Chapter 5: Discussion
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Discussion

5.0 DISCUSSION

The present study was conducted with the Primary objective of assessing the bioequivalence of single oral dose of generic levofloxacin 500 mg tablets with Cravit 500 mg tablet (containing levofloxacin 500 mg) in healthy, adult, human subjects under fasting condition. The secondary objective was to monitor the safety of subjects.

In the present study, a ‘High Performance Liquid Chromatography Tandem Mass Spectrometric Method’ was developed and validated for the Estimation of Levofloxacin in Human K3EDTA Plasma using Levofloxacin-d8 as an Internal Standard. The method was validated in terms of selectivity, selectivity of analyte in presence of concomitant medication (Acetaminophen, Diclofenac, Amoxicillin and Clavulanic acid), sensitivity, linearity of response, carry-over effect in human plasma, precision and accuracy in human plasma, recovery, stability, re-injection reproducibility, dilution integrity, matrix effect, matrix factor, ruggedness, extended precision and accuracy and long term stability.

Selectivity evaluation of Levofloxacin and Levofloxacin-d8 (ISTD) was within the acceptance criteria. Selectivity of Levofloxacin and Levofloxacin-d8 (ISTD) in presence of four different drugs (Acetaminophen, Diclofenac, Amoxicillin and Clavulanic acid) did not show significant interfering peaks at the retention time of Levofloxacin and Levofloxacin-d8 (ISTD). The method was sensitive and the limit of quantitation was 30.8 ng/mL for Levofloxacin. The between-batch or inter-day
precision and accuracy at LOQ QC concentration for Levofloxacin were 3.80% and 97.77%, respectively. The calibration curve was shown to be linear from 30.8 ng/mL to 13073.2 ng/mL for Levofloxacin.

The precision results were within the acceptance criteria of % CV ≤15% at low, middle and high QC concentrations and ≤ 20% at LOQ QC concentration. The accuracy results were within the acceptance criteria of the mean concentrations being within ±15% of the nominal concentration at low, middle and high QC concentrations and within ±20% of the nominal concentration at LOQ QC 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-sequence, two-period, single-dose crossover bioequivalence study under fasting condition. The test and reference formulations of the study drug (levofloxacin) were administered alternately in the two periods of the study, to twenty eight (28) healthy, adult, human, male subjects under fasting condition. The study was designed based on the known pharmacokinetics of Levofloxacin. The study 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. The study drugs (both test and reference) were well tolerated by the study subjects. Two adverse events were reported, during the conduct of the study, which were not serious and resolved without sequelae.

The estimation of Levofloxacin in the samples collected during the study was done using the validated ‘High Performance Liquid Chromatography Tandem Mass...
Spectrometric Method assay. Pharmacokinetic parameters AUC<sub>0-t</sub>, AUC<sub>0→∞</sub>, AUC % Extrapolated, C<sub>max</sub>, T<sub>max</sub>, K<sub>e</sub>, T<sub>1/2</sub> were calculated for Levofloxacin. Non-Compartmental Analysis for deriving pharmacokinetic parameters was performed with WinNonlin version 5.0.1. PK software. A statistical analysis was then performed using the SAS software, version 9.1.3 (SAS Institute Inc., USA).

Pharmacokinetic data analysis of the concentrations, of analyzed samples of study subjects, after administration of the reference product R, provided the following results:

- The mean C<sub>max</sub> ± S.D. of all subjects was 7755.63 (±2091.263) ng/mL in plasma at a mean T<sub>max</sub> ± S.D. of 1.3839 (±0.88235) hours.
- The mean AUC<sub>0-t</sub> ± S.D. was 55031.90388 (±9348.587085) ng.h/mL.
- The mean AUC<sub>0→∞</sub> ± S.D. was 57411.55416 (±10144.842984) ng.h/mL.
- The mean AUC % Extrapolation ± S.D. was 4.03474 (±1.507335) %.
- Mean half-life (t<sub>1/2</sub>) ± S.D. was 7.81149 (±1.005810) hours
- Mean Elimination Rate Constant (K<sub>e</sub>) ± S.D. was 0.09020 (± 0.011924) hours<sup>-1</sup>

Pharmacokinetic data analysis of the concentrations, of analyzed samples of study subjects, after administration of the test product T, provided the following results:

- The mean C<sub>max</sub> ± S.D. of all subjects was 8293.86 (±2620.739) ng/mL in plasma at a mean time T<sub>max</sub> ± S.D. of 1.2976 (±0.85509) hours.
- The mean AUC<sub>0-t</sub> ± S.D. was 58135.18875 (±9828.009857) ng.h/mL.
- The mean AUC<sub>0→∞</sub> ± S.D. was 60584.31569 (±10604.506295) ng.h/mL.
- The mean AUC % Extrapolation ± S.D. was 3.94468 (±1.896302) %.
- Mean half-life (t<sub>1/2</sub>) ± S.D. was 7.75116 (±1.038091) hours
- Mean Elimination Rate Constant (K<sub>e</sub>) ± S.D. was 0.09093 (± 0.011813) hours<sup>-1</sup>
The mean terminal plasma elimination half-life of levofloxacin was within the range from approximately 7 to 8 hours in the study. Mean ± S.D. for terminal plasma elimination half-life were 7.81149 ±1.005810 hours and 7.75116 ± 1.038091 hours for the reference and test product respectively.

The mean $C_{\text{max}}$ ± S.D. values were 7755.63 (±2091.263) ng /mL and 8293.86 (±2620.739) ng /mL for the reference and test product respectively.

The Mean $T_{\text{max}}$ ± S.D. values were 1.3839 (±0.88235) hours and 1.2976 (±0.85509) hours for the reference and test products respectively.

Based on the pharmacokinetic data obtained, the test product [Levofloxacin 500 mg tablets (Loxof tablets 500 mg)] was found bioequivalent to reference product (Cravit® tablets 500 mg) in this study conducted in healthy adult male subjects under fasting condition. The study drugs (both test and reference) were well tolerated by the study subjects. Two adverse events were reported, during the conduct of the study, which were not serious and resolved without sequelae.

Statistical analyses of BE (Bioequivalence) data were based on a statistical model based on measurement of AUC and $C_{\text{max}}$. The model is 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.

In the present study, for the purpose of demonstrating bioequivalence, $C_{\text{max}}$, AUC$_{0\rightarrow t}$ and AUC$_{0\rightarrow \infty}$ were considered as the primary Pharmacokinetic parameters. ANOVA was used to analyze each of these parameters.
Bioequivalence Results and Discussion

On Statistical analyses, the ratios [ T/R(%) ] of least-squares means (with 90% confidence intervals) for the log transformed pharmacokinetic parameters, $C_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ were 105.60% (98.34% – 113.40%), 105.63% (103.30% – 108.02%) and 105.54% (103.20% – 107.94%) respectively, for Levofloxacin.

Intra-subject variability (expressed as % CV) reported for log-transformed data for Levofloxacin was 15.72 %, 4.90 %, 4.93 % for $C_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ respectively.

The power of the test (%) for $C_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ were reported as 99.86 %, 100.00 %, 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 treatment effect and period effect was observed for log-transformed PK parameter $C_{\text{max}}$ (P values for these effects was > 0.05 from the ANOVA model). No significant sequence effect was observed for log-transformed PK parameters $C_{\text{max}}$, $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$ (P values for these effects was > 0.1 from the ANOVA model). However, a significant treatment effect and period effect (P values for these effects were < 0.05 from the ANOVA model) was observed for log-transformed PK parameters $\text{AUC}_{0-t}$ and $\text{AUC}_{0-\infty}$. It should not have any impact on bioequivalence conclusion.

The mean $T_{\text{max}}$ values for reference and test products were 1.3839 hours and 1.2976 hours, respectively for Levofloxacin and statistically, no significant difference in $T_{\text{max}}$ was observed between the test and reference products for Levofloxacin.
The Predose plasma sample were ‘below limit of quantitation’ i.e. No predose plasma concentration of Levofloxacin could be detected during the study, thereby implying that there was no carryover effect and the washout period was adequate.

The 90% confidence intervals for the ratios of Test (T) and Reference (R) product averages (least-squares means) derived from the analysis of log transformed pharmacokinetic parameters $C_{\text{max}}$, $AUC_{0\rightarrow t}$ and $AUC_{0\rightarrow \infty}$ were within 80-125% for Levofloxacin. Based on these results, single oral dose of levofloxacin 500 mg tablet and Cravit 500 mg tablet (containing levofloxacin 500 mg) were demonstrated to be bioequivalent in healthy, adult, human male subjects under fasting condition.