CHAPTER IV

RESULTS AND DISCUSSION

In Vitro Studies

The results from the test for uniformity of weight and content of active ingredient following the United States Pharmacopocia XXII and/or the British Pharmacopoeia 1988 requirments were shown in Tables 2, and 3. The data showed that the weight variation of ciprofloxacin tablet was in the range of limitation (± 5%) and the content of active ingredient were within the United States Pharmacopoeia supplement 1 limits of 90.0-110.0% of the labelled amount (United States Pharmacopoeial Convention, Inc., 1990). Therefore, these results indicated that all various brands were pharmaceutical equivalence.

To control the quality of tablet manufacture, the disintegration and the dissolution test were required. The disintegration time was tested following the British Pharmacopoeia 1988 which stated for film-coated tablet, it would disintegrate completely in distilled water within 30 minutes. All of four brands of ciprofloxacin tablets met the British Pharmacopoeia 1988. The rank orders of disintegration time, maximum to minimum were D > A = B > C as shown in Table 4. Only brand D disintegrated significantly different from brand A did (Tables 5, and 6)

The dissolution test was carried out using the United States Pharmacopoeia XXII method II with distilled

Table 2 Weight Variation of Four Brands of Ciprofloxacin Tablets

Tablet No.	Weight per Tablet (gm)			
	A	B	C	D
1	0.3766	0.3812	0.3980	0.3739
2	0.3815	0.3784	0.4072	0.3686
3	0.3849	0.3790	0.3980	0.3903
4	0.3891	0.3789	0.4140	0.3775
5	0.3905	0.3810	0.3809	0.3847
6	0.3829	0.3803	0.4000	0.3759
7	0.3836	0.3790	0.4030	0.3934
8	0.3877	0.3757	0.4235	0.3739
9	0.3821	0.3759	0.4046	0.3658
10	0.3805	0.3824	0.4087	0.3879
11	0.3858	0.3846	0.4122	0.3783
12	0.3856	0.3789	0.4083	0.3791
13	0.3819	0.3796	0.4046	0.3786
14	0.3842	0.3788	0.4062	0.3789
15	0.3849	0.3813	0.4036	0.3896
16	0.3873	0.3845	0.3909	0.3790
17	0.3869	0.3804	0.3929	0.3632
18	0.3854	0.3837	0.3861	0.3932
19	0.3805	0.3797	0.3865	0.3776
20	0.3846	0.3797	0.3861	0.3848
Mean	0.3843	0.3802	0.4008	0.3802
SD	0.0033	0.0024	0.0109	0.0080

Table 3 Percent Labeled Amount of Four Brands of Ciprofloxacin Tablets

Test No.	% Labeled Amount			
	A	B	C	D
1	105.92	101.68	99.75	108.93
2	105.35	101.21	98.60	108.08
3	107.54	99.82	101.72	108.32
Mean	106.27	100.90	100.02	108.44
SD	1.14	0.97	1.58	0.44

Table 4 Disintegration Time of Four Brands of Ciprofloxacin Tablets

Tablet No.	Time (min)			
	A	B	C	D
1	1.40	1.27	2.05	5.25
2	1.52	1.07	0.97	4.17
3	1.27	1.65	1.00	6.00
4	1.65	1.45	0.87	8.50
5	1.37	1.62	1.55	8.42
6	1.30	1.47	1.40	9.33
Mean	1.42	1.42	1.31	6.95
SD	0.14	0.22	0.45	2.09

Table 5 Analysis of Variance for Disintegration Time of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f[a]	SS[b]	MS[c]	F[d]
Among groups	3	139.30	46.43	40.03
Within groups	20	23.11	1.16	
Total	23	162.41		

$F^{e}_{0.05}(3,20) = 3.10$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 6 Comparison of Disintegration Time of Locally Manufactured Products with Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical significance
B	0	NS
C	-0.15	NS
D	8.68	S

t^{a} (0.05, 20) = 2.086

S = significant at $P < 0.05$

NS = not significant at $P > 0.05$

a = t-value from the table

water as the medium and the amount of ciprofloxacin dissolved from the tablets after 30 minutes should not less than 80% of the labelled amount. The mean percent dissolution of ciprofloxacin from tablet at various times was shown in Table 7 and Figure 2. Results indicated that all brands met the specification.

The dissolution rate constant was obtained from the sigma-minus plot. The rank orders of the mean dissolution rate constant, maximum to minimum, were brands B > C > A > D (Table 8). There were no significant differences of all brands when compared with that of brand A (Tables 9, and 10). It is interesting that brand D had the less dissolution rate constant, although the dissolution rate constant of brand D was not significantly different from others.

There are many factors affect the disintegration of tablets. Some of these factors were the materials and processes used in manufacturing such as diluents, binders, disintegrating agents, lubricant, compaction and compression pressures and coating ingredients espectially the type of film-forming agent to be used (Cadwallader,1975; Gibaldi and Perrier, 1975). These factors might be the cause of slow disintegration of brand D. As can be seen its disintegration time was significantly slower than those of brands A, B and C. However, this was not observed with dissolution test. In practice, the dissolution rate is generally the slowest step following oral administration of a solid dosage form, therefore the disintegrated drug can not instantly total dissolved.This reason explained why disintegration time of ciprofloxacin tablet of brand D is significantly slower than other

Table 7 Dissolution Data of Four Brands of Ciprofloxacin Tablets

Time (min)	Percent Ciprofloxacin Dissolved[a]			
	Brand			
	A	B	C	D
5	49.65 ± 12.25	55.06 ± 10.24	61.40 ± 15.05	25.85 ± 12.96
10	91.65 ± 7.41	84.74 ± 5.98	80.90 ± 5.82	82.53 ± 9.56
15	105.30 ± 6.78	90.84 ± 4.27	88.94 ± 3.55	103.41 ± 3.49
20	107.27 ± 2.26	93.19 ± 2.68	91.53 ± 2.75	107.43 ± 2.49
25	108.94 ± 1.77	92.51 ± 1.97	91.88 ± 2.01	109.39 ± 2.69
30	108.53 ± 2.01	93.55 ± 1.65	92.18 ± 3.17	111.74 ± 3.22
45	109.69 ± 1.75	92.68 ± 3.45	91.73 ± 1.96	109.04 ± 1.59
60	110.51 ± 2.13	90.84 ± 2.36	90.81 ± 2.66	108.15 ± 3.23
90	108.36 ± 2.57	89.06 ± 1.87	91.76 ± 5.68	106.76 ± 1.94
120	106.93 ± 2.36	87.98 ± 1.19	88.13 ± 3.12	105.34 ± 1.42

a = values are mean ± standard deviation (n=6)

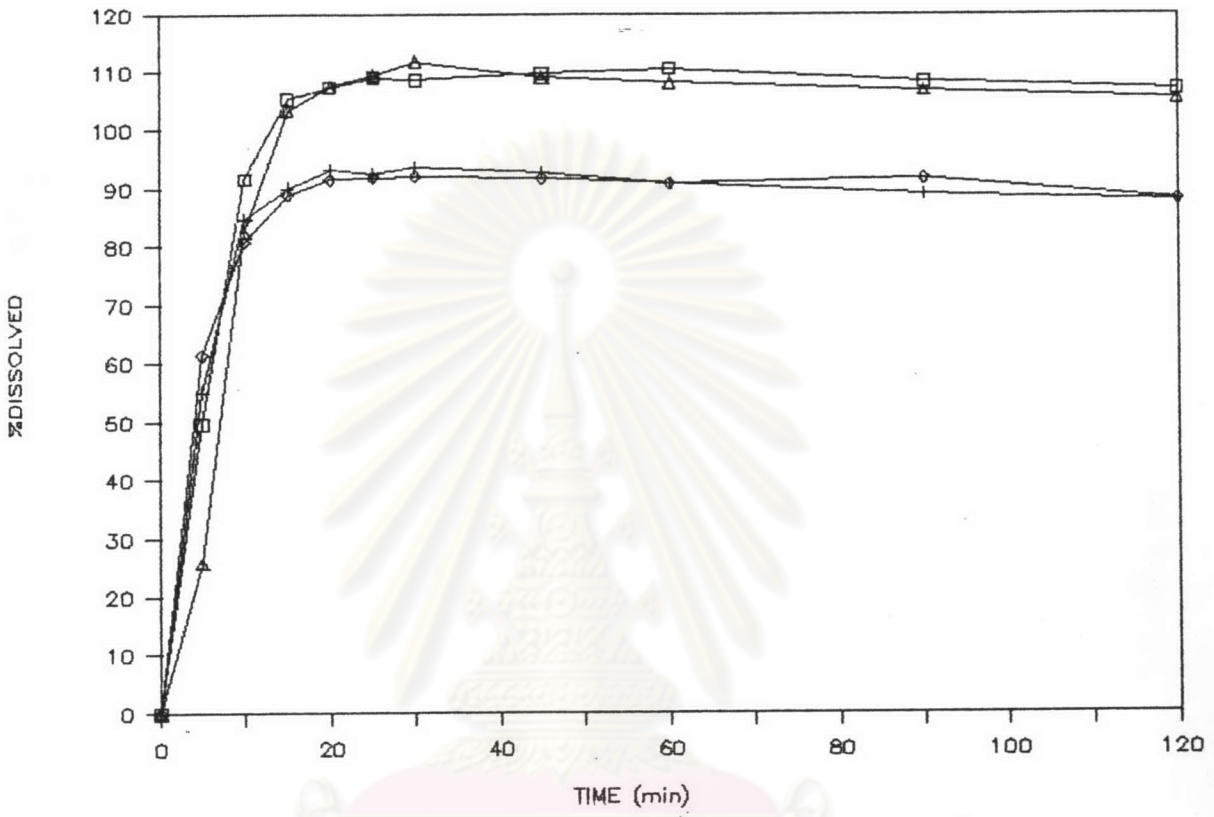


Figure 2 Percent of ciprofloxacin dissolved from four brands of ciprofloxacin tablets in carbondioxide-free water

Key : Brand A (□) , Brand B (+) , Brand C (◊), Brand D (△)

Table 8 Dissolution Rate Constant of Four Brands of Ciprofloxacin Tablets

Tablet No.	Dissolution Rate (hr^{-1})			
	A	B	C	D
1	8.24	12.64	10.38	11.74
2	9.62	12.11	9.20	9.83
3	12.32	8.36	8.90	9.38
4	7.76	5.80	14.32	8.81
5	12.67	9.34	11.60	8.20
6	12.33	24.49	11.92	11.63
Mean	10.49	12.12	11.05	9.93
SD	2.23	6.56	2.01	1.46

Table 9 Analysis of Variance for Dissolution Rate Constant of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	3	15.76	5.25	0.39
Within groups	20	270.82	13.54	
Total	23	286.58		

$F^{e}_{0.05}(3,20) = 3.10$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 10 Comparison of Dissolution Rate constant of Locally Manufactured Products with Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical significance
B	0.77	NS
C	0.26	NS
D	-0.26	NS

t^{a} (0.05, 20) = 2.086

NS = not significant at P > 0.05

a = t-value from the table

brands while the dissolution rate of the drug is not significant difference from others. All of the in vitro results were summarized in Table 11.

In Vivo Studies

1. Analysis of Ciprofloxacin in Plasma Samples

The method used for ciprofloxacin analysis in plasma samples was modified from the methods of Morton et al (1986) and Pauliukonis et al (1984). A protein in plasma was separated by precipitation.

The validation of method was established which included both precision and recovery. A typical chromatogram obtained for the analysis of the blank plasma and plasma sample from a subject taking a single dose of a drug and internal standard (pipemedic acid) was displayed in Fig. 3. The retention times of ciprofloxacin and pipemidic acid were 4.93 minutes and 1.95 minutes, respectively. The sensitivity of this method was 0.15 mcg/ml. A calibration curve of ciprofloxacin in plasma was linear from 0.15 mcg/ml to 2.2 mcg/ml.

To assess the within run (n=3) and between run precision (n=4), the mean, standard deviation and coefficient of variation (C.V.) of the peak were estimated. Tables 12 and 13 illustrated the percentage of coefficient of variation of both the within run and the between run precision, respectively, which varied randomly over the concentration ranges.

The mean percent recoveries for pipemedic acid

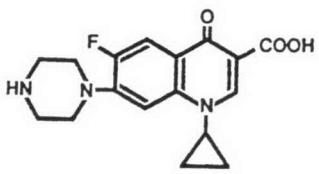
Table 11 Physical Characteristics of In Vitro Studies of Four Brands of Ciprofloxacin Tablets

Brand	Weight variation[a] (g)	%Labeled amount[b]	Disintegration[c] time (min)	% Dissolved[c] at 30 min	Dissolution rate[c] (hr^{-1})
A	0.3843 ± 0.0033	106.27 ± 1.14	1.42 ± 0.14	108.53 ± 2.01	10.49 ± 2.23
B	0.3802 ± 0.0024	100.90 ± 0.97	1.42 ± 0.22	93.55 ± 1.65	12.12 ± 6.56
C	0.4008 ± 0.0109	100.02 ± 1.58	1.31 ± 0.45	92.18 ± 3.17	11.05 ± 2.01
D	0.3802 ± 0.0080	108.44 ± 0.44	6.95 ± 2.09	111.74 ± 3.22	9.93 ± 1.46

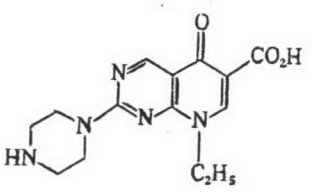
a = values are mean ± standard deviation (n=20)

b = values are mean ± standard deviation (n=3)

c = values are mean ± standard deviation (n=6)



ciprofloxacin



pipemedic acid

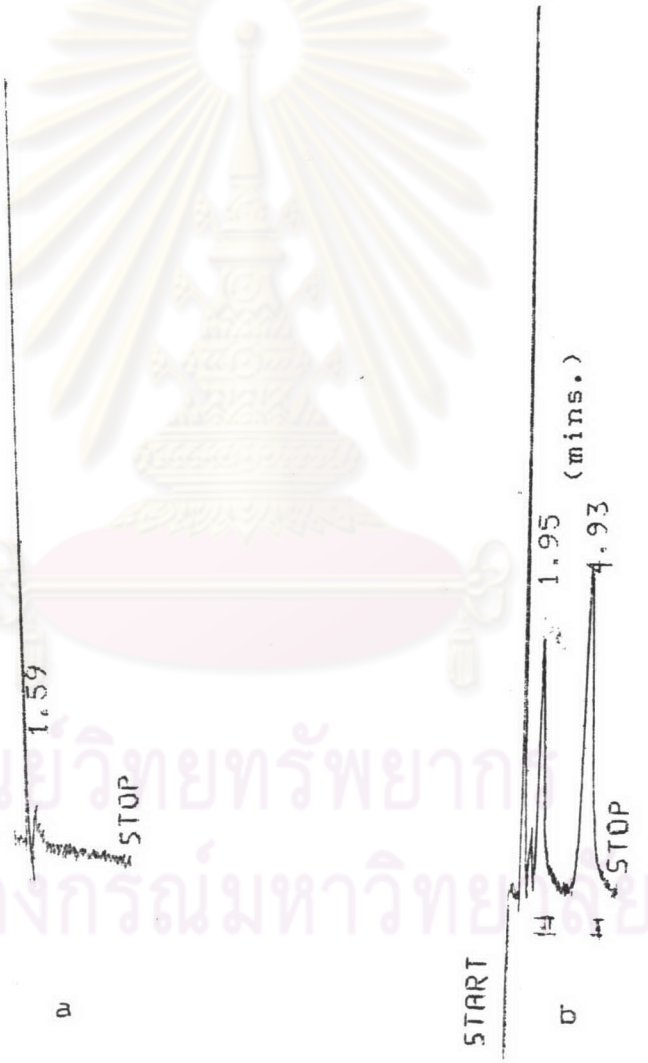


Figure 3 Chromatogram of blank plasma (a) and ciprofloxacin (I) with pipemedic acid (II) in plasma sample (b)

Table 12 Within-Run Precision for Ciprofloxacin from Three Replicated Plasma Standard Curves Obtained in the Same Day.

Concentration (mcg/ml)	Peak Height Ratio (mean ± S.D.)	C.V. (%)
0.15	0.13 ± 0.01	8.66
0.20	0.19 ± 0.02	7.90
0.40	0.32 ± 0.01	1.82
0.60	0.40 ± 0.03	7.07
0.80	0.63 ± 0.02	2.75
1.00	0.76 ± 0.02	2.73
1.40	1.06 ± 0.02	1.45
1.80	1.35 ± 0.03	2.26
2.20	1.71 ± 0.05	2.64

Table 13 Between Run Precision for Ciprofloxacin from Four Replicated Plasma Standard Curves Obtained from Four Different Days.

Concentration (mcg/ml)	Peak Height Ratio (mean ± S.D.)	C.V. (%)
0.15	0.14 ± 0.01	9.15
0.20	0.19 ± 0.02	9.11
0.40	0.31 ± 0.03	8.33
0.60	0.46 ± 0.04	7.65
0.80	0.58 ± 0.03	4.45
1.00	0.73 ± 0.03	3.92
1.40	1.00 ± 0.06	6.28
1.80	1.29 ± 0.07	5.47
2.20	1.51 ± 0.05	8.92

and ciprofloxacin in the concentration range of 0.2 mcg/ml to 2.2 mcg/ml were 83.22 ± 4.87 and 84.81 ± 4.06, respectively as shown in Table 14. The recovery of ciprofloxacin was not dependent on concentrations. Only one of the coefficient of variation of the standard was 10.82 whereas those of others were less than 10%.

2. Clinical Observation

No side effects and/or any indication of intoxication were appeared following oral administration of ciprofloxacin tablets throughout the study.

3. Plasma Ciprofloxacin Level

Plasma ciprofloxacin concentration at each sampling time from 0 to 12 hours after administration of the four brands of 250 mg ciprofloxacin tablets (A, B, C and D) were presented in Tables 15-18 respectively. The plasma ciprofloxacin concentration-time profile of all subjects were shown graphically in Figures 4-15. The mean plasma concentration-time curve for individual product was presented in Fig. 16.

4. Bioequivalence Assessment

The bioavailability of drug depended on both the rate and the extent of its absorption into the systemic circulation. The pharmacokinetic parameters which demonstrated the bioavailability of the drug included the peak plasma concentration, the time to peak plasma concentration, and the area under the plasma concentration-time curve (AUC) (Gibaldi and Perrier, 1975). If the

Table 14 Recovery of Ciprofloxacin from Plasma at Various Concentrations (n=3) and Pipemedic Acid at Concentration of 1.2 mcg/ml (n=15)

Concentration (mcg/ml)	Peak Height Ratio (mean ± S.D.)	C.V. (%)
0.20	88.89 ± 9.62	10.82
0.60	88.54 ± 4.77	5.39
1.00	83.08 ± 3.33	4.01
1.40	84.43 ± 5.50	6.51
2.20	84.83 ± 2.54	3.00
Mean (%)	84.81 ± 4.06	4.79
Pipemedic acid	83.22 ± 4.87	5.86

Table 15 Plasma Ciprofloxacin Concentrations from 12 Subjects Following Administration of Ciprofloxacin Tablet of Brand A

Subject No.	Time (hr)								
	0.5	1	1.5	2	2.5	3	5	8	12
1	0.7879	0.8715	1.8531	1.6343	1.4081	1.1856	0.5169	0.4561	0.1797
2	0.6812	1.1707	1.2385	1.1130	1.1289	0.9018	0.4478	0.2957	0.1610
3	0.4810	1.3850	2.2687	1.5029	1.0437	0.7512	0.3836	0.1688	0.0000
4	0.4110	0.9154	1.1123	0.7599	0.8973	0.8660	0.4596	0.3104	0.1828
5	1.5932	1.6449	0.9628	0.9507	0.7384	0.6182	0.3609	0.2921	0.1350
6	1.3464	2.7778	1.6246	1.1130	0.8336	0.7280	0.4447	0.3044	0.1610
7	1.7713	2.5914	1.7416	1.2208	1.0682	0.8775	0.3687	0.3275	0.1145
8	1.2616	2.1064	1.5976	1.1569	0.9446	0.7826	0.4056	0.2587	0.1345
9	2.7036	1.6929	1.0566	0.9263	0.8042	0.5716	0.4499	0.2427	0.1224
10	0.0000	1.9515	1.4497	0.9922	1.0781	0.8073	0.6018	0.3345	0.1813
11	0.8338	2.3016	1.5029	0.9625	0.8907	0.7076	0.4296	0.2285	0.1044
12	2.3988	1.2723	1.1214	0.8321	0.7063	0.6407	0.3345	0.1847	0.1377
Mean	1.1892	1.7235	1.4608	1.0971	0.9618	0.7865	0.4336	0.2837	0.1345
SD	0.7829	0.6060	0.3686	0.2477	0.1881	0.1566	0.0702	0.0730	0.0479

a = concentration in mcg/ml

Table 16 Plasma Ciprofloxacin Concentrations from 12 Subjects Following Administration of Ciprofloxacin Tablet of Brand B

Subject No.	Time (hr)								
	0.5	1	1.5	2	2.5	3	5	8	12
1	1.1849	2.1841	1.3914	0.9925	0.8896	0.6934	0.4157	0.2540	0.1519
2	0.0000	0.1579	0.3552	0.8255	0.8919	0.7798	0.4561	0.2183	0.1640
3	1.3333	2.3041	1.9966	1.3339	1.0488	0.7771	0.5036	0.2236	0.1094
4	1.2205	1.2049	1.0301	0.9647	0.9138	0.7346	0.6051	0.2127	0.1166
5	0.6108	1.4207	1.2758	1.0353	0.9352	0.6387	0.4243	0.3041	0.1302
6	0.6252	1.8258	0.9898	1.1216	0.9006	0.6911	0.4323	0.2876	0.1224
7	1.5365	1.8960	1.4924	1.1990	1.0305	0.8200	0.3743	0.3497	0.1377
8	1.3798	1.7553	1.5896	1.3081	1.1030	0.8779	0.5832	0.2794	0.1371
9	2.0521	1.7385	1.0692	0.7223	0.6513	0.5898	0.2887	0.2598	0.0863
10	1.8766	1.6736	1.1074	0.8663	0.7977	0.7406	0.3497	0.2876	0.1472
11	2.1779	2.0188	1.4103	1.0722	0.8978	0.8321	0.4955	0.3065	0.1580
12	1.1268	2.7420	1.9175	1.5269	1.0797	0.6765	0.4006	0.2257	0.1460
Mean	1.2604	1.7435	1.3021	1.0807	0.9283	0.7376	0.4441	0.2674	0.1339
SD	0.6044	0.6141	0.4243	0.2224	0.1214	0.0807	0.0879	0.0407	0.0214

a = concentration in mcg/ml

Table 17 Plasma Ciprofloxacin Concentrations from 12 Subjects Following Administration of Ciprofloxacin Tablet of Brand C

Subject No.	Time (hr)								
	0.5	1	1.5	2	2.5	3	5	8	12
1	1.6307	2.9570	2.5627	1.9081	1.4515	1.2364	0.6795	0.4705	0.2947
2	1.2222	1.7354	2.0933	1.4308	1.2195	0.9808	0.5348	0.3609	0.1498
3	1.0419	1.8559	1.6587	1.2938	0.9631	0.7310	0.4191	0.1809	0.0877
4	0.1596	0.5866	1.2046	1.3255	1.3700	1.0672	0.5315	0.2852	0.1369
5	1.7024	2.6666	1.9374	1.3063	1.0698	0.9386	0.5754	0.4885	0.1990
6	0.0944	1.7720	1.5799	1.6199	1.3071	0.8210	0.5612	0.2297	0.0944
7	1.9273	1.7190	1.4538	1.1949	0.9507	0.6083	0.4624	0.3592	0.1369
8	1.6796	2.1557	1.5347	1.0970	0.9325	0.7959	0.4196	0.2308	0.1254
9	2.3609	1.8816	1.4156	1.1473	0.9754	0.6812	0.3504	0.2768	0.1298
10	2.4404	1.4889	1.1331	1.0164	0.4730	0.3733	0.2714	0.2748	0.1169
11	1.7244	1.5722	1.1514	1.0158	0.7923	0.7066	0.4561	0.2531	0.1517
12	1.1742	2.2692	1.5912	1.0114	0.9636	0.8254	0.3107	0.2540	0.1302
Mean	1.4298	1.8883	1.6097	1.2806	1.0390	0.8138	0.4643	0.3054	0.1461
SD	0.7104	0.5763	0.4002	0.2591	0.2584	0.2158	0.1138	0.0918	0.0524

a = concentration in mcg/ml

Table 18 Plasma Ciprofloxacin Concentrations from 12 Subjects Following Administration of Ciprofloxacin Tablet of Brand D

Subject No.	Time (hr)								
	0.5	1	1.5	2	2.5	3	5	8	12
1	1.5075	2.7001	1.8332	1.4397	1.1049	0.9189	0.5753	0.3086	0.1749
2	1.3005	1.7016	1.3609	1.1055	0.9676	0.9068	0.5778	0.2887	0.1369
3	0.0000	0.4615	2.8978	1.7745	0.9450	0.9116	0.4773	0.2434	0.0828
4	0.4492	0.8993	1.1600	1.2839	1.4783	0.9860	0.5512	0.3599	0.1809
5	1.4416	2.4192	1.6161	1.1990	1.0939	0.8431	0.6377	0.3687	0.1918
6	0.5272	2.1440	1.5303	1.3749	1.0934	0.7885	0.4073	0.2233	0.0000
7	1.7027	2.3373	1.6894	1.5546	1.3435	0.8047	0.5734	0.3018	0.1765
8	1.3764	1.6268	1.3658	1.1067	0.9958	0.6400	0.3911	0.2211	0.1104
9	1.4113	2.3560	1.2723	0.9213	0.8321	0.6628	0.4944	0.1945	0.0871
10	1.5029	1.9335	1.1260	1.1126	1.0572	0.6416	0.4347	0.2484	0.1404
11	0.3018	1.6802	1.9672	1.6407	1.2517	1.0453	0.4900	0.2768	0.1431
12	1.0356	1.6296	1.4533	0.9997	0.9197	0.6242	0.3525	0.1460	0.0000
Mean	1.0464	1.8241	1.6060	1.2928	1.0903	0.8145	0.4969	0.2651	0.1187
SD	0.5470	0.6196	0.4606	0.2558	0.1786	0.1393	0.0845	0.0628	0.0630

a = concentration in mcg/ml

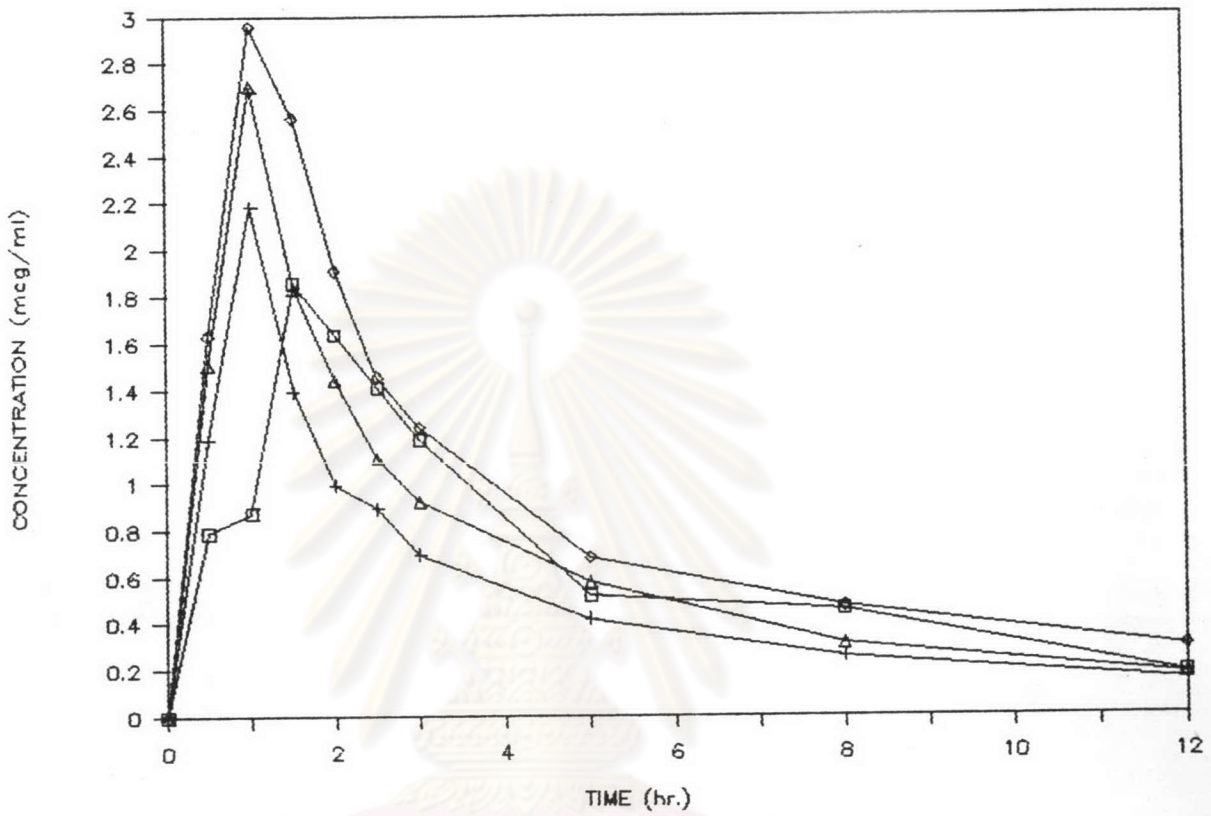


Figure 4 Plasma concentration-time profile of subject no.1 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

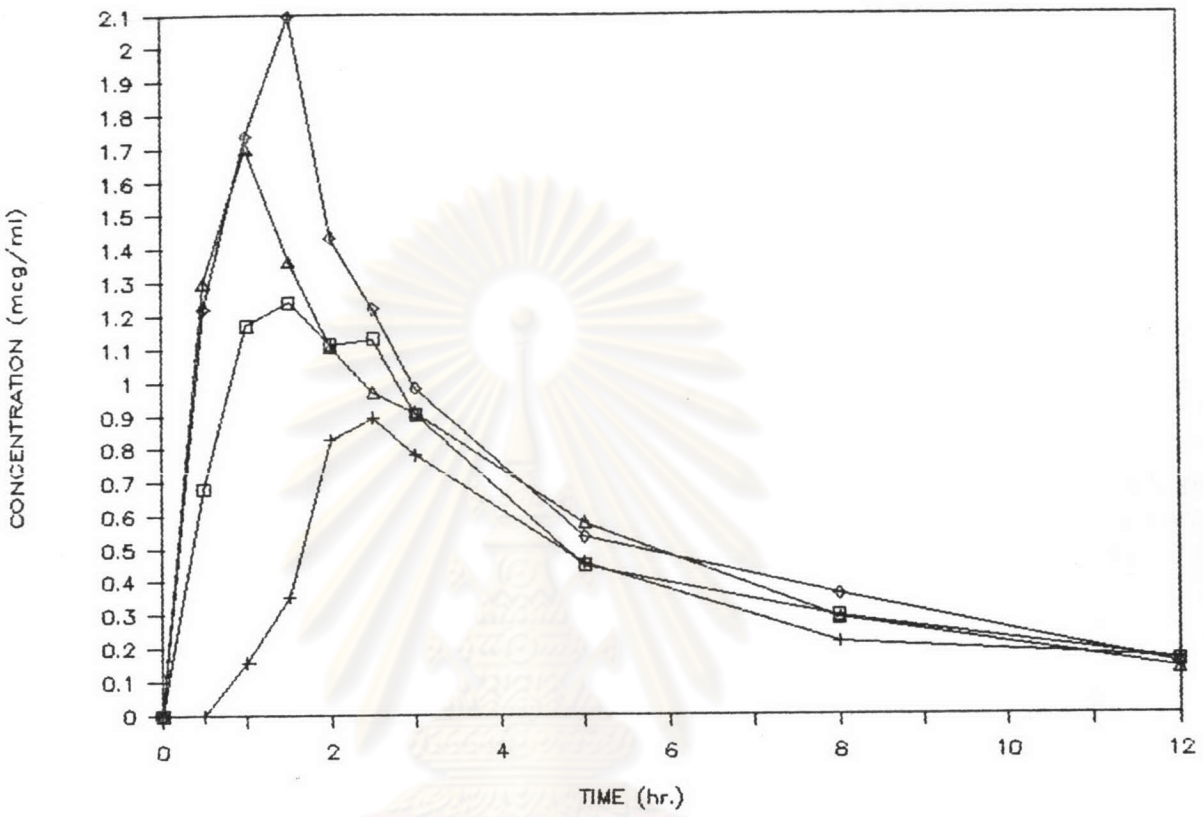


Figure 5 Plasma concentration-time profile of subject no.2 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

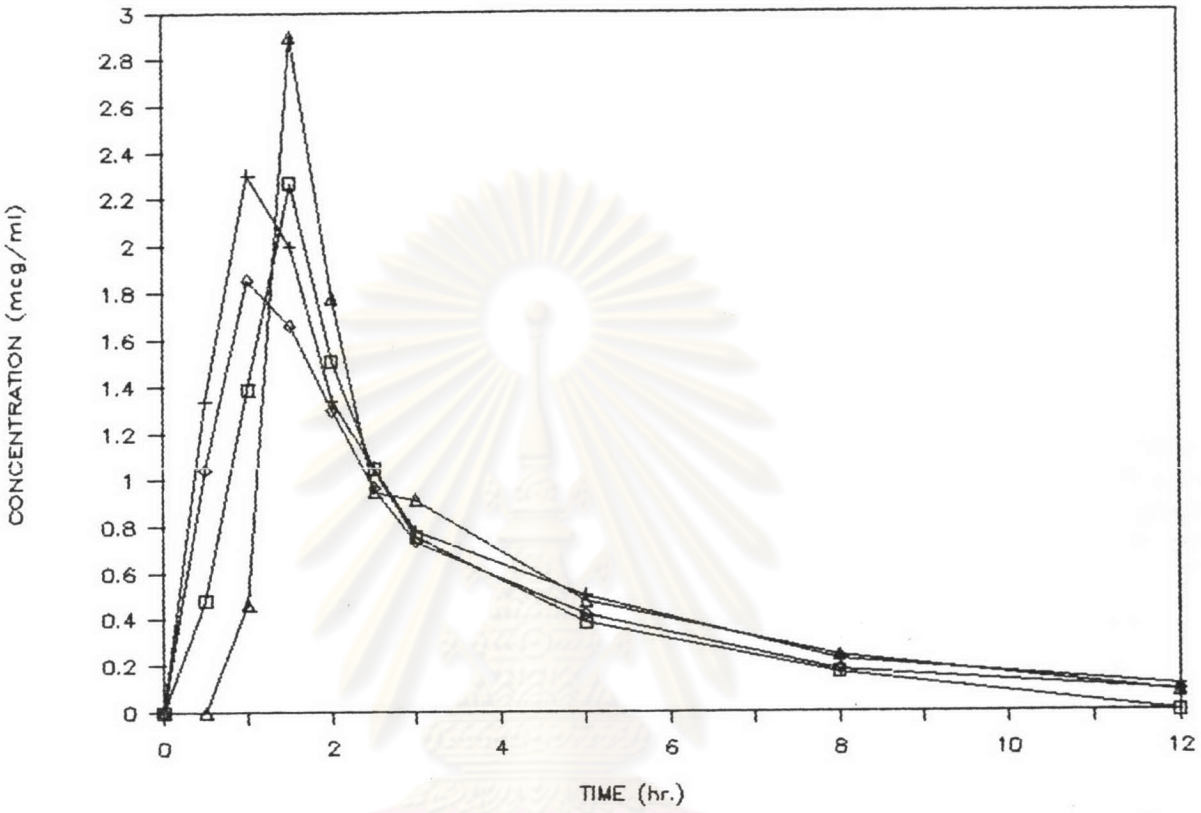


Figure 6 Plasma concentration-time profile of subject no.3 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

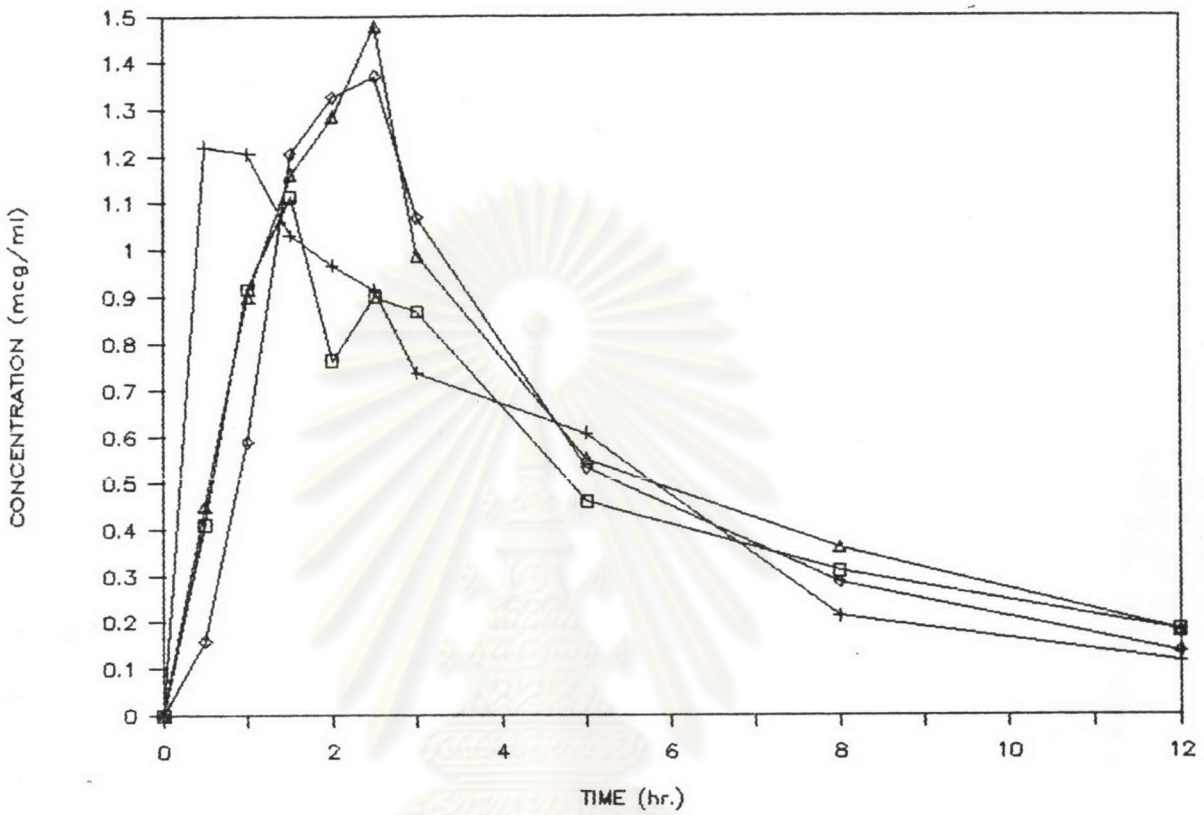


Figure 7 Plasma concentration-time profile of subject no.4 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

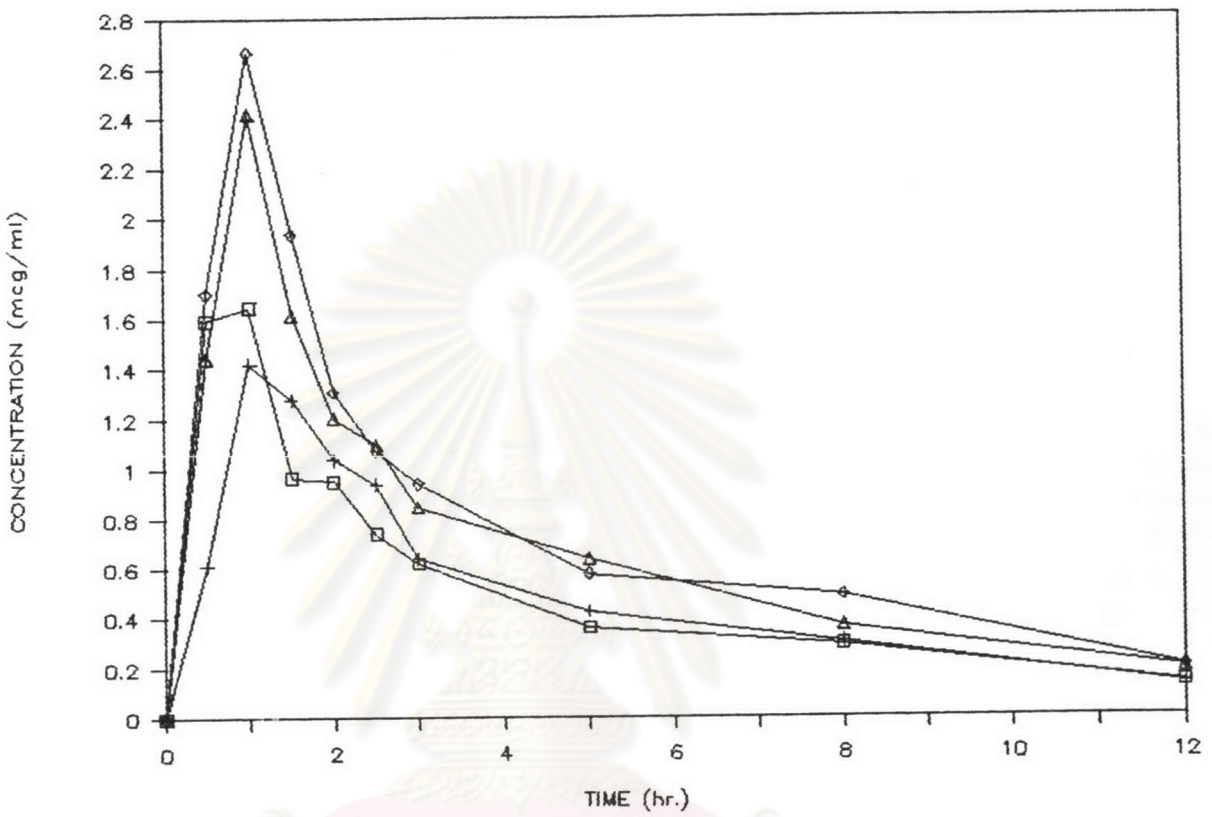


Figure 8 Plasma concentration-time profile of subject no.5 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◇), Brand D (△)

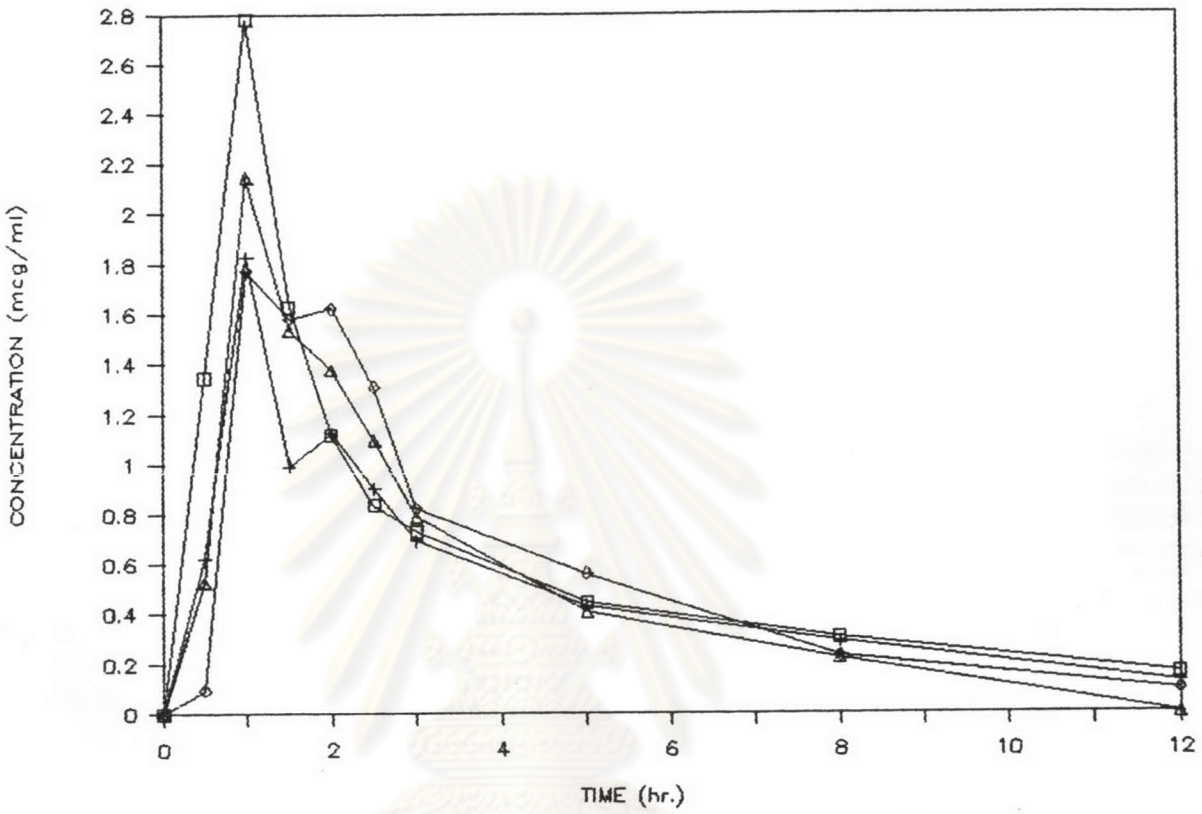


Figure 9 Plasma concentration-time profile of subject no.6 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

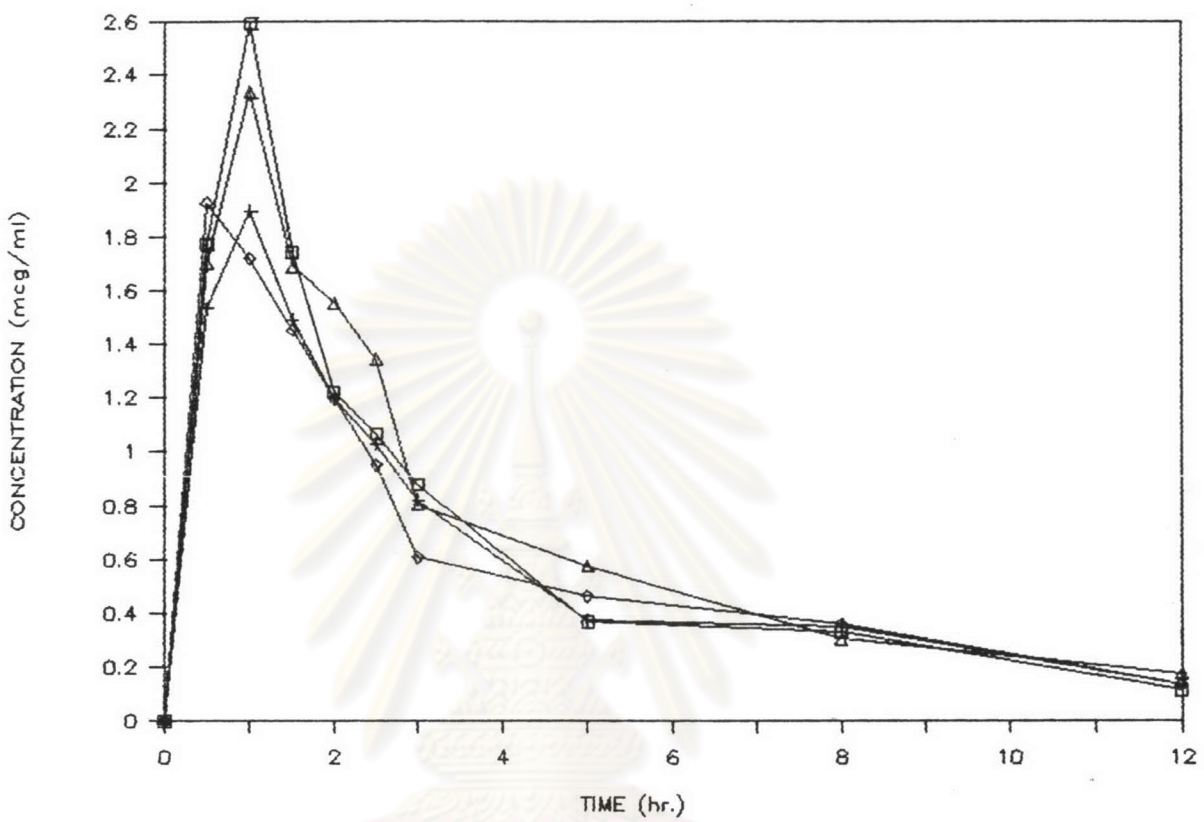


Figure 10 Plasma concentration-time profile of subject no.7 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

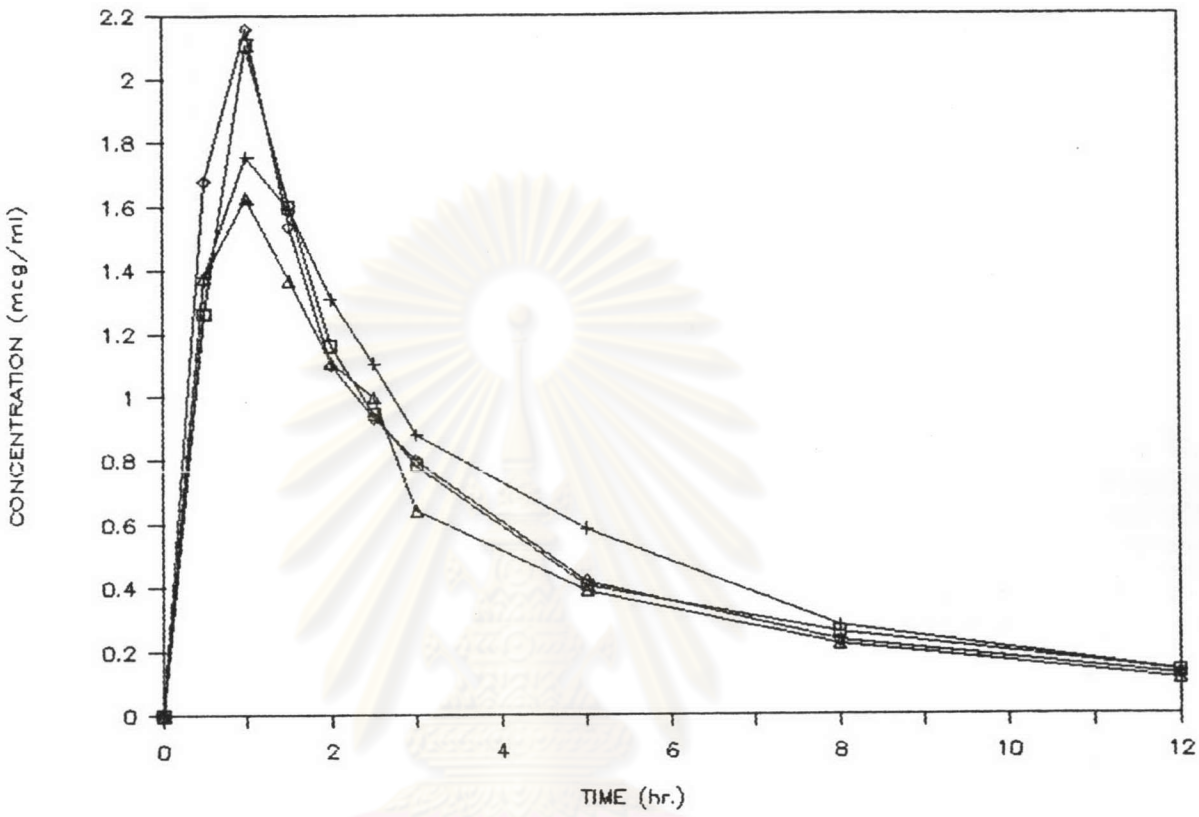


Figure 11 Plasma concentration-time profile of subject no.8 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

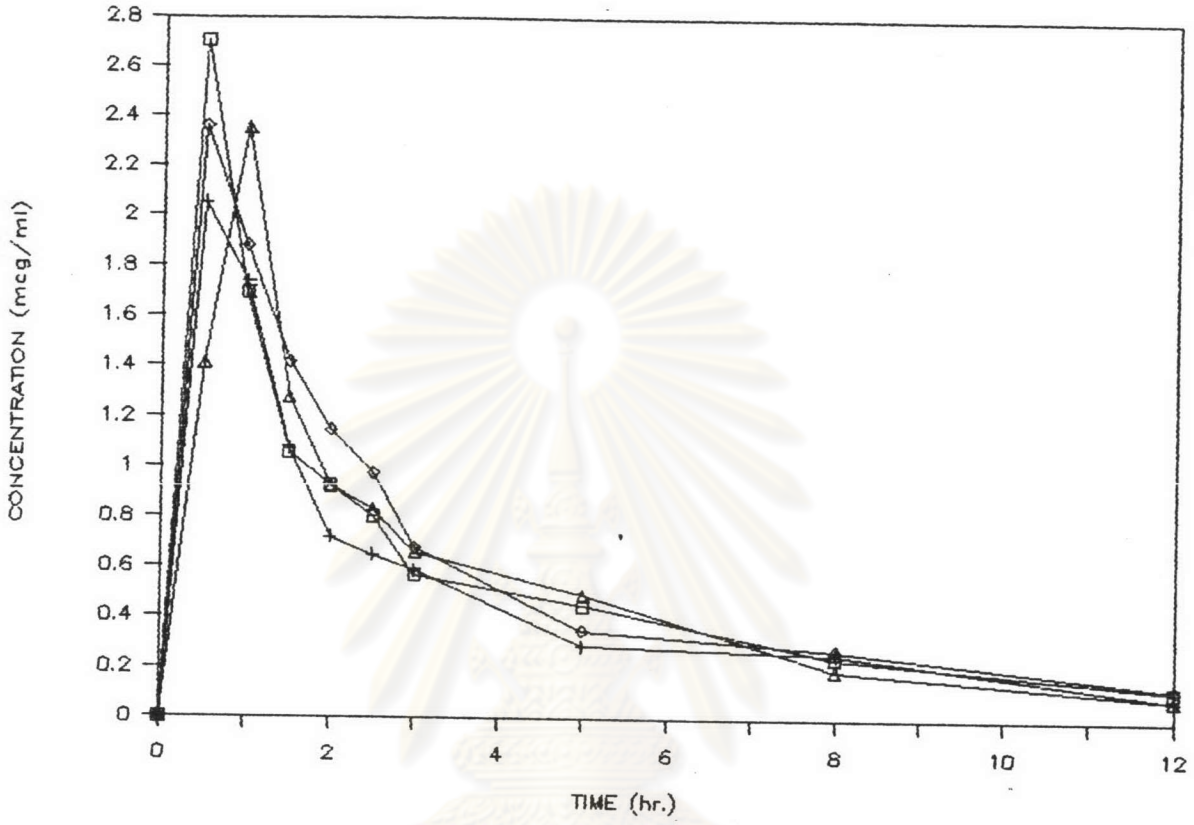


Figure 12 Plasma concentration-time profile of subject no.9 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◇), Brand D (△)

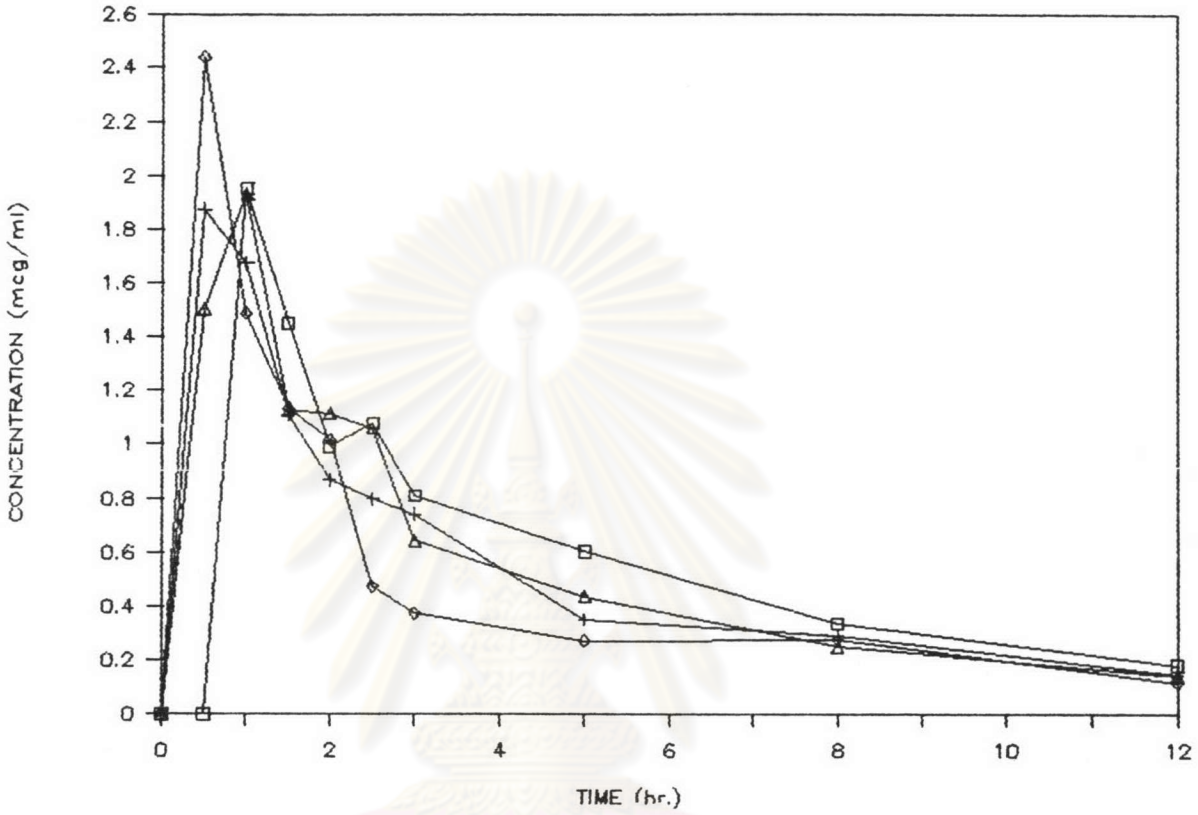


Figure 13 Plasma concentration-time profile of subject no.10 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

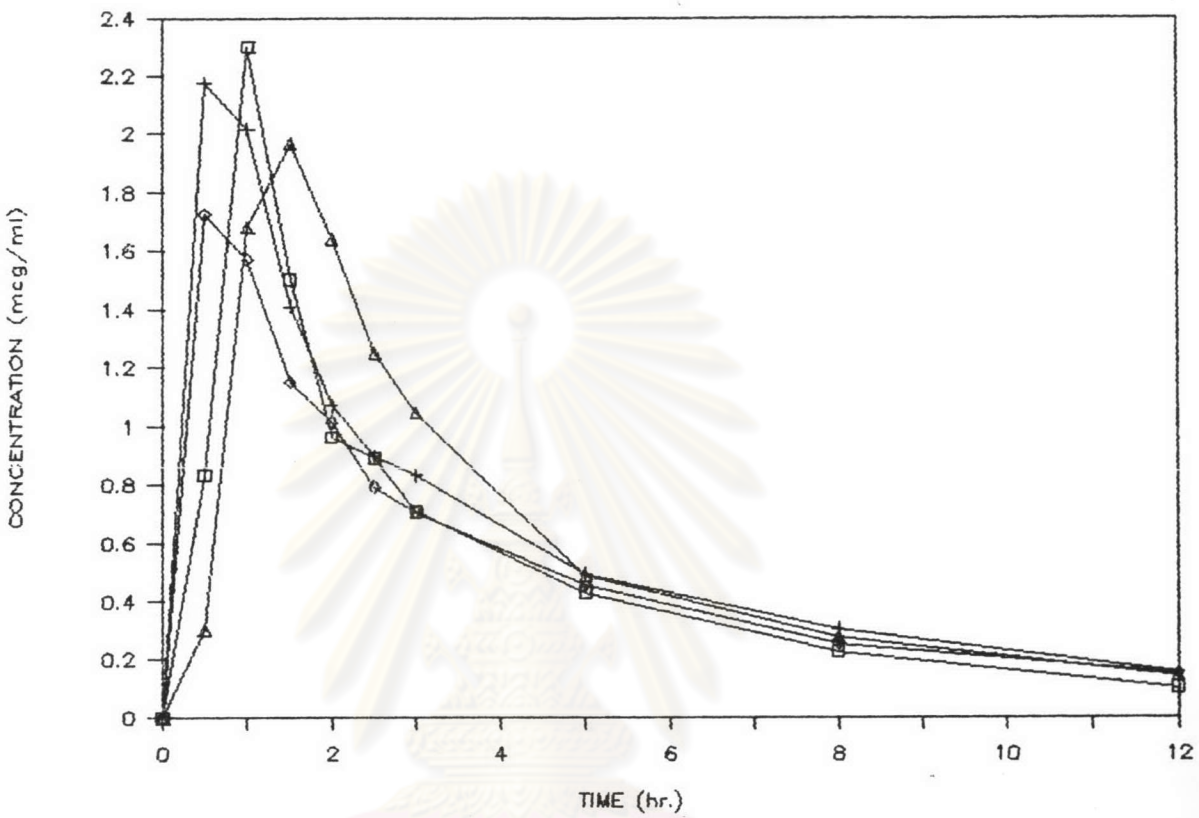


Figure 14 Plasma concentration-time profile of subject no.11 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

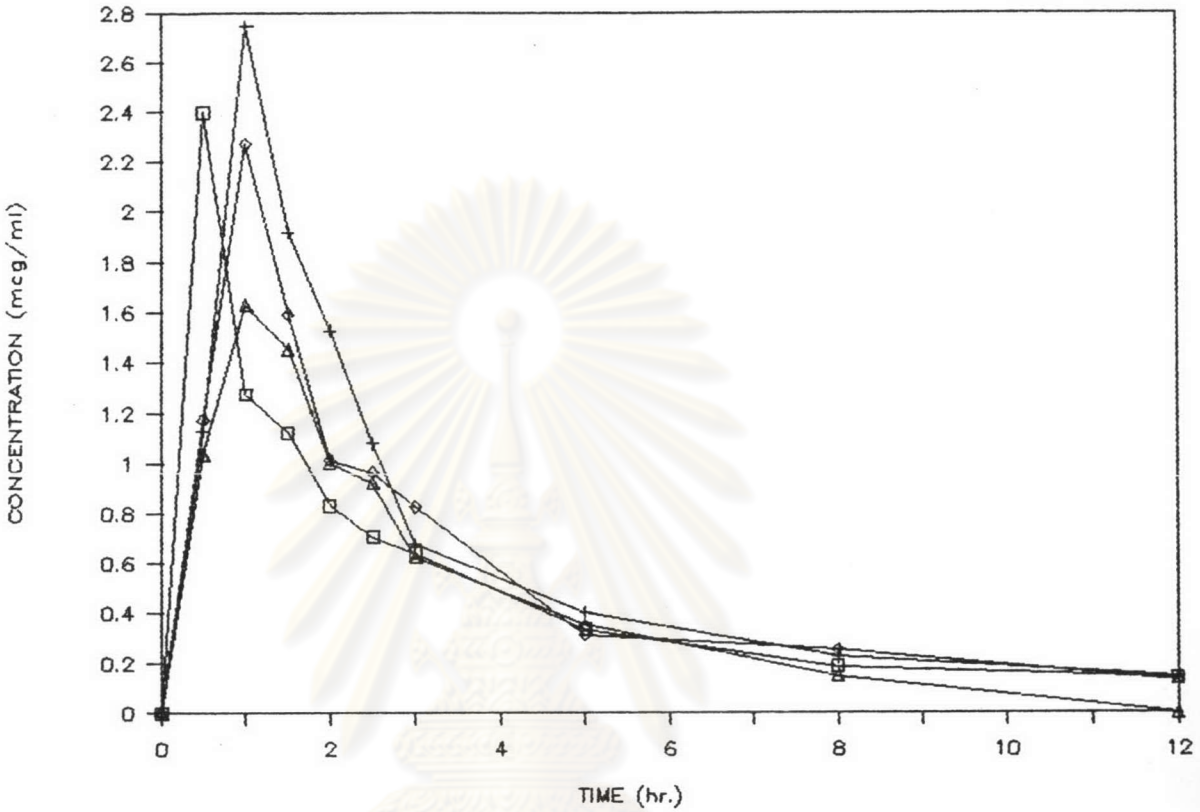


Figure 15 Plasma concentration-time profile of subject no.12 following oral administration of ciprofloxacin tablets

Key : Brand A (□), Brand B (+), Brand C (◊), Brand D (△)

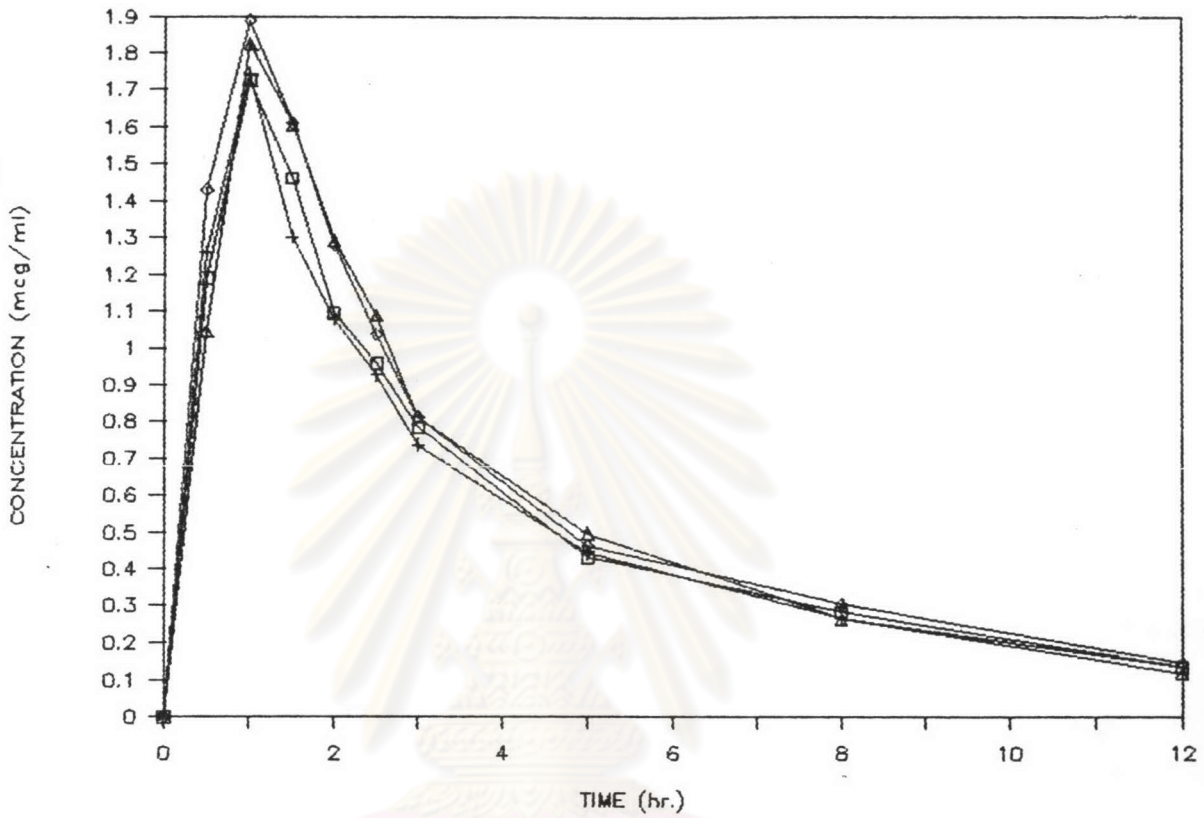


Figure 16 Mean plasma concentration-time profile of 12 subjects following oral administration of ciprofloxacin tablets of brands A, B, C and D
Key : Brand A (□), Brand B (+), Brand C (◇), Brand D (△)

test for differences of these corresponding parameters examined by ANOVA were no significant differences ($P > 0.05$), it could be concluded that all four brands of ciprofloxacin tablets were complete bioequivalence.

4.1 Peak Plasma Concentration

The peak plasma concentration of each subject was directly read from the plasma concentration-time curve and showed in Table 19. The range of peak plasma concentrations were 0.89-2.96, mcg/ml with 2.08 ± 0.52, 1.86 ± 0.48, 2.13 ± 0.42 and 2.10 ± 0.45 mcg/ml, for brands A, B, C and D, respecticely. The rank orders of peak plasma concentrations were brands C > D > A > B. The test for difference among all brands of this parameter were not significant ($p > 0.05$) (Tables 20-21).

4.2 Time to Peak Plasma Level

As seen in Table 22, the time to peak plasma level determined from the plasma concentration curve of 12 subjects ranged from 0.5-2.5 hours with the mean values for brands A, B, C and D were 1.08 ± 0.36, 0.96 ± 0.52, 1.00 ± 0.54 and 1.20 ± 0.44 hours, respectively. The rank orders of time to peak plasma were brands D > A > C > B. These values were also not statistically significant differene as shown in Tables 23 and 24. However, the time to peak plasma of brand D was rather slower than those of other brands.

4.3 Area Under the Plama level Versus Time Curve

From the output of CSTRIP program, the data

Table 19 Peak Plasma Concentration (C_{max}) of Ciprofloxacin Observed Directly from the Plasma Concentration-Time Curve of Each Individual Following Oral Administration of Four Brands of Ciprofloxacin Tablets

Subject No.	C_{max} (mcg/ml)			
	A	B	C	D
1	1.85	2.18	2.96	2.70
2	1.24	0.89	2.09	1.70
3	2.27	2.30	1.86	2.90
4	1.11	1.22	1.37	1.48
5	1.64	1.42	2.67	2.42
6	2.78	1.83	1.77	2.14
7	2.59	1.90	1.93	2.34
8	2.11	1.76	2.16	1.63
9	2.70	2.05	2.36	2.36
10	1.95	1.88	2.44	1.93
11	2.30	2.18	1.72	1.97
12	2.40	2.74	2.27	1.63
Mean	2.08	1.86	2.13	2.10
SD	0.52	0.48	0.42	0.45

Table 20 Analysis of Variance for Peak Plasma Concentration of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f[a]	SS[b]	MS[c]	F[d]
Among groups	3	0.54	0.18	0.75
Within groups	44	10.40	0.24	
Total	47	11.94		

$F^{e}_{0.05}(3,44) = 2.82$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 21 Comparison of Peak Plasma Concentration of 3 Brands (B, C, and D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparion with Brand A	Statistical significance
B	-1.10	NS
C	0.25	NS
D	0.10	NS

t^{a} (0.05, 44) = 2.015

NS = not significant at P > 0.05

a = t-value from the table

Table 22 Time to Peak Plasma Concentration (t_{max}) of Ciprofloxacin Observed Directly from the Plasma Following Oral Administration of Four Brands of Ciprofloxacin Tablets

Subject No.	t_{max} (hr)			
	A	B	C	D
1	1.50	1.00	1.00	1.00
2	1.50	2.50	1.50	1.00
3	1.50	1.00	1.00	1.50
4	1.50	0.50	2.50	2.50
5	1.00	1.00	1.00	1.00
6	1.00	1.00	1.00	1.00
7	1.00	1.00	0.50	1.00
8	1.00	1.00	1.00	1.00
9	0.50	0.50	0.50	1.00
10	1.00	0.50	0.50	1.00
11	1.00	0.50	0.50	1.50
12	0.50	1.00	1.00	1.00
Mean	1.08	0.96	1.00	1.20
SD	0.36	0.52	0.54	0.44

Table 23 Analysis of Variance for Peak Plasma Concentration of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f[a]	SS[b]	MS[c]	F[d]
Among groups	3	0.41	0.14	0.61
Within groups	44	10.31	0.23	
Total	47	10.72		

$$F^{e}_{0.05}(3,44) = 2.82$$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 24 Comparison of Time to Peak Plasma Concentration of 3 Brands (B, C, and D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical significance
B	-0.61	NS
C	-0.41	NS
D	0.61	NS

t^{a} (0.05, 44) = 2.015

NS = not significant at P > 0.05

a = t-value from the table

were well described by a triexponential function. The mean AUC as shown in Table 25 were 6.87 $\pm$ 1.63, 6.59 $\pm$ 2.41, 7.27 $\pm$ 3.00 and 7.42 $\pm$ 1.29 mcg.hr/ml for brands A, B, C and D, respectively. Tables 26 and 27 illustrated that no significant difference ($p > 0.05$) for this value of all brands. This referred that the amount of ciprofloxacin absorbed from every formulas were the same.

The pharmacokinetic parameters ; C_{max}, t_{max} and AUC, which were examined for the differences using ANOVA and student's t-test indicated that all test products did not showed any statistically significant differences among and between each other. Therefore, they were considered to be bioequivalent.

The relative bioavailability as calculated relatively to that of innovator's product were 95.92, 105.82 and 108.07% for brands B, C and D, respectively.

5. Pharmacokinetics of Ciprofloxacin Tablets

The pharmacokinetic model of ciprofloxacin from tablet in this study was judged as a two compartment open model with first order absorption. This is seen by after the drug reached its maximum concentration, it declined rapidly at first and then more slowly later. These results were the same as Borner et al (1986) and Bergan et al (1986) reported previously. Thus, the data were treated according to the two compartment open model without lag time for obtaining the pharmacokinetic parameters.

The pharmacokinetic parameters derived from

Table 25 Area Under the Plasma Concentration-Time Curve (AUC) of Ciprofloxacin Following Oral Administration of Four Brands of Ciprofloxacin Tablets

Subject No.	AUC (mcg.hr/ml)			
	A	B	C	D
1	8.95	7.49	13.54	8.97
2	7.30	1.99	2.14	7.72
3	*0.94	7.29	6.56	5.56
4	6.99	6.52	2.36	7.99
5	6.92	6.85	10.06	8.93
6	7.45	1.82	9.32	7.01
7	7.91	7.98	8.16	9.41
8	7.64	8.02	7.40	6.67
9	7.58	*39.24	8.07	7.04
10	2.25	7.35	5.14	7.29
11	6.22	8.53	7.41	7.27
12	6.36	8.67	7.03	5.21
Mean	6.87	6.59	7.27	7.42
SD	1.63	2.41	3.00	1.29

* EXCLUDED DATA

Table 26 Analysis of Variance for AUC of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f[a]	SS[b]	MS[c]	F[d]
Among groups	3	4.89	1.63	0.32
Within groups	42	213.61	5.09	
Total	45	218.50		

$F^{e}_{0.05}(3,42) = 2.83$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 27 Comparison of Area under the Plasma Concentration Time Curve of 3 Brands (B, C, and D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	-0.29	NS
C	-0.45	NS
D	0.59	NS

t^{a} (0.05, 42) = 2.018

NS = not significant at P > 0.05

a = t-value from the table

Table 28 Absorption Rate Constant (K_a) of Ciprofloxacin from 12 Subjects Following Oral Administration of Four Brands of Ciprofloxacin Tablet

Subject No.	K_a (hr^{-1})			
	A	B	C	D
1	1.06	2.44	2.60	1.94
2	1.44	1.05	2.17	2.94
3	0.94	1.82	1.60	1.57
4	1.45	5.59	1.19	1.10
5	3.70	2.67	2.07	2.26
6	2.05	1.96	6.61	1.98
7	2.22	1.88	2.44	2.62
8	2.65	2.39	2.53	2.32
9	4.61	5.23	4.74	3.40
10	1.60	4.58	3.63	2.82
11	2.78	4.48	4.31	1.64
12	2.96	2.34	1.73	1.86
Mean	2.29	3.04	2.97	2.20
SD	1.06	1.45	1.51	0.63

the plasma ciprofloxacin concentration-time data were illustrated in Tables 28-33 and summerized in Table 34, In Table 28, the absorption rate constants were presented with the mean values of 2.29 $\pm$ 1.06, 3.04 $\pm$ 1.45, 2.97 $\pm$ 1.51 and 2.20 $\pm$ 0.63 for brands A, B, C and D, respectively. The ciprofloxacin biological half-life determined from compartmental analysis for brands A, B, C and D were 3.62 $\pm$ 1.21, 3.55 $\pm$ 1.33, 3.64 $\pm$ 1.57 and 3.81 $\pm$ 0.93 hours as shown in Table 31. Statistical analysis revealed that no differences were observed (Tables 32 and 33) among and between this parameter.

The mean pharmacokinetic parameters of 250 mg ciprofloxacin tablets obtained in the present study were compared with those reported previously (Bergan et al., 1986; Borner et al.,1986; Brittain et al., 1985; Gonzalez et al., 1984). Results in Table 35 indicated that the values of C_{max}, AUC and K_a in this study were greater than those of others. It should be according that the drug was well and rapidly absorbed from the gastroin-testinal tract of Thai subjects. The variables in these parameters may be due to the interpretation of the differences in subject poppulation entered in the studies (ie. the differences in the race, age, weight and normal habits), assay method or the study condition. The other pharmacokinetic parameters, t_{max} and $t_{1/2}$, were similar to those published earlier.

C. In Vitro-In Vivo Correlative Study

The correlation study between the in vitro and the in vivo parameters were shown in Table 36. The correlation coefficients (r) calculated demonstrated that

there were no statistically significant between the in vitro and the in vivo data. This indicated that the in vitro parameters could not be used precisely to predict the bioavailability of ciprofloxacin tablet.

The pharmacokinetic parameters of brand D were not significant difference from those of other brands despite of slow disintegration of tablet. This was probable due to absorption process of the drug into systemic circulation was slower than drug disintegration and/or dissolution.

Table 29 Analysis of Variance for Absorption Rate Constant of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f[a]	SS[b]	MS[c]	F[d]
Among groups	3	4.93	2.31	1.44
Within groups	44	70.58	1.60	
Total	47	77.51		

F^{e} 0.05 (3,44) = 2.82

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 30 Comparison of Absorption Rate Constant of 3 Brands (B, C, and D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical significance
B	1.45	NS
C	1.32	NS
D	-0.17	NS

t^{a} (0.05, 44) = 2.015

NS = not significant at P > 0.05

a = t-value from the table

Table 31 Biological Half-Life ($t_{1/2}$) of Ciprofloxacin Following Oral Administration of Four Brands of Ciprofloxacin Tablets

Subject No.	$t_{1/2}$ (hr)			
	A	B	C	D
1	3.41	5.39	5.81	4.88
2	4.73	1.65	0.70	3.72
3	0.94	3.88	2.77	2.65
4	4.23	3.01	0.74	4.33
5	4.84	3.68	4.00	4.15
6	4.75	0.80	2.81	3.46
7	4.02	4.69	3.90	5.17
8	4.24	3.32	4.54	3.99
9	3.54	*24.75	4.80	3.45
10	1.30	4.24	3.65	4.86
11	3.54	4.24	4.45	2.64
12	3.89	4.13	5.47	2.38
Mean	3.62	3.55	3.64	3.81
SD	1.21	1.33	1.57	0.93

Table 32 Analysis of Variance for Biological Half-life of Four Brands of Ciprofloxacin Tablets

Source of variation	d.f[a]	SS[b]	MS[c]	F[d]
Among groups	3	0.42	0.41	0.08
Within groups	43	74.33	1.73	
Total	46	74.75		

$F^{e}_{0.05}(3,43) = 2.83$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 33 Comparison of Biological Half-Life of 3 Brands (B, C, and D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical significance
B	-0.13	NS
C	0.04	NS
D	-0.66	NS

t^{a} (0.05, 44) = 2.016

NS = not significant at P > 0.05

a = t-value from the table

Table 34 Estimated Pharmacokinetic Parameters (Mean ± SD) from 12 Subjects Following Oral Administration of Four Brands of Ciprofloxacin Tablets

Brand	C_{max} (cg/ml)	t_{max} (hr)	AUC (mc-hr.ml)	K_a (hr^{-1})	$t_{1/2}$ (hr)
A	2.08 ± 0.52	1.08 ± 0.36	6.87 ± 1.63	2.29 ± 1.06	3.62 ± 1.21
B	1.86 ± 0.48	0.96 ± 0.52	6.59 ± 2.41	3.04 ± 1.45	3.55 ± 1.33
C	2.13 ± 0.42	1.00 ± 0.54	7.27 ± 3.00	2.97 ± 1.51	3.64 ± 1.57
D	2.10 ± 0.45	1.20 ± 0.44	7.42 ± 1.29	2.20 ± 0.63	3.81 ± 0.93
F-test	0.75	0.61	0.32	1.44	0.08
Statistical test	NS	NS	NS	NS	NS

NS = not significant at $p > 0.05$

Table 35 Comparison of Pharmacokinetic Parameters Following a Single Dose Oral Administration of 250 mg Ciprofloxacin Tablet

Study	Pharmacokinetic parameters				
	C_{max} (mcg/ml)	t_{max} (hour)	AUC (mcg.hr/ml)	K_a (hour)	$t_{1/2}$ (hour)
Present Study	1.86-2.13	0.96-1.20	6.59-7.42	2.20-3.04	3.55-3.81
Bergan et al. (1986)	1.59	1.25	5.28	3.37	2.79
Borner et al. (1986)	1.04	1.05	4.23	2.03	5.33
Brittain et al.(1985)	1.45	1.00	6.37	-	3.97
Gonzalez et al.(1984)	1.42	1.05	5.43	1.54	4.19

Table 36 In Vitro-In Vivo Correlations

Correlation	df	r	t-value	Statistical significance
Disintegration time vs Dissolution rate	2	- 0.69	-1.35	NS
Disintegration time vs C_{max}	2	0.30	2.25	NS
Disintegration time vs t_{max}	2	0.88	2.62	NS
Disintegration time vs AUC	2	0.66	1.24	NS
Dissolution rate vs C_{max}	2	- 0.81	-0.51	NS
Dissolution rate vs t_{max}	2	- 0.93	-3.58	NS
Dissolution rate vs AUC	2	- 0.76	-1.65	NS

$t_{0.05, 2} = 4.302$

NS : not significant at $p > 0.05$