CHAPTER IV

RESULTS

1. Determination of the sunscreen formulation.

1.1 Physical properties of the sunscreen formulations.

1.1 Physical appearance

All of the prepared sunscreen emulsions had good appearances and stability. They showed no phase separation and no color change during stored in hot air oven at 40 ^{0}C, 50 ^{0}C and 60 ^{0}C for 3 months and Freeze thaw cycle (six cycles) The appearance of the prepared sunscreen emulsions after freshly prepare and after stability test were not different.

1.2 pH values

The pH of the prepared sunscreen emulsions is summarized in Table 13. It is seen that pH values of all preparations are well within the ranges of their requirements

Table 13 The pH values of the prepared sunscreen emulsions.

Formula	Required pH values	Actual pH values
1	7.0 ±0.5	7.1
2	7.0 ±0.5	7.0
3	7.0 ±0.5	6.8
4	7.0 ±0.5	6.7
5	7.0 ±0.5	7.3
6	7.0 ±0.5	7.1
7	7.0 ±0.5	7.4
8	7.0 ±0.5	6.9
9	7.0 ±0.5	6.8
10	7.0 ±0.5	7.1
11	7.0 ±0.5	7.0
12	7.0 ±0.5	7.0
13	7.0 ±0.5	6.9
14	7.0 ±0.5	6.7
15	7.0 ±0.5	6.6
16	7.0 ±0.5	6.7
17	7.0 ±0.5	6.8
18	7.0 ±0.5	7.0
19	7.0 ±0.5	7.3
20	7.0 ±0.5	7.2
21	7.0 ±0.5	7.1
22	7.0 ±0.5	7.0
23	7.0 ±0.5	6.9
24	7.0 ±0.5	7.1

Table 13 The pH values of the prepared sunscreen emulsions (continued).

Formula	Required pH values	Actual pH values
26	7.0 ±0.5	6.8
27	7.0 ±0.5	7.1
28	7.0 ±0.5	6.9
29	7.0 ±0.5	7.2
30	7.0 ±0.5	7.3
31	7.0 ±0.5	7.1
32	7.0 ±0.5	6.9
33	7.0 ±0.5	6.8
34	7.0 ±0.5	6.7
35	7.0 ±0.5	6.6
36	7.0 ±0.5	6.7
Standard homosalate sunscreen	7.0 ±0.5	7.1

2. Analysis of sunscreen agent

2.1 Analysis of homosalate by UV visible spectrophotometry

The content of homosalate in the standard US-FDA sunscreen formulation was determined by using UV visible spectrophotometry at wavelength 306 nm. The UV scanning absorption spectrum of homosalate was shown in Figure 21.

The calibration curve was plotted between the concentrations of homosalate in 1% glacial acetic acid in 95% ethanol and absorbances at wavelength 306 nm as shown in Figure 22. A straight line represented the relationship between the absorbances and the concentrations was fitted using a linear regression analysis program.

2.2 Analysis of octyl dimethyl PABA, octyl methoxycinnamate and oxybenzone by HPLC

The HPLC method employed sulfamerazine as an internal standard. Figure 23, 24 and 25 showed the representative HPLC chromatogram of octyl dimethyl PABA, octyl methoxycinnamate and oxybenzone respectively all of them using sulfamerazine as the internal standard of the analysis. Figure 26 and 27 showed the representative HPLC chromatogram of the combination of octyl dimethyl PABA and oxybenzone, octyl methoxycinnamate and oxybenzone both of them also using sulfamerazine as the internal standard respectively. Figure 28, 29 and 30 showed standard curve of octyl dimethyl PABA, octyl methoxycinnamate and oxybenzone in methanol using sulfamerazine as the internal standard at wavelength 254 nm respectively. Table 15, 16 and 17 showed peak area ratio of sunscreen agent and internal standard in calibration curves shown in Figure 28, 29 and 30 respectively.

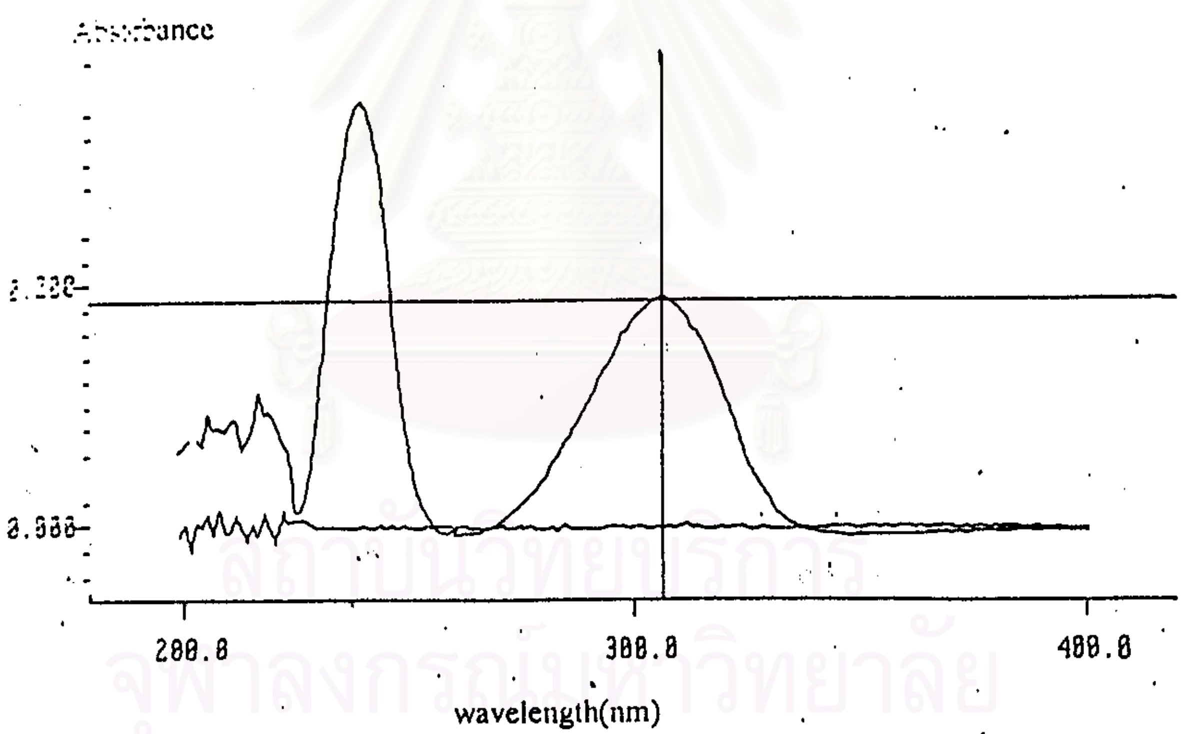

Figure 21 UV scanning absorption spectrum of homosalate.

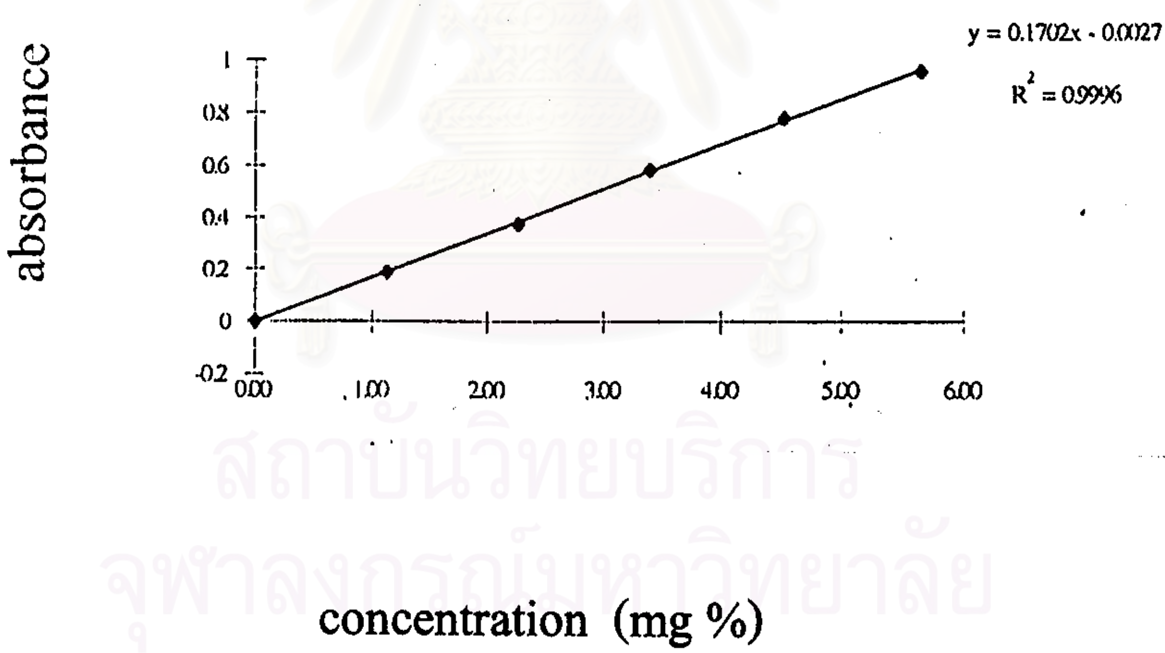

Figure 22 Standard curve of homosalate in 1% glacial acetic acid in 95% ethanol at wavelength 306 nm.

Table 14 Calibration curve data of homosalate in 1% glacial acetic acid in 95% ethanol at wavelength 306 nm.

No.	Homosalate conc[n]	Absorbance (306 nm)	Inversely Estimated Conc. (mg %)	%Theory
1	1.13	0.200	1.12	99.12
2	2.20	0.371	1.98	90.00
3	3.33	0.571	3.15	94.59
4	4.53	0.800	4.44	98.01
5	5.60	0.943	5.45	97.32
			Mean	95.81
			S.D.	5.81
			C.V.	5.81

Linear regression $r^2 = 0.9996$, $y = 0.17027X - 0.0027$

Inversely Estimate

$$\text{Concentration} = \frac{\text{Absorbance} + 0.0027}{0.17027}$$

$$\text{\% Theory} = \frac{\text{Inversely Estimated Conc.} \times 100}{\text{Known Conc.}}$$

$$\text{\% C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100$$

Figure 23 Representative HPLC chromatogram of octyl dimethyl PABA and sulfamerazine as internal standard solution.

S = Sulfamerazine

P = Octyl dimethyl PABA

Figure 24 Representative HPLC chromatogram of octyl methoxycinnamate and sulfamerazine as internal standard solution.

S = Sulfamerazine

C = Octyl methoxycinnamate

Figure 25 Representative HPLC chromatogram of oxybenzone and sulfamerazine as internal standard solution

S = Sulfamerazine

O = Oxybenzone

Figure 26 Representative HPLC chromatogram of octyl dimethyl PABA, oxybenzone and sulfamerazine as internal standard solution.

S = Sulfamerazine

O = Oxybenzone

P = Octyl dimethyl PABA

Figure 27 Representative HPLC chromatogram of octyl methoxycinnamate, oxybenzone and sulfamerazine as sinternal standard solution.

S = Sulfamerazine

O = Oxybenzone

C = Octyl methoxycinnamate

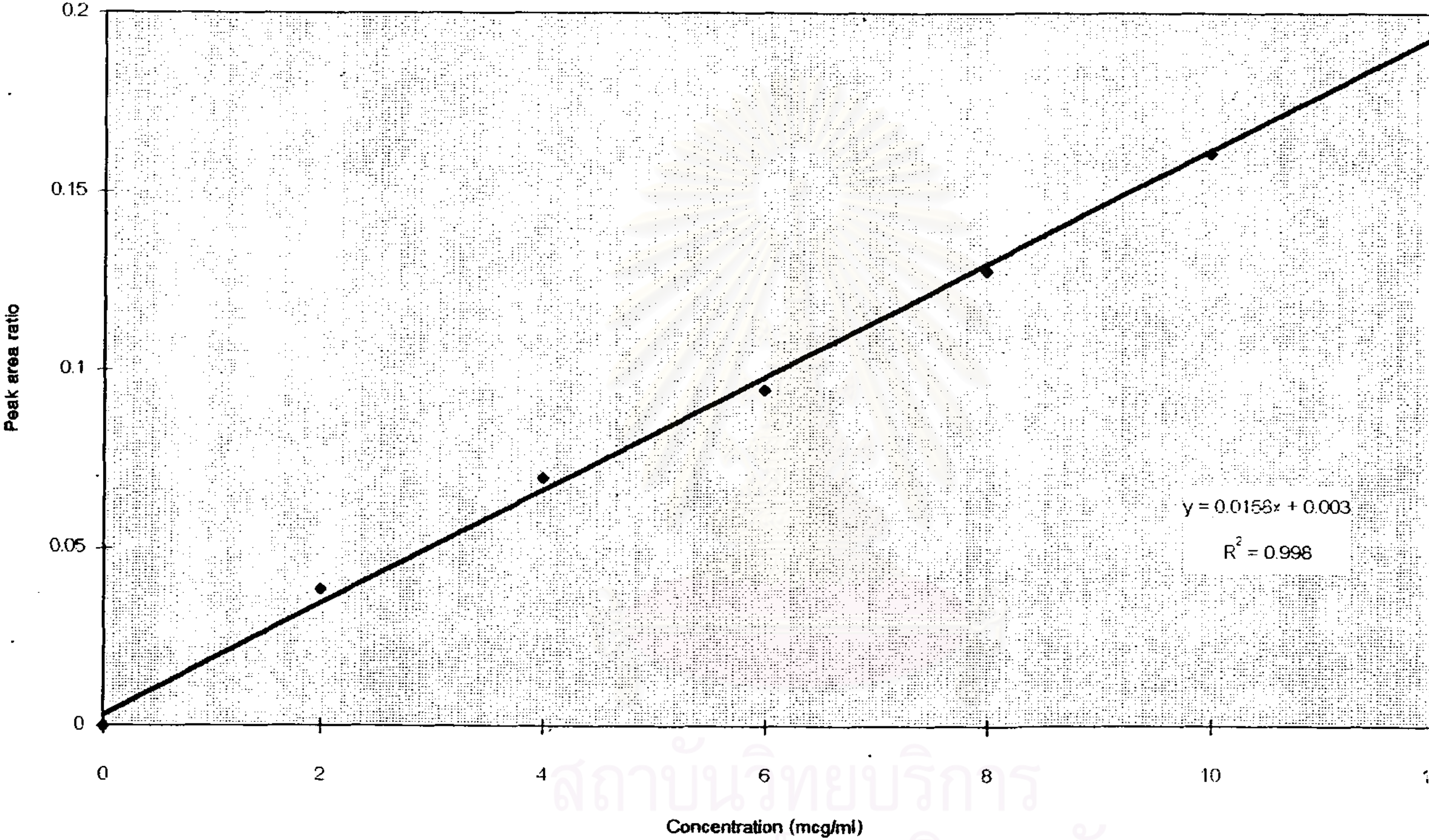

Figure 28 Standard curve of octyl dimethyl PABA in methanol using sulfamerazine as the internal standard at wavelength 254 nm.

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Table 15 Calibration curve data of peak area ratio between octyl dimethyl PABA and sulfamerazine as a function of octyl dimethyl PABA concentration.

No.	Octyl dimethyl PABA Conc. (mcg/ml)	Peak area ratio	Inversely Estimated Conc. (mcg/ml)	%Recovery
1	2.00	0.0385	1.98	98.76
2	4.00	0.0699	3.97	99.25
3	6.00	0.0946	6.01	100.10
4	8.00	0.1280	7.79	97.36
5	10.00	0.1607	9.92	99.18
6	12.00	0.1950	12.04	100.35
			Mean	99.17
			S.D.	1.81
			C.V.	1.81

Linear regression $r^2 = 0.998$, $y = 0.0158X + 0.003$

Inversely Estimate

$$\text{Concentration} = \frac{\text{Peak area ratio} - 0.003}{0.0158}$$

$$\%\ \text{Theory} = \frac{\text{Inversely Estimated Conc.} \times 100}{\text{Known Conc.}}$$

$$\%\ \text{C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100$$

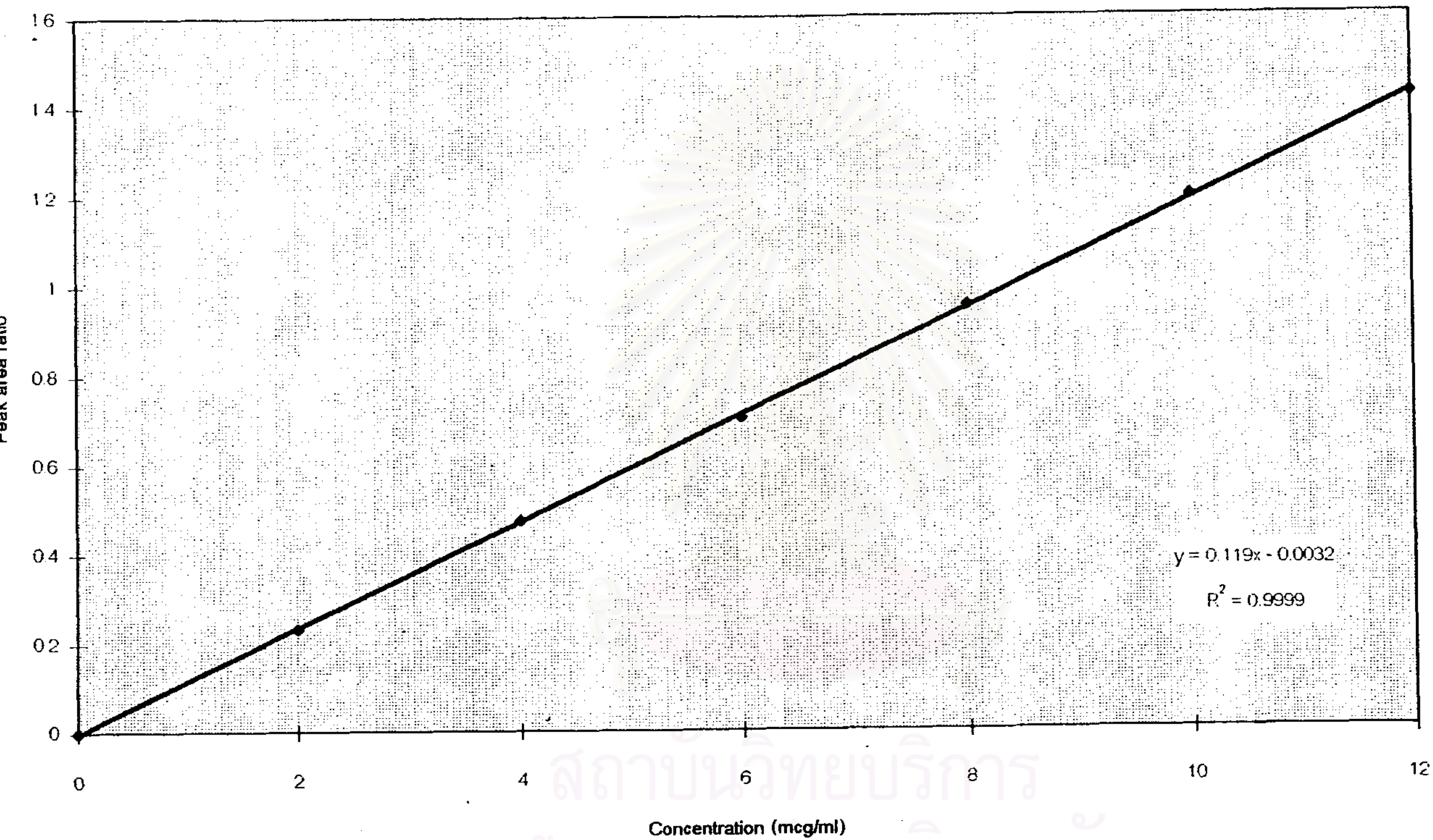

Figure 29 Standard curve of octyl methoxycinnamate in methanol using sulfamerazine as the internal standard at wavelength 254 nm.

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Table 16 Calibration curve data of peak area ratio between octyl methoxycinnamate and sulfamerazine as a function of octyl methoxycinnamate concentration.

No.	Oxybenzone Conc. (mcg/ml)	Peak area ratio	Inversely Estimated Conc. (mg %)	%Recovery
1	2.00	0.2312	1.99	99.35
2	4.00	0.4747	4.00	100.08
3	6.00	0.7031	5.96	99.26
4	8.00	0.9526	7.86	98.28
5	10.00	1.1938	10.03	100.25
6	12.00	1.4204	12.16	101.34
			Mean	99.76
			S.D.	0.50
			C.V.	0.50

Linear regression $r^2 = 0.9999$, $y = 0.025X + 0.0009$

Inversely Estimate

$$\text{Concentration} = \frac{\text{Peak area ratio} - 0.0009}{0.025}$$

$$\text{\% Theory} = \frac{\text{Inversely Estimated Conc.} \times 100}{\text{Known Conc.}}$$

$$\text{\% C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100$$

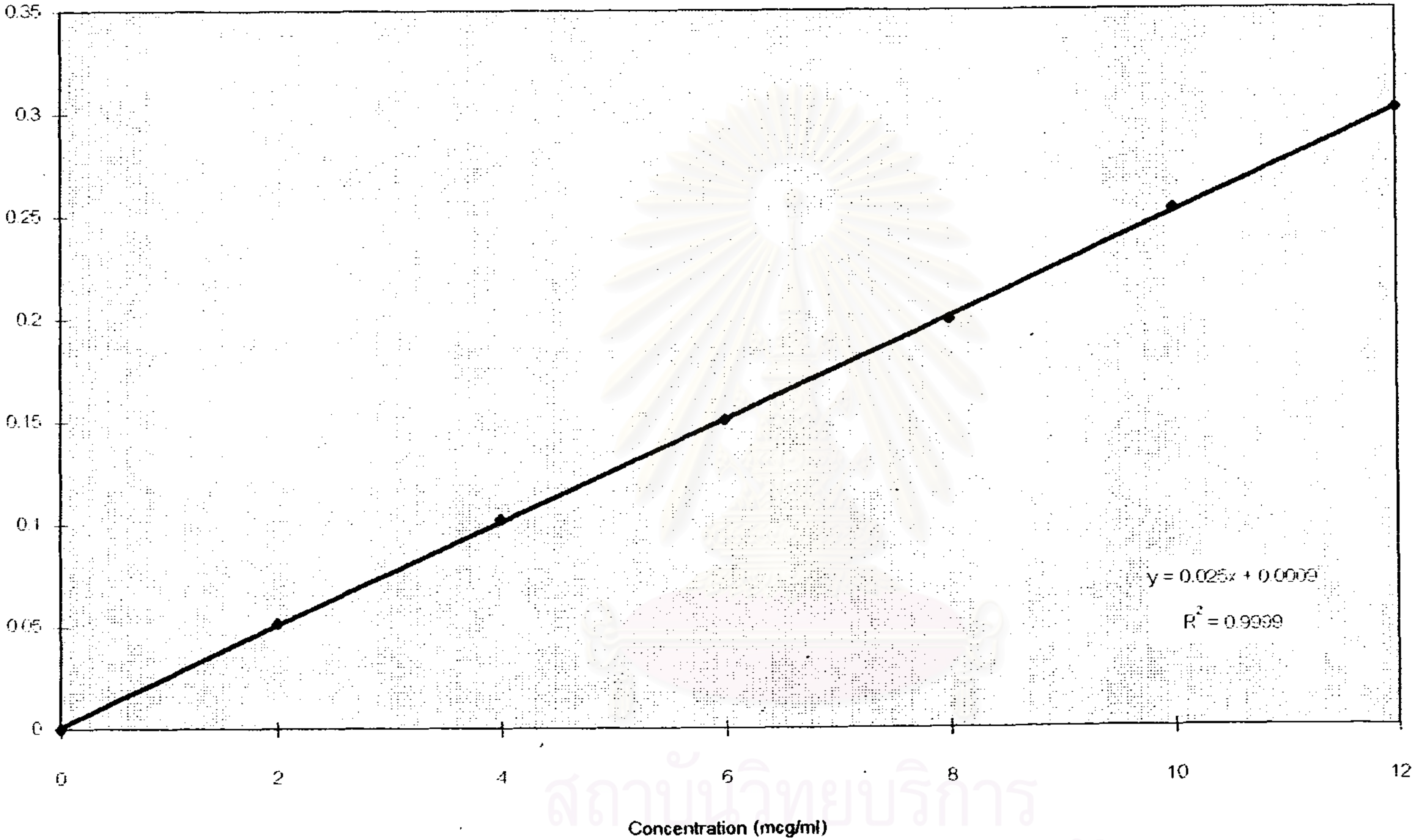

Figure 30 Standard curve of oxybenzone in methanol using sulfamerazine as the internal standard at wavelength 254 nm.

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Table 17 Calibration curve data peak area ratio between oxybenzone and sulfamerazine as a function of oxybenzone concentration.

No.	Octyl methoxycinnamate Conc.(mcg/ml)	Peak area ratio	Inversely Estimated Conc. (mcg/ml)	%Recovery
1	2.00	0.0515	1.95	99.74
2	4.00	0.1025	4.01	100.36
3	6.00	0.1508	5.92	98.59
4	8.00	0.1995	8.10	101.25
5	10.00	0.2526	9.94	99.38
6	12.00	0.3010	11.8	97.95
			Mean	99.95
			S.D.	1.7
			C.V.	1.7

Linear regression $r^2 = 0.9999$, $y = 0.119X-0.0032$

Inversely Estimate

$$\text{Concentration} = \frac{\text{Peak area ratio}+0.0032}{0.119}$$

$$\%\ \text{Theory} = \frac{\text{Inversely Estimated Conc.} \times 100}{\text{Known Conc.}}$$

$$\%\ \text{C.V.} = \frac{\text{S.D.}}{\text{Mean}} \times 100$$

2.3 Content analysis of the prepared sunscreen emulsions

The results of the content analysis of sunscreen agents in each sunscreen preparation are expressed as the percent labeled amount and are shown in Table 18. The amount of the test sunscreen was calculated from the calibration curve.

3. Determination of SPF values obtained from SPF - 290 analyzer

The results of experimental *in vitro* SPF values measured by SPF - 290 analyzer were shown in Table 19. It can be seen that the SPF values can be divided into three group, low - SPF, medium - SPF and high - SPF. The data were shown in Table 20, 21 and 22. The statistical analysis on the SPF values from SPF 290s analyzer in sunscreen emulsions, comparative between oil in water emulsion and water in oil emulsion were shown in Table 23. Seven pairs of comparative between oil in water emulsion and water in oil emulsion were not significant different but eleven pairs of them were significant different. The statistical analysis on the SPF values from SPF 290s analyzer in sunscreen emulsion, comparative between added silicone and none were shown in Table 24. Four pairs of comparative between added silicone and non added silicone showed non significant different but fourteen pairs of them showed significant different.

Table 23 and 24 showed that type of emulsion and the addition of silicone did not show significant different in SPF value when calculated with ANOVA.

Result from SPF values obtained from SPF 290s analyzer, the formula 26, 27, 35 and 36 (High - SPF) was selected to study skin penetration and evaluate of SPF of sunscreen emulsion by *in vivo* method.

Table 18 Content analysis of sunscreen agents in sunscreen emulsions.

Formula and its sunscreen agent	Concentration requirement of sunscreen agent (%w/w)	Analyzed concentration of sunscreen agent (%w/w)[a]	Percent labeled amount
standard homosalate sunscreen	8.0	7.95 ± 0.02	98.20
P Fomula 2	7.0	6.97 ± 0.07	100.43
5	7.0	6.25 ± 0.07	89.29
8	7.0	6.09 ± 0.03	87.00
11	7.0	7.12 ± 0.01	101.71
14	7.0	6.31 ± 0.03	90.14
17	7.0	7.02 ± 0.02	100.29
20	7.0	6.79 ± 0.05	97.00
23	7.0	6.49 ± 0.03	92.71
26	7.0	7.10 ± 0.04	101.43
29	7.0	7.12 ± 0.05	101.71
32	7.0	6.61 ± 0.08	94.43
35	7.0	6.76 ± 0.06	96.57
C Fomula 3	8.0	7.51 ± 0.04	93.88
6	8.0	7.34 ± 0.03	91.75
9	8.0	8.35 ± 0.07	104.38
12	8.0	8.46 ± 0.03	105.75
15	8.0	7.87 ± 0.04	98.38
18	8.0	7.76 ± 0.02	97.00
21	8.0	8.15 ± 0.03	101.88
24	8.0	8.03 ± 0.04	100.38
27	8.0	8.07 ± 0.05	100.88
30	8.0	7.89 ± 0.03	98.63
33	8.0	7.46 ± 0.05	93.25
36	8.0	9.05 ± 0.02	113.13

a : Indicated data are means ± SD, n = 3

P = Octyl dimethyl PABA and C = Octyl methoxycinnamate

Table 18 Content analysis of sunscreen agents in sunscreen emulsions (continued).

Formula and its sunscreen agent	Concentration requirement of sunscreen agent (%w/w)	Analyzed concentration of sunscreen agent (%w/w)[a]	Percent labeled amount
O Fomula 4	3.0	2.80 ± 0.04	93.33
7	3.0	2.56 ± 0.02	85.33
8	3.0	2.53 ± 0.02	84.33
9	3.0	2.68 ± 0.05	89.33
13	3.0	3.09 ± 0.02	103.00
16	3.0	2.71 ± 0.03	90.33
17	3.0	2.65 ± 0.01	88.33
18	3.0	2.49 ± 0.02	83.00
22	3.0	3.70 ± 0.04	113.33
25	3.0	2.73 ± 0.02	91.00
26	3.0	2.96 ± 0.02	98.67
27	3.0	3.05 ± 0.03	101.67
31	3.0	2.83 ± 0.02	94.33
34	3.0	2.65 ± 0.03	88.33
35	3.0	3.14 ± 0.04	104.67
36	3.0	2.73 ± 0.02	91.00

a : Indicated data are means ± SD, n = 3

O = Oxybenzone

Table 19 *In vitro* SPF values obtained from SPF 290s analyzer.

Formula	SPF ± SD	Formula	SPF ± SD
1	2.1 ± 0.2	19	3.0 ± 0.3
2	4.3 ± 0.4	20	4.1 ± 0.2
3	5.9 ± 0.3	21	4.4 ± 0.7
4	2.3 ± 0.1	22	3.7 ± 0.3
5	9.1 ± 1.0	23	8.9 ± 0.5
6	11.6 ± 0.5	24	9.3 ± 1.0
7	8.8 ± 0.6	25	6.1 ± 0.4
8	18.1 ± 1.2	26	20.0 ± 1.6
9	14.8 ± 1.0	27	22.3 ± 0.6
10	2.3 ± 0.1	28	3.8 ± 0.7
11	4.2 ± 0.8	29	4.6 ± 0.6
12	4.0 ± 0.3	30	4.8 ± 0.7
13	3.5 ± 0.2	31	3.7 ± 0.3
14	9.7 ± 1.0	32	6.9 ± 1.2
15	6.5 ± 0.5	33	11.6 ± 1.2
16	13.9 ± 0.6	34	7.6 ± 1.1
17	15.6 ± 0.6	35	18.3 ± 1.1
18	20.0 ± 1.1	36	18.7 ± 0.9

Note : Indicated data are means ± SD, n = 6

Table 20 *In vitro* SPF values obtained from SPF 290s analyzer (low-SPF).

Formula	emulsion base	sunscreen agent[a]	SPF ± SD[b]
1	oil in water	Mi. TiO_2 5%	2.1 ± 0.2
2	oil in water	P 7%	4.3 ± 0.4
3	oil in water	C 8%	5.9 ± 0.3
4	oil in water	O 3%	2.3 ± 0.1
10	water in oil	Mi. TiO_2 5%	2.3 ± 0.1
11	water in oil	P 7%	4.2 ± 0.8
12	water in oil	C 8%	4.0 ± 0.3
13	water in oil	O 3%	3.5 ± 0.2
19	oil in water	Mi. TiO_2 + Silicone	3.0 ± 0.3
20	oil in water	P + Silicone	4.1 ± 0.2
21	oil in water	C + Silicone	4.4 ± 0.7
22	oil in water	O + Silicone	3.7 ± 0.3
28	water in oil	Mi. TiO_2 + Silicone	3.8 ± 0.7
29	water in oil	P + Silicone	4.6 ± 0.6
30	water in oil	C + Silicone	4.8 ± 0.7
31	water in oil	O + Silicone	3.7 ± 0.3

a : Mi. TiO_2 = Micronized Titanium dioxide, P = Octyl dimethyl PABA, C = Octyl methoxycinnamate and O = Oxybenzone

b : Indicated data are means ± SD, n = 6

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Table 21 *In vitro* SPF values obtained from SPF 290s analyzer (medium-SPF).

Formula	emulsion base	sunscreen agent[a]	SPF ± SD[b]
5	oil in water	Mi. TiO_2 + P	9.1 ± 1.0
6	oil in water	Mi. TiO_2 + C	11.6 ± 0.5
7	oil in water	Mi. TiO_2 + O	8.8 ± 0.6
14	water in oil	Mi. TiO_2 + P	9.7 ± 1.0
15	water in oil	Mi. TiO_2 + C	6.5 ± 0.5
16	water in oil	Mi. TiO_2 + O	13.9 ± 0.6
23	oil in water	Mi. TiO_2 + P + Silicone	8.9 ± 0.5
24	oil in water	Mi. TiO_2 + C + Silicone	9.3 ± 1.0
25	oil in water	Mi. TiO_2 + O + Silicone	6.1 ± 0.4
32	water in oil	Mi. TiO_2 + P + Silicone	6.9 ± 1.2
33	water in oil	Mi. TiO_2 + C + Silicone	11.6 ± 1.2
34	water in oil	Mi. TiO_2 + O + Silicone	7.6 ± 1.1

a : Mi. TiO_2 = Micronized Titanium dioxide, P = Octyl dimethyl PABA, C = Octyl methoxycinnamate and O = Oxybenzone

b : Indicated data are means ± SD, n = 6

Table 22 *In vitro* SPF values obtained from SPF 290s analyzer (high-SPF).

Formula	emulsion base	sunscreen agent[a]	SPF ± SD[b]
8	oil in water	Mi. TiO_2 + P + O	18.1 ± 1.2
9	oil in water	Mi. TiO_2 + C + O	14.8 ± 1.0
17	water in oil	Mi. TiO_2 + P + O	15.6 ± 0.6
18	water in oil	Mi. TiO_2 + C + O	20.0 ± 1.1
26	oil in water	Mi. TiO_2 + P + O + Silicone	20.0 ± 1.6
27	oil in water	Mi. TiO_2 + C + O + Silicone	22.3 ± 0.6
35	water in oil	Mi. TiO_2 + P + O + Silicone	18.3 ± 1.1
36	water in oil	Mi. TiO_2 + C + O + Silicone	18.7 ± 0.9

a : Mi. TiO_2 = Micronized Titanium dioxide, P = Octyl dimethyl PABA, C = Octyl methoxycinnamate and O = Oxybenzone

b : Indicated data are means ± SD, n = 6

Table 23 The ANOVA statistic on the SPF value from SPF 290s analyzer in sunscreen emulsions.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	0.0225	1	0.0225	0.00642	0.937075	4.451323
Columns	1207.005	17	71.00028	20.25771	4.71E-08	2.271893
Error	59.5825	17	3.504853			
Total	1266.61	35				

Table 24 The statistical analysis on the SPF values from SPF 290s analyzer in sunscreen emulsions (comparative between oil in water base and water in oil base) $\alpha = 0.05$.

Tested	Formula	number	t-value	Significances
1	VS	10	-2.181	non-significant
2	VS	11	0.2738	non-significant
3	VS	12	10.970	significant
4	VS	13	-13.086	significant
19	VS	28	-2.572	significant
20	VS	29	-1.936	non-significant
21	VS	30	-0.990	non-significant
22	VS	31	0.000	non-significant
5	VS	14	-1.039	non-significant
6	VS	15	17.660	significant
7	VS	16	-14.723	significant
23	VS	32	3.774	significant
24	VS	33	-3.611	significant
25	VS	34	-3.138	significant
8	VS	17	4.571	significant
9	VS	18	-8.567	significant
26	VS	35	2.144	non-significant
27	VS	36	8.152	significant

t_{table} ; $t_{.05}$ df 10 = 2.228

Table 25 The statistical analysis on the SPF values from SPF 290s analyzer in sunscreen emulsions (comparative between added silicone and none) $\alpha = 0.05$.

Tested	Formular	number	t-value	Significances
1	VS	19	-6.110	significant
2	VS	20	1.094	non-significant
3	VS	21	4.837	significant
4	VS	22	4.811	significant
10	VS	28	-5.194	significant
11	VS	29	-0.980	non-significant
12	VS	30	-2.572	significant
13	VS	31	-1.358	non-significant
5	VS	23	0.438	non-significant
6	VS	24	5.038	significant
7	VS	25	9.066	significant
14	VS	32	4.704	significant
15	VS	33	-9.608	significant
16	VS	34	12.312	significant
8	VS	26	-2.849	significant
9	VS	27	-15.753	significant
17	VS	35	-5.277	significant
18	VS	36	2.240	significant

t_{table} ; $t_{.05}$ df 10 = 2.228

4. Determination of *in vitro* skin penetration

In order to investigate a possible influence of the formulation, and to measure the concentration of sunscreen agents in stratum corneum, epidermis, dermis and receptor fluid. Amounts of sunscreen agents recovered at the end of 8 hr. was shown in Table 25. Penetration values of sunscreen agent at each time intervals in receptor fluid was shown in Table 26.

Table 26 Amounts of sunscreen agents recovered at the end of 8 hr.

Sunscreen	agent	% Recovery [a]				
		stratum corneum	epidermis	dermis	receptor fluid	total recovery
Formula 26	P	99.65 ± 0.35	0.68 ± 0.06	0.02 ± 0.01	0 ± 0	100.35 ± 0.42
	O	95.62 ± 0.77	0.32 ± 0.04	0 ± 0	0 ± 0	95.94 ± 0.81
Formula 35	P	94.26 ± 0.34	0.46 ± 0.08	0.04 ± 0.02	0 ± 0	94.76 ± 0.44
	O	99.80 ± 0.42	1.63 ± 0.15	0 ± 0	0 ± 0	101.43 ± 0.57
Formula 27	C	95.45 ± 0.21	1.13 ± 0.02	0.15 ± 0.03	0 ± 0	97.73 ± 0.26
	O	99.23 ± 0.28	0.76 ± 0.03	0.06 ± 0.12	0 ± 0	100.05 ± 0.43
Formula 36	C	100.57 ± 0.67	0.69 ± 0.08	0 ± 0	0 ± 0	101.26 ± 0.75
	O	87.94 ± 0.52	2.06 ± 0.09	0 ± 0	0 ± 0	90.00 ± 0.61

a : Indicated data are means ± SD, n = 3

P = Octyl dimethyl PABA, O = Oxybenzone, C = Octyl methoxycinnamate

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Table 27 Penetration values of sunscreen agents at each time intervals in receptor a fluid.

		Receptor fluid			
		oil in water emulsion base		water in oil emulsion base	
		Formula 26 [b]	Formula 35	Formula 27	Formula 36
2 min	P	0 ± 0	0 ± 0	-	-
	O	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C	-	-	0 ± 0	0 ± 0
0.5 hr	P	0 ± 0	0 ± 0	-	-
	O	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C	-	-	0 ± 0	0 ± 0
2 hr	P	0 ± 0	0 ± 0	-	-
	O	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C	-	-	0 ± 0	0 ± 0
4 hr	P	0 ± 0	0 ± 0	-	-
	O	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C	-	-	0 ± 0	0 ± 0
6 hr	P	0 ± 0	0 ± 0	-	-
	O	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C	-	-	0 ± 0	0 ± 0
8 hr	P	0 ± 0	0 ± 0	-	-
	O	0 ± 0	0 ± 0	0 ± 0	0 ± 0
	C	-	-	0 ± 0	0 ± 0

a : Indicated data are means ± SD, n = 3

b : Formular 26 and 35; sunscreen agents = Octyl dimthyl PABA and Oxybenzone

Formular 27 and 36; sunscreen agents = Octyl methoxycinnamate and Oxybenzone

P = Octyl dimethyl PABA, O = Oxybenzone and C = Octyl methoxycinnamate

SPF values obtained from the US - FDA procedure

The results of experimental *in vivo* SPF values of Formula 26, 27, 35 and 36 measured by the US - FDA procedure were shown in Table 27. It should be noted that these products were tested in Thai volunteers who has only skin type III and IV. In fact, the US - FDA standard specifies the use of skin type I and II, but these skins were virtually impossible to find in Thailand. The correlation between the *in vitro* SPF and the *in vivo* SPF of tested products were shown in Table 28. It can be seen that the correlation coefficient (r) between SPF values obtained from the US - FDA procedure and the SPF 290s analyzer is 0.5658 (Figure 31).

Table 28 The SPF values of sunscreen emulsions measured by the US-FDA procedure.

volunteers no.	skin type	MED_u	SPF			
			Formula 26	Formula 27	Formula 35	Formula 36
1.	3	1.125	14.76	13.45	12.76	14.42
2.	3	1.1.25	13.86	9.76	10.48	13.26
3.	4	1.625	15.04	10.46	11.78	12.14
4.	4	1.625	14.97	12.54	14.54	14.45
5.	4	1.25	14.72	13.75	12.46	12.37
6.	4	1.25	13.45	14.34	14.21	13.04
7.	4	1.25	12.96	13.45	9.91	12.74
8.	4	1.25	11.94	12.86	10.06	9.48
9.	4	1.25	15.17	11.75	11.14	12.63
10.	4	1.25	14.05	10.78	12.21	11.78
11.	4	1.25	14.44	10.46	13.16	10.98

MED_u = minimal erythema dose for unprotected skin

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Table 28 The SPF values of sunscreen emulsions measured by the US-FDA procedure (continued).

volunteers no.	skin type	MED_u	SPF			
			Formula 26	Formula 27	Formula 35	Formula 36
12.	4	1.25	13.65	11.74	10.08	11.16
13.	4	1.50	11.24	12.48	11.17	12.25
14.	4	1.25	10.94	11.47	12.98	10.08
15.	4	1.25	12.54	10.29	13.15	11.72
16.	4	1.50	14.01	10.97	14.14	13.14
17.	4	1.50	14.84	11.45	13.19	10.14
18.	3	1.125	12.15	11.74	14.14	14.73
19.	4	1.250	13.67	12.03	14.74	12.48
20.	3	1.125	14.01	13.14	13.04	12.84
mean SPF			13.62	11.95	12.46	12.45

MED_u = minimal erythema dose for unprotected skin

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Table 29 Comparison between SPF values obtained from US-FDA procedure and SPF-290 analyzer.

Sunscreen emulsions	US-FDA SPF (mean)	SPF-290 analyzer		t-value	$\alpha = 0.05$
		SPF(mean)	range		
Formula 26	13.62	20.0	18.4-21.6	-8.821	significant
Formula 27	11.95	22.3	21.7-22.9	-8.491	significant
Formula 35	12.46	18.3	17.2-19.4	-2.819	significant
Formula 36	12.45	18.7	17.8-19.6	-2.278	significant
Homosalate	4.10	4.2	4.0-4.4	-0.833	non-significant

t_{table} ; $t_{.05}$ df 24 = 2.064

Example Fomula 26 F test $= \dfrac{S_1^2/n_1-1}{S_2^2/n_2-1} = \dfrac{(1.35)^2/(20-1)}{(0.94)^2/(6-1)} = 0.54279$

F_{table} ; $F_{.05}$ df 24 = 1.52 = non-significant

Pearson 's t test ; $t = \dfrac{r}{\sqrt{(1-r^2)/(n-2)}}$

$$= \frac{0.5658}{\sqrt{(1-0.3201)/(4-2)}}$$

$$= \frac{0.5658}{0.5831} = 0.9703$$

t_{table} ; $t_{.05}$ df 2 = 4.303

Note : data from US-FDA procedure and SPF-290 analyzer are different.

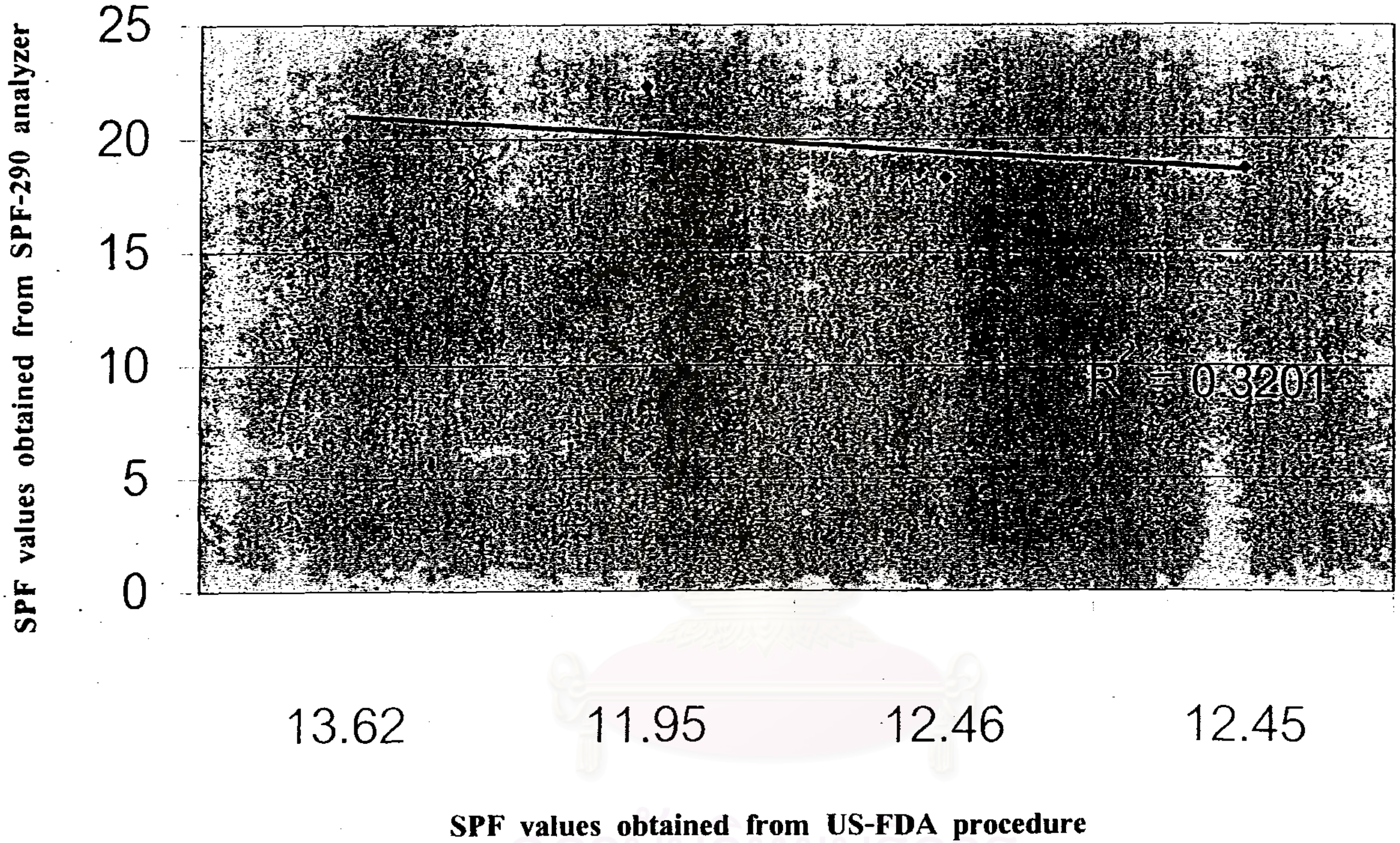

Figure 31 Correlation between SPF values obtained from the US-FDA procedure and SPF-290s analyzer.

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