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Development of the chromatographic fingerprint of Scutellaria barbata D. Don by GC–MS combined with Chemometrics methods

Ruijing Pan, Fangqiu Guo*, Hongmei Lu, Wei-wei Feng, Yi-zeng liang

Research Center of Modernization of Chinese Herbal Medicine, College of Chemistry and Chemical Engineering, Central South University, Changsha, Hunan, PR China

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ABSTRACT

Gas chromatography fingerprint of Scutellaria barbata D. Don (SB) from different origins was studied by gas chromatography–mass spectrometry (GC–MS) and related Chemometrics methods. The constituents of essential oil of Scutellaria barbata D. Don (SB) and its two adulterants Oldenlandia diffusa (OD) and Lobelia chinensis Lour (LCL) were analyzed and compared, 50, 36 and 38 components were identified from SB, OD and LCL, respectively, there were 16 and 18 common components between SB and OD, SB and LCL. Nine different samples collected from different producing areas of SB were studied. The relative standard deviations (RSDs) of retention time and peak area of each component were less than 0.2% and 6%, respectively. The number of common peaks was up to 52 and the un-common peak area was less than 10%. The similarity and difference among SB and its adulterants were also evaluated by correlation coefficient similarity analysis and principal component analysis, the result showed that developed fingerprint characterize the SB from different producing areas and it is useful and feasible for the discrimination of its adulterants.

1. Introduction

Traditional Chinese medicine (TCM) has been proudly used to cure human disease for over a millennium. It plays a more and more important role in modern pharmaceutical industry because of its low toxicity and rare side effects. Different from western medicine [1–4], an herb might consist of hundreds of phytochemicals, depending on the climate, regions of cultivation and seasons of harvest. However, the traditional Chinese medicine (TCM) has not been officially recognized in most countries because of its complicated constituents and uncertainly of its pharmaceutical effects. The efficient quality and quantity data on tradition Chinese medicine are far from sufficient to meet the criteria needed to support its use and quality control [5–8] world-widely.

There are more than 10 thousand kinds of TCM are being used in China, belong to different categories, some kinds TCM of same category have similar chemical constituents and appearance, some even belong to different category can have similar looks or pharmaceutical effects. It leads to the mis-using and mix-using. So it is necessary to build a method for the discrimination of the TCM with similar chemical constituents or appearance.

Nowadays, the construction of chromatographic fingerprint becomes one of the most powerful approaches for quality control of herbal medicine and others [1,9–11]. By definition, a chromatographic fingerprint of a TCM is, in practice, a chromatographic pattern of the extract some common chemical components of pharmacologically active and chemically characteristics [11–13]. This chromatographic fingerprint profile should feature the fundamental attributions of “integrity” and “fuzziness” or “sameness” and “differences” so as to chemically represent the studied samples. With the help of chromatographic fingerprint, the authentication and identification of a TCM can be accurately conducted (integrity), meanwhile the number and/or concentration of chemically characteristic constituents are not similar in different samples (fuzziness) or, chromatographic fingerprint could demonstrate both the “sameness” and “differences” between various samples [14], successfully.

It is well known that chromatography has strong separation ability. In recent years a variety of separation techniques have been developed, such as high-performance liquid chromatography–diode (HPLC–DAD), gas chromatograph combined with mass spectrometry (GC–MS), capillary electrophoresis (CE)–DAD. They are also called hyphenated chromatography and spectrometric approaches. Gas chromatography–mass spectrometry (GC–MS) is the most commonly used technique for the analysis and development of fingerprint of the essential oil of TCMs.

Chemometrics has become a popular method for analysis of complex systems, mainly because it can provide a large number

* Corresponding author. Tel.: +86 731 88836376; fax: +86 731 88879616.
E-mail address: fangqiu1968@yahoo.com (F. Guo).

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Table 1
The information about SB from nine different origins and its 2 adulterants.

Sample No	Name	Origin
1	Oldenlandia diffusa	Hunan province, China
2	Lobelia Chinensis Lour	Hunan province, China
3	Scutellaria barbata D. Don	Anhui province, China
4	Scutellaria barbata D. Don	Guanxi province, China
5	Scutellaria barbata D. Don	Henan province, China
6	Scutellaria barbata D. Don	Hubei province, China
7	Scutellaria barbata D. Don	Hunan province, China
8	Scutellaria barbata D. Don	Jingsu province, China
9	Scutellaria barbata D. Don	Shanxi province, China
10	Scutellaria barbata D. Don	Sichuan province, China
11	Scutellaria barbata D. Don	Zhejing province, China

of information. The development of chromatographic fingerprints aims at evaluating the quality of TCM. To identify the real TCM and the false one, the intuitive choice is comparing the similarities and differences of the chromatographic fingerprints' shape. The commonly used standard for evaluation of similarity of different samples is correlation coefficient. The correlation coefficient can be used for comparing a set of chromatographic fingerprints with the mean-value reference in terms of similarity and dissimilarity. The method of principal components analysis (PCA), one of the most commonly used methods of multivariate data analysis in Chemometrics has been widely applied to analyze the large amount of data obtained from GC–MS [15,16].

Scutellaria barbata D. Don (SB) is one of herbs belonging to Labiatae perennial plants, which is mainly distributed throughout southern China and Korea. This herbal material is known in traditional Chinese medicine as 'Ban-zhi-lian' and has long been used for inflammation, hepatitis, osteomyelitis, gynecological, lung, and rectal tumors in China and Korea. In clinically, there are other herbs similar to SB in appearance and function, such as Oldenlandia diffusa (OD) and Lobelia chinensis Lour (LCL). Though OD belongs to Rubiaceae and LCL belongs to Campanulaceae. There are different pharmacological effects among them according to *Bencaogangmu*.

In this study, we developed a method for investigating the chromatographic fingerprint of SB with GC–MS along with relative retention index (RRI) [17] and related Chemometrics methods. Chromatographic fingerprint based on this method was established for the characteristic analysis of samples from different producing areas. The fingerprint obtained from this method can provide useful information for the evaluation of the difference and similarity in SB grown in the various areas of China and furthermore it is extremely useful for the discrimination of the adulterants such as OD and LCL.

2. Experiments

2.1. Herbal materials and reagents

SB was collected from nine different origins of China, and its adulterants, OD, and LCL were all collected from Hunan province, China. All samples were identified in the pharmacies and shown in Table 1.

All solvents (hexane) were of chromatographic grade and were purchased from Merck (Darm-stadt, Germany). In order to calculate the RRI, a mixture of *n*-alkanes from *n*-octane (C8) to eicosane (C20) which were purchased from Fluka Chemika was used.

2.2. Extraction of essential oils

The dried herbal medicine materials were ground to powder before extraction. Then the essential oil samples were extracted by water distillation for 6 h according to the procedure described in the Chinese Pharmacopoeia [18]. The collected essential oils were stored at −10 °C in the refrigerator prepared for GC–MS analysis.

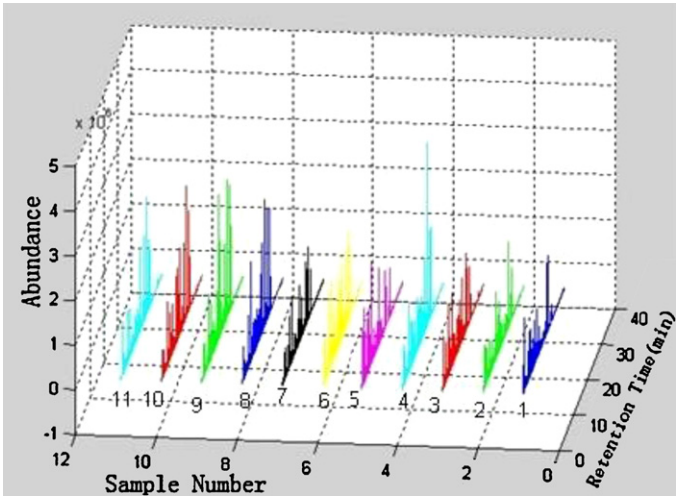

Fig. 1. Fingerprint of SB from nine different origins and its adulterants. Numbers 1–11 stand for the different samples shown in Table 1.

2.3. Instrumentation

GC–MS analysis was performed on a Shimadzu GC–MS–QP 2010 system using DB-5 fused silica column (5% phenyl methyl polysiloxane 30 m × 25 mm id, film thickness 0.25). The column temperature was programmed as follows: initial temperature was 50 °C, holding for 3 min, 10 °C/min to 210 °C, and then 4 °C/min to 260 °C, holding for 1.5 min, the total time was 33 min. Split injection was conducted at a split ratio of 20:1 and high purity helium was used as carrier gas at a flow rate of 3.0 mL/min. The spectrometers were operated in electron-impact (EI) mode, the scan range was 30–500 amu, the ionization energy was 70 eV, and the scan rate was 0.35 s per scan. The ionization source temperature and accelerating voltage were 220 °C and 80 eV, respectively. The injector and detector temperatures were 250 °C and 250 °C, respectively.

2.4. Data analysis methods

Qualitative analysis of SB from nine different origins and its adulterants' main volatile components were obtained through using mass spectrum library searching and mass spectrum similarity comparisons together with RRI information. Quantitative analysis was obtained by area normalization method.

Data analysis was performed by an original software "Extendable Data base for Quality Assessment of Traditional Chinese Herbal Medicine" (It was written by Research Center of Modernization of Chinese Herbal Medicine, Central South University) which is mainly applied to the analysis of Chinese Herbal Medicine based on the Matlab 6.5.

3. Results and discussions

3.1. Characteristic analysis of the chromatographic fingerprint of SB

Nine samples collected from different producing areas were analyzed. The chromatograms are shown in Fig. 1. All the normal samples have high similarity values in retention time while their peak abundances are different. The 52 common peaks of 9 SB samples were assigned as "common peaks" to indicate the similarity

Table 2
Result of qualitative and quantitative of SB, OD and LCL.

ID	I^T	Compound name	Scutellaria barbata D. Don (%) (Hunan)	Oldenlandia diffusa (%) (Hunan)	Lobelia chinensis Lour (%) (Hunan)
1	801	Hexanal	1.91	5.3	2.87
2	812	Cyclopropabenzene	–	–	1.31
3	825	1,3-Octadiene	–	1.27	–
4	831	2-Furancarboxaldehyde	–	–	2.79
5	843	1,2,5,5-Tetramethyl-1,3-cyclopentadiene	0.26	0.61	–
6	853	2-Hexenal	2.77	–	1.51
7	868	1-Hexanol	0.26	–	–
8	888	2-Heptanone	0.34	–	–
9	902	Heptanal	0.58	0.35	0.29
10	917	3-(2-Propenyl)-cyclohexene	–	0.97	–
11	918	4-Ethyl-phenol	–	–	1.96
12	936	2,6,6-Trimethyl-bicyclo[3.1.1]hept-2-ene	–	–	0.96
13	945	α-Pinene	–	1.88	–
14	965	Benzaldehyde	0.34	–	–
15	978	1-Octen-3-one	1.55	–	–
16	981	1-Octen-3-ol	6.21	–	1.13
17	981	β-Pinene	–	0.8	–
18	985	2,5-Octanedione	1.25	1.16	0.65
19	991	2-Pentyl-furan	1.16	3.22	2.16
20	998	3-Octanol	0.61	–	–
21	999	3-Methyl-6-(1-methylethenyl)-cyclohexene	–	–	3.67
22	1004	Octaldehyde	–	1.51	–
23	1027	1-Methyl-2-iso-propylbenzene	–	–	1.51
24	1029	2-Propyl-1-pentanol	–	–	2.49
25	1033	D-Limonene	0.42	0.3	–
26	1036	2-Oxabicyclo[2.2.2]octane	0.78	–	–
27	1047	Benzeneacetaldehyde	–	0.59	0.78
28	1047	1,5-Decadiyne	0.42	–	–
29	1060	2-Octenal	–	0.47	–
30	1069	Acetophenone	0.75	–	–
31	1074	4-Methylbenzaldehyde	–	2.03	0.37
32	1101	1,6-Octadien-3-ol	1.69	–	–
33	1103	2,6-Dimethylocta-2,7-dien-6-ol	5.8	–	–
34	1106	Nonanal	0.71	2.14	1.12
35	1155	1,7,7-Trimethylbicyclo[2.2.1]heptan-2-one	0.34	0.67	–
36	1158	1-Ethenyl-4-methoxybenzee	–	2.23	–
37	1162	(*E*)-2-Nonenal	0.44	1.03	0.48
38	1181	Borneol	–	3.79	–
39	1201	α-Terpineol	1.41	–	0.4
40	1205	1,3-Cyclohexadiene-1-carboxaldehyde	–	–	1.89
41	1205	Safranal	0.28	–	–
42	1208	Decanal	–	0.56	–
43	1224	2,3-Dihydrobenzofuran	–	0.76	–
44	1229	4,8-Dimethyl-1-nonanol	–	0.33	–
45	1253	trans-3,7-Dimethyl-2,6-octadien-1-ol	1.33	0.34	
46	1253	Carvone	–	1.25	–
47	1261	trans-Chrysanthenyl Acetate	–	0.36	–
48	1294	5-Methyl-2-(1-methylethyl)-phenol	–	0.36	–
			–	–	1.12
49	1303	2H-1-Benzopyran	0.36	–	–
50	1303	2-Methyl-5-(1-methylethyl)-Phenol	–	–	1.35
51	1307	1-Methyl-naphthalene	–	–	0.93
52	1318	2-Methoxy-4-vinylphenol	–	4.72	1.33
53	1357	2-Methoxy-4-(2-propenyl)- Phenol	0.43	–	–
54	1385	β-Damascenonel	–	1.74	–
55	1386	Copaene	0.53	–	2.12
56	1395	Cyclobuta[1,2,3,4]dicyclopentene	2.12	–	–
57	1398	α-Gurjenene	–	–	1.47
58	1401	1,2-Dimethoxy-4-(2-propenyl)-benzene	1.17	–	–
59	1416	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)butan-2-one	0.3	–	–
60	1433	β-Caryophyllene	1.55	1.27	2.93
61	1450	trans-Geranylacetone	0.75	1.47	0.51
62	1455	(Z)-β-Farnesene	–	–	1.12
63	1468	α-Guaiene	1.11	–	–
64	1468	Durenol	–	–	0.56
65	1482	Carotene	0.34	–	–
66	1482	B-guaiene	–	–	1.04
67	1486	*E*-β-ionone	1.08	–	–
68	1494	(*Z*,*E*)-α-Farnesene	–	–	0.71
69	1497	Caryophyllene	4.39	–	–
70	1503	Eremophilene	0.51	–	–
71	1509	α-Selinene	1.61	–	–
72	1519	Dicyclohexyl-methanone	0.63	–	–
73	1528	δ-Cadinene	0.56	–	–

Table 2 (*Continued*)

ID	I^T	Compound name	Scutellaria barbata D. Don (%) (Hunan)	Oldenlandia diffusa (%) (Hunan)	Lobelia chinensis Lour (%) (Hunan)
74	1543	1,2,3-Trimethoxy-5-(2-propenyl)-benzene	0.29	–	–
75	1565	(*E*)-Nerolidol	–	–	1.97
76	1592	Spathulenol	0.43	–	–
77	1599	Caryophyllene oxide	1.55	–	1.27
78	1612	Cubenol	0.58	–	–
79	1630	Cedrol	0.71	–	0.58
80	1669	A-cadinol	0.3	–	–
81	1716	*n*-Pentadecana	0.32	0.52	0.24
82	1764	Tetradecanoic acid	0.79	1.6	0.58
83	1842	Hexahydrofarnesyl acetone	4.6	5.53	1.05
84	1861	Pentadecanoic acid	–	1.4	–
85	1923	Hexadecanoic acid, methyl ester	–	0.96	–
86	1978	Hexadecanoic acid	28.6	28.2	31.66
Total			87.22	81.69	80.88

–, not found.

among different samples. RSD values of the relative retention times of 52 common peaks were less than 0.2%, which means the retention time of each component was comparatively stable. The various abundances of the components may be attributed to the climate, regions of cultivation seasons of harvest and the different storage times. Except the common peaks, there are about 10 non-common peaks in different chromatograms. But the non-common peak area is about 7.9%, less than the national standard of 10%.

3.2. Component identification and comparative analysis of the volatile constituents of SB and its adulterants

The essential constituents of SB and its adulterants' were separated and analyzed by GC–MS. The total ion current chromatograph obtained was shown in Fig. 2:

From Fig. 2, we can see the chromatograms of SB, OD, LCL are similar. In order to find the similarity and difference, qualitative and quantitative analysis were performed and the results were listed in Table 2. These compounds were all tentatively identified through mass spectrum library searching and mass spectrum similarity comparisons together with relative retention index information. The results of constituents analyzed are shown in Table 2:

The results showed there were 16 common components between SB and OD, common peak area percent was about 73.47%, 18 between SB and LCL, common peak area percent was about 72.10%. Three of them share 12 common components, which were heptanal, hexanal, 2,5-octanedione, 2-pentyl-furan, nonanal, (*E*)-2-nonenal, β-caryophyllene, trans-geranylacetone, *n*-pentadecana, tetradecanoic acid, hexahydrofarnesyl acetone, hexadecanoic acid. The total proportions of their common constituents were 42.66%, 51.79% and 44.54%, respectively. The non-common peak areas between SB and its adulterants were all higher than 25%, which demonstrated that the adulterants were apparently different from SB by their chemical constituents.

3.3. Similarity analysis

After area normalization and peak alignment, the chromatographic fingerprint of SB from 9 different areas and its adulterants are shown in Fig. 2. The fingerprint of the samples' similarity

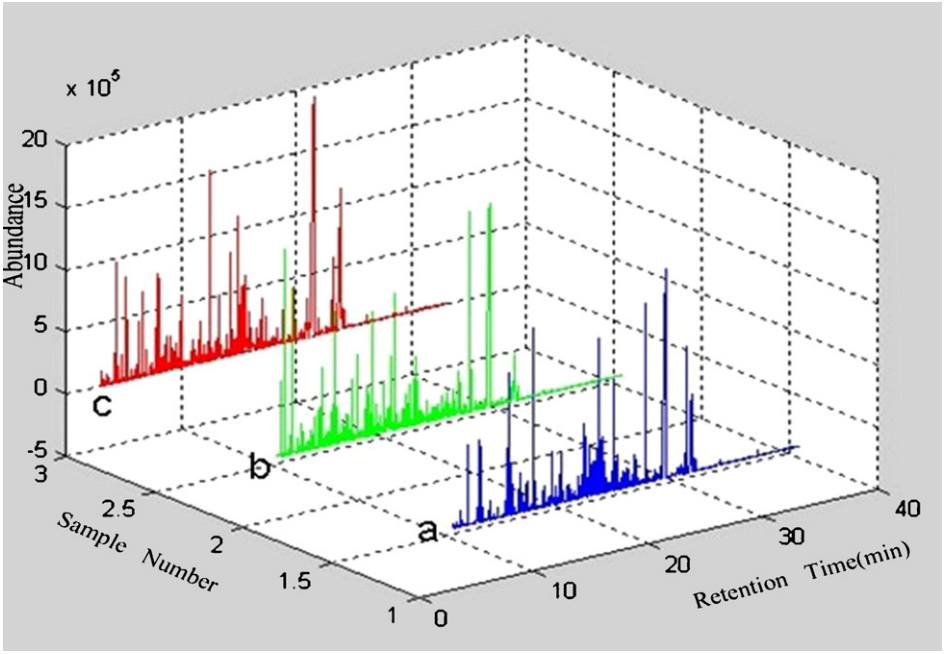

Fig. 2. The chromatograms of SB and its adulterants. (a) SB from Hunan province. (b) OD from Hunan province. (c) LCL from Hunan province.

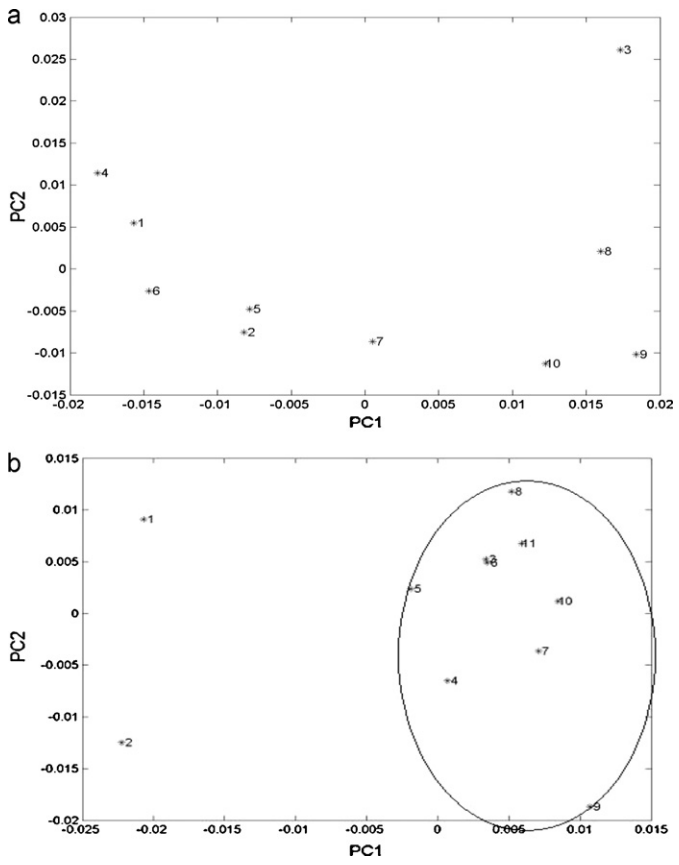

Fig. 3. Distribution of samples on the PCA. Numbers 1–11 stand for the different samples shown in Table 1. (a) The origin data. (b) The new data that picked out hexadecanoic acid.

analysis were performed by "Extendable Data base for Quality Assessment of Traditional Chinese Herbal Medicine" based on Matlab6.5. The program was used to achieve the correlation coefficient between a test sample and a reference sample in evaluating the similarities of different chromatogram. Mean GC–MS chromatogram of SB was taken as a reference sample. The similarity results are shown in Table 3, the similarities of SB from 9 producing areas were higher than 0.7660, and the similarities of OD (Hunan) and LCL (Hunan) with the reference were only 0.4199 and 0.4059, respectively. The correlation coefficients of samples are shown in Table 3:

3.4. PCA analysis

In this paper, PCA pattern recognition method was also used for further discrimination SB from its adulterants. Principal components analysis (PCA) is a method that is to generate new original variables but shows linear combinations of them, and simultaneously capture most features of the original data. Each PC includes a set of scores and loadings [19–21]. PCA describes the variation in date with minimum latent variables. The score values for the first two PCs (PC1 and PC2) are often used to represent the characteristics of the samples. Fig. 3(a) is the plot of PC1 to PC2 of the 11 data of SB and its 2 adulterants. It did not show any resolution of the 11 data.

From the result of identified components, hexadecanoic acid (ID 86 in Table 2) existed in all of samples with very high content, it might be the main reason to affect the PCA result. According to pharmacological research, no evidence shows that hexadecanoic acid is the possible active component. In this paper, the peak of hexadecanoic acid was picked out from the chromatogram and the PCA calculation was reprocessed, Fig. 3(b) is the new result of the PCA profile. The samples outside the circle can be viewed the false ones. From Fig. 3(b), the PC1 values of samples 1 and 2 are far away from the rest of the samples, and the PC1 difference of SB from 9 different origins are less than 0.015, they are comparatively concentrated, samples 8 and 9 are a little deviated from the center of the data, this result is consistence with the result of the similarity of the fingerprints.

Table 3
The correlation coefficient result of samples.

Sample no	Origin	Correlation coefficient
1	Oldenlandia diffusa (Hunan)	0.4199
2	Lobelia chinensis Lour (Hunan)	0.4059
3	Scutellaria barbata D. Don (Anhui)	0.9473
4	Scutellaria barbata D. Don (Guanxi)	0.8851
5	Scutellaria barbata D. Don (Henan)	0.8455
6	Scutellaria barbata D. Don (Hubei)	0.9198
7	Scutellaria barbata D. Don (Hunan)	0.9055
8	Scutellaria barbata D. Don (Jiangsu)	0.7687
9	Scutellariabarbata D. Don (Shanxi)	0.7660
10	Scutellaria barbata D. Don (Sichuan)	0.8688
11	Scutellaria barbata D. Don (Zhejiang)	0.8735

4. Conclusion

The problem of quality control of herbal medicines has been solved to a great extent with the help of chromatographic fingerprint analysis. Similarity evaluation and pattern recognition can be used to discriminate different kinds of samples of herbal medicines investigated. In this paper, SB from 9 different producing areas and 2 common used adulterants of SB were analyzed and evaluated by the developed chromatographic fingerprint, the result represents that the samples of SB from nine different origins were consistent, while there are difference between the SB and its adulterants. Chromatographic fingerprint analysis is an efficient quality control tool for Chinese traditional medicines.

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