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Simultaneous determination of acetylpuerarin and puerarin in rat plasma by liquid chromatography–tandem mass spectrometry: Application to a pharmacokinetic study following intravenous and oral administration

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ABSTRACT

A rapid and sensitive liquid chromatography–tandem mass spectrometric (LC–MS/MS) method was developed and validated for the simultaneous determination of acetylpuerarin (AP) and its major metabolite puerarin (PUE) in rat plasma using genistein as the internal standard (IS). Plasma samples were pretreated by protein precipitation with a mixture of methanol and acetonitrile. Chromatographic separation was performed on a CAPCELL PAK C_{18} MGIII column with a mixture of 0.1% formic acid in water and methanol (35:65, v/v) as the mobile phase. The analytes were detected using a tandem mass spectrometer in the positive ionization and multiple-reaction monitoring mode. The ion transition of m/z 669.4 → 627.3, 417.5 → 297.6 and 271.3 → 153.0 was utilized to quantify AP, PUE and the IS, respectively. The calibration curves showed good linearity over the plasma concentration range of 1–2000 ng/mL for AP and 2.5–5000 ng/mL for PUE. The intra- and inter-day precisions (RSD %) for each analyte were less than 6.91%, and the accuracies ranged from −2.17% to 2.93%. The validated LC–MS/MS method was further successfully applied to a pharmacokinetic study of AP and PUE in rats following intravenous and oral administration.

1. Introduction

Cerebrovascular disease (CVD) is presently one of the leading causes of death and disability throughout the world. In China, stroke is the second leading cause of death and is a major cause of adult disability. Approximately 150 million new strokes occur each year [1]. However, lack of specific and effective drugs remain a great challenge in the treatment of CVD.

Puerarin (PUE, Fig. 1A), an isoflavonoid compound extracted from the Chinese medical herb *Radix puerariae*, is applied clinically in China for the treatment of cardiovascular diseases, such as angina and myocardial infarction [2]. Moreover, PUE has been shown to exert protective effects against cerebrovascular ischemia–reperfusion injury (CIRI) *in vivo* [3–5] and neuron damage induced by oxygen–glucose deprivation *in vitro* [6,7]. However, the clinical application of PUE is greatly limited because of its low oral bioavailability [8,9]. Acetylpuerarin (AP, Fig. 1B) is an acetylated derivative of PUE with higher liposolubility. Previous studies [10–12] have indicated that AP can protect against brain injury in animals, and the mechanisms underlying this protection may involve increases in antioxidase, reductions in the damage induced by oxygen-free radicals, decreases in the calcium concentration in neurons, inhibition of the apoptosis of nerve cells and prevention of the inflammatory response in the ischemic area [13–15]. However, the pharmacokinetics of AP remain unclear.

Many previous reports [16–18] described the determination of PUE in different biological matrix through high-performance liquid chromatography (HPLC) methods. HPLC provided a higher limit of detection, lower sensitivity and longer retention time and was not appropriate for the bio-analysis of PUE due to its poor oral bioavailability. Several recent studies [19,20] used liquid chromatography–tandem mass spectrometry (LC–MS/MS) to quantify PUE in biological samples, and these methods showed

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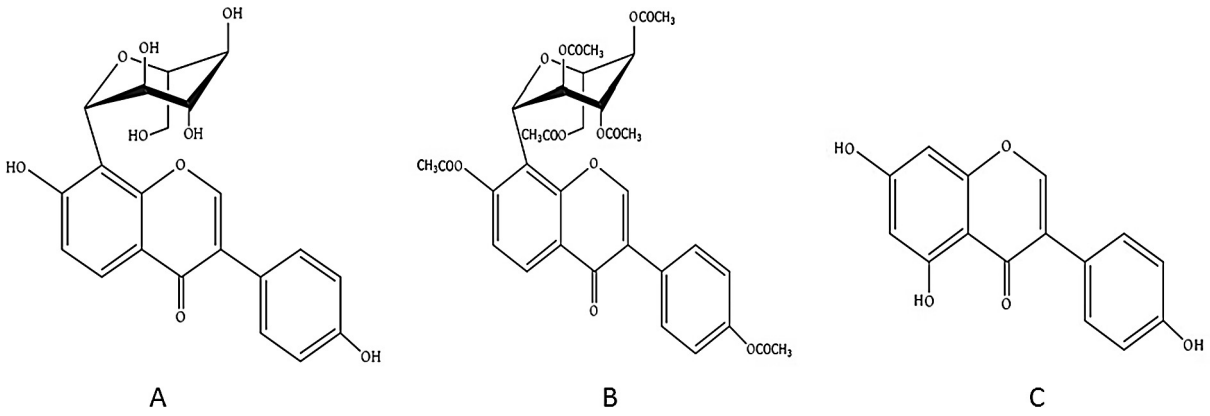

Fig. 1. Chemical structures of (A) puerarin, (B) acetylpuerarin and (C) genistein.

higher sensitivity. However, no previous publication has reported the quantification of AP in a biological matrix.

AP would be hydrolyzed to penta-acetylpuerarin, tetra-acetylpuerarin and PUE in rats *via* stepwise deacetylation, and PUE was found to be the major metabolite [21,22]. Xiang et al. [22] presented an HPLC method to quantify the metabolite PUE in rat plasma following intravenous and oral administration of AP formulations. In their study, the plasma samples were pretreated by liquid–liquid extraction and condensed by solvent evaporation and reconstitution. Accordingly, the procedure was tedious. Furthermore, a simple determination of the metabolite of AP is not sufficient for a pharmacokinetic study of AP. It is thus necessary to simultaneously determine levels of the unchanged drug and its metabolite in rat plasma.

LC–MS/MS is a favorable technology with high specificity and sensitivity as well as a high analytical throughput, and it has become the dominant tool for assaying compounds in complex biological matrix. Therefore, in this study, we aimed to develop a rapid and sensitive LC–MS/MS method for the simultaneous determination of AP and PUE in rat plasma.

The method developed was successfully applied to a pharmacokinetic study of AP and PUE following intravenous and oral administration of AP formulations.

2. Experimental

2.1. Chemicals and reagents

The puerarin standard (Batch no. 110752–200912) was purchased from the National Institutes for Food and Drug Control (Beijing, China). The acetylpuerarin sample (purity ≥ 98%) was a gift from the Shandong Academy of Medical Sciences (Jinan, China). Genistein (purity ≥ 98%, Fig. 1C), which used as an internal standard (IS), was supplied by the Shandong Academy of Sciences (Jinan, China). Chromatographic–grade methanol and acetonitrile were obtained from Fisher Company (USA). The ammonium formate used in this study was of analytical grade, and the water was deionized and double-distilled.

2.2. LC–MS/MS instrument and conditions

The HPLC Agilent 1100 Series (Agilent Technologies, USA) was equipped with the following units: a binary pump, a vacuum degasser, a thermostatted column compartment and an autosampler (140-vial capacity). Chromatographic separation was performed on a CAPCELL PAK C_{18} MGIII column (150 mm × 2.0 mm, 5 μm, SHISEIDO, Japan) by isocratic elution at a flow rate of 0.35 mL/min. The mobile phase consisted of a mixture of 0.1% formic acid in water (containing 5 mM ammonium formate) and methanol (35:65, v/v). The column temperature was maintained at 35 °C.

Mass spectrometric (MS) detection was performed using an API 4000 triple–quadrupole instrument (Applied Biosystems, USA) equipped with an electrospray ionization (ESI) source and set up in the positive ionization mode. The multiple-reaction monitoring (MRM) mode was used for the MS analysis, which was performed by monitoring the ion transitions at m/z 669.4 → 627.3 for AP, 417.5 → 297.6 for PUE and 271.3 → 153.0 for genistein (IS). The system control and data analysis were performed using the Analyst™ software (version 1.3).

2.3. Standard solutions, calibration standards and quality control samples

Stock solutions of AP and PUE were prepared by dissolving the accurately weighed standard compounds in methanol, and serially diluted working solutions were obtained through stepwise dilutions of the stock solution with methanol. The calibration standards were prepared by diluting the corresponding working solutions with blank rat plasma. The final plasma concentrations of the calibration standards were 1, 2, 10, 20, 100, 200, 1000 and 2000 ng/mL for AP and 2.5, 5, 25, 50, 250, 500, 2500 and 5000 ng/mL for PUE.

The IS working solution (100 ng/mL) was prepared in a mixture of methanol and acetonitrile (50:50, v/v). The quality control (QC) samples in plasma were prepared separately at three concentration levels of 2, 100, and 1600 ng/mL for AP and 5, 250, and 4000 ng/mL for PUE.

All of the stock solutions, working solutions, calibration standards and QC samples were stored at −20 °C until use.

2.4. Sample preparation

A plasma sample (50 μL) was pipetted into a micro-tube, and 25 μL of methanol and 150 μL of IS solution were then added to this tube. The mixture was vortexed for 1 min and centrifuged at 10,000 × g for 10 min. Then, 15 μL of the supernatant was injected for analysis.

2.5. Method validation

Method validation was performed according to the FDA Guidance for Industry on Bioanalytical Method Validation, and this process includes an evaluation of the selectivity, linearity, accuracy, precision, extraction recovery, matrix effect, carry-over effect and stability of the method.