5.0 DISCUSSION

Epilepsy is a serious neurological disorder characterized by recurrent, spontaneous seizures. Up to one third of patients with epilepsy will have seizures refractory to all available pharmacologic treatments. In addition, 20-30% of epilepsy patients do not tolerate antiepileptic therapy because of the presence of intolerable neuropsychiatric adverse effects (LaRoche and Helmers, 2004).

In clinical practice, the goal is to maintain AED (Anti Epileptic Drug) concentrations within a target range with minimal fluctuation, thereby optimizing the benefit-to-risk ratio. Peak-to-trough variations found with IR (immediate release) AEDs can be minimized by two approaches: (1) increasing the dosing frequency of the IR formulation to lower the individual doses; and lowering the $C_{\text{max}}$ while raising the $C_{\text{min}}$; or (2) reformulating the AED treatment in a once-daily ER (extended release) preparation to minimize peak-to-trough drug concentration differences. The former strategy may adversely affect patient adherence while the latter may result in more convenience for the patient, and hence positively affect adherence (Leppik and Hovinga, 2013).

Various innovative drug delivery technologies (e.g., Microtrol technology, OROS, DiffCore, hydrophilic matrix) have been developed to minimize the fluctuations in plasma drug levels seen with IR products (Reed et al, 2010) like the Film-coated tablet technology used in Keppra XR (Levetiracetam extended release). Medications used to treat a range of disorders have been prepared using these technologies, and the resulting improvements in Pharmacokinetic profiles have increased patient adherence through simplified dosing schedules (Saini et al, 2009) and have enabled dose increases in an effort to achieve greater efficacy (Miller et al, 2004; Smith et al, 2004).

Poor adherence may be the most important cause of poorly controlled epilepsy. Being a once-daily formulation, Levetiracetam 1000 mg extended release tablet is a better alternative for epilepsy patients since because of the convenience of its once a day dosing, this formulation of Levetiracetam is expected to increase patient compliance and, given the relatively constant plasma concentrations, it may minimize concentration-related adverse effects (diminished incidences of neuropsychiatric adverse events) by eliminating the troughs and peaks of drug concentration in a patient’s blood plasma. Therefore, ultimately this 1000 mg extended release formulation of Levetiracetam can potentially lead to better adherence to the prescribed treatment, which is of critical importance in the overall management of a chronic illness like epilepsy.
In this regard, it is important to note that Keppra XR (Levetiracetam extended release) was approved by the U.S. Food and Drug Administration in September 2008 for use as adjunctive treatment for people with partial-onset seizures who are 16 years of age and older. In an indirect comparison using a meta-analytic approach, the safety profile of Levetiracetam Extended Release was compared to Immediate Release formulation. Researchers conducted a meta-analysis of Phase III data to determine whether Keppra XR is associated with any tolerability advantages versus the same daily dose of levetiracetam IR. While adverse events associated with levetiracetam IR were also observed with Keppra XR, patients treated with Keppra XR once-daily experienced statistically significantly lower rates of adverse events related to nervous system disorders (i.e., headache, somnolence and dizziness) versus levetiracetam IR twice-daily. Keppra XR-treated patients reported numerically lower rates of psychiatric disorders (i.e., nervousness, anxiety and depression) and nutrition/metabolism disorders. No other differences in rates of adverse events were statistically significant [Summary of Keppra XR Data Presented at 2008 American Epilepsy Society Annual Meeting, 2008].

Ideally, an extended release formulation should be bioequivalent to the corresponding immediate release form of the medication when given as directed. Such bioequivalence has advantages, especially in patients who wish to be switched to an extended release product (Leppik and Hovinga, 2013).

The present study was conducted for Indian regulatory submission and as per the ‘Requirements of modified release formulations unlikely to accumulate’ [CDSCO guidelines for the conduct of bioavailability and bioequivalence studies, 2005], when the modified release product is the first market entry of the modified release type, the reference formulation is normally the innovator’s immediate-release formulation and 90% confidence intervals for the ratio of geometric means (Test:Reference drug) of AUC (both AUC\(_{0-\tau}\) and AUC\(_{0-t}\)) determined using log-transformed data should generally be within the range 80 to 125% when the products are compared after single dose administration.

The objective of the present study was to compare the pharmacokinetic profile of a single oral dose of Levetiracetam 1000 mg extended release tablet manufactured by Ranbaxy Laboratories Limited with two oral doses of a Keppra\textsuperscript{TM} 500 mg tablet (each tablet containing Levetiracetam 500 mg) of UCB Pharma Inc., administered twelve hourly, in healthy, adult, male, human subjects under fed condition.
In the present study, an Ultra Performance Liquid Chromatography Mass Spectrometry method was developed and validated for the analysis of Levetiracetam in human K3EDTA plasma using Didanosine as internal standard.

The reference and test formulations of Levetiracetam were administered to 24 healthy, adult, male human, subjects under fed condition followed by estimation of Levetiracetam in human plasma using the validated UPLC MS (Ultra Performance Liquid Chromatography Mass Spectrometry) assay.

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. Many variables affect the analysis of analytes in biological matrix; such as endogenous matrix components, metabolites, decomposition products; and during the actual conduct of the study, concomitant medication and other exogenous xenobiotics. Any method developed for the analysis of analytes in biological matrix must yield consistent results despite the variations in conditions during the course of a project.

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 this validation include (1) accuracy, (2) precision, (3) selectivity, (4) sensitivity, (5) reproducibility, and (6) stability [US Food and Drug Administration’s Guidance for Industry: Bioanalytical Method Validation, 2001].

In the present study, the chromatographic method for the analysis of Levetiracetam in plasma was developed and validated for selectivity, sensitivity, recovery, ruggedness, stability, within and between batch precision and accuracy. The limit of quantitation was 0.482 µg/mL for Levetiracetam. The between-batch or inter-day precision and accuracy ranged from 4.6% to 6.3% and 95.2% to 105.2% respectively. The precision results were within the acceptance criteria of ≤15% at low, middle and high QC concentrations and ≤ 20% at LOQQC concentration. The accuracy results were within the acceptance criteria of ±15% of the nominal concentration at low, middle and high QC concentrations and within ± 20% of the nominal concentration at LOQQC concentration. All other results of validation parameters were in acceptable range as recommended in various regulatory guidelines. So, the method was reliable, reproducible and accurate.
The clinical phase of the study was conducted as an open label, balanced, randomized, two-treatment, two-period, two-sequence, crossover bioequivalence study; and the test and reference formulations of the study drug (Levetiracetam) were administered alternately in the two periods of the study to twenty four (24) healthy, adult, human, male subjects under fed condition. The study was designed based on the known pharmacokinetics of the study drug (Levetiracetam) and complied with all the generally accepted standards, including the national and the international regulatory guidelines for the conduct of bioequivalence studies. Blood samples were collected at predefined time points. Study subjects were monitored throughout the course of the study. Both the test and reference formulations were well tolerated by the study subjects as evidenced by the fact that no adverse event was reported during the course of this study.

This was followed by estimation of Levetiracetam in plasma samples using the validated Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC-MS/MS) assay. Pharmacokinetic parameters $\text{AUC}_{0-t}$, $\text{AUC}_{0-\infty}$, $\text{AUC}_{0-24}$, $\text{AUC \% Extrap}$, $C_{\text{max}}$, $T_{\text{max}}$, $K_{\text{el}}$, $T_{1/2}$ were calculated for Levetiracetam. The Non-Compartmental Analysis for deriving pharmacokinetic parameters was performed with WinNonlin version 5.0.1. A statistical analysis was then performed the SAS system for Windows, release 9.1.3 (SAS Institute Inc., USA).

Pharmacokinetic data obtained from this study showed that the administration of the reference product R (Keppra™ 500 mg tablet) at a 12 hour interval between the 2 doses, showed a maximum concentration ($C_{\text{max}}$) of 26.55 (±2.99) µg /ml in plasma after single dose administration at 17.000 hours ($T_{\text{max}}$). Administration of the test product T (Levetiracetam 1000 mg extended release tablet) showed a maximum concentration ($C_{\text{max}}$) of 35.04 (±5.28) µg /ml in plasma after single dose administration at 20.000 hours ($T_{\text{max}}$).

The higher maximum concentration ($C_{\text{max}}$) in plasma observed in the present trial with the test product (35.04 µg /ml versus 26.55 µg /ml for the reference product) is comfortably within the wide therapeutic dose range of 12-46 µg /ml for Levetiracetam (Krasowski, 2010) and therefore unlikely to have any adverse impact clinically. Additionally, there were no adverse events during the conduct of the study which corresponded to the highest drug concentrations and both the 500 mg immediate release and 1000 mg extended release formulations were found to be safe & well tolerated.
The mean area under the curve (AUC₀₋₂₄) after 24 hrs was 251.60 (±30.77) Hr*ug/ml for the reference product R and 258.51 (±45.67) Hr*ug/ml for the test product T indicating that the administration of 1000 mg extended-release Levetiracetam tablets once daily produced comparable area under the plasma concentration versus time as did the administration of one 500 mg immediate-release tablet twice daily in fed conditions.

The Mean half-lives and Tₘₐₓ values for test product (Levetiracetam 1000 mg extended release tablet) were 8.30 (±0.75) hours and 20.00 hours, respectively. In comparison, the mean half-lives and mean Tₘₐₓ values for reference product (Two 500 mg immediate release tablets, administered at an interval of 12 hours; total dose 1000 mg) were 8.56 (±1.27) hours and 17.00 hours, respectively. These results are consistent with the reported plasma half-life of approximately 7 hours in extended-release Levetiracetam and the finding that the time to peak plasma concentration (Tₘₐₓ) is about 3 hours longer with extended-release Levetiracetam than with immediate-release tablets [US Prescribing Information, KEPPRA XR (Levetiracetam Extended Release), UCB, Inc, 2012].

A recent bioequivalence study was conducted by Rouits et al in the year 2009 with the objectives to compare the relative bioavailability of Levetiracetam extended-release tablets (XR) with immediate release tablets (IR) following single and multiple dosing, to assess the food effect and the dose-proportionality of XR from 1000 to 3000mg. In this study, 1000 mg of levetiracetam XR (two 500 mg XR tablets) was found to be bioequivalent to two 500 mg immediate release (IR) tablets given 12 hours apart, with comparable maximal plasma concentrations (Cₘₐₓ) and area under the plasma concentration vs time (AUC). Under fasting conditions, the median time to maximal plasma concentration (Tₘₐₓ) was 4 hours for levetiracetam XR vs 0.9 hours for levetiracetam IR. Time to peak was further prolonged by 2.5 hours when the XR tablet was given after intake of a high-fat, high-calorie breakfast. However, the AUC and Cₘₐₓ were similar to those in the fasting state.

Based on the pharmacokinetic data obtained, the bioavailability of test product T (Levetiracetam 1000 mg extended release tablet; administered once) is similar to that of the reference product R (Keppra™ 500 mg tablet; administered twice at a gap of 12 hours between the two doses) in the present study conducted in healthy adult, male subjects under fed condition.
Statistical analyses of BE (Bioequivalence) data are typically based on a statistical model used for measurement of AUC and $C_{\text{max}}$. The model is a mixed-effects or two-stage linear model. For a conventional two-treatment, two-period, two-sequence (2 x 2) randomized crossover design, the statistical model typically includes factors accounting for the following sources of variation: sequence, subjects nested in sequences, period, and treatment/formulation. [US Food and Drug Administration's Guidance for Industry: Statistical Approaches to Establishing Bioequivalence, 2001].

A similar approach was used in the present study for the purpose of bioequivalence analysis wherein $C_{\text{max}}$, $\text{AUC}_{0 \to t}$, $\text{AUC}_{0 \to 24}$ and $\text{AUC}_{0 \to \infty}$ were considered as the primary variables. A separate ANOVA model was used to analyze each of the parameters.

After statistical analysis, the 90% confidence intervals for log transformed data for $\text{AUC}_{0 \to t}$ and $\text{AUC}_{0 \to 24}$ for the Test product T and Reference product R (T/R) were 96.33% and 101.58% respectively.

As mentioned earlier, 90% confidence intervals for the ratios of LSM (Least Square Means) for log transformed data were not applicable and were not calculated for $C_{\text{max}}$ and $\text{AUC}_{0 \to \infty}$ since the comparator product in this bioequivalence trial was an immediate release formulation and not an existing & approved modified release formulation.

Intra subject variability (expressed as % CV) reported for log-transformed data for Levetiracetam was 19.9, 8.2, 13.8 and 7.5 for $C_{\text{max}}$, $\text{AUC}_{0 \to t}$, $\text{AUC}_{0 \to 24}$ and $\text{AUC}_{0 \to \infty}$ respectively. Overall, the intra subject variability for both the test and reference products was less than 30%.

The power of the test (%) for $C_{\text{max}}$, $\text{AUC}_{0 \to t}$, $\text{AUC}_{0 \to 24}$ and $\text{AUC}_{0 \to \infty}$ were reported as 96.17, 100.00, 99.91 and 100.00 respectively. Accordingly, the present study confirms that the sample size was adequate since the power of all parameters were above 80%.
Based on the ANOVA results, no significant sequence and treatment effect was observed for ln-transformed PK parameters $C_{\text{max}}$, $AUC_{0-t}$, $AUC_{0-24}$ and $AUC_{0-\infty}$; and no significant period effect was observed for ln-transformed PK parameter $C_{\text{max}}$. A significant treatment effect was observed for ln-transformed PK parameters $C_{\text{max}}$, and significant period effect was observed for ln-transformed PK parameters $AUC_{0-t}$, $AUC_{0-24}$ and $AUC_{0-\infty}$. However, the observed significant treatment and period effect should not have any impact on the bioequivalence conclusion in a bioequivalence trial done in a crossover fashion like the present study. There was no predose concentration observed during the study, thereby implying that there was no carryover effect and the washout period used in the present study was adequate.