BIOAVAILABILITY OF CIPROFLOXACIN TABLETS IN HUMANS AND ITS CORRELATION WITH THE DISSOLUTION RATES

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ABSTRACT

Bioavailabilities of seven tablets of ciprofloxacin were determined. The co-rrelation between in-vivo bioavailability parameters and *in-vitro* dissolution rates were studied. Ciprofloxacin concentration from the blood was determined by microbiological assay technique. The release pattern of ciprofloxacin from tablets, which were determined by dissolution USP paddle method and spectrophotometric method was used to determine the concentration. All the parameters of ciprofloxacin bioavailability i.e., T_{max}, C_{max}, AUC and absorption rate constant (K_a) showed no significant correlation with dissolution rates at T30%, T50%, T90% and dissolution at 30 mins with aims as in-vivo bioequvalence waiver. The value of dissolution test used as quality tool for predicting in-vivo performance of drug product is significantly enhanced, if the *in-vivo – in-vitro* relationship (correlation or association) is established.

Keywords: Ciprofloxacin, bioavailability, dissolution rates and *in-vivo/in-vitro* correlation.

INTRODUCTION

The ciprofloxacin tablets contains ciprofloxacin hydrocholride monohydrate ($C_{17}H_{18}FN_3O_3$. HCl. H_2O, molecular weight 385.8). Ciprofloxacin has uncharacteristic melting point; melting process with decomposition at around 270°C to 385°C. Ciprofloxacin is a quinolone acid derivative that exhibits a rapid onset of action, and lacks cross-reactivity with pencillins, cephalosporins, and aminoglycosides. Four metabolites of ciprofloxacin have been identified in body fluid as desethyleneciprofloxacin sulphociprofloxacin, oxocipro-floxacin and formayl ciprofloxacin (Bayer *et al.*, 1987). As zwitterion Ciprofloxacin, has shown good penetration and accumulation in tissues with a wide distribution throughout the body. Concentrations of fluoroquinolones in tissues were found to be higher than that in plasma. The apparent volume of distribution (Vd) is 2 to 3 L/kg, after a single oral dose of 400 to 500 mg were found to be 1 to 3 μg /ml concentrations in urine range from 100 to 650 μg /ml, which far exceeded the MIC require for most urinary bacterial pathogens (Mandell & Sande *et al.*, 1990). Absorption of Ciprofloxacin after oral administration is rapid and can be satisfactorily described as a zero-order process; peak serum ciprofloxacin concentrations (C_{max}) are reached in approximately 1 to 2 hours. Concomitant administration of food does not cause clinically significant impairment of absorption and may be helpful in minimizing gastric distress caused by the drug. A linear relationship between serum ciprofloxacin concentrations and the dose administered either orally or intravenously has been reported. The absolute bioavailability of ciprofloxacin is approximately 70%. Nonrenal clearance accounts for approximately 33% of the elimination of ciprofloxacin; fecal recovery of ciprofloxacin accounts for approximately 15% of an intravenous dose. Nonrenal elimination includes metabolic degradation, biliary excretion and transluminal secretion across the enteric mucosa. Glomerular filtration and tubular secretion account for approximately 66% of the total serum clearance. The terminal disposition half-life ($t_{1/2}$) is about 3 to 4 hours.

MATERIALS AND METHODS

Bioavailability, bioequivalence and its correlation with dissolution rate of the Ciprofloxacin were investigated in normal healthy male subjects. The protocol adopted for this study was crossover performed as a single-dose on the healthy subjects with adequate washout period and by analyzing the samples by two validated method i.e., UV-VIS spectrophotometric and Microbiological Assay. The study was conducted in accordance with good clinical practice guidelines. Volunteers enrolled for this study were apprised in details about all aspects of the study in easy understandable language and terminologies. Those who agreed voluntarily were registered for further studies. The 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. The 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

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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 (T) manufactured by different pharmaceutical companies i.e., Ciprofloxacin (Test I, Test II, Test III, Test IV, Test V, Test VI & Test VII) and reference standard i. e. market imaging were used for the study of bioequivalence. 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. Water consumption was restricted from 2 hours prior to and for 2 hours after dosing. The volunteers took a standard breakfast two hours following drug administration. Each volunteer was given orally one tablet of 500 mg Ciprofloxacin with 240-250 ml of drinking water.

Table 1: Description of all healthy male volunteers participated in the study of bioequivalence of Test and Reference Tablets of Ciprofloxacin

Parameters	Units	Mean	± SE	Minimum	Maximum
Age	Years	22.3	0.3	18.0	37.0
Weight	Kg	64.9	0.8	40.0	87.0
Height	Cm	169.2	0.9	142.0	202.5
Systolic BP	mmHg	121.0	0.7	104.0	140.0
Diastolic BP	mmHg	80.0	0.8	62.0	100.0
Temperature	^oF	98.1	0.1	95.8	99.2
Body Surface Area (BSA)	m^2	1.7	0.0	1.4	2.0

Table 2: Correlation estimates for dissolution vs bioequivalence and pharmacokinetic parameters

	T 30%	T50%	T90%	Dissolution in 30min
C_{max}	-0.204	-0.205	-0.019	0.173
T_{max}	-0.055	-0.057	-0.067	-0.070
AUC_{0-t}	0.165	0.252	0.231	-0.105
K_a	-0.224	-0.318	-0.135	0.192

Table 3: Dissolution % age profiles of tests and reference formulations

Time Mins.	Standard %age	Test VI %age	Test V %age	Test III %age	Test I %age	Test VII %age	Test II %age	Test IV %age
5	17.5	14.91	24.7	32.76	16.05	20.37	32.1	14.7
10	20.14	19.59	48.9	59.52	42.78	27.76	48.1	30.2
15	57.01	33.55	60.9	72.34	55.23	38.88	63.2	50.9
20	75.31	36.7	76.45	81.93	74.72	57.01	68.54	63.41
25	93.9	44.52	91.08	82.01	87.16	62.99	83.17	81.61
30	95.81	53.66	93.25	76.54	92.46	69.16	88.61	93.94
40	99.88	63.96	94.09	83.15	94.78	84.49	90.74	97.69
60	101.13	80.7	96.01	90.7	86.52	99.07	77.99	98.5
90	104.38	91.37	98.31	92.6	81.86	103.06	81.42	101.23

Table 4: Blood level profiles of different brands

	Standard		Test-I		Test-II		Test-III		Test-IV		Test-V		Test-VI		Test-VII	
0.5	2.96	0.25	2.89	0.27	2.24	0.12	0.94	0.1	3.07	0.23	89	0.12	2.77	0.26	2.98	0.4
1	3.8	0.2	3.95	0.32	3.51	0.21	1.46	0.13	4.95	0.27	1.4	0.15	4.54	0.38	4.68	0.43
1.5	3.47	0.17	3.67	0.28	3.47	0.21	1.61	0.1	4.14	0.22	1.61	0.1	3.77	0.21	3.79	0.31
2	2.99	0.18	3.2	0.28	3.38	0.15	1.18	0.07	3.38	0.17	1.16	0.07	2.84	0.22	3.09	0.27
2.5	2.4	0.13	2.43	0.2	3.04	0.11	0.95	0.07	2.87	0.17	0.96	0.07	2.36	0.17	2.56	0.23
3	1.97	0.11	2.11	0.23	2.67	0.1	0.75	0.07	2.44	0.18	0.75	0.07	2.02	0.18	2.21	0.22
4	1.53	0.09	1.66	0.18	2.24	0.07	0.56	0.43	1.98	0.16	0.57	0.06	1.67	0.14	1.73	0.18
5	1.14	0.05	1.26	0.13	1.75	0.08	0.43	0.03	1.44	0.13	0.45	0.04	1.33	0.1	1.41	0.15
6	0.87	0.04	1	0.11	1.56	0.08	0.35	0.02	1.02	0.1	0.36	0.03	0.91	0.07	1.03	0.1
8	0.56	0.03	0.64	0.08	0.93	0.06	0.24	0.02	0.62	0.06	0.52	0.02	0.6	0.04	0.7	0.06
12	0.25	0.02	0.27	0.03	0.39	0.05	0.13	0.01	0.3	0.06	0.13	0.02	0.39	0.03	0.3	0.02

Subjects were confined to the study unit until 24 hours after dosing. Following a 7-day washout period from the day of Ciprofloxacin dosing subjects were readmitted and received the alternate treatments according to their randomization for treatment sequence. Before drug administration, a control/blank venous blood sample was collected from each volunteer through a sterile venous Branula with in-stopper 18G (1 ®Vasocan, B. Braun Melsungen AG) 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 OC.

Demographic/anthropometric measurements

The age, weight, height, blood pressure (Systolic/ Diastolic) temperature and body surface area (BSA) of each volunteer was also recorded. For the calculation of Body surface area Hue *et al.* (2003) formula was used: BSA = (W 0.425 x H 0.725) x 0.007184 W= Weight H=Height The clinical parameters including Glucose, Blood urea, Serum Creatinine, Cholesterol, Bilirubin total, SGPT, SGOT, and CPK of all the volunteers was also determined. Table 1 Description of all healthy male volunteers participated in the study of bioequivalence of Test and Reference Tablets of Ciprofloxacin Parameters Units Mean ± SE Minimum Maximum Age Years 22.3 0.3 18.0 37.0 Weight Kg 64.9 0.8 40.0 87.0 Height Cm 169.2 0.9 142.0 202.5 Systolic BP mmHg 121.0 0.7 104.0 140.0 Diastolic BP mmHg 80.0 0.8 62.0 100.0 Temperature ºF 98.1 0.1 95.8 99.2 Body Surface Area (BSA) m2 1.7 0.0 1.4 2.0 Two types of analytical methods were used to quantify quinolones (Ciprofloxacin) concentration in plasma and in dissolution test.

Table 5: Table showing the values of C_{max}, AUC_{0-t} and K_a of different brands

C_{max}	AUC_{0-t}	K_a
3.8	15.4	1.75
3.95	16.56	1.83
3.51	19.67	1.42
1.61	5.48	3.19
4.95	15.4	7.25
1.61	5.3	4.77
4.54	16.7	4.35
4.68	16.3	11.36

Table 6: Table showing the values of C_{max}, AUC_{0-t} K_a and 30 min dissolved of different brands with its correlation coefficient

30 min Dissolved %	C_{max}	AUC_{0-t}	K_a
95.81	3.8	15.4	1.75
92.46	3.95	16.56	1.83
53.66	3.51	19.67	1.42
93.25	1.61	5.48	3.19
76.54	4.95	15.4	7.25
69.16	1.61	5.3	4.77
88.61	4.54	16.7	4.35
93.94	4.68	16.3	11.36
Correlation values	0.173	-0.105	0.192

Table 7: Table showing the values of C_{max}, AUC_{0-t} K_a and T30 % of different brands with its correlation coefficient

T30%	C_{max}	AUC_{0-t}	K_a
12.5	3.8	15.4	1.75
7	3.95	16.56	1.83
14	3.51	19.67	1.42
6	1.61	5.48	3.19
5	4.95	15.4	7.25
11	1.61	5.3	4.77
5	4.54	16.7	4.35
10	4.68	16.3	11.36
Correlation values	-0.204	0.165	-0.224

Table 8: Table showing the values of C_{max}, AUC_{0-t} K_a and T50 % of different brands with its correlation coefficient

T50%	C_{max}	AUC_{0-t}	K_a
15	3.8	15.4	1.75
15	3.95	16.56	1.83
30	3.51	19.67	1.42
10	1.61	5.48	3.19
10	4.95	15.4	7.25
20	1.61	5.3	4.77
10	4.54	16.7	4.35
15	4.68	16.3	11.36
Correlation Values	-0.205	0.252	-0.318

a) Microbiological agar diffusion method

To measure microbiologically active drug or its metabolite(s), Ciprofloxacin concentration in the plasma samples was also measured by microbiological assay. The method is reproducible and accurate for plasma/serum. HPLC method measures drug and metabolite(s) without distinguishing the microbiological activity, hence, for most antibacterial drugs a validated microbiological assay procedures is more appropriate for the assay of drugs in biological samples. 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 measured with Zone Reader and the concentrations of Ciprofloxacin in plasma samples was calculated by sample zones 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 inimum and maximum zone sizes recorded during the studies.

Table 9: Table showing the values of C_{max}, AUC_{0-t} K_a and T90 % of different brands with its correlation coefficient

T90%	C_{max}	AUC_{0-t}	K_a
25	3.8	15.4	1.75
30	3.95	16.56	1.83
90	3.51	19.67	1.42
25	1.61	5.48	3.19
60	4.95	15.4	7.25
60	1.61	5.3	4.77
40	4.54	16.7	4.35
Correlation values	-0.019	0.231	-0.135

b) Spectrophotometric method

Spectrophotometric method of analysis was employed to measure the Ciprofloxacin concentration in the dissolution test performed for both test and reference samples at different time interval. The standard Ciprofloxacin concentrations were recorded during the studies. Measuring the direct test and reference solution concentration by using spectrophotometer 160A Shimadzu Corporation Tokyo Japan UV-VIS manual system. The drawn samples by using 1cm quartz cell at approx. 276nm on an absorbance scale 0-1 against the dissolution medium (water). Dissolve approx. 65mg Ciprofloxacin Hcl reference standard in 100ml water. Dilute further one ml of stock solution with dissolution medium to 100ml. Dissolution test was performed according to USP method (Paddle) using water as medium at 37± 0.5 ^{0}C and paddle speed was fixed at 50 rpm. Times for collecting samples were 5, 10, 15, 20, 25, 30, 40, 60 and 90 minutes. 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 logarithmic scale against time. The plasma concentration versus time data was used to calculate parameters of bioavailability and pharmaco-kinetics by PC-Computer Program, APO, MWPHARM version 3.02 a MEDIWARE product Holland. Calculations also included area under curve (AUC_{0-t}) by polyexponential and trapezoidal methods and the regression coefficient of best fit to depict the compartmental analysis. Bioequivalence comparisons were performed on log-transformed data using the two, one-sided test. For the ratios of the mean bioavailability parameters models were used to construct 90% confidence intervals for test versus reference tablets. The term correlation is frequently employed within the pharmaceutical and related sciences to describe the relationship that exists between variables. Mathematically, the term correlation means interdependence between quantitative or qualitative data or relationship between measurable variables and ranks. From biopharmaceutical standpoint, correlation could be referred to as the relationship between appropriate in vitro release characteristics and *in vivo* bioavailability parameters.

RESULTS

In the present study estimated Multiple-level C correlation (Jaber Emami, 2006) values for dissolution T30%age, T50%age, T90%age and 30 minutes % age Dissolved VS C_{max}., Tmax., AUC 0-t and Ka are shown in the Table 6-9. All the brands Cmax bioequivalence parameter correlation (r) value with the K_a pharmacokinetic parameter is 0.381348. The correlation value (r) between C_{max} and AUC_{0-t} is 0.84814. The percent values of Ciprofloxacin in different formulations are measured by spectrophotometeric assay in the USP-NF dissolution apparatus, 900ml water as dissolution medium in each vessel, paddle at speed 50 r.p.m. The samples collected from time range 5, 10, 15, 20, 25, 30, 40, 60, and 90 minutes respectively and shown in the table 3 and graph were depicted in fig. 1. The 50 % age release of Ciprofloxacin (57.01 %) for reference formulation is at 15 minutes and other test brands are (Test VI) 53.66 at 30 minutes, (Test V) 48.9 % age at 10 minutes, Test III 55.23 % age at 15 minutes, (Test I) 55.23% age at 15 minutes, (Test VII) 57.01 % age at 20 minutes Alpine 48.1% age at 10 minutes and (Test IV) 50.9 % age at 15 minutes. Comparison between dissolution profile of test and references to be achieved from simple model independent approach between time 15minutes, 30 minutes , 40 minutes and 60 minutes and percent of released drug by applying a Similarity factor (f1) with values up to 15 (0-15). Difference factor (f2) values greater than 50 (50-100) ensure sameness or equivalence in the table 3. For formulation of (Tet I) the value of f1 is 7.0203 and f2 63.3632 for (Test VI) f1 is 34.4685 and f2 33.6868, for (Test V) f1 is 2.7075 and f2 74.949, for (Test III) f1 is 8.7895 and f2 48.8125, for (Test VII) f1 is 17.5875 and f2 46.0996, for (Test II) f1 is 9.4084 and f2 52.4506 and for (Test IV) f1 is 3.676 and f2 79.1268 were observed. The *in-vivo* data of all the brands Ciprofloxacin concentration from plasma were measured by Bioassay technique. Which were shown in the table 4 and graph was constructed fig. 2. The results all brands C_{max}, T_{max}, AUC_{0-t} and K_a values were shown in table 5 and fig. 3.

$$f_1 = \left\{\left[\sum_{t=1}^{n} |R_t - T_t|\right] \Big/ \left[\sum_{t=1}^{n} R_t\right]\right\} * 100$$

DISCUSSION

The concept and application of the *in vitro-in vivo* correlation (IVIVC) for pharmaceutical dosage forms have been a main focus of attention and consideration of pharmaceutical industry, academia, and regulatory sectors. Development and optimization of formulation is an integral part of manufacturing and marketing of any therapeutic agent which is indeed a time consuming and costly process. Optimization process may require alteration in formulation composition, manufacturing process, equipment and batch sizes. If these types of changes are applied to a formulation, studies in human healthy volunteers may be required to prove that the new formulation is bioequivalent with the old one. Certainly, implementation of these requirements not only halts the marketing of the new formulation but also increases the cost of the optimization processes. It would be, desirable, therefore, to develop *in vitro* tests that reflect bioavailability data. A regulatory guidance for both immediate- and modified-release dosage forms has been,

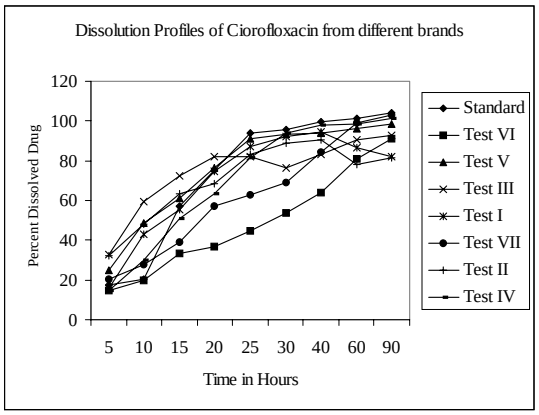

Fig. 1: Graph showing Dissolution % age profiles of tests and reference formulations.

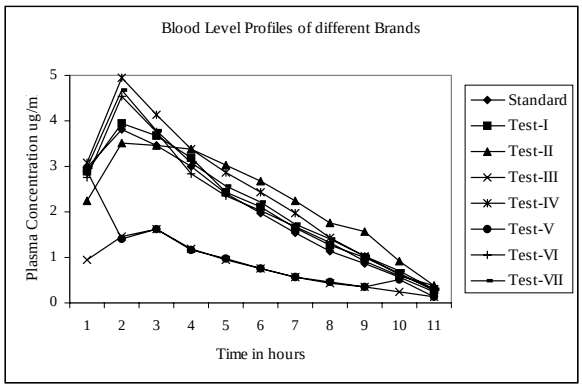

Fig. 2: Graph showing Blood level profiles of tests and standard formulations.

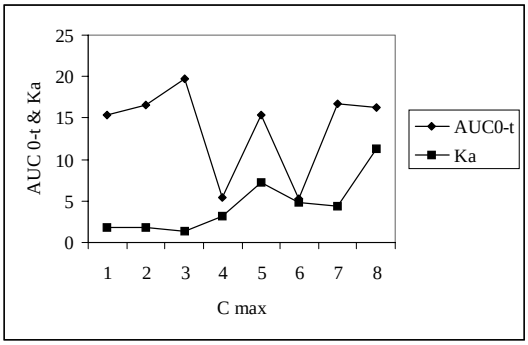

Fig. 3: Graph showing Correlation between C_{max} and AUC_{0-t} and K_a of different brands.

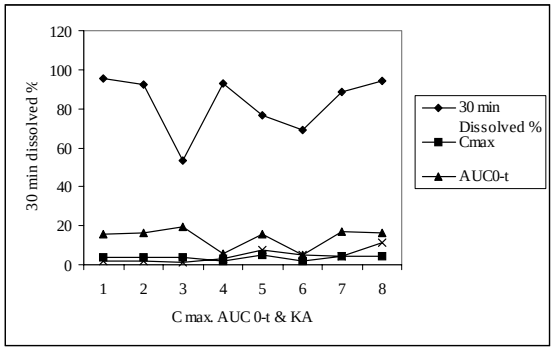

Fig. 4: Graph showing Correlation between 30 min dissolved % C_{max} AUC_{0-t} and K_a of different brands.

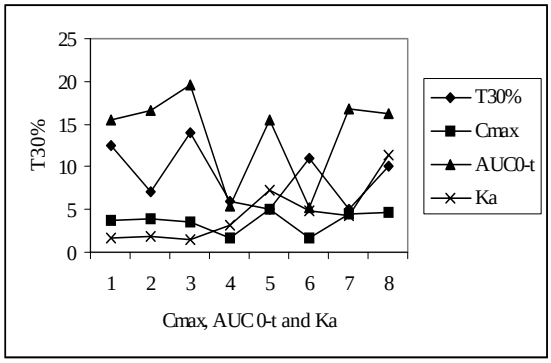

Fig. 5: Graph showing Correlation between T30 C_{max} AUC_{0-t} & K_a of different brands.

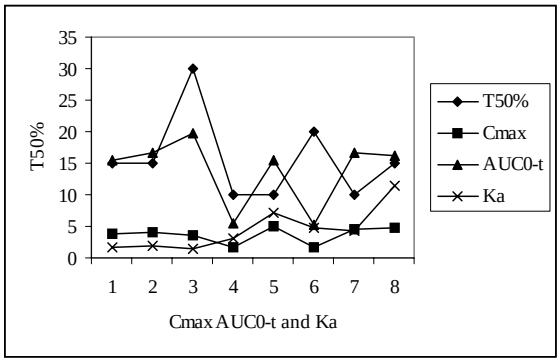

Fig. 6: Graph showing Correlation between T50 C_{max} AUC_{0-t} & K_a of different brands.

therefore, developed by the FDA to minimize the need for bioavailability studies as part of the formulation design and optimization. IVIVC procedures are specific to certain countries but could be adopted or used as the background for regulatory recommendations by other countries. IVIVC can be used in the development of new pharmaceuticals to reduce the number of human studies during the formulation development. The main objective of an IVIVC is to serve as a surrogate for *in vivo* bioavailability and to support biowaivers. IVIVCs could also be employed to establish dissolution specifications and to support and/or validate the use of dissolution methods. This is because the IVIVC includes *in vivo* relevance to *in vitro* dissolution specifications. It can also assist in quality control for certain scale-up and post-approval changes, for instance, to improve formulations or to change production processes. There must be some *in vitro* means of assuring that each batch of the same product will perform identically in vivo. The study estimated correlation values for dissolution T30 %age, T50%age, T90%age and 30 minutes % age Dissolved VS C_{max}, T_{max}, AUC_{0-t} and K_a are shown in the table 2. All the brands C_{max} bioequivalence parameter correlation (r) value with the K_a pharmacokinetic parameter is 0.381348 and regression coefficient (R2) value 0.1362. The correlation value (r) between C_{max} and AUC_{0-t} is 0.84814 and regression coefficient (R2) value -2.5184. if there is good correlation detween *in vitro* testing and *in vivo* testing , then dissolution test can be used as atool far predicting *in vivo* performance of the drug. In the present study there was no correlation or association exist between *vivo* and *vitro* parameters as ciprofloxacin is immediate released drug so whenever any change in ingredients of formulation or manufacturing techniques it must be needed bioequivalence study in the existing environment.

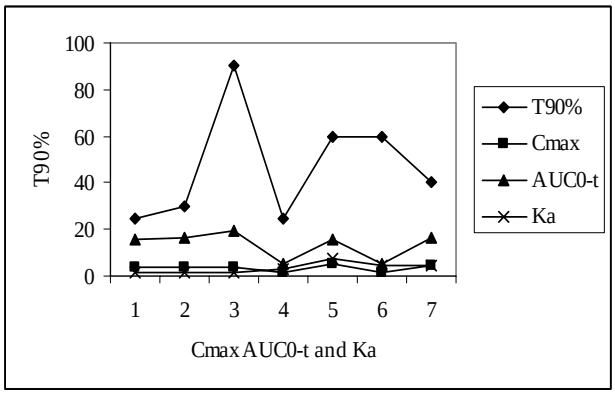

Fig. 7: Graph showing Correlation between T90 C_{max} AUC_{0-t} and K_a of different brands.

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