

Simultaneous Determination of Neohesperidin and Naringin in Rat Plasma After Oral Administration of a Chinese Compound Formulation by UPLC–MS–MS

Chao Li, Chunjuan Yang, Xuling Peng, Zhili Xiong, and Famei Li*

Department of Analytical Chemistry, Shenyang Pharmaceutical University, Shenyang, 110016, P.R. China

Abstract

A sensitive, specific method has been developed for simultaneous determination of neohesperidin and naringin in rat plasma using liquid chromatography–tandem mass spectrometry. With hesperidin as the internal standard, plasma samples were prepared by protein precipitation with methanol. Analysis was carried out on an ACQUITY UPLC BEH C₁₈ column using acetonitrile–water (20:80, v/v) as the mobile phase. Detection was performed by means of electrospray ionization mass spectrometry in negative ion mode with multiple reaction monitoring. Linear calibration curves of neohesperidin and naringin were obtained over the concentration ranges of 15.0–960 ng/mL and 12.0–1200 ng/mL, respectively. The intra- and inter-day precisions were within 9.7% and 7.6% for neohesperidin and 7.8% and 12.9% for naringin. The accuracy was from –4.3% to 0.43% for neohesperidin and from –3.8% to 3.0% for naringin. The validated method was successfully applied to the pharmacokinetic study of neohesperidin and naringin in rats after oral administration of a Chinese compound formulation, gushudan.

Introduction

Determination of multiple components of compound herb medicines entering the body is of great importance for evaluating efficacy and investigating the action mechanism. In recent years, efforts have been made to develop methods applicable to the analysis of components in biological samples.

Gushudan is a Chinese compound formulation composed of *Herba Epimedii*, *Fructus Cnidii*, *Rhizoma Drynariae*, and *Radix Salviae Miltiorrhizae*. It was formed according to “the basic concept of establishing platforms for operational techniques in systems of elaborately selecting small formulations of traditional Chinese medicine” (1). It is a kidney-tonifying and bone-strengthening formulation used for the treatment of osteoporosis. It was found that gushudan formulation inhibited high bone turnover of ovariectomized rats and effectively stimulated bone formation (2). Its main active components are naringin, neohesperidin, epimedin B, epimedin C, icariin, imperatorin, and

osthole. High-performance liquid chromatography (HPLC) and liquid chromatography–tandem mass spectrometry (LC–MS–MS) methods have been developed for the determination of epimedin C, icariin, and osthole (3,4) in rat plasma and their pharmacokinetic study after oral administration of this formulation.

Naringin and neohesperidin are major constituents of *Drynariae Rhizome*. Naringin is a flavonoid existing in many citrus fruits and traditional Chinese medicines. Naringin has been reported to have several pharmacological properties such as antimicrobial, antimutagenic, anticancer, anti-inflammatory, cholesterol lowering, free radical scavenging, and antioxidant effects (5–7). Naringin and neohesperidin offered virtually complete protection against the autophagy-inhibitory effect of okadaic acid (8). In our previous study (9), neohesperidin was found to have activity stimulating proliferation of osteoblastic-like cells. *Drynariae Rhizoma* extract (DR) is a candidate known to be effective for the treatment of inflammation, hyperkemia, arteriosclerosis, and gynecological diseases such as osteoporosis and bone resorption according to ancient Chinese medicinal and herbal literature (10).

Liquid chromatographic methods for determination of naringin in rabbit plasma (11), human plasma (12), human urine (13), and LC–MS–MS methods for detecting naringin in rat serum (14) and in rat plasma (15) have been reported. However, no assay has so far been published for neohesperidin analysis in biological fluids. Herein, for the first time, we described a specific, rapid, and sensitive LC–MS–MS method for simultaneous determination of neohesperidin and naringin concentrations in rat plasma and applied it to the pharmacokinetic study following oral administration of gushudan formulation.

Experimental

Materials and reagents

Herba Epimedii was purchased from Baoji (Shannxi, China). *Fructus Cnidii* was purchased from Henan, China). *Rhizoma Drynariae* was purchased from Hunan, China, and *Radix Salviae*

*Author to whom correspondence should be addressed: email lifamei@syphu.edu.cn.

Miltiorrhizae was obtained from Shenyang (Liaoning, China). The raw materials were identified by Professor Qishi Sun (School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Liaoning, China) as the herb of *Epimedium brevicornum* Maxim., the fruits of *Cnidium monnieri* (L.) Cuss., the Rhizoma of *Drynaria fortunei* (Kunze) J. Sm., and the *Radix* of *Salvia miltiorrhiza* Bge. Naringin and hesperidin were supplied by the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Neoeriocitrin was isolated and purified from *Rhizoma Drynariae* in our laboratory (2). The purity of neoeriocitrin was 98% determined by HPLC. The structures of neoeriocitrin, naringin, and internal standard (IS) are given in Figure 1A–1C. HPLC-grade methanol used for protein precipitation was purchased from Yuwang Chemical Reagent Plant (Shandong, China). HPLC-grade acetonitrile was purchased from Tedia (Fairfield, OH). Water was purified by redistillation and passed through 0.22- μ m membrane filter before use. Wistar rats were obtained from the Experimental Animals Center of Shenyang Pharmaceutical University (Shenyang, China).

Instrumentation and operating conditions

Chromatographic conditions

The chromatography was performed on an Acquity UPLC system (Waters, Milford, MA) with a cooling autosampler and column oven. A Waters Acquity UPLC BEH C₁₈ column (50 mm \times 2.1 mm, 1.7 μ m) was employed. The column temperature was maintained at 40°C. The mobile phase consisted of acetonitrile (20%) and water (80%). The flow rate was set at 0.20 mL/min. The autosampler was conditioned at 4°C, and the injection volume was 10 μ L. The total run time was 2 min for each injection.

Mass spectrometric conditions

Triple-quadrupole tandem mass spectrometric detection was carried out on a Waters Micromass Quattro micro API mass spectrometer with an electrospray ionization (ESI) interface. The ESI source was operated in negative ionization mode. Quantification

was performed using multiple reaction monitoring (MRM) with transitions of m/z 595 \rightarrow 151 for neoeriocitrin, m/z 579 \rightarrow 271 for naringin, m/z 609 \rightarrow 301 for hesperidin (the IS), respectively, with a scan time of 0.1 s per transition. The MS parameters were as follows: capillary voltage 2.7 kV, cone voltage 35 V, source temperature 110°C, and desolvation temperature 350°C. Nitrogen was used as the desolvation and cone gas with a flow rate of 400 L/h and 40 L/h, respectively. Argon was used as the collision gas at a pressure of approximately 0.385 Pa. The optimized collision energy chosen for neoeriocitrin, naringin, and IS was 45 eV, 32 eV, and 30 eV, respectively. All data collected in centroid mode were processed using a Waters MassLynx NT 4.0 software with a QuanLynx program.

Preparation of standard solutions, calibration samples, and quality control samples

Stock solutions of neoeriocitrin and naringin were prepared in methanol at concentrations of 24 μ g/mL and 30 μ g/mL, respectively. Standard solutions were prepared by diluting the stock solutions with methanol. The stock solution of IS (40.0 μ g/mL) was prepared in methanol and then diluted with methanol to prepare the working internal standard solution containing 200 ng/mL of IS. All solutions were stored at 4°C and protected from light. Calibration samples for neoeriocitrin and naringin were prepared by spiking blank rat plasma with appropriate amounts of the standard solutions to achieve the final plasma concentrations of 15.0, 30.0, 120, 240, 480, and 960 ng/mL for neoeriocitrin and 12.0, 24.0, 120, 240, 600, and 1200 ng/mL for naringin. The quality control (QC) samples were prepared at low, medium, and high concentrations of 38.4, 384, and 768 ng/mL for neoeriocitrin and 24.0, 480, and 960 ng/mL for naringin, respectively.

Preparation of drug extract

The dried powder of *Herba Epimedii*, *Fructus Cnidii*, *Rhizoma Drynariae*, and *Radix Salviae Miltiorrhizae* was mixed according to the formulation, extracted with 75% ethanol under thermal reflux for 1.5 h, and then filtered. The extraction was repeated

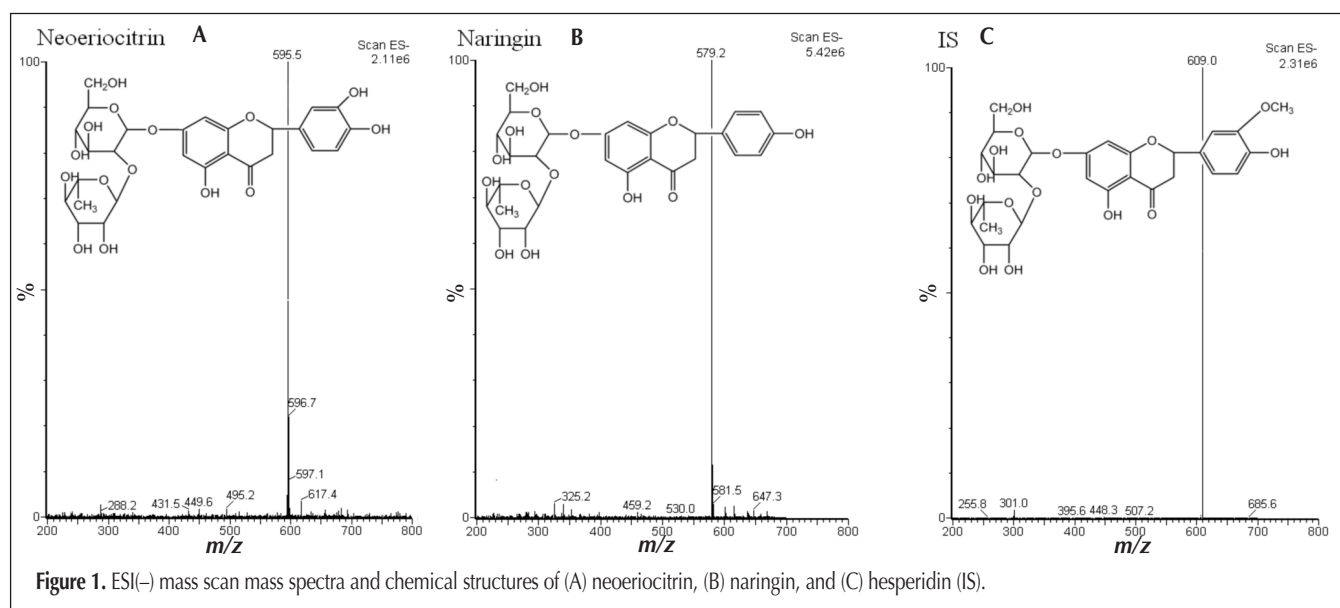


Figure 1. ESI(-) mass scan mass spectra and chemical structures of (A) neoeriocitrin, (B) naringin, and (C) hesperidin (IS).

twice. The extracted solutions were combined, and ethanol was removed under reduced pressure. The residue was dissolved in 0.5% sodium carboxymethyl cellulose to give an extract with a concentration of 3.5 g/mL (expressed as the weight of raw materials in the solution).

Sample preparation

A 100 μ L aliquot of IS solution (200 ng/mL), 50 μ L of acetic acid, and 400 μ L of methanol were added to 100 μ L of rat plasma samples. The sample was vortex-mixed for 60 s and centrifuged at 14,000 \times g for 15 min. The supernatant was transferred into glass vials, and 10 μ L was injected into the LC-MS-MS system for analysis.

Method validation

Selectivity

Selectivity was investigated by comparing chromatograms of blank plasma obtained from six rats with those of standard plasma sample spiked with neoeriocitrin, naringin, and IS and plasma sample after oral administration of this formulation.

The matrix effect on the ionization of analytes was evaluated by comparing peak areas of blank plasma extracts spiked with analytes at three concentration levels (38.4, 384, and 768 ng/mL

for neoeriocitrin and 24.0, 480, and 960 ng/mL for naringin) to those of the standard solutions. The matrix effect of IS was evaluated using the same procedure.

Linearity and lower limit of quantification

Calibration curves were prepared by assaying standard plasma samples at six concentrations over the range of 15.0–960 ng/mL for neoeriocitrin and 12.0–1200 ng/mL for naringin, respectively. The linearity of each calibration curve was determined by plotting the peak area ratios of analyte/IS versus plasma concentrations of neoeriocitrin and naringin. Weighted ($1/\text{conc}^2$) least-squares linear regression analysis was used to determine the slope, intercept, and correlation coefficient.

The lower limit of quantification (LLOQ) is defined as the lowest concentration on the calibration curve at which an acceptable accuracy within $\pm 20\%$ and a precision of less than 20% were obtained.

Precision and accuracy

The precision and accuracy of the method were assessed by determination of QC samples at low, medium, and high concentrations. To determine intra-day precision and accuracy, the assays were carried out on QC samples on the same day. Inter-day precision and accuracy were determined by assaying the QC

Table I. Precision and Accuracy For the Determination of Neoeriocitrin and Naringin in Rat Plasma*

Added C (ng/mL)	Found C (ng/mL)	Intra-run RSD* (%)	Inter-run RSD† (%)	Accuracy RE (%)
<i>Neoeriocitrin</i>				
38.4	38.1 \pm 3.6	9.7	7.6	0.4
384	374 \pm 28	7.5	6.9	-1.2
768	725 \pm 43	5.7	7.1	-4.3
<i>Naringin</i>				
24.0	24.7 \pm 2.5	7.5	12.9	3.0
480	493 \pm 37	7.1	10.1	1.5
960	933 \pm 74	7.8	8.9	-3.8
* n = 6 series per day for 3 days.		† n = 6 series per day for 3 days.		

Table II. The Mean Recoveries of Neoeriocitrin, Naringin, and IS in Rat Plasma (n = 5)

	Concentration (ng/mL)	Recovery (%), mean \pm SD
Neoeriocitrin	38.4	91.7 \pm 7.2
	384	94.5 \pm 3.8
	768	101 \pm 4.8
Naringin	24.0	90.9 \pm 4.7
	480	104 \pm 6.7
	960	97.4 \pm 5.2
IS	200	97.3 \pm 6.1

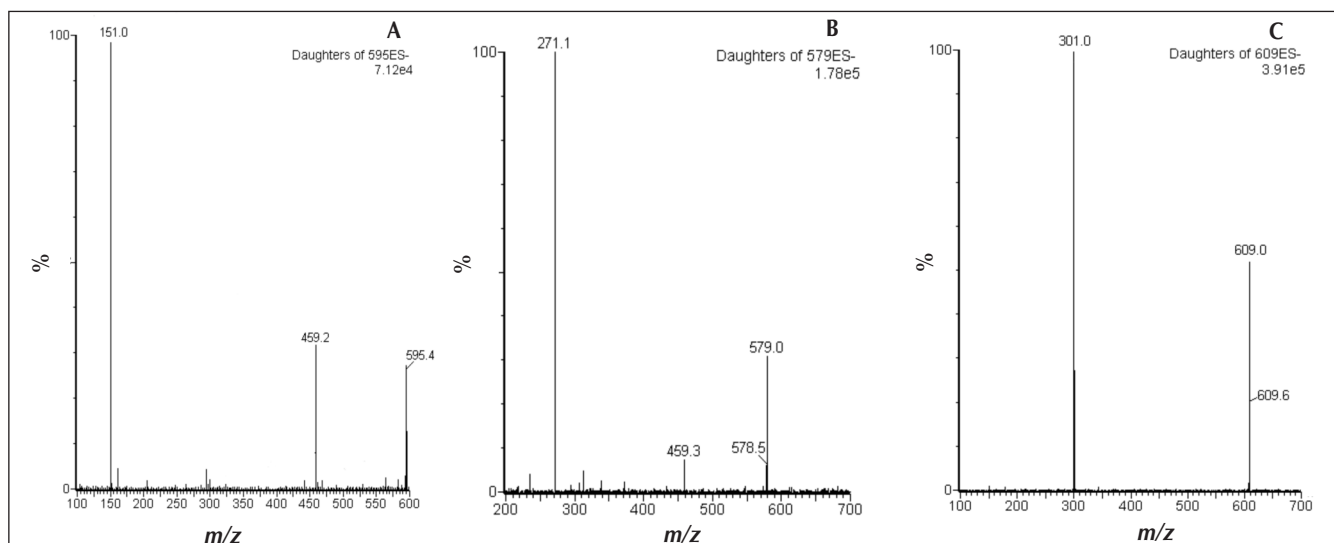


Figure 2. Product ion mass spectra of $[M-H]^-$ of (A) neoeriocitrin, (B) naringin, and (C) hesperidin (IS).

samples over three consecutive days. The concentration of each sample was determined using a calibration curve prepared on the same day. The precision was expressed as relative standard deviation (RSD) and the accuracy as the relative error (RE).

Recovery

The extraction recoveries of neoeriocitrin, naringin, and IS were determined by comparing the peak areas obtained from blank plasma samples spiked with analytes before extraction to those samples to which analytes were added after extraction. This procedure was performed at three QC levels.

Stability

The stability of neoeriocitrin, naringin, and IS stock solutions was evaluated after storage at room temperature for 6 h and at 4°C for 10 days. The stability of neoeriocitrin, naringin, and IS working solutions was investigated at room temperature for 5 h.

Long-term stability of plasma samples was determined by assaying QC samples after storage at -20°C for 10 days. Short-term stability was assessed by analyzing QC samples kept at room temperature for 4 h that exceeded the required time for routine sample preparation. Freeze-thaw stability was investigated after three freeze -20°C-thaw (room temperature) cycles. Post-preparation stability was assessed by analyzing the extracted QC samples kept in the autosampler at 4°C for 8 h.

Application of the analytical method to the pharmacokinetic study of neoeriocitrin and naringin in rats

Six Wistar rats (three males, three females, body weight 250 ± 20 g) were fasted for 12 h prior to administration of the herbal extract. The experimental protocol was approved by the University Ethics Committee for the use of experimental animals and conformed to the Guide for Care and Use of Laboratory Animals. This formulation was given orally at 3 g (expressed as the weight of raw materials approximately containing 15 mg neoeriocitrin and 19 mg naringin)/kg body weight. Animals had free access to water during the experiment. Blood (0.3 mL) was

collected from the suborbital vein before administration and at 0.05, 0.10, 0.17, 0.25, 0.33, 0.5, 0.83, 1.33, 2.0, 2.67, 3.33 h after dosing. Blood samples were collected into heparinized tubes and immediately centrifuged at 8000 g for 10 min. The plasma was transferred into clean tubes and stored at -20°C until analysis.

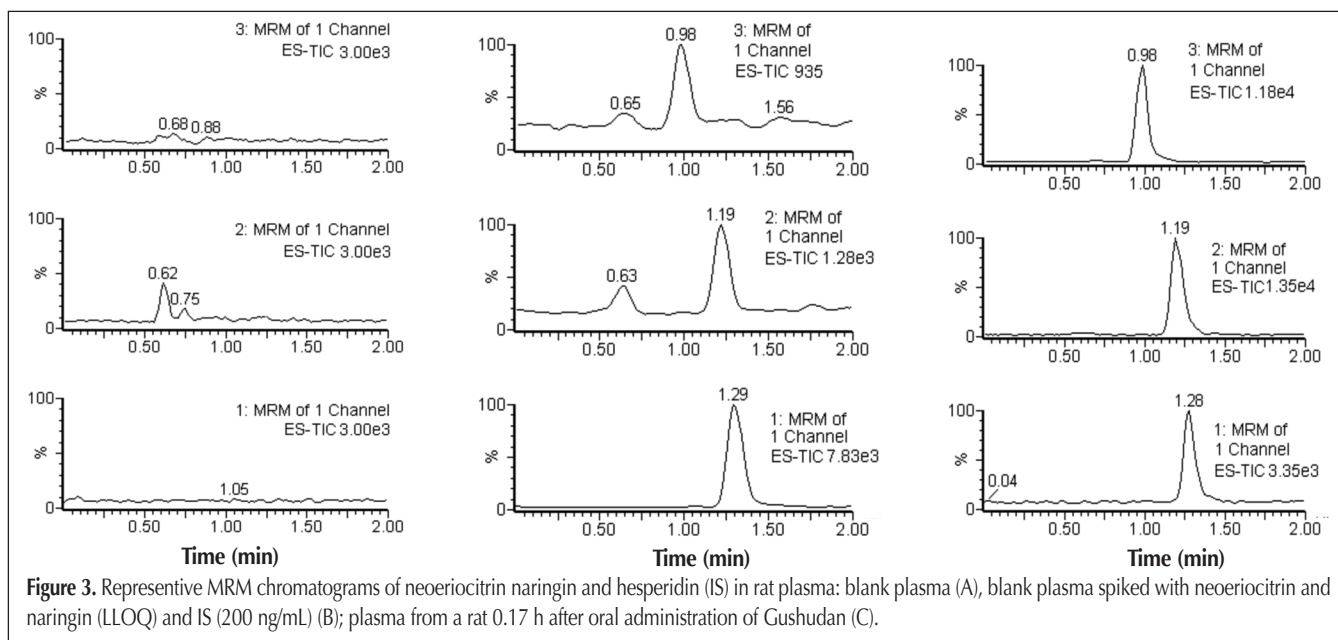
The terminal elimination half-life ($t_{1/2}$) was $0.693/k_e$, where k_e , the elimination rate constant, was calculated by least-square regression of the plot of logarithms of concentration against time for the last four measurable points. Maximum drug plasma concentrations (C_{max}) and time to reach the maximum concentrations (T_{max}) were obtained directly from the observed data. The area under the curve ($AUC_{0-\infty}$) was calculated by the trapezoidal rule between first (0 h) and last sampling time plus C_n/k_e , where C_n is the last measurable concentration, that is $AUC_{0-t} = \sum(C_i + C_{i-1}) \times (t_i - t_{i-1})/2$, $AUC_{0-\infty} = AUC_{0-t} + C_n/k_e$.

Results and Discussion

Chromatography and mass spectrometry

Hesperidin was selected as the internal standard for its structural similarity (Figure 1) to the analytes. It exhibited a reasonable retention time after neoeriocitrin and naringin, a good response under the same ESI ionization conditions as those optimal for analytes, and a high and stable extraction recovery of $97.3 \pm 6.1\%$, which demonstrated its behaviors similar to the analytes during the sample preparation, chromatographic separation, and MS detection.

ESI in both negative and positive ion modes was attempted in the method development. Different buffers of various proportions were used in positive and negative ion mode turning to optimize the response signals of analytes. The tested buffers included 0.1% or 0.2% acetic acid or formic acid, 5 mM ammonium acetate. It was found that the negative ion mode is more sensitive than positive ion mode under all conditions. Using MS scan mode, deprotonated molecular ions $[M-H]^-$ of



neeroiocitrin, naringin, and IS gave signals at m/z 595, 579, and 609, respectively (Figure 1A–1C). In the product ion scan mode, the most abundant product ions were m/z 151 from m/z 595, m/z 271 from m/z 579, and m/z 301 from m/z 609. The MS parameters were optimized to obtain high signal for both precursor and product ions mentioned previously. Figure 2A–2C shows the product ion mass spectra of neeroiocitrin, naringin, and IS in negative ion ESI mode, respectively.

Preparation of plasma samples

Sample preparation is a critical step for accurate and reliable LC–MS–MS assays. The most widely employed biological sample preparation methodologies are liquid–liquid extraction (LLE), protein precipitation (PPT), and solid-phase extraction (SPE). There have been reports on the extraction of naringin with ethyl acetate (14) and SPE (15). But neeroiocitrin is a highly polar compound because of one more hydroxyl in its structure, which makes it difficult to be extracted from plasma by LLE. Its extraction recovery was below 30% when ethyl acetate or diethyl ether was used as the extraction solvent. PPT often provides higher recovery for those compounds of high polarity. Methanol and acetonitrile were investigated as the precipitation reagents during the experiment. Higher extraction recovery of neeroiocitrin was obtained with methanol as a precipitation reagent.

Method validation

Selectivity

Typical chromatograms of neeroiocitrin, naringin, and IS are shown in Figure 3. Three MRM channels were used for recording and the retention times for neeroiocitrin, naringin, and IS were 0.97 min, 1.19 min, and 1.29 min, respectively. Neeroiocitrin, naringin, and IS were detected with good peak shapes, and no interfering peaks were observed at the retention times of the analytes. Due to the high selectivity of MRM mode, there was no interference from other components of the formulation, their metabolites, and endogenous substances.

As for the evaluation of matrix effect, the peak-area ratios of blank plasma extracts spiked with neeroiocitrin, naringin, and IS post-extraction compared to those of the standard solutions were

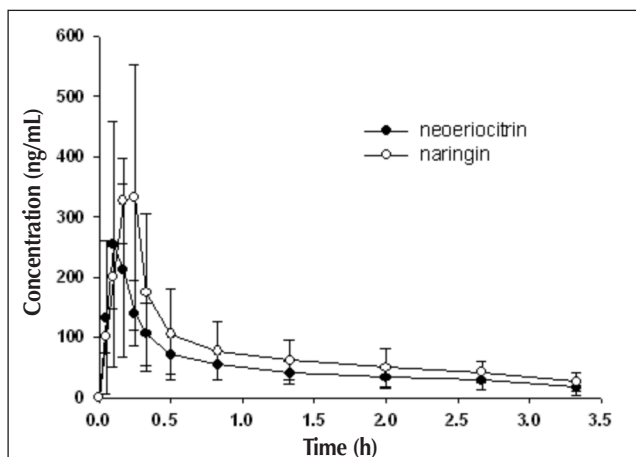


Figure 4. Mean (\pm SD) plasma concentration-time curves of neeroiocitrin and naringin in six rats after oral administration of Gushudan.

within 85–115%, indicating that the developed method was free from matrix effect.

Linearity of calibration curves and lower limits of quantification

Linear responses were obtained in the concentration range from 15.0 to 960 ng/mL for neeroiocitrin and from 12.0 to 1200 ng/mL for naringin. Typical equations for the calibration curves were: $y = 6.29x + 0.0801$, $r = 0.9971$ for neeroiocitrin, and $y = 5.22x + 0.0143$, $r = 0.9965$ for naringin, respectively, where y is the relative peak-area ratio of neeroiocitrin/IS and naringin/IS and x is the concentration of neeroiocitrin and naringin in plasma.

The LLOQ was found to be 15.0 ng/mL for neeroiocitrin and 12.0 ng/mL for naringin with 10 μ L of sample solution injected into the ultra high-performance liquid chromatography column. The final concentration of neeroiocitrin and naringin in the prepared sample solution is approximately 2.5 ng/mL and 2.0 ng/mL, respectively. The precisions (RSD) and accuracies (RE) of LLOQ in this study were less than 14.3% and within \pm 15.8% for neeroiocitrin as well as less than 15.7% and within \pm 12.3% for naringin, respectively. The limit of detection (LOD) defined as the analyte concentration in plasma with a signal-to-noise ratio of 3, which was found to be 5.0 ng/mL and 4.0 ng/mL for neeroiocitrin and naringin, respectively.

Precision, accuracy, recovery, and stability

The data of intra- and inter-day precision and accuracy for neeroiocitrin and naringin quantification are shown in Table I. The data indicates an acceptable precision and accuracy of the present method for determination of neeroiocitrin and naringin in rat plasma.

Table III. Stability of Neeroiocitrin and Naringin in Rat Plasma ($n = 3$)

Conc. (ng/mL)	Accuracy (%), mean \pm SD			
	Short-term stability	Long-term stability	Freeze-thaw stability	Post-preparative stability
<i>Neeroiocitrin</i>				
38.4	102 \pm 2.9	97.4 \pm 3.1	99.9 \pm 4.9	98.8 \pm 6.5
384	91.3 \pm 6.1	92.1 \pm 3.8	96.7 \pm 5.2	100 \pm 9.6
768	90.8 \pm 4.3	89.3 \pm 4.7	97.2 \pm 5.1	92.3 \pm 4.3
<i>Naringin</i>				
24	100 \pm 2.3	95.4 \pm 5.9	103 \pm 8.1	93.2 \pm 6.7
480	92.0 \pm 4.7	90.1 \pm 8.4	100 \pm 8.9	91.5 \pm 4.3
960	91.0 \pm 3.3	93.5 \pm 7.7	94.5 \pm 7.9	92.6 \pm 1.7

Table IV. Pharmacokinetic Parameters* of Neeroiocitrin and Naringin in Rats After Oral Administration of Gushudan ($n = 6$)

Parameters	Neeroiocitrin	Naringin
C_{max} (ng/mL)	236 \pm 67.2	332 \pm 33.2
T_{max} (h)	0.17 \pm 0.04	0.25 \pm 0.03
$t_{1/2}$ (h)	1.62 \pm 0.57	1.88 \pm 0.70
k_e (h^{-1})	0.47 \pm 0.14	0.42 \pm 0.16
$AUC_{0-\infty}$ (ng h/mL)	204 \pm 88	289 \pm 149

* Mean \pm SD.

The mean recoveries of neoeriocitrin and naringin ranged from 91.7% to 101% and from 90.9% to 104%, respectively. The mean recoveries of neoeriocitrin and naringin at three QC levels are shown in Table II. The recovery of IS was $97.3 \pm 6.1\%$.

The stock solutions of neoeriocitrin, naringin, and IS were found to be stable at room temperature for 6 h. The three working solutions were stable at room temperature for 5 h. Table III summarizes the results of short-term stability, long-term stability, freeze-thaw stability of neoeriocitrin and naringin in plasma, and post-preparative stability, which indicated that the analytes were stable under these conditions.

Application to pharmacokinetic study of neoeriocitrin and naringin in rats

This validated method was applied to monitoring the plasma concentrations of neoeriocitrin and naringin in rats after a single oral administration of this formulation at a dose of 12 g/kg body weight. The mean plasma concentration–time curves are illustrated in Figure 4. The pharmacokinetic parameters are presented in Table IV. Neoeriocitrin and naringin were quickly absorbed into the body and could be detected 3 min after oral administration. The T_{\max} of naringin was 15 min, which is faster than that (45 min) reported in the literature (14). This may be due to the different dosage forms (herb extract in CMC versus pure compound in PEG 400) used. The plasma concentration of both neoeriocitrin and naringin declined quickly after T_{\max} .

Conclusions

A sensitive, rapid, specific, and accurate UPLC–MS–MS method was developed for the analysis of neoeriocitrin and naringin in rat plasma with a chromatographic run time of 2 min. The method has advantages of simple sample preparation and satisfactory selectivity and sensitivity with an LLOQ of 15.0 ng/mL for neoeriocitrin and 12.0 ng/mL for naringin. The analytical procedure was successfully applied to the pharmacokinetic study of neoeriocitrin and naringin in rats after oral administration of gushudan formulation, which will play an important role in investigating the action mechanism of gushudan formulation and supply a suitable reference in clinical application of the formulation.

Acknowledgment

This study was supported by the National Natural Science Foundation of China (No. 30472168 and No.20705021).

References

1. M.X. Liang. A Basic Concept of Establishing Platforms for Operational Techniques in Systems of Elaborately Selecting Small Prescriptions of Traditional Chinese Medicine. *World Sci. Technol.* **4**: 31–37 (2002).
2. F.H. Meng. Doctor's thesis, Shenyang Pharm. Univ., China, (2004).
3. Y.B. Li, Z.L. Xiong, and F.M. Li. Determination of epimedin C in rat plasma by reversed-phase high-performance chromatography after oral administration of Herba Epimedii extract. *J. Chromatogr. B* **821**: 235–239 (2005).
4. M. Liu, H.P. Liu, X.M. Lu, C. Li, Z.L. Xiong, and F.M. Li. Simultaneous determination of icariin, icaraside II and osthole in rat plasma after oral administration of the extract of Gushudan (a Chinese compound formulation) by LC–MS/MS. *J. Chromatogr. B* **860**: 113–120 (2007).
5. I. Lambev, I. Krushkov, D. Zheliazkov, and N. Nikolov. Antiexudative effect of naringin in experimental pulmonary edema and peritonitis. *Eksp. Med. Morfol.* **19**: 207–212 (1980).
6. M.J. Martin, E. Marhuenda, C. Perez-Guerrero, and J.M. France. Antiulcer effect of naringin on gastric lesions induced by ethanol in rats. *Pharmacology* **49**: 144–150 (1994).
7. S.M. Jeon, Y. B. Park, and M.S. Choi. Antihypercholesterolemic property of naringin alters plasma and tissue lipids, cholesterol-regulating enzymes, fecal sterol and tissue morphology in rabbits. *Clin. Nutr.* **23**: 1025–1034 (2004).
8. P.B. Gordon, I. Holen, and P.O. Seglen. Protection by naringin and some other flavonoids of hepatocytic autophagy and endocytosis against inhibition by okadaic acid. *J. Biol. Chem.* **270**: 5830–5838 (1995).
9. F.M. Li, F.H. Meng, Z.L. Xiong, Y.B. Li, R. Liu, and H. Liu. Stimulative activity of *Drynaria fortunei* (Kunze) J. Sm. extracts and two of its flavonoids on the proliferation of osteoblastic like cells. *Pharmazie* **61**: 962–965 (2006).
10. X.Z. Shuai. Chinese-English Term of Tradition Chinese Medicine, China, (1983).
11. S.L. Hsiu, T.Y. Huang, Y.C. Hou, D. H. Chin, and D.P. Chao. Comparison of metabolic pharmacokinetics of naringin and naringenin in rabbits. *Life Sci.* **70**: 1481–1489 (2002).
12. K. Ishii, T. Furuta, and Y. Kasuya. Determination of naringin and naringenin in human plasma by high-performance liquid chromatography. *J. Chromatogr. B* **683**: 225–229 (1996).
13. K. Ishii, T. Furuta, and Y. Kasuya. Determination of naringin and naringenin in human urine by high-performance liquid chromatography utilizing solid-phase extraction. *J. Chromatogr. B* **704**: 299–305 (1997).
14. T.Z. Fang, Y.G. Wang, Y. Ma, W.W. Su, Y. Bai, and P.Y. Zhao. A rapid LC/MS/MS quantitation assay for naringin and its two metabolites in rats plasma. *J. Pharm. Biomed. Anal.* **40**: 454–459 (2006).
15. X.L. Li, H.B. Xiao, X.M. Liang, D.Y. Shi, and J.G. Liu. LC–MS/MS determination of naringin, hesperidin and neohesperidin in rat serum after orally administrating the decoction of *Bulpleurum falcatum* L. and *Fractus aurantii*. *J. Pharm. Biomed. Anal.* **34**: 159–166 (2004).

Manuscript received January 3, 2008;
revision received June 17, 2008.