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Short communication

Simultaneous determination of scopoletin, psoralen, bergapten, xanthotoxin, columbianetin acetate, imperatorin, osthole and isoimperatorin in rat plasma by LC–MS/MS for pharmacokinetic studies following oral administration of Radix Angelicae Pubescentis extract

Yan-xu Chang[a,b,*], Qiu-Hong Zhang[a,b], Jin Li[a], Ling Zhang[a,b], Xin-rong Guo[a,b], Jun He[a,b], Peng Zhang[a,b], Lin Ma[a], Yan-ru Deng[a], Bo-li Zhang[a], Xiu-mei Gao[a,*]

[a] Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China
[b] Tianjin Key Laboratory of Phytochemistry and Pharmaceutical Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China

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ABSTRACT

A rapid and sensitive bioassay based on liquid chromatography tandem mass spectrometry (LC–MS/MS) has been developed and validated for the simultaneous determination of eight coumarins in rat plasma. The liquid–liquid extraction method with ethyl acetate was used to prepare the plasma samples after addition of warfarin as an internal standard (IS). Chromatographic separation was performed on an Eclipse plus C18 column (100 mm × 4.6 mm, 1.8 μm) using gradient elution when 1 mM ammonium acetate aqueous solution – acetonitrile was used as the mobile phase. The lower limit of quantitation (LLOQ) of each coumarin was lower than 2.16 ng mL^{-1}.

Intra-day and inter-day precisions were less than 15%. The accuracies were in the range of 88.9–117%. The mean recoveries of coumarins and IS were higher than 84%. The method was successfully applied to a pharmacokinetic study of eight coumarins in rats after oral administration of radix angelicae pubescentis.

1. Introduction

Radix Angelicae Pubescentis (RAP), one of the most commonly used traditional Chinese medicines (TCM), has been widely used to treat rheumatic disease for centuries in China. Previous findings from pharmacological studies suggested that it possessed anti-inflammatory and analgesic activities, and inhibitory effects on 5-lipoxygenase and cyclooxygenase [1].

Coumarins have attracted considerable interest due to their multi-potential health benefits [2]. At present, more than 60 coumarins have been isolated and identified from RAP [3]. Among them, it has been shown that osthole had anti-inflammatory, anti-osteoporosis, anti-hepatitis, anti-allergenic, anticancer and neuroprotective effects [4]; scopoletin possessed anti-inflammatory, anti-proliferative and antioxidant effects [5]; psoralen had anticancer, immunomodulatory and estrogenic properties [6]; columbianetin acetate and imperatorin have been shown to possess the gamma-aminobutyric acid (GABA) receptor modulating activity [7]. In addition, bergapten could also exert anti-proliferative effect and induce apoptotic responses in breast cancer cells [8]. The above studies suggested that coumarins were important constituents contributing to the pharmacological efficacy of RAP. Therefore, it would be useful and informative to characterize the pharmacokinetics of coumarins for assessing fully the pharmacological values of RAP.

Several analytical methods for assessing coumarin in biological fluids have been previously reported [9]. In these studies, the LC–MS methods were used to determine coumarins in rat plasma, urine and bile fluid after oral administration of various TCM extract, but not RAP [10,11]. For the purpose of studying the mechanism of action of RAP, it is necessary to develop a quantitative method for detecting multiple coumarins in biological samples.

In this paper, a sensitive and selective LC–MS/MS method was developed for simultaneous determination of eight coumarins in rat plasma after oral administration of the RAP extract. To our knowledge, it is the first study with detailed pharmacokinetic characterizations of coumarins in RAP extract.

* Corresponding authors at: Tianjin State Key Laboratory of Modern Chinese Medicine, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, China. Tel.: +86 22 59596163; fax: +86 22 59596163.
E-mail addresses: tcmcyx@126.com (Y.-x. Chang), gaoxiumei@tjutcm.edu.cn (X.-m. Gao).

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Table 1
Mass transitions and MS/MS settings for scopoletin, psoralen, bergapten, xanthotoxin, columbianetin acetate, imperatorin, osthole and isoimperatorin.

Compounds	Q1	Q3	Dwell time (ms)	DP (V)	EP (V)	CE (V)	CXP (V)
Scopoletin	193.2	133.0	100	55	9	29	3
Psoralen	187.2	131.1	100	55	6	32	2.5
Bergapten	217.0	202.3	100	55	6	31	18
Xanthotoxin	217.0	202.1	100	58	4	28	16
IS (wafarin)	309.2	163.2	100	55	5	21	14
Columbianetin acetate	289.3	229.1	100	37	3	11	20
Imperatorin	271.3	203.1	100	30	4	16	17
Osthole	245.2	189	100	32	5	16	15
Isoimperatorin	271.2	203.1	100	36	4	17	17

2. Materials and methods

2.1. Chemicals and reagents

Acetonitrile (Fisher technologies Inc., USA) and methanol (Tianjin concord Science Co. Ltd., Tianjin, China) were of HPLC grade. Isoimperatorin, xanthotoxin, psoralen, scopoletin, imperatorin and warfarin (purity > 98%) were purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Osthole, columbianetin acetate and bergapten were isolated previously from the dried RAP in our laboratory. Their structures were confirmed by H NMR, IR, HPLC and MS spectra. The purities were more than 98%. Ammonium acetate was purchased from Tianjin Guangfu Fine Chemicals Inst. (Tianjin, China). Deionized water was purified with a Milli-Q Academic ultra-pure water system (Millipore, Milford, MA, USA).

2.2. Preparation of stock and working solution

A stock solution of each coumarin was prepared in methanol at an appropriate concentration respectively. Internal standard warfarin (IS) solution was prepared in methanol at a concentration of 1000 ng mL^{-1}. Appropriate aliquots of each of the coumarin stock solutions were taken, calculated and mixed together to prepare a mixed stock solution. The mixed stock solution was then diluted with the drug-free rat plasma to span a calibration standard range. All the solutions were stored at 4 °C and brought to room temperature before use.

2.3. Apparatus and chromatographic conditions

The LC–MS/MS equipment included an Agilent 1200 system (Agilent Corp., USA) and an API 3200 triple quadrupole mass spectrometer (Concord, Ontario, Canada). Data acquisition was performed with Analyst 1.4.2 software (AB MDS Sciex). The chromatographic separation was achieved on an Eclipse plus C18 (4.6 mm × 100 mm, 1.8 μm) column with a security guard C18 (2.1 mm × 12.5 mm, 5 μm) column. A linear gradient elution of eluents A (1 mM aqueous ammonium acetate) and B (acetonitrile) was used for the separation, using a B gradient elution of 40% at 0–5 min, 40–70% at 5–10 min, 70–75% at 10–11 min, 75–90% at 11–19 min, 90% at 19–23 min, 90–40% at 23–24 min, and the re-equilibration time of gradient elution was 5 min. The flow rate was set at 0.300 mL min^{-1}. The column oven temperature was set at 20 °C. The injection volume was 5 μL.

The components were detected using electrospray ionization (ESI) in positive ionization mode and quantified by multiple-reaction monitoring (MRM) mode. Mass spectrometry was operated with an optimized spray voltage at +5500 V, turbo spray temperature at 400 °C and curtain gas at 25 psi. The collision gas, nebulizer gas (gas 1) and auxiliary gas (gas 2) were at 5, 6 and 40 psi, respectively. The precursor-to-product ion pairs, declustering potential (DP), collision energy (CE), collision cell exit potential (CXP) and entrance potential (EP) for each coumarin are described in Table 1.

2.4. Preparation of samples and quality control samples

The dried RAP (1 kg) was extracted twice with 95% ethanol of 6 L for 2 h each time. The solution was concentrated to dryness under reduced pressure. To a 100 μL aliquot of rat plasma in a 1.5 mL eppendorf tube, 10 μL IS (1000 ng mL^{-1}) and 1000 μL ethyl acetate were added. Then, the samples were vortexed for 1 min and centrifuged at 14,000 rpm for 10 min at 4 °C. The supernatant fluid was transferred to another 1.5 mL tube, and evaporated to dryness under a constant flow of nitrogen. The residue was dissolved in 100 μL methanol. It was centrifuged at 14,000 rpm for 10 min. 5 μL aliquot of the solution was injected into the LC–MS/MS system for analysis.

Quality control (QC) samples at high, medium and low concentrations were made by spiking appropriate standard solutions into blank rat plasma with the required plasma concentrations following the same sample preparation and operation method described above.

2.5. Method validation

The method was validated in terms of specificity, lower limit of quantification (LLOQ), linearity, accuracy and precision, recovery, matrix effect and stability according to the USFDA guidelines and literature [12]. Short-term stability was evaluated by assessing QC samples after 24 h at room temperature. Freeze–thaw stability was checked after three cycles. Long-term stability was determined by assessing QC samples which had been stored at −20 °C for 30 days. All stability was expressed as the mean of percentage remains.

2.6. Pharmacokinetic study

Twelve male Sprague-Dawley rats (220–240 g) were housed in a cage with unlimited access to food and water except for 12 h before the experiment. They were randomly divided into two groups (I and II). The group I was used to study pharmacokinetic of isoimperatorin, columbianetin acetate and osthole. The group II was selected to clarify pharmacokinetic characterization of the other coumarins. Each rat was given RAP extract at a single dose of 6.0 g kg^{-1} by oral administration. Blood samples (about 250 μL) were immediately collected in heparinized 1.5 mL polythene tubes before dosing and at 0.083, 0.16, 0.25, 0.33, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12 and 24 h after dosing for group I and before dosing and at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h for group II. Blood samples were collected into heparinized tubes and immediately centrifuged at 4000 rpm for 10 min. A fixed volume (100 μL) of plasma was transferred into clean tubes and stored at −20 °C until analysis. Pharmacokinetic parameters were calculated by using non-compartmental pharmacokinetic analysis with the computer program "Drug and Statistics 1.0" (DAS 1.0) (Medical College of Wannan, China).