Pharmacokinetic Study of Two Oral Formulations of Levofloxacin in Healthy Male Volunteers

Maruf Mohammad Akbor[1], Rebeka Sultana[1], Mohammad Abul Kalam Azad[2], A. H. M. Mahbub Latif[3], Md. Ashik Ullah[1] and Abul Hasnat[1]

[1]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Dhaka-1000, Bangladesh
[2]Department of Pharmaceutical Technology, University of Dhaka, Dhaka-1000, Bangladesh
[3]Institute of Statistical Research and Training, University of Dhaka, Dhaka-1000, Bangladesh

ABSTRACT: The objective of this study was to compare different pharmacokinetic parameters of a local ("X") and reference (Tavanic) formulations of levofloxacin 250 mg tablets after oral administration of a single dose under fasting condition. Thirteen blood samples were collected from each of the eight Bangladeshi healthy male volunteers over 24 hours after oral administration of the drugs. Serum levofloxacin concentrations were determined by HPLC assay using UV detection, and pharmacokinetic parameters were determined by the non-compartmental method. Mean ± SD of C_{max}, AUC_{0-24}, $AUC_{0-\alpha}$, T_{max}, $t_{1/2}$, k_{el}, were 4.33 ± 1.16 and 4.56 ± 1.51 µg/mL, 45.90 ± 8.74 and 37.77 ± 9.94 hr-µg /mL, 79.94 ± 32.80 and 66.85 ± 35.43 hr-µg/mL, 1.22 ± 0.49 and 1.28 ± 0.41, 19.90 ± 11.49 and 21.00 ± 16.39 hr, 0.04 ± 0.02 and 0.05 ± 0.03 hr^{-1} for the local ("X") and reference formulation, respectively. From the paired t-test, the *p*-values for two formulations were found to be 0.182, 0.412 and 0.725 for AUC_{0-24}, $AUC_{0-\alpha}$, and C_{max} respectively. The 90% confidence intervals of the mean of the difference between log-transformed values for C_{max} were almost within the bioequivalence accepted range of 80% to 125%, namely: (78.90%, 118.36%); but for AUC_{0-24} and $AUC_{0-\alpha}$ the values were are beyond the acceptable range. (100.83%, 146.52%) and (94.34%, 157.89%) respectively. The results indicate that the two formulations are not bioequivalent for both the rate and extent of absorption.

Key words: Levofloxacin, pharmacokinetic, bioavailability, bioequivalence

INTRODUCTION

Levofloxacin(*(-)-(S)*-9-fluoro-2,3-dihydro-3-methyl-10-(4-methyl-1-piperazinyl)-7-oxo-7H-pyrido [1,2,3-de]-1,4-benzoxazine-6-carboxylic acid hemi-hydrate) is a quinoline carboxylic acid derivative with a broad antibacterial activity against both gram-positive and gram-negative bacteria.[1] It has been shown to be effective against a variety of pathogens such as members of the family *Enterobacteriaceae, Pseudomonas aeruginosa*, and commonly isolated gram-positive organisms such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. It is approximately twice as active as ofloxacin[2] and compared with ciprofloxacin. It has enhanced activity against gram-positive organisms.[2-4]

A single dose pharmacokinetic study of levofloxacin showed that an oral dose of levofloxacin was rapidly and almost completely absorbed. Levofloxacin showed excellent tissue penetration by absorbing two-thirds of those in plasma. The extent of penetration is similar to other fluroquinolones.[5,6] Peak plasma concentrations are usually attained one to two hours after oral dosing.[7,8]

Correspondence to: Abul Hasnat
Tel: 88-02-8612069; Fax: 88-02-8615583
Email: ahasnat99@yahoo.com

Co-administration of levofloxacin with Calcium-fortified orange juice decreases the values of C_{max} and t_{max} of levofloxacin by 14% to 18% and 50% respectively.[9,10]

The aim of this study was to compare the rate and extent of absorption of two formulations of levofloxacin 250 mg tablet; a local (test) formulation: ("X"), manufactured by a well reputed leading Bangladeshi pharmaceutical company and a reference formulation: Tavanic, manufactured by Sanofi-Aventis, UK under the licensing authority of Ortho McNeil, USA; under fasting condition.

MATERIALS AND METHODS

Subjects. Eight healthy, non-smoking, adult Bangladeshi male volunteers were randomly selected in the study. Their mean age ± SD was 25.63 ± 1.41 (24 to 28) years. Their mean body weight and mean height were 69.50 ± 4.72 (60 to 75) kg and 1.74 ± 0.04 (1.68 to 1.80) m respectively, giving a mean body mass index (BMI) of 22.89 ± 1.50 (20.50 to 25.0) kg/m^2. Subjects were selected after examining the medical history, physical check up, chest X-ray, ECG, serological screening for infectious disease, and urine analysis. Participation in the study was limited to those with no evidence of significant abnormal hematology and serum chemistry. Exclusion criteria included any history of a significant gastrointestinal condition that could potentially impair the absorption or disposition of the study medicine, previous history of allergy to any fluoruquinolone, need for any chronic medication (e.g. theophylline, antacid, glibenclamide, phenytoin, iron or vitamins), donation of blood within 30 days preceding the first dose of the study or use of a investigational agent within 30 days of study entry. Potential subjects were also excluded if they use any medication within one day before administration of the first dose. The volunteers were asked to abstain from taking any medication (including over-the-counter drugs) throughout the study; and from smoking, taking alcohol or caffeine or consuming xanthene-containing beverages or food for at least 48 hours prior to, and throughout the study. Any incidence of vomiting or any other adverse events resulted in the exclusion of the subject from the study. They were informed about the risks, benefits, procedures, and aims of the study, as well as their rights as research subjects. The study was conducted according to the Declaration of Helsinki (1964). Each volunteer signed an informed consent document and data collection form before entering the study. Ethical permission was taken to approve the protocol and consent form of this study from Ethical Review Committee of Bangladesh Medical Research Council (BMRC).

Study drugs. The test formulation was ″X″, 250-mg film coated tablet (Batch # 6075), manufactured by well reputed and top leading Bangladeshi pharmaceutical company whereas the reference formulation was Tavanic 250 mg tablets (Batch # 40D878), manufactured by Sanofi-Aventis, UK under the licensing authority of Ortho McNeil, USA.

Study design. In the study, 8 volunteers were selected randomly. All volunteers received single 250 mg film coated tablet of both formulations: Reference formulation (**A)** or Test formulation (**B)**. Volunteers were randomly divided into two groups (Group–1 and 2) consisting of 4 volunteers in each group. Group-1 received treatment **A** followed by treatment **B** with a seven-day washout period. This sequence of treatment is denoted by **AB**. Group-2 received treatment **B** followed by treatment **A** after the same washout period. This sequence of treatment is denoted as **BA.** In the first period, Group-1 received treatment **A** and Group-2 received treatment **B.** In the second period Group-1 received treatment **B** and Group-2 received treatment **A**. This type of study design is known as crossover design in statistical literature.[11] Each volunteer received the treatment with 250 mL of water in the morning after overnight fasting. A standard lunch was allowed after 4 hours of dosing. The volunteers were ambulatory during the study but were prohibited from strenuous activity. Volunteers were monitored constantly for the period of 24 hours by a medical doctor.

Blood sampling. The timing of blood collection was planned according to the previously reported value of time to reach peak serum concentration (T_{max}) and serum elimination half-life ($t_{1/2}$).[12-16] An intravenous cannula was placed into the volunteers' forearm vein before drug administration and left in place until the 24-hour blood sample was collected. Venous blood samples were collected before, and at 0.25, 0.50, 0.75, 1.00, 1.50, 2, 3, 5, 7, 9, 12 and 24 hours after administration of drug. The blood samples were collected in coded, evacuated tubes, kept 30 minutes for clotting and centrifuged at room temperature (3000 rpm for 15 minutes). The serum was collected in coded eppendorf tubes and serum protein was separated by precipitation with ethanol followed by centrifugation at 10,000 rpm for 5 minutes. The serum was collected and stored at −80°C until analyzed.

Levofloxacin level determination by HPLC. Levofloxacin was separated at room temperature on a 5-µm (particle-size), 4.6 X 250-mm Kromasil ODS C_{18} and Kromasil C_{18} 5-µm insert. The compounds of interest were detected by using a UV detector at 293 nm wavelength. The mobile phase consists of 0.05 M (mol/L) citric acid : 1 M ammonium acetate and acetonitrile (77 : 1 : 22 v/v) and was delivered at a flow rate of 1.0 mL/min. Samples were injected in the HPLC system by an autosampler. The retention time was 4.8445 ± 0.0016 minutes. The standard curves were linear over the concentration ranges of 25 to 1000 ng/mL with a mean correlation coefficient of 0.9958. The lower limit of quantification (LLOQ) of levofloxacin in the serum was found to be 25 ng/mL. All the blood samples were analyzed within one week of collection. The precision and accuracy were investigated with quality control (QC) samples at concentrations of 25, 50,100, 250, 500, 1000 ng/mL. The results are shown in the Table 1. The intra-day and inter-day coefficient of variation for five QC samples were satisfactory with R.S.D.(s) less than 9.31 %. The determined values deviated from the declared concentration with relative error less than 15.05 %.

Table 1. Precision and accuracy of the method for the determination of the levofloxacin in human plasma (n = 6)

Concentration (ng/mL)		Relative error* (%)	Intra-day R.S.D. (%)	Inter-day R.S.D. (%)
Added	Found			
25	25.45	3.27	6.90	7.10
50	55.01	0.08	4.82	3.49
100	99.98	3.09	2.00	2.22
250	252.56	15.05	7.48	4.77
500	486.99	-9.77	9.31	6.82
1000	1000.16	5.85	3.01	4.14

*Relative error = (Mean measured concentration – added concentration)*100/ added concentration

Pharmacokinetic analysis. The following pharmacokinetic parameters were directly calculated by the standard non-compartmental analysis: **(a)** Maximum serum concentration (C_{max}), time to reach peak serum concentration (T_{max}). **(b)** The elimination half-life ($t_{1/2}$) was calculated as $t_{1/2} = (\ln 2)/K_{el}$, where K_{el} is the apparent elimination rate constant. K_{el} was calculated by using the software WinNonlin.[17] **(c)** Area under the serum concentration-time curve (AUC_{0-24}), area under the first moment curve (AUMC), mean residence time (MRT) were calculated from the measured levels, from time zero to the time of last quantifiable level, by the linear trapezoidal rule. **(d)** The area under the serum concentration-time curve extrapolated to infinity ($AUC_{0-\alpha}$) was calculated according to the following formula: $AUC_{0-\alpha} = AUC_{0-t} + C_t/K_{el}$, where C_t is the last quantifiable serum level. **(e)** The rate of absorption was evaluated by means of the ratio of C_{max} /$AUC_{0-\alpha}$. Pharmacokinetic parameters were calculated by personal computer using Microsoft Excel (Version 2000) and WinNonlin (Version 2.1).

Statistical Analysis: Let y_{ijk} be the observed value of a pharmacokinetic parameter corresponding to the subject k in period j of group i. The following model is assumed for y_{ijk} :

$$y_{ijk} = \mu + S_{ik} + \Pi_j + \tau_{d[i,j]} + \lambda_{d[i,j-1]} + \varepsilon_{ijk}, \quad (1)''$$

where μ is the general mean, S_{ik} is the random effect of subject k in group i, Π_j is the effect of period j, $\tau_{d[i,j]}$ is the effect of treatment administered in period j of group i, $\lambda_{d[i,j-1]}$ is the carry–over (sequence) effect of the treatment administered in period j−1 of group i with λ[i,0]=0 and ε_{ijk} is the random error term. It is assumed that random terms S_{ik} and ε_{ijk} follow normal distribution with same mean 0 and variance σ^2 and σ^2_s, respectively. Carry–over effect can be tested by comparing corresponding mean sum of squares with the between subject mean sum of squares ($\hat{\sigma}_s^2$) and period of a treatment effects are tested by comparing corresponding mean squares with the within subject mean squares ($\hat{\sigma}^2$).

In our analysis, log–transformed value of the pharmacokinetic parameters AUC_{0-24}, $AUC_{0-\alpha}$, C_{max}, K_{el}, $t_{1/2}$, and $C_{max}/ AUC_{0-\alpha}$ are used in the model (1). The model (1) can be fitted by usual statistical software. We have used statistical software R for fitting the model and drawing inferences about the parameters[18].

Besides fitting the model, we also reported the approximate 90% confidence interval for the difference between two formulations only for the pharmacokinetic parameters AUC_{0-24}, $AUC_{0-\alpha}$, C_{max}, and $C_{max}/ AUC_{0-\alpha}$.

RESULTS AND DISCUSSION

The mean (± SD) serum concentration-time profile of the two formulations, shown in the Figure 1, was closely similar and superimposable.

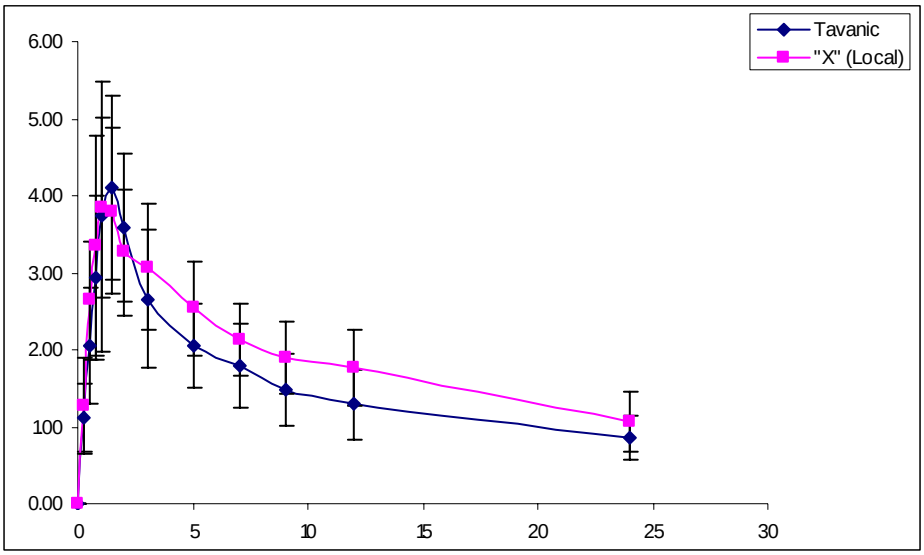

Figure 1. Mean plasma concentrations of levofloxacin at different time intervals after single oral administration of 250 mg tablet of Tavanic and ″X″ Local to 8 healthy male volunteers

Central and dispersion measures for all pharmacokinetic parameters for both formulations are shown in Table 2. From this, the mean values of C_{max} were found to be 4.56 (SD = 1.51) μg/mL for reference product and 4.33 (SD = 1.16) μg/mL for local (test) product. For the mean values of T_{max} (hr) were found to be 1.28 (SD = 0.41) μg/mL for reference product and 1.22 (SD = 0.49) μg/mL for local (test) product. The mean values of AUC_{0-24} were found to be 37.77 (SD = 9.94) μg.hr/mL for reference and 45.90 (SD = 8.74) μg.hr/mL for local product. AUC is important in determining the bioavailability

and bioequivalence of a drug product. For all volunteers the values of AUC_{0-24} were found to be greater than 80% of $AUC_{0-\alpha}$. The mean $AUC_{0-\alpha}$ values were found to be 66.85 (SD = 35.43) µg.hr/mL and 79.94 (SD = 32.80) µg.hr/mL for reference and local product respectively. Other pharmacokinetic parameters such as $t_{1/2,}$ $k_{el,}$ $AUMC_{0-24,}$ $AUMC_{0-\alpha}$ and MRT were also determined.

Table 3 features that the change of $C_{max,}$ AUC_{0-24} and $AUC_{0-\alpha}$ were found to be insignificant (p>0.1). Table 4 shows the ANOVA of the model (1). It shows after controlling the effects of period, sequence, and subject there is no significant difference between the two formulations for all the pharmacokinetic parameters we considered. Period effects were found to be insignificant for all the parameters. The insignificant sequence effects indicate no carry-over effect of the two formulations. Subject variations were also found to be insignificant at 10 percent level of significance.

Table 2. Mean Pharmacokinetic Parameters of Tavanic 250-mg tablet (Reference formulation - A) and "X" 250-mg tablet (Local formulation - B)

Pharmacokinetic parameters (n =8)	Test Formulation **A**						
	Geom. mean	Median	Mean	SD	CV (%)	Min	Max
C_{max} (µg/mL)	4.35	4.44	4.56	1.51	33.23	2.83	7.24
t_{max} (hr)	1.22	1.25	1.28	0.41	32.04	0.75	2.00
AUC_{0-24} (hr µg/mL)	36.68	35.65	37.77	9.94	26.31	26.95	52.64
$AUC_{0-\alpha}$ (hr µg/mL)	60.86	52.90	66.85	35.43	52.99	40.11	146.67
$t_{1/2}$ (hr)	17.45	15.94	21.00	16.40	78.07	6.94	59.93
k_{el} (hr^{-1})	0.04	0.04	0.05	0.03	55.17	0.01	0.10
$AUMC_{0-24}$ (hr^2 µg/mL)	328.10	295.37	339.87	99.31	29.22	245.18	499.28
$AUMC_{0-\alpha}$ (hr^2 µg/mL)	1479.67	1217.20	2583.39	3969.49	153.65	535.42	12337.10
MRT (hr)	24.31	22.19	29.04	22.95	79.03	11.41	84.12
C_{max} /$AUC_{0-\alpha}$	0.07	0.07	0.09	0.04	43.96	0.03	0.15
	Test Formulation **B**						
C_{max} (µg/mL)	4.20	3.91	4.33	1.16	26.77	3.06	6.17
t_{max} (hr)	1.12	1.25	1.22	0.49	40.19	0.50	2.00
AUC_{0-24} (hr µg/mL)	45.14	44.85	45.90	8.74	19.04	31.81	55.82
$AUC_{0-\alpha}$ (hr µg/mL)	74.28	79.49	79.94	32.80	41.04	41.82	141.48
$t_{1/2}$ (hr)	20.01	17.65	23.32	15.01	64.37	10.93	47.21
k_{el} (hr^{-1})	0.03	0.04	0.04	0.02	45.36	0.01	0.06
$AUMC_{0-24}$ (hr^2 µg/mL)	419.65	432.62	433.86	116.82	26.93	285.20	566.19
$AUMC_{0-\alpha}$ (hr^2 µg/mL)	1820.40	1941.14	2633.52	2871.02	109.02	676.51	9438.84
MRT (hr)	24.51	23.55	27.46	16.66	60.66	14.53	66.71
C_{max} /$AUC_{0-\alpha}$	0.06	0.06	0.06	0.03	50.50	0.02	0.11

Table 3. *p*-values for different pharmacokinetic parameters of two formulations calculated by paired *t* test (n=8)

Pharmacokinetic parameter	AUC_{0-24}	$AUC_{0-\alpha}$	C_{max}	t_{max}	k_{el}	$t_{1/2}$	MRT	$AUMC_{0-24}$	$AUMC_{0-\alpha}$
p-values	0.182	0.412	0.725	0.732	0.683	0.882	0.879	0.145	0.978

Table 4. *p*-values for sources of variations obtained from ANOVA

Sources of Variations	AUC_{0-24}	$AUC_{0-\alpha}$	C_{max}	t_{max}	k_{el}	$t_{1/2}$	C_{max} /$AUC_{0-\alpha}$
Formulations	0.3108	0.3880	0.7040	0.6499	0.9265	0.9938	0.6331
Period	0.1625	0.3214	0.8309	0.6499	0.9024	0.9205	0.3265
Sequence	0.7008	0.1878	0.4278	0.7231	0.0871	0.0826	0.1510
Subjects	0.73	0.34	0.48	0.28	0.70	0.69	0.29

Table 5 shows the 90% confidence intervals of the ratios (Test/Reference) between the two formulations regarding $AUC_{0-24,}$ $AUC_{0-\alpha}$, C_{max} and C_{max}/ $AUC_{0-\alpha.}$

Assessment of bioequivalence of local product to reference product is required to exclude any clinically important differences in the rate or extent at which the active entity of the drugs becomes available at the site of action. Two drugs are considered to be bioequivalent if they are pharmaceutically equivalent and their bioavailabilities are so similar that they are unlikely to produce clinically relevant differences in regard to safety and efficacy.[19]

Table 5. Large sample based 90% Confidence Intervals for different pharmacokinetic parameters from log transformed for assessment of bioequivalence.

Test/Reference			
	Log Transformed		
Parameters	Mean Ratio (T/R)	90%	CI
AUC_{0-24}(hr-µg/mL)	121.55 %	146.52%	100.83%
$AUC_{0-\alpha}$(hr-µg/mL)	122.05%	157.89%	94.34%
C_{max} (µg/mL)	96.64 %	118.34%	78.90%
C_{max} /$AUC_{0-\alpha}$	79.18%	108.27%	57.91%

The aim of this study was to compare the bioavailability of two formulations of levofloxacin 250 mg tablet, a local (test) formulation, "X", and a reference formulation, Tavanic. The study revealed that at 90% confidence interval (Table 5) AUC_{0-24}, $AUC_{0-\alpha}$, and C_{max} were found to be (100.83%, 146.52%), (94.34%, 157.89%) and (78.90%, 118.34%) respectively from log-transformed data. The values for C_{max} are found almost within the acceptable range but the values for AUC_{0-24} and $AUC_{0-\alpha}$ are beyond the accepted range of 80% to 125%.[20-21] Moreover, a further evaluation of the rate of absorption was performed by analyzing the C_{max}/ $AUC_{0-\alpha}$, since this parameter has been proposed to better reflect the absorption rate.[22] The 90% confidence intervals for this parameter also indicated bioequivalence.

In conclusion, the two formulations can not be considered bioequivalent in regard to the extent and rate of absorption and therefore not interchangeable.

ACKNOWLEDGEMENT

The authors wish to express their gratefulness to Dr Fakhrul Ahsan, Texas Tech University, USA for providing software support in calculating different pharmacokinetic parameters. The authors also would like to express their gratitude to the authority of NOVO Healthcare and Pharma Ltd., Bangladesh, for their continuous support especially with some chemicals during this thesis work.

REFERENCES

1. Hopper, D.C. and Wolfson, J.S. 1991. Fluoroquinolone antimicrobial agents. *N. Engl. J. Med.* **324**, 384-394.
2. Dholakia, N., Rolston, K.V.I., Ho, D.H., LeBlanc, B. and Bodey, G.P. 1994. Susceptibilities of bacterial isolates from patients with cancer to levofloxacin and other quinolones. *Antimicrob. Agents Chemother.* **38**, 848-852.
3. Fu, K.P., Lafredo, S.C., Foleno, B., Isaacson, D.M., Barrett, J.F., Tobia, A.J. and Rosenthale, M.E. 1992. *In vitro* and in vivo antibacterial activities of levofloxacin (l-ofloxacin), an optically active ofloxacin. *Antimicrob. Agents Chemother.* **36**, 860-866.
4. Marshall, S.A. and Jones., R.N. 1993. The *in vitro* activity of DU-6859a, a new fluropropyl quinolone. *Antimicrob. Agents Chemother.* **37**, 2747-2753.
5. Johnson, J.H., Cooper, M.A., Andrews, J.M. and Wise, R. 1992. Pharmacokinetics and inflammatory fluid penetration of sparfloxacin. *Antimicrob. Agents Chemother.* **36**, 2444-2446.
6. Nye, K., Shi, Y.G., Andrews, J.M., Ashby, J.P. and Wise. R. 1989. The *in vitro* activity, pharmacokinetics and tissue penetration of temafloxacin. *J. Antimicrob. Chemother.* **24**, 415-424.
7. Wolfson, J.S. and Hooper, D.C. 1989. Comparative pharmacokinetics of ofloxacin and ciprofloxacin. *Am. J. Med*. **87**(suppl 6C)**,** 31-36.
8. Fish, D.N. and Chow, A.T. 1997. The clinical pharmacokinetics of levofloxacin. *Clin Pharmacokinetic*. **32**, 101-119.
9. Keller, I., Lubasch, A. and Rau, M. 1999. Comparative pharmacokinetics of ciprofloxacin, gatifloxacin, levofloxacin, moxifloxacin, and trovafloxacin after a single in healthy volunteers [abstract 30]. 39th Interscience Conference on *Antimicrobial Agents and Chemotherapy*, Sep 26-29.

10. Allison, W.W., Jennifer, M.V. and Guy, W.A. 2003. Lack of Bioequivalence when Levofloxacin and Calcium-Fortified Orange Juice are coadministered to healthy volunteers. *J. Clin. Pharmacology* **43**, 539-544.

11. Jones, B. and Kenward, G.M. 2003. *Design and Analysis of Cross-Over Trials.* Second edition. Champman and Hall/CRC, ISBN 0412606402.

12. Food and Drug Administration. Tequin Tablets (gatifloxacin), Tequin Injection (gatifloxacin). 1999. Washington, DC: Department of Health and Human Services.

13. Hutchinson, T.A., Shahan, D.R. and Anderson, M.L. 2001. *Drugdex System*. Englewood, CO: Micromedex.

14. Panacea Biotec. MYGAT (Gatifloxacin infusion- 2 mg/mL). 2003.

15. Nakashima, M., Uematsu, T. and Kosuge, K. 1995. Single and multiple-dose pharmacokinetics of AM-1155, a new 6-fluoro-8-methoxy quinolone, in humans. *Antimicrob. Agents Chemother.* **39**, 2635-2640.

16. Liang, H., Kays, M. and Sowinski, K. 2002. Separation of levofloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, trovafloxacin and cinoxacin by high performance liquid chromatography: application to levofloxacin determination in human plasma. *J. Chromatogr.* **772**, 53-63.

17. Gibaldi, M. and Perrier, D. 1982. *Pharmacokinetics,* 2nd ed. New York. Dekker. pp. 433-434.

18. R Development Core Team. 2005. R : A Language and Environment for Statistical Computing. R foundation for Statistical Computing, Vienna, Austria. (www.R-project.org)

19. Food and Drug Administration. 2002. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products - general considerations. Rockville, Maryland, USA.

20. Hauschke, D., Steinijans, V.W. and Diletti, E.A. 1990. Distribution- free procedure for the statistical analysis of bioequivalence studies. *Int. J. Clin. Pharmacol. Ther. Toxicol.* **28**, 72-78.

21. Food and Drug Administration. 2001. Guidance for industry: bioanalytical method validation. Rockville, Md.

22. Endrenyl, L., Fritsch, S. and Yan, W. 1991. C_{max}/AUC is a clearer measure then C_{max} for absorption rates in investigations of bioequivalence. *Int. J. Clin. Pharmacol. Ther*. **29**, 394-399.