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Original Article

Pharmacokinetic and bioavailability study of angeloylgomisin H in rat plasma by UPLC-MS/MS

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Abstract: Angeloylgomisin H, as a major lignin in the fruits, was reported to have the potential to improve insulin-stimulated glucose uptake by activating PPAR-γ. In this work, a sensitive and selective UPLC-MS/MS method for determination of angeloylgomisin H in rat plasma is developed. After addition of rutin as an internal standard (IS), protein precipitation by acetonitrile was used to prepare samples. Chromatographic separation was achieved on a UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm) with 0.1% formic acid and acetonitrile as the mobile phase with gradient elution. An electrospray ionization source was applied and operated in positive ion mode; multiple reactions monitoring (MRM) mode was used for quantification using target fragment ions m/z 523.2-315.1 for angeloylgomisin H, and m/z 611.1-303.1 for IS. Calibration plots were linear throughout the range 5-2000 ng/mL for angeloylgomisin H in rat plasma. Mean recoveries of angeloylgomisin H in rat plasma ranged from 86.2% to 92.5%. RSD of intra-day and inter-day precision were both < 11%. The accuracy of the method was between 93.0% and 104.1%. The method was successfully applied to pharmacokinetic study of angeloylgomisin H after either oral or intravenous administration. The absolute bioavailability of angeloylgomisin H was reported as high as 4.9%.

Keywords: Angeloylgomisin H, UPLC-MS/MS, pharmacokinetics, rat plasma

Introduction

Fructus Schisandrae chinensis, known as "Wuweizi", is derived from the ripe fruits of Schisandra chinensis (Turcz). Baill and has a long history in health care as a herbal medicine in many East Asian countries such as China, Japan and Korea. Wuweizi has been revealed a widely variety of pharmacological effects including anti-inflammatory [1], antihepatotoxic [2], antioxidant [3], antitumoural [4], sedative and hypnotic activities [5], thereby being officially documented in the Chinese Pharmacopoeia [6]. Modern pharmacological studies demonstrated that most of the biological actions and the pharmacological effects of Wuweizi could be attributed to its lignan constituents which represent approximately 1% of the fruits' composition and comprise over 100 related compounds [7-9]. Angeloylgomisin H (**Figure 1A**), as a major lignin in the fruits, was reported to have the potential to improve insulin-stimulated glucose uptake by activating PPAR-γ [10]. However, even with the comprehensive research on bioactivity, there was little information about its pharmacokinetic profile. Consequently, it is necessary to study and describe the pharmacokinetic properties of angeloylgomisin H.

In recent years, several methods have been reported for quantitation of the angeloylgomisin H in drugs, plant samples and biological fluid, such as HPLC-UV [11], UPLC/Q-TOF MS [12], HPLC-DAD-MS [13] and UPLC-MS/MS [14, 15].

We developed a sensitive, rapid and reliable UPLC-MS/MS method for the quantitative analysis of this bioactive lignan in rat plasma sample, for the further development and rational use of angeloylgomisin H. Furthermore, the validated method was successfully applied to the pharmacokinetic study in rat.

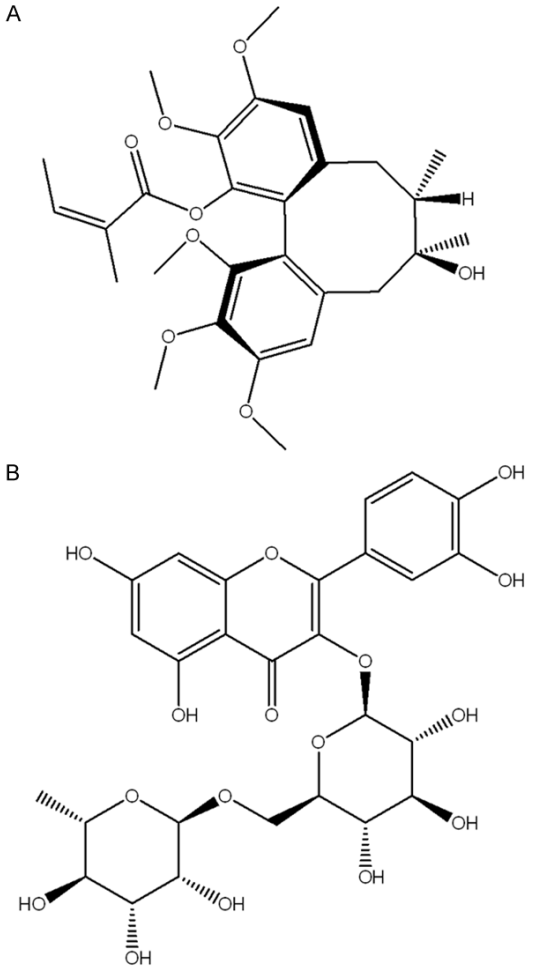


Figure 1. Chemical structure of angeloylgomisin H (A) and rutin (IS, B).

Experimental

Chemicals and reagents

Angeloylgomisin H (purity > 98%, **Figure 1A**) and rutin (IS, purity > 98%, **Figure 1B**) were purchased from the Chengdu Mansite Pharmaceutical CO. LTD. (Chengdu, China). LC-grade acetonitrile and methanol were purchased from Merck Company (Darmstadt, Germany). Ultrapure water was prepared by Millipore Milli-Q purification system (Bedford, MA, USA). Rat blank plasma samples were supplied from drug-free rats (Laboratory Animal Center of Wenzhou Medical University).

Instrumentation and conditions

A UPLC-MS/MS system with ACQUITY I-Class UPLC and a XEVO TQD triple quadrupole mass spectrometer (Waters Corp., Milford, MA, USA), equipped with an electrospray ionization (ESI) interface, was used to analyze the compounds. The UPLC system was comprised of a Binary Solvent Manager (BSM) and a Sample Manager with Flow-Through Needle (SM-FTN). Masslynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control.

Angeloylgomisin H and rutin (IS) were separated using a UPLC BEH C18 column (2.1 mm × 100 mm, 1.7 μm, Waters, USA) maintained at 40°C. The initial mobile phase consisted of acetonitrile and water (containing 0.1% formic acid) with gradient elution at a flow rate of 0.4 mL/min and an injection volume of 2 μL. Elution was in a linear gradient, where the acetonitrile content increased from 20% to 85% between 0 and 1.0 min. The acetonitrile content was maintained at 85% for 1.0 min, then dropped to 20% within 0.5 min. The total run time of the analytes was 3 min.

Mass spectrometric detection was performed on a triple-quadrupole mass spectrometer equipped with an ESI interface in positive mode. Nitrogen was used as the desolvation gas (1000 L/h) and cone gas (50 L/h). Ion monitoring conditions were defined as capillary voltage of 2.5 kV, source temperature of 150°C, and desolvation temperature of 500°C. Multiple reaction monitoring (MRM) modes of m/z 523.2-315.1 for angeloylgomisin H, and m/z 611.1-303.1 for IS were utilized to conduct quantitative analysis, **Figure 2**.

Calibration standards and quality control samples

The stock solutions of angeloylgomisin H (1.0 mg/mL) and rutin (IS) (1.0 mg/mL) were prepared in methanol-water (50:50). The 0.5 μg/mL working standard solution of the IS was prepared from the IS stock solution by dilution with methanol; working solutions for calibration and controls were prepared from stock solutions similarly, using methanol diluent. All of the solutions were stored at 4°C and were brought to room temperature before use.

Angeloylgomisin H calibration standards were prepared by spiking blank rat plasma with appropriate amounts of the working solutions. Calibration plots were offset to range between 5-2000 ng/mL for angeloylgomisin H in rat

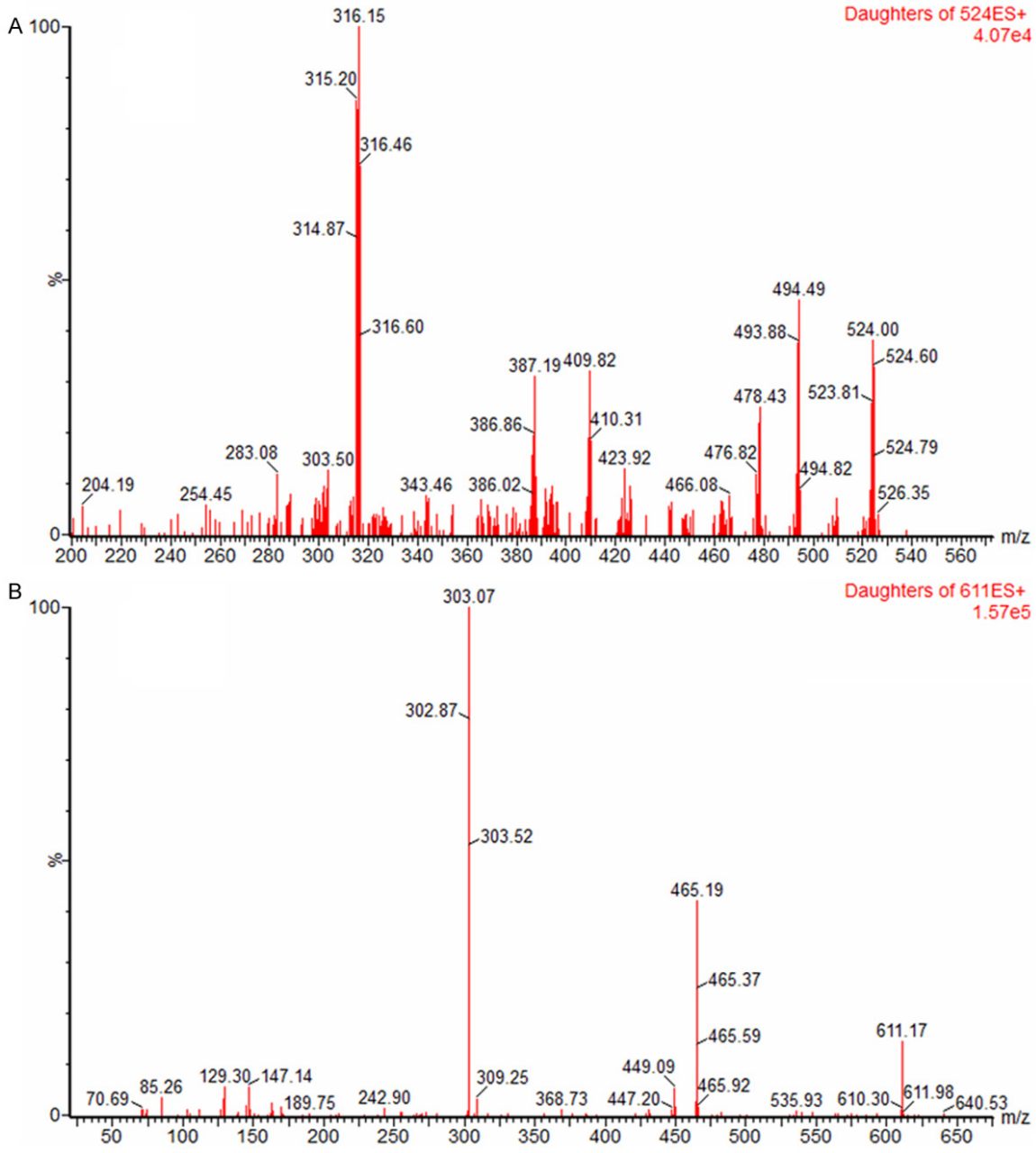
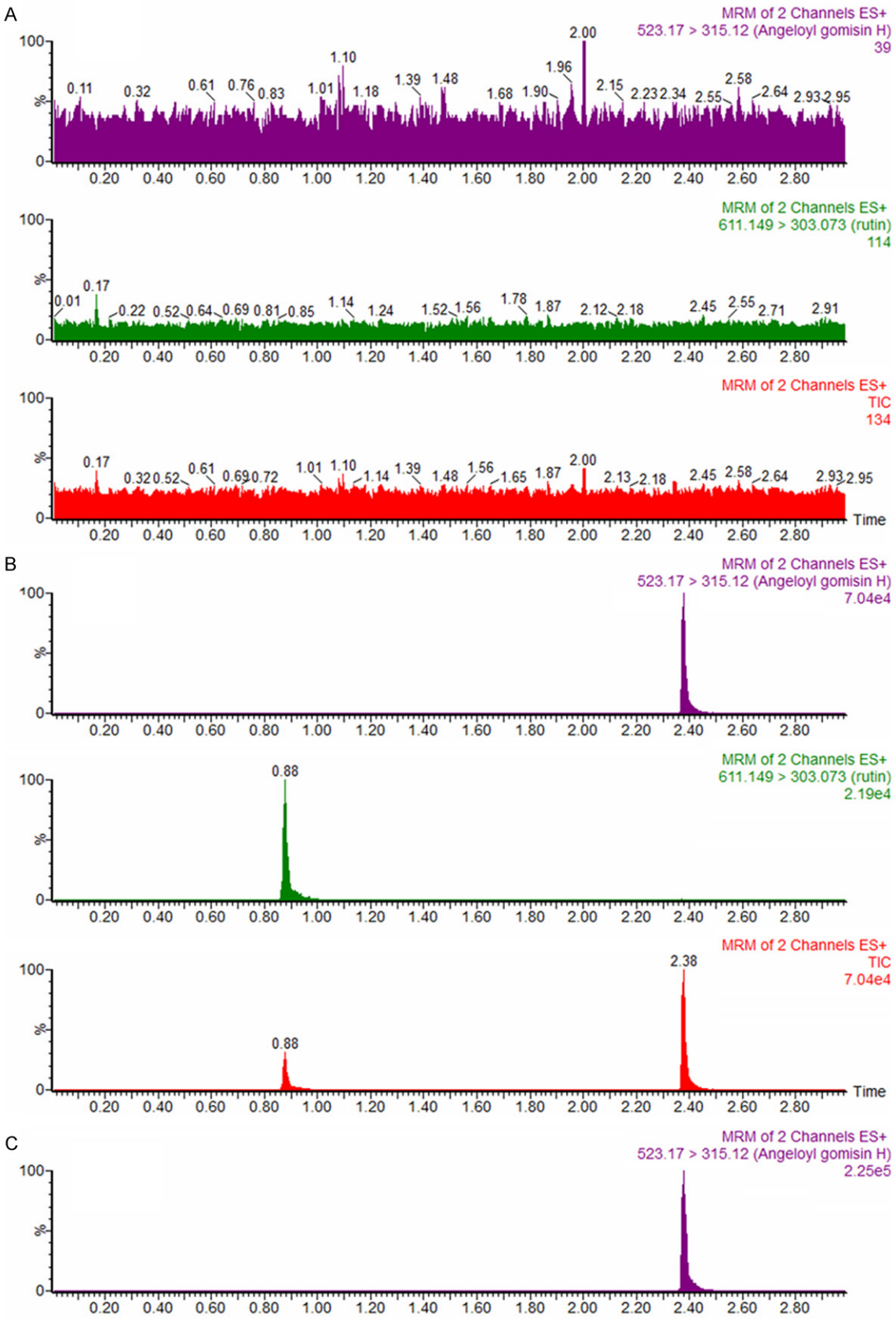


Figure 2. Mass spectrum of angeloylgomisin H (A) and rutin (IS, B).

plasma at 5, 10, 20, 50, 100, 200, 500, 1000, and 2000 ng/mL, each by adding 10 μL of the appropriate working solution to 100 μL of blank rat plasma, followed by short vortex mixing. Quality-control (QC) samples were prepared in the same manner as the calibration standards, in three different plasma concentrations (8, 800, and 1600 ng/mL). The calibration standards and QC samples protein precipitation by acetonitrile before UPLC-MS/MS analysis.

Sample preparation

Before analysis, the plasma sample was thawed to room temperature. An aliquot of 10 μL of the IS working solution (0.5 μg/mL) was added to 100 μL of the collected plasma sample in a 1.5 mL centrifuge tube, followed by the addition of 200 μL of acetonitrile. The tubes were vortex mixed for 1.0 min. After centrifugation at 14900 *g* for 10 min, the supernatant (2


A
MRM of 2 Channels ES+
523.17 > 315.12 (Angeloyl gomisin H)
39
MRM of 2 Channels ES+
611.149 > 303.073 (rutin)
114
MRM of 2 Channels ES+
TIC
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Time
B
MRM of 2 Channels ES+
523.17 > 315.12 (Angeloyl gomisin H)
7.04e4
MRM of 2 Channels ES+
611.149 > 303.073 (rutin)
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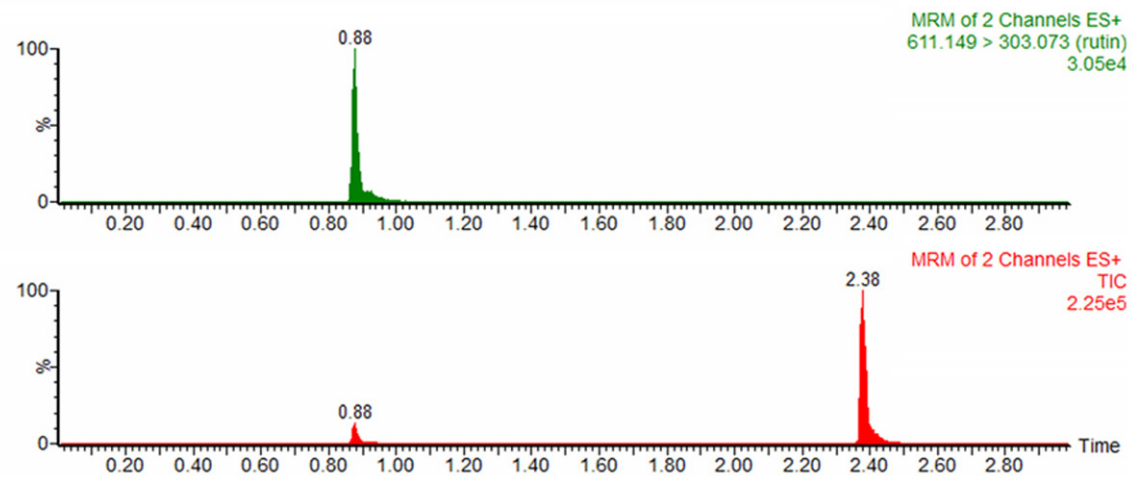

Figure 3. Representative UPLC-MS/MS chromatograms of angeloylgomisin H and rutin (IS), (A) blank plasma; (B) blank plasma spiked with angeloylgomisin H (5 ng/mL) and IS (50 ng/mL); (C) a rat plasma sample 3 h after intravenous administration of single dosage 2 mg/kg angeloylgomisin H.

μL) was injected into the UPLC-MS/MS system for analysis.

Method validation

Rigorous tests for selectivity, linearity, accuracy, precision, recovery, and stability, according to the guidelines set by the United States Food and Drug Administration (FDA) [16] and European Medicines Agency (EMA) [17], were conducted in order to thoroughly validate the proposed bioanalytical method. Validation runs were conducted on three consecutive days. Each validation run consisted of one set of calibration standards and six replicates of QC plasma samples.

The selectivity of the method was evaluated by analyzing blank rat plasma, blank plasma-spiked angeloylgomisin H and IS, and a rat plasma sample.

Calibration curves were constructed by analyzing spiked calibration samples on three separate days. Peak area ratios of angeloylgomisin H-to-IS was plotted against analyte concentrations. Resultant standard curves were well fitted to the equations by linear regression, with a weighting factor of the reciprocal of the concentration ($1/x$) in the concentration range of 5-2000 ng/mL. The LLOQ was defined as the lowest concentration on the calibration curves.

To evaluate the matrix effect, blank rat plasma was extracted and spiked with the analyte at 8, 800, and 1600 ng/mL concentrations (n = 6). The corresponding peak are as were then compared to those of neat standard solutions at equivalent concentrations, this peak area ratio is defined as the matrix effect. The matrix effect of the IS was evaluated at a concentration of 50 ng/mL in a similar manner.

Accuracy and precision were assessed by the determination of QC samples at three concentration levels in six replicates (8, 800, and 1600 ng/mL) over three days of validation testing. The precision is expressed as RSD.

The recovery of angeloylgomisin H was evaluated by comparing the peak area of extracted QC samples with those of reference QC solutions reconstituted in blank plasma extracts (n = 6). The recovery of the IS was determined in the same way.

Carry-over was assessed following injection of a blank plasma sample immediately after 3 repeats of the upper limit of quantification (ULOQ), after which the response was checked for accuracy [25].

Stability values of angeloylgomisin H in rat plasma were evaluated by analyzing three replicates of plasma samples at concentrations of

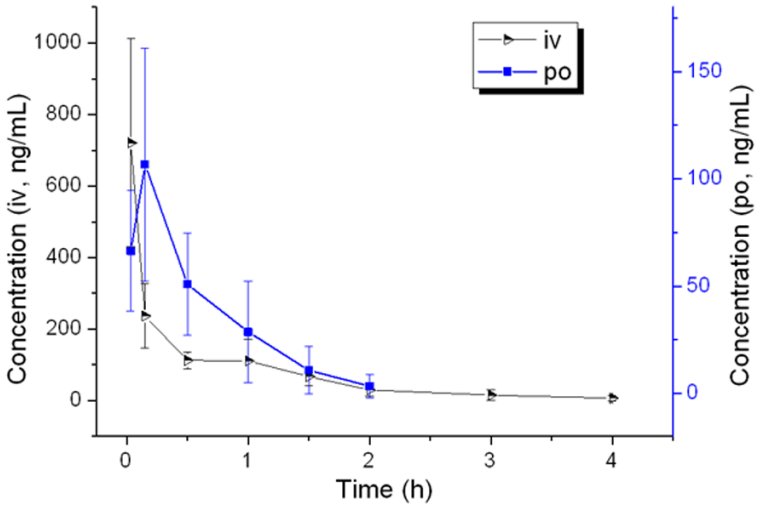

Figure 4. Mean plasma concentration time profile after oral (10 mg/kg, po) and intravenous (2 mg/kg, iv) administration of angeloylgomisin H in rats.

Table 1. Primary pharmacokinetic parameters after oral and intravenous administration of angeloylgomisin H in rats (n = 6)

Parameters	Unit	Mean	SD	Mean	SD
		po 10 mg/kg		iv 2 mg/kg	
$AUC_{(0-t)}$	ng/mL*h	72.1	38.0	295.6	66.8
$AUC_{(0-\infty)}$	ng/mL*h	74.6	40.8	306.2	77.1
$t_{1/2}$	h	0.4	0.1	0.7	0.4
CL	L/h/kg	168.2	80.8	6.9	1.8
V	L/kg	83.4	40.0	6.8	2.8
C_{max}	ng/mL	117.7	37.1	720.8	290.9
Absolute bioavailability/F_{abs}		4.9%			

The maximum plasma concentration (C_{max}) was observed directly from the concentration-time curve. The area under the plasma concentration-time curve (AUC) was estimated by the trapezoidal rule. The plasma clearance (CL), apparent volume of distribution (V), and the half-life ($t_{1/2}$) were estimated using non-compartmental calculations performed with DAS (Drug and statistics) software.

8 or 1600 ng/mL which were all exposed to different conditions. These results were compared with the freshly-prepared plasma samples. Short-term stability was determined after the exposure of the spiked samples to room temperature for 2 h, and the ready-to-inject samples (after protein precipitation,) in the HPLC autosampler at room temperature for 24 h. Freeze/thaw stability was evaluated after three complete freeze/thaw cycles (-20 to 25°C) on consecutive days. Long-term stability was assessed after storage of the standard spiked plasma samples at -20°C for 20 days. The stability of the IS (50 ng/mL) was evaluated similarly [26, 27].

Pharmacokinetic study

Male Sprague-Dawley rats (200-220 g) were obtained from the Laboratory Animal Center of Wenzhou Medical University to study the pharmacokinetics of angeloylgomisin H. All twelve rats were housed at the Laboratory Animal Center of Wenzhou Medical University. All experimental procedures and protocols were reviewed and approved by the Animal Care and Use Committee of Wenzhou Medical University, and were in accordance with the Guide for the Care and Use of Laboratory Animals. Diet was prohibited for 12 h before the experiment but water was freely available. Blood samples (0.3 mL) were collected from the tail vein into heparinized 1.5 mL polythene tubes at 0.0333, 0.15, 0.5, 1, 1.5, 2, 3, 4 h after oral (10 mg/kg) or intravenous (2 mg/kg) administration of angeloylgomisin H. Angeloylgomisin H (40 mg) was dissolved in 4 mL saline with little 0.1% HCl, about 3 mL for oral administration and 1 mL for intravenous administration. The samples were immediately centrifuged at 3000 g for 10 min. The plasma as-obtained (100 μL) was stored at -20°C until analysis.

Plasma angeloylgomisin H concentration versus time data for each rat was analyzed by DAS (Drug and statistics) software (Version 2.0, Wenzhou Medical University). The maximum plasma concentration (C_{max}) was observed directly from the concentration-time curve. The area under the plasma concentration-time curve (AUC) was estimated by the trapezoidal rule. The plasma clearance (CL), apparent volume of distribution (V), and the half-life ($t_{1/2}$) were estimated using non-compartmental calculations performed with DAS software. The

absolute bioavailability (F_{abs}) is the dose-corrected area under curve (AUC) non-intravenous divided by AUC intravenous. The formula for calculating *F* for a drug administered by the oral route (po) is given below.

$$F_{abs} = 100 \times \frac{AUC_{Po} \times Dose_{iv}}{AUC_{iv} \times Dose_{po}}$$

Results and discussion

Selectivity and matrix effect

Figure 3 shows typical chromatograms of a blank plasma sample, a blank plasma sample spiked with angeloylgomisin H and IS, and a plasma sample. There were no interfering endogenous substances observed at the retention time of the angeloylgomisin H and IS.

The matrix effect for angeloylgomisin H at concentrations of 8, 800, and 1600 ng/mL were measured between 98.8% and 109.8% (n = 6). The matrix effect for IS (50 ng/mL) was 95.6% (n = 6). As a result, matrix effect from plasma is considered negligible in this method.

Calibration curve and sensitivity

Linear regressions of the peak area ratios versus concentrations were fitted over the concentration range 5-2000 ng/mL for angeloylgomisin H in rat plasma. The equation utilized to express the calibration curve is: $y = 0.00022381*x+0.00325613$, $r = 0.9955$, where *y* represents the ratios of angeloylgomisin H peak area to that of IS, and *x* represents the plasma concentration. The LLOQ for the determination of angeloylgomisin H in plasma was 5 ng/mL. The precision and accuracy at LLOQ were 12.6% and 90.7%, respectively. The LOD, defined as a signal/noise ratio of 3, was 2 ng/mL for angeloylgomisin H in rat plasma.

Precision, accuracy and recovery

The precision of the method was determined by calculating RSD for QCs at three concentration levels over three days of validation tests. Intra-day precision was 7% or less, and inter-day precision was 11% or less at each QC level. The accuracy of the method ranged from 93.0% to 104.1% at each QC level. Mean recoveries of angeloylgomisin H were higher than 86.2%. The recovery of the IS (50 ng/mL) was 89.6%.

Carry-over

None of the analytes showed any significant peak (≥ 20% of the LLOQ and 5% of the IS) in blank samples injected after the ULOQ samples. Adding 0.5 extra minutes to the end of the gradient elution effectively washed the system between samples, thereby eliminating carry-over [25].

Stability

Results from the auto-sampler showed that the analyte was stable under room temperature, freeze-thaw, and long-term (20 days) conditions, confirmed because the bias in concentrations were within ± 15% of their nominal values. To this effect, the established method is suitable for pharmacokinetic study.

Application

The method was applied to a pharmacokinetic study in rats. The mean plasma concentration-time curve after oral (10 mg/kg) or intravenous (2 mg/kg) administration of angeloylgomisin H is shown in **Figure 4**. Primary pharmacokinetic parameters, based on non-compartment model analysis, are summarized in **Table 1**. The absolute bioavailability of angeloylgomisin H was reported as high as 4.9%, it helps to build a better understanding of the pharmacological features of angeloylgomisin H.

Conclusion

In present study, a simple, precise, and accurate UPLC-MS/MS method for the quantitation of angeloylgomisin H in rat plasma was established, utilizing 100 μL of plasma with an LLOQ of 5 ng/mL. The UPLC-MS/MS method was successfully applied to a pharmacokinetic study of angeloylgomisin H after both oral and intravenous administration. The absolute bioavailability of angeloylgomisin H was identified at 4.9%.

Disclosure of conflict of interest

None.

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Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, Zhejiang, China. Tel: (86) 577 55579095; E-mail: liufulimz@163.com

References

[1] Kang YS, Han MH, Hong SH, Park C, Hwang HJ, Kim BW, Kyoung KH, Choi YW, Kim CM and Choi YH. Anti-inflammatory effects of schisandra chinensis (turcz.) baill fruit through the inactivation of nuclear factor-kappab and mitogen-activated protein kinases signaling pathways in lipopolysaccharide-stimulated murine macrophages. J Cancer Prev 2014; 19: 279-287.

[2] Hancke J, Burgos R and Ahumada F. Schisandra chinensis (Turcz.) baill. Fitoterapia 1999; 70: 451-471.

[3] Kang JS, Han MH, Kim GY, Kim CM, Kim BW, Hwang HJ and Hyun Y. Nrf2-mediated HO-1 induction contributes to antioxidant capacity of a Schisandrae Fructus ethanol extract in C2C12 myoblasts. Nutrients 2014; 6: 5667-5678.

[4] Slaninova I, Brezinova L, Koubikova L and Slanina J. Dibenzocyclooctadiene lignans overcome drug resistance in lung cancer cells-study of structure-activity relationship. Toxicol In Vitro 2009; 23: 1047-1054.

[5] Huang F, Xiong Y, Xu L, Ma S and Dou C. Sedative and hypnotic activities of the ethanol fraction from Fructus Schisandrae in mice and rats. J Ethnopharmacol 2007; 110: 471-475.

[6] Committee SP. Pharmacopoeia of the People's Republic of China. Beijing: People's Medical Publishing House; 2010.

[7] Kohda H, Ozaki M and Namera A. Production of lignans in calluses of Schisandra chinensis. J Nat Med 2012; 66: 373-376.

[8] Liu J, Wang Y and Liu H. Research progress of the chemical components and the pharmacology of schisandra chinensis. Spec Wild Econ Animal Plant Res 2005; 3: 49-53.

[9] Qiu H, Zhao X, Li Z, Wang L and Wang Y. [Study on main pharmacodynamic effects for Schisandra lignans based upon network pharmacology]. Zhongguo Zhong Yao Za Zhi 2015; 40: 522-527.

[10] Kwon DY, Kim da S, Yang HJ and Park S. The lignan-rich fractions of Fructus Schisandrae improve insulin sensitivity via the PPAR-gamma pathways in in vitro and in vivo studies. J Ethnopharmacol 2011; 135: 455-462.

[11] Chen YJ, Shao Q and Qu HB. Simultaneous Determination of Five Lignan Compounds in Tangke Capsules by HPLC. Chin Pharm J 2008; 22: 18.

[12] Zhang WD, Wang Q, Wang Y, Wang XJ, Pu JX, Gu Y and Wang R. Application of ultrahigh-performance liquid chromatography coupled with mass spectrometry for analysis of lignans and quality control of Fructus Schisandrae chinensis. J Sep Sci 2012; 35: 2203-2209.

[13] Liu H, Lai H, Jia X, Liu J, Zhang Z, Qi Y, Zhang J, Song J, Wu C, Zhang B and Xiao P. Comprehensive chemical analysis of Schisandra chinensis by HPLC-DAD-MS combined with chemometrics. Phytomedicine 2013; 20: 1135-1143.

[14] Kim YJ, Lee HJ, Kim CY, Han SY, Chin YW and Choi YH. Simultaneous determination of nine lignans from Schisandra chinensis extract using ultra-performance liquid chromatography with tandem mass spectrometry in rat plasma, urine, and gastrointestinal tract samples: application to the pharmacokinetic study of Schisandra chinensis. J Sep Sci 2014; 37: 2851-2863.

[15] Dou ZH, Luo L, Chen JY, An LP and Yang AH. Analysis of lignans in serum containing drug of Schisandra chinensis by UPLC-MS/MS. Chin J Clin Pharm 2013; 3: 17.

[16] Guidance for industry on Bioanalytical Method Validation. U.S. Department of Health and Human Services Food and Drug Administration 2013.

[17] Guidance on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009 Committee for Medicinal Products for Human Use (CHMP) 2011.

[18] Ma J, Wang S, Huang X, Geng P, Wen C, Zhou Y, Yu L and Wang X. Validated UPLC-MS/MS method for determination of hordenine in rat plasma and its application to pharmacokinetic study. J Pharm Biomed Anal 2015; 111: 131-137.

[19] Wang S, Wu H, Huang X, Geng P, Wen C, Ma J, Zhou Y and Wang X. Determination of N-methylcytisine in rat plasma by UPLC-MS/MS and its application to pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 2015; 990: 118-124.

[20] Wang X, Wang S, Lin F, Zhang Q, Chen H, Wang X, Wen C, Ma J and Hu L. Pharmacokinetics and tissue distribution model of cabozantinib in rat determined by UPLC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 2015; 983-984: 125-131.

[21] Ma J, Ding X, Sun C, Lin C, An X, Lin G, Yang X and Wang X. Development and validation a liquid chromatography mass spectrometry for determination of solasodine in rat plasma and its application to a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 2014; 963: 24-28.

[22] Ma J, Lin C, Wen C, Xiang Z, Yang X and Wang X. Determination of bicuculline in rat plasma by liquid chromatography mass spectrometry

and its application in a pharmacokinetic study. J Chromatogr B Analyt Technol Biomed Life Sci 2014; 953-954: 143-146.

[23] Wen C, Lin C, Cai X, Ma J and Wang X. Determination of sec-O-glucosylhamaudol in rat plasma by gradient elution liquid chromatography-mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 2014; 944: 35-38.

[24] Zhang Q, Wen C, Xiang Z, Ma J and Wang X. Determination of CUDC-101 in rat plasma by liquid chromatography mass spectrometry and its application to a pharmacokinetic study. J Pharm Biomed Anal 2014; 90: 134-138.

[25] Williams JS, Donahue SH, Gao H and Brummel CL. Universal LC-MS method for minimized carryover in a discovery bioanalytical setting. Bioanalysis 2012; 4: 1025-1037.

[26] Du J, Ma Z, Zhang Y, Wang T, Chen X and Zhong D. Simultaneous determination of ornidazole and its main metabolites in human plasma by LC-MS/MS: application to a pharmacokinetic study. Bioanalysis 2014; 6: 2343-2356.

[27] Liu J, Wang L, Hu W, Chen X and Zhong D. Development of a UHPLC-MS/MS method for the determination of plasma histamine in various mammalian species. J Chromatogr B Analyt Technol Biomed Life Sci 2014; 971: 35-42.