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Bioequivalence of Two Different Rivastigmine Hard Capsule Formulations of Two Different Strengths (1.5 mg and 6 mg) in Indian Healthy Human Adult Male Volunteers

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Abstract

Rivastigmine, a butyl- and acetylcholinesterase inhibitor, is approved for symptomatic treatment of Alzheimer's disease (AD). The aim of these studies was to determine the bioequivalence of test and reference formulations of Rivastigmine 1.5 mg and 6 mg Hard Capsule. Both studies were single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design. Rivastigmine 1.5 mg Hard Capsule study was conducted in 36 healthy adult Indian male volunteers under fasting conditions with a washout period of 5 days and Rivastigmine 6 mg Hard Capsule study was conducted in 40 healthy adult Indian male volunteers under fed conditions with a washout period of 7 days. For 1.5 mg study, blood samples for pharmacokinetic profiling were taken post-dose up to 10 h. For 6 mg study, blood samples for pharmacokinetic profiling were taken post-dose up to 12 h. Safety was evaluated through the assessment of adverse events, and laboratory tests. Plasma concentrations of Rivastigmine were determined with a validated LC-MS/MS method. Bioequivalence between the products was determined by calculating 90% confidence intervals (90% CI) for the ratio of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ values for the test and reference products, using logarithmic transformed data. The 90% CI of Rivastigmine were 89.63-113.68, 86.91-103.87 and 87.30-103.80 for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ respectively for Rivastigmine 1.5 mg Hard Capsule study. The 90% CI of Rivastigmine were 93.08-118.44, 94.14-104.46 and 93.77-104.12 for C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ respectively for Rivastigmine 6 mg Hard Capsule study. Since the 90% CI for C_{max} and AUC_{0-t} were within the 80.00-125.00% interval, it was concluded that the test and reference formulations of Rivastigmine 1.5 mg and 6 mg Hard Capsule were bioequivalent in their rate and extent of absorption.

Keywords: Rivastigmine 1.5 mg; Rivastigmine 6 mg; Hard capsule; Bioequivalence; Pharmacokinetics

Abbreviations: AUC_{0-t}: Area Under the Plasma Concentration versus Time Curve from Time 0 to Time t; AUC_{0-inf}: Area Under the Plasma Concentration versus Time Curve from Time 0 Extrapolated to Infinity; BE: Bioequivalence; C_{max}: Maximum Plasma Concentration; cc: Cubic Centimeter; CI: Confidence Interval; CV: Coefficient of Variation; °C: Degree Centigrade; cm: Centimeter; ≥: Greater than or Equal to; h (s): Hour (s); Ke: Elimination Rate Constant; kg (s): Kilogram (s); LC-MS/MS: Liquid Chromatography-Mass Spectroscopy/ Mass Spectroscopy; ≤: Less Than or Equal to; LOQ: Lower Limit of Quantification; min (s): Minute (s); mm: Millimeter; m: Meter; mM: Millimol; µ: Micron; µL: Microliter; ng/mL: Nanogram/Milliliter; %: Percent; PK: Pharmacokinetic; rpm: Rotations Per Minute; SAS: Statistical Analysis Software; T_{max}: Time to Reach C_{max}; $t_{1/2}$: Elimination Half-Life; yr (s): year (s)

Introduction

Alzheimer's disease is the commonest cause of dementia affecting older people. As the disease progresses, people lose the ability to remember, communicate, think clearly and perform the usual daily activities. Their behaviour or personality may also change. In severe Alzheimer's disease, the patients lose the ability to care for themselves and require full time care. Currently, there is no cure available for Alzheimer's disease, but a few pharmacological interventions are available to alleviate symptoms. The symptoms are caused by the loss of a type of nerve cell in the brain called cholinergic neurons. Rivastigmine, an acetylcholine inhibitor, works by increasing the levels of a brain chemical called acetylcholine which allows the nerve cells to communicate. This may improve the symptoms of dementia. Rivastigmine can be taken orally, either as capsules or a liquid, or by applying a patch on the skin [1].

Rivastigmine 1.5 mg and 6 mg hard capsules are indicated in the symptomatic treatment of mild to moderately severe Alzheimer's dementia and severe dementia in patients with idiopathic Parkinson's disease. The most commonly reported Adverse Reactions (ADRs) are gastrointestinal, including nausea (38%) and vomiting (23%) [2,3].

New generic formulations of Rivastigmine 1.5 mg and 6 mg hard capsule were developed having the same composition as innovator brand, Exelon® 1.5 mg and 6 mg hard capsule (containing Rivastigmine hydrogen tartrate equivalent to Rivastigmine 1.5 mg and 6 mg) of Novartis, UK respectively. A single dose of Rivastigmine 1.5 mg and 6 mg hard capsule were evaluated in these studies. The pharmacokinetics of Rivastigmine was evaluated in 36 healthy male volunteers in 1.5 mg study and 40 volunteers in 6 mg study. To support marketing authorization application, two bioequivalence studies were conducted. The aim of these studies was to determine the bioequivalence and to compare the pharmacokinetics of test and reference formulations of Rivastigmine 1.5 mg and 6 mg hard capsule.

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Materials and Methods

Volunteers

In 1.5 mg study, a total of 36 (32 plus up to 4 reserve volunteers) and in 6 mg study, a total of 40 Asian Indian healthy adult human male volunteers who were part of Sitec healthy volunteer pool representing the general population were enrolled. Male volunteers between 18-45 years of age having body weight at least 50 kg and within ±10% of the ideal body weight in relation to height, according to Life Insurance Corporation of India height-weight chart for Indian men and women at the time of screening were enrolled in both the studies.

The volunteers were screened within 21 days prior to study enrolment. The screening procedure included general history (like previous participation in clinical study/blood donation, alcohol and tobacco consumption); demographic data, including name, sex, race, age, body weight (kg), height (m); medical history, physical examination, vital signs measurement, a 12-lead electrocardiogram (ECG), haematology, biochemistry, urine analysis, testing for HIV I and II; hepatitis B and C. Volunteers were judged to be healthy based on acceptable physical examination, and clinical laboratory test results. The clinical investigator reviewed the screening data and performed the physical examinations.

The demographics of all 36 recruited volunteers of 1.5 mg study and 40 volunteers in 6 mg study are summarized in Table 1.

Informed consent and ethical approval

The protocol and informed consent forms (ICFs) were reviewed and approved prior to study initiation by an independent ethics committee. Sitec Labs Institutional Review Board (SLIRB) approved the 1.5 mg study protocol and informed consent forms (ICFs) on 21st August 2006. Dakshata, an Independent Ethics Committee approved the 6 mg study protocol and informed consent forms (ICFs) on 28th October 2009; and amendments to IEC approved protocol and informed consent forms (ICFs) were approved on 18th November 2009 before dosing of period-2. All the volunteers were informed about the purpose, nature, procedure, duration, anticipated risks and discomfort of the study in the language they understand. Adequate time was given to read and understand the ICF and a written informed consent was obtained from each one of them prior to study initiation. This clinical trial was conducted in accordance with the Declaration of Helsinki, good Clinical Practice guidelines and national regulatory requirements [4-7].

Rivastigmine 1.5 mg study was conducted from November to December, 2006 and Rivastigmine 6 mg study was conducted from November to December, 2009.

Study design

1.5 mg study was open label, randomized, single-dose, two-treatment, two-sequence, two-period, cross-over design, bioequivalence study under fasting conditions. A single dose of one test formulation of Rivastigmine 1.5 mg hard capsule was compared with one reference formulation of Exelon® 1.5 mg hard capsule (containing Rivastigmine hydrogen tartrate equivalent to Rivastigmine 1.5 mg) of Novartis, UK.

6 mg study was open label, randomized, single-dose, two-treatment, two-sequence, two-period, cross-over design, bioequivalence study under fed conditions. A single dose of one test formulation of Rivastigmine 6 mg hard capsule was compared with one reference formulation of Exelon® 6 mg hard capsule (containing Rivastigmine hydrogen tartrate equivalent to Rivastigmine 6 mg) of Novartis, UK.

The volunteers did not consume any food and beverages containing xanthine or alcohol (48 h before dosing and throughout the period of sample collection), grapefruit (7 days before dosing and throughout the study), or vitamins (throughout the confinement period). Medications (including herbal and over-the-counter products) were prohibited for the 14 days preceding the study and also during the study.

On check in day, at least 12 h prior to each dosing, all volunteers were screened for cocaine, cannabinoids, benzodiazepines, opioids, amphetamines, barbiturates and alcohol. In 1.5 mg study, a total of 36 volunteers and in 6 mg study a total of 40 volunteers who satisfied all the criteria for inclusion were admitted to the study center in the evening before dosing (Day-1). 1.5 mg study was conducted in two batches (Batch A and Batch B). Batch A consisted of 20 volunteers (subject No. 01-20) and batch B consisted of 16 volunteers (subject No. 21-36). 6 mg study was conducted in one batch of 40 volunteers (subject No. 01-40). On the check–in day, volunteers' belongings were thoroughly checked and they were asked to remove all outer garments and take a shower. Volunteers wore clothing provided by Sitec for the duration of confinement.

Then, they were assigned to each treatment sequence as per the randomization scheme. All study medications were kept in a pharmacy and temperature and humidity were monitored continuously. A SAS generated randomization code was used to ensure balanced permutation of the treatments.

Drug administration

For 1.5 mg study, a single dose of Rivastigmine 1.5 mg hard capsule of the test or reference product was orally administered with 240 mL of water to the volunteers in sitting position after an overnight fast of at least 10 h in each period. No food was permitted until 4 h after dosing. Water was not permitted from 1 h before dosing until 2 h following dosing, but it was allowed at all other times. After administration of the dose of Investigational Product, a mouth check was performed under supervision of quality control personnel to assess the compliance to this procedure. During the trial, the volunteers were to remain ambulatory or seated upright for the first 2 h after drug administration. During housing, post-dose meals were identical for both periods of the study. Lunch, snack and dinner were served at 4.0, 9.0 and 13.0 h, respectively, after dosing. Each dosing period was separated by 5 days.

For 6 mg study, after an overnight fast of at least 10 h, a standardized non-high-fat breakfast was given 30 min prior to dosing. Thereafter, a single dose of Rivastigmine 6 mg hard capsule of the test or reference product was orally administered with 240 mL of water to the volunteers in sitting position. After administration of the dose of Investigational Product, a mouth check was performed under supervision of quality control personnel to assess the compliance to this procedure. All the blood samples were collected at bedside and volunteers were not allowed to walk around unattended to take care of the occurrence of dizziness, somnolence or syncope. Volunteers were advised to remain in supine position and resting on the bed after taking the Investigational Product. Subjects were advised to avoid severe physical exertion. No food was permitted until 4 h after dosing. Water was not permitted from 1 h before dosing until 2 h following dosing, but it was allowed at all other times. After administration of the dose of Investigational Product, a mouth check was performed under supervision of quality control personnel to assess the compliance to this procedure. During housing, post-dose meals were identical for both periods of the study. Lunch, snack, and dinner were served at 4.0 h, 9.0 h and 13.0 h, respectively, after dosing. Each dosing period was separated by 7 days.

	Rivastigmine 1.5 mg			Rivastigmine 6 mg		
	Age (years)	Weight (kg)	Height (m)	Age (years)	Weight (kg)	Height (m)
Number of observations	36	36	36	40	40	40
Mean	27.31	63.19	1.68	26.63	64.55	1.69
Median	28.00	62.00	1.67	27	64.9	1.69
Standard Deviation	5.22	7.58	0.06	5.29	6.74	0.05
Minimum	19.00	52.00	1.59	19	53.1	1.57
Maximum	39.00	82.00	1.90	39	80.1	1.80

Table 1: The demographics of all the recruited subjects in both Rivastigmine 1.5 mg and 6 mg studies are summarized.

Concomitant medication: To prevent nausea and vomiting which are very commonly associated with high doses of Rivastigmine, a single dose of 8 mg Ondansetron (2 mg/mL) injection which was given within 15 min prior to Rivastigmine 6 mg Hard Capsule dosing by slow intravenous injection in period-1. But adverse events like nausea and vomiting were observed in many volunteers (about 25%). So, to further prevent nausea and vomiting in period-2, one dose of 1 mg Granisetron (1 mg/mL) injection after diluting it to a volume of 5 mL and one dose of 50 mg Ranitidine (25 mg/mL) injection were given within 15 min prior to Rivastigmine dosing by slow intravenous injection instead of 8 mg Ondansetron (2 mg/mL) injection.

Concomitant medication was changed in period-2 as per the opinion of Principal Investigator after taking approval from independent ethics committee prior to dosing of period-2 which was agreed by sponsor's medical expert.

Adverse events were monitored throughout the study, until resolution or lost to follow-up. Adverse events were described in terms of severity, seriousness, outcome, action, frequency and relationship to treatments. The principal investigator or sub-investigator was on-site, within the proximity of the subject confinement area till all the volunteers were checked-out from the clinical pharmacology unit. Volunteers were instructed to inform the study physician and/or nurses of any adverse events that occurred during the study.

Blood sampling

For 1.5 mg study, Blood samples (1 × 6 mL) for Rivastigmine analysis were collected via an indwelling catheter (intra-venous) in vacutainers containing (dipotassium ethylene diamine tetra-acetic acid) K_2EDTA anticoagulant at -1.00 h (pre-dose) and at 0.25, 0.50, 0.67, 0.84, 1.00, 1.17, 1.33, 1.50, 2.00, 2.50, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00 and 10.00 h post dose.

For 6 mg study, Blood samples (1 × 6 mL) for Rivastigmine analysis were collected via an indwelling catheter (intra-venous) in vacutainers containing K_2EDTA anticoagulant at -1.50 h (pre-dose) and at 0.33, 0.67, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50, 2.75, 3.00, 3.25, 3.50, 3.75, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00 and 12.00 h post dose.

After blood collection, vacuum collection tubes were inverted gently several times to ensure the mixing of tube content and blood sample. Tubes containing blood samples were immediately placed in an iced water bath at approximate temperature of 8-12°C till they were centrifuged. The blood sample tubes were centrifuged to separate plasma as soon as possible at 3000 rpm for 10 min in a centrifuge set at a temperature of 4°C. Then plasma was stored below -30°C at the clinical unit of Sitec Labs Pvt. Ltd. and then transferred to the bioanalytical facility of Sitec Labs Pvt Ltd under frozen condition and then samples were stored at -70°C or below until sample analysis. The concentration of Rivastigmine was measured in plasma samples of the volunteers. As Rivastigmine is not stable in plasma and undergoes *in vitro* hydrolysis, 50 µL of 0.1 mM Physostigmine solution was added to prevent the process of hydrolysis in pre-cooled blood collection tubes. Physostigmine is a light sensitive material. Therefore, solution was prepared and transferred to tubes in subdued light. Also, all the blood samples were collected and processed in subdued light.

Analytical methods

Plasma concentrations of Rivastigmine were assessed by a method using high-performance liquid chromatography with mass spectrometry detection (LC-MS/MS). An aliquot of 500 µL of human plasma containing the analyte and the internal standard was extracted using solid phase extraction technique. The internal standard for Rivastigmine assay was Tramadol. About 12.5 µL of the internal standard working solution were added to 500 µL of plasma sample. After vortexing the tubes, 500 µL of 0.1% Formic acid solution was added and the tubes were again vortexed. This sample was transferred to a pre-conditioned Waters HLB 1 cc SPE cartridge. After loading the entire sample, low vacuum was applied to the cartridge till the entire sample has flowed out of the cartridge. Subsequently the cartridge was washed with 1 mL of 0.1% Formic acid followed by 1 mL of water. After washing was complete, the analytes were eluted with 0.4 mL of mobile phase under low vacuum. This final extract is transferred to glass vial for analysis using LC-MS/MS.

The extracts were analyzed on the LC-MS/MS system comprising of Agilent 1100 series HPLC and MDS Sciex API-4000 mass spectrometer. Positive ions were monitored in the multiple reaction-monitoring (MRM) modes. Following ion transitions using analyst 1.4.2 were monitored 251.17/206.13 and 264.20/58.20 for Rivastigmine and internal standard respectively. Linearity for Rivastigmine was assessed by plotting area ratios versus standard concentrations and using a linear regression weighted 1/concentration^2. The calibration standard ranges for Rivastigmine for 1.5 mg study and 6 mg study were 0.10-40 ng/ mL and 0.10-60 ng/ mL respectively. The column used for the analysis is BDS Hypersil C8 4.6 × 100 mm, 5 µ and the mobile phase composition was a mixture of methanol and ammonium acetate buffer (70:30). The retention time of Rivastigmine is 1.75 min.

Rivastigmine is not stable in plasma when kept on bench for more than 15 min as it undergoes *in vitro* hydrolysis in plasma. Physostigmine (Eserine) was added to inhibit *in vitro* hydrolysis process. Rivastigmine is stable in plasma for 7 h at room temperature after Physostigmine is added to plasma. Physostigmine is a light sensitive compound and it was stored in a dark place at 2°C to 4°C in the refrigerator. Physostigmine was weighed in subdued light and immediately transferred to an amber colored volumetric flask. Subsequently it was dissolved with methanol to get 0.1 mM solution of Physostigmine. This solution was stored at 2°C to 4°C in refrigerator when not in use. Solution was prepared freshly every day. About 50 µL of this solution was added to 6 mL of blood collected at each interval.

Method validation was performed according to the current international approach and the applicable regulations regarding bio-analytical method validation. The intra-batch and inter-batch accuracy and precision was evaluated at four different concentrations of control samples. The inter-batch accuracy ranged from 100.00 to 104.82% and the inter-batch precision ranged from 3.66-7.42%. The selectivity of the method was assessed by analyzing plasma samples from six sources. Matrix effect was evaluated by performing post-extraction addition and post-column infusion experiments. Stabilities such as stock solution stability, short-term stability of analyte in plasma, freeze-thaw stability, post-preparative stability and long-term stability in plasma were assessed.

During the 6 mg study, some concomitant medications were administered to prevent adverse events. These concomitant medications were: a) Ondansetron injection (2 mg/mL), b) Granisetron injection (1 mg/mL) and c) Ranitidine injection (25 mg/mL). After the completion of BE study, a partial validation was performed to evaluate the effect of above stated concomitant drugs on the quantitation of Rivastigmine. In this partial validation, specificity and selectivity, sensitivity, carry over, solution linearity, precision and accuracy, recovery, dilution integrity, stability (post-preparative stability, freeze-thaw stability, short-term stability and long-term stability and matrix effect were evaluated in presence of the concomitant medication. The inter-batch accuracy ranged from 90.00-106.18% and the inter-batch precision (% CV) ranged from 0.76% to 12.72%. All partial method validation experiments met the acceptance criteria. Rivastigmine was stable in plasma in presence to the above stated concomitant drugs for 482 days when stored at -70°C ± 10°C.

Pharmacokinetic analysis

The following PK parameters were calculated using validated PK software (WinNonlin version 5.0.1 for 1.5 mg study and WinNonlin version 5.2 for 6 mg study). The area under the curve from time zero to the last measurable concentration (AUC_{0-t}) using the linear trapezoidal rule, the area under the curve extrapolated to infinity ($AUC_{0-t} + C_{last}/k_{el}$, where C_{last} is the last measurable plasma concentration), the maximum plasma concentration (C_{max}), and the time to maximum plasma concentration (t_{max}), the terminal rate constant of elimination (k_{el}) and terminal elimination half-life ($t_{1/2}$).

Statistical analysis

A statistical analysis was performed using the NCSS 97 software (Number Cruncher Statistical Systems) for 1.5 mg study. A statistical analysis was performed using the SAS® GLM procedure (SAS® system for windows® release and 9.1.3) for 6 mg study.

Concentration values below the LOQ of the assay for Rivastigmine (0.10 ng/mL for both the studies) were set to zero. Arithmetic means, standard deviations, coefficients of variation, geometric means, median, minimum and maximum values were calculated for the plasma concentrations and the pharmacokinetic parameters. Analyses of variance (ANOVA) were performed on In-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ parameters. The ANOVA model included sequence, formulation, and period as fixed effects, and subject nested within sequence as a random effect. Each ANOVA included calculation of LSM, the difference between formulation LSM, and the standard error associated with this difference. To determine bioequivalence in this comparative bioavailability study, the standard that the 90% confidence interval of the ratios of least-squares means of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of the test to the reference formulation should be within 80.00-125.00% was used. A statistical analysis was performed using the SAS® GLM procedure (SAS® system for windows® release 9.1.3) Geometric least-square means (LSM) as well as ratio of LSM with corresponding 90% confidence intervals (CI's) for the generic and innovator formulations were calculated. In addition, nonparametric methods were used to assess differences in median values of t_{max} between the two formulations and 90% CI's were constructed.

Results

Safety

A total of 36 volunteers were recruited in 1.5 mg study. There was 1 adverse event of mild severity. Overall, 1/36 (2.78%) volunteers experienced an adverse event.

A total of 40 volunteers were recruited in 6 mg study. There were 23 adverse events of mild and moderate severity. Overall, 15/40 (37.50%) volunteers experienced an adverse event in both the periods.

11/40 (27.50%) volunteers experienced an adverse event in period-1. But 10/40 (25.00%) volunteers experienced vomiting of mild to moderate intensity in period-1. 11/40 (27.50%) volunteers experienced an adverse event in period-2. But 5/40 (12.50%) volunteers experienced vomiting of mild intensity in period-2. Therefore, frequency of vomiting was reduced to exactly half in period-2 with severity was reduced to mild in period-2. Mild vomiting was defined as 1-2 episodes in 24 h and moderate vomiting was defined as 3-5 episodes in 24 h.

No deaths or serious adverse events (SAE) occurred during conduct of both the studies. Adverse events of both the studies (1.5 mg and 6 mg) are summarized period-wise in Table 2. Preferred terminology is given for all the adverse events according to MedDRA software (version 18.1).

During vital signs examination, there were no clinically significant deviations observed from the baseline values and no clinically significant changes were noted in post-study clinical laboratory data. All volunteers were found fit in post-study examination. There were no clinically significant changes observed in post-study ECGs when compared with pre-study ECGs.

Pharmacokinetics and statistics

A total of 36 (32 plus 4 reserve) volunteers were recruited in Rivastigmine 1.5 mg study and all 36 volunteers completed the study. The plasma samples of all 36 volunteers were analyzed for Rivastigmine. But data of first 32 volunteers was considered for pharmacokinetic and statistical analysis as per the protocol.

A total of 40 volunteers were recruited in Rivastigmine 6 mg study and all 40 volunteers completed the study. The plasma samples of all volunteers were analyzed for Rivastigmine. 9 volunteers had vomiting within the 2 times of the T_{max}. Therefore, data of 9 volunteers was not considered for final pharmacokinetic and statistical analysis. Data of remaining 31 volunteers was considered for pharmacokinetic and statistical analysis.

Considering the intra-subject CV of about 28.0% for C_{max}, a total of 36 volunteers were recruited in Rivastigmine 1.5 mg study and 40 volunteers were recruited in Rivastigmine 6 mg study to take care of dropout or discontinued volunteers due to adverse events like nausea and vomiting which are very commonly associated with high doses of Rivastigmine.

Adverse Event (Preferred Term as per MedDRA)	Frequency (Percentage)	Relationship	Number of Adverse Events	
			Test product (T)	Reference product (R)
Rivastigmine 1.5 mg (period-1)				
Nausea	2.78%	Related	0	1
Rivastigmine 6 mg (period-1)				
Vomiting	25.00%	Related	3	7
Dizziness	2.50%	Related	1	0
Headache	2.50%	Related	1	0
Rivastigmine 6 mg (period-2)				
Vomiting	12.50%	Related	3	2
Nausea	12.50%	Related	2	3
Dizziness	2.50%	Related	0	1

Table 2: Adverse events of both the Rivastigmine 1.5 mg and 6 mg studies are summarized period-wise.

The blood samples were collected up to 10 h post dose for 1.5 mg study and up to 12 h for 6 mg study. Mean plasma concentration profiles of Rivastigmine under linear over the 10 h pharmacokinetic study (1.5 mg) are presented in Figure 1 and over the 12 h pharmacokinetic study (6 mg) are presented in Figure 2. Overall, mean plasma concentrations of Rivastigmine peaked rapidly and then declined in a mono-exponential manner, with most of the plasma concentration values falling below the LOQ of the assay at 10 h post dose (1.5 mg study) but most of the plasma concentration values not falling below the LOQ of the assay at 12 h post dose (6 mg study). Therefore, 1-2 additional time points may be required after 12 h post dose for 6 mg study. Values below the LOQ were set to zero for pharmacokinetic analysis. Mean plasma concentrations of Rivastigmine following oral administration of these formulations were almost superimposable during the early absorption, distribution, and elimination phases of the products. Ratios of AUC_{0-t}/$AUC_{0-\infty}$ for all the volunteers found to be more than 80% indicating the blood samples collected adequately characterized the pharmacokinetic profile of the drug.

In addition, 32 volunteers provided >90% power to detect a difference of at least 20% in C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ between the two treatments for 1.5 mg study; and 31 volunteers provided >90% power to detect a difference of at least 20% in C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ between the two treatments for 6 mg study.

The statistical results of primary pharmacokinetic parameters of Rivastigmine (1.5 mg and 6 mg) are presented in Table 3. The Geometric mean ratios, 90% CI, power and intra-subject coefficient of variation of test and references for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for Rivastigmine (1.5 mg and 6 mg) are presented in Table 4.

Discussion

In these studies, we investigated the bioequivalence of test and reference formulations of Rivastigmine 1.5 mg Hard Capsule and Rivastigmine 6 mg Hard Capsule.

Assessment of bioequivalence of generic product to reference product is required to exclude any clinically important differences in the rate or extent at which the active entity of the drugs becomes available at the site of action. Two medicinal products containing the same active substance are considered bioequivalent if they are pharmaceutically equivalent or pharmaceutical alternatives and their bio-availabilities (rate and extent) after administration in the same molar dose lie within acceptable predefined limits. These limits are set to ensure comparable *in vivo* performance, i.e., similarity in terms of safety and efficacy [8].

Exelon® (containing Rivastigmine hydrogen tartrate equivalent to Rivastigmine) of Novartis, UK is authorized for marketing 1.5 mg, 3 mg, 4 mg and 6 mg hard capsule. Rivastigmine has been shown to have nonlinear pharmacokinetics. The non-linearity gives about 50% higher AUCs than expected when doubling the dose. According to the Guideline on the Investigation of Bioequivalence CPMP/EWP/QWP/1401/98, the bioequivalence study should in general be conducted at the highest strength for drugs with non-linear pharmacokinetics characterized by a more than proportional increase in AUC with increasing dose over the therapeutic dose range [9]. Therefore, a bioequivalence study comparing test and reference formulation of Rivastigmine 6 mg hard capsule was conducted.

As the pharmacokinetics of Rivastigmine are very variable, a study comparing the lowest strength, 1.5 mg of Rivastigmine hard capsule of test and reference formulation was also conducted.

As per FDA guidance, *in vivo* testing can be waived for 3 mg strength based on: (i) Acceptable bioequivalence studies on the 1.5 mg strength, (ii) Acceptable *in vitro* dissolution testing on the 1.5 mg and 3 mg strengths, and (iii) Proportional similarity in the formulations of the 1.5 mg and 3 mg strengths. Also *in vivo* testing can be waived for 4.5 mg strength based on: (i) Acceptable bioequivalence study on the 6 mg strength, (ii) Acceptable *in vitro* dissolution testing on the 4.5 mg and 6 mg strengths, and (iii) Proportional similarity in the formulations of the 4.5 mg and 6 mg strengths [10]. Therefore, bioequivalence studies on 3 mg and 4.5 mg strength of Rivastigmine Hard Capsule were not conducted.

Rivastigmine 1.5 mg study was conducted under fasting conditions. Rivastigmine is recommended to be administered with meals [2]. FDA guidance recommends Rivastigmine BE studies to be conducted under fed conditions [10]. Administration of Rivastigmine with food delays absorption (t_{max}) by 90 min and lowers C_{max} and increases AUC by approximately 30% [2]. Therefore, Rivastigmine 6 mg study was conducted under fed conditions. Rivastigmine 1.5 mg study should also be conducted under fed conditions.

Both the studies demonstrate generic and innovator formulations of both Rivastigmine (1.5 mg and 6 mg) displayed similar rate and extent of bioavailability of Rivastigmine. The median T_{max} for both test and reference was found to be 1.00 h for 1.5 mg study. The T_{max} is comparable. The median T_{max} for test and reference was found to be 1.25 h and 1.50 h respectively for 6 mg study. Wilcoxon-Mann-Whitney two sample test for difference in median T_{max} was performed using SAS® 9.1.3. Difference between the median T_{max} of Test and Reference product was not significant statistically. The C_{max} was found to be consistent both for test and reference in both the studies indicating

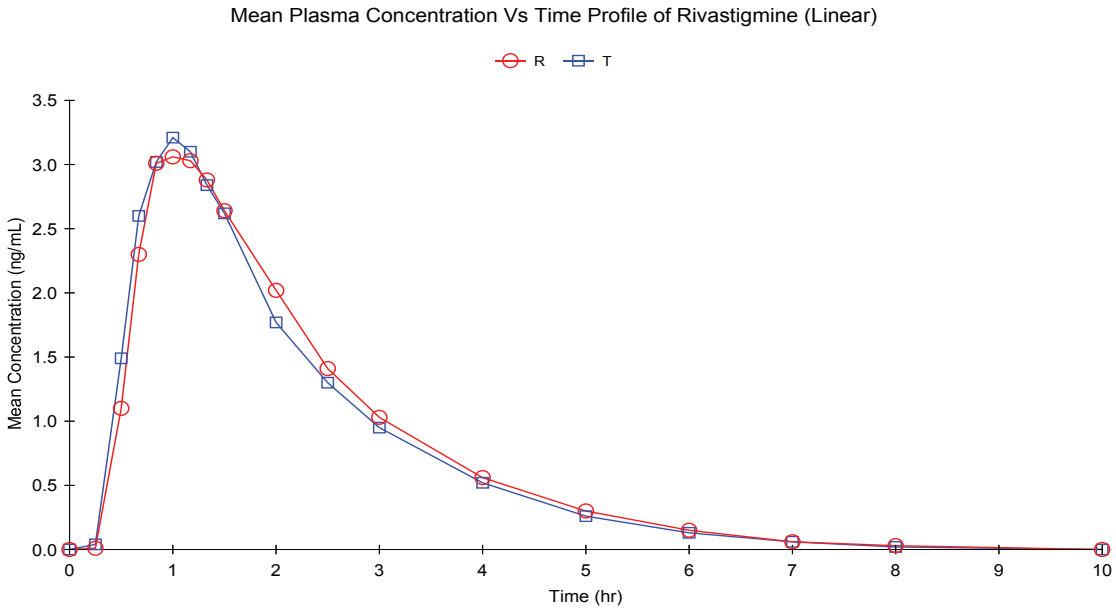

Figure 1: Mean graph (linear) for plasma concentration vs. time profile of Rivastigmine (1.5 mg).

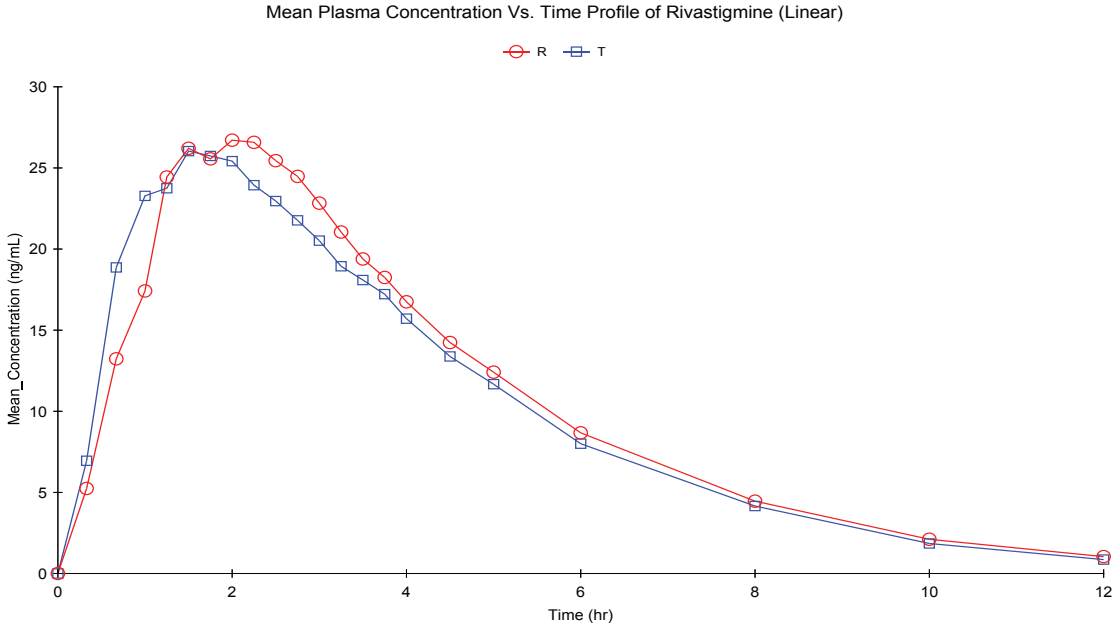

Figure 2: Mean graph (linear) for plasma concentration vs. time profile of Rivastigmine (6 mg).

the attainment of body peak levels similarly. However, the mean data is very much comparable. For the AUC_{0-t} parameter the results found to be similar and not much difference in inter-subject variability. The $T_{1/2}$ values are also comparable and in the elimination phase there is no variation.

The statistical analysis was carried out for both untransformed and log-transformed data. The data showed statistical equivalence for the important pharmacokinetic parameters i.e., C_{max}, AUC_{0-t} and $AUC_{0-\infty}$. A power of >90% was achieved for the pharmacokinetic parameters for 1.5 mg study.

Considering that all 90% CIs of the ratios of the pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were found to be within the predetermined ranges of bioequivalence and that the two one-sided t tests found all of the probability values to be <0.05, the results of both studies satisfied the accepted regulatory requirements to assume bioequivalence.

The intra-subject CV was found to be 28.56% for C_{max}, 21.22% for AUC_{0-t} and 20.61% for $AUC_{0-\infty}$ for log-transformed data for 1.5 mg study. A power of >90% was achieved for the pharmacokinetic parameters for 6 mg study. The intra-subject CV was found to be

Pharmacokinetic Parameters	1.5 mg		6 mg	
	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)	Test (T) (Mean ± SD)	Reference (R) (Mean ± SD)
N	32	32	31	31
C_{max} (ng/mL)	3.63 ± 1.85	3.60 ± 1.96	38.13 ± 11.83	37.23 ± 15.81
AUC_{0-t} (h.ng/mL)	6.82 ± 3.99	7.00 ± 3.72	122.73 ± 43.46	126.40 ± 56.95
$AUC_{0-\infty}$ (h.ng /mL)	7.03 ± 4.01	7.21 ± 3.76	125.08 ± 45.39	129.46 ± 59.94
*T_{max} (h)	1.00 (0.50-1.50)	1.00 (0.67-2.00)	1.25 (0.33-3.75)	1.50 (0.33-2.75)
K_{el} (1/h)	0.669 ± 0.122	0.665 ± 0.091	0.412 ± 0.065	0.406 ± 0.074
$T_{1/2}$ (h)	1.07 ± 0.17	1.06 ± 0.15	1.72 ± 0.27	1.76 ± 0.34

*Median (range)

Table 3: The statistical results of primary pharmacokinetic parameters of Rivastigmine (1.5 mg and 6 mg) are presented.

Pharmacokinetic Parameters	Geometric Mean		*(%)T/R	90% Confidence Interval	Power (%)	Intra subject CV%
	Test	Ref				
Rivastigmine 1.5 mg						
N	32	32	-	-	-	-
C_{max} (ng/ml)	3.23	3.20	100.94	89.63-113.68	92.67	28.56
AUC_{0-t} (h.ng/ml)	5.84	6.15	95.01	86.91-103.87	99.20	21.22
$AUC_{0-\infty}$ (h.ng/ml)	6.07	6.38	95.19	87.30-103.80	99.41	20.61
Rivastigmine 6 mg						
N	31	31	-	-	-	-
C_{max} (ng/ml)	36.49	34.65	105.00	93.08 - 118.44	92.08	28.08
AUC_{0-t} (h.ng/ml)	115.08	115.03	99.17	94.14 - 104.46	100.00	11.94
$AUC_{0-\infty}$ (h.ng/ml)	116.94	117.27	98.81	93.77 - 104.12	100.00	12.01

*(%) T/R is ratio of Test Geometric Mean/Ref Geometric Mean

Table 4: The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ for Rivastigmine (1.5 mg and 6 mg) are presented.

28.08% for C_{max}, 11.94% for AUC_{0-t} and 12.01% for $AUC_{0-\infty}$ for log-transformed data for 6 mg study.

The sample size of 32 volunteers selected for 1.5 mg study and 40 volunteers selected for 6 mg study was considered to be sufficient to provide adequate power to meet bioequivalence criteria. All the volunteers were dosed between 07:00 to 07:06 in both the periods for 1.5 mg study. All the volunteers were dosed between 08:00 to 08:2 in both the periods for 6 mg study.

Ondansetron injection (2 mg/mL), granisetron injection (1 mg/mL) and ranitidine injection (25 mg/mL) were selected to be given as concomitant medications to prevent nausea and vomiting in Rivastigmine 6 mg study. These drugs have no reported pharmacokinetic interaction with Rivastigmine [11-13]. Same was confirmed by partial validation done during bio-analysis. There was no impact of having used different concomitant medications in each period.

During the clinical study, there were no significant protocol/standard operating procedure (SOP) deviations and adverse events were mild to moderate in nature. The volunteers tolerated the study medication well in 1.5 mg study but volunteers did not tolerate the study medication well in 6 mg study. Granisetron was more effective in reducing the frequency and severity of vomiting in 6 mg study in comparison to Ondansetron.

The biological samples were successfully analyzed by LCMS/MS. The quality control data was found to be consistent and precise.

Conclusion

The 90% CI of Rivastigmine for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within 80.00-125.00% for both the studies. Therefore,

- The generic formulation of Rivastigmine 1.5 mg hard capsule was bioequivalent with the innovator formulation of Exelon® 1.5 mg hard capsule (containing Rivastigmine hydrogen tartrate equivalent to Rivastigmine 1.5 mg) of Novartis, UK under fasting conditions.
- The generic formulation of Rivastigmine 6 mg hard capsule was bioequivalent with the innovator formulation of Exelon® 6 mg hard capsule (containing Rivastigmine hydrogen tartrate equivalent to Rivastigmine 1.5 mg) of Novartis, UK under fed conditions.
- Single dose of Rivastigmine 6 mg hard capsule was not well tolerated by healthy human adult male volunteers.

Disclosure

These bioequivalence studies were conducted at Sitec Labs. Pvt. Ltd., Mumbai, India. Dr. Muneesh Garg was the Principal Investigator for the studies, wrote the manuscript, and reviewed and approved the final draft. Dr Raghu Naidu was responsible for the bio-analysis. This publication was supported by Sitec Labs. Pvt. Ltd.

Conflict of Interest

All authors are employees of Sitec Labs. Pvt. Ltd. The authors have indicated that they have no other conflicts of interest regarding the content of the article.

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