CHAPTER IV

RESULTS AND DISCUSSION

1. In Vitro Studies

All three commercial brands of ceftriaxone IM preparations were evaluated following the United States Phamacopoeia (U.S.P. XXIII) requirements. They were tested for weight variation, content of active ingredient (% L.A.) and stability tests, respectively. Results of all tests were summerized in Table 3. Weight variation, claculated in term of ceftriaxone anhydrous (mg)/ ceftriaxone sodium hydrous (g), and the content of active ingredient for all three brands met the requirements of the U.S.P. XXIII within the limits of not less than 776 µg of ceftriaxone anhydrous/mg of ceftriaxone sodium hydrous and 90.0-115.0 percent labeled amount (% L.A.), respectively. Therefore, it was concluded that all three brands were pharmaceutically equivalent.

The stability tests in this study were conducted using the modified method of that for determining of the % L.A. to calculate the remaining amount of active ingredient under specified conditions. Results are shown in Tables 4 and 5. There were no changes in physical appearances of brands A and C but the color of brand B changed from deep yellow to brown on day 7 at 4°C. However, at 30°C all brands were changed in color from day 7. No precipitation and/or crystallization were observed in any brands (Table 4). There were no significantly different changes in active ingredient between days 1 and 7 among the three brands of ceftriaxone IM at 30°C. However, at 4°C, statistical comparison indicated that the change in amount of active ingredient of only brand B was statistically greater than that of brand A ($p < 0.05$) as shown in Tables 6 and 7. The amount of active ingredient of all three brands were not determined on days 14, 21 and 28 at both temperature. This was because of the split of ceftriaxone peak in HPLC chromatogram referring that ceftriaxone was no longer stable.

In summary, each brand of ceftriaxone intramuscular injection resulted in its own stability and physical characteristics after reconstituting, such as color of the solutions and the

```
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WIDTH        10
SLOPE       250
DRIFT         0
MIN AR      200
MIN AR     2000
T DBL         0
LOCK        3.1
STP TM       10
ATTEN         3
SPEED         2
METHOD       41
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2.54
3.49

Blank

STOP

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C-R1A
SMPL #       00
FILE #        2
REPT #     4401
METHOD       41

  #    NAME   TIME       CONC    MK     AREA
  0           2.54    17.5543          1204
  0           3.49    82.4456          5656
         TOTAL        99.9999          6861
START  09.01.10.07.
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STOP 4.95 4.02   (A)   5 µg/ml

```
C-R1A
SMPL #       00
FILE #        2
REPT #     4402
METHOD       41

  #    NAME   TIME       CONC    MK     AREA
  0           4.02      100           17539
         TOTAL          100           17539
START  09.01.10.38.
```

3.08
STOP 5.32   4.05

(A)   50 µg/ml

```
C-R1A
SMPL #       00
FILE #        2
REPT #     4406
METHOD       41

  #    NAME   TIME       CONC    MK     AREA
  0           4.05    99.9999         203635
         TOTAL        99.9999         203635
START  09.01.11.07.
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4.

STOP

(A)   240 µg/ml

```
C-R1A
SMPL #       00
FILE #        2
REPT #     4410
METHOD       41

  #    NAME   TIME       CONC    MK     AREA
  0           4.      99.9999         882197
         TOTAL        99.9999         882197
```

Figure 2   High performance liquid chromatography of ceftriaxone (A) for in vitro studies

Table 3 In vitro studies of three commercial brands of ceftriaxone intramuscular injections

Brand	Weight Variation[a] (n = 10)	Content of Active Ingredient (% L.A.) (n = 3)	Stability Tests Content of Active Ingredient (mg/ml)									
			day (4^{0}C)					day (30^{0}C)				
			1	7	14	21	28	1	7	14	21	28
A	835.58 $\pm$ 9.61	111.51 $\pm$ 1.12	269.73 $\pm$ 12.78 (100%)	268.98 $\pm$ 13.06 (99.72%)	*	*	*	279.45 $\pm$ 2.44 (100%)	217.45 $\pm$ 20.57 (77.95%)	*	*	*
B	837.43 $\pm$ 13.70	113.20 $\pm$ 1.88	260.38 $\pm$ 11.10 (100%)	245.11 $\pm$ 5.02 (94.14%)	*	*	*	255.41 $\pm$ 11.86 (100%)	218.52 $\pm$ 11.40 (85.56%)	*	*	*
C	837.06 $\pm$ 10.25	114.54 $\pm$ 0.83	262.36 $\pm$ 13.22 (100%)	254.99 $\pm$ 6.89 (97.19%)	*	*	*	272.78 $\pm$ 12.83 (100%)	209.86 $\pm$ 25.78 (76.93%)	*	*	*

** All values are presented as mean $\pm$ S.D.

* Unevaluated values (Degradation of active ingredient)

[a] Weight variation presented in term of mg. of ceftriaxone anhydrous/g. of ceftriaxone sodium hydrous

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Table 4 Physical appearances of reconstitued ceftriaxone intramuscular injections

Band	day (4^0C)					day (30^0C)				
	1	7	14	21	28	1	7	14	21	28
A	+	++	++	++	++	+	**	**	**	**
B	++	*	*	*	*	++	**	**	**	**
C	+	++	++	++	++	+	**	**	**	**

+ = yellow

++ = deep yellow

* = brown

** = dark brown

Table 5 Content of active ingredient for stability tests

Sample Number	Content of active ingredient (mg/ml)																	
	4^{0}C									30^{0}C								
	Brand A			Brand B			Brand C			Brand A			Brand B			Brand C		
	day 1	day 7	ΔX	day 1	day 7	ΔX	day 1	day 7	ΔX	day 1	day 7	ΔX	day 1	day 7	ΔX	day 1	day 7	ΔX
1	255.42	254.54	0.88	264.13	248.33	15.80	250.18	247.34	2.84	277.08	194.07	83.01	266.40	218.96	47.44	263.38	180.34	83.04
2	273.76	272.43	1.33	269.13	239.33	29.80	276.42	260.71	15.71	281.96	229.70	52.26	256.98	229.69	27.29	267.56	227.94	39.62
3	280.02	279.97	0.05	247.89	247.67	0.22	260.48	256.91	3.57	279.30	229.69	49.61	242.84	206.91	35.93	287.40	221.30	66.10
Mean	269.73	268.98	0.75	260.38	245.11	15.27	262.36	254.99	7.37	279.45	217.82	61.63	255.41	218.52	36.89	272.78	209.86	62.92
S.D.	12.78	13.06	0.65	11.10	5.02	14.80	13.22	6.89	7.23	2.44	20.57	18.57	11.86	11.40	10.11	12.83	25.78	21.88

ΔX   =   Difference of active ingredient concentration between days 1 and 7

Table 6   Analysis of Variance for stability tests of three brands of ceftriaxone
intramuscular injections stored at 4° C

Source of Variation	d.f[a].	SS[b]	MS[c]	F[d]
Among group	2	543.27	271.64	6.0
Within group	6	317.06	45.29	
Total	8	860.33		

Calculation data from different values between days 1 and 7

$$F^{e}_{0.05} (2, 6) = 5.14$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 7 Comparison of stability tests for each brand of ceftriaxone intramuscular injections with that of the innovator's product (brand A) stored at 4°C

Brand	Δx	Statistical Significance
B	14.52	S
C	6.62	NS

$t_{0.05}(6)$ = 2.447

LSD $(0.05) = t_{0.05} \times S_d = 7.76$

S = Significant difference at P<0.05

NS = Not significant difference at P>0.05

Table 8   Analysis of Variance for stability tests of three brands of ceftriaxone
intramuscular injections stored at 30° C

Source of Variation	d.f[a].	SS[b]	MS[c]	F[d]
Among group	2	1,291.48	645.74	2.09
Within group	6	1,851.58	308.60	
Total	8	3,143.06		

Calculation data from different values between days 1 and 7

$F^{e}_{0.05} (2, 6) = 5.14$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

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ease to form a clear solution. These might be due to ceftriaxone powder itself. Different sources and/or manufacturing processes of the powdered drug could contribute for such properties.

2. In Vivo Studies

2.1 Clinical Observations

No side effects and/or any indication of intoxications were found during administration of a high dose of 1 g. ceftriaxone IM.

2.2 Analysis of ceftriaxone in plasma

Plasma ceftriaxone concentrations were analyzed using high performance liquid chromatography. In this study, the procedure of Demotes-Mainard et al. (1988) was modified for analyzing ceftriaxone concentrations in plasma samples. The method involved precipitation of proteins from plasma using acetonitrile followed by extraction of endogenous compounds using chloroform and injection of the upper aqueous phase into the chromatography.

The mobile phase consisted of methanol and 0.1 M phosphate buffer (pH 3.0±0.1) 25:75 v/v and 0.4% v/v triethylamine. Ceftriaxone always exhibited tailing of the peak in reversed-phase liquid chromatography. Hence, to reduce such effect, methanol was used instead of acetonitrile in the mobile phase.

Typical chromatograms of ceftriaxone and internal standard (ciprofloxacin) are shown in Figure 3. The retention times of ceftriaxone and internal standard were 2.9 and 4.9 minutes, respectively. The method was validated by determining the within-run and between-run precisions. The percent coefficient of variation (% C.V.) in the within-run and between-run precision were 1.39-8.78 percent and 3.02-9.91 percent as shown in Tables 9 and 10, respectively. The efficiency of separating technique used was evaluated by calculating the percentage of recoveries and comparing the peak height obtained from spiked plasmas to those from standard solutions. Results as shown in Table 11 indicated that the analytical method

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ATTEN        2
SLOPE      250
LOCK         2
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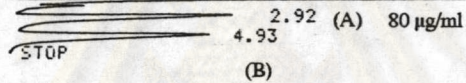
2.09

Plasma blank
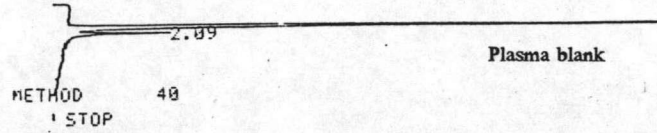
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' STOP
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STP TM       7
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2.99 (A)   10 µg/ml

4.96

STOP

(B)
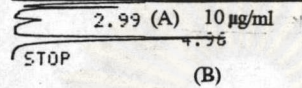
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FILE #      2
REPT #   3892
METHOD   1041

#    NAME    TIME      CONC      MK     HEIGHT
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0            4.96     89.0116            1433
        TOTAL         99.9999            1610
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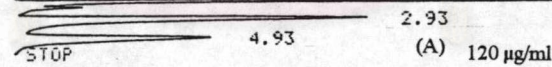
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START   12.12.13.47.
```

2.92 (A)   80 µg/ml

4.93

STOP

(B)

```
C-R1A
SMPL #     00
FILE #      2
REPT #   3895
METHOD   1041

#    NAME    TIME      CONC      MK     HEIGHT
0            2.92     52.564             1479
0            4.93     47.4359    V       1335
        TOTAL         99.9999            2814
```

```
START   12.12.13.54.
```

2.93

4.93   (A)   120 µg/ml

STOP

(B)

```
C-R1A
SMPL #     00
FILE #      2
REPT #   3896
METHOD   1041

#    NAME    TIME      CONC      MK     HEIGHT
0            2.93     64.5997            2403
0            4.93     35.4002    V       1316
        TOTAL         99.9999            3720
```

Figure 3 High performance liquid chromatography of ceftriaxone (A) and internal
standard (ciprofloxacin : B)

Table 9   Within-run precision of ceftriaxone

Concentration (μg/ml)	Average Peak Height Ratio	% C.V.
5	0.0581	4.13
10	0.1235	2.60
20	0.2773	8.78
40	0.5332	5.26
80	1.0394	1.39
120	1.6624	2.53
160	2.0773	2.05
240	3.2507	3.74

(n = 3)

Table 10 Between-run precision of ceftriaxone

Concentration (μg/ml)	Average Peak Height Ratio	% C.V.
5	0.0784	3.28
10	0.1483	6.66
20	0.3121	9.12
40	0.5614	3.02
80	1.0809	5.39
120	1.6901	5.75
160	2.1903	9.91
240	3.3549	7.29

(n = 3)

Table 11 Recoveries of ceftriaxone and internal standard (ciprofloxacin)

Conc. (μg/ml)	Drug Peak Height (c.m.)		% Recovery	Internal Standard Peak Height (c.m.)		% Recovery
	Solution	Plasma		Solution	Plasma	
5	0.1400	0.1400	100.00	6.4500	3.5167	54.52
20	0.6667	0.5467	82.00	6.2400	4.2667	68.38
40	1.5667	1.2833	81.91	7.0833	3.9667	56.00
80	3.1500	3.0133	95.66	6.5500	3.9500	60.31
160	6.1000	5.9585	97.68	5.5900	3.6500	65.30
Mean % Recovery ± S.D.			91.45 ± 8.80			60.90 ± 5.92

% Recovery = $\frac{\text{Peak height from spiked plasma}}{\text{Peak height from solution}} \times 100$

used was independent to concentration. The percentage recoveries of ceftriaxone and internal standard were in the range of 81.91-100.00 percent and 54.52-68.38 percent, respectively.

The calibration curve of plasma levels of ceftriaxone concentration versus peak height ratio of ceftriaxone to internal standard was linear upto 240 μg/ml (Appendix C). The sensitivity of detection was 5 μg/ml.

2.3 Plasma ceftriaxone concentration

The plasma ceftriaxone concentrations from 12 subjects at each sampling time interval upto 24 hr. after intramuscular injections of brands A, B and C are shown in Tables 12 to 14. Individual plasma ceftriaxone concentration-time prefile for each of 12 subjects are shown graphically in Figures 4 to 15. Comparison of the mean plasma ceftriaxone concentration-time profile of each brand from 12 subjects were summarized in Figure 16. The mean plasma ceftriaxone concentration of all three brands showed nonlinear absorption, producing an erratic pattern of early serum levels. This might be explained that ceftriaxone has enterohepatic cycling effects which caused the drug having control release properties with a long half-life. The reabsorption of the drug might be induced a little change on plasma level which showed as split peak (Figure 16).

2.4 Bioequivalent Evaluation

Some physicians assume that the intramuscular route is as reliable as the intravenous and that it results in equally complete bioavailability of the injected drug. Careful studies during the past several years, greatly facilitated by the ability to measure drug concentrations in the blood, have shown that intramuscular injection of drug does not always assume rapid of complete bioavailability. The absorption of intramuscularly injected drugs can be quite slow or incomplete or both. The drug concentrations at the site of action after single or repeated intramuscular injection of some formulations may never equal those obtained after oral or intravenous administration of the same doses. (Greenblatt et al., 1976)

The bioavailability of drug from intramuscular injection dosage form depends on penetration and absorption into the general circulation. These factors can be evaluated by

Table 12 Plasma ceftriaxone concentration (µg/ml) from 12 subjects following intramuscular injection of brand A

Subject Number	Time (hr.)									
	0.25	0.5	1	1.5	2	3	5	8	12	24
1	11.14	20.39	36.50	57.94	62.08	46.46	76.95	26.21	10.17	4.27
2	53.21	87.22	128.30	76.32	120.17	151.98	76.97	61.37	21.82	0.93
3	40.39	57.51	79.40	80.06	110.40	99.84	65.13	51.73	31.10	6.55
4	61.02	90.47	76.73	106.77	138.33	128.55	80.76	72.91	26.62	8.83
5	148.25	159.99	163.35	148.25	145.40	157.23	117.57	86.01	63.92	27.91
6	70.10	111.11	121.71	121.69	115.08	153.86	111.32	95.24	73.72	31.59
7	165.75	165.48	217.09	167.76	164.39	166.82	146.54	90.48	60.08	27.04
8	135.11	116.08	170.36	138.41	132.38	146.23	119.77	126.29	67.21	30.54
9	160.13	214.16	190.11	193.21	247.35	161.18	158.90	97.32	53.65	13.03
10	47.61	79.21	127.26	87.98	110.32	87.93	121.28	73.80	18.77	2.81
11	191.77	127.76	253.74	138.93	205.02	157.45	147.52	90.81	51.17	10.74
12	63.98	122.71	147.52	125.81	194.68	132.03	159.70	110.66	61.78	24.17
Mean	95.62	112.43	143.17	120.26	145.47	132.46	115.20	81.90	45.00	15.70
S.E.M.	17.38	14.93	17.87	11.59	14.54	10.56	9.77	7.77	6.32	3.37

Table 13   Plasma ceftriaxone concentration (μg/ml) from 12 subjects following
intramuscular injection of brand B

Subject	Time (hr.)									
Number	0.25	0.5	1	1.5	2	3	5	8	12	24
1	14.55	30.74	58.80	39.08	75.84	83.75	99.02	99.21	47.24	18.77
2	55.79	56.84	94.62	103.33	134.55	168.22	153.50	101.99	68.87	12.11
3	99.37	126.58	205.82	127.42	170.09	161.64	127.03	106.16	64.51	20.84
4	158.06	200.17	223.17	171.79	217.95	165.43	144.12	145.43	67.70	23.19
5	36.875	37.98	67.40	123.77	117.94	70.17	114.45	67.50	26.76	10.79
6	35.50	82.86	57.35	99.62	123.32	157.99	52.29	68.08	25.45	9.39
7	84.79	65.16	145.81	127.69	194.02	99.73	113.99	68.30	32.50	6.25
8	26.82	63.48	84.82	103.60	108.40	98.24	159.27	31.97	49.76	11.83
9	116.90	174.70	198.13	144.99	173.28	138.32	140.21	96.19	58.26	11.21
10	56.41	78.52	85.80	105.55	99.09	104.98	79.40	86.43	52.57	15.13
11	155.41	195.16	214.38	155.63	180.61	148.72	126.06	125.26	50.45	26.75
12	132.80	155.13	184.79	179.13	177.35	152.28	142.54	126.29	76.22	23.41
Mean	81.11	105.61	135.07	123.47	147.70	129.12	120.99	93.57	51.69	15.81
S.E.M.	14.67	17.81	19.20	10.93	12.59	10.18	9.12	9.06	4.82	1.90

Table 14   Plasma ceftriaxone concentration (μg/ml) from 12 subjects following intramuscular injection of brand C

Subject Number	Time (hr.)									
	0.25	0.5	1	1.5	2	3	5	8	12	24
1	12.15	32.85	37.85	55.95	74.85	61.69	77.66	75.77	79.34	30.42
2	93.16	110.41	142.14	125.86	142.16	151.13	121.97	87.51	63.32	21.62
3	87.23	149.38	145.53	133.95	162.60	168.77	115.10	89.05	78.77	30.23
4	109.65	149.90	201.28	189.72	174.35	185.63	121.21	108.58	93.65	31.41
5	78.85	70.94	170.66	137.32	167.92	145.74	198.67	114.10	85.45	33.88
6	116.11	101.13	209.67	151.98	188.23	154.34	232.26	117.03	64.09	19.16
7	115.17	162.36	232.84	104.85	196.09	192.50	181.67	120.78	56.01	11.92
8	108.42	150.02	227.68	220.96	235.64	190.46	149.05	105.08	79.22	35.97
9	22.59	57.04	85.27	108.06	89.45	52.35	114.48	53.84	26.14	4.92
10	27.67	82.11	95.93	61.78	101.54	91.89	42.10	41.43	9.00	2.47
11	132.94	137.47	83.22	123.67	128.06	131.72	61.18	48.86	15.61	0.20
12	72.75	54.66	100.12	94.39	101.47	84.89	54.42	66.81	37.87	12.36
Mean	81.39	104.86	144.35	125.71	146.86	134.26	122.48	85.74	54.82	19.55
S.E.M.	11.65	12.95	18.64	13.69	14.15	14.41	17.20	8.13	8.26	3.74

PLASMA CEFTRIAXONE CONCENTRATION
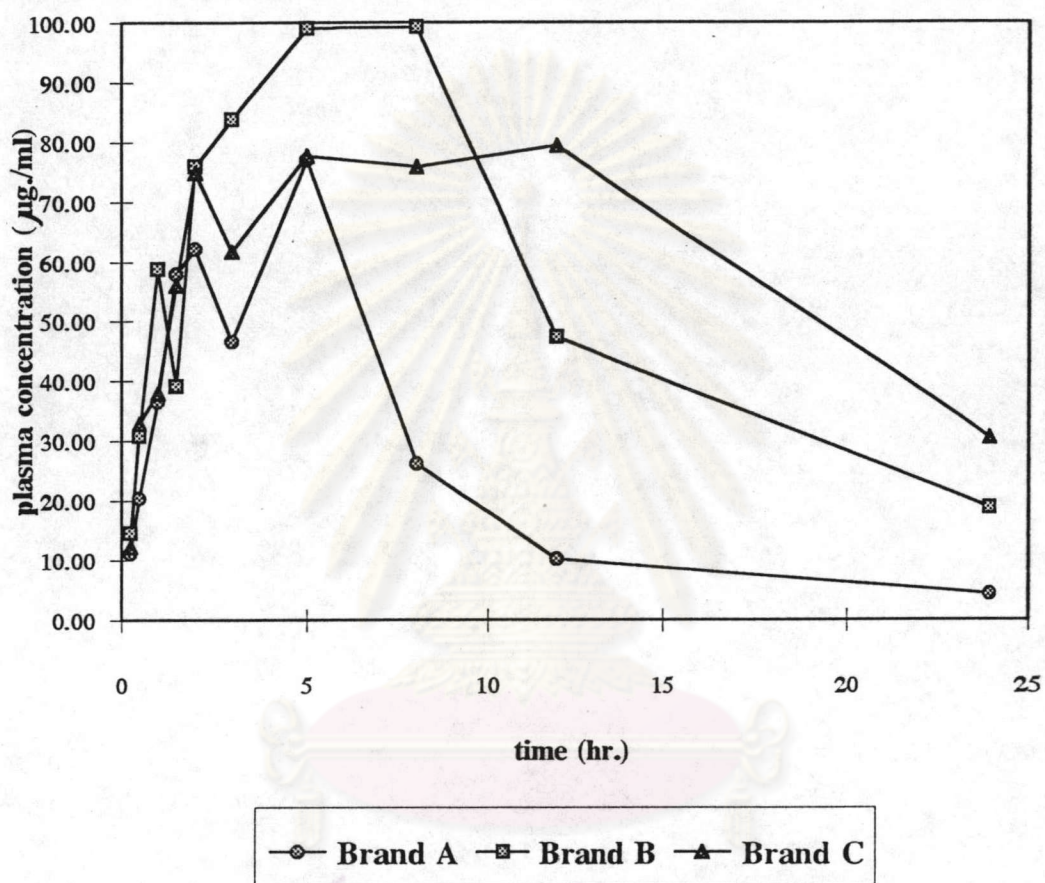
subject no.1

Figure 4 Plasma ceftriaxone concentration-time profile of subject number 1 following intramuscular injection of 1g. ceftriaxone
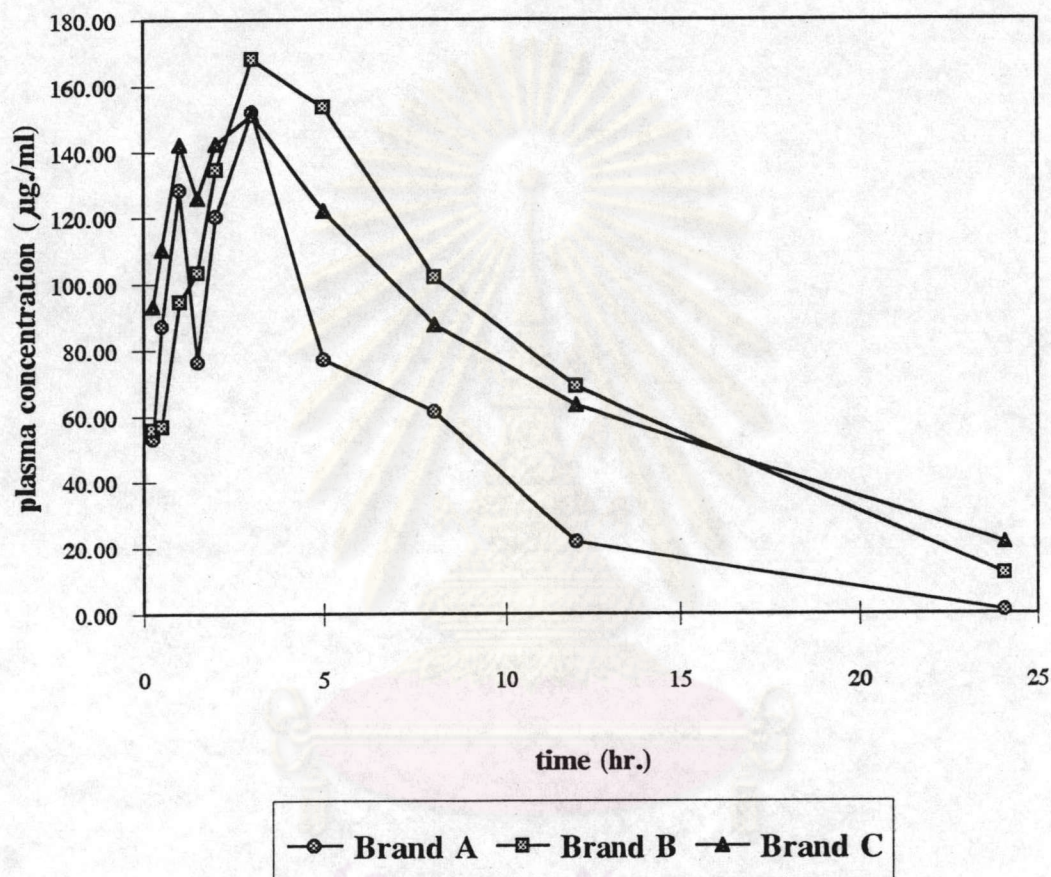
PLASMA CEFTRIAXONE CONCENTRATION

subject no.2

Figure 5 Plasma ceftriaxone concentration-time profile of subject number 2 following
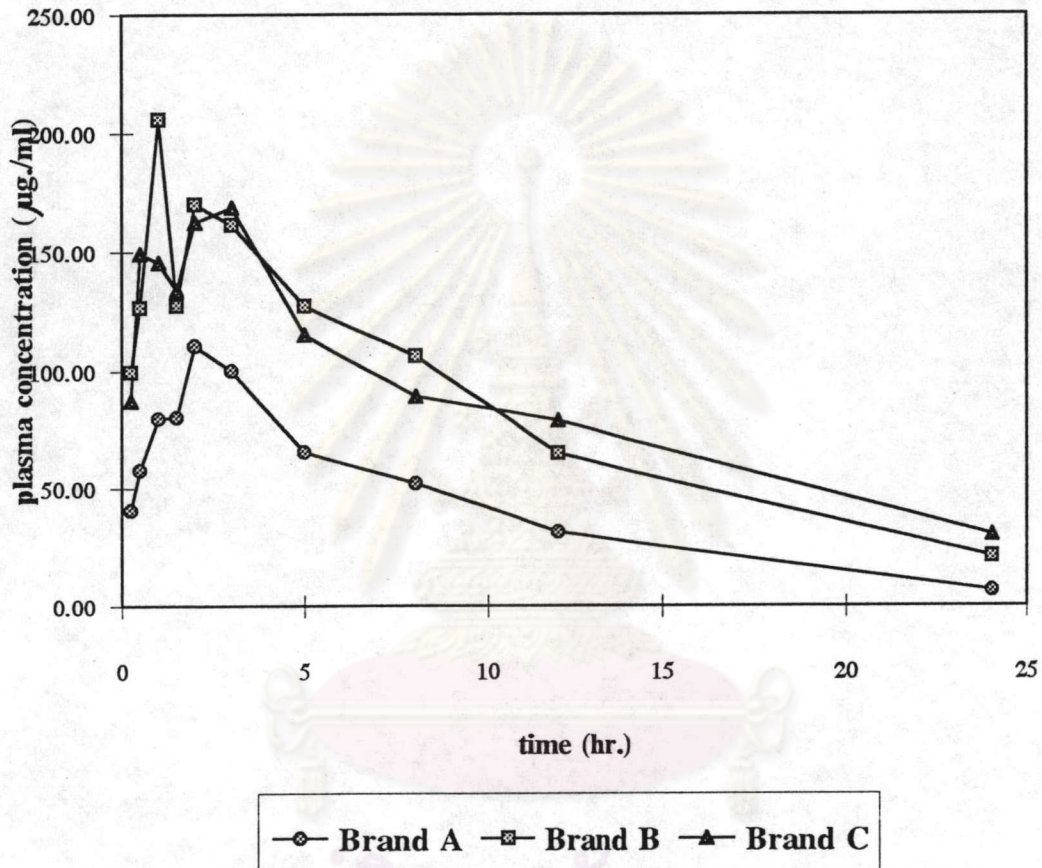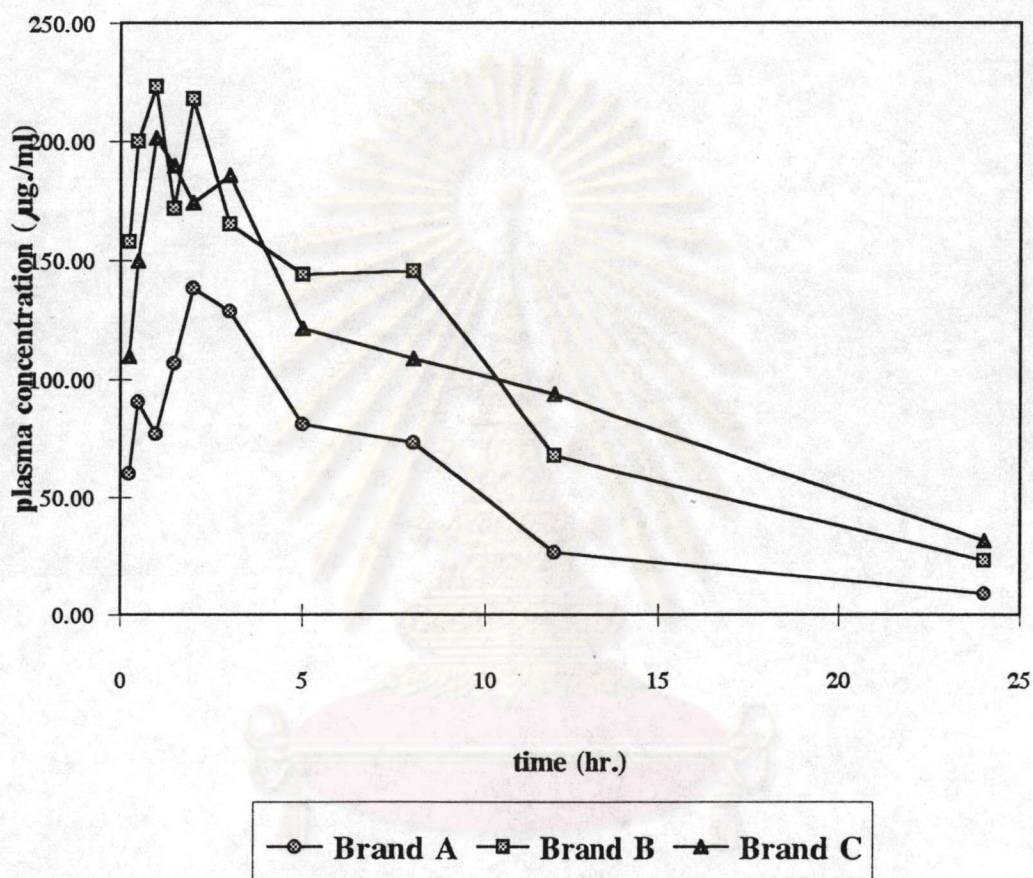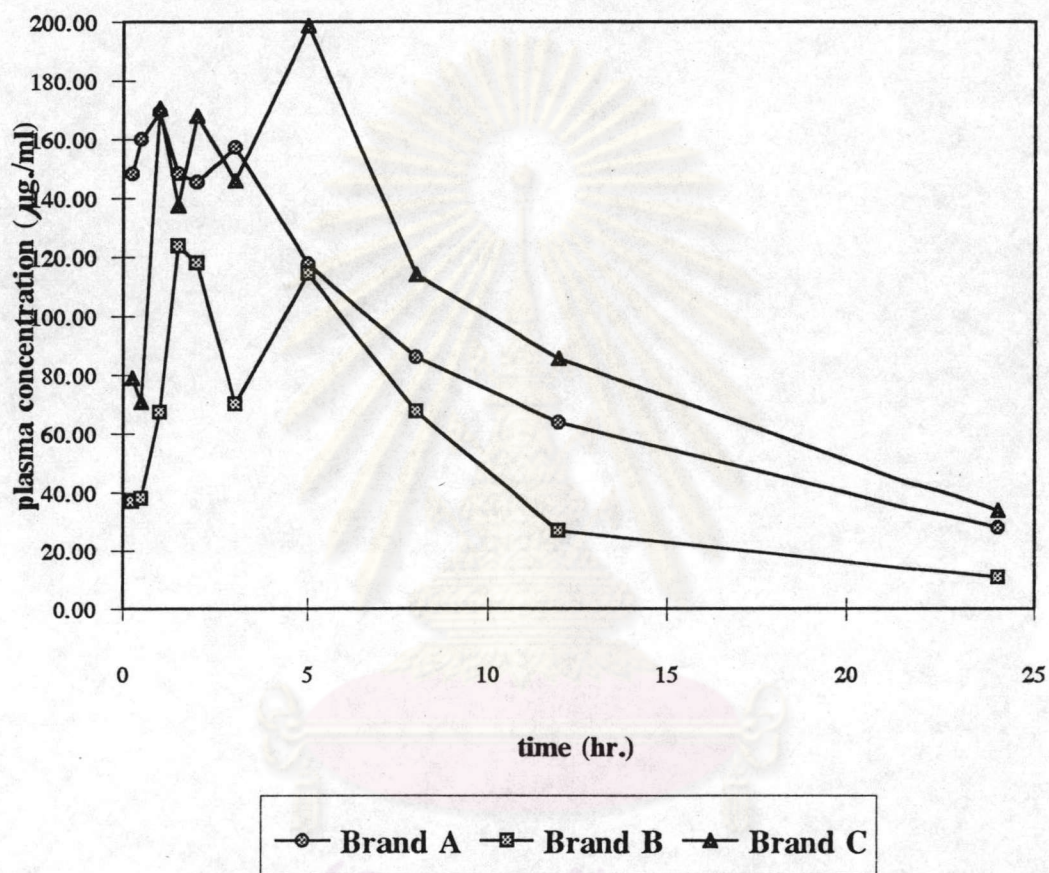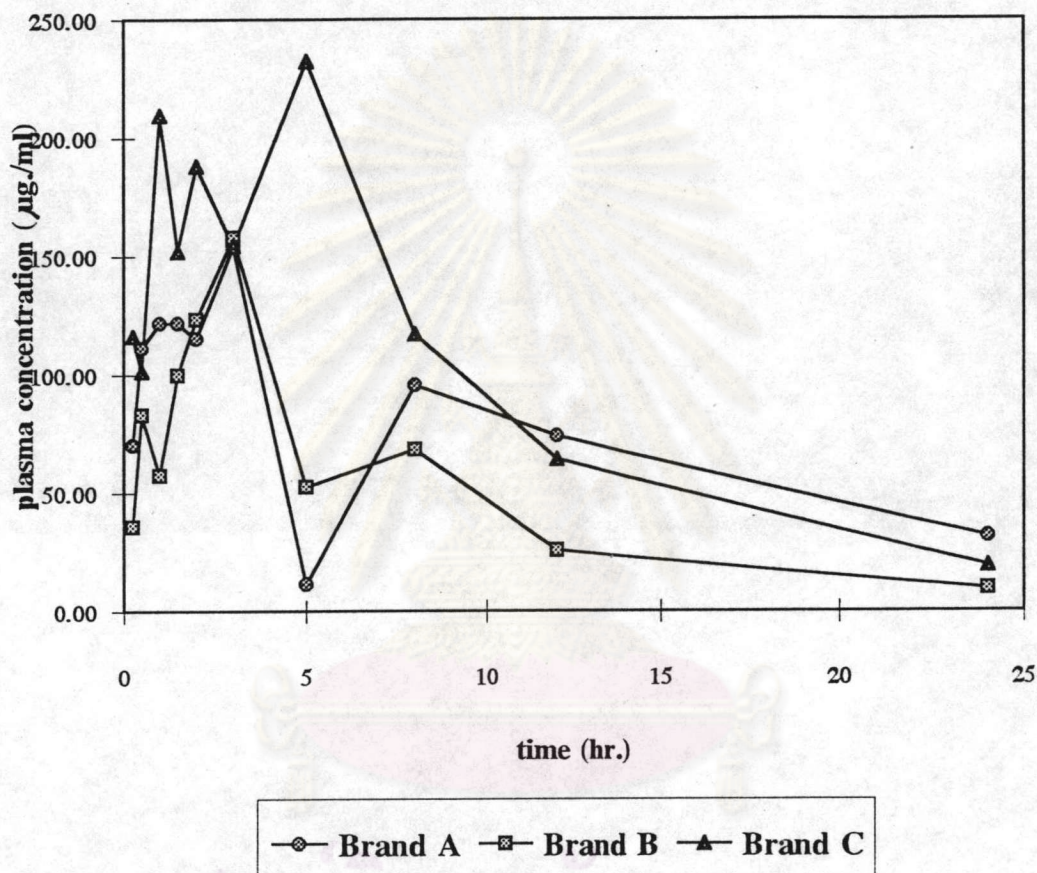intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.3

Figure 6 Plasma ceftriaxone concentration-time profile of subject number 3 following intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

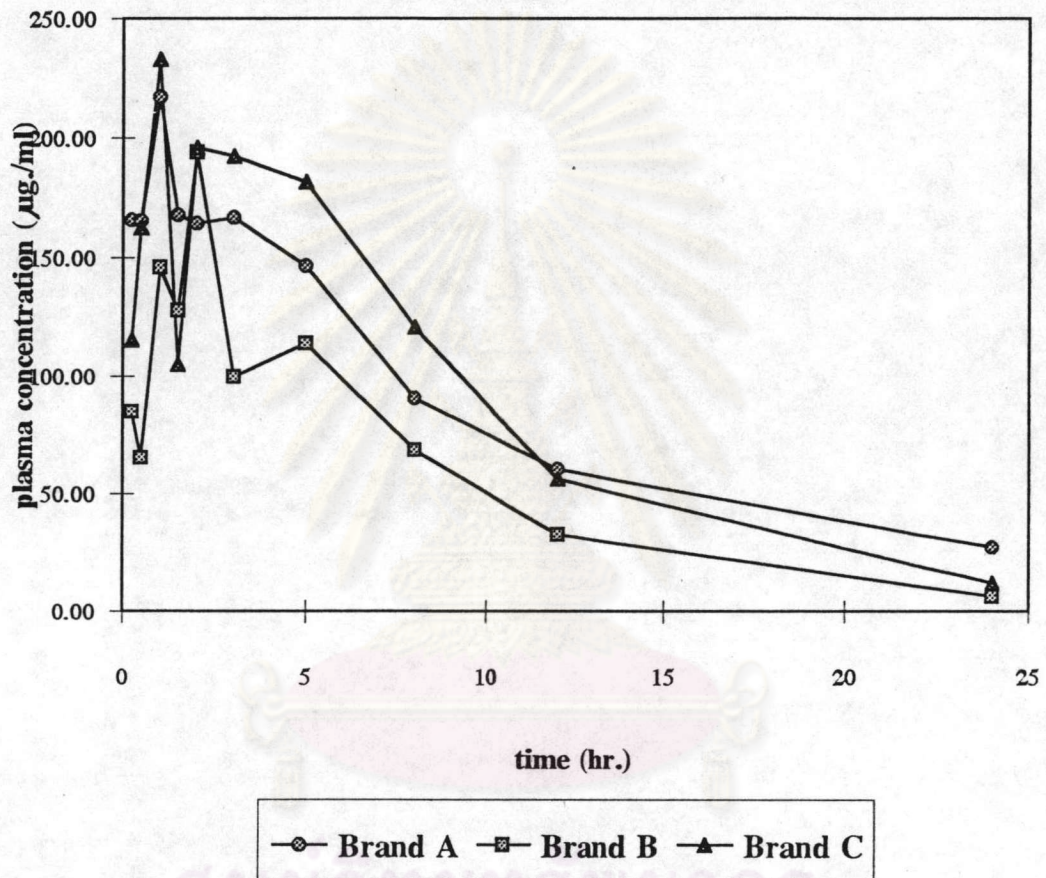## subject no.4

Figure 7 Plasma ceftriaxone concentration-time profile of subject number 4 following intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.5
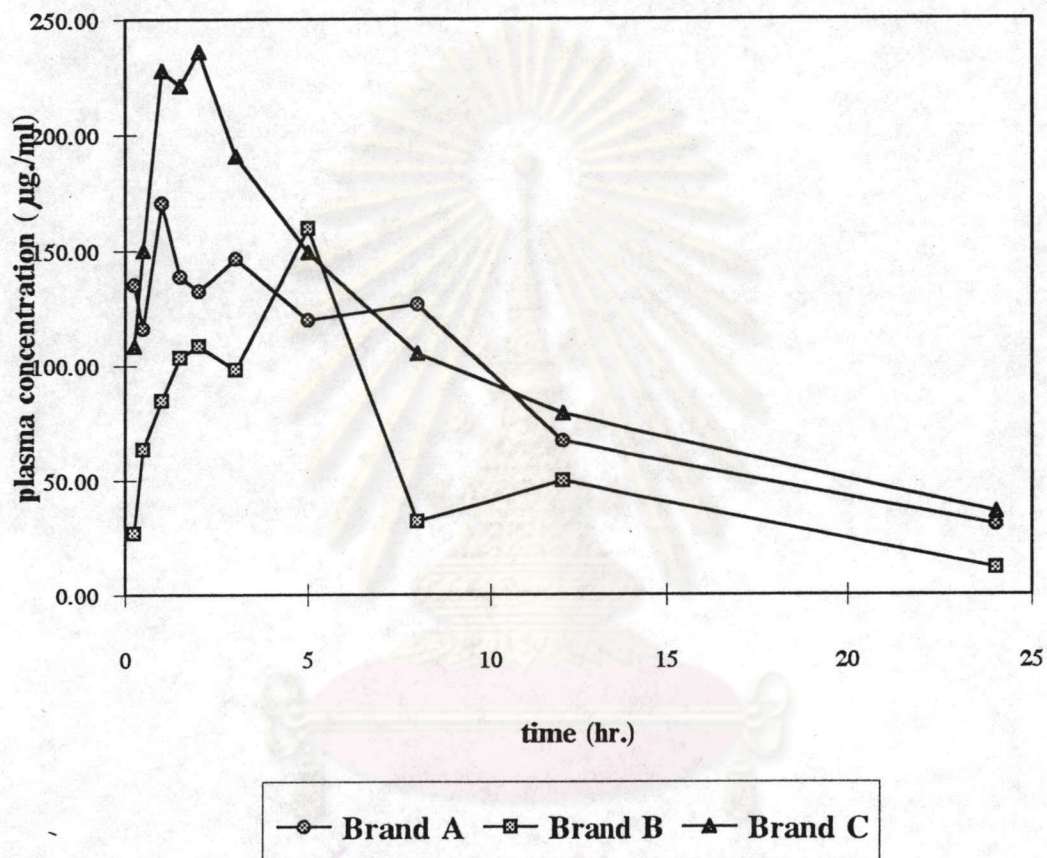

Figure 8 Plasma ceftriaxone concentration-time profile of subject number 5 following intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.6

Figure 9 Plasma ceftriaxone concentration-time profile of subject number 6 folloeing intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.7

Figure 10 Plasma ceftriaxone concentration-time profile of subject number 7 following intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.8

Figure 11 Plasma ceftriaxone concentration-time profile of subject number 8 following intramuscular injection of 1g. ceftriaxone
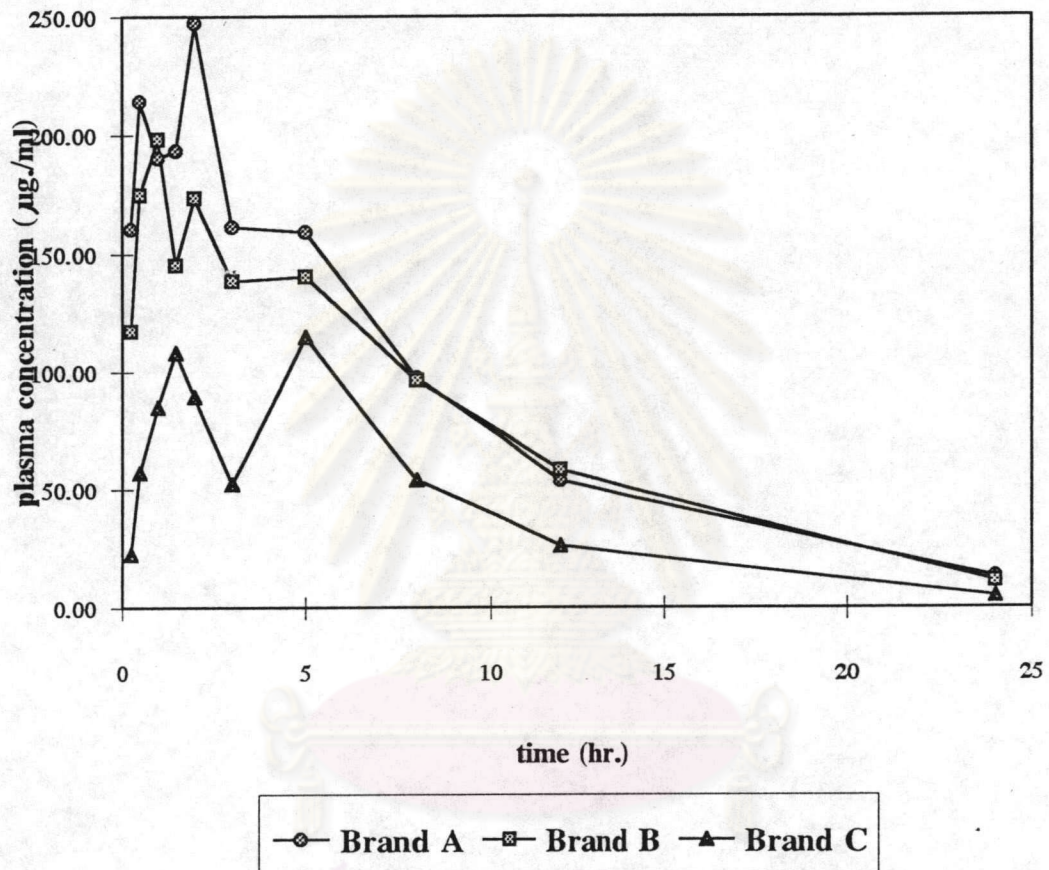
PLASMA CEFTRIAXONE CONCENTRATION

subject no.9

Figure 12 Plasma ceftriaxone concentration-time profile of subject number 9 following intramuscular injection of 1g. ceftriaxone
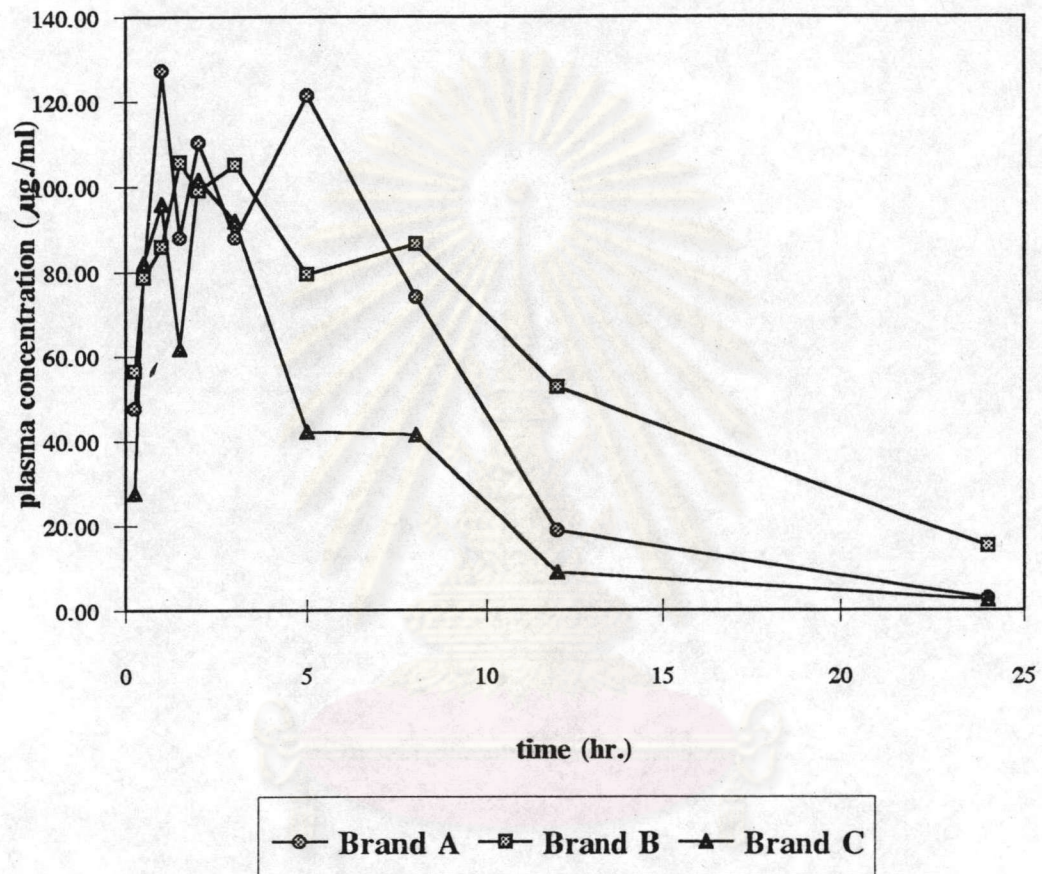
PLASMA CEFTRIAXONE CONCENTRATION

subject no.10

Figure 13 Plasma ceftriaxone concentration-time profile of subject number 10 following intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.11

Figure 14 Plasma ceftriaxone concentration-time profile of subject number 11 following intramuscular injection of 1g. ceftriaxone

PLASMA CEFTRIAXONE CONCENTRATION

subject no.12

Figure 15 Plasma ceftriaxone concentration-time profile of subject number 12 following intramuscular injection of 1g. ceftriaxone
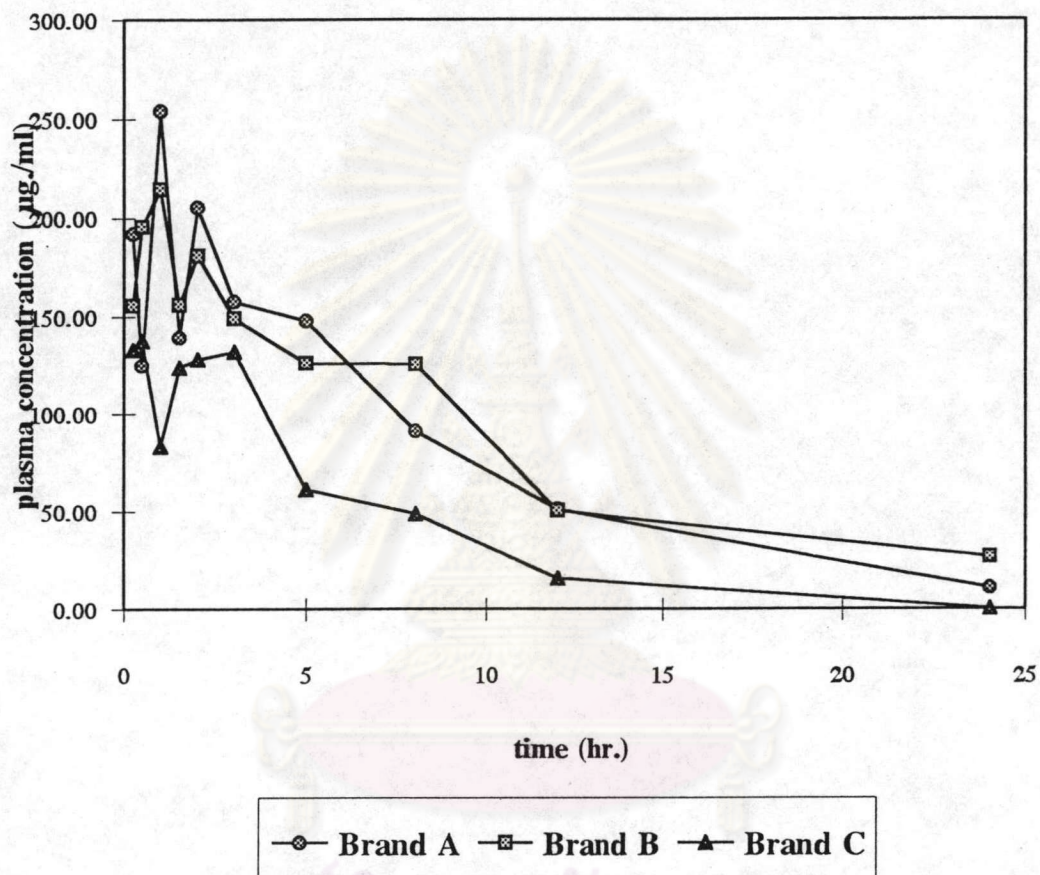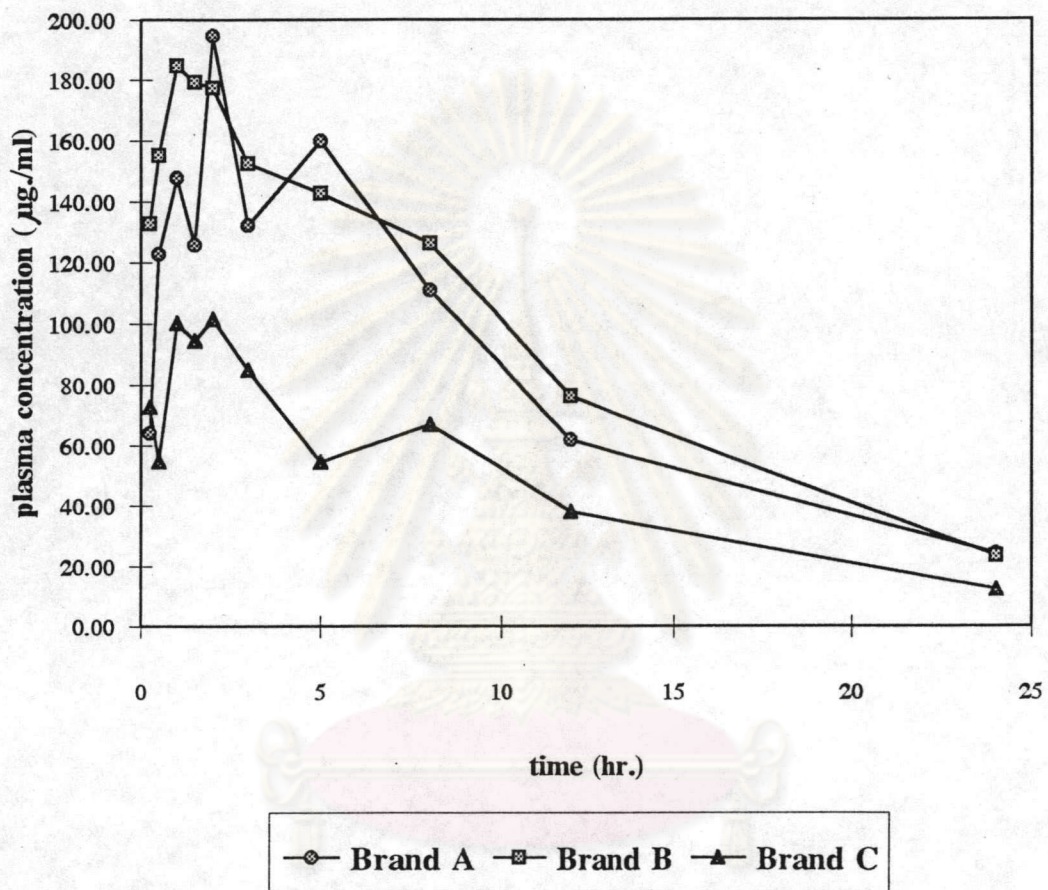
MEAN PLASMA CEFTRIAXONE
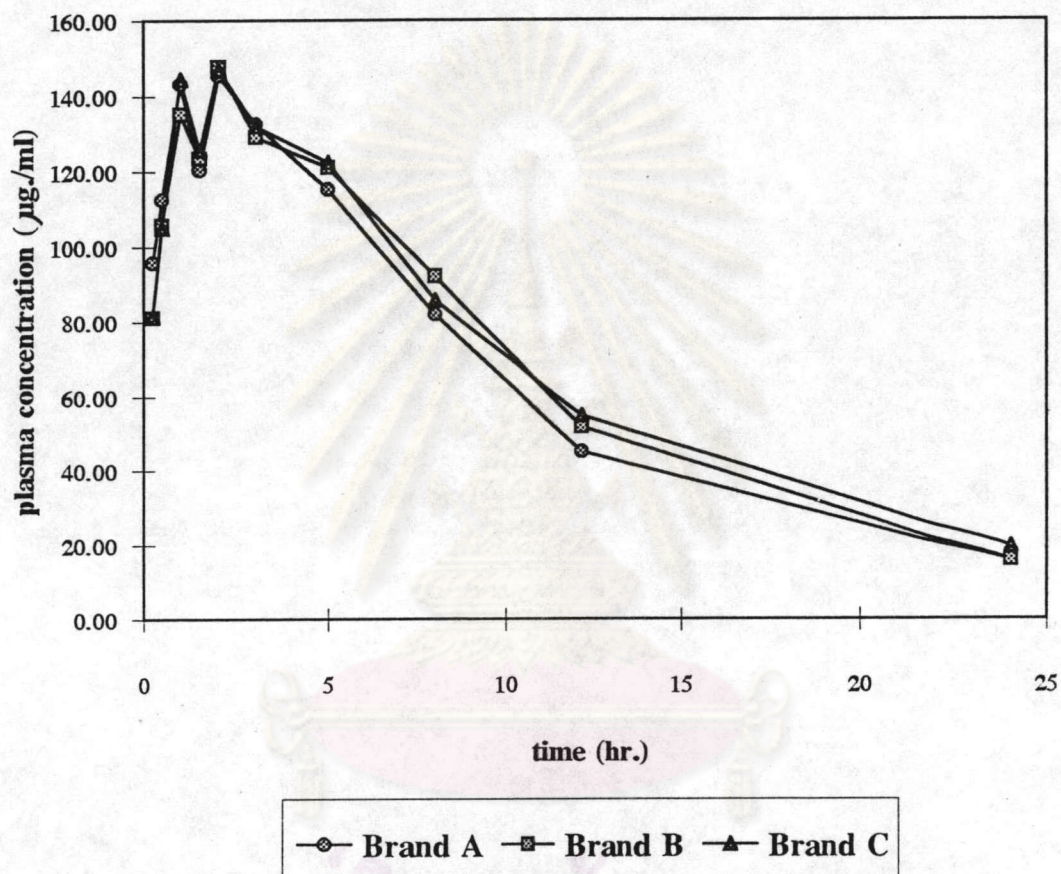
CONCENTRATION

Figure 16 Plasma ceftriaxone concentration-time profile from 12 subjects following intramuscular injection of 1g. ceftriaxone

determining the relevant pharmacokinetic parameters, C_{max}, T_{max} and AUC. These parameters were used to characterize the bioavailability of pharmaceutical formulation after administration. The parameter, C_{max} and T_{max} represented the rate of drug reaching the systemic circulation while the AUC values indicated the extent of absorbed drug entering the systemic circulation.

In the bioequivalence study, if drug products are pharmaceutically equivalent, they are accepted to be bioequivalent if no statistically significant difference in the rate and the extent of drug absorption can be observed (Skelly, 1976 ; Shargel and Yu, 1980).

These relevant pharmacokinetic parameters read from the PCNONLIN computer output were as follows :

2.4.1 Peak Plasma Concentration (C_{max})

Previous reports indicated that the mean peak plasma concentration achieved following intramuscular injection of 1 g. ceftriaxone were 81 ± 13.1 µg/ml (Scully et al., 1984) and 95.2 ± 53.0 µg/ml (Meyers et al., 1983).

In this study, the mean peak plasma ceftriaxone levels for each treatment of brands A, B and C as chown in Table 15 were 145.9 ± 13.03, 150.34 ± 11.07 and 148.29 ± 14.01 µg/ml, respectively. The rank order of these values was brand B>C>A. Statistical comparison as shown in Table 16 indicated that the peak plasma concentrations of all brands were not significantly different from each other (p>0.05).

These calculated C_{max} were almost two times greater than those of the previous studies. The reasons might be the % L.A. of the all test products were closed to upper limits of the U.S.P. XXIII requirements. The Mongolian subjects have smaller bodies than Caucasians, hence, smaller volume of distribution is expected and results in higher plasma drug concentration.

Table 15 Peak plasma concentration (C_{max}) of three brands of ceftriaxone intramuscular injections

Subject Number	C_{max} (μg/ml)		
	Brand A	Brand B	Brand C
1	63.99	98.30	81.68
2	125.60	158.27	145.67
3	97.88	170.99	157.63
4	123.47	210.87	190.15
5	167.0	110.11	173.65
6	132.60	119.73	194.14
7	193.03	149.93	194.51
8	151.56	124.34	224.30
9	219.89	180.10	93.99
10	113.49	103.79	92.59
11	198.54	194.26	135.22
12	163.70	183.43	95.92
Mean	145.90	150.34	148.29
S.E.M.	13.03	11.07	14.01

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Table 16 Analysis of Variance for peak plasma concentration (C_{max}) of three brands
of ceftriaxone intramuscular injections

Source of Variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	118.88	59.44	0.03
Within group	33	64,494.63	1,954.38	
Total	35	64,613.51		

Calculation data from Table 15

$$F^e_{0.05}(2, 33) = 2.99$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

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2.4.2 Time to Peak Plasma Concentration (T_{max})

The time to peak plasma ceftriaxone concentration of each treatment is shown in Table 17. The average times for brands A, B and C were 1.48 ± 0.22, 2.08 ± 0.38 and 1.74 ± 0.33 hr., respectively. The rank order of these values was brand B>C>A. There were no statistically significant difference (p>0.05) among these three brands (Table 18). The time to peak plasma ceftriaxone concentrations in this study agreed well with those found by other reports, which ranging from 1.5-4 hr. (Patel et al., 1982 ; Scully et al., 1984).

T_{max} of brand B was greater than those of brands A and C, this might be due to the difference in solubility of each brand, brand B was the least soluble among the three brands. Drug concentration in the injected solution may change independently of absorption. After injection of a hypertonic solution, water may be drawn to the injection site by osmotic forces. From this reason, the drug of brand B might be separated and/or remained in a very localized depot or precipitate at the injection site, dramatically reducing the absorption rate (Greenblatt et al., 1976).

2.4.3 Area Under the Plasma Concentration Versus Time Curve (AUC)

The means AUC from individual plasma data of brands A, B and C are illustrated in Table 19 as 1769.51 ± 204.97, 1772.49 ± 165.48 and 1982.50 ± 215.02 μg.hr/ml, respectively. The rank order was brand C>B>A. Statistical comparison as observed in Table 20 indicated that there were no significantly difference among all brands (p>0.05).

AUC of brand C was greater than those of the two brands, these results were related to % L.A. of each product, ie. brand C had the highest % L.A.

The values of AUC in this study were greater than those presented by Meyers et al. (1983) (903.5 ± 588.9 μg.hr/ml). The difference might be caused by the difference of the study condition and assay method, and the difference in nationality as previously described.

Table 17 Time to peak plasma concentration (T_{max}) of three brands of ceftriaxone
intramuscular injections

Subject Number	T_{max} (hr.)		
	Brand A	Brand B	Brand C
1	3.16	5.03	5.04
2	1.85	3.57	1.31
3	2.08	1.27	1.21
4	2.02	0.85	1.26
5	0.62	2.73	2.48
6	1.48	2.44	1.78
7	0.72	1.77	1.18
8	0.79	3.04	1.50
9	0.85	0.93	2.02
10	1.62	1.61	1.46
11	0.72	0.71	0.36
12	1.84	1.01	1.32
Mean	1.48	2.08	1.74
S.E.M.	0.22	0.38	0.33

Table 18   Analysis of Variance for time to peak plasma concentration (T_{max}) of three
brands of ceftriaxone intramuscular injections

Source of Variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	2.18	1.09	0.89
Within group	33	40.50	1.23	
Total	35	42.68		

Calculation data from Table 17

$$F^e_{0.05} (2, 33) = 2.99$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

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Table 19 Area under the plasma concentration-time curve (AUC) of three brands of ceftriaxone intramuscular injections

Subject	AUC (µg.hr/ml)		
Number	Brand A	Brand B	Brand C
1	550.17	1389.40	2306.18
2	1080.03	1815.25	2125.29
3	969.03	2205.98	2378.83
4	1192.96	2632.27	2570.46
5	2137.21	1151.45	2812.65
6	2536.32	985.06	2672.48
7	2190.68	1296.91	2514.41
8	2745.71	1193.89	2340.99
9	2151.75	2003.54	1123.93
10	1302.77	1767.29	670.84
11	2044.68	2196.72	1063.55
12	2332.85	2632.11	1210.37
Mean	1769.51	1772.49	1982.50
S.E.M.	204.97	165.48	215.02

Table 20   Analysis of Variance for area under the plasma concentration-time curve (AUC) of three brands of ceftriaxone intramuscular injections

Source of Variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	365232	182616	0.39
Within group	33	1.54×10^7	466295.8	
Total	35	1.58×10^7		

Calculation data from Table 19

$$F^e_{0.05}(2, 33) = 2.99$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

The pharmacokinetic parameters of all brands ; C_{max}, T_{max} and AUC, which were examined for the differences using ANOVA indicated that all test products did not showed any statistically significant differences among each other. Therefore, they were considered bioequivalent.

2.5 Pharmacokinetics of Ceftriaxone Intramuscular Injections

The pharmacokinetic parameters obtained from the PCNONLIN computer program (Figure 19) indicated that pharmacokinetics of ceftriaxone in Thai male healthy volunteers could be explained by a one compartment open model with first-order absorption and first order elimination. This finding agreed with the study of Patel et al. (1982).

2.5.1 Absorption Rate Constant (K_a)

The average absorption rate constant for brands A, B and C were 3.3 ± 0.69, 2.34 ± 0.57 and 3.02 ± 0.92 $hr.^{-1}$, respectively. Results are shown in Table 21. These values agreed with the previous study of Scully et al. 1984 (3.1 ± 0.73 $hr.^{-1}$). There were no statistically significant difference among these three brands ($p>0.05$) (Table 22).

K_a value of brand B was less than those of brands A and C. This might be according to the slowest solubility of brand B relatively to those of the two brands. It could be explained as the same reason as of T_{max} evaluation.

2.5.2 Elimination Rate Constant (K_{el})

The average elimination rate constant for brands A, B and C were 0.12 ± 0.02, 0.12 ± 0.01 and 0.09 ± 0.01 $hr.^{-1}$, respectively. Results are presented in Table 23. Statistical analysis as seen in Table 24 indicated that there were no significant difference among these values ($p>0.05$). The values were closed to those reported by Meyers et al. (1983) (0.13 ± 0.22 $hr.^{-1}$).

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

The mean half-life of ceftriaxone for brands A, B and C were 7.08±0.84, 6.36±0.66 and 8.18±0.85 hr., respectively (Table 25). The values agreed with those investigated by several studies ranging from 5.4-10.9 hr. (Patel et al., 1982 ; Meyers et al., 1983 ; Richards et al., 1984 ; Scully et al., 1984 ; Borner et al., 1985). Statistical analysis showed no significant difference among these values (Table 26).

Table 21   Absorption rate constant (K_a) of three brands of ceftriaxone intramuscular
injections

Subject	K_a (hr.$^{-1}$)		
Number	Brand A	Brand B	Brand C
1	0.32	0.25	0.54
2	1.30	0.47	2.83
3	1.19	2.82	3.22
4	1.22	4.82	2.90
5	7.37	0.76	1.20
6	2.66	0.74	1.80
7	5.81	1.41	3.14
8	5.93	0.55	2.04
9	4.46	4.09	1.40
10	1.90	2.26	1.73
11	5.65	5.93	12.81
12	1.74	4.01	2.66
Mean	3.30	2.34	3.02
S.E.M.	0.69	0.57	0.92

Table 22 Analysis of Variance for absorption rate constant (K_a) of three brands of ceftriaxone intramuscular injections

Source of Variation	d.f[a].	SS[b]	MS[c]	F[d]
Among group	2	5.78	2.89	0.44
Within group	33	217.51	6.59	
Total	35	223.29		

Calculation data from Table 21

$$F^e_{0.05}(2, 33) = 2.99$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 23 Elimination rate constant (K_{el}) of three brands of ceftriaxone intramuscular injections

Subject Number	K_{el} (hr.$^{-1}$)		
	Brand A	Brand B	Brand C
1	0.32	0.15	0.04
2	0.15	0.15	0.08
3	0.13	0.09	0.07
4	0.14	0.09	0.08
5	0.08	0.14	0.07
6	0.06	0.20	0.08
7	0.09	0.15	0.09
8	0.06	0.18	0.11
9	0.11	0.10	0.10
10	0.10	0.07	0.18
11	0.10	0.09	0.13
12	0.08	0.08	0.09
Mean	0.12	0.12	0.09
S.E.M.	0.02	0.01	0.01

Table 24 Analysis of Variance for elimination rate constant (K_{el}) of three brands of ceftriaxone intramuscular injections

Source of Variation	d.f[a].	SS[b]	MS[c]	F[d]
Among group	2	6.44×10^3	3.22×10^3	1.22
Within group	33	8.73×10^2	2.65×10^3	
Total	35	9.38×10^2		

Calculation data from Table 23

$$F^e_{0.05}(2, 33) = 2.99$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

Table 25 Biological half-life ($t_{1/2}$) of three brands of ceftriaxone intramuscular injections

Subject Number	$t_{1/2}$ (hr.)		
	Brand A	Brand B	Brand C
1	2.20	4.55	15.66
2	4.47	4.68	9.16
3	5.20	8.01	9.59
4	5.08	8.04	8.45
5	8.43	4.95	9.34
6	12.19	3.53	8.21
7	7.35	4.59	8.10
8	12.00	3.86	6.10
9	6.17	7.03	6.74
10	6.73	10.62	3.86
11	6.62	7.33	5.20
12	8.50	9.22	7.77
Mean	7.08	6.36	8.18
S.E.M.	0.84	0.66	0.85

Table 26 Analysis of Variance for biological half-life ($t_{1/2}$) of three brands of ceftriaxone intramuscular injections

Source of Variation	d.f.[a]	SS[b]	MS[c]	F[d]
Among group	2	20.05	10.03	1.34
Within group	33	246.73	7.48	
Total	35	266.78		

Calculation data from Table 25

$$F^e_{0.05}(2, 33) = 2.99$$

a = Degree of freedom

b = Sum of square

c = Mean square

d = Variance ratio

e = F value obtained from the table

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Table 27 Estimated pharmacokinetic parameters (Mean+S.E.M.) of three brands of ceftriaxone from 12 subjects following intramuscular injections

Pharmacokinetic Parameters (Mean±S.E.M.)	Products			F-test	Statistical Significance
	Brand A	Brand B	Brand C		
C_{max} (µg/ml)	145.90±13.03[*]	150.34±11.07[*]	148.29±14.01[*]	0.03 (2.824)[**]	NS
T_{max} (hr.)	1.48±0.22	2.08±0.38	1.74±0.33	0.89 (2.824)[**]	NS
AUC (µg.hr/ml)	1769.51±204.97	1772.49±165.48	1982.50±215.02	0.39 (2.824)[**]	NS
K_a (hr^{-1})	3.30±0.69	2.34±0.57	3.02±0.92	0.44 (2.824)[**]	NS
K_{el} (hr.$^{-1}$)	0.12±0.02	0.12±0.01	0.09±0.01	1.22 (2.824)[**]	NS
$t_{1/2}$ (hr.)	7.08±0.84	6.36±0.66	8.18±0.85	1.34 (2.824)[**]	NS

NS = Not significant difference at $p>0.05$

* = Mean±S.E.M.

** = F value obtained from the table