decreases, which may lead to unqualified products. In the year of 2004, just with our suggestions, the preparation procedures, especially the extraction procedure of *Fructus Gardenise*, are adjusted to fit the issued ones. Thus, the samples of group II, which are produced in the adjusted procedures, are of higher quality.

Four conventional 1D chromatographic fingerprints are also applied in the quality control for the same samples. The PCA results are shown in the Fig. 7. Seen from Fig. 7(A)

Table 3
Similarity values of *Qingkailing* injection samples

Sample no.	2D fingerprint	1D fingerprint			
		240 nm	254 nm	280 nm	330 nm
1	0.312	0.334	0.377	0.442	0.313
2	0.340	0.388	0.421	0.370	0.267
3	0.394	0.553	0.408	0.381	0.289
4	0.363	0.441	0.378	0.543	0.469
5	0.352	0.420	0.379	0.499	0.475
6	0.653	0.261	0.411	0.562	0.607
7	0.666	0.347	0.363	0.553	0.454
8	0.704	0.412	0.494	0.558	0.562
9	0.697	0.380	0.654	0.519	0.766
10	0.625	0.519	0.646	0.655	0.835
11	0.887	0.924	0.864	0.811	0.854
12	0.922	0.798	0.787	0.879	0.947
13	0.922	0.949	0.853	0.831	0.878
14	0.916	0.847	0.820	0.807	0.920

and (B), 1D fingerprints at 240 nm and 254 nm can properly discriminate various samples from different manufacturers, but the samples in various procedures couldn't be identified; while fingerprints at 280 and 330 nm can determine neither the manufacturers nor the procedures (shown in Fig. 7(C) and (D)). So that compared with 1D chromatographic fingerprint, 2D fingerprint has much more classification and discrimination power in quality control, and consequently is more likely to reveal the further qualitative characteristics of *Qingkailing* injections.

In this study, similarity assessment was also applied to perform further quality assessment. The similarity values between the fingerprint of each sample and that of AUS were calculated, as shown in Table 3.

From Table 3, it can be easily seen that using 2D fingerprint technique, all the samples are classified into three groups with different quality grades, just the same as the PCA analysis results. Samples 1–5 are classified into group I with the similarities of 0.312–0.392, and group II is composed of samples 6–10, whose similarity values are in a range of 0.653–0.704. The highest similarities (0.887–0.922) are shown in the samples 11–14 of group III. Obviously, the samples of group I are the worst, group II the better, and those of group III are the best. From 1D chromatographic fingerprints, all the samples of group III can be successfully determined as superior products with higher values of similarity, however the quality differences of samples 1–10 produced in different procedures could not be distinguished. Thus, compared with 2D fingerprint, the classification ability and discrimination power of 1D chromatographic fingerprint is limited to perform further quality control.

5. Concluding remarks

In general, with additional spectral information compiled, 2D fingerprint hence can comprehensively and properly reveal the quality characteristics of TCM, and logically it can reach more objective conclusions in the practice of quality control of TCM. The presented approach is equally applicable to other TCM. Suffice it to say that 2D fingerprints would provide a powerful and meaningful tool to comprehensively conduct the quality control of TCM products.

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