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Determination of liquiritigenin by ultra high performance liquid chromatography coupled with triple quadrupole mass spectrometry: Application to a linear pharmacokinetic study of liquiritigenin in rat plasma

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ABSTRACT

A simple, sensitive and rapid ultra high performance liquid chromatography tandem mass spectrometry (UHPLC–MS/MS) method has been developed and validated for the quantification of liquiritigenin, a promising anti-tumor agent. Liquiritigenin and the internal standard were separated on an Agilent Extend C_{18} column and eluted with a gradient mobile phase system of acetonitrile and water. The analysis was performed on a negative ionization electrospray mass spectrometer *via* multiple reaction monitoring (MRM). Transitions of m/z 255.0 → 119.0 for liquiritigenin and m/z 269.0 → 117.0 for the IS were monitored. One-step protein precipitation with acetonitrile was used to remove impurities and extract the analytes from plasma. The method had a chromatographic run time of 4.5 min and a good linearity in the range of 1–1000 ng/mL. The precision (R.S.D.) of intra-day and inter-day ranged from 4.54 to 10.65% and 5.94 to 13.81%, respectively; while the accuracy of intra-day and inter-day ranged from 104.06 to 109.28% and 94.98 to 112.05%. The recovery and stability were also within the acceptable limits. The validated method was applied to a linear pharmacokinetic study of liquiritigenin in rat plasma for the first time.

1. Introduction

Liquiritigenin (7,4′-dyhydroxy flavanone) is a poly phenolic flavanone structure that exists in Radix glycyrrhizae. As an inhibitor of Akt protein kinase and selective estrogen receptor agonist [1], liquiritigenin has been found to have various biological activities, such as anti-inflammatory [2], cytoprotection [3], inhibition of acute hepatic injury [4], and antitumor activity.

Liquiritigenin has been shown to possess significant antitumor activity. It has cytotoxic activity against five human cancer cell lines *in vitro* [5]. In accordance with our previous studies, liquiritigenin inhibits the growth of SGC-7901 and Lovo cells. Time- and dose-dependent inhibition and apoptosis induction were particularly exhibited on SMMC 7721 cells [6]. Liquiritigenin suppresses cancer cell proliferation and angiogenesis, and promotes apoptosis [7,8]. These findings suggested strong cancer preventive effects of liquiritigenin *in vitro*, yet the underlying molecular mechanisms remain unknown. Our previous study also showed that liquiritigenin significantly inhibited the growth of murine H_{22} hepatocarcinoma *in vivo* through a 15-day treatment after tumor inoculation and exhibited potent tumor therapeutic activity [9]. All these results suggested that liquiritigenin could be a promising antitumor agent for human cancers.

To ensure safety and effectiveness of the medication, a novel UHPLC–MS/MS method, aiming at both simplicity and sensitivity, was established for the preclinical linear pharmacokinetic study. Several methods involving high-performance liquid

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chromatography and mass spectrometry have been described previously to analyze liquiritigenin concentration in plasma after administration [10–19]. While these methods provide precise quantitation, the sample preparation and mobile phase composition of such methods are rather complex. A longer time is also required for analysis. In conventional HPLC analysis, liquiritigenin has a long retention time of approximately 9.8 min. By focusing on a single compound rather than multiple constituents in the plasma, shorter analysis time can be achieved by UHPLC–MS/MS.

In the present work, we developed and validated a UHPLC–MS/MS method for the determination and quantitation of liquiritigenin in rat plasma that boasts higher specificity, increased sensitivity, simpler sample preparation, and shorter analysis time. The method has been applied to the pharmacokinetic study involving three-level single-dose intravenous administration of liquiritigenin in rats for the first time.

2. Materials and methods

2.1. Materials and reagents

Liquiritigenin (purity > 98.0%) was provided by Professor Wei Li from Nanjing University of Chinese Medicine. The internal standard, apigenin was purchased from Shanghai Yuanye Biotechnology Co. Ltd. (Shanghai, China), lot number: 20120220. HPLC–MS-grade acetonitrile and water were purchased from ANPEL Scientific Instrument Co. Ltd. (Shanghai, China). HPLC-grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany).

2.2. Instruments

The chromatography system used was composed of a Shimadzu LC-30A series chromatographic system (Shimadzu Corporation UFLC XR, Kyoto, Japan) with a LC-30AD binary pump, a DGU-20A 5R degasser, a SIL-30AC auto sampler and a CTO-30A column oven. The separation was performed using the C_{18} column (Agilent, USA), 2.1 × 100 mm, 1.8 μm, at a temperature of 35 °C. Mass spectrometric detection was performed by using 5500 triple quad tandem mass spectrometer equipped with electrospray ionization (ESI) source (AB SCIEX, Foster City, CA, USA). Peak Genius^2 3030 instrument was used for generating nitrogen gas.

2.3. LC–MS/MS conditions

The mobile phase containing water (A) and acetonitrile (B) was used to achieve separation from endogenous interferences. The gradient program was as follows: 0–1.0 min, 5–50% B, 1.0–3.0 min, 50–90% B, 3.0–4.0 min, 90% B, 4.0–4.1 min, 90–5% B, 4.1–4.5 min, 5% B. The flow rate was set at 0.3 mL/min. The autosampler was conditioned at 4 °C and the injection volume was 2 μL for analysis.

Mass parameters were optimized by infusing neat solutions of liquiritigenin and internal standard (IS) separately into mass with an apparatus syringe pump. A turbo ion spray interface operating in negative ionization mode was used. Typical source conditions were as follows: curtain-gas (CUR), 35; ion source temperature, 500 °C; ion source gas1 (GAS1), 35; ion source gas2 (GAS2), 35; ionspray voltage, 5500 V; declustering potential (DP), −60.73; collision energy (CE), −33.03; entrance potential (EP), −12.87; collision cell exit potential (CXP), −12.96. Data acquisition was performed with Analyst Software (AB SCIEX, Foster City, CA, USA).

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

Standard stock of liquiritigenin of 1.02 mg/mL and IS of 1.06 mg/mL solutions were prepared using 100% methanol. The concentration of working solution for internal standard was 106 ng/mL. All liquiritigenin and IS solutions were stored at 4 °C until needed.

Working solutions of calibration standards (10, 50, 100, 250, 500, 1000, 5000, 10,000 ng/mL) and quality control samples (25, 1000 and 8000 ng/mL) were prepared by diluting the stock solutions with methanol. Calibration standards were prepared by spiking 10 μL of working stock to 90 μL of blank rat plasma to obtain concentrations of 1, 5, 10, 25, 50, 100, 500, 1000 ng/mL. Quality control (QC) samples (of low, medium and high concentration) at 2.5, 100 and 800 ng/mL were prepared in the same way as the calibration standards and stored at −20 °C.

2.5. Sample preparation

100 μL of plasma sample was mixed with 20 μL of the IS working solution (106 ng/mL) and vortexed for 30 s. Then, 400 μL of acetonitrile was added and the mixture was vortexed for 3 min to precipitate protein. These samples were centrifuged at 12,000 rpm for 5 min. 300 μL of the supernatant was then transferred to a new tube and centrifuged further at the same conditions. 2 μL of the sample was injected into the HPLC for analysis.

2.6. Method validation

2.6.1. Specificity

Plasma samples from six different rats were screened for the presence of endogenous components which might interfere with detection of liquiritigenin or the IS. The specificity was investigated by comparing the chromatograms of the blank plasma with plasma samples spiked with liquiritigenin and IS, as well as samples collected from treated rats.

2.6.2. Linearity and lower limit of quantification (LLOQ)

Calibration standards of liquiritigenin at concentrations of 1, 5, 10, 25, 50, 100, 500, 1000 ng/mL were prepared using the standard plasma samples described in Section 2.5. Peak-area ratios of liquiritigenin to IS were utilized for construction of calibration curves, using weighted liner least square regression (weighting: $1/C^2$) of the plasma concentrations and the measured ratios. The linearity of the calibration curve was assessed by plotting the peak-area ratios *versus* the concentrations of liquiritigenin. The lower limit of quantification (LLOQ) was determined as the lowest concentration on the calibration curve at which accuracy (RE) within ±20% and a precision (R.S.D) below 20% can be obtained. The LOD was defined as the plasma concentration that produced a signal-to-noise ratio (S/N) at 3.

2.6.3. Precision and accuracy

The precision and accuracy of the assay were determined at QC samples of low, medium and high concentrations at 2.5, 100 and 800 ng/mL of liquiritigenin, following the steps in Section 2.4. Intra-day precision was determined by repeated analyses of each QC sample for six times during one day. Inter-day precision was determined by repeated analyses of six replicates of each QC sample on three consecutive days. The concentration of each sample was calculated by the calibration curve each day. Precision was assessed by calculating the relative standard deviation (R.S.D) for each concentration level. Accuracy was calculated by comparing the average measurements with the nominal values.