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BIOAVAILABILITY AND BIO EQUIVALENCE STUDY OF FENOFIBRATE SR 250 mg CAPSULES IN HEALTHY HUMAN VOLUNTEERS

G. Prasanna Ramakrishna[*1], Nallamotu Shivakrishna[1], Narayabhatla S V Jahnavi[1], Pavani Marella[1], G. Kiran[1], P. Venkateswara Rao[1]

[1*]Department of Pharmaceutics, A. M. Reddy Memorial College of Pharmacy, Narasaraopet Guntur, Andhra Pradesh- 522601, India.

ABSTRACT

This present bioequivalence study was designed to determine the pharmacokinetic, bioavailability and bioequivalence of Fenofibrate 250 mg Sustained Release Capsules in comparison with Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg). The primary pharmacokinetic parameters (C_{max}, AUC_{0-t} and AUC_{0-inf}) 90%CI were within the 80 to 125% interval required for bioequivalence as stipulated in the current regulations of the USFDA acceptance criteria. The geometric mean ratios (Test/Reference) between the two products of extended-release Fenofibrate SR capsule under fed condition were 104.91% (92.86%-118.53%) and 114.41% (103.43%-124.55%) for C_{max} ratios, 102.24% (95.95%-108.94%) and 105.27% (96.76%-114.53%) for AUC_{0-t} ratios and 101.66% (95.73%-107.97%) and 104.71% (96.13%-114.05%) for AUC_{0-inf} ratios of Fenofibrate. Fourteen volunteers had completed both treatment periods. There was no significant difference of the T_{max} parameter between the two formulations ($p > 0.05$). No serious adverse events related to the study drugs were found.

KEYWORDS

Fenofibrate, Bioavailability, Bioequivalence, Intrasubject Variability

Author for Correspondence:

Prasanna Ramakrishna G,
Department of Pharmaceutics,
A. M. Reddy Memorial College of Pharmacy, Narasaraopet, Guntur, Andhra Pradesh,
India.

Email: prasannaramg@gmail.com.

INTRODUCTION[1, 2]

The concept of Bioavailability and Bioequivalence plays an important role in drug research and development, especially in generic-drug industry. The cost of healthcare has been escalating globally during the last two decades, and this has prompted efforts in most countries to reduce those costs. As a result, generic drug equivalents of brand-name drugs (innovator drugs) are introduced.

Atherosclerosis is a chronic disease characterized by abnormal thickening of the walls of the arteries due to fatty deposits (atheromas) of CHOLESTEROL on the

arterial inner walls. These thicken, forming plaques that narrow the vessel channel (lumen) and impede blood flow. Scarring and calcification make the walls less elastic, raising blood pressure. Eventually plaques may completely block a lumen, or a blood clot (thrombus) may obstruct a narrowed channel. Atherosclerosis of one or more coronary arteries (also called CORONARY HEART DISEASE) can decrease the heart muscle's blood supply, causing ANGINA PECTORIS. Complete blockage causes HEART ATTACK. In the brain, atherosclerosis may result in STROKE. Treatments include drugs that reduce the level of cholesterol and fat in the blood, anticoagulants and other drugs that prevent the formation of blood clots, CORONARY BYPASS, and balloon ANGIOPLASTY.

The lipid-modifying effects of fenofibric acid seen in clinical practice have been explained *in vivo* in transgenic mice and *in vitro* in human hepatocyte cultures by the activation of peroxisome proliferator activated receptor (PPAR). Through this mechanism, Fenofibrate increases lipolysis and elimination of triglyceride-rich particles from plasma by activating lipoprotein lipase and reducing production of Apo protein C-III (an inhibitor of lipoprotein lipase activity). The resulting decrease in triglycerides produces an alteration in the size and composition of LDL from small, dense particles (which are thought to be atherogenic due to their susceptibility to oxidation), to large buoyant particles. These larger particles have a greater affinity for cholesterol receptors and are catabolized rapidly. Activation of PPAR also induces an increase in the synthesis of Apo lipoproteins AI, AII and HDL cholesterol. Fenofibrate also reduces serum uric acid levels in hyperuricemic and normal individuals by increasing the urinary excretion of uric acid.

To assess bioequivalence by comparing the single oral-dose bioavailability of test product Fenofibrate SR 250 mg capsules manufactured by RA Chem Pharma Ltd., FDF Division, India with that of reference product Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg), Solvay Pharmaceuticals, Turkey in healthy, adult, human subjects under fed conditions.

MATERIAL AND METHODS[3, 4, 5]

According to the USFDA Regulatory individual product recommendations, two studies (Fed and Fed Sprinkle) to be done with 250 mg Fenofibrate SR Capsules to obtain marketing authorization in USA.

Study Drugs

Test product: Fenofibrate SR 250 mg capsules manufactured by RA Chem Pharma Ltd., FDF Division, India.

Reference product: Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg), Solvay Pharmaceuticals, Turkey.

Study population

The study was carried out at CR Bios Private Limited, India. The study protocol was approved by the Ethics Committee. In addition, the protocol was performed in accordance with the Declaration of Helsinki Principles as outlined in the ICH-E6 Guidelines for Good Clinical Practice (GCP). All subjects were given a detailed description of the study and written informed consent was obtained prior to the enrollment.

The sample size was estimated based on, Coefficient of variation (C.V.) of the drug, sufficient statistical power to detect 20% difference with the power of 0.8 in C_{max} and AUC between the test and reference product, Regulatory requirements.

Sample size was based on estimates obtained from reported literature and previous studies. Assuming a formulation ratio (T/R) ranging from 0.95-1.05 a sample of 20 subjects including dropouts would be sufficient to show bioequivalence between the two formulations with a power of at least 80%. Hence sample size of 14 subjects was enrolled in the study.

Twenty healthy male volunteers between the ages of 18-45 years with a body mass index between 18.5 kg/m^2 and 24.9 kg/m^2, with body weight equal to or not less than 50 kg were assessed to be in good physical condition by a complete medical screening including a medical history, physical examination and laboratory screening test for hematologic and blood biochemistry parameters. Subjects with a

history of hypersensitivity to any ingredients in the Fenofibrate products and/or related drugs or its constituents or who were taking any medication or alcohol for a 21-day period prior to the study were excluded. Subjects who had a history of cardiovascular, hepatic, renal, gastrointestinal or hematologic disease were excluded from the study.

Study design

The study an Open label, balanced, randomized, two-treatment, two-period, two-sequence, single dose, crossover, comparative oral bioavailability and bioequivalence pilot study of test product Fenofibrate SR 250mg capsule with that of reference product in healthy, adult, human subjects under fed conditions.

Number of subjects: 14 healthy adult human volunteers will be included in the study.

Washout period: At least 07 days will be given as a washout period between each dosing.

Duration of the study: The expected duration of subject participation in a study will be approximately 09 days including washout period of 07 days.

Subjects were asked about their medication history in the past, particularly two weeks before screening date and were instructed not to take any medications (either prescribed or over-the-counter) from the date of screening till completion of the study. If drug therapy other than that specified in the protocol was required prior to or during the study or in the washout period, decisions were taken by the investigator to continue or discontinue the subject.

All subjects were instructed to abstain from any xanthine-containing food or beverages (tea, coffee, chocolates, soft drinks etc.), grapefruit, or alcoholic products at least 48 hours prior to dosing till the end of sampling in each period and were prohibited from consuming above mentioned products, during their in house stay.

The subjects were housed in the clinical facility at Clinical research and bio-sciences clinical department from at least 12 hours prior to drug administration until 24 hours post dose in each study period.

Sample Collection

In each study period, 22 blood samples will be collected from each subject as per the following schedule:

The first blood sample (1 x 10 ml) will be collected within 1 hour prior to dosing (0.00 hour-pre dose). The post-dose blood samples (1x 5 ml each) will be collected at 0.25, 0.50, 0.75, 1.00, 1.25, 1.50,1.75, 2.00, 2.25, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00, 6.00, 8.00, 10.00,12.00, 16.00 and 24.00 hours after dosing. The total volume collected per subject in this study will not exceed 271 ml including 10 ml each for screening, 21 ml for heparinised and 10 ml for post clinical assessment of lab parameters**.**

The first blood sample (1 x 10 ml) will be collected within 1 hour prior to dosing (0.00 hour-predose). The post-dose blood samples (1x 5 ml each) will be collected at the total volume collected per subject in this study will not exceed 271 ml including 10 ml each for screening, 21 ml for heparinised and 10 ml for post clinical assessment of lab parameters. 24ml for heparinized and 8 mL for post clinical assessment of lab parameters.

Blood samples were collected through an indwelling cannula placed in a forearm vein. Heparin lock technique was used to prevent the clotting of the blood. After the collection of blood sample at each time point, sample was transferred to sample collection tubes containing K_2-EDTA as anticoagulant. Before each blood sample was drawn except for predose sample (0.00 hours), 0.5 mL of heparinised blood was withdrawn to prevent heparin in the cannula to interfere with analysis. If for any reason the indwelling cannula was blocked or removed for practical reasons, direct venipuncture were done. Blood samples were collected within + 2 minutes of specified sampling time during housing period.

After collection, blood samples were centrifuged at a temperature of 4±2°C, approximately 4000 rpm for 10 minutes. As soon as possible, the plasma obtained were separated and transferred into two different polypropylene tube / RIA vials. Each tube / vial was labeled (Project No., Period, Subject No., Sampling time point and Aliquot No.). All samples

were stored at a temperature of -20°C or below until analysis.

BIOANALYTICAL AND DATA PROCESSING[7, 8]

Bio analytical Methodology

Validated LCMS/MS method will be employed for the estimation of Fenofibrate in plasma. If any subject from 1 to 7 does not complete the study, then the samples of standby subject would be analyzed. Plasma samples from dropout subjects would not be analyzed, unless such dropouts or withdrawals are due to adverse events related to study drug. In these cases, the samples would be analyzed only for safety issues. During estimation of Fenofibrate quality control samples will be distributed throughout each batch of study samples.

Whenever possible, samples from each subject will be analyzed on the same standard curve. Samples with drug concentration greater than upper limit of the validated range of the analysis may be diluted with the appropriate drug free biological fluid and reanalyzed as per the method validation report. Samples, which are below the lower limit of quantification (LLOQ), will be set to zero for all pharmacokinetic and statistical evaluation and reported as below limit of quantification (BLQ). Any missing sample or un reportable concentration value will be reported as 'missing' and will not be included for pharmacokinetic and statistical analysis. Time point deviations (more than 2 minutes) will be incorporated while PK calculation.

Pharmacokinetic Analysis[10-15]

Pharmacokinetic parameters of Fenofibrate will be calculated using the pk solver.

T_{max}: Time of maximum measured plasma concentration. If maximum value occurs at more than one point, T_{max} is defined as the first point with this value in each period.

C_{max}: Maximum measured plasma concentration following each treatment.

AUC_{0-t}: The area under the plasma concentration versus time curve from time zero to the last measurable concentration, as calculated by the linear trapezoidal method.

$AUC_{0-\infty}$: The area under the plasma concentration versus time curve, from zero to infinity. $AUC_{0-\infty}$is calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant.

K_{el}: Apparent first order elimination or terminal rate constant calculated from semi log plot of the plasma concentration versus time curve. The parameters will be calculated by linear least square regression analysis using at least the last three non-zero plasma concentration.

$T_{1/2}$: Time required for the plasma drug concentration to decrease to one half.

Statistical Analysis

Statistical analysis will be performed on plasma Fenofibrate using the SAS system version 9.1.3. The analysis will include data from subjects 1 to 14, if all these subjects complete the study. In case of dropouts, they will be replaced with standby subjects.

RESULTS

The Least Square Mean, T/R ratio values were within the acceptance limit of 80%- 125% for C_{max}, AUC_{0-t} and $AUC_{0-\alpha}$ for Fenofibrate. Hence, the present study provided firm evidence to support that the in house Fenofibrate SR 250 mg capsule (test product) was bioequivalent with reference product Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg).

In-vivo data was predicted by using Solid Phase Extraction procedure and concentrations were found out through Liquid Chromatography mass Spectroscopy detection instrument. The Pharmacokinetic parameters assessed were AUC_{0-t}, $AUC_{0-\alpha}$, C_{max}, T_{max}, $T_{1/2}$, K_{el}. The bioequivalence criterion was based on the Least Square Mean, T/R ratio values of AUC_{0-t}, $AUC_{0-\alpha}$, and C_{max}, whose acceptance range was in between 80%-125%. The results obtained for Least Square Mean of AUC_{0-t}, $AUC_{0-\alpha}$, C_{max} were, 10.2, 10.4, 8.1 and 10.2, 10.3, 8.1 for test and reference (Secalip®) drugs respectively. The results obtained for T/R ratio of AUC_{0-t}, $AUC_{0-\alpha}$, C_{max} were, 104.2, 106.3, and 104.5.The results are shown in Table No.1-3 and Figure No.2.

DISCUSSION

An observational study was conducted on fenofibrate 250mg comparing it with the reference drug Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg). 14 healthy male volunteers aged between 18-45 years were required for conducting the study. For this purpose, total 34 volunteers were screened one week before starting the study. The process of screening involved collection of demographic details and other details like vitals, medical history, family history, present medication status and personal habits. The screening process also involved the collection of blood samples and urine samples for hematology test, clinical biochemistry, clinical pathology, serology test and urine analysis for drug of abuse. The chest X-ray and ECG were also performed. The volunteers were considered healthy if their report were in normal limits.

During the overall conduct of the study, critical watch was kept at each and every step by principal investigator and clinical investigator to ensure that everything was according to the study protocol, SOPs, Indian guidelines, Regulatory authorities guidelines, ICH guidelines and that there was maximum compliance to GCP guidelines.

Table No.1: Pharmacokinetics Data of Fenofibrate

Subject	Sequence	$AUC_{0-\alpha}$	
		A	**B**
1	AB	19652.27	25718.78
2	BA	29123.7	19707.04
3	BA	45083.82	42418.01
4	AB	43595.3	54691.73
5	BA	29274	27800.45
6	AB	61012.07	32663.8
7	AB	34641.85	27676.95
8	BA	34790.59	32521.38
9	BA	33616.69	23945.67
10	AB	22800.54	23941.12
11	BA	21456.28	29064.74
12	AB	42469.9	41989.55
13	AB	41646.84	51034.78
14	BA	36169.9	31330.24
N		14	14
Mean		35380.98	33178.87
SD		11054.84	10497.61
Min		29123.7	19707.04
Max		45083.82	54691.73

Table No.2: Pharmacokinetics Data of Fenofibrate

Subject	**Sequence**	**AUC_{0-t}**	
		A	**B**
1	AB	17309.98	22714.05
2	BA	25169.17	17258.1
3	BA	40064.17	38306.69
4	AB	38195.14	48077.94
5	BA	24958.27	24351.02
6	AB	47402.24	28366.22
7	AB	28962.71	23660.19
8	BA	31262.19	28342.9
9	BA	27517.46	19851.89
10	AB	19886.71	19847.35
11	BA	16328.46	25681.26
s12	AB	34993.62	35320.81
13	AB	33931.46	42458.22
14	BA	33052.74	27503.02
N		14	14
Mean		29931.02	28695.69
SD		8895.743	9112.213
Min		16328.46	17258.1
Max		47402.24	48077.94

Table No.3: Summary Table (N=14)

Parameter	**AUC_{0-t}**	**$AUC_{0-\alpha}$**	**C_{max}**	**T_{max}**	**T½**	**K_{el}**
Test (A)						
Mean	29931.02	35380.98	3687.772	2.446429	8.5478	0.086489
Co-efficient Variation	29.72081	31.24514	34.22818	42.64154	26.29709	26.56409
Reference (B)						
Mean	28695.69	33178.87	3717.76	2.553571	7.6861	0.09209
Co-efficient Variation	31.75464	31.63944	25.74872	46.9017	15.1634	14.85843
Least square Mean						
Test (A)	10.26274	10.42843	8.195501	-	-	-
Reference (B)	10.22098	10.36681	8.150893	-	-	-
90% Confidence Interval for Test(A)						
Lower Limit	102.2270076	104.2912617	101.7874722	-	-	-
Upper Limit	106.3434326	108.4622972	107.4117814	-	-	-

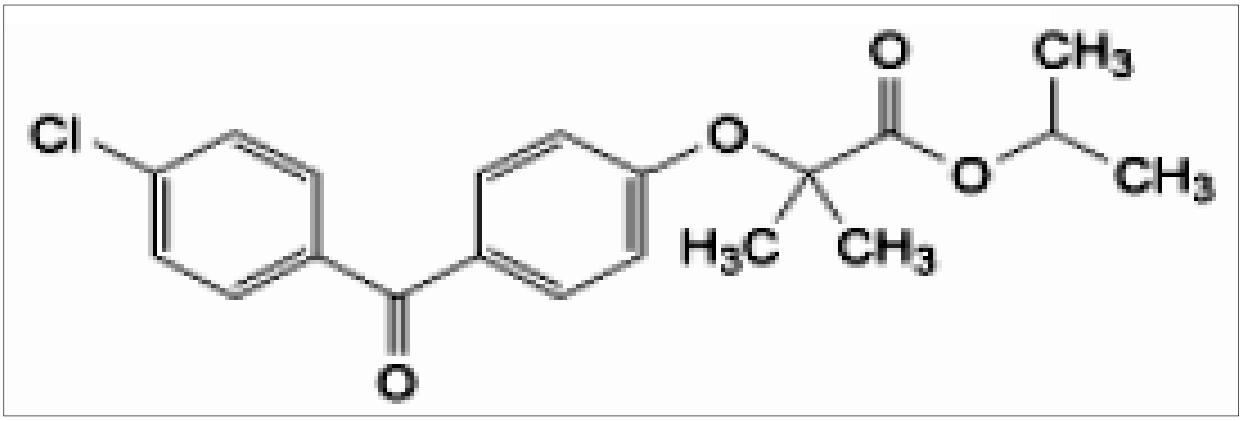

Figure No.1: Chemical structure of Fenofibrate

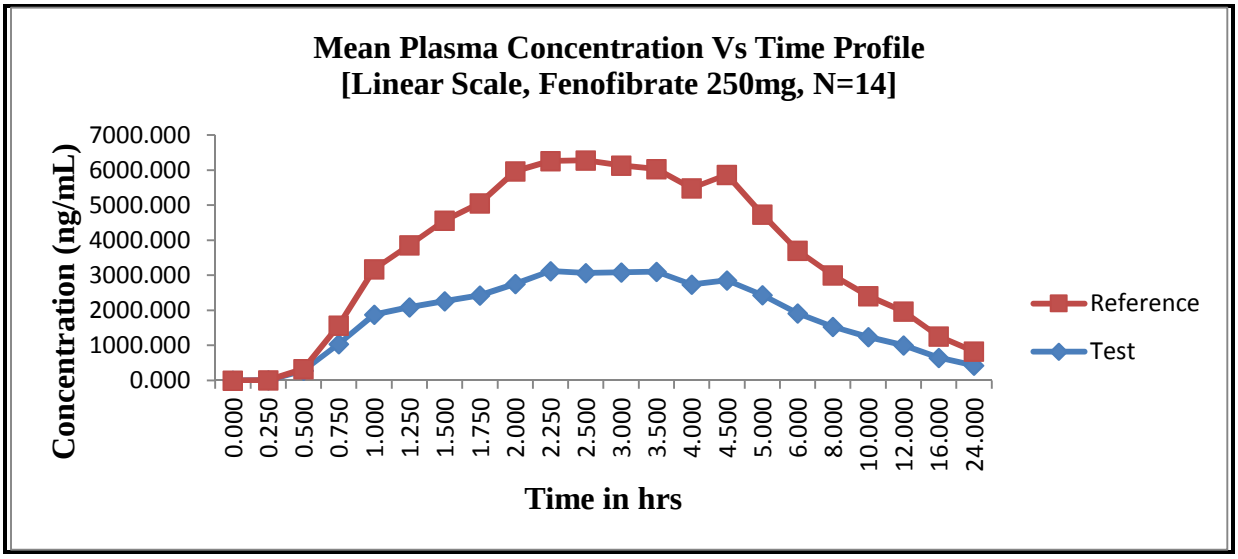

Figure No.2: Mean Plasma Concentration Vs Time Profile

CONCLUSION

The bioavailability and bioequivalence study of Fenofibrate 250 mg SR capsules was conducted on 14 healthy, human, male volunteers in Clinical Research and Biosciences, Hyderabad. Blood samples were collected from the volunteers after single oral dose of test formulation i.e., Fenofibrate 250 mg capsules and reference formulation i.e., Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg) at scheduled time period and blood sample analyzed. From the pharmacokinetic measurement (parameters) the test formulation is bioequivalent with reference formulation. Bioequivalence is evaluated by three parameters viz., C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, out of which AUC_{0-t}, C_{max}, are main paramètres for évaluation. The Pharmacokinetic paramètres (AUC_{0-t} and C_{max}) are with in the acceptable limits of bioéquivalence 80 – 125%. Hence, it is concluded that the test drug of Fenofibrate 250mg SR capsules is bioequivalent with reference drug of Secalip® SR 250 mg capsules (containing Fenofibrate 250 mg).

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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