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BIOAVAILABILITY AND BIOEQUIVALENCE STUDIES OF AGOMELATINE TABLETS 25MG ON HEALTHY HUMAN VOLUNTEERS

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ABSTRACT

This present bioequivalence study was designed to determine the bioavailability and bioequivalence of Agomelatine 25 mg tablets in comparison with VALDOXAN® 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 7 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 $AUC_{0\rightarrow17}$ for AGOMELATINE were 96.01% and100.94 % respectively. Seventeen volunteers had completed all treatment periods. There was no significant difference between the two formulations. No serious adverse events related to the study drug were found.

KEYWORDS

Agomelatine, Bioequivalence, ICH and Pharmacokinetic parameters.

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INTRODUCTION[1,2]

The phenomenal growth in pharmaceutical generic industry has led to various changes in carrying out concerned studies. Also rules governing the studies and methods determining the efficacy have evolved over time by leaps and bounds. Though the crude methods does not change much with time, but the precision and accuracy associated in determining the final result changed. This has led to the entry of various generics with potential and equivalent results over a short period of time thus enabling the

human kind to achieve Drug sufficiency with minimum resources and time.

Various regulatory bodies around the world laid down various guidelines for smooth functioning of Pharmaceutical field. These guidelines are generated in accordance with the International Conference on Harmonization (ICH) guidelines which is said to be linking various regulatory bodies around the world.

India has witnessed a huge change over last 2 decades in various fields including Pharmaceutical sector. This industry particularly flourished owing to the developing trends in Market. India has become a major player in determining global Pharmaceutical markets. Various factors like huge resources, available human talent and intellect are said to have brought these changes thus enabling India to control global markets.

The concept of generic market has brought out a revolutionary change in Indian pharmaceutical markets. Owing to the huge demand, Indian markets quickly adapted for the development of generic markets. Regulatory bodies like DCGI have laid down guidelines in India. Though guidelines operating in India resemble ICH guidelines to a large extent, necessary amendments were made to suit Indian markets.

The concept of Bio-equivalence and Bio-availability developed in 1970's has taken the world by storm. Before the development of these concepts, introduction of a drug into market was a herculean task. Various drugs developed were tested and tried effectively but introduction of generic versions of the same drug posed various challenges in terms of their therapeutic equivalency. Very few methods were available to prove their efficacy but desperate need for drugs had driven the countries to introduce generics without proper testing. Only with the all-round development of technology did remarkable changes have taken place in testing drugs. Since then, there is no turning back in developing generics with proved efficacy and safety. This present study intends to answer the need for proper testing through various critical validated methods for development of a generic.

Bioavailability implies biologically available amount of drug. It is defined as the rate and extent to which the active ingredient or active moiety is absorbed from a drug product and becomes available at the site of action. By definition, when the drug is administered intravenously, its bioavailability is 100% theoretically (slightly variable practically due to intrinsic factors). However when a medication is administered via other routes (such as by mouth), its bioavailability decreases (due to incomplete absorption and first-pass metabolism). Bioequivalence of a drug is achieved if its rate and extent of absorption is not statistically significantly different from those of reference product when administered at same molar dose. Here, now I am going to develop the generic drug AGOMELATINE (25mg) comparing with the reference product VALDOXAN tablets by Conducting bioavailability and bioequivalence Studies in human healthy male volunteers.

Depression is a state of low mood and aversion to activity that can affect a person's thoughts, behavior, feelings and sense of well-being.[1] Depressed people may feel sad, anxious, empty, hopeless, worried, helpless, worthless, guilty, irritable, hurt, or restless.

Signs and Symptoms

People with depressive illnesses do not all experience the same symptoms. The severity, frequency and duration of symptoms will vary depending on the individual and his or her particular illness and Symptoms include persistent sad, anxious or "empty" feelings, feelings of hopelessness and/or pessimism, feelings of guilt, worthlessness and/or helplessness, irritability, restlessness, loss of interest in activities or hobbies once pleasurable, including sex, fatigue and decreased energy, difficulty concentrating, remembering details and making decisions, insomnia, early–morning wakefulness, or excessive sleeping, overeating, or appetite loss, thoughts of suicide, suicide attempts, persistent aches or pains, headaches, cramps or digestive problems that do not ease even with treatment.

Depressions are of different forms like major depressive disorder, psychotic depression,

postpartum depression, seasonal affective disorder, bipolar disorder, dysthymic disorder.

Detection and Treatment[3,4,5]:

Depression, even the most severe cases, is a highly treatable disorder. As with many illnesses, the earlier that treatment can begin, the more effective it is and the greater the likelihood that recurrence can be prevented. The first step to getting appropriate treatment is to visit a doctor. Certain medications, and some medical conditions such as viruses or a thyroid disorder, can cause the same symptoms as depression. A doctor can rule out these possibilities by conducting a physical examination, interview and lab tests. If the doctor can eliminate a medical condition as a cause, he or she should conduct a psychological evaluation or refer the patient to a mental health professional.

The doctor or mental health professional will conduct a complete diagnostic evaluation. He or she should discuss any family history of depression, and get a complete history of symptoms, e.g., when they started, how long they have lasted, their severity, and whether they have occurred before and if so, how they were treated. He or she should also ask if the patient is using alcohol or drugs, and whether the patient is thinking about death or suicide. Once diagnosed, a person with depression can be treated with a number of methods. The most common treatments are medication and psychotherapy.

The drugs used in treatment of depression Amitriptyline, Imipramine, Nortriptyline, Fluvoxamine, Fluoxetine, Buspirone, Isocarboxazib, and Agomelatine.

MATERIAL AND METHODS

Number of Subjects

17 normal healthy adults male subjects.

Investigational Products:

a. Test product (T) Agomelatine tablets 25mg.
b. Reference product (R) Valdoxan tablets 25mg.

Study Facilities:

Clinical and screening facility given by:

Clinsync Clinical Research Pvt.Ltd. Clinical Pharmacology Department, 4-1-1, Hayathnagar, Hyderabad, A.P-501505

Methodology[7]

Check-In

Subjects were admitted and housed in the clinical facility of CR Bio Sciences at 10.5 hours before dosing and were fasted overnight for more than 10 hours. A duty dietician ensured that the food given to subjects does not interfere with the study and was responsible for feeding the subjects as and when scheduled as in protocol. Two pre-dose samples each 6 ml were collected which serve as a control

Housing

Subjects will be admitted and housed in the clinical facility from not less than 12 hours before dosing and will be checked out 24.00 hours after dosing in each period, if the subjects do not suffer from any adverse event.

Dosing

All subjects will be fasted overnight for at least 10.00 hours before scheduled time for dosing. The subjects will be administered as per the randomization schedule, either single dose of one tablet of containing Agomelatine 25 mg or Reference (B): Valdoxan 25 mg (containing 25 mg of Agomelatine) with 240±5 ml of water in each period as per SOP for "Dosing/Administration of Solid Oral Investigational Products" under the supervision of Principal Investigator/ Physician.

Diet and Water

The subjects will receive a standard meal at about 04.00, 08.00, 12.00 and 24.00 hours after dosing in each period. During housing, the meal menu will be same for both the periods. In case, meal and blood sample collection timings coincide, samples will be collected before meal. Drinking water will be restricted from one hour pre-dose till one hour post-dose (except during the administration of the dose) in each period. At all other times, drinking water will be provided ad libitum.

Restrictions

1. Drinking water will not be allowed from one hour before dosing till one hour post-dose.
2. Subjects will be instructed not to chew or crush the tablet but to consume it as whole.

3. Administration of investigational products will be done while the subjects are in sitting posture.

Blood Sample Collection

1. Total Number of Blood Samples: nineteen (19) per period.
2. Volume per each sample: 05 ml
3. Sampling Hours: Pre-dose (before dosing, in the morning of the day of dosing) and at0.25, 0.50, 0.75, 01.00, 1.25, 1.50, 1.75, 02.00, 2.25, 2.50, 2.75, 3.00, 3.50, 4.00, 6.00, 8.00, 10.00, 12.00hr after dosing. There are no ambulatory samples in study.

Wash out Period: 2 periods will be separated by at least 7 days as wash out period.

Clinical Safety Measures

Vital signs (sitting blood pressure, radial pulse rate, respiratory rate and axillary temperature) will be measured at bed side and recorded at the time of check-in, pre-dose, 01.00, 03.00, 05.00, 11.00 hours post dose (within ± 40 minutes of scheduled time of recording), checkout and at last ambulatory sample in each period and/or at termination from the study.

Check-Out

After the In-House sample are collected, volunteer can be checked out along with their belongings and compensation amount decided for the study and the volunteer has to attend the facility on the appropriate time for the ambulatory sample to collect.

Method Development

1. Samples from subject numbers 1-12 will be analyzed if they complete the study as per the approved protocol. In case of dropout/ withdrawal, unbalanced sequences will be considered for calculations.
2. Plasma samples obtained from subjects will be assayed for Agomelatine using a validated LC-MS/MS bio-analytical method. During analysis, standard and quality control samples will be distributed throughout each batch of study samples analyzed.

RESULTS

The present Bioequivalence study was conducted in 17 healthy male volunteers with age between 18 to 55 years and BMI with range 18.9-24.7 $kg/m^{2.}$ The final evaluation was carried out on data obtained from 17 volunteers who completed the study according to protocol. The mean plasma concentrations of Agomelatine for test and reference products on linear is shown in Table No.3-5.

DISCUSSION

Healthy volunteers should be examined by the physician through a medical examination including a review of medical history and results of routine tests of liver, kidney and haematological functions. So17 healthy human adult male volunteers are selected for the test. The subjects should have age between 18-55 years. Height and weight of subjects should preferably have a body mass index within 18.5 – 24.9 kg/m^2, vital signs like seated blood pressure, radial pulse rate, respiratory rate and auxiliary temperature are measured at bedside and recorded at the time check in, pre dose, post dose and check out in each period and/or extermination of the study. Wash out period is 7 days shown in Figure No.1and 2.

Table No.1: Time Table of Events

Day	Hours	Approximate time*	Activities
1	–12.0 or before	20:00 or before	Reporting at clinical facility. Obtaining Informed Consent (Period I only). Urine drug screen, alcohol breath test, clinical examination, vital signs measurement and check-in.
1	–10.0 or before	22:00 or before	Completion of Dinner

1	After Dinner		Bed time
2	–3.0 to –0.5	05:00 to 07:30	Pre-dose vital signs measurement
2	–1.0 to -0.5	07:00 to 07:30	Cannulation and pre-dose (0 hour) blood sample collection
2	- 1.0	07:00	Drinking water restriction starts
2	0.00	08:00	Dosing, Posture restriction starts, After dosing water restriction starts
2	0.25	08:15	Blood sample collection
2	0.50	08:30	Blood sample collection
2	0.75	08:45	Blood sample collection
2	1.00	09:00	Blood sample collection, end of water restriction
2	1.25	09:15	Blood sample collection
2	1.50	09:30	Blood sample collection
2	1.75	09:45	Blood sample collection
2	2.00	10:00	Blood sample collection, end of posture restriction, vital signs measurements
2	2.25	10:15	Blood sample collection
2	2.50	10:30	Blood sample collection
2	2.75	10:45	Blood sample collection
2	3.00	11:00	Blood sample collection
2	3.50	11:30	Blood sample collection
2	4.00	12:00	Blood sample collection, vital signs measurements, Lunch
2	6.00	14:00	Blood sample collection, vital signs measurements

2	8.00	16:00	Blood sample collection, Snacks
2	10.00	18:00	Blood sample collection, vital signs measurements
2	12.00	20:00	Blood sample collection, Dinner
2	Up to 12.5	20:30	Clinical Examination
2	12.5 on wards	20:30 on wards	Check-out, After period 02, Safety assessment (10 mL of blood for haematology (except blood group) and biochemistry), and Payment of participation of fee at the end of the study.

Table No.2: Time points and concentrations of subjects 1 and 2

Time in hours	**Subject 1**		**Subject 2**	
	Period 1	**Period 2**	**Period 1**	**Period 2**
0.00	0.00	0.00	0.00	0.00
0.25	435.56	367.56	378.59	223.45
0.5	598.54	456.87	438.01	368.78
0.75	645.39	496.56	489.42	389.45
1.00	735.89	546.89	548.56	425.46
1.25	788.34	689.54	756.34	538.56
1.50	923.47	724.34	819.89	579.56
1.75	984.67	788.46	886.56	599.67
2.00	999.89	846.57	900.45	609.89
2.25	1005.46	919.54	916.22	688.71
2.50	1013.64	971.46	867.34	632.45
2.75	946.43	900.43	723.67	524.56
3.00	733.46	823.54	678.45	479.65
3.50	683.45	690.43	511.45	432.45
4.00	589.68	553.45	476.89	500.78

6.00	489.32	368.69	379.78	456.98
8.00	267.21	284.56	189.45	389.54
10.00	77.45	15.46	56.53	109.44
12.00	0	0	0	0

Table No .3: Pharmacokinetic Parameters of Agomelatine (Reference)

Subject	**Lambda_z**	**T1/2**	**Tmax**	**Cmax**	**Auc 0-t**	**Auc 0-inf_obs**
1	0.44838	1.54588	2.5	971.46	4594.87	4629.34
2	0.16951	4.08902	2.25	688.71	4219.68	4865.29
3	0.26946	2.57226	2.25	962.53	3710.38	3966.21
4	0.32416	2.13828	2	724.22	3022.80	3095.14
5	0.55990	1.23796	1.5	1155.34	4568.57	4633.70
6	0.60799	1.14006	1.75	1058.85	4385.31	4438.68
7	0.26191	2.64649	1.75	1124.98	4604.10	4968.54
8	0.41093	1.68675	1.25	806.46	2642.69	2726.64
9	0.33325	2.07989	2.75	1066.02	5191.88	5394.22
10	0.25724	2.69450	2.5	813.87	4426.37	4773.58
11	0.31384	2.20858	2.25	1088.53	5011.84	5225.61
12	0.35167	1.97101	1.0	771.23	2527.05	2593.67
13	0.47057	1.47297	2.5	1163.51	5812.54	6002.59
14	0.22682	3.05593	2.75	1216.26	5546.77	6316.49
15	0.31387	2.20833	2.75	668.71	3021.99	3201.77
16	0.33919	2.04347	2.25	1078.52	4309.16	4473.25
17	0.35367	1.96540	1.25	985.14	2656.36	2726.03

Table No.4: Pharmacokinetic properties of Agomelatine (test)

Subject	Lambda_z	T1/2	Tmax	Cmax	Auc 0-t	Auc 0-inf_obs
1	0.46084	1.50407	2.5	1013.64	5213.70	5381.76
2	0.47620	1.45556	2.25	916.22	4232.50	4351.21
3	0.24971	2.77572	2.25	685.61	3658.97	3929.40
4	0.35707	1.94117	2.0	871.74	3671.28	3863.00
5	0.33508	2.06855	1.5	696.34	1934.59	1992.58
6	0.24779	2.79726	1.75	979.17	4502.87	4903.44
7	0.39697	1.74606	1.75	1084.98	5230.01	5480.00
8	0.34079	2.03389	1.25	869.03	2990.24	3090.35
9	0.39101	1.77269	2.75	821.07	4540.79	4769.40
10	0.34998	1.98050	2.5	730.18	3392.20	3491.51
11	0.49532	1.39936	2.25	919.55	4686.19	4822.32
12	0.26470	2.61860	1.0	1095.83	3739.16	4034.17
13	0.34072	2.03430	2.5	1294.01	5427.87	5721.03
14	0.31260	2.21729	2.75	1019.69	6595.61	7278.41
15	0.39796	1.74171	2.75	924.95	4573.94	4793.63
16	0.34271	2.02249	2.25	603.46	3454.25	3742.73
17	0.38388	1.80560	1.25	735.39	2291.84	2324.84

Table No.5: Summary statistics for PK parameters of Agomelatine

Treatment	Statistics	T_{max} (hr)	C_{max} (ng/mL)	AUC_{0-t} (ng.hr/mL)	AUC_{inf} (ng.hr/mL)
R	N	17	17	17	17
	Mean	2.135	939.488	6242.861	6493.626
	SD	1.149	233.736	1404.081	1452.752
	Min	1..0	668.71	2527.05	2593.67

	Median	1.38	942.32	4169.55	4454.58
	Max	2.75	1216.26	5812.54	6316.49
	Geo mean	1.852	914.144	6083.81	6329.54
	%CV	53.8	24.9	22.5	22.4
T	N	17	17	17	17
	Mean	2.167	895.98	6286.581	6571.29
	SD	1.098	186.44	1405.296	1476.663
	Min	1.0	603.46	1934.59	1992.58
	Median	1.38	948.65	3680.58	4635.98
	Max	2.75	1294.01	5427.87	7278.41
	Geo mean	1.897	875.98	6130.6	6407.433
	%CV	50.7	20.8	22.4	22.5

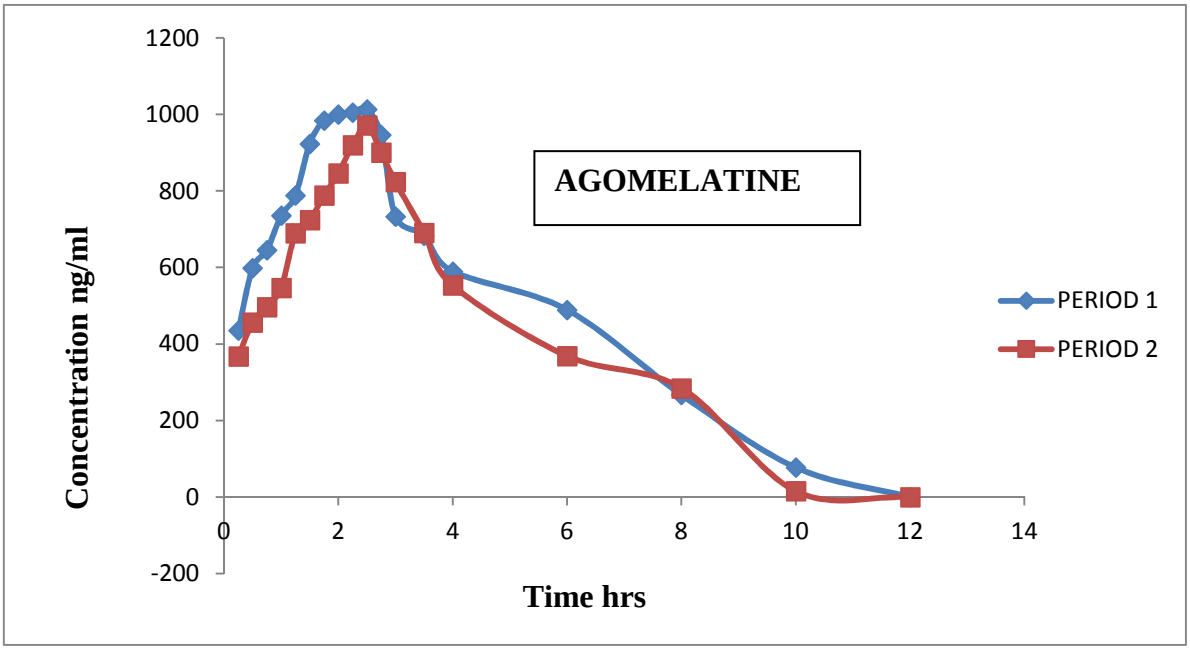

Figure No.1: Linear Concentration Graph Of Subject No: 01

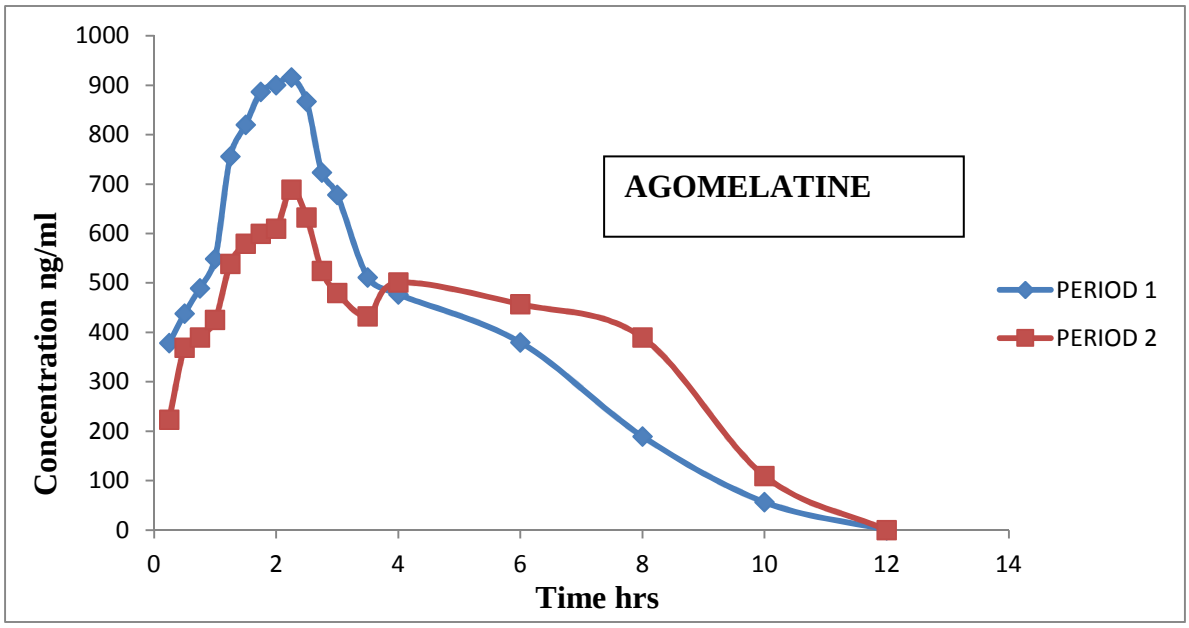

Figure No.2: Linear Concentration Graph Of Subject No: 02

CONCLUSION

The results obtained from 17 subjects in this study confirm that the test formulation, combination of AGOMELATINE 25mg Tablets, M/S Hetero Labs Limited, India when compared with the reference formulation, VALDOXAN (AGOMELATINE) 25 mg Tablets of Les Laboratories Servier 22, rue Garnier 92200 Neuilly – sur – Seine, Frankreich meets the bioequivalence criteria in terms of rate and extent of absorption after administration of single dose as set in the protocol. This test was carried out in 17 healthy volunteers in Clinsync clinical research pvt. Ltd, Hyd.

Pharmacokinetic parameters of test product assessed were within the acceptable limits of Bioequivalence 80-125%. Hence it is inferred that test drug AGOMELATINE 25 mg tablet is bioequivalent to reference drug VALDOXAN (Agomelatine 25mg). If this study had to go through clinical studies, it would have still yielded the same result but with great expenditure and long time. The concept of expenditure testing has taken over the conventional clinical testing BA & BE studies assume that the results observed reflects in the general patient population. The tabulated results were carefully derived, scrutinized and documented at appropriate times without any delay.

Various parameters were assessed in comparison with Standard drug .The final discretion of results in terms of log transformed C_{max}, AUC $_{0-t}$ and AUC $_{0-\infty}$ is found to be in the range of 80-125 % of standard Agomelatine formulation. Confidence interval was maintained at 90%. The significance of errors noted was found to have 0.005 % impact on the final values. Thus safety and protocol instructions were strictly executed. The standard group used served as a positive control to carry out the study effectively.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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