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APPENDICES

APPENDIX A

Table 30. Typical calibration curve data for determination of diltiazem hydrochloride in phosphate buffer pH 7.2 estimated using linear regression[1]

Concentration (mcg/mL)	Absorbance	Inversely Estimated Concentration (mcg/mL)[2]	% Recovery[3]
2	0.098	2.004	100.19
4	0.203	4.004	100.10
6	0.308	6.004	100.06
8	0.413	8.004	100.05
10	0.518	10.004	100.04
12	0.623	12.004	100.03
14	0.728	14.004	100.03
16	0.833	16.004	100.02
18	0.938	18.004	100.02
		Average	100.06
		S.D.	0.05
		% C.V.[4]	0.05

1. $r^2 = 1$, Y = 0.0525 - 0.0072 (Y = Absorbance, X = Known concentration)
2. Inversely estimated concentration = (Absorbance + 0.0072)/0.0525
3. % Recovery = (Inversely estimated concentration / Known concentration) x 100
4. % C.V. = (S.D./Average) x 100

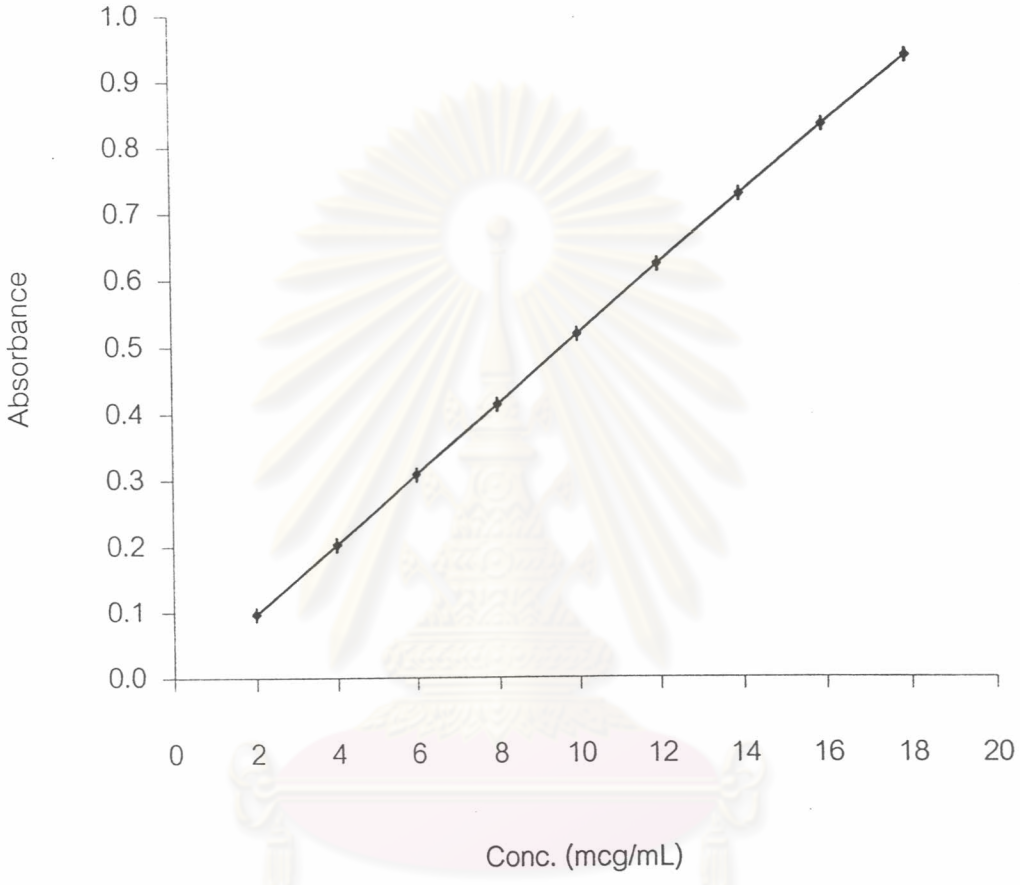

Figure 25. Typical calibration curve for determination of diltiazem hydrochloride in phosphate buffer pH 7.2

Table 31. Typical calibration curve data for determination of diltiazem hydrochloride in 0.1N hydrochloric acid pH 1.2 estimated using linear regression[1]

Concentration (mcg/mL)	Absorbance	Inversely Estimated Concentration (mcg/mL)[2]	% Recovery[3]
2	0.107	2.013	100.66
4	0.212	3.983	99.58
6	0.318	5.972	99.53
8	0.427	8.017	100.21
10	0.531	9.968	99.68
12	0.641	12.032	100.27
14	0.745	13.983	99.88
16	0.853	16.009	100.06
18	0.957	17.961	99.78
		Average	99.96
		S.D.	0.37
		% C.V.[4]	0.37

1. $r^2 = 1$, $Y = 0.0533X - 0.0003$ (Y = Absorbance, X = Known concentration)
2. Inversely estimated concentration = (Absorbance – 0.0003)/0.0533
3. % Recovery = (Inversely estimated concentration / Known concentration) x 100
4. % C.V. = (S.D./Average) x 100

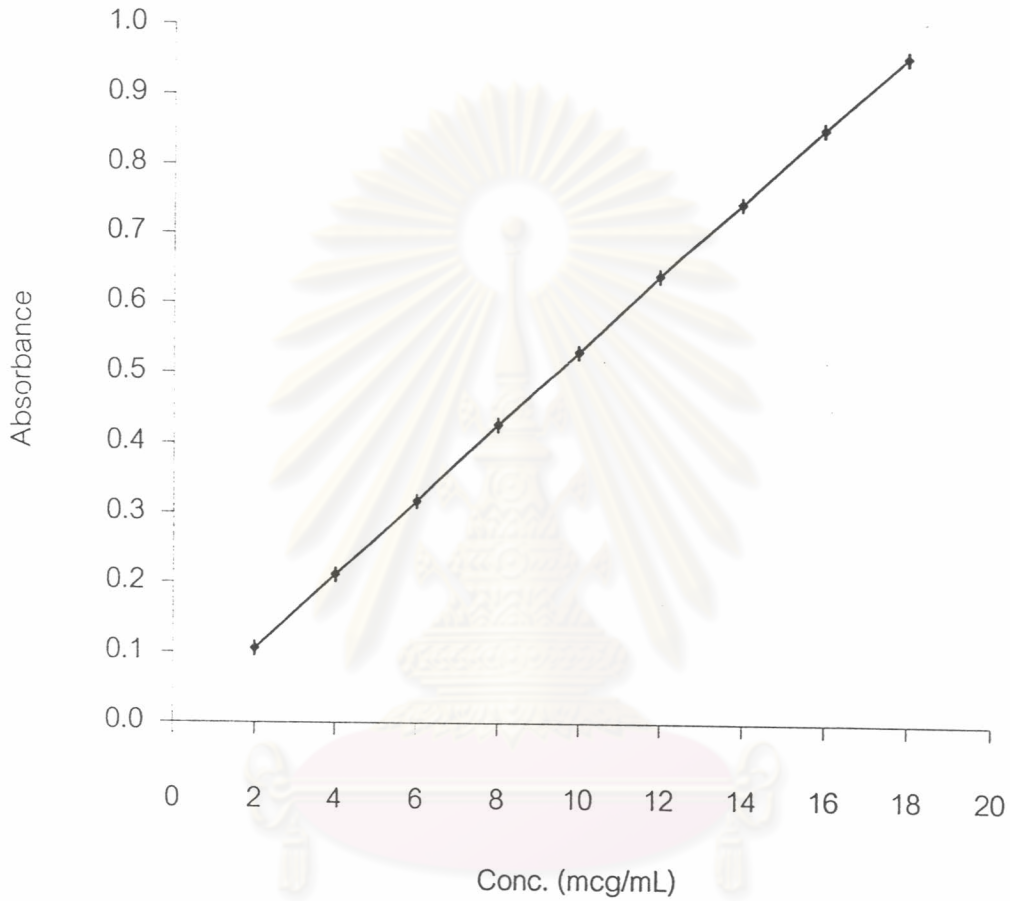

Figure 26. Typical calibration curve for determination of diltiazem hydrochloride in 0.1N hydrochloric acid pH 1.2

Table 32. Typical calibration curve data for determination of diltiazem hydrochloride in water estimated using linear regression[1]

Concentration (mcg/mL)	Absorbance	Inversely Estimated Concentration (mcg/mL)[2]	% Recovery[3]
2	0.110	1.996	99.81
4	0.216	3.996	99.91
6	0.322	5.996	99.94
8	0.428	7.996	99.95
10	0.534	9.996	99.96
12	0.640	11.996	99.97
14	0.746	13.996	99.97
16	0.852	15.996	99.98
18	0.958	17.996	99.98
		Average	99.94
		S.D.	0.05
		% C.V.[4]	0.05

1. $r^2 = 1$, $Y = 0.053X + 0.0042$ (Y = Absorbance, X = Known concentration)
2. Inversely estimated concentration = (Absorbance – 0.0042)/0.053
3. % Recovery = (Inversely estimated concentration / Known concentration) x 100
4. % C.V. = (S.D./Average) x 100

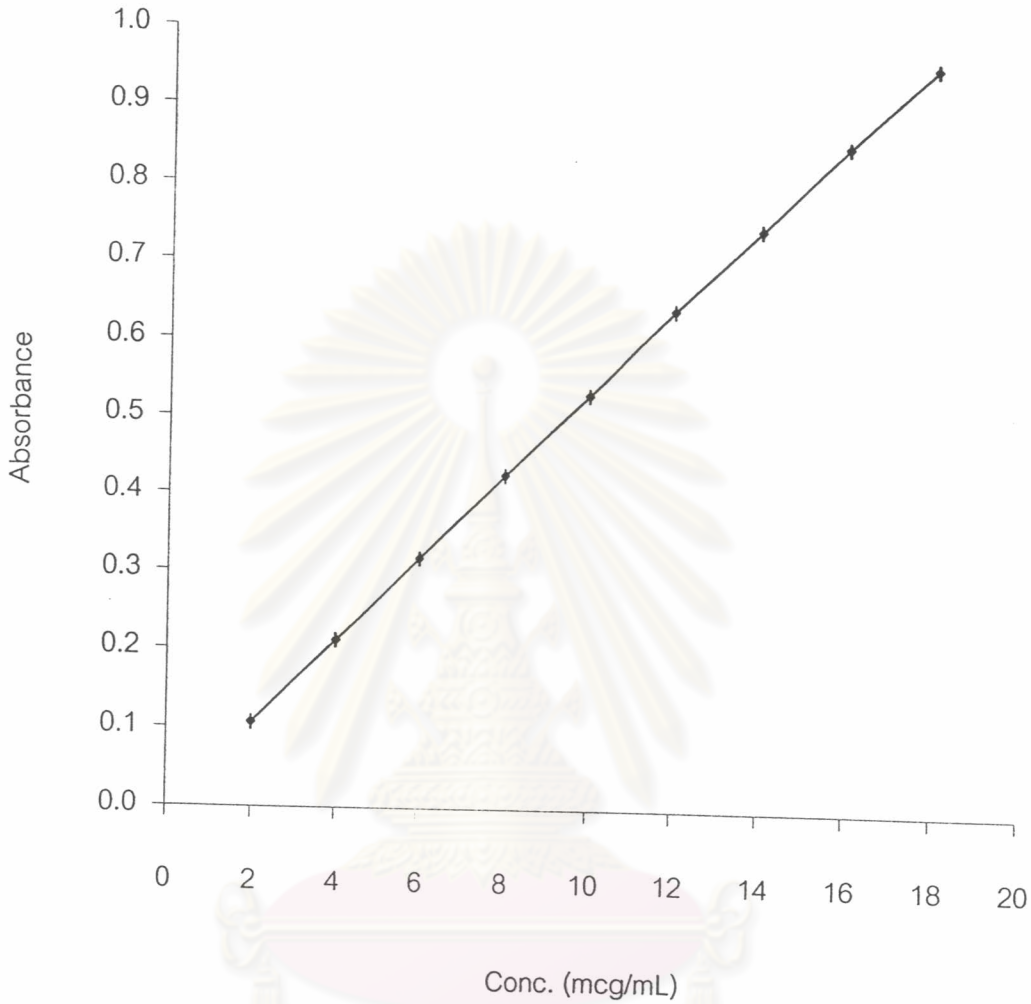

Figure 27. Typical calibration curve for determination of diltiazem hydrochloride in water

Table 33. Accuracy of analytical method for determination of diltiazem hydrochloride in phosphate buffer pH 7.2 (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% Recovery
3	3.07	102.33
9	9.05	100.56
15	15.04	100.27
	Average	101.05
	S.D.	1.12
	% C.V.	1.11

Table 34. Within run precision of analytical method for determination of diltiazem hydrochloride in phosphate buffer pH 7.2 (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% C.V.
3	3.05 ± 0.05	1.57
9	9.06 ± 0.11	1.17
15	15.14 ± 0.22	1.43

Table 35. Between run precision of analytical method for determination of diltiazem hydrochloride in phosphate buffer pH 7.2 (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% C.V.
3	2.90 ± 0.05	1.65
9	8.98 ± 0.08	0.86
15	15.06 ± 0.11	0.76

Table 36. Accuracy of analytical method for determination of diltiazem hydrochloride in 0.1N hydrochloric acid pH 1.2 (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% Recovery
3	2.98	99.33
9	9.06	100.67
15	14.93	99.53
	Average	99.84
	S.D.	0.72
	% C.V.	0.72

Table 37. Within run precision of analytical method for determination of diltiazem hydrochloride in 0.1N hydrochloric acid pH 1.2 (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% C.V.
3	3.07 ± 0.06	1.97
9	8.93 ± 0.17	1.95
15	15.05 ± 0.27	1.77

Table 38. Between run precision of analytical method for determination of diltiazem hydrochloride in 0.1N hydrochloric acid pH 1.2 (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% C.V.
3	3.03 ± 0.06	1.86
9	9.05 ± 0.08	0.84
15	15.05 ± 0.20	1.33

Table 39. Accuracy of analytical method for determination of diltiazem hydrochloride in water (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% Recovery
3	3.05	101.67
9	8.94	99.33
15	14.97	99.80
	Average	100.27
	S.D.	1.23
	% C.V.	1.23

Table 40. Within run precision of analytical method for determination of diltiazem hydrochloride in water (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% C.V.
3	2.89 ± 0.06	1.99
9	9.07 ± 0.17	1.87
15	15.04 ± 0.19	1.28

Table 41. Between run precision of analytical method for determination of diltiazem hydrochloride in water (n=3)

Concentration (mcg/mL)	Inversely Estimated Concentration (mcg/mL)	% C.V.
3	3.08 ± 0.06	1.87
9	9.27 ± 0.16	1.73
15	15.19 ± 0.23	1.52

Table 42. Linearity of analytical method for determination of diltiazem hydrochloride in plasma (n=3)

Concentration (ng/mL)	Peak Area Ratio	Inversely Estimated Concentration (ng/mL)	% Recovery
70	0.038	80.38	114.83
100	0.062	99.00	99.00
200	0.158	173.08	86.54
400	0.416	370.85	92.71
1000	1.243	1007.23	100.72
1500	1.851	1474.92	98.33
2000	2.434	1923.31	96.17
		Average	98.33
		S.D.	8.69
		% C.V.	8.84

$r^2 = 0.9991$, $Y = 0.0013X-0.0665$

where Y = Peak area ratio, X = Concentration of diltiazem hydrochloride in plasma

Table 43. Percent recovery of analytical method for determination of diltiazem hydrochloride in plasma (n=3)

Concentration (ng/mL)	Peak Area Ratio	Inversely Estimated Concentration (ng/mL)	% Recovery
160	0.146	163.46	102.16
800	0.924	761.92	95.24
1600	1.793	1430.39	89.40
		Average	95.60
		S.D.	6.39
		% C.V.	6.68

Table 44. Within run precision of analytical method for determination of diltiazem hydrochloride in plasma (n=3)

Concentration (ng/mL)	Inversely Estimated Concentration (ng/mL)			Average ± S.D.	% C.V.
	1	2	3		
160	155.00	161.15	165.01	160.38 ± 5.044	3.14
800	761.15	734.23	808.08	767.82 ± 37.37	4.87
1600	1444.23	1492.69	1389.62	1442.18 ± 51.57	3.58

Table 45. Between run precision of analytical method for determination of diltiazem hydrochloride in plasma (n=3)

Concentration (ng/mL)	Inversely Estimated Concentration (ng/mL)			Average ± S.D.	% C.V.
	1	2	3		
160	160.38	175.77	169.62	168.59 ± 7.74	4.59
800	681.92	825.77	751.15	752.95 ± 71.94	9.55
1600	1441.92	1670.39	1426.54	1512.95 ± 136.56	9.03

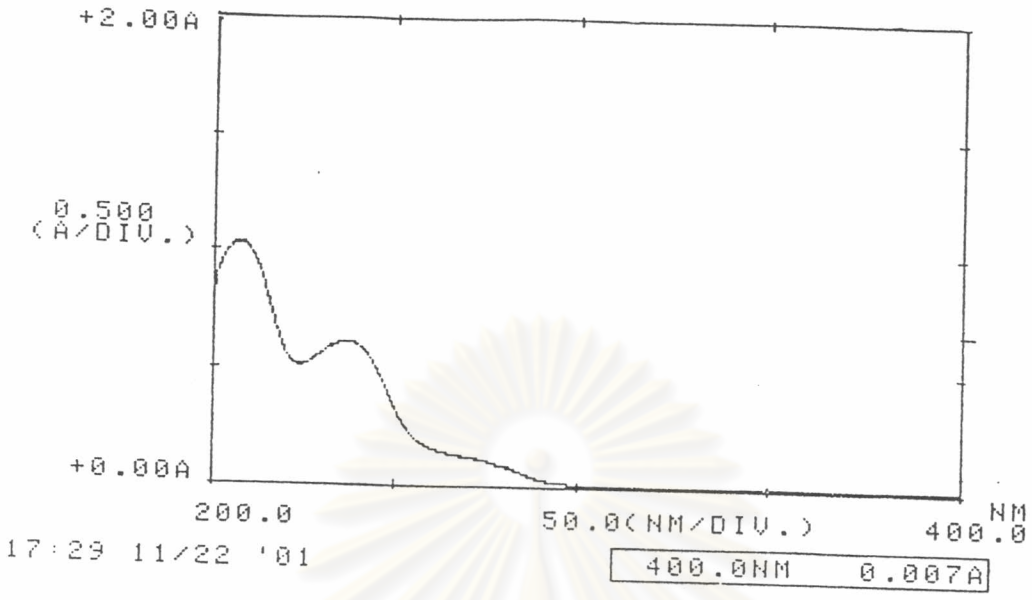

Figure 28. The ultraviolet spectrum of diltiazem hydrochloride at the maximum wavelength of 237 nm. in phosphate buffer pH 7.2

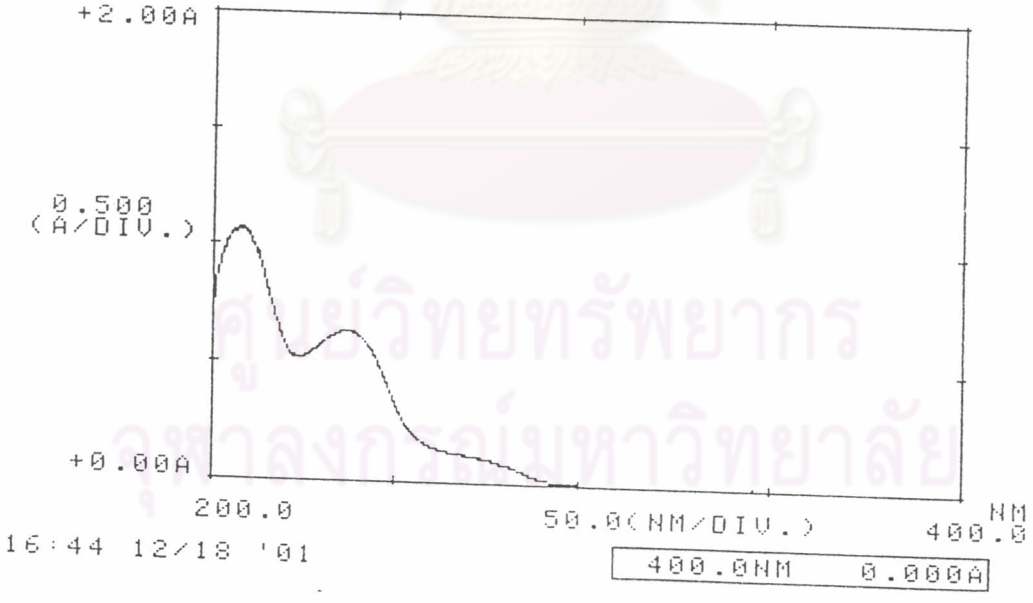

Figure 29. The ultraviolet spectrum of diltiazem hydrochloride at the maximum wavelength of 237 nm. in 0.1N hydrochloric acid pH 1.2

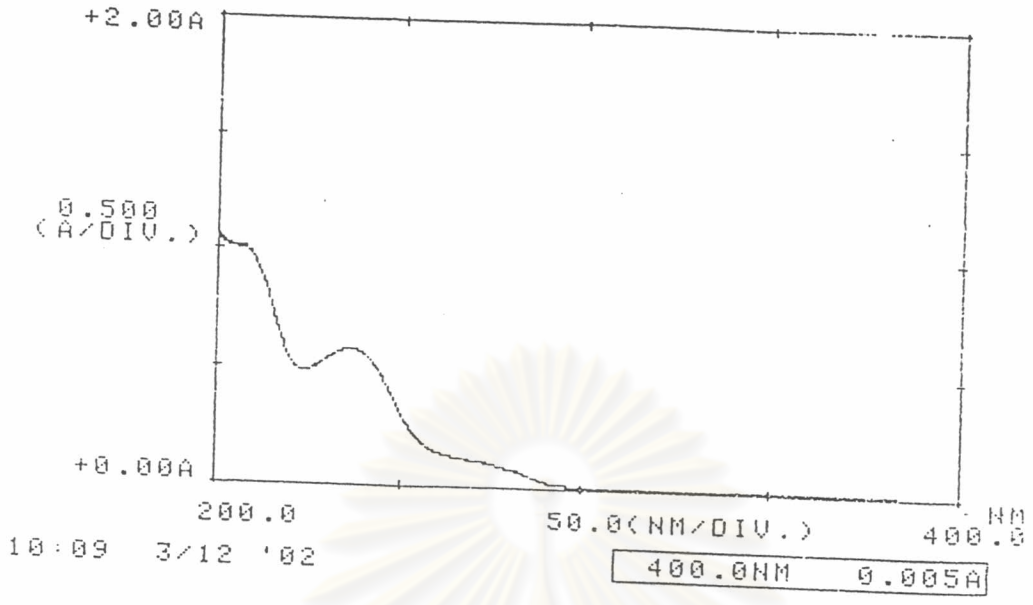

Figure 30. The ultraviolet spectrum of diltiazem hydrochloride at the maximum wavelength of 237 nm. in water

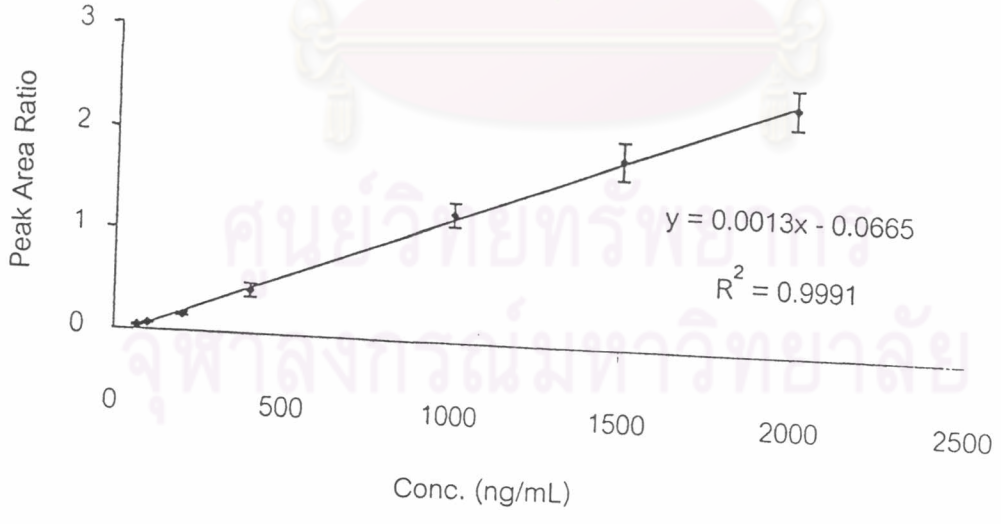

Figure 31. Typical calibration curve for determination of diltiazem hydrochloride in plasma

APPENDIX B

STATISTICS

Average

$$\bar{X} = \sum X/n$$

Standard deviation (S.D.)

$$S.D. = \sqrt{\sum (x - \bar{x})^2 / n-1}$$

Coefficient of variation (C.V.)

$$C.V. = S.D./Average$$

Area under the concentration time curve (AUC_0^{∞})

$$AUC_0^{\infty} = \frac{\sum (C_{n-1} + C_n)(t_n - t_{n-1})}{2} + \frac{\hat{C}}{K}$$

Elimination rate constant (K)

$$K = \frac{\ln C_1 - \ln C_2}{t_2 - t_1}$$

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

$$t_{1/2} = 0.693/K$$

Analysis of variance

The experimental design is as follow :

Sequence	Subject No.	Period			
		1	2	3	4
I	1-3	A	B	C	D
II	4-6	B	C	D	A
III	7-9	C	D	A	B
IV	10-12	D	A	B	C

Where A, B, C and D = formulations 1, 2, 3 and 4, respectively.

In statistical terms the calculations to set up an analysis of variance table are as follow:

Source of Variation	df	SS	MS	Variance Ratio
Total	g.n.t-1	SSTO	-	-
Sequences	g-1	SSG	MSG	MSG / MSS
Subjects(Sequence)	g(n-1)	SSS	MSS	MSS / MSE
Periods	p-1	SSP	MSP	MSP / MSE
Formulations	f-1	SSF	MSF	MSF / MSE
Error	(gn-2)(t-1)	SSE	MSE	-

df = degree of freedom

g = number of groups or treatment sequences

n = number of subjects per group or treatment sequence

t = number of treatment

90% confidence interval

Ln-transformed data

$$90\%\ CI = (\bar{X}_T - \bar{X}_R) \pm (t_{0.1,df} \times S.E)$$

$$S.E. = \sqrt{2MSE/n}$$

$$\%\ lower\ limit = \left\{ e^{\left[(\bar{x}_T - \bar{x}_R) - (t_{0.1,df} \times S.E.)\right]} \right\} \times 100$$

$$\%\ upper\ limit = \left\{ e^{\left[(\bar{x}_T - \bar{x}_R) + (t_{0.1,df} \times S.E.)\right]} \right\} \times 100$$

Calculation of analysis of variance of ln AUC

Sequence	Subject No.	Period				Sum
1	1	7.68	8.29	8.24	8.74	32.93
	2	8.20	7.22	7.72	7.12	30.27
	3	8.70	8.96	7.49	7.89	33.04
2	4	8.12	8.41	6.86	9.00	32.47
	5	8.26	8.24	8.67	8.54	33.70
	6	7.60	8.67	7.70	8.76	32.73
3	7	8.25	8.20	8.70	8.16	33.30
	8	7.79	7.56	8.60	8.29	32.20
	9	7.71	7.38	9.10	7.89	32.07
4	10	7.74	8.40	7.70	8.15	31.98
	11	7.28	7.61	8.03	8.64	31.57
	12	7.39	8.90	7.75	8.76	32.80
Sum		94.80	97.79	96.56	99.92	389.07

1. CT = 3,153.58
2. SSTOT = 14.34
3. SSG = 0.39
4. SSS = 1.87
5. SSP = 1.16
6. SSF = 4.06
7. SSE = 6.86

Calculation of analysis of variance of ln C_{max}

Sequence	Subject No.	Period				Sum
1	1	6.21	6.77	6.98	6.42	26.38
	2	6.18	4.64	5.92	4.95	21.69
	3	7.45	7.82	6.05	6.09	27.40
2	4	6.73	7.11	4.87	7.50	26.22
	5	6.78	7.15	6.46	6.94	27.31
	6	6.15	7.52	6.30	7.24	27.22
3	7	6.81	6.66	6.73	6.74	26.94
	8	6.06	5.98	6.79	6.70	25.53
	9	6.13	5.57	7.52	6.37	25.58
4	10	6.42	7.58	5.75	6.41	26.15
	11	5.59	6.27	6.10	7.42	25.39
	12	5.83	7.59	6.54	7.12	27.08
Sum		76.34	80.66	76.01	79.88	312.89

1. CT = 2039.63
2. SSTOT = 24.72
3. SSG = 1.17
4. SSS = 5.49
5. SSP = 1.43
6. SSF = 7.56
7. SSE = 9.07

Duncan's new multiple ranges test calculation

P	2	3	4	
q (n,df)	2.89	3.04	3.12	
R_p	0.4010	0.4218	0.4329	
Parameter	.....	.....	.....	.....
$\Sigma(R_p$, parameter)	.....	.....	.....	.....

P = Treatment (2, 3, 4,...)

q = Tabulated Values from multiple range test table $\alpha = 0.05$

R_p = q x S

S = $\sqrt{2MSE/n}$

n = Number of subject (= 12)

VITA

Miss Aninda Akkarasrisawad was born on August 3, 1973 in Bangkok. She received Bachelor Degree of Science in Pharmacy from Faculty of Pharmacy, Mahidol University, Bangkok, Thailand in 1997. After graduation, she worked at Kasetsombun Hospital from 1997 to 2000 before entering the Master's degree program in Pharmacy at Chulalongkorn University.