Determination of Puerarin, Daidzein, Baicalin and Wogonin in Composite Preparations by Capillary Electrophoresis

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Summary: A simple method for the simultaneous determination of four bioactive components (puerarin, daidzein, baicalin, and wogonin) in composite preparations by microemulsion capillary electrophoresis with UV detection has been developed for the first time. A running buffer composed of 8% acetonitrile + 4% microemulsion(3.24% n-heptane + 13.24% sodium dodecyl sulfate (SDS) + 26.44% n-butanol + 57.08% distilled water in weight ratio) + 20 mM borax solution was found to be the most suitable for this separation. The limits of detection for four analytes were over the range of 0.50 - 1.2 μg ml^{-1}. In the tested concentration range, linear relationships (correlation coefficients: 0.997 for baicalin, 0.997 for wogonin, 0.998 for daidzein and 0.999 for puerarin) between the peak areas and the concentrations of the analytes were obtained. This method has been successfully applied to simultaneous determination of the four bioactive components with recoveries from 94.6 to 106.3%.

Keywords: Microemulsion; Capillary electrophoresis; Puerarin; Daidzein; Baicalin; Wogonin.

Introduction

Pueraria lobata and scutellaria baicalensis are two commonly used Chinese herbal medicines. Pueraria lobata is the dried root of the leguminous plant-elegant jessamine or kudzu vine. It is used for the treatment of common cold, influenza, exogenous fever, headache, and so on; and it can generate saliva and quench thirst, raise Yang and stop diarrhea. Scutellaria baicalensis is the dried root of radix scutellariae in perennial plant family, with the functions of clearing heat, drying dampness, purging fire, detoxifying, hemostasis and placentation. Gegen Qinlian pellets is a compound preparation composed of pueraria lobata root, scutellaria baicalensis and other herbs. Puerarin and daidzein are two of Pueraria Montana's main pharmacological active ingredients; and they are flavonoid, with antioxidant, anti-inflammatory and antipyretic effects, dilate coronary arteries, and increase coronary blood flow. Baicalin and wogonin are important pharmacologically active ingredients of scutellaria baicalensis georgi. Modern pharmacological studies have shown that baicalin and wogonin have anti-inflammatory, antibacterial, anti-cancer and heat-clearing pharmacological activities [1-9].

Thin-layer chromatography [10], liquid chromatography [11-13] and capillary electrophoresis [14, 15] have been used to determine one or three of these four components (puerarin , daidzein, baicalin, and wogonin). Due to the low separation efficiency and resolution, the application of thin layer chromatography is limited to a certain extent; although liquid chromatography is a mature technology with high precision and accuracy, widely used in the purification and preparation of substances, it has its shortcomings, such as a longer analysis time, consumption of samples and solvents, easy contamination of the column, and so on. Capillary electrophoresis [18-23] is a powerful separative and quantitative technique developed in the 1980s that often provides higher resolving power, shorter analysis times, low sample and solvent consumption and lower operational costs, and so on, has been successfully used in drug analysis, environmental, chemical and other fields. Because many drugs and their active components are hydrophobic substances, it is difficult to separate them by capillary zone electrophoresis or micellar electrophoresis.

Microemulsions are microheterogeneous liquids which have characteristic properties, such as optical transparency, thermodynamic stability, and high solubilization capacity. The microemulsion can dissolve the hydrophobic substance, and microemulsion capillary electrophoresis can effectively separate hydrophobic neutral substances.

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Neutral solutes can be separated by partitioning between the oil core of a droplet and aqueous buffer, and the separation of charged analytes depends on their electrophoretic mobilities, partition coefficient, and electrostatic interaction with the charged microemulsion droplet. Microemulsion electrokinetic chromatography is an electro-driven separation technique based on both hydrophobicity and electrophoretic mobility. Microemulsion electrokinetic chromatography offers the possibility of highly efficient separations of both charged and neutral or hydrophilic and lipophilic solutes [16, 17, 24-31].

So far, there is no report on the simultaneous separation and determination of the four components (puerarin, daidzein, baicalin and wogonin). Capillary electrophoresis was first used to separate the four active components in the composite preparation. The experimental results show that the method provided in this article is simple, reliable, and reproducible.

Experimental

Apparatus

The experiment used the P/ACE 5510 capillary electrophoresis instrument of Beckman Coulter Instrument, Fullerton, CA, USA. The system was controlled by the P/ACE™ workstation, using the Beckman V8.1 gold software; the detector was a photodiode detector with a detection window of 100μm × 200μm. The temperature of the capillary tube was adjusted and controlled by the coolant; and the capillary tube used was the product of Hebei Yongnian Optical Fiber Factory, with an inner diameter of 75μm, a total length of 47 cm, and an effective length of 40 cm. The new capillary was rinsed with 0.1 M hydrochloric acid, 0.1 M sodium hydroxide, and water for 15 min, 15 min, and 10 min before use; between two injections, it was rinsed with 0.1M hydrochloric acid, 0.1M sodium hydroxide, water and running buffer for 0.5 min, 0.5 min, 0.5 min and 2 mins, respectively; and at the end of the experiment, it was rinsed with water for 5 mins. The sample was injected from the anode end, the injection pressure was 0.5 psi, and the injection time was 3 seconds. It was detected from the cathode end with a detection wavelength of 273 nm.

Materials and Reagents

Puerarin (P), daidzein (D), baicalin (B) and wogonin (W) were purchased from China Institute for the Control of Pharmaceutical and Biological Products. Acetonitrile and methanol were purchased from Tianjin No.1 Chemical Plant. Borax ($Na_2B_4O_7.10H_2O$), n-heptane, n-butanol and sodium dodecyl sulfate (SDS) were obtained from Beijing Chemical Reagent Company. All used reagents used are of analytical grade. Gegen Qinlian pellets were purchased from Zhongyou Pharmacy (Lanzhou, China).

Standard Solution Preparation

The stock standard solutions of puerarin (P) (940.0 $\mu g.ml^{-1}$), daidzein (D) (1020.0$\mu g.ml^{-1}$), baicalin (B) (1140.0$\mu g.ml^{-1}$), and wogonin (W) (870.0 $\mu g.ml^{-1}$) were prepared in methanol, with 5.0 mL each of the stock standard solution. Other low-concentration standard solutions were obtained by diluting the stock solution with a microemulsion solution.

Preparation of Microemulsion and Electrolyte

The microemulsion was prepared by mixing 3.24% n-heptane, 13.24% sodium dodecyl sulfate (SDS), 26.44% n-butanol, and 57.08% distilled water in weight ratio; 100 mM borax solution was prepared by dissolving borax in distilled water; and the running buffer solution was prepared by mixing the microemulsion, acetonitrile, and 100 mM borax in water.

Preparation of Sample Solution

The crushed sample Gegen qinlian pellets 1.9867g were weighed and placed in a 25 mL colorimetric tube. 15 mL methanol was added for ultrasonic extraction for 30 mins, and the extraction was repeated three times. The extract was combined for three times and condensed by vacuum distillation so that the final volume was 5 ml. After being filtered by 0.45μm organic filter membrane, it was directly analyzed by capillary electrophoresis.

Results and Discussion

Fig.1 shows the molecular structures of puerarin (P), daidzein (D), baicalin (B) and wogonin (W). It can be seen in the figure that the three analytes had similar structures, but contained different functional groups on the benzene ring. Based on the UV absorption characteristics of the analytes, capillary electrophoresis was chosen to separate, 273nm as the detection wavelength. The

effects of borax concentration, microemulsion addition ratio, acetonitrile content and separation voltage on separation were investigated in this paper.

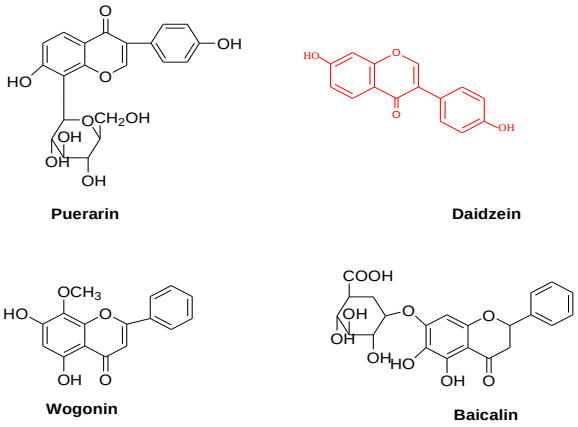
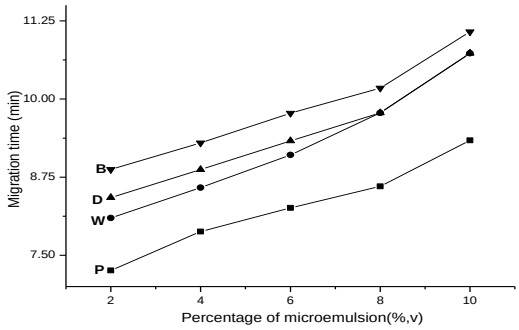


Fig. 1: The Molecular Structures of the Analytes.

The Effect of Microemulsion Addition Ratio on Separation

Fig. 2 shows the effect of the percentage of microemulsionon the separation. It can be seen from the figure that the migration time was increased with the rising of the amount of microemulsion, while the separation between wogonin and daidzein was worsened. On the whole, a better separation could be achieved by the addition of 4% microemulsion.

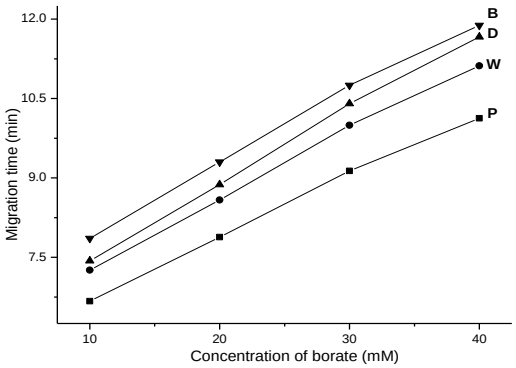


Analytes: P=puerarin, w=wogonin, D= daidzein and B=baicalin.
Buffer: 20 mM borax+2-10% microemulsion(3.24% n-heptane+13.24% sodium dodecyl sulfate (SDS)+26.44% n-butanol+57.08% distilled water in weight ratio).
Capillary length: uncoated fused-silica capillary 47 cm (40 cm injector to detector) × 75 μm i.d..
UV detection wavelength: 273 nm.
Applied voltage: 15 kV.
Capillary temperature: 25 ℃.

Fig. 2: The effect of the percentage of microemulsion on the separation.

The Effect of Borax Concentration on Separation

Fig. 3 shows the effect of borax concentration on separation. It can be seen from the figure that the migration time increased with the rising of borax concentration, which was the result of the increase of ionic strength; the resolution also changed with the increase of concentration, and the separation was better at 20 mM and 30 mM borax; considering the migration time and resolution, 20 mM borax concentration was chosen as the best condition.



Analytes: P=puerarin, w=wogonin, D= daidzein and B=baicalin.
Buffer: 10 – 40 mM borax+4% microemulsion.
Other experimental conditions are the same as Fig.2.

Fig. 3: The Effect of Borax Concentration on Separation.

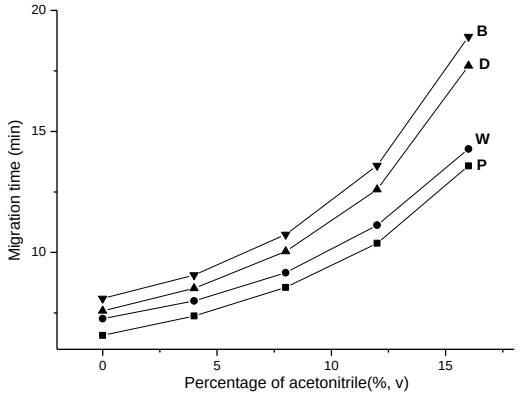
The Effect of Acetonitrile on Separation

Fig. 4 shows the effect of acetonitrile on separation. It can be seen from the figure that when the content of acetonitrile increased, the migration time and resolution of each analyte would rise. It was found in the experiment that: (1) when the content of acetonitrile increased, the tailing of the peaks of wogonin and baicalin could be improved; (2) when analyzing the sample, the interference of other substances on the separated components could be greatly reduced by the addition of acetonitrile; (3) when the content of acetonitrile increased, the ultraviolet absorption of the analyte would be enhanced. In general, 8% acetonitrile was better.

The Effect of Separation Voltage and Capillary Temperature

The effect of voltage was studied in the range of 15-25kV. The experiments have confirmed that the separation time would be shortened when the voltage was increased, but the Joule heat would rise,

causing peak broadening. The effect of the capillary temperature was studied in the range of 16-30℃, and it was found that the increase in temperature could slightly reduce the separation time, but the effect was not significant. Taken together, the separation voltage was 20 kV and the capillary temperature was 20 ℃.



Analytes: P=puerarin, w=wogonin, D= daidzein and B=baicalin.
Buffer: 0-16% acetonitrile + 20 mM borax + 4% microemulsion.
Applied voltage: 20 kV.
Other experimental conditions are the same as **Fig.2.**

Fig. 4: The Effect of Acetonitrile Concentration on Separation.

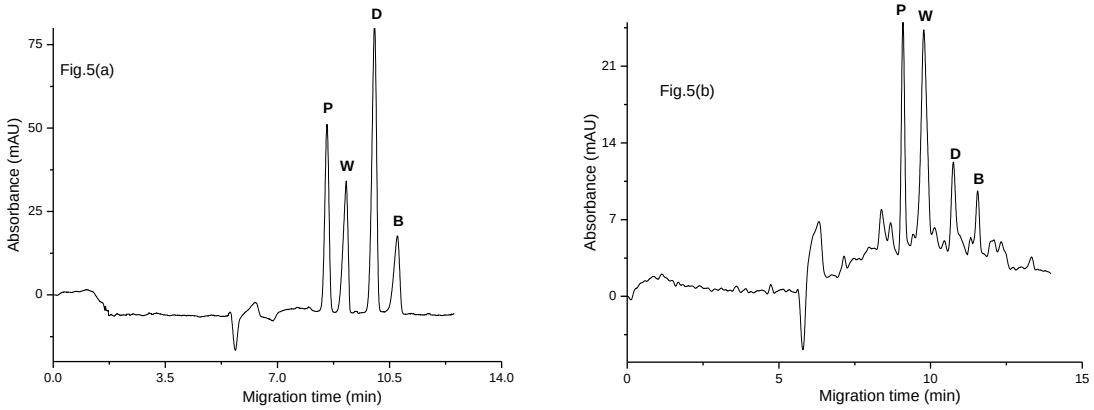
According to the previous experimental results, the best separation conditions were 8% acetonitrile + 4% microemulsion + 20 mM borax solution as running buffer solution; separation voltage 20 kV, capillary temperature 20 ℃. The electropherogram of the standard mixtures obtained in the best experimental conditions is shown in Fig. 5(a).

Regression Equation, Reproducibility and Detection Limit

In the optimal separation conditions, the linear range, standard curve and detection limit of each analyte were listed in Table 1. The detection limit was determined with a signal-to-noise ratio equal to 3. The relative standard deviations (RSD) of migration time and peak height obtained by running the standard solution for 5 consecutive times were respectively 1.4% and 2.1% (P), 1.4% and 2.3% (D), 1.4% and 2.1% (B), 0.14% and 2.6% (W).

Application

In the best experimental conditions, the actual sample (Gegen Qinlian pellets) was analyzed, and the electropherogram was shown in Fig. 5 (b). By comparing the migration time and the standard addition method, the peaks of the three components in the actual sample electropherogram were identified. The content of each component in the sample was listed in Table 2. In this paper, the standard addition method was used to determine the recovery rates of puerarin, daidzein, baicalin, and wogonin. The results were listed in Table-2.



(a) the standard mixture; (b) the actual sample (Gegen Qinlian pellets) extracts.
Peaks: P=puerarin, W=wogonin, D=daidzein and B=baicalin. Peaks were identified by standard addition method.
Buffer: 8% acetonitrile + 20 mM borax + 4% microemulsion.
Applied voltage: 20 kV.
Other experimental condition are the same as Fig.2.

Fig. 5: The Electropherograms of the standard mixtures and the actual sample (Gegen Qinlian pellets) extracts.

Table-1: The Regression Data and Detection Limits.

Compound	Regression equation [a)]	Correlation coefficient	Linear range ($\mu g.ml^{-1}$)	LOD [b)] ($\mu g.ml^{-1}$)
baicalin	**Y=-1.402+274.4X**	**0.997**	**5.9－285.0**	**0.8**
wogonin	**Y=0.923+197.5X**	**0.997**	**9.1－217.5**	**1.2**
daidzein	**Y=-0.965+421.1X**	**0.998**	**5.3－255.0**	**0.6**
puerarin	**Y=-0.663+164.0X**	**0.999**	**4.9－235.0**	**0.5**

a) Y and X were the peak height and concentration ($\mu g.ml^{-1}$) of the analytes, respectively.
b) The limit of detection (LOD) was obtained based on the signal-to-noise ration of 3.

Table-2: The Content and Recovery Rate of Each Component in the Actual Samples.

Compound	Content ($mg.g^{-1}$)	Recovery (%)
baicalin	**0.06**	**106.3**
wogonin	**0.0401**	**97.2**
daidzein	**0.0321**	**105.6**
puerarin	**0.0502**	**94.6**

Conclusion

The new microemulsion capillary electrophoresis method established in this paper has been successfully used for the separation and determination of puerarin, daidzein, wogonin and baicalin in Pueraria lobata Qinlian pellets. The method provided in this article is simple and reproducible, and is expected to be a quality control method for Chinese herbal medicines and their compound preparations containing these four ingredients.

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