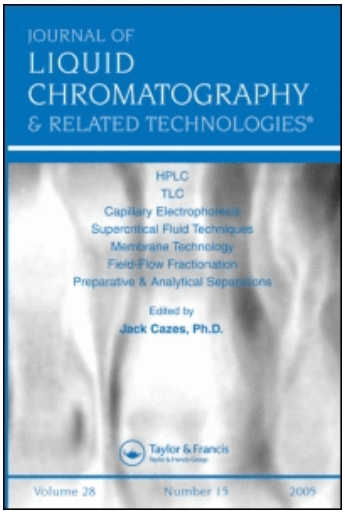

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Isolation of Four Compounds from *Herba Artemisiae Scopariae* by Preparative Column HPLC

Dandan Han[a]; Minglei Tian[a]; Kyung Ho Row[a]
[a] Department of Chemical Engineering, Inha University, Incheon, Korea

To cite this Article Han, Dandan , Tian, Minglei and Row, Kyung Ho(2009) 'Isolation of Four Compounds from *Herba Artemisiae Scopariae* by Preparative Column HPLC', Journal of Liquid Chromatography & Related Technologies, 32: 16, 2407 — 2416
To link to this Article: DOI: 10.1080/10826070903188120
URL: http://dx.doi.org/10.1080/10826070903188120

Journal of Liquid Chromatography & Related Technologies®, 32: 2407–2416, 2009

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826070903188120

Isolation of Four Compounds from *Herba Artemisiae Scopariae* by Preparative Column HPLC

Dandan Han, Minglei Tian, and Kyung Ho Row

Department of Chemical Engineering, Inha University, Incheon, Korea

Abstract: Reversed-phase high-performance liquid chromatography (HPLC) was applied on a preparative scale to purify chlorogenic acid, caffeic acid, scoparone, and rutin from *Herba Artemisiae Scopariae*. By changing the different extraction solvents, procedures, and times, the optimum extraction condition was established using methanol/water (60:40, v/v) as the extraction solvent followed by ultrasonic extraction for 40 min. Extraction amounts of the four compounds were 4.37 mg/g, 0.27 mg/g, 0.56 mg/g, and 0.083 mg/g, respectively. The column with 40/63 μm C_{18} as the particles successfully isolated the extract by using acetonitrile/water/acetic acid (20:80:0.1, v/v/v) as the mobile phase.

Keywords: Extraction and separation, *Herba Artemisiae Scopariae*, Preparative column

INTRODUCTION

Herba Artemisiae Scopariae (HAS) is one of the oldest medicinal herbs in traditional Chinese medicine. Pharmacological tests revealed that HAS has pharmacological activities such as protecting the liver, lowering blood pressure, eliminating fever, sedation, anti-inflammation, antibacteria, anti-pathogenic-microbes, and antitumor action.[1–5] It has been used to treat acute icteric infectious hepatitis, hyperlipidemia, and oral

Correspondence: Prof. Kyung Ho Row, Department of Chemical Engineering, Inha University, 253, Yonghyun-Dong, Nam-Gu, Incheon 402-751, Korea. E-mail: rowkho@inha.ac

ulcers. Recent studies by the State Administration of Traditional Chinese Medicine suggest that it also can be used in combination with *Herba Houttuyniae, Flos Chrysanthemi Indici, Herba Eupatorii*, and *Fructus Tsaoko* for the treatment and prevention of SARS.[6]

Chlorogenic acid, caffeic acid, scoparone, and rutin (the structures are shown in Fig. 1) are the four major active components of the herb, which are often used as the criteria in quality control of HAS.[7,8] There are some reports about the separation of chlorogenic acid, caffeic acid, scoparone, and rutin.[9–11] However, there is no established method for the simultaneous extraction and separation of these four compounds using a preparative column.

Preparative column chromatography is mainly used for analysis, preparation of pure samples, and especially for the isolation of active compounds from natural plants.[12–15] The preparative columns generally have larger diameters than analytical columns in order to handle larger samples. The size of the column depends on the amount of material required in pure form.

The aim of this study was to develop a method to extract chlorogenic acid, caffeic acid, scoparone, and rutin from HAS by RP-HPLC, and to separate the four compounds using a preparative column. Compared with previous reports, the present method is simple, rapid, and can be used to isolate the four compounds from HAS simultaneously.

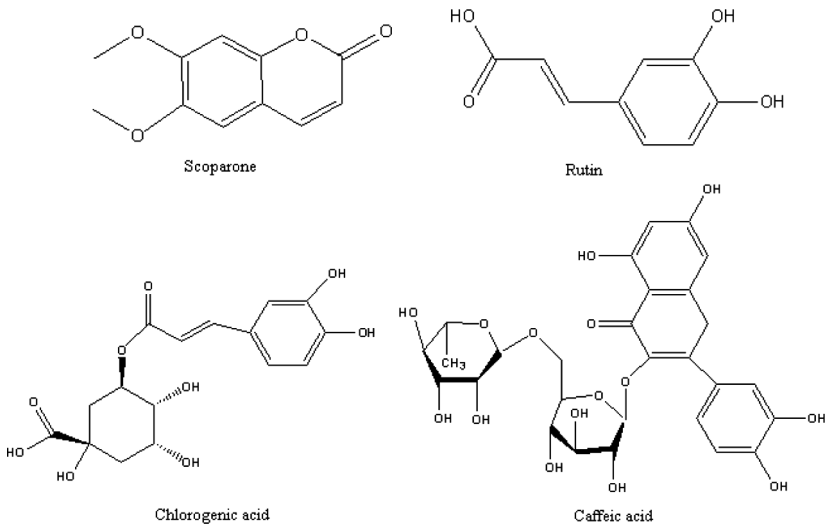

Figure 1. The structures of the four compounds.

EXPERIMENTAL

Apparatus

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) which was set to 325 nm as detection wavelength, and two Reodyne injection valves with 25 μL and 200 μL sample loop were used. Autochro-2000 software (Younglin Co. Ltd., Korea) was used as data acquisition system. The analytical column (C_{18}, 5 μm, 250 mm × 4.6 mm I.D.) was from RStech, Korea.

Chemicals and Materials

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 were used without further purification. HAS 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 the highest grade. Double distilled water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and filter (HA-0.45, Division of Millipore, Waters, U.S.A.) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, Whatman, U.S.A.) before injection into the HPLC system.

Preparation of Standard Solutions and Sample Solution

The stock solution of four compounds, at 1.0 mg/mL, was prepared in methanol. HSA was pulverized and 1.0 g of the powder was extracted with 20.0 mL methanol/water (60:40, v/v) for 40 min in an ultrasonic bath (repeated three times) and extracts were combined. After centrifugation and filtration, the extract was collected and stored for injection.

Column Preparation

An HPLC column was packed by using 40/63 μm C_{18} particles (YMC Co., Kyoto, Japan) as stationary phase. The C_{18} particles were suspended in methanol and degassed by helium. The slurry was pressed into the

hollow HPLC columns (250 mm × 4.6 mm I.D.) using a pump. Then, the packed column was washed with methanol until a stable baseline was observed.

Separation of Extract by Analytical Column and Preparative Column

In order to determine the maximum injection volume of the analytical column, 10, 20, and 50 μL injection volumes were used. Also, different volumes of extracts from HAS were injected into the preparative column.

RESULTS AND DISCUSSION

Optimization of Separation Condition

Different elution methods (isocratic and gradient mode) and different mobile phases (methanol/water/acetic acid and acetonitrile/water/acetic acid, v/v) were examined. On an analytical scale, the analysis was normally performed in gradient mode in order to save time, because scoparone had a much longer retention than the other three compounds. However, it was difficult to separate the four compounds in the preparative column; this resulted from lower column efficiency due to the larger particles so, considering the retention time and resolution, acetonitrile/water/acetic acid (20:80:0.1, v/v/v) by isocratic mode was selected, at a flow rate of 0.5 mL/min.

Optimization of Extraction Conditions

Sample pretreatment is one of the most important procedures for natural plant analysis due to the complexity of the matrices of herbs. Some methods, such as ultrasonics, heat refluxing and dipping, have been used to extract herbal medicines. In our study, all these methods were investigated; the result showed that ultrasonic extraction was an effective extraction method and had the highest extraction efficiency compared with other methods.

The extraction solvent is another key factor in the extraction efficiency. Different solvents, such as water, ethanol, methanol, ethyl acetate, and chloroform were studied. It was found that methanol was the best choice to extract rutin and scoparone, while water was good for extracting chlorogenic acid and caffeic acid. So the extraction efficiencies of methanol–water at different ratios were examined. The results are shown in Fig. 2. Considering the relatively satisfactory extraction

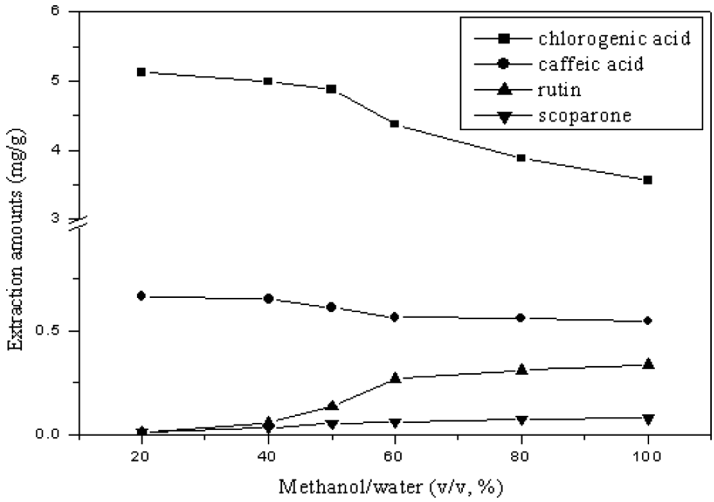

Figure 2. The effect of methanol–water ratios on the extraction amount of chlorogenic acid, caffeic acid, scoparone, and rutin.

efficiencies of these four compounds, methanol/water (60:40, v/v) was selected as the extraction solvent for further studies.

The influence of the different extraction times (20, 30, 40, 50, and 60 min) was examined. As shown in Fig. 3, the extraction efficiency

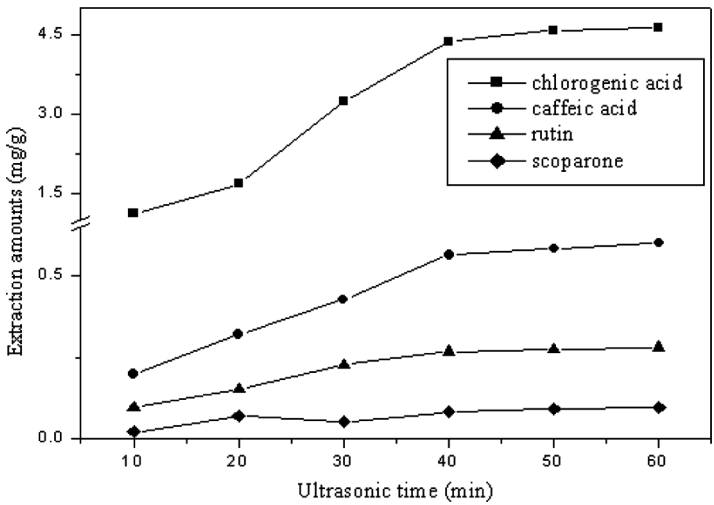

Figure 3. The effect of ultrasonic time on the extraction amount of chlorogenic acid, caffeic acid, scoparone, and rutin.

improved with increasing extraction time, while there was little improvement after 40 min. Therefore, 40 min was considered to be the optimal extract time. So, the optimum extraction method was methanol/water (60:40, v/v) as solvent, ultrasonically extracted for 40 min.

Linearity, Reproducibility and Limits of Detection

A series of mixtures of standard solutions containing chlorogenic acid, caffeic acid, scoparone, and rutin were diluted (0.5, 1.0, 4.0, 8.0, 10.0, and 15.0 μg/mL) with methanol. As a result, linear regression equations ($Y = aX + b$) of the four compounds were obtained within the concentration range studied. Here Y represents the peak area of the analytes, and X represents the concentration of the analytes. The results of regression analyses and the correlation coefficients (r^2) are listed in Table 1. The high correlation coefficient values ($r^2 > 0.9997$) indicated good linearity between their peak areas (y) and investigated compound concentrations (x, μg/mL) in a relatively wide concentration range. The limits of detection (LOD) were also determined with a signal-to-noise ratio of 3 (S/N = 3:1) and the LOD was 0.03 μg/mL for chlorogenic acid, caffeic acid, and scoparone, and 0.14 μg/mL for rutin.

Determination of Four Compounds in Traditional Chinese Herba

The developed HPLC method was applied successfully to the analysis of chlorogenic acid, caffeic acid, scoparone, and rutin in HAS under the optimum conditions. Figure 4 shows the chromatogram of the extract from HAS on the analytical column. Chlorogenic acid, caffeic acid, scoparone, and rutin were obtained with baseline separation.

The recoveries of the four compounds were determined by the method of standard additions. Suitable amounts (about one time of the

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

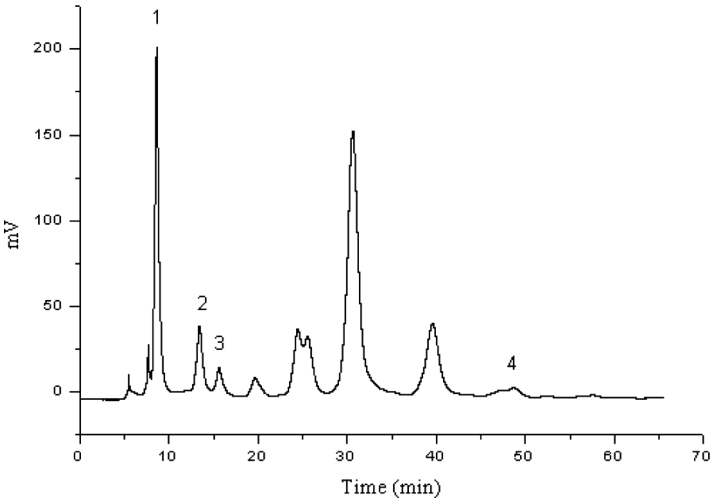

Figure 4. Chromatogram of the extracts of HAS by analytical column (Column: C_{18}, 5 μm, 4.6 * 250 mm, injection volume: 50 μL, Flow rate: 0.5 mL/min, detection wavelength: 325 nm) (1) chlorogenic acid, (2) caffeic acid, (3) scoparone, and (4) rutin.

content) of the four compounds were spiked into a sample of HAS, which had been determined previously. The mixture was extracted and analyzed using the proposed sample preparation procedure. For comparison, an unspiked sample was concurrently prepared and analyzed simultaneously. As shown in Table 2, the mean recoveries of the four compounds were 74.0–113.0%, with RSD values ranging from 1.24 to 3.17% (n = 5).

Effect of Injection Volumes by Different Particle Sizes

Larger injection volumes were used to determine the effect of the injection volumes in the analytical column. The areas of the peaks increased

Table 2. Recovery of the four compounds

Analytes	Original (mg/g)	Added (mg/g)	Found (mg/g)	Recovery (%)	RSD (%) n = 5
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

with increasing injection volume, as shown in Fig. 5. When the injection volume was larger than 50 μL, chlorogenic acid and caffeic acid could be separated well but caffeic acid and rutin could not. Therefore, the maximum injection volume of the analytical column was determined to be less than 50 μL.

For separation of the extract on the preparative column, an isocratic mode has the great advantage of being a simpler apparatus, thus making it particularly useful. In this study, the mobile phase compositions were varied with the particle sizes in order to separate the four compounds on the preparative column. Acetonitrile/water/acetic acid (20:80:0.1, v/v/v) was used as the mobile phase. Figure 6 shows the chromatograms at the particle sizes of 40/63 μm with different injection volumes. With the injection volume increasing, the area of the peaks increased, but the resolution decreased. The four target compounds could be determined until 150 μL extract was injected into the preparative column. As the injection volume became lager than 150 μL, the four compounds could not be separated from each other or interferences. Hence, from the results, it was determined that particle size of 40/63 μm could be used in the preparative column and the maximum injection volume was less than 150 μL.

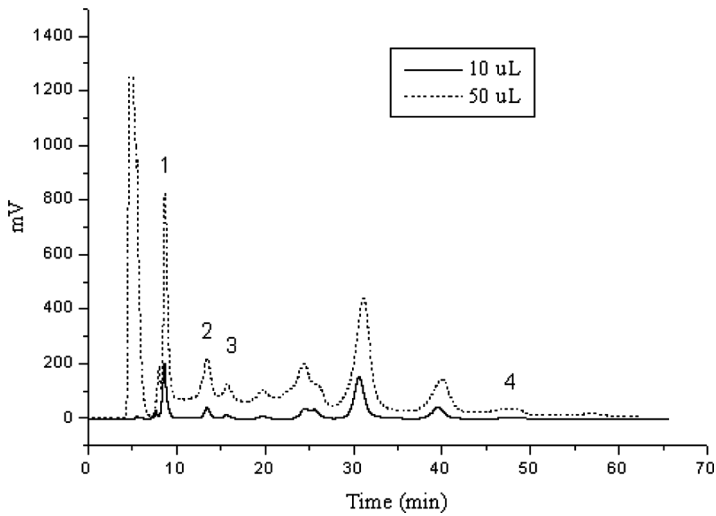

Figure 5. Chromatogram of the extracts of HAS by analytical column with different injection volumes (Column: C_{18}, 5 μm, 4.6 × 250 mm, 10, 20 and 50 μL, FR: 0.5 mL/min, 325 nm) (1) chlorogenic acid, (2) caffeic acid, (3) scoparone, and (4) rutin.

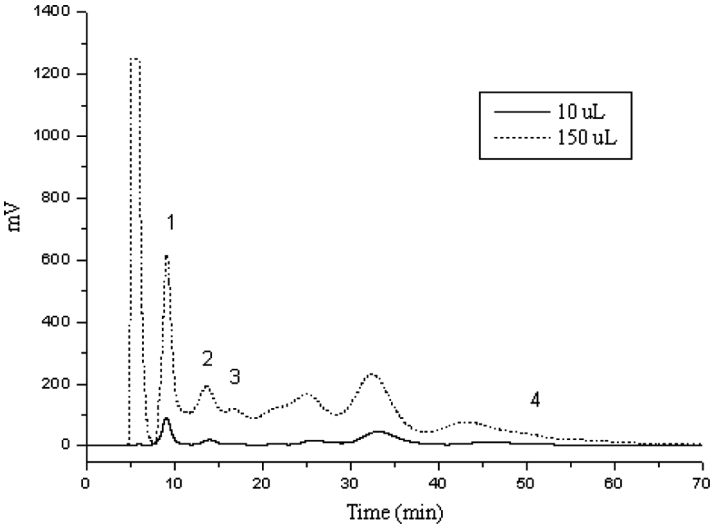

Figure 6. Chromatogram of the extracts of HAS by preparative column with different injecttion volumes (Column: C_{18}, 40/63 μm, 4.6 × 250 mm, 10, 50, and 150 μL, FR: 0.5 mL/min, 325 nm) (1) chlorogenic acid, (2) caffeic acid, (3) scoparone, and (4) rutin.

CONCLUSION

Based on the resolutions and separation times, the optimized separation condition and extraction method were established for the analysis and extraction of chlorogenic acid, caffeic acid, scoparone, and rutin from HAS using a preparative column. The extraction amounts of the four compounds were 4.37 mg/g, 0.27 mg/g, 0.56 mg/g and 0.083 mg/g, respectively. The preparative column with 40/63 μm C_{18} particles can isolate the target compounds well from 150 μL extract from HAS.

ACKNOWLEDGMENT

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, China, 2005; 166.

2. Cha, J.D.; Jeong, M.R.; Jeong, S.I. Planta Medica **2005**, *71* (2), 186–190.
3. Niggeweg, R.; Michael, A.J.; Martin, C. Nature Biotechnol. **2004**, *22* (6), 746–754.
4. Ramezani, M.; Fazli-Bazzaz, B.S.; Saghafi-Khadem, F. Fitoterapia **2004**, *75* (2), 201–203.
5. Zhang, Q.W.; Zhang, Y.X.; Zhang, Y. China J. Chin. Mater. Med. **2002**, *27* (4), 202–204.
6. Zhang, T.; Chen, D.F. J. Ethnopharmacol. **2008**, *117* (2), 351–361.
7. Song, X.J.; Zhang, Y.X.; Zhang, Y.; Zhang, Q.W. China J. Chin. Mater. Med. **2002**, *27* (4), 267–269.
8. Lin, S.; Zhang, Q.W.; Zhang, N.N.; Zhang, Y.X. China J. Chin. Mater. Med. **2005**, *30* (8), 591–594.
9. Tan, X.J.; Li, Q.; Chen, X.H. J. Pharm. Biomed. Anal. **2008**, *47* (4–5), 847–853.
10. Yao, X.; Chen, G. Anal. Bioanal. Chem. **2007**, *388* (2), 475–481.
11. Zhang, Q.W.; Zhang, Y.X.; Zhang, Y.; Liang, G.G. China J. Chin. Mater. Med. **2002**, *27* (1), 23–25.
12. Kang, D.H.; Row, K.H. J. Chromatogr. A **2002**, *949* (1–2), 217–223.
13. Kim, I.J.; Hong, S.B.; Row, K.H. J. Chromatogr. A **2002**, *949* (1–2), 275–280.
14. Kao, T.H.; Lu, Y.F.; Chen, B.H. Eur. Food Res. Technol. **2005**, *221* (3–4), 459–465.
15. Tian, M.L.; Yan, H.Y.; Row, K.H. Biotechnol. Bioproc. Eng. **2008**, *13* (6), 671–676.

Received March 6, 2009
Accepted April 14, 2009
Manuscript 6512