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Determination of dihydromyricetin in rat plasma by LC–MS/MS and its application to a pharmacokinetic study

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ABSTRACT

Ampelopsis grossedentata (Hand.-Mazz.) W.T. Wang has long been used as a traditional Chinese medicinal herb among the indigenous people in the Yangtze River region of China. Dihydromyricetin (DMY) is the most abundant (approximately 30%) and bioactive constituent in *A. grossedentata* (Hand.-Mazz.) W.T. Wang, and recent studies have demonstrated its various pharmacological activities. In the present study, a first specific, sensitive, rapid and reliable LC–MS/MS method for the determination of DMY in rat plasma was developed and validated. The plasma samples were prepared with protein precipitation method, and chromatographic separation was performed on a Welch Ultimate XB-C18 column (50 × 2.1mm, 5 μm) using a gradient elution with water and acetonitrile. The mass spectrometry (MS) analysis was conducted in negative ionization mode with multiple reaction monitoring (MRM) transitions at *m/z* 319.1 → 192.8 for DMY and *m/z* 609.0 → 301.2 for rutin (IS). The plasma concentration profiles and pharmacokinetic parameters were analyzed after oral administration of dextroisomer and racemate DMY at the dose of 100 mg/kg in rats. The method validation was conducted over the calibration range of 10.0–5000 ng/ml with the intra- and inter-day precision and accuracy within 12.0% (RSD) and 5.6% (RE). The recoveries, matrix effect and stability under different conditions were all proved acceptable. The values of T_{max}, $AUC_{0-\infty}$ and Vd were significantly different between the groups of dextroisomer and racemate DMY ($P < 0.05$), and pharmacokinetic results revealed their poor absorptions into blood, probably high tissue distributions and slow elimination processes. The present study will provide helpful information for the further studies and future clinical applications of DMY.

1. Introduction

Ampelopsis grossedentata (Hand.-Mazz.) W.T. Wang, also known as vine tea, is a medicinal herb that distributed widely in southern provinces in the Yangtze River region of China. The tender stems and leaves of vine tea, have been widely utilized not only as a healthy tea, but also as a medicinal herb in traditional Chinese medicine to cure disease both by internal and external administration [1]. For hundreds of years, the Chinese indigenous people such as Tujia, Yao, Dong and Lahu have utilized vine tea for various disease treatments including sore throat, pharyngitis, cold fever and allergenic skin disease. And recent studies have demonstrated its pharmacological effects such as antioxidative, anti-inflammatory, antihypertensive, antiviral and hepatoprotective activities [2]. Dihydromyricetin (DMY), a dihydro flavonol compound found as the most abundant (approximately 30%) and bioactive constituent in vine tea, has been demonstrated to posses numerous pharmacological activities including anti-oxidant [3–5], anti-cancer [6–8], anti-inflammation [9], anti-hypertension [10], anti-fatigue [11], anti-alcoholic intoxication [12] and hepatoprotective effects [13].

Because of these beneficial pharmacological activities, it is of great significance to explore the pharmacokinetic properties of DMY. To the best of our knowledge, even though HPLC-DAD methods have been previously reported for the determination of DMY in rat plasma [14–15], no bioanalytical method with LC–MS/MS has been developed and validated for the quantitation of DMY in biological matrices. In contrast with HPLC-DAD, LC–MS/MS possesses obvious advantages of greater sensitivity, better specificity and more rapid for the biological analysis. In addition, the previous pharmacokinetic studies of DMY with HPLC-DAD methods ignored the fact that DMY possesses two kinds of enantiomers as shown in

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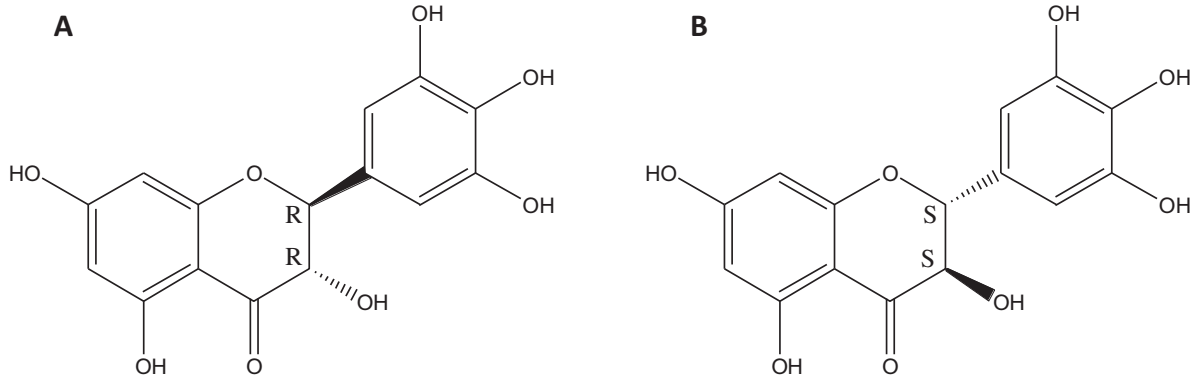

Fig. 1. Chemical structures of (A) dextroisomer DMY and (B) laevoisomer DMY.

Fig. 1. Recently, a method that prevented DMY from racemization during the extraction procedures was developed for the purpose of extracting highly purified home-made dextroisomer DMY from vine tea (patent number: 201410715828.5, State Intellectual Property Office of the P.R.C), while the purifying of laevoisomer DMY by preparative HPLC was unsuccessful due to its instability.

In the present study, a first, specific, sensitive, rapid and reliable LC–MS/MS method was developed and validated for the determination of DMY in rat plasma, and applied to the pharmacokinetic study after oral administration of dextroisomer and racemate DMY.

2. Materials and methods

2.1. Chemicals and reagents

Dextroisomer DMY was prepared in-house and racemate DMY was obtained from Microherb Inc. (Zhangjiajie, China), of which the purity was all greater than 99%. Rutin was purchased from the National Institutes for Food and Drug Control (Beijing, China). HPLC-grade acetonitrile and methanol were purchased from Fisher Scientific (Fairlawn, NJ, USA). Formic acid was obtained from Sinopharm Chemistry Reagent Co., Ltd (Shanghai, China). The deionized water was obtained with a Milli-Q® water purification system (Milford, MA, USA). K_2-EDTA and Vitamin C were both purchased from Ruite Biotechnology Co., Ltd (Guangzhou, China).

2.2. Instrumentation and LC–MS/MS conditions

The HPLC system consisted of a CBM-20A controller, a LC-20AD solvent delivery system, a SIL-20AC autosampler, a CTO-20AC column oven, a DGU-20A degasser from Shimadzu (Kyoto, Japan). The separation process was performed on a Ultimate XB-C18 column (50 × 2.1 mm, 5 μm) from Welch Materials Inc. (Shanghai, China). The mobile phase consisted of water and acetonitrile with a gradient elution of acetonitrile from 10% to 90% (0–2.00 min), 90% (2.00–3.00 min), from 90% to 10% (3.00–3.10 min) and 10% (3.10–3.60 min) with the flow rate of 0.8 min/ml. The temperature of column and autosampler was controlled at 40 °C and 4 °C, respectively.

An API 4000 Triple Quadrupole™ with Turboionspray® source (TIS) from AB SCIEX (Concord, Ontario, Canada) was utilized for the LC-MS/MS analysis with MRM in negative ionization mode. The optimization of MS parameters was accomplished as follows: TIS temperature 550 °C, ionspray voltage −4500 V, curtain gas 25, nebulizing gas 50, TIS gas 50, entrance potential −10 V, collsion cell exit potential −13 V, declustering potential (DP) −82 eV for DMY and −120 eV for IS, collision energy (CE) −15 eV for DMY and −42 eV for IS. The optimization of MS transitions were accomplished as DMY (m/z 319.1 → 192.8) and IS (m/z 609.0 → 301.2) with the dwell times of 100ms. And the MS detection time was limited within 2 min.

2.3. Sample preparation

2.3.1. Preparation of dextroisomer DMY

The preparation procedures of dextroisomer DMY have been described in the previous patent as mentioned above (patent number: 201410715828.5, State Intellectual Property Office of the P.R.C). Briefly, the stems and leaves of vine tea were ultrasonic extracted (1:10, w/v) with 95% ethanol (pH 4.6) in 60 min, and the filtrate was evaporated at 40 °C. Then the boiling water (conductivity of 2.7 μs/cm) was added to the dried powder followed by chilling and crystallization. The crystals were dissolved in the boiling water and treated with activated carbon, and the filtrate was chilled to obtain the crystals. The procedures of recrystallization were conducted several times until the crystals were light yellow. Then the filtrate was extracted with petroleum ether, and the aqueous layer was chilled and filtrated to obtain the final crystals with the optical purity greater than 99.0%.

2.3.2. Preparation of stock solutions, working solutions, calibration standards and QC samples

The stock solutions of DMY were prepared in methanol (containing 3% formic acid and 100 mM Vitamin C) to the concentration of 1.25 mg/ml. The stock solutions of IS were prepared in methanol to the concentration of 0.5 mg/mL. The desired concentration of working solutions for the preparation of calibration standards and QC samples were prepared with serial dilution in methanol–water (50:50, v/v) containing 3% formic acid and 100 mM Vitamin C. The IS working solution of 500 ng/mL was prepared by diluting the IS stock solution with methanol. All the stock and working solutions were properly kept at −20 °C.

Methanol containing 3% formic acid and 100 mM Vitamin C was added to the blank rat plasma (pretreated with K_2-EDTA) at the volume ratio of 1:25. And the treated blank plasma was properly stored at −20 °C.

Calibration standards were prepared by spiking working solutions with appropriate volume ratio into the treated plasma, to yield the concentrations of 10.0, 20.0, 50.0, 250, 500, 2500, 4000 and 5000 ng/mL. Likewise, LLOQ, LQC, MQC and HQC were prepared at the concentrations of 10.0, 30.0, 300 and 3750ng/mL. The spiked volume of working solutions into treated plasma was less than 5% of the final volume. All the calibration standards and QC samples were properly stored at −20 °C.

2.3.3. Plasma sample preparation

A volume of 50.0 μL of each calibration standards, QC samples, blank matrix samples and incurred samples was aliquoted into different tubes. And 50 μL of IS working solution was added to each tube except for the blank samples. Then 400 μL acetonitrile with 1% formic acid was added to each individual samples for the proteins