CHAPTER IV

RESULTS AND DISCUSSION

In Vitro Studies

The eight commercial brands of diclofenac sodium 25 mg. enteric-coated tablets were first tested for uniformity of weight and content of active ingredient. Each of these eight brands met the British Pharmacopoeia requirement (B.P. 1988) for uniformity of weight within the range of limit weight (± 10%). All products were assayed for content of active ingredient. Results indicated that each brand was within the limits of 90.0-110.0 percent labeled amount as shown in Table 4.

Disintegration time was also an important attribute of enteric-coated tablet quality control because disintegration time must take place in the intestinal fluid before the active ingredient of the tablet was dissolved and absorbed but no evidence of disintegration in gastric fluid.

All of these products except brand F of diclofenac sodium enteric-coated tablets met the requirements of the United States Pharmacopoeia XXII for disintegration of enteric-coated tablets in simulated intestinal fluid TS. at 37 ± 2°C. The reason of failing to meet the

specification of brand F was due to the tablets already had cracked in simulated gastric fluid. Disintegration time of these products were ranged from 4.16 to 38.00 minutes. The rank orders in term of mean disintegration time were brands H > E > C > D > G > A > B. Statistical comparison indicated that the disintegration time of all brands except brand B were statistically longer than that of brand A ($p < 0.05$) as shown in Tables 5 and 6.

The dissolution testing of diclofenac sodium enteric-coated tablets was a crucial factor for systemic drug availability because a drug had to, in general, dissolved to solution before it could be absorbed. The dissolution test was performed in simulated intestinal fluid TS without enzyme (pH 7.5 $\pm$ 0.1) using the United States Pharmacopoeia dissolution apparatus type II (paddle method). Figure 3 and Table 7 illustrated the dissolution profiles at various times of all eight brands of diclofenac sodium enteric-coated tablets. Each brand reached the equilibruim state within 60 minutes. The mean percent dissolved of diclofenac sodium from all brands ranged from 91.08 to 106.07 percent at 60 minutes. The dissolution rate constants (K_d) were calculated from the slope of the first order plot between the amount of undissolved diclofenac sodium ($B\alpha - Bt$) versus time in semi-logarithmic scale. The dissolution rate constants of all brands were reported in Table 8. The rank order of all brands in term of dissolution rate constant were brands B > A > G > F > D > E > C > H. Statistical

Table 4 In Vitro Studies of Eight Commercial Brands of Diclofenac Sodium Enteric-Coated Tablets.*

Brand	Weight[a] (mg)	Assay, % of labeled amount[b]	Disintegration time[c] (min.)	Dissolution rate constant[d] ($min.^{-1}$)
A	148.25 ± 1.40	99.70 ± 0.24	8.67 ± 0.51	0.20 ± 0.04
B	152.76 ± 2.48	105.26 ± 0.21	4.16 ± 0.98	0.22 ± 0.09
C	163.92 ± 2.94	101.67 ± 0.21	23.00 ± 1.90	0.08 ± 0.02
D	174.76 ± 2.88	100.81 ± 0.32	14.33 ± 1.21	0.11 ± 0.03
E	167.63 ± 9.45	100.55 ± 0.44	24.17 ± 1.72	0.10 ± 0.03
F	153.09 ± 3.70	108.56 ± 0.58	**	0.17 ± 0.02
G	158.08 ± 2.29	102.26 ± 0.79	12.50 ± 1.38	0.18 ± 0.09
H	143.94 ± 3.49	95.50 ± 0.36	38.00 ± 3.85	0.06 ± 0.01

* All values are presented as mean ± S.D.

a = n = 20 c = n = 6

b = n = 3 d = n = 6

** No data shown because the tablets failed to meet the specification of U.S.P. XXII

Table 5 Analysis of Variance for Disintegration Time of Seven Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	6	4710.00	785.00	211.59
Within group	35	129.83	3.71	
Total	41	4839.83		

* Calculation data from Table 4

F^{e} 0.05 (6, 35) = 2.37

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 Each Brand of Diclofenac Sodium Enteric-Coated Tablets with that of the Innovator's Product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
B	4.05	$p < 0.001$	S
C	12.86	$p < 0.001$	S
D	5.04	$p < 0.001$	S
E	13.94	$p < 0.001$	S
G	3.42	$p < 0.05$	S
H	26.35	$p < 0.001$	S

$t^{a}(0.05, 35) = 2.03$

* S = Significant difference at $p < 0.05$

a = t value obtained from the table

Table 7 Dissolution Profiles of Eight Brands of Diclofenac Sodium Enteric-Coated Tablets in Simulated Intestinal Fluid T.S. without Enzyme (pH 7.5±0.1)

Time (min.)	% Drug Dissolved[a]							
	Brand A	Brand B	Brand C	Brand D	Brand E	Brand F	Brand G	Brand H
5	0.48±0.17	20.65±1.30	1.88±0.32	0.28±0.32	0.54±0.11	10.76±5.27	2.98±0.54	0.69±0.35
10	27.69±8.08	58.85±3.72	5.77±4.51	13.75±5.13	1.18±0.33	46.09±10.62	9.66±3.77	2.80±0.65
15	85.65±6.0	91.00±7.68	24.98±11.41	40.15±7.34	7.84±3.33	71.44±10.85	48.75±18.90	8.20±1.71
20	100.52±1.00	102.48±3.51	46.87±12.42	62.51±9.26	24.53±8.66	89.16±9.08	99.84±11.48	18.48±5.34
25	100.09±1.23	105.04±4.94	64.54±11.99	81.47±8.74	51.17±12.87	99.68±6.20	109.61±2.62	32.96±7.06
30	99.41±1.07	104.41±5.04	78.85±11.36	93.78±6.70	73.32±13.23	102.67±3.00	108.81±2.77	46.41±6.76
40	98.84±1.25	103.64±5.06	98.28±9.23	102.87±1.10	99.15±7.40	102.69±2.69	107.77±2.60	67.77±5.28
50	98.31±1.21	103.28±5.07	103.25±4.44	102.65±1.93	105.29±3.37	102.08±2.40	107.07±2.69	82.72±4.54
60	97.73±1.11	102.46±5.04	103.20±3.69	102.03±2.02	104.86±3.95	101.50±2.36	106.07±2.57	91.08±2.38
75	97.02±1.22	101.68±5.10	102.57±3.78	101.40±1.90	104.31±3.88	100.80±2.32	105.35±2.40	95.30±1.77
90	96.79±1.36	101.05±5.11	102.09±3.82	100.72±1.94	103.71±3.78	100.25±2.37	104.68±2.43	94.93±1.51
105	96.22±1.18	100.77±4.94	101.17±3.71	100.23±1.80	103.14±3.84	99.88±2.63	103.96±2.30	94.23±1.76
120	95.46±1.23	100.19±4.95	100.80±3.53	99.86±1.73	102.53±3.60	99.39±2.48	103.14±2.51	93.49±1.61

[a] All values are presented as mean ± S.D. (n = 6)

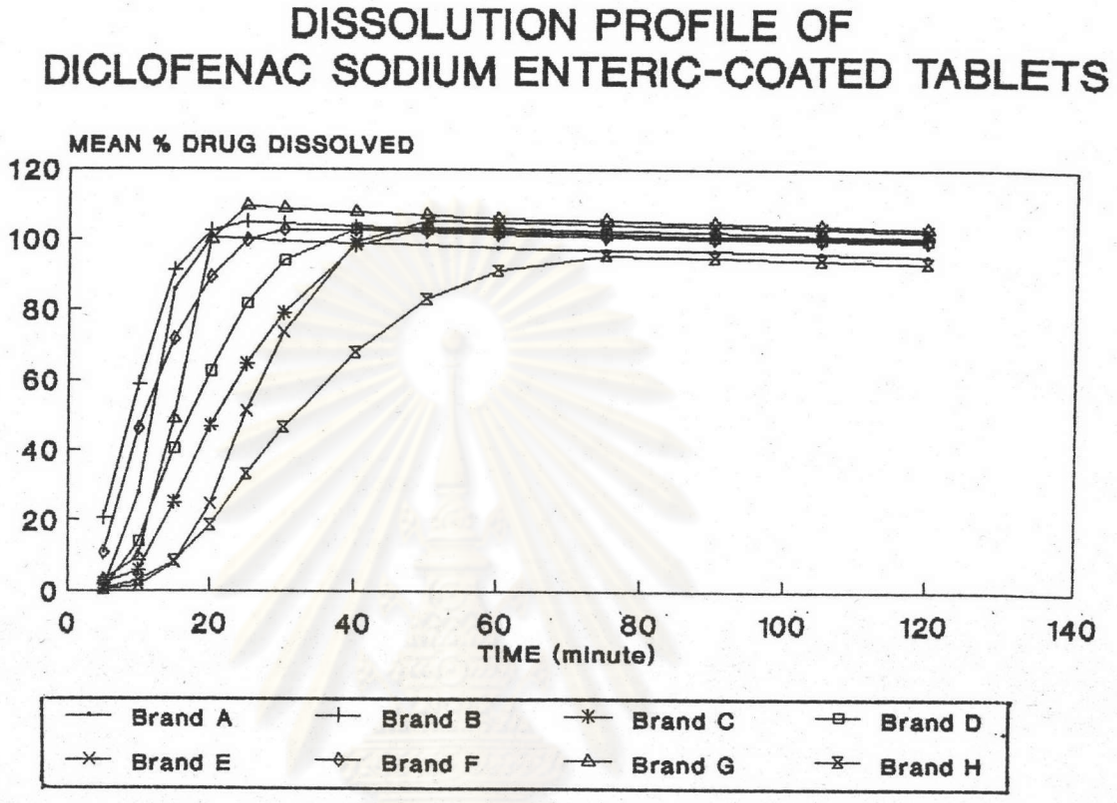

Figure 3 Dissolution profiles of eight commercial brands of diclofenac sodium 25 mg enteric-coated tablets in simulated intestinal fluid T.S. without enzyme (pH 7.5 $\pm$ 0.1)

Table 8 Dissolution Rate Constant (k_d) for Eight Brands of Diclofenac Sodium Enteric-Coated Tablets in Simulated Intestinal Fluid T.S. without Enzyme (pH 7.5±0.1)

Brand	Dissolution Rate Constant (k_d)($min.^{-1}$)						
	Tab 1	Tab 2	Tab 3	Tab 4	Tab 5	Tab 6	Mean ± S.D.
A	0.14	0.17	0.16	0.24	0.24	0.23	0.20 ± 0.04
B	0.14	0.26	0.11	0.36	0.24	0.21	0.22 ± 0.09
C	0.67	0.05	0.10	0.10	0.08	0.10	0.08 ± 0.02
D	0.12	0.08	0.10	0.10	0.18	0.11	0.11 ± 0.03
E	0.13	0.10	0.07	0.13	0.06	0.08	0.10 ± 0.03
F	0.18	0.20	0.16	0.20	0.13	0.17	0.17 ± 0.02
G	0.33	0.12	0.08	0.22	0.20	0.12	0.18 ± 0.09
H	0.06	0.05	0.05	0.05	0.07	0.05	0.06 ± 0.01

Table 9 Analysis of Variance for Dissolution Rate Constant of Eight Brands of Diclofenac Sodium Enteric-Coated Tablets in Simulated Intestinal Fluid TS without Enzyme (pH 7.5 ± 0.1)*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	7	0.1517	0.0217	8.0370
Within group	40	0.1085	0.0027	
Total	47	0.2602		

* Calculation data from Table 8

F^{e} 0.05 (7, 40) = 2.42

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 Each Brand of Diclofenac Sodium Enteric-Coated Tablet with that of The Innovator's Product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
B	0.73	$0.400 < p < 0.500$	NS
C	3.81	$p < 0.001$	S
D	2.78	$0.005 < p < 0.010$	S
E	3.37	$0.001 < p < 0.005$	S
F	0.91	$0.200 < p < 0.400$	NS
G	0.76	$0.400 < p < 0.500$	NS
H	4.74	$p < 0.001$	S

$t^{a}(0.05, 40) = 2.02$

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

a = t value obtained from the table

comparison, as presented in Tables 9 and 10, indicated that the dissolution rate constant of brands B, F, and G did not show statistically significant difference ($p < 0.05$) when compared to that of brand A.

As seen in table 4, different brands of diclofenac sodium enteric-coated tablets resulted in different the disintegration times and dissolution rate. These might presumably be due to different property of coating material solubility in media. In addition diclofenac sodium raw material source, drug formulation, and/or manufacturing process might be contributed for these difference.

Statistical correlation ($p < 0.5$) was found between the disintegration time and the dissolution rate constant of each brand as shown in Table 11. This result indicated that disintegration times were rate limiting step of diclofenac sodium dissolution.

In Vivo Studies

Three brands of diclofenac sodium enteric-coated tablets with maximum, moderate and minimum dissolution rate constant were selected to compare their bioavailabilities with that of the innovator's product (brand A). They were brands B, D and H respectively.

Table 11 In Vitro Parameters Correlation

Correlation	Correlation Coefficient	t value	Statistical Significance
Disintegration Times virsus Dissolution Rate Constants	- 0.92	5.25	S

n = 6

t^{a} 0.05(5) = 2.571

a = t value obtained from the table

S = Significant difference at $p < 0.05$

1. Analysis of Diclofenac Sodium in Plasma Samples.

Several methods have been reported for the measurement of diclofenac sodium in plasma. These include thin layer chromatography (Schumacker *et al.*, 1980), gas chromatography (Geiger *et al.*, 1975; Ikeda *et al.*, 1980) and high performance liquid chromatography (Nielsen-Kudsk, 1980). Among these methods, there were some disadvantages such as; the thin layer and the former high-performance liquid chromatographic method lack the sensitivity required for low concentration determination of diclofenac (less than 100 ng./ml). The gas chromatographic procedures are based on the formation of an indolene derivative, a methyl ester derivative, or an acetylated derivative and subsequent electron capture detection are extremely sensitive. However, the methods require extensive sample clean up and the procedures are extremely tedious.

In this present study, the modified procedure from Chan *et al.* s' method (1982) was employed for analyzing diclofenac sodium concentration in plasma samples. The mixtures of hexane and isopropanol (9:1 V/V) were used as extraction solvent and they exhibited an interfere-free chromatogram in the HPLC system.

The ratio of mobile phase mixture which consisted of methanol, acetonitrile and 0.02 M sodium acetate buffer (pH 7.0) was modified from 25 : 20 : 55 to 21 : 17 : 62.

Typical chromatograms of diclofenac sodium and internal standard were shown in Figure 4. The retention time of diclofenac sodium and internal standard were 5.99 and 7.89 minutes, respectively. The method was validated by determining the within-run and between-run precisions. The percent coefficient of variation (%CV.) in the within-run and between-run precisions were 1.52-13.63 and 1.45 - 8.26% as shown in Tables 12 and 13, respectively. The standard calibration curve of plasma concentration of diclofenac sodium versus peak height ratio of diclofenac sodium to internal standard was linear up to 2.4 mcg./ml. (Appendix B). The efficiency of the separation technique used was evaluated by calculating the percentage of recoveries. This was accomplished by comparing the peak height of the drug obtained from spiked plasma to the peak height that obtained from standard solution which were directly injected to HPLC. Results as shown in Table 14 indicated that the analytical method used is independent to concentration. The percentage recoveries of diclofenac sodium and internal standard were in the range of 66.13-78.72% and 68.42-77.77%, respectively.

2. Plasma Diclofenac Sodium Concentrations

The plasma level of diclofenac sodium at each sampling time ranging from 0 to 10 hours after oral administration of two 25 mg tablet of brands A, B, D and H were shown in Tables 15 to 18, respectively. Individual plasma diclofenac sodium concentration-time profile for each of twelve subjects were shown graphically from

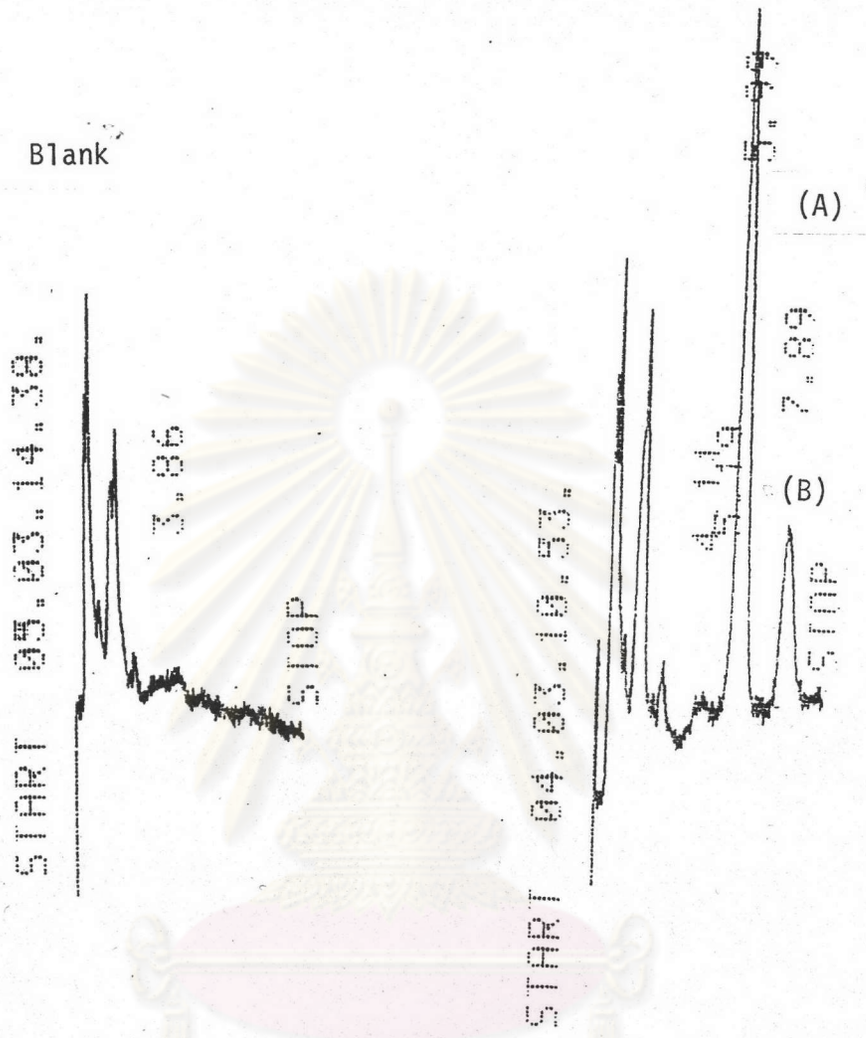

Figure 4 High pressure liquid chromatography of diclofenac sodium (A) and internal standard (B)

Table 12 Within-run Precision of Diclofenac Sodium

Concentration (mcg./ml.)	Peak Height Ratio			Mean ± S.D.	% C.V.
	1	2	3		
0.0499	0.24	0.23	0.28	0.25 ±0.026	10.58
0.0991	0.37	0.47	0.48	0.42 ±0.060	13.63
0.1981	0.69	0.83	0.84	0.79 ±0.084	10.63
0.3963	1.38	1.54	1.60	1.51 ±0.114	7.53
0.7926	2.61	2.90	2.91	2.81 ±0.170	6.06
1.1888	4.04	4.06	4.30	4.13 ±0.145	3.50
1.5851	5.29	5.41	5.44	5.38 ±0.079	1.48
1.9814	6.55	6.72	6.89	6.72 ±0.170	2.53
2.3777	7.92	8.00	8.76	8.23 ±0.464	5.63

n = 3

Table 13 Between-run Precision of Diclofenac Sodium

Concentration (mcg./ml.)	Peak Height Ratio			Mean ± S.D.	% C.V.
	1	2	3		
0.0499	0.24	0.23	0.28	0.25 ± 0.03	10.58
0.0991	0.34	0.48	0.46	0.43 ± 0.07	17.28
0.1981	0.78	0.88	0.83	0.83 ± 0.05	6.13
0.3963	1.34	1.56	1.54	1.48 ± 0.12	8.26
0.7926	2.79	2.91	2.90	2.87 ± 0.07	2.34
1.1888	4.20	4.22	4.11	4.18 ± 0.06	1.45
1.5851	5.16	5.39	5.42	5.32 ± 0.14	2.67
1.9814	6.51	6.80	6.72	6.68 ± 0.15	2.24
2.3777	7.91	8.61	8.00	8.17 ± 0.38	4.67

n = 3

Table 14 Recoveries of Diclofenac Sodium and Internal Standard

Conc. (ng./ml.)	D[a] PH[c] (mm.)		% Recovery[d]	IS[b] PH[c] (mm.)		% Recovery[d]
	Sol[n]	Plasma		Sol[n]	Plasma	
0.0991	14.0	9.5	67.86	16.0	12.0	75.00
0.1981	31.0	20.5	66.13	18.5	13.5	72.97
0.3963	47.0	37.0	78.72	17.0	13.0	76.47
0.7926	82.0	58.0	70.73	18.0	14.0	77.77
1.1888	98.0	72.0	73.47	18.5	13.5	72.97
1.5851	115.0	88.0	76.52	19.0	13.0	68.42
Mean % Recovery ± S.D.			72.24±4.91			73.93±3.30
% C.V.			6.80			4.46

a = Drug = Diclofenac Sodium

b = Internal Standard = Mefenamic acid

c = Peak Height

d = % Recovery

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

Table 15 Plasma Diclofenac Sodium Concentration (mcg/ml.) from 12 Subjects Following Oral Administration of two 25 mg. Diclofenac Sodium Enteric-Coated Tablets of Brand Ang

Subject No.	Time (hr.)									
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	10.0
1	0.0934	1.6302	0.5453	0.3239	0.1772	0.1064	0.0504	0.0261	0.0111	*
2	*	*	0.1043	1.9712	2.4456	0.9363	0.2198	0.0326	*	*
3	0.3120	1.7992	0.6037	0.3295	0.1548	0.1055	0.0595	0.0060	*	*
4	0.7443	0.2137	0.6167	0.2791	0.8400	0.2870	0.0513	*	*	*
5	*	*	0.3343	0.9521	1.7428	1.4092	0.4084	0.2602	0.2001	0.1861
6	0.0729	0.0526	0.0606	2.5874	0.5439	0.2248	0.0624	0.0230	0.0411	0.0152
7	*	*	0.0524	0.6788	1.1990	0.5337	0.1420	0.0727	0.0107	*
8	*	*	0.0506	0.5075	1.4916	0.7097	0.2419	0.0871	0.0321	*
9	0.0428	1.1246	0.3289	0.1413	0.0822	0.0630	0.0495	0.0056	*	*
10	*	*	0.0212	0.0659	0.7827	0.3906	0.4084	0.2140	0.0837	0.1036
11	0.0526	4.0973	1.5225	0.5568	0.2459	0.1513	0.0301	0.0153	0.0230	0.0378
12	1.1497	0.0567	0.8390	2.0304	0.1411	0.4882	0.1911	0.0526	0.0176	0.0007
Mean	0.2056	0.6645	0.4233	0.8692	0.8198	0.4505	0.1596	0.0663	0.0350	0.0286
S.E.M.	0.1060	0.3635	0.1277	0.2445	0.2193	0.1164	0.0397	0.0245	0.0166	0.0168

* Undetectable data

Table 16 Plasma Diclofenac Sodium Concentration (mcg/ml.) from 12 Subjects Following Oral Administration of two 25 mg. Diclofenac Sodium Enteric-Coated 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	10.0
1	1.0977	0.7706	0.2153	0.0632	0.0529	0.0493	0.0019	*	*	*
2	3.6725	2.9766	1.2535	0.7267	0.3032	0.1428	0.1018	0.0099	*	*
3	0.0704	1.2014	0.3597	0.1712	0.1001	0.0824	0.0041	*	*	*
4	1.4189	2.4388	0.2989	0.0915	0.0616	0.0495	0.0041	*	*	*
5	1.4818	2.7093	1.0991	0.5158	0.3287	0.1825	0.1095	0.1415	0.1852	0.0446
6	*	*	0.3802	1.1282	0.3468	0.5196	0.1445	0.2101	0.0841	0.0133
7	0.3236	0.1036	0.1018	0.1007	0.3803	0.5042	0.6685	0.0873	0.0280	*
8	0.2507	0.1973	0.4616	1.1631	3.9256	1.6960	0.3195	0.2637	0.2110	0.1911
9	0.7806	1.2483	1.0964	0.0624	0.1138	0.0508	0.0166	*	*	*
10	0.3936	0.2783	0.0897	0.0727	0.0624	0.0796	1.4113	0.3382	0.2027	0.0325
11	1.2627	1.7185	0.5511	0.2602	0.1802	0.1284	0.0202	0.0046	*	*
12	*	2.0043	0.8238	0.3201	0.1729	0.0885	0.0230	*	*	*
Mean	0.8960	1.3039	0.5609	0.3897	0.5024	0.2978	0.2354	0.0879	0.0593	0.0235
S.E.M.	0.2990	0.3070	0.1177	0.1177	0.3131	0.1358	0.1206	0.0351	0.0255	0.0158

* Undetectable data

Table 17 Plasma Diclofenac Sodium Concentration (mcg/ml.) from 12 Subjects Following Oral Administration of two 25 mg. Diclofenac Sodium Enteric-Coated 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	10.0
1	0.0100	0.9469	1.4276	0.6753	0.2146	0.1181	0.0451	0.0044	*	*
2	*	3.3918	1.8618	0.7602	0.6071	0.2337	0.1119	0.0141	0.0032	*
3	*	2.5136	0.6887	0.3263	0.1848	0.0349	0.0435	0.1284	0.1119	*
4	*	*	0.0641	0.7553	1.7167	0.3326	0.2200	0.0378	*	*
5	0.0141	0.1090	0.1504	0.1060	0.0971	0.1010	1.4612	0.5668	0.6694	0.6486
6	2.8045	0.9336	0.3082	0.1776	0.1507	0.1855	0.0486	0.1298	0.1108	0.0674
7	*	0.0090	0.0152	0.0435	0.0624	0.3295	0.2027	0.0961	0.0632	*
8	*	*	0.4204	1.8476	0.6892	0.4043	0.1496	0.0634	0.0468	0.0093
9	*	*	0.0261	0.0513	0.4050	1.4226	0.1305	0.2391	0.0971	*
10	*	*	0.0819	2.8084	0.8267	0.3319	0.1205	1.1310	*	*
11	*	*	0.0612	0.8993	0.5485	1.0160	0.1673	0.0423	*	*
12	*	*	*	0.0492	0.1468	1.2964	0.1449	0.0828	0.0204	0.0093
Mean	0.2357	0.6587	0.4255	0.7083	0.4708	0.4839	0.2372	0.2113	0.0936	0.0612
S.E.M.	0.2335	0.3303	0.1776	0.2439	0.1353	0.1386	0.1125	0.0945	0.0539	0.0537

* Undetectable data

Table 18 Plasma Diclofenac Sodium Concentration (mcg/ml.) from 12 Subjects Following Oral Administration of two 25 mg. Diclofenac Sodium Enteric-Coated Tablets of Brand H

Subject No.	Time (hr.)									
	0.5	1.0	1.5	2.0	2.5	3.0	4.0	6.0	8.0	10.0
1	*	*	0.0624	0.3879	0.9534	0.5940	0.3560	0.0335	0.0624	*
2	*	0.9941	1.5049	3.0645	1.0899	0.4814	0.1211	0.0664	*	*
3	*	0.1688	0.4141	0.7545	1.0813	0.2507	0.1179	0.3290	0.1415	0.0695
4	0.2444	0.2231	1.4775	1.0407	0.8657	0.2602	0.0485	0.2320	0.4295	0.1754
5	*	*	*	0.0086	0.0868	0.5512	0.9725	0.1626	0.0614	*
6	*	*	*	0.1786	1.0168	1.1874	0.5868	0.3561	0.0377	*
7	*	*	0.0383	0.4893	1.2037	0.9274	0.1314	0.0842	*	*
8	*	*	0.0822	0.4736	1.0596	0.7963	0.2884	0.0996	0.0288	0.0050
9	*	*	1.0170	0.2781	0.0498	0.0202	0.6257	0.0075	*	*
10	0.0176	0.0513	0.1119	0.3375	0.5093	0.5439	0.1472	0.0954	*	*
11	*	*	0.1713	0.4887	0.7786	0.6929	0.5940	0.2011	0.0254	*
12	*	*	0.0857	0.1526	0.9063	0.5421	0.1214	0.1235	0.0160	*
Mean	0.0218	0.1198	0.4138	0.6379	0.8001	0.5706	0.3426	0.1492	0.0669	0.0208
S.E.M.	0.0203	0.0824	0.1665	0.2345	0.1113	0.0907	0.0835	0.1110	0.0350	0.0152

* Undetectable data

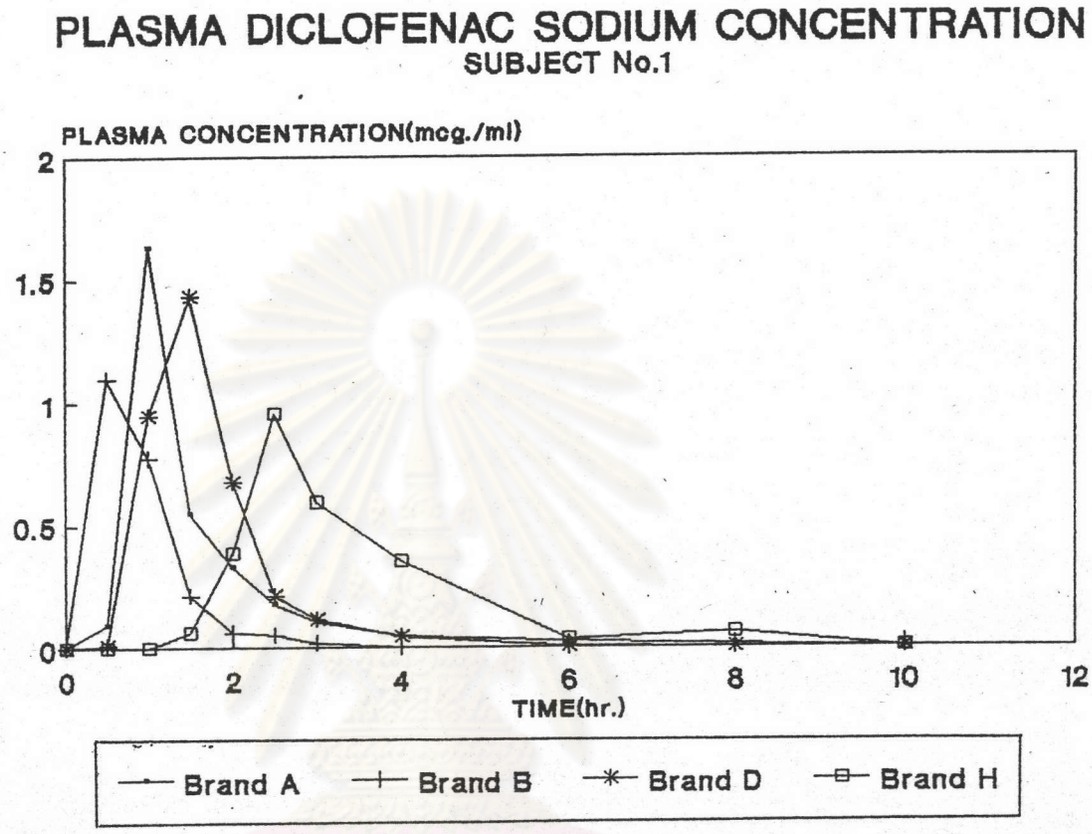

Figure 5 Plasma diclofenac sodium concentration-time profile of subject No.1 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

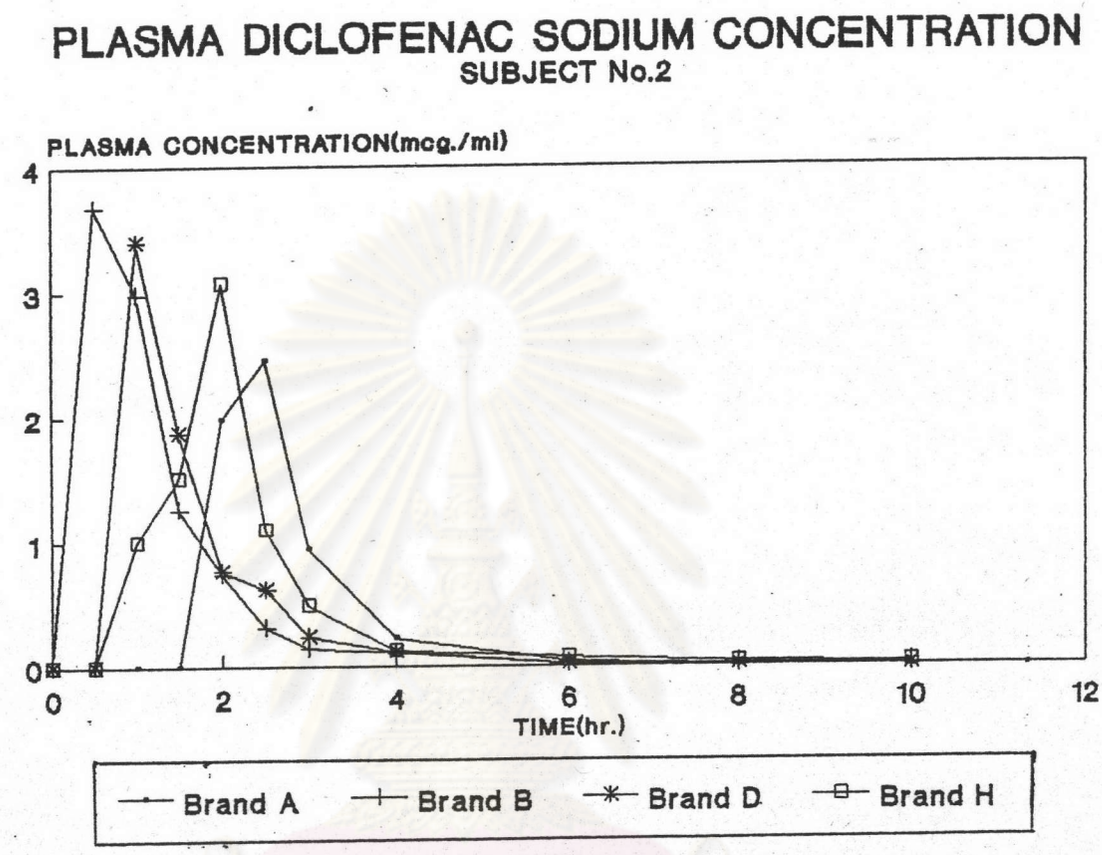

Figure 6 Plasma diclofenac sodium concentration-time profile of subject No. 2 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

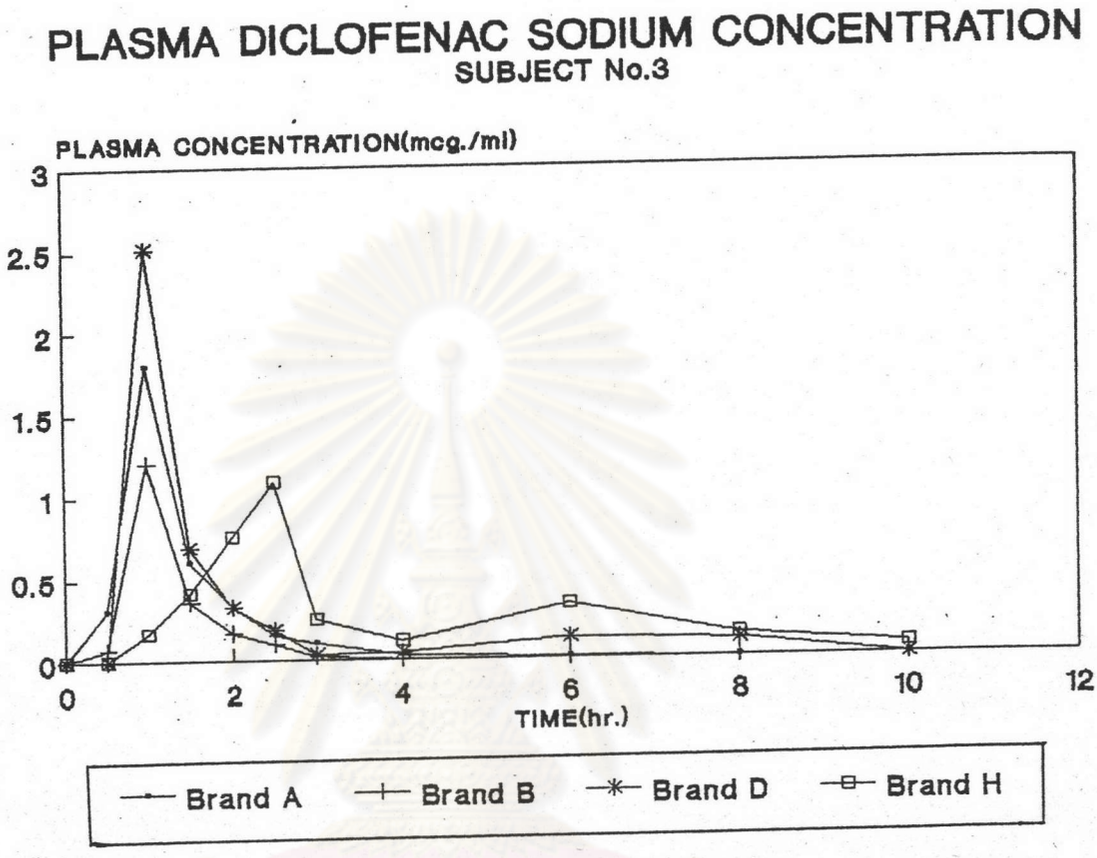

Figure 7 Plasma diclofenac sodium concentration-time profile of subject No. 3 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

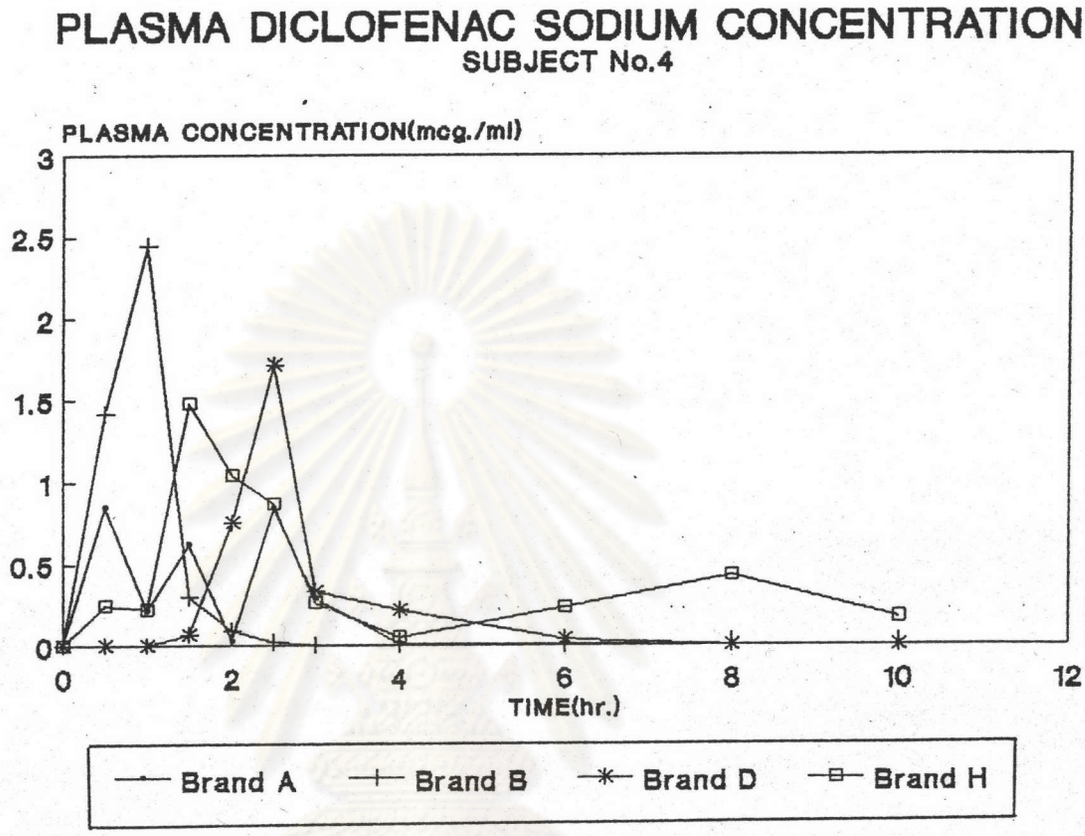

Figure 8 Plasma diclofenac sodium concentration-time profile of subject No. 4 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

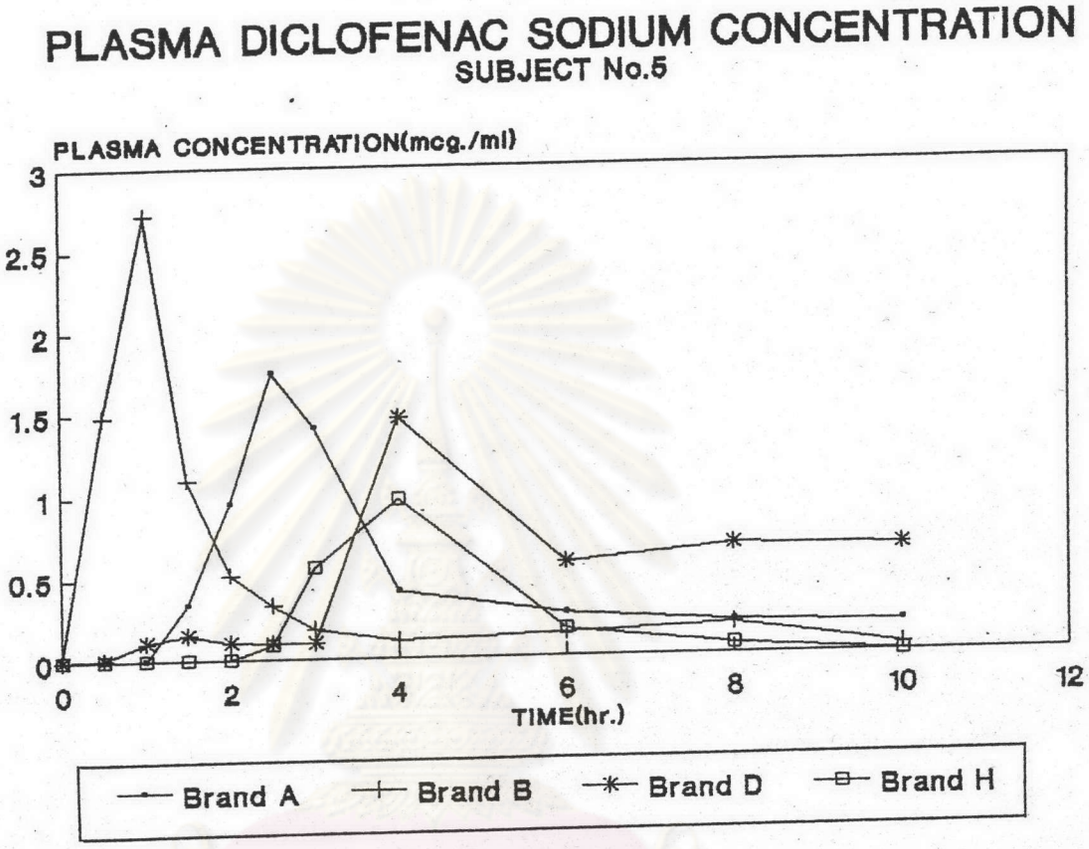

Figure 9 Plasma diclofenac sodium concentration-time profile of subject No. 5 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

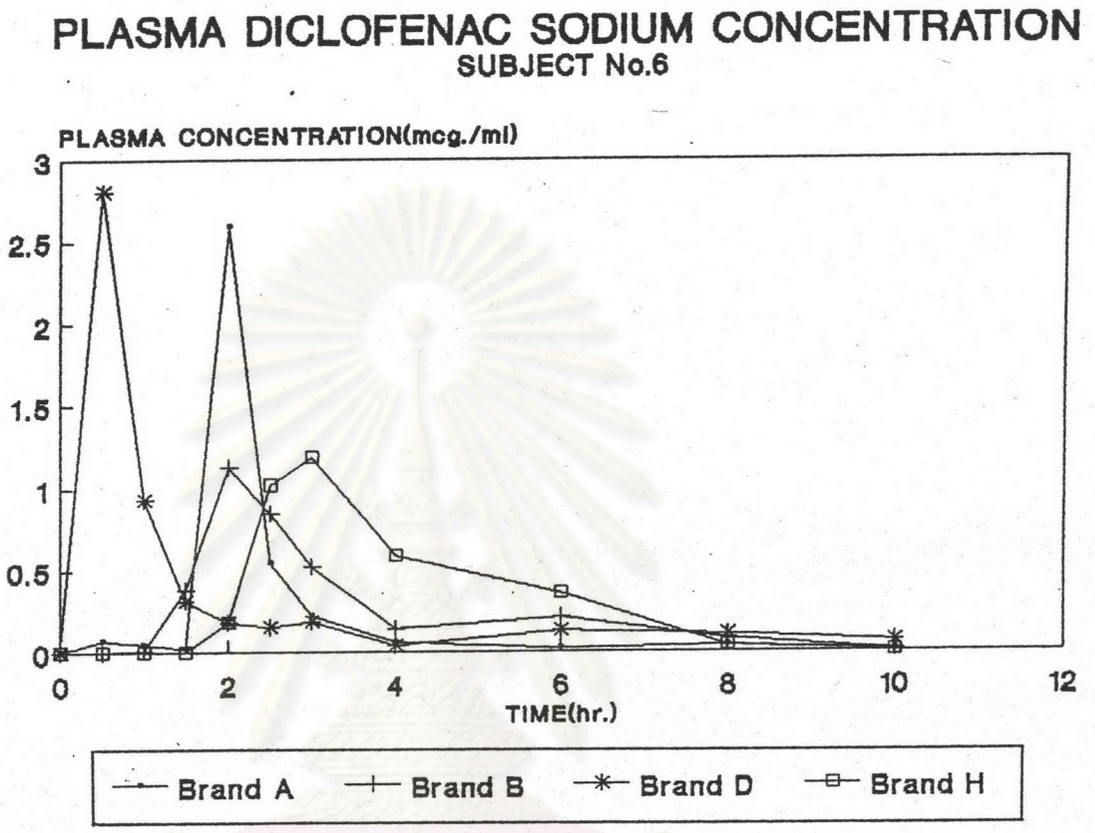

Figure 10 Plasma diclofenac sodium concentration-time profile of subject No. 6 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

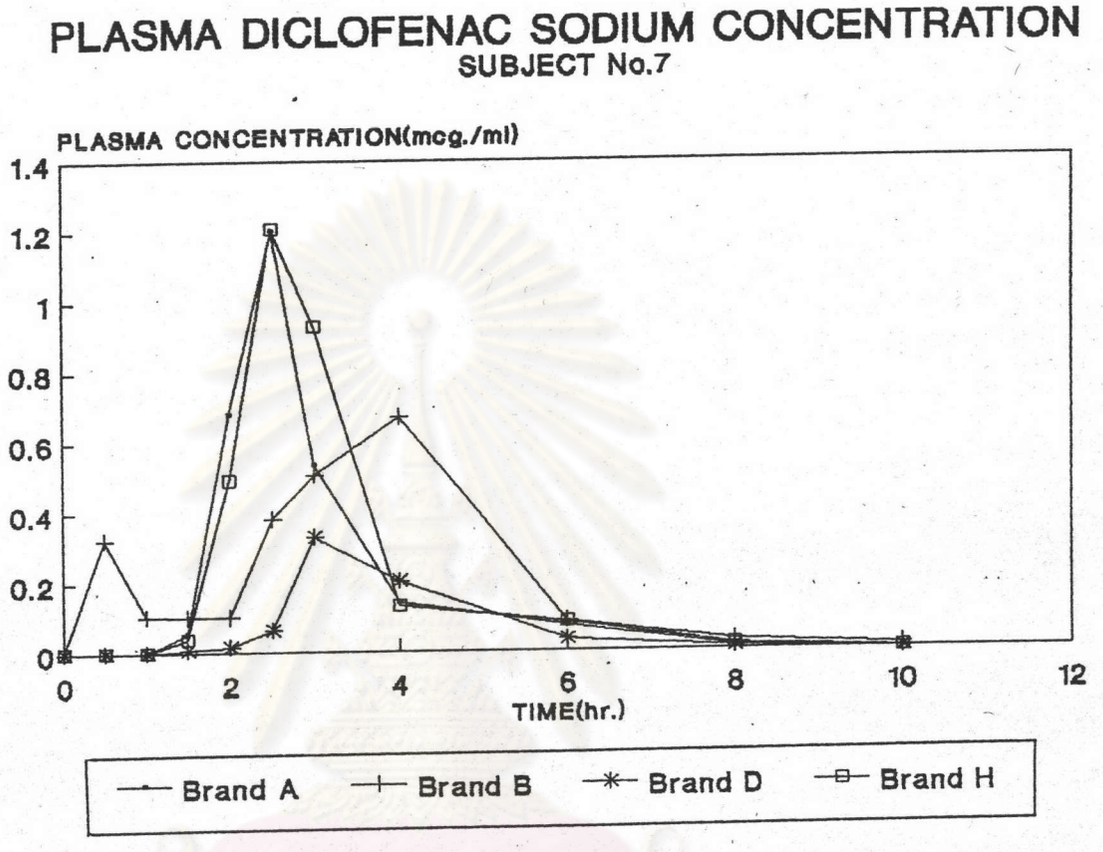

Figure 11 Plasma diclofenac sodium concentration-time profile of subject No. 7 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

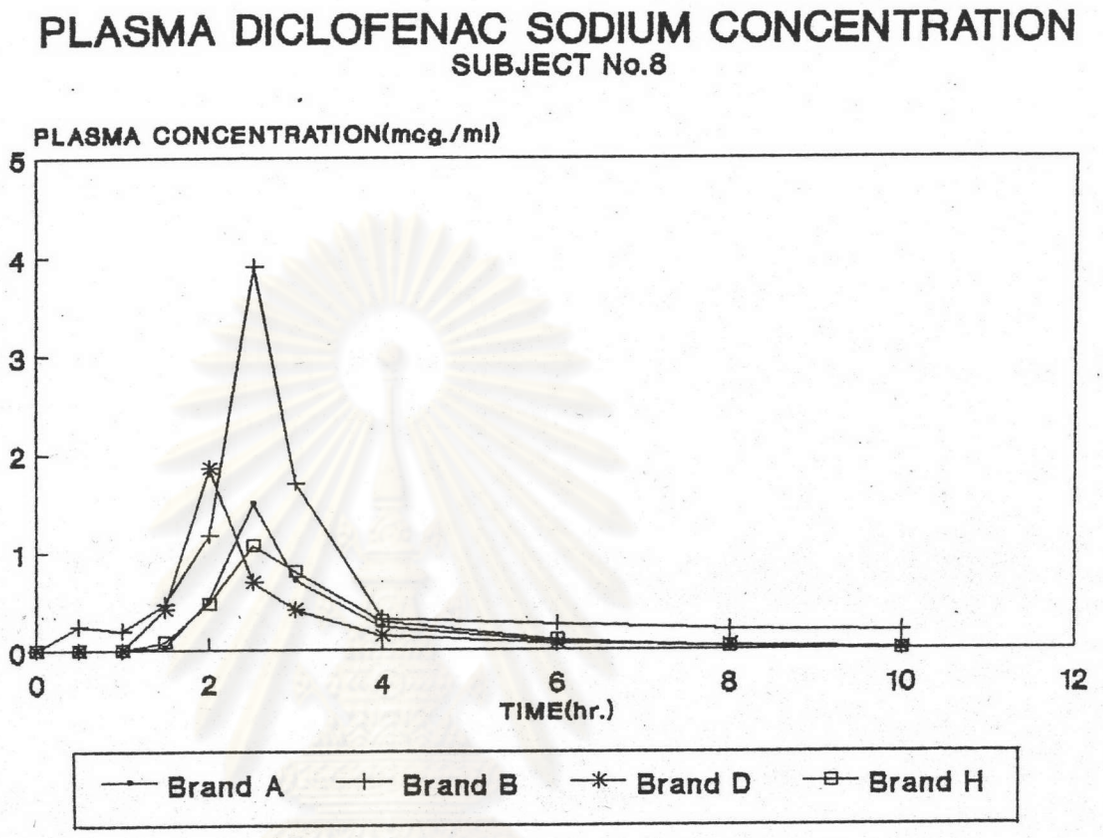

Figure 12 Plasma diclofenac sodium concentration-time profile of subject No. 8 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

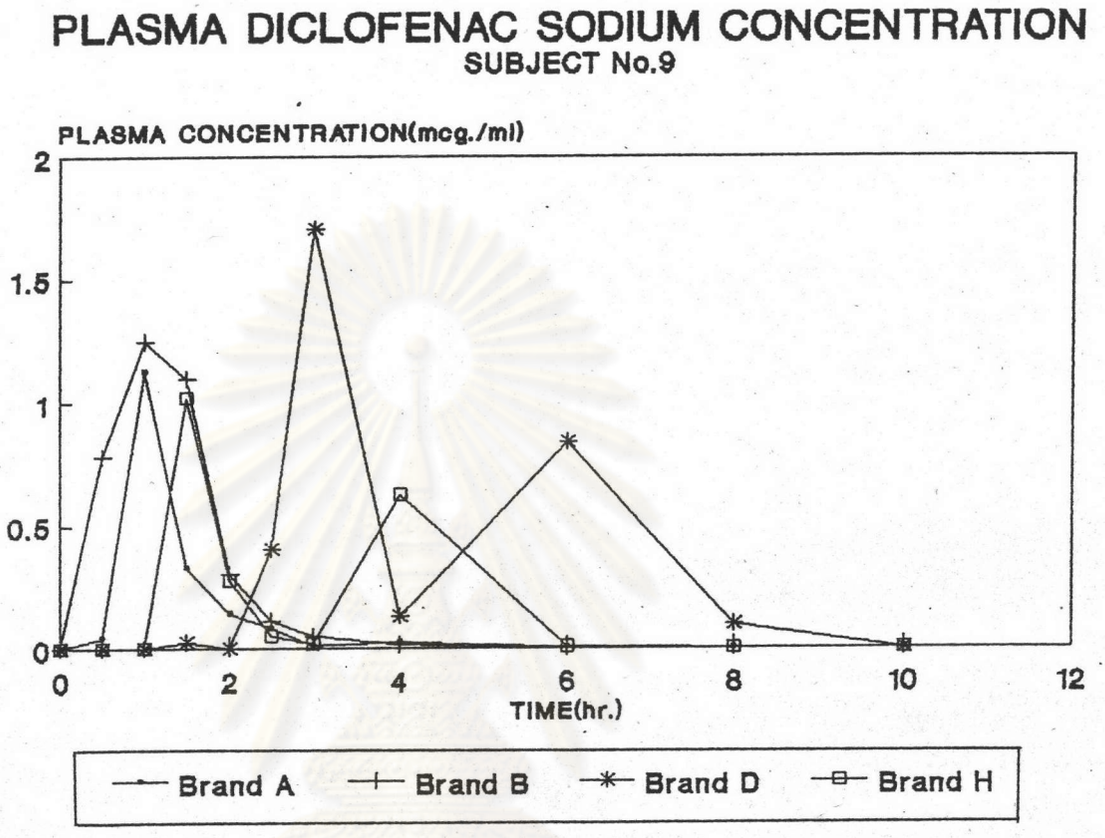

Figure 13 Plasma diclofenac sodium concentration-time profile of subject No. 9 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

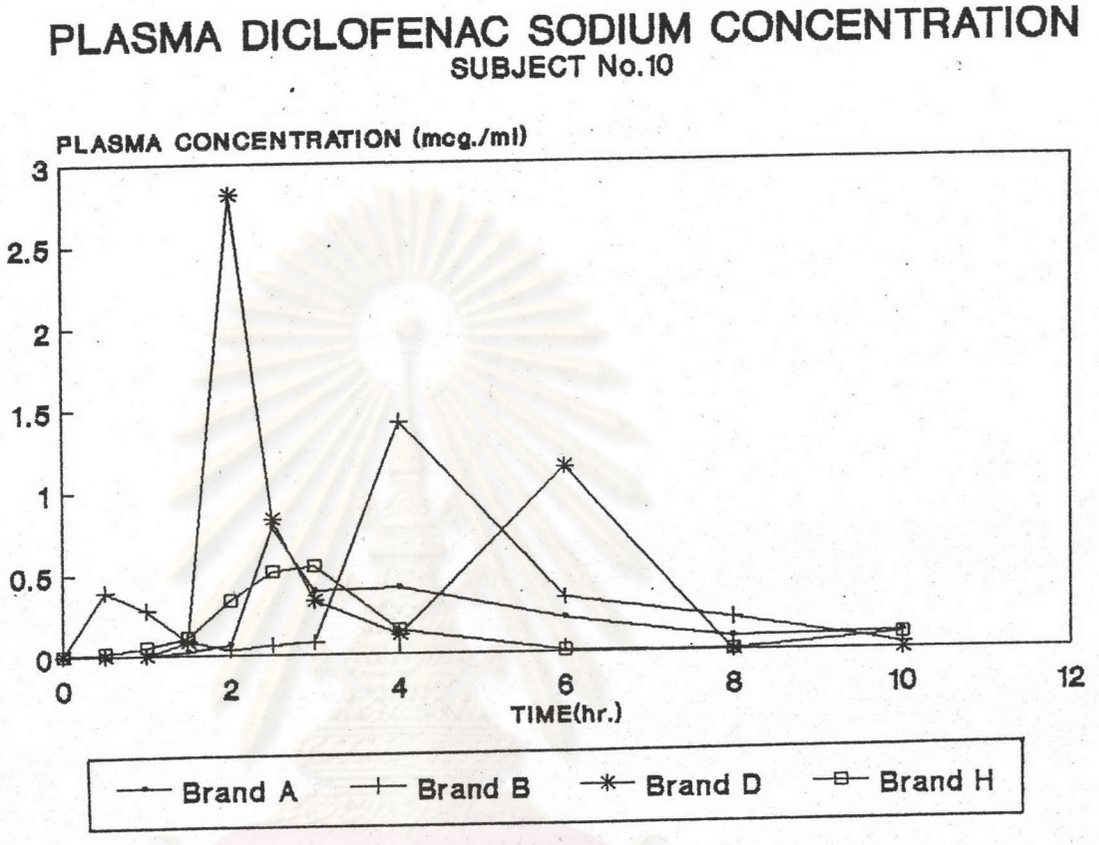

Figure 14 Plasma diclofenac sodium concentration-time profile of subject No. 10 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

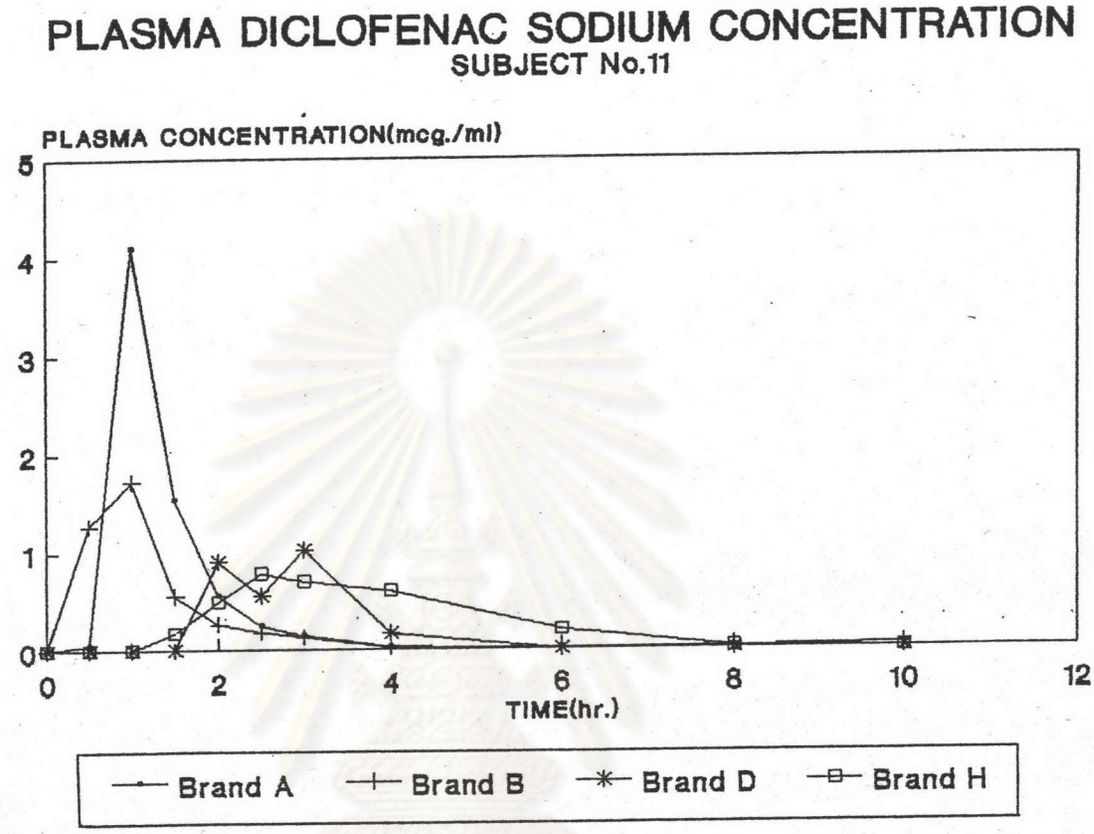

Figure 15 Plasma diclofenac sodium concentration-time profile of subject No. 11 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

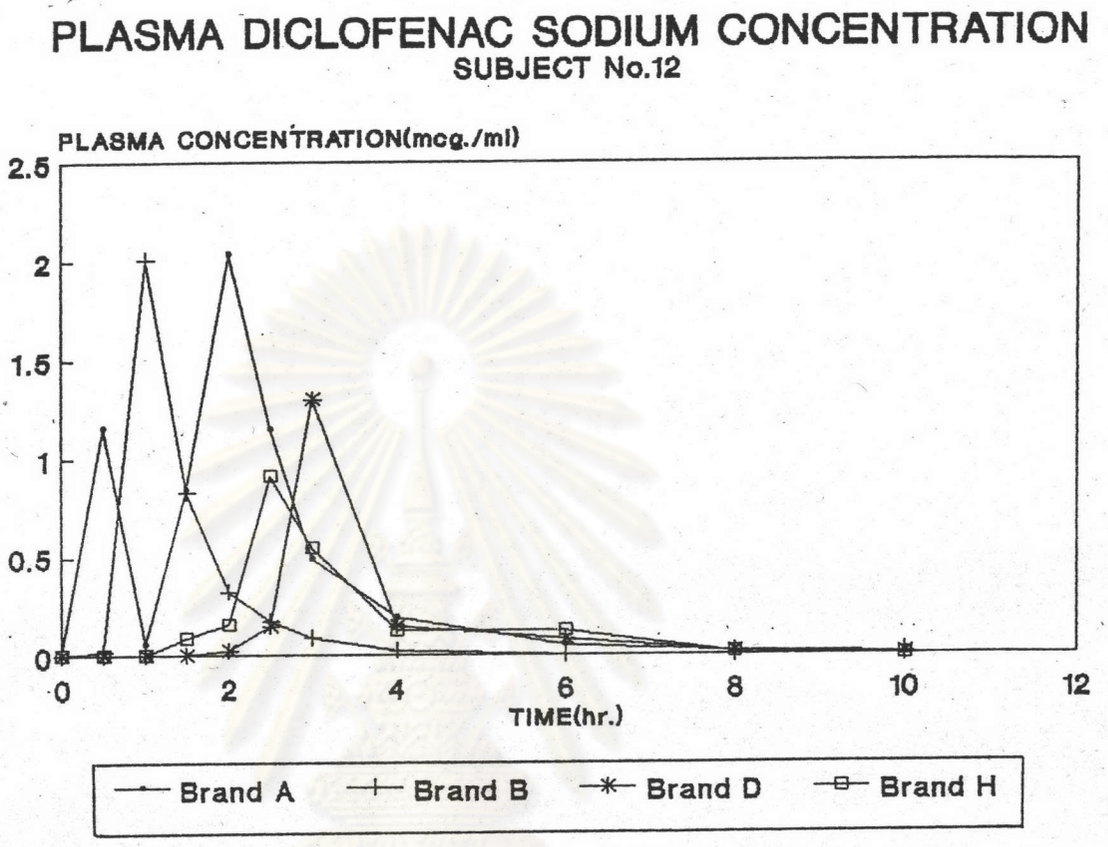

Figure 16 Plasma diclofenac sodium concentration-time profile of subject No. 12 following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

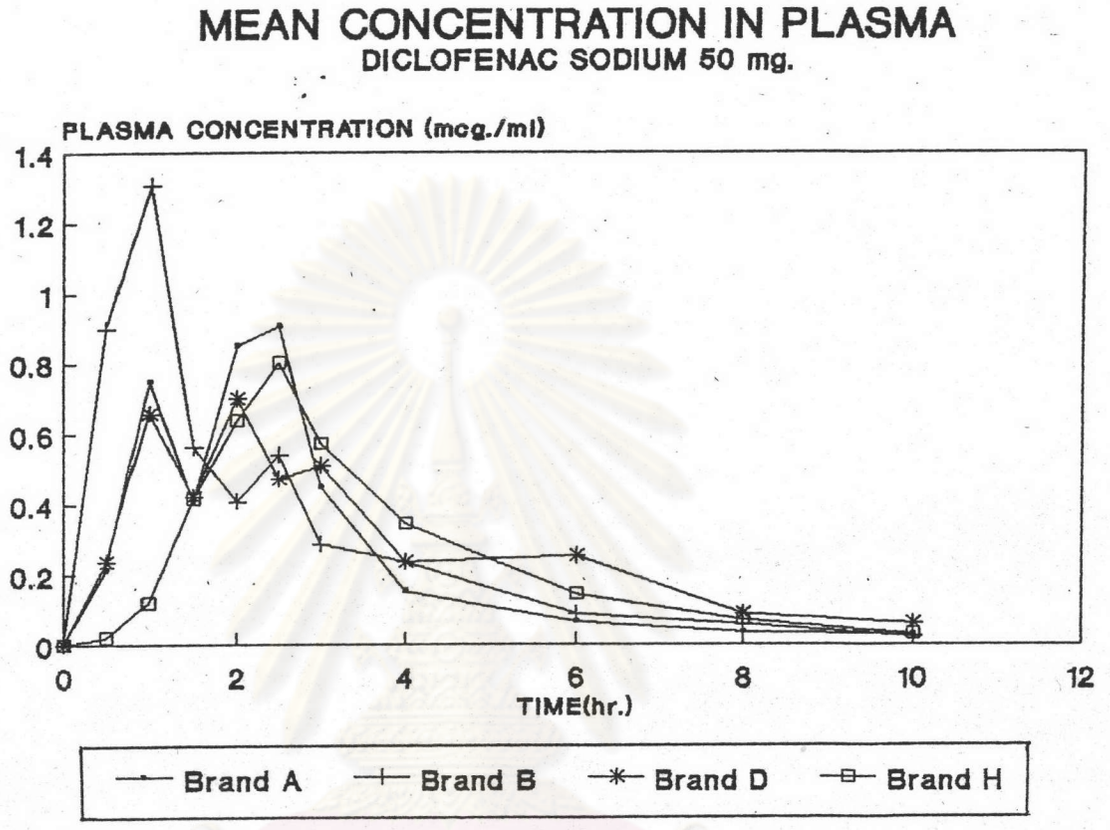

Figure 17 Comparison of mean plasma diclofenac sodium concentration-time profile from 12 subjects following oral administration of two 25 mg diclofenac sodium enteric-coated tablets

Figures 5 to 16. As could be seen from the plots individual plasma concentration-time profile was not smooth, instead it had its own pattern. This was because diclofenac kinetics in the body was high intersubject variation.

Some profiles exhibited two peaks plasma concentration, would probably be the drug possess entero-hepatic cycling effect (Figure 13 to 15). Comparison of the mean plasma concentration-time profile of each brand were summarized in Figure 17.

The plots also indicated that the concentration of the drug were high fluctuation in individual among brands. Due to diclofenac kinetics in the body individual was wide variation, analysis of the data using CSTRIP computer program seemed to be unappropriate which could read to wrong interpretation.

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

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 values indicated the extent of absorbed drug which escaping first pass metabolism and entering the systemic circulation. They can be obtained from the plasma drug concentration-time data. In the bioequivalent study, drug products that are pharmaceutical equivalence are accepted to be bioequivalent if no statistically significant difference in the rate and the extent of drug absorption can be observed (Skelly, 1976; Shargel and Yu, 1980).

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

3.1 Peak Plasma Concentration (C_{max})

Previous report indicated that the mean peak plasma concentration achieved following oral administration of 50 mg diclofenac sodium enteric-coated tablets was about 1.5 mcg./ml. (ranged from 1.4-3.0 mcg./ml.) (Willis and Kendall, 1978; Willis *et al.*, 1979; 1980; 1981) and 0.84 - 1.06 mcg./ml. with a 25 mg enteric-coated tablets (Paton, 1987). In this study, the mean peak plasma diclofenac sodium concentration for brands A, B, D and H were 1.8173 $\pm$ 0.2657, 1.9360 $\pm$ 0.3021, 1.8387 $\pm$ 0.2540 and 1.1897 $\pm$ 0.1831 mcg./ml., respectively. The result from each treatment was shown in Table 19. The rank order of these values were brands B > D > A > H. There were no statistically significant difference ($p > 0.05$) among these value of all brands

(Table 20). This refered that all brands could produce the same intensity of action.

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

The time to peak plasma diclofenac sodium concentration of each individual was presented in Table 21. The average peak times were 1.75 ± 0.23, 1.63 ± 0.36, 2.21 ± 0.30 and 2.50 ± 0.19 hours for brand A, B, D and H, respectively. The time to peak plasma levels in this study agreed with the results presented by other investigators ranging in which it was from 1.5 - 2.5 hrs. (Geiger *et al.*, 1975, Willis *et al.*, 1981).* There were no statistically significant difference ($p > 0.05$) among these values of all brands (Table 22) despite brand D demonstrated highest value.

The results from 3.1 and 3.2 indicated that the rate of diclofenac sodium absorption was similar in each brand despite. Although, the values of t_{max} of brands D and H were slightly higher than others which could result in slow onset of action. The pharmaceutical formulations and/or production processes were possibly thought to be the reason for the lower rate of dissolution of these brands.

3.3 Area Under the Plasma Versus Time Curve (AUC)

The mean $AUC_{0\rightarrow10}$ from individual plasma of all brand were 2.4420 ± 0.3155, 2.8060 ± 0.4798,

Table 19 Peak Plasma Concentration (C_{max}) of Diclofenac Sodium Following Oral Administration of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets

Subject No.	C_{max} (mcg/ml)			
	A	B	D	H
1	1.6302	1.0977	1.4276	0.9534
2	2.4456	3.6725	3.3918	3.0645
3	1.7992	1.2014	2.5136	1.0813
4	0.8400	2.4388	1.7167	1.4775
5	1.7428	2.7093	1.4612	0.9725
6	2.5874	1.1282	2.8045	1.1874
7	1.1990	0.6685	0.3295	1.2037
8	1.4916	3.9256	1.8476	1.0596
9	1.1246	1.2483	1.4226	1.0170
10	0.7827	1.4113	2.8084	0.5439
11	4.0973	1.7185	1.0160	0.7786
12	2.0304	2.0043	1.2964	0.9063
Mean	1.8142	1.9353	1.8363	1.1871
S.E.M.	0.2650	0.3031	0.2536	0.1831

Table 20 Analysis of Variance for Peak Plasma Concentration of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	4.2017	1.4006	1.80
Within group	44	34.3072	0.7797	
Total	47	38.5089		

* The full data were shown in Table 19

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 21 Time to Peak Plasma Concentration (t_{max}) of Diclofenac Sodium Following Oral Administration of Four Different Brand of Diclofenac Sodium Enteric-Coated Tablets

Subject No.	t_{max} (hr.)			
	A	B	D	H
1	1.00	0.50	1.50	2.50
2	2.50	0.50	1.00	2.00
3	1.00	1.00	1.00	2.50
4	0.50	1.00	2.50	1.50
5	2.50	1.00	4.00	4.00
6	2.00	2.00	0.50	3.00
7	2.50	4.00	3.00	2.50
8	2.50	2.50	2.00	2.50
9	1.00	1.00	3.00	1.50
10	2.50	4.00	2.00	3.00
11	1.00	1.00	3.00	2.50
12	2.00	1.00	3.00	2.50
Mean	1.75	1.63	2.21	2.50
S.E.M.	0.23	0.36	0.30	0.19

Table 22 Analysis of Variance of Time to Peak Plasma Concentration of Diclofenac Sodium of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	5.94	1.98	2.13
Within group	44	41.04	0.93	
Total	47	46.98		

* The full data were shown in Table 21

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 Area Under the Plasma Concentration-Time Curve (AUC) of Diclofenac Sodium Following Oral Administration of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets

Subject No.	$AUC_{0\rightarrow10}$ (mcg.hr./ml)			
	A	B	D	H
1	1.6178	1.1397	1.8022	1.8732
2	3.3577	4.7459	3.6677	4.0014
3	1.7800	1.0194	2.4287	2.6542
4	1.6391	2.1982	1.9071	3.8674
5	4.9225	4.2220	6.2755	2.3679
6	2.1200	2.1825	3.0153	3.1561
7	1.7452	2.1165	0.9348	1.9267
8	2.1584	6.4408	2.8134	2.1062
9	0.9926	1.7135	2.1763	1.6412
10	2.4401	4.0101	4.5502	1.3333
11	3.6274	2.1222	1.8521	2.5830
12	2.9033	1.7614	1.4737	1.4400
Mean	2.4420	2.8060	2.7414	2.4126
S.E.M.	0.3155	0.4798	0.4295	0.2554

Table 24 Analysis of Variance for AUC of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	1.47	0.49	0.28
Within group	44	76.50	1.74	
Total	47	77.97		

* The full data were shown in Table 23

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

2.7414 ± 0.4295 and 2.4126 ± 0.2554 mcg.hr./ml. for brands A, B, D and H, respectively as shown in Table 23. The rank order of these values were brands B > D > A > H. This result correlated well with the C_{max} of all brands There were no statistically significant different ($p > 0.05$) among these value of all brands (Table 24). The values of mean AUC in this study was higher than those presented by Willis *et al.*, 1978 (1.5 - 1.9 mcg.hr./ml). This difference may be caused by the differences of the study condition and assay method. The similar AUC value of all brands indicated that the drug were absorbed with the same amount from all formulation into the body.

The principal pharmacokinetic parameters of diclofenac sodium following oral administration of four brands were summarized in Table 33. Statistical analysis of these corresponding parameters among the four brands demonstrated that all brands were bioequivalent in terms of both the rate and the extent of drug absorption.

Although the disintegration time and the dissolution rate were different in each brand, all brands were bioequivalent with each other. This might be due to diclofenac sodium absorption occured slower than the disintegration and/or the dissolution processes of the drug.

4. The Relative Bioavailability

Relative Bioavailability is the availability of a drug product as compared to a recognized standard (innovater's product) (Shargel and Yu, 1980). In this study the mean relative bioavailability calculated by comparing the mean $AUC_{0\rightarrow10}$ of each brand to that of the innovator's product. The value obtained of brand B, D and H relatively to brand A were 114.91%, 112.26% and 98.80%, respectively

5. Pharmacokinetic of Diclofenac Sodium Enteric-Coated Tablets

From the plasma diclofenac sodium concentration-time relationship, results demonstrated that the data were well described by means of biexponential equation. This referred that pharmacokinetic of diclofenac sodium in Thai healthy volunteers could be explained by a one compartment open model.

The pharmacokinetic parameters derived from the model of analysis from plasma concentration-time data of each brand of diclofenac sodium enteric-coated tablets were detailed in Tables 19 to 24.

5.1 Absorption Rate Constant (K_a)

The average absorption rate constant for brands A, B, D and H were 1.1321 ± 0.0978, 1.8850 ± 0.3085, 0.9085 ± 0.1722 and 1.2066 ± 0.1944 $hr.^{-1}$,

Table 25 Absorption Rate Constant (K_a) of Diclofenac Sodium Following Oral Administration of Four Different Brands of 25 mg Diclofenac Sodium Enteric-Coated Tablets

Subject No.	K_a ($hr.^{-1}$)			
	A	B	D	H
1	0.9008	2.3726	1.9182	1.0093
2	1.7543	1.6572	1.6875	3.1255
3	1.5280	2.9720	0.1545	0.6053
4	0.8926	3.4871	1.2633	1.3225
5	0.8465	0.5665	0.2841	0.8351
6	0.9823	0.8828	0.4277	1.0530
7	1.1079	0.8776	1.1699	1.2543
8	1.1530	0.7751	1.0990	1.1679
9	1.3594	2.9001	0.5080	0.7768
10	0.8231	0.8178	0.2153	1.6736
11	0.6977	2.3595	1.3731	0.9656
12	1.5398	2.9517	0.8017	0.6905
Mean	1.1321	1.8850	0.9085	1.2066
S.E.M.	0.0978	0.3085	0.1722	0.1944

Table 26 Analysis of Variance for Absorption Rate Constant of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	6.37	2.12	4.08
Within group	44	22.72	0.52	
Total	47	29.09		

* The full data were shown in Table 25

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 27 Comparison of Absorption Rate Constant of Each Brand of Diclofenac Sodium Enteric-Coated Tablets with that of the Innovator's product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
B	2.59	$0.005 > p > 0.001$	S
D	0.75	$0.500 > p > 0.400$	NS
H	0.27	$p > 0.5$	NS

$t^{a}(0.05, 44) = 2.01$

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

a = t value obtained from the table

Table 28 Elimination Rate Constant (K_{el}) of Diclofenac Sodium Following Oral Administration of Four Different Brands of 25 mg Diclofenac Sodium Enteric-Coated Tablets

Subject No.	K_{el} ($hr.^{-1}$)			
	A	B	D	H
1	0.5662	1.5500	1.2254	0.4333
2	1.0997	1.1392	0.9027	0.6031
3	1.0049	1.328	0.0684	0.3869
4	0.3992	1.876	0.8746	0.1228
5	0.2947	0.2867	0.1570	0.4837
6	0.2734	0.3976	0.2465	0.7403
7	0.7772	0.5176	0.7583	0.8160
8	0.4938	0.3475	0.4701	0.7213
9	0.7502	1.6339	0.3517	0.5801
10	0.2519	0.1200	0.1556	0.4045
11	0.3235	1.1343	0.9343	0.6600
12	0.8029	1.3306	0.5331	0.4989
Mean	0.5865	0.9714	0.5565	0.5376
S.E.M.	0.0851	0.1745	0.1086	0.0551

Table 29 Analysis of Variance for Elimination Rate Constant of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	1.54	0.51	3.19
Within group	44	6.94	0.16	
Total	47	8.48		

* The full data were shown in Table 28

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 30 Comparison of Elimination Rate Constant of Each Brand of Diclofenac Sodium Enteric-Coated Tablets with that of the Innovator's product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
B	2.37	$0.025 > p > 0.010$	S
D	0.19	$p > 0.5$	NS
H	0.30	$p > 0.5$	NS

$t^{a}(0.05, 44) = 2.01$

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

a = t value obtained from the table

Table 31 Biological Half-life ($t_{1/2}$) of Diclofenac Sodium Following Oral Administration of 25 mg Diclofenac Sodium Enteric-Coated Tablets

Subject No.	$t_{1/2}$ (hr.)			
	A	B	D	H
1	1.22	0.45	0.55	1.60
2	0.63	0.61	0.70	1.15
3	0.69	0.52	10.13	1.79
4	1.74	0.37	0.79	5.64
5	2.35	2.42	4.41	1.43
6	2.54	1.74	2.81	0.94
7	0.89	1.34	0.91	0.85
8	1.40	1.99	1.47	0.96
9	0.92	0.42	1.97	1.20
10	2.75	5.78	4.45	1.71
11	2.14	0.61	0.74	1.05
12	0.86	0.52	1.30	1.39
Mean	1.51	1.40	2.52	1.64
S.E.M.	0.22	0.45	0.80	0.38

Table 32 Analysis of Variance for Biological Half-life of Four Different Brands of Diclofenac Sodium Enteric-Coated Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	9.40	3.08	0.98
Within group	44	135.49	3.13	
Total	47	144.89		

* The full data were shown in Table 31

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

respectively as shown in Table 25. The rank order of these values were brands B > H > A > D. Statistical analysis results in Tables 26 and 27 indicated that only the absorption rate constant of brand B exhibited significant higher than that of the innovator's product (brand A) ($p < 0.05$). This was due to higher dissolution of the drug of brand B than any other brands did.

5.2 Elimination Rate Constant (K_{el})

The average elimination rate constant obtained from individual plasma data of brands A, B, D and H were 0.5865 ± 0.0851, 0.9714 ± 0.2093, 0.5565 ± 0.1086 and 0.5376 ± 0.0551 $hr.^{-1}$, respectively (Table 28). The rank order of this value was brand B > A > D > H. Statistical analysis results in Table 29 and 30 indicated that only the elimination rate constant of brand B exhibited significant higher than that of the innovator's product (brand A) ($p < 0.05$).

5.3 Half-life ($t_{1/2}$)

The mean half-life of diclofenac sodium determined for brands A, B, D and H were 1.51 ± 0.22, 1.40 ± 0.45, 2.52 ± 0.80, and 1.64 ± 0.38 hours, respectively (Table 31). The values agreed with the results found by other investigators ranging from 1.2 to 1.8 hours (Kendall *et al.*, 1979; Willis *et al.*, 1979). But Paton (1987) reported the half-life of diclofenac sodium as 0.61 ± 0.09 hr. This difference may be caused by the difference of the study condition, subjects' race and/or

Table 33 Estimated Pharmacokinetic Parameters (Mean ± SEM) of Diclofenac Sodium from Twelve Subjects Following Oral Administration of Four Different Brands of Diclofenac Sodium Enteric-Coated Tables.

Parameters	Brand				F-test	t-test * with respect to brand A
	A	B	D	H		
C_{max} (mcg./ml.)	1.8140 ± 0.2650	1.9353 ± 0.3031	1.8363 ± 0.2536	1.1871 ± 0.1831	1.80 (2.824)**	NS
t_{max} (hr.)	1.75 ± 0.23	1.63 ± 0.36	2.21 ± 0.30	2.50 ± 0.19	2.13 (2.824)**	NS
AUC (mcg.hr./ml.)	2.4420 ± 0.3155	2.8060 ± 0.4798	2.7414 ± 0.4295	2.4126 ± 0.2554	0.28 (2.824)**	NS
$t_{1/2}$ (hr.)	1.51 ± 0.22	1.40 ± 0.45	2.52 ± 0.80	1.64 ± 0.38	1.04 (2.824)**	NS

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

** F value obtained from the table

Table 34 In Vitro - In Vivo Correlations

Correlation (n = 4)	Correlation Coefficient	t value	Statistical* Significance
Disintegration Times versus C_{max}	- 0.9805	7.06	S 0.010<p<0.025
Disintegration Times versus t_{max}	0.9200	3.32	NS 0.050<p<0.100
Disintegration Times versus AUC	- 0.6150	1.10	NS 0.400<p<0.500
Dissolution Rate Constants versus C_{max}	0.8124	1.97	NS 0.100<p<0.200
Dissolution Rate Constants versus t_{max}	- 0.9991	33.32	S p<0.001
Dissolution Rate Constants versus AUC	- 0.3830	0.59	NS p>0.500

$t^{a}_{0.05(2)} = 4.303$

a = t value obtained from the table

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

assay method. Statistical analysis showed no significant differences among these values (Table 32)

6. In Vitro - In Vivo Correlation

The correlation studies between the in vitro and in vivo data for Brands A, B, D, and H were presented in Table 34.

Disintegration time showed statistically significant correlation with the C_{max} ($p < 0.05$).

Dissolution rate constant demonstrated statistically significant correlation ($p < 0.05$) with the t_{max} value. Although the disintegration time was correlative with the C_{max}, this correlation however, seemed to be less important. This was because in fact, disintegration time was a part of dissolution processes. The correlation of dissolution rate constant with the t_{max} values refered that the dissolution rate of the drug could affect the rate of drug absorption. Therefore the dissolution rate constant might be used as the preliminary tool to predict the rate of drug absorption.