CHAPTER II

MATERIALS AND METHODS

Materials

1. Chemicals and reagents

Standard gemfibrozil (distributed by Berlin Pharmaceutical CO. LTD., Thailand) , standard flurbiprofen (Sigma chemical , U.S.A.) , sodium acetate trihydrate and monobasic potassium phosphate (Fluka chemic, Switzerland), sodium hydroxide (Merck , Darmstadt ,Germany) , glacial acetic acid (J.T. Bager ,Phillpsburg , U.S.A.) , and hydrochloric acid (Mallinckrodt chemical , Kentucky, USA) are analytical grade agents , dichloromethane (AJAX chemical , N.S.W. , Australia) , and methanol (Mallinckrodt chemical, Kentucky, U.S.A.) are HPLC grade.

2. Apparatus

2.1 High Performance Liquid Chromatography (Waters , division of Millipore CO., MA, USA) consists of model 510 multiple solvent delivery system, model 740 computing integrator , model 481 UV-spectrophotometric detector and model FP-210 spectrofluorometric detector (Jasco , Japan spectroscopic CO. LTD., Tokyo , Japan)

2.2 fixed loop 10 µl , 20 µl injector port (Rheodyne 7161, CA, USA)

2.3 analytical column : stainless steel dimension (300 x 3.9 mm i.d.) packed with μ-bondapak® C-18 partical size 10 microns (Waters, Millipore CO., MA, USA)

2.4 guard column : stainless steel dimension (20 x 5 mm i.d.) , packed with bondapak® C-18/Coracil particle size 37-50 microns (Waters, Millipore CO., MA, USA)

2.5 spectrophotometer (model-1601, Shimadzu CO. LTD., Tokyo, Japan)

2.6 analytical balance (Mettler , Zuerich , Switzerland)

2.7 vortex-mixer (Vortex-Genic, Scientific industries Inc., USA)

2.8 degasor (model 2200 , Brandson Europa B.V., Netherland)

2.9 magnetic stirrer (Labinco , Netherland)

2.10 pH meter (model SA-520, Orion research Inc., MA , USA)

2.11 centrifuge (Clay-Adam Inc., NY , USA)

2.12 reciprocating shaker (Rotamix® , Heto , Germany)

2.13 water bath(Julabo 20B , Julabo Lab, Seelbach, West Germany)

2.14 autopipette : adjustable volume 10 μl (Biohit Proline®Helsinki, Finland) micropipette 200 μl , 1000 μl (Pipetman®,Gilson medical Electronics, SA, France) and 5 ml (Socorex® , Swiss)

2.15 Disintegration apparatus (model 31-161, Hanson Research CO., CA, USA)

2.16 Dissolution apparatus (model 63-550-001, Hanson Research, CO., CA, USA)

3. Preparation solution

3.1 <u>0.18 M sodium acetate pH 3.8</u>

Dissolve 24.49 g of sodium acetate trihydrate ($CH_3COONa.3H_2O$) in water , make up to a 1000 ml in volumetric flask . This solution is adjusted to pH 3.8 with glacial acetic acid.

3.2 0.2 M phosphate buffer pH 7.5

Dissolve 27.22 g of monobasic potassium phosphate (KH_2PO_4) in water, transfer to a 1000 ml volumetric flask and dilute to volume. Mix a 250.0 ml of monobasic potassium phosphate solution with 12.0 ml of 1N sodium hydroxide solution in a 1000 ml volumetric, and make up to volume with water.

3.3 1 N sodium hydroxide

Dissolve 20.0 g sodium hydroxide in water, make to a 500 ml in volumetric flask .

3.4 1 N hydrochloric acid

Added 8.35 ml of concentrated HCl (37% w/w) into a 100 ml volumetric flask containing water and adjust to volume.

4. Pool serum

The whole blood was generously supplied by the National Blood Center Thai Red Cross Society and was left to be clotted. The clotted blood was centrifuged for 15 min at 3500 rpm to separate serum and then pool serum was kept frozen at -20 °C.

5. Products

Local manufactured product and original product were randomized sampling from the department of pharmacy , Chulalongkorn Hospital. The details of both products were shown in Appendix A.

6. Subjects

The subjects were selected on the basis of inclusion criteria as follows :

(1) Thai male volunteer , age ranged between 18 - 55 years

(2) no history of allergy or hypersensitivity to any drug.

(3) no history of liver disease , renal disease and gastrointestinal tract disorder.

(4) no drug abuse and/or alcohol dependence

(5) healthy condition

Methods

In Vitro Studies

1. Standardization of Products

In this study , the local manufactured product and original product were codified " brand A" and " brand B " , respectively (investigator did not involved in coding this products) and they were standardized in the terms of their content of active ingredient , uniformity of dosage units (weight variation), disintegration and dissolution as follows :

1.1 Content of active ingredient

Determination of the content of active ingredient was performed according to the USP XXIII . The procedure was described as follows :

1.1.1 Preparation of the gemfibrozil solution from capsule

The content of twenty capsules of each brand was removed as completely as possible , and weighed accurately. The

combined content were mixed. An accurately weighed portion of the powder equivalent to about 100 mg of gemfibrozil to a 100 ml volumetric flask, add about 80 ml of methanol, shake to dissolve, and dilute with methanol to volume. The solution was then mixed and filtered. Transfer 5 ml of this clear solution to a 25 ml volumetric flask , dilute with mobile phase to volume , and mix . The final solution was then assayed by isocratic reversed phase high performance liquid chromatographic technique.

1.1.2 Preparation of the standard gemfibrozil solution

Accurately weighed standard gemfibrozil and dissolved in methanol to obtain a solution having a known concentration of approximately 1 mg per ml . Aliquot 5 ml of this solution and make up to a 25 ml in volumetric flask using mobile phase as solvent .

Aliquot 10 µl of sample or standard gemfibrozil solution was injected into HPLC system.

1.1.3 HPLC condition for gemfibrozil analysis

mobile phase ; methanol : glacial acetic acid : deionized water

75 : 1 : 24

flow rate	:	1 ml/min
pressure	:	1000 psi
temperature	:	ambient
detector	:	UV 276 nm
volume injected	:	10 µl
integrator	:	attenuation 2^6 mv/full scale
		chart speed 5 min/cm

The actual content of gemfibrozil was quantified by standard solution

1.1.4 Calculation the % labelled amount of gemfibrozil capsule

From the data of peak responses of brand A or B , and standard solution obtained , the amount in milligram (mg) of gemfibrozil in the portion of the powder either brand A or B can be calculated from the formula 500 C (r_u / r_s) , when C is the concentration in mg per ml of gemfibrozil in standard solution ; r_u and r_s are the peak responses of brand A or B sample and standard solution, respectively.

1.2 Uniformity of dosage units

The uniformity of dosage units of gemfibrozil was determined in the term of weight variation that gemfibrozil in each individual up to 10 capsules were determined according to the USP XXIII as follows :

1.2.1 Preparation of the gemfibrozil solution from capsule

Ten capsules of each brand of gemfibrozil capsules were sampled. Each was individually weighed accurately . The content of each capsule was removed and the weight of emptied shells was individually determined. The content net-weight of each capsule was calculated by subtracting the weight of the shell from the respective gross weight.

The content of gemfibrozil from all 10 capsules were combined and mixed . Accurately weighed portion of the powder-equivalent to approximately 100 mg of gemfibrozil into a 100 ml volumetric flask , disslove with methanol and make to volume. The solution was then mixed and filtered. Aliquot a 5 ml of this solution to

a 25 ml volumetric flask , dilute with mobile phase to volume. This solution was promptly injected into HPLC with injection volume of 10 µl.

1.2.2 Preparation of the standard gemfibrozil solution

The same procedure was already described in 1.1.2.

1.2.3 HPLC condition for gemfibrozil analysis

The same procedure was already described in 1.1.3.

1.2.4 Calculation the % labelled amount of gemfibrozil capsule

The same procedure was already described in 1.1.4.

1.2.5 Calculation of the content of gemfibrozil in each capsule

The content of gemfibrozil in each capsules was calculated from the accurately weighed portion of powder , equivalent to 100 mg of gemfibrozil that was analysed , the actual content of gemfibrozil in the portion of powder calculated from the formula 500C (r_u/r_s) and accurately weight of the contents in each capsule.

1.3 Disintegration Test

The disintegration test of gemfibrozil capsules were determined according to the USP XXIII . The procedure was described as follows :

Place one capsule in each of the six tubes of the basket. A disk was then added to each tube and the apparatus was then operated having water as the medium for maintaining at $37 \pm 2^{\circ}$C. The time that the apparatus started to move until all of capsule have disintegrated

except for the fragments from the capsule shell , was determined as the disintegration time. The mean and standard deviation of disintegration time of each brand was calculated .

1.4 Dissolution Test

The dissolution of gemfibrozil capsule was determined according to the USP XXIII by using the USP-Dissolution Apparatus Type II (paddle). The procedure was described as follows :

Added nine hundred millilitres of deairated dissolution medium (0.2 M phosphate buffer pH 7.5) into the vessel of apparatus and maintained the temperature at $37 \pm 0.5^{\circ}$C. Place 1 capsule of gemfibrozil in the vessel. The apparatus was then immediately operated and maintained speed of rotation at 50 rpm. Five millilitres of dissolution medium was taken from vessel at 45 minutes. This solution was then filtered through a membrane filter (diameter of pore size 0.2 microns) diluted threefold with 1 N sodium hydroxide. Determine the amount of drug dissolved in dissolution medium via spectrophotometer at absorption wavelenghts of 276 nm. The amount of the drug dissolved at 45 minutes was quantified using the calibration curve. The dissolution of each six gemfibrozil capsule of both brands was gradually determined using same procedure.

Preparation of standard gemfibrozil solution for calibration curve

Standard gemfibrozil was dissolved in the dissolution medium at the concentration of 0, 12.5 , 25.0 , 50.0 , 75.0 , 100.0 , 150.0 , 200.0 , 300.0 and 400.0 µg/ml then diluted threefold with 1 N sodium hydroxide.

The absorbance of each concentration was determined using spectrophotometer at 276 nm. The calibration curve of absorbance versus gemfibrozil concentration was constructed using linear regression.

2. Evaluation of the in vitro studies

Both brands of gemfibrozil capsule were evaluated under the procedure of the United State Pharmacopoeia.

The statistical significant difference between brand A and B in the terms of their content of active ingradient , uniformity of dosage units, disintegration and dissolution were determined using unpaired student's t-test at significant level of 0.05. The brand A and B would be concluded to be pharmaceutical equivalent. If no statistically significant difference of these terms were observed.

In Vivo Studies

1. Bioanalytical Methodology

1.1 Determination of gemfibrozil in serum by HPLC

The analytical method for determining gemfibrozil in serum have to be developed for this study. The analytical procedures were described as follows :

1.1.1 Preparation of calibration solution

A stock methanolic solution of gemfibrozil was prepared in the concentration of 2.4 mg/ml once a week. The solution of gemfibrozil used for constructing the calibration curve were prepared by serial dilutions of a stock solution. A series of calibration solution

consisting gemfibrozil in the concentration of 0, 0.0025 , 0.025, 0.15 , 0.3, 0.6 , 1.2 , 2.4 mg/ml were freshly prepared from the stock solution for each analysis. A stock of calibration solution were kept frozen at -20 °C.

1.1.2 Preparation of internal standard solution

A stock of flurbiprofen (internal standard , IS) in methanol was prepared (1 mg/ml) every week and diluted to 0.2 mg/ml before used. This stock of internal standard solution were kept frozen at -20 °C.

1.1.3 Extraction procedure

All frozen serum samples and blank serum were thawed at room temperature just prior to analysis. The extraction was performed in 15 ml glass tube.

The calibration curve was prepared for each assay run by transferring a 10 µl of the calibration solution containing 0.025 - 24 µg of gemfibrozil into a tube containing 0.5 ml of blank human serum. Similarly, 10 µl of methanol was added to the tubes containing 0.5 ml of serum taken from human subjects that had received gemfibrozil. The extraction of samples were performed along with the standard solution. A 10 µl volume of internal standard solution was spiked into each tube with thoroughly vortex-mixed. The extraction was carried out in acidic medium by adding 0.5 ml of 1 N HCl into each tube with gently mixed and then added in a 5.0 ml dichloromethane. The mixture was shaken on a reciprocating shaker for 30 minutes at 45 rpm , centrifuged for 20 minutes at 3,000 rpm. The organic layer was transferred to the another tube and evaporated to dryness under water bath at 60 °C. The separated residue from each tube was reconstitued with 350 µl

of mobile phase , and aliquot of 20 µl was directly injected into the HPLC system.

1.1.4 HPLC condition for serum gemfibrozil analysis

The condition of the HPLC was set up in an isocratic-reverse phase mode. The isocratic mobile phase consisted of methanol and aqueous sodium acetate buffer (pH 3.8 , 0.18 M) in the ratio of 75 : 25 The flow rate was 1.0 ml/min. Spectrofluorometric detector was carried out at excitation and emission wavelengths of 284 and 316 nm , respectively.

1.1.5 Calculation for gemfibrozil in serum sample

A calibration curve was generated for gemfibrozil by using the least square regression analysis of the peak area ratio of gemfibrozil to that of the internal standard against spiked gemfibrozil concentration. The concentration of gemfibrozil in the serum samples were obtained from interpolation of the calibration curve.

1.2 Bioanalytical methods validation

The analytical method developed was validated to ensure the acceptability of the performance. The parameters that essentially determined are accuracy, precision, sensitivity, specificity and linearity.

1.2.1 Accuracy

The accuracy of analytical method developed can be accomplished by analysis of triplicate sets of serum containing spiked known concentration of gemfibrozil. Three different concentrations of

gemfibrozil, 3.0 , 16.0 and 32.0 μg/ml that represent the entired range of calibration curve have been spiked into blank human serum and being analysed along with the series of calibration solution for constructing the calibration curve.

The accuracy was indicated in term of the recovery of gemfibrozil added compared to the analysis value.

$$\% \text{ recovery} = \frac{\text{concentration analysed}}{\text{concentration spiked in}} \times 100$$

The dependence of % recovery upon serum gemfibrozil concentration was tested using analysis of variance (ANOVA). If no statistically significant differences of three different concentrations of gemfibrozil in serum were observed at significant level of 0.05 , it would be concluded that % recovery was not dependent on concentration. The grand total mean of % recovery was then calculated and was designed as the accuracy of the method.

1.2.2 Precision

The precision of the assay was evaluated by analysing replicate calibration curves on the same day (intra-day precision), and on the different day assay (inter-day precision). The peak area ratio (PAR) of gemfibrozil to the internal standard for each concentration was compared over the calibration and relative standard deviation (RSD) for each concentration was determined.

1.2.3 Sensitivity

The sensitivity of the analytical method can be indicated in term of the lowest limit of quantitation (LOQ). The LOQ is the lowest concentration on the calibration curve that can be measured with acceptable precision and variability.

The LOQ was determined by analysing more than triplicate serum containing gemfibrozil at the lowest detectable concentration and determining the ralative standard variation. The concentration of gemfibrozil that can be accepted to be the LOQ should exhibit the RSD of not more than 20%.

1.2.4 Specificity and selectivity

The specificity of the analytical method to identify the peak of gemfibrozil and internal standard in the serum sample can be demonstrated by comparing the retention time of gemfibrozil and internal standard from the observed chromatogram of blank human serum , blank human serum spiked with standard solution of gemfibrozil and internal standard , the standard solution of gemfibrozil and internal standard and also the chromatogram of serum sample from sujects that administration gemfibrozil. The similar time that gemfibrozil and internal standard were eluted from HPLC column without any interference on the chromatogram confirm the specificity of the method.

1.2.5 Linearity

The relationship of response (as PAR) versus concentration was determined using linear regression analysis. The region of which the PAR values is directly related to gemfibrozil concentration , would be

expressed as the linear range of the analysis. The slope and intercept obtained within this linear range was used as the represent calibration equation [PAR = a. (concentration) + b] for determining the concentration of gemfibrozil in serum sample.

1.3 The stability study of gemfibrozil in serum at -20 °C

The stability of gemfibrozil in serum at the storage temperature of -20 °C was determined at three different concentrations of gemfibrozil (3.0 , 12.0 and 24.0 μg/ml) that covered the entired range of drug in serum. The spiked gemfibrozil serum was analysed every week up to seven weeks (42 days) or until the degradation was observed. The dependence of serum gemfibrozil concentration upon storage time was tested using correlation coefficient. The less the relationship observed, the more stable the sample is.

2. Experimental Methodology

2.1 Subjects

The sixteen Thai healthy male volunteers were selected for this study program. Four volunteers for pilot study and the rest for bioavailability study.

Every subject to be selected have to inform his medical history and confirm his fitness by having the physical examination, clinical chemistry and hematological evaluation. Any subject passed the criteria and can confirm his healthy condition , can then enter into this study program. The full detail of the study program was explained to all selected subjects. The written informed consent have to be given by

every subject before starting the study program. Every subject would be well taken care from the physician and nurse during study period.

2.2 Study design

The study was designed into two steps.

The first step was pilot study aimed to determine the appropriate blood sampling time schedule and also to observe the possible side effect if it may accur during experiment.

The second step was the bioavailability study in which the randomized crossover design with double blind technique was conducted. Each subject have to administer the drug in randomized order and elapsed at least one week for washout period before the next administration. The randomized order was obtained by using random table. The treatment schedule was shown in Table 1.

Both pilot and bioavailability study were conducted on the same experimental condition.

The study protocol was approved by the Ethical Committee of the Faculty of Medicine , Chulalongkorn University.

2.3 Experimental condition

Every subject was requested to refrain from other drugs treatment, consumption of alcohol , beverage containing caffeine and no smoking at least two weeks before and throughout the study to eliminate possible drug-induced influence on liver enzyme system. They were restrained from food at least 10 hours before their allocated treatment and

Table 1 Randomized schedule crossover design for bioequivalent study of two brands gemfibrozil capsule in 12 healthy male volunteers

subject No.	drug product	
	study period 1	study period 2
01	B	A
02	B	A
03	B	A
04	A	B
05	A	B
06	A	B
07	B	A
08	B	A
09	A	B
10	A	B
11	B	A
12	A	B

remained fasting 4 hours after drug administration , except water was ad libitum.

On the day of experiment , the catheter was inserted into the forearm vein and was used as route of blood sample collection. The blank blood was collected from each subject before drug administration. Blood sampling schedule begin at 0.16 hour until up to 12 hours after dosing. Every blood sample was left to be clotted at room temperature and then centrifuged to separate serum and kept frozen at -20 °C for subsequent analysis within one week using isocratic high performance liquid chromatographic technique (HPLC).

2.4 Pilot study

The pilot study was planned with three main purposed.

(1) To determine the appropriate sampling time schedule for blood sample.

(2) To prove the reliability of the analytical method in determining gemfibrozil concentration in serum sample.

(3) To observe any side effects that may occur in subject during experiment.

This study was performed in four subjects , two subjects for brand A and the others for brand B. A single dose of 600 mg gemfibrozil was administered to each subject with 200 ml of drinking water.

The blood sample was withdrawn through catheter using disposable syringe. Approximately 5.0 ml of whole blood was collected after discarded the first 2.0 ml portion of blood that may be contaminated with heparin. The catheter was then filled with 0.2 ml heparin in saline (100 U/ml) to prevent blood clotting. Every blood sample was left about

10 minutes for blood clotting, serum was then separated via centrifugation and then kept frozen for analysis.

The sampling time schedule for pilot study was before drug administration and at 0.16 , 0.5 , 0.75 , 1.0 , 1.25 , 1.5 , 1.75 , 2.0 , 2.5 , 3.0 , 4.0, 6.0 , 8.0 and 12.0 hours after dosing.

2.5 Bioavailability study

The overall twelve subjects joined the study. The same procedure in the pilot study was followed in bioavailability study with some changes in sampling time schedule.

By using randomized crossover and double blind technique, every subject have to administer gemfibrozil as a single dose of 600 mg (2 capsules) of both brands with at least one week apart for each administration. The blood sample was collected before drug administration and at 0.5 , 1.0 , 1.25 , 1.5 , 1.75 , 2.0 , 2.5 , 3.5 , 5.0, 7.0 , 9.0 and 12.0 hours after dosing. Serum was separated and kept frozen for subsequent analysis within one week.

3 Data Analysis

3.1 Pharmacokinetics analysis

The serum gemfibrozil concentration-time profiles were generated for each individual subject by plotting the concentration of gemfibrozil analysed against sampling time schedule. The pharmacokinetic parameters were determined by fitting the concentration-time data via compartmental analysis and confirmed with the noncompartmental method.

3.1.1 Compartmental analysis

Gemfibrozil concentration-time data was fitted to model compartment using the RSTRIP program (MicroMath, Inc. USA). The program estimated the exponential parameters by stripping or residual method. The appropriateness of the compartment model proposed for gemfibrozil was confirmed by testing the statistical significant difference of the AUC value determine from the compartmental and the noncomparmental methods at significant level of 0.05. The equations defined serum concentration of gemfibrozil at time t for two , three and four exponentials can be expressed in equation (1) , (2) and (3) , respectively

$$C_t = A_1e^{-k_1t} - A_2e^{-k_2t} \quad \text{(1)}$$

$$C_t = A_1e^{-k_1t} + A_2e^{-k_2t} - A_3e^{-k_3t} \quad \text{(2)}$$

$$C_t = A_1e^{-k_1t} + A_2e^{-k_2t} + A_3e^{-k_3t} - A_4e^{-k_4t} \quad \text{(3)}$$

where, C_t = serum concentration of gemfibrozil at time t

A_1, A_2, A_3, A_4 = amount of gemfibrozil for each exponential

k_1, k_2, k_3, k_4 = rate constant for absorption and elimination

The parameters (A_1, A_2, A_3, A_4, k_1, k_2, k_3, k_4) from stripping were utilized as the initial parameters for programs iteration. The final estimation of the parameters were obtained by iteration until the values were best fitted to the experiment data. The microparameters were then determinted according to the compartment proposed. They were the absorption rate constant (Ka), the elimination rate constant (Ke) , the peak serum concentration (Cmax), the time to peak serum

concentration (Tmax), the area under the concentration-time curve ($AUC_{0-\infty}$), the area under the first moment curve ($AUMC_{0-\infty}$) , the elimination half-life ($T_{1/2}$) and the mean residence time (MRT). Supposing the two-exponential was the best-fitted model , all the aforementioned parameters were calculated by the following equation.

The values of Ka and Ke were obtained from the residual method.

$$Tmax = [(1/k_2-k_1) \ln (k_2/k_1)] + t_0 \quad \text{(4)}$$

$$Cmax = [A_1e^{-k_1 (t_{max} - t_0)} - A_2e^{-k_2 (t_{max} - t_0)}] \quad \text{(5)}$$

$$AUC_{0-\infty} = (A_1 / k_1) + (A_2 / k_2) + C_t / k_1 \quad \text{(6)}$$

$$AUMC_{0-\infty} = (A_1 / k_1^2) + (A_2 / k_2^2) + (t.C_t / k_1) + (C_t / k_1^2) \quad \text{(7)}$$

$$MRT = AUMC_{0-\infty} / AUC_{0-\infty} \quad \text{(8)}$$

$$T_{1/2} = 0.693/k_1 \quad \text{(9)}$$

3.1.2 Noncompartmental analysis

Noncompartmental analysis do not required the assumption of specific compartment or number of exponential. The following pharmacokinetic parameters were calculated utilizing the MKMODEL program (Auckland , New Zealand, 1988). In this program, Cmax and Tmax values were obtained by reading directly from the serum concentration time data. $AUC_{0-\infty}$ and $AUMC_{0-\infty}$ value were calulated by trapezoidal rule.

The pharmacokinetic parameters ($AUC_{0-\infty}$, $AUMC_{0-\infty}$, MRT) can be expressed by the following equations.

$$AUC_{0-\infty} = \sum_{i}^{t} \tfrac{1}{2} (t_2 - t_1) (C_1 + C_2) + Ct/k \quad \text{_____(10)}$$

$$AUMC_{0-\infty} = \sum_{i}^{t} \tfrac{1}{2} (t_2 - t_1) (C_1 + C_2) + t.Ct/k + Ct/k^2 \quad \text{_____(11)}$$

$$MRT = AUMC_{0-\infty} / AUC_{0-\infty} \quad \text{_____ (12)}$$

where, Ct = concentration of gemfibrozil at time t

k = elimination rate constant

3.2 Bioavailability and statistical analysis

The comparative bioavailability of gemfibrozil between two brands were evaluated using the follow parameters, Cmax , Tmax , Ka and $AUC_{0-\infty}$.

The unpaired student ' s t-test was utilized as a tool to test statistical significant differences of the Cmax , Tmax , Ka and $AUC_{0-\infty}$ of two brands of gemfibrozil. The brand A and B would be concluded to be bioequivalence if no statistically significant differences of all four pharmacokinetic parameters , Cmax , Tmax , Ka , $AUC_{0-\infty}$, were observed at significant level of 0.05.

The relative bioavailability of gemfibrozil given at the same dosage level were calculated by

$$F_{rel} = AUC_{test} / AUC_{reference}$$

where ; F_{rel} = Relative bioavailability

AUC_{test} = Area under the concentration time curve of local manufactured product

$AUC_{reference}$ = Area under the concentration time curve of original product