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Bioequivalence study of Ramipril tablets in healthy adult male human subjects under fed condition

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Abstract

Bioequivalence studies compare both the rate and extent of absorption of various multisource drug formulations with the innovator (reference) product, on the basis that if two formulations exhibit similar drug concentration-time profiles in the blood/plasma, they should exhibit similar therapeutic effects. Open label, balanced, randomized, single-dose, two-treatment, two-sequence, two-period crossover oral bioequivalence study of Ramipril 5 Mg Tablets supplied by laboratory comparing with that of Tritace® (containing Ramipril 5 Mg) of Sanofi-Aventis Australia pvt Ltd. Australia in healthy, adult , male, human subjects under fed conditions. To monitor the safety and tolerability of a single dose of the test product as compared to the reference product in healthy adult male human subjects under fed condition. In the following sections, requirements for the design and conduct of comparative bioavailability studies are formulated. Investigator(s) should have appropriate expertise, qualifications and competence to undertake a proposed study and is familiar with pharmacokinetic theories underlying bioavailability studies. The design should be based on a reasonable knowledge of the pharmacodynamics and/or the pharmacokinetics of the active substance in question The aim of a bioequivalence study is to demonstrate equivalence within the acceptance range regarded as clinically relevant. The primary concern in bioequivalence assessment is to limit the risk of erroneously accepting bioequivalence which should not exceed the nominal risk of 5%, and to try to minimize the risk of erroneously rejecting bioequivalence.

Keywords: Bioequivalence study, Ramipril, Bioavailability.

Introduction

The protocol should also specify methods for handling drop-outs and for identifying biologically implausible outliers. *Post hoc* exclusion of outliers is not generally accepted. If modeling assumptions made in the protocol (e.g. for extrapolating AUC to infinity) turn out to be invalid, a revised analysis in addition to the planned analysis (if this is feasible) should be presented and

discussed. To date, most bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Therefore, no specific recommendation is given on this matter. However, studies with replicate design may be helpful for substance with highly variable absorption. The results of *in vitro* dissolution tests, obtained with the batches of test and reference products that were used in the bioavailability or bioequivalence study should be reported. These results should be reported as profiles of amount dissolved versus time for individual dosage units. The specifications for the *in vitro* dissolution of the product should be derived from the dissolution profile of the batch that was found to be bioequivalent to the reference product and would be expected to be similar to those of the reference product. For immediate release products, if the dissolution profile of the test product is dissimilar compared to that of the reference product and the in vivo data remain acceptable, the dissolution test method should be re-evaluated and optimized. In case that no discrimatory test method can be developed this reflects in vivo bioequivalence a different dissolution specification for the test product could be set. The report of a bioavailability or bioequivalence study should give the complete documentation of its protocol, conduct and evaluation complying with GCP-rules. This implies that the authenticity of the whole of the report is attested by the signature of the study monitor. The responsible investigator(s) should sign for their respective sections of the report. Names and affiliations of the responsible investigator (s), site of the study and period of its execution should be stated. The names and batch numbers of the products used in the study as well as the composition(s) of the test product(s) should be given. In addition the applicant may submit a signed statement, confirming the test product is the same as the one which is submitted for marketing authorization. All results should be clearly presented and should include data from subjects who eventually drop-out. Drop-out subjects and withdrawals should be fully documented and accounted for. The method used to derive the pharmacokinetic parameters from the raw data should be specified. The data used to estimate AUC should be reported. If pharmacokinetic models are used to evaluate the parameters the model and computing procedure used should be justified. Deletion of data should be justified. All individual subject data should be given and individual plasma concentration/time curves presented on linear/linear, and log/linear scale. The analytical report should include the results for all standard and quality control samples as well. A representative number of chromatograms or other raw data should be included covering the whole concentration range for all, standard and quality control samples as well as the specimens analyzed. The analytical validation report should be submitted as well. The statistical report should be sufficiently detailed to enable the statistical analyses to be repeated.To date, most bioequivalence studies are designed to evaluate average bioequivalence. Experience with population and individual bioequivalence studies is limited. Therefore, no specific recommendation is given on this matter. However, studies with replicate design may be helpful for substance with highly variable absorption. The fed study is to be designed in such a way that the effects of formulation can be distinguished from other factors. If two formulations are being compared, a randomized two-period, two-sequence crossover study is commonly considered the design of choice. An adequate washout period between periods is needed to avoid drug carryover effects. Replicate studies, although not mandated, offer the advantage of providing a comparison of intra-subject variances for the test and reference products. All facets of the study are to be tightly controlled. The full characteristics, including lot numbers and expiry dates, of the test and reference products shall be known. Normally, subjects fast for 10 hours prior to ingesting a standardized meal. The meal is to provide the greatest changes from the gastrointestinal physiology of a fasting state. A meal with high-fat and high-calorie content is recommended (e.g.

150, 250 and 500-600 calories from protein, carbohydrate, and fat, respectively). The meal shall be ingested over a period of 30 minutes or less. The product dose shall be ingested 30 minutes after start of the meal. Generally, the highest safe strength/dose of the test or reference product will be administered with about 8 ounces (240 mL) of water. Further fluid shall be withheld for about 2 hours; standardized meals will be permitted beginning at four hours after drug administration. All subsequent meals will be carefully standardized. For most drugs, subjects shall not be allowed to recline until at least two hours after product ingestion. Physical activity and posture shall be standardized to limit effects on gastrointestinal blood flow and motility. Blood samples (about 12 to 18, including a pre-dose sample) are to be drawn at appropriate, specified, and carefully recorded times (to capture increasing and decreasing concentrations during the absorption, distribution and elimination phases). The collections shall continue for about three terminal drug half-lives in order to capture at least 80% of the total area. At least three to four samples shall be obtained from the terminal log-linear phase to derive an acceptable estimate of the terminal constant (λz) from linear regression. For long half-life drugs, a truncated AUC (e.g. up to 72 hours) is generally considered adequate. Blood samples or the harvested plasma/serum are to be analyzed for the administered drug or metabolites by means of a validated analytical method. Ramipril 5 mg Tablets and Ramipril 10 mg Tablets contain, respectively, 5 mg and 10 mg of the ACE inhibitor ramipril. The tablets are indicated for the treatment of mild to moderate hypertension; congestive heart failure as adjunctive therapy to diuretics (with or without cardiac glycosides); and to reduce mortality when given to patients surviving acute myocardial infarction with clinical evidence of heart failure. This drug inhibit the Angiotensin converting enzyme (ACE) which hydrolyzes the inactive angiotensin I to active angiotensin II thus inhibits the formation of angiotensin II and decreases of angiotensin mediated secretion of aldosterone from the adrenal cortex.

Materials and Methods

Objective and Purpose

To compare and evaluate the single-dose oral bioequivalence study of Ramipril 5 Mg Tablets supplied by laboratory comparing with that of Tritace® (containing Ramipril 5 Mg) of Sanofi-Aventis Australia pvt Ltd. Australia in healthy, adult , male, human subjects under fed conditions.

Study design

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

Number of Subjects

10 healthy, adult, male, human subjects were enrolled in the study. Being a pilot study, since no definite statistically valid conclusion on bioequivalence is sought, 10 subjects were 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. (Randomization was done in blocks using PROC PLAN such that the design was balanced. The

order of receiving the reference and test formulations for each subject during both the periods of the study was determined according to randomization schedule.

Blinding

The study was comprised of a randomized open label design; as it was needless to design double-blind study for a bioavailability and bioequivalence study. However, analysts were blinded to the sequence of administration of test and reference formulations.

Duration of Study

Subjects went for a screening procedure not earlier than 21 days before the first day of dosing. Total expected duration of the study was of at least 29 days from the day of check-in of the first period till the end of the second 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 was 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 ramipril was about 13-17 hours.The washout period was a minimum of 10 half-lives of the administered drug. A washout period of at least 21 days was 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.

Investigational products

Test Product (T/A)	:	Ramipril 5 Mg Tablets supplied by laboratory
Reference Product (R/B)	:	Tritace® (containing Ramipril 5 Mg) of Sanofi-Aventis Australia pvt Ltd. Australia

Procurement, Storage and Accountability Procedures for Investigational Products

Receipt and storage of investigational products

Adequate supplies of investigational products, for the dose administration and the sample retention purposes, were received by the Principal Investigator from the sponsor. The test and reference formulations were supplied in original market packs or in a sealed packs 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 the 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. Sufficient quantity of the samples were stored as retention samples at the end of the study.

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 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 were 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 after completion of the project.

Maintenance of Randomization Code and Dispensing Record

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

Treatment Schedule:

Table No.1: Randomisation Schedule

Subject No.	Randomization	Period I	Period II
1	RT	R	T
2	TR	T	R
3	RT	R	T
4	TR	T	R
5	TR	T	R
6	RT	R	T
7	RT	R	T
8	TR	T	R
9	TR	T	R
10	RT	R	T

Maintenance of study Treatment Randomization Codes

The randomization schedule was 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 the subjects underwent a screening procedure comprising of 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) was 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 the subjects. A urine screen for the 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 met all of the following criteria:

- Male subjects aged between 18 and 55 years (including both).
- Subjects with a BMI between 18.5- 24.9 kg/m^2
- 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).
- Had a negative urine screen for drugs of abuse (including amphetamines, barbiturates, benzodiazepines, marijuana, cocaine, and morphine).
- Had negative alcohol breath test.
- Subjects willing to adhere to the protocol requirements and to provide written informed consent.
-

Exclusion Criteria
The subjects were excluded from the study, if they met any of the following criteria:

- Hypersensitivity to ramipril 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 97.5^0F or more than 98.9^0F
- 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 withdrew a subject from the study for any of the following:

- The subject suffered from significant inter-current illness or undergoes surgery during the course of the study.
- The subject was non-cooperative and indisciplined
- The subject was found to have entered the study in violation of this protocol.
- If vomiting occured at or before 2 times median t_{max}
- The subject was suffering from any other significant adverse event.
- The subject who required any concomitant medication, which may interfere with the pharmacokinetic property of the study medication.
- The subject was violating any restrictions mentioned in the protocol.
- If it was felt in the investigator's opinion that it is not in the subject's best interest to continue.
- Subject wished to withdraw consent.

Any subject withdrawal during the study along with the reason there of was documented.

Treatment of subjects:

Housing

Subjects were housed in the clinical facility from not less than 10.5 hours pre-dose to ensure 10 hours fasting prior to high fat breakfast and were allowed to 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 was taken till the event subsides.

Diet and Water

All the 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 the subjects were required to undergo fast (overnight) for at least 10 hours prior to high fat breakfast. The subjects received a standard meal 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, the samples were given priority over meal. Drinking water was not be allowed from one hour before and after post-dose (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. The Investigational product (allocated as per the randomization schedule) was administered orally to each subject exactly with in 30 minutes from the scheduled start time of high fat breakfast and the subject was instructed to swallow it with 240 ± 2 mL of water at ambient temperature in sitting posture. The subject was instructed not to chew or crush the tablet but to consume as a whole.

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 (0.0 hr) was collected within one hour prior to the dosing. The post-dose blood samples of 5 mL each was drawn at 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 3.50, 4.00, 5.00, 6.00, 8.00, 12.00, 24.00, 48.00, 72.00, 96.00, 120.00 and 144.00 hours post dose. The samples at 48.00, 72.00, 96.00, 120.00 and 144.00 hours post dose was given on separate visits (i.e. Ambulatory samples).

Sample Collection Procedure

Samples were collected 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 ambulatory samples were collected within ± 2 hours 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 is 31 or above. The deviations greater than mentioned in this protocol from the scheduled sampling time was reported as protocol deviations. 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 the insertion of the cannula was not possible or cannula was blocked, alternatively blood samples were 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 K_2EDTA 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) did not exceed 246 mL for each subject.

Restrictions:

Medication

The 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 was completed. If the drug therapy other than that specified in the protocol was required prior to or during the study or in the washout period, decision was 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

The subjects were fasted overnight from at least 10 hours prior to high fat breakfast till about 4 hours after dosing and drinking water was not allowed from one hour before and after dosing except 240 ± 2mL of dosing water unless clinically indicated.

Sitting Posture

The subject remained 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

The 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 were not 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 were done and if all of these parameters were within normal reference range, along with satisfacirory selection criteria, volunteers were eligible for participating in the study

Table No.2-Blood Test

Blood tests:		
Hematology:	Biochemistry:	Urine analysis:
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
Serology:	Liver Function Tests:	Microscopic examination
HIV(1 & 2) antibodies HBsAg (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 were 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) were measured at 1.00, 2.00, 3.00, 4.00 and 12.00 hours after dosing in each period(Within ± 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 felt 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 was considered as 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 was recorded for each adverse event individually in Adverse Event Reporting Form:

- Type of adverse event
- was 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.3- Treatment Characterization

Causality term	Assessment criteria
Certain	• 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	• 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	• 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	• 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	• Event or laboratory test abnormality • More data for proper assessment needed, or • Additional data under examination
Unassessable/ Unclassifiable	• Report suggesting an adverse reaction • Cannot be judged because information is insufficient or contradictory • Data cannot be supplemented or verified

Intensity of adverse events was 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 specifically asked about any adverse event throughout the study period during the recording of vital signs or clinical examination. A medically qualified designate was available round-the-clock during the period of housing at the clinical facility.

All AEs including both observed and volunteered ones were recorded on the appropriate CRF, irrespective of their association with the investigational products. The Independent Ethics Committee (IEC) was 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 was 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 was suspended or terminated depending upon the seriousness of the AEs.

According to Schedule-Y: In case of Unexpected SAE's, the Sponsor was informed (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 were fatal or life-threatening, Regulatory agencies were 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 met the minimum criteria for expedited reporting.

Follow-up

The subjects were instructed to report at the clinical facility for any adverse events during the washout between the both 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 the adverse events were followed up wherever possible to resolution or until the Clinical Research Physician believed that there was no further change. This involved additional visits.

Sample processing and transfer procedures:

After the collection of blood sample from each subject of that particular time point, the samples were centrifuged at 3000 rpm for 10 minutes at 4^0C. After centrifugation the plasma samples were separated into two respective pre-labeled (Project No., Subject No., Period, Sampling time point and Sample code) ria vials. Ria vials were stored at -40ºC± 10ºC for a maximum period of 12 hours and then they were stored at - 80ºC± 20ºC to bioanalytical department.

Safety data

All subjects who had received at least one dose of study medication was included in the safety evaluation.

Result obtained when evaluating safety and tolerability (adverse events, vital signs) was listed in the report.

Amendment to the protocol

Any significant change in the study procedure or study design was only in effect upon the mutual agreement with the Sponsor, and after obtaining the approval or a favorable opinion from the Independent Ethics Committee (IEC). All such changes were documented in the amended version of the protocol and a list of changes with reference to the previous version was generated.

Source data accessibility:

The Quality Assurance (QA) auditors of Synapse Labs Pvt. Ltd. as well as sponsor's monitors, IEC and Regulatory agency (ies) was have the access to the raw data during inspection and audits.

Quality control and quality assurance audits

The raw data generated during the course of the study as well as reports underwent a thorough quality control check and random quality assurance process for conformance to this protocol and all the governing SOPs by auditors from the Internal Quality Monitors and Internal Quality Assurance department of Synapse Labs Pvt. Ltd. The final report contained a statement for quality assurance duly signed by the Head, Quality Assurance department.

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 were reviewed by the IEC and subjects were not be enrolled into the study until the IEC approved 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 informed the subjects (in English and/or Marathi language understandable by the subject) before initiation of the 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 subject was 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 was 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 randomisation schedule was also be treated as raw data. All the raw data and the transcribed data forms compiled by the study personnel assisting in the study were checked for completeness. All datas related to the project were in the custody of the Principal Investigator or Project Incharge until transferred to archives.

Table No. 4: Subject Participaton Fee

Sr. No.	Reasons of withdrawal from the study	**Compensation**
1.	Principal Investigator / Medical Officer withdraw the subjects from the study based on medical decision.	Full payment
2.	After the initiation of the study, subject withdrew on his own free will	50% proportionate participation dues
3.	The subject withdrew from the study on humanitarian grounds, with the permission of the Principal Investigator / Medical Officer.	100% proportionate participation dues
4.	Subject was dropped from the study due to violation of requirements of the study by the Principal Investigator / Medical Officer after signing the Informed Consent Form but before receiving any medications	No payment
5.	Subject was withdrawn from the study by the Principal Investigator / Medical Officer because of willful misinformation on present and /or past medical illness/history.	No payment

Archiving

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

Insurance policy

Synapse Labs Pvt. Ltd. had an insurance policy to cover the risks to the subjects and/or any other eventualities pertaining to the study.

Confidentiality of data

The data identifying each subject by name was kept confidential and was accessible only to the study personnel and if necessary, to the QA auditors, IEC, Sponsor representative and Regulatory agency (ies).

Bioanalytics and data processing:

Bioanalytical Methodology:

Validated LCMS/MS method was employed for the estimation of Ramipril and Ramiprilate in plasma during estimation of Ramipril and Ramiprilate quality control samples were distributed throughout each batch of study samples.

All the samples from the same subject were analyzed with the same standard curve. Samples with drug concentration greater than the upper limit of the validated range of the analysis were diluted with the appropriate drug free biological fluid and reanalyzed as per the method validation report.

Pharmacokinetic Analysis:

Pharmacokinetic parameters of Ramipril and Ramiprilate were calculated using the SAS® system version 9.1.3.

Statistical analysis:
Statistical analysis was performed on plasma Ramipril and Ramiprilate using the SAS® system version 9.1.3. The analysis included data from subjects 1 to 10. Time point deviations (more than 2 minutes) were incorporated while PK calculations. Samples, which was below the lower limit of quantification (LLOQ), was set to zero for all pharmacokinetic and statistical evaluation and reported as below limit of quantification (BLQ).

Summary Statistics:
The summary statistics (for relevant pharmacokinetic parameters) were reported for both the test and reference products. The reported parameters were the arithmetic means, geometric means, standard deviations and the coefficient of variation for untransformed data and the arithmetic means and the coefficient of variation for the log-transformed (natural) data. The ratio for both the products averages for the relevant pharmacokinetic parameters was also reported.

Analysis of Variance (ANOVA):
The log-transformed pharmacokinetic parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$) were analysed using an ANOVA model with the main effects of sequence, subject nested within sequence, period and 'treatment'. A separate ANOVA model was used to analyze each of the parameters.

A 5% level of significance was used for with-in subject comparison (i.e., period, 'treatment') and between-subject comparison (i.e., sequence). Each analysis of variance was include calculation of mean square error, coefficient of variance and the associated degree of freedom.

90% Confidence Intervals:
A 90% confidence interval for the ratio of both the products averages (geometric means) was calculated by first calculating the 90% confidence interval for the differences in the averages (least square means) of the log-transformed data and then taking the antilogarithms of the obtained confidence limits.

Intra-Subject Variability:
The intra-subject variability for each of the pharmacokinetic parameters reflected the residual variability after accounting for the difference between subjects, periods and 'treatments' and were reported in terms of the overall coefficient of variation (C.V.%), from the ANOVA results using log-transformed data.

Bioequivalence:
The 90 % confidence interval for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ of Ramipril formed the basis for concluding the equivalence of Ramipril in product R and T. The point estimate of the ratio and the confidence intervals were entirely included in the range of 80 – 125 % for AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} log-transformed data.

Acceptance Criteria for Bioequivalence:
The geometric least square mean ratios and the 90% CI of C_{max}, AUC_{0-t}, AUC_{0-inf} for reference and test formulations were considered for evaluating bioequivalence. To be considered bioequivalent ratios & CI was lie between following acceptance range. If the point estimate of the ratio and the confidence intervals were entirely included within the range of 80-125% for

log-transformed Cmax, AUC0-t and AUC0-∞ for Test Drug then the 'test' formulation were considered bioequivalent to the 'reference' formulation.

Table No.5: Acceptance range of Log Transformed Data

Pharmacokinetic Parameters	Acceptance Range of log transformed data
	For 90 % CI (%)
C_{max}	80-125
AUC_{0-t}	80-125
AUC_{0-inf}	80-125

Results and Discussion

A total of 10 adult, healthy, human male subjects were enrolled in the study. A total of 10 subjects completed the study successfully and the data of these 10 subjects were considered to draw statistical conclusions. Pharmacokinetic and statistical analysis was done on 10 subjects.The mean $AUC0_{-t}/AUC_{0-inf}$ ratio for the Test and Reference Products were 97.25% and 97.31% respectivelyRamiprilate was detected in plasma at 1.00 hr. for the Test and 0.00 hr. for the Reference Product. The mean AUC0-t/AUC0-inf ratio for the Test and Reference Products were 97.17% and 97.19% respectivelyIn analysis of Ramipril , there was no significant sequence effect for Log transformed pharmacokinetic parameters Cmax, AUC0-t and AUC0-inf.

Table No. 6: Pharmacokinetic parameters of Ramipril

Formulations		**Cmax (ng/mL)**	**AUC0-t (ng*/hr/mL)**	**AUC0-inf (ng*/hr/mL)**	**Tmax (hrs)**	**Kel (hrs-1)**	**t1/2 (hrs)**	**AUC0-t/ AUC0-inf Ratio**
Test Product A	Least Square Mean	541.66	6932.25	7130.44	5.21	0.11	6.93	97.22
	Arithmetic Mean ± SD	542.86 ± 107.32	6939.69 ± 2122.53	7138.05 ± 2211.09	5.20 ± 1.50	0.10 ± 0.02	6.93 ± 1.61	97.25 ± 1.50
Reference Product B	Least Square Mean	497.86	6924.53	7120.57	4.77	0.10	7.05	97.25
	Arithmetic Mean ± SD	498.45 ± 96.54	6946.24 ± 2140.58	7142.68 ± 2244.43	4.77 ± 0.90	0.10 ± 0.02	7.06 ± 1.65	97.31 ± 1.65
% Ratio (A/B)	Least Square Mean	108.80	100.11	100.14	-	-	-	-
	Arithmetic Mean	108.91	99.91	99.94				

Table No. 7: Pharmacokinetic parameters of Ramiprilate metabolite –M1

Formulations		Cmax (ng/mL)	AUC0-t (ng*/hr/mL)	AUC0-inf (ng*/hr/mL)	Tmax (hrs)	Kel (hrs-1)	t1/2 (hrs)	AUC0-t/ AUC0-inf Ratio
Test Product A	Least Square Mean	79.47	1276.11	1310.58	6.57	0.09	7.91	97.37
	Arithmetic Mean ± SD	79.35 ± 27.97	1272.84 ± 376.21	1307.33 ± 378.59	6.56 ± 1.74	0.09 ± 0.02	7.91 ± 1.56	97.17 ± 2.11
Reference Product B	Least Square Mean	72.76	1283.33	1317.97	7.14	0.09	8.02	97.37
	Arithmetic Mean ± SD	72.60 ± 24.51	1281.86 ± 385.02	1316.69 ± 387.78	7.15 ± 1.79	0.09 ± 0.02	8.04 ± 1.69	97.19 ± 2.14
% Ratio (A/B)	Least Square Mean	109.23	99.44	99.44	-	-	-	-
	Arithmetic Mean	109.30	99.30	99.29				

Table No. 8: Geometric Means & 90 % Confidence interval for Ramipril

Parameters	*Geometric mean		% Ratio	90 % Confidence Interval	
	Test (A)	Reference (B)	A/B	Lower Limit	Upper Limit
AUC0-inf	6816.06	6807.35	100.13	97.88	102.43
AUC0-t	6627.56	6622.14	100.08	97.77	102.45
Cmax	531.14	488.47	108.73	104.68	112.95

There was no significant treatment effect for Log transformed pharmacokinetic parameters AUC0-t and AUC0-infStatistically significant variation was observed for a period for Log transformed $AUC_{0\text{-}t}$ and $AUC_{0\text{-}inf.}$ 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 appeared 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 %.Treatment effect was found to be significant for Log transformed $C_{max,}$ It might be due to low intra subject variability.

Table No. 9: Geometric Means & 90 % Confidence interval for Ramipril

Parameters	*Geometric mean		% Ratio	90 % Confidence Interval	
	Test (A)	Reference (B)	A/B	Lower Limit	Upper Limit
AUC0-inf	1252.45	1256.70	99.66	97.26	102.12
AUC0-t	1216.81	1221.29	99.63	97.18	102.15
Cmax	73.95	68.29	108.29	104.44	112.28

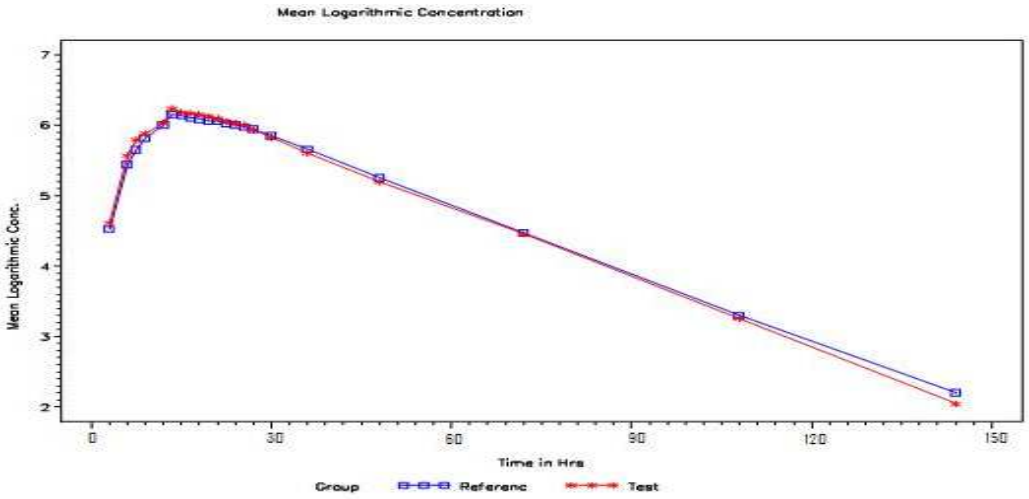

Fig No. 1: Ramipril – Mean log concentration time profile

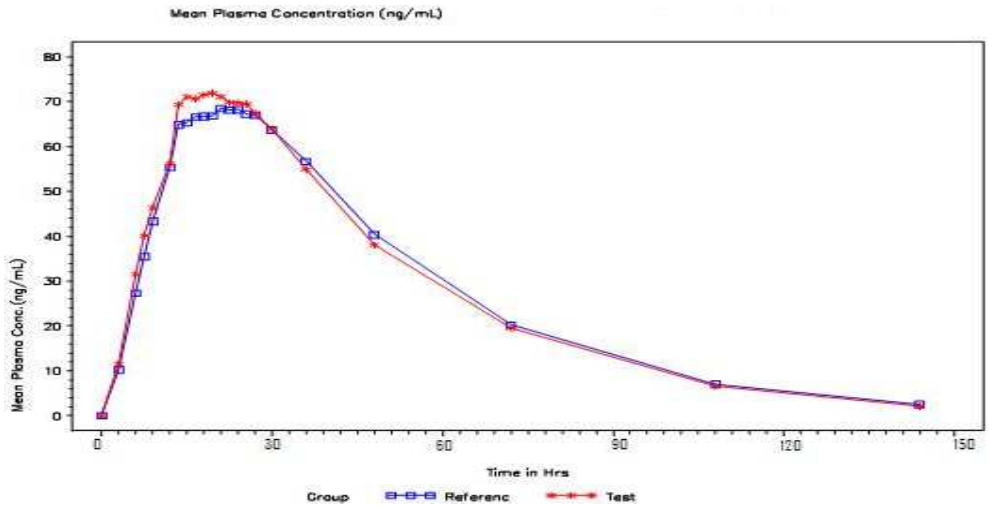

Fig No. 2: Ramiprilate - Mean concentration time profile untransformed

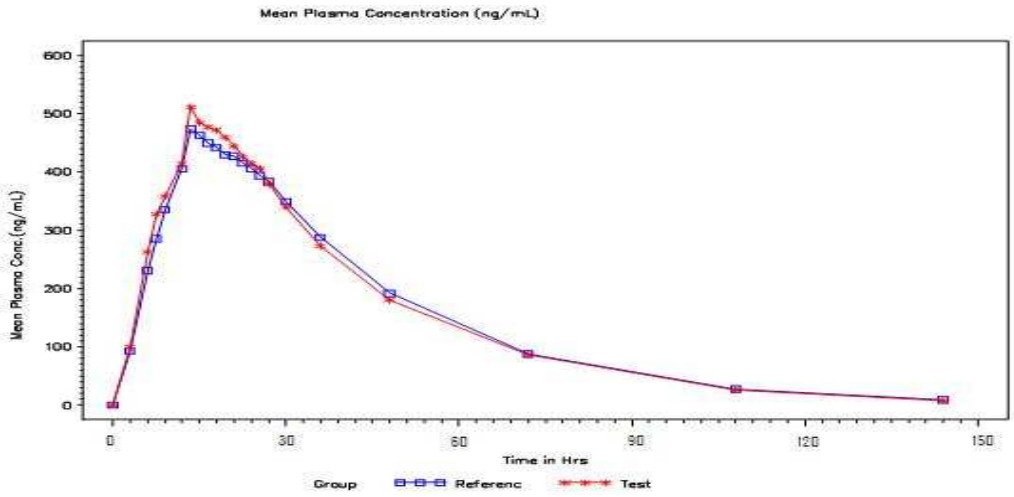

Fig No. 3: Ramipril - Mean Concentration Time Profile - Untransformed

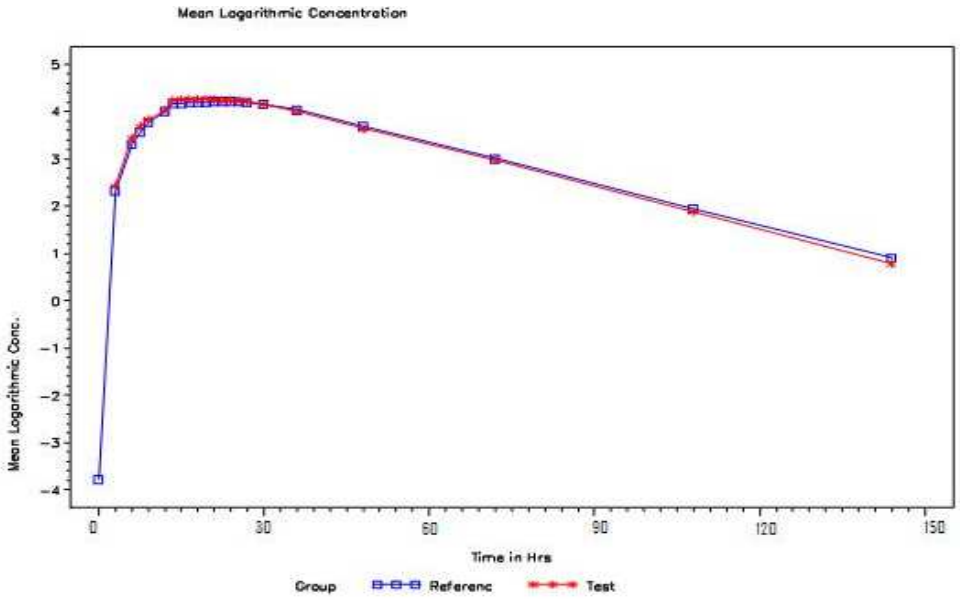

Fig No. 4: Ramiprilate – Mean log concentration time profile

The decision of equivalence was based on the 90% confidence interval based on Schuirmann‘t’ test and the 90% confidence interval was within the equivalence limits hence significant treatment effect can be ignored.In analysis of Ramiprilate , there was no significant period effect for Log transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf}. There was no significant treatment effect for Log transformed pharmacokinetic parameters AUC_{0-t} and $AUC_{0-inf.}$Treatment effect was found to be significant for Log transformed $C_{max,}$ It might be due to low intra subject variability. The decision of equivalence was based on the 90% confidence interval based on Schuirmann‘t’ test and the 90% confidence interval was within the equivalence limits hence significant treatment effect can be ignored. There was no significant sequence effect for Log transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and AUC_{0-inf}.The ratios for Geometric Least Square Means and 90% Confidence Intervals of primary efficacy variables lied between the acceptance ranges 80-125% for Log transformed C_{max}, AUC_{0-t} and AUC_{0-inf} of

RamiprilThe ratios for Geometric Least Square Means and 90% Confidence Intervals of primary efficacy variables lied between the acceptance ranges 80-125% for Log transformed C_{max}, AUC_{0-t} and AUC_{0-inf} of Ramiprilate.

Conclusion

As far as the study was concerned, the drug was well tolerated upon single-dose administration to healthy, adult, male, human subjects. 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 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. The confidence interval of Cmax, AUC0-t, and AUC0-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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