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Short communication

A sensitive LC–MS/MS method for simultaneous determination of amygdalin and paeoniflorin in human plasma and its application

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ABSTRACT

A simple and sensitive HPLC–MS/MS method was developed and fully validated for the simultaneous determination of amygdalin (AD) and paeoniflorin (PF) in human plasma. For both analytes, the method exhibited high sensitivity (LLOQs of 0.6 ng/mL) by selecting the ammonium adduct ions ($[M+NH_4]^+$) as the precursor ions and good linearity over the concentration range of 0.6–2000 ng/mL with the correlation coefficients > 0.9972. The intra- and inter-day precision was lower than 10% in relation to relative standard deviation, while accuracy was within ±2.3% in terms of relative error for both analytes. The developed method was successfully applied to a pilot pharmacokinetic study of AD and PF in healthy volunteers after intravenous infusion administration of Huoxue-Tongluo lyophilized powder for injection.

1. Introduction

Huoxue-Tongluo lyophilized powder for injection (HTLPI), a simplified formulated Chinese medicine derived from the well-known traditional Chinese medicine prescription Tao-Hong-Si-Wu-Tang, is mainly made from the extracts of Persicae Semen and Paeoniae Radix Rubra and used for treating blood-stasis obstruction syndrome in the acute stage of cerebral ischemic stroke. HTLPI has passed the Phase I clinical trial (Chinese CFDA: 2004L01097) and its safety has been verified. The Phase II and III clinical trials are carrying out. Amygdalin (AD) and paeoniflorin (PF), the phytochemical markers for the quality control of Persicae Semen and Paeoniae Radix Rubra (Pharmacopeia of PR China, 2010), are regarded as the principal constituents responsible for the pharmacological activities [1–6] and selected as the indicators in the pharmacokinetic study of HTLPI.

Various chromatographic methods have been published for the determination of AD or PF in animal biological fluids using HPLC-UV [7–9], LC–MS [10,11], LC–MS/MS [12–15], UPLC–MS/MS [16]. However, to the best of our knowledge, there is presently no report on the determination of AD or PF in human plasma for the clinical pharmacokinetic study. In the present investigation, a simple and highly sensitive (LLOQs of 0.6 ng/mL) LC–MS/MS method was developed and fully validated for simultaneous determination of AD and PF in human plasma for the first time. This method was successfully applied to a pilot clinical pharmacokinetic study of AD and PF after intravenous infusion administration of HTLPI to healthy Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Huoxue-Tongluo lyophilized powders for injection (6 g, containing 25.3 mg of amygdalin and 35.8 mg of paeoniflorin) were supplied by Jiangsu Kanion Pharmaceutical Co., Ltd. (Lianyungang, China). Amygdalin (AD, purity 93.6%), paeoniflorin (PF, purity 96.5%) and geniposide (IS, purity 99.7%) were purchased from

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National Institutes for Food and Drug Control (Beijing, China). Methanol and acetonitrile were HPLC grade and purchased from Merck KGaA (Darmstadt, Germany). Ammonium acetate and formic acid (analytical grade) were purchased from Nanjing Chemical Reagents Co., Ltd. (Nanjing, China). Water was prepared with double distillation.

2.2. LC–MS/MS instruments and conditions

The liquid chromatography was performed on an Agilent 1260 Series liquid chromatography (Agilent Technologies, Palo Alto, CA, USA), which comprised an Agilent 1260 binary pump (model G1312B), a vacuum degasser (model G4225A), an autosampler (model G1367E) and an Agilent 1290 temperature controlled column compartment (model G1330B). Chromatographic separation was carried out on a 38 °C Hedera ODS-2 analytical column (2.1 mm × 150 mm, 5 μm; Hanbon Science and Technology, Huai'an, China) with a security Guard-C_{18} column (4 mm × 2.0 mm, 5 μm; Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and 5 mM ammonium acetate buffer solution containing 0.05% formic acid (20:80, v/v) at a flow rate of 300 μL/min. Autosampler temperature was maintained at 6 °C and the injection volume was 10 μL. A 10-port switching valve (Valco, Houston, TX, USA) was used to direct flow eluate to a waste container in the first 1.5 min of the chromatographic run and afterwards to the mass spectrometer. The total run time was 4.5 min.

Mass spectrometric detection was performed on an API 4000 tandem mass spectrometer (Applied Biosystems, Toronto, Canada) equipped with a Turbo-V® ionspray source operation in the positive ESI mode. Quantification was performed using multiple reaction monitoring (MRM) of the transitions of m/z 475.2 → 163.1 for AD, m/z 498.2 → 179.1 for PF and m/z 406.2 → 227.1 for IS, respectively (see Fig. 1). The ion spray temperature and ion spray voltage were maintained at 400 °C and 5500 V, with nebulizer gas (GS1) and heater gas (GS2) set at 70 and 70 psi, respectively. The curtain gas (CUR) was kept at 30 psi and the collision gas (CAD) was 6 psi. The specific parameters for each analyte are displayed in Table 1. The system control and data analysis were performed by AB Sciex Analyst software (version 1.5.2).

2.3. Preparation of calibration standards and quality control samples

The mixture standard stock solution of AD and PF was prepared in methanol at the concentration of 1 mg/mL. Standard working solutions ranged from 0.006 to 20 μg/mL were prepared by diluting the stock solution with methanol for both analytes. Calibration standards were prepared using blank human plasma spiked with 20 μL of mixture working solutions, to yield the concentrations of 0.6, 2, 6, 20, 60, 200, 600, 1200 and 2000 ng/mL for both analytes, respectively. QC samples were prepared in the same way as the calibration samples at three concentration levels: 1.5, 100 and 1500 ng/mL for both analytes. The stock solution (1 mg/mL) of the IS was prepared in methanol and was further diluted with methanol to 1 μg/mL. All the solutions were kept at −20 °C and brought to room temperature before use.

2.4. Sample preparation

All samples were stored in a freezer at −20 °C and allowed to thaw at room temperature by vortex before processing. A 200 μL of plasma sample was mixed with 20 μL IS solution (1 μg/mL). The mixture was deproteinized with 600 μL of methanol, vortexed for 5 min, and then centrifuged at 15,600 rpm for 10 min. An aliquot of 0.2 mL supernatant was diluted by adding 0.2 mL of water. After

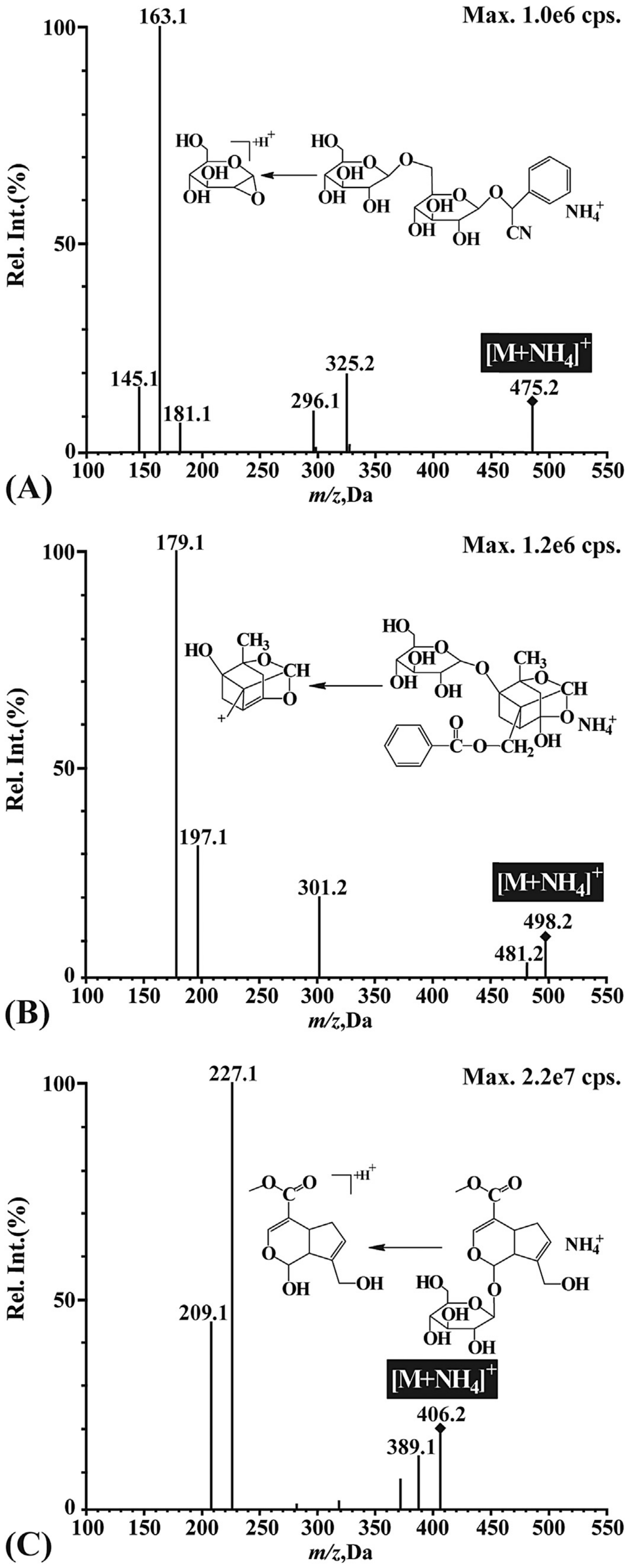

Fig. 1. Positive product ion mass spectra of $[M+NH_4]^+$ of AD (A), PF (B) and IS (C) and their proposed fragmentation patterns.