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## **APPENDIX I**

**UV SPECTRUM OF *C. asiatica* (UK lab.)**

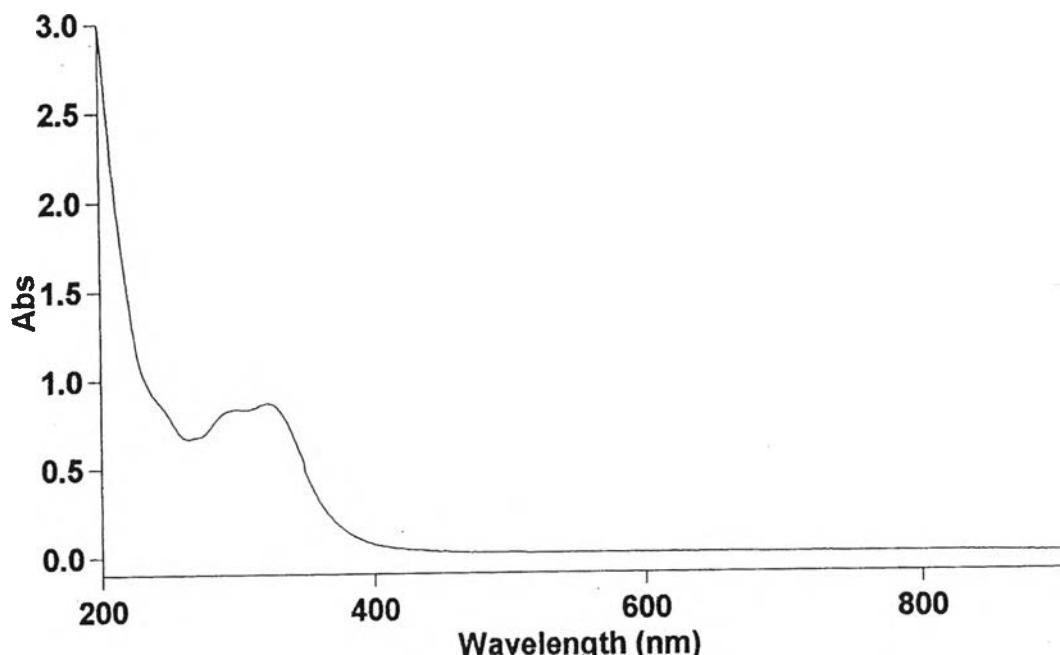


Figure 1A Spectrum of *C. asiatica* fresh leaves  
in ethanol extract (UK lab.)

### Scan Report

Batch ethanol extract  
Software version : 01.00 (6)

#### Peak table

Peak type	peaks
Peak Threshold	0.1000
Range	900.00 nm to 200.00 nm

Wavelength (nm)	Abs
322.00	1.7446
255.00	0.2351
220.00	0.4421

Because of the most widely used detectors for liquid chromatography are based upon absorption of UV or visible radiation. As result for ethanol extract as above the functional groups of **asiaticoside** and other from *C. asiatica* fresh leaves absorb as above region.

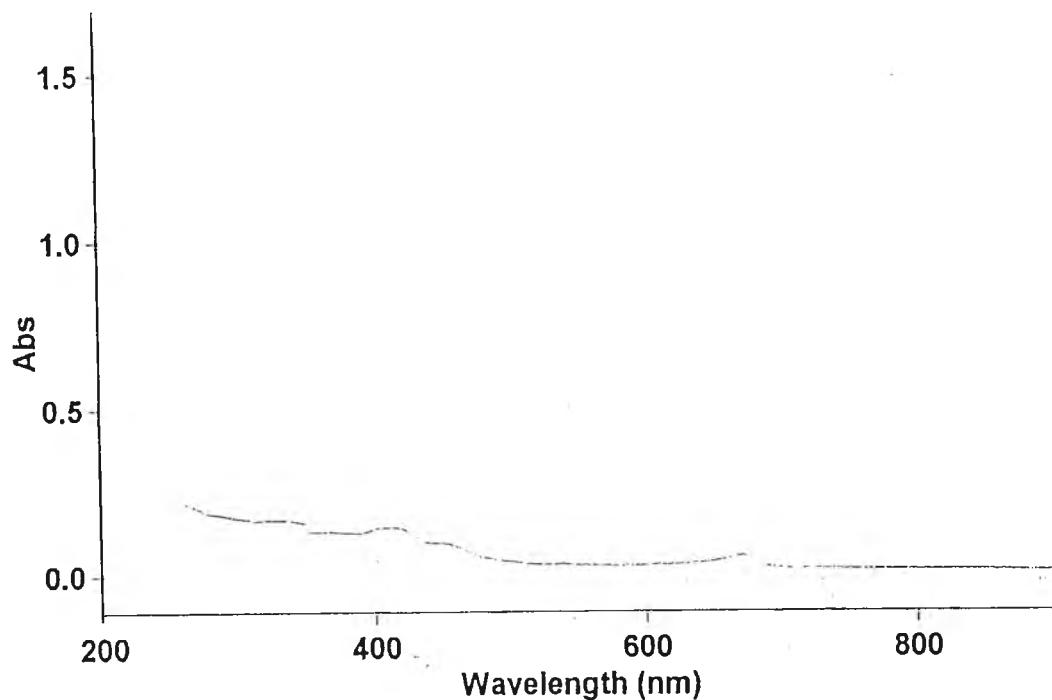


Figure 1B Spectrum of hexane extract from *C. asiatica*  
fresh leaves.(UK lab).

### Scan Report

Batch hexane extract  
Software version : 01.00 (6)

#### Peak table

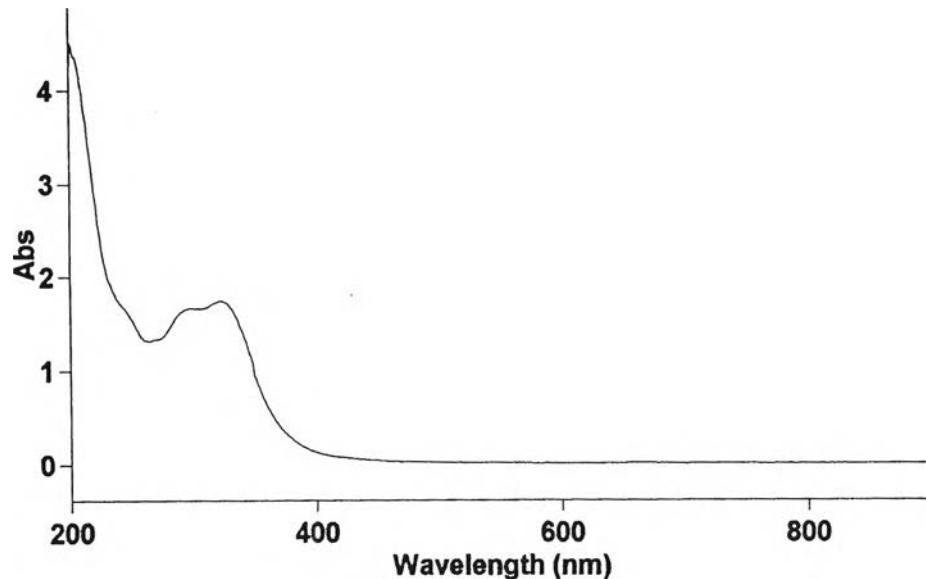
Peak type peaks

Peak Threshold 0.2000  
Range 900.00 nm to 202.00 nm

No peak found above threshold.

For hexane extract, there are no peak absorbtion result. Because of non polarity of hexane. **Asiaticoside** is polarity so the result is as above.

Kingston University  
Department of Chemistry  
Instrument Serial Number EL 9806 3588



### Scan Report Fri 03 Aug 03:46:30 PM 2001

Batch:  
Software version: 01.00(6)  
Operator: Suvipha S.

Sample Name: CA  
Collection Time 03/08/01 15:45:09

#### Peak Table

Peak Type	Peaks
Peak Threshold	0.0100
Range	900.00nm to 200.00nm

Wavelength (nm)	Abs
322.00	1.7446
205.00	4.3648
201.00	4.5043

Figure 1C Spectrum of chloroform extract from *C. asiatica* fresh leaves.(UK lab)

## Scan Report

Batch chloroform extract  
Software version: 01.00 (6)

### Peak table

Peak type peaks

Peak Threshold 0.0100  
Range 900.00 nm to 200.00 nm

Wavelength (nm)	Abs
322.00	1.7446
205.00	4.3648
201.00	4.5043

Figure 1 C shows UV Spectrum of chloroform extract in the second steps in Method 1.2. The spectrum show more than 1 peak, it demonstrate the peak at 201, 205 and 322 nm

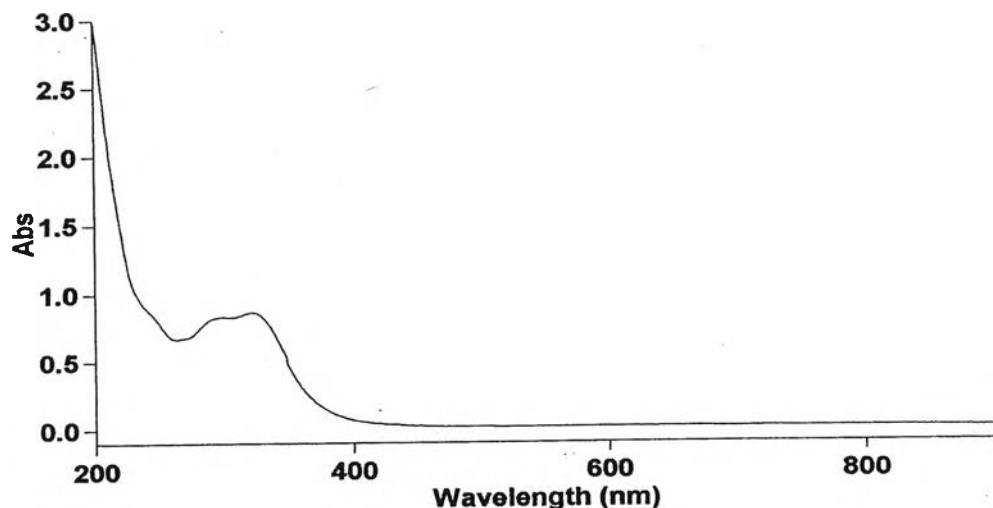


Figure 1D Spectrum of isobuthanol extract from *C. asiatica* fresh leaves.(UK lab).

## Scan Report

Batch isobuthanol extract  
Software version: 01.00 (6)

### Peak table

Peak type peaks

Peak Threshold 0.0100

Range 900.00 nm to 200.00 nm

Wavelength (nm)	Abs
322.00	0.8605
255.00	0.9885
205.00	1.5042

In isobuthanol extract, it present peak at 322 nm. as shown in Figure 1D. From crude extract asiaticoside cannot identify by using UV In isobuthanol extract, it present peak at 322 nm. as shown in spectrophotometer.

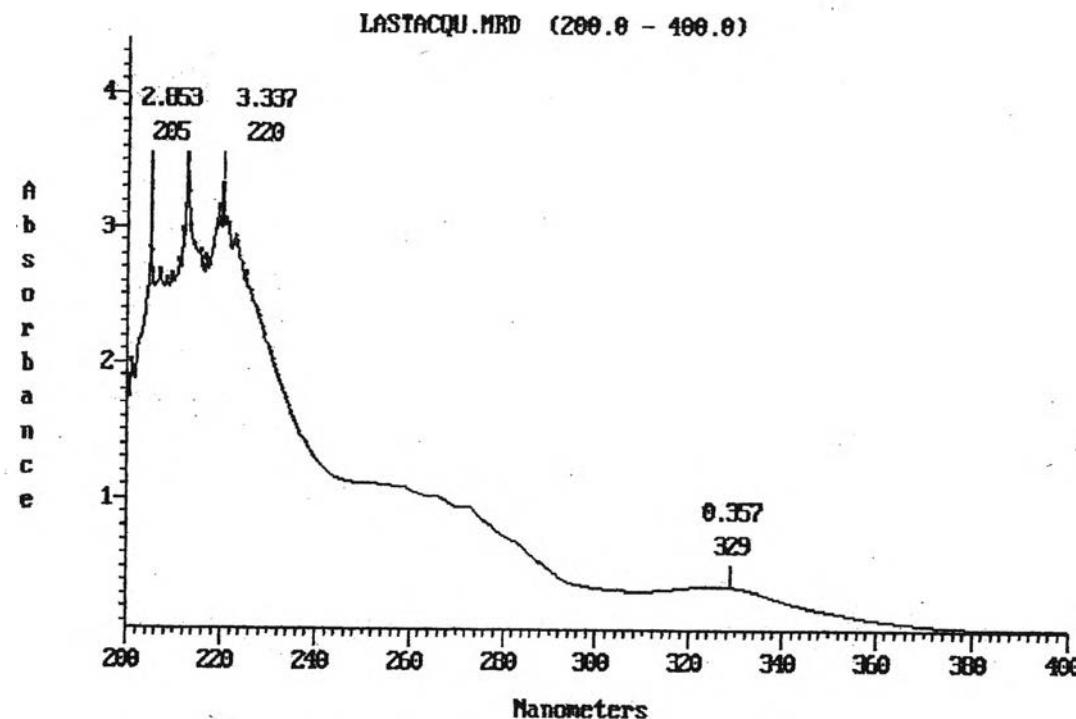


Figure 1 E UV spectrum of purified asiaticoside from *C. asiatica* fresh leaves.

### Scan Report

Batch                    butanol extract  
 Software version:    01.00 (6)

#### Peak table

Peak type            peaks

Peak Threshold    0.0100

Range                200.00 nm to 400.00 nm

Wavelength (nm)	Abs
220.00	3.337
205.00	2.853

This figure we can find that the wavelength of 220 nm.,  
 asiaticoside can absorb as the report result.

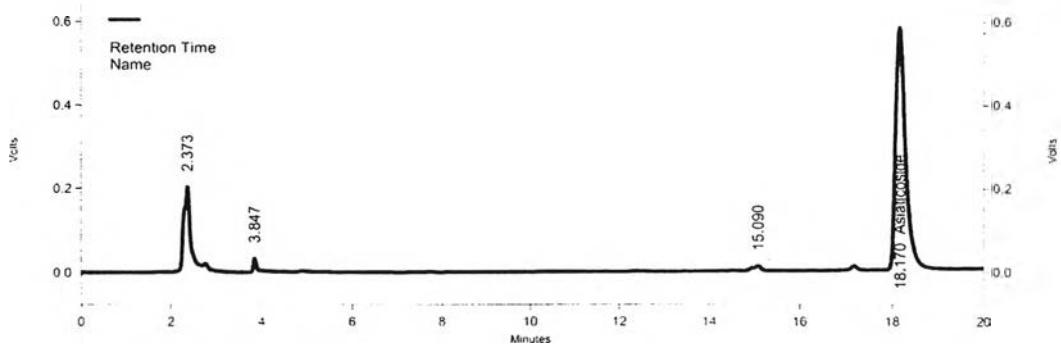
## **APPENDIX II**

### **CHROMATOGRAM OF ASIATICOSIDE**

## External Standard Report

**Asiaticoside from *Centella asiatica* in  
microemulsion gel ( formula 1) for 72 hrs. in Franz diffusion cell**

Method: C:\CLASS-VP\anne\methodAnne.met  
 Data File: C:\CLASS-VP\anne\Suvipha\5.dat  
 User: **Suvipha**  
 Acquired: 4/1/02 1:33:31 AM  
 Printed: 4/16/02 7:15:33 AM



Detector A - 1  
(220nm)

Pk #	Retention Time	Name	Area	ESTD concentration
4	18.170	Asiaticoside	8707124	0.063
Totals			8707124	0.063

Figure 2A Chromatogram of **asiaticoside** from fresh *C. asiatica* leaves in microemulsion gel formulation 1 (72 hrs.)

The retention time of purified **asiaticoside** in the matrix of microemulsion gel for formula 1 is 18.170. Peak area is 8707124 cm<sup>2</sup> when the concentration is about 0.0630 g./ml. That means the penetration in Franz diffusion cell of **asiaticoside** when calculate in flux rate will show most outstanding when compare with other formula. This part of **asiaticoside** is increasing collagen. In the contrary the amount of **asiaticoside** that cover for skin lost water has just a few. When calculate it has about 0.0370 g./ml.

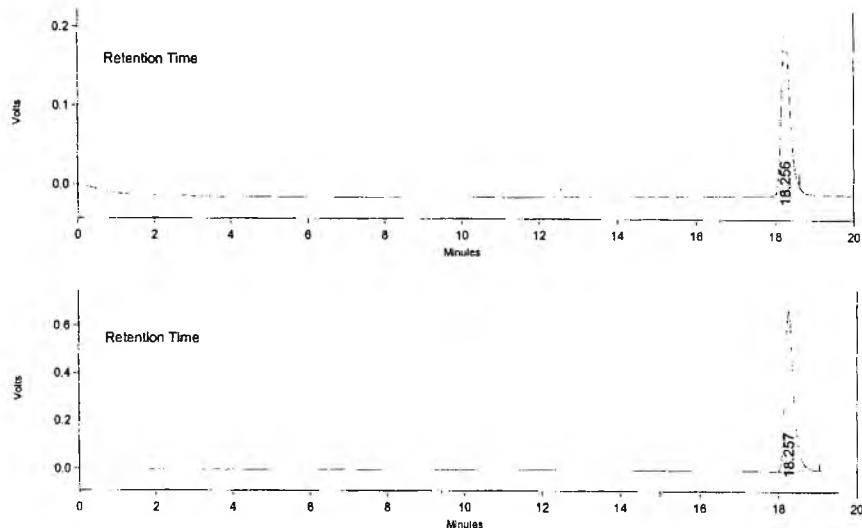
Shimadzu CLASS-VP

**Franz diffusion cell of *Centella asiatica***

(Microemulsion gel formula 1)

**Area % Report**

Method Name: C:\CLASS-VP\anne\methodAnne.met  
 Data Name: C:\CLASS-VP\anne\Suvipha\FranzCen72hr.fo1  
 User: Suvipha  
 Acquired: 4/1/02 4:44:17 AM  
 Printed: 4/1/02 5:08:25 AM

**Detector A - 1 (220nm)**

Pk #	Retention Time	Area	Area %	Height	Height %
1	18.256	2942275	100.000	200827	100.000

Totals

2942275 100.000 200827 100.000

**Detector****A - 2****(255nm)**

Pk #	Retention Time	Area	Area Percent	Height	Height Percent
1	18.257	10478002	100.00	673649	100.00

Totals

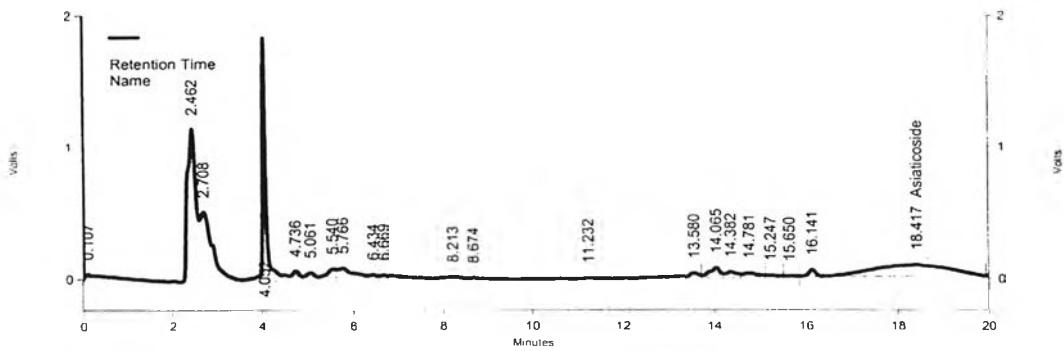
10478002 100.00 673649 100.00

Figure 2B Chromatogram of **asiaticoside** from fresh *C. asiatica* leaves in microemulsion gel formulation 1 (48 hrs.)

## External Standard Report

**Asiaticoside from fresh *Centella asiatica* in microemulsion gel for 72 hrs. in Franz diffusion cell (Formula 1)**

Method: C:\CLASS-VP\anne\methodAnne.met  
 Data File: C:\CLASS-VP\anne\Suvipha\FCASH4-f3-m1-2  
 User: **Suvipha**  
 Acquired: 3/24/02 1:09:08 PM  
 Printed: 4/16/02 9:16:43 AM



Detector A - 1 (220nm)		Retention Time	Name	Area	ESTD concentration
21		18.417	Asiaticoside	10498276	0.069
Totals				10498276	0.069

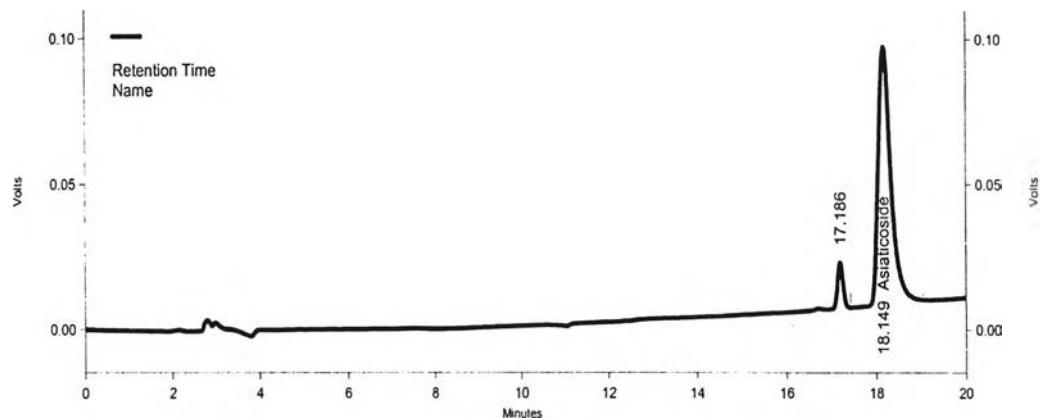
Figure 2 C Chromatogram of asiaticoside in microemulsion gel for 72 hrs.  
 (Formula 1)

We can observe that for concentration of asiaticoside in formula 1, it also get a good result for skin prenetration with Franz diffusion cell.

## External Standard Report

**The purified standard of asiaticoside in concentration  
0.04 g./ml.**

Method: C:\CLASS-VP\anne\A1\Asiaticoside.stand.0.04-1  
 Data File: C:\CLASS-VP\anne\A1\Asiaticoside.stand.0.04-1  
 User: **Suvipha**  
 Acquired: 4/11/02 7:54:00 PM  
 Printed: 5/02 10:11:59 PM



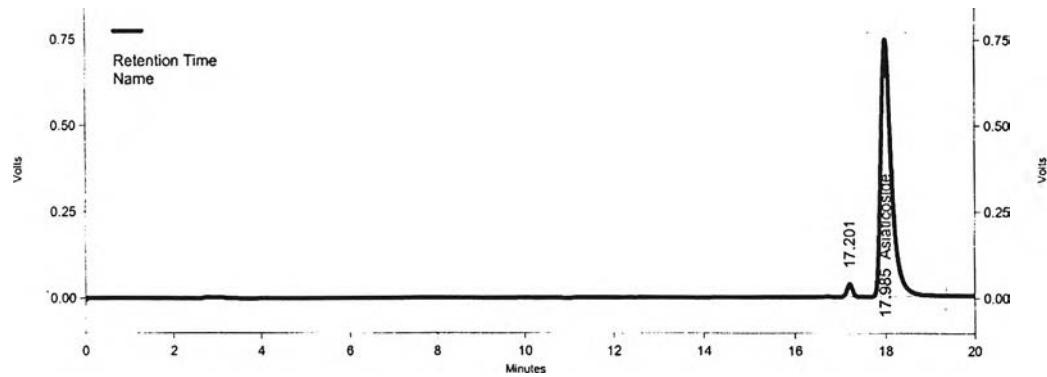
Detector A - 1 (220nm)			
Pk #	Retention Time	Name	Area
2	18.149	Asiaticoside	1720482
Totals			1720482

Figure 2 D Chromatogram of **asiaticoside** from fresh leaves of *C. asiatica*

Asiaticoside peak area  $1720482 \text{ cm}^2$  and retention time 18149 for detector 220 nm.

**External Standard Report****Asiaticoside**

Method: C:\CLASS-VP\anne\methodAnne.met  
Data File: C:\CLASS-VP\anne\A1\Asiaticoside.stand.0.08-3  
User: **Suvipha**  
Acquired: 4/11/02 10:27:00 PM  
Printed: 4/18/02 3:38:36 PM



Detector A - 1  
(220nm)

Pk #	Retention Time	Name	Area	ESTD concentration
2	17.985	Asiaticoside	12038078	0.080 CAL
Totals			12038078	0.080 CAL

Figure 2E The authentic asiaticoside for concentration 0.080 g/ml.

**External Standard Report****Asiaticoside ( Standard 0.08,1 )**

Method: C:\CLASS-VP\anne\methodAnne.met  
 Data File: C:\CLASS-VP\anne\Suvipha\Franz.Cenf1.72h  
 User: **Suvipha**  
 Acquired: 4/11/02 5:07:31 PM  
 Printed: 4/12/02 10:19:25 PM

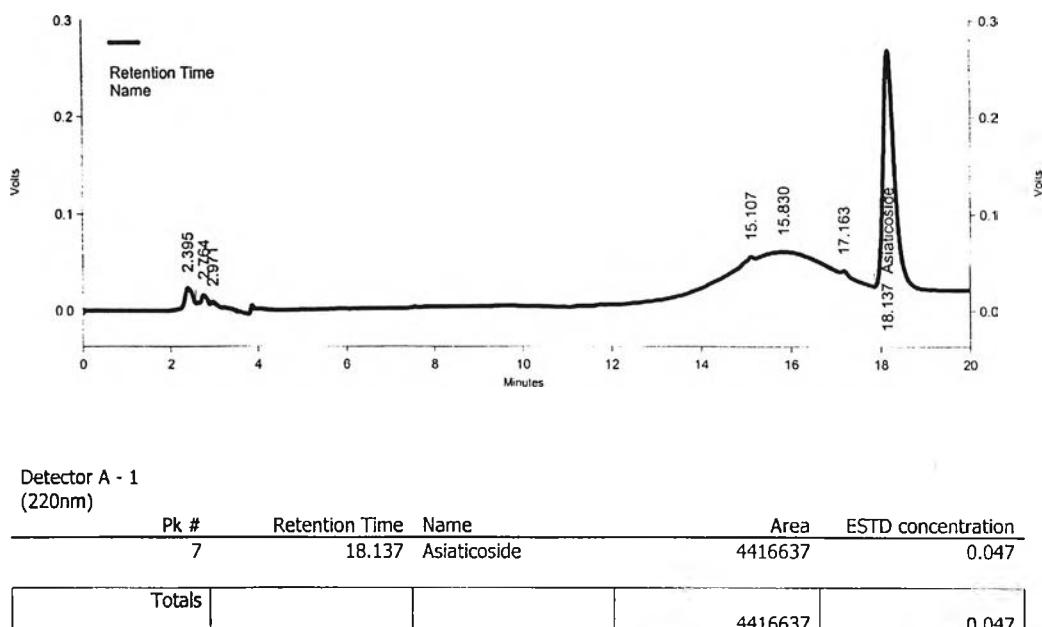


Figure 2 F Chromatogram of **asiaticoside** from fresh *C. asiatica* leaves.

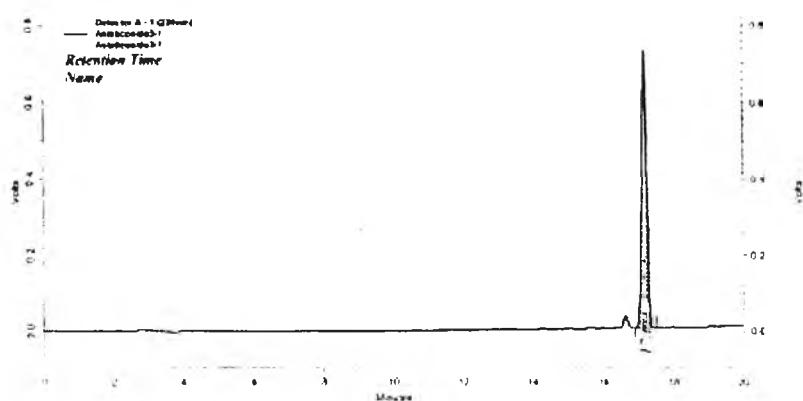
The purified **asiaticoside** in microemulsion gel occurred in the retention time 18.137 with peak area  $4416637 \text{ cm}^2$  and the concentration 0.047 g/ml. Present for the detector 220 nm. Compaired with authentic **asiaticoside** 0.08 g/ml for concentration in figure 2E.

Shimadzu CLASS-VP V 6.10

External Standard Report

**Fresh *Centella asiatica* T2**

Method Name: C:\CLASS-VP\anne\method1.met  
Data Name: C:\CLASS-VP\anne\Suvipha\Asiaticoside3-1  
User: Suvipha  
Acquired: 3/28/02 4:16:28 PM  
Printed: 3/29/02 1:11:58 PM



Detector A -  
1 (220nm)  
Pk # Name Retention Height Area ESTD Units

Shimadzu CLASS-VP V 6.10

External Standard Report

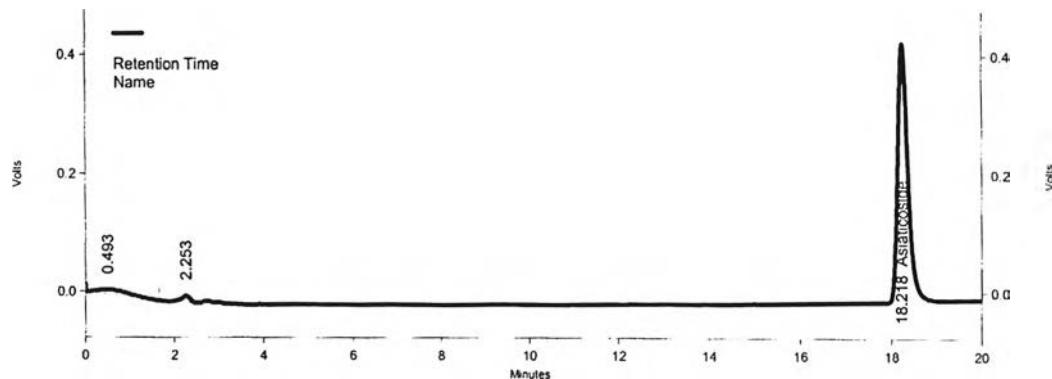
**Fresh *Centella asiatica* T2**

Figure 2 G Chromatogram of standard purified asiaticoside

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**External Standard Report****Asiaticoside**

Method: C:\CLASS-VP\anne\methodAnne.met  
Data File: C:\CLASS-VP\anne\Suvipha\8.dat  
User: **Suvipha**  
Acquired: 4/1/02 3:58:40 AM  
Printed: 5/5/02 6:34:41 PM

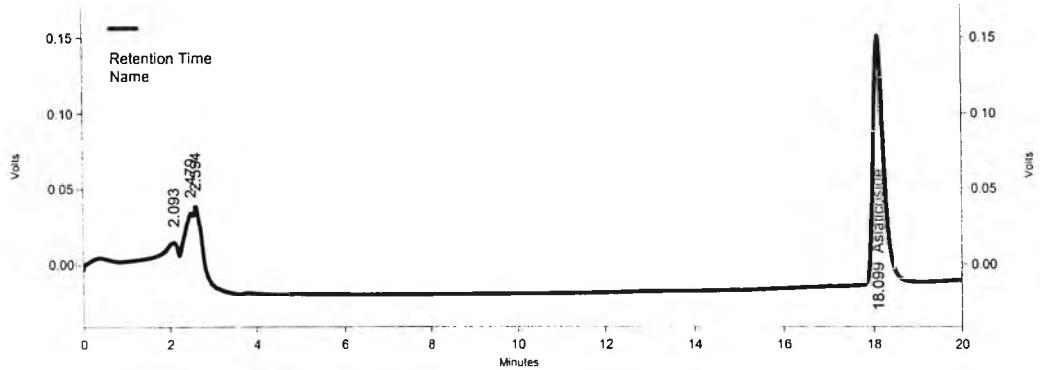


Detector A - 1 (220nm)			
Pk #	Retention Time	Name	Area
3	18.218	Asiaticoside	6769615
Totals			6769615

Figure 2H Chromatogram of asiaticoside from fresh *C.asiatica* leaves extract.

**External Standard Report****Asiaticoside**

Method: C:\CLASS-VP\anne\methodAnne.met  
Data File: C:\CLASS-VP\anne\Suvipha\6.dat  
User: **Suvipha**  
Acquired: 4/1/02 4:21:51 AM  
Printed: 5/5/02 6:39:29 PM



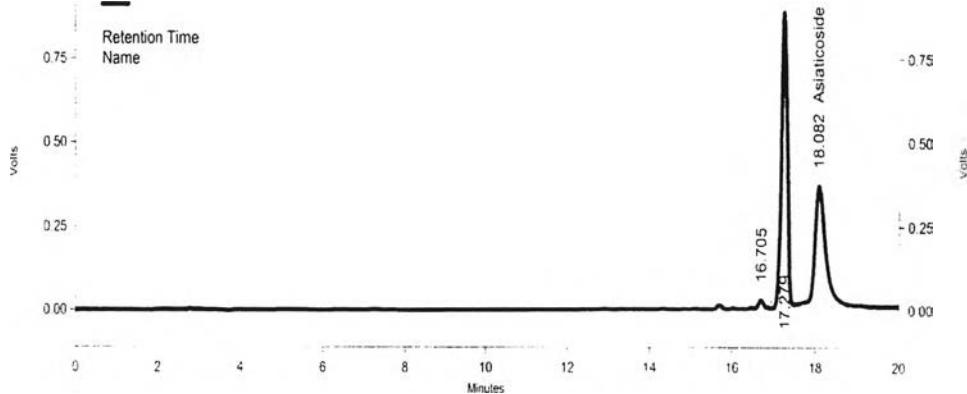
Detector A - 1 (220nm)				
Pk #	Retention Time	Name	Area	
4	18.099	Asiaticoside	2694672	
Totals			2694672	

Figure 2 I Chromatogram of **asiaticoside** from fresh *C. asiatica* leaves in microemulsion gel formula 3

## External Standard Report

**Asiaticoside from fresh *Centella asiatica* in  
microemulsion gel ( formula6) for 72 hrs. in Franz diffusion cell**

Method: C:\CLASS-VP\anne\methodAnne.met  
 Data File: C:\CLASS-VP\anne\Suvipha\Asiaticoside.s  
 User: Suvipha  
 Acquired: 4/11/02 11:28:03 PM  
 Printed: 4/16/02 7:55:00 AM



Detector A - 1  
(220nm)

Pk #	Retention Time	Name	Area	ESTD concentration
3	18.082	Asiaticoside	6980365	0.057
Totals			6980365	0.057

Figure 2 J; Chromatogram of **asiaticoside** from fresh *C. asiatica*

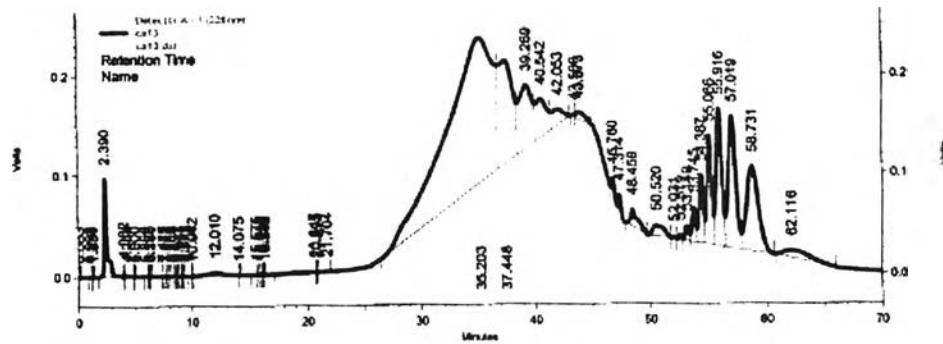
leaves in microemulsion gel formula 6

The **asiaticoside** from fresh *C. asiatica* leaves in microemulsion gel formula 6 for 72 hr. in Franz diffusion cell. The retention time = 18.082 minutes, peak area = 6980365 cm<sup>2</sup>. This formula is quite well for the penetration result that is = 0.057 g. /ml. for concentration of **asiaticoside**.

## External Standard Report

**Asiaticoside**

Method: C:\CLASS-VP\anne\methodAnne.met  
 Data File: C:\CLASS-VP\anne\ca13.dat  
 User: Suvipha  
 Acquired: 1/25/02 10:01:29 PM  
 Printed: 4/18/02 2:55:53 PM



**Asiaticoside**  
 Method: C:\CLASS-VP\anne\methodAnne.met  
 Data File: C:\CLASS-VP\anne\ca13.dat  
 User: Suvipha  
 Acquired: 1/25/02 10:01:29 PM  
 Printed: 4/18/02 2:55:53 PM

Figure 2K Chromatogram of asiaticoside from crude extract

from drug store in formula 1

Microemulsion gel of *C. asiatica* from drug store which is undetectable for skin penetration with Franz diffusion cell.

### **APPENDIX III**

**LOW AND HIGH RESOLUTION LC-MS WITH  
ELECTROSPRAY IONIZATION**

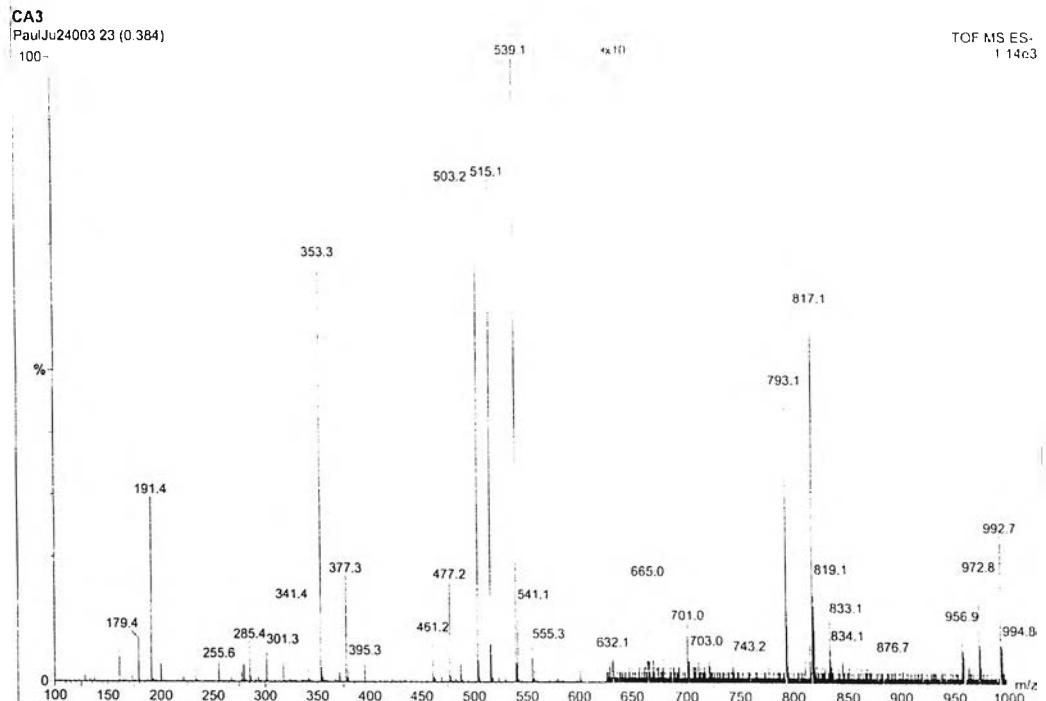


Figure 3 A Low resolution LC-MS with electrospray ionization of *C. asiatica*

fresh leaves.

Asiaticoside in low resolution is 956.9 when compared with authentic  $957.13 \pm 5\%$

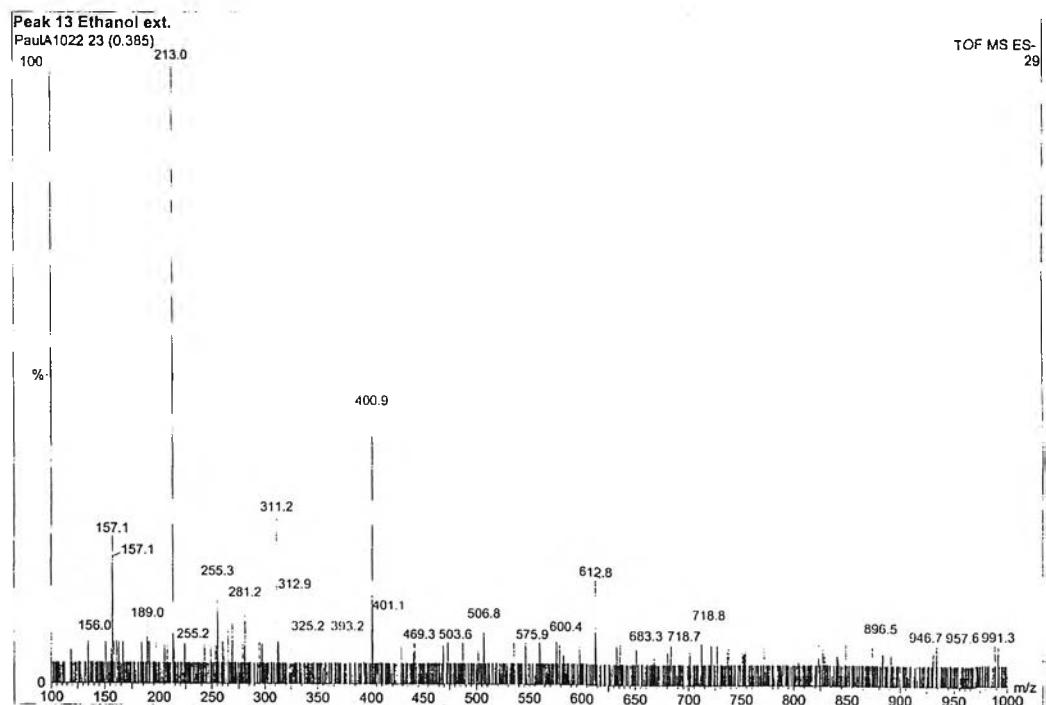


Figure 3 B Low resolution LC-MS with electrospray ionization of *C. asiatica* in ethanol extract.

Asiaticoside can be found in ethanol with MW = 957.6 (authentic asiaticoside, MW is = 957.13  $\pm$  5%)

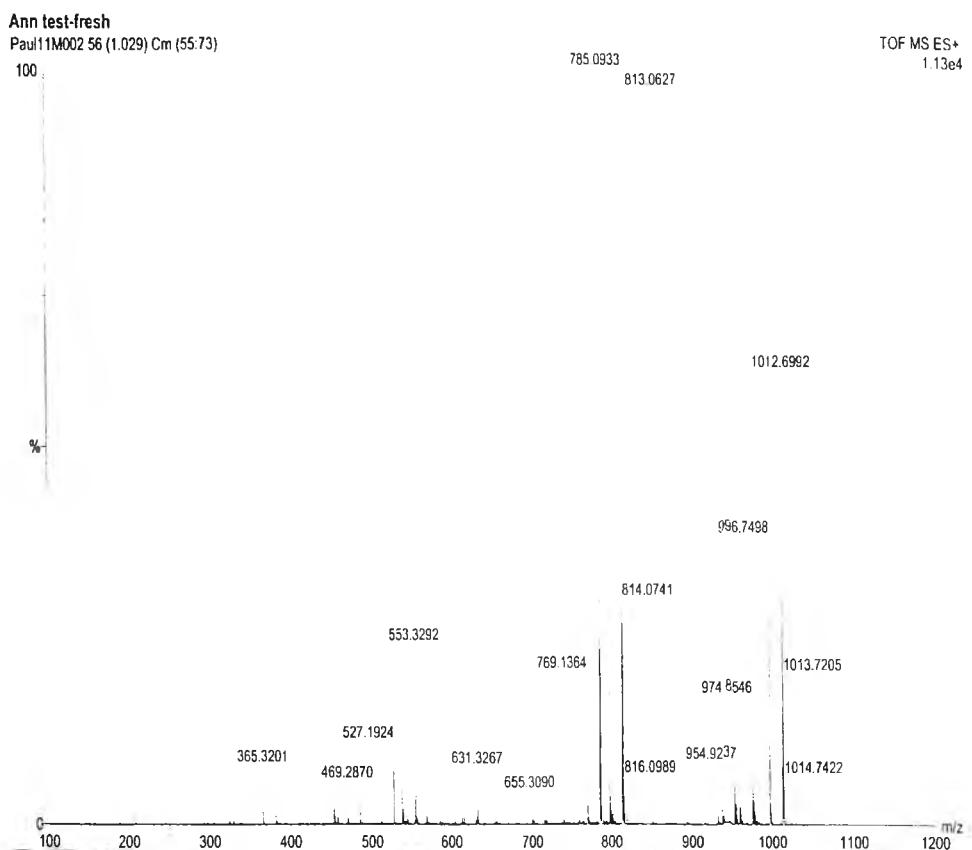


Figure 3C High resolution LC-MS with electrospray ionization of *C. asiatica* fresh leaves.

For high resolution we can get **asiaticoside** with  $\text{Na}^+$  for the molecular weight of  $\text{Na} = 23$  and **asiaticoside** is  $959.13 \pm 5\%$

## **APPENDIX IV**

### **VISCOSITY OF MICROEMULSION GEL**

Table 4 A Viscosity of asiaticoside in microemulsion gel for formula 1

Rheologic Data Output		Rheology International Shannon Ltd.				Tel: +353-61-471632	Fax: +353-61-471042
Operator:	Suvipha	Sample:	Asiaticoside1fo1				
Date:	04-04-2002	Time:	17:11:44				
Method:	Interactive	Viscometer:	H2				
Spindle:	ASTM 7	Version:	Rheologic 1.0				
Data Points. Page: 1							
Reading no.	Shear Rate s <sup>-1</sup>	Shear Stress Nm <sup>-2</sup> x E-07	Viscosity mPas	Time s	% Torque	Temp C	Speed R.P.M.
1	100.00	117,667.20	1,176.67	1.43	0.37	24.98	100.00
2	100.00	141,673.60	1,416.74	2.91	0.44	24.91	100.00
3	100.00	129,670.40	1,296.70	4.39	0.41	24.91	100.00
4	100.00	129,670.40	1,296.70	5.88	0.41	24.91	100.00
5	100.00	129,670.40	1,296.70	7.36	0.41	24.91	100.00
6	100.00	129,670.40	1,296.70	8.84	0.41	24.91	100.00
7	100.00	129,670.40	1,296.70	10.27	0.41	24.91	100.00
8	100.00	129,670.40	1,296.70	11.70	0.41	24.91	100.00
9	100.00	129,670.40	1,296.70	13.13	0.41	24.91	100.00
10	100.00	117,667.20	1,176.67	14.56	0.37	24.91	100.00
11	100.00	117,667.20	1,176.67	15.98	0.37	24.91	100.00
12	100.00	117,667.20	1,176.67	17.41	0.37	24.91	100.00
13	100.00	117,667.20	1,176.67	18.84	0.37	24.91	100.00
14	100.00	105,664.00	1,056.64	20.27	0.33	24.91	100.00
15	100.00	105,664.00	1,056.64	21.70	0.33	24.91	100.00
16	100.00	105,664.00	1,056.64	23.12	0.33	24.91	100.00
17	100.00	105,664.00	1,056.64	24.55	0.33	24.91	100.00
18	100.00	105,664.00	1,056.64	25.98	0.33	24.91	100.00
19	100.00	105,664.00	1,056.64	27.41	0.33	24.91	100.00
20	100.00	105,664.00	1,056.64	28.84	0.33	24.91	100.00
21	100.00	105,664.00	1,056.64	30.26	0.33	24.91	100.00
22	100.00	105,664.00	1,056.64	31.69	0.33	24.91	100.00
23	100.00	105,664.00	1,056.64	33.12	0.33	24.91	100.00
24	100.00	105,664.00	1,056.64	34.55	0.33	24.83	100.00
25	100.00	105,664.00	1,056.64	35.98	0.33	24.83	100.00
26	100.00	105,664.00	1,056.64	37.41	0.33	24.83	100.00
27	100.00	105,664.00	1,056.64	38.84	0.33	24.83	100.00
28	100.00	105,664.00	1,056.64	40.43	0.33	24.83	100.00
29	100.00	105,664.00	1,056.64	41.91	0.33	24.83	100.00
30	100.00	105,664.00	1,056.64	43.39	0.33	24.83	100.00
31	100.00	105,664.00	1,056.64	44.87	0.33	24.83	100.00
32	100.00	105,664.00	1,056.64	46.36	0.33	24.83	100.00
33	100.00	105,664.00	1,056.64	47.84	0.33	24.83	100.00
34	100.00	105,664.00	1,056.64	49.32	0.33	24.83	100.00
35	100.00	105,664.00	1,056.64	50.81	0.33	24.83	100.00
36	100.00	105,664.00	1,056.64	52.29	0.33	24.83	100.00
37	100.00	105,664.00	1,056.64	53.77	0.33	24.83	100.00
Rheologic Data Output		Rheology International Shannon Ltd.		Tel: +353-61-471632		Fax: +353-61-471042	
Operator:	Suvipha	Sample:	Asiaticoside1fo1				
Date:	04-04-2002	Time:	17:11:44				

Table 4B Viscosity of asiaticoside in microemulsion gel for formula 2

Rheologic Data Output		Rheology International Shannon Ltd.				Tel: +353-61-471632	Fax: +353-61-47104
Operator:	Suvipha	Sample:	Asiaticoside.fo2				
Date:	04-04-2002	Time:	18:24:03				
Method:	Interactive	Viscometer:	H2				
Spindle:	ASTM 7	Version:	Rheologic 1.0				
Data Points. Page: 1							
Reading no.	Shear Rate s-1	Shear Stress Nm^-2 x E-07	Viscosity mPas	Time s	% Torque	Temp C	Speed R.P.M.
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	100.00	7,163,545.60	71,635.46	4.77	22.39	24.46	100.00
3	100.00	4,366,800.00	43,668.00	10.20	13.65	24.39	100.00
4	100.00	3,766,640.00	37,666.40	15.64	11.77	24.39	100.00
5	100.00	3,490,566.40	34,905.66	21.08	10.91	24.46	100.00
6	100.00	3,106,464.00	31,064.64	26.52	9.71	24.46	100.00
7	100.00	2,806,384.00	28,063.84	31.96	8.77	24.46	100.00
8	100.00	2,614,332.80	26,143.33	37.39	8.17	24.46	100.00
9	100.00	2,434,284.80	24,342.85	42.83	7.61	24.46	100.00
10	100.00	2,338,259.20	23,382.59	48.32	7.31	24.46	100.00
11	100.00	2,134,204.80	21,342.05	53.82	6.67	24.46	100.00
12	100.00	1,966,160.00	19,661.60	59.25	6.14	24.46	100.00
13	100.00	1,786,112.00	17,861.12	64.69	5.58	24.46	100.00
14	100.00	1,630,070.40	16,300.70	70.13	5.09	24.46	100.00
15	100.00	1,474,028.80	14,740.29	75.57	4.61	24.46	100.00
16	100.00	1,366,000.00	13,660.00	81.00	4.27	24.46	100.00
17	100.00	1,245,968.00	12,459.68	86.44	3.89	24.46	100.00
18	100.00	1,137,939.20	11,379.39	91.93	3.56	24.46	100.00
19	100.00	1,053,916.80	10,539.17	97.43	3.29	24.46	100.00
20	100.00	981,897.60	9,818.98	102.86	3.07	24.46	100.00
21	100.00	10,248,368.00	102,483.68	108.30	32.03	24.46	100.00
22	100.00	19,154,742.40	191,547.42	113.74	59.86	24.46	100.00
23	100.00	18,326,521.60	183,265.22	119.18	57.27	24.46	100.00
24	100.00	18,482,563.20	184,825.63	124.61	57.76	24.46	100.00
25	100.00	18,482,563.20	184,825.63	130.05	57.76	24.46	100.00

Rheologic Data Output		Rheology International Shannon Ltd.		Tel: +353-61-471632	Fax: +353-61-47104
Operator:	Suvipha	Sample:	Asiaticoside.fo2		
Date:	04-04-2002	Time:	18:24:03		

Table 4C Viscosity of asiaticoside in microemulsion gel for formula 3

Rheologic Data Output	Rheology International Shannon Ltd.		Tel: +353-61-471632	Fax: +353-61-471042
Operator:	Suvipha	Sample:	Cenofo3	
Date:	04-04-2002	Time:	22:34:59	
Method:	Interactive	Viscometer:	H2	
Spindle:	ASTM 7	Version:	Rheologic 1.0	

Data Points. Page: 1

Reading no.	Shear Rate s <sup>-1</sup>	Shear Stress Nm <sup>-2</sup> x E-07	Viscosity mPas	Time s	% Torque	Temp C	Speed R.P.M.
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	100.00	165,680.00	1,656.80	5.49	0.52	26.10	100.00
3	100.00	153,676.80	1,536.77	10.93	0.48	26.10	100.00
4	100.00	153,676.80	1,536.77	16.37	0.48	26.10	100.00
5	100.00	165,680.00	1,656.80	21.81	0.52	26.10	100.00
6	100.00	165,680.00	1,656.80	27.24	0.52	26.10	100.00
7	100.00	165,680.00	1,656.80	32.68	0.52	26.10	100.00
8	100.00	165,680.00	1,656.80	38.12	0.52	26.10	100.00
9	100.00	165,680.00	1,656.80	43.61	0.52	26.10	100.00
10	100.00	153,676.80	1,536.77	49.10	0.48	26.10	100.00
11	100.00	153,676.80	1,536.77	54.60	0.48	26.10	100.00

Rheologic Data Output	Rheology International Shannon Ltd.		Tel: +353-61-471632	Fax: +353-61-471042
Operator:	Suvipha	Sample:	Cenofo3	

Table 4D Viscosity of asiaticoside in microemulsion gel for formula 4

Rheologic Data Output		Rheology International Shannon Ltd.			Tel: +353-61-471632		Fax: +353-61-47104:	
Operator:	SSSSS	Sample:	CENFO4					
Date:	04-04-2002	Time:	16:14:46					
Method:	Interactive	Viscometer:	H2					
Spindle:	ASTM 7	Version:	Rheologic 1.0					
Data Points. Page: 1								
Reading no.	Shear Rate s <sup>-1</sup>	Shear Stress Nm <sup>-2</sup> x 10 <sup>-7</sup>	Viscosity mPas	Time s	% Torque	Temp C	Speed R.P.M.	
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
2	100.00	1,654,076.80	16,540.77	0.79	5.17	24.83	100.00	
3	100.00	4,486,832.00	44,868.32	2.22	14.02	24.83	100.00	
4	100.00	6,063,257.60	60,832.58	3.65	19.01	24.83	100.00	
5	100.00	6,455,356.80	64,553.57	5.08	20.17	24.83	100.00	
6	100.00	6,275,308.80	62,753.09	6.51	19.61	24.83	100.00	
7	100.00	6,023,241.60	60,232.42	7.93	18.82	24.83	100.00	
8	100.00	5,807,184.00	58,071.84	9.36	18.15	24.83	100.00	
9	100.00	5,699,155.20	56,991.55	10.79	17.81	24.83	100.00	
10	100.00	5,639,139.20	56,391.39	12.22	17.62	24.83	100.00	
11	100.00	5,555,116.80	55,551.17	13.65	17.36	24.83	100.00	
12	100.00	5,483,097.60	54,830.98	15.07	17.13	24.83	100.00	
13	100.00	5,603,129.60	56,031.30	16.50	17.51	24.83	100.00	
14	100.00	5,675,148.80	56,751.49	17.93	17.73	24.83	100.00	
15	100.00	5,615,132.80	56,151.33	19.36	17.55	24.83	100.00	
16	100.00	5,471,094.40	54,710.94	20.84	17.10	24.83	100.00	
17	100.00	5,363,065.60	53,630.66	22.32	16.76	24.83	100.00	
18	100.00	5,135,004.80	51,350.05	23.81	16.05	24.83	100.00	
19	100.00	4,858,931.20	48,589.31	25.29	15.18	24.83	100.00	
20	100.00	5,351,062.40	53,510.62	26.77	16.72	24.83	100.00	
21	100.00	5,723,161.60	57,231.62	28.26	17.88	24.83	100.00	
22	100.00	5,699,155.20	56,991.55	29.74	17.81	24.83	100.00	
23	100.00	5,615,132.80	56,151.33	31.22	17.55	24.83	100.00	
24	100.00	5,483,097.60	54,830.98	32.70	17.13	24.83	100.00	
25	100.00	5,327,056.00	53,270.56	34.19	16.65	24.83	100.00	
26	100.00	5,219,027.20	52,190.27	35.67	16.31	24.83	100.00	
27	100.00	5,110,998.40	51,109.98	37.15	15.97	24.83	100.00	
28	100.00	5,002,969.60	50,029.70	38.64	15.63	24.83	100.00	
29	100.00	4,870,934.40	48,709.34	40.12	15.22	24.83	100.00	
30	100.00	4,786,912.00	47,869.12	41.60	14.96	24.83	100.00	
31	100.00	4,726,896.00	47,268.96	43.03	14.77	24.83	100.00	
32	100.00	4,690,886.40	46,908.86	44.46	14.66	24.83	100.00	
33	100.00	4,642,873.60	46,428.74	45.89	14.51	24.83	100.00	
34	100.00	4,606,864.00	46,068.64	47.32	14.40	24.83	100.00	
35	100.00	4,558,851.20	45,588.51	48.74	14.25	24.83	100.00	
36	100.00	4,522,841.60	45,228.42	50.17	14.13	24.83	100.00	
37	100.00	4,486,832.00	44,868.32	51.60	14.02	24.83	100.00	
38	100.00	4,450,822.40	44,508.22	53.03	13.91	24.83	100.00	
39	100.00	4,414,812.80	44,148.13	54.46	13.80	24.83	100.00	
40	100.00	4,378,803.20	43,788.03	55.88	13.68	24.83	100.00	
41	100.00	4,354,796.80	43,547.97	57.31	13.61	24.83	100.00	
42	100.00	4,390,806.40	43,908.06	58.74	13.72	24.83	100.00	
43	100.00	4,426,816.00	44,268.16	60.17	13.83	24.83	100.00	
44	100.00	4,438,819.20	44,388.19	61.60	13.87	24.83	100.00	

Rheologic Data Output	Rheology International Shannon Ltd.	Tel: +353-61-471632	Fax: +353-61-47104:
Operator:	SSSSS	Sample:	CENFO4

Table 4E Viscosity of asiaticoside in microemulsion gel for formula 5

Rheologic Data Output      Rheology International Shannon Ltd.      Tel: +353-61-471632      Fax: +353-61-471042

Operator: Sumpha Sample: A station side to 5

Date: 04-04-2002 Time: 22:52:30

Method: Interactive Viscrometer: H2

Spindle: ASTM 7 Version: Rheologic 1.0

Data Points, Page: 1

Reading no.	Shear Rate s-1	Shear Stress Nm <sup>-2</sup> x E-07	Viscosity mPas	Time s	% Torque	Temp C	Speed R.P.M.
1	100.00	381,737.60	3,817.38	4.98	1.19	24.98	100.00
2	100.00	381,737.60	3,817.38	10.47	1.19	24.98	100.00
3	100.00	381,737.60	3,817.38	15.96	1.19	24.98	100.00
4	100.00	369,734.40	3,697.34	21.40	1.16	24.98	100.00
5	100.00	345,728.00	3,457.28	26.84	1.08	24.98	100.00
6	100.00	333,724.80	3,337.25	32.28	1.04	25.06	100.00
7	100.00	309,718.40	3,097.18	37.71	0.97	25.06	100.00
8	100.00	285,712.00	2,857.12	43.15	0.89	25.06	100.00
9	100.00	249,702.40	2,497.02	48.59	0.78	24.98	100.00
10	100.00	225,696.00	2,256.96	54.08	0.71	24.98	100.00

Rheologic Data Output      Rheology International Shannon Ltd.      Tel: +353-61-471632      Fax: +353-61-471042

Operator: Sungha Sample: Astaticoside.s05

Date: 04-04-2002 Time: 22:52:30

Method: Interactive Viscrometer: H2

**Spindle:**

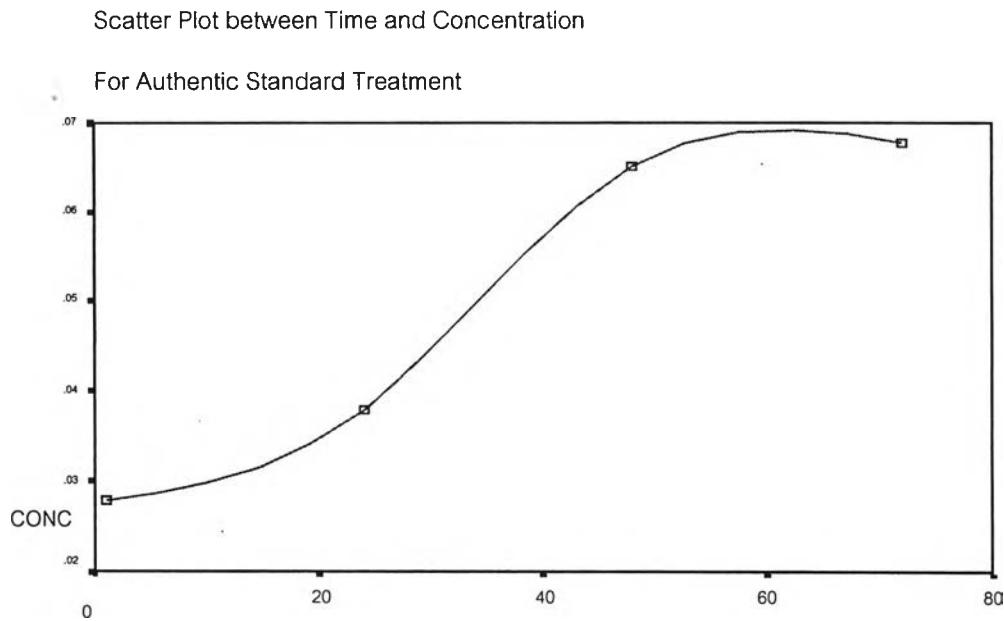
Table 4F Viscosity of asiaticoside in microemulsion gel for formula 6

Rheologic Data Output		Rheology International Shannon Ltd.				Tel: +353-61-471632	Fax: +353-61-4710
Operator:	Sunipha	Sample:	Cen.fo6				
Date:	04-04-2002	Time:	23:33:29				
Method:	Interactive	Viscometer:	H2				
Spindle:	ASTM 7	Version:	Rheologic 1.0				
Data Points. Page: 1							
Reading no.	Shear Rate s <sup>-1</sup>	Shear Stress Nm <sup>-2</sup> x E-07	Viscosity mPas	Time s	% Torque	Temp C	Speed R.P.M.
1	0.00	0.00	0.00	0.00	0.00	0.00	0.00
2	100.00	3,430,550.40	34,305.50	5.14	10.72	24.46	100.00
3	100.00	3,430,550.40	34,305.50	10.52	10.72	24.46	100.00
4	100.00	2,746,368.00	27,463.68	15.96	8.58	24.46	100.00
5	100.00	2,338,259.20	23,382.59	21.40	7.31	24.46	100.00
6	100.00	2,122,201.60	21,222.02	26.84	6.63	24.46	100.00
7	100.00	2,014,172.80	20,141.73	32.27	6.29	24.46	100.00
8	100.00	1,894,140.80	18,941.41	37.77	5.92	24.46	100.00
9	100.00	1,798,115.20	17,981.15	43.26	5.62	24.46	100.00
10	100.00	1,714,092.80	17,140.93	48.75	5.36	24.46	100.00
11	100.00	1,618,067.20	16,180.67	54.19	5.06	24.46	100.00
12	100.00	1,546,048.00	15,460.48	59.63	4.83	24.46	100.00

## **APPENDIX V**

### **STATISTIC FOR PERMEABILITY STUDY**

## Statistic Analysis



VAR00003, Time

**Dependent variable.. CONC**

**Independent variable: TIME**

**Method.. QUADRATI**

R Square .92944

Adjusted R Square .78833

Standard Error .00909

**Analysis of Variance:**

	DF	Sum of Squares	Mean Square
Regression	2	.00108804	.00054402
Residuals	1	.00008259	.00008259

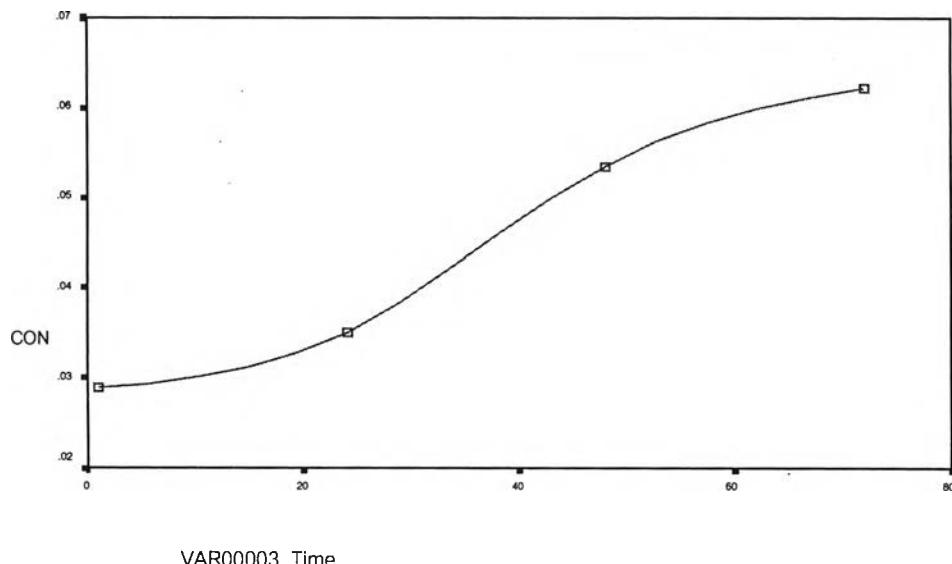
F = 6.58662      Signif F = .2656

----- Variables in the Equation -----

<b>Variable</b>	<b>B</b>	<b>SE B</b>	<b>Beta</b>	<b>T</b>	<b>Sig T</b>
VAR00003	.000886	.000619	1.372266	1.430	.8471
VAR00003**2	-3.67108125E-06	8.1427E-06	.432563	-.451	.9326
(Constant)	.024988	.009268		2.696	.8065

Scatter Plot between Time and Concentration

For Formula 1



**Dependent variable.. CONC      Independent variable: TIME**

**Method.. QUADRATI**

R Square .96836

Adjusted R Square .90507

Standard Error .00481

**Analysis of Variance:**

	<b>DF</b>	<b>Sum of Squares</b>	<b>Mean Square</b>
Regression	2	.00070856	.00035428
Residuals	1	.00002315	.00002315

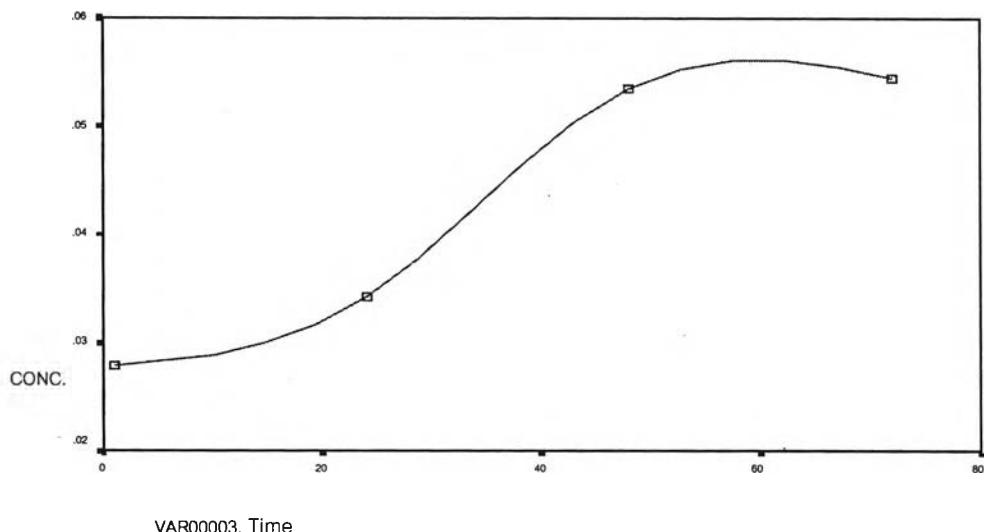
F = 15.30067 Signif F = .1779

**----- Variables in the Equation -----**

<b>Variable</b>	<b>B</b>	<b>SE B</b>	<b>Beta</b>	<b>T</b>	<b>Sig T</b>
VAR00003	.000438	.000328	.858885	1.337	.8522
VAR00003**2	8.69425312E-07	4.3113E-06	.129577	.202	.9683
(Constant)	.027352	.004907		5.574	.7783

Scatter Plot between Time and Concentration

For Formula 3



**Dependent variable.. CONC      Independent variable: TIME**

**Method.. QUADRATI**

R Square .91551

Adjusted R Square .74652

Standard Error .00677

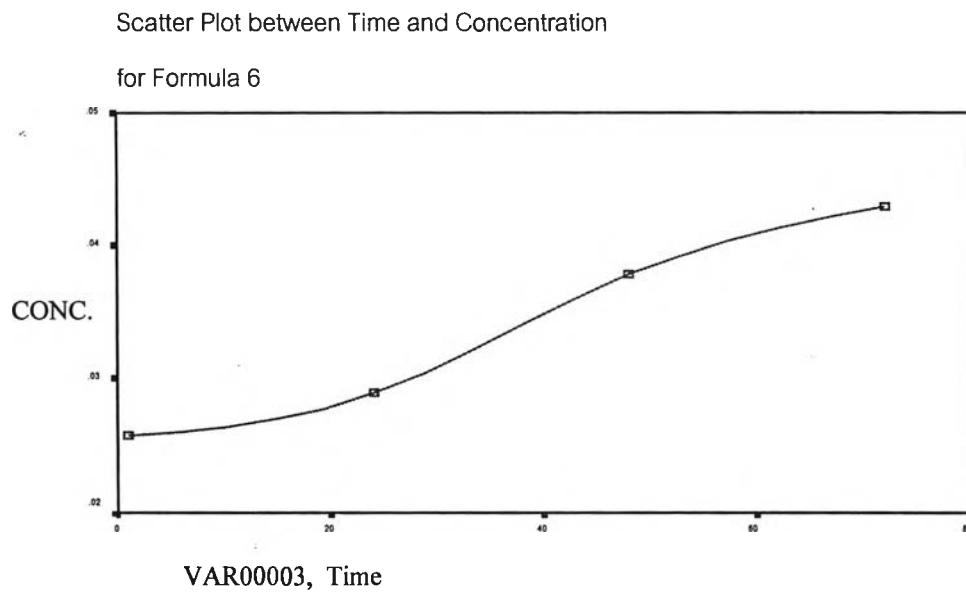
**Analysis of Variance:**

	<b>DF</b>	<b>Sum of Squares</b>	<b>Mean Square</b>
Regression	2	.00049691	.00024845
Residuals	1	.00004586	.00004586

F = 5.41770      Signif F = .2907

----- **Variables in the Equation** -----

<b>Variable</b>	<b>B</b>	<b>SE B</b>	<b>Beta</b>	<b>T</b>	<b>Sig T</b>
VAR00003	.000612	.000462	1.392218	1.326	.8528
VAR00003**2	-2.67008718E-06	6.0675E-06	-.462045	-.440	.9340
(Constant)	.025803	.006906		3.736	.7916



**Dependent variable.. CONC**

**Method.. QUADRATI**

R Square .97728

Adjusted R Square .93184

Standard Error .00207

#### **Analysis of Variance:**

	DF	Sum of Squares	Mean Square
Regression	2	.00018404	.00009202
Residuals	1	.00000428	.00000428

F = 21.50832 Signif F = .1507

----- **Variables in the Equation** -----

<b>Variable</b>	<b>B</b>	<b>SE B</b>	<b>Beta</b>	<b>T</b>	<b>Sig T</b>
VAR00003	.000199	.000141	.770401	1.415	.8479
VAR00003**2	7.65899931E-07	1.8532E-06	.225004	.413	.9376
(Constant)	.025041	.002109		11.871	.7634

**Comparative R<sup>2</sup> in microemulsion gel**

1) Authentic **asiaticoside**

$$\text{Conc} = 0.024988 + 0.006886 * \text{time} + -3.67108125 \times 10^{-6} * \text{time}^2$$

$$R^2 = 0.92944$$

2) Microemulsion gel Formula 1

$$\text{Conc} = 0.027352 + 0.00438 * \text{time} + 8.69425312 \times 10^{-7} * \text{time}^2$$

$$R^2 = 0.96836$$

3) Microemulsion gel Formula 6

$$\text{Conc} = 0.025041 + 0.000199 * \text{time} + 7.6559993 \times 10^{-7} * \text{time}^2$$

$$R^2 = 0.97728$$

4) Microemulsion gel Formula 3

$$\text{Conc} = 0.025803 + 0.000612 * \text{time} + 2.6700817 \times 10^{-6} * \text{time}^2$$

$$R^2 = 0.91551$$

As the statistic analysis can conclude the flux rate of **asiaticoside** in microemulsion gel formula 1 is the best formula when compared with other formulations.

## VITA

Mrs. Suvipha Sermboonsang was born on June 7, 1957 in Bangkok, Thailand. She received her Bachelor Degree of Science from Kasetsart University, Bangkok, Thailand in 1982. After graduation, she had worked in Department of Medical Professional Product, Johnson & Johnson (Thailand) Ltd., during 1982 –1986; and Organics Department, East Asiatic Company Ltd. (Thailand), during 1986-1990. Then she worked as the director of Research and Development, Cosmetic Department of CNN International Co. Ltd., during 1996-2000 before enrolling the Master's Degree in Pharmaceutical Technology (International) Program, Faculty of Pharmaceutical Sciences, at Chulalongkorn University.

