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**Research article**
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# Determination of active components in Chinese medicinal preparations by capillary electrophoresis

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**Abstract:** The determination of paeoniforin, paeonol, and censenoside $Rg_1$ in traditional Chinese medicinal preparations, Tze Po San Pien pills and Liuwen Dihuang pills has been investigated by micellar electrokinetic capillary electrophoresis with borate buffer (20 mM), sodium dodecylsulfate (30 mM) and acetonitrile (20%) as background electrolytes (pH 9.30), 20 kV applied voltage and 203 nm UV detection. The effects of SDS concentration, borate, buffer pH, and organic modifier on electrophoretic behavior and separation are discussed. Regression equations revealed linear relationships between the peak-area of each component and the content with the correlation coefficients from 0.9982 to 0.9999. In addition, the levels of the active compounds in two kinds of traditional Chinese medicinal preparations were easily determined with the recoveries from 93.1% to 108.2%.


*Keywords: Micellar electrokinetic capillary electrophoresis, paeonol, paeoniforin, censenoside $Rg_1$, Chinese medicinal preparations*

## 1 Introduction

Traditional Chinese medicines are usually administered in the form of a multicomponent prescription, and each component may show a complicated profile of respective constituents. Tze Po San Pien pills and Liuwen Dihuang pills are included in these types of traditional Chinese medicinal preparations. Tze Po San Pien pills represent the oldest

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and most exemplary general tonic pills among all Chinese formulas. These pills have been adjusted and balanced over the years to form tonic pills which contain more than 42 kinds of medicinal materials including penis of Callorhinus ursinus, Radix Ginseng, Cortex Moutan Rsdicis etc.. Personal testimonies illustrate their effectiveness in combating general weakness and fatigue, while improving the functions of kidney and heart [1]. Paeonol, paeoniforin, and censenoside $Rg_1$ are important bioactive compounds and can be used to roughly estimate the quality of the pills. Liuwen Dihuang pills are composed of six kinds of herbs including Cortex Moutan Rsdicis; pharmacologically these pills have demonstrated that they can also improve the functions of kidney and combat general weakness and fatigue. They are used to treat dizziness, tinnitus, night sweats and spermatorrhea [2]. Paeonol and paeoniforin are the bioactive components of Liuwen Dihuang pills. Therefore, a simple and rapid method for the determination of the content of the three compounds is required.

Thin-layer chromatography (TLC) is the conventional method for analyzing traditional Chinese medicinal preparations; it has been used to identify Liuwen Dihuang pills [3, 4] and San Pien pills [5]. However, this method can't be applied to simultaneously analyze several components in a single crude herb or in a medicinal preparation composed of several crude materials. Generally, high-performance liquid chromatography (HPLC) has been employed to analyze crude herbs or several marker components in a Chinese medicinal preparation including Liuwen Dihuang pills [6, 7]. However, these methods usually consume a lot of materials as large quantitites of organic reagents are often required. The development of high-performance capillary electrophoresis has been reviewed many times [8], and clearly, it continues to be a very active research area in the field of separation science as this technique often provides higher resolving power, shorter analysis time and lower operating cost than TLC and HPLC. The micellar electrokinetic capillary electrophoresis (MEKC) analytical method presented herein possesses the above advantages. Consequently, MEKC has been employed to analyze Chinese crude herbs and respective medicinal preparations [9–11]. But a simultaneous determination of several bioactive components in traditional Chinese medicinal preparations mentioned above has not been reported.

In this study, based on the systematic investigation of the influence of the concentration of sodium dodecylsulfate (SDS), borax, buffer pH, and organic modifier, a simple and rapid method to identify, separate and determine the three bioactive compounds in Tze Po San Pien Pills has been developed. This study will focus on a simple and rapid method to identify, separate, and determine the three bioactive compounds in Tze Po San Pien pills. Meanwhile, this method can also be used to determine paeonol and paeoniforin in Liuwen Dihuang pills.

## 2 Experiment

### 2.1 Apparatus

A LUMEX CAPEL 105 Capillary Electrophoresis System (LUMEX Ltd., 19, Moskovsky Pr., St., Petersburg, 198005, Russia) was used, controlled by a personal computer. Capillary electrophoresis was performed using a 50.0 cm (40.5 cm to the detector) × 75 $\mu$m I.D. fused silica capillary (Yongnian Photoconductive Fibre Factory, Hebei Province, China). Samples were introduced to the capillary by pressure injection at 30 mbar for 5 s. Direct UV detection was employed at a wavelength of 203 nm in order to detect the three components simultaneously. All operations were conducted at 25 °. A pH/Ion 510 Bench pH/Ion/mV Meter (Oakton Instruments, Vernon Hills, IL60061, USA) was used for pH measurements. Methanol was used as an electroosmotic flow (EOF) marker.

### 2.2 Materials and reagents

Paeonol, paeoniforin and censenoside $Rg_1$ were purchased from the National Institute for Control of Pharmaceutical and Products, Beijing, China. Their structures are shown in Fig. 1. Tze Po San Pien pills were manufactured by Yantai Zhongya Parmaceutical CO. LTD. Yantai, China. Liuwen Dihuang pills were manufactured by Jiangsu Kangyuan Medicine Factory, Lianyungang, China. All other chemicals were of analytical reagent grade.

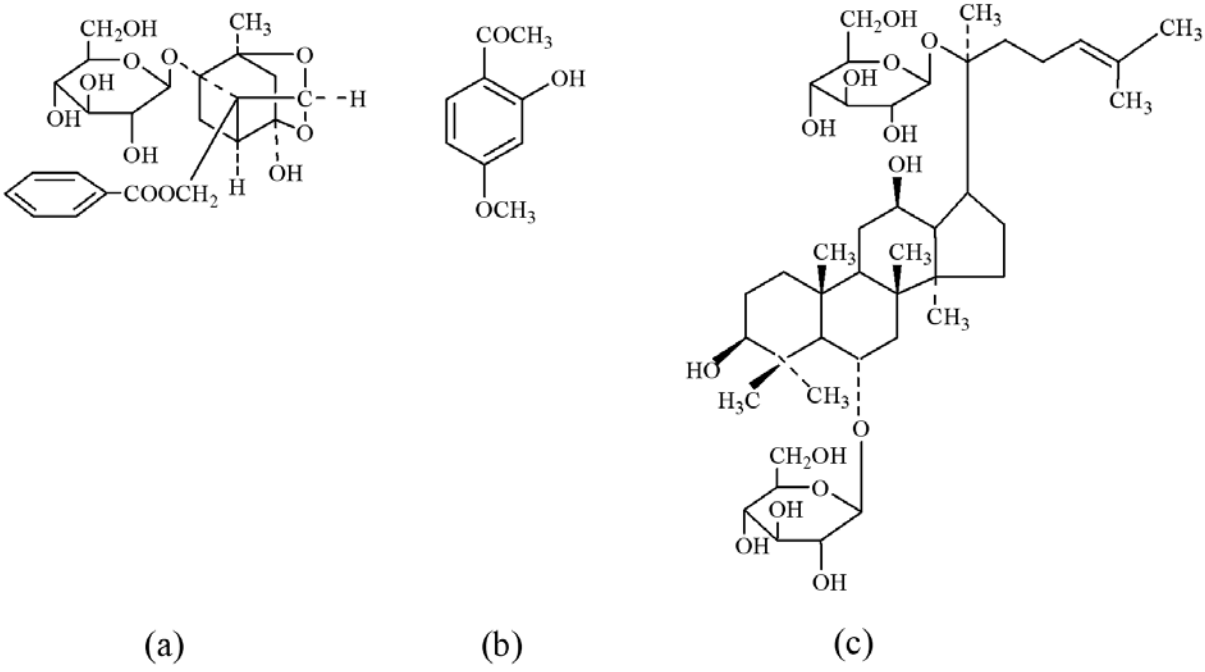


**Fig. 1** Molecular structures of the analytes. (a) Paeoniforin, (b) Paeonol, (c) censenoside $Rg_1$.

### 2.3 Procedure

Buffer solutions were prepared by diluting 0.1 M borate and 0.2 M SDS with distilled water and adjusted to the desired pH with 0.5 M NaOH or HCl.

Standard solutions, 1.0 mg/mL of paeonol, paeoniforin and 2.0 mg/mL of censenoside $Rg_1$ were prepared in 50% methanol (v/v). Less concentrated standard solutions were prepared by diluting the stock solutions with 50% methanol (v/v).

1.0 g of pulverized Tze Po San Pien pills and 1.0 g of pulverized Liuwei Dihuang pills were extracted with 10 mL of methanol in an ultrasonic bath for 30 min, respectively. The extracted solution was then filtered through a paper filter. The extraction procedure was repeated three times. The total extracted solution was concentrated to near-dryness and 50% methanol (v/v) was added to dissolve the residue to a final volume of 1 mL. All the buffers, standard and sample solutions were filtered through 0.45 $\mu$m membrane filters before they were used.

## 3 Results and Discussion

### 3.1 Optimization of analytical condition

The structure of the three analytes suggested that they could be analyzed both as anions and neutral analytes. So a micellar electrokinetic capillary electrophoresis method was selected as a separation mode; borax and SDS were selected as background electrolytes. The separation was achieved by optimizing the concentration of SDS, borax, buffer pH, and organic modifier.

In MEKC, the first parameter with the greatest influence on the resolution of the analytes was the surfactant concentration. To optimize the effect of SDS concentration on the migration time and separation efficiency, 20 mM borax buffer at pH 9.30 with different SDS concentrations ranging from 10 to 40 mM were used to separate the analytes. The results showed that the migration time of all components increased with the increasing SDS concentration. Paeoniforin and the electroosmotic flow (EOF) marker had almost the same migration time when SDS concentration was 10 mM; a full separation of the three analytes was obtained when the SDS concentration was $\geq$30 mM. Therefore, 30 mM was selected for its shorter migration period.

The effects of the borax concentration on the migration time were investigated with SDS concentrations of 30 mM at a pH of 9.30. The results indicated that when the borax concentration varied from 10 mM to 40 mM, the migration time of the three analytes increased. A full separation of paeoniforin and the EOF marker was achieved when the borax concentration was increased to 20 mM. A high concentration of a background electrolyte also increased the current. Thus, 20 mM was selected for a good peak shape and shorter migration time.

To verify the effect of the buffer pH on migration behavior, experiments were performed with 20 mM borax, 30 mM SDS and different pH values (10.00, 9.30, 8.50, 8.00,

7.50) as background electrolytes. It is known that pH affects both the charge of the analytes and the EOF. It can be determined from the results that a full separation of the components was obtained at different pH values. When pH changed from 10.00 to 7.50, the migration time of censenoside $Rg_1$decreased from 19.8 min to 15.7 min. Meanwhile the pH value influenced the peak shape of paeonol and censenoside $Rg_1$ significantly. At pH levels of 10.00, 8.50, 8.00 and 7.50, the peak of paeonol branched and the peak of censenoside $Rg_1$ broadened. So pH 9.30 was selected for a shorter separation time and a relatively good peak shape.

It has been reported that the addition of an organic modifier to the buffer is an important parameter to improve the separation selectivity, efficiency and resolution [12]. These result in the modification of the partition coefficient, mobile phase polarity and electroosmotic flow. The effects of organic modifiers investigated herein indicated that a full separation with a good peak shape couldn't be obtained without a modifier or with methanol as the modifier. But it was obtained with the modifier acetonitrile from 10% to 30%. The result is shown in Fig. 2. It can be seen from Fig. 2 that the migration time of paeoniforin and paeonol increased slightly, while that of censenoside $Rg_1$ decreased rapidly with the increased modifier concentration. So when the concentration of acetonitrile was 30%, the peak sequence of paeonol and censenoside $Rg_1$ reversed. When the concentration of acetonitrile was 20%, a full separation and good peak shape was obtained whereby the running time was less than 10 min. So the 20% acetonitrile modifier was selected.

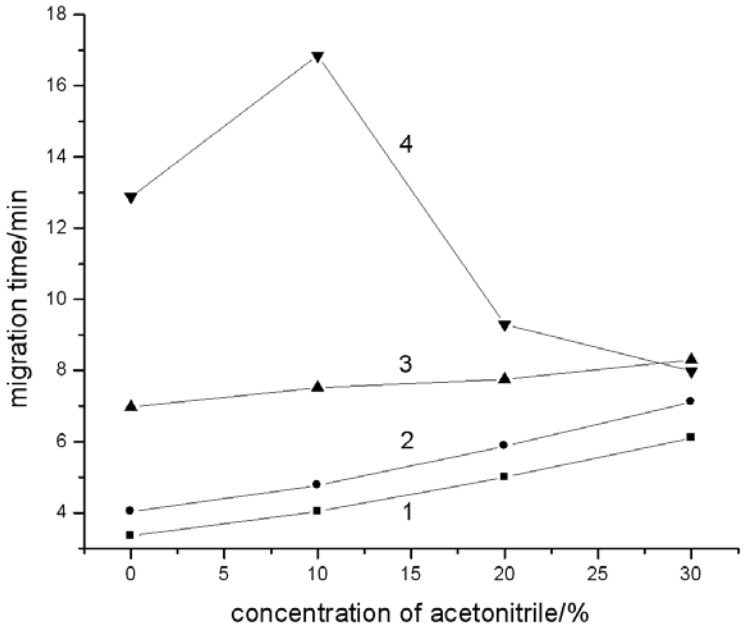


**Fig. 2** Effect of acetonitrile concentration on the migration time: 1 = EOF marker; 2 = paeoniforin; 3 = paeonol; 4 = censenoside $Rg_1$. Analytical conditions: 20 mM borax, 30 mM SDS, pH 9.30, applied voltage 20 kV, sample time 5 s, detection UV 203 nm.

According to the factors mentioned above, the optimum separation conditions used in this work were 20 mM borax, 30 mM SDS and 20% acetonitrile at a pH level of 9.30. A typical electropherogram of the three standard components is shown in Fig. 3.

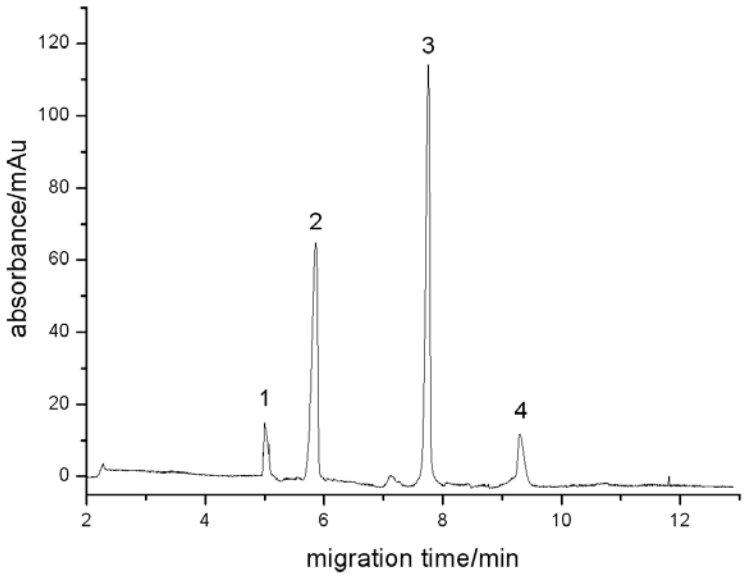


**Fig. 3** Capillary electropherogram of a standard mixture. Peaks: 1 = EOF marker; 2 = paeoniforin; 3 = paeonol; 4 = censenoside $Rg_1$. Analytical conditions: 20 mM borax, 30 mM SDS, pH 9.30, applied voltage 20 kV, sample time 5 s, detection UV 203 nm.

## 3.2 Calibration

Calibration curves were constructed in the concentration ranges from 100.0 to 1000.0 $\mu$g/mL for paeoniforin and paeonol, and 200.0 to 2000.0 $\mu$g/mL for censenoside $Rg_1$. The linear regression equations and correlation coefficients were: y = 625.6x – 27.703 (r = 0.9999) for paeoniforin, y = 1583x + 80.438 (r = 0.9982) for paeonol, y = 37.601x + 30.853 (r = 0.9995) for censenoside $Rg_1$, where y is the peak area (mAU·s) and x is the concentration of the analytes (mg/mL) in the sample.

## 3.3 System suitability test

The method was validated for reproducibility of the migration time and the peak area of the analyses. The relative standard deviation (RSD) values of the migration time and the peak area for five replicate injections intra-day were 1.23% and 2.02% for paeoniforin, 1.45% and 2.11% for paeonol, 1.26% and 3.88% for censenoside $Rg_1$. The RSD values inter-day were 3.17% and 3.62% for paeoniforin, 2.42% and 2.96% for paeonol, 2.73% and 4.21% for censenoside $Rg_1$, respectively.

The recovery methods were determined by the addition of the standard analytes with the results ranging from 93.1 - 108.2% for Tze Po San Pien pills and 94.2 - 107.4% for Liuwen Dihuang pills.

### 3.4 Applications

Sample extract solutions were injected and separated under optimum conditions described above. A typical electropherogram was shown in Fig. 4. It was observed that paeoniforin, paeonol, censenoside $Rg_1$ and other unknown compounds were completely separated The limits of detection were 21.2 $\mu$g/mL for paeoniforin, 9.0 $\mu$g/mL for paeonol and 132.5 $\mu$g/mL for censenoside $Rg_1$, respectively. Peaks were identified by adding standard analytes. The analytical results were summarized in Table 1.

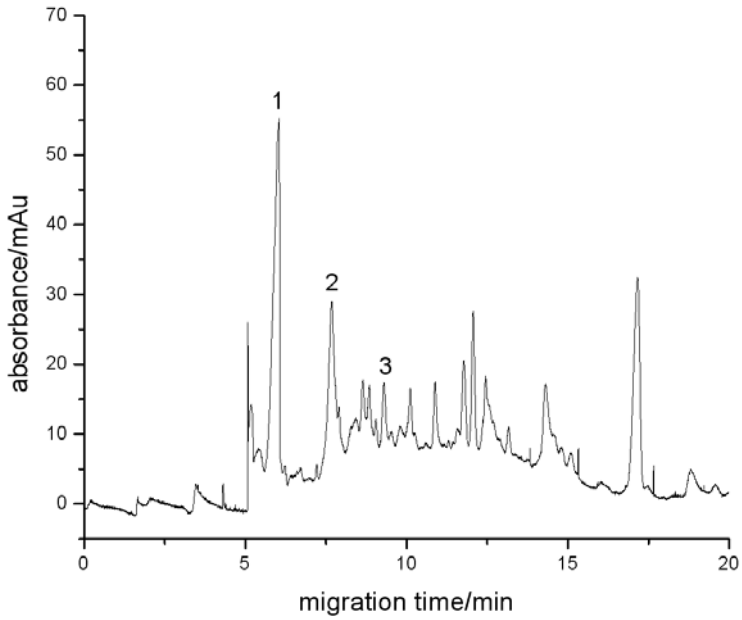


**Fig. 4** Capillary electropherogram of Tze Po San Pien Pills. Peaks: 1 = paeoniforin; 2 = paeonol; 3 = censenoside $Rg_1$. Analytical conditions: 20 mM borax, 30 mM SDS, pH 9.30, applied voltage 20 kV, sample time 5 s, detection UV 203 nm.

## 4 Conclusions

Results demonstrated that the MEKC method is a useful, simple and rapid technique for the identification, separation and determination of paeoniforin, paeonol and censenoside $Rg_1$ in Chinese medicinal preparations. Operating parameters such as buffer pH, SDS concentration, borax concentration, type and percentage of organic modifier have been optimized. In comparison to other chromatographic methods, where expensive columns

**Table 1** Content of the analytes in Chinese medicinal preparations (n = 5).

| Sample | Component | Content (mg/g) | RSD ( % ) |
|---|---|---|---|
| Tze Po San Pien Pills | paeoniforin | 0.57 | 2.21 |
| | paeonol | 0.23 | 3.72 |
| | censenoside $Rg_1$ | 0.66 | 3.60 |
| Liuwen Dihuang pills | paeoniforin | 0.21 | 4.18 |
| | paeonol | 0.45 | 3.86 |

and solvents are needed, the proposed MEKC method is a good alternative for the simultaneous analysis of bioactive components in traditional Chinese medicinal preparations. Furthermore, the method also promises to be applicable to the quality control of traditional Chinese medicine.

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