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Short Communication

Rapid and sensitive HPLC–MS/MS method for quantitative determination of lycorine from the plasma of rats

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ABSTRACT

A simple, rapid and sensitive high-performance liquid chromatography–tandem mass spectrometric (HPLC–MS/MS) method was developed and validated for determining lycorine in rat plasma. Plasma samples were prepared by a simple protein precipitation with methanol containing dextrorphan as internal standard. The chromatographic separation was performed on a Kromasil 60-5CN column (3 μm, 2.1 mm × 150 mm) with the mobile phase of methanol/water (containing 0.1% formic acid) (40:60, v/v) at a flow rate of 0.2 mL/min, the total analytical runtime was 5 min. The detection was performed on a triple quadrupole tandem mass spectrometer equipped with Electronic Spray Ion by selected reaction monitoring (SRM) of the transitions at m/z 288.1 → 147.1 for lycorine and m/z 258.1 → 157.2 for dextrorphan, respectively. The calibration curve was linear over the range of 1–1000 ng/mL with the lower limit of quantification of 1 ng/mL for lycorine. The intra- and inter-day precision (R.S.D.%) were less than 8.5% and accuracy (R.E.%) was within ±7.0%. Lycorine was sufficiently stable under all relevant analytical conditions. This method was successfully applied to the pharmacokinetic study of lycorine in rats after intraperitoneal administration with different doses of 5, 10 and 20 mg/kg.

1. Introduction

Lycorine is the most abundant alkaloid isolated from the *Amaryllidaceae* family of plants [1]. Pervious studies reported that lycorine possessed various biological activities including anti-inflammatory, myocardial preservation, antioxidant, antiviral and anti-malarial action and hepatoprotective effect [2–4]. In particular, lycorine showed strong cytotoxicity toward a variety of cancer cell lines, such as lymphoma, multiple melanoma, carcinoma, myeloma, leukemia and so on [5]. Moreover, further studies provided a mechanistic insight into its anticancer properties. Evdokimov et al. reported that lycorine showed cytostatic effects by targeting the actin cytoskeleton in cancer cells [6]. The suppression of neovascularization and the regulations of apoptosis as well as cell cycle arrest were also closely associated with the anticancer effect of lycorine [7–9]. Many factors thus seemed involved in its anticancer properties. Due to its potential anti-cancer activity, lycorine is considered as a promising candidate for the new anticancer drug development.

Despite extensive researches in the pharmacological activities of lycorine, little is known about its pharmacokinetics. It is necessary to develop a reliable method for the pharmacokinetic study of lycorine. To the best of our knowledge, only one LC–MS method has been reported for the quantitative bioanalysis of lycorine in mice plasma. However, this method had several disadvantages, such as long analytical time, large volume of plasma and complicated liquid–liquid extraction procedure [10], which was unsuitable for analyzing large numbers of samples. Until now, no full validated LC–MS/MS method for quantification of lycorine in rat plasma has been reported.

In this study, we developed a simple, rapid and sensitive LC–MS/MS method for quantitative analysis of lycorine in rats. The validation results showed higher sensitivity (an LLOQ as low as 1 ng/mL), shorter analytical time (5 min per sample), smaller volume of plasma (20 μL) and simpler sample preparation procedure (protein precipitation). This method was then successfully applied to a preclinical pharmacokinetic study in rats after intraperitoneal administration of lycorine with different doses of 5, 10 and 20 mg/kg.

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2. Experimental

2.1. Materials and reagents

Lycorine (purity: 99%) was supplied by Aladdin reagent (Shanghai, China). Dextrorphan (IS, purity: 98%) was purchased from Toronto Research Chemicals Inc. (Canada). HPLC grade of methanol was obtained from CNW Technologies GmbH (Dusseldorf, Germany). Deionized water was produced using a Milli-Q system (Millipore, USA).

2.2. Instruments and chromatographic-mass conditions

The LC–MS/MS system consisted of a Accela Surveyor autosampler, a Accela 1250 pump, a TSQ Quantum Access TM triple quadrupole mass spectrometer with an electrospray ionization (ESI) source and Xcalibur 1.6 software for data acquisition and processing (Thermo Finnigan, USA). The chromatographic separation was achieved on a Kromasil 60-5CN column (3 μm, 2.1 mm × 150 mm, Kromasil), which was maintained at 35 °C. Methanol and water containing 0.1% formic acid (40:60, v/v) were used as the mobile phase at a flow rate of 0.2 mL/min. The total analytical runtime was 5 min. The sample volume injected was 10 μL.

The mass spectrometer was operated in positive ESI mode. Quantification was performed using selected reaction monitoring (SRM) mode of the transitions m/z 288.1 → 147.1 with a collision energy (CE) of 28 eV for lycorine and m/z 258.1 → 157.2 with CE of 37 eV for dextrorphan. The mass spectrometric condition was optimized as follows: spray voltage, 3500 V; vaporizer temperature, 300 °C; sheath gas pressure, 30 psi; auxiliary gas pressure, 5 arb; collision gas pressure, 1.5 mTorr; capillary temperature, 350 °C, respectively.

2.3. Preparation of stock solutions, calibration standards and quality control samples

Stock solutions of lycorine and IS were dissolved by methanol at the concentration of 1 mg/mL. Working standards were prepared by the dilution of stock solution in methanol to obtain the desired concentrations of 10, 20, 100, 500, 1000, 2500, 5000 and 10,000 ng/mL for lycorine. The working solution of IS (450 ng/mL) was obtained by diluting of stock solution with methanol. All working solutions were stored at −20 °C until use.

Calibration curves were prepared individually by mixing 2 μL of IS working solution (450 ng/mL), 2 μL of working standards, 20 μL of blank rat plasma and 200 μL of methanol. The quality control (QC) samples (2, 250 and 800 ng/mL for lycorine) were prepared in a manner similar to that used for preparation of the calibrator samples.

2.4. Sample preparation

A 2 μL of IS working solution (450 ng/mL) and 202 μL of methanol were added to 20 μL of plasma sample. The mixture was vortexed for 30 s followed by centrifugation at 14,000 rpm for 5 min at room temperature. A 10 μL aliquot of each supernatant was injected into the LC–MS/MS system for the analysis.

2.5. Method validation

2.5.1. Selectivity

Selectivity was evaluated by analyzing the plasma samples collected from six rats to investigate the chromatographic interferences for lycorine and IS from endogenous plasma components. Chromatographic peaks of analytes were identified on the basis of retention times and their SRM responses.

2.5.2. Linearity, accuracy and precision

The linearity of this method for the determination of lycorine was evaluated by a calibration curve in the range of 1–1000 ng/mL. The calibration curve was determined by plotting the peak area ratio (analyte/internal standard) versus the nominal concentration of analyte in plasma with weighted ($1/x^2$) least square liner regression. The calibration curve requires a correlation coefficient (r^2) of 0.99 or better.

The accuracy and precision of assay were determined from QC samples. Intra-day precision and accuracy were evaluated by analysis of five replicates of QC samples at three levels (2, 250 and 800 ng/mL) on the same day. Inter-day precision and accuracy were determined by analyzing the three levels of QC samples on five consecutive days. The precision of lycorine determination was depicted as the relative standard deviation (R.S.D.%) and accuracy was expressed as relative error (R.E.%). The acceptance criteria for intra- and inter-day precision were required to below 15% and accuracy must be within ±15%, except for LLOQ, where it should not exceed ±20% of the accuracy as well as precision.

2.5.3. Matrix effect and recovery

The matrix effect was evaluated by comparing the peak areas of analytes spiked in deproteinized samples of blank plasma from six rats with those of standard solutions at equivalent concentrations. Experiments were performed at three QC levels of lycorine in five replicates.

The recovery was calculated by comparing the peak area of the precipitated plasma samples with that of the analyte spiked to the post-precipitated blank samples at three QC levels. The recoveries of lycorine in rat plasma were examined at least five times.

2.5.4. Stability

Short-term, long-term, three freeze–thaws and post-preparative stabilities of lycorine in rat plasma were tested using three levels of QC samples. Short-term stability was determined by analyzing fresh plasma samples kept at room temperature for 24 h before treatment. Long-term stability was examined by assaying plasma samples stored at −20 °C for 10 d. Freeze–thaw stability was investigated by plasma samples after three freeze/thaw cycles (−20 to 24 °C). Post-preparative stability was studied by analyzing the extracted QC samples left in auto-sampler vials at ambient temperature for 24 h. Additionally, dilution stability was assessed using a spiked sample the concentration of which was higher than the upper limit of quantification samples (ULOQ). The result was obtained by comparing the back-calculated value of the sample after being diluted 10 times with the nominal value.

2.6. Pharmacokinetic study

All animal protocols were approved by Institute Animal Care and Welfare Committee. Sprague-Dawley rats (adult male), weighing 200 ± 20 g, were obtained from Jiangsu Institute of Parasitic Diseases. The rats were quarantined for 1 week prior to the study and maintained on a 12 h light/12 h dark cycle at 22 ± 1 °C and at 60% relative humidity. Animals had free access to the SPF nutritionally balanced diet (Beijing HFK Bioscience Co., Ltd) and water prior to the study.

After intraperitoneal administration of 5, 10 and 20 mg/kg of lycorine to rats, approximately 0.2 mL blood samples were collected in heparinized 1.5 mL polythene tubes by orbital bleeding via capillary tubes at 0.08, 0.17, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 12 and 24 h after dosing. The blood samples were immediately centrifuged