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APPENDICES

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APPENDIX A

TEST PRODUCTS

Table 28 Test Products

Brand name	Manufacturer	Batch No.	Mfg. Date	Exp. Date
Cef-3	Siam Pharmaceuticals	3COXG 066	13-7-94	13-7-97
Rocephin	Roche	1634	02-94	02-97
Tricephin	Atlantic	93329	9-9-93	9-9-96

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APPENDIX B

REAGENT PREPARATIONS

1. pH 7.0 Buffer

Dissolve 13.6 g. of dibasic potassium phosphate and 4.0 g. of monobasic potassium phosphate in water to make a 1000 ml solution. Adjust to pH 7.0±0.1 with ortho-phosphoric acid or 10 N potassium hydroxide.

2. Stock Solution

Stock solutions of ceftriaxone and ciprofloxacin (1 mg/ml) were prepared in tripply distilled water. They were stored at -20°C without degradation for 12 months (Demotes - Mainard et al., 1988).

3. Internal Standard

Dissolve 770 mg. of ammonium acetate in a 100 ml volumetric flask, add to volume with water. Adjust to pH 5.0±0.1 with acetic acid. Pipette 8 ml of ciprofloxacin stock solution and place in a 100 ml volumetric flask, adjust with 0.1 M ammonium acetate buffer pH 5.0 to volume.

4. Mobile Phase for In Vivo Stedies

Pipette 4 ml of triethylamine and transfer into 750 ml of tripply distilled water, adjust to pH 3.0±0.1 with ortho-phosphoric acid. Mix with 250 ml methanol, filter and degass before use.

APPENDIX C

CALIBRATION CURVE DETERMINATION

The typical calibration curves data for ceftriaxone concentrations in mobile phase (pH 7.0+0.1) and human plasma are represented in Tables 29, 30 and Figures 17, 18, respectively.

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Table 29 Typical calibration curve data for ceftriaxone concentrations in mobile phase (pH 7.0±0.1) for stability tests. Estimated using linear regression[a]

Standard No.	Concentration (µg/ml)	Peak Area	Inversely[b] Estimated Concentration (µg/ml)	% Theory[c]
1	5	23786	4.8601	92.20
2	10	47442	9.8638	98.64
3	20	94264	19.8053	99.03
4	40	191005	40.3458	100.86
5	80	379532	80.3758	100.47
6	120	565582	119.8779	99.90
7	160	754110	159.9071	99.94
8	240	1131165	239.9653	99.99
			Mean	99.50
			S.D.	1.17
			% C.V.[d]	1.18

a. $r^2 = 0.999$, $y = 4709.76\ x + 985.91$

b. Inversely Estimated Concentration = $\frac{\text{Peak area - 985.91}}{4709.76}$

c. % Theory = $\frac{\text{Inversely Estimated Concentration}}{\text{Known Concentration}} \times 100$

d. % C.V. = $\frac{\text{S.D.}}{\text{Mean}} \times 100$

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CALIBRATION CURVE OF CEFTRIAXONE

In Mobile Phase

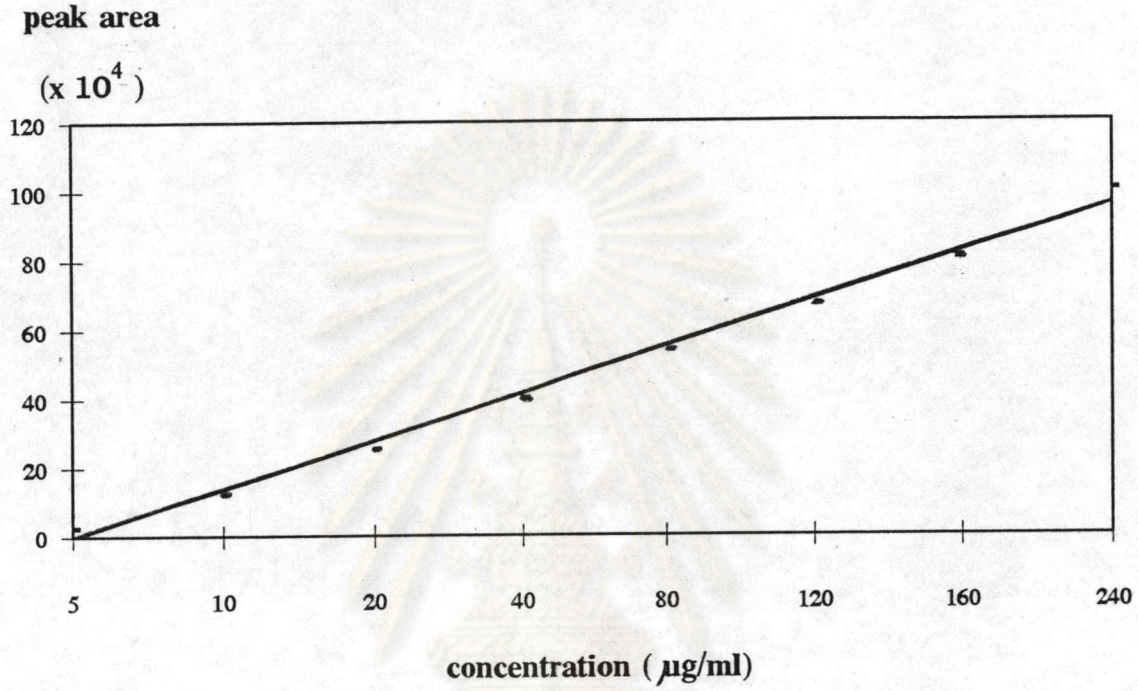

Figure 17 Calibration curve of ceftriaxone in mobile phase (pH 7.0+0.1)

Table 30 Typical calibration curve data for ceftriaxone concentrations in human phasma. Estimated using linear regression[a]

Standard No.	Concentration (μg/ml)	Peak Height Ratio	Inversely[c] Estimated Concentration (μg/ml)	% Theory[c]
1	5	0.0581	4.9556	99.11
2	10	0.1235	9.8000	98.00
3	20	0.2773	21.1926	105.96
4	40	0.5332	40.1481	100.37
5	80	1.0394	77.6444	97.06
6	120	1.6624	123.7926	103.16
7	160	2.0773	154.5259	96.58
8	240	3.2507	241.4444	100.60
			Mean	100.11
			S.D.	3.19
			% C.V.[d]	3.18

a. $r^2 = 0.999$, $y = 0.0135\ x - 0.0088$

b. Inversely Estimated Concentration = $\frac{\text{Peak Height Ratio} + 0.0088}{0.0135}$

c. % Theory = $\frac{\text{Inversely Estimated Concentration}}{\text{Known Concentration}} \times 100$

d. % C.V. = $\frac{\text{S.D.}}{\text{Mean}} \times 100$

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CALIBRATION CURVE OF CEFTRIAXONE

In Human Plasma

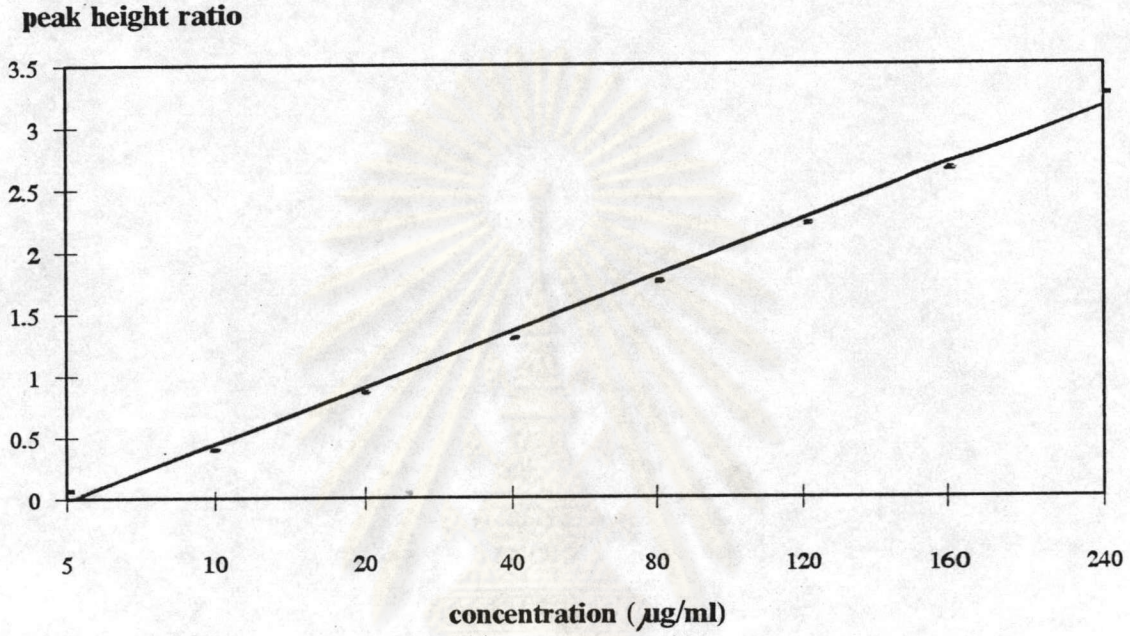

Figure 18 Calibration curve of ceftriaxone in human plasma

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APPENDIX D

SUBJECTS

Table 31 Demographic Data

Subject No.	Age (yr.)	Weight (Kg)	Height (cm).
1	56	100	178
2	33	60	170
3	29	55	161
4	24	52	161
5	29	68	173
6	29	63	170
7	24	45	162
8	33	69	168
9	23	51	156
10	45	73	172
11	32	52	160
12	31	55	165
Mean	32.33	61.92	166.33
S.D.	9.45	14.66	6.51

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Table 32 Biochemical laboratory results

Clinical Test	Normal Range	Subject Number											
		1	2	3	4	5	6	7	8	9	10	11	12
AP	20-90 U/L	62	44	43	68	72	56	38	45	61	49	43	42
ASAT	14-33 U/L	24	33	19	18	31	27	16	24	26	15	20	15
ALAT	6-36 U/L	35	33	10	21	10	24	9	22	12	20	17	10
Urea	2.4-6.1 mmol/L	4.8	4.1	5.9	5.0	4.4	3.6	5.0	4.1	4.3	5.0	4.3	3.6
Cr.	53-115 µmol/L	88	98	99	112	99	78	105	113	93	93	97	87
Total - Bilirubin	3.4-17.1 µmol/L	8.0	17.3	16.3	15.2	12.0	14.1	13.6	10.1	10.8	3.6	6.8	12.3
Direct - Bilirubin	0.0-3.4 µmol/L	1.0	1.1	2.4	4.0	2.5	2.4	2.1	1.1	1.9	0.1	0.6	2.2
Urea/Cr.	47-56	54.5	41.8	53.6	41.0	44.4	46.2	47.6	36.3	46.2	53.8	44.3	41.4
Indirect - Bilirubin	2-12 µmol/L	7.0	10.2	10.9	11.2	11.7	7.7	11.5	9.0	8.9	3.5	6.2	10.1

AP = Alkaline Phosphatase

ASAT = Aspartate Aminotransferase

ALAT = Alanine Aminotransferase

Cr. = Creatinine

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Table 33 Biochemical laboratory results

Clinical Test	Normal Range	Subject Number											
		1	2	3	4	5	6	7	8	9	10	11	12
Neutrophils	50-70%	47	40	33	39	64	46	57	42	54	61	43	47
Lymphocytes	20-40%	33	32	39	41	20	41	34	41	35	32	30	35
Monocytes	0-7%	5	5	6	8	1	7	5	6	4	5	3	4
Eosinophils	0-5%	4	2	3	2	4	5	3	3	4	2	4	4
Basophils	0-1%	1	1	1	1	1	1	1	1	1	1	1	1
Hgb	14.0-18.0 g/dl	14.0	17.4	15.1	14.6	16.3	14.2	16.5	12.7	15.3	14.1	15.2	16.4
Hct	39-49%	43.5	54.7	45.8	45.3	49.0	48.3	53.1	39.7	44.0	45.2	45.3	53.0
WBC	3.2-9.8x10^3/µl	9.3	7.7	7.2	9.6	8.7	9.2	6.5	7.4	8.5	9.7	7.7	7.5
RBC	4.3-5.9x10^6/µl	5.3	5.8	5.0	5.5	5.9	5.1	5.7	4.7	4.8	5.3	5.0	5.6
MCV	80-94 fl	81.6	93.6	92.1	83.1	82.5	94.3	93.1	84.4	91.2	85.1	90.3	94.7
MCH	27-31 pg	26.3	29.8	30.4	26.9	27.3	27.7	29.0	26.9	31.7	26.5	30.3	29.3
MCHC	33-37 g/dl	32.2	31.8	33.0	32.4	33.1	29.4	31.2	31.9	34.8	31.1	33.5	30.9
PLT	150-350x10^3/µl	297	240	212	457	251	249	204	267	355	216	225	254
ABO group		O	B	B	O	B	A	B	O	A	A	B	B

Hgb = Hemoglobin
Hct = Hematocrit
WBC = White Blood Cell
RBC = Red Blood Cell
MCV = Mean Corpuscular Volume
MCH = Mean Corpuscular Hemoglobin
MCHC = Mean Corpuscular Hemoglobin Concentration
PLT = Platelet
ABO = Blood Group

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APPENDIX E

COMPARTMENTAL ANALYSIS

The PCNONLIN computer program output used for analyzing data was shown in Figure 19

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Figure 19 PCNONLIN computer program output

```
 PCNONLIN NONLINEAR ESTIMATION PROGRAM  V01-E

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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 1000
init 3.8572, 0.2871, 0.2042
nobs 10
data
begin


PCNONLIN NONLINEAR ESTIMATION PROGRAM

PARAMETER  ESTIMATE        STANDARD      95% CONFIDENCE LIMITS
                           ERROR
VOLUME        7.458210      1.219214      4.575203     10.341216 UNIVARIAT
                                          2.928995     11.987424 PLANAR

K01           1.904070       .847905       -.100921      3.909062 UNIVARIAT
                                         -1.245780      5.053920 PLANAR

K10            .102920       .040427        .007324       .198515 UNIVARIAT
                                          -.047262       .253101 PLANAR

AUC        1302.769319    368.525011
K01-HL         .364034       .161947
K10-HL        6.734845      2.642824
TMAX          1.619965       .437523
CMAX        113.489892     11.215020
```

APPENDIX F

STATISTICS

1. Mean ($\bar{x}$)

$$\bar{x} = \frac{\sum x}{N}$$

2. Standard deviation

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

3. Standard error of mean (S.E.M.)

$$S.E.M. = \frac{S.D.}{\sqrt{N}}$$

4. Testing the difference among treatment means

Completely randomized design

	Treatments			Total	Mean
	1	2	3.....k		
	X_{11}	X_{12}	X_{13}.....X_{1k}	T_1	X_1
	X_{21}	X_{22}	X_{23}.....X_{2k}	T_2	X_2
	.....	.....	.....	.....	.....
	X_{n1}	X_{n2}	X_{2n}.....X_{2k}	T_2	X_2
Total	T_1	T_2	T_3.....T_k	T	X
Mean	X_1	X_2	X_3.....X_k		

where T = Total of all observations

X = Overall mean

k = Number of treatments

n = Number of sampling units in each treatment

$\mu_1, \mu_2, \mu_3, \ldots\ldots, \mu_k$ = Population mean

The null hypothesis $H_0 : \mu_1 = \mu_2 = \ldots\ldots \mu_k$

The alternative hypothesis $H_a : \mu_1 \neq \mu_2 \neq \ldots\ldots \mu_k$

Analysis of variance (ANOVA) for testing differences among treatment mean

Source of variation	d.f.	SS	MS	F
Among group	k - 1	SS_{among}	MS_{among}	F_T
Within group	$\sum n - k$	SS_{within}	MS_{within}	
Total	$\sum n - 1$	SS_{total}		

where : d.f. = Degree of freedom

SS = Sum of Square

MS = Mean Square

F_T = Variance ratio

Sum of Spuares :

1. Complete a correction term (C.T.)

$$C.T. = \frac{T^2}{\sum n}$$

2. Total sum of square (SS_{total})

$$SS_{total} = \sum_{I=1}^{k} \left(\sum_{j=1}^{n} X^2_{ij}\right) - C.T.$$

3. The among group sum of squares (SS_{among})

$$SS_{among} = \sum_{i=1}^{k} (T_i^2 / n_i) - C.T.$$

4. The within group sum of squares (SS_{within})

$$SS_{within} = SS_{total} - SS_{among}$$

$$\text{Mean squares} = \frac{\text{Sum of squares}}{\text{Degree of freedom}}$$

$$\text{Variance ratio} = \frac{\text{Among group mean squares}}{\text{Within group mean squares}}$$

F has (k-1), ($\sum$n-k) degree of freedom

If F value calculated is less then $F_{0.05}$, the null hypothesis is accepted and the alternative hypothesis is rejected. If F value is greater than $F_{0.05}$, the alternative hypothesis stands which shows that there are significant differences among treatment means ($p<0.05$).

5. Testing the difference of two means

If the result of the difference testing among treatment means by analysis of variance is significance ($p<0.05$), the testing of difference between the mean of the reference treatment and the each other treatment mean is performed by Least Significant Different (L.S.D.)

$X_1 - X_2$ = difference of the two means

$t_{0.05}$ has ($\sum$n-k) degree of freedom

L.S.D. = $t_{0.05} X S_d$

where $S_d = \sqrt{2 MS_{(within)}/n}$

If the difference of the two means is greater than L.S.D. calculated, it indicated that there is statistically significant difference of these means ($p<0.05$).

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หอสมุดกลาง สถาบันวิทยบริการ จุฬาลงกรณ์มหาวิทยาลัย

VITAE

Miss Orawan Srisakulchai was born on December 17th, 1965 in Bangkok. She received a Bachelor of Science in Pharmacy in 1989 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University. She is a pharmacist in Department of Health, The Bangkok Metropolitan Administration, Bangkok, Thailand.

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