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Research Article

STEADY-STATE PHARMACOKINETICS OF IMMEDIATE-RELEASE AND CONTROLLED-RELEASE METRONIDAZOLE TABLETS

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ABSTRACT

The objective of the study was to evaluate the plasma levels of metronidazole in Controlled Release (CR) tablets given once daily and comparing it with Immediate Release (IR) metronidazole tablets given thrice daily, in twelve healthy adult male human subjects, dosed under fasting conditions for 5 days till a steady state was reached. On the 6th day, after dosing, the elimination of the drug was studied.

A high-performance liquid chromatographic (HPLC) method for the pharmacokinetic analysis of metronidazole was developed to quantify metronidazole in plasma samples. Extraction of analyte and Internal Standard (Tinidazole) from plasma involved protein precipitation with 20% trichloroacetic acid solution in methanol. Chromatographic separation was achieved with Kromasil-100 C_{18} (250 × 4.6 mm) 5 µm column and 50 mM KH_2PO_4 with 0.1% Triethylamine (pH 6.0 with dil. H_3PO_4): acetonitrile (85:15, v/v) as the mobile phase with flow rate 1ml/min and UV detection at 320 nm. The calibration curve was linear in the concentration range 2.0 – 80.0 µg/ml. When the pharmacokinetic parameters of metronidazole in the two formulations were calculated and compared statistically using analysis of variance (ANOVA), they were similar, without any statistically significant difference. Pharmacokinetic parameters including $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, C_{max}, T_{max}, $T_{1/2}$ and K_{el} were determined during saturation phase and elimination phase. The test and reference preparations of metronidazole were found to have comparable bioavailability.

Keywords*:* Protein precipitation, Bioavailability, Metronidazole, Steady-state, Pharmacokinetics.

INTRODUCTION

Metronidazole [2-Methyl-5-nitroimidazole-1-ethanol] is a nitroimidazole derivative, which is widely used in the long term therapy for trichomoniasis, amoebiasis and giardiasis[1]. Plasma half-life of metronidazole is 8 hours and longer in neonates and patients with renal failure[2-4]. Since its biological half-life is about 8 hours; multiple daily dosing (approximately 3 - 4 times/day) is necessary for the maintenance of its therapeutic effect throughout the day. Controlled release systems are designed to release the drug for longer periods of time as compared to immediate release and have predictable release kinetics. The advantages of controlled release over immediate release tablets include reduced dosing frequency, better patient compliance, reduced GI side effects, less fluctuating plasma drug levels, improved efficacy/safety ratio, more uniform drug effect and at times can reduce the total dose required[5-8].

Till date, large number of HPLC methods involving liquid extraction and solid phase extraction for the determination of metronidazole in body fluids have been described[9-19]. Subsequently, Galmeir et al[20] and Emami et al[21] have reported HPLC methods involving protein precipitation. These methods are comparatively simpler, faster and reliable. Referring to the available literature, we report a similar method with an application to estimate the drug in the subject samples collected to study comparative bioavailability of IR and CR formulations.

We report a randomized, open - label, two - way crossover pharmacokinetic study to compare the systemic availability and steady-state pharmacokinetics of a controlled-release metronidazole formulation with that of a conventional metronidazole immediate-release tablet, in healthy volunteers.

MATERIALS AND METHODS

Materials

Metronidazole (99.75%) was obtained from the Nicholas Piramal India Ltd., India. Tinidazole (99.70%) was supplied by Aarti drugs Ltd., India. Methanol and acetonitrile of HPLC grade were purchased from E-Merck. (India). Trichloroacetic acid of analytical grade was also purchased from E-Merck (India). Potassium dihydrogen orthophosphate (KH_2PO_4) and Orthophosporic acid (H_3PO_4) of analytical grade were procured from Qualigens. Triethylamine (TEA) of analytical grade was procured from S. D. Fine Chem. Ltd. HPLC grade water, used for dilution, was prepared in-house using 'mini quartz distiller' of Qualigens.

Instruments and method

Chromatographic separation was performed on HPLC system (Agilent 1100 series) equipped with G1379A degasser, a G1311A quaternary pump, a G1330B autosampler, a G1330B autosampler thermostat-ALS Therm, a G1316A column oven (COLCOM) and a G1314A UV – Visible detector. The data acquisition was carried on Chemstation software version 10.01.

The HPLC column used was Kromasil 100 C_{18} (250 × 4.6 mm) 5 µm. The mobile phase consisted of 50 mM KH_2PO_4 with 0.1% TEA (pH 6.0 with dil. H_3PO_4): acetonitrile (85:15, v/v). The flow rate was 1ml/min and the eluate was monitored with UV detection at 320 nm.

Study products

The developed method was applied to study steady state pharmacokinetics of two tablet formulations of metronidazole in 12 healthy subjects, under fasting conditions: metronidazole CR (600 mg × 2) tablets (Test Preparation) and metronidazole IR (400 mg × 3) tablets (Standard Preparation) both manufactured by Nicholas Piramal India Ltd, Mumbai-63, Maharashtra, India. The study was conducted at C. B. Patel Research centre in accordance with the principles stated in the declaration of Helsinki. Approval was obtained from the ethics committee of the C. B. Patel Research Centre's SPC Biokinetic Study Centre (Ref.: METZ-SS\100907\Nic).

Drug administration and blood sampling

The study was based on open label, balanced, randomized, two treatment, two sequences, two periods, single dose, and crossover design. Randomized subjects received three 400 mg tablets of Metronidazole IR, at 8 hours interval and two 600 mg tablets of Metronidazole CR once daily for successive 5 days till a steady state was achieved. Blood samples were analysed for 5 days to confirm plasma saturation. On sixth day after dosing (test/standard), blood samples were drawn at 2, 4, 6, 8, 12, 16, and 24 hours interval to study elimination of drug post steady state. After a washout period of seven days, the volunteers were crossed over with identical

treatment. Approximately 5 ml of blood samples were drawn into heparinized tubes. The blood samples were centrifuged at 3000 rpm for 15 min. Plasma samples were separated and kept frozen at -20 ºC until quantitative analysis.

Pharmacokinetics and statistical analysis

Pharmacokinetic analysis was performed by use of Winolin version 5.2 computer program. Data obtained from individual volunteers was subjected to non-compartmental pharmacokinetic analysis. Various pharmacokinetic parameters such as area under curve (AUC), peak plasma concentration (C_{max}), time to reach the peak (T_{max}), elimination rate constant (K_{el}), elimination half-life ($T_{1/2}$) and absorption efficiency were determined for each volunteer. The elimination rate constant (K_{el}) was obtained as the slope of the linear regression of the log-transformed concentration versus time data in the terminal portion of the curve. AUC_{0-t} was determined by linear trapezoidal rule. $AUC_{0-\infty}$ was calculated as $AUC_{0-t}+C_{last}/K_{el}$, where C_{last} is the last measured concentration. Pharmacokinetic parameters such as C_{max} and T_{max} were determined by the inspection of the individual plasma-concentration time profiles.

Standard preparation and quality control samples

The stock solutions of analyte (metronidazole) and IS (tinidazole) were prepared by dissolving accurately weighed standard compound in methanol to give final concentration of 2 mg/ml and 1 mg/ml respectively. The stock solution was diluted to suitable concentrations using acetonitrile and HPLC grade water (80:20, v/v) for spiking into plasma to obtain calibration curve (CC) standards, with concentrations of 2.0, 4.0, 8.0, 10.0, 20.0, 40.0, 60.0 and 80.0 µg/ml. The linear regression of the peak area ratio of analyte/IS vs. concentration was obtained. A weighted ($1/concentration^2$) equation was used to obtain calibration curve. The regression equation of the calibration curve was then used to calculate the plasma concentration. The back calculated values of the concentrations were statistically evaluated.

Quality control samples were prepared using the stock solution. Four levels of QC samples in plasma were 2.0 µg/ml (lower limit of quantitation), 6.0 µg/ml (low), 35.0 µg/ml (medium), and 65.0 µg/ml (high) for the analyte. All stock solutions and working standard solutions were stored in refrigerator below −20 °C.

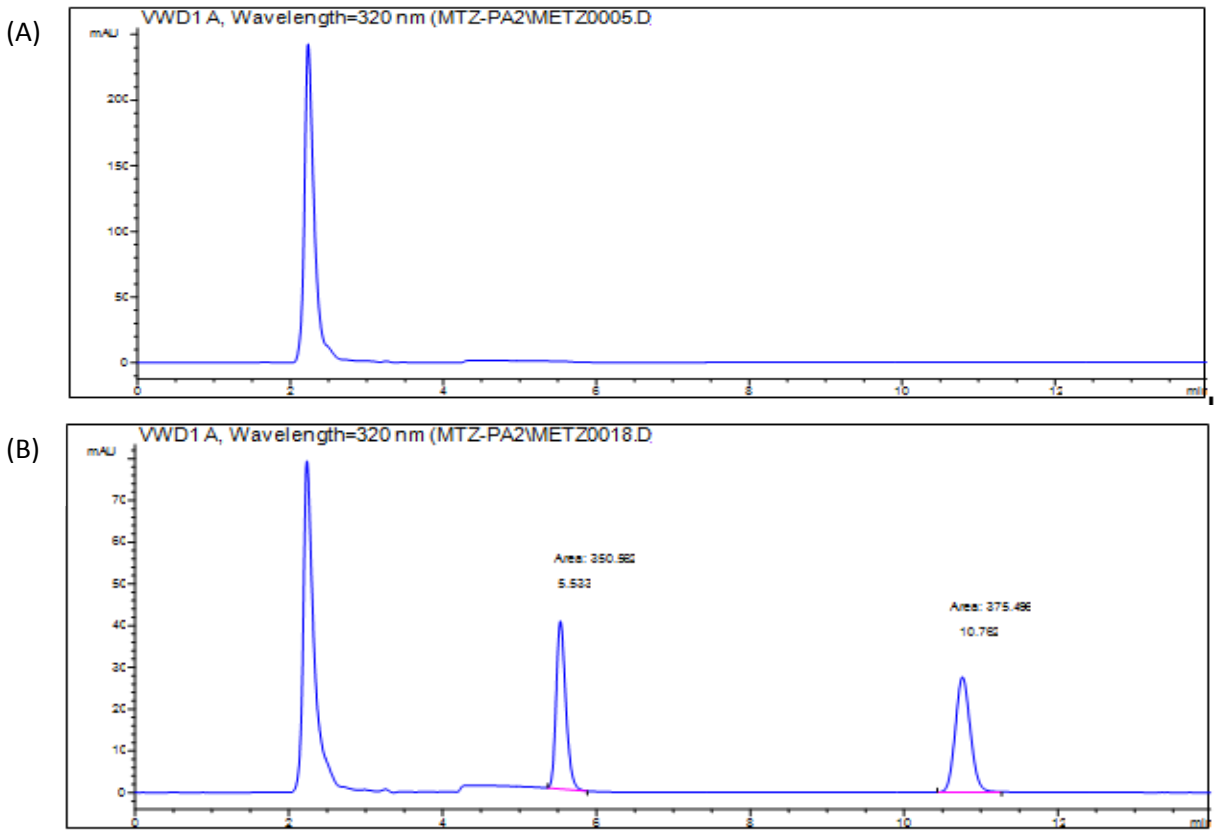
Sample preparation

Frozen human plasma samples were thawed at ambient temperature and 1 ml of samples were placed in glass tubes. 50 µl of internal standard, working solution (1 mg/ml Tinidazole), was added and vortex mixed for 30 s. 50 µl of HPLC grade water and 1000 µl of 20% Trichloroacetic acid solution, in methanol, were added. After a thorough vortex mixing for 60 s, samples were centrifuged at 4000 rpm for 10 min. 0.5 ml of supernatant was collected and 5 µl was injected into HPLC system.

RESULTS AND DISCUSSION

Method validation

Fig. 1 shows representative chromatograms of extracted blank plasma samples. The retention times for metronidazole and tinidazole (I.S.) were 5.7 and 11.3 min, respectively. No endogenous interference was observed with either metronidazole or the I.S. The calibration curve was drawn by plotting the ratio of area under the peak versus concentration, which was linear over the range of 2.0–80.0 µg/ml with mean correlation coefficient 0.9995. The intra-day and inter-day precision of the assay was estimated by analyzing four different concentrations of metronidazole in plasma (values are given in Table 1). The results show good reproducibility for the proposed method, with coefficients of variation (CV) for intra-day and inter-day less than 3.48 and 3.34 %, respectively. The limit of quantification (LOQ) for metronidazole was 2.0 µg/ml (CV < 3.38%). Recovery was estimated by comparing the peak area ratio of the metronidazole to I.S. in the samples that were spiked with the analytes prior to extraction, with the samples to which the analytes were added post-extraction. The mean overall recovery of metronidazole was 62.93% with a precision of 2.074% and that for I.S., it was 13.9% with a precision of 1.660%. These results indicate that the LC analytical method developed during the present study is appropriate for the pharmacokinetic analysis of metronidazole, with good sensitivity and precision.



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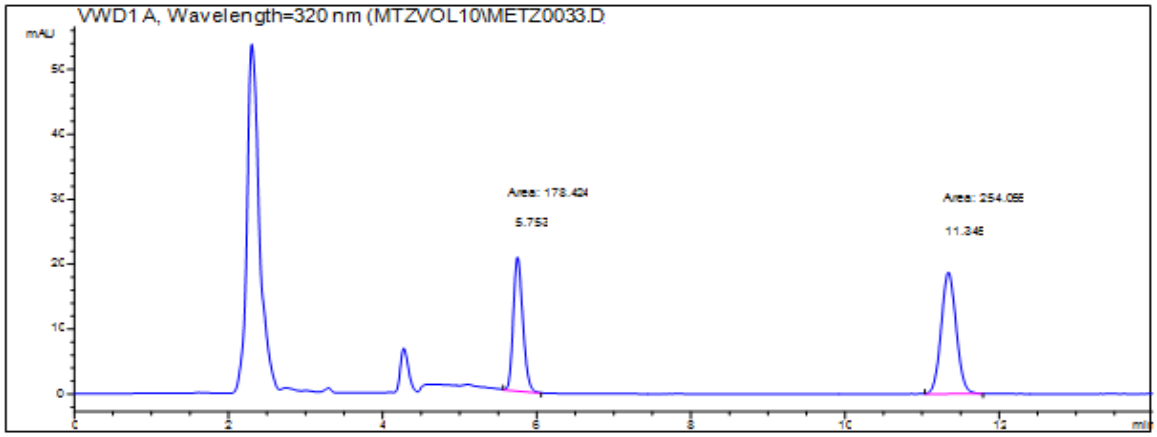

Fig. 1: Representative chromatograms of plasma extracts. (A) Drug-free human plasma; (B) blank plasma spiked with 35.30µg/ml metronidazole and I.S; (C) Plasma extract from a subject 4 hours post dose(600mg x 2 Metronidazole), value found = 22.53µg/ml metronidazole in plasma. Metronidazole – 5.75 min, Tinidazole (I.S.) – 11.34 min

Table 1: Intra-day and inter-day coefficient of variation and accuracy for the determination of metronidazole in human plasma

Theoretical concentration (µg/ml)	Intra-day (n=6)			Inter-day (n=18)		
	Mean concentration (µg/ml)	CV (%)	Accuracy (%)	Mean concentration (µg/ml)	CV (%)	Accuracy (%)
2.0	2.06	2.75	100.80	2.024	3.34	99.17
6.0	6.18	1.27	101.80	6.141	1.08	101.13
35.0	36.80	3.48	104.24	37.01	2.83	104.84
65.0	68.29	2.79	104.46	68.46	2.14	104.72

Bioavailability Study

Metronidazole CR (600 mg × 2) tablets (Test preparation) and Metronidazole IR (400 mg × 3) Flagyl tablets (Standard preparation) produced a steady state within 5 days. The comparative graph for mean (± S. D.) plasma concentration-time profiles obtained during saturation phase and after a single oral dose on 6th day of either formulation in tablet form during elimination phase is shown in Fig. 2

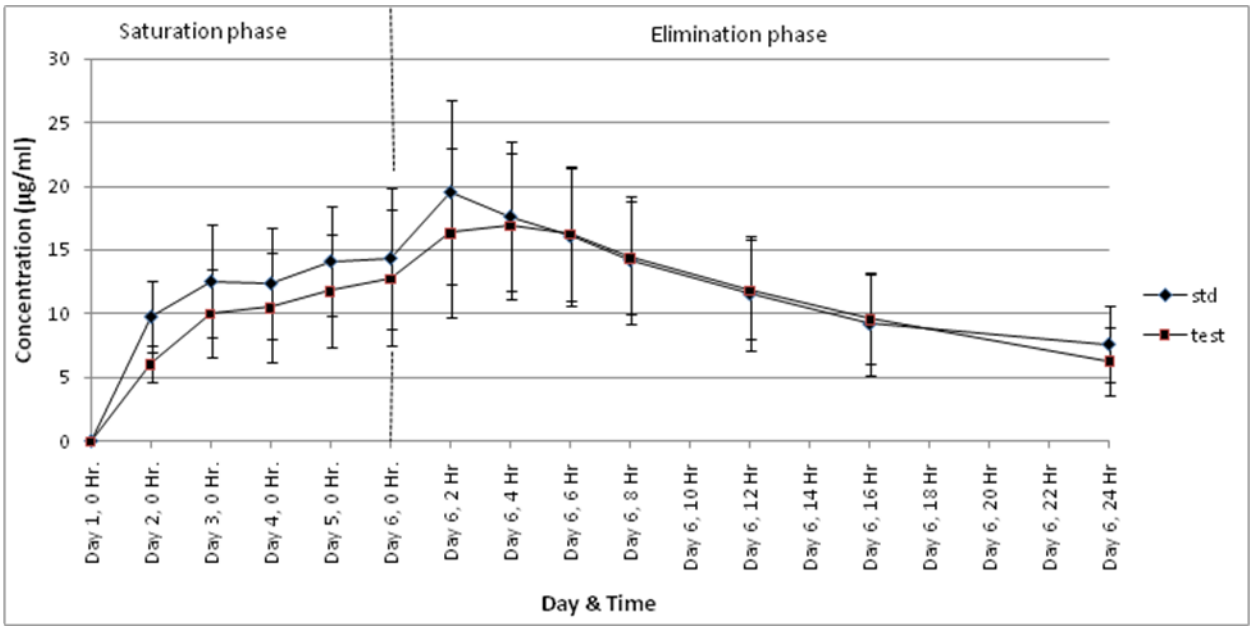

Fig. 2: The comparative mean (± S.D.) plasma concentrations of Metronidazole vs. Day and Time in Twelve healthy human subjects during saturation phase for 5 days and after administration of single dose of 600 mg (Test) and 400 mg (Standard) during elimination phase on 6th day

Intake of either formulation produced similar plasma concentration-time profiles curve. The pharmacokinetic parameters of elimination phase of the two formulations are shown in Table 2. Pharmacokinetic parameters values obtained for the Test formulations were close to those of the standard formulation and there were no statistically significant differences

between the two products. AUC $_{(o-t)}$ obtained for standard and test formulation during saturation phase is 284.77 µg.h/ml and 292.12 µg.h/ml respectively. During elimination phase the standard formulation showed mean peak plasma concentration (C_{max}) 21.71 µg/ml and that of the test formulation was 16.09 µg/ml. Similarly, mean T_{max} value for standard formulation is 2.16 h and that of the test formulation is 4.0 h. The difference was expected as the test preparation was controlled release and standard preparation was instant release preparation. The two brands of metronidazole were well tolerated by the volunteers in both phases of the study, with no adverse reactions and all volunteers completed the study.

Table 2: Mean pharmacokinetic parameters for metronidazole in Test and Standard preparation during elimination phase

Pharmacokinetic parameters	TEST Mean ± S. D.	Standard Mean ± S. D.
C_{max} (µg/ml)	16.096 ± 5.487	21.717 ± 6.515
T_{max} (h)	4.00 ± 1.907	2.167 ± 0.578
$T_{1/2}$ (h)	15.413 ± 5.1461	25.228 ± 29.9679
AUC_{0-t} (µg.h/ml)	283.418 ± 106.099	296.766 ± 93.812
$AUC_{0-\infty}$ (µg.h/ml)	458.215 ± 226.58	545.419 ± 303.530
K-elimination	0.0498 ± 0.0162	0.0428 ± 0.0160

CONCLUSIONS

The developed HPLC method for the determination of metronidazole was fast, precise and sensitive. The percent bioavailability of the standard preparation (metronidazole IR 400 mg tablets) of Nicholas Piramal India Ltd., (100%), with that of the test preparation metronidazole CR 600 mg × 2 tablets (O.D.) of Nicholas Piramal India Ltd., was found to be 95.50% at t = 24 h which was well within the pharmaceutical tolerance limits of 80 – 120%. We can conclude that the Test preparation has comparable bioavailability with that of Standard formulation (Flagyl, manufactured by then Nicholas Piramal India Ltd and now Abbott Healthcare Private Limited) with respect to metronidazole.

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