Quantification of Fargesin in Mouse Plasma Using Liquid Chromatography-High Resolution Mass Spectrometry: Application to Pharmacokinetics of Fargesin in Mice

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Abstract : Fargesin, a tetrahydrofurofuranoid lignan isolated from Flos Magnoliae, shows anti-inflammatory, anti-oxidative, anti-allergic, and anti-hypertensive activities. To evaluate the pharmacokinetics of fargesin in mice, a sensitive, simple, and selective liquid chromatography-high resolution mass spectrometric method using electrospray ionization and parallel reaction monitoring mode was developed and validated for the quantification of fargesin in mouse plasma. Protein precipitation of 6 μL mouse plasma with methanol was used as sample clean-up procedure. The standard curve was linear over the range of 0.2–500 ng/mL in mouse plasma with the lower limit of quantification level at 0.2 ng/mL. The intra- and inter-day coefficient variations and accuracies for fargesin at four quality control concentrations including were 3.6-11.3% and 90.0-106.6%, respectively. Intra-venously injected fargesin disappeared rapidly from the plasma with high clearance values (53.2-55.5 mL/min/kg) at 1, 2, and 4 mg/kg doses. Absolute bioavailability of fargesin was 4.1-9.6% after oral administration of fargesin at doses of 1, 2, and 4 mg/ kg to mice.

Keywords : fargesin, LC-HRMS, mouse plasma, pharmacokinetics

Introduction

Flos Magnoliae (Chinese name: Xin-yi) has been traditionally used for the treatment of allergic rhinitis, sinusitis, and headaches.[1-3] Fargesin (Figure 1A), a tetrahydrofurofuranoid lignan isolated from Flos Magnoliae, shows therapeutic effects for allergy, inflammatory diseases, hypertension, osteoarthritis, and atherosclerosis in the experimental animals by attenuating inducible nitric oxide synthase,[4,5] lipoxygenase,[6] various signaling pathways such as MAPK, CDK2/Cyclin E, PKC-dependent AP-1, and NF-κB,[7-11] ORAI1 channel,[2] reverse cholesterol transport,[12] oxidative stress,[13, 14] apoptosis,[13] lipid and glucose metabolism,[15,16] and melanin synthesis.[17]

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Fargesin inhibited CYP2C9-catalyzed diclofenac 4′-hydroxylation (K_i, 16.3 μM), UGT1A1-mediated SN-38 glucuronidaton (K_i, 25.3 μM), and UGT1A3-mediated chenodeoxycholic acid 24-acyl-glucuronidation activities (K_i, 24.5 μM) and showed the mechanism-based inhibition of CYP2C19-catalyzed [S]-mephenytoin 4′-hydroxylation (K_i, 3.7 μM), CYP2C8-catalyzed amodiaquine N-deethylation (K_i, 10.7 μM), and CYP3A4-catalyzed midazolam 1′-hydroxylation (K_i, 23.0 μM) human liver microsomes.[18,19] For the *in vivo* prediction of fargesin-induced drug interaction potential from *in vitro* data, the information regarding fargesin pharmacokinetics in the animals or humans is necessary. However, there are a few reports on the pharmacokinetics of fargesin after oral administration of purified extract of Flos Magnoliae or fargesin in the rats using high-performance liquid chromatography (HPLC) with atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS/MS)[18] or ultraviolet detection.[20,21]

We have developed a rapid, simple, and sensitive LC-high resolution mass spectrometric method (LC-HRMS) for the quantification of fargesin in mouse plasma samples using the least mouse plasma volume (6 μL) and successfully applied the method to evaluate the pharmacokinetics of fargesin after intravenous and oral administration of fargesin at 1, 2, and 4 mg/kg dose in male ICR mice.

Experimental

Materials

Fargesin (purity, 98%) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Magnolin (purity, 98.9%; internal standard) were obtained from PhytoLab GmbH & Co. (Vestenbergsgreuth, Germany). Water and methanol (LC-MS grade) were supplied by Fisher Scientific Co. (Fair Lawn, NJ, USA). All other chemicals used were of the highest quality available.

Sample preparation

Standard stock solution was prepared separately by dissolving fargesin (1 mg) in 1 mL of dimethyl sulfoxide and was diluted with methanol for the preparation of standard solutions (2.4 to 6000 ng/mL). The internal standard (IS) working solution (magnolin, 10 ng/mL) was prepared by diluting an aliquot of the stock solution with methanol. All standard solutions were stored at 4°C in darkness for 4 weeks.

Mouse plasma calibration standards for fargesin were prepared at eight concentration levels: 0.2, 0.4, 1, 5, 25, 100, 250, and 500 ng/mL. QC samples for fargesin were prepared at the concentrations of 0.2, 0.6, 20, and 450 ng/mL in drug-free mouse plasma and stored at -80°C until analyzed.

A 6 μL aliquot of mouse plasma sample was mixed with 18 μL of magnolin (IS, 10 ng/mL) in methanol. The mixture was vortexed and centrifuged at 13,500 rpm for 5 min. An aliquot of each supernatant was transferred to autosampler vial, and 5 μL was injected in the LC-HRMS system for analysis.

LC-HRMS analysis

Plasma concentrations of fargesin were analyzed by an LC-HRMS system coupled with Nexera X2 UPLC (Shimadzu, Kyoto, Japan) and Q-Exactive Orbitrap mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The separation was performed on a Halo C18 column (2.1 × 100 mm, 2.7 μm; Advanced Material Technology, Wilmington, DE, USA) using a gradient elution of 10 mM ammonium formate in 5% methanol (mobile phase A) and 95% methanol (mobile phase B), with flow rate of 0.3 mL/min: 20% mobile phase B for 0.5 min, 20 to 98% mobile phase B for 2.5 min, 98% mobile phase for 3 min, 98% to 20% mobile phase B for 0.2 min, 20% mobile phase B for 2.8 min. The column and autosampler were maintained at 40°C and 4°C, respectively. Heated electrospray ionization source settings in positive ion mode were spray voltage, 3.50 kV; sheath gas, 40 (arbitrary units); auxiliary gas, 10 (arbitrary units); capillary gas heater temperature, 250°C; and auxiliary gas heater temperatures, 200°C, respectively. Nitrogen gas (purity 99.999%) was used for higher-energy collision dissociation, and the collision energies for fragmentation of fargesin and magnolin (IS) were 25 and 40 eV, respectively. Parallel reaction monitoring (PRM) transitions were m/z 388.17547→135.04407 for fargesin and m/z 417.19022→219.10136 for the magnolin (IS). Xcalibur software (version 3.1.66.10, Thermo Fisher Scientific Inc.) was used for LC-HRMS system control and data processing.

Method validation

Method validation was performed according to the methods set out in the FDA Guidance on Bioanalytical Method Validation (https://www.fda.gov/media/70858/download). The intra- and inter-day precisions and accuracies were evaluated by analyzing batches of calibration standards and QC samples (0.2, 0.6, 20, and 450 ng/mL) in five replicates on three different days. Accuracy was defined as the proximity of the measured mean value to the theoretical value and precision was defined as the coefficient of variation (CV, %) of the measured concentrations. LLOQ value was defined as the lowest amount of fargesin in a mouse plasma sample that could be quantified as follows: signal-to-noise ratio, > 5; CV, < 20%; accuracy, 80-120%.

The stability of fargesin in mouse plasma was evaluated by analyzing low and high QC samples in triplicate: post-preparation sample stability in the autosampler at 4°C for 24 h; short-term storage stability following storage of plasma samples at room temperature for 2 h; three freeze–thaw cycles, and long-term storage stability following the storage for 28 days at -80°C.

The recovery of fargesin were determined by comparing the peak areas of the extract of fargesin-spiked plasma with those of fargesin-spiked post-extraction into six different blank mouse plasma extracts at 0.6, 20, and 450 ng/mL levels.

Pharmacokinetic study of fargesin in mice

Male ICR mice (8 weeks of age weighing 26.4 – 41.6 g) were purchased from Samtako Inc (Osan, Korea). All experimental procedures involving animal care were approved by the Institutional Animal Care and Use Committee of The Catholic University of Korea (approval number 2021-004-01). All mice were allowed unrestricted access to water and food before experiment. They were housed under suitable and standard housing conditions at a temperature of 23 ± 2°C, with relative humidity of 55 ± 10%, 12 h light/12 h dark cycle.

Fargesin in dimethylsulfoxide:propylene glycol:water (1:6:3, $v/v/v$) was administered by the bolus injection via tail vein of mice for intravenous study and using oral gavage for the oral study at doses of 1, 2 and 4 mg/kg (n = 6)(administration volume, 3 mL/kg). Blood sample (approximately 20 μL) was collected from the retro-orbital plexus under light anesthesia with isoflurane at 2 (intravenous study only), 5, 15, 30, 45 and 60 min and 1.5, 2, 3, 4, 6, 8, 10, and 24 h after drug administration. Plasma samples were harvested by centrifugation at 3000×g for 5 min and stored at -80°C until analysis.

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Fargesin in dimethylsulfoxide:propylene glycol:water (1:6:3, *v/v/v*) was administered by bolus injection into the tail vein at 4 mg/kg dose ($n = 3$) and by oral administration at 4 mg/kg dose ($n = 3$) to male ICR mice. Mice were returned to metabolic cages and urine and feces samples were collected individually for 48 hours. Urine and feces samples were stored in -80°C until analysis.

Pharmacokinetic parameters, including the area under the plasma concentration-time curve during the period of observation (AUC_{last}), the area under the plasma concentration-time curve to infinite time (AUC_{inf}), the terminal half-life ($t_{1/2}$), clearance (CL), volume of distribution at steady state (V_{ss}), and mean residence time (MRT), were analyzed using noncompartmental analysis (Phoenix WinNonlin 6.3; Pharsight, Mountain View, CA, USA). C_{max} and the time to reach C_{max} (T_{max}) were obtained directly from the experimental data. The extent of absolute oral bioavailability (F) was estimated by dividing AUC_{last} at each oral dose by AUC_{last} at intravenous administration. Each value is expressed as the mean ± standard deviation (SD). Statistical comparisons of pharmacokinetic variables were performed by one-way ANOVA followed by Tukey test. The values were treated as statistically significant when *p-value* < 0.05.

Results and Discussion

LC-HRMS analysis

The positive electrospray ionization of fargesin formed $[M+NH_4]^+$ ion at *m/z* 388.17547 instead of $[M+H]^+$ ion, and therefore, $[M+NH_4]^+$ ion was selected as the precursor ion and produced the intense product ion at *m/z* 135.04410 (Figure 1A). Magnolin (IS) showed $[M+H]^+$ ion at *m/z* 417.19022 and the intense product ion at *m/z* 219.10136 in MS/MS spectra (Figure 1B). PRM mode was used for the quantification of the analytes due to the high selectivity
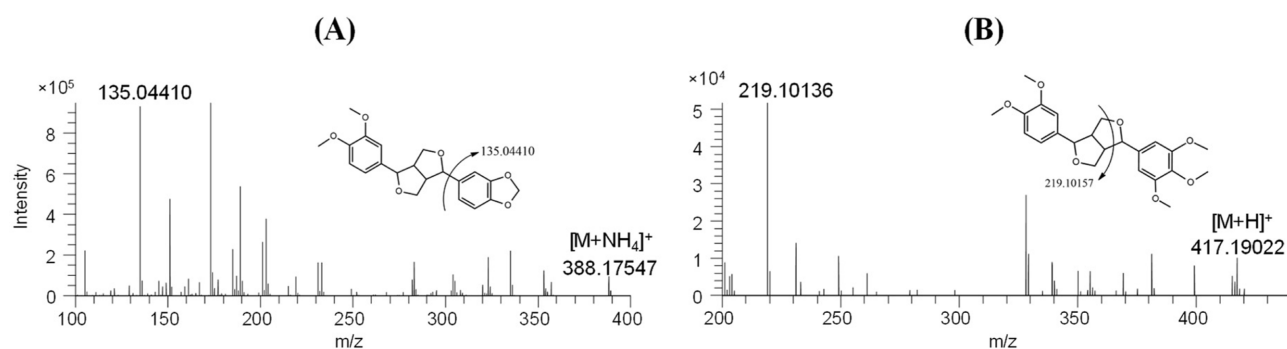

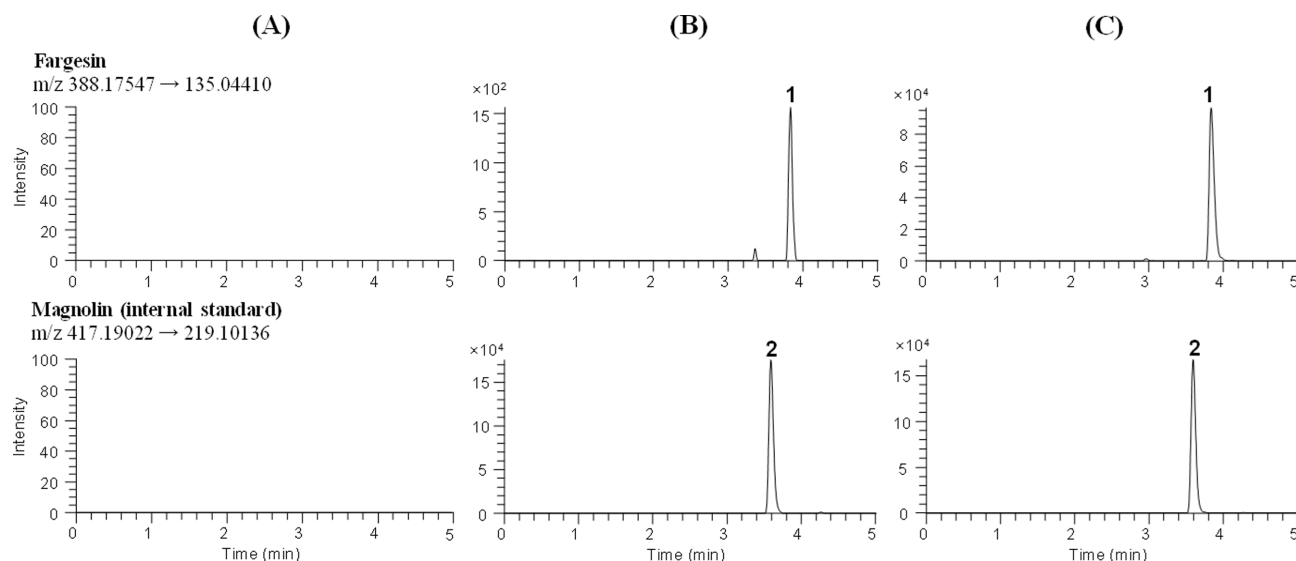**Figure 1.** Product ion spectra of (A) fargesin and (B) magnolin.

Figure 2. Representative parallel reaction monitoring chromatograms of (A) mouse blank plasma; (B) mouse plasma spiked with fargesin at LLOQ level (0.2 ng/mL); and (C) mouse plasma obtained 5 min after oral administration of fargesin at a dose of 1 mg/kg to a male ICR mouse. 1, fargesin (3.83 min); 2, magnolin (3.59 min, internal standard).

and sensitivity (Figure 2). Electrospray ionization mode yielded better sensitivity compared to APCI ionization[20] for the quantification of fargesin.

Analysis of blank plasma samples obtained from 40 mice revealed no significant interference peaks in the retention times of the analytes, indicating good method selectivity of the present method (Figure 2A). Figure 2B presents a typical PRM chromatogram of mouse plasma sample spiked with fargesin at 0.2 ng/mL. Figure 2C presents representative PRM chromatograms of a plasma sample obtained 5 min after intravenous administration of fargesin at a dose of 1 mg/kg in a mouse.

Method validation

Calibration curve for fargesin in mouse plasma was linear over the concentration ranges of 0.2–500 ng/mL with the coefficient of determination of 0.9977 using linear regression analysis with a weighting of 1/concentration (Table 1). The CV and accuracy of the calculated concentrations were 4.2% to 15.0% and from 95.0% to 103.6%, respectively, for eight calibration points. The CV value for the regression line slopes of fargesin was 0.7%, indicating good method repeatability.

The intra- and inter-day CV and accuracy values for fargesin in LLOQ, low, medium, and high QC samples ranged from 3.6% to 11.3% and from 90.0% to 106.6%, respectively (Table 2), indicating that the accuracy and precision of this method are acceptable.

Matrix effects of fargesin and magnolin (IS) were 91.7%-107.6% at 0.6, 20, and 450 ng/mL and 110.6%, respectively, indicating a little matrix effect (Table 3). The average recoveries of fargesin and magnolin (IS) in mouse plasma were 88.4%-98.1% at three concentrations and 95.1±3.2%, respectively (Table 3), indicating that the protein precipitation using methanol was suitable as sample preparation.

Table 1. Calculated concentrations of fargesin in calibration standards prepared with mouse plasma ($n = 3$).

Variables	Theoretical concentrations (ng/mL)								slope	r^2
	0.2	0.4	1	5	25	100	250	500		
Mean (ng/mL)	0.20	0.38	0.97	4.9	25.4	101.3	259.1	489.3	0.04655	0.9977
Accuracy (%)	100.0	95.0	97.0	98.0	101.6	101.3	103.6	97.9	-	-
CV (%)	15.0	13.2	12.0	4.7	7.8	7.2	4.7	4.2	0.7	0.3

Table 2. Precision (CV, %) and accuracy of fargesin in mouse plasma QC samples.

Variables	Intra-day ($n = 5$)				Inter-day ($n = 15$)			
QC (ng/mL)	0.2	0.6	20	450	0.2	0.6	20	450
Mean (ng/mL)	0.19	0.63	18.7	479.7	0.18	0.60	20.0	470.4
CV (%)	10.1	7.0	5.5	3.6	8.9	11.3	9.9	6.3
Accuracy (%)	95.5	105.6	93.7	106.6	90.0	100.0	100.0	104.5

Table 3. Matrix effects and recoveries of fargesin and magnolin (IS) in mouse plasma samples ($n = 6$).

Analytes (ng/mL)	Matrix effect (%)		Recovery (mean ± SD, %)
	Mean	CV	
Fargesin			
0.6	107.3	5.3	90.8 ± 4.1
20	107.6	3.4	88.4 ± 3.7
450	91.7	11.3	98.1 ± 4.4
Magnolin (IS, 10)	110.6	6.8	95.1 ± 3.2

Table 4. Post-preparation, short-term, long-term, and freeze–thaw stabilities of fargesin in mouse plasma QC samples ($n = 3$).

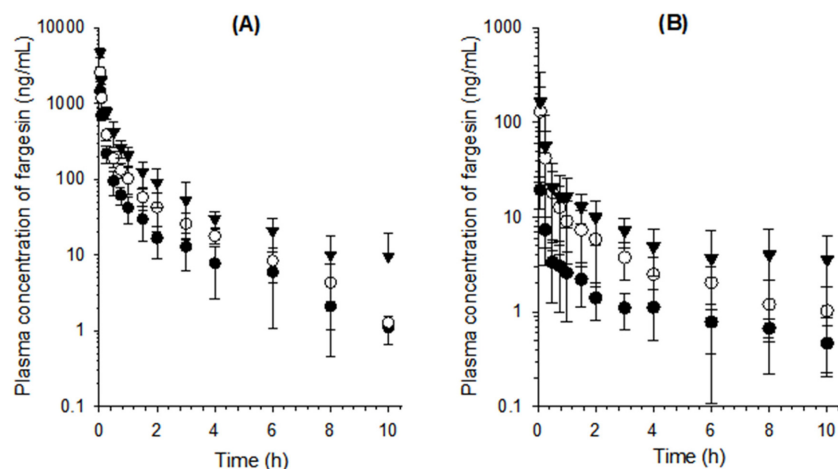| Stability conditions	fargesin concentration (ng/mL)			
	0.6		450	
	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
Post-preparation for 24 h at 4°C	100.3	8.2	105.5	4.9
Short-term storage for 2 h at room temperature	93.5	9.0	96.9	0.6
Long-term storage for 28 days at -80°C	105.1	1.7	92.6	0.5
Three freeze–thaw cycles of –80°C to room temperature	99.6	4.3	89.6	4.0

Figure 3. Mean plasma concentration-time profiles of fargesin after (A) an intravenous injection and (B) an oral administration at doses of 1 (●), 2 (○), and 4 (▼) mg/kg to male ICR mice. Data are represented as mean ± SD ($n = 6$).

Table 5. Mean pharmacokinetic parameters of fargesin after its intravenous injection and oral administration at 1, 2, and 4 mg/kg doses to male ICR mice. Data are represented as mean ± SD ($n = 6$).

Pharmacokinetic parameters	1 mg/kg	2 mg/kg	4 mg/kg
Intravenous injection			
C_0 (ng/mL)	2481.6 ± 1154.8	4461.0 ± 1007.7	8398.9 ± 1811.8
AUC_{last} (ng·min/mL)[a]	19776.0 ± 5038.4	37038.3 ± 6673.7	72486.9 ± 10273.8
AUC_{inf} (ng·min/mL)	19994.8 ± 5229.1	37943.2 ± 7706.0	73457.0 ± 10691.3
CL (mL/min/kg)	53.2 ± 15.0	54.5 ± 10.2	55.5 ± 8.8
V_{ss} (mL/kg)	2763.0 ± 516.2	3536.3 ± 588.6	3897.9 ± 1199.7
$t_{1/2}$ (min)	94.6 ± 21.2	119.2 ± 63.9	84.7 ± 16.7
MRT (min)	50.4 ± 16.2	53.9 ± 7.4	62.2 ± 11.7
Oral administration			
C_{max} (ng/mL)[a]	19.4 ± 7.2	130.3 ± 106.9	192.0 ± 178.0
T_{max} (min)	5.0	5.0	5.0
AUC_{last} (ng·min/mL)[a]	802.9 ± 240.7	3164.1 ± 1733.2	6953.8 ± 3847.1[b]
AUC_{inf} (ng·min/mL)	877.6 ± 272.5	3553.8 ± 1581.3	7888.1 ± 3989.7
$t_{1/2}$ (min)	108.8 ± 57.4	140.0 ± 108.4	123.8 ± 60.2
F (%)	4.1 ± 1.2	8.5 ± 4.7	9.6 ± 5.3

[a] Dose normalized (1 mg/kg) AUC_{last} and C_{max} were compared for statistical analysis.
[b] Significantly different ($p < 0.05$) from 1 mg/kg.

Three freeze-thaw cycles, short-term storage at room temperature, long-term storage for 28 days at -80°C, and post-preparation stability for 24 h in 4°C autosampler showed negligible effect on the stability of fargesin (Table 4).

Pharmacokinetics of fargesin in male ICR mice

After intravenous injection of fargesin at doses of 1, 2, and 4 mg/kg to male ICR mice, the mean plasma concentration-time curves are shown in Figure 3A. The pharmacokinetics of intravenously injected fargesin showed a linear kinetics in the dose range of 1–4 mg/kg, which was evidenced by

the dose proportional increase of AUC and dose independent CL (53.2-55.5 mL/min/kg), V_{ss} (2763.0-3897.9 mL/kg), and $t_{1/2}$ (84.7-119.2 min) (Table 4). The cumulative fecal excretion of fargesin for 48 h following its intravenous injection at 4 mg/kg was 0.014 ± 0.017% of the dose but it was not excreted in urine, indicating that high systemic clearance (53.2-55.5 mL/min/kg) of fargesin may result from the metabolism.

After oral administration of fargesin at doses of 1, 2, and 4 mg/kg to male ICR mice, the mean plasma concentration-time curves and pharmacokinetic parameters are shown in

Figure 3B and Table 5, respectively. Fargesin was rapidly absorbed after oral administration based on its T_{max} at the first blood sampling time point (5 min). The dose normalized C_{max} and $t_{1/2}$ (108.8-140.0 min) values of fargesin were comparable among three doses studied (Table 5). However, dose normalized AUC_{last} of fargesin at 4 mg/kg (1738.4 ± 961.8 ng·min/mL) was significantly larger than that at 1 mg/kg (802.9 ± 240.7 ng·min/mL). The absolute oral bioavailability of fargesin was 4.0-9.6% for oral dose examined. The cumulative fecal recovery of fargesin after its oral administration at 4 mg/kg dose was 0.089 ± 0.045% of the dose without urinary excretion. Based on these results, low F may be due to the extensive fargesin metabolism.

Conclusions

A sensitive, simple, and reproducible LC-HRMS method using protein precipitation as a sample clean-up procedure was developed for the determination of fargesin with LLOQ level of 0.2 ng/mL in 6 μL of mouse plasma. We evaluated the plasma concentrations of fargesin using this method and the pharmacokinetic parameters of fargesin after intravenous and oral administration of fargesin at doses of 1, 2, and 4 mg/kg to male ICR mice.

Acknowledgements

References

1. Shen, Y.; Li, C. G.; Zhou, S. F.; Pang, E. C.; Story, D. F.; Xue, C. C. *Curr. Med. Chem.* **2008**, 15, 1616, DOI: 10.2174/092986708784911515.
2. Hong, P. T. L.; Kim, H. J.; Kim, W. K.; Nam, J. H. *Korean J. Physiol. Pharmacol.* **2021**, 25, 251, DOI: 10.4196/kjpp.2021.25.3.251.
3. Kim, H. J.; Nam, Y. R.; Nam, J. H. *Am. J. Chin. Med.* **2018**, 46, 1079, DOI: 10.1142/S0192415X18500568.
4. Baek, J. A.; Lee, Y. D.; Lee, C. B.; Go, H. K.; Kim, J. P.; Seo, J. J.; Rhee, Y. K.; Kim, A. M.; Na, D. J. *Nitric Oxide* **2009**, 20, 122, DOI: 10.1016/j.niox.2008.10.003.
5. Kim, J. S.; Kim, J. Y.; Lee, H. J.; Lim, H. J.; Lee, D. Y.; Kim, D. H.; Ryu, J. H. *Phytother. Res.* **2010**, 24, 748, DOI: 10.1002/ptr.3028.
6. Lim, H.; Son, K. H.; Bae, K. H.; Hung, T. M.; Kim, Y. S.; Kim, H. P. *Phytother. Res.* **2009**, 23, 1489, DOI: 10.1002/ptr.2783.
7. Pham, T. H.; Kim, M. S.; Le, M. Q.; Song, Y. S.; Bak, Y.; Ryu, H. W.; Oh, S. R.; Yoon, D. Y. *Phytomedicine* **2017**, 24, 96, DOI:10.1016/j.phymed.2016.11.014.
8. Yue, B.; Ren, Y. J.; Zhang, J. J.; Luo, X. P.; Yu, Z. L.; Ren, G. Y.; Sun, A. N.; Deng, C.; Wang, Z. T.; Dou, W. *Molecules* **2018**, 23, 1380, DOI:10.3390/molecules23061380.
9. Lu, J.; Zhang. H.; Pan. J.; Hu, Z.; Liu, L.; Liu, Y.; Yu, X.; Bai, X.; Cai, D.; Zhang, H. *Arthritis Res. Ther.* **2021**, 23, 142, DOI: 10.1186/s13075-021-02512-z.
10. Jun, A. Y.; Kim, H. J.; Park, K. K.; Son, K. H.; Lee, D. H.; Woo, M. H.; Chung, W. Y. *Invest. New Drugs* **2014**, 32, 1, DOI: 10.1007/s10637-013-9969-0.
11. Lee, G. E.; Lee, C. J.; An, H. J.; Kang, H. C.; Lee, H. S.; Lee, J. Y.; Oh, S. R.; Cho, S. J.; Kim, D. J.; Cho, Y. Y. *Int. J. Mol. Sci.* **2021**, 22, 2073, DOI: 10.3390/ijms22042073.
12. Wang, G.; Gao, J. H.; He, L. H.; Yu, X. H.; Zhao, Z. W.; Zou, J.; Wen, F. J.; Zhou, L.; Wan, X. J.; Tang, C. K. *Biochim. Biophys. Acta Mol. Cell. Biol. Lipids* **2020**, 1865, 158633, DOI: 10.1016/j.bbalip.2020.158633.
13. Wang, X.; Cheng, Y.; Xue, H.; Yue, Y.; Zhang, W.; Li, X. *Fitoterapia* **2015**, 105, 16, DOI: 10.1016/j.fitote.2015.05.016.
14. Sha, S.; Xu, D.; Wang, Y.; Zhao, W.; Li, X. *Can. J. Physiol. Pharmacol.* **2016**, 94, 900, DOI:10.1139/cjpp-2015-0615.
15. Lee, Y. S.; Cha, B. Y.; Choi, S. S.; Harada, Y.; Choi, B. K.; Yonezawa, T.; Teruya, T.; Nagai, K.; Woo, J. T. *Biofactors* **2012**, 38, 300, DOI:10.1002/biof.1022.
16. Choi, S. S.; Cha, B. Y.; Choi, B. K.; Lee, Y. S.; Yonezawa, T.; Teruya, T.; Nagai, K.; Woo, J. T. *J. Nat. Med.* **2013**, 67, 320, DOI: 10.1007/s11418-012-0685-4.
17. Fu, T.; Chai, B.; Shi, Y.; Dang, Y.; Ye, X. *J. Dermatol. Sci.* **2019**, 94, 213, DOI: 10.1016/j.jdermsci.2019.03.004.
18. Kim, J. H.; Kwon, S. S.; Jeong, H. U.; Lee, H. S. *Int. J. Mol. Sci.* **2017**, 18, 952, DOI: 10.3390/ijms18050952.
19. Park, R.; Park, E. J.; Cho, Y. J.; Lee, J. Y.; Kang, H. C.; Song, I. S.; Lee, H. S. *Pharmaceutics* **2021**, 13, 187, DOI: 10.3390/pharmaceutics13020187.
20. Jeong, J. H.; Kim, D. K.; Ji, H. Y.; Oh, S. R.; Lee, H. K.; Lee, H. S. *Biomed. Chromatogr.* **2011**, 25, 879, DOI: 10.1002/bmc.1538.
21. Zhang, Z.L., X.; Cao, W.; Xiao, X.; Qu, J.; Zhao, M.; Li, X., *Curr. Pharm. Anal.* **2016**, 12, 379, DOI: 10.2174/1573412912666151211191600.