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APPENDICES

APPENDIX A

1. Calibration curve of glibenclamide for solubility study

The concentration (mg/ml) versus peak area of glibenclamide in various concentrations ranging from 4.5 to 33.6 µg/ml of glibenclamide reference standard in methanol were prepared and analyzed. The linear equation of the curve obtained by plotting the peak area at each level prepared versus the concentrations of each standard. The standard curve of glibenclamide are illustrated in Figure 38

Table 13 Data of peak area of glibenclamide in methanol, in various concentrations.

No.	Standard Conc.	Peak area 1	2	3	Average	% RSD
1	0.0045	241,771	253,822	245,084	246,892	2.522
2	0.0090	461,521	471,761	474,428	471,014	1.16
3	0.0112	580,904	584,196	587,700	584,237	0.582
4	0.0179	924,454	937,484	929,353	930,430	0.707
5	0.0224	1,164,091	1,159,728	1,170,981	1,164,933	0.487
6	0.0336	1,780,139	1,767,086	1,772,556	1,773,260	0.37

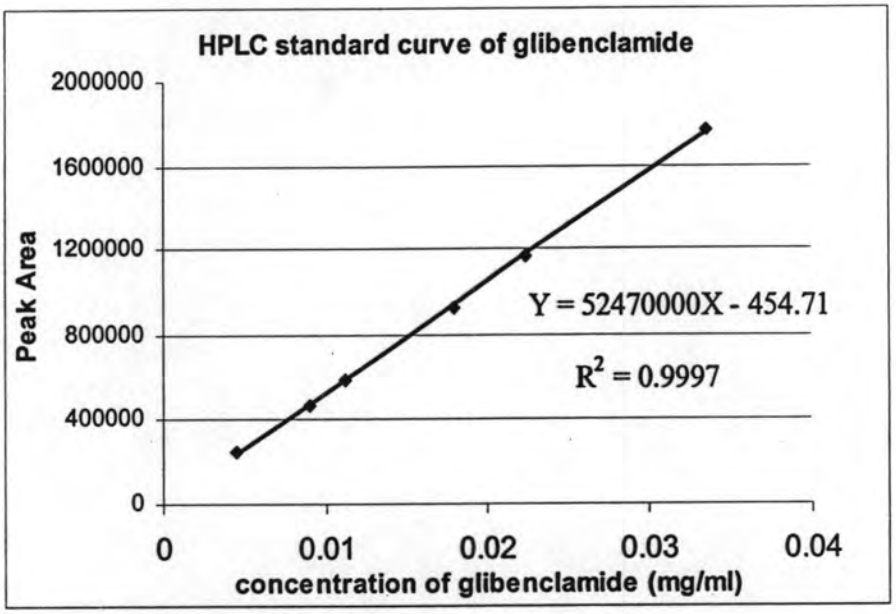

Figure 38 Standard curve of glibenclamide in methanol by HPLC method, UV detector at 227nm

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. Precision of the method was expressed as the percentage of relative standard deviation (%RSD) and the data were shown in Table 14.

Table 14 Precision of method validation of glibenclamide

sample	1	2	3	4	5	6	Average	% RSD
1st day	462168	463127	463304	468701	469830	469861	466165	0.785
2nd day	461521	471761	474428	468001	476415	473956	471014	1.160
3rd day	460428	461428	467132	461846	468554	469162	464758	0.849

The HPLC chromatograms of crystalline glibenclamide had a peak at retention times 9.237 minutes, as shown in Figure 39. On the other hand, the glibenclamide after post process melting and quench cooling was present peaks at retention times 3.871minutes and 9.419 minutes. As a result, peak retention time at 3.8 minutes showed that chemical degradation had occurred.

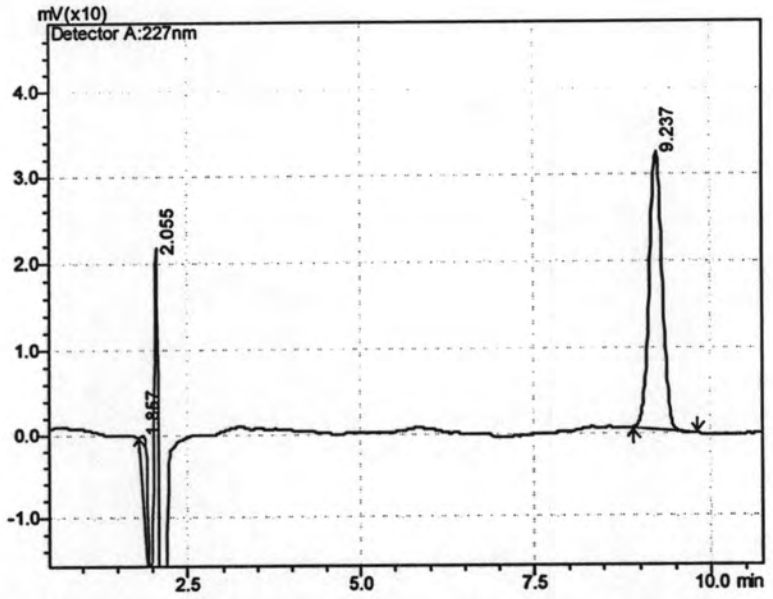

Figure 39 HPLC chromatogram of crystalline glibenclamide raw material.

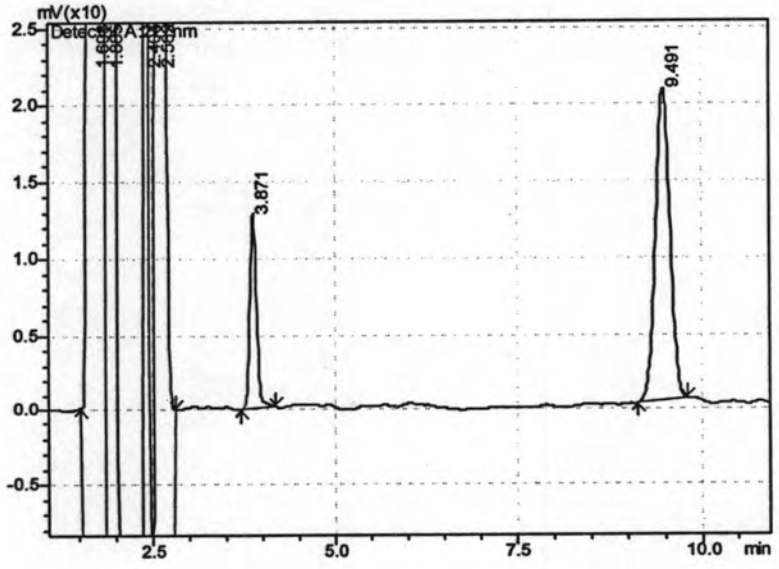

Figure 40 HPLC chromatogram of glibenclamide after melting and quench cooling technique.

The resolution between impurity and glibenclamide peak was 21.53. Tailing factor of impurity peak was 1.155 and glibenclamide was 0.966.The resolution (>1.5) and tailing factor (<2.0) indicated the good system suitability of this method as shown in Table 15.

Table 15 Tailing factor and resolution of impurity and glibenclamide of this method.

	Sample	1	2	3	4	5	6	Average	% RSD
Tailing factor	Impurity	1.181	1.177	1.149	1.146	1.147	1.127	1.155	1.79
	glibenclamide	0.964	0.964	0.977	0.964	0.964	0.961	0.966	0.59
Resolution between peak		21.435	21.584	21.617	21.499	21.579	21.467	21.530	0.34

2. The solubility profile of amorphous glibenclamide

The solubility profile of amorphous glibenclamide was investigated by shaking samples of 100 mg amorphous glibenclamide powder in 200 ml phosphate buffer pH 7.4 in sealed Erlenmeyer flasks at 37°C±0.5°C. The samples are withdrawn, the solid is filtered and the liquid assayed at times 1, 3, 6, 9, 18, 30, 42, 54, 72 and 96 hours. Samples were prepared in triplicate. Each sample was assayed using the HPLC method.

Table 16 Data of the solubility of amorphous glibenclamide at difference of time.

Time (hours)	Concentrations of glibenclamide (mg/ml)				SD
	1	2	3	Average	
0	0.00	0.00	0.00	0.00	0.00
1	0.0860	0.0869	0.0767	0.0832	0.0057
3	0.1006	0.1043	0.1616	0.1222	0.0342
6	0.1316	0.1225	0.1726	0.1422	0.0267
9	0.1654	0.1594	0.1798	0.1682	0.0105
18	0.2364	0.2207	0.1972	0.2181	0.0197
30	0.2420	0.2391	0.2312	0.2374	0.0056
42	0.2411	0.2374	0.2435	0.2407	0.0031
54	0.2450	0.2402	0.2385	0.2412	0.0034
72	0.2490	0.2418	0.2402	0.2437	0.0047
96	0.2501	0.2397	0.2394	0.2431	0.0061

3. The equilibrium solubility of amorphous glibenclamide

The equilibrium solubility of glibenclamide powder and tablet post storage at 50°C and 60°C in different time. Samples approximately 5 mg of glibenclamide were placed in to 10 ml phosphate buffer (pH 7.4) in Erlenmeyer flasks. The flasks were agitated at 100 strokes/minute in a thermostated shaking water bath adjusted to 37±0.5°C. After 48 hours, the sample were collected and filtered through a 0.45μm Millipore filter. The concentration of glibenclamide in each collected sample was assayed by HPLC method. The results are shown in Tables 17, 18 and 19.

Table 17 The initial equilibrium solubility of crystalline and amorphous glibenclamide. Samples were prepared in triplicate and assayed by HPLC method.

sample	Peak area of GB	DF	Conc. * DF	Average (mg/ml)	SD	Peak area of impurity
Crystalline GB at initial	696784	1	0.01329	0.01274	0.0018	Not found
	742248	1	0.01415			Not found
	564958	1	0.01078			Not found
Amorphous GB After process melting and quench cooling	538127	25	0.25661	0.26661	0.0098	100166
	579346	25	0.27625			133035
	559870	25	0.26697			139712

Note: GB= glibenclamide, DF= dilution factor

Table 18 The equilibriums solubility of amorphous glibenclamide powder and tablets after storage at 50°C for 1 to 4 weeks.

sample	Peak area of GB	DF	Conc. * DF	Average (mg/ml)	SD	Peak area of impurity
amorphous GB powder / week 1 at 50°C	391662	25	0.18683	0.17463	0.01745	96324
	382412	25	0.18242			90152
	324102	25	0.15464			81492
amorphous GB powder / week 2 at 50°C	358663	10	0.06844	0.06301	0.00477	86436
	320043	10	0.06108			85732
	311723	10	0.05950			83924
amorphous GB powder / week 3 at 50°C	280375	10	0.05352	0.05569	0.00200	60430
	301122	10	0.05748			77012
	293745	10	0.05607			76530
amorphous GB powder / week 4 at 50°C	274075	10	0.05232	0.05267	0.00050	73759
	278943	10	0.05325			74920
	274702	10	0.05244			71628
amorphous GB tablets / week 1 at 50°C	313261	25	0.14947	0.14708	0.00494	70713
	296322	25	0.14140			64874
	315140	25	0.15037			70956
amorphous GB tablets / week 2 at 50°C	297220	10	0.05673	0.06541	0.00863	68519
	387788	10	0.07399			97183
	343201	10	0.06550			90456
amorphous GB tablets / week 3 at 50°C	308140	10	0.05881	0.05631	0.00400	72561
	306038	10	0.05841			76808
	270759	10	0.05169			52159
amorphous GB tablets / week 4 at 50°C	303662	10	0.05796	0.05577	0.00296	90313
	298284	10	0.05694			81662
	274505	10	0.05240			61118

Note: GB= glibenclamide, DF= dilution factor

Table 19 The equilibriums solubility of amorphous glibenclamide powder and tablets after storage at 60°C for 1 to 4 weeks. Samples were prepared in triplicate and assayed by HPLC method.

sample	Peak area of GB	DF	Conc. * DF	Average (mg/ml)	SD	Peak area of impurity
amorphous GB powder / week 1 at 60°C	492024	10	0.09386	0.09510	0.00187	117727
	509835	10	0.09725			120071
	493763	10	0.09419			119387
amorphous GB powder / week 2 at 60°C	313251	10	0.05979	0.05753	0.00222	71727
	290015	10	0.05536			72513
	300987	10	0.05745			79387
amorphous GB powder / week 3 at 60°C	298623	10	0.05700	0.05652	0.00111	72484
	289425	10	0.05525			70106
	300241	10	0.05731			71118
amorphous GB powder / week 4 at 60°C	291783	10	0.05570	0.05668	0.00097	77541
	301989	10	0.05764			79664
	296986	10	0.05669			79278
amorphous GB tablets / week 1 at 60°C	405421	10	0.07735	0.08421	0.01340	69972
	396294	10	0.07561			78413
	522406	10	0.09965			119775
amorphous GB tablets / week 2 at 60°C	292975	10	0.05592	0.05363	0.00247	59733
	282571	10	0.05394			58823
	267206	10	0.05101			57697
amorphous GB tablets / week 3 at 60°C	501197	5	0.04780	0.04363	0.00458	136459
	464973	5	0.04435			112229
	406024	5	0.03873			96553
amorphous GB tablets / week 4 at 60°C	558095	5	0.05323	0.04880	0.00564	134342
	531913	5	0.05073			146463
	444945	5	0.04244			103213

Note: GB= glibenclamide, DF= dilution factor

4. Chemical degradation determination of glibenclamide

Percentage of chemical degradation of solidified glibenclamide powder was analyzed by using HPLC and calculated as following;

$$\% \text{ drug degradation} = \frac{(\%\text{recovery at time 0} - \%\text{recovery after process})}{\%\text{ recovery at time 0}} \times 100$$

(The equation referring to Eq.2 in method part)

The same equation was also used to determine the extent of chemical degradation of amorphous glibenclamide tablets after storage at different temperatures. The results are shown in Tables 20, 21 and 22.

Table 20 Assay of crystalline glibenclamide at initial.

sample	Sample weight (mg)	Peak area GB	average	SD	% recovery after process	% drug degradation	Peak area of impurity
Crystalline GB at initial	30.0	628317	631581	5588	100	0	Not found
	30.0	628392					
	30.0	638033					

The crystalline glibenclamide used as reference standard for calculation percentage of drug degradation. In this case, the % recovery at time 0 is 100 %

$$\% \text{ recovery after process} = \frac{(\text{Area of GB peak at time t/ sample weight})}{(\text{Area of GB peak at time 0/standard weight})} \times 100 \text{ --Eq. 10}$$

Where, In this case, Area of GB peak at time 0 is 631581 and standard weight is 30.0 milligrams.

Table 21 Percentage of drug degradation of amorphous glibenclamide powder and tablets after storage at temperature 50°C for 1 to 4 weeks.

sample	Sample weight (mg)	Peak area GB	% recovery after process	% drug degradation	Average	SD	Peak area of impurity
Amorphous GB	30.1	496303	78.32	21.68			110421
After process melting and quench cooling	30.0	495281	78.42	21.58	22.18	0.95	113456
	30.1	486242	76.73	23.27			112354
amorphous GB powder / week 1 at 50°C	30.2	492384	77.44	22.56			124342
	30.2	482723	75.92	24.08	24.09	1.54	120325
	30.1	471245	74.37	25.63			113874
amorphous GB powder / week 2 at 50°C	30.1	487912	77.00	23.00			113421
	30.4	476323	74.43	25.57	24.31	1.29	110874
	30.2	480892	75.64	24.36			112472
amorphous GB powder / week 3 at 50°C	30.1	493427	77.87	22.13			112483
	30.2	502352	79.01	20.99	21.80	0.71	103532
	30.2	494154	77.72	22.28			122743
amorphous GB powder / week 4 at 50°C	30.1	481212	75.94	24.06			123822
	30.0	483341	76.53	23.47	24.43	1.19	118732
	30.1	470422	74.24	25.76			121874
amorphous GB tablets / week 1 at 50°C	30.0	489212	77.46	22.54			112832
	30.0	487832	77.24	22.76	23.22	0.98	109753
	30.1	479423	75.66	24.34			110732
amorphous GB tablets / week 2 at 50°C	30.0	498212	78.88	21.12			112762
	30.2	510792	80.34	19.66	20.73	0.94	102453
	30.1	498002	78.59	21.41			121123
amorphous GB tablets / week 3 at 50°C	30.0	464232	73.50	26.50			123423
	30.4	479423	74.91	25.09	25.37	1.02	111232
	30.1	478342	75.49	24.51			113242
amorphous GB tablets / week 4 at 50°C	30.2	474373	74.61	25.39			127432
	30.1	473202	74.67	25.33	25.72	0.62	118389
	30.2	467743	73.57	26.43			112837

Table 22 Percentage of drug degradation of amorphous glibenclamide powder and tablets after storage at temperature 60°C for 1 to 4 weeks.

sample	Sample weight (mg)	Peak area GB	% recovery after process	% drug degradation	Average	SD	Peak area of impurity
amorphous GB powder / week 1 at 60°C	30.3	482112	75.58	24.42	22.83	1.53	112454
	30.0	488223	77.30	22.70			110475
	30.1	498322	78.64	21.36			110353
amorphous GB powder / week 2 at 60°C	30.0	492423	77.97	22.03	23.72	2.01	114837
	30.0	485244	76.83	23.17			116452
	30.1	469287	74.06	25.94			122354
amorphous GB powder / week 3 at 60°C	30.2	467832	73.58	26.42	26.19	1.89	116893
	30.0	454985	72.04	27.96			124892
	30.0	478739	75.80	24.20			120016
amorphous GB powder / week 4 at 60°C	30.1	456823	72.09	27.91	26.57	1.42	127738
	30.2	476323	74.92	25.08			118938
	30.2	465832	73.27	26.73			120102
amorphous GB tablets / week 1 at 60°C	30.0	462721	73.26	26.74	25.31	1.32	119223
	30.2	482356	75.87	24.13			110113
	30.2	476432	74.94	25.06			116893
amorphous GB tablets / week 2 at 60°C	30.1	467245	73.73	26.27	26.99	1.55	113489
	30.3	454352	71.23	28.77			124383
	30.0	467782	74.07	25.93			112984
amorphous GB tablets / week 3 at 60°C	30.0	473993	75.05	24.95	24.45	0.77	113880
	30.1	476300	75.16	24.84			113452
	30.2	486003	76.44	23.56			110953
amorphous GB tablets / week 4 at 60°C	30.2	468903	73.75	26.25	25.90	2.30	124925
	30.2	457734	71.99	28.01			120058
	30.2	486933	76.56	23.44			112329

APPENDIX B

1. Aspirin calibration curve

The concentration (mg/ml) versus peak area of aspirin in various concentrations ranging from 1 to 5 mg/ml of aspirin reference standard in acetonitrile: methanol: phosphoric acid (92:8:0.5) were prepared and analyzed. The linear equation of the curve obtained by plotting the peak area at each level prepared versus the concentrations of each standard. The standard curve of aspirin are illustrated in Figure 41

Table 23 The peak area of acetylsalicylic acid in acetonitrile: methanol: phosphoric acid (92:8:0.5), in various concentrations.

No.	Standard Conc.(mg/ml)	Peak area of Acetylsalicylic acid (Aspirin) 1	2	3	Average	% RSD
1	1.007	1013270	1014586	1013897	1013918	0.065
2	1.611	1560237	1564964	1563021	1562741	0.152
3	2.014	1892822	1899267	1898056	1896715	0.181
4	3.020	2743532	2748689	2748652	2746958	0.108
5	4.027	3557181	3549804	3552041	3553009	0.106
6	5.034	4382047	4378707	4379980	4380245	0.038

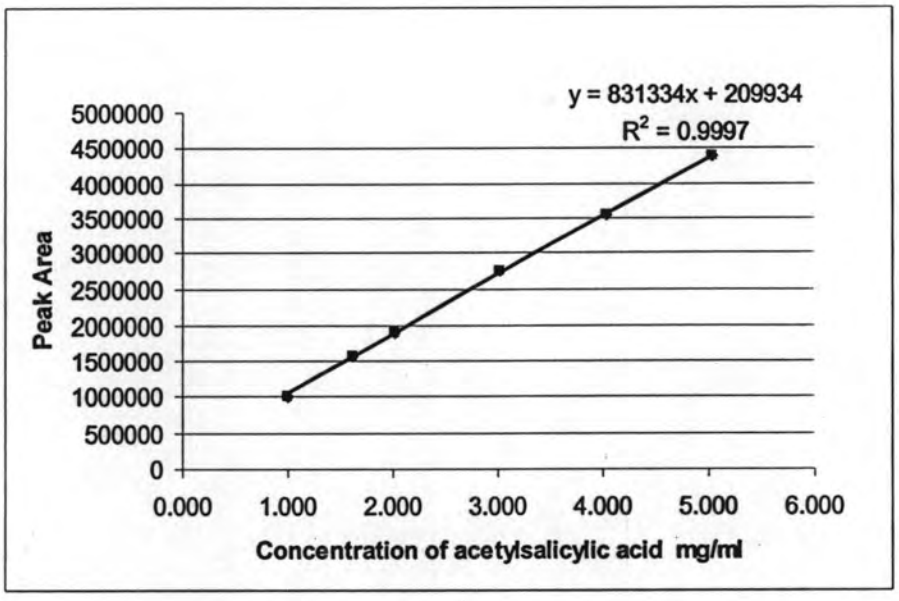

Figure 41 Standard curve of acetylsalicylic acid in acetonitrile: methanol: phosphoric acid (92:8:0.5) by HPLC method, UV detector at 295nm.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. Precision of the method was expressed as the percentage of relative standard deviation (%RSD) and the data were shown in Tables 24.

Table 24 Precision of method validation of aspirin

sample	1	2	3	4	5	6	Average	% RSD
1st day	2761068	2756157	2762981	2769496	2761727	2754885	2761052	0.190
2nd day	2843820	2829172	2840113	2825004	2837644	2811158	2831152	0.425
3rd day	2804592	2815193	2818649	2814727	2811068	2823986	2814703	0.234

2. Calibration curve of Salicylic acid

The concentration (mcg/ml) versus peak area of salicylic acid in various concentrations ranging from 3 to 18 µg/ml of salicylic acid reference standard in acetonitrile: methanol: phosphoric acid (92:8:0.5) were prepared and analyzed. The linear equation of the curve obtained by plotting the peak area at each level prepared versus the concentrations of each standard. The standard curve of salicylic acid are illustrated in Figure 42

Table 25 The peak area of salicylic acid in acetonitrile: methanol: phosphoric acid (92:8:0.5), in various concentrations.

No.	Standard Conc.(mcg/ml)	Peak area of Salicylic acid (SA) 1	2	3	Average	% RSD
1	3.020	62913	59775	62753	61814	2.859
2	6.040	124283	123046	124126	123818	0.544
3	9.060	191529	192293	192695	192172	0.350
4	12.080	257081	256757	258967	257602	0.230
5	15.100	324312	323530	323768	323870	0.368
6	18.120	389146	389945	391212	390101	0.103

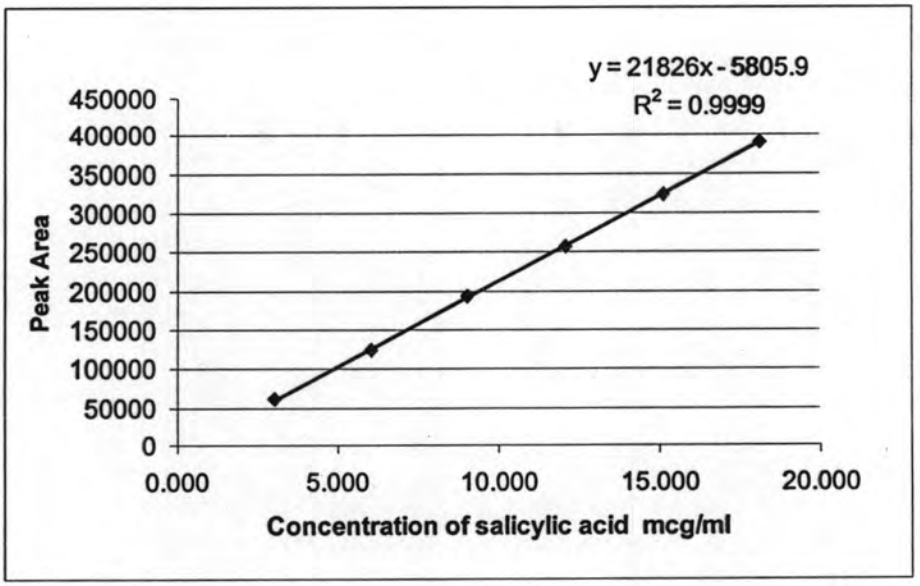

Figure 42 Standard curve of salicylic acid in acetonitrile: methanol: phosphoric acid (92:8:0.5) by HPLC method, UV detector at 295nm.

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogeneous sample. Precision of the method was expressed as the percentage of relative standard deviation (%RSD) and the data were shown in Tables 26.

Table 26 Precision of method validation of salicylic acid

sample	1	2	3	4	5	6	Average	% RSD
1st day	124283	123046	124126	124095	124121	124188	123977	0.372
2nd day	125997	126438	126201	126402	125964	126632	126272	0.209
3rd day	126012	125873	124991	126004	125896	125943	125787	0.313

The resolution between aspirin and salicylic acid peak was 5.67. Tailing factor of aspirin peak was 1.016 and salicylic acid was 1.447.The resolution (>1.5) and tailing factor (<2.0) indicated the good system suitability of this method as shown in Table 27

Table 27 Tailing factor and resolution of aspirin and salicylic acid of this method.

	Sample	1	2	3	4	5	6	Average	% RSD
Tailing factor	aspirin	1.017	1.015	1.048	1.005	1.01	1.003	1.016	1.62
	salicylic acid	1.467	1.479	1.428	1.441	1.458	1.409	1.447	1.80
Resolution between peak		5.611	5.567	5.611	5.652	5.776	5.775	5.665	1.58

The equation from the standard calibration of salicylic acid was used to calculate the aspirin remained at time t.

3. Calculations for stability of aspirin

Table 28 Calculated values of salicylic acid in aspirin tablet after storage at temperature 30°C / 75%RH

Time (wks)	wt.	Area SA	Conc. SA	Conc. *DF	SA (mg)	% w/w SA	Ave.	% w/w A_{deg}	% w/w A_{rem}	A_{rem} / A_0	Ln (A_{rem} /A_0)
0	120.7	26645	1.4868	49.56	0.050	0.041					
0	120.2	25402	1.4298	47.66	0.048	0.040	0.040	0.0519	85.3649	0.999393	-0.00061
0	121.4	24875	1.4057	46.86	0.047	0.039					
2	119.8	23032	1.3213	44.04	0.044	0.037					
2	120.3	24198	1.3747	45.82	0.046	0.038	0.038	0.0492	85.3676	0.999424	-0.00058
2	120.0	24342	1.3813	46.04	0.046	0.038					
4	119.3	24532	1.3900	46.33	0.046	0.039					
4	120.2	24762	1.4005	46.68	0.047	0.039	0.039	0.0511	85.3657	0.999402	-0.0006
4	119.4	25389	1.4293	47.64	0.048	0.040					
6	119.8	23406	1.3384	44.61	0.045	0.037					
6	120.3	25811	1.4486	48.29	0.048	0.040	0.040	0.0518	85.3650	0.999393	-0.00061
6	119.5	26954	1.5010	50.03	0.050	0.042					
8	119.4	25461	1.4326	47.75	0.048	0.040					
8	120.2	25631	1.4403	48.01	0.048	0.040	0.040	0.0518	85.3650	0.999394	-0.00061
8	119.8	24880	1.4059	46.86	0.047	0.039					

* DF =dilution factor in this case =33.33

Table 29 Calculated values of salicylic acid in aspirin tablet after storage at temperature 37°C / 75%RH

Time (wks)	wt.	Area SA	Conc. SA	Conc. *DF	SA (mg)	% w/w SA	Ave.	% w/w A_{degd}	% w/w A_{rem}	A_{rem} / A_0	Ln (A_{rem} /A_0)
0	120.7	26645	1.4868	49.56	0.050	0.041					
0	120.2	25402	1.4298	47.66	0.048	0.040	0.040	0.0519	85.3649	0.999393	-0.00061
0	121.4	24875	1.4057	46.86	0.047	0.039					
2	120.7	22447	1.2945	43.15	0.043	0.036					
2	120.1	21008	1.2285	40.95	0.041	0.034	0.036	0.0464	85.3704	0.999457	-0.00054
2	120.8	23347	1.3357	44.52	0.045	0.037					
4	120.8	26679	1.4884	49.61	0.050	0.041					
4	120.3	26831	1.4953	49.84	0.050	0.041	0.042	0.0544	85.3624	0.999363	-0.00064
4	120.0	27710	1.5356	51.19	0.051	0.043					
6	119.0	32865	1.7718	59.06	0.059	0.050					
6	120.2	32901	1.7734	59.11	0.059	0.049	0.049	0.0644	85.3524	0.999247	-0.00075
6	120.1	32891	1.7730	59.10	0.059	0.049					
8	119.2	35906	1.9111	63.70	0.064	0.053					
8	120.1	36012	1.9160	63.87	0.064	0.053	0.052	0.0683	85.3485	0.999200	-0.0008
8	119.3	33711	1.8105	60.35	0.060	0.051					

* DF =dilution factor in this case =33.33

Table 30 Calculated values of salicylic acid in aspirin tablet after storage at temperature 45°C / 75%RH

Time (wks)	wt.	Area SA	Conc. SA	Conc. *DF	SA (mg)	% w/w SA	Ave.	% w/w A_{degd}	% w/w A_{rem}	A_{rem} / A_0	Ln (A_{rem} /A_0)
0	120.7	26645	1.4868	49.56	0.050	0.041					
0	120.2	25402	1.4298	47.66	0.048	0.040	0.040	0.0519	85.3649	0.999393	-0.00061
0	121.4	24875	1.4057	46.86	0.047	0.039					
2	118.8	28178	1.5570	51.90	0.052	0.044					
2	120.1	29032	1.5962	53.21	0.053	0.044	0.044	0.0576	85.3592	0.999326	-0.00067
2	120.4	29191	1.6035	53.45	0.053	0.044					
4	120.1	37303	1.9751	65.84	0.066	0.055					
4	120.2	37564	1.9871	66.24	0.066	0.055	0.055	0.0722	85.3446	0.999155	-0.00085
4	119.7	38174	2.0150	67.17	0.067	0.056					
6	120.1	64428	3.2179	107.26	0.107	0.089					
6	120.3	64612	3.2263	107.54	0.108	0.089	0.090	0.1168	85.3000	0.998633	-0.00137
6	119.0	64287	3.2114	107.05	0.107	0.090					
8	119.9	119253	5.7298	190.99	0.191	0.159					
8	120.1	122673	5.8865	196.22	0.196	0.163	0.161	0.2106	85.2062	0.997534	-0.00247
8	119.9	121187	5.8184	193.95	0.194	0.162					

* DF =dilution factor in this case =33.33

Table 31 Calculated values of salicylic acid in aspirin tablet after storage at temperature 50°C / 75%RH

Time (wks)	wt.	Area SA	Conc. SA	Conc. *DF	SA (mg)	% w/w SA	Ave.	% w/w A_{degd}	% w/w A_{rem}	A_{rem} / A_0	Ln (A_{rem} /A_0)
0	120.7	26645	1.4868	49.56	0.050	0.041					
0	120.2	25402	1.4298	47.66	0.048	0.040	0.040	0.0519	85.3649	0.999393	-0.00061
0	121.4	24875	1.4057	46.86	0.047	0.039					
2	120.4	44123	2.2876	76.25	0.076	0.063					
2	120.1	40621	2.1271	70.90	0.071	0.059	0.061	0.0797	85.3371	0.999067	-0.00093
2	118.6	41454	2.1653	72.18	0.072	0.061					
4	120.2	76613	3.7762	125.87	0.126	0.105					
4	120.1	79103	3.8903	129.68	0.130	0.108	0.108	0.1405	85.2763	0.998355	-0.00165
4	120.3	81176	3.9852	132.84	0.133	0.110					
6	120.1	168626	7.9919	266.40	0.266	0.222					
6	120.3	173873	8.2323	274.41	0.274	0.228	0.226	0.2944	85.1224	0.996553	-0.00345
6	119.5	171986	8.1459	271.53	0.272	0.227					
8	119.8	328912	15.3357	511.19	0.511	0.427					
8	120.1	348612	16.2383	541.28	0.541	0.451	0.440	0.5735	84.8433	0.993286	-0.00674
8	119.9	340936	15.8866	529.55	0.530	0.442					

* DF =dilution factor in this case =33.33

Table 32 Calculated values of salicylic acid in aspirin tablet after storage at temperature 60°C /75%RH

Time (wks)	wt.	Area SA	Conc. SA	Conc. *DF	SA (mg)	% w/w SA	Ave.	% w/w A_{degd}	% w/w A_{rem}	A_{rem} / A_0	Ln (A_{rem} /A_0)
0	120.7	26645	1.4868	49.56	0.050	0.041					
0	120.2	25402	1.4298	47.66	0.048	0.040	0.040	0.0519	85.3649	0.999393	-0.00061
0	121.4	24875	1.4057	46.86	0.047	0.039					
2	120.2	175956	8.3278	277.59	0.278	0.231					
2	120.0	170021	8.0558	268.53	0.269	0.224	0.225	0.2937	85.1231	0.996561	-0.00344
2	120.9	169084	8.0129	267.10	0.267	0.221					
4	120.5	521967	24.1809	806.03	0.806	0.669					
4	120.3	538295	24.9290	830.97	0.831	0.691	0.688	0.8973	84.5195	0.989495	-0.01056
4	120.6	550295	25.4788	849.29	0.849	0.704					
6	118.9	1147983	52.8630	1762.10	1.762	1.482					
6	120.2	1162359	53.5217	1784.06	1.784	1.484	1.486	1.9378	83.4790	0.977313	-0.02295
6	118.6	1152014	53.0477	1768.26	1.768	1.491					
8	120.3	2016754	92.6675	3088.92	3.089	2.568					
8	120.1	2025612	93.0733	3102.44	3.102	2.583	2.583	3.3694	82.0474	0.960554	-0.04025
8	119.8	2032860	93.4054	3113.51	3.114	2.599					

* DF =dilution factor in this case =33.33

APPENDIX C

The thin films were kept in well close container with silica gel at 25°C and protected from light for 2 months. The a* value of each formulation was evaluated for stability study. The results are shown in Tables 33 and 34.

Table 33 The a* value and standard deviation for film formulations F1-F4 at ambient condition and protected from light for 2 months.

initial	SD	Control F1-F4	SD	F1	SD	F2	SD	F3	SD	F4	SD
48.73	1.4	27.03	1.4	28.6	1.2	30.3	1.5	31.97	1.7	33.27	1.3

Table 34 The a* value and standard deviation for film formulations F5-F8 at ambient condition and protected from light for 2 months.

initial	SD	Control F5-F8	SD	F5	SD	F6	SD	F7	SD	F8	SD
51.00	1.3	39.50	1.9	39.83	1.1	40.73	1.4	42.03	1.7	42.53	1.5

The thin films were kept at 60°C/20%relative humidity. The changes in color of thin films as indicators were evaluated as function of duration of temperature exposure at 3, 6, 9, 12, 15, 18, 21 and 24 days and the experiment was done in triplicate.

Table 35 Data of a* value and standard deviation for films formulations F1-F4 at condition 60°C/20% relative humidity.

Time days	Control F1-F4	SD	End	SD	F1	SD	F2	SD	F3	SD	F4	SD
0	48.7	1.5	-1.7	3.5	48.7	1.5	48.7	1.5	48.7	1.5	48.7	1.5
3	44.7	2.3	-1.7	3.5	36.0	2.3	23.2	1.8	19.9	1.7	18.5	1.6
6	30.3	2.0	-1.7	3.5	19.5	3.2	17.7	2.6	12.6	2.6	9.9	1.9
9	21.8	2.3	-1.7	3.5	12.6	3.2	8.8	2.7	10.0	3.5	3.0	2.4
12	20.7	1.7	-1.7	3.5	7.5	2.2	7.0	2.1	5.9	3.7	0.9	2.1
15	20.0	1.5	-1.7	3.5	4.5	2.1	5.0	1.6	2.9	2.9	-0.3	2.1
18	19.0	1.9	-1.7	3.5	-0.2	2.4	-0.8	3.1	-2.1	1.5	-2.1	2.2
21	18.5	1.6	-1.7	3.5	-1.8	2.2	-2.5	2.3	-2.4	1.9	-2.6	2.6
24	17.9	2.2	-1.7	3.5	-3.2	1.9	-3.6	1.9	-3.3	2.0	-3.5	2.3

Table 36 Data of a* value and standard deviation for films formulations F5-F8 at condition 60°C/20% relative humidity.

Time days	Control F5-F8	SD	End	SD	F5	SD	F6	SD	F7	SD	F8	SD
0	51.0	1.3	-3.1	2.3	51.0	1.3	51.0	1.3	51.0	1.3	51.0	1.3
3	46.6	2.4	-3.1	2.3	39.7	2.0	37.0	2.7	31.5	2.6	28.8	1.8
6	42.3	2.2	-3.1	2.3	32.7	2.8	20.6	1.8	17.3	2.6	11.4	1.9
9	40.2	2.1	-3.1	2.3	23.2	2.1	16.8	2.1	10.4	1.8	5.7	2.2
12	39.3	2.3	-3.1	2.3	15.4	3.2	9.0	1.8	3.2	2.2	-0.5	2.9
15	38.6	2.8	-3.1	2.3	8.2	2.1	4.2	2.3	-3.3	2.3	-3.2	2.2
18	37.9	2.3	-3.1	2.3	4.9	1.1	-2.2	1.7	-4.1	1.8	-3.7	2.0
21	37.4	2.5	-3.1	2.3	-0.8	2.2	-3.1	2.0	-3.9	1.6	-3.8	1.7
24	37.0	2.2	-3.1	2.3	-3.1	2.0	-3.4	2.2	-3.5	2.2	-3.7	2.3

The average a*value and times of each formulations had plotted graphs. Methods used to compare color change data are, Statistical Methods (one-way ANOVA) and model Independent Methods (Difference factor (fl), Similarity factor (f2))

The area under the curve calculated by integration of a* value from time 0 to 24 days for each films formulations. The mean area under the curves were evaluated by one-way ANOVA. The out put for film formulations F1 toF4 are shown in Tables 37 and 38. Film formulations F5 to F8 are shown in Tables 39 and 40.

Table 37 Area under the curve of film formulations F1–F4 were calculated by integration of a* value from time 0 to 24 days for each formulation.

Formulation	N	Mean	Std. Deviation	Std. Error	Min.	Maximum
control	3	746.270	6.855	3.958	738.510	751.500
F1	3	423.550	15.157	8.751	407.100	436.950
F2	3	363.490	11.586	6.689	353.220	376.050
F3	3	328.650	10.678	6.165	317.550	338.850
F4	3	269.650	5.336	3.081	263.850	274.350

The homogeneity of variances shows that no significant differences (p value = 0.481). Levene's test assesses this assumption. It tests the null hypothesis that the population variances are equal. The variance of data in groups should be the same.

Table 38 The statistical results were compare means area between formulations at fixed red cabbage extract in concentration 0.2%w/w. (One-way ANOVA).

Multiple Comparisons				
Dependent Variable: AREA				
	FORMULATION	FORMULATION	Mean Difference	Sig.
Scheffe	Control F1-F4	F1	322.72	0.001
	Control F1-F4	F2	382.78	0.001
	Control F1-F4	F3	417.62	0.001
	Control F1-F4	F4	476.62	0.001
	F1	F2	60.06	0.001
	F1	F3	94.9	0.001
	F1	F4	153.9	0.001
	F2	F3	34.84	0.001
	F2	F4	93.84	0.001
	F3	F4	59	0.001

As the results, one-way ANOVA showed significant differences between the groups at the 0.05 level. It could be conclude that the color change of each film formulations were difference.

Table 39 Area under the curve of film formulations F5–F8 were calculated by integration of a* value from time 0 to 24 days for each formulation.

Formulation	N	Mean	Std. Deviation	Std. Error	Min.	Maximum
control	3	1098.850	7.575	4.373	1090.200	1104.300
F5	3	562.100	3.305	1.908	558.300	564.300
F6	3	438.300	8.079	4.664	432.150	447.450
F7	3	344.250	8.555	4.939	336.450	353.400
F8	3	295.800	6.920	3.995	289.200	303.000

The homogeneity of variances shows that no significant differences (p value = 0.610). Levene's test assesses this assumption. It tests the null hypothesis that the population variances are equal. The variance of data in groups should be the same.

Table 40 The statistical results were compare means area between formulations at fixed red cabbage extract in concentration 0.5%w/w. (One-way ANOVA).

Multiple Comparisons				
Dependent Variable: AREA				
	FORMULATION	FORMULATION	Mean Difference	Sig.
Scheffe	Control F5–F8	F5	536.75	0.001
	Control F5–F8	F6	660.55	0.001
	Control F5–F8	F7	754.6	0.001
	Control F5–F8	F8	803.05	0.001
	F5	F6	123.8	0.001
	F5	F7	217.85	0.001
	F5	F8	266.3	0.001
	F6	F7	94.05	0.001
	F6	F8	142.5	0.001
	F7	F8	48.45	0.001

As the results in Table 40, one-way ANOVA showed significant differences between the groups at the 0.05 level. It could be conclude that the color change of each film formulations were difference.

The model independent methods using Difference factor (f1) and Similarity factor (f2) was utilized to compare reference color and the color test profile. Table 42 is show the difference between each thin film formulations.

Table 41 Data f_1 and f_2 between film formulations at time 3 days to 24 days of test.

Reference Formulation	Test Formulation	Difference factor (f_1)	Similarity factor (f_2)
Control F1-F4	Control F5-F8	65	39
Control F1-F4	F1	61	40
Control F1-F4	F2	72	38
Control F1-F4	F3	77	36
Control F1-F4	F4	88	34
F1	F2	28	66
F1	F3	42	60
F1	F4	54	68
F2	F3	27	79
F2	F4	57	65
F3	F4	45	72
Control F5-F8	F5	62	28
Control F5-F8	F6	75	25
Control F5-F8	F7	85	23
Control F5-F8	F8	90	22
F5	F6	34	60
F5	F7	60	49
F5	F8	74	44
F6	F7	40	66
F6	F8	61	57
F7	F8	37	74

VITA

Miss Duchdoune Nitayavardhana was born on July 8th 1980, in Bangkok, Thailand. She received a Bachelor of Pharmacy Degree from the Faculty of Pharmaceutical Sciences, Chulalongkorn University in 2001.