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Simultaneous determination of ten bioactive constituents of Sanjie Zhentong Capsule in rat plasma by ultra-high-performance liquid chromatography tandem mass spectrometry and its application to a pharmacokinetic study

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ABSTRACT

Sanjie Zhentong capsule, a well-known traditional Chinese medicine prescription, are used for the treatment of endometriosis-related diseases. In this study, a simple, rapid and sensitive ultra-high-performance liquid chromatography-tandem mass spectrometry (UHPLC–MS/MS) method was developed for the simultaneous determination of ten bioactive constituents, including peimine, peiminine, peimisine, loureirin A, loureirin B, 7,4′-dihydroxyflavone, pterostilbene, ginsenoside Rg1, ginsenoside Rb1, and notoginsenoside R1 in rat plasma after oral administration of Sanjie Zhentong capsule. The sample preparations for protein removal was accomplished using a simple methanol precipitation method. The analytes were completely separated from the endogenous compounds on an Agilent Poroshell 120 SB-C18 column (4.6 mm × 150 mm, 2.7 μm) using an isocratic elution with methanol – 0.1% formic acid aqueous (4/1, v/v) as a mobile phase. The single-run analysis time was as short as 14.0 min. The inter-day and intra-day precision of the quality control samples exhibited relative standard deviations (RSD) <9.5% and the accuracy values ranged from −8.6% to 15.0%. The lower limits of quantification (LLOQ) were 10, 10, 10, 10, 10, 10, 5, 10, 10 and 20 ng/mL for peimine, peiminine, peimisine, loureirin A, loureirin B, 7,4′-dihydroxyflavone, pterostilbene, ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1, respectively. The analytical method was successfully applied to a pharmacokinetic study of the multi-components after oral administration of Sanjie Zhentong Capsule in rats.

1. Introduction

Dysmenorrhea is the most common gynaecological disorder in young women and usually begins during or just before the menstrual period [1,2]. Dysmenorrhea is presumed to be caused by an excess of prostanoids, and possibly other eicosanoids, released from the endometrium during menstruation [3]. The standard treatment for dysmenorrhea is nonsteroidal anti-inflammatory drugs (NSAIDs) or oral contraceptives (OCs) [4,5]. Traditional Chinese medicine prescription, prepared with a specific combination of different herbs as a formula, may consist of multiple components which are responsible for the medicinal therapeutic efficacy by synergistic or antagonistic interaction [6,7]. Sanjie Zhentong Capsule (SZC), as a well-known traditional Chinese medicine prescription (TCMP), has special clinical efficacy in the treatment of gynaecological problems such as oophoritic cyst, endometriosis, hysteromyoma, and especially dysmenorrhea in traditional Chinese medicines (TCM) [8]. The prescription consists of four botanical drug materials, including *Notoginseng Radix Et Rhizoma*, *Fritillaria Thunbergii Bulbus*, *Coicis Semen*, and *Dragon's Blood*. The

Abbreviations: IS, internal standard; MRM, multiple reaction monitoring mode; SD, Sprague Dawley; SIM, selected ion monitoring; SZC, Sanjie Zhentong Capsule; TCMP, traditional Chinese medicine prescription.

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majority of prescriptions of TCM are compound preparation, which exert therapeutic efficacies through multiple components on multiple targets. The study of pharmacokinetic parameters of SZC will be helpful to evaluate the efficacy and safety in clinical application.

To our knowledge, the studies about determination of bioactive compounds in SZC were not reported in vivo. Many pharmacokinetic studies on the active components from the extracts of *Notoginseng Radix Et Rhizoma* as well as their individual compounds have been investigated [9–11]. However, only a few studies focused on other ingredients deriving from *Fritillaria Thunbergii Bulbus* [12] and *Dragon's Blood* [13] in biological samples. As we all know, one or some ingredients in single herb are not enough to represent the pharmacokinetic profiles of the whole compound prescriptions in vivo. We screened out ten compounds as bioactive ingredients according to previous investigations to explore their pharmacokinetic profiles in rat plasma after oral administration, including the ginsenosides, e.g. ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1 (deriving from *Notoginseng Radix Et Rhizoma*), peimine, peiminine, peimisine (deriving from *Fritillaria Thunbergii Bulbus*), loureirin A, loureirin B, 7,4′-dihydroxyflavone and pterostilbene (deriving from *Dragon's Blood*) [9–19]. And the pharmacokinetic studies of constituents of pterostilbene and 7,4′-dihydroxyflavone were firstly reported in vivo.

LC–MS is highly selective method in selected ion monitoring (SIM) and in multiple reaction monitoring mode (MRM). In order to achieve good sensitivity and selectivity, liquid chromatography via an electrospray ionization interface has been employed in pharmacokinetic study. In this study, we developed a selective and sensitive LC–MS/MS methodology for the simultaneous determination of ten bioactive constituents, including peimine, peiminine, peimisine, loureirin A, loureirin B, 7,4′-dihydroxyflavone, pterostilbene, ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1 in rat plasma for the first time. The validated method was successfully applied to pharmacokinetic study of SZC after oral administration.

2. Experimental

2.1. Materials and reagents

The reference standards of peimine, peiminine, peimisine, loureirin A, ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1 and wogonin (internal standard) were purchased from the National Institute for Control of Pharmaceutical and Biological Products (Beijing, China) (purity >98.0%). The reference standards of loureirin B, 7,4′-dihydroxyflavone and pterostilbene were obtained from Kaimi Co. Ltd. (Shanghai, China) (purity >98.0%). Sanjie Zhentong Capsules (Batch number: 140906) were supplied by Kanion Co., Ltd. (Jiangsu, China). HPLC-grade methanol and acetonitrile were purchased from Merck Millipore (Darmstadt, GER). Formic acid was purchased from TEDIA Co. (Fairfield, USA). Deionized water was prepared by passing distilled water through a Milli-Q system (Millipore, Milford, USA).

2.2. Chromatographic conditions

Liquid chromatography was performed on a Shimadzu Series 30AD UHPLC system (Kyoto, Japan) consisting of a binary pump solvent management system, an online degasser, and an autosampler. Chromatographic separation was performed under an Agilent Poroshell 120 SB-C18 column (4.6 mm × 150 mm, 2.7 μm) using an isocratic elution with methanol – 0.1% formic acid aqueous (4:1, v/v) as mobile phase and maintained at 40 °C. The flow rate was 0.4 mL/min. The autosampler was conditioned at 4 °C and the sample volume injected was 5 μL. Injection wash solvents were methanol–water (10:90, v/v) and methanol–water (90:10, v/v) for weak and strong wash, respectively.

Table 1
List of the analytes and the Corresponding MRM Parameters.

Analyte	Precursor	Production	CE	DP	Ret. time
Ginsenoside Rg1	823.7 $[M+Na]^+$	643.4	49	157	4.0
Ginsenoside Rb1	1131.6 $[M+Na]^+$	365.5	84	185	6.0
Notoginsenoside R1	955.5 $[M+Na]^+$	775.5	57	176	3.8
Pterostilbene	255.1 $[M+H]^+$	196.9	39.6	86.9	6.2
Peimine	432.2 $[M+H]^+$	414.4	46	107	2.8
Peiminine	430.2 $[M+H]^+$	412.2	47	129	2.8
Peimisine	428.2 $[M+H]^+$	114.2	45	132	2.8
Loureirin A	285.1 $[M-H]^-$	133.9	−37	−109	5.5
Loureirin B	315.1 $[M-H]^-$	133.9	−37	−101	5.5
7,4′-dihydroxyflavone	253.1 $[M-H]^-$	116.8	−33	−100	8.6

2.3. Mass spectrometric conditions

Mass analysis was carried out with an Applied Biosystems API 4000+ triple quadrupole mass spectrometer with electrospray ionization interface source (Foster City, USA) operating in the positive and negative ionization mode. The operation conditions were as follows: ion spray voltage, 5.5 kV; curtain gas, 35 psi; ion source gas 1, gas 2 both at 50 psi; source temperature at 500 °C. The optimal MRM parameters were conducted using multiple reaction monitoring, and summarized in Table 1.

2.4. Standard solutions preparation

Stock standard solutions of peimine, peiminine, peimisine, loureirin A, loureirin B, 7,4′-dihydroxyflavone, pterostilbene, ginsenoside Rg1, ginsenoside Rb1, notoginsenoside R1 and wogonin (IS) were prepared by dissolving the accurately weighed the standard reference compounds in methanol, respectively. A mixed stock solution was obtained by mixing all the stock solutions above, and given a final concentration of 40 μg/mL.

The calibration standard solutions of the analytes were prepared by serially diluting the mixed stock solution with methanol to provide six standards of desired concentration. And the IS solution of wogonin was diluted with methanol to give a concentration of 200 ng/mL. All the solutions were stored at −20 °C and brought to room temperature before use. The low, middle and high concentration of quality control (QC) standard work solutions were prepared in the same manner, as shown in Table 3.

2.5. Sample preparation

After thawing at room temperature, 50 μL of rat plasma samples or spiked standard or quality control plasma samples were transferred to 1.5 mL centrifuge tubes. 25 μL of the IS solution (200 ng/mL) was added and the tubes were vortex mixed for 1 min. The protein in the plasma samples was precipitated by 200 μL methanol. The samples were then vortexed for 2.0 min, and then centrifuged at 12,000 rpm for 10 min. The supernatant was evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted with 100 μL of methanol-water (4:1, v/v), then vortexed for 3 min and centrifuged at 12,000 rpm for 5 min prior to analysis by LC–MS/MS.

2.6. Method validation

2.6.1. Selectivity

The selectivity was assessed by comparing the chromatograms of six different batches of blank rat plasma with the plasma samples spiked with the analytes and IS at the concentration of lower limit