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Development of high performance liquid chromatography/electrospray ionization mass spectrometry for assay of ginkgolic acid (15:1) in rat plasma and its application to pharmacokinetics study

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ABSTRACT

A highly sensitive HPLC–ESI-MS method has been developed and validated for the quantification of ginkgolic acid (15:1) in a small quantity of rat plasma (50 μL) using its homologous compound ginkgolic acid (17:1) as an internal standard. GA (15:1) and GA (17:1) were extracted from biological matrix by direct protein precipitation with 5-fold volume of methanol and separated on an Elite hypersil BDS C_{18} column (2.1 × 100 mm, 3 μm), eluted with acetonitrile:water (92:8, v/v, containing 0.3% glacial acetic acid). Linear range was 8–1000 ng/mL with the square regression coefficient (r^2) of 0.996. The lowest concentration (8 ng/mL) in the calibration curve was estimated as LLOQ with both deviation of accuracy and RSD of precision <20% (n = 6). The intra- and inter-day precision ranged from 3.6% to 9.9%, and the intra- and inter-day accuracy was between 89.9% and 101.3%. This method was successfully applied to study pharmacokinetics of GA (15:1) in rats after oral administration at a dose of 10 mg/kg. GA (15:1) pharmacokinetic parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-12h} are 1552.9 ± 241.0 ng/mL, 0.9 ± 0.7 h, 5.5 ± 2.6 h, 3356.0 ± 795.3 ng h/mL, respectively.

1. Introduction

Ginkgolic acids (GA) is a series of structurally related n-alkyl phenolic acid compounds and extensively exists in leaves, nuts and external seed coat of *Ginkgo biloba* L. The alkyl side chain in molecular structures varies from 13 to 17 carbons in length with 0–2 double bonds [1]. It was reported that this kind of compounds could cause contact allergenic [2] and cytotoxic [3], etc. Thus, the content of total ginkgolic acids in Ginkgo biloba preparations is limited to less than 10 mg/kg according to China Pharmacopoeia (2010) [4]. Besides their toxicities, people gradually pay more attention on their pharmacological activities, including antitumour [5] and antidepressant [6] activities, etc. Recently, it was revealed that GA (15:1) could inhibit the P-gp mediated efflux of daunorubicin in KB-C2 cells [7].

In our previous study, we found that GA (15:1) underwent extensively phase I metabolism in rat liver microsome [8], in which cytochrome P450 enzymes might play an important role. Furthermore, co-incubation with selective CYP inhibitors (α-naphthoflavone and ketoconazole) could decrease the cytotoxicity of GA (15:1) in primary rat hepatocytes; meanwhile, pretreatment with selective CYP inducers (β-naphthoflavone and rifampin) could increase the cytotoxicity of GA (15:1) in HepG2 cells. Our results showed that HepG2 cells are more sensitive to the cytotoxicity of GA (15:1) than normal cells as primary rat hepatocytes, and CYP 1A and CYP 3A might be involved in transforming GA (15:1) to more toxic metabolites in HepG2 cells [9].

To our knowledge, there is no report about pharmacokinetics of GA (15:1) in vivo, even though several methods for quality control have been established to determine the content of GA (15:1) or quantify the total ginkgolic acids in *Ginkgo biloba* L. leaf extracts or its phytopharmaceutical preparations by high performance liquid chromatography with ultraviolet detection (HPLC–UV) [10], liquid chromatography–mass spectrometry (LC–MS) [11], enzyme-linked immunosorbent assay (ELISA) [12] and ^{1}H NMR spectrometry [13]. Therefore, in this work, we have developed and validated a highly sensitive HPLC/MS bioanalytical method to the estimation of GA (15:1) in rat plasma for the first time. This analytical method has been applied to study the pharmacokinetics of GA (15:1).

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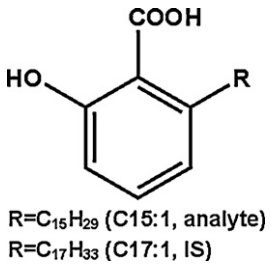

Fig. 1. Chemical structure of GA (15:1, $C_{22}H_{34}O_3$) and GA (17:1, $C_{24}H_{38}O_3$).

2. Experimental

2.1. Chemicals and reagents

GA (15:1) and GA (17:1, internal standard) were isolated and purified from external seed coat of Ginkgo biloba in our laboratory. The dried external seed coat of Ginkgo biloba (200 g) was ground to powder and then extracted with petroleum ether (60–90 °C, 1 L) for 1 h under reflux. The extract was concentrated under vacuum at 50 °C and subjected to silica gel column chromatography (10 × 70 cm, 200–300 mesh). The column was washed with petroleum ether, and then eluted with petroleum ether–ethyl acetate (9:1), and each fraction contained 100 mL eluent. The fractions containing ginkgolic acids were put together and evaporated to dryness under vacuum at 50 °C and further purified by reversed phase preparative HPLC, which was performed on a C_{18} chromatographic column (20 × 250 mm, 10 μm; Waters, USA) at ambient temperature, and a Waters 600 liquid chromatograph apparatus equipped with a Waters 2487 UV detector (Waters, Milford, MA, USA) was used. The mobile phase consisted of methanol and water (87:13, containing 0.1% glacial acetic acid) and ran at a flow rate of 8 mL/min, and the detection wavelength was set at 310 nm. The operation was controlled by a Millennium^{32} workstation (Waters, Milford, MA, USA). The chemical structure of GA (15:1) and GA (17:1) was identified by ^{1}H NMR and ^{13}C NMR comparing with those of reference data [14]. The purity of both compounds (>99.0%) was determined by UPLC–PDA-MS analysis. Chemical structure of both compounds is shown in Fig. 1.

HPLC grade acetonitrile and glacial acetic acid were purchased from TEDIA Inc. (Fairfield, USA). Ultra-pure water (18.2 MΩ) was obtained from an ELGA-purelab Ultra system (High Wycombe, UK). All other reagents were of analytical grade from the chemical reagent company of Ludu, Shanghai.

2.2. Animals

Male Sprague–Dawley rats (180–200 g, 7–8 weeks old) were obtained from the Animal Center of Zhejiang Academy of Medical Sciences (Hangzhou, China). Before experiments, the rats were acclimatized to new environment for one week, and during this time, the room temperature was maintained at 22 ± 2 °C with relative humidity of 50 ± 10%. They had free access to water and rodent chow all the time, until 12 h prior to administration, during which only water could be available. The study was approved by the Animal Ethics Committee of Zhejiang University.

2.3. Instrumentation and chromatographic conditions

A Waters ACQUITY^{TM} TQD with an ultra performance liquid chromatography (Waters, Milford, MA, USA) was used. Chromatographic separation was achieved on an Elite hypersil BDS C_{18} column (2.1 × 100 mm, 3 μm; Dalian, China). The system was run in isocratic mode with mobile phase consisting of acetonitrile and water in ratio of 92:8 (v/v), both solvents contained 0.3% glacial acetic acid. Mobile phase was delivered at a flow rate of 0.2 mL/min and introduced into ESI source with no split, and effluent of 0–2.0 min from chromatographic column was switched to waste before it flew into ion source. The temperature of the sample manager was maintained at 20 °C. The volume of needle wash was 600 μL for both strong needle wash solution (acetonitrile/water; 80/20; v/v) and weak needle wash solution (acetonitrile/water; 20/80; v/v).

The mass spectrometer was operated at ESI negative ion mode and detection of the ions was performed in selected ion recording (SIR) mode, m/z 345.3 $[M-H]^-$ for GA (15:1) and m/z 373.3 $[M-H]^-$ for GA (17:1), respectively (Fig. 2). The optimized mass spectrometric parameters for the HPLC–MS analysis of both compounds (analyte and internal standard) were as follows: nitrogen was used as desolvation gas (550 L/h), and the source and desolvation gas temperature were set at 120 and 350 °C, respectively. Capillary voltage was set at 3.0 kV, and cone voltage, extractor and RF lens voltage were of 60, 3 and 0.1 V, respectively. Quadrupoles Q1 and Q3 were set on unit resolution. Data acquisition and processing were performed using Masslynx 4.1 software (Micromass, Manchester, UK) and Microsoft Excel 2003.

2.4. Preparation of stock and standard solutions

Primary stock solutions of the analyte and IS were prepared in methanol (200 μg/mL) respectively, and stored at −20 °C. Gradient dilutions were made with methanol for analyte to produce working stock solution of 40, 80, 200, 400, 1000, 2000, 5000 ng/mL which were used to prepare standards for the calibration curve (CC) and quality control (QC) samples. A working stock solution of IS (80 ng/mL) was prepared in methanol from primary stock solution (200 μg/mL). Calibration samples were prepared by spiking 50 μL drug-free rat plasma with working solution of analyte (10 μL) to obtain concentrations of calibration samples range from 8 to 1000 ng/mL. QC samples were prepared at three concentration levels of 12 ng/mL (QC low), 160 ng/mL (QC medium) and 800 ng/mL (QC high).

2.5. Sample preparation

Rat plasma protein was directly precipitated by 5-fold volume methanol. To 50 μL plasma aliquot in 1.5 mL polyethylene tube, 150 μL IS working stock solution and extra volume of 100 μL methanol was added. After vortexed for 1 min, the plasma sample was centrifuged for 10 min at 12,000 rpm. The supernatant was transferred to another polyethylene tube and evaporated to dryness under vacuum at room temperature. Finally, the residue was reconstituted in 100 μL mobile phase, followed by vortexing for 1 min and centrifuged for 10 min at 12,000 rpm, and 7 μL supernatant was injected for HPLC–MS analysis.

2.6. Method validation

A full validation was performed for the assay of GA (15:1) in rat plasma according to FDA guidelines [15].

2.6.1. Selectivity and sensitivity

The selectivity of the method was evaluated by comparing rat plasma chromatogram from six different sources to investigate the potential interferences at the retention windows of analyte and IS. The limit of detection (LOD) was estimated as the plasma concentration of GA (15:1) with a signal to noise (S/N) ratio of 3.

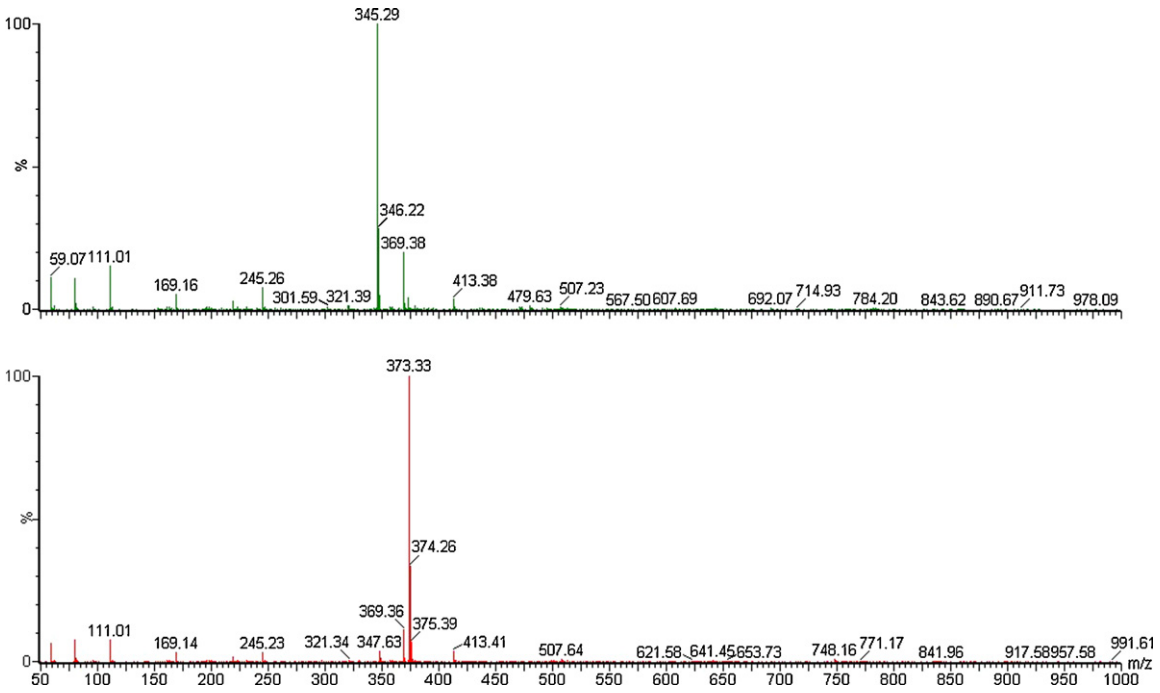

Fig. 2. Full scan ion spectra of GA (15:1, *m/z* 345.3) and GA (17:1, *m/z* 373.3).

2.6.2. Matrix effect

The matrix effect of endogenous constituents in plasma on the ionization of analyte and IS was determined by comparing the responses of post-extracted QC samples ($n = 6$) with the response of analytes from neat standard samples dissolved in mobile phase at equivalent concentrations. The matrix effect over analyte was determined at LLOQ (8 ng/mL), QC low (12 ng/mL), QC medium (160 ng/mL) and QC high (800 ng/mL) concentration levels. The matrix effect on IS was determined at a single concentration of 240 ng/mL.

2.6.3. Recovery

The recovery of analyte and IS was determined by comparing the responses of the analytes from QC samples ($n = 6$) with the responses of analytes spiked in post-extracted blank rat plasma at equivalent concentrations. The recovery of analyte was determined at LLOQ (8 ng/mL), QC low (12 ng/mL), QC medium (160 ng/mL) and QC high (800 ng/mL) concentration levels, and the recovery of the IS was determined at a single concentration of 240 ng/mL.

2.6.4. Calibration curve

The calibration curve was obtained by plotting the ratio of peak area of analyte to that of IS against the nominal concentration of calibration standards, including 8, 16, 40, 80, 200, 400 and 1000 ng/mL. The standard curve was fitted to linear regression ($y = ax + b$) using $1/x$ as weighting factor.

2.6.5. Accuracy and precision

The intra-day accuracy and precision were evaluated by QC samples at four concentration levels, i.e., 8, 12, 160 and 800 ng/mL, $n = 6$ for each concentration. The inter-day accuracy and precision were determined by analyzing the four levels QC samples on three different days.

2.6.6. Stability

The stability of analyte in rat plasma was estimated by QC samples at three concentration levels, i.e., QC low (12 ng/mL), QC medium (160 ng/mL) and QC high (800 ng/mL), using six replicates for each concentration level. The stability experiments of analyte included (1) freeze and thaw stability of analyte in rat plasma after three freeze–thaw cycles; (2) short-term temperature stability of analyte in rat plasma at room temperature for 8 h; (3) long-term stability of analyte in rat plasma for 40 days at −20 °C; (4) post-preparative stability of analyte in auto-sampler at 20 °C for 24 h.

2.6.7. Dilution tests

In some cases when plasma sample concentrations are expected to be higher than the upper limit of quantitation (ULOQ, 1000 ng/mL), dilution with blank rat plasma is required. Dilution experiments were completed by five times dilution of plasma samples (HQCs) containing 4000 ng/mL GA (15:1) with blank rat plasma to obtain samples concentrations of 800 ng/mL in six replicates.

2.7. Pharmacokinetics study

The bioanalytical method developed and validated above has been applied to study the pharmacokinetics of GA (15:1) in rat with oral administration. The dosage form of GA (15:1) was prepared as follows: 10 mg of GA (15:1) accurately weighed was dissolved in 0.5 mL anhydrous ethanol firstly and then diluted to 10 mL with water to get 1 mg/mL suspension solution. Male SD rats ($n = 5$) were administered orally with GA (15:1) at a dose of 10 mg/kg. Blood samples (150 μL) were collected from tail vein of rats into heparinized centrifuge tube at 0 h before dosing and 0.083, 0.167, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0, 12.0 h post-dosing. Plasma was harvested by centrifugation at 4000 rpm for 10 min and stored at −20 °C until analysis.

Rat plasma samples (50 μL) were thawed at room temperature and spiked with IS and processed as described above, then analyzed along with calibration standard samples and QC samples in duplicate at three concentration levels. The criteria for acceptance of analytical runs are two out of six QC samples were permitted beyond 15% of the nominal concentration but should not in the same concentration level. Data was processed by non-compartmental model using DAS 2.0 software (Mathematical Pharmacology Professional Committee of China, Shanghai, China).

3. Results and discussion

3.1. HPLC–MS system

To optimize ESI conditions for detection of GA (15:1) and GA (17:1), positive or negative ion detection mode was tried. Both compounds had good responses in negative ion detection mode with low background noise level, thus detection was finally operated in negative ion mode in this study. MS detector parameters for SIR mode were auto-tuned by directly infusing 1 μg/mL GA (15:1) or GA (17:1) methanol solution into ion source, and fine tuning was done to cone voltage and desolvation gas temperature by manual manipulation, considering that these two parameters played an important role on the ion response of GA (15:1) or GA (17:1). In multi-reactive monitor (MRM) mode, both GA (15:1) and GA (17:1) could obtain stable daughter ions of *m/z* 301 and *m/z* 329, respectively, after loss of one molecule of carbon dioxide from precursor ion $[M-H]^-$.

The mobile phase has been tried with acetonitrile/water and methanol/water binary solvent system. GA (15:1) could obtain better response in acetonitrile/water than that in methanol/water solvent system. The mobile phase containing 0.3% (v/v) glacial acetic acid could improve symmetry of peak shapes. In order to avoid carryover influence on low concentration samples determination, the volume of both weak and strong needle wash solution was increased from 200 μL (default settings) to 600 μL, which proved to be valid, and there was no distinct residue to be detected after ULOQ determination.

Table 1
Recovery of GA (15:1) and GA (17:1) from rat plasma and matrix effects on GA (15:1) and GA (17:1) ($n = 6$).

Concentration (ng/mL)	Recovery (mean ± SD, %)	Matrix effects[a] (mean ± SD, %)
GA (15:1)		
8	99.0 ± 7.0	−4.8 ± 1.6
12	89.6 ± 5.1	−2.0 ± 4.2
160	91.6 ± 5.9	0.1 ± 3.0
800	90.9 ± 3.2	−5.2 ± 3.9
GA (17:1, IS)		
240	98.9 ± 4.8	−2.3 ± 5.1

[a] Ionization suppression extent was expressed as minus (−) mean value and ionization enhancement extent was expressed as plus (+) mean value.

3.2. Validation procedures

3.2.1. Recovery

Ginkgolic acids are highly nonpolar compounds, but have good solubility in both polarity and non-polarity solvent, such as methanol and petroleum ether. Initially, we tried liquid–liquid extraction (LLE) method with ethyl acetate or petroleum ether, and protein precipitation (PP) method with methanol or acetonitrile. LLE method was proved to be tedious and laboursome, while in PP method, GA (15:1) and GA (17:1) showed better solubility in methanol than in acetonitrile, although the protein precipitation efficiency of acetonitrile is better than methanol as we know. Therefore, in this study, sample preparation was carried out by direct

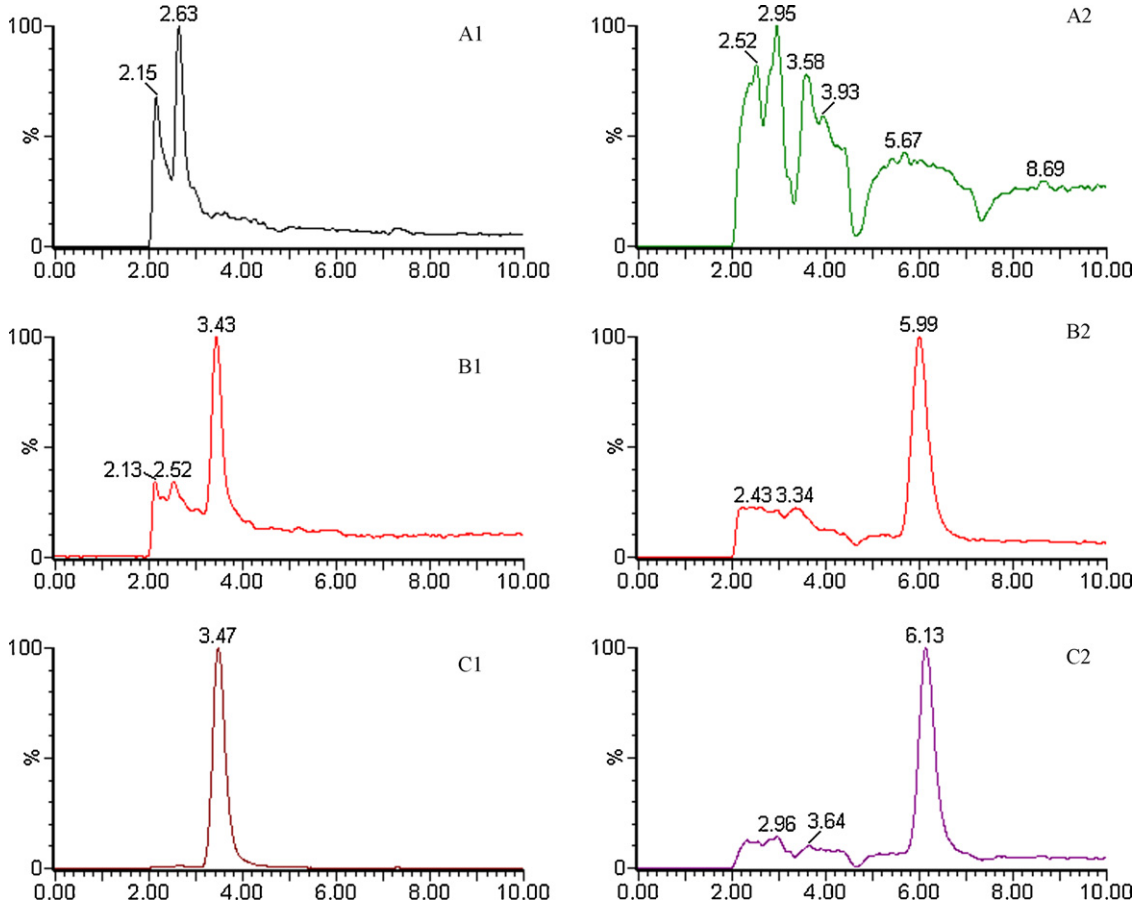

Fig. 3. Typical SIR chromatograms of GA (15:1) and GA (17:1, IS). Rat blank plasma (A1, A2), rat blank plasma spiked with GA (15:1) at LLOQ (8 ng/mL) and GA (17:1, 240 ng/mL), (B1) GA (15:1), retention time 3.43 min; (B2) IS, 5.99 min; Rat plasma sample at 1 h time point after oral administration of 10 mg/kg GA (15:1) spiked with GA (17:1, 240 ng/mL), (C1) GA (15:1), retention time 3.47 min; (C2) IS, 6.13 min.

Table 2
Precision and accuracy data of back-calculated concentrations of calibration standard for GA (15:1) in rat plasma ($n = 3$).

Nominal concentration (ng/mL)	Observed concentration (mean ± SD, ng/mL)	Precision (%)	Accuracy (%)
8	7.9 ± 0.4	−5.1	98.8
16	14.9 ± 0.8	−5.4	93.1
40	39.8 ± 2.1	−5.3	99.5
80	81.8 ± 4.1	5.0	102.3
200	209.8 ± 21.8	10.4	104.9
400	421.8 ± 24.9	5.9	105.5
1000	968.1 ± 17.9	−1.8	96.8

Table 3
Intra- and inter-day precision and accuracy for GA (15:1) in rat plasma.

QC (ng/mL)	Observed concentration (mean ± SD, ng/mL)	Precision (%)	Accuracy (%)
Intra-day (n = 6)			
8	8.1 ± 0.8	9.9	101.3
12	11.2 ± 0.5	4.5	93.3
160	150.9 ± 5.6	3.7	94.3
800	718.8 ± 26.2	3.6	89.9
Inter-day (n = 3)			
8	7.8 ± 0.3	3.8	97.5
12	11.8 ± 0.6	5.1	98.3
160	157.0 ± 5.8	3.7	98.1
800	737.7 ± 48.3	6.5	92.2

precipitation of protein with 5-fold volume methanol to differentiate analyte and IS with other endogenous apolarity substances in rat plasma, and the effluent of 0–2.0 min from chromatographic column switched to waste could refrain ion source from pollution, avoiding ion suppress effect on analyte and IS. This method proved to be simple, valid and might be preferred strategy for this kind of compounds to be extracted from biological matrix. The results of the comparison of pre-extracted standards versus post-extracted plasma standards were evaluated for GA (15:1) at 8, 12, 160 and 800 ng/mL, and the absolute recovery ranged from 89.6% to 99.0%. The absolute recovery of IS at 240 ng/mL was 98.9% (Table 1).

3.2.2. Matrix effect and specificity

The matrix effect was evaluated by analyzing LLOQ (8 ng/mL), QC low (12 ng/mL), QC medium (160 ng/mL) and QC high (800 ng/mL) samples. Mean matrix effect values were −4.8%, −2.0%, 0.1% and −5.2%, respectively. Matrix effect on IS was −2.3% at concentration of 240 ng/mL (Table 1).

The specificity in the present study has been assessed by comparing the chromatograms of spiked samples at LLOQ concentration level with those of blank plasma samples from six different rats. Fig. 3 exhibited an overlaid chromatogram for the blank rat plasma, blank rat plasma spiked with GA (15:1) at LLOQ and IS and rat plasma sample obtained at 1 h after oral administration of GA (15:1) spiked with IS. The retention time of analyte and IS was 3.47 and 6.13 min, respectively. The total chromatographic run time was 10 min. Obviously, there was no interference in retention windows of both analyte and IS.

3.2.3. Calibration curve

The plasma calibration curve was constructed by plotting the peak-area ratios (peak area analyte/peak area IS) versus analyte concentrations of 8, 16, 40, 80, 200, 400 and 1000 ng/mL and fitted to $y = ax + b$ using weighting factor ($1/x$). Typical standard curve was $y = 0.004301x + 0.002896$, and the square regression coefficient (r^2, $n = 3$) was found to be ≥ 0.996. The lowest concentration (8 ng/mL) was estimated as LLOQ with both deviation of accuracy and RSD of precision <20% and the ratio of signal to noise (S/N) at LLOQ was >10. The LOD of GA (15:1) in rat plasma was evaluated to be 1.6 ng/mL ($n = 5$, S/N ratio > 3) in this study. The accuracy observed for the mean of back-calculated concentrations for three calibration curves was 93.1–105.5% with RSD ranged from −1.8% to 10.4% (Table 2).

3.2.4. Accuracy and precision

Accuracy and precision data for intra- and inter-day plasma samples were listed in Table 3. The intra-day accuracy (expressed as percent of nominal values) ranged from 89.9% to 101.3% and the inter-day accuracy ranged from 92.2% to 98.3%. The intra-day precision ranged from 3.6% to 9.9%, and the inter-day precision ranged

Table 4
Stability of GA (15:1) in rat plasma ($n = 6$).

Nominal concentration (ng/mL)	Stability	Mean ± SD (ng/mL)	Precision (%)	Accuracy (%)
12	24 h (auto-sampler)	12.2 ± 0.4	3.3	101.7
	8 h (bench-top)	11.4 ± 0.8	7.0	95.0
	3rd freeze–thaw	11.6 ± 1.2	10.3	96.7
	40 days at −20 °C	10.9 ± 0.2	1.8	90.8
160	24 h (auto-sampler)	150.3 ± 9.3	6.2	93.9
	8 h (bench-top)	160.8 ± 6.4	4.0	100.5
	3rd freeze–thaw	146.3 ± 3.6	2.5	91.4
	40 days at −20 °C	142.1 ± 4.5	3.2	88.8
800	24 h (auto-sampler)	842.0 ± 94.3	11.2	105.3
	8 h (bench-top)	712.2 ± 10.7	1.5	89.0
	3rd freeze–thaw	771.2 ± 30.1	3.9	96.4
	40 days at −20 °C	721.9 ± 47.5	6.6	90.2

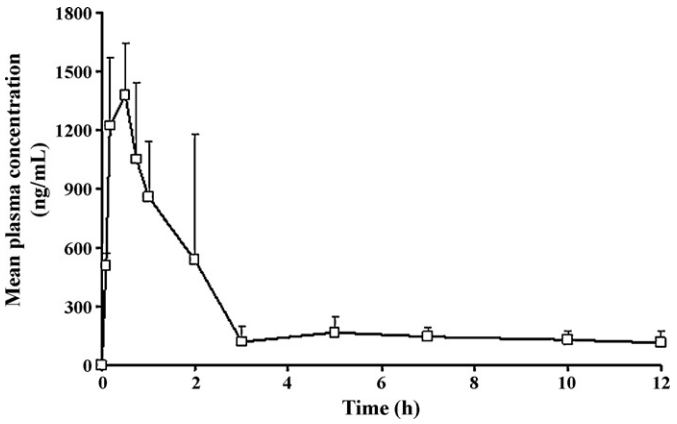

Fig. 4. Mean plasma concentration–time profile of GA (15:1) in rat plasma after oral administration at a dose of 10 mg/kg ($n = 5$).

Table 5
Pharmacokinetic parameters of GA (15:1) after oral administration at a dose of 10 mg/kg to rats ($n = 5$).

Pharmacokinetic parameters	Mean ± SD
AUC_{0-t} (ng h/mL)	3356.0 ± 795.3
$AUC_{0-\infty}$ (ng h/mL)	4394.6 ± 1311.1
$t_{1/2}$ (h)	5.5 ± 2.6
CLz/F (L/h/kg)	2.4 ± 0.7
Vz/F (L/kg)	17.5 ± 3.9
C_{max} (ng/mL)	1552.9 ± 241.0
T_{max} (h)	0.9 ± 0.7

from 3.7% to 6.5%. The assay values on both intra- and inter-day were found to be within the acceptance criteria.

3.2.5. Stability

The stability experiment was performed by using QC samples at concentrations of 12, 160 and 800 ng/mL. The results indicated that GA (15:1) was stable in auto-sampler (24 h) at 20 °C, bench-top (8 h) at room temperature, repeated three freeze/thaw cycles and frozen condition at −20 °C for 40 days (Table 4) and it would satisfy a routine pharmacokinetics study.

3.2.6. Dilution integrity

Dilution experiments were carried out by five times dilution with blank rat plasma at six replicates. The accuracy (%) observed for the mean back-calculated concentrations for diluted HQCs was 98.3%. The precision (RSD %) for diluted HQCs was 4.7%. The results suggested that re-analysis of samples whose concentrations were above ULOQ by appropriate dilution could be fulfilled.

3.3. Application

We have succeeded in applying this established HPLC–MS method to study pharmacokinetics of GA (15:1) in rats. The mean plasma concentration versus time profile of GA (15:1) was shown in Fig. 4. The non-compartmental pharmacokinetic parameters of GA (15:1) were summarized in Table 5. Maximum concentration in plasma (C_{max} 1552.9 ± 241.0 ng/mL) was achieved at 0.9 ± 0.7 h (T_{max}). The half-life ($t_{1/2}$) of GA (15:1) was 5.5 ± 2.6 h, while the AUC_{0-t} in 12 h and $AUC_{0-\infty}$ were 3356.0 ± 795.3 and 4394.6 ± 1311.1 ng h/mL, respectively. The ratio of mean value of AUC_{0-t} to that of $AUC_{0-\infty}$ was 76.4%.

4. Conclusions

We have developed and validated a simple and highly sensitive HPLC–MS assay for determination of GA (15:1) in a small quantity of rat plasma (50 μL) using its homologous compound of GA (17:1) as IS for the first time and successfully applied this method to study pharmacokinetics of GA (15:1) in rats after oral administration at a dose of 10 mg/kg. The results show that GA (15:1) pharmacokinetic parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-12h} are 1552.9 ± 241.0 ng/mL, 0.9 ± 0.7 h, 5.5 ± 2.6 h, 3356.0 ± 795.3 ng h/mL, respectively. The present method can be used for determination of ginkgolic acids in biological samples.

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