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Short communication

Determination of active ingredients in Huangdan Yinchen Keli by CZE with amperometric detection

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Abstract

A simple, reliable and reproducible method, based on capillary zone electrophoresis with amperometric detection (CZE-AD), has been developed for simultaneous determination of five active ingredients in complicated traditional Chinese medicines including chlorogenic acid, caffeic acid, aloe-emodin, emodin and rhein. A carbon-disk electrode was used as working electrode. The optimal conditions of CZE detection were 30 mM borate solution (pH 9.5) as running buffer, 18 kV as separation voltage and 1.00 V (versus Ag/AgCl) as detection potential. There was excellent linearity between current response and analyte concentration over two orders of magnitude, while the limits of detection were 1.1×10^{-7}, 3.0×10^{-7}, 1.5×10^{-7}, 2.9×10^{-7}, 1.4×10^{-6} mol/l for aloe-emodin, emodin, rhein, chlorogenic acid and caffeic acid, respectively ($S/N = 3$). The utility of this method was demonstrated by monitoring a kind of complicated Chinese medicine named Huangdan Yinchen Keli. Two samples manufactured by different companies were monitored with satisfactory results.

Keywords: Capillary electrophoresis; Amperometric detection; Chlorogenic acid; Caffeic acid; Aloe-emodin; Emodin; Rhein

1. Introduction

It is well known that compound Chinese herb medicines usually consist of several herbs and each has many active ingredients, which has made it a hard work for the quantitative analysis and quality control. Although there have been many investigations focused on determining traditional Chinese medicines, we still have much to do in the field of assay for active ingredients in complicated preparations, which have broader range of therapeutic applications. It is necessary to develop more sensitive, more simple, and efficient methods for the determination of active ingredients in traditional Chinese medicines.

Chlorogenic acid and caffeic acid are the main active ingredients in many traditional Chinese herbs such as herba artemisiae, Taraxacum sinicum Kitag, Polygonum aviculare L., etc. Some related investigations [1,2] show that chlorogenic acid and caffeic acid have many physiological activities such as anti-inflammatory, anti-bacteria, antioxidative and other protective effects. Rhein, emodin and aloe-emodin are the active ingredients of Polygonum multiforum Thunb, Rhubarb Root, Cassia Tora Seed, etc. They also possess anti-bacteria, lapactic, hypoten-

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sive, blood-lipid lowering functions. Herbalist doctors always use these two kinds of herbs together in order to clear away the heat and aurigo. An ancient Chinese medical book named '*Shanghanlun*' provided a prescription which mainly included herba artemisiae and Rhubarb Root for the treating of hepatitis. At present time, there are still widely clinical applications of complicated medicines made of these herbs. Huangdan Yinchen Keli is one of the most widely used in Chinese medical market that mainly consist of these two herbs.

Chlorogenic acid has been determined by high-performance liquid chromatograph [3–7], chemiluminescence detection [8,9], thin-layer chromatograph [10–13]. Capillary Electrophoresis (CE) with UV-Vis detector has also been employed for the detection of it [14,15]. Rhein, emodin and aloe-emodin were determined by Sheu and Chen [16] using micellar electrokinetic capillary electrophoresis with UV-Vis method in Chinese herbal medicines [16], but the detection limits were only as low as 1×10^{-5} mol/l. Yang and Wang have also used reversed-phase high performance liquid chromatography to determine aloe-emodin and emodin in Cassia Tora Seed [17]. Compared to above methods, CZE-AD performs the merits of both CZE and AD with higher sensitivity and better selectivity. In this paper, the simple, sensitive, reliable and efficient CZE-AD method is described for the simultaneous determination of chlorogenic acid, caffeic acid, aloe-emodin, emodin and rhein in a kind of complicated Chinese medicine named Huangdan Yinchen Keli. They could be perfectly separated within 18 min and the detection limits were ranged from 1.1×10^{-7} to 1.4×10^{-6} mol/l for all compounds.

2. Experimental

2.1. Reagents and solutions

The standard samples of chlorogenic acid, caffeic acid, emodin, rhein, aloe-emodin (their structures showed in Fig. 1) were provided by National Institute for the Control of Pharmaceuticals and Biological Products (Beijing, PR China). All aqueous solutions were made up in doubly distilled water. Other chemicals were of analytical grade.

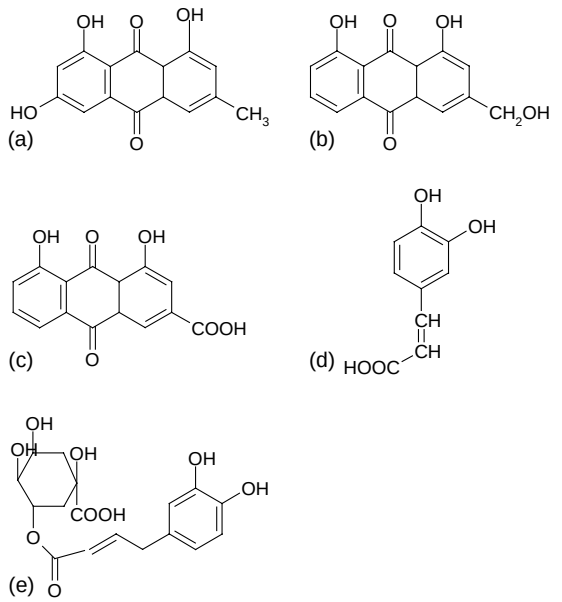

Fig. 1. Molecular structures of emodin (a), eloe-emodin (b), rhein (c), caffeic acid (d), and chlorogenic acid (e).

Stock solutions of emodin, rhein and aloe-emodin (1×10^{-4} mol/l) were prepared in 1:9 (v/v) 0.1 mol/l NaOH–0.03 mol/l borate buffer (BB, pH 9.5) mixture and diluted to the desired concentration with the running buffer just prior to use. The solutions were kept at 4 °C in a refrigerator and were stable for at least 1 month. NaOH in the stock solutions was used to enhance the solubility of the investigated analytes. Accurately weighed chlorogenic acid and caffeic acid were carefully dissolved with ethanol (1×10^{-4} mol/l) and diluted to the desired concentration with the running buffer just prior to use.

2.2. Apparatus

The CZE system with wall-jet amperometric detection assembly was constructed in the laboratory and was similar to that described previously [18,19]. Electrophoresis in the capillary was driven by a ±30 kV high-voltage supplier (Shanghai Institute of Nuclear Research, China). The separations were proceeded in a 70 cm long, o.d. 360 μm, i.d. 25 μm, polyimide-coated fused silica capillary (Polymicro Technologies, Phoenix, AZ). The injector electrode was kept at high positive voltage, the electrochem-

ical cell for detection was kept at ground and samples were all injected electro-kinetically, applying 18 kV for 10 s. Preparations of carbon working electrodes were the same as that described previously [19]. A three-electrode system, which consisted of a carbon disk working electrode (300 μm), an Ag/AgCl (3 mol/l) reference electrode and a platinum wire counter electrode, were used in this experiment.

2.3. Sample preparation

Two samples of Huangdan Yinchen Keli were respectively obtained from Tianmushan Pharmaceutical Ltd., Zhejiang, China (sample 1) and Jing'an Pharmaceutical Ltd., Shanghai, China (sample 2). Accurate weights of the powder (2.406 g for the sample 1 and 1.403 g for the sample 2) were leached with 15 ml ethanol for 24 h, respectively. Then the mixtures were filtered through a paper filter and the residues were washed twice with 10 ml ethanol. The extracts and washings were combined and then diluted to 50 ml in a volumetric flask with ethanol. The extracts were further diluted with ethanol and NaOH prior to their analysis. Peak identification was performed by standard addition method.

2.4. Procedures

CZE was performed at the separation voltage of 18 kV with a 30 mM borate buffer (pH 9.5) used as running buffer. The potential applied to the working electrode was 1.00 V (versus Ag/AgCl). Samples were electrokinetically injected at 18 kV for 10 s. Moreover, sample solutions, standard solutions and the running buffer were all filtered through an injection cellulose acetate filter (0.22 μm) prior to their use.

3. Results and discussion

3.1. Effect of potential applied to working electrode

Since all the five ingredients can be electrochemically oxidized at a relatively moderate potential, electrochemical detection was used in this work. The hydrodynamic voltammograms (HDVs) of chlorogenic acid, caffeic acid, aloe-emodin, emodin and rhein in separation medium as 30 mM borate buffer were illustrated in Fig. 2. When the applied potential exceeded +0.75 V (versus Ag/AgCl), the current responses of all analytes increased rapidly; when the potential surpassed +1.00 V (versus Ag/AgCl), the current responses increase much more slowly. Although an applied potential greater than +1.00 V (versus Ag/AgCl) resulted in larger peak currents, the baseline noise increased strongly. Thus, the applied potential of the working electrode was maintained at +1.00 V (versus Ag/AgCl), where the *S*/*N* ratio is the highest. The working electrodes showed good stability and high reproducibility at the optimum potential for more than 4 weeks.

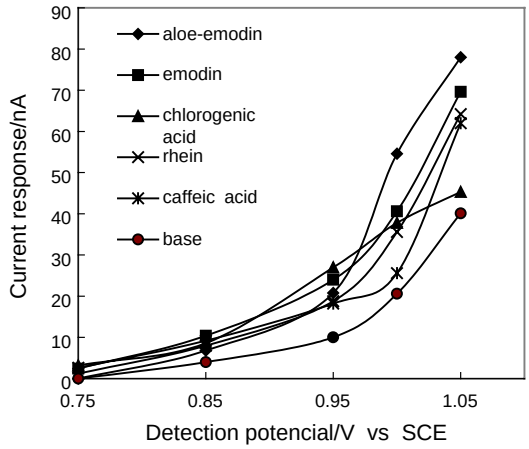

Fig. 2. The hydrodynamic voltammograms of the five standard samples. Fused-silica capillary: 25 μm i.d. × 70 cm; working electrode: 300 μm carbon disk electrode; separation medium: 30 mM borate buffer solution (pH 9.5); separation voltage: 18 kV; injection: 18 kV × 10 s.

3.2. Effects of pH and concentration of running buffer

In order to improve resolution and sensitivity, borate buffer and phosphate buffer (PB) were respectively employed in this study, and the former got better results. The dependence of the current responses of the five ingredients on pH was studied in the pH 8.00–10.00. The experimental result showed that in the pH 8.0–9.0, the response of aloe-emodin was poor, while in the range of 9.3–9.7, the five analytes

can be well separated within a relatively short time, so pH 9.5 was chosen. The effect of the running solution concentration was also examined with the borate buffer pH as 9.5. The results indicated that with the increase of the concentration of borate buffer, the peak currents of the analytes increased and the peak shapes changed from flat to sharp. The migration time increased too. This is because the ionic strength of borate buffer increases, which will result in the decrease in electroosmotic flow in the capillary. So the migration time was prolonged. The electric current in the capillary also increased with the increase of the concentration of borate buffer, which causes the Joule heating [20]. The peak exhibited a significant broadening. In our experiments, 30 mM borate buffer was chosen as the running solution.

3.3. Effect of separation voltage and sampling time

The dependence of the migration velocities of the analytes on the applied potential was examined for various voltages. The results showed that with the increase of the separation voltage, the migration time decreased, the peak shape became sharper and the electric current in the capillary increased. When the current in the capillary is too high, the peak shape will broaden too much. In order to obtain higher separation efficiency and to save analysis time, 18 kV was used as the separation voltage.

The amount of electrokinetic sampling was also tested by changing the sampling time for 5, 8, 10, 12, 15 s at 18 kV. The result showed that the peak current was relatively higher as injection time was longer. When the injection time was more than 10 s, the height of the peak current changed slowly, but the peak exhibited a significant broadening. In our experiments, 10 s was chosen as the sampling time.

Through the experiments above, the optimum conditions for determining emodin, aloe-emodin, rhein, chlorogenic acid and caffeic acid were detection potential 1.00 V (versus Ag/AgCl, 3 mol/l), separation voltage 18 kV, electrokinetic sampling time 10 s at 18 kV and 30 mM borate buffer (pH 9.5). The typical electropherograms for a standard mixture solution are shown in Fig. 3. A base-lined separation for all the five analytes can be achieved within 18 min.

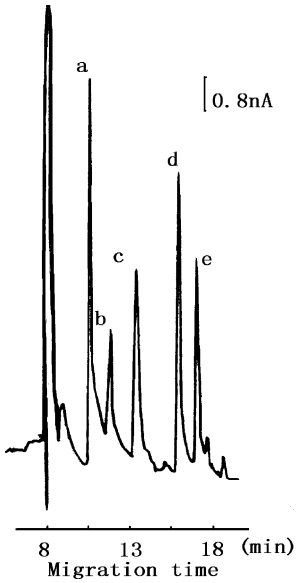

Fig. 3. Typical electropherograms of the standard mixture, obtained under the optimum conditions. (a) Aloe-emodin (2.2×10^{-5} mol/l); (b) emodin (7.7×10^{-5} mol/l); (c) chlorogenic acid (3.1×10^{-5} mol/l); (d) rhein (5.5×10^{-5} mol/l); and (e) caffeic acid (3.0×10^{-4} mol/l). Working potential: +1.00 V (vs. Ag/AgCl); other conditions as same as in Fig. 2.

3.4. Reproducibility, linearity and detection limit of aloe-emodin, emodin, rhein, caffeic acid and chlorogenic acid

A standard mixture solution of 2.2×10^{-5} mol/l aloe-emodin, 7.7×10^{-5} mol/l emodin, 5.5×10^{-5} mol/l rhein, 3.1×10^{-5} mol/l chlorogenic acid and 3.0×10^{-4} mol/l caffeic acid were analyzed for five times to determine the reproducibility of the current response and migration time under the optimum conditions in this experiment. The relative standard deviations (R.S.D.s) of the peak currents and migration time were 0.71 and 1.05% for aloe-emodin, 2.96 and 2.36% for emodin, 2.66 and 3.12% for rhein, 5.73 and 3.54% for chlorogenic acid, 1.79 and 2.68% for caffeic acid.

A series of the standard mixture solutions of these five analytes were tested to determine the linearity between the analyte concentration and current response. The results of regression analysis on calibration curves and detection limits are presented in Table 1. The detection limits were evaluated

Table 1
The regression equations and detection limits

Analyte	Regression equation C (mol/l); I (nA)	Correlating coefficient (R)	Linear range ($\times 10^{-5}$ mol/l)	Detection limit ($\times 10^{-7}$ mol/l)
Aloe-emodin	$I = 3.85 \times 10^5 C + 0.082$	0.9981	8.2–0.032	1.1
Emodin	$I = 4.18 \times 10^4 C + 0.099$	0.9993	11.3–0.12	3.0
Rhein	$I = 2.11 \times 10^5 C - 0.015$	0.9986	9.7–0.046	1.5
Chlorogenic acid	$I = 8.12 \times 10^4 C - 0.024$	0.9996	11–0.086	2.9
Caffeic acid	$I = 1.55 \times 10^4 C + 0.053$	0.9989	55–0.43	14

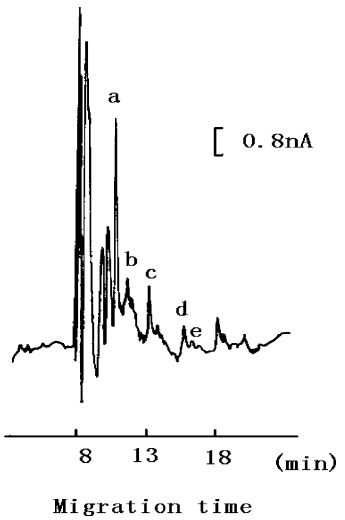

Fig. 4. Electropherograms of samples under the optimum conditions. (a) Aloe-emodin; (b) emodin; (c) chlorogenic acid; (d) rhein; and (e) caffeic acid.

on the basis of a signal-to-noise ratio of 3. The calibration curves exhibited an excellent linear behavior over the concentration range of about two orders of magnitude with the detection limits ranged from 1.1×10^{-7} to 1.4×10^{-6} mol/l for all compounds.

3.5. Application and recovery

Under the optimum conditions, CZE-ED was applied for the determination of aloe-emodin, emodin, rhein, chlorogenic acid and caffeic acid in two samples of compound Chinese medicines from different companies according to the procedures described above. Typical electropherograms of the diluted extracts are shown in Fig. 4. The two samples from different companies have similar electropherograms, which shows that they have similar ingredients. The assay results are summarized in Table 2.

Accurate amounts of the five ingredients were added to the diluted extract of sample 1 solutions, and the recovery values were obtained using their peak currents from the calibration curves under the same conditions. The average recoveries are 96.3, 105.9, 100.8, 90.9 and 97.2% for aloe-emodin, emodin, chlorogenic acid, rhein and caffeic acid, respectively (with the R.S.D.s for them 3.6, 4.3, 4.7, 5.1, and 3.4%, respectively, $n = 5$). The results indicated that this method was reliable, accurate and reproducible for all analytes.

4. Conclusion

The experimental results showed that CZE-AD method was practical for simultaneous determination

Table 2
The concentrations of the analytes in the preparations (mg/g) ($n = 5$)[a]

Sample	Aloe-emodin	Emodin	Rhein	Chlorogenic acid	Caffeic acid
Sample 1	0.52 (5.6)[b]	1.6 (1.8)	0.41 (4.3)	0.41 (2.5)	0.65 (4.6)
Sample 2	0.36 (4.2)	1.0 (1.2)	0.25 (5.1)	0.31 (2.6)	0.34 (3.7)

[a] Working potential is 1.00 V (vs. Ag/AgCl), other conditions are the same as Fig. 2.
[b] The data in the brackets refer to the R.S.D. (%).

of aloe-emodin, emodin, rhein, chlorogenic acid, caffeic acid. This method is easy to manipulate and can be applicable to determination and quality control for relative medicines.

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