5.0 Discussion

Neurological disorders like Parkinson’s cause significant morbidity, mortality, disability, socioeconomic losses and reduce the quality of life. The incidence of Parkinson disease has been estimated to be 4.5-21 cases per 100,000 population per year, and estimates of prevalence range from 18 to 328 cases per 100,000 population, with most studies yielding a prevalence of approximately 120 cases per 100,000 population. The global anti-parkinson’s market registered a growth rate of 18.9% from 2006-2007 representing sales valued at $3.7bn in 2007. This growth rate is robust when compared to its average growth rate of 11.8% observed for the period of 2005-2006. The growth of the parkinson’s disease (PD) market was mainly driven by newly approved drug AZILECT (Rasagiline), which registered sales growth of 196.9%. AZILECT accrued sales of $114m in 2007, which was $38m in previous year. AZILECT was approved by the FDA in May 2006 as a once-daily oral treatment of PD. The drug is also approved as adjunct therapy to levodopa in moderate-to-advance PD. The growth of AZILECT shows it is a promising molecule of the PD market, so as it comes off patent there would be onset of heavy generic competition.102

A generic copy of reference drug must contain identical amounts of the same active ingredients in the same dose formulation and route of administration, as well as meet standards for purity, quality and identity. Some inactive ingredients such as binders and fillers are allowed to differ, but must occur in the similar ratio to the active compound as that observed in the brand name drug.129 Recently, a commentary was published on the implications of the pharmaceutical alternatives (medicinal products with different salts) for safety and efficacy. These products can have drastic differences in solubility, dissolution, toxicity profile, polymorphic phenomenon and stability issues in formulations. FDA recommended that establishing bioequivalence between pharmaceutical alternatives is not sufficient for therapeutic equivalence and additional data is required. Different manufactures use various methods, salts, polymorph etc to formulate these products so many of these marketed formulations may differ in the rate and extent of absorption.127

Rasagiline mesylate is an irreversible, monoamine oxidase type B inhibitor (MAO-B). Rasagiline mesylate is indicated for the treatment of idiopathic Parkinson’s disease as initial monotherapy and as adjunct therapy to Levodopa. Rasagiline offers an advantage over the first-generation MAO-B inhibitor selegiline in its lack of amphetamine metabolites, which may interfere with neuroprotection. Rasagiline has minor adverse effect profile.21
As per regulatory requirements, reasonable assurance has to be provided that various products, containing same active ingredients, marketed by different licensees, are clinically equivalent and interchangeable. The bioavailability of an active substance from a pharmaceutical product should be known and reproducible. Both bioavailability and bioequivalence focus on the release of a drug substance from its dosage form and subsequent absorption into the systemic circulation. In bioequivalence studies, an applicant compares the systemic exposure profile of a test drug product to that of a reference drug product (RLD). For two orally administered drug products to be bioequivalent, the active drug ingredient or active moiety in the test product must exhibit the same rate and extent of absorption as the reference drug product. Several in vivo and in vitro methods can be used to measure BA and to establish BE. These include pharmacokinetic, pharmacodynamic, clinical, and in vitro studies.

BA and BE, which are expressed in terms of rate and extent of absorption of the active ingredient or moiety to the site of action, emphasize the use of pharmacokinetic measures in an accessible biological matrix such as blood, plasma, and/or serum to indicate release of the drug substance from the drug product into the systemic circulation. This approach rests on an understanding that measuring the active moiety or ingredient at the site of action is generally not possible and, furthermore, that some relationship exists between the efficacy/safety and concentration of active moiety and/or its important metabolite or metabolites in the systemic circulation. In pharmacokinetic study, clearance, volume of distribution, and absorption, as determined by physiological variables (e.g. gastric emptying, motility, pH), are assumed to have less interoccasion variability compared to the variability arising from formulation performance. Therefore, differences between two products because of formulation factors can be determined.\(^\text{127}\)

Regulatory guidelines recommend sponsors to ensure that bioanalytical methods for BA and BE studies are accurate, precise, selective, sensitive, and reproducible. It is recommended to perform a pilot study (n= 8-12) before a pivotal study. Pilot study can be used to assess variability, optimize sample time points, optimize bioanalytical methodology etc.

For in vivo pharmacokinetic BE studies, the following general approaches are recommended:

- The test or reference products can be administered with about 8 ounces (240 milliliters) of water to an appropriate number of subjects under fasting conditions, unless the study is a food-effect BA and BE study or label claim mentions otherwise.
• Generally, the highest marketed strength can be administered as a single unit. If warranted for analytical reasons, multiple units of the highest strength can be administered, providing the total single-dose remains within the labeled dose range.

• An adequate washout period (e.g., more than 5 half lives of the moieties to be measured) should separate each treatment.

• The drug content of the test product cannot differ from that of the reference listed product by more than 5 percent.

• It is recommended that under normal circumstances, blood, rather than urine or tissue, be used. In most cases, drug, or metabolites are measured in serum or plasma. However, in certain cases, whole blood may be more appropriate for analysis. Blood samples be drawn at appropriate times to describe the absorption, distribution, and elimination phases of the drug.

• Data from subjects who experience emesis during the course of a BE study for immediate-release products be deleted from statistical analysis if vomiting occurs at or before 2 times median $T_{max}$.

The following pharmacokinetic information is recommended for submission:

• Plasma concentrations and time points

• Period, sequence, treatment effect

• $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{\text{max}}$, $T_{\text{max}}$, $\lambda z$, and $t_{1/2}$

• Intersubject, intrasubject, and/or total variability

• $C_{\text{min}}$ (concentration at the end of a dosing interval), $C_{\text{av}}$ (average concentration during a dosing interval), degree of fluctuation $[(C_{\text{max}}-C_{\text{min}})/C_{\text{av}}]$, and swing $[(C_{\text{max}}-C_{\text{min}})/C_{\text{min}}]$ if steady-state studies are employed.

In addition, it is recommended to provide the following statistical information for $AUC_{0-t}$, $AUC_{0-\infty}$, and $C_{\text{max}}$:

• Geometric mean

• Arithmetic mean

• Ratio of means

• Confidence intervals
The logarithmic transformed data of pharmacokinetic parameters should be used for BE demonstration.

The confidence interval (CI) values should not be rounded off; therefore, to pass a CI limit of 80 to 125, the value would be at least 80.00 and not more than 125.00.

Keeping in mind the global regulatory requirements, the present study was undertaken to evaluate whether the generic brand of rasagiline could replace safely and successfully the innovator/reference drug product. The objective was to prove bioequivalence between Ranbaxy’s test formulation and Teva Neuroscience’s innovator formulation. This study was a two way crossover, using healthy adult human subjects (N=18) and dosing was done under fasting conditions.

The discussion part was mainly divided into three sections:

- Clinical Part
- Bioanalytical Part
- PK/SAS Evaluation

**Clinical Part**

The present study was started with preparation of study protocol. Protocol described the objective, design, methodology, statistical considerations and organization of a trial. The purpose of protocol was to describe the study in detail and was focused around the specific objectives. The protocol was carefully designed to safeguard the health of the participants as well as answer specific research questions. The protocol described the types of subjects who participated in the study; the schedule of tests, procedures, medications, and dosages; and the length of the study.

The study protocol was presented before IRB for approval. An IRB/IEC is responsible to safeguard the rights, safety, and well-being of all trial subjects. IRB approval was ensured by obtaining the statement from the IRB that the trial protocol is organized and operates according to GCP and the applicable laws and regulations. Documented IRB approval/favorable opinion with date was obtained with a current copy of protocol, written informed consent form(s), any other written information to be provided to subjects, subject recruiting procedures, and documents related to payments and compensation available to the subjects, and any other documents that the IRB may have requested.
The clinical study was carried out in accordance with ICH Good Clinical Practices. This study was designed based on the known pharmacokinetics of the study drug, Rasagiline on the generally accepted standards for the conduct of bioequivalence studies.

Each of the subjects was required to read and understand the information before giving his consent to participate in the study by signing the informed consent form. The signed original copy was retained and one signed copy was given to the study subject for the record.

The study was conducted by using an open label; balanced, randomized, cross over design in healthy, male volunteers under fasting conditions. An open-label trial is a type of clinical trial in which both the researchers and participants know which treatment is being administered. Open-label trials may be appropriate for comparing two very similar treatments to determine which is most effective. Balanced, means that all subjects received the same number of treatments and that all subjects participate for the same number of periods. In fact, each subject receives all treatments. Balanced designs are useful in eliminating the possibility of Hawthorne effects. Randomization is of central importance in clinical trials. A good clinical trial minimizes variability of the evaluation and provides an unbiased evaluation of the intervention by avoiding confounding from other factors. Randomization insures that each patient has an equal chance of receiving any of the treatments under study. Randomization eliminates selection bias, it balances arms with respect to prognostic variables (Known and Unknown) and it also forms basis for statistical tests. Cross over design was selected because it produces within subject comparisons, whereas the parallel design produces between subject comparisons. In cross over design each subject act as his or her own control and they can produce statistically and clinically valid results with fewer subjects than would be required with a parallel design. Cross over design is able to discover small differences between interventions.

The clinical examination of the subjects was performed by a physician. Clinical examination with medical history was performed at screening and brief clinical examination after admission and at discharge in each period. Only subjects who had clinically normal laboratory profiles as well as fulfilled the inclusion and exclusion criteria were enrolled in the study. The clinical examination of all the subjects was found to be normal in both periods of the study.

A single oral dose of two tablets of rasagiline 1 mg of either test or two tablets of Azilect® of reference formulation was administered during each period of the study. The order of
receiving the test and reference products for each subject was determined according to a SAS generated randomization schedule. During the study, the assigned doses were administered under the supervision of trained study personnel. The oral cavity was checked after dosing to ensure compliance with the treatment.

The subjects were administered two tablets of 1 mg of rasagiline in order to ensure sensitivity of analytical method. If single tablet of 1 mg rasagiline taken then the plasma drug concentration was too low, this was in ng/ml. Rationale of taking two tablets instead of one was only the sensitivity of the validated analytical method. As per reported literature, the C_max for rasagiline 1 mg under fasting conditions is ~10 ng/ml and accordingly the desired LOQ for bioanalytical method should have been around 10 pg/ml which was difficult to achieve, hence it was decided to dose two tablets of rasagiline. The washout period selected in the present study was 7 days between two periods, which was chosen based on the half life of rasagiline. Rasagiline is a new molecule and all the studies reported in literature were conducted in caucasian population, there was no data reported on asian population. So be on the safer side we decided to take washout period of 7 days. In the present study no predose concentration were observed in any of the subjects in period two, which confirms that the washout period selected was adequate.

The present study was carried out in fasting condition. As per EMEA requirements, only a single study conducted in the fasting state is required assuming that it is the most sensitive condition to detect formulation differences. Administration of rasagiline with food does not have any pharmacodynamic affect on the product (Azilect Product Monograph). Therefore fasting condition was selected for the conduct of study.

Selection of sampling point is very crucial in the design of study. According to regulatory guidance the blood sampling period in single dose study of an immediate release product should extend to at last three elimination half lives. Sampling should be continued for a sufficient period to ensure that the area extrapolated from the time of the last measured concentration to infinite time is only a small percentage (normally less than 20%) of total AUC. There should be at least three sampling points during the absorption phase, three to four at the projected T_max and four points during the elimination phase. Considering this fact, extensive sampling timepoints were selected.

Since this study was on rasagiline used in parkinson’s disease, so specific inclusion and exclusion criteria was set for the study. Healthy subjects between the age range of 18-45
years were included. Subjects excluded from the study were those who had history of hypersensitivity to rasagiline mesylate and/or other related drugs like MAO inhibitors, history of hypertensive crisis precipitated by intake of tyramine rich foods and/ or medications, history of arthalgia, history of dyspepsia, history of depression, and history of recurrent episodes of headache. The subjects who didn’t fulfill the inclusion and exclusion criteria were not enrolled in the study.

In present study plasma was selected as biological matrix instead of whole blood. Because biological samples are extremely complex matrices comprised of many components that can interfere with good separations and good mass spectrometer signals, so selection of appropriate biological matrix is an important aspect of bioanalytical estimation. Plasma was selected as the biological matrix for the estimation of rasagiline. Reason being that rasagiline is present in plasma and harvesting of plasma is simpler than serum. Besides most of the compounds are found to be stable in plasma than the whole blood.

In present study both the test and reference drug were administered with 240 ml of water. US FDA guidance states that drug should be administered with 8 ounces (240 ml) of water, while EU guidance states that it should be taken with 150 ml water, ANVISA recommends with 200 ml of water, Japanese guidance recommends with 100-200 ml (normally 150 ml) of water. The quantity 240 ml comes from 8 ounce which is equivalent to 1 standard cup of water. It is assumed that normally a patient consumes 1 cup of water to take any orally administered formulation. Moreover, 240 ml is the optimal volume for the dissolution of a tablet in humans.

In the interest of subject’s safety and acceptable standards of medical care the investigators or medical officers were permitted to prescribe treatment(s) at their discretion, the details of which were recorded in concomitant medication form.

In a placebo controlled clinical trial by innovator on administration of 1mg/ day rasagiline tablets the adverse event with at least 2% difference over placebo reported were headache (14.1%), depression (5.4%), hallucination (1.3%), musculoskeletal pain (6.7%), neck pain (2.7%) and influenza (4.7%). At the dose 1 mg/day rasagiline was well tolerated.¹² Adverse event monitoring was done throughout the study. No adverse events were observed during the present study. The test and reference product were well tolerated by the study subjects in the study.
Bioanalytical Part-

In a pharmaceutical industry which develops generic versions of molecules, the timelines for conducting bioequivalence studies are very short. Mass spectrometry is preferred because of short run times and specificity. Hundreds of samples are analyzed everyday with thousands lying for analysis. Also the bioequivalence studies, which are submitted to various regulatory agencies, are to be conducted as per the regulatory requirements. The method used for analysis of plasma samples for BA/BE (bioavailability/bioequivalence) studies should not only be precise and accurate, but should be also completely validated with respect to current GLP requirements.

Analytical methods employed for the quantitative determination of drugs and their metabolites in biological samples are the key determinants in generating reproducible and reliable data which in turn are used in the evaluation and interpretation of bioavailability, bioequivalence, and pharmacokinetic findings. It is essential to employ well-characterized and fully validated analytical methods to yield reliable results which can be satisfactorily interpreted.

A number of HPLC (high performance liquid chromatography) methods (M V Lakshmi et al., 2010; Napa D. Raj et al., 2012; S Kathrivel et al., 2012; R Narendra Kumar et al. 2010; Venkatesh D et al., 2012; M Fernandez et al., 2009; K R Jayavarapu et al., 2012) for the estimation of rasagiline are available in the literature; run time varies from 3.0 to 12 minutes. Methods using mass spectrometry (Ma J. et al., 2008; Song M. et al., 2008; P R Ravi et al., 2012; R K Konda et al., 2012) as detection are also available in the literature. Since the HPLC methods have disadvantages of longer run times, LC-MS/MS as an instrument has become the method of choice.

It is usually recommended to develop and validate a method for analyte estimation in the same lab conditions where analysis has to be performed. In the present bioequivalence study, LC/MS/MS method was developed for the analysis of Rasagiline in human plasma using stable labelled rasagiline as the internal standard. The mobile phase was consisted of acetonitrile and 0.1 % formic acid. The sample preparation of plasma was done by liquid liquid extraction using methyl tert-butyl ether (MTBE). The separation was performed on Phenomenex: Luna 5µm, C18 column. The retention time for Rasagiline and IS was 2.3 mins. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode.
using the transition: m/z 172.2→m/z 117.2 for rasagiline, and m/z 175.2→m/z 117.2 for IS, respectively, with a dwell time of 300ms and 150ms respectively.

Advantage of our method over the methods in literature is that we used stable labelled rasagiline as the internal standard. An internal standard is meant to correct for variability in dilutions, evaporation, degradation, recovery, adsorption, derivatization, and instrumental parameters such as injection volume. With LC-mass spectrometry (MS) for quantitative bioanalysis, the purpose of an internal standard has become mainly to correct for errors of detection. This method offers other advantages in terms of a simple liquid–liquid extraction without any other cleanup procedures and with a short retention time (2.3 min), which is important for large batches of clinical investigation samples.

Regulatory guidelines state that selective and sensitive analytical methods for the quantitative evaluation of drugs and their metabolites (analytes) are critical for the successful conduct of preclinical and/or biopharmaceutics and clinical pharmacology studies. Full validation of a bioanalytical method should be carried out before use. Bioanalytical method validation includes all of the procedures that demonstrate that a particular method used for quantitative measurement of analytes in a given biological matrix, such as blood, plasma, serum, or urine, is reliable and reproducible for the intended use.

The fundamental parameters for validation include (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability.

In the present study the method was validated in terms of selectivity, precision, accuracy, linearity, recovery, dilution integrity, ruggedness and stability studies. Stability studies includes freeze-thaw, bench top, in-injector, long term and stock solution. Before the initiation of unknown plasma samples of a biostudy, validation was completed and all validation parameters met acceptance criteria. The acceptance criteria of international regulatory standards were followed. Selectivity using eight lots of plasma (which includes 6 normal, 1 haemolysed and 1 lipemic human plasma) was evaluated and none shows interferences at drug and internal standard retention times. Method was found to be precise and accurate. This was confirmed by four precision and accuracy batches. The intra assay precision was less than 4.4% and inter assay precision was less than 5.5%. intra assay accuracy was 96.6% to 101.8% and inter assay accuracy was 98.1% to 101.1%. Matrix effect evaluation was performed on six lots of plasma batches and matrix effect accuracy was
98.2% and CV (%) was 4.9%. Rasagiline recovery was 80.6% and was found consistent between low, middle and high concentration. Rasagiline was found stable during three freeze thaw cycles. Bench top stability was performed for four hour and rasagiline was found stable for that period. Rasagiline sample extract, when kept in auto-sampler at 5 °C rasagiline was found quiet stable for 72 h. Stock solution stability and long term stability were performed and both the results met the acceptance criteria.

During actual conduct of the study, all the above mentioned parameters were kept in mind. Subject samples did not exceed three freeze thaw cycles. Processing of each subject took 3-4 hours which is well within our bench top stability data. Auto-sampler time did not exceed 72 hr during subject analysis. From period I dosing till final analysis, the storage of samples does not exceed 50 days. No concomitant medication was used for all completed subjects. Samples from single subject were run against one calibration curve along with two sets of quality control samples (QC_Low, QC_Mid and QC_High) interspersed between subject samples. System suitability was run on each day before initiation of run. There were no batch failures and no identified repeats in the study. The final concentration data was used for pharmacokinetic and statistical analysis.

Regulatory guideline states assays of all samples of an analyte in a biological matrix should be completed within the time period for which stability data are available. A calibration curve should be generated for each analyte to assay samples in each analytical run and should be used to calculate the concentration of the analyte in the unknown samples in the run. The spiked samples can contain more than one analyte. An analytical run can consist of QC samples, calibration standards, and either all the processed samples to be analyzed as one batch or a batch composed of processed unknown samples of one or more volunteers in a study. The calibration (standard) curve should cover the expected unknown sample concentration range in addition to a calibrator sample at LLOQ. Estimation of concentration in unknown samples by extrapolation of standard curves below LLOQ or above the highest standard is not recommended. Instead, the standard curve should be redefined or samples with higher concentration should be diluted and reassayed. It is preferable to analyze all study samples from a subject in a single run. The QC samples in duplicate at three concentrations (one near the LLOQ, one in midrange, and one close to the high end of the range) should be incorporated in each assay run. The number of QC samples (in multiples of three) will depend on the total number of samples in the run. The results of the QC samples provide the basis of accepting or rejecting the run. At least four of every six QC samples should be within
15% of their respective nominal value. Two of the six QC samples may be outside the 15% of their respective nominal value, but not both at the same concentration.

**PK/SAS Evaluation**

Rasagiline is a highly soluble substance, according to the Biopharmaceutics Classification System. Rasagiline is rapidly absorbed following oral administration, with an absolute bioavailability of approximately 36%, indicating that rasagiline undergoes a significant first-pass metabolism. Maximum plasma concentrations of rasagiline (C_{max} ~10 ng/mL) are reached at approximately 0.5 hours after oral administration (2 mg) in healthy subjects. The pharmacokinetics of 2 mg rasagiline administered following a high fat meal or under fasted conditions was compared. Administration of rasagiline with a high fat meal resulted in a decrease in extent of absorption (C_{max}) and systemic exposure (AUC) by about 60% and 20%, respectively. These changes are considered clinically insignificant (since AUC is not substantially affected) and rasagiline may be administered with or without food.

A pharmacokinetic analysis of plasma conc. was performed using standard non-compartmental methods in WinNonlin®, Version 5.0.1, software. The mean pharmacokinetic parameters of T_{max} (hrs), C_{max} (ng/ml), AUC_{0-t} (ng.hr/ml), and AUC_{0-∞} (ng.hr/ml) after administration of two tablets of Rasagiline 1mg both for reference and test (Reference R, Test T) was determined.

Literature states the maximum plasma concentrations of rasagiline (C_{max} ~10 ng/mL) are reached at approximately 0.5 hours after oral administration (2 mg) in healthy subjects. The half life of Rasagiline was reported 1.5 to 3.0 hr.

In the present study, mean time to peak plasma concentrations i.e T_{max} were 0.425±0.133 h and 0.366±0.095 hr for the products test and reference respectively and the values were inline with reported literature. Mean peak plasma concentrations i.e C_{max} were 13.87 (±5.24) ng/mL and 14.98 (±2.26) ng/ml for the products test and reference respectively. Mean area under curve till last measurable concentration i.e. AUC_{0-t} were 11.35±4.11ng.h/mL and 11.40±1.86 ng.h/mL for the products test and reference respectively. Mean AUC_{0-∞} were 11.56±4.16 ng.h/mL and 11.59±1.89 ng.h/mL for the products test and reference respectively. Mean elimination half life were 2.75±1.35 and 2.54±0.87 hr for the products test and reference respectively and are within reported literature values.
In a multiple-dose studies by Thébault JJ et al. (2004) of orally administered rasagiline 2 mg in healthy subjects after daily dosing for 10 days, $C_{\text{max}}$ was reported 17.6 (3.5) ng/ml, $AUC_{0-24\text{h}}$ was 20 (4.8) ng.h/ml, time to reach $C_{\text{max}}$ i.e. $T_{\text{max}}$ was 0.4 (0.2) hr, and mean half life $t_{1/2}$ was 2.1 (1.1) hr. Since rasagiline is an irreversible MAO-B inhibitor, the plasma $t_{1/2}$ does not correlate with duration of symptomatic effect.

Statistical analysis was performed using SAS system for Windows, release 9.1.3 (SAS Institute Inc., USA). The summary statistics i.e. mean, co-efficient of variation, ratios of least square means (LSM) of the test and the reference product, 90% confidence intervals for log-transformed pharmacokinetic parameters were determined.

US FDA and other international regulatory agencies recommends that to establish BE the calculated 90% confidence interval should fall within a BE limit of 80% to 125% using logarithm transformed data of $C_{\text{max}}$ and $AUC$.

The 90% confidence interval for log transformed T/R ratios for $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were 74.73-100.81, 87.78-103.15 and 87.96-103.39 respectively. The ratios of mean expressed as percentage of LSM for the reference formulation (T/R %) for $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ were 86.80, 95.15 and 95.36 respectively. In present study the maximum concentration ($C_{\text{max}}$) in plasma after rasagiline administration attained by test T was lower than reference R. While in case of area under curve ($AUC$) the values obtained by test T are near about similar to that obtained by reference R. T/R ratio of least squares mean for $C_{\text{max}}$ and $AUC$ values of test T are within the stated regulatory bioequivalence range of 80-125% (FDA, DCGI). 90% confidence intervals for T/R ratio lies within the range of 80-125% in case of area under curve ($AUC$) but in case of $C_{\text{max}}$ it was below the lower limit.

p values were calculated for the pharmacokinetic parameters $C_{\text{max}}$ and $AUC$. The sequence effect was tested at the 10% level of significance using the subjects nested within sequence mean square as the error term. All other main effects were tested at the 5% level of significance against the residual error (mean square error) from the ANOVA model as the error term. There was no significant treatment effect for $C_{\text{max}}$ and $AUC$, while non significant period effect for $C_{\text{max}}$ and significant period effect for $AUC$. The sequence effect was significant for all pharmacokinetic parameters. In bioequivalence study the period and treatment effect have no major importance, while sequence effect has its importance therefore it is tested at the 10% level of significance. In present study no carryover effect was observed during predose sampling, suggests that sufficient washout period was taken between each
period of the study. Regulatory guideline states that wash out period should be ideally equal to or more than five half life’s of the moiety to be measured, the half life of our drug was 2.5 hr and washout period in present study was selected 7 days which is adequate. Therefore there will be no impact of significant sequence effect on the conclusion of study.

In the present study the power of the test for $C_{\text{max}}$ was low while for AUC it was within the acceptable limit of $\geq 80\%$. The power for $C_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ were 68.20\%, 99.22\% and 99.21\% respectively. The overall intra subject variability for $C_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ was 26.2, 13.9 and 14.0 respectively. Highly variable drug products are considered to be drugs and drug products exhibiting intra-subject variability greater than 30\% coefficient of variation in the pharmacokinetic measures, AUC and/or $C_{\text{max}}$. Due to this high variability, large sample size may be needed in BE studies to give adequate statistical power to meet FDA BE limits, and thus designing BE studies for HVDs is challenging. In present study the intra-subject variability was less than 30\%, suggesting that this drug does not fall under high variability category. Even though the power of AUC was adequate but it was little lesser for $C_{\text{max}}$. Based on both intra-subject variability and power, we can conclude that the conclusion drawn from the present study is accurate and chances of improvement in the results are minimal even if sample size is increased.

Even though the 90\% CI’s for $C_{\text{max}}$ T/R ratios are not falling within 80-125\% limit, but it is also a fact that $C_{\text{max}}$ is evaluated only from a single point and usually varies for many molecules. AUC is calculated taking in account many timepoints and represents total exposure. Drug products which are safe, the elevated $C_{\text{max}}$ values are not a concern. However, drug molecules which fall under NTI’s (narrow therapeutic index drugs), the $C_{\text{max}}$ (even though single value) is of much importance and may lead to toxicity.

Based on the above discussion we can say that the results do favour for using this test product (evaluated in this study) as an alternate therapy for Rasagiline reference drug because this molecule is a safe drug with minor adverse events. However as per regulatory requirements, this test product is not bioequivalent to reference one and hence may require re-formulation.