ORIGINAL ARTICLE

Chromatographic fingerprinting and quantitative analysis for the quality evaluation of Xinkeshu tablet

Pei Wang[a], Lian Li[a], Hailong Yang[a], Shijuan Cheng[b], Yingzi Zeng[b], Lei Nie[a,*], Hengchang Zang[a,*]

[a]National Glycoengineering Research Center and School of Pharmaceutical Sciences, Shandong University, No. 44 Wenhuaxi Road, Jinan 250012, China
[b]Shandong Wohua Pharmaceutical Limited Company, No. 519 Liyuan Road, Weifang 261205, China

Abstract A simple, sensitive and accurate method based on high performance liquid chromatography (HPLC) with diode array detector (DAD) was developed and validated for systematic quality evaluation of one type of traditional Chinese medicine preparations named Xinkeshu (XKS) tablet. In this study, the chromatographic fingerprints of XKS tablet were developed first, 23 peaks were selected as the common peaks to evaluate the similarities among different batches of XKS samples, which were manufactured in a long time span of three years. Additionally, simultaneous quantification of six markers in XKS tablet, including Danshensu, Protocatechuic aldehyde, Puerarin, Daidzin, Salvianolic acid B and Daidzein, was performed. The validation results showed that the developed method was specific, accurate, precise and robust. The preliminary explanation on why a close similarity between fingerprints did not exactly mean similar contents of chemical components in samples was given. The contribution of each chromatographic peak to similarity was also evaluated. The developed method offers an efficient, reliable and practical approach for systematic quality evaluation of XKS tablet.

*Corresponding authors. Tel./fax: +86 531 88380268.
  E-mail addresses: nielei2004@sdu.edu.cn (L. Nie), zanghcw@126.com (H. Zang)

1. Introduction

During the last decade, traditional Chinese medicines (TCMs) have attracted more and more attention because of huge medicinal and economic values. Since pharmacological and biological active constituents of TCMs are usually very complex, particular concern has focused on their quality, safety and efficacy [1,2]. It is important to note that, the characteristics of multi-target and synergistic action of TCMs come from their multiple constituents, and if only few

constituents are emphasized, the holistic nature of TCMs will be neglected. Thus, how to control the quality more effectively and systematically is one of the urgent issues in application and development of TCMs [3].

In general, the chromatographic fingerprint is considered to be one of the most important and acceptable approaches for quality evaluation of herbal preparations during the last decade [4,5]. This technique puts emphasis on systematic characterization of a complex matrix with a quantitative degree of reliability [6]. It was usually used to evaluate authenticity, superiority and stability of Chinese medicinal materials, TCM preparations and their semi-finished products [7]. To facilitate the requirement of quality assurance (QA) approaches for such complex products such as TCMs, the Food and Drug Administration (FDA) and World Health Organization (WHO) both have accepted chromatographic fingerprint as a strategy to assess the consistency of botanical drugs [8,9]. In 2004, the State Food and Drug Administration of China (SFDA) also clearly required that all the injections made from herbal medicines or their raw materials should be standardized by chromatographic fingerprints [10].

Although chromatographic fingerprint technique has been accepted as one of the most rational and powerful approaches for quality evaluation of TCMs, it still has some limitations. For instance, a close similarity between fingerprints does not exactly mean similar contents of chemical components in samples, because the contents may be affected by the instability of the preparation and diverse sources of herbal medicines [11]. Therefore, for systemic quality evaluation of TCMs, the chemical pattern analysis recognition methods such as simultaneous quantification of multi-ingredients should be taken into consideration.

Xinkeshu (XKS) tablet, is a traditional Chinese medicine for the treatment of coronary heart and cerebrovascular disease [12]. It is a really complex matrix, which comprises five medicinal materials or extracts thereof, including *Salvia miltiorrhiza Bge*, *Radix Puerariae*, *Hawthorn*, *Panax Notoginseng* and *Radix Aucklandiae*. All of these medicinal materials collected in the Chinese Pharmacopoeia(edition 2010, volume 1) were used in one formula and had significant therapeutic effects in clinical practice [13,14]. According to the theory and hypothesis of TCMs, those five herbs play different roles in the prescription of XKS. Among them, *Salvia miltiorrhiza Bge* and *Radix Puerariae* are major herbs in content, and they play the important role of "King" and "minister", respectively [15]. For instance, Puerarin is required to be quantified by HPLC–UV for quality control of XKS in the Chinese Pharmacopoeia [11]. So far quite few approaches have been developed for the determination of the bioactive constituents in XKS tablet. The reports published just mainly concentrated on determination of individual or several components in XKS [16,17]. Yet, these methods applied to quality control have apparent limitations, because the determination of just one or two components is very difficult to reflect the intrinsic quality of XKS, and is insufficient to reveal the complexity and synergic effect of this formula. A recent report concerning qualitative and quantitative analysis of XKS preparation was published [18]. The focal point of the report was mainly on simultaneous identification and quantification of multi-constituent in XKS rather than HPLC-fingerprint analysis for quality assessment. The developed method was based on liquid chromatography coupled with linear trap quadrupole and high resolution mass analyzer-orbitrap

(LC-LTQ-Orbitrap). Because LTQ-Orbitrap mass spectrometry system (Thermo-Fisher Scientific, Bremen, Germany) is very expensive, most of pharmaceutical companies in China cannot afford to be equipped with such a precious mass spectrometer. Therefore, the application of the HPLC method based on this MS system will be still limited. To the best of our knowledge, no literature has been concerned on application of chromatographic fingerprint technology as a quality control method to the quality assurance of XKS tablet. Considering applicability and popularization, a simple and rapid method based on general LC system is quite necessary to be developed for the quality of XKS.

In the present study, a specific, practical and quantifiable HPLC-fingerprint approach to quality assessment of different batches of XKS tablet was developed. Quantification of six bioactive components in 15 commercial samples of XKS tablet was also carried out. Additionally, the preliminary explanation on why the close similarity between chromatographic fingerprints could not reflect the similar contents of the important components was given. A valuable insight from different angles into the application of chromatographic fingerprint and quantification of active constituents to quality evaluation of XKS tablet may be provided.

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile and methanol (HPLC grade) were purchased from Merck Company Inc. (Merk, Darmstadt, Germany). Formic acid (HPLC grade) was purchased from Tedia Company Inc. (Tedia Way, Fairfield USA). Ultrapure water was purified with a Milli-Q system (Millipore, Bedford, USA). All other reagents were of analytical grade.

Reference compounds including Danshensu, Puerarin and Daidzin were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and Protocatechuic aldehyde, Salvianolic acid B and Daidzein were obtained from National Pharmaceutical Engineering Center for Solid Preparation in Chinese Herb Medicine (Jiangxi Herb-fine Hi-tech Co. Ltd., China). All of the six reference compounds have a purity of more than 98%. Their chemical structures are presented in Fig. 1. Fifteen batches of XKS were provided by Shandong Wohua Pharmaceutical Limited Company in China.

2.2. Sample preparation

Each appropriate amount of standard that accurately weighed for quantification was dissolved in 70% methanol as stock solutions, respectively. A certain amount of the above stock solutions were mixed well and diluted to an appropriate concentration as standard solutions, which were stable at least for 2 weeks under 4 °C.

The outer coats of XKS tablets were removed. All the samples were milled into the homogeneous size. The pulverized powder of 100 mg for each sample was accurately weighed and ultrasonically extracted with 5 mL 70% methanol for 20 min in a conical flask at room temperature. This sample solution (4 mL) was transferred to 10 mL centrifuge tube and centrifuged at 3000 rpm for 10 min. The supernatant was
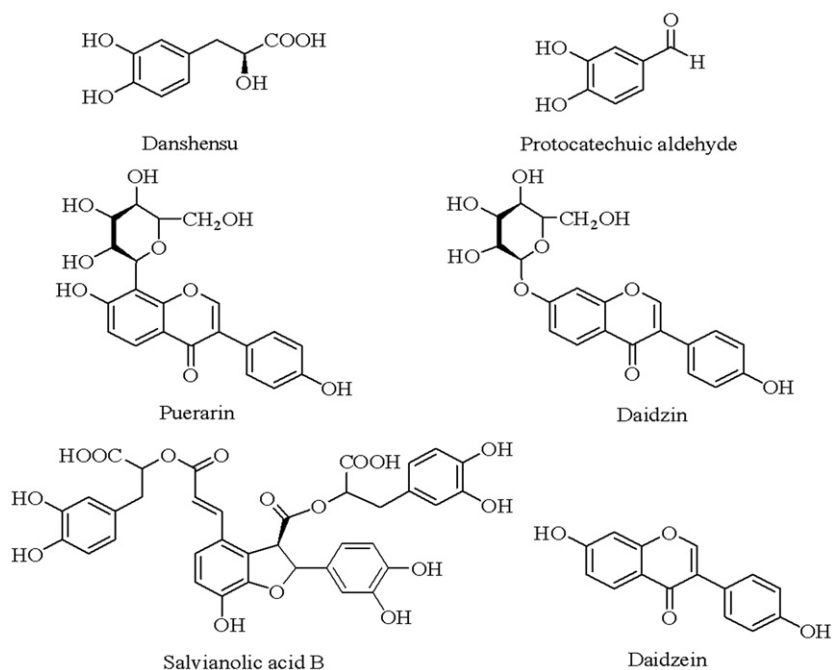
Figure 1 Molecular structures of the six compounds.

further filtered through a 0.45 μm membrane filter before HPLC injection.

2.3. HPLC analysis

An Agilent 1200 series HPLC system (Agilent Corp., Santa Clara, CA, USA) with a diode array detector (DAD) was used to acquire chromatograms and UV spectra. All the chromatographic analysis was performed on a Phenomenex C_{18} column (250 mm × 4.6 mm i.d. 5 μm) protected by a pre-column (12.5 mm × 4.6 mm i.d. 5 μm) of the same material. The mobile phase was composed of acetonitrile (A) and 0.05% phosphoric acid in water (B). The conditions of solvent gradient elution were 8–12% (A) in 0–21 min, 12–17% (A) in 21–31 min, 17–38% (A) in 31–55 min, 38–90% (A) in 55–65 min, 90% (A) in 65–75 min, at a flow rate of 1.0 mL/min. A pre-equilibration period of 10 min was set between individual runs. For the determination of the major constituents in XKS tablet, the UV detector was set at 278 nm with full spectral scanning from 190 nm to 400 nm. The column temperature was maintained at 30 °C, and all the injection volumes of sample solutions were fixed at 10 μL.

2.4. Data analysis

The software "Similarity Evaluation System for Chromatographic Fingerprint of Traditional Chinese Medicine (Version 2004A)" recommended by SFDA was used to calculate the similarity between a test sample and a reference one for similarity evaluation of different chromatographic fingerprints. All the calculations about the contribution of each chromatographic peak to similarity were carried out in the software system MATLAB 2009B (The Math Works, Inc., Natick, MA, USA).

3. Results and discussion

3.1. Procedure of sample preparation and optimization of chromatographic conditions

To obtain as many peaks as possible in chromatographic fingerprint, some factors involved in extraction procedure, such as extraction time and solvents (ethanol or methanol), were investigated. According to HPLC fingerprints detected, the procedure of 20 min of extraction and 70% methanol was adopted since the fingerprints had clear characteristics as follows: more peaks with high response, little interference and good peak shape.

To achieve a satisfactory level of resolution (R_S), separations were carried out on a Phenomenex Luna C_{18} reversed-phase column. Various compositions of mobile phase were tried to inhibit the ionization of acidic ingredients in XKS tablet extracts for improving the peak shape and restraining the peak tailing. The DAD detector was employed to select appropriate wavelength for detection. After the optimization of HPLC conditions, the R_S values for the six marker compounds were, in generally, higher than 1.5. Acetonitrile-water with 0.05% phosphoric acid was selected as the solvent of mobile phase since the best separation ability and acceptable tailing factor within running time of 75 min by gradient elution were achieved. Detection wavelength was set at 278 nm by comparing all the compounds and the UV characteristic spectra of reference compounds. The chromatogram of six reference compounds and the representative chromatographic fingerprint were shown in Fig. 2.

3.2. Validation of HPLC–DAD quantitative methods

3.2.1. Linearity, limit of detection, limit of quantification
The linearity of six marker compounds was performed with six different concentrations of chemical markers under the
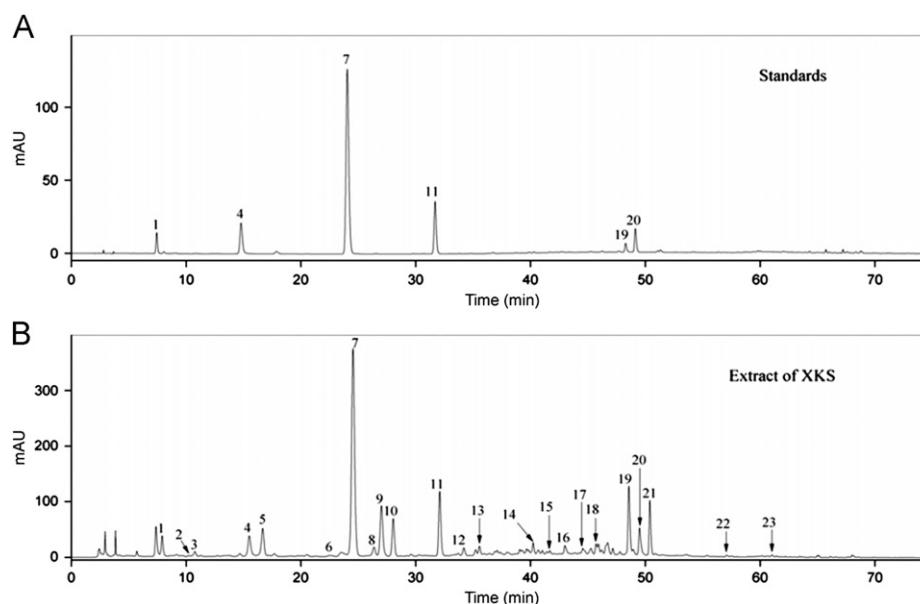
Figure 2 The HPLC–UV chromatogram of (A) 6 mixed standards and (B) the representative HPLC-fingerprint of extract of XKS. The numbers from 1 to 23 correspond to the common peaks.

Table 1 Calibration curves, LOD and LOQ for the six marker compounds obtained with the optimized HPLC–DAD method.

Analyte	Calibration curve[a]	Correlation coefficient (r^2)	Linear range (µg/mL)	LOD [b] (µg/mL)	LOQ [c] (µg/mL)
Danshensu	$y = 6.154\,x - 3.7725$	0.9996	0.64–159.20	0.14	0.43
Protocatechuic aldehyde	$y = 42.792x - 4.2797$	1.0000	0.72–35.90	0.17	0.52
Puerarin	$y = 15.470x - 5.9912$	1.0000	2.31–577.20	0.22	0.63
Daidzin	$y = 17.595x - 1.6224$	1.0000	0.56–112.20	0.18	0.53
Salvianolic acid B	$y = 9.320x - 7.0816$	0.9999	1.13–141.40	0.27	0.81
Daidzein	$y = 28.743x - 2.1099$	1.0000	0.53–26.50	0.14	0.42

[a]The calibration curves were constructed by six different concentrations of marker compounds.
[b]LOD refers to the limits of detection.
[c]LOQ refers to the limits of quantification.

optimal separation conditions and UV detection (278 nm). Each concentration was analyzed in triplicate. The chromatogram of the 6 mixed standards is shown in Fig. 2A. Calibration curves were constructed by plotting the integrated peak areas (Y) of chromatography versus the corresponding concentrations of the injected standard solutions (X). The calculated results are summarized in Table 1. Good linear calibrations ($r^2 >$ 0.9996) for all the analytes were achieved in a relatively wide concentration range. The limits of detection (LOD) and quantification (LOQ) were determined at a signal-to-noise ratio (S/N) of 3 and 10, respectively (see Table 1).

3.2.2. Precision and repeatability
The measurement of intra- and inter-day variability was utilized to assess the precision of the analytical method established. The intra-day variation was determined by analyzing the known concentrations of six analytes in six replicates during a single day, while inter-day variation was determined in duplicated on three consecutive days, respectively. To confirm the repeatability, five independently prepared working solutions of XKS with the same amount were analyzed. The results of precision and repeatability are summarized in Table 2. The overall intra- and inter-day variations were less than 3.6% (ranging 0.2–3.6%), indicating that satisfactory precision of the instrumentation and stability of the samples were achieved. Furthermore, the analytical method developed had a good repeatability with RSD less than 1.9% ($n = 5$) for six analytes.

3.2.3. Recoveries
Three different quantities (low, medium and high) of the authentic standards were added into the sample (batch no. 100932) to evaluate the accuracy of the developed analytical method. The mixtures were extracted and quantified as described before. Then, the quantity of each component was subsequently calculated from the corresponding calibration curves. The results are summarized in Table 3. The method had a satisfactory accuracy with the overall recovery from 98.6% to 103% for the six marker compounds.

3.3. Sample analysis of XKS

For the simultaneous determination of six major components in Chinese medicine XKS from different batches, the proposed

Table 2 Precision and repeatability of the six marker compounds obtained with the optimized HPLC–DAD method.

Analyte	Precision				Repeatability ($n=5$)	
	Intra-day ($n=6$)		Inter-day ($n=6$)			
	Mean (µg/mL)	RSD (%)	Mean (µg/mL)	RSD (%)	Mean (µg/mL)	RSD (%)
Danshensu	81.13	0.9	80.91	2.5	91.27	1.9
Protocatechuic aldehyde	19.52	0.6	19.61	0.7	21.40	0.9
Puerarin	299.63	0.3	297.22	0.9	314.11	0.9
Daidzin	55.30	0.2	55.15	0.5	64.09	0.8
Salvianolic acid B	68.96	0.3	68.91	0.7	94.85	1.2
Daidzein	12.87	0.9	12.44	3.6	13.62	1.8

Table 3 Recoveries of the six marker compounds obtained with the optimized HPLC–DAD method.

Analyte[a]	Initial amount (mg)	Added amount (mg)	Detected amount (mg)	Recovery (%)	RSD (%)
Danshensu	0.201	0.096	0.299	102.9	2.1
		0.191	0.389	98.6	3.0
		0.287	0.479	97.1	1.1
Protocatechuic aldehyde	0.047	0.022	0.069	97.1	2.5
		0.043	0.090	99.0	0.7
		0.066	0.113	101.2	2.7
Puerarin	0.767	0.346	1.110	99.4	1.1
		0.693	1.455	99.6	1.9
		1.039	1.796	99.2	0.7
Daidzin	0.142	0.067	0.209	98.4	0.6
		0.135	0.277	99.4	1.9
		0.202	0.346	100.4	1.6
Salvianolic acid B	0.173	0.085	0.261	100.9	3.2
		0.170	0.348	101.2	1.0
		0.255	0.434	101.6	2.0
Daidzein	0.033	0.016	0.049	103.0	3.6
		0.032	0.065	101.5	1.2
		0.048	0.081	101.0	2.1

[a]Triplicate assays at each concentration level.

HPLC–DAD method was applied by comparing the retention times and on-line UV spectra with those of standards. Each sample was determined in triplicate.

The results summarized in Table 4 revealed that the contents were obviously different among batches. According to literatures [19–21], the variation of contents of TCMs was mainly ascribed to the different quality of raw materials, the varying production procedure, storage, etc. The similar results were also confirmed in our study. For instance, the puerarin, daidzin and daidzein contents were close from batch to batch in samples 6–8 or samples 12–15, which were prepared with similar *Radix Puerariae* material. However, if the analyzed samples were produced in a large time span of three years, the content of some marker components had a big fluctuation with high RSD values (for example, the RSD value of Daidzein was up to 27.4%. See Table 4).

3.4. Chromatographic fingerprint analysis

To obtain the standardized fingerprint, fifteen batches of XKS tablets which were manufactured in different years were analyzed with the developed HPLC–DAD method. As shown in Fig. 2B, 23 peaks (denoted from 1 to 23) in the representative fingerprint were assigned as "common peaks", which were existed in all 15 batches of samples (Fig. 2B). The peak of Puerarin (peak **7**), which was symmetrical and located in the middle of the chromatogram, was selected as a reference peak. The sum of all the 23 peak areas accounted for more than 90% of the sum of total peak areas in the chromatogram.

The "common peaks" 1, 4, 5, 7, 11, 16 and 19 were adopted as reference peaks to match the chromatographic fingerprints of different batches for similarity evaluation when using the software "Similarity Evaluation System for Chromatographic Fingerprint of TCM". The standard chromatographic fingerprint shown in Fig. 3 was derived from the fingerprints of 15-batch samples by average method. The method validation of fingerprint analysis was performed based on the relative retention time (the ratio of retention time of each peak to that of the reference standard peak) and the relative peak area (the ratio of peak area of each peak to that of the reference standard peak). Their relative standard deviation (RSD) values of the relative retention time were less than 0.7%, which demonstrated good stability and reproducibility of the fingerprint analysis by HPLC. The similarity between standard chromatographic fingerprint and 15 batches of XKS

Table 4 Contents in mg/g of the six marker compounds in XKS collected from different batches.

No.	Batch no.	Danshensu	Protocatechuic aldehyde	Puerarin	Daidzin	Salvianolic acid B	Daidzein
1	080321	6.087 [a]	0.986	16.591	3.415	3.650	0.478
2	080445	4.051	0.767	18.955	3.489	4.021	0.734
3	080563	3.418	0.727	18.703	3.486	3.210	0.789
4	080676	3.838	0.745	16.589	3.418	3.306	0.616
5	081043	5.166	0.892	17.532	3.138	3.174	0.544
6	090518	3.588	0.723	16.752	2.916	2.708	0.556
7	090627	2.991	0.669	16.772	2.859	3.195	0.581
8	090737	3.392	0.730	15.418	2.595	3.911	0.585
9	090946	3.592	0.768	14.467	2.608	4.356	0.573
10	091272	2.934	0.631	20.312	3.871	5.895	0.644
11	100952	3.123	0.716	21.984	4.459	4.674	0.893
12	101067	2.479	0.714	22.614	4.715	2.344	0.974
13	101182	3.581	0.875	20.649	4.279	4.286	0.948
14	101291	4.430	0.970	20.456	4.189	5.425	1.000
15	101296	4.438	1.058	21.323	3.947	5.399	1.102
Average		3.807	0.798	18.608	3.559	3.970	0.734
RSD (%)		24.4	16.0	13.6	18.9	26.3	27.4

Values are expressed in mean ($n=3$).

[a]Data calculated as average of three replicates (RSD<5%).
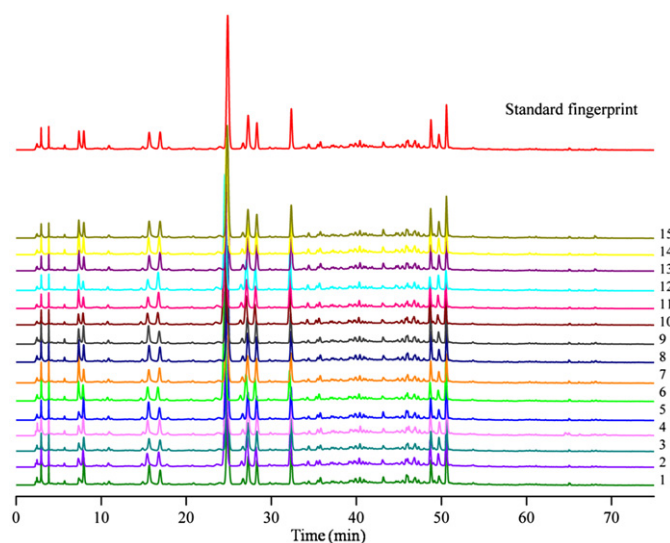
Figure 3 HPLC fingerprints of 15 commercial samples of XKS from different batches and their standard fingerprint.

Table 5 The fingerprint similarities of XKS collected from different batches.

Sample number [a]	Similarities [b]	Sample number	Similarities	Sample number	Similarities
1	0.985	6	0.998	11	0.998
2	0.999	7	0.997	12	0.993
3	0.998	8	0.996	13	0.999
4	0.999	9	0.998	14	0.997
5	0.996	10	0.996	15	0.998

[a]The sample numbers from 1 to 15 is corresponding to different batch no. of XKS.

[b]Similarity calculated by cosine of the angle between the different-batch sample fingerprints and the standard fingerprint generated by average method.

fingerprints were very close to 1 (Table 5), which suggested that the chromatographic fingerprints of different batches of XKS samples were consistent. However, the RSD values of the contents of the six marker compounds among different batches were very high (13.6–27.4%, Table 5). Obviously, a close similarity between fingerprints does not exactly mean

that the XKS samples have similar contents of chemical components, since the contents may be affected by the instability of the preparation and diverse sources of herbal medicines.

According to the results of chromatographic fingerprinting and quantitative analysis of XKS tablet, the similarity between chromatographic fingerprints could not comprehensively reflect the similarity in the contents of a few important components. Similar conclusion has also been confirmed by literature [22]. The reasons resulting in inconsistency of the quality assessment between fingerprints and the contents of several key compounds were preliminarily explained from the following different aspects.

The similarity is the overall evaluation of the fingerprints, while it is ambiguous in content of component. The similarity is a measure of shape closeness of the two profiles [23]. However, the comparison of component contents refers to the difference of components in content. Although the component contents may vary greatly, but the areas of different peaks in fingerprints simultaneously increase or decrease and the shapes of profiles keep the same, then the similarity will be unchanged. If the difference of component contents is very great and then can affect the shapes of the fingerprint profiles, the similarity between fingerprints will change accordingly.

About similarity calculation, two methods were usually used in software mentioned above. One is cosine of the angle (r^{cos}) that is often used, the other is correlation coefficient (r).

They are respectively calculated by Eqs. (1) and (2) as follows:

$$r^{cos} = \frac{\sum_{i=1}^{n} x_i \cdot y_i}{\sqrt{\sum_{i=1}^{n} x_i^2 \sum_{i=1}^{n} y_i^2}} \qquad (1)$$

$$r = \frac{\sum_{i=1}^{n} (x_i - \overline{x}) \cdot (y_i - \overline{y})}{\sqrt{\sum_{i=1}^{n} (x_i - \overline{x})^2 \sum_{i=1}^{n} (y_i - \overline{y})^2}} \qquad (2)$$

where x_i and y_i refer to the chromatographic peak areas of peak i in the two fingerprints which are compared, respectively. n is the number of the chromatographic peaks. $\overline{x}$ and $\overline{y}$ are the average of n chromatographic peak areas of the two fingerprints in the comparison, respectively.

According to Eqs. (1) and (2), if the chromatographic peak areas are different, their contributions to r^{cos} and r will be various. In the present study, a total of 83 peaks in the fingerprints after matching were acquired. To investigate the contribution of each chromatographic peak to the similarity, each peak was deleted in turn, and then the similarities of each batch fingerprint with other batch fingerprints were calculated by r^{cos} and r, respectively. The average value of r^{cos} or r was regarded as the similarity between this batch with other ones. So the similarity values of 15 batches were achieved. The mean
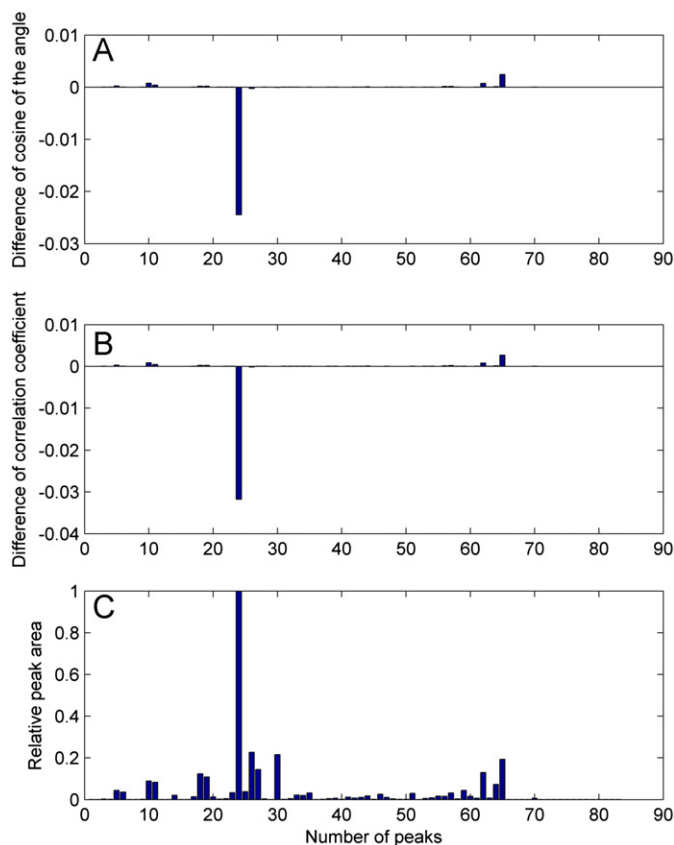

Figure 4 The contribution of each chromatographic peak to the similarity of the 15 batches of XKS. (A) and (B) refer to the difference of the r^{cos} or r before and after each peak was deleted in turn, and (C) refers to the average relative peak areas in the chromatographic fingerprints of the 15 batches of XKS (The peak of Puerarin was used as the reference peak).

of 15-batch similarity values was calculated again and the corresponding average considered as the global similarity of 15 batches was obtained. The difference of the global similarity before and after each chromatographic peak removed was used to evaluate the contribution of each peak to similarity. The results are shown in Fig. 4.

As shown in Fig. 4, before and after each peak was deleted, the change trend of the global similarity value of r^{cos} and r was very similar. In general, the larger the peak area is, the greater contribution to similarity is. Among all the peaks, the peak area of Puerarin is the largest (peak 24, Fig. 4C), and its contribution to similarity is the biggest. While some peaks with relative small peak areas have little or even neglected influence on similarity.

Similarity calculation is based on the chromatographic peak area rather than the content of compound. Because the sensitivity of detector response varies in different compounds, the size of different chromatographic peak areas cannot accurately indicate that the size of the contents of various compounds. Therefore, the similarity between fingerprints cannot exactly reflect the real change in the content of compounds.

Although similarity analysis is ambiguous in content of component, it is still a fast, useful and convenient technique for a preliminary analysis of fingerprints in data set.

4. Conclusion

Although the XKS tablet was officially recorded in Chinese Pharmacopoeia and used more and more widely, the method for evaluating and ensuring its quality still needs to be improved. In the present work, the developed method could provide a systematic quality control including chromatographic fingerprint analysis and simultaneous quantization of markers. The chromatographic fingerprint analysis of herbal products could serve as an efficient tool for quality control and preliminary evaluation of quality consistency of herbal preparations. As a supplement to fingerprint analysis, quantitative analysis of the six marker compounds in fifteen different batches of XKS tablet produced in a long time span of three years was performed. The results of chromatographic fingerprint analysis and quantification of six marker compounds indicated that the samples from different batches shared a satisfactory similarity, but large variations appeared in the contents of constitutes among different batches, the reasons were tentatively discussed above in details. We recommend that a minimum limit on these six marker compounds should be necessary for quality control according to further statistical analysis based on the adequate quantities of samples. The developed method offered an efficient and reliable approach for systematic quality evaluation of XKS tablet.

References

[1] P.S. Xie, A.Y. Leung, Understanding the traditional aspect of Chinese medicine in order to achieve meaningful quality control of Chinese materia medica, J. Chromatogr. A 1216 (11) (2009) 1933–1940.

[2] J. Qiu, China plans to modernize traditional medicine, Nature 446 (7136) (2007) 590–591.

[3] Y.Z. Liang, P. Xie, K. Chan, Quality control of herbal medicines, J. Chromatogr. B 812 (1-2) (2004) 53–70.

[4] X.M. Liang, Y. Jin, Y.P. Wang, et al., Qualitative and quantitative analysis in quality control of traditional Chinese medicines, J. Chromatogr. A 1216 (11) (2009) 2033–2044.

[5] P. Xie, S. Chen, Y.Z. Liang, et al., Chromatographic fingerprint analysis—a rational approach for quality assessment of traditional Chinese herbal medicine, J. Chromatogr. A 1112 (1-2) (2006) 171–180.

[6] D.Z. Yang, Y.Q. An, X.L. Jiang, et al., Development of a novel method combining HPLC fingerprint and multi-ingredients quantitative analysis for quality evaluation of traditional Chinese medicine preparation, Talanta 85 (2) (2011) 885–890.

[7] P. Drasar, J. Moravcova, Recent advances in analysis of Chinese medical plants and traditional medicines, J. Chromatogr. B 812 (1-2) (2004) 3–21.

[8] FDA Guidance for Industry—Botanical Drug Products (Draft Guidance), US Food and Drug Administration, Rockville, 2000, pp. 4.

[9] World Health Organization, Guidelines for the Assessment of Herbal Medicines, Munich, 28.6.1991, WHO, Geneva, 1991.

[10] State Food and Drug Administration of China, Note for Studying Fingerprint of Traditional Chinese Medicine Injections (Draft), Shanghai, 2000.

[11] Y. Jiang, B. David, P. Tu, et al., Recent analytical approaches in quality control of traditional Chinese medicines—a review, Anal. Chim. Acta 657 (1) (2010) 9–18.

[12] Chinese Pharmacopoeia Commission. Pharmacopoeia of the People's Republic of China, vol. 1, Chienical Industry Press, Beijin, 2010. pp. 604.

[13] Y.G. Li, L. Song, M. Liu, et al., Advancement in analysis of Salviae miltiorrhizae Radix et Rhizoma (Danshen), J. Chromatogr. A 1216 (11) (2009) 1941–1953.

[14] K.C. Shum, F. Chen, S.L. Li, et al., Authentication of Radix Aucklandiae and its substitutes by GC–MS and hierarchical clustering analysis, J. Sep. Sci. 30 (18) (2007) 3233–3239.

[15] M. Peng, S.W. Ding, Prescription analysis of Xinkeshu tablets, Tradit. Chin. Med. J. 6 (6) (2007) 62–63.

[16] H.Z. Wei, Z.H. Hang, J.G. Cui, et al., Determination of Salvianolic acid B in Xinkeshu tablet and Xinkeshu capsule by HPLC, Chin. Tradit. Herb. Drugs 38 (8) (2007) 1996–1997.

[17] J.T. He, Z.H. Shi, J. Yan, et al., Simultaneous separation and determination of four bioactive constituents in traditional Chinese medicinal tablet Xinkeshu by HPLC–DAD, Chin. J. Chem. 22 (10) (2004) 1211–1214.

[18] J.B. Peng, H.M. Jia, Y.T. Liu, et al., Qualitative and quantitative characterization of chemical constituents in Xin-Ke-Shu preparations by liquid chromatography coupled with a LTQ Orbitrap mass spectrometer, J. Pharm. Biomed. Anal. 55 (5) (2011) 984–995.

[19] C.Z. Liu, H.Y. Zhou, Q. Yan, Fingerprint analysis of Dioscorea nipponica by high-performance liquid chromatography with evaporative light scattering detection, Anal. Chim. Acta 582 (1) (2007) 61–68.

[20] R. Liu, J. Zhang, M. Liang, et al., Simultaneous analysis of eight bioactive compounds in Danning tablet by HPLC-ESI-MS and HPLC–UV, J. Pharm. Biomed. Anal. 43 (3) (2007) 1007–1012.

[21] C. Feng, Y.L. Cai, J.L. Ruan, Simultaneous determination of 10 active components in traditional Chinese medicine, J. Pharm. Biomed. Anal. 47 (2) (2008) 442–447.

[22] C. Niu, J. Zhang, Quantitative analysis and chromatographic fingerprinting of the semen zizyphi spinosae by ultra-high-performance liquid chromatography coupled with diode-array detector, J. Sep. Sci. 34 (21) (2011) 2989–2996.

[23] A.C. Rencher, Methods of Multivariate Analysis, John Wiley & Sons, Inc. Publication, 2002, pp. 454.