Short communication

Determination of geniposidic acid in rat plasma by LC–MS/MS and its application to in vivo pharmacokinetic studies

Xia Zheng, Xiao-Tao Huang, Neng Li, Ying-Yi Li, Sui-Qing Mi, Ning-Sheng Wang, Chang-Hui Liu*

Institute of Clinical Pharmacology, Guangzhou University of Chinese Medicine, No. 12, Jichang Road, Guangzhou 510405, China

ARTICLE INFO

Article history:
Received 28 November 2011
Accepted 17 January 2012
Available online 24 January 2012

Keywords:
Geniposidic acid
LC–MS/MS
Rat plasma
Pharmacokinetics

ABSTRACT

A simple, rapid and sensitive method for the determination of geniposidic acid (GSA) in rat plasma was developed using liquid chromatography tandem mass spectrometry (LC–MS/MS). Geniposide (GS) was used as the internal standard. Rat plasma pretreated by solid-phase extraction (SPE) was analyzed by LC–MS/MS with negative ion mode electrospray ionization (ESI) in the multiple reaction monitoring (MRM) mode. The analytical column was C8 column and the mobile phase was methanol (A) and water (B). The flow rate was set at 0.8 mL/min with split ratio of 1:3, the total run time was 15 min. The MS/MS ion transitions monitored were m/z 373.3–211.1 for GSA and m/z 387.3–225.3 for GS. The quantification limit was 5 ng/mL within a linear range of 10–4000 ng/mL. The inter-day and intra-day accuracy and precision were within ±10%. The method was fully validated for its sensitivity, selectivity, matrix effect, stability study and recovery. The data indicate that our LC–MS/MS assay is an effective method for the pharmacokinetics study of GSA in rat plasma.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Eucommia ulmoides Oliver (EuO), known as “Du-zhong” in Chinese, is a notable traditional Chinese medicine and widely used for treating lower back pain, knee pain, and the liver, kidney, and spleen disorders in China. Recently, EuO has been reported to have antihypertensive, antioxidative, antimutagenic effects, antigastric ulcer effects and to enhance collagen synthesis [1–8]. Geniposidic acid (GSA, Fig. 1) is the main iridoid glucoside extracted from EuO as a food additive for specific health use to prevent hypertension in Japan due to the intense hypertensive activity in clinical use [9]. In addition, GSA has been shown to have the antitumor and anticarcinogenic activity [10], inhibit low-density lipoproteins (LDL)-oxidation [11], increase the synthesis of collagen [12] and improve the turnover rate of the corneal layer of the skin [13]. However, although GSA has been studied for therapeutic use for many years, its pharmacokinetic profile has not been investigated.

Several analytical methods for the quantification of GSA have been reported, such as high-performance liquid chromatography with ultraviolet detection (HPLC-UV) [14], diode-array detection (DAD) [15,16], capillary electrophoresis (CE) [17] and high-performance liquid chromatography–tandem mass spectrometry (LC–MS/MS) [18]. However, to the best of our knowledge, the use of LC–MS/MS for the determination and quantitation of GSA in rat plasma has not been demonstrated. Therefore, the purpose of this study was to develop a sensitive and highly selective LC–MS/MS method suitable for the quantification of GSA in rat plasma. The method has been successfully applied to the pharmacokinetic evaluation of GSA using the rat as an animal model.

2. Experimental

2.1. Chemicals and reagents

Geniposidic acid (>98% pure, GSA) and Geniposide (>98% pure, GS) were purchased from Chendu King-tiger Pharm-Chem Tech Co. Ltd. (Chendu, China). Methanol, HPLC grade, was purchased from Merck (Darmstadt, Germany). Water was purified with the Milli-Q Plus system (Millipore, Bedford, MA, USA). Freshly obtained drug free rat plasma was collected from male Sprague-Dawley rats in our laboratory and stored at −20 °C prior to use.

2.2. LC–MS/MS condition

An Agilent 1200 series liquid chromatographic system, interfaced to a API4000 triple quadruple mass spectrometer and coupled with electro-spray ionization (ESI), was used for plasma sample determinations. The analytes of GSA and GS were separated on a Zorbax XDB-C8 column (150 mm × 4.6 mm, 5 μm, Agilent Technologies, USA). The mobile phase was methanol (A) and water (B). The solvent A was held at the initial condition of 5% (v/v) for 0.1 min and maintained for 3 min then linearly increased to 90% by 0.5 min
and maintained for 3.5 min then returned to 5% by 1 min, and held for 7 min. The flow rate was set at 0.8 mL/min with split ratio of 1:3, the total run time was 15 min. The column temperature was maintained at 40 °C using a column heater. The tandem MS detections were carried out with negative electrospray ionization and multiple reaction monitoring (MRM) detection mode which controlled by Analyst (version 1.5.1) operating software. The product ion scan spectra of [M−H] ions for both GSA and GS are shown in Fig. 1. The MRM transitions selected for determination were m/z 373.3→211.1 for GSA and m/z 387.3→225.3 for GS. The ionspray voltage was set to −4500 V, and the probe temperature was set at 300 °C. Nitrogen was used as the collision gas. And the nebulizer (GS1), curtain, and turbo gas (GS2) were set to 30, 35, and 25 psi, respectively. Dwell times were set to 200 ms for each transition. Compounds parameters, viz., declustering potential (DP), entrance potential (EP), collision energy (CE) and collision exit potential (CXP) were −77.7, −9.8, −18.5, −10 V and −52.4, −9.2, −8.9, −15 V for GSA and GS, respectively.

2.3. Animals and pharmacokinetic analysis

Male Sprague-Dawley rats (300 ± 20 g) were purchased from Medical Experimental Animal Center of Guangdong Province and acclimatized to our animal house for at least 3 days prior to the experiments. The animal experiments were performed in accordance with the Guidelines for animal Experiments of Guangzhou University of Chinese Medicine.

A 0.25 mL aliquot of blood sample was collected into heparinized tubes from the femoral artery at 0 (to serve as control), 0.03, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, and 12 h after a single dose by oral administration of 30 mg/kg GSA to rats (n = 8). The blood samples were centrifuged at 3000 × g for 10 min, and the plasma samples were stored at −20 °C until LC–MS/MS analysis.

The total area under the plasma concentration–time curve from time zero to time infinity (AU_{0→∞}) was calculated using the trapezoidal rule–extrapolation method [19]. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were determined by obtained from the experimental data. The elimination rate constant (K_{el}) was estimated from the slope of the regression line of best fit, and the half-life (t_{1/2}) of the drug was obtained by 0.693/K_{el}.

2.4. Preparation of calibration standards and quality control (QC) samples

Separate stock solutions of GSA and internal standard GS were prepared by dissolving 10 mg of each substance in 10 mL of methanol:water (50:50, v/v). A series of working standard solutions of GSA ranging from 100 to 40,000 ng/mL and the internal GS solution at 2500 ng/mL were prepared by diluting their stock methanol:water (50:50, v/v). All the solutions were kept at 4 °C and were brought to room temperature before use. The plasma calibration standards of GSA were prepared as follows: 10 μL of the working solution was added to 1.5 mL ependorf centrifuge tubes and evaporated dryness by a gentle stream of nitrogen, and then 100 μL of blank rat plasma was added to obtain the concentrations of 10, 20, 50, 100, 200, 500, 1000, 2000 and 4000 ng/mL. The QC samples were prepared in a similar manner at low, medium and high GSA levels (20, 200, 4000 ng/mL).

2.5. Sample preparation

The solid-phase extraction (SPE) cartridges (ProElut PXK 60 mg/3 mL 50/ pkg, Dikma Technologie, USA) were washed with 1 mL of methanol followed by 2 mL of water. For sample preparation, 100 μL aliquot of plasma was added into 1.5 mL ependorf centrifuge tubes, then 10 μL of working solution and 200 μL water were added. After vortexed for 30 s the samples were spiked into the cartridge under vacuum, then washed with 300 μL of water. A 1.5 mL clean tube was positioned below the SPE cartridge and the compounds were eluted with 1 mL of methanol. The eluent was evaporated dryness by a gentle stream of nitrogen at 37 °C. The residues were reconstituted in 100 μL of the mobile phase and 5 μL was injected onto analytical column.

2.6. Method validation

The analytical method was validated following the criteria suggested by the US Food and Drug Administration (FDA) bioanalytical method validation guidance [20].

The specificity of the method was established by comparing the chromatograms of blank plasma samples from six different sources with those spiked with analytes to find out interference from endogenous components.

2.6.1. Matrix effect

An assessment of matrix effect was done by comparing the peak areas of analytes in extracted samples of blank plasma spiked with the test compounds with the corresponding peak areas obtained by injection of standard solutions at appropriate concentration. Six different sources of blank plasma were used to assess the matrix effect. The matrix effect for GSA was determined at low, medium and high concentrations, viz., 20, 200 and 4000 ng/mL whereas the matrix effect over the IS was determined at a single concentration of 250 ng/mL.

2.6.2. Calibration curve and lower limit of quantification

Calibration curves were prepared by spiking pooled blank plasma with an appropriate amount of working solution to produce the calibration curve points equivalent to 10, 20, 50, 100, 200, 500, 1000, 2000 and 4000 ng/mL of GSA and 250 ng/mL of IS Calibration curves were calculated using weighted (1/x^2) linear regression
of internal ratios (analyte/IS peak areas) versus analyte concentrations. Lower limit of quantification, LLOQ, was defined as the lowest plasma concentration in the calibration curve that can be quantitatively measured with acceptable precision and accuracy (within 20%).

2.6.3. Accuracy and precision

QC plasma samples containing low, medium, and high (20, 200, 4000 ng/mL) concentrations of GSA were used to evaluate the accuracy, and precision of the assay method. For the evaluation of intra-day accuracy and precision, five aliquots of each sample were analyzed using a single calibration curve. For inter-day accuracy and precision, five aliquots of each sample were analyzed in three consecutive assays. The accuracy of the method was expressed as the relative error (RE%) obtained by calculating the percentage difference between the measured and spiked concentration over that of the spiked value. The precision was expressed as relative standard deviation (RSD).

2.6.4. Stability

Three aliquots of the low, medium and high (20, 200, 4000 ng/mL) concentration QC samples were used to test the stability. The short-term stability was determined with untreated QC samples stored for 24 h at room temperature. The long-term stability tested by assaying the untreated QC samples after 30 days of storage at −80 °C. The freeze–thaw stability was determined after three freeze–thaw cycles (−20 °C to room temperature as one cycle).

2.6.5. Recovery

The recovery studies were conducted at low, medium, and high (20, 200, 4000 ng/mL) concentration levels. The extraction recovery was determined by comparing the response ratio of extracted plasma QC samples with those of extracted blank plasma spiked with corresponding concentrations.

3. Results and discussion

3.1. LC–MS/MS method

The chromatographic run time for the extracted plasma samples was 15 min. The retention times for GSA and the internal standard were 6.16 and 6.20 min, respectively. Fig. 2 shows the representative LC–MS/MS chromatograms of blank plasma (A), blank plasma spiked with GSA at concentration of 100 ng/mL (B), and the plasma at 1 h of a rat following an single oral administration of GSA at 30 mg/kg (C). The separation of GSA and the internal standard without any interference from endogenous plasma components.

3.2. Matrix effect

In the present work, the matrix effect was evaluated by analyzing the low, medium, and high (20, 200, 4000 ng/mL) QC plasma samples. The average matrix effect values were 104.5%, 98.2% and 95.8% for GSA at low, medium and high QC, respectively. The matrix
3.3. Calibration curve and lower limit of quantification

The plasma calibration curve was constructed using nine calibration standards (viz., 10–4000 ng/mL). A typical equation of the calibration curve was obtained as follows: \( y = 4.9 \times 10^{-3} + 4.6 \times 10^{-2} (r = 0.9994, n = 5) \), where \( y \) is the peak-area ratio of GSA to IS and \( x \) is the plasma concentration of GSA, respectively. The LOQ for GSA was 5 ng/mL, which is sufficient for rat pharmacokinetic studies following oral administration of GSA.

3.4. Accuracy and precision

The intra-day and inter-day accuracy and precision in rat plasma were evaluated using the low, medium, and high QC spiked plasma samples. The intra-day (\( n = 5 \)) and inter-day (\( n = 15 \)) results obtained when the QC samples were analyzed for GSA are summarized in Table 1. The assay values for both occasions (intra- and inter-day) were found to be within the accepted variable limits. The data indicated that the present method has a satisfactory accuracy and precision.

3.5. Stability

Three concentration QC samples were analyzed in five replicates used for studying the possible conditions to which the samples might be exposed during storage and handling. All results of the stability tests are summarized in Table 2. The precisions were ranging from 3.8% to 5.2% and the accuracy was ranging from 94.7% to 105.8%. The results shown that GSA to be stable throughout all the stability tests, which suggested that this analytical method was applicable for routine analysis.

3.6. Extraction recovery

The extraction recovery was determined in five replicates by comparing the peak areas of the extracted plasma at 20, 200 and 4000 ng/mL with those obtained from the direct injection of standard solutions without preparation at the same concentrations. In our experiments we try to use different extraction methods to test the recovery. Initially, protein precipitation using acidified acetone-trile gave strong interferences. Secondly, liquid–liquid extraction with various organic solvents such as n-butyl alcohol and diethyl ether and their mixtures resulted in non-reproducible recoveries and interferences from the sample matrix. Results showed that the extracted recovery was below to the LLOQ when using protein precipitation by acetone-trile or liquid–liquid extraction with ethylacetate in the plasma concentration of GSA below 200 ng/mL. Therefore, it is not advisable for the plasma samples consisting of GSA to be pretreated by liquid–liquid extraction using organic solvents because the GSA is a strongly hydrophilic compound, which renders it extremely difficult to extract from aqueous biological media and results in low recovery. Subsequently, SPE was investigated as samples pre-treatment technique. Various SPE columns, such as C2, C8, C18, and cation exchanges (ProElut PXC 60 mg/3 mL, Dikma Technologie, USA) were tested the extraction recovery of GSA in rat plasma. Interestingly, the extracted recovery with C2, C8 and C18 SPE were less than 50% but high than 80% with PXC SPE cartridge in all plasma concentration of GSA. Thus, good extraction recovery of GSA was found only using the PXC SPE cartridges. All results of the extraction recovery tests are summarized in Table 3.

3.7. Pharmacokinetic studies

The mean plasma concentration versus time curves is presented in Fig. 3. The major pharmacokinetic parameters of GSA were calculated by non-compartment model and are demonstrated
in Table 4. The successful application of LC/MS/MS method to pharmacokinetic study of GSA indicated that the established analytical method was suitable and sufficient for pharmacokinetic study.

4. Conclusions

A sensitive, selective and rapid LC–MS/MS method using solid-phase extraction and sample analysis on C8 column was developed for the quantification of GSA in rat plasma. This method was proved to be linear over the range of 10–4000 ng/mL. The method was applied to the pharmacokinetic study of GSA in rats and may also be applied, with minor changes, to other biological samples.

Acknowledgements

We would like to thank the National Natural Science Foundation of China (Grant No. 81102883) and the National Natural Science Foundation of Guangdong Province (Grant No. S201110005540) for financial support for this research.

References