BIOEQUIVALENCE STUDY OF TWO ORAL EXTENDED RELEASE FORMULATIONS OF FELODIPINE 10 MG TABLETS IN HEALTHY HUMAN VOLUNTEERS UNDER FED CONDITION

1Patel Devang S.*, 1Shanker Neeraj, 1Shah Sweety K., 1Thakkar Vaishali K, 1Mehta Niral N, 1Srivstava Ambrish K., 2Singh Sanjay, 2Patel Chitrang G

1Torrent Pharmaceuticals Limited, Torrent Research Centre, Village Bhat, District – Gandhinagar – 382428, Gujarat, India
2Department of Pharmaceutics, Institute of Technology - Banaras Hindu University, Varanasi – 221005, U. P.

ABSTRACT
A bioequivalence study between Felodipine Extended Release 10 mg tablet (Test, Torrent Pharmaceuticals Ltd., India) and Felodipine Extended Release 10mg Tablet (Reference, Mylan Pharmaceuticals Inc, USA) as reference was carried out in 56 healthy human volunteers under fed condition. The study design used was an open label, randomised, 2-period, 2-treatment, 2-sequence, crossover, single-dose bioequivalence study. Blood samples were taken before, and up to 120 hrs after drug administration. The estimation of felodipine in plasma samples was carried out by a validated LC-MS/MS method. The pharmacokinetic parameters, $C_{\text{max}}$ and $T_{\text{max}}$, were calculated directly from plasma concentration, $K_{el}$ was estimated by log-linear regression, and the Area Under Curve (AUC) was calculated by the linear trapezoidal rule. The parameter $C_{\text{max}}$, $\text{AUC}_{0-4}$ and $\text{AUC}_{0-\infty}$ were tested for bioequivalence after log-transformation of data, while the differences of $T_{\text{max}}$ were evaluated non-parametrically. The 90% confidence intervals of the mean values for $C_{\text{max}}$, $\text{AUC}_{0-4}$ and $\text{AUC}_{0-\infty}$ were 98.73-117.33%, 96.79-111.80% and 96.81-111.64% respectively. All of these values were within the bioequivalence acceptance range of 80-125%. There was no statistically significant difference between two formulations of felodipine under fed condition. We found that both formulations of felodipine were bioequivalent and therefore interchangeable in clinical practice.

Keywords: Anti hypertensive, Felodipine, Pharmacokinetic, Bio equivalence study.

INTRODUCTION
Hypertension has become a major cause of morbidity and mortality worldwide and it is now ranked third as a cause of disability-adjusted life years. The World Health Report states that elevated blood pressure alone contributes to about 50% of
cardiovascular disease (CVD) worldwide. So the effective treatment of hypertension is necessary.\cite{1,2}

Felodipine is used as an antihypertensive drug that belongs to second generation calcium channel blocker (CCB). Felodipine have anti-anginal activity. Structurally it is a dihydropyridine derivative and its chemically describe as \( \pm \) ethyl methyl 4-(2,3-dichlorophenyl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate.

Felodipine effectively reduces blood pressure in hypertensive patients as monotherapy and in combination with other antihypertensive agents.\cite{3,4} Felodipine is orally absorbed and undergoes extensive first pass metabolism. Felodipine is greater than 99% bound to plasma proteins. Felodipine is highly lipophilic and its IR formulation is given twice a daily for the treatment of hypertension. \( C_{\text{max}} \) of felodipine immediate release is 2.5 to 5 hrs and mean terminal half life is around 11-16 hrs. About 70% of a given dose is excreted as metabolites in the urine; the remaining fraction is excreted in the faeces. Less than 0.5% of a dose is recovered unchanged in urine. The recommended starting dose of felodipine is 5mg once daily. If necessary the dose may be further increased or another antihypertensive agent added. The usual maintenance dose is 5 - 10 mg once daily. Doses higher than 20mg daily are not usually needed.\cite{4,5,8} Fast release of felodipine after application followed high drug plasma concentration may cause other significant adverse effects. The most dangerous are the rapid drop in systemic blood pressure and reflux tachycardia. Reflex tachycardia is act desired in patients with angina pectoris and heart failure. So to overcome such type of adverse effects it should be used in extended dosage form.\cite{6} It was reported that the bioavailability of felodipine is greatly influenced by food. When administered either with a high fat or carbohydrate diet, \( C_{\text{max}} \) is increased by approximately 60%; AUC is unchanged. The bioavailability of felodipine was increased approximately two-fold when taken with grapefruit juice. Orange juice does not affect the kinetic of felodipine.\cite{4,5,7}

**RATIONALE OF STUDY**

In the process of development of a new modified release formulation of any product, in order to obtain the marketing authorization, it is important to investigate the relative bioavailability of the new product in comparison with a market standard. The efficacy and safety of Felodipine Extended Release 10mg Tablet (Reference, Mylan
Pharmaceuticals Inc, USA) has already been proved in clinical trials. Hence, this drug served as reference and basis for comparison with the test product (Felodipine Extended Release 10mg Tablet, Torrent Pharmaceuticals Limited, India). This study was conducted with the aim to investigate whether differences concerning rate and extent of absorption and safety exist between the test product and the reference product.

**MATERIALS AND METHODS**

**Subjects**

Fifty six healthy male volunteers 18-42 years and Body Mass Index (BMI) of 18.16-26.81 kg/m$^2$ with minimum of 50 kg weight were included in the study. None of the volunteers had any acute or chronic gastrointestinal, cardiovascular, hepatic, renal, endocrine or metabolic disease. The use of any other concomitant medication was forbidden one week before and during the study period. Volunteers were instructed to avoid coffee, tea or alcoholic beverage and tobacco for 48hr prior to the start of each treatment period until completion of both periods of the study. All subjects had given written informed consent before enrolment in the study.

The design of study was adequate to determine the pharmacokinetic end points of the test and reference formulations. The wash out period of 21 days was sufficient to avoid any carry over effects. The study was performed in compliance with ICH- GCP guidelines$^{[9]}$, World Medical Association Declaration of Helsinki Seoul, 2008$^{[10]}$ and Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products- General Considerations, Revision 1, March 2003.$^{[11]}$

**Study design**

This study was monocentric, open-label, randomised, 2-period, 2-treatment, 2-sequence, crossover single dose bioequivalence study under fed condition. Selection of subjects was based on inclusion and exclusion criteria as per approved protocol. Volunteers were randomly assigned to one of the two groups taking either the test formulation or the reference formulation. The volunteers were administered a single oral dose of the test or reference drug in each period. After supervised overnight fast of at least 10 hours before breakfast, volunteers were administered a single oral dose (Felodipine ER 10mg tablet) of either test or reference drug with 240ml drinking water at room temperature in each period 30 minutes after serving of a high fat high-calorie meal. Water
was permitted ad libitum until 1.0 hour before dosing and again 2.0 hours after dosing. Volunteers received lunch at around 5.0 hours, snacks at around 9.0 hours, dinner at around 13.0 hours and breakfast (Day 2) at around 24.0 hours post dose in both the periods. During the clinic residential stay food and water intake was standardized and was identical in both the periods. Sampling was done up to 120.0 hrs such that plasma concentration could be measured for more than 4 half-lives. A total of 56 volunteers were enrolled and amongst them 51 volunteers had completed the clinical phase according to the study protocol. During period I, 56 volunteers were reported while in period II, 51 volunteers were reported. Subjects were randomly assigned to one of the possible sequences of administration of the study product in consecutive order following the randomization code according to SAS® generated randomization schedule. The study was approved by Institutional Ethical Committee of Torrent Research Centre, Gandhinagar, (INDIA).

**Figure 1**

Flow chart summarizes the volunteer’s disposition

**Blood sampling**

A series 24 x 6 ml venous blood samples were collected over a 120.00 hours period of time. The indwelling intravenous cannula was used for collection of pre-dose
and up to 24.00 hours blood sample by syringe and transferred into tubes which contain
diluted heparin in normal saline. Heparin-lock technique was used to prevent clotting of
the blood in the indwelling cannula. Blood collected in the tubes were shaken gently to
ensure the proper mixing with anticoagulant. Sampling was related to drug administration
time. In the morning of dosing day after recording vitals, a pre-dose blood sample was
taken. Other blood samples were withdrawn at following times: 0.5, 1.0, 1.5, 2.0, 2.5, 3.0,
3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0, 8.0, 10.0, 12.0, 18.0, 24.0, 36.0, 48.0, 72.0, 96.0 and 120.0
hours post dose in each period. Volunteers came for ambulatory sample at 36.0, 48.0,
72.0, 96.0 and 120.0 hours post dose. Ambulatory samples were collected by direct
veinpuncture. Blood sampling up to ± 2 minutes of the planned time of in-house
sampling and ±1 hrs in ambulatory sample was considered as an acceptable deviation.

**Analytical method**

The concentration levels in plasma were determined by a validated LC-MS/MS
method for samples from 56 volunteers in the Bioanalytical department of Torrent
Pharmaceuticals Ltd., India. The analyst did not have access to the randomization
schedule during the course of the analysis.

A LC-MS/MS method for estimation of Felodipine in human plasma has been
developed and validated. Sample preparation process was accomplished by using solid
phase extraction (SPE) method. The extracted sample was separated using a Betabasic
C8, 100 X 4.6, 5 μ. The composition of mobile phase was Methanol: (0.1%v/v)Ammonia
in water [90: 10 % v/v]. The flow rate was maintained at 0.7 ml/min.. Clopidogrel was
used as internal standards for Felodipine. The analysis was performed by TSQ Quantum
LC-MS/MS. Chromatograms were acquired using the computer based LCquan 2.5.6
software. The standard curve employed for ranged from 0.050 ng/ml to 10.000 ng/ml.
The lower limit of quantification was 0.050 ng/ml for Felodipine. The standard curve was
linear and coefficient of correlation (r) was consistently greater than 0.9960 during the
course of validation. The concentration of quality control samples were 0.050 ng/ml
(LLOQ), 0.150 ng/ml (LQC), 3.500 ng/ml (MQC) and 7.500 ng/ml (HQC) for
Felodipine. The concentration of the unknown sample was calculated using regression
analysis of spiked plasma calibration standards with the reciprocal of the product
concentration. The method was linear from 0.100 ng/ml to 30.000 ng/ml. In this method
clopidogrel was used as an internal standard and recovery was 82.40%. Recovery of felodipine from human plasma found to be 97.67% the results of all stability parameters were found to be within admissible limits. Intra and inter batch precision and accuracy was determined by analyzing five batches of the above quality control samples. The method was found to be adequate with respect to precision and accuracy. The results of the precision and Accuracy batch showed that the change in calibration curve range was within the earlier validated range, hence that did not affect the validity of the method for felodipine.

**Pharmacokinetic and Statistical Analysis**

The pharmacokinetic parameters used for the determination of bioavailability-bioequivalence of felodipine were evaluated assuming non-compartment model, linear elimination kinetics.

The pharmacokinetic parameters \([T_{\text{max}}, C_{\text{max}}, AUC_{(0-t)}, AUC_{(0\text{-inf})}, AUC_{\%\text{Extrap}}, K_{el}\text{ and } T_{\text{half}}]\) were to be calculated for those volunteers who completed both the periods of study.

Maximum plasma concentration \((C_{\text{max}})\) and time to maximum concentration \((T_{\text{max}})\) were calculated directly from experimental data. Area under the plasma concentration-time curve from zero to the last measurable concentration \((AUC_{0-t})\) was calculated using the linear trapezoidal (Linear Interpolation) method. Area under plasma concentration-time curve from zero to infinite time \((AUC_{0\text{-inf}})\) was calculated by addition of \(AUC_{0-t}\) value to the last measurable plasma concentration divided by terminal elimination rate constant \((\lambda z)\). Terminal elimination rate constant \((\lambda z)\) was calculated as the negative slope of the log-linear terminal portion of the plasma concentration-time curve. The elimination half-life \((\text{Half-life})\) was obtained by dividing 0.693 by \(\lambda z\). The results of pharmacokinetic calculations were presented as mean ± SD. Non-compartmental method (Model 200 of WinNonlin® 5.3) was used to estimate pharmacokinetic parameters of Felodipine ER.

Statistical analysis was to be performed using the SAS® software version 9.1.3 (SAS Institute Inc., Cary NC, USA). The following summary statistics for the pharmacokinetic parameters were calculated for both the Test and Reference products: N (No. of Subjects), Arithmetic Mean (Mean), Standard Deviation (SD), Minimum,
Maximum, Median and Percentage Coefficient of Variation (CV%). Additionally Geometric Mean (GM) was calculated for $C_{\text{max}}$ and AUC$_{0-\tau}$.

The log-transformed pharmacokinetic parameters [$C_{\text{max}}$, AUC$_{(0-t)}$ and AUC$_{(0-inf)}$] of Felodipine ER were subjected to analysis of variance (ANOVA) with the main effects of sequence, formulation and period at 5% level of significance. The percentage geometric least square mean (LSM) ratio of test and reference values was expressed as point estimates of relative bioavailability. Average bioequivalence was evaluated based on the 90% CI for the intra-individual mean ratio of log-transformed [$C_{\text{max}}$, AUC$_{(0-t)}$ and AUC$_{(0-inf)}$] of Felodipine ER for test and reference formulation were found within the accepted bioequivalence range of 80.00%-125.00%.

**RESULTS**

The present bioequivalence study was conducted in 56 healthy male volunteers and the final evaluation was carried out on data obtained from 51 volunteers who completed the study according to protocol. Detailed demographic data is presented in table-1.

**Table 1: Overall demographic profile of volunteers enrolled in the study (N=56)**

<table>
<thead>
<tr>
<th>Asian, Males</th>
<th>Age (Years)</th>
<th>Height (cm)</th>
<th>Weight (Kg)</th>
<th>BMI (kg/m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>28.88</td>
<td>168.74</td>
<td>61.16</td>
<td>21.50</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>5.9</td>
<td>5.7</td>
<td>6.7</td>
<td>2.3</td>
</tr>
<tr>
<td><strong>Max</strong></td>
<td>42</td>
<td>181.50</td>
<td>74.06</td>
<td>26.81</td>
</tr>
<tr>
<td><strong>Min</strong></td>
<td>18</td>
<td>159.00</td>
<td>50.05</td>
<td>18.16</td>
</tr>
<tr>
<td><strong>%CV</strong></td>
<td>20.5</td>
<td>3.4</td>
<td>10.9</td>
<td>10.9</td>
</tr>
</tbody>
</table>

The mean plasma concentrations of Felodipine ER for test and reference products on linear scales are shown in figure-2.
Mean Plasma Concentration Vs. Time Curve \([N=51]\)

- **A(Test)**
- **B(Ref)**

**Figure 2**
Linear Mean plasma concentration vs. time curve

Various parameters were evaluated during study are mentioned in table-2.

### Table 2: Summary of Pharmacokinetic parameters of Felodipine ER

<table>
<thead>
<tr>
<th>PK Parameters [(N=51)](^1)</th>
<th>Test</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2^{T_{\text{max}}}(\text{hr}))</td>
<td>Mean 4.50</td>
<td>Mean 5.00</td>
</tr>
<tr>
<td>(C_{\text{max}}(\text{ng/mL}))</td>
<td>8.732 3.98</td>
<td>8.171 3.76</td>
</tr>
<tr>
<td>(\text{AUC}_{(0-t)}(\text{hr*ng/mL}))</td>
<td>50.889 24.70</td>
<td>49.937 26.31</td>
</tr>
<tr>
<td>(\text{AUC}_{(0-inf)}(\text{hr*ng/mL}))</td>
<td>54.335 26.32</td>
<td>53.285 28.05</td>
</tr>
<tr>
<td>(K_{\text{el}}(1/\text{hr}))</td>
<td>0.071 0.05</td>
<td>0.070 0.05</td>
</tr>
<tr>
<td>(T_{\text{half}}(\text{hr}))</td>
<td>17.30 16.5</td>
<td>16.33 11.0</td>
</tr>
</tbody>
</table>

\(^1\)Total evaluated subjects for Pharmacokinetic and statistical analyses.

\(^2\)For \(T_{\text{max}}\), Median is presented instead of Arithmetic Mean & Range (Min-Max) is presented instead of Standard Deviation.
The median time to reach peak plasma concentrations ($T_{\text{max}}$) was found to be 4.50hr with range (2.50hr-6.00hr) for test formulation and 5.00hr with range (1.50hr-6.00hr) for reference formulation.

There was no serious adverse event reported in the study. 20 adverse events (During study adverse event + Post study lab investigation considered as adverse event) were reported in total 17 volunteers during entire duration of the study.

Out of these total 14 AEs reported during study; In Period-I total 04 (Headache) AEs were reported. While, In Period-II total 10 AEs (Headache) were reported. Total eight adverse events of headache were reported after administration of test drug and six adverse events of headache were reported after administration of reference drug. All during study adverse events were mild in severity and resolved completely.

Total 06 adverse events were reported as post study lab abnormalities. All the post study lab abnormalities were mild in severity and unlikely related with study drug except the abnormality in SGPT in one volunteer with possible relation to study drug.

**DISCUSSION**

The study was conducted as a monocentric, open-label, randomized, two-period, two-treatment, two-sequence crossover, single dose bioequivalence study under fed condition.

The primary objective of the study was to assess the bioequivalence of Felodipine Extended Release 10mg Tablet (Test formulation, Torrent Pharmaceuticals Ltd., India) versus Felodipine Extended Release 10mg Tablet (Reference, Mylan Pharmaceuticals Inc, USA) in healthy human volunteers after administration of single tablet of either test or reference formulation under fed condition during two period study and to evaluate the safety of Felodipine Extended Release 10mg Tablet in healthy human volunteers.

A total of 56 healthy, adult male volunteers were enrolled in the study and the samples of all these volunteers were analyzed. Out of that total 51 volunteers had completed the study. The 90% confidence intervals are based on the data of 51 study completers.

The analytical methods LC-MS/MS allowed specific and sensitive determination of felodipine in plasma and showed linearity between 0.100 ng/ml to 30.000 ng/ml for
felodipine. Simultaneously, the validation parameters of the method fulfilled international requirements for method validation.

As shown in the result, Mean peak concentrations ($C_{\text{max}}$), Mean area under the curve $\text{AUC}_{(0-t)}$ and Mean $\text{AUC}_{(0-\text{inf})}$ have no statistical significant difference for sequence and formulation except period effect at 5% level of significance.

As the cross-over design exploits the fact that period effect is orthogonal to treatment effect i.e., the true treatment effect is not affected if a statistically significant period effect is encountered. Therefore even though ANOVA reveals a significant period effect for log-transformed $C_{\text{max}}$, $\text{AUC}_{(0-t)}$ and $\text{AUC}_{(0-\text{inf})}$, it shall not influence the bioequivalence assessment of Felodipine ER.

Ratio analysis of untransformed and difference of log transformed primary pharmacokinetic parameters [$C_{\text{max}}$, $\text{AUC}_{(0-t)}$ and $\text{AUC}_{(0-\text{inf})}$] for Felodipine ER for test & reference formulation were calculated. The percentage geometric LSM ratio was expressed as point estimates of relative bioavailability for Felodipine ER.

The percentage geometric least square mean (LSM) ratio was found to be 107.63, 104.03 and 103.96 for $C_{\text{max}}$, $\text{AUC}_{(0-t)}$ and $\text{AUC}_{(0-\text{inf})}$ respectively.

Actual values of secondary pharmacokinetic parameter $T_{\text{max}}$ were compared for test and reference using non-parametric Wilcoxon Signed Rank test for Felodipine ER. No statistically significant difference found between formulations (p-value = 0.46). Also, there was no serious adverse event reported in the study.

The 90% confidence interval for an intra individual means ratio for log-transformed [$C_{\text{max}}$, $\text{AUC}_{(0-t)}$ and $\text{AUC}_{(0-\text{inf})}$] of Felodipine ER of the test to the reference formulation were within the range 80.00%-125.00%. Hence, the test formulation was bioequivalent to reference formulation.

Based on the results obtained in this study, the Felodipine Extended Release 10mg Tablet (Test formulation: Torrent Pharmaceuticals Ltd., India) is bioequivalent to that of Felodipine Extended Release 10mg Tablet (Reference, Mylan Pharmaceuticals Inc, USA) in healthy human volunteers under fed condition. Both the products were well tolerated.
ACKNOWLEDGEMENT

The study was conducted by Torrent Research Centre, Torrent Pharmaceuticals Ltd., Gujarat, India.

REFERENCES:


8. Package insert of PLENDIL® ER Tablet- Mylan Pharmaceuticals Ltd. (Felodipine Extended Release tablet 10mg).


11. Bioavailability and Bioequivalence Studies for Orally Administered Drug Products — General Considerations; Guidance for Industry, Center for Drug Evaluation and Research (CDER); March 2003, Revision 1.

For Correspondence:
Devang S Patel
Torrent Pharmaceuticals Limited,
Torrent Research Centre, Village Bhat,
District – Gandhinagar – 382428, Gujarat, India
E mail: devangrx@gmail.com