Asian Journal of Chemistry Vol. 22, No. 4 (2010), 2711-2716

Determination of Four Bioactive Compounds in *Herba artemisiae scopariae*

D.D. HAN and K.H. ROW*
Department of Chemical Engineering, Inha University, Incheon-402-751, South Korea
Fax: (82)(328)720959; Tel: (82)(328)607470; E-mail: rowkho@inha.ac.kr

A simple HPLC method was developed for the determination of chlorogenic acid, caffeic acid, scoparone and rutin in *Herba artemisiae scopariae*. Chromatographic separation was carried out on a C_{18} column (250 mm × 4.6 mm, 5 μm) with gradient elution. The mobile phase consisted of acetonitrile:water:acetic acid (25:75:0.1, v/v/v) (A) and acetonitrile/water (65:35, v/v) (B). The UV detection wavelength was set to 325 nm. Under optimal conditions, the four compounds were baseline separated within 20 min. The calibration curve was linear over the following range: 1-100 μg/mL for chlorogenic acid, caffeic acid and scoparone and 5-500 μg/mL for rutin. The correlation coefficient of chlorogenic acid, caffeic acid, scoparone and rutin was 0.999, 0.999, 0.999 and 0.998, respectively. The detection limit (S/N = 3:1) was 0.03 μg/mL for chlorogenic acid, scoparone and caffeic acid and 0.14 μg/mL for rutin. This method is simple and sensitive and has been applied successfully to determine the level of chlorogenic acid, caffeic acid and scoparone in *Herba artemisiae scopariae*.

Key Words: *Herba artemisiae scopariae*, Bioactive components, HPLC.

INTRODUCTION

Herba artemisiae scopariae is a widely used traditional Chinese medicine that is prepared from the dried sprout of *Artemisia scoparia waldst.* et Kit. It is often used as an important ingredient in many traditional prescriptions[1-3]. Besides having a cholagogic effect, it also has other pharmacological actions, such as protecting the liver, lowering blood pressure, eliminating fever, sedation and antiinflammation, antibacteria, antipathogenic-microbes and antitumor action[4,5]. It has many clinical applications in the treatment of acute icteric infectious hepatitis, hyperlipemia and oral ulcers[6,7]. Recent investigations by the state administration of traditional Chinese medicine suggest that it can also be used in combination with *Herba houttuyniae, Flos chrysanthemi indici, Herba eupatorii* and *Fructus tsaoko* for the treatment and prevention of SARS[8]. Hence, it is essential to establish a rapid, simple and accurate approach for determining bioactive components.

Chlorogenic acid, caffeic acid, scoparone and rutin (the structures are shown in Fig. 1) are four important constituents in *Herba artemisiae scopariae*. 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[1,2]. So far, many studies have examined chlorogenic acid in *Herba artemisiae scopariae*[9-11]. However, a single or a few marker compounds cannot accurately reflect the quality of *Herba artemisiae scopariae* due to the many constituents involved in its therapeutic effect.

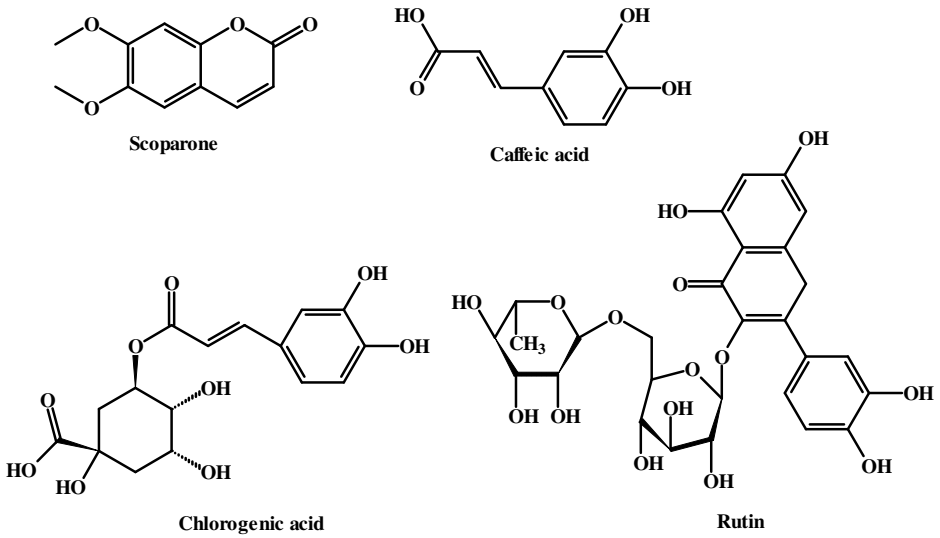

Fig. 1. Structures of four compounds

In this study, a simple and rapid HPLC method was developed to determine these four components simultaneously in *Herba artemisiae scopariae*. This method provides a way of evaluating the quality of *Herba artemisiae scopariae* in market-places and is an alternative approach for quality control in factories producing medicines as well as in investigations of other related plants.

EXPERIMENTAL

HPLC analysis was performed using a liquid chromatography system containing a waters 600s multisolvent delivery system and a waters 616 pump (waters, Milford, MA, USA), a waters 486 tunable absorbance UV detector (waters, Milford, MA, USA) and a Rheodyne injection valve (20 μL sample loop). Autochro-2000 software (Younglin Co. Ltd., Korea) was used as data acquisition system. The analytical column (250 mm × 4.6 mm i.d.) was packed with C_{18} stationary phase (Particle size 5 μm, R. Stech, Korea).

Chlorogenic acid, caffeic acid, scoparone and rutin 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 and acetic

acid were obtained from Duksan Pure Chemical Co., Ltd. (Ansan, Korea). All the other reagents used in the experiment were of the highest grade commercially available. Double distilled water was filtered with a vacuum pump (Division of Millipore, waters, USA) and filter (HA-0.45, division of millipore, waters, USA) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, Whatman, USA) before injection into the HPLC system.

Preparation of standard solutions and sample solution: The stock solution of four compounds at 1.0 mg/mL were first prepared in methanol and according to requirement to diluted to different concentrations with methanol.

Herba artemisiae scopariae was pulverized and 0.5 g of the resultant powder was weighed and extracted with 20.0 mL methanol/water (60:40, v/v) for 40 min in an ultrasonic bath. After centrifugation, the extract was collected as stock solution.

RESULTS AND DISCUSSION

Effect of the mobile phase: The mobile phase plays a key role in HLPC. It directly effects the retention time and resolution of the analytes. Therefore, it is important to choose a suitable mobile phase. Different compositions of mobile phase were examined, such as methanol/water, acetonitrile/water and *n*-propanol/water. The optimum mobile phase was acetonitrile/water when a short analysis time and high resolution were considered. In this experiment, acetonitrile/water with different compositions (20:80, 25:75, 40:60, v/v) were studied. The result showed that the analysis time decreased with increasing acetonitrile concentration. However, chlorogenic acid, caffeic acid and scoparone could not be baseline separated. Considering both the resolution and the analysis time as a whole, acetonitrile/water (25:75, v/v) was selected for subsequent investigations.

Effect of mobile phase additives: It was difficult to separate chlorogenic acid, caffeic acid and scoparone without additives to the mobile phase. Therefore, 0.1 % (v/v) acetic acid was added to improve separation. Different concentrations of acetic acid and trifluoroacetate were added to the mobile phase to determine the influence of these additives. The result showed that there were little difference in retention time and resolution with increasing acid concentration. Hence, acetonitrile/water/acetic acid (25:75:0.1, v/v/v) was selected for the experiment.

Gradient eluted program: When acetonitrile:water:acetic acid (25:75:0.1, v/v/v) was used as the mobile phase, the retention time of scoparone was 15 min longer than caffeic acid. Therefore, a gradient eluted program was applied to shorten the time. The best resolution and shortest analysis time was obtained when the mobile phase was changed linearly from acetonitrile:water:acetic acid (25:75:0.1, v/v/v) (A) to acetonitrile/water (65:35, v/v) (B) within 10 min.

Optimization of extraction condition: A sample pretreatment is one of the most important procedures for Chinese medicine analysis on account of the complexity of the matrices of herbs. Some methods, such as ultrasonics, microwave, heat refluxing and dipping have been used to extract herbal medicine. In preliminary

studies in the selection of extraction solvents, methanol, which can effectively extract a wide variety of compounds with different polarities, was found to be the best choice. The extraction efficiencies of methanol-water at different ratios were examined with ultrasonic extraction. The results showed that different methanol-water ratios significantly affected the extraction efficiencies of chlorogenic acid and rutin (Fig. 2). The content of rutin decreased gradually with decreasing methanol-water ratios. Considering the relatively satisfactory extraction efficiencies of these four compounds, methanol:water (60:40, v/v) was selected as the extraction solvent for further study.

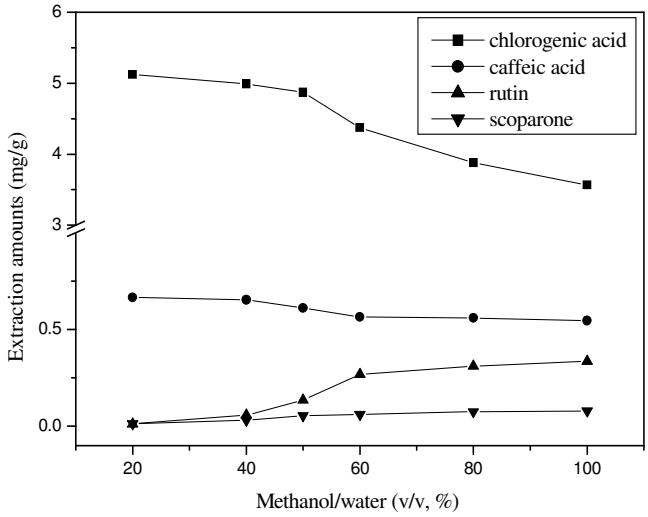

Fig. 2. Effect of methanol-water ratios on extraction amount

The influence of the different extraction times (20, 30, 40, 50 and 60 min) was examined. As shown in Fig. 3, the extraction efficiency improved increasing extraction time, while there was little improvement after 40 min. Therefore, 40 min was considered the optimal time.

Linearity, reproducibility and limits of detection: A series of standard solutions containing chlorogenic acid, caffeic acid, scoparone and rutin at six concentrations (0.5, 1.0, 4.0, 8.0, 10 and 15 μg/mL) were obtained by mixing the appropriate amount of stock solution (1 mg/mL). Each concentration was analyzed in triplicate. As a result, linear regression equations (Y = aX + b) of the four compounds were obtained within the concentration range investigated. Here Y represents the peak area of the analytes and X represents the concentration of the analytes. The results are listed in Table-1. The detection limit (S/N = 3:1) was 0.03 μg/mL for chlorogenic acid, caffeic acid and scoparone and 0.14 μg/mL for rutin. The results showed good precision with a relative standard deviation (RSD) for chlorogenic acid, caffeic acid, scoparone and rutin of 3.67, 3.21, 1.24 and 2.35 %, respectively.

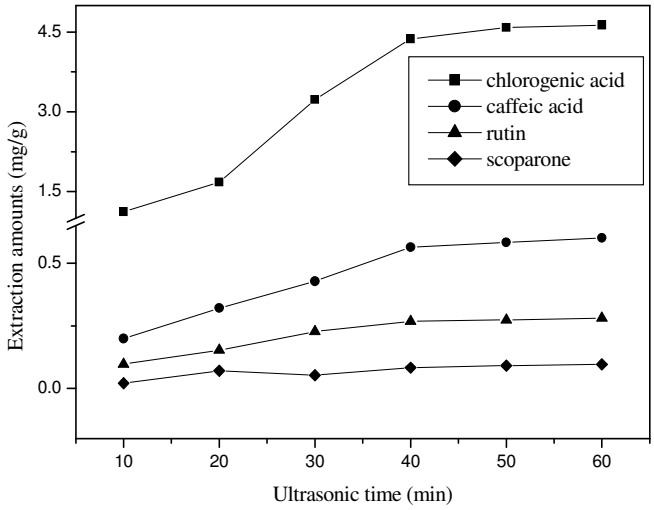

Fig. 3. Effect of ultrasonic time on extraction amount

TABLE-1
REGRESSION EQUATIONS AND DETECTION LIMITS OF CHLOROGENIC ACID, CAFFEIC ACID, SCOPARONE AND RUTIN

Sample	Regression equation	Linear range (μg/mL)	r^2	Detection limit (μg/mL)
Chlorogenic acid	Y=25544X – 41.56	1.0-100.0	0.9999	0.03
Rutin	Y=11320X – 63.93	5.0-500.0	0.9997	0.14
Caffeic acid	Y=52782X + 19.15	1.0-100.0	0.9998	0.03
Scoparone	Y=26076X – 18.64	1.0-100.0	0.9980	0.03

Determination of four compounds in traditional Chinese medicines: The developed HPLC method was applied successfully to the analysis of chlorogenic acid, caffeic acid, scoparone and rutin in *Herba artemisiae scopariae* under the optimum conditions. Fig. 4 showed the chromatograms of *Herba artemisiae scopariae*. Chlorogenic acid, caffeic acid, scoparone, rutin and other unknown sample matrix components could be baseline resolved within 20 min. Table-2 shows the contents of the four compounds and recoveries in *Herba artemisiae scopariae*.

Conclusion

The developed HPLC method offers a simple and reliable approach for the determination of chlorogenic acid, caffeic acid, scoparone and rutin in traditional Chinese herbal medicines in terms of both chromatographic conditions and sample preparation. These four compounds were resolved in 20 min. The calibration curve was linear over the range, 1-100 μg/mL, for chlorogenic acid, caffeic acid and scoparone and 5-500 μg/mL for rutin with a correlation coefficient of 0.999, 0.999, 0.999 and 0.998, respectively. The detection limit (S/N = 3:1) was 0.03 μg/mL for chlorogenic acid, caffeic acid and scoparone and 0.14 μg/mL for rutin. This method is considered suitable for evaluating the quality of *Herba artemisiae scopariae*.

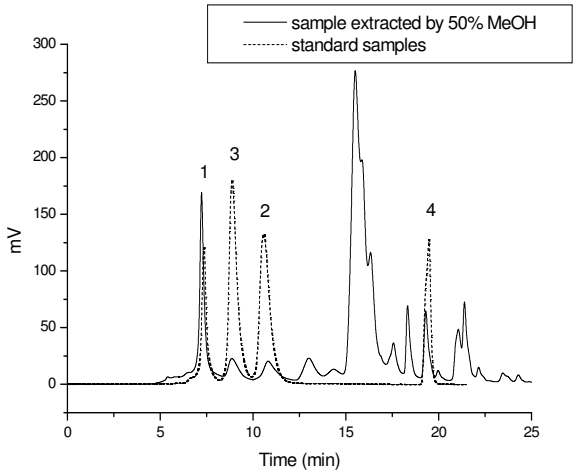

Fig. 4. Chromatograms of standard samples and the extract from natural plant. (Flow rate: 0.5 mL/min, inject volume: 5 μL, UV wavelength: 325 nm, 1: Chlorogenic acid, 2: Caffeic acid, 3: Rutin, 4: Scoparone)

TABLE-2
RECOVERY OF THE FOUR CHEMICAL COMPOUNDS

Analytes	Original (mg/g)	Added (mg/g)	Found (mg/g)	Recovery (%)	RSD (%)
Chlorogenic acid	4.373	5.000	8.943	89.4	3.67
Rutin	0.268	0.100	0.381	113.0	2.35
Caffeic acid	0.564	0.500	0.972	81.6	3.21
Scoparone	0.083	0.050	0.120	74.0	1.24

ACKNOWLEDGEMENT

This research was supported by Basic Science Research Program through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (2009-0072787).

REFERENCES

1. Committee of National Pharmacopoeia: Pharmacopoeia of People's Republic of China, Press of Chemical Industry, Beijing, p.166 (2005).
2. J.D. Cha, M.R. Jeong and S.I. Jeong, *Planta Med.*, **71**, 186 (2005).
3. T. Sawa, M. Nakao and T. Akaike, *J. Agric. Food Chem*., **47**, 397 (1999).
4. R. Niggeweg, A.J. Michael and C. Martin, *Nature Biotech.*, **22**, 746 (2004).
5. M. Ramezani, B.S. Fazli-Bazzaz and F. Saghafi-Khadem, *Fitoterapia*, **75**, 201 (2004).
6. Q.W. Zhang, Y.X. Zhang and Y. Zhang, *China J. Chin. Mater. Med*., **27**, 202 (2002).
7. T. Zhang and D.F. Chen, *J. Ethnopharmacol*., **117**, 351 (2008).
8. X.J. Song, Y.X. Zhang and Y. Zhang, *China J. Chin. Mater. Med*., **27**, 267 (2002).
9. S. Lin, Q.W. Zhang and N.N. Zhang, *China J. Chin. Mater. Med*., **30**, 591 (2005).
10. X.J. Tan, Q. Li and X.H Chen, *J. Pharm. Biomed. Anal*., **47**, 847 (2008).
11. X. Yao and G. Chen, *Anal. Bioanal. Chem*., **388**, 475 (2007).

(*Received*: 26 March 2009; *Accepted*: 14 December 2009) AJC-8189