

## **REPORT**

# **Pharmacokinetic and drug interaction studies of Pefloxacin with paracetamol (NNAID) in healthy volunteers in Pakistan**

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**Abstract:** In the present study, the pharmacokinetic and drug interaction evaluation of two drugs pefloxacin and paracetamol was carried out by a single-dose, two-treatment and two-sequence crossover design. Total fifteen healthy volunteers participated out of which ten completed the study. All were male volunteers, aged 22.36 years (means), with a mean weight of 76.45±12.05 Kg. The washout period between treatments was 5 week. Initially the method utilized for quantitative analysis of the drug was developed which was further validated. The study involved plasma protein precipitation with ethyl acetate and detection was done at 275nm. The retention time for pefloxacin 18±1 min and paracetamol were approximately 6±1 min, respectively. The calibration curve for pefloxacin was linear in the concentration range of 0.125-12.0µg/ml with  $r^2=0.9987$  in plasma. Standard concentration solution was maintained on the same temperature as that of volunteer's samples to optimize the periods for the determination of drug concentration in the plasma samples. Blood samples were collected from volunteers at different time intervals. The pharmacokinetics and drug interaction studies were anticipated by plotting concentration versus time-profiles. The value of  $AUC_{0-\infty}$  in control was 67.355±3.174µg.h/ml, in treatment 61.242±3.868µg.h/ml along with relative bioavailability =91.395±4.864. Under the control and treatment condition the mean maximum plasma concentrations were found to be 4.679±0.248 µg/ml and 4.6595±0.266 µg/ml respectively. The average  $T_{max}$  for plasma concentrations was 1.819±0.1743hr and 1.605±0.1134hr respectively. The biological half-lives in the two phases of studies were found to be 7.953±0.33hr in control and 7.7257±0.355hr in treatment. No significant effect were observed on the bioavailability and pharmacokinetics of pefloxacin by the concomitant administration with paracetamol, however very minor effect were observed that might be related with inter-individual variation in human volunteers. This pharmacokinetic studies also indicated that the level of drug ( $C_{max}$ ) do not differ from previous studies in different races.

**Keywords:** HPLC method development; pefloxacin; pharmacokinetics; drug interaction, paracetamol and bioavailability.

## **INTRODUCTION**

There are several therapeutic misadventures that had occurred in the bioavailability studies of various drugs such as phenytoin, primidone, and digoxin that made it necessary to evaluate the performance of dosage form in delivering the active substance to the systemic circulation and thereby to the site of action. The pharmacokinetics and bioavailability studies are done to ensure that the active substance is being released from pharmaceutical product at the optimum level. These pharmacokinetics data are usually used to establish the therapeutic profile.

Pefloxacin mesylate is a fluoroquinolone antibiotic. This is rapidly growing class of antibacterial agent that has proved useful in the treatment of many soft tissues infections, urinary tract infection, gastrointestinal tract infection, respiratory tract infection, ulcers, skin infection, endocarditis, septicaemia and toxic shock syndrome etc. This newer fluoroquinolone also display favorable

pharmacokinetics profiles, including excellent tissues penetration and convenient once a day administration. Increased drug availability in the tissue results in increased activity against the enzyme target DNA gyrase. This exhibits a linear correlation between the MIC against *Escherichia coli* and interaction with gyrase as measured either by inhibition of super coiling or cleavable complex formation (Crumplin, 1990). The gram-positive potency of this agent is also enhanced over that of the first generation quinolone agents.

The pharmacokinetics studies is a major area of concern for the majority of under developed countries such as Pakistan where doctors are prescribing medicines according to the pharmacokinetic literature available from developed countries. Many scientists have worked on the racial aspect of pharmacokinetics. Zhou *et al.* in 1989 studied various pharmacokinetic parameters of propranolol on Caucasians and Chinese population. Zhou *et al.* in 1993 observed 50% difference in clearance of

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morphine in Caucasians comparing the Asians. The other drugs such as nifedipine, alprazolam, and omeprazole showed significant difference due to lower hepatic metabolism of these drugs in Asians. It is also observed that some significant therapeutic variations are found in different brands of the drug even though they have the same generic active ingredient this can be due to the fact that they are manufactured by different pharmaceutical companies. This holds true especially for pefloxacin (an antibiotic) which is marketed by different (ten local) companies in Pakistan and not a single clinical study has been done about this drug in Pakistani population.

Under these circumstances the clinical studies of pefloxacin is mandatory because it is still under the FDA consideration. There are no licensed uses for pefloxacin in the United States. The licensed uses in various other countries are also quite limited. It is considered as a drug of last resort when all other antibiotics are failed. Pefloxacin has interaction with a number of other drugs as well as with a number of herbal and natural supplements, and certain thyroid medications. Therefore, the pharmacokinetic analysis in local population is important to adjust the dose properly as well as to evaluate the loading dose, maintenance dose, and adverse effects.

The erratic absorption of pefloxacin mesylate and the unpredictable bioavailability has been reported in several studies for large unpredictable variation in the blood levels after oral administration of the drug (Gonzalez and Henwood 1989; Montay *et al.*, 1984; Webberley *et al.*, 1987; Wolff *et al.*, 1984). This gives a strong rationale to support the need for a bioavailability study of pefloxacin mesylate along with the influence of other medication. The present study has been done with paracetamol. It is a very common and popular OTC drug. The chemical name is acetaminophen, NSAID, Nor-Narcotics anti-inflammatory drug. Paracetamol due to its cyclooxygenase inhibition property is sometimes grouped together with the NSAIDs. Paracetamol, however, does not have any significant anti-inflammatory properties and is not a true NSAID.

## MATERIAL AND METHODS

### Drug and reagents

Pefloxacin mesylate (reference powder) were obtained from the courtesy of Sami Pharmaceutical (Pvt) Ltd. Karachi. Test tablets (Code: PEF<sub>D</sub>) containing pefloxacin; paracetamol containing tablets (Code: PARA). All other reagents such as acetonitrile; ortho-phosphoric acid (89%); potassium hydroxide (KOH); ethyl acetate and distilled water were analytical grade. Heparin injection (Shenzhen China) and human blood free from drug obtained from Baqai Medical University.

### Subject

The total fifteen (15) healthy volunteer participated in this study. Ten (10) completed the study. Five (5) dropped out as a result of noncompliance. All were male volunteers, aged from twenty to twenty four years (20-24 years with mean age =22.36years), with a mean weight of 76.45±12.05 Kg. A written consent was taken from these volunteers.

### Study drugs

The tested drug was PEF<sub>D</sub> tablet (400mg) of film-coated pefloxacin. The concurrent formulation was a 500mg paracetamol (acetaminophen) tablet.

### Study design

The study was a single-dose, two-treatment, two-sequence crossover design. An equal number of volunteers were assigned to each sequence. The washout period between treatments was 5 week or 35 days.

### Procedure

All volunteers received a single 400mg dose of pefloxacin mesylate orally with 240ml water, after fasting for at least 10 hours, during each of the two (2) trial periods.

The treatment was as follows:

- Treatment 1: 400mg pefloxacin tablet was taken alone with water.
- Treatment 2: 400mg pefloxacin tablet + 500mg paracetamol tablet, taken concomitantly with water.

The human bioavailability and pharmacokinetics study was performed at Department of Pharmaceutics, Faculty of Pharmacy, at University of Karachi. Analysis of the pefloxacin plasma concentration level was conducted by HPLC.

The volunteers were reported to the investigator after an overnight fast, on the morning of the both experiment days. Volunteers were asked to abstain from using ethanol, caffeine, chocolate, and tea for at least 24 hours before each dose. All volunteers abstained from smoking and exercise. The dietary regimen was similar for all volunteers in both trial periods.

Within the 7 days before the first administration, every volunteer verified his compliance with the inclusion and exclusion criteria. Table 1 shows the mean demographic data of the ten (10) volunteers who completed the study. One week before enrollment a complete physical examination was performed.

### Collection and storage of sample

Blood was taken from an indwelling intravenous cannula immediately before the administration of the dose and after 0.5, 1, 1.5, 2, 3, 5, 7, 8, 24, 32 hours post dose. The

collected blood samples were immediately heparinized centrifuged at 5000 rev/min for 5 minutes. The separated plasma was then immediately frozen at -20°C until assay.

According to the ICH (1996) guideline, the stability study should continue for at least the same length of time as that for which the study samples are to be stored. Therefore standard concentration solution was maintained on the same temperature as that of volunteer's samples to optimize the periods for the determination of drug concentration in the plasma samples (Shahnaz *et al.*; 2009). Their potency was checked from time to time which showed that concentration of drug in samples was maintained almost for (3) three weeks as reported by Abanmi *et al.* (1996).

#### Sample preparation

Plasma samples were analyzed for unchanged pefloxacin by reversed phase high performance liquid chromatography (HPLC) assays. The HPLC method (Shahnaz *et al.*; 2009) which was used, for extraction and analysis of pefloxacin was modified and validated form of Al. Obaidy *et al.* (1995). Ethyl acetate was used as precipitating agent (deproteinizing agent) and the ultraviolet detection at 275nm was used.

In this study, pefloxacin was administered alone and with paracetamol. The paracetamol concentrations were not analyzed because the emphasis was on determining the effect of paracetamol on the bioavailability of pefloxacin in human.

#### Preparation of mobile phase

Mobile phase consisted of 87% 0.025M orthophosphoric acid and 13% v/v acetonitrile adjusted to pH=2.9. The mobile phase was degassed and filtered by 0.45µm Millipore filter paper.

#### Analysis of plasma sample

Plasma concentrations of pefloxacin mesylate were measured by HPLC (LC-10ATVP Shimadzu liquid chromatograph).

#### Chromatographic condition

HPLC consisted of double piston, 2-pump system. Injector (Rheodyne, Japan) with 20µl loops size. Ultraviolet detector with wavelength at 275nm, a prepacked stainless steel column Shim-pack CLC-ODS (6.0mm ID x 15cm) column protected with Octa decyl Silane guard column. The system was operated at room temperature. Flow rate was 1ml/min.

#### Extraction

In a centrifuge tube, 1000µl (1ml) of plasma sample was mixed with equal volume (1000µl) of ethyl acetate. Vortex mixing for 1min and centrifuged for 5minutes at 5000rpm, resulted in deproteinization. Supernatant layer was collected and transferred to a 25ml beaker (Pyrex 25ml capacity). The plasma also underwent the same extraction procedure and then it was evaporated to the organic phase by drying in open air at room temperature. The residue was reconstituted in 500µl (0.5ml) of mobile phase and filtered with 0.45µm Millipore filter paper and 20µl of filtrate was injected into the HPLC system. The concentration of pefloxacin was calculated from the standard curve of pefloxacin peak.

#### Standard curve

Prior to sample analysis the solutions of known concentrations (0.25µg/ml, 0.5µg/ml, 1.0µg/ml, 4.0µg/ml, 8.0µg/ml and 12.0µg/ml) were analyzed and then standard curve of peak area were plotted versus their respective concentrations of pefloxacin. The values of regression coefficient 1 ( $r^2=0.9978$ ) was plotted which showed a slope of the curve indicating the precision and reproducibility of the assay procedure (fig. 2).

#### Calculation of pharmacokinetic parameters

Following pharmacokinetic parameters were calculated for assessing the bioavailability of the drug product.

#### Statistical evaluation of the data

Statistical evaluation was performed on the estimated pharmacokinetic parameters. The pharmacokinetic parameters values were calculated manually. An analysis

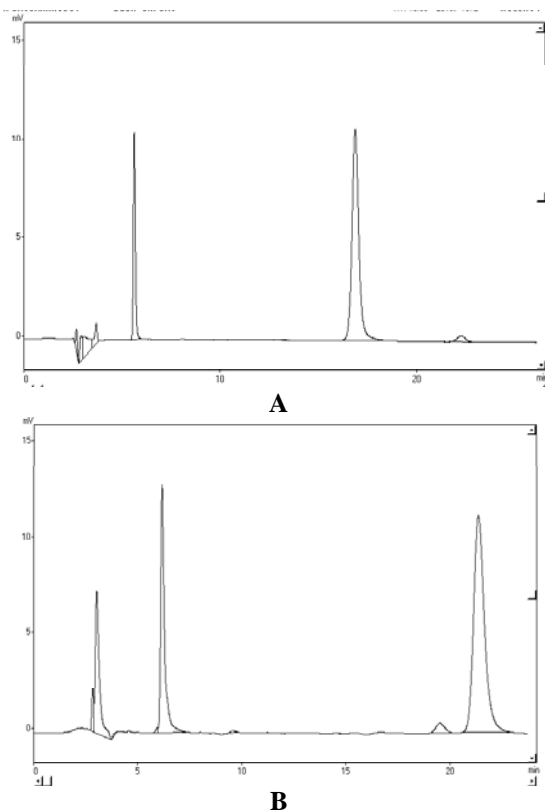
Parameters	Formula	Parameters	Formula
$C_{max}$	$C_{max} = \frac{FK_a D}{Vd(k_a - k_{el})} [e^{k_{el}t} - e^{k_a t}]$	Half Life	$t_{1/2} = 0.693 / k_{el}$
$T_{max}$	$T_{max} = \frac{2.3 \log(k_a / k)}{K_a k}$	Volume of Distribution	$V_d = \frac{\text{Dose}}{\text{Drug conc.}}$
AUC	$AUC = (1/2) (C_1 + C_2) (t_2 - t_1) + (1/2) (C_2 + C_3)(t_3 - t_2) + \dots + (1/2) (C_{n-1} + C_n) (t_n - t_{n-1})$	Total Clearance	$Cl_{tot} = \frac{k_{el} \cdot V_d}{60} [\text{ml/min}]$
Elimination rate constant	$(k_{el}) = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$	Mean Residence Time	$MRT = \frac{AUMC}{AUC} [h]$
Absorption rate constant	$(k_a) = \frac{\ln C_{1diff} - \ln C_{2diff}}{t_2 - t_1}$	Variance of Residence Time	$VRT = \frac{AUMC}{AUC} - MRT^2$

of variance (ANOVA) was used to determine the statistical differences inherent in the pharmacokinetic parameters. A statistical differences between the pharmacokinetic parameters obtained from two or more drug products is considered significant if there is a probability of less than 1 in 20 times or 0.05 probability ( $p \leq 0.05$ ).

For oral dosage forms, the bioavailability of the drug is most often described by measurement of the area under the plasma drug concentration- time curve (AUC), the time for peak drug concentration,  $T_{max}$ , and the peak drug concentration,  $C_{max}$ , whereas  $T_{max}$  and  $C_{max}$  relate to the rate of systemic drug absorption.

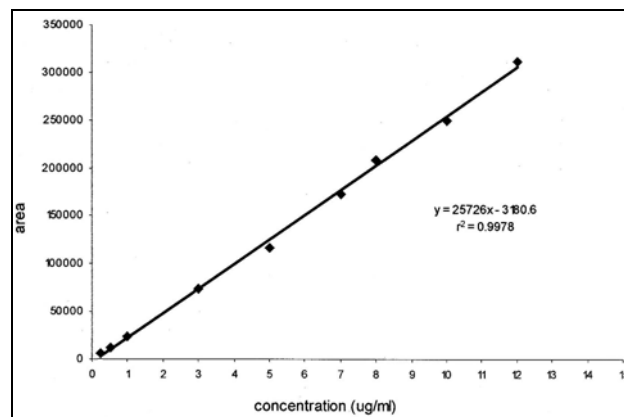
## RESULTS

The main objective of the present study was to determine whether the pharmacokinetics parameters varied with the concurrent administration of paracetamol or not. A good separation between pefloxacin and paracetamol was achieved by use of the mobile phase containing 87% 0.025M orthophosphoric acid and 13% v/v acetonitrile adjusted to pH; 2.9. Retention times were 17.34 and 5.828 min respectively, and changed less than 3% in both intra-day and inter-day analyses (fig. 1).



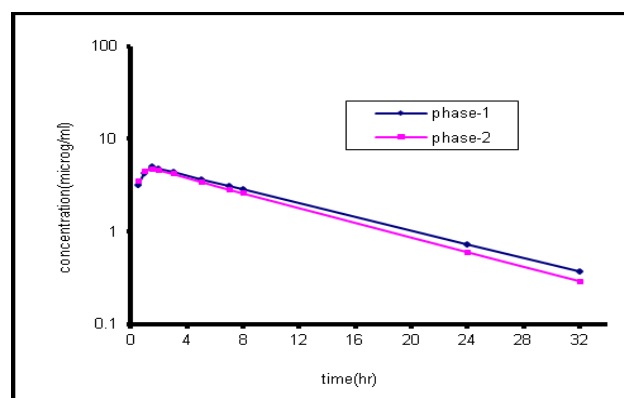
**Fig. 1:** A: Chromatogram of pefloxacin with paracetamol in mobile phase shows good separation, B: Chromatogram of pefloxacin with paracetamol in plasma shows good separation.

The calibration curve of pefloxacin was obtained before the HPLC analysis over the concentration ranges of 0.25-12.0  $\mu\text{g/ml}$  (fig. 2). The chromatographic system was validated first (Shahnaz *et al.*, 2009) than used for the determination of  $C_{max}$ ,  $T_{max}$  and area under the curve (AUC) for the pharmacokinetic analysis that made it precise, accurate and acceptable.



**Fig. 2:** Graphical presentation of peak area vs. standard concentration of pefloxacin.

The concentrations of pefloxacin (alone) and in combination of paracetamol (concomitant administration) in healthy volunteers were determined up to 32 hours. The mean concentration-time profile with Mean  $\pm$  SEM values for ten (10) healthy human volunteers after administration of a single dose of PEF<sub>D</sub> 400mg dose (alone) and concomitant administration of PEF<sub>D</sub> 400mg and PARA 500mg dose (paracetamol tablet) in fasting conditions showed that there is no significant effect of paracetamol on the pharmacokinetics of pefloxacin (fig. 3) (table 1).



**Fig. 3:** Comparisons of two phases

Statistical difference in the two phases of drug pharmacokinetics that in controlled and treated phases were compared by analysis of variance followed by f-ratio, t- test paired and t-test unpaired where the probability was less than 1 in 20 times or 0.05 ( $p < 0.05$ ).

**Table 1:** Mean plasma pharmacokinetic parameters obtained for pefloxacin (control) and pefloxacin plus paracetamol (treated) after a single oral administration.

Parameters		Units	Control group	Treated group
Maximum plasma Concentration	$C_{max}$	$\mu\text{g/ml}$	4.679 $\pm$ 0.248	4.6595 $\pm$ 0.266
Time to peak concentration	$T_{max}$	H	1.819 $\pm$ 0.1743	1.605 $\pm$ 0.1134
Area under plasma conc.-time curve from 0 to 32h post drug admin	$AUC_{0-t}$	$\mu\text{g. H/ml}$	63.0103 $\pm$ 2.89	57.937 $\pm$ 3.597
Area under plasma conc-time curve from 0 to infinity post drug admin.	$AUC_{0\rightarrow\infty}$	$\mu\text{g. H/ml}$	67.355 $\pm$ 3.174	61.326 $\pm$ 3.908
Elimination rate constant	$K_{el}$	$\text{Hr}^{-1}$	0.08835 $\pm$ 0.0035	0.0913 $\pm$ 0.00393
Absorption rate constant	$K_a$	$\text{Hr}^{-1}$	1.9499 $\pm$ 0.2413	2.26 $\pm$ 0.2142
Elimination Half Life	$T_{1/2\beta}$	H	7.953 $\pm$ 0.33	7.7257 $\pm$ 0.355
Absorption Half Life	$T_{1/2\alpha}$	H	0.407 $\pm$ 0.049	0.33 $\pm$ 0.028
Volume of Distribution	$V_d$	Lit	74.868 $\pm$ 3.797	76.464 $\pm$ 4.0435
Total Clearance	$Cl_{tot}$	Lit/hr	6.5799 $\pm$ 0.3653	6.9869 $\pm$ 0.5016
Mean Residence Time	MRT	Hr	11.934 $\pm$ 0.518	11.625 $\pm$ 0.516

Values are expressed as mean  $\pm$  SE (n=10)

## DISCUSSION

Ten (10) healthy volunteers (male) ranging in age from 20-24 years of age (mean 20.36 $\pm$ 1.319) completed the two phases of the study. The comparison of plasma concentrations after a single 400mg of pefloxacin oral administration and concomitant administration of pefloxacin (400mg) + paracetamol (500mg), for the entire 32 hours study period on ten volunteers (Mean  $\pm$  SEM) values indicated that the pattern of absorption of pefloxacin follows the one-compartment model. This finding is similar to that of Malik *et al.*, (2002); Nikolaidis *et al.*, (1991); Barre *et al.*, (1984).

In this study, area under the plasma levels versus time curves (AUC) were computed. The  $AUC_{0-\infty}$  (Mean  $\pm$  SEM) in control was found to be 67.355 $\pm$ 3.174 $\mu\text{g.h/ml}$ , whereas in treatment it appeared as 61.326 $\pm$ 3.908 $\mu\text{g.h/ml}$  (table 2) along with relative bioavailability (Mean  $\pm$  SEM= 91.395  $\pm$  4.904). This extent of absorption is close to Montay *et al.*, (1984); Ulrich *et al.*, (1994); Lepeleire *et al.*, (1988). The relative bioavailability of pefloxacin was obtained by dividing the  $AUC_{0-\infty}$  after single administration of pefloxacin in both treatment and control condition. The result of this study shows that the bioavailability of pefloxacin is not affected by the paracetamol. In the present study the range of relative bioavailability was from 64.489-112.071%. This indicated that the effective concentration of pefloxacin at the site of action is reached without any interference.

The Mean maximum plasma concentrations (Mean  $\pm$  SEM) in both i.e. control and treatment condition were 4.679 $\pm$ 0.248  $\mu\text{g/ml}$  and 4.6595 $\pm$ 0.266  $\mu\text{g/ml}$  respectively (table: 1). These values are closed with those reported by Gonzalez and Henwood, 1989; Montay *et al.*, 1984. In control condition the highest  $C_{max}$  obtained in volunteer P<sub>4</sub>

which is 6.105 $\mu\text{g/ml}$  (close to the result of Webberley *et al.*, 1987;  $C_{max}$  =6.6 $\mu\text{g/ml}$ ) and the lowest in volunteer P<sub>7</sub> which is 3.9244  $\mu\text{g/ml}$  (close to the result of Montay *et al.*, 1984; Barre *et al.*, 1984; Gonzalez and Henwood, 1989; Lepeleire 1988;  $C_{max}$  =3.77-3.84 and 4.3, 4.09 $\mu\text{g/ml}$  respectively). A large variation in the  $C_{max}$  values have been reported by other workers after the oral administration of pefloxacin tablets 400mg. It means that the value of  $C_{max}$  of pefloxacin found in Pakistani subjects in the present work matches to those found in the studies of pefloxacin in other countries. Whereas this inter-individual variation might be due to the numbers of different factors such as physiological variances, body weight, height, etc.

The Mean  $T_{max}$  for plasma concentrations was 1.819  $\pm$  0.1743 hr and 1.605  $\pm$  0.1134 hr respectively (table 1). This time is close with those reported by Gonzalez and Henwood, 1989;  $T_{max}$  =1-1.5 hr and Neuman, 1988 reported  $T_{max}$  = 1-3hr. It is also similar to the reported value given in the book "The Quinolones" edited by Vincent T. Andriole (1998) that is 1-2hr after oral administration. Whereas the absorption half-lives with its mean  $\pm$  SEM in both the states of control and treatment were found to be 0.407 $\pm$ 0.049hr and 0.33 $\pm$ 0.028hr respectively (table 1). It indicates that the  $T_{max}$  is dependent on the absorption rate constant and this is confirmed by the fact that the drug was absorbed rapidly in treatment state as compared with the control state. The  $T_{max}$  in treatment condition is slightly reduced. In volunteers P<sub>1</sub>  $T_{max}$  reduced from 2.474 hr to 1.907, P<sub>3</sub> from 2.743hr to 2.082, and P<sub>9</sub> from 1.615hr to 1.178hr P<sub>10</sub> from 2.101 hr to 1.67hr. The possible reason may be that in the present study volunteers were allowed to move freely. The posture affected the gastric emptying; therefore in the treatment state a marginal decrease in  $T_{max}$  has been found.

Shargel (1993) reported that if the elimination rate constant is kept at  $0.1\text{hr}^{-1}$  and the  $k_a$  changes from 0.2 to  $0.6\text{hr}^{-1}$  (absorption rate increases), then the  $T_{\max}$  becomes shorter. So in present study the absorption rate is slightly higher in the treatment state (table 1). The mean absorption rate constant  $k_a$  (Mean  $\pm$  SEM) in both states is  $1.9499 \pm 0.2413\text{hr}^{-1}$  and  $2.26 \pm 0.2142\text{hr}^{-1}$  respectively.

In the present study the pefloxacin and paracetamol were used for observing the effect of paracetamol on the bioavailability of pefloxacin because liver metabolizes both these drugs. Therefore the increase in absorption rate constant may occur due to the competition between drugs for common binding sites on plasma proteins or may be due to the physiological variability of subjects participating in the studies.

The plasma levels of drug was calculated against the time profiles in the post-absorptive phases and it was found that the value of correlation coefficient was greater than  $r^2 = 0.9$ . This showed the linear nature of drug with the dose administered. Although volume of distribution was evaluated for any difference in control and treatment state. The mean volume of distribution (Mean  $\pm$  SEM) was  $74.868 \pm 3.797$  litre and  $76.464 \pm 4.0435$  litre respectively (table 1).

Total body clearance,  $Cl_T$ , is a more useful index of measurement of drug removal compared to the elimination half-life,  $T_{1/2}$ . Total body clearance takes into account changes in both the apparent volume of distribution, and half-life. Total clearance (Mean  $\pm$  SEM) in the two phases of studies (table 1) was found to be  $6.5799 \pm 0.365$  litre/hr in control and  $6.9869 \pm 0.5016$  lit/hr in treatment. This total clearance is similar to what has been reported by Petitjean *et al.* (1993).

The non-compartmental analysis of the data was also performed in order to detect effect of paracetamol on the pefloxacin, which might not be reflected from compartmental analysis. The values of Mean Residence Time (MRT) in control and treatment states (Mean  $\pm$  SEM) were found to be  $11.934 \pm 0.518$  hr and  $11.625 \pm 0.516$  hr respectively (table 1), apparently resembling values bearing no significant difference. This value is very close with those reported by Bouvet *et al.*, (1992). The Mean Residence Time (MRT) describes the average time for all the drug molecules to reside in the body. This high value of MRT may support the longer biological half-life of the pefloxacin, a pharmacokinetics property that offers the possibility of once-daily dose.

The statistical bioequivalence tests of pharmacokinetics parameters are concluded equal along with the results of ANOVA, f-ratio, t-tests (paired and unpaired). An intra-individual comparison of pharmacokinetics parameters of pefloxacin (control and treatment) was done at the level

of 0.05. That indicates the pharmacokinetics of the treated group is not found to be significantly different to that of the control group.

## CONCLUSION

No significant effect were observed on the bioavailability and pharmacokinetics of pefloxacin by the concomitant administration with paracetamol, however very minor effect were observed that might be related with inter-individual variation in human volunteers. Although the pharmacokinetic responses of pefloxacin is very erratic but the present study indicated that the level of drug ( $C_{\max}$ ) in Pakistani volunteers are not differ from previous studies in different races.

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