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Quantitation of the flavonoid wogonin and its major metabolite wogonin-7β-D-glucuronide in rat plasma by liquid chromatography–tandem mass spectrometry

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Abstract

This study described the application of liquid chromatography–tandem mass spectrometry for the quantitation of wogonin and its major metabolite in rat plasma. Only one conjugated metabolite with glucuronic acid was identified by chromatographic and electrospray multi-stage mass spectrometric assay. A derivatization reaction with 2-chlorethanol further demonstrated that the metabolite was wogonin-7β-D-glucuronide (W-7-G), not wogonin-5β-D-glucuronide. Other conjugated metabolites, e.g., sulfates and glucosides, were not detected. The plasma concentration of free wogonin was determined using atmospheric pressure chemical ionization source in the selected reaction monitoring mode. The method had a lower limit of quantitation of 0.25 ng/ml for wogonin, which offered increased sensitivity, selectivity and speed of analysis over an existing method. Incubation of the plasma samples with β-glucuronidase allows the quantitation of W-7-G. This quantitation method was successfully applied to a preclinical pharmacokinetic study of wogonin and its major metabolite, W-7-G, after an oral administration of 5 mg/kg wogonin to rats.

Keywords: Wogonin; Wogonin-7β-D-glucuronide

1. Introduction

Some naturally occurring flavonoids exhibit potential beneficial properties to human health with regard to the prevention of free radical pathologies such as inflammation and coronary heart disease [1–4]. Wogonin (Fig. 1), 5,7-dihydroxy-8-methoxyflavone, is one of the major bioactive flavonoid aglycones isolated from the root of *Scutellaria baicalensis* Georgi (Fam. Labiatae). It has been commonly used in traditional Chinese medicine as a remedy for the treatment of fever, cough, inflammation and hypotension. In recent years, wogonin has attracted substantial interest as it could modulate drug-metabolizing enzymes (suppressing CYP 2E1, 3A, 1A2 and UGT activities) [5] and possesses cancer-preventive effect [6].

With the increasing significance of a potential beneficial role of wogonin in human health, there is a growing demand for research on its absorption, metabolism and excretion. But few studies have been performed due to the lack of methodologies that meet the requirements of sensitivity and specificity

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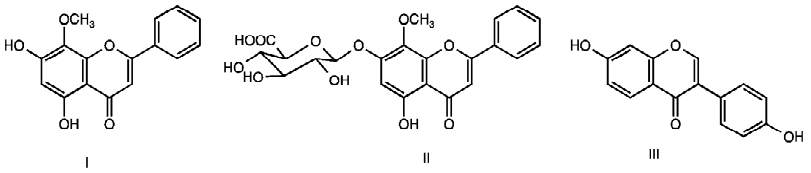

Fig. 1. The structures of wogonin (I), wogonin-7β-D-glucuronide (W-7-G, II) and daidzein (internal standard, III).

for the determination in biological fluids. Only a high-performance liquid chromatography (HPLC)–UV method was reported to determine wogonin in rat plasma after an intravenous administration [7]. The method with the lower limit of quantitation of 50 ng/ml could not offer the sensitivity necessary for the study of the pharmacokinetics of wogonin following oral administration.

Liquid chromatography with mass spectrometry (LC–MS) has been widely employed for the analysis of drug compounds in biological fluids because of its excellent specificity, speed, and sensitivity. It has been reported that 12 dietary flavonoid glycosides and aglycones in human urine were identified and quantified by LC–MS [8].

In the present study, we firstly identified wogonin metabolites in rat plasma with a liquid chromatography–ion trap mass spectrometry method. On this basis, we developed a rapid, selective and sufficiently sensitive liquid chromatography–triple quadrupole mass spectrometry method to determine wogonin and its major metabolite in rat plasma. The methods were successfully used in a preclinical pharmacokinetic study with rats.

2. Experimental

2.1. Materials

Wogonin was a kind gift of the Hong Kong University of Science and Technology (Hong Kong, China). Wogonin-7β-D-glucuronide was isolated from *Radix scutellariae*, and its structure was confirmed with ^{1}H- and ^{13}C-nuclear magnetic resonance spectroscopy in our laboratory. Daidzein (internal standard, I.S.) was supplied by the Liaoning Institute for the Control of Pharmaceutical Products (Shenyang, China). β-D-Glucuronidase (EC 3.2.1.31) was purchased from Sigma (St. Louis, MO, USA). Methanol and acetonitrile were of HPLC-grade, and other chemicals used were of analytical grade. Distilled water, prepared from demineralized water, was used throughout the study.

2.2. Instrumentation

A Shimadzu LC-10AD pump (Kyoto, Japan) was used for solvent delivery. A Finnigan LCQ ion trap mass spectrometer (San Jose, CA, USA) with an electrospray ionization (ESI) source was used for identification of wogonin metabolites and derivatives. A Finnigan TSQ triple quadrupole tandem mass spectrometer with an atmospheric pressure chemical ionization (APCI) interface was used for quantitative analysis of wogonin in rat plasma. Data acquisition was performed with Xcalibur 1.1 software (Finnigan). Peak integration and calibration were performed using Finnigan LCQuan software.

2.3. Chromatographic conditions

Chromatographic separation was achieved on a 250×4.6 mm, 5 μm Diamonsil C_{18} column (Dikma, Beijing, China) with a 4×3.0 mm SecurityGuard C_{18} (5 μm) guard column (Phenomenex, Torrance, CA, USA). The column temperature was maintained at 25 °C. The mobile phase consisted of acetonitrile–water–formic acid (80:20:1, v/v) at a flow-rate of 0.8 ml/min.

2.4. MS conditions

The mass spectrometer was operated in the positive ion detection mode with the corona discharge current set at 5 μA. The vaporizer temperature was 400 °C. Nitrogen was used as the sheath gas (70

p.s.i.) and the auxiliary gas (3 l/min) to assist with nebulization (1 p.s.i.=6894.76 Pa). The interface capillary temperature was heated to 250 °C. Collision induced dissociation (CID) studies were performed using argon with a collision cell gas pressure of 1.9 Pa and collision energies of 35 eV. The Quantitation was performed by selected reaction monitoring (SRM) of the transitions m/z 285→270 for wogonin and m/z 255→199 for the internal standard, respectively, with a scan time of 0.3 s per transition.

2.5. Preparation of standard and quality control samples

Stock solutions of wogonin and internal standard were prepared in methanol (1 mg/ml). A series of standard working solutions with concentrations in the range of 0.5–2000 ng/ml for wogonin were obtained by further dilution of the stock solution with the mobile phase. A 1000 ng/ml internal standard working solution was prepared by diluting the stock standard solution of daidzein with the mobile phase.

The standard working solutions (50 μl) were used to spike blank plasma sample (100 μl) either for calibration curves of wogonin or for quality control (QC) in prestudy validation and during the pharmacokinetic study.

All the solutions were stored at 4 °C and were brought to room temperature before use.

2.6. Sample preparation

2.6.1. Determination of free wogonin in rat plasma

To a 100 μl aliquot of rat plasma, 50 μl of the internal standard and 100 μl of 50 m*M* $NH_4H_2PO_4$ buffer (pH 5.0) were added. This mixture was extracted with 2 ml of diethyl ether–*n*-hexane (4:1, v/v) by shaking for 10 min. The organic and aqueous phases were separated by centrifugation at 2000 *g* for 5 min. The upper organic phase was transferred to another tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was dissolved in 100 μl of the mobile phase, and vortex mixed. A 20 μl aliquot of the solution was injected into the LC–MS–MS system for analysis.

2.6.2. Determination of the conjugated metabolite of wogonin in rat plasma

Rat plasma (100 μl) was mixed with 100 μl of β-D-glucuronidase (400 units/ml in 50 m*M* $NH_4H_2PO_4$ buffer, pH 5.0), followed by incubation in a water bath at 37 °C overnight (16 h) [9]. After enzymatic hydrolysis, total wogonin including free and conjugated wogonin was determined as described above.

2.7. Method validation

2.7.1. Confirmation of the major metabolite in rat plasma

To confirm the structure of major metabolite in rat plasma sample, a derivatization reaction [10] with three samples was carried out as follows: 100 μl aliquots of wogonin stock solution (400 μg/ml) were evaporated to dryness at 40 °C. The residue was dissolved in 100 μl of 2 *M* NaOH. To this solution, 25 μl of 2-chlorethanol was added. The reaction was carried out at 75 °C for 2 h. The reaction mixture was then extracted with ethyl acetate. The organic layer was evaporated to dryness. The residue was dissolved in 200 μl of methanol. Aliquots (20 μl) of the solution were analyzed by LC–MS^n. Chromatographic separation was achieved on a Diamonsil C_{18} column (250×4.6 mm) with a mobile phase consisting of acetonitrile–water–formic acid (65:35:1, v/v). The flow-rate was 0.5 ml/min.

The hydroxyethyl derivative of wogonin-7β-D-glucuronide (W-7-G) was synthesized by reaction of W-7-G (instead of wogonin) and 2-chlorethanol as described above. The reference compound of W-7-G was obtained from *S. radix*. After the derivatization, the reaction mixture was adjusted to pH 5 with 50 m*M* H_3PO_4, then incubated with 200 μl of β-D-glucuronidase at 37 °C for 24 h. After the incubation, the mixture was extracted and analyzed by LC–MS^n as described above.

Plasma samples 1 h after administration of 5 mg/kg wogonin to rats (1 ml) were extracted as described in Section 2.6.1. But the organic layer was discarded and the aqueous layer was further treated by a solid-phase extraction (SPE) procedure. Then the methanol eluate was evaporated to dryness and reacted with 2-chlorethanol in 2 *M* NaOH solution as described above. The reaction mixture was hydro-

Table 1
Summary of precision and accuracy from QC samples of rat plasma extracts before enzymatic hydrolysis ($n=3$ day, six replicates per day)

Added Conc. (ng/ml)	Found Conc. (ng/ml)	Intra-run RSD (%)	Inter-run RSD (%)	Relative error (%)
0.50	0.51	2.2	5.9	1.3
4.00	3.96	10.6	6.2	−1.0
20.0	19.9	13.1	7.3	−0.3

lyzed with β-D-glucuronidase, and analyzed by LC–MS^n.

2.7.2. Quantitation of wogonin and its major metabolite in rat plasma

During prestudy validation, the calibration curves were defined in three runs based on triplicate assays of the spiked plasma samples, and quality control (QC) samples from three concentrations (see Tables 1 and 2) were determined in replicates ($n=6$) on the same day to calculate the accuracy and precision of the method. Calibration curves were constructed using a $1/x^2$ weighted linear regression of the peak-area ratios of the analyte to internal standard versus the plasma concentrations of the analyte. During routine analysis, each analytical run included a set of calibration samples, a set of QC samples in duplicate and unknowns.

The matrix effect (co-eluting, undetected endogenous matrix compounds that may influence ionization of the analyte) was investigated by extracting ''blank'' biological fluids from six different sources, reconstituting the final extract in the mobile phase containing a known amount of analyte, analyzing the reconstituted extracts and then comparing the peak areas of analyte.

The extraction recovery of wogonin was determined by comparing the peak area ratios of the analyte to the internal standard between the pre-extraction spike and the post-extraction spike of the analyte, with the internal standard spiked post-extraction in both cases.

The stability of wogonin in rat plasma was determined under a variety of storage and process conditions. The freeze–thaw stability was evaluated by analyzing QC samples at concentrations of 5, 100 and 1000 ng/ml after undergoing three freeze (−20 °C)–thaw (room temperature) cycles. The bench-top storage stability was assessed by placing QC samples at room temperature for a fixed period of time before being extracted and analyzed. The stability of wogonin in the reconstitution mobile phase was determined.

2.8. Application of the LC–MS–MS quantitative analysis

Male Wistar rats (68–90 days old, 250–280 g) were purchased from the Laboratory Animal Center of Shenyang Pharmaceutical University. Before the day of administration, the rats were fasted overnight but were allowed water ad libitum. The solution of wogonin was administered to rats (5 mg/kg) by oral gavage. Blood samples were withdrawn from the tail vein at 0.08, 0.25, 0.5, 1, 1.5, 2, 3, 5, 8, 12, 24, 36 and 48 h after administration. Within 0.5 h of collection, the heparinized blood was centrifuged for 10 min at 2000 *g*, and plasma was obtained and stored frozen at −20 °C until analysis.

Table 2
Summary of precision and accuracy from QC samples of rat plasma extracts after enzymatic hydrolysis ($n=3$ day, six replicates per day)

Added Conc. (ng/ml)	Found Conc. (ng/ml)	Intra-run RSD (%)	Inter-run RSD (%)	Relative error (%)
5.00	5.05	8.7	7.4	1.0
100	99.7	5.4	7.1	−0.3
1000	1007	1.1	5.5	0.7

3. Results and discussion

3.1. Identification of wogonin metabolites in rat plasma

Studies on the metabolism of wogonin in vivo were not reported until now. In this experiment, we firstly identified the major metabolites of wogonin in rat using an ion trap mass spectrometer. Compared with the APCI source, ESI was more suitable to analyze heat-labile, high-molecular-mass or highly polar compounds. In order to profile for potential metabolites of wogonin, especially conjugated metabolites, the plasma samples after an administration of 5 mg/kg wogonin to Wistar rats were extracted by SPE and analyzed using LC–MS^n. The mobile phase consisted of acetonitrile–water–formic acid (65:35:1, v/v) at a flow-rate of 0.5 ml/min. Under the conditions, only the metabolite M-1 was observed (retention time 6.2 min) by comparison of blank plasma (see Fig. 2). The retention time on HPLC and multiple mass spectra of M-1 were identical to those of the reference compound of W-7-G. The MS–MS spectrum gave abundant ion at m/z 285, which was produced by neutral loss of the glucuronic acid moiety (see Fig. 2). Further full scan MS^3 spectrum of m/z 461→285 produced the product ion m/z 270, which was identical to that produced from MS–MS spectrum of wogonin. Wogonin has two potential sites for glucuronidation, one at the phenolic 5-OH group and one at phenolic 7-OH group. The existence of wogonin-5β-D-glucuronide (W-5-G) in the rat plasma sample could not be excluded at this stage.

Only W-7-G was found and extracted from *S. radix*, and W-5-G was not found [11]. Derivatization of flavonoids with 2-chlorethanol also showed the same tendency, i.e., the 5-OH group is much more difficult to react than the 7-OH group [10]. Obviously, the 5-OH group is easily to form an intramolecular hydrogen bond with the carbonyl group, so that it shows weak chemical as well as biochemical reactivity.

To confirm the structure of M-1, a hydroxyethyl derivatization was carried out. Fig. 3 shows the selected ion monitoring (SIM) results for hydroxyethyl wogonin from the reaction products of W-7-G, wogonin, and the plasma sample by monitoring the $[M+H]^+$ ion at m/z 329. W-7-G only has one site (phenolic 5-OH group) free for hydroxyethylation.

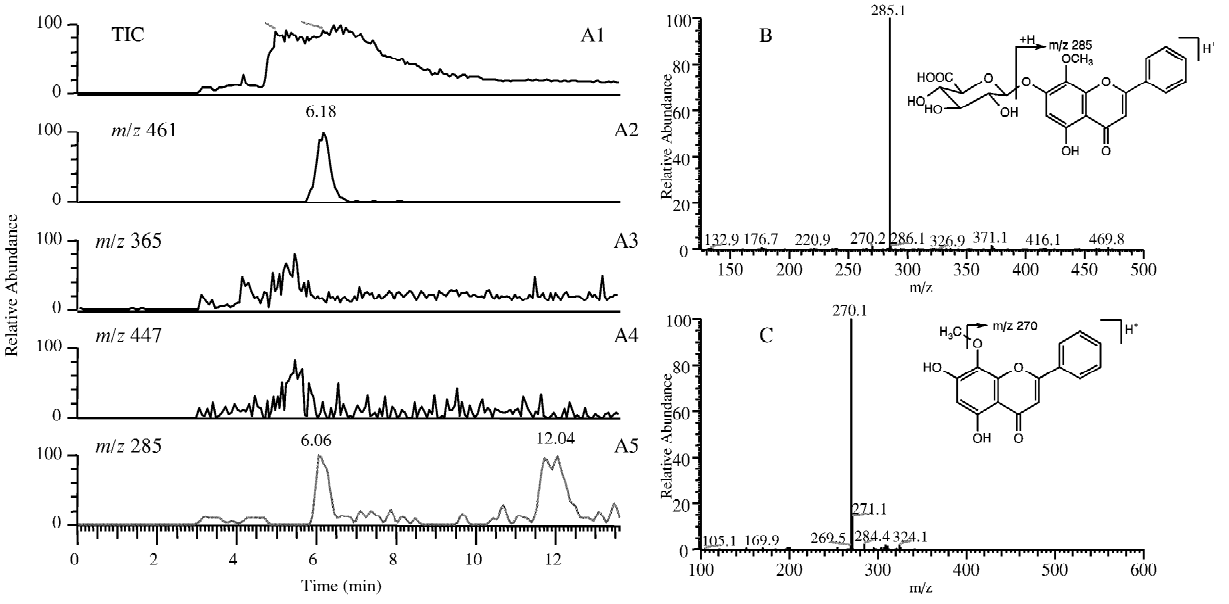

Fig. 2. LC–MS^n analysis of wogonin and its conjugated metabolites in plasma after an administration of 5 mg/kg wogonin to a Wistar rat. (A) Total ion current and selected ion monitoring chromatograms (A2, wogonin glucuronide; A3, wogonin sulfate; A4, wogonin glucoside; A5, free wogonin); (B) full scan MS–MS spectrum of peak at 6.18 min; (C) full scan MS^3 spectrum of peak at 6.18 min.

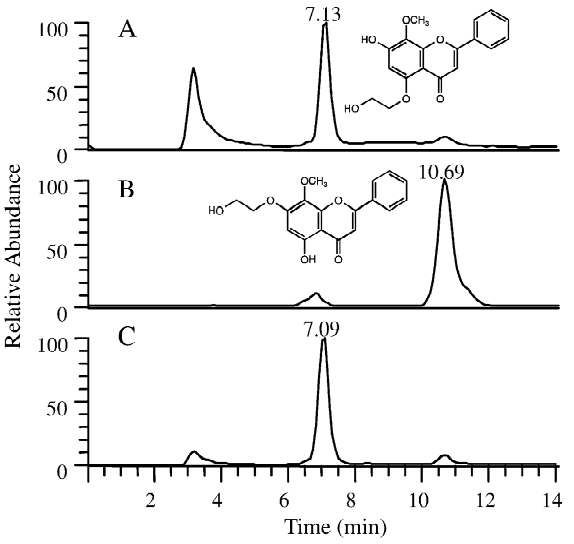

Fig. 3. SIM chromatograms of hydroxyethyl wogonin ($[M+H]^+=329$): (A) from hydrolyzed product of W-7-G hydroxyethyl derivative; (B) from wogonin hydroxyethyl derivative; (C) from hydrolyzed product of derivatized plasma sample 1 h after an administration of 5 mg/kg wogonin to a Wistar rat.

So only a peak ($t_R=7.1$ min) was found after hydroxyethyl W-7-G was hydrolyzed by β-D-glucuronidase, which corresponded to 5-hydroxyethyl wogonin. Although wogonin has two phenolic hydroxyl groups for derivatization reaction, only one peak ($t_R=10.7$ min) was found in its reaction product at m/z 329 (a further product was dihydroxyethyl wogonin, not shown in this chromatogram). Compared with the result of W-7-G, the peak should correspond to 7-hydroxyethyl wogonin. The chromatographic difference between derivatives of 5-OH and 7-OH substituted wogonin was obvious. From the rat plasma sample containing M-1 after derivatization and enzymatic hydrolysis, only one peak was found in the SIM chromatogram, which corresponded to 5-hydroxyethyl wogonin. Therefore we could infer that M-1 was a glucuronide metabolite conjugated with 7-OH group of wogonin but not with 5-OH group.

In addition, other conjugated metabolites of wogonin such as sulfate and glucoside could not be found in plasma samples by SIM for m/z 365 and 447, respectively (see Fig. 2). Under the LC–MS^n conditions, the retention time of wogonin was ca. 12 min. It exhibited the protonated molecular ion $[M+H]^+$ at m/z 285. The MS–MS spectrum produced characteristic fragment ions of m/z 270, which can be explained by the loss of a methyl group from the precursor ion. Plasma concentrations of free wogonin in rat were found to be significantly lower than those of its glucuronide metabolite from the SIM chromatogram (see Fig. 2).

3.2. Optimization of LC–MS–MS for quantitative analysis

APCI was chosen to quantify wogonin in plasma in preference to ESI due to significantly lower levels of chemical noise and its compatibility with a conventional HPLC system (i.e., high flow-rate of 0.8 ml/min). Similar to the ESI source, the protonated molecular ions $[M+H]^+$ were the most abundant ions in the APCI full-scan Q1 spectra of wogonin and daidzein. The $[M+H]^+$ ions were therefore selected as the precursor ion. Fig. 4 displays the product ion spectra of wogonin and the internal standard. In the selected conditions, $[M+H]^+$ ion for wogonin was reduced by more than 95%, which resulted in maximum intensity for the product ions at m/z 270. While the maximum intensity for product ion at m/z 199 could be obtained when $[M+H]^+$ ion for I.S. was reduced by more than 50%. These major fragment ions were chosen in the SRM acquisition.

The chromatographic conditions were optimized in view of achieving maximum peak responses, namely by not interfering with the ionization process of the analytes in the APCI source. Although using short HPLC columns can reduce chromatographic time, significant ion suppression could be observed in the experiment. Therefore, a 250×4.6 mm column was selected to achieve an efficient chromatographic separation of the analyte from endogenous plasma components for eliminating matrix effect and ion suppression from endogenous substances.

Different liquid–liquid extraction (LLE) conditions were evaluated including different aqueous pH buffers and organic extraction solvents. The pH control by the phosphate buffer solution could increase the extraction recovery of the analyte, with concomitant lowering of the plasma background and thus higher sensitivity could be achieved. In this work, a pH 5.0 phosphate buffer solution was found

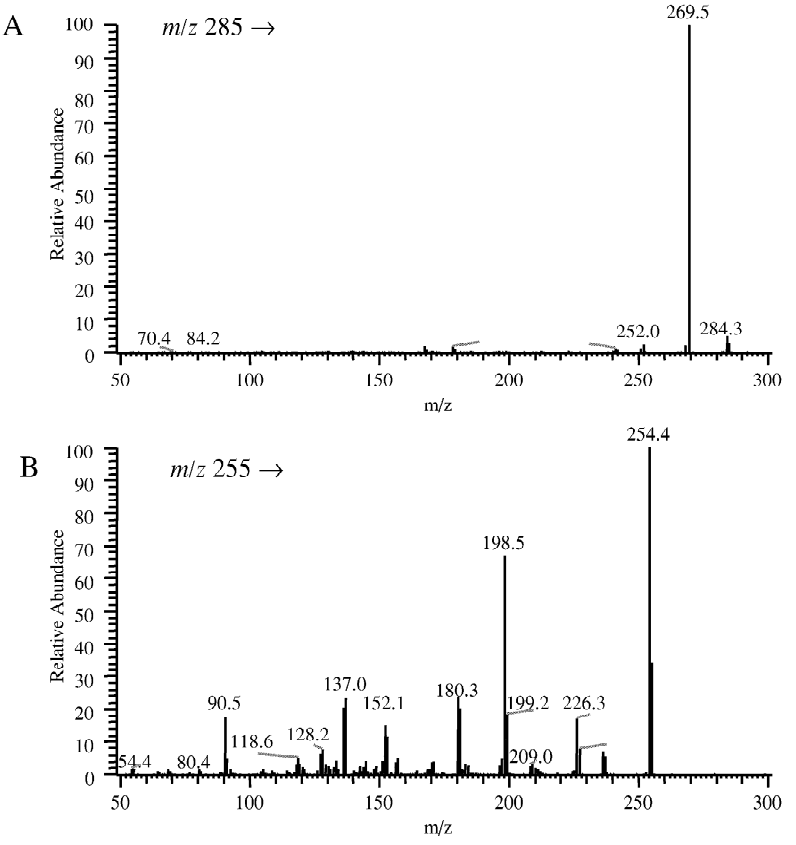

Fig. 4. Full-scan product ion spectra of $[M+H]^+$ of (A) wogonin and (B) daidzein.

the optimal. Four organic extraction solvents were evaluated: ethyl acetate, diethyl ether, diethyl ether–dichloromethane (2:1, v/v) and diethyl ether–*n*-hexane (4:1). Among them, diethyl ether–*n*-hexane clearly yielded the highest recovery.

3.3. Method validation

3.3.1. Assay specificity

The specificity of the method was demonstrated by comparing chromatograms of six independent plasma samples from rats—each as a blank sample and a spiked sample. Fig. 5 demonstrates that no interferences from endogenous substances with the analyte and internal standard were detected. Typical retention times for wogonin and the internal standard were 4.9 and 3.1 min, respectively.

No matrix effect was observed for the six different plasma pools. The peak areas of the six reconstituted samples had an RSD of 5.1%, indicating that the extracts were ''clean'' with no co-eluting ''unseen'' compounds that could influence the ionization of wogonin.

3.3.2. Linearity of calibration curves and lower limit of quantitation

Linear calibration curves were obtained over the concentration range of 0.25–20 ng/ml for wogonin in rat plasma. The typical equation of calibration curves was: $y=3.897\cdot10^{-5}+1.997\cdot10^{-3}x$, $r=0.9965$.

The lower limit of quantitation (LLOQ) for determination of free wogonin in plasma, defined as the lowest concentration analyzed with an accuracy $\leq$15% and a precision $\leq$15%, was 0.25 ng/ml. The limit is sufficient for pharmacokinetic study of free wogonin after an oral administration to rats.

It was found that the concentrations of W-7-G

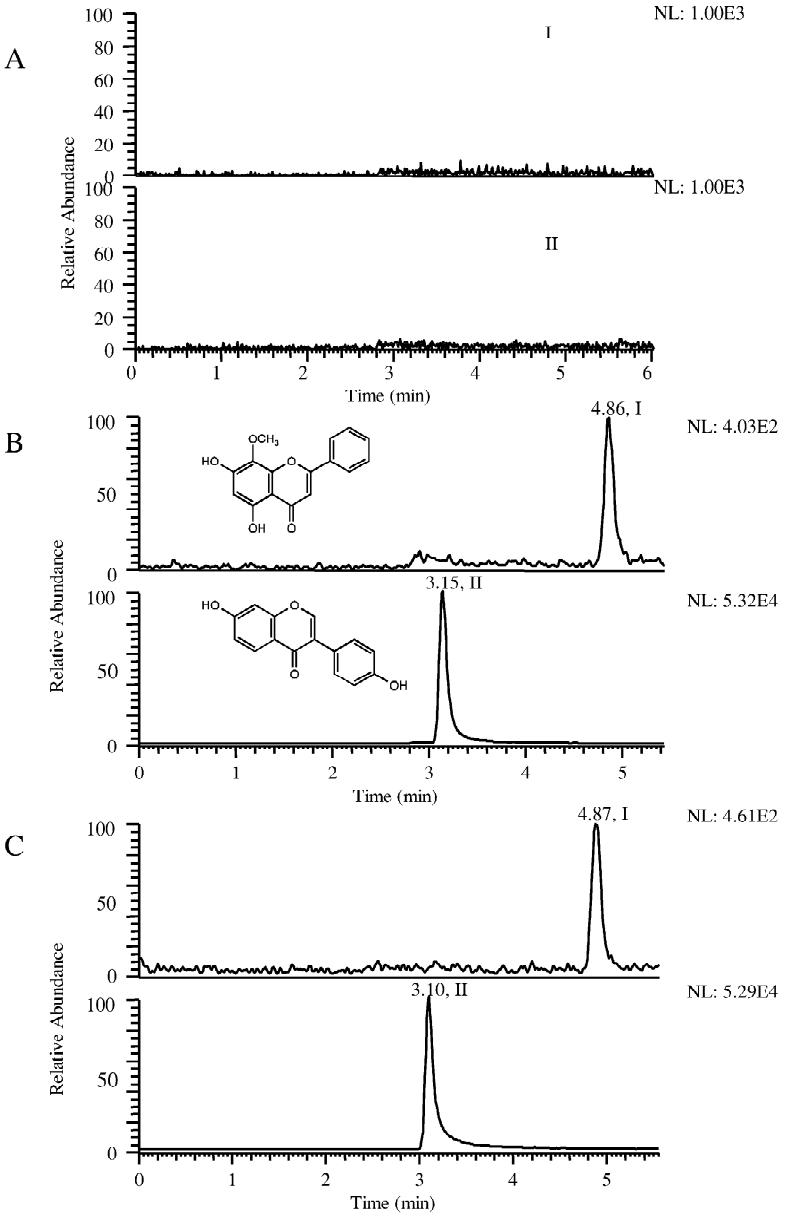

Fig. 5. Representative SRM chromatograms of: (A) a blank rat plasma sample; (B) a blank plasma sample spiked with 2.0 ng/ml wogonin and the internal standard daidzein (I.S., 1000 ng/ml); (C) plasma sample from a Wistar rat 0.5 h after an oral administration of wogonin (5 mg/kg). Peak I, wogonin; Peak II, I.S.

were about 100-times higher than those of free wogonin in rat plasma. Linear calibration curves were constructed over the concentration range of 5–1000 ng/ml to determine total wogonin after enzymatic hydrolysis in rat plasma. Typical equation of calibration curves was: $y = -1.275 \cdot 10^{-4} + 2.009 \cdot 10^{-3}x$, $r = 0.9995$. Concentrations of W-7-G in rat plasma were calculated as the difference between wogonin concentrations before and after enzymatic hydrolysis and expressed as wogonin equivalents.

3.3.3. Assay precision and accuracy

The data from QC samples in validation were examined by a one-way analysis of variance (ANOVA) to estimate the inter- and intra-run precision and accuracy of the method. The results are presented in Tables 1 and 2 for determination of wogonin before/after enzymatic hydrolysis, respectively. The intra-run precisions, calculated from QC samples, were less than 14 and 9% for each QC level of free and total wogonin, respectively. The inter-run precision, calculated from QC samples, was less than 8% for each QC level. The accuracy as determined from QC samples was within ±2% for each QC level.

3.3.4. Extraction recovery

The analyte recoveries under the LLE conditions were 72.9±6.8, 72.5±4.3, 70.5±5.6, and 72.4±5.5% at concentrations of 0.5, 5.0, 100.0, and 1000.0 ng/ml, respectively. The recovery of the internal standard averaged 65% in rat plasma.

3.3.5. Analyte stability

The stability of wogonin in plasma and mobile phase were investigated. Wogonin was found to be stable after three freeze–thaw cycles in plasma. The accuracy calculated from QC samples ranged from −9.8 to 9.5%. The analyte was also shown to be stable in rat plasma at room temperature for at least 0.5 h (relative error, RE<8%) and in the reconstitution mobile phase for 24 h (RE<6.7%).

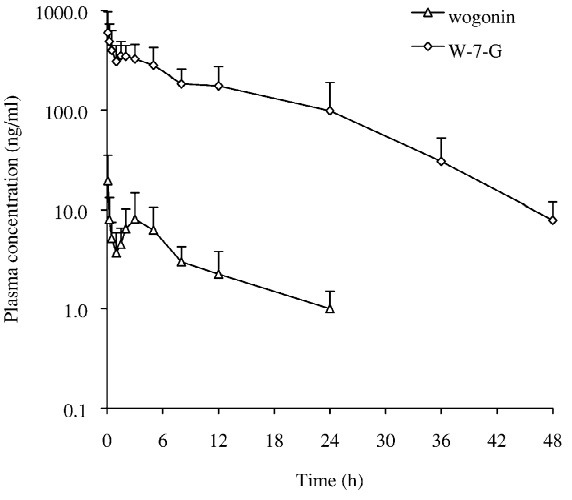

Fig. 6. Mean plasma concentration–time profile of wogonin and its major metabolite wogonin-7β-D-glucuronide (W-7-G) after an oral administration of 5 mg/kg wogonin to eight Wistar rats. Plasma concentrations of W-7-G in rat were expressed as wogonin equivalents.

3.4. Application of analytical method in pharmacokinetic studies

After a single oral administration of 5 mg/kg wogonin to eight Wistar rats, plasma concentrations of wogonin and its glucuronide conjugate were determined by the described LC–APCI-MS–MS method. Fig. 6 shows mean plasma concentration–time curves of wogonin and its metabolite after an oral administration ($n=8$). Plasma concentrations of free wogonin in rat were detectable for at least 24 h.

4. Conclusion

In this study, the use of LC–ESI-MS in multi-stage full scan mode allowed one to identify the conjugated metabolites of wogonin in a complex biological matrix. Only W-7-G was observed as major metabolite in rat plasma. The use of LC–APCI-MS–MS in the SRM mode gave specific, quantitative levels as low as 0.25 ng/ml. The quantitative method was validated with respect to linearity, precision and accuracy, and analysis of real samples was demonstrated. Application with modification to the assay should allow the developed methodology to be used for the determination of different flavonoids in plasma.

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References

[1] M.G.L. Hertog, E.J.M. Feskens, P.C.H. Hollman, M.B. Katan, D. Kromhout, Lancet 342 (1993) 1007.

[2] P.J. Knekt, R. Jaervinen, A. Reunanen, J. Maatela, Br. Med. J. 312 (1996) 478.
[3] S.O. Keli, M.G.L. Hertog, E.J.M. Feskens, D. Kromhout, Arch. Intern. Med. 156 (1996) 637.
[4] L. LeMarchand, S.P. Murphy, J.H. Hankin, L.R. Wilkens, L.N. Kolonel, J. Natl. Cancer Inst. 92 (2000) 154.
[5] Y.F. Ueng, C.C. Shyu, Y.L. Lin, S.S. Park, J.F. Liao, C.F. Chen, Life Sci. 67 (2000) 2189.
[6] H. Yano, A. Mizoguchi, K. Fukuda, M. Haramaki, S. Ogasawara, S. Monosaki, M. Kojiro, Cancer Res. 54 (1994) 448.
[7] T.H. Tsai, C.J. Chou, T.R. Tsai, C.F. Chen, Planta Med. 62 (1996) 263.
[8] S.E. Nielsen, R. Freese, C. Cornett, L.O. Dragsted, Anal. Chem. 72 (2000) 1503.
[9] D.F. Zhong, X.Y. Chen, J. Chromatogr. B 721 (1999) 67.
[10] P. Von Dittrich, J. Ostrowski, E. Beubler, E. Schraven, W. Kukovetz, Arzneim.-Forsch. 35 (1985) 765.
[11] F. Ikegami, K. Matsunae, M. Hisamitsu, T. Kurihara, T. Yamamoto, I. Murakoshi, Biol. Pharm. Bull. 18 (1995) 1531.