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Optimized Extraction of Two Organic Acids from *Herba artemisiae scopariae* with Reversed-Phase High Performance Liquid Chromatography Separation

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Extraction of chlorogenic acid and caffeic acid from *Herba artemisiae scopariae* was developed by liquid-liquid extraction with liquid chromatography separation. By utilizing different extraction solvents, extraction time and extraction temperature, the optimum extraction conditions were established. The extract was separated by a C_{18} column with a mobile phase consisting of acetonitrile-water (20:80, v/v). 4.78 mg/g of chlorogenic acid and 0.26 mg/g of caffeic acid were obtained. This method is simple and sensitive and has been applied successfully to determine the component of chlorogenic acid and caffeic acid in *Herba artemisiae scopariae*.

Key Words: Chlorogenic acid, Caffeic acid, *Herba artemisiae scopariae*.

INTRODUCTION

Herba artemisiae scopariae (HAS) is prepared from the dried sprout of *Artemisia scoparia*. It is a widely used traditional medicine and often used as an important ingredient in many traditional prescriptions[1,2]. It has both a cholagogic effect and other pharmacological actions, such as protecting the liver, lowering blood pressure, eliminating fever, sedation, antiinflammation, antibacteria, antipathogenic-microbes and antitumor action[3-5]. It has many clinical applications in the treatment of acute icteric infectious hepatitis, hyperlipemia and oral ulcers[6]. Recent investigations by the State Administration of traditional Chinese medicine suggest that it also can be used for the treatment and prevention of SARS[7,8].

Two of the major active compounds in the herb are chlorogenic acid and caffeic acid (Fig. 1). They bear a close relationship with the quality of the herbal drug and a higher content can indicate a better quality of the crude drugs. The chlorogenic acid has an especially broad range of physiological activities, such as antitumor, antiinflammatory[9], antibacterial and antioxidant activities[10]. In order to evaluate the quality of *Herba artemisiae scopariae* in the market, it is necessary to establish a rapid, simple and accurate process for extraction and separation.

Han *et al.*[11] already did some researches about *Herba artemisiae scopariae* extraction and separation with high-performance liquid chromatography (HPLC) but the extract amount was not high enough. Hence, in this study, a simple and convenient extraction process to extract the two organic acids from *Herba artemisiae scopariae* by liquid-liquid extraction followed with RP-HPLC analysis was optimized.

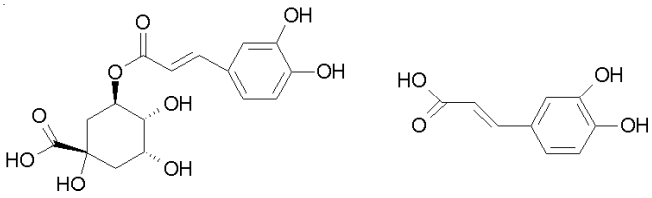

Fig. 1. Molecular structures of two target compounds

EXPERIMENTAL

Chlorogenic acid and caffeic acid were obtained from the National Institute for the Control of Pharmaceuticals and Biological Products of China, Beijing, China and used without further purification. *Herba artemisiae scopariae* was obtained from Wanbaotang Drugstore, Baoding, China. Acetonitrile, acetic acid and other common organic compounds were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). Distilled water was filtered using a vacuum pump (Division of Millipore, Waters, USA) and a filter (HA 0.45, Division of Millipore, USA) before use. All other solvents used in the experiment were HPLC or analytical grade. All the samples were filtered by using a filter (MFS 25, 0.2 μm TF, Whatman, USA) before injection into the HPLC system.

Chromatographic conditions: Chromatography was performed with a Waters 600 s multisolvent delivery system,

a Waters 616 liquid chromatography and a Waters 2487 variable wavelength, dual-channel UV detector (Waters Associates, Milford, MA, USA). A syringe with 25 µL injection volume and 20 µL sample loop were used. Data processing was performed with Millennium 3.2 software. Compounds were separated on a 150 mm × 4.6 mm, 5 µm particle, OptimaPak C_{18} column (RStech, Daejeon, Korea). Acetonitrile/water/acetic acid (20:80:0.1, v/v/v) was used as the mobile phase at a flow rate of 0.5 mL/min and the detection was carried out at a wavelength of 320 nm. The solution of two target compounds (0.25 mg/mL) was prepared in 1 mL of methanol.

Extraction conditions: 5 g of *Herba artemisiae scopariae* powder was extracted with 50.0 mL of different solvent, such as water, methanol, ethanol and ethyl acetate. Different temperature (35, 45, 55, 65 and 75 ºC) with different dipping time (10, 20, 30, 60, 90, 120 and 180 min) were evaluated. In addition, ultrasonic method was invited. 5.0 g of *Herba artemisiae scopariae* powder was extracted with 50 mL of water by ultrasonic bath for 2, 5, 10, 20, 40 and 60 min. After centrifugation and filtration, the extract was collected and stored for injection.

RESULTS AND DISCUSSION

Effect of different extraction solvents: Table-1 shows the extracted amounts of two target compounds by the different solvents, respectively. Fig. 1 shows, both of the two acid compounds have several hydroxyls, which make them easily dissolved and extracted by polar solvents. Also chlorogenic acid has higher polarity than caffeic acid. So using water as extractant can get the highest amount from *Herba artemisiae scopariae* than that of using organic solvents. Hence, water was selected as the optimized extraction solvent.

TABLE-1
EXTRACTION AMOUNTS OF TWO TARGET COMPOUNDS BY DIFFERENT SOLVENTS

Solvent	Extraction amount (mg/g)			
	Water	Methanol	Ethanol	Ethyl acetate
Chlorogenic acid	1.70	0.50	0.30	0.01
Caffeic acid	0.05	0.01	0.01	0.01

Effect of dipping time: The influence of the different dipping times (10, 20, 30, 60, 90, 120 and 180 min) was examined. As shown in Fig. 2, the extracted amount was decreased before 1 h. The cell wall of *Herba artemisiae scopariae* contained large amount of cellulose, the -OH groups on cellulose had interaction with target compounds. In this case, small amount of organic acids was remained on cell wall. With the extraction time increasing, cell wall was destroyed so the interaction was decreased. Then more amounts of organic acids were moved to water. After 90 min, there was little improvement of extracted amount. Therefore, 90 min was considered to be the optimal dipping time.

Effect of temperature: The -OH groups on cellulose had interaction with target compounds. When the temperature was lower than 30 ºC, the interaction was strong that some amounts of target compounds adsorbed on cell wall. In Fig. 3, the extracted amount in water was decreased. With the temperature increasing, the interaction was destroyed and more amounts of organic acids were moved to water. The extracted amount was increased until 65 ºC. In this case, 65 ºC was the optimized temperature of water extraction and the chromatogram of the extract was shown in Fig. 4.

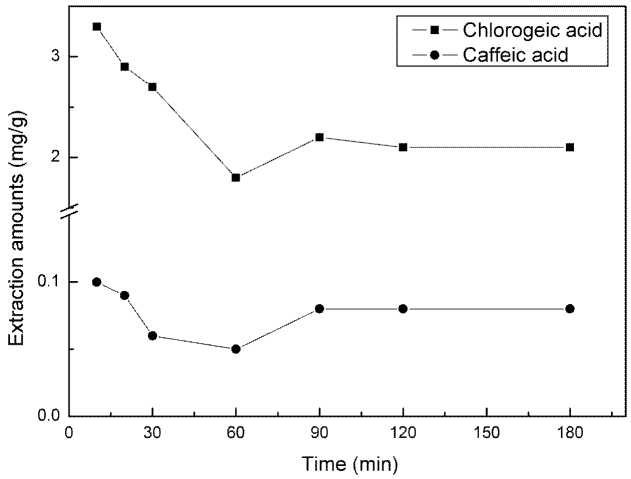

Fig. 2. Extracted amounts of two target compounds with different dipping time

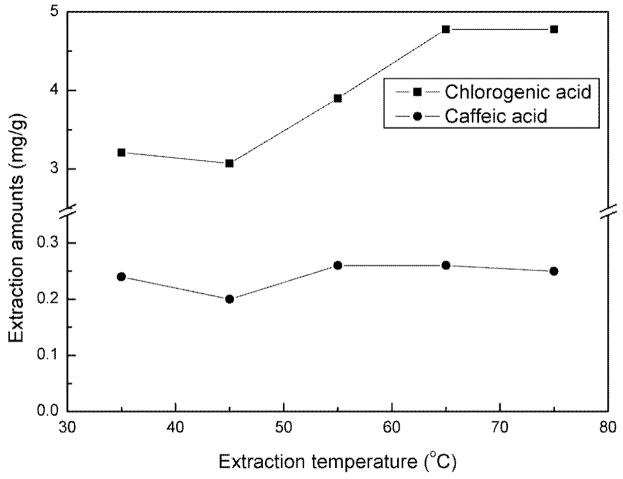

Fig. 3. Extracted amounts of two target compounds with different temperature

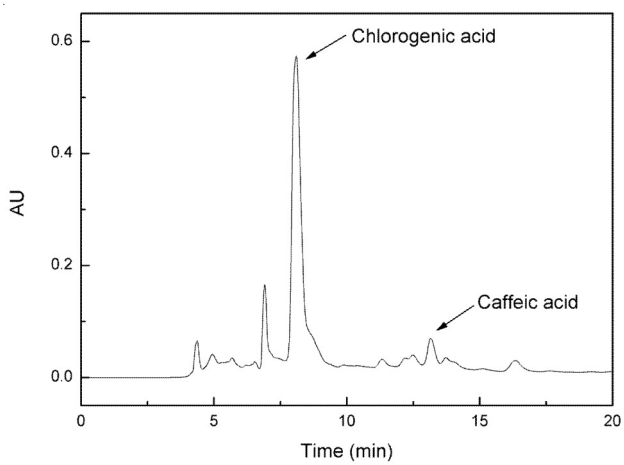

Fig. 4. Chromatogram of extract sample

TABLE-2
RESOLUTION (R) AND RETENTION FACTOR (k) OF TWO TARGET COMPOUNDS IN EXTRACTION SOLVENTS (DIFFERENT EXTRACTION TIMES)

	Extraction time (min)						
	10	20	30	60	90	120	180
Resolution (error %)	6.76 (± 8.55)	6.18 (± 6.71)	6.31 (± 3.52)	6.86 (± 6.91)	6.97 (± 1.69)	7.44 (± 4.67)	7.01 (± 6.05)
k_{CGA}	2.70	2.71	2.75	2.74	2.82	2.79	2.80
k_{CA}	5.22	5.23	5.33	5.31	5.51	5.44	5.49

Effect of ultrasonic extraction: Fig. 5 shows the extracted amounts of two target compounds with different ultrasonic times. The extraction amount was increased with the extraction time increasing. However, the extracted amounts of the two target compounds were much less than the dipping method. Therefore, ultrasonic method was not considered in this experiment.

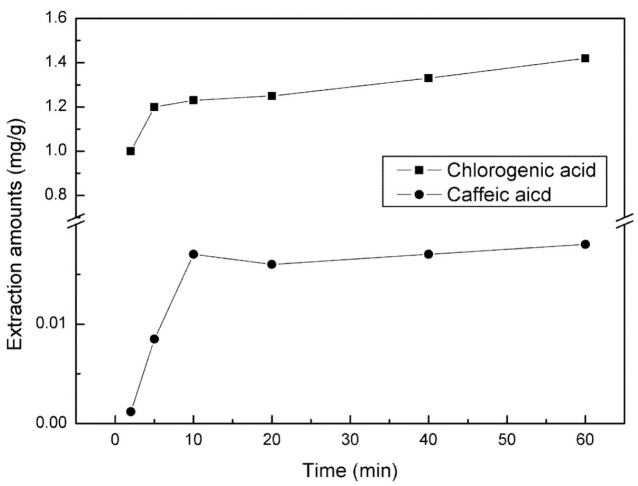

Fig. 5. Extracted amounts of two target compounds with different ultrasonic time

Linearity and reproducibility: A series of mixtures of standard solutions containing chlorogenic acid and caffeic acid were diluted (0.5, 1.0, 4.0, 8.0, 10.0 and 15.0 mg/mL) with methanol. As a result, linear regression equations of the two compounds were Y = 25544x - 41.56 (r^2 = 0.999) for chlorogenic acid and Y = 52782x + 19.15 (r^2 = 0.998) for caffeic acid. Assays of repeatability calculated as relative standard deviations (RSDs) were performed by injecting standard solutions 5 times in a 5-day period. The RSDs lower than 8.55 % showed acceptable precision and accuracy results (Table-2).

Conclusion

The optimized separation condition and extraction method were established for the analysis and extraction of chlorogenic acid and caffeic acid from *Herba artemisiae scopariae* using RP-HPLC. The extraction amounts of the two compounds were 4.78 mg/g for chlorogenic acid and 0.26 mg/g for caffeic acid. The low deviation error demonstrated the method to be a viable alternative tool for further researches.

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