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Bioequivalence Study of antiulcer drug Lansoprazole delayed release capsules 30mg in healthy adult male human subjects under fed condition

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Abstract

Bioequivalence assessments necessary in abbreviated new drug application submissions to establish bioequivalence between a pharmaceutically equivalent generic drug product (T) and the corresponding reference listed drug (reference listed drug). Together with the determination of pharmaceutical equivalence, bioequivalence is a primary element in the determination of therapeutic equivalence. To assess bioavailability of Drug of 'Test' product comparing with 'Reference' product in normal, adult, human subjects under fasting or fed conditions. To monitor adverse events and ensure safety of the subjects. To further investigate the source of the observed variability in the C_{max} of 'Drug'. To evaluate the suitability of different study designs and statistical approaches for the assessment of bioequivalence between different (Test & Reference) 'Drug' tablet formulations. To monitor the safety and tolerability of a single dose of the test product as compared to the reference product in healthy human subjects. A randomized, single dose, open label, three treatment, three sequence, three period crossover bioequivalence study of Lansoprazole 30 mg Delayed Release Capsules of XXXX Limited, India comparing with that of Prevacid® (containing Lansoprazole 30 mg Delayed Release Capsules) in healthy, adult, male, human subjects under fed conditions.

Keywords: Lansoprazole, Bioequivalence study, Delayed Release capsules, adult male human subjects.

INTRODUCTION

Multisource pharmaceutical products need to conform to the same standards of quality, efficacy and safety as required of the originator's (comparator) product. Specifically, the multisource product should be therapeutically equivalent and interchangeable with the comparator product. Testing the bioequivalence between a product and a suitable comparator (pharmaceutically

equivalent or a pharmaceutical alternative) in a bioequivalence and bioavailability study with a limited number of subjects is one way of demonstrating therapeutic equivalence without having to perform a clinical trial involving many patients. In such a bioequivalence and bioavailability study any statement about the safety and efficacy of the test product will be a prediction based on measurement of systemic concentrations, assuming that essentially similar plasma concentrations of the drug will result in essentially similar concentrations at the site of action, and thus an essentially similar therapeutic outcome. The bioequivalence study thus provides indirect evidence of the efficacy and safety of a multisource drug product. Often this will be the only evidence that the product is safe and efficacious. It is therefore crucial that the bioequivalence study is performed in an appropriate manner. Several guidance documents stress the importance of onsite inspections to verify compliance with standards of good clinical practice. Studies that measure the bioavailability and/or establish bioequivalence of a product are important elements in the support of investigational new drugs, new drug application, abbreviated new drug application, and their supplements. As part of investigational new drugs and new drug application for orally administered drug products, bioavailability studies focus on determining the process by which a drug is released from the oral dosage form and moves to the site of action. bioavailability data help the sponsor/applicant estimate the fraction of the drug absorbed, as well as its subsequent distribution and elimination. Bioavailability can be documented by establishing a systemic exposure profile obtained by measuring drug and/or metabolite concentration in systemic circulation over time. The systemic exposure profile determined during clinical trials in the investigational new drugs period can serve as a benchmark for subsequent bioequivalence studies. Studies to establish bioequivalence between two products are important for certain changes prior to approval in a pioneer product in new drug application submissions and in the presence of certain post approval changes in new drug applications and abbreviated new drug application. In bioequivalence studies, an applicant compares the systemic exposure profile of a test drug product to that of the reference drug product. For two orally administered drug products to be bioequivalent, the active drug substance and/or active moiety in the test product should exhibit the same rate and extent of absorption as the reference drug product.

MATERIALS AND METHODS

Objective and Purpose

To compare and evaluate the single-dose oral bioequivalence study of Lansoprazole Delayed Release capsules USP 30 mg of XXXX Limited., India comparing with that of Prevacid® (containing Lansoprazole Delayed Release capsules 30 mg) Distributed by TAP Pharmaceuticals Inc, U.S.A. in healthy, adult, male, human subjects under fed conditions.

Study Design

Open label, balanced, randomized, three-treatment, three-period, three-sequence, single dose, crossover, bioequivalence study in healthy, adult, male human subjects under fed conditions.

Number of Subjects

12 healthy, adult, male, human subjects were enrolled in the study. Being a pilot study, since no definite statistically valid conclusion on bioequivalence is sought, 12 subjects would be dosed at the beginning of study as per sponsor's requirement.

Randomization Method

Randomization was carried out using SAS (SAS Institute Inc., USA) Version 9.1.3. Randomization was done in blocks using PROC PLAN such that the design is balanced. The order of receiving the reference and test formulations for each subject during all the periods of the study was determined according to randomisation schedule.

Blinding

This study was comprise of a randomised open label design; as it is needless to design double-blind study for a bioavailability and bioequivalence study. However, analysts would be blinded to the sequence of administration of test and reference formulations.

Duration of Study

Subjects were undergo a screening procedure not earlier than 21 days before the first day of dosing. Total expected duration of the study would be of at least 23 days from the day of check-in of the first period till the end of the third period. Upon entering into the study, the subjects were confined in the clinical facility of Synapse labs Pvt. Ltd. to ensure 10 hours overnight fasting prior to high fat breakfast and till 24 hours post-dose blood sample collection in all the periods.

Washout Period

The administration of each product is followed by a sufficiently long period of time to ensure complete elimination of the drug (washout period) before the next administration. The mean elimination half-life of lansoprazole is about 1.5 hour. The washout period was a minimum of 10 half-lives of the administered drug. A washout period of at least 10 days were kept between each dosing periods which was sufficient enough to ensure complete elimination of the drug.

Termination of the Study

The sponsor reserves the right to discontinue the study at any time. The Principal Investigator reserves the right to discontinue the study for safety reasons at any time. The Independent Ethics Committee (IEC) may ask to terminate the study, if there are major violations of the ethical considerations or due to any serious adverse event(s). Reasons for the termination of the study was provided to the subjects.

Table-1 Investigational Products

Test Product (T ₁)	:	Lansoprazole Delayed Release capsules USP 30 mg of XXXX Limited, India
Test Product (T ₂)	:	Lansoprazole Delayed Release capsules USP 30 mg of XXXX Limited, India
Reference Product (R)	:	Prevacid [®] (containing Lansoprazole Delayed Release capsules 30 mg) Distributed by TAP Pharmaceuticals Inc, U.S.A.

Procurement, Storage and Accountability Procedures for Investigational Products**Receipt and storage of investigational products**

Adequate supplies of investigational products, for dose administration and sample retention purposes, were received by the Principal Investigator from the sponsor. The test and reference

formulations were supplied in original market pack or in a sealed pack along with their certificates of analysis (COA) and the details of the product (Product name, Strength, No. of dosage units, Manufacturer, Batch or Lot No., Expiry date and storage condition). After receipt of the investigational products, they were transferred to the pharmacy. The investigational products were stored as per the storage condition supplied along with the investigational products. If sufficient quantity of samples was available they were stored as retention samples at the end of the study. Other wise they would be sent back to sponsor.

Accountability of investigational products

Accountability for the investigational products were documented in the respective "Investigational Product Accountability Record" for the test and reference formulations.

Dispensing

The pharmacy custodian were dispensed a quantity of the test and reference formulations sufficient for dosing for the period as per the randomization schedule and the remaining Investigational Products

(If sufficient quantity available) would be kept in their original containers as retention samples after completion of the project. The dispensed doses were transferred to the dispensing sachets, pre-labeled "For Clinical Research Use Only", and with information about Project No., Batch /Lot No., Subject No., Period, Product type (Test or Reference), Sponsor's Name and Storage condition.

Handling of Unused Samples

The dispensed but un-dosed investigational products were retained along with the remaining Investigational Products (If sufficient quantity available) after completion of the project. Other wise they would be sent back to sponsor.

Maintenance of Randomization Code and Dispensing Record

The randomization code and investigational product dispensing record was kept in the pharmacy under controlled access. The personnel involved in dispensing of investigational products (the dispenser) and the Principal Investigator was accountable for ensuring compliance to the randomization schedule.

Maintenance of study Treatment Randomization Codes

The randomization schedule would be made available to the clinical research physicians or the Sponsor and Independent Ethics Committee in case of any serious adverse event in consultation with the Principal Investigator to ascertain the treatment allocation.

Selection and Withdrawal Of Subjects

All subjects were undergone a screening procedure comprising clinical examination, recording of electrocardiogram and laboratory investigations of blood as well as urine less than 21 days prior to first dosing (Annexure-III). Chest X-Ray (P/A view) were taken not more than 6 months prior to the dosing of first period of the study. An alcohol breath test was performed at check-in of each period for subjects. A urine screen for drugs of abuse was performed before check-in of each period for subjects. The subjects were selected on the basis of the following inclusion and exclusion criteria.

Inclusion Criteria

The subjects were selected for study participation, if they meet all of the following criteria:

- Male subjects aged between 18 and 45 years (including both).
- Subjects with a BMI between 18.5- 24.9 kg/m²
- Subjects with normal health as determined by personal medical history, clinical examination and laboratory examinations including serological tests are within the clinically acceptable normal range.
- Subjects having normal 12-lead electrocardiogram (ECG).
- Subjects having normal chest X-Ray (P/A view).
- Have a negative urine screen for drugs of abuse (including amphetamines, barbiturates, benzodiazepines, tetra hydro cannabinoids, cocaine, and morphine).
- Have negative alcohol breath test.
- Subjects willing to adhere to the protocol requirements and to provide written informed consent.

Exclusion Criteria

The subjects will be excluded from the study, if they meet any of the following criteria:

- Hypersensitivity to lansoprazole or related drugs
- History or presence of significant cardiovascular, pulmonary, hepatic, renal, gastrointestinal, endocrine, immunological, dermatological, neurological or psychiatric disease or disorder
- Any treatment which could bring about induction or inhibition of hepatic microsomal enzyme system within 1 month of starting of study
- History or presence of significant alcoholism or drug abuse in the past one year
- History or presence of significant smoking (more than 10 cigarettes or beedi's/day or consumption of tobacco products)
- History or presence of significant asthma, urticaria or other allergic reactions
- History or presence of significant gastric and/or duodenal ulceration
- History or presence of significant thyroid disease, adrenal dysfunction, organic intracranial lesion such as pituitary tumour
- History or presence of cancer
- Difficulty with donating blood
- Difficulty in swallowing solids like tablets or capsules
- Systolic blood pressure less than 90 mm Hg or more than 140 mm Hg
- Diastolic blood pressure less than 60 mm Hg or more than 90 mm Hg
- Pulse rate less than 60/minute or more than 100/minute
- Oral temperature less than 97.5⁰F or more than 98.9⁰F
- Respiratory rate less than 16/minute or more than 20/minute
- Use of any prescribed medication during last two weeks or OTC medicinal products and grapefruit juice during the last one week prior to initiation of study.
- Major illness during 3 months before screening.
- Participation in a drug research study within past 3 months.
- Donation of blood in the past 3 months before screening.

Withdrawal Criteria

The Principal Investigator may withdraw a subject from the study for any of the following:

- The subject suffers from significant inter-current illness or undergoes surgery during the course of the study.
- The subject is non-cooperative and undisciplined
- The subject found to have entered the study in violation of this protocol.
- If vomiting occurs at any point during the study.
- The subject suffering from any other significant adverse event.
- The subject who requires any concomitant medication, which may interfere with the pharmacokinetic property of the study medication.
- The subject violating any restrictions mentioned in the protocol.
- If it is felt in the investigator's opinion that it is not in the subject's best interest to continue.
- Subject wishes to withdraw consent.

Any subject withdrawal during the study along with the reason thereof would be documented.

Treatment of Subjects**Housing**

Subjects were housed in the clinical facility from not less than 12.0 hours pre-dose to ensure 10 hours fasting prior to high fat breakfast and were leave the facility after 24 hours post-dose sample in each period, if the subjects did not suffer from any adverse event. In case of any adverse event, necessary action would be taken till the event subsides.

Diet and Water

All subjects were instructed to abstain from xanthine containing food or beverages, cigarettes and tobacco products for at least 48 hours prior to dosing and throughout their stay in the facility. All subjects were required to fast (overnight) for at least 10 hours prior to high fat breakfast (as per Annexure VIII). The subjects were receive a standard meal on the day of check-in before dosing and at about 4, 8, 12 and 24 hours after dosing in each period. During housing, the meal menu was identical for all periods. In case, meal and blood sample collection times coincide, samples was given the priority over meal. Drinking water was not be allowed from one hour before and after dosing (except for 240 ± 2 mL of drinking water given for dosing). Before and after that, drinking water was allowed at all times.

Dosing

The subjects were fasted overnight for at least 10 hours prior to high fat breakfast (as per Annexure VIII). Investigational product (allocated as per the randomisation schedule) would be administered orally to each subject exactly within 30 minutes after the scheduled start time of high fat breakfast and the subjects were instructed to swallow it with 240 ± 2 mL of water at ambient temperature in sitting posture. The subjects were instructed not to chew or crush the capsule but to consume as a whole.

Treatment Schedule:**Table No.2- Randomization**

Subject No.	Randomization	Period I	Period II	Period III
1	RT1T2	R	T1	T2
2	T2RT1	T2	R	T1
3	T2RT1	T2	R	T1
4	T1T2R	T1	T2	R
5	RT1T2	R	T1	T2
6	T1T2R	T1	T2	R
7	T1T2R	T1	T2	R
8	RT1T2	R	T1	T2
9	T1T2R	T1	T2	R
10	T2RT1	T2	R	T1
11	T2RT1	T2	R	T1
12	RT1T2	R	T1	T2

Dosing Compliance

Compliance for dosing was assessed by a thorough check of the oral cavity using torch immediately after dosing and sticking the duplicate label of dispensed container on the 'Dosing' section of individual Case Report Form (CRF).

Sampling Schedule

The sampling schedule was planned to provide an adequate estimation of C_{max} and to cover the plasma concentration-time curve long enough to provide a reliable estimate of the extent of absorption. A total of twenty two blood samples were collected from each subject during each period. The pre-dose blood sample of 5 mL (0.00 hr) was collected within one hour prior to the dosing. The post-dose blood samples of 5 mL each was drawn at 0.67, 1.00, 1.33, 1.67, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 5.50, 6.00, 7.00, 8.00, 10.00, 12.00, 14.00, 16.00, 20.00 and 24.00 hours post- dose

Sample Collection Procedure

Samples were collected in dark room using monochromatic light (sodium vapour lamps) through an indwelling cannula placed in a forearm vein using disposable syringe or thorough fresh vein puncture with disposable syringes and needles. The pre-dose blood sample was collected at the time of cannulation; the post-dose in-house samples were collected within ± 2 minutes from the scheduled sampling time. The time of collection of each blood sample (as displayed in the centrally synchronized digital clock) was recorded in hh:mm format in the 'Blood Sample Collection' section of individual CRF at the end of each blood sample collection procedure. The time displayed in hh:mm:ss format of digital clock was rounded to the next minute, if the display in seconds was 30 or above. The deviations greater than mentioned in this protocol from the scheduled sampling time was reported as protocol deviations. In case of protocol deviations of

samples collection, actual time of sample collection was taken into consideration for pharmacokinetic calculations. Intravenous indwelling cannula was kept in situ as long as possible by injecting, 0.5 mL of 5 IU/mL of heparin in normal saline solution to maintain the cannula patent. While sampling through the cannula, blood samples were collected after discarding the first 0.5 mL of heparinised blood from the cannula. If insertion of cannula was not possible or cannula was blocked, alternatively blood samples might be drawn by a fresh venipuncture using a pair of disposable sterile syringe and a needle. The blood samples were collected in pre-labeled (Project No., Subject No., Period, Sampling time point and Sample code) 6mL vacutainers containing K₂EDTA as anticoagulant.

Blood Loss

The total blood loss combining all the periods (including 0.5 mL of discarded heparinised blood prior to each post-dose sample collected through cannula, 10 mL of blood drawn for screening) would not exceed 371.5 mL for each subject.

Restrictions

Medication

Subjects were instructed not to consume any prescribed medications beginning two weeks prior to and no OTC medications beginning one week prior to initiation of study and until after the study is completed. If drug therapy other than that specified in the protocol is required prior to or during the study or in the washout period, decision should be taken by the Principal Investigator whether to continue or discontinue the subject on the basis of the following:

- The pharmacology and pharmacokinetics of the non-study medication.
- The likelihood of drug-drug interaction, thereby affecting pharmacokinetic comparison of the investigational products.
- The time and duration of administration of the non-study medication.
- The clinical judgment about the subject.

Diet and Water

Subjects were fasted overnight from at least 10 hours prior to high fat breakfast (as per Annexure VIII) till about 4 hours after dosing and drinking water will not be allowed from one hour before and after dosing except 240 ± 2mL of dosing water unless clinically indicated.

Sitting Posture

The subject was remain in sitting posture for at least 2 hours after the administration of investigational product unless clinically indicated. Thereafter, the subjects were allowed to engage in normal activities while avoiding severe physical exertion.

Others

Subjects were instructed during screening to refrain from smoking, chewing tobacco, pan or pan masala, gutkha, masala (containing beetle nut and tobacco) and from consuming any alcoholic products, xanthine-containing foods or beverages and fruit juice for 48 hours prior to dosing till the completion of study. They would not be allowed to smoke, chew tobacco, pan or pan masala, gutkha, masala (containing tobacco and supari (beetle nut) and to have any xanthine-containing

food and/or beverages (like chocolate, tea, coffee or cola drinks) or fruit juice from check-in till checkout in each period.

Assessment of Efficacy

Being a comparative bioavailability study, the pharmacokinetic parameters C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, T_{max} , $t_{1/2}$ and K_{el} and residual area of the test and reference formulations were assessed for efficacy.

Assessment of Safety

Eligibility Assessments

The eligibility assessments were conducted before the entry of the subjects into the study as per selection and withdrawal criteria of the subjects (as per section no.: 10.0). Clinical laboratory tests mentioned below are done and if all of these parameters are within normal reference range, along with satisfactory selection criteria, volunteers were eligible for participating in the study.

Table No.3-Blood Test

Blood tests:		Urine analysis:
Hematology:	Biochemistry:	
Haemoglobin RBC WBC and Platelet count Differential count Peripheral smear	Random Blood Glucose Blood urea Serum creatinine Serum sodium and potassium Serum uric acid Serum amylase Serum total cholesterol Serum triglycerides	pH Specific gravity Protein Glucose Ketones Bilirubin Urobilinogen Blood Nitrites Microscopic examination
Serology:	<u>Liver Function Tests:</u>	
HIV(1&2) antibodies HBsA (Hepatitis B surface antigen) HCV antibodies VDRL	Total bilirubin Direct bilirubin SGOT (AST) SGPT (ALT) Serum alkaline phosphatase Total protein Serum albumin	

Recording of Vital Signs and Clinical Examination

Clinical Examination along with vital signs (sitting blood pressure, radial pulse rate, respiratory rate and oral temperature) measurement was carried out and recorded at check-in, before dosing of Investigational product (in the morning of the day of dosing) and at checkout and/or at the termination of the study. Vital signs (sitting blood pressure and radial pulse rate) will be measured at 1.00, 2.00, 3.00 and 4.00 hours after dosing in each period (Within \pm 40 minutes variation of scheduled time). Clinical examination and measurement of vital signs may also be carried out at any time during the conduct of the study, if the clinical research physician feels it necessary. Subjects were questioned for well being at the time of clinical examinations and

recording of vital signs. In case of abnormality during pre-dose vital signs recording, medical opinion was taken whether to dose the subject or not.

Handling and Reporting of Adverse Events and Serious Adverse Events

An adverse event (AE) is any untoward medical occurrence or clinical investigation in a subject after administration of a pharmaceutical product and that does not necessarily have a causal relationship with the administered product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not causally related to the medicinal (investigational) product.

Adverse Drug Reaction (ADR): All noxious and unintended responses to a medical product related to any dose should be considered adverse drug reactions.

Unexpected Adverse Drug Reaction: An adverse reaction, the nature or severity of which is not consistent with the applicable product information.

Serious Adverse Event (SAE): A serious adverse event (experience) or reaction is any medical occurrence that at any dose:

- Results in death,
- Is life-threatening,
- Requires in-patient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.

The following information has to be recorded for each adverse event individually in Adverse Event Reporting Form:

- Type of adverse event
- Is it serious or non-serious?
- Date and time of onset/reporting
- Date and time of resolution
- Severity (mild, moderate or severe)
- Association with the study medication (unassessable, conditional, unlikely possible, probable or certain)
- Action taken
- Outcome of adverse event (resolved or unresolved)
- Further, details of the AE, if any

The causality assessment to the study treatment is characterized as:

Table No.4- Treatment Characterization

Causality term	Assessment criteria
Certain	<ul style="list-style-type: none"> • Event or laboratory test abnormality, with plausible time relationship to drug intake • Cannot be explained by disease or other drugs • Response to withdrawal plausible (pharmacologically, pathologically) • Event definitive pharmacologically or phenomenological (i.e. an objective and specific medical disorder or a recognized pharmacological phenomenon) • Rechallenge satisfactory, if necessary

Probable / Likely	<ul style="list-style-type: none"> • Event or laboratory test abnormality, with reasonable time relationship to drug intake • Unlikely to be attributed to disease or other drugs • Response to withdrawal clinically reasonable • Rechallenge not required
Possible	<ul style="list-style-type: none"> • Event or laboratory test abnormality, with reasonable time relationship to drug intake • Could also be explained by disease or other drugs • Information on drug withdrawal may be lacking or unclear
Unlikely	<ul style="list-style-type: none"> • Event or laboratory test abnormality, with a time to drug intake that makes a relationship improbable (but not impossible) • Disease or other drugs provide plausible explanations
Conditional / Unclassified	<ul style="list-style-type: none"> • Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable/ Unclassifiable	<ul style="list-style-type: none"> • Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified

Intensity of adverse events would be assessed as following:

- Mild: An adverse event, usually transient in nature and generally not interfering with normal activities.
- Moderate: An adverse event, which is sufficiently discomforting to interfere with normal activities.
- Severe: An adverse event, which is in incapacitating and prevents normal activities.

Subjects were monitored throughout the study period for adverse events. Subjects were instructed to bring to the notice of any study personnel of any adverse event that may occur during their stay at the clinical facility. Subjects were also be specifically asked about any adverse events throughout the study period during the recording of vital signs or clinical examination. A medically qualified designate will be available round-the-clock during the period of housing at the clinical facility. All AEs including both observed and volunteered ones was recorded on the appropriate CRF, irrespective of its association with the investigational products. The Independent Ethics Committee (IEC) will be informed regarding the AE as necessary. Any SAE was reported to Secretary/Chairman, IEC /IRB within 24 hours from the time the SAE is identified, either by telephone/telephonic facsimile transmission or by e-mail and a detailed report is sent within 07 days or next meeting (which ever comes first), followed by regular updates. Each AE was evaluated for duration, severity and action taken, outcome and association with the investigational product. The study might be suspended or terminated depending upon the seriousness of the AEs. According to Schedule-Y: In case of Unexpected SAE's, the Sponsor would inform (e.g. by telephone, facsimile transmission or by e-mail) the Drugs Controller General of India (DCGI) as soon as possible but not later than 14 calendar days after first knowledge of SAE. According to ICH E2A guidelines: In case of Unexpected SAE's that are fatal or life-threatening, Regulatory agencies should be notified (e.g., by telephone, facsimile transmission, or in writing) as soon as possible but no later than 7 calendar days after first knowledge by the sponsor that a case qualifies, followed by as complete a report as possible within 8 additional calendar days. Unexpected SAE's that are not fatal or life-threatening must

be filed as soon as possible but no later than 15 calendar days after first knowledge by the sponsor that the case meets the minimum criteria for expedited reporting.

Follow-up

Subjects were instructed to report at the clinical facility for any adverse events during the washout between all the periods. All the adverse events were treated by the clinical research physician at the clinical facility or in a nearby hospital (Noble Hospital, Ruby Hospital). All adverse events were followed up wherever possible to resolution or until the Medical Officer/Physician believes that there will be no further change. This may involve additional visits.

Sample Processing and Transfer Procedures

After collection of blood sample from each subject of that particular time point, the samples were centrifuged at 3000 rpm for 10 minutes at 4⁰C. After centrifugation the plasma samples were separated into two aliquots of 950µl each and transferred into respective pre-labeled (Project No., Subject No., Period, Sampling time point and Sample code) ria vials containing 50 µl of 0.5 M Sodium Carbonate. Samples were processed in dark room using monochromatic light (sodium vapour lamps). Ria vials were vortexed for proper mixing and would be stored at -40°C± 10°C for a maximum period of 12 hours and then they were stored at - 55°C until analysis.

Ethics

Independent Ethics Committee

This protocol and corresponding informed consent form (ICF) (containing information about the study to be given to the subjects) to be used to obtain written informed consent of study subjects will be reviewed by the IEC and subjects will not be enrolled into the study until the IEC approves the protocol and the ICF.

The study was conducted as per the ICMR Guidelines for Biomedical Research on Human Subjects, ICH-GCP Guidelines and in accordance with the Declaration of Helsinki.

Written Informed Consent

The Principal Investigator or designated study personnel was inform the subjects (in English and / or Marathi language understandable by the subject) before initiation of study through an oral presentation regarding the purpose, procedures to be carried out, investigational products, potential hazards and rights of the study subjects. The subjects were required to understand and sign the ICF prior to check-in for the study in the first period and the signed ICF was filed in the respective study file.

Subject Participation Fee

The subjects were paid an adequate (IEC approved) participation fee on account of their participation in the study. In case of dropout / withdrawal of a subject before completion of the study, the subjects were paid pro-rated participation fees depending upon the extent of participation and any controversy pertaining to this was forwarded to the IEC and the decision of the IEC would be final as well as binding on both the subjects and Synapse Labs Pvt. Ltd

Data Handling and Record Keeping

All clinical data generated during the conduct of the study was directly entered in the respective CRFs. The computer-generated randomization schedule was also be treated as raw data. All raw data and transcribed data forms compiled by the study personnel assisting in the study were checked for completeness. All data related to the project was in the custody of the Principal Investigator or Project In charge until transferred to archives.

Archiving

All raw data generated in connection with this study, together with a copy of this protocol, signed ICFs and the final report was archived according to the ICH guidelines for good clinical practice.

RESULTS AND DISCUSSION**Statistics Theory**

After the completion of the bioanalytical phase data was sent to the statistical department and it has been processed further for obtaining the results. ANOVA was performed on log transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf}. To conclude Bioequivalence, two one sided 90% confidence intervals were calculated for test by reference ratio of geometric least square mean of C_{max}, AUC_{0-t} and AUC_{0-inf}. T_{max} was evaluated by nonparametric Wilcoxon test. All pharmacokinetics and statistical analysis was performed by SAS® 9.1. Total 12 subjects completed the both periods of study successfully. In statistical analysis of Lansoprazole, there was no significant sequence and treatment effect for Log transformed Pharmacokinetic parameters AUC_{0-t} and AUC_{0-inf}. Significant period effect for Log transformed Pharmacokinetic parameters AUC_{0-t} and AUC_{0-inf} was observed. During the study, clinical conditions were kept equivalent in both the periods of the study. Also no pre-dose concentrations were observed. Since the period effect was not coupled with the sequence effect and had no impact on the power; it appears to be insignificant in nature and the decision of equivalence is based on the 90% confidence interval by Schuirmann's two one sided 't' test and the 90 % CI is within the acceptance criteria i.e., 80 % to 125 %. Ratios for Geometric Least Square Means should be lies within the acceptance criteria of 80-125% for Log transformed C_{max}, AUC_{0-t} and AUC_{0-inf}. 90 % Confidence Interval of primary efficacy variables should lie between the acceptance ranges 80-125% for Log transformed C_{max}, AUC_{0-t} and AUC_{0-inf} of Lansoprazole. Efficacy Results: The 90 % confidence intervals of Lansoprazole Log-transformed parameters are summarized below:

Table No.5-90 % Confidence Interval for ratio of Geometric Means of Test A and Reference C

Parameters	*Geometric mean		% Ratio	90 % Confidence Interval for Log-transformed data	
	Test (A)	Reference (C)		A/C	Lower Limit
AUC _{0-inf}	3362.39	3198.99	105.11	94.87	116.45
AUC _{0-t}	3333.98	3162.40	105.43	95.35	116.56
C _{max}	1530.48	1380.50	110.86	101.53	121.06

Table No.6-90 % Confidence Interval for ratio of Geometric Means of Test B and Reference C

Parameters	*Geometric mean		% Ratio	90 % Confidence Interval for Log-transformed data	
	Test (B)	Reference (C)		B/C	Lower Limit
AUC _{0-inf}	3523.16	3198.99	110.13	99.41	122.02
AUC _{0-t}	3484.61	3162.40	110.19	99.66	121.83
C _{max}	1590.40	1380.50	115.20	105.50	123.80

Fig.No.16- Mean graph of Lansoprazole for Test products (A and B) Vs Reference product C For Un-transformed data

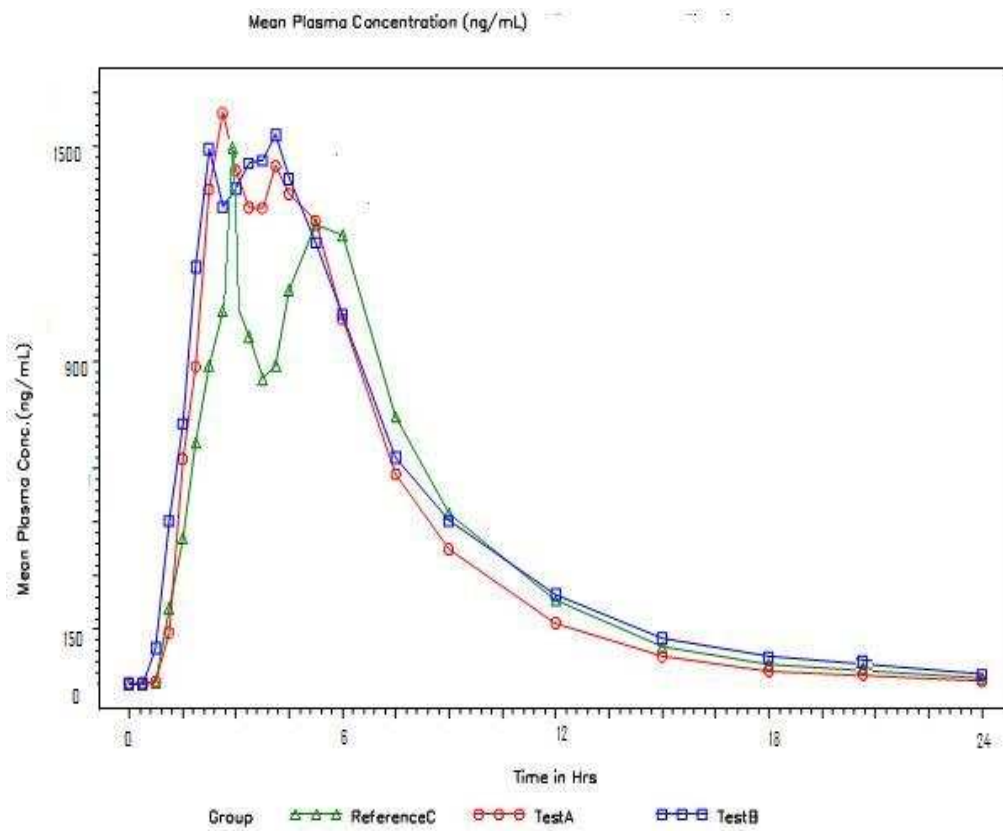
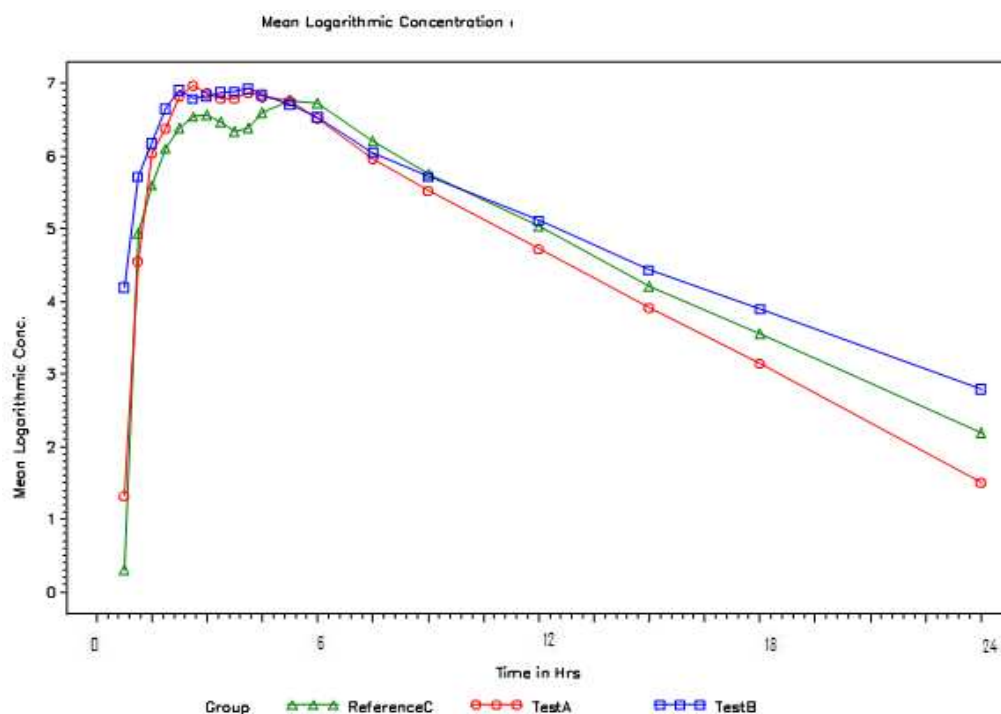


Fig.No.17- Mean graph of Lansoprazole for Test products (A and B) vs Reference product C For Log-transformed data

In statistical analysis of Lansoprazole, there was no significant sequence and treatment effect for Log transformed Pharmacokinetic parameters AUC_{0-t} and AUC_{0-inf}. Significant period effect for Log transformed Pharmacokinetic parameters AUC_{0-t} and AUC_{0-inf} was observed. During the study, clinical conditions were kept equivalent in both the periods of the study. Also no pre-dose concentrations were observed. Since the period effect was not coupled with the sequence effect and had no impact on the power; it appears to be insignificant in nature and the decision of equivalence is based on the 90% confidence interval by Schuirmann's two one sided 't' test and the 90% CI is within the acceptance criteria i.e., 80% to 125%. The geometric least square means for log-transformed C_{max} were 1543.48 ng/mL for Test Product A and 1380.50 ng/mL for Reference Product C. The ratio estimate of Test and Reference Products was 110.86%. The 90% confidence interval for log-transformed data for C_{max} (as a measure of rate of absorption) of Test Product compared to that of the Reference Product was 101.53- 121.08%. The geometric least square means for log-transformed C_{max} were 1590.40 ng/mL for Test Product B and 1380.50 ng/mL for Reference Product C. The ratio estimate of Test and Reference Products was 110.20%. The 90% confidence interval for log-transformed data for C_{max} (as a measure of rate of absorption) of Test Product compared to that of the Reference Product was 105.50 – 123.80%. The geometric least square means for log-transformed AUC_{0-t} were 3333.98 ng*hr/mL for Test Product A and 1162.40 ng*hr/mL for Reference Product C. The ratio estimate of Test and Reference Products was 105.43%. The 90% confidence interval for log-transformed data for AUC_{0-t} (as a measure of extent of absorption) of Test Product compared to that of the Reference Product was 95.35 – 116.56%. The geometric least square means for log-transformed AUC_{0-t} were 3484.61 ng*hr/mL for Test Product B and 3162.40 ng*hr/mL for Reference Product C.

The ratio estimate of Test and Reference Products was 110.19%. The 90% confidence interval for log-transformed data for AUC_{0-t} (as a measure of extent of absorption) of Test Product compared to that of the Reference Product was 99.66- 121.83%. The geometric least square means for log-transformed AUC_{0-inf} were 3362.39 ng*hr/mL for Test Product A and 3198.99 ng*hr/mL for Reference Product C. The ratio estimate of Test and Reference Products was 105.11 %. The 90% confidence interval for log-transformed data for AUC_{0-inf} (as a measure of extent of absorption) of Test Product compared to that of the Reference Product was 94.87- 116.43%. The geometric least square means for log-transformed AUC_{0-inf} were 3523.16ng*hr/mL for Test Product B and 3198.99 ng*hr/mL for Reference Product C. The ratio estimate of Test and Reference Products was 110.13 %. The 90% confidence interval for log-transformed data for AUC_{0-inf} (as a measure of extent of absorption) of Test Product compared to that of the Reference Product was 99.47- 122.02%.

CONCLUSION

Safety conclusions

As far as the study was concerned, the drug was well tolerated upon single-dose administration to healthy, adult, male, human subjects.

Adverse Events

There were no adverse events during the study. There were no deaths and other serious adverse events reported during the study. Vital signs, physical findings and other observations related to safety. Sitting blood pressure, radial pulse rate was measured and recorded at check in, before dosing, at 1, 2, 3, 4, 12 hours post dosing, & at check out.

Tests for consumption of drugs of abuse and sample for alcohol consumption were done at the check in. The Physician did clinical examination of the subjects at the time of check in and check out. After dosing, adverse event monitoring was done throughout the study. Blood samples of about 5 ml each were collected from all the study subjects who participated in the study for post study safety assessment at the end of the study. Values for the laboratory parameters tested were found clinically non-significant for all the subjects. All the above subjects were examined by the doctor on duty at the time of check-out from clinical facility and were found clinically asymptomatic.

Clinical laboratory evaluation

Clinical laboratory evaluation was carried out at screening and found within normal limits. Post study safety evaluation of each of the subjects dosed was carried out at the end of the study and found within normal limits.

Pharmacokinetic conclusions

The confidence interval of C_{max}, AUC_{0-t}, and AUC_{0-inf} of Ramipril was within the bioequivalence acceptance limits of 80 -125%. Hence the Test Product is bioequivalent to Reference Product.

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