5.0 DISCUSSION

The use of relatively inexpensive generic drugs is a topical theme in pharmaceutical policy and regulation internationally. The greater use of generic prescription medications has been widely advocated as a policy solution to rising healthcare costs (Kohl and Shrank, 2007; Kesselheim and Choudhry, 2008).

The essential attribute of generic drugs is that they cost less than their original brand equivalents. Public and private third-party payers therefore increasingly encourage or mandate the use of generics through measures such as generic prescribing and generic substitution (Jacobzone S, 2000). Reference pricing schemes, taking advantage of the price competition made possible by the market entry of generics, have been introduced, or are under consideration, in many countries. The encouragement of generics through reference-based pricing – now ‘one of the preferred models for drug expenditure control’ internationally (Lopez-Casanovas and Puig-Junoy 2000) – is based on the principle that a drug’s benefits should be compared systematically to alternative drug treatments.

Bioequivalence needs to be established when a new generic formulation is tested against the innovator’s marketed product.

The present study was undertaken to determine and compare the bioequivalence between a generic version of Esomeprazole magnesium which could replace safely and successfully the innovator drug product (NEXIUM® 40 mg). Hence, the bioequivalence study of esomeprazole magnesium for delayed release oral suspension (containing esomeprazole 40 mg) of Ranbaxy Laboratories Limited was conducted using NEXIUM®40 mg (esomeprazole magnesium) for delayed- release oral suspension (containing esomeprazole 40 mg) of AstraZeneca LP, as Reference product, following single dose oral administration in healthy, adult, human male subjects under fasting conditions.

The conduct of bioavailability studies in man requires that a drug product be administered to a group of individuals and that the time-course of the concentration of the drug in the blood be evaluated either directly or indirectly.
It is necessary, therefore, that there be available (i) analytical methods for
determining the concentration of the active ingredient in body fluids; (ii)
standardized procedures for administering the drug product and obtaining
appropriate blood and/or urine samples; and (iii) adequate methods for
statistical analysis and interpretation of the results.

In the present study, first a liquid chromatography mass spectrometric (LC-
MS/MS) method was developed for the analysis of Esomeprazole in plasma.
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 in
the actual 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 (Food and Drug Administration's Guidance
for Industry: Bioanalytical Method Validation).

The method developed for the analysis of Esomeprazole was validated for
selectivity, sensitivity, recovery, ruggedness, and stabilities. The limit of
quantitation was 9.78 ng/mL for Esomeprazole. The between batch precision
and accuracy at LOQQC concentration for Esomeprazole using internal
standard ratio method was 9.7% and 101.2%, respectively. This was within the
acceptance criteria of ≤20% and 80-120% for between batch precision and
accuracy, respectively at LOQQC concentration. This is in line with the FDA's
Guidance for Industry: Bioanalytical Method Validation. The calibration was
shown to be linear from 9.78 ng/mL to 4011.75 ng/mL for Esomeprazole.
Between batch precision and accuracy ranged from 4.1% to 9.7% and 101.2%
to 104.4% respectively. Hultman et al., (2007) described a LC-MS/MS method developed for quantitative determination of esomeprazole, and its two main metabolites 5-hydroxyesomeprazole and omeprazole sulphone in 25 _L human, rat, or dog plasma. The linearity range was 20–20,000 nmol/L for esomeprazole and omeprazole sulphone, and 20–4000 nmol/L for 5-hydroxyesomeprazole. The extraction recoveries ranged between 80 and 105%. The intra- and inter-day imprecision were less than 9.5% with accuracy between 97.7% and 100.1% for all analytes (Hultman et al., 2007).

All other results of validation parameters were in acceptable range as recommended in regulatory guidelines. The method was therefore reliable, reproducible, and accurate and validated for the purpose of this study.

In the second step, the test and reference formulations were administered in the clinical phase of the study comprising of eighteen (18) healthy, adult, human, male subjects under fasting condition. The study was conducted as an open label, balanced, randomized, two treatment, two period, single dose crossover study. The study was designed based on the known pharmacokinetics of the study drug, esomeprazole 40 mg on 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. No adverse event was reported during the course of study.

This was followed by estimation of esomeprazole in plasma samples using the validated liquid chromatography mass spectrometric (LC-MS/MS) assay. Pharmacokinetic parameters \( \text{AUC}_{0-t}, \text{AUC}_{0-24}, \text{AUC} \% \text{ Extrap}, \text{C}_{\text{max}}, \text{T}_{\text{max}}, \text{K}_{\text{el}}, \text{T}_{1/2} \) were calculated for esomeprazole using WinNonlin-PK Software, version 5.0.1. A statistical analysis was performed on plasma esomeprazole using the SAS system.

Pharmacokinetic profile in the present study revealed that maximum concentration mean \( \text{C}_{\text{max}} \) in plasma after administration of the test formulation, was 1304.50 \( \pm 431.44 \) ng/ml which was in close proximity to the \( \text{C}_{\text{max}} \) of the reference formulation, i.e. 1270.71 \( \pm 400.66 \) ng/ml.
For the test formulation, the area under the curve (AUC$_{0-t}$) was 3267.95 (±1642.43) ng.hr/ml and AUC$_{0-24}$ was 3287.17 (±1647.09) ng.hr/ml.

For the reference formulation, the area under the curve (AUC$_{0-t}$) was 3356.94 (±1634.99) ng.hr/ml and AUC$_{0-24}$ was 3377.01 (±1641.28) ng.hr/ml.

The 90% confidence intervals for log transformed data for $C_{max}$, AUC$_{0-t}$, AUC$_{0-24}$ and AUC$_{0-\infty}$ for the test product vs. reference (T/R) were 102.58% (90.97%–115.67%), 96.86% (87.20%–107.60%), 96.94% (87.31%–107.63%) and 96.88% (87.29%–107.54%) respectively.

Similar results were reported in a bioequivalence study conducted in healthy Bangladeshi male volunteers by Ullah et al.,(2010). The study was conducted as an open label, randomized, two way crossover study in 24 subjects comparing two formulations of Esomeprazole 40 mg.90% Confidence Interval for the test/reference ratios of the log transformed AUC$_{0-\infty}$ and $C_{max}$ were 92.92% (84.02%–102.76%) and 102.36% (85.96%–121.90%) respectively, which were within the FDA limits for establishing bioequivalence. No adverse events were reported by the subjects during the study.

Nazi M. et al. (2011) conducted a bioequivalence study between a single capsule formulation of esomeprazole 40 mg and acetylcalicylic acid 325mg and the monotherapies given separately. This was an open label, randomized, single dose,2-stage group sequential design,2-way crossover study in 49 healthy subjects.90 % CI for the ratio of AUC and $C_{max}$ of esomeprazole between single therapy and monotherapy was 97%(90.0%–104.0%) and 99% (90.0%–109.0% respectively and was concluded to be bioequivalent.

The intra subject variability for the pharmacokinetic parameters, $C_{max}$, AUC$_{0-t}$, AUC$_{0-24}$, and AUC$_{0-\infty}$ was reported for log-transformed data. Overall, the intrasubject variability (expressed as % CV) for both the test and reference products was less than 30%. Intra subject variability was 20.9, 18.2, 18.1 and 18.1 for $C_{max}$, AUC$_{0-t}$ AUC$_{0-24}$ and AUC$_{0-\infty}$ respectively.

The power of test was more than 80% for all pharmacokinetic parameters as recommended in the regulatory guidelines.

The BE study should have 80 or 90% power to conclude bioequivalence.
between the two formulations (Anderson and Hauck, 1990). Power of the test for Cmax, AUC0-1, AUC0-24 and AUC0-∞ was found to be 86.11%, 93.41%, 93.58% and 93.70% respectively. This shows the sample size considered for this study was adequate to conclude bioequivalence.

To establish bioequivalence, the calculated 90% Confidence Interval for AUCs and Cmax should fall within the bioequivalence range, usually 80 – 125 % (FDA, DCGI Guidances on Bioavailability and Bioequivalence studies).

Two drug products will be considered bioequivalent drug products if they are pharmaceutical equivalents or pharmaceutical alternatives whose rate and extent of absorption do not show a significant difference when administered at the same molar dose of the active moiety under similar experimental conditions, either single dose or multiple dose.

Based on the results obtained in this study, the test formulation, Esomeprazole magnesium for delayed release oral suspension (containing esomeprazole 40 mg) of Ranbaxy Laboratories Limited, India are bioequivalent with the reference formulation, Nexium ® 40 mg (esomeprazole magnesium) for delayed release oral suspension (containing esomeprazole 40 mg) of AstraZeneca LP, in healthy, adult, human male subjects under fasting condition.