High-performance liquid chromatography determination and pharmacokinetics of coumarin compounds after oral administration of Samul-Tang to rats

Youn-Hwan Hwang, Won-Kyung Cho, Doorye Jang, Jeong-Ho Ha, Jin Yeul Ma

Korean Medicine-Based Herbal Drug Development Group, Korea Institute of Oriental Medicine, South Korea

Submitted: 05-04-2013 Revised: 01-05-2013 Published: 07-02-2014

Access this article online
Website: www.phcog.com
DOI: 10.4103/0973-1296.126656
Quick Response Code:

ABSTRACT

Background: Samul-tang has been traditionally used for the treatment of cardiovascular, gynecologic, cutaneous, and chronic inflammation disorders. Although coumarin compounds do have various pharmacological activities and the same may be present in Samul-tang, however there is little information about it. **Objective:** A simple and sensitive high-performance liquid chromatography (HPLC) method has been developed for the determination of nodakenin, nodakenetin, decursin, decursinol, and decursinol angelate in rat plasma. To obtain a better understanding for pharmacological properties of Samul-tang, pharmacokinetic study of coumarin compounds was performed after oral administration of Samul-tang in rats. **Materials and Methods:** Chromatographic separation of the analytes was successfully achieved on a Phenomenex Luna C_{18} column (4.6 mm × 250 mm, 5 μm) using a mobile phase composed of acetonitrile water with a gradient elution at a flow rate of 1 mL/min. Noncompartmental analysis was performed. **Results:** Calibration curves for all analytes had good linearity ($r^2 < 0.999$) in a wide linear range. The lower limit of quantification (LLOQ) ranged from 0.05 to 0.1 μg/mL. The variation of intra- and interday assay was less than 15%. Nodakenin, nodakenetin, and decursinol were determined in rat plasma after oral administration of Samul-tang. **Conclusion:** This developed and validated HPLC method was successfully applied to the pharmacokinetic study of three coumarin compounds in rats, given as a single oral administration of Samul-tang. These pharmacokinetic data of the nodakenin, nodakenetin, and decursinol could offer a new point of view to evaluate the pharmacological effects of Samul-tang.

Key words: Decursinol, nodakenin, nodakenetin, pharmacokinetics, rat

INTRODUCTION

Samul-tang (*Si-Wu-tang* in Chinese, *Shimotsu-to in* Japanese); composed of *Angelicae gigantis* Radix, Cnidii Rhizoma, Paeonia Radix, and Rehmanniae Radix in the Korean Pharmacopoeia; has been traditionally used to treat cardiovascular, gynecologic, cutaneous, and chronic inflammation disorders.[1] Recently, Samul-tang was reported to exert anti-inflammatory, antiallergic, and antipruritic properties.[2,3] Major bioactive components in Samul-tang are well-known to contain phenolics (e.g. gallic acid, ferulic acid), terpene glycosides (e.g. albiflorin, paeoniflorin, paenol), phthalides (e.g. Z-ligustilide, senkyunolide A, ligustrazine, butylphthalide), and iridoid glycosides (e.g. catalpol).[1,4] However, although herbal medicines in Samul-tang contain various coumarin compounds, there is little information about them. Especially, many researchers found that *Angelica gigantis* Radix contain coumarin compounds such as nodakenin, nodakenetin, decursinol, decursinol angelate, and decursin.[5-7] These coumarin compounds have various biological properties including anticancer, anti-inflammatory, antibacterial, and antioxidant activities.[8-10] To provide helpful information for the pharmacological and clinical effect of Samul-tang, the pharmacokinetic study of coumarin compounds and a quantitative analytical method for the determination of coumarin compounds are required.

Address for correspondence:
Dr. Jin Yeul Ma, 1672 Yuseongdae-ro, Yuseong-gu, Daejeon - 305811, South Korea. E-mail: jyma@kiom.re.kr

According to the literature, several high-performance liquid chromatography ultraviolet (HPLC-UV)[11] and HPLC/electrospray ionization mass spectrometry (ESI-MS) method[7,12] were used to determine the coumarin compounds in single herbs and to analyze their preparation. In addition, several HPLC-UV[13-16] and HPLC/ESI-MS method[5] to determine nodakenin, nodakenetin, and decursin/decursinol angelate were developed and validated in animal plasma and tissue. However, there is no analytical method to determine five coumarin compounds simultaneously and to apply the pharmacokinetics of those compounds, although the mixture of those compounds in single herbal medicines and formulations were commonly used. In this study, a simple and sensitive HPLC-UV method for determining five coumarin compounds in rat plasma was developed, validated, and successfully applied to pharmacokinetic study of coumarins in Samul-tang.

MATERIALS AND METHODS

Chemicals and reagents

Nodakenin, nodakenetin, decursinol, and decursin and decursinol angelate obtained from Korea Food and Drug Administration (KFDA, Osong, South Korea). Isoliquiritin used as an internal standard (IS) were purchased from Wako Chemicals (Osaka, Japan). The chemical structures of five coumarin compounds and IS are shown in Figure 1. HPLC-grade acetonitrile, methanol, and water were purchased from J.T. Baker Inc. (Philipsburg, NJ, USA). Other chemicals with analytical grade were purchased from Sigma-Aldrich Inc.

All herbal medicines were purchased from Yeongcheon traditional herbal market (Yeongcheon, South Korea). Samul-tang was prepared in accordance with the method previously described.[17] Brownish powder (403.5 ± 5.4 g) of Samul-tang was obtained and stored at 4°C before use. The amounts of nodakenin, nodakenetin, decursinol, and decursin/decursinol angelate in Samul-tang were 2.29 ± 0.14, 0.08 ± 0.01, 0.094 ± 0.01, and 5.24 ± 0.12 mg/g extract, respectively, according to previous report.[7]

Chromatographic condition and sample preparation

Plasma concentrations of five coumarin compounds were determined using a HPLC system (Lachrom Elite, Hitach High-Technologies Corp, Tokyo, Japan) equipped an binary pump, an autosampler, a column oven, and a diode array detector. The analytes and IS were separated on a Phenomenex Luna C_{18} column (4.6 mm × 250 mm, 5.0 μm, Torrance, CA, USA) and eluted with a gradient of deionized water (A) and acetonitrile (B). The gradient elution was programmed as follows: 35-50% (v/v) B at 0-6 min; 50-100% B at 6-14 min; 100-100% B at 14.0-18.0 min. The column temperature was 30°C and the flow rate was 1.0 mL/min.

The analytes and IS in rat plasma was extracted with a liquid-liquid extraction (LLE) method. One hundred microliters of plasma samples, 100 μL of acetonitrile, and 10 μL of IS (2.0 μg/mL) were mixed. The mixture was vortexed for 5 min and then centrifuged at 10,000 g for 10 min. Then 20 μL of aliquot was injected into the HPLC system. All analytes were detected at 330 nm.

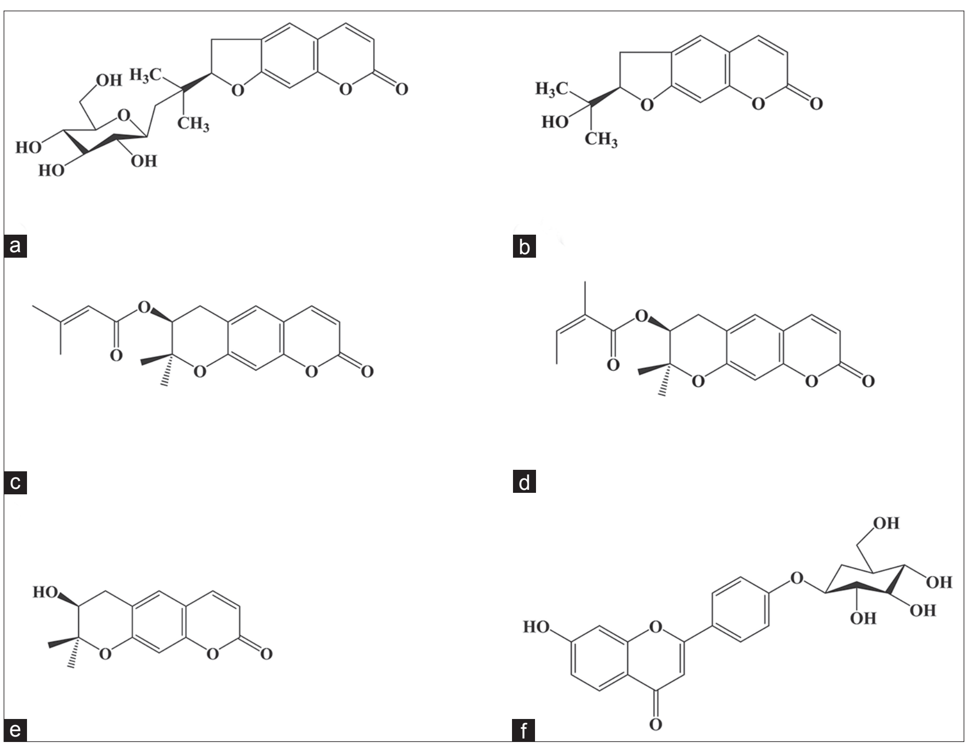

Figure 1: Chemical structures of the five coumarin compounds ((a) nodakenin; (b) nodakenetin; (c) decursin; (d) decursinol angelate; (e) decursinol) and isoliquiritin ((f), IS)

Preparation of stock solution, calibration samples, and quality control samples

Standard stock solutions of analytes and IS were prepared by dissolving 1 mg/mL in methanol. Especially, decursin and decursinol angelate (1:1, v/v) were mixed at each concentration of 0.5 mg/mL. Working solutions were freshly prepared by serial dilutions in methanol. Calibration samples were prepared by 10 μL of working solutions in 90 μL of drug-free rat plasma to obtain final concentrations in the range of 0.1-10.0 μg/mL for nodakenin and 0.05-10.0 μg/mL for nodakenetin, decursinol, and decursin/decursinol angelate. QC samples were prepared at low, medium, and high concentration of 0.1, 0.5, and 5.0 μg/mL, respectively, in the same manner.

Method validation

Drug-free rat plasma were analyzed for the determination of any endogenous interferences at the peak region of each analyte and IS comparing the plasma spiked analytes and IS.

The calibration curves were constructed with linear least squares regression and demonstrated the linearity of this method, based on the peak area ratio of analytes and IS. The lower limit of quantification (LLOQ, signal-to-noise ratio >10) was defined as the lowest concentration of analytes the lowest concentration at which the analytes can be quantified with an accuracy of ± 15% and a precision ≤20%.

QC samples for the determination of intraday accuracy and precision were freshly prepared and analyzed on the same day. For interday accuracy and precision, the analyses of QC samples were repeated for four consecutive days. Accuracy was calculated as the percent error (bias, %) between the measured concentration and the nominal concentration of QC samples. Precision expressed as relative standard deviation (RSD, %).

Recoveries of analytes were determined by comparing the peak area ratio of analytes and IS of QC samples to that of intact working standard in methanol. QC samples at three concentrations were used by analyzing in triplicate. Stability of analytes in rat plasma was tested with QC samples which were stored under different temperature conditions (room temperature and -20°C) for the desired time. Subsequently, postpreparative stability was evaluated using the prepared samples after storage at 4°C for 24 h.

Application of pharmacokinetics

Animal experiment was approved from the Institutional Animal Care and Use Committee of Korea Institute of Oriental Medicine (Daejeon, South Korea). Ten male Sprague-Dawley rats (260-280 g) were obtained from Samtaco (Osan, South Korea). After acclimation in a controlled environment for 1 week, rats were randomly divided into two-dose groups ($n = 5$). After the preparation of Samul-tang in distilled water, the animals were administered by single oral gavage (2.5 or 10 g/kg). Blood samples (300 μL) were collected from the caudal vein at 0, 20, and 40 min and 1, 2, 3, 4, 6, 8, 12, 14, 18, 20, 24, 30, 36, and 48 h after administration. The blood samples were collected in ethylediaminetetraacetic acid (EDTA)-contained tubes and centrifuged at 4,000 × g for 10 min. The plasma samples were stored at -20°C prior to uses and were analyzed within 2 weeks after plasma sampling. The noncompartmental pharmacokinetic analysis was performed using PKSolver program.[18]

RESULTS AND DISCUSSION

Optimization of chromatographic condition and sample preparation

Several mobile phases were applied to separate analytes and IS in biological matrix including deionized water, 0.1% trifluoroacetic acid (TFA), 0.1% acetic acid, acetonitrile, and methanol. Finally, deionized water and acetonitrile were chosen as mobile phases with a gradient elution because of good separation and short chromatographic cycle. In addition, Gemini C_{18}(4.6 mm × 100 mm, 3.0 μm, Phenomenex, CA, USA), Agilent Eclips Plus C_{18}(4.6 mm × 100 mm, 3.5 μm, Agilent, Santa Clara, CA, USA), and TSKgel ODS-100V C_{18} (4.6 mm × 250 mm, 5.0 μm, TOSOH, Tokyo, Japan) column were compared. The Phenomenex Luna C_{18} column was selected on the basis of relatively short retention time, good peak shape, and excellent selectivity. The wavelength (330 nm) of detection and IS was chosen, based on UV spectrum of analytes and validation procedures.

Validation of HPLC method

Selectivity

The HPLC chromatograms of a blank plasma sample, a blank plasma sample spiked with analytes, and plasma sample after oral administration of Samul-tang were shown in Figure 2. Each peak of analytes in plasma was identified by comparing retention time and UV spectra of each standard. The retention times of nodakenin, nodakenetin, decursinol, decursin/decursinol angelate, and IS were 4.407, 7.893, 8.527, 13.107, and 11.147 min, respectively. There was no endogenous interference during the elution of all analytes and IS.

Linearity and LLOQ

Calibration curves with good linearity were covered in a wide linear range. The mean regression equations were $y = 0.605x + 0.0098$ ($r^2 < 0.999$) for nodakenin, $y = 1.002x + 0.0216$ ($r^2 < 0.999$) for nodakenetin,

$y = 0.987x + 0.0195$ (r^2 <0.999) for decursinol, and $y = 0.926x + 0.0188$ (r^2 <0.999) for decursin/decursinol angelate. The LLOQ of nodakenin, nodakenetin, decursinol, and decursin/decursinol angelate were 0.1, 0.05, 0.05, and 0.05 μg/mL, respectively. In this study, the calibration curves were in a sufficient range and sensitivity for pharmacokinetics of five coumarin compounds.

Accuracy and precision

In the present study, intra- and interday accuracy and precision with QC samples at three concentrations (0.1, 0.5, and 5.0 μg/mL) were evaluated and summarized in Table 1. The results indicated that this HPLC method is accurate and highly reproducible.

Recovery and stability

In this study, LLE method was used for the preparation of plasma sample. Various solvents, such as methanol, acetonitrile, ethyl acetate, and dichloromethane were tested for the extraction of analytes and IS from rat plasma. Consequently, acetonitrile showed relatively good recoveries from endogenous interferences in biological matrix. Absolute recoveries of all the analytes were 61.1 ± 3.2% for nodakenin, 67.3 ± 5.1% for nodakenetin, 65.7 ± 3.6% for decursinol, and 56.9 ± 4.3% for decursin/decursinol angelate. These results suggest no relevant difference of extraction recoveries at different concentration levels of all the analytes.

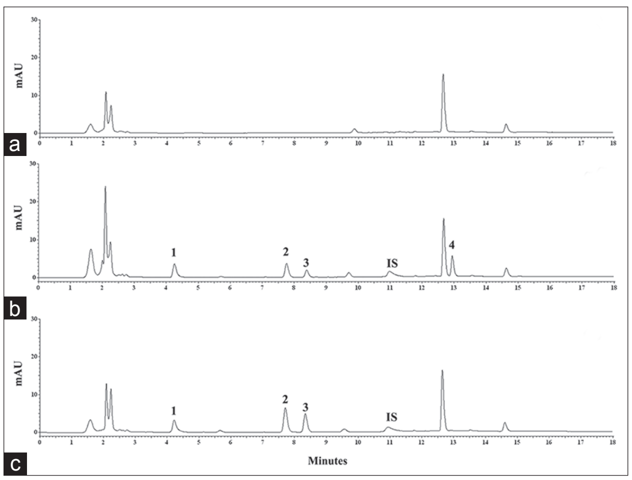

Figure 2: Representative chromatogram of coumarin compounds (1, nodakenin; 2, nodakenetin; 3, decursinol; 4, decursin/decursinol angelate). (a) blank plasma; (b) blank plasma spiked with the five coumarins; (c) plasma sample of the rat at 4 h after oral administration of Samul-tang

All stability tests were carried out using QC samples at three concentrations (0.1, 0.5, and 5.0 μg/mL). The recovered percentage of all the analytes were 97.8 ± 7.1% for nodakenin, 96.8 ± 6.3% for nodakenetin, 89.9 ± 10.6% for decursinol, and 95.2 ± 5.3% for decursin/decursinol angelate after 12 h storage at room temperature; and 99.0 ± 4.4% for nodakenin, 97.6 ± 6.8% for nodakenetin, 100.9 ± 5.9% for decursinol and 95.7 ± 5.9% for decursin/decursinol angelate after 4-weeks storage at -20°C. The postpreparative stability of analytes were 100.5 ± 6.1% for nodakenin, 101.1 ± 6.6% for nodakenetin, 97.8 ± 5.4% for decursinol, and 100.6 ± 6.5% for decursin/decursinol angelate after 24 h storage at 4°C. These results mean that the analytes did not markedly degrade during extraction procedures and storage in this study. The stability of all analytes is consistent with other reports.[14,15,19]

Pharmacokinetic study

The mean plasma concentration-time curves and pharmacokinetic parameters of coumarin compounds after oral administration of Samul-tang are shown in Figure 3 and Table 2, respectively. The T_{max} of nodakenin, nodakenetin, and decursinol were 9.20, 4.4, and 2.4 h, respectively. The mean C_{max} of nodakenin (1.26 versus 2.89 μg/mL), nodakenetin (0.9 versus 3.13 μg/mL), and decursinol (0.59 versus 3.09 μg/mL) significantly increased in accordance with oral doses of Samul-tang. The increases

Table 1: Precision and accuracy for coumarin compounds in rat plasma

Compounds	Nominal concentration	Intraday		Inter day	
	(μg/mL)	Precision (RSD, %)	Accuracy (bias, %)	Precision (RSD, %)	Accuracy (bias, %)
Nodakenin	5.0	6.0	−2.7	3.4	2.8
	1.0	7.6	2.4	4.9	−4.6
	0.5	14.1	6.6	11.0	−1.3
Nodakenetin	5.0	6.5	−3.5	10.2	−2
	1.0	7.7	−2.4	7.1	1.8
	0.5	12.7	5.4	13.3	−3.2
Decursinol	5.0	9.3	−7.3	4.6	3.3
	1.0	9.2	−3.9	5.0	−2.9
	0.5	13.8	9.6	11.1	−6.8
Decursin/decursinol angelate	5.0	4.5	0.4	8.0	−4.6
	1.0	5.2	0.1	6.2	−1.9
	0.5	8.8	2.4	14.1	2.6

RSD: Relative standard deviation

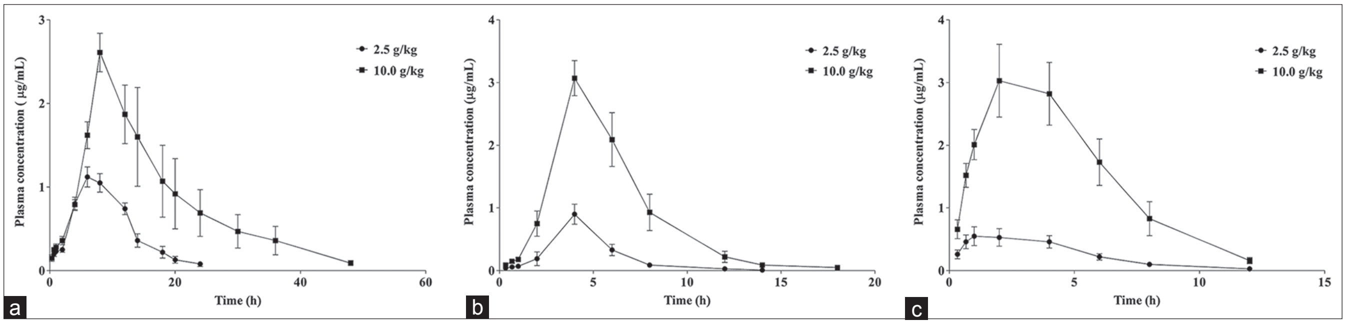

Figure 3: Plasma concentration-time curves of nodakenin (a), nodakenetin (b) and decursinol (c) after oral administraion of Samul-tang (2.5 or 10.0 g/kg) in rats.

Table 2: Pharmacokinetics parameters of nodakenin, nodakenetin, and decursinol after oral administration of Samul-tang in rats

Parameters	Nodakenin		Nodakenetin		Decursinol	
	2.5 g/kg	10.0 g/kg	2.5 g/kg	10.0 g/kg	2.5 g/kg	10.0 g/kg
λ_z	0.16±0.02	0.11±0.01	0.46±0.08	0.45±0.10	0.34±0.04	0.41±0.07
$t_{1/2\lambda z}$	4.55±0.46	6.23±0.51	1.69±0.31	2.12±0.70	2.18±0.26	1.99±0.46
T_{max} (h)	6.80	9.20	4.00	4.40	1.13	2.40
C_{max} (μg/mL)	1.26±0.07	2.89±0.35	0.90±0.16	3.13±0.30	0.59±0.14	3.09±0.57
$AUC_{0\rightarrow t}$ (h·μg/mL)	12.16±0.96	38.70±9.91	3.17±0.71	15.38±2.07	3.10±0.68	18.50±3.31
$AUC_{0\rightarrow\infty}$ (h·μg/mL)	12.78±1.21	39.71±9.98	3.21±0.73	15.59±2.01	3.18±0.71	19.06±3.21
MRT (h)	9.05±0.45	14.23±1.42	4.69±0.17	5.58±0.30	3.67±0.19	4.20±0.20

C_{max}: Peak plasma concentration; T_{max}: Time of peak concentration; $t_{1/2\lambda z}$: Terminal elimination half-life; AUC: Area under the concentration-time curve; MRT: Mean residence time

of $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow\infty}$ of nodakenin, nodakenetin, and decursinol were also dependent on the oral dose. Other pharmacokinetic parameters are consistent with previously reported reports.[14,20] This proposed method was successfully applied for the pharmacokinetic study of coumarin compounds in Samul-tang.

Unfortunately, decursin/decursinol angelate were not detected in our study, despite to their relatively high contents in Samul-tang with respect to other coumarin compounds. Kim *et al.*,[7] have reported that decursin and decursinol angelate from *A. gigas* is almost completely absorbed into blood stream with their parent forms via gastrointestinal tract and mainly excreted into feces through bile. On contrary, Park *et al.*,[21] demonstrated extensive hepatic first-pass metabolism of decursin. Similar to our results, decursin in plasma was rapidly decreased after intravenous administration and only decursinol was detected after oral administration of decursin. Therefore, decursin/decursinol angelate in Samul-tang could be natural prodrugs of decursinol.

To our knowledge, albiflorin and paeoniflorin as main active compounds in Samul-tang have been centrally studied and evaluated their anticoagulation effects and pharmacokinetic properties. Apart from aforementioned compounds, however, we found the absorption and pharmacokinetic characteristics of nodakenin, nodakenetin, and decursinol to have various biological properties after oral administration of Samul-tang, which is not reported yet. Nodakenin and nodakenetin, its aglycone, has antioxidant and anti-inflammatory effect and amelioration of scopolamine-induced learning and memory impairments.[22-24] Decursinol is well-known for its anticancer and analgesic effect, protection of septic shock, and activation of serotonergic system.[10,25,26] In addition, the decursin and nodakenin from the roots of *A. gigas* exhibited antiplatelet aggregation and blood coagulation.[8] Therefore, pharmacokinetic data of the coumarins in this study could provide helpful information to evaluate the pharmacological effects of Samul-tang.

CONCLUSION

A simple and sensitive HPLC method with a simple LLE procedure was developed and validated for the determination of nodakenin, nodakenetin, decursinol, and decursin/decursinol angelate. This method was not only accurate and precise, but also successfully applied to a pharmacokinetic study of nodakenin, nodakenetin, and decursinol in rats.

REFERENCES

1. Wang ZJ, Wo SK, Wang L, Lau CB, Lee VH, Chow MS, *et al.* Simultaneous quantification of active components in the

herbs and products of Si-Wu-Tang by high performance liquid chromatography-mass spectrometry. J Pharm Biomed Anal 2009;50:232-44.

2. Dai Y, But PP, Chan YP, Matsuda H, Kubo M. Antipruritic and antiinflammatory effects of aqueous extract from Si-Wu-Tang. Biol Pharm Bull 2002;25:1175-8.
3. Tahara E, Satoh T, Toriizuka K, Nagai H, Nunome S, Shimada Y, *et al*. Effect of Shimotsu-to (a Kampo medicine, Si-Wu-Tang) and its constituents on triphasic skin reaction in passively sensitized mice. J Ethnopharmacol 1999;68:219-28.
4. Tang Y, Zhu M, Yu S, Hua Y, Duan JA, Su S, *et al*. Identification and comparative quantification of bio-active phthalides in essential oils from si-wu-tang, fo-shou-san, radix angelica and rhizoma chuanxiong. Molecules 2010;15:341-51.
5. Kim MR, Abd El-Aty AM, Kim IS, Shim JH. Determination of volatile flavor components in danggui cultivars by solvent free injection and hydrodistillation followed by gas chromatographic-mass spectrometric analysis. J Chromatogr A 2006;1116:259-64.
6. Avula B, Joshi VC, Reddy VL, Choi YW, Khan IA. Simultaneous determination of eight coumarins in *Angelica gigas* and in various other Angelica species by high performance liquid chromatography and comparative micro-morphology study of *Angelica* species. Planta Med 2007;73:1509-16.
7. Ahn MJ, Lee MK, Kim YC, Sung SH. The simultaneous determination of coumarins in *Angelica gigas* root by high performance liquid chromatography-diode array detector coupled with electrospray ionization/mass spectrometry. J Pharm Biomed Anal 2008;46:258-66.
8. Lee S, Shin DS, Kim JS, Oh KB, Kang SS. Antibacterial coumarins from *Angelica gigas* roots. Arch Pharm Res 2003;26:449-52.
9. Li L, Li W, Jung SW, Lee YW, Kim YH. Protective effects of decursin and decursinol angelate against amyloid beta-protein-induced oxidative stress in the PC12 cell line: The role of Nrf2 and antioxidant enzymes. Biosci Biotechnol Biochem 2011;75:434-42.
10. Seo YJ, Kwon MS, Park SH, Sim YB, Choi SM, Huh GH, *et al*. The analgesic effect of decursinol. Arch Pharm Res 2009;32:937-43.
11. Sheng YX, Li L, Wang Q, Guo HZ, Guo DA. Simultaneous determination of gallic acid, albiflorin, paeoniflorin, ferulic acid and benzoic acid in Si-Wu decoction by high-performance liquid chromatography DAD method. J Pharm Biomed Anal 2005;37:805-10.
12. Concannon S, Ramachandran VN, Smyth WF. A study of the electrospray ionisation of selected coumarin derivatives and their subsequent fragmentation using an ion trap mass spectrometer. Rapid Commun Mass Spectrom 2000;14:1157-66.
13. Liu Z, Li F. Development and validation of a reliable high-performance liquid chromatographic method for determination of nodakenin in rat plasma and its application to pharmacokinetic study. Biomed Chromatogr 2011;25:1076-80.
14. Zhang P, Li F, Yang XW. Determination and pharmacokinetic study of nodakenin in rat plasma by RP-HPLC method. Biomed Chromatogr 2008;22:758-62.
15. Li L, Zhang J, Shaik AA, Zhang Y, Wang L, Xing C, *et al*. Quantitative determination of decursin, decursinol angelate, and decursinol in mouse plasma and tumor tissue using liquid-liquid extraction and HPLC. Planta Med 2012;78:252-9.
16. Zhang P, Yang XW. Biotransformation of nodakenin and simultaneous quantification of nodakenin and its aglycone in incubated system of human intestinal bacteria by HPLC method. J Asian Nat Prod Res 2009;11:371-9.
17. Hwang YH, Kim T, Cho WK, Jang D, Ha JH, Ma JY. Food- and gender-dependent pharmacokinetics of paeoniflorin after oral administration with Samul-tang in rats. J Ethnopharmacol 2012;142:161-7.
18. Zhang Y, Huo M, Zhou J, Xie S. PKSolver: An add-in program for pharmacokinetic and pharmacodynamic data analysis in Microsoft Excel. Comput Methods Programs Biomed 2010;99:306-14.
19. Zhang P, Yang XW. A new metabolite of nodakenetin by rat liver microsomes and its quantification by RP-HPLC method. Biomed Chromatogr 2010;24:216-21.
20. Song JS, Chae JW, Lee KR, Lee BH, Choi EJ, Ahn SH, *et al*. Pharmacokinetic characterization of decursinol derived from *Angelica gigas Nakai* in rats. Xenobiotica 2011;41:895-02.
21. Park HS, Kim B, Oh JH, Kim YC, Lee YJ. First-pass metabolism of decursin, a bioactive compound of *Angelica gigas*, in rats. Planta Med 2012;78:909-13.
22. Kim DH, Kim do Y, Kim YC, Jung JW, Lee S, Yoon BH, *et al*. Nodakenin, a coumarin compound, ameliorates scopolamine-induced memory disruption in mice. Life Sci 2007;80:1944-50.
23. Rim HK, Cho W, Sung SH, Lee KT. Nodakenin suppresses lipopolysaccharide-induced inflammatory responses in macrophage cells by inhibiting tumor necrosis factor receptor-associated factor 6 and nuclear factor-kappaB pathways and protects mice from lethal endotoxin shock. J Pharmacol Exp Ther 2012;342:654-64.
24. Zhao D, Islam MN, Ahn BR, Jung HA, Kim BW, Choi JS. *In vitro* antioxidant and anti-inflammatory activities of *Angelica decursiva*. Arch Pharm Res 2012;35:179-92.
25. Abdullayeva R, Ganiyev M, Asmetov V. Effects of diumancal and decursinol on 5-hydroxytryptamine level in rat brain. Georgian Med News 2009:84-8.
26. Jung JS, Yan JJ, Song DK. Protective effect of decursinol on mouse models of sepsis: Enhancement of interleukin-10. Korean J Physiol Pharmacol 2008;12:79-81.

Cite this article as: Hwang Y, Cho W, Jang D, Ha J, Ma JY. High-performance liquid chromatography determination and pharmacokinetics of coumarin compounds after oral administration of Samul-Tang to rats. Phcog Mag 2014;10:34-9.

Source of Support: This work was supported by the grant (No. K13050) from the Korea Institute of Oriental Medicine funded by the Ministry of Education, Science and Technology (MEST), the Republic of Korea, **Conflict of Interest:** None declared.