REFERENCE


ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย
## APPENDIX A

### TEST PRODUCTS

<table>
<thead>
<tr>
<th>Brand name</th>
<th>Manufacturer/Distributor</th>
<th>Mfd. date</th>
<th>Batch no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cifran 250</td>
<td>Raubaxy</td>
<td>0-1-90</td>
<td>000190</td>
</tr>
<tr>
<td>Cilab</td>
<td>Biolab</td>
<td>4-6-90</td>
<td>005236</td>
</tr>
<tr>
<td>Cifroxin</td>
<td>Siam Pharmaceutical Co. Ltd.</td>
<td>20-6-90</td>
<td>T22 TF 16</td>
</tr>
<tr>
<td>Ciprobay</td>
<td>Bayer</td>
<td>30-5-90</td>
<td>477 F</td>
</tr>
</tbody>
</table>

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย
APPENDIX B

SUBJECTS

Table 37 Demographic Data

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Age (yr.)</th>
<th>Weight (kg.)</th>
<th>Height (cm.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>34</td>
<td>60.0</td>
<td>169</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>58</td>
<td>52.0</td>
<td>170</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>24</td>
<td>55.0</td>
<td>171</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>30</td>
<td>70.0</td>
<td>177</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>33</td>
<td>50.0</td>
<td>171</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>26</td>
<td>58.0</td>
<td>178</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>30</td>
<td>57.0</td>
<td>161</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>28</td>
<td>50.5</td>
<td>153</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>38</td>
<td>62.0</td>
<td>171</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>36</td>
<td>62.0</td>
<td>170</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>37</td>
<td>63.0</td>
<td>165</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>27</td>
<td>54.0</td>
<td>163</td>
</tr>
</tbody>
</table>

| Mean        | 33.42 | 57.79 | 168.25 |
| SD          | 8.96  | 5.93  | 6.90   |
APPENDIX C

CALIBRATION CURVE

Table 38 Calibration Curve Data of Ciprofloxacin in Carbodioxide Free Water

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration (mcg/ml)</th>
<th>Absorbance at 275 nm</th>
<th>%Theory</th>
<th>Concentration (mcg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.4941</td>
<td>0.060</td>
<td>0.5142</td>
<td>104.07</td>
</tr>
<tr>
<td>2</td>
<td>0.7905</td>
<td>0.084</td>
<td>0.7922</td>
<td>100.22</td>
</tr>
<tr>
<td>3</td>
<td>0.9881</td>
<td>0.102</td>
<td>1.0006</td>
<td>101.27</td>
</tr>
<tr>
<td>4</td>
<td>1.4822</td>
<td>0.140</td>
<td>1.4407</td>
<td>97.20</td>
</tr>
<tr>
<td>5</td>
<td>1.9762</td>
<td>0.198</td>
<td>1.9734</td>
<td>99.86</td>
</tr>
<tr>
<td>6</td>
<td>2.9643</td>
<td>0.268</td>
<td>2.9231</td>
<td>98.61</td>
</tr>
<tr>
<td>7</td>
<td>3.9524</td>
<td>0.355</td>
<td>4.0465</td>
<td>102.38</td>
</tr>
<tr>
<td>8</td>
<td>4.9405</td>
<td>0.438</td>
<td>4.8919</td>
<td>99.02</td>
</tr>
<tr>
<td>9</td>
<td>5.9286</td>
<td>0.528</td>
<td>5.9342</td>
<td>100.09</td>
</tr>
</tbody>
</table>

Mean 100.30
SD 2.06
C.V. 2.05%

a. $r^2 = 0.999$, $y = 0.0156 + 0.0863 \times x$

b. Inversely estimated concentration = $\frac{\text{Absorbance} - 0.0156}{0.0863}$

c. % Theory = $\frac{\text{Inversely estimated concentration}}{\text{Known concentration}} \times 100$

d. % C.V. = $\frac{\text{SD}}{\text{Mean}} \times 100$
**Figure 17** Calibration curve of ciprofloxacin in carbon dioxide-free water
Table 39  Calibration Curve Data of Ciprofloxacin Spiked in Drug Free Plasma

<table>
<thead>
<tr>
<th>No.</th>
<th>Concentration (mcg/ml)</th>
<th>Peak Height</th>
<th>Inversely Estimated Ratio</th>
<th>Concentration (mcg/ml)</th>
<th>%Theory</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.15</td>
<td>0.14</td>
<td>0.1514</td>
<td>100.93</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.17</td>
<td>0.1976</td>
<td>98.80</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.40</td>
<td>0.28</td>
<td>0.3670</td>
<td>91.75</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.60</td>
<td>0.44</td>
<td>0.6133</td>
<td>102.22</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.80</td>
<td>0.57</td>
<td>0.8134</td>
<td>101.68</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.00</td>
<td>0.74</td>
<td>1.0716</td>
<td>107.16</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.40</td>
<td>1.01</td>
<td>1.4908</td>
<td>106.49</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.80</td>
<td>1.24</td>
<td>1.7988</td>
<td>99.93</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.20</td>
<td>1.41</td>
<td>2.1067</td>
<td>95.76</td>
<td></td>
</tr>
</tbody>
</table>

Mean 100.52
SD 4.83
C.V. 4.81%

a. \( r^2 = 0.994 \)
\[ y = 0.0416 + 0.6495x \]
b. Inversely estimated concentration = Peak height ratio - 0.0416
\[ 0.6495 \]
c. % Theory = \( \frac{\text{Inversely estimated concentration}}{\text{Known concentration}} \times 100 \)
d. % C.V. = \( \frac{\text{SD}}{\text{Mean}} \) x 100
Figure 18  Calibration curve of ciprofloxacin in drug-free plasma
APPENDIX D

COMPARTMENTAL ANALYSIS

The pharmacokinetic analysis of individual plasma ciprofloxacin levels from each treatment was performed using the CSTRIP, a fortran iv computer program for obtaining polyexponential parameter estimates (Sedmen and Wagner, 1976).

The analysis indicated that a triexponential equation:

\[ C_t = Ae^{-\alpha t} + Be^{-\beta t} + le^{-\kappa t} \]

Where \( C_t \) is the plasma concentration at time \( t \), \( A, B \) and \( I \) are the coefficients with no lag time well described the concentration-time curve for ciprofloxacin.

Thus, the pharmacokinetic model of these data was the two compartment open model with first order absorption and elimination as shown in Figure 19.
where $D$ is the amount of drug administration.

$x_s, x_1, x_2$ are the amount of drug in gut, central and peripheral compartment, respectively.

$c_1, c_2$ are the concentration of drug in central and in peripheral compartment, respectively.

$v_1, v_2$ are the volume of distribution of drug in central and in peripheral compartment, respectively.

$k_a$ is the absorption rate constant.

$k_{12}, k_{21}$ are distribution rate constant for transfer of drug from central to peripheral and peripheral to central compartment, respectively.

$k_{e1}$ is the elimination rate constant of drug from central compartment.

Figure 19  Diagram of two compartment open model with first order absorption and elimination
The parameter estimates were directly obtained from the CSTRIP program as seen from the output in Figure 20 were

\[ \begin{align*}
A &= 2.2059 \\
B &= 0.7999 \\
I &= -3.0059 \\
\alpha &= 1.0245 \text{ hour}^{-1} \\
\beta &= 0.1433 \text{ hour}^{-1} \\
K &= 3.6980 \text{ hour}^{-1}
\end{align*} \]

Hence, an equation to describe the model was

\[ C_t = 2.2059e^{-1.0245t} + 0.7999e^{-0.1433t} - 3.0059e^{-3.6980t} \]
Figure 20 The output of CSTRIP computer program for analysis of ciprofloxacin concentration-time data
APPENDIX E

STATISTICS

1. **Mean** (x)

\[ \bar{x} = \frac{\sum x}{N} \]

2. **Standard deviation** (S.D.)

\[ S.D. = \sqrt{\frac{\sum (x - \bar{x})^2}{N-1}} \]

3. **Standard error of the mean** (SEM)

\[ SEM = \frac{S.D.}{\sqrt{N}} \]

4. **Testing the difference of the two mean** by Student’s t-test

Let \( \mu_1, \mu_2 \) = Population means

\( \bar{x}_1, \bar{x}_2 \) = Sample means

\( \sigma_1, \sigma_2 \) = Population variances

\( N_1, N_2 \) = Sample size

The null hypothesis \( H_0 \) = \( \mu_1 = \mu_2 \)

The alternative hypothesis \( H_a \) = \( \mu_1 \neq \mu_2 \)

The statistic \( t \) is given as

\[ t = \frac{(\bar{x}_1 - \bar{x}_2) - (\mu_1 - \mu_2)}{S_p} \]
4.1 If $\sigma_1^2 \neq \sigma_2^2$, the statistic is given as

$$
(t - \mu_1 - \mu_2) = \frac{(\bar{x}_1 - \bar{x}_2)}{S_p}
$$

Where $S_p^2$ is the pooled variance:

$$
S_p^2 = \frac{(S_1^2 + S_2^2)}{N_1 + N_2}
$$

with degree of freedom: d.f. = $N_1 + N_2$

$$
\left(\frac{S_1^2}{N_1}\right) + \left(\frac{S_2^2}{N_2}\right)
$$

4.2 If $\sigma_1^2 = \sigma_2^2$, the statistic $t$ for this case is

$$
(t - \mu_1 - \mu_2) = \frac{(\bar{x}_1 - \bar{x}_2)}{S_p}
$$

Where the pooled variance is:

$$
S_p^2 = \frac{1 + 1}{N_1 + N_2} \left[\frac{(N_1 - 1) S_1^2 + (N_2 - 1) S_2^2}{N_1 N_2 N_1 + N_2 - 2}
$$

with degree of freedom: d.f. = $N_1 + N_2 - 2$

This $t$ value is compared with $t_{(\alpha, \beta)}$, which is obtained from the table for $\alpha/2$

If $t > t_{(\alpha, \beta)}$, the null hypothesis that $\mu_1 = \mu_2$ is rejected and the alternative hypothesis is accepted. If $t$ is not significant, the null hypothesis stands.
5. **Analysis of variance (ANOVA)**

Analysis of Variance for completely Randomized Design

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Sum of Squares</th>
<th>d.f.</th>
<th>Mean Square</th>
<th>Variation Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups (Treatment)</td>
<td>$\sum_{j=1}^{k} n_j (X_{i,j} - \bar{X}_{..})^2$</td>
<td>$k-1$</td>
<td>$SS_{\text{among}}$</td>
<td>$V.R. = \frac{MS_{\text{among}}}{MS_{\text{within}}}$</td>
</tr>
<tr>
<td>Within groups (Error)</td>
<td>$\sum_{j=1}^{k} \sum_{i=1}^{n_j} (X_{i,j} - \bar{X}_{.j})^2$</td>
<td>$N-k$</td>
<td>$SS_{\text{within}}$</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>$\sum_{j=1}^{k} \sum_{i=1}^{n_j} (X_{i,j} - \bar{X}_{..})^2$</td>
<td>$N-1$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Where: $X_{i,j} =$ observed value i at Treatment j

- $i = 1, 2, \ldots, n_j$
- $j = 1, 2, \ldots, k$

- $T_{.j} = \sum_{i=1}^{n_j} X_{i,j}$
- $\bar{X}_{.j} = \frac{T_{.j}}{n_j}$
- $T_{..} = \sum_{j=1}^{k} T_{.j}$
\[ \bar{X} = \frac{T}{N} \]

\[ N = \sum_{j=1}^{k} n_j \]

The V.R. value is compared with the critical value, \( F \), which is obtained from table at degree of freedom \( (k-1) \) and \( (N-k) \).

In this study "\( k \)" represents number of brands studied
"\( N \)" represents total number of samples
If \( F > F_{(k-1), (N-k)} \), the null hypothesis that
\[ \mu_1 = \mu_2 = \mu_3 = \ldots = \mu_k \]

is rejected and the alternative hypothesis is accepted. If \( F \) is not significant, the null hypothesis stands.

6. Correlation coefficient test

The correlation coefficient is a quantitative measure of the relationship of correlation between two variable, \( x \) and \( y \)

\[ r = \frac{\Sigma xy - \frac{\Sigma x \Sigma y}{N}}{\sqrt{(\Sigma x^2 - (\Sigma x)^2)(\Sigma y^2 - (\Sigma y)^2)}} \]

where \( r \) = the correlation coefficient
\( N \) = the number of \( x \) and \( y \) pairs

Test of zero Correlation
Let \( \rho \) = the true correlation coefficient, estimated by \( r \)
The null hypothesis \(H_0: \mu = 0\)
The alternative hypothesis \(H_a: \mu \neq 0\)

\[ t_{n-2} = \left| \frac{r \sqrt{N - 2}}{\sqrt{1 - r^2}} \right| \]

The value of \(t\) is referred to a \(t\) distribution with \((N-2)\) degree of freedom. If \(t > t_{tab}\), we reject the null hypothesis and accept the alternative hypothesis. If \(t\) is not significant, the null hypothesis stands.
VITAE

Miss Srisutha Peemanee was born on June 13th, 1966, in Bangkok. She received a Bachelor of Science in Pharmacy in 1989 from the Faculty of Pharmacy, Chiang Mai University.

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย