CHAPTER IV

RESULTS AND DISCUSSION

A. Formulation development and *in vitro* evaluation

1. Formulation development

1.1 Preliminary study

One hundred and twenty milligram diltiazem hydrochloride controlled release tablet was formulated and pressed using hydraulic press. Diameter of punch was 1.21 cm. Weight of tablet was approximately 375 mg. Hardness of tablets were in the range of 6-8 kps. Tablets obtained were white and flat-faced round. Thickness of tablet in preliminary study ranged between 2.80-3.25 mm depended on types of filler. Thickness of tablet with Tablettose® was greater than that with Emcompress® because the density of Tablettose® powder was less than that of Emcompress® (Wade and Weller, 1994). The weight variations for all formulations were in the range of 365-380 mg. The average weight variations and hardness of each formulation were summarized in Table 4. Percent drug content of diltiazem hydrochloride matrixs in each formulation was presented in Table 5. Contents of some formulations were less than 100%. This might be due to weight of tablet was less than 375 mg which resulted in less drug content. However, the average drug contents were in the range of 90-110% and conformed general specifications as specified in the USP 24.

For dissolution test, drug release of each formulation was tested up to 12 hours in phosphate buffer pH 7.2 (U.S.P. 24, 2000). The release profile of test formulation was compared to Cardil® using difference factor and similarity factor. The release profiles of the drug and the data of their average percent drug release in phosphate buffer pH 7.2 were shown in Figure 4 and Table 6, respectively. It was found that the release rate of diltiazem hydrochloride decreased as polymer concentration increased. This might be

due to an increase in viscosity of the hydrate layer as polymer concentration was raised. This result agreed with previous report that the more content of polymer incorporated, the less of drug release would occured (Vinny and Joel, 1993). At low polymer concentration (10% mixed polymers), diltiazem hydrochloride released from tablet containing Emcompress® was significantly less than that with Tablettose® because Emcompress® was an insoluble excipient whereas Tablettose® was a soluble excipient. At higher polymer concentration (20% and 30% mixed polymers), drug release from tablets with both excipients was similar. This might be due to effect of mixed polymer was more predominant than that obtained from types of excipient. Difference factor calculated for diltiazem hydrochloride tablets with 0% polymer in Emcompress® (0% P/E), 5% HPMC in Emcompress® (5% H/E), 5% XG in Emcompress® (5% X/E), 10% mixed polymers in Emcompress® (10% P/E), 20% mixed polymers in Emcompress® (20% P/E), 30% mixed polymers in Emcompress® (30% P/E), 0% polymer in Tablettose® (0% P/T), 5% HPMC in Tablettose® (5% H/T), 5% XG in Tablettose® (5% X/T), 10% mixed polymers in Tablettose® (10% P/T), 20% mixed polymers in Tablettose® (20% P/T) and 30% mixed polymers in Tablettose® (30% P/T) vs Cardil® were 180.68, 165.04, 58.90, 26.47, 11.17, 32.21, 441.51, 414.63, 209.93, 153.32, 5.06 and 28.34 %, respectively whereas similarity factor of these corresponding formulations vs Cardil® were 13.23, 19.20, 36.98, 50.39, 70.31, 48.55, 11.41, 12.61, 14.21, 16.09, 81.94 and 50.72 %, respectively as shown in Table 7.

From this result as shown in Figure 4 and Table 7, tablets with 10% P/E, 20% P/E and 20% P/T were selected based on similarity values as well as less amount of polymer being used to test in scale up study

Table 4. Weight variation and hardness of diltiazem hydrochloride matrix tablets during preliminary study (n=20)

Formulations	Weight variation (mg) (Average ± S.D.)	Hardness (kp) (Average ± S.D.)
0% P/T	379.45 ± 3.56	6.89 ± 0.49
0% P/E	369.22 ± 4.79	6.97 ± 0.25
5% H/T	378.93 ± 5.01	7.23 ± 0.35
5% H/E	367.89 ± 4.68	7.46 ± 0.31
5% X/T	373.67 ± 3.46	7.04 ± 0.48
5% X/E	371.89 ± 4.01	7.29 ± 0.30
10% P/T	376.79 ± 3.89	7.18 ± 0.32
10% P/E	370.87 ± 4.35	7.65 ± 0.47
20% P/T	369.98 ± 4.13	7.72 ± 0.52
20% P/E	375.87 ± 4.23	6.93 ± 0.30
30% P/T	378.49 ± 3.83	7.07 ± 0.35
30% P/E	376.30 ± 3.72	7.14 ± 0.47

Table 5. Percent drug contents of diltiazem hydrochloride matrix tablets of each formulation during preliminary study (n=3)

Formulations	% Drug content	Formulations	% Drug content
0% P/T	103.69 ± 3.29	10% P/T	100.45 ±1.23
0% P/E	94.47 ± 2.41	10% P/E	98.83 ± 2.89
5% H/T	102.38 ± 3.17	20% P/T	96.79 ± 2.75
5% H/E	95.98 ±3.06	20% P/E	101.28 ± 3.36
5% X/T	99.05 ± 2.90	30% P/T	102.93 ± 3.28
5% X/E	99.29 ±1.58	30% P/E	99.78 ± 3.15

Table 6. Average percent drug release of each formulation in phosphate buffer pH 7.2 during preliminary study (n=6)

Time	Cardil®			0% P/E			0% P/T			5% H/E			5% H/T			5% X/E		
(hr.)	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.
0.25	8.40	0.23	2.80	40.12	1.81	4.51	71.82	2.15	2.99	22.42	2.05	9.16	55.13	6.82	12.37	15.89	2.65	16.66
0.5	11.77	0.20	1.68	55.21	2.06	3.73	102.61	3.64	3.55	35.80	2.36	6.59	94.89	4.70	4.95	21.78	2.75	12.61
0.75	14.69	0.19	1.32	62.86	2.95	4.69	103.91	2.97	2.86	40.37	2.36	5.83	102.04	2.68	2.62	26.36	2.08	7.87
1	16.15	0.38	2.36	68.74	1.40	2.04	103.31	2.53	2.45	50.16	1.87	3.73	101.51	3.84	3.78	30.28	1.79	5.91
2	21.03	0.42	2.02	83.84	1.13	1.34	102.83	3.64	3.54	62.35	2.82	4.52	100.94	2.98	2.95	39.96	0.47	1.18
3	25.17	0.69	2.72	90.91	2.74	3.01	101.85	3.29	3.23	75.48	2.79	3.70	99.63	3.18	3.19	46.03	0.91	1.98
4	30.01	1.00	3.32	92.92	1.64	1.77	100.43	2.78	2.77	83.31	3.26	3.92	98.53	2.96	3.00	48.89	1.57	3.21
5	33.94	1.09	3.22	93.67	1.50	1.60	-	-	-	90.62	2.27	2.50	-	-	-	53.30	1.25	2.35
6	37.76	1.07	2.83	96.52	1.24	1.29	-	-	-	94.71	2.83	2.99	-	-	-	54.69	2.45	4.49
7	40.83	1.12	2.75	100.02	1.15	1.15	-	-	-	96.29	2.86	2.97	-	-	-	58.12	2.13	3.67
8	43.63	1.34	3.08	99.85	1.23	1.23	-	-	-	97.18	2.70	2.78	-	-	-	65.42	2.57	3.92
10	49.60	1.36	2.75	98.75	2.56	2.59	-	-	-	-	-	-	-	-	-	72.36	3.54	4.90
12	53.61	0.97	1.80	98.23	3.75	3.82	-	-	-	-	-	-	-	-	-	79.26	2.30	2.90

where 0% P/E = Emcompress® formulation without polymer

0% P/T = Tablettose® formulation without polymer

5% H/E = 5% HPMC in Emcompress® formulation

5% H/T = 5% HPMC in Tablettose® formulation

5% X/E = 5% XG in Emcompress® formulation

Table 6. Average percent drug release of each formulation in phosphate buffer pH 7.2 during preliminary study (n=6) (cont.)

Time	10% P/E			20% P/E			30% P/E			10% P/T			20% P/T			30% P/T		
(hr.)	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.
0.25	6.90	0.43	6.23	5.63	0.25	4.49	5.18	0.03	0.49	24.75	0.13	0.51	8.00	0.18	2.21	5.09	0.20	3.97
0.5	11.90	1.34	11.25	8.61	0.03	0.29	7.65	0.03	0.33	45.36	1.46	3.23	11.74	0.56	4.73	8.16	0.10	1.24
0.75	15.04	0.98	6.55	11.11	0.08	0.68	9.41	0.25	2.68	47.61	1.31	2.76	14.31	0.66	4.59	9.08	0.13	1.39
1	17.74	0.86	4.84	13.45	0.10	0.75	10.79	0.38	3.51	55.51	1.62	2.91	16.75	0.83	4.97	12.52	0.71	5.65
2	26.51	0.35	1.33	20.04	0.10	0.50	14.91	0.51	3.39	83.09	1.82	2.19	21.11	0.51	2.39	16.26	0.67	4.13
3	33.04	0.20	0.61	24.79	0.45	1.83	18.79	0.78	4.17	92.37	1.82	1.97	26.65	0.86	3.22	18.87	0.69	3.68
4	38.90	0.40	1.04	28.40	0.71	2.49	21.04	1.21	5.76	92.23	0.40	0.44	30.76	0.61	1.97	24.65	1.46	5.94
5	42.86	0.56	1.30	31.01	1.36	4.40	23.01	1.16	5.05	92.51	2.02	2.18	34.61	0.61	1.75	25.01	1.36	5.45
6	46.15	0.05	0.11	34.08	1.77	5.19	25.29	1.67	6.59	90.16	0.10	0.11	36.36	1.06	2.92	27.40	1.82	6.64
7	49.69	0.00	0.00	37.26	3.13	8.41	27.11	1.21	4.47	89.80	0.20	0.22	38.19	1.01	2.65	29.44	2.17	7.38
8	58.87	0.71	1.20	37.83	2.63	6.94	28.86	1.36	4.72	88.87	0.51	0.57	39.94	1.06	2.66	30.47	2.53	8.29
10	63.73	1.11	1.74	43.73	4.34	9.93	32.26	1.31	4.07	88.01	0.51	0.57	46.94	1.21	2.58	33.44	3.18	9.52
12	73.09	1.01	1.38	46.37	4.85	10.46	36.94	0.81	2.19	85.94	0.81	0.94	48.51	1.41	2.92	35.76	3.54	9.89

where 5% X/T = 5% XG in Tablettose® formulation

10% P/E = 10% mixed polymers (5% HPMC and 5% XG) in Emcompress® formulation

10% P/T = 10% mixed polymers (5% HPMC and 5% XG) in Tablettose® formulation

20% P/E = 20% mixed polymers (10% HPMC and 10% XG) in Emcompress® formulation

20% P/T = 20% mixed polymers (10% HPMC and 10% XG) in Tablettose® formulation

30% P/E = 30% mixed polymers (15% HPMC and 15% XG) in Emcompress® formulation

30% P/T = 30% mixed polymers (15% HPMC and 15% XG) in Tablettose® formulation

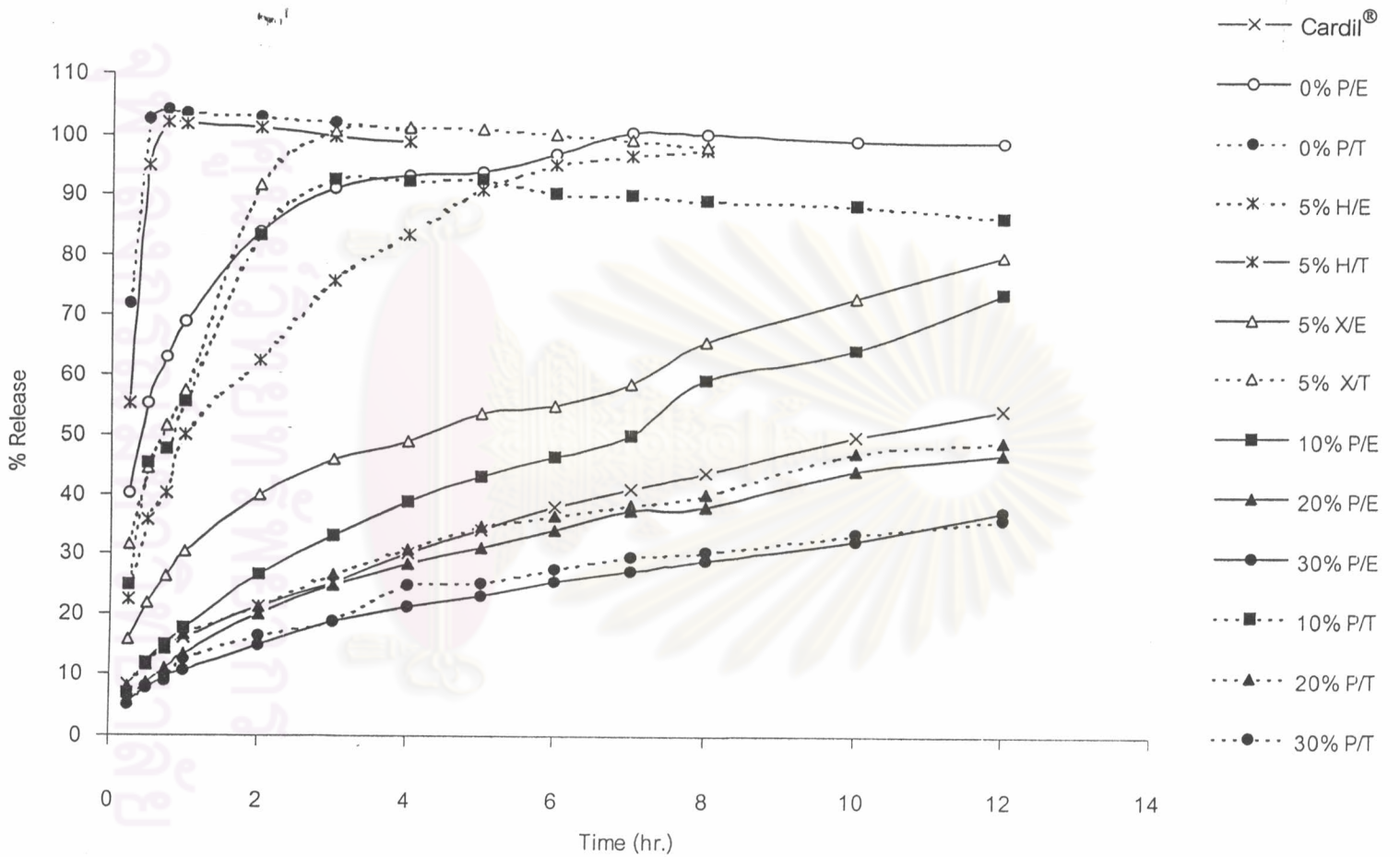

Figure 4. Drug release profiles of diltiazem hydrochloride of each formulation in phosphate buffer pH 7.2 during preliminary study

Table 7. Difference factor (f_1) and similarity factor (f_2) in phosphate buffer pH 7.2 of each formulation during preliminary study

Formulations	f_1	f_2
0% P/T	441.51	11.41
0% P/E	180.68	13.23
5% H/T	414.63	12.61
5% H/E	165.04	19.20
5% X/T	209.93	14.21
5% X/E	58.90	36.98
10% P/T	153.32	16.09
10% P/E	26.47	50.39
20% P/T	5.06	81.94
20% P/E	11.17	70.31
30% P/T	28.34	50.72
30% P/E	32.21	48.55

1.1 Scale up study

Diltiazem hydrochloride controlled release tablets according to the selected formulations in preliminary study (10% P/E, 20% P/E and 20% P/T formulations) were prepared using single punch tabletting machine. Diameter of the punch was 0.91 cm. Hardness of tablets were in the range of 6-8 kps. It was found that drug released of 10% P/E, 20% P/E and 20% P/T formulations in phosphate buffer pH 7.2 differed from those in preliminary study. It was because the surface area of tablet using hydraulic press was greater than that using single punch tabletting machine. Hence, decreasing in medium contact for tablets using single punch tabletting machine were observed. Thus, difference factor and similarity factor of 10% P/E, 20% P/E and 20% P/T formulations in scale up study were remarkably different as seen in Table 10 from those in preliminary study. Therefore, concentration of polymer in both formulations (10% P/E

and 20% P/T) was varied. Results obtained showed that changing polymer concentration of Emcompress® formulation from 10% to 7% and Tablettose® formulation from 20% to 15% produced considerably better values of difference and similarity factor (Table 10).

Furthermore, drug release profile of 10% mixed polymers in Tablettose® and Emcompress® in the proportion of 1:2 was studied. Result in Table 10 showed that its profile was similar to that of Cardil®.

From above results, formulations with 7% mixed polymers (3.5% HPMC and 3.5% XG) in Emcompress® (7% P/E), 10% mixed polymers (5% HPMC and 5% XG) in Emcompress® and Tablettose® in the proportion of 2:1 (10% P/E+T) and 15 % mixed polymers (7.5% HPMC and 7.5% XG) in Tablettose® (15% P/T) were selected for *in vitro* study.

2. *In vitro* study

2.1 Weight variation, hardness and thickness of diltiazem hydrochloride matrices

Data of weight variation, hardness and thickness of diltiazem hydrochloride matrices tablets were shown in Table 8. Weight of tablets containing 7% P/E, 10% P/E+T and 15% P/T were 371.25±3.52, 373.90±3.83 and 372.55±3.10 mg, respectively. The variation was less than 6%. This might be occurred during production. Hardness of them was in the range of 6.75-7.00 kps and thickness of tablets with 7% P/E, 10% P/E+T and 15% P/T were 3.11, 3.25 and 3.45 mm., respectively. Thickness of tablet with Tablettose® was greater than that with Emcompress® since the density of Tablettose® powder was less than that of Emcompress® (Wade and Weller, 1994).

2.2 Tablet friability

Results of tablet friability of all three formulations were resented in Table 8. Percent weight loss of tablet containing 7% P/E, 10% P/E+T and 15% P/T were 0.77, 0.59 and 0.40 %, respectively. The weight loss of all formulations were in the acceptance criteria that was less than 1% of the weight of the tablets being tested (USP 24, 2000). This referred that the hardness of tablet was suitable.

2.3 Content of active ingredient

The content of diltiazem hydrochloride of each formulation was determined following the method as described previously. Results were shown in Table 9. Content of formulation with 7% P/E, 10% P/E+T and 15% P/T was 98.23, 97.34 and 98.89 %, respectively. All of them had average content less than 100%. This might be due to flowability of powder into feedshoe was not uniformly consistent. However the average drug content was in the range of 90-110%.

2.4 Dissolution profile

Each formulation was studied for dissolution profiles in three media, phosphate buffer pH 7.2, water and 0.1N hydrochloric acid pH 1.2 (Figures 6-8). Results in Tables 10-13 showed that drug released profiles of all formulations were similar to that of Cardil®.

Apart from what being found as the best three formulations, the experiment was also attempted to test the effects of polymer and filler on drug release. The results were summarized as follows :

Effect of polymer

According to previous work, the substitution of HPMC with XG could reduce the initial burst drug release for insoluble drug (Parinda, 2000). For soluble drug (diltiazem hydrochloride), dissolution profiles in water of tablets containing 7% mixed polymers in Emcompress® and 15% mixed polymers in Tablettose® were selected to compare with those with 7% HPMC in Emcompress® (7% H/E) and 15% HPMC in Tablettose® (15% H/T), respectively. From Figure 9, initial drug release of tablet composed of XG in both formulations (7% P/E and 15% P/T) was less than that with only HPMC (7% P/E and 15% P/T). It was because matrix containing of XG could maintain matrix integrity at the beginning of dissolution time. This result is consistent with previous report (Talukdar et al, 1996). Since XG is hydrated quickly and gel layer is formed immediately, it makes the pathlength of drug diffusion longer than that of HPMC only. Consequently, the substitution of HPMC with XG could reduce the initial burst drug release for soluble drug as well.

Effect of filler

For the effect of filler, soluble filler (Tablettose®) or insoluble filler (Emcompress®) was studied. The release profiles of tablets with 10% mixed polymers in Tablettose® (10% P/T) and 10% mixed polymers in Emcompress® (10% P/E) in water were demonstrated in Figure 10. Release of diltiazem hydrochloride from 10% P/T tablet was more than that of 10% P/E tablet. Reason was that Emcompress® did not dissolve in water and thus caused an increase in the tortuosity of matrix. Therefore the percent drug released from Emcompress® matrix was less than that of Tablettose®. All these three formulations were subjects to be further *in vivo* investigated.

Table 8. Weight variation, hardness, thickness and tablet friability of diltiazem hydrochloride matrices during scale up study (n=20)

Formulations	Weight variation (mg) (Average ± S.D.)	Hardness (kps) (Average ± S.D.)	Thickness (mm) (Average ± S.D.)	Tablet friability (%)
7% P/E	371.25 ± 3.52	6.88 ± 0.46	3.11 ± 0.01	0.77
10% P/E+T	373.90 ± 3.83	6.75 ± 0.39	3.25 ± 0.01	0.59
15% P/T	372.55 ± 3.10	7.00 ± 0.47	3.44 ± 0.01	0.40

Table 9. Percent drug content of diltiazem hydrochloride matrices (Average ± S.D.) of each formulation during scale up study (n=20)

Formulations	% Drug Content
7% P/E	98.23 ± 0.95
10% P/E+T	97.34 ± 1.45
15% P/T	96.89 ± 2.35

Table 10. Difference factor (f_1) and similarity factor (f_2) in phosphate buffer pH 7.2, 0.1N hydrochloric acid pH 1.2 and water of each formulation compared to Cardil® during scale up study

Formulations	Phosphate Buffer pH 7.2		0.1N Hydrochloric Acid pH 1.2		Water	
	f_1	f_2	f_1	f_2	f_1	f_2
7% P/E	10.94	68.78	9.27	58.03	7.02	64.80
10% P/E	11.54	65.88	10.80	54.78	9.37	63.31
	(26.47)*	(50.39)*				
20% P/E	29.06	51.48	24.84	40.37	16.21	49.67
	(11.17)*	(70.31)*				
10% P/E+T	6.08	81.21	7.30	65.18	8.43	63.08
15% P/T	7.03	78.64	8.23	62.08	13.37	51.22
20% P/T	17.17	60.48	11.48	59.55	14.08	50.98
	(5.06)*	(81.94)*				

* = Preliminary data

Table 11. Average percent drug release of each formulation in phosphate buffer pH 7.2 in scale up study (n=12)

Time	Cardil®			7% P/E			10% P/E+T			15% P/T		
(hr.)	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.
0.25	8.38	0.23	2.80	7.84	1.53	19.49	8.15	1.57	19.21	7.93	3.29	41.45
0.5	11.74	0.20	1.69	12.13	1.93	15.95	12.45	2.02	16.24	13.55	1.72	12.67
0.75	14.65	0.19	1.32	14.69	1.09	7.45	15.69	2.82	17.96	15.72	1.75	11.15
1	16.10	0.38	2.37	17.35	1.97	11.37	17.98	3.21	17.86	18.40	3.74	20.35
2	20.96	0.42	2.02	25.41	1.75	6.89	24.38	3.91	16.04	24.99	2.93	11.73
3	25.09	0.69	2.73	31.60	1.28	4.05	28.37	4.46	15.71	29.83	2.65	8.87
4	29.91	1.00	3.33	36.33	1.92	5.30	32.41	4.95	15.28	33.56	2.13	6.34
5	33.83	1.09	3.23	40.06	2.29	5.72	35.46	6.65	18.74	36.61	2.35	6.41
6	37.64	1.07	2.84	43.29	2.90	6.71	38.80	7.50	19.33	39.72	2.13	5.37
7	40.70	1.12	2.76	45.67	3.27	7.16	41.27	8.82	21.37	41.53	2.44	5.88
8	43.50	1.34	3.09	47.24	3.07	6.50	43.13	9.24	21.43	43.61	1.91	4.38
10	49.44	1.36	2.76	49.88	3.54	7.10	46.20	9.62	20.83	47.86	2.29	4.77
12	53.44	0.97	1.81	51.95	3.59	6.90	50.05	8.14	16.27	51.79	0.50	0.97

where 7% P/E = 7% mixed polymers (3.5% HPMC and 3.5% XG) in Emcompress® formulation

10% P/E+T = 10% mixed polymers (5% HPMC and 5% XG) in Emcompress® and Tablettose® in the proportion of 2:1 formulation

15% P/T = 15% mixed polymers (7.5% HPMC and 7.5% XG) in Tablettose® formulation

Table 12. Average percent drug release of each formulation in 0.1 N hydrochloric acid pH 1.2 in scale up study (n=12)

Time	Cardil®			7% P/E			10% P/E+T			15% P/T		
(hr.)	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.
0.25	18.65	0.19	1.00	16.17	1.10	6.82	12.58	1.63	12.96	11.14	2.35	21.08
0.5	26.51	0.30	1.12	26.12	1.15	4.39	20.19	1.84	9.10	17.72	1.94	10.95
0.75	32.00	0.21	0.65	33.15	1.04	3.14	27.51	2.98	10.83	24.39	1.32	5.40
1	36.48	0.16	0.43	38.46	0.99	2.59	37.65	7.10	18.85	33.26	5.63	16.91
2	47.98	0.23	0.48	52.95	0.48	0.91	44.31	2.70	6.10	40.55	1.77	4.35
3	57.11	0.41	0.73	63.76	0.56	0.87	54.51	2.82	5.18	50.43	1.91	3.80
4	66.33	5.04	7.60	71.22	0.81	1.14	63.53	5.68	8.94	58.08	2.23	3.85
5	69.92	0.52	0.74	81.66	2.03	2.48	72.76	4.68	6.43	66.50	3.62	5.45
6	74.51	0.35	0.47	85.13	0.69	0.82	79.08	3.56	4.50	70.81	4.05	5.72
7	78.22	0.81	1.04	90.09	0.76	0.85	86.38	1.66	1.92	78.51	5.47	6.97
8	81.87	0.37	0.46	90.90	1.26	1.39	90.08	1.98	2.20	85.66	2.49	2.91
10	87.56	0.48	0.55	92.05	1.96	2.13	91.82	0.73	0.79	91.40	1.23	1.35
12	91.35	0.83	0.91	92.52	1.77	1.91	92.27	1.02	1.10	91.86	1.19	1.29

where 7% P/E = 7% mixed polymers (3.5% HPMC and 3.5% XG) in Emcompress® formulation

10% P/E+T = 10% mixed polymers (5% HPMC and 5% XG) in Emcompress® and Tablettose® in the proportion of 2:1 formulation

15% P/T = 15% mixed polymers (7.5% HPMC and 7.5% XG) in Tablettose® formulation

Table 13. Average percent drug release of each formulation in water in scale up study (n=12)

Time	Cardil®			7% P/E			10% P/E+T			15% P/T		
(hr.)	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.	Average	S.D.	% C.V.
0.25	15.91	1.13	7.10	14.41	0.89	6.15	13.01	2.19	16.81	15.41	1.88	12.23
0.5	23.03	1.05	4.57	22.85	1.07	4.67	20.24	2.23	11.01	23.59	2.07	8.80
0.75	28.50	0.98	3.44	29.24	1.52	5.18	25.84	2.18	8.44	29.41	2.17	7.38
1	32.92	0.82	2.50	34.34	1.53	4.46	30.27	2.67	8.81	33.28	2.20	6.62
2	45.05	1.33	2.96	49.17	1.92	3.91	43.16	2.92	6.76	46.05	2.61	5.66
3	54.56	2.49	4.56	60.04	3.21	5.34	53.30	3.33	6.25	57.98	4.92	8.49
4	59.48	2.81	4.72	67.61	2.35	3.47	61.61	3.81	6.18	63.48	6.53	10.29
5	63.79	2.34	3.67	73.29	1.71	2.33	70.61	5.19	7.35	74.15	5.90	7.96
6	68.10	2.79	4.09	76.37	1.71	2.24	77.25	3.51	4.55	83.06	2.81	3.39
7	71.37	3.35	4.70	77.80	1.42	1.83	80.93	0.85	1.05	87.60	0.64	0.73
8	74.18	3.04	4.09	78.37	1.57	2.00	81.95	0.76	0.93	89.09	0.48	0.54
10	77.85	2.83	3.64	79.38	1.68	2.12	83.07	0.64	0.77	89.72	1.06	1.18
12	79.71	1.95	2.44	79.80	2.03	2.54	83.29	0.52	0.62	90.20	1.88	2.08

where 7% P/E = 7% mixed polymers (3.5% HPMC and 3.5% XG) in Emcompress® formulation

10% P/E+T = 10% mixed polymers (5% HPMC and 5% XG) in Emcompress® and Tablettose® in the proportion of 2:1 formulation

15% P/T = 15% mixed polymers (7.5% HPMC and 7.5% XG) in Tablettose® formulation

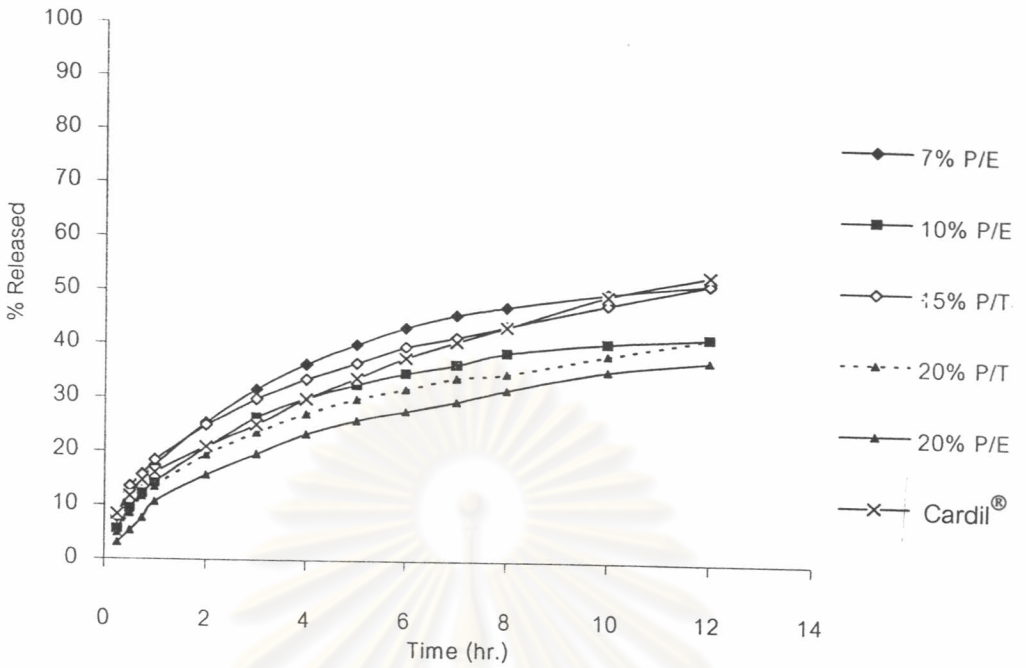

Figure 5. Drug release profiles of diltiazem hydrochloride of each formulation in phosphate buffer pH 7.2 during varied concentration of polymers

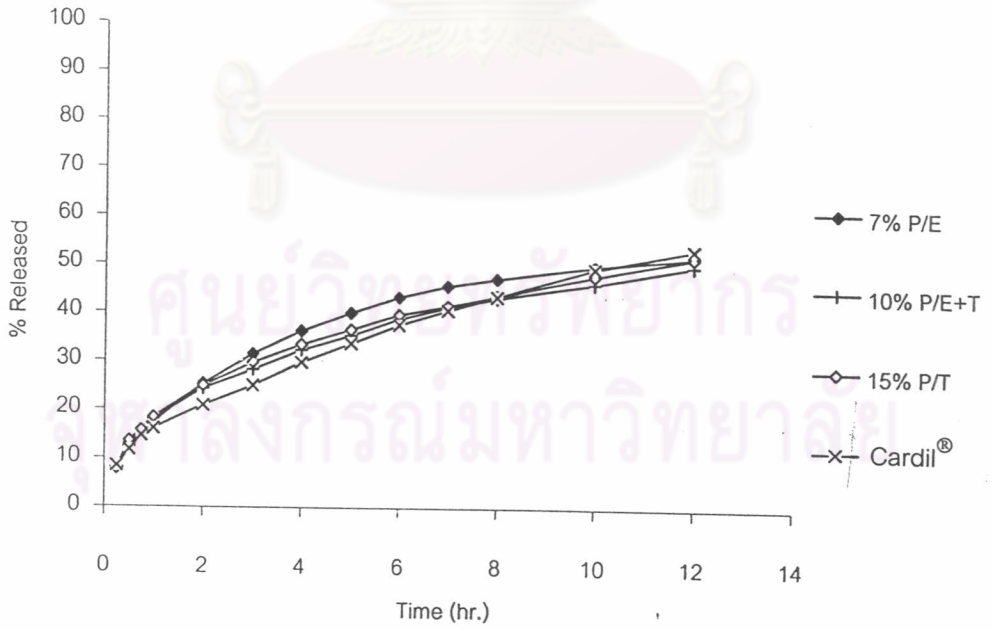

Figure 6. Drug release profiles of diltiazem hydrochloride of each formulation in phosphate buffer pH 7.2 in scale up study

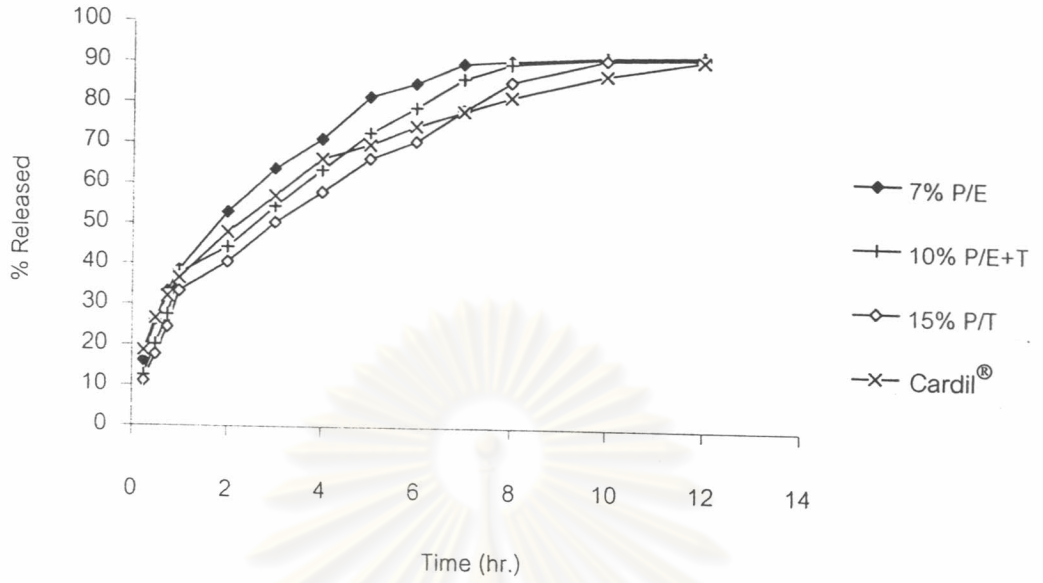

Figure 7. Drug release profiles of diltiazem hydrochloride of each formulation in 0.1 N hydrochloric acid pH 1.2 in scale up study

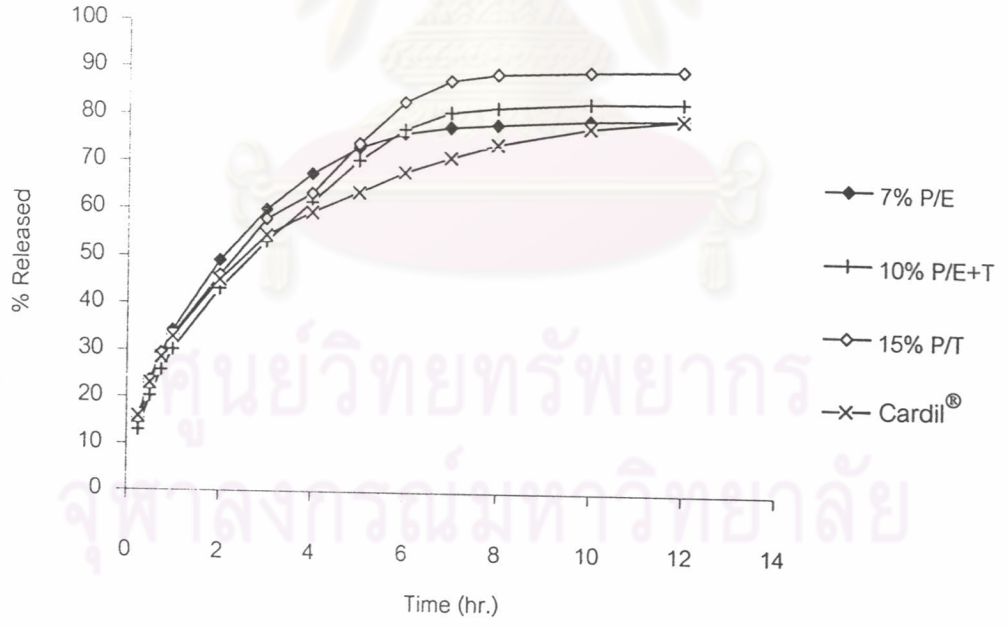

Figure 8. Drug release profiles of diltiazem hydrochloride of each formulation in water in scale up study

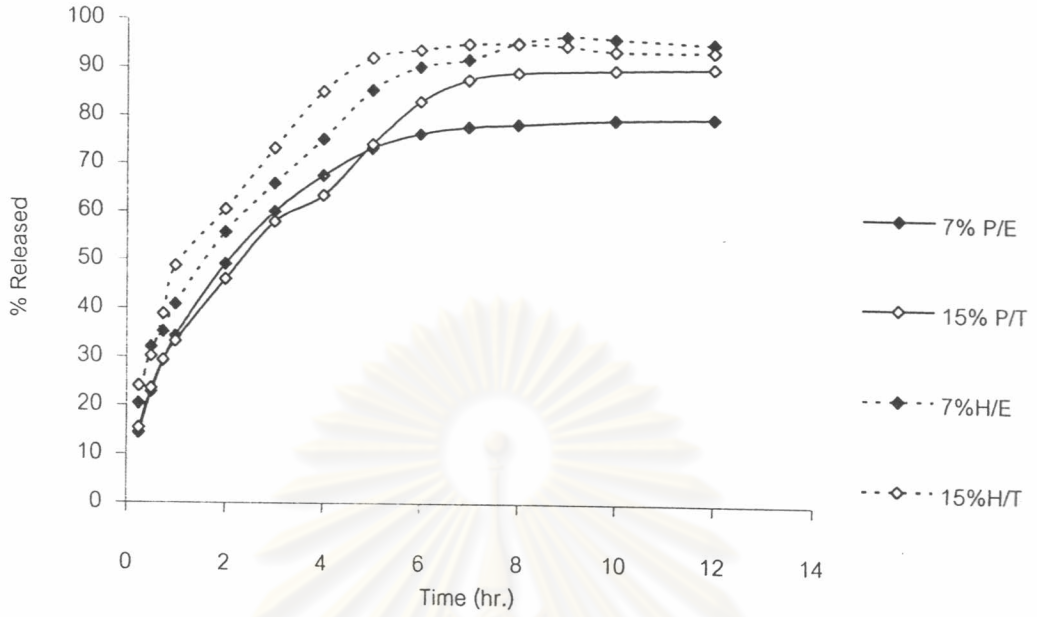

Figure 9. Effect of polymer on drug release profiles of diltiazem hydrochloride tablets in water

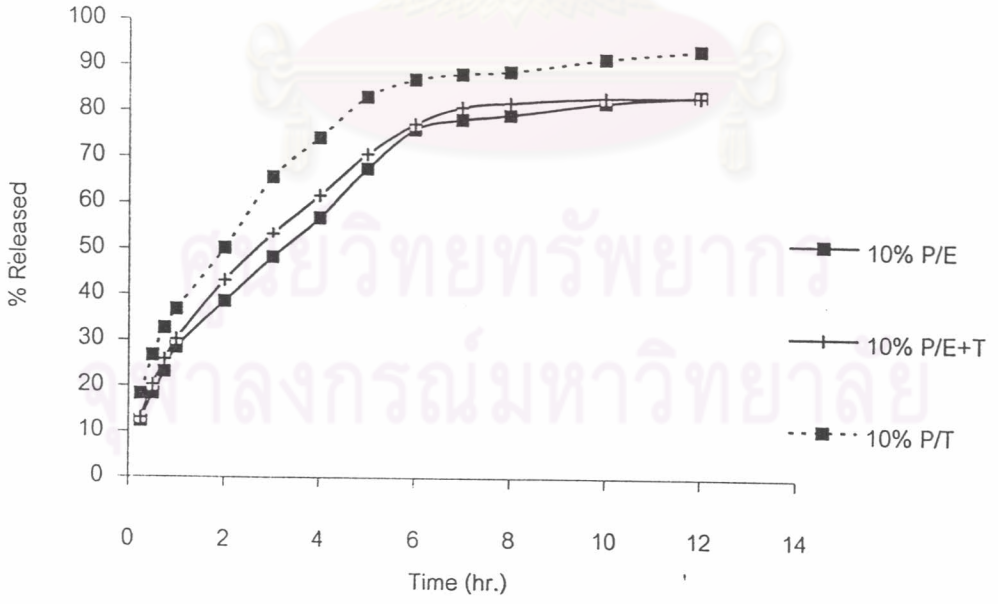

Figure 10. Effect of filler on drug release profiles of diltiazem hydrochloride tablets in water

B. *In vivo* study

1. Assay validation

Chromatograms of blank plasma, diltiazem hydrochloride and ethylparaben were shown in Figure 11. The retention time of diltiazem hydrochloride and ethylparaben were 8.8 and 10.9 minutes, respectively. No any interference peaks of plasma protein or endogenous substances were observed.

The calibration curve of peak area ratio of diltiazem hydrochloride to ethylparaben versus plasma diltiazem hydrochloride concentrations was linear covered the range of concentrations used with the coefficient of determination (r^2) of 0.9991 as shown in Table 43 and Figure 29 in Appendix A. The method of analysis was validated by determining the accuracy, the within run and between run precisions. The values of accuracy and precision were accessible in Tables 44-46 in Appendix A. Percent recovery for accuracy was between 89.40 to 102.16% and the average recovery was 95.60%. The percent coefficient of variations (% C.V.) in the within run and between run precision were less than 15%. These results were within acceptance criteria for accuracy and precisions. The lower limit of quantification was 70 ng/mL.

2. Plasma diltiazem hydrochloride concentrations

Twelve healthy male rabbits were used as subjects in this study. The plasma concentrations of diltiazem hydrochloride at each sampling time interval ranging from 0 to 18 hours after oral administration of two 120 mg diltiazem hydrochloride tablets containing 7% P/E, 10% P/E+T, 15% P/T and Cardil® were shown in Tables 14-17. The plasma concentration at time zero was zero. Plasma concentration-time profiles of each subject were demonstrated in Figures 12-23. The average plasma concentration-time profile of each formulation was displayed in Figure 24.

The plasma concentration-time profiles were widely different among the formulations studied in each subject. Some concentration-time profiles exhibited two or three peak plasma concentrations. These might be due to variations originated from difficulty of administration, for examples. (a) The total dose was two tablets, however, only one tablet could be administered at a time. (b) The first tablet was easily swallowed whereas the second one still remained in the oral cavity for a period of time. (c) Some rabbits refreshed to drink water after introducing the first tablet. So, the second tablet might be deposited at any places of the upper part of the gastrointestinal tract for sometimes. Thus, the first tablet might be readily absorbed prior to the second one reached the absorption sites. Furthermore, the intra and inter subject variability might be contributed. It was due to diltiazem hydrochloride was mainly and highly metabolized in the liver (Homsy et al, 1995).

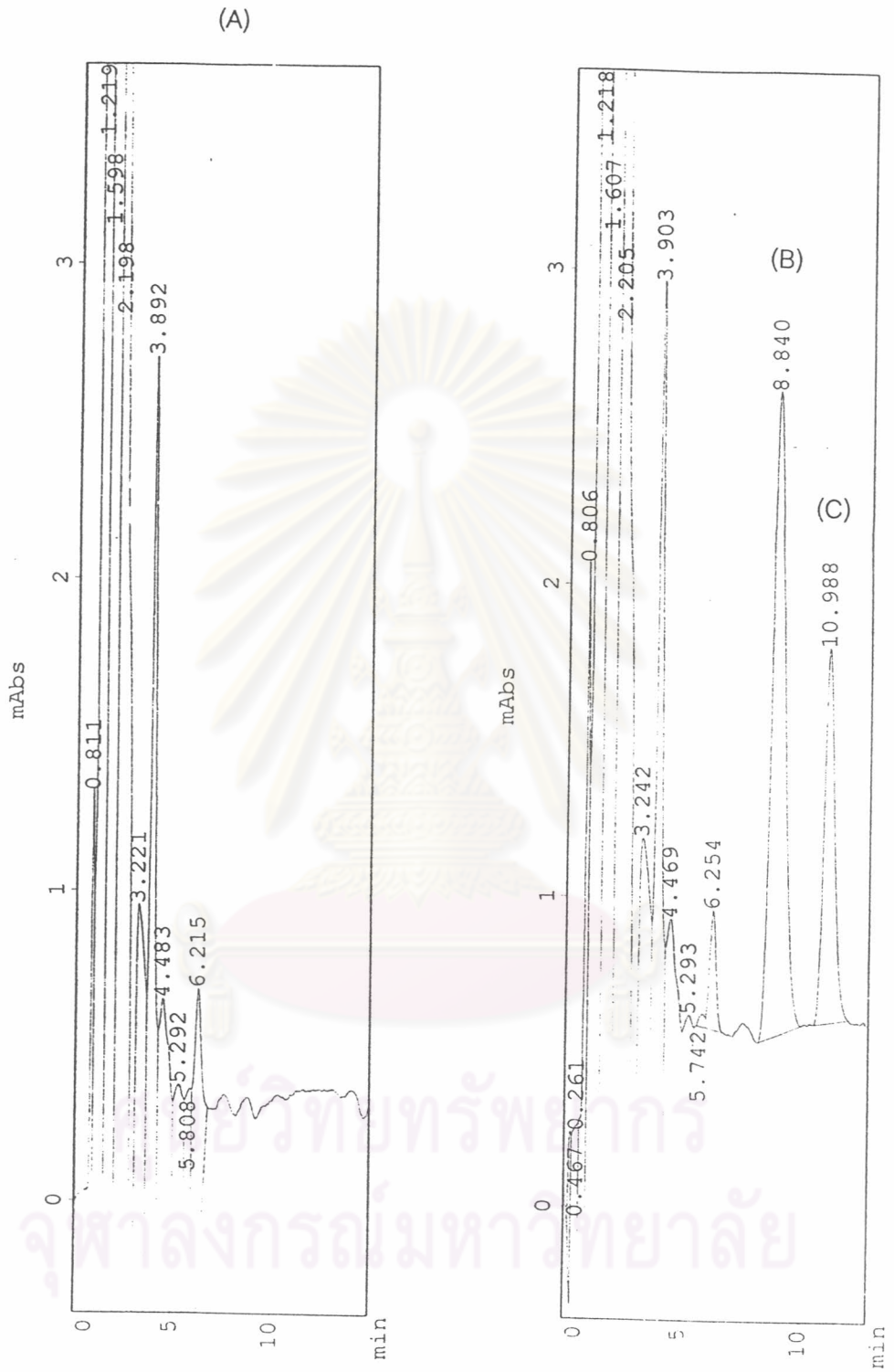

Figure 11. High performance liquid chromatograms of blank plasma (A), peak of diltiazem hydrochloride (B) and ethylparaben (C)

Table 14. Plasma diltiazem hydrochloride concentration (ng/mL) from 12 rabbits following oral administration of 2 x 120 mg of 7% mixed polymers in Emcompress® tablets

Subject No.	Time (hr.)											
	1	2	3	4	5	6	7	8	10	12	15	18
1	70.70	149.37	274.04	386.30	481.14	498.25	122.13	*	*	*	*	*
2	79.55	130.59	156.51	243.59	382.34	325.24	316.53	483.69	219.99	*	*	*
3	271.36	230.46	1716.74	1451.31	674.76	484.33	295.76	202.11	131.31	*	*	*
4	1062.93	1467.93	1812.68	1271.93	867.72	526.41	314.26	199.44	113.07	*	*	*
5	95.12	106.12	626.95	546.75	680.87	1027.29	576.23	421.08	198.03	*	*	*
6	326.32	354.14	391.15	855.02	1390.64	1370.99	691.33	330.01	*	*	*	*
7	289.43	804.92	840.23	605.76	472.73	374.25	325.35	608.02	254.18	*	*	*
8	203.76	366.13	343.38	612.99	889.01	878.94	740.67	332.24	196.52	*	*	*
9	96.27	150.50	495.02	1074.68	1837.18	1122.33	902.86	813.99	384.21	*	*	*
10	88.25	93.31	96.17	1948.86	910.31	424.97	326.71	125.36	106.76	*	*	*
11	70.55	81.31	71.59	192.00	320.10	528.19	256.47	141.24	78.26	*	*	*
12	392.17	1979.86	802.58	531.45	1387.39	917.09	465.45	235.24	*	*	*	*
Average	253.87	492.89	635.59	810.05	857.85	706.52	444.48	324.37	140.19	-	-	-
S.D.	279.29	618.54	584.53	531.16	466.86	350.01	230.62	213.80	94.56	-	-	-
% C.V.	110.02	125.49	91.97	65.57	54.42	49.54	51.89	65.91	67.45	-	-	-

* = < Lower Limit of Quantification

Table 15. Plasma diltiazem hydrochloride concentration (ng/mL) from 12 rabbits following oral administration of 2 x 120 mg of 10% mixed polymers in Emcompress® and Tablettose® in proportional of 2:1 tablets

Subject No.	Time (hr.)											
	1	2	3	4	5	6	7	8	10	12	15	18
1	305.81	322.19	546.95	873.20	358.86	349.66	467.81	219.08	*	*	*	*
2	75.07	81.53	71.50	103.80	86.09	76.54	94.07	80.30	75.74	*	*	*
3	187.80	2482.00	975.68	792.43	310.20	1365.20	751.49	329.14	110.01	*	*	*
4	361.25	540.30	838.44	540.34	352.51	211.38	163.97	149.29	106.44	*	*	*
5	284.95	766.71	875.54	757.65	381.52	259.80	165.62	101.74	*	*	*	*
6	85.03	171.17	285.70	470.35	365.97	225.77	103.98	81.82	*	*	*	*
7	103.86	319.24	499.49	842.82	584.34	296.06	187.35	134.68	108.46	*	*	*
8	160.21	689.02	813.24	298.22	412.33	446.74	368.13	255.83	107.49	*	*	*
9	151.62	348.68	581.81	360.76	466.93	329.46	130.69	77.46	*	*	*	*
10	189.65	358.10	241.65	313.93	310.98	231.52	145.73	97.60	*	*	*	*
11	322.70	447.76	271.77	158.40	171.18	303.38	283.17	196.67	143.27	*	*	*
12	267.53	212.52	262.9	691.43	234.56	186.49	141.48	*	*	*	*	*
Average	207.96	561.60	522.06	516.94	336.29	356.83	250.29	156.69	108.57	-	-	-
S.D.	93.77	610.14	286.88	260.26	125.70	316.66	185.96	79.67	19.53	-	-	-
% C.V.	45.09	108.64	54.95	50.35	37.38	88.74	74.30	50.85	17.99	-	-	-

* = < Lower Limit of Quantification

Table 16. Plasma diltiazem hydrochloride concentration (ng/mL) from 12 rabbits following oral administration of 2 x 120 mg of 15% mixed polymers in Tablettose® tablets

Subject No.	Time (hr.)											
	1	2	3	4	5	6	7	8	10	12	15	18
1	383.69	436.20	1072.80	777.39	330.30	157.27	155.77	140.67	76.02	*	*	*
2	94.12	91.47	185.10	352.60	373.30	319.80	222.20	149.80	81.89	*	*	*
3	70.96	80.40	113.10	243.20	288.30	423.10	319.40	88.59	*	*	*	*
4	581.86	1226.28	903.47	319.86	531.35	300.82	178.82	101.01	73.84	*	*	*
5	79.67	1151.40	1272.30	512.62	264.43	165.09	100.57	72.37	*	*	*	*
6	377.86	1850.60	833.45	920.14	571.08	305.54	186.70	140.11	128.03	*	*	*
7	235.23	361.11	668.44	904.37	546.07	420.36	230.52	130.46	74.66	*	*	*
8	143.86	427.05	308.18	241.90	245.51	177.69	188.94	147.29	81.91	*	*	*
9	88.72	171.65	339.11	459.90	393.54	186.70	191.16	87.39	*	*	*	*
10	177.29	144.22	158.79	219.20	195.96	324.81	475.38	604.92	172.6	*	*	*
11	91.88	87.58	1673.68	1398.67	892.88	578.20	299.26	214.15	80.16	*	*	*
12	388.77	899.90	1164.90	1235.60	1076.80	448.76	355.91	206.62	*	*	*	*
Average	226.16	577.32	724.44	632.12	475.79	317.35	242.05	173.62	96.14	-	-	-
S.D.	160.71	550.77	489.04	389.83	258.50	126.81	98.79	136.71	33.33	-	-	-
% C.V.	71.06	95.40	67.51	61.67	54.33	39.96	40.82	78.74	34.67	-	-	-

* = < Lower Limit of Quantification

Table 17. Plasma diltiazem hydrochloride concentration (ng/mL) from 12 rabbits following oral administration of 2 x 120 mg of Cardil® tablets

Subject No.	Time (hr.)											
	1	2	3	4	5	6	7	8	10	12	15	18
1	350.91	493.85	615.75	421.80	582.71	328.60	445.33	300.15	175.02	*	*	*
2	123.27	136.96	98.07	91.15	140.78	117.38	85.28	73.60	*	*	*	*
3	169.78	420.34	364.81	418.61	440.82	375.88	153.18	110.56	*	*	*	*
4	95.65	93.55	81.23	130.30	83.11	84.07	78.19	76.07	*	*	*	*
5	336.57	567.12	636.13	487.02	503.04	525.97	430.93	240.76	142.36	*	*	*
6	207.21	545.68	437.31	281.88	174.62	154.63	123.38	71.13	*	*	*	*
7	417.34	748.29	778.49	483.40	288.73	249.06	160.23	125.05	80.70	*	*	*
8	161.10	396.93	259.09	239.36	172.05	138.83	115.33	98.13	*	*	*	*
9	83.03	262.19	158.47	154.03	126.40	112.91	113.41	98.01	74.01	*	*	*
10	214.58	616.39	236.99	260.85	176.92	180.18	184.48	124.03	*	*	*	*
11	141.80	184.00	153.90	225.60	268.70	96.23	84.26	74.87	*	*	*	*
12	142.39	339.67	123.25	197.39	204.47	190.65	86.20	75.43	*	*	*	*
Average	203.64	400.41	328.62	282.62	243.64	212.87	171.68	122.32	118.023	-	-	-
S.D.	103.42	195.55	227.62	132.04	163.31	128.79	123.42	69.86	42.34	-	-	-
% C.V.	50.79	48.84	69.27	46.72	67.03	60.50	71.89	57.11	35.88	-	-	-

* = < Lower Limit of Quantification

3. Pharmacokinetic and bioequivalence study

3.1 Bioequivalence evaluation

The pharmacokinetic parameters, AUC and C_{max} were used to characterize the bioavailability of the formulation. All of them were derived from plasma concentration-time profile. In the bioequivalence study, the formulations that are pharmaceutical equivalents should not show statistically significant difference in the term of the rate and the extent of drug absorption (Shargel and Yu,1980) as well as the ratios of individual parameter of test formulations relative to the reference product were contained within 80-125% based on ln-transformed data of 90% confidence interval.

3.1.1 Area under the plasma concentration versus time curve (AUC)

The average AUC values of the formulations with 7% P/E, 10% P/E+T, 15% P/T and Cardil® were 5451.63 ± 2166.42, 3364 ± 1630.10, 3825.08 ± 1521.89 and 2626.76 ± 1735.66 ng-hr./mL, respectively as shown in Table 18. Result in Table 19 showed that there were statistically significant difference ($p<0.05$) among formulations. The tablet with 7% P/E exhibited the maximum extent of drug absorption followed by those with 15% P/T, 10% P/E+T and Cardil®. It might be due to the three formulations except Cardil®consisted of at least one water soluble polymer that could facilitate more drug released. On the other hand, Cardil® might contain some other type of polymer that could affect both drug release and absorption providing less extent of the drug in blood stream. These results were well correlative with *in vitro* dissolutions of these products in terms of the higher amount of drug was released, the greater amount of drug was absorbed. Comparison of the AUC values using Duncan's new multiple ranges test (Table 20) revealed that only 7%P/E formulation differed from Cardil®. The 90% confidence interval presented in Table 29 for AUC of individual test formulation relative to Cardil® was not in the range of 80-125% based on ln-transformed data (181.19-309.50%, 109.56-186.54% and 132.59-225.74%, respectively). This implied that they were not bioequivalent to Cardil® with respect to the extent of drug absorption. The

highly variation of plasma drug concentration from each formulation as seen from % C.V. might be responsible for these results as well as the small number of subjects being used might contribute.

3.1.2 Peak plasma concentration (C_{max})

From data in Tables 21-22, the average peak plasma concentration of formulations with 7%P/E, 10% P/E+T, 15% P/T and Cardil® were 1246.05 ± 598.57, 777.86 ± 591.68, 960.33 ± 509.49 and 430.99 ± 209.67 ng/mL, respectively. These results were in accordance with the AUCs. It was concentration of polymer and filler in each formulation affected absorption process as seen when polymer concentration was increased (10% P/E+T and 15% P/T), peak plasma concentration was declined. Statistically significant difference ($p<0.05$) among all formulations were observed. Duncan's multiple ranges test (Table 23) was performed and found that none of formulation was similar to Cardil®. The 90% confidence interval reported in Table 29 for C_{max} of 7% P/E, 10% P/E+T and 15% P/T formulations relative to Cardil® was not in the range of 80-125% based on ln-transformed data (215.21-395.53%, 105.45-193.80% and 163.24-300.02%, respectively). These referred that all test formulations were bioinequivalent to Cardil® in term of the rate of drug absorption.

Results of these finding suggested that all test formulations were not bioequivalent to Cardil®. However at least one of these formulated product with 10% P/E+T was as closely bioavailable as Cardil®.

3.1.3 Time to peak plasma concentration (t_{max})

The average time to peak plasma concentration were 4.50, 3.10, 3.58 and 3.17 hr. for 7% P/E, 10% P/E+T, 15% P/T formulations and Cardil®, respectively. It implied that the drug from 10% P/E+T tablet and Cardil® were as fast as and more rapidly absorbed into the systemic circulation than those observed from the 15% P/T and 7%

P/E formulations. There were statistically significant difference ($p<0.05$) among all formulations (Tables 24-25). Duncan's new multiple ranges test was calculated and found that only 7% P/E formulation was different from Cardil® (Table 26).

The elimination half-life ($t_{1/2}$) of the drug in all formulations studied were also determined (Tables 27-28). The $t_{1/2}$ values of 7% P/E, 10% P/E+T, 15% P/T formulations and Cardil® were 1.36 $\pm$ 0.52, 1.52 $\pm$ 0.52, 1.17 $\pm$ 0.33 and 1.74 $\pm$ 0.52 hr, respectively. It was found that there were no statistically significant difference ($p>0.05$) among these values. However, between subject variations were observed. This indicated that the drug was equally eliminated.

From this study, 10% P/E+T formulation was the most similar to Cardil®. Further study should be done in terms of slight modification of this formulation and proof its avalability relative to Cardil® in human subjects.

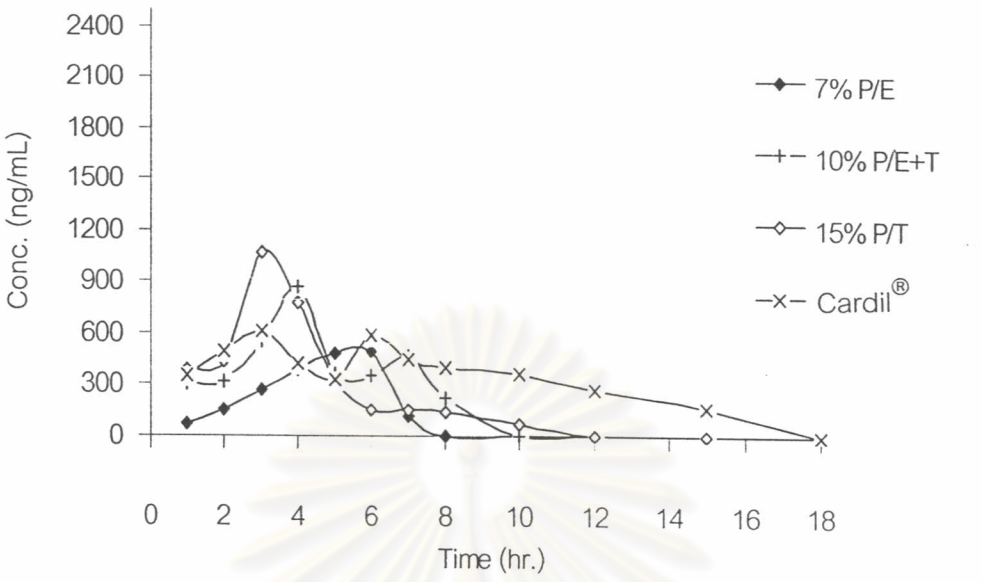

Figure 12. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.1

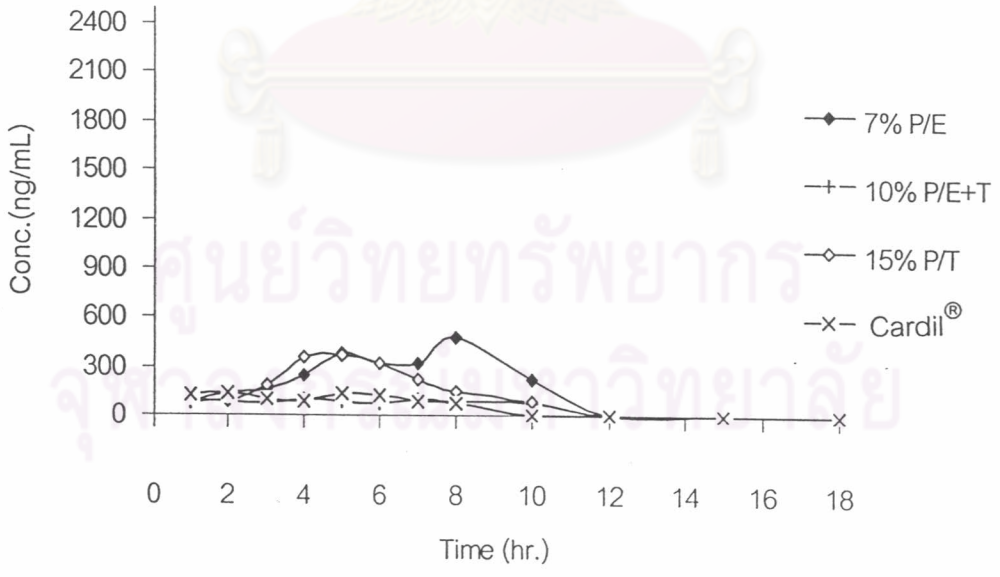

Figure 13. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.2

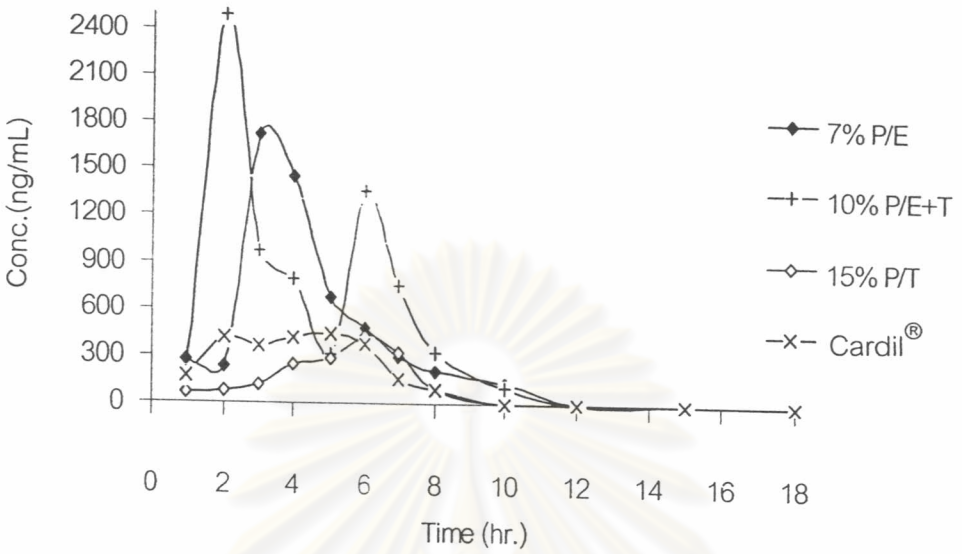

Figure 14. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.3

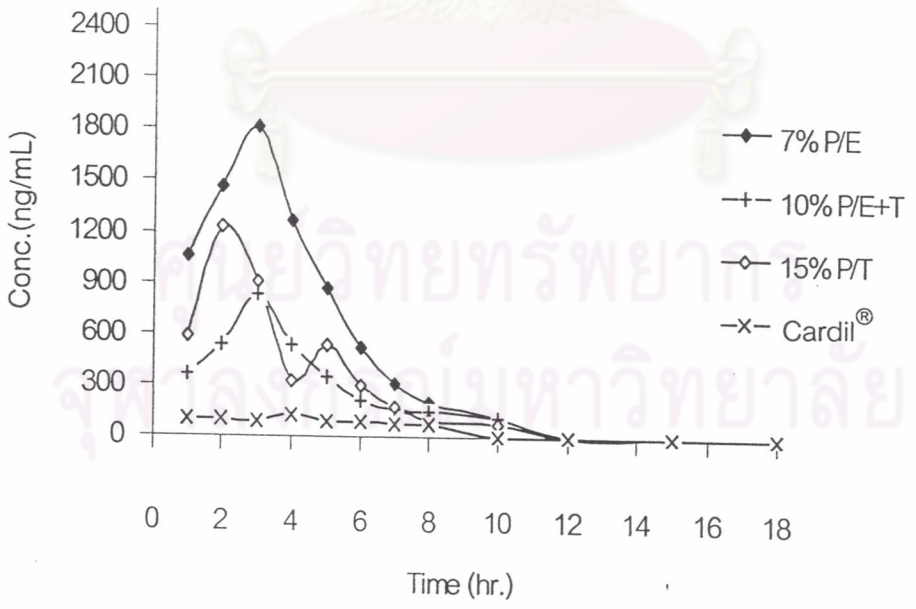

Figure 15. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.4

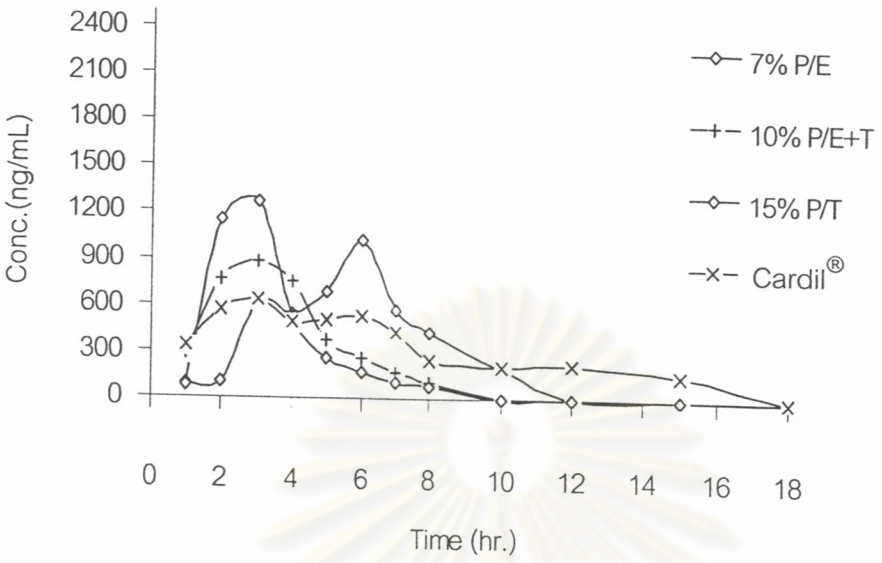

Figure 16. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.5

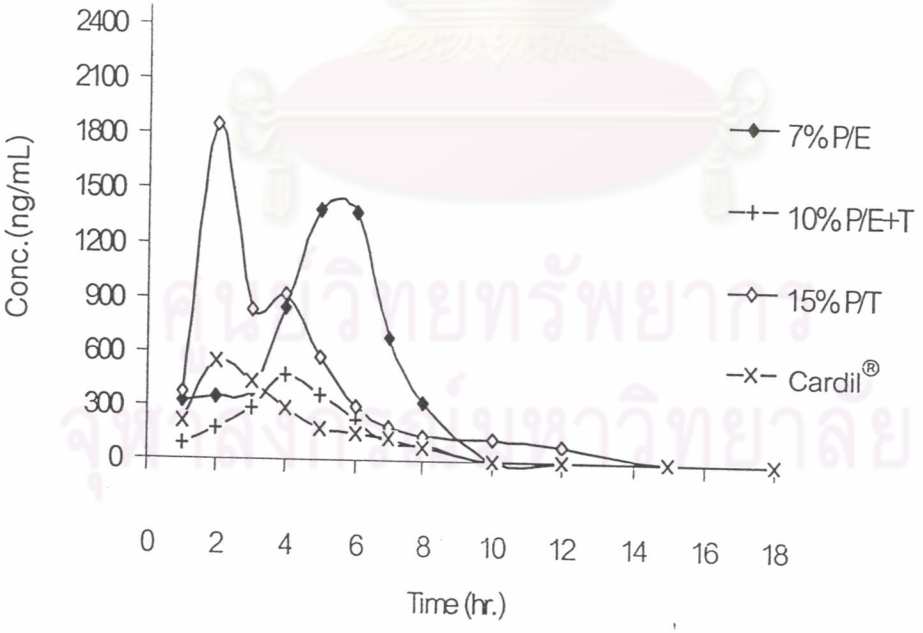

Figure 17. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.6

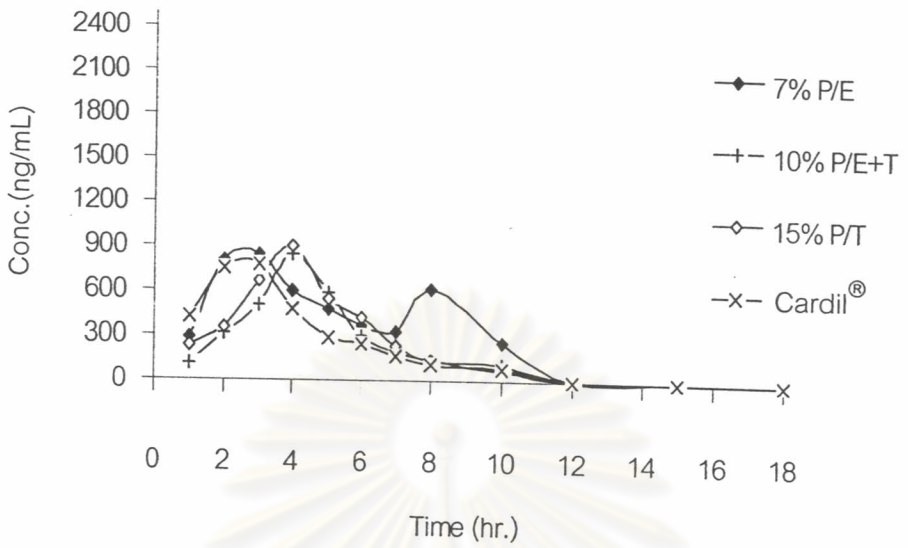

Figure 18. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.7

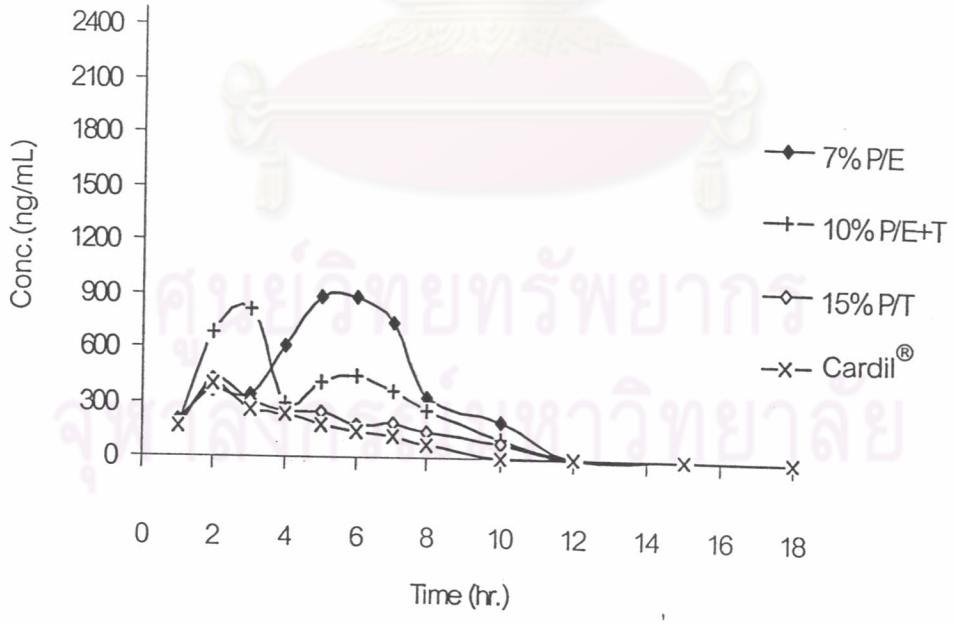

Figure 19. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.8

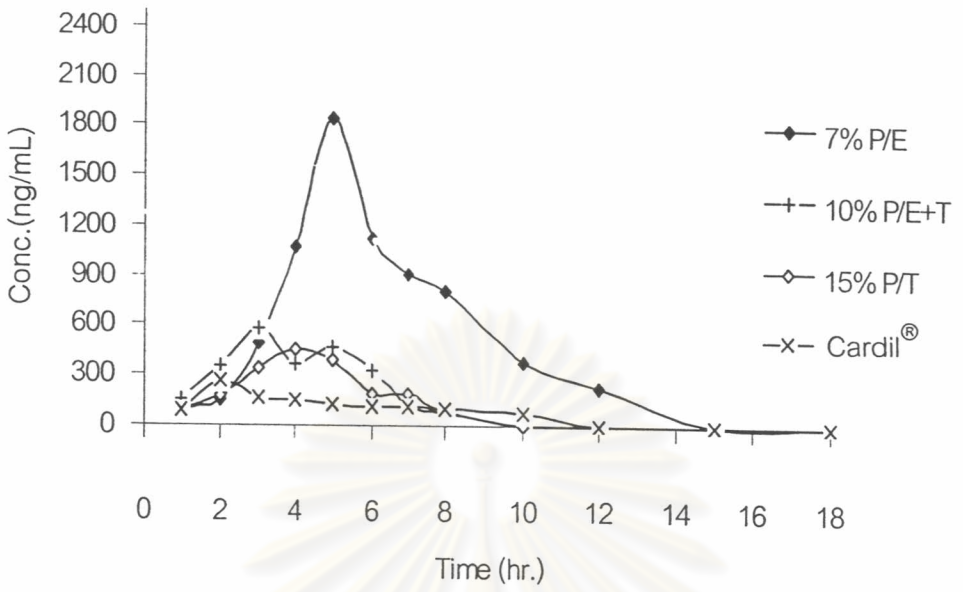

Figure 20. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.9

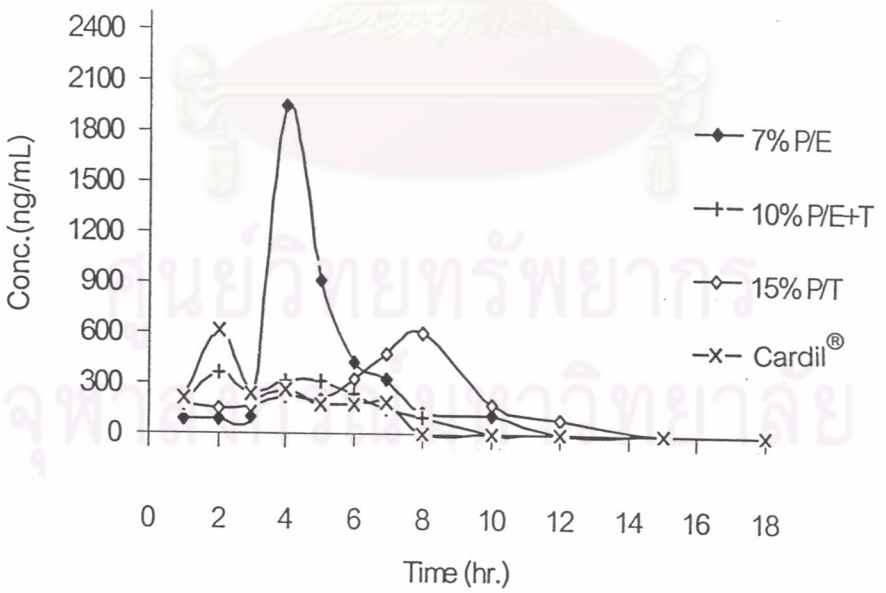

Figure 21. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.10

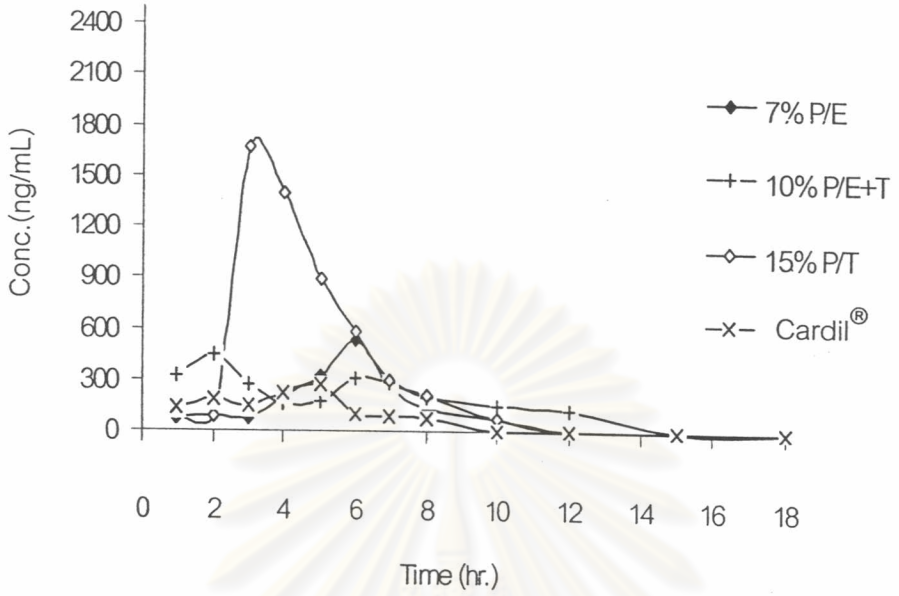

Figure 22. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.11

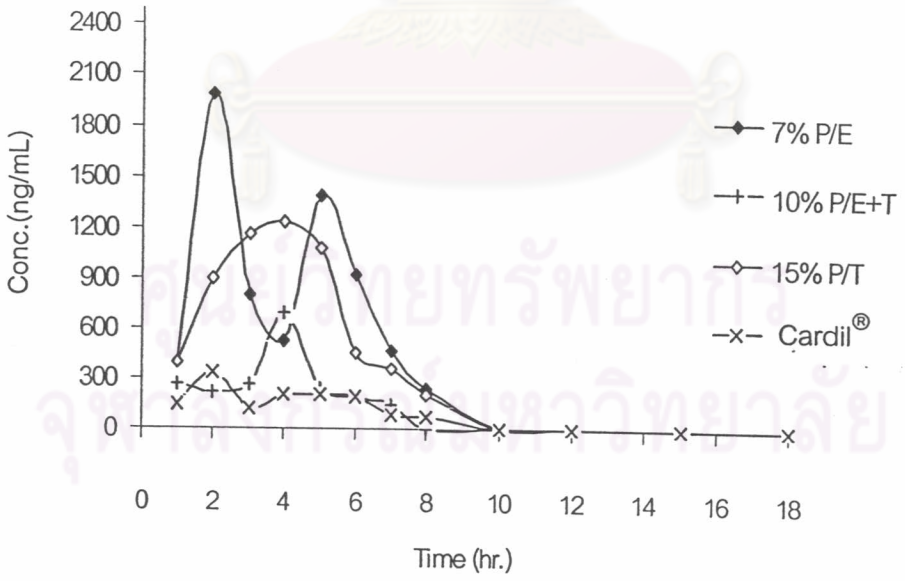

Figure 23. Plasma concentration-time profiles of diltiazem hydrochloride in rabbit no.12

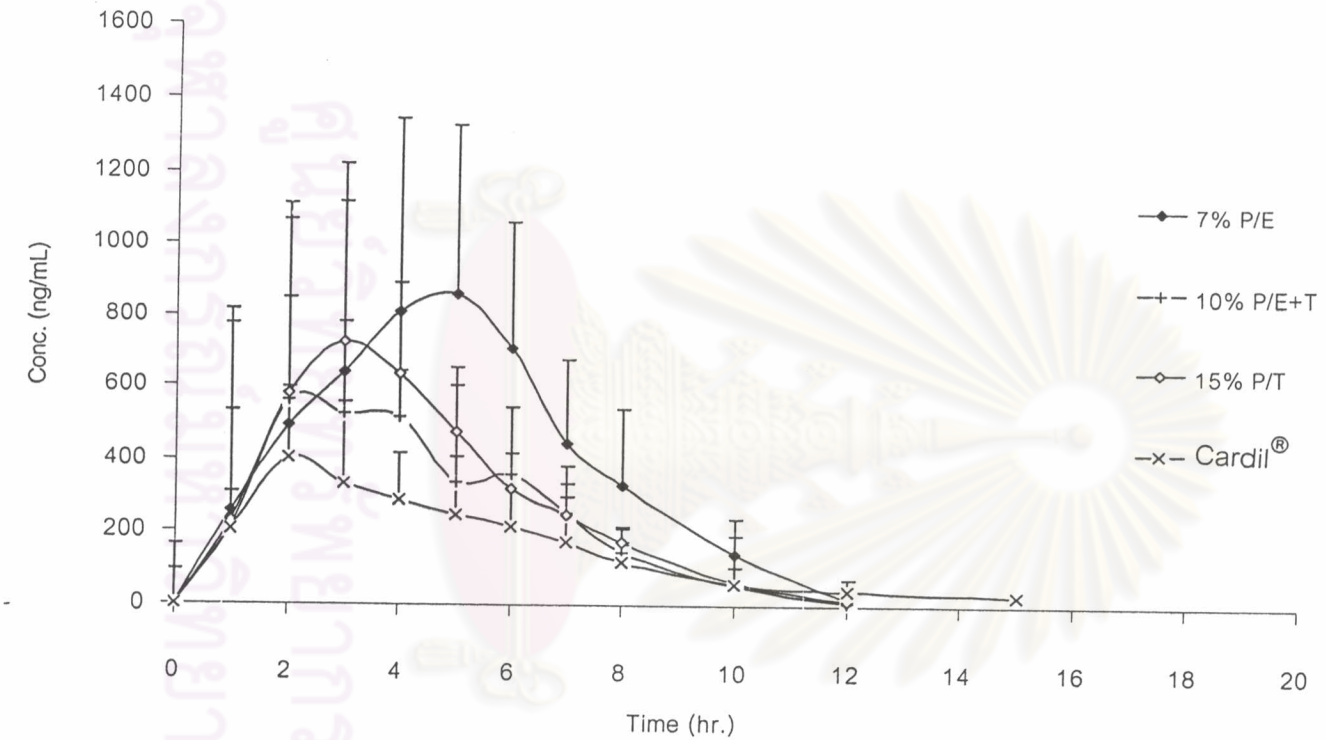

Figure 24. Average plasma concentration of diltiazem hydrochloride of each formulation in 12 rabbits following oral administration of 2 x 120 mg diltiazem hydrochloride tablets

Table 18. Area under the plasma concentration-time curve (AUC) of diltiazem hydrochloride following oral administration of four formulations of 2 x 120 mg diltiazem hydrochloride tablets

Subject No.	AUC (ng-hr/mL)			
	7% P/E	10% P/E+T	15% P/T	Cardil®
1.	2156.40	3966.46	3777.83	6222.16
2.	3645.98	1365.46	2261.04	1237.84
3.	5988.94	7753.15	1795.07	2669.36
4.	8084.72	3686.91	4478.40	950.38
5.	5103.92	3865.57	3781.60	5806.59
6.	6367.157	1994.30	5847.08	2209.24
7.	5980.33	3487.88	3842.78	3624.60
8.	5443.44	3970.99	2421.25	1847.80
9.	8938.31	2668.40	2224.30	1600.04
10.	4448.33	2201.03	3462.25	2289.66
11.	2023.52	3087.19	5669.66	1443.17
12.	7329.36	2325.17	6339.73	1620.33
Average	5459.20	3364.38	3825.08	2626.76
S.D.	2153.65	1630.10	1521.89	1735.66
% C.V.	39.45	48.45	39.79	66.08

Table 19. Analysis of variance for ln AUC of four formulations of 2 x 120 mg diltiazem hydrochloride tablets for crossover design at $\alpha = 0.05$

Source of Variance	df	SS	MS	Fcal	Ftable	Significance Level
Total	47	14.38	--			
Sequences	3	0.40	0.13	0.58	4.07	NS
Subjects(Sequence)	8	1.85	0.23	1.00	2.27	NS
Periods	3	1.18	0.39	1.70	2.92	NS
Formulations	3	4.02	1.34	5.80	2.92	S
Error	30	6.93	0.23			

NS = Not significant difference at $p>0.05$

S = Significant difference at $p<0.05$

df = Degree of freedom

SS = Sum of square

MS = Mean square

Fcal = Variance ratio

Ftable = F value obtained from the table

Table 20. Duncan's new multiple ranges test calculation for ln AUC

Cardil®	10% P/E+T	15% P/T	7% P/E
7.87	8.12	8.25	8.60

90% C.I. of formulation

7% P/E vs. Cardil® = 181.19-309.50%

10% P/E+T vs. Cardil® = 109.56-186.54%

15% P/T vs. Cardil® = 132.59-225.74%

Table 21. Peak plasma concentration (C_{max}) of diltiazem hydrochloride following oral administration of four formulations of 2 x 120 mg diltiazem hydrochloride tablets

Subject No.	C_{max} (ng/mL)			
	7% P/E	10% P/E+T	15% P/T	Cardil®
1.	498.25	873.20	1072.80	615.75
2.	483.69	103.80	373.30	140.78
3.	1716.74	2482.00	423.10	440.82
4.	1812.68	838.44	1226.28	130.30
5.	1027.29	875.54	1272.30	636.13
6.	1390.64	470.35	1850.60	545.68
7.	840.23	842.82	904.37	778.49
8.	889.01	813.24	427.05	396.93
9.	1837.18	581.81	459.90	262.19
10.	1948.86	313.93	604.92	616.39
11.	528.19	447.76	1673.68	268.70
12.	1979.86	691.43	1235.60	339.67
Average	1246.05	777.86	960.33	430.99
S.D.	598.57	591.68	509.50	209.67
% C.V.	48.04	76.06	53.05	48.65

Table 22. Analysis of variance for ln C_{max} of four formulations of 2 x 120 mg diltiazem hydrochloride tablets for crossover design at $\alpha = 0.05$

Source of Variance	df	SS	MS	Fcal	Ftable	Significance Level
Total	47	24.72	--			
Sequences	3	1.17	0.39	0.57	4.07	NS
Subjects(Sequence)	8	5.49	0.69	2.27	2.27	NS
Periods	3	1.43	0.48	1.58	2.92	NS
Formulations	3	7.56	2.52	8.34	2.92	S
Error	30	9.07	0.30			

NS = Not significant difference at $p>0.05$

S = Significant difference at $p<0.05$

df = Degree of freedom

SS = Sum of square

MS = Mean square

Fcal = Variance ratio

Ftable = F value obtained from the table

Table 23. Duncan's new multiple ranges test calculation for ln C_{max}

Cardil®	10% P/E+T	15% P/T	7% P/E
6.06	6.66	6.87	7.13

90% C.I. of formulation

7% P/E vs. Cardil® = 215.21-395.53%

10% P/E+T vs. Cardil® = 105,45-193.80%

15% P/T vs. Cardil® = 163.24-300.02%

Table 24. Time to peak plasma concentration (t_{max}) of diltiazem hydrochloride following oral administration of four formulations of 2 x 120 mg diltiazem hydrochloride tablets

Subject No.	t_{max} (hr.)			
	7% P/E	10% P/E+T	15% P/T	Cardil®
1.	6.00	4.00	3.00	3.00
2.	5.00	4.00	5.00	5.00
3.	4.00	2.00	6.00	5.00
4.	3.00	3.00	2.00	4.00
5.	6.00	3.00	2.00	3.00
6.	5.00	4.00	2.00	2.00
7.	3.00	4.00	4.00	3.00
8.	5.00	3.00	2.00	2.00
9.	5.00	3.00	4.00	2.00
10.	4.00	2.00	6.00	2.00
11.	6.00	2.00	3.00	5.00
12.	2.00	4.00	4.00	2.00
Average	4.50	3.10	3.58	3.17
S.D.	1.31	0.83	1.51	1.27
% C.V.	29.21	26.36	42.00	40.02

Table 25. Analysis of variance for t_{max} of four formulations of 2 x 120 mg diltiazem hydrochloride tablets for crossover design at $\alpha = 0.05$

Source of Variance	df	SS	MS	Fcal	Ftable	Significance Level
Total	47	83.48	-			
Sequences	3	8.90	2.97	5.47	4.07	NS
Subjects(Sequence)	8	4.33	0.54	0.35	2.27	NS
Periods	3	9.56	3.19	2.06	2.92	NS
Formulations	3	14.23	4.74	3.06	2.92	S
Error	30	46.46	1.55			

NS = Not significant difference at $p>0.05$

S = Significant difference at $p<0.05$

df = Degree of freedom

SS = Sum of square

MS = Mean square

Fcal = Variance ratio

Ftable = F value obtained from the table

Table 26. Duncan's new multiple ranges test calculation for t_{max}

10% P/E+T	Cardil®	15% P/T	7% P/E
3.10	3.17	3.58	4.50

Table 27. Half-life ($t_{1/2}$) of diltiazem hydrochloride following oral administration of four formulations of 2 x 120 mg diltiazem hydrochloride tablets

Subject No.	$t_{1/2}$ (hr.)			
	7% P/E	10% P/E+T	15% P/T	Cardil®
1.	0.99	1.33	0.89	1.66
2.	2.76	4.71	1.54	2.86
3.	1.08	0.86	1.09	1.14
4.	0.92	1.21	0.93	2.01
5.	1.32	1.06	0.90	1.70
6.	1.15	1.21	0.89	1.22
7.	1.90	1.22	0.95	1.04
8.	1.46	1.04	1.80	1.69
9.	1.47	1.14	1.52	2.45
10.	0.95	1.50	1.51	1.74
11.	1.19	1.43	0.92	1.75
12.	1.13	1.51	1.16	1.64
Average	1.36	1.52	1.17	1.74
S.D.	0.52	1.02	0.33	0.52
% C.V.	38.31	67.54	27.95	29.72

Table 28. Analysis of variance for $t_{1/2}$ of four formulations of 2 x 120 mg diltiazem hydrochloride tablets for crossover design at $\alpha = 0.05$

Source of Variance	df	SS	MS	Fcal	Ftable	Significance Level
Total	47	20.74	-			
Sequences	3	1.81	0.60	0.52	4.07	NS
Subjects(Sequence)	8	9.39	1.17	4.87	2.27	S
Periods	3	0.21	0.07	0.29	2.92	NS
Formulations	3	2.10	0.70	2.91	2.92	NS
Error	30	7.23	0.24			

NS = Not significant difference at $p>0.05$

S = Significant difference at $p<0.05$

df = Degree of freedom

SS = Sum of square

MS = Mean square

Fcal = Variance ratio

Ftable = F value obtained from the table

Table 29. Pharmacokinetic parameters (Average ± S.D.) of diltiazem hydrochloride of 12 rabbits following oral administration of 2 x120 mg diltiazem hydrochloride tablets

Parameters	Formulations			
	7% P/E	10% P/E+T	15% P/T	Cardil®
AUC (ng-hr./mL)	5459.20 ± 2153.65 (181.19 - 309.50%)*	3364.38 ± 1630.10 (109.56 - 186.54%)*	3825.08 ± 1521.89 (132.59 - 225.74%)*	2626.76 ± 1735.66
C_{max} (ng/mL)	1246.05 ± 598.57 (215.21 - 395.53%)*	777.86 ± 591.68 (105.45 - 193.80%)*	960.33 ± 509.49 (163.24 - 300.02%)*	430.99 ± 209.67
t_{max} (hr.)	4.50 ± 1.31	3.10 ± 0.83	3.58 ± 1.51	3.17 ± 1.27
$t_{1/2}$ (hr.)	1.36 ± 38.31	1.52 ± 67.54	1.17 ± 27.95	1.74 ± 29.72

* = 90% Confidence interval of parameter ratio of test formulation to reference product (Cardil®)