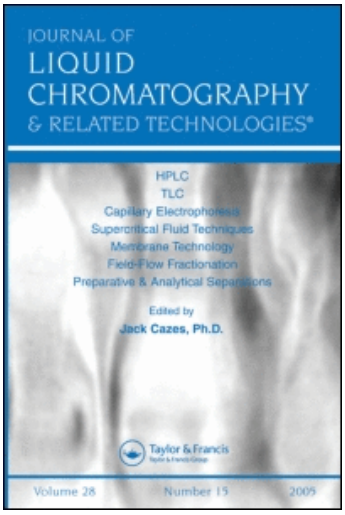

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Digitized Chromatographic Fingerprint Spectrum of Water Soluble Components of *Salvia Miltiorrhiza* Bunge by High Speed Countercurrent Chromatography

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Abstract: Water soluble components are a major part of active components of *Salvia miltiorrhiza* Bunge, a very important species of TCM, whose quality control is valuable. Twelve water extracted crude samples of *Salvia miltiorrhiza* Bunge were analyzed and fingerprints of them were developed by high speed countercurrent chromatography (HSCCC). Three major components in each sample were identified. Their digitized chromatographic fingerprint spectrum (DCFS) were developed by an improved method with relative retention time, relative peak area, relative standard deviation of relative retention time, relative standard deviation of relative peak area, difference rate, and fingerprint characteristic. HSCCC-DCFS was a quantitative and intuitive method for the fingerprinting of water soluble components of *Salvia miltiorrhiza* Bunge.

Keywords: High speed countercurrent chromatography (HSCCC), Digitized chromatographic fingerprint spectrum (DCFS), 3,4-Dihydroxyphenyllactic acid, Salvianolic acid B, Protocatechualdehyde, *Salvia miltiorrhiza* Bunge

INTRODUCTION

Salvia miltiorrhiza Bunge, a popular traditional Chinese medicinal plant, has been used extensively for the treatment of coronary heart disease, cerebrovascular disease, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrhea, and neuroasthenic insomnia and cytotoxicity against human tumor cell

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lines.[1–3] Decoction has been the most popular preparation of TCM from more than two thousand years ago, which is the original preparation of *Salvia miltiorrhiza* Bunge. The largest part of the active components of the decoction is water soluble polyphenols that possess a variety of biological activities, including antioxidant, antiplatelet, antitumor, and antiviral activity.[4,5] 3,4-Dihydroxyphenyllactic acid, also known as danshensu (Chinese word to mean literally the element of Danshen) and salvianolic acid B (lithospermic acid B) are major active components of polyphenols in *Salvia miltiorrhiza* Bunge. 3,4-Dihydroxyphenyllactic acid was first isolated from *Salvia miltiorrhiza* Bunge and found to be a coronary vasodilator and to scavenge the free oxygen radicals.[6] Salvianolic acid B has significant scavenging effects on oxygen free radicals and protective effects on heart and brain injuries induced by ischemia reperfusion.[7] Protocatechualdehyde (protocatechuic aldehyde) is normally used as a symbolic component in analysis of the related preparation of *Salvia miltiorrhiza* Bunge.

Active components of TCM are influenced by its growing conditions, the seasons when plants are harvested, the process methods and storage duration, which make quality control essentially necessary for its application.[8] As a very important species of TCM, it is of great value to develop different analytical methods for quality control of *Salvia miltiorrhiza* Bunge. With the development of technologies of instrumental analysis and information processing, fingerprinting, normally referring to chemical fingerprinting, becomes a popular and authoritative method of the quality control of TCM, which is accepted or recommended by authorities, including State Food and Drug Administration (SFDA) of China, Food and Drug Administration (FDA) of United States, the European Agency for the Evaluation of Medicinal Products (EMEA), herbal pharmacopoeia of Britain and India, as well as the World Health Organization (WHO).[9] Chromatography, including thin-layer chromatography (TLC), high performance liquid chromatography (HPLC), gas chromatography (GC), and high performance capillary electrophoresis (HPCE), is widely applied[10–12] and is recommended for the quality control of TCM by Chinese Pharmacopoeia (2005 edition).[13] In our previous research, another chromatographic method, high speed countercurrent chromatography was forwarded for fingerprinting of TCM.[14] High speed countercurrent chromatography (HSCCC) is a liquid-liquid partition chromatography without any solid matrix, which eliminates the irreversible adsorption of samples on solid support. Its special structure makes it easy to analyze and separate samples with high viscosity and is easily adsorbed. It has been widely used for separation and purification of several hundreds of low molecular weight compounds including polyphenols, alkaloids, terpenoids, lignanoids, coumarin, et al.

The expression of the original chromatographic fingerprint is a chromatogram, which is called image chromatographic fingerprint (ICF). ICF itself was influenced by operation factors. Digitized chromatographic fingerprint spectrum (DCFS), as an evaluation method, was forwarded.[14] By DCFS, a

complicated chromatogram of ICF is converted into intuitive numbers. A reference peak is chosen in common peaks of chromatograms to introduce relative values, including relative retention time (RRT) and relative peak area (RPA), which eliminates the negative effects of operation factors on integral stability and reproducibility of fingerprinting and facilitates quantitative and quick comparison of different samples. A series of parameters, rules, and formulae are employed in DCFS to form a whole methodology. The key part of original DCFS was a table that includes RRT and RPA of chromatographic peaks of different batches of samples. The original DCFS method was improved in our research by introducing relative standard deviation (RSD) of RRT as well as RSD and difference rate (DR) of RPA into the table of DCFS to make it more intuitive for quantitative comparison of any parts of samples. HSCCC-DCFS has not been reported so far.

HSCCC-DCFS was used for water soluble components of *Salvia miltiorrhiza* Bunge in this report, which supplied an alternative quality control method of raw material and related preparation of *Salvia miltiorrhiza* Bunge.

EXPERIMENTAL

Reagents

Analytical grade n-hexane, ethyl acetate, methanol, and acetic acid for HSCCC separation were from Atoz Fine Chemicals Co. Ltd., Tianjin, China. Methanol of HPLC grade methanol and acetic acid was obtained from Concord Tech Co. Ltd., Tianjin, China. All aqueous solutions were prepared with pure water produced by Milli-Q system (18 MΩ, Milipore, Bedford, MA, USA).

Nine samples (no. 1 to no. 9) of twelve batches of raw material *Salvia miltiorrhiza* Bunge were supplied by Tianjin TASLY Group Company from different plantations of trueborn plant area, Shangnan, Shanxi province. Samples no. 10, no. 11, and no. 12 were obtained from Changzhi (Shanxi province), Weifang (Shandong province) and Jiangsu province.

Standard reference materials of 3,4-dihydroxyphenyllactic acid (110763-200504), salvianolic acid B (111562-200506), and protocatechualdehyde (0810-200004) were supplied by the State Food and Drug Administration of China (SFDA) (Figure 1).

Apparatus

HSCCC (TBE-300) is from Tauto Biotech, Shanghai, China, with three preparative coils connected in series (diameter of 2.6 mm, total volume 300 mL) and a 20 mL sample loop . The revolution radius or the distance between the holder axis and central axis of the centrifuge (R) was 5 cm, and the β value varied from 0.5 at the internal terminal to 0.8 at the

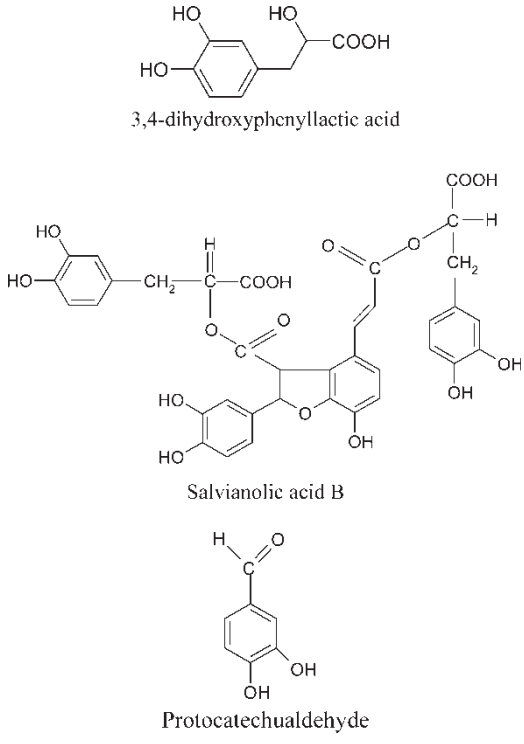

Figure 1. Structures of 3,4-dihydroxyphenyllactic acid, salvianolic acid B and protocatechualdehyde.

external terminal ($\beta = r/R$, where r is the distance from the coil to the holder shaft). The HSCCC systems are equipped with a Model S constant flow pump, a Model 8823A UV monitor operating at 280 nm, and a Model 3057 recorder.

Preparation of Water Extracted Crude Sample of *Salvia Miltiorrhiza* Bunge

Raw material *Salvia miltiorrhiza* Bunge was ground into powder and decocted with water for 4 hours. The decoction was dried into powder. The water extracted crude sample (100 mg) of *Salvia miltiorrhiza* Bunge was dissolved in 5 mL lower phase of the solvent system in an ultrasonic bath.

Preparation of Solvent System for HSCCC Separation

A hydrophilic organic/aqueous solvent system was prepared by thoroughly mixing organic solvents and water in a separatory funnel at room temperature.

Two phases was separated just before use. The optimized solvent system of water soluble components of *Salvia miltiorrhiza* Bunge was n-hexane-ethyl acetate-methanol-acetic acid-water (1:6:1.5:1.5:8).

HSCCC Operation

The coiled column of the HSCCC was filled with the upper phase of the solvent system. Then, the apparatus was rotated at 850 rpm and at temperature of 26°C, and at the same time, the lower phase of the solvent system was pumped through the column at a flow rate of 1.5 mL/min. After the mobile phase emerged in the effluent and hydrodynamic equilibrium was established in the column, 5 mL of the sample solution containing 100 mg of the water extracted crude sample of *Salvia miltiorrhiza* Bunge was injected through the valve. Retention of stationary phase was 50%. The effluent was monitored with a UV-Vis detector at 280 nm and the peak fractions were collected, respectively.

HPLC Analysis

The water extracted crude sample and fractions separated by HSCCC were analyzed by the HPLC system (10 Avp, Shimadzu, Japan) composed of two pumps, UV detector, oven, system controller, and 20 μL sample loop. The column used was Ultrasphere C18 column (250 × 4.6 mm I.D., 5 μm, Shimadzu, Japan). The mobile phase was solvent A (methanol-water-acetic acid = 10:89.2:0.8) and solvent B (methanol-water-acetic acid = 89.2:10:0.8) in the gradient mode as follows: 0–60 min, 0–60% B. The flow rate was 1.0 mL/min and temperature was 40°C. The effluent was monitored at 280 nm. The crude sample and three kinds of reference material were dissolved with solvent A.

RESULTS AND DISCUSSION

Development of Image Chromatographic Fingerprint of Water Soluble Components of *Salvia Miltiorrhiza* Bunge by High Speed Countercurrent Chromatography (HSCCC)

A water extracted crude sample of *Salvia miltiorrhiza* Bunge was separated successfully by HSCCC with the solvent system composed of n-hexane-ethyl acetate-methanol-acetic acid-water (1 : 6:1.5 : 1.5 : 8), at a flow rate of 1.5 mL/min and at a speed of 850 r/min. Optimization of the separation condition was presented in detail in our previous report.[15] Major active components, 3,4-dihydroxyphenyllactic acid, salvianolic acid B and protocatechualdehyde, were identified by mixing the fraction with corresponding standard reference material. This optimal method was applied to analyze twelve water extracted

crude samples of *Salvia miltiorrhiza* Bunge. Three major active components in the twelve crude samples were identified in the above way.

Twelve crude samples were analyzed by HSCCC and nine peak fractions were eluted from each sample (as shown in Figure 2). The RSD of retention

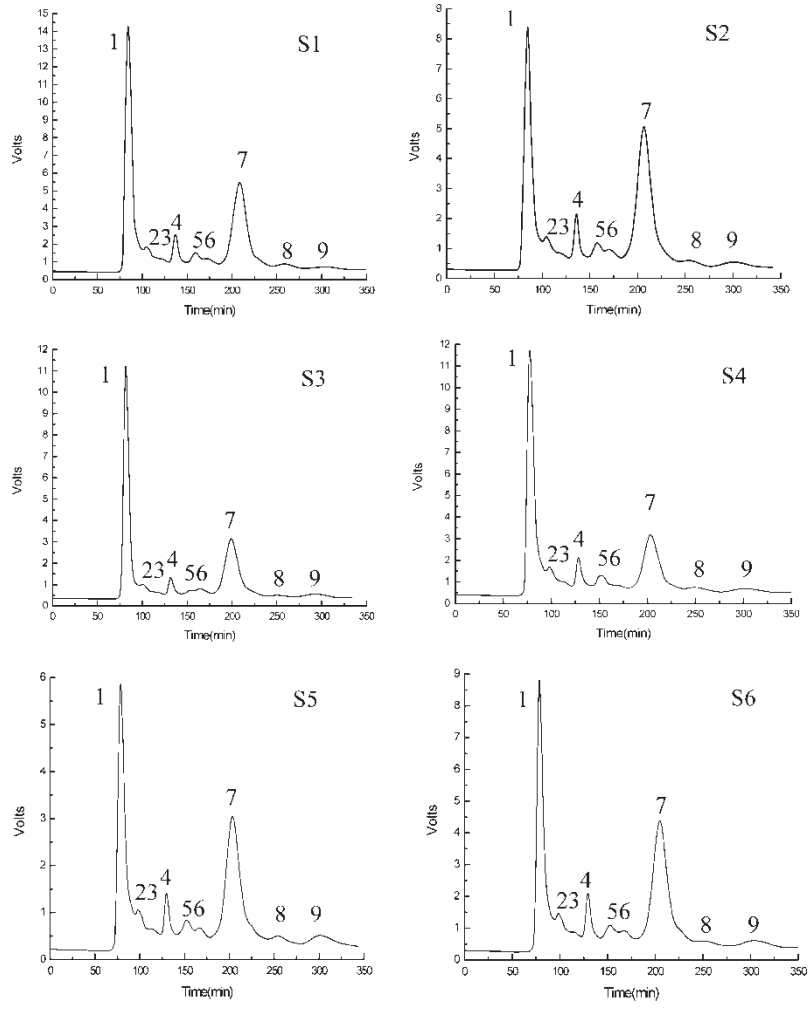

Figure 2. Fingerprint of water soluble components of *Salvia miltiorrhiza* Bunge by HSCCC. Peak no. 2: 3,4-dihydroxyphenyllactic acid; Peak no. 7: salvianolic acid B; Peak no. 8: protocatechualdehyde. Experimental conditions for HSCCC are as follows: apparatus: TBE-300A with 200 mL column; retention of stationary phase: 50%; solvent system: n-hexane-ethyl acetate-methanol-acetic acid-water (1:6:1.5:1.5:8); mobile phase: lower phase; elution mode: head to tail; flow rate: 1.5 mL/min; revolution: 850 r/min; temperature: 26°C; detection wavelength: 280 nm; sample loading: 100 mg/5 mL.
(*continued*)

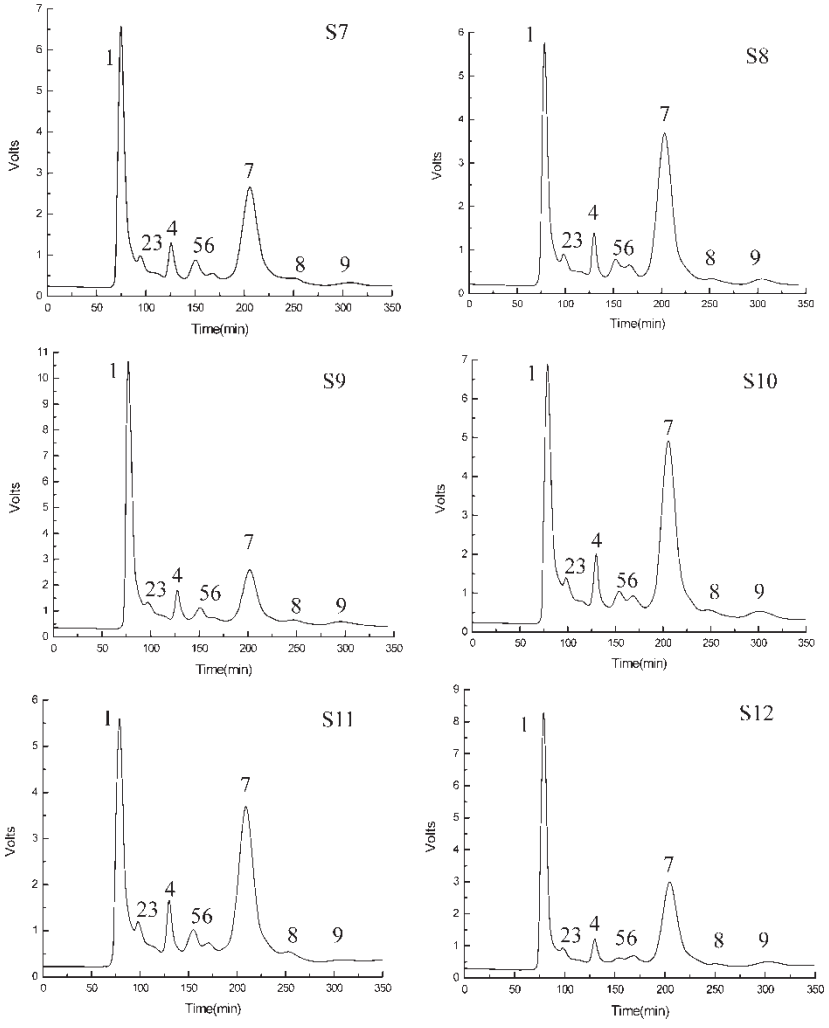

Figure 2. Continued.

time of corresponding peaks in HSCCC analyses was in the range of 0.39–2.5%, which was within the methodological error range of HSCCC (3%).[16] This led to the conclusion that the corresponding peak fractions in the twelve samples were the same. This conclusion was confirmed by HPLC analysis of peak fractions from HSCCC separation. Peak fraction no. 2, no. 7, and no. 8 in each sample were 3,4-dihydroxyphenyllactic acid, salvianolic acid B, and protocatechualdehyde, respectively.

It was observed that corresponding peaks in different samples had different contents (Table 1), which confirmed the importance of quality control of TCM.

Table 1. Peak area percent of peak fractions in twelve water extracted crude samples of *Salvia miltiorrhiza* Bunge by HSCCC

	Peak area (%)								
	P1	P2	P3	P4	P5	P6	P7	P8	P9
S1	41.26	4.78	3.07	6.02	4.46	3.22	31.46	3.37	2.36
S2	33.49	4.41	2.92	6.93	4.63	3.65	37.76	3.10	3.10
S3	48.12	3.24	2.63	4.70	2.25	3.80	30.52	2.02	2.72
S4	44.73	5.64	3.45	7.10	5.41	2.99	24.59	3.30	2.80
S5	32.45	5.24	2.97	5.82	4.76	3.81	36.10	4.02	4.82
S6	33.00	5.25	3.29	7.27	4.40	3.94	36.60	2.99	3.25
S7	36.11	4.55	3.31	6.56	5.58	3.51	32.65	4.49	3.25
S8	29.80	4.10	3.34	6.03	4.92	3.81	42.92	2.75	2.34
S9	46.03	4.74	3.35	6.88	5.63	2.37	25.38	3.02	2.60
S10	27.42	5.18	3.41	7.55	5.10	4.49	41.14	2.76	2.95
S11	30.07	5.09	3.23	6.86	5.98	4.07	39.67	3.72	1.32
S12	41.62	3.57	2.64	5.06	3.08	3.85	34.69	2.20	3.30

It was difficult to compare these twelve chromatograms quantitatively and directly. Also, there were negative effects of operation factors on the integral stability and reproducibility of ICF. HSCCC-DCFS was, consequently, brought forward.

Development of Digitized Chromatographic Fingerprint Spectrum of Water Soluble Components of *Salvia Miltiorrhiza* Bunge by High Speed Countercurrent Chromatography (HSCCC-DCFS)

The relative value that was calculated based on the reference peak is the basis of DCFS, which realizes quantitative comparison. Salvianolic acid B is the major active component of water soluble components of *Salvia miltiorrhiza* Bunge, and it appeared in all twelve samples. Resolutions between its peak and adjacent peaks were very good. Based on the above reasons, the peak of salvianolic acid B (peak no. 7) was chosen as the reference peak and its relative retention time (RRT) and relative peak area (RPA) were set to 1. Retention time and peak area of other peaks were divided by those of the reference peak, respectively, to get their own RRT and RPA (Table 2). In between, peak no. 2 and 8, were 3,4-dihydroxyphenyllactic acid and protocatechualdehyde, respectively. Quantitative comparison became very easy with relative values. For example, RPA of peak no. 2 of sample no. 4 was 0.24 that was higher than that of other eleven samples. It indicated that if peak fraction 2 (3,4-dihydroxyphenyllactic acid) was the target compound, sample no. 4 could be better for preparation of 3,4-dihydroxyphenyllactic acid than other samples.

Table 2. Digitized chromatographic fingerprint spectrum of water soluble components of *Salvia miltiorrhiza* Bunge by HSCCC

	P1	P2	P3	P4	P5	P6	P7	P8	P9
RRT									
S1	0.40	0.50	0.59	0.66	0.77	0.84	1.00	1.23	1.48
S2	0.40	0.51	0.58	0.66	0.76	0.82	1.00	1.23	1.47
S3	0.41	0.50	0.59	0.66	0.76	0.83	1.00	1.24	1.46
S4	0.38	0.48	0.56	0.63	0.75	0.82	1.00	1.22	1.48
S5	0.39	0.49	0.57	0.64	0.75	0.82	1.00	1.24	1.49
S6	0.38	0.48	0.56	0.63	0.75	0.82	1.00	1.24	1.48
S7	0.38	0.46	0.55	0.61	0.73	0.82	1.00	1.21	1.49
S8	0.39	0.48	0.57	0.64	0.75	0.82	1.00	1.24	1.49
S9	0.39	0.48	0.56	0.63	0.75	0.83	1.00	1.22	1.46
S10	0.38	0.48	0.56	0.63	0.75	0.82	1.00	1.22	1.48
S11	0.40	0.49	0.58	0.66	0.78	0.86	1.00	1.19	1.55
S12	0.38	0.48	0.56	0.63	0.75	0.82	1.00	1.22	1.48
Average	0.388	0.486	0.569	0.64	0.754	0.827	1.00	1.225	1.484
RSD of RRT (%)	2.88	2.70	2.30	2.58	1.64	1.49	0.00	1.23	1.56
RPA									
S1	1.31	0.16	0.10	0.19	0.14	0.10	1.00	0.11	0.08
S2	0.89	0.12	0.08	0.18	0.12	0.10	1.00	0.08	0.08
S3	1.58	0.11	0.09	0.15	0.07	0.12	1.00	0.07	0.09
S4	1.82	0.23	0.14	0.29	0.22	0.12	1.00	0.13	0.11
S5	0.90	0.15	0.08	0.16	0.13	0.11	1.00	0.11	0.13
S6	0.90	0.14	0.09	0.20	0.12	0.11	1.00	0.08	0.09
S7	1.10	0.14	0.10	0.20	0.17	0.11	1.00	0.14	0.10
S8	0.69	0.10	0.08	0.14	0.11	0.09	1.00	0.06	0.05
S9	1.81	0.19	0.13	0.27	0.22	0.09	1.00	0.12	0.10
S10	0.67	0.13	0.08	0.18	0.12	0.11	1.00	0.07	0.07
S11	0.76	0.13	0.08	0.17	0.15	0.10	1.00	0.09	0.03
S12	1.20	0.10	0.08	0.15	0.09	0.11	1.00	0.06	0.10
RSD of RPA (%)	36.44	26.72	21.93	24.48	33.32	9.41	0.00	29.73	31.21
DR									
S2	0.34	0.25	0.23	0.07	0.16	0.08	0.03	0.25	0.06
S3	0.27	0.57	0.46	0.51	0.68	0.26	0.39	0.62	0.28
S4	0.17	0.09	0.13	0.09	0.07	0.28	0.40	0.25	0.09
S5	0.56	0.39	0.46	0.46	0.40	0.34	0.36	0.33	0.14
S6	0.38	0.15	0.17	0.07	0.24	0.06	0.10	0.32	0.06
S7	0.50	0.46	0.38	0.38	0.28	0.38	0.41	0.24	0.21
S8	0.64	0.57	0.45	0.50	0.44	0.40	0.31	0.59	0.50
S9	0.29	0.37	0.31	0.27	0.20	0.53	0.49	0.43	0.30
S10	0.49	0.17	0.14	0.03	0.12	0.07	0.01	0.37	0.04
S11	0.56	0.36	0.37	0.31	0.19	0.24	0.24	0.33	0.66
S12	0.46	0.60	0.54	0.55	0.63	0.36	0.41	0.65	0.25

For easy and intuitive evaluation of fingerprinting, RSDs of RRT and RPA were introduced into our HSCCC-DCFS method. The error range of HSCCC-DCFS was settled based on RSD of RRT and RPA in our previous research as follows: RSD of RRT was 3% and RSD of RPA was 8%. In this HSCCC-DCFS of water soluble components of *Salvia miltiorrhiza* Bunge, RSD of RRT was less than 2.88%, which was within the error range, meaning that corresponding peaks from different samples were the same. RSD of RPA was in the range of 9.41–36.44%, which was out of the error range. This means that contents of corresponding peaks from different samples were different, which confirmed the necessity of fingerprinting of TCM. The difference rate specially showed degree of difference between the standard sample and test sample. Calculation of DR is shown in Formula 1. In this study, sample no. 1 was supposed to be the standard sample of *Salvia miltiorrhiza* Bunge and the DR of test samples were calculated.

$$\text{Difference rate} = \left|A_I^S - A_I^T\right| / A_I^S$$

Formula 1: Calculation of difference rate

A_I^S: peak area of standard sample, A_I^T: peak area of test sample

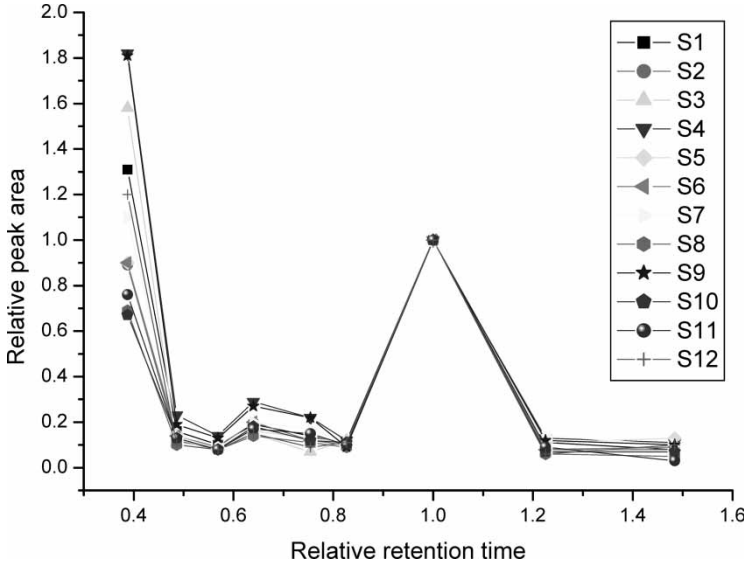

Figure 3. Characteristic fingerprint curve of water soluble components of *Salvia miltiorrhiza* Bunge by HSCCC.

The total difference rate showed general difference between standard sample and test sample. Its calculation is shown in Formula 2.

$$\text{Total difference rate} = \frac{\sum\left(\left|A_I^S - A_I^T\right|/A_I^S\right)}{n}$$

Formula 2: Total calculation of difference rate

A_I^S: peak area of standard sample, A_I^T: peak area of test sample

The total difference rates of sample no. 2 to sample no. 12 were 0.16, 0.45, 0.17, 0.38, 0.17, 0.36, 0.49, 0.36, 0.16, 0.36, and 0.49. It showed that, in general, the largest differences were in sample no. 8 and no. 12 from the standard sample.

The characteristic fingerprint curve was an intuititive way to show the difference of samples in one reference frame. This was plotted using RRT as x-axis and RPA as y-axis (as shown in Figure 3). Each curve here included information of one chromatogram of ICF.

CONCLUSION

Image chromatographic fingerprinting of twelve water extracted crude samples of *Salvia miltiorrhiza* Bunge was developed by HSCCC and three major components in each sample were identified. The HSCCC-DCFS was developed by an improved DCFS method with RRT, RPA, RSD of RRT, RSD of RPA, DR and fingerprint characteristics. It was proven that HSCCC-DCFS was a quantitative and intuititive method for fingerprinting of water soluble components of *Salvia miltiorrhiza* Bunge.

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REFERENCES

1. Lu, Y.R.; Foo, L.Y. Polyphenolics of *Salvia*-a review. Phytochemistry **2002**, *59*, 117–140.
2. Lin, G.C.; Chang, W.L. Diterpenoids from *Salvia miltiorrhiza*. Phytochemistry **2000**, *53*, 951–953.
3. Wu, C.T.; Mulabagal, V.; Nalawade, S.M.; Chen, C.L.; Yang, T.F.; Tsay, H.S. Isolation and quantitative analysis of cryptotanshinone, an active quinoid diterpene formed in callus of *Salvia mitiorrhiza* Bunge. Biol. Pharm. Bull. **2003**, *26*, 845–848.

4. Yuan, J.P.; Chen, H.; Chen, F. Simultaneous determination of rosmarinic acid, lithospermic acid B, and related phenolics in *Salvia mitiorrhiza* by HPLC. J. Agric. Food Chem. **1998**, *46*, 2651.
5. Petersen, M.; Simmonds, M.S.J. Rosmarinic acid. Phytochemistry **2003**, *62*, 121–125.
6. Zhang, J.L.; Cui, M.; He, Y.; Yu, H.L.; Guo, D.A. Chemical fingerprint and metabolic fingerprint analysis of Danshen injection by HPLC-UV and HPLC-MS methods. J. Pharm. Biomed. Anal. **2005**, *36*, 1029–1035.
7. Lin, Y.H.; Liu, A.H.; Wu, H.L.; Westernbroek, C.; Song, Q.L.; Yu, H.M.; Horst, G.J.T.; Li, X.J. Salvianolic acid B, an antioxidant from *Salvia mitiorrhiza*, prevents Aβ_{25-35}-induced reduction in BPRP in PC12 cells. Biochem. Biophys. Res. Comm. **2006**, *348*, 593–599.
8. Drašar, P.; Moravcova, J. Recent advances in analysis of Chinese medical plants and traditional medicines. J. Chromatogr. B **2004**, *812*, 3–21.
9. Dniel, K.W.; Chau, M.F.T. Chemical information of Chinese medicines: A challenge to chemist. Chemo. Intell. Lab. Sys. **2006**, *82*, 210–217.
10. Wang, X.H.; An, R.; Zou, Y.; Wang, Z.H.; Hong, X.K.; Li, G.W. Application of chromatograph-digitized fingerprint spectrum in identification of medicinal materials and quantitative control of Shuanghuanglian preparation. Chinese Trad. Herb. Drugs **2002**, *33*, 115–118.
11. Huang, T.J.; Zhang, Z.X.; Xiang, B.R.; An, D.K. Standardization and digitization of fingerprint gas chromatograms of essential oil of *curcuma longa*. Chinese Trad. Herb. Drugs **2003**, *34*, 793–797.
12. Song, G.Q.; Lin, J.M.; Qu, F.; Dong, W.J. Study of tea digitized chromatographic fingerprint spectra using micellar electrokinetic chromatography. Chinese J. Chem. **2003**, *21*, 1325–1329.
13. Committee of National Pharmacopoeia. In: *Pharmacopoeia of P.R. China*; Press of Chemical Industry: Beijing, 2005.
14. Gu, M.; Ouyang, F.; Su, Z.G. Comparison of high speed counter current chromatography and high performance liquid chromatography on fingerprinting of chinese traditional medicine. J. Chromatogr. A **2004**, *1022*, 139–144.
15. Gu, M.; Ouyang, F. One-step separation and purification of 3,4-dihydroxyphenyllactic acid, salvianolic acid B and protocatechualdehyde from *Salvia miltiorrhiza Bunge* by high speed countercurrent chromatography. J. Chromatogr. A **2007**, *1140*, 107–111.
16. Gu, M. Separation and Fingerprinting of Natural Products by High speed Countercurrent Chromatography. Dissertation of Ph.D. candidate of Institute of Process Engineering, Chinese Academy of Sciences, 2004.

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