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Short communication

Simultaneous determination of pharmacologically active ingredients in *Rhodiola* by capillary chromatography with electrochemical detection

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Abstract

Rhodiola, in which there are abundant pharmacologically active ingredients, is one of the functional adaptogenic agent that aid specific bodily functions to adapt to the changes and stress of life in addition to being tonic. In an attempt to qualitatively and quantitatively determine the pharmacologically active ingredients in *Rhodiola*, a new method based on capillary electrophoresis with electrochemical detection (CE–ED) has been developed. The effects of working electrode potential, pH and concentration of running buffer, separation voltage, applied potential and injection time on CE–ED were investigated. Under the optimum conditions, the analytes could be well separated within 24 min at the separation voltage of 18 kV in a 80 mmol L^{-1} borax running buffer (pH 9.0). Good linear relationship was established between peak current and concentration of analytes over two orders of magnitude with detection limits (S/N = 3) ranged from 3.16×10^{-7} to 1.11×10^{-7} g mL^{-1} for all target ingredients. This proposed method has been successfully applied for the analysis of real samples, with satisfactory results.

Keywords: Capillary electrophoresis; Electrochemical detection; *Rhodiola*; Pharmacologically active ingredients

1. Introduction

People are becoming increasingly interested in traditional Chinese medicines because of their low toxicity and good therapeutic performance. It has been reported that 90% of the world's population uses some sorts of traditional Chinese medicines and medicinal plants [1].

Rhodiola, one of the most popular traditional Chinese medicines, mainly distributes in north atmosphere with about 90 species recorded in the world and above 70 species found in China [2]. Because of its potent biological activity [3–14], *Rhodiola* has been widely used to increase physical endurance, work productivity, longevity and to treat fatigue, asthma, haemorrhage, anaemia, impotence and nervous system disorders for a long time in China, Serbia, Mongolia, Russia, etc. [14,15].

Chemical constituent investigations show that not only salidroside but also catechol, ferulic acid, salicylic acid, quercetin, 4-hydroxybenzoic acid, caffeic acid, gallic acid and 3,4-dihydroxybenzoic acid are the important bioactive constituents of *Rhodiola* [12,13,16–19]. Many methods have been reported for the determination of the salidroside in recent years [19–28]. However, currently available methods concerning the determination of catechol, ferulic acid, salicylic acid, quercetin, 4-hydroxybenzoic acid, caffeic acid, gallic acid and 3,4-dihydroxybenzoic acid in *Rhodiola* are quite limited [27], especially, the method for the simultaneous determination of multiple biologically active constituents in *Rhodiola* has not been reported. Hence, it is necessary to develop some simple, economical and efficient methods for the analysis of multiple potentially biological active constituents in *Rhodiola*.

Capillary electrophoresis (CE) is increasingly recognized as an important analytical separation technique for its speed, efficiency, reproducibility, ultra-small sample volume, and little consumption of solvent. In addition, with electrochemical

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detection (ED), CE–ED offers high sensitivity and good selectivity for electroactive analytes. In this work, a simple and rapid CE–ED method for the simultaneous determination of biologically active ingredients in *Rhodiola* has been developed for the first time, which can offer an excellent alternative method in quality control for medicinal manufacturers and the constituent analysis of plants.

2. Experimental

2.1. Apparatus

The laboratory-built CE–ED system was used in this work has been constructed and described previously [29,30].

2.2. Reagents and solutions

Catechol, ferulic acid, salicylic acid, 4-hydroxybenzoic acid, caffeic acid, gallic acid and 3,4-dihydroxybenzoic acid were all purchased from Sigma (St. Louis, MO, USA), quercetin was obtained from Shanghai Reagent Factory (Shanghai, China), and were all used as received. The root of *Rhodiola*, purchased from Crude Drug Company in Shanghai (China). Stock solutions of eight analytes (1.00×10^{-3} g mL^{-1}, each) were prepared in anhydrous ethanol (A.R. grade), and were diluted to the desired concentration with the running buffer. Before use, all solutions were filtered through 0.22 μm nylon filters.

2.3. Sample preparation

The sample was ground into powder in a mortar and accurately weighed, then extracted with 10 mL anhydrous ethanol (analytical-reagent grade) and water (4:1, v/v) for 2 h in an ultrasonic bath. Extracted sample was centrifuged by a desk centrifuge first, and then filtered through a 0.22 μm syringe filter. After filtration, the solution was injected directly to the CE–ED system for analysis. Before use, all sample solutions were stored at 4 °C in the dark.

3. Results and discussion

In electrochemical detection, the potential applied to the working electrode directly affects the sensitivity, detection limit and stability of this method. Therefore, hydrodynamic voltammetry was investigated to obtain optimum detection results. As shown in Fig. 1, the peak current of each analyte increases with the rising of applied potential. However, when applied potential is greater than +950 mV (versus SCE), although the peak current of the analytes still increases, both the baseline noise and the background current increase substantially, which is a big disadvantage for sensitive and stable detection. Therefore, the potential applied to the working electrode is maintained at +950 mV (versus SCE).

In addition, the effect of H_3BO_3–$Na_2B_4O_7$ running buffer pH on the migration time of the analytes was investigated in the pH range of 7.4–9.2. At pH 9.0, all analytes can be well separated within a relatively short time. Besides the pH value, the running buffer concentration is also an important parameter. The effect of the running buffer concentration on migration time was also studied ranging from 20 to 100 mmol L^{-1}(at pH 9.0), and the optimum running buffer concentration is 80 mmol L^{-1} (pH 9.0).

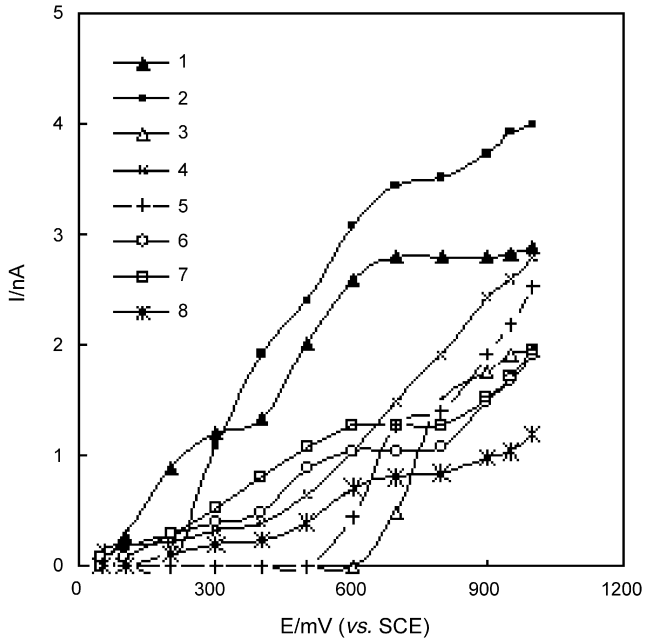

Fig. 1. Hydrodynamic voltammograms (HDVs) of catechol (1), ferulic acid (2), salicylic acid (3), quercetin (4), 4-hydroxybenzoic acid (5), caffeic acid (6), gallic acid (7) and 3,4-dihydroxybenzoic acid (8) in CE–ED. Fused-silica capillary: 25 μm i.d. 75 cm; working electrode: 300 μm diameter carbon disk electrode; running buffer: 80 mmol L^{-1} (pH 9.0); separation voltage: 18 kV; injection time: 8 s/18 kV; concentrations of analytes: 1.0×10^{-5} g mL^{-1}, each.

In addition to the running buffer and applied potential, the effect of separation voltage and injection time on CE–ED was also investigated. Under the optimum conditions, eight analytes can be well separated within 24 min at the separation voltage of 18 kV in a 75 cm length capillary with injection time of 8 s (at 18 kV), and the typical electropherogram for a standard mixture solution was shown in Fig. 2A.

To determine the linear range of CE–ED response to the eight analytes, a series of standard solutions from 1.0×10^{-6} to 1.0×10^{-4} g mL^{-1} were tested. The calibration curves exhibit very good linear behavior over the concentration range 1.0×10^{-6} to 1.0×10^{-4} g mL^{-1} for catechol, ferulic acid and quercetin, 1.0×10^{-6} to 5.0×10^{-5} g mL^{-1} for salicylic acid, 4-hydroxybenzoic acid, caffeic acid, gallic acid and 3,4-dihydroxybenzoic acid, respectively. The linear equations are: $Y = 2.86 \times 10^5 X + 1.17$ ($R = 0.9919$, catechol), $Y = 4.09 \times 10^5 X + 1.09$ ($R = 0.9916$, ferulic acid), $Y = 1.97 \times 10^5 X + 0.19$ ($R = 0.9999$, salicylic acid), $Y = 1.96 \times 10^5 X + 0.50$ ($R = 0.9921$, quercetin), $Y = 2.92 \times 10^5 X + 0.33$ ($R = 0.9993$, 4-hydroxybenzoic acid), $Y = 2.09 \times 10^5 X + 0.03$ ($R = 0.9981$, caffeic acid), $Y = 1.80 \times 10^5 X + 0.19$ ($R = 0.9988$, gallic acid), $Y = 1.07 \times 10^5 X - 0.03$ ($R = 0.9968$, 3,4-dihydroxybenzoic acid), where Y is the peak current (nA), X is the concentration of analyte (g mL^{-1}), and R is the correlation coefficient. The determination limits (S/N = 3) of the above analytes in turn are 1.20×10^{-7}, 1.11×10^{-7}, 1.62×10^{-7},

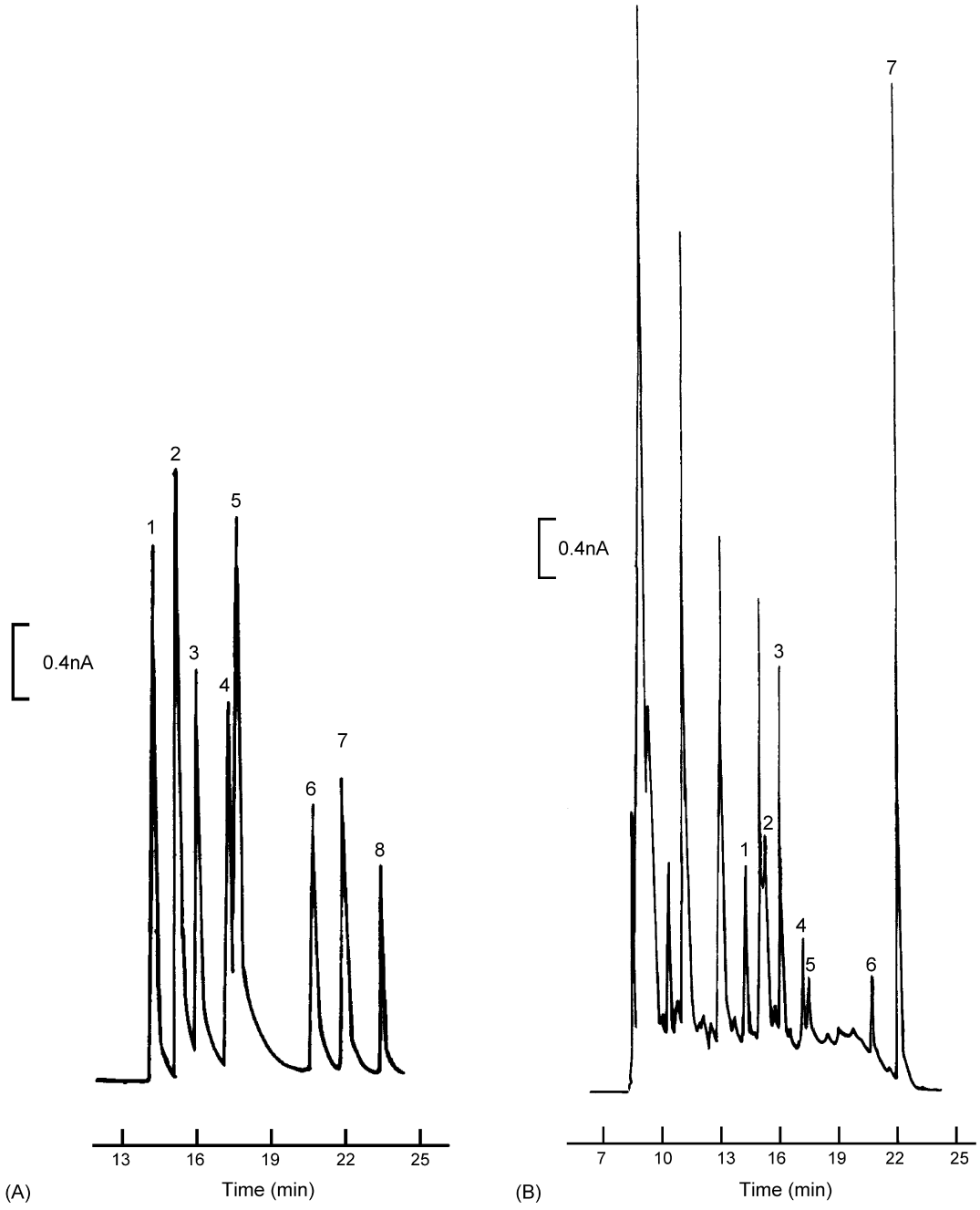

Fig. 2. The electropherograms of standard solution (A), real sample (B). Peak identification: (1) catechol; (2) ferulic acid; (3) salicylic acid; (4) quercetin; (5) 4-hydroxybenzoic acid; (6) caffeic acid; (7) gallic acid; (8) 3,4-dihydroxybenzoic acid. Experiment conditions are the same as in Fig. 1. Working electrode potential: +950 mV (vs. SCE).

1.70×10^{-7}, 1.22×10^{-7}, 2.73×10^{-7}, 2.40×10^{-7} and 3.16×10^{-7} g mL^{-1}.

The reproducibility of the peak current and migration time is estimated by making repetitive injections of a standard mixture solution (1.0×10^{-5} g mL^{-1} for each analyte) under the selected optimum conditions ($n = 7$). The relative standard derivations (RSDs) of the peak current and migration time are 3.55 and 1.12% for catechol, 1.61 and 0.83% for ferulic acid, 2.86 and 3.04% for salicylic acid, 3.74 and 3.16% for quercetin, 0.56 and 2.95% for 4-hydroxybenzoic acid, 1.79 and 1.20% for caffeic acid, 1.89 and 1.34% for gallic acid, 2.90 and 2.59% for 3,4-dihydroxybenzoic acid, respectively.

To further evaluate the precision and accuracy of the method, the recovery was determined by comparing the increase of the peak height before and after the addition of standards ($n = 3$), and the results were within 97.0–103.4%, which indicate the method is sufficiently accurate for the simultaneous determination of the above analytes.

The proposed procedure was applied for the determination of active ingredients in *Rhodiola* sample. Typical electrophero-

Table 1
Assay results for real samples ($n = 3$)

Ingredients	Found ($\mu g\ g^{-1}$)	RSD (%)
Sample: *Rhodiola*		
Catechol	423.20	2.1
Ferulic acid	103.68	4.1
Salicylic acid	1224.4	3.9
Quercetin	370.30	3.3
4-Hydroxybenzoic acid	131.66	3.1
Caffeic acid	394.98	3.7
Gallic acid	4334.4	2.3
3,4-Dihydroxybenzoic acid	N.F.[a]	

CE–ED conditions are the same as Fig. 2.
[a] N.F. refers to not found.

grams of real sample is shown in Fig. 2B. By the migration time of analytes compared with the electropherogram of the standard mixture solution in Fig. 2A (1.0×10^{-5} g mL^{-1}, each), the active ingredients in *Rhodiola* sample can be identified and determined, and 3,4-dihydroxybenzoic acid was not found in the given real sample. The assay results (Table 1) show that the method is accurate, sensitive and efficient, which can offer an excellent alternative method in quality control for medicinal manufacturers and the constituent analysis of plants.

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