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Simultaneous determination of imperatorin and its metabolite xanthotoxol in rat plasma by using HPLC–ESI-MS coupled with hollow fiber liquid phase microextraction

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ABSTRACT

The objective of the present study was to develop a new method for the simultaneous quantitation of imperatorin and its metabolite xanthotoxol in rat plasma. The samples were prepared with hollow fiber liquid phase microextraction (HF-LPME). The optimized extraction procedure was acquired by assessing extraction solvent, length of the fiber, agitation rate, extraction temperature and time. A comparison of sample pretreatment ways between HF-LPME and deproteinization with methanol was performed, which demonstrated less ion suppression and better sensitivity of HF-LPME. Analytes were separated on a C_{18} column with a gradient elution consisted of methanol and water containing 1 mmol/L ammonium acetate. The detection was accomplished by electrospray ionization (ESI) source operating in the positive ionization mode. Selected-multiple-reaction monitoring (SMRM) scanning was employed, which guaranteed a higher sensitivity compared with MRM mode. Calibration curves were linear over investigated ranges with correlation coefficients greater than 0.9979. Precision varied from 0.26% to 14%, and the accuracy varied within ±5.5%. The developed method was successfully applied to the pharmacokinetic research of imperatorin and its metabolite xanthotoxol after oral administration of imperatorin to rats.

1. Introduction

Imperatorin, an active natural furocoumarin, originates from many medicinal plants such as *Glehnia littoralis* Fr. Schmidt ex Miq and *Peucedanum Praeruptorum* Dunn. It has been reported that imperatorin possesses many pharmacologic actions including anti-convulsion [1], anti-inflammatory [2], vasodilation [3], inhibiting the activity of cytochrome P450 enzyme [4] and anti-hypertension [5].

Evaluation of the pharmacokinetic properties of imperatorin is quite necessary due to the high importance in determining whether imperatorin can be pursued as a chemical entity. To date, some studies on imperatorin pharmacokinetics in rat plasma [5–9] and rat liver [9] after oral administration of imperatorin have been reported. There have also been a few reports on imperatorin metabolites. In 2012, two metabolites of imperatorin, imperatorin hydroxylate and imperatorin epoxide, were found and quantitated in dog plasma after intravenous administration of imperatorin [10]. Recently, 51 metabolites of imperatorin have been identified in rat urine in our lab [11], in which we found that xanthotoxol displayed obvious and stronger response among these metabolites. Moreover, during our preliminary experiment, xanthotoxol was also mainly detected in rat plasma after oral administration of imperatorin. These suggested that xanthotoxol was one of the main metabolites of imperatorin and it would be informative to employ xanthotoxol as one of marker compounds for pharmacokinetic study of imperatorin. Until now, xanthotoxol has not yet been determined either alone or along with imperatorin in rat plasma.

Some methods, including HPLC-UV [6,7,9], GC–MS [5] and LC–MS [8,10], have been developed to quantitate imperatorin and metabolites in plasma after oral administration of imperatorin. Among these methods, LC–MS method was more attractive than other methods due to its high sensitivity and specificity. Nevertheless, this method was still faced with the challenge of matrix effect [12–14]. Various sample preparation methods, such as liquid–liquid extraction (LLE) [15–17] and solid-phase extraction (SPE) [18–20] have been established to reduce the matrix effect. However, they also suffer from some drawbacks, such as high labor intensity, high cost and large mounts of toxic solvent. In the year of

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1999, HF-LPME was introduced [21] and afterwards, this method was widely applied to the analysis of biological samples [22–34] because it not only can avoid above shortcomings but also has the advantage of excellent sample cleanup ability.

In the present study, a HPLC–ESI-MS coupled with HF-LPME method was first developed to the determination of imperatorin and xanthotoxol in rat plasma. The extraction procedure was optimized by assessing extraction solvent, length of the fiber, agitation rate, extraction temperature and time. A comparison of sample pretreatment ways between HF-LPME and deproteinization with methanol was performed, which demonstrated less ion suppression and better sensitivity of HF-LPME. The method was then applied to the pharmacokinetic study of imperatorin and its metabolite xanthotoxol after oral administration of imperatorin.

2. Experimental

2.1. Reagents and materials

Imperatorin (purity ≥ 98%) was purchased from Shanghai Sunny Biotech Co., Ltd. Xanthotoxol (purity ≥ 98%) was purchased from Shanghai Tauto Biotech (Shanghai, China) Scopoletin (purity ≥ 98%) was obtained from National Institutes for Food and Drug Control, Beijing, China. *n*-Propanol, *n*-butanol *n*-pentanol and acetone were bought from Tianjin Yongda Chemical Reagent Co., Ltd. Hexylalcohol, *n*-heptanol and *n*-octyl alcohol were bought from Tianjin Forever Chemical Co., Ltd. Acetic ether was bought from Tianjin Hedong District Red Rock Reagent Factory. HPLC grade methanol was provided by J.T. Baker. Ammonium acetate and formic acid were from Dikma Technologies Incorporation. Ultrapurified water was purchased from Hangzhou Wahaha Group Co. Ltd. Polyvinylidene difluoride hollow fiber (pore: 0.2 μm; inner diameter: 0.7 mm) was provided by Tianjin Motmo Membrane Technology Co. Ltd.

2.2. Instrumentation and conditions

2.2.1. Liquid chromatography

Agilent 1200 liquid chromatography system (Agilent, USA) equipped with a quaternary solvent delivery system, an autosampler and a column compartment was used. The analytes were separated on an Agilent Zorbax SB-C_{18} column (150 mm × 4.6 mm, 5 μm). The column temperature was maintained at 25 °C. The mobile phase consisted of methanol (A) and water containing 1 mmol/L ammonium acetate (B). The gradient condition was as follows: 50% A linear gradient to 95% A over 5 min, held at 95% A for the next 3.5 min, and returned to 50% A over 0.1 min. The flow rate was set at 0.8 mL/min and the injection volume was 10 μL.

2.2.2. Mass spectrometer

The mass spectrometer was a QTRAP™ 3200 with Turbo V sources and Turbo Ionspray interface from Applied Biosystems (Applied Biosystems, Foster City, CA, USA). The turboionspray interface operated in positive ionization mode was used. Typical source conditions were set as follows: ion spray needle voltage 5500 V; turbo spray temperature 650 °C; nebulizer gas 60 psi; heater gas 65 psi; curtain gas 25 psi. Interface heater was on. Nitrogen was used in all cases. Analytes were quantified under selected-multiple-reaction monitoring (SMRM) mode using the following precursor-to-product ion pair and parameters: Imperatorin, *m*/*z* 271.1 → 203.2 with DP 25 V and CE 15 eV, and the selected time was 7.32 min; Xanthotoxol, *m*/*z* 203.2 → 147.1 with DP 20 V and CE 32 eV, and the selected time was 4.38 min; Scopoletin (IS) *m*/*z* 193.1 → 133.1 with DP 49 V and CE 29 eV, and the selected time was 3.48 min; the detection windows of all the analytes were 60 s. All of the dwell time was set at 50 ms. Applied Biosystems/MDS Sciex Analyst software (versions 1.5.2) was used for data acquisition and processing.

2.3. Preparation of standard solution

The appropriate amount of imperatorin and xanthotoxol was exactly weighed and dissolved respectively in methanol to prepare stock standard solution. Then, the two stock solutions were mixed and diluted with methanol to prepare a final mixed standard solution containing 4890 ng/mL of imperatorin and 706.6 ng/mL of xanthotoxol, respectively. A series of working solutions of the two analytes were freshly prepared by diluting mixed standard solution with methanol at the appropriate ratios to yield concentration from 38.20 to 4890 ng/mL for imperatorin and from 5.520 to 706.6 ng/mL for xanthotoxol, respectively. The internal standard solution was prepared by dissolving scopoletin with methanol to 617.3 ng/mL. For the validation of the method, three concentration levels of standard solution containing imperatorin (76.40, 305.6 and 2445 ng/mL) and xanthotoxol (11.04, 44.16 and 353.3 ng/mL) were used for preparing QC plasma samples.

2.4. Animals

Six male Sprague–Dawley rats (weighed 250 ± 20 g, Experimental Animal Research Center, Hebei Medical University, China) were used in this study. The rats were housed under controlled environmental conditions (temperature: 25 ± 2 °C; humidity: 55 ± 5%; 12-h dark/12-hlight cycle) for at least 5 days before the experiments. The animals were fed with food and water ad libitum and were fasted overnight prior to the test. All animal experiments were carried out according to the Guidelines for the Care and Use of Laboratory Animals, and approved by the Animal Ethics Committee of Hebei Medical University.

2.5. Drug administration and blood sampling

Imperatorin was suspended in 0.3% CMC-Na and orally administrated to rats at a dose of 80 mg/kg. Blood samples were collected at 0.083, 0.33, 0.67, 1, 1.5, 2, 4, 6, 9, 12 and 24 h after dosing, 0.3 mL of venous blood samples were collected at the rat's medial angle of eye with heparinized capillary and then put in 1.5 mL heparinized tube. The blood samples were then centrifuged at 4000 rpm for 5 min at room temperature, and the plasma was separated and stored at −80 °C for later analysis.

2.6. Pretreatment procedure of the samples

2.6.1. Real samples

20 μL of internal standard solution was placed in a sample vial with a screw top and evaporated to dryness at room temperature under a gentle stream of nitrogen. 100 μL of thawed plasma sample was added to the vial and then diluted with 2% NaCl solution to a total volume of 1.5 mL. After the mixture was vortexed for 2 min (served as aqueous B), a magnetic stirrer bar was put into the vial.

Before using, the hollow fiber was ultrasonically cleaned in acetone-water (50:50) for 10 min in order to remove any contaminants and then dried in air. Immediately afterwards, the hollow fiber was cut manually into 5 cm length pieces, and then the pieces were immersed in *n*-heptanol (organic solvent, acceptor phase) for around 1 min in order to impregnate its pores with organic solvent. Then the fiber paper was employed to blot the organic solvent on the ektexine gently. 20 μL of *n*-heptanol was injected into the hollow fiber using a microsyringe and a knot was tied at the length of 5 cm. Then the part of fiber under the knot was then immersed in the diluted plasma sample, afterwards, the sample vial was stirred at room temperature with a magnetic stirrer to facilitate for 50 min.