Available online at www.jpbms.info

ISSN NO- 2230 - 7885

Research Article

JPBMS

JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Bioequivalence and Pharmacokinetic Evaluation of Two Formulations of Fenofibrate 145 Mg in Healthy Indian Subjects.

Shep Dhaneshwar[1], Ojha Ashwini [2], Patel Sweta[3], Nivsarkar Manish[4], *Jaiswal Vijaya[5] and Padh Harish[6]

[1]Sr. Manager, Medical Services, Troikaa Pharmaceuticals Ltd., Ahmedabad, India.

[2]Scientist A, Department of Bio-analytical, B.V. Patel PERD Centre, Ahmedabad, India

[3]Research Assistant, Department of Biostatistics, B.V. Patel PERD Centre, Ahmedabad,India.

[4]Joint Director, Department of Pharmacology and Toxicology, B.V. Patel PERD Centre, Ahmedabad, India.

[5]Vice-President, Medical Services, Troikaa Pharmaceuticals Ltd, Ahmedabad, India.

[6]Vice chancellor, Sardar Patel University, Vallabh Vidyanagar, India.

Abstract:

The prodrug fenofibrate, a synthetic phenoxy-isobutyric acid derivative, is rapidly hydrolyzed in vivo to form fenofibric acid, which alters plasma lipid levels by activating the peroxisome proliferator-activated receptor alpha. The aim of this study was to compare the bioavailability and tolerability of 2 oral formulations of fenofibrate 145 mg. The study was designed as a single-dose, randomized, single-label, 2-period crossover study in healthy Indian adult volunteers. Subjects received 1 tablet of each fenofibrate 145 mg formulation. Study drugs were administered with 240 mL of water after standardized meal on each of 2 treatment days separated by a 2-week washout period. After study drug administration, serial blood samples were collected over a period of 96 hours. Plasma was analyzed for fenofibric acid concentration using a validated high-performance liquid chromatography method. Pharmacokinetic (PK) parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, $AUC_{0-\infty}$, and k_{el}, were determined for the 2 fenofibrate formulations. The formulations were to be considered bioequivalent if the log-transformed ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125%. A total of 18 subjects were enrolled. No significant differences were found based on analysis of variance, with mean values and 90% confidence intervals of test/reference ratios for these parameters as follows: C_{max}, 6.40 versus 7.12 µg/mL (81.71 – 103.05); AUC_{0-t}, 139.57 versus 153.50 µg.hr/mL (85.56 – 102.02); and $AUC_{0-\infty}$, 147.49 versus 161.25 µg.hr/mL (83.44 – 105.87). In these healthy adult Indian volunteers, results from the PK analysis suggested that the test and reference formulations of fenofibrate 145 mg tablets were bioequivalent. Both the formulations were well tolerated.

Key words : Bioequivalence, Fenofibrate, Pharmacokinetics.

Introduction:

Fenofibrate is a lipid-lowering agent introduced internationally in 1975 and now used in >80 countries. [1,2] It has become one of the world's most widely prescribed pharmacologic treatments for hypercholesterolemia, combined dyslipidemia, remnant hyperlipidemia, endogenous hyperlipemia (hypertriglyceridemia), and mixed hyperlipemia (Frederickson types IIa, IIb, III, IV, and V dyslipidemia, respectively).[1,2]

Fenofibrate is a prodrug.[3,4] After oral administration, it is rapidly converted through hydrolysis of the ester bond to its active form and major metabolite, fenofibric acid. Plasma levels of fenofibric acid peak 6 to 8 hours after oral administration, and food enhances its absorption.[5-7] The extent of absorption of fenofibrate tablets is increased approximately 35% under fed as compared to fasting conditions.[3] Steady-state plasma levels are reached within 5 days of dosing, and no accumulation has been observed in healthy volunteers following multiple doses.[3] Fenofibric acid is metabolized by the hepatic cytochrome P (CYP)-450 3A4 isozyme and has a half-life ($t_{1/2}$) of 20 hours, which allows once-daily administration.[3,8] Fenofibrate is mainly excreted in urine as metabolites, primarily fenofibric acid and fenofibric acid glucuronide.

Since fenofibrate was first made commercially available, its main drawback has been the low bioavailability of the active metabolite, fenofibric acid, when the prodrug is taken orally on an empty stomach.[2,9-12] Fenofibrate is virtually insoluble in water and is highly lipophilic, hence it is poorly absorbed when taken orally, especially under fasting conditions.[1,8] In contrast, its absorption is substantially increased in the presence of food.[1,8,12] Therefore, product labeling of formulations marketed to date have mandated administering the drug with meals, even for newer fenofibrate formulations such as micronized capsule and a microcoated tablet, that were introduced to improve bioavailability.[1,8,9]

The objective of this study was to compare the bioavailability of the Test formulation of fenofibrate 145 mg tablet (Troikaa Pharmaceuticals Ltd, India) with reference formulation of fenofibrate 145 mg (Ranbaxy pharmaceuticals Ltd, India) in healthy Indian adult male volunteers under fed condition. The reference formulation is the pioneer brand in India.

Material and methods:

The study was carried out at the B. V. Patel Pharmaceutical Education and Research Development centre, Ahmedabad. All the subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was approved by the institutional ethics committee and was conducted in accordance with good clinical practice and the declaration of Helsinki.

Study Subjects

The study population consisted of 18, adult, male healthy Indian subjects with mean BMI 21.7 (range 19.14 – 24.21), a mean age of 32.2 years (range 25 - 44), mean weight of 59.8 kg (range 48 - 69) and a mean height of 165.6 cm. (range 154 - 177)

Design

The study was designed as Single labeled, Balanced, Randomized, Two- Treatment, Two-Sequence, Two Period, Single Dose, Crossover Bioequivalence study with a 14 days washout period.

The volunteers were administered one of the two study drugs after standardized meal. The dose administration was performed as per the randomization schedule generated at B.V. Patel PERD Centre, Ahmedabad. Subjects received single oral doses of the test formulation (fenofibrate 145 mg, Troikaa Pharmaceuticals Ltd. India) and reference formulation (fenofibrate 145 mg of Ranbaxy pharmaceuticals Ltd, India,).

Blood sampling

A total of 16 blood samples were collected during each period. Blood samples were collected through an indwelling cannula placed in the forearm vein using disposable syringe or with disposable syringes and needles. 6 mL of blood samples (including 0.2 mL discarded heparinised blood) were withdrawn at pre-dose and 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 10.0, 14.0, 24.0, 36.0, 48.0, 72.0 and 96.0 hrs following drug administration in each period. After centrifugation, plasma separated from blood samples and was stored at –20 ± 5°C for interim storage and then at –80 ± 4°C until analysis.

Method of analysis

Aliquots of 0.5 ml plasma were pippeted into the conical tubes and 50µl of 100µg/ml of internal standard (mefenamic acid) was added into it and vortexed for 15 s. Twenty five (25) microlitres of 10% perchloric acid was added to this and vortexed for 15 s. In this mixture 5 ml of ethyl acetate was added and vortexed and then tubes were kept on extractor for 10 min. After extraction, the organic layer was centrifuged at 2500 rpm at 6°C for 10 min. The organic supernatant was transferred in another tubes and kept for evaporation under nitrogen gas till dryness. Then the sample was reconstituted in 100µl mobile phase and 70µl injected into the system.

Fenofibric acid was chromatographed on a reverse phase Flexit-Jour, Kromasil, RP-18 column (150 mm x 4.00 mm, 5 µm) and guard column C18 (Corasil) maintained at room temperature. The mobile phase consisting of a v/v mixture of 0.01 mM potassium phosphate (pH 3.0): acetonitrile (45:55), pH adjusted to 3.0 with phosphoric acid, was pumped at a flow rate of 1.0 ml/min. Borwin software was used for the data analysis. The retention time for drug and internal standard were 4.0 and 6.8 min, respectively detected by UV detector at 287 nm. On each analysis day, a standard curve at the beginning and three quality control samples (high, mid and low) at the beginning, after each subject each period and end of sample assay were also analyzed.

Method validation

The method was validated on Jasco HPLC (Japan) consisting of PU-980 pump, AS-950-10 autosampler and UV-975 which included Accuracy, Precision, Linearity, Limit of Quantification, Inter- and Intra-day variations, Stability and Recovery. Eight point calibration curve was linear over the range 0.1 – 16 µg/ml. The regression equation was $y = 0.1863(\pm 0.0053)x + 0.0023(\pm 0.00072)$ where y is the peak area ratio of analyte to internal standard and x is the concentration of the analyte. The correlation coefficient was >0.999 on all the days. The lowest concentration in the calibration curve was the lowest limit of quantitation. The within-batch and between-batches accuracy and precision were checked using blank plasma spiked with analyte at three concentrations within the range and at the lowest calibrator. Six replicates were analysed at each concentration. The accuracy ranged from 96 to 109% and the precision ranged from 2 to 7%. The percent recovery of drug (high, mid, low) and internal standard was 75.3%, 77.5%, 66.4% (high, mid, low) and 71.9%, respectively. Freeze-thaw cycle showed that the drug was stable up to three cycles. Post-processing autosampler stability showed that the drug was stable for 24 hours at 4 ^{o} C. The analyte was stable in plasma at room temperature for a minimum of 6 hours and at -80 ^{o} C for a minimum period of one month.

Pharmacokinetic and Statistical Analyses

Maximal plasma concentration (C_{max}) and time to reach the peak concentration (T_{max}) were obtained directly by the visual inspection of each subject's plasma concentration-time profile. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (K_{el}). The elimination half-life was obtained from the formula, $t_{1/2} = ln(2)/ K_{el}$. The AUC_{0-t} from time zero to the last quantifiable point (Ct) was calculated using the trapezoidal rule and the extrapolated AUC from Ct to infinity ($AUC_{0-\infty}$) was determined as Ct/ K_{el}. The area under the plasma concentration-time from 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant. To test the bioequivalence of the test and reference formulations, analysis of variance (ANOVA) for the crossover design was conducted on log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. The formulations were to be considered bioequivalent if the log transformed ratios (test/reference) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125% and if P was >0.05 for the 90% confidence intervals.

Safety and tolerability

General clinical safety was assessed *via* physical

examinations and vital signs conducted at screening and at the end of the study. Clinical laboratory tests and ECGs were also conducted at screening, before dosing within each treatment period, and at the end of the study. Adverse events were assessed for severity and relationship to treatment through out the study.

Result:

Pharmacokinetic Analysis

The mean plasma concentration–time curves of test and reference formulation of fenofibrate each administered as a single 145 mg oral dose to 18 healthy Indian male volunteers are shown in the figure 1. The primary PK parameters for both drugs are listed in ***Table 1***

Table 1: **Summary of pharmacokinetic parameters of Fenofibrate, following administration of the reference and test formulations**

Products	Reference						Test					
Parameters	C_{max}	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	$t_{1/2}$	K_{el}	C_{max}	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	$t_{1/2}$	K_{el}
	(µg/mL)	(h)	(µg.h/mL)	(µg.h/mL)	(h)	(h^{-1})	(µg/mL)	(h)	(µg.h/mL)	(µg.h/mL)	(h)	(h^{-1})
MEAN	7.12	4.33	153.50	161.25	29.32	0.03	6.40	4.39	139.57	147.49	30.16	0.03
SD	2.42	1.03	58.96	64.10	7.23	0.01	1.92	1.04	43.24	47.98	7.81	0.01
SEM	0.57	0.24	13.90	15.11	1.70	0.00	0.45	0.24	10.19	11.31	1.84	0.00
%CV	34.00	23.75	38.41	39.75	24.66	33.48	29.98	23.63	30.98	32.53	25.90	42.91

C_{max}: Maximum measured plasma concentration; T_{max}: Time of maximum measured plasma concentration; AUC_{0-t}: The area under the plasma concentration versus time curve from time zero to the last measurable concentration; $AUC_{0-\infty}$: The area under the plasma concentration verses time curve from zero to infinity; $t_{1/2}$: Time required for the plasma drug concentration to decrease by one half; K_{el}: Apparent first order elimination or terminal rate constant; SEM: Standard error of mean; %CV: Coefficient of variation; Test: Troikaa Pharmaceuticals Ltd. India, Reference: Ranbaxy Pharmaceuticals Ltd, India

The mean (SD) C_{max} values of the test and reference formulations were 6.40 (1.92) and 7.12 (2.42) µg/mL, respectively. The mean (SD) T_{max} values were 4.39 (1.04) and 4.33 (1.03) hours. Results for the extent of absorption, as determined from mean (SD) AUC_{0-t} and $AUC_{0-\infty}$ values, were 139.57 (43.24) and 147.49 (47.98) µg/mL/h respectively, after administration of the test formulation and 153.50 (58.96) and 161.25 (64.10) µg/mL/h after administration of the reference formulation. The mean (SD) $t_{1/2}$ was 30.16 (7.81) hours for the test formulation and 29.32 (7.23) hours for the reference formulation. On ANOVA, no period, formulation or sequence effects were observed for any PK property. The 90% confidence intervals of the ratios (test vs reference) for the natural log (*ln*)-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ are shown in ***Table 2*** and summary statistics are shown in ***Table 3.*** The 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 81.71 to 103.05, 85.56 to 102.02 and 83.44 to 105.87 respectively, meeting the predetermined criteria for bioequivalence.

Table 2 : **Point estimate and 90% confidence intervals for the ratio of the products averages of Test and Reference formulations**

Parameter	Point Estimate Test: Reference	Lower Confidence Limit	Upper Confidence Limit
C_{max}	0.90	81.71	103.05
AUC_{0-t}	0.909	85.56	102.02
$AUC_{0-\infty}$	0.915	83.44	105.87

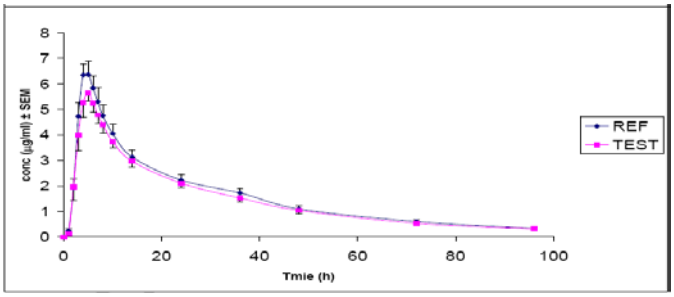
C_{max}: Maximum measured plasma concentration; AUC_{0-t}: The area under the plasma concentration versus time curve from time zero to the last measurable concentration; $AUC_{0-\infty}$: The area under the plasma concentration verses time curve from zero to infinity

Table 3: **Summary statistics of fenofibrate in 18 healthy, adult, subjects under fed conditions.**

Parameters Summary statistics	Product	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)
Geometric Mean	Test Reference	6.05 6.58	126.60 135.44	132.96 141.32
Least Square Mean (LSM)	Test Reference	6.05 6.58	126.60 135.44	132.96 141.32
LSM Ratio B/A %		91.9	93.5	94.1
90 % Confidence Interval : B/A Lower Limit Upper Limit		 81.71 103.05	 85.56 102.02	 83.44 105.87
p - value (ANOVA) Period Formulation Sequence		 >0.05 >0.05 >0.05	 >0.05 >0.05 >0.05	 >0.05 >0.05 >0.05
Intra-subject Variability: CV(%)		20.2	15.25	20.7

A: Reference Product; B: Test Product; ANOVA: Analysis of variance; B/A: Bioavailability ratio Test/Reference ; %CV: Coefficient of variance.

Figure 1: **Linear plot of mean fenofibric acid concentration versus time in 18 healthy, adult, subjects under fed condition**



Safety and tolerability

All 18 subjects completed the study and there were no premature withdrawals, replacements or death during the study. No serious adverse events were recorded, and there were no clinically significant changes in vital signs, clinical laboratory variables, ECG parameters or physical examination findings during the study. There were no adverse events reported during the study.

Discussion:

Optimization of lipid management is a crucial aspect in the treatment of cardiovascular disease. Currently, HMG-CO reductase inhibitors (statins) are a mainstay of therapy. While this class of drugs has proven efficacy at lowering low-density lipoprotein cholesterol, their effects on other important lipid parameters, such as high-density lipoprotein cholesterol and triglycerides, are less robust.[13] This is especially relevant in the Indian context as a combination of hypertriglyceridemia, low levels of HDL-cholesterol and high levels of small dense lowdensity lipoprotein, termed as "atherogenic dyslipidemia', is particularly seen in Asian Indians.[14]

Fenofibrate exerts a favorable effect on the atherogenic lipid profile of mixed dyslipidemia and can effectively reduce cardiovascular disease. It may be used in combination with statins in patients with mixed dyslipidemias not at goals on statin and as a mono-therapy for patients intolerant to or with a contraindication to statin therapy.[15,16]

This study examined the PK properties and bioequivalence of the test and reference formulations of fenofibrate 145 mg in healthy Indian adult male volunteers. All the 18 subjects completed the study and were included for both statistical and analytical analysis. Based on repeated measures of ANOVA subject, period, treatment and interaction term (period × treatment) has non-significant difference. The P-values suggest that there is no significant difference. The 90% CI for all the pharmacokinetic parameters were within bioequivalence acceptance criteria suggesting that the formulations were bioequivalent.

The absorption of fenofibrate was the same for the two formulations and the peak was obtained at the same time for both the formulations. The mean C_{max} of the test was 6.40 µg/mL, which was comparable to that of the reference formulation 7.12 µg/mL as there was no statistical significant difference. Guivarc'h PH et al reported mean C_{max} 9.98 µg/mL and 7.72 µg/mL for 160 mg tablet and 200 mg capsule of fenofibrate respectively under fed conditions.[1] The mean T_{max} of the test was 4.39 hours, which was comparable to that of the reference formulation (4.33 hours). Guichard J. P. et al reported mean T_{max} 4 h and 4.1 h for two different formulations of fenofibrate.[17] The mean $t_{1/2}$ obtained in this study was 30.16 hours for the test formulation, which was comparable to that of the reference formulation (29.32 hours). The half-life values for both the products were longer in comparison to reported half-life of 20 hours.[3]

Fenofibrate is generally well-tolerated with a safety profile comparable to placebo. In the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study, which included nearly 5,000 patients receiving the active drug, the safety profile of fenofibrate matched that of a placebo over an average of 5-years follow-up.[18] In the present study both formulations were well tolerated and no adverse events were reported during the study.

Conclusions:

This study did not find any statistically significant differences in C_{max} or AUC values between the test and reference formulations of oral fenofibrate 145 mg in this population of healthy adult Indian volunteers. On that basis, and according to both the rate and extent of absorption, the test and reference formulations were bioequivalent. Both formulations were well tolerated.

Acknowledgements:

We are thankful to Troikaa Pharmaceuticals Ltd., Ahmedabad for providing the drug samples. We are also grateful to clinical staff of B.V. Patel PERD Centre for assistance in the conduct of pharmacokinetic study.

Declaration of Interest:

Ms. Ashwini Ojha, Ms. Sweta Patel, Dr. Manish Nivsarkar and are employees of B.V. Patel PERD Centre, Ahmedabad, Dr. Harish Padh is Vice Chancellor of Sardar Patel, University; Vallabh Vidyanagar and Mr. Dhaneshwar Shep and Dr. Vijaya Jaiswal are employees of Troikaa Pharmaceuticals Ltd. Ahmedabad. The authors state no financial conflict of interest.

References:

1. Guivarc'h PH, Vachon MG, Fordyce D. A new fenofibrate formulation: results of six single-dose, clinical studies of bioavailability under fed and fasting conditions. Clin Ther. 2004;26(9):1456-69.
2. Nearing GM, Ormrod D. Micronised fenofibrate: An updated review of its clinical efficacy m the management of dyslipidaemia. Drugs. 2002;62:1909-1944.
3. Najib J. Fenofibrate in the treatment of dyslipidemia: a review of the data as they relate to the new suprabioavailable tablet formulation. Clin Ther. 2002;24(12):2022-50.
4. Chapman MJ. Pharmacology of fenofibrate. Am J Med. 1987;83:21-25.
5. Balfour JA, McTavish D, Heel RC. Fenofibrate. A review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in dyslipidemia. Drugs. 1990;40:260-290.
6. Adkins JC, Faulds D. Micronised fenofibrate: A review of its pharmacodynamic properties and clinical efficacy in the management of dyslipidaemia. Drugs. 1997;54:615-633.

7. Hunninghake DB. Treatment of Hypertriglyceridemia with fenofibrate. Practical Cardiol. 1989;15:38-54.
8. Konstantinos Tziomalos and Vasilios G Athyros. Fenofibrate: a novel formulation (Triglide™) in the treatment of lipid disorders: a review. Int J Nanomedicine. 2006; 1(2):129–147
9. Adkins JC, Faulds D. Micronised fenofibrate: A review of its pharmacodynamic properties and clinical efficacy in the management of dyslipidaemia. Drugs. 1997;54:615-633.
10. Guay DR. Update on fenofibrate. Cardiovasc Drug Rev. 2002;20:281-302.
11. Ramjattan BR, Callaghan DJ, Theiss U. Efficacy and tolerability of a "suprabioavailable" formulation of fenofibrate in patients with dyslipidemia: A pooled analysis of two open-label trials. Clin Ther. 2002;24:1105-1116.
12. Munoz A, Guichard JP, Reginauh P Micronised fenofibrate. Atherosclerosis. 1994;110(Supp1):S45-S48.
13. Schima SM, Maciejewski SR, Hilleman DE, Williams MA, Mohiuddin SM. Fibrate therapy in the management of dyslipidemias, alone and in combination with statins: role of delayed-release fenofibric acid. Expert Opin Pharmacother. 2010;11(5):731-8.
14. A Misra, Kalpana Luthra, NK Vikram. Dyslipidemia in Asian Indians: Determinants and Significance. JAPI. 2004;52:137-142
15. Farnier M. Update on the clinical utility of fenofibrate in mixed dyslipidemias: mechanisms of action and rational prescribing.Vasc Health Risk Manag. 2008;4(5):991-1000.
16. Abourbih S, Filion KB, Joseph L, Schiffrin EL, Rinfret S, Poirier P, Pilote L, Genest J, Eisenberg MJ. Effect of fibrates on lipid profiles and cardiovascular outcomes: a systematic review. Am J Med. 2009;122(10):962.e1-8.
17. J. P. Guichard, R. Levy-Prades Sauron. A comparison of the bioavailability of standard or micronized formulations of fenofibrate. Current Therapeutic Research 1993;54(5):610-14
18. Paul L. Drury, Ryan DuBosar, Tracy DuBosar, Conni Bergmann Koury. Fenofibrate May Have Potential Role in Slowing the Progression of Nephropathy in Diabetes. Review of endocrinology. 2008;september:suppl:1-13. (http://www.reviewofendo.com/articles/0908/0908_supp.pdf. Accessed on October 4, 2010)

***Correspondence Author :**
Dr. Vijaya Jaiswal
Vice-President, Medical Services,
Troikaa Pharmaceuticals Ltd, Commerce House-1, Satya Marg,
Bodakdev, Ahmedabad – 54, India, Phone No- 91-79-26856242-45,
Fax : 91-79-26856246
E-mail: medicalservices@troikaapharma.com

Source of support:

Study was sponsored by Troikaa Pharmaceuticals Ltd, Ahmedabad, India.

Conflict of interest: None.

Registration no. : **NA**