International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

EVALUATION OF PHARMACOKINETICS AND LIVER FUNCTION EVALUATED IN RATS FOLLOWING THE CO-ADMINISTRATION OF CAPTOPRIL AND CIPROFLOXACIN

Essam Ezzeldin*[1, 2] and Hebatalla Ibrahim Ahmed[3]

[1]Drug Bioavailability Center, National Organization for Drug Control and Research, (NODCAR), P.O.Box 29 Cairo, Egypt
[2]Drug Bioavailability Laboratory, College of Pharmacy, King Saud University, P.O. Box 22452, Riyadh 11495, Saudi Arabia
[3]Pharmacology and Toxicology Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

***Corresponding author e-mail:** ezzeldin24@hotmail.com

ABSTRACT

Simultaneous co-administration of multiple drugs is highly probable to allow effective treatment of a disease or multiple disease symptoms. Ciprofloxacin antibiotic is in use worldwide and it is possible to be used in cardiovascular patient's treatment. The objective of this study was to investigate the biochemical and pharmacokinetic interactions between ciprofloxacin and captopril in rats. The study was conducted in six groups of rats that received captopril 7.5 mg/kg, ciprofloxacin 50 and 100 mg/kg alone or co-administered with captopril 7.5 mg/kg for 28 days. Captopril and Ciprofloxacin plasma levels were determined by validated HPLC methods. Liver function biomarkers were also assessed. Ciprofloxacin significantly increased the C_{max} AUC_{0-12} and AUC_{0-inf} and prolonged $t_{1/2}$ and MRT of captopril. Conversely, captopril had no significance effect on the ciprofloxacin. Ciprofloxacin elevated the liver function biomarkers. Therefore the clinical significant of this work should be taken into consideration when these two agents are concomitantly administered.

Keywords Ciprofloxacin; captopril; pharmacokinetics; drug-drug interaction; Rats

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors including captopril are widely used to treat several heart diseases including hypertension and heart failure [1 - 8]. Bacterial infections may occasionally occur in cardiovascular patients; consequently they have to be treated with one of the antibiotics in combination with cardiovascular therapy. Ciprofloxacin, a fluoroquinolone antibiotic, is widely used in clinical practice all over the world.

It is effective against a large number of bacteria, some of which tend to be resistant to other commonly used antibiotics. It is used to treat a wide range of infections, including the chest [9], the gastrointestinal system and used for a long term for treatment of urinary tract infections [10 - 11]. During the concurrent use of some drugs, these drugs may exert their effects independently or may interfere or interact with each other in biopharmaceutical, biochemical or in pharmacological point of view.

In a previous in-vitro study of drug-drug interaction between ciprofloxacin and captopril it was found ciprofloxacin increases captopril concentrations [12]. However, to date there has been no study aimed to determine any potential for pharmacokinetic interaction of these two drugs in-vivo. The objective of this study was to provide such data so we sought to compare the pharmacokinetics of multiple doses of ciprofloxacin administered alone or co-administered with captopril of both drugs and assess the possible interaction between them.

MATERIAL AND METHODS

Animals: Male Sprague-Dawley rats, weighing 200 ±15 g obtained from the animal house of National Organization for Drug Control and Research (NODCAR), Egypt were utilized in this work. Rats were housed under constant temperature and humidity with a 12-hour in dark to12-hour in light cycle. They were given free food and water and received drugs daily for 28 days.

Drugs and chemicals: Pure powder of captopril, ciprofloxacin and internal standards (Thiopental and carbamazepine) were obtained from internal references standard unit, National Organization for Drug Control and Research, Egypt, acetonitrile were purchased from E-Merck (Darmstadt, Germany) Potassium dihydrogen phosphate (ADWIC, Egypt), Monobromobiamine, orthophosphoric acid, triethylamines (SIGMA-ALDRICH, St. Louis, USA). Trichloroacetic acid, perchloric acid and methanol from Labscan, Ireland. Deionized water was obtained from a Milli-Q water purification system (MILLIPORE, FRANCE**).**

Study design: Forty-eight rats were divided equally into six groups, 8 animals each. One of these groups served as a control group (group 1) and the other groups treated orally with captopril 7.5 mg/kg (group 2), ciprofloxacin 50 and 100 mg/kg alone (groups 3 and 4, respectively) or co-administered with captopril 7.5 mg/kg (group 5 and 6, respectively) for 28 days. Captopril and ciprofloxacin doses used in this study were equivalent to the human therapeutic dose range of these drugs [6, 13-14]. Each of captopril and ciprofloxacin was made into suspension with 1% tween 80/water. The drug administered in a total volume of 10 ml/kg.

Sample collection: Under light ether anaesthesia, blood samples, from the retro-orbital vein, were collected at 0.0 (pre-dose), 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12 hours post-dose into heparinized tubes. Plasma samples were prepared by centrifugation for 5 minutes at 2500 rpm at room temperature and were stored deep frozen at approximately -80ºC until the drug bioanalysis. For analysis of captopril, before samples storage, 0.1 ml of 0.1M phosphate buffer (pH: 7.5) was added to 200µl plasma immediately after its separation and after shaking for 30 second, 10µl of Monobromobiamine solution in acetonitrile (4mg/ml) was added and allowed to stand for 30 min at room temperature.

Analytical method: The HPLC methods validations were carried out according to bioanalytical method validation guideline of Food and Drug Administration [15]. The plasma calibrations curves were constructed in range of 10 – 2000 ng/ml for captopril and 0.05 – 10 µg/ml for ciprofloxacin. The HPLC instrument composed of Waters pump controlled by 610 Waters controller, 717 Waters autosampler, 486 UV detector and 474 fluorescence detector. The system was controlled and monitored by a single computer operated with Millennium software (Waters, USA). For the data acquisition and integration, Waters millennium software operated by Pentium III (450 MHz) processor (Compaq, UK) was used.

Captopril assay: The concentrations of captopril were determined by development of previously published method Tache *et al.*, [16] using reversed phase HPLC coupled with fluorescence detector. The extraction procedure involved protein precipitation by 70% trichloroacetic acid following the addition of 10 µl of thiopental solution (100 µg/ml) as internal standard. After brief mixing for 30 second and centrifugation for 5 minutes at 5000 rpm, 200 µl of supernatant was injected into HPLC system, onto Novapack® C_{18} column (39 X 150mm, 4µm). The column temperature maintained at 50 °C. The mobile phase was composed of distilled water, acetonitrile, trifluoroacetic acid (80:20:0.1) at a flow rate of 1.3 ml/min. The fluorescence detector was adjusted at excitation/ emission values of 400/480 nm, respectively.

Ciprofloxacin assay: The concentrations of ciprofloxacin were determined by new developed reversed phase HPLC using UV detector. Ciprofloxacin extracted from plasma according to the method of Maya *et al.* [17] by protein precipitation using 70% perchloric acid after addition of 10 µl of carbamazepine (200 µg/ml) as internal standard. After centrifugation for 10 minutes at 3000 rpm, 100 µl of the supernatant were injected to HPLC instrument. The separation was achieved using Thermo®, C_{18} (39 X 150mm, 5µm) (Thermo, UK) and the mobile phase consisted of distilled water, acetonitrile, ortho-phosphoric acid and triethylamine (860:140:6.3) at a flow rate of 2 ml/min. The column effluent was monitored by UV detector at a value of 294 nm.

Biochemical analysis:The determination of plasma creatinine concentration achieved by the kinetic Jaffe reaction [18], Plasma alkaline phosphatase (ALP) was determined by kinetic method according to Wenger *et al* [19]. Albumin estimated in plasma by Gendler method [20] and urea by the method of Jung *et al.* [21]. For the biochemical assay, Shimadzu, UV 160 Spectrophotometer was used.

Pharmacokinetic analysis: Non-compartmental pharmacokinetic analysis was performed by using Kinetica-4.3 (InnaPhase Corp., Philadelphia, PA, USA). The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (t_{max}) were observed from the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$.

Statistical analysis: All results are expressed as mean ± S D. The coefficient of variation (CV %) was calculated for the obtained parameters. Statistical significant for selected pharmacokinetic parameters was assessed using an analysis of variance (ANOVA) of log-transformed pharmacokinetics data. Since the T_{max} data were not normally distributed, they were compared using the Friedman's nonparametric test [22]. To assess if ciprofloxacin had an effect on captopril and if captopril had an effect on it FDA recommendations, 90% confidence intervals (two 1-sided tests) were computed for the difference between the test (co-dosing) and reference (captopril or ciprofloxacin alone) for pharmacokinetics parameters values especially for C_{max} and AUC [23]. For C_{max} and AUC, no drug interaction effect was assumed if the 90% confidence interval was between 0.8 – 1.25 for the log-transformed.

For biochemical parameters Statistical analysis was performed using SPSS statistical software (SPSS Inc Chicago, IL). Means were used and data were analyzed by one-way analysis of variance (ANOVA). If the test showed a significant difference, the least significant difference test was used as a Post hoc Tukey's test for multiple comparisons. The differences were considered significant if the probability was associated with $p<0.05$.

RESULTS

Partial validation for the methods of Tache et al. [18] for determination of captopril and to the method Maya et al. [19] for determination of ciprofloxacin were carried out according to FDA bioanalytical method validation. The validation of the methods used in this work was achieved due to the slight modification in analytical methods and to the change of matrix species (plasma) from human to rats. Standard curve of captopril and ciprofloxacin were prepared in methanol and plasma. Using standard weighted square method, all standard curves were linear over the range of 10 – 2000 ng/ml for captopril and from 0.05 – 10.00 µg/ml for ciprofloxacin.

Typical chromatograms of drug-free plasma spiked with captopril or ciprofloxacin and its corresponding internal standard are shown in Fig.1 and 2, respectively. Comparison of the assays showed the absence of any endogenous interfering peak at the retention times for each of captopril or ciprofloxacin and its internal standard. The present method for the separation of captopril and the internal standard showed retention time of about 5.243 min. and 6.397 min. respectively.

The retention time separation of ciprofloxacin and carbamazepine (internal standard) was 4.012 min and 6.979 min, respectively. The correlation coefficients were 0.997 and 0.999 for plasma calibration curves of captopril and ciprofloxacin, respectively (Figs. 3& 4). Both the intra-day and inter-day accuracy values were all within 95.3 – 112.06% and 90.1 – 112.0% for captopril respectively (tables 1 & 2) and within 87.0 – 105.8% and 96.1 – 111.9% for ciprofloxacin respectively (tables 3 & 4), respectively at the range of concentrations determined, while all the coefficient of variation (CV%) values were less than 10% at the same concentrations. In addition, the averages of absolute recovery values were 88.62% for captopril and 91.13% for ciprofloxacin.

The low limits of quantitation for captopril and ciprofloxacin were 10 ng/ml and 0.05 µg/ml, respectively. Co-administration of ciprofloxacin significantly enhanced the rate and extent of captopril bioavailability, resulting in significantly higher C_{max} values, in comparison with administration of captopril alone (Table 5 & 6). The mean captopril C_{max} when administered in combination (test) with 50 mg and 100 mg ciprofloxacin (443.302 and 694.801ng/mL) was approximately 28.71 and 101.7333% higher respectively than the mean C_{max} when administered alone (references)(344.422 ng/ml), and the 90% confidence interval for the test/reference ratio for the log-transformed C_{max} (0.298 - 12.793 and 0.117 - 0.151, respectively) was not entirely contained within the 0.8 – 1.25 range, indicating that administration of captopril in combination with ciprofloxacin significantly increased the rate of absorption of captopril. The mean captopril $AUC_{0\text{-}inf}$ when administered in combination with 50 mg and 100 mg ciprofloxacin (832.875 and 1131.356 ng.h/mL) was approximately 42.29 and 93.29 % higher respectively than the mean AUC_{inf} when administered alone (585.316 ng/ml), and the 90% confidence interval for the test/reference ratio for the log-transformed C_{max} (0.744 - 1.777 and

0.067 - 0.596, respectively) was not entirely contained within the 0.8 – 1.25 range, indicating that administration of captopril in combination with ciprofloxacin significantly increased the extent of absorption of captopril. Co-administration of captopril and with dose levels used ciprofloxacin had no effect on the pharmacokinetics of ciprofloxacin The 90% confidence intervals for the log-transformed Cmax and AUC for ciprofloxacin are entirely contained within the 0.80– 0.125 range (Tables 7-8). Captopril also had no effect on the elimination half life and mean residence time of ciprofloxacin. Administration of ciprofloxacin in the used dose levels caused significant liver injury in rats as indicated by increase in plasma ALT and AST activities, albumin and alkaline phosphatase while the changes induced by captopril were insignificant (table 9).

DISCUSSION

Pharmacokinetic analysis showed that concomitant administration of captopril and ciprofloxacin increased the plasma captopril levels, increased the area under time concentration curve as well as prolonged half life time and residence time of captopril. On the other hand, captopril effects on pharmacokinetics of ciprofloxacin were not statistically significant. The increase in captopril level was ciprofloxacin-dose dependent. This effect may be due to displacement of captopril from its binding site to plasma proteins by ciprofloxacin as ciprofloxacin bind strongly to plasma proteins than captopril. This attribution is in agreement with the results obtained by Mahbubul *et al*. [12] who found that free concentration of captopril increases with elevation of ciprofloxacin concentration in their *in vitro* study of interaction between ciprofloxacin and captopril at binding site of bovine plasma albumin. Ciprofloxacin affects liver function in dose dependent manner. Administration of ciprofloxacin in the used dose levels caused significant liver injury in rats These results are in consistent with the increase of ALT ana AST activities, albumin and alkaline phosphatase in dose dependent fashion. The dose dependent alteration of liver function induced by ciprofloxacin may be due to oxidative stress in hepatic tissue induced by ciprofloxacin [24].

CONCLUSION

Chronic or long using of captopril for the treatment of hypertension draws attention to taking into account the effects may occur in the body's physiology when administered concurrently with antibiotic as ciprofloxacin especially when used for long periods.

Table 1: Intra-day Repeatability of Captopril

Parameters	Intra-day							
	Theoretical Concentration (ng/ml)							
	10	20	50	100	200	500	1000	2000
	Concentration found (ng/ml)							
Mean	11.2	19.4	54.9	104.4	212.2	507.3	953.2	2072.3
CV %	3.6	5.3	8.4	8.4	5.4	8.4	9.7	1.9
Accuracy (%)	112.0	97.0	109.8	104.4	106.1	101.5	95.3	103.6
Maximum	11.6	20.8	59.2	124.5	224.9	543.5	1132.1	2119.7
Minimum	10.5	17.9	46.9	86.6	196.0	440.6	796.6	2022.8

Table 2: Inter-day Repeatability of Captopril

Parameters	Inter-day							
	Theoretical Concentration (ng/ml)							
	10	20	50	100	200	500	1000	2000
	Concentration found (ng/ml)							
Mean	11.2	18.8	54.3	102.6	210.3	503.7	901.4	2011.6
CV %	5.3	7.1	9.3	9.1	8.9	9.7	8.2	6.5
Accuracy (%)	112.0	94.0	108.6	102.6	105.1	100.4	90.1	100.6
Maximum	12.8	20.8	64.4	124.5	240.7	617.3	1132.1	2258.8
Minimum	10.5	16.1	42.2	77.9	176.4	396.6	716.9	1820.5

Table 3: Intra-day Repeatability of Ciprofloxacin

Parameters	Intra-day							
	Concentration Added (µg/ml)							
	0.05	0.10	0.20	0.50	1.00	2.00	5.00	10.00
	Concentration found (µg/ml)							
Mean	0.044	0.087	0.174	0.495	0.994	2.117	4.977	9.990
CV %	9.7	9.6	7.1	6.3	4.5	3.7	1.6	3.3
Accuracy (%)	88.0	87.0	87.0	99.0	99.4	105.8	99.5	99.9
Maximum	0.049	0.099	0.191	0.542	1.072	2.241	5.063	10.411
Minimum	0.038	0.071	0.161	0.464	0.962	2.048	4.876	9.502

Table 4: Inter-day Repeatability of Ciprofloxacin

Parameters	Inter-days							
	Concentration Added (µg/ml)							
	0.050	0.10	0.20	0.50	1.00	2.00	5.00	10.00
	Concentration found (µg/ml)							
Mean	0.049	0.096	0.199	0.548	1.066	2.238	5.530	10.731
CV (%)	8.8	9.5	9.7	9.1	7.4	5.1	8.4	6.8
Accuracy (%)	98.0	96.1	99.5	109.6	106.6	111.9	110.6	107.3
Maximum	0.066	0.134	0.250	0.683	1.291	2.446	6.533	12.840
Minimum	0.034	0.067	0.161	0.464	0.712	2.048	4.836	9.499

Table5: Pharmacokinetics of Captopril with and without Coadministration of 50 mg Ciprofloxacin

Parameters	Captopril	Captopril and Ciprofloxacin	% Change	90% C.I
C_{max} (ng/ml)	344.422 ± 310.25	443.302 ± 455.29*	28.71	0.298 - 12.793
t_{max} (hour)	0.5 ± 0.36	0.5 ± 0.15	-	_
AUC_{0-12} (ng.h/ml)	470.619 ± 83.62	597.298 ± 231.96*	26.92	1.173 - 1.913
AUC_{0-inf} (ng.h/ml)	585.316 ± 134.6	832.875 ± 209.52*	42.29	0.744 - 1.777
$t_{1/2}$ (hour)	5.394 ± 3.20	6.135 ±6.066*	13.74	1.057 - 6.895
MRT (hour)	3.721 ± 2.44	6.056 ± 3.58*	62.75195	1.298 - 7.781

*: Statistically significant in comparison to corresponding value of captopril-treated alone.

Table 6: Pharmacokinetics of Captopril with and without Coadministration of 100 mg Ciprofloxacin

Parameters	Captopril	Captopril and Ciprofloxacin	% Change	90% C.I
C_{max} (ng/ml)	344.422 ± 310.25	694.801 ± 330.65*	101.73	0.117 - 0.151
t_{max} (hour)	0.5 ± 0.36	0.5 ± 0.43	-	-
AUC_{0-12} (ng.h/ml)	470.619 ± 83.62	992.490 ± 1105.87*	110.89	0.105 – 0.398
AUC_{0-inf} (ng.h/ml)	585.316 ± 134.66	1131.356 ± 254.61*	93.29	0.067 – 0.596
$t_{1/2}$ (hour)	5.394 ± 3.20	8.444 ± 4.60*	56.54	1.744 – 13.858
MRT (hour)	3.721 ± 2.44	6.586 ± 3.96*	76.99	0.293 – 1.978

*: Statistically significant in comparison to corresponding value of captopril-treated alone.

Table 7: Pharmacokinetics of 50 mg Ciprofloxacin with and without Coadministration of 7.5 mg Captopril

Parameters	Ciprofloxacin	Ciprofloxacin and Captopril	% Change	90% C.I
C_{max} (µg/ml)	1.929 ±0.33	1.952 ±0.35	1.19	0.942 - 1.017
t_{max} (hour)	0.50 ± 0.38	0.75 ± 0.65	_	_
AUC_{0-12} (µg.h/ml)	6.649 ± 0.65	7.017 ± 0.81	5.53	0.821 - 0.857
AUC_{0-inf} (µg.h/ml)	8.110 ± 0.91	8.119 ± 0.78	0.11	0.885 - 1.117
$t_{1/2}$ (hour)	4.500 ± 1.91	4.915 ± 1.53	9.22	0.369 - 1.078
MRT (hour)	5.931 ± 2.04	6.059 ± 1.77	2.15	0.366 - 0.886

Table 8: Pharmacokinetics of 100 mg Ciprofloxacin with and without Coadministration of 7.5 mg Captopril

Parameters	Ciprofloxacin	Ciprofloxacin and Captopril	% Change	90% C.I
C_{max} (µg/ml)	2.506 ± 0.23	2.423 ± 0.96	-3.31	0.940 - 1.018
t_{max} (hour)	0.5± 0.18	0.5 ± 4.53	_	_
AUC_{0-12} (µg.h/ml)	8.198± 1.77	8.310±1.06	1.37	0.820 - 0.858
AUC_{0-inf} (µg.h/ml)	9.741 ± 1.51	10.380 ± 3.55	6.56	0.983 - 1.006
$t_{1/2}$ (hour)	14.117 ± 16.17	15.045 ± 1.53	6.17	0.489 - 0.812
MRT (hour)	16.233 ±22.81	17.718 ± 14.28	9.15	0.480 - 0.676

Table 9: Evaluation of the Effect of Captopril and Ciprofloxacin and its Combination on Liver Aspartase Aminotransferase (AST) and Analine Transferase (ALT) Activities, Albumin and Alkaline Phosphatase

Treatment	AST (g/dl)	ALT (g/dl)	Albumin (g/dl)	Alkaline Phosphatase (IU/L)
Control	7.29 ± 2.67	17.00 ± 2.45	3.73 ± 0.52	167.43 ± 0.34
Captopril (7.5 mg/kg)	8.63± 3.25	18.25 ± 3.26	4.03 ± 3.25	167.41 ± 32.25
Ciprofloxacin (50 mg/kg)	9.500 ± 5.86	17.33 ± 5.24	3.40 ± 0.56	194.67 ± 77.80
Captopril (7.5 mg/kg) and. Ciprofloxacin (50 mg/ml)	10.17 ± 1.81	23.00 ± 3.27	3.177 ± 0.07	240.29 ± 49.92
Ciprofloxacin (100 mg/kg)	13.57 ± 0.055*	24.71 ± 2.14*	3.087 ± 0.146*	244.0 ± 10.88 *
Captopril (7.5 mg/kg) and Ciprofloxacin	14.20 ± 0.45*	28.80 ± 3.49*	3.00 ± 0.15*	234.43 ± 9.67*

*: Significant in comparison to control group $P < 0.5$

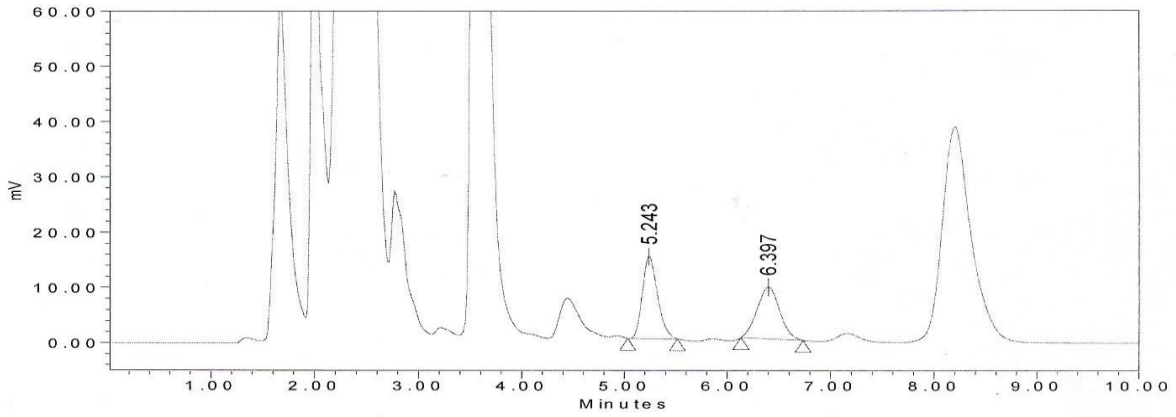

Fig.1: HPLC Chromatogram of Captopril (5.243min) and Internal Standard (Thiopental) (6.397min) Extracted from Spiked Plasma.

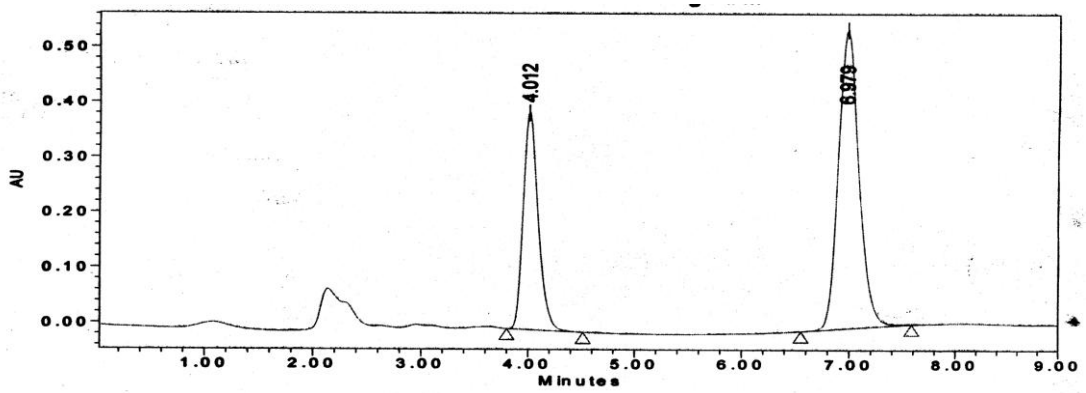

Fig.2: HPLC Chromatogram of Ciprofloxacin (4.012 min) and Internal Standard (Carbamazepine) (6.979 min).

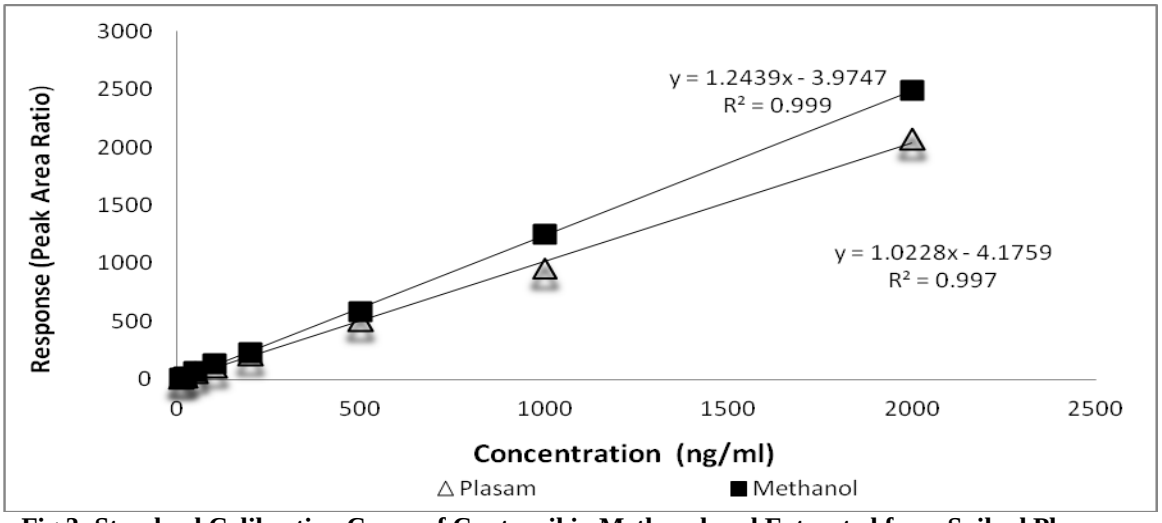

Fig 3: Standard Calibration Curve of Captopril in Methanol and Extracted from Spiked Plasma.

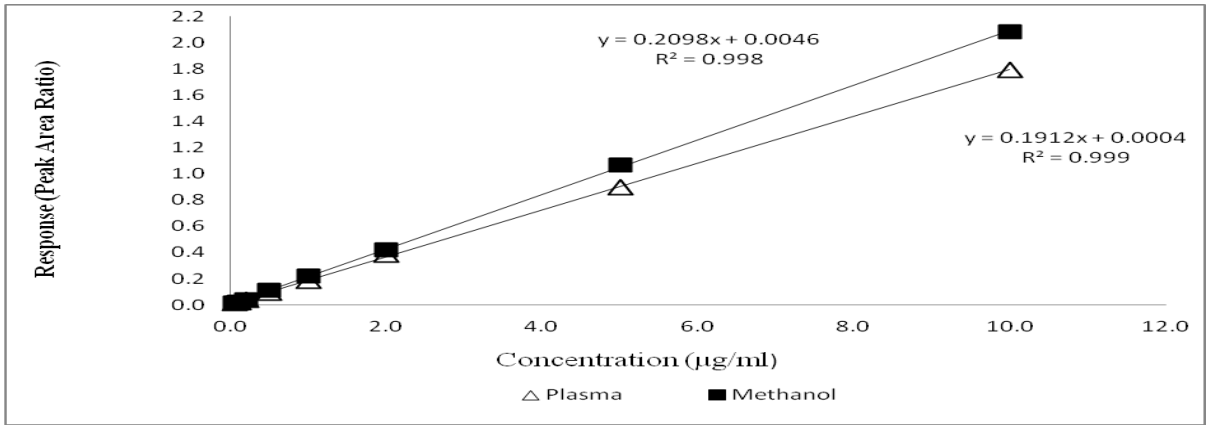

Fig. 4: Standard calibration Curve of Ciprofloxacin in Methanol and Extracted from Spiked Plasma.

REFERENCES

1. Forey K, Analytical profiles of drug substances, vol. 11, Academic Press, New York, USA 1982.
2. Albert X, Huertas I, Pereir II, Sanfélix J, Gosalbes V, Perrota C. Cochrane. Database. Syst. Rev. 2004 ;(3):CD001209.
3. Weir MR. Clin Ther, 2007; 29: 1803–24.
4. Heran BS, Wong MM, Heran IK, Wright JM. Cochrane. Database Syst Rev, 2008; 4 (1):358 - 62.
5. Mancia G, Giannattasio C, Seravalle G, Quarti-Trevano F, Grassi G. J Hum Hyper, 2009; 23:570–7.
6. Simko F, Simko J, Fabryova M. Cardiovasc Drugs Ther, 2003; 17(3):287-90.
7. Brooks WW, Bing OH, Robinson KG, Slawsky MT, Chaletsky DM, Conrad, CH. Circulation, 1997; 199 (96): 4002–10.
8. Peng H, Carretero OA, Vuljaj N, Liao TD, Motivala, A, Peterson EL. Rhaleb NE. Circulation, 2005; 112: 2436–45.
9. Scully BE, Nakatomi M, Ores C, Davidson S, Neu HC. Am J Med, 1987; 82: 196 – 201.
10. Meropol SB, Chan KA, Chen Z, Finkelstein JA, Hennessy S, Lautenbach E, Platt R, Schech SD, Shatin D, Metlay JP, Pharmacoepidemiol, 2008; 17(5):523-32.
11. Krcméry S, Hromec J, Gábrisová Z, Tahotný R. Vnitr Lek, 2010; 56: 955-60.
12. Mahbubul ASM, Jamil AM, Nadim RM, Fatima I, Firoj A, Shilpi, JA. Pak .J. Biol. Sci, 2004; 7 (1): 79-81.
13. Kazerani H, Hajimoradi B, Amini A, Naseri MH, Moharamzad Y. Singapore Med. J, 2009; 50(4): 400 – 2.
14. Gajjar, DA, LaCreta FP, Kollia GD, Stolz RR, Berger S, Smith WB, Swingle M, Grasela DM. Pharmacotherapy, 2000 (20); 76S-86S.
15. US DHHS, FDA, CDER, Guidance for Industry Bioanalytical Method Validation. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Veterinary Medicine (2001).
16. Tache F, Farca A, Medvedovici A, David V. J Pharm Biomed Anal, 2008; 28: 549 – 57.
17. Maya MT, Gonçalves NJ, Silva NB, Morais JA. J. Chromatogr. B Biomed. Sci. Appl, 2001; 5, 755(1-2): 305- 9.
18. Murry RL. Creatinine. In: Pesce, AJ,. Kaplan and LA (eds), Methods in Clinical Chemistry, St. Louis; CV Mosby Co: 1987, pp.10-17.
19. Wenger C. Alkaline phsphatase. In: Kaplan A, (eds), Clin Chem, St Louis, Toronto; C.V. Mosby Co:1984, pp. 1094 -8.
20. Gendler S. Uric acid. In: Kaplan A (eds). Clin Chem, St Louis, Toronto; C.V. Mosby Co: 1984, pp: 1268 – 73.
21. Jung D, Biggs H, Erikson J, Ledyard PU. Clin Chem. 1975; 21(8):1136-40.
22. Sokal RR., Rohlf FJ. Biometry: The Principles and Practice of Statistics in Biological Research. In: Freeman WH. (eds). San Francisco, USA, 1995, pp. 440–2.
23. Steinijans VW, Hartmann M, Huber R, Radtke HW. Intl J Clin. Pharmacol Ther Toxicol, 1991; 29: 323–8.
24. AGürbay A, Hincal F. Drug Chem. Toxicol, 2004; 27(3): 233-42.