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Fingerprinting of *Salvia miltiorrhiza* Bunge by non-aqueous capillary electrophoresis compared with high-speed counter-current chromatography

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Abstract

The component of the traditional Chinese medicine (TCM) can be influenced by soil, climate, and growth stage, and fingerprint is an important means in its quality control. Our previous studies showed that high-speed counter-current chromatography (HSCCC) was helpful in the development of fingerprint of TCM. Since the HSCCC method is new, it is necessary to compare it with conventional ones, such as high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC) and high-performance capillary electrophoresis (HPCE). Comparison with HPLC was conducted in our previous study. In this study, HSCCC was compared with non-aqueous capillary electrophoresis (NACE). With NACE, seven stable components were separated within 55 min, respectively, from three crude samples of *Salvia miltiorrhiza* Bunge from different growth locations. In HSCCC separation, 12 components were separated, respectively, with good correspondence and precision within 13 h. Both NACE and HSCCC were effective in showing whole concentration distribution of all kinds of constituents. Principles of these two methods were very different, which led to different elution sequences and relative contents of peaks. HSCCC showed better performance in analysis of tanshinones, which made its fingerprint containing more chemical information than that of NACE. It was further proven that HSCCC could be a feasible and cost-effective method in the development of the fingerprint of TCM.

Keywords: Non-aqueous capillary electrophoresis (NACE); High-speed counter-current chromatography (HSCCC); Fingerprint; *Salvia miltiorrhiza* Bunge; Tanshinones

1. Introduction

The active components of traditional Chinese medicine (TCM) are influenced by its growing soil and climate, and the growth stage when harvested [1]. Fingerprint becomes a popular method in the quality control of TCM [2].

Chromatography, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), is recommended for the quality control of TCM in Chinese Pharmacopoeia (2000 edition) [1]. High-performance capillary electrophoresis (HPCE), as an established analytical technique in several other areas, has recently been accepted as an effective method by regulatory authorities such as State Food and Drug Administration of China and US Food and Drug Administration. HPCE is an automated analytical technique that separates species by applying voltage across buffer filled capillaries. It is generally used for ion separation, which move at different speed depending on their size and charge. However, non-aqueous capillary electrophoresis (NACE) has been developed rapidly in

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the past few years. NACE has proven to be a very promising approach for the separation of a large range of compounds, including both ionic and neutral hydrophobic compounds that are difficult to separate in aqueous electrolytes. The use of organic solvents as separation medium opens up a new way to improve the separation selectivity [3].

High-speed counter-current chromatography (HSCCC) is a liquid–liquid partition chromatography without any solid matrix, which eliminates the irreversible adsorption of samples on solid support [4,5]. Its special structure makes it easy to analyze and separate samples with high viscosity. It has been applied to develop fingerprint of TCM in our study [6]. As a new method, it is therefore necessary to compare HSCCC with conventional approaches, for example HPLC, HPCE and TLC, to evaluate its feasibility. Comparison with HPLC has been involved in our previous report.

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 [7]. *S. miltiorrhiza* Bunge also showed significant cytotoxicity against human tumor cell lines [8]. There are two kinds of major active constituents in *S. miltiorrhiza* Bunge, tanshinones and polyphenolics. Since it is impossible to separate these two kinds of compounds with same condition, only tanshinones were involved in this study. Cryptotanshinone, tanshinone I and tanshinone IIA are major active components. Cryptotanshinone is usually used against inflammation, tanshinone I for therapy of angina pectoris and tanshinone IIA for improving blood circulation.

HPCE has been reported in analysis of hydrophilic components of *S. miltiorrhiza* Bunge in English and Chinese journals [9–11], but no report for analysis of hydrophobic components of *S. miltiorrhiza* Bunge was found.

Separation and purification of *S. miltiorrhiza* Bunge by different types of counter-current chromatography has been reported. Tian prepared four tanshinones by HSCCC [12] and eight tanshinones by multidimensional counter-current chromatography [13]. Li and Chen [14] separated eight tanshinones and one water-soluble phenolic compound by HSCCC [15]. However, 12 components, more than other reports, were prepared by HSCCC in our study [6].

In this study, NACE was applied to develop fingerprint of *S. miltiorrhiza* Bunge and the results were compared with those of HSCCC.

2. Materials and methods

2.1. Materials

Methanol of HPLC grade was obtained from Concord Tech. Co. Tianjin, China. Disodium tetraborate and sodium deoxycholate of analytical-grade were obtained from current commercial sources in China. Analytical-grade ethanol and *n*-hexane were from Atoz Fine Chemicals Tianjin, China. All aqueous solutions were prepared with pure water produced by Milli-Q system (18 MΩ, Milipore, Bedford, MA, USA). Three standard samples, including cryptotanshinone, tanshinone I and tanshinone IIA were supplied by the State Food and Drug Administration of China (SFDA).

2.2. Non-aqueous capillary electrophoresis

NACE experiments were carried out with a Beckman P/ACE 5500 system (Beckman Instruments, Fullerton, CA, USA) equipped with a UV detector and a power supply able to deliver up to 30 kV. Data were processed by P/ACE software. Bare fused-silica capillaries, 57 cm (50 cm from inlet to detector) × 75 μm i.d., were from Yongnian Optic Fiber Work, Hebei, China. Samples were introduced under 0.5 psi for 3 s (1 psi = 6894.76 Pa). Separation was performed under 25 kV at 25 °C. Absorbance detection was carried out at 280 nm. The acquisition rate was 10 points/s.

The final non-aqueous separation electrolyte was 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05. Prior to each sample injection, the capillary was rinsed with, consecutively, water, methanol and buffer for 3 min. Samples were diluted with separation electrolyte for injection.

2.3. High-speed counter-current chromatography

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 internal terminal to 0.8 at the 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.

Solvent system A was *n*-hexane–ethanol–water (10:5.5:4.5, v/v/v) and solvent system B was *n*-hexane–ethanol–water (10:7:3, v/v/v) [6]. Each mixture was equilibrated thoroughly in a funnel at room temperature. The upper phase and lower phase were separated before use.

Preparative separation was performed by stepwise elution with solvent systems A and B in sequence. First, the coiled column was filled with the upper phase of solvent system A. Then, the apparatus was rotated at 900 rpm, and at the same time, the lower phase of solvent system A was pumped through the column at a flow rate of 2.0 ml/min. After the mobile phase emerged in the effluent, and hydrodynamic equilibrium was established in the column, 6 ml of the sample solution containing 100 mg of crude tanshinones was injected through the valve. The effluent was monitored with a UV–vis detector at 280 nm and the peak fractions were collected, respectively. After 470 min of elution, the mobile phase was changed to the lower phase of system B to the end [6].

2.4. Crude sample preparation

The powdered dried roots (20 g) of *S. miltiorrhiza* Bunge from Hebei, Shandong and Jiangsu province were added to 50 ml *n*-hexane–ethanol (1:1, v/v). The mixture was shaken for 45 min before centrifugation at 10 000 × *g* for 10 min, and the supernatant was saved. Repeat the above procedure once and combine the supernatants. The extract was diluted with water at a ratio of 1:2 and equilibrated for 2 h. The organic phase was separated and washed with 30% aqueous ethanol until the water phase was almost colorless. The organic extracts were dried by rotary vaporization at 40 °C to yield the final crude samples. Hundred mg crude sample was dissolved in the lower phase of solvent system A for HSCCC separation [6].

2.5. Standard sample preparation

Standard samples (1 mg/ml) of tanshinone I, tanshinone IIA and cryptotanshinone were prepared with methanol and were diluted with electrolyte at a ratio of 1:2.

3. Results and discussion

3.1. Fingerprinting of tanshinones in S. miltiorrhiza Bunge by NACE

Micellar electrokinetic chromatography (MEKC) had ever been applied in the analysis of tanshinones in our study. Organic modifier was necessary in MEKC separation, which led to tailing peak and low repeatability. NACE was more preferable for the separation of diterpenoids than MEKC.

Methanol was chosen as electrolyte according to conditions applied in HPLC analysis [12,16]. Since there was no reference about separation of tanshinones of *Salvia* on NACE, three factors were optimized: concentration of disodium tetraborate, pH values and concentration of sodium deoxycholate.

Four concentrations, 80 mM, 100 mM, 125 mM and 150 mM of disodium tetraborate, which had evident effect on resolution of peak fractions, were applied to separate crude samples from Hebei province first. Methanol with pH value of 9.16 served as electrolyte. Only five peak fractions were eluted with 80 mM disodium tetraborate, in which tanshinone I and tanshinone IIA were not eluted separately. Although number of eluted peaks increased with 100 mM, 125 mM, 150 mM disodium tetraborate, major active components were not separated effectively. Disodium tetraborate (150 mM) was chosen for further experiment. But there was distinguished solvent peak in the chromatogram of blank, which had negative effect on fingerprint (Fig. 1A).

Four pH values, 9.16, 9.66, 10.05 and 11.3, were tried in separation of mixture of the three standard samples. pH value had great effect on the resolution of peaks. Resolution of tanshinone IIA and tanshinone I was only 0.2 with pH 9.16, and resolution increased from 0.2 to 0.96, when pH value changed from 9.16 to 10.05. Since pH values 10.05 and 11.3 led to similar resolution of the two tanshinones, pH 10.05 was chosen for further optimization.

Four concentrations of sodium deoxycholate, 10 mM, 20 mM, 40 mM and 50 mM were applied to separate mixture of the three standard samples with 125 mM disodium tetraborate in methanol solvent, pH 10.05. Sodium deoxycholate was advantageous to improve resolution of peaks. Resolutions of tanshinone IIA and tanshinone I were, respectively, 1.0, 1.0, 2.0, 2.2 with 10 mM, 20 mM, 40 mM and 50 mM sodium deoxycholate in electrolyte. The two major active components were separated effectively when concentration of sodium deoxycholate increased to 50 mM. There was no distinguished solvent peak in the chromatogram of blank with 50 mM sodium deoxycholate in electrolyte (Fig. 1B).

The optimized non-aqueous separation electrolyte was 125 mM disodium tetraborate and 50 mM sodium deoxycholate in methanol solvent (pH 10.05). Prior to each sample injection, the capillary was rinsed with water, consecutively,

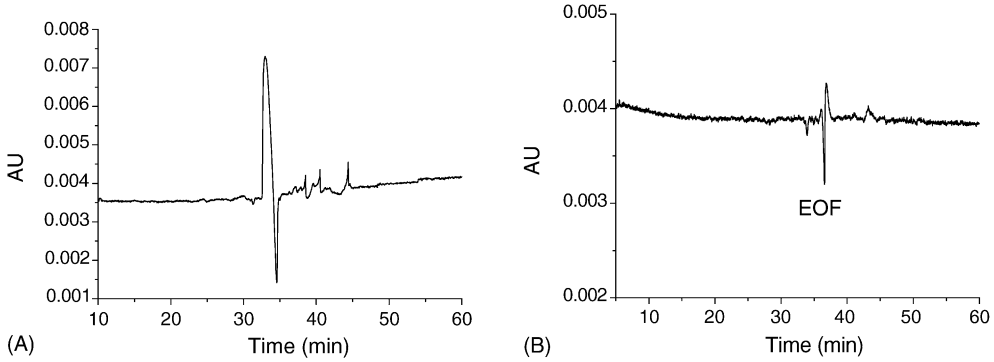

Fig. 1. Effect of different separation electrolytes in NACE analysis. (A) Separation electrolyte: 125 mM disodium tetraborate in methanol solvent; (B) separation electrolyte: 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent. Conditions: bare fused-silica capillaries, 57 cm × 75 μm i.d.; samples, diluted with separation electrolyte for injection, introduced under 0.5 psi for 3 s; voltage, 25 kV; temperature, 25 °C; detection, 280 nm; acquisition rate, 10 points/s.

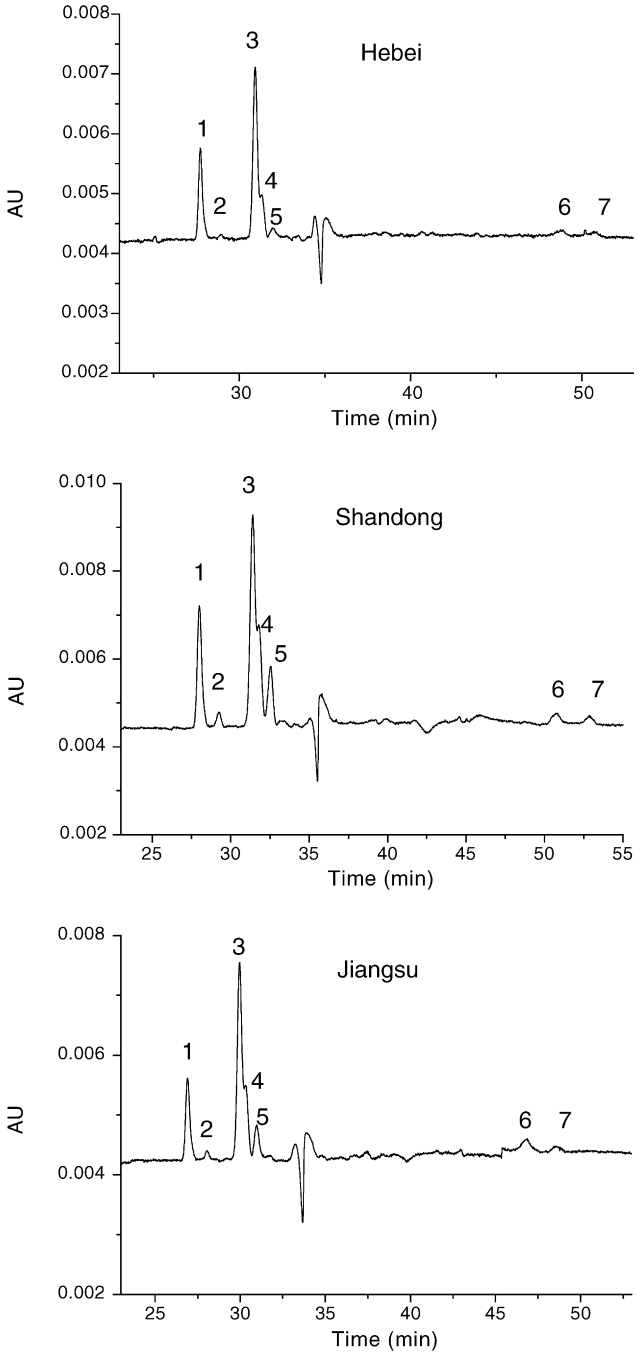

Fig. 2. Chromatograms of crude samples of *S. miltiorrhiza* Bunge from different provinces by NACE analysis. Conditions: bare fused-silica capillaries, 57 cm × 75 μm i.d.; samples, diluted with separation electrolyte for injection, introduced under 0.5 psi for 3 s; separation electrolyte, 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05; voltage, 25 kV; temperature, 25 °C; detection, 280 nm; acquisition rate, 10 points/s. Peaks (1) cryptotanshinone; (3) tanshinone IIA; (5) tanshinone I.

methanol and buffer for 3 min. Crude samples of *S. miltiorrhiza* Bunge from three different growth locations were prepared in accordance with the method described in Section 2.4. Samples were diluted with separation electrolyte, respectively, and injected directly without further treatment.

Table 1
Migration times of peaks in NACE analysis

Peak no.	Hebei (min)	Shandong (min)	Jiangsu (min)	R.S.D. (%)
1	27.72	28.01	26.90	2.1
2	28.90	29.23	28.06	2.7
3	30.93	31.40	29.97	2.4
4	31.29	31.78	30.31	2.4
5	31.92	32.55	30.97	2.5
6	48.55	50.72	46.76	4.1
7	50.60	52.82	48.42	4.4

Conditions: bare fused-silica capillaries: 57 cm × 75 μm i.d.; samples: diluted with separation electrolyte for injection, introduced under 0.5 psi for 3 s; separation electrolyte: 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05; voltage: 25 kV; temperature: 25 °C; detection: 280 nm; acquisition rate: 10 points/s.

Since most of tanshinones has maximum absorption within the wavelength of 265–295 nm, and 280 nm was applied successfully in HPLC analysis and HSCCC separation [6], 280 nm was also chosen for NACE analysis.

Three samples of *S. miltiorrhiza* Bunge were separated effectively on NACE with optimized conditions, as shown in Fig. 2. Compared with chromatogram of blank (Fig. 1B), solvent peaks were in the range of 33–37 min, which could not be involved in the fingerprint.

There were seven distinct and stable peaks respectively, in the three samples as labeled in the chromatograms (Fig. 2). Relative standard deviations (R.S.D.s) of migration times of the corresponding peaks in the three crude samples were calculated (Table 1). The average R.S.D. was 2.9%, which showed preferable correspondence of them. The seven peaks were defined as common peaks in the fingerprint.

Each peak area of peak 1 in the three crude samples was set to 1, and the other peak areas were expressed as relative values (Table 2). For example, relative peak areas of peak 3 in three crude samples were, respectively, 2.08, 1.98 and 2.57, which showed great difference in samples from different locations. Therefore, it was important to develop fingerprint to control the quality of TCM. It was proven that NACE could be an effective method in fingerprinting.

Table 2
Relative peak areas of three crude samples in NACE analysis

Peak no.	Hebei	Shandong	Jiangsu
1	1	1	1
2	0.06	0.11	0.08
3	2.08	1.98	2.57
4	0.39	0.72	0.77
5	0.14	0.52	0.51
6	0.21	0.13	0.21
7	0.09	0.07	0.08

Conditions: bare fused-silica capillaries: 57 cm × 75 μm i.d.; samples: diluted with separation electrolyte for injection, introduced under 0.5 psi for 3 s; separation electrolyte: 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05; voltage: 25 kV; temperature: 25 °C; detection: 280 nm; acquisition rate: 10 points/s.

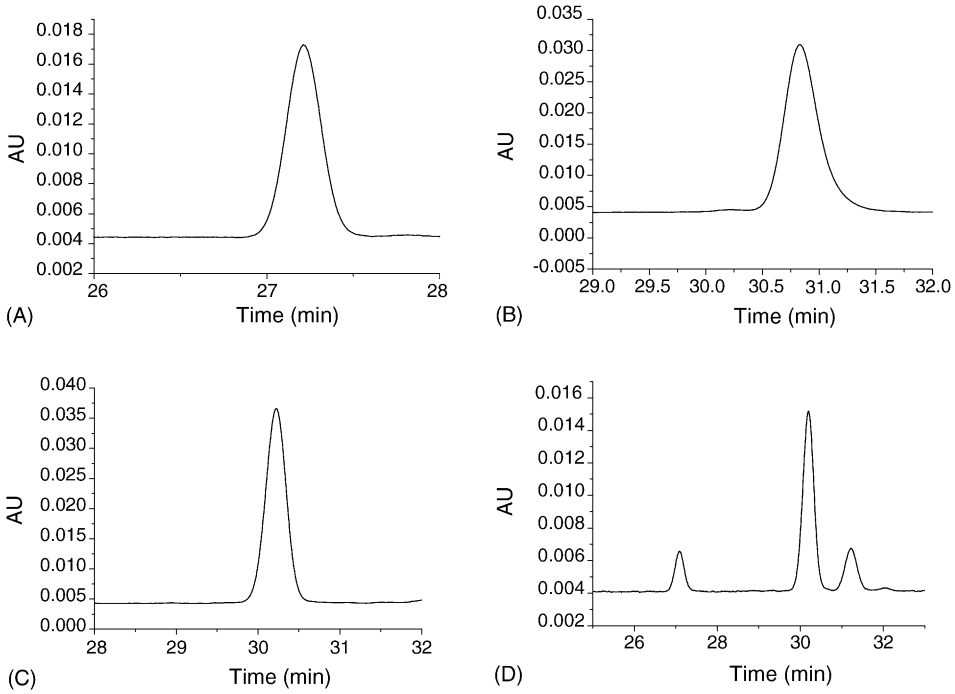

Fig. 3. Chromatograms of three standard samples by NACE analysis: (A) standard sample of cryptotanshinone; (B) standard sample of tanshinone I, (C) standard sample of tanshinone IIA, (D) mixture of the three standard samples. Conditions: bare fused-silica capillaries: 57 cm × 75 μm i.d.; samples: diluted with separation electrolyte for injection, introduced under 0.5 psi for 3 s; separation electrolyte: 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05; voltage, 25 kV; temperature, 25 °C; detection, 280 nm; acquisition rate, 10 points/s.

3.2. Identification of major active constituents in NACE analysis

Cryptotanshinone, tanshinone I and tanshinone IIA were major active constituents in *S. miltiorrhiza* Bunge, and needed to be identified in the fingerprint. Each standard sample and the mixture of the three standard samples were separated respectively, on NACE under the same conditions (Fig. 3). Migration times of standard sample and the corresponding peaks in crude samples were very similar (average of R.S.D. was 1.18%) as shown in Table 3. It could be inferred that peak 1 was cryptotanshinone (M_r 296), peak 3 was tanshinone IIA (M_r 294) and peak 5 was tanshinone I (M_r 276).

Table 3
Migration times of standard samples in NACE analysis

Standard sample	Migration time (min)			R.S.D. (%)
	1	2	3	
Cryptotanshinone	27.22	27.12	27.72	0.95
Tanshinone I	30.83	31.17	31.92	1.54
Tanshinone IIA	30.25	30.28	30.93	1.00

1: Analysis of only one standard sample, 2: analysis of mixture of standard samples, 3: analysis of the corresponding peak in crude sample, conditions: bare fused-silica capillaries: 57 cm × 75 μm i.d.; samples: diluted with separation electrolyte for injection, introduced under 0.5 psi and 3 s; separation electrolyte: 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05; voltage: 25 kV; temperature: 25 °C; detection: 280 nm; acquisition rate: 10 points/s.

3.3. Fingerprinting of tanshinones in S. miltiorrhiza Bunge by HSCCC

Stepwise elution showed better performance than one-step elution in previous study [12]. Stepwise elution strategy was applied in our study as described [14]: 0–210 min, in solvent system A; then in solvent system B. Although 12 peaks were eluted within 9 h (Fig. 4), resolution of peaks 9 and 10 was only 0.7. An optimized stepwise elution strategy was performed for better resolution: 0–470 min, in solvent system A; then in solvent system B. HSCCC system was performed

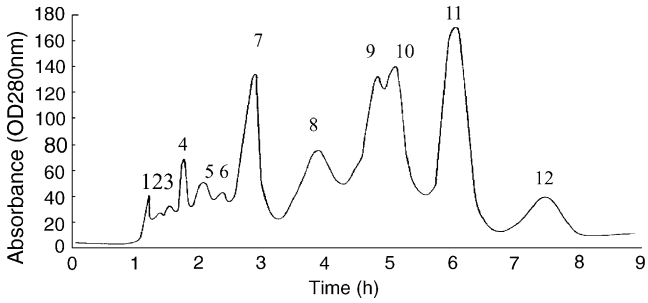

Fig. 4. Chromatogram of crude sample of *S. miltiorrhiza* Bunge from Hebei province by HSCCC with stepwise elution. Conditions: column: multilayer coil of 2.6 mm i.d. tube with a total capacity of 300 ml; rotary speed, 900 rpm; stationary phase: the upper phase of solvent system A; mobile phase: 0–210 min, the lower phase of solvent system A and after 210 min, the lower phase of solvent system B; flow rate: 2 ml/min; detection at 280 nm; sample size, 100 mg; retention of stationary phase, 78.8%.

at a speed of 900 rpm and at a flow rate of 2 ml/min [6]. Retention of the stationary phase was 78.8%, which assured the resolution of separation. Twelve distinct peak fractions were eluted respectively, from the three crude samples within 13 h (Fig. 5). More peak fractions were eluted in our studies than previous reports [8,9] when an optimized elution strategy was applied in separation of tanshinones from *S. miltiorrhiza* Bunge by HSCCC. It was proven that the corresponding peaks in the three crude samples were the same constituents in our previous study [6]. The average of R.S.D. of retention time was 2.7% as referred in our report [6].

Each peak area of peak 7 in three crude samples was set to 1 and the other peak areas were expressed by relative values (Table 4). For example, relative peak areas of peak 11 in three crude samples were respectively, 2.05, 3.78 and 3.17, which showed great difference in samples from different locations.

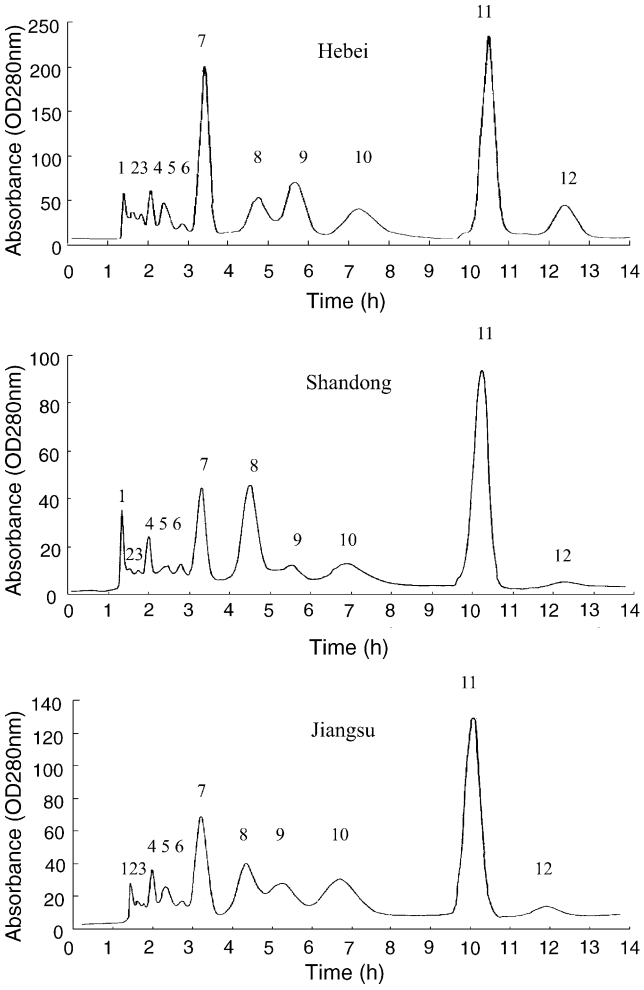

Fig. 5. Chromatograms of crude samples of *S. miltiorrhiza* Bunge from three different provinces by HSCCC with optimized stepwise elution. Conditions: column: multilayer coil of 2.6 mm i.d. tube with a total capacity of 300 ml; rotary speed, 900 rpm; stationary phase: the upper phase of solvent system A; mobile phase, 0–470 min, the lower phase of solvent system A and after 470 min, the lower phase of solvent system B; flow rate: 2 ml/min; detection at 280 nm; sample size, 100 mg; retention of stationary phase, 78.8%. Peaks (7) cryptotanshinone; (8) tanshinone I; (11) tanshinone IIA.

Cryptotanshinone, tanshinoneI and tanshinoneIIA were also identified in the fingerprint of *S. miltiorrhiza* Bunge developed by HSCCC. Analysis was performed by liquid chromatography–mass spectrometry (LC–MS). It was concluded from ultraviolet absorption spectra, retention times in LC analysis and mass spectrograms, that fractions 7, 8 and 11 were cryptotanshinone, tanshinone I and tanshinone IIA, respectively [17].

3.4. Comparisons of NACE and HSCCC

Both NACE and HSCCC could display the whole concentration distribution of different kinds of components, which is the most important character of fingerprint. Differences of TCM from different locations could be shown directly by NACE and HSCCC in two aspects, one was qualitative, characterized by the retention times, the other was quantitative, characterized by peak areas. In other words, differences of kinds and contents of components could be shown by both methods. Moreover, strict sample pretreatment was not necessary for both methods, which made their operations easier than that of HPLC.

The principles of the two methods were very different. CE is an automated analytical technique that separated species by applying voltage across buffer filled capillaries. When the voltage is applied, different size and charge of the components result in different migration speed, which makes the components separated. HSCCC is a unique liquid–liquid partition chromatography with no solid support matrix. Separation on HSCCC is depending on the partition coefficient of different components in the two-phase liquid–liquid extraction system. The principle differences led to different separation performance, elution sequence and relative peak area. HSCCC showed advantageous over NACE in analysis of tanshinones. Fingerprint developed by HSCCC included 12 peaks, which contained more chemical information

Table 4
Relative peak areas of three crude samples in HSCCC separation

Peak no.	Hebei	Shandong	Jiangsu
1	0.1	0.22	0.09
2	0.01	0.01	0.01
3	0.02	0.01	0.01
4	0.10	0.19	0.15
5	0.12	0.08	0.13
6	0.03	0.07	0.02
7	1	1	1
8	0.27	1.56	0.59
9	0.46	0.13	0.28
10	0.54	0.62	0.89
11	2.05	3.77	3.17
12	0.42	0.10	0.21

Conditions: column: multilayer coil of 2.6 mm i.d. tube with a total capacity of 300 ml; rotary speed: 900 rpm; stationary phase: the upper phase of solvent system A; mobile phase: 0–470 min, the lower phase of solvent system A and after 470 min, the lower phase of solvent system B; flow rate: 2 ml/min; detection at 280 nm; sample size: 100 mg; retention of stationary phase:78.8%.

Table 5
Differences between HSCCC and NACE

Content of comparison	NACE	HSCCC
Principle	Separation is performed according to different speeds depending on size and charge of constituent when the voltage is applied	Liquid–liquid extraction is performed according to their partition coefficients
Scale	Analytical	Semi-preparative
Volume of separation column	10 nl	300 ml
Temperature	25 °C	Non-controlled
Sample pre-treatment	Dilute with running buffer	Load directly
Mass of crude sample	ng	mg–g
Volume of crude sample	2 nl	2 ml
Common peaks	7	12
Non-common peak	More than 3	0
Run time	55 min	13 h
Sequence of elution peaks	CPTT, T IIA and T I	CPTT, T I and T IIA
Relative content	T I/CPTT = 0.39, 0.72, 0.77 T; IIA/CPTT = 2.08, 1.98, 2.57	T I/CPTT = 0.27, 1.56, 0.59 T; IIA/CPTT = 2.05, 3.77, 3.17
Average of R.S.D. of retention time or migration time (%)	2.9	2.7
Cost of the apparatus	More than US$ 50,000	US$ 12,000

Cryptotanshinone: CPTT; tanshinone IIA: T IIA; tanshinone I: T I. Conditions of NACE: bare fused-silica capillaries: 57 cm × 75 μm i.d.; samples: diluted with separation electrolyte for injection, introduced under 0.5 psi and 3 s; separation electrolyte: 125 mM disodium tetraborate, 50 mM sodium deoxycholate in methanol solvent, pH 10.05; voltage: at 25 kV; temperature: at 25 °C; detection: at 280 nm; acquisition rate: 10 points/s. Conditions of HSCCC: column: multilayer coil of 2.6 mm i.d. tube with a total capacity of 300 mL; rotary speed: 900 rpm; stationary phase: the upper phase of solvent system A; mobile phase: 0–470 min, the lower phase of solvent system A and after 470 min, the lower phase of solvent system B; flow rate: 2 ml/min; detection at 280 nm; sample size: 100 mg; retention of stationary phase: 78.8%.

than that of NACE. And there was no non-common peak in HSCCC fingerprint. Elution sequences of peaks were different in the two methods: cryptotanshinone, tanshinone IIA and tanshinone I in NACE analysis; cryptotanshinone, tanshinone I and tanshinone IIA in HSCCC separation. Relative contents of these three components were different: tanshinone I/cryptotanshinone = 0.39, 0.72, 0.77 and tanshinone IIA/cryptotanshinone = 2.08, 1.98, 2.57 in NACE analysis; tanshinone I/cryptotanshinone = 0.27, 1.56, 0.59 and tanshinone IIA/cryptotanshione = 2.05, 3.77, 3.17 in HSCCC separation (Table 5).

Although the average R.S.D. of elution time in HSCCC separation, 2.7%, was not so good as that in HPLC analysis (0.13%), it was somewhat better than that in NACE analysis (2.9%). The sample loaded on NACE was very small (2 nl), which led to relatively low stability. Anyway precisions of HSCCC and NACE were preferable for fingerprinting.

It is a pity that it was a semi-preparative HSCCC that be compared with the analytical NACE in our study. If analytical HSCCC was applied and separation temperature was controlled, the precision should be better and the running time could be shortened greatly.

4. Conclusion

There are complicated constituents in TCM. Since each method has its own advantages and disadvantages, it is necessary to develop more approaches in fingerprinting of TCM. Both HSCCC and NACE were effective in showing the concentration distribution of different kinds of components. Differences of kinds and contents of components were shown by both methods. The great difference in principles results in different separation performance, elution sequence and relative contents of peaks of these two methods. HSCCC showed better performance on fingerprinting of tanshinones, which contained more chemical information than that of NACE. It was further proven that HSCCC could be a feasible and cost-effective method for development of fingerprint of TCM based on the comparison with NACE.

HSCCC, as a new method in fingerprinting, should be further studied in following aspects: accuracy, repeatability, detection limit, application range and other factors.

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