Comparison of High-Pressure Liquid Chromatography and microbiological assay for determination of ciprofloxacin tablets in human plasma employed in bioequivalence and pharmacokinetics study

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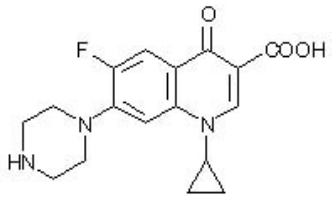
Abstract: Ciprofloxacin was given orally to 28 healthy male volunteers for single oral dose of 500mg; Plasma samples were collected at different time's interval between 0 and 12h and analyzed both by high pressure liquid chromatography and by a microbiological assay. The detection limits (LOD) were 0.02ug/ml and 0.1ug/ml, for both methods respectively. For each method, coefficients of variation (R^2) were 0.9995 and 0.9918 in plasma and limit of quantitation (LOQ).02 and 0.5ug/ml. The Comparison of means maximum concentration 2.68 *u*g/ml at 1.5 hr for test and 2.43 *u*g/ml are attain in HPLC method of Reference at 2hrs respectively. The plasma concentrations measured by microbiological assay of reference tablet are 3.95μg/ml (mean ± SE) at 1 hour and 3.80μg/ml (mean ± SE) at 1 hour. The concentrations in plasma measured by microbiological method were markedly higher than the high-pressure liquid chromatography values which indicates the presence of antimicrobially active metabolites. The mean ± SE values of pharmacokinetic parameters calculated by HPLC method, for total area under the curve (AUC 0-∞) were 13.11, and 11.91 h.mg/l for both test and reference tablets respectively. The mean ± SE values of clearance measured in l/h were 44.91 and 48.42 respectively. The elimination rate constant Kel [l/h] showed 0.17 l/h for test and 0.15 l/h reference tablets and likewise, absorption half-life expressed in hours shown 0.67 h for test and 1.04 h for reference respectively. The Mean Residence Time for test is 5.48 h and 5.49 h for reference. The mean ± SE values of pharmacokinetic parameters (Microbiological assay) for total area under the curve (AUC 0-∞) were 22.11 and 19.33 h.mg/l for both test and reference tablets respectively. The mean ± SE values of clearance measured in l/h were 29.02 and 31.63 respectively. The elimination rate constant Kel [l/h] showed 0.21 l/h for test and 0.20 l/h reference tablets and likewise, absorption half-life expressed in hours shown 0.86h for test and 0.56 h for reference respectively. The Mean Residence Time for test is 5.27 h and 4.67 h for reference. Significant difference observed between two methods.

Keywords: HPLC, microbiological assay, ciprofloxacin, bioequivalence, pharmacokinetics.

INTRODUCTION



Ciprofloxacin (C_{17} H_{18} F N_3 O_3)

Prevention of diseases is a consistent attempt of scientist to enhance the quality of health and life. Antibiotics play a significant role to check susceptible/infectious diseases and are one of the extensively used/prescribed drugs especially in the developing world. Development of antibiotics resistant bacteria is a problem continuously inciting the scientists to modify the existing or develop newer remedies, which has resulted in a constant flow of the products in the market. Quinolones the especially ciprofloxacin are intended for use by the susceptible disease specialists, primary care practitioners, pharmacologists, pharmacists, advance nurse clinicians, nurse practitioners, and other physicians involved in the treatment of patients with upper & lower respiratory tract and other infections. Fluoroquinolones have become an extremely important addition to our armamentarium against infection. The role of the newest generation of fluoroquinolones, derivatives released in the late 1990s, is still being developed. The inclusion of the fluorine atom further improves potency; enhances antimicrobial activity; and alters pharmacokinetics properties, which provide tremendous therapeutic advantages. Quinolones especially fluorinated derivatives were introduced in 1980s, (Ciprofloxacin, 1987) and are freely available in Pakistan. Fluoroquinolones derivatives are useful in the treatment of infections caused by some gram negative and gram positive organisms Thomas *et al*. (2001). Quinolones are composed of a bicyclic aromatic core, a dual-ring chemical structure. Some have a quinolone

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nucleus; others have a napthyridone nucleus. Their mechanism of action is unique among available antibiotics, suggesting that cross-resistance to the fluoroquinolones can be minimized. The quinolone with unique structure also inhibits relaxation of super-coiled necessary for DNA replication and increases double-stranded DNA breakage. Absorption of fluoroquinolones from GI tact is rapid and absorption in humans is greater than 70% Walker *et al*. (1987). The chemical name of the ciprofloxacin is 1-Cyclo-Propyl-6-Fluoro-1,4-dihydro-4-oxo- 7-(Piperazin-1-yl)-quinoline-3-Carboxylic Acid. The tablet contains ciprofloxacin hydrochloride monohydrate ($C_{17}H_{18}FN_3O_3$. HCl. H_2O), molecular weight 385.8). The intravenous formulation contains ciprofloxacin lactate; the starting material is ciprofloxacin ($C_{17}H_{18}FN_3O_3$, molecular weight 331.4). Ciprofloxacin has uncharacteristic melting range with decomposition at around 270°C. Ciprofloxacin is a quinolone acid derivative that shows a rapid onset of action, and lack of cross-reactivity with pencillins, cephalosporins, and aminoglycosides. Four metabolites of ciprofloxacin were identified during the study in body fluid as desethyleneciprofloxacin, sulphociprofloxacin, oxocipro-floxacin and formaylciprofloxacin.

Ciprofloxacin as zwitterion, has good penetration ability, accumulated in tissues and widely distributed throughout the body. It is observed that concentrations of fluoroquinolone derivatives are higher in tissue than plasma.

MATERIALS AND METHODS

Pharmacokinetics, bioavailability and bioequivalence of the ciprofloxacin were investigated in normal healthy male subjects. The protocol adopted for this study was crossover designed as a single-dose on the healthy male subjects with 7 days washout period and by analyzing the samples by two validated methods i.e. High performance liquid chromatography and Microbiological Assay. The study was conducted in accordance with good clinical practice guidelines. The young healthy male volunteers who presented themselves for the studies were helped to fill out the questionnaire covering details of their history. This history included complete address, demographic information, smoking, blood donation, food, medication, allergies, surgery if any and disease status. More than 19 years of age, only healthy non-smoker subjects with homogenous age and body weight were enrolled for the study. Each volunteer voluntarily signed the "Informed Consent Form" at the time of registration and dully approved by an Institutional Ethics Committee constituted by head of the centre (59^{th} WMA General Assembly, Seoul, October 2008). The young healthy male volunteers 28 in number were randomly divided into 2 groups 14 each in Group 1 and 2. A conventional non-replicate crossover design, two formulations, two periods, and two sequences cross over design were used. After an overnight fast of at least 8-12 hours, subjects were randomized to receive a single dose of one tablet of 500 mg Ciprofloxacin standard or test tablet with 240 ml of water.

Ciprofloxacin tablets as test drug and reference standard Ciprofloxacin i.e. market imaging were used for the study. The label information of the tablets used is given below. Subjects were admitted at the Study Center and were briefed about the study protocol by a Medical Expert on the day prior to drug administration (Day 0). Subjects fasted overnight for at least 10 hours prior to morning dosing and continued to fast for an additional 2 hours after dosing.

1. Drug test
Ciprofloxacin 500 mg tablets
B. No. Test (ready for marketing)
Mfg. 01-2002 Exp.12-2005
Disintegration Time=7-10 mins
Hardness= 9-12 N Friability = 0.01%

2. Drug reference
Ciprofloxacin 500 mg tablets
Batch No. 132 - B
Mfg. Date 05-2001 Exp. Date 04-2006
Disintegration Time= 3-4 mins
Hardness= 35-42 N Friability = 0.01%

Sample collection and handling
Before drug administration, a control/blank venous blood sample was collected from each volunteer through a sterile venous Branula with in-stopper 18G aseptically inserted in the vein of left arm. Before drug administration (predose) venous blood about 5-ml was collected from each volunteer. Following drug administration, serial blood samples were drawn at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 8.0, and 12 hours in the heparinized tubes. Heparinized blood samples from various volunteers were centrifuged and the plasma was separated and stored until the day of analysis at -20^{o}C.

Analytical methods
Two types of analytical methods were used to quantify quinolones (Ciprofloxacin) concentration in plasma.

High pressure liquid chromatography method (HPLC)
This method was employed in the analysis of ciprofloxacin in plasma due to relative simple sample preparation step and only isocratic chromatographic elution that separate and quantitate in just 10 minutes of total run. The normal chromatograms of ciprofloxacin having average retention time 6.35 minutes fig.5 In order to demonstrate acceptable measurement precision, five runs of 0.5 ug/ml solution were made and the relative standard deviations were not more than 2.0%. Limit of

detection (LOD) was established by stepwise dilution of a known standard of 0.2 ug/ml until the point at which a real positive response is no longer detected. LOD was calculated by dilution method on the basis of signal noise ratio (S/N) of 3 using dilutions in the range of 0.010-0.040 µg/ml and that was 0.02 µg/ml. Limit of quantification (LOQ) is the lowest concentration points of the linear range i.e. 0.2 ug/ml with relative standard deviation value of 10.0%. LOQ was determined by dilution method using signal noise ratio (S/N) of 10 from sample dilutions in the range of 0.05-0.5 µg/ml that was 0.200 µg/ml. The regression coefficient (R^2) is 0.9995.

HPLC separation was performed using a LC-10As, Liquid Pump single plunger pump, CDQR system with flow rate range 0.01-5 ml/min (0-400 kgf/cm^2) and 5.01-99.9 ml/min (10-200 kgf/cm^2) with timer programme control of flow rate. SPD-10AV-UV-VIS spectro-phtometric detector wavelength 190-900nm with range 0.0001 - 2.56 AUFS Auto-sampler SIL-10A Auto injector for 220 v with sample rack and 1.5 ml sample vial/cap. DATA processor, C-R7A, chromatopac, with 40MB hard Disc Drive, thermal chart paper. The N-N-Dimethylformamide (6%v/v), Sodium dihydrogen phosphate monobasic (NaH_2PO_4) (0.01 M) Merck, Acetonitrile (15% v/v) HPLC grade Merck, Phosphoric Acid (Fluka) and Distilled water. (6:79:15) pH=3.0 was used as mobile phase. Heparinized blood samples from various volunteers were centrifuged and the plasma was separated and stored at -20°C until the day of analysis. The frozen plasma was allowed to thaw at room temperature and extracted. One ml of plasma sample was transferred into a test tube, 0.5 ml of different concentration of ciprofloxacin standard preparations was added and to it 1ml of acetonitrile was then added and the mixture was vortexed for 5 minutes, then the samples were centrifuged and the supernated solution was transferred into HPLC vials for chromatography analysis. Aliquots (20µl) of ciprofloxacin reference standard and plasma spiked samples, were injected in to the bondapack C_{18} column at constant temperature 50ºC, and flow rate was adjusted at 1 ml/minute. The mobile phase composition: Acetonitrile: NN Dimethyl formamide: 0.01 M Sodium Dihydrogen Phosphate Dihydrate (15:6:79) and pH3.0 was adjusted by adding phosphoric Acid (85%).

Microbiological agar diffusion method

The ciprofloxacin concentration in the plasma samples was also measured by microbiological assay. The method is reproducible and accurate for plasma. For assay of ciprofloxacin, the Disc Agar Diffusion Method was standardized and validated for accuracy and precision by using Streptococcus faecalis as test organism by the method of Arret *et al.*, 1971. The concentration of Ciprofloxacin in plasma was determined at least in duplicate. The zones of inhibition were estimated with help of Zone Reader and the concentrations of ciprofloxacin in plasma samples was calculated with standard curve equation formula for at least duplicate analysis of standard ciprofloxacin. The standards were run with each analysis. The concentrations of standard ciprofloxacin in plasma and minimum and maximum zone sizes recorded during the studies were LOQ 0.5ug/ml and LOD 0.1ug/ml.The regression equation and regression coefficient (R^2) is 0.9918.

STATISTICAL ANALYSIS

Pharmacokinetics/bioavailability parameters

The ciprofloxacin plasma concentration as a function of time data and the graphics were computed using software Microsoft Excel 7.0. The plasma concentration of ciprofloxacin from each volunteer was plotted on a semi

Table 1: Comparisons of plasma concentration of ciprofloxacin after an oral dose of 500 mg test tablet and reference tablet Ciprofloxacin measured by both high pressure liquid chromatography method and microbiological assay in healthy male volunteers

Time Hours	0.5	1	1.5	2	2.5	3	4	5	6	8	12
Plasma Concentration µg/ml by HPLC & Microbiological assay											
Test HPLC Mean ± SE	0.56 ± 0.04	1.65 ± 0.07	2.68 ± 0.08	2.39 ± 0.05	1.89 ± 0.05	1.48 ± 0.05	1.08 ± 0.03	0.82 ± 0.03	0.67 ± 0.02	0.40 ± 0.01	0.19 ± 0.01
Test Micro Mean ± SE	2.89 ± 0.27	3.95 ± 0.32	3.67 ± 0.28	3.20 ± 0.28	2.43 ± 0.20	2.11 ± 0.23	1.66 ± 0.18	1.26 ± 0.13	1.00 ± 0.11	0.64 ± 0.08	0.27 ± 0.03
Ratio T/T	0.194	0.42	0.73	0.75	0.78	0.70	0.65	0.65	0.67	0.63	0.70
Reference HPLC Mean ± SE	0.39 ± 0.02	1.04 ± 0.03	1.84 ± 0.03	2.43 ± 0.05	2.10 ± 0.02	1.65 ± 0.03	1.13 ± 0.03	0.88 ± 0.03	0.68 ± 0.02	0.40 ± 0.02	0.15 ± 0.01
Reference Micro Mean ±SE	2.96 ± 0.25	3.80 ± 0.20	3.47 ± 0.17	2.99 ± 0.18	2.40 ± 0.13	1.97 ± 0.11	1.53 ± 0.09	1.14 ± 0.05	0.87 ± 0.04	0.56 ± 0.03	0.25 ± 0.02
Ratio R/R	0.32	0.27	0.53	0.81	0.88	0.84	0.74	0.77	0.78	0.72	0.6

logarithmic scale against time. The plasma concentration versus time data was used to calculate parameters of bioavailability and pharmacokinetics by PC-Computer Program, APO, MWPHARM version 3.02 a MEDIWARE product Holland (1987). Calculations also included area under curve (AUC) from time t to ∞ (infinity) by polyexponential and trapezoidal methods and the regression coefficient of best fit to depict the compartmental analysis.

RESULTS

The comparative mean ± SE "bioequivalence" parameters of ciprofloxacin 500 mg Test and Reference (Brayer) tablets given orally as 500 mg dose to 14 volunteers by both methods have been presented in the table1.

The area under the plasma concentration versus time curve (AUC inf) for the test tablets calculated through HPLC and microbiological assay methods are 13.11 ± 0.27 and 22.11 ± 1.94 h.mg/l with a ratio (T/R) is about 0.59, with correlation value 0.32805while, the reference are 11.91 ± 0.26 and 19.33 ± 0.75 with ratio (T/R) 0.62 with correlation value 0.126949 respectively as shown in table 4. The Values for the time to peak concentration Tmax in hours were 1.71 ± 0.02 and 2.00 ± 0.04 for test with ratio (T/R) between two methods is 0.855 & correlation value 0.344854. For reference the ratio of both methods is 0.72 & correlation 0.213922 respectively. The peak drug concentration (Cmax) expressed in mg/L for test drugs was 2.28 ± 0.04 HPLC and for the microbiological assay 4.09 ± 0.33 with correlation values 0.558052 for test and for reference about 0.197795. The difference in the log C_{max} [Ln(C_{max})] was nearly -40.15 %. The area under the plasma concentration versus time curve (AUC) measured by HPLC and microbiological (Test–A) and Reference R1 Tablets given to 14 volunteers on a linear scale.

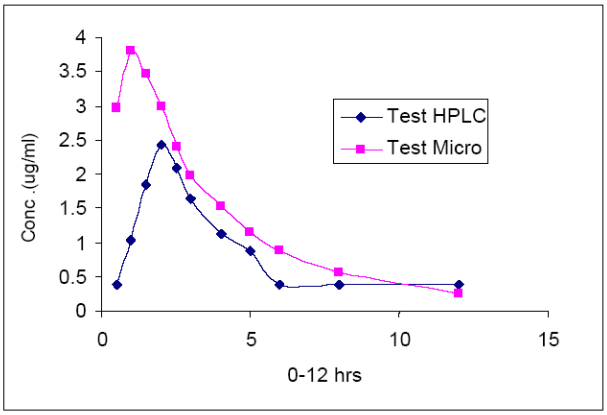

Fig. 2: Figure showing values of the plasma concentration measured by HPLC and Microbiological assay of ciprofloxacin after 500 mg oral dose of Test -A Tablets given to 14 volunteers on a linear scale.

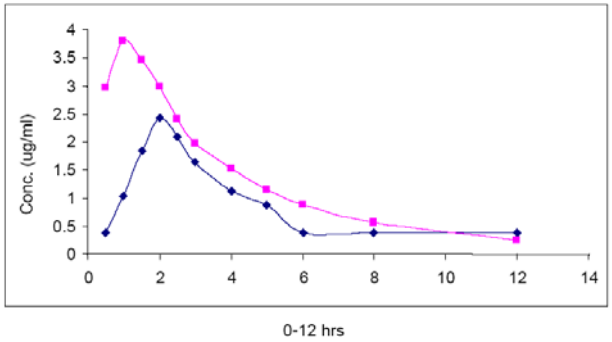

Fig 3: Figure showing values of the plasma concentration measured by HPLC and Microbiological assay of ciprofloxacin after 500 mg oral dose of Reference R1 Tablets given to 14 volunteers on a linear scale.

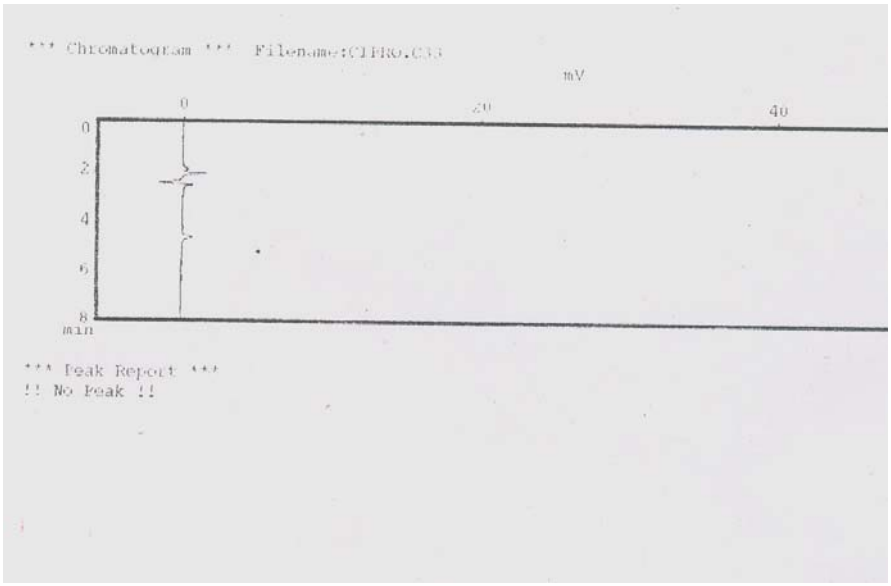

Fig. 4: Standard Chromatogram of plasma sample.

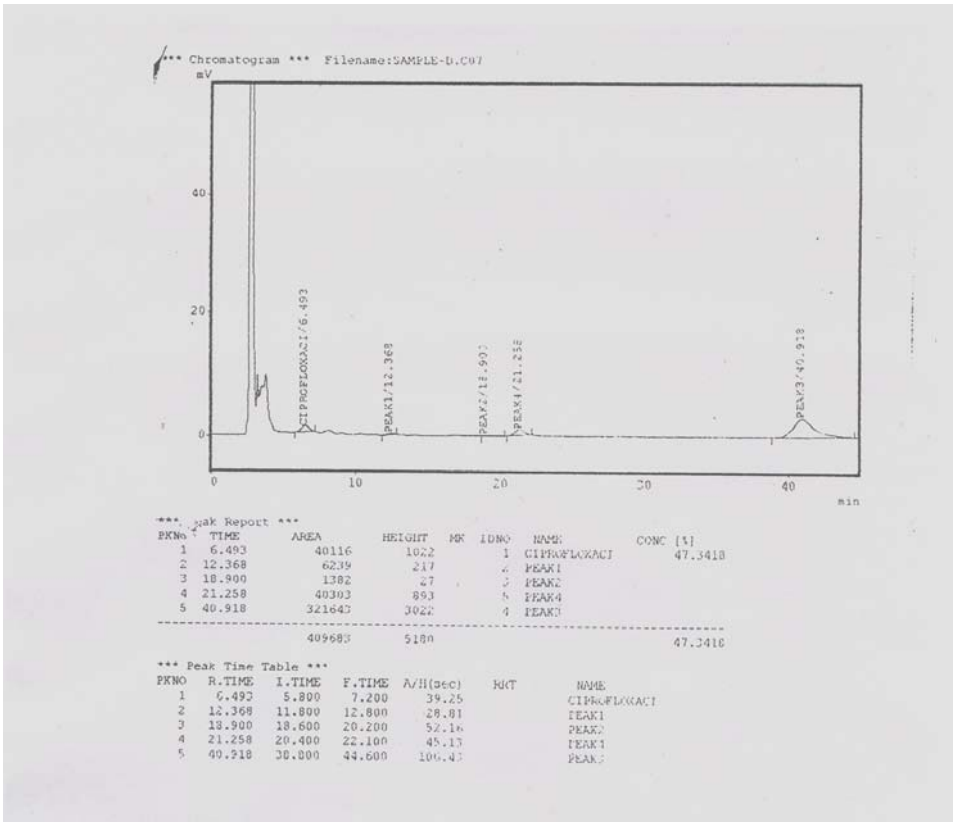

Fig. 5: Chromatogram of ciprofloxacin extracted from plasma sample.

respectively in table 2. The correlation values for pharmacokinetics parameter are reflected in table 4. The mean ± SE values of pharmacokinetic parameters (Microbiological assay) for total area under the curve (AUC 0-∞) were 22.11 ± 1.94, and 19.33 ± 0.75 h.mg/l for both test and reference tablets respectively. The mean ± SE values of clearance measured in l/h were 29.02 ± 1.70 and 31.63 ± 1.39 respectively. The elimination rate constant Kel [l/h] showed 0.21 ± 0.03 l/h for test and 0.20 ± 0.01 l/h reference tablets and likewise, absorption half-life expressed in hours shown 0.86 ± 0.26h for test and 0.56 ± 0.17h for reference respectively. The Mean Residence Time for test is 5.27 ± 0.44 h and 4.67 ± 0.22 h for reference.

DISCUSSION

In the present study the only T_{max} results are comparable between HPLC and bioassay results for ciprofloxacin and rest of other Parameters of bioequivalence and pharmacokinetics were not comparable. Ziegler *et al.* (1983) found no significant differences in plasma or in urine. In contrast, Wingender *et al* (1984) reported higher values for urine samples when determined by a microbiological assay. The urinary recovery of ciprofloxacin in these studies was about 30%, which is comparable to the results of Crump *et al.* (1983) who used a microbiological assay with Iso-Sensitest agar (pH 7.4) (Oxoid) as the nutrient base.

The Ciprofloxacin Test peak concentration as detected by high performance liquid chromatography in plasma was 2.68 μg/ml for test drug at 1.5 h and 2.43 μg/ml at 2 h for reference tablet respectively, while the concentration for test and reference tablet as measured by microbiological assay in plasma was attained after 1 hour of drug administration (3.80 and 3.95 μg/ml) respectively (table 3). After 12 hrs of sampling the plasma levels of the drug by microbiological assay fell to 0.25 and 0.27 μg/ml for both test and reference product and for HPLC is 0.19 and 0.15. These were compared with study conducted by Borner et al. (1986) studied pharmacokinetics of ciprofloxacin in 16 healthy male and female volunteers. HPLC and microbiological assay were used for determining drug concentrations in serum. After 60-75min of oral administration (750mg dose) the mean peak concentrations were 0.37±0.49 mg/l (100 mg dose) and 1.97±0.50 (750 mg dose) After 12 hrs serum concentrations were 0.15±0.05 mg/l. Catchpole et al, (1994) observed the pharmacokinetics comparison of ciprofloxacin following single doses of 400 mg i.v. and 750 mg post oral in six healthy volunteers. Concentrations of ciprofloxacin were measured in plasma; by microbiological assay and high performance liquid

chromatography (HPLC). Mean peak plasma concentration was 6.7±1.4 mg/L after i.v. and 3.9±1.7 mg/L after oral administration. The success of antibacterial therapy exhibiting time dependent activity is highly correlated with the time at which plasma or biological fluid concentrations of antibiotic exceed minimum inhibitory concentration (MIC) (Rao et al. 2002). Previous studies suggest that fluoroquinolones kill bacteria in a concentration-dependent manner and area under inhibitory curve (AUIC) calculated by AUC/MIC is highly correlated with the outcome of successful treatment (Drusano et al. 1993, Ali Abadi and Lees. 1997). For effective eradication of bacteria and good clinical therapy, it has been suggested that an AUIC ≥ 100 is required for gram-negative bacteria and ≥ 30 is needed for gram-positive organisms (Nightingale et al, 2000; Walker and Wright. 1987). Although MIC values of ciprofloxacin for many pathogens of genus Pasturella, Escherichia, Haemophilus, Moraexella, and Salmonella are reported to be in the range of 0.01-0.06 µg/ml (Bottner et al. 1995). On the basis of MIC reported for highly sensitive pathogens (0.01-0.06 µg/ml) and AUC (22.11 ± 1.94, and 19.33 ± 0.75 h.mg/L) determined in the present study, AUIC would be much greater than 100 for effective eradication of bacteria and good clinical therapy but no correlation exist both method of analysis.

CONCLUSION

The ciprofloxacin concentration in the plasma samples are measured by HPLC and microbiological assay. Both the methods are reproducible and accurate. HPLC method measures drug and their metabolite(s) without distinguishing their microbiological activity, hence, for most antibacterial drugs a validated microbiological assay procedures is more appropriate for the assay of drugs in biological samples. There is significant difference observed in both methods and microbiological assay shows higher concentration of ciprofloxacin contents therefore no correlation existed by comparing the AUC(h.mg/L), Cmax(mg/L), half life ½ (h), clearance CL (l/h), T_{max} (h), Volume of distribution [l] and MRT (h) for both the methods.

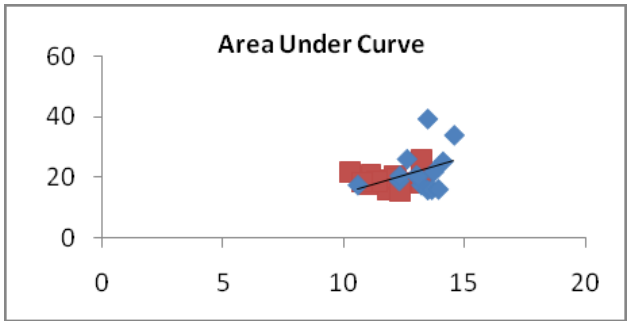

Fig. 6: Correlation diagrams between HPLC V:S Microbiological assay methods for both test &reference tablets [For Area Under Cure (Scatter plots)]

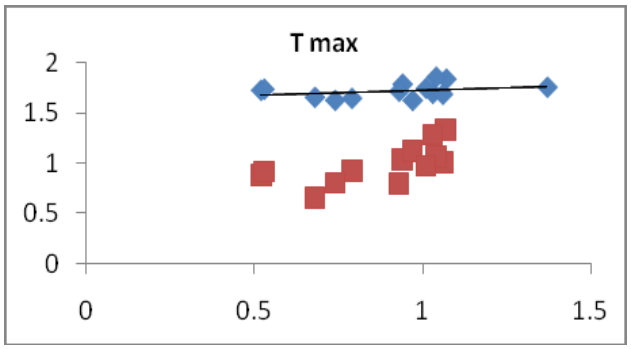

Fig. 7: Correlation diagrams between HPLC V:S Microbiological assay methods for both test &reference tablets [For Tmax (scatter plots)]

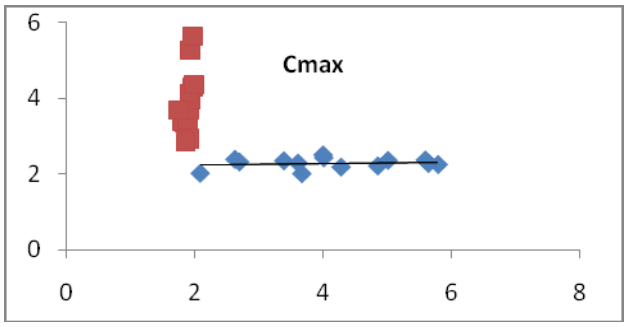

Fig. 8: Correlation diagrams between HPLC V:S Microbiological assay methods for both test &reference tablets [For Cmax (scatter plots)]

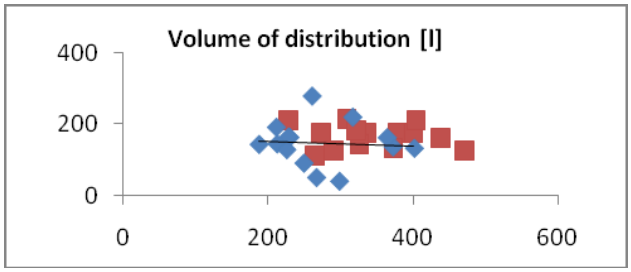

Fig. 9: Correlation diagrams between HPLC V:S Microbiological assay methods for both test &reference tablets [For Volume of distribution (scatter plots)]

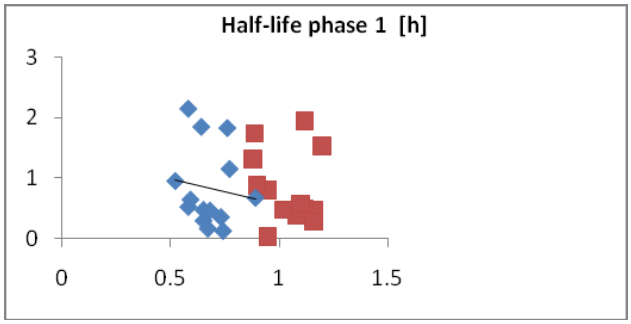

Fig. 10: Correlation diagrams between HPLC V:S Microbiological assay methods for both test and reference tablets [For Half-life phase 1(scatter plots)]

Table 2: Comparison of pharmacokinetics/ Bioavailability parameters of ciprofloxacin after oral dose of 500 mg tablets of test (Test-A) and reference R1 Tablets (Ciprofloxacin) measured by high pressure liquid chromatography and Microbiological assay

Kinetic Parameters	AUC inf	Ln (AUC inf)	AUC polyexponential (t= 12)	AUC trapezoidal rule (t= 12)	(AUCTail)	Ln (AUCTail)	AUCxtr %	Clearance (CL) [l/h]	Half-life phase 1 [h]	Half-life phase 2 [h]	Beta [1/h]	Mean Residence Time (MRT) [h]	Absorption rate constant (ka) [1/h]	Absorption half-life [h]
Plasma Concentration µg/ml by HPLC & Microbiological assay														
Test HPLC Mean ± SE	13.11 ± 0.27	2.57 ± 0.21	10.1 ± 0.21	10.1 ± 0.19	1.93 ± 0.12	0.63 ± 0.07	14.6 ± 0.77	44.9 ± 0.93	0.68 ± 0.03	4.22 ± 0.27	0.17 ± 0.01	5.48 ± 0.16	1.06 ± 0.05	0.67 ± 0.03
Test Micro Mean ± SE	22.11 ± 1.94	3.06 ± 0.08	16.5 ± 1.27	16.5 ± 1.38	4.08 ± 0.81	1.22 ± 0.16	16.9 ± 1.78	29.0 ± 1.70	0.83 ± 0.19	3.92 ± 0.45	0.21 ± 0.03	5.26 ± 0.44	1.83 ± 0.31	0.86 ± 0.26
Ratio T/R	0.59	0.84	0.61	0.61	0.47	0.52	0.86	1.55	0.82	1.08	0.81	1.04	0.58	0.78
Reference HPLC Mean ± SE	11.91 ± 0.26	2.47 ± 0.02	9.63 ± 0.19	9.66 ± 0.18	1.51 ± 0.09	0.39 ± 0.06	12.70 ± 0.71	48.42 ± 1.18	1.05 ± 0.03	4.97 ± 0.31	0.15 ± 0.01	5.49 ± 0.08	0.68 ± 0.02	1.04 ± 0.03
Reference Micro Mean ± SE	19.33 ± 0.75	2.95 ± 0.04	15.1 ± 0.61	15.4 ± 0.60	3.15 ± 0.19	1.13 ± 0.06	16.4 ± 1.03	31.6 ± 1.39	0.81 ± 0.16	3.72 ± 0.29	0.20 ± 0.01	4.67 ± 0.22	1.75 ± 0.27	0.56 ± 0.17
Ratio T/R	0.62	0.84	0.64	0.63	0.48	0.35	0.77	1.53	1.30	1.34	0.75	1.18	0.39	1.86

Table 3: Mean ± SE Bioequivalence parameters for test (Test-A) and reference R1 Tablets of 500 mg ciprofloxacin measured by High Pressure liquid chromatography method and Microbiological assay

Bioequivalence parameters	Units	Test Mean ± SE	Test Mean ± SE	Ratio T/R	% Difference
		HPLC	Microbiological assay		
AUC*	h.mg/l	11.2 ± 0.22	18.0 ± 1.17	0.62	-37.94
Ln(AUC)*	h.mg/l	2.41 ± 0.02	2.87 ± 0.06	0.84	-16.03
Tmax*	H	1.71 ± 0.02	0.91 ± 0.06	1.88	87.91
Cmax*	mg/L	2.28 ± 0.04	4.09 ± 0.33	0.56	-44.25
Ln(Cmax)*	mg/L	0.82 ± 0.02	1.37 ± 0.09	.60	-40.15
		Reference Mean ± SE HPLC	Microbiological assay		
AUC*	h.mg/l	10.4 ± 0.26	16.2 ± 0.72	0.64	-35.72
Ln(AUC)*	h.mg/l	2.34 ± 0.02	2.77 ± 0.04	0.85	15.52
Tmax*	H	2.00 ± 0.04	0.97 ± 0.06	0.72	106.19
Cmax*	mg/L	1.90 ± 0.02	3.89 ± 0.22	0.49	-51.16
Ln(Cmax)*	mg/L	0.64 ± 0.01	1.34 ± 0.05	0.48	-52.24

Table 4: Correlation (r) between Bioequivalence & pharmacokinetics parameters for Test and Reference Tablets of 500 mg Ciprofloxacin measured by High Pressure liquid chromatography method and Microbiological assay

Parameters	Correlation (r) for Reference Tablets of HPLC V:S Microbiological methods	Correlation (r) for Test Tablets of HPLC V:S Microbiological methods
Total Area Under the Curve (AUC) [h.mg/l]	0.126949	0.32805
Volume of distribution [l]	-0.13217	-0.06683
Mean Residence Time (MRT) [h]	-0.33024	-0.02278
Half-life phase 1 [h]	-0.17275	-0.12108
Clearance (CL) [l/h]	-0.06861	0.43102
T_{max} [h]	0.213922	0.344854
C_{max} [ug/ml]	0.197795	0.558052

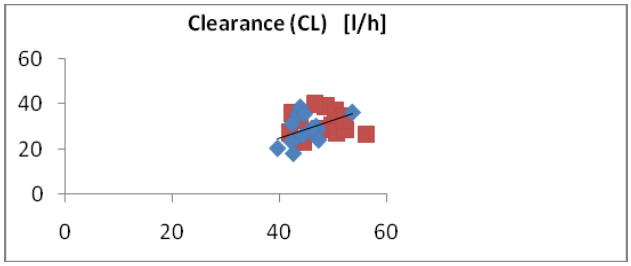

Fig. 11: Correlation diagrams between HPLC V:S Microbiological assay methods for both test &reference tablets [Clearance (scatter plots)]

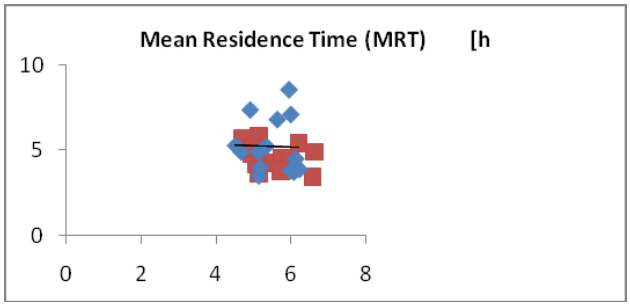

Fig. 12: Correlation diagrams between HPLC V: S Microbiological assay methods for both test &reference tablets [Mean Residence time (scatter plots)]

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