Determination of Nifuratel in Human Plasma by HPLC and Study on Its Pharmacokinetics

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Abstract

A simple and sensitive reversed-phase high-performance liquid chromatography method using UV detection is established for the determination of nifuratel in human plasma and applied to a study of its pharmacokinetics. Plasma samples are extracted with ethyl acetate. A C_{18} column and a mobile phase of 0.01M (pH 7) phosphate buffer (KH_2PO_4) and acetonitrile (61:39, v/v) are used. Analysis is run at a flow rate of 1.0 mL/min with the detector operated at a wavelength of 367 nm. The calibration curve is linear over a concentration range of 0.2–40 ng/mL with a correlation coefficient of 0.9996. The limit of detection is 0.1 ng/mL. The mean absolute recovery value is greater than 80%. The intraday precision (relative standard deviation) ranges from 1.89% to 7.32%, and the interday precision ranges from 1.71% to 7.83%. The results show that the area under the plasma concentration-time curve, time to maximum observed plasma concentration, maximum concentration reached in the concentration profile, and elimination half-life between the testing tablets and reference tablets have no significant difference ($P > 0.05$). Relative bioavailability is 104.0% ± 16.5%.

Introduction

The active principle of Inimur, nifuratel [5-([methylthio] methyl)-3-([5-nitrofurfurylidene] amino)-2-oxazolidinone] (Figure 1), is a chemotherapeutic agent synthetized in the research laboratories of Poli Industria Chimica (Milano, Italy). Nifuratel exerts an intense and efficacious action against the bacterial, protozoan, and fungal species that attack the female genital tract and does not give rise to unwanted immediate or long-term side effects (1). Nifuratel is mostly eliminated through the renal emunctory and has a strong antibacterial action in the urinary tract. It is, therefore, prescribed for treatment of urethritis, cystitis, pyelitis, and urinary septic retentions of various etiological and preparatory stages of surgical operations on the urinary tract. Liu Jin-ai (2) and Zou Li (3) have established high-performance liquid chromatography (HPLC) method to determine the content and the related substances of nifuratel. Guinebault et al. used nifuratel as internal standard (IS) for the measurement of nifuroxazide in plasma (4). However, there was no previous report of the analytical method of the determination of nifuratel in human plasma. This paper describes a simple, selective, and highly sensitive method using HPLC coupled with a UV detector for the determination of nifuratel in human plasma for the first time; it was successfully applied to a study of its pharmacokinetics and bioavailability.

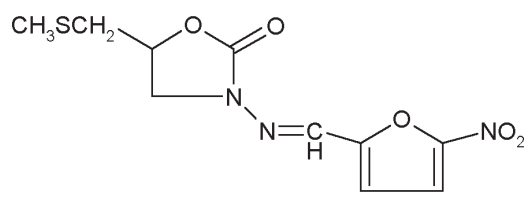

Figure 1. Chemical structure of nifuratel.

Experimental

Materials and reagents

A nifuratel test table was provided by Nanjing Life-Origin (Nanjing, China). A nifuratel reference table was purchased from Monsanto Italiana S.p.A (Milan, Italy). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany). Other analytical-grade reagents used were purchased from (Nanjing Chemical Reagent Factory No. 1 (Nanjing, China).

Chromatographic system

The HPLC system was constructed from an LC-10ATVP pump with SPD-10A vp (Shimadzu, Kyoto, Japan) UV detector set at 367 nm on a N2000 workstation (Zhejiang University Star Instrument Technology Co., Ltd., Zhejiang, China). Chromatographic separation was carried out at room temperature with an analytical column (Hanbang C_{18}, 150- × 2.0-mm, 5 µm). The mobile phase

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consisted of a mixture of acetonitrile–10 mmol/L phosphate buffer (KH_2PO_4) (39:61, v/v) adjusted to pH 7.0 with a sodium hydrate solution. The mobile phase was prepared daily, filtered under vacuum through a 0.45-µm membrane filter, and degassed before use. The flow rate was set at 1.0 mL/min.

Preparation of stock solutions

Primary stock solutions of nifuratel were prepared by dissolving 10.0 mg nifuratel in 10 mL of acetonitrile. They were stored in brown quantitative bottles at –4°C because nifuratel decomposes under light. Working solutions of nifuratel were prepared in the solvent (acetonitrile) by an appropriate dilution of 20.0, 100.0, and 1000.0 ng/mL.

The IS stock solution was prepared by dissolving 10.0 mg of Dipyridamole in 10 mL methanol solvent, producing a concentration of 1.0 mg/mL. A 1-mL sample of this primary stock solution was diluted in methanol to produce a final concentration of 10 µg/mL.

Blood sample preparation

A 1-mL sample of plasma was transferred to a 10-mL glass centrifuge tube, mixed with 50 µL of IS solution (10 µg/mL) and 50 µL sodium hydroxide solution (0.2 mol/L), followed by 5 mL of ethyl acetate. The sample was vortexed for 3 min and centrifuged at a speed of 4000 rpm for 10 min. The organic layer was transferred to another 10-mL centrifuge tube and evaporated to dryness in a water bath at 40°C under a stream of nitrogen. The residue was reconstituted in 50 µL of 70% acetonitrile (acetonitrile–water, 70:30, v/v); after a high-speed centrifugation of 16,000 rpm, a 20-µL aliquot was injected into the HPLC for analysis. All operations were perfomed in a darkroom because nifuratel is susceptible to photochemical decomposition.

Calibration curves and quality control samples

Calibration curves were prepared by spiking different 1-mL sample plasma portions with the proper volume of the previously mentioned working solutions to produce the calibration curve points equivalent to 0.2, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 40.0 ng/mL of nifuratel. Each sample contains 50 µL of 10 µg/mL of IS. Samples containing zero plasma that were used in each run were prepared containing 50 µL of a 10-µg/mL sample of IS. In each run, a blank plasma sample (no IS) was also analyzed.

Quality control (QC) samples for the determination of accuracy and precision of the method were prepared also at low (0.5 ng/mL), medium (5.0 ng/mL), and high (20.0 ng/mL) concentrations.

Method validation

The method's specificity was evaluated by screening six different batches of healthy human plasma. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic and spectroscopic conditions and compared with those obtained in an aqueous solution of the analyte at a concentration near the lower limit of quantitation (LLOQ) (5).

Linearity was tested for the range of concentrations of 0.20–40.0 ng/mL. For the determination of linearity, standard calibration curves of at least seven points (non-zero standards) were used. In addition, a blank plasma sample was also analyzed to confirm the absence of interferences, this sample was not used to construct the calibration curve. The acceptance criteria for the correlation coefficient was 0.998 or more, otherwise the calibration curve was rejected. Five replicate analyses were performed.

The intraday precision was measured by analyzing five spiked samples of nifuratel at each QC level (0.5, 5.0, and 20.0 ng/mL). The interday precision was determined over 5 days by analyzing 15 QC samples. The acceptance criteria for precision and accuracy deviation values should be within 15% of the actual values.

The extraction yield (or absolute recovery) was determined by comparing the nifuratel IS peak area ratios obtained following the outlined extraction procedure with those obtained from the unextracted pure authentic standard solution. This procedure was repeated for the three different concentrations of nifuratel added, namely 0.50, 5.0, and 20.0 ng/mL.

For sensitivity determination, the lowest standard concentration in the calibration curve was considered as the LLOQ and was to meet the following criteria: LLOQ response should be 10 times the response of the blank and the LLOQ response should be identifiable, discrete, and reproducible with a precision of 20%.The limit of of detection (LOD) is defined as the sample concentration resulting in a peak area of three times the noise level.

The freezing test is essential because nifuratel is an unstable chemical. To evaluate stability on repeat analysis of samples, freeze-and-thaw stability was determined for three concentrations of nifuratel in plasma. QC plasma samples were tested after three freeze (–20°C) and thaw (room temperature) cycles.

Clinical study

This was an open, randomized, and balanced crossover study in 24 healthy Chinese volunteers. All volunteers were separated randomly into two groups and required to fast for 12 h. Each volunteer took a single 400 mg oral dose of nifuratel in a test or reference tablet. Blood samples (5 mL) for assay of plasma concentration of nifuratel were collected predose and then at 0, 0.33, 0.66, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, and 11 h after oral administration of the tablets. Blood was taken using lithium heparin tubes and centrifuged at 3000 rpm^{-1} for 10 min to obtain plasma for assay of nifuratel, which was then frozen at –20°C until analysis.

Results and Discussion

Selection of IS

IS was applied to get high accuracy when the nifuratel was determined in human plasma. Furazolidone, nitrofurantoin, and dipyridamole were investigated to find the most suitable one. Furazolidone and nitrofurantoin are very similar to nifuratel in terms of chemical structure, but they are not stable. Also, their retention times were not optimal. Dipyridamole was adopted in the end because of its stability, suitability of retention action, good extraction efficiency, and lower endogenous interference.

Separation

Nifuratel and IS were well separated from the biological background under the described chromatographic conditions with retention times of 6.6 and 9.5 min, respectively. The peaks had a

good shape and resolved completely. No interference from the plasma matrix was observed. The typical chromatograms of blank plasma, blank plasma spiked with nifuratel and IS, as well as the volunteer's plasma, are shown in Figure 2.

Assay performance

Assay performance of the present method was assessed by the following criteria: linearity, precision, recovery, stability, LOD, and LOQ.

The calibration curve was obtained by analyzing five samples for each of the eight test concentrations (0.2, 0.5, 1.0, 2.5, 5.0, 10.0, 20.0, and 40.0 ng/mL). The curve was linear over the whole range tested (0.2–40.0 ng/mL) and described by

$$f = 0.011 + 0.0283\,C \qquad \text{Eq. 1}$$

where r is 0.9996, n is 5, and f corresponds to the peak area ratio of nifuratel and the IS and C to the concentration of nifuratel added to the whole range.

To evaluate the precision of the method, samples at three

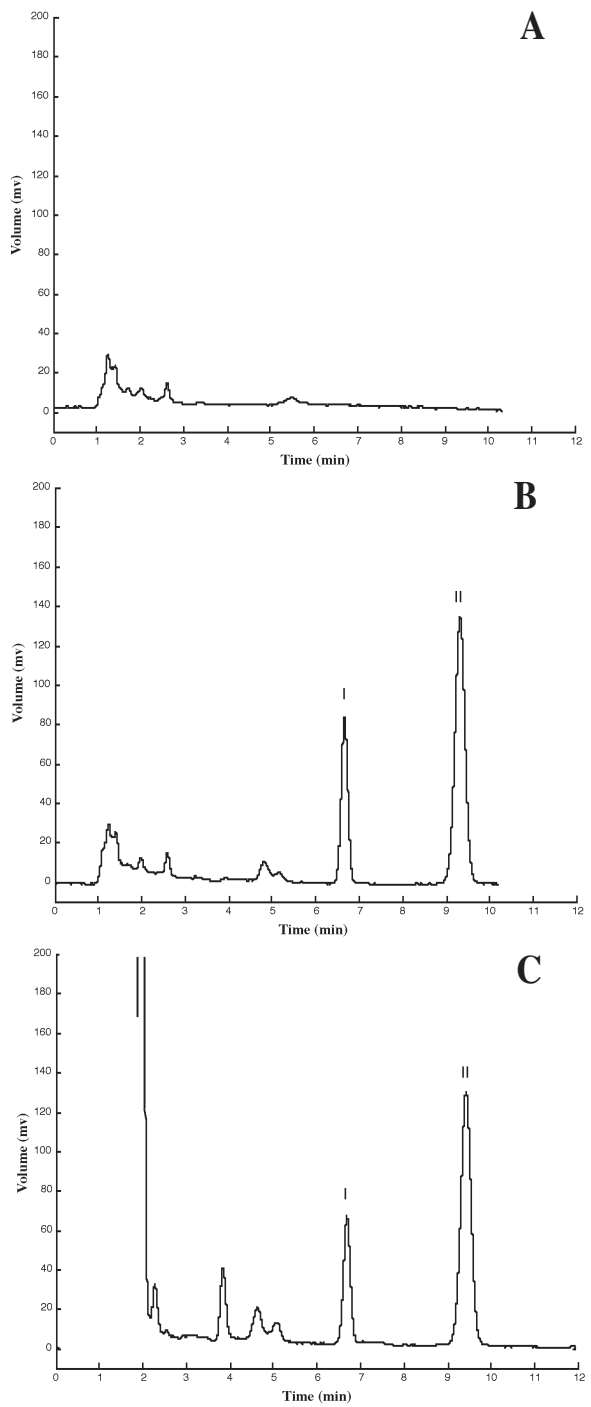

Figure 2 Blank plasma (A), plasma spiked with nifuratel standard (B), and subject plasma after oral nifuratel (C) [nifuratel (I) and IS (II)].

Table I. Precision of the Method with Determination of Nifuratel (n = 5)

Added concentration (ng/mL)	Intraday		Interday	
	Mean (ng/mL)	RSD (%)	Mean (ng/mL)	RSD (%)
0.5	0.4994	7.32	0.5031	7.83
5.0	4.9872	2.28	4.9570	2.62
20.0	19.8365	1.89	19.6388	1.71

Table II. Extraction of Recovery of Nifuratel in Human Plasma (n = 5)

Added (ng/mL)	Recovery (%)	RSD (%)
0.5	89.9 ± 4.0	4.5
5.0	92.6 ± 2.2	2.3
20.0	94.7 ± 0.7	0.8

Table III. Results of Freeze and Thaw Stability Study

Concentration (ng/mL)	Mean (ng/mL)	RSD (%)
0.5	0.49	7.23
5.0	4.87	2.84
20.0	19.91	1.18

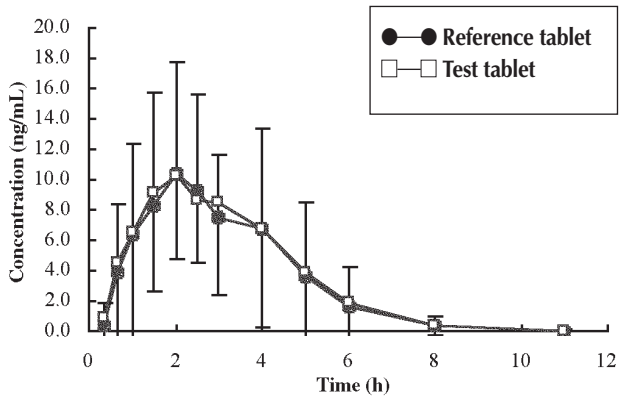

Figure 3 Plasma concentration relationships of nifuratel after adminstration of a 400-mg dose in 24 volunteers ($\bar{x} \pm s$).

Table IV. Pharmacokinetic Parameters of Nifuratel in 24 Volunteers Following Oral Administration ($\bar{x} \pm s$)

Parameters	Test	Reference
$AUC_{0-11\ h}$/μg × h × L^{-1}	38.50 ± 23.81	36.58 ± 20.85
AUC0–∞/μg × h × L^{-1}	39.67 ± 24.33	37.61 ± 21.07
Cmax/μg × L^{-1}	15.26 ± 8.43	14.89 ± 8.07
Tmax/h	2.2 ± 1.1	2.4 ± 0.9
$T_{1/2}$/h	0.89 ± 0.35	0.94 ± 0.39

different concentrations were analyzed both on the same day and different days. The results are shown in Table I.

The absolute recovery of nifuratel was determined at three different concentration levels (low, medium, and high) by comparing extracted versus unextracted samples. The results are shown in Table II. Freezing test data listed in Table III shows that nifuratel and IS were stable at least within 10 days under said conditions. The LOD and LOQ in this investigation were 0.1 and 0.2 ng/mL, respectively.

Pharmacokinetic study

The method was applied to the analysis of plasma samples obtained after the administration of a single oral dose of 400.0 mg nifuratel preparation to 24 healthy volunteers participating in bioequivalence studies. Figure 3 depicts the mean (24 volunteers) plasma concentration-time curve of nifuratel after oral administration of test tablets and reference tablets. As is shown, the two curves are almost overlapping.

Pharmacokinetic parameters of the test tablet and the reference tablet are listed in Table IV. The results demonstrate that the two formulations of nifuratel were bioequivalent by variance analysis, test of confidence interval and 90% confidence limit. The relative bioavailability of the test table was 104.0% ± 16.5%.

Conclusion

The described method was established as a rapid, sensitive, and selective reverse-phase HPLC analysis of nifuratel in human plasma with UV detection. A simple two-step analytical procedure involving liquid–liquid extraction and evaporation followed by injection of the clean extract into the HPLC. Plasma levels obtained after oral administration were low but sufficient enough to allow the calculation of the pharmacokinetic parameters probably because of rapid metabolization.

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