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APPENDICES

APPENDIX A

CALCULATION OF THE DISPLACEMENT VALUES

The method for determination of the amount of base in preparation of medicated suppositories required the following steps:

1. Determine the average blank weight, A, per mold using the suppository base of interest.
2. Weigh the quantity of suppository base necessary for 10 suppositories.
3. Weigh 1.0 g of medication.

The weight of medication per suppository, B, is then equal to 1g/10 supp = 0.1 g/suppo.

4. Melt the suppository base and incorporate the medication, mix, pour into molds and remove from the molds.
5. Weigh the 10 suppositories and determine the average weight (C).
6. Determine the displacement value as follows:

$$\text{Displacement value} = \frac{B}{A - C + B}$$

Where A = average weight of blank,
B = weight of medication per suppository, and
C = average weight of medicated suppository.

An example for calculation of the displacement value of ketoprofen in Suppocire ®AM was illustrated as follow:

1. A = (average blank weight) is 1.9565 g (n=10).
2. B = (weight of medication per suppository) = 0.1 g.

3. C = (average weight of medicated suppository) = 1.9663 g (n=10).

$$\text{Displacement value} = \frac{B}{A - C + B}$$

$$= \frac{0.1}{(1.9565-1.9663+0.1)} = 1.109$$

The displacement value of ketoprofen in Suppocire ®AM was 1.109.

APPENDIX B

REAGENT PREPARATION

Phosphate buffer pH 7.2

Dissolved 27 g of potassium dihydrogen phosphate in water and adjust to 1 liter, take 50 mL of this solution to mix with 34.7 mL of 0.2 N sodium hydroxide solution and adjust the resulting solution with water to a pH 7.2±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 a pH of 4.2±0.02.

APPENDIX C

VALIDATION OF ANALYTICAL METHOD FOR *IN VITRO* STUDIES

1. Accuracy.

Table 39 Accuracy of analytical method for determination of ketoprofen in phosphate buffer pH 7.2 at λ = 260 nm.

Known concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
3.2	3.16	98.81
4.8	4.77	99.41
6.4	6.43	100.46
8.0	8.01	100.15
9.6	9.64	100.46
11.2	11.23	100.29
12.8	12.84	100.33
14.4	14.28	99.40

Mean % recovery = 99.91, S.D.=0.78, %C.V. = 0.78

Table 40 Accuracy of analytical method for determination of ketoprofen in chloroform at λ = 255 nm.

Known concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
2.4	2.36	98.14
4.8	4.81	100.27
7.2	7.26	100.51
9.6	9.62	100.19
12.0	11.95	99.61

Mean % recovery = 99.81, S.D.=1.26, %C.V. = 1.26.

Table 41 Accuracy of analytical method for determination of ketoprofen in methanol at λ = 255 nm.

Known concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
3.2	3.21	100.27
4.8	4.78	99.49
6.4	6.41	100.22
8.0	7.98	99.76
9.6	9.62	100.19
11.2	11.26	100.56
12.8	12.77	99.78
14.4	14.42	100.19

Mean % recovery = 100.06, S.D.=0.62, %C.V. = 0.62

2. Precision.

2.1 Within Run Precision.

Table 42 Within run precision of analytical method for determination of ketoprofen in phosphate buffer pH 7.2 at $\lambda = 260$ nm.

Concentration (μg/mL)	Absorbance Mean ± S.D.	%C.V.
3.2	0.212 ± 0.002	0.94
4.8	0.312 ± 0.002	0.64
6.4	0.415 ± 0.002	0.48
8.0	0.514 ± 0.003	0.58
9.6	0.614 ± 0.002	0.33
11.2	0.713 ± 0.004	0.56
12.8	0.810 ± 0.005	0.62
14.4	0.904 ± 0.002	0.22

Table 43 Within run precision of analytical method for determination of ketoprofen in chloroform at λ = 255 nm.

Concentration (μg/mL)	Absorbance Mean ± S.D.	%C.V.
2.4	0.177 ± 0.007	3.79
4.8	0.343± 0.005	1.47
7.2	0.508 ± 0.006	1.20
9.6	0.669 ± 0.013	1.94
12.0	0.832 ± 0.009	1.10

Table 44 Within run precision of analytical method for determination of ketoprofen in methanol at λ = 255 nm.

Concentration (μg/mL)	Absorbance Mean ± S.D.	%C.V.
3.2	0.211 ± 0.005	2.37
4.8	0.316 ± 0.003	0.95
6.4	0.425 ± 0.007	1.65
8.0	0.526 ± 0.005	0.95
9.6	0.634± 0.007	1.10
11.2	0.734 ± 0.011	1.50
12.8	0.835 ± 0.006	0.72
14.4	0.648 ± 0.009	0.95

2.2 Between Run Precision.

Table 45 Between run precision of analytical method for determination of ketoprofen in phosphate buffer pH 7.2 at λ = 260 nm.

Concentration (μg/mL)	Absorbance Mean ± S.D.	% C.V.
3.2	0.209 ± 0.003	1.44
4.8	0.311 ± 0.003	1.01
6.4	0.413 ± 0.004	1.04
8.0	0.512 ± 0.004	0.87
9.6	0.613 ± 0.005	0.88
11.2	0.713 ± 0.007	0.98
12.8	0.811 ± 0.007	0.85
14.4	0.907 ± 0.007	0.77

Table 46 Between run precision of analytical method for determination of ketoprofen in chloroform at $\lambda = 255$ nm.

Concentration (μg/mL)	Absorbance Mean ± S.D.	% C.V.
2.4	0.175 ± 0.010	5.78
4.8	0.335 ± 0.010	2.96
7.2	0.503 ± 0.012	2.34
9.6	0.664 ± 0.015	2.22
12.0	0.803 ± 0.018	2.19

Table 47 Between run precision of analytical method for determination of ketoprofen in methanol at $\lambda = 255$ nm.

Concentration (μg/mL)	Absorbance Mean ± S.D.	% C.V.
3.2	0.208 ± 0.007	3.27
4.8	0.349 ± 0.004	1.28
6.4	0.427 ± 0.007	1.60
8.0	0.533 ± 0.009	1.72
9.6	0.638 ± 0.010	1.53
11.2	0.745 ± 0.011	1.51
12.8	0.847 ± 0.012	1.39
14.4	0.954 ± 0.010	1.05

3. Calibration curves.

Table 48 Typical calibration curve data for determination of ketoprofen in phosphate buffer pH 7.2 estimated using linear regression [1].

Concentration (μg/mL)	Absorbance (λ = 260 nm)	Inversely estimated conc [2]	% Recovery [3]
3.2	0.210	3.18	99.23
4.8	0.310	4.78	99.64
6.4	0.412	6.42	100.36
8.0	0.512	8.03	100.38
9.6	0.612	9.64	100.40
11.2	0.710	11.21	100.12
12.8	0.810	12.82	100.17
14.4	0.906	14.36	99.76
		Mean	100.01
		S.D.	0.42
		%C.V. [4]	0.42

1. $r^2 = 1$, Y=0.0622X + 0.0125 (Y=Absorbance, X=Known conc.)

2. Inversely estimated concentration = (Absorbance - 0.0125)/ 0.0622

3. %Recovery = (Inversely estimated concentration / Known concentration)x100

4. % C.V.= (S.D./Mean)x100

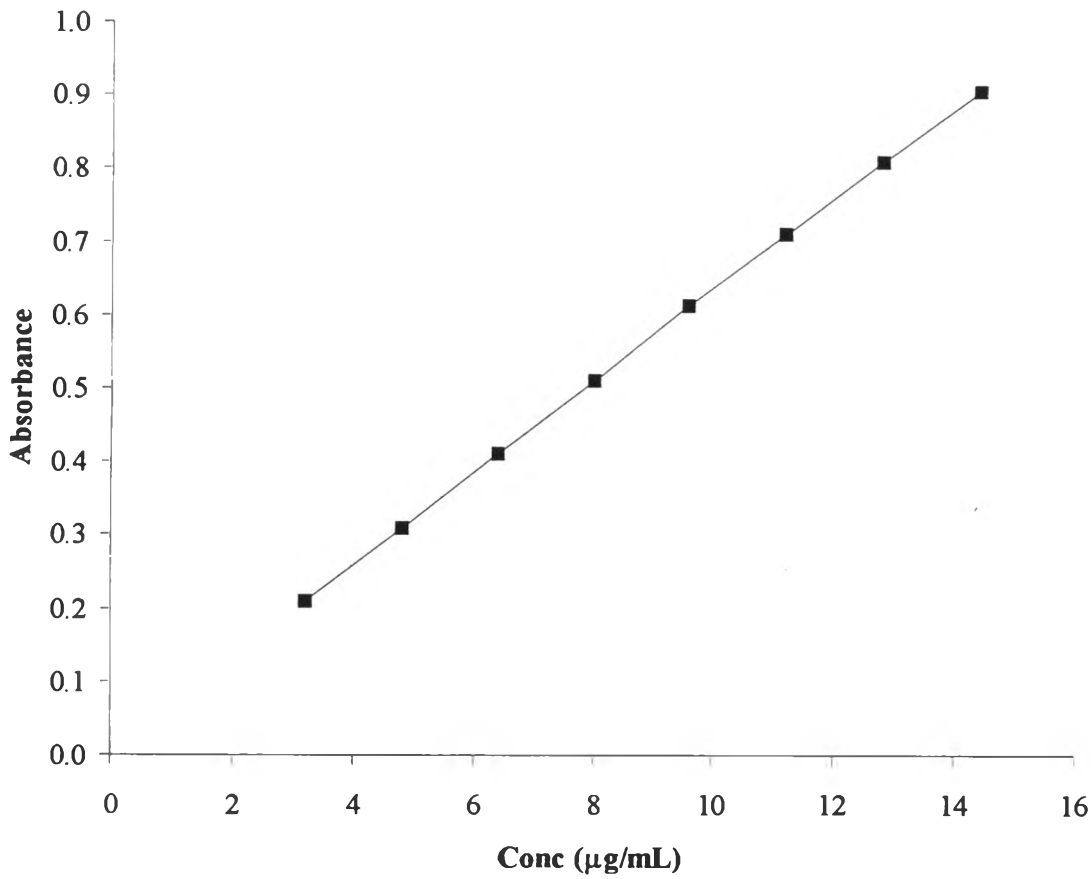


Figure 23 Typical calibration curve for determination of ketoprofen in phosphate buffer pH7.2 at $\lambda = 260$ nm.

Table 49 Typical calibration curve data for determination of ketoprofen in chloroform estimated using linear regression [1].

Concentration (μg/mL)	Absorbance (λ = 255 nm)	Inversely estimated conc[2]	% Recovery[3]
2.4	0.184	2.40	100.19
4.8	0.343	4.76	99.17
7.2	0.512	7.26	100.88
9.6	0.666	9.55	99.43
12.0	0.832	12.00	100.04
		Mean	99.94
		S.D.	0.67
		%C.V.[4]	0.68

1. $r^2 = 0.9999$, Y=0.0675X + 0.0217 (Y=Absorbance, X=Known conc.)
2. Inversely estimated concentration = (Absorbance - 0.0217)/ 0.0675
3. %Recovery = (Inversely estimated concentration / Known concentration)x100
4. % C.V.= (S.D./Mean)x100

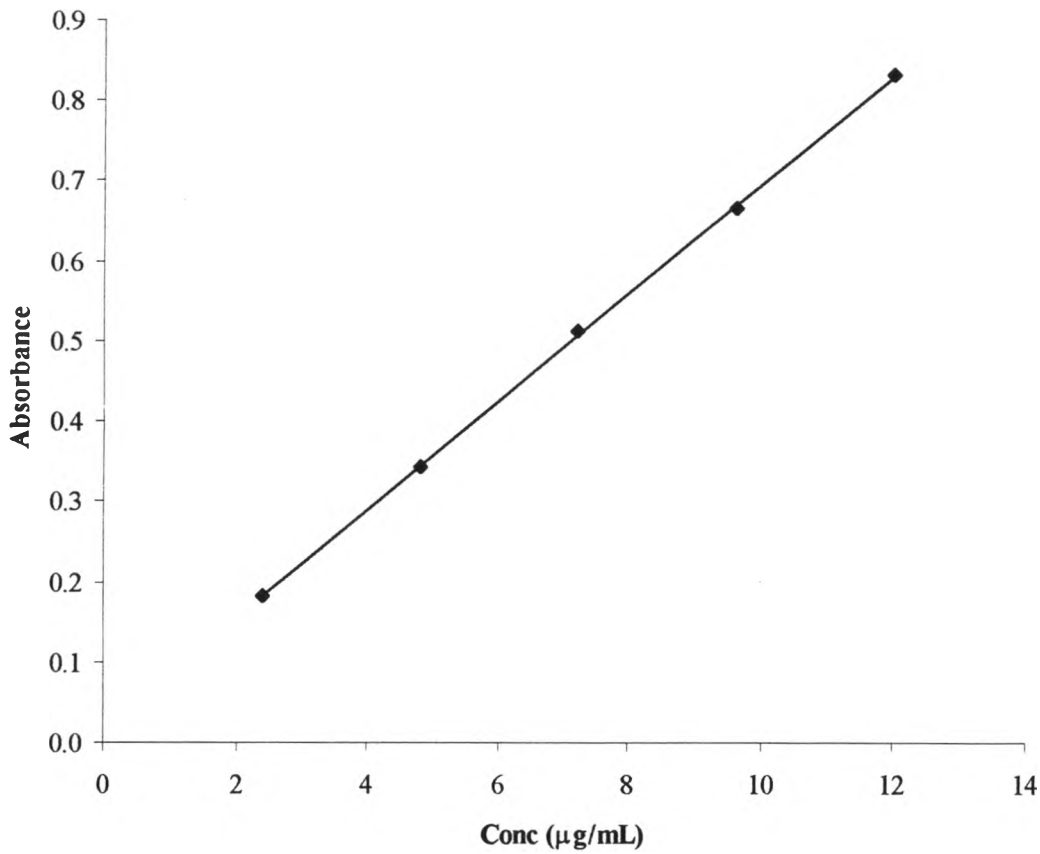


Figure 24 Typical calibration curve for determination of ketoprofen in chloroform at λ= 255 nm.

Table 50 Typical calibration curve data for determination of ketoprofen in methanol estimated using linear regression [1].

Concentration (μg/mL)	Absorbance (λ = 255nm)	Inversely estimated conc[2]	% Recovery[3]
3.2	0.208	3.20	100.14
4.8	0.311	4.80	99.97
6.4	0.413	6.38	99.65
8.0	0.517	7.99	99.85
9.6	0.625	9.66	100.62
11.2	0.726	11.22	100.20
12.8	0.828	12.80	100.01
14.4	0.930	14.38	99.87
		Mean	100.04
		S.D.	0.29
		%C.V.[4]	0.29

1. $r^2 = 1$, Y=0.0646X + 0.001 (Y=Absorbance, X= Known conc.)
2. Inversely estimated concentration = (Absorbance - 0.001)/ 0.0646
3. %Recovery = (Inversely estimated concentration / Known concentration)x100
4. % C.V.= (S.D./Mean)x100

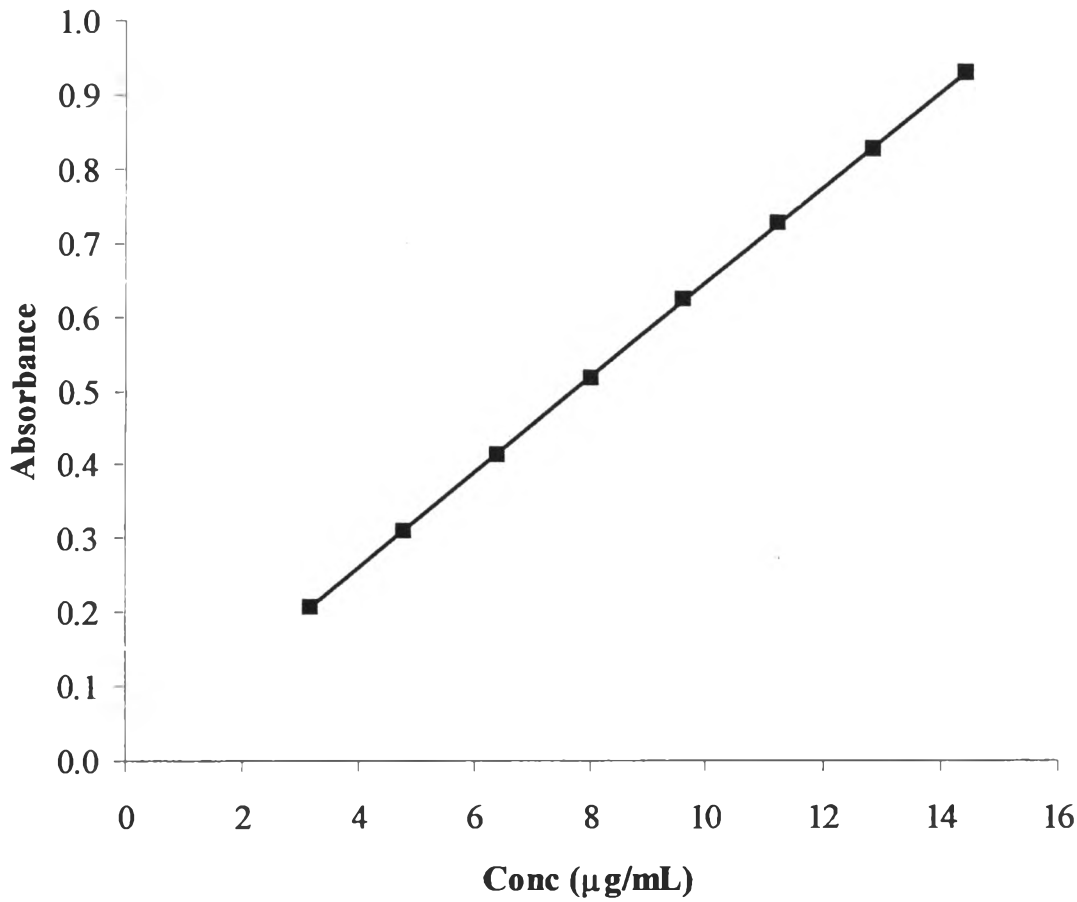


Figure 25 Typical calibration curve for determination of ketoprofen in methanol at $\lambda = 255$ nm.

APPENDIX D

CALCULATION OF RELEASE RATE CONSTANT

The release rate constant is calculated according to sigma-minus method as shown in equation

$$\ln (X_\alpha - X_t) = -Kt + \ln X_\alpha$$

where X_α = The amount of drug released at infinity (t_α).

X_t = The amount of drug released at time t.

K = The release rate constant.

t = Time.

A linear curve is obtained by plotting the natural logarithm of the amount of unreleased ketoprofen ($X_\alpha - X_t$) versus time. The release rate constant is obtained from the slope of this curve.

An example: using the release data in Table 51, the release rate constant was determined as follow:

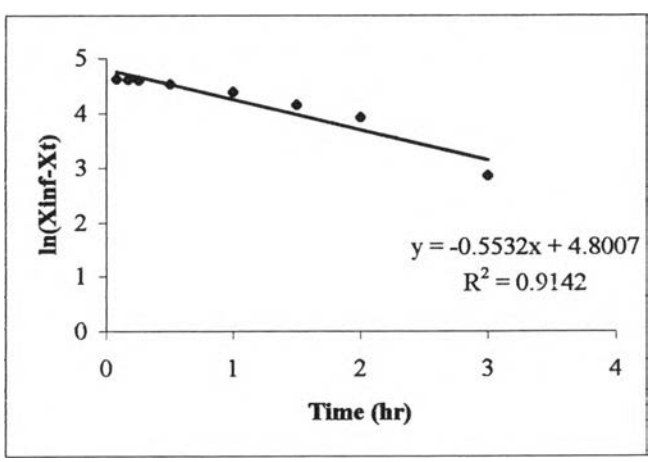
1. Construct the relevant data and
2. Determine the amount of unreleased ketoprofen ($X_\alpha - X_t$) and then plot ($X_\alpha - X_t$) on the natural log scale versus time.

The release rate constant obtained from this data is 0.553 hr^{-1}.

Table 51 Typical data for determination of the release rate constant according to sigma-minus method.

Time (hr)	X_t(%)	X_α-X_t %)	ln(X_α-X_t)
0.08	0.923	102.229	4.627
0.17	2.139	101.013	4.615
0.25	3.739	99.413	4.599
0.5	10.107	93.045	4.533
1	22.672	80.480	4.388
1.5	39.472	63.680	4.154
2	52.272	50.880	3.929
3	85.712	17.400	2.859
4	103.152*	-	-
5	106.192	-	-
6	107.632	-	-

* X_α



The release rate constant was 0.553 hr^{-1}

APPENDIX E

VALIDATION OF ANALYTICAL METHOD FOR *IN VIVO* STUDIES

1. Accuracy

Table 52 Accuracy of analytical method for determination of ketoprofen in rabbit plasma.

Concentration (μg/mL)	Inversely estimated concentration (μg/mL)	% Recovery
5	5.17	103.34
10	9.81	98.08
20	19.95	99.76
50	49.23	98.46
100	103.83	103.83
150	142.53	95.02
200	203.89	101.94

2. Precision.

2.1 Within Run Precision.

Table 53 Within run precision of analytical method for determination of ketoprofen in rabbit plasma.

Concentration (μg/mL)	PAR (Ketoprofen / Diclofenac sodium) Mean ± S.D.	% C.V.
5	0.34 ± 0.01	3.46
10	0.66 ± 0.02	2.55
20	1.39 ± 0.08	5.48
50	3.43 ± 0.14	4.00
100	7.12 ± 0.21	2.94
150	10.44 ± 0.21	2.01
200	13.92 ± 0.37	2.69

2.2 Between Run Precision.

Table 54 Between run precision of analytical method for determination of ketoprofen in rabbit plasma.

Concentration (μg/mL)	PAR (Ketoprofen / Diclofenac sodium) Mean ± S.D.	% C.V.
5	0.35 ± 0.04	10.98
10	0.67 ± 0.03	4.58
20	1.41 ± 0.04	2.90
50	3.49 ± 0.12	3.29
100	7.20 ± 0.10	1.33
150	10.08 ± 0.77	7.62
200	14.62 ± 0.82	5.61

3. Calibration curve.

Table 55 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[3]
2	0.134	2.23	111.36
5	0.343	5.16	103.17
10	0.677	9.84	98.43
20	1.341	19.16	95.78
50	3.479	49.14	98.28
100	7.362	103.60	103.60
150	10.486	147.42	98.28
200	14.263	200.39	100.20
		Mean	101.14
		S.D.	4.90
		%C.V.[4]	4.84

1. $r^2 = 0.9995$, Y=0.0713x-0.0248 (Y=PAR, X = Known conc.).
2. Inversely estimated concentration = (PAR + 0.0248)/ 0.0713.
3. % Recovery = (Inversely estimated concentration / Known concentration)x100
4. % C.V.= (S.D./Mean)x100

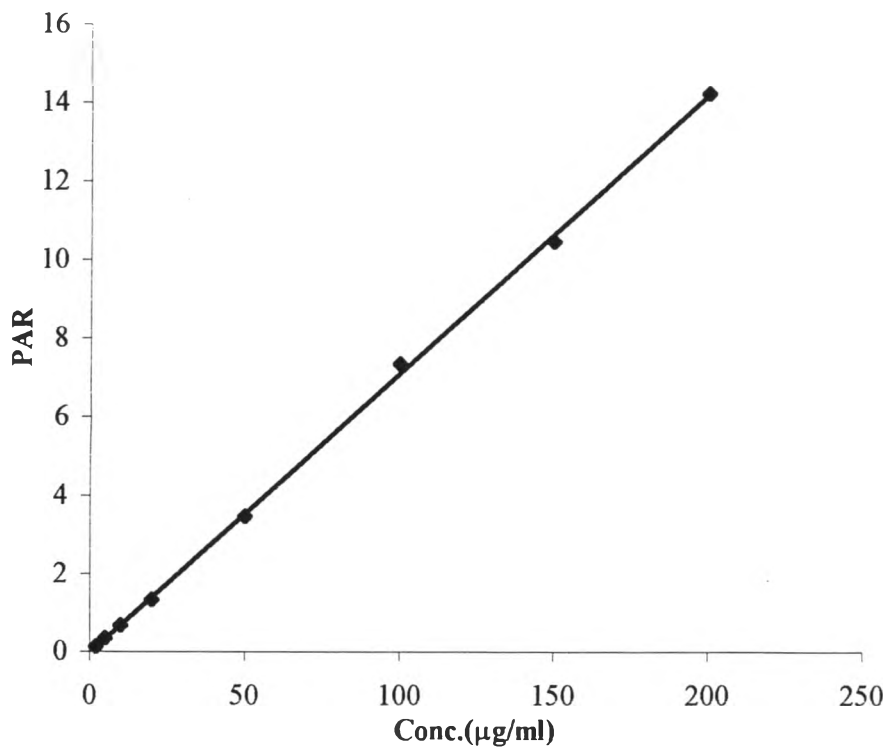


Figure 26 Typical calibration curve for determination of ketoprofen in rabbit plasma.

APPENDIX F

STATISTICS

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

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

2. Standard deviation (S.D.)

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

3. Coefficient of variation (C.V.)

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

4. Non-compartmental 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)

$$AUC = AUC_{o\text{-}t^*} + AUC_{t^*\text{-}\alpha}$$

Where $AUC_{t^*\text{-}\alpha} = C^* / \lambda_n$

Where λ_n is 2.303 times the slope of the terminal exponential phase of a plot log drug concentration versus time.

4.2 Area under the moment curve (AUMC).

The same approach must be used to estimate total AUMC.

$$AUMC = AUMC_{0-t^*} + AUMC_{t^*-\alpha}$$

The area under the moment curve from t* to infinity is estimated as

$$AUMC_{t^*-\alpha} = t^*C^*/\lambda_n + C^*/(\lambda_n)^2$$

4.3 Clearance (CL/F)

$$CL/F = Dose/AUC$$

4.4 Mean residence time (MRT).

Represents the time for 63.2% of the administered dose to be eliminated.

$$MRT = AUMC/AUC$$

4.5 Volume of distribution (V_d/F).

$$V_d/F = CL/\lambda_n$$

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

$$t_{1/2} = 0.693 . MRT_{non\ iv}$$

5. Analysis of variance for three way crossover design.

The experimental plan is:

Sequence	Subjects /Group	Treatment		
		Period I	Period II	Period III
I	1,2,3	A	B	C
II	4,5,6	B	C	A
III	7,8,9	C	A	B

Where A = Eudragit S-100, B = Suppocire ®AM and C = Hydroxypropyl methylcellulose phthalate (HP55).

In statistical terms the calculations to set up an analysis of variance table are as follow:

Source of variation	d.f.
Total	g.n.t -1
Sequences	g-1
Subjects(sequences)	g(n-1)
Period	w-1
Formulation	t-1
Error	(gn-2)(t-1)

Where

C.T. = Correction term = $(\sum x)^2$ / g.n.t

n = number of subjects per group or treatment sequence (n=3).

t = number of treatments (t=3)

g = number of groups or treatment sequences (g=3)

w = number of time periods (w=3).

gn = total number of subjects (gn=9).

Data presented below are individual subject of the log of peak plasma concentration (log C_{max}) of ketoprofen after administration of 100 mg prolonged release ketoprofen rectal suppositories.

Sequence	Subject	FormulationA	FormulationB	FormulationC	Subject total
I	1,2,3	2.01 periodI 2.32 sum 2.13 6.46	2.06 periodII 2.39 sum 2.42 6.87	2.11 periodIII 2.36 sum 1.98 6.45	6.18 7.07 6.53
II	4,5,6	2.25 periodIII 2.18 sum 2.19 6.62	2.32 periodI 2.33 sum 1.56 6.21	2.19 periodII 2.14 sum 2.11 6.44	6.76 6.65 5.86
III	7,8,9	1.51 periodII 2.19 sum 2.31 6.01	2.13 periodIII 2.25 sum 2.21 6.59	1.79 periodI 2.16 sum 2.17 6.12	5.43 6.60 6.69
Formulation total		19.09	19.67	19.01	57.77

Period I = 6.46 + 6.21 + 6.12 = 18.79

Period II = 6.87 + 6.44 + 6.01 = 19.32

Period III = 6.45 + 6.62 + 6.59 = 19.66

1. Correction term (C.T.) = $(57.77)^2 / 27$ = 123.61
2. SS_{total} = $[(2.01)^2+(2.32)^2+\ldots+(2.17)^2]$ –C.T. = 1.26
3. $SS_{sequence}$ =[$(6.18+7.07+6.53)^2+(6.76+6.65+5.86)^2+(5.43+6.60+6.69)^2$]-C.T. = 0.06
4. $SS_{sub(seq)}$ = $[(6.18)^2+(7.07)^2+\ldots+(6.69)^2]/3$–C.T. = 0.68
5. SS_{period} =[$(18.79)^2+(19.32)^2+(19.66)^2$]/9 –C.T. = 0.04
6. $SS_{treatment}$ =[$(19.09)^2+(19.67)^2+(19.01)^2$]/9 –C.T. = 0.03
7. $SS_{residual}$ = 1.26-(0.06+0.68+0.04+0.03) = 0.45

Analysis of variance table for three way crossover design:

Source of variation	d.f	SS	MS	F_{ratio}	F_{table}	Sig.level
Total	26	1.26	0.048	-		
Sequence	2	0.06	0.03	0.03/0.113 = 0.265	5.14	NS
Subject(seq)	6	0.68	0.113	0.113/0.032 = 3.531	2.64	S
Period	2	0.04	0.02	0.02/0.032 = 0.625	3.68	NS
Treatment	2	0.03	0.015	0.015/0.032 = 0.469	3.68	NS
Error	14	0.45	0.032	-		

Where F_{table} obtained from the table of F ratio for 0.05 level of significance. The test showed that there are no significant differences in C_{max} value among three formulations.

6. Construction of 90% confidence interval (Two one-sided test).

The confidence interval (CI) for the difference of two means has the form:

$$\mu_{T,obs} - \mu_{R,obs} \pm t_{0.95(v)}\, s \sqrt{2/n} \qquad \text{Eq . 1}$$

where

$\mu_{T,obs}$	=	observed mean for the test treatment
$\mu_{R,obs}$	=	observed mean for the reference treatment
ν	=	the degree of freedom associated with the "error" mean square
$t_{0.95(\nu)}$	=	the point that probability of 0.05 in the upper tail of the Student's t distribution with ν degrees of freedom
s	=	the square root of the "error" mean square from the crossover design analysis of variance
n	=	the total number of subjects participating in the crossover design
$s\sqrt{2/n}$	=	standard error of the estimate

The two one-sided tests procedure consists of a pair of ordinary one-sided t-tests. Since the nominal confidence level of each one-sided test is $\alpha = 0.05$, the two one-sided tests procedure is operationally equivalent to the ordinary (shortest) the $1 - 2(\alpha)$ (or 90%) confidence interval. By this procedure, if test and reference products are not bioequivalent (i.e., means differ by more than 20%), there is a 5% (not a 10%) chance of concluding that they are bioequivalent.

The foregoing confidence interval equation (Eq. 1) applies to a balanced crossover study in which

1. There is an equal number of subjects in each treatment-administration sequence.
2. There are no missing observations from any subject.

An example : Computation of the 90% confidence interval for the difference of C_{max} means of the rectal suppository with Eudragit S-100 and Suppocire ®AM .

The following data were obtained following analysis of variance performed.

$\mu_{T,obs}$ = 2.12 μg/mL

$\mu_{R,obs}$ = 2.19 μg/mL

ν = 14

$t_{0.95(14)}$ = 1.761

s = 0.2449

n = 9

$s\sqrt{2/n}$ = $0.2449\sqrt{2/9}$ = 0.1155

the first of the two confidence interval equations (Eq. 1) may be applied. Substituting the foregoing data into this equation:

= (2.12 – 2.19) ± 1.761 (0.1155) μg/mL

= -0.07 ± 0.20 μg/mL

= -0.27 μg/mL ; 0.13 μg/mL

Therefore, the 90% confidence interval extends from –0.27 μg/mL below to +0.13 μg/mL above the observed reference treatment mean value. As indicated earlier, confidence interval (CI) approval criteria are based upon percentage differences from the references from the reference treatment mean value (taken as 100%). Thus, converting to percentages:

$$\text{The lower CI limit} = \frac{2.19 - 0.27}{2.19} \times 100 = 87.67\%$$

$$\text{The upper CI limit} = \frac{2.19 + 0.13}{2.19} \times 100 = 105.94\%$$

90% CI of suppository with Eudragit S-100 = 87.67 – 105.94, This could be concluded that the suppository with Eudragit S-100 and Suppocire® AM were bioequivalent in term of the rate and extent of drug absorption into systemic circulation.

VITA

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