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APPENDICES

APPENDIX A

TLC plate of Asiaticoside and R_f value

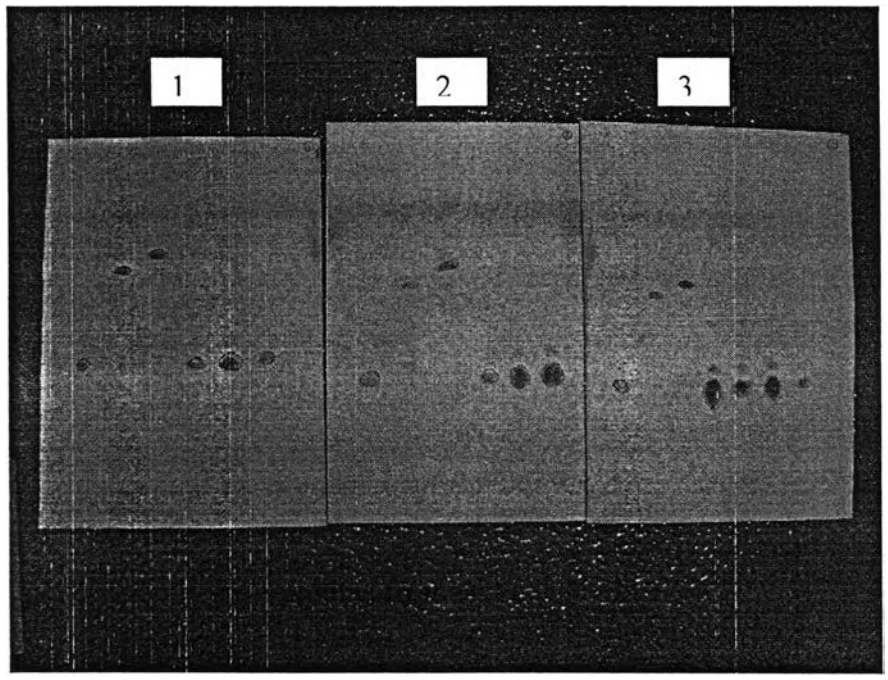

Figure 63 TLC plate for identification asiaticoside (From left → right , Plate No.1 asiaticoside , madecassic acid , asiatic acid, recrystallized product from methyl alcohol , recrystallized product from ethyl alcohol, recrystallized product from n-proyl alcohol : Plate No.2 asiaticoside, madecassic acid, asiatic acid, recrystallized product from isopropyl alcohol, recrystallized product from 1-butyl alcohol, recrystallized product from 2-butyl alcohol : Plate No.3 asiaticoside , madecassic acid , asiatic acid, recrystallized product from acetone, recrystallized product from methyl alcohol/acetonitrile, recrystallized product from methyl alcohol/water and asiaticoside)

The Rf values were calculated from following equation and the data were presented in Table 5

$$R_f \text{ value} = \frac{\text{Distance moved by the solute}}{\text{Distance moved by mobile-phase front}}$$

Table 7 The R_f values of asiaticoside , madecassic acid , asiatic acid, recrystallized asiaticoside from various solvents

Substance	Distance moved by solute(cm)	Distance moved by mobile phase front(cm)	R_f value
Asiaticoside	2.9	7.0	0.41
Madecassic acid	5.2	7.0	0.74
Asiatic acid	5.6	7.0	0.80
product from methyl alcohol	2.9	7.0	0.41
product from ethyl alcohol	2.9	7.0	0.41
product from n-proyl alcohol	2.9	7.0	0.41
product from isopropyl alcohol	2.9	7.0	0.41
product from 1-butyl alcohol	2.9	7.0	0.41
product from 2-butyl alcohol	2.9	7.0	0.41
product from acetone	2.8	7.0	0.40
product from methyl alcohol / acetonitrile	2.9	7.0	0.41
product from methyl alcohol / water	2.8	7.0	0.40

APPENDIX B

Standard curve of asiaticoside

Table 8 Concentration and Peak Area data for calibration curve of asiaticoside

Conc. (mg/ml)	Peak area			Average Peak area	SD
0.2728	897336	886731	888435	890834	5694.99
0.5456	1777697	1777576	1771162	1775478	3738.54
0.8184	2606344	2617222	2634946	2619503	14434.77
1.0912	3458048	3466018	3485206	3469757	13959.81
1.3640	4363728	4345095	4330447	4346423	16680.22

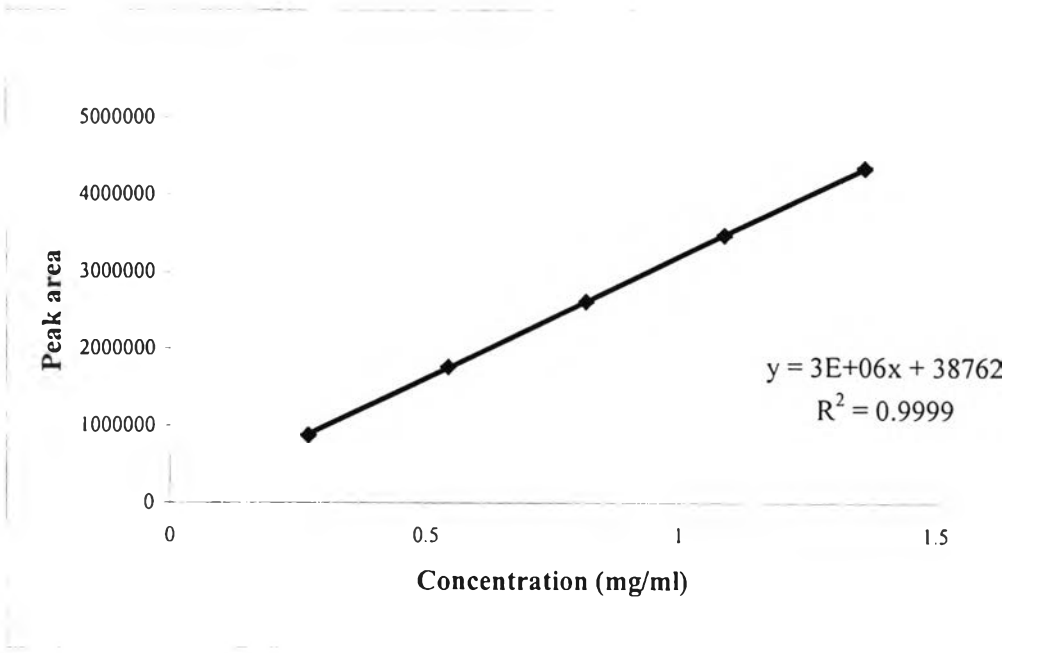

Figure 64 Calibration curve of Asiaticoside from HPLC analysis

Table 9 Solubility data of Asiaticoside I in water 37 ± 2 °C

Time (mins)	Concentration(mg/ml) 1	2	3	Average Concentration (mg/ml)	SD
5	0.4284	0.4020	0.4196	0.4167	0.0134
10	0.4738	0.4237	0.4504	0.4493	0.0251
15	0.4861	0.4416	0.4578	0.4619	0.0225
20	0.4879	0.4452	0.4567	0.4633	0.0221
25	0.4520	0.4457	0.4560	0.4513	0.0051
40	0.4576	0.4437	0.4863	0.4625	0.0217
60	0.4898	0.4605	0.4651	0.4718	0.0158
90	0.4744	0.4668	0.4663	0.4692	0.0045
120	0.4700	0.4655	0.4683	0.4679	0.0023
180	0.4634	0.4681	0.4662	0.4659	0.0024
240	0.4719	0.4638	0.4653	0.4668	0.0045
300	0.4766	0.4599	0.4687	0.4684	0.0084
360	0.4476	0.4548	0.4608	0.4544	0.0066
480	0.4387	0.4430	0.4470	0.4429	0.0042
600	0.4374	0.4390	0.4415	0.4393	0.0021

Table 10 Solubility data of asiaticoside II in water 37 ± 2 °C

Time (mins)	Concentration(mg/ml) 1	2	3	Average Concentration (mins)	SD
5	0.5391	0.5136	0.5348	0.5292	0.0137
10	0.5862	0.5472	0.5429	0.5588	0.0238
15	0.5486	0.5535	0.5689	0.5570	0.0106
20	0.5517	0.5548	0.5738	0.5601	0.0120
25	0.5531	0.5535	0.5619	0.5561	0.0050
40	0.5332	0.5486	0.5412	0.5410	0.0077
60	0.5198	0.5435	0.5563	0.5399	0.0185
90	0.4924	0.5274	0.4987	0.5062	0.0187
120	0.5048	0.5151	0.4737	0.4978	0.0215
180	0.4990	0.4727	0.4902	0.4873	0.0134
240	0.4774	0.4419	0.4853	0.4682	0.0231
300	0.4656	0.4804	0.4814	0.4758	0.0088
360	0.4791	0.4796	0.4634	0.4741	0.0093
480	0.4507	0.4741	0.4653	0.4634	0.0118
600	0.4453	0.4582	0.4679	0.4572	0.0113

APPENDIX C

Method validation for assay asiaticoside

Linearity study

Table 11 Concentration and Peak Area data for calibration curve of asiaticoside (between days)

Conc. (mg/ml)	Average Peak area			SD
	1 day	2 days	3 days	
0.2728	890834	878187	887462	6549.08
0.5456	1775478	1762574	1812499	25915.16
0.8184	2619503	2600661	2672194	37077.24
1.0912	3469757	3465614	3542449	43214.40
1.3640	4346423	4317277	4407452	46017.31

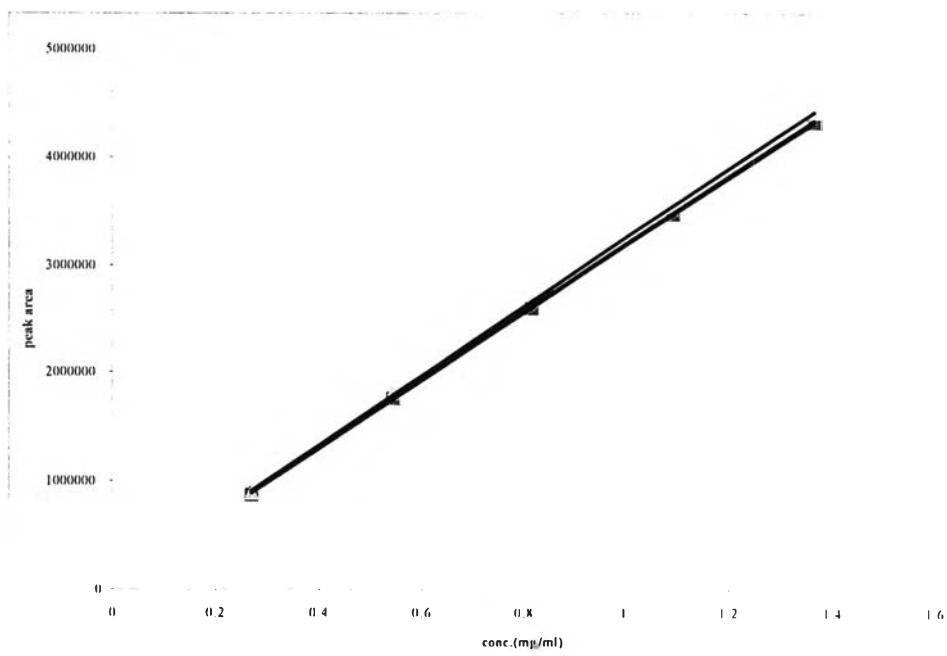

Figure 65 Linearity curve of asiaticoside (between day)

Equation for 1 day : $Y = 3 \times 10^6X + 38762$, $r^2 = 0.9999$

Equation for 2 days: $Y = 3 \times 10^6X + 30497$, $r^2 = 0.9999$

Equation for 3 days: $Y = 3 \times 10^6X + 33432$, $r^2 = 0.9999$

Precision study

Table 12 Peak area of asiaticoside standard solution concentration 1.0 mg/ml

Injection No.	Peak area
1	3458048
2	3466018
3	3485206
4	3478781
5	3480676
average	3473746
%RSD	0.33

APPENDIX D

XRPD patterns of asiaticoside I and asiatocoside II and Incompatability testing at 18 weeks

This XRPD patterns using Joel X-ray diffractometer (JDX-3530) at 30 mA and 40 kV with CuK∝ radiation. The samples were scanned with the diffraction angle increasing from 5° to 50°, 2θ,with a step size of 5° and count time of 1 minute.

Sample preparation

The samples were mounts onto the glass slide by vasaline, and then pressed the samples until it was a smooth surface by using other slide.

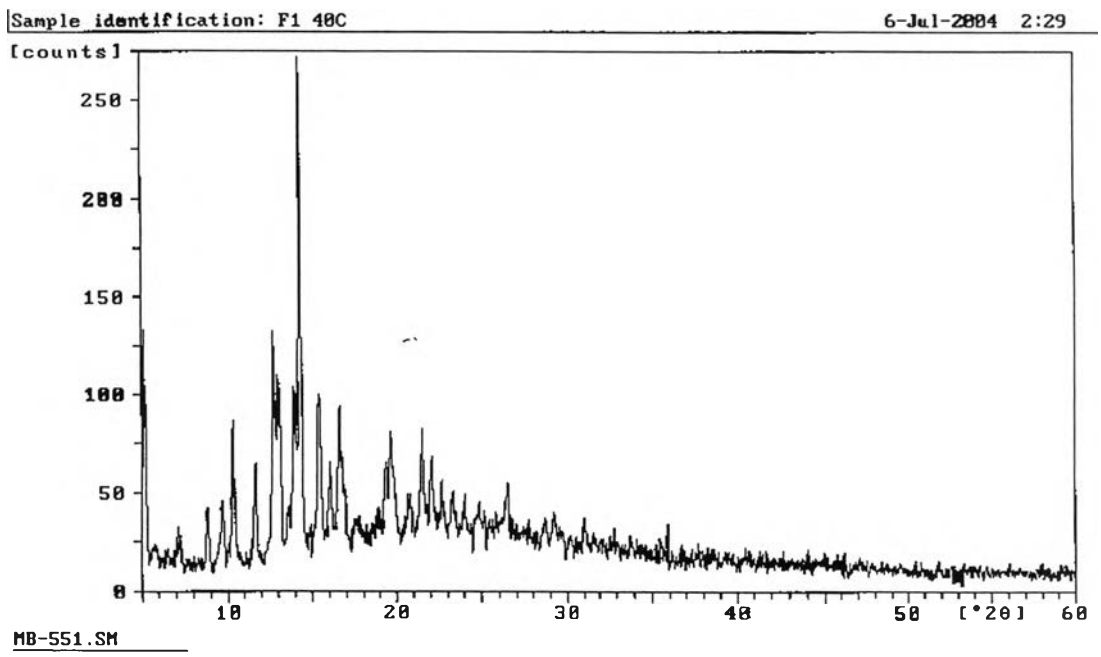

Figure 66 XRPD pattern of asiaticoside I after stored in 40° C and 62%RH at 18 weeks

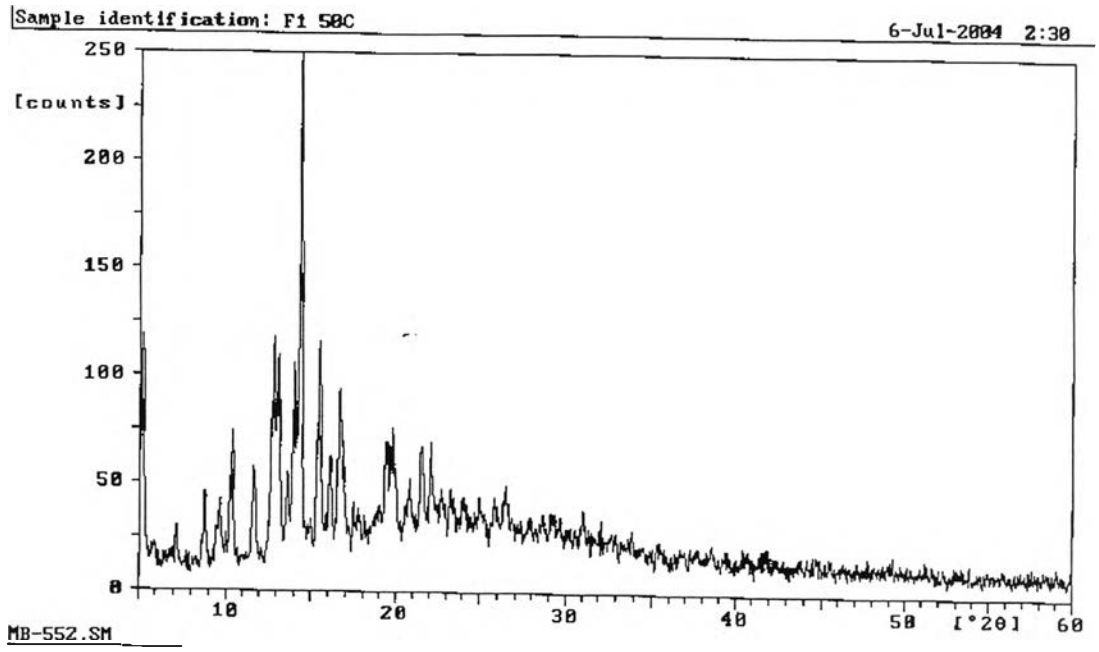

Figure 67 XRPD pattern of asiaticoside I after stored in 50° C, 55-65%RH at 18 weeks

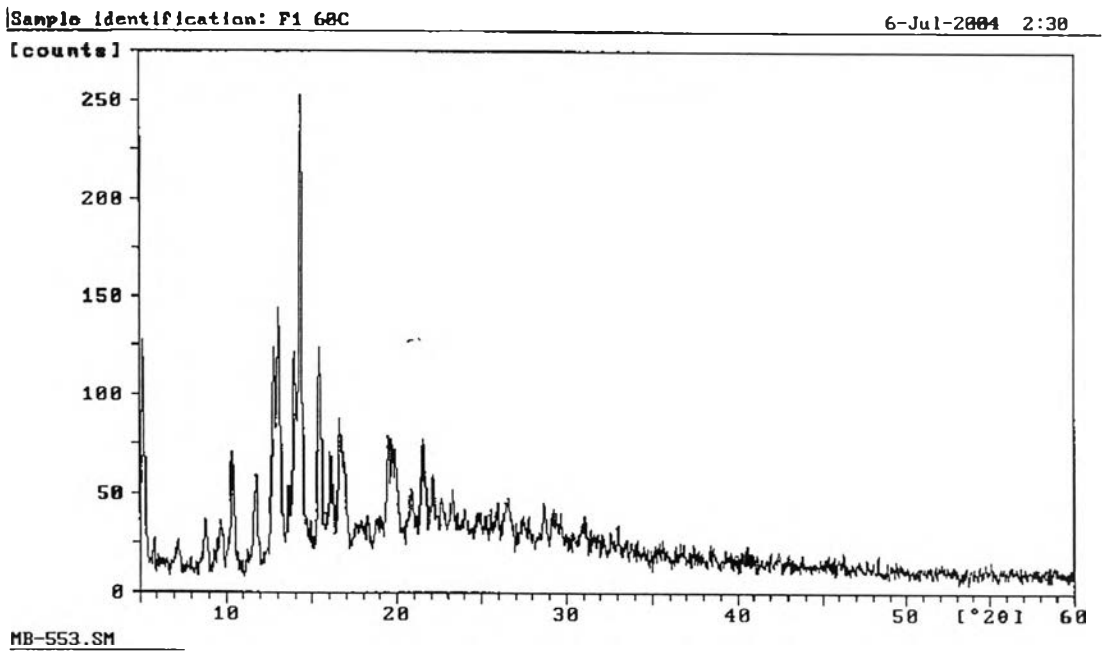

Figure 68 XRPD pattern of asiaticoside I after stored in 60° C, 55-65%RH at 18 weeks

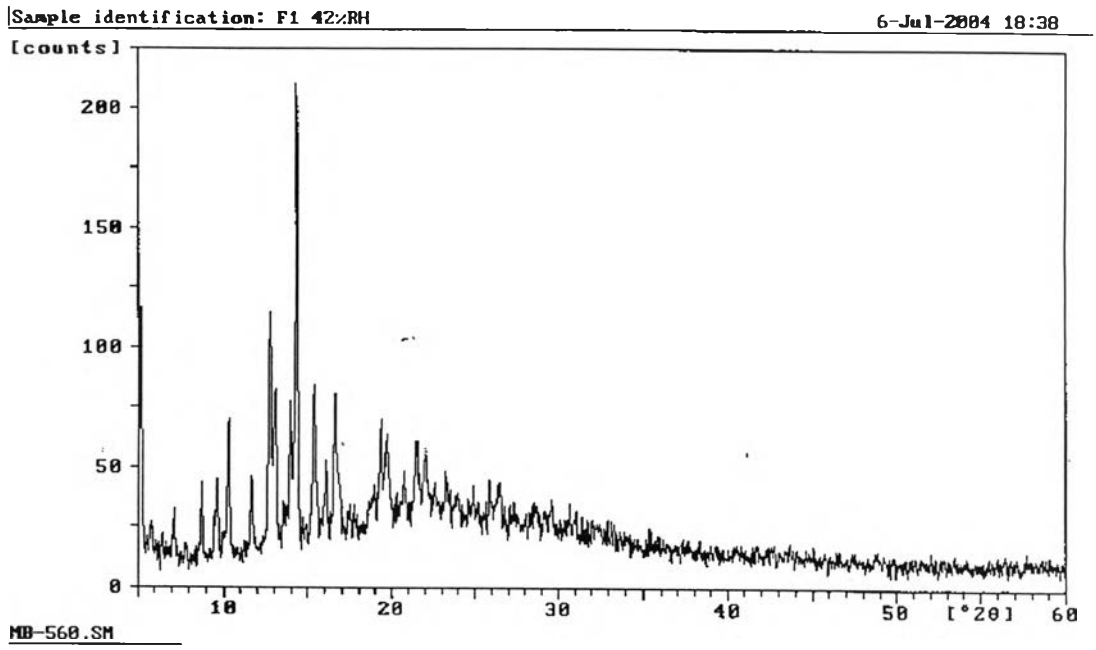

Figure 69 XRPD pattern of asiaticoside I after stored in 42%RH , 40°C at 18 weeks

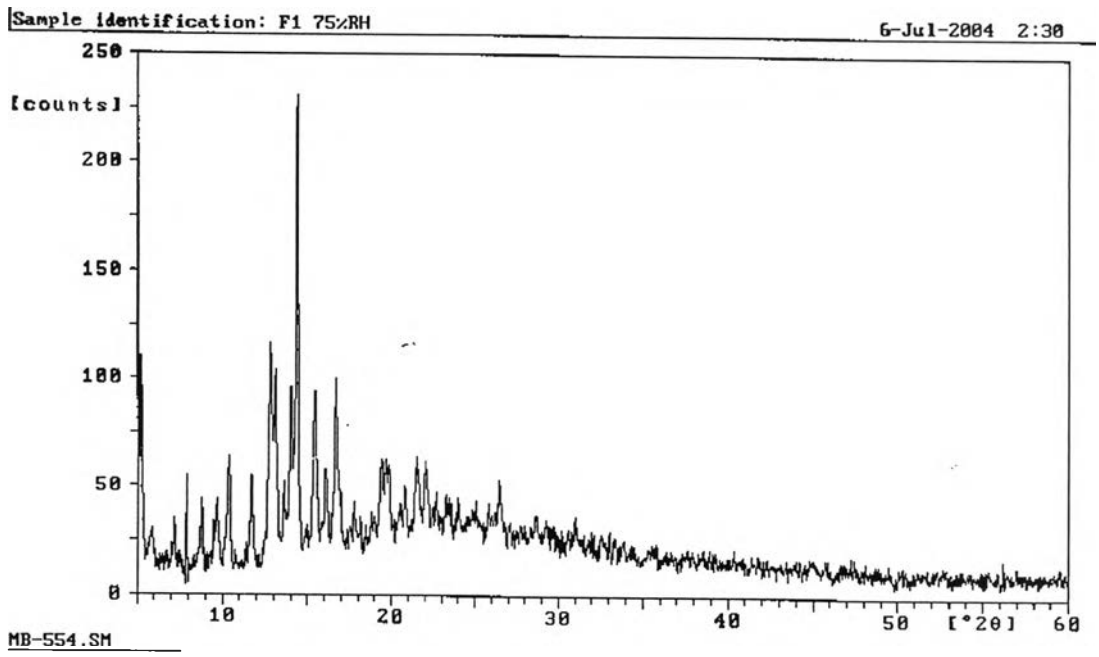

Figure 70 XRPD pattern of asiaticoside I after stored in 75%RH, 40°C at 18 weeks

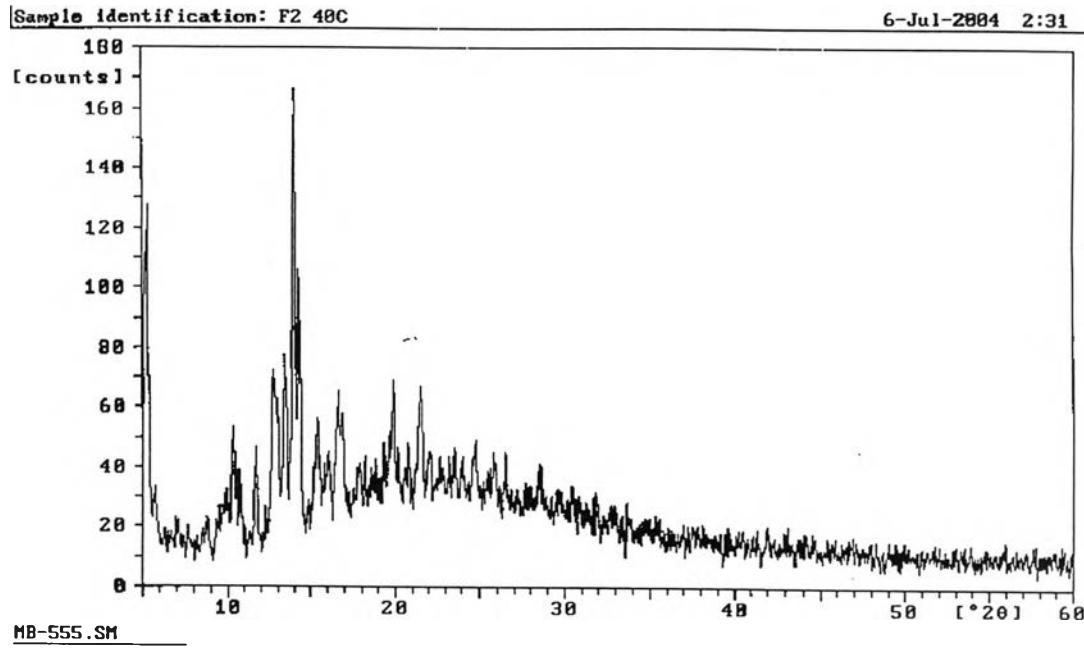

Figure 71 XRPD pattern of asiaticoside II after stored in 40° C and 62%RH at 18 weeks

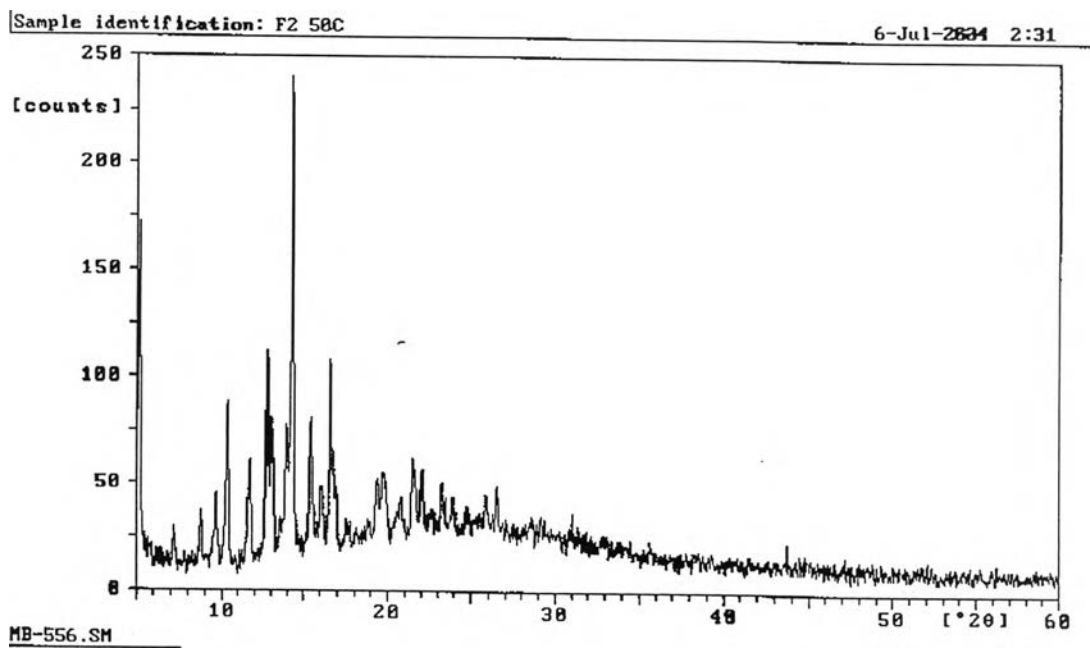

Figure 72 XRPD pattern of asiaticoside II after stored in 50° C , 55-65%RH at 18 weeks

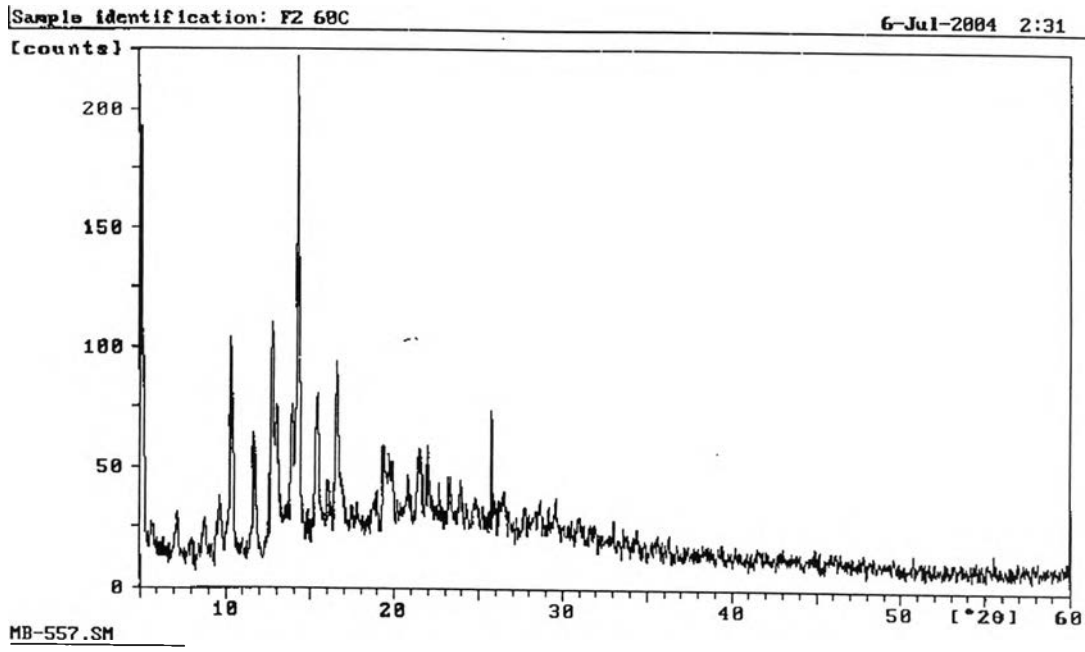

Figure 73 XRPD pattern of asiaticoside II after stored in 60° C, 55-65%RH at 18 weeks

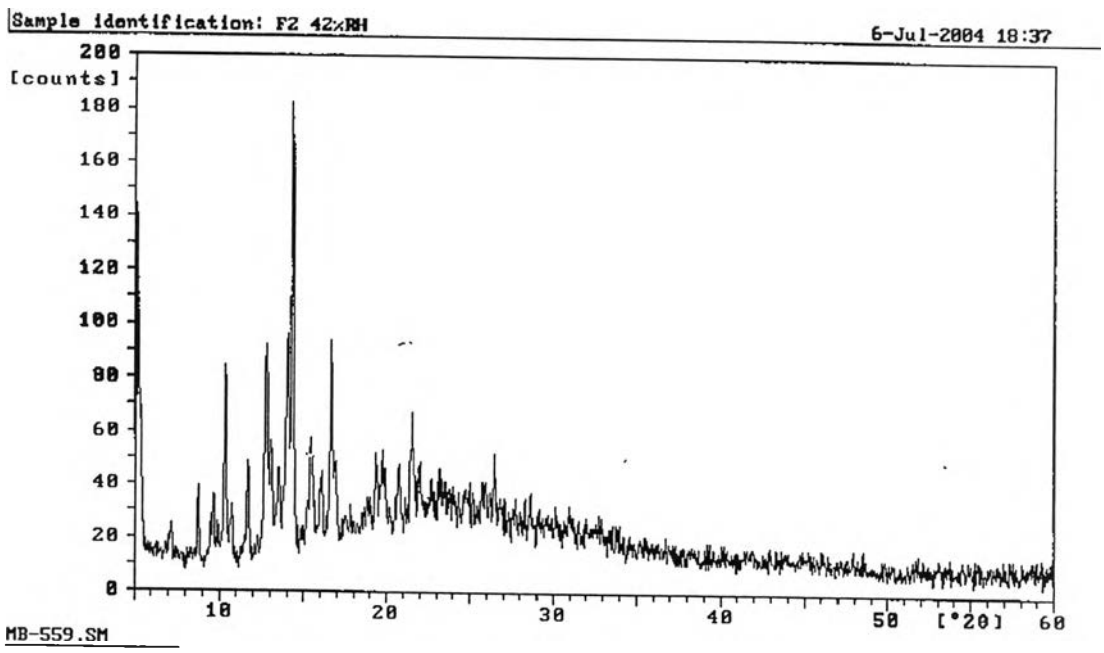

Figure 74 XRPD pattern of asiaticoside II after stored in 42%RH, 40°C at 18 weeks

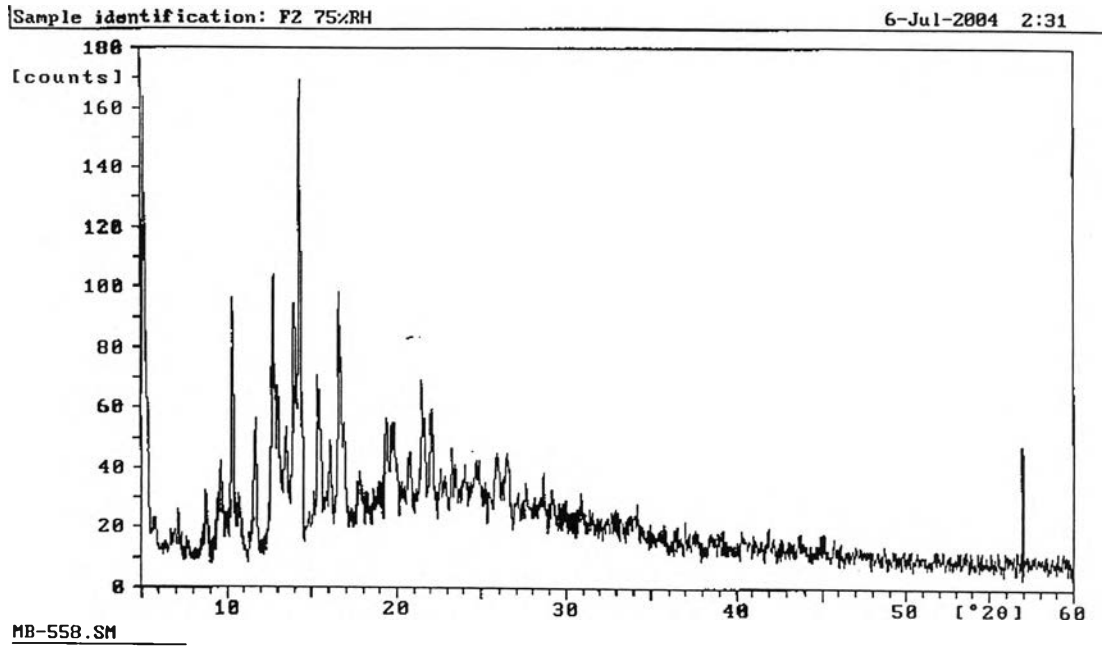

Figure 75 XRPD pattern of asiaticoside II after stored in75%RH, 40°C at 18 weeks

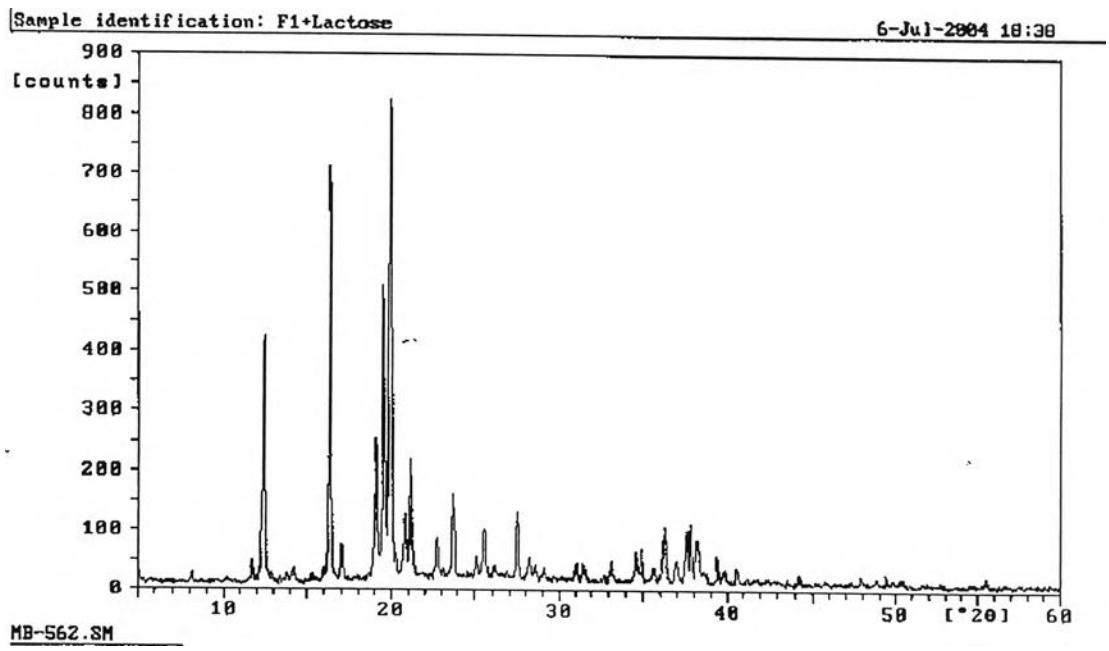

Figure 76 XRPD pattern of mixture of asiaticoside I and lactose after stored in 40° C and 75%RH at 18 weeks

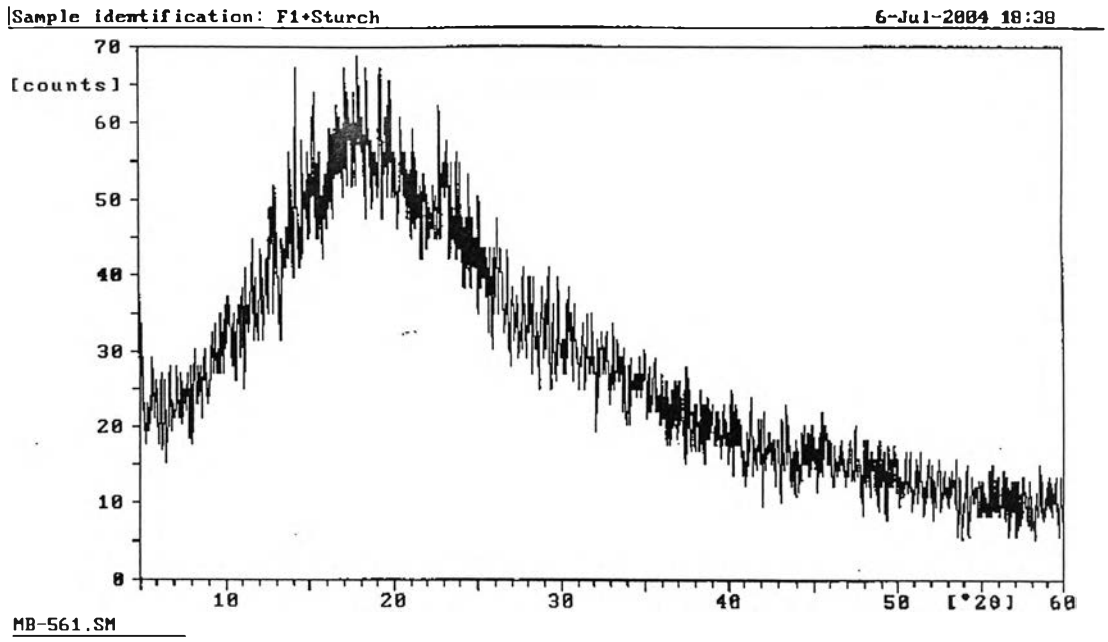

Figure 77 XRPD pattern of mixture of asiaticoside I and pregellatinized starch after stored in 40° C and 75%RH at 18 weeks

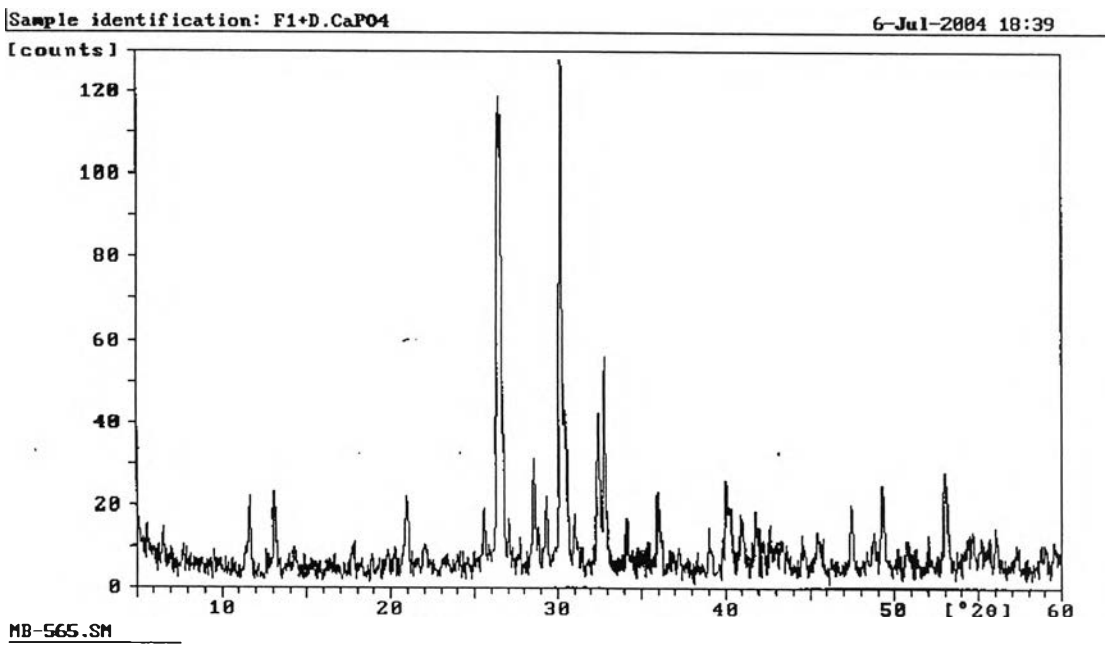

Figure 78 XRPD pattern of mixture of asiaticoside I and dibasic calcium phosphate after stored in 40° C and 75%RH at 18 weeks

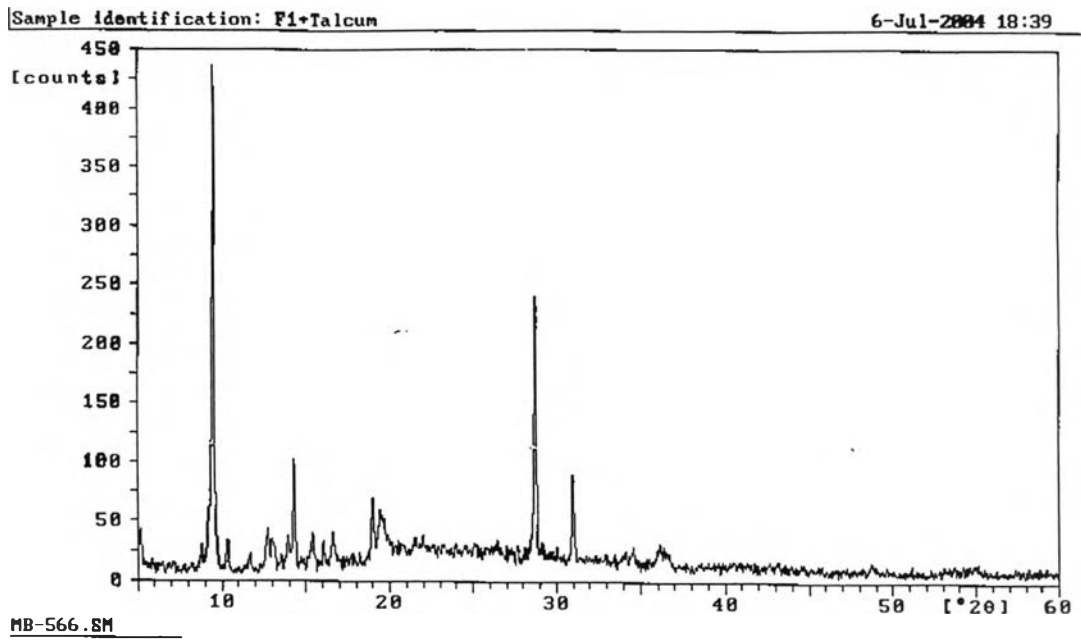

Figure 79 XRPD pattern of mixture of asiaticoside I and talcum after stored in 40° C and 75%RH at 18 weeks

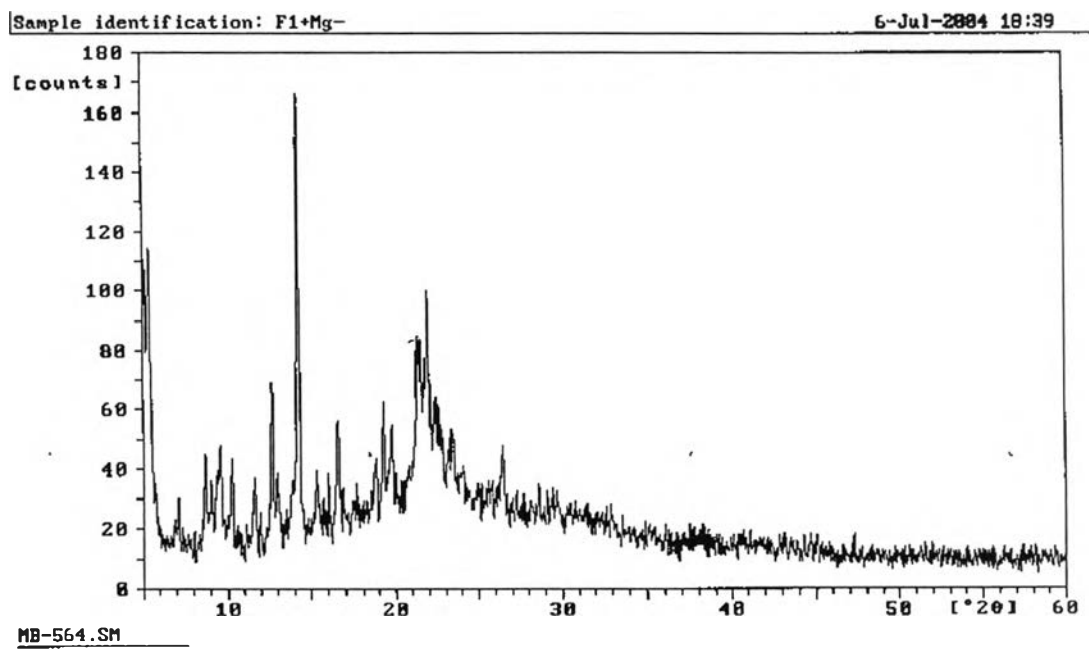

Figure 80 XRPD pattern of mixture of asiaticoside I and magnesium stearate after stored in 40° C and 75%RH at 18 weeks

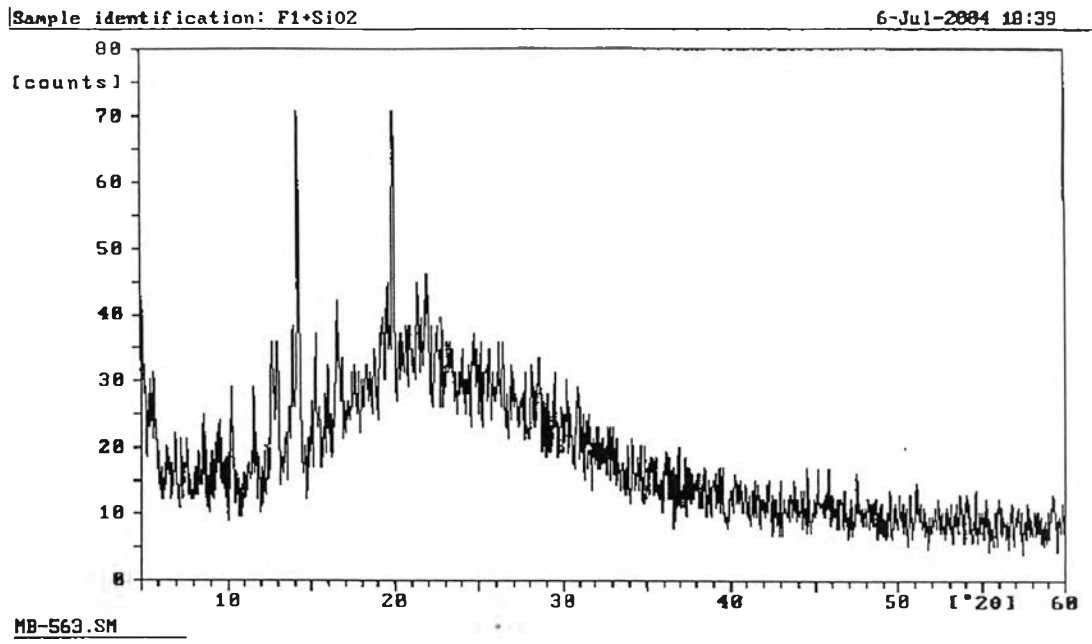

Figure 81 XRPD pattern of mixture of asiaticoside I and silicon dioxide after stored in 40° C and 75%RH at 18 weeks

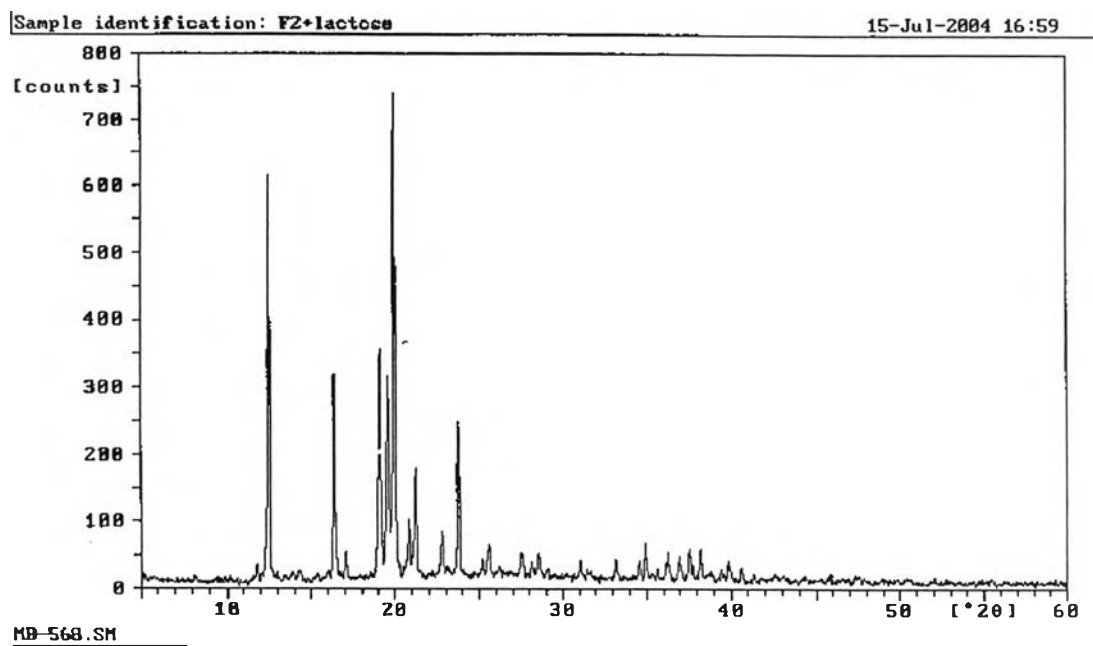

Figure 82 XRPD pattern of mixture of asiaticoside II and lactose after stored in 40° C and 75%RH at 18 weeks

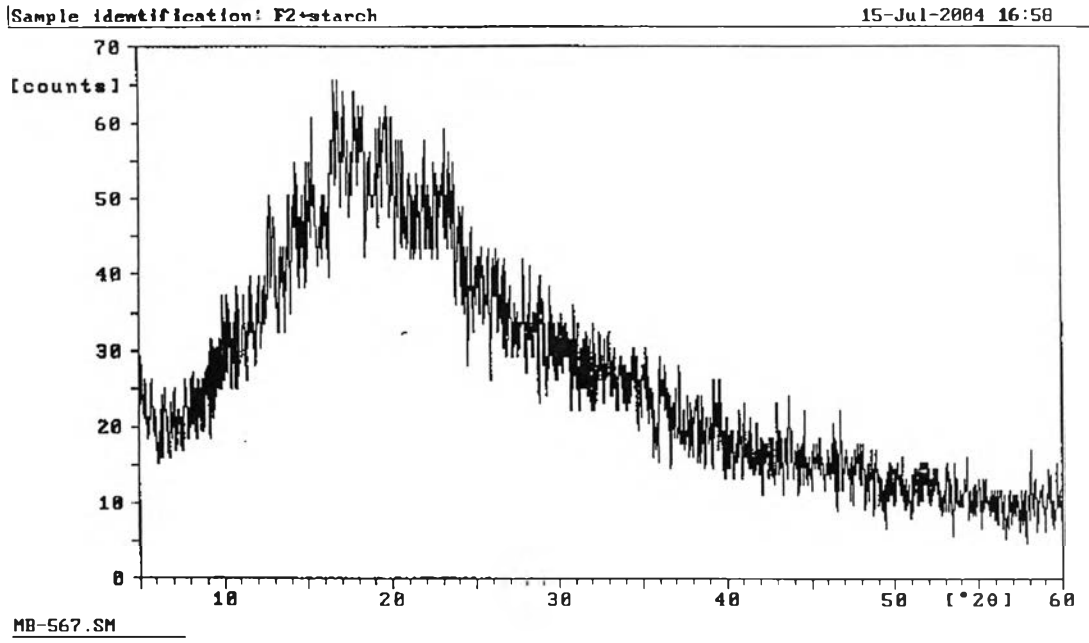

Figure 83 XRPD pattern of mixture of asiaticoside II and pregelatinized starch after stored in 40° C and 75%RH at 18 weeks

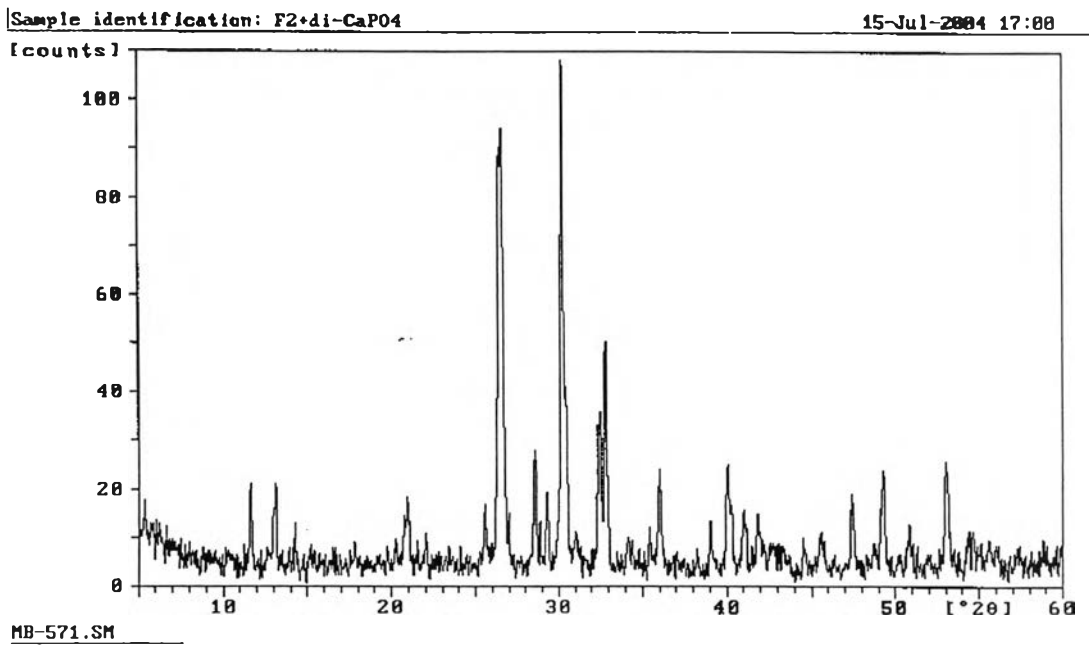

Figure 84 XRPD pattern of mixture of asiaticoside II and dibasic calcium phosphate after stored in 40° C and 75%RH at 18 weeks

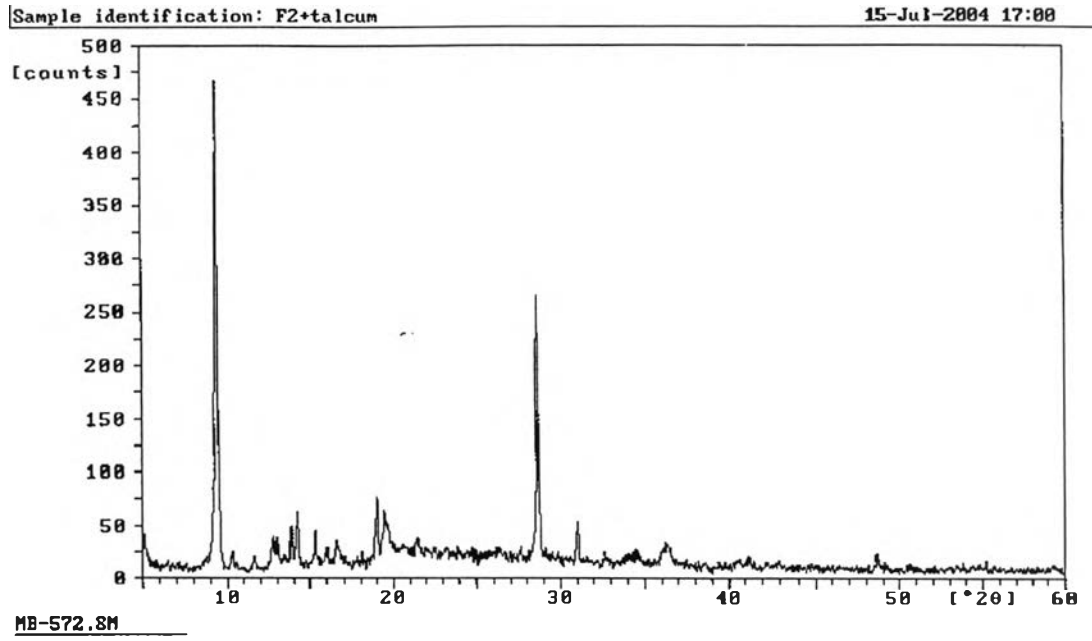

Figure 85 XRPD pattern of mixture of asiaticoside II and talcum after stored in 40° C and 75%RH at 18 weeks

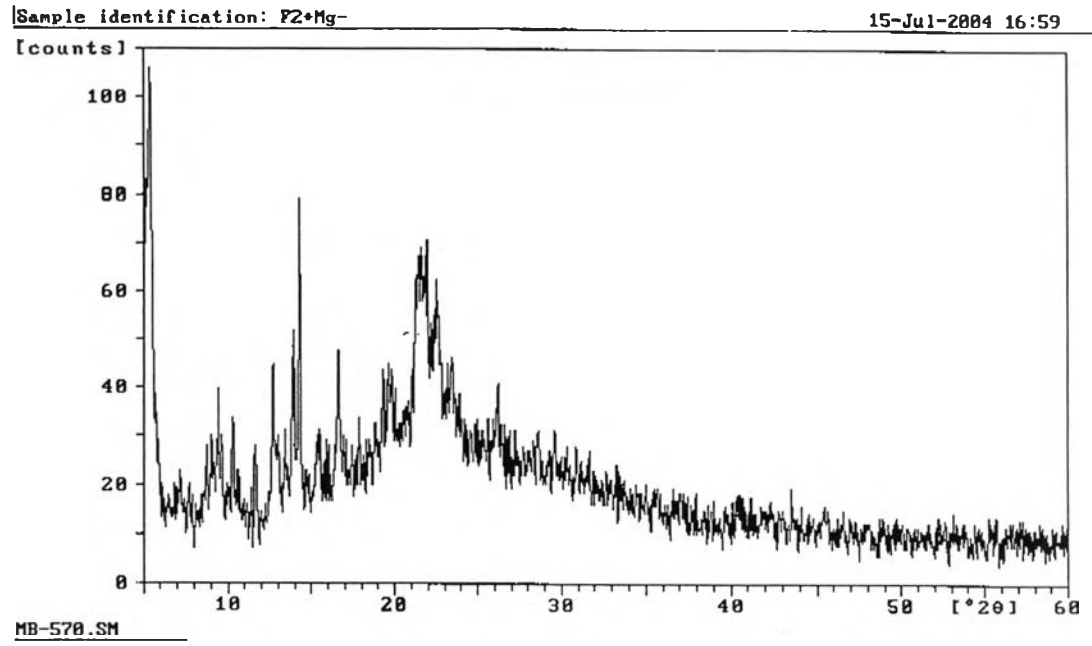

Figure 86 XRPD pattern of mixture of asiaticoside II and magnesium stearate after stored in 40° C and 75%RH at 18 weeks

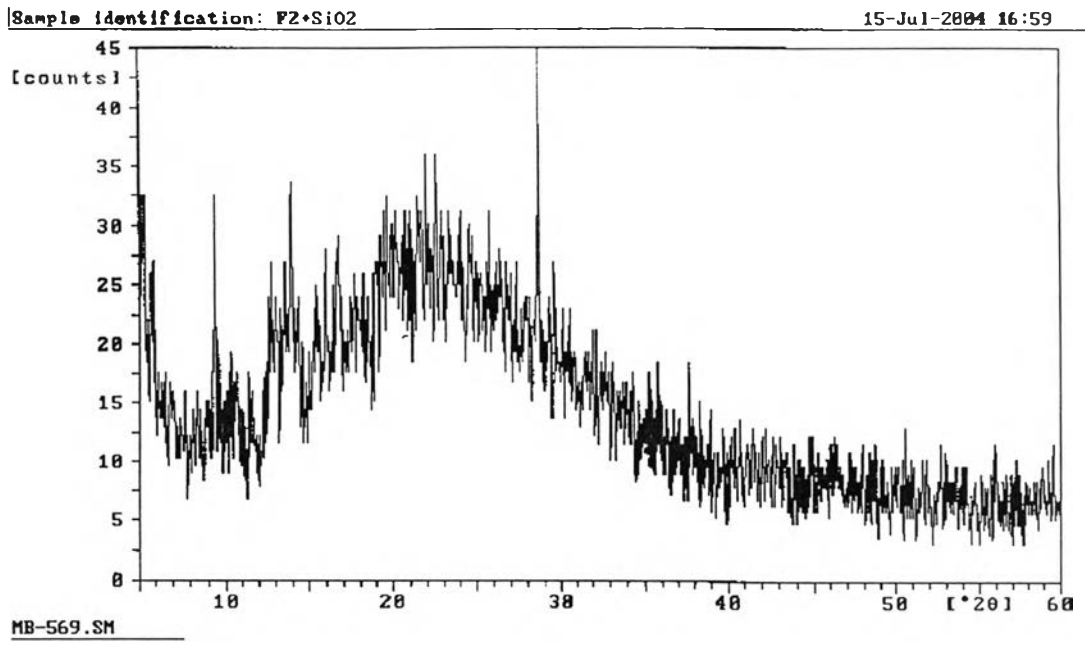

Figure 87 XRPD pattern of mixture of asiatiocoside II and silicon dioxide after stored in 40° C and 75%RH at 18 weeks

APPENDIX E

Karl Fischer Titration

Table 13 Titer of Karl Fischer reagent

Weight of water (g)	Volume of Karl Fischer reagent (ml)	Titer (mg/ml)
0.0215	4.1300	5.206
0.0211	4.0620	5.194
0.0200	4.0000	5.000
	average	5.133
	SD	0.12

APPENDIX F

TGA thermogram of asiaticoside I and asiaticoside II

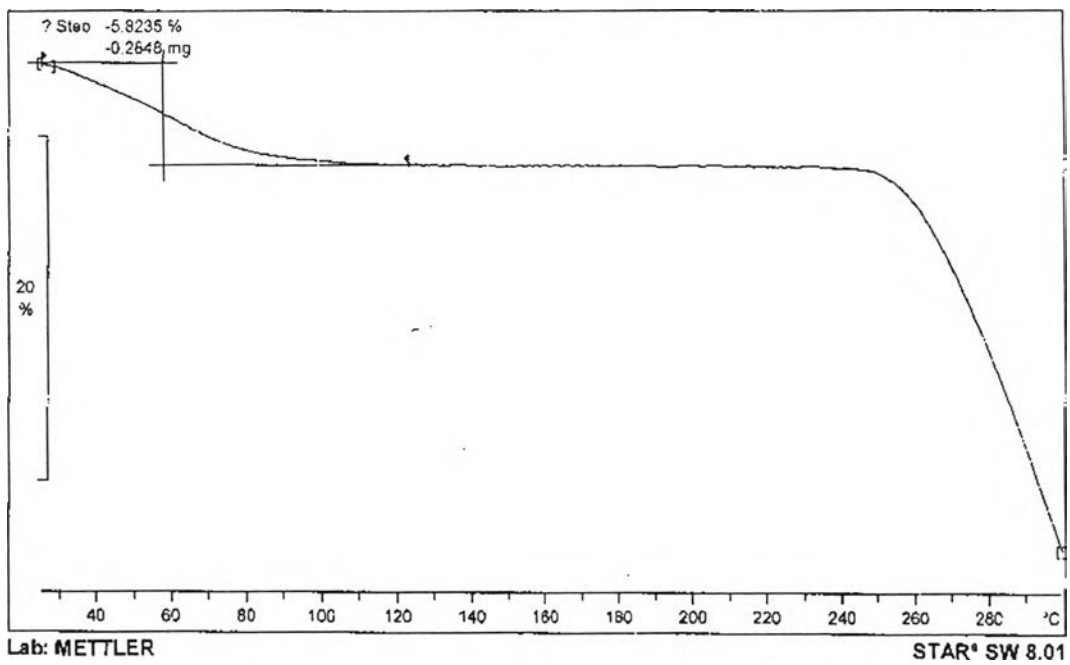

Figure 88 TGA thermogram if asiaticoside I at scanning rate 5°C/min, from 25 - 300°C

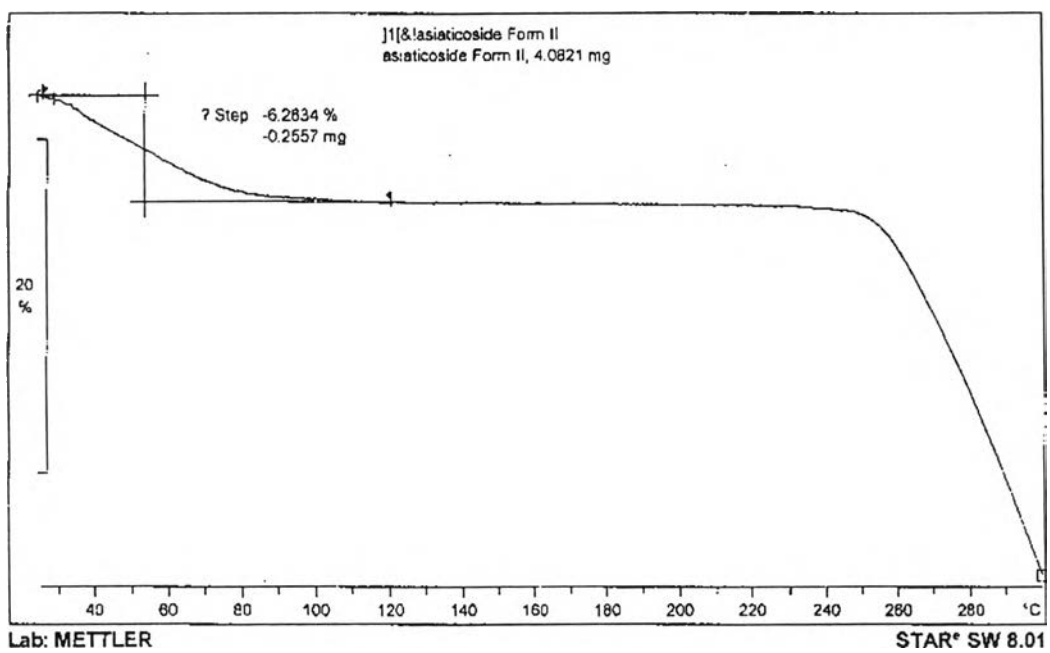

Figure 89 TGA thermogram if asiaticoside II at scanning rate 5°C/min, from 25 - 300°C

APPENDIX G

DSC thermogram of Incompability studies

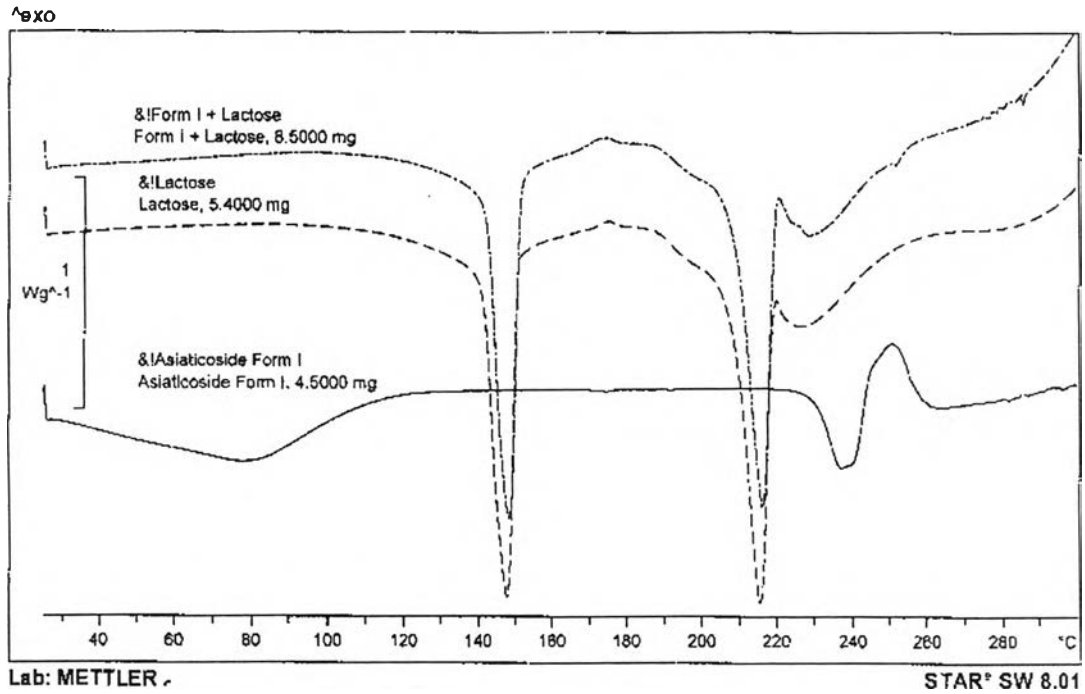

Figure 90 DSC thermogram of asiaticoside I and spray dried lactose mixture at scanning rate 5°C/min, from 25 - 300°C

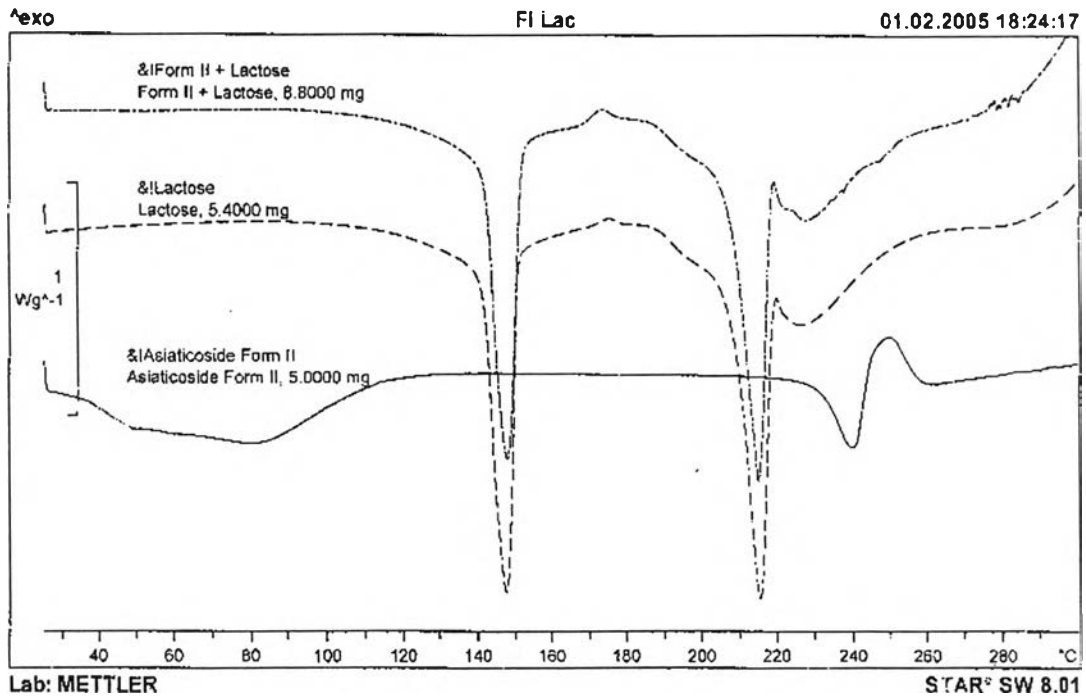

Figure 91 DSC thermogram of asiaticoside II and spray dried lactose mixture at scanning rate 5°C/min, from 25 - 300°C

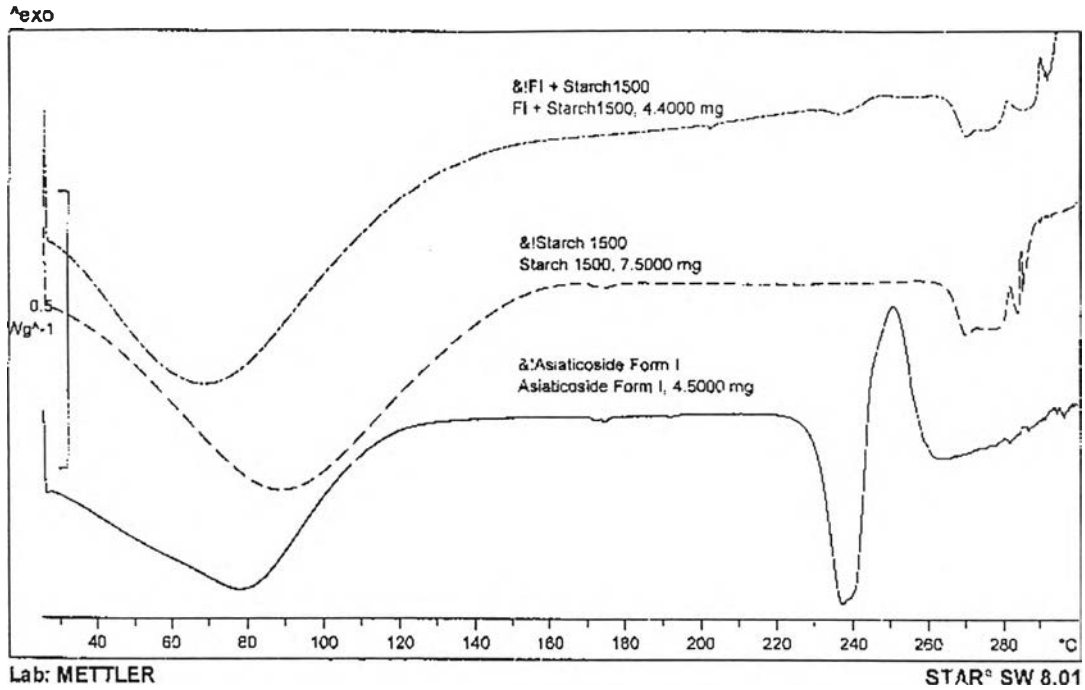

Figure 92 DSC thermogram of asiaticoside I and pregellatinized starch mixture at scanning rate 5°C/min, from 25 - 300°C

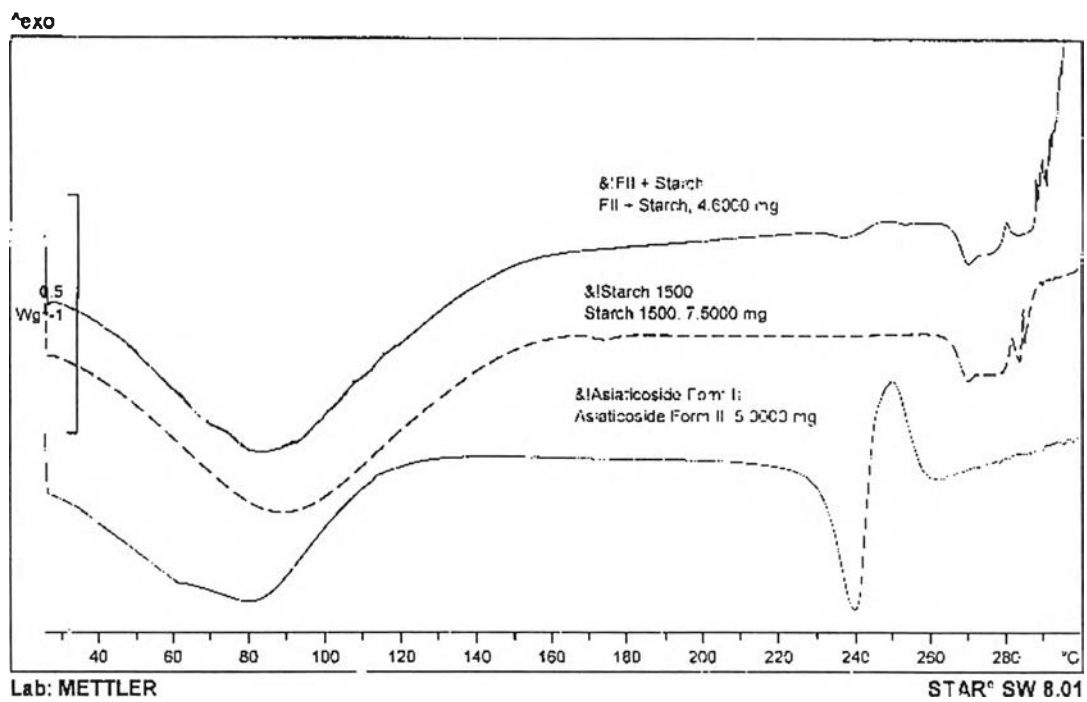

Figure 93 DSC thermogram of asiaticoside II and pregelatinized starch mixture at scanning rate 5°C/min, from 25 - 300°C

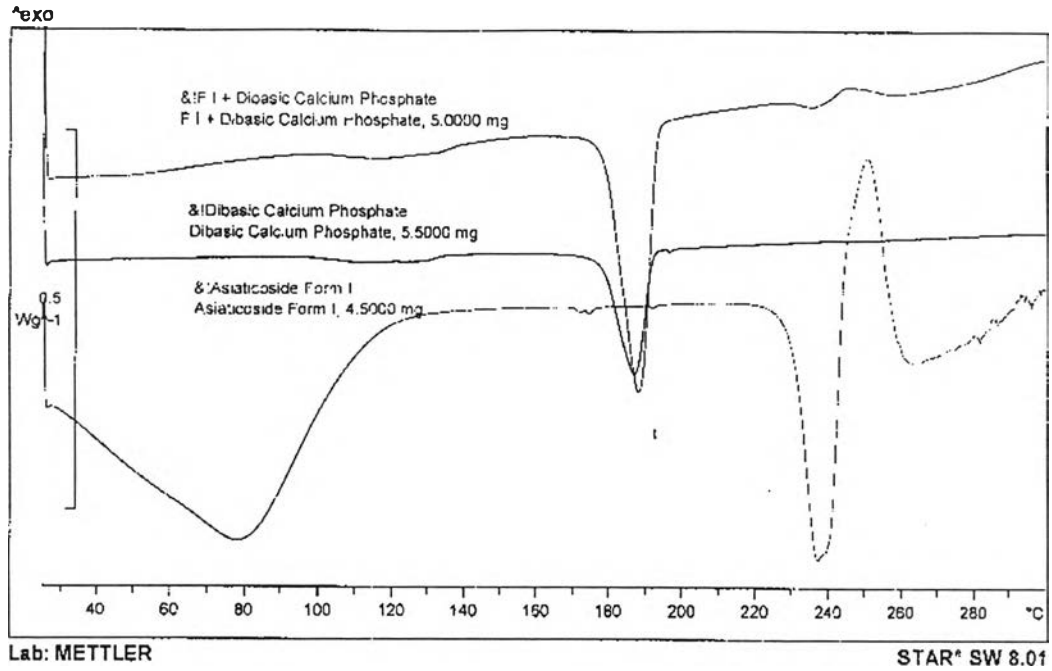

Figure 94 DSC thermogram of asiaticoside I and dibasic calcium phosphate mixture at scanning rate 5°C/min, from 25 - 300°C

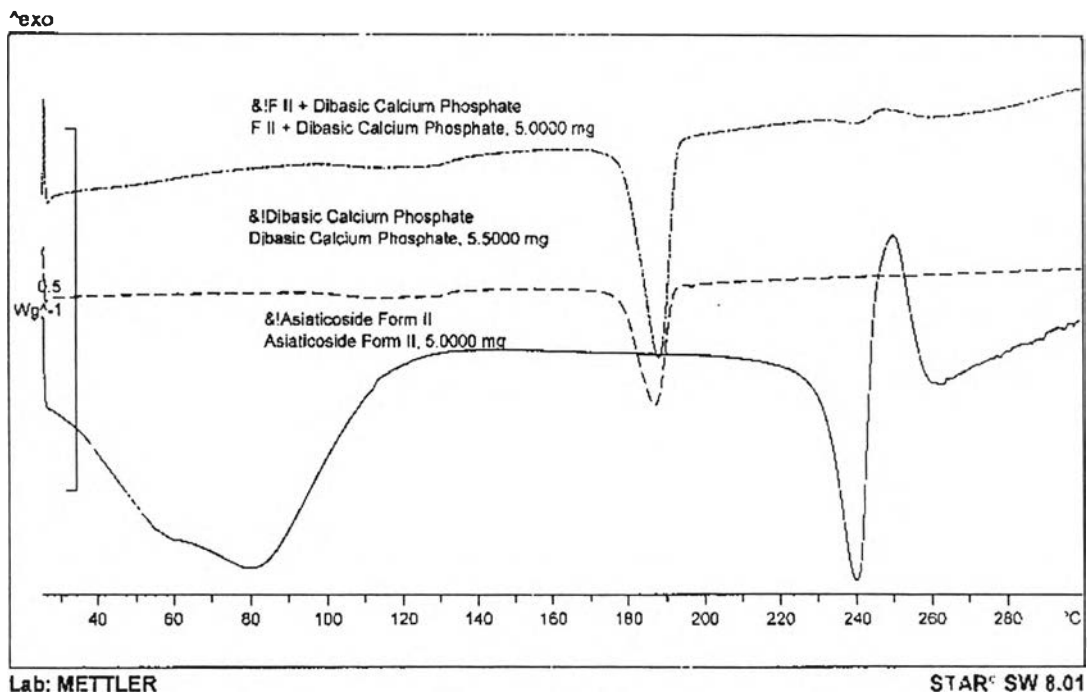

Figure 95 DSC thermogram of asiaticoside II and dibasic calcium phosphate mixture at scanning rate 5°C/min, from 25 - 300°C

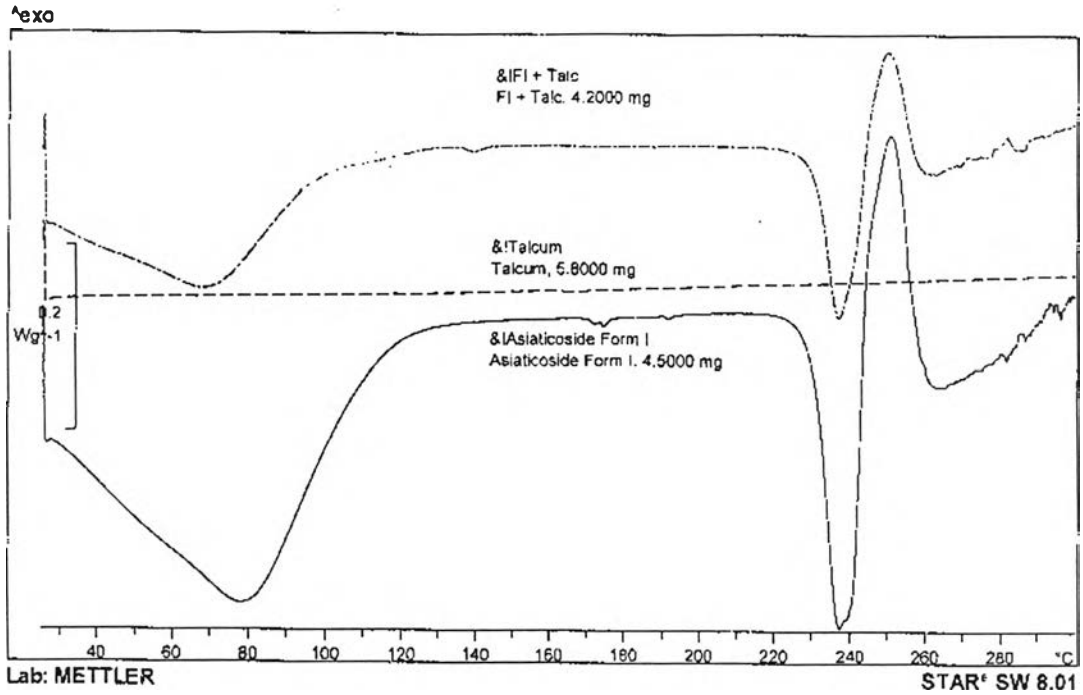

Figure 96 DSC thermogram of asiaticoside I and talcum mixture at scanning rate 5°C/min, from 25 - 300°C

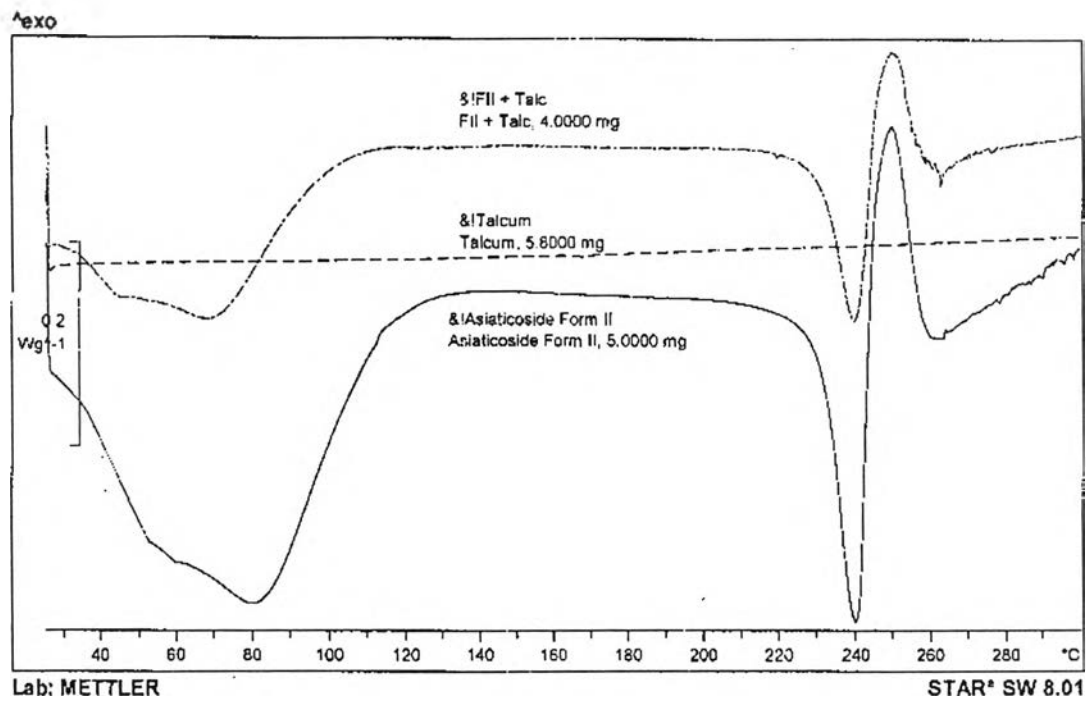

Figure 97 DSC thermogram of asiaticoside II and talcum mixture at scanning rate 5°C/min, from 25 - 300°C

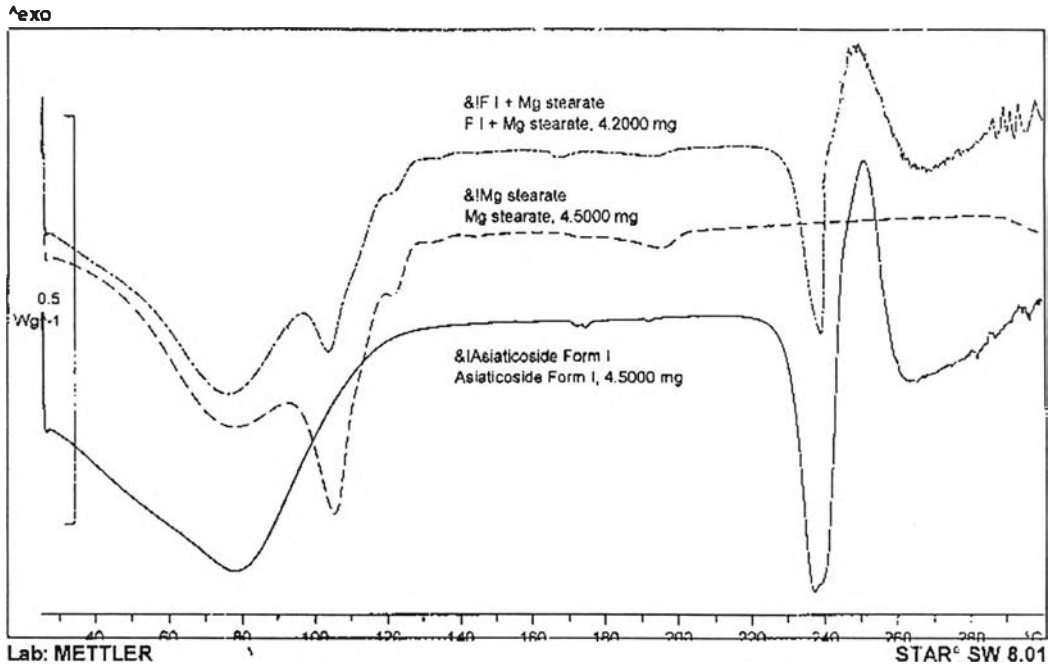

Figure 98 DSC thermogram of asiaticoside I and magnesium stearate mixture at scanning rate 5°C/min, from 25 - 300°C

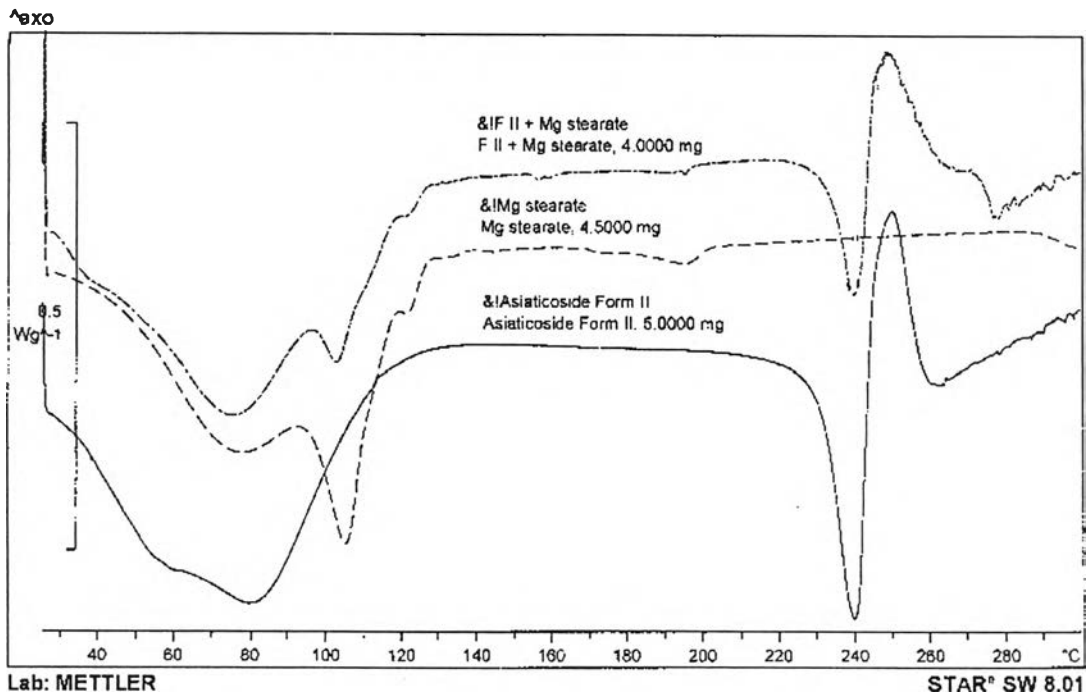

Figure 99 DSC thermogram of asiaticoside II and magnesium stearate mixture at scanning rate 5°C/min, from 25 - 300°C

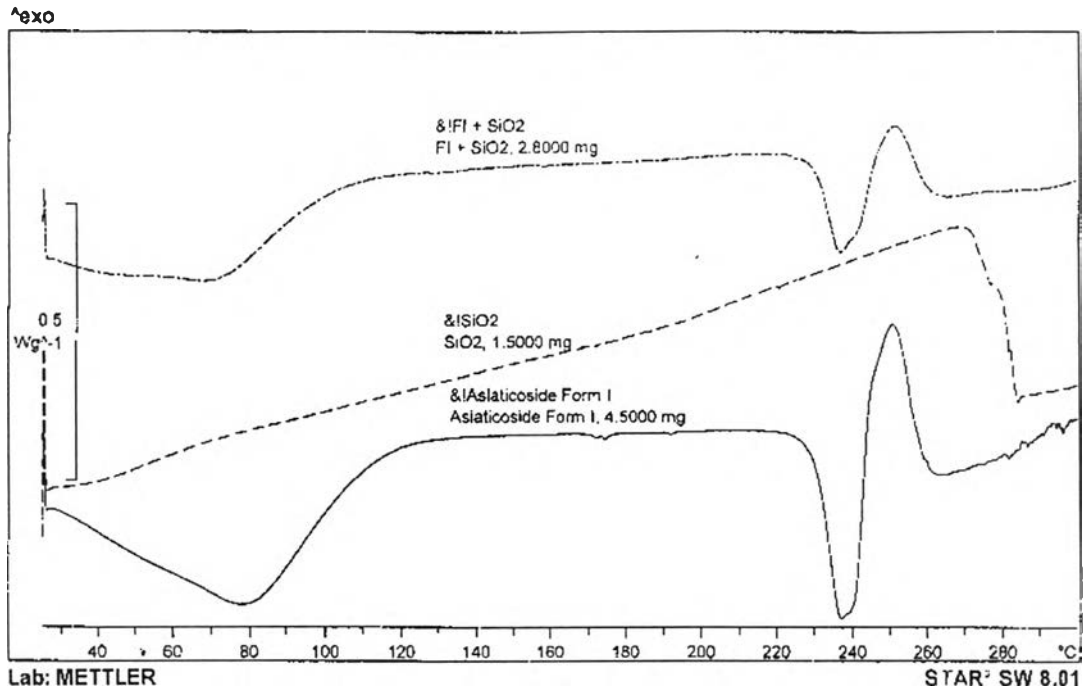

Figure 100 DSC thermogram of asiaticoside I and silicon dioxide mixture at scanning rate 5°C/min, from 25 - 300°C

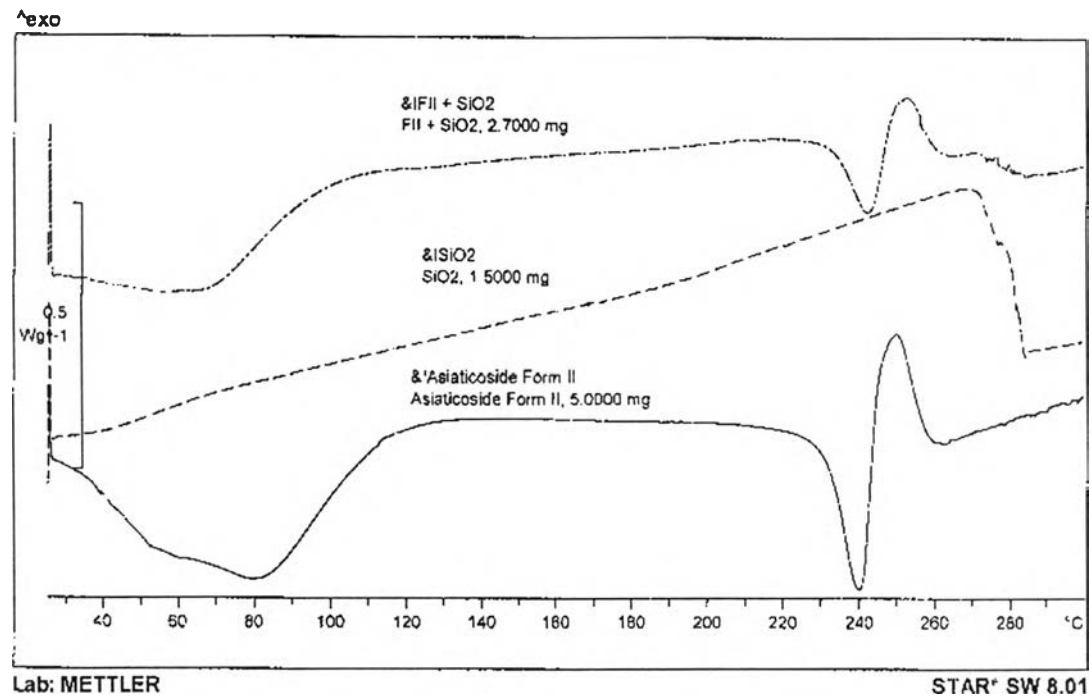

Figure 101 DSC thermogram of asiaticoside II and silicon dioxide mixture at scanning rate 5°C/min, from 25 - 300°C

VITA

Miss Supawadee surangkul was born in January 26, 1977 in Nakormratchasima, Thailand, She guaduated Bachelor degree of Science in Pharmacy from the Faculty of Pharmaceutical sciences, Khon Kaen University,Thailand in 1999. After graduated, she works in Department of Medical Science , Ministry of Public Health. In 2002, she entred the Master's Degree program in department of Manufacturing pharmacy at Chulalongkorn University.