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Short communication

Application of non-aqueous micellar electrokinetic chromatography to the analysis of active components in radix *Salviae miltiorrhizae* and its medicinal preparations

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Abstract

A simple, economical and effective non-aqueous micellar electrokinetic chromatography (NAMEKC) method was developed for simultaneous assay of three bioactive components (cryptotanshinone, tanshinone IIA and tanshinone I) in radix *Salviae miltiorrhizae* and its medicinal preparations for the first time. After optimization of separation conditions, a buffer of 140 mmol l^{-1} sodium cholate (SC) in methanol was selected for the separation of the three tanshinones, but baseline separation of tanshinone I and tanshinone IIA in practical samples was not achieved. Therefore, second-order derivative electropherograms were applied for resolving overlapping peaks. Regression equations revealed good linear relationships (correlation coefficients 0.995–0.999) between peak heights in second-order derivative electropherograms and concentrations of the three analytes. The recoveries of three constituents ranged from 91.3 to 105.7%. The results indicated that baseline separation of the analytes was hard to be achieved in practical samples sometimes and second-order derivative electropherograms was applicable for the resolving and analysis of overlapping peaks.
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Keywords: Tanshinones; Non-aqueous micellar electrokinetic chromatography; Sodium cholate; Second-order derivative electropherograms

1. Introduction

Salvia miltiorrhizae Bunge, Dan-Shen in Chinese, is a well-known traditional Chinese medicinal herb used for the treatment of various kinds of diseases especially for coronary disorders [1–3]. The major active constituents of this herb are tanshinones, a group of abietane-type diterpenes [4]. Pharmacological tests revealed that all these components have an anti-cancer effect, and among these tanshinones, tanshinone I (T_I), tanshinone IIA (T_{IIA}) and cryptotanshinone (CT) are the most effective components and the quality of radix *S. miltiorrhizae* has been controlled in terms of the level of these compounds. The chemical structures of the three tanshinones are given in Fig. 1.

Several analytical methods have been reported for the determinations of tanshinone species, Therein thin-layer chromatography (TLC) [5] and high performance liquid chromatography (HPLC) [6–9], which are most widely used in pharmaceutical analysis in real world due to its satisfactory reproducibility and high sensibility. In recent years, non-aqueous capillary electrophoresis (NACE) has been widely used in the analysis of pharmaceuticals [10–16]. Advantages of NACE are the following: different selectivity, high efficiency, short analysis times, better solubility and stability of some compounds in organic solvent comparing with those in water. However, to our best knowledge, there are only some researches on radix *S. miltiorrhizae* by CE methods [2,17], and more recently, we have also reported a NACE

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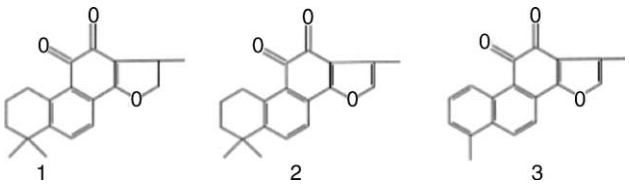

Fig. 1. Structures of the analytes: (**1**) cryptotanshinone; (**2**) tanshinone IIA; (**3**) tanshinone I.

method with a buffer of methanol–acetonitrile mixture containing ammonium acetate and acetic acid for the separation and determination of the three active components in radix*S. miltiorrhizae* and its pharmaceutical preparations [18]. Yet, baseline separation of tanshinone I and tanshinone IIA in standard solutions was not achieved in our previous paper, and also taken more time. For these reasons, and considering the speciality of poorly water-soluble and structural similarity of investigated tanshinones, we developed a simple, economical and effective non-aqueous micellar electrokinetic chromatography (NAMEKC) method for the simultaneous assay of three bioactive components (T_I, T_{IIA} and CT) in radix *S. miltiorrhizae* and its pharmaceutical preparations for the first time in this paper. The results reveal that the baseline separation of the three tanshinones investigated in standard solutions can be achieved in a shorter migration time than that in our previous paper (23 min).

2. Materials and methods

2.1. Materials

Tanshinone I, tanshinone IIA and cryptotanshinone were obtained from the National Institute for the Control of Pharmaceutical and Bio-products of China (Beijing, China). Sodium cholate (SC) was purchased from Serva Feinbiochemica (Heidelberg, NY). Methanol was purchased from Shanghai Zhenxing First Chemical Factory. Dan-Shen herbs and traditional Chinese medicinal preparations were purchased from Zhongyou pharmaceutical store, Lanzhou, China. All reagents were of analytical grade, unless otherwise specified.

Stock solutions of T_I (200 mg l^{-1}), T_{IIA} (600 mg l^{-1}) and CT (600 mg l^{-1}) were prepared in a mixture of methanol and chloroform (75:25, v:v). Analytical solutions were prepared from them by appropriate dilution with the mixture solvents. The running buffer was prepared by 17.5 ml 0.2 mol l^{-1} sodium cholate (methanol medium) in a 25 ml flask and diluted to 25 ml with methanol. All solutions and running buffers were filtered with 0.45 μm disposable filters and degassed by sonication for 10 min prior to use. As a solution electrolysis can alter the running buffer and subsequently change the electroosmotic flow (EOF), the separation buffer was replaced every 3–5 runs.

2.2. Apparatus and methods

The experiments were performed on a P/ACE5510 system (Beckman Coulter Instrument, Fullerton, CA, USA) equipped with P/ACE diode-array detector. The system was controlled by P/ACE station software. The separation was carried out on a 47 cm (40 cm to the detector) × 75 μm i.d. fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China) housed in a cartridge with a 800 μm × 100 μm detector window. Capillaries were rinsed with 0.5 mol l^{-1} sodium hydroxide for 15 min before they were used the first time to activate the silanol groups at the inner surface. Each day, the capillary was conditioned by flushing with 0.1 mol l^{-1} sodium hydroxide, followed by water and methanol for 5 min, respectively. This flushing procedure is expected to remove any trace of water from the capillary [19]. Between two runs, the capillary was equilibrated with run buffers for 4 min. When not in use, the capillary was washed with methanol, water and then stored dry. The capillary was maintained at 25 °C. The sample was injected by applying a pressure of 0.5 psi for 2 s.

2.3. Sample preparation

The two herbal medicines *S. miltiorrhizae* (S1, from Hebei), *S. przewalskii* (S2, from Gansu) and the medicinal preparations Huoxue Tongmai Tablet (S3) were finely powered and ground, and then 0.6000, 0.6000, 1.0000 g powder were respectively weighed and extracted with 10 ml of a mixture of methanol and chloroform (75:25, v:v) in an ultrasonic bath for 1 h (for S1 and S2) and 30 min (for S3). After being centrifuged, the extracts were concentrated to 5 ml, respectively. The solutions were passed through a 0.45 μm membrane filter, and were injected directly into the capillary electrophoresis system.

3. Results and discussion

3.1. Determination of critical micelle concentration (CMC*) of SC in methanol

Sodium cholate (SC), a bile salt, is a natural anionic surfactant which has a more polar structure than sodium dodecyl sulfate (SDS) and is found in biological components. It dissolves with difficulty in ethanol or acetonitrile, but it is easily dissolved in methanol and is commonly used to improve the selectivity in NACE [20,21]. It is not only a biological surfactant, but also a good electrolyte [22]. Herein, we firstly obtained the CMC* value (111 mM) of SC in methanol using the method proposed by Cifuentes et al. [23]. The plot of electric current versus concentration of SC under electrophoretic conditions is shown in Fig. 2, where the current was measured at 20 kV. The concentration of SC in methanol was in the range of 40–160 mM. The

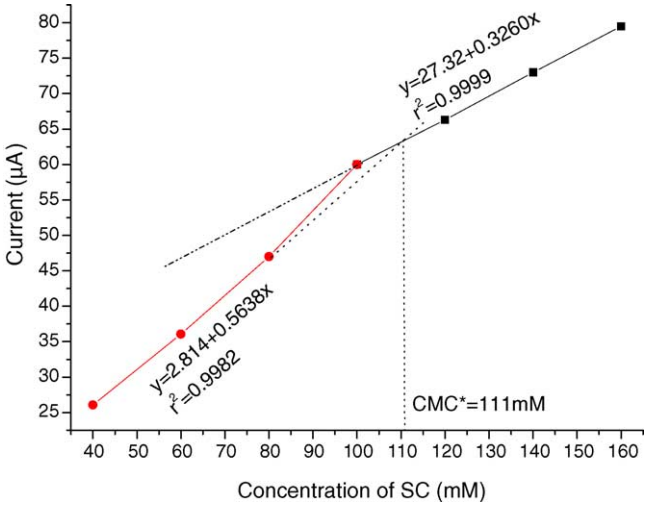

Fig. 2. Plots of electric current vs. concentration of CS (mmol l^{-1}). Electrophoretic medium: different concentration of SC in methanol as running buffer. Experimental conditions: uncoated fused-silica capillary 47 cm (40 cm injector to detector) × 75 μm i.d., applied voltage 20 kV, capillary temperature 25 °C.

concentration at the cross point was considered to be the CMC* value.

3.2. Effect of SC concentration

To compare the effect of CMC* on the separation, SC with varying concentrations (ranging from 40 to 160 mM) were chosen for examination. It was found that the resolution increased with the increasing of SC concentration, and that the separation was not completely until the concentration of SC was beyond the value for CMC* (111 mM). A higher concentration of SC (>120 mM) resulted in better separation (Rs(**1**,**2**) = 3.4 and Rs(**2**,**3**) = 1.1 when concentration of SC was 120 mM) although the migration time was longer. Considering the problem of Joule heating and band broadening phenomenon that caused by higher ionic strength, and as a compromise of separation time and resolutions, 140 mmol l^{-1} sodium cholate (Rs(**1**,**2**) = 6.0 and Rs(**2**,**3**) = 2.3) was selected as the optimum for subsequent experiments.

3.3. Effect of applied voltage and capillary temperature

High voltage was required in CE to reduce the analysis time. In this paper, the effect of applied voltage was tested in the range of 20–27.5 kV. The results showed that the migration times of the three compounds investigated were shortened with increased applied voltage, but obvious decrease in resolution of T_I and T_{IIA} was also found. Furthermore, breakdown of electric current occurred sometimes during the experiments when the voltage was higher than 25 kV. Therefore, 25 kV was selected for relatively good separation of the two compounds with shorter analysis time and acceptable electric current (<100 μA).

The influence of the capillary temperature was also studied with optimized electrophoretic medium between 15 and 27.5 °C. As the temperature increased, the migration times of the analytes decreased due to the reduction of the electrolyte viscosity. The separation was found to be affected slightly by temperature variations. When the temperature was higher than 25 °C, breakdown of electric current was often observed. Therefore 25 °C was selected as the optimum.

According to the above results, baseline resolution of the three tanshinones was obtained under optimum separation conditions. Yet, complete baseline separations between analytes and sample matrices in practical samples were not obtained in their determination. Therefore, the second-order derivative electropherograms [18,24] were introduced for their quantitative analysis and the results were satisfactory.

3.4. Linearity, precision and accuracy

The linear relationships between the concentration of the three tanshinones and the corresponding peak height were investigated under the optimum separation conditions. All calibration curves were found linear over the investigated range of 4.69–600 mg l^{-1} for **1** and **2**, and 1.56–200 mg l^{-1} for **3**. The relevant values are summarized in Table 1.

The precision and accuracy of the proposed method, evaluated as relative standard derivation (R.S.D.%) and the percentage deviation of observed concentration from theoretical concentration, on the basis of the three different standard mixture solutions of 200, 100 and 50 mg l^{-1} of T_I, T_{II} and CT for five replicate injections under the optimum condition was 0.52–1.64 and 0.47–2.31% (intra-day), 1.06–2.07 and 3.75–6.08% (inter-day), respectively.

3.5. Application

To demonstrate the potential of the method in the analysis of tanshinone samples, two medicinal plants of genus *Salvia* (S1, S2) and Chinese medicinal preparations (S3) were analyzed under the optimum separation conditions. The peaks

Table 1
The regression data and the detection limits

Analyte	Regression equation[a]	Correlation coefficient	Linear range (mg l^{-1})	Detection limit[b] (mg l^{-1})
1	$Y = -0.96 + 0.30X$	0.998	4.69–600	0.26
2	$Y = 1.55 + 0.11X$	0.999	4.69–600	0.75
3	$Y = 3.33 + 0.28X$	0.995	1.56–200	0.29

[a] Y and X stand for the second-order derivative peak-height and the concentration (mg l^{-1}) of the analytes, respectively.

[b] The detection limit was defined as the concentration where the signal-to-noise ratio is 3.

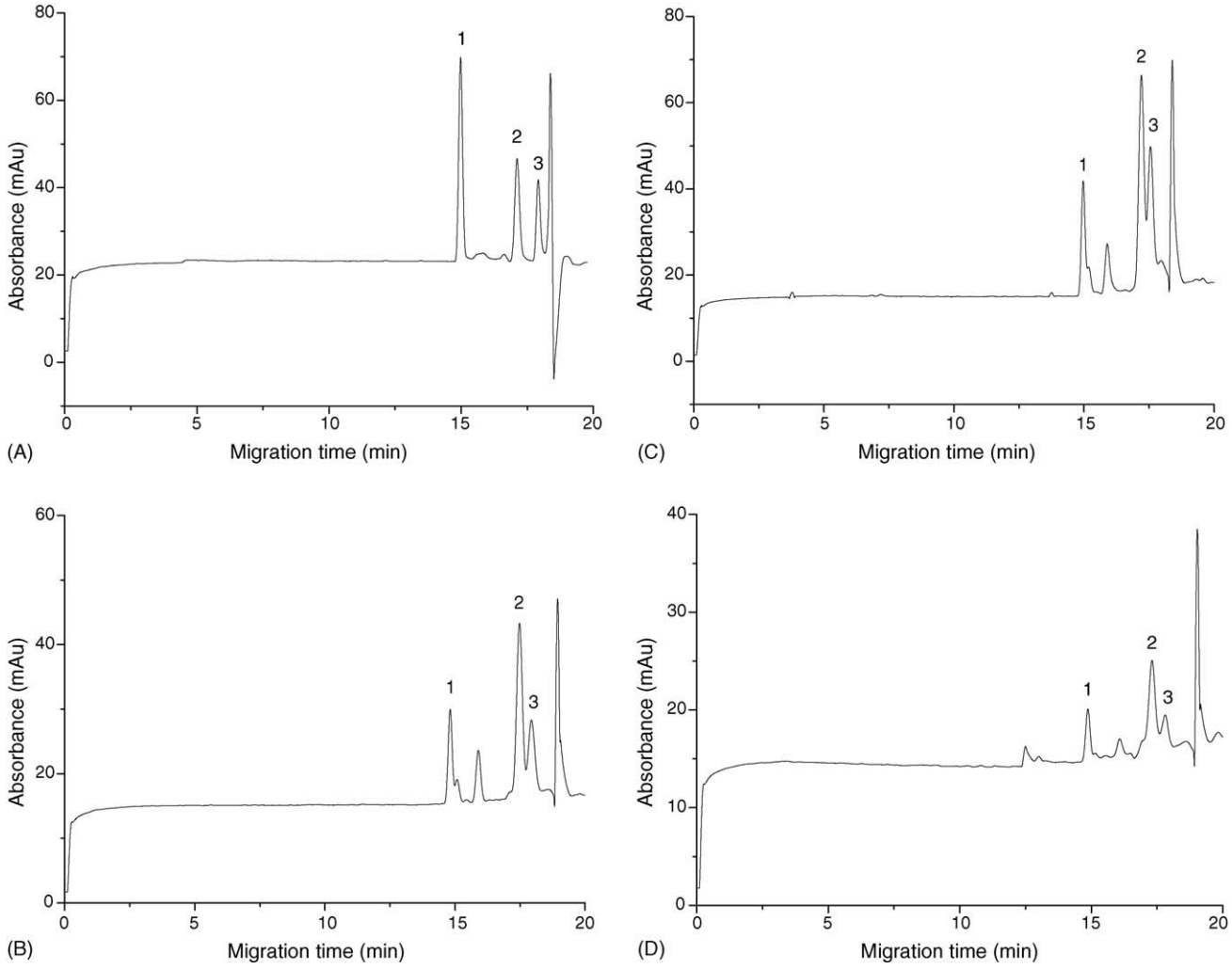

Fig. 3. Electropherograms of the standards and the samples. (A) Standards (300 mg l^{-1} CT, 300 mg l^{-1} T_{IIA} and 100 mg l^{-1} T_I); (B) S1; (C) S2; (D) S3. Running buffer: 140 mmol l^{-1} SC in methanol. UV detection at 275 nm. Applied voltage 25 kV. Other CE conditions see Fig. 2.

were identified with comparing the migration times and the spectra of the separated compounds and standards, and spiking standards to the sample solutions. The electropherograms and the corresponding second-order derivative electropherograms are shown in Figs. 3, 4 and 5, respectively. The resulting contents of the three tanshinones in samples obtained from the calibration curves (heights in second-order derivative electropherograms versus concentrations of the three analytes) are listed in Table 2, which match well with the results of our previous paper. The recovery of the method was

Table 2
Determination results and average recoveries of the tanshinones in the samples ($n = 3$, mg g^{-1})

Sample	Compounds	Content[a]	Added (mg l^{-1})	Recovery (%)
S. miltiorrhizae (S1)	**1**	0.79 (0.62)	200, 100, 50	102.7
	2	2.0 (2.1)	300, 200, 100	93.5
	3	0.31 (0.51)	100, 50, 25	103.2
S. prezawalskii (S2)	**1**	1.5 (1.4)	300, 150, 75	91.3
	2	4.3 (4.7)	600, 300, 150	102.5
	3	10 (1.1)	300, 150, 75	104.2
Huoxue Tongmai Tablet (S3)	**1**	0.16	100, 50, 25	105.7
	2	0.39	100, 50, 25	98.8
	3	0.014	10, 5, 2.5	93.55

[a] The values in parenthesis are the determination results of the tanshinones in the same samples obtained from our previous paper.

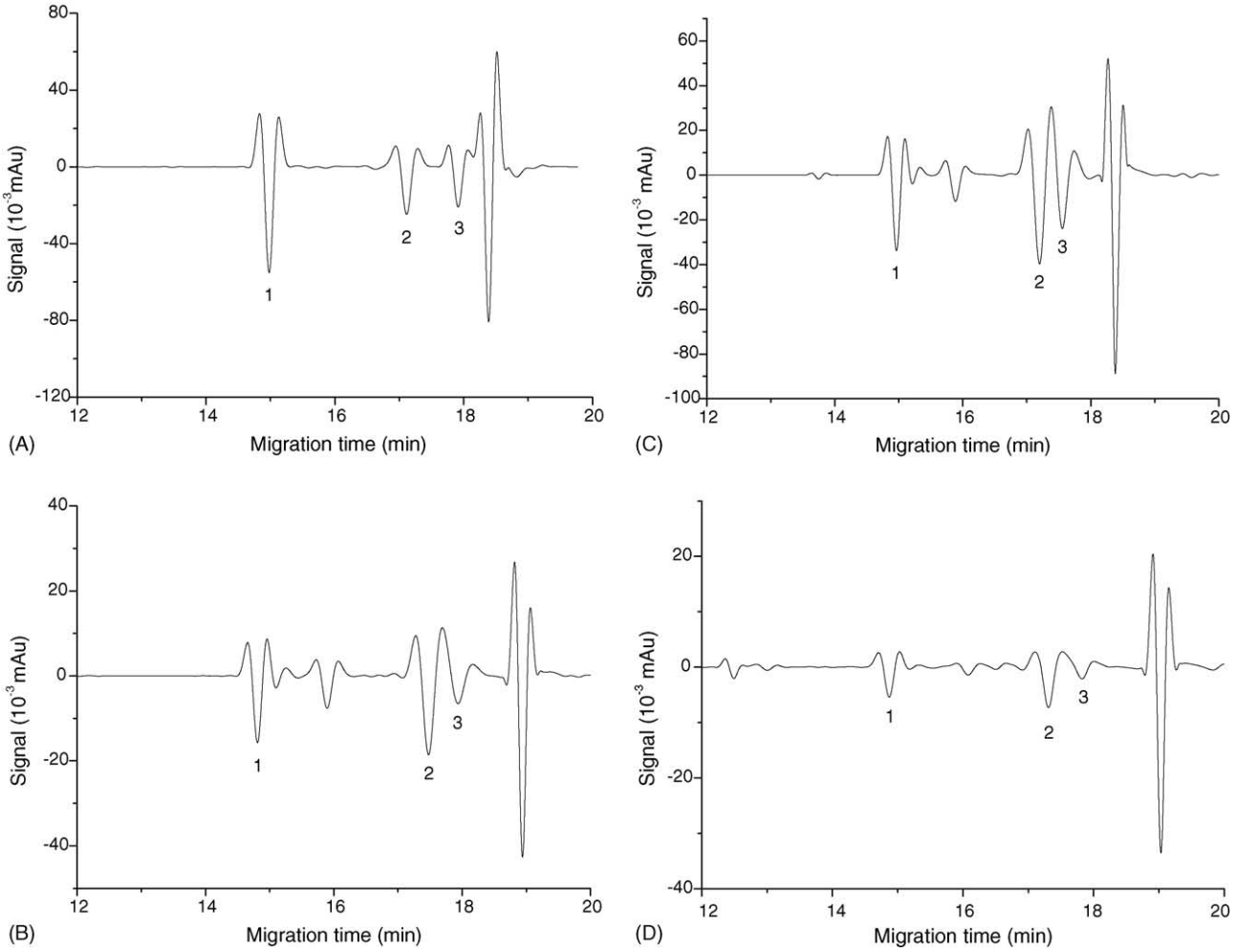

Fig. 4. Second-order derivative electropherograms corresponding to Fig. 3.

determined with the standard addition method for the three compounds in the three sample solutions, respectively, and the results were also given in Table 2.

4. Conclusions

A simple, economical and effective non-aqueous micellar electrokinetic chromatography (NAMEKC) method was developed for simultaneous assay of three bioactive components (T_I, T_{IIA} and CT) in radix *S. miltiorrhizae* and its medicinal preparations for the first time. Comparing with the method proposed in our previous paper, the method has higher efficiency and is more simple and time saving. Though baseline separation of T_I and T_{IIA} was not achieved in the practical samples under optimum experiment conditions, but the use of second-order derivative electropherograms made the simultaneous determination of the three compounds possible in shorter time. The experimental results proved also that the second-order derivative electropherogram is effective in determination of a low-content component and of those not fully separated from adjacent ones. This new method is promising for the quality control of Dan-Shen herbs and their pharmaceutical preparations.

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