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Analysis of Ferulic Acid, Rosemarinic Acid and Caffeic Acid in *Rosmarnus officinalis* L. by Capillary Zone Electrophoresis

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A capillary zone electrophoretic method has been developed to determine ferulic acid, rosemarinic acid and caffeic acid in *Rosmarnus officinalis* L. The effects of buffer pH and concentration and applied voltage on separation were investigated. The optimum electrophoretic conditions are as follows: 40 mmol L^{-1} Borax-40 mmol L^{-1} NaH_2PO_4 (pH 8.0) as the running buffer and applied voltage of 25 kV. Under the optimized conditions, a good linearity between the peak area and the concentration was found in the ranges of 10-200, 20-400 and 20-400 mg mL^{-1} for rosemarinic acid, ferulic acid and caffeic acid, respetively. The relative standard deviation in migration time and peak area was 0.68 and 1.37 % for ferulic acid, 0.51 and 1.98 % for rosemarinic acid, 0.76 and 1.86 % for caffeic acid. The detection limits of the three analytes ranged from 3.1 to 5.6 mg mL^{-1}. The recoveries of three analytes ranged between 92.5 and 102.5 %.

Keywords: Capillary zone electrophoresis, Ferulic acid, Rosemarinic acid, Caffeic acid.

INTRODUCTION

Rosmarinus officinalis L., native to the Mediterranean basin, belongs to Rosmarinus genus, Lamiaceae plants. It has the functions of antioxidant, antivirus, antitumour, liver protection and has been widely used in food, medicine and drinks[1,2]. Ferulic acid, rosemarinic acid and caffeice acid are three active constituents of *Rosmarinus officinalis* L.[1,3-5]. Ferulic acid can inhibit the accumulation of platelets and the release of 3H-5HT from platelets. Rosmarinic acid has the functions of anti-oxidant, immune suppression, anti-inflammation, anti-thrombus, antiplatelet, antianaphylaxis and the neurons protection. Caffeic acid has the functions of antibacterial, antivirus and antivenom. So, analysis of ferulic acid, rosemarinic acid and caffeice acid in *Rosmarinus officinalis* L. is very important.

In *Rosmarinus officinalis* L. analysis, the normal methods is high performance liquid chromatography (HPLC)[1,3]. With the advantage of high identification power, high performance liquid chromatography-mass spectrometry(HPLC-MS) has also been used for this purpose[4,5]. High performance liquid chromatography has the shortcomings of low efficiency and resolution and HPLC-MS requires the instruments of high cost, which is not available in many ordinary laboratories. Capillary electrophoresis (CE) has the advantages of low consumption of samples and chemicals, short analysis times and high resolution and has been widely applied in natural products analysis[6-20].

Herein, a capillary zone electrophoretic method has been developed to determine ferulic acid, rosemarinic acid and caffeic acid in *Rosmarinus officinalis* L. The method is accurate, reproducible and cost effective and the recovery is also satisfactory.

EXPERIMENTAL

Capillary electrophoresis analysis were carried out in a P/ACE MDQ capillary electrophoresis system with a photodiode array detector for absorbance measurements at 254 nm (Beckman Coulter, Fullerton, CA, USA). Uncoated fused-silica capillaries was purchased from Polymicro Technologies, Phoenix, AZ, USA. The dimensions of the capillary were 60.2 cm × 50 mm i.d. The effective length of the capillary was 50 cm. The temperature of the capillary was kept at 25 °C. The capillary electrophoresis system was interfaced with a Computer 32 Karat Software (Version 7.0) of Beckman was used for data acquisition.

The BGE was comprised of 40 mM of Borax-40 mM NaH_2PO_4 (pH 8.0). The buffer solutions were prepared freshly each day, sonicated for 5 min and filtered through a 0.45 μm membrane filter before use.

Ferulic acid and and caffeic acid were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Rosemarinic acid obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical reagent grade. Analytes Standard solutions of 1 mg mL^{-1} were prepared in methanol. All stock solutions were stored in a refrigerator. Buffer solutions were prepared with Borax and NaH_2PO_4 (concentration range: 10-50 mmol L^{-1}) by dissolving them in 18.2 mΩ·cm ultrapure water. All buffer solutions were filtered through a 0.45 µm membrane filter and degassed by ultrasonication for approximately 10 min before use.

Electrophoretic procedure: The capillary was conditioned daily by washing with 0.1 mol L^{-1} sodium hydroxide for 10 min, with water for 10 min and with the running buffer for 10 min sequentially. Between consecutive analysis, the capillary was rinsed with water for 3 min, with 0.1 mol L^{-1} sodium hydroxide for 3 min, with water for 4 min, with running buffer for 4 min sequentially to maintain proper reproducibility of run-to-run injections. Duplicate injection of the solutions were performed and average peak areas were used for the quantification. Peak identification was conducted by spiking the sample with the analytes to be identified. Comparing the on-line ultraviolet spectrum of real sample with that of the standard solution also served as a complementary method for peak identification in this work.

Sample preparation: A sample of *Rosmarnus officinalis* L. was purchased from a local drug store. It was dried at 60 °C oven for 6 h and then was pulverized. Thereafter, 0.5000 g of the powder was extracted with 15 mL of methanol by ultrasonication at room temperature for 20 min, then centrifuged at 3000 rpm for 10 min. The extraction process was repeated three times. Extracts were combined and diluted to 50 mL.

RESULTS AND DISCUSSION

Effect of buffer pH and buffer concentration: Buffer pH is a very important factor for separation optimization in capillary zone electrophoresis as it can affect the electroosmotic flow (EOF) and the ionization of the analytes[21]. The effect of buffer pH on the separation was investigated in the pH 7-9 range. As shown in Fig. 1a, migration time and resolution of the three analytes increased with the increase of the buffer pH in the pH 7-8 range. After pH is higher than 8, the migration time of the three analytes generally decreased with the increase of the buffer pH. At the same time, the resolution between rosemarinic acid and caffeic acid decreased with the increase of the buffer pH. When the buffer pH is 8, the optimum separation between the three analytes was achieved. So pH 8 was selected.

Keep the buffer pH at 8 and other conditions the same as the pH optimization, the influence of buffer concentration was investigated in the 10-50 mmol L^{-1} concentration range. As indicated in Fig. 1b, the migration time and the resolution of the three analytes increased with increasing buffer concenration, the three analytes can be well separated when buffer concentraton is higher than 40 mmol L^{-1}. The results also showed that the capillary current increased with the increase of the buffer concentration, which will increase the Joule heating effect and in the long run sacrify the detection limits. In consideration of resolution, analysis time and detection limits, 40 mmol L^{-1} was adopted.

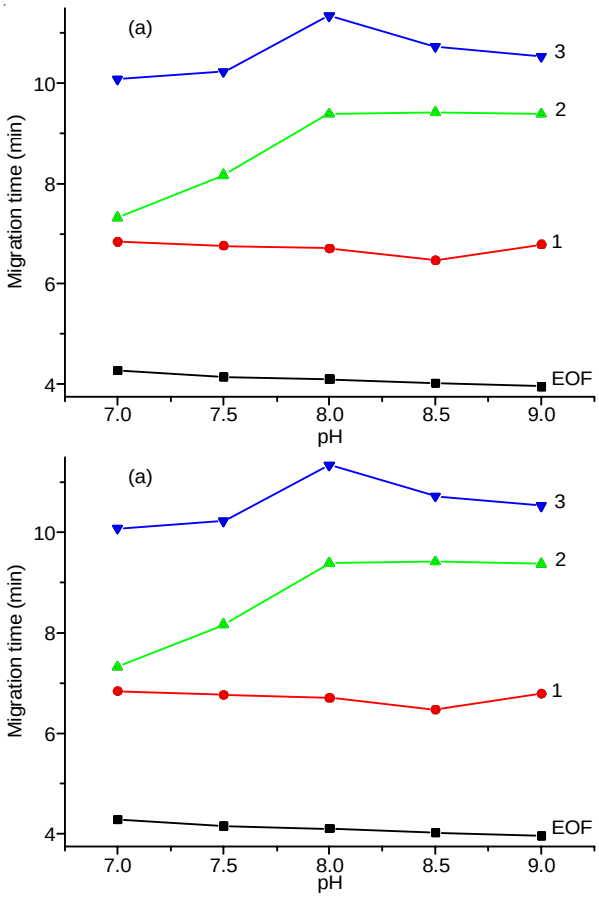


Fig. 1. Effects of pH; (a) and buffer concentration; (b) on the migration time of the analytes: peak designation: 1. ferulic acid; 2. rosemarinic acid; 3. Caffeic acid. Conditions: 60.2 cm × 50 µm (50.2 cm to detector) fused silica capillary, BGE 40 mM $Na_2B_2O_7$-NaH_2PO_4 (pH 7.0-9.0) for a and 10-50 mM $Na_2B_2O_7$-NaH_2PO_4 (pH 8.0) for b; voltage, 25 kV; detection was at 254 nm. Sample: hydrodynamic injection of 100 µg mL^{-1} of each analyte for 5 s at 0.5 psi

Effect of applied voltage: The effect of applied voltage on the separation was examined in the range of 15-30 kV. The results showed that with the increase of applied voltage, the migration time of the three analytes decreased, which results in shorter analysis time and an improvement of the efficiency. However, the baseline noise increased apparently when the applied voltage exceeded 25 kV, which can make the detection limits deteriorate. This was due to the pronounced Joule heating caused by the applied voltage increase. So, 25 kV was selected.

Validation of the method: Under the optimized conditions, a good separation of the three analytes was achieved in 12 min. Fig. 2(a) shows a typical electropherogram of the three analytes. The linearity of the three analytes in standard solutions was investigated. The calibration graphs were plotted by peak area against concentration. The detection limits were acquired based on three times noise. The calibration and detection limits results, were summarized in Table-1. The reproducibility is estimated by making five replicate injection of a standard mixture solution under the selected optimum conditions. As shown in Table-2, the relative standard deviation of the three analytes based on migration time and peak area were in the 0.51-0.76 and 1.37-1.98 % range, respectively.

TABLE-1
REGRESSION EQUATIONS AND DETECTION LIMITS[a]

Compound	Regression equation[b]	Correlation coefficient	Linear range (μg mL^{-1})	Detection limit[c] (μg mL^{-1})
Ferulic acid	Y=63.86X - 103.56	0.9993	10-200	3.1
Rosemarinic acid	Y=50.57X + 65.48	0.9986	20-400	5.6
Caffeic acid	Y=128.05X - 67.60	0.9990	20-400	4.4

Notes: [a]Capillary electrophoresis conditions are the same as in Fig. 2; [b]In the regression equation, the X value is the concentration of analytes (μg mL^{-1}), the y value is the peak area; [c]The detection limit is evaluated on the basis of a signal-to-noise ratio of 3

TABLE-2
REPRODUCIBILITY OF THE PEAK AREA AND MIGRATION TIME OF THE COMPOUNDS (n = 5)

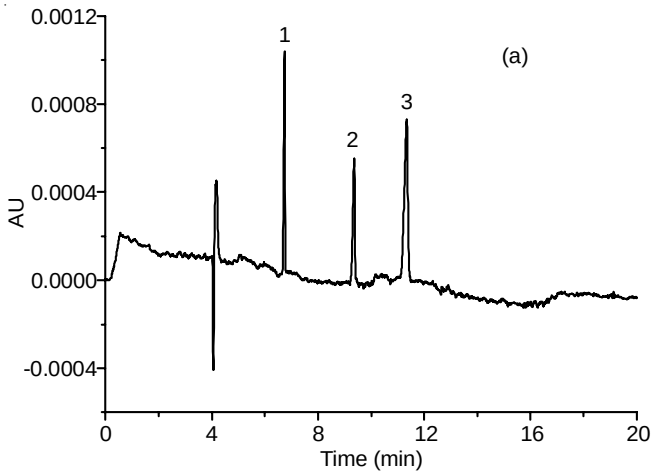
Compound	Concentration (μg mL^{-1})	Migration time (min)		Peak area (μAU sec^{-1})	
		Mean	R.S.D. (%)	Mean	R.S.D. (%)
Ferulic acid	50	6.787	0.68	3033	1.37
Rosemarinic acid	50	9.446	0.51	2462	1.98
Caffeic acid	50	11.450	0.76	6548	1.86

Results of sample analysis and recovery: The developed method was applied to determine ferulic acid, rosemarinic acid and caffeic acid in *Rosmarnus officinalis* L. Electropherogram of *Rosmarnus officinalis* L. extracts is shown in Fig. 2b and the analysis results are shown in Table-3.

TABLE-3
SAMPLE ANALYSIS AND RECOVERY EXPERIMENTS (n = 5)

Compounds	Original amount (mg g^{-1})	Added amount (mg g^{-1})	Found (mg g^{-1})	Recovery (%)	R.S.D. (%)
Ferulic acid	22.8	10.0	32.1	92.5	3.5
Rosemarinic acid	26.7	10.0	36.2	95.3	3.7
Caffeic acid	18.6	10.0	29.2	105.2	5.0

Accurate amount of ferulic acid, rosemarinic acid and caffeic acid were added to *Rosmarnus officinalis* L. to do recovery experiments and the recovery value were achieved by the corresponding calibration curve under the same conditions. The recoveries of the three analytes were shown in Table-3 and were in the 92.5-105.2 % range, which were satisfactory.



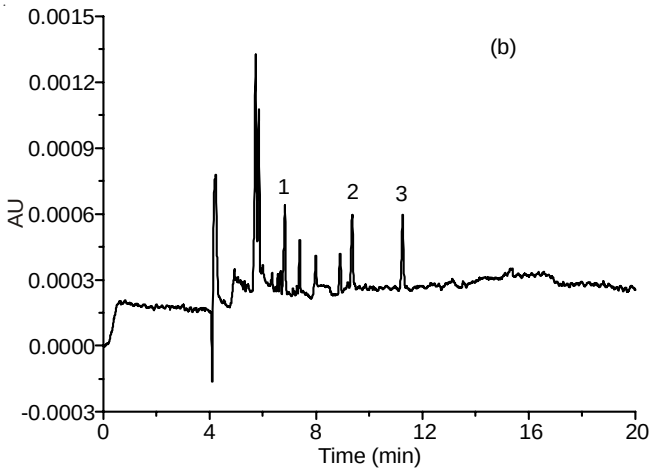


Fig. 2. Electropherogram of standard mixture (a) and the rosemary sample (b) Conditions: 60.2 cm × 50 μm (50.2 cm to detector) fused silica capillary, BGE 40 mM $Na_2B_2O_7$-NaH_2PO_4 (pH 8.0); voltage, 25 kV; detection was at 254 nm. Sample: hydrodynamic injection of 100 μg mL^{-1} standards mixture or the sample for 5 s at 0.5 psi. peak designation the same as in Fig. 1

Conclusion

A method of determination of ferulic acid, rosemarinic acid and caffeic acid in *Rosmarnus officinalis* L. by capillary zone electrophoresis was developed. The method was accurate and fast and is hopeful to be used for the quality control of *Rosmarnus officinalis* L. and its products.

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