CHAPTER IV

RESULTS AND DISCUSSION

In Vitro Studies

As a basis of pharmaceutical quality control, physical and chemical analysis of the drugs in any dosage forms must be processed in order to assure that the drugs meet the standard requirements. In this study, all seven brands of glibenclamide tablets were analyzed following the requirement specified in the official compendial monograph in the British Pharmacopoeia 1988 (London Her Majesty's Stationary Office, 1988). Results of all tests were summarized in Table 2. Each brand was tested for content of active ingredient and uniformity of content. The content of active ingredient of all brands were within the limits 95.0-105.0 percent labeled amount. Uniformity of content of all brands complied with the test limit which stated that not more than one individual value of the tested values was outside the limits 85.0-115.0 percent of the average value and none of the tested values was outside the limits 75.0-125.0 percent of the average value.

Weight variation was tested to confirm the uniformity of tablet weights. All brands passed the test requirement which specified that not more than two of the individual tablet weights deviated from the average

weight by more than 7.5 percent deviation and none deviated from the average weight by more than 15 percent.

Disintegration time was also an important attribute of tablet quality control because disintegration time must take place before the active ingredient of the tablet can dissolve and be absorbed. All brands disintegrated within 15 minutes as specified in the British Pharmacopoeia 1988 as general requirement for uncoated tablet. Disintegration time ranged from 1.22 to 8.31 minutes. The rank order in term of mean disintegration time were brands E > B > C > F > G > D > A. Statistical comparison indicated that the disintegration time of brands B and E were statistically longer than that of brand A ($p < 0.05$) as shown in Tables 3 and 4.

In summary, all brands of glibenclamide tablets met the official compendial standard of potency, uniformity of content, weight variation and disintegration time so that they were all pharmaceutical equivalence.

Although the dissolution testing of glibenclamide tablets was not official in the British Pharmacopoeia 1988, it was an appropriate crucial factor for systemic drug availability because a drug had to, in general, dissolve 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

Table 2 In Vitro Studies of Seven Commercial Brands of Glibenclamide Tablets*

Brand	Weight[a] (gm.)	% Labeled amount[b]	Uniformity of[c] Content	Disintegration[d] Time (minutes)
A	0.1602±0.0024	103.44±0.34	102.85±1.31	1.22±0.12
B	0.1616±0.0046	101.90±1.17	106.21±2.95	6.34±0.54
C	0.1659±0.0031	98.29±0.38	100.00±3.16	2.44±0.52
D	0.1696±0.0021	99.99±0.30	103.09±1.58	1.80±0.53
E	0.1607±0.0015	101.09±0.61	97.74±3.75	8.31±3.21
F	0.1626±0.0035	101.91±0.51	101.57±8.71	2.38±0.86
G	0.1597±0.0041	103.19±0.62	103.82±2.96	2.36±1.61

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

a : n = 20 c : n = 10

b : n = 3 d : n = 6

Table 3 Analysis of Variance for Disintegration Time of Seven Brands of Glibenclamide Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	6	257.41	42.90	20.73
Within group	35	72.31	2.07	
Total	41	329.72		

* Calculation data from Table 2

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

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 4 Comparison of Disintegration Time of Each Brand of Glibenclamide Tablets with that of the Innovator's product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
B	6.17	$p < 0.001$	S
C	1.47	$0.100<p<0.200$	NS
D	0.70	$0.400<p<0.500$	NS
E	8.54	$p < 0.001$	S
F	1.40	$0.100<p<0.200$	NS
G	1.37	$0.100<p<0.200$	NS

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

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

a = t value obtained from the table

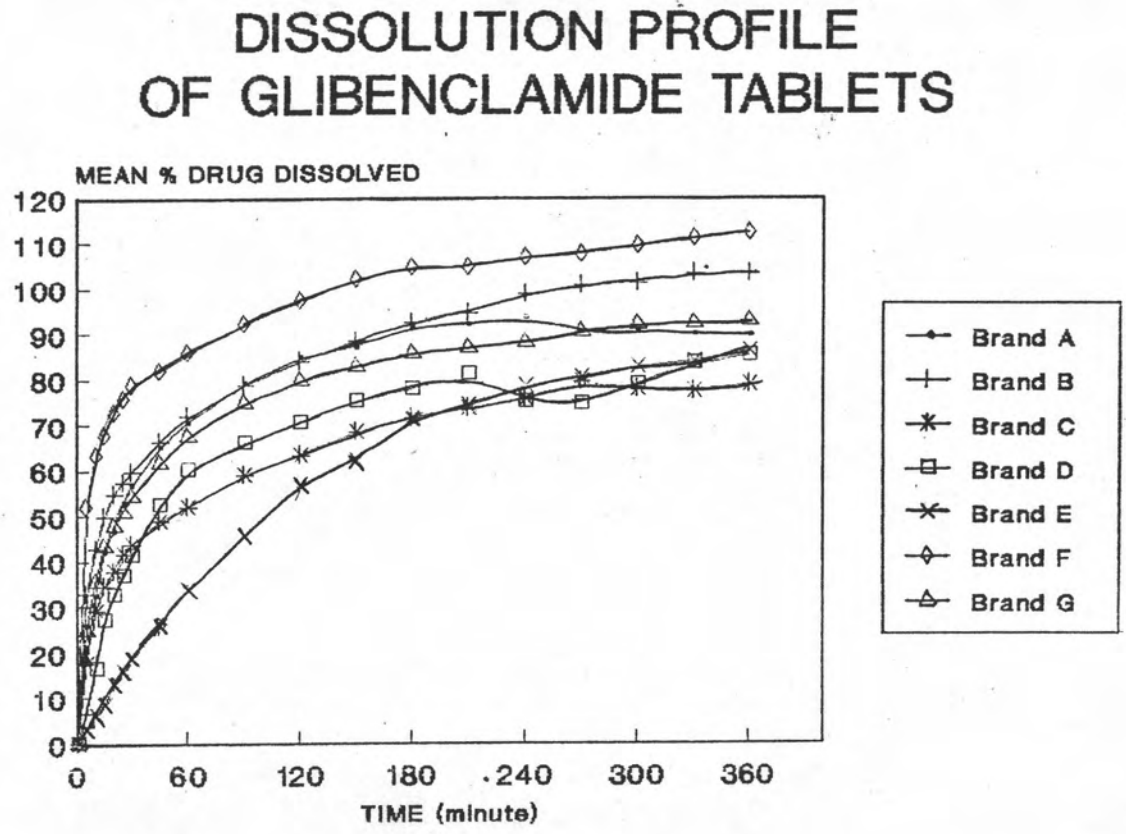

Figure 2 Dissolution profile of seven commercial brands of glibenclamide tablets in simulated intestinal fluid TS without enzyme (pH 7.5±0.1)

Table 5 Dissolution Data of Seven Brands of Glibenclamide Tablets in Simulated Intestinal Fluid TS without Enzyme (pH 7.5±0.1)

Brand	Percent Glibenclamide Dissolved*						
Time(min)	A	B	C	D	E	F	G
5	18.58±2.31	32.81±4.05	17.60±1.16	4.93±2.95	3.05±1.97	51.99±1.64	24.62±3.04
10	32.39±1.71	42.80±2.02	29.09±1.06	16.42±3.85	6.15±1.77	63.21±2.49	35.58±1.31
15	42.14±2.32	49.83±2.93	34.58±1.80	26.93±4.24	8.45±1.97	67.74±1.87	43.32±3.11
20	46.66±1.05	54.73±2.20	37.96±1.01	32.74±3.60	12.87±2.96	72.71±2.59	47.73±1.50
25	52.99±1.37	57.45±2.34	41.51±1.44	36.95±4.56	15.69±2.69	75.71±2.86	50.70±1.84
30	56.61±1.13	59.64±1.51	43.67±0.79	41.27±3.55	18.61±2.76	78.70±3.35	54.35±1.65
45	65.21±1.55	66.36±1.67	48.96±1.40	52.44±3.53	25.88±3.84	81.92±3.26	61.51±1.94
60	70.57±1.48	71.89±3.00	51.92±1.47	60.16±2.04	33.85±4.89	85.75±3.75	67.49±1.76
90	79.30±1.86	78.68±1.81	59.08±1.95	66.08±2.16	45.58±4.79	92.15±4.30	74.79±1.32
120	84.77±1.40	83.93±2.33	63.33±2.16	70.50±2.39	56.86±3.62	97.26±5.63	79.71±2.21

* All values are presented as mean ± S.D. (n=6)

(continued)

(continued)

Table 5 Dissolution Data of Seven Brands of Glibenclamide Tablets in Simulated Intestinal Fluid TS without Enzyme (pH 7.5±0.1)

Brand	Percent Glibenclamide Dissolved*						
Time(min)	A	B	C	D	E	F	G
150	87.06±1.70	88.39±1.95	68.34±2.10	75.27±2.11	62.01±4.16	102.01±6.21	82.64±1.96
180	91.42±2.09	92.32±1.97	71.58±1.57	77.81±2.72	70.88±4.19	104.72±5.73	85.54±2.67
210	91.94±1.25	94.51±2.28	73.46±2.21	81.01±3.01	74.36±4.85	104.54±5.67	86.77±1.76
240	93.54±1.59	98.62±3.20	75.51±2.20	75.02±2.45	78.40±4.48	106.84±6.90	87.96±0.76
270	90.16±1.30	100.60±2.50	79.37±2.90	74.57±2.67	80.49±4.29	107.35±6.51	90.35±3.28
300	90.51±1.21	101.19±2.84	77.91±2.18	78.75±1.78	82.71±5.53	109.32±8.14	92.02±3.15
330	89.88±1.06	103.11±2.31	77.39±2.31	83.45±3.22	83.93±5.50	110.97±7.81	92.09±5.18
360	89.61±1.44	103.25±3.11	78.68±1.88	85.01±2.93	86.02±5.75	112.14±4.58	92.61±6.79

* All values are presented as mean ± S.D. (n=6)

Table 6 Dissolution Rate Constant of Seven Brands of Glibenclamide Tablets

Brand	Dissolution Rate Constant[a] (K_d) (hr^{-1})
A	1.14±0.17
B	0.70±0.03
C	0.68±0.12
D	1.06±0.13
E	0.69±0.10
F	0.90±0.15
G	0.92±0.21

a = values are presented as mean ± S.D.

n = 6

Table 7 Analysis of Variance for Dissolution Rate Constant of Seven Brands of Glibenclamide 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	6	1.24	0.21	10.5
Within group	35	0.82	0.02	
Total	41	2.06		

* Calculation data from Table 6

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

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 8 Comparison of Dissolution Rate Constant of Each Brand of Glibenclamide Tablets with that of the Innovator's product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
B	5.50	$p < 0.001$	S
C	5.75	$p < 0.001$	S
D	1.00	$0.200 < p < 0.400$	NS
E	5.63	$p < 0.001$	S
F	3.00	$0.001 < p < 0.005$	S
G	2.75	$0.005 < p < 0.010$	S

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

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

a = t value obtained from the table

method). Since glibenclamide is a weak acid and it exhibits very low solubility in water thus, its solubility is increased as the pH of the aqueous medium increased. Figure 2 and Table 5 illustrated the dissolution profiles at various times of all seven brands of glibenclamide tablets. Each brands reached the equilibrium state within about 360 minutes. The mean percent dissolved of glibenclamide from all brands ranged from 78.63 to 112.14 percent at 360 minutes. The dissolution rate constants (K_d) were calculated from the slope of the first order plot between the amount of undissolved glibenclamide ($B_\infty - B_t$) versus time in semi-logarithmic scale. The dissolution rate constants of all brands were reported in Table 6. The rank order of all brands in term of dissolution rate constant were brands A > D > G > F > B > E > C. Statistical comparison, as presented in Tables 7 and 8, indicated that only the dissolution rate constant of brand D did not show statistically significant difference ($p < 0.05$) when compared to that of brand A.

No statistical correlation ($p > 0.05$) was found between the disintegration time and the dissolution rate constant of each brand as shown in Table 9.

For the further in vivo studies, three brands according to different dissolution characteristics; high, medium and low dissolution rate constants; were selected to assess the bioavailability studies relatively to the innovator's product (brand A).

Table 9 In Vitro Parameters Correlation

Correlation	Correlation Coefficient	t value	Statistical Significance*
Disintegration Times versus Dissolution Rate Constants	-0.73	2.35	NS (0.05<p<0.10)

n = 6

$$t^{a}_{0.05(5)} = 2.571$$

a = t value obtained from the table

NS = Not significant difference at $p > 0.05$

In Vivo Studies

Three brands of glibenclamide 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 D, F and C, respectively.

1. Analysis of Glibenclamide in Plasma Samples

Owing to the low therapeutic dose of glibenclamide (2.5 - 20 mg.), UV spectrophotometric, fluorometric and colorimetric methods were not sufficiently sensitive and selective for determination of plasma levels of glibenclamide. Recently several methods using HPLC had been developed (Uihlein, 1982; Adams et al., 1982; Potter and Hulm,1983; Zecca et al., 1985; Emilsson et al., 1986). Among these methods, there were some disadvantages, such as the following :

The study of Uihlein (1982) detected the serum glibenclamide levels at 200 nm. with UV detector which had some endogenous interferences. Inappropriate solvent using for extraction of the drug from plasma samples, e.g. diethyl ether in which the drug was practically insoluble (Florey ed., 1981; Moffat ed., 1986) was described in the study of Uihlein (1982) and Potter and Hulm (1983). Peak interferances had developed in the method of Adams et al. (1982). Besides, derivatization method that took many steps for sample preparation had been reported by Zecca et

al. (1985).

In this present study, the modified of Emilsson method (1986) was employed for analyzing the glibenclamide concentration in plasma samples. The mixture of benzene and dichloromethane (3:1 V/V) was used for separation in stead of using only benzene in order to improve the extraction of the drug. The ratio of mobile phase mixture which consisted of acetonitrile and 0.01 M phosphate buffer (pH 3.5) was modified from 50 : 50 to 46 : 54 and tetrahydrofuran was added to improve the polarity and to decrease the endogenous peak interferences.

Typical chromatogram of glibenclamide and internal standard was shown in Figure 3. The retention time of glibenclamide and internal standard were 14.74 and 4.59 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 0.42-3.59% and 0.36-8.72% as shown in Tables 10 and 11, respectively. The standard calibration curve of plasma levels of glibenclamide concentration versus peak height ratio of glibenclamide to internal standard was linear up to 320 ng./ml. (Appendix D). The efficiency of the separation technique used was evaluated by calculating the percentage of recoveries comparing the peak height obtained from spiked plasma to the peak height from standard solution

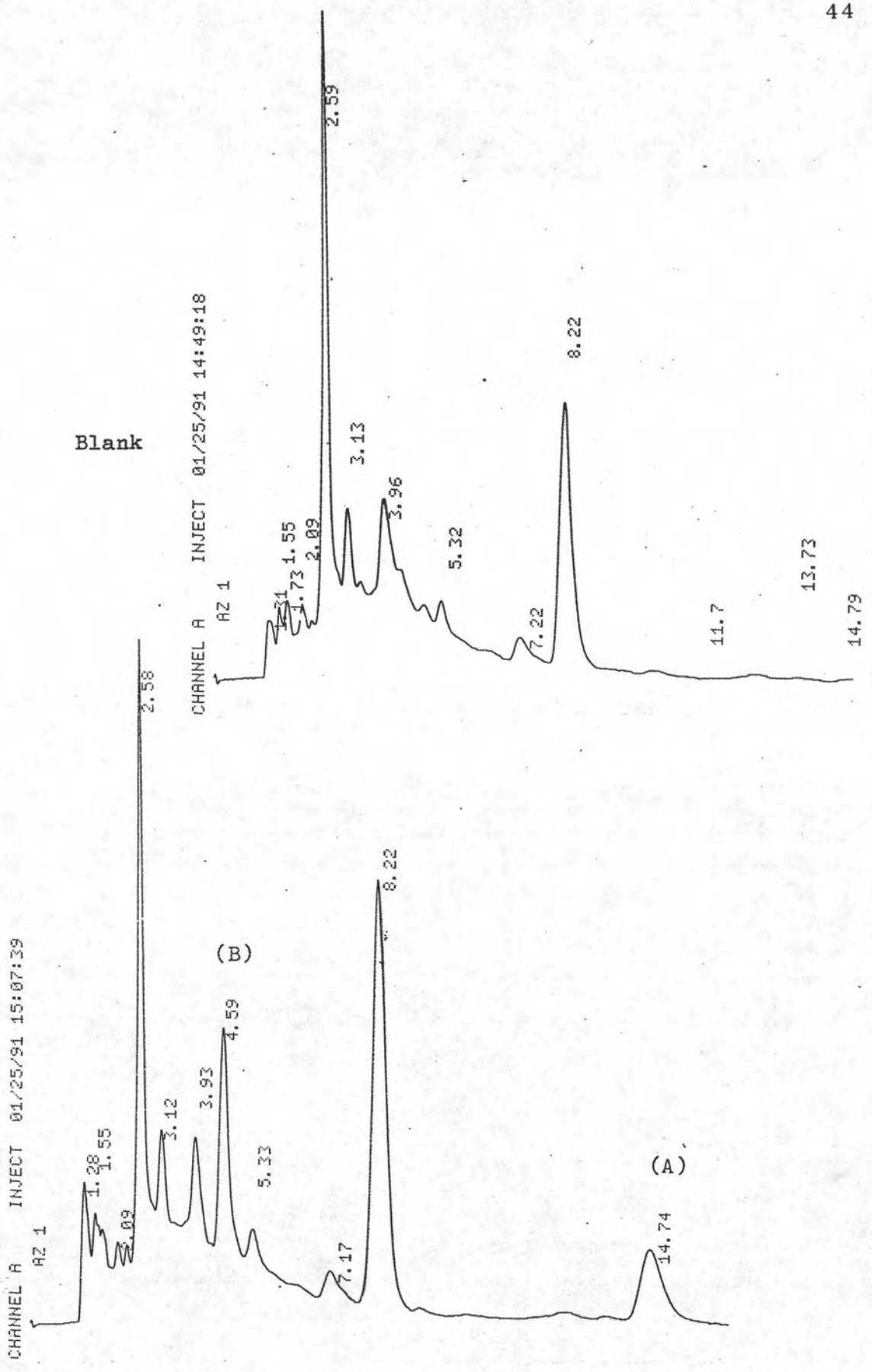

Figure 3 High pressure liquid chromatography of glibenclamide (A) and internal standard (B)

Table 10 Within-run Precision of Glibenclamide

Concentration (ng./ml.)	Average Peak Height Ratio	% C.V.
21.48	0.020	0.42
42.95	0.039	1.09
85.91	0.085	3.59
128.9	0.137	2.79
214.8	0.225	1.01
257.7	0.268	2.69
300.7	0.312	1.24
343.6	0.359	2.23

n = 3

Table 11 Between-run Precision of Glibenclamide

Concentration (ng./ml.)	Average Peak Height Ratio	% C.V.
21.28	0.020	0.36
42.56	0.037	2.55
85.11	0.087	8.72
127.7	0.128	5.79
212.8	0.206	1.23
255.3	0.220	5.84
297.9	0.268	7.75
340.4	0.332	5.14

n = 3

Table 12 Recovery of Glibenclamide 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	
20.00	1.50	1.00	66.67	48.0	41.0	85.42
40.00	2.00	1.50	75.00	52.0	42.0	80.77
120.0	6.00	4.00	66.67	51.0	42.0	82.35
240.0	13.0	8.50	65.38	50.0	43.0	86.00
280.0	15.0	11.5	76.67	51.0	45.5	89.22
320.0	17.0	13.5	79.41	50.0	44.0	88.00
Mean % Recovery			71.63			85.29

a = Drug = Glibenclamide

b = Internal Standard = Glipizide

c = Peak Height

d = % Recovery

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

directly injected to HPLC. The results as shown in Table 12 indicated that the analytical method used is independent of concentration. The percentage recoveries of glibenclamide and internal standard were in the range of 65.38-79.41% and 80.79-89.22%, respectively.

2. Plasma Glibenclamide Levels

The plasma level of glibenclamide at each sampling time ranging from 0 to 10 hours after oral administration of tablets of brands A, C, D and F were shown in Tables 13 to 16, respectively. Individual plasma glibenclamide concentration-time profile for each of twelve subjects were shown graphically from Figures 4 to 15. The plasma glibenclamide concentration at the time before dosing was equal zero. The plasma glibenclamide concentration in some subjects at 0.5, 1.0, 1.5 hrs. after dosing were equal zero. This might be due to the recovery of glibenclamide in this assayed method was 71.63%. Comparison of the mean plasma concentration-time profile of each brand were summarized in Figure 16.

3. Bioavailability Evaluation

The pharmacokinetic parameters, C_{max}, t_{max} and AUC are used to characterize the bioavilability 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

Table 13 Plasma Glibenclamide Concentrations (ng./ml.) from 12 Subjects Following Oral Administration of Glibenclamide 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	10.0
1	0.000	23.68	74.34	241.6	297.3	226.0	105.2	50.00	23.27	0.000
2	0.000	0.000	76.46	97.38	114.5	165.9	118.8	65.83	44.43	21.43
3	0.000	0.000	42.52	150.9	197.0	165.3	118.4	63.48	45.25	25.60
4	0.000	16.97	27.28	94.43	180.4	204.5	179.3	77.45	50.80	19.83
5	0.000	66.65	97.38	241.8	299.9	276.7	179.3	107.3	59.80	39.64
6	0.000	0.000	0.000	78.44	97.38	151.5	276.5	140.2	74.64	54.75
7	0.000	37.60	82.26	132.3	115.8	70.37	51.02	34.88	28.77	0.000
8	0.000	37.60	57.53	157.2	171.4	177.1	131.3	56.10	27.45	0.000
9	0.000	0.000	23.28	63.11	87.28	65.83	60.59	39.64	19.53	0.000
10	31.09	50.45	234.9	294.8	254.8	242.8	144.6	60.59	51.61	43.64
11	44.17	86.34	114.5	127.3	103.5	96.18	56.10	34.88	25.96	0.000
12	0.000	37.49	75.03	188.0	218.6	252.4	191.2	72.99	63.11	41.91
Mean	6.271	29.73	75.45	155.6	178.1	174.6	134.4	66.95	42.88	20.57
S.E.M.	4.304	8.218	17.33	21.01	22.23	20.27	18.96	8.881	5.152	5.943

Table 14 Plasma Concentrations of Glibenclamide (ng./ml.) from 12 Subjects Following Oral Administration of Glibenclamide 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	10.0
1	0.000	0.000	28.38	52.21	71.07	81.54	74.00	39.64	21.43	0.000
2	0.000	20.13	31.32	45.80	68.39	79.44	83.74	62.25	43.13	21.43
3	0.000	43.13	69.84	139.8	141.27	130.2	84.40	44.43	31.32	0.000
4	0.000	0.000	23.68	45.11	43.77	39.17	32.27	20.76	0.000	0.000
5	0.000	28.77	42.52	54.48	84.40	71.37	43.43	31.09	22.15	0.000
6	0.000	20.13	41.03	62.25	71.07	91.24	80.48	50.12	29.17	0.000
7	0.000	0.000	0.000	56.10	67.77	68.79	44.43	43.77	31.32	0.000
8	0.000	0.000	23.68	45.11	53.70	45.11	41.32	19.83	0.000	0.000
9	17.92	41.03	62.25	72.02	93.24	80.48	50.12	29.17	18.69	18.43
10	0.000	0.000	0.000	43.77	45.80	71.07	87.28	76.09	55.28	28.77
11	20.76	42.52	43.13	54.48	84.07	70.37	43.77	22.13	0.000	0.000
12	0.000	0.000	23.68	43.77	67.77	89.80	63.11	48.02	22.13	0.000
Mean	3.223	16.31	32.45	59.58	74.36	76.55	60.70	40.61	22.89	5.719
S.E.M.	2.180	5.364	6.111	7.701	7.462	6.665	5.851	4.990	4.949	3.058

Table 15 Plasma Concentrations of Glibenclamide (ng./ml.) from 12 Subjects Following Oral Administration of Glibenclamide 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	19.25	57.53	87.42	185.9	155.2	129.2	84.25	36.19	34.88	26.92
2	0.000	0.000	36.65	95.01	123.4	144.4	117.3	81.54	56.45	27.10
3	0.000	53.71	91.12	112.8	116.2	86.07	67.77	48.02	20.76	0.000
4	52.21	71.54	81.54	72.78	108.7	113.8	92.01	56.10	36.65	0.000
5	0.000	48.81	65.83	137.8	169.7	163.4	112.8	65.83	44.43	21.43
6	0.000	0.000	36.65	74.34	84.40	139.3	150.9	138.2	136.0	69.83
7	0.000	0.000	40.19	67.77	86.07	93.86	81.54	51.50	30.87	0.000
8	0.000	21.77	59.80	94.49	165.3	188.7	182.1	159.05	67.49	17.20
9	0.000	45.80	65.83	137.8	167.9	129.2	71.07	56.10	43.13	28.77
10	0.000	0.000	20.13	63.99	109.7	199.2	135.8	89.80	56.10	20.76
11	0.000	40.19	84.89	109.7	120.9	126.1	74.00	43.13	30.87	0.000
12	99.24	109.4	129.7	144.4	241.3	241.9	231.2	216.94	183.90	148.0
Mean	14.22	37.39	66.64	108.1	137.4	146.3	116.7	86.86	62.21	30.00
S.E.M.	8.926	9.949	8.821	10.83	12.86	13.06	14.60	16.14	14.02	12.14

Table 16 Plasma Concentrations of Glibenclamide (ng./ml.) from 12 Subjects Following Oral Administration of Glibenclamide Tablets of Brand F

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	0.000	0.000	36.19	72.02	148.2	188.38	146.3	68.79	48.81	0.000
2	0.000	29.58	40.19	105.2	108.2	97.38	82.63	40.75	19.53	0.000
3	0.000	16.31	43.77	65.83	82.26	102.5	83.74	63.11	31.32	0.000
4	0.000	0.000	21.09	44.43	78.29	88.52	75.03	56.10	34.88	0.000
5	68.79	93.24	109.15	135.4	144.6	174.9	146.3	126.3	93.35	73.75
6	0.000	0.000	23.68	52.95	77.18	81.54	51.50	36.19	18.97	0.000
7	0.000	0.000	0.000	39.11	90.82	126.7	93.24	50.12	19.83	0.000
8	0.000	21.43	44.43	65.83	90.82	108.2	83.74	63.99	31.32	0.000
9	0.000	51.50	72.99	123.4	142.53	125.7	93.36	65.83	48.81	28.77
10	0.000	23.68	52.95	70.14	80.48	81.54	83.74	45.80	22.13	0.000
11	0.000	20.76	21.77	39.17	44.43	54.48	44.77	31.32	21.43	0.000
12	0.000	24.68	32.51	51.62	87.53	89.66	81.54	56.10	43.13	28.77
Mean	5.733	23.43	41.56	72.10	97.95	110.0	88.82	58.70	36.10	10.94
S.E.M.	5.732	7.764	8.104	9.356	9.229	11.24	8.850	7.069	6.120	6.547

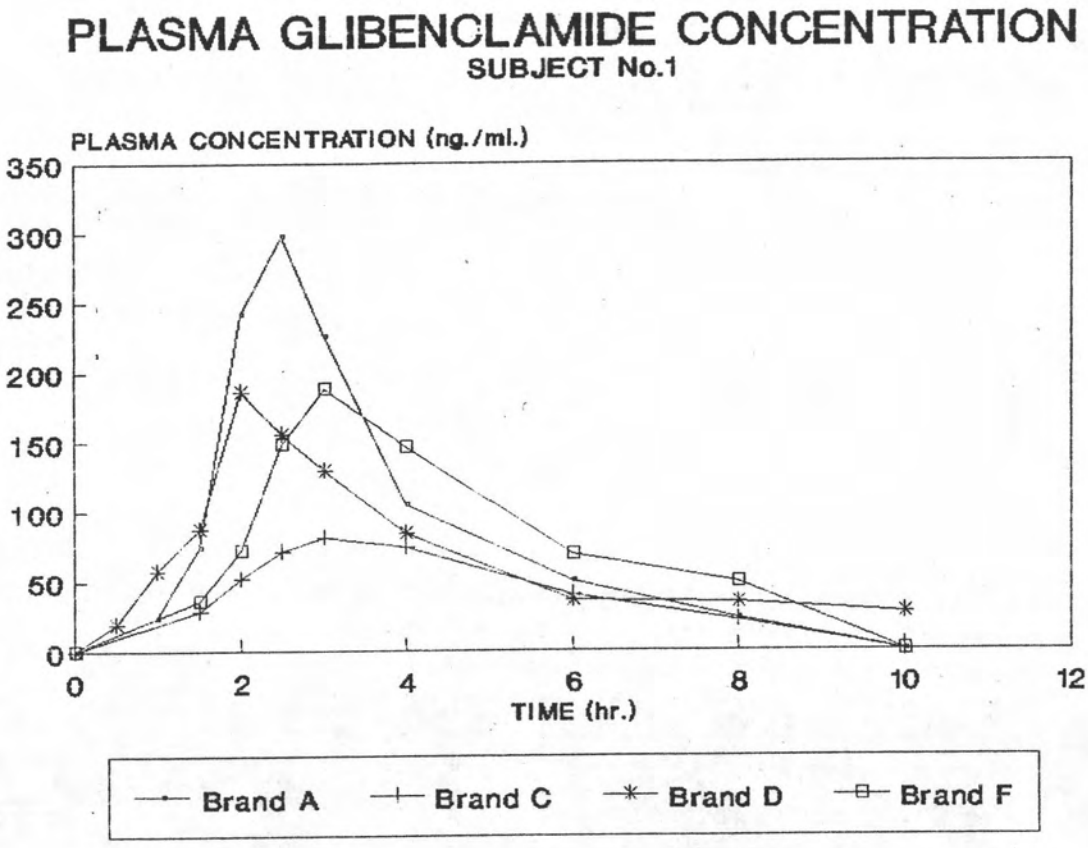

Figure 4 Plasma glibenclamide concentration-time profile of subject No.1 following oral administration of glibenclamide tablets

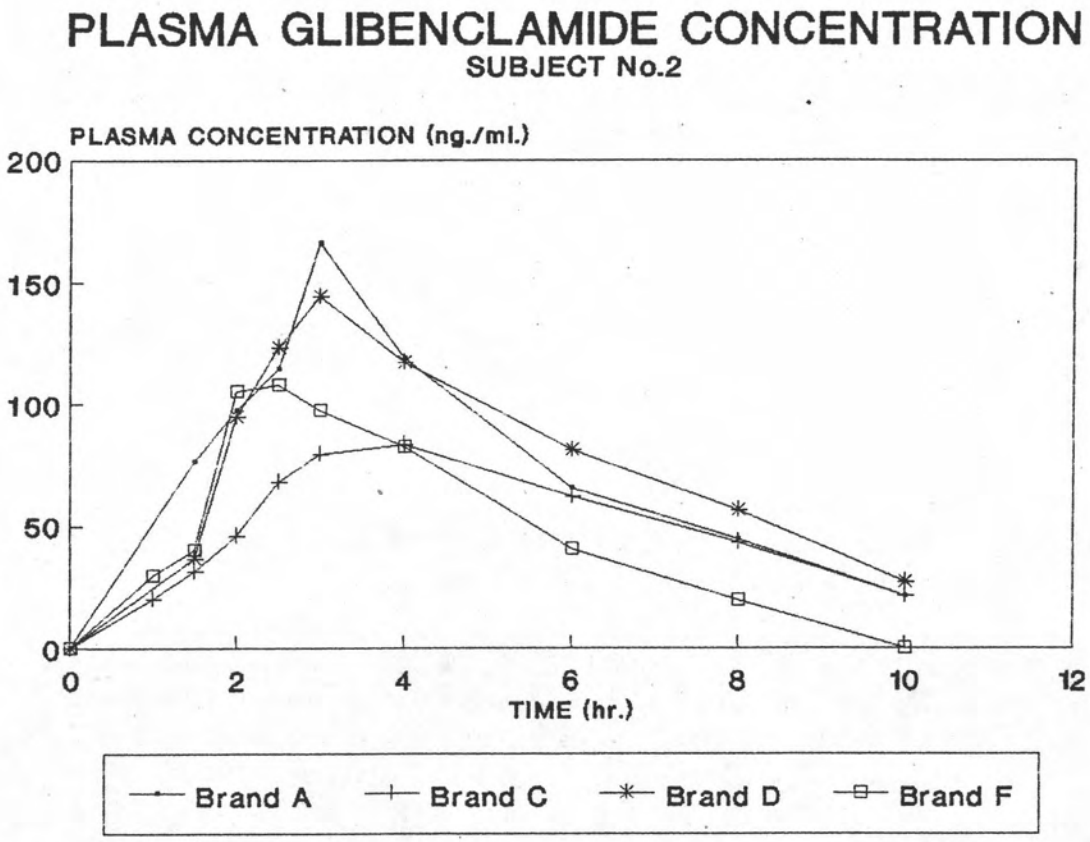

Figure 5 Plasma glibenclamide concentration-time profile of subject No.2 following oral administration of glibenclamide tablets

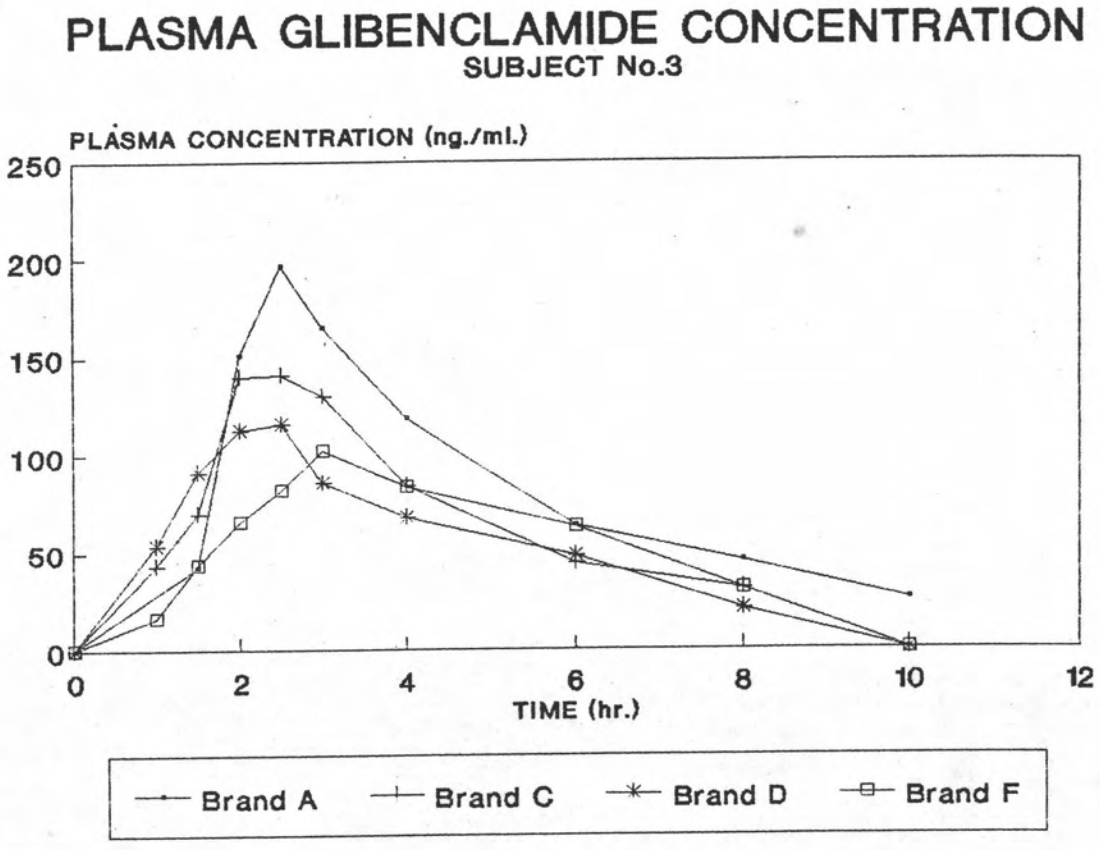

Figure 6 Plasma glibenclamide concentration-time profile of subject No.3 following oral administration of glibenclamide tablets

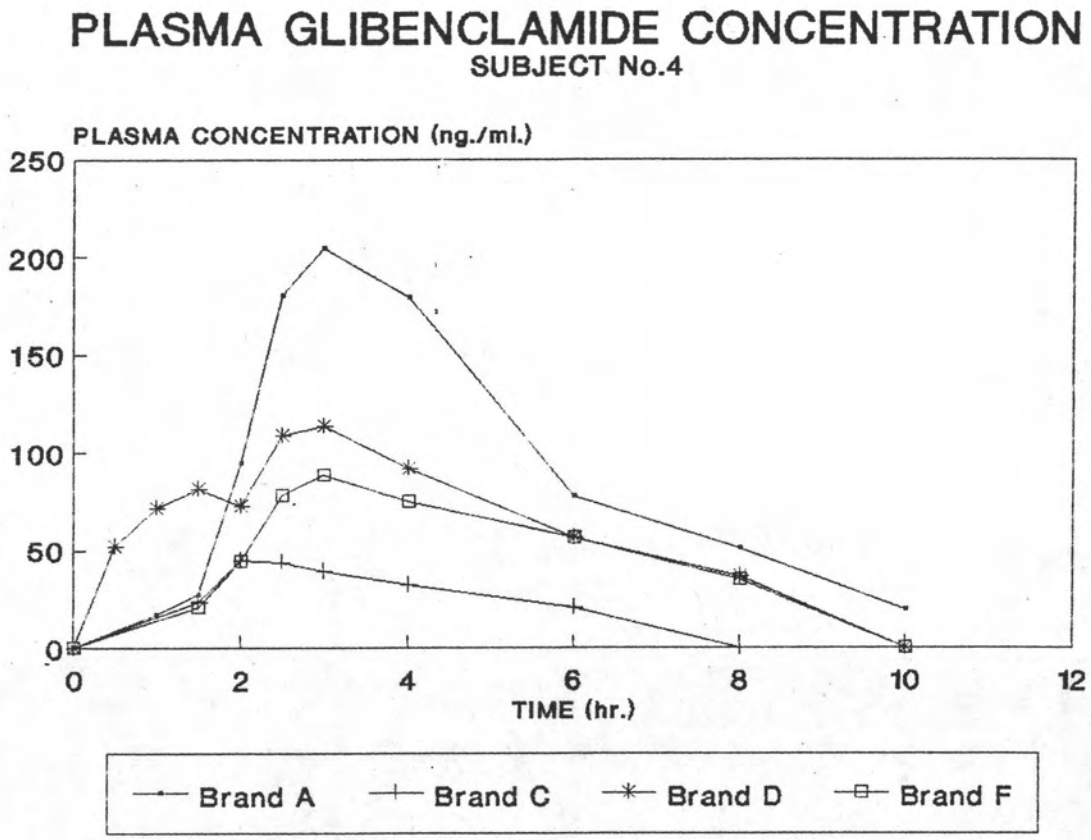

Figure 7 Plasma glibenclamide concentration-time profile of subject No.4 following oral administration of glibenclamide tablets

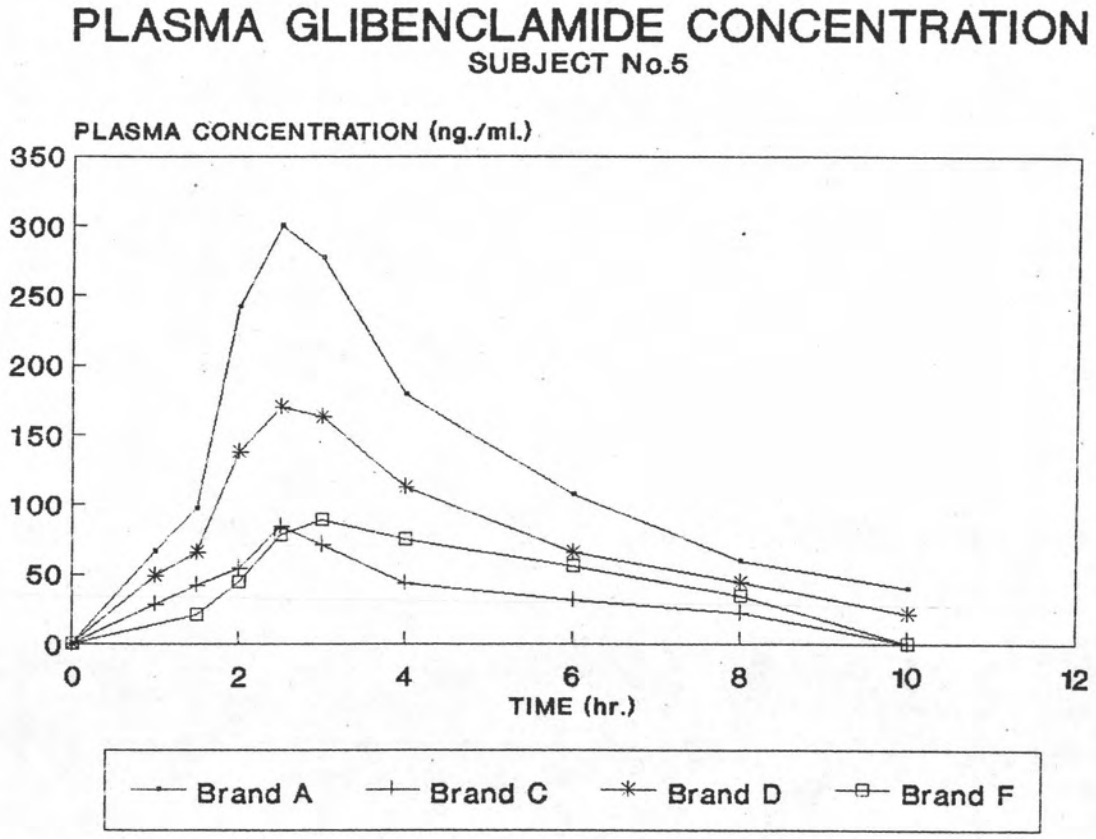

Figure 8 Plasma glibenclamide concentration-time profile of subject No.5 following oral administration of glibenclamide tablets

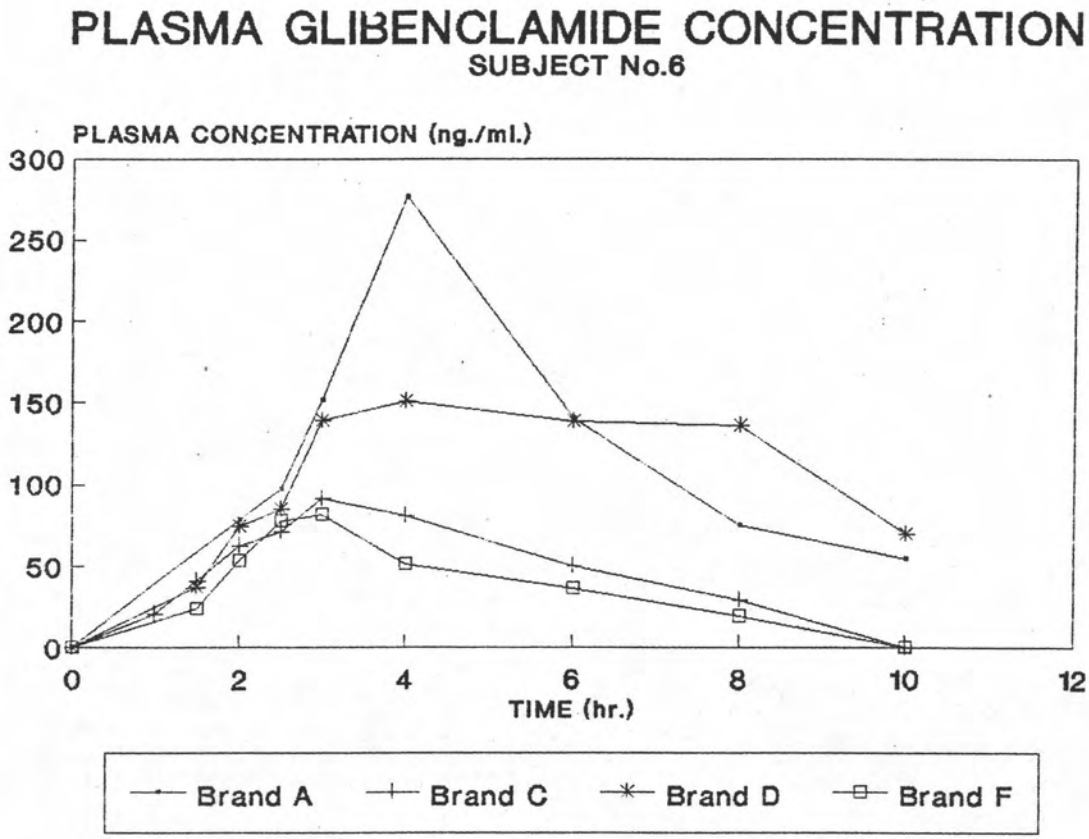

Figure 9 Plasma glibenclamide concentration-time profile of subject No.6 following oral administration of glibenclamide tablets

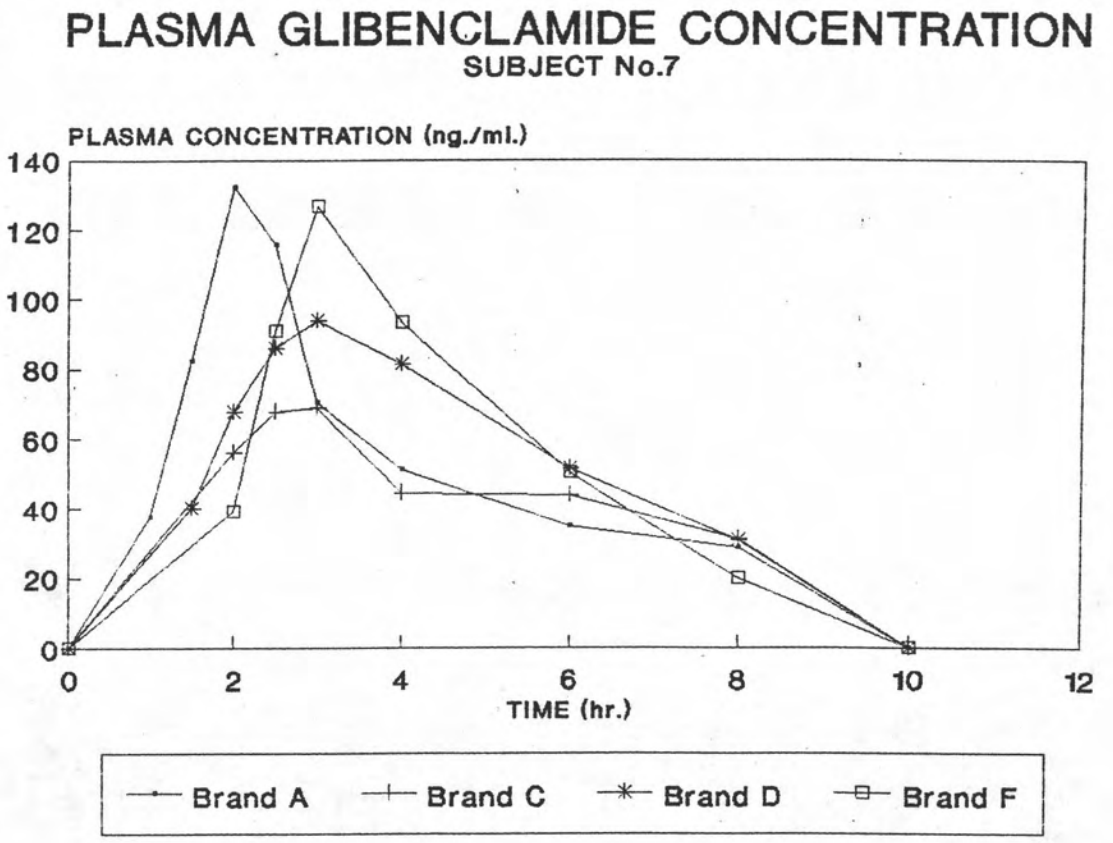

Figure 10 Plasma glibenclamide concentration-time profile of subject No.7 following oral administration of glibenclamide tablets

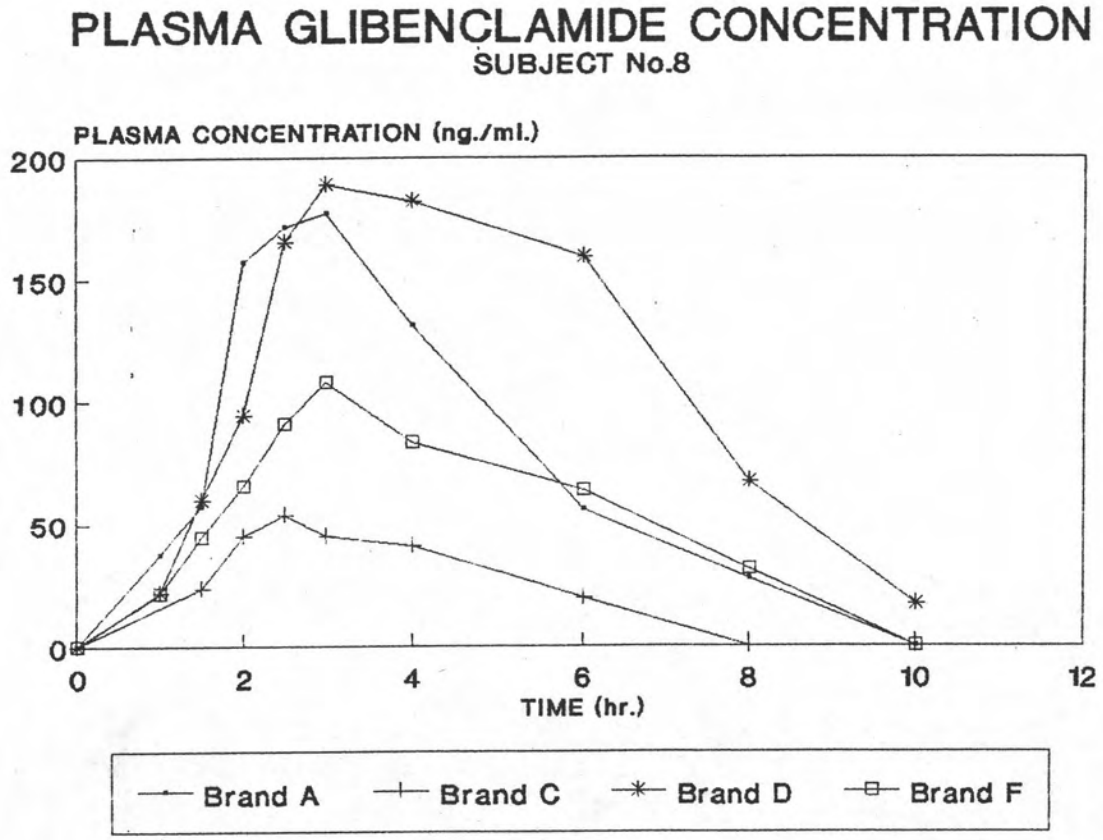

Figure 11 Plasma glibenclamide concentration-time profile of subject No.8 following oral administration of glibenclamide tablets

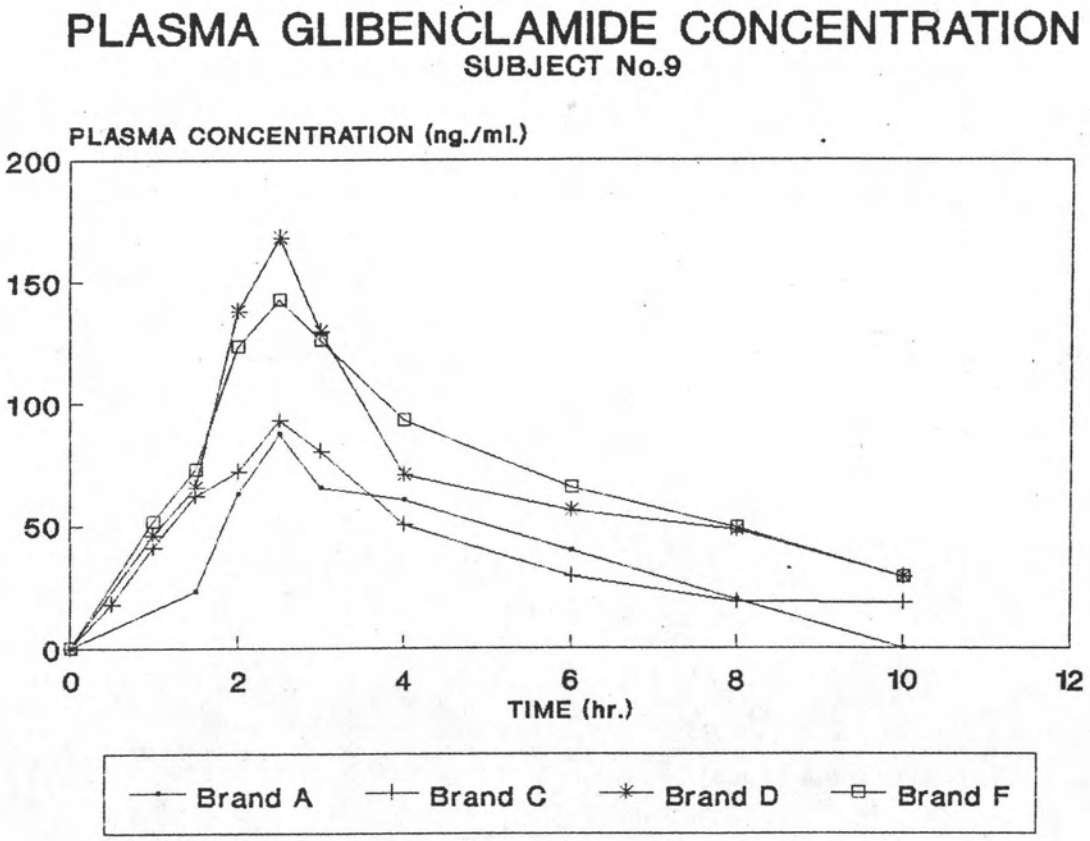

Figure 12 Plasma glibenclamide concentration-time profile of subject No.9 following oral administration of glibenclamide tablets

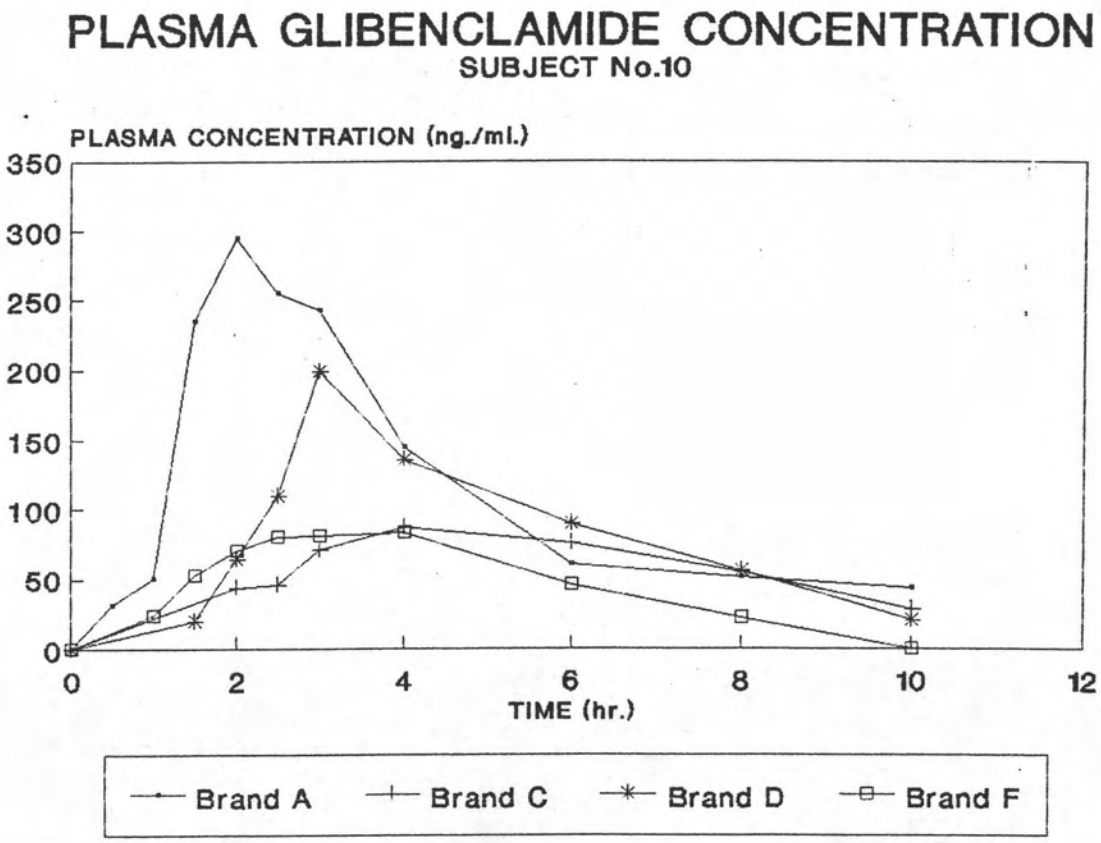

Figure 13 Plasma glibenclamide concentration-time profile of subject No.10 following oral administration of glibenclamide tablets

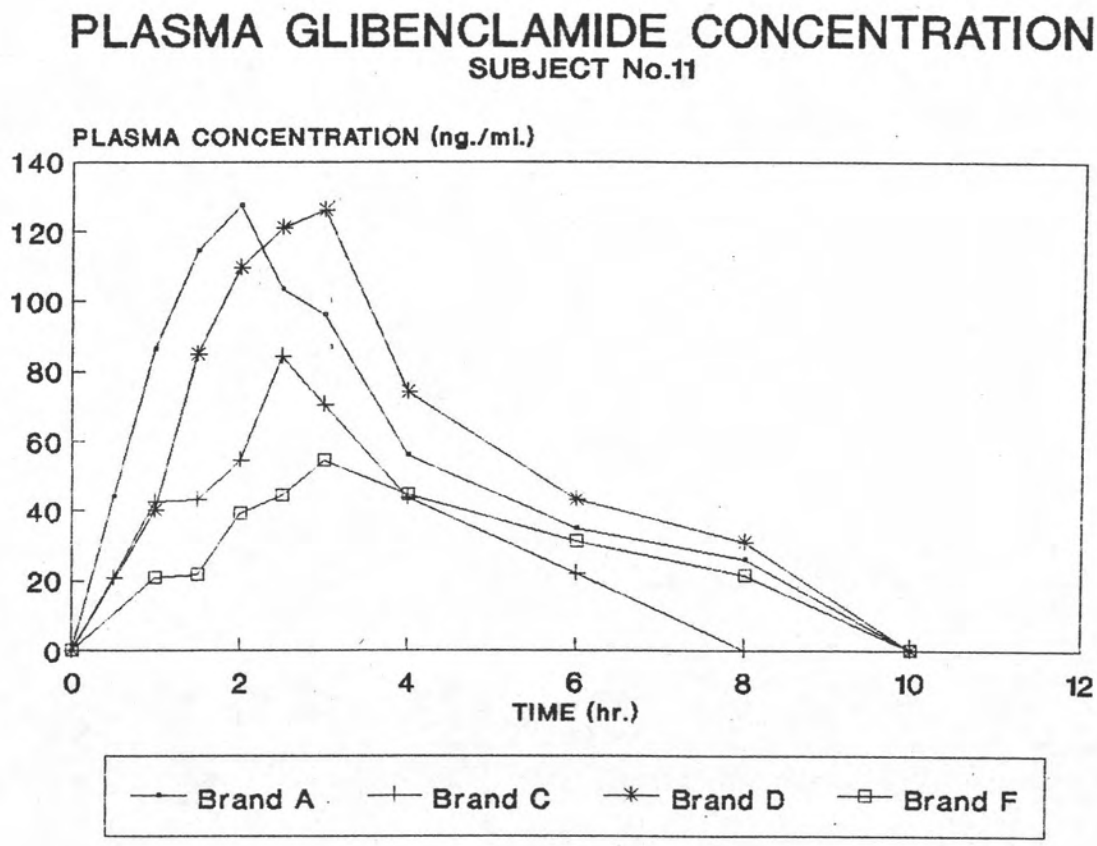

Figure 14 Plasma glibenclamide concentration-time profile of subject No.11 following oral administration of glibenclamide tablets

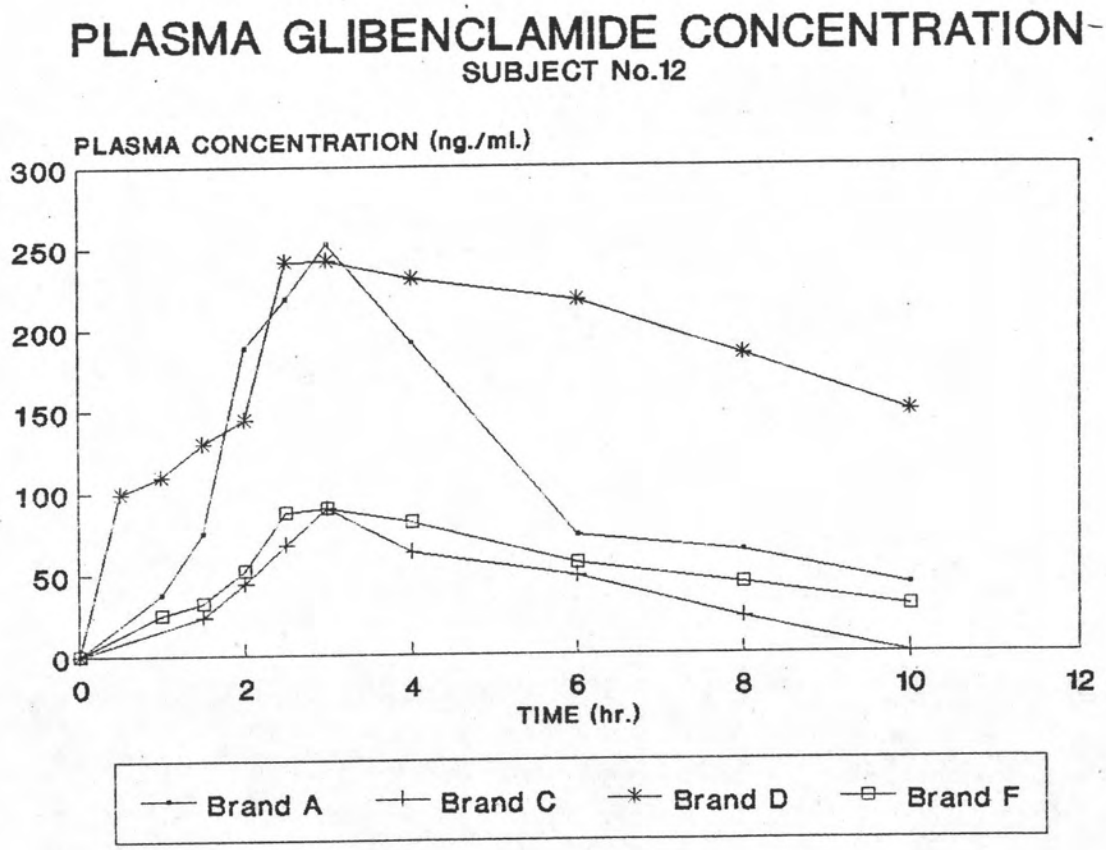

Figure 15 Plasma glibenclamide concentration-time profile of subject No.12 following oral administration of glibenclamide tablets

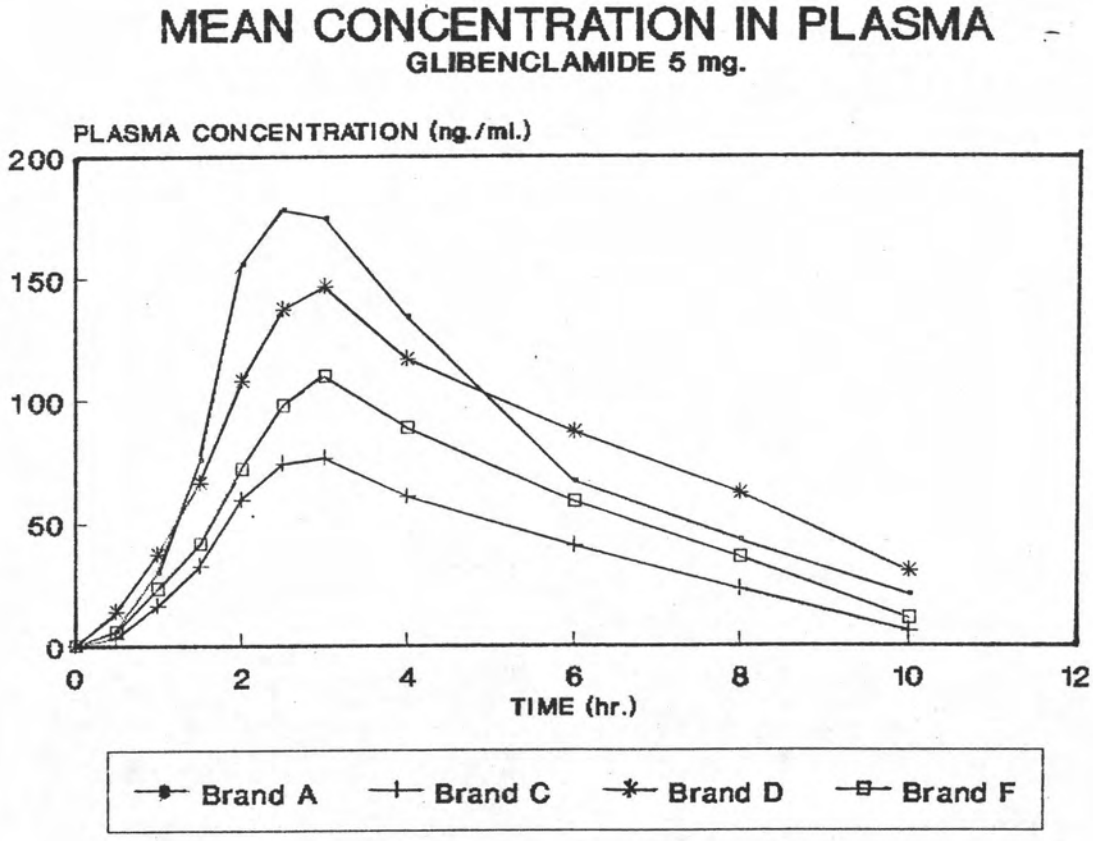

Figure 16 Comparison of mean plasma glibenclamide concentration-time profile from 12 subjects following oral administration of glibenclamide tablets

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 bioequivalence 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 calculated from the biexponential equation were obtained for bioavailability comparison as follows :

3.1 Peak Plasma Concentration (C_{max})

Previous reports indicated that mean peak plasma concentration achieved following oral administrtion of 5 mg. glibenclamide tablets varied widely ranging from 50.3 to 189.6 ng./ml. (Ings et al., 1981; Chalk et al., 1986; Shaheen et al., 1987; El-Sayed et al., 1989). In this study, the mean peak plasma glibenclamide level for each treatment shown in Table 17 for brands A, C, D and F were 138.1 $\pm$ 13.73, 62.26 $\pm$ 5.77,119.9 $\pm$ 11.62 and 82.93 $\pm$ 7.63, respectively. The rank order of these values were brands A > D > F > C. Statistical comparison indicated that the peak plasma concentrations obtained from brands C and F were significantly lower than that from the innovator's product ($p < 0.05$) as shown in Tables 18 and 19.

Table 17 Peak Plasma Concentration (C_{max}) of Glibenclamide Following Oral Administration of Four Different Brands of Glibenclamide Tablets

Subject No.	C_{max} (ng./ml.)			
	A	C	D	F
1	186.3	59.55	90.91	114.7
2	126.6	69.44	129.9	86.38
3	121.0	121.5	102.4	73.52
4	136.4	41.96	98.67	65.60
5	201.4	53.45	117.7	143.2
6	119.6	65.45	123.7	65.12
7	77.36	61.31	76.95	63.25
8	125.2	39.73	157.9	78.75
9	52.28	68.39	122.3	110.9
10	208.9	59.92	85.08	77.53
11	109.3	50.84	99.95	41.38
12	192.9	55.58	233.1	74.78
Mean	138.1	62.26	119.9	82.93
S.E.M.	13.73	5.77	11.62	7.63

Table 18 Analysis of Variance for Peak Plasma Concentration of Four Different Brands of Glibenclamide Tablets*

Source of Variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	42,708.26	14,236.09	10.48
Within group	44	59,754.77	1,358.06	
Total	47	102,463.03		

* The full data were shown in Table 17

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

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 19 Comparison of Peak Plasma Concentration of three Differrent Brands of Glibenclamide Tablets with that of the Innovator's product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
C	5.04	$p < 0.001$	S
D	1.21	$0.200 < p < 0.400$	NS
F	3.67	$p < 0.001$	S

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

a = t value obtained from the table

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

Table 20 Time to Peak Plasma Concentration (t_{max}) of Glibenclamide Following Oral Administration of Four Different Brands of Glibenclamide Tablets

Subject No.	t_{max} (hr.)			
	A	C	D	F
1	1.87	2.64	2.42	2.75
2	2.44	3.11	2.85	2.13
3	2.51	1.93	1.87	2.83
4	2.55	2.06	2.26	3.09
5	2.36	2.02	2.40	3.00
6	3.55	2.80	4.34	2.32
7	2.13	2.53	2.67	2.64
8	2.18	2.07	2.57	2.73
9	2.67	2.08	2.02	2.36
10	1.93	3.93	3.02	2.41
11	1.51	1.88	2.07	2.86
12	2.33	2.83	3.66	2.93
Mean	2.34	2.49	2.68	2.67
S.E.M.	0.14	0.17	0.20	0.08

Table 21 Analysis of Variance of Time to Peak Plasma Concentration of Glibenclamide of Four Different Brands of Glibenclamide Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	0.97	0.32	1.03
Within group	44	13.60	0.31	
Total	47	14.57		

* The full data were shown in Table 20

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

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

The differences in solubility characteristic of glibenclamide in these formulations may affect the amount of drug absorbed, leading to the differences of the C_{max} and AUC values (as seen in Tables 23 and 24).

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

The time to peak plasma glibenclamide level of each individual was presented in Table 20. The average peak times were 2.34 ± 0.14, 2.49 ± 0.17, 2.68 ± 0.20 and 2.67 ± 0.08 hours for brands A, C, D and F, respectively. There was lag time (averaged 0.43 hour for all brands) that was similar to the reports of Fucella et al. (1973) which was 0.5-1 hour and Chalk et al. (1986) which was 1 - 2 hours. The appearance of lag time may be due to the slow dissolution behaviour of glibenclamide from formulations. The time to peak plasma levels in this study agreed with the results found by other investigators ranging from 2-4 hours (Fucella et al., 1973; Ings et al., 1981; Chalk et al., 1986; Shaheen et al., 1987; El-Sayed et al., 1989). There were no statistically significant difference ($p > 0.05$) among these values of all brands. (Table 21)

3.3 Area Under the Plasma Versus Time Curve (AUC)

The means AUC from individual plasma date of each brand were 843.8 ± 82.27, 449.02 ± 38.19, 784.1 ± 95.31 and 650.6 ± 101.2 ng.hr./ml. for brands A, C, D and F, respectively as shown in Table 22. The rank order

Table 22 Area Under the Plasma Concentration-Time Curve (AUC) of Glibenclamide Following Oral Administration of Four Different Brands of Glibenclamide Tablets

Subject No.	AUC (ng.hr./ml.)			
	A	C	D	F
1	810.9	396.7	641.5	781.8
2	787.4	565.9	813.5	483.2
3	798.4	677.0	533.9	564.0
4	821.8	269.4	699.4	580.3
5	1273.8	420.5	764.5	1706.9
6	1025.4	498.8	1665.1	398.2
7	495.0	559.2	553.7	376.3
8	677.5	253.9	912.1	581.2
9	373.0	459.1	821.5	830.4
10	1245.4	608.6	607.7	458.5
11	584.9	283.3	611.7	357.6
12	1231.8	396.0	3398.70*	689.2
Mean	843.8	449.0	784.1	650.6
S.E.M.	82.27	38.19	95.31	101.2

* Excluded data

Table 23 Analysis of Variance for AUC of Four Different Brands of Glibenclamide Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	1,091,569.09	363,856.36	4.28
Within group	43	3,657,570.30	85,059.77	
Total	46	4,749,139.39		

* The full data were shown in Table 22

$$F^{e}_{0.05(3,43)} = 2.828$$

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 AUC of the Three Different Brands with that of the Innovator's product (brand A)

Brand	t value (calculated)	p value	Statistical Significance*
C	3.32	$0.001<p<0.005$	S
D	0.49	$p > 0.500$	NS
F	1.62	$0.100<p<0.200$	NS

$$t^{a}_{0.05(43)} = 2.017$$

a = t value obtained from the table

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$

of this value was brands A > D > F > C. Statistical analysis results in Tables 23 and 24 indicated that only the AUC of brand C exhibited significant lower than that of the innovator's product (brand A) ($p < 0.05$). The values of AUC in this study was higher than those presented by Ings et al. (1981) (502 $\pm$ 43.30 ng.hr./ml.) and lower than those reported by Shaheen et al. (1987) (826 $\pm$ 77 ng.hr./ml.). This differences may be caused by the differences of the study condition and assay method.

The principal pharmacokinetic parameters of glibenclamide following oral administration of four brands were summarized in Table 31. Statistical analysis of these corresponding parameters among the four brands demonstrated that brand C produced the peak plasma concentration and the area under the plasma concentration-time curve significant lower than those of brand A ($p < 0.05$), leading to be bioinequivalent in term of the extent but not the rate of absorption. Meanwhile, brand F produced only peak plasma concentration significant lower than that of brand A so that it was almost bioequivalent to brand A if the AUC and t_{max} values were the parameters represented the bioavailability in this condition. It was only brand D showed complete bioequivalence to brand A in term of both the rate and the extent of drug absorption.

4. The Relative Bioavailability

Relative Bioavailability is the availability of a drug product as compared to a recognized standard (innovator's product) (Shargel and Yu, 1980). In this study the mean relative bioavailability calculated by comparing the mean AUC of each brand to that of the innovator's product. The values obtained of brands C, D and F relatively to brand A were 67.16%, 104.09% and 82.95%, respectively.

5. Pharmacokinetic of Glibenclamide Tablets

Using the CSTRIP computer program to analyze the plasma glibenclamide concentration-time relationship (Appendix E), the results obtained demonstrated that the data were well described by means of biexponential equation. This referred that pharmacokinetic of glibenclamide in Thai healty volunteers could be explained by a one compartment open model. This finding agreed with the study of Chalk et al. (1986).

The pharmacokinetic parameters derived from the model of analysis from plasma concentration-time data of each brand of glibenclamide tablets were detailed in Tables 25 to 30.

5.1 Absorption Rate Constant (K_a)

The average absorption rate constant for brands A, C, D and F were 0.92 $\pm$ 0.07, 0.87 $\pm$ 0.09, 0.75 $\pm$

Table 25 Absorption Rate Constant (K_a) of Glibenclamide Following Oral Administration of Four Different Brands of Glibenclamide Tablets

Subject No.	K_a (hr^{-1})			
	A	C	D	F
1	1.02	0.73	0.71	0.71
2	0.92	0.61	0.58	0.96
3	0.81	1.19	0.67	0.69
4	0.72	1.18	0.85	0.73
5	0.84	1.57	0.80	0.71
6	0.49	0.67	0.51	1.02
7	0.97	0.77	0.78	0.64
8	0.84	0.77	0.62	0.73
9	0.75	1.04	1.36	0.99
10	1.15	0.47	0.53	0.73
11	1.54	0.78	0.98	0.74
12	0.99	0.67	0.65	0.58
Mean	0.92	0.87	0.75	0.77
S.E.M.	0.07	0.09	0.06	0.04

Table 26 Analysis of Variance for Absorption Rate Constant of Four Different Brands of Glibenclamide Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	0.24	0.08	1.33
Within group	44	2.64	0.06	
Total	47	2.88		

* 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 Elimination Rate Constant (K_{el}) of Glibenclamide Following Oral Administration of Four Different Brands of Glibenclamide Tablets

Subject No.	K_{el} (hr^{-1})			
	A	C	D	F
1	0.46	0.28	0.25	0.27
2	0.28	0.22	0.37	0.39
3	0.27	0.29	0.42	0.23
4	0.33	0.21	0.23	0.18
5	0.28	0.17	0.27	0.12
6	0.24	0.23	0.12	0.27
7	0.25	0.17	0.23	0.37
8	0.35	0.28	0.40	0.24
9	0.25	0.23	0.21	0.20
10	0.26	0.18	0.30	0.33
11	0.27	0.34	0.27	0.19
12	0.26	0.26	0.10	0.16
Mean	0.29	0.24	0.26	0.25
S.E.M.	0.02	0.01	0.03	0.02

Table 28 Analysis of Variance for Elimination Rate Constant of Four Different Brands of Glibenclamide Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	0.02	0.0067	1.14
Within group	44	0.26	0.0059	
Total	47	0.28		

* The full data were shown in Table 27

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

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

0.06 and 0.77 ± 0.04 hr^{-1}, respectively (Table 25). These values agreed with the previous study of Chalk et al. (1986) which the range of the absorption rate constant was 0.57 to 2.0 hr^{-1}. No statistical difference was found among these values ($p > 0.05$) as shown in Table 26.

5.2 Elimination Rate Constant (K_{el})

The average elimination rate constant obtained from individual plasma data of brands A, C, D and F were 0.29 ± 0.02, 0.24 ± 0.01, 0.26 ± 0.03 and 0.25 ± 0.02 hr^{-1}, respectively (Table 27). There were no statistical differences among these values ($p > 0.05$) (Table 28). The values agreed with those reported by Chalk et al. (1986) (0.18-0.72 hr^{-1}) and El-Sayed et al. (1989) (0.339 ± 0.035 hr^{-1}).

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

The mean half-life of glibenclamide determined for brands A, C, D and F were 2.46 ± 0.11, 3.04 ± 0.19, 3.17 ± 0.47 nad 3.18 ± 0.30 hours, respectively (Table 29). The values agreed with those investigated by El-Sayed et al. (1989) that the half-life of glibenclamide was reported as 2.34 ± 0.242 hr. and Shaheen et al. (1987) as 2.8-3.3 hr. But from the study of Ings et al. (1981), they reported the half-life of glibenclamide was 1.59 ± 0.41 hr. This difference may be caused by the differences of the study condition and assay method. Statistical analysis showed no significant differences among these

values (Table 30).

6. <u>In Vitro - In Vivo Correlation</u>

The correlation studies between the in vitro and in vitro data for brands A, C, D and F were presented in Table 32.

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

Dissolution rate constant demonstrated statistically significant correlation ($p < 0.05$) with both the C_{max} and the AUC values. 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 with both the C_{max} and the AUC values referred that the dissolution rate of the drug could affect the amount of drug absorption. Therefore the dissolution rate constant might be used as the preliminary tool to predict the extent of drug absorption.

Table 29 Biological Half-life ($t_{1/2}$) of Glibenclamide Following Oral Administration of Glibenclamide Tablets

Subject No.	$t_{1/2}$ (hr.)			
	A	C	D	F
1	1.51	2.50	2.76	2.53
2	2.47	3.11	1.89	2.20
3	2.60	2.41	1.65	3.03
4	2.09	3.26	3.02	3.79
5	2.50	4.08	2.55	5.75
6	2.95	2.98	5.95	2.53
7	2.74	4.14	3.07	1.89
8	1.96	2.49	1.75	2.94
9	2.83	3.01	3.23	3.41
10	2.65	3.80	2.30	2.08
11	2.55	2.03	2.59	3.74
12	2.69	2.65	7.25	4.26
Mean	2.46	3.04	3.17	3.18
S.E.M.	0.11	0.19	0.47	0.30

Table 30 Analysis of Variance for Biological Half-life of Four Different Brands of Glibenclamide Tablets*

Source of variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	3	4.15	1.38	1.16
Within group	44	52.24	1.19	
Total	47	56.39		

* The full data were shown in Table29

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

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 31 Estimated Pharmacokinetic Parameters (Mean±SEM) of Glibenclamide from Twelve Subjects Following Oral Administration of Four Different Brands of Glibenclamide Tablets

Parameters	Brand				F-test	t-test* with respect to brand A
	A	C	D	F		
C_{max} (ng./ml.)	138.1±13.73	62.26±5.77	119.9±11.62	82.93±7.63	10.48 (2.824)**	S (A=D > C,F)
t_{max} (hr.)	2.34±0.14	2.49±0.17	2.68±0.20	2.67±0.08	1.03 (2.824)**	NS
AUC (ng.hr./ml.)	843.8±82.27	449.0±38.19	784.1±95.31	650.6±101.2	4.28 (2.828)**	S (A=D=F > C)
K_a (hr^{-1})	0.92±0.07	0.87±0.09	0.75±0.06	0.77±0.04	1.33 (2.824)*	NS
K_{el} (hr^{-1})	0.29±0.02	0.24±0.01	0.26±0.03	0.25±0.02	1.14 (2.824)**	NS
$t_{1/2}$ (hr.)	2.46±0.11	3.04±0.19	3.17±0.47	3.18±0.30	1.16 (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 32 In Vitro-In Vivo Correlations

Correlation (n=4)	Correlation Coefficient	t value	Statistical Significance*
Disintegration Times versus C_{max}	-0.96	4.85	S (0.025<p<0.050)
Disintegration Times versus t_{max}	0.56	0.96	NS (0.400<p<0.500)
Disintegration Times versus AUC	-0.87	2.50	NS (0.050<p<0.100)
Dissolution Rate Constants versus C_{max}	0.98	6.96	S (0.010<p<0.025)
Dissolution Rate Constants versus t_{max}	-0.16	0.23	NS (P>0.500)
Dissolution Rate Constants versus AUC	0.99	9.92	S (p=0.010)

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

a = t value obtained from the table

* S = Significant difference at $p < 0.05$

NS = Not significant difference at $p > 0.05$