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Differentiation of Swertia Mussotii Franch from Artemisiae Capillaris Herba by capillary electrophoresis with electrochemical detection

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Abstract

A high-performance capillary electrophoresis (CE) with electrochemical detection (ED) method is developed for differentiation of Swertia Mussotii Franch from Artemisiae Capillaris Herba in this work. Swertia Mussotii Franch contains a great deal of swertiamarin and mangiferin that are not present in Artemisiae Capillaris Herba, whereas Artemisiae Capillaris Herba consists of abundant chlorogentic acid. Therefore, determining their swertiamarin, mangiferin and chlorogentic acid contents can differentiate these two crude herbs. Operated in a wall-jet configuration, a 300 μm diameter carbon-disk electrode was used as the working electrode, which exhibits good response at +1000 mV (versus SCE) for the three analytes. With a separation voltage of 14 kV, the three analytes were separated within 14 min in a 52 cm length capillary in 50 mmol/l borax buffer (pH 9.2). The system was demonstrated good stability and reproducibility with an R.S.D. of less than 5% for both migration time and peak current. This method was successfully used to analyze and identify the crude herbs with satisfactory assay results.

Keywords: Swertia Mussotii Franch; Artemisiae Capillaris Herba; Capillary electrophoresis; Electrochemical detection

1. Introduction

Swertia Mussotii Franch, referred to as “Yinchen” in Chinese, is an important crude drug in Tibetan medicines. It is a kind of special plant on the Qinghai–Tibet Plateau, and grows in the highland areas at an elevation of 3200–4200 m. Swertia Mussotii Franch belongs to the family of radix gentianae, the genera of Swertia [1]. Swertiamarin and mangiferin [2], as the main active ingredients isolated from Swertia Mussotii Franch have a wide range of therapeutic effects; their molecular structures are shown in Fig. 1. Pharmacological studies reveal that swertiamarin has anticonvulsant, depressant action [3] and inhibition of human DNA ligase I [4]. Mangiferin also has anti-diabetic [5], anti-cancer [6], immunomodulatory [7] and antioxidant activity [8], and inhibition of a variety of enzymes [9]. Swertia Mussotii Franch is often used to treat the febrile diseases in liver and gallbladder. Modern research has shown Swertia Mussotii Franch may promote the regeneration of the liver, repair the fibrillation of the liver, clean the deposit of fat within the liver [10] and protect liver from experimental damage by CCl_4 and TTA [11]. Compared with Swertia Mussotii Franch, Artemisiae Capillaris Herba has the same name in Chinese, and it is also commonly used to treat disease in liver and gallbladder in traditional Chinese medicines [12]. Therefore, these two medicinal herbs are easily confused with each other. Artemisiae Capillaris Herba belongs to family of Compositae, and its active ingredients contain coumarins, chromones, flavones and carboxylic acid [1]. Chlorogentic acid is customarily used as a quality control marker for Artemisiae Capillaris Herba herbs, its molecular structure illustrated in Fig. 1. Chlorogentic acid has special biological activities such as antibacterial, anti-inflammatory, antispasmotic, antioxidation and inhabition of various enzymes [13]. In China, the crude drugs were usually identified by the experts. In order to rapid and objective differentiation of these herbs, it is indispensable to establish a simple and accurate analytical method.

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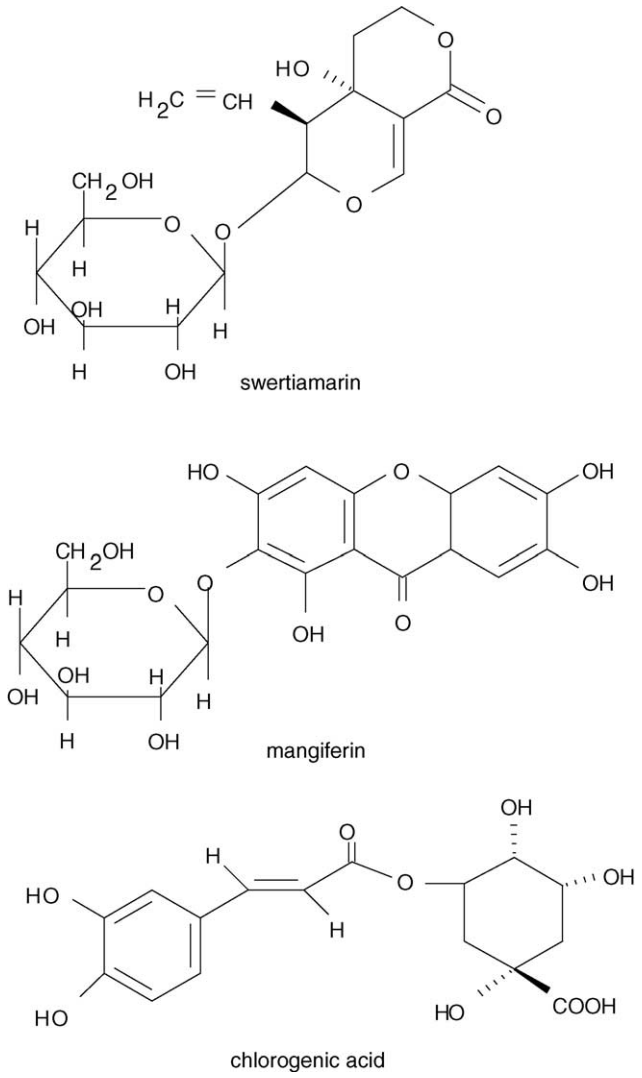

Fig. 1. Molecular structure of swertiamarin, mangiferin and chlorogenic acid.

Though Swertia Mussotii Franch and Artemisiae Capillaris Herba have the same names in Chinese and similar therapeutical function, in fact, they belong to different families, and their active ingredients are quite distinct from each other. Since Swertia Mussotii Franch contains swertiamarin and mangiferin, and Artemisiae Capillaris Herba consists of chlorogentic acid, the simplest method for differentiating between two crude drugs is to identify and determine these active constitutes. It is reported that high-performance liquid chromatography (HPLC) [14–16] and thin layer chromatography (TLC) [17,18] methods have been applied for determination of swertiamarin and mangiferin. Many kinds of methods including HPLC [19,20], capillary electrophoresis (CE) [21,22], chemiluminescence [23], flow injection (FI) [24], UV spectrometry [25], polarography [26] has also been employed to determine chlorogentic acid. At present, the commonly used method for analysis herbal medicines is HPLC. However, owing the complexity of herbal medicines, the use of HPLC is suffered from its column being easily contaminated and hard to regenerate. Compared with HPLC, CE is a developing separation technique characterized with high efficiency, short analysis time, low consumption, simple sample pretreatment, multiple modes to be chosen and ease of clearing up the contaminants in capillary. Therefore, CE is ideal for analyzing herbal medicines. In addition, with electrochemical detection (ED), CE–ED offers high sensitivity and good selectivity for electroactive species. In this work we successively developed a simple, rapid, and dependable method for differentiation of Swertia Mussotii Franch from Artemisiae Capillaris Herba by CE–ED.

2. Experimental

2.1. Apparatus

In this work, a CE–ED system was constructed, and is similar to that described previously [27]. 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 52 cm length of 25 mm i.d. and 360 mm o.d. fused silica capillary (Polymicro Technologies, Phoenix, AZ, USA) was used for the separation. Samples were all injected electrokinetically, applying 14 kV for 10 s.

A carbon-disk electrode with 300 μm diameter was employed as the working electrode. Before use, the surface of the carbon-disk electrode was polished with emery sand paper, sonicated in deionized water, and then positioned carefully opposite the capillary outlet with the aid of an Oriel Corporation (Stratford, CT, USA) Model 14901 micropositioner. A three-electrode cell system consisting of a carbon-disk working electrode, a platinum auxiliary electrode and a saturated calomel electrode (SCE) 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

Swertiamarin, mangiferin and chlorogentic acid were purchased from Chinese Chemicals and Biological Material Institute (Beijing, China), Sigma (St. Louis, MO, USA), Aldrich (Milwaukee, WI, USA), respectively, and were used as received. Swertia Mussotii Franch herbs and its medicinal preparation Dida capsules were purchased from Xining Pharmaceutical Factory (Xining, China) and Xian Tianyuan Pharmaceutical Co. (Xian, China), respectively. Artemisiae Capillaris Herba herbs were purchased from a local drug store (Wuxi, China). Stock solutions of swertiamarin (7.5×10^{-3} mol/l), mangiferin (5.00×10^{-3} mol/l) and chlorogentic acid (5.00×10^{-3} mol/l) were prepared in 50% methanol 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

2.5 g of dried Swertia Mussotii Franch and Artemisiae Capillaris Herba herbs were ground into powder and accurately weighed, respectively. Each weighed sample was soaked with 10 ml methanol for 24 h in dark, then was added 5 ml 100 mol/l running buffer and extracted for 30 min in an ultrasonic bath. The extract was then filtered through a filter paper. The extraction procedure was repeated three times. Next, a total of extracted solutions were diluted with 50 mol/l the running buffer to 25 ml in volume. To determine swertiamarin and mangiferin in sample, 10 ml-extracted solution of Swertia Mussotii Franch herbs was again diluted with 50mmol/l running buffer to 100 ml. After filtered through 0.22 μm nylon filter, all sample solution can be directly injected electrokinetically to the CE–ED system for analysis.

3. Results and discussion

3.1. Optimum conditions for the determination of swertiamarin, mangiferin and chlorogentic acid

Borate buffer was employed as the running buffer in this experiment because borate can chelate with mangiferin to form more soluble complex anions. As expected, the pH value of the running buffer directly affects the electroosmotic flow (EOF) and the migration velocity of the analytes. The pH dependence of the migration time was investigated in the pH range of 8.7–9.5. When pH value lowers than 9.0, solvent peak and swertiamarin cannot be separated completely. From pH 9.2 to 9.5, baseline separation of solvent and the analytes can be achieved. However, higher pH value results in long analysis time and easy oxidation of the analytes. Based on experiments, 50 mmol/l borax (pH 9.2) is chosen as the running buffer in considering the resolution, sensitivity and analysis time.

Since phenolic hydroxyl groups in mangiferin and chlorogentic acid can be oxidized electrochemically in moderate potential, and ethenyl in swertiamarin may be oxidized to form hydroxyl in higher potential, electrochemical detection was used in this work. The potential applied to the working electrode directly affects 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 +0.75 V (versus SCE), oxidation current of mangiferin increase rapidly; when the applied potential passes +0.95 V (versus SCE), however, the peak current of mangiferin increase much slower. On the other hand, the peak current of swertiamarin increase with the applied potential increase when the applied potential greater than +0.85 V (versus SCE), and the peak current of chlorogentic acid increase slowly from 0.75 to 1.00 V. Although an applied potential greater than +1.00 V (versus SCE) produces larger oxidation current for swertiamarin, both the baseline noise and the background current increase very strongly, which is obviously a disadvantage for sensitive and stable detection. Therefore, +1.00 V (versus SCE) was selected as the applied potential.

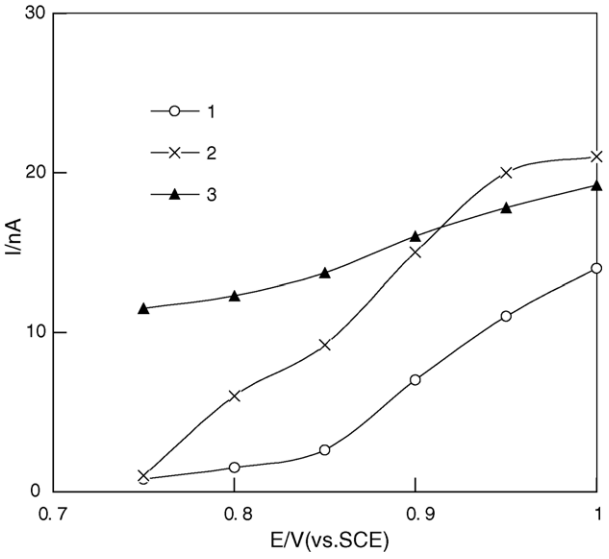

Fig. 2. Hydrodynamic voltammograms (HDVs) of swertiamarin (1), mangiferin (2) and chlorogenic acid (3). Fused-silica capillary: 25 μm i.d. × 50 cm; concentrations of swertiamarin, mangiferin and chlorogenic acid: 7.5×10^{-4}, 1.0×10^{-4} and 5×10^{-4} mol/l, respectively. Working electrode: 300 μm diameter carbon disk electrode; running buffer: 50 mmol/l borate (pH 9.2); separation voltage: 14 kV; injection time: 14 kV/10 s.

Higher separation voltages give shorter migration time for all analytes. However when the separation voltage exceeds 16 kV, separation of solvent peak and swertiamarin cannot be achieved, besides, baseline noise becomes larger. Six-

Table 1
The regression equations and detection limits[a]

Compound	Regression equation[b]	Correlation coefficient	Linear range (mol/l)	Detection limit (mol/l)
Swertiamarin	$y = 2.14 \times 10^4 x - 0.57$	0.9995	3×10^{-5} to 3×10^{-3}	1.1×10^{-5}
Mangiferin	$y = 1.67 \times 10^5 x + 0.70$	0.9995	5×10^{-6} to 5×10^{-4}	1.0×10^{-6}
Chlorogenic acid	$y = 4.42 \times 10^4 x + 0.79$	0.9998	5×10^{-6} to 2×10^{-3}	1.5×10^{-6}

[a] CE–ED condition are same as Fig. 3.
[b] In the regression equation, the x value is the concentration of analytes (mol/l), the y value is the peak current (nA).

teen kilovolts is chosen as the optimum separation voltage, at which good separation can be obtained for all analytes within 14 min. The injection time affects both peak current and peak shape. Ten seconds (14 kV) is selected as the optimum injection time by considering the peak broadening and sensitivity.

Under the above selected conditions, the typical electropherogram for a standard solution of the three analytes is shown in Fig. 2, we can see that satisfactory separation can be achieved within 14 min.

3.2. Reproducibility, linearity, detection limit of the three analytes

The reproducibility of the peak current and migration time is estimated by making repetitive injections of a standard mixture solution (7.5×10^{-4} mol/l for swertiamarin, 1.0×10^{-4} mol/l for mangiferin and 5×10^{-4} mol/l for chlorogenic acid) under the selected optimum conditions. The relative standard derivations (R.S.D.s) of migration time and peak current are 1.1 and 1.2% for swertiamarin, 0.9 and 1.7% for mangiferin, 0.8 and 1.0% for chlorogentic acid, respectively ($n = 7$). The high reproducibility indicates that the system is stable, and this method is dependable.

To determine the linearity of swertiamarin, mangiferin and chlorogentic acid, a series of standard solutions 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 summarized in Table 1.

3.3. Differentiation of Swertia Mussotii Franch from Artemisiae Capillaris Herba

Under the optimum conditions, the extracted solutions of Swertia Mussotii Franch and Artemisiae Capillaris Herba were determined by CE–ED according to the procedures described above. Typical electropherograms of Swertia Mussotii Franch (A) and Artemisiae Capillaris Herba (B) are shown in Fig. 3. By comparing with the electropherogram of the standard solution (Fig. 4), we can see that swertiamarin (peak 1) and mangiferin (peak 2) are present in Swertia Mussotii Franch herbs, however, no peaks of swertiamarin and mangiferin can be found in electropherogram of Artemisiae Capillaris Herbawe herbs. On the other hand, chlorogrntic acid (peak 3) is present in Artemisiae Capillaris Herba, but it is not detected in Swertia Mussotii Franch. Therefore, Swertia Mussotii Franch can be easily differentiated from Artemisiae Capillaris Herba by comparing their electropherograms.

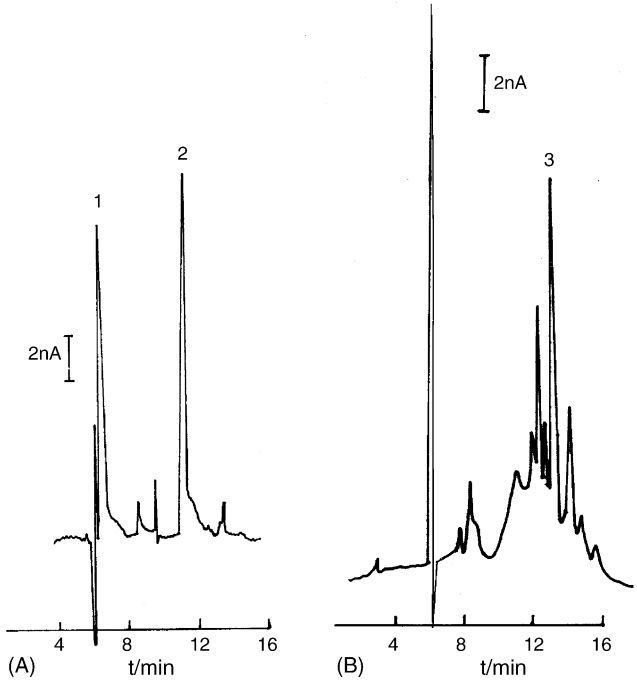

Fig. 3. The electropherograms of actual samples: Swertia Mussoti Franch herbs (A) and Artemisiae Capillaris Herba (B). Peak identification: (1) swertiamarin; (2) mangiferin; (3) chlorogenic acid. Working potential is 1.00 V (vs. SCE).

Table 2
Assay results for two medicinal herbs[a] ($n = 3$)

Sample	Ingredients	Found (mg/g)	R.S.D. (%)
Swertia Mussoti Franch	Swertiamarin	26.9	3.1
	Mangiferin	3.9	3.4
Artemisiae Capillaris Herba	Chlorogenic acid	2.06	2.5

[a] CE–ED condition are same as Fig. 3.

The contents of swertiamarin and mangiferin in Swertia Mussotii Franch and that of chlorogrntic acid in Artemisiae Capillaris Herba have been determined. The assay results are listed in Table 2. 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 3. The above assay results indicate that this method is accurate, sensitive and reproducible.

Table 3
Determination results of recovery in this method ($n = 3$)

Ingredient	Original amount (mol/l)	Added amount (mol/l)	Found (mol/l)	Recovery (%)	R.S.D. (%)
Swertiamarin	7.6×10^{-4}	3.0×10^{-4}	10.9×10^{-5}	102.8	3.6
Mangiferin	9.4×10^{-5}	5.0×10^{-5}	13.9×10^{-5}	96.5	3.2
Chlorogentic acid	2.9×10^{-4}	3.0×10^{-4}	6.1×10^{-4}	103.4	2.9

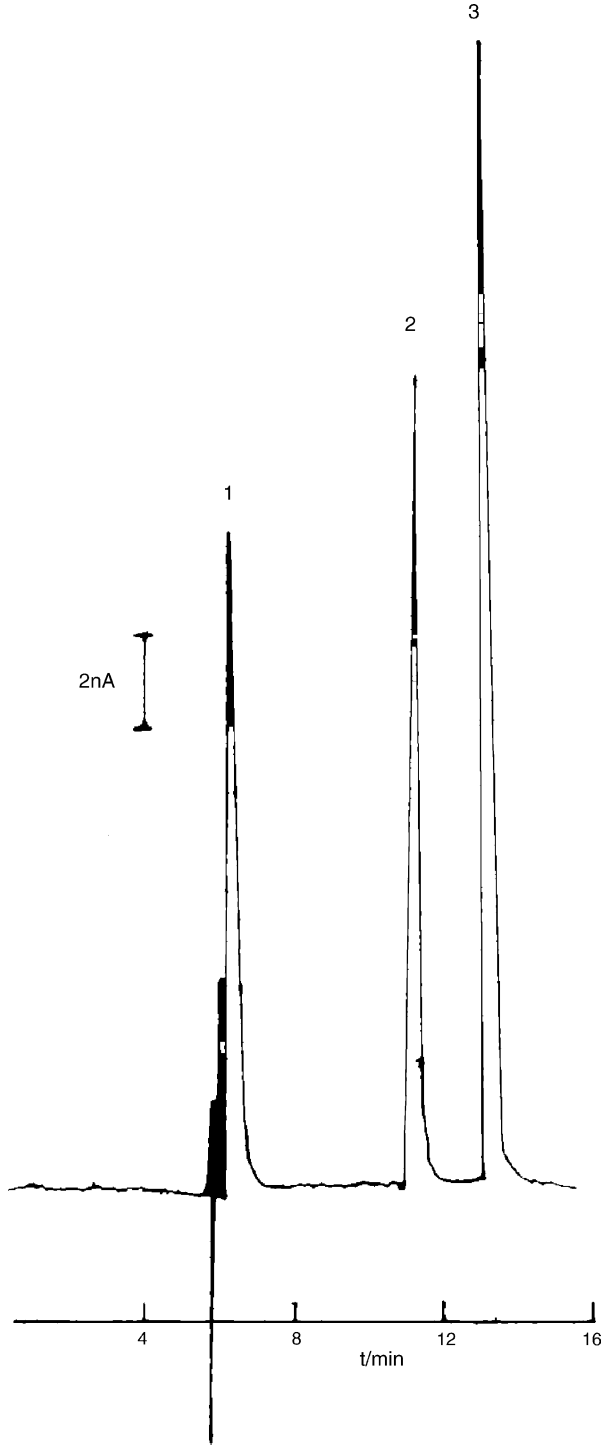

Fig. 4. The electropherogram of standard solution containing swertiamarin (7.5×10^{-4} mol/l), mangiferin (1.0×10^{-4} mol/l) and chlorogenic acid (5×10^{-4} mol/l). Peak identifications and determination conditions are the same as in Fig. 3. Other conditions are the same as in Fig. 2.

As well known, the separation of active ingredients of herbal medicines is often a challenging task owing to their complicated compositions. Even though CE with high efficiency, peak overlapping in the electropherogram often occurs because there are numerous coexistent compounds. The electrochemical detection used in this work can provide a high selectivity as only electroactive substances can be detected. Therefore, with electrochemical detection, the electropherogram is simpler, and identification of marker substances in the herbs becomes easier than with UV detection usually as the prime detector. In this work, many coexistent constitutes such as oleanolic in Swertia Mussotii Franch and coumarins, chromones in Artemisiae Capillaris Herba do not interfere with identification of swertiamarin, mangiferin and chlorogentic acid, as they could not be oxidized on a carbon electrode under the selected conditions.

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