CHAPTER IV

DISCUSSION AND CONCLUSION

In vitro study

The confirmation of pharmaceutical equivalence of gemfibrozil capsules between brand A and brand B is necessary before conducting the bioequivalent study. Pharmaceutical equivalence can be determined via the in vitro study following the requirement of the United State Pharmacopoeia. For this study, the content of active ingredient, uniformity of dosage units (weight variation) , disintegration and dissolution of both brand A and B were determined according to the monograph of gemfibrozil in the USP XXIII. The identical results were observed from both brand A and B which were confirmed to be statistically nonsignificant difference between brand A and B ($p>0.05$) as already shown from Table 2-5.

From table 2 , eventhough the percent labelled amount of gemfibrozil in brand B (99.08 ± 1.59%) was more than that in brand A (96.60 ± 1.14%) but the difference did not reach statistical significance ($p>0.05$). Moreover, they were lied within the acceptable range of percent labelled amount that was claimed to be 90-110%.

Amount of gemfibrozil contained in each individual capsule up to 10 capsules in either brand A or brand B were rather uniformed as illustrated in Table 3. They all lied within the range of 80-110% of

labelled claim with the RSD not more than 6.0%. Therefore, the amount of gemfibrozil in both brand A and brand B were weighed equivalent.

Gemfibrozil capsule can disintegrated in water within only less than six minutes. The identical results of brand A and brand B have already shown in Table 4. In addition, gemfibrozil from either brand can be well dissolved more than 85% within 45 minutes as restricted in the USP XXIII. These obtained results significantly confirmed the pharmaceutical equivalence of both brands and led to the allowable bioequivalent study in vivo.

In vivo studies

The bioequivalence of gemfibrozil was studied in vivo having healthy male volunteer as subjects. Before performancing the experiment, the analytical method for quantifying gemfibrozil in serum have to be developed and validated. The analytical method was used in the pilot study. Finally the well approved analytical method and also the well-set human study were really conducted to obtain the reliable and valuable bioequivalent results.

1. Analysis of gemfibrozil in serum

The analytical methods for determining gemfibrozil in plasma/serum have already been described (Hengy and Kölle, 1985 ; Randinitis, Parker and Kinkel, 1986). These methods utilized the high performance liquid chromatography (HPLC) and prepared samples by extracting gemfibrozil from serum and quantitated the amount of gemfibrozil by UV-detector.

These methods were firstly tried in this study, but they could not be practiced for analysing serum gemfibrozil samples due to the markly interfered endogeneous substances from serum. Although such methods were modified, the interfering peak from endogeneous substances still could not be avoided. Therefore, the analytical method was developed and validated for using in this study. This method based on high performance liquid chromatographic technique with fluorometric detection having flurbiprofen as internal standard. Gemfibrozil and internal standard were extracted from acidified serum into dichlorometane and resulting residue was analysed on HPLC-column with methanol/acetate buffer pH 3.8 75:25 (v/v) as mobile phase.

The chromatograms of gemfibrozil and internal standard were shown in Figure 3. Gemfibrozil and internal standard were approximately eluted at 9.2 and 5.4 minutes , respectively without any interferences from endogenous substance and/or gemfibrozil metabolites. The retention time of gemfibrozil and internal standard were all identical whether from chromatograms of standard solution, serum spiked or serum sample. This confirmed the specificity of the analytical method developed. The representative calibration curve of gemfibrozil was linear in the concentration range of 0-48 μg/ml (Figure 4). The lowest limit of quantitation (LOQ) for the analysis of gemfibrozil was determined to be 0.05 μg/ml that sensitive enough for pharmacokinetic study. The analytical recovery representing the accuracy of method was 96.05% (Table 6). This recovery value can be expected to be appropriated enough for analysis of gemfibrozil in serum. For the precision of analytical method, it is reflected by the data obtained from the intra-day and inter-day analyses of spiked serum. The data were shown in Table 7 and 8 that the RSD values of intra-day and inter-day precision were within

the range of 2.35 to 8.14 and 3.32 to 18.31, respectively. Therefore, this analytical method was precised enough to be used in analysis of gemfibrozil in serum samples.

Moreover, the stability of gemfibrozil in serum at storage condition was studied up to 42 days. The result was postulated that serum sample containing gemfibrozil can be stored at -20 °C up to 42 days without any degradation observed.

All of these data clearly confirmed the appropriateness of analytical method for determining gemfibrozil in serum sample.

2. Pilot study

According to the serum gemfibrozil concentration-time profiles graphically shown from Figure 8 to Figure 11, three main purposed in doing the pilot study can be solved. The appropriated blood sampling time was rescheduled in bioavailability study to be 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. Since, the maximum concentration of gemfibrozil observed (Cmax) were ranged from 32.78 - 48.99 μg/ml, the concentration range of gemfibrozil in calibration curve was then extented from 0-40 μg/ml to 0-48 μg/ml to ensure that they can cover the whole range of gemfibrozil detection serum sample.

All four volunteers in pilot study did not show any adverse side effect during experiment. Therefore, the same 600 mg single dose of gemfibrozil (2 capsules) was used in bioavailability study.

3. Bioavailability study

3.1 Pharmacokinetic of gemfibrozil

Generally , the pharmacokinetic of drug can be specified either by compartmental (model dependent) or noncompartmental (model independent) analysis. In addition , if the compartment model is proposed, it is usually proven by the noncomparment determination. In this study , the pharmacokinetic of gemfibrozil can be specified via RSTRIP program to be one compartment open model in which it was confirmed in noncompartmental analysis as shown in Table 15.

According to the one compartment open model proposed for gemfibrozil in Thai subjects , the absorption , distribution and elimination of gemfibrozil can then be explained. After oral administration of 600 mg single dose of gemfibrozil , the drug can reach the maximum concentration (Tmax) within 2.5 hrs (range 1.34 to 2.36 hrs) at the concentration (Cmax) ranged from 11.05 to 49.37 μg/ml , as displayed in Table 10. The Tmax and Cmax values were detected directly from the real data observed from subjects rather than those from the fitted values in RSTRIP program. By comparing to the previous report of Okerholm (1976) , the mean Cmax value from the study was 26 μg/ml that was much closer to the mean Cmax value calculating from the RSTRIP program (28.32 ± 9.211μg/ml).

Gemfibrozil can be absorbed readily with the mean absorption rate constant (Ka) of 0.8415 ± 0.1819 hr^{-1} and then rapid declined exponentially with the mean elimination rate constant (Ke) of 0.6307 ± 0.1353 hr^{-1} and the mean elimination half-life of 1.15 ± 0.227 hrs (range 0.79 to 1.53 hrs).

Presently , the elimination half-life of gemfibrozil in human is still ambiguous. There were reports described the elimination of gemfibrozil in both monoexponential and biexponential declined. Previous investigators explained the elimination half-life at 1.5 - 2.0 hrs and terminal elimination half-life at 6.5 - 7.9 hrs , these studies declared the elimination of gemfibrozil as monoexponential at 1.5 hrs (Okerholm , 1976 ; Warner Lambert , 1982 cite in Todd and Ward , 1988 ; Knauf , 1990) . For this study, the concentration-time profiles generated seem to involve biexponential elimination in most of subjects and some in monoexponential. Therefore, the RSTRIP program was utilized for fitting all of the concentration-time data of gemfibrozil in this study. Moreover , to ensure the result of data fitting, the MKMODEL program for determining the model independent parameters was also used in this study. The outcome of statistical analysis confirmed that gemfibrozil exhibit monoexponential declined in all of the subjects studied except only one subject (A11) as shown in Table 15. Therefore , according to all the afore-mentioned result , gemfibrozil's pharmacokinetic in Thai subjects could be mostly explained as one compartment open model with the elimination as monoexponential declined. Nevertheless, the result of this study should not considered as absolute conclusion , more subjects may be needed for more precised explanation.

3.2 Relative bioavailability

In assessing the bioequivalence of drug products , the rate and extent of drug reaching the systemic circulation after an administered dosage form must be involved . The parameter used to indicate the extent of drug absorption is the area under the concentration-time curve

(AUC) and the parameters used to indicate the rate of drug absorption is absorption rate constant (Ka) and the time to peak serum concentration (Tmax) while the peak serum concentration (Cmax) involved both rate and extent of drug absorption.

From Table 16 to 19 , the peak serum gemfibrozil concentration (Cmax) for brand A and B was 39.65 ± 9.402 μg/ml and 39.06 ± 10.25 μg/ml, the time to peak serum concentration (Tmax) was 2.06 ± 0.355 hr and 1.92 ± 0.358 hr , the absorption rate constant (Ka) was 0.8351 ± 0.1869 hr^{-1} and 0.8478 ± 0.1765 hr^{-1} and the area under the concentration-time curve ($AUC_{0-\infty}$) was calculated to be 113.94 ± 18.232 μg/ml.hr, and 103.92 ± 26.037 μg/ml.hr, respectively. No statistically significant differences were observed for all four parameters , Cmax , Tmax , Ka and $AUC_{0-\infty}$ ($p>0.05$). The relative bioavailability of gemfibrozil for brand A and B was calculated to be 1.09 or 109% . This value is acceptable criteria by FDA in which noted the bioavailability difference is within the ± 20%. (Gennaro, 1995). Therefore, it can be concluded that both brands gemfibrozil was bioequivalence in term of rate and extent of absorption as summarized in Table 20.

CONCLUSION

1. Local manufactured and original products in the form of capsule containing 300 mg of gemfibrozil used in this study met the requirement of the United State Pharmacopoeia XXIII in terms of their content of active ingredient , the uniformity of dosage units and the dissolution of drug.

2. The content of active ingredient , the uniformity of dosage units, the disintegration and dissolution time were proven to similar with no statistically significant differences between local manufactured and original products ($P>0.05$) indicating that two products were both pharmaceutical equivalents.

3. The analytical method was developed for analysing gemfibrozil in serum samples. The method based on reversed phase high performance liquid chromatography and prepared serum sample by liquid extraction. The amount of gemfibrozil was quantified by spectrofluorometric detector with excitation and emission wavelength at 284 and 316 nm, respectively. The developed analytical method was validated in their parameters of accuracy , precision , sensitivity , specificity and linearity. The results of validation confirmed the suitability of this analytical method to be used in analysis of gemfibrozil in serum samples.

4. The stability of gemfibrozil in serum at -20 °C (storage condition) were determined every 7 days through a 42 days period. The result postulated that serum gemfibrozil can be stored at -20 °C up to 42 days with no degradation observed.

5. The bioavailability of local manufactured and original products of gemfibrozil were studied in 12 Thai healthy male volunteers. Single oral dose of 600 mg gemfibrozil was administered to subject in a crossover design with double blind technique. Serum gemfibrozil concentration was analysed via a high performance liquid chromatographic technique (HPLC). Individual serum gemfibrozil concentration- time profile was assessed for pharmacokinetic parameters.

In this bioequivalent study, the peak serum concentration (Cmax), the time to peak serum concentration (Tmax) were directly detected from the concentration-time data. The area under the concentration-time curve ($AUC_{0-\infty}$) was calculated by MKMODEL program using trapezoidal rule. The absorption rate constant (Ka) can be determined by method of residuals via the RSTRIP program. No statistically difference of the Cmax , Tmax , Ka and $AUC_{0-\infty}$ values were observed between local manufactured and original products ($p>0.05$). The relative bioavailability of drug was calculated to be 1.09 or 109%. All of these data referred that local manufactured and original products were bioequivalence in term of rate and extent of drug absorption into systemic circulation.

6. The pharmacokinetics of gemfibrozil was also described in these 12 Thai healthy male volunteers. Gemfibrozil's pharmacokinetic follows the one compartment model with the Cmax and Tmax values of 39.35 ± 9.420 μg/ml and 1.99 ± 0.364 hrs , respectively. The rate constant of absorption (Ka) and elimination (Ke) were 0.8415 ± 0.1819 hr^{-1} and 0.6307 ± 0.1353 hr^{-1}, respectively, the area under the concentration-time curve ($AUC_{0-\infty}$) and the mean residence time (MRT) were 108.93 ± 23.028 μg/ml.hr and 3.18 ± 0.457 hrs., respectively.