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ORAL BIO-EQUIVALENCE STUDY OF FIXED DOSE COMBINATION OF OLMESARTAN 40mg+AMLODIPINE 10mg+ HYDROCHLOROTHIAZIDE 25mg TABLETS IN HEALTHY HUMAN VOLUNTEERS

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ABSTRACT

This present bioequivalence study was designed to determine the bioavailability and bioequivalence of Olmesartan 40mg+Amlodipine 10mg+ Hydrochlorothiazide 25mg tablets in comparison with TRIBENZOR® tablets after single dose administration under fasting conditions in healthy adult male subjects. Therefore the design of an open label, balanced, randomized, single dose, two-treatment, two-period crossover study with a wash-out period of at least 15 days was used. The pharmacokinetic parameters including C_{max}, AUC_{0-t}, AUC_{0-inf} and T_{max} were analyzed using the non-compartmental model. Drug safety and tolerability were assessed. The primary pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) 90%CI were within the 80 to 125% interval required for bioequivalence as stipulated in the current regulations of the USFDA acceptance criteria. The 90% confidence intervals for the ratios of the ln-transformed Cmax and $AUC_{0\rightarrow72}$ for Olmesartan were 98.788%-99.602% and 98.637%-98.835% respectively and for Amlodipine were 99.976%-101.918% and 104.957%-111.670% respectively and for hydrochlorothiazide were 98.082%-99.623% and 95.301%-98.406% respectively. Eighteen volunteers had completed all treatment periods. The pharmacokinetic parameters of test product assessed were within the acceptable limits of Bioequivalence 80-125%.

KEYWORDS

Bio-equivalence, Hydrochlothiazide, Amlodipine, Olmesartan.

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INTRODUCTION[1, 2]

Hypertension is the term used to describe high blood pressure. Blood pressure is a measurement of the force against the walls of your arteries as your heart pumps blood through your body. Normal blood pressure is when your blood pressure is lower than 120/80 mmHg most of the time. High blood pressure (hypertension) is when your blood pressure is 140/90 mmHg or above most of the time. The top

number is called the systolic blood pressure, and the bottom number is called the diastolic blood pressure.

The active ingredients of Tribenzor target three separate mechanisms involved in blood pressure regulation. Specifically, amlodipine blocks the contractile effects of calcium on cardiac and vascular smooth muscle cells; Olmesartan medoxomil blocks the vasoconstriction and sodium retaining effects of angiotensin II on cardiac, vascular smooth muscle, adrenal and renal cells; and hydrochlorothiazide directly promotes the excretion of sodium and chloride in the kidney leading to reductions in intravascular volume.

Amlodipine is a dihydropyridine calcium channel blocker that inhibits the trans membrane influx of calcium ions into vascular smooth muscle and cardiac muscle. Experimental data suggests that amlodipine binds to both dihydropyridine and nonhydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extracellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells[3].

Hydrochlorothiazide is a thiazide diuretic. Thiazides affect the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride in approximately equivalent amounts. Indirectly, the diuretic action of hydrochlorothiazide reduces plasma volume, with consequent increases in plasma renin activity, increases in aldosterone secretion, increases in urinary potassium loss, and decreases in serum potassium. The renin-aldosterone link is mediated by angiotensin II, so co-administration of an angiotensin II receptor antagonist tends to reverse the potassium loss associated with these diuretics[4].

The objective of this study is to assess the Relative bioavailability of Olmesartan40mg+Amlodipine 10mg+ Hydrochlorothiazide 25mg Tablets and Tribezor® tablets (containing Olmesartan40mg+ Amlodipine 10mg+ Hydrochlorothiazide 25mg) in 18 Normal, Healthy, Adult, Human Male Subjects under fasting conditions.

MATERIAL AND METHODS[5, 6]

Study Drugs

Test (T): Olmesartan40mg+Amlodipine 10mg+ Hydrochlorothiazide 25mg Tablets.

Reference (R): TRIBENZOR® tablets (containing 100mg of Olmesartan40mg +Amlodipine 10mg+ Hydrochlorothiazide 25mg).

Study Population

The study was carried out at Clinsync Clinical Research Private Limited, India. The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP). All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment.

The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in C_{max} and AUC between the test and reference product, Regulatory requirements.

Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 18 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 18 subjects was enrolled in the study.

Twenty healthy male volunteers between the ages of 18-55 years with a body mass index between18.5 and 24.9 kg/m^2, with body weight equal to or not less than 45 kgs were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination and laboratory screening test for hematologic and blood biochemistry parameters.

Study Design

An Open label, Balanced, Randomized, Single Dose, Two-Treatment, Two- period, crossover Oral Bioequivalence study in 18 Normal, Healthy, Adult, and Human Male Subjects under fasting conditions.

As per the randomization schedule, single dose of test (T) or reference product (R) will be administered to each subject with 240± 2ml of water at ambient temperature in each period.

Subjects will be instructed not to chew or crush the Tablet but to consume it as a whole. Compliance for Dosing will be assessed by a thorough check of the oral cavity immediately after dosing. The Subjects will be admitted and housed in the clinical facility from not less than 12.00 hours pre-dose till at least 72.00 hours post-dose in each period.

Subjects will be fasted for at least 10hours prior to scheduled time for dosing. Drinking water will be prohibited for one hour before and one hour after dosing. At other times, drinking water will be provided ad libitum. Meals or snacks will be provided at 4^{th} hr lunch, 8^{th} hr snacks, 12^{th} hr dinner and 24^{th} hour breakfast, 28^{th}hr lunch 32^{nd}hr snacks, 36^{th}hr dinner, 48^{th}hr breakfast, 52^{nd}hr lunch, 56^{th} hr snacks, 60^{th}hr dinner, 72^{nd}hr breakfast check out, after dosing in each period.

Sample Collection

- Total Number of Blood Samples: Twenty eight samples (28) per period from each subject.
- Volume per each sample: 06 ml. Sampling Hours: pre-dose (with in 01 hr prior to dosing) 0.50, 1.00, 1.33, 1.66, 2.00, 2.33, 2.66, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 6.50, 7.00, 7.50, 8.00, 8.50, 9.00, 10.00, 12.00,16.00, 24.00, 36.00, 48.00 and 72.00 hours after dosing.
- The total blood loss combining all the periods will not exceed 385ml (including 336ml for pharmacokinetic analysis, including 24ml of discarded heparinised blood prior to each post-dose sample collected through cannula about 15ml blood collected for pre-study screening and about up 10ml blood collected for post safety assessment).
- After collection of blood samples from all the subjects at each time point, samples will be centrifuged under refrigeration with machine set at 3000 RPM for 10 minutes and 4ºC.

After centrifugation, the plasma samples will be separated and transferred into respective pre-labeled RIA vials in duplicate double aliquot tubes. 1ml of plasma will be transferred into Aliquot-I and rest of the plasma into Aliquot-II. These polypropylene tubes will then be stored upright in a box containing dry ice or in a freezer at a temperature below -20°C for a maximum period of 12 hours and then they will be stored at -70°C ±15°C at the clinical and analytical sites. The plasma samples will be transferred to Clinse Lab Pvt. Ltd for analysis after completion of clinical phase.

LC-MS METHOD FOR ANALYSIS[6, 7, 8]

Tribenzor, a commercial formulation containing a combination of HCT, OLM and AML has been taken up for checking the applicability of the proposed method to the formulation. Twenty tablets were weighed, finely powdered and an accurately weighed sample of powdered tablets equivalent to 40mg OLM, 10mg AML and 25mg HCT was extracted with small amount of diluent in a 100 ml volumetric flask. The solution was shaken well and allowed to stand for 15 min with intermittent sonication to ensure complete solubility of drug. The contents were made up to the mark with the diluent and filtered through a 0.45µ membrane filter. From the filtrate, dilution was made in a 10 ml volumetric flask to get 40µg/ml Olmesartan, 2µg/ml amlodipine and 25µg/ml hydrochlorothiazide respectively. Now the sample of 20µl was injected and chromatographed.

Pharmacokinetic and statistical analysis

For the purpose of Average Bioequivalence analysis C_{max}, AUC_{0-t} and AUC_{0-inf} were considered as the primary variables and T_{max}, $t_{1/2}$ and K_{el} were considered as the secondary variables. General Linear Model for analysis of variance (ANOVA) for crossover design was performed for log-transformed data and used to assess the effect of formulations, periods, sequences and subjects nested in sequence on these parameters. The difference between two related parameters was considered statistically significant for a *p*-value equal to or less than 0.05. 90% confidence interval (CI) for the ratios of

geometric mean Test/Reference (T/R) for C_{max}, AUC_{0-t} and AUC_{0-inf} was calculated based on least squares means from the ANOVA of log-transformed data.

The 90% geometric CI of the ratio (T/R) of least squares means from the ANOVA of the log-transformed C_{max}, AUC_{0-t} and AUC_{0-inf} should be within 80.00% to 125.00%.

RESULTS

The present Bioequivalence study was conducted in 18 healthy male volunteers with age between 19 to 43 years and BMI with range 18.9-24.7 $kg/m^{2.}$ The final evaluation was carried out on data obtained from 18 volunteers who completed the study according to protocol. The mean plasma concentrations of Olmesartan, Amlodipine, hydrochlorothiazide for test and reference products on linear. The results are shown in Table No.1-7 and Figure No.1-6.

Tolerability

Total fifteen volunteers were dosed at least once. All the volunteers were included in the safety evaluation. Of the 18 subjects enrolled, 18 volunteers received both the test and reference products during the study. Total five adverse event were reported in the study which included head ache, dizziness and myalgia. The adverse event was mild in nature and resolved completely before discharge of volunteer. There were no trends towards clinically significant changes in laboratory safety parameters. Overall the Olmesartan 40mg + Amlodipine 10mg + Hydrochlorothiazide 25 mg was well tolerated as a single, oral dose (1 X 40mg+10 mg+25mg) administered under fasting conditions.

Table No.1: Demograhic Characteristics

Category		Treatment		Total
		Test (T)	Reference (R)	
Age (years)	Mean ± SD	30.13±8.17	30.13±8.17	23.84 ± 4.14
	Range	19-43	19-43	18.0 – 36.0
	Median	28	28	23.0
	N	30	15	40
Age Groups	< 18	00	00	00
	18 – 40	26	13	39
	41 – 64	8	4	12
	65 – 75	00	00	00
	> 75	00	00	00
Gender	Female	00	00	00
	Male	30	18	45
Race	American	00	00	00
	Hispanic	00	00	00
	Caucasian	00	00	00

	Asian	30	18	45
Height (cm)	Mean ± SD	164.12 ± 5.69	166.84 ± 4.89	165.48 ± 5.57
	Range	155.0 – 175.0	159.0 – 176.0	155.0 – 176.0
	Median	162.00	168.0	165.0
	N	20	20	40
Weight (kg)	Mean ± SD	58.96 ± 6.24	61.56 ± 6.43	60.26 ± 6.41
	Range	52.0 – 70.0	52.0 – 77.0	52.0 – 77.0
	Median	58.0	59.0	59.0
	N	20	20	40
BMI (kg/m^2)	Mean ± SD	21.86 ± 1.46	22.10 ± 1.79	21.98 ± 1.62
	Range	20.1 – 24.8	20.0 – 24.9	20.0 – 24.9
	Median	22.00	21.60	21.80
	N	20	20	40

Table No.2: Summary statistics for PK parameters of Olmesartan

Treatment	**Statistics**	**T_{max}**	**C_{max}**	**AUC_{0-t}**	**AUC_{inf}**
R	N	18	18	18	18
	Mean	2.988333	2422.464	13995.05	14086.3
	SD	0.58862	508.3058	4251.265	4360.955
	Min	2	1224.73	7036.704	7159.016
	Median	3	2436.661	12601.49	12608.13
	Max	4.5	3658.628	25075.56	25193.88
	Geo mean	2.937579	2366.103	13436	13506.73
	%CV	19.69727	20.98301	30.37691	30.95884
T	N	18	18	18	18
	Mean	2.988889	2369.617	13275.15	13414.53
	SD	0.391676	466.0373	4293.396	4354.542
	Min	2.33	1360.36	7182.199	7196.552
	Median	3	2387.293	12012.59	12127.3
	Max	3.5	3238.548	23757.98	23767.69
	Geo mean	2.964235	2322.468	12677.48	12800.49
	%CV	13.10442	19.6672	32.34159	32.46139

Table No.3: Summary statistics for PK parameters for Bioequivalence of Olmesartan

Parameter	C_{max}	AUC_{0-t}	$AUC_{0-\alpha}$
Lsm-A	3.36595	4.103033	4.107227
Lsm-B	3.374034	4.12827	4.13055
Difference (Lcm-A, Lcm-B)	-0.00808	-0.02524	-0.02332
T / R Ratio	99.19486	97.50786	97.69463
Variation (A & B)	0.000448	0.004861	0.005291
Mean Standard Error (A & B)	0.000895	0.009721	0.010581
Standard Error (A & B)	0.002351	0.007746	0.008082
ISCV	60.68022	60.9486	60.97481
Lower CL (A & B)	98.78863	98.63737	98.58033
Upper CL (A & B)	99.60277	98.83556	99.08287

Table No.4: Summary statistics for PK parameters of Amlodipine

Treatment	Statistics	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	AUC_{inf} (ng.hr/mL)
R	N	18	18	18	18
	Mean	6.777778	11.63017	114.3522	116.8806
	SD	0.491762	3.152052	51.56063	52.26593
	Min	6	5.424	30.00364	30.0213
	Median	7	11.163	101.1714	113.6987
	Max	7.5	16.51	211.3415	214.1666

	Geo mean	6.760735	11.19416	102.6248	104.7661
	%CV	7.255508	27.10238	45.08933	44.71737
T	N	18	18	18	18
	Mean	6.666667	11.67667	136.477	148.5901
	SD	0.514496	2.404065	58.57142	73.84583
	Min	5	6.542	30.52587	30.53102
	Median	7	11.322	140.2349	143.3334
	Max	7	16.898	266.6931	345.3834
	Geo mean	6.645571	11.43877	123.2069	131.2596
	%CV	7.717436	20.58863	42.91671	49.69768

Table No.5: Summary statistics for PK parameters for Bioequivalence of Amlodipine

Parameter	**C_{max}**	**AUC_{0-t}**	**$AUC_{0-\alpha}$**
Lsm-A	1.058379	2.090635	2.118131
Lsm-B	1.048992	2.011252	2.020221
Difference (Lcm-A, Lcm-B)	0.009388	0.079383	0.09791
T / R Ratio	100.9432	108.2619	110.2864
Variation (A & B)	0.002459	0.02554	0.028411
Mean Standard Error (A & B)	0.004917	0.05108	0.056822
Standard Error (A & B)	0.005509	0.017757	0.018728
ISCV	60.80238	62.22209	62.40099
Lower CL (A & B)	99.97687	104.9571	106.7386
Upper CL (A & B)	101.9188	111.6707	113.9521

Table No.6: Summary statistics for PK parameters of hydrochlorothiazide

Treatment	Statistics	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	AUC_{inf} (ng.hr/mL)
R	N	18	18	18	18
	Mean	2.33	121.7106	695.1759	697.2147
	SD	0.253098	32.3019	468.0051	469.5103
	Min	2	74.4	269.5362	269.5376
	Median	2.33	124.67	555.6398	556.4351
	Max	2.66	191.84	2199.786	2201.386
	Geo mean	2.316922	117.7182	589.8236	591.0946
	%CV	10.86259	26.53993	67.32182	67.34085
T	N	18	18	18	18
	Mean	2.256667	125.4706	735.8426	686.3731
	SD	0.213431	33.55199	397.134	437.4457
	Min	2	69.35	231.306	0.999816
	Median	2.33	132.455	622.6309	600.9629
	Max	2.66	191.84	1592.466	1661.582
	Geo mean	2.247186	120.896	635.0605	434.6783
	%CV	9.457815	26.74093	53.96997	63.73293

Table No.7: Summary statistics for PK parameters for Bioequivalence of Hydrochlorothiazide

Parameter	C_{max}	AUC_{0-t}	$AUC_{0-\alpha}$
Lsm-A	2.070843	2.770722	2.771657
Lsm-B	2.082412	2.802815	2.638168
Difference (Lcm-A, Lcm-B)	-0.01157	-0.03209	0.133489

T / R Ratio	98.84983	96.84165	114.2809
Variation (A & B)	0.001614	0.006829	0.380735
Mean Standard Error (A & B)	0.003227632	0.013657745	0.761470071
Standard Error (A & B)	0.004463592	0.009181888	0.068559701
ISCV	60.75102788	6.10686755	88.75725943
Lower CL (A & B)	98.08249562	95.30160962	101.3887325
Upper CL (A & B)	99.62316737	98.40658453	108.8123235

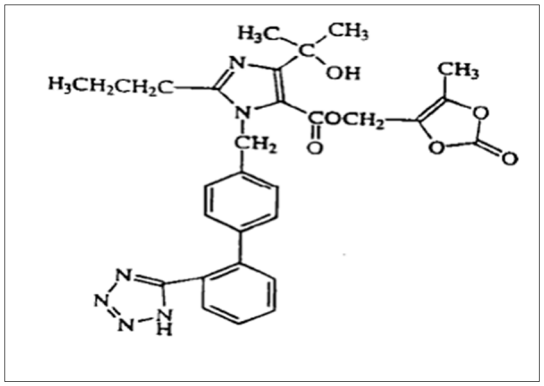

Figure No.1: OLMESARTAN MEDOXOMIL

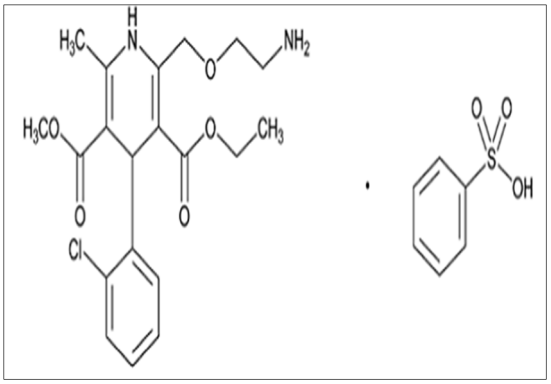

Figure No.2: AMLODIPINE

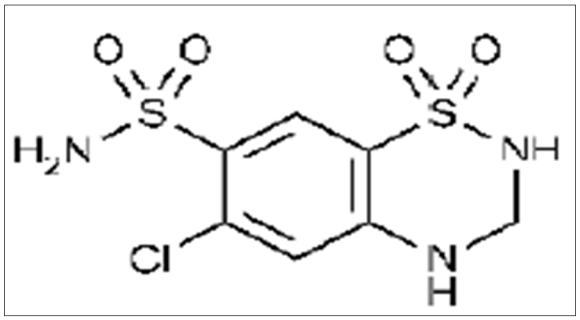

Figure No.3: Hydrochlorthiazide

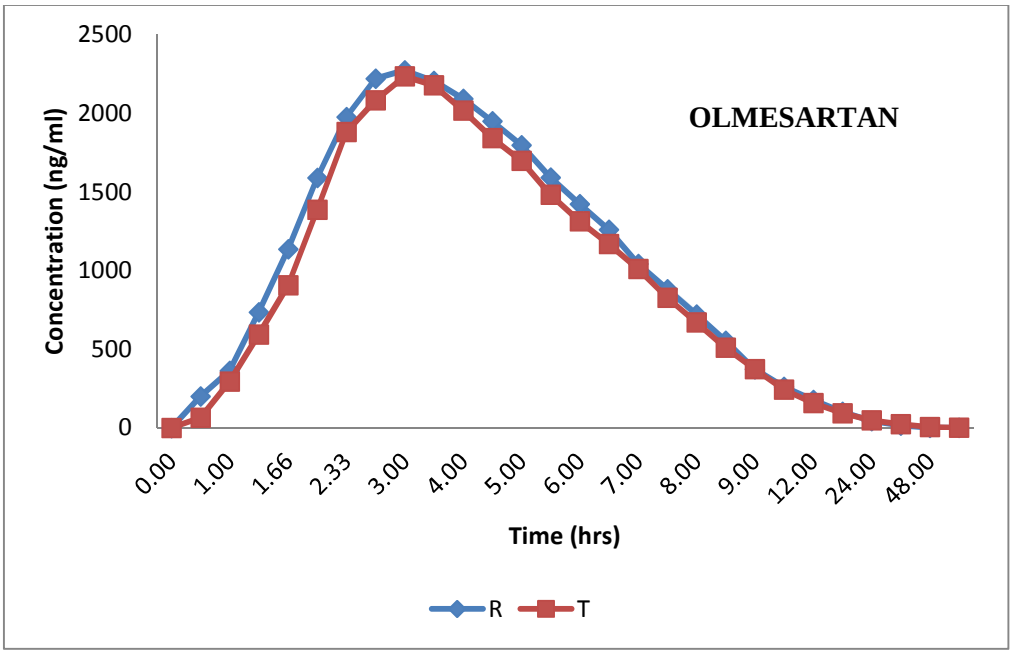

Figure No.4: Mean Concentration Graph of Olmesartan

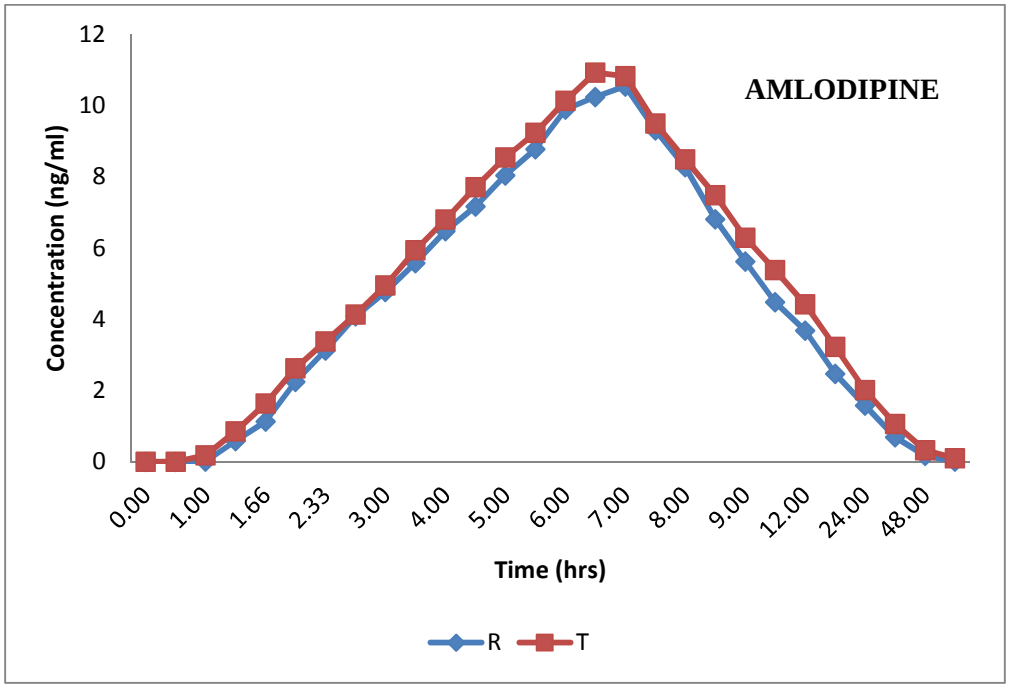

Figure No.5: Mean Concentration Graph of Amlodipine

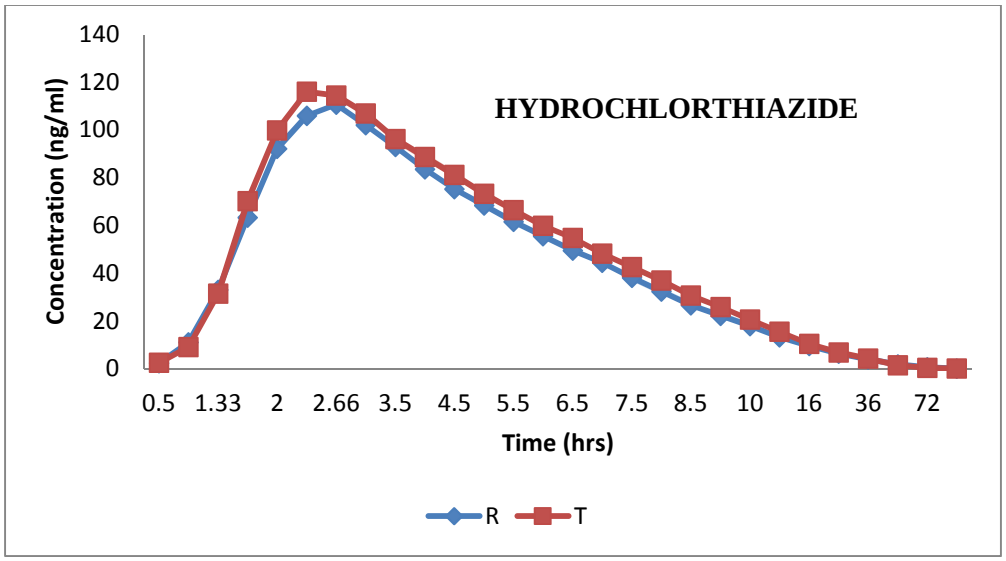

Figure No.6: Mean Concentration Graph of Hydrochlorthiazide

CONCLUSION

Bioequivalence is evaluated by three parameters viz., C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, out of which AUC_{0-t}, $C_{max,}$ are main parameters for évaluation. The Pharmacokinetic parameters (AUC_{0-t} and C_{max}) are with in the acceptable limits of bioéquivalence 80-125%. Hence it is concluded that single dose bioéquivalence study of test drug of Olmesartan 40mg + Amlodipine 10mg + Hydrochlorothiazide 25mg tablet is bioequivalent with reference drug of Tribenzor® (Olmesartan 40mg + Amlodipine 10mg + Hydrochlorothiazide 25mg) in terms of rate and extent of absorption after administration of single dose as set in the protocol.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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