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APPENDICES

APPENDIX A

TEST PRODUCTS

Table 33 Test Products

Brand name	Manufacturer	Mfg.date	Batch.No.
BENLACON	H.K.Pharmaceuticals Co., Ltd.	24-4-90	7526
DAONIL	Hoechst Pharamceuticals (Thailand) Ltd.	14-3-90	30 CO
DIABENOL	Greater Pharma	28-6-90	112015
EUGLUCON	F.E. Zuellig (Bangkok) Ltd.	2-8-89	96131
EUNIL	Medico	22-5-90	150 AE
GLIBENCLAMIDE TABLETS	G.P.O.	29-11-90	331109
SUGRIL	Siam Pharmaceuticals Co., Ltd .	4-5-90	20TE074 & C642/90

APPENDIX B

REAGENT PREPARATIONS

0.1 M Methanolic Hydrochloric Acid

Dilute 8.5 ml. of conc. hydrochloric acid to 1000 ml. with methanol.

Simulated Intestinal Fluid TS Without Pancreatin

Dissolve 6.8 gm. of monobasic potassium phosphate in 250 ml. of water, mix and add 190 ml. of 0.2 N sodium hydroxide and 400 ml. of water, mix. Adjust the resulting solution with 0.2 N sodium hydroxide to a pH of 7.5 $\pm$ 0.1. Dilute with water to 1000 ml.

0.01 M Phosphate Buffer (pH 3.5)

Dissolve 1.36 gm. of monobasic potassium phosphate in 900 ml. of water. Adjust pH to 3.5 by adding dropwise of 85% phosphoric acid. Dilute with water to 1000 ml.

APPENDIX C

SUBJECTS

Table 34 Demographic Data

Subject No.	Age (yr.)	Weight (kg.)	Height (cm.)
1	29	51	153
2	38	65	163
3	30	58	161
4	34	50	174
5	24	59	167
6	30	70	177
7	41	52	162
8	39	62	171
9	27	59	178
10	24	55	171
11	24	56	170
12	31	76	161
Mean	30.92	59.42	167.33
S.D.	5.96	7.82	7.50

APPENDIX D

CALIBRATION CURVE DETERMINATION

The typical calibration curves data for glibenclamide concentrations in simulated intestinal fluid TS without enzyme (pH 7.5+0.1) and human plasma are represented in Tables 35, 36 and Figures 17, 18, respectively.

Table 35 Typical Calibration Curve Data for Glibenclamide Concentrations in Simulated Intestinal Fluid TS without enzyme (pH 7.5±0.1) Estimated Using Linear Regression[a].

Standard No.	Conc. (ug./ml.)	Absorbance at 207 nm.	Inversely[b] Estimated Conc. (ug./ml.)	% Theory[c]
1	0.4976	0.056	0.4791	96.28
2	0.9953	0.097	0.9548	95.93
3	1.4929	0.144	1.5000	100.48
4	1.9906	0.183	1.9524	98.08
5	3.9812	0.360	4.0058	100.62
6	5.9718	0.543	6.1288	102.63
7	7.9624	0.701	7.9617	99.99
8	9.9529	0.864	9.8527	98.99
			Mean	99.13
			S.D.	2.28
			C.V.[d]	2.30

a. r^2 =0.999, Y = 0.0862X + 0.0147

b. Inversely Estimated Conc. = $\frac{\text{Absorbance} - 0.0147}{0.0862}$

c. % Theory = $\frac{\text{Inversely Estimated Conc.}}{\text{Known Conc.}} \times 100$

d. % C.V. = $\frac{\text{S.D.}}{\text{Mean}} \times 100$

Table 36 Typical Calibration Curve Data for Glibenclamide Concentrations in Human Plasma Estimated Using Linear Regression[a]

Standard No.	Conc. (ng./ml.)	Peak Height Ratio	Inversely[b] Estimated Conc. (ng./ml.)	% Theory[c]
1	21.4769	0.020	22.3122	103.89
2	42.9538	0.039	40.3141	93.85
3	85.9075	0.085	83.8978	97.66
4	128.8613	0.137	133.1663	103.34
5	214.7688	0.225	216.5437	100.83
6	257.7226	0.268	257.2849	99.83
7	300.6763	0.312	298.9736	99.43
8	343.6301	0.359	343.5047	99.96
			Mean	99.85
			S.D.	3.17
			C.V.[d]	3.17

a. $r^2 = 0.999$, $Y = 1.0554 \times 10^{-3} X - 3.5492 \times 10^{-3}$

b. Inversely Estimated

$$\text{Concentration} = \frac{\text{Peak Height Ratio} + 3.5492 \times 10^{-3}}{1.0554 \times 10^{-3}}$$

c. $$\%\ \text{Theory} = \frac{\text{Inversely Estimated Conc.}}{\text{Known Conc.}} \times 100$$

d. $$\%\ \text{C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100$$

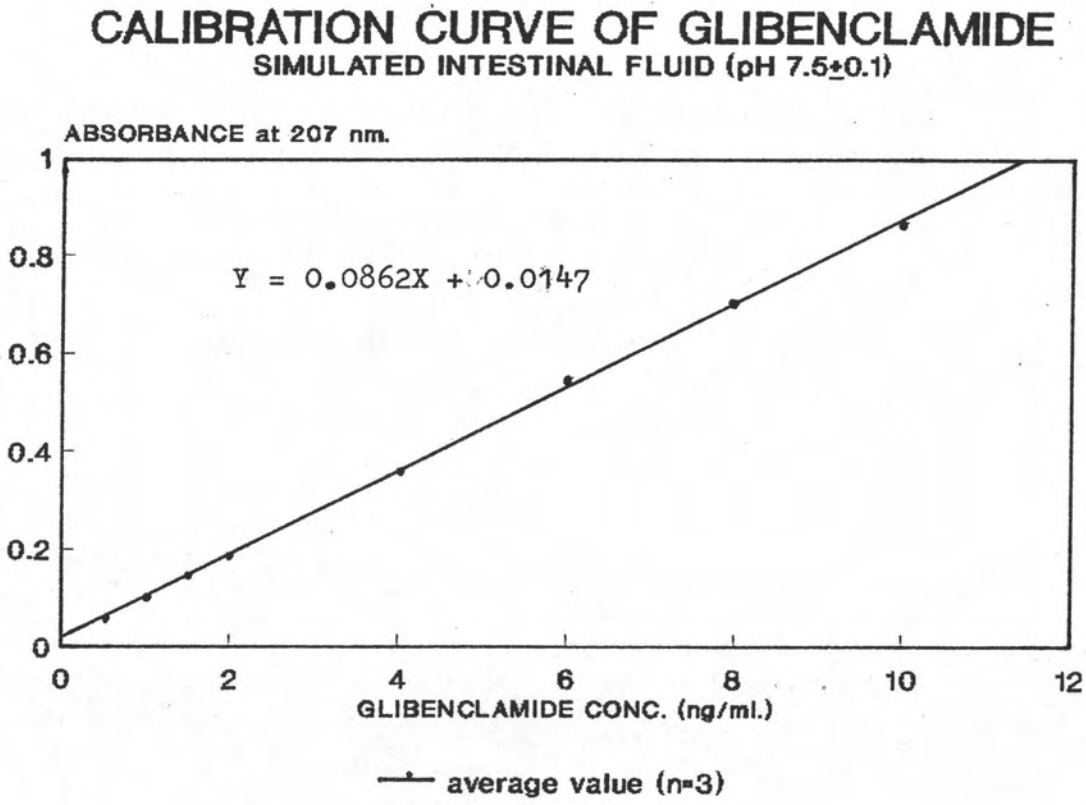

Figure 17 Calibration curve of glibenclamide in simulated intestinal fluid TS without enzyme (pH 7.5±0.1)

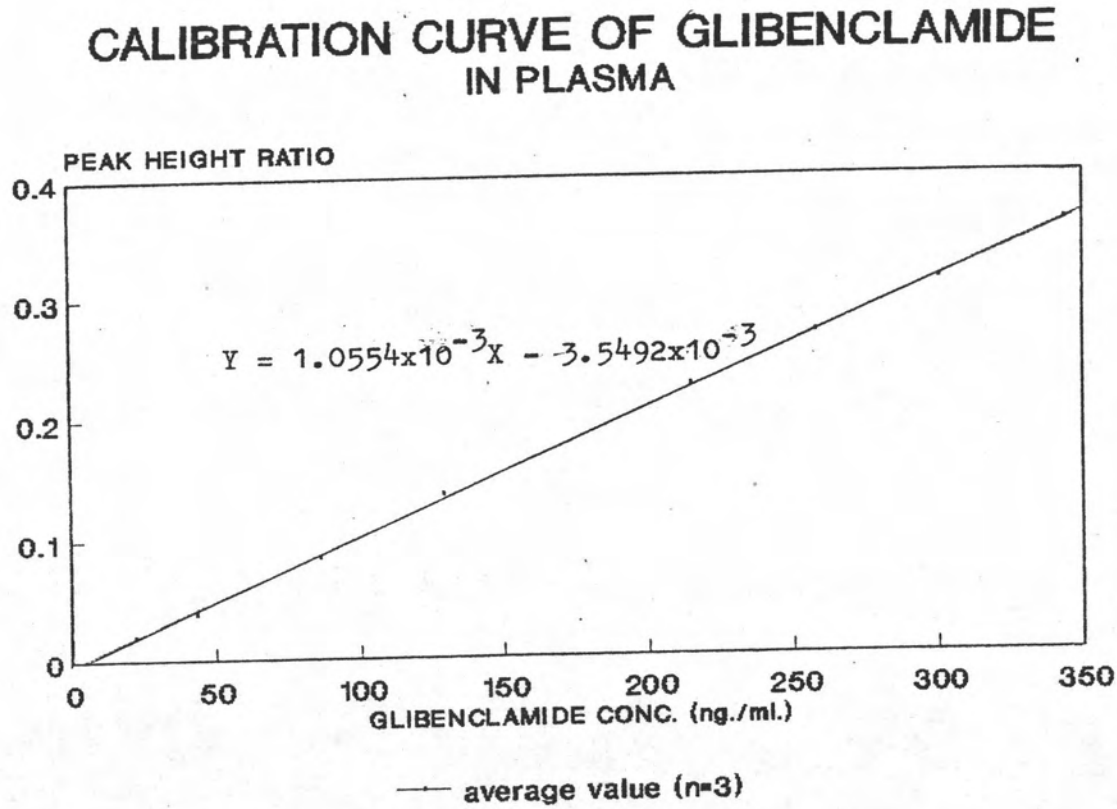

Figure 18 Calibration curve of glibenclamide in human plasma

APPENDIX E

COMPARTMENTAL ANALYSIS

The CSTRIP computer program output used for analyzing data was shown in Figure 19.

A_1, A_2 = the ordinate intercept constants

$B_1 = K_{el}$

$B_2 = K_a$

Figure 19 CSTRIP COMPUTER PROGRAM OUTPUT

```
.....CURVE STRIPPING.....

**********************************

DATA SET NUMBER  1

THE NUMBER OF EXPONENTIALS = 2
SUMMARY OF EXPONENTIAL STRIPPING

THE NUMBER OF POINTS IN THE EXPONENTIAL PHASES (LAST TO FIRST)
      L1=     5
      L2=     6

THE BEST ESTIMATES OF THE COEFFICIENTS AND EXPONENTS ARE
 A1= 0.247763E+03      B1= 0.120552E+00
 A2=-0.247763E+03      B2= 0.711263E+00
 F= 0.142384E+00

NO LAG TIME WAS NEEDED TO DESCRIBE THESE DATA
 THEREFORE, THE SUM OF THE EXPONENTIAL TERMS WAS FORCED THROUGH ZER

 R SQUARE(2) =   0.93845
```

NO.	TIME	C(OBS)	C(EST)	% DEV
1	0.0000	0.0000	0.0000	0.00
2	0.5000	68.7380	59.6552	13.28
3	1.0000	93.2378	97.9677	-5.07
4	1.5000	109.1533	121.5293	-11.34
5	2.0000	135.4406	134.9462	0.37
6	2.5000	144.6203	141.4353	2.20
7	3.0000	174.9254	143.2407	18.11
8	4.0000	146.2894	138.5711	5.28
9	6.0000	126.2609	116.7290	7.55
10	8.0000	93.3507	93.6115	-0.28
11	10.0000	73.7445	74.0122	-0.36

APPENDIX F

STATISTICS

1. Mean (X)

$$X = \frac{\Sigma X}{N}$$

2. Standard deviation

$$S.D. = \sqrt{\frac{\Sigma (X - X)^2}{N-1}}$$

3. Standard error of mean (S.E.M.)

$$S.E.M. = \frac{S.D.}{\sqrt{N}}$$

4. Testing the difference among treatment means

Completely randomized design

Treatments					Total	Mean
1	2	3	.....	k		
X_{11}	X_{12}	X_{13}	.....	X_{1k}	T_1	X_1
X_{21}	X_{22}	X_{23}	.....	X_{2k}	T_2	X_2
.	.	.	.....		...	...
X_{n1}	X_{n2}	X_{n3}	.....	X_{nk}	T_n	X_n
Total T_1	T_2	T_3	.....	T_k	T	X
Mean X_1	X_2	X_3	.....	X_k		

where T = Total of all observations

X = Overall mean

k = Number of treatments

n = Number of sampling units in each treatment

$u_1, u_2, u_3, \ldots u_k$ = Population mean

The null hypothesis $H_o : u_1 = u_2 = \ldots\ldots u_k$

The alternative hypothesis $H_a : u_1 \neq u_2 \neq \ldots\ldots u_k$

Analysis of variance (ANOVA) for testing differences among treatment mean

Source of variation	d.f.	SS	MS	F
Among group	k-1	SS_{among}	MS_{among}	F_T
Within group	$\Sigma n-k$	SS_{within}	MS_{within}	
Total	$\Sigma n -1$	SS_{total}		

where : d.f.= Degree of freedom

SS = Sum of Square

MS = Mean square

F_T = Variance ratio

Sum of squares :

1. Complete a correction term (C.T.)

$$C.T. = \frac{T^2}{\Sigma n}$$

2. Total sum of squares (SS_{total})

$$SS_{total} = \sum_{i=1}^{k} \left(\sum_{j=1}^{n} x^2_{ij} \right) - C.T.$$

3. The among group sum of squares (SS_{among})

$$SS_{among} = \sum_{i=1}^{k} \frac{(T^2_i)}{n_i} - C.T.$$

4. The within group sum of squares (SS_{within})

$$SS_{within} = SS_{total} - SS_{among}$$

$$\text{Mean squares} = \frac{\text{Sum of squares}}{\text{Degree of freedom}}$$

$$\text{Variance ratio} = \frac{\text{Among group mean squares}}{\text{Within group mean squares}}$$

F has (k-1), (Σ n - k) degree of freedom.

If F value calculated is less than $F_{0.05}$, the null hypothesis is accepted and the alternative hypothesis is rejected. If F value is greater than $F_{0.05}$, the alternative hypothesis stands which shows that there are significant differences among treatment means ($p < 0.05$).

5. Testing the difference of two means

If the result of the difference testing among treatment means by analysis of variance is significant ($p < 0.05$), the testing of difference between the mean of the reference treatment and the each other treatment mean is performed by t-test.

The null hypothesis : $H_o : u_1 = u_2$

The alternative hypothesis : $H_a : u_1 \neq u_2$

$$t = \frac{x_1 - x_2}{S_d}$$

where $x_1 - x_2$ = difference of the two means

S_d^2 = pooled error variance

when n in each treatment is equal,

$$S_d = \sqrt{\frac{2\ MS_{within}}{n}}$$

when n in each treatment is not equal,

$$S_d = \sqrt{MS_{within}\frac{(n_1+n_2)}{n_1 n_2}}$$

where n_1, n_2 = number of samples in treatment 1, 2 respectively

$t_{0.05}$ has (Σ n - k) degree of freedom.

If t value calculated is greater than $t_{0.05}$ from the table, it indicated that there is statistically significant difference of these means ($p < 0.05$).

6. Correlation coefficient test

The correlation coefficient is a quantitative measure of the relationship of correlation between two variables, x and y.

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

where r = Correlation coefficient

N = the number of x and y pairs

Test of Zero Correlation

Let = the true correlation coefficient, estimated by r

The null hypothesis $H_o = \rho = 0$

The alternativ hypothesis $H_a = \rho \neq 0$

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

The value of $t_{o.o5}$ is referred to a t distribution with (N - 2) degree of freedom. If t calculated is greater than $t_{o.o5}$, the null hypothesis is rejected and the alternative hypothesis is accepted. If t is not significant, the null hypothesis stands.

VITAE

Miss Suwanna Champreeda was born on February 25th 1964, in Bangkok. She received a Bachelor of Science in Pharmacy (second class honors) in 1987 from the Faculty of Pharmacy, Mahidol University.