CHAPTER IV

RESULTS AND DISCUSSION

A. Pharmaceutical Equivalence and *In Vitro* Dissolution Testing

The two brands of 5 mg glipizide tablets were analyzed for Pharmaceutical equivalence following the monograph of the United States Pharmacopoeia 27. All related results were as follows:

1. Identification

Chromotograms of assay for glipizide are displayed in Figure 2. As can be seen, the retention time of the major peak in the chromatograms of the assay preparation (A and B) correspond to that in the chromatogram of the standard preparation as obtained in the assay. This indicates that glipizide were found in both products.

2. Uniformity of Dosage Units

Due to the dosage unit of the products to be determined is less than 50 mg, content uniformity method is procedure of choice. Results are presented in Table 3. the amount of the active ingredient in each of the 10 dosage units of test and innovator's product lie within the range of 85.0% to 115.0% of the label claim and relative standard deviation is less than 6.0% are obtained. These meet the criteria of USP 27 specifications. The data also show that the two brands contain uniformly active ingredient. This is observed by the standard deviations are quite low, indicating they were well manufactured, especially mixing process for low dosage unit.

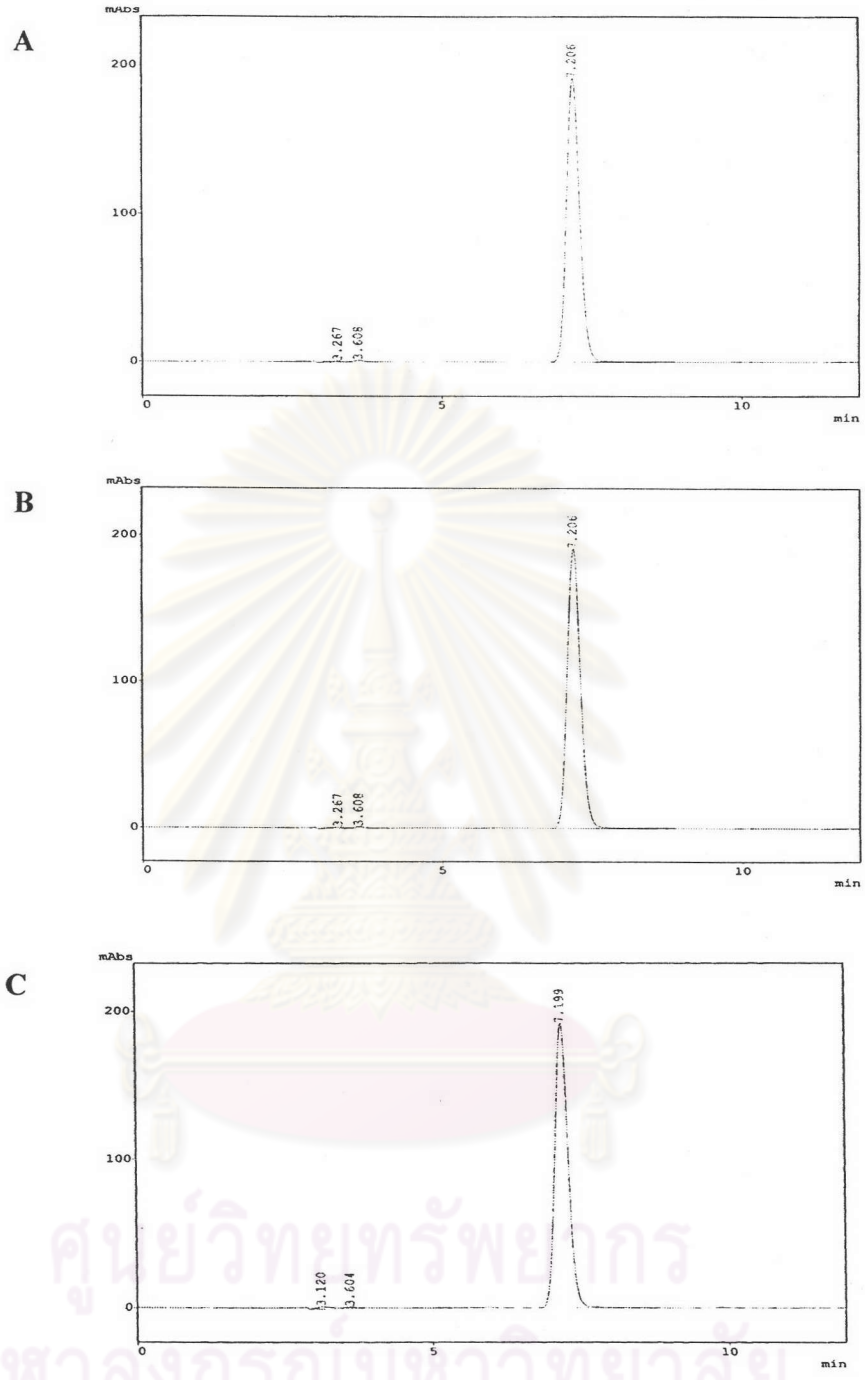


Figure 2 Chromatograms of Glipizide in (A) Assay Preparation of Test Product, (B) Assay Preparation of Innovator's Product and (C) Standard Preparation.

Table 3 Uniformity of Dosage Units of 5 mg Glipizide Tablets of Test and Innovator's Products

Tablet no.	% Labeled Amount	
	Test Product	Innovator's Product
1	99.93	99.87
2	99.78	103.36
3	99.56	100.74
4	99.58	102.92
5	99.59	102.92
6	99.54	103.70
7	99.63	100.82
8	99.66	100.41
9	99.66	99.58
10	99.40	100.27
Mean	99.63	101.46
S.D.	0.14	1.58
%R.S.D	0.14	1.56

3. Assay for Content of Active Ingredient

Both brands were analyzed for content of active ingredient and found as shown in Table 4 that glipizide in both formulations are within the limit of 90.0-110.0% as stated in USP 27 specification. The percent contents of glipizide in the test and innovator's product are very similar and less than one percent difference (99.14 vs 98.74). They are readily eligible for *in vivo* bioequivalence study.

Table 4 Assay of 5 mg Glipizide Tablets of Test and Innovator's Products

No.	% Labeled Amount	
	Test Product	Innovator's Product
1	99.30	98.91
2	98.97	98.57
Mean	99.14	98.74
S.D.	0.23	0.24
%R.S.D.	0.24	0.24

4. *In Vitro* Dissolution Testing

Typical standard/calibration curve data and the plot for determining glipizide dissolved are illustrated in Table 5 and Figure 3. The data showed that the relation between absorbance versus concentrations computed using linear regression analysis was highly correlative with coefficient determination of 0.9999 and percent recovery of each concentration closed to 100. The percents glipizide dissolved from 5 mg glipizide tablets are presented in Tables 6 and 7 for test and innovator's product, respectively. It can be seen that both products rapidly dissolved reaching 85 percent of glipizide dissolution in simulated intestinal fluid within 5 minutes after starting the test. Percent glipizide dissolved increased with time. At 45 minutes, percent drug dissolved from each unit of both products were greater than the specified value of USP requirement (Q=80%), referring they passed the test for single point dissolution. As shown in Figure 4, dissolution profiles for the two brands were also conducted and compared. Comparisons were made and reported in Table 8. The different factor (f_1) and similarity factor (f_2) calculated using model independent approach were found to be 2.83% and 69.74%, respectively and fell within the acceptance criteria. This demonstrates that the dissolution profiles of test and innovator's product are complete equivalence.

Table 5 Calibration Curve for Determination of Glipizide Concentrations in Phosphate Buffer pH 6.8 Estimated Using Linear Regression Analysis

Standard no.	Known Concentration (μg/mL)	Absorbance	Estimated Concentration (μg/mL)	Mean of Estimated Concentration*	S.D.	%C.V.	% Recovery*
1	1	0.0248 0.0249 0.0250	1.00 1.00 1.00	1.00	0.01	0.52	100.19
2	2	0.0471 0.0472 0.0482	1.98 1.97 2.02	1.99	0.02	1.06	99.57
3	3	0.0693 0.0693 0.0702	2.96 2.95 2.97	2.96	0.01	0.48	98.64
4	4	0.0924 0.0940 0.0922	3.97 4.03 3.93	3.98	0.05	1.23	99.46
5	5	0.1144 0.1151 0.1144	4.94 4.96 4.90	4.93	0.03	0.60	98.63
6	6	0.1379 0.1370 0.1389	5.97 5.92 5.97	5.95	0.03	0.50	99.19
7	7	0.1586 0.1597 0.1611	6.88 6.91 6.93	6.91	0.03	0.38	98.70
8	8	0.1835 0.1835 0.1849	7.97 7.96 7.97	7.97	0.01	0.10	99.59

* n = 3

Where $r^2 = 0.9999$ $y = 0.0226x + 0.0020$

Estimated concentration $= \frac{[\text{Absorbance} - 0.0020]}{0.0226}$ and %Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

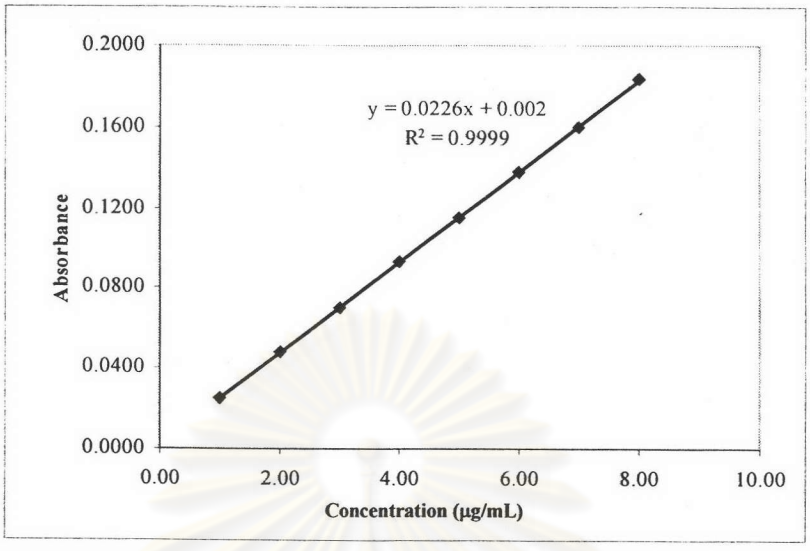


Figure 3 Calibration Curve for Determination of Glipizide in Dissolution Medium.

Table 6 Dissolution Data of 5 mg Glipizide Tablets of Test Product

Vessel No.	Time (min)							
	5	10	15	20	30	45	60	90
1	83.43	97.10	102.04	103.63	107.15	107.15	105.65	105.48
2	82.63	97.72	103.45	105.57	107.77	107.42	110.07	106.45
3	81.48	97.10	101.95	104.07	105.21	105.21	105.13	105.21
4	91.01	103.63	107.42	108.74	109.98	110.33	110.42	109.09
5	87.39	102.66	108.04	108.83	111.92	110.68	115.18	110.07
6	84.66	98.77	102.74	105.13	106.18	106.45	106.18	105.48
7	83.52	95.61	99.35	100.74	102.22	101.78	101.09	102.48
8	82.99	94.39	99.87	100.74	104.74	102.82	103.00	101.69
9	92.21	101.61	107.00	107.35	109.44	109.70	108.57	109.44
10	86.91	99.78	106.13	107.52	109.87	108.83	107.26	107.52
11	83.69	96.48	102.13	103.17	105.00	104.74	105.52	103.78
12	86.12	97.78	103.78	106.83	107.52	107.26	107.00	107.00
Mean	85.50	98.55	103.66	105.19	107.25	106.86	107.09	106.14
S.D.	3.36	2.85	2.90	2.80	2.75	2.84	3.69	2.67
%C.V.	3.93	2.89	2.79	2.66	2.56	2.66	3.44	2.51

Table 7 Dissolution Data of 5 mg Glipizide Tablets of Innovator's Product

Vessel No.	Time (min)							
	5	10	15	20	30	45	60	90
1	96.22	109.54	109.45	108.74	108.39	107.51	106.80	105.21
2	85.37	103.89	110.07	105.04	103.89	102.74	101.42	100.71
3	91.28	106.36	108.74	109.98	110.07	108.74	108.92	106.98
4	87.13	109.80	111.04	110.68	109.45	108.12	107.77	106.54
5	96.39	109.45	110.42	110.24	109.45	109.54	108.83	108.39
6	88.72	105.13	105.57	104.51	104.68	104.33	103.18	103.89
7	82.38	101.69	102.82	103.09	103.17	101.87	100.04	105.52
8	91.87	105.09	105.35	105.00	103.78	104.91	103.17	102.48
9	100.04	108.83	108.39	108.04	107.26	105.96	105.96	104.65
10	89.69	108.39	109.78	111.87	111.61	110.39	109.00	107.87
11	82.56	105.61	113.61	110.48	110.74	107.52	107.43	106.04
12	87.86	106.30	108.22	107.00	107.43	104.74	103.17	102.30
Mean	89.96	106.67	108.62	107.89	107.49	106.36	105.48	105.05
S.D.	5.52	2.56	2.89	2.90	2.95	2.70	3.14	2.35
%C.V.	6.13	2.40	2.66	2.69	2.75	2.54	2.98	2.24

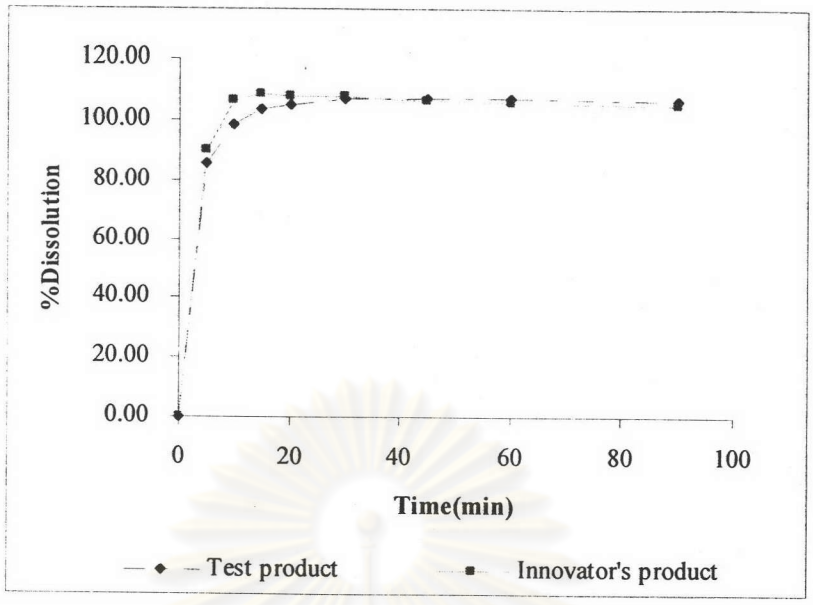


Figure 4 Mean Dissolution Profiles of 5 mg Glipizide Tablet of Test and Innovator's Products

Table 8 Dissolution Profiles Comparison Data of 5 mg Glipizide Tablets of Test and Innovator's Product

Time	$\%\bar{X}_R$ Dissolved	$\%\bar{X}_T$ Dissolved	$\lvert\bar{X}_R-\bar{X}_T\rvert$	$(\bar{X}_R-\bar{X}_T)^2$
5	89.96	85.50	4.45	19.84
10	106.67	98.55	8.12	65.97
15	108.62	103.66	4.96	24.63
20	107.89	105.19	2.70	7.28
30	107.49	107.25	0.24	0.06
45	106.36	106.86	0.50	0.25
60	105.48	107.09	1.61	2.60
90	105.05	106.14	1.09	1.19
$\sum$	837.52	-	23.69	121.82

$f_1 = 2.83\%$

$f_2 = 69.74\%$

5. Evaluation of *In Vitro* Studies

All *in vitro* studies revealed that the two brands of 5 mg glipizide tablet complied the specified requirements of USP 27 and Thai-FDA in all essential aspects. Thus, it can be concluded that both products tested are pharmaceutically equivalent.

B. Bioanalytical Method for Determining Glipizide in Plasma

Analytical methods used for the quantitative determination of drugs in biological samples are important to generate reproducible and reliable data before using in the evaluation and interpretation of bioavailability, pharmacokinetic findings and bioequivalence. It is essential to perform fully validated analytical methods to yield reliable results.

1. Selectivity/ Specificity

Chromatograms of blank plasma from 6 sources are shown in Figure 5. Under the analysis procedures, glipizide and internal standard (gliclazide) were eluted with retention times of approximately 8 – 9 and 12 – 13 minutes, respectively as displayed in Figures 6 and 7. As can be seen from Figures 5 to 7, no any interferent peaks due to the presence of plasma proteins and/or endogenous substances affecting the peaks of glipizide and internal standard are observed. This indicates the selectivity/ specificity of the analytical method.

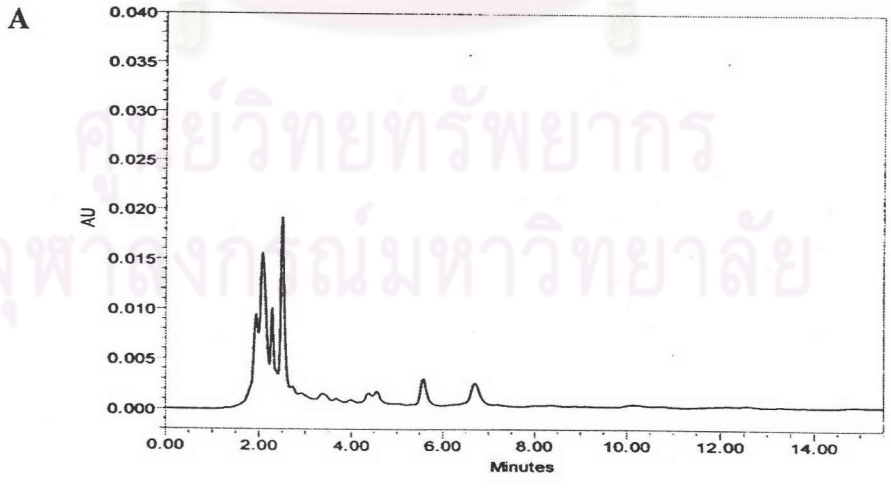


Figure 5 Chromatograms of Blank Plasma from Six Sources (A-F).

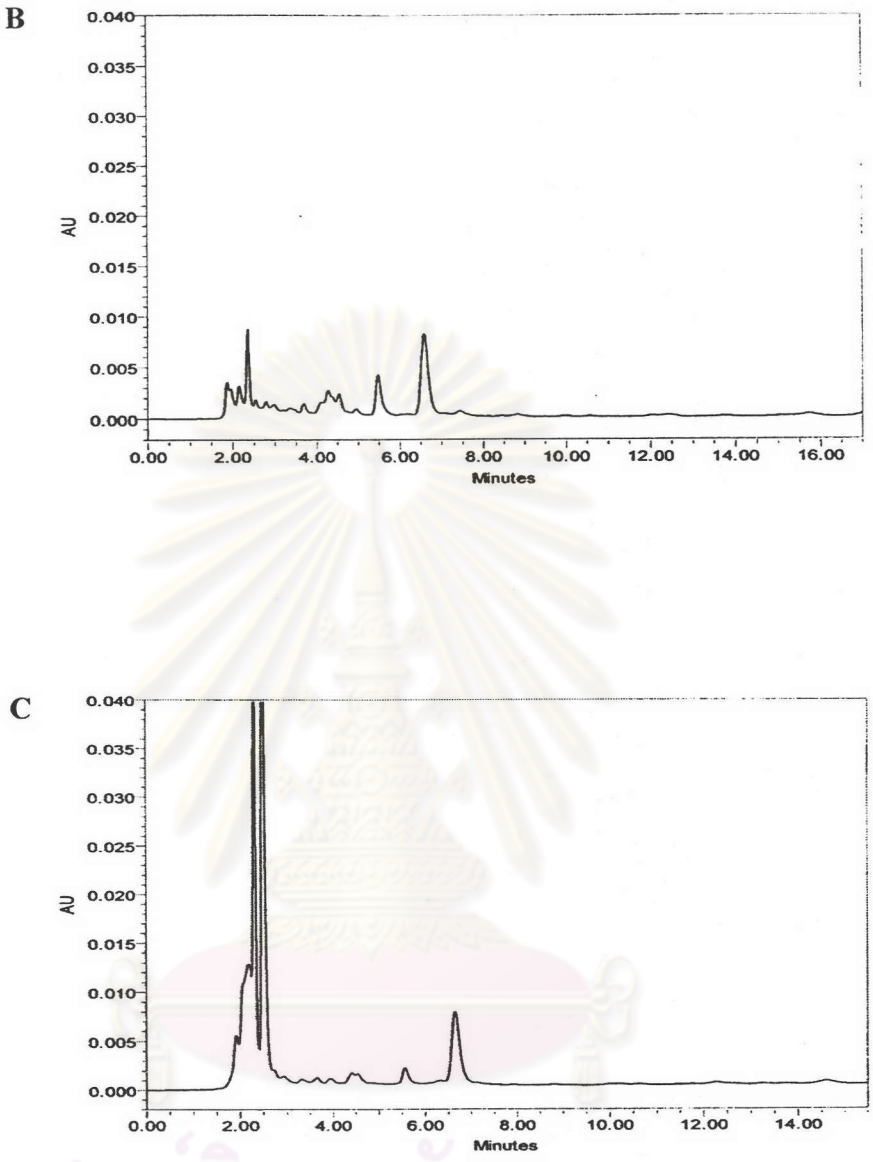


Figure 5 Chromatograms of Blank Plasma from Six Sources (A-F) (cont.).

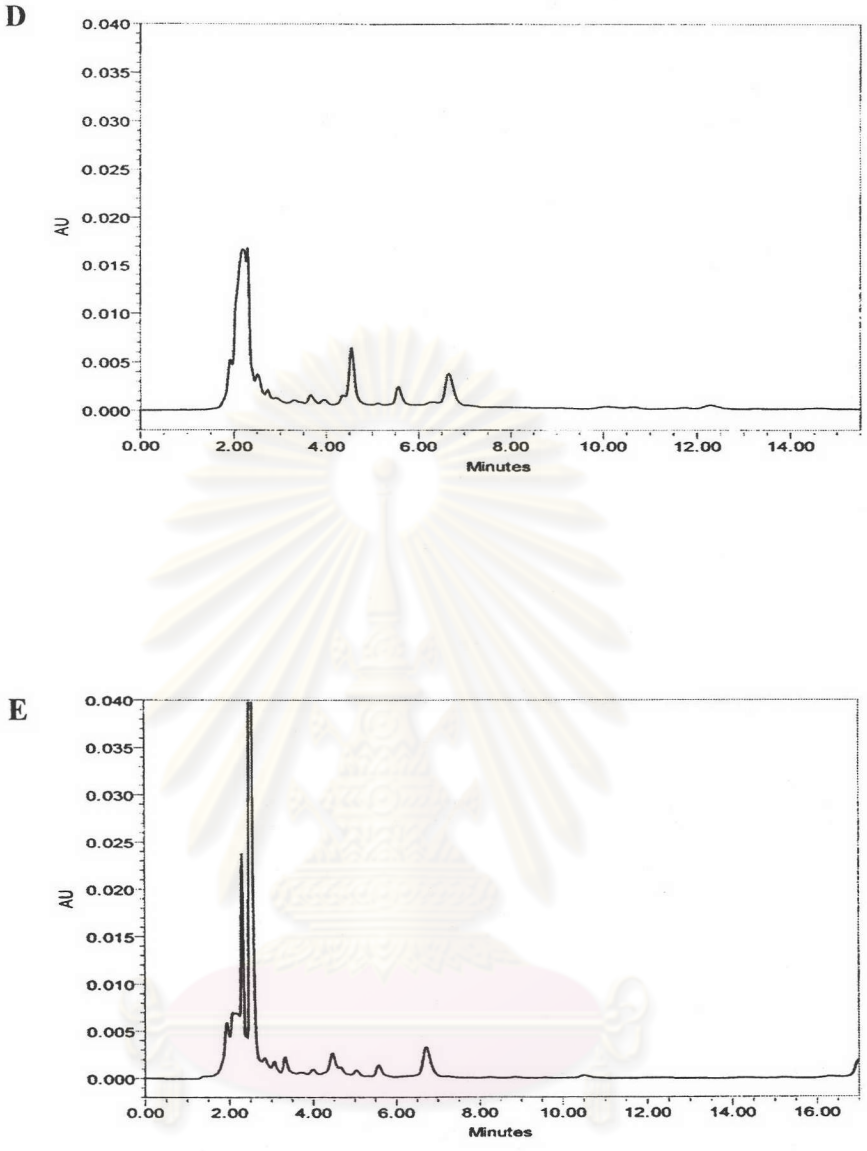


Figure 5 Chromatograms of Blank Plasma from Six Sources (A-F) (cont.).

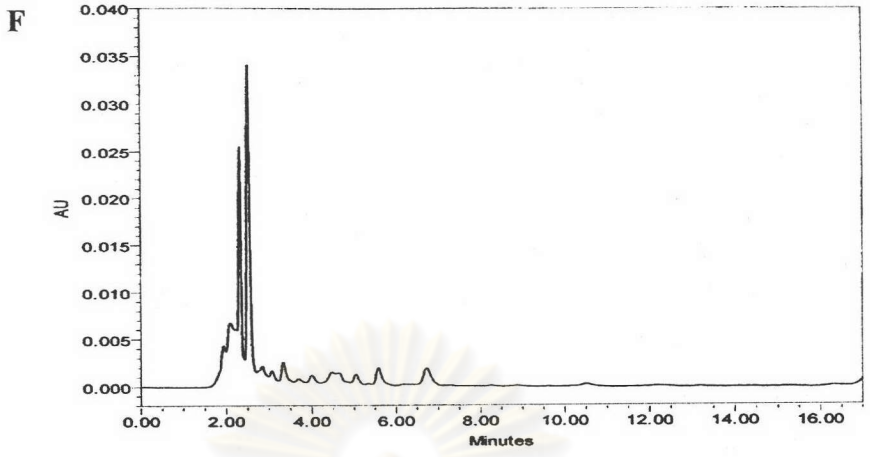


Figure 5 Chromatograms of Blank Plasma from Six Sources (A-F). (cont.)

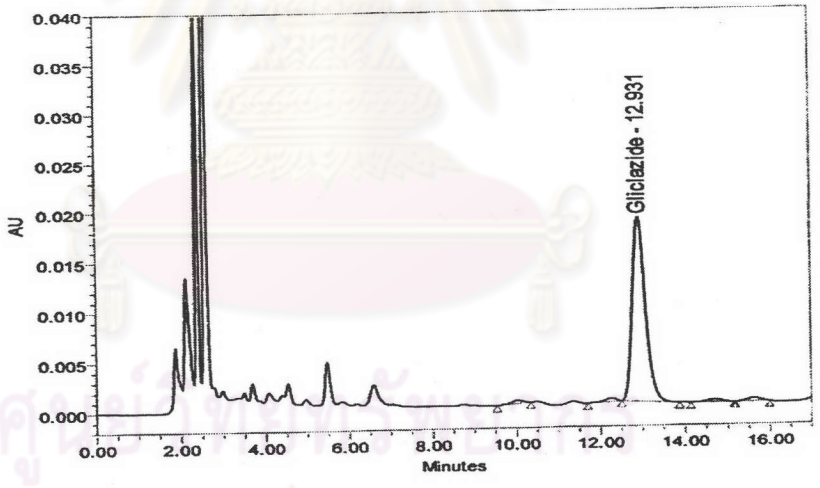


Figure 6 Chromatogram of Plasma Spiked with Gliclazide (Internal Standard).

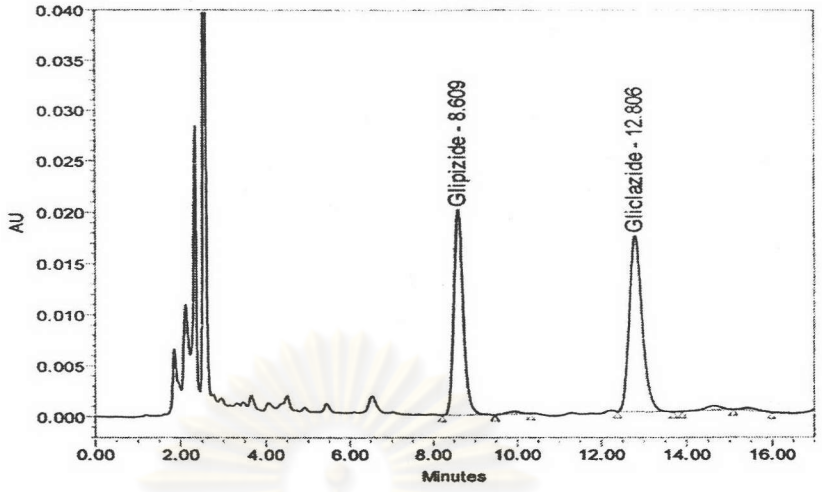


Figure 7 Chromatogram of Plasma Spiked with Glipizide 600 ng/mL and Internal Standard.

2. Linearity and Lower Limit of Quantification

Typical linearity data and the plots utilizing linear regression analysis are presented in Tables 9-13 and Figures 8-12. These demonstrate that an eight points calibration curve of peak area ratio of glipizide to gliclazide was linear covered the range of concentrations employed (20-1000 ng/mL) with the coefficient of determination of better than 0.999. The percent deviation from nominal concentration of each level except the first one was within $\pm$ 15%. The lower limit of quantification which served as the first concentration in a series of standard solutions was found to be 20 ng/mL with accuracy in term of percent recovery within $\pm$ 20% and precision in term of percent coefficient of variation less than 20% as shown in Table 14.

Table 9 Linearity of Curve No. 1 of Analytical Method for Determination of Glipizide in Plasma

Standard no.	Known Concentration (ng/mL)	Peak Area Ratio	Estimated Concentration (ng/mL)	%Recovery
1	20	0.03434	20.82	104.10
2	25	0.04232	25.97	103.89
3	50	0.07907	49.70	99.40
4	100	0.15193	96.73	96.73
5	200	0.29882	191.57	95.78
6	400	0.62201	400.21	100.05
7	600	0.96282	620.23	103.37
8	1000	1.53523	989.77	98.98

Where; r^2 = 0.9993 y = 0.0015x + 0.0021

Estimated concentration = $\frac{\text{[Peak area ratio - 0.0021]}}{0.0015}$

%Recovery = $\frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

Table 10 Linearity of Curve No. 2 of Analytical Method for Determination of Glipizide in Plasma

Standard no.	Known Concentration (ng/mL)	Peak Area Ratio	Estimated Concentration (ng/mL)	%Recovery
1	20	0.03547	23.45	117.27
2	25	0.03948	25.94	103.78
3	50	0.08053	51.44	102.87
4	100	0.16614	104.59	104.59
5	200	0.30744	192.32	96.16
6	400	0.61180	381.29	95.32
7	600	0.99454	618.93	103.16
8	1000	1.60351	997.04	99.70

Where; $r^2 = 0.9991$ $y = 0.0016x - 0.0023$

Estimated concentration $= \frac{[\text{Peak area ratio} + 0.0023]}{0.0016}$

%Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

Table 11 Linearity of Curve No. 3 of Analytical Method for Determination of Glipizide in Plasma

Standard no.	Known Concentration (ng/mL)	Peak Area Ratio	Estimated Concentration (ng/mL)	%Recovery
1	20	0.028543	18.99	94.96
2	25	0.037972	26.21	104.84
3	50	0.067931	49.15	98.29
4	100	0.132498	98.58	98.58
5	200	0.267216	201.72	100.86
6	400	0.546796	415.76	103.94
7	600	0.758299	577.68	96.28
8	1000	1.318966	1006.92	100.69

Where; $r^2 = 0.9991$ $y = 0.0013x + 0.0037$

Estimated concentration $= \frac{[\text{Peak area ratio} - 0.0037]}{0.0013}$

%Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

Table 12 Linearity of Curve No. 4 of Analytical Method for Determination of Glipizide in Plasma

Standard no.	Known Concentration (ng/mL)	Peak Area Ratio	Estimated Concentration (ng/mL)	%Recovery
1	20	0.03085	19.71	98.57
2	25	0.03455	22.24	88.96
3	50	0.06928	45.91	91.81
4	100	0.15963	107.49	107.49
5	200	0.30818	208.72	104.36
6	400	0.57422	390.04	97.51
7	600	0.87908	597.81	99.63
8	1000	1.47375	1003.09	100.31

Where; $r^2 = 0.9997$ $y = 0.0015x + 0.0019$

Estimated concentration $= \frac{[\text{Peak area ratio} - 0.0019]}{0.0015}$

%Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

Table 13 Linearity of Curve No. 5 of Analytical Method for Determination of Glipizide in Plasma

Standard no.	Known Concentration (ng/mL)	Peak Area Ratio	Estimated Concentration (ng/mL)	%Recovery
1	20	0.03552	18.70	93.49
2	25	0.04381	24.67	98.68
3	50	0.07324	45.86	91.72
4	100	0.15116	101.97	101.97
5	200	0.28610	199.15	99.58
6	400	0.56777	402.00	100.50
7	600	0.85395	608.08	101.35
8	1000	1.39061	994.56	99.46

Where; $r^2 = 0.9999$ $y = 0.0014x + 0.0096$

Estimated concentration $= \frac{[\text{Peak area ratio} - 0.0096]}{0.0014}$

%Recovery $= \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$

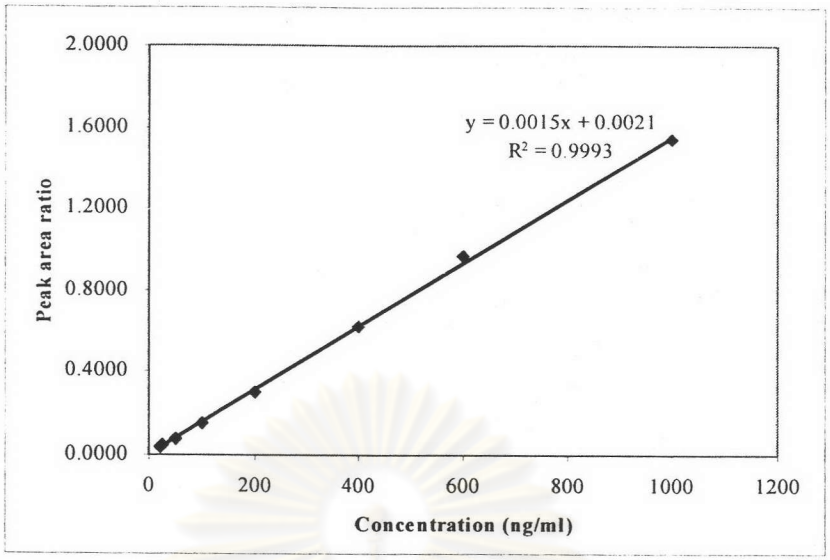


Figure 8 Linearity Curve No. 1 of Analytical Method for Determination of Glipizide in Plasma

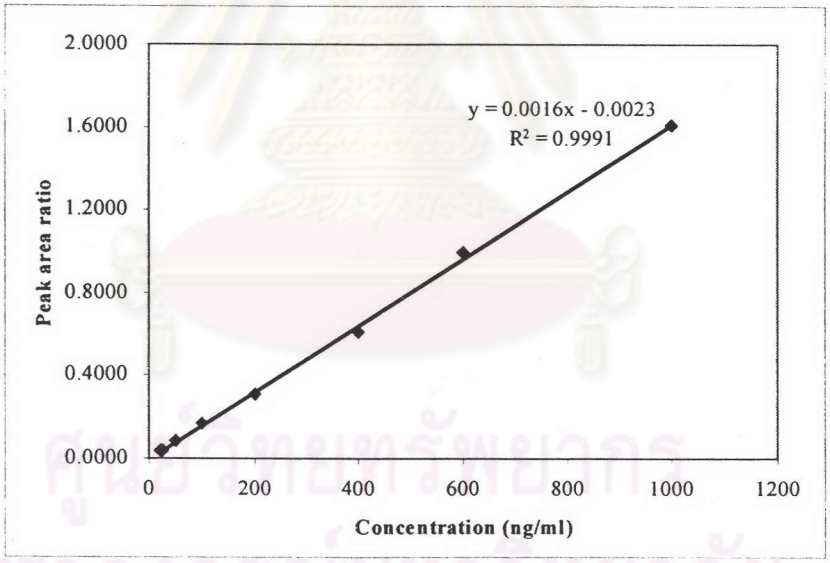


Figure 9 Linearity Curve No. 2 of Analytical Method for Determination of Glipizide in Plasma

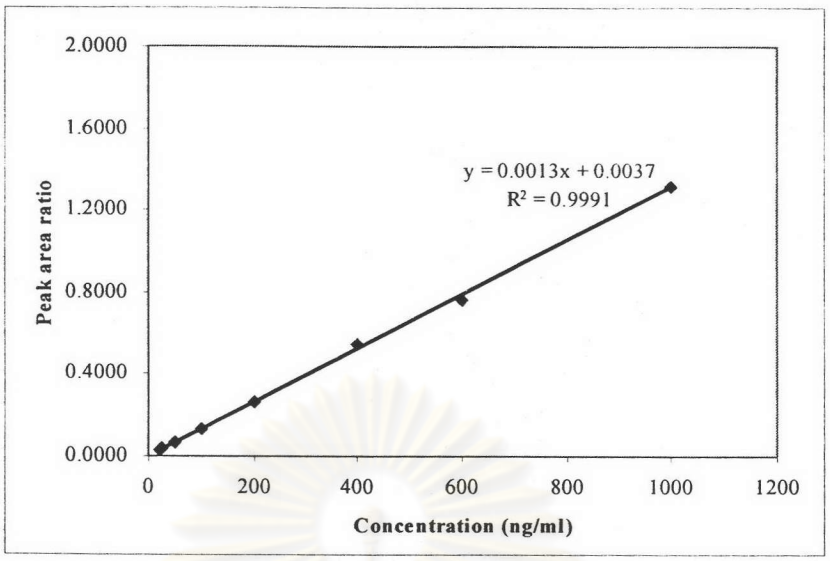


Figure 10 Linearity Curve No. 3 of Analytical Method for Determination of Glipizide in Plasma

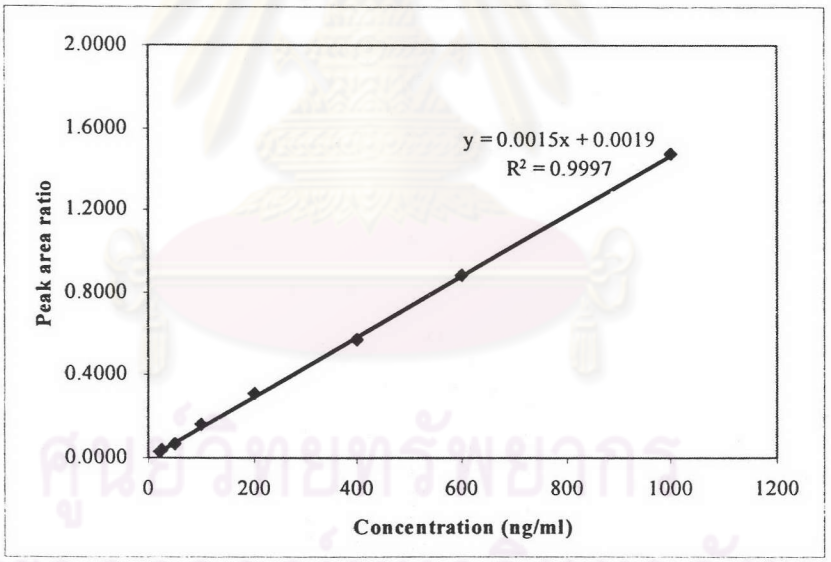


Figure 11 Linearity Curve No. 4 of Analytical Method for Determination of Glipizide in Plasma

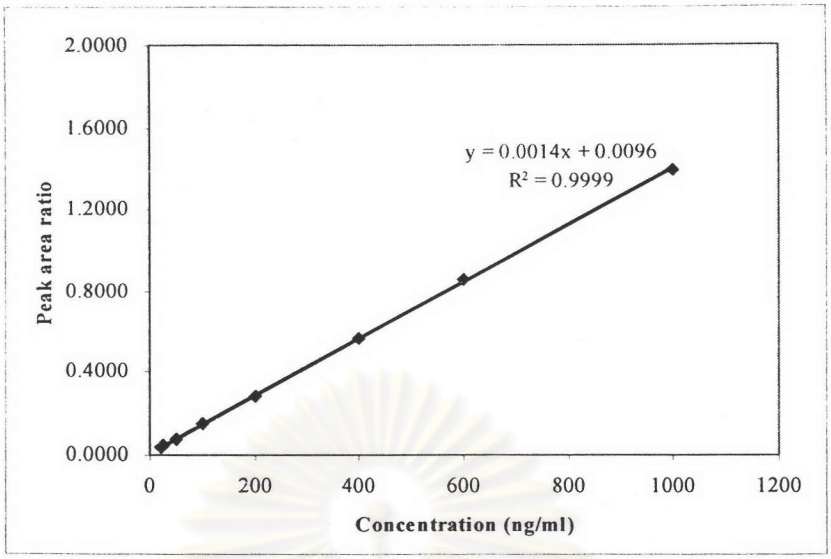


Figure 12 Linearity Curve No. 5 of Analytical Method for Determination of Glipizide in Plasma

Table 14 Lower Limit of Quantification of Analytical Method for Determination of Glipizide in Plasma

Replication no.	Known Concentration (ng/mL)	Estimated Concentration (ng/mL)	%Recovery
1	20.00	20.82	104.10
2	20.00	23.45	117.27
3	20.00	18.99	94.96
4	20.00	19.71	98.57
5	20.00	18.70	93.49
Mean		20.33	101.68
S.D.		1.92	9.63
%C.V.		9.46	9.47

3. Accuracy, Within-run and Between-run Precisions

These were accomplished using 3 quality control concentrations. Results are reported in Tables 15 to 17, respectively. As observed, the accuracy of the method was between 96.07 – 100.05% meanwhile those for within-run and between-run precisions were less than 15% of coefficient of variations. These values agreed with recommended limits of acceptance.

4. Recovery of Extraction

Results are reported in Table 18. High recoveries were obtained for glipizide ranging from 94.42 – 102.98%, indicating specificity of the extraction procedure. However, the extraction recovery of internal standard was slightly low with value of about 64% but its peak was consistent and reproducible. Generally, the recovery of extraction of analytes need not be 100%, but the extent of recovery should be constant in terms of consistency, precision and reproducibility. Besides, recovery of extraction of the drug found in this study is superior to that using liquid-liquid extraction technique reported previously (Emilsson, 1987). Thus, the extraction method used is appropriate for the purpose of this study.

Table 15 Accuracy of Analytical Method for Determination of Glipizide in Plasma

Known Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	S.D.	%C.V.	Mean of %Recovery*
60	56.97 59.20 60.99 57.32 55.41	57.98	2.16	3.72	96.63
500	454.69 477.35 447.21 544.00 478.51	480.35	38.15	7.94	96.07
900	885.68 875.43 901.20 957.62 882.25	900.44	33.33	3.70	100.05

* n = 5

Where; % Recovery = $\frac{\text{Estimated concentration}}{\text{Known concentration}}$ x 100

Table 16 Within-run Precision of Analytical Method for Determination Glipizide in Plasma

Known Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	S.D.	%C.V.
60	68.21 59.79 59.74 58.14 56.65	60.51	4.50	7.44
500	444.28 496.88 507.28 478.51 454.18	476.23	26.94	5.66
900	945.99 954.01 891.87 923.01 912.31	925.44	25.22	2.73

* n = 5

Table 17 Between-run Precision of Analytical Method for Determination of Glipizide in Plasma

Known Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	S.D.	%C.V.
60	68.21 56.97 59.61 69.63 61.46	63.18	5.50	8.71
500	444.28 454.69 480.90 476.73 503.04	471.93	23.09	4.89
900	946.00 885.68 807.45 829.57 883.31	870.40	54.19	6.23

* n = 5

Table 18 Recovery of Extraction of Analytical Method for Determination of Glipizide in Plasma

Active Ingredient	Known Concentration (ng/mL)	Peak Area		%Recovery	Mean of % Recovery*
		Extracted	Unextracted		
Glipizide	60	26665	28818	92.53	94.42
		26493	28425	93.20	
		30453	27448	110.95	
		25863	30078	85.99	
		27453	30689	89.46	
	500	216124	228988	94.38	96.31
		224428	232179	96.66	
		195882	226078	86.64	
		236990	229442	103.29	
		243255	241896	100.56	
	900	416498	447038	93.17	102.98
		441076	433485	101.75	
		450145	456506	98.61	
		473732	415600	113.99	
		476359	443598	107.39	
Gliclazide	1200	247507	402192	61.54	63.56
		236598	400160	59.13	
		263966	396274	66.61	
		238599	384619	62.04	
		262039	382387	68.53	

* n = 5

Where; % Recovery of extraction

$$= \frac{\text{PA of glipizide or int. std. of extracted samples from plasma}}{\text{PA of glipizide or int. std. of unextracted samples from mobile phase}} \times 100$$

5. Stability

All stability data are presented in Tables 19 to 23. Elaboration of these for more details is as follows:

5.1 Freeze-thaw stability

After completion of 3 freeze-thaw cycle, glipizide was still stable in plasma under this stressed condition. This is observed in Table 19, the percent deviation of glipizide concentrations compared to those before processing was within the acceptable ranges. However, the low QC sample appeared to be less resistant to this.

5.2 Long-term stability

The data in Table 20 shows that only percent deviation of low QC sample after storing at -20°C for 6 weeks was lower than -15%. This suggests that plasma containing glipizide can be kept at -20°C for only 4 weeks. However, this time period of storing is sufficient since all plasma glipizide samples can be completely analyzed within 1 month.

5.3 Short-term room temperature stability

Results demonstrates that the two QC samples were found to be well stable after they were thawed and kept at room temperature for 12 hours. No tendency of degradation of glipizide could be observed. Percent deviation of glipizide at both levels was slightly changed from those at time zero. This finding is beneficial for sample preparation.

5.4 Post-preparative stability

It can be seen in Table 22 that the processed plasma samples could be stored in autosampler at 8°C upto 24 hours without any problems. All percent deviations of glipizide were within acceptance range. This accelerates analysis of the samples to be quickly complete in a short period of time.

5.5 Stock solution stability

Results obtained in Table 23 indicates that both glipizide and gliclazide stock solutions in methanol after keeping in refrigerator were stable for 2 months. This facilitates processing samples in all aspects.

Table 19 Freeze-thaw Stability of Analytical Method for Determination of Glipizide in Plasma

Cycle	Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	Mean of %Deviation*
0	60	64.45 71.59 73.17	69.74	-
	900	964.11 1007.45 1018.06	996.54	-
3	60	63.90 65.75 63.77	64.48	-7.54
	900	917.29 878.87 827.94	874.70	-12.23

* n = 3

where; $$\% \text{ Deviation} = \frac{(\text{Est.conc.}_{\text{cycle 3}} - \text{Est.init.conc.}_{\text{cycle 0}}) \times 100}{\text{Est.init.conc.}_{\text{cycle 0}}}$$

Table 20 Long-term Stability of Analytical Method for Determination of Glipizide in Plasma

Time (week)	Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	Mean of % Deviation*
0	60	69.63 67.05 69.13	68.60	-
	900	829.57 862.67 844.70	845.65	-
2	60	67.63 70.13 69.38	69.05	0.66
	900	920.25 959.41 884.46	921.37	8.95
4	60	59.23 61.55 59.21	60.00	-12.54
	900	827.21 813.66 835.30	825.39	-2.40
6	60	56.40 55.10 57.51	56.33	-17.88
	900	881.70 872.01 865.54	873.08	3.24

* n = 3

where; $$\% \text{ Deviation} = \frac{(\text{Est.conc.}_{\text{week n}} - \text{Est.init.conc.}_{\text{week 0}}) \times 100}{(\text{Est.init.conc.}_{\text{week 0}})}$$

Table 21 Short-term Room Temperature Stability of Analytical Method for Determination of Glipizide in Plasma

Time (hour)	Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	Mean of % Deviation*
0	60	57.09 60.07 53.99	57.05	-
	900	865.53 843.49 914.67	874.56	-
4	60	60.17 57.29 57.94	58.47	2.49
	900	925.86 881.07 863.60	890.18	1.77
8	60	61.35 61.43 62.09	61.62	8.02
	900	946.42 861.78 872.13	893.44	2.16
12	60	56.90 59.51 55.86	57.42	0.66
	900	837.53 879.44 804.03	840.33	-3.91

* n = 3

where; % Deviation = $\frac{(\text{Est.conc.}_{\text{hour n}} - \text{Est.init.conc.}_{\text{hour 0}}) \times 100}{\text{Est.init.}_{\text{hour 0}}}$

Table 22 Post-preparative (Autosampler) Stability of Analytical Method for Determination of Glipizide in Plasma

Time (hour)	Concentration (ng/mL)	Estimated Concentration (ng/mL)	Mean of Estimated Concentration* (ng/mL)	Mean of % Deviation*
0	60	59.61 57.17 62.34	59.71	-
	900	807.45 831.70 794.08	811.08	-
6	60	56.38 56.49 59.51	57.46	-3.77
	900	801.87 839.62 855.97	832.49	2.64
12	60	60.09 54.24 57.53	57.29	-4.05
	900	844.65 820.12 800.61	821.80	1.32
24	60	59.22 48.78 59.84	55.95	-6.30
	900	781.10 792.12 782.64	785.29	-3.18

* n = 3

Where; % Deviation = $\frac{(\text{Est.conc.}_{\text{hour n}} - \text{Est.init.conc.}_{\text{hour 0}}) \times 100}{\text{Est.init.conc.}_{\text{hour 0}}}$

Table 23 Stock Solution Stability of Analytical Method for Determination of Glipizide in Plasma

Active Ingredient	Time (week)	Peak Area	Mean of Peak Area*	Mean of % Deviation*
Glipizide	0	9720739 9733905 9730663	9728436	-
	2	9745909 9751844 9764905	9754219	0.27
	4	9777070 9826908 9857854	9820611	0.95
	8	10167316 10176440 10184514	10176090	4.60
Gliclazide	0	7551123 7573865 7580347	7568445	-
	2	7584618 7602170 7615395	7600728	0.43
	4	7454988 7457535 7609679	7507401	-0.81
	8	7768854 7816142 7843445	7809480	3.18

* n = 3

Where; $\%\ \text{Deviation} = \dfrac{(\text{Peak Area}_{\text{week n}} - \text{Init. Peak Area}_{\text{week 0}}) \times 100}{\text{Init. Peak Area}_{\text{week 0}}}$

6. Evaluation of Bioanalytical Method

Method validations for analysis of glipizide in plasma are established. It has been proven to be specific, accurate and precise with the need of internal standard. The lower limit of quantification and stability data obtained in this finding can be successfully applied in bioequivalence study of glipizide without any limitations.

C. *In Vivo* Bioequivalence Studies

1. Drug-products

The same brands and batch number of two products of 5 mg glipizide tablet which have been tested for *in vitro* properties were subjects for *in vivo* bioequivalence study.

2. Subjects

Twelve healthy Thai male subjects participated the experiment. Their demographic data as well as chemical and hematologic laboratory tests are presented in Tables 24, 48 and 49, respectively. These data confirm their healthy as well as the requirements for aging and BMI values of all subjects of Thai FDA.

3. Plasma Glipizide Concentrations

The plasma glipizide concentrations at each sampling time upto 12 hours of 12 subjects following oral administration of glipizide tablets of test and innovator's product in a replicated crossover manner were collected and assayed. Typical chromatogram of analysis for these plasma samples is illustrated in Figure 13 as well as the QC samples at 3 levels in duplicate incorporated in each analytical run for accepting or rejecting the data is reported in Table 25. Both of these results conform the acceptance criteria.

Table 24 Demographic Data of Subjects Participated in This Study

Subject no.	Age (years)	Weight (kg)	Height (m)	BMI (kg/m^2)
1	22	58	1.68	20.5
2	21	64	1.67	22.9
3	33	57	1.68	20.2
4	30	65	1.70	22.5
5	19	52	1.70	18.0
6	25	63	1.75	20.6
7	19	73	1.79	22.8
8	28	49	1.59	19.4
9	19	53	1.63	19.9
10	30	62	1.72	21.0
11	32	54	1.67	19.4
12	21	54	1.64	20.1
Mean	24.92	58.67	1.69	20.61
S.D.	5.40	6.88	0.05	1.50
%C.V.	21.68	11.73	3.18	7.27
Min	19	49	1.59	18.0
Max	33	73	1.79	22.9

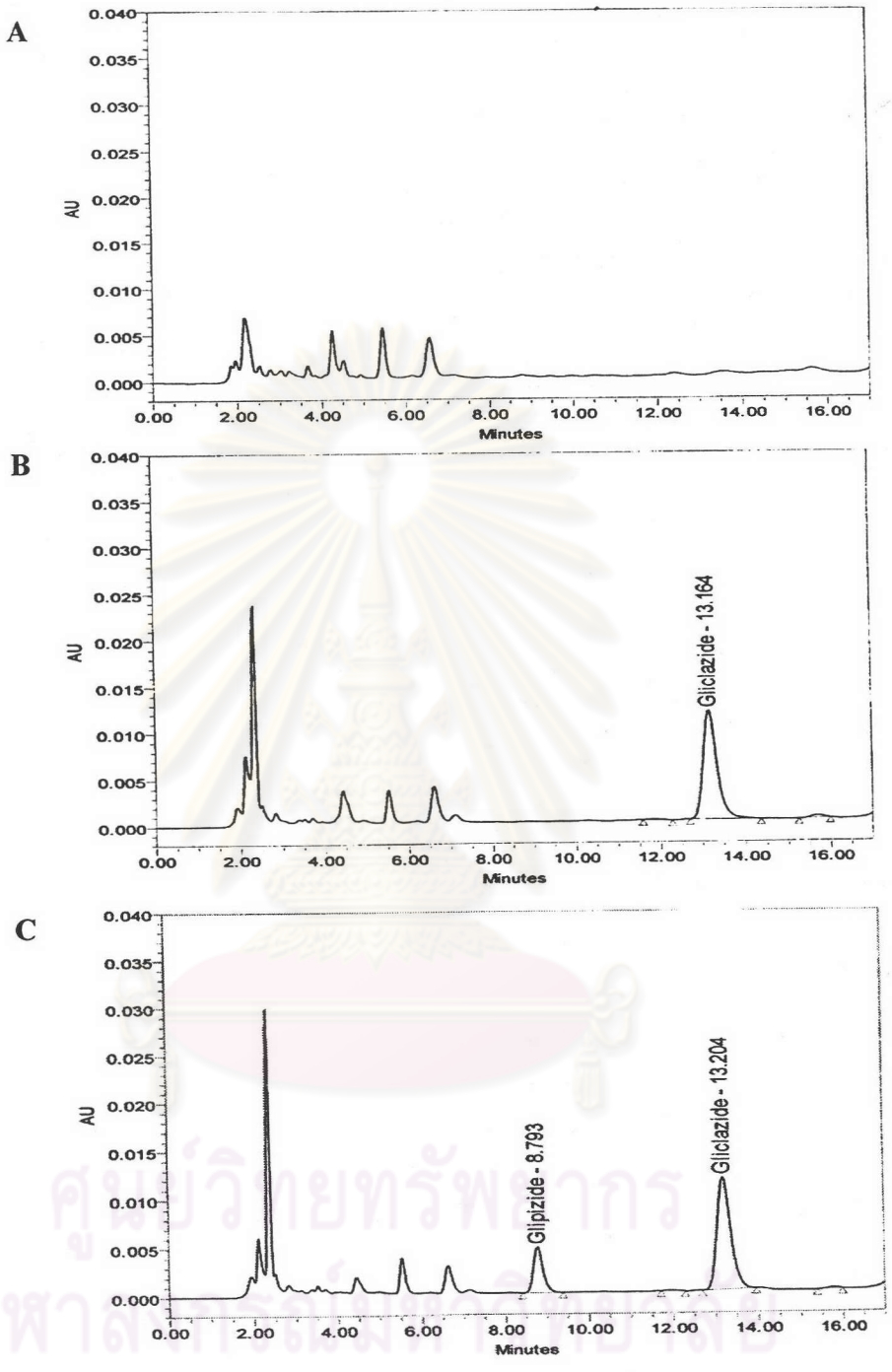


Figure 13 Chromatogram of Subject no. 9 for (A) Blank Plasma, (B) Plasma Sample Spiked at 0 hr with Gliclazide (Internal Standard) 20 ng/mL, (C) Plasma Sample at 6 hrs Spiked with Gliclazide (Internal Standard) 20 ng/mL.

Table 25 QC Samples for Accepting an Analytical Run for Determination of Glipizide in Plasma

QC Samples (ng/mL)	Estimated Concentraion (ng/mL)	%Recovery	%Deviation
60	63.87	106.45	6.45
	53.66	89.43	-10.57
500	459.99	92.00	-8.00
	512.28	102.46	2.46
900	998.40	110.93	10.93
	863.95	95.99	-4.01

where; $$\% \text{ Recovery} = \frac{\text{Estimated concentration}}{\text{Known concentration}} \times 100$$

$$\% \text{ Deviation} = \frac{(\text{Estimated conc.} - \text{Known conc.})}{\text{Known conc.}} \times 100$$

Glipizide plasma concentration-time profiles of individual subject for test and innovator's product are reported in Tables 26 and 27, respectively. It is clearly observed from data presented in both tables that there are wide variations of plasma glipizide concentrations among subjects, especially those in the first crossover study of the two brands. Absorption of the drug was also erratic. This is proven by the AUC_{0-12} and C_{max} values of each brand are much less than its corresponding value in the second time. Besides, the appearance of glipizide in plasma of subject no. 6 and no. 10 was delayed and started at 2.5 and 3.5 hours after administration of test products as well as irregular plasma glipizide concentration of subject no. 9 was taken place following innovator's product dosing. In addition, glucose solution, a nutrient which was also administered to monitor blood sugar, was able to affect gastric emptying time by slowing down the movement of the stomach. These may be contributed to the variability.

Generally, a well defined pharmacokinetics of particular drug need plasma drug concentration-time at least 8 points. In this study, although subjects no. 6, 10 (received test product) and 9 (received innovator's product) participated in the first crossover study had less, their data were also included for evaluation. This is because the C_{max}, AUC_{0-12}, and $AUC_{0-\infty}$ calculated from them were closed to others with full concentration-time points.

The plots of plasma glipizide concentration-time profile as pairwise comparison of test and innovator's product of each individual for the first and second crossover study are shown in Figures 14 to 25. The mean plasma drug concentration-time of all subject for each treatment is plotted and illustrated in Figure 26.

4. Subjects Monitoring

Although the study was conducted in subjects with fasting condition, no adverse effects and/or any intoxications due to glipizide with the dose administered were observed (Table 50). No subjects withdrew from the experiment.

Table 26 Concentration (ng/mL) of Glipizide in Plasma of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablet of Test Products

Subject no.	Product code	Time (hrs)											
		0.5	1	1.5	2	2.5	3	3.5	4	6	8	10	12
1	Test 1	49.67	92.33	128.79	200.51	238.63	218.98	260.79	303.50	235.76	195.03	161.08	134.54
	Test 2	299.03	412.69	511.76	485.71	431.22	438.53	492.13	513.44	387.07	313.54	186.16	148.62
2	Test 1	48.52	121.42	193.75	213.77	195.11	177.06	206.32	174.00	106.63	81.18	55.92	46.07
	Test 2	59.01	78.45	88.38	96.85	228.74	223.88	287.75	223.43	124.84	98.89	69.20	55.22
3	Test 1	36.77	52.51	63.67	60.74	57.36	59.08	66.55	56.71	113.85	131.30	115.88	106.94
	Test 2	49.65	75.55	76.44	137.78	137.27	134.07	109.72	103.05	161.03	130.86	230.55	147.57
4	Test 1	*	173.90	167.35	265.10	236.61	189.29	215.74	198.45	118.27	83.09	66.76	65.03
	Test 2	*	23.71	24.11	26.45	115.31	82.49	75.40	75.58	42.68	27.82	22.19	*
5	Test 1	31.43	28.50	49.29	131.04	104.26	81.75	92.99	81.82	137.59	181.14	161.32	150.10
	Test 2	113.11	135.03	152.42	138.85	93.61	107.50	129.46	139.06	196.86	155.46	129.38	86.25
6	Test 1	*	*	*	*	35.48	31.30	36.25	61.71	238.14	154.67	110.74	78.80
	Test 2	76.51	124.23	123.50	159.87	183.48	216.83	315.33	342.50	220.14	161.00	120.51	89.72
7	Test 1	85.94	216.53	347.97	320.36	269.57	234.38	225.55	193.44	122.77	106.44	85.39	81.87
	Test 2	30.31	27.18	152.50	141.28	178.18	171.93	155.13	223.41	169.51	100.65	76.85	51.77
8	Test 1	32.05	86.71	125.02	117.55	113.98	86.20	76.40	72.77	380.61	238.63	151.25	84.32
	Test 2	51.87	49.42	66.41	69.02	59.70	51.27	62.11	45.43	393.77	452.61	347.89	240.52
9	Test 1	*	30.03	54.49	52.43	65.45	88.06	78.95	198.55	237.63	243.51	207.99	168.42
	Test 2	52.22	55.88	58.95	60.13	60.29	86.01	350.88	404.68	233.11	188.87	126.84	78.66
10	Test 1	*	*	*	*	*	*	65.06	134.06	184.27	114.15	69.20	62.97
	Test 2	48.59	60.28	77.79	80.99	105.45	102.73	101.33	96.72	150.95	204.13	133.39	71.32
11	Test 1	368.80	441.79	342.06	285.07	222.81	187.99	166.52	145.93	107.75	86.40	70.41	68.06
	Test 2	102.50	675.50	587.41	499.03	490.10	414.86	352.02	298.87	184.94	178.62	161.93	86.29
12	Test 1	25.95	74.66	130.60	136.35	127.66	158.29	310.61	249.51	116.86	80.95	50.51	43.22
	Test 2	33.06	78.80	147.33	291.37	292.70	297.52	319.73	314.58	139.99	142.29	105.24	61.78
Mean	-	66.46	129.80	152.92	165.43	168.46	160.00	189.70	193.80	187.71	160.47	125.69	92.00
S.D.	-	88.61	161.20	150.32	134.80	119.78	110.13	122.58	121.16	91.99	89.12	70.49	50.90
%C.V.	-	133.32	124.20	98.30	81.49	71.11	68.83	64.62	62.52	49.01	55.54	56.08	55.33

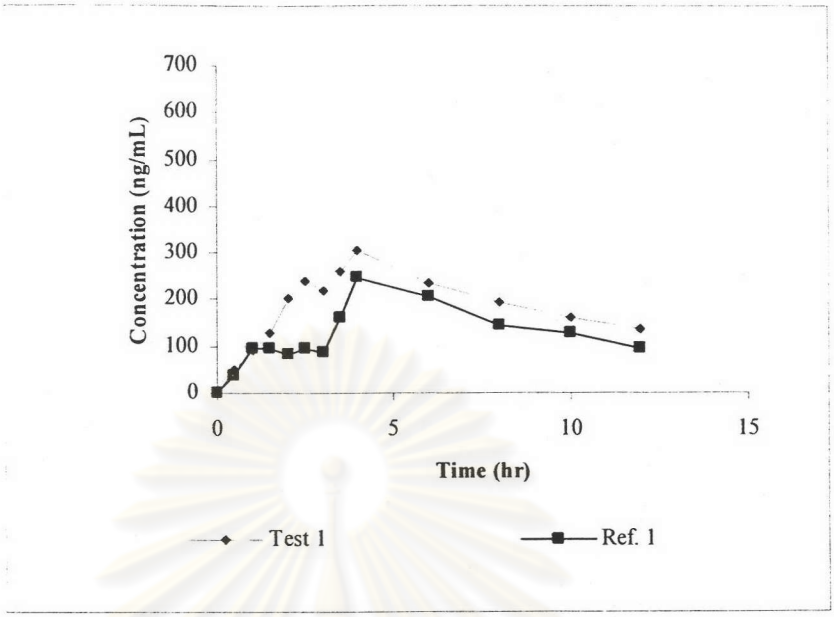
* = Glipizide Concentration < LLOQ (20 ng/mL)

Table 27 Concentration (ng/mL) of Glipizide in Plasma of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablet of Innovator's Products

Subject no.	Product code	Time (hrs)											
		0.5	1	1.5	2	2.5	3	3.5	4	6	8	10	12
1	Ref. 1	39.00	92.78	95.70	84.23	94.22	86.03	159.13	248.12	206.40	143.76	126.35	92.89
	Ref. 2	20.58	53.94	105.03	125.62	177.55	228.79	225.67	394.65	275.19	169.68	143.43	81.88
2	Ref. 1	71.67	67.74	93.02	106.34	118.87	109.84	104.30	97.32	193.04	112.37	92.72	76.70
	Ref. 2	196.74	220.82	441.48	532.99	359.74	307.20	305.42	273.14	160.44	92.01	80.59	*
3	Ref. 1	25.33	59.36	34.92	38.54	68.28	70.85	78.72	68.37	103.23	173.27	129.24	116.73
	Ref. 2	*	34.07	43.49	55.52	51.44	62.28	61.26	86.30	119.11	196.09	217.46	189.96
4	Ref. 1	72.65	144.85	273.14	345.47	304.07	278.95	216.17	199.91	115.50	88.19	75.15	71.84
	Ref. 2	138.00	232.29	365.80	443.41	499.80	419.17	378.41	284.10	210.67	199.62	144.51	87.04
5	Ref. 1	74.10	104.42	102.90	55.97	59.44	57.47	58.20	93.53	209.88	155.54	93.29	67.84
	Ref. 2	32.22	40.06	41.45	43.80	40.44	139.96	146.32	115.71	198.57	186.32	152.05	128.63
6	Ref. 1	140.28	284.28	301.26	280.23	228.63	200.51	174.02	148.27	97.34	78.10	48.16	43.01
	Ref. 2	105.47	206.19	259.35	174.86	274.63	269.77	220.28	204.44	134.85	96.85	68.58	45.17
7	Ref. 1	32.61	96.17	116.02	69.72	155.96	125.39	111.57	118.99	158.42	96.19	70.28	46.07
	Ref. 2	34.82	112.73	161.61	222.73	198.29	228.61	229.41	177.89	104.66	78.11	55.84	36.09
8	Ref. 1	52.77	96.72	199.48	244.90	250.12	212.25	211.77	163.69	162.84	147.68	153.87	155.03
	Ref. 2	131.15	239.42	258.53	238.57	212.09	189.22	154.56	143.93	171.06	184.68	128.77	111.58
9	Ref. 1	*	20.24	23.19	*	*	21.77	*	*	181.32	163.00	176.00	160.26
	Ref. 2	36.85	47.31	55.38	65.67	271.86	439.61	421.87	504.93	308.04	274.91	222.29	145.94
10	Ref. 1	32.81	56.42	71.17	69.93	65.81	90.64	127.48	136.44	193.13	161.09	112.47	82.64
	Ref. 2	91.12	163.36	142.20	346.69	139.78	138.48	175.05	149.70	89.06	57.33	42.22	23.10
11	Ref. 1	*	*	*	230.71	280.26	290.99	271.95	235.48	149.32	111.55	86.61	63.94
	Ref. 2	*	355.89	367.39	385.66	299.90	230.14	221.49	269.63	117.33	95.37	79.79	60.48
12	Ref. 1	53.85	294.76	355.71	343.87	256.22	205.45	186.10	148.52	101.35	77.61	60.88	55.78
	Ref. 2	103.99	262.31	373.36	368.92	392.76	330.65	249.52	236.79	135.04	113.63	89.89	60.97
Mean	-	61.92	136.92	178.40	203.10	200.01	197.25	187.03	187.49	162.32	135.54	110.44	83.52
S.D.	-	52.25	100.84	134.77	150.37	125.74	112.25	98.46	108.74	55.97	52.44	49.82	46.75
%C.V.	-	84.38	73.65	75.55	74.04	62.87	56.91	52.64	58.00	34.48	38.69	45.11	56.00

* = Glipizide Concentration < LLOQ (20 ng/mL)

A



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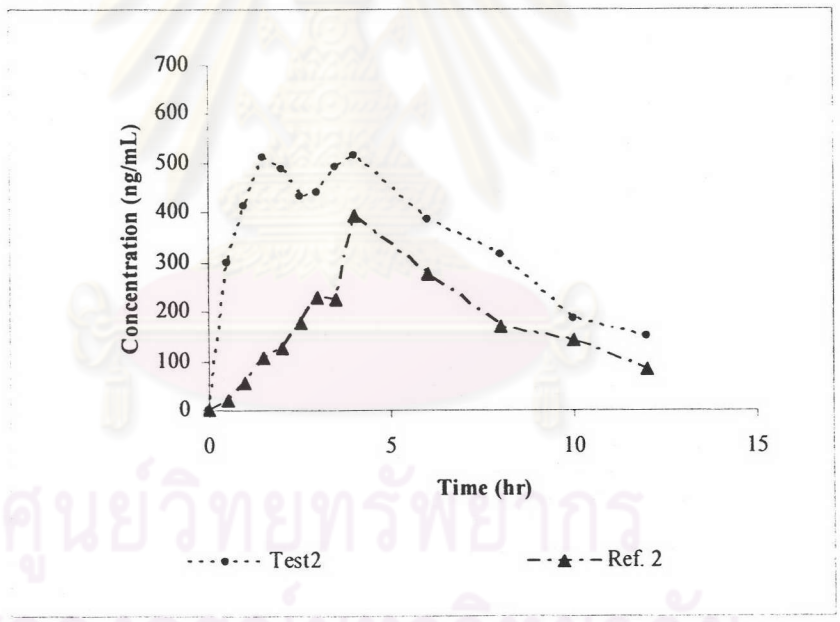
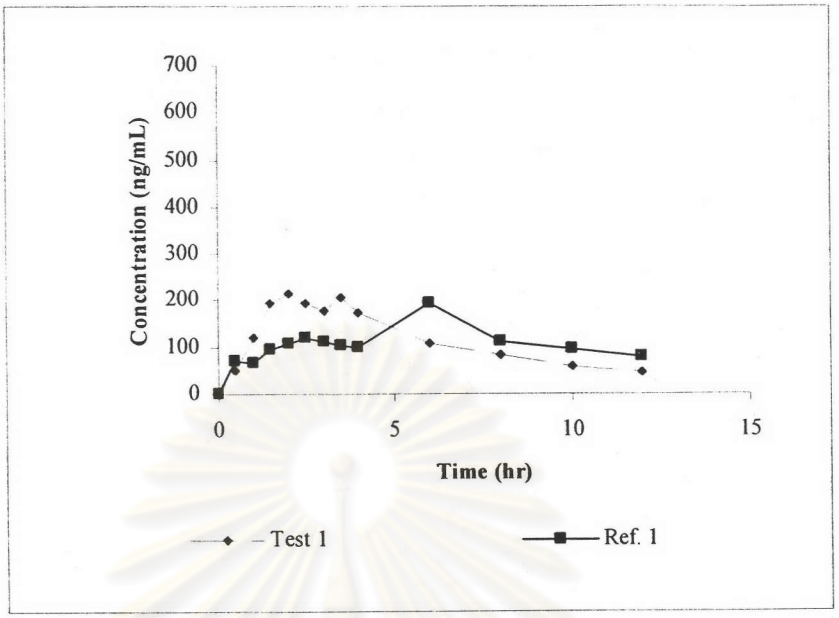


Figure 14 Plasma Glipizide Concentration-time Profiles of Subject No.1 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



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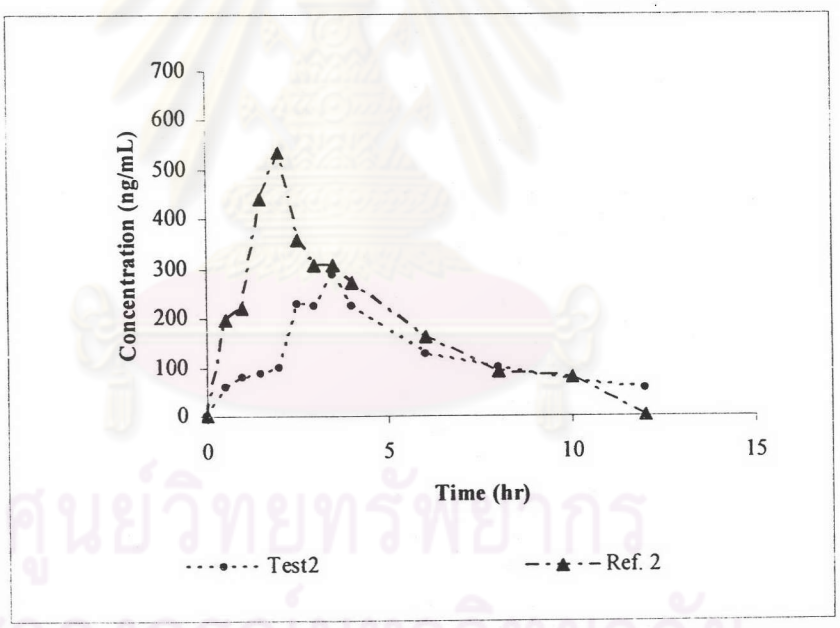
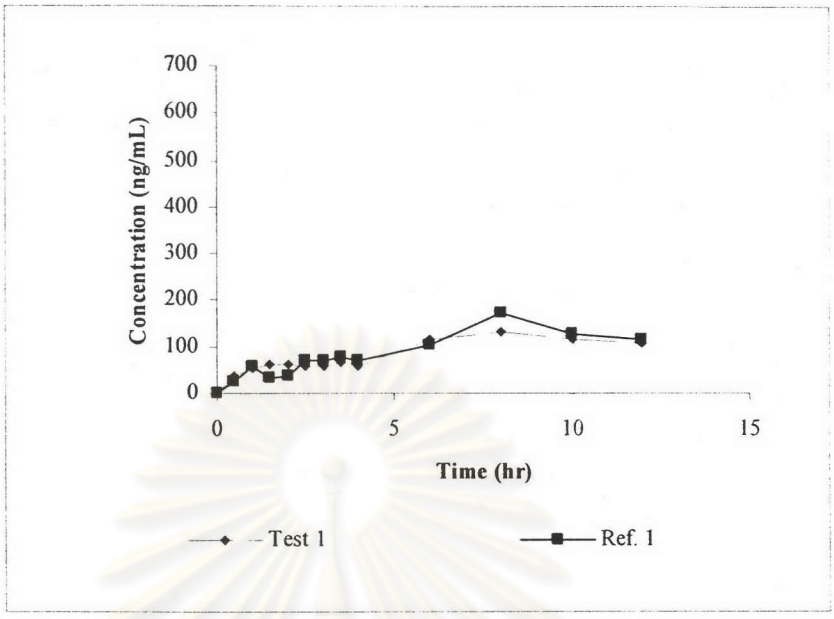


Figure 15 Plasma Glipizide Concentration-time Profiles of Subject No.2 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



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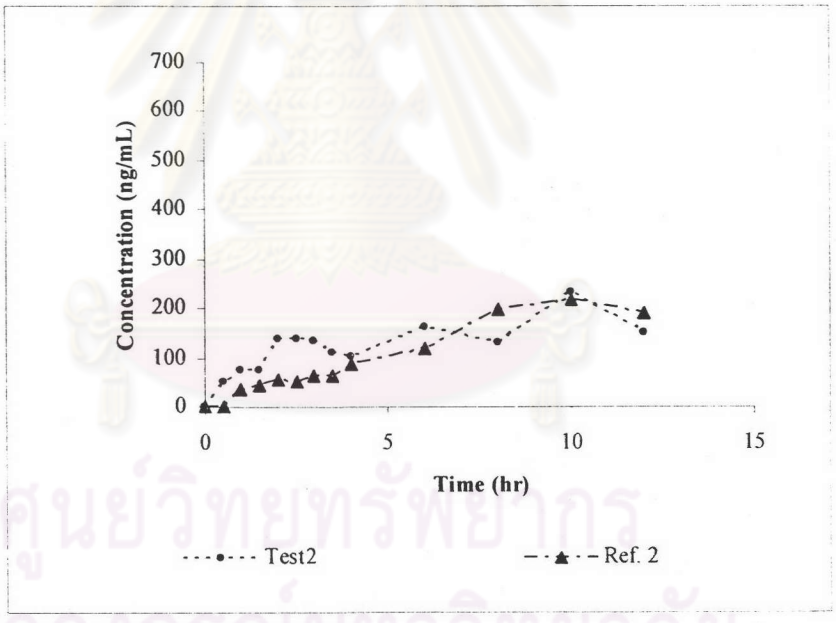
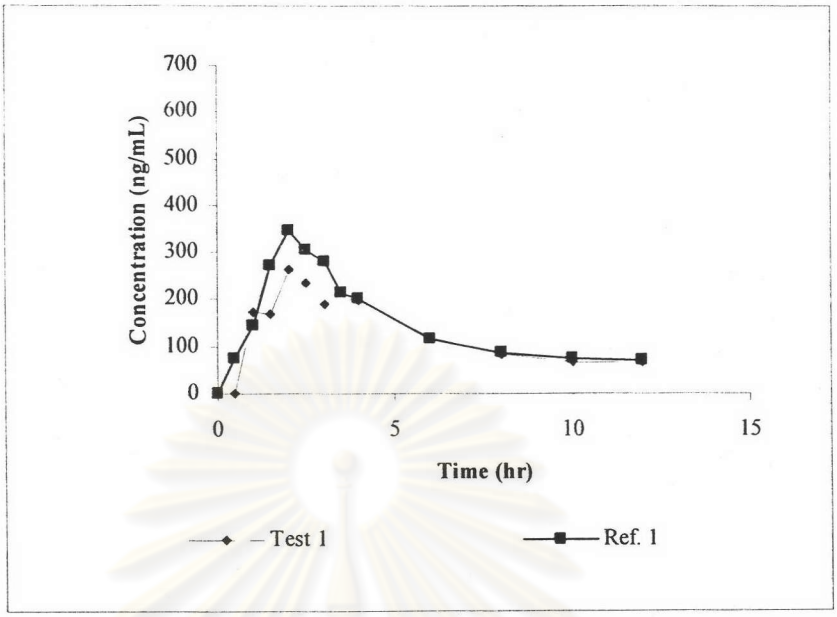


Figure 16 Plasma Glipizide Concentration-time Profiles of Subject No.3 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

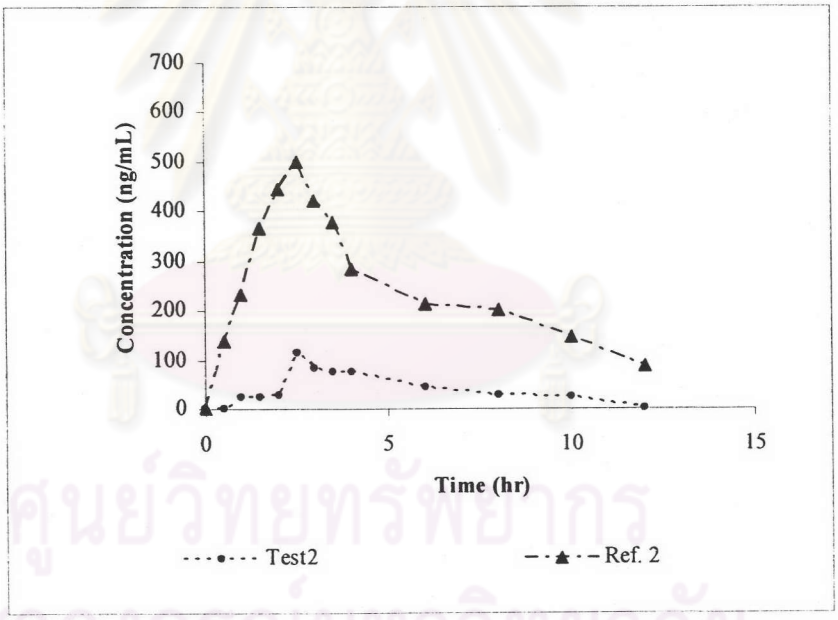
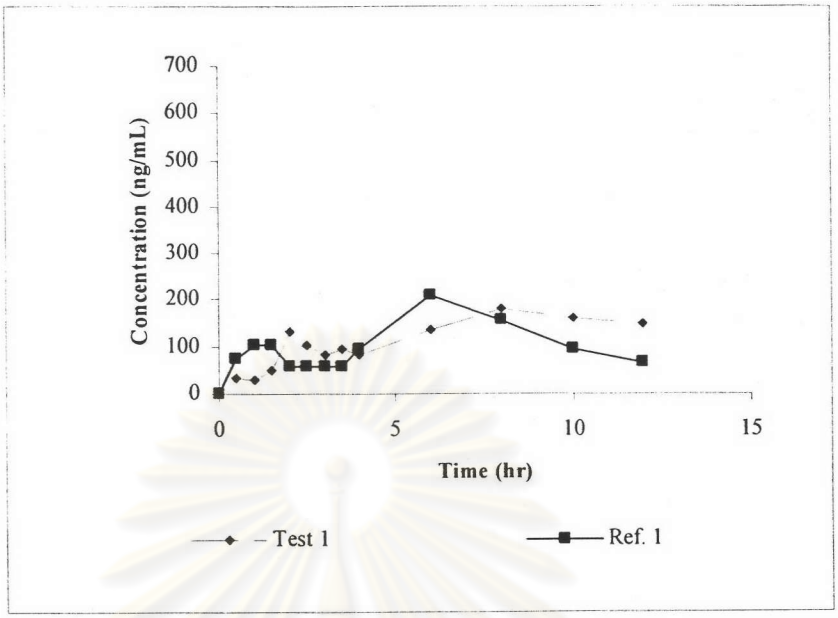


Figure 17 Plasma Glipizide Concentration-time Profiles of Subject No.4 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

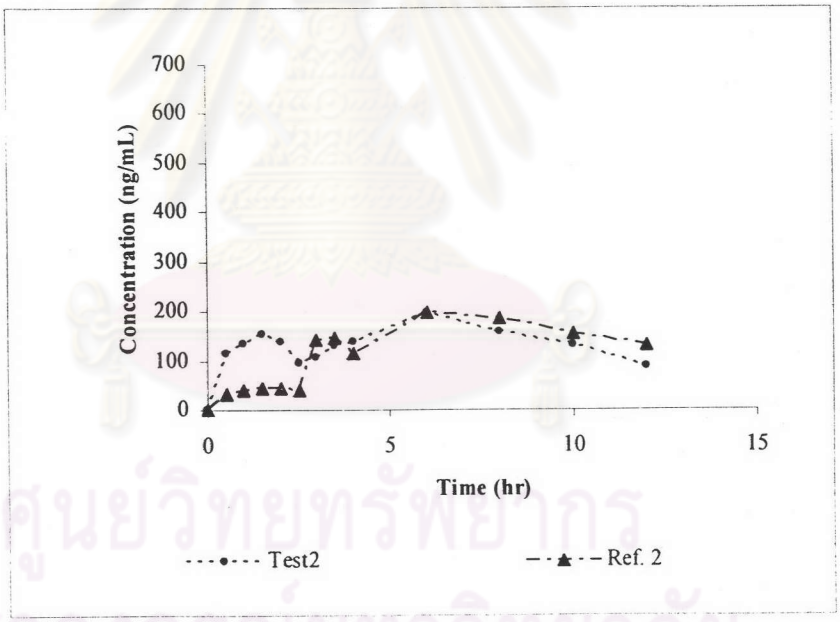
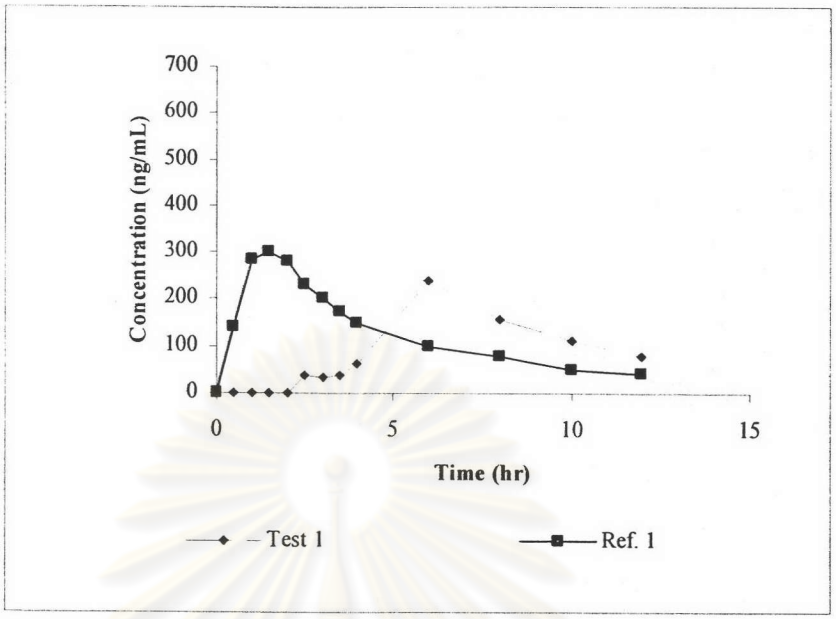


Figure 18 Plasma Glipizide Concentration-time Profiles of Subject No.5 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

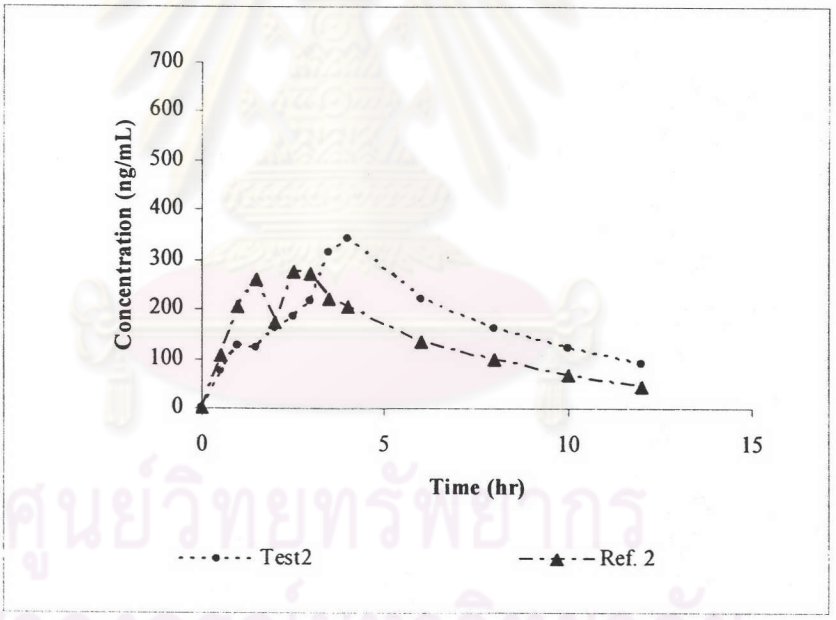
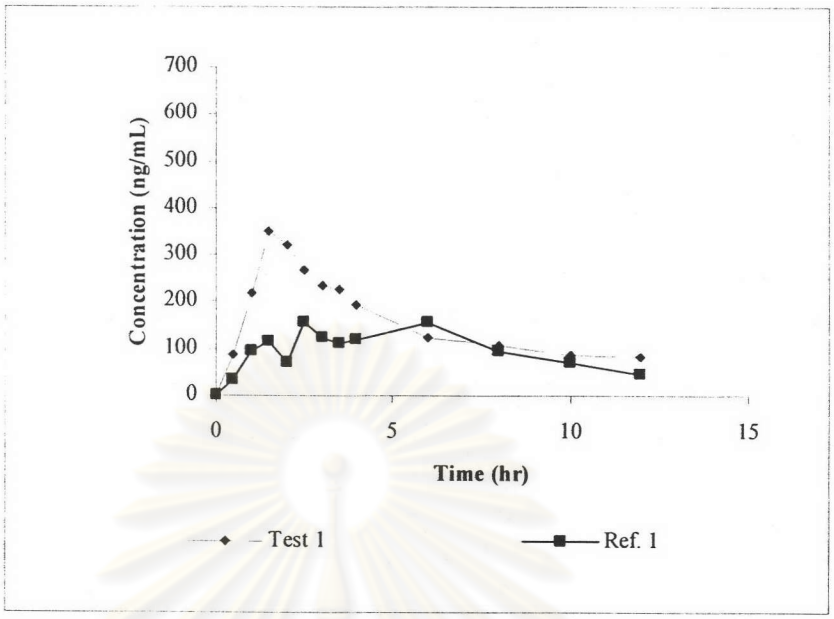


Figure 19 Plasma Glipizide Concentration-time Profiles of Subject No.6 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

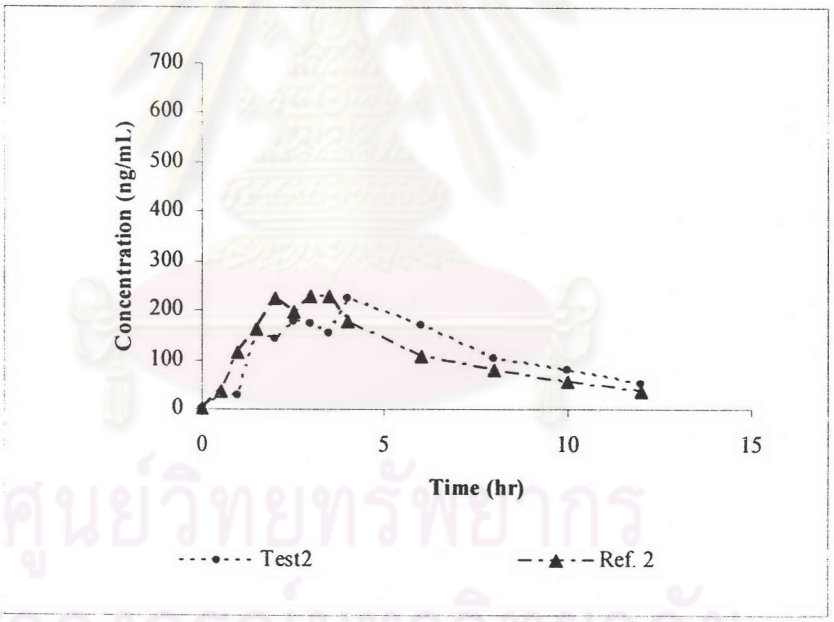
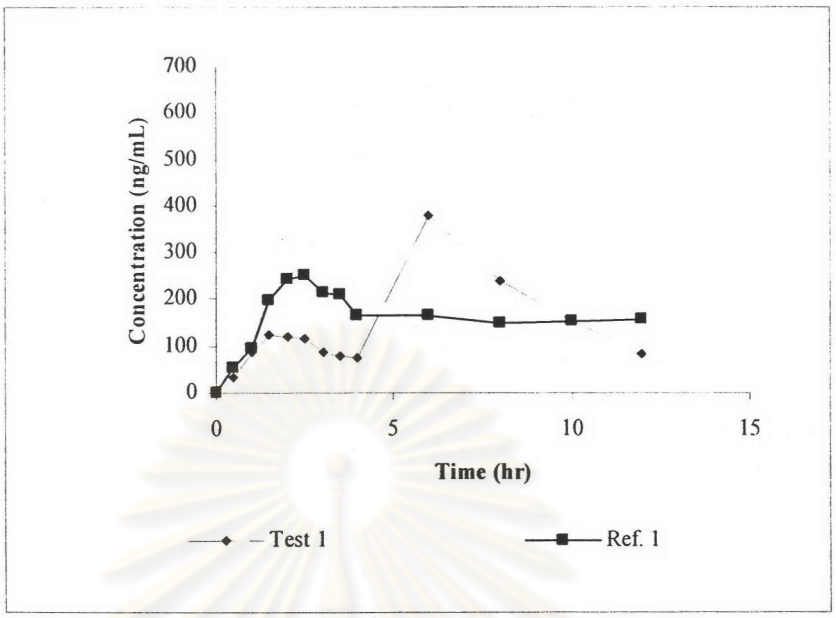


Figure 20 Plasma Glipizide Concentration-time Profiles of Subject No.7 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

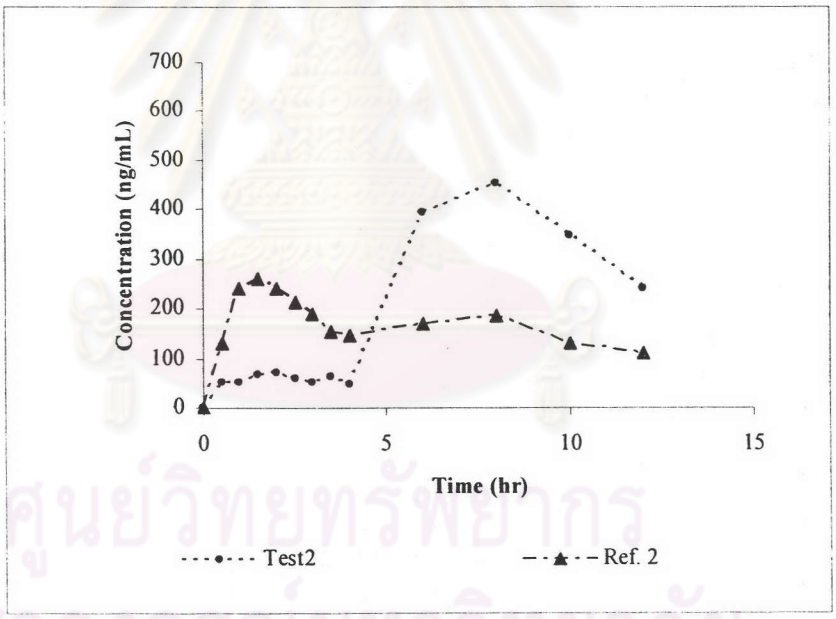
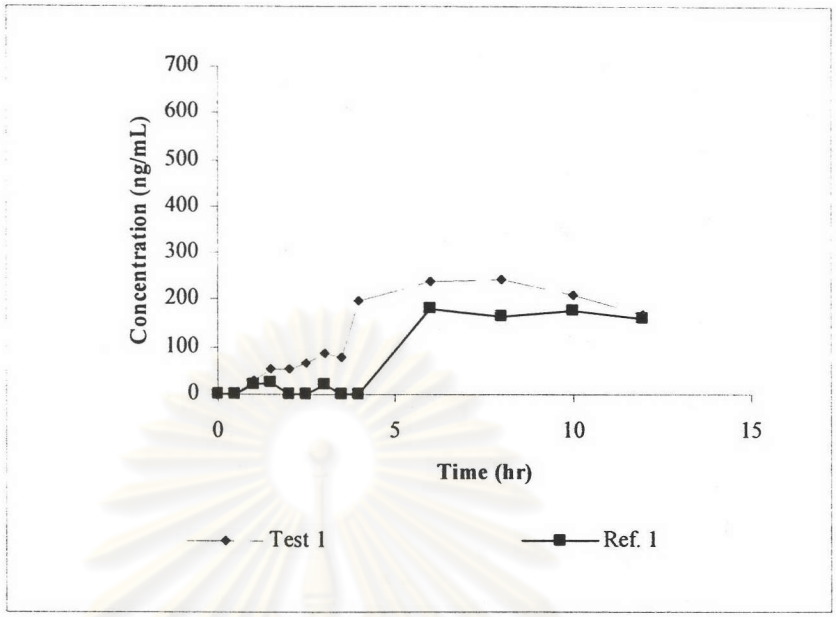


Figure 21 Plasma Glipizide Concentration-time Profiles of Subject No.8 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

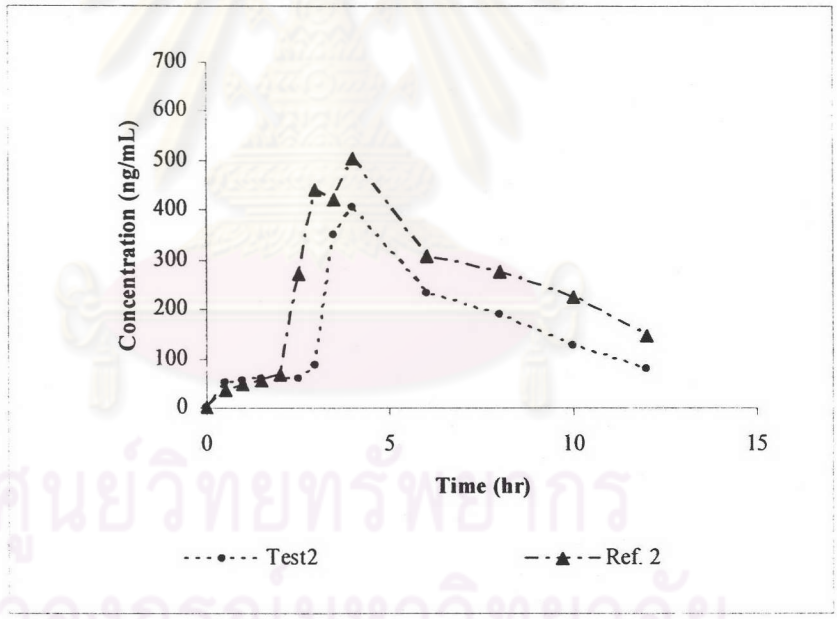
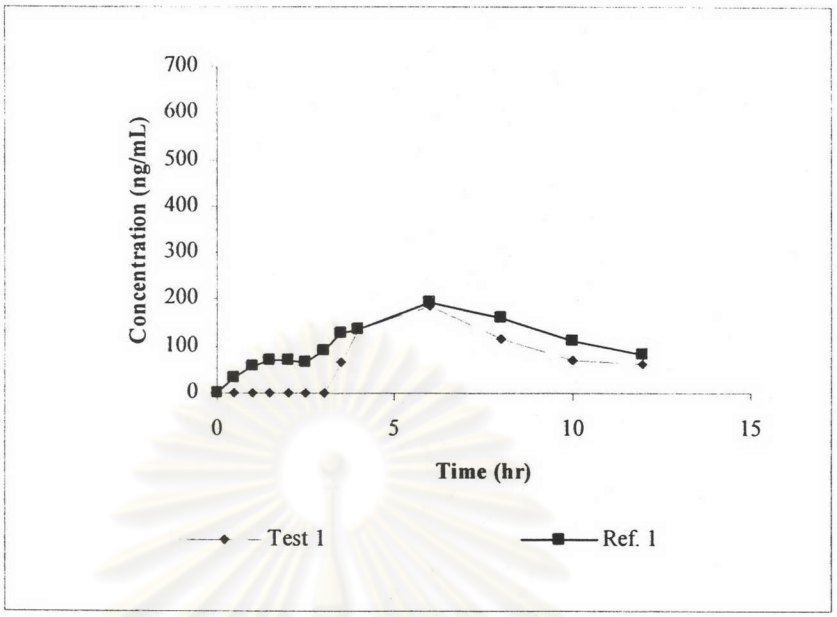


Figure 22 Plasma Glipizide Concentration-time Profiles of Subject No.9 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

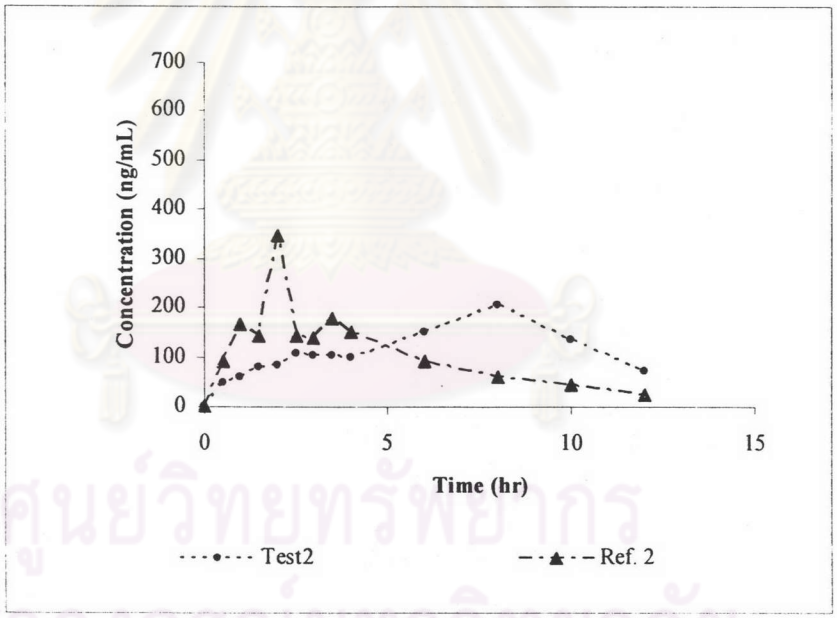
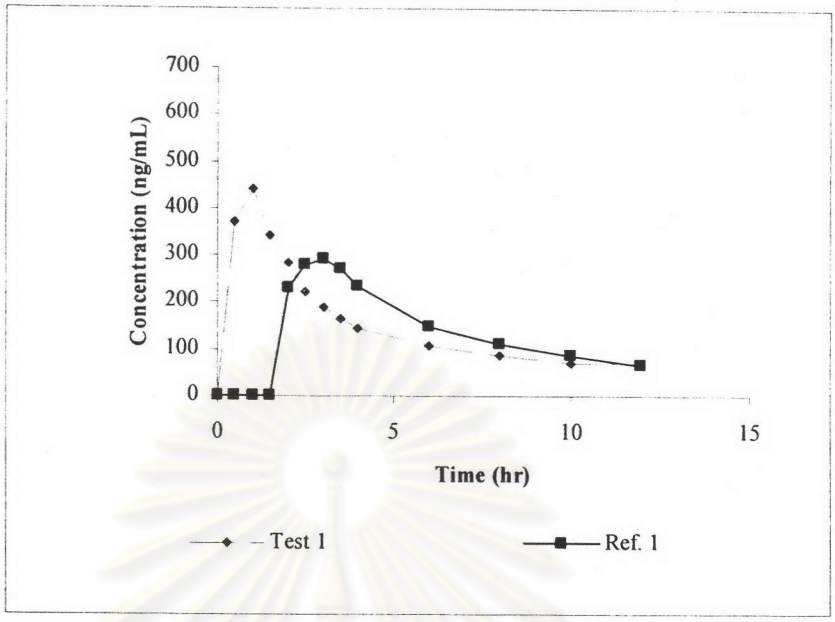


Figure 23 Plasma Glipizide Concentration-time Profiles of Subject No.10 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

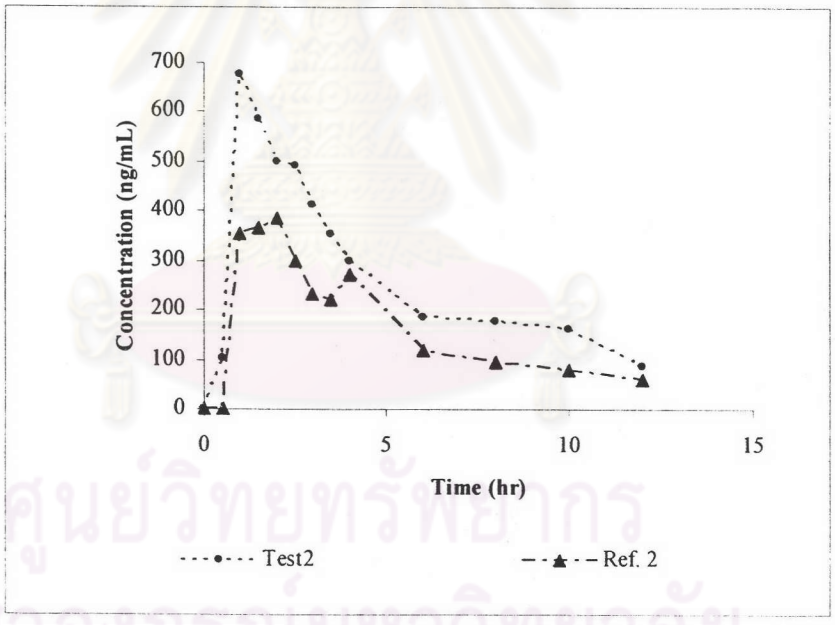
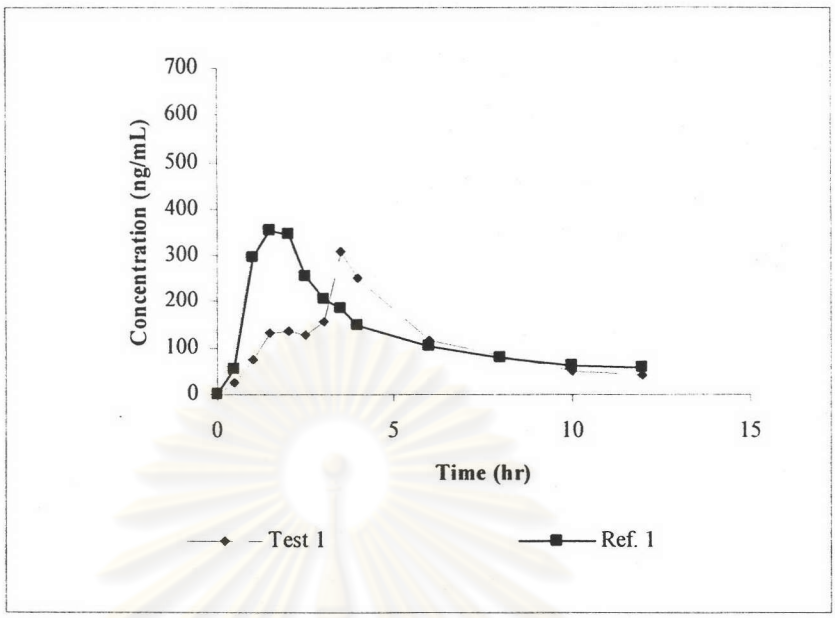


Figure 24 Plasma Glipizide Concentration-time Profiles of Subject No.11 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

A



B

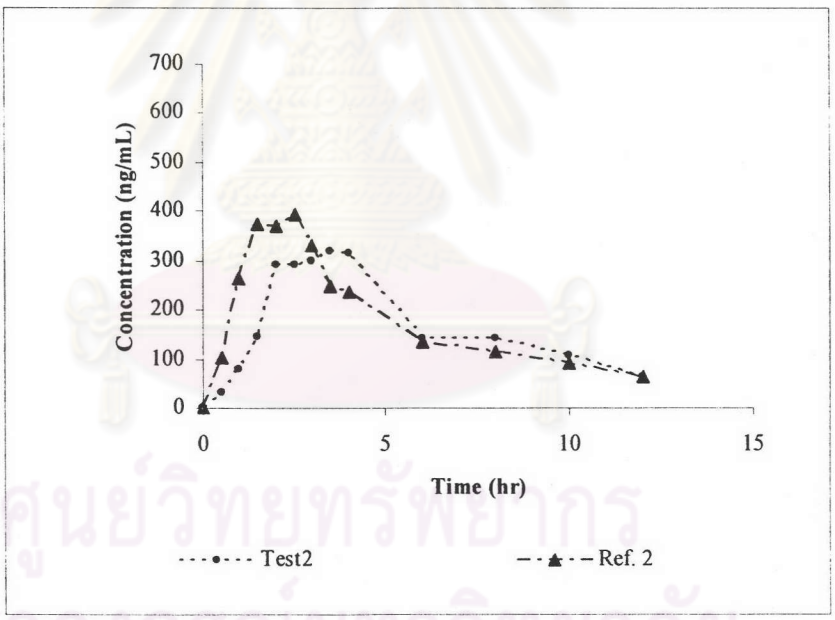


Figure 25 Plasma Glipizide Concentration-time Profiles of Subject No.12 Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Product

A = 1st Crossover Study

B = 2nd Crossover Study

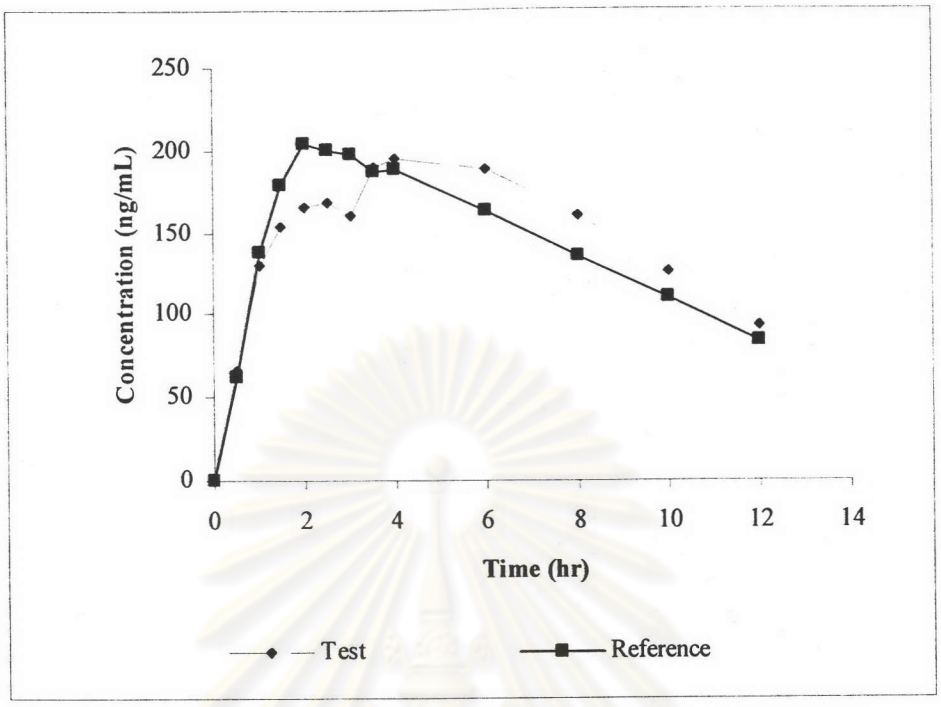


Figure 26 Mean Plasma Glipizide Concentration-time Profiles of 12 Subjects Following Oral Administration of 5mg Glipizide Tablets of Test and Innovator's Product

5. Pharmacokinetic Parameters

In this study, after oral administration of 5 mg glipizide tablet, the pharmacokinetics parameters were observed and calculated from plasma glipizide concentration-time curve. Principal pharmacokinetic parameters for bioequivalence evaluation. They are elaborated in detailed as follows:

5.1 Area Under the Plasma Concentration-time Curves (AUC)

The AUC_{0-12} and $AUC_{0-\infty}$ of all subjects for test and innovator's product are shown in Tables 28 and 29, respectively. As mentioned earlier, there are wide variations of plasma glipizide concentrations among subjects. The $AUC_{0-\infty}$ values obtained for both products are also affected. This is seen by the $AUC_{0-12}/AUC_{0-\infty}$ ratios of some subjects are less than 80%, reflecting the time for collecting blood samples were not complete. In general, the time of 3 $t_{1/2}$ of the drug is sufficient to obtain a well defined pharmacokinetic of particular drug. This finding suggests that for a drug with wide variations, collecting time for plasma sample should be extend and longer than 3 $t_{1/2}$ of the drug. After performing the second crossover study, this parameter appears to be normalized in all subjects resulted in regular values. In most cases, the ratios of $AUC_{0-12}/AUC_{0-\infty}$ are greater than 80%. The mean AUC_{0-12} values were 1797.44 and 1713.40 ng.hr./mL for test and innovator's product, respectively and those corresponding mean $AUC_{0-\infty}$ values were 2520.75 and 2553.72 ng.hr./mL. The AUC_{0-12} and $AUC_{0-\infty}$ of test product were similar to those of innovator's product. However, AUC_{0-12} of test product appeared to be slightly higher than those of innovator's product due to test product contains slightly on average more active moiety as seen in Table 4. Analysis of variance based on log-transformed data of AUC_{0-12} and $AUC_{0-\infty}$ are presented in Tables 32 and 33, respectively. Results reveal that the formulation effect shows no significant difference ($p>0.05$). This implies that the total amounts of drug absorption from both brands are equal. The interaction of subject and formulation is negligible.

5.2 Peak Plasma Concentration (C_{max})

The mean peak plasma concentration for test and innovator's product were 300.34 and 297.37 ng/mL, respectively as shown in Table 30. These values were

similar due to their similarity in percent content of active ingredient and uniformity of active ingredient in dosage units. Generally, this term is related to absorption of the drug which in turn affected by dissolution. In this study, dissolution of two brands are rapidly as presented in Tables 6 and 7. The drug was promptly available to be absorbed staring from the first 5 minutes. This suggests that absorption should be rate-limiting step. Both formulations could be absorbed with the same rate as seen the same C_{max} values are results. Analysis of variance as presented in Table 34 revealed the same results as seen in the case of AUCs.

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

The mean time to peak plasma concentration for test and innovator's product were 4.77 and 3.96 as shown in Table 31, respectively. The t_{max} value of test product was slightly higher than that of innovator's product. As seen from the data reported that the t_{max} values of both formulations are attained in approximately 4-6 hours. This implies that absorption of glipizide into blood circulation is quite slow, supporting rate controlling process as mentioned previously. The shorter t_{max} assessed after an innovator's product administration may be due to a better physicochemical properties of glipizide as well as a good grade of fillers being used in the formula. Analysis of variance of t_{max} in Table 35 presented that the formulation effect had showed no significant difference ($p>0.05$), demonstrating the two t_{max} values are equal.

Table 28 Area Under the Plasma Glipizide Concentration-time Curves from 0 to 12 hr (AUC_{0-12}) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	AUC_{0-12} (ng.hr/mL)			
	Test.1	Test.2	Ref.1	Ref.2
1	2292.51	4099.50	1681.61	2220.38
2	1329.01	1451.90	1330.50	2109.11
3	1098.23	1681.50	1201.67	1517.19
4	1473.33	431.40	1697.06	2790.21
5	1472.11	1658.47	1358.42	1589.27
6	1214.55	2121.02	1480.15	1656.56
7	1803.02	1453.31	1198.31	1329.77
8	2035.22	2890.75	1922.43	1972.28
9	1979.58	2044.33	1233.50	3056.86
10	998.32	1457.74	1443.70	1185.79
11	1787.11	3071.57	1590.21	1912.73
12	1333.81	1960.30	1569.09	2074.83
Arithmetic Mean	1797.44		1713.40	
S.D.	752.10		481.68	
%C.V.	41.84		28.11	
Min	431.40		1185.79	
Max	4099.50		3056.86	
Geometric Mean	1653.49		1657.51	
S.D.	1.54		1.29	

Table 29 Area Under the Plasma Glipizide Concentration-time Curves from 0 to Infinite Time ($AUC_{0-\infty}$) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	$AUC_{0-\infty}$ (ng.hr/mL)			
	Test.1	Test.2	Ref.1	Ref.2
1	3632.57	4975.54	2285.48	2650.80
2	1607.94	1778.10	1848.53	2483.28
3	3182.59	2343.00	2383.80	4327.23
4	1902.66	534.95	2125.04	3376.48
5	4666.31	2083.88	1685.46	2977.91
6	1646.10	2667.81	1709.71	1901.33
7	2367.69	1723.78	1448.63	1518.80
8	2359.43	4194.07	5509.61	2858.04
9	3575.75	2474.49	4654.61	3750.49
10	1336.71	1729.03	1938.95	1294.06
11	2205.44	3616.90	1974.06	2271.13
12	1583.95	2309.28	1889.08	2426.73
Arithmetic Mean	2520.75		2553.72	
S.D.	1110.88		1073.33	
%C.V.	43.67		42.03	
Min	534.95		1294.06	
Max	4975.54		5509.61	
Geometric Mean	2283.40		2375.96	
S.D.	1.61		1.46	

Table 30 Peak Plasma Glipizide Concentrations (C_{max}) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	C_{max} (ng/mL)			
	Test.1	Test.2	Ref.1	Ref.2
1	303.50	513.44	248.12	394.65
2	213.77	287.75	193.04	532.99
3	131.30	230.55	173.27	217.46
4	265.10	115.31	345.47	499.80
5	181.14	196.86	209.88	198.57
6	238.14	342.50	301.26	274.63
7	347.97	223.41	158.42	229.41
8	380.61	452.61	250.12	258.53
9	243.51	404.68	181.32	504.93
10	184.27	204.13	193.13	346.69
11	441.79	675.50	290.99	385.66
12	310.61	319.73	355.71	392.76
Arithmetic Mean	300.34		297.37	
S.D.	129.96		110.17	
%C.V.	43.27		37.05	
Min	115.31		158.42	
Max	675.50		532.99	
Geometric Mean	275.93		279.36	
S.D.	1.52		1.43	

Table 31 Time to Peak Plasma Glipizide Concentrations (t_{max}) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	t_{max} (hr)			
	Test.1	Test.2	Ref.1	Ref.2
1	4	4	4	4
2	2	3.5	6	2
3	8	10	8	10
4	2	2.5	2	2.5
5	8	6	6	6
6	6	4	1.5	2.5
7	1.5	4	6	3.5
8	6	8	2.5	1.5
9	8	4	6	4
10	6	8	6	2
11	1	1	3	2
12	3.5	3.5	1.5	2.5
Arithmetic Mean	4.77		3.96	
S.D.	2.58		2.29	
%C.V.	54.06		57.94	
Min	1.00		1.50	
Max	10.00		10.00	

Table 32 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Ln Area Under the Plasma Glipizide Concentration-time Curves from 0 to 12 hr (Ln AUC_{0-12}) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Ln AUC_{0-12} Means

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	5.84890	-	-	-	-
Sequence	1	0.14196	0.14196	0.7617	4.96	NS
Subject (Sequence)	10	1.86380	0.18638	2.2401	2.30	NS
Period	3	0.95543	0.31848	3.8277	3.05	S
Formulation	1	0.00007	0.00007	0.0009	4.30	NS
Subj. x Form. (Seq.)	10	1.05720	0.10572	1.2706	2.30	NS
Error	22	1.83050	0.08320	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
S = Significant difference at $p < 0.05$
NS = Not significant difference at $p > 0.05$

Product	Mean Ln AUC_{0-12}	90% Confidence Interval
Test (T)	7.4106	86.5-115.1
Innovator's (R)	7.4131	
T/R	0.9976	

Table 33 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Ln Area Under the Plasma Glipizide Concentration-time Curves from 0 to Infinite Time (Ln $AUC_{0-\infty}$) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Ln $AUC_{0-\infty}$ Means

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	8.54470	-	-	-	-
Sequence	1	0.98933	0.98933	3.2993	4.96	NS
Subject (Sequence)	10	2.99860	0.29986	2.5819	2.30	S
Period	3	0.66135	0.22045	1.8981	3.05	NS
Formulation	1	0.01895	0.01895	0.1631	4.30	NS
Subj. x Form. (Seq.)	10	1.32130	0.13213	1.1376	2.30	NS
Error	22	2.55520	0.11614	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
S = Significant difference at $p < 0.05$
NS = Not significant difference at $p > 0.05$

Product	Mean Ln $AUC_{0-\infty}$	90% Confidence Interval
Test (T)	7.7334	81.2-113.8
Innovator's (R)	7.7732	
T/R	0.9610	

Table 34 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Ln Peak Plasma Glipizide Concentrations (Ln C_{max}) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products and 90% Confidence Interval for the Ratio of Ln C_{max} Means

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	7.03640	-	-	-	-
Sequence	1	0.03966	0.03966	0.1445	4.96	NS
Subject (Sequence)	10	2.74450	0.27445	3.3234	2.30	S
Period	3	1.16070	0.38690	4.6850	3.05	S
Formulation	1	0.00183	0.00183	0.0222	4.30	NS
Subj. x Form. (Seq.)	10	1.27290	0.12729	1.5414	2.30	NS
Error	22	1.81680	0.08258	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
S = Significant difference at $p < 0.05$
NS = Not significant difference at $p > 0.05$

Product	Mean Ln C_{max}	90% Confidence Interval
Test (T)	5.6202	85.7-113.9
Innovator's (R)	5.6325	
T/R	0.9877	

Table 35 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Time to Peak Plasma Glipizide Concentrations (t_{max}) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products and Their Differences

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	281.870	-	-	-	-
Sequence	1	24.7970	24.7970	1.6062	4.96	NS
Subject (Sequence)	10	154.390	15.4390	7.5566	2.30	S
Period	3	16.2240	5.40800	2.6470	3.05	NS
Formulation	1	7.92190	7.92190	3.8774	4.30	NS
Subj. x Form. (Seq.)	10	33.5940	3.35940	1.6443	2.30	NS
Error	22	44.9480	2.04310	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
S = Significant difference at $p < 0.05$
NS = Not significant difference at $p > 0.05$

Difference of t_{max} means = (4.77-3.96) x 100/3.96
= 20.45%

5.4 Related Pharmacokinetic Parameters

The pharmacokinetic parameters such as V_d/F, CL/F, MRT, K_e, and $t_{1/2}$ were shown in Tables 36 to 40, respectively and summarized in Table 46. Analysis of variance of these parameters are presented in Tables 41 to 45. The V_d/F, CL/F, MRT, K_e, and $t_{1/2}$ values of both formulations show no statistical difference ($p>0.05$) with respect to their formulations which is the principal source to be concerned.

Table 36 Apparent Volume of Distribution/ Fraction of Dose to be Absorbed (V_d/F) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	V_d/F(L)			
	Test.1	Test.2	Ref.1	Ref.2
1	13.7109	5.9235	14.2217	9.9155
2	18.8276	16.6116	18.2686	9.3483
3	30.6213	9.5660	21.2415	17.0927
4	17.3493	43.6151	14.0170	9.9744
5	22.8025	11.8343	14.3035	18.1262
6	16.6346	11.4222	15.6088	14.2505
7	14.5651	15.1542	18.7584	17.2427
8	8.1484	6.4600	20.9985	13.8877
9	13.2528	11.0498	22.9335	6.3363
10	20.1006	10.9999	15.4538	18.1103
11	13.9347	8.7364	15.2059	13.0460
12	18.2699	12.2304	15.1835	11.8919
Arithmetic Mean	15.49		15.23	
S.D.	8.13		3.99	
%C.V.	52.46		26.21	
Min	5.9235		6.3363	
Max	43.6153		22.9335	

Table 37 Clearance/ Fraction of Dose to be Absorbed (CL/F) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	CL/F (L/hr)			
	Test.1	Test.2	Ref.1	Ref.2
1	1.3764	1.0049	2.1877	1.8862
2	3.1096	2.8120	2.7049	2.0135
3	1.5710	2.1340	2.0975	1.1555
4	2.6279	9.3467	2.3529	1.4808
5	1.0715	2.3994	2.9665	1.6790
6	3.0375	1.8742	2.9245	2.6297
7	2.1118	2.9006	3.4515	3.2921
8	2.1192	1.1922	0.9075	1.7495
9	1.3983	2.0206	1.0742	1.3332
10	3.7405	2.8918	2.5787	3.8638
11	2.2671	1.3824	2.5329	2.2015
12	3.1567	2.1652	2.6468	2.0604
Arithmetic Mean	2.49		2.24	
S.D.	1.64		0.76	
%C.V.	65.85		34.14	
Min	1.0049		0.9075	
Max	9.3467		3.8638	

Table 38 Mean Residence Time (MRT) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	MRT (hr)			
	Test.1	Test.2	Ref.1	Ref.2
1	11.8843	7.3601	9.4374	7.7206
2	7.1260	7.5519	9.6373	5.4431
3	23.0664	9.6613	14.6513	20.1714
4	8.0297	6.6024	7.3715	7.3584
5	25.0512	8.2656	8.3787	14.3730
6	10.0225	8.1532	5.9617	6.3112
7	8.1509	7.3139	7.6961	6.3238
8	7.8796	10.7669	24.9708	10.0441
9	13.6093	8.0022	26.7201	8.0829
10	9.6061	8.1068	9.4537	5.3394
11	6.7686	6.6327	7.9316	6.5936
12	7.0343	7.0826	6.6110	6.4430
Arithmetic Mean	9.74		10.13	
S.D.	4.75		5.92	
%C.V.	48.73		58.42	
Min	6.6024		5.3394	
Max	25.0512		26.7201	

Table 39 Elimination Rate Constant (K_e) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	K_e (hr^{-1})			
	Test.1	Test.2	Ref.1	Ref.2
1	0.1004	0.1696	0.1538	0.1902
2	0.1652	0.1693	0.1481	0.2154
3	0.0513	0.2231	0.0987	0.0676
4	0.1515	0.2143	0.1679	0.1485
5	0.0470	0.2027	0.2074	0.0926
6	0.1826	0.1641	0.1874	0.1845
7	0.1450	0.1914	0.1840	0.1909
8	0.2601	0.1845	0.0432	0.1260
9	0.1055	0.1829	0.0468	0.2104
10	0.1861	0.2629	0.1669	0.2133
11	0.1627	0.1582	0.1666	0.1688
12	0.1728	0.1770	0.1743	0.1733
Arithmetic Mean	0.17		0.16	
S.D.	0.05		0.05	
%C.V.	31.23		32.71	
Min	0.0470		0.0432	
Max	0.2629		0.2154	

Table 40 Elimination Half-Life ($t_{1/2}$) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Subject no.	$t_{1/2}$ (hr)			
	Test.1	Test.2	Ref.1	Ref.2
1	6.9031	4.0849	4.5050	3.6430
2	4.1959	4.0938	4.6805	3.2175
3	13.5073	3.1065	7.0181	10.2514
4	4.5752	3.2338	4.1284	4.6678
5	14.7475	3.4181	3.3414	7.4814
6	3.7952	4.2234	3.6988	3.7553
7	4.7797	3.6206	3.7663	3.6297
8	2.6647	3.7552	16.0351	5.5013
9	6.5681	3.7897	14.7950	3.2937
10	3.7240	2.6360	4.1530	3.2482
11	4.2595	4.3796	4.1604	4.1066
12	4.0109	3.9145	3.9755	3.9998
Arithmetic Mean	4.92		5.46	
S.D.	3.01		3.47	
%C.V.	61.14		63.50	
Min	2.6360		3.2175	
Max	14.7475		16.0351	

Table 41 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Appearant Volume of Distribution/ Fraction of Dose to be Absorbed (V_d/F) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	1886.50	-	-	-	-
Sequence	1	6.81490	6.81490	0.1776	4.96	NS
Subject (Sequence)	10	383.800	38.3800	1.0806	2.30	NS
Period	3	253.370	84.4550	2.3779	3.05	NS
Formulation	1	0.85473	0.85473	0.0241	4.30	NS
Subj. x Form. (Seq.)	10	460.280	46.0280	1.2959	2.30	NS
Error	22	781.370	35.5170	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
NS = Not significant difference at $p > 0.05$

Table 42 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Clearance/ Fraction of Dose to be Absorbed (CL/F) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	75.9220	-	-	-	-
Sequence	1	7.12300	7.12300	3.7234	4.96	NS
Subject (Sequence)	10	19.1310	1.91310	1.4083	2.30	NS
Period	3	4.43570	1.47860	1.0885	3.05	NS
Formulation	1	0.73525	0.73525	0.5413	4.30	NS
Subj. x Form. (Seq.)	10	14.6120	1.46120	1.0757	2.30	NS
Error	22	29.8850	1.35840	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
NS = Not significant difference at $p > 0.05$

Table 43 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Mean Residence Time (MRT) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	1324.60	-	-	-	-
Sequence	1	104.840	104.840	2.2640	4.96	NS
Subject (Sequence)	10	463.090	46.3090	2.4001	2.30	S
Period	3	180.080	60.0270	3.1110	3.05	S
Formulation	1	1.80080	1.80080	0.0933	4.30	NS
Subj. x Form. (Seq.)	10	150.330	15.0330	0.7791	2.30	NS
Error	22	424.490	19.2950	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
S = Significant difference at $p < 0.05$
NS = Not significant difference at $p > 0.05$

Table 44 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Elimination Rate Constant (K_e) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	0.12450	-	-	-	-
Sequence	1	0.01085	0.01085	6.1964	4.96	S
Subject (Sequence)	10	0.01751	0.00175	0.7848	2.30	NS
Period	3	0.02336	0.00779	3.4919	3.05	S
Formulation	1	0.00192	0.00192	0.8610	4.30	NS
Subj. x Form. (Seq.)	10	0.02179	0.00218	0.9771	2.30	NS
Error	22	0.04907	0.00223	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
S = Significant difference at $p < 0.05$
NS = Not significant difference at $p > 0.05$

Table 45 Analysis of Variance for a Replicated Crossover Design at $\alpha = 0.05$ of Elimination Half-Life ($t_{1/2}$) of Glipizide of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products

Source of Variation	d.f.	SS	MS	F-ratio	F-table	Significance Level
Total	47	487.890	-	-	-	-
Sequence	1	28.9430	28.9430	2.7838	4.96	NS
Subject (Sequence)	10	103.970	10.3970	1.1676	2.30	NS
Period	3	78.3400	26.1130	2.9326	3.05	NS
Formulation	1	3.55670	3.55670	0.3994	4.30	NS
Subj. x Form. (Seq.)	10	77.1850	7.71850	0.8668	2.30	NS
Error	22	195.900	8.90460	-	-	-

Where; Subj. x Form. (Seq.) = Subject x Formulations (Sequence)
d.f. = Degree of freedom
SS = Sum of squares
MS = Mean square
NS = Not significant difference at $p > 0.05$

Table 46 Summary of Pharmacokinetic Parameters of Glipizide ($\overline{X} \pm$ S.D.) of 12 Subjects Following Oral Administration of 5 mg Glipizide Tablets of Test and Innovator's Products in a Replicated Crossover Design

Parameters	Product		90% Confidence Interval
	Test Product	Innovator's Product	
AUC_{0-12} (ng.hr/mL)	1797.44 ± 752.10	1713.40 ± 481.68	86.5-115.1
$AUC_{0-\infty}$ (ng.hr/mL)	2520.75 ± 1110.88	2553.72 ±1073.33	81.2-113.8
C_{max} (ng/mL)	300.34 ± 129.96	297.37 ± 110.17	85.7-113.9
t_{max} (hr)	4.77 ± 2.58	3.96 ± 2.29	-
V_d/F (L)	15.49 ± 8.13	15.23 ± 3.99	-
CL/F (L/hr)	2.49 ± 1.64	2.24 ± 0.76	-
MRT (hr)	9.74 ± 4.75	10.13 ± 5.92	-
K_e (hr^{-1})	0.17 ± 0.05	0.16 ± 0.05	-
$t_{1/2}$ (hr)	4.92 ± 3.01	5.46 ± 3.47	-

6. Bioequivalence Study

At present, only average bioequivalence study estimates are used to establish bioequivalence of a generic drug products relative to an innovator's product in Thailand. The purpose of a bioequivalence study is to compare bioavailability between two formulations of the same drug with respect to the rate and extent of absorption. AUC is used as a measure of the extent of absorption, whereas C_{max} and t_{max} are used as an indication of drug absorption rate.

Two drug-products that are pharmaceutically equivalent are accepted to be bioequivalent when the ratios of the average AUC and C_{max} values based on log-transformed data of test product relative to innovator's product are contained within 80-125% of 90% confidence interval. Regarding analysis of t_{max}, the difference between untransformed data of test product and innovator's product are calculated but it is not taken to be considerably inclusive for bioequivalence decision.

In this study, bioequivalence analysis after log-transformed data of AUC_{0-12} and $AUC_{0-\infty}$ showed that 90% confidence interval of ratios for the two values of test product to innovator's product were 86.5-115.1% and 81.2-113.8%, respectively. These values lied within the bioequivalence criteria of 80-125% as shown in Tables 32 and 33. Therefore, the results could be considered that the test product was bioequivalent to the innovator's product with respect to the extent of drug absorption. According to the log-transformed data of C_{max}, the comparison of the test product versus innovator's product resulted in 90% confidence intervals of 85.7-113.9% as presented in Table 34, which showed that the test product was bioequivalent with the innovator's product within the acceptance criteria of 80-125%, regarding to the rate of drug absorption.

The difference of t_{max} values of test and innovator's product were 20.45%. However, this parameter was not essential for evaluation of bioequivalence study.

Replicated crossover design is successfully proven to be a suitable mean for conducting the bioequivalence of a high variability drug-product in individual like glipizide.

7. Comparison of Pharmacokinetic Parameters of Glipizide

The pharmacokinetic parameters obtained from this study were compared to those of previous studies. The comparisons are presented in Table 47.

Comparative of pharmacokinetic parameters of glipizide in healthy Thai male volunteers found in this experiment agreed and closed to those previously reported.

Table 47 Comparison of Pharmacokinetic Parameters

Parameters	References	Values
AUC_{0-12} (ng.hr/mL)	Present Study* Zmeili et al. (1995)	1713.4-1797.4 2169.9-2278.8
$AUC_{0-\infty}$ (ng.hr/mL)	Present Study* Kobylinska et al. (2000)	2520.8-2553.7 2011.5-2126.1
C_{max} (ng/mL)	Present Study* McEvoy (2001) Balant (1981); Zmeili et al. (1995); Kobylinska et al. (2000)	297-300 310-450 450-500
t_{max} (hr)	Present Study* Brogden et al. (1979); Balant (1981); Marchetti et al. (1989); Zmeili et al. (1995); Kobylinska et al. (2000); McEvoy (2001)	4-4.8 1-3
V_d (L)	Present Study* Brogden et al. (1979)	15.2-15.5 11.5-25
CL (L/hr)	Present Study* Brogden et al. (1979); McEvoy (2001)	2.2-2.5 2.4-3.0
K_e (hr^{-1})	Present Study* Zmeili et al. (1995); Kobyliska et al. (2000)	0.16-0.17 0.2-0.25
$t_{1/2}$ (hr)	Present Study* McEvoy (2001)	4.9-5.5 3-4.7

*Data of present study are the mean value of test and innovator's product.