

## CHAPTER III

### RESULT AND DISCUSSION

1. To set up the analytical method for determining curcumin in human plasma by HPLC

#### 1.1 To determine the detection wavelength for HPLC

Curcuminoids methanolic solution exhibit the maximum absorption wavelength at 420 and 282 nm (Figure 3). Due to higher absorptivity of curcumin at 420 nm than at 282 nm, the wavelength 420 nm was used as the detector wavelength of curcumin while the wavelength of 282 nm was used for IS and possible metabolite detection.

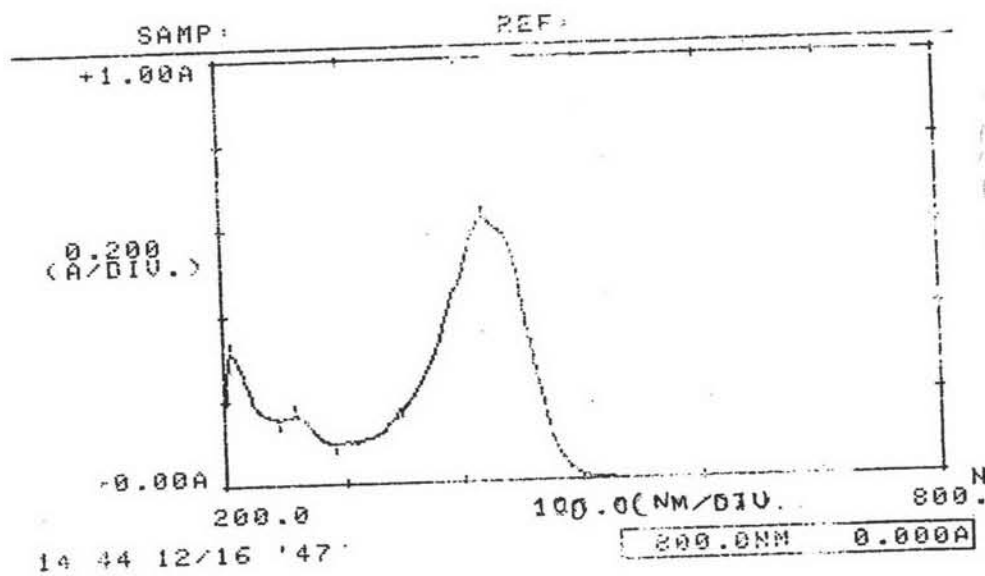


Figure 3. UV spectrum of methanolic curcuminoids solution

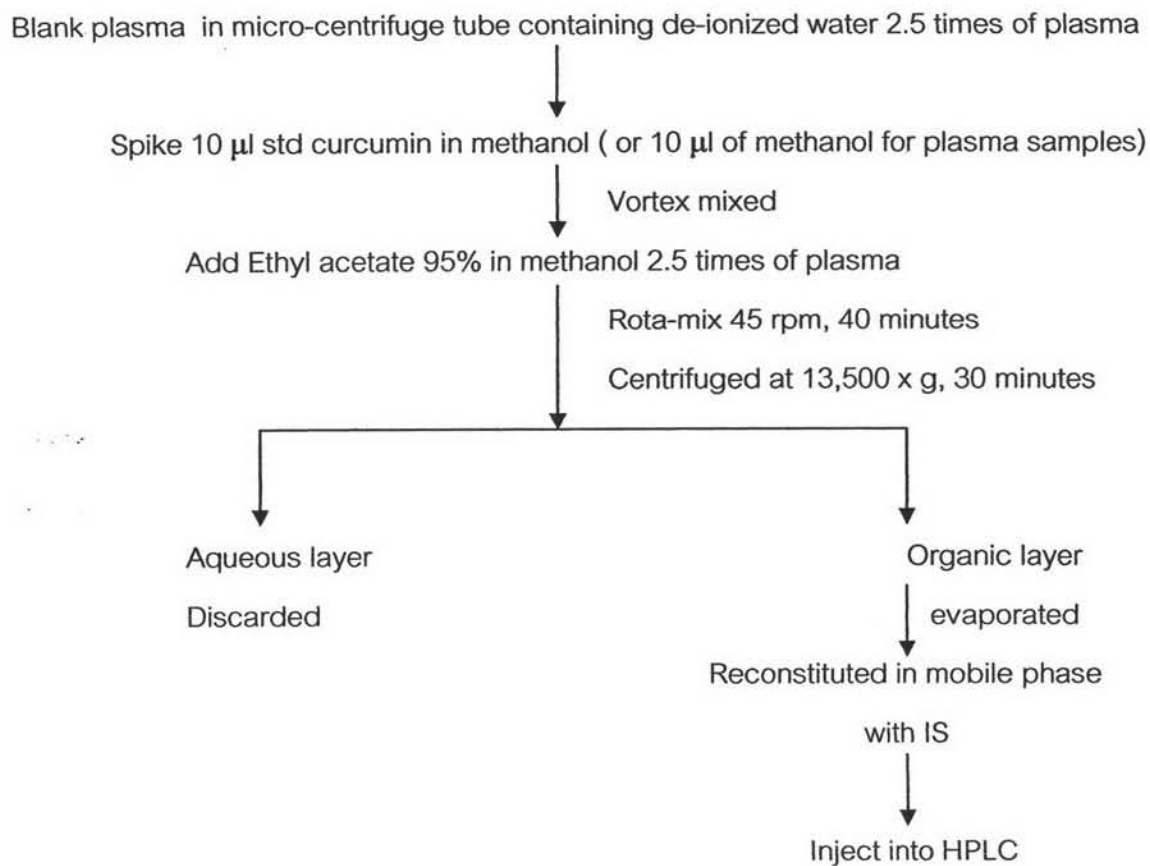
### 1.2 Sample preparation

As showed in Table 4, Figure 5 the extracted time of 40 minutes was the most appropriate for all three compounds such that the percentage recoveries was 94.48%, 98.35% and 98.04% for curcumin, demethoxycurcumin and bisdemethoxycurcumin, respectively. Therefore, this extraction time would be used in sample preparation (scheme 1)

### 1.3 Internal standard selection

By using the HPLC condition of curcumin, only mefenamic acid showed the later retention time than curcumin with the well shape peak. Therefore, mefenamic acid was selected at the IS for curcumin at the detector wavelength of 282 nm (Figure 4).

## Scheme 1.

HPLC condition

Analytical column	: Water SymmetryShield®, C <sub>18</sub> , (150 x 3.0 mm, i.d.) 5 $\mu\text{m}$
Guard column	: Corosil® C18 (20 x 2.0 mm, i.d.) 37-50 $\mu\text{m}$
Mobile phase	: ACN : MeOH : H <sub>2</sub> O : Acetic acid (41 : 23 : 36 : 1)
Flow rate	: 0.5 ml/min
Detector	: 420, 282 nm

**Table 4. Extraction efficiency of curcuminoids at different extraction time**

Curcuminoids	%recovery $\pm$ SD (n=3)			
	Extraction time (minutes)			
	10	20	30	40
Curcumin	69.50 $\pm$ 5.35	76.53 $\pm$ 5.17	85.86 $\pm$ 2.51	94.48 $\pm$ 1.15
Demethoxycurcumin	76.30 $\pm$ 4.12	82.37 $\pm$ 3.76	95.84 $\pm$ 2.46	98.35 $\pm$ 0.95
Bisdemethoxycurcumin	75.99 $\pm$ 5.69	88.86 $\pm$ 5.28	96.11 $\pm$ 1.69	98.04 $\pm$ 1.01

**Table 5. The retention time of internal standard selection under curcumin HPLC condition**

1.0 $\mu$ g/ml methanol	Retention time (minute) (n=1)
Curcumin	7.24
Demethoxycurcumin	8.46
Bisdemethoxycurcumin	9.87
Chloramphenicol	2.23
Diclofenac	7.67
Naproxen	4.12
Furosemide	2.68
Mefemic acid	12.84

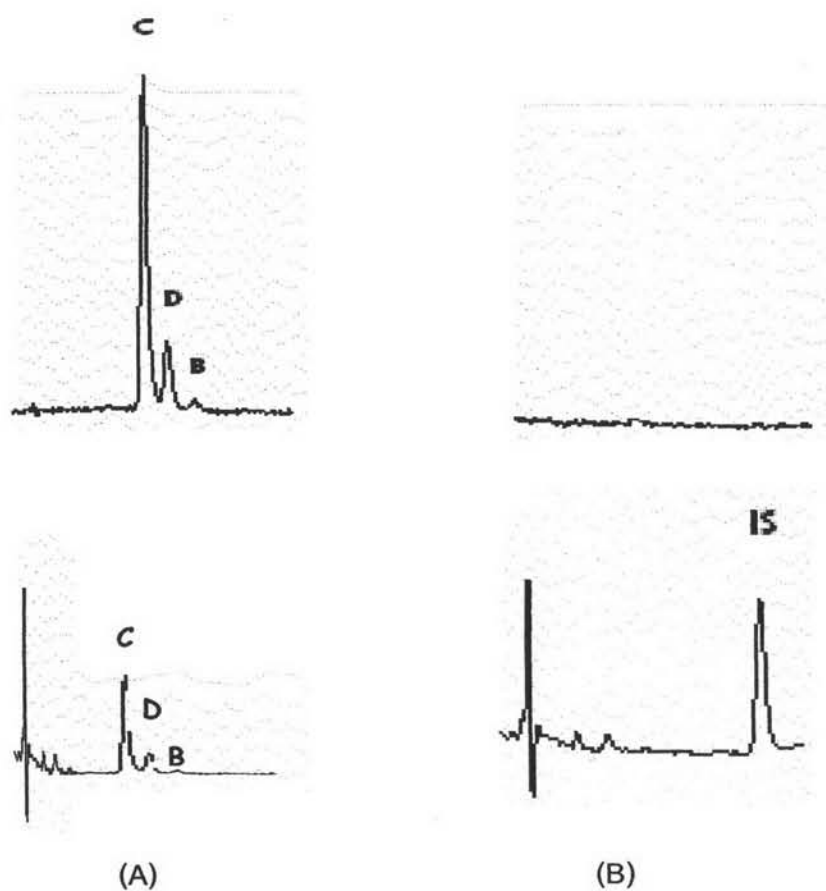


Figure 4. Chromatograms of curcuminoids and mefenamic acid

(A): Standard solution of curcuminoids 1.0 ug/ml

(B): Standard solution of mefenamic acid 0.10 ug/ml

Above: 420 nm, Bottom: 282 nm

C: curcumin, D: demethoxycurcumin, B: bisdemethoxycurcumin, IS: internal standard

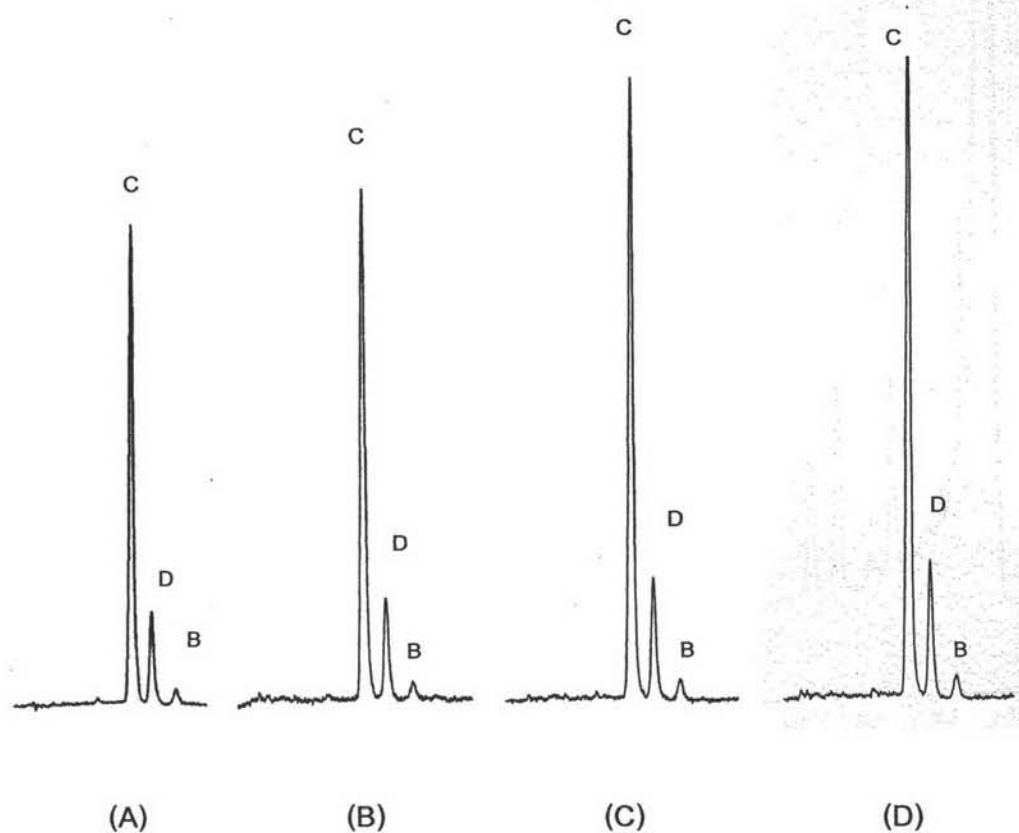


Figure 5. Chromatogram of curcuminoids from Standard plasma spiked with curcuminoids 0.80 ug/ml at different extraction time

(A) : at 10 minutes, (B) : at 20 minutes, (C) : at 30 minutes, (D) : at 40 minutes

C: curcumin, D: demethoxycurcumin, B: bisdemethoxycurcumin

## 2. To perform the bioanalytical method validation

### 2.1 Linearity

The relationship between the concentration of spiked curcumin and PAR could be explained as the linear pattern with the linear range of 0.01-1.0  $\mu\text{g/ml}$ . Figure 6 displayed the represent calibration curve of curcumin with the regression equation of  $\text{PAR} = 11.48\text{Conc.} + 0.0275$ ,  $R^2 = 0.9982$ . To confirm the linear pattern, the percentage of relative standard deviation (%RSD) of slope and  $R^2$  were determined to be 5.10 and 0.08, respectively (Table 6).

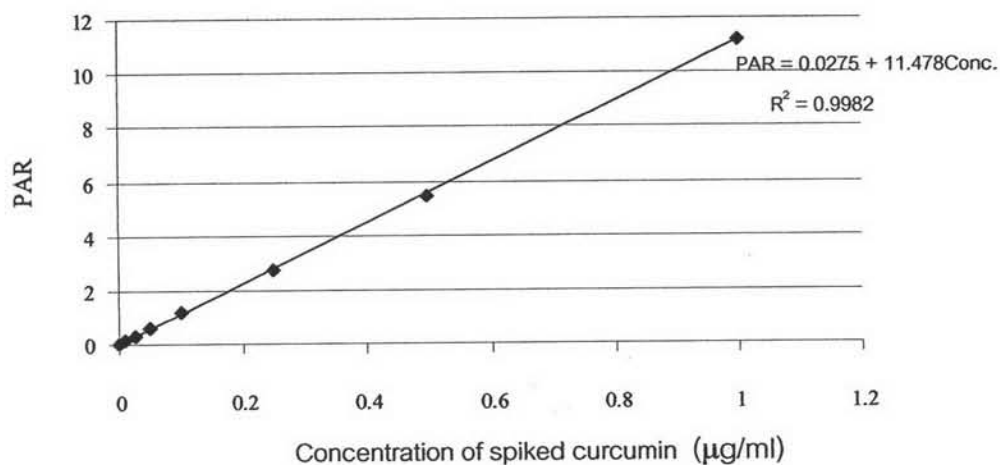


Figure 6. The represent calibration curve of curcumin

Table 6. Reproducibility of curcumin calibration curve

N	Parameters of calibration curve		
	Slope	Intercept	$R^2$
1	10.386	0.0362	0.9994
2	11.155	0.0030	0.9997
3	11.478	0.0275	0.9982
Mean	11.006	0.0222	0.9991
SD	0.56	0.017	0.0008
%RSD	5.10	-	0.08

Since %RSD of the slope and  $R^2$  were all less than 15%. This confirmed the linear range of the calibration curve was then confirmed.

## 2.2 Accuracy and precision

### 2.2.1 The intra-day accuracy and precision

The percentage of bias in the same day assay of spiked curcumin in plasma were ranged from -14.64 to +14.83% for the three plasma curcumin concentrations covered the concentration range in plasma that possibly found in the body as shown in Table 7. The %RSD were between 4.23 and 10.74% (Table 8). Therefore, the method for curcumin analysis in plasma was accurate and precise enough for applicable use.

### 2.2.2 The inter-day accuracy and precision

The percentage of bias at six difference days of spiked curcumin in plasma were ranged from -9.79 to +6.80% and the percentage of relative standard deviation were between 3.64 and 5.61 as displayed in Table 9 and 10, respectively.

Both accuracy and precision of either intra-day or inter-day were within the limit range ( $\pm 15\%$  of %bias and within 15% of %RSD); therefore, this analytical method revealed the acceptable accuracy and precision.



Table 7. The intra-day accuracy of analysis of curcumin in spiked plasma

Spiked curcumin concentration ( $\mu\text{g/ml}$ )	%Bias (n=6)						Mean
	1	2	3	4	5	6	
0.04	+1.47	+2.40	+7.68	+6.97	-1.25	-3.15	+2.35
0.40	-9.94	-10.87	+14.83	-10.89	-8.47	-9.14	-5.75
0.80	-14.64	+10.30	-11.03	-9.44	-14.63	-11.36	-8.47

Table 8. The intra-day precision of analysis of curcumin in spiked plasma

Spiked curcumin concentration ( $\mu\text{g/ml}$ )	Analyzed curcumin concentration (n=6)						Mean $\pm$ SD	%RSD
	1	2	3	4	5	6		
0.04	0.041	0.041	0.043	0.043	0.039	0.039	0.041 $\pm$ 0.002	4.23
0.40	0.360	0.356	0.459	0.356	0.366	0.363	0.377 $\pm$ 0.040	10.74
0.80	0.683	0.882	0.712	0.724	0.683	0.709	0.732 $\pm$ 0.070	10.29

Table 9. The inter-day accuracy of analysis of curcumin in spiked plasma

Spiked curcumin concentration ( $\mu\text{g/ml}$ )	%Bias						Mean
	1	2	3	4	5	6	
0.04	+6.66	-1.12	+0.25	-9.79	-5.41	-2.75	-2.03
0.40	-3.83	+3.86	+1.55	+2.78	-1.31	+6.48	+1.59
0.80	-1.32	+5.03	+5.89	+5.15	-2.34	+6.80	+3.20

Table 10. The inter-day precision of analysis of curcumin in spiked plasma

Spiked curcumin concentration ( $\mu\text{g/ml}$ )	Analyzed curcumin concentration						Mean $\pm$ SD	%RSD
	1	2	3	4	5	6		
0.04	0.043	0.040	0.040	0.036	0.038	0.039	0.039 $\pm$ 0.002	5.61
0.40	0.385	0.415	0.406	0.411	0.395	0.426	0.406 $\pm$ 0.010	3.64
0.80	0.789	0.840	0.847	0.841	0.781	0.854	0.826 $\pm$ 0.030	3.83

### 2.3 Sensitivity

The limit of quantitation (LOQ) of curcumin in plasma was determined to be 0.01  $\mu\text{g/ml}$ , as exhibited in Table 11. It was the lowest concentration of curcumin that the %bias and %RSD of analysis were less than  $\pm 20\%$  and 20%, respectively. This concentration of curcumin was used as the initial concentration in the calibration curve.

### 2.4 Specificity

As shown in Figure 7, the specificity of the analytical method was clearly presented in which curcuminoids in standard solution, spiked curcuminoids and IS in blank plasma gave the identical retention times. They were eluted 7.6, 8.9 and 10.5 and 12.8 minutes for curcumin, demethoxycurcumin, bisdemethoxycurcumin and IS, respectively. The presented chromatograms showed that both curcuminoids and IS were well resolved from each other without any detectable interference, whether in spiked standard plasma or sample plasma from volunteer following curcuminoids administration.

Table 11. The limit of quantitation (LOQ) for curcumin in spiked plasma

Concentration of curcumin ( $\mu\text{g/ml}$ )	N	Analyzed concentration of curcumin ( $\mu\text{g/ml}$ )	%Bias
0.01	1	0.0110	-3.60
	2	0.0128	+12.30
	3	0.0134	+17.50
	4	0.0128	+12.00
	5	0.0126	+10.20
	6	0.0127	+11.30
Mean		0.0126	+9.95
SD		0.0079	
%RSD		6.31	

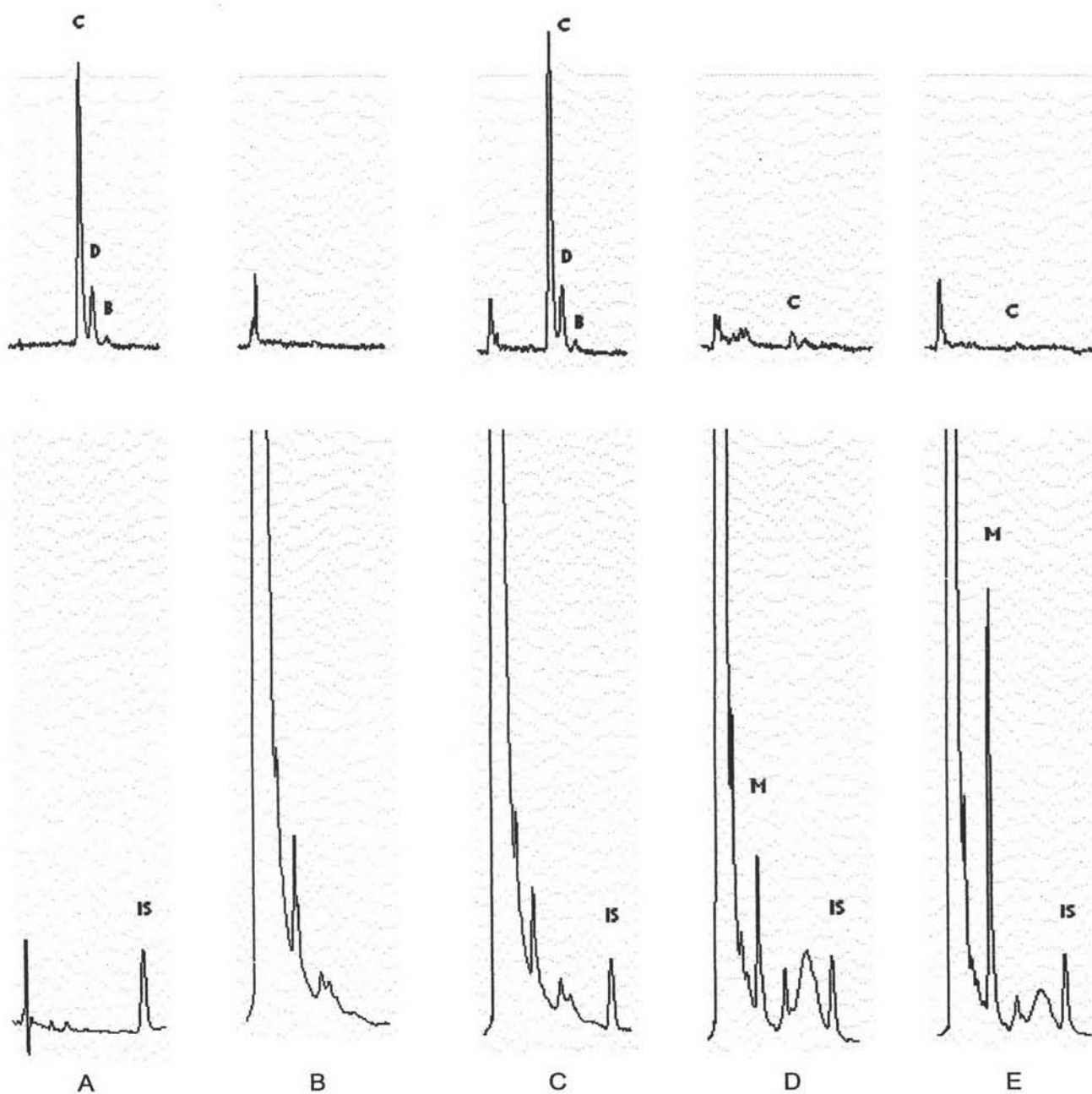


Figure 7. Chromatogram of curcuminoids and IS

Above: detector wavelength 420 nm, Bottom: detector wavelength 282 nm

A : Standard solution of curcuminoids 1.0 ug/ml and IS 0.10 ug/ml

B : Blank plasma

C : Standard plasma spiked with curcuminoids and IS

D : Curcumin plasma sample from subject at 3 hours after oral administration

E : Curcumin plasma sample from subject at 8 hours after oral administration

IS = internal standard; C = curcumin; D = demethoxycurcumin;

B = bisdemethoxycurcumin ; M = metabolite

## 2.5 Stability of plasma sample , in-processed analyte and stock solution

### 2.5.1 Stability of curcumin in plasma sample

#### *Stability of curcumin in plasma sample at room temperature (23±2 °C)*

As shown in Table 12 and 13 plasma curcumin was stable at room temperature up to 8 hours with the percentages of curcumin remained in the range of 102.2 to 103.3%.

#### *Stability of curcumin in plasma sample at storage temperature (-47±1 °C)*

Curcumin could still be stable in plasma at the freezing temperature (-48±1 °C) to less than 15 days. The percentage recoveries of both 0.04 µg/ml and 0.80 µg/ml were 98.12% and 101.5%, respectively (Table 14 and 15).

#### *Stability of curcumin in plasma sample under the freeze-thaw cycle*

As demonstrated in Table 16 and 17, curcumin in plasma could be stable even after three cycles of freeze and thawed. The percentage of average remaining of curcumin (0.04 µg/ml) from the first to third cycle were determined to be 98.97, 96.69 and 94.55, respectively and for 0.08 µg/ml were 95.60 , 95.33 and 97.82, respectively. Thus, plasma sample could be used and restored in the frozen temperature up to three times.

Table12. Stability of curcumin 0.04 µg/ml in plasma at room temperature (23±2 °C)

Storage time at room temperature (hrs.)	%remained (n=3)			Mean±SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0±0.00	0.00
3	109.5	97.71	101.6	102.9±6.03	5.86
6	103.1	106.1	103.7	104.3±1.57	1.50
8	108.1	109.4	92.33	103.3±9.52	9.21

Table13. Stability of curcumin 0.08 µg/ml in plasma at room temperature (23±2 °C)

Storage time at room temperature (hrs.)	%remained (n=3)			Mean±SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0±0.00	0.00
3	100.6	99.38	104.6	101.5±2.73	2.69
6	104.0	97.61	96.96	99.53±3.90	3.92
8	100.1	101.9	104.5	102.2±2.23	2.18

**Table 14. Stability of curcumin 0.04 µg/ml in plasma stored at -47±1 °C**

Storage time in freezer (days.)	%remained (n=3)			Mean±SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0±0.00	0.00
4	103.9	103.5	105.3	104.3±0.95	0.91
6	102.7	99.53	98.22	100.1±2.30	2.30
11	96.08	101.9	90.36	96.11±5.76	5.99
15	92.40	107.3	94.67	98.12±8.02	8.17

**Table 15. Stability of curcumin 0.08 µg/ml in plasma stored at -47±1 °C**

Storage time in freezer (days.)	%remained (n=3)			Mean±SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0±0.00	0.00
4	94.88	97.66	93.81	95.45±1.99	2.08
6	93.31	97.46	90.16	93.64±3.66	3.91
11	96.01	91.13	92.05	93.06±2.59	2.79
15	106.9	96.49	101.1	101.5±5.21	5.13



Table 16. Stability of curcumin 0.04 µg/ml in plasma under the freeze-thaw cycle

Number of freeze and thaw cycle	%remained (n=3)			Mean±SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0±0.00	0.00
1	98.28	92.94	105.7	98.97±6.40	6.46
2	90.44	104.7	94.92	96.69±7.30	7.55
3	92.40	98.35	92.89	94.55±3.30	3.49

Table 17. Stability of curcumin 0.08 µg/ml in plasma under the freeze-thaw cycle

Number of freeze and thaw cycle	%remained (n=3)			Mean±SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0±0.00	0.00
1	98.76	92.68	95.36	95.60±3.05	3.19
2	94.22	95.86	95.92	95.33±0.96	1.01
3	99.77	94.93	98.76	97.82±2.55	2.61

### 2.5.2 Stability of in-processed analyte in the autosampler

For in-processed analyte that was transferred into the autosampler (4 °C) waiting for injection into HPLC , curcumin could be withstood only within 16 hours (Table 18 and 19). The percentage of average remaining of curcumin were observed to be between 98.73 and 102.16%. Therefore, it was necessary to manage the number of processed analyte in autosampler to be injected within less than 16 hours to ensure no occurrence of decomposition.

### 2.5.3 Stability of curcumin or IS stock solution

As shown in Table 20, after 15 days storage at  $-18\pm 2^{\circ}\text{C}$  and protected from light , the percentage of average remaining of curcumin and IS calculated from peak area were ranged between 94.82 to 98.94 and 94.64 to 101.72, respectively. The results indicated that methanolic stock solution of both curcumin and IS were stable at least 15 days in these conditions.

Table 18. Stability of curcumin 0.04  $\mu\text{g/ml}$  in-processed analyte in the autosampler ( $4^\circ\text{C}$ )

Number of freeze and thaw cycle	%remained (n=3)			Mean $\pm$ SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0 $\pm$ 0.00	0.00
16	92.62	105.5	98.03	98.73 $\pm$ 6.48	6.57

Table 19. Stability of curcumin 0.08  $\mu\text{g/ml}$  in-processed analyte in the autosampler ( $4^\circ\text{C}$ )

Number of freeze and thaw cycle	%remained (n=3)			Mean $\pm$ SD	%RSD
	1	2	3		
0	100.0	100.0	100.0	100.0 $\pm$ 0.00	0.00
16	104.2	98.10	104.20	102.2 $\pm$ 3.52	3.44

Table 20. Stability of stock standard methanolic curcumin or IS solution stored at  $-18\pm 2^{\circ}\text{C}$

Storage time in freezer (days)	curcumin (1.5 $\mu\text{g/ml}$ ) (n=3)		IS (0.48 $\mu\text{g/ml}$ ) (n=3)	
	%recovery		%recovery	
	Mean $\pm$ SD	%RSD	Mean $\pm$ SD	%RSD
0	100 $\pm$ 0.00	0.00	100 $\pm$ 0.00	0.00
2	98.94 $\pm$ 1.37	1.38	100.5 $\pm$ 0.22	0.22
4	96.46 $\pm$ 1.34	1.38	101.7 $\pm$ 4.27	4.20
6	96.45 $\pm$ 1.53	1.58	100.5 $\pm$ 1.36	1.36
11	94.82 $\pm$ 0.45	0.48	95.84 $\pm$ 2.35	2.45
15	96.56 $\pm$ 3.95	4.09	94.64 $\pm$ 0.84	0.89

### 3. To determine the content of curcumin in curcuminoids tablets

#### 3.1 To confirm the proportional ratio of curcuminoids tablet and extracted turmeric powder

The proportional ratio of curcumin: demethoxycurcumin: bisdemethoxycurcumin in turmeric was 0.90: 0.08: 0.02. Meanwhile the proportional ratio of curcuminoids component in tablet was 0.89: 0.09 :0.01. No difference in the chromatogram was observed as showed in Figure 8. Therefore, the manufacturing process was not effect their component.

#### 3.2 Weight variation

In this study, weight individually 10 whole tablets due to have a few curcuminoids tablets. The criteria in USP 24, the weight of tablet was higher than 324 mg used not more than 5% difference of average weight, then curcuminoids tablet weight within 430.35-475.65 mg, not more than 2 of the tablets differ from the average weight (Table 21).

#### 3.3 The content of curcumin in curcuminoids tablets

The content of curcumin, demethoxycurcumin and bisdemethoxycurcumin were determined to be 88.22, 88.00 and 88.48%, respectively (Table 22).

Table 21. Weight of curcuminoids tablets

No. Tablet	Weight of tablet (g)	
	Pre-sliced film coat	Post-sliced film coat
1	0.4596	0.4070
2	0.4479	0.4011
3	0.4473	0.4037
4	0.4557	0.4095
5	0.4527	0.4089
6	0.4494	0.4006
7	0.4546	0.4083
8	0.4542	0.4044
9	0.4528	0.3964
10	0.4559	0.4090
Mean	0.4530	0.4049
SD	0.85	1.09
RSD	0.0039	0.0044

Table 22. Content of curcumin in tablets

Sample	%Analysis of content
1	88.19
2	88.00
3	88.48
Mean	88.22
SD	0.24
RSD	0.27

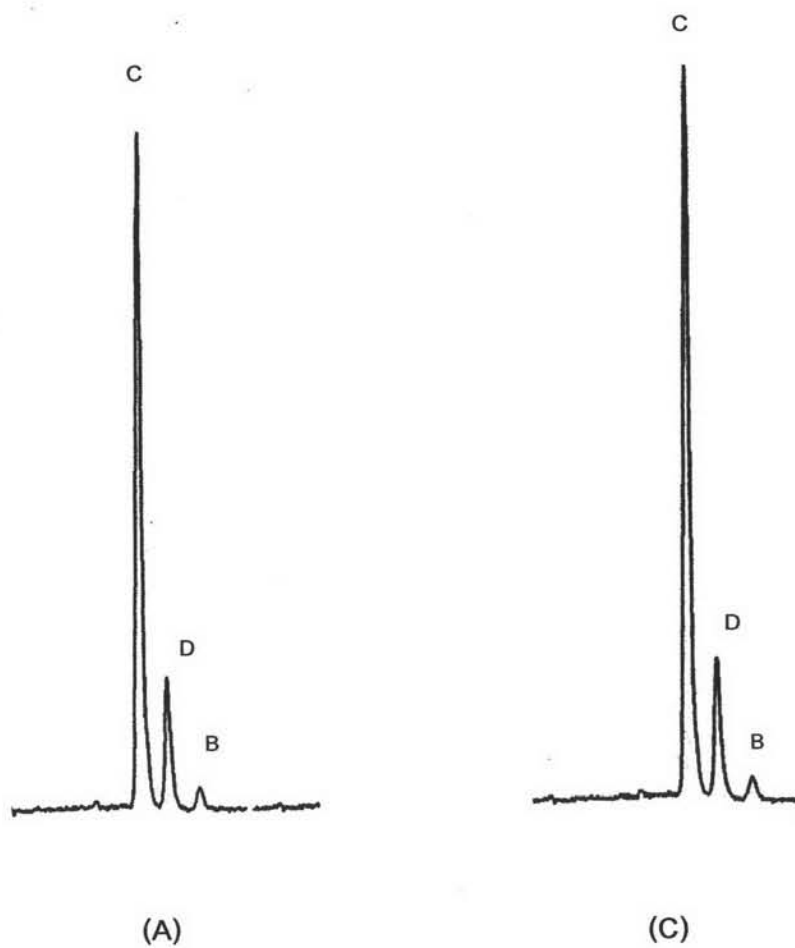


Figure 8. Chromatograms of curcuminoids tablets and tumeric extracted powder

A : curcuminoids tablets

B : tumeric extracted powder

#### 4. Pharmacokinetic study of curcumin in Thai healthy volunteers

##### 4.1 Volunteer characteristics

Physical characters of all volunteers were displayed in Table 23 They were considered healthy from blood chemistry examinations (appendix A). Every subject strictly followed the experiment recommendation. No any adverse effects were observed during the study. Dark yellow feces were noticed in every subject when administered curcuminoids tablets up to two days. This would probably due to curcumin elimination after absorption as has been reported elsewhere (Sharma, R.A., 2004).

Table 23. Physical characters of 12 volunteers

Subject code	age (years)	height (m)	weight (kg)	BMI (kg/m <sup>2</sup> )
S01	36	1.70	72	24.91
S02	23	1.77	70	22.34
S03	22	1.69	55	19.26
S04	30	1.58	55	22.03
S05	29	1.66	58	21.05
S07	21	1.78	86	27.14
S08	21	1.78	78	24.62
S09	21	1.79	80	26.12
S10	21	1.68	58	20.55
S11	21	1.65	62	22.77
S12	24	1.70	64	22.14
S13	21	1.70	60	20.76
Mean±SD	24.17±4.90	1.71±0.06	66.50±10.51	22.81±2.41



#### 4.2 Plasma curcumin concentration determination

According to the analytical method used, curcumin plasma samples could be mostly detected till 6-8 hours after drug administration. For the very late point data at 12 hours, plasma sample needed to be more concentrated for detection. Demethoxycurcumin and bisdemethoxycurcumin could hardly be detected. Curcumin concentration was determined from the calibration equation such that curcumin concentration – time profile of each subject was portrayed (appendix B). The mean concentration – time profile was also displayed in Figure 9.

At detector wavelength of 282 nm, the metabolite peak was observed (Figure 7) as other previous report (Cheng, A.L., 2001; Sharma, R.A., 2004). The metabolite peaks were observed at approximately 3 hours after oral administration of curcuminoids tablets. However, the analytical method utilized was unable to resolve all metabolite peaks. This finding is vital and have to be further studied.

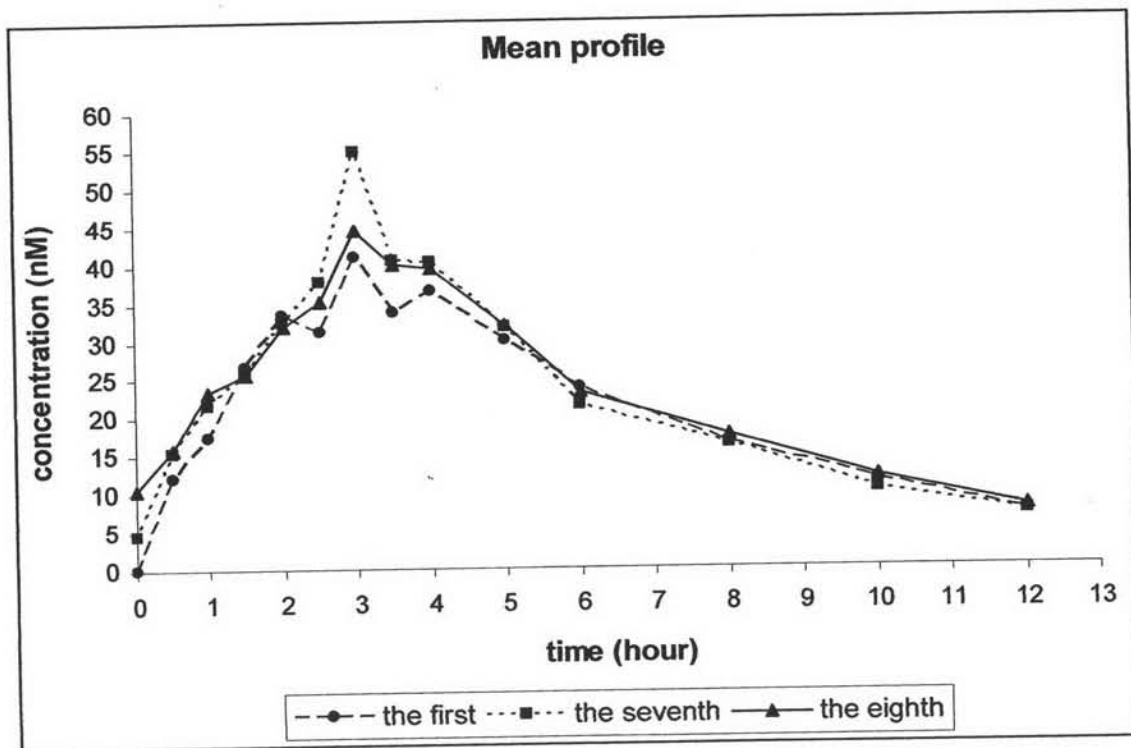


Figure 9. Mean concentration – time profiles of subject following the first, seventh and eighth day of oral administration of curcuminoids tablets

### 4.3 Pharmacokinetic parameters determination

The  $T_{max}$  and  $C_{max}$  values of plasma curcumin in each subject on the first, seventh and eighth day of experiment were directly determined from the concentration – time profiles as showed in Table 24. No statistically significant difference of the  $T_{max}$  or the  $C_{max}$  value in these three different days was observed, at significant level of 0.05 with the p-values of 0.25 and 0.45, respectively. This  $T_{max}$  value was little difference to Cheng' s study that the curcumin tablet was used in pre-malignant lesion patients (Cheng et al., 2001). The  $C_{max}$  values of curcumin were only within 36 to 152 nM, even though the administered dose of curcuminoids was 6 grams. It would then supported other studies even in animal or patient that curcumin concentration was almost undetectable at the dose less than 4 grams (Cheng et al., 2001).

In addition, no statistically significant difference of the first, seventh and eighth day for the area under the concentration – time curve (AUC) of curcumin and elimination half-life,  $t_{1/2}$ , were observed (Table 25). This result confirmed no curcumin accumulation during these eight days of drug administration.

The MRT value that represented the time for 63.20% of the administered dose to be eliminated also showed non significant different among these three days ( $p = 0.73$ ). It implied the independency of curcumin in circulation during eight days of repetitive dosing

The apparent volume of distribution at steady state ( $V_{ss}$ ) of all subjects were in the range of 216 – 537 L. No statistically significant difference of the  $V_{ss}$  value among three days were observed ( $p = 0.78$ ). In addition, the observed clearance was determined to be 40.23 – 78.57 L/hr with the significantly different of 0.29 within three day of blood sampling. These  $V_{ss}$  and Cl/F values would possibly be the parameter of curcumin for Thai people.

The inter-subject variation on each pharmacokinetic parameter was also indicated illustrated in Table 24 to Table 26. As for the informations of curcumin pharmacokinetic in Thais, the 95% confident interval of pharmacokinetic parameters were tabulated in Table 27.

Due to the very short elimination half-life of curcumin, the accumulation of curcumin would not be the problem. The average accumulation value (R) was determined to be  $1.09 \pm 0.06$  with the mean steady-state curcumin concentration of  $12.98 \pm 0.44$  nM. Although the dosage of 6 grams of curcuminoids would not harm the consumers, the concentration of detected metabolite would be the other concern.

These pharmacokinetic parameters of curcumin in Thai subjects would be meaningful in managing dose recommendation for further used of curcumin as supplement or drug therapy. However, the metabolite peaks of curcumin should not be ignored in explanation the full profile for curcumin. It is therefore suggested for further studies.

Table 24. The  $T_{max}$  and  $C_{max}$  values of plasma curcumin among three days

Subject	$T_{max}$ (hour)			$C_{max}$ (nM)		
	Day-1	Day-7	Day-8	Day-1	Day-7	Day-8
1	1.5	3.0	3.0	36.33	46.77	48.26
2	4.0	5.0	3.5	68.80	61.36	87.12
3	3.0	3.0	3.5	55.57	152.64	56.58
4	2.5	2.0	4.0	57.56	56.25	55.33
5	2.0	3.0	3.0	69.38	83.97	67.47
6	3.5	3.0	3.5	38.12	35.43	42.45
7	3.5	4.0	4.0	44.78	55.95	51.14
8	4.0	4.0	4.0	44.97	45.98	55.35
9	3.0	3.0	3.0	34.65	46.79	52.04
10	3.0	3.0	4.0	49.27	49.08	45.14
11	3.0	3.0	3.0	44.35	58.04	43.40
12	3.0	3.0	3.0	68.89	46.17	38.40
Mean	3.0	3.25	3.46	51.06	61.54	53.56
SE	0.21	0.22	0.13	3.70	8.97	3.79
ANOVA ( $\alpha=0.05$ )	NS ( $p = 0.25$ )			NS ( $p = 0.45$ )		

Table 25. The  $AUC_{0-\infty}$ ,  $MRT_{0-\infty}$  and  $t_{1/2}$  values of curcumin in among three days

Subject	$AUC_{0-\infty}$ (nM.hr)			$MRT_{0-\infty}$ (hour)			$t_{1/2}$ (hours)		
	Day-1	Day-7	Day-8	Day-1	Day-7	Day-8	Day-1	Day-7	Day-8
1	291.3	280.1	282.1	6.93	7.65	9.29	3.34	4.58	6.36
2	299.3	337.4	363.2	6.15	6.27	6.01	2.94	3.27	2.86
3	302.3	405.3	294.6	6.38	5.37	6.50	3.73	3.23	3.99
4	284.2	303.8	301.8	6.24	8.18	6.55	3.58	6.60	4.07
5	389.8	370.5	347.5	6.80	7.29	9.15	3.67	4.97	6.35
6	264.7	207.5	269.9	7.02	5.90	6.36	3.39	3.83	3.52
7	257.8	354.9	349.8	7.22	7.46	7.55	4.26	4.23	4.19
8	289.6	288.0	362.5	6.11	6.06	5.76	2.74	3.00	2.81
9	199.4	241.5	264.3	5.54	4.94	5.16	3.00	2.48	2.94
10	251.4	283.2	318.4	5.95	6.58	6.18	3.06	3.74	3.07
11	313.0	320.2	307.2	7.44	6.74	7.67	4.08	4.05	4.16
12	307.2	311.7	276.8	7.53	8.87	7.31	5.13	6.50	4.07
Mean	287.5	308.7	311.5	6.61	6.78	6.96	3.58	4.20	4.03
SE	13.01	15.79	10.48	0.18	0.33	0.37	0.19	0.37	0.35
ANOVA ( $\alpha=0.05$ )	NS ( $p = 0.39$ )			NS ( $p = 0.73$ )			NS ( $p = 0.50$ )		

Table 26. The  $V_{ss}$ ,  $Cl/F$  and  $C_{ss,av}$  values of curcumin in among three days

Subject	$V_{ss}$ (L)			$Cl/F$ (L/hr)			$C_{ss,av}$ (nM)
	Day-1	Day-7	Day-8	Day-1	Day-7	Day-8	
1	388	445	537	56.00	58.22	57.81	11.75
2	335	303	270	54.47	48.32	44.89	15.13
3	344	216	359	53.93	40.23	55.35	12.28
4	358	439	354	57.38	53.67	54.09	12.58
5	284	321	429	41.83	44.01	46.91	14.48
6	432	462	385	61.60	78.57	60.40	11.25
7	457	342	352	63.24	45.94	46.60	14.58
8	344	343	259	56.30	56.60	44.98	15.10
9	453	333	318	81.75	67.52	61.69	11.01
10	386	379	316	64.85	57.58	51.20	13.27
11	388	343	330	52.10	50.91	43.03	12.80
12	400	464	430	53.07	52.31	58.91	11.53
Mean	381	366	362	58.04	54.49	52.16	12.98
SE	14.72	21.64	22.14	2.76	3.04	1.94	0.44
ANOVA ( $\alpha=0.05$ )	NS ( $p = 0.78$ )			NS ( $p = 0.29$ )			-

Table 27. Pharmacokinetic parameters of curcumin in Thai subjects at 95% confidence interval

Pharmacokinetic parameter	95%confidence interval
$T_{max}$ (hr)	2.46 – 5.51
$C_{max}$ (nM)	48.37 – 62.40
$AUC_{0-\infty}$ (nM.hr)	287.0 – 318.1
MRT (hr)	6.43 – 7.13
$t_{1/2}$ (hr)	3.57 – 4.31
$V_{ss}$ (L)	347 – 392
CL (L/hr)	51.80 – 58.00
$C_{ss}$ (nM)	12.02 – 13.94