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ORIGINAL ARTICLE

Determination of Isoorientin Levels in Rat Plasma by Ultra-High Performance Liquid Chromatography Coupled with Diode Array Detector and Its Application to a Pharmacokinetic Study

Doan Nguyen Kieu Trang*, Yu Chul Kim[1]*, Seung-Hwan Kwon[2], Choon-Gon Jang[2], Han-Joo Maeng

Gachon Institute of Pharmaceutical Sciences, College of Pharmacy, Gachon University, Yeonsu-gu, Incheon, Republic of Korea, [1]Department of Pharmaceutical Engineering, Inje University, Gimhae, Republic of Korea, [2]Department of Pharmacology, School of Pharmacy, Sungkyunkwan University, Suwon, Republic of Korea
*These authors contributed equally to this work

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ABSTRACT

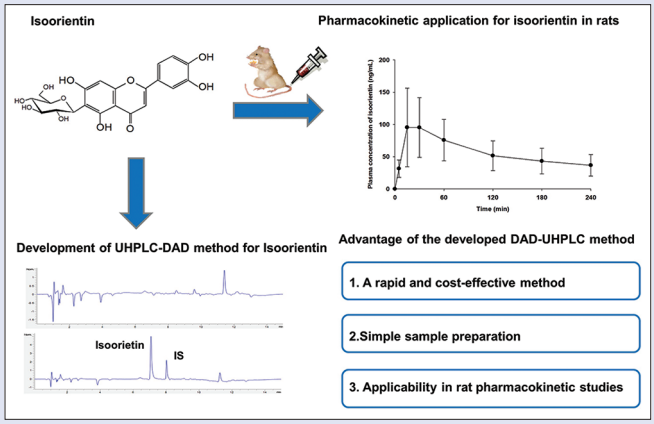
Background: Isoorientin is a C-glycosylflavone and a pharmacologically active ingredient found in various medicinal plants. **Objective:** The aim of this study is to develop a specific and reproducible ultra-high-performance liquid chromatography-diode array detector (UHPLC-DAD) based method to quantify isoorientin in rat plasma and to apply the devised method to a pharmacokinetic study in rats. **Materials and Methods:** Simple protein precipitation with methanol was utilized to extract isoorientin and rutin (the internal standard) from rat plasma. Analytes were separated on an UHPLC Phenomenex Luna Omega Polar C18 column (100 mm × 2.1 mm, 1.6 μm) by gradient elution using a mobile phase containing 1% aqueous acetic acid and 100% acetonitrile at the flow rate of 0.25 mL/min. **Results:** The developed UHPLC-DAD method showed good linearity (R^2 = 0.9993) over the concentration range 20–5000 ng/mL with a lower limit of quantification of 20 ng/mL. Intra- and inter-day precisions were <10.7% and accuracy were within the range of 89.4%–101.3%. The recovery of isoorientin from plasma was in acceptable range (89.4%–95.5%). The devised method was successfully applied to a pharmacokinetic study after a single oral administration of isoorientin to rats. **Conclusion:** This is the first report of a simple, rapid, and cost-effective UHPLC-DAD-based method for quantifying isoorientin in rat plasma and its application to a pharmacokinetic study.

Key words: Isoorientin, oral, pharmacokinetics, plasma, rat, ultra-high-performance liquid chromatography-diode array detector

SUMMARY

- A simple, rapid, and cost-effective ultra-high-performance liquid chromatography-diode array detector (UHPLC-DAD) method was developed and validated for the quantitation of isoorientin
- This method showed good linearity, accuracy, and precision suitable for the determination of isoorientin in rat plasma
- The developed UHPLC-DAD method successfully applied to an oral pharmacokinetic study in rats.

Abbreviations used: UHPLC: Ultra-high-performance liquid chromatography; DAD: Diode array detector; LC-MS/MS: Liquid chromatography-tandem mass spectrometry; LLOQ: Lower limit of quantification; IS: Internal standard; QC: Quality control; RSD: Relative standard deviation; C_{max}: The maximum plasma concentration; T_{max}: The time point to reach the maximum concentration; AUC_{last}: The area under the curve from time zero to the last measurable point, AUC_{inf}: The area under the curve from time zero to infinity; k_e: Terminal elimination rate constant; $t_{1/2}$: Terminal half-life; CL/F: Oral clearance; V_d/F: Apparent volume of distribution after oral administration; SD: Standard deviation; SPE: Solid-phase extraction.



Correspondence:

Dr. Han-Joo Maeng,
Gachon Institute of Pharmaceutical Sciences, College of Pharmacy,
Gachon University 191 Hambakmoei-ro,
Yeonsu-gu, Incheon 21936, Republic of Korea.
E-mail: hjmaeng@gachon.ac.kr
Dr. Choon-Gon Jang,
Department of Pharmacology,
School of Pharmacy, School of Pharmacy,
Sungkyunkwan University, Suwon 16419,
Republic of Korea.
E-mail: jang@skku.edu
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INTRODUCTION

Flavonoids are natural polyphenolic compounds and are widely distributed in plants and have a diphenylpropane (C6-C3-C6 skeleton) consisting of two phenyl rings and heterocyclic ring. The flavonoids are divided into the following subclasses according to oxidative status and the number and types of substituents on the heterocyclic ring, flavones, flavonols, flavanones, flavanols, dihydroflavonols, anthocyanidins, and isoflavones.[1] Flavonoids have received widespread attention because they have various health benefits and pharmacological effects.[2]

Most flavonoids exist as glycosides in plants, typically as O- or C-glycosides, though a small number are aglycones.[3] In some cases, glycosyl flavonoids

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have better pharmacological effects or pharmacokinetic properties than their aglycones, although it is difficult to draw general conclusions regarding the impact of glycosylation.[3,4] Isoorientin (also called homoorientin, 6-C-beta-D-glucopyranosyl-3',4',5,7-tetrahydroxyflavone) is a flavone C-glycoside (luteolin-6-C-glucoside) [Figure 1a] that is present in a variety of medicinal plants, including *Jatropha ciliata*, *Arum palaestinum*, *Desmodium styracifolium*, *Swertia pseudochinensis, Cymbopogon citrates*, *Gentiana olivieri*, *Fagopyrum esculentum*, and *Patrinia villosa*.[5-12] Recent publications have reported isoorientin has diverse pharmacological effects, which include anti-inflammatory,[13-15] antioxidant,[15,16] anti-Alzheimer's disease,[15] anti-diabetic,[15] hepato-protective,[17] and anticancer effects[18] *in vitro* and/or *in vivo*. Furthermore, isoorientin has been used in nutraceutical products made from plants, and thus, its pharmacokinetics and pharmacodynamics are of considerable research interest.

Several reports have been issued on the pharmacokinetics of isoorientin in rats.[8,19-22] In these studies, the active substances were administered as components of plant extracts, with the exception of a study in which pure isoorientin was administered by intravenous injection (5, 10, and 15 mg/kg) or orally (150 mg/kg).[21] It was reported that the average intake of flavonols and flavones in the Netherlands was 23 mg/day, whereas Americans consumed about 115 mg/day of flavonol and flavone aglycones.[23] As isoorientin is one of the flavones contained in functional products, it is likely to be taken routinely in low amounts. In order to investigate the pharmacokinetics of isoorientin, we carried out a pharmacokinetic study by administrating isoorientin orally to rats.

Several methods have been proposed for determining isoorientin levels in complex matrices like plasma. High-performance liquid chromatography (HPLC) is readily available in most laboratories, but we found a reported HPLC method for isoorientin quantification in rat plasma was not sufficiently sensitive for quantification purposes (i.e., lower limit of quantification [LLOQ] of 4000 ng/mL).[21] Although a small number of liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have been reported to quantify isoorientin in plasma with excellent sensitivity,[8,20,22] such methods are limited to research and clinical facilities because of the high cost and complexities of LC-MS/MS systems. On the other hand, ultra-HPLC (UHPLC) has been utilized extensively because of its short run times, excellent separation efficiencies, low solvent consumption, and low cost.[24] Therefore, we undertook to develop and validate an UHPLC-diode array detector (DAD)-based method for the determination of isoorientin in rat plasma. The validated UHPLC-DAD method developed during the present study was found to be superior to previously described HPLC methods in terms of simplicity, cost-efficiency, and sensitivity, and was successfully applied to a pharmacokinetic study of orally administered isoorientin in Sprague-Dawley (SD) rats.

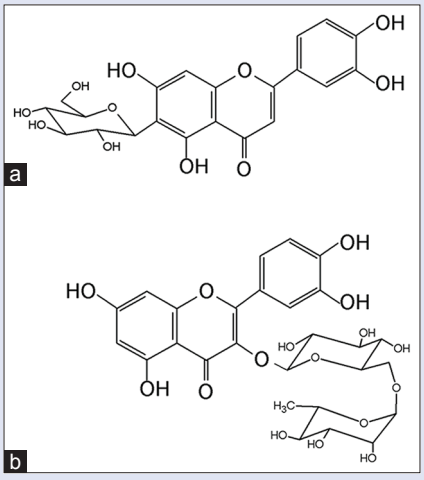

Figure 1: Chemical structures of isoorientin (a) and rutin (b, internal standard)

MATERIALS AND METHODS

Chemicals and reagents

Isoorientin (99% purity by HPLC Figure 1a) was provided from Extrasynthese Co., Lyon, France. Rutin (the internal standard [IS] Figure 1b) and acetic acid (≥99%) were purchased from Sigma-Aldrich Co., St. Louis, MO, USA. HPLC grade acetonitrile and methanol were obtained from Honeywell Burdick and Jackson Co., Ulsan, Korea. Physiological saline was produced by Daehan Pharm. Co. Ltd., Gyeonggi-do, Korea. Dimethyl sulfoxide (DMSO) and propylene glycol were obtained from Duksan Pure Chemicals (Gyeonggi-do, Korea).

Animals

Male SD rats (8 weeks, 260–280 g) were purchased from Nara Biotech (Korea). Rats were housed in cages under climate-controlled conditions and a 12 h dark/light cycle with free access to food and water. Animals were acclimated for at least 5 days in the laboratory prior to experiments, and all animal experiments were performed in accordance with the Guidelines for Animal Care and Use issued by Gachon University.

Instrument and chromatographic conditions

An Agilent 1290 Infinite II LC system (Agilent Technologies, Santa Clara, CA, USA) equipped with an autosampler, a 1290 Infinite binary pump and a DAD was used for the UHPLC analysis. Separations were performed using a Phenomenex Luna Omega Polar C18 (100 mm × 2.1 mm, 1.6 μm) column (Phenomenex, Torrance, CA, USA) at 25°C. Gradient elution was performed at 0.25 ml/min using 1% acetic acid (solvent A) and acetonitrile (solvent B) as follows: (1) solvent B was set to 14% in the first 3.5 min, (2) a linear gradient was run to 24% B from 3.5 to 5 min and then to 30% B at 6.5 min, (3) solvent B was maintained at 30% from 6.5 to 10 min, and (4) kept at 14% for until 15 min. A detection wavelength of 350 nm was chosen because isoorientin absorbed maximally at this wavelength.

Preparation of stock and working solutions

Stock solutions were prepared by dissolving isoorientin or rutin in methanol at a concentration of 1 mg/mL. Subsequently, the stock solution of isoorientin was diluted with methanol into a series of working solutions of concentration 0.2, 0.5, 1, 2, 5, 10, 20, and 50 μg/mL. In addition, a 250 ng/mL stock solution of the IS (rutin) in methanol was prepared. Quality control (QC) working solutions of concentration 0.2, 0.4, 8, and 40 μg/mL were prepared from isoorientin stock solution. All solutions were stored at −20°C and brought to room temperature before use.

Preparation of plasma standards and quality control samples

Plasma standards were prepared as follows. Blank plasma (90 μL) was transferred into 1.5 mL centrifuge tubes, and then 10 μL aliquots of isoorientin working solutions were added to achieve concentrations of 20, 50, 100, 200, 500, 1000, 2000, and 5000 ng/mL. In the same manner, QC samples were prepared in blank plasma at four isoorientin concentrations, i.e., at 20 ng/mL (LLOQ), 40 ng/mL (low), 800 ng/mL (medium), and 4000 ng/mL (high).

Sample preparation

IS solutions (200 µL) were added to 100 µL plasma samples, QC samples or plasma standards. Mixtures were vortexed for 1 min and then centrifuged at 14,000 rpm for 15 min at 4°C. Supernatants were filtered through Phenex RC membrane (0.2 µm) and 5 µL aliquots of filtrates were injected directly onto the analytical column.

Method validation

The validation procedure was conducted in accord with the Guideline for Industry: Bioanalytical Method Validation, U.S. Food and Drug Administration, in terms of selectivity, linearity, sensitivity, precision, accuracy, recovery, and stability.[25]

Selectivity

Selectivity was evaluated using blank rat plasma collected from five different sources. Results obtained for blank plasma, blank plasma spiked with IS only, blank plasma containing isoorientin and IS, and plasma from pharmacokinetic study were compared to confirm the absence of interference with isoorientin and IS in chromatograms.

Linearity

Plasma standards were prepared at isoorientin concentrations ranging from 20 to 5000 ng/mL. Calibration curves were constructed by plotting isoorientin to IS peak area ratios (Y-axis) versus isoorientin concentration (X-axis) using six different replications. Linearity was assessed using coefficients of determination (R^2) obtained by weighted (1/x) least-squares linear regression analysis.

Sensitivity

LLOQ was defined as the lowest concentration on the calibration curve that could be quantified with an accuracy of within 80%–120% and with a precision (relative standard deviation [RSD%]) not exceeding 20%. The signal-to-noise ratio (S/N ratio) threshold of LLOQ samples was taken to be >5.[26]

Precision and accuracy

Intraday precision and accuracy were assessed using QC samples at LLOQ, low, middle, and high isoorientin concentrations in five replicate samples, whereas interday precision and accuracy were determined using data obtained on 5 consecutive days. Precision was expressed as relative standard deviation and accuracy by expressing determined concentrations as percentages of true concentrations.

Recovery and extraction efficiency

The recoveries of isoorientin and IS were determined by comparing peak areas of extracted plasma QC samples with those of standard solutions in methanol at the same concentration.[27]

Extraction efficiencies (EEs) were determined by comparing peak areas of extracted plasma QC samples with those of samples spiked after protein precipitation of plasma. Both recovery and extraction efficiency were determined using three replicates at four different QC concentration levels.

Stability

The stability of isoorientin in rat plasma was examined using QC samples at four concentration levels under different storage conditions. Short-term stability was assessed after 6 h at room temperature and long-term stability after 4 weeks storage at −20°C. Freeze-thaw stability was evaluated after three complete freeze-thaw cycles (−20°C/room temperature). To assess autosampler stability, QC samples after protein precipitation were stored in the autosampler of the UPLC instrument at 4°C for 24 h. Additionally, the stabilities of isoorientin and IS in stock solutions were assessed by storing stock solutions at room temperature for 6 h and at −20°C for 4 weeks. Stability was defined as percentage of concentrations of samples under storage to those of freshly prepared samples. The acceptance criterion for stability was that the response of the stored samples was within 15% to that of fresh samples.[28]

Pharmacokinetic study

To evaluate the relevance of developed method, a pharmacokinetic study was performed by orally administrating isoorientin to SD rats. Rats were fasted for 12 h with free access to water prior to the experiment. In each animal, a femoral artery was cannulated with a polyethylene tube (PE50; Clay Adams, Becton Dickinson and Company, Franklin Lakes, NJ, USA) for blood sampling. Isoorientin was dissolved in a mixture of DMSO, propylene glycol and physiological saline (5:50:45) to obtain solution of 2.5 mg/mL. Rats were administered isoorientin solution by oral gavage once at 5 mg/kg. Considering clinically relevant plasma concentration reported,[29] the oral dose (i.e., 5 mg/kg) was chosen in this study. Blood samples (~220 µL) were collected at 0, 5, 15, 30, 60, 120, 180, 240, 360, and 480 min after dosing. After each time point, the volume of blood collected was replaced with an equal volume of saline containing 25 IU/mL heparin to compensate blood loss. Blood samples were centrifuged immediately at 14,000 rpm for 15 min at 4°C to obtain plasma samples, which were then stored at −20°C until required for analysis.

Data analysis

Pharmacokinetic parameters were calculated by noncompartmental analysis using WinNonlin® software (Ver. 5.0.1, Pharsight Co., Mountain View, CA, USA). Based on the plasma concentration-time profile of isoorientin in each animal, the maximum plasma concentration (C_{max}) and the time point to reach the maximum concentration (T_{max}) were determined. The area under the curve from time zero to the last measurable point (AUC_{last}) and the area under the curve from time zero to infinity (AUC_{inf}) were calculated using the linear trapezoidal rule. Terminal elimination rate constant (k_e) was determined from the slope of the terminal phase in log concentration-time curve. The terminal half-life ($t_{1/2}$) was determined as $0.693/k_e$. Oral clearance and apparent volume of distribution were also calculated. Results are reported as mean ± standard deviation.

RESULTS AND DISCUSSION

Optimization of chromatographic conditions

Chromatographic conditions were optimized based on peak shapes and column longevity. Flavonoid glycosides are polar compounds and eluted with broad tails from a reverse phase C18 column filled with 4 µm or 5 µm particles.[30] Therefore, two UHPLC columns containing smaller particles, that is, an Agilent Zorbax Plus C18 (50 mm × 2.1 mm, 1.8 µm) and a Phenomenex Luna Omega Polar C18 (100 mm × 2.1 mm, 1.6 µm), column were used. Isoorientin eluting from an Agilent Zorbax Plus C18 column produced a broad, asymmetrical peak, which became narrower and Gaussian when the Phenomenex Luna Omega polar C18 was used. This improvement of peak performance could be attributed to the polar modified surfaces of particles, which interact with the hydrophilic moieties of polar compounds. Regarding the mobile phase, acetic acid solutions of concentration 0.1%, 0.5%, and 1% were examined in an effort to improve peak shapes, and 1% acetic acid (pH 2.7) was chosen because it produced sharp, symmetrical peaks and improved column longevity. Acetonitrile, instead of methanol at a flow rate of 0.25 mL/min was selected because it enabled analysis at an acceptable column pressure and time. The gradient elution program mentioned above was used to separate isoorientin and the IS from peaks generated by

plasma and to reduce the run time to 15 min. After plasma protein was precipitated with methanol and centrifuged, white particles were observed in supernatant. For this reason, an UHPLC Fully Porous Polar C18 guard column (internal diameter 2.1 mm) was used and supernatants were filtered after centrifugation to protect the main column. The UHPLC method enabled separation of isoorientin and IS, which had retention times of ~7.2 and 8.1 min, respectively. Namely, the flow rate (0.25 mL/min) required was lower than that required for other methods (0.5 mL/min) and total run time each sample (15 min) was shorter (17 or 25 min including column re-equilibration), which reduce solvent usage, time and analysis costs.

Method validation

Selectivity

Figure 2 shows typical chromatograms of blank plasma, plasma spiked with only IS, plasma spiked with isoorientin at LLOQ, plasma spiked with isoorientin at 1 μg/mL, and plasma from a rat collected 15 min after dosing. No interfering peak was observed at the retention times of isoorientin or IS in five different sources of blank plasma.

Linearity

The linearity of responses to isoorientin in plasma was investigated. Calibration curves of isoorientin in rat plasma were linear over the concentration range 20–5000 ng/mL and the coefficient of determination R^2 was 0.9993 ± 0.0005 (n = 6). Slopes and y-intercepts were calculated by weighted (1/x) least-squares regression analysis. The mean regression equation for isoorientin concentration in plasma was $y = (0.004202 \pm 0.000267)x + (0.020414 \pm 0.009664)$. Regression analysis showed accuracies of isoorientin concentration determinations in the calibration curve ranged from 90.0% to 113%.

Sensitivity

Noise levels of blank plasma and the heights of isoorientin were measured automatically using Agilent OpenLab software (Agilent Technologies, Santa Clara, CA, USA) to determine S/N ratios. The LLOQ for isoorientin was 20 ng/mL using an S/N ratio threshold of >5, a precision of 10.7%, and an accuracy in the range 83.1%–116.9%. These results satisfied the criterion for LLOQ as detailed in FDA Guideline for Industry: Bioanalytical method validation.[24] Furthermore, despite its simplicity, the sensitivity of the assay was better than that reported for a previously described method, that is, it has a LLOQ of 20 ng/mL as compared with a previously described LLOQ of 4000 ng/mL.[21] Although a lower LLOQ (5 ng/mL) was reported in another study that used human plasma,[29] the assay using solid-phase extraction (SPE) required a large plasma volume (0.5 mL) which is not practical for pharmacokinetic studies in rats.

Precision and accuracy

Table 1 summarizes the precision and accuracy data of the devised UHPLC-DAD method based on analysis of QC samples at four concentrations (20, 40, 800, and 4000 ng/mL) in five replicates. Intra-day precision for the analysis of isoorientin in plasma was <2.1% and the inter-day precision for 5 consecutive days was not >10.7% as determined using RSD. Moreover, accuracy for the determination of isoorientin in intra-day study varied from 89.4% to 101.3%, while the inter-day accuracy ranged from 96.3% to 99.9%. These results show the reproducibility of our method conforms with FDA Guidance for Industry: Bioanalytical Methods Validation.

Recovery and extraction efficiency

Recoveries and EEs of QC samples are presented in Table 2. Recovery of isoorientin at four concentration levels ranged from

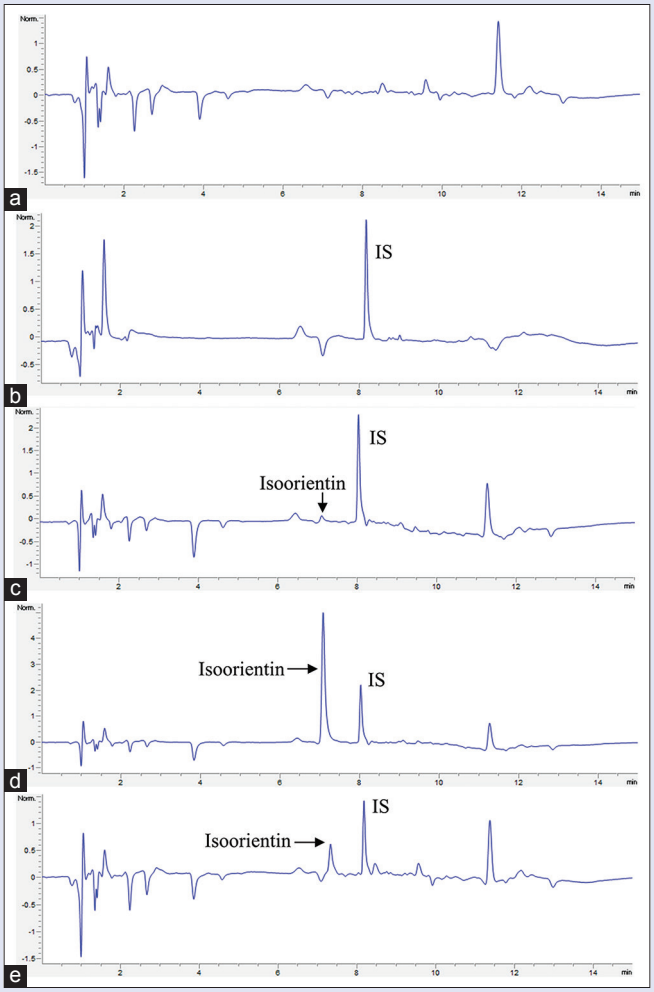

Figure 2: Representative high-performance liquid chromatograms of isoorientin in rat plasma. (a) Blank plasma. (b) Blank plasma spiked with only internal standard. (c) Blank plasma containing isoorientin at its lower limit of quantification and internal standard. (d) Blank plasma containing isoorientin at 1000 ng/mL and internal standard, and (e) a plasma sample collected from a rat 15 min after isoorientin was administered orally at 5 mg/kg

Table 1: Precision and accuracy data for the quantification of isoorientin

Nominal concentration (ng/mL)	Intra-day (n=5)			Inter-day (n=25)		
	Average (%)	Precision (RSD %)	Accuracy (%)	Average	Precision (RSD %)	Accuracy(%)
20	18.1	1.1	90.6	20.0	10.7	99.9
40	35.8	2.1	89.4	38.8	9.5	97.1
800	779	1.3	97.3	770	8.6	96.3
4000	4051	0.02	101.3	3996	10.0	99.9

RSD: Relative standard deviation

89.4% to 95.5% with a RSD% of <9.1%, whereas recovery of the IS at 250 ng/mL was 98.4% with a RSD% of 0.9%. The consistent EEs of QC samples (91.3%–93.5%) demonstrated protein precipitation did not cause significant variations in extraction efficiency at concentration levels of 20, 40, 800, and 4000 ng/mL. Taken together, these observations show the sample preparation procedure is simple and highly efficient.

Stability

Storage and temperature stability tests simulated the conditions likely to be encountered during analysis. According to Table 3, stability results were with acceptable limits, that is, within 15% of the response of freshly prepared samples. The stability of isoorientin at different concentrations in plasma ranged from 98.5% to 101.2% after 6 h at room temperature and from 89.5% to 98.1% after 4 weeks at −20°C. The stability of isoorientin after three free-thaw cycles was 90.4%–100.8%. QC samples after protein precipitation returned an accuracy of 98.4%–104.9% after being stored in the autosampler at 4°C for 24 h. The peak area response of isoorientin in stock solutions after short- and long-term storage were 102.9% and 105.0%, respectively, as compared with responses of fresh solutions. Those values of IS after short- and long-term storage were 95.8% and 103.8%, respectively. The above results indicate isoorientin and IS were stable under the various conditions tested.

Pharmacokinetics analysis

The validated UHPLC-DAD method was applied to a pharmacokinetic study of isoorientin in SD rats following a single oral administration at 5 mg/kg. The mean plasma concentration-time curve for isoorientin is presented in Figure 3. Pharmacokinetic parameters were determined from the curve and presented in Table 4. Isoorientin was rapidly absorbed and reached a maximum plasma concentration of 0.102 ± 0.057 mg/mL at 20.0 ± 8.7 min after administration. After reaching peak concentration, the concentration of isoorientin declined in an near biphasic manner with a terminal half-life of 262 ± 14 min. Based on these results, pharmacokinetic properties of isoorientin were found to be similar to those reported by previous pharmacokinetic studies although the dosages used differed.[21,22] Namely, the terminal half-life for oral dose of 150 mg/kg was found to be about 373 min, whereas that of oral dose of 0.5 mg/kg was around 209 min.[21,22] Moreover, T_{max} from previous literatures was comparable to that of our present observation (20 min vs. 30 min or 35 min).[21,22]

CONCLUSION

This is the first report on a UHPLC-DAD based method for the quantification of isoorientin in rat plasma. As compared with the conventional HPLC method previously reported,[21,29] the developed method has the advantages of straightforward sample preparation, a short analysis time, and high sensitivity. Our method involves protein precipitation, which is considerably less complex and time consuming than the solvent evaporation/reconstitution[21] or SPE[29] required by other methods. In addition, when validated according to current regulatory guidelines, the devised method was found to have sufficient linearity, accuracy and precision, and was successfully applied to pharmacokinetic study after isoorientin was orally administered to rats.

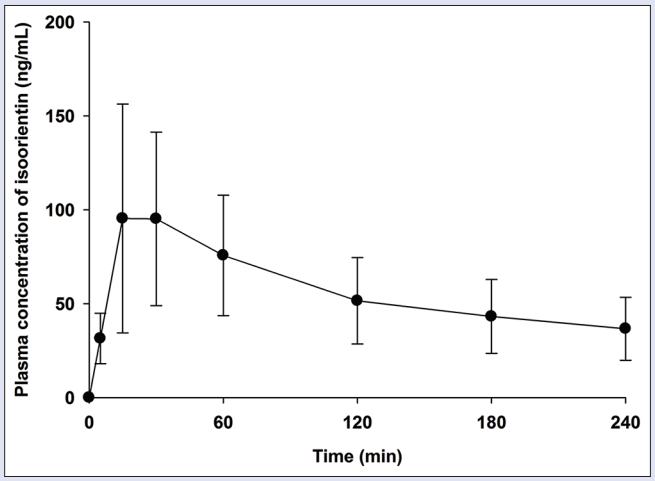

Figure 3: Mean plasma concentration-time profile of isoorientin after the single oral administration of 5 mg/kg to rats (*n* = 4, mean ± standard deviation)

Table 2: Recovery and extraction efficiency data for the quantification of isoorientin (*n*=3)

	Nominal concentration (ng/mL)	Recovery (%)	RSD (%)	Extraction efficiency (%)	RSD (%)
Isoorientin	20	95.5	9.1	93.5	7.9
	40	91.6	2.8	91.3	4.0
	800	91.6	2.3	91.6	2.4
	4000	89.4	0.5	92.8	0.9
IS	250	98.4	0.9		

RSD: Relative standard deviation; IS: Internal standard

Table 3: Stability data for the quantification of isoorientin (*n*=3)

	Nominal concentration (ng/mL)	Freeze-thaw stability (3 cycles)	Autosampler stability (24 h at 4°C)	Short-term stability (6 h at room temperature)	Long-term stability (4 weeks at −20°C)
QC samples	20	90.4±4.4	104.9±6.6	99.2±5.7	95.0±3.6
	40	96.9±5.9	101.0±4.2	101.2±3.0	98.1±0.8
	800	98.1±0.4	99.2±0.9	98.5±0.7	90.1±1.3
	4000	100.8±0.9	98.4±0.4	98.9±1.1	89.5±0.3
Stock solution					
Isoorientin	200			102.9±4.1	105.0±0.7
Rutin	300			95.8±6.4	103.8±2.7

QC: Quality control

Table 4: Pharmacokinetic parameters of isoorientin following oral administration of isoorientin at a dose of 5 mg/kg in rats (*n*=4, mean±standard deviation)

Parameters	Isoorientin
T_{max} (min)	20.0±8.7
C_{max} (μg/mL)	0.102±0.057
$t_{1/2}$ (min)	262±14
AUC_{last} (μg min/mL)	21.6±10.3
AUC_{inf} (μg min/mL)	33.6±16.3
CL/F (mL/min/kg)	171±69
V_d/F (L/kg)	64.9±28.3

C_{max}: The maximum plasma concentration; T_{max}: The time point to reach the maximum concentration; AUC_{last}: The area under the curve from time zero to the last measurable point; AUC_{inf}: The area under the curve from time zero to infinity; $t_{1/2}$: Terminal half-life; CL/F: Oral clearance; V_d/F: Apparent volume of distribution after oral administration

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Conflicts of interest

There is no conflicts of interest.

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