CHAPTER III

RESULTS AND DISCUSSION

Part 1 Analytical Method for Theophylline

1. The Investigation for Optimum UV Detector Wavelength

As shown in Figure 1 and 2, both of theophylline aqueous solution and IS_1 solution in methanol exhibited a maximum absorption at wavelength 272 nm. with the absorptivity of 638 and 450, respectively. The internal standard spectrum has another maximum absorption at 205 nm. which is in the UV cut off region of methanol. Therefore the UV determination wavelength for HPLC analysis in this study was adjusted to 272 nm.

2. The Internal Standard Selection

Three chemical compounds which were studied as the internal standard for theophylline analysis were β-Hydroxyethyl theophylline (IS_1), β-Hydroxypropyltheophylline (IS_2) and 8-Chlorotheophylline (IS_3). From Figure 3A., good resolution was clearly detected between theophylline and each internal standard studied. The retention times for theophylline, IS_1,IS_2 and IS_3 were approximately 6.45, 7.47, 10.91, and 11.81 minutes, respectively. Figure 3B. demonstrated the

Figure 1 The UV spectrum of theophylline aqueous solution

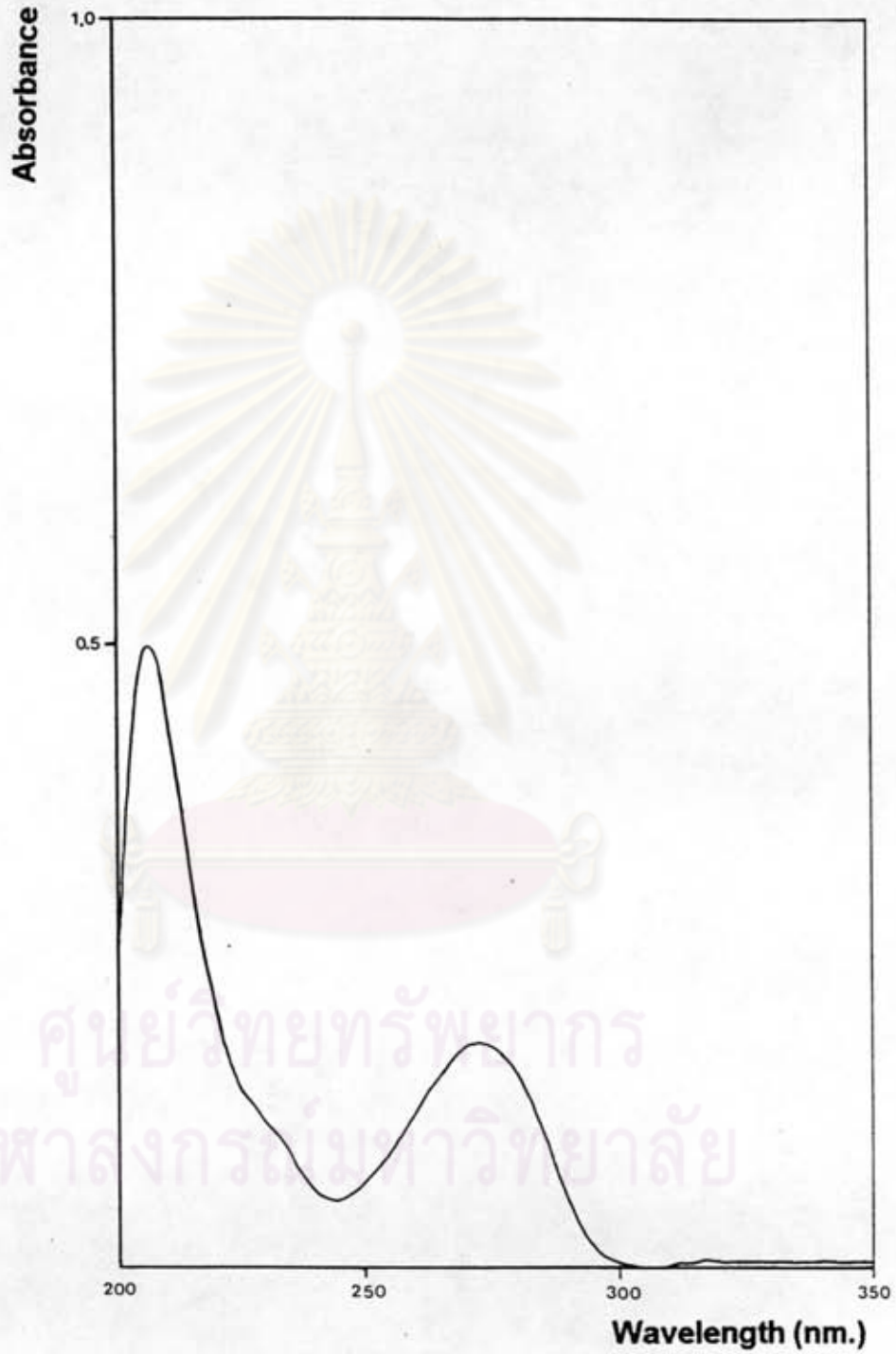

Figure 2 The UV spectrum of β-Hydroxyethyltheophylline (IS) in methanol

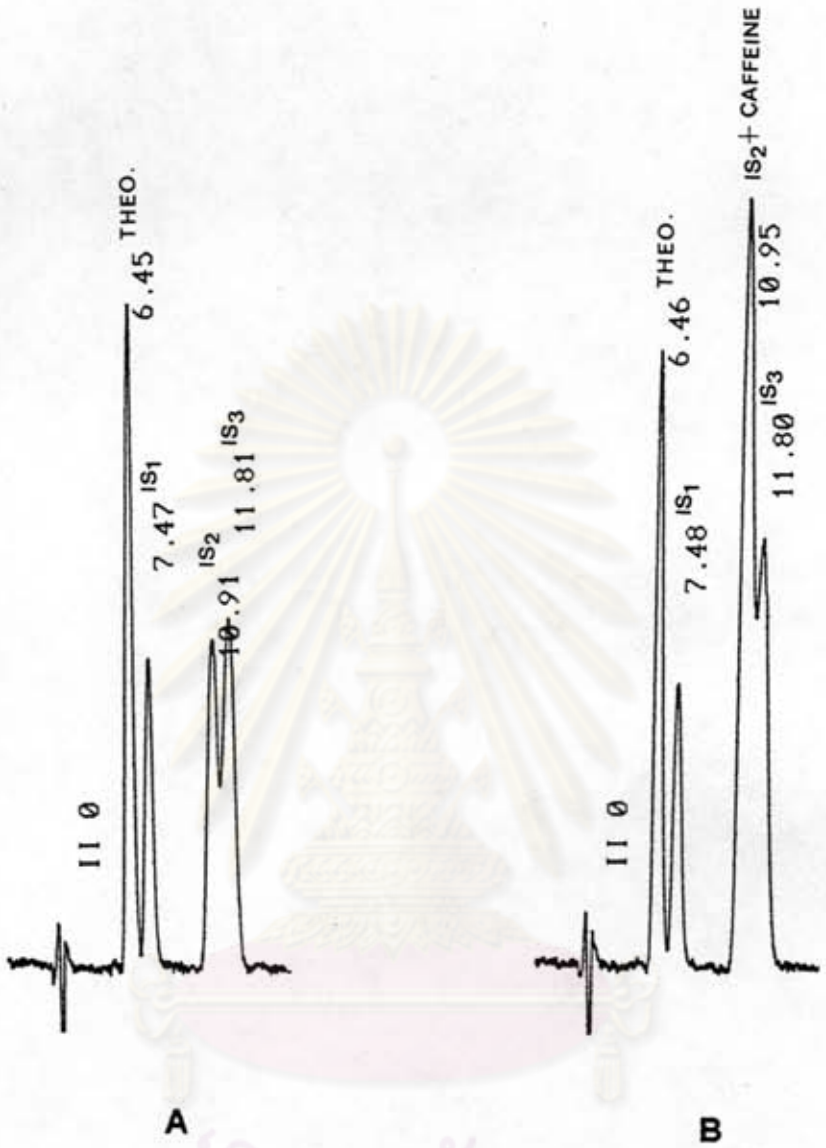

Figure 3 Chromatograms for the internal standard selection

A. Chromatogram of theophylline and its homologues (IS_1, IS_2, and IS_3)

B. Chromatogram of theophylline, its homologues (IS_1, IS_2, and IS_3) and caffeine

(Analytical procedure and HPLC condition refer ed to page 18-19)

concentration of theophylline = 0.5 mg./ml.

concentration of IS_1, IS_2, IS_3 = 0.35 mg./ml

concentration of caffeine = 0.4 mg./ml.

contamination of caffeine with the IS_2 in which both were co-eluted at 10.95 minutes.

Since IS_3 was eluted with rather long retention time compared to IS_1, so IS_1 would be more benificial for this theophylline analysis. Therefore, in all three compounds studied, the most appropriate IS selected for theophylline analysis would be β-Hydroxyethyltheophylline (IS_1).

3. Validation of the Analytical Method

3.1 Linearity

The plasma and saliva calibration curves for theophylline were linear over the concentration ranges of 0.0-20.0 mcg./ml. and 0.0-10.0 mcg./ml., respectively. These wide analytical ranges are sufficient in covering the usual theophylline concentration detected following drug administration. The linear regression equation correlated the peak area ratio (PAR) of theophylline to plasma theophylline concentration was created to be:

$$y = 0.1548\,x - 0.003616, \quad r = 0.9999 \qquad (1)$$

and for theophylline concentration in saliva, the equation was created to be:

$$y = 0.3586\,x - 0.02117, \quad r = 0.9994 \qquad (2)$$

where y represents peak area ratio (PAR) of theophylline to IS and x represents theophylline concentration in plasma or saliva.

The calibration curves provided from the regression were shown in Figure 4 and 5, respectively.

3.2 Lower Limit of Detection (LLD)

The lowest concentration of theophylline to be detected by the UV detector of HPLC at 272 nm. was determined to be 200 ng./ml. in either plasma or saliva media. At this concentration, the signal per noise ratios (S/N) of spiked theophylline in plasma and in saliva experimented in ten-replicated samples were shown in Table 2. With the S/N mean values for plasma and saliva as 2.57 $\pm$ 0.21; %CV=8.05 and 2.62 $\pm$ 0.24; %CV=9.01 respectively, this concentration value is quantified to be the LLD of theophylline both in plasma and in saliva for this analytical method.

3.3 Specificity

The chromatograms of theophylline and IS_1 in various types of solution were shown in Figure 6 and 7, respectively. The specificity of the analytical method was clearly revealed in which the retention time of theophylline in standard solution or spiked plasma, spiked saliva or volunteer's plasma, volunteer's saliva or patient's plasma and patient's saliva were all approximately 6.2-6.4 minutes. In the

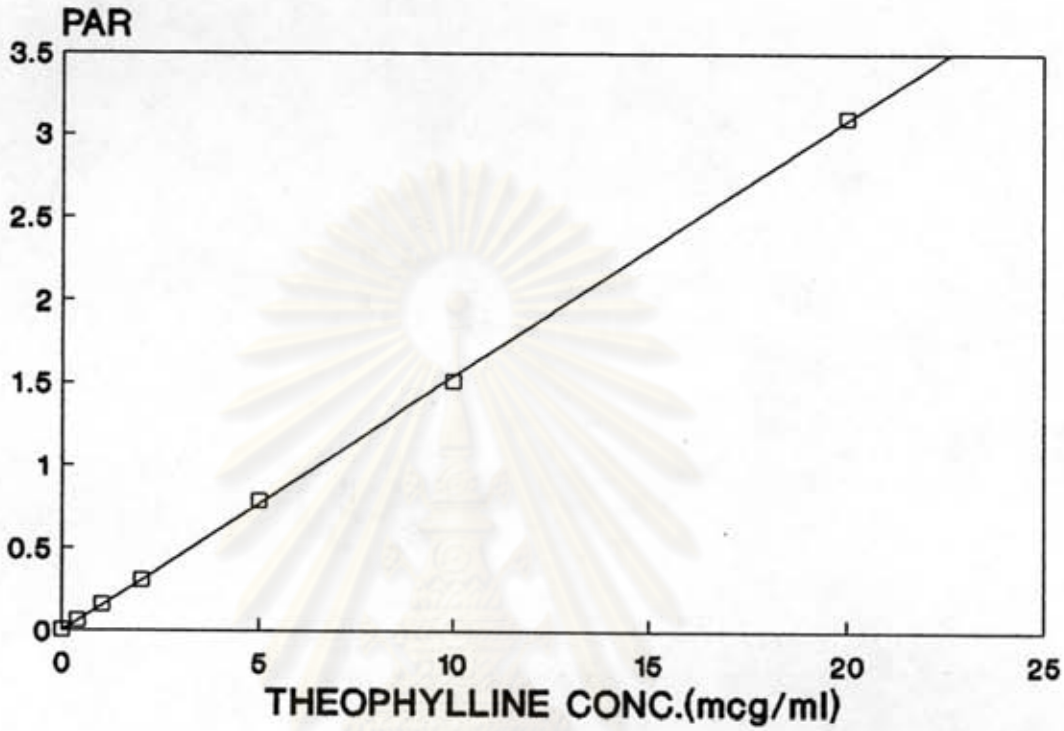

Figure 4 Calibration curve of spiked theophylline in plasma

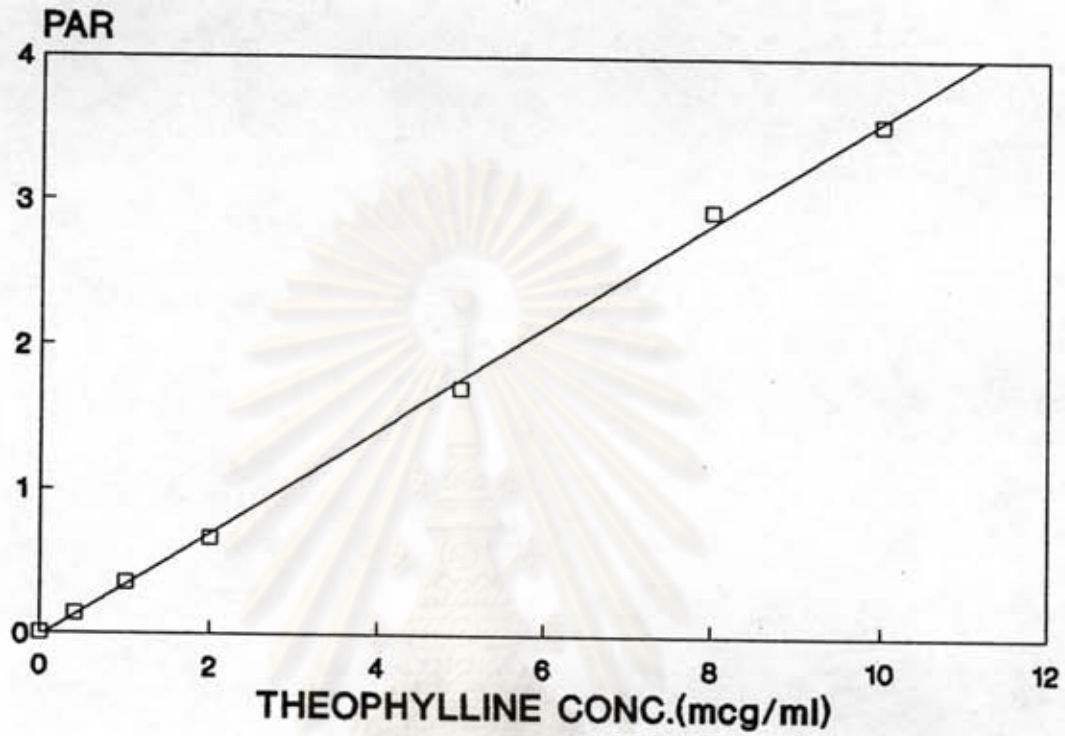

Figure 5 Calibration curve of spiked theophylline in saliva

Table 2 Signal to noise ratio (S/N) of spiked theophylline in plasma and saliva at theophylline concentration of 200 ng/ml

NUMBER	S/N (PLASMA)	S/N (SALIVA)
1	2.75	2.25
2	2.75	2.62
3	2.88	2.88
4	2.62	2.62
5	2.38	2.50
6	2.48	2.38
7	2.38	2.50
8	2.25	2.62
9	2.48	3.00
10	2.75	2.88
$\bar{X}$ (S.D)	2.57 (0.21)	2.62 (0.24)
% C.V.	8.05	9.01

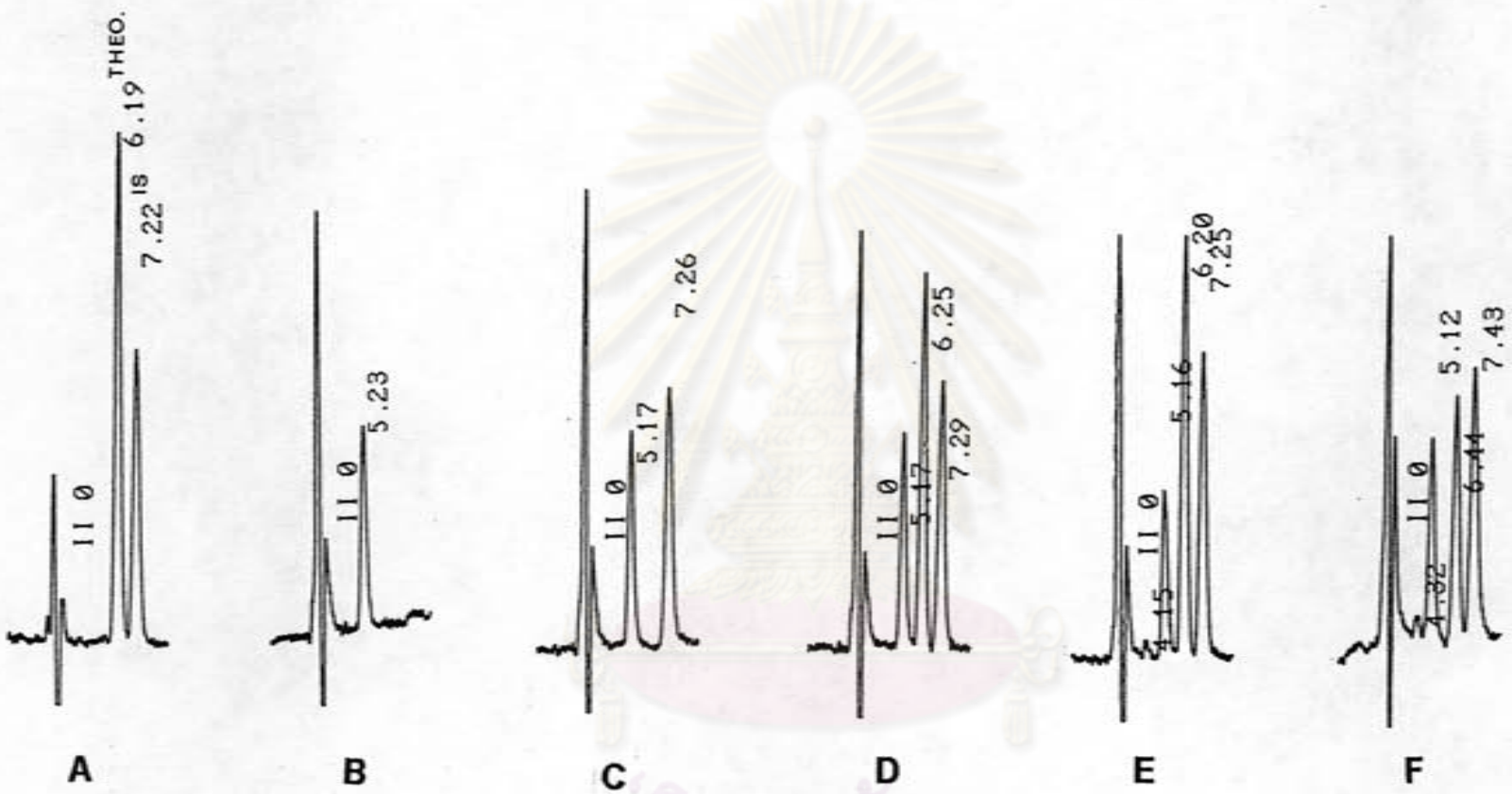

Figure 6 Chromatogram showed the specificity of the analytical method for theophylline in plasma
A. Typical chromatogram of theophylline and IS in standard solution at concentration of 10.0 and 4.0 mcg/ml, respectively
B, C. Typical chromatogram of blank plasma and blank plasma with IS
D. Typical chromatogram of spiked theophylline (10.0 mcg/ml) and IS in plasma
E, F. Typical chromatogram of theophylline and IS from volunteer and patient plasma at theophylline concentration of 10.70 and 4.724 mcg/ml, respectively

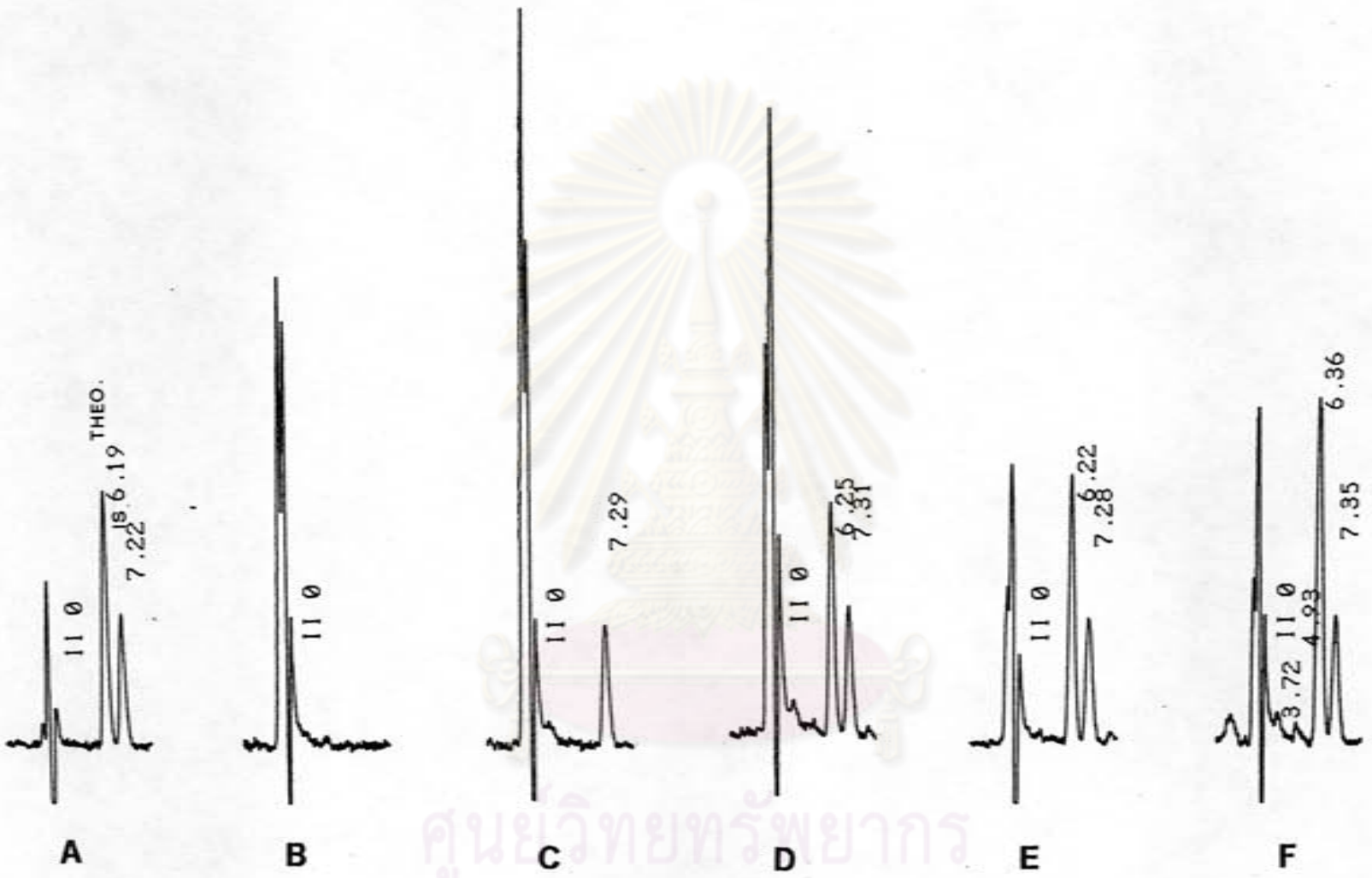

Figure 7 Chromatogram showed the specificity of the analytical method for theophylline in saliva
- A. Typical chromatogram of theophylline and IS in standard solution at concentration of 5.0 and 2.0 mcg/ml, respectively
- B, C. Typical chromatogram of blank saliva and blank saliva with IS
- D. Typical chromatogram of spiked theophylline (5.0 mcg/ml) and IS in saliva
- E, F. Typical chromatogram of theophylline and IS from volunteer and patient saliva at thephylline concentration of 5.265 and 7.091 mcg/ml, respectively

addition, the retention time for IS_1 in aforementioned mixture was approximately 7.2-7.4 minutes. Both theophylline and IS_1 were well resolved at their specified retention times without any observed interference or endogenous peak.

3.4 Accuracy

3.4.1 Physical Recovery

As established in Table 3 and 4, the percentage physical recoveries covering the concentration range for theophylline and its IS in plasma were ranged from 91.54 to 100.13% and 95.36 to 101.32%, respectively. Accordingly, for theophylline and IS_1 in saliva, the percentage physical recoveries were ranged from 92.33 to 102.46% and 97.78 to 103.06%, respectively, as demonstrated in Table 5 and 6. The physical recovery values of both theophylline and its IS compounds appeared to be independent of theophylline concentration , indicating the efficient seperation as well as stability of both compounds in plasma and saliva during the sample preparation procedure. Therefore, the grand total mean values of the percentage physical recovery for theophylline and IS_1 in plasma and saliva can be calculated to be 95.62 $\pm$ 5.56% and 99.07 $\pm$ 6.49% for theophylline in plasma and saliva , 99.09 $\pm$ 5.19% and 99.98 $\pm$ 4.13% for IS_1 in plasma and saliva, respectively.

Table 3 Physical recoveries of theophylline in plasma

conc.* (mcg/ml)	1	2	3	4	5	$\bar{X}$	S.D.	%C.V.
0.4	97.02	83.45	92.33	101.25	83.67	91.54	7.94	8.67
1.0	104.75	92.23	95.25	104.21	104.21	100.13	5.93	5.92
2.0	97.95	98.17	86.10	89.32	91.75	92.66	5.32	5.74
5.0	93.84	91.06	99.54	95.77	99.10	95.86	3.58	3.73
10.0	100.43	98.54	94.62	97.96	97.54	97.82	2.10	2.15
20.0	93.20	97.60	100.38	89.78	97.43	95.68	4.18	4.37
			TOTAL	MEAN		95.62	5.56	5.81

* concentration of theophylline in plasma

Table 4 Physical recoveries of β-Hydroxyethyltheophylline (IS_1) in plasma

conc.* (mcg/ml)	1	2	3	4	5	$\bar{X}$	S.D.	%C.V.
0.4	97.40	109.05	101.29	101.48	97.37	101.32	4.76	4.70
1.0	93.33	101.46	106.50	98.39	100.44	100.02	4.79	4.79
2.0	99.43	100.66	94.98	94.66	100.45	98.04	2.97	3.03
5.0	100.47	104.04	100.66	89.87	100.49	99.11	5.38	5.43
10.0	103.60	92.91	107.42	98.16	101.40	100.70	5.50	5.46
20.0	96.28	85.35	105.68	94.60	94.91	95.36	7.22	7.57
TOTAL MEAN						99.09	5.19	5.24

* concentration of theophylline in plasma

Table 5 Physical recoveries of theophylline in saliva

conc.* (mcg/ml)	1	2	3	4	5	$\bar{X}$	S.D.	%C.V.
0.4	99.64	81.13	90.95	93.19	96.76	92.33	7.09	7.68
1.0	102.65	99.76	89.21	84.35	105.56	96.31	9.10	9.45
2.0	96.40	96.74	96.40	108.00	104.39	100.39	5.46	5.44
5.0	94.64	102.30	104.99	103.31	107.08	102.46	4.73	4.62
8.0	95.44	99.55	103.03	106.10	103.78	101.58	4.16	4.10
10.0	98.30	103.55	101.34	103.59	100.11	101.38	2.27	2.24
TOTAL MEAN						99.07	6.49	6.55

* concentration of theophylline in saliva

Table 6 Physical recoveries of β- Hydroxyethyltheophylline (IS_1) in saliva

conc.* (mcg/ml)	1	2	3	4	5	$\bar{X}$	S.D.	%C.V.
0.4	96.73	96.09	101.36	96.25	110.40	100.17	6.12	6.11
1.0	104.24	98.85	102.90	95.63	100.79	100.48	3.40	3.38
2.0	96.12	97.04	99.40	99.10	99.77	98.29	1.61	1.64
5.0	98.40	97.56	100.07	104.04	100.53	100.12	2.50	2.50
8.0	93.65	97.38	104.91	98.48	94.48	97.78	4.46	4.56
10.0	96.31	100.66	103.45	109.18	105.72	103.06	4.90	4.75
TOTAL MEAN						99.98	4.13	4.13

* concentration of theophylline in saliva

3.4.2 Analytical Recovery

The analytical recoveries of theophylline determination in plasma and saliva were shown in Table 7 and 8. The mean percentage analytical recoveries at the low, medium and high concentration range for theophylline in plasma and saliva were 99.82 ± 2.31 and 96.60 ± 3.79, respectively. More than 95% of the analytical recoveries of both plasma and saliva reveals the exactness of the analytical method in theophylline analysis. Since in general, the overall recovery of the assay in biologic sample for varying concentrations of added authentic analyte taken through the entire procedure should be at least 75-80 %. (Silva,1985)

3.5 Precision

The percentage coefficient of variation (%CV) of the within-run precision(n=3) for theophylline in plasma and saliva as shown in Table 9 and 10 were in the range of 1.10 - 6.32 and 0.29 - 7.60, respectively. For the between-run precision (n=6) within four months as shown in table 11 and 12, %CV of theophylline in plasma and saliva were in the range of 3.58 - 8.00 and 5.10 - 10.26, respectively. With the %C.V. values, they veried randomly over the concentration range and not more than ± 15% which was suggested by US.FDA. (Shah et. al., 1992) Consequently, this analytical method should reveal the precise theophylline concentration not matter whenever the samples were analyzed.

Table 7 Analytical recoveries of theophylline in plasma

added conc.* (mcg/ml)	analytical conc. (mcg/ml)	% analytical recovery			
	n=3	n=3	$\bar{X}$	S.D.	%C.V.
0.400	0.389	97.25			
0.400	0.389	97.25	99.58	4.04	4.06
0.400	0.417	104.25			
5.00	4.98	99.60			
5.00	4.89	97.80	99.53	1.70	1.71
5.00	5.06	101.20			
20.0	20.3	101.50			
20.0	19.8	99.00	100.33	1.26	1.26
20.0	20.1	100.50			
	TOTAL	MEAN	99.82	2.31	2.31

* concentration of theophylline in plasma

Table 8 Analytical recoveries of theophylline in saliva

added conc.* (mcg/ml)	analytical conc. (mcg/ml)	% analytical recovery			
	n=3	n=3	$\bar{X}$	S.D.	%C.V.
0.400	0.375	93.75			
0.400	0.369	92.25	94.00	1.89	2.01
0.400	0.384	96.00			
5.00	4.81	96.20			
5.00	4.64	92.80	94.47	1.70	1.80
5.00	4.72	94.40			
10.0	10.2	102.00			
10.0	10.1	101.00	101.33	0.58	0.57
10.0	10.1	101.00			
	TOTAL	MEAN	96.60	3.79	3.92

* concentration of theophylline in saliva

Table 9 Within-run precision for theophylline in plasma (n=3)

conc.* (mcg/ml)	PAR 1	PAR 2	PAR 3	$\bar{X}$	S.D.	%C.V.
0.4	0.0562	0.0588	0.0547	0.0566	0.0017	3.00
1.0	0.1411	0.1516	0.1454	0.1460	0.0053	3.63
2.0	0.3008	0.3156	0.3159	0.3108	0.0086	2.77
5.0	0.8566	0.8429	0.8609	0.8535	0.0094	1.10
10.0	1.5844	1.5687	1.5273	1.5601	0.0295	1.89
20.0	2.7706	3.1052	3.0904	2.9887	0.1890	6.32

* concentration of theophylline in plasma

Table 10 Within-run precision for theophylline in saliva (n=3)

conc.* (mcg/ml)	PAR 1	PAR 2	PAR 3	$\bar{X}$	S.D.	%C.V.
0.4	0.1191	0.1382	0.1333	0.1302	0.0099	7.60
1.0	0.4030	0.3670	0.3489	0.3730	0.0275	7.37
2.0	0.7244	0.7188	0.6640	0.7024	0.0334	4.67
5.0	1.6955	1.6484	1.7059	1.6833	0.0306	1.82
8.0	2.7411	3.0082	2.9314	2.8936	0.1375	4.75
10.0	3.5564	3.5414	3.5364	3.5447	0.0104	0.29

* concentration of theophylline in saliva

Table 11 Between-run precision for theophylline in plasma (n=6)

conc.*mcg/ml	PAR 1	PAR 2	PAR 3	PAR 4	PAR 5	PAR 6	$\bar{X}$	S.D.	%CV
0.4	0.0502	0.0536	0.0597	0.0506	0.0550	0.0558	0.0542	0.0035	6.46
1.0	0.1586	0.1463	0.1338	0.1566	0.1517	0.1546	0.1503	0.0091	6.05
2.0	0.2873	0.2809	0.2955	0.2760	0.2983	0.3024	0.2901	0.0104	3.58
5.0	0.6631	0.7966	0.7380	0.7902	0.8408	0.7907	0.7699	0.0616	8.00
10.0	1.4359	1.6135	1.4344	1.5431	1.5233	1.5120	1.5104	0.0681	4.51
20.0	3.1001	3.2133	2.9116	2.7984	3.1245	3.1042	3.0420	0.1548	5.09

*concentration of theophylline in plasma

Table 12 Between-run precision for theophylline in saliva (n=6)

conc.* (mcg/ml)	PAR 1	PAR 2	PAR 3	PAR 4	PAR 5	PAR 6	$\bar{X}$	S.D.	%C.V
0.4	0.1038	0.1125	0.1018	0.1162	0.1222	0.1333	0.1150	0.0118	10.26
1.0	0.3517	0.3276	0.2979	0.2911	0.2917	0.3489	0.3182	0.0283	8.89
2.0	0.6755	0.6980	0.6884	0.6656	0.5696	0.6640	0.6602	0.0463	7.01
5.0	1.7829	1.9532	1.5283	1.5503	1.5821	1.7059	1.6838	0.1644	9.76
8.0	2.6812	3.1554	2.8024	2.4790	2.6146	2.9314	2.7773	0.2417	8.70
10.0	3.6969	3.7654	3.2410	3.6190	3.5704	3.5364	3.5715	0.1822	5.10

* concentration of theophylline in saliva

The lower limit of quantitation of the method was determined to be the lowest concentration on the calibration curve showing an acceptable percentage of coefficient of variation in the within-run and between-run analysis. Therefore, the lower limit of quantitation for theophylline in both plasma and saliva was 0.4 mcg./ml., based on 500 mcl. of samples

Part 2. Experiment in Volunteers

The plasma and saliva theophylline concentration -time profiles for each subject after single oral administration of theophylline tablets in the dose of 5 mg./kg. were graphically shown from Figure 8 to 43. (Individual data of theophylline concentration in plasma and saliva were tabulated in Appendix B and C, respectively) According to the dose used in this study that is the usual recommended dose for bronchial asthma therapy (Piafsky and Ogilvie, 1975;Rall, 1985), none of the subject showed any sign of adverse reaction form the drug.

Peak Theophylline Concentration and Time to Reach Peak Concentration

The individual maximum or peak concentration of theophylline (Cmax) from either plasma or saliva profile was seperately tabulated for male and female subjects concomitantly with its time values in Table 13 and 14, respectivity. The mean peak plasma theophylline concentration for male and female subjects were 10.23 $\pm$ 1.56 and 12.47 $\pm$ 2.08 mcg./ml. with the time values (Tmax) of 1.8 $\pm$ 0.9 and 1.8 $\pm$ 0.9 hour, respectively. Meanwhile, the mean peak saliva theophylline concentration for male and female subjects were 6.03 $\pm$ 0.83 and 7.70 $\pm$ 1.65 mcg/ml with the time values (Tmax) of 1.9 $\pm$ 1.2 and 1.6 $\pm$ 1.0 hour, respectively.

By Student's unpaired-t-test (α=0.05), the Cmax value of females' plasma theophylline concentration showed the statistically significant higher than the Cmax value from males (p = 0.001) as shown in Table

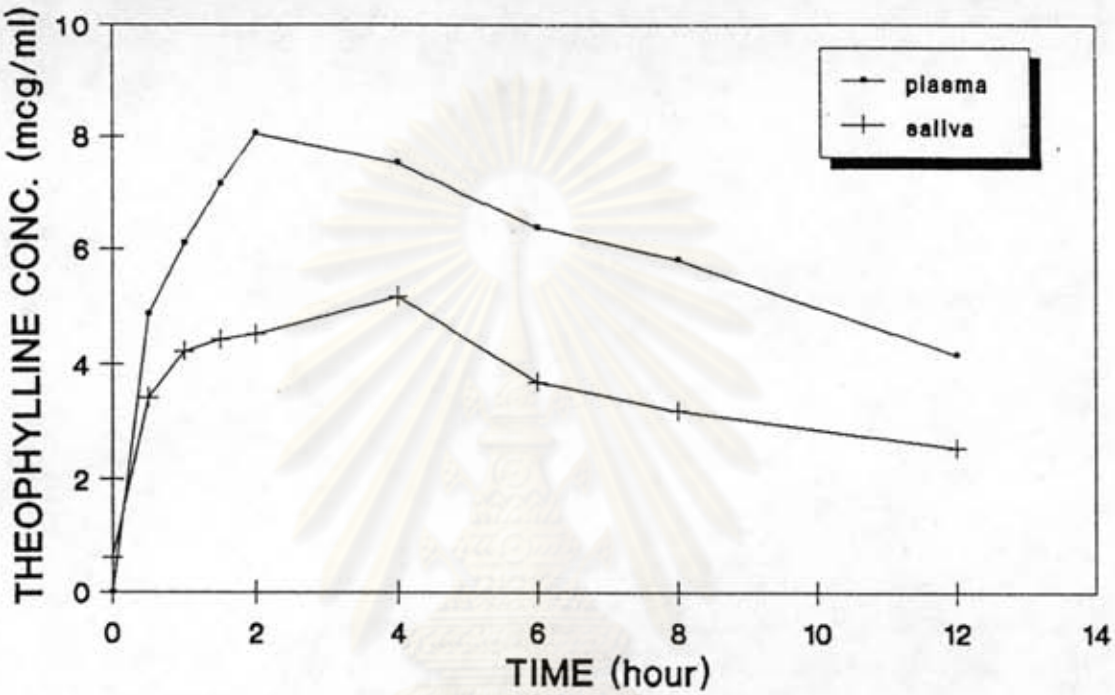

Figure 8 **The plasma and saliva theophylline concentration-time profile of subject No.1**

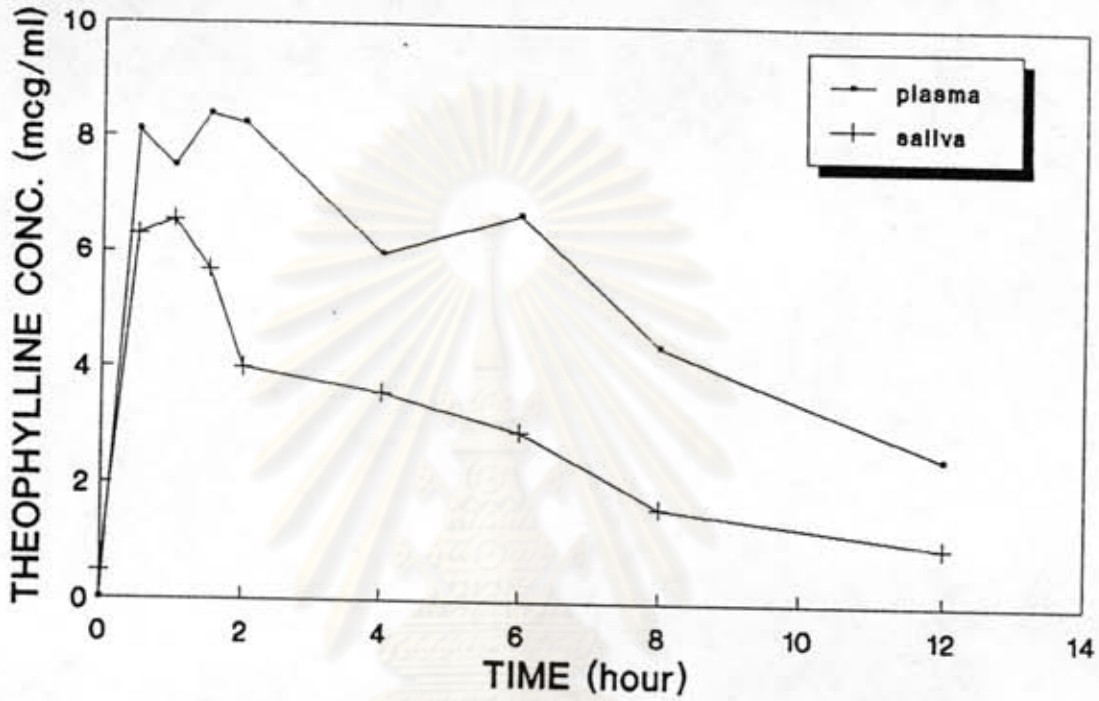

Figure 9 The plasma and saliva theophylline concentration-time profile of subject No.2

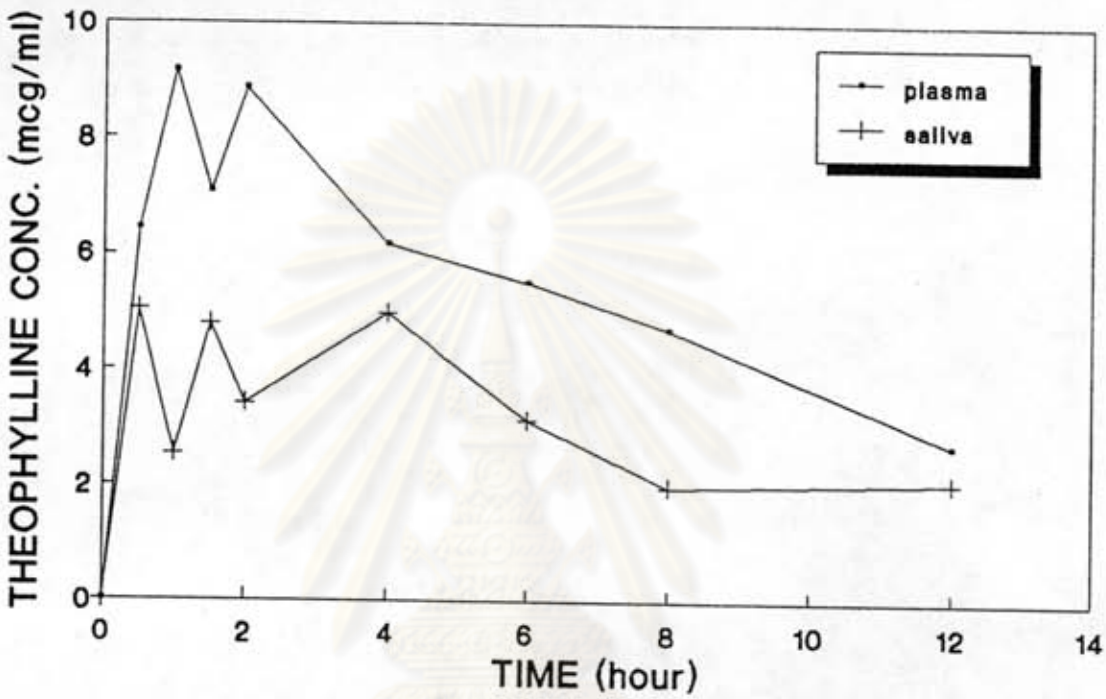

Figure 10 The plasma and saliva theophylline concentration-time profile of subject No.3

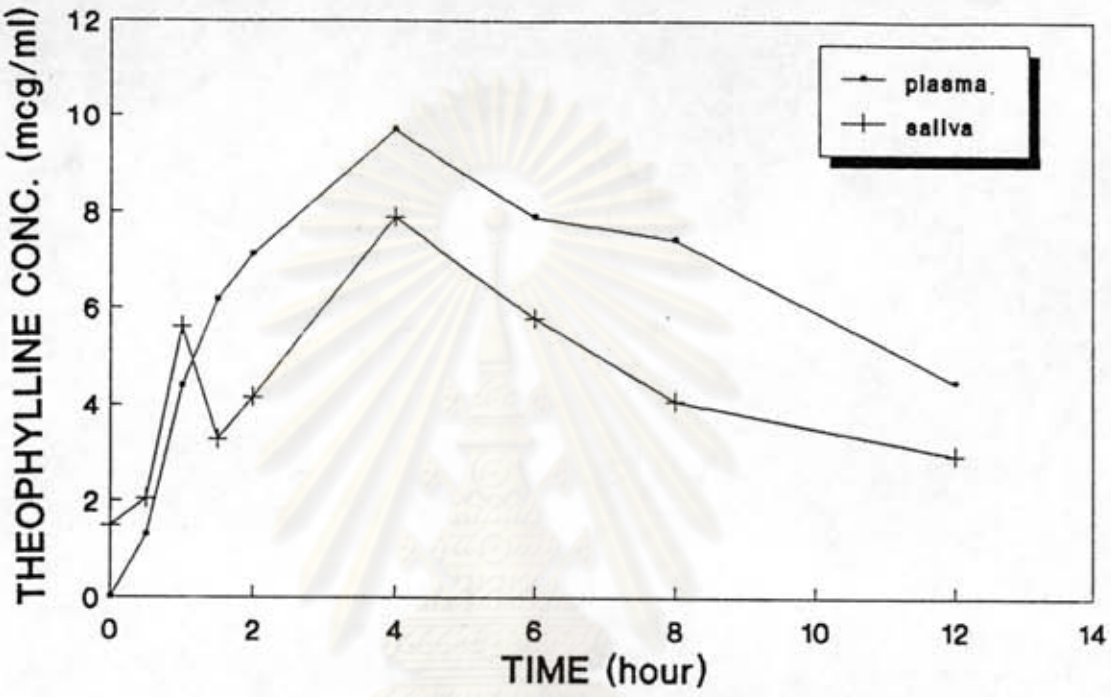

Figure 11 The plasma and saliva theophylline concentration-time profile of subject No.4

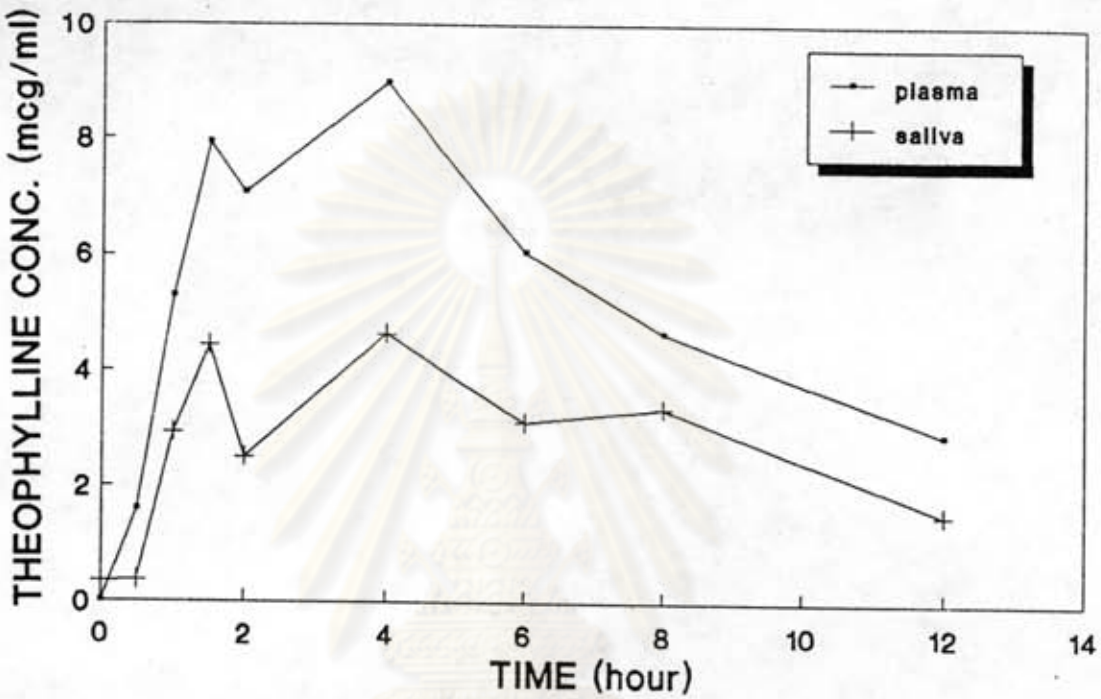

Figure 12 The plasma and saliva theophylline concentration-time profile of subject No.5

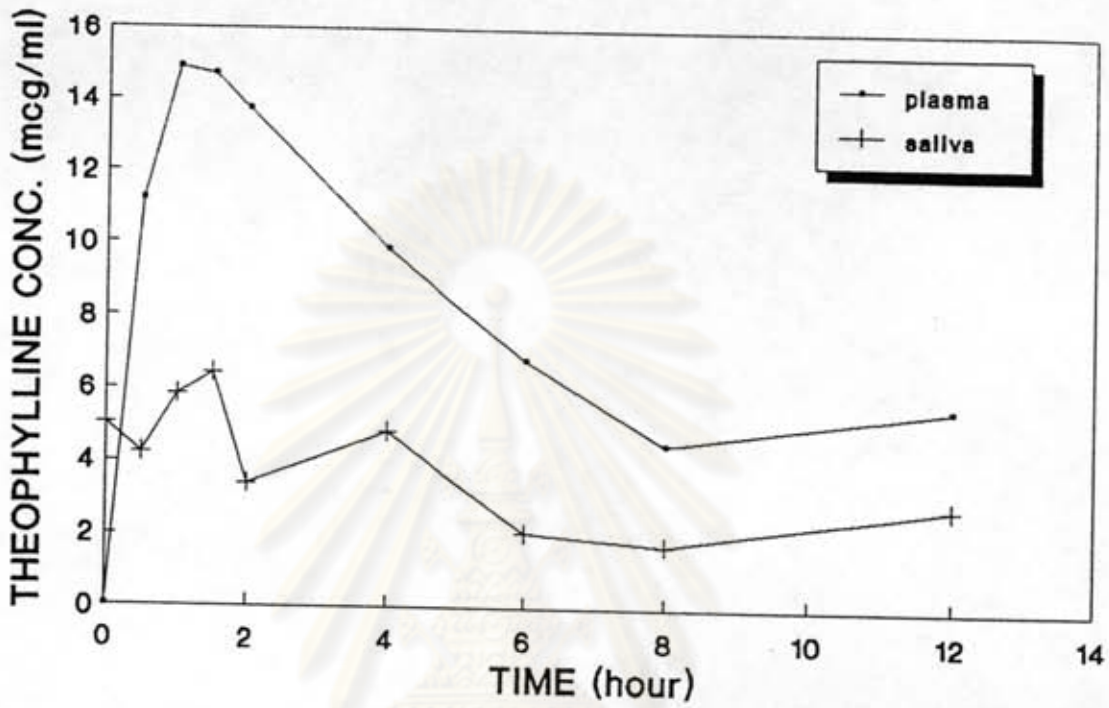

Figure 13 The plasma and saliva theophylline concentration-time profile of subject No.6

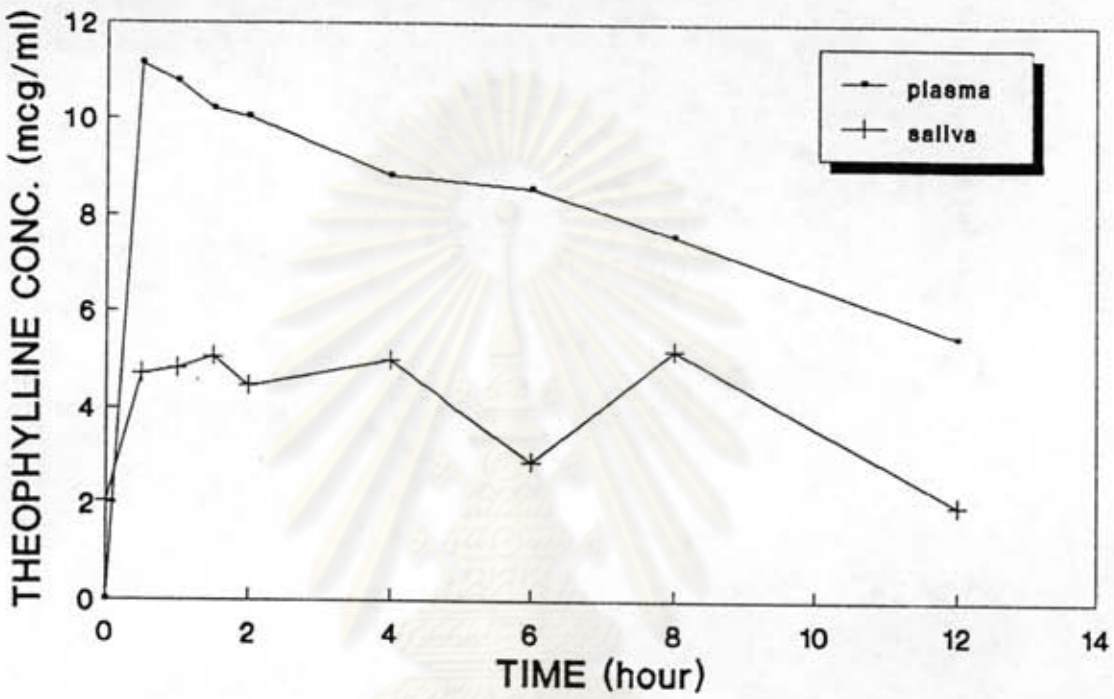

Figure 14 **The plasma and saliva theophylline concentration-time profile of subject No.7**

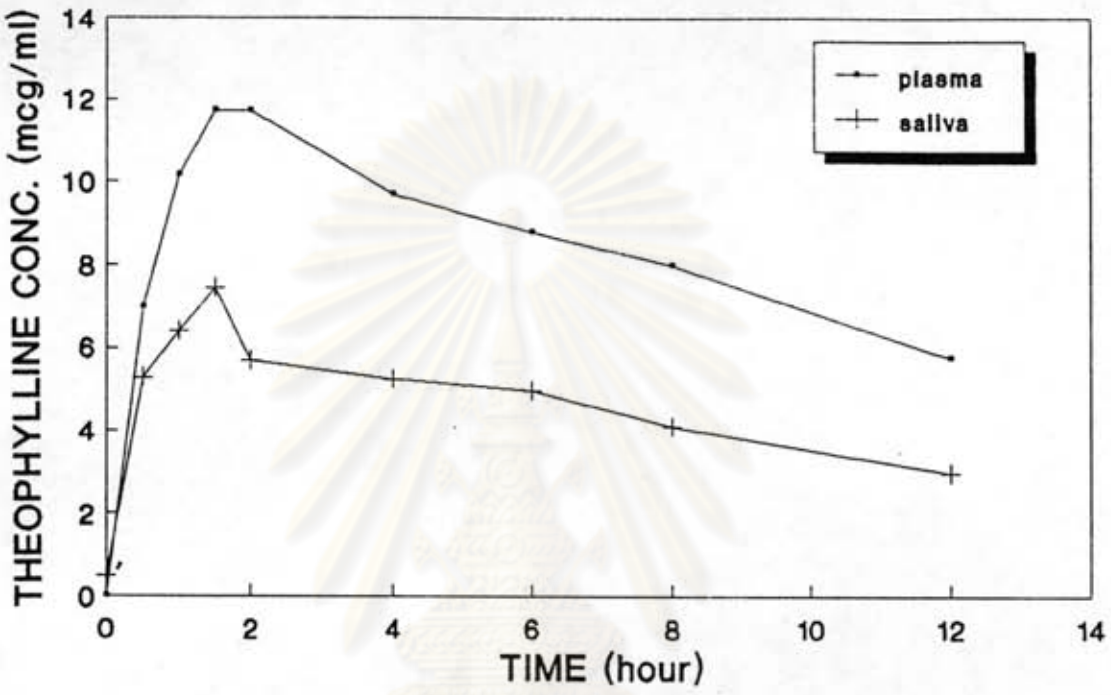

Figure 15 The plasma and saliva theophylline concentration-time profile of subject No.8

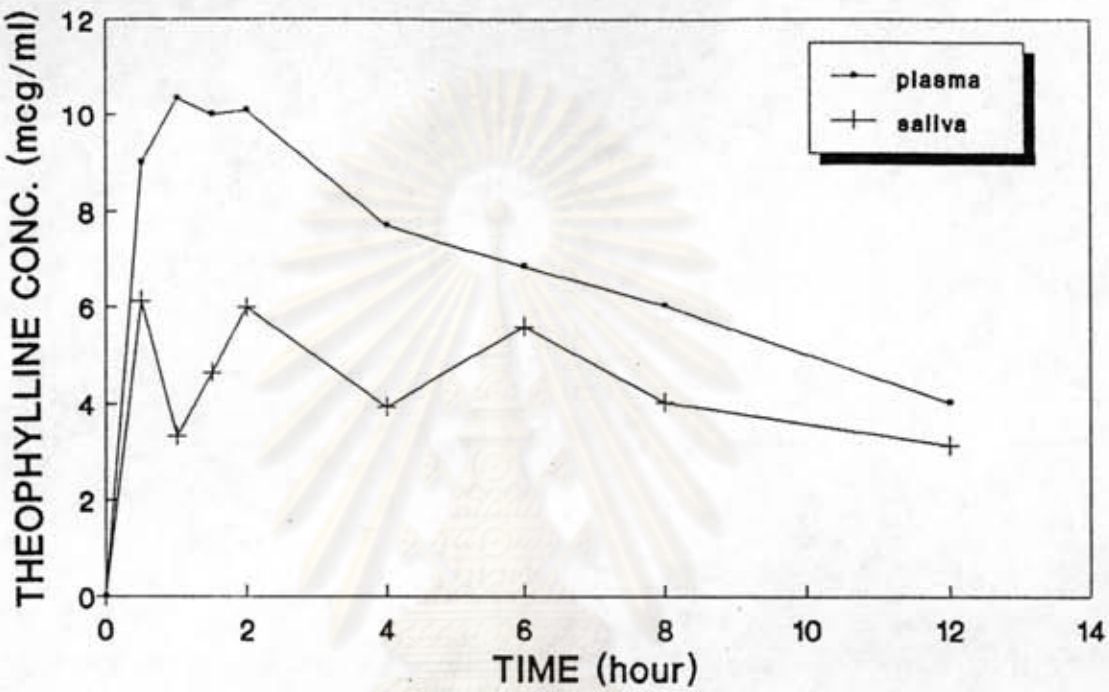

Figure 16 The plasma and saliva Theophylline concentration-time profile of subject No.9

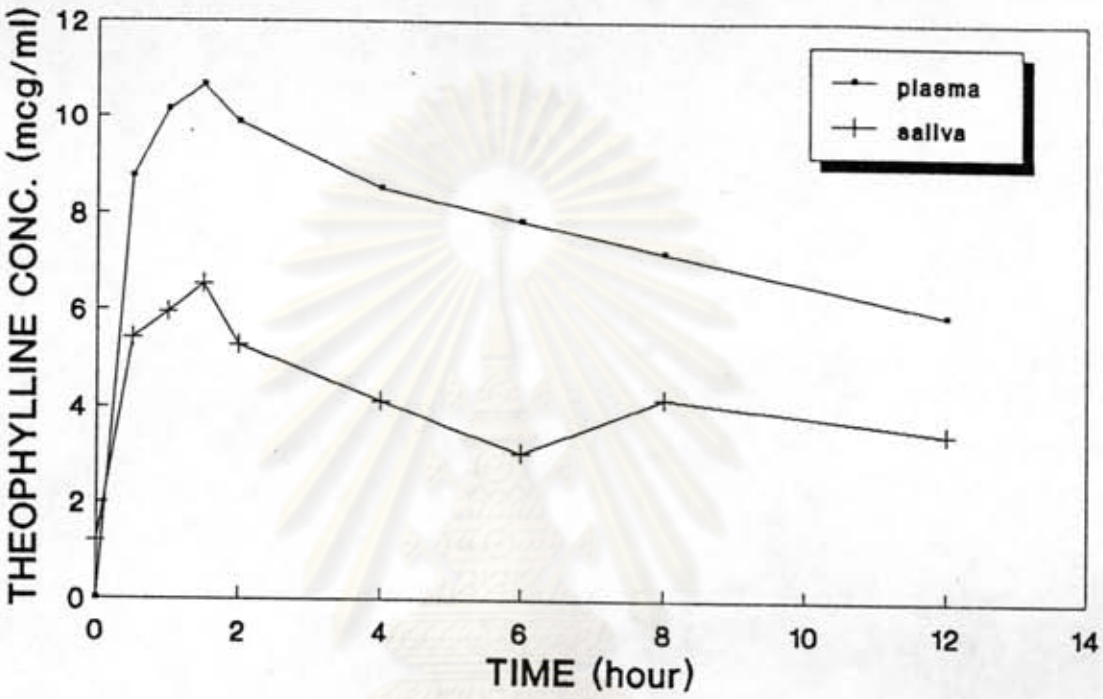

Figure 17 The plasma and saliva theophylline concentration-time profile of subject No.10

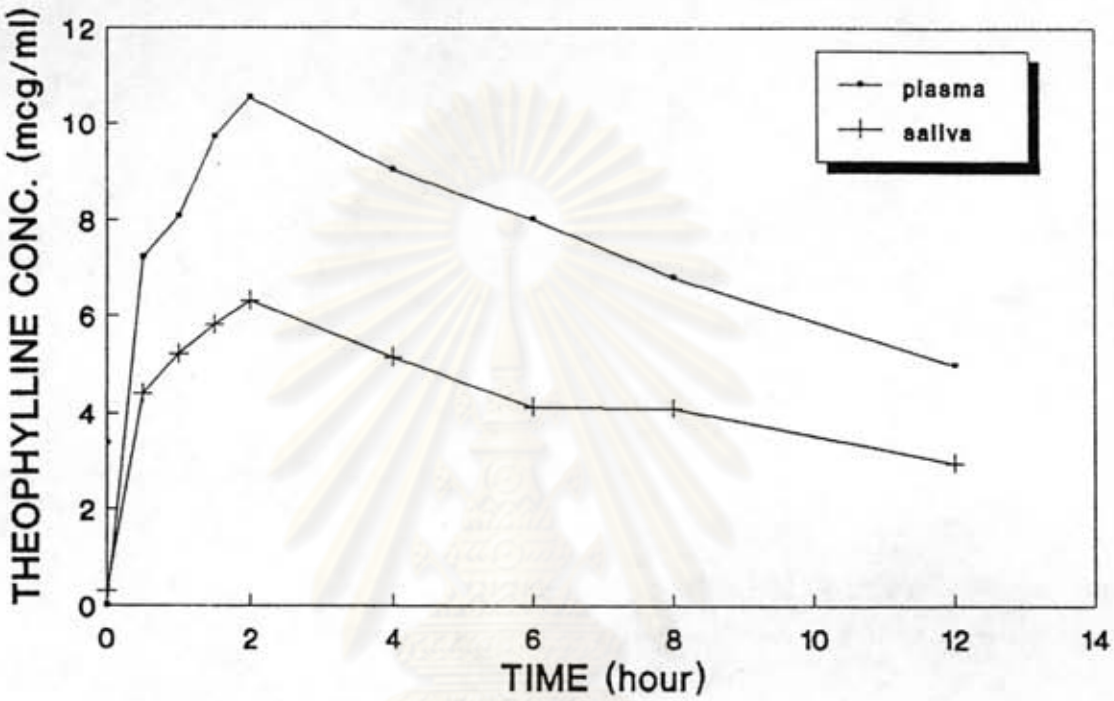

Figure 18 The plasma and saliva theophylline concentration-time profile of subject No.11

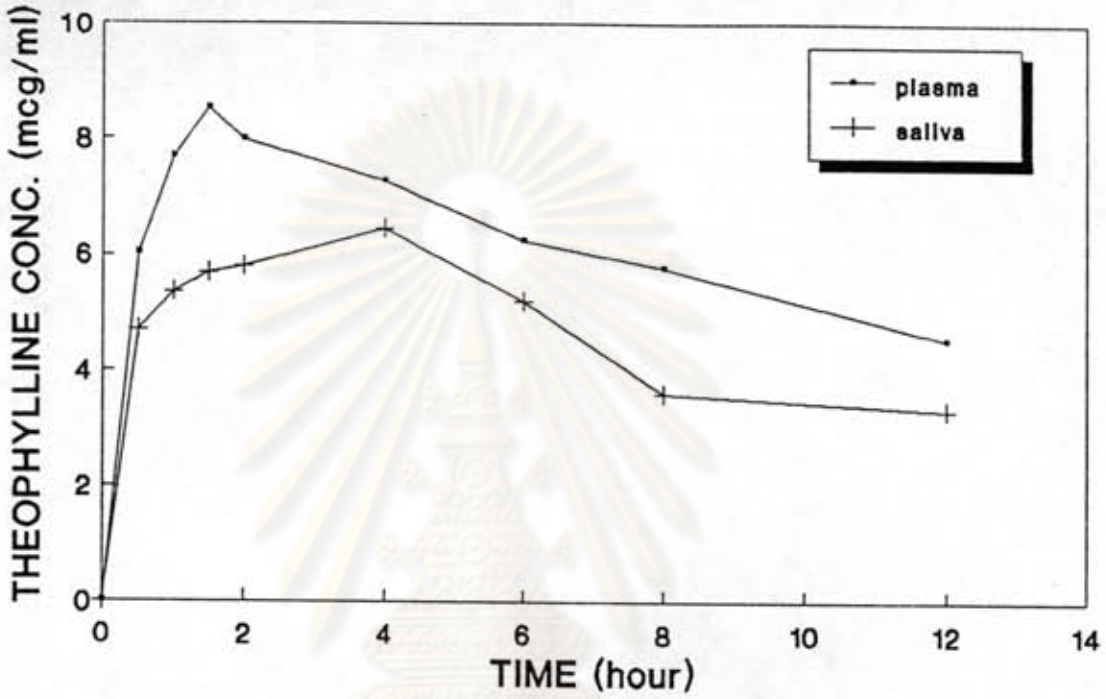

Figure 19 The plasma and saliva theophylline concentration-time profile of subject No.12

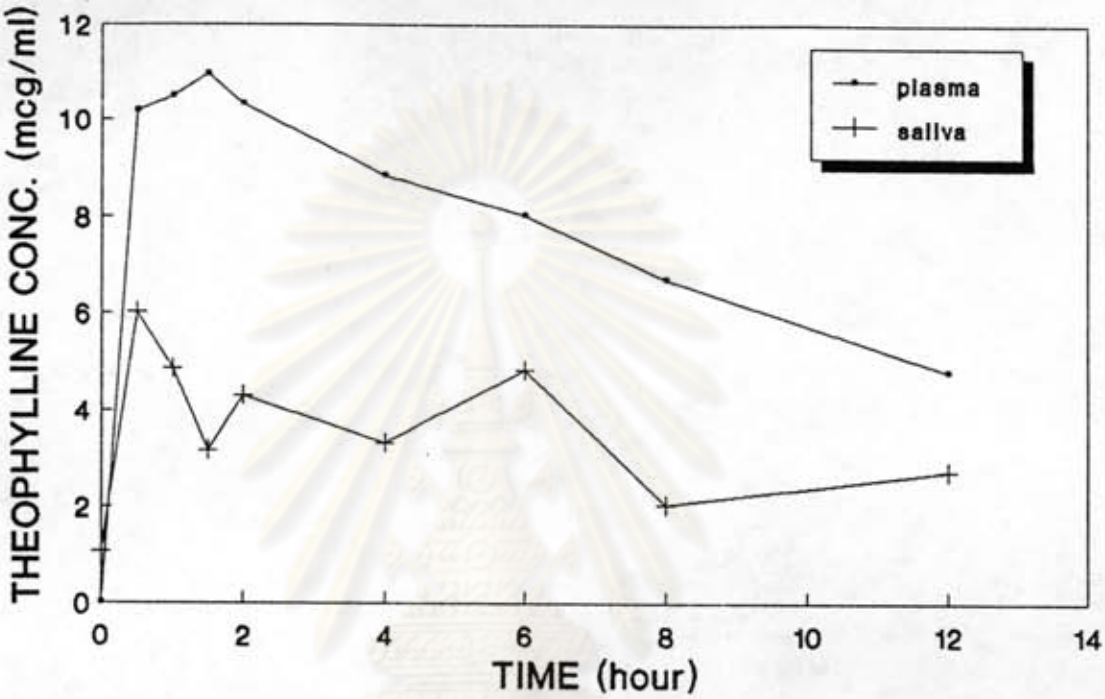

Figure 20 The plasma and saliva theophylline concentration-time profile of subject No.13

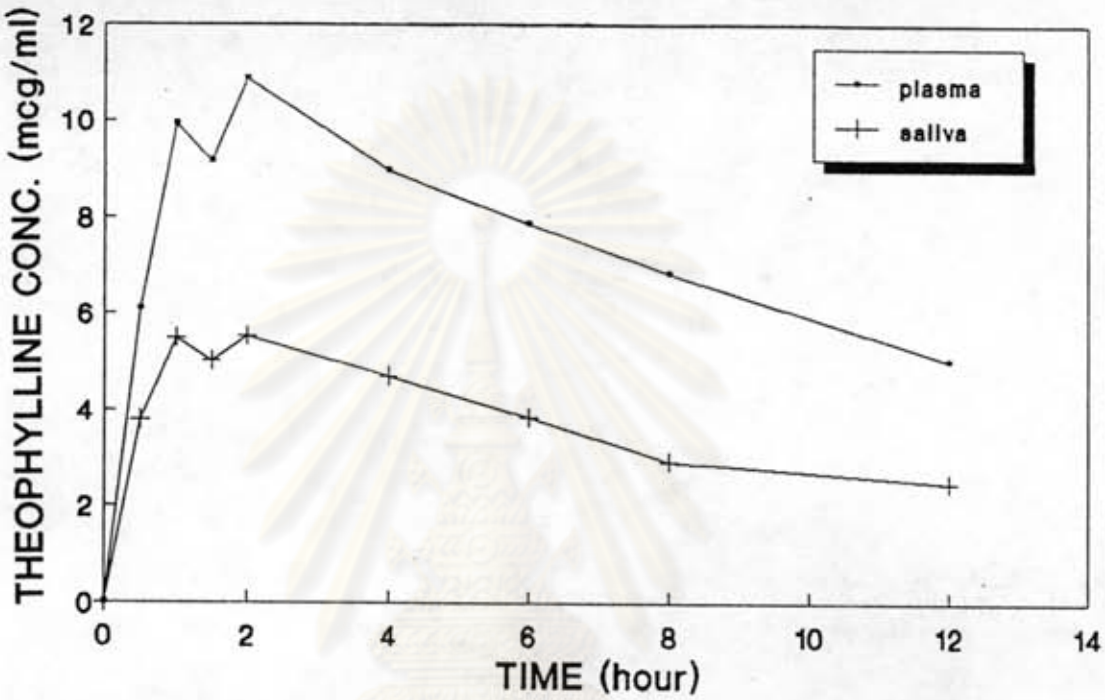

Figure 21 The plasma and saliva theophylline concentration-time profile of subject No.14

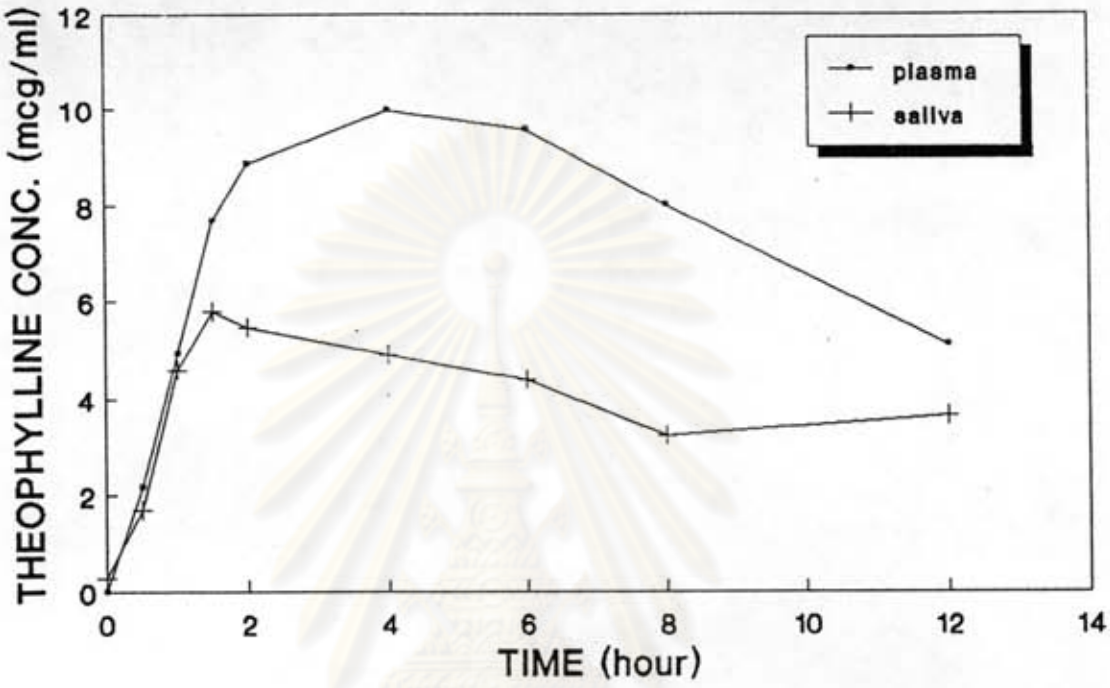

Figure 22 The plasma and saliva theophylline concentration-time profile of subject No.15

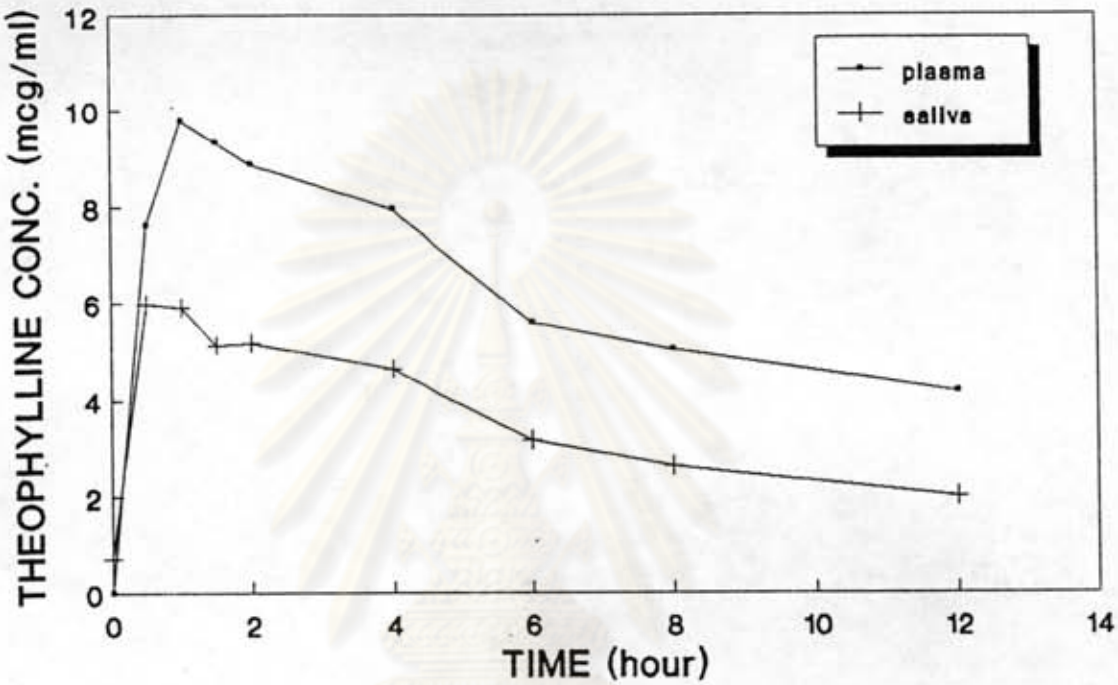

Figure 23 The plasma and saliva theophylline concentration-time profile of subject No.16

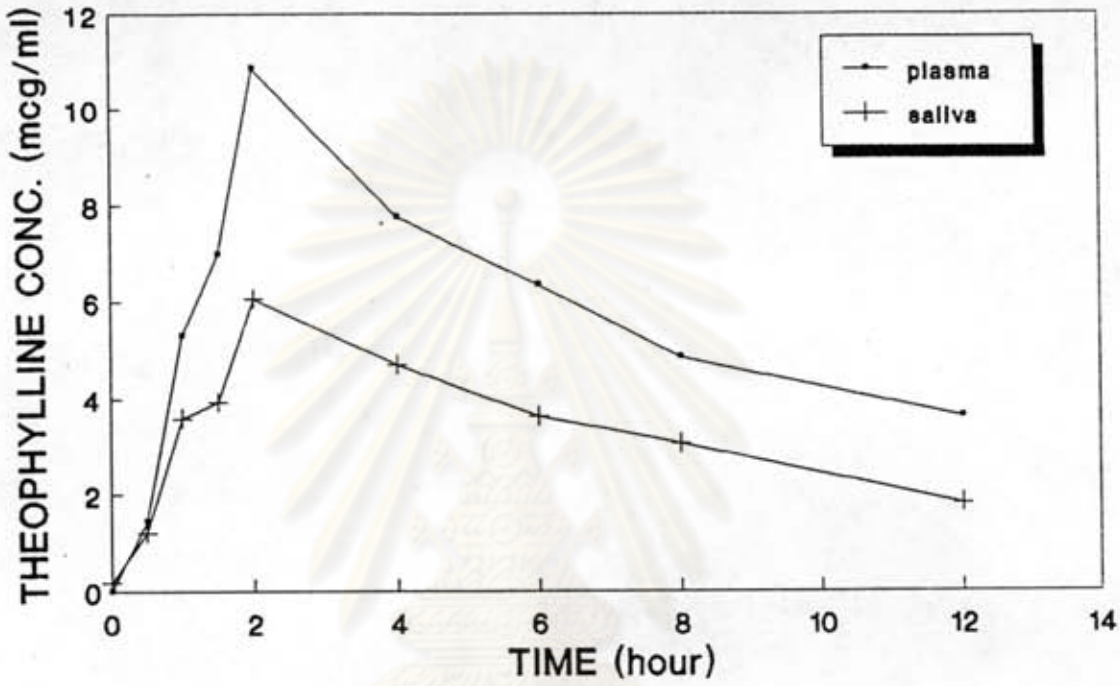

Figure 24 The plasma and saliva theophylline concentration-time profile of subject No.17

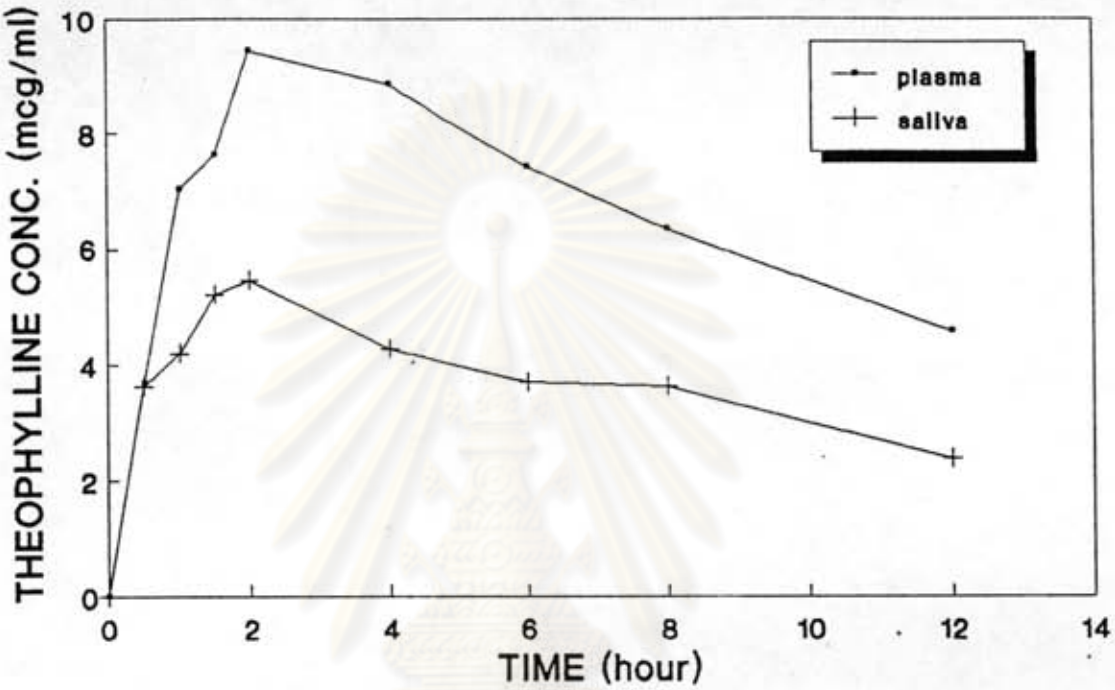

Figure 25 The plasma and saliva theophylline concentration-time profile of subject No.18

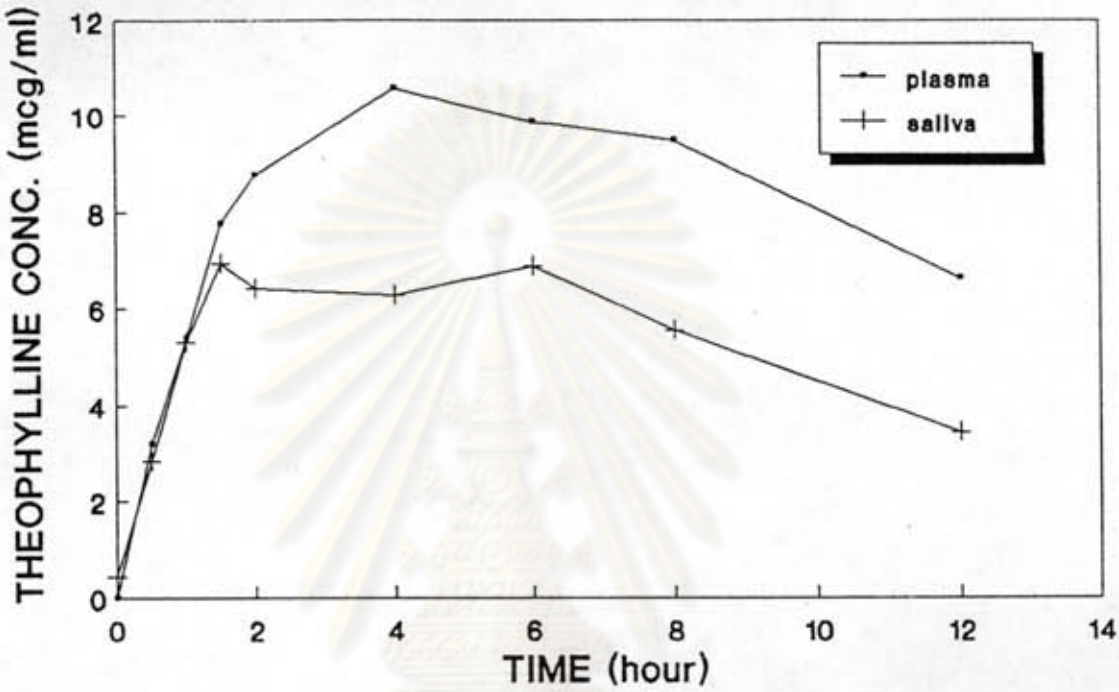

Figure 26 The plasma and saliva theophylline concentration-time profile of subject No. 19

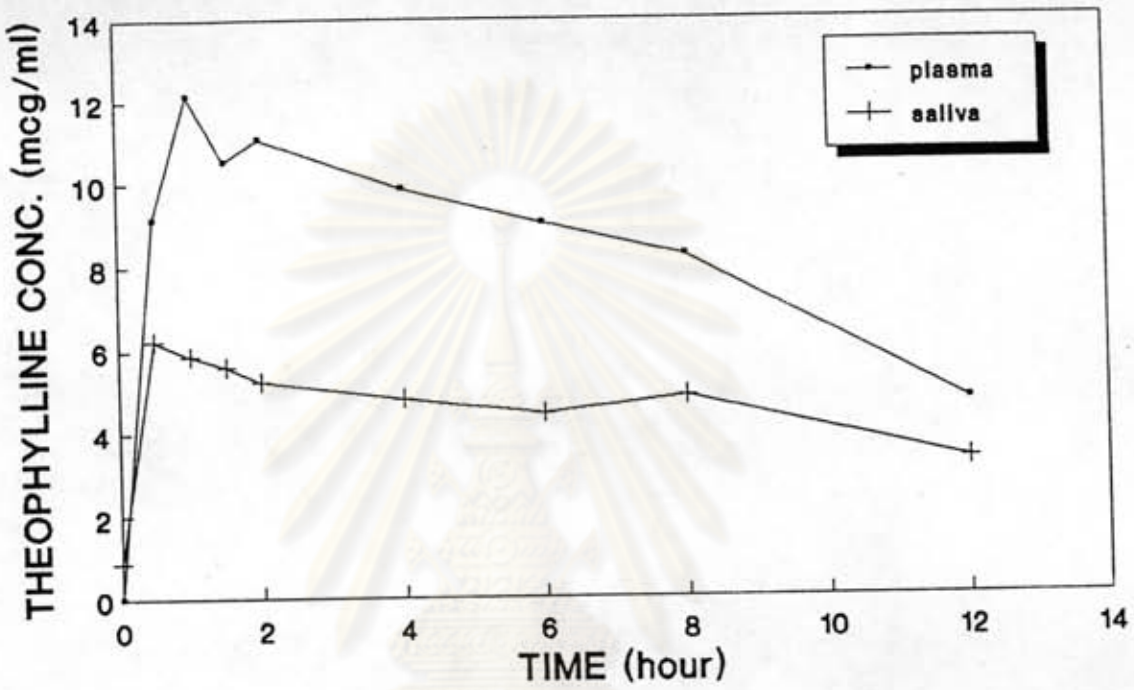

Figure 27 The plasma and saliva theophylline concentration-time profile of subject No.20

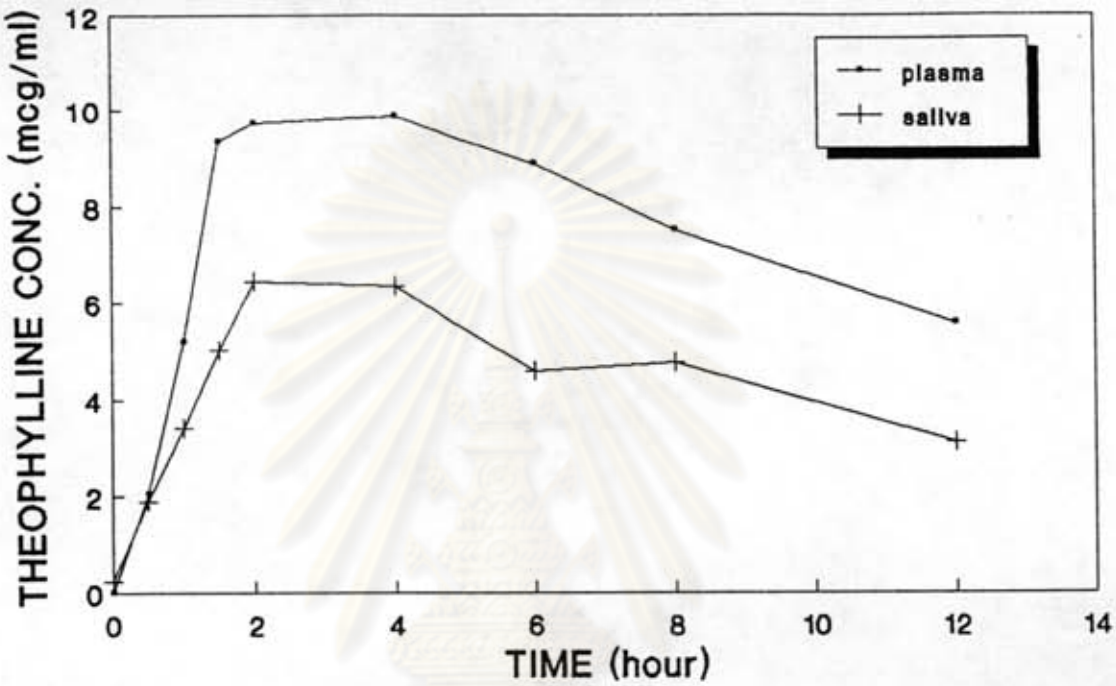

Figure 28 The plasma and saliva theophylline concentration-time profile of subject No.21

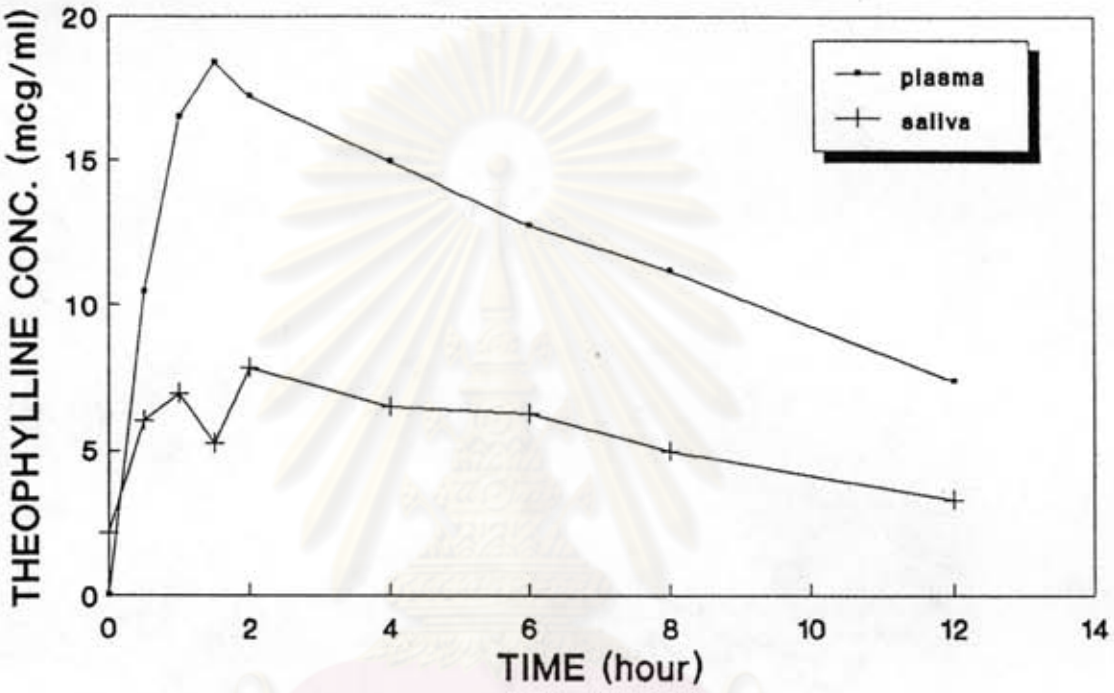

Figure 29 The plasma and saliva theophylline concentration-time profile of subject No.22

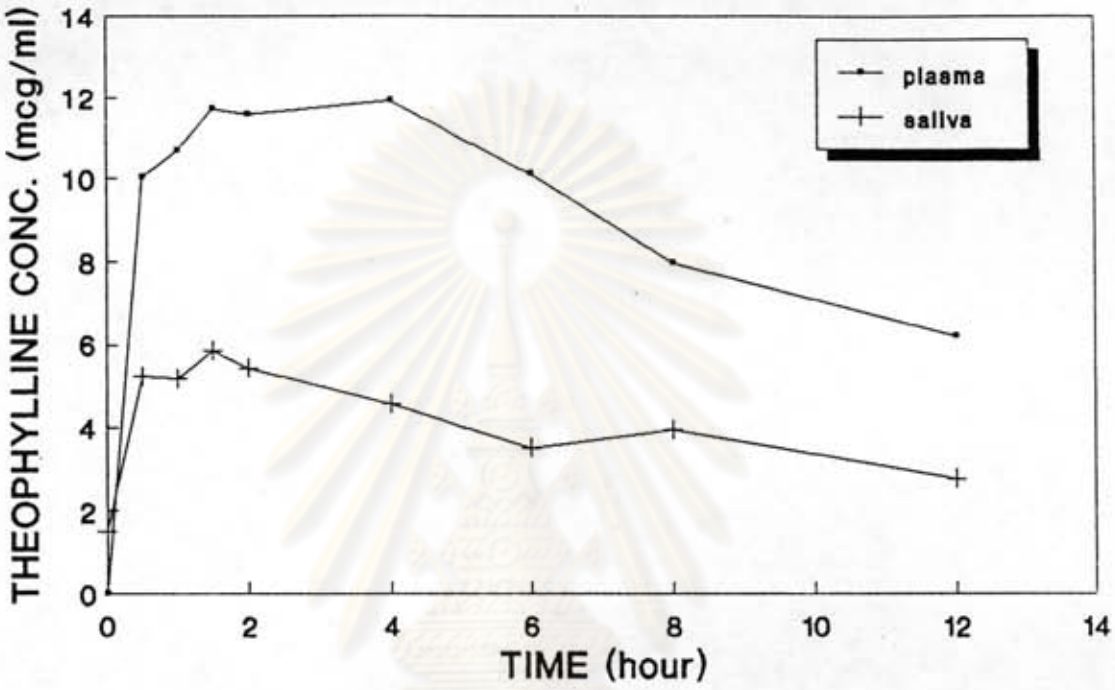

Figure 30 The plasma and saliva theophylline concentration-time profile of subject No.23

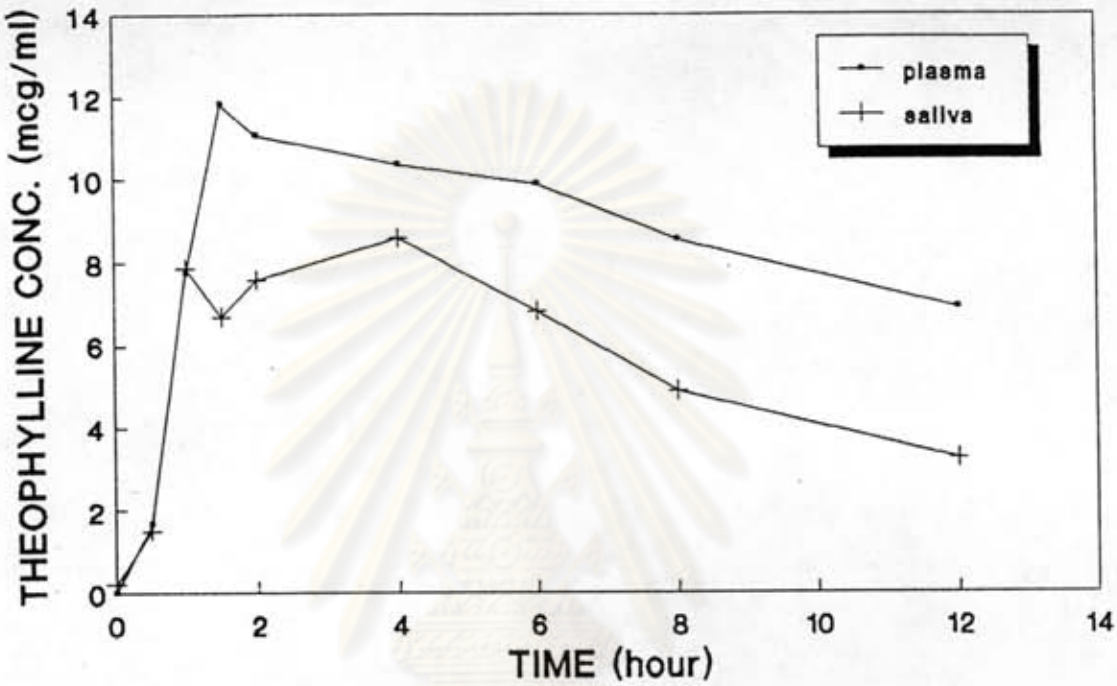

Figure 31 The plasma and saliva theophylline concentration-time profile of subject No.24

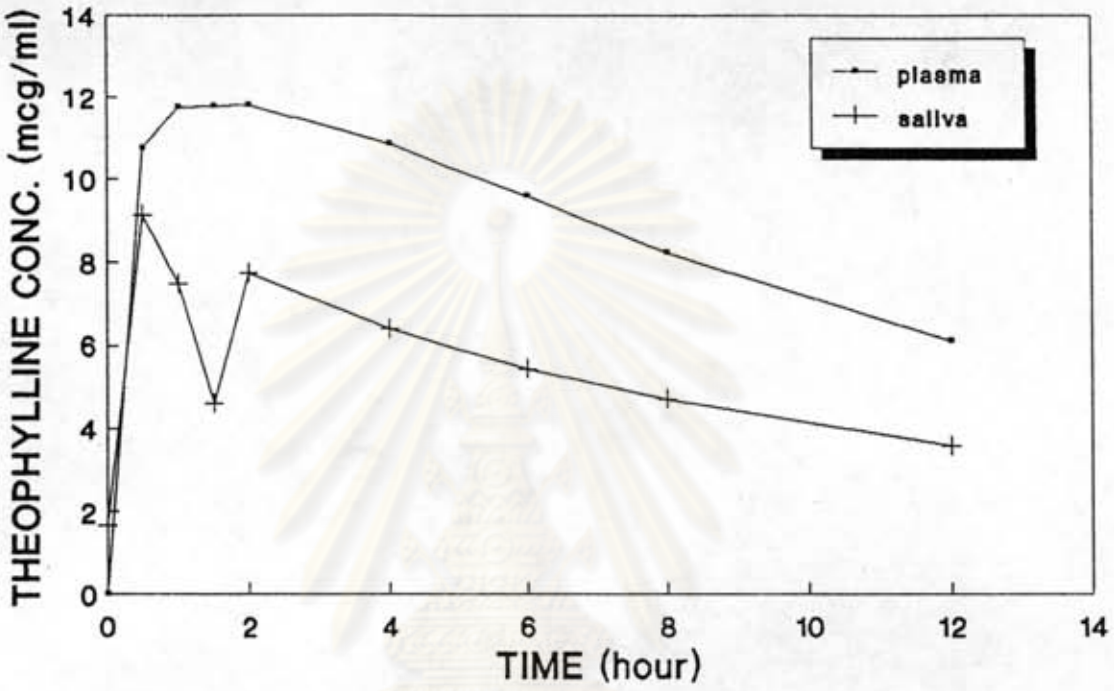

Figure 32 The plasma and saliva theophylline concentration-time profile of subject No.25

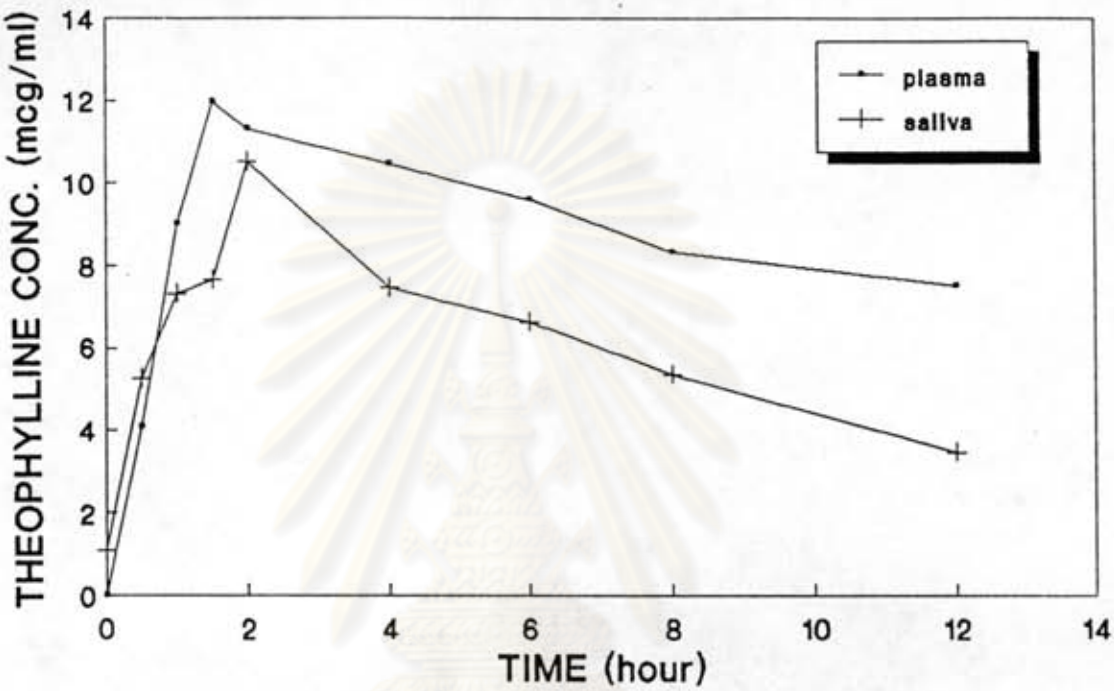

Figure 33 The plasma and saliva theophylline concentration-time profile of subject No.26

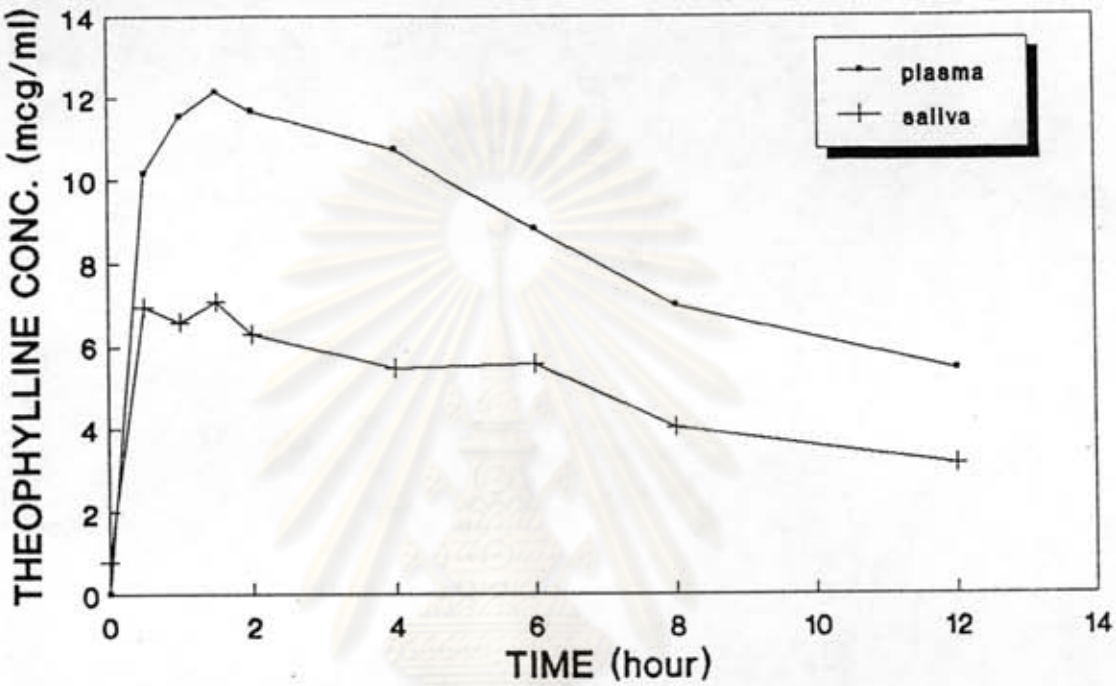

Figure 34 The plasma and saliva theophylline concentration-time profile of subject No.27

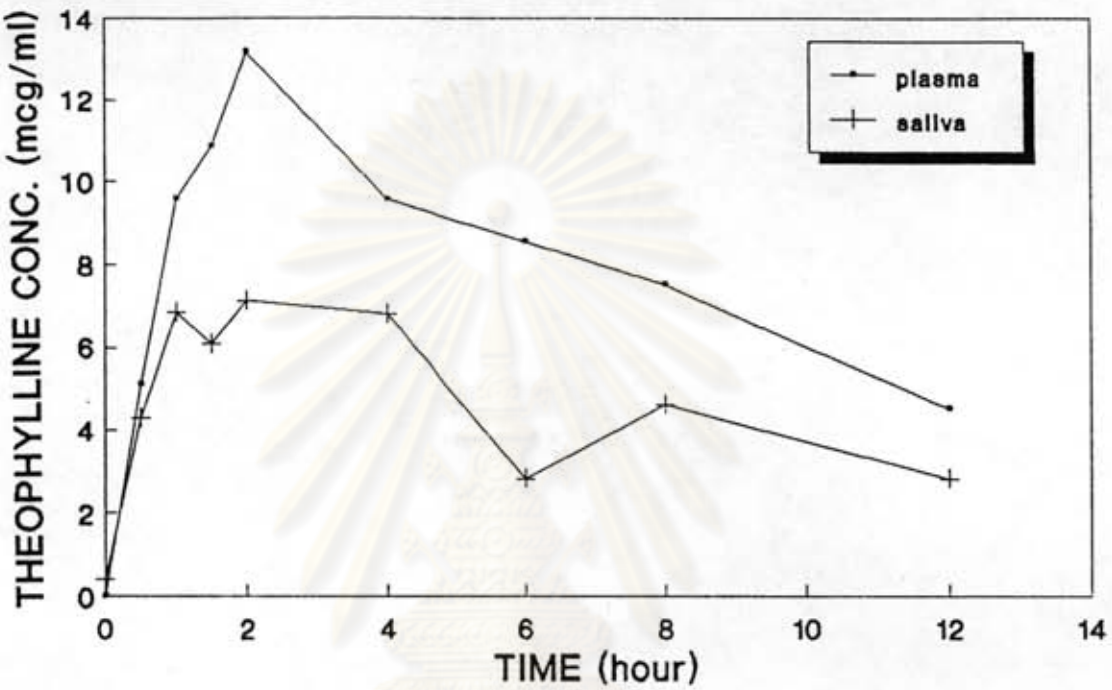

Figure 35 The plasma and saliva theophylline concentration-time profile of subject No.28

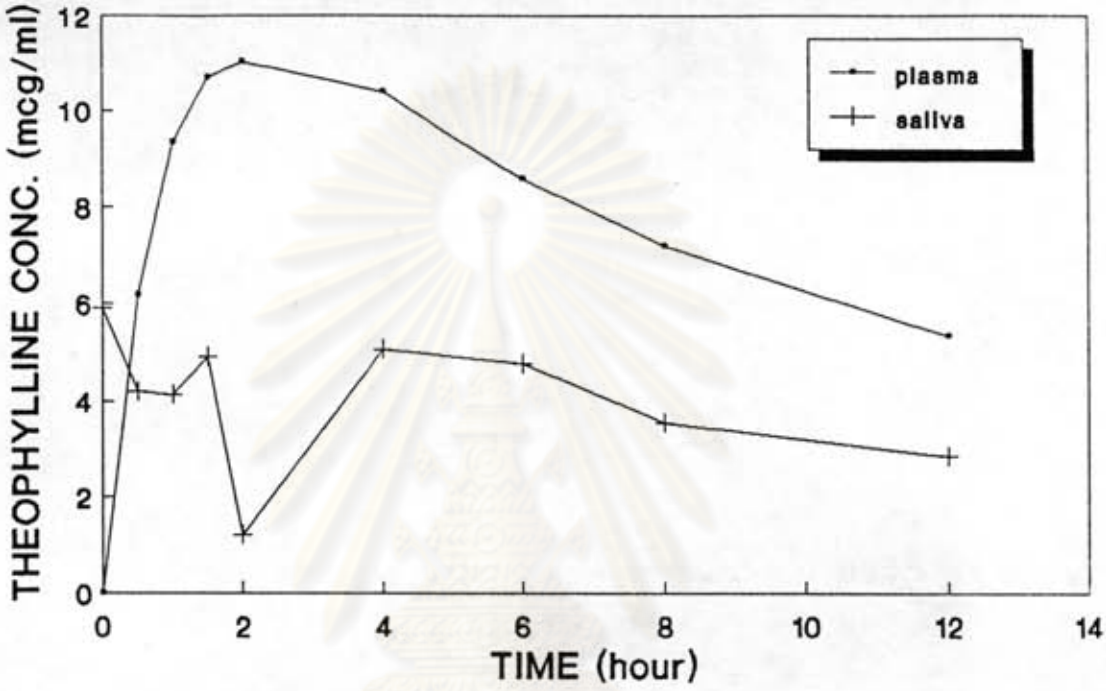

Figure 36 The plasma and saliva theophylline concentration-time profile of subject No.29

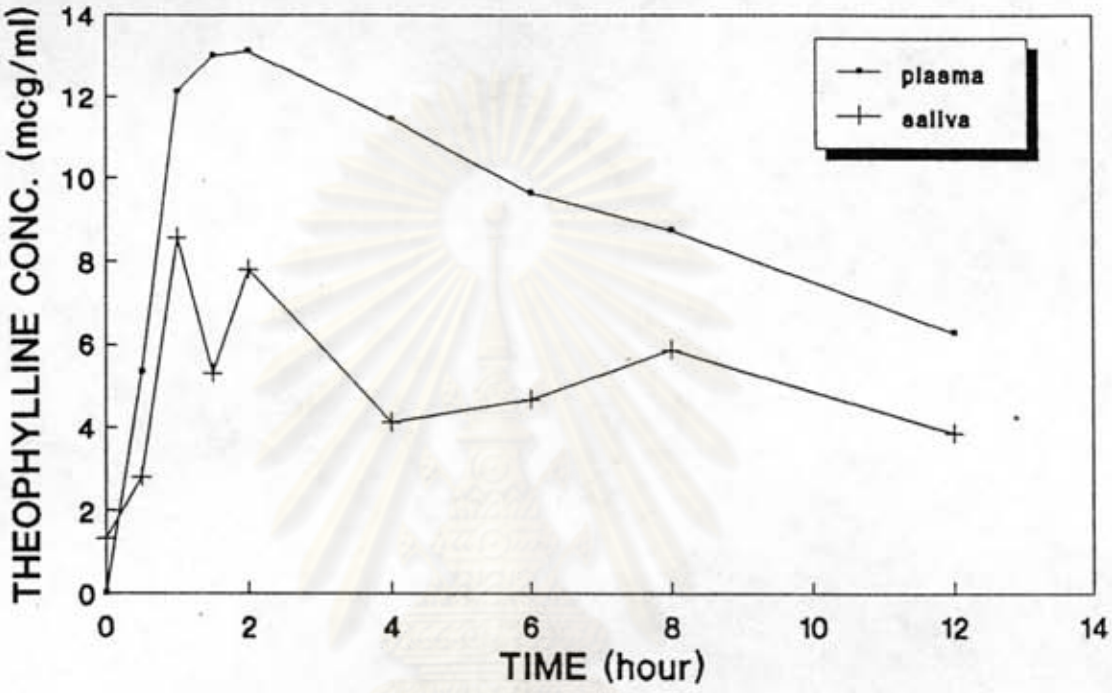

Figure 37 The plasma and saliva theophylline concentration-time profile of subject No.30

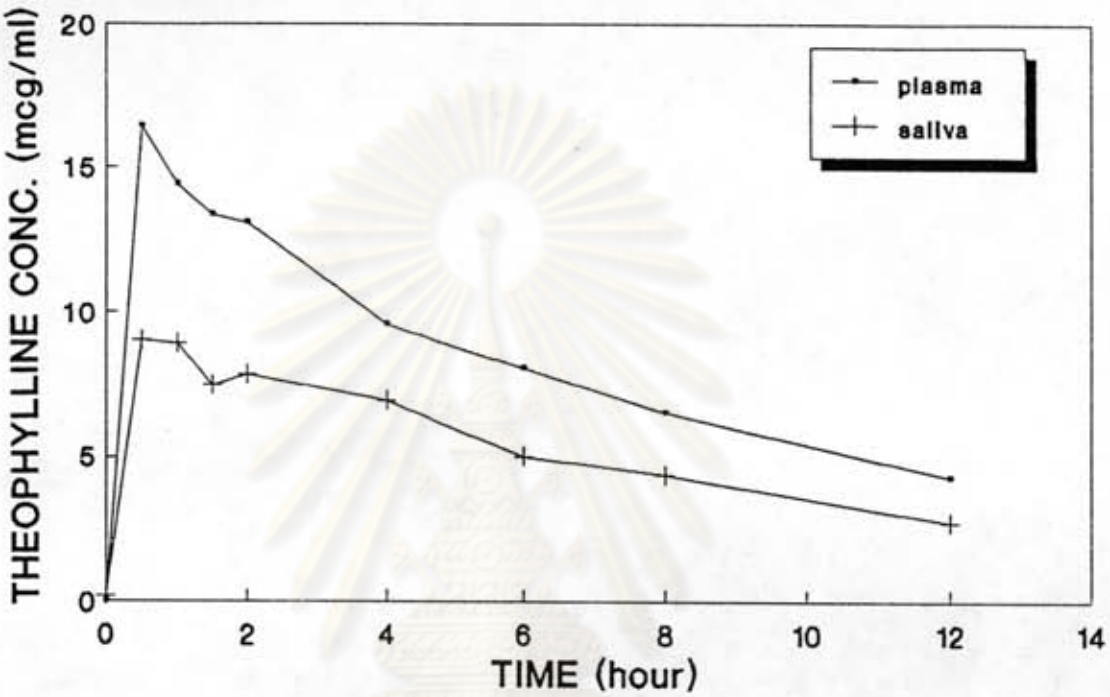

Figure 38 The plasma and saliva theophylline concentration-time profile of subject No.31

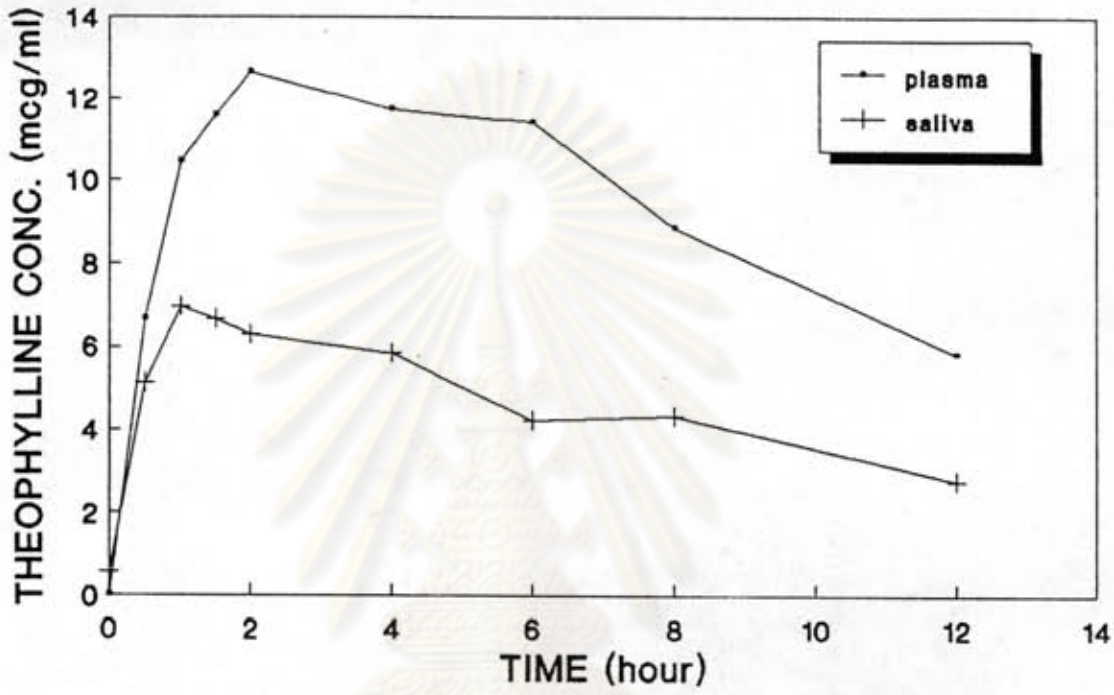

Figure 39 The plasma and saliva theophylline concentration-time profile of subject No.32

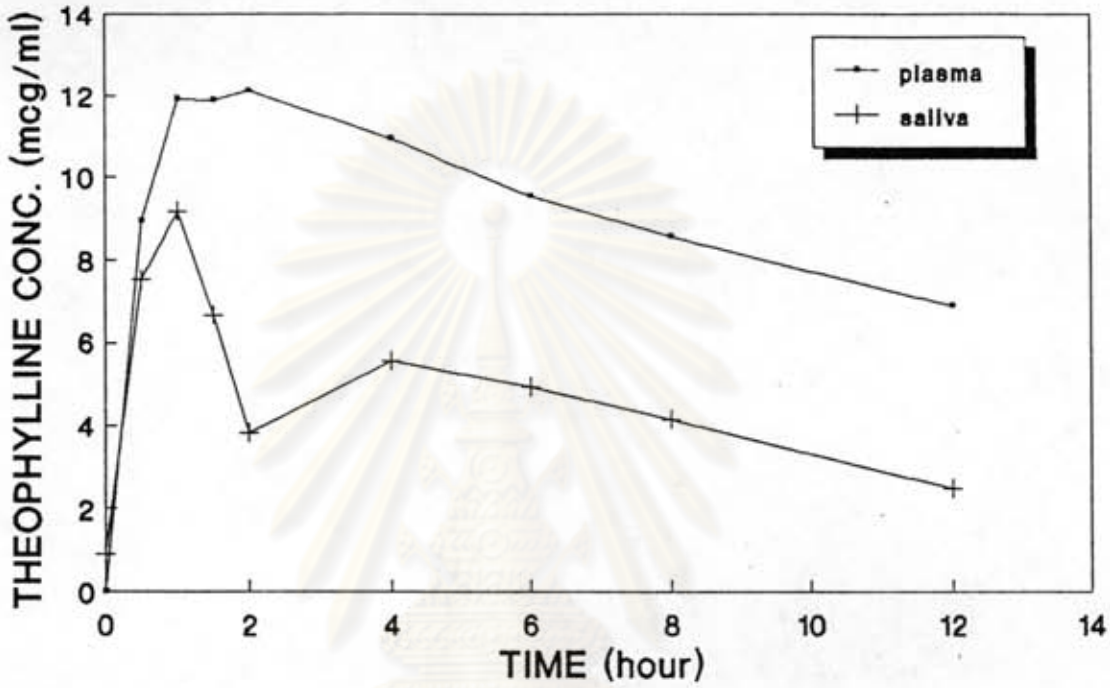

Figure 40 The plasma and saliva theophylline concentration-time profile of subject No.33

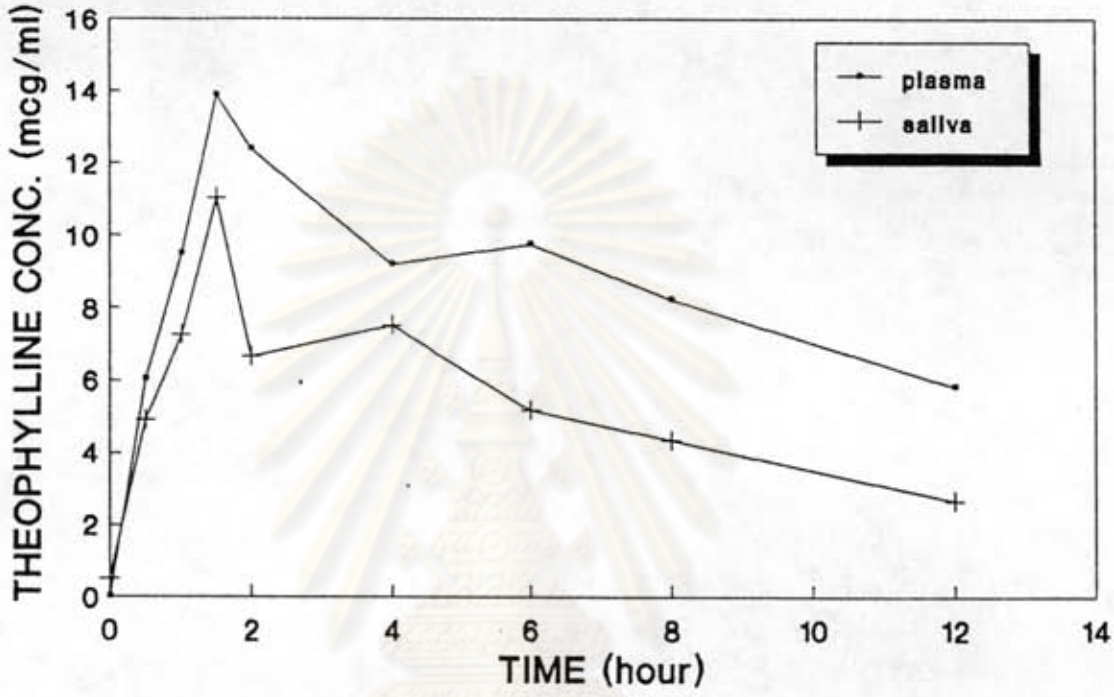

Figure 41 The plasma and saliva theophylline concentration-time profile of subject No.34

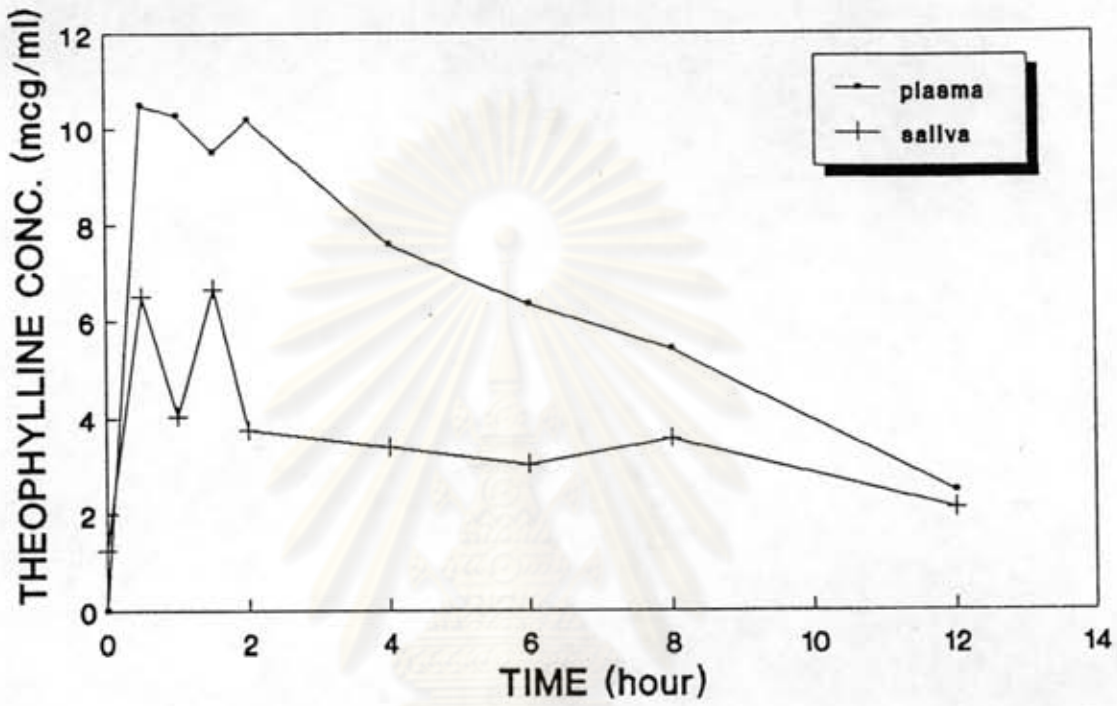

Figure 42 The plasma and saliva theophylline concentration-time profile of subject No.35

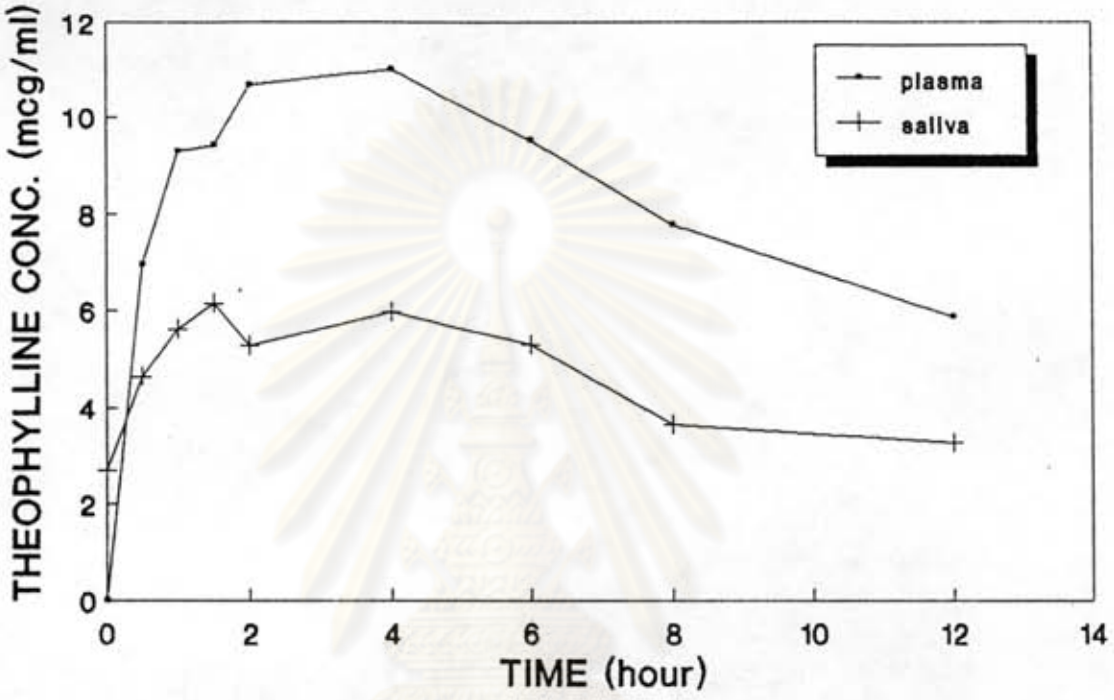

Figure 43 The plasma and saliva theophylline concentration-time profile of subject No.36

Table 13 Cmax and Tmax of theophylline in plasma and saliva of male subjects

male subject	plasma		saliva	
	Cmax (mcg/ml)	Tmax (hour)	Cmax (mcg/ml)	Tmax (hour)
1	8.05	2.0	5.19	4.0
2	8.39	1.5	6.55	1.0
3	9.16	1.0	5.05	0.5
4	9.72	4.0	7.92	4.0
5	8.97	4.0	4.66	4.0
6	14.91	1.0	6.43	1.5
7	11.16	0.5	5.08	1.5
8	11.74	1.5	7.44	1.5
9	10.34	1.0	6.13	0.5
10	10.65	1.5	6.55	1.5
11	10.55	2.0	6.32	2.0
12	8.52	1.5	6.45	4.0
13	10.98	1.5	6.03	0.5
14	10.89	2.0	5.55	2.0
15	10.00	2.0	5.81	1.5
16	9.79	1.5	5.91	1.0
17	10.85	2.0	6.07	2.0
18	9.44	2.0	5.45	2.0
$\bar{X}$	10.23	1.8	6.03	1.9
S.D.	1.56	0.9	0.83	1.2

Table 14 Cmax and Tmax of theophylline in plasma and saliva of female subjects

female subject	plasma		saliva	
	Cmax (mcg/ml)	Tmax (hour)	Cmax (mcg/ml)	Tmax (hour)
1	10.60	4.0	6.94	1.5
2	12.14	1.0	6.22	0.5
3	9.89	4.0	6.45	2.0
4	18.39	1.5	7.85	2.0
5	11.74	1.5	5.86	1.5
6	11.84	1.5	8.61	4.0
7	11.81	2.0	9.15	0.5
8	11.96	1.5	10.54	2.0
9	12.14	1.5	7.09	1.5
10	13.16	2.0	7.13	2.0
11	11.03	2.0	5.08	4.0
12	13.11	2.0	8.59	1.0
13	16.43	0.5	9.05	0.5
14	12.64	2.0	6.97	1.0
15	12.13	2.0	9.17	1.0
16	13.88	1.5	11.04	1.5
17	10.51	0.5	6.68	1.5
18	11.01	2.0	6.17	1.5
$\bar{X}$	12.47	1.8	7.70	1.6
S.D.	2.08	0.9	1.65	1.0

15. Also, the Cmax value of females' saliva was significant higher than the Cmax value of males' saliva ($p = 0.001$).

The extracellular fluid space of female and male was described for this result. Because, females commonly had a greater accumulation of fat tissue than males. (Novak, 1972). Fat tissue had a smaller proportion of water compared to muscle tissue. Thus, the females had a smaller proportion of total body water to total body weight compared to that of the males (Shargel and Yu, 1985). Therefore, at the approximately same administered dose, The higher Cmax value of theophylline in female plasma is accounting for the higher Cmax value in female saliva comparing to male. However, it is realized that the proportion between the Cmax value of theophylline in plasma and saliva of male is similar to that of female.

Nevertheless the time to reach peak concentration (Tmax) of theophylline in either plasma and saliva showed statistically non-significant difference between male and female, as detailed in Table 16.

Additionally, the Tmax value for plasma theophylline coincided with the Tmax value for saliva theophylline in such the way that no statistically significant difference was observed ($p = 0.908$). The small Tmax value for theophylline in either plasma and saliva sample from this study reflects the reality that conventional theophylline tablet is very fast dissolved and completely absorbed after administration (Reynold, 1989).

Table 15 Comparative data of Cmax values between male and female using Student's t-test

parameter	Cmax (plasma)		Cmax (saliva)	
	male	female	male	female
$\bar{X}$	10.23	12.47	6.03	7.70
S.D.	1.56	2.08	0.83	1.65
S.E.M.	0.37	0.49	0.20	0.39
n	18	18	18	18
d.f (n-2)	34		34	
t Table (α=0.05)	$\pm$ 2.034		$\pm$ 2.034	
t Calculate	-3.65		-3.84	
statistical significance	*S (p=0.001)		*S (p=0.001)	

*S = Significant difference

Table 16 Comparative data of Tmax values between male and female and between plasma and saliva, using Student's t-test

parameter	Tmax (plasma)		Tmax (saliva)	
	male	female	male	female
$\bar{X}$	1.8	1.8	1.9	1.6
S.D.	0.9	0.9	1.2	1.0
S.E.M.	0.2	0.2	0.3	0.2
n	18	18	18	18
d.f (n-2)	34		34	
t Table (α=0.05)	$\pm$ 2.034		$\pm$ 2.034	
t Calculate	-0.09		0.82	
statistical significance	*NS (p=0.928)		*NS (p=0.420)	
$\bar{X}$ total	1.8		1.8	
S.D.	0.9		1.1	
S.E.M.	0.2		0.2	
n	36		36	
d.f (n-2)	70			
t Table (α=0.05)	$\pm$ 1.997			
t Calculate	0.12			
statistical significant	*NS (p=0.908)			

*NS = Non-significant difference

Plasma and Saliva Theophylline Concentration-Time Profile

As could be suspected from the data (Appendix B and C) or graphical profiles (Figure 8 - 43), there are two, interesting issues occurred within the first hour following drug administration. Firstly, theophylline concentration could be detected from saliva sample at zero time (immediately after drug administration) in most subjects. Secondly, the detected concentration of theophylline in saliva and plasma sample were almost at the same level during the first hour after drug administration. Results from this study correspond with the other previous reports (Glynn and Bastain, 1973; Koysooko et al. 1974; Welch et al., 1975; Wan, Matin and Azarnoff, 1978; Posti, 1982) such that the reasons could be well explained.

For the first issue about the detection of theophylline is saliva at zero sampling time, the possibility of the concentration in oral cavity by the ingested drug due to the retention of drug in oral mucosa and being released in saliva during the first period of study has already been reported (Koysooko et al., 1974; Chiou, Chang and Peng, 1976; Wan et al, 1978; Paxton and Foote, 1979; Posti, 1982). For the second issue, the rather high theophylline level in saliva during the first hour after drug administration corresponds to the absorption phase of drug in the body. As has been documented by Killman and Thaysen(1955) , during absorption process, drug concentration in arterial blood is higher than that in peripheral venous blood. The body behaves as two distinct pool consisting of highly perfused tissue e.g. saliva glands that equilibrate almost instantaneously with drug and a less highly perfused peripheral

pool that equilibrates more slowly. This leads to possible higher drug concentration in saliva which simultaneously determined with that in plasma during absorption phase. Posti(1982) who directed paracetamol solution in subjects' stomach avoiding buccal contamination for saliva sample also observed the relatively high concentration of drug in saliva during the absorption phase. This would also be affirmed with the other mentioned studies.

It may then be concluded that, eventhough the dosage form of theophylline in this study is a conventional tablet one, it would be very rapidly dissolved so that some amount of drug can still be remained in buccal cavity in most of subjects and contaminated with the first saliva sample. Whether, is there any contamination at zero sampling time for saliva, the rather high theophylline concentration in saliva would probably due to the high arterial concentration of drug during absorption phase.

Beyond the absorption phase of the drug as shown in Figure 8-43, the concentration in saliva and plasma for each individual demonstrated some intersubject variations. This kind of variation can be considered as the usual performance of such a drug that major elimination is by biotransformation in the liver, like theophylline. (Piafsky and Ogilvie, 1975) Some fluctuations in theophylline concentration during sampling period, especially for saliva sample were observed in subjects No. 2 (Figure 9), 3(Figure 10), 4 (Figure 11), 5(Figure 12), 6(Figure 13), 7(Figure 14), 9(Figure 16), 13(Figure 20), 25(Figure 32), 28(Figure 35), 29(Figure 36), 30(Figure 37) and 35(Figure

42). With these observation, subject No. 2-7 were discovered to be the smoking habit subjects. However a consistantly seperate levels of theophylline in plasma and saliva for male and female subjects are clearly shown up to twelve hours of sampling schedule, as shown in Figure 44 and 45. This reveals some kind of fixed proportional concentration between plasma and saliva sample that would possibly be determined.

Storage Period of Plasma and Saliva Samples at - 15°C

Eventhough, all of the samples obtained from subjects were analyzed within one week, there should always be questions whether how long could the rest of samples be kept. Since the rest of the samples had often been useful for data check-up, this study has also determined of sample kept-up time.

It was determined that more than 10% of theophylline concentration changed when the either plasma and saliva samples be kept up to 15 days after sample analysis, compared to the initial concentrations. (data not shown)

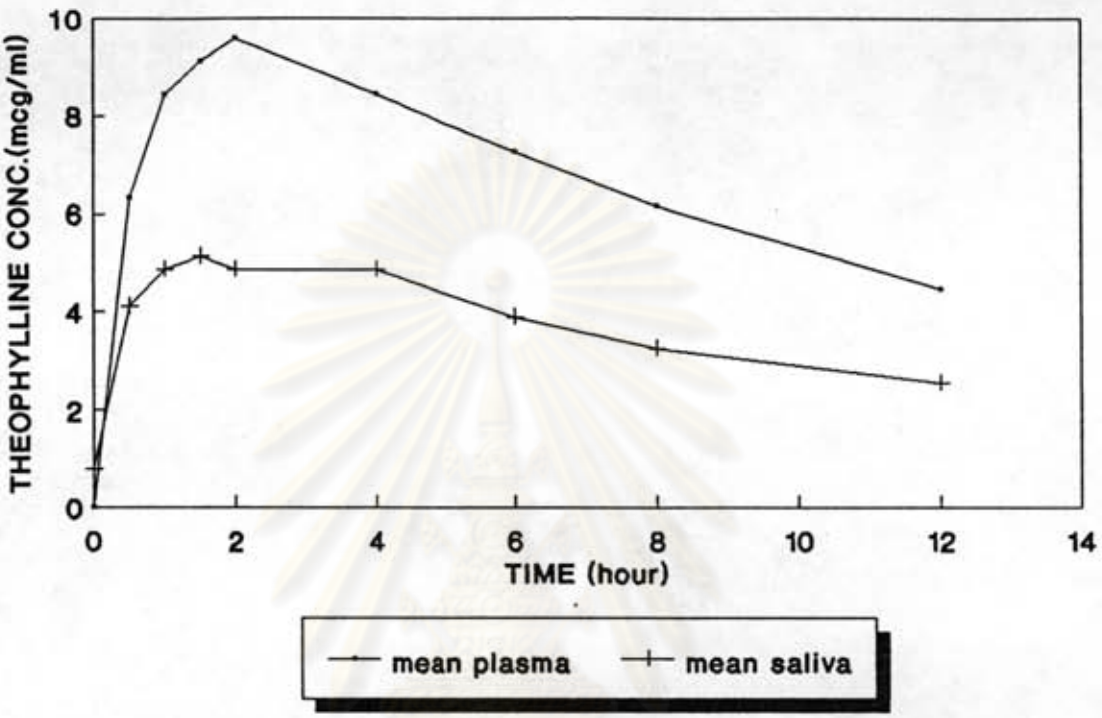

Figure 44 **The average plasma and saliva theophylline concentration-time profile of male volunteers**

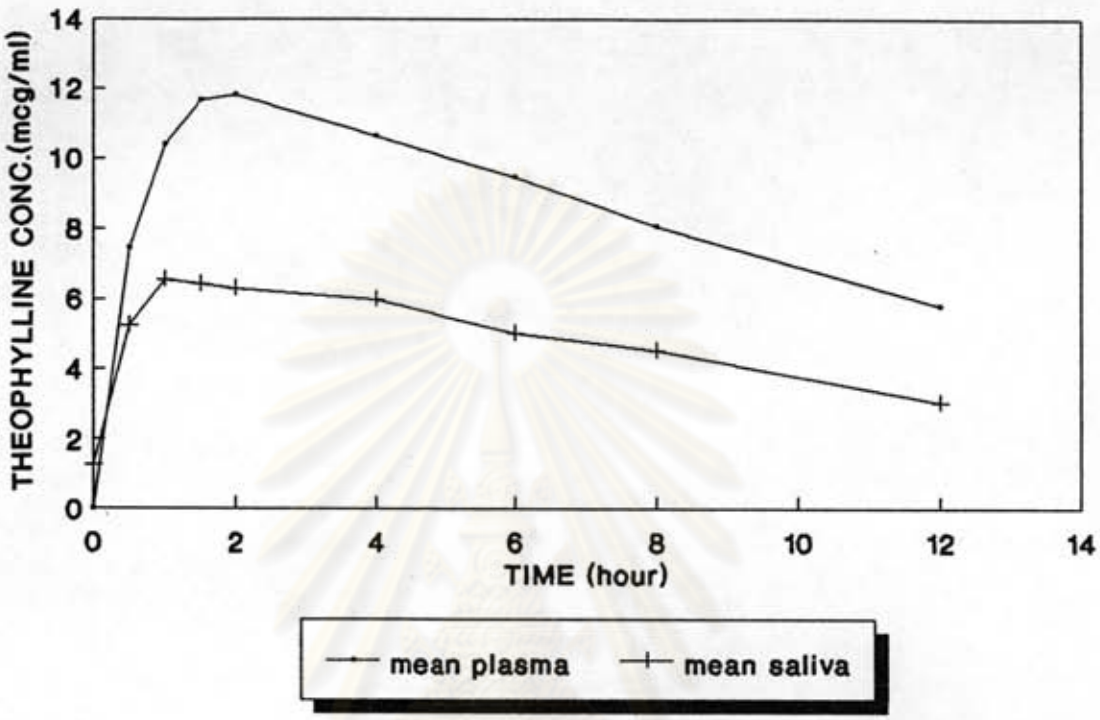

Figure 45 The average plasma and saliva theophylline concentration-time profile of female volunteers

Part 3 Data Analysis

The determination for the correlation between the plasma and salivary theophylline concentration is shown in Figure 46. All data points obtained through-out the experiment were included in this analysis. There is a highly statistically significant ($p<0.001$) relationship between the concentration of theophylline in plasma and saliva ($r = 0.81$).

As shown in Table 17, The variation in the saliva-plasma concentration ratio of theophylline in all subjects ($n = 324$) was analysed and showed the statistically independent upon the sex including smoking habit at significant level equal to 0.05 ($p = 0.767$). No interactions between time and sex with the ratio value was observed ($p=0.961$). However, the ratio value showed highly significant dependence upon sampling time ($p < 0.001$). It was finally detected after Scheffe procedure that the saliva-plasma concentration ratio at 0.5 hour after administration is the main factor of difference. By excluding the saliva-plasma ratio data at $t = 0.5$ hour, no statistically significant difference among sex and sampling time to the saliva-plasma ratio was observed. Therefore, the grand total mean of the saliva-plasma ratio was calculated as the representative saliva-plasma concentration ratio of theophylline for Thais in which it is equal to 0.57 ± 0.14.

The value of saliva-plasma concentration ratio determined in this study is less than that reported for Thai asthmatic children that was 0.62 (Montri Tuchinda, 1987) but agrees with the other previous

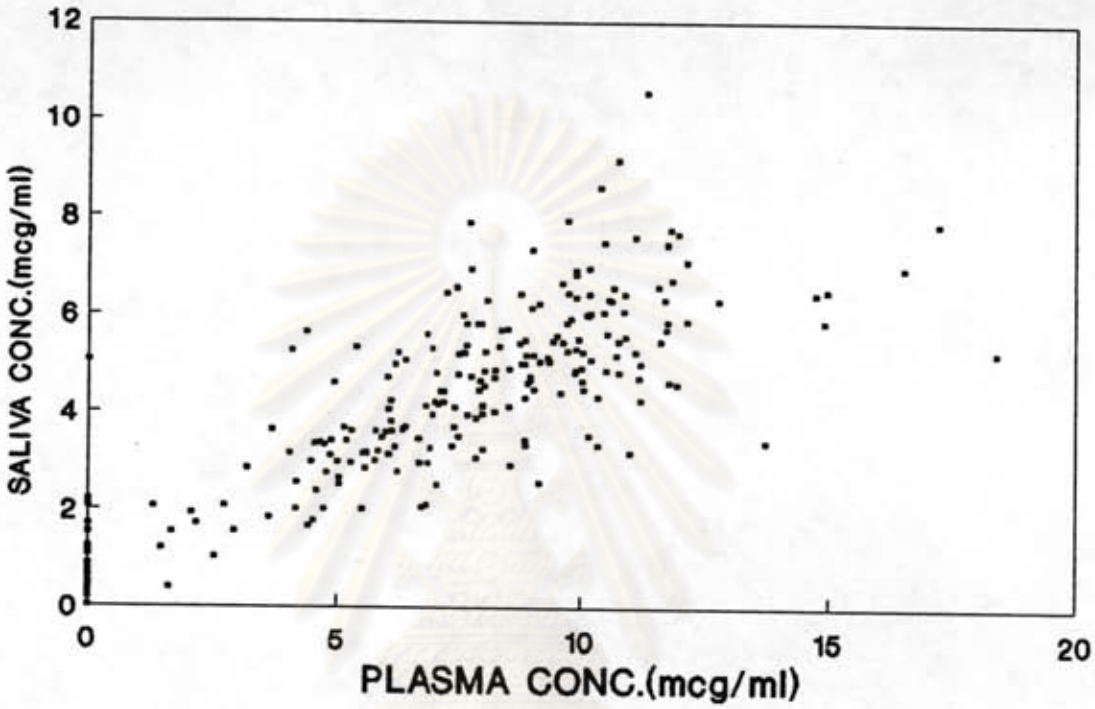

Figure 46 The correlation between saliva and plasma theophylline concentration of all data points (n=324)

Table 17 **The two-way ANOVA output of the saliva-plasma theophylline concentration ratio by using statistical package SPSS/PC+**

* * * A N A L Y S I S O F V A R I A N C E * * *

RATIO
BY TIME
SEX

Source of Variation	Sum of Squares	DF	Mean Square	F	Signif of F
Main Effects	12.355	10	1.236	55.292	.000
TIME	12.337	8	1.542	69.011	.000
SEX	.012	2	.006	.265	.767
2-way Interactions	.167	16	.010	.467	.961
TIME SEX	.167	16	.010	.467	.961
Explained	12.522	26	.482	21.554	.000
Residual	6.637	297	.022		
Total	19.159	323	.059		

reports in which the saliva-plasma ratio was between 0.46-0.58. (Koysooko, 1974; Culig, Johnson and Turner, 1982) Thus, race may not be the affected factor to the saliva-plasma ratio of theophylline as that of the other pharmacokinetic parameters e.g. elimination half-life.

Part 4 Application to Patients

As tabulated in Table 18, theophylline concentration in patients' saliva sample was used for calculating the plasma theophylline concentration in each individual via the saliva-plasma concentration ratio determined from this study. The calculated concentration values of theophylline in plasma were normalized by plasma theophylline concentration obtaining from analysis of patient plasma sample in which they are in the range of 0.950 to 1.676 as demonstrate in Table 18. The linear relationship between calculated plasma theophylline concentration and analyzed plasma theophylline concentration was observed with the r value of 0.9540 at $p < 0.001$ (Figure 47) The represent linear equation was determinated to be

$$y = 1.308 x + 0.3591 \qquad (3)$$

where y represents calculated plasma theophylline concentration

x represents analyzed plasma theophylline concentration

The rather wide range of normalized calculated plasma theophylline concentration and the rather high value of slope obtained from patient subjects indicated that the direct use of saliva-plasma ratio

via saliva sample may cause the overestimation of theophylline concentration in patient plasma. However this result is just the very preliminary study in patient but not the absolute conclusion for use of this saliva-plasma ratio, this application to patients is done in order to propose the restricted quideline for further study. The overestimation outcome would probably be due to the small number of patients used and the non-restricted limitation of the out-patients in this study. The only restriction for the participating out-patients in this study was that they must be on theophylline therapy and it does not matter whether any conditions which the patients were. Therefore, as shown in Table 18, the time between sample collection and the last administered dose in each patient was then quite diverse. Moreover, patient compliance, concurrent illness, concomitant drug, severity of disease etc. were all ignored from the study. As detected from patient numbers 5,8 and 12 unless the analysis is not correct, patient's condition must be carefully verified before making any conclusion on the normalized calculated value.

Besides these aforementioned contexts happening in this study, the saliva-plasma concentration ratio of theophylline has been proven as a helpful parameter in predicting plasma theophylline concentration from saliva sample (Levy et al., 1974; Johnson et al. 1975; Eney and Goldstein, 1976; Galent et al, 1977; Ohmori, 1986; Aviram et al., 1987)

It is anyhow the advantage of this study in opening up the idea of using saliva-plasma concentration ratio of theophylline for Thai patient in accordance with the therapeutic theophylline monitoring in

Thailand. Further study in determination of the correction factor coupling with the saliva-plasma concentration ratio of theophylline is fully recommended.

Table 18 The individual data of application to patients

No.	sex	age (year)	body wt. (kg)	dosage regisea	sampling tine after last dose (hr.)	analyzed saliva conc. (μg/ml)	analyzed plasaa conc. (μg/ml)	calculated plasaa conc. (μg/ml)	calculated conc. / analyzed conc.
1	F	7	24	Quibron TSR 1/2 tab. morning 2/3 tab. evening	4.0	2.979	4.218	5.266	1.221
2	M	8	23	Quibron TSR 1/2 tab.x2	6.5	4.789	6.699	8.402	1.254
3	F	12	24	Theodur 200 mg. 1 tab. x2	8.0	7.091	8.789	12.44	1.415
4	F	10	22	Theodur 200 mg. 1/2 tab. x2	7.0	8.403	10.64	14.74	1.385
5	F	10	30	Aminophylline 100 mg.1 tab. q 6 hr.	3.0	6.858	7.316	12.03	1.644
6	F	11	36	Theodur 200 mg. 1 tab. x2	7.0	4.015	4.724	7.044	1.491
7	F	15	42	Theodur 300 mg. 1 tab. x2	6.0	5.437	10.04	9.538	0.950
8	F	31	44	Theodur 200 mg. 1 tab. x2	14.0	2.751	2.880	4.826	1.676
9	F	58	55	Aminophylline 100 mg. 1x3	6.0	5.175	7.160	9.079	1.268
10	M	36	60	Aminophylline 100 mg. 1x4	6.0	4.623	6.217	8.110	1.304
11	M	56	55	Theodur 300 mg. 1 tab. x2	4.0	13.52	17.09	23.72	1.388
12	M	60	50	Theodur 200 mg. 1 tab. x2	6.0	5.053	5.445	8.865	1.628
								$\bar{X}$	1.385
								S.D.	0.208

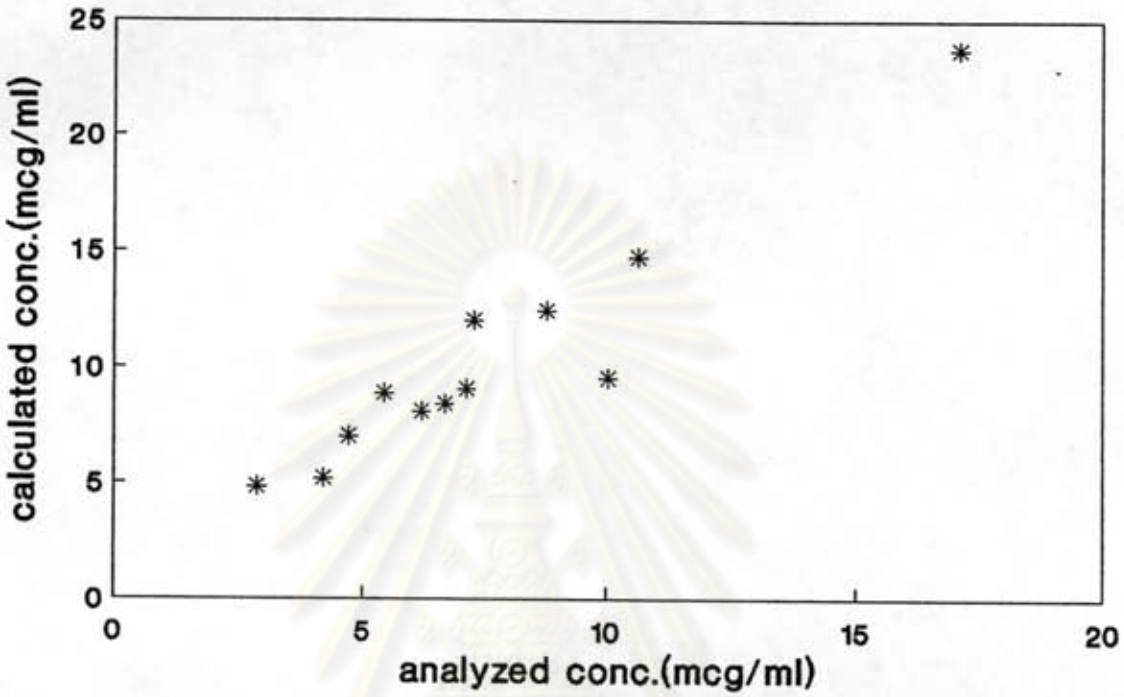

Figure 47 The correlation between analyzed and calculated theophylline concentration in plasma