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Title: Pharmacokinetic Investigation of a Potential Drug Interaction: Aminophylline and Prednisone

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Six subjects were administered oral aminophylline alone and with oral prednisone as well as prednisone alone. Although half of the subjects exhibited at least 25% reduction in theophylline bioavailability with concomitant prednisone based on uncorrected AUC calculation, the average relative bioavailability of theophylline was 100% when adjusted for changes in elimination rate constant (f_{adj} = 1.00). Differences in mean peak theophylline concentrations and mean time to individual peaks were small and not significantly different. Plasma concentrations of prednisolone from prednisone were decreased somewhat by concomitant aminophylline administration. The difference in mean peak concentrations (-9.7%) was statistically significant but time to peak was not affected. Total absorption based on uncorrected AUC calculations was not affected (f_{rel} = 0.98) but adjustment

for changes in elimination rate constants resulted in a small but statistically significant decrease in prednisolone bioavailability when prednisone was administered with aminophylline (f_{adj} = 0.93).

Pharmacokinetic Investigation of a Potential Drug Interaction: Aminophylline and Prednisone

by

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INTRODUCTION

In 1978 Ayres[1] et al. reported three cases suggesting an interaction involving aminophylline and prednisone. Two cases described apparently reduced bioavailability of prednisolone from prednisone tablets when aminophylline tablets were given concomitantly. The third case described a decrease in half-life of theophylline from aminophylline tablets when prednisone tablets were given concomitantly.

Aminophylline, a salt form of theophylline, is widely used in the treatment of reversible obstructive pulmonary disease. The effectiveness of theophylline is related to plasma concentration,[2] as is toxicity.[3] For drugs with minimum effective concentrations approaching toxic concentrations, like theophylline, factors altering bioavailability or elimination kinetics are important considerations. Several such factors have been identified for theophylline. Bioavailability can be affected by product formulation,[4] diet, and fluid intake.[5] Theophylline elimination can be influenced by age,[6] diet,[7] obesity,[8] disease,[9-11] smoking[12,13] and concurrent administration of other drugs[14-17] or vaccines.[18] Addition or subtraction of one or more of these factors may cause theophylline concentrations to fall below minimum effective con-

centrations or rise to toxic concentrations.

Prednisone, a synthetic corticosteroid, is often added to theophylline therapy during acute exacerbations of asthma. It is also indicated in a wide range of immune disorders so that coadministration with theophylline products is not uncommon. Prednisone is biotransformed *in vivo* to prednisolone which is considered to be the active metabolite.[19] As with theophylline, various factors have been described which can alter bioavailability of prednisone or elimination of prednisolone.[20-25] Although relationships between plasma concentrations and therapeutic or toxic effects are not well described for these steroids, alteration in bioavailability or elimination can adversly affect therapy. Differences in bioavailability of different brands of prednisone tablets have resulted in problems clinically.[20,21] These differences appeared to be related to *in vitro* dissolution characteristics of the tablets and could be correlated to different rates of appearance of prednisolone in the systemic circulation.[22] Enzyme induction resulting in an increased elimination of prednisolone has been related to decreased effectiveness.[23,24] Inhibition of metabolism of prednisolone has also been described.[25]

If the effects observed and reported earlier as a potential interaction do exist, then patients requiring one of these drugs chronically would have a risk of

adverse drug reactions if the other drug was added intermittently.

This study was undertaken to measure bioavailability and pharmacokinetic parameters of both theophylline (from aminophylline) and prednisolone (from prednisone) when given individually and in combination with each other.

METHODS

Approval was granted by the Committee for Protection of Human Subjects of Oregon State University and six healthy nonsmoking male subjects consented to participate. All subjects were between 21 and 35 years old and within 15% of their ideal body weight.[a] Alcohol was forbidden during the entire study period. Beginning 48 hours before each study day all subjects abstained from xanthine containing foods and beverages. Subjects fasted from approximately 10 hours before until 4 hours following the beginning of each treatment. Water was allowed ad lib during this period. No other dietary restrictions were imposed. Subjects were requested to avoid heavy exercise on the study days.

Treatments were separated by 7 days. Treatment schedules and doses administered are shown in Table 1. The 3 way crossover design allowed each subject to serve as his own control. Samples of blood were collected over 24 hours following each dose at times 0.0, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 18.0 and 24.0 hours. Approximately 10 ml of blood was withdrawn at each sample time and placed in heparinized vacuum containers. Plasma was harvested immediately and frozen until time of assay.

Plasma samples were assayed for theophylline by an adaptation of the method of Desiraju and Sugita.[26] The

standard curve was constructed of 18 points over a range of 0 to 16 mcg/ml (R^2 =0.9971). Mean inverse estimate of known concentrations was 100% of theory with a coefficient of variation in percent (CV%) equal to 9.3%.

Plasma steroid concentrations were assayed by the method of Loo and Jordan.[27] The standard curve for prednisolone was constructed of 21 points over a range of 0 to 1000 ng/ml (R^2 = 0.9946). Mean inverse estimate was 97% of theory with 6.4% CV. Prednisone was also eluted on these chromatograms. A standard curve for prednisone was constructed of 26 points over a range of 0 to 100 ng/ml (R^2 = 0.9277). Mean inverse estimate was 101.8% of theory but precision was poor (CV = 27%). Data were pooled from samples assayed on 5 different days. Standard curves for individual days (n ranged from 4 to 7) had a weighted mean inverse estimate of 100.2%.

Several standards used in the construction of standard curves for theophylline were spiked with prednisone and prednisolone. Similarly, several steroid standards were spiked with theophylline. No interference in either assay was detected.

Individual plasma concentration vs. time curves for theophylline and prednisolone were fit to a one compartment open model with first order absorption and elimination.[5,28,29] Points were weighted in the regression by the factor $1/y2$. Curves for mean data vs. time were

similarly fit using a weighting factor of $1/\sigma^2$. Fits were accomplished using Expfit[30], a public procedure of the Prophet system. The data were well described by the model (mean R^2 for individual theophylline data = 0.9925 and for prednisolone data = 0.9949).

Area under the plasma concentration vs. time curve up to the time of the last sample (AUC_{O-T}) was determined by the trapezoidal rule. AUC from this time to infinity ($AUC_{T-\infty}$) was estimated by the quotient of the estimated concentration at the time of the last sample and the elimination rate constant (C_p/k_{el}). Total AUC ($AUC_{O-\infty}$) was determined by the sum of these areas.

Differences in mean plasma concentrations and parameter estimates were tested with a "t" statistic for paired data. Differences occurring at $\alpha < 0.05$ were considered significant.

RESULTS

Mean concentrations of theophylline at each sampling time are shown in Table 2. Concentrations from time one hour and thereafter during treatment A (control) are greater than during treatment B but only 2 of 10 of the differences are statistically significant. Individual pharmacokinetic bioavailability parameters are shown in Table 3. Peak concentration and time to peak are very similar for both treatments. Theophylline elimination rate constants tended to increase with prednisone administration but the difference was not statistically significant. $AUC_{0-\infty}$ for theophylline tended to decrease with prednisone administration but this difference was not statistically significant. Curves fit to the mean theophylline concentration *vs.* time data are shown in Figure 1.

Mean concentrations of prednisolone at each sampling time are shown in Table 4. Mean concentrations at all sample times are very similar except at 1.0 and 2.0 hours, near the peak of the mean curves. Differences in prednisolone concentration after administration of prednisone with aminophylline at these sampling times were depressed statistically significantly compared to after administration of prednisone alone, (14.6% and 7.5% at 1.0 and 2.0 hours respectively). Individual bioavialability and phar-

macokinetic parameters for prednisolone are shown in Table 5. The mean of the peak prednisolone concentrations observed in treatment C (control) was greater than that from treatment B. This difference (9.8%) was statistically significant. Differences in time to peak, elimination rate constant and $AUC_{0-\infty}$ were not statistically significant. Curves fit to the mean prednisolone concentration vs. time data are shown in Figure 2.

Plasma prednisone concentrations are shown in Table 6. Concentrations were higher in treatment C (control) than in treatment B at most sampling times. However, differences in mean concentrations were not statistically significant at any of the sampling times.

Estimates of relative bioavailability (f_{rel}) for theophylline (from aminophylline) and prednisolone (from prednisone) are shown in Table 7. Although half the subjects exhibited at least 25% reduction in $AUC_{0-\infty}$ for theophylline when prednisone was administered concomitantly the difference between the mean f_{rel} (0.87) and a relatively complete bioavailability (1.0) was not statistically significant. The tendency towards an increased elimination rate of theophylline when administered concomitantly with prednisone (Table 3) could contribute to the decrease in f_{rel} (Table 7, column 2). To determine the extent of this contribution f_{rel} was adjusted (f_{adj}) as suggested by Gibaldi,[32] to compensate for intrasubject

variability in elimination rate constant between treatments. The results of this correction are also shown in Table 7 (column 3). This adjustment shows that the seemingly altered bioavailability based on $AUC_{0-\infty}$ ratios (f_{rel} = 0.87) is really not correct and the relative theophylline bioavailability from the aminophylline was not affected by concomitant prednisone (f_{adj} = 1.00, Table 7).

For prednisolone relative bioavailability following prednisone administration alone compared to concomitant administration with aminophylline, the initial estimates were near unity (f_{rel} = 0.98) and had a small range (Table 7, column 4). When the above correction for f_{rel} based on k_{el} variation between treatments was applied to prednisolone data, a small reduction in relative bioavailability was revealed (f_{adj} = 0.93). This reduction, although small, was highly statistically significant ($p < 0.01$).

DISCUSSION AND CONCLUSIONS

The relative bioavailability of theophylline from aminophylline was not statistically significantly affected by concomitant oral prednisone administration. Time to peak concentration, and individual peak concentrations measured were very similar for both treatments. However, there were considerable differences in plasma concentration *vs.* time profiles for theophylline and therefore differences in $AUC_{0-\infty}$ ratios. These differences were due to a variation of the elimination rate of theophylline in the individual subjects which may or may not have been due to the concomitant prednisone. Overall these differences were not statistically significant. The tendency for the elimination of theophylline to increase with concomitant prednisone administration is consistant with an earlier report,[1] but can not necessarly be attributed to prednisone treatment. This is especially true since the range of variability reported here is within the range of intrasubject variability previously reported.[33-35] Since the results do not attain statistical significance, one cannot conclude that concomitant prednisone administration affects the bioavailability or pharmacokinetic parameters of theophylline following administration of aminophylline. Consideration of the trends displayed in this report suggest that a repeat study with higher doses of con-

comitant administration of both drugs as reported earlier might be more revealing. Also, assuming the means and standard deviations of theophylline parameters observed here would hold in similar studies with larger populations, then a doubling of sample size would produce statistical significance in the altered $AUC_{0-\infty}$ ratios ($\alpha = 0.05$, $\beta = 0.03$)[36] without any change in study design. Of course, statistically significant effects do not necessarily mean there will be clinically significant effects. Based on the data herein, one must conclude that prednisone administered concomitantly with aminophylline in the single doses employed does not have clinically significant effects on theophylline bioavailability or pharmacokinetics.

Bioavailability of prednisolone from prednisone was statistically significantly affected by concurrent administration of aminophylline. Peak concentration and $AUC_{0-\infty}$ ratios when adjusted for changes in k_{el} were both statistically significantly depressed. The plasma prednisone concentration relationship to treatment is consistent with decreased absorption of prednisone resulting in decreased bioavailability of prednisolone rather than decreased conversion of prednisone to prednisolone but differences in mean prednisone concentrations were not statistically significant.

Differences observed in prednisolone pharmacokinetic

and bioavailability parameters were small and, in light of the paucity of information on pharmacodynamics of prednisolone, are hard to interpret. Again, as in the case of theophylline, additional study of this situation with higher and/or prolonged doses may yield more information. For the single doses studied, one must conclude that aminophylline administered concomitantly with prednisone does not clinically significantly affect prednisolone bioavailability or pharmacokinetics.

Until the influence which these drugs have on one another is further characterized, alteration of drug therapy when these two agents are administered concomitantly should be done with some caution in case the trends in Table 3 or Table 5 develop in the specific patient being treated.

ENDNOTES

[a]Ideal body weight (males)= 50kg + 2.3kg/inch over 5'

TABLE 1. Treatment Schedule and Doses Administered

Subject No.	Week 1	Week 2	Week 3
1,2	A[a]	B[b]	C[c]
3,4	B	C	A
5,6	C	A	B

[a]200 mg aminophylline (1 x 200 mg Aminophyllin® tablet, Searle, lot #978-121) and 240 ml water.

[b]200 mg aminophylline (1 x 200 mg Aminophyllin® tablet, Searle, lot #978-121) and 20 mg prednisone (4 x 5 mg Deltasone® tablets, Upjohn, lot #465F9) and 240 mg water.

[c]20 mg prednisone (4 x 5 mg Deltasone® tablets, Upjohn, lot #465F9) and 240 mg water.

TABLE 2. Comparison of Plasma Theophylline Concentrations After Administering Aminophylline Alone or With Prednisone

Time (hours)	Mean Theophylline Concentration (mcg/ml) A[a]	B[b]	Significance[c]
0.0	0.0	0.0	--
0.25	1.44 (0.35)[d]	1.51 (0.46)	NS
0.50	3.62 (0.60)	4.41 (0.31)	NS
1.0	5.12 (0.30)	5.07 (0.26)	NS
2.0	4.67 (0.09)	4.53 (0.36)	NS
3.0	4.26 (0.27)	3.75 (0.26)	S
4.0	3.70 (0.20)	3.36 (0.26)	NS
6.0	3.04 (0.16)	2.73 (0.21)	NS
8.0	2.42 (0.18)	2.16 (0.20)	NS
12.0	1.63 (0.11)	1.26 (0.18)	S
18.0	0.78 (0.10)	0.58 (0.15)	NS
24.0	0.44 (0.08)	0.28 (0.06)	NS

[a]Treatment A (Control)

[b]Treatment B (prednisone administered concomitantly)

[c]Results of paired "t" test (S = $p < 0.05$; NS = $p \geq 0.05$)

[d]Numbers in parenthesis represent standard error of the mean

TABLE 3. Comparison of Individual Pharmacokinetic and Bioavailability Parameters for Theophylline When Administered Alone or With Prednisone

	Peak Concentration (mcg/ml)			Time of Peak (hrs)		k_{el} (hr^{-1})[d]			AUC (mcg/ml·hr)[e]		
Subject	A[a]	B[b]	% Change[c]	A	B	A	B	% Change[c]	A	B	% Change[c]
1	4.57	5.48	19.9	2.0	1.0	0.1047	0.1042	- 0.5	53	57	7.6
2	4.74	5.23	10.3	1.0	0.5	0.1305	0.1919	47.1	39	29	-25.6
3	6.30	5.04	-20.0	1.0	1.0	0.1309	0.1076	-17.8	61	45	-26.2
4	4.87	5.85	20.1	1.0	1.0	0.1270	0.1335	5.12	50	53	6.0
5	5.52	3.98	-27.9	0.5	1.0	0.0953	0.1201	26.0	54	40	-25.9
6	5.08	5.33	4.9	2.0	2.0	0.0947	0.1289	36.1	66	56	-15.2
$\bar{x}$[f]	5.18	5.15	- 0.58	1.25	1.08	0.1139	0.1310	15.0	54	47	-12.96
SD[g]	0.64	0.63		0.61	0.49	0.0175	0.0320		9.1	10.7	
Significance[h]	NS			NS		NS			NS		

[a]Treatment A (control), [b]Treatment B (Prednisone administered concomitantly), [c]% Change is calculated: (B-A)/A, [d]Elimination rate constant obtained by computer fitting of data using PROPHET, [e]Area under the curve from time 0 to infinity, [f]Mean, [g]Standard deviation, [h]Based on paired t test (S = $p < 0.05$, NS = $p \geq 0.05$).

TABLE 4. Comparison of Plasma Prednisolone Concentrations After Administration of Prednisone With Aminophylline or Alone

	Mean Prednisolone Concentration (ng/ml)		
Time (hrs)	B[a]	C[b]	Significance[c]
0.0	0.00	0.00	--
0.25	124 (28)[d]	75 (12)	NS
0.50	250 (20)	230 (22)	NS
1.0	298 (14)	349 (13)	S
2.0	284 (12)	307 (17)	S
3.0	273 (21)	264 (21)	NS
4.0	224 (21)	223 (19)	NS
6.0	137 (12)	137 (11)	NS
8.0	82 (9)	80 (4)	NS
12.0	35 (4)	34 (6)	NS

[a]Treatment B (Aminophylline administered concomitantly)

[b]Treatment C (Control)

[c]Results of paired "t" test (S = $p < 0.05$; NS = $p \geq 0.05$)

[d]Numbers in parentheses represent standard error of the mean

TABLE 5. Comparison of Individual Bioavailability and Pharmacokinetic Parameters For Prednisolone After Administering Prednisone With Aminophylline or Alone

	Peak Concentration (mcg/ml)			Time of Peak (hrs)		k_{el} (hr^{-1})[d]			AUC (mcg/ml·hr)[e]		
Subject	B[a]	C[b]	% Change[c]	B	C	B	C	% Change[c]	B	C	% Change[c]
1	301	394	-23.6	2.0	1.0	0.2364	0.2480	- 4.7	1953	2069	- 5.6
2	313	340	- 7.9	1.0	1.0	0.2331	0.2490	- 6.4	1628	1621	- 0.4
3	347	348	- 0.3	3.0	1.0	0.2496	0.2718	- 8.2	2059	2045	0.7
4	350	376	- 6.9	1.0	1.0	0.2414	0.2218	8.8	1916	2168	-11.6
5	288	316	- 8.9	1.0	1.0	0.2020	0.2136	- 5.4	1847	1802	2.5
6	339	376	- 9.8	2.0	2.0	0.2057	0.2282	- 9.9	2471	2446	1.0
$\bar{x}$[f]	323	358	- 9.8	1.7	1.2	0.2280	0.2387	- 4.5	1979	2025	- 2.3
SD[g]	26	29		0.7	0.2	0.0196	0.0215		281	287	
Significance[h]	S			NS		NS			NS		

[a]Treatment B, [b]Treatment C, [c]% Change is calculated: (B-C)/C, [d]Elimination rate constant obtained by computer fitting of data using PROPHET, [e]Area under the curve from time 0 to infinity, [f]Mean, [g]Standard deviation,

[h]Based on paired t test (S = $p < 0.05$, NS = $p \geq 0.05$).

TABLE 6. Comparison of Plasma Prednisone Concentrations Following Administration of Prednisone With Aminophylline or Alone

	Mean Prednisone Concentration (ng/ml)		
Time (hrs)	B[a]	C[b]	Significance[c]
0.0	0.00	0.00	--
0.25	2.1 (2.1)[d]	3.9 (2.9)	NS
0.50	15.5 (4.0)	10.3 (3.9)	NS
1.0	23.9 (6.0)	27.9 (4.6)	NS
2.0	27.0 (5.8)	34.7 (3.3)	NS
3.0	36.6 (2.7)	42.8 (3.7)	NS
4.0	36.7 (4.4)	38.4 (3.0)	NS
6.0	23.4 (3.0)	25.2 (3.3)	NS
8.0	10.3 (4.1)	15.9 (3.4)	NS
12.0	0.9 (0.9)	3.9 (2.5)	NS

[a]Treatment B (Aminophylline administered concomitantly)

[b]Treatment C (Control)

[c]Results of paired "t" test (S = $p < 0.05$; NS = $p \geq 0.05$)

[d]Numbers in parentheses represent standard error of the mean

TABLE 7. Estimation of Relative Bioavailability For Theophylline and Prednisolone (From Prednisone)

	Theophylline		Prednisolone	
Subject	f_{rel}[a]	f_{adj}[b]	f_{rel}	f_{adj}
1	1.08	1.08	0.94	0.90
2	0.74	1.09	1.00	0.94
3	0.74	0.61	1.01	0.93
4	1.06	1.11	0.88	0.96
5	0.74	0.93	1.02	0.96
6	0.85	1.16	1.01	0.91
X[c]	0.87	1.00	0.98	0.93
SD[d]	0.16	0.20	0.06	0.03
Significance[e]	NS	NS	NS	S

[a]Estimate of relative bioavailability $\frac{AUC_T}{AUC_C}$ Where $AUC_T = AUC_{0-\infty}$ for the treatment period and $AUC_C = AUC_{0-\infty}$ for the control period.

[b]Estimate of relative bioavailability adjusted with respect to elimination rate, $f_{rel} \cdot (f_{rel}) \times (k_{el-T}/k_{el-C})$. Where k_{el-T} is k_{el} from the treatment period and k_{el-C} is k_{el} from the control period

[c]Mean

[d]Standard Deviation

[e]Results of two tailed "t" test (S = $p < 0.05$; NS = $p \geq 0.05$)

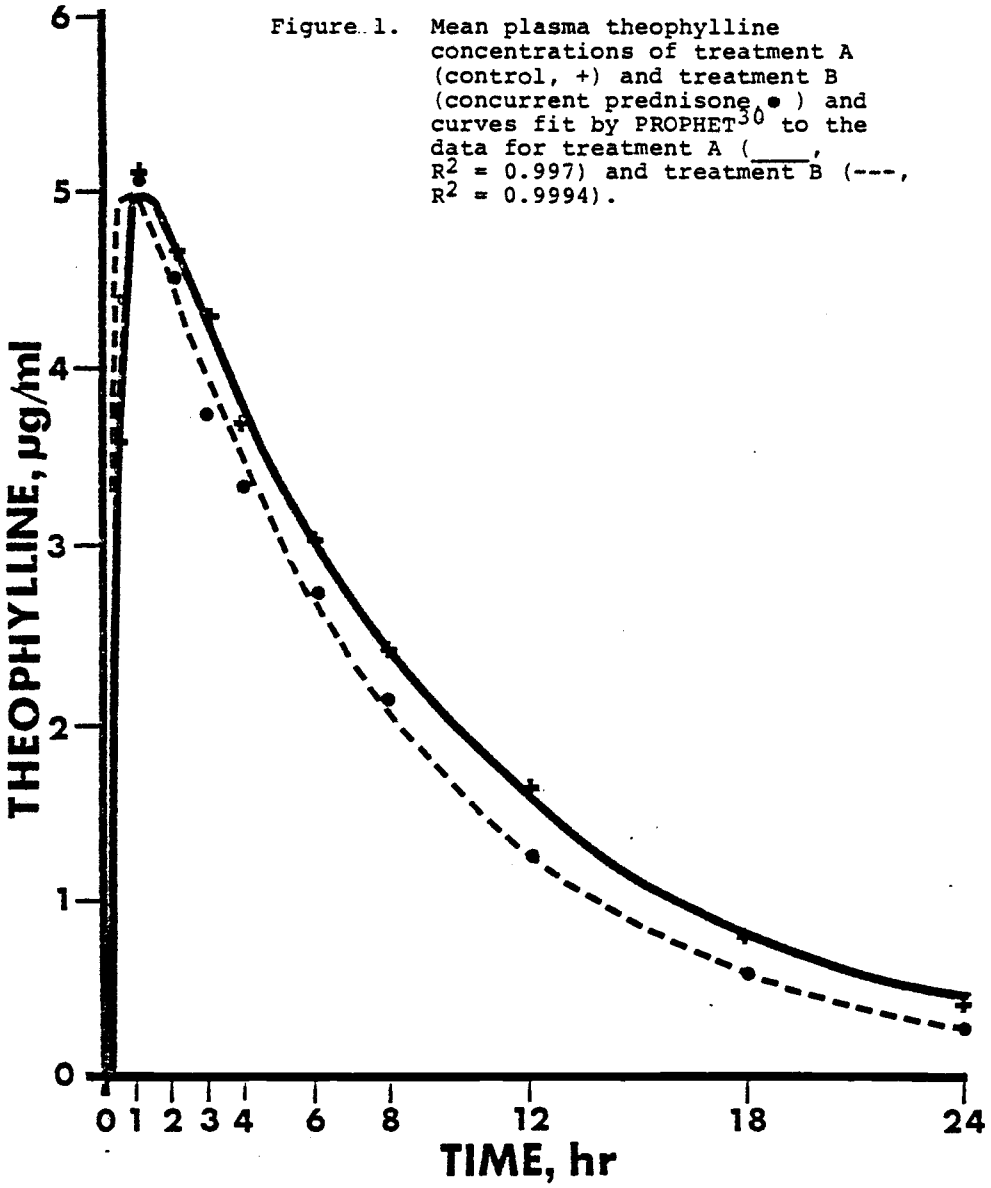

Figure 1. Mean plasma theophylline concentrations of treatment A (control, +) and treatment B (concurrent prednisone, •) and curves fit by PROPHET[30] to the data for treatment A (____, R^2 = 0.997) and treatment B (---, R^2 = 0.9994).

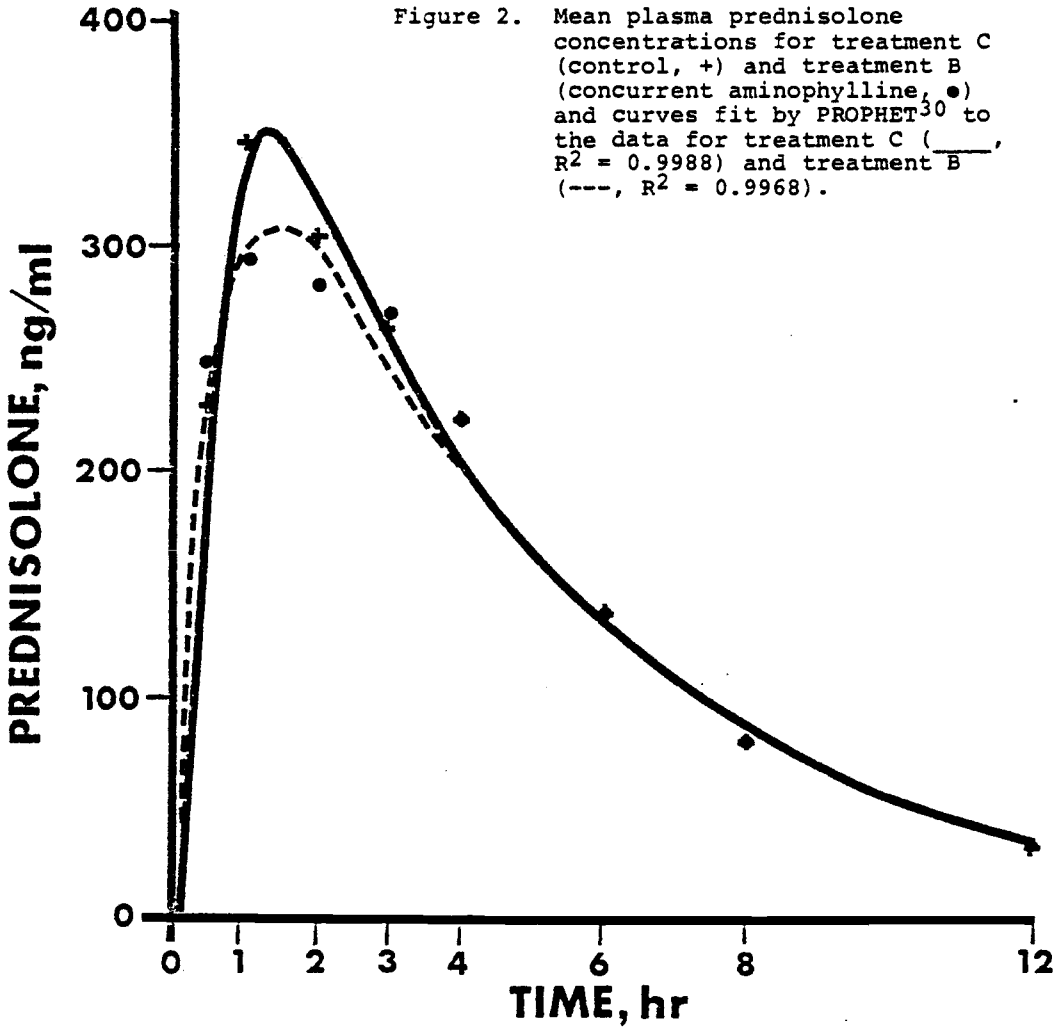

Figure 2. Mean plasma prednisolone concentrations for treatment C (control, +) and treatment B (concurrent aminophylline, •) and curves fit by PROPHET[30] to the data for treatment C (____, R^2 = 0.9988) and treatment B (---, R^2 = 0.9968).

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APPENDICES

APPENDIX I - THEOPHYLLINE ASSAY PROCEDURE

A. INTERNAL STANDARD SOLUTION

β-Hydroxyethyltheophylline (100 mg) is dissolved in 100 ml distilled water. One ml of this solution is transfered to a 100 ml volumetric flask and acetonitrile (chromatographic grade) is added to bring to 100 ml volume. Final concentration = 10 mcg/ml. STORE IN REFRIGERATOR.

B. SAMPLE PREPARATION

1) Vortex each plasma sample 10-15 sec.

2) Combine 0.3 ml plasma with 0.3 ml internal standard solution in pointed centrifuge tube.

3) Vortex 10-15 sec.

4) Centrifuge @ 2000 RPM X 10 min.

5) Separate supernatant from pellet and store on ice until injection.

C. CHROMATOGRAPH SPECIFICATIONS

1) Mobile Phase

a) add 0.82 gm NaOAc to a 1.0 L volumetric flask and qs to 1.0 L with distilled water

b.) adjust pH to 6.6 with HAc or NAOH

c) add 70 ml chromatographic grade acetonitrile to a 1.0 L volumetric flask.

d) qs to 1.0 L using aqueous solution (above)

e) filter and de-gas

Appendix I (con't)

C. (con't)

2) Pump (Waters Assoc. Inc, Model V # M-6000) at 2 ml/min

3) Column/Pre-column

a) Waters C_{18} μ-Bondapak column

b) C_{18} μ-Bondapak pre-column also used.

4) Detector (Waters Assoc Inc. Model 440) = 280 nm

Sensitivity = 0.005

5) Recorder - mV = 10, paper speed = 16 /hr

D. Injection

a) Injector - Waters Assoc Inc, model # UK6

b) Injection volume - 25 μl

APPENDIX II - THEOPHYLLINE STANDARD CURVES[1] (pg 83)

	ACTUAL[2]	DRUG[3]	IS[4]	PHR[5]	INV[6]	%THEO[7]
1	0.42	19	202	0.094	0.46	109.5
2	0.64	29	196	0.148	0.79	124.5
3	0.85	33	205	0.161	0.87	102.7
4	1.06	42	211	0.199	1.10	104.0
5	2.12	65	177	0.367	2.12	100.1
6	4.24	153	211	0.725	4.29	101.3
7	8.47	214	136	1.573	9.44	111.4
8	12.71	195	96	2.031	12.22	96.1
9	16.95	186	66	2.818	16.99	100.3
10	0.42	14	187	0.075	0.35	82.0
11	0.64	22	177	0.124	0.65	101.9
12	0.85	27	180	0.150	0.80	94.8
13	1.06	34	194	0.175	0.96	90.3
14	2.12	63	192	0.328	1.88	89.0
15	4.24	129	188	0.686	4.06	95.8
16	8.47	142	100	1.420	8.51	100.4
17	12.71	188	93	2.022	12.16	95.7
18	16.95	120	42	2.857	17.23	101.7

$\bar{X}$ = 100.1[8]

VAR = 86.4[9]

S.D. = 9.30[10]

CV% = 9.29[11]

FOOTNOTES FOR THEOPHYLLINE STANDARD CURVE

1 Results of regression of PHR on actual:
R^2 = 0.9971; Intercept = 0.017; Slope = 0.165.

2 Actual concentration of sample (mcg/ml)

3 Drug - Height of drug peak on chromatogram (mm)

4 IS - Height of internal standard peak on chromatogram (mm)

5 PHR - Peak Height Ratio (Drug/IS)

6 Inverse - Inversly estimated concentration calculated from PHR and regression parameters by the equation:
Inverse = (PHR - Intercept)/slope

7 %THEO - Percent of theoretical concentration:
(Inverse/Actual)100 = %THEO

8 Mean percent of theoretical concentration

9 Variance of the mean

10 Standard deviation of the mean

11 Coeffecient of variation as a percent of the mean

APPENDIX II (con't)

DETERMINATION OF FACTOR TO ACCOUNT FOR 'AGING' OF THEOPHYLLINE SAMPLES

Approximately one years time separated the freezing of the samples and their final assay for theophylline. To determine what, if any, allowance should be given to account for degradation. The following was done.

5 Spiked plasma samples that had been used in early workup of the standard curve and had been frozen with the subjects samples were reassayed. Their initial labeled concentrations and inversely estimated concentrations are shown below.

	LABEL (mcg/ml)	INVERSE (mcg/ml)	%THEO
1	1	0.77	77.3
2	2	1.52	75.8
3	3	2.51	83.7
4	4	3.33	83.3
5	6	4.91	81.8

$\bar{X}$	80.4
VAR	12.75
S.D.	3.57

APPENDIX II (con't)

Based on this observation a factor of 1/0.80 would be used to scale up theophylline concentrations determined from subjects samples to account for degradation of the drug in samples over time.

Summary:

Plasma theophylline concentrations from subjects samples were determined by the equation:

INVERSE = (PHR - 0.17)/(0.165 x 0.80)

TABLE AII-1: PLASMA THEOPHYLLINE CONCENTRATIONS FOR SUBJECT 1

SAMPLE #	DRUG Pk	ISPk	PHR	INVERSE
TREATMENT A				
1	---	---	---	0
2	54	193	.280	2.12
3	75	167	.449	3.41
4	MISSING			
5	88	146	.603	4.57
6	94	174	.540	4.10
7	85	166	.512	3.88
8	59	148	.399	3.03
9	44	156	.282	2.14
10	40	171	.230	1.78
11	27	189	.143	1.08
12	11	199	.055	0.42
TREATMENT B				
1	---	---	---	0
2	71	151	.470	3.57
3	99	166	.596	4.52
4	104	144	.722	5.48
5	103	170	.606	4.60
6	77	142	.542	4.11
7	99	200	.495	3.76
8	75	176	.426	3.23
9	54	156	.346	2.63
10	37	158	.234	1.78
11	24	161	.149	1.13
12	10	176	.057	0.43

TABLE AII-2: PLASMA THEOPHYLLINE CONCENTRATIONS FOR SUBJECT 2

SAMPLE #	DRUG Pk	ISPk	PHR	INVERSE
TREATMENT A				
1	---	---	---	0
2	9	140	.064	0.49
3	49	159	.308	2.34
4	110	176	.625	4.74
5	91	156	.583	4.42
6	74	156	.474	3.60
7	67	160	.419	3.18
8	51	161	.317	2.01
9	MISSING			
10	26	167	.156	1.18
11	10	164	.061	0.46
12	5	138	.036	0.27
TREATMENT B				
1	---	---	---	0
2	21	146	.144	1.09
3	93	135	.689	5.23
4	87	137	.635	4.82
5	67	134	.500	3.79
6	78	185	.422	3.20
7	48	131	.366	2.78
8	36	133	.271	2.06
9	26	145	.179	1.36
10	12	161	.075	0.57
11	---	---	---	0
12	---	---	---	0

TABLE AII-3: PLASMA THEOPHYLLINE CONCENTRATIONS FOR SUBJECT 3

SAMPLE #	DRUG Pk	ISPk	PHR	INVERSE
TREATMENT A				
1	---	---	---	0
2	46	157	.292	2.21
3	83	133	.624	4.73
4	142	171	.830	6.30
5	79	129	.612	4.64
6	123	175	.702	5.33
7	98	168	.583	4.42
8	70	164	.426	3.23
9	61	152	.401	3.04
10	38	169	.224	1.70
11	12	145	.082	0.02
12	---	---	---	0
TREATMENT B				
1	---	---	---	0
2	7	179	.039	0.30
3	69	158	.437	3.32
4	101	152	.665	5.05
5	94	153	.614	4.66
6	MISSING			
7	48	140	.343	2.60
8	42	141	.298	2.26
9	41	165	.249	1.89
10	27	194	.139	1.05
11	15	168	.089	0.68
12	7	132	.053	0.40

TABLE AII-4: PLASMA THEOPHYLLINE CONCENTRATIONS FOR SUBJECT 4

SAMPLE #	DRUG Pk	ISPk	PHR	INVERSE
TREATMENT A				
1	---	---	---	0
2	---	---	---	0
3	44	207	.212	1.61
4	117	182	.642	4.87
5	108	174	.620	4.70
6	102	183	.557	4.23
7	85	187	.454	3.44
8	65	174	.373	2.83
9	52	181	.287	2.18
10	36	189	.190	1.44
11	16	197	.081	.61
12	8	215	.037	.28
TREATMENT B				
1	---	---	---	0
2	39	164	.238	1.81
3	117	169	.692	5.25
4	131	170	.771	5.85
5	118	161	.733	5.56
6	78	162	.481	3.65
7	83	161	.516	3.91
8	65	159	.409	3.18
9	51	152	.336	2.55
10	20	145	.136	1.05
11	13	181	.072	0.55
12	8	198	.040	0.30

TABLE AII-5: PLASMA THEOPHYLLINE CONCENTRATIONS FOR SUBJECT 5

SAMPLE #	DRUG Pk	ISPk	PHR	INVERSE
TREATMENT A				
1	---	---	---	0
2	37	173	.213	1.62
3	91	125	.728	5.52
4	80	127	.629	4.77
5	82	135	.607	4.61
6	80	168	.476	3.61
7	62	144	.430	3.26
8	74	168	.440	3.34
9	45	157	.286	2.17
10	36	150	.240	1.82
11	17	155	.109	0.83
12	13	162	.080	0.61
TREATMENT B				
1	---	---	---	0
2	28	155	.180	1.37
3	73	140	.521	3.95
4	72	137	.525	3.98
5	61	143	.426	3.23
6	61	142	.429	3.25
7	58	146	.397	3.01
8	60	182	.329	2.50
9	49	178	.275	2.09
10	30	155	.193	1.46
11	11	165	.066	0.50
12	5	151	.033	0.25

TABLE AII-6: PLASMA THEOPHYLLINE CONCENTRATIONS FOR SUBJECT 6

SAMPLE #	DRUG Pk	ISPk	PHR	INVERSE
TREATMENT A				
1	---	---	---	0
2	20	190	.105	0.80
3	101	186	.543	4.12
4	115	178	.646	4.90
5	128	191	.670	5.08
6	109	175	.622	4.72
7	105	200	.525	3.98
8	73	161	.453	3.44
9	66	193	.342	2.59
10	40	165	.242	1.84
11	25	182	.137	1.04
12	14	169	.083	0.63
TREATMENT B				
1	---	---	---	0
2	25	195	.128	0.97
3	106	191	.555	4.21
4	118	171	.690	5.23
5	116	165	.703	5.33
6	110	184	.598	4.54
7	97	180	.539	4.09
8	78	187	.417	3.16
9	59	183	.322	2.44
10	34	159	.214	1.62
11	13	158	.082	0.62
12	7	169	.041	0.31

APPENDIX III. STEROID ASSAY PROCEDURE

A. Internal Standard Solution
Methylene Chloride containing β-hydroxyethyltheophylline in a concentration of 400 ng/ml.

B. Sample Preparation
1) Vortex each plasma sample 10 - 15 sec
2) Combine 2.4 ml plasma with 8 ml internal standard in a 50 ml erlenmyer flask
3) Shake gently for 15 min
4) Transfer to centrifuge tube and spin @ 2000 RPM x 10 min
5) Aspirate top layer (aqueous and particulate)
6) Transfer 6.4 ml of organic solution to a pointed centrifuge tube with screw cap tops.
7) Evaporate to dryness under N_2 stream (gently warm tips of centrifuge tubes in water bath @ 50°C)
8) Reconstitute with 50 µl mobile phase
9) Tightly cap tubes and wrap with parafilm and store on ice until injection

C. Chromatograph Specification
1) Mobile phase - mix

Glacial Acetic Acid	2.0 ml
Ethanol	50.0 ml
Methylene Chloride	300.0 ml
Hexane	qs 1.0 l

filter and de-gas
place mobile phase reservoir on a magnetic stirrer and keep a bar spinning slowly in the solution

2) Pump (Water Assoc Inc, Model # M6000) 3 ml/mn
3) Column/precolumn - normal phase µ-porasil columns
4) Detector - (Waters Associate Inc, Model # 440) = 254 Sensitivity = 0.005
5) Recorder - MV = 10, paper speed = 8"/hr

D. Injection
1) Injection Waters Assoc Inc, Model # UK6
2) Injection volume = 25 µl

E. Glassware Preparation
All glassware were prepared for use by soaking in No-Chro-Mix® according to manufacturers recommended procedures

APPENDIX IV PREDNISONE STANDARD CURVE[1]

Actual[2]	Drug[3]	IS[4]	PHR[5]	INV[6]	%THEO[7]	INV_p[16]	$\%THEO_p$[17]
20.2	8.5	66	0.1288	16.3	80.66	34.8	172.0
30.3	8.5	42	0.2024	39.4	130.2	54.2	179.1
70.	18.0	66	0.2727	61.5	87.05	72.9	103.1
90.9	26.0	74	0.3514	86.3	94.93	93.8	103.2
101.0	26.0	61	0.4262	109.8	108.73	113.6	113.5

$\bar{X}$ = 100.31[8]
Var = 388.8[9]
S dev = 19.7[10]
CV% = 19.6[11]

20.2	5	78	0.0641	19.71	97.59	17.6	87.1
30.3	9	82	0.1096	30.27	99.89	29	97.9
50.5	20	98	0.2041	52.19	103.34	54.7	108.4
70.7	20	72	0.2778	69.28	97.99	74.3	105.1
101.0	37	8	0.4157	101.27	100.27	110.8	109.8

$\bar{X}$ = 99.8[12]
Var = 5.23
S dev = 2.29
CV% = 2.29

25.25	11	101	0.1089	27.60	109.31	29.5	116.7
50.48	25	143	0.1748	44.03	87.22	47.0	93.0
75.72	34.5	10	0.3255	81.59	107.75	86.9	114.8
100.96	42	16	0.3962	99.21	98.27	105.7	104.7

$\bar{X}$ = 100.86[13]
Var = 103.8
S dev = 10.1
CV% = 11.04

APPENDIX IV (con't)

Actual[2]	Drug[3]	IS[4]	PHR[5]	INV[6]	%THEO[7]	INV_p[16]	$\%THEO_p$[17]
30.3	7	125	0.0560	32.20	106.27	15.44	50.96
40.4	13	135	0.0963	41.96	103.86	26.13	64.68
50.5	16	122	0.1311	50.38	99.78	35.36	70.02
60.6	21	126	0.1667	59.01	97.37	44.80	73.93
70.7	28	130	0.2154	70.80	100.14	57.72	81.64
80.8	30	133	0.2256	73.27	90.68	60.34	74.79
101.0	27	74	0.3649	106.99	105.94	97.38	96.41

$\bar{X}$ = 100.58[14]
Var = 30.17
S dev = 5.49
CV% = 5.46

19.54	6.5	82	0.0793	19.43	99.45	21.62	110.65
29.31	13.0	22	0.1066	26.60	90.74	28.86	98.47
39.08	16.0	12	0.1429	36.12	92.43	38.49	98.49
48.85	24.0	23	0.1951	49.84	102.03	52.34	107.14
58.62	23.0	93	0.2473	63.54	108.39	66.18	112.90

$\bar{X}$ = 98.61[15]
Var = 52.04
S dev = 7.21
CV% = 7.31

STATISTICS OF POOLED CURVE

$\bar{X}$ = 101.82[18]
Var = 757.35
S dev = 27.52
CV% = 27.1

FOOTNOTES FOR APPENDIX IV

1. See footnotes 8,12,13,14 for individual regression parameters and 17 for pooled regression parameters

2. Actual prednisone concentration of sample (ng/ml)

3. Drug - Height of drug peak on chromatogram (mm)

4. IS - Height of Internal Standard Peak (mm)

5. PHR - Peak Height Ratio (DRUG/IS)

6. Inverse - Inversely estimated concentration

7. % THEO Percent of theoretical concentration: (Inverse/Actual)100 = %THEO

8. $\bar{X}$ - Mean %THEO based on regression analysis of 5 points.

   Results of regression of PHR on actual: $R^2 = 0.9486$; Intercept = 0.07699; Slope = 0.00318

9. VAR - Variance of the mean

10. SDEV - Standard deviation of the mean

11. CV% - Coeffecient of variation as a percent of the mean

12. $\bar{X}$ - Mean %THEO based on regression analysis of 5 points.
    Results of regression of PHR on actual:
    $R^2 = 0.9988$; Intercept = $^{-}0.0289$; Slope = 0.004311

13. $\bar{X}$ - Mean %THEO based on regression analysis of 4 points.
    Results of regression of PHR on actual:
    $R^2 = 0.9741$; Intercept = $^{-}0.0018$; Slope = 0.00401

14. $\bar{X}$ - Mean %THEO based on regression analysis of 7 points.
    Results of regression of PHR on actual:
    $R^2 = 0.9723$; Intercept = $^{-}0.0770$; Slope = 0.00413

15. $\bar{X}$ - Mean %THEO based on regression analysis of 5 points.
    Results of regression of PHR on actual:
    $R^2 = 0.9849$; Intercept = 0.0052; Slope = 0.00381

16. INV_p - Inversly estimated concentrations based on pooled standard curve

17. $\%THEO_p$ - Percent of the theoretical concentrations for pooled standard curve

18. $\bar{X}$ - Mean %THEO based on regression analysis of 26 points.

    Results of regression of PHR on actual:
    $R^2 = 0.9277$; intercept = $^{-}0.00221$;
    Slope = 0.00377

TABLE AIV-1 INDIVIDUAL PLASMA PREDNISONE CONCENTRATIONS FOR SUBJECT 1

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	96	---	0
2	(3)	94	---	0
3	10	119	0.0840	22.87
4	9	107	0.0841	22.89
5	11	98.5	0.1117	30.22
6	15	112	0.1339	36.10
7	14	111.5	0.1256	33.92
8	10	131.5	0.0760	20.75
9	---	---	---	0
10	---	---	---	0
11	---	---	---	0
12	---	---	---	0
Treatment C				
1	0	108	---	0
2	7	148	0.0473	13.13
3	12	128	0.0938	24.47
4	21	119	0.1765	47.40
5	23	129	0.1783	47.88
6	---	---	---	MISSING
7	23	122	0.1885	50.59
8	17	120	0.1417	38.17
9	12	121	0.0992	26.80
10	7	114	0.0614	16.87
11	6	137	0.0438	12.20
12	8	114	0.0702	19.21

TABLE AIV-2 INDIVIDUAL PLASMA PREDNISONE CONCENTRATIONS FOR SUBJECT 2

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	98	---	0
2	0	101	---	0
3	8	96.5	0.0829	22.58
4	19	110.5	0.1719	46.18
5	20	131	0.1527	41.09
6	24	135	0.1778	47.74
7	20	115	0.0739	46.71
8	11	130	0.0846	23.03
9	5	118	0.0424	11.83
10	0	115	---	0
11	0	110	---	0
12	0	100	---	0
TREATMENT C				
1	0	110	---	0
2	4	110	0.0364	10.24
3	6	141	0.04255	11.87
4	9	110	0.0818	22.28
5	15	112	0.1339	36.10
6	20	117	0.1709	45.92
7	16	99.5	0.1608	43.24
8	8	91	0.0879	23.90
9	3	110	0.0273	7.82
10	0	120	---	0
11	0	105	---	0
12	0	110	---	0

TABLE AIV-3 INDIVIDUAL PLASMA PREDNISONE CONCENTRATIONS FOR SUBJECT 3

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	115	---	0
2	0	94	---	0
3	4	125	0.0320	9.07
4	9	109	0.0826	22.50
5	12	100	0.1200	32.42
6	14	98	0.1429	38.49
7	16	85	0.1882	50.51
8	11	86	0.1279	34.52
9	10	127	0.0787	21.46
10	2	114	---	0
11	0	108	---	0
12	0	93	---	0
Treatment C				
1	0	62	---	0
2	0	125.5	---	0
3	0	113	---	0
4	10	115.5	0.0866	23.56
5	13	124	0.1048	28.39
6	7.5	67	0.1119	30.28
7	16	127	0.1260	34.01
8	10	91	0.1099	29.74
9	5	115	0.0435	12.13
10	0	145	---	0
11	0	113	---	0
12	0	130	---	0

TABLE AIV-4 INDIVIDUAL PLASMA PREDNISONE CONCENTRATIONS FOR SUBJECT 4

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	128	---	0
2	5	113	0.0442	12.31
3	12	133	0.0902	24.51
4	12	127	0.0945	25.65
5	14	154.5	0.0906	21.62
6	14	116.5	0.1207	32.46
7	13	122	0.1066	28.85
8	7	138	0.0507	14.05
9	3	140.5	0.0214	6.25
10	0	100	---	0
11	0	133	---	0
12	0	103	---	0
Treatment C				
1	0	133	---	0
2	0	121	---	0
3	4	117.5	0.0340	9.61
4	13	134	0.0970	26.32
5	16	139	0.1151	31.12
6	15	121	0.1240	33.48
7	18.5	143	0.1296	34.96
8	12	128	0.0938	25.47
9	12	132	0.0909	24.70
10	0	127	---	0
11	0	---	---	0
12	0	113	---	0

TABLE AIV-5 INDIVIDUAL PLASMA PREDNISONE CONCENTRATIONS FOR SUBJECT 5

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	162	---	0
2	0	112.5	---	0
3	6	118	0.0508	14.07
4	12	125	0.0960	26.05
5	14	112	0.1250	33.73
6	18	133	0.1353	36.48
7	11	138	0.0797	21.73
8	9	130	0.0692	18.94
9	0	106	---	0
10	0	116	---	0
11	0	111	---	0
12	0	112	---	0
Treatment C				
1	0	119	---	0
2	0	113	---	0
3	6	113.5	0.0529	14.62
4	13	106	0.1227	33.13
5	14	98	0.1429	38.49
6	25.5	130	0.1962	52.63
7	7	110	0.0636	29.70*
8	5	99	0.0505	10.71*
9	6.5	135	0.0481	7.23*
10	0	98	---	0
11	---	---	---	0
12	0	222	---	0

* Calculated by separate standard curve

TABLE AIV-6 INDIVIDUAL PLASMA PREDNISONE CONCENTRATIONS FOR SUBJECT 6

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	149	---	0
2	0	125	---	0
3	0	123	---	0
4	0	183	---	0
5	0	118	---	0
6	15	145	0.1034	28.01
7	9	63	0.1429	38.48
8	7	65	0.1077	29.15
9	8	97	0.0825	22.47
10	3	159	0.0189	5.60
11	0	113	---	0
12	0	163	---	0
Treatment C				
1	0	106	---	0
2	0	139	---	0
3	0	128	---	0
4	6	114	0.0526	14.55
5	13	136	0.0956	25.94
6	23	141	0.1631	43.85
7	27	144	0.1875	50.32
8	18	138	0.1304	35.18
9	12	121	0.0992	26.89
10	5	124	0.0403	11.28
11	---	127	---	0
12	---	132	---	0

APPENDIX V PREDNISOLONE STANDARD CURVE[1]

Actual[2]	Drug[3]	IS[4]	PHR[5]	Inverse[6]	%THEO[7]
402.66	31	40	0.7750	404	100.23
604.00	82	66	1.2424	642	106.30
805.33	118	74	1.5946	822	102.04
1016.66	125	61	2.0492	1054	103.64
62.90	10.5	101	0.1040	61	97.34
251.60	67	143	0.4685	247	98.25
629.00	138.5	106	1.3066	675	107.28
1006.40	199	106	1.8774	966	95.99
100.66	21	131	0.1603	90	89.36
151.00	37	125	0.2960	159	105.42
201.33	50	135	0.3704	197	97.92
251.67	51	122	0.4180	221	87.98
302.00	66	126	0.5238	275	91.19
503.33	122	130	0.9385	487	96.75
704.67	173	133	1.3008	672	95.34
1006.67	137	74	1.8514	953	94.64
97.74	15	82	0.1829	101	103.83
146.12	30	122	0.2459	134	91.45
194.80	36	112	0.3214	172	88.37
243.80	40	93	0.4301	228	93.36
292.20	60	123	0.4878	257	87.97

$\bar{X}$	=	96.88[8]
VAR	=	38.81[9]
SD	=	6.23[10]
CV%	=	6.43[11]

1- Results of regression of PHR on actual:
R^2 = 0.9946; Intercept = 0.016; Slope = 0.00196

2- Actual concentration of sample (ng/ml)

3- 11 See Appendix II

PREDONISOLONE STANDARD CURVE (con't)

Subject 5 TxB samples 7-12.

These samples were not treated correctly during the extraction phase. The result was that a double volume of internal standard solution was added. A separate standard curve was constructed to analyze these points.

PREDNISOLONE STANDARD CURVE (2x IS)[1]

Actual[2]	Drug[3]	IS[4]	PHR[5]	Inverse[6]	%THEO[7]
40.26	6.0	194	0.0392	33.63	83.52
80.53	8.0	110	0.07272	96.01	119.22
161.06	26	235	0.11063	152.60	94.75
302.00	51	238	0.21428	307.30	101.75

$\bar{X} = 99.81$[8]

1) Results of regression or PHR on actual:
$R^2 = 0.9955$; Intercept = 0.00839; Slope = 0.00067

2) Actual concentration of sample (ng/ml)

3-8) See Appendix II

TABLE: AV-1 INDIVIDUAL PLASMA PREDNISOLONE CONCENTRATIONS FOR SUBJECT 1

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	96	0	0
2	39	94	0.4149	219.85
3	61.5	119	0.5168	271.84
4	59	107	0.5514	289.49
5	56.5	985	0.5736	300.81
6	58	112	0.5179	272.40
7	42	111.5	0.3767	200.36
8	33	131.5	0.2510	136.22
9	---	---	---	MISSING
10	3	96	0.0313	24.13
11	--	---	---	0
12	---	---	---	0
Treatment C				
1	0	108	---	0
2	11	148	0.0743	46.07
3	60.5	128	0.4727	249.34
4	90	119	0.7563	394.03
5	77	129	0.5969	312.74
6	---	---	---	MISSING
7	53	122	0.4344	229.80
8	32.5	120	0.2708	146.33
9	19	121	0.1570	88.27
10	4.5	114	0.0395	28.32
11	2	137	---	0
12	0	114	---	0

TABLE: AV-2 INDIVIDUAL PLASMA PREDNISOLONE CONCENTRATIONS FOR SUBJECT 2

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	98	---	0
2	28	101	0.2772	149.59
3	56.5	96.5	0.5855	306.89
4	66	110.5	0.5973	312.91
5	65	131	0.4962	261.33
6	53	135	0.3926	208.50
7	35	115	0.3043	163.42
8	22	130	0.1692	94.49
9	12	118	0.1017	60.05
10	4	115	0.0348	25.92
11	---	110	---	0
12	---	100	---	0
Treatment C				
1	0	110	---	0
2	11	110	0.1000	59.18
3	55	141	0.3901	207.19
4	71.5	110	0.6500	339.80
5	58	112	0.5179	272.40
6	51	117	0.4359	230.56
7	31	99.5	0.3116	167.14
8	16	91	0.1758	97.86
9	13	110	0.1182	68.47
10	3.5	120	---	0
11	0	105	---	0
12	0	110	---	0

TABLE: AV-3 INDIVIDUAL PLASMA PREDNISOLONE CONCENTRATIONS FOR SUBJECT 3

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	115	---	0
2	4	94	0.0426	29.90
3	40	125	0.3200	171.43
4	50	109	0.4587	242.19
5	53	100	0.5300	278.57
6	65	98	0.6633	346.58
7	46	85	0.5412	284.29
8	23	86	0.2674	144.59
9	19.5	127	0.1535	86.48
10	6	114	0.0526	35.00
11	0	108	---	0
12	0	93	----	0
Treatment C				
1	0	62	---	0
2	15	125.5	0.1195	69.13
3	30	113	0.2655	143.62
4	77	115.5	0.6667	348.32
5	76.5	124	0.6169	322.91
6	37	67	0.5522	289.90
7	56	127	0.4409	233.11
8	23	91	0.2527	137.09
9	14.5	115	0.1261	72.5
10	6	145	0.0414	29.29
11	3	113	0.0265	21.68
12	0	130	---	0

TABLE: AV-4 INDIVIDUAL PLASMA PREDNISOLONE CONCENTRATIONS FOR SUBJECT 4

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	128	---	0
2	33.5	113	0.2965	159.44
3	59.5	133	0.4474	236.43
4	85	127	0.6693	349.64
5	77	154.5	0.4984	262.45
6	57.5	116.5	0.4936	260.00
7	49	122	0.4016	213.06
8	36	138	0.2609	141.28
9	18	140.5	0.1281	73.52
10	4	100	0.0400	28.57
11	0	133	---	0
12	0	103	---	0
Treatment C				
1	0	133	---	0
2	12	121	0.0992	58.78
3	47	117.5	0.4000	212.25
4	96.5	134	0.7201	375.51
5	80	139	0.5755	301.79
6	58	121	0.4793	252.70
7	58	143	0.4056	215.10
8	32	128	0.2500	135.71
9	20	132	0.1515	85.46
10	---	127	---	0
11	---	---	---	0
12	---	---	---	0

TABLE: AV-5 INDIVIDUAL PLASMA PREDNISOLONE CONCENTRATIONS FOR SUBJECT 5

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	162	---	0
2	25	112.5	0.2222	121.53
3	63.5	118	0.5381	282.70
4	68.5	125	0.5480	287.76
5	56	112	0.5000	263.27
6	58	133	0.4361	230.66
7	49	138	0.3551	189.34
8	29	130	0.2231	121.99
9	14	106	0.1321	75.56
10	6	116	0.0517	34.54
11	0	111	---	0
12	0	112	---	0
Treatment C				
1	0	119	---	0
2	27	113	0.2389	130.05
3	62	113.5	0.5463	286.89
4	64	106	0.6037	316.17
5	47.5	98	0.4847	256.58
6	53	130	0.4077	216.17
7	15	110	0.1364	191.06*
8	9	99	0.0909	123.15*
9	7.5	135	0.0556	70.46*
10	0	98	---	0
11	0	---	---	0
12	0	---	---	0

* Calculated by separate standard curve

TABLE: AV-6 INDIVIDUAL PLASMA PREDNISOLONE CONCENTRATIONS FOR SUBJECT 6

Sample #	Drug PK	IS Pk	PHR	Inverse
Treatment B				
1	0	149	---	0
2	14	125	0.1120	65.31
3	53	123	0.4309	228.01
4	106	183	0.5792	303.67
5	76	118	0.6441	336.79
6	88	145	0.6069	317.81
7	35	63	0.5556	291.63
8	22	65	0.3385	180.87
9	20	97	0.2062	113.37
10	13	159	0.0818	49.90
11	0	113	---	0
12	0	163	---	0
Treatment C				
1	0	106	---	0
2	21	139	0.1511	85.26
3	68	128	0.5313	279.23
4	70	114	0.6140	321.43
5	98	136	0.7206	375.82
6	89	141	0.6312	330.20
7	83	144	0.5764	302.25
8	47	138	0.3406	181.94
9	20	121	0.1653	92.50
10	9	124	0.0726	45.20
11	0	127	---	0
12	---	132	---	0