CHAPTER IV

RESULTS AND DISCUSSION

1. In Vitro Studies

The concentrations of roxithromycin in the tablet were determined according to the modified method using high performance liquid chromatography from that of Oliveira, Bergold, and Schanpoval (1996). Typical chromatograms of internal standard (clarithromycin) and roxithromycin are shown in Figure 2. The retention times of internal standard and roxithromycin were 9.55 and 11.63 minutes, respectively. The method was validated for the within-run and between-run precisions. The percent coefficient of variation (%C.V.) in the within-run and between-run precisions were 0.63-2.48% and 0.84-7.51% as shown in Tables 2 and 3, respectively. The accuracy was evaluated by calculating the percent recovery of roxithromycin. Results as shown in Table 4, the percent recovery of roxithromycin was in the range of 99.34 to 100.39%.

All three commercial brands of 150 mg roxithromycin tablets were first tested for uniformity of dosage units and content of active ingredient. Each of these three brands met the United States Pharmacopoeia 1995 (USP XXIII) for content uniformity within the range of 85.0-115.0% of the labeled claim and the percent coefficient of variation (%C.V.) was less than 6.0%. All products were assayed for content of active ingredient and found that each brand was within the limits of 90.0-110.0 percent labeled amount. The percent labeled amount (%L.A.) of the all products were closed to upper limits of the USP XXIII requirements. The reason might be the manufacturer would like to keep longer of the shelf-life and antibacterial activity of drug. The disintegration testing of all products revealed that all products were able to disintegrate within 30 minutes. Brand A showed fastest disintegration followed by brands B and C, respectively. Results of all test were summarized in Table 5.

The dissolution testing of roxithromycin tablets was a crucial factor for systemic drug availability because the drug was practically insoluble in water. Since

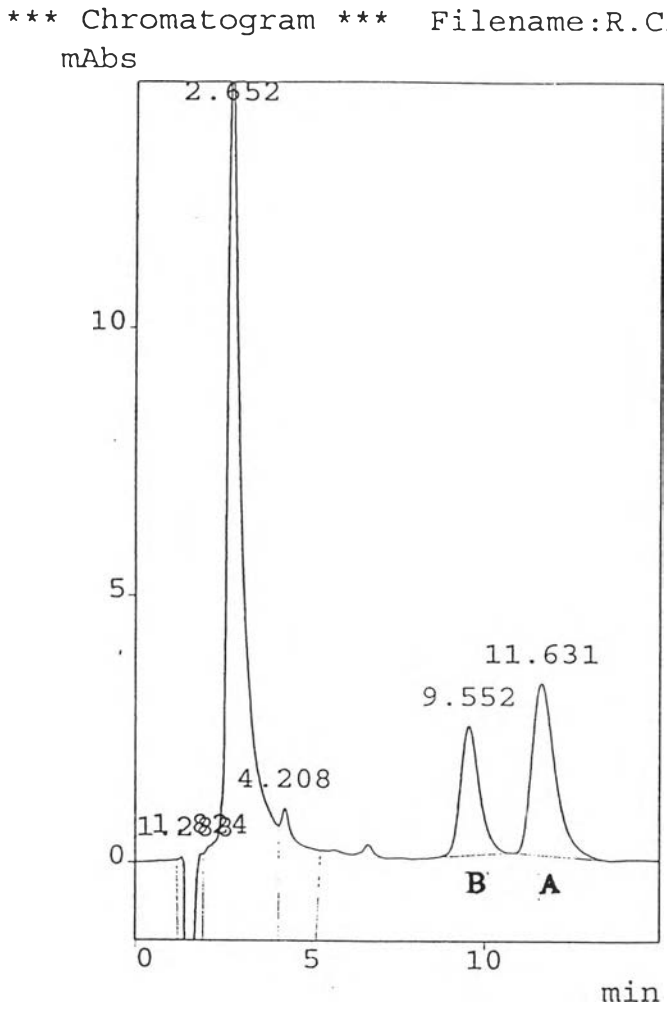

Figure 2 High Performance Liquid Chromatography of Roxithromycin (A) and Internal Standard (Clarithromycin:B) for In Vitro Studies

Table 2 Within-Run Precision of Roxithromycin for In Vitro Studies.

Concentration (μg/mL)	Peak height ratio			Mean±S.D.	% C.V.
	1	2	3		
10	0.2528	0.2436	0.2518	0.2494±0.0050	2.02
20	0.5022	0.4810	0.4815	0.4882±0.0121	2.48
40	0.9722	0.9930	0.9838	0.9830±0.0104	1.06
50	1.2560	1.2709	1.2569	1.2613±0.0084	0.67
60	1.4904	1.4771	1.4725	1.4800±0.0093	0.63
80	2.0082	2.0112	1.9834	2.0009±0.0152	0.76
100	2.5718	2.6746	2.6242	2.6235±0.0514	1.96

n = 3

Table 3 Between-Run Precision of Roxithromycin for In Vitro Studies.

Concentration (μg/mL)	Peak height ratio			Mean±S.D.	% C.V.
	1	2	3		
10	0.2825	0.2510	0.2898	0.2744±0.0206	7.51
20	0.5234	0.5748	0.5406	0.5463±0.0262	4.79
40	1.0032	1.1001	1.0610	1.0548±0.0487	4.62
50	1.2911	1.3099	1.3103	1.3038±0.0110	0.84
60	1.5491	1.5859	1.5456	1.5602±0.0223	1.43
80	2.0180	2.0395	2.0904	2.0493±0.0372	1.81
100	2.6235	2.5619	2.5903	2.5919±0.00308	1.19

n = 3

Table 4 Percent Recovery of Roxithromycin for In Vitro Studies Estimated Using Linear Regression[1]

Standard No.	Concentration (μg/mL)	Peak height ratio	Inversely estimated concentration[2] (μg/mL)	% Recovery[3]
1	10.00	0.2517	9.9336	99.34
2	20.00	0.4788	19.9823	99.91
3	40.00	0.9347	40.1549	100.39
4	50.00	1.1564	49.9646	99.93
5	60.00	1.3846	60.0619	100.10
6	80.00	1.8369	80.0752	100.09
7	100.00	2.2864	99.9646	99.96

Mean	99.96
S.D.	0.32
% C.V.[4]	0.32

1. $r^2 = 0.9999$, $y = 0.0226x + 0.0272$
2. Inversely estimated concentration $= \frac{\text{peak height ratio} - 0.0272}{0.0226}$
3. % Recovery $= \frac{\text{Inversely estimated concentration} \times 100}{\text{Known Concentration}}$
4. % C.V. $= \frac{\text{S.D.} \times 100}{\text{Mean}}$

Table 5 In Vitro Studies of Three Brands of 150 mg Roxithromycin Tablets

Brand	Content uniformity[a] range (%)	% L.A.	Content of active ingredient[b](% L.A.)	Disintegration time[c](min)
A	108.39-114.84	110.55±1.89	105.85±1.98	1.72±0.18
B	101.41-113.23	107.56±4.26	109.93±1.22	2.10±0.12
C	107.47-112.97	110.22±1.90	107.35±0.95	2.64±0.39

All values are presented as mean ± S.D.

a, n = 10

b, n = 3

c, n = 6

the method of dissolution testing for roxithromycin was not described elsewhere, the new procedure was tried out. The United States Pharmacopoeia paddle method using phosphate buffer pH 7.4±0.1, 900 mL was found to be the most reasonable mean.

Table 6 and Figure 3 illustrated the dissolution profiles at various sampling times of all products of roxithromycin tablets. The mean percent dissolved of roxithromycin from all brands were greater than 80 percent at 45 minutes. The dissolution rate constants (K_d) were calculated from the slope of the first order plot between the amount of undissolved roxithromycin (B_α-B_t) versus time in semi-logarithmic scale and results were reported in Table 7. The rank order of these dissolution rate constants for all brands were brands A>B>C. All sampling times, the mean percent dissolved of roxithromycin from brand A was higher than brands B and C (Table 6 and Figure 3), it might be due to the difference in formulation and manufacturing process as well as the contribution of disintegration results as seen by the two tests were highly correlated. Statistical comparison, as presented in Tables 8 and 9, indicated that with respect to brand A, dissolution rate constant of brand B was not statistically significant difference ($p>0.05$) while the value of brand C was statistically lower ($p<0.05$).

2. In Vivo Studies

2.1 Analysis of Roxithromycin in Plasma

Plasma Roxithromycin concentrations were analyzed by microbiological agar diffusion assay. In this study, the procedure of Campa et al. (1990) and Barry (1986) was modified for analyzing roxithromycin concentrations in plasma samples. The method involved precipitation of proteins from plasma and the drug was extracted using absolute ethanol.

The assay method was validated by determining the within-run and between-run precisions as well as accuracy. The percent coefficient of variation (%C.V.) in the within-run and between-run precisions were 0.22-1.63 % and 0.76-2.98 % as shown in Tables 10 and 11, respectively. The percent recovery for accuracy

Table 6 Dissolution Profiles of Three Brands of 150 mg Roxithromycin Tablets in Phosphate Buffer Solution (pH 7.4±0.1)

Time	% Drug Dissolved		
(min.)	Brand A	Brand B	Brand C
5	51.58±4.08	37.96±6.38	22.68±4.16
10	72.98±3.42	49.60±6.14	54.16±2.25
15	85.41±3.05	62.24±2.76	64.45±1.98
20	93.10±3.11	69.90±2.02	71.38±1.47
30	102.47±3.57	79.14±1.58	79.42±1.15
45	109.93±2.88	88.93±2.30	86.61±1.57
60	113.11±3.31	93.72±3.62	90.47±1.33
90	115.75±2.57	99.90±3.34	96.41±1.18
120	116.30±2.95	103.12±3.02	100.10±0.87
150	117.88±2.04	103.92±4.81	102.01±0.99
180	117.30±2.37	104.79±4.17	104.60±1.17

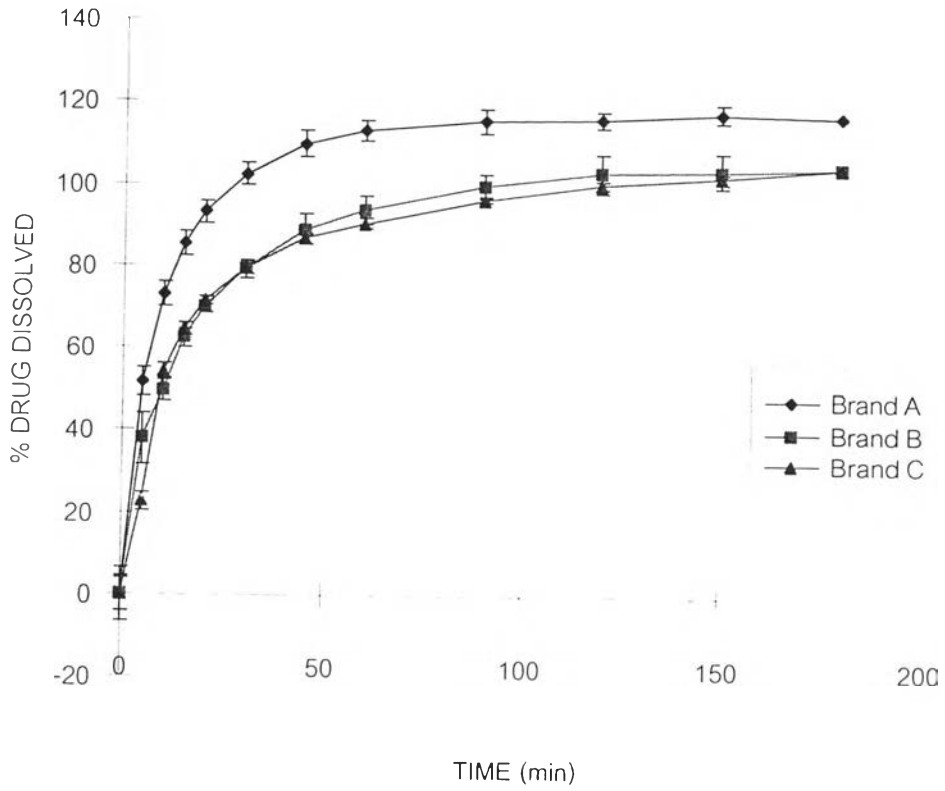

Figure 3 Dissolution Profiles of Three Brands of 150 mg Roxithromycin Tablets in Phosphate Buffer Solution (pH 7.4±0.1)

Table 7 Dissolution Rate Constant(K_d) of Three Brands of 150 mg Roxithromycin Tablets in Phosphate Buffer Solution (pH 7.4±0.1)

Tablet No.	Dissolution rate constant (K_d) (hr^{-1})		
	Brand A	Brand B	Brand C
1	1.57	1.47	1.30
2	1.48	1.68	1.26
3	1.96	1.61	1.43
4	1.90	1.84	1.12
5	2.33	1.69	1.42
6	1.84	2.06	1.31
Mean	1.85	1.73	1.31
S.D.	0.31	0.20	0.12

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Table 8 Analysis of Variance for Dissolution Rate Constant of Three Commercial Brands of 150 mg Roxithromycin Tablets in Phosphate Buffer Solution (pH 7.4±0.1)

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	0.9728	0.4864	9.92
Within group	15	0.7352	0.0490	
Total	17	1.7080		

F^{e} 0.05(2, 15) = 3.68

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 9 Comparison of Dissolution Rate Constant of Each Brand of 150 mg Roxithromycin Tablet with that of the Innovator's Product (Brand A)

Brand	$\bar{x}_1 - \bar{x}_2$[a]	L.S.D[b]. (calculated)	Statistical significant
B	0.12	0.2012	NS
C	0.54	0.2012	S

$t^c (0.05, 15) = 2.131$

S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

a = Difference of the two means

b = Least Significant Difference

c = t value obtained from the table

Table 10 Within-Run Precision of Roxithromycin for In Vivo Studies

Concentration	The mean diameter of inhibition zone (mm)			Mean±S.D.	%C.V.
(μg/mL)	1	2	3		
0.50	15.42	15.93	15.70	15.68±0.26	1.63
1.00	18.13	18.43	18.27	18.28±0.15	0.82
3.00	21.30	22.23	22.23	21.92±0.54	2.45
5.00	23.40	23.00	23.13	23.18±0.20	0.88
7.00	24.28	24.20	24.30	24.26±0.05	0.22
10.00	25.83	25.77	25.60	25.73±0.12	0.46

n = 3

Table 11 Between-Run Precision of Roxithromycin for In Vivo Studies

Concentration	The mean diameter of inhibition zone (mm)			Mean±S.D.	%C.V.
(μg/mL)	1	2	3		
0.50	15.90	15.67	15.73	15.77±0.12	0.76
1.00	18.53	18.00	18.13	18.22±0.28	1.54
3.00	22.40	21.83	21.10	21.78±0.65	2.98
5.00	23.70	23.43	23.20	23.44±0.25	1.07
7.00	25.17	24.13	24.20	24.50±0.58	2.36
10.00	26.73	25.37	25.73	25.94±0.70	2.70

n = 3

Table 12 Percent Recovery of Roxithromycin for In Vivo Studies Estimated Using Linear Regression[1]

Standard No.	Concentration (μg/ml)	Logarithm of concentration	Inhibition zone diameter (mm)	Inversely estimated concentration[2] (μg/ml)	% Recovery[3]
1	0.50	-0.3010	15.74	0.4957	99.14
2	1.00	0	18.13	1.0171	101.71
3	3.00	0.4771	21.81	3.0761	102.54
4	5.00	0.6990	23.22	4.7000	94.00
5	7.00	0.8451	24.48	6.8659	98.08
6	10.00	1.0000	25.85	10.3657	103.66

Mean	99.86
S.D.	3.55
% C.V.[4]	3.56

1. r^2 = 0.9990, log y = 0.1306x - 2.3604
   : x = Inhibition zone diameter, y = plasma roxithromycin concentration
2. Inversely estimated concentration
   = antilog [(Inhibition zone diameter)*0.1306 - 2.3604]
3. % Recovery = $\frac{\text{Inversely estimated concentration}}{\text{Known Concentration}} \times 100$
4. % C.V. = $\frac{\text{S.D.}}{\text{Mean}} \times 100$

was 99.86 as report in Table 12. The calibration curve of the inhibition zone diameters versus the logarithm of plasma concentration of roxithromycin was linear up to 10 μg/mL (Appendix B, Table 32 and Figure 19).

2.2 Plasma Roxithromycin Concentrations

The plasma concentration of roxithromycin at each sampling time interval ranging from 0 to 24 hours after oral administration of 150 mg roxithromycin tablet of brands A,B and C are shown in Tables 13 to 15, respectively. Individual plasma roxithromycin concentration-time profile for each of twelve subjects were shown graphically from Figures 4 to 15. Comparison of the mean plasma roxithromycin concentration profile of each brand from twelve subjects were illustrated in Figure 16.

The plasma roxithromycin concentration in some subject of all three brands showed irregular absorption phase as seen by occurring two or three peak concentrations. This might be explained that roxithromycin has enterohepatic cycling affecting the reabsorption of drug which was repeated until it was totally eliminated. The mean plasma roxithromycin concentration of brands A and B showed more than one peak concentration while brand C did not. This might be due to the intersubject variations.

3. Bioavailability Evaluation

The pharmacokinetic parameters, C_{max}, t_{max} and AUC are used to characterize the bioavailability of pharmaceutical formulation after administration. The parameters, C_{max} and t_{max} represented the rate of drug reaching the systemic circulation 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 absorption can be observed (Shargel and Yu, 1980) as well as the ratios of individual parameter of the test products relatively to the reference product were

Table 13 Plasma Roxithromycin Concentration (μg/mL) from 12 Subjects Following Oral Administration of 150 mg Roxithromycin Tablets of Brand A

Subject No.	Time (hr.)										
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	12.0	24.0
1	3.4205	4.5813	5.8444	3.8321	4.7325	2.9556	2.5127	1.7298	1.3781	0.6638	0.4876
2	2.8583	3.4310	4.5583	3.1636	3.7974	2.2865	2.8009	1.3768	0.7800	0.3913	0.2217
3	2.7536	3.9676	4.7624	3.5848	2.3890	2.8100	2.1584	1.5288	0.9586	0.7826	0.6012
4	6.8745	8.7698	4.5075	4.0892	3.4205	3.8321	3.3654	2.6813	1.8161	0.9961	0.6531
5	5.2608	5.8417	5.6200	4.9608	4.8604	4.2638	3.6905	2.5124	1.9231	1.2618	0.5807
6	7.5001	4.7817	6.6511	4.8365	6.3072	5.7475	6.0609	4.5864	3.5640	3.1210	0.9081
7	4.3734	3.5320	4.2608	4.4166	3.6698	2.9436	3.4934	1.7769	1.9892	1.7419	0.8100
8	4.9012	8.4463	6.0609	6.5634	5.2375	4.8365	4.1243	2.9206	2.8065	1.4452	0.5200
9	3.6095	4.9680	4.6201	4.3594	3.2137	5.5799	3.4557	2.6609	1.9054	1.0974	0.4526
10	2.8351	3.4241	3.5776	4.6449	4.7818	4.6449	3.0931	2.2150	1.6092	1.1524	0.7564
11	5.9136	8.6260	9.2755	8.5016	8.5016	6.8377	4.2966	3.2137	2.8200	1.6007	0.7202
12	5.1144	4.0541	5.2651	4.7562	7.0392	5.1144	4.1292	4.0541	2.3349	1.6007	0.6139
Mean	4.6179	5.3686	5.4170	4.8091	4.8292	4.3210	3.5984	2.6047	1.9904	1.3212	0.6105
S.E.M.	0.4618	0.6012	0.4306	0.4161	0.5058	0.4029	0.2935	0.2862	0.2312	0.2018	0.0526

Concentrations in all subjects = 0 at t = 0

Table 14 Plasma Roxithromycin Concentration (μg/mL) from 12 Subjects Following Oral Administration of 150 mg Roxithromycin Tablets of Brand B

Subject No.	Time (hr.)										
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	12.0	24.0
1	4.8365	8.2250	4.8365	4.9012	4.7098	5.5969	5.3784	4.0700	2.2695	1.5650	0.5783
2	4.0700	6.7400	6.2240	4.5864	6.2240	5.1684	3.7088	2.6614	1.9098	1.1532	0.4931
3	5.9810	6.1400	4.4074	4.1794	5.8243	4.9667	4.4663	2.9596	2.2695	1.4645	0.4931
4	5.8076	5.4346	7.4951	7.4951	8.1165	8.0095	7.3963	6.1419	3.9111	3.1210	0.8386
5	5.2398	6.0585	4.6652	3.5922	3.0178	3.1982	4.8026	2.6099	1.5031	0.8169	0.4986
6	3.6095	4.3594	4.8964	5.5033	5.3421	4.9680	4.9680	2.9886	2.1092	1.7464	0.9770
7	1.9054	3.6095	5.1144	4.4878	3.8254	3.6095	3.7159	1.9333	1.6241	1.1297	0.5709
8	1.5776	4.8258	3.9957	3.2137	3.0767	2.0788	1.7464	1.2874	1.0974	1.0205	0.4526
9	1.9439	4.2891	6.5679	4.6517	5.3617	4.4667	3.5014	1.8291	1.3220	0.6115	0.3913
10	4.6925	5.2542	5.3783	4.3683	4.3283	4.2500	3.8083	2.6742	1.8042	1.2525	0.5250
11	4.3770	6.5679	7.8838	4.4667	2.8583	3.0377	2.6355	2.5307	1.9049	1.0792	0.3395
12	3.3621	5.0450	6.3067	3.9547	3.2946	2.9767	2.5307	2.1083	1.1945	0.8123	0.4158
Mean	3.9502	5.5458	5.6476	4.6167	4.6650	4.3606	4.0549	2.8162	1.9099	1.3144	0.5478
S.E.M.	0.4363	0.3683	0.3576	0.3130	0.4597	0.4532	0.4335	0.3639	0.2149	0.1896	0.0533

Concentrations in all subjects = 0 at t = 0

Table 15 Plasma Roxithromycin Concentration (μg/mL) from 12 Subjects Following Oral Administration of 150 mg Roxithromycin Tablets of Brand C

Subject No.	Time (hr.)										
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	12.0	24.0
1	2.9886	4.6201	4.2346	4.2346	3.6623	3.1673	3.1217	2.0193	1.2506	0.7858	0.3858
2	6.5142	5.0160	5.7162	5.6337	4.7328	4.1532	4.0341	2.8473	2.5719	1.8417	1.1079
3	2.9886	3.4557	3.7702	5.0406	4.2346	3.5062	2.7393	1.9902	1.5776	1.2148	0.4796
4	1.8779	6.4519	8.2583	7.2466	5.9136	4.0541	3.2137	2.3690	1.5104	1.0355	0.4592
5	3.1842	4.8775	5.4233	5.1667	4.5700	3.9733	3.6583	2.8108	1.8925	1.3708	0.5675
6	4.2894	6.3070	8.2109	7.1237	5.6986	4.5586	5.8154	5.0454	3.0379	1.6195	0.7961
7	3.1000	3.3621	4.1185	3.9547	3.7211	3.3621	2.8009	1.9838	1.2955	0.7960	0.3913
8	3.7974	6.3067	8.0454	6.1800	6.0558	3.5731	3.1000	1.9838	1.0155	0.6906	0.3682
9	3.5170	5.3075	4.2919	3.2049	2.8440	3.3351	2.9596	2.0408	1.7403	0.9576	0.4494
10	2.1460	3.6982	3.4606	5.0856	4.3368	4.2778	4.1125	3.5069	2.3238	1.9298	0.4788
11	0.8386	2.6263	4.5260	4.4074	5.4503	4.1795	4.1795	2.7330	2.2100	1.7174	0.7247
12	6.9213	9.0259	8.0095	6.1449	6.3072	5.0330	4.4663	3.9633	2.4576	2.4576	0.9450
Mean	3.5136	5.0879	5.6721	5.2853	4.7939	3.9311	3.6834	2.7745	1.9070	1.3681	0.5961
S.E.M.	0.5069	0.5103	0.5546	0.3590	0.3165	0.1613	0.2583	0.2790	0.1782	0.1593	0.0701

Concentrations in all subjects = 0 at t = 0

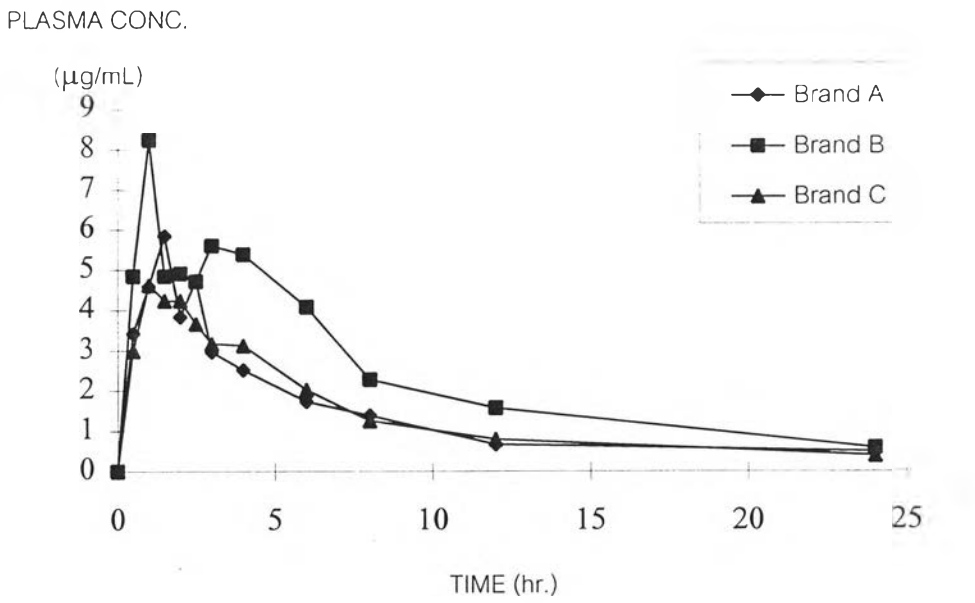

Figure 4 Plasma Roxithromycin Concentration-time Profile of Subject No.1 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

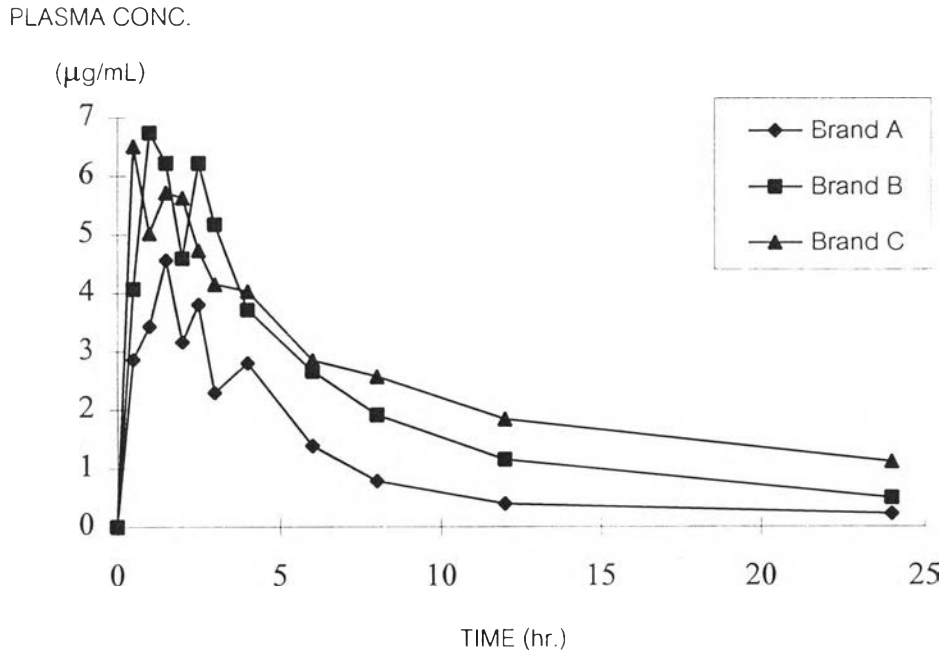

Figure 5 Plasma Roxithromycin Concentration-time Profile of Subject No.2 Following Oral Administration of Three Brands of 150 mg Roxithromycin tablets

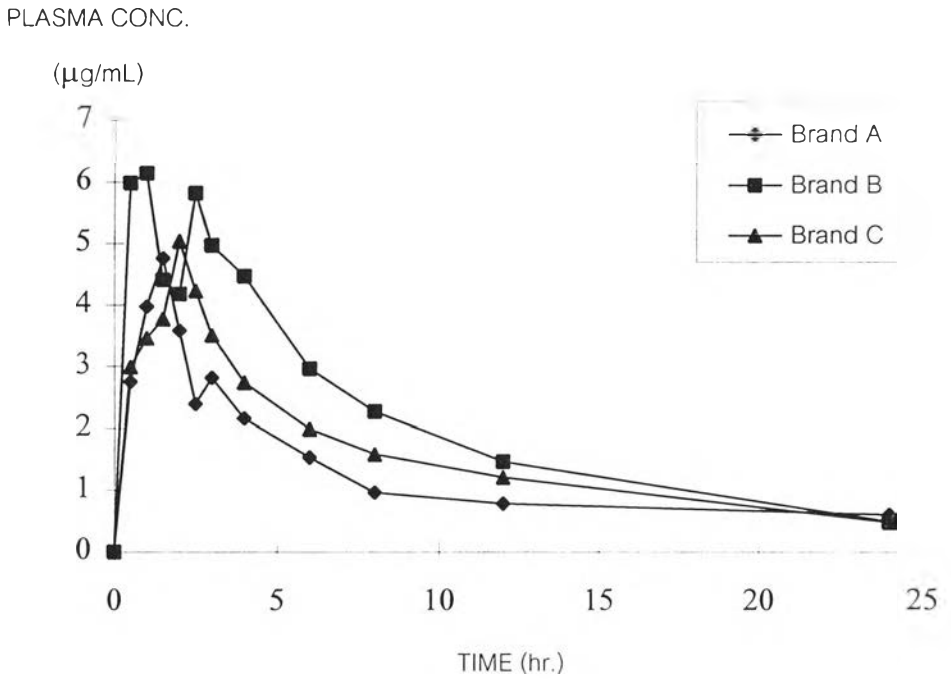

Figure 6 Plasma Roxithromycin Concentration-time Profile of Subject No.3 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

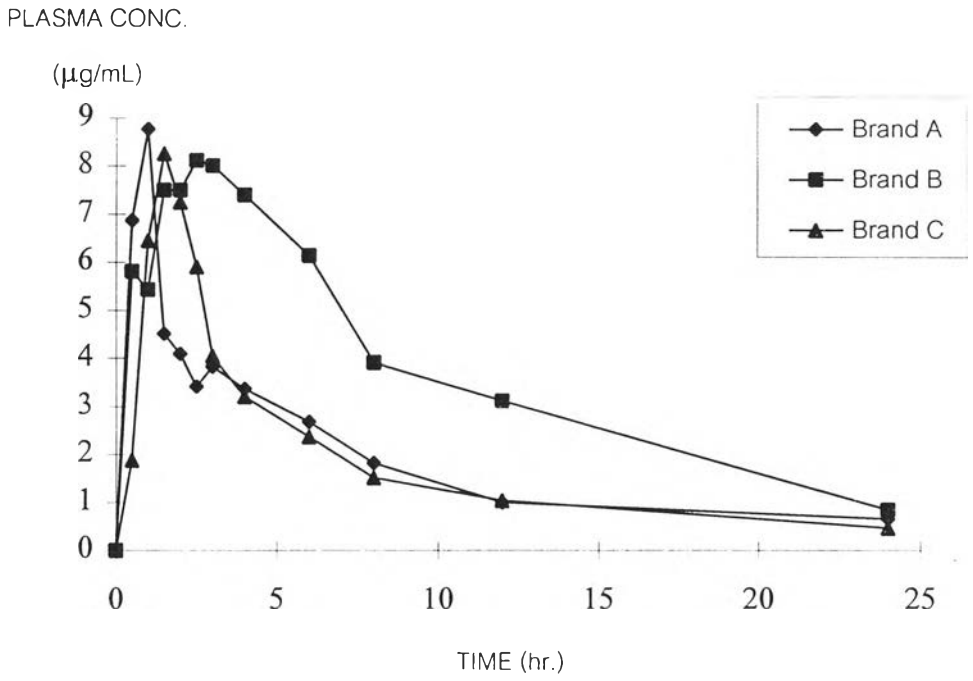

Figure 7 Plasma Roxithromycin Concentration-time Profile of Subject No.4 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

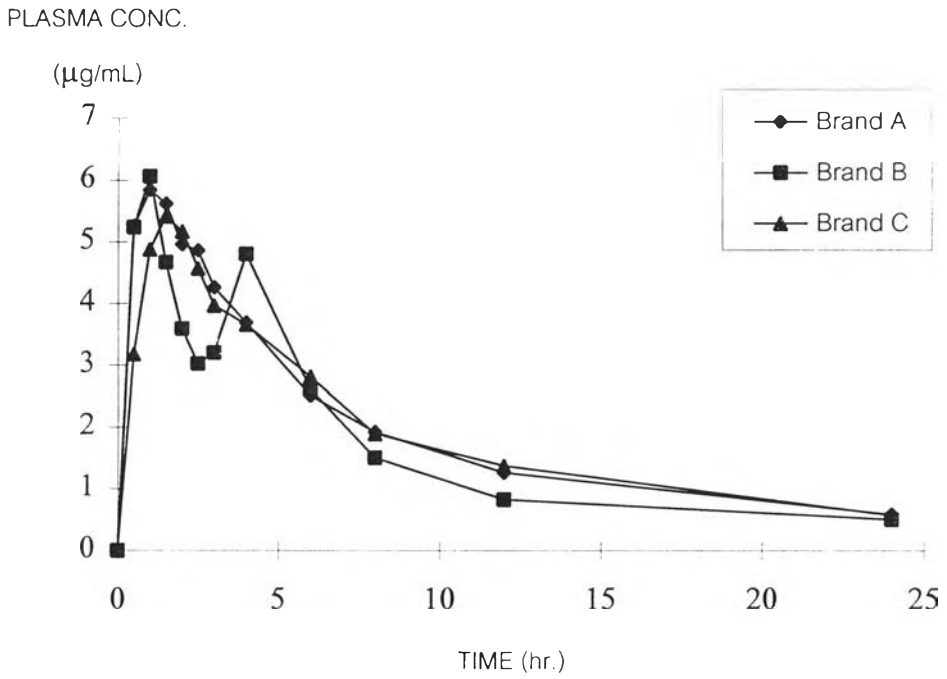

Figure 8 Plasma Roxithromycin Concentration-time Profile of Subject No.5 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

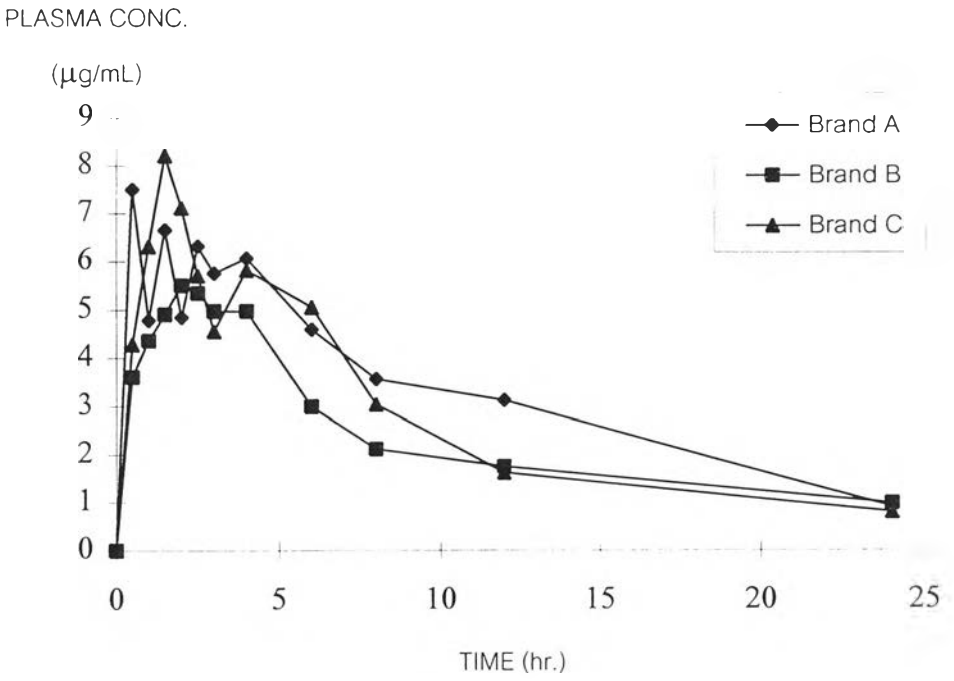

Figure 9 Plasma Roxithromycin Concentration-time Profile of Subject No.6 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

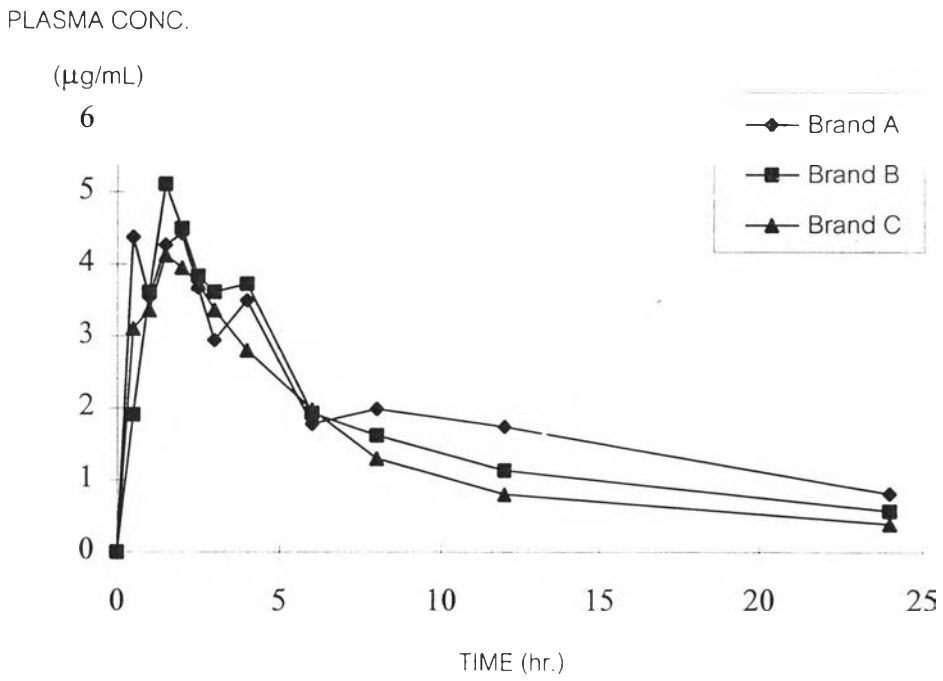

Figure 10 Plasma Roxithromycin Concentration-time Profile of Subject No.7 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

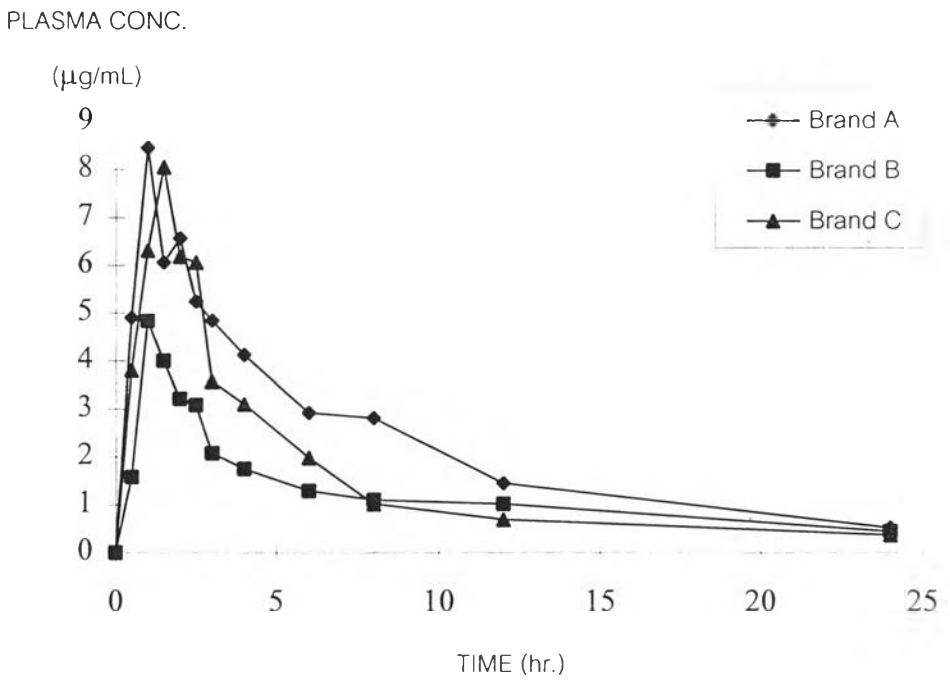

Figure 11 Plasma Roxithromycin Concentration-time Profile of Subject No.8 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

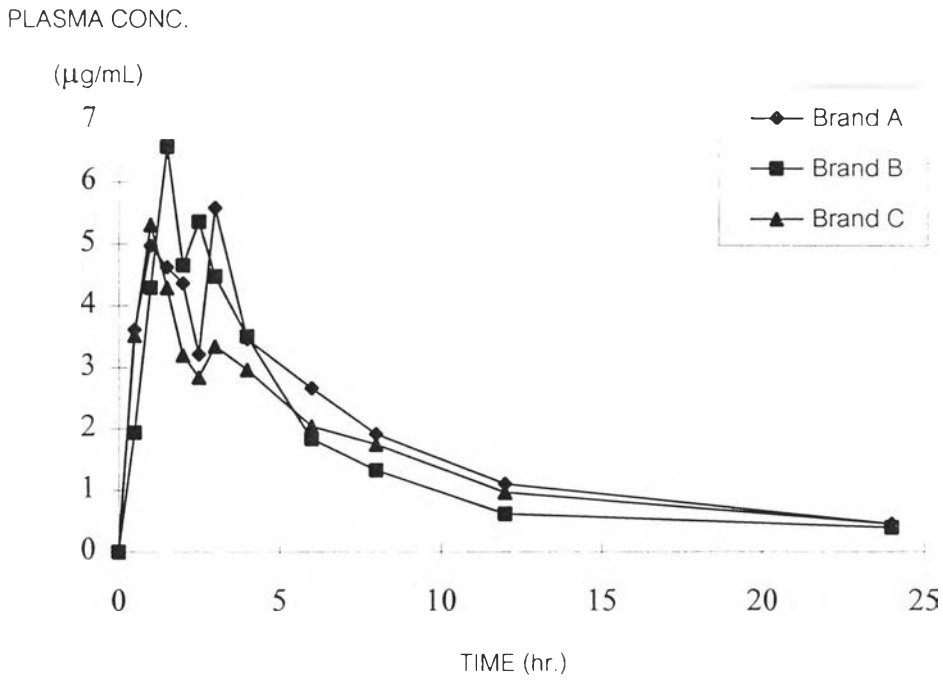

Figure 12 Plasma Roxithromycin Concentration-time Profile of Subject No.9 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

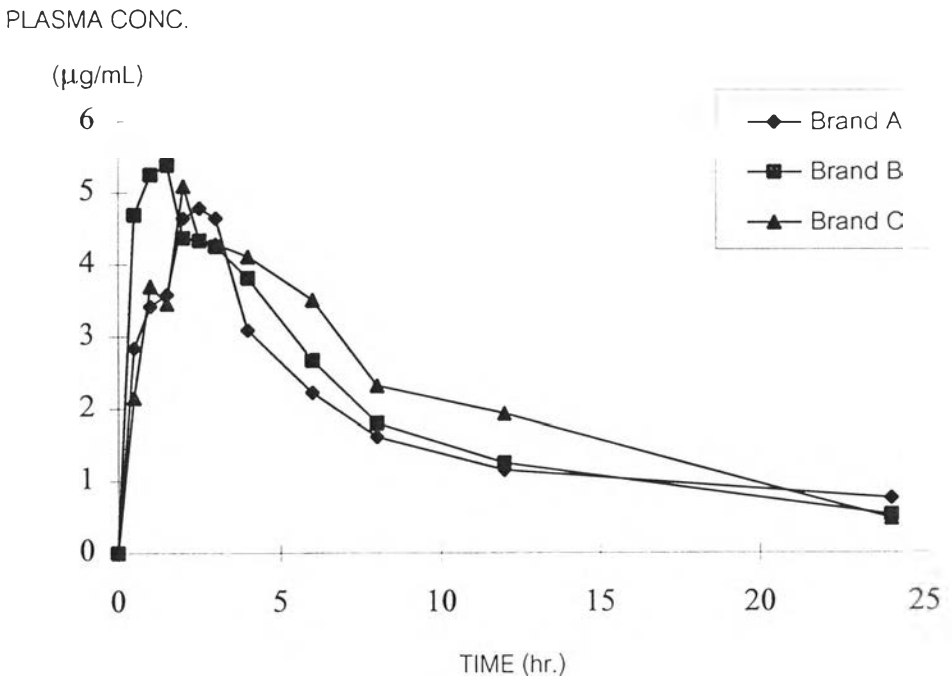

Figure 13 Plasma Roxithromycin Concentration-time Profile of Subject No.10 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

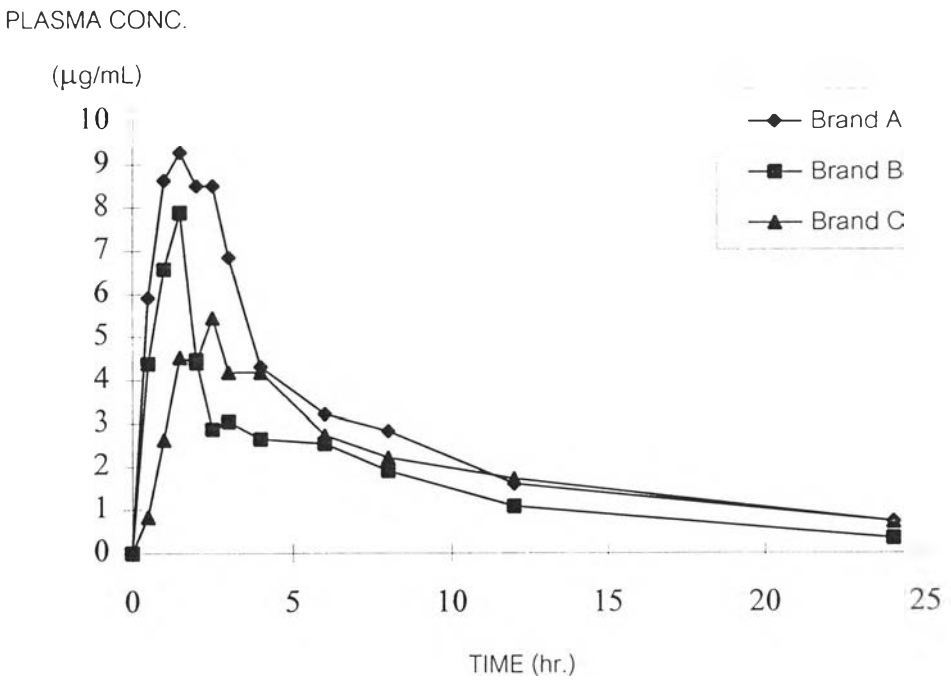

Figure 14 Plasma Roxithromycin Concentration-time Profile of Subject No.11 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

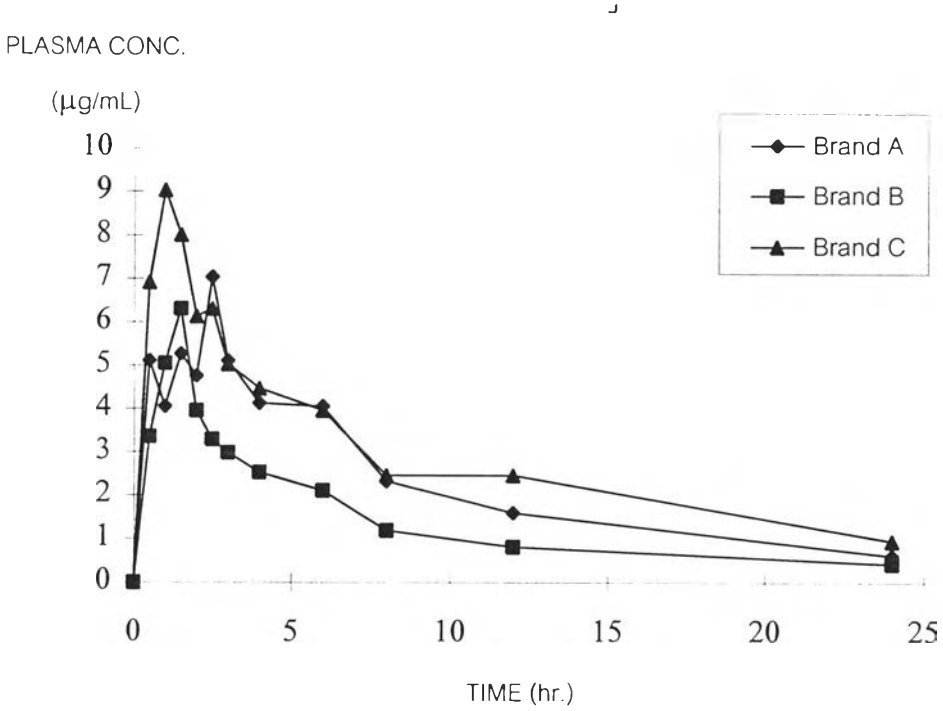

Figure 15 Plasma Roxithromycin Concentration-time Profile of Subject No.12 Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

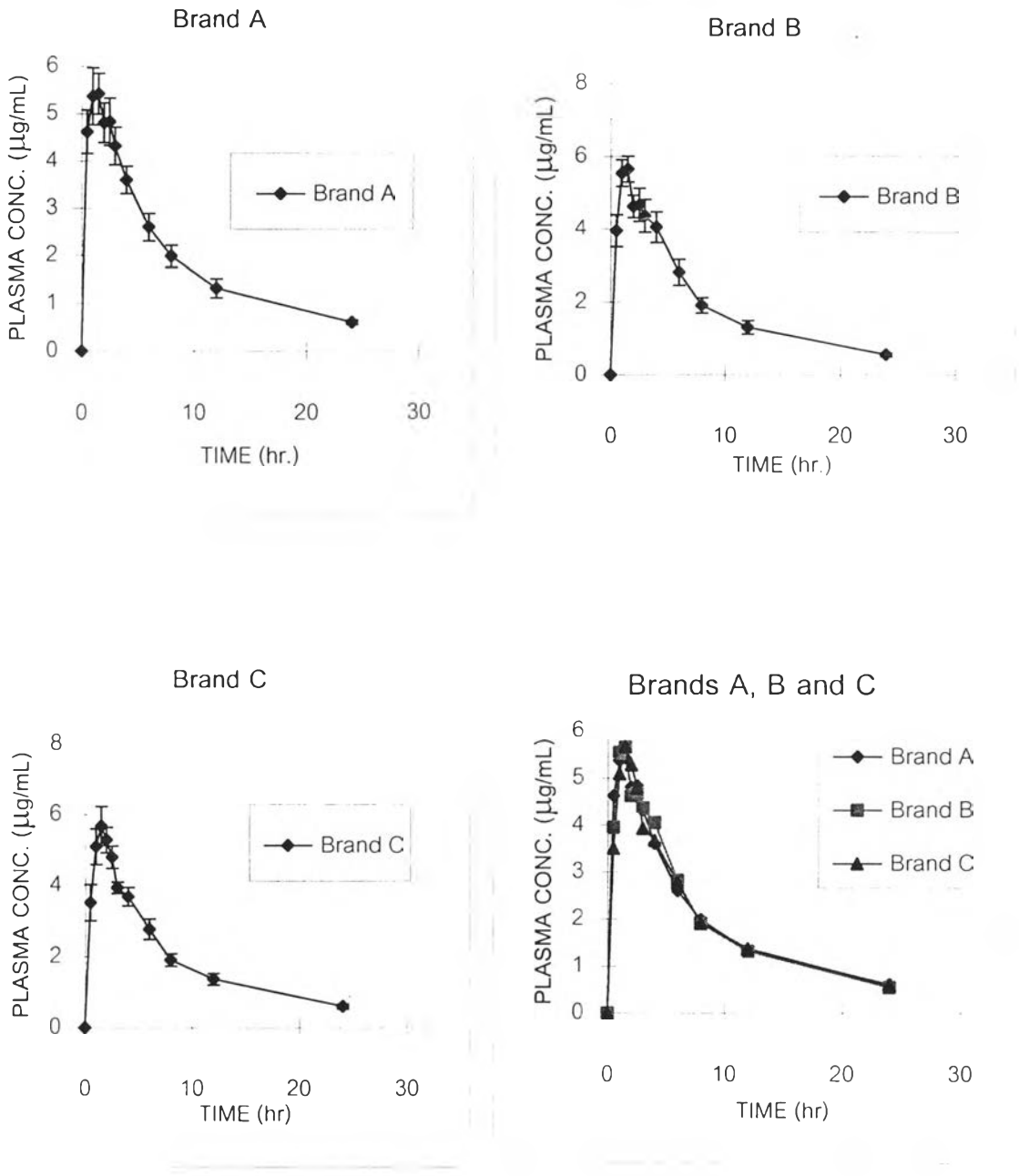

Figure 16 Comparison of Mean Plasma Roxithromycin Concentration-time Profile from 12 Subjects Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

contained within 80-125% of 90% confidence interval (The United States Pharmacopoeia XXIII).

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 achieved following oral administration of 150 mg roxithromycin was about 6.6-7.9 μg/mL (Kees et al., 1988). In this study, the mean peak plasma roxithromycin concentration for brands A, B and C were 6.35±0.52, 6.40±0.33 and 6.26±0.49 μg/mL, respectively, as seen in Table 16. The rank orders of these values were B > A > C. Table 17 showed that there were no statistically significant difference ($p>0.05$) between all three brands, eventhough the intersubject variations was rather large ($p<0.05$). The ratio of C_{max} of brands B and C relatively to brand A were 96-109 and 92-111%, respectively.

The plasma profiles obtained showed more than one peak thus the C_{max} was selected from the highest peak plasma concentration. These observed C_{max} were less than those of the previous studies. The reasons might be the difference of assay method as well as race and biological behavior of subjects. In Thais, the drug may be metabolized rapidly than foreigner.

The C_{max} of brand C was slightly lower than those of the two brands, these might be the drug was less absorbed than others.

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

The time to peak plasma roxithromycin concentration of each individual was presented in Table 18. The average times were 1.46±0.18, 1.42±0.14 and 1.46± 0.16 hours for brands A, B and C, respectively. The rank order of these values were B < A = C. The t_{max} of brand B was lower than those of brands A and C, indicated that the drug of brand B was absorbed into the systemic circulation rapidly than others.

Table 16 Peak Plasma Concentration (C_{max}) of Roxithromycin Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Subject No.	C_{max} (μg/mL)		
	Brand A	Brand B	Brand C
1	5.84	8.22	4.62
2	4.56	6.74	6.51
3	4.76	6.14	5.04
4	8.77	8.12	8.26
5	5.84	6.06	5.42
6	7.50	5.50	8.21
7	4.41	5.11	4.12
8	8.45	4.82	8.05
9	4.97	6.57	5.31
10	4.78	5.38	5.08
11	9.28	7.88	5.45
12	7.04	6.31	9.03
Mean	6.35	6.40	6.26
S.E.M.	0.52	0.33	0.49

Table 17 Analysis of Variance for Peak Plasma Concentration of Three Brands of 150 mg Roxithromycin Tablets for Three-Way Crossover Study

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	Significance Level
Subjects	9	44.58	4.95	3.46	S
Groups	2	0.83	0.42	0.29	NS
Subjects/Group	7	43.75	6.25	4.37	S
Weeks	2	4.92	2.46	1.72	NS
Treatments	2	0.13	0.06	0.04	NS
Residual	22	31.50	1.43		
Total	35	81.13			

F^e 0.05(2, 22) = 3.49

F^e 0.05(7, 22) = 2.51

F^e 0.05(9, 22) = 2.39

NS = Not significant difference at $p>0.05$

S = Significant difference at $p<0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 18 Time to Peak Plasma Concentration (t_{max}) of Roxithromycin Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Subject No.	t_{max} (hr)		
	Brand A	Brand B	Brand C
1	1.50	1.00	1.00
2	1.50	1.00	0.50
3	1.50	1.00	2.00
4	1.00	2.50	1.50
5	1.00	1.00	1.50
6	0.50	2.00	1.50
7	2.00	1.50	1.50
8	1.00	1.00	1.50
9	1.00	1.50	1.00
10	2.50	1.50	2.00
11	1.50	1.50	2.50
12	2.50	1.50	1.00
Mean	1.46	1.42	1.46
S.E.M.	0.18	0.14	0.16

There were no statistically significant difference ($p>0.05$) among these values of all three brands (Table 19). The ratio of t_{max} of brands B and C relatively to brand A were 85-120 and 82-118%, respectively. The time to peak plasma roxithromycin concentration in this study agreed well with those found by other reports, which ranging from 1.5-1.93 hours (Young et al., 1989). The study also indicated that dissolution process of the drug was not the rate limiting step of drug absorption. As seen in Table 9, brand C had K_d statistically lower than others, it still produced similar the time to peak value like any other brands did.

3.3 Area Under the Plasma versus Time Curve (AUC)

The mean AUC from individual plasma of all brands were 53.39±4.58, 52.89±4.64 and 53.18±4.32 μg hr/mL for brands A, B and C, respectively, as shown in Table 20. The rank order of these values were A > C > B. According to Table 21, there were no statistically significant difference ($p>0.05$) between all three brands. The ratio of AUC of brands B and C relatively to brand A were 88-113 and 87-114%, respectively.

For the antibacterial drug such as roxithromycin, the AUC reflected the total amount of active drug that reached to the systemic circulation and produced clinical efficacy. The values of AUC in this study were lower than those presented by Young et al.,1989 (72.6-81.0 μg hr/mL). The difference might be caused by the difference of assay method as well as race and biological behavior of subjects as the same results as the C_{max}.

AUC of brand A was slightly greater than those of two brands, although it had lowest of %L.A.. These results indicated that AUC was not related to %L.A. and C_{max}.

The principal pharmacokinetic parameters of roxithromycin followingoral administration of three brands were summarized in Table 22. The 90% confidence interval based on ratio of bioavailability parameters (C_{max}, t_{max} and AUC) were fully

Table 19 Analysis of Variance for Time to Peak Plasma Concentration of Roxithromycin of Three Brands of 150 mg Roxithromycin Tablets for Three-Way Crossover Study

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	Significance Level
Subjects	9	3.39	0.38	1.41	NS
Groups	2	0.89	0.44	1.63	NS
Subjects/Group	7	2.50	0.36	1.33	NS
Weeks	2	0.52	0.26	0.96	NS
Treatments	2	0.01	0.005	0.02	NS
Residual	22	5.97	0.27		
Total	35	9.89			

F^e 0.05(2, 22) = 3.49

F^e 0.05(7, 22) = 2.51

F^e 0.05(9 , 22) = 2.39

NS = Not significant difference at p>0.05

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 20 Area Under the Plasma Concentration-Time Curve(AUC) of Roxithromycin Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Subject No.	AUC (μg hr/mL)		
	Brand A	Brand B	Brand C
1	37.53	62.46	36.85
2	30.98	50.93	72.88
3	36.88	55.89	42.56
4	51.03	96.50	46.76
5	52.00	44.74	51.38
6	89.50	64.62	72.74
7	56.55	44.81	35.97
8	58.84	39.56	45.01
9	46.81	41.98	40.83
10	49.79	49.76	55.90
11	70.24	43.63	57.08
12	60.58	39.85	80.17
Mean	53.39	52.89	53.18
S.E.M.	4.58	4.64	4.32

Table 21 Analysis of Variance for AUC of Three Brands of 150 mg Roxithromycin Tablets for Three-Way Crossover Study

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]	Significance Level
Subjects	9	3077.70	341.97	1.69	NS
Groups	2	49.70	24.85	0.12	NS
Subjects/Group	7	3028.00	432.57	2.14	NS
Weeks	2	548.70	274.35	1.36	NS
Treatments	2	1.52	0.76	0.004	NS
Residual	22	4441.90	201.90		
Total	35	8069.82			

F^e 0.05(2, 22) = 3.49

F^e 0.05(7, 22) = 2.51

F^e 0.05(9, 22) = 2.39

NS = Not significant difference at $p>0.05$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 22 Principal Pharmacokinetic Parameters (Mean±S.E.M.) of Roxithromycin from Twelve Subjects Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Parameter	Brand			F-test	t-test with
	A	B	C		respect to brand A
C_{max} (μg/mL)	6.35±0.52	6.40±0.33 (96-109)*	6.26±0.49 (92-111)*	0.0269	NS
t_{max} (hr)	1.46±0.18	1.42±0.14 (85-120)*	1.46±0.16 (82-118)*	0.0232	NS
AUC (μg hr/mL)	53.39±4.58	52.89±4.64 (88-113)*	53.18±4.32 (87-114)*	0.0031	NS

NS = Not significant difference at $p > 0.05$

F value obtained from the table = 3.28

* 90% Confidence interval of parameter ratio of tested product and innovator's product

contained within a practical bioequivalence range (80-125%). According to guideline for bioequivalence in USP XXIII, it could be concluded that brands B and C were bioequivalent to the innovator's product (brand A) in terms of both the rate (C_{max} and t_{max}) and the extent of the drug absorption (AUC) into systemic circulation.

4. Pharmacokinetics of Roxithromycin Tablets

The pharmacokinetics of roxithromycin following oral administration of 150 mg roxithromycin tablet in healthy Thai male volunteers, which the result revealed that roxithromycin had biphasic elimination manner. The elimination phase of the concentration-time profile showed a fast declining initial phase and a slowly elimination phase thereafter. It could be explained by a two compartment model with first-order absorption and first-order elimination. This finding agreed with the study of Campa et al.,1990.

4.1 Absorption Rate Constant (K_a)

The average absorption rate constants for brands A, B and C were 0.58± 0.10, 0.66±0.07 and 0.54±0.08 hr^{-1}, respectively as shown in Table 23. The rank order of these values were brands B>A>C. Statistical analysis results in Table 24 indicated that there were no significant difference ($p > 0.05$) among these values of all brands. Absorption of drug appeared to be independent of drug dissolution. This was seen by, even though brand A showed higher dissolution rate than brand B did, the rate of drug absorption from brand B appeared to be greater.

4.2 Elimination Rate Constant (K_e)

The average elimination rate constant obtained from individual plasma data of brands A, B and C were 0.10±0.005, 0.11±0.004 and 0.10±0.005 hr^{-1}, respectively (Table 25). There were no statistical difference among these values of all three brands ($p > 0.05$) as shown in Table 26, indicating that the drug was eliminated from the body with the same rate although the drug showed intersubject variations.

Table 23 Absorption Rate Constant (K_a) of Roxithromycin Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Subject No.	K_a (hr^{-1})		
	Brand A	Brand B	Brand C
1	0.25	0.90	0.38
2	0.23	0.64	0.39
3	0.29	0.91	0.26
4	0.94	0.74	1.24
5	0.80	0.92	0.82
6	0.58	0.41	0.43
7	0.91	0.47	0.40
8	0.37	0.72	0.39
9	0.32	0.34	0.24
10	0.54	0.85	0.76
11	0.36	0.76	0.57
12	1.33	0.28	0.55
Mean	0.58	0.66	0.54
S.E.M.	0.10	0.07	0.08

Table 24 Analysis of Variance for Absorption Rate Constant of Three Brands of 150 mg Roxithromycin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	0.0997	0.0499	0.5862
Within group	33	2.8070	0.0851	
Total	35	2.9067		

F^{e} 0.05(2, 33) = 3.2849

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 25 Elimination Rate Constant (K_e) of Roxithromycin Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Subject No.	K_e (hr^{-1})		
	Brand A	Brand B	Brand C
1	0.11	0.10	0.11
2	0.08	0.11	0.10
3	0.14	0.11	0.08
4	0.10	0.11	0.12
5	0.10	0.11	0.10
6	0.09	0.08	0.10
7	0.07	0.10	0.11
8	0.12	0.09	0.14
9	0.10	0.13	0.10
10	0.08	0.10	0.10
11	0.12	0.12	0.09
12	0.12	0.11	0.09
Mean	0.10	0.11	0.10
S.E.M.	0.005	0.004	0.005

Table 26 Analysis of Variance for Elimination Rate Constant of Three Brands of 150 mg Roxithromycin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	0.00012	0.00006	0.2351
Within group	33	0.00820	0.00025	
Total	35	0.00832		

$F^{e}\ 0.05(2, 33) = 3.2849$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

4.3 Elimination half-life ($t_{1/2}$)

The mean elimination half-life of roxithromycin determined for brands A, B and C were 7.01±0.38, 6.60±0.23 and 6.78±0.31 hr, respectively (Table 27). Statistical analysis showed no significant difference among these values (Table 28). The values disagreed with the results found by other investigators ranging from 8.4-15.5 hours (Tremblay et al.,1988). This might be due to the metabolism of drug in Thais were rapidly than foreigner.

The pharmacokinetic parameters of roxithromycin from twelve subjects following oral administration of three brands were summarized in Table 29.

Table 27 Elimination Half-life ($t_{1/2}$) of Roxithromycin Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Subject No.	$t_{1/2}$ (hr)		
	Brand A	Brand B	Brand C
1	6.41	6.59	6.05
2	8.09	6.09	6.89
3	5.03	6.32	9.17
4	6.98	6.51	5.54
5	6.76	6.38	6.85
6	7.76	8.53	6.78
7	9.96	7.08	6.27
8	5.97	7.33	5.09
9	6.93	5.34	6.79
10	8.26	6.88	6.64
11	6.01	5.87	7.81
12	5.97	6.26	7.51
Mean	7.01	6.60	6.78
S.E.M.	0.38	0.23	0.31

Table 28 Analysis of Variance for Elimination Half-life of Three Brands of 150 mg Roxithromycin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	1.0186	0.5093	0.4284
Within group	33	39.2338	1.1889	
Total	35	40.2524		

$F^{e}\ 0.05(2, 33) = 3.2849$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 29 Estimated Pharmacokinetic Parameters (Mean±S.E.M.) of Roxithromycin from Twelve Subjects Following Oral Administration of Three Brands of 150 mg Roxithromycin Tablets

Parameter	Brand			F-test	t-test with
	A	B	C		respect to brand A
K_a (hr^{-1})	0.58±0.10	0.66±0.07	0.54±0.08	0.5862	NS
K_e (hr^{-1})	0.10±0.005	0.11±0.004	0.10±0.005	0.2351	NS
$t_{1/2}$ (hr)	7.01±0.38	6.60±0.23	6.78±0.31	0.4284	NS

NS = Not significant difference at $p > 0.05$

F value obtained from the table = 3.28