Journal of Chromatography B, 988 (2015) 8–12

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short Communication

Bioavailability and pharmacokinetics profile of helicid in beagle dogs using gradient elution high performance liquid chromatography electrospray ionization mass spectrometry

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ARTICLE INFO

Article history:
Received 23 July 2014
Accepted 1 February 2015
Available online 11 February 2015

Keywords:
Helicid
HPLC–ESI-MS
Pharmacokinetics
Bioavailability

ABSTRACT

A simple, sensitive and reliable gradient elution high performance liquid chromatography electrospray ionization mass spectrometry (HPLC–ESI-MS) method was developed for quantifying helicid in dog plasma. The limit of detection (LOD) and the lower limit of quantitation (LLOQ) were 0.3 and 1 ng/mL, respectively. This method was validated for selectivity, linearity, accuracy and precision, extraction recoveries, matrix effects, carry-over, cross-talk, dilution integrity, stability and incurred sample reanalysis (ISR). Bioavailability and pharmacokinetic parameters of helicid in beagle dogs were researched from a two period crossover design study. After intravenous administration (i.v.), helicid had a mean (±SD) $AUC_{0-\infty}$ of 12062.06 ± 2482.69 ng/mL h and terminal half-life ($t_{1/2z}$) of 2.91 ± 1.37 h, while C_{max} was 35613.23 ± 8157.18 ng/mL. Following intragastric gavage administration (i.g.), $AUC_{0-\infty}$ was 7589.16 ± 1797.20 ng/mL h along with a longer $t_{1/2z}$ of 4.10 ± 4.35 h. C_{max} was researched at 0.58 ± 0.20 h. The absolute bioavailability (*F*) of helicid was 15.74 ± 1.87%.

1. Introduction

Helicid (4-formylphenyl β-D-glucopyranoside, molecular structure seen in Fig. 1a), the major active component of the fruits of *Helicid hilagirica Beed*, is often used in the clinic to treat neurasthenic syndromes, vascular headache, and trigeminal neuralgia [1]. It drew more and more attention from scientists for its high efficacy and low toxicity [2,3]. However, not much work concerning dog pharmacokinetics (PK) studies of helicid has been done up to now. Only one article, early in 2006, concerning helicid dog PK studies was reported by Liu et al. [4] In that research, a liquid chromatography/ultra-violet/electrospray ionization-ion trap mass spectrometry method (LC/UV/ESI-ITMS) was developed using external standard method for determination of helicidum (helicid) and its metabolites in dog plasma with a LLOQ of 30 ng/mL and the run time up to 11.5 min [4].

In our previous studies, we already validated helicid detection methods in both rat biomatrices and human plasma [5–7]. Nevertheless, the foundation of a quantitation method has a direct relationship with the biological matrix. Researchers cannot simply shift one detection method in one matrix to another.

Herein, we report the development and validation of a gradient elution HPLC–ESI-MS method of helicid in dog plasma with highly improved sensitivity of LOD 0.3 ng/mL and LLOQ 1 ng/mL compared to Liu et al. [4] and successful application of this method to explore the bioavailability and pharmacokinetic characteristics of helicid in a two-period crossover design study of intravenous and intragastric administration in beagle dogs.

2. Experimental

2.1. Chemicals and reagents

Helicid (Batch No. 040801) was kindly provided by Kun Ming Baker Norton Co., Ltd. Bergeninum (Batch No. 1532-200202) was purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). The purities of all chemicals were above 99.9%. All other chemicals and solvents were of analytical grade.

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http://dx.doi.org/10.1016/j.jchromb.2015.02.003
1570-0232/

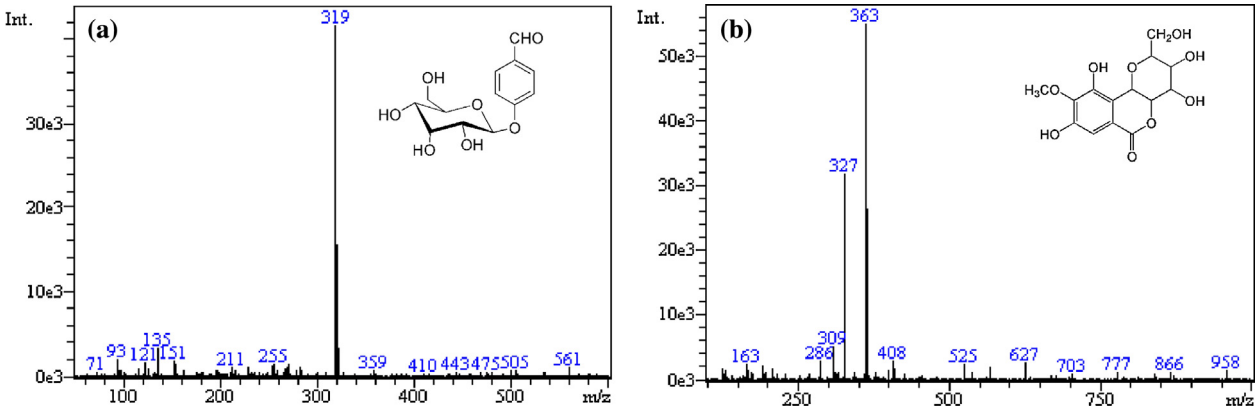

Fig. 1. Negative ion electrospray mass spectrum obtained in scan mode from standard samples of helicid (a, 500 ng/mL) and bergeninum (b, 200 ng/mL), respectively, with abundance of $[M+Cl]^-$.

2.2. Animals

Three male and three female beagle dogs (Certificate No. SCXK (SU) 2005-0003) weighing 12.97 ± 1.12 kg were purchased from An-limo laboratory animal center (Nanjing, China). All the studies were conducted under the ethics approval obtained from The Institutional Animal Care and Use Committee at China Pharmaceutical University. All the animals were maintained according to the Chinese government guidelines for care and use of laboratory animals under a constant temperature at 22 ± 1 °C, 12 h light/12 h dark cycle and 10–15 air changes per hour. Filtered tap water and a standard animal diet were available ad libitum.

2.3. Instruments and analytical conditions

Quantitative analysis was performed on a Shimadzu 2010A HPLC-MS system with an ESI interface, as well as a Shimadzu LCMS solution Workstation (ver. 2.02) for data acquisition. Liquid chromatographic separations were achieved using a Luna C_{18} column (150 mm × 2.00 mm, 5 μm, S/N: 372232-9, Phenomenex, USA). The column and autosampler tray temperatures were set at 40 °C and 4 °C, respectively. The mobile phase was made up of acetonitrile (solvent B) and water containing 500 μmol/L ammonium chloride (solvent A) at a flow rate of 0.2 mL/min from separate pumps. Gradient elution program were as follows: 0.03–2.0 min, B: 12%; 2.0–2.2 min, B: 12–60%; 2.2–5.0 min, B: 60%; 5.0–5.2 min, B: 60–12%; 5.2–8.0 min, B: 12%. A flow-switching technique was used by commanding the flow channel selection valve. The liquid was only loaded to MS around the times when the target peaks emerged (2–6 min). The optimized MS parameters were selected as follows: CDL (curved desolvation line) temperature, 250 °C; the block temperature, 200 °C; the probe temperature, 250 °C; detector gain, 1.6 kV; probe voltage, −4.5 kV; CDL voltage, −25 V; Q-array DC (direct current) voltage, 0 V; RF (radio frequency) voltage, 150 V. Nitrogen (99.9%, Gas Supplier Center of Nanjing University), served as nebulizer gas (flow rate: 1.5 L/min) and curtain gas (pressure: 1 MPa). Mass spectra were obtained at a dwell time of 0.2 and 1 s for SIM and scan mode, respectively. The MS acquisition was performed in SIM mode of negative ions. The chlorinated adducts of molecular ions $[M+Cl]^-$ of helicid at *m/z* 319.00 and IS at *m/z* 363.05 were monitored.

2.4. Study design

Six beagle dogs were randomly assigned to two groups and applied to a self-control crossover study, including two periods [8–10]. Three were treated i.g. after i.v., and the others were i.v. after i.g. Seven days were the washout phase between periods. During each period, the dogs were fasted overnight and 3 h after dosing, with free access to water. The helicid dosages were 20 mg/kg for i.g. and 5 mg/kg for i.v. bolus. i.v. was conducted using a 10 mL syringe with 22-gauge needle via the hindlimb vein. A 3.5 mL volume of blood was collected from the forelimb vein prior to dosing and at 5 min, 10 min, 15 min, 30 min, 45 min 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h post-dose for i.g. groups and 2 min, 8 min, 15 min, 30 min, 1 h, 1.5 h, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h post-dose for i.v. groups. Blood was harvested in heparinized centrifuge tubes. Plasma was separated and stored at −20 °C until analysis after the centrifugation at 4500 rpm for 10 min at 4 °C (TGL 16C, Medical centrifuge Co., Beijing, China).

2.5. Sample preparation

The standard stock solutions of helicid (10 mg/mL) were prepared in deionized water, while bergeninum (1 mg/mL) in methanol. Appropriate serial dilutions of the stock solution were made in deionized water for spiking blank biomatrices. Internal standard working solution was prepared by diluting internal standard stock solution with methanol. All solutions were stored at 4 °C. Aliquots (10 μL) of the appropriately diluted stock solution of helicid were added to 90 μL amounts of blank dog plasma to yield working standards of the desired plasma concentrations of 1–1000 ng/mL at nine concentration levels. Quality control (QC) samples (1, 100, 1000 ng/mL) were prepared in a similar way. An aliquot (100 μL) of the plasma, spiked with internal standard working solution (2.5 μg/mL, 20 μL) was vortex-mixed for 30 s, and extracted with *n*-butanol (1 mL) using a vortex mixer (Scientific Industries Inc., USA) for 3 min. Then the tubes were centrifuged at 24,000 rpm at 4 °C for 10 min (Micromax RF, Thermo Electron Corporation, USA). All the upper organic phase of each tube was evaporated to dryness in the Thermo Savant SPD 2010 SpeedVac System (Thermo Electron Corporation, USA). Residue was immediately reconstituted in 100 μL water and centrifuged at 24,000 rpm at 4 °C for 10 min. The supernatant (80 μL) was pipetted to an autosampler vial, and 20 μL was injected onto column for analysis.

2.6. Method validation

Chromatographic comparison of blank dog plasma, blank dog plasma spiked with standard and incurred dog plasma samples was conducted to evaluate specificity and selectivity of the method. Calibration curves were constructed by plotting the peak area ratio (analyte/IS) against concentration. Calibration curves with