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Pharmacokinetics and tissue distribution study of Isovitexin in rats by HPLC-MS/MS

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ABSTRACT

A sensitive and credible high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method was established and validated for the determination of isovitexin in rat plasma and various tissues (including heart, liver, lung, kidney, stomach, intestine, muscle, brain and cerebellum). The samples were prepared with methanol by liquid–liquid extraction, and puerarin was used as the internal standard. The chromatographic separation was carried out on an Agilent Poroshell 120 EC-C_{18} column (4.6 mm × 50 mm, 2.7 μm) with a mobile phase consisting of acetonitrile and 0.1% formic acid (21:79, v/v). The MS analysis was performed by multiple reaction monitoring (MRM) with electronic spray ionization source (ESI^-) for quantitative response of isovitexin (431.0→311.0) and puerarin (415.1→295.0). The linearity of isovitexin in all the biosamples was good, with correlation coefficients greater than 0.9912 within the corresponding concentration range. The intra- and inter-day precisions in plasma and various tissues were less than 11.80%, and the accuracy (RE %) ranged from −4.89% to 4.78%. The extraction recoveries were in the range of 72.70%–90.81%. The present method was successfully applied to pharmacokinetics and tissue distribution of isovitexin in rats after tail vein injection with 2.0 mg/kg of the compound. The pharmacokinetic parameters were demonstrated as followed: the half-life ($t_{1/2}$) was 1.05 ± 0.325 h, the apparent volume of mean residual time (MRT) was 1.229 ± 0.429 h, and the area under the curve (AUC) was 11.39 ± 5.05 μg/mL/h. The results of tissue distribution showed that the main tissue depots for isovitexin in rats were kidney, intestine and liver. The results provided a meaningful insight for the further pharmacological investigation of isovitexin.

1. Introduction

Isovitexin, apigenin-6-C-β-D-glucopyranoside, is a typical flavone C-glucoside widely distributed in vegetables, fruits and herbal drugs, such as the leaves of *Desmodium adscendens* (Sw.) DC. [1], the fruits of *Livistona chinensis* R. Br. [2], Herba Desmodii Styracifolii [3], etc. A large number of reports have indicated that isovitexin exhibits various biological activities, including anti-oxidation [4–6], anti-bacteria [7], memory modulation [8], anti-pathogens [9,10], α-glucosidase inhibition [11], and so on. Just in light of these various beneficial effects, great potential of isovitexin being developed as a novel food additive or drug candidate has been demonstrated.

It is well known that good understanding of pharmacokinetics and tissue distribution in vivo is crucial to the accurate interpretation of the action mechanism of a drug candidate. Up to now, it has been confirmed that isovitexin can be assimilated into rat blood after oral administration of some herbal materials (*Santalum album* L. leaves extract, Herba Desmodii Styracifolii extract and *Swertia pseudochinensis* extract) [3,12,13], and exhibits considerable interaction with the human serum albumin (an extracellular protein functions as a carrier of various drugs in blood plasma) [14]. However, there are few reports on the pharmacokinetic and tissue distribution of isovitexin in rat after intravenous administration as a single compound.

Due to its high sensitivity and selectivity, HPLC-MS has become one of the most efficient methods for analyzing the complex biosamples [15,16]. Apart from the MS detection technology, the

Abbreviations: IS, internal standard; MRM, multiple reaction monitoring; SD, Sprague Dawley; ESI, electrospray ionization; LLOQ, lower limit of quantification; QC, quality control; CE, collision energy; RSD, relative standard deviation; RE, relative error; MRT, mean residual time; CL, clearance rate; AUC, area under the curve.

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chromatographic columns with high separation performance are essential for determination of trace analytes in the complex biological samples. Poroshell 120 EC-C_{18} column is a recently developed column packed with 2.7 μm separation materials (1.7 μm solid core and 0.5 μm porous outer layer). Compared with the common columns, the column can achieve high resolution and speed separations within the pressure range of common LC system, which might help to shorten the analyzing time and increase the detecting sensitivity and selectivity.

In the present study, a sensitive and credible HPLC-MS/MS method (using Poroshell 120 EC-C_{18} column) was established and validated for the determination of isovitexin in rat plasma and various tissues. The method was then successfully applied to pharmacokinetics and tissue distribution of isovitexin in Sprague-Dawley rats after tail intravenous (i.v.) administration.

2. Materials and methods

2.1. Chemicals and materials

Isovitexin and puerarin (purity over 98%) were purchased from Shunbo Biotech CO., Ltd. (Shanghai, China). HPLC grade methanol and acetonitrile were purchased from Merck (Darmstadt, Germany). Chromatographic pure water was from J.T. Baker (J.T. Baker Chemicals, USA). The other chemicals used in this study were all of analytical grade.

2.2. Instrumentations and HPLC–MS/MS conditions

The HPLC-MS/MS method was performed with an Agilent 1200 Series system (Agilent Technologies, USA), which mainly consisted of a G1312B binary pump, a G1367D autosampler SL Plus, a G1322A vacuum degasser, a G1316B column heater and a G6410B triple quadrapole mass spectrometer with an electrospray ionization (ESI) source.

The separation was performed on an Agilent Poroshell 120 EC-C_{18} column (Agilent Technologies, USA) with a mobile phase consisting of acetonitrile-0.1% formic acid water (21:79, v/v) at a flow rate of 0.3 mL/min. The temperature of column was maintained at 36 °C. The injection volume was 40 μL.

Mass spectrometric detection was operated on a triple quadrapole mass spectrometer with ESI source in negative ionization mode. High purity nitrogen was used as the nebulizing and drying gas. Quantification was performed with multiple reactions monitoring (MRM) mode. The transitions of isovitexin and puerarin (IS) were *m/z* 431.0→311.0 and *m/z* 415.1→295.0 respectively (Fig. 1). The fragmentor and collision energy (CE) were optimized as 160 V and 20 eV for isovitexin, 180 V and 22 eV for puerarin. The other MS parameters were as follows: ion spray voltage 4000 V; source temperature 350 °C; dwell time 200 ms; nebulizer gas pressure 35 psi; desolvation gas flow 10 L/min. Under these optimal conditions, isovitexin and puerarin were separated and detected efficiently in only 4.8 min.

2.3. Collection and treatment of the plasma and tissues

Male Sprague Dawley rats (SD rats, 200 ± 15 g, 6–8 weeks) were provided by Tianjin Institute of Materia Medica (Tianjin, China). All the rats were kept in environmentally controlled cages (23 ± 2 °C; 12 h light/dark cycle and relative humidity 50%) at least 5 days prior to the experiments, and had free access to standard laboratory diet and water. Before experiments, all the rats were fasted for overnight but allowed free access to water. All the animal experimental procedures were performed according to National Guidelines on the Proper Care and Use of Animals in Laboratory Research.

Blood sample was obtained from rat orbital vein, and put into the heparinized centrifuge tubes. Instantly, the blood sample was centrifuged at 6000 rpm for 10 min (4 °C), the supernatant was gathered as the plasma. Then, the rat was sacrificed quickly by decapitation on the ice. Various tissues (including heart, liver, lung, kidney, stomach, intestine, muscle, brain and cerebrum) were harvested and rinsed with ice-cold 0.9% NaCl to remove the superficial blood. After being blotted dry with filter paper, certain equal amounts of tissues were weighed accurately and homogenized in 0.9% NaCl to prepare homogenates (0.2 g/mL)[17].

2.4. Standard and sample preparation

2.4.1. Preparation of stock and working solutions4

The stock solutions were prepared by dissolving isovitexin and puerarin in methanol to be 1.0 and 2.0 mg/mL, respectively. Subsequently, the stock working solution of isovitexin was serially diluted with methanol into a linear concentration gradient: 0.1, 0.2, 0.4, 1.0, 2.0, 5.0, 10.0, 25.0 μg/mL. A 200 ng/mL solution for puerarin (IS) was prepared in methanol. All the solutions were stored at 4 °C in the dark and brought to room temperature before use.

2.4.2. Preparation of calibration standards and quality control (QC) samples

Calibration standards were prepared as followed: 20 μL of the corresponding standard working solution was transferred into a 1.5 mL Eppendorf tube, and was evaporated to dryness by nitrogen at 40 °C. Then, 200 μL of blank plasma or various tissue homogenates (including heart, liver, spleen, lung, kidney, stomach, intestine, brain and cerebellum) was added. The calibration standards were prepared at concentrations of 10, 20, 40, 100, 200, 500, 1000, 2500 ng/mL in plasma, and 10, 20, 40, 100, 200, 500, 1000 ng/mL in the various tissues. In the same way, the quality control (QC) samples were independently prepared with blank plasma or tissue homogenate, and their concentrations were 10 ng/mL (low), 200 ng/mL (medium), and 1000 ng/mL (high). Moreover, the concentration of IS in all the samples was 20 ng/mL.

2.4.3. Sample treatment

A simple liquid–liquid extraction method was carried out to extract isovitexin from QC samples, calibration standards, and all the bio-samples (including plasma samples and tissue homogenate samples). All of the 200 μL samples was added 20 μL IS (200 ng/mL). Then, sample was mixed with 800 μL methanol and vortexed for 5 min, and then centrifuged at 14,000 rpm for 10 min. The supernatant was collected and transferred into a 1.5 mL Eppendorf tube. After being dried with nitrogen, the residue was mixed with 400 μL methanol and treated as previous procedure again. The final residue was stored at −75 °C and dissolved to be corresponding concentration with 21% acetonitrile before analysis.

2.5. Method validation

In accordance with the FDA bioanalytical method validation guide [18], the HPLC-MS/MS method was validated in terms of specificity, linearity, sensitivity, precision, accuracy, matrix effect, extraction recovery and stability.

2.5.1. Selectivity

The selectivity was evaluated by analyzing the blank biological samples (plasma and various tissue homogenates), blank biological matrix samples spiked with isovitexin, and actual bio-samples after intravenous administration of isovitexin, respectively.