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Analysis of Phenolic Compounds in *Taraxaeum mongolicum Hand Mazz* by α-Cyclodextrin Modified Capillary Zone Electrophoresis After Microwave-Assisted Extraction

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An α-cyclodextrin (α-CD) modified capillary zone electrophoretic (CZE) method has been developed to determine 4 kinds of phenolic compounds (chlrorogenic acid, isoquercitrin, quercetin and caffeic acid) in *Taraxaeum mongolicum Hand Mazz*. The effects of buffer pH and concentration, applied voltage and α-CD concentration on separation were investigated. The optimum electrophoretic conditions are as follows: 50 mmol L^{-1} boric acid-50 mmol L^{-1} borax (pH 9.0)-4 mmol L^{-1} α-cyclodextrin as the running buffer, applied voltage of 25 kV and capillary temperature of 25 ºC. Under the optimized conditions, a good linearity between the peak area and the concentration was found in the range of 3-200, 3-200, 1-100 and 1-200 μg mL^{-1} for chlorogenic acid, isoquercitrin, quercetin and caffeic acid, respetively. The relative standard deviation in migration time and peak area was 0.19 and 1.79 % for chlorogenic acid, 0.34 and 2.58 % for isoquercerin, 0.20 and 2.78 % for quercetin, 0.23 and 2.65 % for caffeic acid. The detection limits of the 4 phenolic compounds ranged from 0.20 to 0.56 μg mL^{-1}. The recoveries of the 4 phenolic compounds ranged between 92.6 and 106.0 %.

Key Words: *Taraxaeum mongolicum Hand Mazz*, α-Cyclodextrin, Capillary zone electrophoresis, Microwave-assisted extraction.

INTRODUCTION

Phenolic compounds are widely spread group of antioxidants presented in plants and their derived products[1]. They are strong antioxidants and free radical scavengers *in vitro*, which have anti-inflammatory, antibacterial, antifungal, antitumour and many other pharmacological effects[2-4]. Phernolic acids and flavonoids are two classes of phenolic compounds and are active constituents of many medicinal plants, so accurate determination of them is very important.

The analytical methods for phenolic compounds analysis in medicinal plants include gas chromatography (GC)[5], thin layer chromatography (TLC)[6,7], high performance liquid chromatography (HPLC)[8-11], high performance liquid chromatography-mass spectrometry (HPLC-MS)[12], gas chromatography-mass spectrometry (GC-MS)[13]

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and capillary electrophoresis (CE)[2,14-16]. GC, TLC and HPLC all suffers from the limitation of insufficient resolving power and complexity of process. Although GC-MS and LC-MS have the advantage of high identification power, the expense of instruments and maintenance are very high. Compared with the above methods, capillary electrophoresis (CE) has the advantages of rapidity, high efficiency, low consumption of sample and the reagents, longevity and easy to elute of the capillary column.

Taraxaeum mongolicum Hand Mazz is a traditional Chinese herbal medicine. It has the functions of clearing heat and detoxifcating, detumescence and removing stasis, diuresis and freeing strangury and can be used to treat acute mastitis, scrofula, sore throat, chronic infection, damp fever and jaundice and many other illness[16,17]. Quercetin and isoquercitrin are two flavonoids occurred in *Taraxaeum mongolicum Hand Mazz*[18,19]. Quercetin has the function of eliminating sputum, relieving cough, lowering blood pressure and blood fat. Isoquercitrin has the function of lowering pressure, anti-inflammation and killing anthelminth. Caffeic acid and chlorogenic acid are two kinds of phenolic acids occurred in *Taraxaeum mongolicum Hand Mazz*[19]. Caffeic acid has the function of antibacteria and antivirus. Chlorogenic acid has the function of antibacteria, purging the liver and stopping bleeding. The structures of chlorogenic acid, isoquercitrin, quercetin and caffeic acid are shown in Fig. 1. Accurate determination of the four phenolic compounds is very important for the quality control and medicinal preparation development of *Taraxaeum mongolicum Hand Mazz*. Hitherto, TLC[6], HPLC[10,11] and CE[16] has been applied to phenolic compounds analysis in *Taraxaeum mongolicum Hand Mazz*. Nevertheless, only one or two phenolic compounds were determined and the analytical efficiency was relatively low.

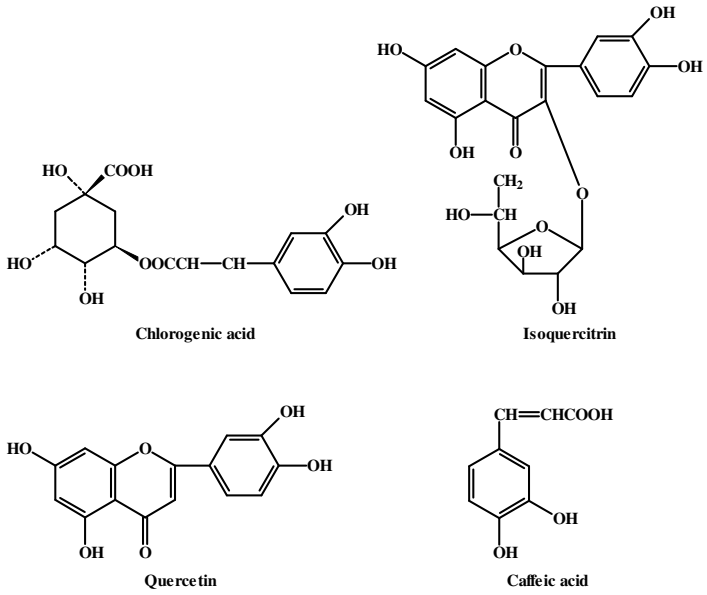


Fig. 1. Molecular structures of the analytes

In medicinal plant analysis, the commonly employed extraction approaches were hot solvent extraction (HSE) and ultrasonic extraction. However, these extraction techniques have the shortcomings of low efficiency, time and solvent consuming and involving lengthy operation techniques. Microwave-assisted extraction (MAE), utilizes microwave energy, is a non-ionizing radiation that cause molecular motion by migration of ions and rotation of dipoles but does not include changes in molecular structure, to heat the solvent and the sample for extracting analytes. Compared with the conventional extraction methods, MAE can considerably reduce both extraction time and solvent consumption[20] and has begun to be utilized to extract active constituents from medicinal plants in recent years[21].

In this paper, an α-cyclodextrin (α-CD) modified capillary zone electrophoretic (CZE) method has been developed to determine chlorogenic acid, isoquercitrin, quercetin and caffeic acid in *Taraxaeum mongolicum Hand Mazz* after microwave-assisted extraction. The method has the advantages of accuracy, rapidity and low cost and important for the quality control of *Taraxaeum mongolicum Hand Mazz* and its medicinal preparations.

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 purchased from Yongnian Optical Fiber Plant(Yongnian, Hebei Province, China) was used. The dimensions of the capillary were 60.2 cm × 50 μm i.d. The effective length of the capillary was 50 cm. The temperature of the capillary was kept at 25 ºC. The applied voltage was 25 kV. Samples were introduced under pressure (5s, 0.5 psi). Capillary electrophoresis system was interfaced with a computer. 32 karat software (version 7.0) of Beckman was used for data acquisition. Samples was prepared in a XH-100A microwave extractor (Beijing Xianghu Science and Technology Development Co., Beijing, China). A pHS-3C pH meter (Leici Instrumentation Factory, Shanghai, China) was employed with a precision of ± 0.02 pH unit. The ultrapure water, used throughout, was prepared with a milli-Q system (Millipore, Bedford, MA, USA).

Chlorogenic acid and caffeic acid were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Isoquercitrin and quercetin were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical reagent grade. Analytes standard solutions of 1.0 mg mL^{-1} were prepared in methanol. All stock solutions were stored in refrigerator with temperature at 4 ºC. Buffer solutions were prepared with borax-boric acid (concentration range: 30-70 mmol L^{-1}) and α-cyclodextrin (concentration range: 0-8 mmol L^{-1}) by dissolving them in 18 MΩ/cm ultrapure water. The final pH values were adjusted with 0.1 mol L^{-1} sodium hydroxide and 0.1 mol L^{-1} HCl.

All buffer solutions were filtered through a 0.45 μm membrane filter and degassed by ultrasonication for *ca.* 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 *Taraxaeum mongolicum Hand Mazz* was purchased from a local drug store. It was dried at 60 °C oven for 6 h and then was pulverized. 5.000 g of the powder was dispersed in 50 mL of methanol and was allowed to expose to the 400 W microwave irradiation in the microware extractor for 5 min. After cooling, the extracts was transferred to a 10 0 mL volumetric flask and diluted to mark with methanol.

RESULTS AND DISCUSSION

Effects of buffer pH and buffer concentration: Structures of the four analytes suggested that they could be analyzed as anions. Borax-Boric acid buffer was employed as the running buffer in this work because it can chelate with the analytes to form more soluble complex anions[22]. Buffer pH can affect zeta potential, electroosmotic flow and the charge state of the analytes, which will affect the migration time of the analytes and the separation efficiency[23]. The effect of buffer pH on the separation was investigated in the pH 8.2-9.2 range. As shown in Fig. 2(a), the migration time

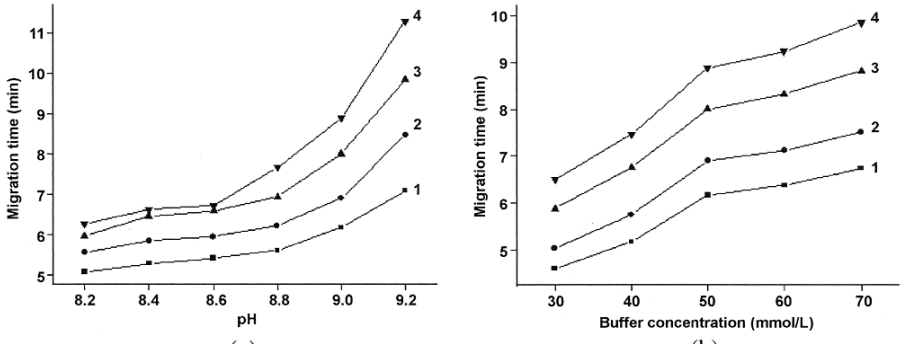


Fig. 2. Effects on migration time of the four analytes by buffer pH (a) and concentration (b): 1. chlrogenic acid; 2. isoquercitrin; 3. quercetin; 4. caffeic acid (The meaning of 1, 2, 3, 4 were the same in the Figures of the whole paper)

of all the four analytes increase with the increase of the buffer pH. The resolution of the four analytes decrease with the increase of the buffer pH in the pH 8.2~8.6 range. When the buffer pH is higher than 8.6, the resolution of the four analytes enhanced with the pH increase. In consideration of the analysis time and good separation, pH 9.0 was chosen as a compromise.

Keep the buffer pH at 9.0 and other conditions the same as the pH optimization, the influence of buffer concentration was investigated in the 30-70 mmol L^{-1} concentration range. As indicated in Fig. 2(b), the migration time and the resolution of the 4 analytes increase with increasing buffer concenration. The four analytes can be well separated when buffer concentraton is higher than 40 mmol L^{-1}. The results also showed that the capillary current increase with the increase of the buffer concentration, which will increase the Joule heating effect and in the long run sacrifice the detection limits. In consideration of resolution, analysis time and detection limits, 50 mmol L^{-1} was selected as the optimum buffer concentration.

Effect of applied voltage: The effect of applied voltage on the separation was examined in the range of 19-27 kV. As shown in Fig. 3, with the increase of applied voltage, the migration time of the four analytes decreased, which results in shorter analysis time and an improvement of the efficiency. At the same time, the resolution didn't improve significantly. 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 as the applied voltage, which combined sufficient separation, moderate analysis time and adequate detection limits.

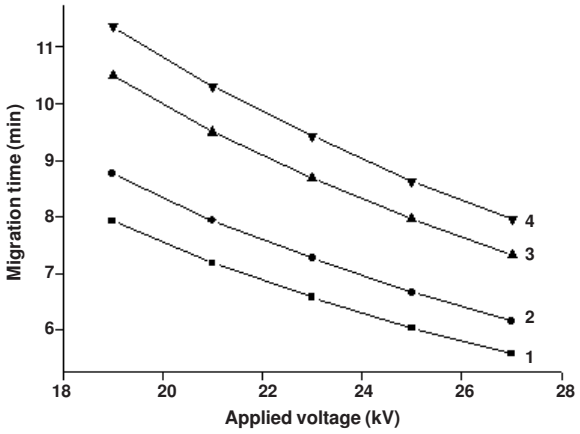


Fig. 3. Effects of applied voltage on migration time of the 4 analytes

Effect of α-cyclodextrin (α-CD)concentration: Incorporation of α-CD into the running buffer can improve the separation selectivity of some analytes. The reason for the improve-ment in separation selectivity are that α-CD has a special

cavity structure, hydrophobic compounds can get into the inside cavity of α-CD, thus the inclusion-complexes are formed. Different inclusion-complexes have different stability, hence the separation selectivity was improved[24]. In an attempt to attain improvement in the separation of the four analytes, the effect of α-cyclodextrin (α-CD) concentration was examined in the 0-8 mmol L^{-1} concentration range. As shown in Fig. 4, the migration time of the four compounds decreased significantly with the increase of the α-CD concentration in the 0-2 mmol L^{-1} concentration range. The change in the migration time of the four analytes become mild after the α-CD concentration is higher than 2 mmol L^{-1}. The separation of the four analytes first reduced (in the 0-2 mmol L^{-1} range), then improved (in the 2-4 mmol L^{-1} range) and then decreased (in the 4-8 mmol L^{-1} range) with the increase of the α-CD concentration. Simultaneously, the peak shapes of the 4 analytes improved with the increase of the α-CD concentration. As shown in Fig. 5, the analysis time decreased apparently with the increase of α-CD concentration, which greatly increased the analysis efficiency. In consideration of separation, peak shape and sensitivity, 4 mmol L^{-1} α-CD was adopted in the further experiments.

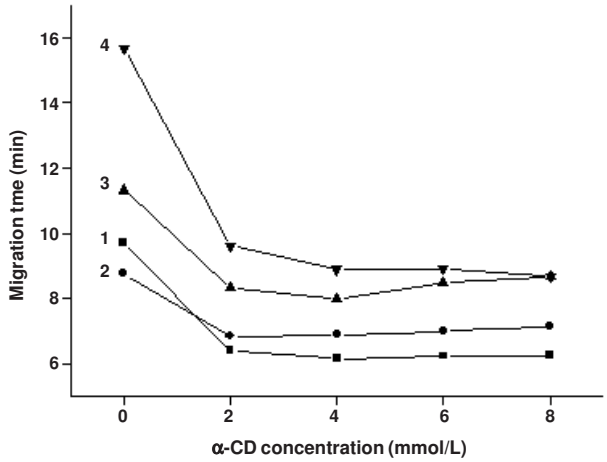
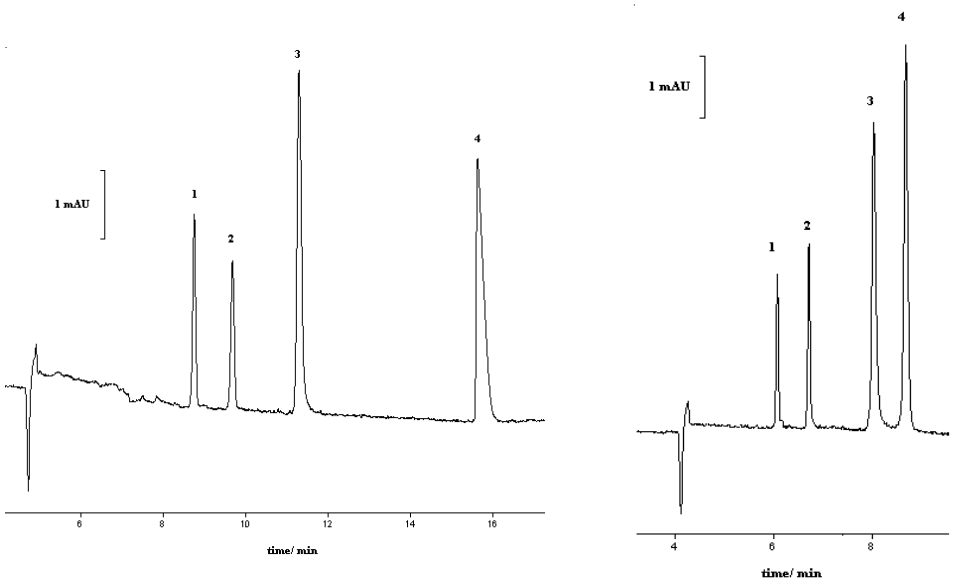


Fig. 4. Effects of α-CD concentration on migration time of the analytes

Validation of the method: Under the optimized conditions, a good separation of the four analytes was achieved in 9 min, Fig. 5(b) shows a typical electropherogram of a the 4 analytes. The linearity of the 4 analytes in standard solutions was investigated. The calibration graphs were plotted by peak area (y, μAU s^{-1}) against concentration (x, μg mL^{-1}). The detection limits were acquired based on 3 times noise. The calibration and detection limits results, were all summarized in Table-1, which were satisfactory. The reproducibility is estimated by making eight replicate injection of a standard mixture solution under the selected optimum conditions. As shown in Table-2, the relative standard deviation of the four analytes based on migration time and peak area were in the 0.19-0.34 and 1.79-2.78 % range, respectively.



(a) (b)

Fig. 5. Differences between electropherogram of the compounds without α-CD (a) and with 4 mmol L^{-1} α-CD (b): other conditions: 50 mmol L^{-1} borax-boric acid (pH 9.0); applied voltage, 25 kV; temperature, 25 ºC; UV detection wavelenghth, 254 nm

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})
Chlorogenic acid	Y=125.1601X+89.2688	0.9999	3-200	0.56
isoquercitrin	Y=126.1429X+97.2234	0.9999	3-200	0.56
quercetin	Y=391.2951X+341.9326	0.9998	1-100	0.20
Caffeic acid	Y=389.1630X+331.3372	0.9994	1-100	0.21

[a]CE conditions are the same as in Fig. 5(b)
[b]In the regression equation, the X value is the concentration of analytes (μg mL^{-1}), the y value is the peak area (μAU s^{-1})
[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 = 8)

Compound	Concentration (μg mL^{-1})	Migration time (min)		Peak area (μAU s^{-1})	
		Mean	RSD (%)	Mean	RSD (%)
Chlorogenic acid	10	6.171	0.19	1367	1.79
isoquercitrin	10	6.896	0.34	1289	2.58
quercetin	10	8.008	0.20	4350	2.78
Caffeic acid	10	8.897	0.23	4284	2.65

Results of sample analysis and recovery: The developed method was applied to determine chlorogenic acid, isoquercitrin, quercetin and caffeic acid in *Taraxaeum mongolicum Hand Mazz*. Electropherogram of *Taraxaeum mongolicum Hand Mazz* extracts was shown in Fig. 6 and the analysis results were shown in Table-3.

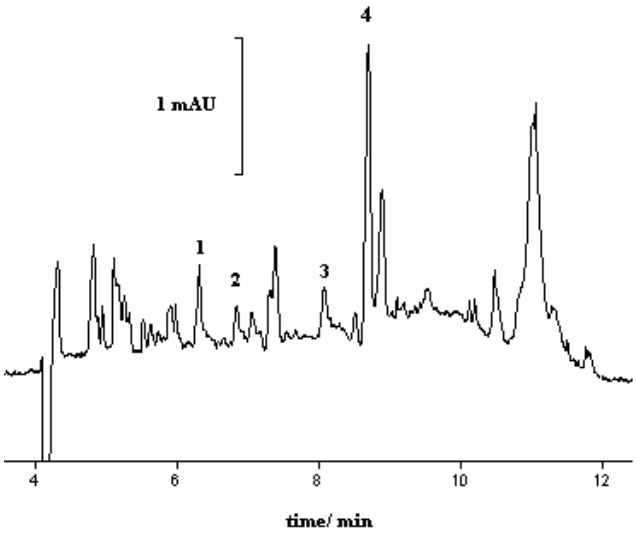


Fig. 6. Electropherogram of the *Taraxacum mongolicum Hand Mazz* extracts. Peak identification and determination conditions are the same as that in Fig. 5(b)

TABLE-3
RESULTS OF *Taraxacum mongolicum Hand Mazz* SAMPLE ANALYSIS (n = 5)

Component	Found (mg g^{-1})	Average (mg g^{-1})	RSD (%)
Chlorogenic acid	0.26, 0.23, 0.24, 0.25, 0.24	0.240	3.81
isoquercitrin	0.11, 0.12, 0.11, 0.11, 0.12	0.110	3.09
quercetin	0.061, 0.064, 0.064, 0.061, 0.064	0.063	2.23
Caffeic acid	0.51, 0.45, 0.48, 0.45, 0.48	0.480	4.84

Accurate amount of chlorogenic acid, isoquercitrin, quercetin and caffeic acid were added to *Taraxaeum mongolicum Hand Mazz* sample to do recovery experiments and the recovery value were achieved by the corresponding calibration curve under the same conditions. The recoveries of the 4 analytes were shown in Table-4 and were in the 92.6-106.0 % range, which were satisfactory.

Conclusion

A method of determination of chlorogenic acid, isoquercitrin, quercetin and caffeic acid in *Taraxaeum mongolicum Hand Mazz* by α-cyclodextrin modified capillary zone electrophoresis after microwave-assisited extraction was developed. The method was accurate and fast and could be effective for quality control of *Taraxaeum mongolicum Hand Mazz* and its medicinal preparations.

TABLE-4
RECOVERIES IN THIS METHOD (n = 5)

Compounds	Original amount (mg g^{-1})	Added amount (mg g^{-1})	Found (mg g^{-1})	Recovery (%)	RSD (%)
Chlorogenic acid	0.240	0.50	0.70	92.6	2.07
isoquercitrin	0.110	0.50	0.64	106.0	3.69
quercetin	0.063	0.50	0.58	103.1	4.03
Caffeic acid	0.480	0.50	0.97	97.3	2.85

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