

# Average bioequivalence of single 500 mg doses of two oral formulations of levofloxacin: a randomized, open-label, two-period crossover study in healthy adult Brazilian volunteers

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Average bioequivalence of two 500 mg levofloxacin formulations available in Brazil, Tavanic<sup>®</sup> (Sanofi-Aventis Farmacêutica Ltda, Brazil, reference product) and Levaquin<sup>®</sup> (Janssen-Cilag Farmacêutica Ltda, Brazil, test product) was evaluated by means of a randomized, open-label, 2-way crossover study performed in 26 healthy Brazilian volunteers under fasting conditions. A single dose of 500 mg levofloxacin tablets was orally administered, and blood samples were collected over a period of 48 hours. Levofloxacin plasmatic concentrations were determined using a validated HPLC method. Pharmacokinetic parameters  $C_{max}$ ,  $T_{max}$ ,  $K_{el}$ ,  $T_{1/2el}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  were calculated using noncompartmental analysis. Bioequivalence was determined by calculating 90% confidence intervals (90% CI) for the ratio of  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  values for test and reference products, using logarithmic transformed data. Tolerability was assessed by monitoring vital signs and laboratory analysis results, by subject interviews and by spontaneous report of adverse events. 90% CIs for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  were 92.1% - 108.2%, 90.7% - 98.0%, and 94.8% - 100.0%, respectively. Observed adverse events were nausea and headache. It was concluded that Tavanic<sup>®</sup> and Levaquin<sup>®</sup> are bioequivalent, since 90% CIs are within the 80% - 125% interval proposed by regulatory agencies.

**Uniterms:** Levofloxacin/bioequivalence. Pharmacokinetics. High-performance liquid chromatography/qualitative analysis. Tavanic<sup>®</sup>/bioequivalence. Levaquin<sup>®</sup>/bioequivalence.

A bioequivalência média de duas formulações de levofloxacino disponíveis no Brasil, Tavanic<sup>®</sup> (Sanofi-Aventis Farmacêutica Ltda, Brasil, produto referência) e Levaquin<sup>®</sup> (Janssen-Cilag Farmacêutica Ltda, Brasil, produto teste) foi determinada por meio da realização de ensaio aleatório, aberto, cruzado, com dois períodos e duas sequências, em 26 voluntários sadios em condições de jejum. Amostras de sangue dos voluntários foram obtidas ao longo de um período de 48 horas após administração de dose única de 500 mg de levofloxacino. As concentrações plasmáticas do fármaco foram determinadas por método cromatográfico validado. Os parâmetros farmacocinéticos  $C_{max}$ ,  $T_{max}$ ,  $K_{el}$ ,  $T_{1/2el}$ ,  $AUC_{0-t}$  e  $AUC_{0-inf}$  foram calculados por análise não compartimental. A bioequivalência foi determinada pelo cálculo de intervalos de confiança 90% (IC 90%) para as razões entre os valores de  $C_{max}$ ,  $AUC_{0-t}$  e  $AUC_{0-inf}$  obtidos para os produtos teste e referência, usando dados transformados logaritmicamente. A tolerabilidade foi avaliada pelo acompanhamento dos sinais vitais e resultados de exames laboratoriais, por consultas e por relato espontâneo dos voluntários. ICs 90% para  $C_{max}$ ,  $AUC_{0-t}$  e  $AUC_{0-inf}$  foram 92.1% - 108.2%, 90.7% - 98.0%, e 94.8% - 100.0%, respectivamente. Os eventos adversos observados foram náusea e cefaleia. Concluiu-se que os produtos Tavanic<sup>®</sup> e Levaquin<sup>®</sup> são bioequivalentes, uma vez que os ICs 90% estão dentro da faixa de 80%-125% proposta pelas agências reguladoras.

**Unitermos:** Levofloxacino/bioequivalência. Farmacocinética. Cromatografia líquida de alta eficiência/análise qualitativa. Tavanic<sup>®</sup>/bioequivalência. Levaquin<sup>®</sup>/bioequivalência.

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## INTRODUCTION

Levofloxacin is the synthetic L-isomer of the racemic quinolone ofloxacin (Anderson, Perry, 2008; Siewert, 2006). The drug is administered to treat infectious diseases, e.g. community acquired and nosocomial pneumonia, skin and skin structure infections and urinary tract infections (Siewert, 2006). It has a broad spectrum antibacterial profile including Gram-positive as well as Gram-negative bacteria, and atypical organisms (Anderson, Perry, 2008; Croom, Goa, 2003; Noreddin, Haynes, Zhanel, 2005; Takahashi, Hayakawa, Akimoto, 2003). Levofloxacin is well tolerated, and is associated with few of the phototoxic, cardiac or hepatic adverse events seen with some other quinolones. Adverse events occurring in  $\geq 2\%$  of patients receiving once-daily levofloxacin 750 mg for 5 days or once-daily levofloxacin 500 mg for 10 days are nausea, headache, diarrhea, insomnia, constipation, abdominal pain, dizziness, dyspepsia and vomiting. Rare adverse effects associated with levofloxacin include photosensitivity/phototoxicity reactions after exposure to the sun or ultraviolet (UV) light, *C. difficile* diarrhoea which may vary in severity from mild to fatal colitis, tendon ruptures, sensory or sensorimotor axonal poly-neuropathy, and hypersensitivity (Anderson, Perry, 2008; Croom, Goa, 2003).

Oral levofloxacin undergoes complete absorption, and the time to maximum concentration ( $T_{max}$ ) occurs 1-2 hours after a 500 or 750 mg oral dose, in subjects with normal renal function. The absolute bioavailability is 99% or greater and the oral solution or tablet formulation have the same bioavailability as the intravenous formulation (Anderson, Perry, 2008). Levofloxacin serum half-life is 6-7 hours (Lee *et al.*, 1997; Lubasch *et al.*, 2000; Wagenlehner *et al.*, 2006). It has a large volume of distribution allowing for penetration in various cells and tissues, including alveolar cells or macrophages and paranasal sinuses mucosa, where it can achieve concentrations that surpass plasma levels after 2-4 hours of administration (Anderson, Perry, 2008; Croom, Goa, 2003). Pharmacokinetics of levofloxacin is not affected by age, gender, race, HIV (human immunodeficiency virus) status or the presence of serious community-acquired bacterial infections (Anderson, Perry, 2008). However,  $T_{max}$  and maximum plasma concentration ( $C_{max}$ ) were affected by consumption of a high-fat meal, although the extent of absorption was not affected (Rodvold, Neuhauser, 2001). Because renal clearance accounts for most of total body clearance of levofloxacin, its pharmacokinetics is affected by renal impairment but not by hepatic impairment (Rodvold, Neuhauser, 2001).

Levofloxacin and newer quinolones, when compared with older agents such as ciprofloxacin, achieve higher peak serum concentrations ( $C_{max}$ ), resulting in higher  $C_{max}/MIC$  (minimum inhibitory concentration) ratios, which result in excellent bacterial killing (Noreddin, Haynes, Zhanel, 2005).

The bioavailability of a drug product is defined as the rate and extent to which the active ingredient or therapeutic moiety is absorbed and becomes available at the site of drug action. Two drug products are considered to be bioequivalent if they are pharmaceutical equivalents (i.e., similar dosage forms made, perhaps, by different manufacturers) or pharmaceutical alternatives (i.e., different dosage forms) and if their rates and extents of absorption do not show a significant difference when administered at the same molar dose of the therapeutic moiety under similar experimental conditions (Chow, Liu, 2000).

Bioavailability and bioequivalence studies provide important information in the overall set of data that ensure the availability of safe and effective medicines to patients and clinicians. These studies gained increasing attention during the last decades, after it became evident that marketed products having same amounts of the same drug may exhibit marked differences between their therapeutic responses. In many instances, these differences were correlated successfully to dissimilar drug blood levels caused mainly by impaired absorption (Abdou, 1989; Chen *et al.*, 2001).

According to international criteria (Agência Nacional de Vigilância Sanitária, 2006; European Medicines Agency, 2010; FDA, 2001), average bioequivalence between two pharmaceutical products is determined by comparing its bioavailabilities through the assessment of rate and extension of drug absorption and through the calculation of 90% confidence intervals (90% CI) for the ratio of  $C_{max}$ , and AUC (area under the plasma concentration-time curve) values for both products, using logarithmic transformed data. The products are considered bioequivalent if all 90% CI fall within 80%-125%.

Especially in antimicrobial therapy, bioequivalence studies are very important, since the dosage form must provide effective plasma concentration, thereby assuring the elimination of the microorganism, which causes the infection. Should the plasma concentration fall below the level required to ensure efficacy, the infection will not be eradicated and the risk of developing resistance to the drug may increase (Porta, Chang, Storpirtis, 2005).

Several Brazilian pharmaceutical industries currently produce or import levofloxacin tablets formulations. These formulations must prove bioequivalence to the reference product to be marketed in Brazil. Thus, the aim

of the present study was to compare the pharmacokinetic profile and to evaluate the bioequivalence of two 500 mg levofloxacin formulations available in Brazil, Tavanic<sup>®</sup> (Sanofi-Aventis Farmacêutica Ltda, Brazil, reference product) and Levaquin<sup>®</sup> (Janssen-Cilag Farmacêutica Ltda, Brazil, test product) for regulatory approval of Levaquin<sup>®</sup>.

## SUBJECTS AND METHODS

### Ethics committee review and selection of volunteers

The study was carried out in accordance with the principles of the Declaration of Helsinki and its amendments (World Medical Association, 2008) and the International Conference on Harmonization Guideline for Good Clinical Practice (International Conference on Harmonization, 1996). The protocol was approved by the Ethics Committee of the College of Medical Sciences of the University of Campinas.

Medical history, physical examination, 12-lead electrocardiography, and laboratory tests (hematology, blood biochemistry, hepatic function and urinalysis) were carried out prior to the beginning of the study and at its conclusion. Inclusion criteria were weight within 15% of ideal body weight, absence of heart, kidneys, neurological or metabolic diseases and no history of drug hypersensitivity. Exclusion criteria were abnormal values on physical examination, electrocardiography or laboratory tests, ongoing pharmacological treatment and pregnancy. Volunteers were instructed to abstain from taking any medication one week before and during the study.

### Study design and drug administration

The bioequivalence of levofloxacin tablet at a single dose of 500 mg was assessed under fasting conditions, in an open, randomized, 2x2 crossover trial, with a washout period of 6 days. Although  $T_{max}$  and  $C_{max}$  could be affected by consumption of a high-fat meal, effect of food was not tested, since it is not required in Brazil for registration of immediate release oral formulations.

Subjects were admitted into hospital at 9:00p.m., the day before the drug administration and were randomly assigned to group A or B. During the first period, volunteers from group A received a single 500 mg dose of reference product (Tavanic<sup>®</sup>, Sanofi-Aventis Farmacêutica Ltda, Brazil), while volunteers from group B received a single 500 mg dose of test product (Levaquin<sup>®</sup>, Janssen-Cilag Farmacêutica Ltda, Brazil), according to a

randomization schedule. In the second study period, the order was reversed.

The products were administered in the morning with 200 mL of water after a 10 hours fasting period. No food was allowed for 4 hours after ingestion of the dose. Subjects were provided with standard meals 4 hours (lunch), 7 hours (snack) and 10 hours (supper) after drug administration in each treatment. Volunteers did not ingest any alcoholic drink, coffee or xanthine containing drinks or foods during the trial.

### Tolerability

Tolerability was assessed by a clinician by monitoring vital signs (blood pressure in the upper arm using sphygmomanometry, and heart rate) at 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 e 48.0 hours after drug administration, by interviewing subjects at each sampling time and at each meal, and by registering adverse events spontaneously reported during the course of the study.

### Sample collection and processing

Sample collection was achieved by means of an indwelling venous cannula of the cubital vein maintained with 1 ml of heparin diluted with normal saline solution to a final concentration of 50 mg/L. Eight milliliters of venous blood were collected to heparinized tubes according to a time schedule which included a blank before-drug sample just prior to dosing (predose) and samples at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0 and 48.0 hours after drug administration. One milliliter of blood was collected and discarded before each sample collection. Plasma was immediately separated by centrifugation at 1900 g for 10 min under refrigeration (4 °C). Plasma samples were divided into two aliquots, transferred to labeled tubes, and stored at -20 °C until analytic assay.

### Analysis of plasma samples

For the determination of levofloxacin plasmatic levels, an accurate, precise and sensitive high performance liquid chromatography (HPLC) method with UV detection was developed and validated, based on previously described methods (Almeida *et al.*, 2005; Baietto *et al.*, 2009; Chien *et al.*, 1997, 1998; Conte *et al.*, 2006; Djabarouti *et al.*, 2004; Ji *et al.*, 2006; Lee *et al.*, 1997; Liang, Kays, Sowinski, 2002; Lubasch *et al.*, 2000; Nemutlu *et al.*, 2007; Schulte *et al.*, 2006; Siewert, 2006; Wagenlehner *et al.*, 2006; Wong, Juzwin, Flor, 1997; Zhou *et al.*, 2007).

Metronidazol was used as internal standard (IS). Briefly, 250  $\mu\text{L}$  of plasma and 25  $\mu\text{L}$  of IS solution (80  $\mu\text{g}/\text{mL}$ ) were added to a 8 mL glass test tube and vortex-mixed for 30 seconds; after this procedure, a 2 mL volume of acetonitrile was added for protein precipitation. Samples were centrifuged for 10 minutes at 1900 g. The supernatant layer was filtered through a Millex GV 0.45  $\mu\text{m}$  filter unit into an 8 mL conical glass tube and solvent was evaporated under a nitrogen stream while immersed in a 40  $^{\circ}\text{C}$  water bath. Each sample was reconstituted with 250  $\mu\text{L}$  of mobile phase and vortex-mixed for 30 seconds. The samples were transferred to auto-sampler vials and 25  $\mu\text{L}$  were injected into the HPLC system. Chromatographic separation was performed in a Gemini C18 column, 150  $\times$  4.6 mm I.D., 5  $\mu\text{m}$  particle size (Phenomenex<sup>®</sup>, Torrance, USA), protected with an AJO-4287 C18 guard cartridge, 5  $\times$  4.6 mm I.D., 5  $\mu\text{m}$  particle size (Phenomenex<sup>®</sup>, Torrance, USA). The mobile phase consisted of a solution of sodium dihydrogen phosphate monohydrate (25 mM) and acetonitrile in the ratio of 87:13 (v/v), without pH adjustment, filtered through a 0.22  $\mu\text{m}$  filter and ultrasonically degassed for 15 min. The chromatographic column was maintained at 40  $^{\circ}\text{C}$  during the analysis. The mobile phase flow-rate was set at 1.2 mL/min and the detection wavelength, at 280 nm.

Method validation was achieved through determination of specificity, recovery, linearity, lower limit of quantification (LLOQ), precision, accuracy and stability using standard plasma samples (0.25  $\mu\text{g}/\text{mL}$  to 10.00  $\mu\text{g}/\text{mL}$ ) and quality control plasma samples (0.75  $\mu\text{g}/\text{mL}$ , 4.00  $\mu\text{g}/\text{mL}$  and 8.00  $\mu\text{g}/\text{mL}$ ). Preparation of calibration standard plasma samples was accomplished daily by spiking drug-free plasma with known amounts of levofloxacin. Quality control plasma samples were prepared by adding known amounts of levofloxacin to drug-free plasma, aliquotation and storage at -20  $^{\circ}\text{C}$ . Specificity was investigated by analyzing four normal plasma samples, one hemolised plasma sample and one lipemic plasma sample for interference of endogenous compounds. The anticoagulant (heparin) interference was also verified during this stage. Recoveries of levofloxacin and IS were evaluated by comparing chromatographic peak areas from standard samples which did not undergo sample pre-treatment and from extracted plasma samples at quality control concentrations. The linearity was determined by the calibration curve ranging from 0.25  $\mu\text{g}/\text{mL}$  to 10.00  $\mu\text{g}/\text{mL}$ , using least-squares linear regression analysis. The LLOQ was the smallest analytical concentration which could be measured with accuracy and precision still better than 20%. Intra- and inter-assay

precision and accuracy were determined by repeated analysis of quality control plasma samples on the same day and on different days. Stability of levofloxacin in plasma was evaluated after three freezing-thaw cycles and after storage at -20  $^{\circ}\text{C}$  for 63 days. Additionally, stability of processed plasma samples during storage in the auto sampler for 24 and 48 hours at room temperature was determined.

## Pharmacokinetic and statistical analysis

The following pharmacokinetic parameters were calculated using a non-compartmental model:  $C_{\text{max}}$ ,  $T_{\text{max}}$ ,  $\text{AUC}_{0-t}$  (area under the plasma concentration-time curve from time zero to sample time of the last measurable levofloxacin concentration),  $\text{AUC}_{0-\text{inf}}$  (area under the plasma concentration-time curve from time zero to infinity),  $K_{\text{el}}$  (elimination rate constant) and  $T_{1/2\text{el}}$  (elimination half-life).  $C_{\text{max}}$  and  $T_{\text{max}}$  were obtained directly from the concentration-time curve.  $\text{AUC}_{0-t}$  was calculated using the linear trapezoidal method.  $K_{\text{el}}$  was calculated by applying a log-linear regression analysis to at least the last three quantifiable concentrations of levofloxacin and  $T_{1/2\text{el}}$  was calculated as  $0.693/K_{\text{el}}$  (Dipiro *et al.*, 1996).  $\text{AUC}_{0-\text{inf}}$  was calculated as  $\text{AUC}_{0-t} + C_t/K_{\text{el}}$ , where  $C_t$  is the last measurable levofloxacin concentration (Dipiro *et al.*, 1996).

A sample size of 26 volunteers was chosen to evaluate bioequivalence with 80% power and to account for dropouts or withdrawals.

Analysis of variance (ANOVA) was performed on logarithmic transformed values of the pharmacokinetic parameters  $C_{\text{max}}$ ,  $\text{AUC}_{0-t}$  and  $\text{AUC}_{0-\text{inf}}$ . The ANOVA model included sequence, formulation, and period as fixed effects and subjects nested within sequence as random effect. These effects were tested at the 5% level of significance (Chow, Liu, 2000).

Bioequivalence between the products was determined by calculating 90% confidence intervals (90% CI) for the ratio of  $C_{\text{max}}$  and  $\text{AUC}_{0-t}$  values for the test and reference products, using logarithmic transformed data. The products were considered bioequivalent if the 90% CI for  $\text{AUC}_{0-t}$  and  $C_{\text{max}}$  fell within 80-125% (Agência Nacional de Vigilância Sanitária, 2006; European Medicines Agency, 2010; FDA, 2001).

## RESULTS

### Tolerability

A total of 26 healthy volunteers, 13 females and 13 males, were included in the study after signing a consent



form. The mean (range) age, weight, and height of the subjects were 33 (21-43) years; 68 (49-86) kg; and 166 (146-178) cm respectively. During the first period of the study, six volunteers from group A (reference product) and three from group B (test product) reported headache and one volunteer from group A reported nausea. During the second period, headache was reported by one volunteer from group A (test product) and two from group B (reference product) and nausea was reported by one volunteer from group B. No clinical abnormalities were found at the conclusion of the study.

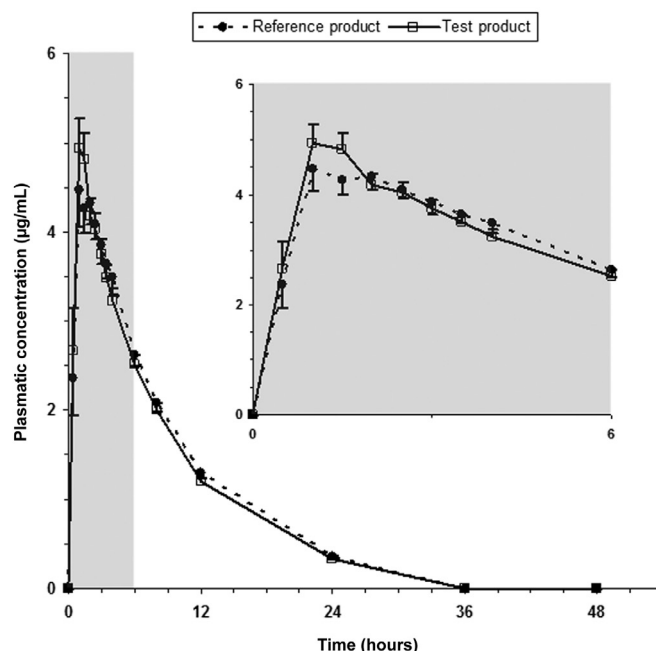
### Method validation and drug concentration in plasma

The HPLC method for levofloxacin quantification was specific for levofloxacin and IS, with total run time of 10 minutes, and was linear in the range of 0.25 µg/mL to 10.00 µg/mL. Intra and inter-assay accuracy ranged from 94.8% to 100.6%, and from 95.2% to 105.9%, respectively. Precision, measured as percent relative standard deviation (RSD%), was between 1.1% and 1.8%, intra-assay, and between 1.9% and 6.3%, inter-assay. Mean recovery was 83.3% for levofloxacin and 86.4% for IS. The processed samples were stable at room temperature for at least 48 hours. Levofloxacin concentrations were not altered after three freeze-thaw cycles and plasma samples were stable for at least 63 days when stored at -20 °C.

Average plasma concentrations of levofloxacin after administration of test and reference products to 26 healthy human volunteers are shown in Figure 1.

### Pharmacokinetic parameters

The mean values for  $C_{max}$ ,  $T_{max}$ ,  $K_{el}$ ,  $T_{1/2el}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  of the two products did not differ significantly, suggesting that plasma profiles generated by the test product are comparable to those produced by the reference



**FIGURE 1** - Average plasma concentrations of levofloxacin after oral administration of reference (Tavanic<sup>®</sup>, Sanofi-Aventis Farmacêutica Ltda, Brazil) and test (Levaquin<sup>®</sup>, Janssen-Cilag Farmacêutica Ltda, Brazil) products to 26 healthy volunteers. Bars indicate mean standard errors (lower bars for reference product and upper bars for test product).

product. Pharmacokinetic parameters values are listed in Table I.

ANOVA detected the occurrence of period effect for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  and product effect for  $AUC_{0-t}$  (Table II). The 90% confidence intervals for the ratio of  $C_{max}$  (92.1-108.2 %),  $AUC_{0-t}$  (90.7-98.0 %) and  $AUC_{0-inf}$  (94.8-100.0 %) values for test and reference products are within the 80-125% interval proposed by United States Food and Drug Administration (FDA) (2001), European Medicines Agency (EMA) (2010) and Brazilian Agência Nacional de Vigilância Sanitária (ANVISA) (2006) (Table II). Power of statistic test was 0.99 for  $C_{max}$ , 1.00 for  $AUC_{0-t}$  and 1.00 for  $AUC_{0-inf}$ .

**TABLE I** - Average (range) pharmacokinetic parameters after oral administration of reference (Tavanic<sup>®</sup>, Sanofi-Aventis Farmacêutica Ltda, Brazil) and test (Levaquin<sup>®</sup>, Janssen-Cilag Farmacêutica Ltda, Brazil) products to 26 healthy volunteers

Pharmacokinetic parameter	Test Product	Reference Product
$C_{max}$ (µg/mL)	5.67 (2.63-8.07)	5.69 (2.90-9.12)
$T_{max}$ (h)	1.29 (0.50-3.00)	1.60 (0.50-4.00)
$K_{el}$ (h <sup>-1</sup> )	0.11 (0.07-0.19)	0.11 (0.07-0.16)
$T_{1/2el}$ (h)	6.69 (3.71-9.49)	6.55 (4.28-9.63)
$AUC_{0-t}$ (µg h/mL)	39.22 (23.84-52.89)	41.52 (26.53-56.60)
$AUC_{0-inf}$ (µg h/mL)	44.24 (27.64-57.26)	45.50 (31.03-61.98)

**TABLE II** - Analysis of variance (ANOVA) for the assessment of the product, sequence and period effects, and 90% confidence intervals (90% CI) for the ratio of  $C_{\max}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  values for the test and reference products, using logarithmic transformed data, after oral administration of reference (Tavanic<sup>®</sup>, Sanofi-Aventis Farmacêutica Ltda, Brazil) and test (Levaquin<sup>®</sup>, Janssen-Cilag Farmacêutica Ltda, Brazil) products to 26 healthy volunteers ( $\alpha=0.05$ )

Pharmacokinetic parameter	ANOVA (p value)			90% CI
	Variation source			
	Product	Sequence	Period	
$C_{\max}$	0.9696	0.5758	0.0217	92.1%-108.2 %
$AUC_{0-t}$	0.0165	0.7999	0.0035	90.7%-98.0 %
$AUC_{0-inf}$	0.0966	0.7604	0.0003	94.8%-100.0 %

## DISCUSSION

Bioequivalence of drug products is usually assessed through open-label, randomized crossover studies (Eradiri *et al.*, 2007; Howard *et al.*, 2005; Schützer *et al.*, 2004). End-points in bioequivalence trials ( $C_{\max}$  and  $AUC_{0-inf}$ ) are objective assessments, obtained from drug plasma concentration-time profiles. These assessments will not be affected by the knowledge of the treatment by the volunteer or the researcher. Since, in this case, it is not possible that unconscious bias will affect the observation, an open-label design can be used. In a crossover design, each subject serves as his or her own control, thus allowing a within subject comparison between formulations. It also removes the inter-subject variability from the comparison between formulations and, with a proper randomization of subjects to the sequence of formulation administrations, it provides the best unbiased estimates for the differences between formulations (Chow, Liu, 2000).

High-performance liquid chromatography (HPLC) methods with ultraviolet (UV) (Baietto *et al.*, 2009; Chien *et al.*, 1998; Djabarouti *et al.*, 2004; Lee *et al.*, 1997; Liang, Kays, Sowinski, 2002; Nemutlu *et al.*, 2007; Wong, Juzwin, Flor, 1997), fluorescence (Almeida *et al.*, 2005; Chien *et al.*, 1997; Lubasch *et al.*, 2000; Schulte *et al.*, 2006; Siewert, 2006; Wagenlehner *et al.*, 2006; Zhou *et al.*, 2007) or mass spectrometry (Conte *et al.*, 2006; Ji *et al.*, 2006) detection for analysis of levofloxacin in biological fluids have been published. Sample preparation procedures include protein precipitation with acetonitrile, methanol, trifluoroacetic acid or perchloric acid (Baietto *et al.*, 2009; Liang, Kays, Sowinski, 2002; Lubasch *et al.*, 2000; Schulte *et al.*, 2006; Siewert, 2006; Wagenlehner *et al.*, 2006), liquid-liquid extraction (Chien *et al.*, 1997, 1998; Lee *et al.*, 1997; Wong, Juzwin, Flor, 1997; Zhou *et al.*, 2007), solid-phase extraction (Djabarouti *et al.*, 2004; Nemutlu *et al.*, 2007) and

ultrafiltration (Liang, Kays, Sowinski, 2002). Most of the methods employ isocratic mobile phase, but the use of mobile phase gradient is also described (Baietto *et al.*, 2009; Nemutlu *et al.*, 2007; Schulte *et al.*, 2006; Siewert, 2006). Although all of these methods are successful in the determination of levofloxacin, some of them have limitations including long chromatographic running time (Baietto *et al.*, 2009; Siewert, 2006), and complicated and time-consuming sample pretreatment procedures (Chien *et al.*, 1997).

The method used in this study has advantages when compared with others published methods: sample pre-treatment requires a one-step preparation, adding acetonitrile for protein precipitation; the chromatographic analysis was done in isocratic conditions; and the running time was 10 minutes for each sample.

The method showed good specificity, sensitivity, linearity, precision and accuracy over the entire concentration range achieved after oral administration of tablets containing 500 mg of levofloxacin, thereby enabling its use in bioequivalence trials of this kind of formulations.

The average plasmatic decay curves obtained for the test product (Levaquin<sup>®</sup>) and reference product (Tavanic<sup>®</sup>) have the same pattern.

Levofloxacin was absorbed rapidly, achieving mean peak plasma level between 1 and 2 hours after drug administration for both test and reference products. Mean values for pharmacokinetic parameters  $C_{\max}$  (reference: 5.69  $\mu\text{g/mL}$ ; test: 5.67  $\mu\text{g/mL}$ ),  $T_{\max}$  (reference: 1.60 h; test: 1.29 h),  $AUC_{0-t}$  (reference: 41.52  $\mu\text{g}\cdot\text{h/mL}$ ; test: 39.22  $\mu\text{g}\cdot\text{h/mL}$ ),  $AUC_{0-inf}$  (reference: 45.50  $\mu\text{g}\cdot\text{h/mL}$ ; test: 44.24  $\mu\text{g}\cdot\text{h/mL}$ ) and  $T_{1/2el}$  (reference: 6.55 hours; test: 6.69 hours) were similar to that reported by other authors (Amsden, Whitaker, Johnson, 2003; Chien *et al.*, 1997; Lee *et al.*, 1997; Lubasch *et al.*, 2000; Wagenlehner *et al.*, 2006). ANOVA revealed the absence of sequence effect for  $C_{\max}$ ,  $AUC_{0-t}$  e  $AUC_{0-inf}$ . Period

effect was observed for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$ , and product effect was observed for  $AUC_{0-t}$ . Regarding product effect, these findings do not mean that the formulations evaluated are not bioequivalent, since bioequivalence between two products is defined in terms of 90% CI. In this case, the occurrence of product effect indicates that there is a statistically significant difference between bioavailabilities of test and reference products regarding extension of drug absorption, but there is no difference regarding absorption rate, since no product effect was observed for  $C_{max}$ . This difference in extension of drug absorption is not considered to be clinically important, since 90% CI is within the limits established by regulatory agencies (Pabst, Jaeger, 1990; Westlake, 1979). A period effect measures the differences between study periods. A well-run study with consistent treatment of subjects should produce no significant period effects. However, a significant period effect does not invalidate the study, but the cause should be investigated (Ormsby, 1994). Period effect may be due to a too short washout between periods (residual or carry-over effects), or they may reflect differing procedures of sample workup or storage, because for each subject usually all samples of all periods are analyzed within the same run. They also may be provoked by acclimatization or even weather conditions, or they may reflect an effect of the diet (Pabst, Jaeger, 1990). In the present study, predose samples did not exhibit any detectable levofloxacin level in all subjects, so the occurrence of residual or carry-over effects can be excluded. Also, procedures regarding volunteers' diet and rest, drug administration and sample workup and storage were the same in the two periods of the study. On the basis of these considerations, the present bioequivalence study is acceptable, despite the period effect.

The 90% CI for the ratio of  $C_{max}$  (92.1%-108.2 %),  $AUC_{0-t}$  (90.7%-98.0 %) and  $AUC_{0-inf}$  (94.8%-100.0 %) values for the test and reference products are within the 80-125 % interval proposed by regulatory agencies, therefore test and reference products are bioequivalent according to average bioequivalence criteria, even if product effect have been detected in ANOVA.

## CONCLUSIONS

Based on the pharmacokinetic and statistical results of this study, it was concluded that Tavanic® (Sanofi-Aventis Farmacêutica Ltda, Brazil) and Levaquin® (Janssen-Cilag Farmacêutica Ltda, Brazil) are bioequivalent. Although a statistically significant difference between bioavailabilities of test and reference products regarding extension of

drug absorption was detected through ANOVA, this was not considered to be clinically important, since the 90% CI of the ratios for  $C_{max}$ ,  $AUC_{0-t}$  and  $AUC_{0-inf}$  were found to be within the regulatory agencies proposed acceptance limits (80-125%).

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