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BIOEQUIVALENCE STUDY OF FLUVASTATIN ER TABLETS 80 MGUNDER FASTING CONDITIONS IN HEALTHY VOLUNTEERS

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Abstract

The bioequivalence of test formulation of Fluvastatin ER tablets 80mg of Beijing Sciecure Pharmaceutical Co., LTD, China was evaluated with respect to the corresponding reference drug formulation, of Lescol®XL (Fluvastatin sodium) 80mg Extended Release Tabletsof Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936.

This study was an open-label, randomized, single oral dose, two treatment, two sequence, two period, two way crossover bioequivalence study was performed under fasting conditions with 36 healthy adult, human male participants. There was a seven-day washout period. The study formulations were administered after an overnight fast of at least 10-hours before dosing in each period, and blood samples were collected at 0.00 hrs (pre dose), 00.33, 00.67, 01.00, 01.50, 02.00, 02.50, 03.00, 03.50, 04.00, 05.00, 06.00, 08.00, 10.00, 12.00, 14.00, 16.00 and 18.00 hours (post dose) after administration.

The concentrations of Fluvastatin in K_3EDTA human plasma was estimated using precise andaccurate LC-MS/MS procedure.The GM Ratio and the 90% CI of Ratio estimates of Cmax, $AUC_{0-t,}$ and $AUC_{0-\infty}$values of the

Fluvastatin ER Tablets 80 mg (Test formulation) over those ofLescol®XL (Fluvastatin sodium80 mg) Extended Release Tablets(Reference formulation) were 92.24 [83.60, 101.77], 91.48 [83.12, 100.67] and 93.59 [85.04, 103.00], respectively.

Based on results, it's been concluded that the two formulations of FluvastatinERTablets 80 mg were bioequivalent in terms of the rate and extent of absorption.

Keywords: Fluvastatin; Pharmacokinetic; Bioavailability; Bioequivalence.

Introduction

Bioavailability means the rate and extent to which the active substance or therapeutic moiety is absorbed from a pharmaceu

form and becomes available at the site of action. Two medicinal products are bioequivalence if they are pharmaceu

equivalents or alternatives and if their bioavailability after administration in the same molar dose are similar to such degree

their effects, with respect to both efficacy and safety, are essentially the same[1,2,3]

Fluvastatin ER tablets 80mg is a medicine used to treat raised levels of fats in the blood in adults, in particular total choles

and LDL cholesterol, which is associated with an increased risk of heart disease and stroke.[2]

- in adult patients with high blood levels of cholesterol

- in adult patients with high blood levels of both cholesterol and triglycerides

The objective of this study is to assess the single dose bioequivalence of Fluvastatin ER Tablets 80mg of Beijing

Sciecure Pharmaceutical Co.,LTD, China and Lescol® XL (Fluvastatin sodium 80 mg) Extended Release Tablets of

Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936 in healthy, adult, human study participants

under fasting conditions. Also to monitor clinical status, adverse events and laboratory investigations and assess relative

safety and tolerance of Fluvastatin formulations under fasting conditions.

Materials and Methods

The study protocol (FLU/CR/052/14-15: Version: 02; issue Date: 16 December 2015) and the informed consent

documents were reviewed and approved by Naithika Independent Ethics Committee, Hyderabad, India and also no

objection letter has been obtained from Drugs Controller General of India.

The study was performed in accordance with the protocol and complied with all the obligations ofinvestigators and all

other current pertinent requirements of CDSCO Schedule Y and itsamendments, ICMR Ethical Guidelines for

Biomedical Research on Human Participant(2006), ICH Guidelines for Good Clinical Practice, and Declaration of

Helsinki, and inaccordance with regulatory and GCP guidelines.

Before performing any study specific procedures, all potential volunteers were explained in nontechnical terms,

regarding the purpose, procedures to be carried out, potential hazards and rights of the volunteers during the course of

the study.All study related procedures wereperformed after the participant had signed the informed consent form.The

clinical phase of this study was performed in February of 2016.

Inclusion Criteria / Exclusion Criteria

Healthy Indian adults who were aged 19 – 43yeares were enrolled in this study. The range of body mass index was 19.0 - 24.9. The health conditions of all of the candidates were evaluated.

The evaluation included an interview and a physical examination (blood pressure [BP], heart rate, weight, height, body temperature and respiratory rate). The diagnostic testing that included a ECG and chest X-ray. The laboratory tests included hematology, blood chemistry, urinalysis, tests for alcohol and drug-abuse. The employed serological tests included hepatitis B and C, RPR for syphilis, as well as HIV 1 & 2.

Systolic and diastolic BP was determined. The BP reading was taken with the subject in a seated position. Subjects were excluded if their laboratory values were considerably outside of the reference range and/or if all tests were not completed.

Prior to the enrollment of the subjects, the laboratory data were reviewed and approved by the clinicians.

Study Design and Drug Administration

This was an open-label, randomized, single oral dose, two treatments, two sequences, two periods, two way crossover bioequivalence study. Single oral dose of either test or reference product was administered with a wash out period of 07 days.

Single oral dose of Fluvastatin ER Tablets 80mg of Beijing Sciecure Pharmaceutical Co., LTD, China was administered orally with 240 ± 2 mL of drinking water at room temperature.

Single oral dose of Lescol® XL (Fluvastatin sodium 80 mg) Extended Release Tablets of Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936 was administered orally with 240 ± 2 mL of drinking water at room temperature.

Study participants were randomized to the 2 treatment sequences (AB and BA) in a random order according to a randomization schedule (using SAS®9.2, Enterprise Guide version 4.2).

Only thosevolunteers who tested negative in Urine Drug Screen for drugs of abuse (Benzodiazepines,Cannabinoids, Amphetamines, Cocaines, Barbiturates and Opiates) and Breath alcohol test werecontinued for drug administration. All the admitted study participants were served dinner on D-1(i.e 11.00 hrspredose)

The blood samples were collected as per the following schedule in each period:

The predose samples (2 x 4 mL) were collected within 1 hour prior to drug administration (0.0hour) and the others (4 mL each) at 00.33, 00.67, 01.00, 01.50, 02.00, 02.50, 03.00, 03.50, 04.00,05.00, 06.00, 08.00, 10.00 12.00, 14.00, 16.00 and 18.00 hours post dose.

Bloodsamples were centrifuged at 3800 rpm for 10 minutes at 10°C ± 2°C, separated the plasma samples and stored at -20°C ± 4°C uptothe completion of analysis.

Subjects were refrained from water intake for 1.00 hr before dosing until 1.00 hr after dosing except whileadministration of the dose. After dosing, the participants remained in sitting position for first fourhours. For all participants, additionally 120 mL of water were given at 1.00, 2.00, and 3.00 hourspost dose in each period.

Urination and Defecation was documented during posture restriction on dosing day in CRFs forboth periods of the study.

On dosing days, meals with standardized menu were given as per table1, Meal schedule to all studyparticipants.

Table 1:Meal schedule.

Day	Breakfast	Lunch	Dinner
D - 1	-	-	11 hrs pre dose
Dosing day (D)	4 hrs post dose	8 hrs post Dose	13 hrs post dose

Participants were allowed to engage in normal activities without any physical exertion throughout their stay in the clinical unit in both study periods. Measuring of vitals has been performed as per table 2, Vitals schedule. Study participants were asked for well being at the timeof recording the vital signs and at the time of check-out.

Table 2:Vitals schedule.

Time relative to dosing minute	At the time of admission	0.00 (within 90 minutes prior to dosing)	2.00	4.00	6.00	12.00	24.00
BP	√	√	√	√	√	√	√
Pulse Rate	√	√	√	√	√	√	√
Temperature	√	√				√	√
Respiratory Rate	√						√

Participant 35 had Glucose Increased (i.e. 290 mg/dL, Reference Range: 70 – 140 mg/dL), duringpost-clinical assessment. As per repeat lab test, the abnormallaboratory value was not within reference range (i.e., 148mg/dL) but declining trend was noticed.Hence, the abnormal laboratory value was considered as clinically non significant by the medicalinvestigator. The event was judged as mild in severity. The outcome of the event was categorized asresolved with no sequelae and considered as unlikely related to the study drug by the investigator.

Chemicals

Fluvastatin sodium working standard (M.Wt. 433.45), Fluvastatin- d7 sodium Salt working standard (As ISTD, M.Wt. 440.49). K_3 EDTA human plasma, Formic Acid, Water, Methanol

Method and Sample Preparation

The concentrations of Fluvastatin in K_3EDTA human plasma was estimated by using SPE method to precise and accurate LC-MS/MS procedure. The method: Vortex the thawed samples of blank, calibration curve standards, quality control samples to ensure complete mixing of contents. Add 25 µL of 60% methanol in water solution to a ria vial which is labeled as plasma blank. Add 25 µL of ISTD from Fluvastatin-d7 (approximately 2.000 µg/ml.) to all pre labeled ria vials (except plasma blank). Transfer 0.250 mL of plasma sample in a pre labeled ria vial from respective samples and vortex the samples. Add 0.250 mL of 0.1% Formic acid solution to all the samples and vortex the samples to ensure complete mixing of contents.

Chromatographic Conditions

The selected analytical column was Chromolith®RP- 18e, 4.6 x 100 mm. Fluvastatin and the internal standard were eluted with a mobile phase consisting of a mixture (20:80 v/v) of 0.1 % Formic Acid Buffer : Methanol. The flow rate was 1.000 ml/min. The column oven temperature was 40°C and analyte was detected by LC-MS/MS (HPLC: Agilent 1100 series, Mass: API 4000): Ion Source: Turbo Ion spray. The spectrometric conditions were,Polarity is Negative ion mode, collision energy (-23.0 V). Typical retention times for Fluvastatin (1.90 to 2.80 minutes) and the internal standard (1.90 to 2.80 minutes). The peak area was measured for calculation of the peak area ratio of Fluvastatin with respect to the internal standard, and the concentration was calculated.

Taken OROCHEM 30mg, 1mL, CELERITY DELUXE (DVB-LP) Cartridges on to a positive pressure processor manifold andfollowed procedures as below

➤ Conditioning: Added 1.000 mL of Methanol followed by 1.000 mL of water

➤ Load: Applied the total volume ofprepared sample under positive pressure.

➤ Rinsing: Rinsed the cartridge with 1.000 mL of water twice. Allowed to dry underpositive pressure forapproximately 2.00 minutes.

➤ Elution: Eluted the drug with 1.000 mL of Methanol and allowed to dry underpositive pressure for approximately 2.00 minutes. Evaporated the samples under astream of nitrogen at 50°C. Reconstituted the residue with 0.250 mL of mobilephase. Loaded the samples into auto injector vials. Injected 20 µL, of the sample onto LC-MS/MS system.

Method Validation

The analytical method was validated according to the guidelines[5]. The selectivity of the present method was established by checking the blank K_3EDTA human plasma, K_3EDTA Lipemic plasma and K_3EDTA Haemolytic plasma obtained from 8 different blood donors. As these blood samples were collected from 8 different people, all possible K_3EDTA human plasma profiles will be included which may contain any interfering compounds that elute along Fluvastatin and ISTD (Fluvastatin-d_7).

Also spiked six samples at LLOQ concentration of Fluvastatin&ISTD (Fluvastatin-d_7) in plasma of one donor from above plasmas except Haemolytic and Lipemic plasma. Compared the response of analyte&ISTD in blank: plasma, if any, with the mean response of LLOQ concentration injected. There were no significant interfering peaks found at Fluvastatin retention time and Internalstandard (Fluvastatin-d_7) retention time in the plasma blanks.

The results of system suitability were within the acceptance criteria. The specificity of the method established, as there were no significant interfering peaks obtained at Fluvastatin retention time due to ISTD and also at internal standard retention time due to Fluvastatin. There was no significant carryover observed. The lower limit of quantitation (LLOQ) was found to be 1.001ng/mL for Fluvastatin.

The % Accuracy was 96.20% and CV% was 7.11%. The average percentage recovery of the Fluvastatin was 64.40% and that of the ISTD (Fluvastatin-d_7) was found to be 54.91%. The method was found to be linear between the range of 1.001ng/mL to 500.295ng/mL for Fluvastatin. This method was considered to be suitable by the investigators for evaluating the bioequivalence of fluvastatin. The acceptance criteria for the approval of the analytical runs and the QC samples, as well as the criteria for performing sample reanalysis, were in accordance with the guidelines[5].

Pharmacokinetic and Statistical Analysis

A total of36 healthy adult human study participants were dosed in this study.The sample size calculation for Fluvasatin was based on the pilot study results. T/R ratio(109%), Significance Level (0.05), Power (90%) Bioequivalence Limits(80.00-125.00%), ISCV of Fluvastatin(18.18%). Based on this, a sample size of 32 study participants would besufficient to establish bioequivalence between two formulations with adequatepower. However, considering the ~ 15% dropout rate, a sample size of 36 wasconsidered for the study.

Pharmacokinetic parameters of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $AUC0-t/AUC_{0-\infty}$,kel,T_{max}, $t_{1/2}$ were calculated for Fluvastatin using Phoenix®WinNonlin® Version 6.3.

A linear mixed effects model that includes fixed effects terms for Sequence, Treatment, Period and a random effects term for Subject (Sequence) were used. Within the framework of this model and consistent with the two one-sided tests for bioequivalence, 90% confidence intervals for the difference between test and reference treatment least-squares means for the comparisons Treatment A (Test) vs. Treatment B (Reference) was calculated for ln-transformed C_{max}, $AUC_{0-t}$and $AUC_{0-\infty}$of Fluvastatin.

The differences and the confidence intervals were exponentiated to obtain point estimates of the ratio of the test over reference geometric means and the 90% CI for the ratio, respectively.

Results

Table 3 shows the demographic characteristics for a total of 36 subjects, who were enrolled in the study.

Table 3: Demographic characteristics of subjects.

|	Age	Weight (kg)	Height (cms)	BMI (kg/m^2)
Mean (SD)	29.2 (6.77)	63.9 (6.78)	167.0 (6.02)	22.9 (1.69)
Median	27.0	64.7	168.5	22.9
CV %	23.2	10.6	3.6	7.4
Range	19.0 - 43.0	55.0 - 76.0	156.0 - 179.5	19.0 - 24.9

BMI = Body mass index

SD = Standard deviation

Participant number 7 was withdrawn from the study in Period-II due to non compliance (late arrival) to the protocol requirements and Participants number 24, 27, 33 & 34 were not reported for Period-II participation due to personal reasons. Thus, the sample size for the bioequivalence evaluation was reduced from 36 subjects to 31 subjects.

Pharmacokinetic Parameters

Mean plasma concentration-time curves of the two formulations are shown in Figure 1. The results suggest that the two formulations have comparable mean plasma concentration-time curves.

Figure 1: Mean Plasma Fluvastatin Concentration Vs. Time Plots-Linear Scale
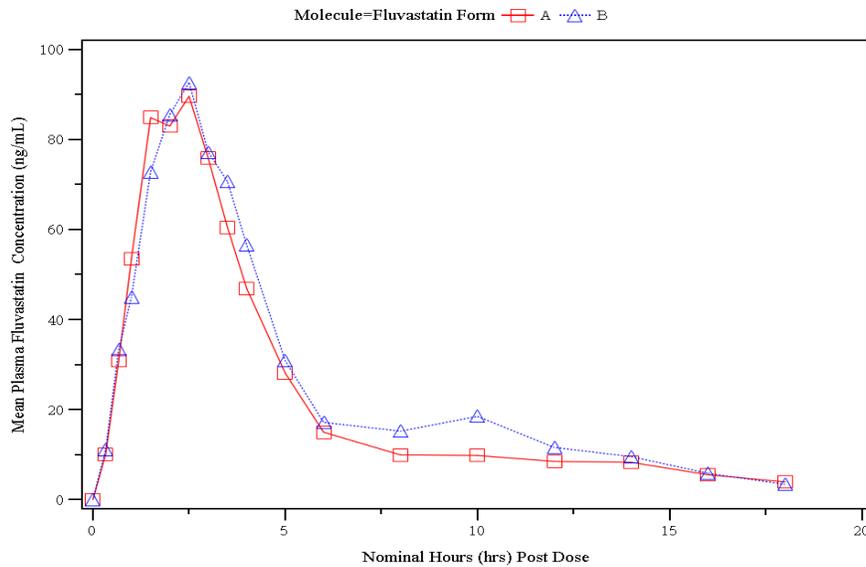
Table 4: shows the Mean (SD) of Pharmacokinetic Parameters of Fluvastatin.

PK Parameter (Units)	Fluvastatin	
	Treatment A (Test) (N=31)	Treatment B (Reference) (N=31)
Cmax (ng/mL)	115.239 (44.1236)	122.242 (41.8765)
AUC_{0-t} (ng.hr/mL)	405.918 (152.8210)	455.956 (209.6927)
$AUC_{0-\infty}$ (ng.hr/mL)	434.836 (174.7888)	476.442 (226.3273)
T_{max}(hr)	2.00 (1.00, 5.00)	2.50 (1.00, 10.00)
$t_{1/2}$	3.431 (1.8777)	2.893 (1.3039)
Kel (1/hr)	0.260 (0.1290)	0.285 (0.1226)
AUC_Ratio	94.33 (6.713)	96.28 (3.542)

Cmax: Maximum plasma drug concentration.

AUC_{0-t}:AUC from time 0 (baseline) to the last measurable concentration.

$AUC_{0-\infty}$: AUC from baseline extrapolated to infinity

Test   : FluvastatinER Tablets 80mg of Beijing Sciecure Pharmaceutical Co., Ltd, Beijing, China

Reference:Lescol® XL (Fluvastatin sodium80 mg) Extended Release Tablets, Novartis Pharmaceuticals Corporation

East Hanover, New Jersey 07936

Table 4: Pharmacokinetic parameters of a test formulation and a reference of Fluvastatin ER tablets 80 mg after a single-dose administration of Fluvastin ER tablets 80 in healthy adult subjects (n = 31). Values are mean (SD)

Table 5: shows the statistical analysis results for the assessment of bioequivalence based on pharmacokinetic parameters of Fluvastatinunder fasting conditions (N=31).

PK Parameter	Least Squares Geometric Means of Treatment		Comparison	Ratio	Intrasubject % CV	90% CI of Ratio
	Treatment A	Treatment B				
log(Cmax) (ng/mL)	106.5123	115.4694	A Vs B	92.24	23.07	(83.60, 101.77)
log(AUC_{0-t}) (ng.hr/mL)	378.6797	413.9585	A Vs B	91.48	22.46	(83.12, 100.67)
log($AUC_{0-\infty}$) (ng.hr/mL)	402.5772	430.1492	A Vs B	93.59	22.47	(85.04, 103.00)

Table 5: shows the bioequivalence statistics using the log-transformed data for C_{max}, $AUC_{0-t}$and $AUC_{0-\infty}$: geometric means, geometric mean ratios (test/reference), 90% CI, and the intra-subject %CV.

The 90% CIs for FluvastatinC_{max}, AUC_{0-t} and $AUC_{0-\infty}$were 83.60% to 101.77%, 83.12% to 100.67% and 85.04% to 103.00% respectively. The 90% CIs of the geometric mean ratios of the three parameters fell within the predetermined range of 80% to 125%. Therefore, these results indicate that the bioequivalence criteria were met.

Safety Conclusions

Vital signs (blood pressure, pulse rate, respiratory rate and temperature) were measured in study participants and recorded at the time of admission into CPU in each period and as per the scheduled timings mentioned in table 2 and also were recorded as and when required during the study. At the time of study exit clinical laboratory (Hematology and Serum chemistry) investigations were performed to determine any abnormality. Abnormal Laboratory values were assessed in each individual case to determine their clinical significance and comparative evaluation was done between test and reference formulation.

01 AE was reported in 01 participant. AE was observed during post clinical assessment. AE was considered as mild in severity and judged to be unlikely related to the study drug. Event was resolved with no sequelae.

Based on evaluation of clinical laboratory results and vital signs examination, it was concluded that both the test and reference formulation were well tolerated and were found to be safe.

Discussion &Conclusions

All of the 90% CIs of the geometric mean ratios of the pharmacokinetic parameters (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) were found to be within the predetermined range of bioequivalence criteria. These results indicate that the bioequivalence criteria were met.

The study evaluated the bioequivalence of 80 mg oral formulations of Fluvastatin ER Tablets (Test formulation), Manufactured by: Beijing Sciecure Pharmaceutical Co., LTD, China and Lescol®XL (Fluvastatin sodium) Extended Release Tablets (Reference formulation), Distributed by Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936 in healthy, adult, human male study participants. Each participant was given the test and the reference product based on a randomized, two way crossover design in fasting state with a washout period of 07 days.

The GM Ratio and the 90% CI of Ratio estimates of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ values of the Fluvastatin ER Tablets 80 mg (Test formulation) over those of the Lescol®XL (Fluvastatin sodium 80mg) Extended Release Tablets (Reference formulation) were 92.24 [83.60, 101.77], 91.48 [83.12, 100.67] and 93.59 [85.04, 103.00], respectively.

Based on the results, it'sbeen concluded that the two formulations of Fluvastatin ER Tablets 80 mg were bioequivalent in terms of the rate and extent of absorption.

Overall, a single oral dose of Fluvastatin ER Tablets 80 mg (Test formulation), Manufactured by: Beijing Sciecure Pharmaceutical Co., LTD, China and Lescol® XL (Fluvastatin sodium 80 mg) Extended Release Tablets (Reference formulation), Distributed by Novartis Pharmaceuticals Corporation East Hanover, New Jersey 07936 when given under fasting condition appears to have been equally tolerated by both groups comprising of 36 healthy, adult male human study participants.

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