An improved HPLC method for determination of nifuratel in human plasma and its application to pharmacokinetics studies

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SUMMARY

A rapid, simple and sensitive high-performance liquid chromatography (HPLC) method was established for the quantification of nifuratel in human plasma and applied to a study of its pharmacokinetics. A test and a reference formulation were investigated and compared, and the study group consisted of 24 healthy male volunteers. The analytical technique was based on a single extraction of the drug from the plasma with chloroform, using ornidazole as internal standard (IS). The chromatographic system consisted of a 5-μm 4.6 mmX250 mm C₁₈ analytical column and the mobile phase consisted of methanol and purified water (45:55, v/v). Nifuratel and ornidazole concentrations were detected by ultraviolet (UV) absorbance at a wavelength of 254 nm. The lower limit of detection and quantification was 0.5 ng·ml⁻¹, and the calibration curves were linear over a concentration range of 0.5–160 ng·ml⁻¹ nifuratel in the plasma. The results showed that the area under the plasma concentration-time curve (AUC), time to maximum observed plasma concentration (Tₘ₉⁰), maximum concentration reached in the concentration profile (Cₘ₉⁰), and elimination half-life (t½) between the test tablets and the reference tablets demonstrated no significant difference (P>0.05). The relative bioavailability amounted to 103.13%±8.73%.

INTRODUCTION

Nifuratel, [5-([methylthio]methyl)-3-([5-nitrofururylidene] amino)-2-oxazolidinone], is a nitrofuran derivative, with broad-spectrum antibiotic activity; it has an inhibitory action on the bacterial enzymatic system, in particular against Gram-negative anaerobes. Nifuratel was first synthesized by Poli Chimical Co. (Italy), and has been found to effectively treat various vaginal infections, including Trichomonas- and Candida- induced infections [1]. Nifuratel is mostly eliminated via the renal system and has a strong antibacterial action in the urinary system. Therefore, it can be used for the treatment of urethritis, cystitis and pyelitis [2]. Guinebault et al. used nifuratel as internal standard (IS) for the measurement of nifuroxazide levels in the plasma [3]. Liu et al. developed a high-performance liquid chromatography (HPLC) method to determine the content and derivatives of nifuratel [4]. However, these specific methods are not available for the determination of nifuratel in human plasma in most laboratories. This paper describes a simple, selective, and highly sensi-
tive method using HPLC coupled to an ultraviolet (UV) detector, and which was successfully applied to a study of the relative bioavailability and pharmacokinetics of nifuratel.

**EXPERIMENTAL PROCEDURES**

**Chemicals and reagents**

Nifuratel test tablets (Lot No. 040401) and ornidazole (Purity 99.8%, Lot No. 000401) were obtained from Luyinlihua Medicine Technology Development Co. Ltd (Beijing, China). Nifuratel reference tablets (Purity 99.5%, Lot No. X960527) were manufactured by Baker Norton Co. Ltd (Hongkong, People’s Republic of China). Chromatographic grade methanol and chloroform were purchased from Tedia Co. Inc.

**Instruments and chromatographic conditions**

All experiments were performed using an HPLC system (Agilent 1100 series, USA) equipped with a single pump, an autosampler and an Agilent dual model wavelength absorbance detector. Separation was performed on an analytical column (4.6 mmX250 mm ID HYPERSIL ODS2 C18; 5-μm particle size). The wavelength was set at 254 nm. The mobile phase consisted of methanol and purified water (45:55, v/v) at a flow rate of 1.0 ml-min⁻¹. The mobile phase was prepared daily and degassed by ultrasonification before use, and not subsequently reused.

**Standard solutions**

Stock solution (1 mg·ml⁻¹) and appropriate dilutions of nifuratel and ornidazole were prepared in methanol and stored at 4 °C.

**Sample preparation**

To 1 ml of plasma in a 10-ml glass centrifuge tube was added 100 μl of ornidazole as IS (1.0 μg·ml⁻¹) and 4 ml of chloroform. After mixing for 1 min, the mixture centrifuged for 5 min at 5000g. The organic layer was transferred to another 5 ml centrifuge tube and dried in a water bath at 37°C under nitrogen. The residue was reconstituted in 100 μl of mobile phase, then 100 μl of this solution was transferred to an injection vial and 20 μl was injected into the HPLC. All operations were performed in a dark chamber because nifuratel is subject to photochemical decomposition.

**Biological samples**

The study was conducted as a randomized trial in a two-way crossover design. The test or reference formulation of nifuratel was administered in a single dose of 400 mg to 24 healthy male volunteers after overnight fasting. A number of blood samples (4 ml) were collected in heparinized tubes at 0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, and 12.0 h after dosing. Plasma samples were frozen immediately and stored at -60°C until analysis.

**Calibration curves and quality control samples**

Blank plasma was prepared from heparinized whole blood samples collected from the 24 healthy volunteers and stored at -20°C. After thawing, a stock solution of nifuratel was added to yield final concentrations of 0.5, 1, 2, 4, 10, 40, 80, and 160 ng·ml⁻¹. IS solution was added. Then the samples were prepared for analysis as described above. Quality control (QC) samples for determination of the accuracy and precision of the method were also prepared at low (1 ng·ml⁻¹), medium (80 ng·ml⁻¹), and high (160 ng·ml⁻¹) concentrations.

**Method Validation**

Specificity was assessed by examining peak interference from endogenous substances. This was carried out by inspecting chromatograms of blank and spiked plasma samples.

Sensitivity was determined in terms of the limit of quantification (LOQ) which was taken as being the lowest concentration in the calibration range. It was designed to yield a precision of <20% relative standard deviation (RSD), and an accuracy of between 80 and 120% of the theoretical value, with a signal to noise ratio of over 10.

Linearity was determined by a calibration curve with the peak area ratio of standard nifuratel to ornidazole in the concentration range of 0.5-160 ng·ml⁻¹ for nifuratel.

Intra- and inter-assay precision was determined from QC samples spiked with three different concentrations of nifuratel (1, 80 and 160 ng·ml⁻¹). Each concentration was analyzed five times in one day or on five consecutive days. Concentrations were determined from different calibration curves, which