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Simultaneous determination of ten active constituents of Yankening Capsule in rat plasma by ultra high performance liquid chromatography with tandem mass spectrometry

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ABSTRACT

An ultra high performance liquid chromatography with tandem mass spectrometry (U-HPLC–MS/MS) method was developed for simultaneous determination and pharmacokinetic study of ten active constituents, phellodendrine, coptisine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and emodin in rat plasma after oral administration of Yankening Capsule. After mixing with two internal standards tetrahydropalmatine and rutin, plasma samples were pretreated by protein precipitation with anhydrous ethanol–acetonitrile (9:1, v/v). The U-HPLC separation was carried on a ZORBAX RRHD Eclipse Plus C_{18} column (2.1 mm × 50 mm, 1.8 μm) with gradient elution using a mobile phase composed of methanol and water (containing 0.3% formic acid) at a flow rate of 0.3 mL min^{-1}. The detection was performed on a triple quadrupole tandem mass spectrometer by multiple reaction monitoring via electrospray ionization source with positive–negative ionization mode. The calibration curves of ten analytes showed good linearity ($r > 0.9979$). The lower limits of quantification of phellodendrine, coptisine, jatrorrhizine, berberine, palmatine, baicalin, wogonoside, baicalein, wogonin and emodin were 0.50, 0.50, 0.30, 0.30, 0.30, 10, 3.0, 8.0, 1.0, 8.0 μg L^{-1}, respectively. The relative standard deviation of intra-day precision and inter-day precision were in the range from 1.13% to 5.96% and from 0.65% to 8.85%, respectively. The matrix effects of all analytes were found to be within the acceptable range with a range of 89.99–109.3%. The method is reliable and rapid and has been applied successfully to pharmacokinetic study of the ten active constituents in rat plasma after oral administration of Yankening Capsule.

1. Introduction

Traditional Chinese Medicine (TCM) has been widely used in China for thousands of years. Yankening Capsule, composed of five herbs including *Cortex phellodendri*, *Rheum officinale*, *Radix scutellariae*, *Radix isatidis* and *Rhizoma coptidis*, is a classic prescription of traditional Chinese medicine [1]. Yankening Capsule, that possessed the effect of clearing away the heat evil in the pericardium and anti-inflammatory, is commonly used to treat acute tonsillitis, bacterial pneumonia, acute conjunctivitis, otitis media, furuncle carbuncle scrofula, acute mastitis, enteritis, bacterial diarrhea and acute urinary tract infections, as described in the Pharmacopoeia of People's Republic of China [1]. Previous study demonstrated that *C. phellodendri* has anti-bacterial, anti-diarrheal and anti-inflammatory effects [2–4]. *R. coptidis* exhibited anti-bacterial, anti-virus and anti-cancer activities [5–7]. Because of bioactive alkaloids, both *C. phellodendri* and *R. coptidis* have been widely used as an anti-inflammatory drug in the treatment of diseases, Phellodendrine is known as the main effective constituents of *C. phellodendri*. In addition to phellodendrine, four other bioactive alkaloids, coptisine, jatrorrhizine, berberine and palmatine, are also present in *R. Coptidis* and *C. phellodendri* [8,9]. Both *R. scutellariae* and *R. officinale* own the efficacy of cooling blood and detoxification [10]. Flavonoids are the main bioactive constituents of *R. Scutellariae*, such as baicalin, wogonoside, baicalein and wogonin [11,12], while emodin, aloe-emodin and rhein are the main active constituents of *R. officinale* [13,14].

Pharmacokinetic studies are useful to explain and predict a valuable of events related to the efficacy and toxicity of drugs. Therefore it is valuable to perform pharmacokinetic studies for evaluating the rationality and compatibility of herbs or prescriptions in general, which have influences on the clinical effects of

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TCM and its rational dosage regiments. So far, *C. phellodendri*, *R. coptidis*, *R. scutellariae* and *R. officinale* as common herbs used in TCM, there are many pharmacokinetic researches about the active constituents in rats [15–18]. Pharmacokinetic study of the five alkaloids (phellodendrine, coptisine, jatrorrhizine, berberine, palmatine) has been reported in different TCM, such as Wuji Pill [19], Er Miao San [20], Huang-Lian-Jie-Du-Tang [21], Fuzi Xiexin Tang [22] and Tang-Min-Ling-Pill [23]. Furthermore, several analytical techniques, such as high performance liquid chromatography coupled with UV [24], diode array and evaporative light scatting detectors [25], fluorescence detection [26] have been reported to quantify active ingredients mentioned above, as well as high-performance capillary electrophoresis [27] techniques. However these methods were insufficient sensitivity or specificity for pharmacokinetic research.

The chemical structures of phellodendrine, coptisine, jatrorrhizine, berberine and palmatine are very similar, as well as the chemical structures of baicalin, wogonoside, baicalein and wogonin, while emodin is anthraquinones compound (Fig. 1). Because of their biological significance, these compounds can be used as quality chemical markers of the Medicine (Yankening Capsule). Up to now rare reports have been seen on pharmacological studies of Yankening Capsule. So it is urgent to establish a sensitive and rapid method for simultaneous determination of the active constituents and to conduct the pharmacokinetic research of multiple active constituents after oral administration of Yankening Capsule. In this paper, a selective and sensitive method is presented for simultaneous determination of the 10 main active constituents in rat plasma by ultra performance liquid chromatography with tandem mass spectrometry (U-HPLC–MS/MS) and pharmacokinetic study after oral administration of Yankening Capsule.

2. Experimental

2.1. Chemicals and reagents

The standard substances of phellodendrine, coptisine, jatrorrhizine, berberine, palmatine, emodin and internal standards of tetrahydropalmatine and rutin with a purity of over 98% were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Standard substances of baicalin, wogonoside, baicalein, wogonin with a purity of over 98% were purchased from Sichuan Weikeqi Bio-tech Co., Ltd. (Sichuan, China). The Yankening Capsule (Batch No.: ZLA1305) was supplied by Yunnan Baiyao Group Co., Ltd. (Yunnan, China).

HPLC grade acetonitrile, methanol and formic acid used for U-HPLC–MS/MS measurement were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Ultra-pure water used throughout the experiment was obtained from a Milli-Q water purification system (Millipore Corporation, Billerica, USA). Anhydrous ethanol and other chemicals were all analytical reagents.

The blank rat plasma was obtained by drawing blood from venous plexus of eyes of rats to a heparinized tube. Plasma was separated by centrifugation at 1672 × *g* for 20 min and then stored at −20 °C.

2.2. Animals

Sprague-Dawley (SD) rats, weighing 280 ± 20 g (certificate NO. SCXK (Guang Xi) 2009–0002), were provided by the Animal Center of Guangxi Medical University. The experimental protocols were approved by Laboratory Animal Care Committee of Guangxi Department of Science and Technology. The rats were raised in an air-conditioned room at temperature of 23 ± 2 °C and a relative humidity of 50 ± 10% with a 12 h dark–light cycle and allowed food and water spontaneously. They were acclimatized to the facilities for 3 days, and then the rats fasted only with free access to water 12 h before the experiments. The experiments were carried out in accordance with the guidelines issued by the Animal Ethical Committee of Guangxi Medical University.

2.3. Instrument and chromatographic conditions

2.3.1. Chromatographic conditions

Chromatographic analysis was performed on an Agilent 1290 U-HPLC system (Agilent Co., USA), consisting of a binary pump solvent management system, an online degasser, and an autosampler. Separation was carried on a ZORBAX RRHD Eclipse Plus C_{18} column (2.1 mm × 50 mm, 1.8 μm) at room temperature. The mobile phase was composed of A (0.3% formic acid aqueous, v/v) and B (methanol). A linear gradient elution at a flow rate of 0.3 mL min^{-1} was run at 30–32% B over 0–8 min, 50–64% B over 8.1–13 min, and the composition was maintained at 100% B for 5 min and then returned to initial condition. All analytes were eluted rapidly within 14 min. A volume of 1 μL of sample solution or standard solution was injected into system for analysis.

2.3.2. Mass spectrometric condition

Mass spectrometric detection was carried out on triple quadrupole 6460 mass spectrometer (Agilent Co., USA) equipped with an electrospray ionization source (ESI) with the spray voltage set at 4000 V. Nitrogen was used as nebulizer gas and nebulizer pressure was set at 2.8×10^5 Pa. Desolvation gas (nitrogen) was heated to 300 °C and delivered at a flow rate of 10 L min^{-1}. For collision-induced dissociation (CID), high purity nitrogen was used as collision gas. The temperature of sheath gas (nitrogen) was 360 °C with the flow rate of 12 L min^{-1}. Capillary voltage and nozzle voltage was set at 4000 V (ESI^+) or 3000 V (ESI^-) and 0 V, respectively. ESI–MS/MS was operated in positive mode for the analysis of alkaloids and flavonoids (ESI^+), in negative mode for emodin (ESI^-). Multiple reaction monitoring (MRM) was used to perform mass spectrometric quantification. The MRM analysis was conducted by monitoring the precursor ion to product ion transitions of *m/z* 342.1–265.1 for phellodendrine, *m/z* 320.1–292.1 for coptisine, *m/z* 338.1–322.1 for jatrorrhizine, *m/z* 336.1–320.1 for berberine, *m/z* 352.1–336.1 for palmatine, *m/z* 447.1–271.0 for baicalin, *m/z* 461.0–285.0 for wogonoside, *m/z* 271.1–123.0 for baicalein, *m/z* 285.1–270.1 for wogonin, *m/z* 269.1–225.1 for emodin, *m/z* 356.1–192.1 for tetrahydropalmatine (IS) and *m/z* 611.2–302.9 for rutin (IS), respectively (Fig. 2). Table 1 shows the optimized MRM parameters for the analytes and IS.

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

Stock solutions for phellodendrine (1.0 mg mL^{-1}), coptisine (0.2 mg mL^{-1}), jatrorrhizine (1.0 mg mL^{-1}), berberine (1.0 mg mL^{-1}), palmatine (1.0 mg mL^{-1}), baicalin (1.0 mg mL^{-1}), wogonoside (0.25 mg mL^{-1}), baicalein (1.0 mg mL^{-1}), wogonin (1.0 mg mL^{-1}), emodin (1.0 mg mL^{-1}), tetrahydropalmatine (1.0 mg mL^{-1}, IS) and rutin (1.0 mg mL^{-1}, IS) were separately prepared by dissolving the accurately weighed standard reference compounds in methanol. All standard solutions were stored at 4 °C in refrigerator. For calibration standards, stock solutions were diluted with methanol to produce standard working solutions at concentrations of 0.5, 4, 30, 200, 400, 800, 1500 and 7000 μg L^{-1} for phellodendrine, 0.5, 4, 30, 200, 400, 800, 3000 and 4000 μg L^{-1} for coptisine, 0.3, 3, 30, 200, 400, 800, 1500 and 4000 μg L^{-1} for jatrorrhizine and palmatine, 0.3, 3, 30, 200, 400, 800, 1500 and 3000 μg L^{-1} for berberine, 10, 50, 100, 200, 400, 800, 1500 and 6000 μg·L^{-1} for baicalin, 3, 20, 100, 400, 800, 1500, 3000