CHAPTER IV

RESULTS AND DISCUSSION

In Vitro Studies

Regarding to fundamental in determining dosage form effectiveness, all six commercial brands of norfloxacin tablets were tested for uniformity of weight and content of active ingredient. Results summarized in Table 2 revealed that each brand met the United State Pharmacopoeial requirement for uniformity of weight within the range of limitation (± 5%). The content of active ingredient of all brands were within the 90-110% limits as specified by the United State Pharmacopoeia XXII monograph (United States Pharmacopoeial Convention, Inc., 1990). Therefore these data indicated that all various brands were pharmaceutically equivalent.

The purpose of disintegration test is to monitor uniform tablet and do serve as a component in the overall quality control of tablet manufacture (Shargel and Yu, 1985). Neither the United State Pharmacopoeia XXII nor the British Pharmacopoeia 1988 contains a disintegration time specification for norfloxacin tablet. However, disintegration time requirement is currently official for film coated tablet under the British Pharmacopoeia 1988. Most of the norfloxacin tablets disintegrated completely in distilled water within limit time (less than 60

minutes) except for those of brand C in which the disintegration time was over one hour, evenafter it was placed in 0.1 N Hydrochloric acid, the disintegration time was still exceeding an hour as seen in Table 2. The rank orders in term of mean disintegration time were brands C > D > F > E > B > A. Statistical comparison indicated that when compared with brand A, the disintegration times of brands C, D and F were significantly longer ($p < 0.05$) as shown in Tables 3 and 4.

Many factors affect the rate of tablet disintegration for example differences in composition of tablet (eg. diluent, filler, type of lubricant, surfactant and binder etc.) manufacturing method, compressional force, hardness, concentration of disintegrant and method of addition, type and composition of coating, age of finished product and storage conditions, etc (Niazi, 1979; Shargel and Yu, 1985).

The disintegration of tablet although is the important process of the rate disintegration is unlikely to be the rate limiting step in the absorption of drug administered (Gilbaldi, 1984; Shargel, 1985). Only the disintegration of the tablet does not assure the ultimate dissolution which may be retarded by hydrophobic lubricants in the formulation (Niazi, 1979). Disintegration test does not guarantee that the drug will in effect dissolve, therefore a dissolution test performed on the drug product is a better guide to its

Table 2 Physical Characteristics of In Vitro studies of six Commercial Brands of Norfloxacin Tablets

Brand	Weight[a] (g)	% Labelled[b] amount	Disintegration[c] time (min)	% Dissolved[c] at 30 min	Dissolution[c] rate (hr^{-1})
A	0.6441±0.0037	103.28±0.63	1.79±0.29	123.19±5.40	12.77±4.13
B	0.5634±0.0073	101.92±0.13	3.96±0.32	97.14±3.34	13.97±5.65
C	0.8220±0.0114	110.87±1.05	63.54±7.18	9.20±1.41	0.52±0.11
D	0.5376±0.0181	104.98±5.06	6.86±3.77	114.61±10.36	12.69±6.18
E	0.6447±0.0096	98.15±0.70	5.78±0.70	98.31±14.36	5.60±2.53
F	0.7225±0.0041	105.50±4.49	6.37±1.29	107.58±4.04	9.48±1.63

a = values are mean ± standard deviation (n=20)
b = values are mean ± standard deviation (n = 3)
c = values are mean ± standard deviation (n = 6)

Table 3 Analysis of Variance for Disintegration Time of Six Brands of Norfloxacin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	5	17266.33	3453.26	253.41
Within groups	30	408.82	13.63	
Total	35	17675.15		

$$F^{e}_{0.05(5,30)} = 2.53$$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 4 Comparison of Disintegration Time of Locally Manufactured Products with Innovator's Product (Brand A) in buffer pH 4.0. Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	1.0228	NS
C	28.9776	S
D	2.3835	S
E	1.8721	NS
F	2.1536	S

$$t^{a}_{(0.05,30)} = 2.042$$

S = significant at $p < 0.05$

NS = not significant at $p > 0.05$

a = t - value from the table

subsequent bioavailability. Dissolution rate testing is the most sensitive and reliable mean to assure product quality and uniformity, assure bioequivalence among generic drug products and rotational for use in correlating or predicting in-vivo drug products bioavailability behavior. Consequently the FDA bioavailability/bioequivalency regulations rely on in-vitro dissolution rate studies to assure lot to lot bioequivalence (Smolen and Ball, 1984).

The dissolution test according to the Method II in the United State Pharmacopoeia XXII, the compendial monograph dissolution requirement that the amount of norfloxacin dissolved from the tablets after 30 minutes should not less than 80% of the labelled amount, was used in this study. Figure 2 and Table 5, illustrated the dissolution profiles at various times of all six brands of norfloxacin tablets in buffer pH 4.0. Brands A, B, D, E and F reached the equibilrium state within 120 minutes whereas the equilibrium state of brand C extended to 270 minutes. Results from Table 5 demonstrated that only brand C failed to meet the specification. The percent dissolved at 30 minute of brand C was only 9%.

The dissolution rate constants (K_d) were calculated from the slope of the first order plot between the amount of undissolved norfloxacin ($B_\infty - B_t$) versus time in semi-logarithmic scale and the corresponding values were reported in Table 2. Rank order of six

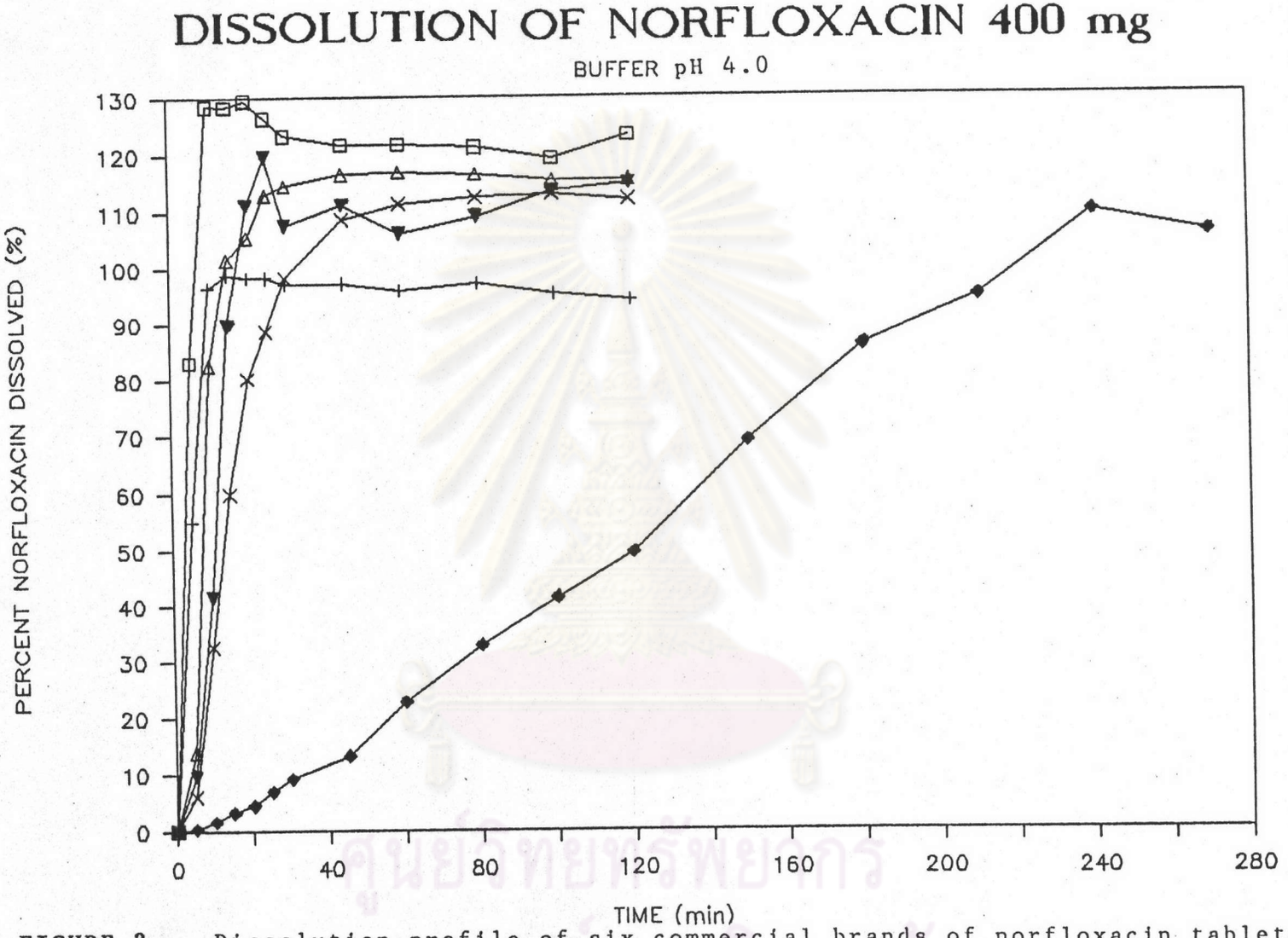


FIGURE 2 Dissolution profile of six commercial brands of norfloxacin tablets in buffer pH 4.0

KEY : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)
Brand E (×) , Brand F (▼)

Table 5 Dissolution Data of Six Brands of Norfloxacin Tablets in buffer pH 4.0

Brand / Time (min)	Percent Norfloxacin Dissolved[a]					
	A	B	C	D	E	F
5	83.19±33.01	54.92±17.70	0.30± 0.05	13.85±13.35	6.09± 6.91	9.62± 5.59
10	128.41± 4.69	96.43± 5.17	1.59± 0.13	82.66±31.91	32.59±21.84	41.55±20.02
15	128.32± 3.61	98.92± 5.54	3.17± 0.43	101.60±33.41	59.85±27.59	89.78±12.56
20	129.44± 4.62	98.39± 4.91	4.38± 0.65	105.54±26.95	80.33±17.59	111.21± 4.74
25	126.36± 3.87	98.30± 4.38	6.87± 1.08	113.03±16.72	88.77±18.80	119.66± 7.15
30	123.19± 5.40	97.14± 3.34	9.20± 1.41	114.61±10.36	98.31±14.36	107.58± 4.04
45	121.70± 1.98	97.23± 2.62	13.30± 1.89	116.57± 3.06	108.58± 7.02	111.21± 4.49
60	121.70± 2.20	95.89± 2.72	22.72± 3.04	116.85± 2.18	111.30± 5.80	106.12± 6.34
80	121.14± 3.22	97.14± 2.89	32.80± 4.51	116.38± 1.70	112.39± 7.00	109.03± 5.56
100	119.09± 4.52	95.18± 2.11	41.37± 5.29	115.36± 9.17	112.93± 6.75	113.57± 2.93
120	123.10± 4.64	94.02± 2.02	49.37± 7.47	115.45± 1.80	111.94±10.99	114.75± 1.79
150			68.85±12.11			
180			85.81±11.76			
210			94.55±11.76			
240			109.63± 8.88			
270			105.80± 4.87			

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

brands in terms of dissolution rate constant were brands B > A > D > F > E > C. Comparison of the dissolution rate constants by analysis of variance and student's t-test were presented in Tables 6 and 7. Dissolution rate of brands C and E were statistically significant lower than that of brand A ($p < 0.05$) whereas the others were not. The reason that brand C showed poor dissolution kinetic might be the same factors which affect the disintegration time especially variations in the formulation and manufacturing process. This means that brand C was different in the rate and extent of dissolution from the innovator's product (brand A).

Gibaldi (1984) proposed that there are examples of drug products which fail to meet the compendial standards for dissolution but nevertheless are relatively well absorbed. In contrast to this study, Smolen and Ball (1984) stated that pharmaceutical products which meet identical the United State Pharmacopoeia dissolution testing requirements can differ significantly in their bioavailabilities.

In the present study, three locally different brands based on high, medium and low dissolution characteristics were selected to assess bioavailability relative to innovator's product.

Table 6 Analysis of Variance for Dissolution Rate Constant of Six Commercial Brands of Norfloxacin Tablets in buffer pH 4.0

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	5	816.54	163.31	6.28
Within groups	30	779 .63	25.99	
Total	35	1596.17		

$F^{e}_{0.05(5,30)} = 2.53$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 7 Comparison of Dissolution Rate Constant of Locally Manufactured Products with Innovator's Product (Brand A) in buffer pH 4.0. Using Student's t - test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	0.4077	NS
C	4.1621	S
D	0.0272	NS
E	2.4361	S
F	1.1178	NS

$t^{a}_{(0.05,30)} = 2.042$

S = significant at $p < 0.05$

NS = not significant at $p > 0.05$

a = t - value from the table

In Vivo Studies

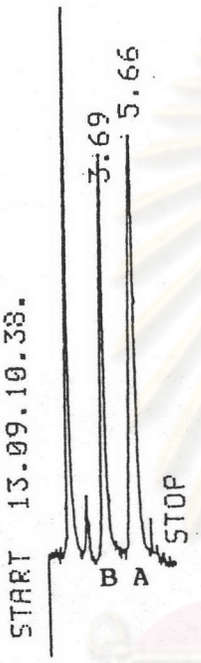
Although dissolution test may assess bioavailability, however such tests are useful mainly as means of screening preliminary formulations and as a routine quality control procedure. The FDA has indicated clearly that in-vitro testing alone often dose not ensure bioequivalence. Judgement of bioequivalency should be based on studies of bioavailability and clinical responses in humans (Smolen and Ball, 1984). Certainly, in this research the bioavailability study was carried out in healthy volunteer. Three local manufactured brands of norfloxacin tablets which showed maximum (brands B), moderate (brand D) and minimum (brand C) dissolution values were compared to the innovator's product (brand A).

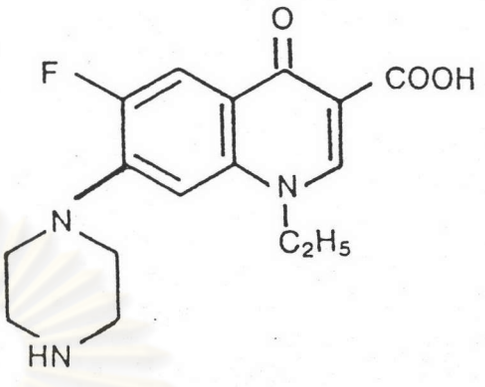
1. **Analysis of Norfloxacin in Plasma Samples**

Several high-pressure liquid chromatographic methods have already been described for the determination of norfloxacin. Those methods consisted many steps for sample preparation (Boppana and Swanson, 1982; Montay and Tassel, 1985). Besides, most of the method described have not incorporated an internal standard (Boppana and Swanson, 1982; Forchetti et al., 1984). In this study the method employs a single-step protein precipitation of norfloxacin and the internal standard was used in the analysis. According to the discovery of Montay and

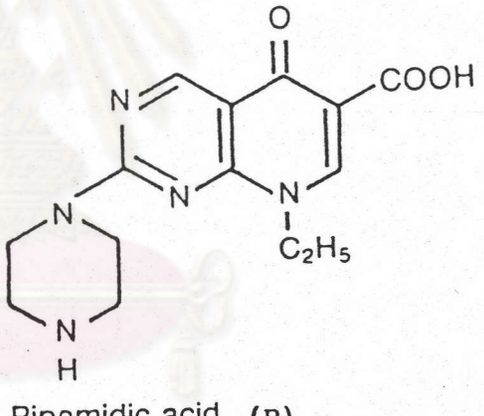
Tassel (1985) that the use of fluorometric detection is disadvantageous since the alkaline pH of the mobile phase dramatically decreases the fluorescent properties of norfloxacin. Thus, during the development for improving the assay, an acidic mobile phase on C_{18} reversed phase-ion-pairing HPLC sorbents was also investigated and found that this acidic mobile phase enables the fluorometric detection.

Typical chromatograms of norfloxacin and internal standard are shown in Figure 3. The retention times of norfloxacin and internal standard were 5.66 and 3.69 minutes, respectively. Minimum detectable concentrations was 0.1 µg/ml. The absolute recovery of norfloxacin and internal standard in plasma was evaluated by comparing the peak height from methanol - extracted with the peak height from aqueous solutions. Percent recovery calculated by dividing the height of the plasma sample peak by that of the aqueous sample peak for norfloxacin and internal standard averaged 104.67 ± 9.84 and 101.00 ± 12.08 %, respectively (n = 3). The HPLC readily provided linear results in the normal analytical range. Calibration curves for norfloxacin in plasma were linear up to at least 2.5 µg/ml and typical least-square regression lines for peak height ratio were Y = 0.7233 X + 0.0084 (r^2 = 0.999) (Y = peak height ratio, X = concentration in µg/ml).





Norfloxacin (A)



Pipemidic acid (B)

Figure 3 High Pressure Liquid Chromatograms of Plasma spiked with 1.5 µg/ml of Norfloxacin (A) and 2.5 µg/ml of Internal Standard Pipemidic acid (B)

2. **Clinical Observation**

No side effects and/or any indication of intoxications were associated following oral administration of norfloxacin tablets to volunteers throughout the study.

3. **Plasma Norfloxacin Level**

The plasma levels of norfloxacin at each sampling time ranging from 0 to 12 hours after administration of brands A, B, C and D are depicted in Tables 8 to 11, respectively. Individual plasma norfloxacin concentration-time profile for 12 subjects were shown graphically in Figures 4-15. As can be seen after achievement of peak concentrations, all plasma concentration versus time curves exhibit an exponential decline over time. Comparison of the mean plasma concentration-time profile for each product were summarized in Figures 16 and 17.

4. **Bioavailability Evaluation**

Bioavailability is an absolute term that indicates the measurement of both rate and amount (extent) of drug reaching the systemic circulation after an administered dosage form. These factors can be evaluated by determining pharmacokinetic parameters derived from plasma concentration versus time curves. In bioequivalence study, a drug in similar dosage forms meet

Table 8 Plasma Norfloxacin Concentrations* (ug/ml) from 12 Subjects Following Oral Administration of 400 mg. Norfloxacin 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
1	0.0000	0.3541	1.1163	1.3691	1.4298	1.0464	0.4919	0.3383	0.2581	0.1878
2	0.0895	0.0182	0.0182	0.2137	1.1203	0.8187	0.4935	0.3181	0.2021	0.1569
3	0.7566	1.4552	1.6137	1.6938	1.2200	0.7711	0.6380	0.4768	0.3918	0.1974
4	0.4078	1.4177	1.1091	0.8302	0.7674	0.7042	0.6276	0.3749	0.2788	0.1800
5	0.7367	1.3868	0.8939	0.9019	0.7391	0.5594	0.4743	0.4020	0.3660	0.2220
6	0.8350	2.0701	1.8207	1.5410	1.1580	1.2031	1.0012	0.6835	0.5666	0.3579
7	0.3723	2.1559	2.7087	3.0112	2.5378	1.7267	1.2389	0.8514	0.5783	0.3376
8	0.3933	0.5715	0.4030	0.4611	0.3719	0.3651	0.2408	0.2063	0.2023	0.1938
9	1.9603	3.9772	2.9336	1.8048	1.5057	1.1984	0.9472	0.6794	0.3550	0.2630
10	0.2105	1.7408	2.9792	2.4079	1.5280	1.1584	0.9102	0.6117	0.5083	0.2369
11	0.8524	1.4381	2.0576	2.0117	2.1948	1.5200	1.1961	0.7884	0.6331	0.3015
12	0.1905	0.5945	0.6334	0.4319	0.4559	0.3633	0.2968	0.2330	0.2062	0.1114
MEAN	0.5686	1.4317	1.5239	1.3899	1.2524	0.9529	0.7131	0.4970	0.3789	0.2288
SEM	0.1455	0.2899	0.2756	0.2365	0.1797	0.1195	0.0932	0.0605	0.0436	0.0205

* concentration = 0 at t = 0 for every subjects

Table 9 Plasma Norfloxacin Concentrations* (µg/ml) from 12 Subjects Following Oral Administration of 400 mg Norfloxacin 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
1	1.4865	2.5229	1.4045	1.3819	1.0522	0.7161	0.5640	0.4066	0.3169	0.1983
2	1.0899	1.9367	2.4761	1.9734	1.7051	1.3936	0.8094	0.4977	0.5737	0.2067
3	0.2616	0.8047	1.3136	1.7301	2.0878	1.6534	1.0376	0.8402	0.6249	0.4293
4	0.5731	1.0827	1.0849	0.7824	0.7478	0.6780	0.6113	0.4372	0.3255	0.1429
5	0.3655	2.2150	2.1780	1.5789	1.4629	1.3408	1.0840	0.6721	0.5694	0.3268
6	1.4022	2.9975	2.1954	1.7359	1.4782	1.4753	1.1752	0.8574	0.6145	0.4986
7	0.1967	1.0655	1.6780	1.9930	1.4909	1.2343	1.0536	0.6423	0.5018	0.2613
8	1.8753	1.7048	1.8179	1.8712	1.0280	1.2489	0.6993	0.6221	0.5220	0.3023
9	0.0182	1.2949	1.0452	0.9326	0.8950	0.7041	0.6296	0.3725	0.2898	0.1914
10	0.1180	0.2087	0.2652	0.1934	0.3114	0.2081	0.1934	0.1169	0.0845	0.1030
11	0.1180	0.5177	0.5318	0.9218	1.4214	1.5982	0.9997	0.5809	0.4381	0.2431
12	1.9750	3.0772	2.1366	1.2328	1.0429	0.7755	0.5850	0.5269	0.4194	0.1813
MEAN	0.7817	1.6190	1.5106	1.3606	1.2270	1.0855	0.7869	0.5477	0.4401	0.2571
SEM	0.2047	0.2618	0.1922	0.1548	0.1312	0.1253	0.0801	0.0568	0.0447	0.0321

* concentration = 0 at t = 0 for every subjects

Table 10 Plasma Norfloxacin Concentrations* (µg/ml) from 12 Subjects Following Oral Administration of 400 mg Norfloxacin 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
1	0.0000	0.1063	0.1233	0.0929	0.1011	0.0778	0.0985	0.0798	0.0000	0.0000
2	0.0784	0.1701	0.1403	0.1495	0.1961	0.1526	0.0819	0.0791	0.0569	0.0056
3	0.2622	0.3730	0.2816	0.2221	0.2094	0.1980	0.1381	0.0954	0.0704	0.1073
4	0.0000	0.1049	0.2598	0.3640	0.6082	0.4390	0.2505	0.1835	0.1673	0.0913
5	0.1011	0.1610	0.1108	0.1110	0.1232	0.0791	0.0939	0.0753	0.0056	0.0000
6	0.0529	0.1345	0.1312	0.1227	0.1198	0.1073	0.1081	0.0703	0.0833	0.0000
7	0.1563	0.2100	0.1837	0.1378	0.1105	0.1350	0.0828	0.0887	0.0450	0.0000
8	0.2818	1.0806	1.2012	1.8296	1.7248	1.2424	0.8902	0.6848	0.5107	0.3126
9	0.1106	0.6114	1.0152	1.5618	1.2171	1.3389	0.7046	0.5761	0.3260	0.2159
10	0.0000	0.0000	0.0000	0.1297	0.3572	0.3917	0.8094	0.9102	0.4900	0.2506
11	0.0000	0.4385	0.6048	0.6326	0.4833	0.3872	0.3466	0.1869	0.1160	0.0896
12	0.0000	0.3536	0.4625	0.5056	0.4430	0.2558	0.3431	0.1658	0.1080	0.0712
MEAN	0.0869	0.3120	0.3762	0.4883	0.4745	0.4004	0.3290	0.2663	0.1649	0.0953
SEM	0.0280	0.0820	0.1054	0.1637	0.1398	0.1201	0.0837	0.0797	0.0494	0.0302

* concentration = 0 at t = 0 for every subjects

Table 11 Plasma Norfloxacin Concentrations* (µg/ml) from 12 Subjects Following Oral Administration of 400 mg Norfloxacin Tablets of Brand D

Subject No.	Time (hr)									
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	12.0
1	1.2794	2.3017	2.4933	1.7086	1.3685	1.0238	0.8124	0.4952	0.3359	0.1910
2	0.1545	0.6452	1.0423	2.1583	1.9272	1.9612	0.9848	0.7246	0.6362	0.3365
3	0.2356	3.2105	1.9744	1.9362	1.3248	1.1090	0.7677	0.6150	0.4121	0.2722
4	0.0000	0.3029	0.8090	1.2041	1.4013	1.6235	0.6058	0.6112	0.4293	0.2167
5	0.5731	0.8873	0.8981	0.9078	0.8560	0.6749	0.5396	0.3566	0.2864	0.1555
6	0.2910	1.5122	2.4910	2.6868	2.2245	2.4603	1.6090	0.9069	0.6998	0.4159
7	0.1327	1.4488	1.9439	1.8361	1.2927	1.1842	0.7913	0.4984	0.4386	0.2819
8	1.0402	1.8577	2.1077	1.8497	1.4662	1.2334	0.8475	0.6560	0.5829	0.2928
9	1.1858	3.3040	2.2742	1.4817	1.2753	0.9036	0.6853	0.4297	0.3648	0.2571
10	0.4867	1.0095	1.5111	1.4389	1.2250	0.9894	0.6476	0.4609	0.4351	0.2187
11	0.0000	0.0000	0.3424	1.0427	1.1427	1.5986	0.8563	0.5418	0.4356	0.2194
12	0.1980	0.7577	0.9302	1.0884	0.8579	0.9252	0.6156	0.4235	0.3173	0.1859
MEAN	0.4652	1.4369	1.5681	1.6116	1.3635	1.3073	0.8136	0.5600	0.4478	0.2536
SEM	0.1268	0.2943	0.2044	0.1439	0.1076	0.1417	0.0777	0.0423	0.0355	0.0200

* concentration = 0 at t = 0 for every subjects

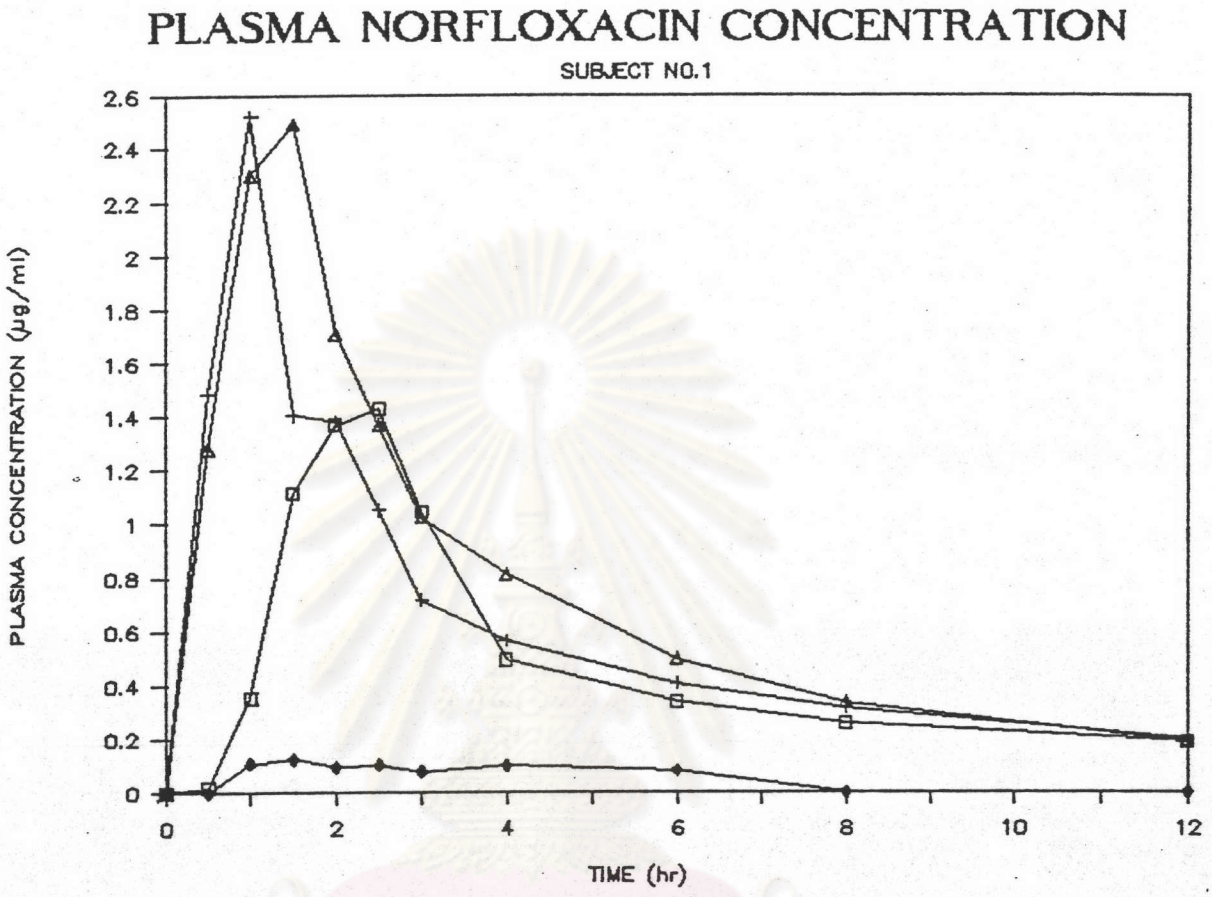


Figure 4 Plasma Norfloxacin Concentration-time Profile of Subject No.1 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (◆) , Brand D (Δ)

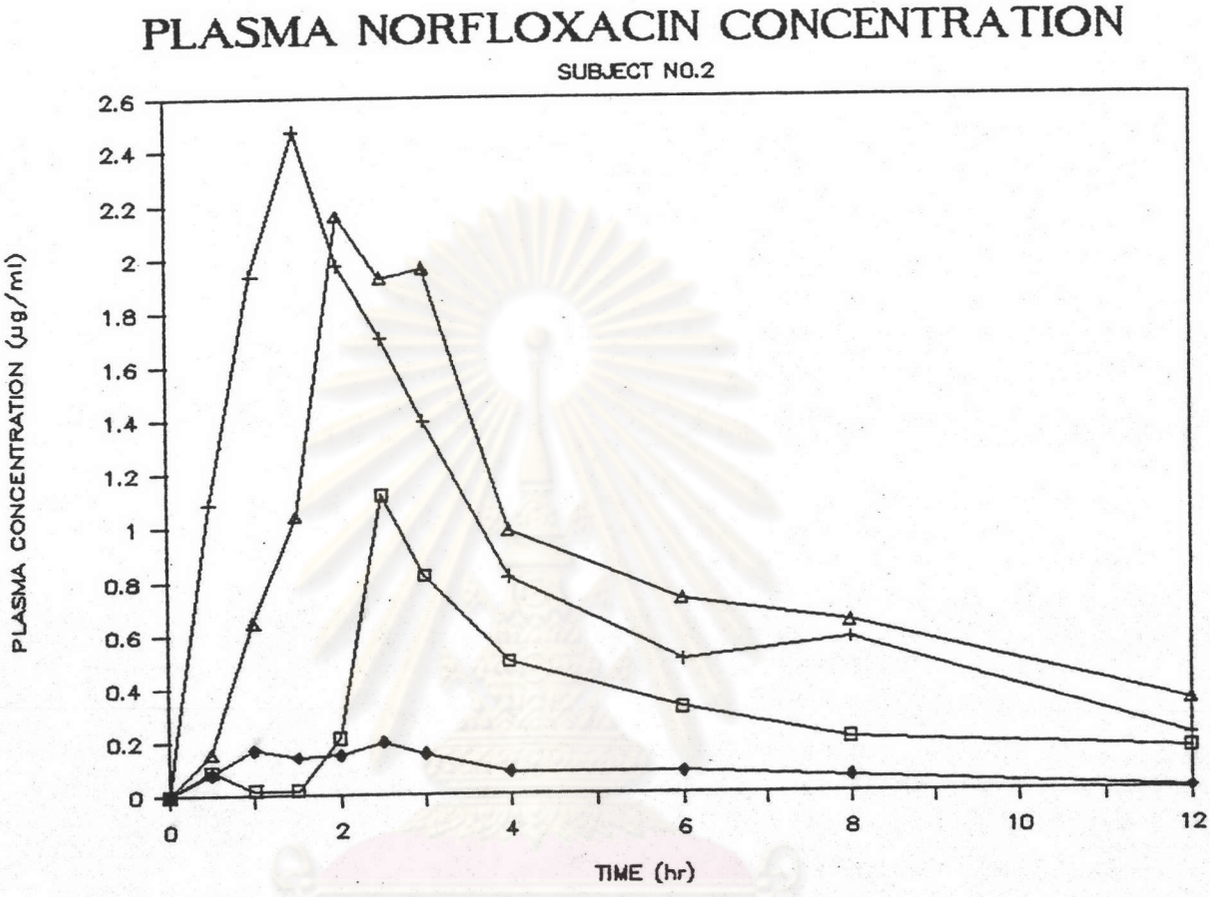


Figure 5 Plasma Norfloxacin Concentration-time Profile of Subject No.2 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

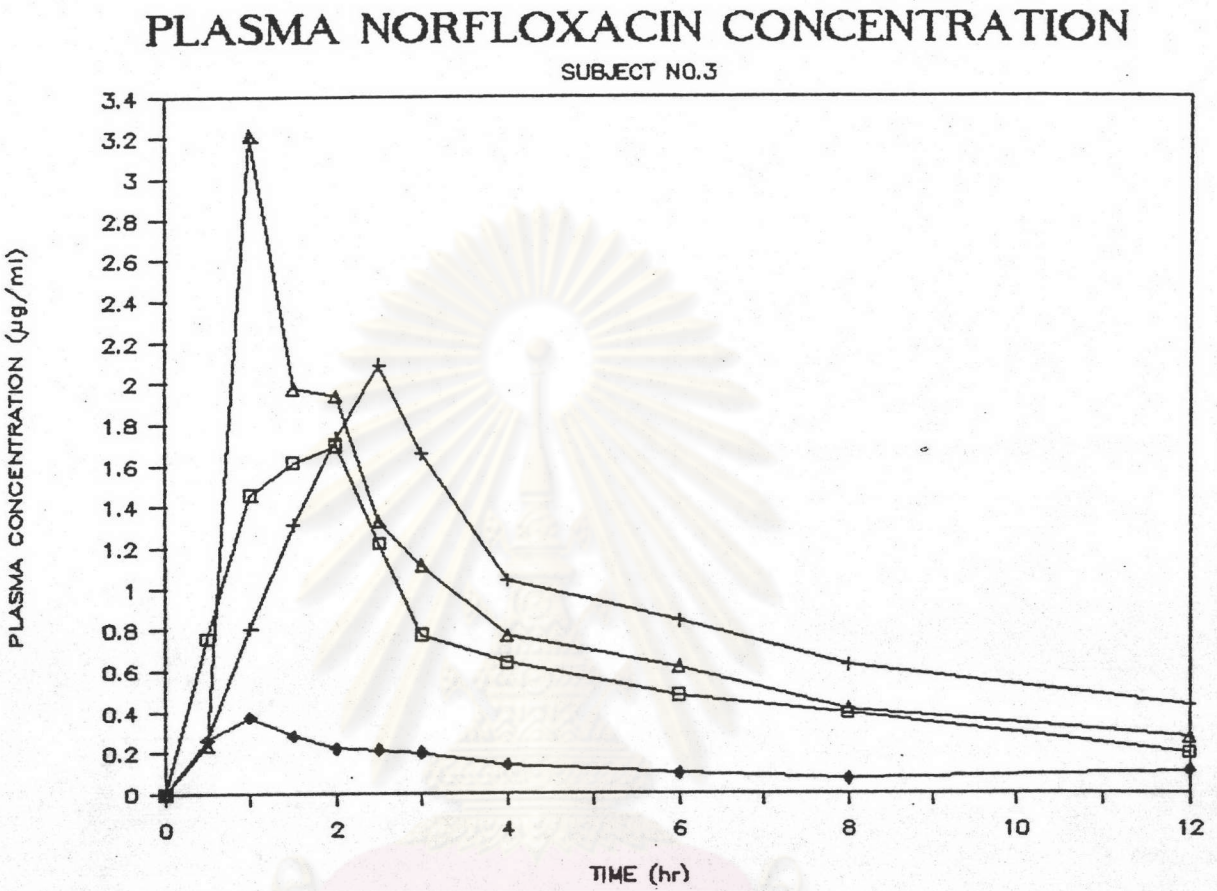


Figure 6 Plasma Norfloxacin Concentration-time Profile of Subject No.3 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

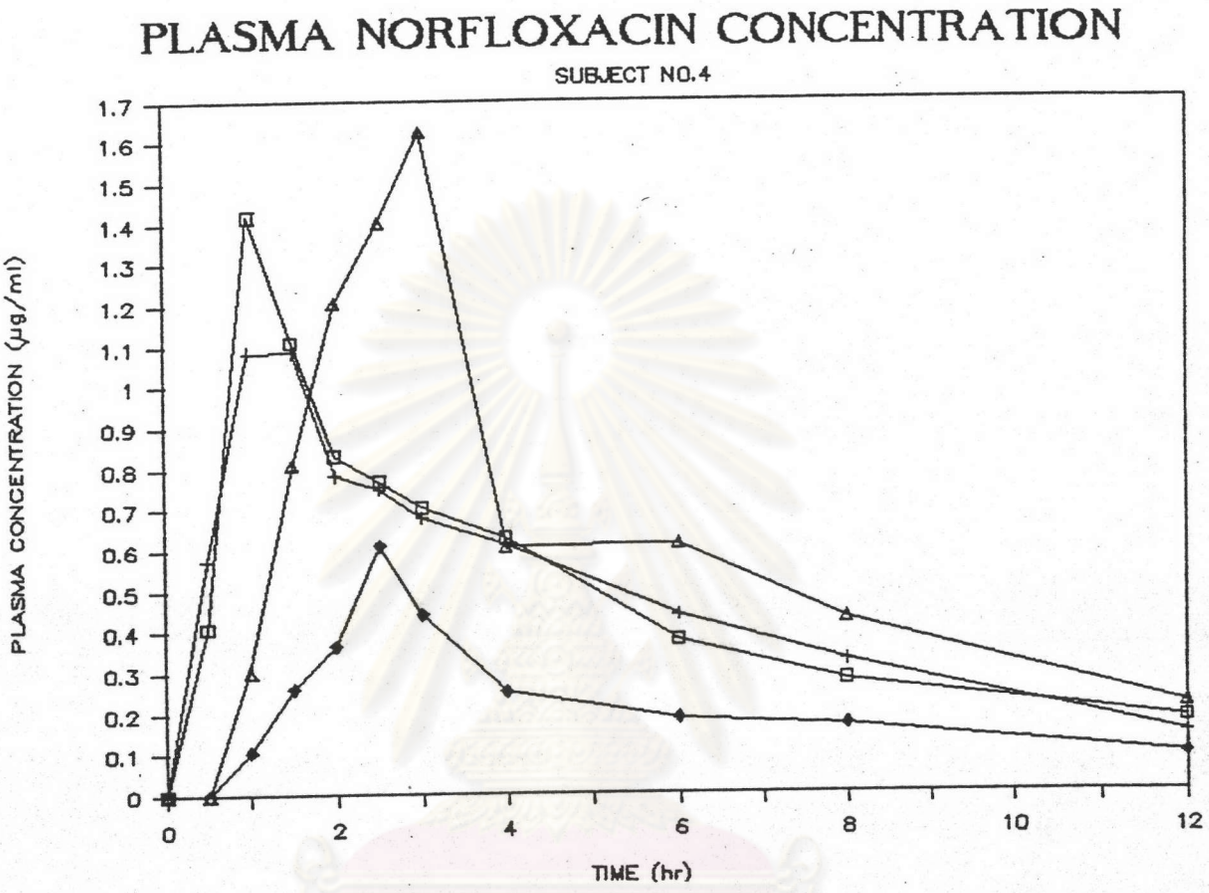


Figure 7 Plasma Norfloxacin Concentration-time Profile of Subject No.4 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

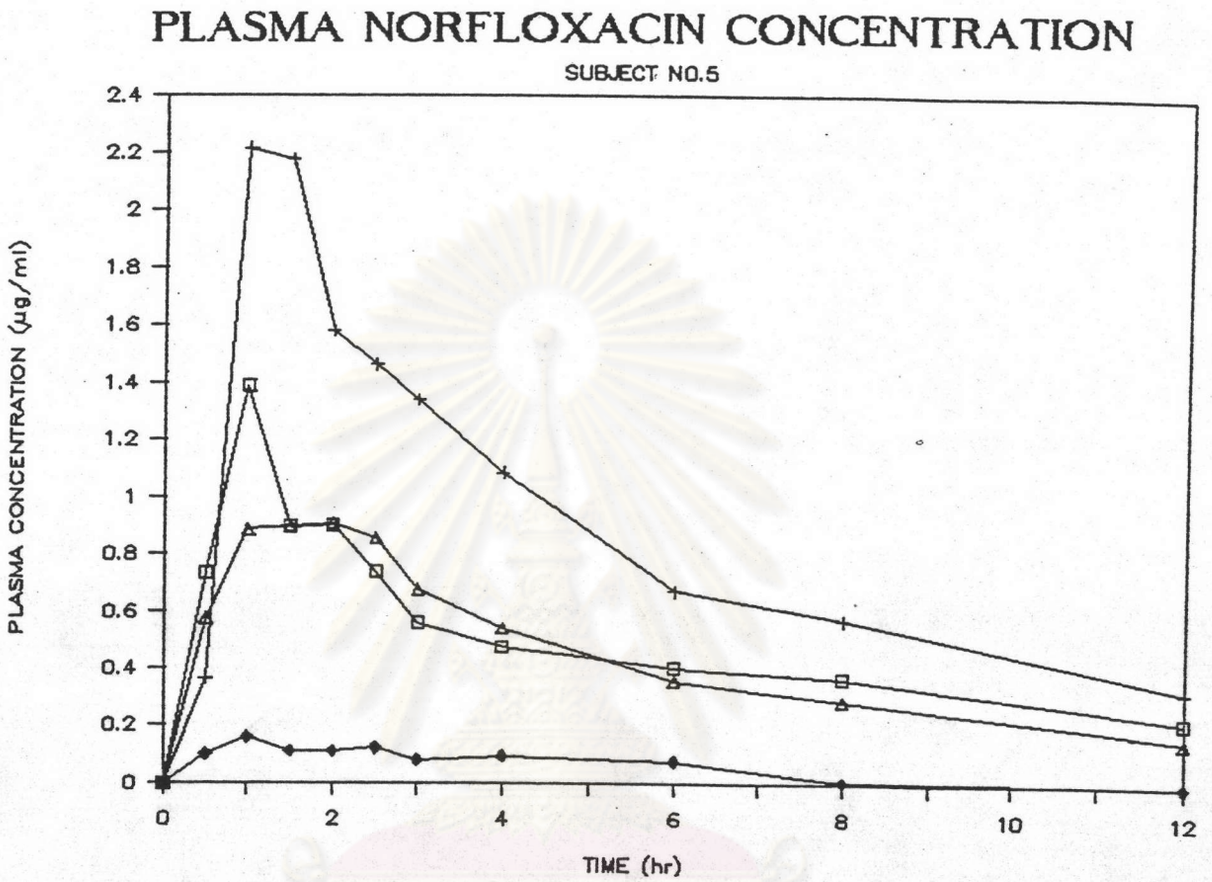


Figure 8 Plasma Norfloxacin Concentration-time Profile of Subject No.5 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

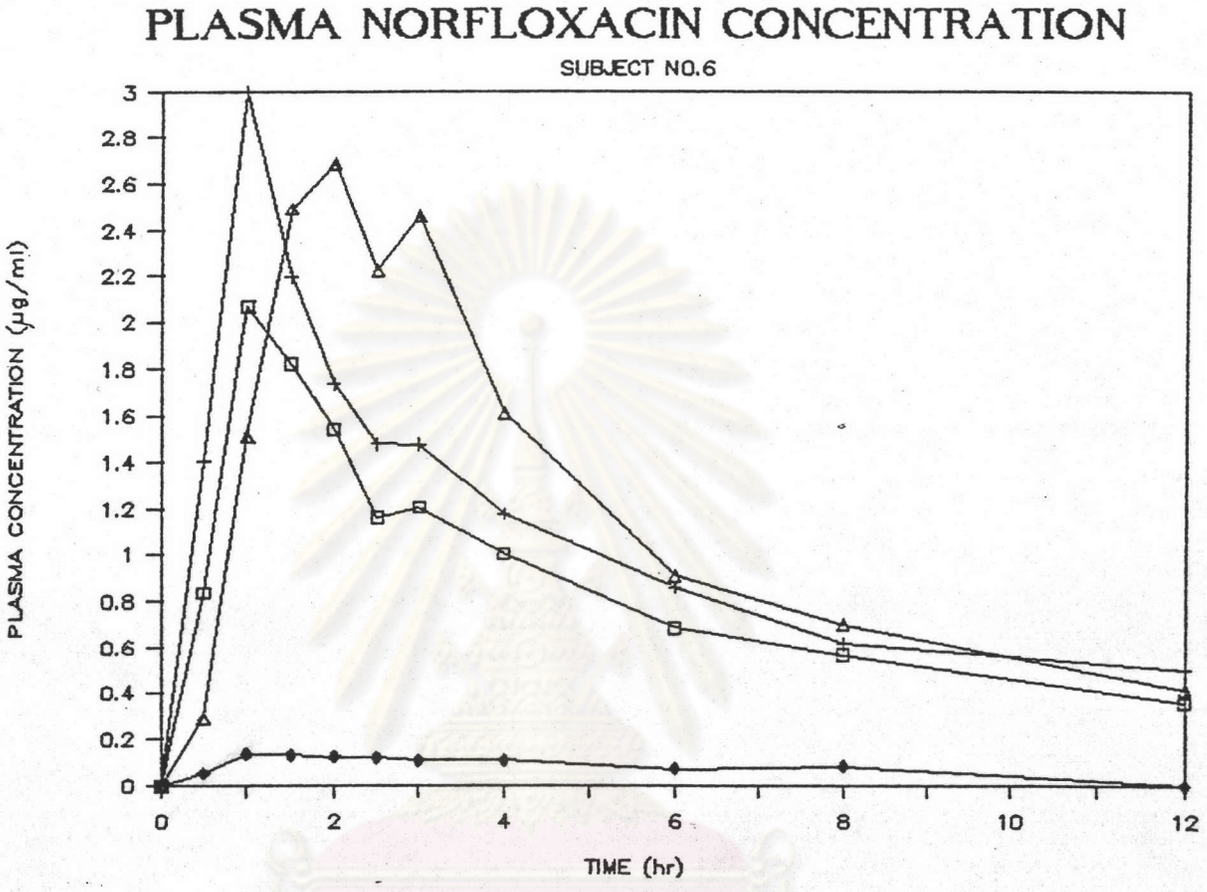


Figure 9 Plasma Norfloxacin Concentration-time Profile of Subject No.6 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

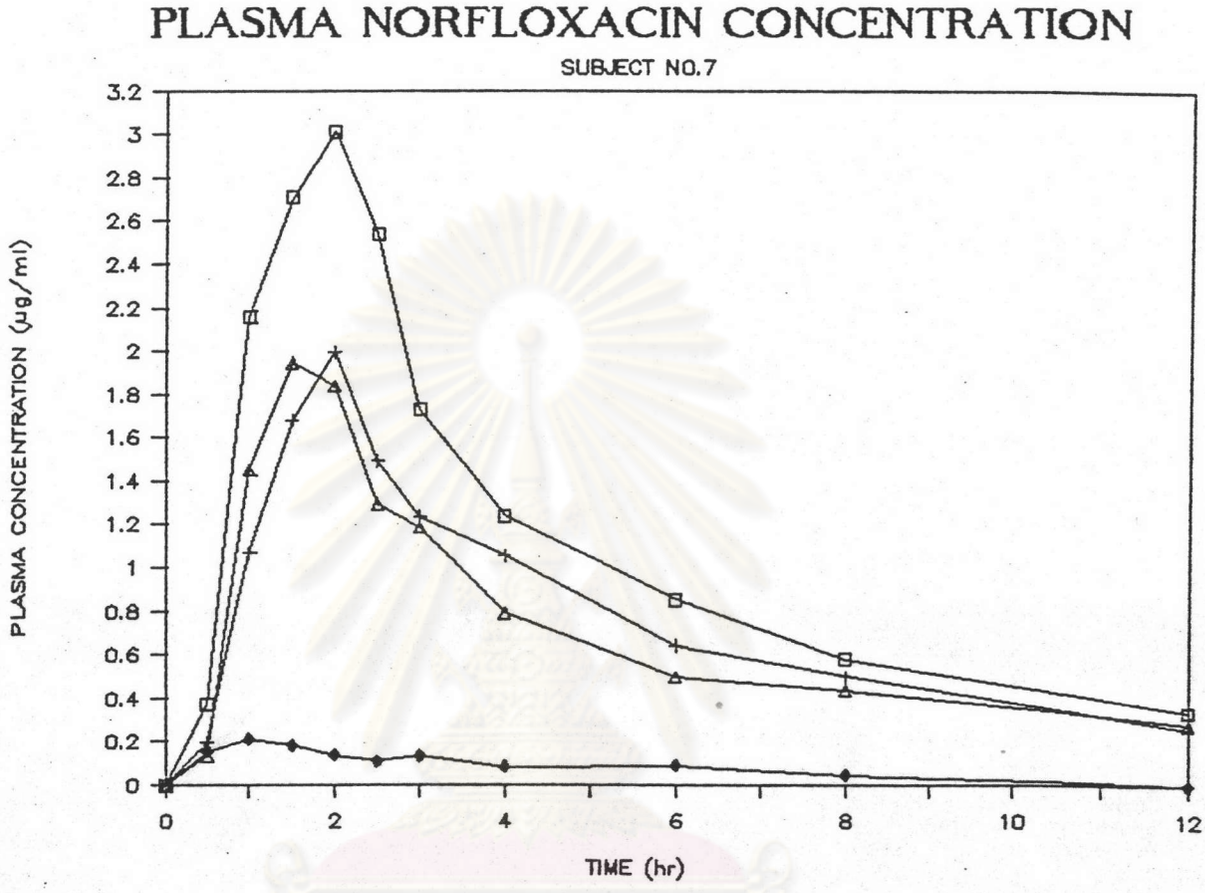


Figure 10 Plasma Norfloxacin Concentration-time Profile of Subject No.7 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

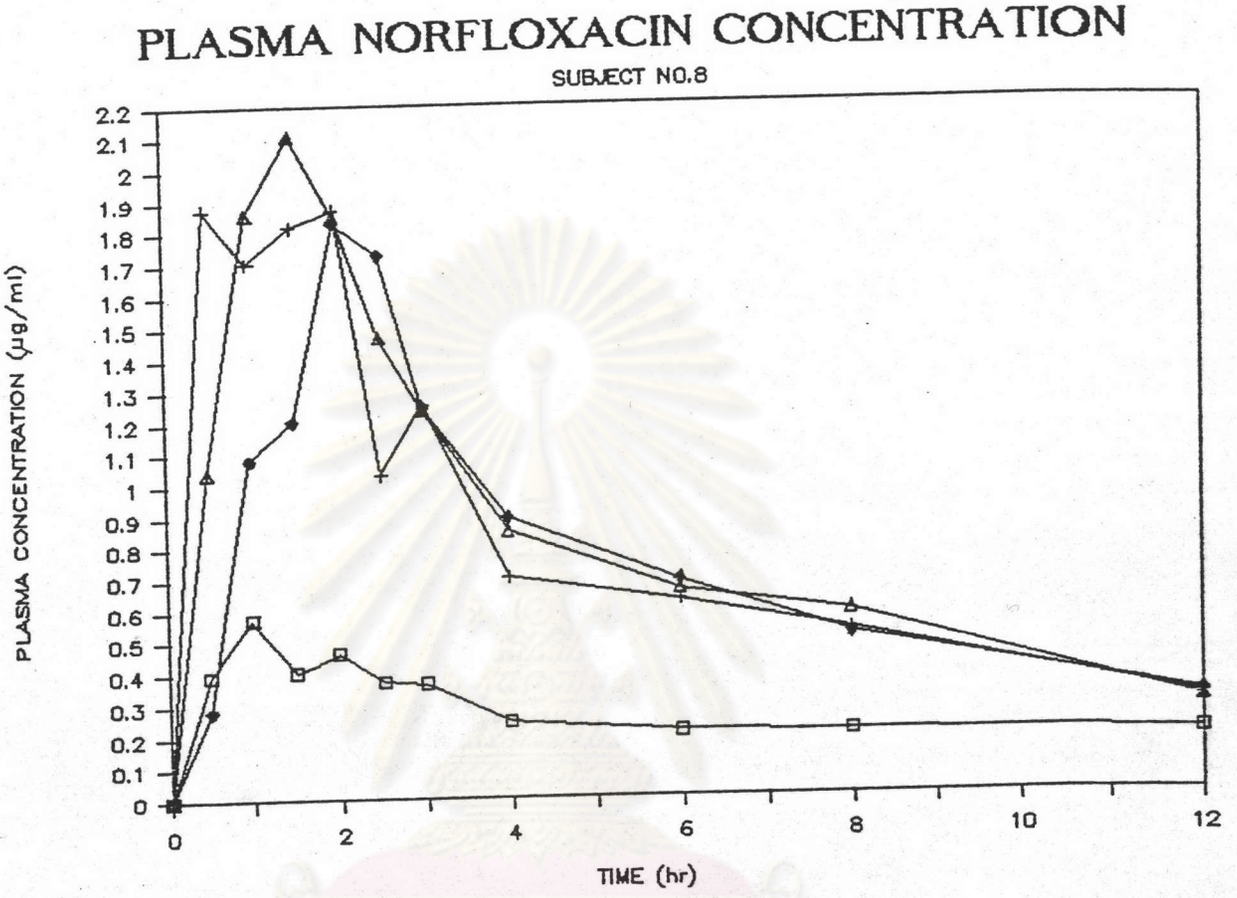


Figure 11 Plasma Norfloxacin Concentration-time Profile of Subject No.8 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

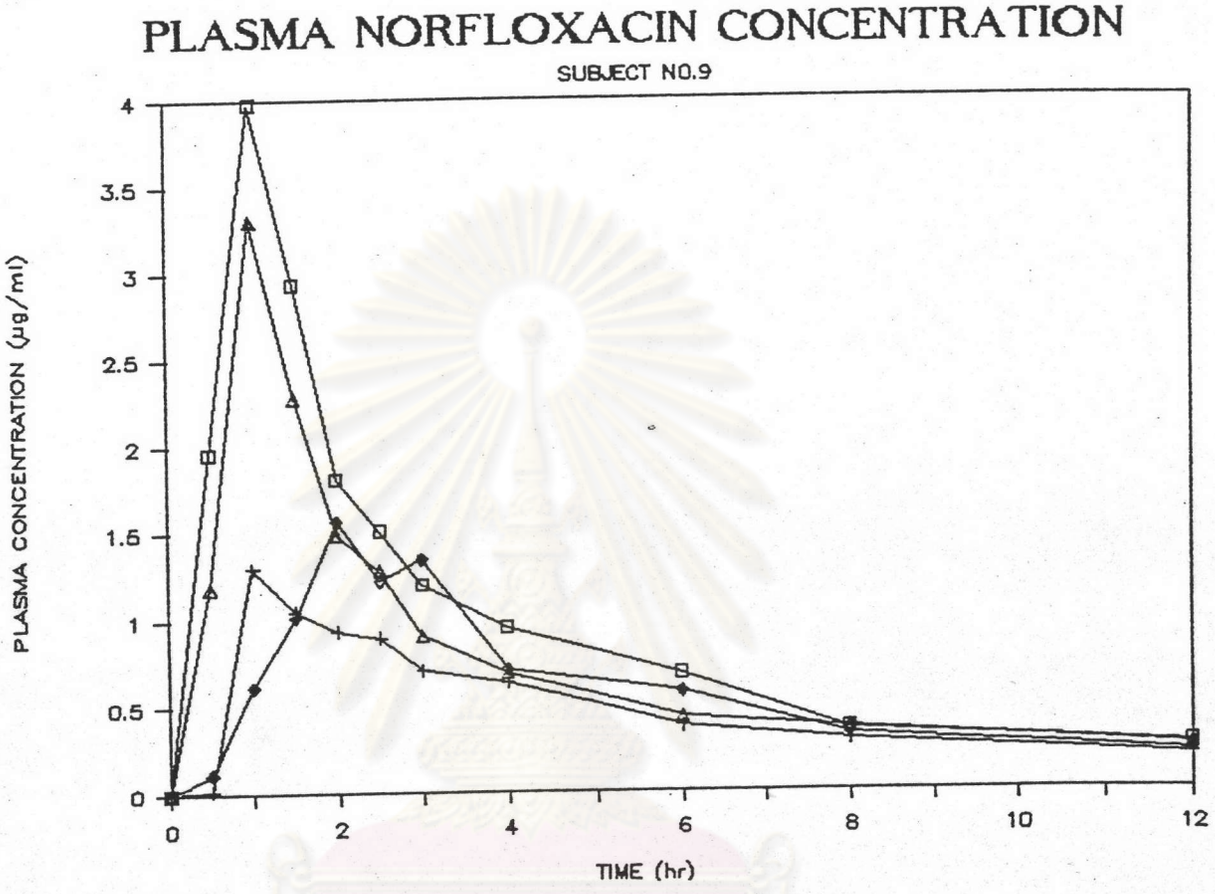


Figure 12 Plasma Norfloxacin Concentration-time Profile of Subject No.9 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

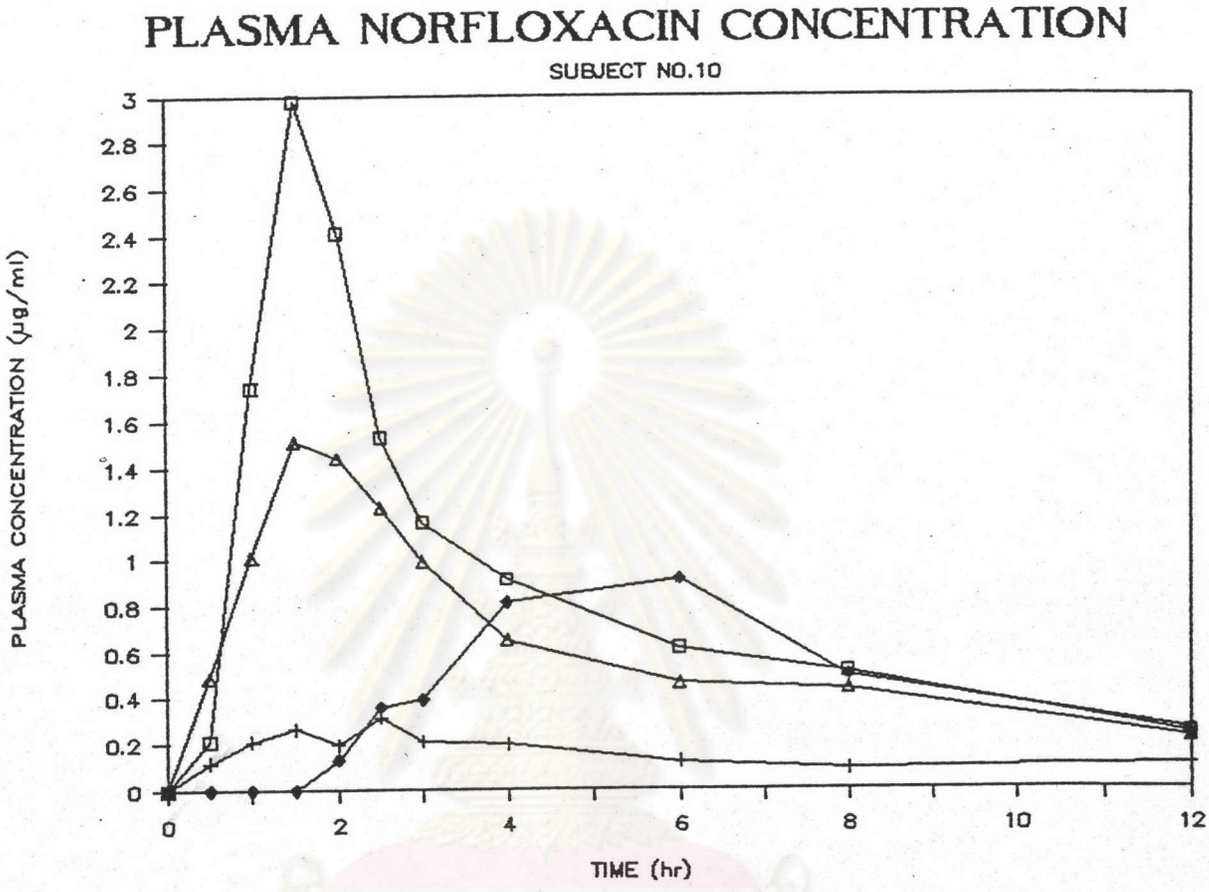


Figure 13 Plasma Norfloxacin Concentration-time Profile of Subject No.10 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

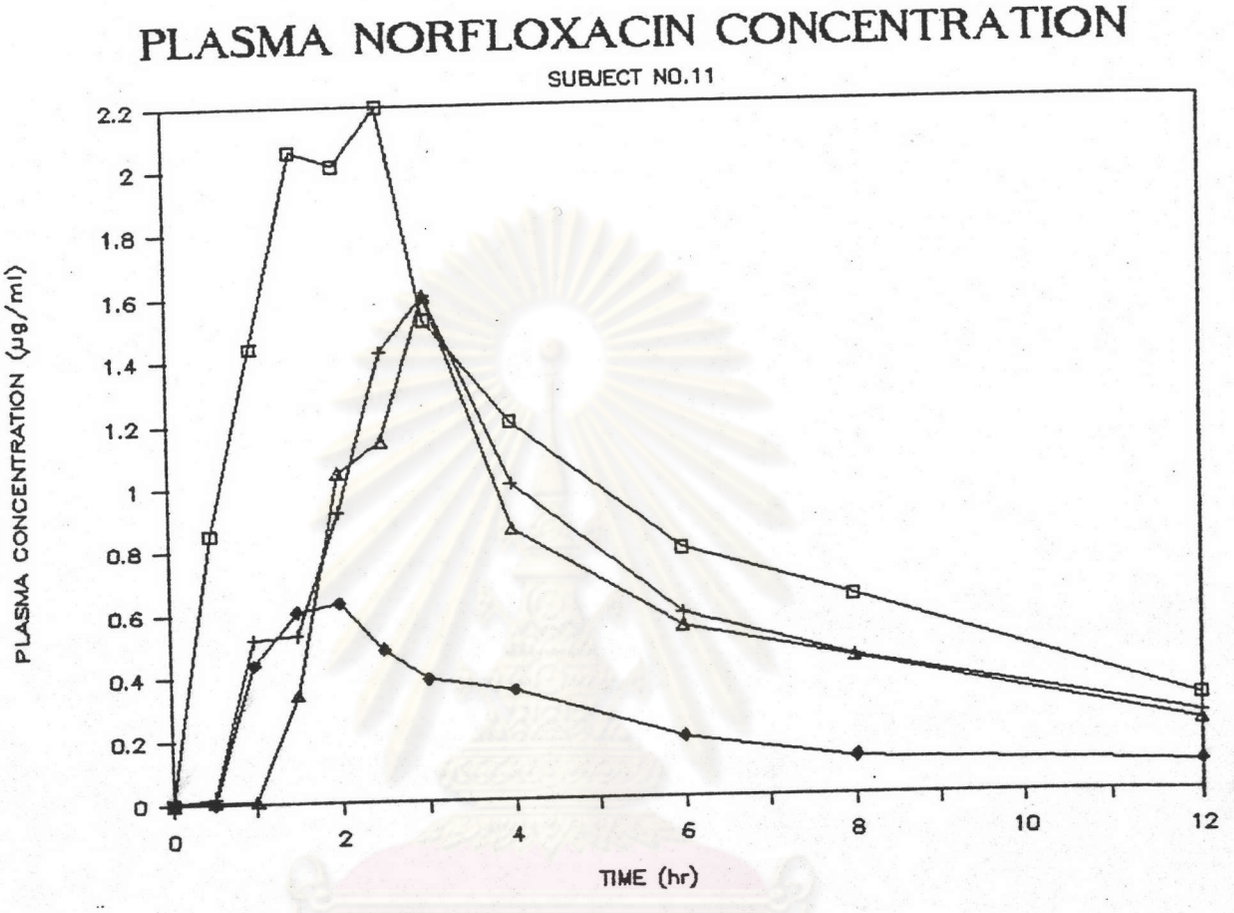


Figure 14 Plasma Norfloxacin Concentration-time Profile of Subject No.11 Following Oral Administration of 400 mg Norfloxacin Tablet

Key : Brand A (□) , Brand B (+) , Brand C (♦) , Brand D (Δ)

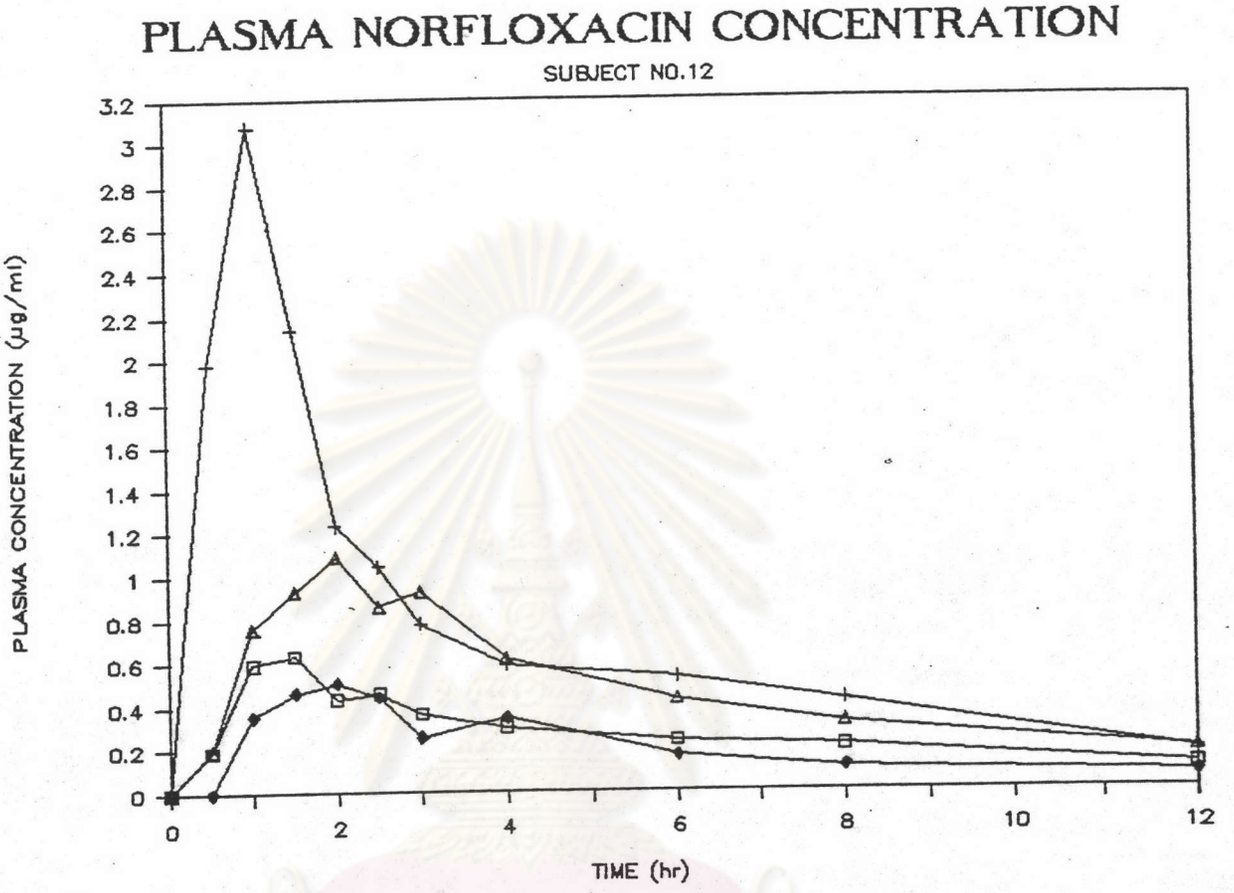


Figure 15 Plasma Norfloxacin Concentration-time Profile of Subject No.12 Following Oral Administration of 400 mg Norfloxacin Tablet

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

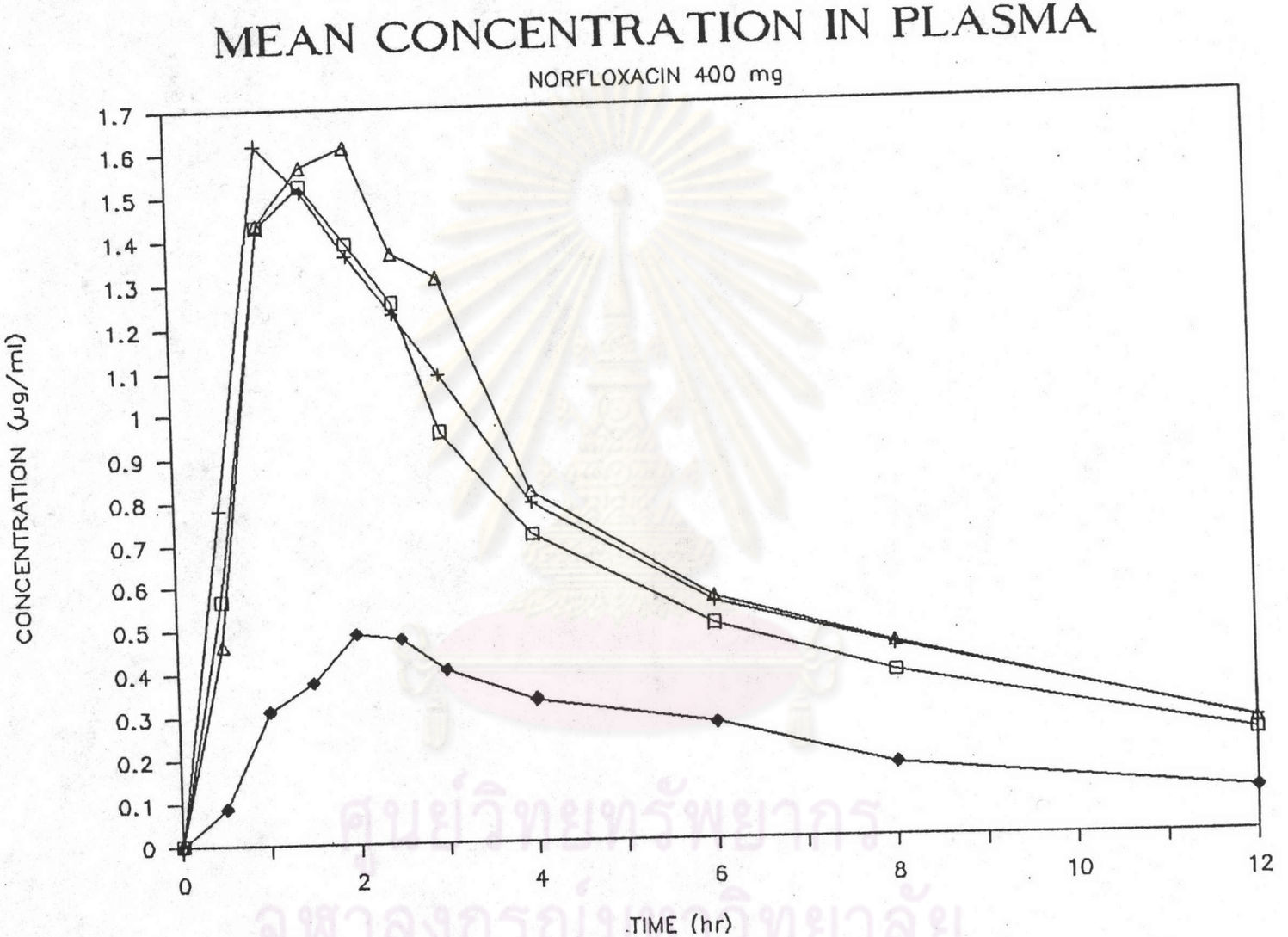


FIGURE 16 Comparison of the mean plasma norfloxacin concentration-time profile of four different brands following oral administration of 400 mg norfloxacin tablet to 12 subjects

KEY : Brand A (□) , Brand B (+) , Brand C (◆) , Brand D (Δ)

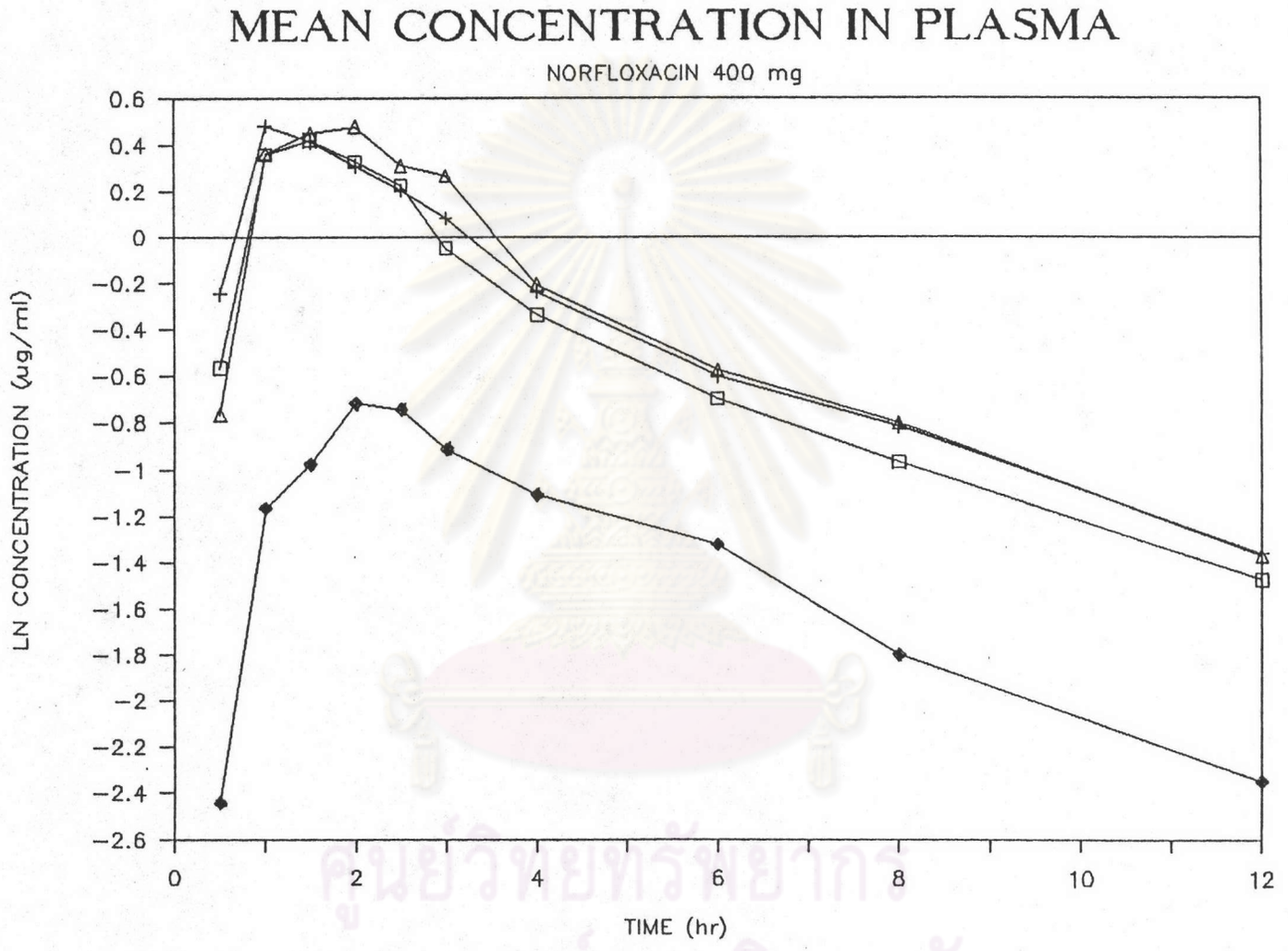


FIGURE 17 Semilogarithmic plot of comparison of the mean plasma norfloxacin concentration-time profile of four different brands following oral administration of 400 mg norfloxacin tablet to 12 subjects

KEY : Brand A (□) , Brand B (+) , Brand C (◆) , Brand D (Δ)

the bioequivalence if it reaches the general circulation at the same relative rate and the same relative extent, such that the plasma level profiles of the drug obtained using the two dosage forms are, within reason, "superimposable" (Disanto, 1985).

The relevant pharmacokinetic parameters for bioavailability comparison obtained from this study were :

4.1 **Peak Plasma Concentration**

Previous reports indicated that the mean peak plasma concentration achieved following oral administration of 400 mg. norfloxacin tablets varied widely ranging from 1.3 to 1.9 µg/ml (Swanson et al., 1983; Lode et al., 1987; McEvoy, ed., 1989). In this present study, the mean peak plasma levels for each treatment of norfloxacin tablets were observed from the plasma concentration versus time curves of 12 subjects as shown in Table 12. The mean value for brands A, B, C and D were 1.87 ± 0.28, 1.96 ± 0.22, 0.60 ± 0.16 and 2.05 ± 0.21 µg/ml, respectively. The rank order of peak plasma norfloxacin concentration was brands D > B > A > C (at $p < 0.05$). Statistical result indicated that only brand C had a peak plasma concentration significantly lower than the innovator's product, brand A ($p < 0.05$) [Tables 13 and 14]. This could be due to the product brand C exhibited longer disintegration time and slower rate of dissolution.

Table 12 Peak Plasma concentration (C_{max}) of Norfloxacin Observed Directly from the Plasma Concentration-Time Curve of Each Individual Following 400 mg Oral Administration of Four Different Brands of Norfloxacin Tablets.

Subject No.	C_{max} (µg/ml)			
	A	B	C	D
1	1.43	2.52	0.12	2.49
2	1.12	2.48	0.20	2.16
3	1.69	2.09	0.37	3.21
4	1.42	1.08	0.61	1.62
5	1.38	2.22	0.16	0.91
6	2.07	2.99	0.13	2.69
7	3.01	1.99	0.21	1.94
8	0.57	1.88	1.83	2.11
9	3.98	1.29	1.56	3.30
10	2.98	0.31	0.90	1.51
11	2.19	1.59	0.63	1.60
12	0.63	3.08	0.51	1.09
MEAN	1.87	1.96	0.60	2.05
SEM	0.28	0.22	0.16	0.21

Table 13 Analysis of Variance for Peak Plasma Concentration of Four Commercial Norfloxacin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	3	16.83	5.61	8.65
Within groups	44	28.54	0.65	
Total	47	45.38		

$F^{e}_{0.05(5,30)} = 2.824$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 14 Comparison of Peak Plasma Concentration of 3 Different Brands (B, C, D) with the Innovator's Product (Brand A). Using Student's t -test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	0.2734	NS
C	3.8585	S
D	0.5468	NS

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

S = significant at $p < 0.05$

NS = not significant at $p > 0.05$

a = t - value from the table

4.2 Time to Peak Plasma Level

The time to peak plasma norfloxacin level also obtained by reading directly from plasma concentration time curve of each individual as shown in Table 15. The average peak times were 1.63 ± 0.18, 1.52 ± 0.20, 1.81 ± 0.20 and 1.66 ± 0.11 hr. for brands A, B, C and D, respectively. There were no staistically significant difference among these brands for this parameter (Tables 16 and 17). Similar results were found by other investigators which were reported in the range of 1 to 2 hr. (Swanson et al., 1983; Adhami et al., 1984; Holmes et al., 1985).

4.3 Area under Plasma Versus Time Curve

The mean AUC_o^∞ estimated by CSTRIP program from individual plasma data for four different brands of each formulation as shown in Table 18 were 8.62 ± 1.03, 10.42 ± 0.94, 4.35 ± 0.93 and 9.98 ± 0.81 μg.hr/ml for brands A, B, C and D, respectively. Statistical analysis in Tables 19 and 20 indicated that there were no significant difference among brands A, B and D except those with brand C ($p < 0.05$). This indicates that the extent of drug absorbed from brand C was statistically significant less than any other brands. The values of AUC_o^∞ in this study were higher than those previously presented by Wise et al (1984) as 5.4 ± 1.7 μg.hr/ml.

Table 15 Time to Peak Plasma Concentration (T_{max}) of Norfloxacin Observed Directly from the Plasma Concentration-Time Curve of Each Individual Following 400 mg Oral Administration of Four Different Brands of Norfloxacin Tablets

Subject No.	T_{max} (hr)			
	A	B	C	D
1	2.5	1.0	1.5	1.5
2	2.5	1.5	2.5	2.0
3	2.0	2.5	1.0	1.0
4	1.0	1.5	2.5	1.9
5	1.0	1.0	1.0	2.0
6	1.0	1.0	1.0	2.0
7	2.0	2.0	1.0	1.5
8	1.0	0.5	2.0	1.5
9	1.0	1.0	2.0	1.0
10	1.5	2.5	3.2	1.5
11	2.5	2.7	2.0	2.0
12	1.5	1.0	2.0	2.0
MEAN	1.63	1.52	1.81	1.66
SEM	0.18	0.20	0.20	0.11

Table 16 Analysis of Variance for Time to Peak Plasma Concentration of Four Commercial Norfloxacin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	3	0.54	0.18	0.44
Within groups	44	17.98	0.41	
Total	47	18.52		

$F^{e}_{0.05\ (3,44)} = 2.824$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 17 Comparison of Time to Peak Concentration of 3 Different Brands (B, C, D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	0.4208	NS
C	0.6885	NS
D	0.1147	NS

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

NS = not significant at $p > 0.05$

a = t - value from the table

Table 18 Area Under the Plasma Concentration-Time Curve (AUC_0^∞) of Norfloxacin from 12 Subjects Following 400 mg Oral Administration of Four Different Brands of Norfloxacin Tablets

Subject No.	$[AUC]_0^\infty$ (ug.hr/ml)			
	A	B	C	D
1	7.94	8.34	8.45	9.85
2	3.52	9.75	0.81	10.47
3	2.20	12.78	4.22	10.68
4	7.17	5.63	1.83	7.99
5	8.33	10.66	2.44	6.26
6	11.94	18.94	1.53	17.17
7	12.93	10.73	0.94	10.18
8	12.71	12.82	10.53	12.44
9	12.63	7.54	9.07	11.39
10	9.45	10.38	5.78	8.96
11	10.32	7.62	3.89	7.70
12	4.33	9.89	2.74	6.66
MEAN	8.62	10.42	4.35	9.98
SEM	1.03	0.94	0.93	0.81

Table 19 Analysis of Variance for $[AUC]_0^\infty$ of Four Commercial Norfloxacin Tablets.

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	3	276.09	92.03	8.06
Within groups	44	502.27	11.42	
Total	47	778.35		

$F^{e}_{0.05\ (3,44)} = 2.824$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 20 Comparison of $[AUC]_0^\infty$ of 3 Different Brands (B, C, D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	1.3057	NS
C	3.0957	S
D	0.9859	NS

$t^a_{(0.05,44)} = 2.015$

S = significant at $p < 0.05$

NS = not significant at $p > 0.05$

a = t - value from the table

4.4 The Relative Bioavailability

Absolute bioavailability of norfloxacin is unknown because an intravenous data is not currently available. Relative bioavailability is a relative amount of drug which compared to that of a standard (innovator's product) (Shargel and Yu, 1985). In this study, the mean relative bioavailabilities calculated using equation (3) as described under CHAPTER III (MATERIAL AND METHOD). The values of brands B, C and D relatively to brand A were 120.88, 50.46 and 115.77%, respectively.

The principal pharmacokinetic parameters of norfloxacin following oral administration of four brands were summarized in Table 27. Statistical analysis of these parameters among four brands revealed that only brand C produced peak plasma concentration and area under the plasma-time curve significantly lower than those of brand A, referring to bioinequivalence in term of the extent of absorption but not the rate, meanwhile C_{max}, T_{max} and AUC of brands A, B and D were similar and these values were not different greater than 20% among and between each others. These implied that brands A, B and D were completely bioequivalent in terms of both the rate and extent of drug absorption.

5. Pharmacokinetics of Norfloxacin Tablets

The in vivo result was best described by compartmental method. Using the computer CSTRIP program (Appendix E), results obtained demonstrated that most data were well described by a triexponential equation referring that pharmacokinetics of norfloxacin tablet in Thai healthy volunteers could be explained in terms of a two-compartment open model.

The pharmacokinetic parameters of norfloxacin derived from model of analysis from plasma concentration time data were detailed in Tables 21 to 26 and summarized in Table 27.

5.1 Absorption Rate Constant

The first order absorption rate constant obtained from individual plasma data of 12 subjecs were presented in Table 21. The average absorption rate constant for brands A, B, C and D were 1.49 ± 0.13, 1.96 ± 0.28, 1.63 ± 0.20 and 1.46 ± 0.12 hr^{-1}, respectively. This value agreed with previous report by Adhami et al (1984) that the range of this absorption rate constant was between 1.8 to 5.54 hr^{-1}. No statistically significance was observed among these values (Tables 22 and 23), indicating the drug from all products tested was absorbed into blood circulation with the similar rate.

5.2 Half-life

Adhami et al (1984) reported that the half-life of norfloxacin ranged from 3 to 4 hours while Eandi et al (1983) founded that the elimination half-life of norfloxacin were between 2.8 to 5.9 hours. Also Holmes et al (1985) showed this parameter ranging from 3.5 to 6.5 hours. In this study some datas were excluded because of high intrasubject variation. Deleting these datas did not affect the results. As can be seen the concentration of the drug could not be detected at time zero following administration the tablet a week later (Tables 8 to 11). Similar results were obtained from this study such that in Thai healthy volunteers, the norfloxacin half-life in plasma determined from compartmental analysis for brands A, B, C and D were 5.36 $\pm$ 0.74, 4.52 $\pm$ 0.71, 3.98 $\pm$ 0.94 and 4.76 $\pm$ 0.32 hours, respectively (Table 24). These values were not statistically significant difference among and between each other as shown in Tables 25 to 26. This result expressed that there were no difference in the elimination process among various marketed products.

The pharmacokinetic parameters obtained from this study were slightly different from those reported by other investigators. The factors possibly responsible for these may be due to interpretation of the differences in subject population, (i.e. the differences in their race, age, weight and normal habits), study conditions,

Table 21 Absorption Rate Constant (K_a) of Norfloxacin from 12 Subjects Following 400 mg Oral Administration of Four Different Brands of Norfloxacin Tablets

Subject No.	K_a (hr^{-1})			
	A	B	C	D
1	0.99	2.12	2.22	1.72
2	0.82	1.44	1.13	0.63
3	1.52	1.22	2.37	1.66
4	1.33	3.45	0.43	1.11
5	2.03	1.87	2.36	1.95
6	1.17	2.77	2.27	1.34
7	1.37	1.43	2.30	1.36
8	2.14	4.06	1.24	1.65
9	1.92	1.59	1.59	2.03
10	1.15	0.95	0.43	1.79
11	1.11	0.62	1.85	0.96
12	2.28	2.05	1.39	1.31
MEAN	1.49	1.96	1.63	1.46
SEM	0.13	0.28	0.20	0.12

Table 22 Analysis of Variance for Absorption Rate Constant of Four Commercial Norfloxacin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	3	1.94	0.65	1.32
Within groups	44	21.57	0.49	
Total	47	23.52		

$F^{e}_{0.05\ (3,44)} = 2.824$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 23 Comparison of Absorption Rate Constants of 3 Different Brands (B, C, D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	1.6441	NS
C	0.4897	NS
D	0.1049	NS

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

NS = not significant at $p > 0.05$

a = t - value from the table

and difference in assay methodology as well as data analysis.

6. <u>In vitro-In vivo Correlative Study</u>

Table 28 presents the correlative studies between the in vitro and the in vivo data for brands A, B, C and D. As can be seen, the disintegration times were statistically significant correlation with the dissolution rate constants and the C_{max} value whereas the dissolution rate constants were statistically significant correlation with the C_{max} and the AUC values. However these correlations seemed to be meaningless. This was because when brand C was excluded from this study due to the disintegration time and dissolution rate constant were very poor. There were no statistically significant correlation between the in vitro parameters with any of the in vivo parameters (Table 29), unless the disintegration times versus the C_{max} appeared to be correlative ($0.05 < p < 0.1$). This indicated that the disintegration time might affect only the rate of absorption. However the in vitro parameters obtained in this investigation might not be used precisely to predict the bioavailability of norfloxacin tablet.

Table 24 Plasma Half-life ($t_{1/2}$) of Norfloxacin from 12 Subjects Following 400 mg Oral Administration of Four Different Brands of Norfloxacin Tablets

Subject No.	$t_{1/2}$ (hr)			
	A	B	C	D
1	5.92	5.70	77.17*	4.67
2	3.94	5.46	2.97	4.00
3	4.72	8.10	23.55*	5.07
4	6.33	4.17	4.12	3.47
5	7.16	5.58	19.68*	4.37
6	4.99	14.48*	9.26	5.22
7	4.21	4.57	3.12	5.55
8	35.98*	6.47	5.47	4.32
9	10.77	6.68	6.42	7.76
10	4.16	62.12*	3.23	4.45
11	4.69	3.49	9.48	3.36
12	7.39	4.01	3.75	4.84
MEAN	5.36	4.52	3.98	4.76
SEM	0.74	0.71	0.94	0.32

* Excluded data

Table 25 Analysis of Variance for Plasma Half - life of Four Commercial Norfloxacin Tablets

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among groups	3	11.63	3.88	0.63
Within groups	44	270.16	6.14	
Total	47	281.8022		

$F^{e}_{0.05\ (3,44)} = 2.824$

a = degree of freedom

b = sum of square

c = mean square

d = variance ratio

e = F value obtained from the table

Table 26 Comparison of Plasma Half-life of 3 Different Brands (B, C, D) with the Innovator's Product (Brand A). Using Student's t-test

Brand	t value (calculated) comparison with Brand A	Statistical Significance
B	0.8303	NS
C	1.3642	NS
D	0.5931	NS

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

NS = not significant at $p > 0.05$

a = t - value from the table

Table 27 Estimated Pharmacokinetic Parameters (MEAN ± SEM) of Norfloxacin from 12 Subjects Following Oral Administration of 400 mg of 4 Different Brands of Norfloxacin Tablets

Brand	C_{max} (µg/ml)	T_{max} (hr)	$[AUC]_o^{\infty}$ (µg.hr/ml)	K_a (hr^{-1})	half-life (hr)
A	1.87 ± 0.28	1.63 ± 0.18	8.62 ± 1.03	1.49 ± 0.13	5.36 ± 0.74
B	1.96 ± 0.22	1.52 ± 0.20	10.42 ± 0.94	1.96 ± 0.28	4.52 ± 0.71
C	0.60 ± 0.16	1.81 ± 0.20	4.35 ± 0.93	1.63 ± 0.20	3.98 ± 0.94
D	2.05 ± 0.21	1.66 ± 0.11	9.98 ± 0.81	1.46 ± 0.12	4.76 ± 0.32
F-test	8.65	0.44	8.06	1.32	0.63
Statistical Significance	S	NS	S	NS	NS

$F_{0.05\ (3,44)} = 2.82$

S = significant at $p < 0.05$

NS = not significant at $p > 0.05$

Table 28 In Vitro-IN Vivo Correlations (I)

Correlation	Degree of freedom[b]	Correlation Coefficient	t-value (calculated)	Statistical Significance
Disintegration Times versus Dissolution Rate Constants	4	- 0.99	- 13.06	S
Disintegration Times versus C_{max}	2	- 0.98	- 7.89	S
Disintegration Times versus T_{max}	2	0.87	2.52	NS
Disintegration Times versus $[AUC]_o^\infty$	2	- 0.95	- 4.11	NS
Dissolution Rate Constants versus C_{max}	2	0.99	9.64	S
Dissolution Rate Constants versus T_{max}	2	- 0.91	- 3.03	NS
Dissolution Rate Constants versus $[AUC]_o^\infty$	2	0.97	6.01	S

$$t^{a}_{(0.05,4)} = 2.7764$$

$$t^{a}_{(0.05,2)} = 4.3027$$

a : t-value from the table

b : degree of freedom = number of pairs -2

S : significant at $p < 0.05$

NS : not significant at $p > 0.05$

Table 29 In Vitro-IN Vivo Correlations (II)

Correlation	Degree of freedom[b]	Correlation Coefficient	t-value (calculated)	Statistical Significance
Disintegration Times versus Dissolution Rate Constants	3	- 0.42	- 0.80	NS
Disintegration Times versus C_{max}	1	0.99	12.22	S
Disintegration Times versus T_{max}	1	0.28	0.29	NS
Disintegration Times versus $[AUC]_o^\infty$	1	0.66	0.89	NS
Dissolution Rate Constants versus C_{max}	1	- 0.05	- 0.05	NS
Dissolution Rate Constants versus T_{max}	1	- 0.98	- 6.66	NS
Dissolution Rate Constants versus $[AUC]_o^\infty$	1	0.64	0.84	NS

$$t^{a}_{(0.05,3)} = 3.182$$

$$t^{a}_{(0.05,1)} = 12.706$$

a : t-value from the table

b : degree of freedom = number of pairs -2

S : significant at $0.05 < p < 0.1$

NS : not significant at $p > 0.05$