

## CHAPTER 4

### Results

The chromatograms obtained from the HPLC system are very good. Peaks of carbamazepine and propylparaben in plasma and saliva were well separated with retention time about 3.2 minutes and 4.0 minutes, respectively. (Figure 6)

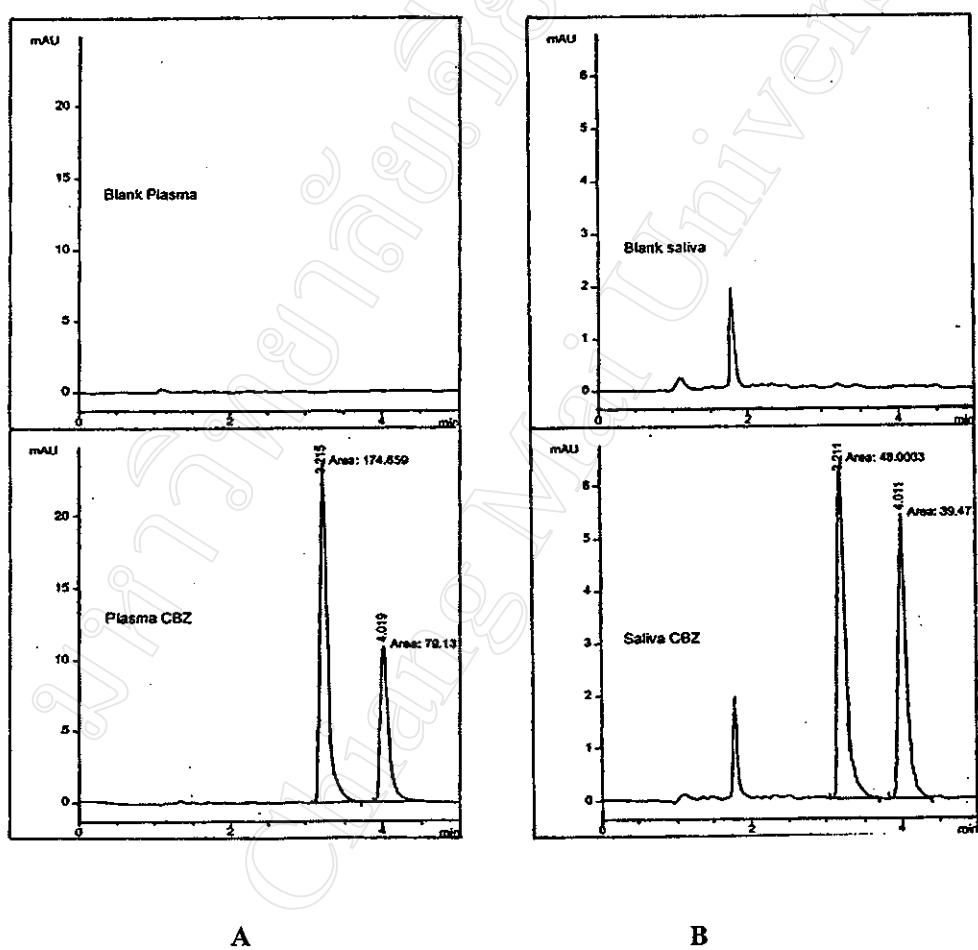


Figure 6 Chromatograms of A) plasma carbamazepine and B) saliva carbamazepine (RT  $\approx$  3.2 min) and propylparaben (RT  $\approx$  4.0 min) as internal standard.

#### 4.1 Precision of the assay procedure

The precision of this analysis method was satisfactory since the percentage relative standard deviation (%RSD) of the intraday variation for CBZ in plasma and saliva, (as shown in table 3 and 4, figure 7 and 8) were in the range of 1.3074 – 2.8934 % and 1.1022 – 7.1236 %, respectively. The %RSD of interday variation for CBZ in plasma and saliva (as shown in table 5 and 6, figure 9 and 10) were in the range of 3.1224 – 7.4443 % and 1.8621 - 4.9125 %, respectively. The limit of detection of this method was 50 ng/ml.

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Table 3 Intraday variation of analysis method for CBZ in plasma

Conc.(mcg/ml)	CBZ/PP Peak Area Ratio					
	1	2	3	Average	SD	%RSD
0	0	0	0	0	0	0
0.5	0.2291	0.2217	0.2174	0.2227	0.0059	2.6672
1.0	0.4341	0.4180	0.4102	0.4208	0.0122	2.8934
2.0	0.9667	0.9867	0.9630	0.9721	0.0127	1.3074
6.0	2.6472	2.6714	2.7527	2.6904	0.0553	2.0543
12.0	5.5255	5.5470	5.3695	5.4807	0.0969	1.7673
Correlation	0.9997	0.9997	0.9998			
Slope	2.1827	2.1707	2.2260			

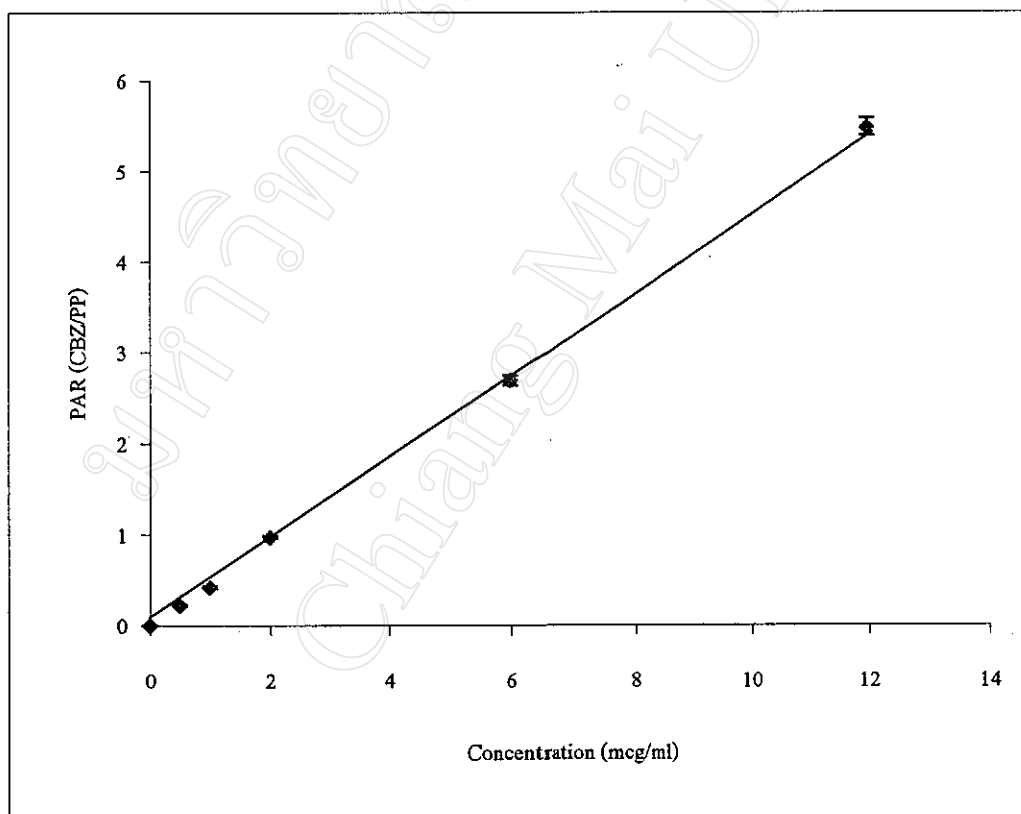


Figure 7 The intraday variation of analysis method for CBZ in plasma

Table 4 Intraday variation of analysis method for CBZ in saliva

Conc.(ng/ml)	CBZ/PP Peak Area Ratio					
	1	2	3	Average	SD	%RSD
0	0	0	0	0	0	0
50	0.0468	0.0474	0.0482	0.0475	0.0007	1.4797
100	0.0971	0.0904	0.0842	0.0906	0.0065	7.1236
500	0.4333	0.4240	0.4549	0.4374	0.0159	3.6243
1000	0.8737	0.8712	0.897	0.8806	0.0142	1.6158
2000	1.8002	1.8403	1.8192	1.8199	0.0201	1.1022
Correlation	0.9998	0.9995	1.0000			
Slope	1115.9854	1091.6863	1100.5172			

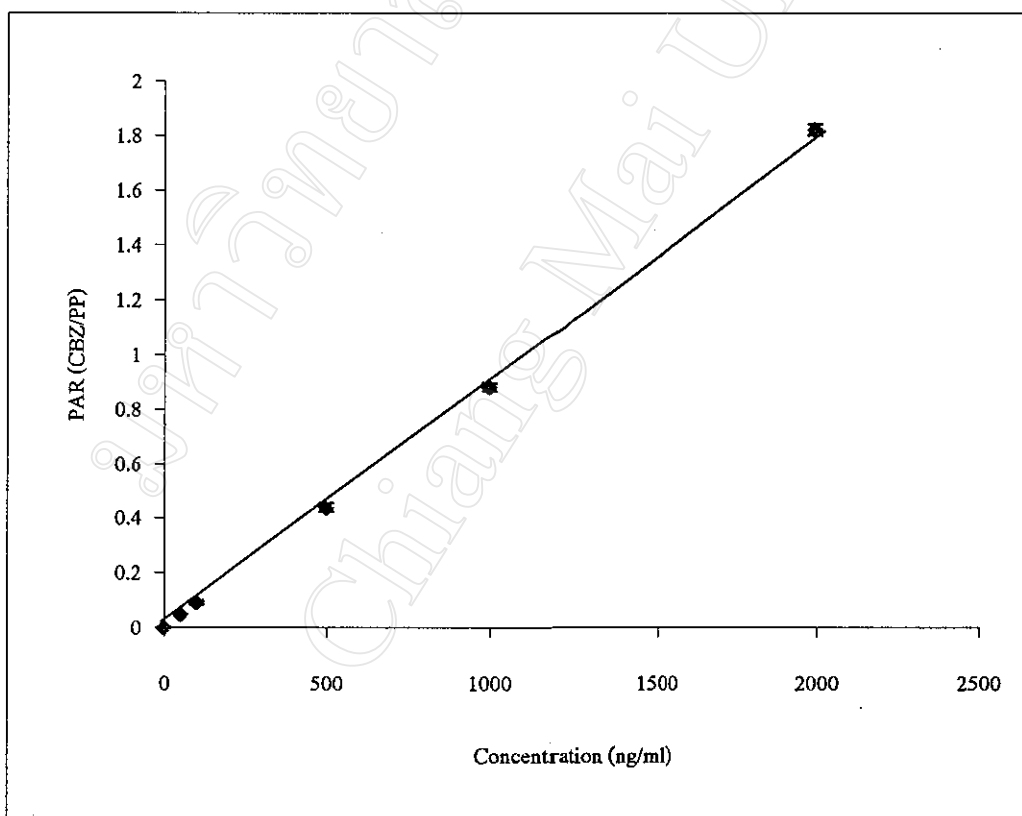


Figure 8 The intraday variation of analysis method for CBZ in saliva

Table 5 Interday variation of analysis method for CBZ in plasma

Conc.(mcg/ml)	CBZ/PP Peak Area Ratio					
	Day-1	Day-2	Day-3	Average	SD	%RSD
0	0	0	0	0	0	0
0.5	0.2227	0.2297	0.2047	0.2190	0.0129	5.8903
1.0	0.4208	0.4330	0.4068	0.4202	0.0131	3.1224
2.0	0.9721	0.8689	0.9164	0.9191	0.0517	5.6208
6.0	2.6904	2.6228	2.3332	2.5488	0.1897	7.4443
12.0	5.4807	5.4661	4.9532	5.3000	0.3005	5.6689
Correlation	0.9998	0.9998	0.9993			
Slope	2.1934	2.2010	2.4449			

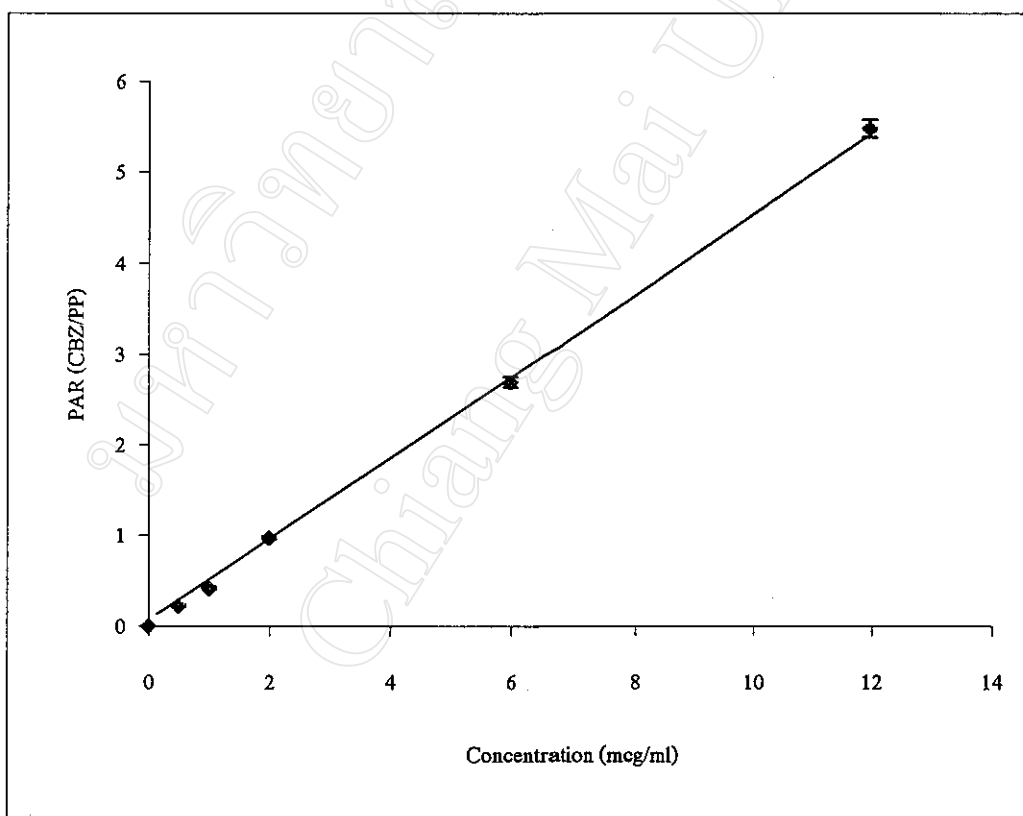


Figure 9 The interday variation of analysis method for CBZ in plasma

Table 6 Interday variation of analysis method for CBZ in saliva

Conc.(ng/ml)	CBZ/PP Peak Area Ratio					
	Day-1	Day-2	Day-3	Average	SD	%RSD
0	0	0	0	0	0	0
50	0.0475	0.0508	0.0480	0.0488	0.0018	3.6471
100	0.0906	0.0944	0.0941	0.0930	0.0021	2.2709
500	0.4374	0.4483	0.4617	0.4491	0.0122	2.7100
1000	0.8806	0.9207	0.9712	0.9242	0.0454	4.9125
2000	1.8199	1.8832	1.8285	1.8439	0.0343	1.8621
Correlation	0.9998	0.9999	0.9995			
Slope	1102.7983	1064.7081	1085.2857			

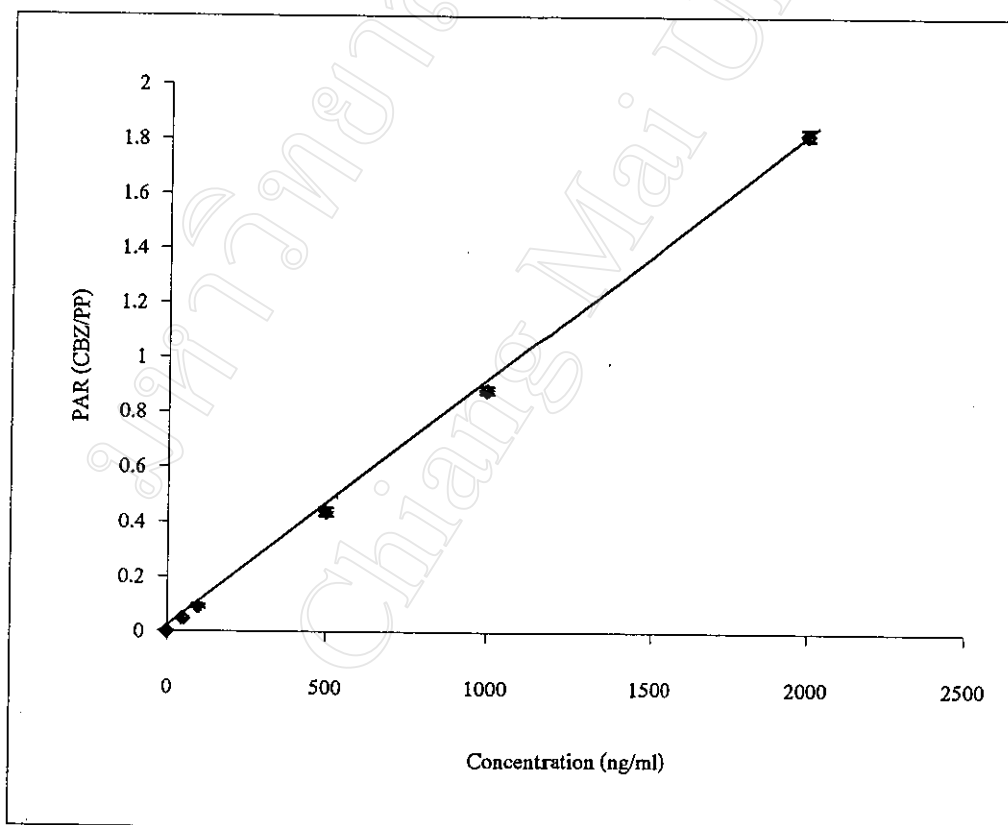


Figure 10 The interday variation of analysis method for CBZ in saliva

#### 4.2 Accuracy of the assay procedure

The percent recoveries of analysis method for CBZ in plasma and saliva were 93.88 to 98.15 % (Table 7) and 95.32 to 103.43 %, respectively (Table 8).

Table 7 Percent recoveries of analysis method for CBZ in plasma

[PCBZ] (mcg/ml)	Percent recovery					
	1	2	3	Average	SD	%RSD
0.5	96.84	96.44	101.19	98.15	2.63	2.68
1.0	98.65	96.45	95.14	96.74	1.77	1.83
2.0	93.68	93.89	94.06	93.88	0.19	0.20
6.0	95.91	95.36	95.38	95.55	0.31	0.33
12.0	94.21	99.57	96.18	96.65	2.71	2.81

Table 8 Percent recoveries of analysis method for CBZ in saliva

[SCBZ] (ng/ml)	Percent recovery					
	1	2	3	Average	SD	%RSD
50	94.63	95.50	95.82	95.32	0.62	0.65
100	97.45	95.34	97.43	96.74	1.22	1.26
500	96.56	98.98	98.56	98.03	1.30	1.32
1000	96.98	99.77	99.40	98.71	1.52	1.54
2000	102.78	103.63	103.90	103.43	0.59	0.57

### 4.3 Stabilities of CBZ in frozen plasma and saliva

The stabilities of CBZ in frozen plasma and saliva were evaluated as previously described and the results are shown in table 9 and 10. Saliva and plasma CBZ levels were not affected by storing the samples for 7 days at  $-40^{\circ}\text{C}$  ( $p > 0.05$  both plasma and saliva samples).

Table 9 Stability of CBZ in frozen plasma.

[PCBZ] (mcg/ml)	CBZ/PP peak area ratio in plasma		p-value
	Day 0 (n=3)	Day 7 (n= 3)	
0.5	$0.2356 \pm 0.0015$	$0.2266 \pm 0.0244$	0.567
1.0	$0.4261 \pm 0.0644$	$0.4118 \pm 0.0405$	0.488
2.0	$0.8719 \pm 0.0491$	$0.8547 \pm 0.0422$	0.104
6.0	$2.7333 \pm 0.0318$	$2.5715 \pm 0.1829$	0.209
12.0	$5.2979 \pm 0.2772$	$5.1985 \pm 0.2515$	0.113

Table 10 Stability of CBZ in frozen saliva.

[SCBZ] (ng/ml)	CBZ/PP peak area ratio in saliva		p-value
	Day 0 (n = 3)	Day 7 (n = 3)	
50	$0.0460 \pm 0.0001$	$0.0464 \pm 0.0003$	0.059
100	$0.0940 \pm 0.0042$	$0.0929 \pm 0.0062$	0.414
500	$0.4784 \pm 0.0038$	$0.4751 \pm 0.0058$	0.126
1000	$0.9595 \pm 0.0125$	$0.9229 \pm 0.0344$	0.102
2000	$1.8440 \pm 0.0370$	$1.8168 \pm 0.0355$	0.318



#### 4.4 The correlation between plasma and saliva CBZ concentration

The age of 50 volunteers (27 male and 23 female) were between 10 and 74 years which had plasma concentration range of 0.3015 – 11.2509 mcg/ml and saliva concentration range of 0.0847 – 3.0366 mcg/ml.

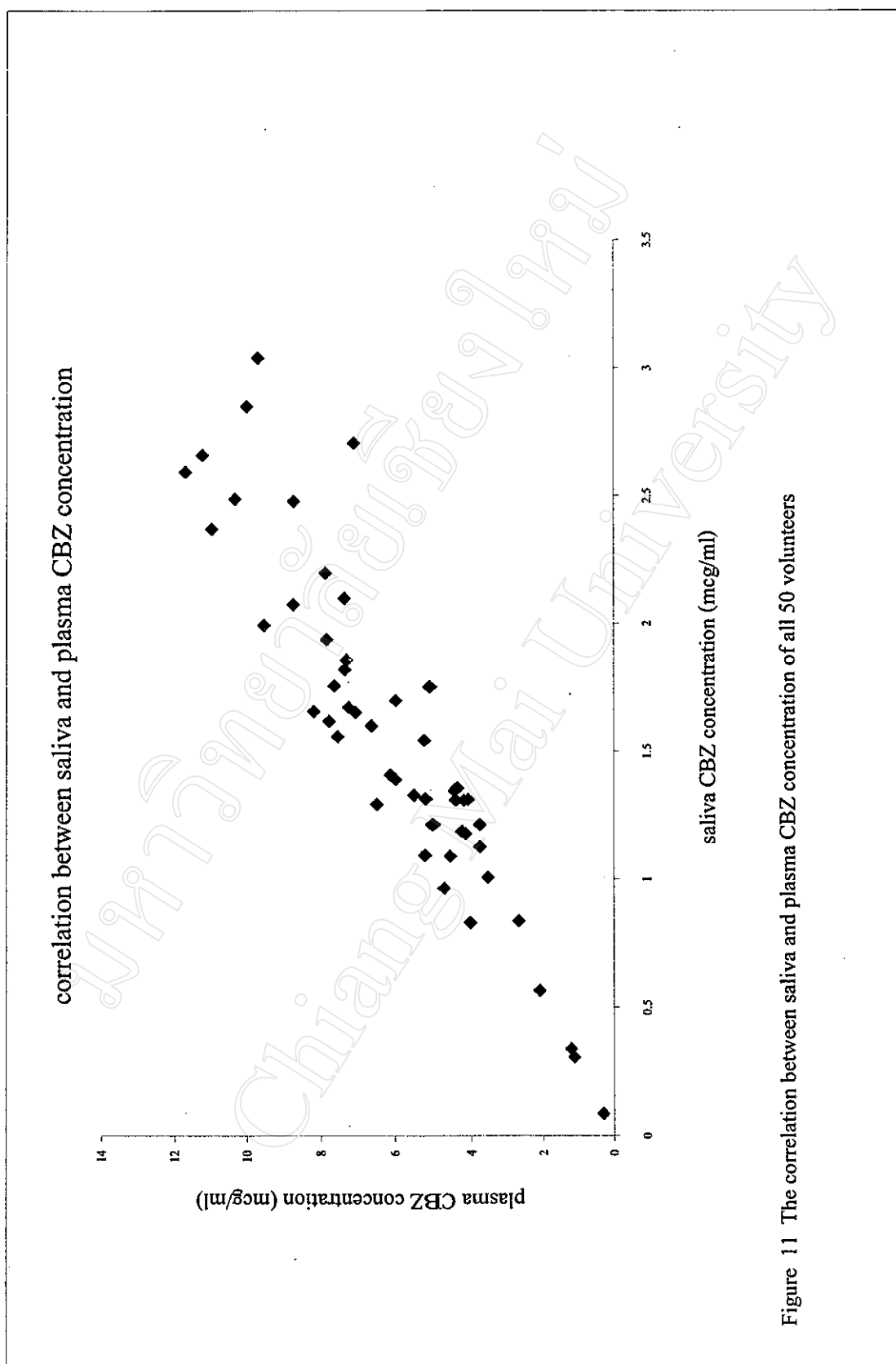
The correlation between the plasma and salivary CBZ concentration was very high linear relationship ( $r = 0.918$  at  $p < 0.001$ ), as show in figure 11. The relationship between plasma carbamazepine concentration [PCBZ] and saliva carbamazepine concentration [SCBZ] can be discribed by the below equation:

$$[\text{PCBZ}] = 3.7326 [\text{SCBZ}] + 0.2787$$

Where

[PCBZ] = plasma CBZ concentration (mcg/ml)

[SCBZ] = salivary CBZ concentration (mcg/ml)



#### 4.5 Application to patients

The calculated [PCBZ] from [SCBZ] of each patient by using the equation and actual [PCBZ] was demonstrated in table 11. When compared the calculated and actual plasma CBZ concentration values, the percent accuracy was  $103.32 \pm 4.83 \%$  with no statistic differences ( $p > 0.05$ ).

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Table 11 The individual data of application to patient

patient	sex	age	body weight	other drugs	[SCBZ] (mcg/ml)	calculated [PCBZ] (mcg/ml)	actual [PCBZ] (mcg/ml)	%accuracy
1	female	32	48	flunarizine tizanidine hydrochloride	1.0118	4.0553	3.9387	102.9615
2	female	71	51	FBC cinnarizine amitriptyline neuviplex	1.5088	5.9104	5.8027	101.8568
3	male	14	68		2.1281	8.222	8.2969	99.0978
4	male	55	74	clonazepam	2.1798	8.415	8.6826	96.9182
5	male	22	60	aspirin 60 mg	1.5713	6.1437	5.6224	109.2725
6	male	52	56	chlorpromazine 50 mg AMA folic acid benzhexol phenytoin	2.404	9.2519	9.4222	98.1922

Table 11 (Continued)

patient	sex	age	body weight	other drugs	[SCBZ] (mcg/ml)	calculated [PCBZ] (mcg/ml)	actual [PCBZ] (mcg/ml)	%accuracy
7	male	38	59	paracetamol 500 mg	1.0705	4.2744	3.8622	110.6739
8	female	48	52	felodipine aspirin 300 mg clonazepam 0.5 mg	1.4306	5.6186	5.5716	100.8428
9	female	27	52	imipramine	1.2036	4.7713	4.5304	105.3165
10	male	79	65	cinnarizine cardiprin diazepam 5 mg	1.9107	7.4106	6.8531	108.1347
							average	103.3267
							SD	4.8326
							%RSD	4.677