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Short communication

Determination of four polyphenolic active ingredients from a pharmaceutical preparation by capillary zone electrophoresis with amperometric detection

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ABSTRACT

The simultaneous determination of four active ingredients in Nao Xue Shuan Tablets, including ferulic acid, protocatechuic aldehyde, caffeic acid and protocatechuic acid was performed by capillary zone electrophoresis with amperometric detection (CZE-AD). The effects of working electrode potential, pH and concentration of running buffer, separation voltage and injection time on CZE-AD were investigated. Under the optimum conditions, the four analytes could be perfectly separated within 18 min. A 300 μm diameter carbon-disc electrode had a good response at +0.95 V (vs. SCE) for the four analytes. The response was linear over three orders of magnitude with detection limits (S/N = 3) as low as 10^{-8} or 10^{-9} g/mL for the analytes. The assay results were satisfactory with recoveries in the range of 85.2–93.0% and RSDs less than 3.3%.

1. Introduction

Nao Xue Shuan Tablets, which is a kind of compound Chinese medicinal prescription preparations embodied by Drug Standards, Issued by Ministry of Public Health of P.R. China, is an effective remedy for cerebral thrombosis [1]. In recent years, it has gradually become routine and even standard for the treatment of cerebral thrombosis in China. However, there are few reports for the quality control of this drug [2,3]. Therefore, it's of great significance to establish a reliable method to monitor it.

Recently, ferulic acid (FA), protocatechuic aldehyde (PAH), caffeic acid (CA) and protocatechuic acid (PA), which are four main bioactive ingredients in Nao Xue Shuan Tablets, have attracted more and more attention owing to their broad physiological activities [4]. So far, some methods, such as HPLC [5], RP-HPLC [6], GC [7] and TLC [8] have been reported for the determination of these ingredients. Nevertheless, every method has its shortcomings, such as time-consuming, easy contamination and lower sensitivity, etc. Due to its advantages in high speed, high separation efficiency and good reproducibility, capillary electrophoresis is becoming increasingly recognized as an important analytical separation technique. Capillary electrophoresis with UV–vis [9], chemiluminescence [10] and amperometric [11,12] detection has been employed for the detection of these ingredients.

Because FA, PAH, CA and PA were all electroactive, we developed a reliable method of capillary zone electrophoresis with amperometric detection (CZE-AD) to determine these four ingredients in Nao Xue Shuan Tablets. Due to the fact that only electroactive constituents could be detected, the interferences of the coexistent inactive substances were eliminated. In combination with the amperometric detection, the CZE offered high sensitivity and good selectivity for the analytes. This method could also be applied to the quality control of relative samples.

2. Experimental

2.1. Apparatus

In this work, a laboratory-built capillary electrophoresis system with a wall-jet amperometric detection was employed, as described previously [11,13]. The apparatus, the carbon-disc electrode and the capillary were prepared exactly the same as described in our former work [11].

2.2. Reagents

Ferulic acid, protocatechuic aldehyde, caffeic acid and protocatechuic acid were purchased from Shanghai Institute for Drug Control (Shanghai, China). The stock standard solutions of the analytes were prepared with ethanol and diluted with running buffer to the needed concentration in CZE experiments. After filtered through a 0.22 μm syringe filter and sonicated for 5 min to remove bubbles, all solutions could be injected directly to the CZE-AD system

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for analysis. Before use, all solutions were stored in the dark at 4 °C.

2.3. Sample preparation

Nao Xue Shuan Tablets were purchased from a drugstore in Shanghai (Shanghai, China). One gram of dried Nao Xue Shuan Tablets was ground into powder and accurately weighed. The weighed sample was extracted with 20 mL anhydrous methanol-distilled water (4:1, v/v) for 30 min in an ultrasonic bath. Then, the extract was filtered through a 0.22 μm syringe filter and the filter was rinsed with the same solution for three times. Next, a total of extracted solutions were diluted with the same solution to 25 mL. Before use, all sample solutions were stored in the dark at 4 °C.

3. Results and discussion

3.1. Effect of the applied potential

The potential applied to the working electrode, directly affects the sensitivity, detection limits and stability. Fig. 1 illustrated the hydrodynamic voltammetry. The applied potential to the working electrode was maintained at +0.95 V (vs. SCE).

3.2. Effects of the pH value and buffer concentration

Borate buffer was employed as the running buffer. The pH dependence of the migration time was investigated in the pH range of 7.2–8.2, and good separation of the analytes could be achieved at pH 7.6 (1.76 mL 0.05 M borax and 10 mL solution with 0.2 M boric acid and 0.05 M sodium chloride were mixed together, and diluted to 100 mL).

Besides pH value, the concentration of the running buffer which affects peak height and theoretical plate number is also an important parameter. The effect of the running buffer concentration on the migration time was studied, and the optimum running buffer concentration was 20 mM (take boric acid as standard).

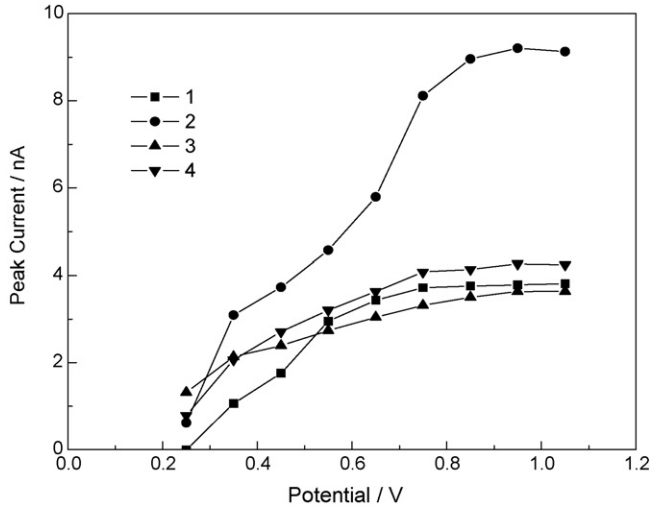

Fig. 1. Hydrodynamic voltammograms (HDV) for 1 = ferulic acid, 2 = protocatechuic aldehyde, 3 = caffeic acid, 4 = protocatechuic acid in CE. Experimental conditions: Fused-silica capillary: 25 μm i.d. 75 cm; working electrode: 300 μm diameter carbon disk electrode; running buffer: 20 mM borate buffer (pH 7.6); separation voltage: 18 kV; electrokinetic injection time: 8 s (18 kV); concentration: 5.0×10^{-6} g/mL for ferulic acid and protocatechuic aldehyde; 1.0×10^{-5} g/mL for caffeic acid and protocatechuic acid.

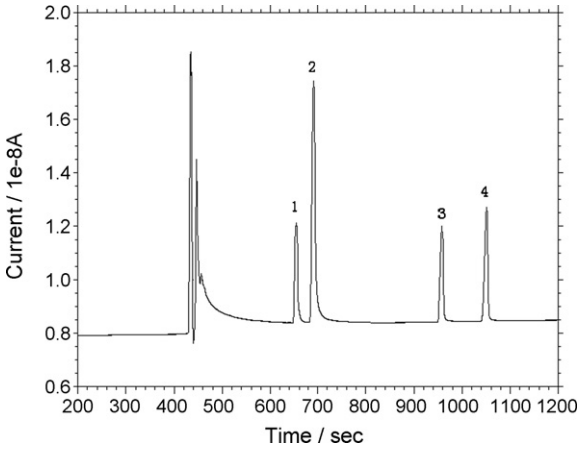

Fig. 2. The electropherogram of the standard mixture solution (5.0×10^{-6} g/mL for ferulic acid and protocatechuic aldehyde, 1.0×10^{-5} g/mL for caffeic acid and protocatechuic acid). Working potential: +0.95 V (vs. SCE); other conditions are the same as in Fig. 1. Peak identification: 1 = ferulic acid, 2 = protocatechuic aldehyde, 3 = caffeic acid, 4 = protocatechuic acid.

3.3. Effect of separation voltage and injection time

For a given capillary length, the separation voltage determines the electric field strength, which affects both the velocity of electroosmotic flow and the migration velocity of the analytes, which in turn determines the migration time of the analytes. In this work, the separation voltage selected was 18 kV.

The injection time determines the amount of sampling and affects both the peak current and the peak shape. The effect of injection time on the peak current was studied and 8 s was selected as the optimum injection time.

Through the experiments above, the optimum conditions for ferulic acid, protocatechuic aldehyde, caffeic acid and protocatechuic acid were validated. A 20 mM borate buffer (pH 7.6) was used as the running buffer at a separation voltage of 18 kV. The potential applied to the working electrode was +0.95 V (vs. SCE). Samples were injected electrokinetically at 18 kV for 8 s. The typical electropherogram for the standard solution of the analytes was shown in Fig. 2 and a perfect separation could be achieved within 18 min.

4. Method validation

4.1. Stability and reproducibility

The stability of the standard and the sample solutions was determined by monitoring the peak currents of the standard mixture solutions and the sample solutions over a period of 1 day. The results showed that the peak current and the migration time of the analytes were almost unchanged(RSDs < 3.4%) and no significant degradation was observed within the given period, indicating that the solutions were stable for at least 24 h.

The reproducibility of the peak current and the migration time was estimated by making repetitive injections of a standard mixture solution under the optimum conditions. The RSDs of the peak current and the migration time were respectively 2.6% and 1.8% for ferulic acid, 2.9% and 2.1% for protocatechuic aldehyde, 2.3% and 1.9% for caffeic acid, 3.2% and 2.4% for protocatechuic acid ($n = 7$).

4.2. Linearity and limit of detection

To determine the linearity of peak area with concentration for the four analytes, a series of mixed standard solutions was tested. The calibration curves exhibited excellent linear behavior over

Table 1
Results of regression analysis on calibration and detection limits

Compound	Regression equation[a]	Correlation coefficient	Linear range (μg/mL)	Detection limits[b] (μg/mL)
Ferulic acid	$y = 5832.2x - 0.1476$	0.9992	0.05–5.0	0.01
Protocatechuic aldehyde	$y = 14225x - 0.8327$	0.9995	0.05–5.0	0.006
Caffeic acid	$y = 2573.4x - 0.0869$	0.9996	0.1–10.0	0.03
Protocatechuic acid	$y = 3147.3x + 0.144$	0.9994	0.1–10.0	0.03

[a] y and x are the peak area (nQ) and concentration (mg/ml) of the analytes, respectively.
[b] The detection limits are corresponding to concentrations giving signal to noise ratio of 3.

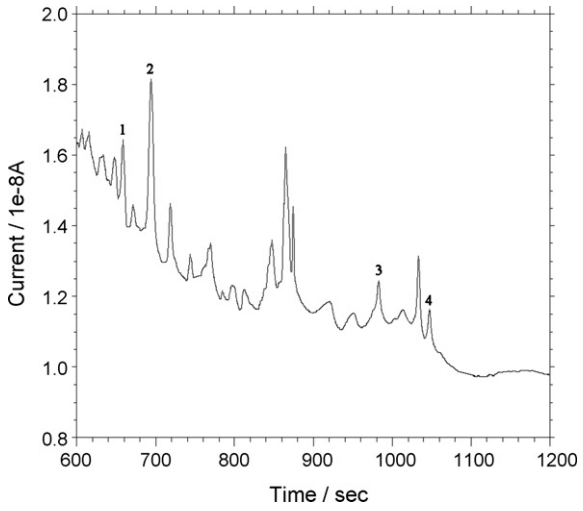

Fig. 3. The typical electropherogram of Nao Xue Shuan Tablets. Peak identification: 1 = ferulic acid, 2 = protocatechuic aldehyde, 3 = caffeic acid, 4 = protocatechuic acid.

Table 2
Assay results for Nao Xue Shuan Tablets from three different manufacturers ($n = 3$)

Sample	Ingredients	Found (μg/mL)	RSD (%)
A	Ferulic acid	1.86	2.4
	Protocatechuic aldehyde	2.27	1.8
	Caffeic acid	1.99	2.7
	Protocatechuic acid	1.63	2.9
B	Ferulic acid	1.97	2.5
	Protocatechuic aldehyde	2.23	2.7
	Caffeic acid	1.81	2.7
	Protocatechuic acid	2.34	3.0
C	Ferulic acid	0.87	2.8
	Protocatechuic aldehyde	1.86	2.1
	Caffeic acid	1.94	2.6
	Protocatechuic acid	2.55	3.2

three orders of magnitude with detection limits (S/N = 3) ranging from 0.006 to 0.03 μg/mL for the analytes. The results of regression analysis on the calibration curves and the detection limits were listed in Table 1.

4.3. Sample analysis and recovery

Under the optimum conditions, determination of ferulic acid, protocatechuic aldehyde, caffeic acid and protocatechuic acid in Nao Xue Shuan Tablets was carried out. The typical electropherogram obtained from Nao Xue Shuan Tablets was shown in Fig. 3. By adding the standard solutions into the actual samples respectively, the four ingredients could be qualitatively determined, and the results were listed in Table 2.

The recovery and reproducibility were also determined to evaluate the precision and accuracy of this method. The average recoveries and RSDs were respectively 86.0% and 3.3% for ferulic acid, 93.0% and 2.9% for protocatechuic aldehyde, 85.2% and 2.3% for caffeic acid, 89.2% and 3.1% for protocatechuic acid ($n = 3$).

5. Concluding remarks

In this work, capillary zone electrophoresis with amperometric detection was applied for the simultaneous determination of four active ingredients in Nao Xue Shuan Tablets. The result was satisfactory with LODs (S/N = 3) ranging from 0.006 to 0.03 μg/mL for the analytes and RSDs less than 3.2%. The results demonstrated that this method was accurate, sensitive and reproducible, and it could be applied to the quality control of practical samples.

Acknowledgement

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