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Statistical Design Based on 90 % Confidence Intervals Analysis of Bioequivalence Studies of Sustained Release Capsules of Metoprolol Tartrate on Healthy Human Volunteers

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The bioequivalence study of formulations of sustained release coated granules equivalent to100 mg metoprolol tartrate (TEST-C1) with the reference formulation (Metolar XR 100SR) tablets of Cipla Ltd. was done. The study was typical two-period, randomized, two-way complete crossover design in 6 healthy, male human volunteers. There was 2 dosing sessions with a washout period of 7 days between the two sessions. Drug plasma samples were collected over a 24 h period after administration. Subsequently, plasma concentrations of drug were analyzed by using HPLC/UV. Pharmacokinetic parameters were determined by using non-compartmental analysis. The results showed that 90 % confidence intervals of the peak concentration (C_{max}) and the area under the concentration-time curve (AUC) of reference and test were within the range 80-125 %. Consequently the bioequivalence of these two preparations can be concluded.

Keywords: Metoprolol tartrate, Bioequivalence study, Confidence intervals.

INTRODUCTION

Metoprolol tartrate (MT), a β-blocker being highly soluble, permeable (class I substance) is absorbed completely through the whole intestinal track within 2-4 h. It is subjected to extensive first pass metabolism. Its low biological availability (~ 50 %), quick absorption and elimination (3-4 h) necessitate the administering of conventional immediate release (IR) up to 4 times daily [1]. To overcome this problem sustained release formulation is developed that enables less frequent dosing. In the present study, matrix granules of metoprolol tartrate have been formed by suitable combination of hydroxypropyl methyl cellulose (HPMC) and ethyl cellulose (EC). Eudragit® RL and RS were chosen to form coating on the granules to extend duration of drug release which acts as a delivery device with the objective of releasing the drug into the patient body at a predetermined rate, or at specific time or with specific release profile. The usual goal of an oral sustained release product is to maintain therapeutic blood levels over a sustained period. For this, drug must enter in the circulation of approximately the same rate of which it is eliminated out of the body [2].

The pharmacokinetics studies of of metoprolol, its tartrate, succinate salts and other formulations have been thoroughly reviewed. Metoprolol is absorbed after oral administration. Only negligible amounts of metoprolol are absorbed in the stomach and the duodenum, jejunum, ileum and colon have similar capacities for absorption by first-order kinetics. Regional absorption decreases in anatomical order with approximately two-thirds of the amount of metoprolol leaving the stomach being absorbed in the duodenum.

The systemic bioavailability varies considerably (range in healthy volunteers is 30 to 75 %) owing to extensive pre-systemic metabolism in the liver. However, due to the large therapeutic window and common individual dose adjustments, these differences among subjects are not clinically relevant. Peak plasma concentrations are achieved within 2 to 3 h after drug administration and bioavailability may be increased (average 40 % increase in AUC) by food intake. Metoprolol is known for its high inter-subject variability. For any given dose, there is a10-20 fold variation in total plasma concentration between individuals as a consequence of pre-systemic metabolism, which ranges from 5 to 50 % or more [3-5]. To determine bioequivalence, pharmacokinetic studies are conducted each of the formulation are administered in a cross-over study to volunteer subjects, generally healthy individuals but occasionally in patients. Serum/plasma samples are obtained at regular

intervals and assayed for parent drug (or occasionally metabolite) concentration [6,7].

EXPERIMENTAL

The aim and objective of the present study was to evaluate the pharmacokinetic parameters and to compare the oral bioavailability of single dose of sustained release (SR) coated granules equivalent to100 mg metoprolol tartrate (TEST formulation-C1) with the reference formulation Metolar XR 100SR tablets of Cipla Ltd., India.

Ethics review procedure: The *in vivo* study was executed as per guidelines proposed by DCGI (Drugs Control General of India, New Delhi). These guidelines describes the requirements of the U.S. Code of Federal Regulations (Title 21, Part 56), (The Declarations of Helsinki and the Canadian MRC Guidelines) [8-10]. The protocol and the informed consent form for the healthy volunteers were submitted to the 'Institutional Ethical Committee of Jadavpur University, India' prior to the initiation of the study. The study was started after receiving the approval of the Ethical Committee.

Design of experiment: The bioequivalence study of TEST preparation and REFERENCE preparation was assessed utilizing a typical two-period, randomized, two-way complete crossover design in 6 healthy male human volunteers. There were 2 dosing sessions with a washout period of 7 days between the two dosing sessions. All the volunteers participated in two dosing sessions. In each dosing session, volunteers took either the test preparation or reference preparations only on the study day, as per the randomization code [11,12]. Design of experiment was presented in Table-1.

TABLE-1
DESIGN OF BIOAVAILABILITY STUDY OF SR COATED GRANULES CONTAINING 100 mg METOPROLOL TARTRATE BOTH FOR REFERENCE (A)* AND TEST SAMPLE* (C1) (B)

Subject No.	Period I	Period II
1	A	B
2	A	B
3	B	A
4	B	A
5	A	B
6	B	A

Mode of treatment: Neither the volunteers nor 'the physician and nursing staff in charge' of the clinical aspects were informed regarding the sequence of administration. The investigator preparing the drugs for administration is the only person who was aware of the code. Total six male non-smoking volunteers were enrolled for the study based on their laboratory tests (serum chemistry, hematology and urine analysis), medical history, physical examination and HIV screening. No alcohol or concomitant medication was allowed 72 h prior to the initial administration of dose and for the entire course of the study. Subjects fasted for 12 h prior to administration of drugs. Formulations were given to the volunteers according to the protocol. Blood samples were obtained at seventeen time points from pre dose (0 h) until 24 h post dose (0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 8, 10, 12, 18 and 24). The plasma samples were stored at –20 °C until assayed. The pharmacokinetic parameters for metoprolol tartrate were determined by 'zero-moment non compartmental method'. The maximum plasma concentration (C_{max}) and time to reach maximum plasma concentration (T_{max}) were directly obtained from the plasma concentration *vs.* time data. Area under the plasma concentration-time curve from time zero to last concentration time point (AUC_{0-t}) was determined by trapezoidal method. Area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$) was determined by the following equation:

$$AUC_{0-\infty} = AUC_{0-t} + \frac{C_t}{K_e}$$

where K_e = elimination rate constant of single dose, which is estimated as a slope of the straight line by plotting the concentration (C_{max} to last concentration) against corresponding time on a semi-logarithm graph paper and C_t is last quantifiable concentration. The elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$ [13].

Drug administration to the human volunteers: The volunteers were randomized on the previous day of Phase I. In period I, each volunteer received either the TEST preparation or the REFERENCE preparations as a single dose at a fixed time. In period II, this order was reversed as per the rule of randomization. For accurate sampling time for every sample, study medications were administered at intervals of 2 min to groups of 2 subjects. Study medication was given with 240 mL water at room temperature.

Blood collection from human volunteers: All the volunteers were assembled at 6.00 a.m. on the study day 1 of each session, after overnight fasting of at least 10 h. Their TPR, BP were recorded and an indwelling intravenous cannula was introduced with strict aseptic precautions in the anticubital vein for blood collection. The volunteers received either of the study preparations (REFERENCE/TEST) according to their code nos. with 240 mL. of water. The exact clock time was calculated according to the drug administration schedule. The first blood sample (t = 0) was collected immediately prior to drug administration. The exact time of collection of all blood samples was recorded and reported for each subject. Any deviation from the sampling schedule was recorded in the subject's sampling time sheet. A total of 12 blood samples were collected from anticubital vein at 0 h. (before drug administration) 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 6.0, 9.0, 12.0, 15.0, 18.0 and 24.0 h in coded centrifuge tubes containing EDTA. Blood samples were centrifuged immediately, the plasma separated into duplicate polypropylene tubes containing EDTA and stored in a deep freezer maintained at -20 °C. The tubes were labeled with volunteer code number, sampling time and study date. The concentration of metoprolol tartrate in blood samples were analyzed by HPLC method as described earlier [14-16]. Fig. 1 shows HPLC chromatogram for standard drug.

Dietary control of the volunteers during *in vivo* study: A standardized breakfast, lunch and dinner were served to subjects at 3, 6-8 and 14 h respectively after drug ingestion. Water was provided *ad libitum* until 1 h pre-dose. Fluid intake was controlled and consistent for the first 3 h following drug administration as follows: drug was given with 240 mL of water at room temperature and no fluids except one cup of non-

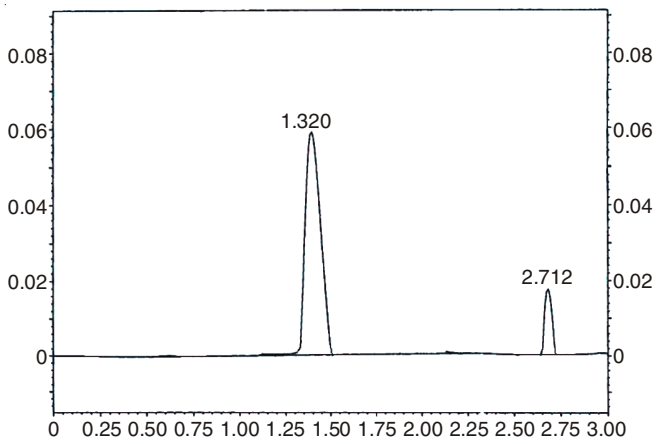

Fig. 1. Representative chromatogram obtained during quantification of metoprolol tartrate (t_R = 1.3 min) in human plasma with pinacidil monohydrate (t_R = 2.7 min)

caffeine-containing soft drink was allowed till 3 h post dose. On the study day volunteers were permitted normal activities, excluding strenuous exercise [17].

Records of adverse events during *in vivo* study: Abnormal symptoms/signs or adverse reactions if any were monitored, during the study period and for 1 week after the study period and if noticed, their details were entered in the case report sheets and tabulated at the end of the study. Medical and nursing personnel supervised all critical stages of the study.

Evaluation of pharmacokinetic parameters of SR coated granules containing 100 mg metoprolol tartrate: The plasma levels produced by the administration of the studied drug (metoprolol tartrate) in each volunteer were used to establish the pharmacokinetic profile of TEST and REFERENCE preparations.

The AUC_{o-t} was determined by the trapezoidal method. Area under the plasma concentration-time curve from time zero to infinity (∞), $AUC_{0-\infty}$, was determined by the following equation:

$$AUC_{o-\infty} = AUC_{o-t} + C\ (t)/K_e$$

where K_e, is elimination rate constant.

Statistical analysis of pharmacokinetic parameters obtained from *in vivo* study: Usual descriptive analysis including the mean and standard deviation (SD) were used for variables such as the height, weight and age. These statistical parameters including coefficient of variance (CV) were used to describe plasma concentrations at each individual time point as well as the pharmacokinetic parameters. Following statistical tests were applied on untransformed [t_{max}, C_{max}, $AUC_{(o-t)}$, $AUC_{(o-\alpha)}$] and log-transformed pharmacokinetic data [$C_{max,}$ $AUC_{(0-t),}$ $AUC_{(0-\alpha)}$]. ANOVA of t_{max}, C_{max}, $AUC_{(o-t)}$, $AUC_{(o-\alpha)}$ were subjected to a one way ANOVA accounting for subjects, period and treatment. 90 % confidence interval (CI) consistent with two-one sided t-test with the significance level of 5 % for untransformed and log transformed parameters [t_{max}, $C_{max,}$ $AUC_{(o-t)}$, $AUC_{(o-\alpha)}$].

Relative bioavailability of metoprolol tartrate in TEST preparation was calculated taking bioavailability of metoprolol tartrate in REFERENCE preparation as 100 % [18-20].

RESULTS AND DISCUSSION

Pharmacokinetic parameters: Plasma concentration data are tabulated in Tables 2 and 3. Administration of the REFERENCE preparation as a single dose in the fasting state produced the maximum plasma concentration of 122.017 ± 0.983 ng/mL (C_{max}) at the time 3.417 ± 0.204 h (t_{max}) whereas the TEST preparation as a single dose in the fasting state produced the maximum plasma concentration 123.950 ± 1.223 ng/mL (C_{max}) at the time 3.33 ± 0.257 h (t_{max}) Administration of the REFERENCE preparations produced the area under plasma concentration time curve (AUC_{0-t}) 1177.056 ± 15.909 ng h/mL whereas administration of the TEST preparation produced the area under plasma concentration curve (AUC_{0-t}) 1172.96 ± 14.246 ng h/mL (Tables 4 and 5). When administered as a single dose, in the fasting state, the REFERENCE preparation produced the area under plasma concentration time curve up to infinity ($AUC_{0-\alpha}$) 1186.633+16.057 ng h/mL whereas administration of the TEST preparation produced area under plasma concentration time curve up to infinity ($AUC_{0-\alpha}$) 1177.855 ± 17.036 (Table-4). Administration of the REFERENCE preparation produced the plasma elimination half-life, ($t_{1/2}$) 6.421 ± 0.116 h whereas administration of the TEST preparation produced the plasma elimination half-life ($t_{1/2}$) 5.145 ± 1.104 h. Administration of the REFERENCE preparation produced the plasma elimination constant (K_{el}) 0.108 ± 0.002 h^{-1} whereas administration of the TEST preparation produced the plasma elimination constant (k_{el}) 0.140 ± 0.030 1 h^{-1}. On the basis of comparison of the $AUC_{0-\alpha}$, the relative bioavailability of metoprolol tartrate in the TEST preparation was 99.26 % to that of the REFERENCE preparation. The advantage of new formulation is that it is in capsule form, which is more flexible than tablet dosage form.

TABLE-2
BIOAVAILABILITY STUDY OF SR COATED GRANULES CONTAINING 100 mg METOPROLOL TARTRATE: PLASMA CONCENTRATION OF REFERENCE PREPARATION FOR 6 VOLUNTEERS

Volunteer No.	Time (h)													
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	8.0	10.0	12.0	18.0	24.0
	Plasma concentration (ng/mL)													
1	0	63.23	77.10	98.21	99.13	117.00	120.10	121.20	117.90	65.23	51.78	40.12	12.80	3.10
2	0	63.23	77.10	102.12	103.20	121.10	122.10	123.10	116.10	66.20	52.10	41.10	13.20	2.90
3	0	66.40	75.21	102.20	104.00	117.00	121.12	123.20	103.20	67.12	50.12	39.12	12.80	2.82
4	0	65.80	74.10	98.10	100.80	116.30	121.00	122.30	118.70	68.21	51.78	40.12	12.80	3.10
5	0	61.20	75.40	99.10	99.13	117.00	120.10	121.20	117.90	67.23	50.12	39.12	12.20	2.70
6	0	65.12	74.20	97.10	98.21	118.10	121.10	119.30	118.20	64.30	49.32	37.21	11.98	2.90
Mean	0	64.16	75.52	99.47	100.75	117.75	120.92	121.72	115.33	66.38	50.87	39.47	12.63	2.92
S.D.	0	1.96	1.33	2.18	2.38	1.74	0.75	1.47	6.01	1.44	1.16	1.33	0.45	0.16
C.V.	0	3.05	1.76	2.19	2.36	1.48	0.62	1.21	5.21	2.16	2.27	3.37	3.57	0.00

TABLE-3
PLASMA CONCENTRATION OF METOPROLOL TARTRATE (ng/mL) IN TEST PREPARATION FOR 6 VOLUNTEERS

Volunteer No.	Time (h)													
	0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	8.0	10.0	12.0	18.0	24.0
	Plasma concentration (ng/mL)													
1	0	65.80	74.10	98.10	100.80	116.30	121.000	122.30	118.70	68.21	51.78	40.12	12.80	3.10
2	0	64.21	75.21	97.21	101.20	117.20	122.200	123.20	119.10	67.21	52.21	39.21	13.20	2.30
3	0	63.21	74.21	101.32	103.20	118.20	123.300	124.30	121.10	65.00	54.21	38.21	12.78	1.198
4	0	65.12	76.21	102.30	103.90	121.21	124.320	125.30	118.90	67.21	56.56	38.32	12.12	2.30
5	0	66.32	75.23	103.20	104.21	119.30	125.300	123.40	119.23	67.20	58.90	39.21	11.32	2.40
6	0	65.23	76.23	104.23	103.20	121.20	123.300	121.00	117.30	65.32	59.23	41.20	9.90	1.32
Mean	0	64.98	75.20	101.06	102.75	118.90	123.24	123.25	119.06	66.69	55.48	39.38	12.02	2.10
S.D.	0	1.12	0.92	2.82	1.42	2.05	1.52	1.50	1.22	1.25	3.25	1.13	1.23	0.72
C.V.	0	1.72	1.23	2.79	1.38	1.72	1.23	1.22	1.02	1.88	5.86	2.88	10.24	34.24

TABLE-4
BIOAVAILABILITY STUDY OF SR COATED GRANULES CONTAINING 100 mg METOPROLOL TARTRATE: PHARMACOKINETIC PARAMETERS OF METOPROLOL TARTRATE IN REFERENCE (A) AND TEST (B) PREPARATIONS

Volunteer No.	K_{el} (h^{-1})		AUC_{0-t} (ng h/mL)		$AUC_{0-\infty}$ (ng h/mL)		$t_{1/2}$ (h)		T_{max} (h)		C_{max} (ng/mL)	
	A	B	A	B	A	B	A	B	A	B	A	B
1	0.105	0.129	1182.530	1188.915	1192.981	1199.019	6.584	6.366	3.5	3.5	121.2	122.300
2	0.108	0.125	1195.000	1165.374	1204.230	1166.028	6.397	6.25	3.5	3.5	123.1	123.200
3	0.111	0.132	1177.976	1152.758	1186.987	1153.380	6.245	6.12	3.5	3.5	123.2	124.300
4	0.109	0.123	1187.820	1181.139	1197.924	1181.688	6.366	6.30	3.5	3.5	122.3	125.300
5	0.108	0.125	1168.990	1185.305	1178.278	1193.144	6.437	6.036	3.5	3.0	121.2	125.300
6	0.107	0.123	1150.019	1164.285	1159.396	1173.868	6.498	6.037	3.0	3.0	121.1	123.300
Mean	0.108	0.126	1177.056	1172.962	1186.633	1177.855	6.421	6.184	3.417	3.333	122.017	123.950
SD	0.002	0.003	15.904	14.246	16.057	17.036	0.116	0.140	0.204	0.258	0.983	1.223
CV %	1.815	2.854	1.351	1.215	1.353	1.446	1.810	2.269	5.974	7.746	0.81	0.99

TABLE-5
BIOAVAILABILITY STUDY OF SR COATED GRANULES CONTAINING 100 mg METOPROLOL TARTRATE: PHARMACOKINETIC PARAMETERS OF METOPROLOL TARTRATE WITH THE REFERENCE (A) AND TEST (B) PREPARATION

Pharmacokinetic parameters	REFERENCE preparation (A) Mean ± S.D.	TEST preparation (B) Mean ± S.D.
C_{max} (ng/mL)	122.017±0.983	123.950±1.223
t_{max} (h)	3.417±0.204	3.333±0.258
AUC_{0-t} (ng h/mL)	1177.056±15.904	1172.962±14.246
$AUC_{0-\infty}$ (ng h/mL)	1186.633±16.057	1177.855±17.036
k_{el} (h^{-1})	0.108±0.002	0.140±0.030
$t_{1/2}$ (h)	6.421±0.116	5.145±1.104
Relative bioavailability (%)	100	99.26

Statistical inference for bioequivalent study: ANOVA (Subject, period, treatment) was applied to the C_{max}, ln C_{max}, AUC_{0-t} and ln $AUC_{0-\alpha}$ values. No significant difference was found statistically for the treatments, subject and period values of C_{max}, ln C_{max}, AUC_{0-t} and ln AUC_{0-t}, $AUC_{0-\alpha}$ and ln $AUC_{0-\alpha}$. Table-7 shows confidence limit and ANOVA Table-8.

Adverse reactions: None of the volunteers complained of any adverse reaction on the pharmacokinetic profile days.

The elimination rate constant value is calculated by considering the last six plasma concentrations as shown in Table-6. Finally, ln conc. drug *vs*. time was plotted (Fig. 2) and the slope is considered elimination rate constant is 0.108.

$AUC_{0-\alpha}$ was calculated by the formula:

$$AUC_{0-\alpha} = AUC_{0-t} + C_t/k_{el} = 1188.915 + 1.1/0.108 = 1199.09$$

TABLE-6
LAST SIX PLASMA DRUG CONCENTRATIONS OF VOLUNTEER No. 1

Time (h)	Plama drug concentration (ng/mL)	ln plasma drug concentration
7	76.21	4.333492688
8	68.21	4.222591182
10	51.78	3.947003974
12	40.12	3.691874963
18	12.80	2.549445171
24	3.10	1.131402111
48	1.10	0.095310180

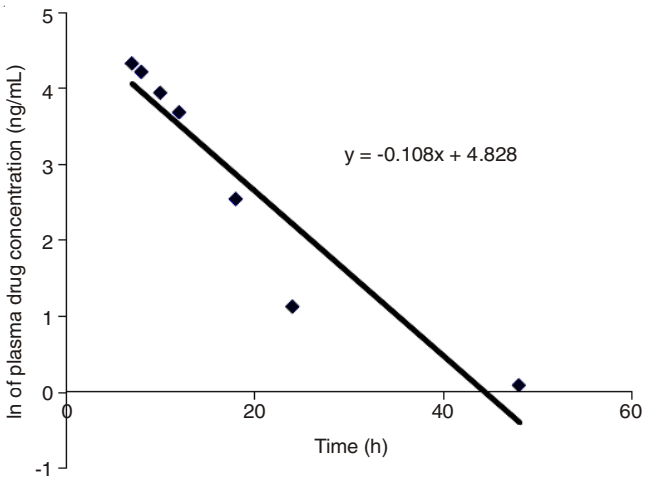

Fig. 2. Determination of K_{el}

Finally, $t_{1/2}$ was calculated according to the following formula: $t_{1/2} = 0.693/0.108 = 6.366$.

TABLE-8
ANOVA SUMMARY FOR PHARMACOKINETIC PARAMETERS OF METOPROLOL TARTRATE

Parameter	Source	Degree of freedom	Sum of squares	Mean sum of squares	Mean sum of squares/Error	Probability
C_{max}	Subjects	5	6.9767	1.3953	1.2477	Non significant
	Treatment	1	11.2133	11.2133	10.0268	Significant
	Period	1	0.8533	0.8533	0.7630	Non significant
	Error	4	4.4733	1.1183		–
	Total	11	23.5167			–
ln C_{max}	Subjects	5	0.0005	0.0001	1.2584	Non significant
	Treatment	1	0.0007	0.0007	10.1061	Significant
	Period	1	0.0001	0.0001	0.7740	Non significant
	Error	4	0.0003	0.0001		–
	Total	11	0.0016			–
AUC_{0-24}	Subjects	5	1295.3888	259.0778	1.1771	Non significant
	Treatment	1	50.2624	50.2624	0.2284	Non significant
	Period	1	103.7544	103.7544	0.4714	Non significant
	Error	4	880.3682	220.0920		–
	Total	11	2329.7738			–
ln AUC_{0-24}	Subjects	5	0.0009	0.0002	1.1792	Non significant
	Treatment	1	0.0000	0.0000	0.2252	Non significant
	Period	1	0.0001	0.0001	0.4651	Non significant.
	Error	4	0.0006	0.0002		–
	Total	11	0.0017			–
$AUC_{0-\infty}$	Subjects	5	1311.8914	262.3783	0.8289	Non significant
	Treatment	1	231.1589	231.1589	0.7302	Non significant
	Period	1	162.2921	162.2921	0.5127	Non significant
	Error	4	1266.1926	316.5481		–
	Total	11	2971.5350			–
ln $AUC_{0-\infty}$	Subjects	5	0.0009	0.0002	0.8276	Non significant
	Treatment	1	0.0002	0.0002	0.7265	Non significant
	Period	1	0.0001	0.0001	0.5012	Non significant
	Error	4	0.0009	0.0002		–
	Total	11	0.0021			–

This bioequivalent study analyzed the comparative bioavailability of 100 mg metoprolol tartrate (TEST-C1) with the reference formulation (Metolar XR 100SR) tablets of Cipla Ltd. This entire study was based on robust statistical and scientific data. Various bioavailability paramaters like C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ were analyzed to access comparative bioavailability study which is purely based on not only extent of absorption but also the rate of absorption. In addition the logarithm of rate and extent of the bioavailability studies in various extents. Parameters are also studied to explore the bioequivalence study. The bioavailability parameters were also treatedon the light of modern statistical concept of ANOVA. The F value derived from the ANOVA statistical concept also indicates that minute differences of the parameters are statistically insignificant. The estimated t_{max}, C_{max}, half-life of the test and the reference formulations in this study are consistent with other investigations [4]. The measured bio-availability parameters showed following oral administration of both formulations (test and reference) were not significantly different and also maintained 90 % confidence interval within 0.8-1.25 for the log transformed values (Table-7).

TABLE-7
90 % CONFIDENCE INTERVAL OF GEOMETRIC MEAN RATIO FOR METOPROLOL TARTRATE WITH THE TEST AND REFERENCE PREPARATION

C_{max}	Untransformed data	1.0067-1.0249
	ln transformed data	1.0014-1.0051
AUC_{0-24}	Untransformed data	0.9833-1.0096
	ln transformed data	0.9976-1.0013
$AUC_{0-\infty}$	Untransformed data	0.9769-1.0082
	ln transformed data	0.9967-1.0012

Conclusion

The statistical analysis of bioavailability of both REFERENCE and TEST brands reveals that bioavailability and pharmacokinetic parameters are within the acceptable limit. Therefore, on the basis of the values of pharmacokinetic and bioavailability parameters, it can be said that the formulated drugs are bioequivalent to the innovator product sample and can be used as pharmaceutical substitute with each other.

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