CHAPTER IV

RESULTS AND DISCUSSION

1. In Vitro Studies

All four commercial brands of theophylline sustained-release tablets were first tested for weight variation, identification, and assay. Each of these four brands met the United States Pharmacopoeia requirement (USP XXII) for identification compared with standard theophylline, UV absorption profiles as shown in Figure 5. And for weight variation within the range of limit weight (± 5 %). All products were assayed for content of active ingredient and the results indicated that each brand was within the limits of 90-110 percent labelled amount as shown in Table 3.

Neither the United States Pharmacopoeia XXII nor the British Pharmacopoeia 1988 contains a dissolution rate constant specification for theophylline sustained-release tablet. The dissolution testing of theophylline sustained-release tablets was a crucial factor for systemic drug availability because the drug was practically insoluble in water. Since the method of dissolution testing for theophylline described in Al-Angary et al.'s report correlates with the in vivo studies, the modified procedure was found to be the most reasonable mean.

Table 4 and Figure 6 illustrated the dissolution profiles at various sampling times of all four brands of theophylline sustained-release tablets. Only brand C reached the equilibrium state within 2 hours, while others (brands A, B, and D) established after 6 hours. The mean percent dissolved of theophylline from all brands ranged from 92.26 to 102.10 percent at 12 hours. The dissolution rate constants

(K_d) were calculated from the slope of the first order plot between the amount of undissolved theophylline ($\beta_\infty - \beta_t$) versus time in semi-logarithmic scale and results were reported in Table 5. The rank order of these dissolution rate constants for all brands were brands C>A>B>D. Statistical comparison, as presented in Table 6 and 7 , indicated that the dissolution rate constant of brands A, D were no statistically significant different ($p > 0.05$) when compared to that of brand B while brand C had the value greater than that of brand B ($p > 0.05$).

As seen in Table 6 and 7 different brands of theophylline sustained-release tablets resulted in different dissolution rates. This was very common and always seen when a practically insoluble drug was manufactured as a sustained-release tablet. The major factor might be due to the various inert substances and methods of formulation used by each individual manufacturer. In addition, source of theophylline raw material, drug formulation, and/or manufacturing process might contributed to these difference. However, these problems could be overcome if formulator(s) selected appropriate added substances for making the drug in this kind of dosage form.

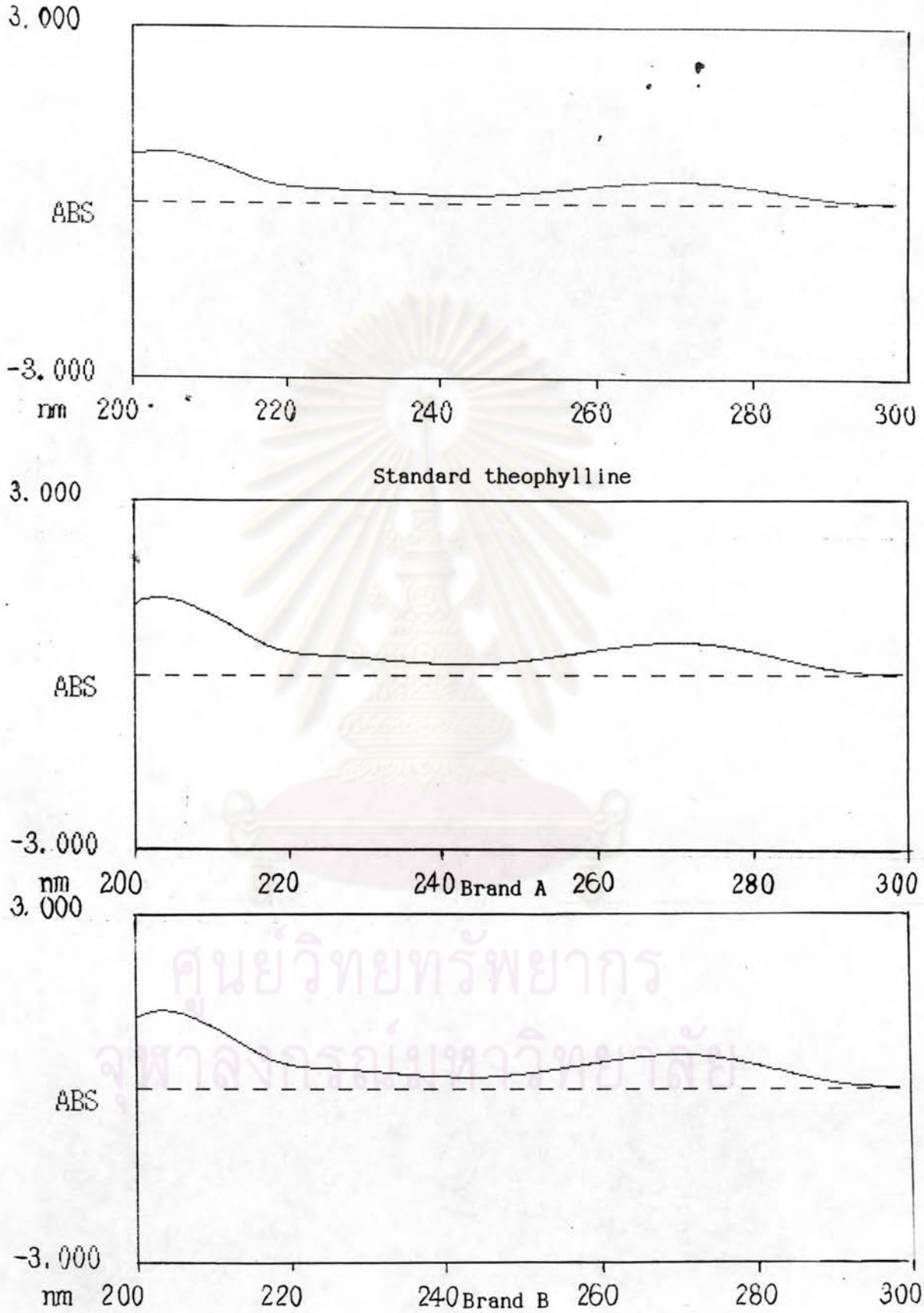
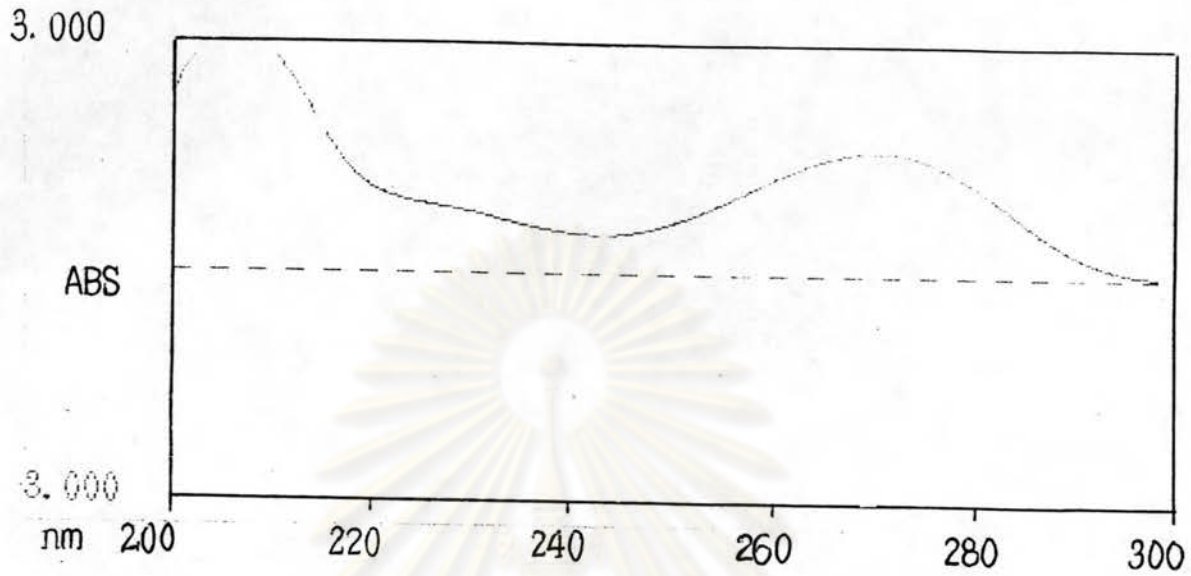
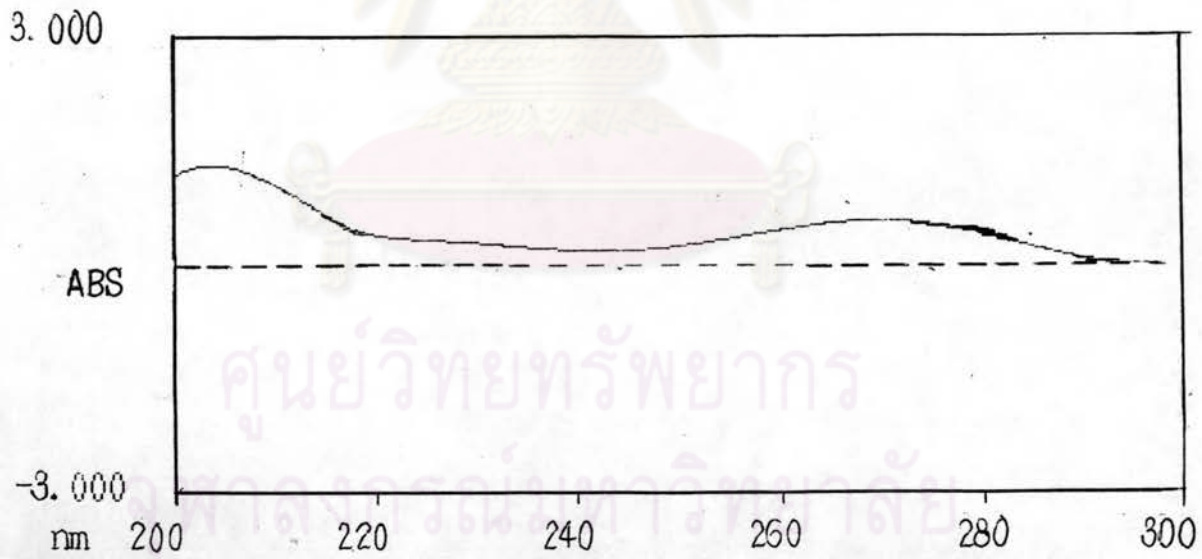


Figure 5 UV Absorption profile



Brand C



Brand D

Table 3 In Vitro Studies of Four Commercial Brands of Theophylline Sustained-release Tablets

Brand	Weight (mg) (n =20)	Content of Active Integredient (% L.A.) (n = 5)	Dissolution Rate Constant (min^{-1}) (n = 6)
A	0.4939 ± 0.0049	109.35 ± 0.36	0.0029 ± 0.0090
B	0.4225 ± 0.0046	103.08 ± 0.66	0.0026 ± 0.0006
C	0.3007 ± 0.0031	101.72 ± 0.58	0.0190 ± 0.0005
D	0.6843 ± 0.0092	103.35 ± 0.76	0.0017 ± 0.0005

All value are presented as mean ± S.D.

Table 4 % Drug Dissolved of Four Branes of Theophylline Sustained-release Tablets in Simulated Gastric and Intestinal Fluid Followed by Modified Al-Angary et al. 's method

Time (min)	% Drug Dissolved			
	Brand A	Brand B	Brand C	Brand D
0	0.00	0.00	0.00	0.00
32.5	16.18 ± 0.64	12.12 ± 0.80	47.14 ± 3.36	6.38 ± 0.40
47.5	20.28 ± 0.60	15.00 ± 1.30	62.70 ± 3.64	9.90 ± 0.60
62.5	23.26 ± 0.40	16.30 ± 1.52	76.06 ± 2.86	12.36 ± 0.60
122.5	38.78 ± 2.36	24.80 ± 3.07	99.20 ± 0.92	33.51 ± 1.26
182.5	55.26 ± 13.28	29.03 ± 9.51	100.10 ± 2.00	46.84 ± 15.43
242.5	71.53 ± 18.92	55.64 ± 14.24	100.10 ± 2.00	80.79 ± 3.00
302.5	78.58 ± 17.70	68.26 ± 13.59	100.10 ± 2.00	90.80 ± 3.49
362.5	86.20 ± 9.50	77.16 ± 11.54	100.10 ± 2.00	91.66 ± 1.69
482.5	94.08 ± 1.94	91.16 ± 7.30	100.10 ± 2.00	94.08 ± 2.25
602.5	94.14 ± 2.00	97.12 ± 4.10	100.10 ± 2.00	94.90 ± 1.63
722.5	94.14 ± 1.88	100.07 ± 1.05	100.10 ± 2.00	94.54 ± 1.63
962.5				97.56 ± 2.25
1442.5				98.80 ± 0.92

DISSOLUTION PROFILES OF Theophylline sustained-release Tablets

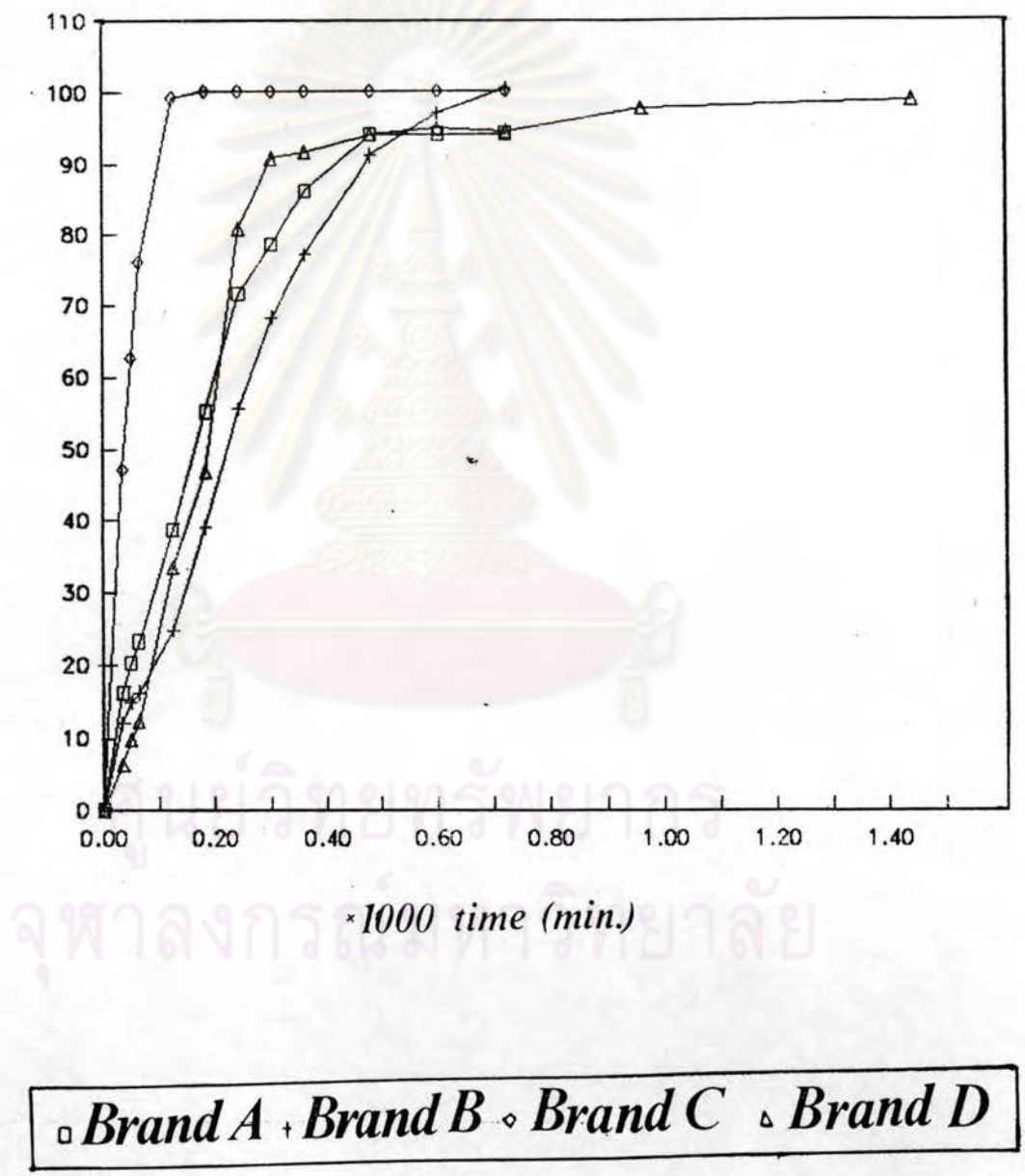


Figure 6 Dissolution Profiles of Four Commercial Brands of Theophylline Sustained-release Tablets in Simulated Gastric and Intestinal Fluids

Table 5 Dissolution Rate Constants (K_d) of Four Brands of Theophylline Sustained-release Tablets in Simulated Gastric and Intestinal Fluids

Sample	Dissolution Rate Constant (K_d) (min^{-1})			
	Brand A	Brand B	Brand C	Brand D
1	0.0021	0.0036	0.017	0.0020
2	0.0042	0.0024	0.020	0.0025
3	0.0021	0.0028	0.022	0.0017
4	0.0026	0.0022	0.025	0.0014
5	0.0026	0.0029	0.010	0.0013
6	0.0036	0.0020	0.022	0.0012
Mean	0.0029	0.0026	0.019	0.0017
S.D.	0.0009	0.0006	0.0053	0.0005

Table 6 Analysis of Variance for Dissolution Rate Constant of Four Commercial Brands of Theophylline Sustained-release Tablets in Simulated Gastric and Intestinal Fluids

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	0.0012	0.00043	61.43
Within group	20	0.00014	0.000007	
Total	23	0.0014		

F 0.05 (3,20) = 3.10

a = Degree of freedom

b = Sum of Square

c = Mean Square

d = Variance ratio

Table 7 Comparison of Dissolution Rate Constant of Each Brand of Theophylline Sustained-release Tablets with that of The Reference 's Product (Brand B)

Brand	$\Delta \bar{x}$	Statistical Significance
A	0.0003	NS
C	0.0164	S
D	0.0009	NS

$t_{(0.05,\ 20)} = 2.086$

$LSD_{(0.05)} = t_{.05} \times S_d = 0.0035$

S = Significant difference at $P < 0.05$

NS = Not signficant difference at $P > 0.05$

2. In Vivo Studies

2.1 Analysis of theophylline in plasma

Plasma theophylline concentrations were analyzed using high performance liquid chromatography following the procedure modified from those of Jung (1989). Zinc sulfate solution was used to precipitate plasma protein and it exhibited an interfere free chromatogram in the HPLC systems (Figure 7).

The ratio of mobile phase mixture which consists of acetonitrile and 0.01 N sodium acetate buffer (pH 4.0) was 11:89.

Typical chromatograms of theophylline and internal standard (etofylline) are shown in figure 8 and 9. The retention time of internal standard and theophylline were 6.5 and 5.5 minutes, respectively.

The calibration curve of peak area ratio of theophylline was linear up to 20 mcg/ml (Appendix B).

The efficacy of seperation technique used was evaluated by calculating the percentage of recoveries. This was accomplished by comparing the peak area ratio of the drug obtained from spiked plasma to the peak area ratio that obtained from standard solution directly injected to HPLC. Results as shown in Table 8 indicated that the analytical method used was independent to concentration. The percentage recoveries of theophylline and internal standard were in the range 83.16 ± 1.87 % and 89.86 ± 0.09 % , respectively.

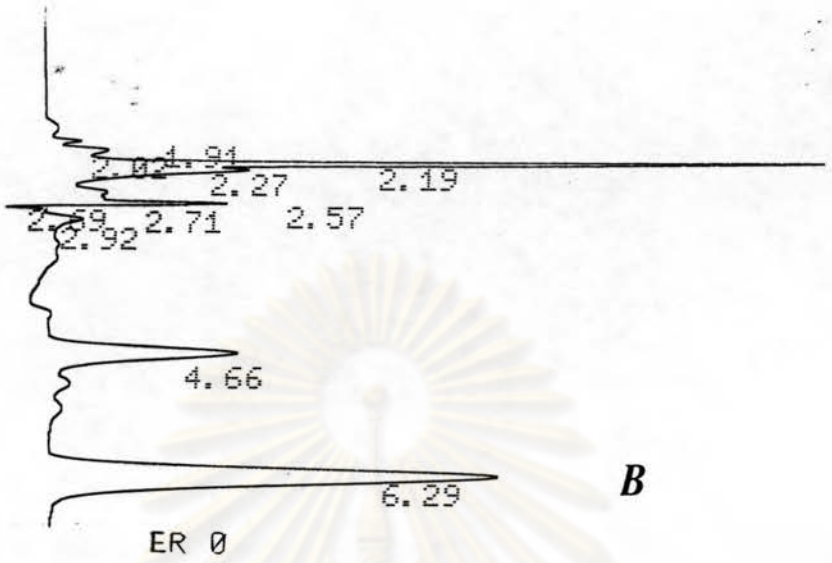


Figure 7 High Performance Liquid Chromatogram of Blank Plasma Sample Spiked with Internal Standard (B) Extracted with Zinc Sulfate and Methanol

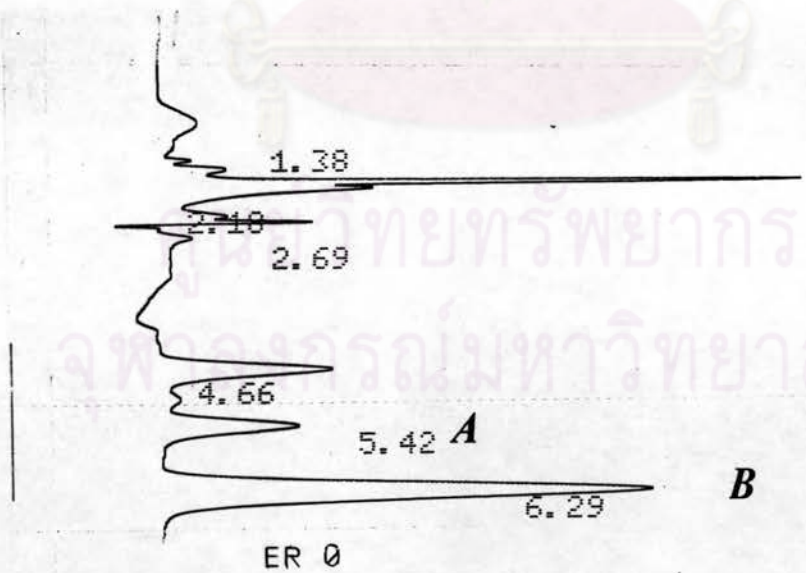


Figure 8 High Performance Liquid Chromatogram of Theophylline (A) and internal Standard (Etofylline) (B)

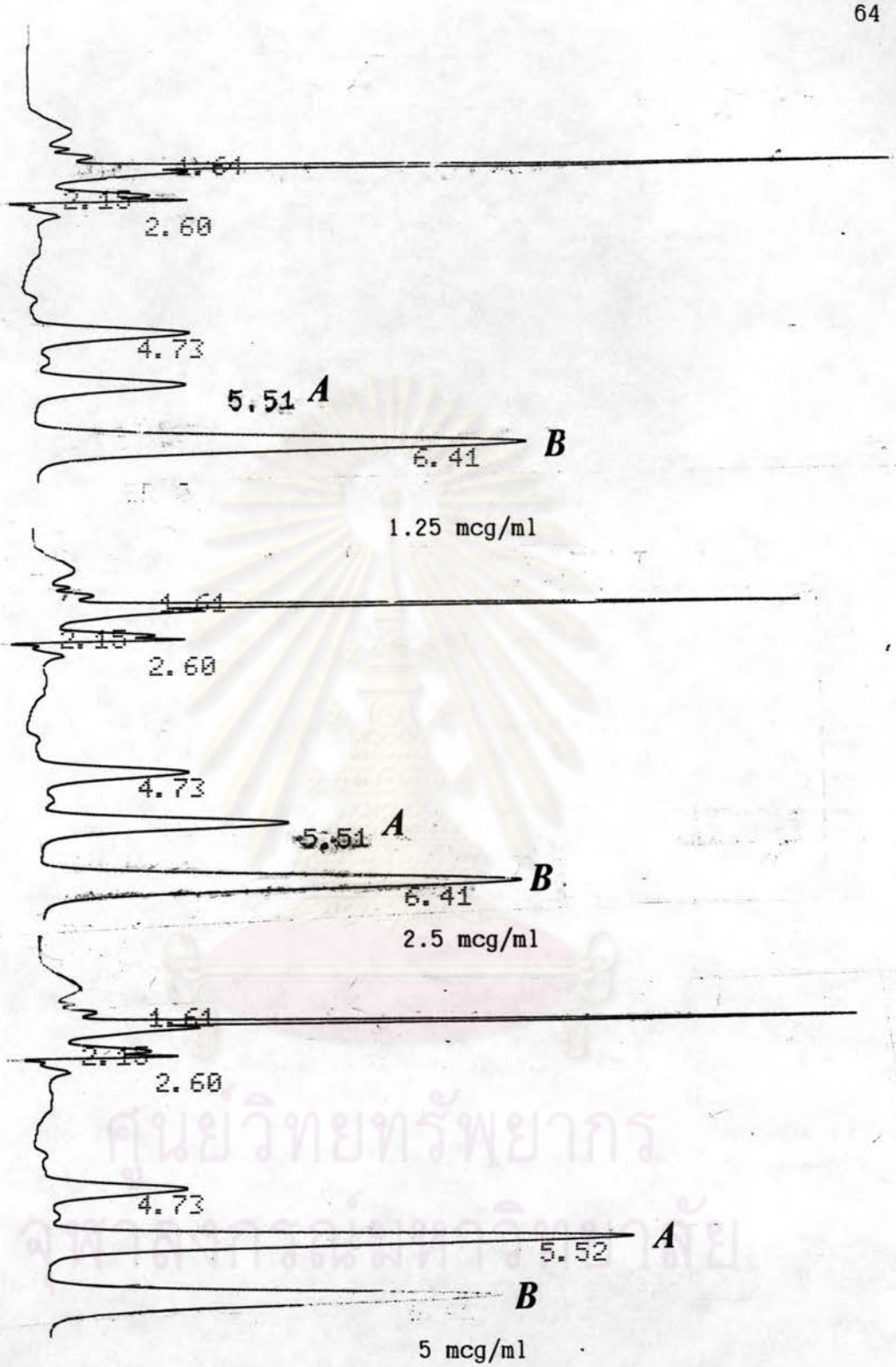


Figure 9 High Performance Liquid Chromatogram of Theophylline (A) and Internal standard (etofylline) (B) at different concentrations

Table 8 Recoveries of Theophyliine and Internal Standard (Etofylline)

Conc. (mcg/ml)	Theophylline Peak area ratio[a]		% Recovery[b]	Etofylline peak area ratio[a]		% Recovery[b]
	Sol[n]	Plasma		Sol[n]	Plasma	
1.25	0.4760	0.3993	83.89	0.3641	0.3277	90.00
2.50	0.8268	0.6949	84.05	0.5124	0.4602	89.81
5.00	1.0245	0.8664	84.57	0.8865	0.7969	89.89
7.50	1.1560	0.9236	79.90	1.1257	1.0110	89.81
10.00	1.7490	1.4588	83.41	1.3743	1.2340	89.79
Mean % Recovery ± S.D.			83.16 ± 1.87			89.86 ± 0.09
% C.V.			2.25			0.10

a = mean peak area ratio from each concentration (n=3)

b = % Recovery

$$= \frac{\text{Peak area ratio from spiked plasma}}{\text{Peak area from solution}} \times 100$$

2.2 Plasma Theophylline Concentration

The plasma concentration of theophylline at each sampling time interval ranging from 0 to 12 hours after oral administration of theophylline sustained-release tablets of brand A, B, C and from 0 to 24 hours of brand D was shown in Appendix F, and data after dose normalized calculation was shown in Table 9 to 21, respectively. Individual plasma theophylline concentration-time profile after dose normalized calculation for each of thirteen subjects were shown graphically from Figure 10 to 22. Comparison of the mean plasma theophylline concentration profiles of each brand from thirteen subjects were illustrated in Figure 23.

The plots indicated that the concentrations of the drug were high fluctuation in individual among brands. This was clearly seen in subjectes no.1, 4, 5, 7, 10 and 12. Reasons might be due to theophylline kinetics in the body was wide variation in individual.

Hence, observed relevant pharmacokinetic parameters, C_{max}, t_{max}, %fluctuation as well as the AUC calculateds using trapezoidal rule of each individual would be used for comparative bioavailability study.

3. Bioavailability Evaluation

The pharmacokinetic parameters, C_{max}, t_{max}, %fluctuation and AUC are used to characterize the bioavailability of pharmaceutical formulation after administration. The parameters C_{max}, t_{max}, and %fluctuation represented the rate of drug reaching the systemic circulation and the rate of drug elimination from the body that can be predicting whether the serum concentrations within the therapeutic

Table 9 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	3.24	3.12	3.39	3.48	4.10	4.76	4.30	4.66	4.42	3.16
2	2.92	3.34	3.06	3.48	2.70	3.02	3.26	2.90	3.11	2.84
3	5.12	5.26	5.60	5.50	6.32	5.60	5.52	5.70	4.24	3.40
4	2.72	2.74	3.42	3.46	3.84	4.00	4.54	5.02	4.08	2.64
5	3.22	2.94	3.24	3.44	4.22	5.56	4.48	4.16	3.40	2.22
6	4.18	4.00	4.20	4.14	4.12	4.04	3.98	4.16	5.12	4.44
7	3.10	3.10	3.34	3.14	3.36	4.70	5.36	4.26	3.42	2.08
8	4.66	5.88	6.00	5.76	5.53	6.86	5.86	6.56	6.12	4.20
9	3.00	3.34	3.48	4.00	3.38	3.38	3.52	3.24	2.94	2.32
10	4.80	4.58	4.70	4.32	4.72	5.62	5.20	5.60	4.64	3.92
11	3.60	4.36	6.20	5.92	5.44	7.36	11.68	11.76	8.76	7.40
12	4.24	4.48	5.00	4.80	5.20	4.60	5.40	6.72	7.72	7.64
13	2.22	1.84	1.84	1.82	2.06	1.96	2.28	1.62	1.52	1.20
Mean	3.62	3.77	4.11	4.10	4.23	4.73	5.03	5.10	4.58	3.65
S.E.M.	0.25	0.31	0.36	0.32	0.33	0.42	0.62	0.68	0.55	0.54

* already dose normalized calculation to 200 mg twice daily

Table 10 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	4.12	5.08	5.16	4.68	5.88	5.28	7.02	6.16	5.10	4.00
12	5.64	5.00	4.30	4.88	5.38	5.82	6.88	5.82	5.00	4.62
13	1.30	1.96	2.14	2.08	1.64	2.26	2.26	2.20	1.52	1.04
Mean	3.98	4.08	4.15	4.19	4.19	4.54	4.84	4.81	4.47	3.49
S.E.M.	0.34	0.29	0.32	0.25	0.33	0.33	0.39	0.34	0.40	0.30

* already dose normalized calculation to 200 mg twice daily

Table 11 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	7.34	7.12	7.56	7.84	8.14	8.04	8.84	8.88	9.80	6.34
12	6.80	8.16	8.92	8.80	8.64	9.24	9.50	10.24	9.32	7.56
13	2.58	2.86	3.56	3.30	4.56	3.66	3.10	3.10	2.06	1.24
Mean	3.75	4.28	4.31	4.47	4.89	5.26	5.40	4.99	4.51	3.30
S.E.M.	0.55	0.60	0.62	0.57	0.55	0.57	0.64	0.68	0.73	0.53

* already dose normalized calculation to 200 mg twice daily

Table 12 Plasma Theophylline Concentration*(mcg/ml) from 13 Subjects Following Oral Administration of Theophylline Sustained-release Tablets of Brand D

Subject	Time (hr.)											
No.	0	0.25	0.5	1.0	2.0	3.0	4.0	6.0	8.0	12.0	16.0	24.0
1	2.12	2.76	1.96	2.76	2.68	2.72	2.92	6.00	5.68	4.52	3.44	2.20
2	3.28	3.44	3.20	3.00	3.32	3.92	3.64	5.68	7.04	4.92	3.52	1.28
3	2.72	2.68	2.36	3.20	3.04	3.52	3.64	3.52	3.20	3.72	2.80	2.60
4	2.00	2.10	1.80	1.88	1.96	2.48	2.72	2.44	2.80	3.76	2.80	1.28
5	1.76	1.92	1.80	2.40	2.80	3.08	2.96	3.44	4.20	3.72	2.80	2.24
6	3.68	2.88	3.12	3.40	3.32	3.44	4.04	4.08	4.00	4.20	4.24	2.84
7	2.78	2.72	2.80	3.00	3.44	3.60	4.56	4.44	5.48	5.28	5.24	3.60
8	2.32	2.64	2.96	2.96	3.68	3.16	3.20	3.28	3.68	2.80	2.32	1.44
9	2.60	2.92	2.36	3.52	3.64	4.04	4.78	5.24	5.52	4.68	4.52	3.20
10	1.36	2.20	2.08	2.40	2.24	2.72	3.36	3.56	3.96	3.28	2.92	1.64
11	4.20	4.92	4.48	4.92	6.24	5.64	6.16	6.84	7.52	7.32	6.48	5.52
12	5.68	6.12	5.44	6.08	5.76	6.24	8.96	11.96	12.44	9.68	7.08	5.52
13	2.56	2.76	3.04	3.00	2.96	3.92	4.40	4.88	3.76	3.36	2.72	1.72
Mean	2.85	3.08	2.96	3.28	3.46	3.72	4.24	5.02	5.32	4.72	3.92	2.66
S.E.M.	0.32	0.32	0.30	0.30	0.34	0.34	0.48	0.68	0.72	0.52	0.42	0.38

* already dose normalized calculation to 400 mg once daily

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 1

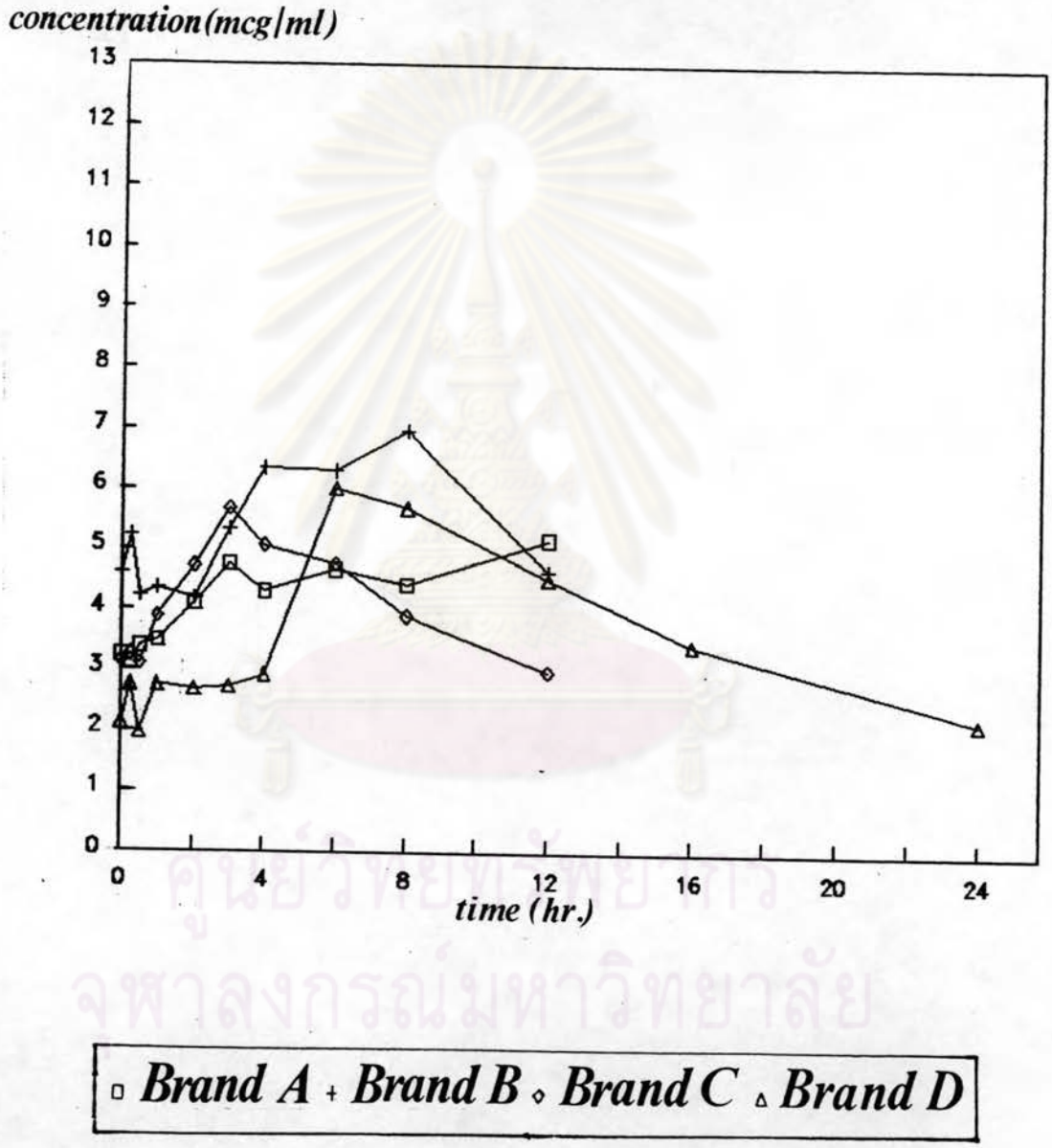


Figure 10 Plasma Theophylline Concentration-Time Profile of Subject No.1 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 2

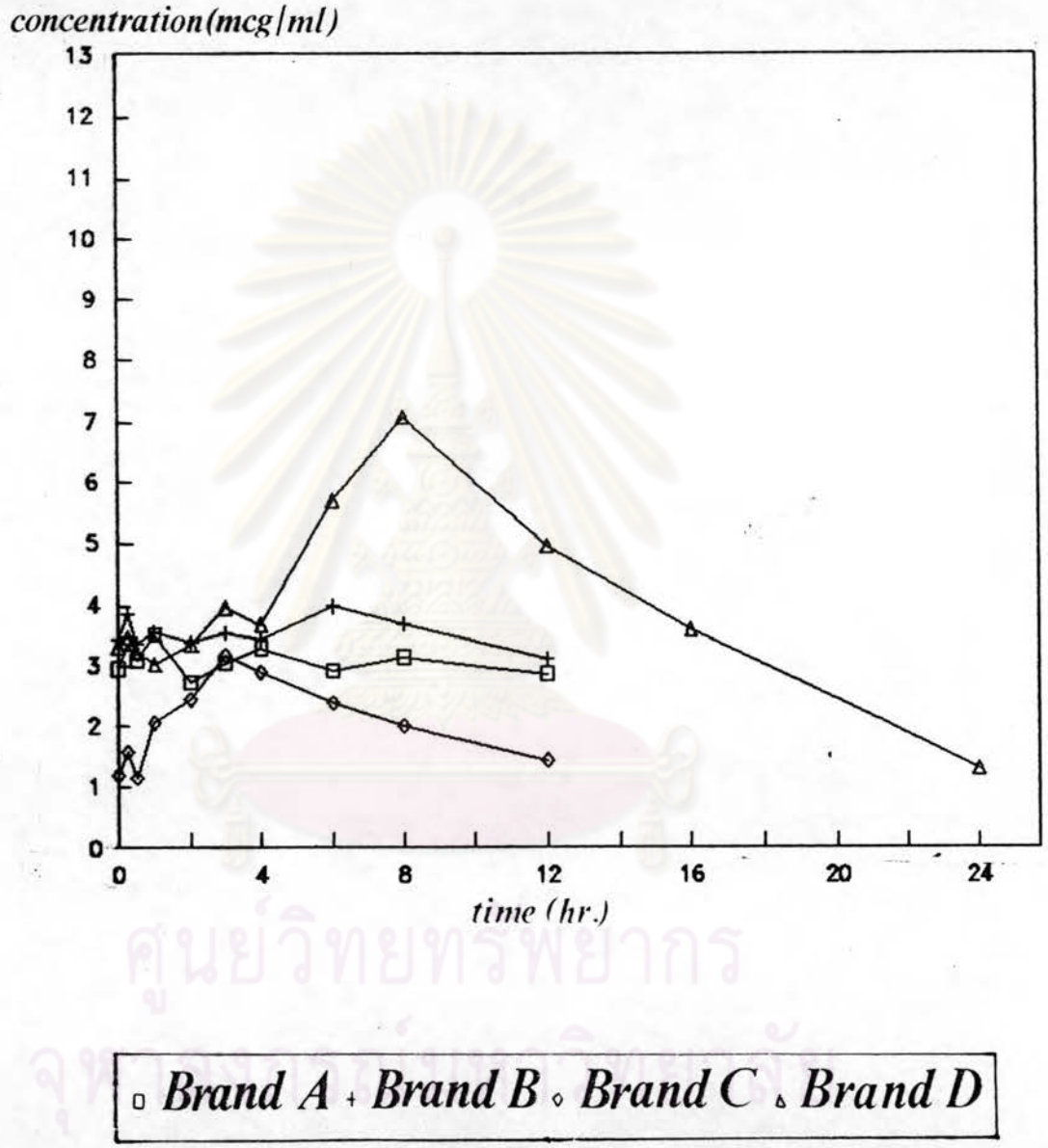


Figure 11 Plasma Theophylline Concentration-Time Profile of Subject No.2 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 3

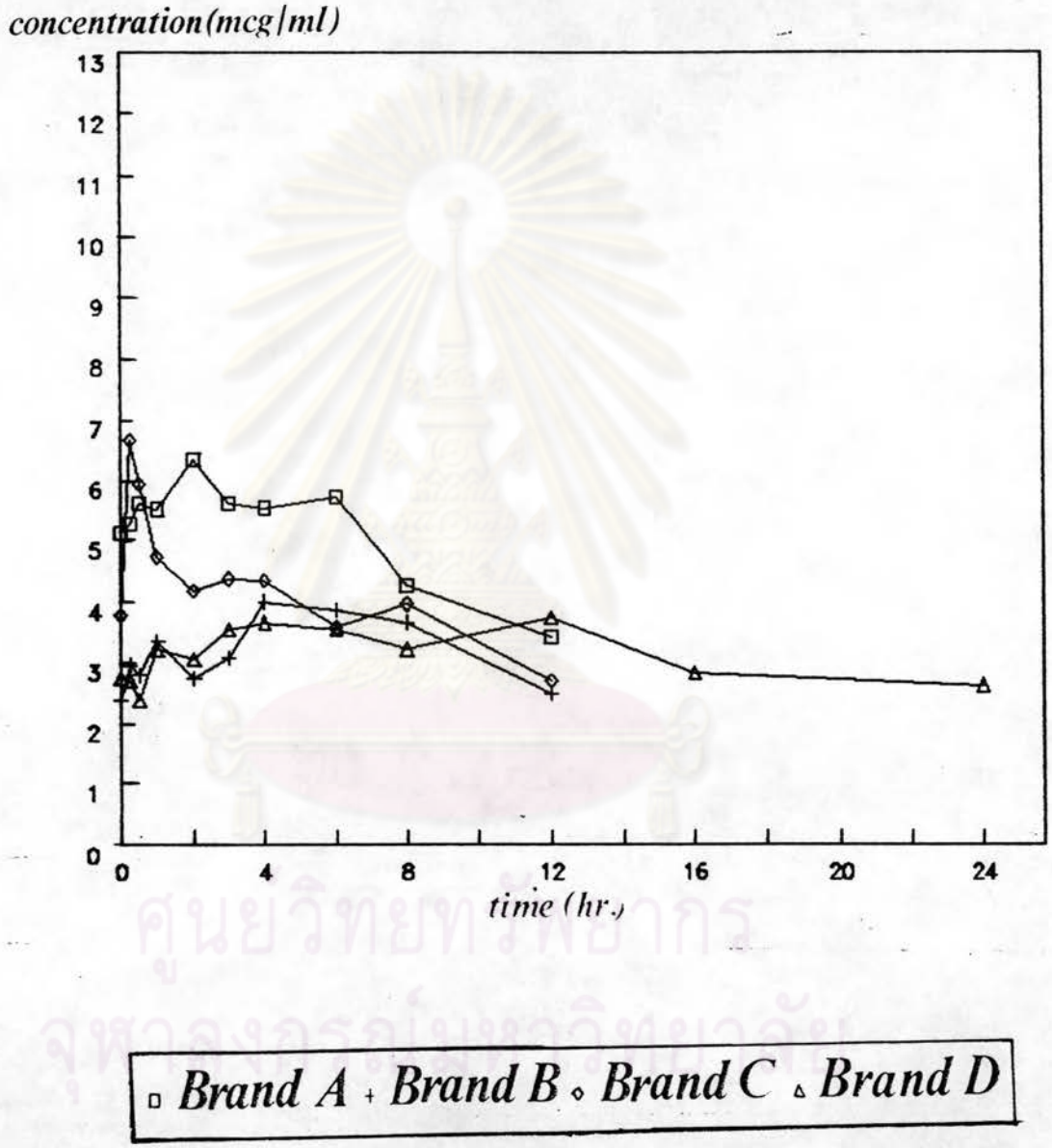


Figure 12 Plasma Theophylline Concentration-Time Profile of Subject No.3 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 4

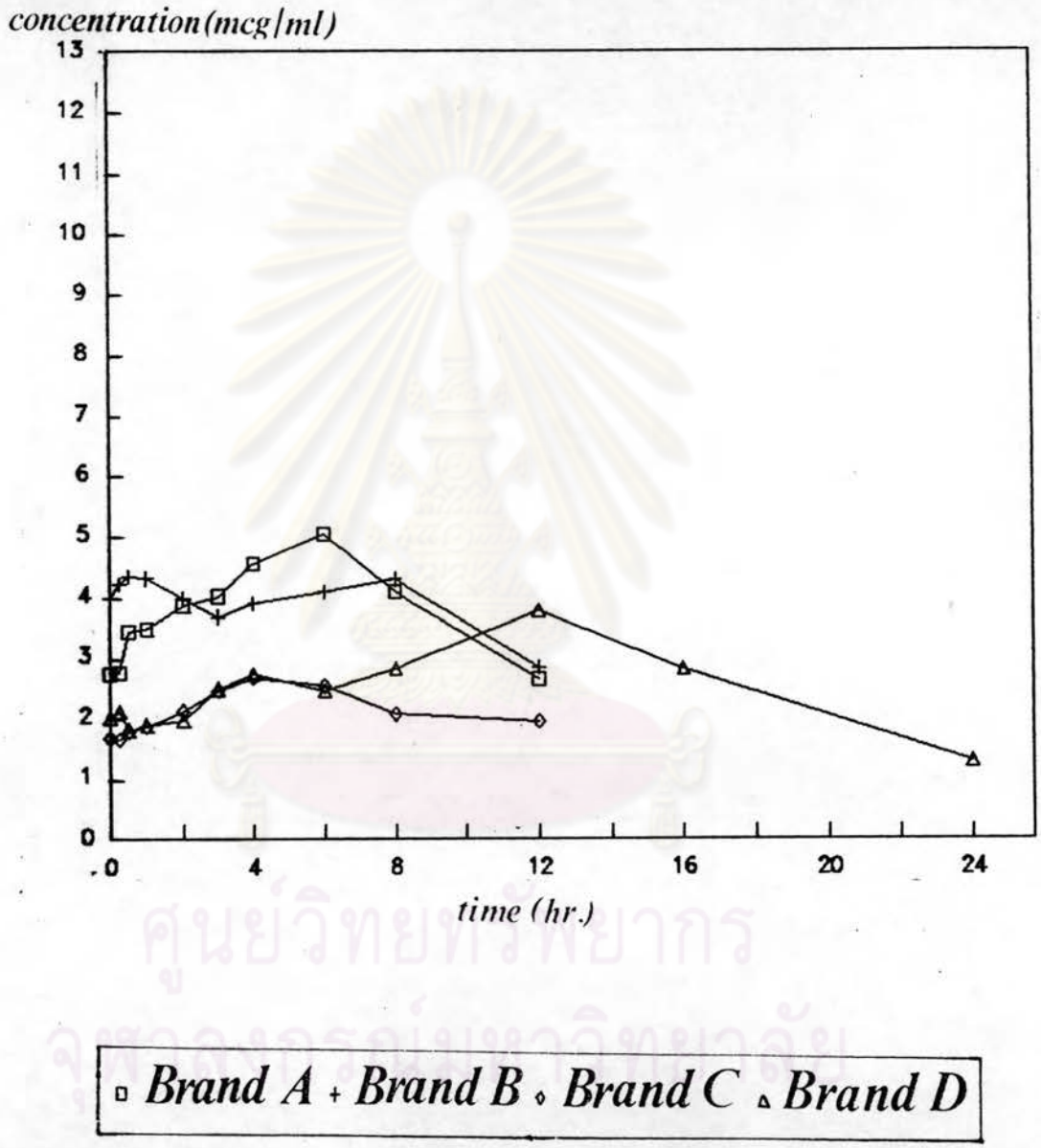


Figure 13 Plasma Theophylline Concentration-Time Profile of Subject No.4 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 5

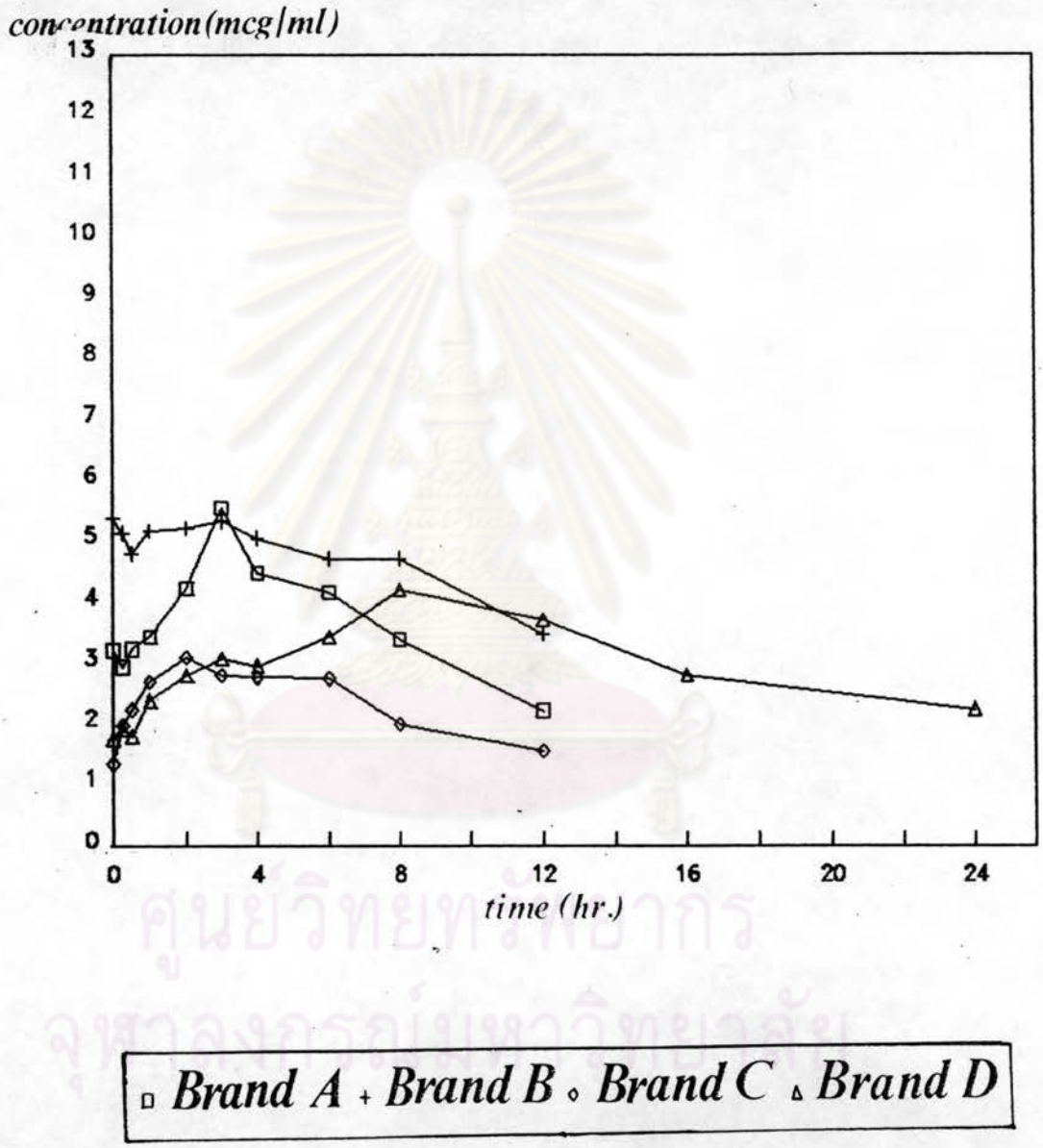


Figure 14 Plasma Theophylline Concentration-Time Profile of Subject No.5 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 6

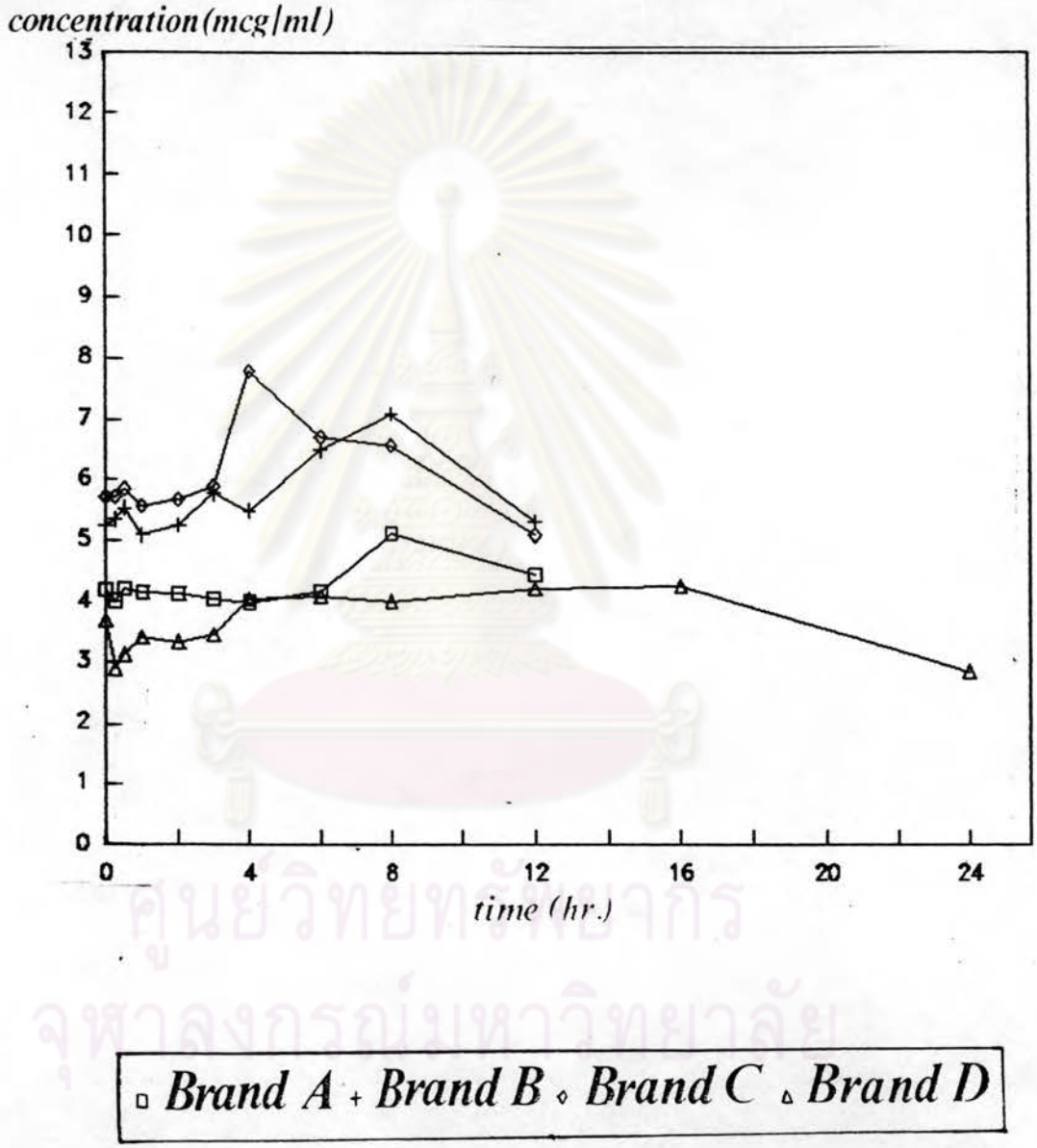


Figure 15 Plasma Theophylline Concentration-Time Profile of Subject No.6 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 7

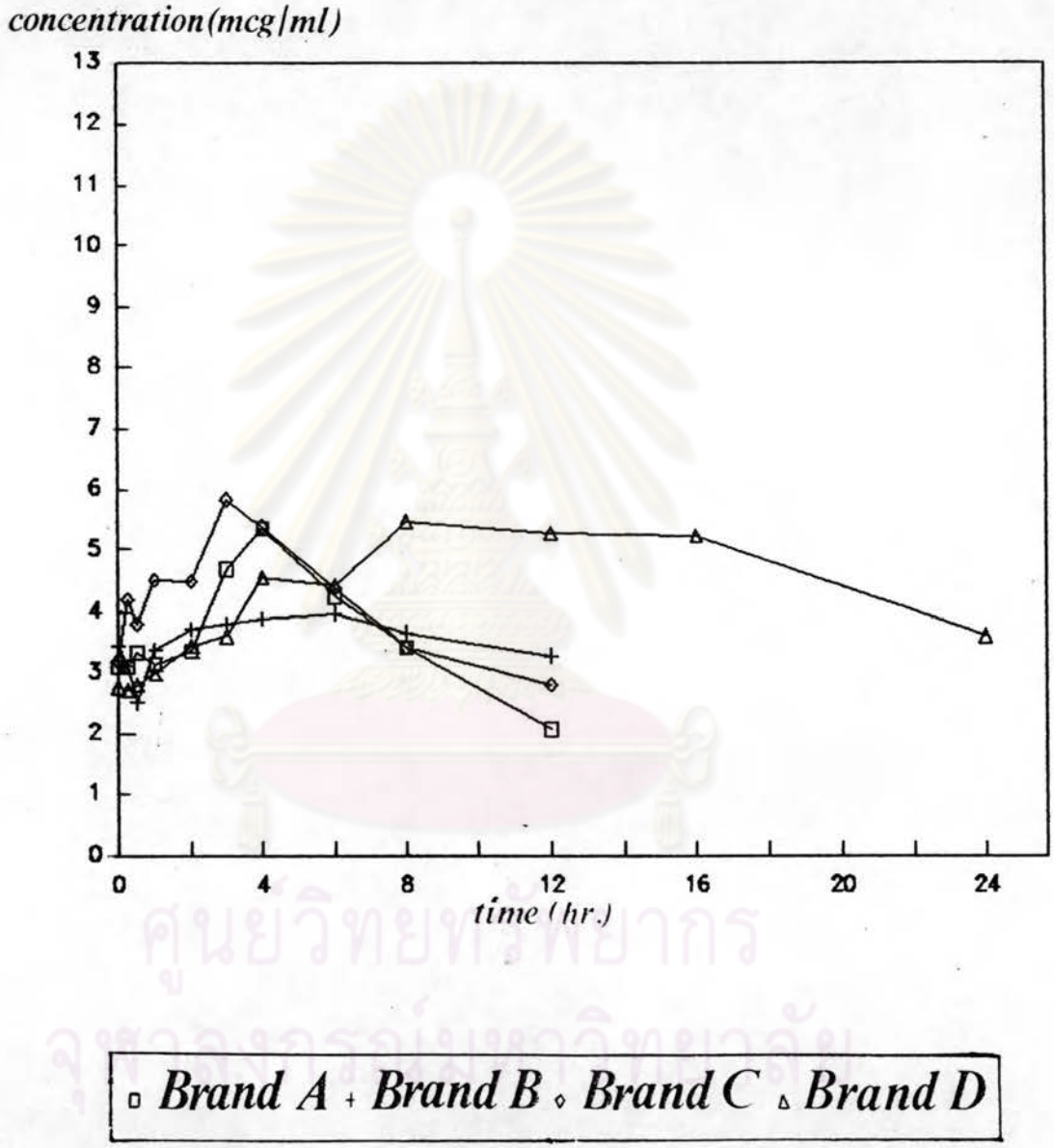


Figure 16 Plasma Theophylline Concentration-Time Profile of Subject No.7 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No.8

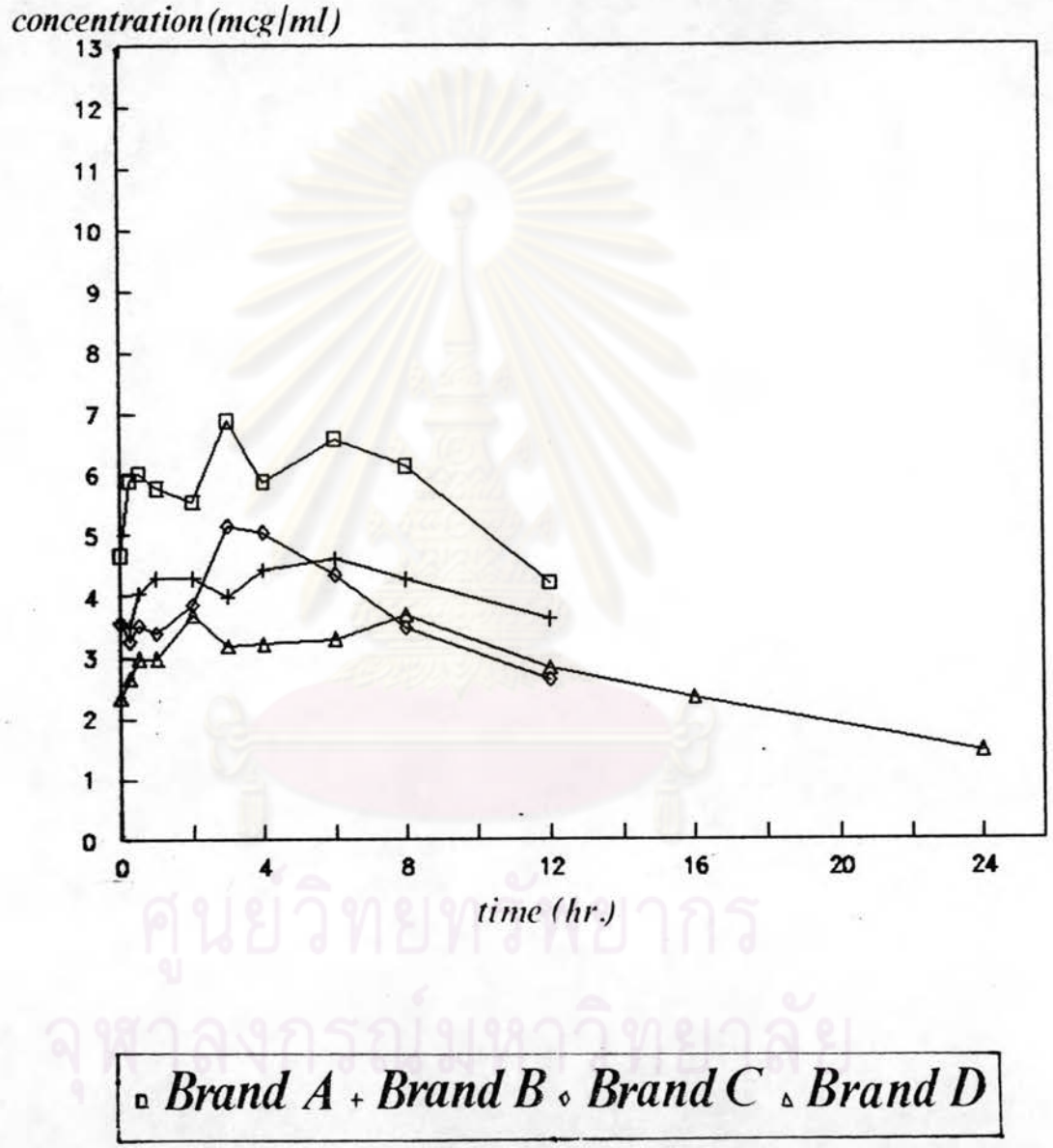


Figure 17 Plasma Theophylline Concentration-Time Profile of Subject No.8 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 9

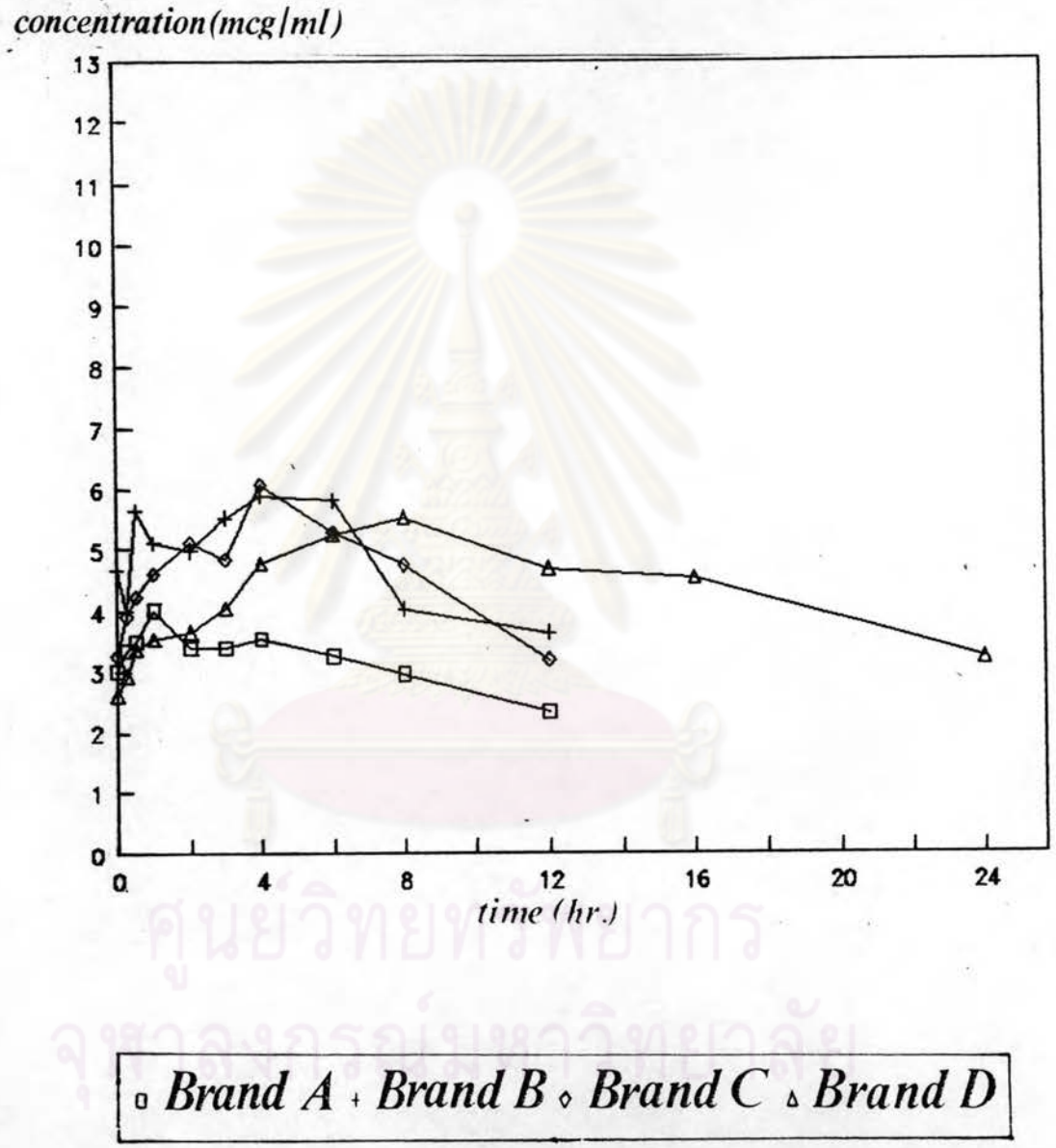


Figure 18 Plasma Theophylline Concentration-Time Profile of Subject No.9 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 10

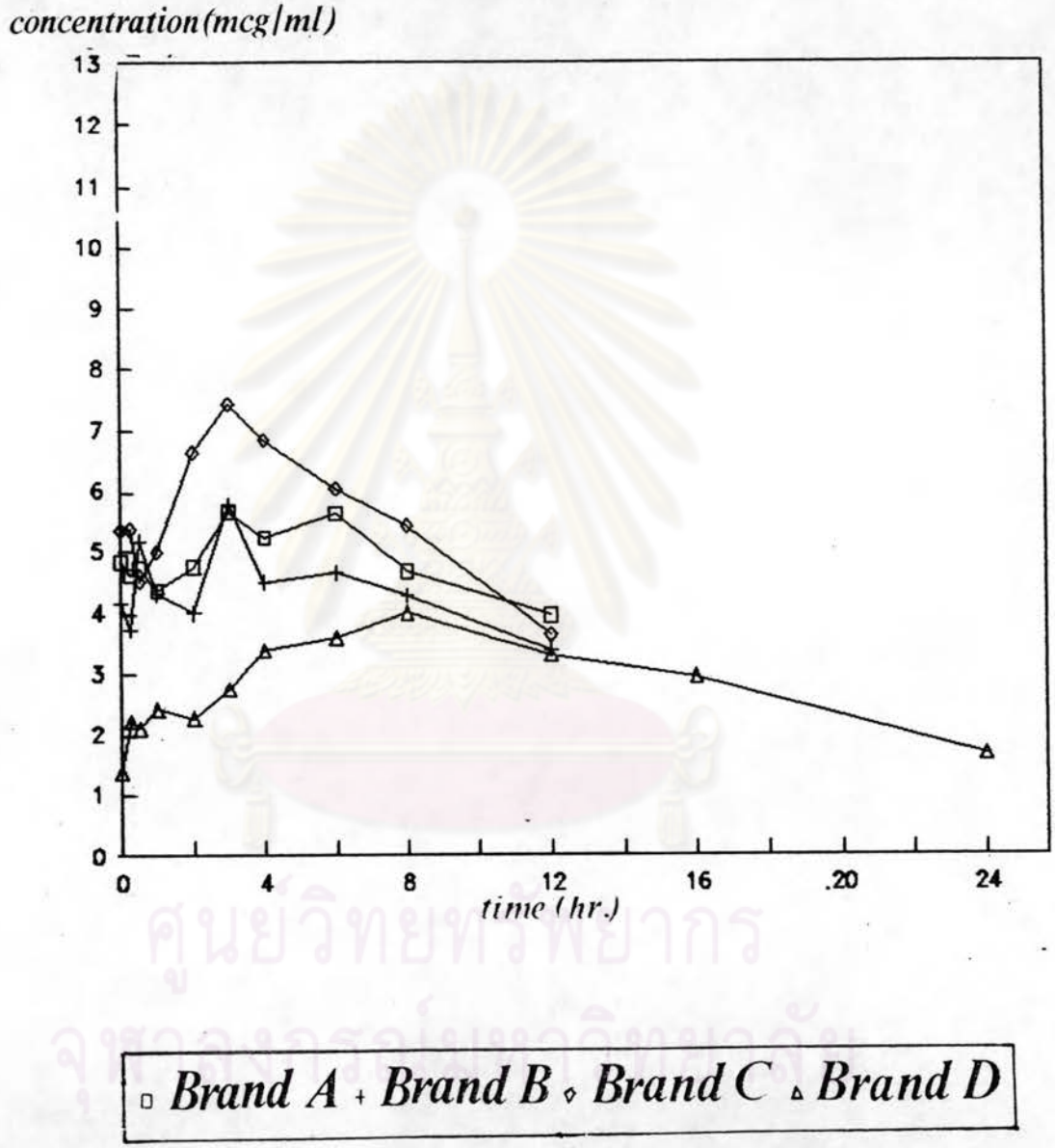


Figure 19 Plasma Theophylline Concentration-Time Profile of Subject No.10 Following Oral Administration of Theophylline Sustained-release Tablets.

PLASMA THEOPHYLLINE CONCENTRATION

Subject No. 11

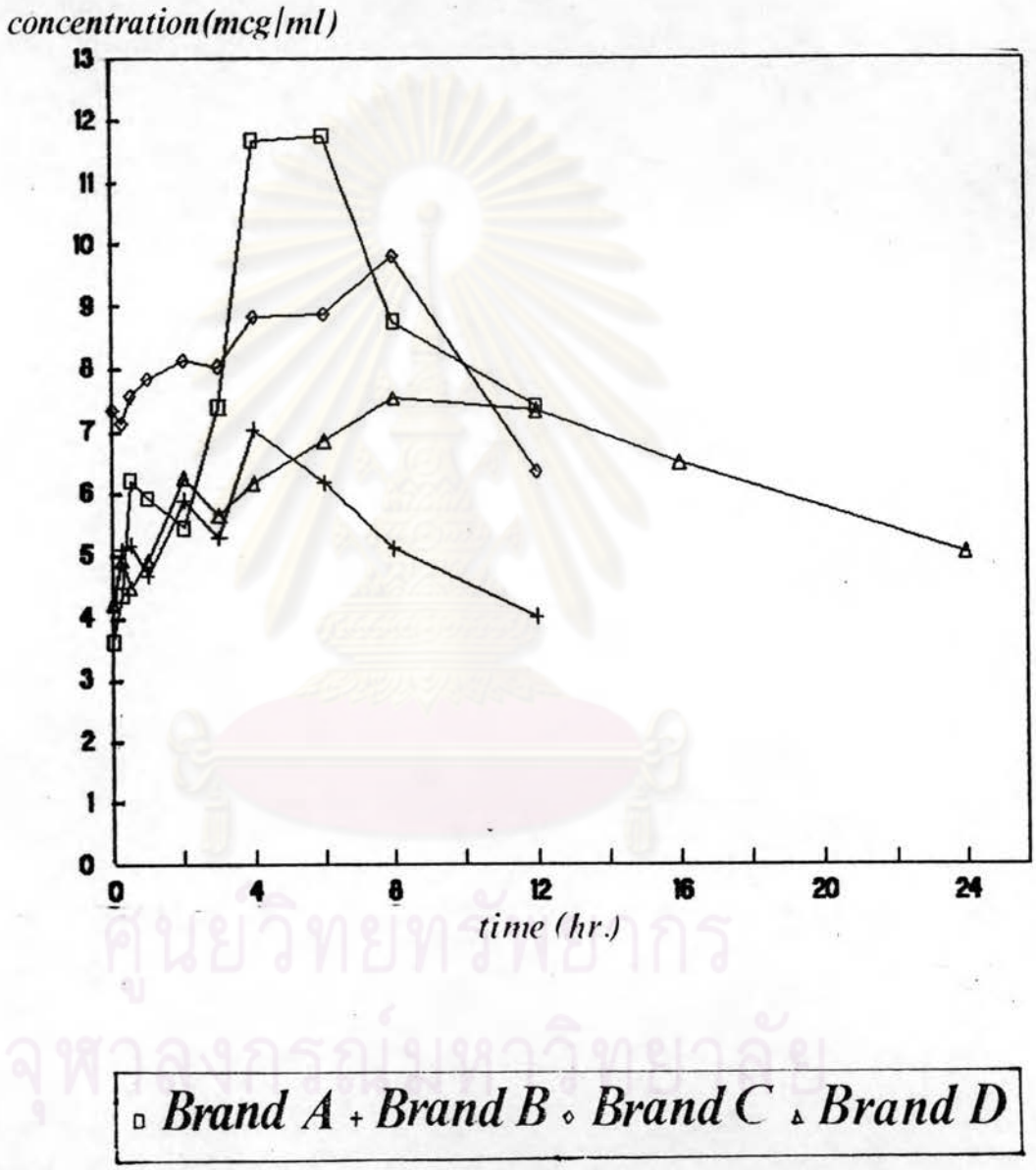


Figure 20 Plasma Theophylline Concentration-Time Profile of Subject No.11 Following Oral Administration of Theophylline Sustained-release Tablets.

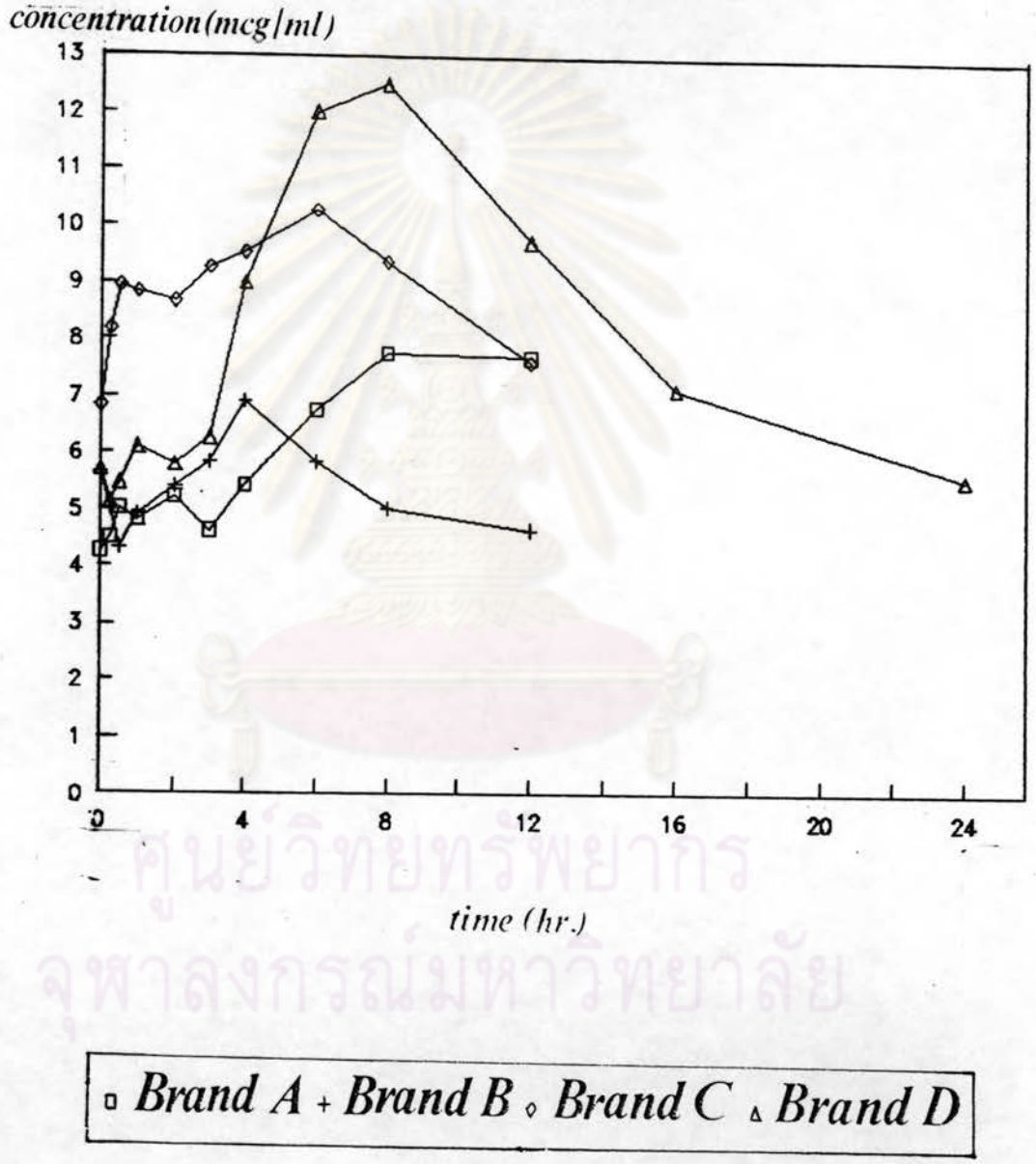


Figure 21 Plasma Theophylline Concentration-Time Profile of Subject No.12 Following Oral Administration of Theophylline Sustained-release Tablets.

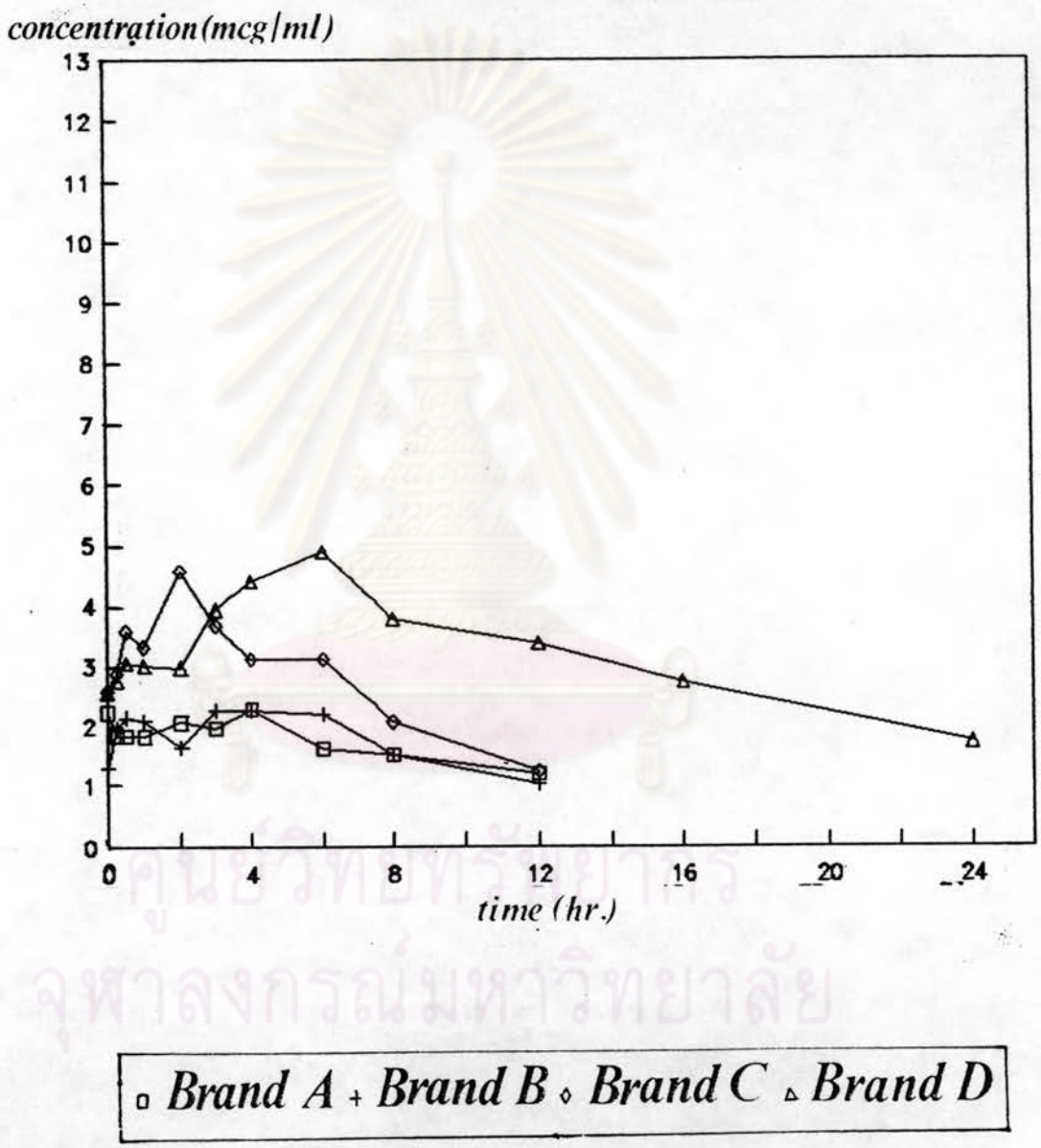


Figure 22 Plasma Theophylline Concentration-Time Profile of Subject No.13 Following Oral Administration of Theophylline Sustained-release Tablets.

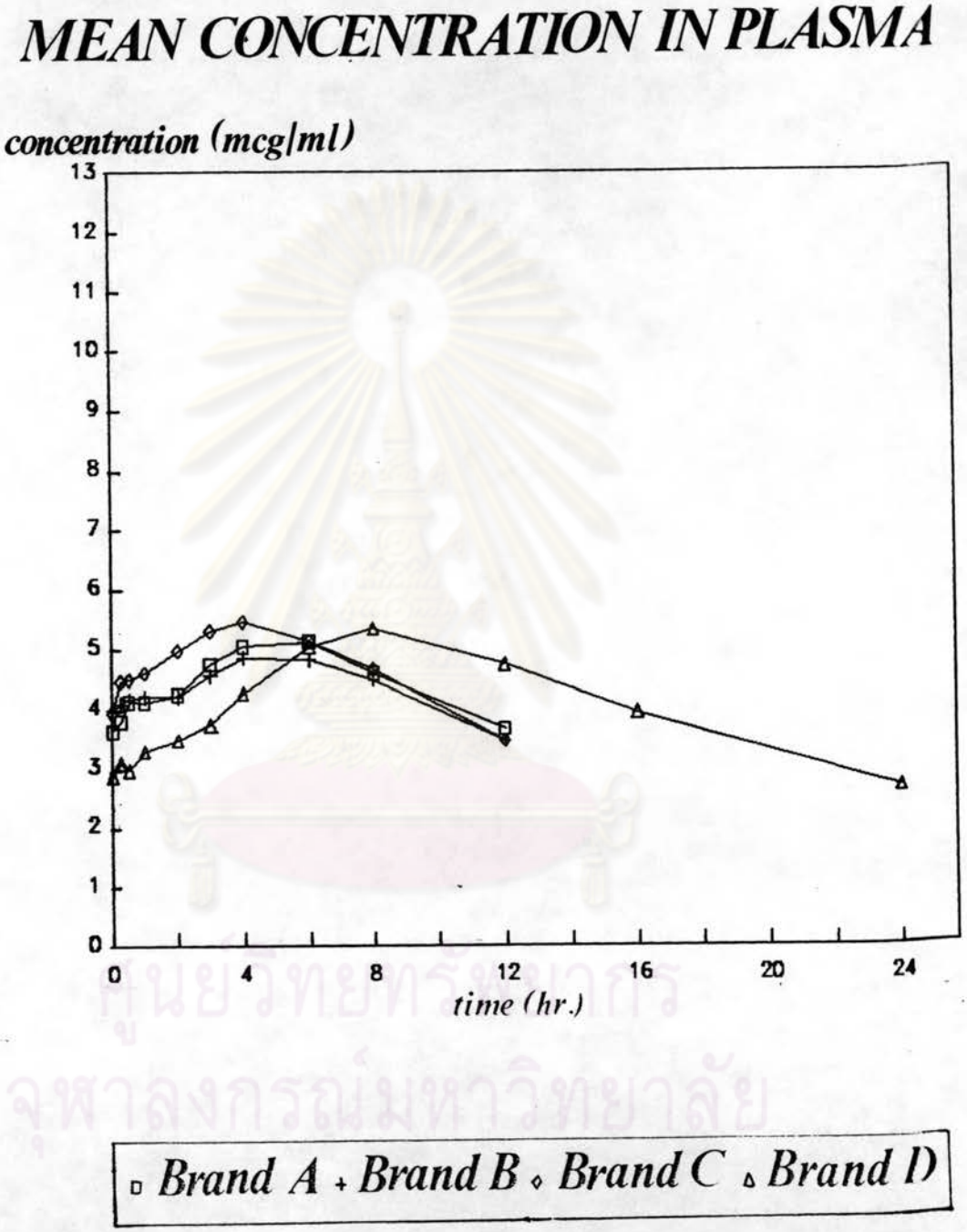


Figure 23 Comparison of Mean Plasma Theophylline Concentration-time Profile from 13 subjects Following Oral Administration of Theophylline Sustained-release Tablets.

range or not. While the AUC value indicated the extent of absorbed drug entering the systemic circulation. They are derived from plasma drug concentration-time data. In the bioequivalence study, drug products that are pharmaceutically equivalent are accepted to be bioequivalent if no statistically significant difference in the rate and the extent of drug absorbtion can be observed (Skelly, 1976; Shargel and Yu, 1980).

The relevant pharmacokinetic parameters obtained for bioavailability comparison are as follows:

3.1 Peak Plasma Concentration (C_{max})

Previous reports indicated that the mean peak plasma concentration achived following oral administration of 7.3 mg/kg theophylline elixir was about 10-20 mcg/ml and of 7.6 mg/kg for theophylline tablets (Weinberger, Hendeles et al.,1981)

In this study, the mean peak plasma theophylline concentration for brands A, B, C, and D after normalized dose to 400 mg per day (200 mg twice a day or 400 mg once a day) were 5.68 $\pm$ 0.64, 5.23 $\pm$ 0.42, 5.99 $\pm$ 0.66 and 5.57 $\pm$ 0.68 mcg/ml, respective, as seen in Table 13. The rank order of these values were C>A>D>B. Tables 14 and 15 showed that there were no statistically significant difference ($p>0.05$) between all of these brands.

This might be said that brands A, B, C,and D could produce the same intensity of action. It indicated that the drug was much absorbed from the dosage form even in the dosage form D that K_d of this brand was lower than those of brand A,B and C.

3.2 Time to Peak Plasma Concentration (t_{max})

The time to peak plasma theophylline concentration of each individual was presented in Table 16. The average times were 4.0 ± 0.65, 4.65 ± 0.58, 3.56 ± 0.52, and 8.92 ± 0.77 hours for brands A,B,C and D, respectively. The rank orders of this value were brands D>B>A>C. There were no statistically significant difference ($p>0.05$) between those of brands B and C or brands B and A while the t_{max} value of brand D was significantly more ($p<0.05$) than that of brand B (Table 17 and 18), refering that brands A and C could produce the same onset of action as brand B while the onset produced by brand D would be longer. Moreover, the study also pointed out that dissolution process of the drug was the rate-limiting step of drug absorption. As seen in Table 3 brand D had K_d statistically lower than others. However, for sustained release product and/or drug which intended to be used to maintain drug level, the onset of the drug id usually considered to be clinically less significant.

3.3 Area Under the Plasma Versus Time Curve (AUC)

The mean $AUC_{0\rightarrow\infty}$ from individual plasma of all brands were 102.48 ± 9.21, 104.34 ± 7.68, 109.60 ± 7.27, and 97.28 ± 10.86 mcg x hr/ml for brands A, B, C and D, respectively as shown in Table 22. The rank order of these values were brand C>B>A>D. This result correlated well with the C_{max} and t_{max} of brands A, B, C and D. According to Tables 23 and 24 there were no statistically significant differences ($p>0.05$)in these brands. This clearly demonstrated that for practically insoluble drug, better formulation could provide more drug absorption into systemic circulation and the appropriate

formulation for sustained-release preparations should be alter only in rate of absorption, not in content.

The principal pharmacokinetic parameters of theophylline following oral administration of four brands were summerized in Table 28. Statistical analysis of these corresponding parameters among the four brands demonstrated that brands A,B and C were bioequivalent to the reference's product (brand B) in terms of both the rate and the extent of drug absorption. The information from Table 28 showed that the C_{max} of brand D was non significantly lower than brand B, whereas % fluctuation and t_{max} were higher than those of brand B, referring that the two brands were bioeinquivalent with respect to the rate of drug absorption into general circulation and the capacity to remain in therapeutic range.

3.4 %fluctuation

The % fluctuation for brands A, B, C, and D were 89.67 $\pm$ 15.99, 58.72 $\pm$ 6.32, 88.36 $\pm$ 12.95 and 155.72 $\pm$ 28.14 mcg/ml, respective, as seen in Table 19. The rank order of these values were D>A>C>B. Tables 20 and 21 showed that there were no statistically significant difference ($p>0.05$) between brands A and B or C and B, but the %fluctuation of brand D was significantly higher ($p<0.05$) than that of the reference's product (brand B).

Thus, it might be concluded that brands A, B, and C could produce the same intensity of action. Brand D had the %fluctuation values more than any other brands produced in all subjects as observed in Figure 9 to 21. It indicated that this brand of higher had chance to be out of therapeutic range. The main factor contributed to this result was probably due to the difference of formulation which is

formulated to be once-a-day preparation and poor dissolution properties of this brand as could be seen in Table 3 that K_d of brands D was lower than those of brand A, B and C.

4. The Relative Bioavailability

Relative bioavailability is the availability of a drug product as compared to a recognized standard (reference's product) (Shargel and Yu, 1980). In this study the mean AUC $_{0 \rightarrow \infty}$ of brand A, C and D was compared to that ofbrand B. The values obtained of brands A, C and D relative to brand B were 98.22%, 105.04% and 93.23%, respectively. This figures suggested that all brands have nearly the same bioavailability.

5. Therapeutic Factor

As seen in Table 9 to 13, peak plasma concentration of all brands in each volunteer was not in therapeutic range 10-20 mcg/ml that was proposed for theophylline (Mitenko, and Ogilvie,1973). In this study, dosage for each subject was 200 mg/day. Thus, to adjust dose reaching therapeutic range, should be increase by multiply factor with C_{max} and C_{min} to be in the range. These factors for each subject are shown in Table 25.

The factor for brand A, B, C and D were 3.46 ± 0.49, 3.69 ± 0.48, 3.58 ± 0.46, and 3.54 ± 0.37, respective, as seen in Table 25,26,27 and 28. From these factor, suitable dosage regimen for each subject can calculate as shown in Table 25,26,27 and 28.

Thus, appropriate dosage for brand A,B,C and D was 200-1600 mg,

400-1600 mg, 300-1400 twice daily and 200-1000 mg once daily, respectively that should be maintain serum theophylline concentrations in therapeutic range as much as they can, for brand A 6 subjects can adjust dose to be in therapeutic range, brand B 9 subjects can adjust dose to be in therapeutic range, brand C 5 subjects can adjust dose to be in therapeutic range , but for brand D only 2 subjects can adjust dose to be in therapeutic range. It is happened from % fluctuation more than 100%.

6. In Vitro-In Vivo Correlation

The correlation studies between the in vitro and in vivo data for brand A, B, C and D were presented in Table 29. Results demonstrated that there were no statistically significant correlation between the two data ($p>0.05$). Therefore, the dissolution rate constant could not be used as the preliminary tool to predict the bioavailability of theophylline sustained-release tablet .

Table 13 Peak Plasma Concentration*(C_{max}) of Theophylline Following Oral Administration of Four Difference Brands of Theophylline Sustained-release Tablets

Subject No.	C_{max} (mcg/ml)			
	Brand A	Brand B	Brand C	Brand D
1	4.76	6.95	5.66	6.00
2	3.48	3.94	3.14	7.04
3	6.32	3.98	6.64	3.72
4	5.02	4.32	2.66	3.76
5	5.56	5.34	3.10	4.20
6	5.12	7.08	7.78	4.24
7	5.36	3.98	5.84	5.48
8	6.86	4.60	5.14	3.68
9	4.00	5.88	6.06	5.52
10	5.62	5.74	7.04	3.96
11	11.76	7.02	9.08	7.52
12	7.72	6.88	10.24	12.44
13	2.28	2.26	3.66	4.88
Mean	5.68	5.23	5.85	5.57
S.E.M.	0.64	0.42	0.65	0.68

* already dose normalized calculation to 400 mg per day

Table 14 Analysis of Variance for Peak Plasma Concentration of Four Difference Brands of Theophylline Sustained-release Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	2.68	0.89	0.19
Within group	48	227.99	4.75	
Total	51	230.67		

F 0.05 (3,48) = 2.60

a = Degree of freedom

b = Sum of Square

c = Mean Square

d = Variance ratio

Table 15 Comparison of Peak Plasma Concentration (C_{max}) of Each Brand of Theophylline Sustained-release Tablets with that of The Reference 's Product (Brand B)

Brand	$\Delta \bar{x}$	Statistical Significance
A	0.45	NS
C	0.62	NS
D	0.34	NS

t (0.05, 48) = 2.0176

LSD (0.05) = $t_{.05} \times S_d$ = 1.22

S = Significant difference (P ‹ 0.05)

NS = Not signficant difference (P › 0.05)

Table 16 Time of Peak Plasma Concentration (t_{max}) of Theophylline Following Oral Administration of Four Difference Brands of Theophylline Sustained-release Tablets

Subject No.	t_{max} (hr.)			
	Brand A	Brand B	Brand C	Brand D
1	3.0	8.0	3.0	6.0
2	1.0	6.0	3.0	8.0
3	2.0	4.0	0.25	12.0
4	6.0	0.5	4.0	12.0
5	3.0	3.0	2.0	8.0
6	8.0	8.0	4.0	16.0
7	4.0	6.0	3.0	8.0
8	3.0	6.0	3.0	8.0
9	1.0	4.0	4.0	8.0
10	3.0	3.0	3.0	8.0
11	6.0	4.0	8.0	8.0
12	8.0	4.0	6.0	8.0
13	4.0	4.0	3.0	6.0
Mean	4.0	4.65	3.56	8.92
S.E.M.	0.65	0.58	0.52	0.77

Table 17 Analysis of Variance for Time to Peak Plasma Concentration of Four Difference Brands of Theophylline Sustained-release Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	237.50	79.17	15.01
Within group	48	253.13	5.274	
Total	51	490.63		

F 0.05 (3,48) = 2.60

a = Degree of freedom

b = Sum of Square

c = Mean Square

d = Variance ratio

Table 18 Comparison of Time to Peak Plasma Concentration (t_{max}) of Each Brand of Theophylline Sustained-release Tablets with that of The Reference 's Product (Brand B)

Brand	$\Delta \bar{x}$	Statistical Significance
A	0.65	NS
C	1.09	NS
D	5.36	S

$t\ (0.05,\ 48) = 2.0176$

$LSD\ (0.05) = t_{.05} \times S_d = 1.285$

S = Significant difference ($P < 0.05$)

NS = Not signficant difference ($P > 0.05$)

Table 19 Percent Fluctuation of Theophylline Following Oral Administration of Four Difference Brands of Theophylline Sustained-release Tablets

Subject No.	% Fluctuation			
	Brand A	Brand B	Brand C	Brand D
1	52.56	66.27	88.67	183.02
2	22.54	27.92	175.44	450.00
3	85.88	68.64	147.76	57.63
4	90.15	53.19	62.20	193.75
5	150.45	53.45	131.34	138.64
6	28.64	39.37	53.15	49.30
7	157.69	57.94	62.50	101.47
8	63.33	32.18	58.64	155.57
9	72.41	47.00	90.57	112.31
10	43.37	55.14	105.56	191.18
11	226.67	75.50	54.57	79.05
12	82.08	69.46	50.59	128.68
13	90.00	117.31	195.16	183.72
Mean	89.67	58.72	98.16	155.72
S.E.M.	15.99	6.32	13.74	28.14

Table 20 Analysis of Variance for Percent Fluctuation of Four Difference Brands of Theophylline Sustained-release Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	253754.52	84584.84	20.39
Within group	48	199097.18	4147.86	
Total	51	452851.52		

F 0.05 (3,48) = 2.60

a = Degree of freedom

b = Sum of Square

c = Mean Square

d = Variance ratio

Table 21 Comparison of Percent Fluctuation of Each Brand of Theophylline Sustained-release Tablets with that of The Reference 's Product (Brand B)

Brand	$\Delta \bar{x}$	Statistical Significance
A	30.95	NS
C	39.44	S
D	97.00	S

t (0.05, 48) = 2.0176

LSD (0.05) = $t_{.05} \times S_d$ = 36.04

S = Significant difference ($P < 0.05$)

NS = Not signficant difference ($P > 0.05$)

Table 22 Area Under the Plasma Contration-Time Curve (AUC) of Theophylline Following Oral Administration of Four Difference Brands of Theophylline Sustained-release Tablets

Subject No.	AUC (mcgxhr/ml)			
	Brand A	Brand B	Brand C	Brand D
1	98.56	136.86	100.94	90.12
2	72.76	84.46	71.86	95.98
3	118.60	79.82	78.62	65.06
4	94.22	92.92	52.66	61.62
5	88.78	112.70	56.50	73.60
6	103.78	143.78	144.26	91.50
7	87.62	27.26	98.72	110.46
8	139.36	100.22	88.38	64.40
9	75.00	116.40	113.16	105.32
10	115.78	103.30	136.00	69.50
11	173.84	129.08	201.40	152.82
12	123.08	127.02	217.06	198.80
13	40.90	42.70	65.16	75.44
Mean	102.48	104.34	109.60	97.28
S.E.M.	9.21	7.68	14.54	10.86

* normalized dose to 400 mg per day (all brands)

Table 23 Analysis of Variance for Area Under the Plasma-Concentration Curve (AUC) of Four Difference Brands of Theophylline Sustained-release Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	1211.15	403.72	0.24
Within group	48	79660.94	1659.60	
Total	51	80872.10		

F 0.05 (3,48) = 2.60

a = Degree of freedom

b = Sum of Square

c = Mean Square

d = Variance ratio

Table 24 Comparison of Area Under the Plasma-Concentration Curve (AUC) of Each Brand of Theophylline Sustained-release Tablets with that of The Reference 's Product (Brand B)

Brand	$\Delta \bar{x}$	Statistical Significance
A	1.86	NS
C	5.26	NS
D	7.06	NS

t (0.05, 48) = 2.0176

LSD (0.05) = $t_{.05} \times S_d$ = 22.80

S = Significant difference ($P < 0.05$)

NS = Not signficant difference ($P > 0.05$)

Table 25 Recommended Dosage Regimen for Brand A

Subject No.	Experimental dose normalized		Calculated dose normalized		
	peak	trough	Factor	peak	trough
1	4.76	3.12	4	19.04	12.48
2	3.48	2.70	5	17.40	13.50
3	6.32	3.40	3	18.96	10.20
4	5.02	2.64	3	15.06	7.92
5	5.56	2.22	3	16.68	6.66
6	5.12	3.98	3	15.36	11.94
7	5.36	2.08	3	16.08	6.24
8	6.86	4.20	2	13.76	8.40
9	4.00	2.32	5	20.00	11.60
10	5.62	3.92	3	16.86	11.76
11	11.76	3.60	1	11.76	3.60
12	7.72	4.24	2	15.44	8.48
13	2.28	1.20	8	18.24	9.60
Mean	5.68	3.05	3.46	16.51	9.41
S.E.M.	0.64	0.26	0.49	0.64	0.80

number of subjects that can be normalized to therapeutic range (10-20 mcg/ml)

= 6

Table 26 Recommended Dosage Regimen for Brand B

Subject No.	Experimental dose normalized		Calculated dose normalized		
	peak	trough	Factor	peak	trough
1	6.95	4.18	2	13.90	8.36
2	3.94	3.08	5	19.70	15.40
3	3.98	2.36	5	19.90	11.80
4	4.32	2.82	4	17.28	11.28
5	5.34	3.48	3	16.02	10.44
6	7.08	5.08	2	14.16	10.16
7	3.98	2.52	5	19.90	12.60
8	4.60	3.48	4	18.40	13.92
9	5.88	3.62	3	17.64	10.86
10	5.74	3.36	3	17.22	10.08
11	7.02	4.00	2	14.04	8.00
12	6.88	4.30	2	13.76	8.60
13	2.26	1.04	8	18.08	8.32
Mean	5.23	3.33	3.69	16.92	10.76
S.E.M.	0.42	0.28	0.48	0.65	0.63

number of subjects that can be normalized to therapeutic range (10-20 mcg/ml)

= 9

Table 27 Recommended Dosage Regimen for Brand C

Subject No.	Experimental dose normalized		Calculated dose normalized		
	peak	trough	Factor	peak	trough
1	5.66	3.00	3.5	19.81	10.50
2	3.14	1.14	5	15.70	5.70
3	6.64	2.68	3	19.92	8.04
4	2.66	1.64	7	18.62	11.48
5	3.10	1.34	6	18.60	8.04
6	7.78	5.08	2	15.56	10.16
7	5.84	2.80	3	17.52	8.40
8	5.40	2.62	3.5	18.90	9.17
9	6.06	3.18	3	18.18	9.54
10	7.04	3.60	2.5	17.60	9.00
11	9.08	6.34	2	18.16	12.68
12	10.24	6.80	1.5	15.36	10.20
13	3.66	1.24	5	18.30	6.20
Mean	5.99	3.19	3.62	18.17	9.16
S.E.M.	0.66	0.52	0.46	0.40	0.54

number of subjects that can be normalized to therapeutic range (10-20 mcg/ml)

= 5

Table 28 Recommended Dosage Regimen for Brand D

Subject No.	Experimental dose normalized		Calculated dose normalized		
	peak	trough	Factor	peak	trough
1	6.00	1.96	3	18.00	5.88
2	7.04	1.28	2	14.08	2.56
3	3.72	2.36	5	18.60	11.80
4	3.76	1.28	5	18.80	6.40
5	4.20	1.76	4	16.80	7.04
6	4.24	3.12	4	16.96	12.48
7	5.48	2.72	3	16.44	8.16
8	3.68	1.44	5	18.40	7.20
9	5.52	2.30	3	16.56	7.08
10	3.96	1.36	5	19.80	6.80
11	7.52	4.20	2	15.04	8.40
12	12.44	5.44	1	12.44	5.44
13	4.88	1.72	4	19.52	6.88
Mean	5.57	2.38	3.54	17.03	7.39
S.E.M.	0.68	0.35	0.37	0.60	0.71

number of subjects that can be normalized to therapeutic range (10-20 mcg/ml)

= 2

Table 29 Estimated Pharmacokinetic Parameters (Mean ± S.E.M.) of Theophylline from Thirteen Subjects Following Oral Administration of Four Different Brands of Theophylline Sustained-release

Parameters	Brand				F-test	t-test* with respect to brand B
	A	B	C	D		
C_{max} (mcg/ml)	5.68±0.64	5.23±0.42	5.85±0.65	5.57±0.68	0.19 (2.60)**	NS
t_{max} (hr.)	4.0±0.65	4.65±0.58	3.56±0.52	8.92±0.77	15.01 (2.60)**	A=B=C<D
AUC (mcg hr/ml)	102.5±9.21	104.3±7.68	109.6±14.5	97.28±10.9	0.24 (2.60)**	NS
% fluctuation	89.67±15.9	58.72±6.32	98.16±13.7	155.7±28.1	20.39 (2.60)**	A=B<C<D

* S =Significant difference at $p < 0.05$, NS = Not significant difference at $p > 0.05$

** F value obtained from the table

Table 30 In Vitro-In Vivo Correlations

Correlation (n=4)	Correlation Coefficient	t value	Statistical Significance
1) Dissolution Rate Constants versus C_{max}	0.003	0.0042	NS
2) Dissolution Rate Constants versus t_{max}	-0.028	-0.040	NS
3) Dissolution Rate Constants versus AUC	0.012	0.017	NS
4) Dissolution Rate Constants versus %fluctuation	-0.0014	-0.0020	NS

t (0.05,2) = 4.30

a = t value obtained from the table

S = Significant difference ($p < 0.05$)

NS = Not Significant difference ($p > 0.05$)