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APPENDICES

APPENDIX A

TEST PRODUCTS

Table 35 Test Products

Brand name	Manufacturer	Mfg. date	Batch no.
DICLOSIAN	Asian	6-3-91	T91060
DIFENO	Milano	11-3-34	341053
DOSANAC	Siam Bhaesach	9-1-91	23UA004
INFLANAC	Biolab Co., Ltd.	5-3-88	802077
PETORAN	Chemephand Medical	6-11-90	155AM
PUTAREN	Thai P.D. Chemical	18-9-33	SPM
VOLTA	Trustman	20-3-34	889-34-24
VOLTAREN	Ciba-geigy	16-11-90	26-073

APPENDIX B

CALIBRATION CURVE DETERMINATION

The typical calibration curves data for diclofenac sodium concentrations in simulated intestinal fluid TS without pancreatin (pH 7.5 ± 0.1), 0.01 N methanolic sodium hydroxide and human plasma are presented in Tables 36, 37, 38 and Figures 18, 19, 20 respectively.

Table 36 Typical Calibration Curve Data for Diclofenac Sodium Concentrations in Simulated Intestinal Fluid TS without Pancreatin (pH 7.5 ± 0.1) Estimated Using Linear Regression [1]

Standard No.	Concentration (mcg./ml.)	Absorbance at 280	Inversely estimated concentration (mcg./ml.) [2]	% Theory [3]
1	2.5	0.076	2.45	97.85
2	5	0.154	4.99	99.74
3	10	0.317	10.29	102.96
4	15	0.465	15.12	100.78
5	20	0.615	20.00	100.00
6	25	0.769	25.02	100.08
7	30	0.917	29.84	99.47
			Mean	101.13
			S.D.	1.54
			C.V. [4]	1.52%

1. $r^2 = 0.999, \quad Y = 0.0307X + 0.0009$
2. Inversely estimated concentration $= \dfrac{\text{Absorbance} - 0.0009}{3.07 \times 10^{-2}}$
3. % Theory $= \dfrac{\text{Inversely estimated concentration}}{\text{Known concentration}} \times 100$
4. % C.V. $= \dfrac{\text{S.D.}}{\text{Mean}} \times 100$

Table 37 Typical Calibration Curve Data for Diclofenac Sodium Concentrations in 0.01 N Methanolic Sodium Hydroxide Solution Estimated Using Linear Regression [1]

Standard No.	Concentration (mcg./ml.)	Absorbance at λ 280	Inversely estimated concentration (mcg./ml.) [2]	% Theory [3]
1	2.50	0.127	2.25	90.00
2	5.00	0.233	4.97	99.49
3	7.50	0.330	7.47	99.57
4	10.00	0.428	9.99	99.87
5	12.50	0.548	13.07	104.58
6	15.00	0.606	14.56	97.09
7	20.00	0.814	19.91	99.55
8	25.00	1.019	25.18	100.72
			Mean	98.86
			S.D.	4.14
			C.V. [4]	4.18%

1. $r^2 = 0.999 \quad Y = 0.0389X + 0.395$
2. Inversely estimated concentration $= \dfrac{\text{Absorbance} - 0.395}{3.89 \times 10^{-2}}$
3. % Theory $= \dfrac{\text{Inversely estimated concentration}}{\text{Known concentration}} \times 100$
4. % C.V. $= \dfrac{\text{S.D.}}{\text{Mean}} \times 100$

Table 38 Typical Calibration Curve Data for Diclofenac Sodium Concentrations in Human Plasma Estimated Using Linear Regression [1]

Standard No.	Concentration (mcg./ml.)	Peak height ratio	Inversely [2] estimated concentration (mcg./ml.)	% Theory [3]
1	0.0499	0.249	0.0375	75.15
2	0.0991	0.442	0.0947	94.71
3	0.1981	0.785	0.1964	99.15
4	0.3963	1.506	0.4102	103.51
5	0.7926	2.806	0.7957	100.39
6	0.1888	4.132	1.1889	100.01
7	1.5851	5.379	1.5587	98.33
8	1.9814	6.719	1.9560	98.72
9	2.3772	8.223	2.4020	101.04
			Mean	96.78
			S.D.	8.45
			C.V. [4]	8.73%

1. $r^2 = 0.999$, $Y = 3.3723X + 0.1226$

2. $\text{Inversely Estimated Concentration} = \dfrac{\text{Peak Height Ratio} - 0.1226}{3.3723}$

3. $\% \text{ Theory} = \dfrac{\text{Inversely Estimated Concentration}}{\text{Known concentration}} \times 100$

4. $\% \text{ C.V.} = \dfrac{\text{S.D.}}{\text{Mean}} \times 100$

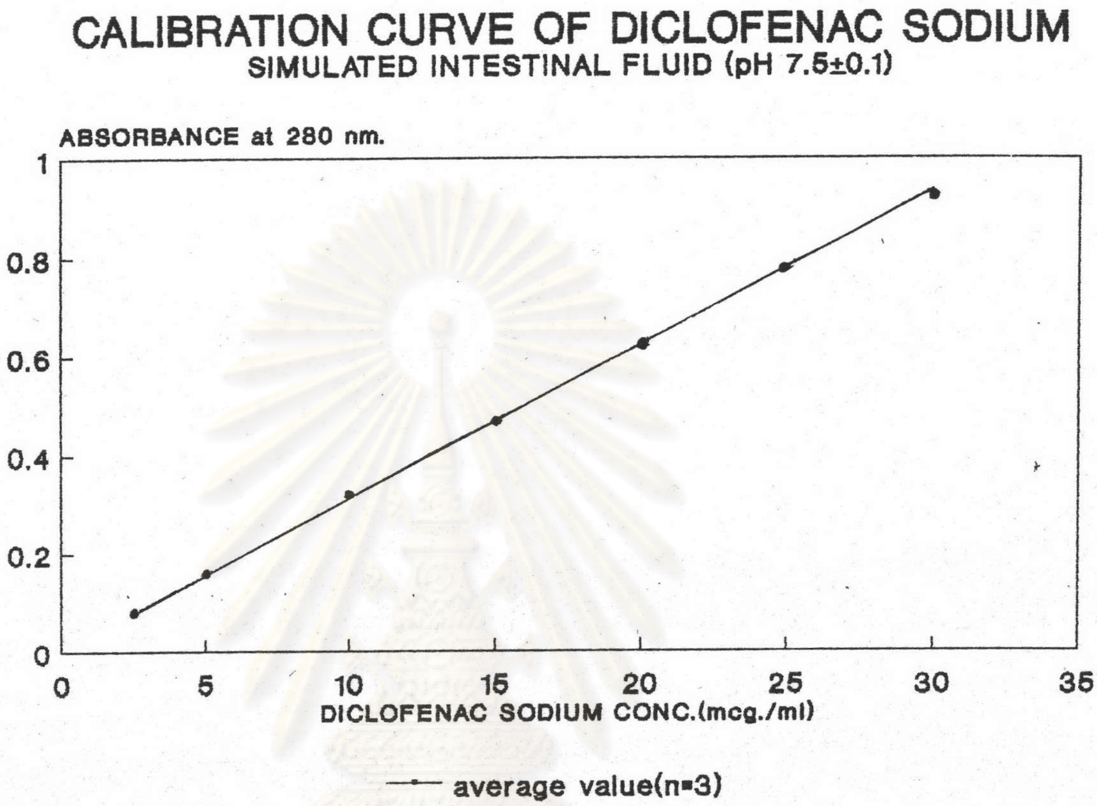

Figure 18 Calibration curve of diclofenac sodium in simulated intestinal fluid T.S. without enzyme (pH 7.5 ± 0.1)

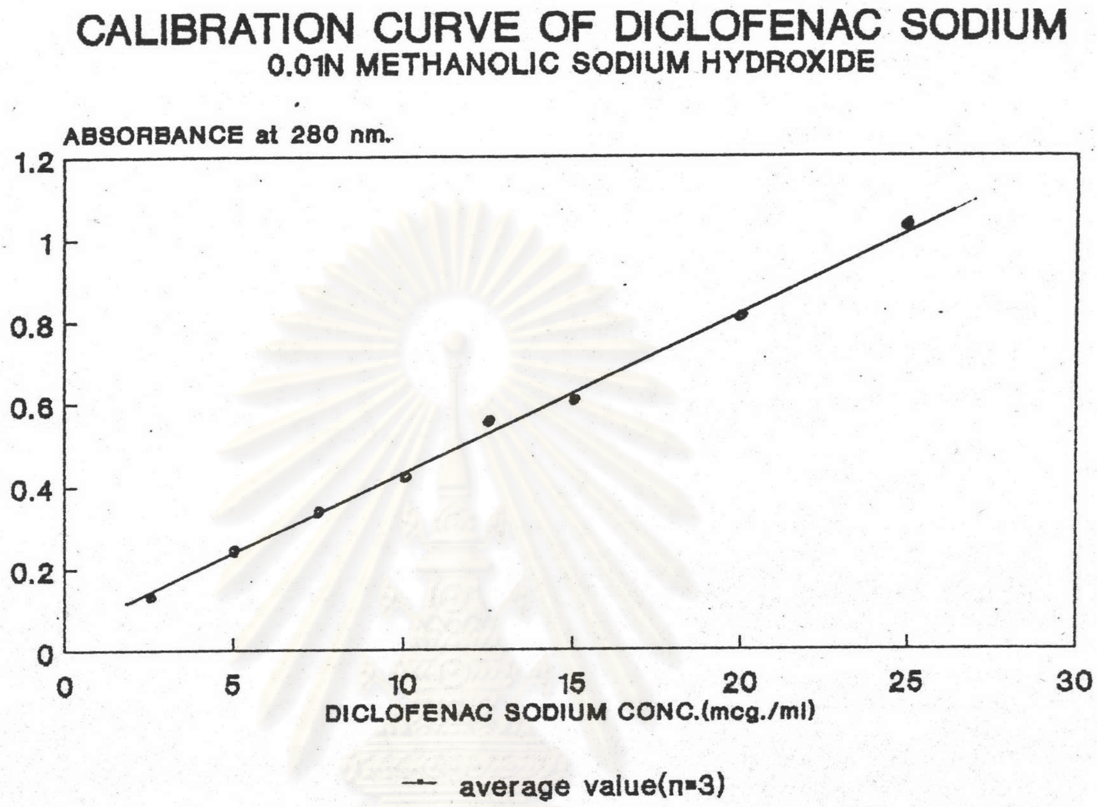

Figure 19 Calibration curve of diclofenac sodium in 0.01 N methanolic sodium hydroxide

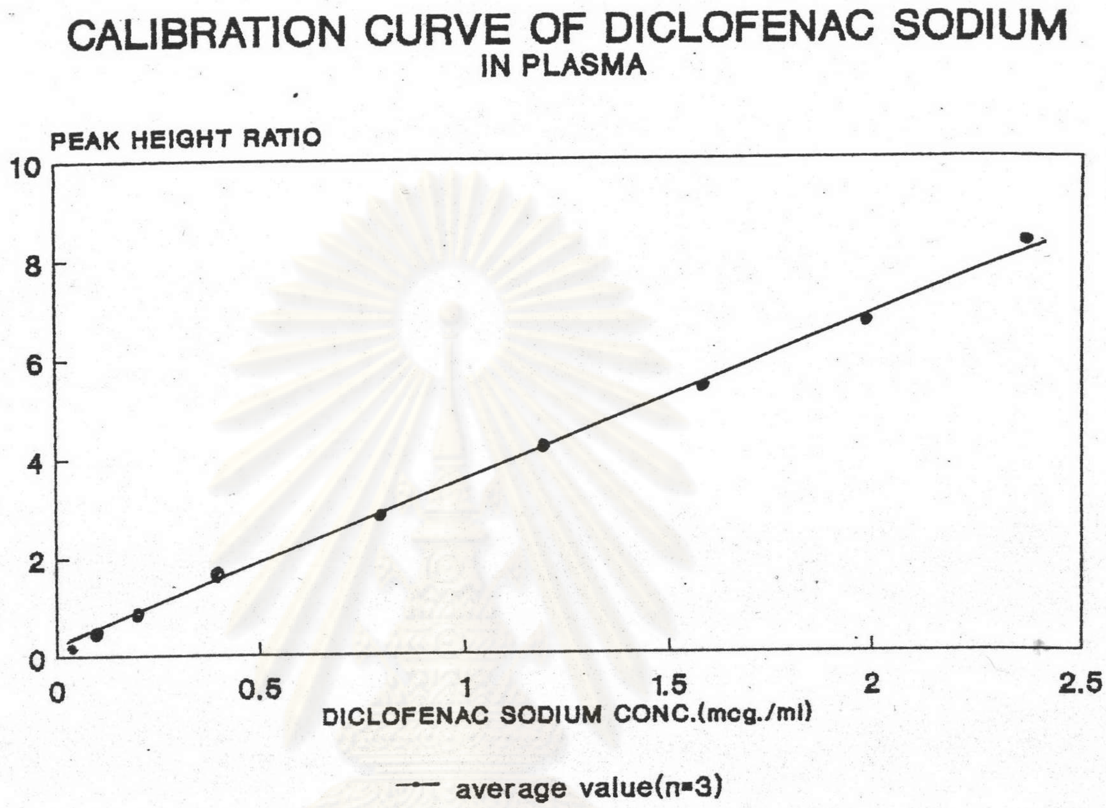

Figure 20 Calibration curve of diclofenac sodium in human plasma

APPENDIX C

REAGENT PREPARATIONS

Simulated Intestinal Fluid TS Without Pancreatin

Dissolve 6-8 g 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.

0.02 M Acetate Buffer (pH 7)

Dissolve 1.64 g of sodium acetate, anhydrous in 500 ml. of water, adjust to pH 7.0 with glacial acetic acid, and dilute with water to 1000 ml.

0.01 N Methanolic Sodium Hydroxide

Dissolve 0.4 g of sodium hydroxide anhydrous in 500 ml of methanol, mix. Dilute with methanol to 1,000 ml.

APPENDIX D

SUBJECTS

Table 39 Demographic data

Subject No.	Age (yr.)	Weight (kg.)	Height (cm.)
1	19	57	166
2	27	52	163
3	38	67	162
4	32	47	157
5	40	62	171
6	25	55	171
7	34	51	172
8	31	70	177
9	31	60	161
10	21	57	165
11	40	67	167
12	41	67	168
Mean	31.58	59.33	166.67
S.D.	7.46	7.40	5.55

APPENDIX E

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

Treatments			Total	Mean
	1	2	3...........k		
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

$\mu_1, \mu_2, \mu_3, \ldots\ldots \mu_k$ = Population mean

The null hypothesis $H_o : \mu_1 = \mu_2 = \ldots\ldots\ldots\ldots \mu_k$

The alternative hypothesis $H_a : \mu_1 = \mu_2 = \ldots\ldots \mu_k$

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	Σn-k	SS_{within}	MS_{within}	
Total	Σn-1	SS_{total}		

where : d.f. = Degree of freedom

SS = Sum of Square

MS = Mean Square

F_T = Variance ratio

Sum of Squares :

1. Compute a correlation term (C.T.)

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

2. Total sum of square (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})

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

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 $(k-1), (\Sigma n-k)$ 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 : $H_o : \mu_1 = \mu_2$

The alternative hypothesis : $H_a : \mu_1 = \mu_2$

$$t = \frac{\overline{X}_1 - \overline{X}_2}{S_{\bar{d}}}$$

where $\overline{X}_1 - \overline{X}_2$ = difference of the two means

$S_{\bar{d}}^2$ = pooled error variance

when n in each treatment is equal,

$$S_{\bar{d}} = \sqrt{\frac{2MS_{within}}{n}}$$

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 $(\Sigma n-k)$ 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.

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

where r = Correlation coefficient

N = the number of x and y pairs

Test of Zero Correlation

Let ρ = the true correlation coefficient, estmated by r

The null hypothesis $H_0 = \rho = 0$

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

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

The value of $t_{0.05}$ is referred to a t distribution with (N-2) degree of freedom. If t calculated is greater than $t_{0.05}$, the null hypothesis is rejected. If t is not significant, the null hypothesis stands.

APPENDIX F

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= 9
L2= 2

THE BEST ESTIMATES OF THE COEFFICIENTS AND EXPONENTS ARE
A1= 0.376750E+01 B1= 0.181170E+01
A2=-0.376750E+01 B2= 0.437258E+01
F= 0.442199E-01

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

R SQUARE(2) = 0.96862

NO.	TIME	C(OBS)	C(EST)	% DEV
1	0.0000	0.0000	0.0000	0.00
2	0.5000	1.0996	1.0996	0.00
3	1.0000	0.7729	0.5680	26.51
4	1.5000	0.2161	0.2435	-12.66
5	2.0000	0.0639	0.1000	-56.43
6	2.5000	0.0536	0.0406	24.29
7	3.0000	0.0193	0.0164	14.91
8	4.0000	0.0025	0.0027	-7.36
9	6.0000	0.0000	0.0001	-100.00
10	8.0000	0.0000	0.0000	-100.00
11	10.0000	0.0000	0.0000	-100.00

THE NUMBER OF EXPONENTIALS = 3
SUMMARY OF EXPONENTIAL STRIPPING

THIS SET OF DATA CAN NOT BE DESCRIBED BY THE SUM OF 3 EXPONENTIALS

THE NUMBER OF EXPONENTIALS = 4
SUMMARY OF EXPONENTIAL STRIPPING

THIS SET OF DATA CAN NOT BE DESCRIBED BY THE SUM OF 4 EXPONENTIALS

VITAE

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