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Short communication

Simultaneous determination of mangiferin and neomangiferin in rat plasma by UPLC–MS/MS and its application for pharmacokinetic study

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ABSTRACT

In this study, a sensitive and rapid ultra performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS) method was developed to determine mangiferin and neomangiferin in rat plasma simultaneously. Chromatographic separation was carried out on an Acquity UPLC BEH C18 column and mass spectrometric analysis was performed using a Xevo TQD triple quadruple mass spectrometer coupled with an electrospray ionization (ESI) source. The MRM transitions of m/z 423.2 → 303.1 and m/z 585.0 → 273.1 were used to quantify for mangiferin and neomangiferin, respectively. The linearity of this method was found to be within the concentration range of 5–2000 ng/mL for mangiferin, and 2–1000 ng/mL for neomangiferin in rat plasma, respectively. Only 3.0 min was needed for an analytical run. This assay was used to support a preclinical study to investigate the pharmacokinetics of mangiferin and neomangiferin in rats.

1. Introduction

Anemarrhena asphodeloides Bunge. (Asparagaceae) yields *Rhizoma anemarrhenae*, which has been commonly used in Asian countries for hundreds of years as Traditional Chinese Medicine (TCM) and officially listed in the Chinese Pharmacopoeia. Modern research found that *Rhizoma anemarrhenae* has the bioactive effects of anti-pathogenic microorganism, hypoglycemic effects, anti-inflammatory, antipyretic effects and anti-platelet aggregation [1,2]. Mangiferin and neomangiferin are the major bioactive constituents of *Rhizoma anemarrhenae*. The chemical structures of mangiferin and neomangiferin are shown in Fig. 1. It has been shown that mangiferin and neomangiferin have many beneficial biological activities, including anti-inflammatory, anti-oxidant, and anti-diabetic effects [3–5]. Moreover, recent studies have indicated that mangiferin may lower triglycerides and total cholesterol levels [6–8], offer neuroprotection [9,10], promote urate excretion [11] and inhibit the development of tumor [12–14]. In addition, neomangiferin has beneficial effect on high fat diet-induced non-alcoholic fatty liver disease [15].

Up to now, there have several analytical methods for the simultaneous determination of mangiferin and neomangiferin in biological samples, mainly including LC–MS/MS [16–18]. However, to our knowledge, their pharmacokinetics in biological fluid are not elucidated clearly. Even above LC–MS/MS publications had reported the methods for the simultaneous determination of mangiferin and neomangiferin with other substances in rats after consumption of crude herb extracts, their pharmacokinetic properties after given with pure substances still remain unknown so far [16–18]. Moreover, these methods generally had poor assay specificity and long run times. Since a very large number of samples are generated in pharmacokinetic assays, most of the above mentioned methods do not meet the criteria for this kind of study.

As a result of recent advances in analysis techniques, ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC–MS/MS) has emerged as an efficient analytical tool with improved sensitivity, selectivity, and specificity. In the present work, a highly rugged, selective and rapid UPLC–MS/MS method has been developed and fully validated as per the USFDA guidelines for the simultaneous measurement of mangiferin and neomangiferin in rat plasma using diazepam as internal standard

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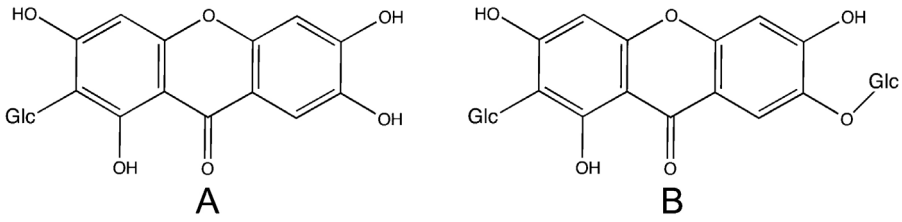

Fig. 1. The chemical structures of the analytes in the present study: (A) mangiferin; (B) neomangiferin.

(IS). The method was free from endogenous matrix interference and was successfully applied to a pharmacokinetic study in rats.

2. Materials and methods

2.1. Chemicals and reagents

Mangiferin (purity 98.0%) and neomangiferin (purity 98.0%) were purchased from Chengdu Mansite Pharmaceutical CO., LTD. (Chengdu, China). Diazepam (internal standard, IS, purity 98.0%) was obtained from Sigma (St. Louis, MO, USA). Formic acid was analytical grade and purchased from the Beijing Chemical Reagents Company (Beijing, China). Acetonitrile and methanol were of HPLC grade and were purchased from Merck Company (Darmstadt, Germany). HPLC grade water was obtained using a Milli Q system (Millipore, Bedford, MA, USA).

2.2. UPLC–MS/MS conditions

Liquid chromatography was performed on an Acquity ultra performance liquid chromatography (UPLC) unit (Waters Corp., Milford, MA, USA) with an Acquity BEH C18 column (2.1 mm × 50 mm, 1.7 μm particle size) and inline 0.2 μm stainless steel frit filter. A gradient program was employed with the mobile phase, combining solvent A (0.1% formic acid in water) and solvent B (acetonitrile) as follows: 10–10% B (0–0.5 min), 10–95% B (0.5–1.0 min), 95–95% B (1.0–2.0 min), 95–10% B (2.0–2.1 min), 10–10% B (2.1–3.0 min). The flow rate was 0.40 mL/min and the injection volume was 6 μL. The column and sample temperature were maintained at 40 °C and 4 °C, respectively.

A Xevo TQD triple quadruple mass spectrometer equipped with an electrospray ionization (ESI) source (Waters Corp.) was used for mass spectrometric detection. The quantitative analysis of mangiferin and neomangiferin in rat plasma was performed using multiple reaction monitoring (MRM) method. The MRM transitions were m/z 423.2 → 303.1, m/z 585.0 → 273.1, and m/z 285.1 → 193.2 for mangiferin, neomangiferin and IS, respectively. The Masslynx 4.1 software (Waters Corp.) was used for data acquisition and instrument control.

2.3. Standard solutions, calibration standards and quality control (QC) sample

The stock solutions of mangiferin and neomangiferin used to make the calibration standards and quality control (QC) samples were prepared by dissolving 10 mg each compound in 10 mL methanol to obtain a concentration of 1.00 mg/mL of each compound. The stock solutions were further diluted with methanol to obtain working solutions at several concentration levels. Calibration standards and QC samples in plasma were prepared by diluting the corresponding working solutions with blank rat plasma. Final concentrations of the calibration standards were 5, 10, 20, 50, 100, 200, 500, 1000 and 2000 ng/mL for mangiferin, and 2, 5, 10, 20, 50, 100, 200, 500 and 1000 ng/mL for neomangiferin in rat plasma, respectively. The concentrations of QC samples in plasma were 10, 160, and 1600 ng/mL for mangiferin, and 4, 80, and 800 ng/mL for neomangiferin, respectively. IS stock solution was made at an initial concentration of 1.00 mg/mL. The IS working solution (300 ng/mL) was made from the stock solution using acetonitrile for dilution. All stock solutions, working solutions, calibration standards and QCs were immediately stored at −80 °C.

2.4. Sample preparation

Before analysis, the plasma sample was thawed to room temperature. In a 1.5 mL centrifuge tube, an aliquot of 200 μL of the IS working solution (300 ng/mL in acetonitrile) was added to 100 μL of collected plasma sample. The tubes were vortex mixed for 1.0 min

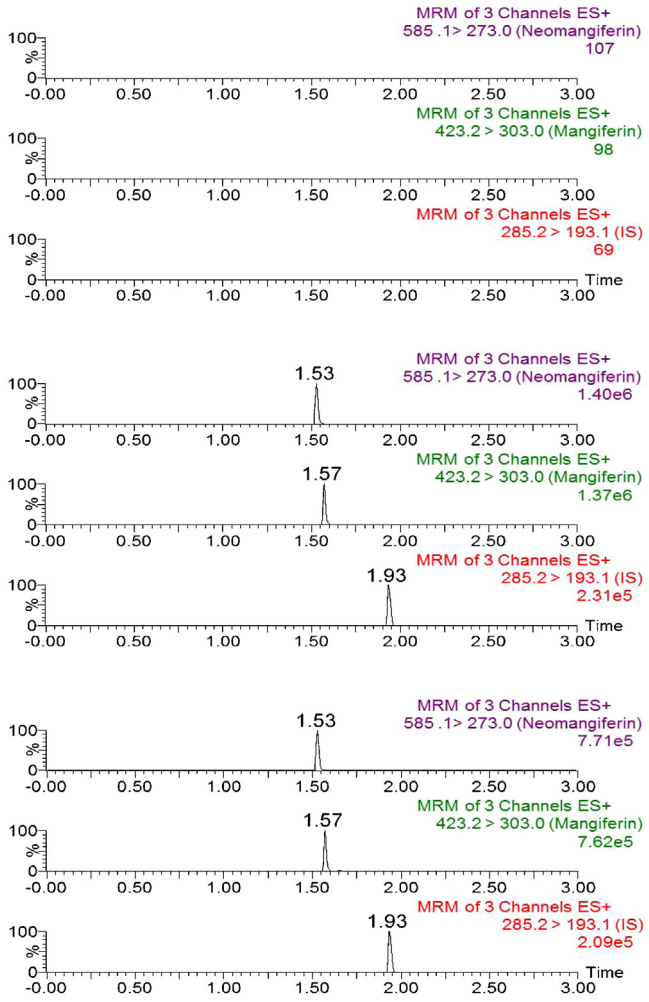

Fig. 2. Representative chromatograms of mangiferin, neomangiferin and IS in rat plasma samples. (A) a blank plasma sample; (B) a blank plasma sample spiked with mangiferin, neomangiferin and IS; (C) a plasma sample from a rat 0.5 h after an intravenous co-administration of mangiferin and neomangiferin.