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APPENDICES

APPENDIX A

TEST PRODUCTS

Table 54 Test Product Information

Trade name	Manufacturers	Mfg. Date	Exp.Date	Batch no.
Docline ®	Atlantic	31/10/88	31/10/92	881093
Doxylin ®	T.P. Drug Laboratories	-	12/92	331040
Medomycin ®	Medochemie (Cyprus)*	1/88	1/92	2103
Servidoxyne 100®	Mulpro Co.Ltd.	19/5/87	5/90	019-08
Siadocin®	Siam Bheasach Co.Ltd.	2/9/88	30/11/91	11R177
Specy®	Pharspec Co. Ltd.	3/1/89	3/92	P088752
Torymycin®	Chinta Trading Co.Ltd.	4/1/89	2/91	742395
Vibramycin®	Pfizer International Co. Ltd. (Phillipine)	-	5/93	809-58008

* Distributed by Biopharm

APPENDIX B

PREPARATION OF REAGENTS

1. 0.1 M Monobasic sodium phosphate:

11.998 g of Monobasic sodium phosphate was dissolved in 1000 ml of distilled water.

2. 0.1 M Sodium sulfite buffer pH 6.2

1.2604 g of sodium sulfite was dissolved in 100 ml of distilled water and adjusted pH to 6.2 with sulfuric acid

3. 0.1 M Sodium citrate:

29.41 g of Sodium citrate was dissolved in 1000 ml of distilled water.

APPENDIX C

DETERMINATION OF DOXYCYCLINE CONTENT IN CAPSULES (40)

Prepare a standard doxycycline solution by dissolving an accurately weighed quantity of standard doxycycline hydrochloride in water to obtain a solution having a known concentration of about 1000 µg ($C_{22}H_{24}N_2O_8$) per ml

Calculate the quantity, in mg, of doxycycline ($C_{22}H_{24}N_2O_8$) in the portion of capsules taken by the formula; $0.05\ C(r_u/r_s)$ in which C is the concentration, in µg/ml of doxycycline ($C_{22}H_{24}N_2O_8$) in the standard preparation, and r_u and r_s are the peak responses obtained from capsules and the standard solution, respectively.

APPENDIX D

CRITERIA FOR DETERMINATION OF CONTENT UNIFORMITY (41)

The requirements for content uniformity are met if the amount of the active ingredient is not less than nine of ten capsules lies within the range of 85.0 percent to 115.0 percent of the label claim and no capsule is outside the range of 75.0 percent to 125.0 percent of label claim and the relative standard deviation of the ten capsules is less than or equal to 6.0 percent.

If two or three capsules are outside the range of 85.0 percent to 115.0 percent of label claim but not outside the range of 75.0 percent to 125.0 percent of label claim, or if the relative standard deviation is greater than 6.0 percent or if both conditions prevail, twenty additional units are tested. The requirements are met if not more than three units of the thirty are outside the range of 85.0 percent to 115.0 percent of label claim and no capsule is outside the range of 75.0 percent to 125.0 percent of label claim, and the relative standard deviation of the thirty capsules does not exceed 7.8 percent.

$$\text{Relative standard deviation} = \frac{100\ S}{\overline{X}}$$

$$\text{Wher } S = \frac{\sum (x_i - \overline{X})^2}{n - 1}$$

$\overline{X}$ = mean of the values obtained from the capsules tested, expressed as a percentage of label claim

n = number of capsules tested.

$x_1, x_2, x_3 \ldots\ldots x_n$ = individual values (x_i) of the capsules tested, expressed as a percentage of the label claim.

APPENDIX E

SUBJECTS

Table 55 Physiological Characteristics of the Subjects.

Subject No.	Age (year)	Height (cm)	Weight (Kg)
1	20	170	65
2	19	175	58
3	21	165	52.5
4	23	168	51
5	21	160	48
6	20	165	47
7	18	168	57
8	20	165	52
9	22	176	77
10	23	165	62
11	18	164	64
12	23	173	46
13	18	171	60
14	18	164	55
15	18	166	51
16	18	164	56
17	18	157	50
18	23	165	50
19	18	170	52
20	24	173	59
range	18.24	157-176	46-77
mean	20.15	167.20	55.62
S.D.	2.18	4.86	7.47

Table 56 Biochemical Laboratory Results

Test	Normal value	Subject									
		1	2	3	4	5	6	7	8	9	10
Glucose	65-110 mg/dl	82	87	88	82	80	92	86	75	86	89
Creatinine	0.5-2.0 mg/dl	1.1	1.1	0.9	0.9	0.9	1.0	1.0	0.9	1.1	1.1
Uric acid	2.4-7.0 mg/dl	5.3	6.4	5.6	4.1	4.2	6.4	5.6	5.0	5.6	6.6
Total bilirubin	0.0-1.0 mg/dl	0.59	0.61	0.46	0.76	0.73	0.52	0.65	0.44	0.5	0.65
Direct bilirubin	0.0-0.4 mg/dl	0.26	0.17	0.24	0.20	0.23	0.25	0.24	0.20	0.35	0.24
Alk.phosphatase	98 - 279 U/L	160	181	191	138	128	108	162	148	149	128
SGOT	0 - 38 U/L	21	20	15	21	17	20	17	17	19	18
SGPT	0 - 38 U/L	18	12	10	8	8	13	11	10	20	11
Cholesterol	150-250 mg/dl	146	185	159	164	197	244	150	179	244	225
Triglycerides	40-155 mg/dl	57	33	68	36	48	48	50	57	120	74

Table 56 (cont) : Biochemical Laboratory Results

Test	Normal value	Subject									
		11	12	13	14	15	16	17	18	19	20
Glucose	65-110 mg/dl	80	80	88	78	87	77	74	88	82	94
Creatinine	0.5-2.0 mg/dl	1.0	1.0	1.0	0.9	0.9	0.9	0.9	0.9	0.8	0.8
Uric acid	2.4-7.0 mg/dl	5.8	6.1	5.2	4.6	6.4	6.7	4.7	5.4	4.1	4.7
Total bilirubin	0.0-1.0 mg/dl	0.75	0.83	1.06	0.95	1.13	0.66	0.7	0.98	0.79	0.66
Direct bilirubin	0.0-0.4 mg/dl	0.24	0.27	0.23	0.28	0.24	0.24	0.23	0.26	0.22	0.19
Alk.phosphatase	98 - 279 U/L	185	154	293	180	193	146	52	155	436	228
SGOT	0 - 38 U/L	13	17	20	22	15	18	39	17	16	28
SGPT	0 - 38 U/L	12	11	8	20	9	12	86	13	5	37
Cholesterol	150-250 mg/dl	210	142	142	196	122	196	148	174	126	242
Triglycerides	40-155 mg/dl	76	45	40	88	69	45	58	63	37	57

Table 57 Hematological Laboratory Results

Test	Normal value	Subject									
		1	2	3	4	5	6	7	8	9	10
Hemoglobin	14-18 gm%	11.2	13.6	15.2	13.2	15.6	14.1	13.9	14.8	14.9	15.2
W.B.C	4500-11000 cell/mm^3	8,400	4,100	11,000	7,600	7,600	5,300	5,100	5,200	4,900	8,500
Neutrophils	40 - 60%	62	55	77	50	60	38	59	65	43	48
Eosinophils	1 - 3 %	5	2	-	1	13	3	2	1	4	6
Basophils	0 - 1 %	-	-	-	-	-	-	-	-	-	-
Lymphocytes	20 - 40 %	27	43	23	43	27	54	39	31	50	45
Monocytes	4 - 8 %	6	-	-	6	-	5	-	3	3	1

Table 57 (cont) : Hematological Laboratory Results

Test	Normal value	Subject									
		11	12	13	14	15	16	17	18	19	20
Hemoglobin	14-18 gm%	16.6	15.4	13.0	15.8	12.9	13.6	15.8	15.6	14.2	14.3
W.B.C	4500-11000 cell/mm^3	7,300	7,000	8,100	6,600	7,100	6,800	5,700	5,900	4,900	4,800
Neutrophils	40 - 60%	64	37	60	50	45	58	44	48	47	63
Eosinophils	1 - 3 %	3	2	4	14	13	16	9	2	2	5
Basophils	0 - 1 %	1	-	-	1	1	-	-	-	-	-
Lymphocytes	20 - 40 %	32	31	33	26	40	26	43	43	47	32
Monocytes	4 - 8 %	-	-	3	9	1	-	4	7	4	-

Table 58 Urinalysis Results

Test	Subject									
	1	2	3	4	5	6	7	8	9	10
Microscopic : R.B.C	0	0	0	0	0	0-1	0-1	0	2-3	0
W.B.C	0	0-1	1-2	0	2-3	1-2	1-2	1-2	0	0-1
Epithelium	1-2	0-1	0-1	2-3	3-5	0	1-2	2-3	2-3	0
Casts	-	-	-	-	-	-	-	-	-	Ca oxalate 1+
Crystals	-	-	-	-	Amorphous 1+	-	-	-	-	-
Organism	-	-	bact 1+	-	-	-	-	bact 1+	-	-
Others	-	-	mucous 1+	-	mucous 1+	mocous 2+	mosous 1+	-	-	-
Chemistry : Albumin	-ve	-ve	-ve	-ve	-ve	Trace	-ve	-ve	-ve	-ve
Sugar	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Occult blood	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Note : -ve = negative , + ve = positive

TABLE 58 (cont) : Urinalysis Results

Test	Subject									
	11	12	13	14	15	16	17	18	19	20
Microscopic : R.B.C	0	0	0	0	0	0	5-7	0	0	3-5
W.B.C	0	1-2	1-2	2-3	0	0	0	0	1-2	3-5
Epithelium	1-2	1-2	2-3	2-3	1-2	1-2	1-2	1-2	2-3	2-3
Casts	-	-	-	-	-	-	-	-	-	-
Crystals	-	-	-	-	-	-	-	-	-	-
Organism	-	-	-	-	bact 1+	-	-	-	bact 1+	-
Others	mucous 2+	mucous 2+	-	mucous 2+	-	-	mosous 2+	-	-	-
Chemistry : Albumin	-ve	Trace	-ve	Trace	-ve	-ve	Trace	-ve	-ve	-ve
Sugar	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Occult blood	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve

Note: -ve = negative, +ve = poositive

APPENDIX F

CALIBRATION CURVE DETERMINATION

The calibration curve and data for doxycycline concentrations in dissolution medium and human plasma were presented in tables 59, 60, and figures 6, 7 respectively.

Table 59 Calibration Curve Data for Doxycycline Concentrations in Water Estimated Using Linear Regression[1]

Standard No.	Conc. [µg/ml]	Absorbance at 276 nm	Inversely estimated[2] concentration [µg/ml]	% THEORY[3]
1	0.761	0.079	0.723	94.97
2	1.522	0.115	1.613	106.03
3	3.043	0.168	2.919	95.91
4	4.565	0.240	4.709	103.15
5	6.086	0.293	6.000	98.57
6	7.608	0.363	7.738	101.71
7	9.130	0.419	9.130	100.00
8	10.651	0.471	10.405	97.68
9	12.173	0.538	12.075	99.19
10	13.695	0.619	14.086	102.86
11	15.216	0.668	15.292	100.50
12	16.738	0.717	16.498	98.57
13	18.259	0.785	18.193	99.64
14	19.781	0.843	19.616	99.17
15	21.303	0.920	21.534	101.09
			Mean	99.94
			S.D.	2.73
			C.V.[4]	2.73

1. $R^2 = 0.999, \quad Y = 0.0404\,X + 0.0500$
2. Inversely estimated concentration = [Absorbance - 0.0500]/0.0404
3. $\%\ \text{Theory} = \dfrac{\text{Inversely estimated concentration} \times 100}{\text{Known concentration}}$
4. $\%\ \text{C.V.} = \dfrac{\text{S.D.} \times 100}{\text{mean}}$

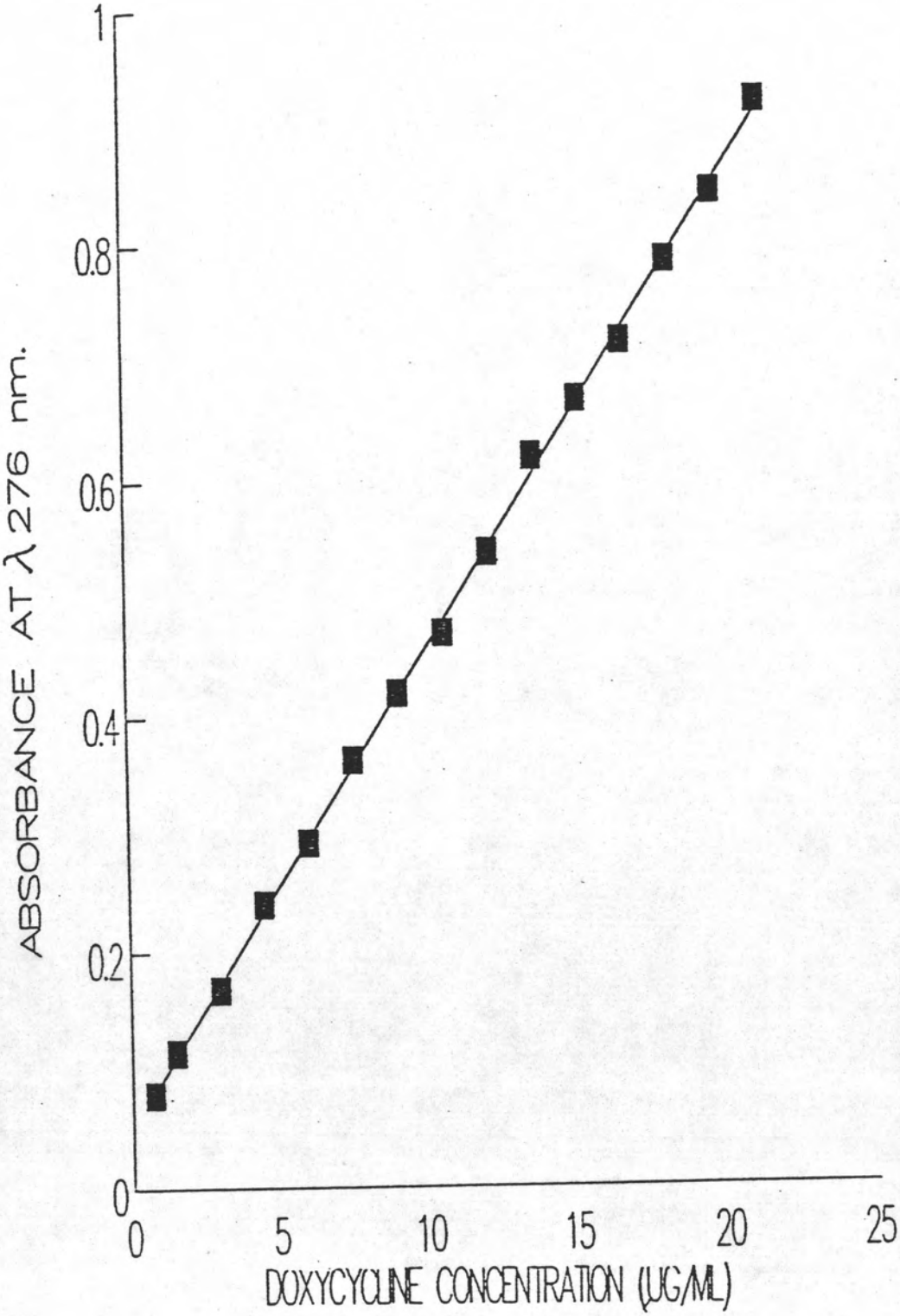


Figure 6 Calibration Curve for Doxycycline Concentrations in Deionized Water

Table 60 Calibration Curve Data for Doxycycline Concentrations in Human Plasma Estimated Using Linear Regression[1]

Standard No.	Concentration (µg/ml)	Peak height Ratio[a]	Inversely estimated[2] concentration(µg/ml)	% Theory[3]
1	0	0	-0.113	-
2	0.10	0.483	0.105	105.88
3	0.20	0.656	0.184	92.19
4	0.50	1.380	0.512	102.55
5	1.00	2.319	0.938	93.88
6	1.50	4.197	1.790	119.37
7	2.00	5.013	2.160	108.03
8	2.50	5.144	2.219	88.79
			Mean	101.53
			S.D	9.91
			C.V.4	9.76

1. R^2 = 0.968, Y = 2.2048 X + 0.2496
2. Inversely estimated concentration = [Peak height ratio - 0.2496]/2.2048
3. % Theory = $\frac{\text{Inversely estimated concentration} \times 100}{\text{Known concentration}}$
4. % C.V. = $\frac{\text{S.D.} \times 100}{\text{mean}}$

a = Doxycycline/Tetracycline

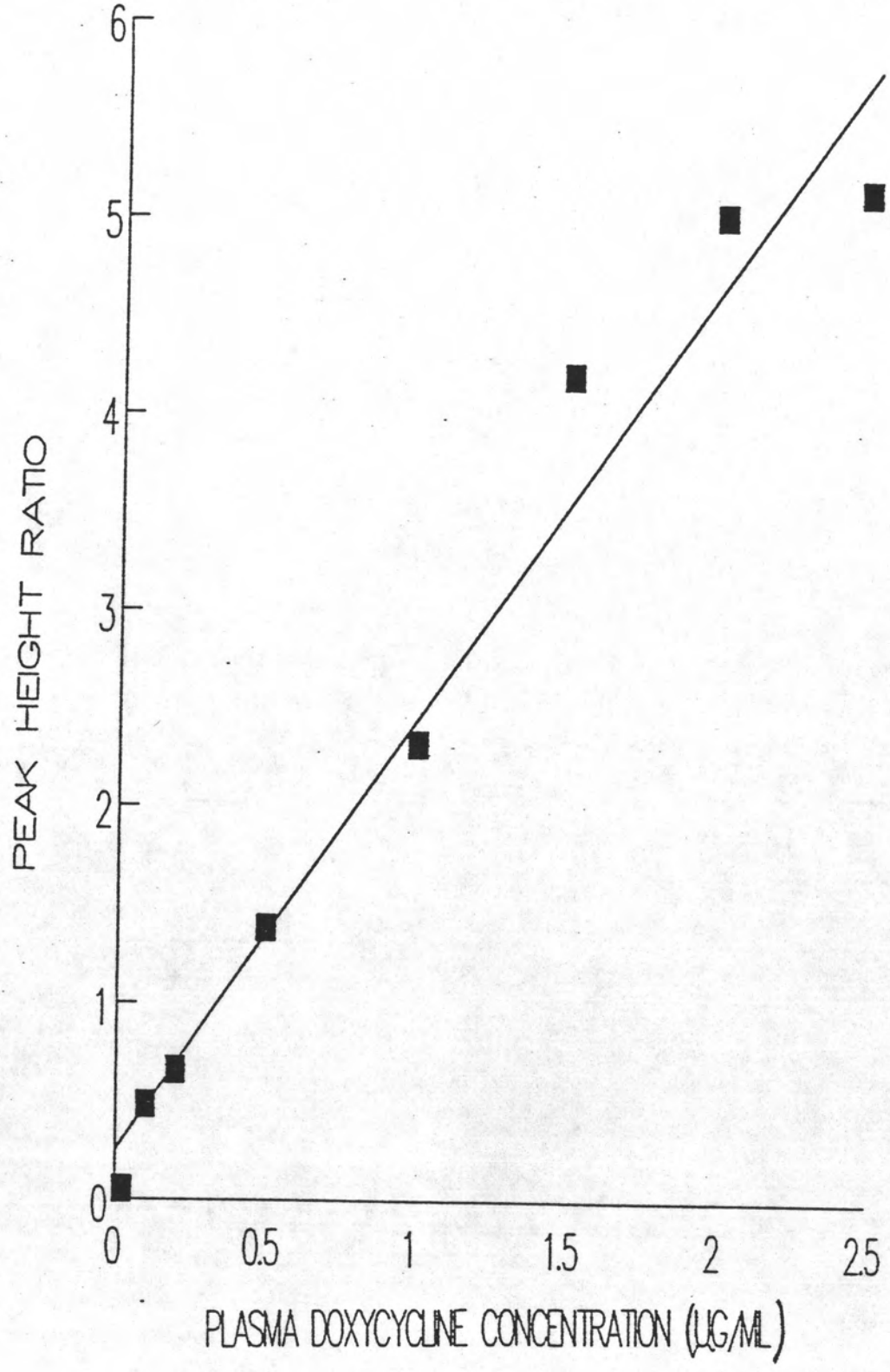


Figure 7 Calibration Curve for Doxycycline Concentration in Human Plasma

APPENDIX G

DATA OF DOXYCYCLINE DISSOLVED IN MEDIUM OF BRAND A TO H

The dissolution data of all eight commercial brands of doxycycline capsules [A, B, C, D, E, F, G, and H] were presented in table 61 to table 68 respectively.

Table 61 Dissolution Data of Brand A in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	2.65	3.74	2.85	6.52	3.67	9.80	4.87	2.54
10.0	15.44	21.79	20.32	25.20	17.88	28.62	21.54	4.39
15.0	92.53	90.09	85.70	87.33	88.96	93.18	89.63	2.66
20.0	94.48	94.81	90.09	89.12	94.16	101.15	93.97	3.90
25.0	92.37	96.44	92.86	93.51	94.48	80.50	91.69	5.18
30.0	97.90	92.70	88.47	93.67	96.11	99.36	94.70	3.60
45.0	91.07	90.91	92.05	91.72	95.79	95.62	92.86	2.05
60.0	85.38	92.53	87.33	93.02	90.74	88.79	89.63	2.75
90.0	81.96	91.72	83.91	91.56	87.65	95.46	88.71	4.70
120.0	86.84	90.26	85.54	88.79	86.35	83.43	86.87	2.20

Table 62 Dissolution Data of Brand B in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	50.74	28.94	29.10	37.72	59.35	36.91	40.46	11.14
10.0	103.76	91.56	100.67	97.41	109.12	95.79	99.72	5.67
15.0	106.19	107.66	108.15	100.67	107.50	95.79	104.32	4.57
20.0	114.65	101.15	108.47	99.53	111.56	99.69	105.84	6.01
25.0	106.19	101.15	113.03	103.27	112.05	89.93	104.24	7.71
30.0	111.72	100.99	107.66	104.89	94.97	97.41	102.94	5.79
45.0	93.67	99.53	110.42	105.38	100.99	99.53	101.59	5.23
60.0	106.36	100.01	104.41	96.92	105.38	92.21	100.88	5.07
90.0	114.65	108.47	110.10	99.53	101.64	102.45	106.14	5.34
120.0	100.67	101.97	103.27	100.34	100.34	97.74	100.17	1.69

Table 63 Dissolution Data of Brand C in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	3.25	2.05	3.61	6.79	12.09	15.30	7.18	4.91
10.0	61.79	58.87	69.76	72.37	59.84	58.22	63.48	5.53
15.0	81.96	82.12	88.14	87.49	81.47	81.96	83.86	2.81
20.0	83.59	83.75	91.07	87.00	83.26	86.52	85.87	2.75
25.0	89.44	84.89	90.58	88.96	80.01	87.33	86.87	3.56
30.0	90.09	93.67	95.14	94.97	91.56	90.26	92.61	2.08
45.0	87.98	94.65	89.77	90.74	94.81	89.44	91.23	2.60
60.0	91.88	87.00	95.14	88.79	90.42	89.28	90.42	2.58
90.0	90.74	90.74	88.96	73.67	83.91	85.54	85.59	5.91
120.0	81.47	84.73	91.07	76.76	82.94	85.38	83.72	4.32

Table 64 Dissolution Data of Brand D in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	1.56	1.34	0.62	1.94	0.89	0.98	1.22	0.44
10.0	46.02	31.87	53.99	77.25	62.28	56.10	54.58	13.93
15.0	75.62	75.94	82.45	84.24	94.97	88.30	83.59	6.78
20.0	90.42	77.73	81.47	83.43	94.97	92.70	86.79	6.28
25.0	93.51	89.61	84.73	88.79	97.74	100.67	92.51	5.44
30.0	92.70	91.56	80.82	97.58	102.62	102.13	94.57	7.44
45.0	97.58	92.37	90.91	80.82	97.58	102.62	93.64	6.89
60.0	95.67	93.56	92.34	85.97	97.34	100.78	94.28	4.60
90.0	92.05	91.23	90.91	86.84	97.25	94.81	92.18	3.26
120.0	93.51	92.05	93.51	85.54	93.35	98.39	92.72	3.78

Table 65 Dissolution Data of Brand E in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	12.07	13.23	15.17	24.55	21.62	20.16	17.80	4.59
10.0	62.45	67.32	67.16	69.76	69.76	54.48	65.16	5.36
15.0	97.90	99.69	91.07	95.95	95.79	78.87	93.21	6.93
20.0	88.96	89.44	97.09	88.63	91.88	88.30	90.72	3.08
25.0	101.32	97.74	93.35	94.16	93.51	95.46	95.92	2.83
30.0	100.34	94.65	77.25	94.65	98.71	94.97	93.43	7.56
45.0	91.72	94.48	89.12	94.32	97.41	87.82	92.48	3.30
60.0	98.23	80.66	87.82	90.42	94.97	93.02	90.85	5.62
90.0	95.14	90.26	90.42	93.83	97.09	86.68	92.24	3.48
120.0	94.81	97.41	83.10	83.91	74.48	87.82	86.92	7.65

Table 66 Dissolution Data of Brand F in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	9.89	34.85	24.27	29.39	26.72	24.72	24.97	7.62
10.0	43.09	72.69	54.31	57.57	73.02	64.56	60.87	10.57
15.0	85.05	101.64	79.36	98.39	96.76	92.70	92.32	7.79
20.0	86.35	100.83	95.14	100.01	103.76	107.66	98.96	6.79
25.0	93.83	102.62	101.32	108.47	96.27	88.79	98.55	6.39
30.0	93.67	109.12	102.94	100.01	103.59	109.61	103.16	5.44
45.0	93.35	108.80	94.97	103.92	97.09	102.29	100.07	5.42
60.0	103.27	85.38	85.54	105.06	83.59	104.41	94.54	9.74
90.0	97.41	105.71	104.57	99.53	82.12	107.33	99.45	8.48
120.0	89.28	109.12	96.44	93.67	84.24	99.69	95.41	7.88

Table 67 Dissolution Data of Brand G in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	3.18	5.41	3.25	3.63	5.63	4.76	4.31	1.00
10.0	55.61	51.39	61.96	71.23	73.34	65.05	63.10	7.85
15.0	76.43	59.03	66.35	73.67	86.03	71.23	72.12	8.37
20.0	82.77	105.38	94.48	97.90	90.91	82.45	92.32	8.13
25.0	84.74	108.31	87.00	97.41	95.30	81.96	92.45	8.98
30.0	81.80	107.33	93.02	98.23	93.83	88.79	93.83	7.88
45.0	82.77	109.29	99.04	98.88	96.76	83.26	94.99	9.36
60.0	82.45	87.49	99.53	89.12	107.66	81.80	91.35	9.34
90.0	85.05	102.29	99.20	101.32	97.58	85.70	95.19	7.10
120.0	83.59	94.81	93.51	92.70	89.12	74.16	87.98	7.20

Table 68 Dissolution Data of Brand H in Water

Time (Min) \ Capsule No.	Percent of doxycycline dissolved							
	1	2	3	4	5	6	Mean	S.D.
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
5.0	1.13	0.89	1.60	1.20	0.82	1.58	1.21	0.30
10.0	82.45	44.39	66.84	66.19	34.96	60.33	59.19	15.58
15.0	97.58	92.05	88.63	95.46	85.38	92.05	91.86	4.04
20.0	94.32	91.39	92.70	93.18	91.88	91.38	92.48	1.05
25.0	96.44	91.88	86.19	98.39	93.83	91.23	92.99	3.92
30.0	93.18	94.16	84.56	99.04	93.02	94.00	92.99	4.28
45.0	101.15	95.30	91.72	99.36	94.97	89.93	95.41	3.93
60.0	97.58	92.70	82.77	97.74	88.47	89.44	91.45	5.27
90.0	92.05	94.00	79.52	91.88	89.44	85.54	88.74	4.90
120.0	87.17	91.39	87.00	90.42	85.70	88.47	88.36	1.99

APPENDIX H

DETERMINATION OF DISSOLUTION RATE CONSTANT

In calculation of dissolution rate constant, it is assumed that the dissolution process is the first order rate process, then

$$\ln [B_\infty - B_t] = \ln B_\infty - Kt$$

where B_∞ = The maximum amount of drug dissolved from the capsule.

B_t = The amount of drug dissolved at time t.

A plot of the natrual logarithm of variable Y where Y is $[B_\infty - Bt]$ versus time, as shown in figure 8, should be linear with the slope K. The rate constant may be calculated using linear regression.

Example of determination of dissolution rate constant was shown in table 69. The dissolution rate constant was calculated from the time ranged 5-20 minutes and the slope obtained from linear regression was 0.159 with r^2 [0.994].

Table 69 Example for Dissolution Rate Constant Calculation Using Data of the Sixth Capsule of Brand E.

Time [min]	B_t [Percent dissolved]	$B_\infty - B_t$
0	0.00	95.46
5.0	20.16	75.30
10.0	54.48	40.98
15.0	78.87	16.57
20.0	88.30	7.16
25.0	95.46*	0.00
30.0	94.97	
45.0	87.82	

* B_∞ = 95.46 %

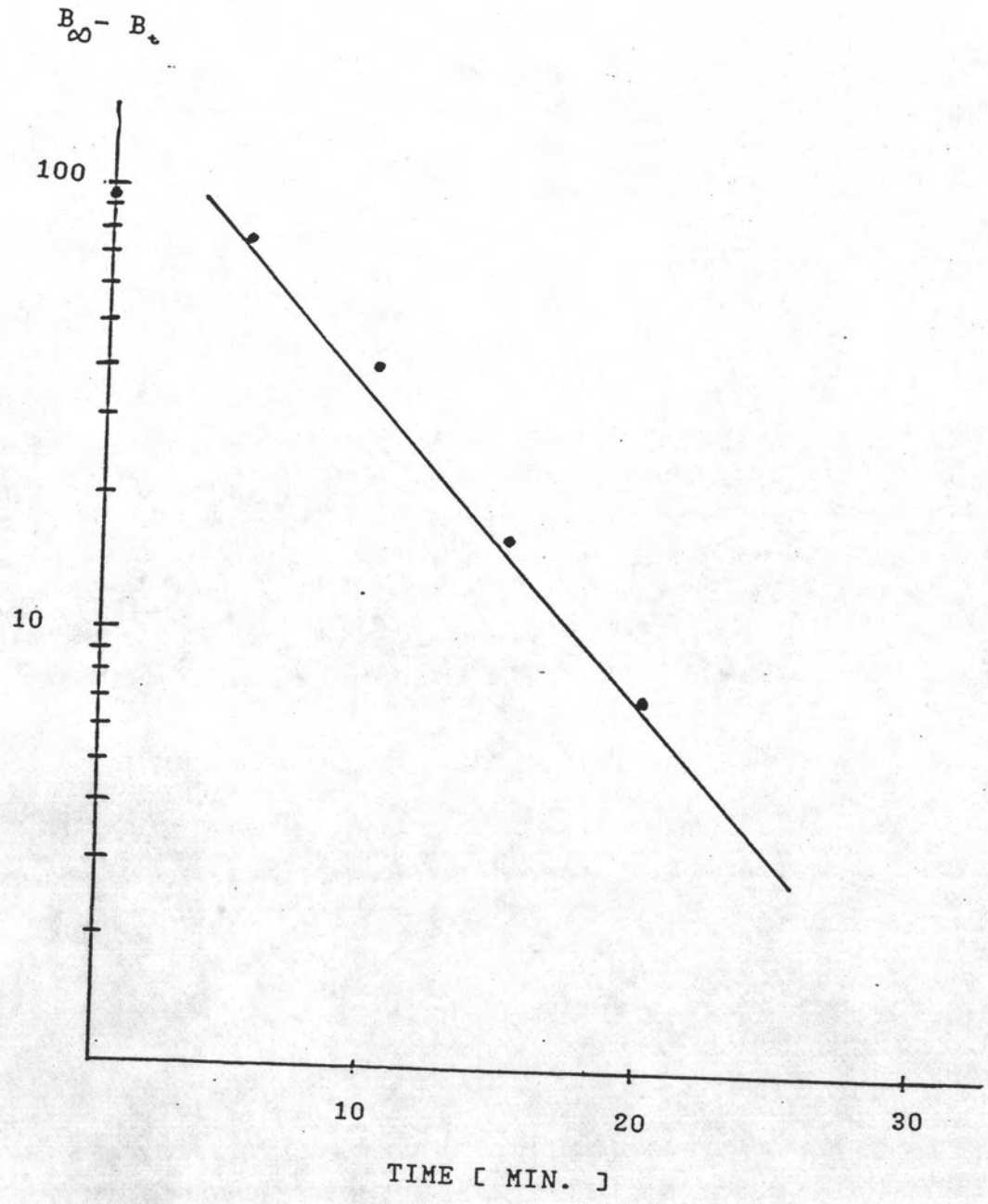


Figure 8 First Order Plot for Dissolution Rate Constant Using the data of the Sixth Capsule of Brand E.

APPENDIX I

VALIDATION OF DOXYCYCLINE ANALYSIS IN PLASMA

The within - run precision and between - run precision of doxycycline analysis in plasma were shown in table 70 and 71 respectively. While percent recoveries of doxycycline and tetracycline in human plasma were presented in table 72.

Table 70 Within - Run Precision for Doxycycline Analysis in Plasma (n=3)

Concentration (µg/ml)	Peak Heigh Ratio D^1/IS^2 [mean ± S.D.]	C.V. (%)
0.10	0.663 ± 0.097	14.59
0.20	0.919 ± 0.078	8.45
0.50	1.263 ± 0.096	7.61
1.00	2.239 ± 0.081	3.60
1.50	3.139 ± 0.275	8.76
2.00	4.945 ± 0.359	7.26
2.50	5.715 ± 0.437	7.65

1. Doxycycline
2. Tetracycline

Table 71 Between - Run Precision for Doxycycline Analysis in Plasma (n = 6)

Concentration (µg/ml)	Peak Heigh Ratio D^1/IS^2 [mean ± S.D.]	C.V. (%)
0.10	0.713 ± 0.106	14.94
0.20	0.932 ± 0.111	11.96
0.50	1.380 ± 0.237	17.20
1.00	2.239 ± 0.231	10.31
1.50	3.158 ± 0.334	10.58
2.00	4.317 ± 0.434	10.07
2.50	5.056 ± 0.381	7.53

1. Doxycycline
2. Tetracycline

Table 72 Recovery of Doxycycline and Tetracycline form Plasma

Concentration (μg/ml)	Doxycycline		Tetracycline	
	Recovery (mean ± S.D.)	C.V. (%)	Recovery (mean ± S.D.)	C.V. (%)
0.10	96.36 ± 3.15	3.27	35.79 ± 3.63	10.15
0.20	103.47 ± 3.76	3.63	50.40 ± 5.14	10.73
0.50	84.21 ± 10.29	12.22	40.77 ± 5.28	12.95
1.00	86.04 ± 3.47	4.04	38.53 ± 3.34	8.66
1.50	80.52 ± 2.83	3.51	37.77 ± 3.51	9.29
2.00	77.81 ± 1.12	1.44	43.26 ± 1.91	4.42
2.50	81.44 ± 4.76	5.84	48.01 ± 1.88	3.91

APPENDIX J

ADDITIONAL DATA OF DOXYCYCLINE IN PLASMA

Comparison of plasma doxycycline concentration-time profiles among four different brands of each subject following oral 100 mg doxycycline capsules were illustrated in figure 9 to 28 respectively.

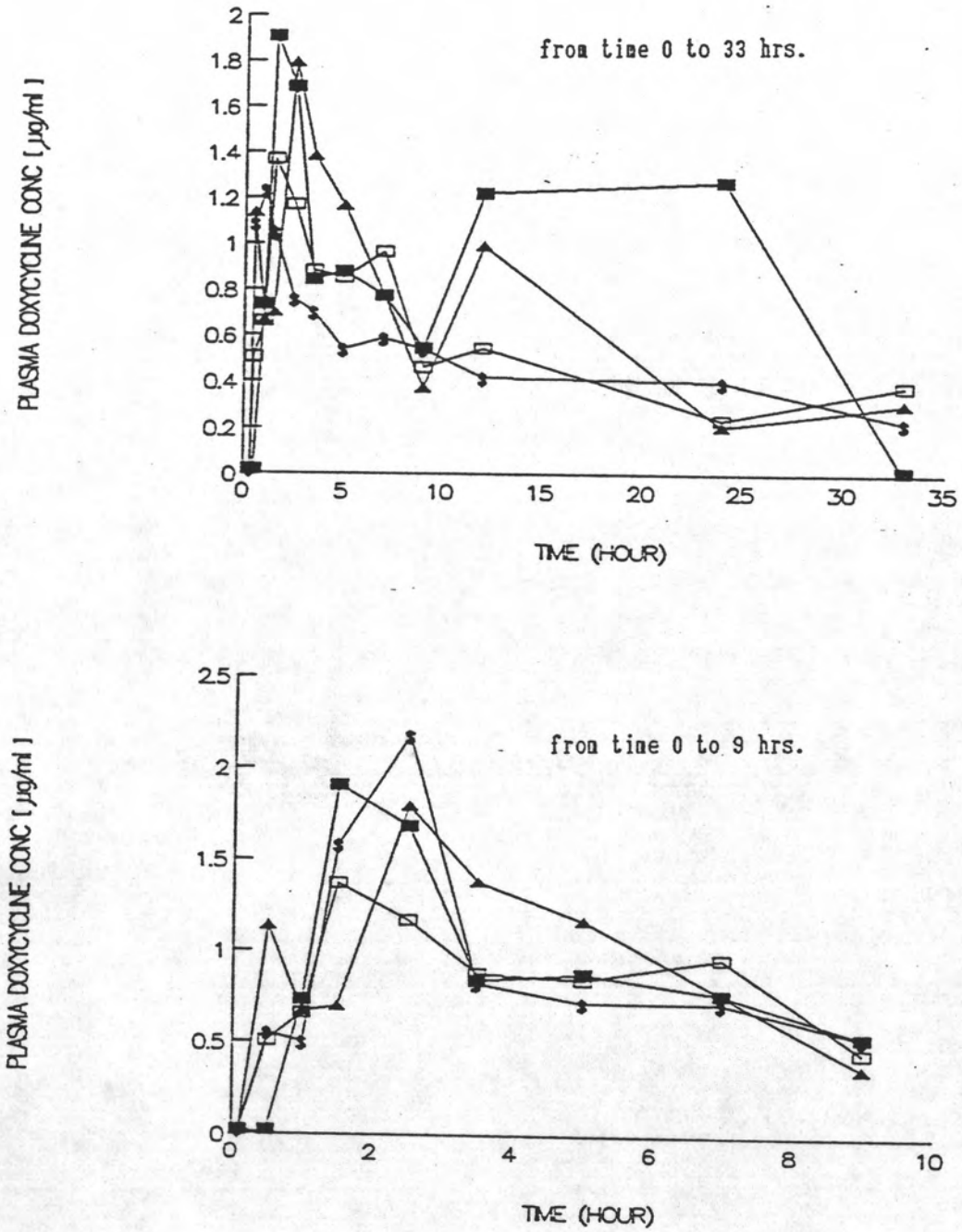
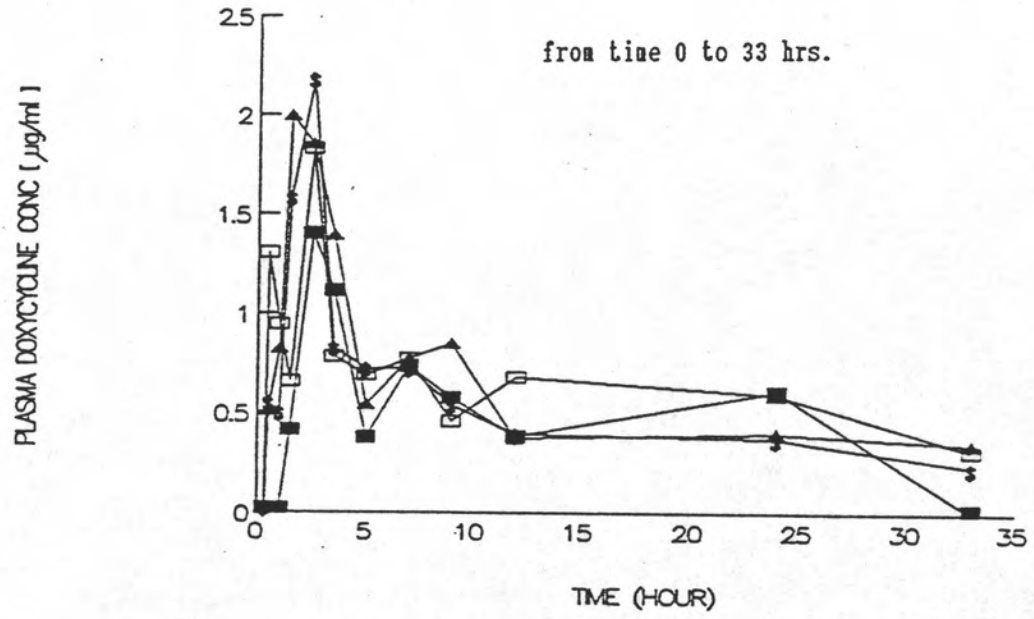


FIGURE 9 Plasma Doxycycline Concentration-Time Profile of the First Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]



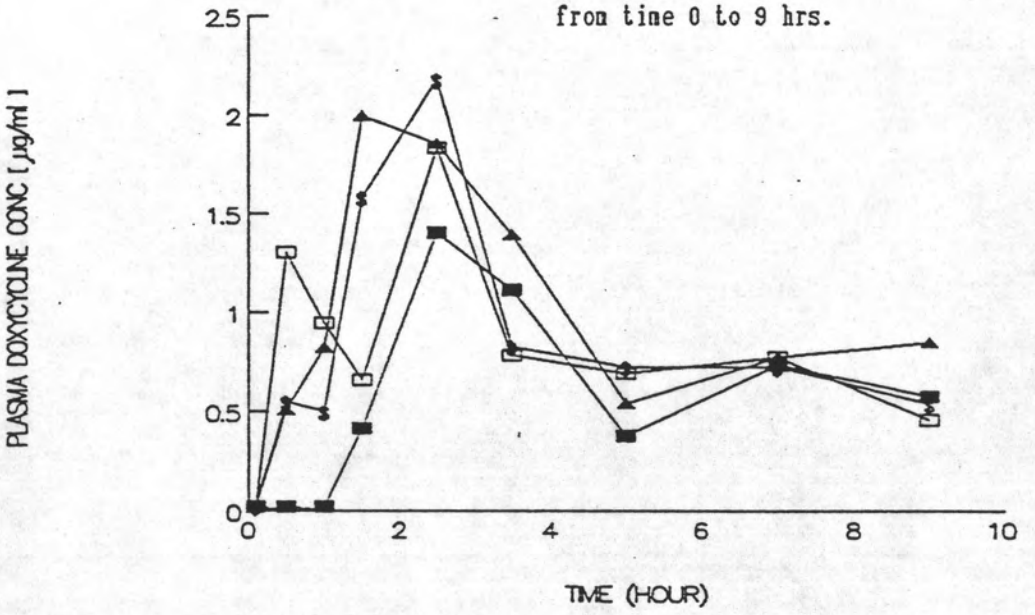


FIGURE 10 Plasma Doxycycline Concentration-Time Profile of the Second Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

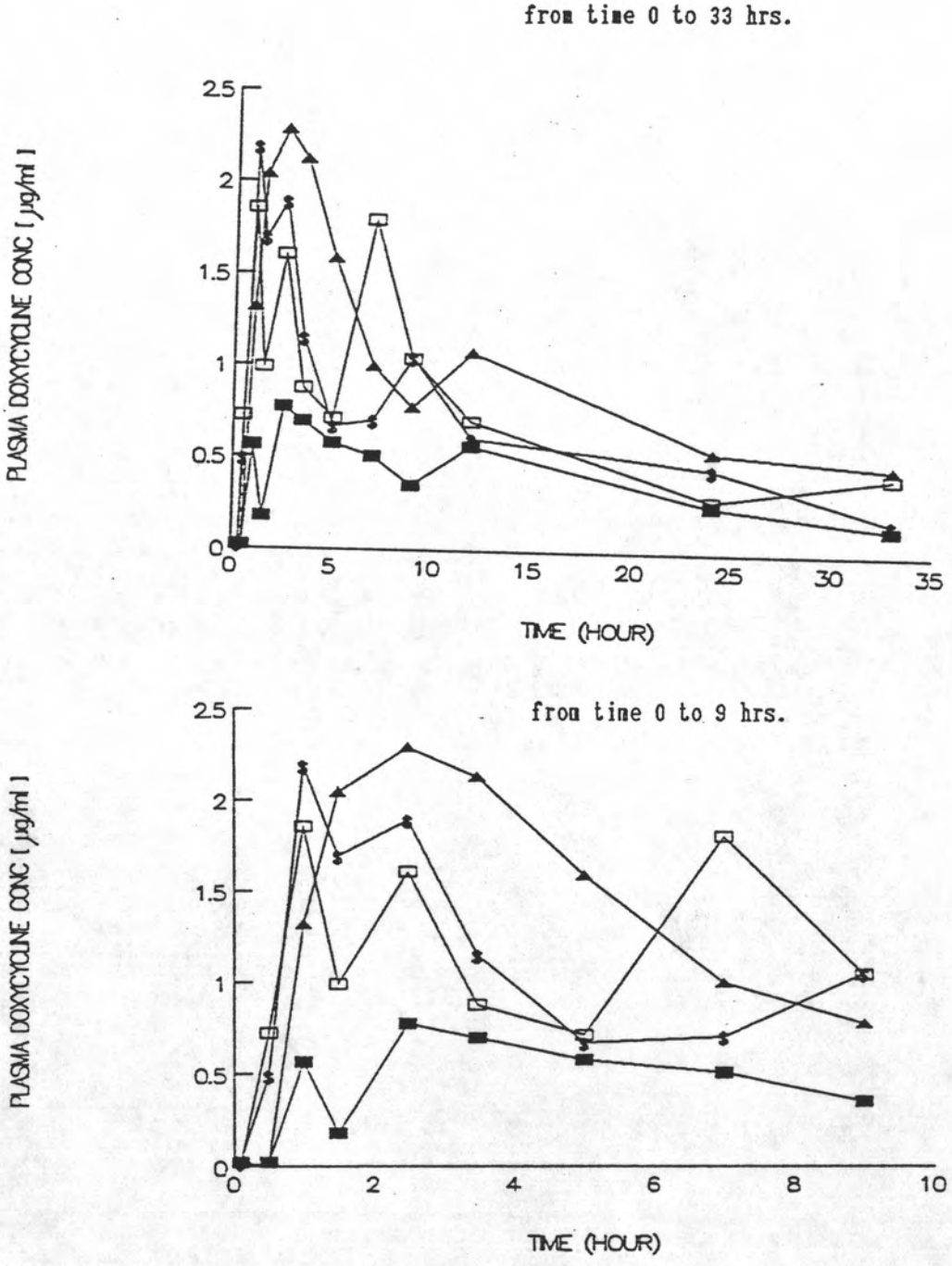


FIGURE 11 Plasma Doxycycline Concentration-Time Profile of the Third Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

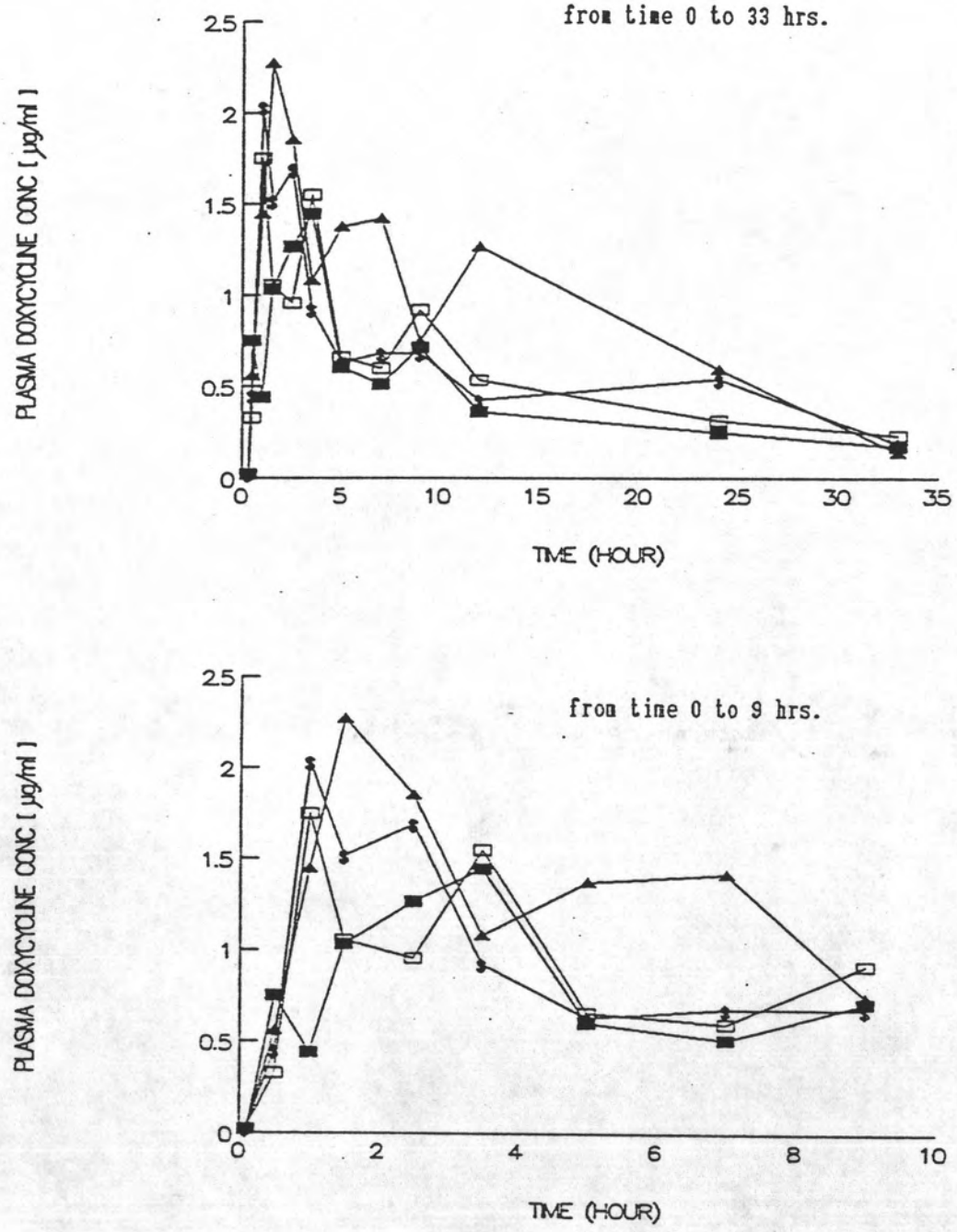


FIGURE 12 Plasma Doxycycline Concentration-Time Profile of the Fourth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

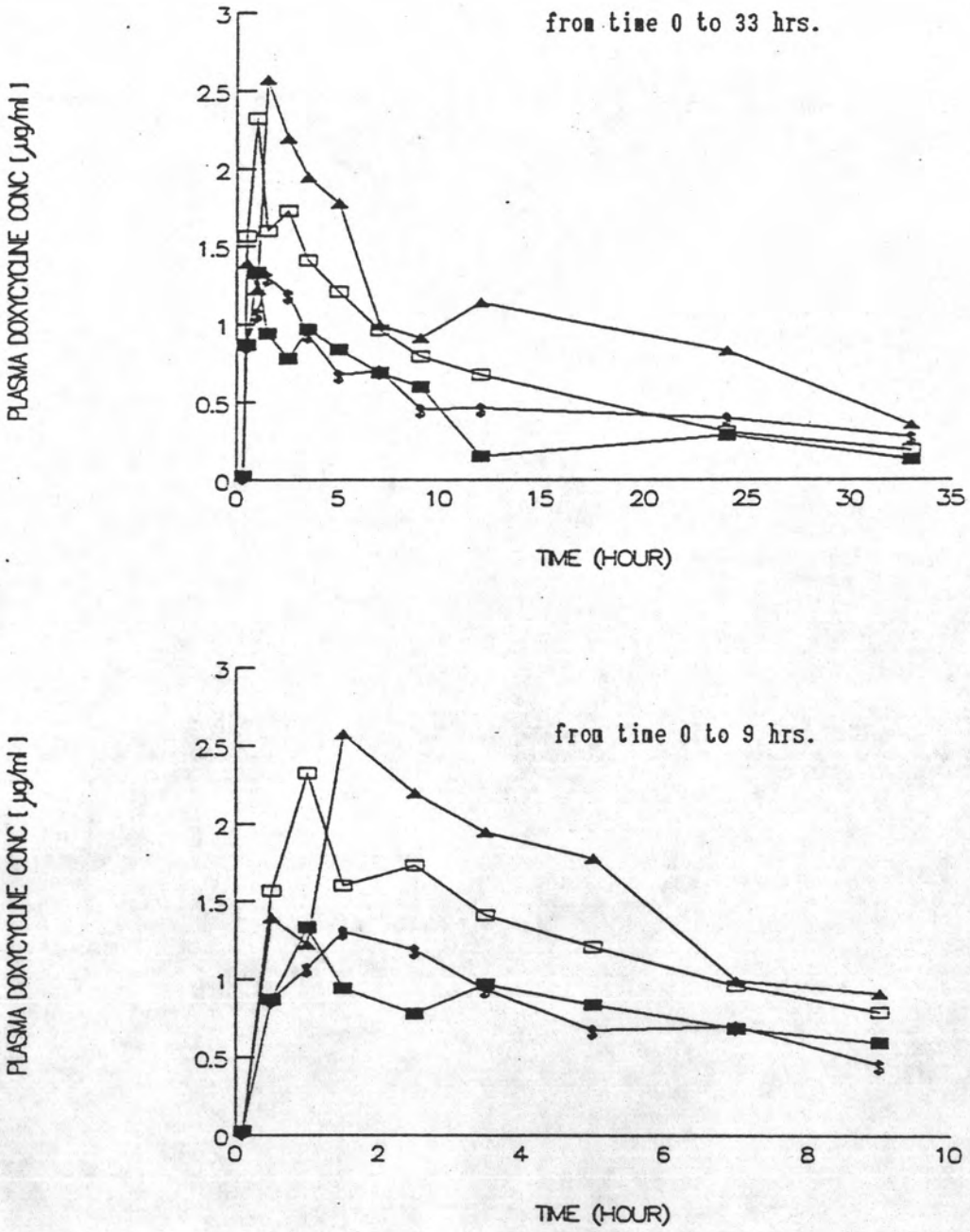


FIGURE 13 Plasma Doxycycline Concentration-Time Profile of the Fifth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

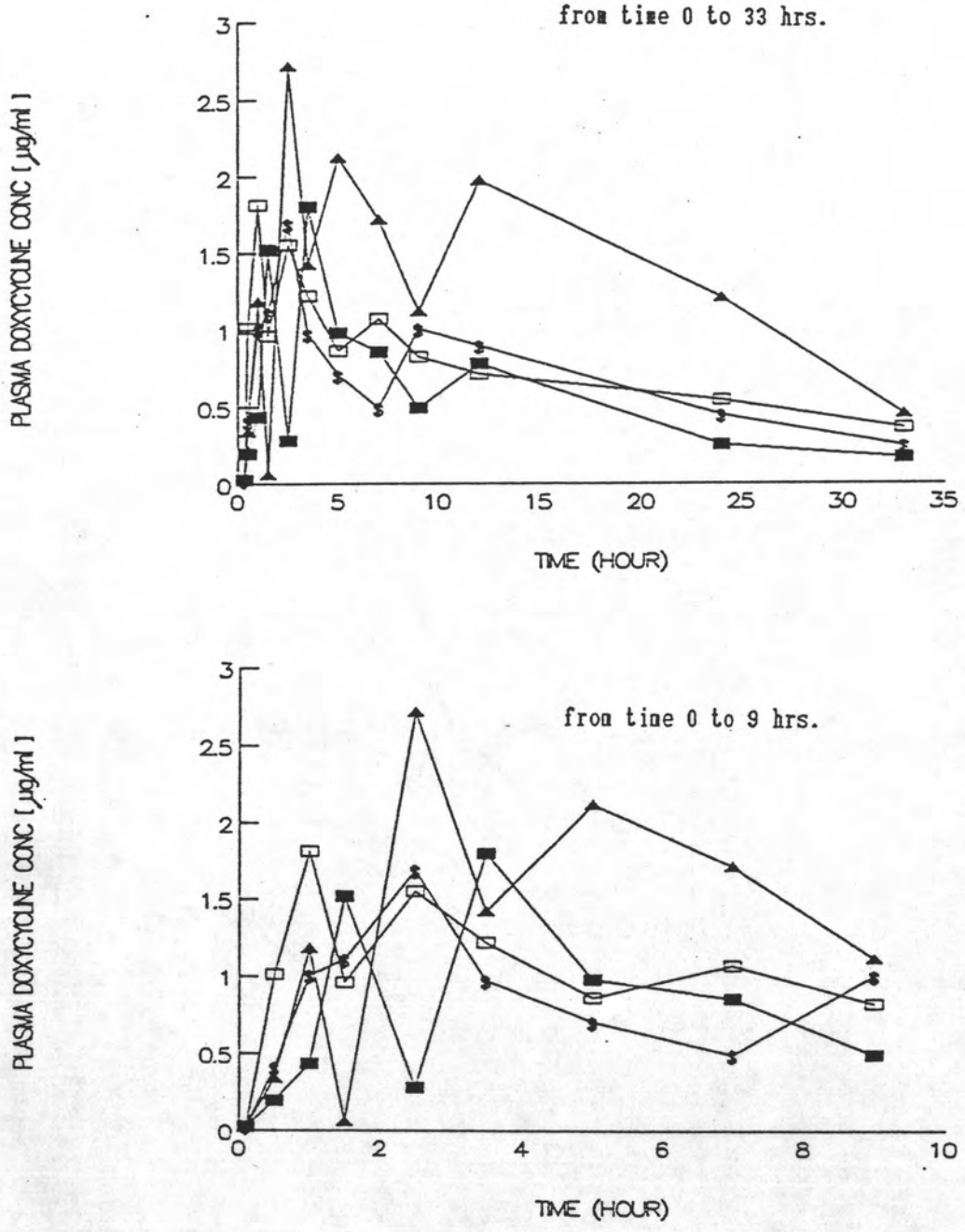


FIGURE 14 Plasma Doxycycline Concentration-Time Profile of the Sixth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

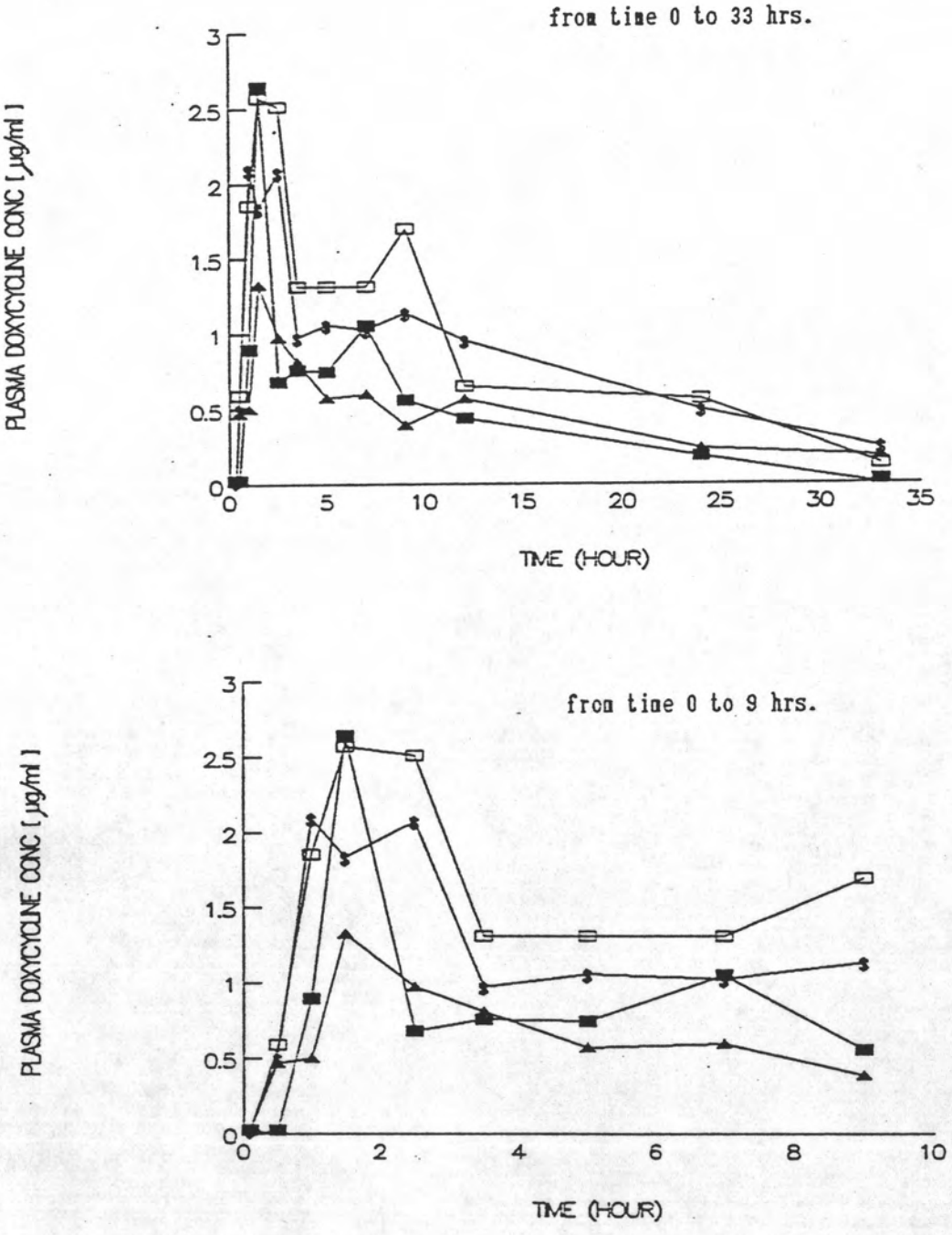


FIGURE 15 Plasma Doxycycline Concentration-Time Profile of the Seventh Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [S], Brand C [▲] and Brand D [□]

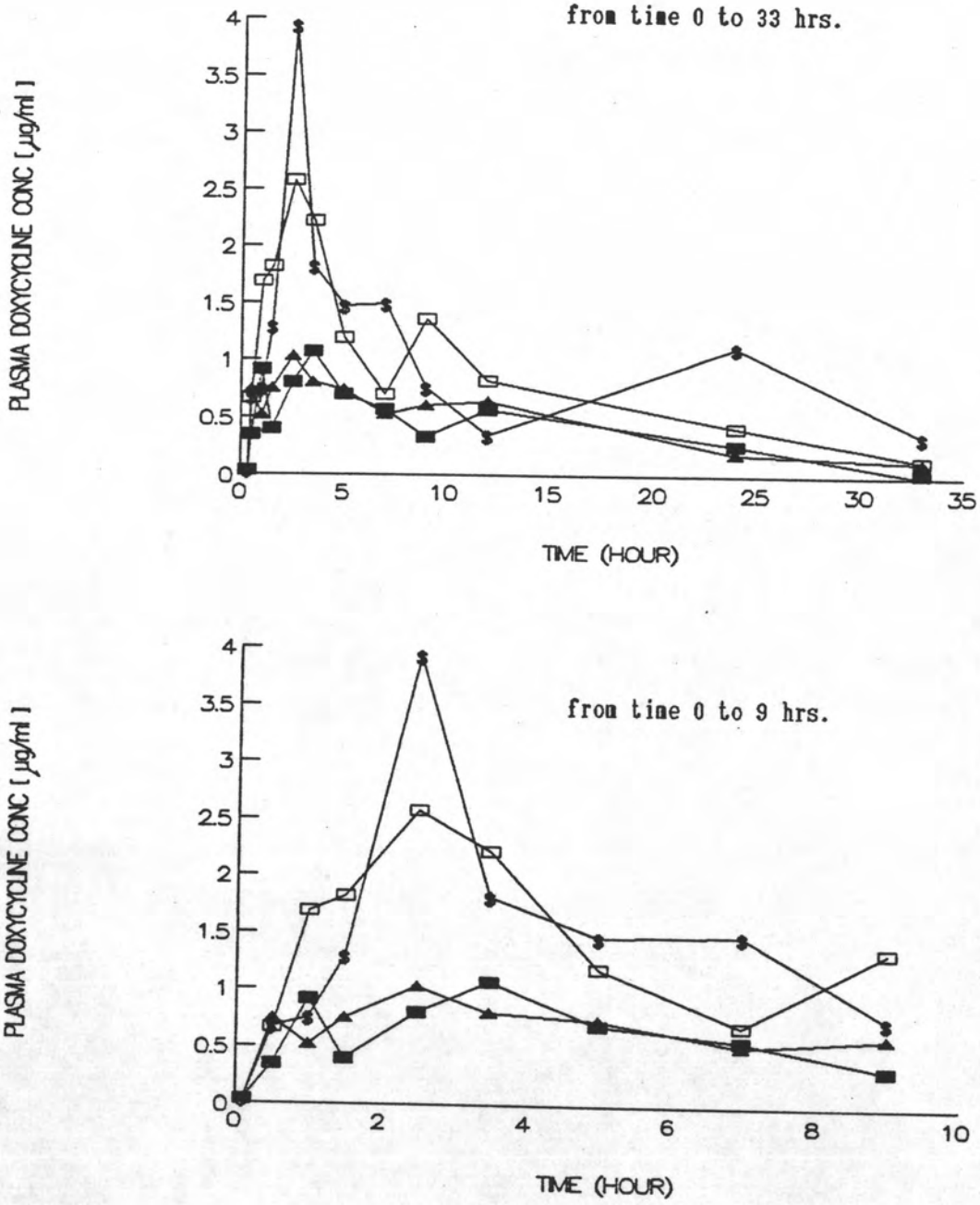
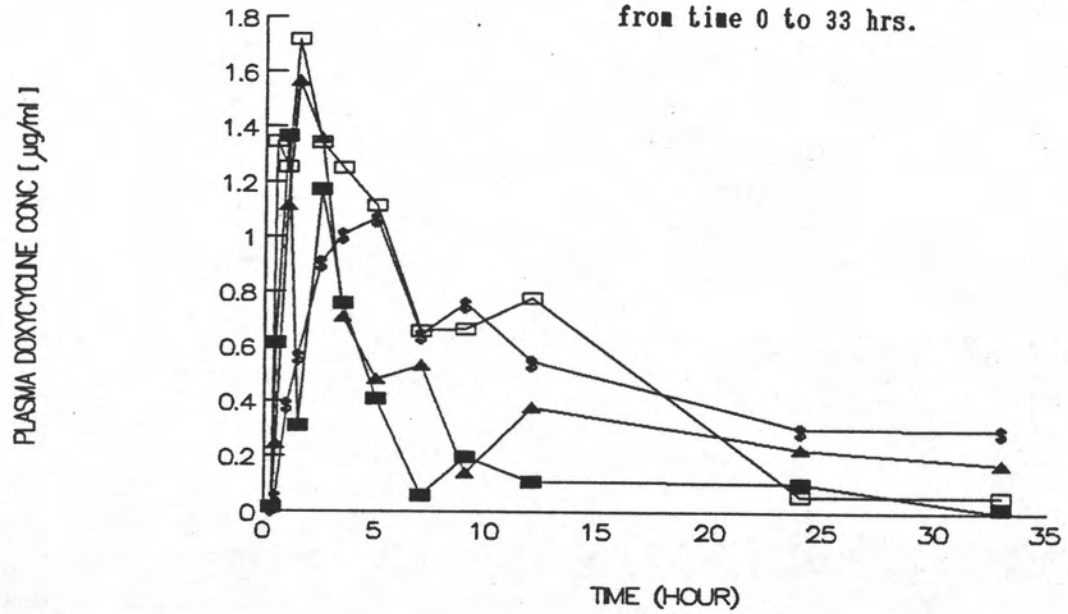


FIGURE 16 Plasma Doxycycline Concentration-Time Profile of the Eighth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]



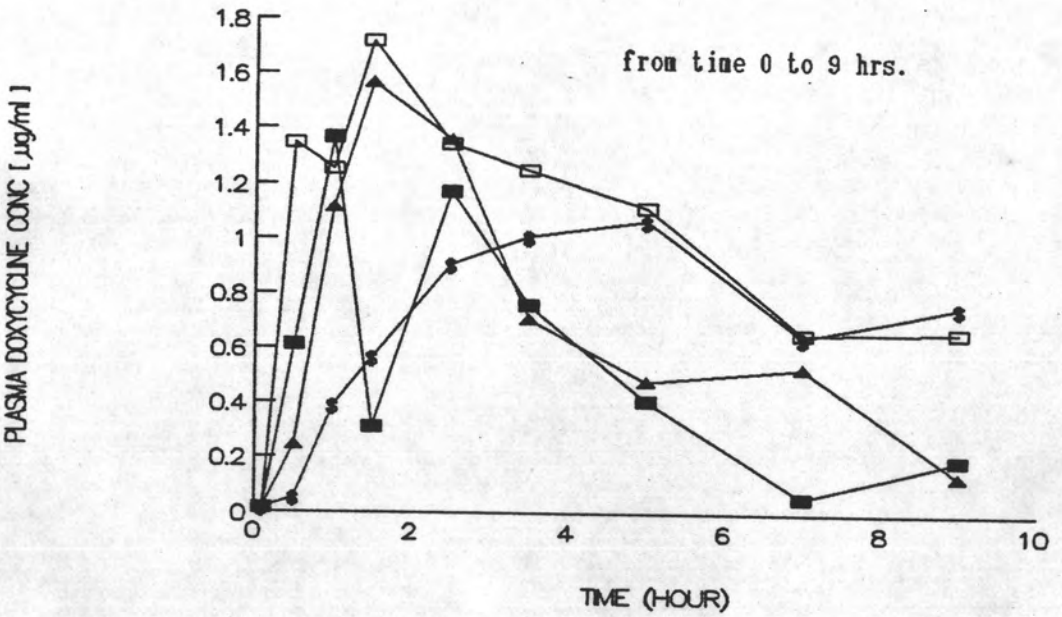


FIGURE 17 Plasma Doxycycline Concentration-Time Profile of the Ninth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

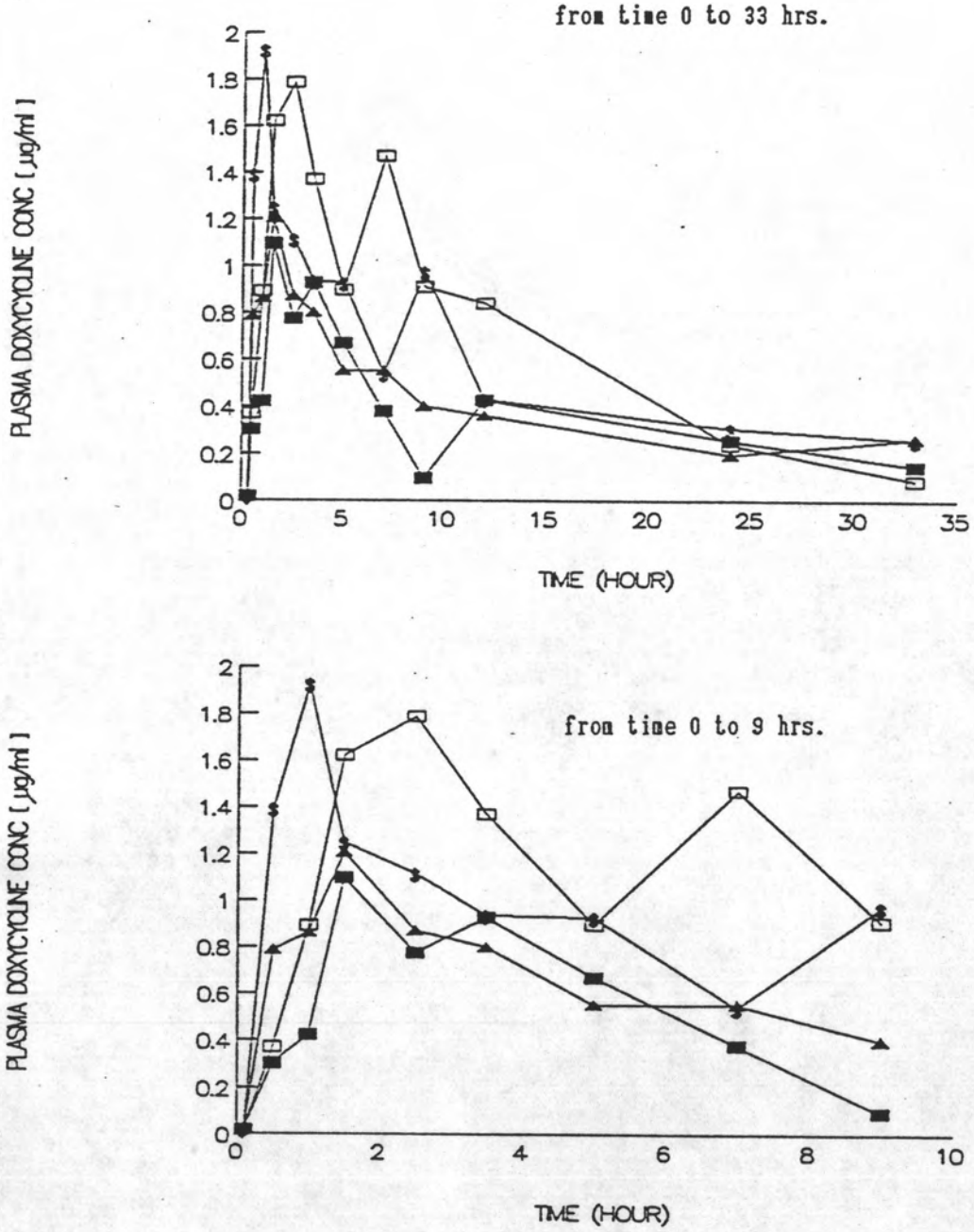


FIGURE 18 Plasma Doxycycline Concentration-Time Profile of the Tenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [⫽], Brand C [▲] and Brand D [□]

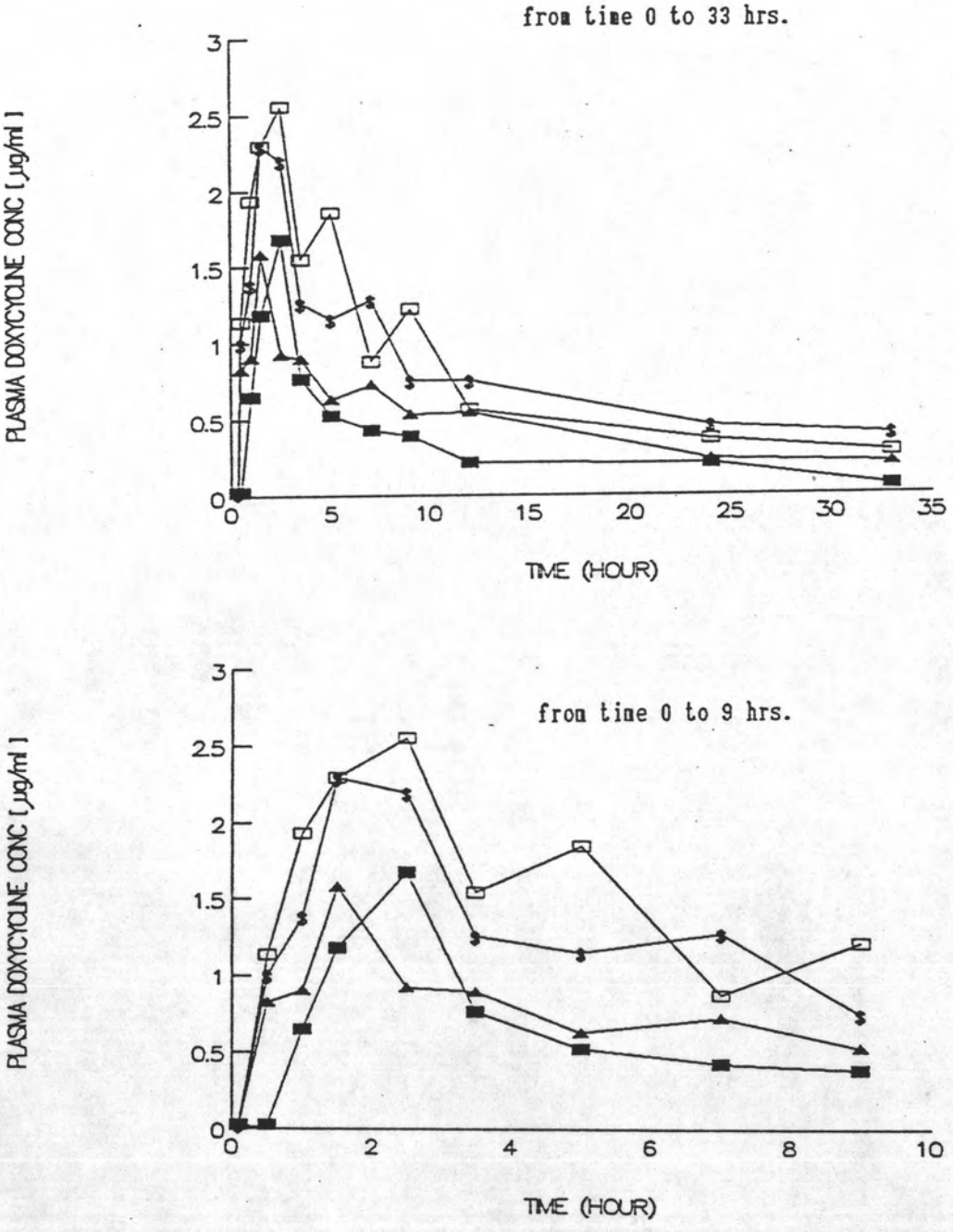


FIGURE 19 Plasma Doxycycline Concentration-Time Profile of the Eleventh Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [▨], Brand C [▲] and Brand D [□]

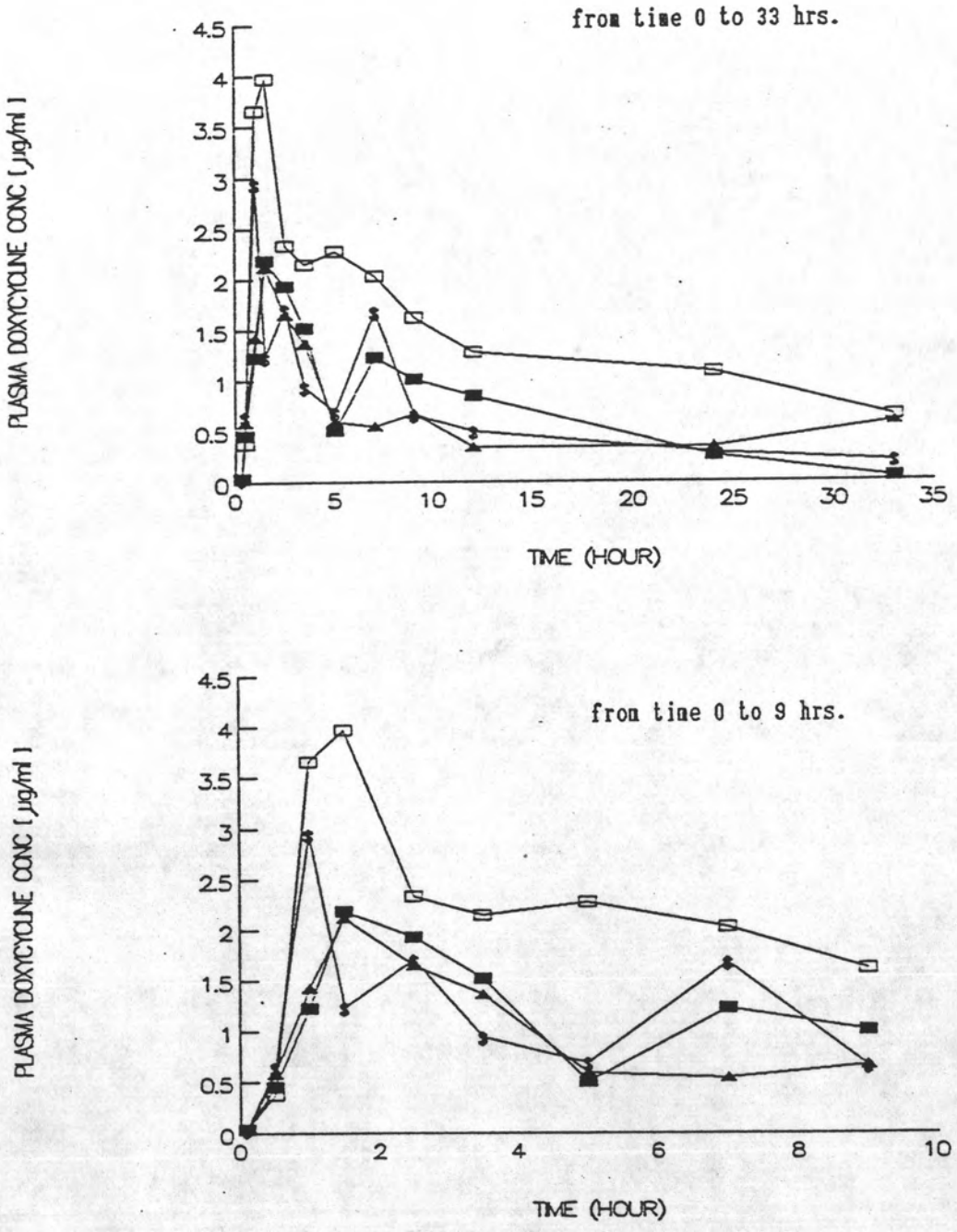


FIGURE 20 Plasma Doxycycline Concentration-Time Profile of the Twelfth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [∮], Brand C [▲] and Brand D [□]

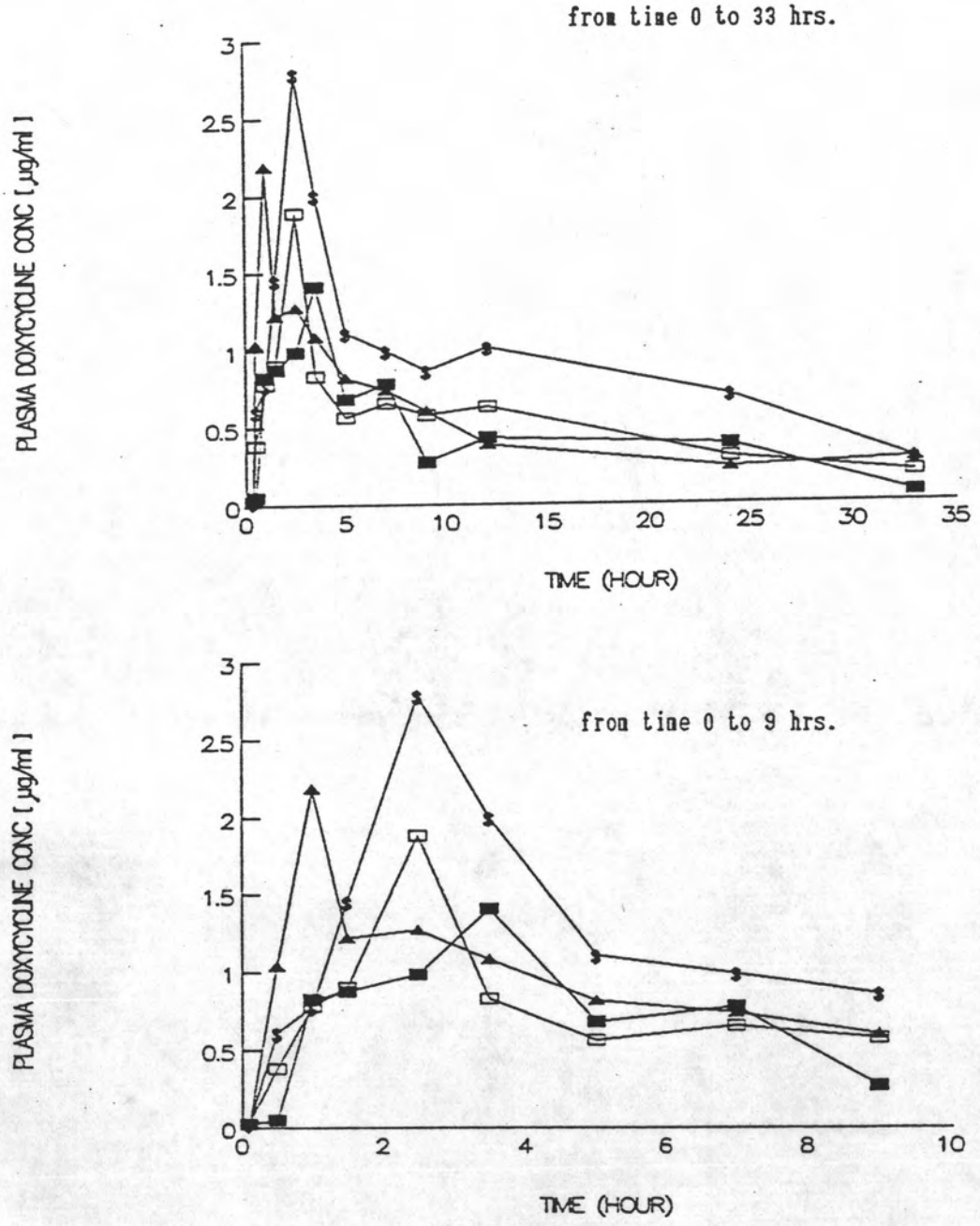


FIGURE 21 Plasma Doxycycline Concentration-Time Profile of the Thirteenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [⫽], Brand C [▲] and Brand D [□]

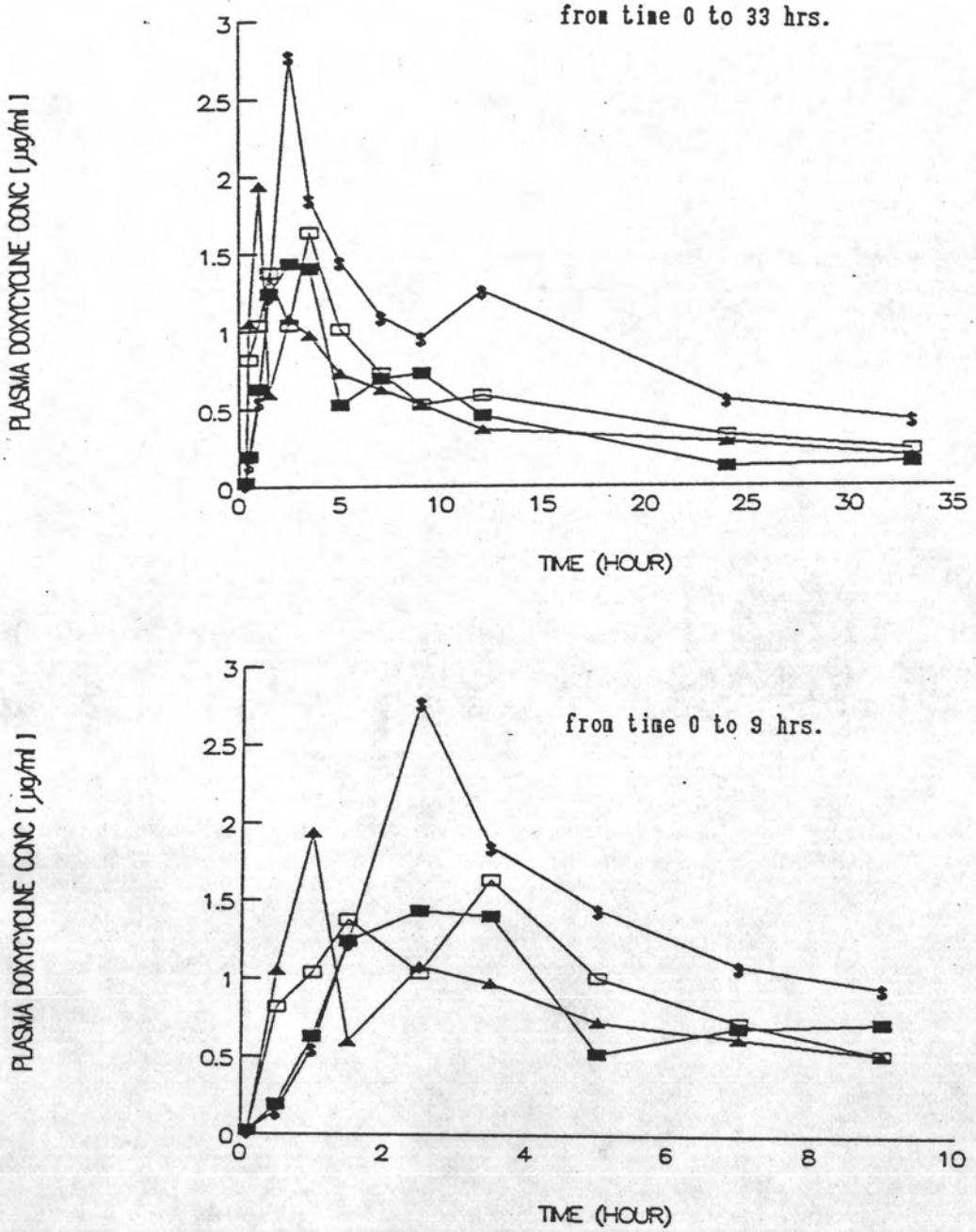


FIGURE 22 Plasma Doxycycline Concentration-Time Profile of the Fourteenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [⋕], Brand C [▲] and Brand D [▭]

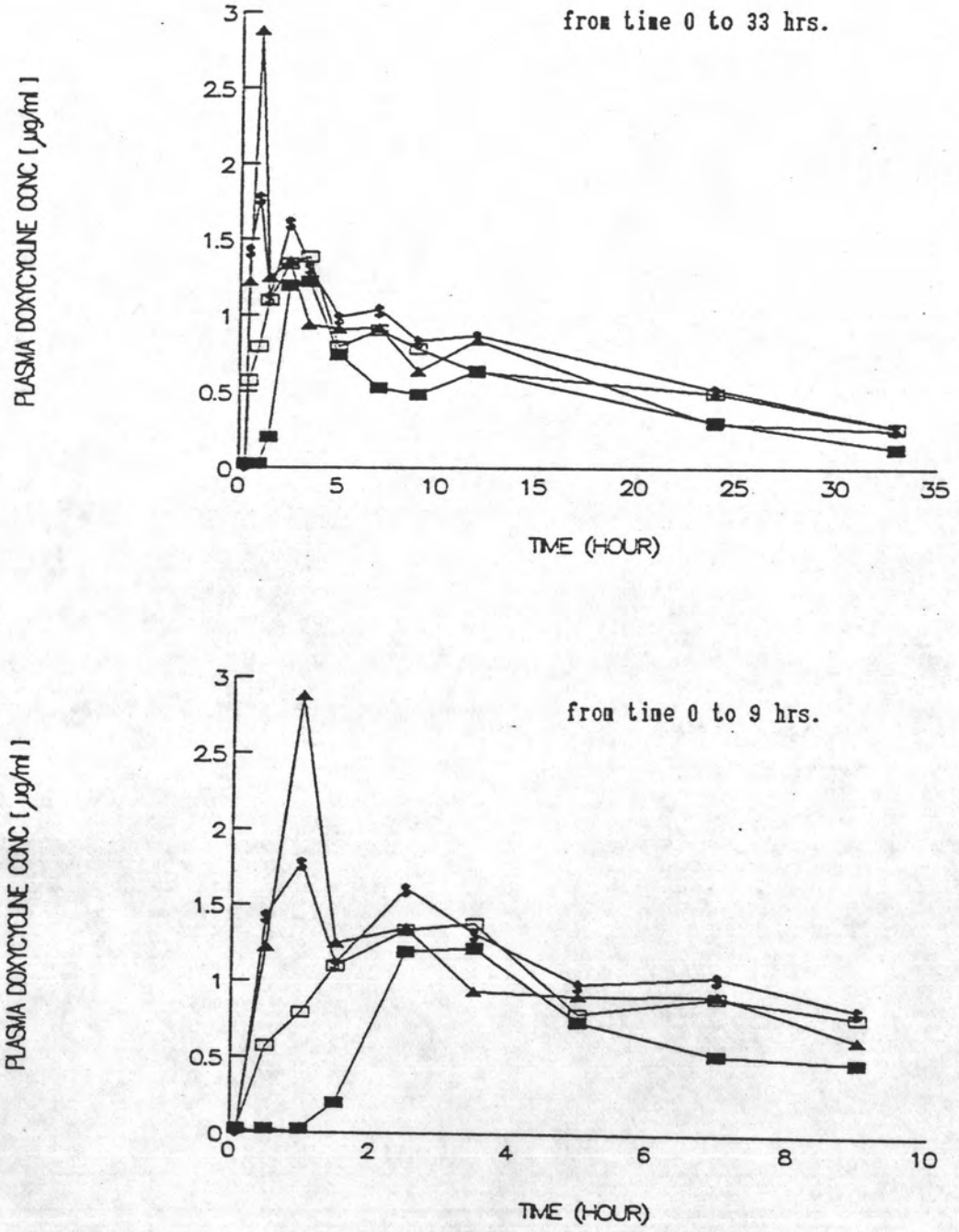


FIGURE 23 Plasma Doxycycline Concentration-Time Profile of the Fifteenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

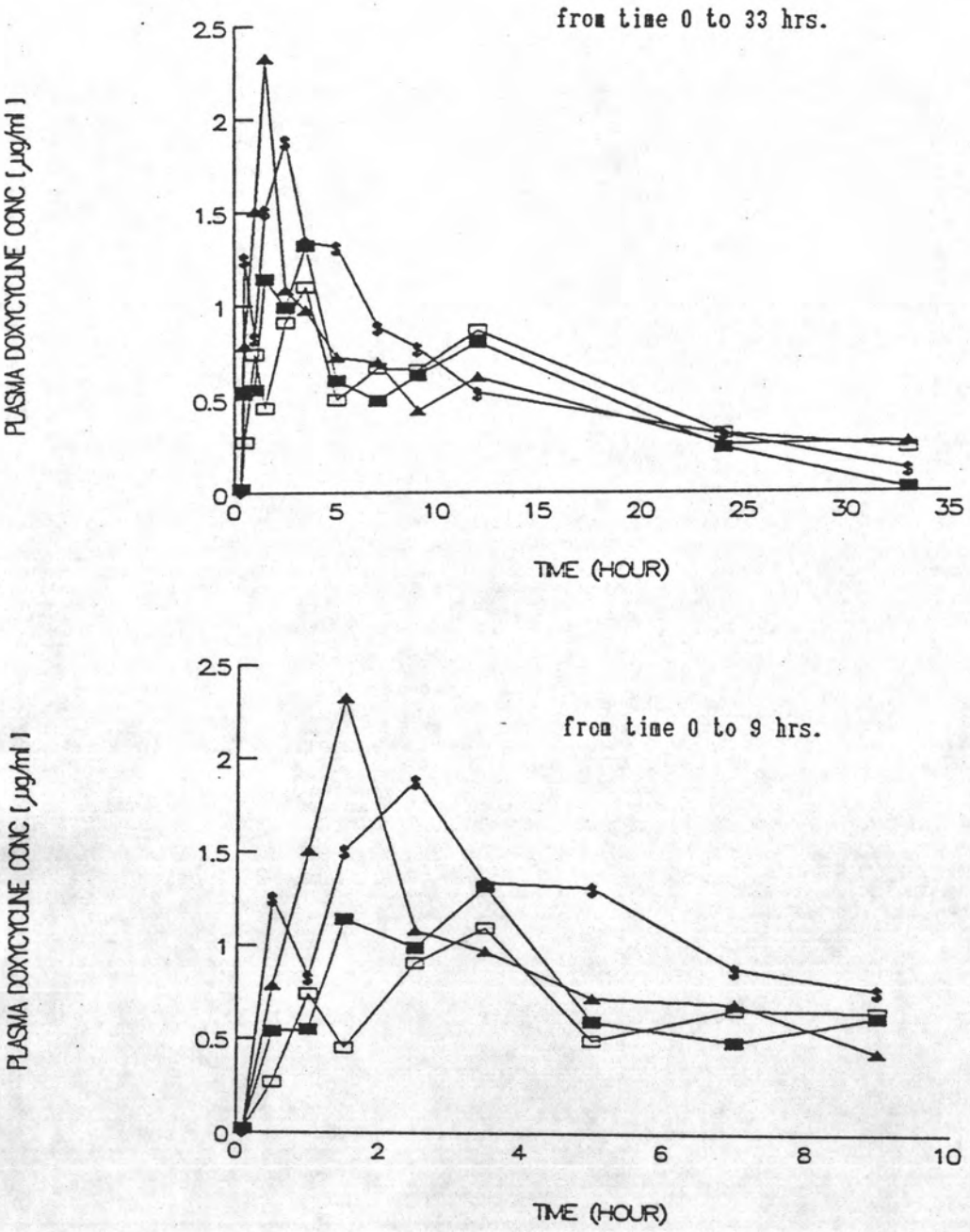


FIGURE 24 Plasma Doxycycline Concentration-Time Profile of the Sixteenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [∮], Brand C [▲] and Brand D [□]

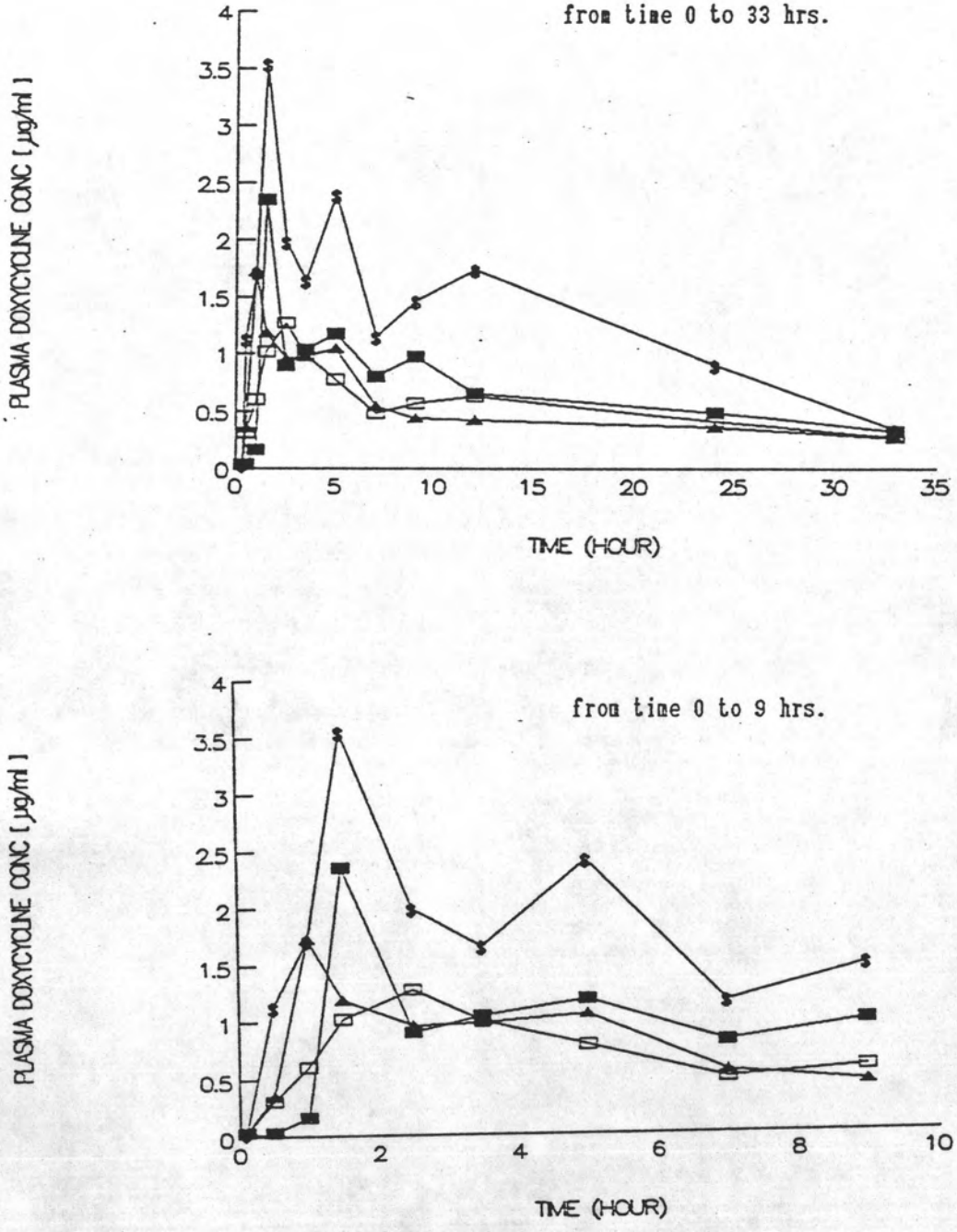


FIGURE 25 Plasma Doxycycline Concentration-Time Profile of the Seventeenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [⫽], Brand C [▲] and Brand D [□]

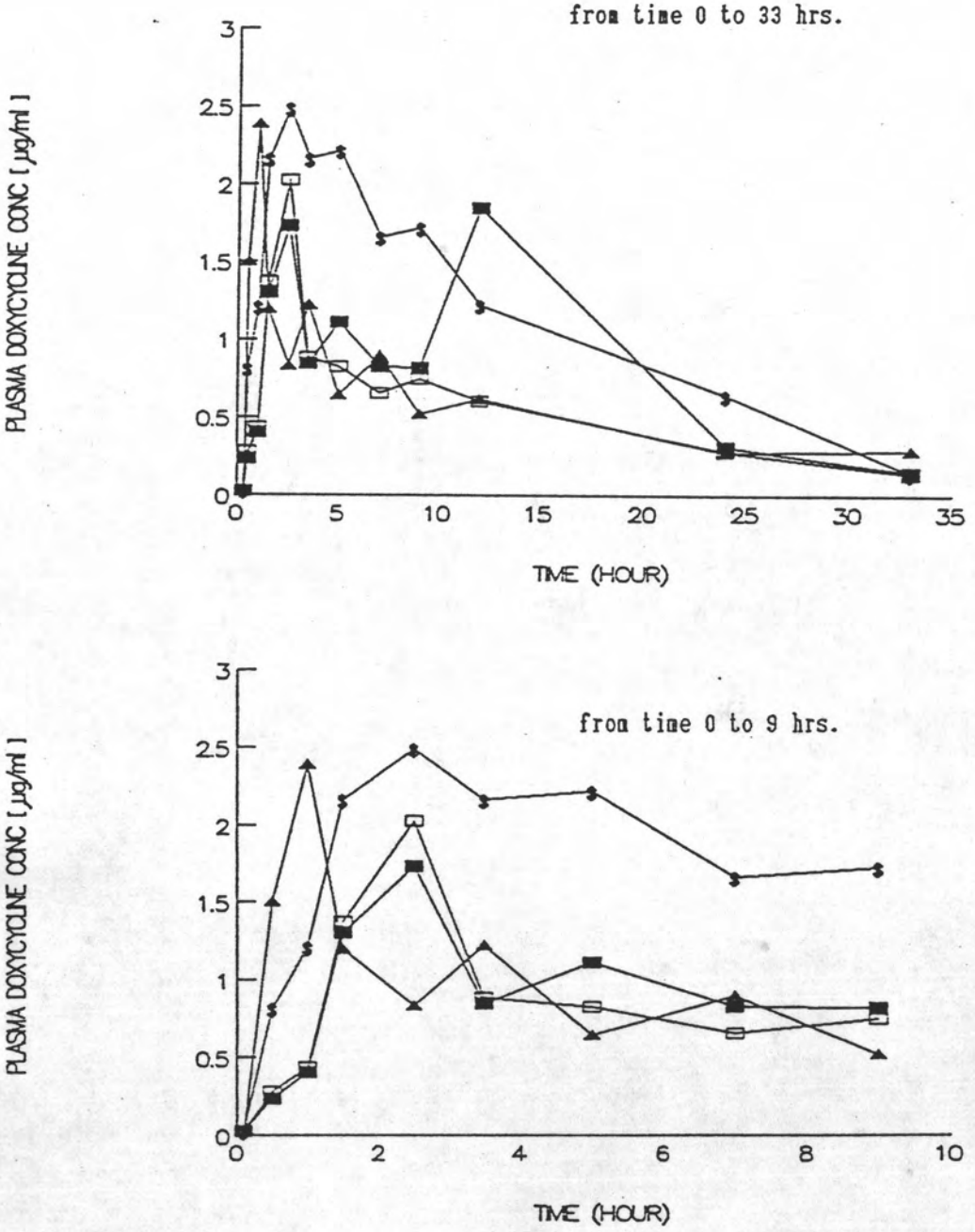
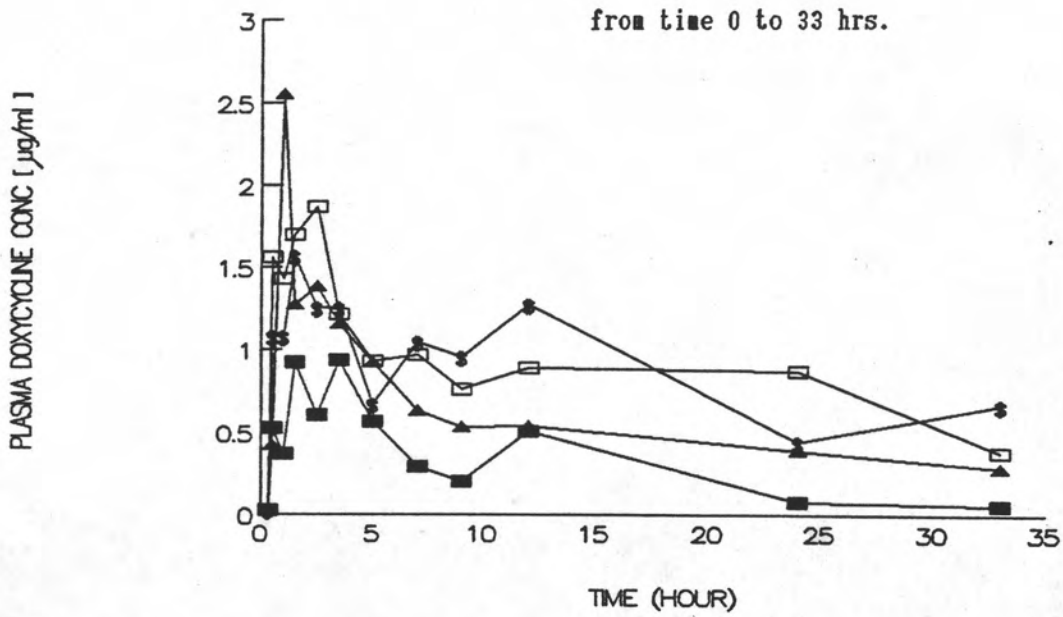


FIGURE 26 Plasma Doxycycline Concentration-Time Profile of the Eighteenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]



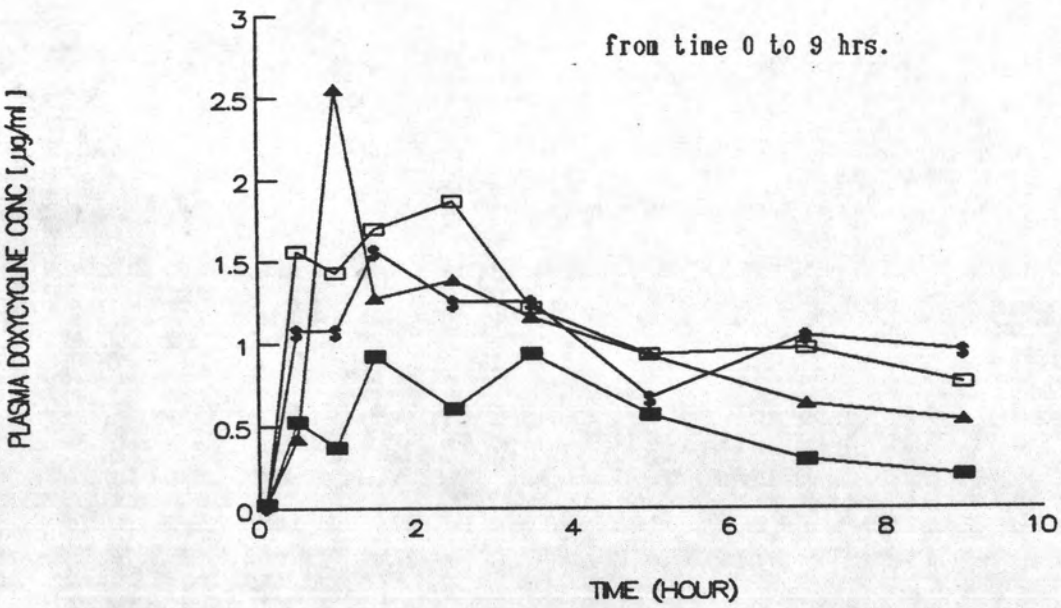


FIGURE 27 Plasma Doxycycline Concentration-Time Profile of the Nineteenth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [$], Brand C [▲] and Brand D [□]

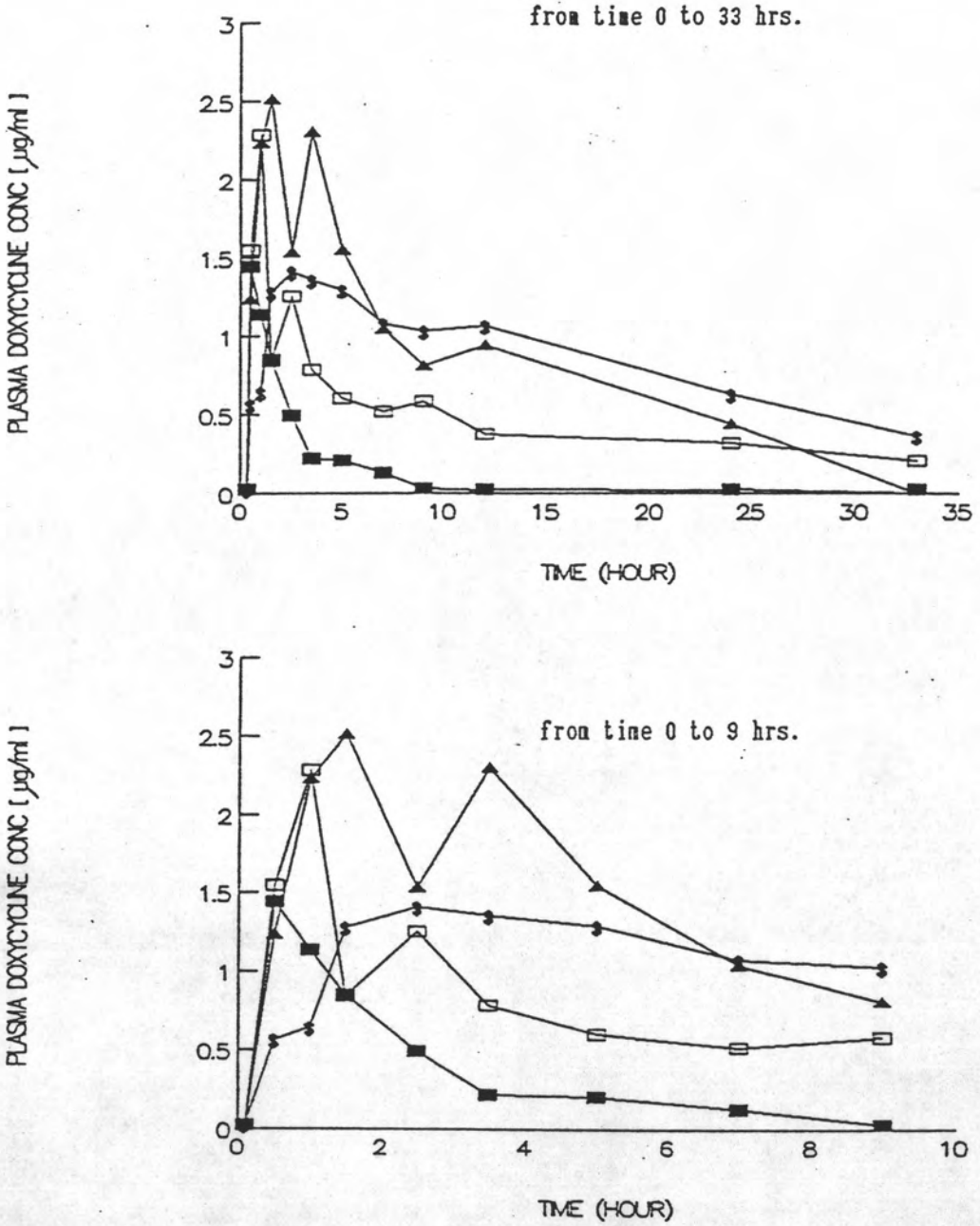


FIGURE 28 Plasma Doxycycline Concentration-Time Profile of the Twentieth Subject Following Oral Administration 100 mg of Doxycycline Capsules of Brand A [■], Brand B [S], Brand C [▲] and Brand D [□]

APPENDIX K

RESIDUAL METHOD AND OUTPUT OF CSTRIP PROGRAM

In order to determine whether a given set of data may be described by such a polyexponential equation the usual procedure is to perform an operation called by "stripping" or method of "residuals". This method provide the initial parameters [A1, A2, Ka, Ke].

In this study, the CSTRIP program was used to estimate the initial polyexponential parameters by stripping method. An example of calculation from data sets in the eighteenth subject receiving brand B was illustrated in table 73 and figure 29. The output of CSTRIP program was shown in figure 30.

Table 73 Stripping Biexponential from Set of the Plasma Doxycycline Concentration in the Eighteenth Subject Following a single Dose of 100 mg Doxycycline Capsule of Brand B.

Time (hours)	C_{obs} (µg/ml)	$C_t = 3.65e^{-0.091t}$	$R_1 = C_{obs} - C_t$		C_{est}	$\frac{C_{est}}{C_{obs}} \times 100$
0	0.00	3.65	-3.65		-	-
0.5	0.81	3.49	-2.68		0.89	109.67
1.0	1.21	3.33	-2.13	2	1.49	123.06
1.5	2.16	3.18	-1.02		1.87	86.60
2.5	2.49	2.91	-0.42		2.24	90.17
3.5	2.16	2.65	-0.49		2.32	107.43
5.0	2.22				2.20	98.98
7.0	1.66				1.90	114.71
9.0	1.72	1			1.61	93.54
12.0	1.22				1.23	100.84
24.0	0.64				0.42	64.68
33.0	0.14				0.18	133.81
					Mean	102.14
					SD	18.86
					CV	188.46

1. $\ln C_t = \ln 3.65 - 0.091t$ Ke
2. $\ln R_1 = \ln 3.65 - 0.683t$ Ka

$Cest = 3.65e^{-0.091t} - 3.65e^{-0.683t}$

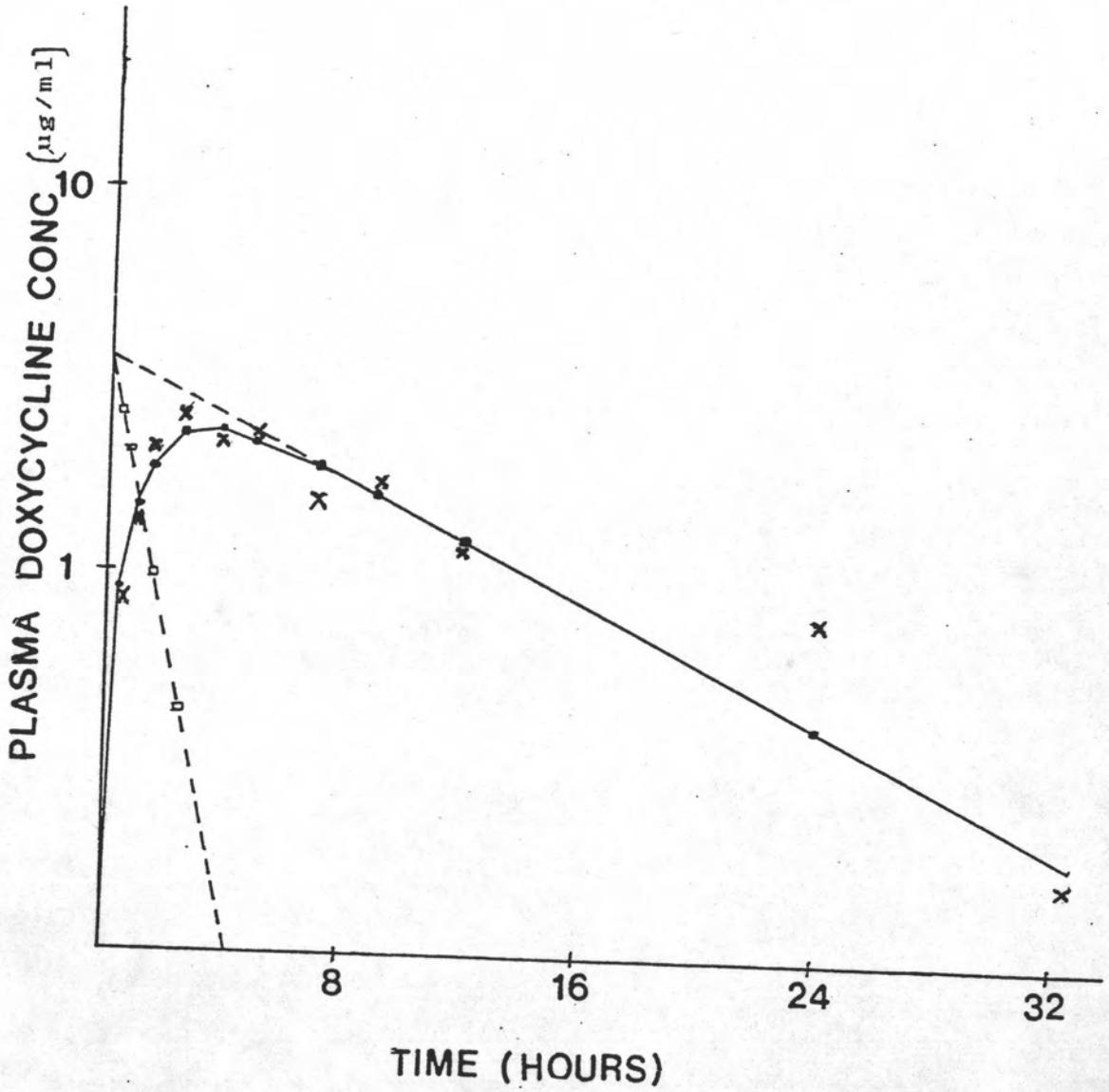


Figure 29 Graphical Technique of Calculation Estimated Parameters in Plasma Data by the Method of Residual.

× real values —●— estimated values

```
.........CURVE STRIPPING.........

**********************************

DATA SET NUMBER  1


THE NUMBER OF EXPONENTIALS = 2
SUMMARY OF EXPONENTIAL STRIPPING

THE NUMBER OF POINTS IN THE EXPONENTIAL PHASES (LAST TO FIRST)
     L1=     6
     L2=     6


THE BEST ESTIMATES OF THE COEFFICIENTS AND EXPONENTS ARE
 A1= 0.364943E+01      B1= 0.905737E-01
 A2=-0.364943E+01      B2= 0.680371E+00
 F= 0.379280E+00


NO LAG TIME WAS NEEDED TO DESCRIBE THESE DATA
THEREFORE, THE SUM OF THE EXPONENTIAL TERMS WAS FORCED THROUGH ZERO

R SQUARE(2) =   0.99685

 NO.        TIME        C(OBS)        C(EST)        % DEV
   1        0.0000       0.0000        0.0000        0.00
   2        0.5000       0.8122        0.8308       -2.37
   3        1.0000       1.2069        1.4852      -23.06
   4        1.5000       2.1601        1.8705       13.40
   5        2.5000       2.4686        2.2439        9.33
   6        3.5000       2.1601        2.3206       -7.43
   7        5.0000       2.2214        2.1987        1.02
   8        7.0000       1.6604        1.9047      -14.71
   9        9.0000       1.7181        1.8071        5.46
  10       12.0000       1.2195        1.2298       -0.84
  11       24.0000       0.6418        0.4151       35.32
  12       36.0000       0.1373        0.1837      -33.81
```

Figure 30 The Output of CSTRIP Program

APPENDIX L

THE OUTPUT OF PCNONLIN PROGRAM

```
PCNONLIN NONLINEAR ESTIMATION PROGRAM V01-E
****  COPYRIGHT 1984,1985  ****
FOR INFORMATION CONTACT - STATISTICAL CONSULTANTS INC.
                    1-606-252-3890
LISTING OF INPUT COMMANDS

model 3, 'nlin.lib'
MODEL 3
REMARK  ONE COMPARTMENT MODEL - FIRST ORDER INPUT AND OUTPUT
REMA
REMA  NO.   PARAMETER     CONSTANT      SECONDARY PARM.
REMA  ---   ---------     --------      ---------------
REMA  1      VOLUME         DOSE           AUC
REMA  2       K01                        K01 HALF LIFE
REMA  3       K10                        K10 HALF LIFE
REMA  4                                    TMAX
REMA  5                                    CMAX
REMA************************************************************
REMA          I------------------I
REMA          I                  I
REMA  K01 --> I  COMPARTMENT 1   I ---> K10
REMA          I                  I
REMA          I------------------I
REMA************************************************************
COMM
NPARM 3
```

```
NCON 1
NSEC 5
PNAMES 'VOLUME', 'K01', 'K10'
SNAMES 'AUC', 'K01-HL', 'K10-HL', 'TMAX', 'CMAX'
END
TEMP
D=CON(1)
V=P(1)
K01=P(2)
K10=P(3)
T=X
END
FUNC1
COEF=D*K01/(V*(K01-K10))
F=COEF*(DEXP(-K10*T)-DEXP(-K01*T))
END
SECO
S(1)=D/V/K10
S(2)=-DLOG(.5)/K01
S(3)=-DLOG(.5)/K10
TMAX=(DLOG(K01/K10)/(K01-K10))
S(4)=TMAX
S(5)=(D/V)*DEXP(-K10*TMAX)
END
EOM
cons 100
init 42.97611, 1.56192, .081136
nobs 12
data
begin
```

PCNONLIN NONLINEAR ESTIMATION PROGRAM

ITERATION	WEIGHTED SS	VOLUME	K01	K10
0	1.90587	42.98	1.562	.8114E-01
1	1.89975	42.96	1.519	.7813E-01
2	1.88943	42.12	1.392	.8270E-01
3	1.88084	41.91	1.468	.8270E-01
4	1.87724	41.51	1.395	.8405E-01
5	1.86992	40.71	1.422	.8728E-01
6	1.86876	40.66	1.388	.8763E-01
7	1.86781	40.29	1.390	.8940E-01
8	1.86749	40.29	1.374	.8924E-01
9	1.86725	40.05	1.358	.9024E-01

CONVERGENCE ACHIEVED

RELATIVE CHANGE IN WEIGHTED SUM OF SQUARES LESS THAN .000100

9	1.86722	40.05	1.361	.9038E-01

PCNONLIN NONLINEAR ESTIMATION PROGRAM

PARAMETER	ESTIMATE	STANDARD ERROR	95% CONFIDENCE LIMITS	
VOLUME	40.053072	8.135213	21.649785	58.456359 UNIVARIATE
		11.714000	68.392144 PLANAR	
K01	1.361477	.724109	-.276585	2.999540 UNIVARIATE
		-1.160961	3.883916 PLANAR	
K10	.090377	.039955	-.000007	.180762 UNIVARIATE
		-.048805	.229559 PLANAR	

PCNONLIN NONLINEAR ESTIMATION PROGRAM

CORRELATION MATRIX OF THE ESTIMATES

1.00000

.75866 1.00000

-.84051 -.64524 1.00000

EIGENVALUES OF (A TRANSPOSE A) MATRIX

NUMBER	EIGENVALUE
1	442.9
2	.9365
3	.3121E-02

PCNONLIN NONLINEAR ESTIMATION PROGRAM

SUMMARY OF NONLINEAR ESTIMATION

FUNCTION 1

X	OBSERVED Y	CALCULATED Y	RESIDUAL	WEIGHT	SD-YHAT	STANDARIZED RESIDUAL
.0000	.0000	.0000	.0000	1.000	.0000	.0000
.5000	.5892	1.202	-.6131	1.000	.3081	-1.346
1.000	1.853	1.758	.9483E-01	1.000	.2813	.2082
1.500	2.570	1.988	.5819	1.000	.2295	1.277
2.500	2.510	2.044	.4659	1.000	.2468	1.023

3.500	1.315	1.926	-.6117	1.000	.2520	-1.343
5.000	1.315	1.699	-.3845	1.000	.2144	-.8441
7.000	1.315	1.420	-.1058	1.000	.1959	-.2323
9.000	1.699	1.186	.5133	1.000	.2185	1.127
12.00	.6497	.9040	-.2543	1.000	.2560	-.5584
24.00	.5611	.3056	.2555	1.000	.2270	.5609
33.00	.1306	.1355	-.4897E-02	1.000	.1487	-.1075E-01

CORRECTED SUM OF SQUARED OBSERVATIONS = 7.97522

WEIGHTED CORRECTED SUM OF SQUARED OBSERVATIONS = 7.97522

SUM OF SQUARED RESIDUALS = 1.86722

SUM OF WEIGHTED SQUARED RESIDUALS = 1.86722

S = .455488 WITH 9 DEGREES OF FREEDOM

CORRELATION (Y,YHAT) = .875

PCNONLIN NONLINEAR ESTIMATION PROGRAM

SUMMARY OF ESTIMATED SECONDARY PARAMETERS

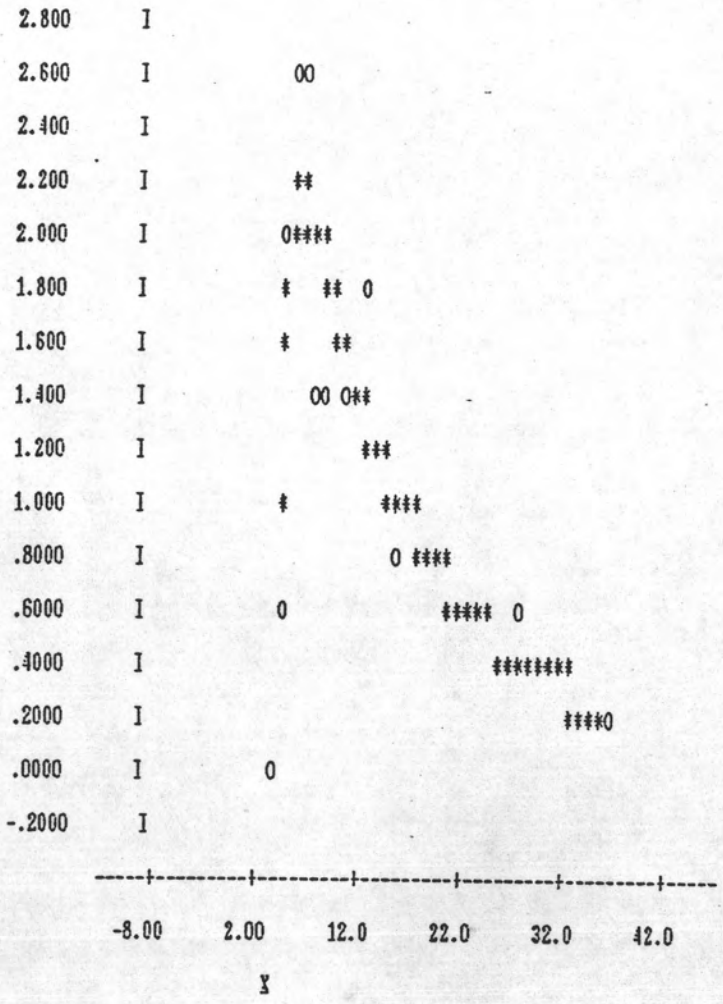
PARAMETER	ESTIMATE	STANDARD ERROR
AUC	27.625106	8.081467
K01-HL	.509114	.270504
K10-HL	7.669468	3.387172
TMAX	2.133845	.651746
CMAX	2.058779	.233971

PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION 1

PLOT OF X VS. OBSERVED Y AND CALCULATED Y

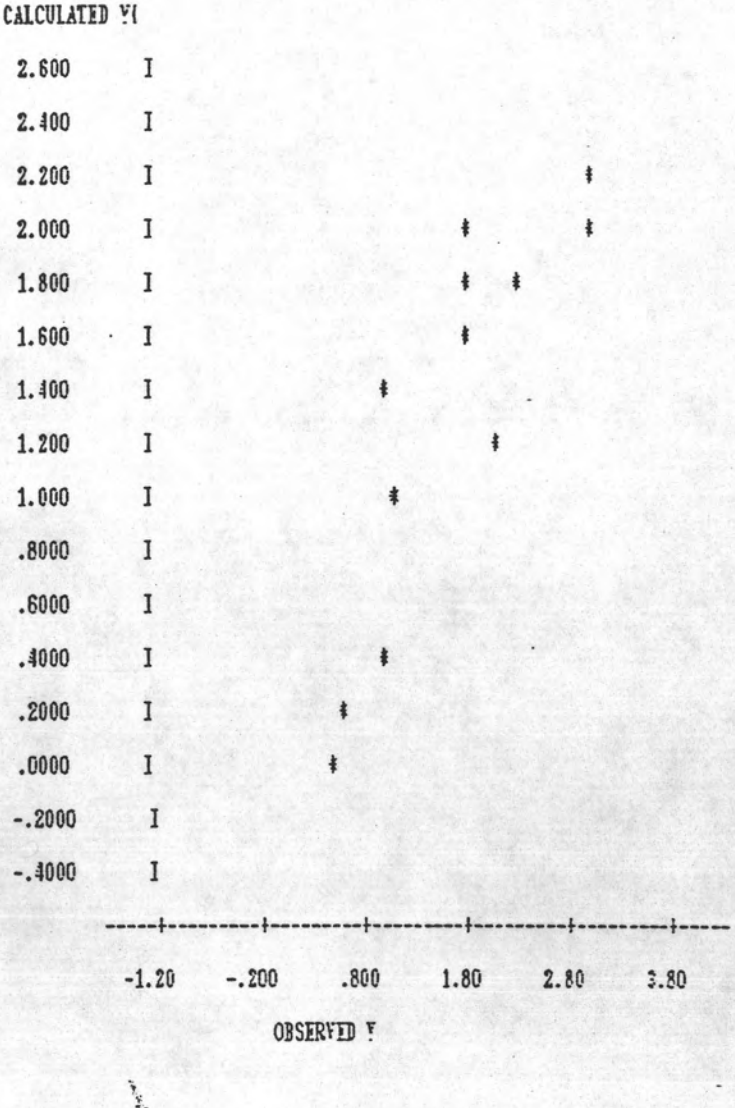
*** ARE CALCULATED POINTS, OOO ARE OBSERVED POINTS



PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION 1

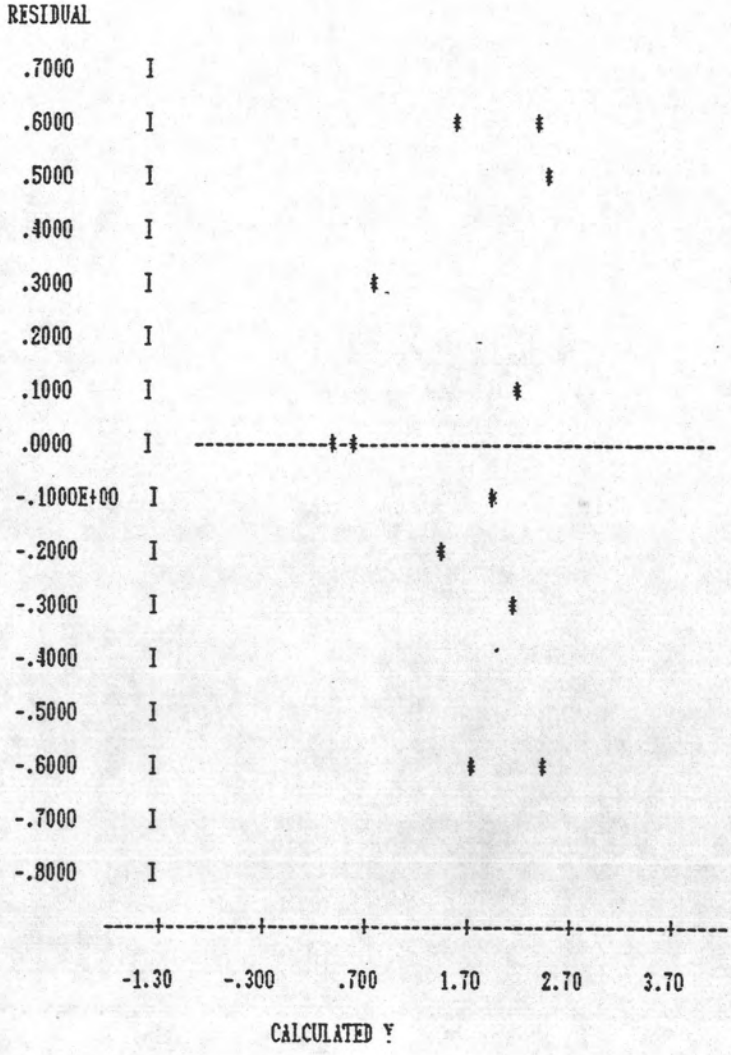
PLOT OF OBSERVED Y VS. CALCULATED Y



PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION 1

PLOT OF CALCULATED Y VS. RESIDUAL

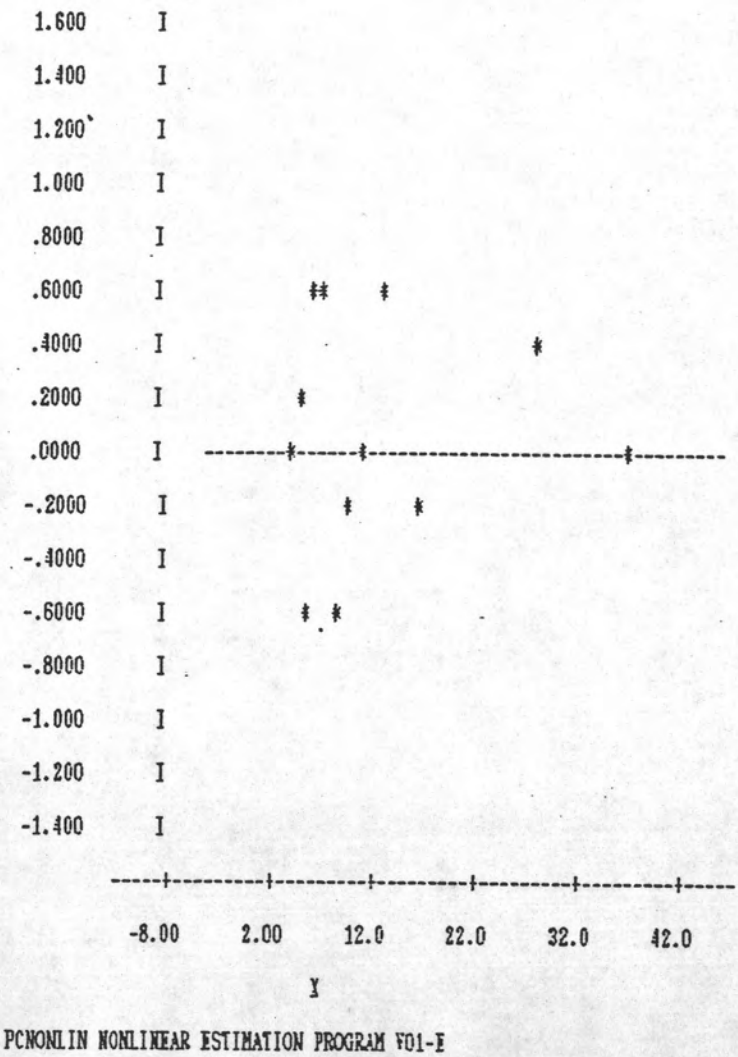


PCNONLIN NONLINEAR ESTIMATION PROGRAM

FUNCTION E

PLOT OF X VS. RESIDUAL V

RESIDUAL



PCNONLIN NONLINEAR ESTIMATION PROGRAM V01-E

LISTING OF INPUT COMMANDS

finish

APPENDIX M

STATISTICS

1. Mean ($\overline{X}$)

$$\overline{X} = \frac{\sum x}{N}$$

2. Standard deviation (S.D.)

$$S.D. = \sqrt{\frac{(X - \overline{X})^2}{N - 1}}$$

3. Standard error of the mean (SEM)

$$SEM = \frac{S.D.}{\sqrt{N}}$$

4. Coefficient of Variation

$$C.V. = \frac{S.D.}{\overline{X}} \times 100$$

5. Testing the difference of two means, by Student's t-test

Let μ_1, μ_2 = Population means

X_1, X_2 = Sample means

σ_1, σ_2 = Population variances

N_1, N_2 = Sample size

The null hypothesis Ho : $\mu_1 = \mu_2$

The alternative hypothesis Ha : $\mu_1 \neq \mu_2$

The statistic t is given as $t = \dfrac{(\overline{X}_1 - \overline{X}_2) - (\mu_1 - \mu_2)}{Sp}$

First homogeneity of variance is tested using the F test, which is defined as follow:

$$F = \frac{(S_1)^2}{(S_2)^2}$$

where $(S_1)^2$ = the larger of the two sample variances
$(S_2)^2$ = the samller of the two sample variances

With this test, the null hypothesis of no difference between the two population variances is evaluated. If the F is not significant the null hypothesis stands.

5.1 If $\sigma_1^2 \neq \sigma_2^2$, the statistic t is given as

$$t = \frac{\overline{X}_1 - \overline{X}_2}{S_P}$$

where S_p^2 is the pooled variance :

$$S_p^2 = \frac{(S_1)^2}{N_1} + \frac{(S_2)^2}{N_2}$$

with degree of freedom, d.f. :

$$\text{d.f.} = \frac{\left[\frac{S_1^2}{N_1} + \frac{S_2^2}{N_2}\right]^2}{\frac{\left[\frac{S_2}{N_1}\right]^2}{N_1 - 1} + \frac{\left[\frac{S_2^2}{N_2}\right]^2}{N_2 - 1}}$$

5.2 If $\sigma_1^2 = \sigma_2^2$ the statistic t for this case is

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S_P}$$

where the pooled variance is

$$S_P^2 = \left[\frac{1}{N_1} + \frac{1}{N_2}\right]\left[\frac{(N_1 - 1)\, S_1^2 + (N_2 - 1)S_2^2}{N_1 + N_2 - 2}\right]$$

with degree of freedom

$$\text{d.f.} = N_1 + N_2 - 2$$

This t value is compared with $t_{(\alpha, ab)}$ which is obtained from the table for $\frac{\alpha}{2}$

If $t > t_{(\alpha, ab)}$, the null hypothesis that $\mu_1 = \mu_2$ is rejected and the alternative hypothesis is accepted. If t is not significant, the null hypothesis stands.

6. Analysis of variance (ANOVA)

Analysis of Variance of Completely Randomized Design

Source of Variation	Sum of Squares	d.f.	Mean Square	Variation
Among groups (Treatment)	$\sum_{j=1}^{k} n_j \, (X._j - X..)^2$	k -1	$\frac{\text{SS among}}{k - 1}$	V.R. = $\frac{\text{MS among}}{\text{MS within}}$
Within groups (Error)	$\sum_{j=1}^{k} \sum_{j=1}^{n_j} (X_{ij}-X._j)^2$	N-k	$\frac{\text{SS within}}{N - k}$	
Total	$\sum_{j=1}^{k} \sum_{j=1}^{n_j} (X_{ij}-X..)^2$	N-1		

where X_{ij} = Observed value i at treatment j

$i = 1, 2, \ldots, n$

$j = 1, 2, \ldots, k$

$$T._j = \sum_{i=1}^{n_j} X_{ij}$$

$$\overline{X}._j = \frac{T._j}{n_j}$$

$$T.. = \sum_{j=1}^{k} T._j$$

$$\overline{X} = \frac{T..}{N}$$

$$N = \sum_{j=1}^{k} n_j$$

The V.R. value is compared with the critical value, F, which is obtained from the table at degree of freedom (k-1) and (N-k)

If $F > F(tab)$, the null hypothesis that $\mu_1 = \mu_2 = \mu_3 = \ldots = \mu_k$ is rejected and the alternative hypothesis is accepted. If F is not significant, the null hypothesis stands.

7. Correlation coefficient test

The correlation coefficient is a quantitative measure of the relationship of correlation between two vaviables, x and y

$$r = \frac{N\sum XY - \sum X \sum Y}{\sqrt{[N\sum x^2 - (\sum x)^2][N\sum y^2 - (\sum y)^2]}}$$

where r = Correlation coefficient

N = The number of x and y pairs

Test of zero correlation

Let ρ = the true correlation coefficient, estimated by r

The null hypothesis Ho : $\rho = 0$

The alternative hypothesis Ha : $\rho \neq 0$

$$t_{N-2} = \frac{\left| r\sqrt{N-2} \right|}{\sqrt{1-r^2}}$$

The value of t is refered to a t distribution with (N - 2) degree of freedom. If $t > t(tab)$, we reject the null hypothesis and accept the alternative hypothesis. If t is not significant, the null hypothesis stands.

VITAE

Miss Somsri Rienniyom was born on May 3, 1958 in Bangkok. She graduated with Bachelor Degree of Science in Pharmacy in 1982 from the Faculty of Pharmacy, Mahidol University. She has worked at the Food and Drug Administration, Ministry of Public Health.