Chapter 5
Pharmacokinetic and Statistical Analysis
5.1 Introduction

The objective of this chapter was the application of the methods developed in previous chapters for the pharmacokinetic analysis of total, unconjugated and conjugated EZM in human plasma samples. A bioequivalence study was designed and conducted to evaluate the comparative oral bioavailability between the generic and branded drug product of EZM. The comparative oral bioavailability was evaluated between the single dose of Ezetimibe Tablets 10 mg (Each tablet contains 10 mg EZM) manufactured by Macleods Pharmaceuticals Ltd., India with Zetia® Tablets 10 mg (Each Tablet contains 10 mg EZM) manufactured by Schering Corporation, USA. The study was performed in healthy, adult, human subjects under fasting and fed conditions. The study was also aimed at monitoring the safety and tolerability of single oral dose of EZM Tablets 10 mg in the given study conditions. Primary pharmacokinetic parameters like $C_{\text{max}}$, AUC$_{0-t}$ and AUC$_{0-\infty}$ and secondary pharmacokinetic parameters like $T_{\text{max}}$, $T_{1/2}$ and $K_{el}$ were calculated.

The chapter consists of bioequivalence study design and conduct, total EZM estimated by HPLC, total EZM estimated by LC-MS/MS, unconjugated EZM estimated by LC-MS/MS and conjugated EZM estimated by LC-MS/MS. HPLC is used to assess total EZM concentrations from 6 healthy volunteers under fasting conditions as per the protocol given in section 5.2. LC-MS/MS has been applied for the estimation of total and unconjugated EZM, from 12 healthy human subjects under fasting and fed conditions as per the protocol mentioned in following section.

5.2 Bioequivalence Study Design and Conduct

5.2.1 Study Design

The study was an open label, balanced, analyst blind, randomized, two-treatment, two-period, two-sequence, single dose crossover bioequivalence study on 12 healthy, adult, human subjects under fasting conditions. Similarly, the study was conducted under fed conditions.
5.2.2 Investigational Products

**Test formulation (A)**: Ezetimibe Tablets (Each tablet contains 10 mg EZM)
- Batch number: VBP (1114) 054D
- Manufacturing Date: March 2011
- Expiry Date: February 2013
- Manufactured by: Macleods Pharmaceuticals Ltd., India
- Dose: 1 tablet
- Mode of Administration: Administered orally with 240 mL of drinking water.

**Reference formulation (B)**: Zetia® Tablets (Each tablet contains 10 mg EZM)
- Lot number: 9EZP587B1
- Manufacturing Date: Not Available
- Expiry Date: November 2012
- Manufactured by: Schering Corporation, USA
- Distributed by: Merck/Schering-Plough Pharmaceuticals, USA
- Dose: 1 tablet
- Mode of Administration: Administered orally with 240 mL of drinking water.

5.2.3 Ethical Conduct of the Study

The study was conducted in accordance with the principles of the Declaration of Helsinki, ICH GCP, National Regulations (ICMR Guidelines), Indian GCP, WHO
guidelines, South African guidelines (MCC) and schedule Y of Indian Drugs and Cosmetics Act.

5.2.4 Study Population

5.2.4.1 Number of Subjects

The numbers of subjects admitted for the study were 12.

5.2.4.2 Selection Criteria

Healthy human subjects within the age range of 18 to 45 years with body-mass index (BMI) between 18.50 kg/m$^2$ and 29.99 kg/m$^2$ (both inclusive) with body weight not less than 50 kg for males and 45 kg for females were selected. During the screening and complying with inclusion and exclusion criteria, the subjects underwent normal physical examination and were checked for any significant disease, clinically significant laboratory values and clinically significant medical history.

5.2.4.3 Subject information and consent

Before admission of volunteers, on the pre-study day, they were given a verbal presentation of the information on the study with a written document in the language that they can understand best. The document described the purpose, procedures and risks of the study together with a description of obligations of the subjects. Volunteers gave their written consent for participating in the study by signing the informed consent form.

5.2.5 Study Conduct

The work was approved and subjected to review by Independent Ethics Committee, an independent body comprising of members-physician, pharmacologist, social worker, lawyer, educationist, philosopher and a layperson.
5.2.6. Justification for Study Design

USFDA has recommended the two way crossover study for EZM in its draft guidance, hence the same design was used (Draft Guidance on Ezetimibe, USFDA, 2008). The half life of EZM is around 22 hours hence the sampling points were selected up to 96 hours. The study is recommended in both fasting and fed conditions to check its effect on the rate and extent of absorption of drug.

5.2.7 Study Conditions

5.2.7.1 Dose Administration

An oral dose of Reference formulation (R) and Test formulation (T) was administered as per the randomization schedule. Subjects received the alternate treatment in both the periods each of fasting and fed state, at the end of the study. An oral dose of reference or test formulation was administered at 0.00 hour during each period with 240 mL of water at room temperature.

1) Fasting Conditions: The dose was administered after an overnight fast of 10 hours in each period. Fasting was continued for four hours post-dose later which the meals were provided at specified intervals. Drinking water was disallowed for one hour pre-dose and one hour post-dose.

2) Fed Conditions: The dose was administered after a high fat breakfast that was served to subjects after overnight fast of 10 hours in each period. The meals were provided after 4 hrs of dosing at specified intervals. Drinking water was disallowed for one hour post-dose.

5.2.7.2 Schedule for Blood sample Collection

Blood samples (1 × 5 mL) were collected in 5 mL blood collection tube containing K$_2$EDTA as an anticoagulant. The venous blood samples were withdrawn pre-dose and at 0.33, 0.67, 1.00, 1.33, 1.67, 2.00, 2.33, 2.67, 3.00, 4.00, 5.00, 6.00, 7.00, 8.00, 9.00, 10.00, 11.00, 12.00, 14.00, 18.00, 24.00, 48.00, 72.00 and 96.00 hours post-dose.
The blood samples collected at each time point was centrifuged at 4°C at 4000 rpm for 10 minutes to separate plasma. The separated plasma was transferred in prelabeled polypropylene tubes and stored at -20°C until analysis.

The washout period of 14 days was kept from the completion of dosing between two periods.

### 5.2.7.3 Safety Assessment

In each period, subject questionnaire and vital signs (blood pressure, temperature and pulse rate) were done at the time of check-in, pre-dose and at 1.00, 3.00, 5.00, 9.00, 24.00, 48.00, 72.00 and 96.00 hours post-dose. The breath of the subjects was checked to see whether they have consumed alcohol or not at the time of check-in using breath alcohol analyzer. Medical examination, ECG and clinical laboratory tests were performed to cater to the post-study safety assessments.

### 5.2.7.4 Missing Samples

There were 10 missing samples including both fasting and fed studies, because the subjects did not report to facility for ambulatory visit.

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Period</th>
<th>Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fasting state</td>
<td></td>
</tr>
<tr>
<td>01</td>
<td>I</td>
<td>72 &amp; 96</td>
</tr>
<tr>
<td>03</td>
<td>I</td>
<td>96</td>
</tr>
<tr>
<td>10</td>
<td>I</td>
<td>72 &amp; 96</td>
</tr>
<tr>
<td>11</td>
<td>I</td>
<td>96</td>
</tr>
<tr>
<td>01</td>
<td>II</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Fed state</td>
<td></td>
</tr>
<tr>
<td>05</td>
<td>I</td>
<td>96</td>
</tr>
<tr>
<td>11</td>
<td>I</td>
<td>96</td>
</tr>
<tr>
<td>04</td>
<td>I</td>
<td>48</td>
</tr>
</tbody>
</table>
5.2.8 Bioanalytical Methodology

The high performance liquid chromatographic method developed in chapter 3, section I, was used to analyze total EZM in 6 healthy human subjects, studied under fasting conditions. The subjects used for the application of liquid chromatographic method were different from those used in the liquid chromatography-tandem mass spectrometric method.

The liquid chromatography-tandem mass spectrometric method developed for total EZM and unconjugated EZM in chapter 4, section I and II were used to analyze the human plasma samples received from 12 healthy subjects under fasting and fed state.

5.2.9 Statistical Methods (Garcia 2009)

The log-transformed pharmacokinetic parameters (C\text{max}, AUC\text{0-\text{t}} and AUC\text{0-\infty}) were analyzed using an unpaired student \(t\)-test in Graphpad Prism version 5.0 (Graphpad, USA). 90% confidence interval for the ratio of both the product averages (Geometric Mean) of C\text{max}, AUC\text{0-\text{t}} and AUC\text{0-\infty} for test was calculated.

i. \(C_{\text{max}}\) and \(T_{\text{max}}\) were obtained directly from the pharmacokinetic profiles of individual subjects.

ii. AUC\text{0-\text{t}} was calculated by the linear trapezoidal rule using the formula-

\[
AUC_{0-t} = \sum_{i=1}^{t} \left( \frac{C_i + C_i-1}{2} \right) (T_i - T_{i-1})
\]

iii. During the elimination phase, the plot of log transformed concentration of drug against time gives a straight line through the data. The slope of this regression line in the elimination phase is related to the elimination rate constant (\(\lambda\)) by the equation as follows-

\[
\lambda = 2.303 \times \text{slope}
\]
iv. The elimination half-life of the drug \((T_{1/2})\) was calculated by using the formula –

\[
T_{1/2} = \ln 2 / \lambda = 0.693 / \lambda
\]

v. AUC\(_{0-\infty}\) which is the total amount of drug present in the plasma was obtained by extending the ‘plasma concentration over time’ profile to infinity. Assuming that the exponential elimination process will continue beyond the last observed concentration at time \(t\), the extended area after \(t\) is \(C_T / \lambda\). This gives -

\[
\text{AUC}_{0-\infty} = \text{AUC}_{0-t} + \text{AUC}_{t-\infty} = \text{AUC}_{0-t} + C_T / \lambda
\]

Where,

- \(C_T\) is last measurable concentration at time ‘\(T\)’
- \(\lambda\) is elimination rate constant

vi. Mean Square Error (MSE) is the ratio of sum of squares to the degree of freedom (DF), multiplied by 2. Sum of Squares is given by the equation –

\[
SS = \sum (x - \bar{x})^2
\]

Therefore, MSE = \((SS/DF) \times 2\)

vii. 90\% Confidence Interval (CI) was calculated by the use of following formula –

\[
90\% \text{ CI} = F \pm t_{(0.1, n_1 + n_2 - 2)} \times \sqrt{\text{Sigma}^2(d) \times (1/n_1 + 1/n_2)}
\]

Where,

- \(F\) = Least Square Mean of treatment A – Least Square mean of treatment B
- \(t\) value is obtained in \(t\)-student tables with 0.1 alpha and \(n_1 + n_2 - 2\) degrees of freedom
- \(\text{Sigma}^2(d)\) is the ratio of sum of squares to the degree of freedom.
viii. Intra-subject Coefficient of Variation (CV) was calculated using the formula –

$$\text{Intra – subject CV} = 100 \times \sqrt{\text{MSE}} - 1$$

5.3 Results and Discussion

5.3.1 Total EZM estimated by High Performance Liquid Chromatography (HPLC)

The method developed using HPLC was linear in the concentration range of 30 – 500 ng/mL. Thus, in order to quantitate nanogram levels below 30 ng/mL, a fixed concentration of 50 ng/mL of EZM was spiked in all the real samples. Thus, modifying the concentration of real samples allowed us to quantitate total EZM in the concentration range of 30 – 500 ng/mL. The concentrations obtained after back calculation, underwent subtraction of 39.5 (accounting for the recovery of the method), after which the final concentrations were subjected to pharmacokinetics and statistical analysis. Thus the extraction procedure used for the analysis of subject samples is as given below:

Final Extraction Procedure

- 50 µL of TAM (6000 ng/mL) was taken in pre-labelled glass centrifuge tube as an internal standard (IS), except in blank plasma samples wherein 50 µL of mobile phase was added.

- 50 µL of EZM (1000 ng/mL) was added, except in blank plasma samples wherein 50 µL of mobile phase was added.

- 950 µL of the thawed plasma samples of EZM-G, were added into the tubes and vortexed to mix.

- 2000 µL of 0.5 M sodium acetate solution (pH 4.50 with glacial acetic acid) was added and vortexed.
- 100 µL of 10% β-glucuronidase (final concentration in plasma; 1457 units/mL) solution was added and mixed thoroughly. The reaction mixture was incubated in water bath maintained at 50 - 55°C, for approximately 6 hours.

- 2000 µL of 0.1 M sodium borate solution (pH 9.80 with triethylamine) was added and mixed.

- The product (EZM) formed was extracted with 5.0 mL of tert-butyl methyl ether. Samples were thoroughly mixed for 90 seconds and centrifuged for 10 minutes at 3000 rpm.

- The organic layer of 4.5 mL was separated and evaporated to dryness at 40°C using stream of nitrogen gas.

The samples were reconstituted with 100 µL of mobile phase. Samples were injected into the HPLC system for analysis.

5.3.1.1 Pharmacokinetic Profiles

Figure 5-1A: Mean ± SEM Plasma Concentrations of Total EZM under fasting conditions in 6 healthy human subjects
5.3.1.2 Statistical Outlier(s)

Subject outliers are defined as subjects having discordant values of one or more pharmacokinetic parameters when compared with other values for the rest of the subjects in a study. The existence of an outlier could be indicative of the product failure or presence of subpopulation wherein the relative bioavailability of the two products is markedly different than it is for the majority of the population. In such case, the two products might not be comparable for the outlier detected, even though they might be bioequivalent in the majority of the population (Guidance for Industry-Statistical approaches to establishing bioequivalence, USFDA, 2001).

Grubb’s test was carried out to detect outliers for pharmacokinetic parameters like $C_{\text{max}}$, $AUC_{0-t}$ and $AUC_{0-\infty}$. Subject # 5 was detected to be an outlier for $AUC_{0-t}$ and $AUC_{0-\infty}$ at significance level of 0.05.

5.3.1.3 Pharmacokinetic Parameters

Table 5-1A: Pharmacokinetic parameters for total EZM estimated by HPLC including statistical outlier (subject # 5)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>Arithmetic Mean (AM)</th>
<th>Standard Deviation (SD)</th>
<th>Coefficient of Variance (%CV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>52.58</td>
<td>15.00</td>
<td>28.54</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hrs.)</td>
<td>1.83</td>
<td>1.11</td>
<td>60.68</td>
</tr>
<tr>
<td>$AUC_{0-t}$ (ng.hr/mL)</td>
<td>917.74</td>
<td>832.74</td>
<td>90.74</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (ng.hr/mL)</td>
<td>1075.22</td>
<td>1057.42</td>
<td>98.34</td>
</tr>
<tr>
<td>$K_e$</td>
<td>0.0370</td>
<td>0.0100</td>
<td>26.96</td>
</tr>
<tr>
<td>$T_{1/2}$ (hrs.)</td>
<td>19.98</td>
<td>5.68</td>
<td>28.40</td>
</tr>
</tbody>
</table>
Table 5-1B: Pharmacokinetic parameters for total EZM estimated by HPLC excluding statistical outlier (subject #5)

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>AM</th>
<th>SD</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (ng/mL)</td>
<td>47.86</td>
<td>10.69</td>
<td>22.34</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hrs.)</td>
<td>1.93</td>
<td>1.21</td>
<td>62.72</td>
</tr>
<tr>
<td>$\text{AUC}_{0-t}$ (ng.hr/mL)</td>
<td>580.49</td>
<td>117.36</td>
<td>20.22</td>
</tr>
<tr>
<td>$\text{AUC}_{0-\infty}$ (ng.hr/mL)</td>
<td>647.56</td>
<td>161.28</td>
<td>24.90</td>
</tr>
<tr>
<td>$K_{\text{el}}$</td>
<td>0.0395</td>
<td>0.0089</td>
<td>22.46</td>
</tr>
<tr>
<td>$T_{1/2}$ (hrs.)</td>
<td>18.34</td>
<td>4.51</td>
<td>24.58</td>
</tr>
</tbody>
</table>

Calculations of Pharmacokinetic Parameters:

Note: Calculations to estimate pharmacokinetic parameters are described for volunteer # 1. Likewise the calculations were carried out for the rest of the volunteers in current chapter. Later AM, SD and %CV were calculated.

i. $C_{\text{max}}$ and $T_{\text{max}}$ were obtained directly from the pharmacokinetic profile. Therefore from Figure 5-1B, the $C_{\text{max}}$ obtained was 59.17 ng/mL at $T_{\text{max}}$ of 1.33 hours. Likewise $C_{\text{max}}$ and $T_{\text{max}}$ were calculated for other five volunteers and mean was reported.

Figure 5-1B: Plasma concentration time curve of total EZM in volunteer # 1, under fasting conditions
ii. \( \text{AUC}_{0-t} \) was calculated by the linear trapezoidal rule using the formula as given in section 5.2.9. Using the given formula, \( \text{AUC}_{0-t} \) was calculated in a table given below-

**Table 5-1C: Calculation of \( \text{AUC}_{0-t} \) for volunteer # 1**

<table>
<thead>
<tr>
<th>Time 'T' (hr)</th>
<th>Concentration 'C' (ng/mL)</th>
<th>Delta Time ( T_i - T_{i-1} )</th>
<th>Average of concentration ( \frac{C_i + C_{i-1}}{2} )</th>
<th>([\text{AUC}] = \Delta T \times \text{Average of concentration} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>0.33</td>
<td>23.56</td>
<td>0.33</td>
<td>11.78</td>
<td>3.89</td>
</tr>
<tr>
<td>0.67</td>
<td>18.52</td>
<td>0.34</td>
<td>21.04</td>
<td>7.15</td>
</tr>
<tr>
<td>1.00</td>
<td>7.42</td>
<td>0.33</td>
<td>12.97</td>
<td>4.28</td>
</tr>
<tr>
<td>1.33</td>
<td>59.17</td>
<td>0.33</td>
<td>33.29</td>
<td>10.99</td>
</tr>
<tr>
<td>1.67</td>
<td>30.48</td>
<td>0.34</td>
<td>44.82</td>
<td>15.24</td>
</tr>
<tr>
<td>2.00</td>
<td>5.53</td>
<td>0.33</td>
<td>18.01</td>
<td>5.94</td>
</tr>
<tr>
<td>2.50</td>
<td>18.92</td>
<td>0.50</td>
<td>12.23</td>
<td>6.11</td>
</tr>
<tr>
<td>3.00</td>
<td>22.85</td>
<td>0.50</td>
<td>20.89</td>
<td>10.44</td>
</tr>
<tr>
<td>3.50</td>
<td>27.52</td>
<td>0.50</td>
<td>25.18</td>
<td>12.59</td>
</tr>
<tr>
<td>4.00</td>
<td>54.08</td>
<td>0.50</td>
<td>40.80</td>
<td>20.40</td>
</tr>
<tr>
<td>4.50</td>
<td>36.94</td>
<td>0.50</td>
<td>45.51</td>
<td>22.76</td>
</tr>
<tr>
<td>5.00</td>
<td>46.47</td>
<td>0.50</td>
<td>41.71</td>
<td>20.85</td>
</tr>
<tr>
<td>5.50</td>
<td>12.97</td>
<td>0.50</td>
<td>29.72</td>
<td>14.86</td>
</tr>
<tr>
<td>6.00</td>
<td>25.25</td>
<td>0.50</td>
<td>19.11</td>
<td>9.55</td>
</tr>
<tr>
<td>7.00</td>
<td>8.28</td>
<td>1.00</td>
<td>16.77</td>
<td>16.77</td>
</tr>
<tr>
<td>8.00</td>
<td>26.75</td>
<td>1.00</td>
<td>17.51</td>
<td>17.51</td>
</tr>
<tr>
<td>9.00</td>
<td>24.55</td>
<td>1.00</td>
<td>25.65</td>
<td>25.65</td>
</tr>
<tr>
<td>10.00</td>
<td>21.54</td>
<td>1.00</td>
<td>23.04</td>
<td>23.04</td>
</tr>
<tr>
<td>12.00</td>
<td>14.89</td>
<td>2.00</td>
<td>18.22</td>
<td>36.43</td>
</tr>
<tr>
<td>14.00</td>
<td>13.05</td>
<td>2.00</td>
<td>13.97</td>
<td>27.94</td>
</tr>
<tr>
<td>16.00</td>
<td>5.16</td>
<td>2.00</td>
<td>9.10</td>
<td>18.20</td>
</tr>
<tr>
<td>24.00</td>
<td>2.55</td>
<td>8.00</td>
<td>3.86</td>
<td>30.84</td>
</tr>
<tr>
<td>48.00</td>
<td>1.86</td>
<td>24.00</td>
<td>2.20</td>
<td>52.92</td>
</tr>
<tr>
<td>72.00</td>
<td>1.01</td>
<td>24.00</td>
<td>1.43</td>
<td>34.33</td>
</tr>
</tbody>
</table>

\[ \sum = 448.70 \]
\[ AUC_{0-72} = \sum_{0}^{72} AUC = 448.70 \text{ ng.hr/mL} \]

iii. The elimination rate constant (\(\lambda\)) is calculated as described in section 5.2.9. From figure 5-1C, slope is 0.0173. Thus, \(\lambda = 2.303 \times 0.0173 = 0.0398\)

![Plasma concentration-time curve](image)

**Figure 5-1C: Elimination phase of the plot of log transformed concentration of drug against time for volunteer # 1, under fasting conditions**

iv. The elimination half-life of the drug (\(T_{1/2}\)) was calculated by using the formula –

\[ T_{1/2} = \frac{0.693}{\lambda} \]

\[ = \frac{0.693}{0.0398} \]

\[ = 17.39 \text{ hrs.} \]

v. \(AUC_{0-\infty} = AUC_{0-72} + C_{72} / \lambda \)

\[ = 448.70 + \left(\frac{1.01}{0.0398}\right) \]

\[ = 474.05 \text{ ng.hr/mL} \]
5.3.1.4 Discussion

Figure 5-1A shows mean ± SEM plasma-concentration time profiles for 6 healthy human subjects under fasting conditions. The profiles exhibited multiple plasma peaks until 15 hours, later which elimination pattern is seen. The typical pharmacokinetic characteristic of multiple peak phenomenon in the plasma concentration-time profile is associated with enterohepatic circulation which apparently results in the prolongation of the elimination half-life (Lehr et al., 2009; Plusquellec et al., 1995), as evident from Table 5-1A and 5-1B. Thus, the pharmacological effect of drug and its metabolite is prolonged (Roberts et al., 2002; Shou et al., 2005). The plasma profile obtained for the five out of six subjects is in agreement with the findings reported by Yamamoto, et al. 2007. Figure 5-1A shows multiple secondary peaks in the concentration time profiles, which were observed at approximately 2 hours, 4 hours and 14 hours.

The pharmacokinetic parameters like C\text{max}, T\text{max} and T\text{1/2} were found to be comparable with those reported in the literature. The reported values of C\text{max} are 54.7 ± 20.7 ng/mL, 39.8 ± 17.4 ng/mL, 70.20 ng/mL with a T\text{max} of 1.3 ± 0.7 hour, 1 hour, 1 hour (Bae et al., 2012; Li et al 2006; Oliveira et al., 2006), respectively. The maximum concentration achieved by HPLC method for 6 healthy human subjects is 47.86 ± 10.69 ng/mL, at a T\text{max} of 1.9 ± 1.2 hours. The T\text{1/2} as obtained by Bae et al., 2012, is 15.5 ± 7.9 hour; equivalent to T\text{1/2} of 18.34 ± 4.51 hour, as obtained in the above method. The large deviations obtained for each of the pharmacokinetic parameter may be attributed to the large inter-individual variation shown by EZM (Hegele et al., 2005).

Therefore, the application of the HPLC method for EZM, in pharmacokinetics, is justified.
5.3.2 Total EZM estimated by Liquid Chromatography-Tandem Mass Spectrometric (LC-MS/MS) method under fasting and fed conditions

5.3.2.1 Pharmacokinetic Profiles

Figure 5-2A: Mean ± SEM Plasma Concentration of Total EZM under fasting conditions in 12 healthy human subjects, receiving treatment A & B

Figure 5-2B: Mean ± SEM Plasma Concentration of Total EZM under fed conditions in 12 healthy human subjects, receiving treatment A & B
5.3.2.2 Statistical Outlier(s)

No statistical outlier was found when Grubb’s test was applied for both the treatments, under fasting and fed state, for either of the treatments.

5.3.2.3 Pharmacokinetic Parameters

Table 5-2A: Pharmacokinetic parameters for total EZM estimated by LC-MS/MS method under fasting conditions

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hrs.)</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; (ng.hr/mL)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng.hr/mL)</th>
<th>K&lt;sub&gt;e&lt;/sub&gt;l</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>96.65</td>
<td>1.39</td>
<td>1079.14</td>
<td>1141.22</td>
<td>0.0522</td>
<td>14.75</td>
</tr>
<tr>
<td>SD</td>
<td>38.91</td>
<td>0.74</td>
<td>603.42</td>
<td>603.41</td>
<td>0.0182</td>
<td>4.82</td>
</tr>
<tr>
<td>%CV</td>
<td>40.26</td>
<td>53.92</td>
<td>55.92</td>
<td>52.87</td>
<td>34.90</td>
<td>32.72</td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>87.37</td>
<td>1.11</td>
<td>873.89</td>
<td>902.84</td>
<td>0.0664</td>
<td>11.78</td>
</tr>
<tr>
<td>SD</td>
<td>52.27</td>
<td>0.62</td>
<td>486.53</td>
<td>497.74</td>
<td>0.0289</td>
<td>3.74</td>
</tr>
<tr>
<td>%CV</td>
<td>59.82</td>
<td>56.23</td>
<td>55.67</td>
<td>55.13</td>
<td>43.56</td>
<td>31.80</td>
</tr>
</tbody>
</table>

Table 5-2B: Pharmacokinetic parameters for total EZM estimated by LC-MS/MS method under fed conditions

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hrs.)</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt; (ng.hr/mL)</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt; (ng.hr/mL)</th>
<th>K&lt;sub&gt;e&lt;/sub&gt;l</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>137.64</td>
<td>1.99</td>
<td>882.94</td>
<td>971.22</td>
<td>0.0598</td>
<td>16.17</td>
</tr>
<tr>
<td>SD</td>
<td>50.08</td>
<td>0.93</td>
<td>386.52</td>
<td>442.08</td>
<td>0.0359</td>
<td>9.39</td>
</tr>
<tr>
<td>%CV</td>
<td>36.38</td>
<td>46.59</td>
<td>43.78</td>
<td>45.52</td>
<td>60.10</td>
<td>58.10</td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>139.82</td>
<td>2.75</td>
<td>875.61</td>
<td>954.16</td>
<td>0.0408</td>
<td>19.35</td>
</tr>
<tr>
<td>SD</td>
<td>76.92</td>
<td>1.19</td>
<td>354.35</td>
<td>343.00</td>
<td>0.0159</td>
<td>7.08</td>
</tr>
<tr>
<td>%CV</td>
<td>55.01</td>
<td>43.56</td>
<td>40.46</td>
<td>35.94</td>
<td>38.82</td>
<td>36.59</td>
</tr>
</tbody>
</table>
Table 5-2C: Summary statistics of pharmacokinetic parameters of Total EZM under fasting conditions

<table>
<thead>
<tr>
<th>Product/Statistics</th>
<th>$C_{\text{max}}$</th>
<th>$\text{AUC}_{0-t}$</th>
<th>$\text{AUC}_{0-\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untransformed Treatment A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>96.65</td>
<td>1079.14</td>
<td>1141.22</td>
</tr>
<tr>
<td>% CV</td>
<td>40.26</td>
<td>55.92</td>
<td>52.87</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Untransformed Treatment B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>87.37</td>
<td>873.89</td>
<td>902.84</td>
</tr>
<tr>
<td>% CV</td>
<td>59.82</td>
<td>55.67</td>
<td>55.13</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ratio of Arithmetic Mean (% Bioavailability)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>110.62</td>
<td>123.48</td>
<td>126.40</td>
</tr>
<tr>
<td><strong>Ratio (%) for Mean AUC$<em>{0-t}$ to Mean AUC$</em>{0-\infty}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>94.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>96.79</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log transformed (Log$_{10}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>1.9562</td>
<td>2.9712</td>
<td>3.0052</td>
</tr>
<tr>
<td>Treatment B</td>
<td>1.8688</td>
<td>2.8768</td>
<td>2.8914</td>
</tr>
<tr>
<td><strong>Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>90.40</td>
<td>935.84</td>
<td>1012.04</td>
</tr>
<tr>
<td>Treatment B</td>
<td>73.92</td>
<td>753.00</td>
<td>778.75</td>
</tr>
<tr>
<td><strong>Ratio of Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>122.29</td>
<td>124.28</td>
<td>129.96</td>
</tr>
<tr>
<td><strong>90% Confidence Interval (A/B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit (%)</td>
<td>97.09</td>
<td>104.52</td>
<td>106.89</td>
</tr>
<tr>
<td>Upper limit (%)</td>
<td>122.67</td>
<td>115.55</td>
<td>117.48</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intra-subject CV (%)</td>
<td>15.91</td>
<td>6.78</td>
<td>6.39</td>
</tr>
<tr>
<td>Mean Square Error (MSE)</td>
<td>0.0250</td>
<td>0.0046</td>
<td>0.0041</td>
</tr>
</tbody>
</table>
Calculations for statistical analysis:

Note: Calculations are shown for $C_{\text{max}}$. Likewise calculations were done for AUC’s. Hereforth, the statistical analysis was done in the same manner as shown for Table 5-6.

i. Untransformed AM of $C_{\text{max}}$ for treatment A, was calculated by taking the mean of $C_{\text{max}}$ values obtained for 12 volunteers. Similarly AM was calculated for treatment B.

ii. Ratio of AM (% Bioavailability) = ($96.65/87.37$) × 100 = 110.62%.

iii. Log transformed ($\log_{10}$) for treatment A, was calculated by taking mean of $\log_{10}$ of $C_{\text{max}}$ values obtained for 12 volunteers. Similar calculations were done for treatment B.

iv. Geometric mean for treatment A is the antilog of the log transformed $C_{\text{max}}$ concentration following treatment A. Similar calculations were performed for treatment B.

v. Ratio of geometric mean = ($90.40/73.92$) × 100 = 122.29%.

vi. For the calculation of $F$, Least Square Mean for treatment A and B were calculated as follows-
Table 5-2D: Calculation of point estimate for $C_{\text{max}}$ of total EZM under fasting conditions

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sequence</th>
<th>$C_{\text{max}}$ Concentrations</th>
<th>Log$_{10}$ Concentration</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Period-1</td>
<td>Period-2</td>
<td>Period-1 (P1)</td>
</tr>
<tr>
<td>1</td>
<td>AB</td>
<td>63.16</td>
<td>77.47</td>
<td>1.8004</td>
</tr>
<tr>
<td>4</td>
<td>AB</td>
<td>182.83</td>
<td>127.37</td>
<td>2.2620</td>
</tr>
<tr>
<td>5</td>
<td>AB</td>
<td>66.65</td>
<td>39.14</td>
<td>1.8238</td>
</tr>
<tr>
<td>7</td>
<td>AB</td>
<td>147.50</td>
<td>85.67</td>
<td>2.1688</td>
</tr>
<tr>
<td>8</td>
<td>AB</td>
<td>71.41</td>
<td>94.60</td>
<td>1.8538</td>
</tr>
<tr>
<td>9</td>
<td>AB</td>
<td>122.43</td>
<td>93.02</td>
<td>2.0879</td>
</tr>
<tr>
<td>2</td>
<td>BA</td>
<td>210.00</td>
<td>107.62</td>
<td>2.3222</td>
</tr>
<tr>
<td>3</td>
<td>BA</td>
<td>26.10</td>
<td>54.71</td>
<td>1.4166</td>
</tr>
<tr>
<td>6</td>
<td>BA</td>
<td>30.73</td>
<td>69.24</td>
<td>1.4876</td>
</tr>
<tr>
<td>10</td>
<td>BA</td>
<td>66.33</td>
<td>74.26</td>
<td>1.8217</td>
</tr>
<tr>
<td>11</td>
<td>BA</td>
<td>58.85</td>
<td>109.12</td>
<td>1.7697</td>
</tr>
<tr>
<td>12</td>
<td>BA</td>
<td>139.20</td>
<td>90.92</td>
<td>2.1436</td>
</tr>
<tr>
<td>Arithmeti</td>
<td></td>
<td></td>
<td></td>
<td>1.9995</td>
</tr>
<tr>
<td>c mean</td>
<td></td>
<td></td>
<td></td>
<td>1.8269</td>
</tr>
</tbody>
</table>

Least Square Mean (LSM) of Treatment A = (Arithmetic mean of period-1 + Arithmetic mean of period-2) / 2

LSM of treatment A = (1.9995 + 1.9129) / 2

= 1.9562

LSM of Treatment B = (Arithmetic mean of period-1 + Arithmetic mean of period-2) / 2

LSM of treatment B = (1.8269 + 1.9107) / 2

= 1.8688
F = LSM of treatment A – LSM of treatment B

= 1.9562 – 1.8688

F = 0.0874

vii. From section 5.2.9, MSE = (SS/DF) × 2

Where SS = \sum (x-\bar{x})^2 which is calculated as follows—

Table 5-2E: Calculation of SS for C_{max} of total EZM under fasting conditions

<table>
<thead>
<tr>
<th>Log_{10} Concentration</th>
<th>x = (P2-P1)/2</th>
<th>Mean \bar{x}</th>
<th>(x-\bar{x})</th>
<th>(x-\bar{x})^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period-1 (P1)</td>
<td>Period-2 (P2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.8004</td>
<td>1.8891</td>
<td>0.0443</td>
<td>-0.0444</td>
<td>0.0887</td>
</tr>
<tr>
<td>2.2620</td>
<td>2.1051</td>
<td>-0.0785</td>
<td>-0.0341</td>
<td>0.0012</td>
</tr>
<tr>
<td>1.8238</td>
<td>1.5926</td>
<td>-0.1156</td>
<td>-0.0712</td>
<td>0.0051</td>
</tr>
<tr>
<td>2.1688</td>
<td>1.9328</td>
<td>-0.1180</td>
<td>-0.0736</td>
<td>0.0054</td>
</tr>
<tr>
<td>1.8538</td>
<td>1.9759</td>
<td>0.0611</td>
<td>0.1054</td>
<td>0.0111</td>
</tr>
<tr>
<td>2.0879</td>
<td>1.9686</td>
<td>-0.0597</td>
<td>-0.0153</td>
<td>0.0002</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.3222</td>
<td>2.0319</td>
<td>-0.1452</td>
<td>0.0430</td>
<td>-0.1882</td>
</tr>
<tr>
<td>1.4166</td>
<td>1.7381</td>
<td>0.1607</td>
<td>0.1177</td>
<td>0.0139</td>
</tr>
<tr>
<td>1.4876</td>
<td>1.8404</td>
<td>0.1764</td>
<td>0.1334</td>
<td>0.0178</td>
</tr>
<tr>
<td>1.8217</td>
<td>1.8708</td>
<td>0.0245</td>
<td>-0.0185</td>
<td>0.0003</td>
</tr>
<tr>
<td>1.7697</td>
<td>2.0379</td>
<td>0.1341</td>
<td>0.0911</td>
<td>0.0083</td>
</tr>
<tr>
<td>2.1436</td>
<td>1.9587</td>
<td>-0.0925</td>
<td>-0.1355</td>
<td>0.0184</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>\sum(x-\bar{x})^2</td>
<td>0.1249</td>
</tr>
</tbody>
</table>

Note: P1 and P2 values in above table are obtained from Table 5-2D.

Thus, SS = 0.1249

DF = (n_1+n_2-2) = 6+6-2 = 10

Therefore, MSE = (0.1249/10) × 2

MSE = 0.0250
viii. Intra−subject CV = \(100 \times \sqrt{e^{\text{MSE}} - 1}\)
\[= 100 \times \sqrt{e^{0.0250} - 1}\]
\[= 15.91\%\]

ix. From section 5.2.9,
\[90\% \text{ CI} = \left[ F \pm t_{(0.1, n_1+n_2-2)} \times \sqrt{\Sigma^2(d) \times \left(1/n_1 + 1/n_2\right)}\right] \times 100\]
Where,
\[F = 0.0874\]  
---(Refer point vi)
\[t_{(0.1, n_1+n_2-2)} = t_{(0.1, 10)} = 1.8125\]  
---(t-value is obtained in t-student table with 0.1 alpha and 10 DF)
\[\Sigma^2(d) = SS/DF = 0.1249/10 = 0.01249\]  
---(Refer point vii)
\[(1/n_1+1/n_2) = (1/6 + 1/6) = 0.3333\]

Therefore, 90% CI = \([0.0874 \pm 1.8125 \times \sqrt{0.01249 \times 0.3333}] \times 100\]
\[= [0.0874 \pm 1.8125 \times \sqrt{0.0042}] \times 100\]
\[= [0.0874 \pm 1.8125 \times 0.0645] \times 100\]
90% CI = \([0.0874 \pm 0.1170] \times 100\)

Upper Limit = (0.0874 + 0.1170) \times 100; Lower Limit = (0.0874 – 0.1170) \times 100

Upper Limit = 0.2044 \times 100; Lower Limit = -0.0296 \times 100

Since logarithmic values were used to calculate 90% CI, back transform the limits with \(e^{\text{Upper Limit}}\) and \(e^{\text{Lower Limit}}\)

Upper Limit = \(e^{0.2044} = 1.2267\)

Lower Limit = \(e^{-0.0296} = 0.9709\)

Thus, 90% Upper Limit = 1.2267 \times 100 = 122.67\%

and 90% Lower Limit = 0.9709 \times 100 = 97.09\%
Table 5-2F: Summary statistics of pharmacokinetic parameters of Total EZM under fed conditions

<table>
<thead>
<tr>
<th>Product/Statistics</th>
<th>$C_{\text{max}}$</th>
<th>AUC$_{0-t}$</th>
<th>AUC$_{0-\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untransformed Treatment A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>137.64</td>
<td>882.94</td>
<td>971.22</td>
</tr>
<tr>
<td>% CV</td>
<td>36.38</td>
<td>43.78</td>
<td>45.52</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Untransformed Treatment B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>139.82</td>
<td>875.61</td>
<td>954.16</td>
</tr>
<tr>
<td>% CV</td>
<td>55.01</td>
<td>40.46</td>
<td>35.94</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ratio of Arithmetic Mean (% Bioavailability)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>98.44</td>
<td>100.84</td>
<td>101.78</td>
</tr>
<tr>
<td><strong>Ratio (%) for Mean AUC$<em>{0-t}$ to Mean AUC$</em>{0-\infty}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>90.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>91.76</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log transformed (Log$_{10}$)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>2.1039</td>
<td>2.8945</td>
<td>2.9322</td>
</tr>
<tr>
<td>Treatment B</td>
<td>2.0847</td>
<td>2.9019</td>
<td>2.9492</td>
</tr>
<tr>
<td><strong>Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>127.02</td>
<td>784.33</td>
<td>855.46</td>
</tr>
<tr>
<td>Treatment B</td>
<td>121.53</td>
<td>797.81</td>
<td>889.61</td>
</tr>
<tr>
<td><strong>Ratio of Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>104.52</td>
<td>98.31</td>
<td>96.16</td>
</tr>
<tr>
<td><strong>90% Confidence Interval (A/B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit (%)</td>
<td>92.58</td>
<td>93.24</td>
<td>91.95</td>
</tr>
<tr>
<td>Upper limit (%)</td>
<td>112.24</td>
<td>105.68</td>
<td>105.12</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intra-subject CV (%)</td>
<td>13.06</td>
<td>8.48</td>
<td>9.06</td>
</tr>
<tr>
<td>Mean Square Error (MSE)</td>
<td>0.0169</td>
<td>0.0072</td>
<td>0.0082</td>
</tr>
</tbody>
</table>
5.3.2.4 Discussion

Figure 5-2A and 5-2B shows mean ± SEM plasma concentration of Total EZM, receiving treatment A and B, under fasting and fed conditions, respectively. The plasma concentration-time curves showed multiple peaks, as seen in section 5.3.1, and are in accordance with the findings of Ezzet et al., 2001. In case of fasting condition, for treatment A, the C\text{max} concentrations varied from 54.71 to 182.83 ng/mL while for treatment B, the C\text{max} concentrations ranged from 30.73 to 210 ng/mL. Thus, large standard deviations are observed which is a result of large inter-individual variation. The mean pharmacokinetic parameters obtained for treatment A and B are indicated in Table 5-2A. The C\text{max} and T\text{max} values obtained for both the treatments, i.e., rate of absorption, are identical. However, the extent of absorption, i.e., AUC\text{0-t} and AUC\text{0-∞} values differ for both the treatments. When statistically analyzed, the ratio of arithmetic mean of product A to arithmetic mean of product B, gives comparable result for C\text{max} and AUC\text{0-t} values. For AUC\text{0-∞} the ratio exceeds 125%. Also when the data is log-transformed, the ratio of geometric mean of product A to product B showed same fate for C\text{max}, AUC\text{0-t}, and AUC\text{0-∞}. The statistical analysis for pharmacokinetic parameters of total EZM under fasting conditions for treatment A and B are presented in Table 5-2C. The ratio of geometric mean of the pharmacokinetic parameters of product A to product B, gives comparable result for C\text{max} and AUC\text{0-t} while for AUC\text{0-∞} the ratio falls outside the acceptance criteria.

Similarly, in case of fed condition, for treatment A, the C\text{max} concentration varied from 46.76 to 218.74 ng/mL while for treatment B, the C\text{max} concentrations ranged from 50.09 to 285.71 ng/mL, thus indicating large inter-individual variation. The mean pharmacokinetic parameters obtained for treatment A, are comparable to those obtained after receiving treatment B as indicated in Table 5-2B. The statistical analysis for pharmacokinetic parameters of total EZM under fed conditions for treatment A and B are presented in Table 5-2F. Unlike the fasting state, fed state showed comparable values of C\text{max}, AUC\text{0-t}, and AUC\text{0-∞}, for both the products. When the pharmacokinetic parameters obtained in fed state are compared with those obtained in fasting state, the AUC’s match.
except for $C_{\text{max}}$, where a significant increase is seen in the fed state, as evident from Table 5-2C and 5-2F.

The ratio of AUC$_{0-t}$ to AUC$_{0-\infty}$ was found to be more than 80%, thus indicating that the schedule for blood sample collection was appropriate and the method sensitivity was adequate. The intra-subject variability observed for all the three pharmacokinetic parameters were less than 30%, indicating that the drug is not a highly variable drug (WHO 2010). The lower values obtained for MSE are an indication of greater power of analysis. The calculated 90% confidence interval for the ratios of geometric means of the measures for A and B product falls within a BE limit of 80 – 125%, for both fasting and fed state.

Thus the two products under investigation have comparable bioavailability under fasting and fed conditions.
5.3.3 Unconjugated EZM estimated by Liquid Chromatography-Tandem Mass Spectrometric Method under fasting and fed conditions

5.3.3.1 Pharmacokinetic Profiles

Figure 5-3A: Mean ± SEM Plasma Concentration of unconjugated EZM under fasting conditions in 12 healthy human subjects, receiving treatment A & B

Figure 5-3B: Mean ± SEM Plasma Concentration of unconjugated EZM under fed conditions in 12 healthy human subjects, receiving treatment A & B
5.3.3.2 Statistical Outlier(s)

Although, subject 8, receiving treatment B, under fasting condition, was detected to be an outlier, it is considered for the statistical analysis.

5.3.3.3 Pharmacokinetic Parameters

**Table 5-3A: Pharmacokinetic parameters for unconjugated EZM estimated by LC-MS/MS method under fasting conditions**

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C(_{\text{max}}) (pg/mL)</th>
<th>T(_{\text{max}}) (hrs.)</th>
<th>AUC(_{0-t}) (pg.hr/mL)</th>
<th>AUC(_{0-\infty}) (pg.hr/mL)</th>
<th>K(_{el})</th>
<th>T(_{1/2}) (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>4507.5</td>
<td>7.19</td>
<td>97184.79</td>
<td>99881.72</td>
<td>0.0585</td>
<td>12.90</td>
</tr>
<tr>
<td>SD</td>
<td>1741.11</td>
<td>4.69</td>
<td>35572.18</td>
<td>36679.25</td>
<td>0.0186</td>
<td>3.82</td>
</tr>
<tr>
<td>%CV</td>
<td>38.62</td>
<td>65.28</td>
<td>36.60</td>
<td>36.72</td>
<td>31.74</td>
<td>29.62</td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>4348.12</td>
<td>8.97</td>
<td>83719.26</td>
<td>86645.54</td>
<td>0.0645</td>
<td>12.27</td>
</tr>
<tr>
<td>SD</td>
<td>2394.75</td>
<td>4.68</td>
<td>40676.75</td>
<td>41384.19</td>
<td>0.0303</td>
<td>4.00</td>
</tr>
<tr>
<td>%CV</td>
<td>55.08</td>
<td>52.21</td>
<td>48.58</td>
<td>47.76</td>
<td>46.93</td>
<td>32.56</td>
</tr>
</tbody>
</table>

**Table 5-3B: Pharmacokinetic parameters for unconjugated EZM estimated by LC-MS/MS method under fed conditions**

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C(_{\text{max}}) (pg/mL)</th>
<th>T(_{\text{max}}) (hrs.)</th>
<th>AUC(_{0-t}) (pg.hr/mL)</th>
<th>AUC(_{0-\infty}) (pg.hr/mL)</th>
<th>K(_{el})</th>
<th>T(_{1/2}) (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>5608.85</td>
<td>3.22</td>
<td>79852.56</td>
<td>85077.90</td>
<td>0.0662</td>
<td>14.28</td>
</tr>
<tr>
<td>SD</td>
<td>2831.84</td>
<td>3.29</td>
<td>44748.42</td>
<td>49506.73</td>
<td>0.0372</td>
<td>8.20</td>
</tr>
<tr>
<td>%CV</td>
<td>50.48</td>
<td>102.27</td>
<td>56.04</td>
<td>58.18</td>
<td>56.34</td>
<td>57.39</td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>6007.58</td>
<td>3.64</td>
<td>75823.09</td>
<td>80219.29</td>
<td>0.0558</td>
<td>17.03</td>
</tr>
<tr>
<td>SD</td>
<td>3957.83</td>
<td>3.50</td>
<td>34369.44</td>
<td>37089.39</td>
<td>0.0426</td>
<td>8.79</td>
</tr>
<tr>
<td>%CV</td>
<td>65.88</td>
<td>96.26</td>
<td>45.32</td>
<td>46.24</td>
<td>76.51</td>
<td>51.63</td>
</tr>
</tbody>
</table>
Table 5-3C: Summary statistics of pharmacokinetic parameters of unconjugated EZM under fasting conditions

<table>
<thead>
<tr>
<th>Product/Statistics</th>
<th>$C_{\text{max}}$</th>
<th>$\text{AUC}_{0-t}$</th>
<th>$\text{AUC}_{0-\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untransformed Treatment A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>4507.50</td>
<td>97184.79</td>
<td>99881.72</td>
</tr>
<tr>
<td>% CV</td>
<td>38.62</td>
<td>36.60</td>
<td>36.72</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Untransformed Treatment B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>4348.12</td>
<td>83719.26</td>
<td>86645.54</td>
</tr>
<tr>
<td>% CV</td>
<td>55.08</td>
<td>48.58</td>
<td>47.76</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ratio of Arithmetic Mean (% Bioavailability)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>103.66</td>
<td>116.08</td>
<td>115.28</td>
</tr>
<tr>
<td><strong>Ratio (%) for Mean $\text{AUC}<em>{0-t}$ to Mean $\text{AUC}</em>{0-\infty}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>97.29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>96.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log transformed (Log_{10})</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>3.6202</td>
<td>4.9583</td>
<td>4.9700</td>
</tr>
<tr>
<td>Treatment B</td>
<td>3.5731</td>
<td>4.8561</td>
<td>4.8746</td>
</tr>
<tr>
<td><strong>Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>4170.61</td>
<td>90844.78</td>
<td>93325.43</td>
</tr>
<tr>
<td>Treatment B</td>
<td>3741.96</td>
<td>71795.96</td>
<td>74920.38</td>
</tr>
<tr>
<td><strong>Ratio of Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>111.45</td>
<td>126.53</td>
<td>124.56</td>
</tr>
<tr>
<td><strong>90% Confidence Interval (A/B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit (%)</td>
<td>95.61</td>
<td>102.32</td>
<td>102.29</td>
</tr>
<tr>
<td>Upper limit (%)</td>
<td>114.94</td>
<td>119.90</td>
<td>118.32</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intra-subject CV (%)</td>
<td>12.49</td>
<td>10.74</td>
<td>9.86</td>
</tr>
<tr>
<td>Mean Square Error (MSE)</td>
<td>0.0155</td>
<td>0.0115</td>
<td>0.0097</td>
</tr>
</tbody>
</table>
Table 5-3D: Summary statistics of pharmacokinetic parameters of unconjugated EZM under fed conditions

<table>
<thead>
<tr>
<th>Product/Statistics</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;0-t&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;0-∞&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untransformed Treatment A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>5608.85</td>
<td>79852.56</td>
<td>85077.90</td>
</tr>
<tr>
<td>% CV</td>
<td>50.48</td>
<td>56.03</td>
<td>58.18</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Untransformed Treatment B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>6007.58</td>
<td>75823.09</td>
<td>80219.29</td>
</tr>
<tr>
<td>% CV</td>
<td>65.88</td>
<td>45.32</td>
<td>46.24</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Ratio of Arithmetic Mean (% Bioavailability)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>93.36</td>
<td>105.31</td>
<td>106.06</td>
</tr>
<tr>
<td>Ratio (%) for Mean AUC&lt;sub&gt;0-t&lt;/sub&gt; to Mean AUC&lt;sub&gt;0-∞&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>93.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>94.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log transformed (Log&lt;sub&gt;10&lt;/sub&gt;)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>3.6922</td>
<td>4.8398</td>
<td>4.8647</td>
</tr>
<tr>
<td>Treatment B</td>
<td>3.7011</td>
<td>4.8331</td>
<td>4.8564</td>
</tr>
<tr>
<td>Geometric Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>4922.66</td>
<td>69151.24</td>
<td>73231.84</td>
</tr>
<tr>
<td>Treatment B</td>
<td>5024.28</td>
<td>68092.61</td>
<td>71845.57</td>
</tr>
<tr>
<td>Ratio of Geometric Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>97.98</td>
<td>101.55</td>
<td>101.92</td>
</tr>
<tr>
<td>90% Confidence Interval (A/B)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit (%)</td>
<td>88.37</td>
<td>92.14</td>
<td>92.29</td>
</tr>
<tr>
<td>Upper limit (%)</td>
<td>111.16</td>
<td>109.99</td>
<td>110.18</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intra-subject CV (%)</td>
<td>15.59</td>
<td>12.01</td>
<td>12.01</td>
</tr>
<tr>
<td>Mean Square Error (MSE)</td>
<td>0.0240</td>
<td>0.0143</td>
<td>0.0143</td>
</tr>
</tbody>
</table>
5.3.3.4 Discussion

The mean ± SEM plasma concentration of unconjugated EZM, receiving treatment A and B, studied under fasting and fed conditions, are shown in figure 5-3A and 5-3B, respectively. The plasma concentration-time curve shows multiple peaks as seen in section 5.3.1. In case of fasting condition, for treatment A, the C\text{max} concentrations varied from 1776.6 to 7492.9 pg/mL while for treatment B, the C\text{max} concentrations ranged from 908.4 to 10382.7 pg/mL. The mean pharmacokinetic parameters obtained for treatment A, are comparable to those obtained after receiving treatment B as indicated in Table 5-3A. The A to B ratio was 103.66 for C\text{max}, 116.08 for AUC\text{0-t} and 115.28 for AUC\text{0-∞}. The statistical analysis for pharmacokinetic parameters of unconjugated EZM under fasting conditions for treatment A and B are presented in Table 5-3C. 90% CI was 95.61 to 114.94% for C\text{max}, 102.32 to 119.90% for AUC\text{0-t} and 102.29 to 118.32% for AUC\text{0-∞}.

Similarly, in case of fed condition, for treatment A, the C\text{max} concentration varied from 1686.1 to 10265.9 pg/mL while for treatment B, the C\text{max} concentrations ranged from 1998.8 to 15223.8 pg/mL. The mean pharmacokinetic parameters obtained for treatment A, are comparable to those obtained after receiving treatment B as indicated in Table 5-3B. The statistical analysis for pharmacokinetic parameters of unconjugated EZM under fed conditions for treatment A and B are presented in Table 5-3D. As mentioned in section 5.3.2.4, the drug is not a highly variable drug as seen from the intra-subject variability values mentioned in Table 5-3C and 5-3D. The calculated 90% confidence interval for the ratios of geometric means of treatment A and B product falls within a BE limit of 80 – 125%, under fast and fed state. Unlike total EZM, the increase in C\text{max} concentration in fed state is not significant as compared to fasting state.

Thus as seen from the results in the current section, the unconjugated EZM levels obtained for product A and B, are identical. The two products under investigation have comparable bioavailability and that the food has no significant effect on the bioavailability of the drug as noticed from the unconjugated EZM pharmacokinetics.
5.3.4 Conjugated EZM estimated by Liquid Chromatography-Tandem Mass Spectrometric Method under fasting conditions

The concentrations of conjugated EZM (EZM-G) were measured by subtracting the concentrations of unconjugated EZM from total EZM. The pharmacokinetic parameters were estimated for the concentrations achieved and later subjected to statistical analysis.

5.3.4.1 Pharmacokinetic Profiles

Figure 5-4A: Mean ± SEM Plasma Concentration of conjugated EZM under fasting conditions in 12 healthy human subjects, receiving treatment A & B
Figure 5-4B: Mean ± SEM Plasma Concentration of conjugated EZM under fed conditions in 12 healthy human subjects, receiving treatment A & B

5.3.4.2 Statistical Outlier(s)

Using Grubb’s test at 0.05% significance level, no statistical outlier was detected.

5.3.4.3 Pharmacokinetic Parameters

Table 5-4A: Pharmacokinetic parameters for conjugated EZM (EZM-G) estimated by LC-MS/MS method under fasting conditions

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C\text{\textsubscript{max}} (ng/mL)</th>
<th>T\text{\textsubscript{max}} (hrs.)</th>
<th>AUC\textsubscript{0-\textsubscript{t}} (ng.hr/mL)</th>
<th>AUC\textsubscript{0-\textsubscript{\infty}} (ng.hr/mL)</th>
<th>K\text{\textsubscript{el}}</th>
<th>T\text{\textsubscript{1/2}} (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>93.50</td>
<td>1.36</td>
<td>985.27</td>
<td>1042.58</td>
<td>0.0508</td>
<td>14.99</td>
</tr>
<tr>
<td>SD</td>
<td>37.60</td>
<td>0.74</td>
<td>567.59</td>
<td>566.42</td>
<td>0.0169</td>
<td>4.61</td>
</tr>
<tr>
<td>%CV</td>
<td>40.21</td>
<td>54.67</td>
<td>57.61</td>
<td>54.33</td>
<td>33.32</td>
<td>30.75</td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>85.08</td>
<td>1.11</td>
<td>786.17</td>
<td>823.04</td>
<td>0.0653</td>
<td>12.15</td>
</tr>
<tr>
<td>SD</td>
<td>51.13</td>
<td>0.63</td>
<td>442.57</td>
<td>471.09</td>
<td>0.0298</td>
<td>4.17</td>
</tr>
<tr>
<td>%CV</td>
<td>60.09</td>
<td>56.24</td>
<td>56.29</td>
<td>57.24</td>
<td>45.68</td>
<td>34.34</td>
</tr>
</tbody>
</table>
Table 5-4B: Pharmacokinetic parameters for conjugated EZM (EZM-G) estimated by LC-MS/MS method under fed conditions

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameters</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; (ng/mL)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (hrs.)</th>
<th>AUC&lt;sub&gt;0→t&lt;/sub&gt; (ng.hr/mL)</th>
<th>AUC&lt;sub&gt;0→∞&lt;/sub&gt; (ng.hr/mL)</th>
<th>K&lt;sub&gt;el&lt;/sub&gt;</th>
<th>T&lt;sub&gt;1/2&lt;/sub&gt; (hrs.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>130.74</td>
<td>2.08</td>
<td>806.03</td>
<td>884.60</td>
<td>0.0594</td>
<td>16.19</td>
</tr>
<tr>
<td>SD</td>
<td>52.13</td>
<td>1.15</td>
<td>358.95</td>
<td>410.36</td>
<td>0.0352</td>
<td>9.38</td>
</tr>
<tr>
<td>%CV</td>
<td>39.87</td>
<td>55.06</td>
<td>44.53</td>
<td>46.38</td>
<td>59.18</td>
<td>57.90</td>
</tr>
<tr>
<td>Treatment B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM</td>
<td>133.98</td>
<td>2.75</td>
<td>803.45</td>
<td>862.05</td>
<td>0.0616</td>
<td>16.24</td>
</tr>
<tr>
<td>SD</td>
<td>75.01</td>
<td>1.20</td>
<td>343.02</td>
<td>345.92</td>
<td>0.0641</td>
<td>6.55</td>
</tr>
<tr>
<td>%CV</td>
<td>55.98</td>
<td>43.56</td>
<td>42.69</td>
<td>40.12</td>
<td>104.05</td>
<td>40.34</td>
</tr>
</tbody>
</table>
Table 5-4C: Summary statistics of pharmacokinetic parameters of conjugated EZM under fasting conditions

<table>
<thead>
<tr>
<th>Product/Statistics</th>
<th>C&lt;sub&gt;max&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;0→t&lt;/sub&gt;</th>
<th>AUC&lt;sub&gt;0→∞&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untransformed Treatment A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>93.50</td>
<td>985.27</td>
<td>1042.58</td>
</tr>
<tr>
<td>% CV</td>
<td>40.21</td>
<td>57.61</td>
<td>54.33</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Untransformed Treatment B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>85.08</td>
<td>786.17</td>
<td>823.04</td>
</tr>
<tr>
<td>% CV</td>
<td>60.09</td>
<td>56.29</td>
<td>57.24</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ratio of Arithmetic Mean (% Bioavailability)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>109.89</td>
<td>125.32</td>
<td>126.67</td>
</tr>
<tr>
<td><strong>Ratio (%) for Mean AUC&lt;sub&gt;0→t&lt;/sub&gt; to Mean AUC&lt;sub&gt;0→∞&lt;/sub&gt;</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>94.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>95.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log transformed (Log&lt;sub&gt;10&lt;/sub&gt;)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>1.9422</td>
<td>2.9284</td>
<td>2.9637</td>
</tr>
<tr>
<td>Treatment B</td>
<td>1.8576</td>
<td>2.8303</td>
<td>2.8497</td>
</tr>
<tr>
<td><strong>Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>87.54</td>
<td>848.00</td>
<td>919.81</td>
</tr>
<tr>
<td>Treatment B</td>
<td>72.04</td>
<td>676.55</td>
<td>707.46</td>
</tr>
<tr>
<td><strong>Ratio of Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>121.52</td>
<td>125.34</td>
<td>130.02</td>
</tr>
<tr>
<td><strong>90% Confidence Interval (A/B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit (%)</td>
<td>96.95</td>
<td>105.09</td>
<td>106.92</td>
</tr>
<tr>
<td>Upper limit (%)</td>
<td>122.16</td>
<td>115.78</td>
<td>117.48</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intra-subject CV (%)</td>
<td>15.71</td>
<td>6.55</td>
<td>6.36</td>
</tr>
<tr>
<td>Mean Square Error (MSE)</td>
<td>0.0244</td>
<td>0.0043</td>
<td>0.0040</td>
</tr>
</tbody>
</table>
## Table 5-4D: Summary statistics of pharmacokinetic parameters of conjugated EZM under fed conditions

<table>
<thead>
<tr>
<th>Product/Statistics</th>
<th>$C_{\text{max}}$</th>
<th>$\text{AUC}_{0-t}$</th>
<th>$\text{AUC}_{0-\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Untransformed Treatment A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>130.74</td>
<td>806.03</td>
<td>884.60</td>
</tr>
<tr>
<td>% CV</td>
<td>39.87</td>
<td>44.53</td>
<td>46.38</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Untransformed Treatment B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arithmetic Mean (AM)</td>
<td>133.98</td>
<td>803.45</td>
<td>862.05</td>
</tr>
<tr>
<td>% CV</td>
<td>55.98</td>
<td>42.69</td>
<td>40.12</td>
</tr>
<tr>
<td>N</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td><strong>Ratio of Arithmetic Mean (% Bioavailability)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>97.58</td>
<td>100.32</td>
<td>102.62</td>
</tr>
<tr>
<td><strong>Ratio (%) for Mean $\text{AUC}<em>{0-t}$ to Mean $\text{AUC}</em>{0-\infty}$</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>91.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>93.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Log transformed (Log_{10})</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>2.0655</td>
<td>2.8529</td>
<td>2.8893</td>
</tr>
<tr>
<td>Treatment B</td>
<td>2.0644</td>
<td>2.8614</td>
<td>2.8949</td>
</tr>
<tr>
<td><strong>Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment A</td>
<td>116.28</td>
<td>712.68</td>
<td>774.99</td>
</tr>
<tr>
<td>Treatment B</td>
<td>115.98</td>
<td>726.78</td>
<td>785.05</td>
</tr>
<tr>
<td><strong>Ratio of Geometric Mean</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/B (%)</td>
<td>100.26</td>
<td>98.06</td>
<td>98.72</td>
</tr>
<tr>
<td><strong>90% Confidence Interval (A/B)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lower limit (%)</td>
<td>90.65</td>
<td>93.23</td>
<td>93.36</td>
</tr>
<tr>
<td>Upper limit (%)</td>
<td>110.57</td>
<td>105.45</td>
<td>105.93</td>
</tr>
<tr>
<td>Degree of freedom</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Intra-subject CV (%)</td>
<td>13.48</td>
<td>8.33</td>
<td>8.55</td>
</tr>
<tr>
<td>Mean Square Error (MSE)</td>
<td>0.0180</td>
<td>0.0069</td>
<td>0.0073</td>
</tr>
</tbody>
</table>
### 5.3.4.4 Discussion

Figure 5-4A and 5-4B shows mean ± SEM plasma concentration of conjugated EZM, receiving treatment A and B, under fasting and fed conditions, respectively. The plot is characterized by multiple peaks, as seen for unconjugated and total EZM. In case of fasting condition, for treatment A, the $C_{\text{max}}$ concentrations varied from 52.93 to 179.91 ng/mL while for treatment B, the $C_{\text{max}}$ concentrations ranged from 25.65 to 205.92 ng/mL. The mean pharmacokinetic parameters obtained for treatment A, are comparable to those obtained after receiving treatment B as indicated in Table 5-4A. Similarly, in case if fed condition, for treatment A, the $C_{\text{max}}$ concentration varied from 46.76 to 218.74 ng/mL while for treatment B, the $C_{\text{max}}$ concentrations ranged from 50.09 to 285.71 ng/mL. The mean pharmacokinetic parameters obtained for treatment A, are comparable to those obtained after receiving treatment B as indicated in Table 5-4B.

The mean $C_{\text{max}}$ for product A and B were 93.50 and 85.08 ng/mL in fasting state; 130.74 and 133.98 ng/mL in fed state, respectively. The mean AUC$_{0-t}$ for product A and B were 985.27 and 786.17 ng.hr/mL in fasting state while 806.03 and 803.45 ng.hr/mL in fed state. The mean AUC$_{0-\infty}$ for product A and B were 1042.58 and 826.17 ng.hr/mL in fasting state while 884.60 and 862.05 ng.hr/mL, in fed state. The statistical analysis for pharmacokinetic parameters of conjugated EZM under fasting and fed conditions for treatment A and B are presented in Table 5-4C and 5-4D, respectively. Under fasting conditions, 90% CI was 96.95 to 122.16% for $C_{\text{max}}$, 105.09 to 115.78% for AUC$_{0-t}$ and 106.92 to 117.48% for AUC$_{0-\infty}$. Under fed conditions, 90% CI was 90.65 to 110.57 for $C_{\text{max}}$, 93.23 to 105.45% for AUC$_{0-t}$ and 93.36 to 105.93% for AUC$_{0-\infty}$. Thus, the calculated 90% confidence interval for the ratios of geometric means of the treatment A and B falls within a BE limit of 80 – 125%.

When pharmacokinetic parameters obtained under fasting state are compared with those obtained in fed state, for either of the formulation, a significant difference is observed for $C_{\text{max}}$ as evident from Tables 5-4C and 5-4D for both the products. Thus the two products under investigation have comparable bioavailability under both fasting and fed conditions.
5.4 Conclusions

The methods developed in previous chapters (Chapter 3 & 4) are successfully applied for the pharmacokinetic determination of unconjugated, conjugated and total EZM from healthy human subjects. The application of the HPLC method in pharmacokinetics of total EZM, showed results comparable to those reported in literature. The methods developed and validated for the determination of unconjugated and total EZM in the earlier chapters, were applied to study pharmacokinetics of unconjugated and total EZM. The two products namely Ezetimibe Tablets 10 mg (Each tablet contains 10 mg EZM) manufactured by Macleods Pharmaceuticals Ltd., India and Zetia® Tablets 10 mg (Each Tablet contains 10 mg EZM) manufactured by Schering Corporation, USA; were investigated to study the comparative bioavailability at pilot scale. The two products were studied under fasting and fed state to check the effect of food on rate and extent of absorption.

The results obtained in the current chapter are an indication that the two investigational products have comparable bioavailability in both fasting and fed states. From the findings of the current chapter, after the oral administration of EZM, drug undergoes extensive glucuronidation with peak concentration achieved for the glucuronide between 1 to 2 hours. Since the drug is reported to show enterohepatic recycling, multiple peaks were observed in plasma concentration-time curve, and thus large $T_{1/2}$ values are observed. The exposure of unconjugated EZM represented approximately 9% of the exposure of total EZM. Both the formulations were well tolerated following a single dose administration of the investigational products. No adverse events occurred during conduct of the study. No serious clinical adverse events causing death, disability or hospitalization of the subjects were encountered.

Thus the methods have proved their applicability in bioanalysis of EZM for pharmacokinetic studies and can be employed for routine use.