REFERENCE

Al-Angary, A.A., Khidr, S.H., Mahrous, G.M., and Gouda, M.W. 1990. In-vitro dissolution and in- vivo absorption of marketed sustained release theophylline preparations. Int.J.Pharm. 65 : R5-R8.

Bakris, G.L. et al.1990. Effects of theophylline on erythropoietin production in normal subjects and in patients with erythrocytosis after renal transplantation. N.Engl.J.Med. 323 :86-90.

Baptista, R.J, and Driscoll, D.F. 1984. Theophylline Pharmacotheraphy. Am. Pharm. NS 24 : 57-65.

British Thoracic society. 1990. Guidelines for management of asthma in adults: I-Chronic persistent asthma. Br.Med.J. 301 :651-653.

Budavari, S. The Merck Index 11th ed. (an encyclopedia of chemicals, drugs and biologicals. U.S.A., Merck & Co., Inc., pp.1461-1462.

Buss, D.C. et al. 1989. The effect of theophylline on thyrotoxic tremor. Br.J.Clin.Pharmacol. 28 :103-107.

Glynn-Barnhart, A.G., Hill, M., and Szefler S.J. 1988. Sustained release Theophylline preparations practical recommendations for prescribing and therapeutic drug monitoring. Drugs 35 : 711-726.

Hardy, C., Schofield, O., George, C.F.,1983. Allergy to aminophylline. Br.Med.J. 286 :2051-2052.

Harris, M.C., Baumgart, S., Rooklin, A.R., Fox, W.W.1983. Successful extubation of infants with respiratory distress syndrome using aminophylline. J.Pediatr. 103 :303-305.

Hendeles, L., Weinberger, M.,and Bighley, L.1977. Absolute bioavailability of oral theophylline. Am.J.Hosp.Pharm. 34 : 525-527.

Hendeles, L., Iafrate, R.P., and Weinberger, M.1984. A clinical and pharmacokinetic basis for the selection and use of slow release theophylline products. Clin.Pharmacokin. 9 : 95-135.

Hendeles, L., Massanari, M., and Weinberger,M. Theophylline. In W.E. Evans, J.J.Schentag, and W.J. JusKo (eds.), Applied Pharmacokinetics. Principles of therapeutic drug monitoring. Second edition, pp.1105-1188. Washington: Applied Therapeutics, 1986.

Henkin, R.I.1991. Hyposmia in woman with asthma and allergic rhinitis. JAMA 299: 583.

Iafrate, R.P., Messey , K.L., and Hendeles, L. 1986. Current concepts in clinical therapeutics : Asthma. Clin. Pharm. 5 ;206-227.

Jacobs, M.H., Senior, R.M., and Kessler G. 1976. Clinical experience with theophylline. Relationships between dosage, serum

concentration and toxicity. JAMA 235: 1983-1986.

Jenne, J.W., et al. 1972. Pharmacokinetics of theophylline : Application to adjustment of the clinical dose of aminophylline. Clin.Pharmacol.Ther. 13 :349-360.

Jonkman, J.H.J., and Upton R.A. 1984. Pharmacokinetic drug interactions with theophylline. Clin.Pharmacokin. 9 : 309-334.

Jung, M.1989. Effects of smoking, sex and age on theophylline. Pharmacokinetics in Thais. (Thesis).

Kriter, K.E., Blanchard, J.1989. Management of apnea in infants. Clin. Pharm. 8 :577-587.

Lesko, L.J.1979. Dose-dependent elimination kinetics of theophylline. Clin.Pharmacokin. 4 : 449-459.

Mally, J.1989. Aminophylline and essential tremor. Lancet 2 : 278-279.

Matthay, R.A., Berger, H.J., Ross, D., Jacob, L., Alexander,G., Burry, Z.L.1982. Improvement in cardiac performance by oral long-acting theophylline in chronic obstructive pulmonary disease. Am.Heart.J. 104 : 1022-1026.

Murciano, D., Aubier,M., Lecocguic,Y., Pariente, R.1984. Effects of theophylline on diaphragmatic strength and fatique in patients with chronic obstructive pulmonary disease. N.Eng.J. Med. 311: 349-353.

Owens, G.R., and Tannenbaum, R.1981. Theophylline-induced urinary retention. Ann.Intern.Med.94 : 212-213.

Prakash, M., and WashBurne, J.D.1981. Theophylline and urinary retention. Ann.Intern.Med. 94: 823.

Reynolds, J.E.F. Martindale ,The Extra Pharmacopoeia,30th ed.1994, London, The Pharmaceutical press, pp.1319-1327

Roberto, M., German, C.L., and Elliot, E.F. 1970. Pharmacologic effects of intravenously administered aminophylline in asthmatic children. J.Pediatr. 76 : 777-782.

Tang-Liu, D.D., Williams, R.L., Riegelman, S.1982. Nonlinear theophylline elimination. Clin.Pharmacol.Ther. 31 : 359-369.

Upton, R.A., Sansom, L., Guentert, T.W., et al. 1980. Evaluation of absorption from 15 commercial theophylline products indicating deficiencies in currently applied bioavailability criteria. J.Pharmacokin.Biopharm. 8 :229-242.

Weinberger, M., et al. 1981. Relationship of formuldtion and dosing interval to fluctuation of serum theophylline concentration in children with chronic asthma. J.Pediatr. 99 : 145-152.

Weinberger, M.,et al. 1987. Effects of theophylline on learning and behavior: Reason for concern or concern without reason? J. Pediatr. 111: 471-474.

APPENDICES

APPENDIX A

TEST PRODUCTS

Table 31 Test Products

Brand name	Weight/tablet (mg)	Manufacturer	Mfg.date	Batch No.
Nuelin-SR	250	Riker Lab.	21/04/9	7854A
Quibron-T/SR	300	Bristol-Myer	30/04/92	MDE97
Theo-Dur	200	Astra	15/10/92	SK390
Theo-24	300	Searle	11/92	2F257

APPENDIX B

CALIBRATION CURVE DETERMINATION

The typical calibration curves data for theophylline concentrations in Simulated Gastric Fluid, Simulated Intestinal Fluid and human plasma are presented in Table 31, 32 and 33 and Figure 22, 23 and 24 respectively.

Table 32 Typical Calibration Curve Data for Theophylline Concentrations in Simulated Gastric Fluid, pH 1.2 Estimated Using Linear Regression

Standard No.	Concentration (mcg/ml)	Absorbance at 270 nm	Inversely estimated concentration (mcg/ml)	% Theory[2]
1	1.00	0.0037	1.009	100.90
2	2.00	0.0570	2.02	101.00
3	5.00	0.2176	5.04	100.80
4	10.00	0.4850	10.09	100.90
5	14.00	0.6989	14.13	100.93
6	16.00	0.8058	16.14	100.88
7	20.00	1.0197	20.18	100.90
			Mean	100.90
			S.D.	0.0596
			C.V.[4]	0.059 %

1. $r^2 = 0.999$, $y = 0.053 x - 0.0498$

2. Inversely estimated concentration $= \frac{(\text{Absorbance} + 0.0498)}{0.053}$

3. %theory $= \frac{(\text{Inversely estimated concentration} \times 100)}{\text{Known Concentration}}$

4. %C.V. = (S.D./mean) x 100

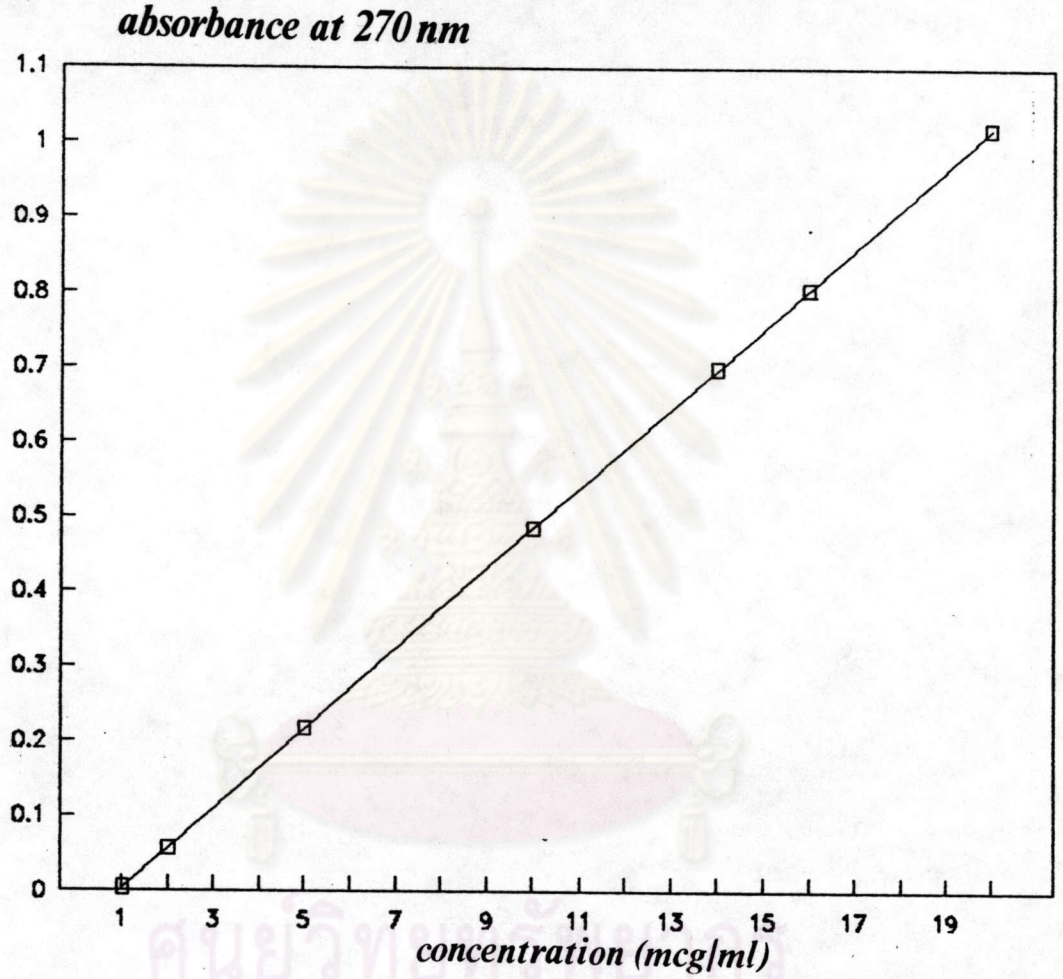

Figure 24 Calibration Curve of Theophylline in Simulated Gastric Fluid, pH 1.2.

Table 33 Typical Calibration Curve Data for Theophylline Concentrations in Simulated Intestinal Fluid, pH 7.5 Estimated Using Linear Regression

Standard No.	Concentration (mcg/ml)	Absorbance at 270 nm	Inversely estimated concentration (mcg/ml)	% Theory[2]
1	1.00	0.0000	1.16	116.00
2	2.00	0.0402	1.93	96.50
3	5.00	0.1964	4.94	98.80
4	10.00	0.4503	9.87	98.70
5	14.00	0.6642	13.99	99.93
6	16.00	0.7682	16.02	100.12
7	20.00	0.9763	20.04	100.20
			Mean	101.46
			S.D.	6.54
			C.V.[4]	6.44%

1. $r^2 = 0.9998$, $y = 0.0517 x - 0.0598$

2. Inversely estimated concentration $= \frac{(\text{Absorbance} + 0.0598)}{0.0517}$

3. %theory $= \frac{(\text{Inversely estimated concentration} \times 100)}{\text{Known Concentration}}$

4. %C.V. = (S.D./mean) x 100

CALIBRATION CURVE OF THEOPHYLLINE

In Simulated Intestinal Fluid

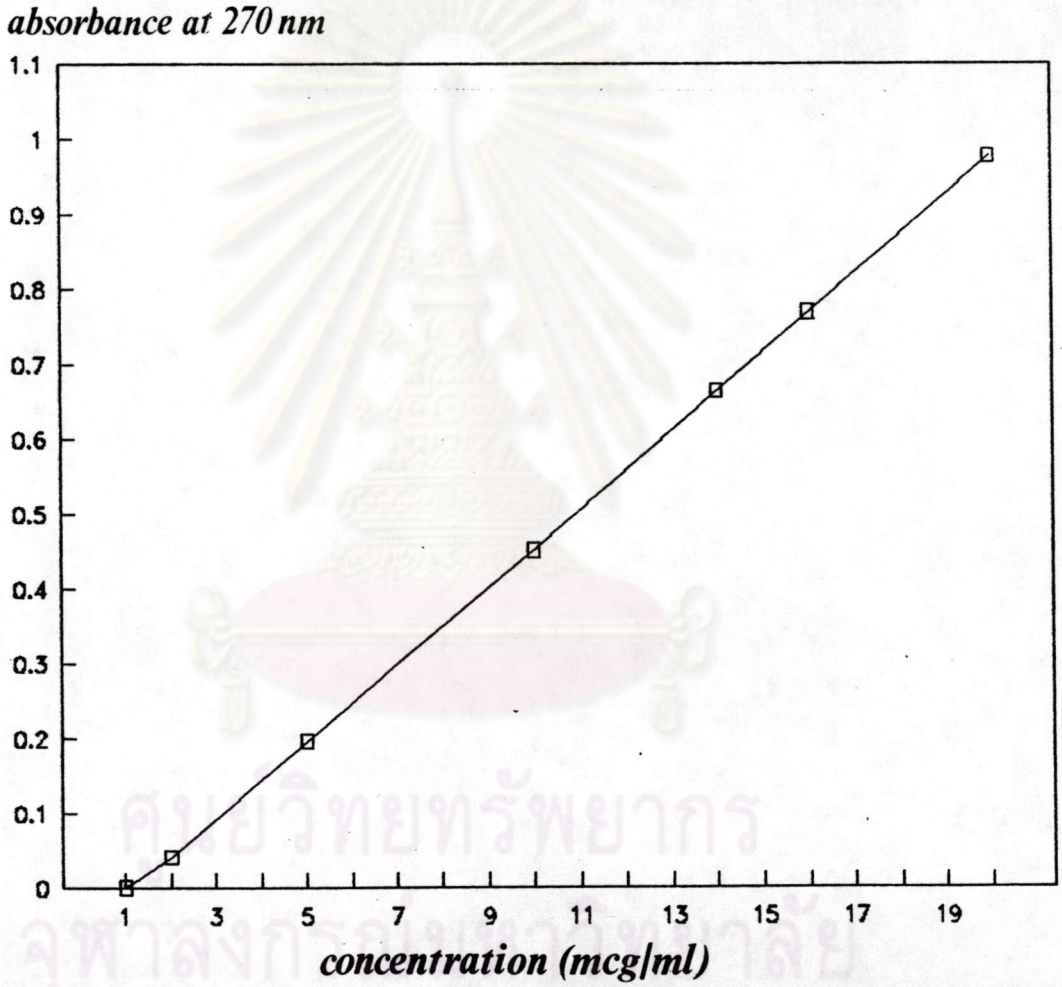

Figure 25 Calibration Curve of Theophylline in Simulated Intestinal Fluid, pH 7.5.

Table 34 Typical Calibration Curve Data for Theophylline Concentrations in Human Plasma Estimated Using Linear Regression

Standard No.	Concentration (mcg/ml)	Peak area ratio	Inversely estimated concentration (mcg/ml)	% Theory[2]
1	1.25	0.2290	1.25	100.25
2	2.50	0.4454	2.50	100.16
3	5.00	0.8800	5.02	100.32
4	7.50	1.3132	7.52	100.27
5	10.00	1.7465	10.02	100.25
6	15.00	2.6138	15.04	100.26
7	20.00	3.4819	20.20	100.98
			Mean	100.36
			S.D.	0.279
			C.V.[4]	0.278 %

1. $r^2 = 0.999$, $y = 0.173 x + 0.0122$

2. Inversely estimated concentration = $\frac{\text{(Peak area ratio - 0.0122)}}{0.173}$

3. %theory = $\frac{\text{(Inversely estimated concentration x 100)}}{\text{Known Concentration}}$

4. %C.V. = (S.D./mean) x 100

CALIBRATION CURVE OF THEOPHYLLINE

In plasma

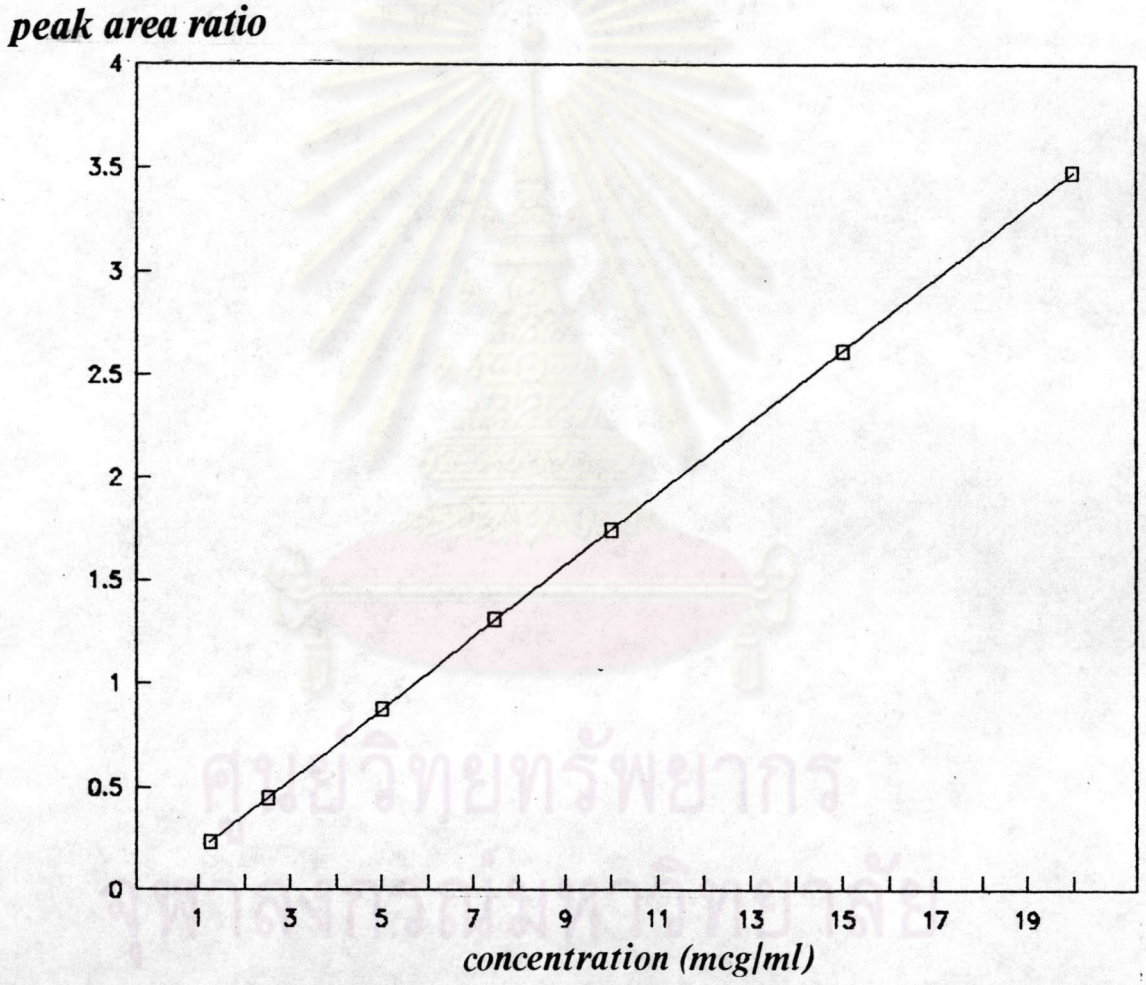

Figure 26 Calibration Curve of Theophylline in Human Plasma.

APPENDIX C

REAGENT PREPARATIONS

1. Phosphate buffer (for assay)

Dissolve monobasic potassium phosphate 1.36 g in 1000 ml of water,mix and adjust pH to 6.50 $\pm$ 0.05 with 1 M potassium hydroxide.

2. Mobile phase (for assay)

Mix prepared phosphate buffer (for assay) with methanol in 7:3 ratio. Filter and degas.

3. Simulated Gastric Fluid, pH 1.2

Dissolve 2 g of sodium chloride in 7.0 ml of hydrochloric acid. Adjust volume to 2500 ml with water. Adjust pH to 1.2.

4. Simulated Intestinal Fluid, pH 7.5

Dissolve 6.8 g of monobasic potassium phosphate in 625 ml of water, mix and add 475 ml of 0.2 N sodium hydroxide. Then add 1000 ml of water, mix. Adjust pH to 7.5 $\pm$ 0.1 with 0.2 N sodium hydroxide.

5. 0.01 N Sodium acetate buffer, pH 4.0

Dissolve 136.1 g of sodium acetate,trihydrate in 1000 ml of water. Adjust pH to 4.0 with glacial acetic acid

APPENDIX D

SUBJECTS

Table 35 Demographic Data

Subject No.	Age (yr.)	Height (cm)	Weight (kg)
1	28	160	56
2	30	164	60
3	21	170	58
4	21	168	58
5	25	160	56
6	35	168	60
7	23	165	56
8	20	165	58
9	38	156	56
10	45	160	70
11	35	160	44
12	22	160	45
13	24	161	56
Mean	28.23	162.85	56.38
S.D.	7.84	4.14	6.47

APPENDIX E

STATISTICS

1. Mean ($\bar{x}$)

$$\bar{x} = \frac{\Sigma x}{N}$$

2. Standard deviation

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

3. Standard error of mean

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

4. Testing the difference among treatment means

complete randomized design

Treatments					Total	Mean
1	2	3	.....	k		
X_{11}	X_{12}	X_{13}	.....	X_{1k}	T_1	$\bar{X}_1$
X_{21}	X_{22}	X_{23}	.....	X_{2k}	T_2	$\bar{X}_2$
X_{31}	X_{32}	X_{33}	.....	X_{3k}	T_3	$\bar{X}_3$
.	.	.....			.....	.....
X_{n1}	X_{n2}	X_{n3}	.....	X_{nk}	T_n	$\bar{X}_n$

Treatments			Total	Mean
1	2	3.....k		
Total T_1	T_2	T_3..... T_k	T	$\bar{X}$
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 $H_o : \mu_1 = \mu_2 = \ldots\ldots\ldots \mu_k$

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

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_I
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 correction 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 (SSamong)

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

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

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

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

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

F has (k-1), (Σn-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 that $F_{0.05}$, 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 Least Significant Different (LSD).

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

$t_{0.05}$ has ($\Sigma n-k$) degree of freedom

$$L.S.D. = t_{0.05} \times S_d$$

where $S_d = \sqrt{2\ MS_{(within)} / n}$

If the difference of the two means is greater than L.S.D. calculated, 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.

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

where r = Correlation coefficient

N = the number of x and y pairs

Test of Zero Correlation

Let ρ = the true correlation coefficient, estimated by r

The null hypothesis $H_o = \rho = 0$

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 that $t_{0.05}$, the null hypothesis is rejected. If t is not significant, the null hypothesis is accepted.

APPENDIX E

Table 36 Plasma Theophylline Concentration(mcg/ml) from 13 Subjects Following Oral Administration of Theophylline Sustained-release Tablets of Brand A

Subject No.	Time (hr.)									
	0	0.25	0.5	1.0	2.0	3.0	4.0	6.0	8.0	12.0
1	4.05	3.90	4.24	4.35	5.12	5.95	5.38	5.82	5.52	3.95
2	3.65	4.18	3.82	4.35	3.64	3.78	4.08	3.62	3.89	3.55
3	6.40	6.58	7.00	6.88	7.90	7.00	6.90	7.12	5.30	4.25
4	3.40	3.42	4.28	4.32	4.80	5.00	5.68	6.28	5.10	3.30
5	4.02	3.68	4.05	4.30	5.28	6.95	5.60	5.20	4.25	2.78
6	5.22	5.00	5.25	5.18	5.15	5.05	4.98	5.20	6.40	5.55
7	3.88	3.88	4.18	3.92	4.20	5.88	6.70	5.32	4.28	2.60
8	5.82	7.35	7.50	7.20	6.91	8.58	7.32	8.20	7.65	5.25
9	3.75	4.18	4.35	5.00	4.22	4.22	4.40	4.05	3.68	2.90
10	6.00	5.72	5.88	5.40	5.90	7.02	6.50	7.00	5.80	4.90
11	4.50	5.45	7.75	7.40	6.80	9.20	14.60	14.70	10.95	9.25
12	5.30	5.60	6.25	6.00	6.50	5.75	6.75	8.40	9.65	9.55
13	2.78	2.30	2.30	2.28	2.58	2.45	2.85	2.02	1.90	1.50

Table 37 Plasma Theophylline Concentration (mg/ml) from 13 Subjects Following Oral Administration of Theophylline Sustained-release Tablets of Brand B

Subject No.	Time (hr.)									
	0	0.25	0.5	1.0	2.0	3.0	4.0	6.0	8.0	12.0
1	4.60	5.22	4.22	4.35	4.18	5.32	6.33	6.29	6.95	4.64
2	3.40	3.82	3.34	3.52	3.36	3.50	3.40	3.94	3.65	3.08
3	2.36	2.94	2.78	3.34	2.72	3.06	3.98	3.84	3.64	2.48
4	3.96	4.20	4.32	4.30	3.96	3.66	3.88	4.08	4.28	2.82
5	5.38	5.14	4.80	5.18	5.22	5.34	5.06	4.72	4.72	3.48
6	5.24	5.34	5.52	5.08	5.24	5.76	5.48	6.48	7.08	5.30
7	3.44	3.12	2.52	3.38	3.72	3.80	3.88	3.98	3.66	3.28
8	3.56	3.48	4.04	4.28	4.28	3.98	4.42	4.60	4.26	3.60
9	4.68	4.00	5.64	5.12	4.98	5.52	5.88	5.80	4.02	3.62
10	4.12	3.70	5.14	4.28	3.96	5.74	4.46	4.62	4.24	3.36
11	2.06	2.54	2.58	2.34	2.94	2.64	3.51	3.08	2.55	2.00
12	2.82	2.50	2.15	2.44	2.69	2.91	3.44	2.91	2.50	2.31
13	1.30	1.96	2.14	2.08	1.64	2.26	2.26	2.20	1.52	1.04

Table 38 Plasma Theophylline Concentration (mcg/ml) from 13 Subjects Following Oral Administration of Theophylline Sustained-release Tablets of Brand C

Subject No.	Time (hr.)									
	0	0.25	0.5	1.0	2.0	3.0	4.0	6.0	8.0	12.0
1	3.14	3.26	3.10	3.88	4.72	5.66	5.06	4.76	3.90	3.00
2	1.18	1.56	1.14	2.04	2.42	3.14	2.86	2.36	1.98	1.42
3	3.76	6.64	5.92	4.72	4.16	4.36	4.32	3.56	3.94	2.68
4	1.66	1.64	1.78	1.86	2.12	2.44	2.66	2.52	2.06	1.94
5	1.34	1.98	2.24	2.70	3.10	2.80	2.78	2.76	2.00	1.56
6	5.70	5.70	5.84	5.56	5.66	5.88	7.78	6.70	6.56	5.08
7	3.20	4.20	3.80	4.52	4.50	5.84	5.40	4.40	3.42	2.80
8	3.54	3.24	3.50	3.38	3.84	5.14	5.04	4.34	3.46	2.62
9	3.24	3.90	4.22	4.60	5.10	4.84	6.06	5.20	4.74	3.18
10	5.32	5.34	4.48	4.96	6.60	7.40	6.80	6.00	5.40	3.60
11	5.50	5.34	5.67	5.88	6.10	6.03	6.63	6.60	7.35	4.76
12	5.10	6.12	6.69	6.60	6.48	6.93	7.12	7.684	6.99	0.93
13	2.58	2.86	3.56	3.30	4.56	3.66	3.10	3.10	2.06	1.24

Table 39 Plasma Theophylline Concentration (mcg/ml) from 13 Subjects Following Oral Administration of Theophylline Sustained-release Tablets of Brand D

Subject No.	Time (hr.)											
	0	0.25	0.5	1.0	2.0	3.0	4.0	6.0	8.0	12.0	16.0	24.0
1	3.18	4.14	2.94	4.14	4.02	4.08	4.38	9.00	8.52	6.78	5.16	3.30
2	4.92	5.16	4.80	4.50	4.98	5.88	5.46	8.52	10.56	7.38	5.28	1.92
3	4.08	4.02	3.54	4.80	4.56	5.28	5.46	5.28	4.80	5.58	4.20	3.90
4	3.00	3.15	2.70	2.82	2.94	3.72	4.08	3.66	4.20	5.64	4.20	1.92
5	2.64	2.88	2.70	3.60	4.20	4.62	4.44	5.16	6.30	5.58	4.20	3.36
6	5.52	4.32	4.68	5.10	4.98	5.16	6.06	6.12	6.00	6.30	6.36	4.26
7	4.17	4.08	4.20	4.50	5.16	5.40	6.84	6.66	8.22	7.92	7.86	5.40
8	3.48	3.96	4.44	4.44	5.52	4.74	4.80	4.92	5.52	4.20	3.48	2.16
9	3.90	4.38	3.54	5.28	5.46	6.06	7.17	7.86	8.28	7.02	6.78	4.80
10	2.04	3.30	3.12	3.60	3.36	4.08	5.04	5.34	5.94	4.92	4.38	2.46
11	6.30	7.38	6.72	7.38	9.36	8.46	9.24	10.26	11.28	10.98	9.72	8.28
12	8.52	9.18	8.16	9.12	8.64	9.36	12.44	17.94	18.66	14.52	10.62	8.28
13	3.84	4.14	4.56	4.50	4.44	5.88	6.60	7.32	5.64	5.04	4.08	2.58

VITAE

Name	Miss Panit Somhom
Birth Date	September 9, 1967
Education	Bachelor of Science in Pharmacy from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.
Position	Medical Scientist, Drug Analysis Division, Department of Medical Sciences, Ministry of Public Health,Bangkok, Thailand.