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Development of the chromatographic fingerprint of herbal preparations Shuang–Huang–Lian oral liquid

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Abstract

A simple, reliable and reproducible method, based on high performance liquid chromatography (HPLC), for developing chromatographic fingerprint of complex herbal medicine Shuang–Huang–Lian (SHL) oral liquid was described. Ten batches of SHL obtained from different pharmaceutical factories were used to establish the fingerprint. In addition, the contents of baicalin and chlorogenic acid, which are two marker constituents in the preparations, were also determined. Chromatographic fingerprint, together with the contents of the markers were applied for quality control of SHL. SHL comprises three kinds of medicinal herbs: *Fols Lonicerae*, *Radix Scutellariae* and *Fructus Forsythiae*. According to State Food and Drug Administration (SFDA) requirement, the chromatographic fingerprints of *Fols Lonicerae*, *Radix Scutellariae* and *Fructus Forsythiae*, the raw materials of SHL preparations, were also established. The data of fingerprints of SHL and its raw herbs established by HPLC were all processed with two kinds of mathematic methods including correlation coefficient and cosine value of vectorial angle to validate their similarities. In conclusion, fingerprints of *Radix Scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae* are suitable for identification, differentiation of their geographic origins and quality control. The similarity of 10 batches of SHL oral liquid was more than 0.988, which showed the preparations from different pharmaceutical factories were consistent.

Keywords: Fingerprint; Shuang–Huang–Lian; High performance liquid chromatography; Traditional Chinese medicines

1. Introduction

Traditional Chinese medicines (TCMs) have been extensively used to prevent and cure human disease for over a millennium. Because of its low toxicity and effective therapeutical performance, TCMs have attracted considerable attention in many fields. It is well known that the therapeutic effect of the herbal medicine is based on the synergistic effect of their constituents, which makes TCM different from Western medicines [1–4]. Traditionally, the contents of active components in crude herbs were used to evaluate the quality of the raw plant materials. An herbal medicine may consist of hundreds of phytochemicals, and their contents vary depending on climate, regions of cultivation and seasons of harvest. Moreover, these ingredients have significant concentration differences. So it becomes difficult or impossible in most case to identify the most of biologically active compounds, and to separate them from a large amount of proteins, sugars or tannins, which does not contribute to the pharmaceutical effect [5–7]. Without doubt, quality control of a compound medicine consisting of more than two kinds of crude herbs is more complicated. Furthermore, according to Chinese medicine theory, the whole of components in crude herbs are responsible for the beneficial effects. Conventional research focuses mainly on determination of the active components, while fingerprinting can offer integral characterization of a complex system with a quantitative degree of reliability. In this respect fingerprint has gained more and more attention among all the quality control systems [8–10]. Fingerprint is a kind of method to show chemical information of medicines with spectrograms, chromatograms and other graphs by analytical techniques [11,12]. Both Food and Drug Administration (FDA) [13] and European Medicines Agency (EMEA) [14] clearly denoted that the appropriate fingerprint chromatogram should be applied to assess the consistency of the botanical drugs.

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In 2004, State Food and Drug Administration (SFDA) also required that all the injections made from herbal medicines and their raw materials should be standardized by chromatographic fingerprint [15]. Fingerprint analysis in medicinal herbs is an efficient measurement on identifying and assessing the stability of the crude herbs. However, fingerprint analysis only shows the result of similarity calculated based on the relative value with the selected marker compound as reference standard, and does not display the absolute quantity. Obviously, quantitative determination of some marker components is necessary. In this work, chromatographic fingerprint, together with the contents of marker constituents was applied for quality control of TCM.

Shuang–Huang–Lian (SHL) oral liquid is a kind of compound herbal medicine often used to treat upper respiratory illness caused by virus or bacterial infection, such as tonsillitis, pharyngitis, pneumonia, acute enteritis, viral dysentery, etc. The compound medicine comprises three kinds of herbs: *Radix Scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae*. *Chinese pharmacopoeia* [16] records the formula of SHL oral liquid as follows: 375 g of *Radix Scutellariae*, 375 g of *Fols Lonicerae* and 750 g of *Fructus Forsythiae* were decocted, concentrated, extracted with ethanol, distilled to eliminate the solvent and the residue were dissolved and diluted with water to 1000 ml in volume. Baicalin, chlorogenic acid and forysthin are the marker compounds came from *Radix Scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae*, respectively. Baicalin has antiviral activity, and is known as the key active component of SHL preparations.

High performance liquid chromatography (HPLC) is regarded as a prime technique applied to develop fingerprint of crude herbs due to precision, sensitivity and reproducibility [17,18]. It is reported that the fingerprint of *Radix Scutellariae* and *Fols Lonicerae*, have been established with HPLC [19,20]. Yet, the fingerprints of compound herbal medicines have not been fully explored [21,22], especially the correlation between fingerprint of the preparations and that of their raw herbs. In this work, we firstly develop a simple, reliable and reproducible method to establish characteristic fingerprints of *Radix Scutellariae*, *Fols Lonicerae*, *Fructus Forsythiae* and their pharmaceutical preparations SHL, and to determine the marker substance baicalin and chlorogenic acid. Both the chromatographic fingerprint and contents of the markers were applied for quality control of TCM.

2. Experimental

2.1. Apparatus

The HPLC apparatus was a Waters 1525 Binary HPLC Pump system (Waters, America) equipped with a photodiode array detector (Waters 2996). The column was a Lichrospher C_{18} column (250 mm × 4.6 mm i.d., 5 μm). Data acquisition and processing were performed by Empower software.

2.2. Reagents and solution

Baicalin, chlorogenic acid and standard sample of *Radix Scutellariae* (1#) were purchased from the Chinese Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). As shown in Table 1, *Radix Scutellariae* and *Fols Lonicerae* samples were collected from different regions in China, and *Fructus Forsythiae* was collected only from Shanxi province (China). All of the raw medicinal herbs were verified by Professor Zhongdong Wang (Luoyang Botanize Institute, Luoyang, China). Ten batches of SHL samples were obtained from different pharmaceutical factories in China, and also shown in Table 1. Chromatographic grade methanol was purchased from Hanbang Sci. & Tech. Co. Ltd. (Jiangsu, China). Other chemicals were of analytical-reagent grade. All aqueous solutions were made up in deionized water. Stock solutions of baicalin and chlorogenic acid were prepared in methanol and were diluted to the desired concentration. Before use, all solutions were filtered through 0.22 μm nylon filters.

2.3. Sample preparation

All kinds of *Radix Scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae* samples were kept in the desiccator. About 0.30 g of dried herbal samples were ground into powder and accurately weighed, then extracted with 10 ml water for 40 s in microwave oven at mid-fire level (Panasonic, Shanghai, China). After centrifugation for about 20 min, the supernatant was filtered through

Table 1
The source of *Radix Scutellariae*, *Fols Lonicerae*, *Fructus Forsythiae* and SHL preparations

Sample number	Raw herbal medicine (growth region (China))			SHL preparation	
	Radix Scutellariae	*Fols Lonicerae*	*Fructus Forsythiae*	Pharmaceutical factory (China)	Lot No.
1#	Standard	Henan	Shanxi	Sanjing	04110251
2#	Anhui	Shandong	Shanxi	Funsen	040315
3#	Anhui	Shandong	Shanxi	Sanjing	04120343
4#	Jiangsu	Shanxi	Shanxi	Huili	04101026
5#	Neimeng	Sichuan		Sanjing	04101652
6#	Shanxi	Sichuan		Sanjing	04120548
7#	Shanxi	Suzhou		White Swan	041023
8#	Shanghai	Wuxi		Ruige	041006
9#	Yunnan			Qingan	030811
10#	Yunnan			Ruige	041006

Table 2
Solvent gradients

Time (min)	A (%)	B (%)
0	12	88
6	12	88
9	34	66
25	45	55
50	45	55
50.01	53	47
70	60	40
70.01	12	88
90	12	88

a 0.22 μm nylon filter, then directly injected into the HPLC system. A 1.0 ml SHL oral liquid were diluted with water to 10.0 ml in a volumetric flask, filtered through a 0.22 μm nylon filter and then injected into the HPLC system.

3. Results and discussion

3.1. Optimization of the chromatographic conditions

Optimization of parameters in HPLC was done through investigating the influence of the mobile phase and detection wavelength, because these two parameters play a key role on resolution and sensitivity. In this work, we chose a mixture of methanol and water as the mobile phase. Considering the presence of flavonoids in the herbal extraction, a little amount of H_3PO_4 was added to the mobile phase to reduce the ionization and lower the polarity of these compounds. The optimum mobile phase was achieved with A (methanol) and solvent B ($H_2O + 0.1\%$ H_3PO_4) in the gradient mode shown as in Table 2. The flow-rate was 0.6 ml/min. In order to obtain a large amount of detectable peaks on the HPLC chromatogram, the spectra of all peaks in the chromatogram of SHL were investigated with photodiode array detection. The result was shown in Fig. 1, and 254 nm was selected as detection wavelength.

Table 3
The regression equations and detection limits for baicalin and chlorogenic acid

Compound	Regression equation	Correlation coefficient	Linear range (mg/ml)	Detection limit (mg/ml)
Baicalin	$y = 70.2x + 7.40$	0.9991	1.60–0.10	0.06
Chlorogenic acid	$y = 98.1x + 6.54$	0.9990	1.20–0.06	0.02

The y value is the peak area of analytes; the x value is the concentration of the analytes (mg/ml).

3.2. Determination of baicalin and chlorogenic acid in SHL oral liquid

3.2.1. Reproducibility, linearity and detection limit of baicalin and chlorogenic acid

In traditional quality control system, baicalin and chlorogenic acid are used as the marker substances to evaluate the quality of SHL preparations. So the contents of the baicalin and chlorogenic acid in SHL preparations were determined at the above-mentioned optimum conditions. The quantitative method was assessed by reproducibility, linearity and detection limit. The reproducibility was estimated by making repetitive injections of a standard mixture solution (0.10 mg/ml for each) under the optimum conditions ($n = 7$). R.S.D. values of the retention time and the peak area were 0.5 and 1.7, and 0.7 and 1.2% for baicalin and chlorogenic acid, respectively. To determine the linearity equations and linear scope for the analytes, a series of mixed standard solutions ranged from 0.01 to 2.00 mg/ml were tested. The detection limit was also evaluated on the basis of a signal-to-noise ratio of 3. The results are summarized in Table 3.

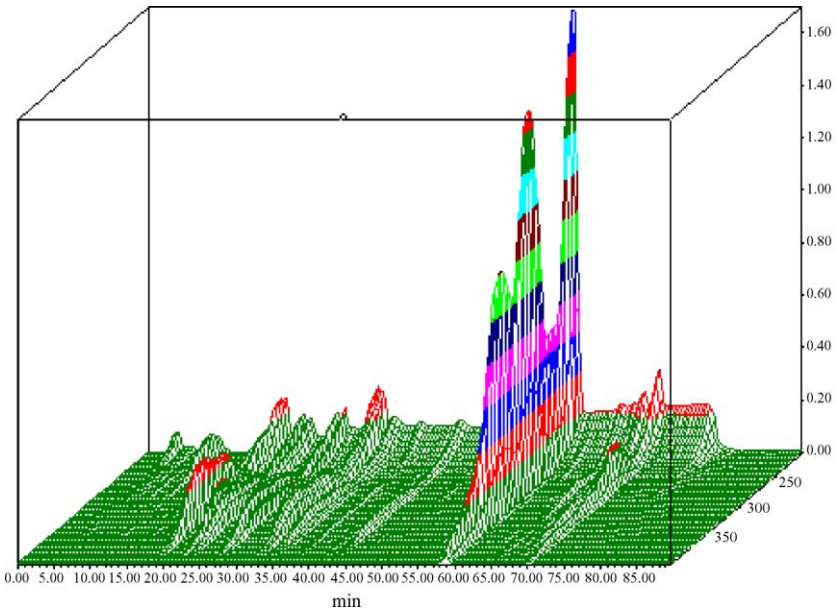

Fig. 1. 3D chromatogram of SHL oral liquid (sample 1#). HPLC conditions: Lichrospher C_{18} column (250 mm × 4.6 mm i.d., 5 μm). The optimum separation mobile phase was A (methyl alcohol) and solvent B ($H_2O + 0.1\%$ H_3PO_4) in the gradient mode shown in Table 2. The flow-rate was 1.0 ml/min.

Table 4
The contents of baicalin and chlorogenic acid in SHL samples

SHL samples Lot No.	Chlorogenic acid (mg/ml)	R.S.D. (%) ($n=3$)	Baicalin (mg/ml)	R.S.D. (%) ($n=3$)
04110251	5.72	1.16	18.9	2.10
040315	4.51	0.59	14.5	0.69
04120343	3.75	1.07	12.5	1.25
04101026	4.34	2.04	15.5	3.13
04101652	4.39	1.24	18.6	0.99
04120548	5.57	1.17	19.4	1.21
041023	6.05	2.49	23.3	3.02
041006	3.73	1.86	21.1	2.18
030811	3.59	2.53	17.5	3.55
041006	3.83	1.87	15.8	1.37

3.2.2. Sample analysis and recovery

Ten batches of SHL preparations were determined by HPLC under the optimum conditions and the assay results are listed in Table 4. Recoveries were also determined to evaluate the precision and accuracy of the method. By standard addition mixture standard solution to sample 1#, recoveries was determined and the average values were 98.4 and 96.3% for baicalin and chlorogenic acid, respectively ($n=3$), with R.S.D. values less than 3%. The assay results indicate that this method is accurate, sensitive and reproducible, and it is a useful method for quantitative analysis of baicalin and chlorogenic acid in SHL samples.

3.3. Fingerprint of compound herbal medicine SHL

According to the definition of fingerprints of TCM, a chromatographic fingerprint is in practice a chromatographic pattern of some common kinds of pharmacologically active and chemically characteristic components in the TCM. This chromatographic profile should feature the fundamental attributions of "integrity" and "fuzziness", in other words, "sameness" and "differences". It is suggested that with the help of chromatographic fingerprints, the authentication and identification of herbal medicines can be accurately conducted, even if the quantity of the chemically characteristic constituents are not exactly the same among different samples, which belong to the same kind of TCM. The chromatographic fingerprints could demonstrate both the "sameness" and "differences" between various samples successfully. With HPLC method, 10 batches of samples from different factories in China were analyzed in the optimum conditions. The average chromatogram from the 10 batches was regarded as the standardized characteristic fingerprint of SHL. Peaks existed in all chromatograms of 10 samples were assigned as "common peaks", indicating the sameness among various samples. The chromatograms of SHL from the 10 samples consisted of 30 common peaks within 90 min, shown in Fig. 2. Among these components, baicalin indicated a high and stable content, therefore it was chosen as the reference substance. All common peaks' relative retention time and relative peak area were obtained with reference to this substance. As shown in Table A1, R.S.D. values of the relative retention time of 30 common peaks in 10 batches samples were less than 1.0%, which means the common peaks were in good correspondence in all samples. Moreover, such low R.S.D. valves demonstrate that the fingerprint developed by HPLC had good stability and reproducibility. So, the peak profile of the 30 components made up the fingerprint of SHL. Besides the common peaks, there are about 25–30 non-common peaks in each chromatogram, which

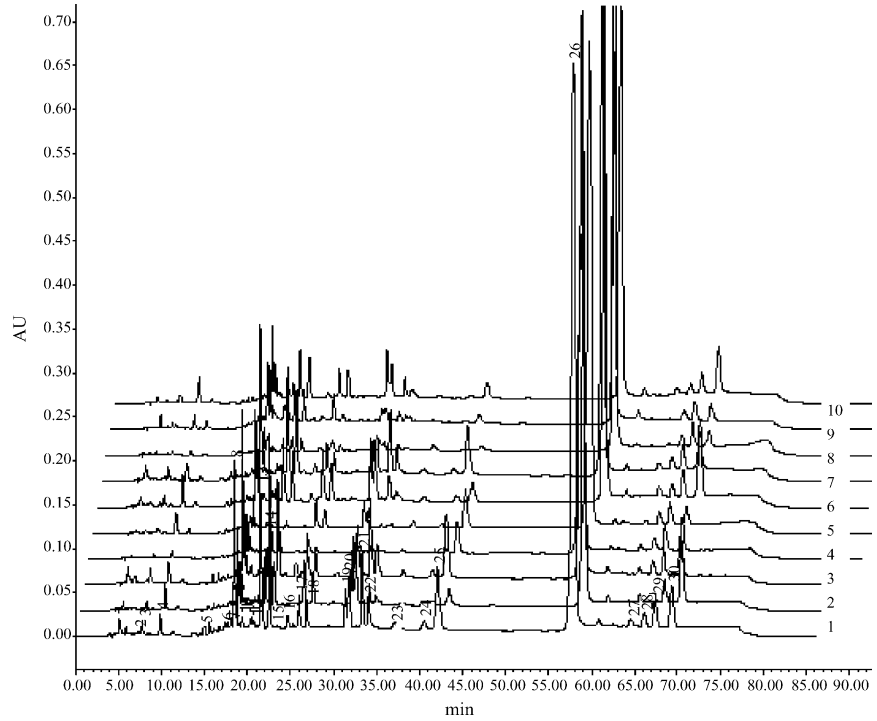

Fig. 2. Chromatogram of SHL oral liquid from different factories. The effluent was monitored at 254 nm, the other conditions are the same as in Fig. 1. Numbers 1–10 stand for the different samples shown in Table 1.

Table 5
Various kinds of extraction methods and the results

The number of extraction method	Extraction method	Extraction solvent	Extraction time	The content of baicalin (mg/ml)	R.S.D. (%) ($n = 3$)	Peak number (area more than 5% in total area)
1	Microwave	Water	40 s	3.86	2.1	6
2	Refluxing	25% ethanol	30 min	3.06	1.6	4
3	Refluxing	50% ethanol	30 min	3.19	1.1	4
4	Refluxing	75% ethanol	30 min	3.86	2.3	4
5	Refluxing	100% ethanol	30 min	1.15	1.0	4
6	Ultrasonic	25% ethanol	30 min	0.22	1.3	5
7	Ultrasonic	50% ethanol	30 min	1.36	0.9	5
8	Ultrasonic	75% ethanol	30 min	2.71	2.5	4
9	Ultrasonic	100% ethanol	30 min	0.35	0.6	6
10	Ultrasonic	Water	30 min	0.16	0.4	6
11	Refluxing	Water	30 min	2.51	1.7	3

HPLC conditions are the same as in Fig. 1.

represents the fuzziness among the same kind of TCM. The non-common peak area is about 6.9%, less than the national standard of 10%.

SFDA suggested that all of herbal chromatograms should be evaluated by their similarities, which come from the calculation on the correlative coefficient and/or angle cosine value of original data [23–25]. With two different mathematic methods including correlation coefficient and the included angle cosine calculated with the software of Excel 2002 [26], the data of fingerprints of 10 batches samples were processed to analyze similarity among these samples. According to the relative peak areas of 30 common peaks in the chromatograms of 10 samples, the similarity analysis was conducted, and the results are shown in Table 6. All of values of correlation coefficient and the included angle cosine of the samples are more than 0.988. It indicates the quality of SHL oral liquid was stable and the products from different pharmaceutical factory were consistent.

3.4. Fingerprints of Radix Scutellariae, Fols Lonicerae and Fructus Forsythiae

3.4.1. Optimization of the extraction method

According to Chinese medicine theory, *Radix Scutellariae* plays a dominating role in SHL compound medicine. So *Radix Scutellariae* was chosen as extracted object for ascertains the extracted method.

A good extraction method not only requires complete isolation of active components from the matrix, but also gains comprehensive chemical profile, i.e. the more and the larger the peaks in the chromatograms, the better the extraction method. In this work, the contents of baicalin, commonly as quality marker of *Radix Scutellariae*, and the number of peaks were determined by HPLC to evaluate the extraction efficiency. Eleven extraction methods with different kinds of extraction ways and solvents shown in Table 5 in detail, and chromatograms of extract of *Radix Scutellariae* with these extraction methods shown in Fig. 3

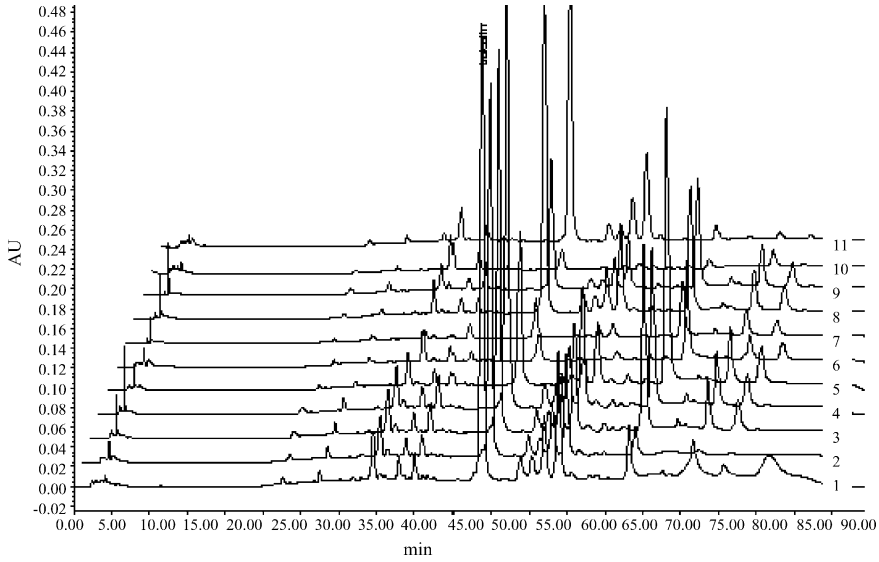

Fig. 3. Chromatogram of *Radix Scutellariae* with different extraction method. HPLC conditions are the same as in Fig. 2. Numbers 1–11 stand for the different extraction methods shown in Table 3.

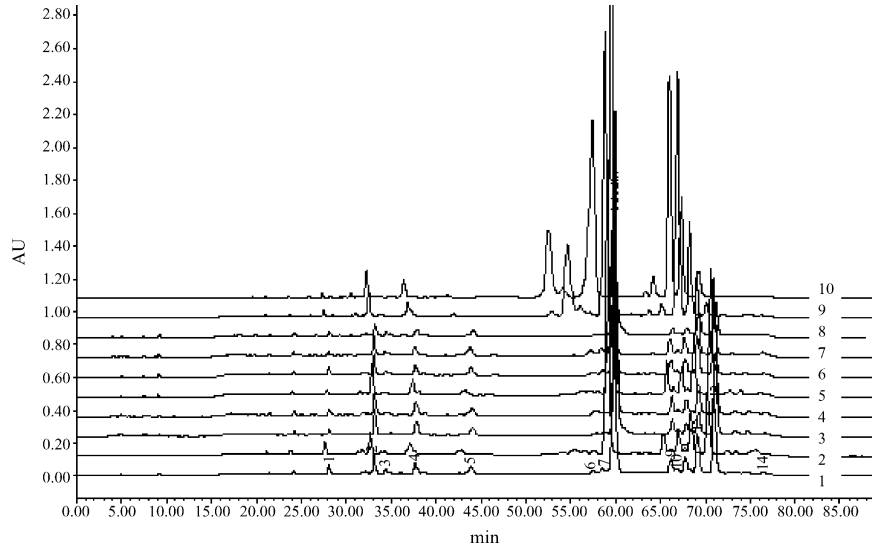

Fig. 4. Chromatograms of *Radix Scutellariae* from different regions. HPLC conditions are the same as in Fig. 2. Numbers 1–10 stand for the different samples shown in Table 1.

were investigated to choose the appropriate method. The content of baicalin and the number of peaks in the chromatograms with various kinds of extraction methods were also shown in Table 5.

As shown in Table 5, the highest content of baicalin in *Radix Scutellariae* extract had been obtained with microwave and 75% ethanol refluxing methods, which means both methods have the better extraction efficiency. Furthermore, according to the character of fingerprint [15], the number of peaks in the chromatogram is a key factor in choosing the extraction method. In this respect, microwave extraction is better than 75% ethanol refluxing method. Moreover, microwave extraction is convenient and rapid. So it was selected as the optimum extraction method in this experiment.

3.4.2. Development of fingerprint of Radix Scutellariae, Fols Lonicerae and Fructus Forsythiaeee

Ten batches of *Radix Scutellariae*, eight batches of *Fols Lonicerae* and four batches of *Fructus Forsythiae* were extracted with the optimum method. The extracts were analyzed with HPLC in the optimum chromatographic conditions.

In the fingerprint of *Radix Scutellariae*, the peaks which areas are more than 0.5% in the chromatogram of standard herb (sample 1#) was selected as the common peaks in this work. As shown in Fig. 4, there are 14 common peaks within 80 min in the fingerprint. Nine samples obtained from different places of cultivation were analyzed with HPLC, and the whole chromatograms, compared with the standard, could provide useful means of identifying and assessing *Radix Scutellariae.* Baicalin as the reference standard, the relative retention time and the relative peak area of common peaks were shown in Tables A3 and A4. As shown in the tables, R.S.D. values of the relative retention time of 14 peaks in the samples are less than 2.6%. According to the relative peak area, correlation coefficient and the included angle cosine were also calculated for similarity analysis among 10 samples. As shown in Fig. 4, the peak of No. 12 was not existed in the chromatogram of sample 9#, nor was No. 10 peak in sample 10#, so relative peak areas of these two peaks were supposed as 0. Without doubt, the similarity between samples 9#, 10# and the other eight samples must be poor, which is testified by the result of similarity analysis shown in Table 6. Though the 10 batches of samples belong to the same family and the same genera, samples 1#–8# are referred as *S. baicalensis* Georgi, and both sample 9# (wild) and sample 10# are referred as *S. amoena* Wright, which belong to two different species of *Radix Scutel-*

Table 6
The similarity analysis of SHL oral liquid, *Radix Scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae*

	The similarity	Sample number									
		1#	2#	3#	4#	5#	6#	7#	8#	9#	10#
SHL	The included angle cosine	0.999	0.991	0.989	0.989	0.999	0.988	0.988	0.995	0.999	1
	Correlation coefficient	0.999	0.994	0.992	0.992	1	0.99	0.991	0.997	0.999	1
Radix Scutellariae	The included angle cosine	0.997	0.996	0.998	0.999	0.977	0.999	0.998	0.997	0.845	0.783
	Correlation coefficient	0.996	0.996	0.997	0.998	0.973	0.999	0.998	0.997	0.810	0.733
Fols Lonicerae	The included angle cosine	0.932	0.940	0.954	0.862	0.938	0.948	0.933	0.860		
	Correlation coefficient	0.903	0.920	0.938	0.796	0.911	0.957	0.907	0.803		
Fructus Forsythiae	The included angle cosine	0.965	0.874	0.951	0.950						
	Correlation coefficient	0.976	0.811	0.967	0.957						

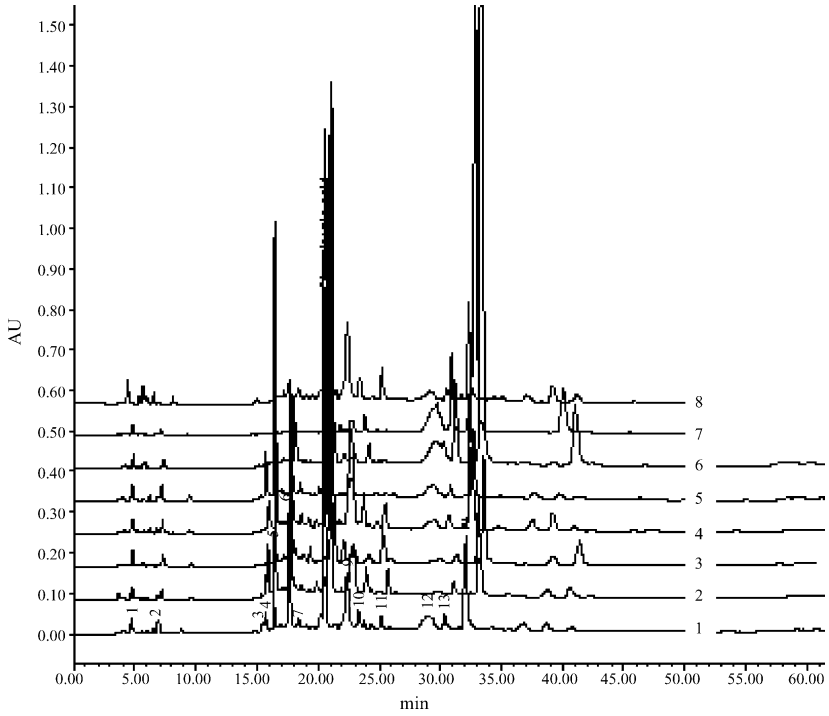

Fig. 5. Chromatograms of *Fols Lonicerae* from different regions. HPLC conditions are the same as in Fig. 2. Numbers 1–8 stand for the different samples shown in Table 1.

lariae. Since samples 9# and 10# are obviously different from the standard sample, they could not be used as the raw material of SHL. Otherwise, the quality of SHL preparations would be unstable.

The chromatograms of 8 batches of *Fols Lonicerae* consist of 15 common peaks within 60 min, as shown in Fig. 5. Chlorogenic acid was chosen as the reference standard, the relative retention time and the relative peak area of common peaks were calculated and displayed in Tables A5 and A6. As shown in the tables, R.S.D. values of the relative retention time of 15 peaks in 8 samples are less than 1.0%, the extremely low values of R.S.D. show that the fingerprint developed by HPLC is precise, reproducible and reliable. According to relative peak area, correlation coefficient and the included angle cosine were also calculated for similarity analysis among eight samples of *Fols Lonicerae*. As shown in Table 6, the values of correlation coefficient and included angle cosine is lower than those of *Radix Scutellariae*. It was indicated that geographical origins has more significantly influence on *Fols Lonicerae* than on *Radix Scutellariae*.

The chromatograms of 4 batches of *Fructus Forsythiae* were shown in Fig. 6, and there are 14 common peaks in the

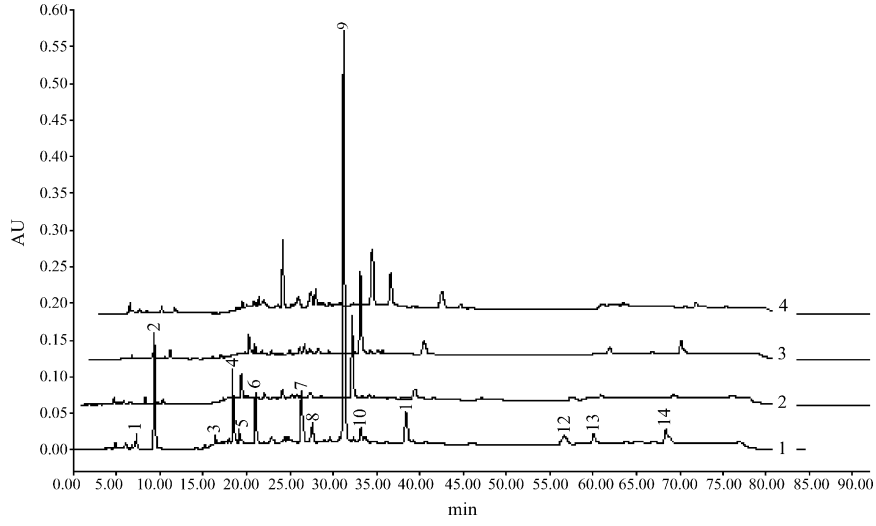

Fig. 6. Chromatograms of *Fructus Forsythiae* from Shanxi (China). HPLC conditions are the same as in Fig. 2.

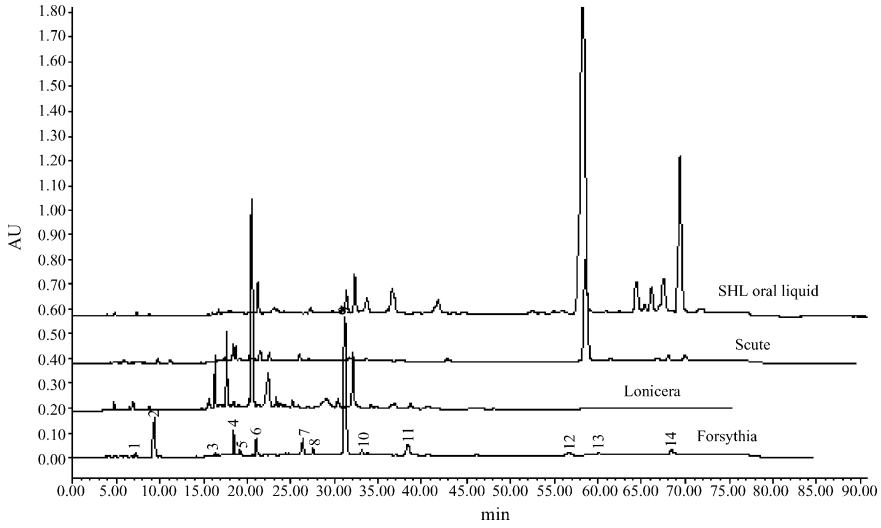

Fig. 7. Chromatograms of *Radix scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae* merged in one. HPLC conditions are the same as in Fig. 2.

fingerprint. Peak No. 9 was chosen as the reference standard. As shown in Table 6, although *Fructus Forsythiae* was collected from the same province in China, however, they were not similar. It is conjectured that the chemical ingredients profile of the medicinal herbs may be extraordinary influenced with the climate, seasons of harvest and the way of processing and storage, besides growth place.

As above-mentioned, the quality of raw herbal medicines varies with many factors. Therefore, it is necessary to develop the fingerprints of raw medicinal herbs to ensure the quality of raw materials. Only when the quality of raw herbal materials is being met, can the quality of herbal preparations be monitored for stability and consistency.

3.5. The correlation between SHL preparations and their raw herbal medicines

The chromatograms of *Radix Scutellariae*, *Fols Lonicerae*, *Fructus Forsythiae* and SHL oral liquid were merged in one chart, shown in Fig. 7. Comparing the relative retention time of peaks in SHL fingerprint with that in *Radix Scutellariae*, it could be found that there were six peaks in SHL fingerprint found in the *Radix Scutellariae*. The relative retention time of SHL was again calculated with the chlorogenic acid as the reference standard, and then compared with that of *Fols Lonicerae*, it was found that five common peaks in SHL could be found in the common peaks in *Fols Lonicerae* fingerprint. Peak No. 22 was chosen as the reference substance, which also exists in the *Fructus Forsythiae*, it was found that four common peaks in SHL correspond to the common peaks in *Fructus Forsythiae*. The correspondence of peaks between in SHL and in raw herbs was shown in details in Table 7.

The main common peaks in SHL preparations fingerprint could be found in their raw herbal fingerprints. Nevertheless, 16 common peaks in the SHL was not found in the common peaks of 3 raw medicines, and more than half of common peaks in the raw medicines were not found in that of SHL preparations, which seems to give illogical results. In fact, it is impossible that the SHL fingerprint completely accords with the fingerprints of its raw medicines. First, in this work, the peaks are assigned as the common peaks only if their areas are more than 0.5%. By checking up the original data carefully, most of common peaks of SHL, not found in common peaks, are appeared in non-common peaks of raw medicines. Though those peaks in the raw herbs are too small to be selected as the common peaks, they may be larger than 0.5% in SHL preparation if they exist in the all raw materials, and their contents should be added after extraction. Second, manufacture process of SHL preparations in pharmaceutical factories must be different from extraction of raw medicines. Loss of peaks in SHL fingerprint means that some chemical substances in raw materials could be destroyed or removed in manufacture process. It needs further studies whether the loss affects the curative effects of SHL preparations. Finally, but most importantly, the chemical substances among these three raw herbs may react with each other, which leads to the difference of chemical profile between SHL preparations

Table 7
The correspondence of peaks between in SHL and in raw herbs

Medicines	Peak No.														
SHL	1	2	3	10	13	16	19	20	22	23	26	27	28	29	30
Radix Scutellariae								2			8	9	11	12	13
Fols Lonicerae		2	4	7	8	10									
Fructus Forsythiae	1						5		8	9					

and their raw medicines. Chinese medicine theory emphasizes compatibility of several kinds of TCM to enhance remedy, lower toxicity and prevent side effect. Essentially, the positive effect of compatibility of medicines results from the chemical reactions among these phytochemicals. From the point of this view, further research on difference of fingerprints between compound medicines and its raw materials will help us understand the theory of Chinese medicine.

4. Conclusion

A HPLC method was developed for fingerprint analysis of compound medicine SHL oral liquid. The average fingerprint of 10 batches of samples from different pharmaceutical factories was obtained, 30 common peaks represents the major constituents of this TCM constituents. The similarity of 10 batches of SHL oral liquid was more than 0.988, which shows the preparations from different pharmaceutical factories were consistent. However, the contents of baicalin and chlorogenic of 10 samples were different, which indicates there are differences in quality among these products. So, it is suggested that the fingerprint, together with contents of markers, are used to identify and assess the SHL preparations and applied for control quality of this TCM. The fingerprints of *Radix Scutellariae*, *Fols Lonicerae* and *Fructus Forsythiae* were also established by HPLC for quality control of raw materials, and the main common peaks in SHL preparations fingerprint could be found in their raw herbal fingerprints, showing there is a sufficient correlation between them. The results demonstrate that the method is feasible for comprehensive quality evaluation of SHL oral liquid.

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Appendix A

See Tables A1–A8.

Table A1
The relative retention time in HPLC fingerprints of SHL oral liquid HPLC conditions are the same as in Fig. 1

Peak number	Sample number										R.S.D. (%)
	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	
1	0.130	0.130	0.131	0.131	0.130	0.133	0.133	0.131	0.132	0.133	1.0
2	0.167	0.166	0.168	0.168	0.167	0.170	0.169	0.167	0.170	0.171	0.91
3	0.191	0.192	0.193	0.194	0.193	0.197	0.196	0.193	0.193	0.195	0.90
4	0.265	0.264	0.265	0.266	0.264	0.269	0.269	0.268	0.270	0.272	1.0
5	0.276	0.272	0.273	0.274	0.275	0.277	0.278	0.273	0.279	0.275	0.80
6	0.285	0.283	0.283	0.285	0.283	0.28	0.281	0.286	0.285	0.285	0.60
7	0.296	0.288	0.288	0.289	0.287	0.288	0.289	0.291	0.290	0.290	0.90
8	0.300	0.300	0.300	0.301	0.305	0.301	0.301	0.300	0.295	0.300	0.80
9	0.307	0.306	0.307	0.308	0.305	0.305	0.306	0.305	0.303	0.308	0.51
10	0.313	0.313	0.313	0.314	0.312	0.311	0.312	0.311	0.313	0.313	0.32
11	0.319	0.318	0.318	0.320	0.317	0.317	0.318	0.317	0.319	0.319	0.30
12	0.325	0.324	0.324	0.325	0.323	0.323	0.324	0.323	0.326	0.325	0.30
13	0.328	0.328	0.329	0.330	0.328	0.328	0.329	0.329	0.331	0.331	0.30
14	0.346	0.346	0.346	0.347	0.346	0.342	0.342	0.35	0.341	0.347	0.81
15	0.353	0.353	0.354	0.354	0.353	0.358	0.352	0.358	0.352	0.359	0.70
16	0.367	0.367	0.367	0.368	0.366	0.364	0.364	0.371	0.371	0.364	0.70
17	0.384	0.384	0.385	0.385	0.384	0.389	0.389	0.389	0.387	0.378	0.80
18	0.421	0.435	0.436	0.436	0.441	0.438	0.438	0.44	0.445	0.431	1.40
19	0.444	0.444	0.445	0.445	0.445	0.449	0.449	0.449	0.445	0.445	0.50
20	0.462	0.461	0.462	0.462	0.461	0.465	0.464	0.467	0.462	0.454	0.70
21	0.54	0.539	0.54	0.54	0.539	0.541	0.541	0.546	0.541	0.55	0.60
22	0.547	0.547	0.548	0.549	0.547	0.549	0.549	0.555	0.55	0.558	0.70
23	0.588	0.587	0.589	0.588	0.588	0.589	0.588	0.593	0.587	0.582	0.40
24	0.641	0.642	0.644	0.64	0.644	0.643	0.641	0.645	0.636	0.644	0.40
25	0.733	0.736	0.74	0.733	0.739	0.731	0.727	0.731	0.723	0.725	0.70
26	1	1	1	1	1	1	1	1	1	1	0
27	1.048	1.047	1.043	1.048	1.044	1.048	1.051	1.046	1.06	1.075	0.90
28	1.139	1.138	1.134	1.139	1.133	1.139	1.142	1.141	1.148	1.137	0.40
29	1.161	1.16	1.158	1.161	1.154	1.161	1.164	1.162	1.168	1.167	0.30
30	1.193	1.193	1.188	1.195	1.186	1.194	1.198	1.195	1.204	1.191	0.40

Table A2
The relative areas of the common peaks in HPLC fingerprints of SHL oral liquid and similarity analysis

Peak number	Sample number									
	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#
1	0.311	0.219	0.228	0.218	0.465	0.105	0.129	0.150	0.126	1.997
2	0.682	0.803	0.780	0.783	0.723	0.152	0.171	0.749	0.644	10.25
3	0.234	0.175	0.164	0.163	0.195	0.058	0.056	0.674	0.139	2.212
4	0.396	0.159	0.158	0.130	0.355	0.067	0.066	0.115	0.047	1.229
5	0.313	0.213	0.126	0.123	0.301	0.038	0.034	0.327	0.045	0.193
6	0.220	0.13	0.178	0.083	0.294	0.040	0.037	0.45	0.111	1.158
7	0.236	0.093	0.081	0.051	0.291	0.067	0.061	1.400	0.055	1.102
8	0.352	0.175	0.162	0.103	0.496	0.13	0.124	0.463	0.092	3.746
9	1.517	0.236	0.229	0.137	0.496	0.072	0.073	0.489	0.431	1.821
10	0.623	1.575	1.56	1.493	2.866	0.117	0.109	2.45	0.14	2.408
11	3.290	0.808	0.991	0.89	1.835	0.996	0.885	2.102	0.213	5.386
12	2.685	0.192	0.192	0.115	0.613	0.23	0.232	0.534	1.664	11.26
13	0.654	0.23	0.328	0.115	1.191	0.064	0.061	0.516	1.038	1.723
14	0.751	0.349	0.31	0.205	1.497	0.039	0.039	1.032	0.203	2.101
15	0.779	0.639	0.481	0.35	0.736	0.124	0.163	1.06	0.198	5.073
16	2.769	1.507	1.526	1.433	2.308	0.052	0.05	1.61	0.218	4.245
17	2.374	1.256	1.237	1.201	1.694	0.81	0.747	1.609	1.225	3.297
18	0.105	0.226	0.244	0.254	0.585	0.138	0.139	0.163	0.123	2.086
19	2.196	1.05	1.388	1.487	1.979	0.341	0.294	1.91	0.045	15.6
20	0.646	0.906	0.963	1.017	0.855	0.206	0.232	0.453	0.68	9.844
21	1.216	1.658	1.744	1.725	1.513	0.331	0.333	0.671	0.955	15.62
22	0.817	1.329	1.413	1.452	1.57	0.464	0.493	1.237	0.631	12.49
23	0.387	0.191	0.499	0.216	0.154	0.354	0.366	0.379	0.513	1.009
24	0.73	0.176	0.212	0.178	1.407	0.103	0.094	0.33	0.491	4.9
25	1	1	1	1	1	1	1	1	1	1
26	105.1	24.28	25.28	25.64	154.8	10.36	10.03	37.5	54.69	612.6
27	0.349	0.297	0.241	0.235	0.478	0.065	0.062	0.526	0.109	0.202
28	2.34	0.567	0.592	0.615	2.952	0.207	0.194	1.037	0.558	11.01
29	1.498	1.637	3.792	2.367	7.835	0.591	0.428	1.527	1.593	21.99
30	6.291	2.774	3.602	3.816	5.201	0.690	0.618	1.788	3.017	19.86
The included angle cosine	0.999	0.991	0.989	0.989	0.999	0.988	0.988	0.995	0.999	1
Correlation coefficient	0.999	0.994	0.992	0.992	1	0.99	0.991	0.997	0.999	1

HPLC conditions are the same as in Fig. 1.

Table A3
The relative retention time in HPLC fingerprints of *Radix Scutellariae*

Peak number	Sample number										R.S.D. (%)
	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#	
1	0.403	0.412	0.404	0.404	0.403	0.400	0.403	0.404	0.412	0.403	0.98
2	0.467	0.478	0.509	0.469	0.469	0.470	0.468	0.469	0.476	0.467	2.60
3	0.554	0.549	0.553	0.553	0.554	0.550	0.553	0.553	0.548	0.553	0.40
4	0.578	0.584	0.575	0.574	0.574	0.580	0.580	0.574	0.582	0.577	0.60
5	0.628	0.633	0.631	0.630	0.630	0.630	0.628	0.630	0.634	0.626	0.40
6	0.718	0.713	0.713	0.711	0.710	0.770	0.724	0.711	0.718	0.713	2.50
7	0.960	0.946	0.964	0.96	0.958	0.960	0.957	0.964	0.943	0.953	0.70
8	1	1	1	1	1	1	1	1	1	1	0
9	1.105	1.100	1.106	1.105	1.105	1.100	1.105	1.105	1.102	1.106	0.20
10	1.121	1.115	1.117	1.117	1.119	1.120	1.118	1.116	1.118	–	0.17
11	1.134	1.129	1.132	1.132	1.132	1.130	1.133	1.131	1.132	1.137	0.20
12	1.158	1.165	1.155	1.155	1.156	1.150	1.156	1.154	–	1.159	0.35
13	1.190	1.199	1.186	1.185	1.186	1.190	1.188	1.186	1.172	1.191	0.50
14	1.228	1.237	1.221	1.221	1.221	1.220	1.224	1.222	1.206	1.220	0.60

HPLC conditions are the same as in Fig. 1.

Table A4
The relative areas of the common peaks in HPLC fingerprints of *Radix Scutellariae* and similarity analysis

Peak number	Sample number									
	1#	2#	3#	4#	5#	6#	7#	8#	9#	10#
1	0.537	1.377	1.133	0.395	0.234	0.377	0.444	0.349	0.060	0.070
2	0.645	1.772	0.282	1.065	0.254	0.499	1.697	0.538	0.150	0.223
3	4.695	1.745	8.600	2.636	1.633	3.075	3.500	2.787	0.071	1.324
4	2.544	4.354	0.455	0.637	0.356	0.271	0.463	0.171	0.131	0.147
5	4.919	0.947	7.034	1.950	1.189	2.361	3.687	2.171	0.924	1.054
6	3.296	1.032	0.625	0.344	0.108	0.141	1.760	0.192	0.212	0.246
7	1	1	1	1	1	1	1	1	1	1
8	93.50	166.1	123.6	45.37	26.28	68.84	66.74	46.11	19.28	18.79
9	5.123	0.252	7.649	2.644	1.679	4.619	4.081	2.734	0.283	0.771
10	1.372	8.936	2.236	0.833	0.533	1.096	0.857	0.742	1.250	0
11	4.468	3.587	5.328	2.833	1.912	3.970	4.829	2.176	0.064	15.04
12	6.407	25.97	12.44	6.984	9.364	8.498	9.319	4.737	0	5.732
13	27.35	48.33	36.76	13.00	8.440	18.68	14.91	9.783	6.151	0.816
14	0.562	1.103	5.669	0.436	0.282	1.569	0.557	0.649	1.613	0.021
The included angle cosine	0.997	0.996	0.998	0.999	0.977	0.999	0.998	0.997	0.845	0.783
Correlation coefficient	0.996	0.996	0.997	0.998	0.973	0.999	0.998	0.997	0.810	0.733

HPLC conditions are the same as in Fig. 1.

Table A5
The relative retention time in HPLC fingerprints of *Fols Lonicerae*

Peak number	Sample number								R.S.D. (%)
	1#	2#	3#	4#	5#	6#	7#	8#	
1	0.230	0.231	0.231	0.233	0.232	0.229	0.232	0.226	0.90
2	0.273	0.271	0.274	0.276	0.275	0.270	0.268	0.273	0.90
3	0.755	0.759	0.752	0.759	0.747	0.754	0.756	0.759	0.50
4	0.796	0.801	0.806	0.793	0.785	0.799	0.802	0.792	0.80
5	0.811	0.814	0.817	0.831	0.813	0.815	0.814	0.814	0.70
6	0.858	0.858	0.860	0.855	0.859	0.858	0.859	0.862	0.30
7	0.971	0.981	0.982	0.970	0.980	0.980	0.982	0.967	0.60
8	1	1	1	1	1	1	1	1	0
9	1.090	1.088	1.087	1.087	1.085	1.087	1.088	1.071	0.50
10	1.136	1.142	1.138	1.136	1.142	1.143	1.139	1.141	0.20
11	1.159	1.162	1.159	1.157	1.161	1.163	1.160	1.169	0.30
12	1.416	1.418	1.409	1.410	1.403	1.418	1.422	1.397	0.005
13	1.479	1.483	1.475	1.507	1.477	1.484	1.479	1.475	0.006
14	1.535	1.550	1.565	1.555	1.548	1.556	1.542	1.547	0.006
15	1.986	1.937	1.967	1.962	1.942	1.959	1.978	1.971	0.009

HPLC conditions are the same as in Fig. 1.

Table A6
The relative areas of the common peaks in HPLC fingerprints of *Fols Lonicerae* and similarity analysis

Peak number	Sample number								Average
	1#	2#	3#	4#	5#	6#	7#	8#	
1	0.119	0.077	0.257	0.050	0.389	0.479	0.440	0.001	0.189
2	0.014	0.022	0.032	0.014	0.084	0.199	0.154	0.087	0.064
3	0.072	0.433	0.985	0.080	0.199	0.118	0.006	0.011	0.205
4	0.422	0.075	0.147	0.359	0.362	0.165	0.009	0.047	0.165
5	0.041	0.099	0.232	0.051	0.795	0.338	0.058	0.043	0.174
6	0.967	0.203	0.442	0.362	1.567	0.775	1.054	0.045	0.557
7	0.043	0.201	0.522	0.035	0.53	0.177	0.197	0.097	0.206
8	2.534	2.649	6.572	1.611	10.32	13.52	9.316	1.494	5.078
9	1	1	1	1	1	1	1	1	1
10	0.197	0.288	0.172	0.240	0.820	0.456	0.790	0.028	0.350
11	0.095	0.059	0.105	0.059	0.073	0.110	0.049	0.065	0.071
12	0.529	0.134	1.400	0.292	4.230	0.431	5.779	0.217	1.402
13	0.178	0.165	0.381	0.130	4.267	0.421	4.588	0.067	1.095
14	0.043	0.037	2.086	0.926	0.837	0.268	0.636	0.780	0.571
15	0.059	0.144	0.026	0.052	5.363	1.491	0.155	0.099	0.754
The included angle cosine	0.932	0.940	0.954	0.862	0.938	0.948	0.933	0.860	1
Correlation coefficient	0.903	0.920	0.938	0.796	0.911	0.957	0.907	0.803	1

HPLC conditions are the same as in Fig. 1.

Table A7
The relative retention time in HPLC fingerprints of *Fructus Forsythiae*

Peak number	Sample number				Average	R.S.D. (%)
	1#	2#	3#	4#		
1	0.234	0.233	0.231	0.232	0.233	0.13
2	0.302	0.279	0.299	0.298	0.295	1.05
3	0.539	0.527	0.523	0.547	0.534	1.1
4	0.591	0.586	0.591	0.589	0.589	0.24
5	0.615	0.607	0.614	0.611	0.612	0.36
6	0.677	0.674	0.675	0.673	0.675	0.17
7	0.845	0.844	0.845	0.843	0.844	0.1
8	0.884	0.884	0.884	0.883	0.884	0.05
9	1	1	1	1	1	0
10	1.064	1.068	1.064	1.065	1.065	0.19
11	1.233	1.254	1.232	1.236	1.239	1.03
12	1.817	1.839	1.819	1.834	1.827	1.09
13	1.921	1.893	1.927	1.92	1.915	1.52
14	2.187	2.18	2.195	2.184	2.187	0.64

HPLC conditions are the same as in Fig. 1.

Table A8
The relative areas of the common peaks in HPLC fingerprints of *Fructus Forsythiae* and similarity analysis

Peak number	Sample number			
	1#	2#	3#	4#
1	0.054	0.09	0.032	0.052
2	0.062	0.102	0.253	0.099
3	0.079	0.194	0.009	0.054
4	0.213	0.192	0.104	0.164
5	0.075	0.269	0.03	0.116
6	0.076	0.989	0.097	0.025
7	0.079	0.097	0.123	0.068
8	0.027	0.042	0.046	0.028
9	1	1	1	1
10	0.047	0.677	0.045	0.043
11	0.206	0.484	0.125	0.263
12	0.088	0.073	0.057	0.072
13	0.043	0.025	0.026	0.117
14	0.047	0.072	0.063	0.228
The included angle cosine	0.965	0.874	0.951	0.950
Correlation coefficient	0.976	0.811	0.967	0.957

HPLC conditions are the same as in Fig. 1.

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