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BIOAVAILABILITY STUDY OF TABLET BEZAFIBRATE 200 MG (LIPOCOR ®)

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ABSTRACT

In order to determine the ultimate quality of any formulated dosage form and rationalize the therapeutic plan as well as to individualize the prescription, *in vivo* measurement of drug is the modem and specialized expertise of the clinical/research area of pharmacy practice, which provides effectiveness and assures the safety of drugs.

All pharmacological, therapeutic or toxic responses are subject to reaching of drug at the site of action through connective tissue. Other than physico chemical properties of drug, there are numerous factors from manufacturing process to biochemical behaviour of the individual which resist in the absorption, distribution, metabolism and elimination of drugs in the biological system. Bezafibrate Tablet 200 mg (Lipocor®) an oral conventional formulation manufactured by Efroze Chemical Industries (Pvt.) Ltd. was investigated for bioavailability followed by pharmacokinetic studies on adult, male, healthy, human local population. For this purpose, a sensitive, specific and validated method was used for the estimation of bezafibrate in blood. HPLC was performed on a reversed phase C_{18} column (flow rate 1.5 ml/min, UV=230 nm) with 0.02 M buffer of KH_2PO_4 (Adjusted pH 3.5 with Phosphoric Acid) and Methanol (40: 60) whereas extraction of the drug from the plasma was carried out by deproteinization of plasma according to classical method described in previous studies (Obaid A. *et al*., 1999).

Peak level (T_{max}) of Bezafibrate Tablet 200 mg (Lipocor®) was observed at about 1.42 ± 0.53 hours after the dose and practically free Bezafibrate Tablet 200 mg (Lipocor®) could be detected in blood after 9 hours. C_{max} of the investigated formulation of Lipocor® was 1732 ± 374.2 ng/ml. Area under curve (AUC) was 5198.65 ± 1231.8 ng. hr/ml.

INTRODUCTION

Bezafibrate 200 mg Tablet:

Beginning with the description of clofibrate in 1962, derivatives of fibric acid (fibrates) have been used clinically to treat dyslipidaemias. Subsequently, gemfibrozil, fenofibrate, bezafibrate, ciprofibrate and long-acting forms of gemfibrozil, fenofibrate and bezafibrate have been developed. Bezafibrate is a synthetic chemical.

Pharmacokinetics:

Pharmacokinetic is the study of drug movement in the body over the time during the drug's absorption, distribution and elimination (excretion and biotransformation).

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Bioavailability:

The concept of bioavailability developed in last three decades and has become more important in order to ensure ultimate quality of product. This concept is based on assumption that the measurement of certain parameters like serially obtained blood or urine concentration of drug following drug administration can be correlated with clinical efficacy.

The bioavailability is defined as: "it is the degree to which the drug is absorbed from the pharmaceutical formulation into the body". For drug products not intended to be absorbed into the blood stream, bioavailability may be assessed by measurements intended to reflect the rate and extent to which the active ingredient or moiety may become available at the site of action (FDA-USA, 1997).

The extent of absorption is measured by the bioavailability or fraction of dose absorbed measured by the area under the concentration-time profile (AUC), whereas the rate of absorption is roughly assessed by measuring the maximum plasma or blood level, C_{max} of a compound. (Mangione R.A., 1998). Although C_{max} is a function of both the rate and extent of absorption, and some have argued that T_{max} would be a more pure measure of the rate of absorption, C_{max} is a clinically relevant parameter in most cases.

Many excipients can affect the absorption of drugs. In addition, any sensitivity the patient may have to the various colorants and preservatives present must be taken into account.

Need of Bioavailability Study:

When the patient on a sustained drug formulation ends, other companies are allowed to market generic formulation of the drug if the company can prove the bioequivalence (FDA, 1992 and FDA, 1997 Draft Guidance for Industry).

Bioequivalence:

Two or more chemically or generic equivalent products of the same preparation can be said to be bioequivalent, if they do not differ (<20%) significantly in their bioavailability characteristics. This bioequivalent drug is assumed that they will be therapeutically equivalent and can be used interchangeably (FDA, 1997 Draft Guidance for Industry).

MATERIAL AND METHOD

In vivo trial was conducted according to international guidelines for *in vivo* studies. The bioavailability of Lipocor® was evaluated by plotting blood free concentration profile of drug at different intervals after oral administration followed by pharmacokinetic calculations.

Subjects:

A subject panel of 14 healthy adults, male, human volunteers of an average age of 21.4 years, ranging from 18.5 years to 27 years, and an average weight of 58.1 kg ranging from 49 kg to 72 kg participated in the study.

Selection of Volunteers:

Selection criteria for volunteer inclusion in this study was:

- No history of allergic tendencies and reaction to fibric acid derivatives or any other ingredient used in the formulation of Lipocor® tab 200 mg.
- With normal blood counts, normal liver and kidney function tests, and without abnormalities in physiology, urine and blood analysis.

- Volunteers should be free of any treatment or any drug for at least a month prior to study.
- Absence of any chronic or pathological disease.
- The panel members were given a general medical examination to establish good health.

Exclusion Criteria:

Subjects with any current or past medical condition that might significantly affect their pharmacokinetic and pharmacodynamics response to the administered drug were the limiting factors in the study.

All volunteers were thoroughly informed about the aims and objectives of the study, the drug to be tested, and the hazards/side effects of the drug bezafibrate and also their rights to separate themselves from the study at any stage without mentioning any reason. Informed consent was also obtained from each subject to participate in the study.

Restrictions:

No volunteer was allowed to take any prescription or OTC drug one week prior to dosing and during the study period, in order to avoid interference with the kinetic behaviour of bezafibrate in the body or in the determination of drug in the blood. The volunteers were instructed to report the investigator about the illness/side effects and the treatment undertaken.

No volunteer took any drug for at least one month prior to and during the study.

Study Design:

Study was designed according to described objectives and conducted on a single occasion. Volunteers were given a single dose of test drug i.e. Lipocor® 200 mg tablet manufactured by Efroze Chemical Industries Pvt., Ltd. with water just after a controlled breakfast (FDA, 1997, Draft Guidance for Industry).

Single Dose-Single Period Study:

In this study, each subject is successively exposed to a single treatment on a single occasion. Blood samples (3 ml) were collected through intravenous route at 0.0, 0.5, 1.0, 1.50 and 2.5, 3.5, 5.5 and 9.0 hours interval after drug administration. Drug was administered according to the study design. A controlled non-fatty diet, juices, refreshment and milk were served to all volunteers.

Sample Extraction:

Extraction of drug was carried out by deproteinization of plasma for this purpose samples of serum were mixed with equal quantity of extraction medium in eppendorff tube followed by vortex mixing for 1 minute and centrifuged for 5 minutes at 3000 rpm. The supernatant layer was transferred to another eppendorff tube.

Identification and Estimation of Drug in Serum:

The method modified in Efroze Research Laboratory was used in the determination of bezafibrate. This method is highly specific and was validated for the purpose.

Liquid Chromatography:

Mobile Phase:

Phosphate buffer (0.02M)	=	400ml (pH adjusted to 3.5 with phosphoric acid)
Methanol	=	600ml

Instrument:

HPLC-l0Avp attached with class VP recorder (Shimadzu) was used.

20-ul samples were injected into C18 u Bondapak 3.9-mm X 300-mm column, signals were observed at 230 nm.

Standard Solutions:

The standards of bezafibrate (200, 100, 50, 10, 01, 0.8, 0.6, 0.4, 0.2 ug/ml of plasma) were prepared by diluting the stock solution 20 mg/ml human serum (Table 1).

Procedure:

The volunteers were asked to report to the investigator after an overnight (12 hours) fast. Drug was administered to all volunteers according to the study design. Blood samples were collected through disposable syringes under aseptic technique, at different intervals of time. The samples were centrifuged, serum collected and immediately frozen at $-70^{o}C$ until analysis. A controlled breakfast, lunch and juices were served to all volunteers at different times as specified in study design. Vital signs of volunteers including blood pressure, respiratory rate, oral temperature and pulse rate were checked pre dosing and closely monitored throughout the day by a consulting physician. The serum level of Bezafibrate was measured using high-pressure liquid chromatography technique.

All chemicals used in the study were procured from Merck, a reliable company in the manufacturing and marketing of high-grade chemicals.

RESULTS

Table 1
Peak area against standard concentration of Bezafibrate
on HPLC technique

Concentration (ug/ml)	Peak Area
200	18020591
100	9216636
50	5543634
10	1086923
1	111036
0.8	79064
0.6	66057
0.4	46499
0.2	22889

Table 2
Blood free concentration (ng/ml) of Bezafibrate following a single oral dose of 200 mg tablet (Lipocor®) when given to healthy human volunteers (N=14 ± SD)

Subject	0 hours	0.5 hours	1 hours	1.5 hours	2.5 hours	3.5 hours	5.5 hours	9 hours	AUC (ng. hr/ml)
A	0	623	1272	1968	1651	1064	329	111	6769.50
B	0	45	383	1368	1271	568	164	39	3882.25
C	0	136	1450	1512	1183	815	134	30	4753.50
D	0	846	1220	1406	967	499	128	38	4221.50
E	0	1712	2262	1316	834	559	315	42	5586.25
F	0	682	1416	745	742	424	76	22	3233.25
G	0	15	133	270	1205	982	468	102	4420.00
H	0	28	1360	2205	1543	820	368	164	6419.75
I	0	1043	1723	1407	1211	623	189	68	5222.50
J	0	571	1899	1855	1527	1064	468	179	7349.50
K	0	1019	1500	1364	930	383	93	11	4062.00
L	0	107	1709	2266	1521	660	193	41	5721.00
N	0	390	2103	2055	1307	759	227	48	5941.50
MEAN	0	555.2	1417.7	1518.2	1222.5	709.2	242.5	68.8	5198.65
±SD	0.0	510.8	604.5	570.3	290.4	230.3	134.4	53.9	1231.8

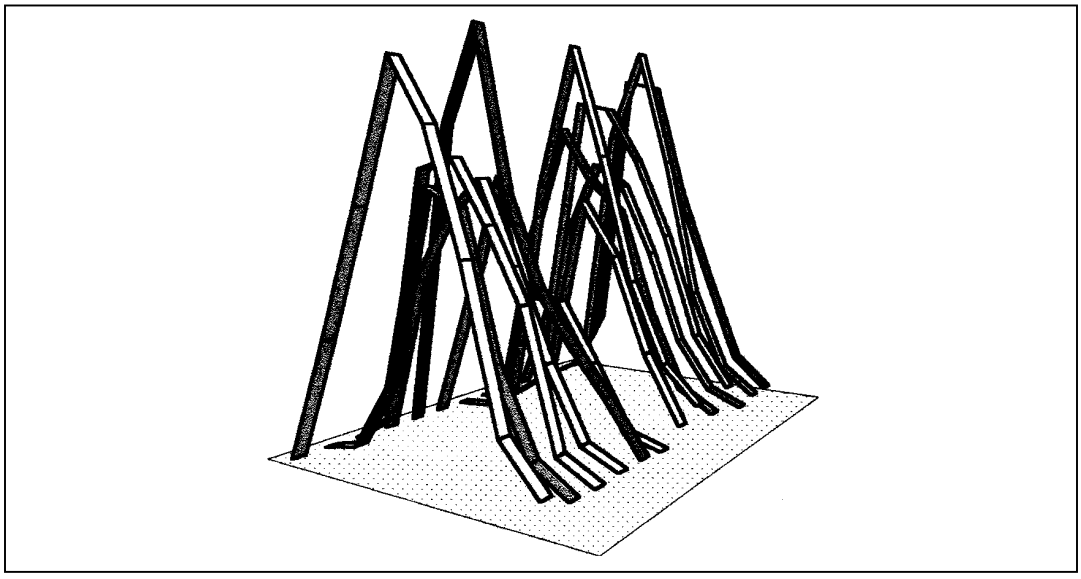

Fig. 1: Inter individual variation in blood free profile of Bezafibrate when given 200 mg orally to the healthy, human volunteers (N=14).

Table 3
Pharmacokinetic parameters of individual healthy, human volunteers after the oral administration of a single dose of tablet Bezafibrate 200 mg (Lipocor®) manufactured by Efroze Chemical Industries (Pvt.) Ltd.

Subject Identity	C_{max} (ng/ml)	T_{max} (ng/hours)	MRT	AUC (ng.hr/ml)	Extent of Bioavailability
A	1651	2.5	155.75	6769.50	0.24388803
B	1368	1.5	011.25	3882.25	0.35237298
C	1512	1.5	034.00	4753.50	0.31808141
D	1406	1.5	211.50	4221.50	0.33305697
E	2262	1.0	428.00	5586.25	0.40492280
F	1416	1.0	170.50	3233.25	0.43794943
G	1205	2.5	003.75	4420.00	0.27262443
H	2205	1.5	007.00	6419.75	0.34347132
I	1723	1.0	260.75	5222.50	0.32991862
J	1899	1.0	142.75	7349.50	0.25838492
K	1500	1.0	254.75	4062.00	0.36927622
L	2266	1.5	026.75	5721.00	0.39608460
N	2103	1.0	097.50	5941.50	0.35395102
Mean	1732	1.42	138.79	5198.65	0.33316342
± SD	374.2	0.53	127.69	1231.8	0.05718165

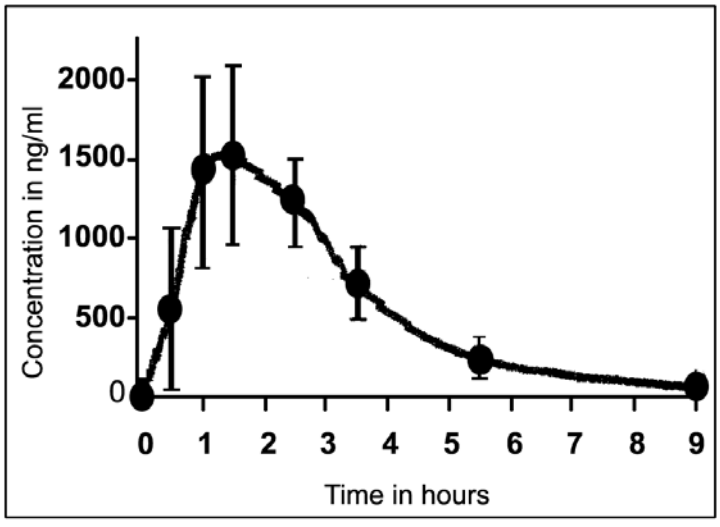

Fig. 2: Mean (N=14) ± SD blood free concentration of Bezafibrate 200 mg when given orally to the healthy, human volunteers.

Table 4
Mean pharmacokinetic values of tablet Bezafibrate after oral administration of 200 mg dose, showing the mean SD ± Biokinetic behavior of Pakistani Population towards Tab Lipocor® manufactured by Efroze Chemical Industries

Parameters	K	t ½ Hours
Elimination	0.0062	1.86
Distribution	0.0149	0.775
Absorption	0.022	0.525

Pharmacokinetic and Statistical Analysis:

Data Analysis:
Chromatograms of all samples were read/calculated in the context of standard calibration curve (as showed in table 1) after system suitability test.

Statistical Analysis:
The plasma drug concentration levels were monitored and analyzed statistically in order to evaluate bioavailability, pharmacokinetic and other parameters of the studied formulation. MS Excel 98 program was used for the calculation of peak concentration (C_{max}), time to peak concentration (T_{max}) while area under the curve (AUC) was calculated by trapezoidal method. Other pharmacokinetic values were calculated by manual technique using basic mathematical tools.

DISCUSSION AND CONCLUSION

Three basic following pharmacokinetic parameters were calculated for the tested product i.e. Tablet Bezafibrate 200mg (a conventional release oral formulation).

1) Area under curve (AUC)
2) Peak Concentration C_{max}
3) Time to peak concentration T_{max}
4) Elimination ½ life
5) Distribution ½ life
6) Absorption ½ life
7) Approx. Mean residence time (MRT)
8) Extent of bioavailability

1. AUC:
5198.65 ng.hr/ml ± 1231.8 was observed as shown in Table 2 and 3 and Fig. 1 and 2.

2. C_{max}:
1732 ng/ml ± 374.2 was observed as shown in Table 3 and Fig. 2 after administration of 200 mg conventional release oral formulation of bezafibrate to healthy human volunteers.

3. T_{max}:
1.42 hours ± 0.53 was observed in this study in Table 3 and Fig 2.

4. Elimination ½ life (1.86 hours)

5. **Distribution ½ life** (46.5 minutes)
6. **Absorption ½ life** (31.5 minutes)
7. **(MRT) Approx. Mean residence time** (138.79 $\pm$ 128.69)
8. **Extent of bioavailability** (0.333 $\pm$ 0.057)

All values calculated in our study are comparable with previous studies and safety/therapeutic data indicating effectiveness of the studied drug (Monk, J.P., Todd, P.A., 1987; Miller, D.B., Spence, 1998, Hoffman, A. *et al.,* 1999 etc).

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