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Short communication

Determination of active ingredients of *Rhododendron dauricum* L. by capillary electrophoresis with electrochemical detection

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Abstract

High-performance capillary electrophoresis with electrochemical detection was employed to analyse active ingredients of *Rhododendron dauricum* L., an important crude herb frequently used in Chinese medicines. Farrerol, quercetin, syringic acid, vanillic acid, 4-hydroxybenzoic acid, protocatechuic acid are major important active ingredients. Operated in a wall-jet configuration, a 300-μm diameter carbon-disk electrode was used as the working electrode, which exhibits a good response at +950 mV (vs. saturated calomel electrodes) for six analytes. Under the optimum conditions, the analytes were baseline separated within 16 min in a borax buffer (pH 8.7). Notably, excellent linearity was obtained over two orders of magnitude with detection limits ($S/N=3$) ranged from $9\cdot10^{-7}$ to $3.0\cdot10^{-6}$ *M* for all analytes. This method was successfully used in the analysis of *Rhododendron dauricum* L. with relatively simple extraction procedures, and the assay results were satisfactory.

Keywords: *Rhododendron dauricum* L.; Plant materials; Farrerol; Quercetin; Phenolic acids

1. Introduction

Traditional Chinese medicine has been extensively used to prevent and cure human diseases for over a millennium in oriental countries. Because of its low toxicity and good therapeutical performance, traditional Chinese medicine has attracted considerable attention in many fields [1]. Work on analysis of active ingredients of medicine herbs and search for alternative drugs has become increasingly important. However, it is often a challenging task to do so because of the diversity of compositions, the significant concentration difference of active ingredients, as well as effects of many factors such as climates, regions of growth and seasons of harvest on the contents of active ingredients in medicine herbs. Capillary electrophoresis (CE) is becoming increasingly recognised as an important analytical separation technique due to its speed, efficiency, reproducibility, ultra-small sample volume, and ease of clearing up the contaminants. In combination with electrochemical detection (ED), CE offers high sensitivity and good selectivity for electroactive species. However to our knowledge, so far this technique has not been fully explored, and its application to the analysis of *Rhododendron dauricum* L. samples has not been conducted.

Rhododendron dauricum L. is a type of medicinal herb often used to cure chronic tracheitic. Its leaves contain farrerol, quercetin, syringic acid, vanillic acid, 4-hydroxy benzoic acid and protocatechuic acid

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[2]. The molecular structures of these compounds are shown in Fig. 1. Some related investigations show that farrerol, quercetin and other flavonols have a broad range of physiological activities such as anti-inflammatory [3], anti-bacterial [4] and antioxidant activity for scavenging radicals [5] and inhibition of a variety of enzymes [6]. Pathological experiments have demonstrated that farrerol relieves coughs and moves phlegm. Farrerol has been synthesised chemically as an antibechic [7]. Syringic acid, vanillic acid, 4-hydroxybenzoic acid and protocatechuic acid are four kinds of phenolic acids, which also anti-bacterial, anti-inflammatory, anti-oxidant and styptic activities [8]. In order to estimate the quality of *Rhododendron dauricum* L., it is necessary to develop a method to assay all the constituents mentioned above, yet which is simple and reliable. It is reported that quercetin in plants [9,10] and four phenolic acids in red wine [11] have been analysed by HPLC and CE with UV detection, and farrerol in *Rhododendron dauricum* L. has been determined by TLC [12]. In this work we developed a simple and rapid method to determine six active ingredients of *Rhododendron dauricum* L. by CE–ED.

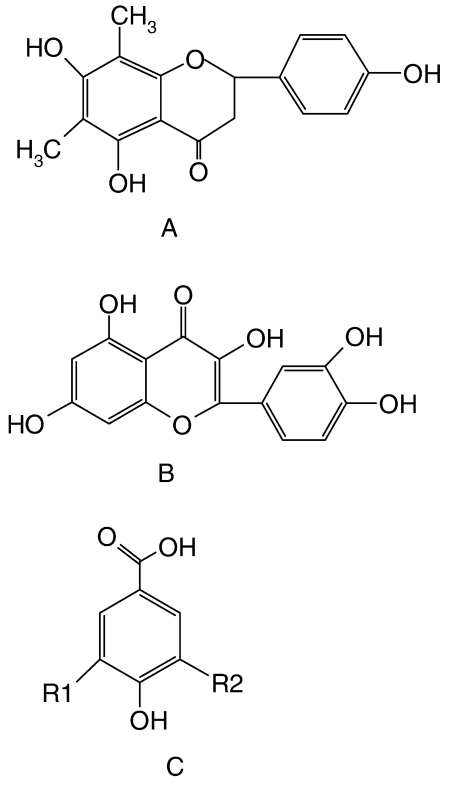

Fig. 1. Molecular structures of the analytes: (A) farrerol; (B) quercetin; (C) syringic acid (R1=R2=OCH_3), vanillic acid (R1=OCH_3, R2=H), 4-hydroxy benzoic acid (R1=R2=H), protocatechuic acid (R1=OH, R2=H).

2. Experimental

2.1. Apparatus

A laboratory-built CE–ED system [13] was employed. A 30-kV high-voltage power supply (Shanghai Institute of Nuclear Research, China) provided a voltage between the ends of the capillary. The inlet end of the capillary was held at a positive potential and the outlet end was maintained at ground. A 50 cm×25 μm I.D. fused-silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used for the separation. Samples were all injected electrokinetically, applying 14 kV for 6 s.

A carbon-disk electrode of 300 μm diameter (Shanghai Shangyuan Stationary Factory, Shanghai, China) was employed as the working electrode as described previously [14]. Before use, the surface of the carbon-disk electrode was polished with emery sand paper, sonicated in deionised water, and then positioned carefully opposite the capillary outlet with the aid of an Oriel (Stratford, CT, USA) Model 14901 micropositioner. A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode and a SCE (saturated calomel electrode) reference electrode was used in combination with a BAS LC-4C amperometric detector (Biochemical System, West Lafayette, IN, USA). The electropherograms were recorded using a chart recorder (Shanghai Dahua Instrument Factory, China).

2.2. Reagents

Farrerol was purchased from Chinese Chemicals and Biological Drugs Institute (Beijing, China), syringic acid was purchased from Merck (Darmstadt, Germany), and vanillic acid, 4-hydroxybenzoic acid, quercetin and protocatechuic acid were obtained from Shanghai Reagent Factory (Shanghai, China),

and used as received. *Rhododendron dauricum* L. leaves were purchased from a drug store in Jilin (China). Stock solutions of six analytes ($1.00 \cdot 10^{-3}$ *M* each) were prepared in methanol and were diluted to the desired concentration with the running buffer (H_3BO_3–$Na_2B_4O_7$ buffer ranging from 25 to 100 m*M* with a pH value from 7.8 to 9.0). Before use, all solutions were filtered through 0.22-μm nylon filters.

2.3. Sample preparation

About 1 g of dried *Rhododendron dauricum L.* leaves was ground into powder in a mortar, sonicated with 25 ml methanol and the running buffer (1:1) for 30 min. Next it was filtered through a filter paper, then through a 0.22-μm syringe filter, and made up to 100 ml in volume. The sample solution was stored in the dark.

3. Results and discussion

3.1. Effect of the potential applied to the working electrode

Since the phenolic hydroxy groups of six analytes can be readily oxidised electrochemically, ED was based on this feature. The potential applied to the working electrode directly affects the sensitivity and detection limit of this method. In order to obtain best detection results, hydrodynamic voltammetry was conducted to find this optimum potential. As shown in Fig. 2, when the applied potential exceeds +0.60 V (vs. SCE), all six analytes can generate oxidation current at the working electrode, and oxidation currents of analytes increase rapidly except vanillic acid and protocatechuic acid. Although an applied potential greater than +0.95 V (vs. SCE) produces even larger oxidation currents for all analytes, both the baseline noise and the background current increase very strongly, which is obviously a disadvantage for sensitive and stable detection. Therefore, an applied potential of +0.95 V (vs. SCE) was selected, where the background current is not too high, and the *S*/*N* ratio is the highest.

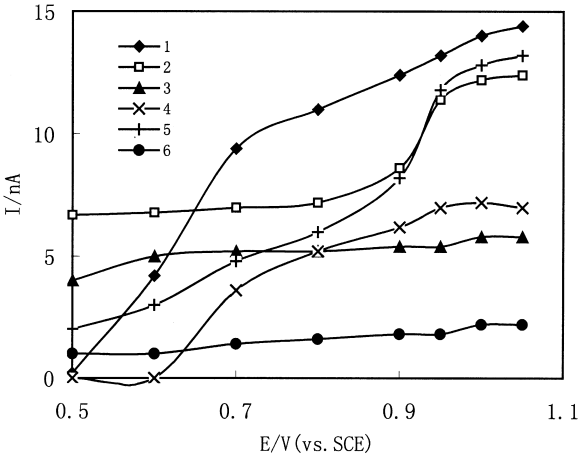

Fig. 2. Hydrodynamic voltammograms (HDVs) of farrerol (1), syringic acid (2), vanillic acid (3), 4-hydroxybenzoic acid (4), quercetin (5) and protocatechuic acid (6) in CE. Fused-silica capillary: 50 cm×25 μm I.D.; working electrode: 300 μm diameter carbon disk electrode; running buffer: 50 m*M* (pH 8.7); separation voltage: 14 kV; injection time: 14 kV/6 s; concentrations of six analytes: $1.0 \cdot 10^{-4}$ *M* each.

3.2. Effects of the pH value and the buffer concentration

Borate buffer was employed as the running buffer in this work because borate can chelate with the analytes to form more soluble complex anions [15]. The pH dependence of the migration time was investigated in the pH range of 7.8–9.0. As shown in Fig. 3, the migration time of all analytes increases with increasing pH value, baseline separation of the analytes can be achieved from pH 8.0 to 8.7. When pH is lower than 8.0, peaks are too broad to be separated completely; when pH value is smaller than 8.4, farrerol and syringic acid cannot be separated from their adjacent unknown peaks, so pH 8.7 was selected as the optimum pH value.

Besides the pH value, the running buffer concentration is also an important parameter. The effect of the running buffer concentration on migration time was studied; the optimum running buffer concentration lies between 50 and 75 m*M*.

3.3. Effects of separation voltage and injection time

The influence of the separation voltage on the

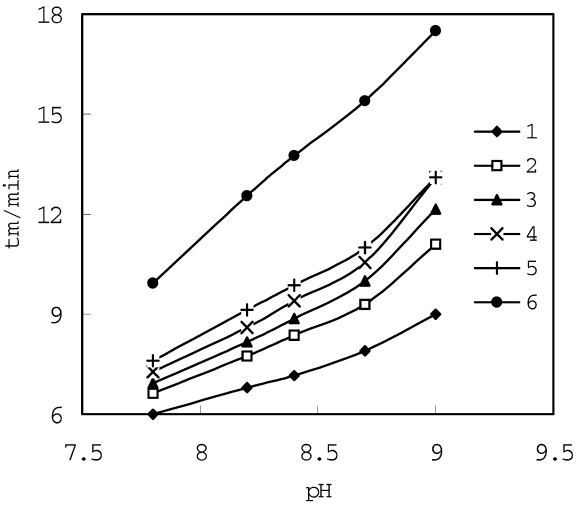

Fig. 3. Effect of pH on migration time. Working electrode potential is 0.95 V (vs. SCE); other conditions as in Fig. 2.

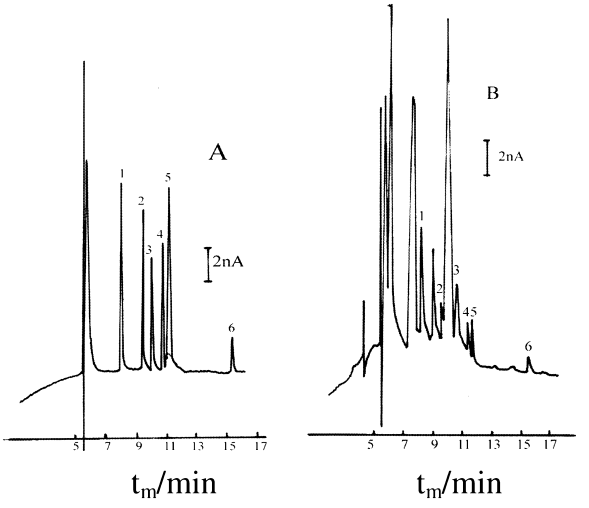

Fig. 4. Electropherograms of standard solution ($1.0\cdot10^{-4}$ *M* of each analyte) (A) and *Rhododendron dauricum* L. herb (B). Peaks: 1=farrerol, 2=syringic acid, 3=vanillic acid, 4=4-hydroxybenzoic acid, 5=quercetin, 6=protocatechuic acid. Working electrode potential is 0.95 V (vs. SCE); other conditions as in Fig. 2.

migration time of the analyte was also studied. Higher separation voltages give shorter migration time for all analytes. However, when the separation voltage exceeded 16 kV, baseline separation of 4-hydroxybenzoic acid and quercetin could not be achieved, and the baseline noise became larger. Therefore the optimum separation voltage is 14 kV, at which good separation can be obtained for all analytes within 16 min.

The injection time affects both the peak current and peak shape. The effect of injection time on peak current was studied by varying injection time from 2 to 10 s at 14 kV. When the injection time is longer than 6 s, the peak current levels off and peak broadening becomes more severe. In this experiment, 6 s (14 kV) is selected as the optimum injection time.

Through the experiments above, the optimum conditions for determining farrerol, syringic acid, vanillic acid, 4-hydroxybenzoic acid, quercetin and protocatechuic acid were decided. The typical electropherogram for a standard solution of the six analytes is shown in Fig. 4A, we can see that baseline separation can be achieved within 16 min.

3.4. Reproducibility, linearity, detection limit of the six analytes

The reproducibility of the peak currents is estimated by making repetitive injections of a standard mixture solution ($1.0\cdot10^{-4}$ *M* for each analyte) under the selected optimum conditions. The relative standard derivations (RSDs) of the peak current are 2.6, 3.0, 4.2, 3.9, 3.3 and 4.0% for farrerol, syringic acid, vanillic acid, 4-hydroxybenzoic acid, quercetin and protocatechuic acid, respectively ($n=7$).

To determine the linearity of farrerol, syringic acid, vanillic acid, 4-hydroxybenzoic acid, quercetin and protocatechuic acid, a series of standard solutions from $1.0\cdot10^{-6}$ to $1.0\cdot10^{-3}$ *M* were tested. The detection limit is evaluated on the basis of a signal-to-noise ratio of 3. The results of regression analysis on calibration curves and detection limits are summarised in Table 1.

3.5. Sample analysis and recovery

Active ingredients in *Rhododendron dauricum* L. were determined by CE–ED under the optimum conditions. A typical electropherogram of *Rhododendron dauricum* L. is shown in Fig. 4B. By comparing with the electropherogram of the standard solution (Fig. 4A), the active ingredients namely farrerol, syringic acid, vanillic acid, 4-hydroxybenzoic acid, quercetin and protocatechuic acid in *Rhododendron dauricum* L. can be determined; the contents are 148,

Table 1
Regression equations and detection limits[a]

Compound	Regression equation[b]	Correlation coefficient	Linear range (M)	Detection limit (10^{-6} M)
Farrerol	$y = 1.09 \cdot 10^5 x + 0.33$	0.9997	$1 \cdot 10^{-6}$–$5 \cdot 10^{-4}$	0.9
Syringic acid	$y = 9.61 \cdot 10^4 x - 0.08$	0.9995	$2 \cdot 10^{-6}$–$5 \cdot 10^{-4}$	1.4
Vanillic acid	$y = 7.18 \cdot 10^4 x - 0.38$	0.9990	$2 \cdot 10^{-6}$–$5 \cdot 10^{-4}$	1.5
4-Hydroxybenzoic acid	$y = 7.79 \cdot 10^4 x - 0.27$	0.9993	$2 \cdot 10^{-6}$–$5 \cdot 10^{-4}$	1.4
Quercetin	$y = 1.07 \cdot 10^5 x + 0.08$	0.9997	$2 \cdot 10^{-6}$–$5 \cdot 10^{-4}$	1.0
Protocatechuic acid	$y = 1.68 \cdot 10^4 x + 0.26$	0.9988	$5 \cdot 10^{-6}$–$5 \cdot 10^{-4}$	3.0

[a] CE–ED conditions as in Fig. 4.
[b] In the regression equations, the x value is the concentration of analytes (M), the y value is the peak current (nA).

Table 2
Determination results of recovery in this method ($n=3$)[a]

Ingredient	Amount (mg/l)			Recovery (%)	RSD (%)
	Original	Added	Found		
Farrerol	14.85	11.00	25.24	97.7	1.9
Syringic acid	4.36	7.93	12.41	101.5	4.2
Vanillic acid	7.90	6.73	14.91	104	3.9
4-Hydroxybenzoic acid	3.04	5.52	8.71	102.7	4.5
Quercetin	5.13	12.08	17.00	98.3	2.6
Protocatechuic acid	5.54	6.16	11.98	104.5	4.6

[a] CE–ED conditions as in Fig. 4.

44, 79, 30, 51 and 55 μg/g, respectively. The corresponding RSD values are 3.3, 3.1, 4.0, 4.1, 3.8 and 4.9%, respectively ($n=3$). The recovery and reproducibility experiments under the optimum conditions were also conducted to evaluate the precision and accuracy of the method. Recovery was determined by standard addition method, and the results are listed in Table 2. The above assay results indicate that this method is accurate, sensitive and reproducible, providing a useful quantitation method for the analyses of traditional Chinese medicines.

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