

Quantitative analysis and chromatographic fingerprinting for the quality evaluation of *Scutellaria baicalensis* Georgi using capillary electrophoresis

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Abstract

Quantitative analysis and chromatographic fingerprinting for the quality evaluation of a Chinese herb *Scutellaria baicalensis* Georgi using capillary electrophoresis (CE) technique was developed. The separation was performed with a 50.0 cm (42.0 cm to the detector window) \times 75 μ m i.d. fused-silica capillary, and the CE fingerprint condition was optimized using the combination of central composite design and multivariate analysis. The optimized buffer system containing 15 mM borate, 40 mM phosphate, 15 mM SDS, 15% (v/v) acetonitrile and 7.5% (v/v) 2-propanol was employed for the method development, and the baseline separation was achieved within 15 min. The determination of the major active components (Baicalin, Baicalein and Wogonin) was carried out using the optimized CE condition. Good linear relationships were provided over the investigated concentration ranges (the values of R^2 : 0.9997 for Baicalin, 0.9992 for Baicalein, and 0.9983 for Wogonin, respectively). The average recoveries of these target components ranged between 96.1–105.6%, 98.6–105.2%, and 96.3–105.0%, respectively. CE fingerprints combined with the quantitative analysis can be used for the quality evaluation of *S. baicalensis*.

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1. Introduction

Herbal medicines have a long history in medical practice and health care especially in some Asian and African countries. During the last two decades, herbal medicines have expanded globally and gained considerable attention because of low toxicity and good therapeutical performance [1]. Unfortunately, the quantification and quality of safety and efficacy data about the herbal medicines are far from sufficiency to satisfy the regulation supporting the world widely uses of these medicines. One of the main reasons lies on the lack of reliable and acceptable strategy for the quality evaluation of the herbal medicines [2–5].

Generally, the current existing approaches for the quality evaluation of the herbal medicines come from a long-standing

practice used on western medicines. However, for the herbal medicines the safety and efficacy are not just attributed to one or several particular compounds, because it is well known that a herbal medicine is one complex mixture containing dozens, even hundreds of chemical components which are usually responsible for the therapeutic effects. Furthermore, the interactions among these components make their relationship to the safety and efficacy much more complicated than that occurs in the western medicines. Therefore, quality evaluation of the herbal medicines, unlike that of the western medicines, is beyond the ability of routine analysis, which focuses on the single or a few marker components. Taking into account the direct quantification of as many as possible components is in common thought to evaluate the quality of the herbal medicines. However, it would be impossible because most reference components are not commercially available; moreover, most of the herbal medicines are so complex that the procedure of separation, identification and determination of numbers of components is rather tedious for the pharmaceutical quality evaluation.

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Chromatographic fingerprinting emphasizes on the systemic characterization of composition of the complex chemical mixture and appears to offer a more logical tool for the quality evaluation of the herbal medicines [6–10]. Nowadays, this technique has been suggested to check the authenticity or quality evaluation of the herbal medicines by WHO [11], and it is also required by the State Food and Drug Administration of China to standardize the injections made from the herbal medicines [12]. High-performance liquid chromatography (HPLC) is the commonly used technique for fingerprinting due to its high resolution, reproducibility and reliability [6–10,13–15]. Capillary electrophoresis (CE) is becoming increasingly recognized as an important separation technique, due to its high resolution, small sample volumes, extraordinary small solvents consumption and rapid separation with high efficiency. Recently, several studies on the chromatographic fingerprinting using CE technique have been reported [16–18].

Scutellaria baicalensis Georgi (Labiatae) is one commonly used herbal medicine in China and other East Asian countries, and it has been officially listed in the Chinese Pharmacopoeia for a long time [19]. Its root has been used for the treatment of bronchitis, hepatitis, tumors, and inflammatory diseases. Some concentrated composite herbal preparations containing this herb as a major ingredient are widely used in China [19]. The main active components are many kinds of flavones compounds such as Baicalin, Baicalein and Wogonin (their chemical structures have been presented in Fig. 1). Baicalin is the most abundant component, and it has anti-inflammatory [20], anti-HIV [21], anti-tumor [22] and anti-SARS coronavirus effects [23]. The activity of baicalein is similar with that of baicalin. Wogonin possesses anti-respiratory syncytial virus [24] and anti-tumor effects [22].

Although chromatographic fingerprinting can provide the global chemical patterns of a herbal medicine through the form of a chromatogram, quantitative analysis of markers or of substance with known therapeutic activity will also provide useful information for the quality evaluation of the herbal medicines [4,25]. The phenolics of the crude extract of *S. baicalensis* were ever examined by micellar electrokinetic chromatography (MEKC) with UV-DAD [26], and two trihydroxyflavones compounds were separated. In the present study, quantitative analysis and chromatographic fingerprint with CE technique was developed for the quality evaluation of *S. baicalensis*. The roots

of *S. baicalensis* (34 samples in all), collected from different geographical origins of China, were analyzed. The combination of central composite design and multivariate analysis was employed to find an optimal CE condition. Three active components (Baicalin, Baicalein and Wogonin) were determined with the optimized CE condition. As a result, the combination of quantitative analysis and chromatographic fingerprinting offers more valuable information for the quality evaluation of the herbal medicines.

2. Materials and methods

2.1. Instrumentation

All the CE experiments were performed on a HP^{3D}CE system (Agilent Technologies, Waldbronn, Germany) equipped with a diode-array detector. Instrumental control and data acquisition were conducted with CE ChemStation software (Agilent Technologies). Capillary electrophoresis was performed using a 50 cm (42 cm from the inlet to the detector) × 75 μm i.d. fused-silica capillary (Yongnian Photoconductive Fiber Factory, Hebei, China). Matlab 7.0 (The Mathworks, Natick, USA) was used for the data treatment. Computer-aided similarity evaluation system for chromatographic fingerprints (SFDA, China), programming by C language and Visual Basic, was used for the similarity analysis.

2.2. Chemicals

Baicalin, Baicalein and Wogonin were all purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Stock standard solutions were prepared with HPLC grade methanol from Merck (Darmstadt, Germany), and stored at 4 °C. Working standard solutions were prepared by diluting with an appropriate volume of methanol to the desired concentrations. Sodium dodecyl sulphate (SDS) was purchased from Sigma (St. Louis, MO, USA). Both acetonitrile and 2-propanol, used as the organic modifiers, were HPLC grade and purchased from Merck. Borate and phosphate were of analytical grade and purchased from Hangzhou Reagent Company (Zhejiang, China). Other reagents were all of analytical grade. Water used throughout the experiments was generated by a Milli-Q academic water purification system (Milford, MA, USA).

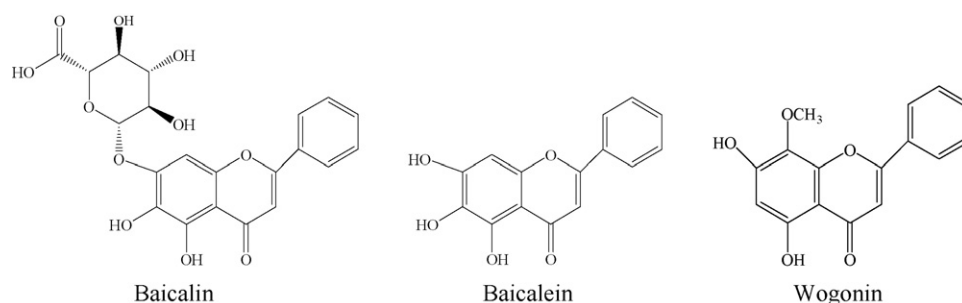


Fig. 1. The chemical structures of Baicalin, Baicalein and Wogonin.

2.3. Materials

Thirty-four raw herb samples from nine different provinces of China were investigated in this study (Table 1). All of them were identified by Associate Professor Xin-yue Xiao and Associate Chief Pharmacist Ji Zhang (National Institute for the Control of Pharmaceutical and Biological Products, China) according to their morphological characteristics. The samples were stored in the laboratory of Department of Chinese Medicine Science and Engineering, Zhejiang University, China.

2.4. Sample preparation

A 1.0 g powder of dried materials was extracted with 10.0 mL ethanol aqueous solution (70%, v/v) in an ultrasonic water bath for 30 min. The extract was repeated twice; then, the extracted solutions were combined together and filtrated through analytical filter papers. After centrifugation (10 000 rpm for 5 min), the supernatant was collected and diluted with ethanol aqueous solution (70%, v/v) to 50 mL. Each sample was prepared with the protocol mentioned above for CE analysis. All solutions were ultrasonically degassed and filtered through a 0.45 μm syringe filter before injection.

Table 1
Raw materials investigated in this study

| Sample no. | Origin | Purchasing time | Species |
|------------|-------------------|-----------------|---|
| 01 | Chende, Hebei | April 2001 | <i>S. baicalensis</i> Georgi |
| 02 | Luochuan, Shaanxi | August 2000 | <i>S. baicalensis</i> Georgi |
| 03 | Weichang, Hebei | April 2001 | <i>S. baicalensis</i> Georgi |
| 04 | Inner Mongolia | August 2000 | <i>S. baicalensis</i> Georgi |
| 05 | Inner Mongolia | August 2000 | <i>S. baicalensis</i> Georgi |
| 06 | Unknown | August 2000 | <i>S. baicalensis</i> Georgi |
| 07 | Unknown | August 2000 | <i>S. baicalensis</i> Georgi |
| 08 | Unknown | August 2000 | <i>S. baicalensis</i> Georgi |
| 09 | Ankang, Shaanxi | June 2001 | <i>S. baicalensis</i> Georgi |
| 10 | Baishan, Shaanxi | June 2001 | <i>S. baicalensis</i> Georgi |
| 11 | Anguo, Hebei | May 2001 | <i>S. baicalensis</i> Georgi |
| 12 | Anguo, Hebei | May 2001 | <i>S. baicalensis</i> Georgi |
| 13 | Inner Mongolia | August 2000 | <i>S. baicalensis</i> Georgi |
| 14 | Unknown | April 2001 | <i>S. baicalensis</i> Georgi |
| 15 | Dingxi, Gansu | May 2001 | <i>S. rehderiana</i> Diels |
| 16 | Longxi, Gansu | June 2001 | Unknown |
| 17 | Shanxi | June 2001 | <i>S. baicalensis</i> Georgi |
| 18 | Baiquan, Henan | July 2001 | <i>S. baicalensis</i> Georgi |
| 19 | Inner Mongolia | August 2000 | <i>S. baicalensis</i> Georgi |
| 20 | Unknown | July 2001 | <i>S. baicalensis</i> Georgi |
| 21 | Inner Mongolia | August 2000 | <i>S. baicalensis</i> Georgi |
| 22 | Anguo, Hebei | May 2001 | <i>S. baicalensis</i> Georgi |
| 23 | Lintao, Gansu | May 2001 | <i>S. rehderiana</i> Diels |
| 24 | Lintao, Gansu | May 2001 | <i>S. rehderiana</i> Diels |
| 25 | Chende, Hebei | December 2000 | <i>S. baicalensis</i> Georgi ^a |
| 26 | Anguo, Hebei | December 2000 | <i>S. baicalensis</i> Georgi* |
| 27 | Anguo, Hebei | December 2000 | <i>S. baicalensis</i> Georgi ^a |
| 28 | Juxian, Shandong | December 2000 | <i>S. baicalensis</i> Georgi ^a |
| 29 | Yishui, Shandong | December 2000 | <i>S. baicalensis</i> Georgi ^a |
| 30 | Chende, Hebei | December 2000 | <i>S. baicalensis</i> Georgi ^a |
| 31 | Datong, Shanxi | July 2001 | <i>S. viscidula</i> Bunge |
| 32 | Yunan | August 2001 | <i>S. amoena</i> CH Wright |
| 33 | Yunan | August 2001 | <i>S. amoena</i> CH Wright |
| 34 | Ningxia | August 2001 | <i>S. rehderiana</i> Diels |

^a The corresponding samples were not wild.

2.5. Electrophoretic procedures

Prior to the first use, the new capillary was conditioned with 1 M NaOH for 30 min and 0.1 M NaOH for 10 min, followed by the demineralized water for 5 min and the running buffer for 5 min, respectively. Between runs, the capillary was washed with 0.1 M NaOH for 1 min, followed by the demineralized water for 1 min, and then equilibrated with the running buffer for 2 min. Each day, the capillary was first conditioned by flushing with 1 M NaOH for 10 min and the demineralized water for 10 min, respectively. The capillary was reconditioned with 1 M NaOH for 3 min and the demineralized water for 2 min after every five runs. The running buffer was refreshed after every five runs to ensure the good reproducibility. Electrophoretogram was obtained with a wavelength set at 280 nm [19]. The sample solution was injected hydrodynamically at 30 mbar for 3 s.

3. Results and discussion

3.1. Optimization of CE condition

3.1.1. Preliminary investigations

The raw material of *S. baicalensis* from Inner Mongolia (sample no.: 04) was used as a typical sample for the optimization procedure. Preliminary experiments with different buffers showed that capillary zone electrophoresis (CZE) gave poor resolution, due to the fact that most components in this raw material were chemically in neutral character. Therefore, micellar electrokinetic capillary chromatography (MEKC) mode was employed for the further investigation. The mixture of 20 mM borate and 30 mM phosphate was chosen as the aqueous buffer because it provided a relative better separation. SDS was chosen as the surfactant. Organic modifiers were commonly used for method development of MEKC. It was shown that the simultaneous addition of acetonitrile and 2-propanol into the buffer offered a better separation. However, some components were still not well separated, and the running time (about 25 min) was too long for CE analysis. The preliminary studies showed that the separation of multiple components in the sample solution was sensitive to the experimental conditions, especially to the applied buffer system.

3.1.2. Central composite design

Five experimental parameters, including the concentration of borate and phosphate, the concentration of SDS, the proportions of acetonitrile and 2-propanol in the buffer, were selected for the experimental design. These five factors displayed much more pronounced effects on the performance of separation compared to the temperature, voltage and injection condition. Considering the quality and time of the separation, the applied voltage, temperature and pressure injection condition were set at 20 kV, 25 °C and 30 mbar \times 3 s, respectively.

The central composite design (CCD) was performed to systematically investigate the effects of the selected five factors on the performance of separation. The upper and lower levels of the factors shown in Table 2 were determined by the preliminary experiments. The star points were located at $+\alpha$ and $-\alpha$ away

Table 2
Experimental factors and levels in the CCD for optimizing CE separation

| Factors | Coded | Levels | | | | |
|----------------------|-------|--------|---------|------|-----------------------|-----------------------|
| | | Low | Central | High | Star point °C (−α) | Star point °C (+α) |
| Borate (mM) | x_1 | 15 | 20 | 25 | 10 | 30 |
| Phosphate (mM) | x_2 | 20 | 30 | 40 | 10 | 50 |
| SDS (mM) | x_3 | 40 | 60 | 80 | 20 | 100 |
| Acetonitrile (% v/v) | x_4 | 5 | 10 | 15 | 0 | 20 |
| 2-Propanol (% v/v) | x_5 | 2.5 | 5 | 7.5 | 0 | 10 |

from the centroid in the experimental domain. The axial distance α set at 2 was in order to establish the rotatability condition. Thus, this design could equally generate information in all directions. The experimental conditions of CCD and the corresponding resolutions of main co-possessing peaks in the chromatogram were presented in Table 3. Total 33 experiments were involved in the matrix of CCD including 7 additional experiments carried out at the centre point to estimate the overall error. The experiments were performed randomly in order to avoid systematic error.

3.1.3. Multivariate analysis

The CCD design permits the response surface to be modeled by fitting the second-order regression models, which can be shown as

$$y = b_0 + \sum_{i=1}^n b_i x_i + \sum_{i,j=1}^n b_{ij} x_i x_j + E \quad (1)$$

Table 3
Experimental parameters for CCD and the corresponding responses

| No. | Variables | | | | | Responses | | | | | |
|-------|------------|------------|------------|---------------|---------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | x_1 (mM) | x_2 (mM) | x_3 (mM) | x_4 (% v/v) | x_5 (% v/v) | Rs _{1/2} | Rs _{3/4} | Rs _{4/5} | Rs _{5/6} | Rs _{6/7} | Rs _{7/8} |
| 1 | 25.0 | 40.0 | 80.0 | 15.0 | 7.5 | 0.58 | 13.91 | 1.89 | 13.53 | 6.92 | 11.75 |
| 2 | 25.0 | 40.0 | 80.0 | 5.0 | 2.5 | 0.00 | 8.19 | 5.40 | 5.12 | 8.26 | 11.86 |
| 3 | 25.0 | 40.0 | 40.0 | 15.0 | 2.5 | 0.00 | 2.99 | 10.64 | 4.39 | 2.23 | 8.93 |
| 4 | 25.0 | 40.0 | 40.0 | 5.0 | 7.5 | 0.00 | 3.80 | 6.81 | 2.58 | 6.23 | 18.54 |
| 5 | 25.0 | 20.0 | 80.0 | 15.0 | 2.5 | 1.39 | 6.67 | 2.02 | 2.73 | 2.08 | 11.05 |
| 6 | 25.0 | 20.0 | 80.0 | 5.0 | 7.5 | 2.43 | 10.85 | 8.70 | 6.68 | 3.27 | 5.04 |
| 7 | 25.0 | 20.0 | 40.0 | 15.0 | 7.5 | 2.17 | 2.07 | 15.27 | 6.48 | 1.23 | 21.32 |
| 8 | 25.0 | 20.0 | 40.0 | 5.0 | 2.5 | 1.23 | 2.39 | 4.30 | 3.12 | 0.80 | 9.83 |
| 9 | 15.0 | 40.0 | 80.0 | 15.0 | 2.5 | 3.60 | 9.42 | 5.06 | 13.27 | 35.49 | 14.76 |
| 10 | 15.0 | 40.0 | 80.0 | 5.0 | 7.5 | 3.36 | 7.90 | 7.90 | 16.51 | 17.71 | 22.20 |
| 11 | 15.0 | 40.0 | 40.0 | 15.0 | 7.5 | 2.01 | 3.47 | 4.60 | 3.89 | 6.91 | 9.29 |
| 12 | 15.0 | 40.0 | 40.0 | 5.0 | 2.5 | 2.42 | 5.68 | 0.33 | 5.61 | 32.52 | 0.60 |
| 13 | 15.0 | 20.0 | 80.0 | 15.0 | 7.5 | 0.00 | 9.09 | 4.39 | 3.82 | 1.24 | 3.08 |
| 14 | 15.0 | 20.0 | 80.0 | 5.0 | 2.5 | 0.00 | 5.30 | 5.47 | 4.38 | 5.50 | 10.72 |
| 15 | 15.0 | 20.0 | 40.0 | 15.0 | 2.5 | 0.47 | 2.64 | 5.75 | 2.98 | 1.05 | 6.43 |
| 16 | 15.0 | 20.0 | 40.0 | 5.0 | 7.5 | 0.00 | 1.93 | 3.59 | 1.79 | 0.23 | 7.17 |
| 17 | 30.0 | 30.0 | 60.0 | 10.0 | 5 | 0.90 | 9.60 | 3.29 | 5.58 | 0.39 | 5.90 |
| 18 | 10.0 | 30.0 | 60.0 | 10.0 | 5 | 2.73 | 8.84 | 5.06 | 12.17 | 17.40 | 2.97 |
| 19 | 20.0 | 50.0 | 60.0 | 10.0 | 5 | 3.87 | 14.29 | 9.85 | 17.33 | 26.80 | 13.97 |
| 20 | 20.0 | 10.0 | 60.0 | 10.0 | 5 | 0.96 | 5.71 | 0.57 | 1.42 | 1.18 | 8.31 |
| 21 | 20.0 | 30.0 | 100.0 | 10.0 | 5 | 0.82 | 3.99 | 17.08 | 1.00 | 7.72 | 19.91 |
| 22 | 20.0 | 30.0 | 20.0 | 10.0 | 5 | 0.00 | 2.11 | 7.05 | 3.98 | 1.44 | 11.75 |
| 23 | 20.0 | 30.0 | 60.0 | 20.0 | 5 | 0.00 | 7.97 | 9.98 | 3.88 | 5.25 | 1.31 |
| 24 | 20.0 | 30.0 | 60.0 | 0.0 | 5 | 0.00 | 6.60 | 6.04 | 6.28 | 5.80 | 10.53 |
| 25 | 20.0 | 30.0 | 60.0 | 10.0 | 10 | 0.00 | 10.27 | 2.02 | 5.62 | 0.74 | 5.40 |
| 26 | 20.0 | 30.0 | 60.0 | 10.0 | 0 | 0.00 | 9.19 | 5.93 | 4.01 | 7.99 | 8.73 |
| 27–33 | 20.0 | 30.0 | 60.0 | 10.0 | 5 | 0.00 | 12.69 | 1.61 | 4.59 | 4.39 | 8.98 |

where b is the regression coefficient, n the number of the variables, and E is the experimental error.

The square term of each variable describes the non-linear effects on the responses, while the cross terms of two different variables exhibit their interaction effects. The regression coefficient plots with confidence intervals for all six responses were shown in Fig. 2. The coefficients of the models described the relationship between the variables and the corresponding response. In this study, the effect of a variable was denoted by a coefficient bar and the 95% confidence limits of an error bar. A regression coefficient smaller than the error bar interval indicated that the variation in the response caused by the change in that variable was smaller than that of the experimental error, which meant that the effect of that variable could be considered to be negligible. The positive or negative coefficient in the chart showed that the corresponding variable possessed a positive or negative effect on the response, respectively.

The developed quadratic models could be visualized in the three-dimensional response surfaces through mapping against two significant variables while the others kept constant. The response surfaces for the resolutions of six critical peak pairs shown in Fig. 3 were developed according to the regression coefficient plots (shown in Fig. 2). For the selection of the overall optimal point, balancing of all these effects was necessary. For example, even though high resolution Rs_{1/2} was obtained at high concentration of borate or phosphate, a poor separation of the other components would be given.

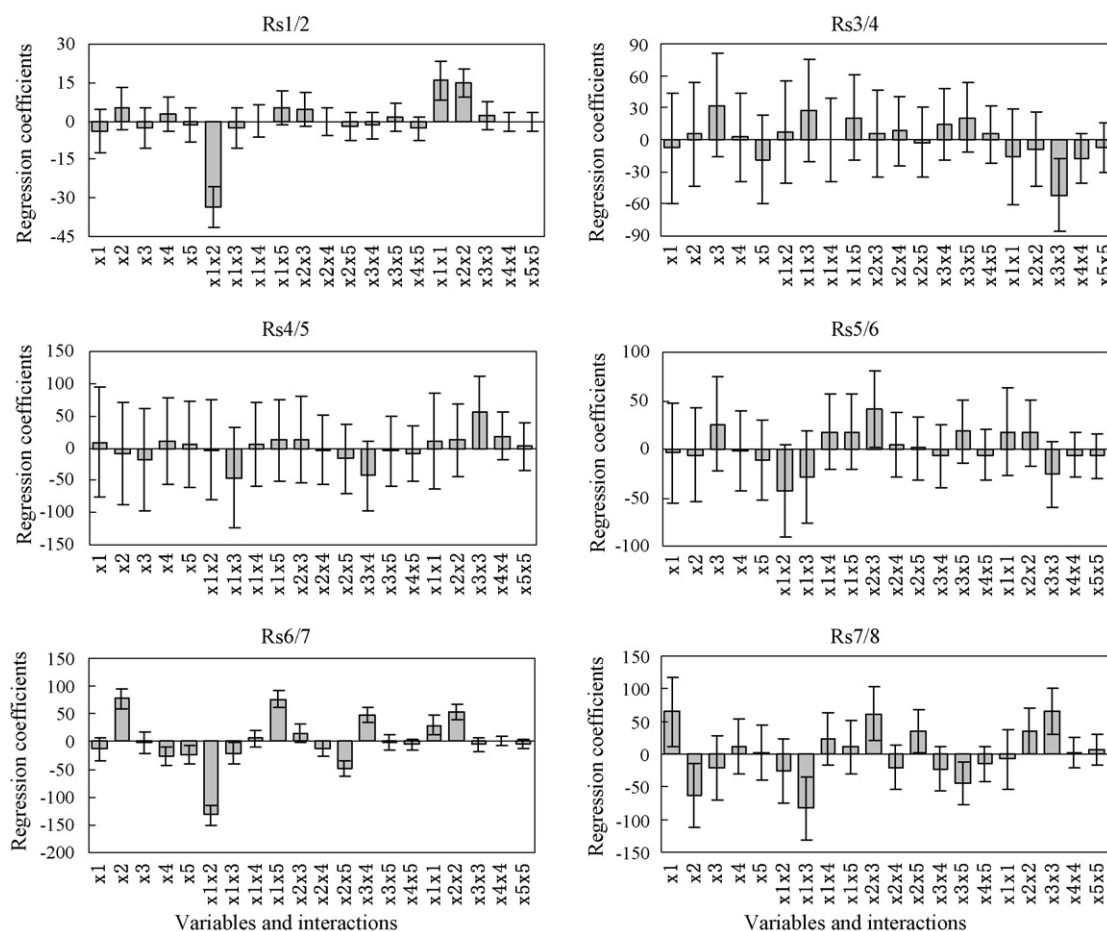


Fig. 2. Regression coefficient plots for the resolutions of six critical peak pairs.

3.1.4. Optimized result

Considering the total resolution of the CE separation and the running time, the optimal buffer system containing 15 mM borate, 40 mM phosphate, 15 mM SDS, 15% acetonitrile and 7.5% 2-propanol was employed for the analysis. The typical MEKC chromatograms of the standard and sample solutions using the optimized buffer condition were shown in Fig. 4. The peaks of Baicalin, Baicalein and Wogonin presented in the chromatogram of the sample solutions were identified by comparing the mobility times and the characteristic of the UV spectra of the peaks with those presented in the standard solution.

3.2. Method validation

3.2.1. Injection repeatability and analysis repeatability

The repeatability of the developed MEKC method was expressed as relative standard deviation (R.S.D.). Both the injection repeatability and analysis repeatability were investigated, and both the R.S.D. values of migration time and peak areas of the co-possessing peaks in the MEKC profiles were calculated. The injection repeatability was carried out by the injection of continuous 6 times using the same sample, while the analysis repeatability was examined by the injection of 6 samples, which were prepared using the same sample preparation procedure. The R.S.D. value of analysis repeatability (below 9.0%) was a

little higher than that of the injection repeatability (below 5.0%), and it should be attributed to some error introduced in the sample preparation steps.

3.2.2. Intra-day and inter-day precision

The intra-assay precision was performed by analyzing the sample solutions with the interval of 4 h in 1 day, and the inter-assay precision was examined over 6 days by the analysis of the samples prepared each day. The R.S.D. values of the peak areas of the investigated chromatographic peaks in the intra-assay and inter-assay examinations were both below 9.0%. The result showed that a satisfactory precision was obtained for the separation of multiple components by the developed method.

3.2.3. Linearity, range and limits of detection

A series of the standard mixture solutions of Baicalin, Baicalein and Wogonin were used to determine the linearity and the linearity range of the analytes in the developed method. The calibration curves for these components were assessed at 7 concentration levels. Triplicate injections were performed at each concentration and the average value was used to establish the standard curves. The linearity of each standard curve was confirmed by plotting the peak area (y) and the corresponding concentration (x , mg/L) of the analyte. The linear regression

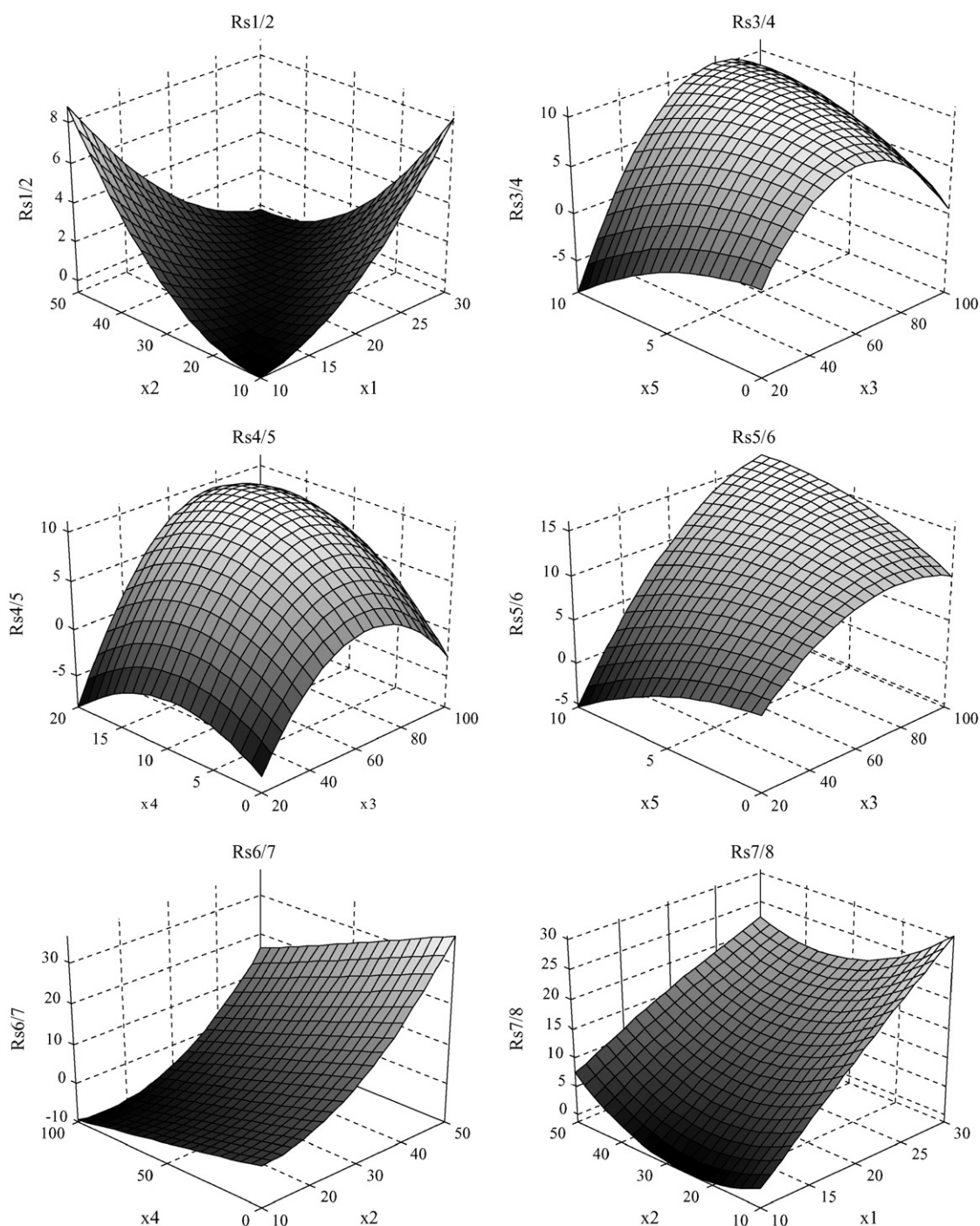


Fig. 3. Response surface plots of the investigated variables and the resolutions of six critical peak pairs.

equations, correlation coefficients and the limits of detection of the analytes were all shown in Table 4.

3.2.4. Recovery test

Because of the complexity of the herbal medicines, there has neither a standard method for the determination of these active components nor a standard reference. Therefore, recovery of the standard from samples is generally used to evaluate the accuracy of the newly developed method. The recoveries of this method for three investigated components at three concentration levels were from 96.1% to 105.6% with the R.S.D. value below 5.1%.

Table 4
Regression equations and the detection limits ($n=3$)

| Analytes | Regression equation | Correlation coefficient (R^2) | Linear range (mg/L) | Limit of detection (mg/L) |
|-----------|---------------------|-----------------------------------|---------------------|---------------------------|
| Baicalin | $y=0.5330x-3.6551$ | 0.9997 | 20.6–990 | 1.79 |
| Baicalein | $y=0.7680x-12.473$ | 0.9992 | 20.8–500 | 1.19 |
| Wogonin | $y=1.1671x-6.0015$ | 0.9983 | 12.3–197 | 0.78 |

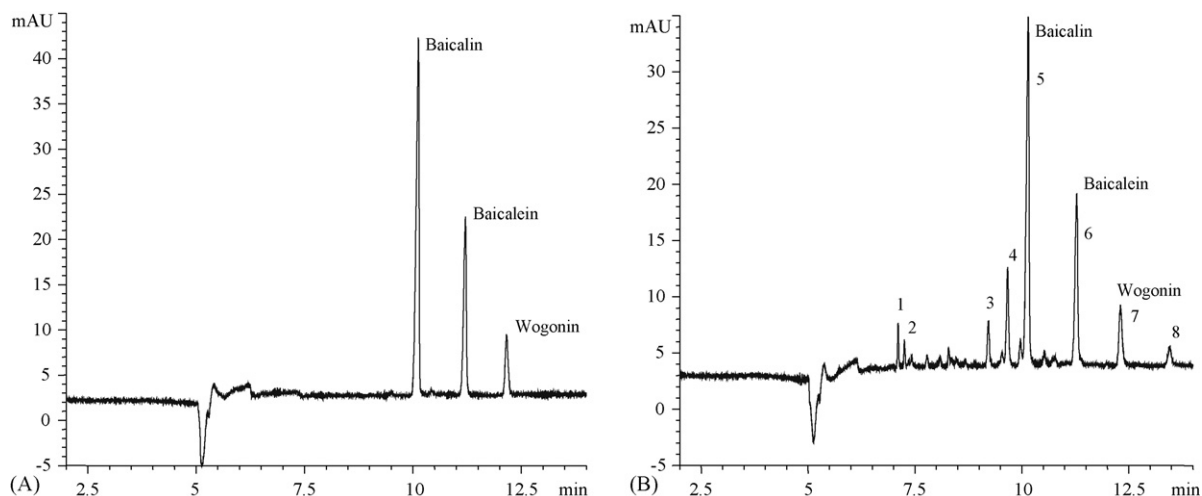


Fig. 4. Typical CE chromatograms of the standard (A) and real sample solution (B) using the optimized experimental conditions. Buffer system: 15 mM borate, 40 mM phosphate, 40 mM SDS, 15% (v/v) acetonitrile and 7.5% (v/v) 2-propanol. Capillary: 50.0 cm (42.0 cm to the detector window) \times 75 μ m i.d.; the applied voltage: 20 kV; temperature: 25 $^{\circ}$ C; detection wavelength: 280 nm; sample injection time 3 s with a 30 mbar pressure.

3.3. Analysis of samples

3.3.1. Quantitative analysis

The developed MEKC method was applied to the determination of Baicalin, Baicalein and Wogonin in the herb samples collected from the different geographical origins of China. The results (shown in Table 5) were corresponding to those of previous HPLC study [27]. However, optimization of a MEKC method for the separation of the complex chemical systems was much easier than that of HPLC. In addition, the analysis efficiency of MEKC was higher than that of HPLC, while the consumption of both reagent and solvent by MEKC was much smaller. Therefore, the developed MEKC method was more applicable to the quantitative analysis of these active components contained in the herb samples investigated. The analytical result showed that there were large variations in the contents of these three active components among the investigated herb samples. These variations might be due to the different cultivation, storage methods, species and geographical origins of these herb samples.

3.3.2. Comparative analysis of chromatographic fingerprints

The co-possessing peaks presented in the chromatograms of these herb samples were considered as the fingerprint peaks, which were employed to the comparative analysis of the samples from different origins. The relative area values of eight co-possessing peaks in each electrophoretogram were used to construct an eight-dimensional vector to characterize the chemical pattern of the fingerprint, and a simple representation to calculate the similarity was given as below [28]:

$$\sigma(X, Y) = \frac{2XY^T}{XX^T + YY^T} \quad (2)$$

where σ was the value of similarity; X and Y two vectors consisted of the co-possessing peaks in their fingerprints; T was the note of transposition for the vector.

The mean fingerprints of the samples from the same origins worked as the references to calculate the similarity, and the results of comparative analysis were shown in Table 6. It was presented that the similarity of the samples from the same origin

Table 5
Determination of three active components in different samples ($n = 3$)

| Sample no. | Baicalin (mg/g) | Baicalein (mg/g) | Wogonin (mg/g) |
|------------|-----------------|------------------|----------------|
| 01 | 72.50 | 4.63 | 1.81 |
| 02 | 84.69 | 5.74 | 1.89 |
| 03 | 54.60 | 4.20 | 1.64 |
| 04 | 38.24 | 15.12 | 4.80 |
| 05 | 24.74 | 6.87 | 2.11 |
| 06 | 78.58 | 5.71 | 2.22 |
| 07 | 79.98 | 9.60 | 3.48 |
| 08 | 58.58 | 3.38 | 1.94 |
| 09 | 48.72 | 7.97 | 3.04 |
| 10 | 26.09 | 7.05 | 2.50 |
| 11 | 86.26 | 2.02 | 0.64 |
| 12 | 102.21 | 3.95 | 0.78 |
| 13 | 69.28 | 2.34 | 0.49 |
| 14 | 67.73 | 1.53 | 0.36 |
| 15 | 66.66 | 6.04 | 1.75 |
| 16 | 73.84 | 14.90 | 4.35 |
| 17 | 93.10 | 6.87 | 2.29 |
| 18 | 37.15 | 11.42 | 4.14 |
| 19 | 44.58 | 5.30 | 2.02 |
| 20 | 31.27 | 3.98 | 1.41 |
| 21 | 57.49 | 5.28 | 1.92 |
| 22 | 128.81 | 4.39 | 0.78 |
| 23 | 73.21 | 9.00 | 2.42 |
| 24 | 59.00 | 4.09 | 1.49 |
| 25 | 141.99 | 4.07 | 0.70 |
| 26 | 71.29 | 2.71 | 0.38 |
| 27 | 73.57 | 3.06 | 0.37 |
| 28 | 97.58 | 2.98 | 0.55 |
| 29 | 63.27 | 3.13 | 1.50 |
| 30 | 134.41 | 4.80 | 1.44 |
| 31 | 143.56 | 4.69 | 0.59 |
| 32 | 112.03 | 7.80 | 0.94 |
| 33 | 106.93 | 8.09 | 0.72 |
| 34 | 70.56 | 6.97 | 1.40 |

Table 6
The similarity of the samples investigated

| Origin of the samples | Similarity of the chromatographic fingerprint | | | | | |
|-----------------------|---|--------|--------|---------|----------|--------|
| | Inner Mongolia | Gansu | Hebei | Shaanxi | Shandong | Yunnan |
| Inner Mongolia | 0.9544 | 0.9875 | 0.8777 | 0.9659 | 0.9878 | 0.8987 |
| Inner Mongolia | 0.9933 | 0.9831 | 0.7993 | 0.9166 | 0.9472 | 0.8241 |
| Inner Mongolia | 0.9223 | 0.8530 | 0.5868 | 0.7269 | 0.7449 | 0.6297 |
| Inner Mongolia | 0.9855 | 0.9150 | 0.6689 | 0.8061 | 0.8464 | 0.7017 |
| Dingxi, Gansu | 0.9710 | 0.9978 | 0.8622 | 0.9606 | 0.9800 | 0.8901 |
| Longxi, Gansu | 0.9311 | 0.9857 | 0.8855 | 0.9686 | 0.9631 | 0.9166 |
| Lintao, Gansu | 0.9460 | 0.9968 | 0.8978 | 0.9796 | 0.9870 | 0.9244 |
| Lintao, Gansu | 0.9890 | 0.9841 | 0.8075 | 0.9225 | 0.9537 | 0.8363 |
| Anguo, Hebei | 0.8799 | 0.9602 | 0.9581 | 0.9950 | 0.9962 | 0.9593 |
| Anguo, Hebei | 0.8117 | 0.9179 | 0.9918 | 0.9879 | 0.9714 | 0.9859 |
| Chengde, Hebei | 0.6516 | 0.7789 | 0.9804 | 0.8973 | 0.8586 | 0.9545 |
| Chengde, Hebei | 0.6804 | 0.8067 | 0.9897 | 0.9192 | 0.8823 | 0.9676 |
| Luochuan, Shaanxi | 0.8986 | 0.9754 | 0.9493 | 0.9987 | 0.9964 | 0.9623 |
| Shaanxi | 0.8604 | 0.9543 | 0.9732 | 0.9988 | 0.9871 | 0.9811 |
| Juxian, Shandong | 0.8311 | 0.9307 | 0.9845 | 0.9923 | 0.9806 | 0.9803 |
| Yishui, Shandong | 0.9726 | 0.9880 | 0.8401 | 0.9428 | 0.9710 | 0.8676 |
| Yunnan | 0.7706 | 0.8888 | 0.9859 | 0.9677 | 0.9406 | 0.9997 |
| Yunnan | 0.7940 | 0.9076 | 0.9847 | 0.9780 | 0.9545 | 0.9997 |

was all higher than 0.9, which meant that the holistic quality of these samples was quite similar. Also, it was indicated that the pattern difference of the samples from Inner Mongolia, Gansu and Shandong (Yishui) was almost undistinguished. However, the samples from Hebei, Shaanxi and Yunnan were easy to be identified from those from Inner Mongolia with their CE fingerprints.

4. Conclusion

In the present study, an improved method using quantitative analysis and chromatographic fingerprinting with capillary electrophoresis was developed for the quality evaluation of Chinese herb *S. baicalensis*. Three major active components (Baicalin, Baicalein and Wogonin) contained in these herb samples were well determined using the CE condition optimized by the combination of central composite design and multivariate analysis. The proposed method was applicable to the quality evaluation of *S. baicalensis*.

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