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APPENDICES

APPENDIX A

TEST PRODUCTS

Table 30 Test Products

Brand name	Manufacturer/Distributer	Batch No.	Mfg. Date	Exp. Date
Rothricin	Siam Bheasach, Co., Ltd.	872470	08-97	08-00
Roxtrocin	Greater Pharma, Co., Ltd.	20203	07-97	07-00
Rulid	Les Laboratories Roussel	497	2-3-97	1-3-00

APPENDIX B

CALIBRATION CURVE DETERMINATION

The typical calibration curves data for roxithromycin concentrations in mobile phase, phosphate buffer(pH 7.4±0.1) and human plasma are presented in Tables 31-33 and Figures 17-19, respectively.

Table 31 Typical Calibration Curve Data for Roxithromycin Concentrations in Mobile Phase Estimated Using Linear Regression[1]

Standard No.	Concentration (μg/mL)	Peak height ratio	Inversely estimated concentration[2] (μg/mL)	% Recovery[3]
1	10.00	0.2625	10.16	100.16
2	20.00	0.5234	20.55	102.75
3	40.00	1.0033	39.67	99.18
4	50.00	1.2624	50.00	100.00
5	60.00	1.5124	59.96	99.93
6	80.00	2.0101	79.78	99.73
7	100.00	2.5242	100.27	100.27

* Each data point was determined tripicately

Mean	100.29
S.D.	1.15
% C.V.[4]	1.15

1. $r^2 = 0.9999, \quad y = 0.0251x + 0.0075$
2. Inversely estimated concentration $= \dfrac{\text{peak height ratio} - 0.0075}{0.0251}$
3. % Recovery $= \dfrac{\text{Inversely estimated concentration}}{\text{Known Concentration}} \times 100$
4. % C.V. $= \dfrac{\text{S.D.}}{\text{Mean}} \times 100$

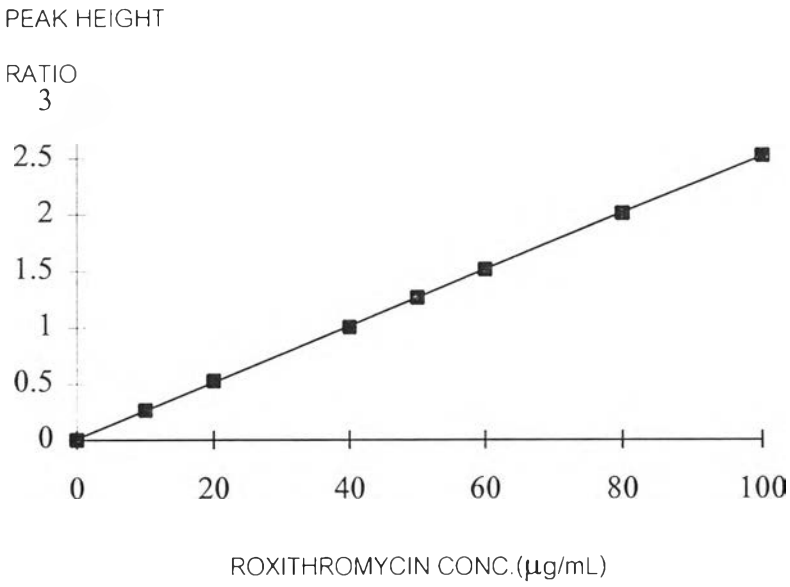

Figure 17 Calibration Curve of Roxithromycin in Mobile Phase

Table 32 Typical Calibration Curve Data for Roxithromycin Concentrations in Phosphate Buffer (pH 7.4±0.1) Estimated Using Linear Regression

Standard No.	Concentration (μg/mL)	Peak height ratio	Inversely estimated concentration[2] (μg/mL)	% Recovery[3]
1	10.00	0.3253	10.28	102.80
2	20.00	0.6149	19.59	97.94
3	40.00	1.2689	40.62	101.54
4	50.00	1.5614	50.02	100.04
5	60.00	1.8763	60.15	100.25
6	80.00	2.4853	79.73	99.66
7	100.00	3.1218	100.20	100.20

* Each data point was determined tripicately

Mean	100.35
S.D.	1.62
% C.V.[4]	1.62

1. $r^2 = 0.9999, \quad y = 0.0311x + 0.0057$
2. Inversely estimated concentration $= \dfrac{\text{peak height ratio} - 0.0057}{0.0311}$
3. % Recovery $= \dfrac{\text{Inversely estimated concentration}}{\text{Known Concentration}} \times 100$
4. % C.V. $= \dfrac{\text{S.D.}}{\text{Mean}} \times 100$

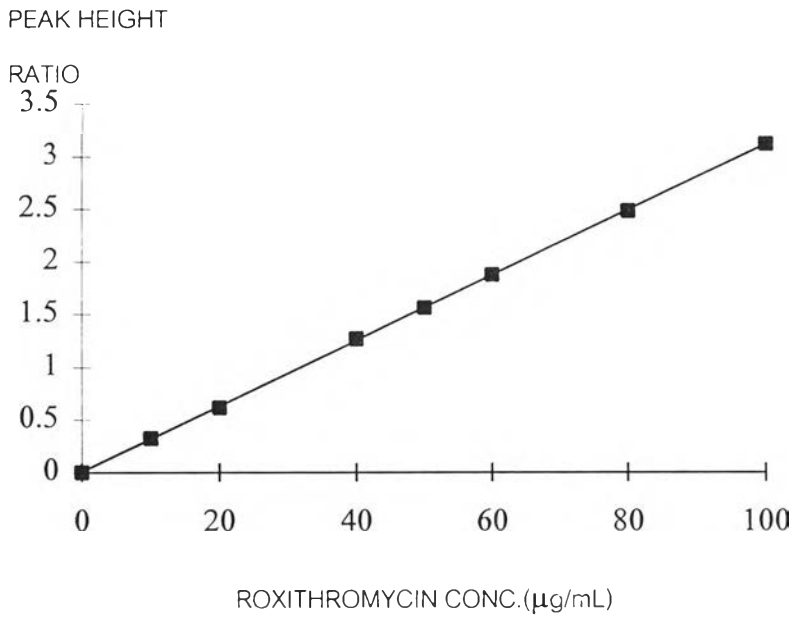

Figure 18 Calibration Curve of Roxithromycin in Phosphate Buffer Solution (pH 7.4 ± 0.1)

Table 33 Typical Calibration Curve Data for Roxithromycin Concentrations in Human Plasma Estimated Using Linear Regression[1]

Standard No.	Concentration (μg/mL)	Logarithm of concentration	Inhibition zone diameter (mm)	Inversely estimated concentration[2] (μg/mL)	% Recovery[3]
1	0.50	-0.3010	15.74	0.4957	99.14
2	1.00	0	18.13	1.0171	101.71
3	3.00	0.4771	21.81	3.0761	102.54
4	5.00	0.6990	23.22	4.7000	94.00
5	7.00	0.8451	24.48	6.8659	98.08
6	10.00	1.0000	25.85	10.3657	103.66

* Each data point was determined tripicately

Mean	99.86
S.D.	3.55
% C.V.[4]	3.56

1. $r^2 = 0.9990$, $\log y = 0.1306x - 2.3604$
   : x = Inhibition zone diameter, y = plasma roxithromycin concentration
2. Inversely estimated concentration
   = antilog [(Inhibition zone diameter)*0.1306 - 2.3604]
3. $\%\ \text{Recovery} = \frac{\text{Inversely estimated concentration}}{\text{Known Concentration}} \times 100$
4. $\%\ \text{C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100$

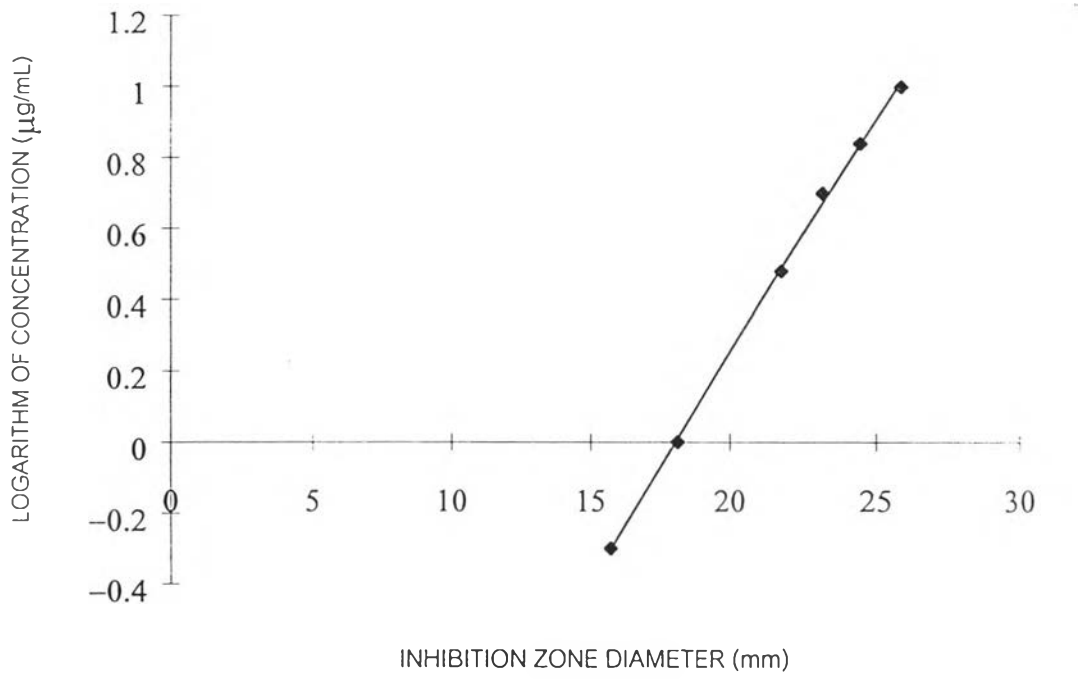

Figure 19 Calibration Curve of Roxithromycin in Human Plasma

APPENDIX C

REAGENT PREPARATIONS

1. Mobile Phase for In Vitro Studies

Prepare a mixture of methanol and 0.067 M monobasic potassium phosphate (560:440), adjust with phosphoric acid to a pH of 4.0, filter through a filter having a porosity of 0.45 μm and degass before use.

2. 0.05 M Phosphate Buffer (pH 7.4)

Dissolve 6.8045 g of monobasic potassium phosphate in 900 mL of water, adjust with phosphoric acid or sodium hydroxide to a pH 7.4 and dilute with water to 1,000 mL.

3. 0.1 M Potassium Phosphate Buffer (pH 8.0)

Dissolve 16.73 g of dibasic potassium phosphate and 0.523 g of monobasic potassium phosphate in distilled water to 1,000 mL, adjust with 18 N phosphoric acid or 10 N potassium hydroxide to yield a pH 7.9 to 8.1 after sterilization.

4. Stock Solution

Stock solutions of roxithromycin and clarithromycin (1 mg/mL) were prepared in methanol. They were stored at -20 °C without degradation for 12 months. (Demotes-Mainard, et al., 1989)

APPENDIX D

SUBJECTS

Table 34 Demographic Data

Subject No.	Age (yr)	Height (cm)	Weight (kg)
1	23	173	60
2	23	175	60
3	31	168	75
4	35	162	54
5	32	160	50
6	40	161	57
7	26	171	62
8	26	160	58
9	31	174	58
10	28	164	55
11	43	168	55
12	33	175	75
Mean	30.92	167.58	59.92
S.D.	6.27	5.99	7.73

Table 35 Hematological and Blood Biochemical Tests of Subjects

Clinical Test	Normal Range	Subject Number											
		1	2	3	4	5	6	7	8	9	10	11	12
AP	20-90 U/L	71	58	68	72	64	62	36	72	39	67	60	60
AST	14-33 U/L	19	23	60	17	19	21	15	33	20	48	18	14
ALT	6-36 U/L	11	21	102	10	12	14	15	60	24	69	17	6
Albumin	32-55 g/L	39.3	49.1	48.0	48.4	52.9	51.9	51.9	49.1	53.1	53.8	48.4	46.4
Total protein	60-83 g/L	64.7	79.2	71.0	76.2	77.6	80.4	73.0	81.4	79.5	75.3	75.5	73.7
Creatinine	53-115 μmol/L	95	76	81	83	66	87	64	79	82	78	93	82
Hemoglobin	14.0-18.0 g/dL	13.2	13.4	14.6	13.8	11.2	13.9	14.6	15.9	15.7	13.5	14.6	16.8
Hematocrit	40-45%	42.0	40.0	43.1	40.3	34.0	40.7	43.3	46.8	46.5	42.0	44.7	43.7
White Blood Cell	$4.8\text{-}11.0*10^3/\mu L$	5.11	6.00	7.31	8.08	7.09	5.60	5.63	7.50	9.18	5.11	9.30	7.90
Red Blood Cell	$5.0\text{-}5.5*10^6/\mu L$	3.85	5.64	4.79	4.80	4.73	4.57	4.76	5.44	5.09	4.96	5.61	5.98
Platelet	$150\text{-}350*10^3/\mu L$	216	244	210	209	325	291	243	257	305	210	296	171

AP = Alkaline Phosphatase
AST = Aspartate Aminotransferase
ALT = Alanine Aminotransferase

VITAE

Mr. Chaiyut Tayavitit was born on April 23, 1970 in Bangkok. He received a Bachelor of Pharmacy in 1993 from Faculty of Pharmacy, Silpakorn University. He is a pharmacist in Pathum Thani hospital, Pathum Thani, Thailand.