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APPENDICES

APPENDIX A

REAGENT PREPARATION

Phosphate buffer pH 7.4

Dissolved 27 g of potassium dihydrogen phosphate in water and adjust to 1 liter. Take 50 mL of this solution to mix with 39.5 mL of 0.2 M sodium hydroxide solution and diluting to 200 mL with water to a pH 7.4 $\pm$ 0.02.

Sodium acetate buffer pH 4.2

Dissolved 1.6256 g of sodium acetate trihydrate in water, mix with 2.4 mL of glacial acetic acid, adjust with water to 500 ml and to pH of 4.2 $\pm$ 0.02.

APPENDIX B

VALIDATION OF ANALYTICAL METHOD FOR *IN VITRO* STUDIES

1. Accuracy

Table 28. Accuracy of analytical method for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm

Concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
4	4.03	100.80
9	8.93	99.23
15	15.03	100.22

Mean % recovery = 100.09, S.D. = 0.79, C.V. = 0.79%

* Each data was determined using three determinations per concentration.

Table 29. Accuracy of analytical method for determination of ketoprofen in 75% methanol at λ = 258 nm

Concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
4	3.95	98.80
10	10.01	100.11
14	14.04	100.27

Mean % recovery = 99.73, S.D. = 0.81, C.V. = 0.81%

* Each data was determined using three determinations per concentration.

2. Precision

2.1 Within Run Precision

Table 30. Within run precision of analytical method for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
4	4.044 ± 0.048	1.18
9	8.954 ± 0.053	0.59
15	15.003 ± 0.031	0.21

* Each data was determined using three determinations per concentration.

Table 31. Within run precision of analytical method for determination of ketoprofen in 75% methanol at λ = 258 nm

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
4	4.008 ± 0.058	1.46
10	10.104 ± 0.081	0.80
14	14.055 ± 0.077	0.55

* Each data was determined using three determinations per concentration.

2.2 Between Run Precision

Table 32. Between run precision of analytical method for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
4	4.029 ± 0.071	1.76
9	8.973 ± 0.063	0.70
15	15.040 ± 0.076	0.51

* Each data was determined using three determinations per concentration.

Table 33. Between run precision of analytical method for determination of ketoprofen in 75% methanol at λ = 258 nm

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
4	3.950 ± 0.073	1.86
10	9.967 ± 0.052	0.52
14	14.068 ± 0.085	0.61

* Each data was determined using three determinations per concentration.

3. Calibration curve

Table 34. Typical calibration curve data for determination of ketoprofen in phosphate buffer pH 7.4 estimated using linear regression[1]

Concentration (μg/mL)	Absorbance (λ = 260 nm)	Inversely estimated concentration (μg/mL)[2].	% Recovery[3]
3	0.182	3.02	100.53
4.6	0.284	4.59	99.82
6.2	0.384	6.16	99.32
7.8	0.491	7.82	100.28
9.4	0.594	9.42	100.25
11.0	0.697	11.02	100.18
12.6	0.801	12.63	100.26
14.2	0.901	14.26	99.99
15.8	1.004	15.79	99.93
		Mean	100.06
		S.D.	0.37
		% C.V.[4]	0.37

1. $r^2 = 1$, $Y = 0.0643x - 0.0116$

2. Inversely estimated concentration = (Absorbance + 0.0116)/ 0.0643

3. % Recovery = (Inversely estimated concentration / Known concentration) x 100

4. % C.V. = (S.D./ Mean) X 100

* Each data was determined tripicately

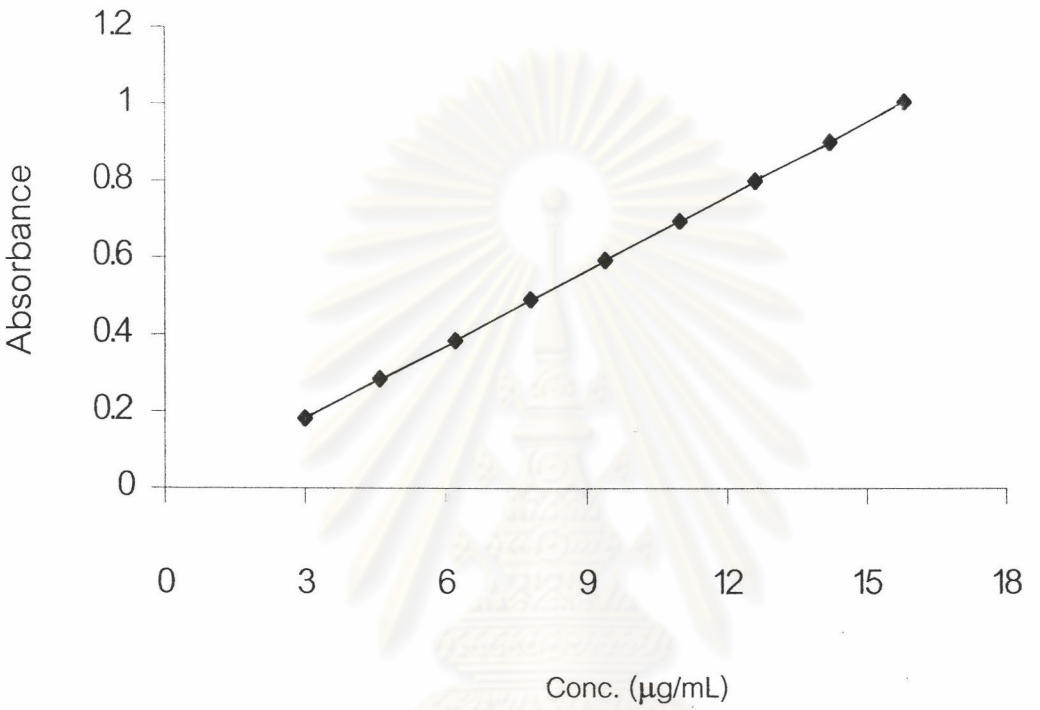

Figure 26. Typical calibration curve for determination of ketoprofen in phosphate buffer pH 7.4 at λ = 260 nm

Table 35. Typical calibration curve data for determination of ketoprofen in 75%methanol estimated using linear regression[1]

Concentration (μg/mL)	Absorbance (λ = 260 nm)	Inversely estimated concentration (μg/mL)[2].	% Recovery[3]
3	0.193	3.02	100.83
5	0.322	4.98	99.50
7	0.453	6.98	99.65
9	0.588	9.03	100.30
11	0.719	11.01	100.08
13	0.848	12.97	99.76
15	0.982	15.02	100.10
		Mean	100.03
		S.D.	0.45
		% C.V.[4]	0.45

2. $r^2 = 1$, $Y = 0.0658x - 0.0057$

2. Inversely estimated concentration = (Absorbance + 0.0057)/ 0.0658

3. % Recovery = (Inversely estimated concentration / Known concentration) x 100

4. % C.V. = (S.D./ Mean) X 100

* Each data was determined tripicately

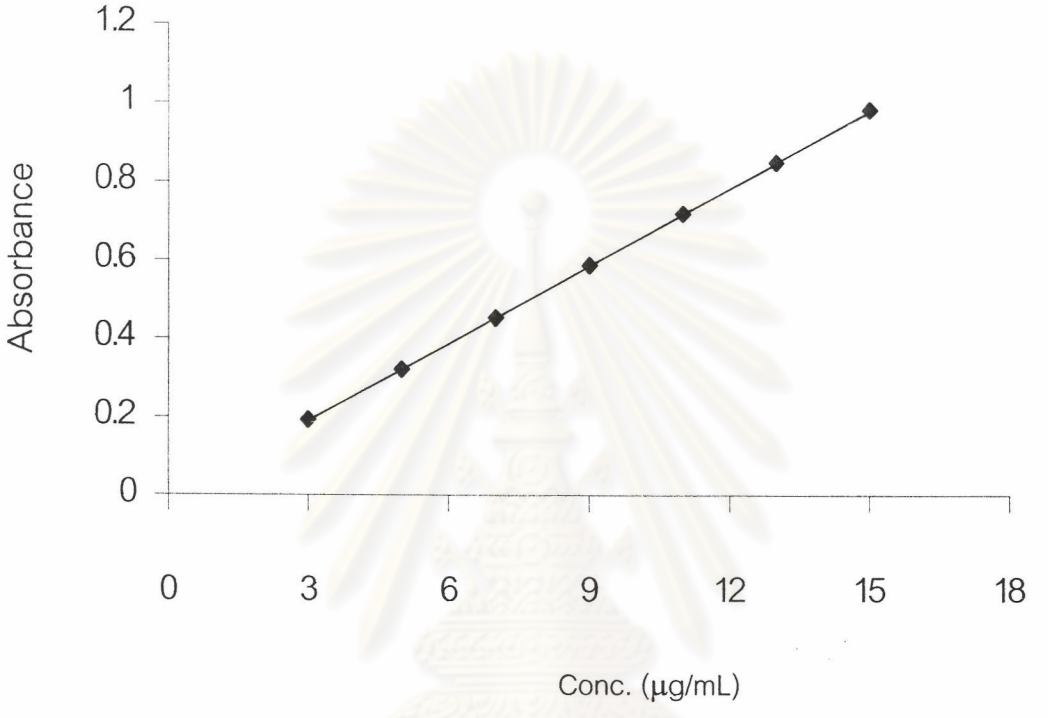

Figure 27. Typical calibration curve for determination of ketoprofen in 75% methanol at 258 nm

APPENDIX C

VALIDATION OF ANALYTICAL METHOD FOR STABILITY STUDIES

1. Accuracy

Table 36. Accuracy of analytical method for determination of ketoprofen in preparation

Concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
15	14.64	97.59
35	34.59	98.83
65	64.53	99.28

Mean % recovery = 98.57, S.D. = 0.88, C.V. = 0.89%

* Each data was determined using three determinations per concentration.

2. Precision

2.1 Within Run Precision

Table 37. Within run precision of analytical method for determination of ketoprofen in preparation

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
15	15.382 ± 0.130	0.85
35	34.883 ± 0.229	0.66
65	64.751 ± 0.514	0.79

* Each data was determined using three determinations per concentration.

2.2 Between run precision

Table 38. Between run precision of analytical method for determination of ketoprofen in preparation

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
15	15.049 ± 0.246	1.63
35	34.769 ± 0.303	0.87
65	64.510 ± 0.766	1.19

* Each data was determined using three determinations per concentration.

Table 39. Linear regression for determination of ketoprofen in preparation at 0, 0.5, 1, 1.5, 2, 2.5 and 3 months

Time (month)	Linear regression	
0	Y = 0.0175X + 0.0075	; r^2 = 1
0.5	Y = 0.0169X + 0.0237	; r^2 = 0.9996
1	Y = 0.0168X - 0.0074	; r^2 = 0.9999
1.5	Y = 0.0151X + 0.0108	; r^2 = 0.9996
2	Y = 0.0157X - 0.0008	; r^2 = 1
2.5	Y = 0.0162X + 0.0010	; r^2 = 0.9997
3	Y = 0.0160X + 0.0029	; r^2 = 1

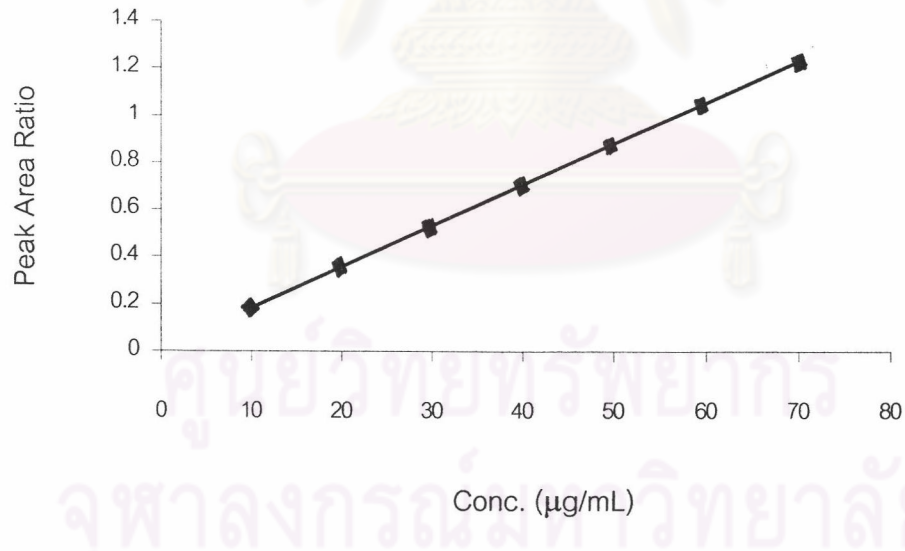

Figure 28. Typical calibration curve for determination of ketoprofen in formulation at 2 months

APPENDIX D

VALIDATION OF ANALYTICAL METHOD FOR *IN VIVO* STUDIES

1. Accuracy

Table 40. Accuracy of analytical method for determination of ketoprofen in rabbit plasma

Concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
10	10.89	108.94
150	156.38	104.26
270	276.74	102.50

Mean % recovery = 105.23, S.D. = 3.33, C.V. = 3.16%

* Each data was determined using three determinations per concentration.

2. Precision

2.1 Within run precision

Table 41. Within run precision of analytical method for determination of ketoprofen in rabbit plasma

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
10	10.756 ± 0.508	4.73
150	152.896 ± 3.065	2.01
270	273.537 ± 5.870	2.14

* Each data was determined using three determinations per concentration.

2.2 Between run precision

Table 42. Between run precision of analytical method for determination of ketoprofen in rabbit plasma

Concentration (μg/mL)	Estimated concentration Mean ± S.D.	% C.V.
10	10.643 ± 0.400	3.76
150	153.308 ± 3.964	2.59
270	267.646 ± 8.691	3.25

* Each data was determined using three determinations per concentration.

3. Calibration curve

Table 43. Typical calibration curve data for determination of ketoprofen in rabbit plasma estimated using linear regression[1]

Concentration (μg/mL)	PAR	Inversely estimated concentration (μg/mL).[2]	% Recovery
2	0.0156	2.29	114.54
40	0.315	40.70	101.75
80	0.617	79.39	99.24
120	0.928	119.30	99.42
160	1.250	160.52	100.33
200	1.557	199.89	99.95
240	1.883	214.71	100.71
280	2.181	279.97	99.99
		Mean	102.07
		S.D.	5.35
		% C.V.	5.27

1. $r^2 = 0.9999$, $Y = 0.0078X - 0.0023$

2. Inversely estimated concentration = (PAR + 0.0023)/ 0.0078

3. % Recovery = (Inversely estimated concentration / Known concentration) x 100

4. % C.V. = (S.D./ Mean) X 100

* Each data point was determined tripicately

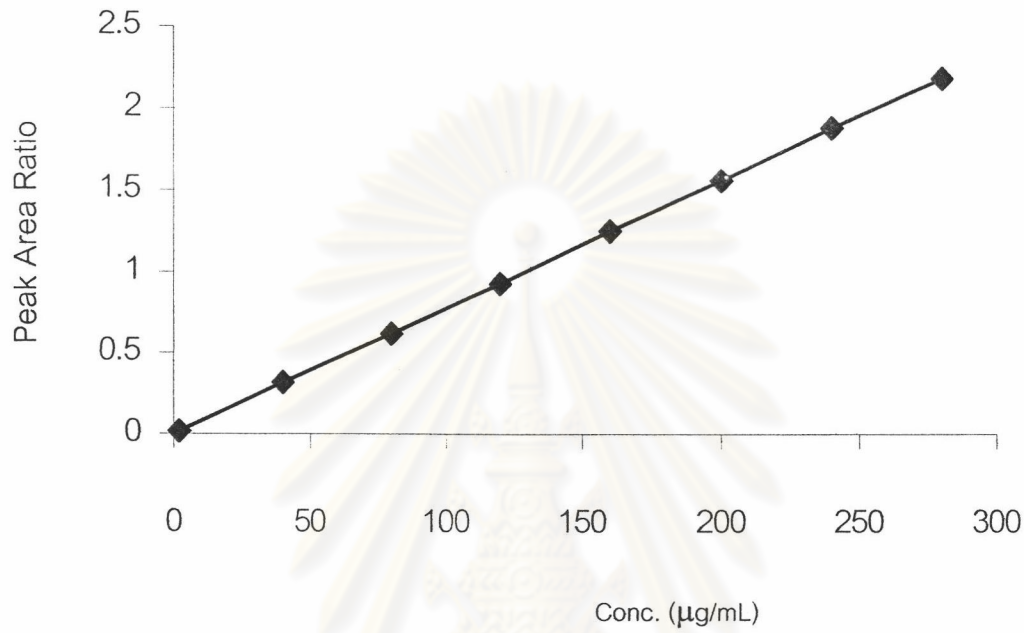

Figure 29. Typical calibration curve data for determination of ketoprofen in rabbit plasma

APPENDIX E

STATISTICS

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

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

2. Standard deviation

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

3. **Coefficient of variation (C.V.)**

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

4. **Non-compartment method.**

In single dose pharmacokinetic study, blood sampling is stopped at some time (t*) when drug concentration (C*) is measurable. Pharmacokinetic parameters are calculated as follow:

4.1 Area under the concentration time curve (AUC).

$$AUC = AUC_{0-t^*} + AUC_{t^*-\infty}$$

$$AUC_{t^*-\infty} = C^*/\lambda$$

Where λ is the slope of the terminal exponential phase of a plot of natural log drug concentration versus time.

4.6 Elimination half life ($t_{1/2}$)

$$t_{1/2} = 0.693 / \lambda$$

5. Analytical of variance for complete randomized block design

In statistic terms the calculation to set up analysis of variance table are as follow:

Souse of variation	d.f.	Sum of Square	Mean square
Total	rp - 1	SStotal	-
Block	r – 1	SSblock	MSblock
Formulation	p – 1	SSformulation	MSformulation
Error	(r – 1)(p – 1)	SSerror	MSerror

Where

C.T. = Correction term = $(\sum x)^2 / rp$

p = number of formulation (p = 2)

r = total number of subjects (r = 12) = number of block

Data presented are individual subject of the C_{max} of ketoprofen after rectal administration of 100 mg ketoprofen coated rectat hard gelatin capsule.

Subject	20% Tween 80®	20% DMI	Sum	Mean
1	236.123	139.864	375.987	187.993
2	166.063	173.430	339.492	169.746
3	246.541	135.863	382.405	191.202
4	288.993	256.093	545.086	272.543
5	312.352	237.603	549.955	274.978
6	218.767	180.144	398.911	199.456
7	203.094	286.025	489.119	244.560
8	229.586	227.073	456.659	228.330
9	247.637	133.734	381.371	190.685
10	233.435	212.183	445.618	222.809
11	235.907	180.811	416.718	208.359
12	244.240	164.678	408.919	204.459
Sum	2862.739	2327.501	5190.240	
Mean	238.562	193.958		216.260

1. Correction term $= (5190.240)^2/24 = 1122441.30$
2. SStotal $= [(236.123)^2+(166.063)^2+.....(408.919)^2]-C.T. = 54370.28$
5. SSreplete $= [(375.987)^2+(339.492)^2+ ....(408.919)^2]/ 2 - C.T. = 24667.08$
6. SSformulation $= [(2862.739)^2+(2327.501)^2]/ 12 - C.T. = 11936.64$
7. SSresidual $= 0.551- (0.030+0.088+0.023+0.294) = 17766.56$
8. MS $= SS/df$

Source of variation	d.f.	SS	MS	F ratio	F table	Sig.level $\alpha = 0.05$
Total	23	54370.28	-	-	-	
Block	11	24667.08	2242.46	1.39	2.82	NS
Formulation	1	11936.64	11936.64	7.39	4.84	S
Error	11	17766.56	1615.14	-	-	

Where : F table obtained from the table of F ratio for 0.05 level of significance. The test showed that there are significant differences in C_{max} value in both formulations.

6. **Relative bioavailability**

$$\text{Relative bioavailability} = (AUC_{test} / AUC_{ref}) \times (Dose_{ref} / Dose_{test}) \times 100$$

VITA

Miss Mallika Laoweerathum was born on June 7, 1974 in Ayutthaya, Thailand. She received her Bachelor Degree of Science in Pharmacy from the faculty of Pharmacy, Mahidol University, Bangkok, Thailand in 1996. After graduation, she worked in Chaiyapoom Hospital, Bumnejnarong Hospital, Chaiyapoom and Bangpahun Hospital, Ayutthaya in 1996, 1997 and 1999, respectively before entering the Master's Degree program in Pharmacy at Chulalongkorn University.