



## Bioequivalence Between Two Metronidazole Formulations

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**SUMMARY.** Two tablet formulations of 400 mg metronidazole were evaluated for their bioequivalence in twenty three healthy male volunteers (metronidazole, from EMS-Sigma Pharma, Brazil, as the test formulations versus Flagyl® from Rhodia, Brazil, as the reference formulation). A single 400 mg oral dose of each preparation was administered in a randomized two-way crossover design with a seven-day interval between doses. Metronidazole plasma concentrations were determined by the HPLC-UV detection. Pharmacokinetic parameters obtained included  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ ,  $C_{max}$ ,  $T_{max}$ ,  $t_{1/2}$ , and  $K_e$ . Geometric mean of metronidazole / Flagyl® 400 mg individual percent ratio was 91.04% for  $AUC_{0-t}$ , 92.05% for  $AUC_{0-\infty}$ , and 98.09% for  $C_{max}$ . The 90% confidence intervals were 85.12 - 97.38%, 85.90 - 98.64% and 90.19 - 106.69 respectively. Since the 90% CI for the  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , and  $C_{max}$  were within the 80-125% interval proposed by ANVISA and by the Food and Drug Administration, and it was concluded that metronidazole 400 mg tablet from EMS-Sigma Pharma was bioequivalent to Flagyl® tablet 400 mg with regard to both the rate and extent of absorption.

**RESUMEN.** "Estudio de Bioequivalencia de Dos Formulaciones de Metronidazol". Se realizó un estudio de bioequivalencia entre dos formulaciones de Metronidazol 400 mg en comprimidos (EMS-Sigma Pharma como ensayo versus Flagyl - Rhodia como referencia). Una dosis única de 400 mg de cada formulación fue administrada en dos períodos cruzados con un intervalo de siete días entre los dos períodos, a un total de 23 voluntarios jóvenes y sanos. Se utilizó un ensayo por HPLC-UV para determinar las concentraciones plasmáticas de Metronidazol. Los parámetros farmacocinéticos determinados fueron: área bajo la curva de concentraciones vs. tiempo de cero a t ( $ABC_{0-t}$ ), área bajo la curva de concentraciones vs. tiempo desde cero a infinito ( $ABC_{0-\infty}$ ), concentración plasmática máxima ( $C_{max}$ ), tiempo máximo ( $T_{max}$ ), tiempo de vida media ( $t_{1/2}$ ) y constante de velocidad de absorción ( $K_e$ ). La razón de los promedios geométricos del Metronidazole EMS / Flagyl 400 mg fueron 91,04% para  $ABC_{0-t}$ , 92,05% para  $ABC_{0-\infty}$  y 98,09% para  $C_{max}$ . Los intervalos de confianza de 90% fueran 85,12 - 97,38%, 85,90 - 98,64% y 90,19 - 106,69 respectivamente. Los IC de 90% para  $ABC_{0-t}$ ,  $ABC_{0-\infty}$  y  $C_{max}$  estaban en el rango de 80-125% como ANVISA y la FDA recomienda. En base a nuestros resultados se concluye que las dos formulaciones son bioequivalentes, asumiéndose que tendrían igual eficacia clínica.

### INTRODUCTION

Metronidazole [1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole], CAS 443-48-1, is active against several protozoa including *Entamoeba histolyti* - *ca*, *Giardia lamblia*, *Trichomonas vaginalis*, *Balantidium coli* and *Blastocystis hominis*. It also has activity against *Gardnerella vaginalis*, *Helicobacter pylori* and some *Spirochaetes*. Metronidazole has well-established bactericidal activity against obligate anaerobic bacteria *in vitro*, including the Gram-negative organisms

*Bacteroides fragilis* and other *Bacteroides spp.*, *Fusobacterium spp.* and *Veillonella spp.*, and the Gram-positive organisms *Clostridium difficile*, *Clostridium perfringens*, besides *Peptococcus spp.* and *Peptostreptococcus spp.* <sup>1-5</sup>.

Bioequivalence and bioavailability of drug products are usually assessed by means of univariate statistical analysis of the important parameters, such as the peak drug concentration ( $C_{max}$ ), the time to reach this concentration ( $T_{max}$ ) and the area under the drug concentra-

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tion curve (AUC). Each parameter is tested separately by ANOVA - Analysis of variance and the 90% confidence interval method<sup>1,6</sup>, among others.

This paper compares the pharmacokinetic profile of a metronidazole 400 mg tablet test formulation from EMS-Sigma Pharma, to a metronidazole reference formulation (Flagyl® 400 mg - Rhodia), Using analysis of variance (ANOVA).

## EXPERIMENTAL

### Subjects

Twenty three healthy male volunteers ranging in age from 18 to 42 years (mean  $\pm$  SD: 24.57  $\pm$  6.81), and in body weight from 53 to 80 Kg (mean  $\pm$  SD: 66.17  $\pm$  8.04) participated in the study (Twenty four volunteers were recruited, but one volunteer does not participated). All subjects had a normal medical history and revealed no abnormalities on physical examination, routine laboratory tests, and electrocardiogram. Written informed consent was obtained from each subject and the clinical protocol was approved by the Human Ethics Committee of the Pernambuco Federal University, Brazil.

### Clinical Protocol

All subjects were nonsmokers and were instructed not to drink caffeine or alcohol containing beverages for at least 10 hours before and during the study day. The study was performed according to an open, randomized, two-period crossover design with an one-week washout period between each treatment. During each period, the subjects were hospitalized at 10 p.m., having already eaten a normal evening meal. After a 10-hour fast, they received a single oral dose of Metronidazole 400 mg EMS-Sigma Pharma or Flagyl® 400 mg - Rhodia tablets with 200 ml tap water. After fasting for 4 hours after drug administration, they received a standardized meal. An indwelling venous catheter was then introduced and kept into each subject with a diluted heparin solution. After zero-time blood samples, 5ml blood samples were collected into EDTA - containing tubes at 0.50, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0 and 36.0 h. Samples were centrifuged at 4000 rpm at room temperature for five minutes, then plasma was removed and frozen until time of assay.

### Material

Metronidazole and tinidazole were purchased from LAPPS (UFRS - Brazil) and Sigma, respectively. Analytical grade phosphoric acid,

potassium carbonate, potassium dihydrogen phosphate, trichloroacetic acid (TCA) and HPLC - grade tetrahydrofuran were purchased from Merck. HPLC - grade acetonitrile and methanol were obtained from Carlo Erba. Metronidazole 400 mg tablets (lot n° 993166, expiration date 06/2001) were used as the test formulation (EMS-Sigma Pharma, Brazil) and Flagyl® 400 mg tablets (lot n° 505, expiration date 10/2001) as the reference formulation.

### Equipment

A Waters high performance liquid chromatography was used (Waters, Division of Millipore, Milford, MA, U.S.A.), which included: a Waters Model 510 solvent delivery pump, a Waters model 717 autosampler and a Waters Model 490E ultraviolet (UV) - visible detector.

### Assay Validation

The HPLC method was specific and yields a quantification limit of 0.2  $\mu$ g/ml. Calibration curves were linear in the range from 0.2 to 20.0  $\mu$ g/ml. The recovery was 93%  $\pm$  6% S.D. (n=6) at the level of 0.5  $\mu$ g/ml. The precision and accuracy of this method were satisfactory with coefficients of variation ranging from 0.6 to 5.93% at the levels of 0.5, 10 and 16  $\mu$ g/ml.

### Plasma Metronidazole Levels

Plasma concentrations were measured by HPLC with UV detection, as described by some authors<sup>7</sup>. The aliquots of plasma (500  $\mu$ L) were pipetted into microtubes. After the addition of 40  $\mu$ L of the internal standard solution (tinidazole 10  $\mu$ g/mL, ethanol solution), deproteination was carried out by adding 100  $\mu$ L of TCA 25%. The tubes were vortexed for 2 min. following centrifugation for 5 min at 2000 rpm. The supernatant layer was transferred to a microtube containing 5 mg of anhydrous potassium carbonate. Each tube was vortex mixed for 30 seconds, followed by centrifugation at 2000 rpm for 5 min. A 10  $\mu$ L aliquot of the supernatant layer was then injected into the HPLC apparatus. Chromatographic analyses were performed with a Waters spherisorb 5  $\mu$ m ODS 2 150 x 4.6 mm column. The mobile phase was composed of dipotassium hydrogen phosphate buffer (5 mM, pH 4.7) - methanol - tetrahydrofuran (82.5/16.5/1). Mean metronidazole plasma concentrations obtained after a single oral administration of each metronidazole tablet formulation are plotted against time.

Parameter	Metronidazole - EMS		Flagyl® - Rhodia	
	GM	90% CI	GM	90% CI
AUC <sub>0-t</sub> (µg.h.mL <sup>-1</sup> )	208.53	195.71 - 222.18	228.22	212.16 - 245.49
AUC <sub>0-∞</sub> (µg.h.mL <sup>-1</sup> )	228.06	213.82 - 243.17	246.81	227.88 - 267.32
C <sub>max</sub> (µg.mL <sup>-1</sup> )	19.56	18.02 - 21.22	19.84	18.03 - 21.82
K <sub>e</sub> (h <sup>-1</sup> )	0.0786	0.0652 - 0.0948	0.0731	0.0687 - 0.0778
t <sub>1/2</sub> (h)	9.74	9.06 - 10.47	9.47	8.90 - 10.08
T <sub>max</sub> (h)	1 (median)	0.5 - 4 (range)	1 (median)	0.5 - 4 (range)

**Table 1.** Mean pharmacokinetic parameters for the 23 volunteers after administration of the two metronidazole formulations. GM - geometric mean; CI - confidence interval; AUC - area under drug concentration curve; C<sub>max</sub> - peak drug concentration; K<sub>e</sub> - elimination rate constant; t<sub>1/2</sub> - terminal half-time; T<sub>max</sub> - time for reach peak concentration.

### Pharmacokinetic analysis

Comparison between the absorption rates was carried out regarding both the maximum plasma concentration (C<sub>max</sub>) and its corresponding occurrence time (t<sub>max</sub>). These parameters were obtained directly from the experimental volunteer curves. The relative extent of absorption, i.e. the amount of drug reaching the systemic circulation, was expressed by the area under the metronidazole plasma concentration *vs.* time curves from 0-36 h (AUC<sub>0-t</sub> and AUC<sub>0-∞</sub>). AUC<sub>0-t</sub> parameter was calculated by application of the linear trapezoid rule. AUC<sub>0-∞</sub> parameter was estimated by assuming an exponential profile kinetic after oral metronidazole dose, *i.e.* AUC<sub>0-∞</sub> = AUC<sub>0-last</sub> + C<sub>last</sub> / K<sub>e</sub>, where C<sub>last</sub> refers to the last measurable point at the plasma concentration-time curve. The elimination rate constant (K<sub>e</sub>) was estimated by linear regression from the points describing the elimination phase in a log-linear plot. Half-life (t<sub>1/2</sub>) was derived from this rate constante (t<sub>1/2</sub> = 0.693 / K<sub>e</sub>).

### Statistical analysis

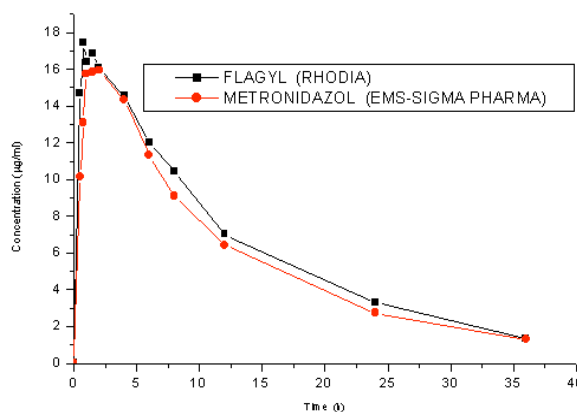
Analysis of variance (ANOVA) between both formulations was assessed by calculating the standard 90% confidence intervals of the geometric mean ratio test/reference, for AUC<sub>0-t</sub> and C<sub>max</sub> data with a parametric test (ANOVA for log-transformed data). The software used was the Program for two-Period Crossover Studies<sup>8</sup>, version 6.21, employed by our group in the lamivudine bioequivalence study<sup>9</sup> and WinNonlin<sup>10</sup>.

## RESULTS AND DISCUSSION

Metronidazole tablets were rapidly absorbed after oral administration, and well tolerated by the subjects. No clinical side effect was observed in any volunteer during the entire study.

For the standard curves for metronidazole in plasma, the coefficient of determination for adjusted experimental points was 0.9996. All the standard curve data indicated that the proposed HPLC method had good precision and accuracy, and metronidazole maintained stability in plasma under storage and assay conditions. Fig. 1 depicts the corresponding average plasma concentration-time curve for the metronidazole test and reference formulations.

A good agreement between test and reference mean pharmacokinetic parameters is observed (Table 1). The geometric means of C<sub>max</sub> were 19.56 µg/ml (18.02 - 21.22 µg/ml with 90% CI) and 19.84 µg/ml (18.03 - 21.82 µg/ml with 90% CI) for the test and reference metronidazole formulations, respectively. Geometric mean values of AUC<sub>0-t</sub> were 208.53 µg.h/ml (195.71 - 222.18 µg.h/ml with 90% CI) and 228.22 µg.h/ml (212.16 - 245.49 µg.h/ml with 90% CI) for the test and reference products, respectively.



**Figure 1.** Mean plasma concentration versus time curves after single dose administration of two metronidazole 400 mg tablets: Test (Metronidazole from EMS-Sigma Pharma) and Reference (Flagyl from Rhodia).

Parameter	GM (%)	90% CI
AUC <sub>0-t</sub>	91.04	85.12 - 97.38
AUC <sub>0-</sub>	92.05	85.90 - 98.64
C <sub>max</sub>	98.09	90.19 - 106.69

**Table 2.** Statistical analysis for AUC<sub>0-t</sub>, AUC<sub>0-</sub>, C<sub>max</sub>, percent Test/Reference ratios of geometric means. GM - geometric mean; AUC - area under drug concentration curve; C<sub>max</sub> - peak drug concentration.

Analysis of variance was carried out supposing normal-distributed parameters (Table 2). ANOVA results of pharmacokinetic parameters indicated that there were no significant effects of formulation on any parameter.

The present study clearly demonstrated that pharmacokinetic parameters for metronidazole after oral administration of the test and reference formulations are comparable. ANOVA statistics indicated no significant effect of formulation on any parameters. The non-parametric bioequivalence  $\pm 20\%$  criterion was respected for the test formulation. According to plasma concentration levels, the bioequivalence of both products was established based on AUC<sub>0-t</sub>, AUC<sub>0-</sub> and C<sub>max</sub> parameters for a confidence interval of 90%.

## CONCLUSION

It was found that metronidazole 400 mg, from EMS - Sigma Pharma and Flagyl® 400 mg - from Rhodia, showed similar bioavailability, either in the rate as in the extent of absorption, after single dose administration. Then, according to the Brazil Agência Nacional de Vigilância

Sanitária (ANVISA) - Ministry of Healthy and FDA both formulations should be assessed as bioequivalent<sup>6,11,12</sup>.

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