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High performance liquid chromatographic method for the determination of cinepazide maleate and its application to a pharmacokinetic study in rats

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ABSTRACT

A simple and reliable high performance liquid chromatographic (HPLC) method has been developed and validated to quantify cinepazide maleate, a calcium blocker, in rat plasma. Cinepazide maleate and Tinidazole (internal standard) have been extracted by a simple liquid–liquid extraction before injection into chromatographic system. Chromatographic separation was achieved on a reversed phase C_{18} column with a mobile phase consisted of a water mixture of 10 mM potassium dihydrogen phosphate (pH = 4.5):methanol (40:60, v/v), pumped at flow rate of 1.0 mL/min, and detected at 303 nm. The method exhibited a linear range of 0.12–120 μg/mL in blank rat plasma, with the lower detection limit of 0.06 μg/mL. The method was statistically validated for linearity, accuracy, precision, selectivity and stability following FDA guidelines. The intra- and inter-assay coefficients of variation did not exceed ±15% from the nominal concentration. The accuracy of cinepazide maleate was within ±15% of the theoretical value. The assay has been applied successfully in a pharmacokinetic study of cinepazide maleate after a single intravenous at three doses in rat. And cinepazide maleate injection can improve the bioavailability of cinepazide maleate greatly, and has a dose-dependence profile in rats.

1. Introduction

Cinepazide maleate, 1-[(l-pyrrolidinylcarbonyl)-methyl]-4-(3,4,5-trimethoxycynnamoyl) piperazine maleate (Fig. 1), a calcium blocker, has a vasodilator action and could increase the blood flow through the brain and peripheral organs such as cardiac muscle, skeletal muscle and kidney [1]. And at present, cinepazide maleate has been widely used as vasodilator in peripheral vascular disorders in China [2–10]. There has the pharmacokinetic information of cinepazide maleate with the oral administration [11], but till date, the pharmacokinetic of cinepazide maleate with intravenous administration is still unclear. To characterize pharmacokinetic profiles or evaluate formulation bioavailability of cinepazide maleate, it is necessary to development an accurate, sensitive and affordable bioanalytical method for the quantification of cinepazide maleate in plasma.

An analytical method have been published for the quantification of cinepazide maleate in plasma using high-performance liquid chromatography–tandem mass spectrometry (LC/MS/MS) [11] with an excellent sensitivity (LLOQ was 1 ng/mL) and fast analysis time. But LC/MS/MS requires relatively expensive instrumentation and highly skilled technical expertise. These may not be readily available and/or affordable for most laboratories in resource-limited settings. In such settings, selective and sensitive HPLC methods are preferable to more expensive LC/MS/MS techniques.

In this paper, a fast, simple, selective and sensitive HPLC method for the determination of cinepazide maleate in rat plasma after a simple liquid–liquid extraction procedure was developed and

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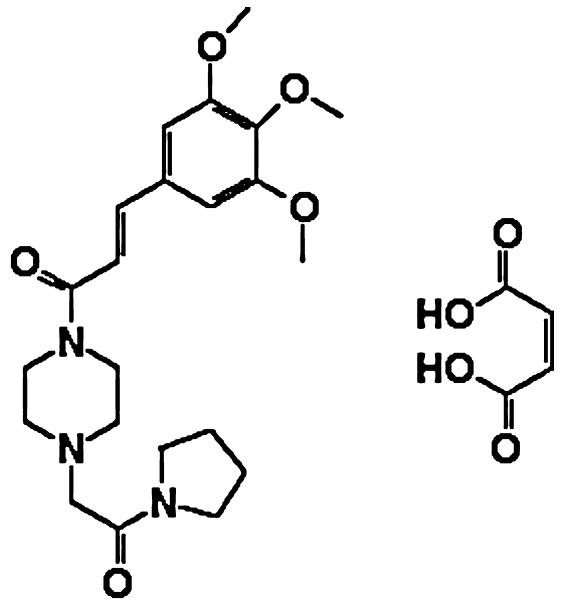

Fig. 1. Chemical structure of cinepazide maleate.

validated. Furthermore, pharmacokinetic of cinepazide maleate after intravenous administration on rats was carried out by applying this validated HPLC method.

2. Materials and methods

2.1. Reagents and materials

Cinepazide maleate and tinidazole (>98.0% both purity) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Cinepazide Maleate Injection was supplied by Beijing Sihuan pharmaceutical Co. Ltd. The structure of cinepazide Maleate was described in Fig. 1. HPLC-grade methanol and ethyl acetate was purchased from Fisher scientific (Pittsburgh, PA, USA). Analytical grade potassium dihydrogen phosphate was purchased from BDH Chemicals (Poole, UK). Ultra pure water with a sensitivity of 18 μΩ was prepared by a Millipore Milli-Q purification system (Bedford, MA, USA). All other chemicals were of analytical grade and used without further purification.

2.2. Apparatus

Analyses were performed on an Agilent 1200 series (Agilent Technologies, USA) liquid chromatographic system which was composed of a binary pump, a variable wavelength detector, an autosampler, a column compartment and a computer system for data acquisition (Agilent Chemstation). The data were collected with an Empower pro Chromatography Manager for data acquisition analysis.

The HPLC separation was performed with an Inspire C_{18} analytical column (150 mm × 4.6 mm, 5 μm; Dikma, Beijing, China). All solutions were degassed by ultrasonication (China, Shanghail) and filtered through a 0.45 μm millex filter (Millipore).

The samples were centrifuged by Jouan GR 412 (Saint Mazaire Cedex, France). All pharmacokinetic parameters were calculated using the pharmacokinetic software WinNonlin (version 6.2, Pharsight Corp., Mountain View, CA, USA) using non-compartmental method.

2.3. Chromatographic conditions

The mobile phase consisted of a mixture of 10 mM potassium dihydrogen phosphate (pH = 4.5):methanol (40:60, v/v), which was freshly prepared and then filtered and degassed, delivered at a flow-rate of 1.0 mL/min. Column temperature was maintained a room temperature (25 ± 2 °C). The injection volume was 20 μL, and the detection wavelength was set at 303 nm.

2.4. Animals

Sprague-Dawley rats (250 ± 20 g) were obtained from the animal lab of the Fourth Military Medical University (Xi'an, China). They were kept in an environmentally controlled breeding room for 5 days before starting the experiments and fed with standard laboratory food and water. Animal welfare and experimental procedures were strictly in accordance with the guide for the care and use of laboratory animals and the related ethical regulations of the Fourth Military Medical University. The study was approved by the Animal Ethics Committee of the Fourth Military Medical University.

2.5. Preparation of standard solutions

Standard stock solutions of cinepazide maleate was prepared by dissolving appropriate amount in methanol to yield concentration of 32 mg/mL, and tinidazole as IS was prepared by dissolving appropriate amount in methanol and little acetone mixture to yield concentration of 9 mg/mL. Working standard solutions of cinepazide maleate (4000, 2000, 800, 400, 80, 40, 8, and 4 μg/mL) were prepared by serial dilution of the stock standard solution with water. Working standard solution of the IS (400 μg/mL) was obtained by diluting the stock solution with water. The working solutions were stable at −20 °C, protected from light and brought to room temperature before use. The stock solutions were stable for at least two months when stored in refrigerator, and no evidence of degradation of the analyte was observed on the chromatograms during this period.

2.6. Preparation of samples

Rat plasmas (160 μL) were spiked with 20 μL cinepazide maleate and 20 μL IS working solutions to yield cinepazide maleate concentrations of 120, 60, 24, 12, 2.4, 1.2, 0.24 and 0.12 μg/mL and IS (12 μg/mL). Then, ethyl acetate (800 μL) was added to the plasma samples as an extracted solvent. Each tube was vortexed for 3 min and then centrifuged at 16,000 rpm for 10 min. The supernatant was transferred 600 μL to 100 × mm borosilicate glass tubes (James A. Jobling and Company Ltd., Sunderland, UK). The organic extraction process was repeated collecting organic supernatant into the same glass tube. Samples were evaporated to dryness under a stream of nitrogen at 40 °C and reconstituted in 400 μL of mobile phase, vertex mixed. The supernatants were transferred to glass tubes with caps (Waters Inc., Millipore, MA, USA) and a 20 μL volume was injected into the HPLC system. Blank rat plasma samples were processed in the same manner using water rather than cinepazide maleate and IS. The accuracy and precision of the method was calculated. Three pools of quality control (QC, Table 2) samples for cinepazide maleate were prepared in rat plasma at concentrations of 0.3, 15 and 75 μg/mL.

2.7. Bioanalytical method of validation

2.7.1. Selectivity

To investigate the potential interferences by endogenous compounds co-eluting with cinepazide maleate and IS, blank rat plasma from six different sources were tested. Chromatographic peaks of