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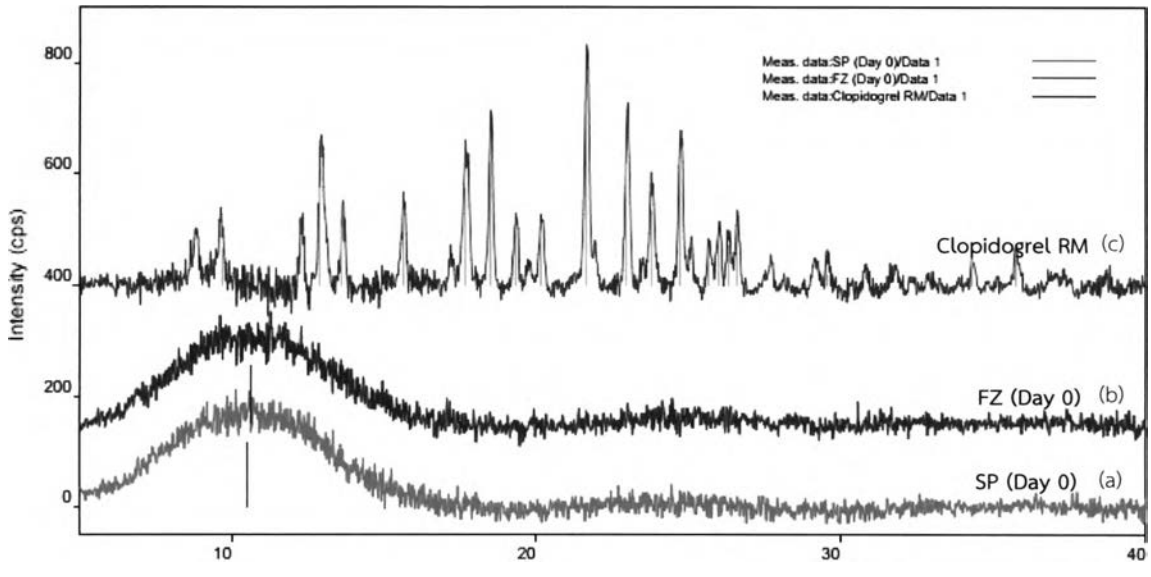
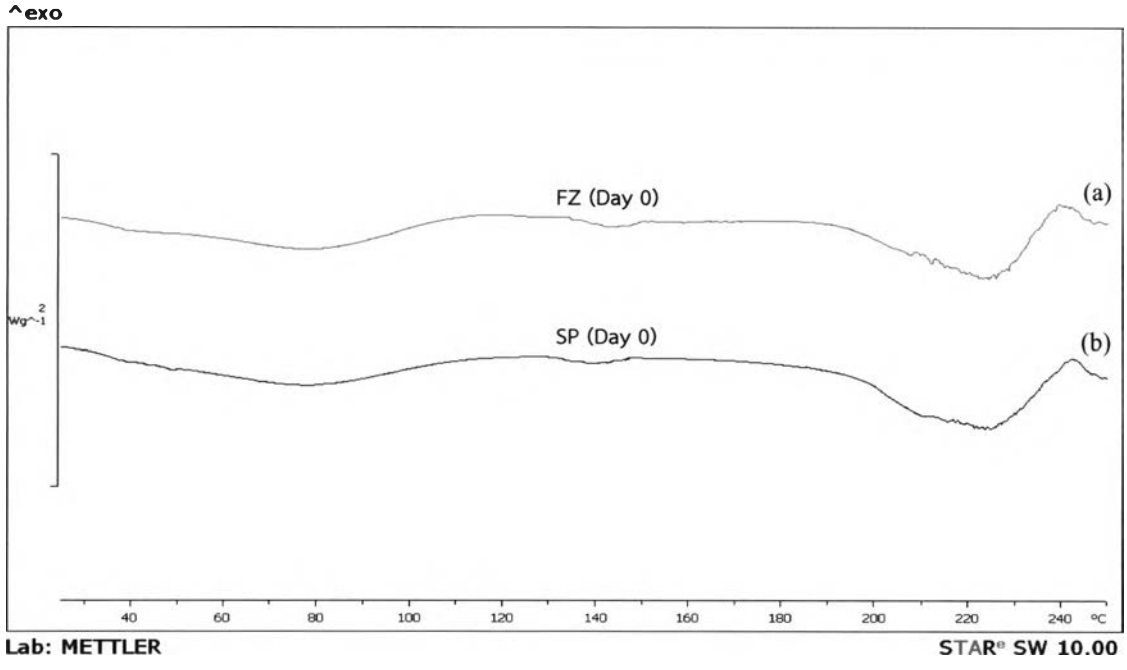
APPENDICES

APPENDIX A



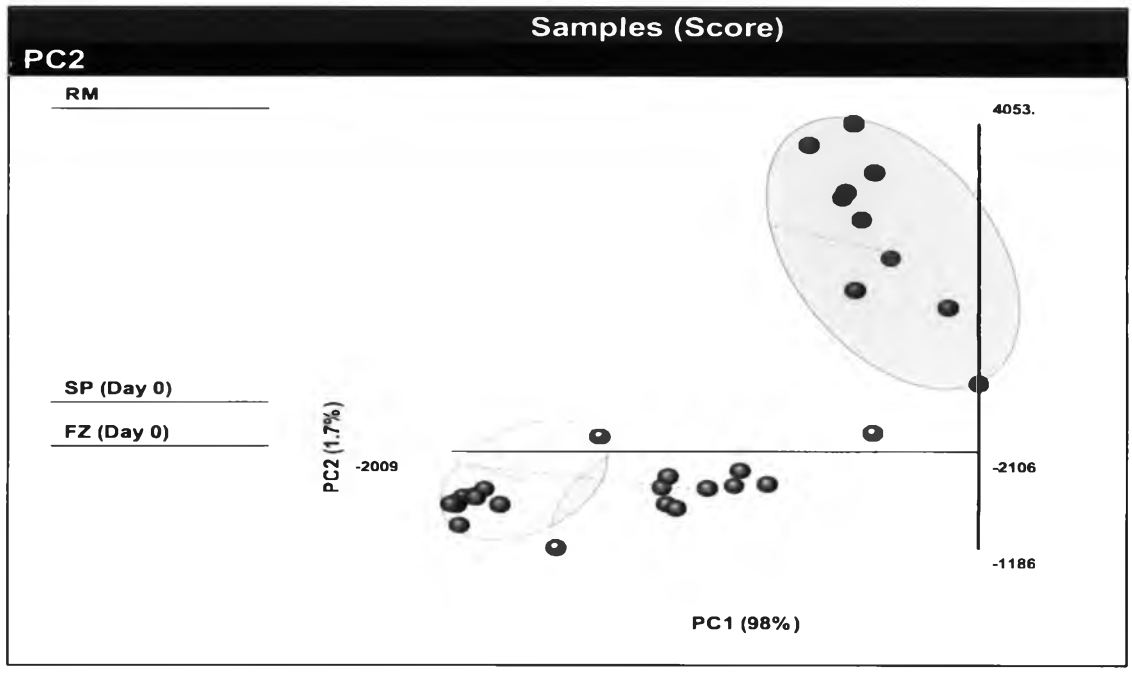
PXRD diffractograms of amorphous clopidogrel 2nd batch prepared by spray drying method (a) and freeze drying method (b) compare to clopidogrel RM (c)

APPENDIX B



DSC thermograms of initially prepared spray dried (a) and freeze dried clopidogrel (b) (2^{nd} batch)

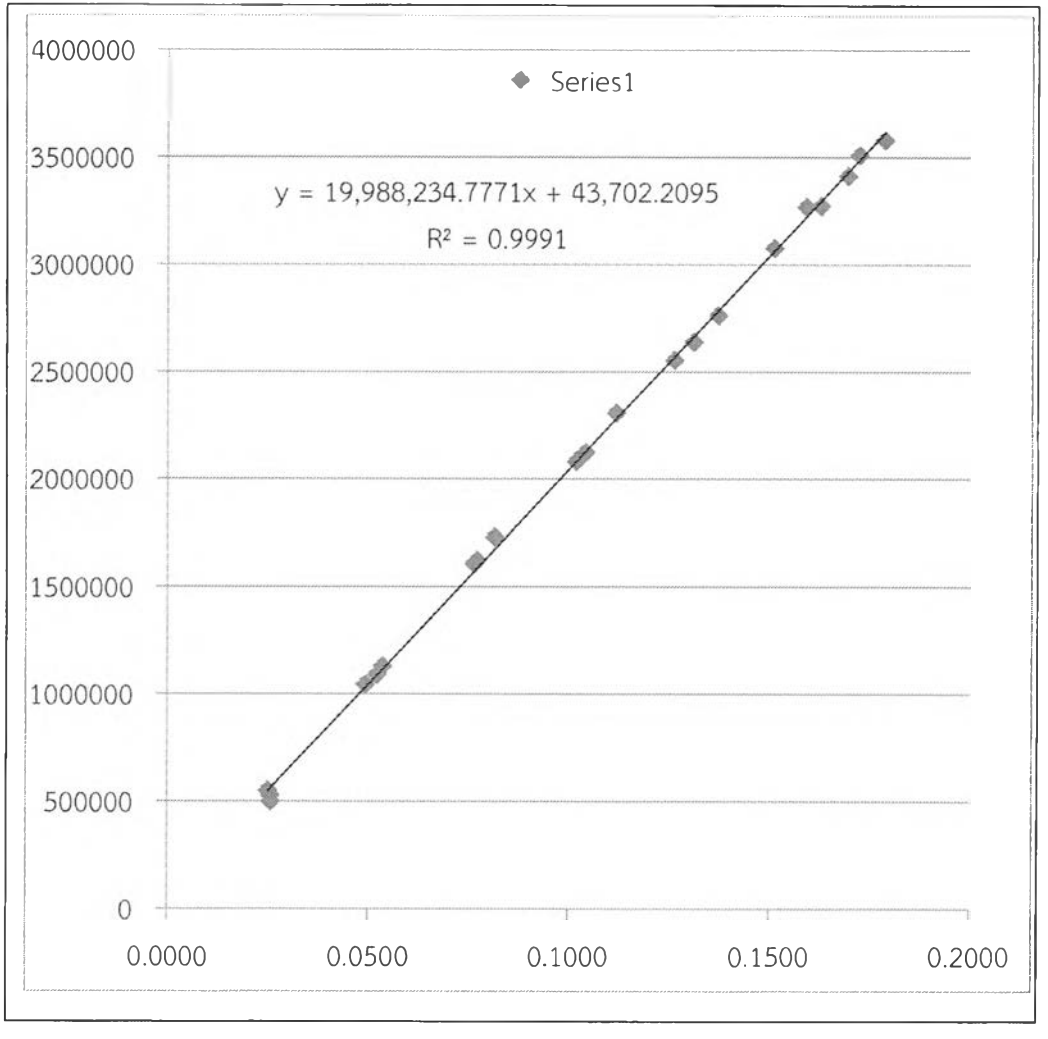
APPENDIX C



PCA of initial samples prepared by spray drying and freeze drying compare to clopidogrel RM obtained by Multibase program (2nd batch)

Linearity and Range

Std-Clopidogerl		
Conc. (mg/ml)		Area
0.025	0.0259	527753
	0.0253	548043
	0.0260	498857
0.050	0.0494	1042992
	0.0536	1129362
	0.0522	1090972
0.075	0.0772	1622496
	0.0816	1730037
	0.0764	1607193
0.100	0.1044	2124783
	0.1120	2307230
	0.1020	2081571
0.125	0.1264	2553025
	0.1312	2638394
	0.1372	2762477
0.150	0.1512	3075976
	0.1591	3269942
	0.1628	3272746
0.175	0.1788	3580481
	0.1696	3412437
	0.1724	3509795



Regression equation: $y = (19,988,234.7771)x + 43702.2095$

The correlation coefficient: $R^2 = 0.9991$

Accuracy

Sample solution	No.	Weight (mg)	Conc. Add (mg/ml)	Area	Conc. found (mg/ml)	% Recovery
80% of the test concentration	1	3.98	0.07960	1646570	0.08019	100.74
	2	4.13	0.08260	1675031	0.08161	98.81
	3	3.88	0.07760	1619781	0.07885	101.61
100% of the test concentration	1	5.15	0.10300	2118533	0.10380	100.78
	2	5.12	0.10240	2128358	0.10429	101.85
	3	5.01	0.10020	2080935	0.10192	101.72
120% of the test concentration	1	5.96	0.11920	2457759	0.12077	101.32
	2	6.08	0.12160	2463165	0.12104	99.54
	3	6.11	0.12220	2533967	0.12459	101.95
					Mean	**100.92**

Average %Recovery = 100.92%

Specification:

- Average %Recovery between 98 and 102%

Precision (Related substances)

Std-Clopidogrel Related Cpd A			Std-Clopidogrel Related Cpd B1 & B2					Std-Clopidogrel			Std-Clopidogrel Related Cpd C		
Injection No.	t_R (mins)	Area (r_s)	Injection No.	t_R (mins)	Area (r_s)	t_R (mins)	Area (r_s)	Injection No.	t_R (mins)	Area (r_s)	Injection No.	t_R (mins)	Area (r_s)
1	3.875	58088	1	8.000	46710	10.983	45261	1	9.917	10579	1	17.150	78043
2	3.867	59816	2	8.075	45292	11.167	48920	2	9.900	11097	2	17.158	78011
3	3.867	58158	3	8.142	43288	11.250	48901	3	9.892	11173	3	17.208	77500
4	3.858	58531	4	8.125	46026	11.200	49522	4	9.892	11324	4	17.208	77436
5	3.867	58735	5	8.125	43634	11.275	49071	5	9.900	11479	5	17.258	77751
6	3.858	58735	6	8.158	43693	11.317	49560	6	9.917	11695	6	17.092	77679
Average	3.865	58677.2	Average	8.104	44773.8	11.199	48539.2	Average	9.903	11224.5	Average	17.179	77736.7
SD	-	623.16	SD	-	1432.50	-	1631.76	SD	-	382.52	SD	-	252.60
%RSD	-	1.06	%RSD	-	3.20	-	3.36	%RSD	-	3.41	%RSD	-	0.32

Item / Compound	Related A	Related B1	Related B2	Clopidogrel	Related C
Retention time (t_R) (mins)	3.865	8.104	11.199	9.903	17.179
Relative retention time (RRT)	0.39	0.82	1.13	1.00	1.73
Resolution (R_s) between B1 and clopidogrel: NLT 2.5	2.61				
Relative standard deviation (RSD) : NMT 15% for each peak	1.06	3.20	3.36	3.41	0.32

Specification (USP 35)

System suitability solution::

- The relative retention times are about 0.5 for clopidogrel related cpd A, 0.8 and 1.2 for the two enantiomers of clopidogrel related cpd B, 1.0 for clopidogrel and 2.0 for clopidogrel related cpd C
- The resolution (R) between clopidogrel and the first enantiomer of clopidogrel related cpd B is greater than 2.5

Standard solution:

- The relative standard deviation (RSD) for replicate injections is not more than 15% for each peak

Precision (Assay)

Std-Clopidogrel		
Injection No.	t_R (mins)	Area (r_s)
1	8.819	2408533
2	8.801	2429964
3	8.803	2428358
4	8.789	2430935
5	8.784	2441902
6	8.764	2430549
Average	8.793	2428373.5
SD	-	10867.29
%RSD	-	0.45

Std-Clopidogrel Related Cpd B1 & B2					Std-Clopidogrel		
Injection No.	t_R (mins)	Area (r_s)	t_R (mins)	Area (r_s)	Injection No.	t_R (mins)	Area (r_s)
1	6.917	28424	9.042	29588	1	8.000	11287
2	6.917	28450	9.025	29711	2	7.992	11514
3	6.900	28486	9.008	29574	3	8.000	11218
4	6.883	28599	9.000	28995	4	7.992	10907
5	6.892	28310	8.992	29434	5	7.992	11330
6	6.883	28591	9.008	29491	6	7.975	11162
Average	6.899	28476.7	9.013	29465.5	Average	7.992	11236.3
SD	-	108.98	-	249.02	SD	-	201.38
%RSD	-	0.38	-	0.85	%RSD	-	1.79

Item / Compound	Related B1	Related B2	Clopidogrel
Retention time (t_R) (mins)	6.899	9.013	7.992
Relative retention time (RRT)	0.86	1.13	1.00
Resolution (R_S) between B1 and clopidogrel: NLT 2.5		2.91	
Relative standard deviation (RSD) : NMT 1.0%			0.45

Specification (USP 35)

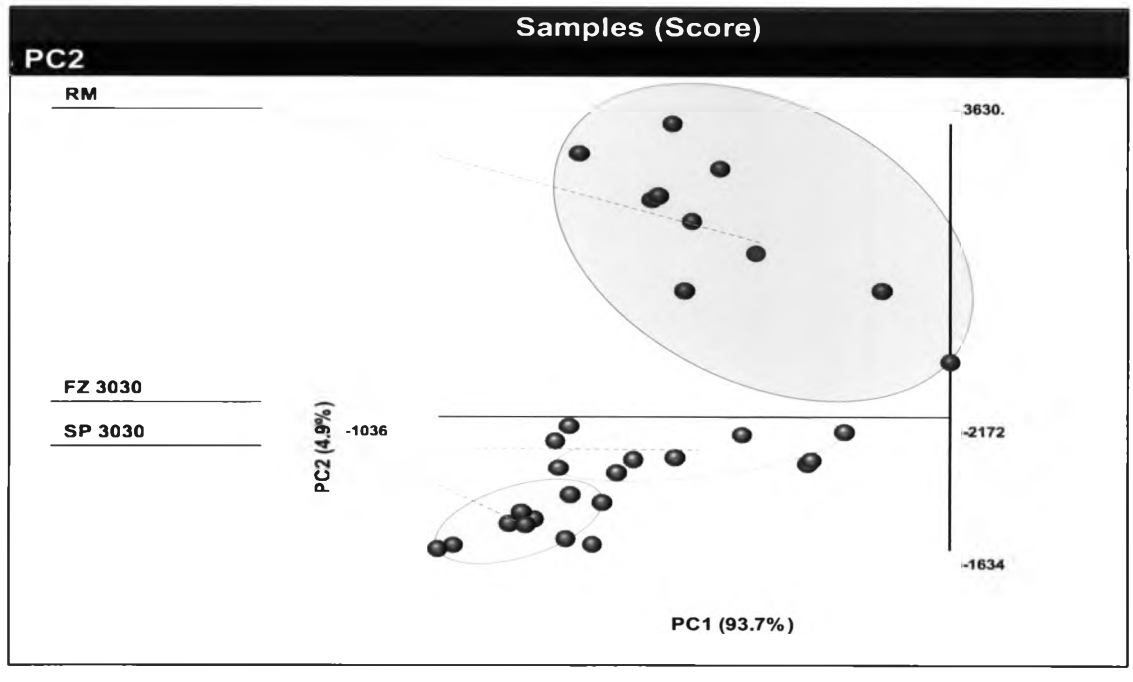
System suitability solution::

- The relative retention times are about 0.8 and 1.2 for the two enantiomers of clopidogrel related cpd B and 1.0 for clopidogrel
- The resolution (R) between clopidogrel and the first enantiomer of clopidogrel related cpd B is greater than 2.5

Standard preparation:

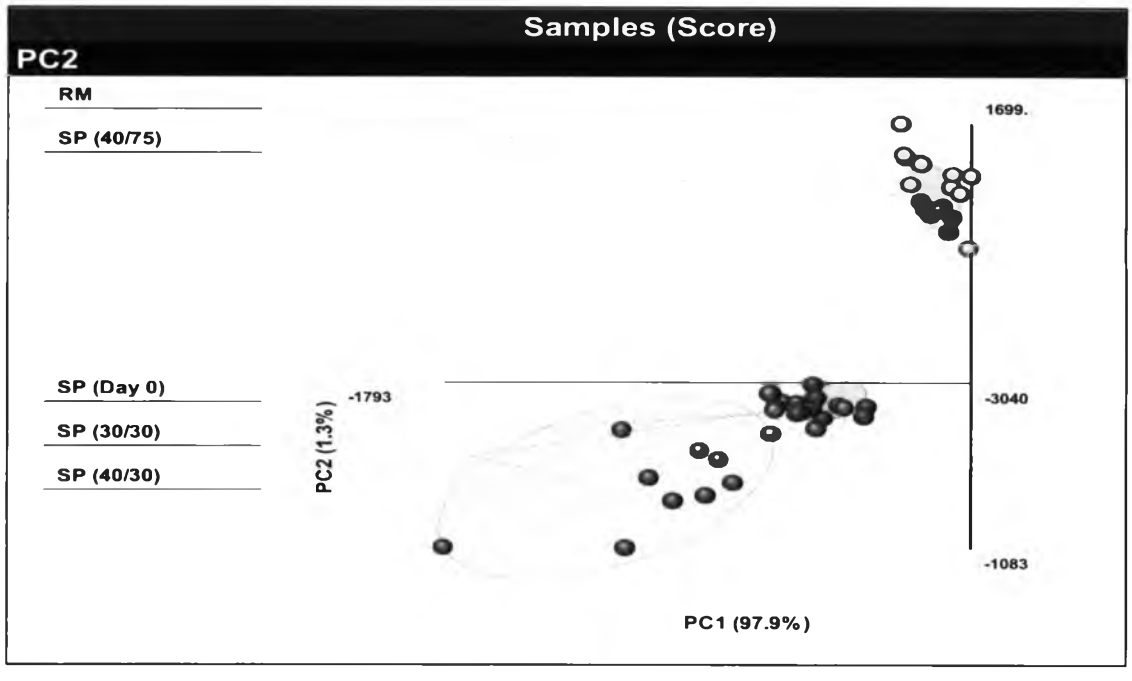
- The relative standard deviation (RSD) for replicate injections determined from clopidogrel bisulfate is not more than 1.0%

APPENDIX E

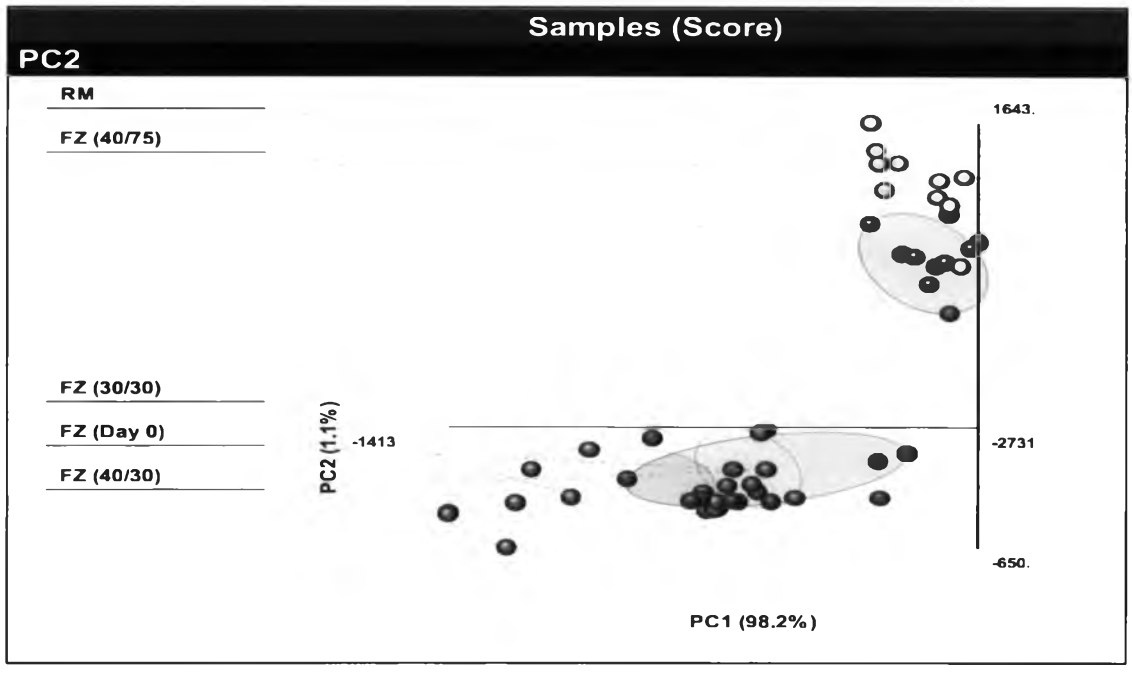


PCA of spray dried and freeze dried samples stored at 30°C 30%RH for 7 days compare to clopidogrel RM obtained by and Multibase program (2nd batch)

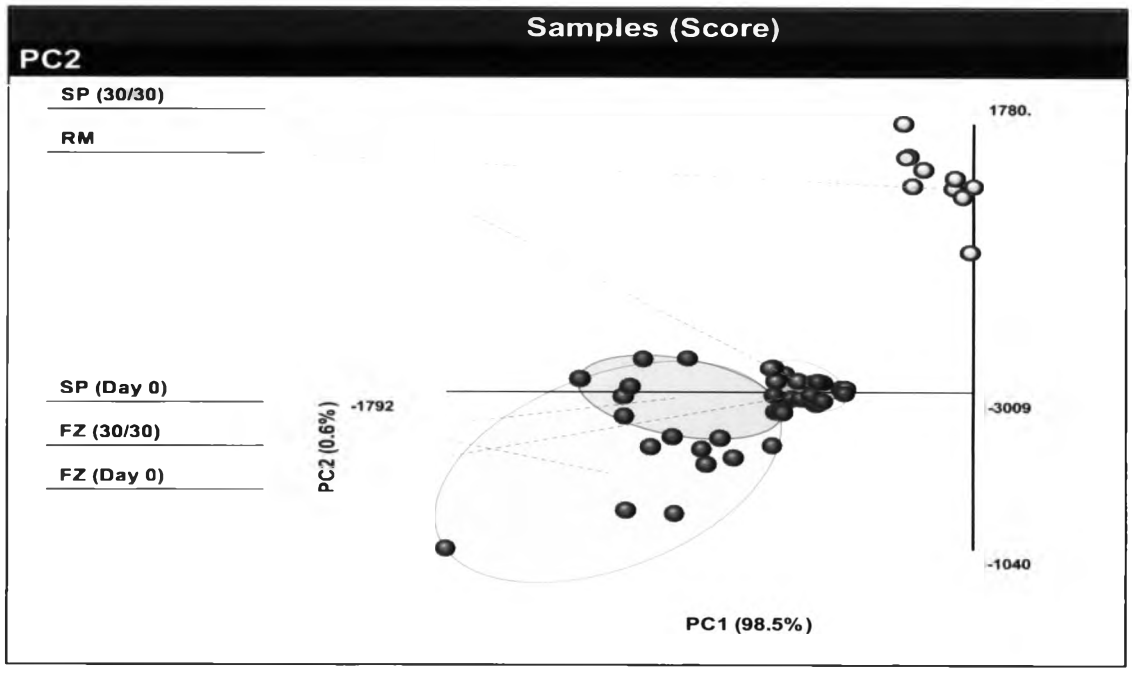
APPENDIX F



PCA of $SP^{7}_{30/30}$, $SP^{7}_{40/30}$, $SP^{7}_{40/75}$ and RM (Figure 85) compare to SP^{0}



PCA of $FZ^{7}_{30/30}$, $FZ^{7}_{40/30}$, $FZ^{7}_{40/75}$ and RM (Figure 86) compare to FZ^{0}



PCA of $SP^{7}_{30/30}$, $FZ^{7}_{30/30}$ and RM (Figure 97) compare to SP^{0} and FZ^{0}

APPENDIX G

Clopidogrel Related Compound A (Spray dried samples)

Descriptives

Data1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	11	.174022	.0563400	.0169871	.136172	.211871	.0840	.2586
2	11	.346037	.2403325	.0724630	.184580	.507495	.1596	.9914
3	11	1.009422	.6961938	.2099103	.541712	1.477131	.2066	2.5136
Total	33	.509827	.5516242	.0960255	.314229	.705424	.0840	2.5136

Test of Homogeneity of Variances

Data1

Levene Statistic	df1	df2	Sig.
11.282	2	30	.000

ANOVA

Data1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.281	2	2.141	11.769	.000
Within Groups	5.456	30	.182		
Total	9.737	32			

Multiple Comparisons

Dependent Variable: Data1

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett T3	1	2	-.1720155	.0744274	.112	-.378930	.034898
		3	-.8354003*	.2105965	.007	-1.429788	-.241013
	2	1	.1720155	.0744274	.112	-.034898	.378930
		3	-.6633847*	.2220658	.032	-1.270948	-.055822
	3	1	.8354003*	.2105965	.007	.241013	1.429788
		2	.6633847*	.2220658	.032	.055822	1.270948

*. The mean difference is significant at the 0.05 level.

Conclusion (Clopidogrel Related Compound A obtained from spray drying method)

- Storage at 30°C 30%RH and 40°C 75%RH have significant difference (P=0.007)
- Storage at 40°C 30%RH and 40°C 75%RH have significant difference (P=0.032)
- Storage at 30°C 30%RH and 40°C 30%RH have not significant difference

Clopidogrel Related Compound A (Freeze dried samples)

Descriptives

Data1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	11	.286479	.1428016	.0430563	.190543	.382414	.1234	.6592
2	11	.645650	.5481467	.1652724	.277400	1.013899	.2370	1.9509
3	11	1.097531	.6831655	.2059822	.638574	1.556487	.2370	2.3252
Total	33	.676553	.5997241	.1043986	.463900	.889206	.1234	2.3252

Test of Homogeneity of Variances

Data1

Levene Statistic	df1	df2	Sig.
8.348	2	30	.001

ANOVA

Data1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.634	2	1.817	6.921	.003
Within Groups	7.876	30	.263		
Total	11.509	32			

Post Hoc Tests

Multiple Comparisons

Dependent Variable: Data1

	(I) Group	(J) Group	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Dunnett T3	1	2	-.3591707	.1707888	.157	-.832292	.113951
		3	-.8110517*	.2104341	.008	-1.397981	-.224122
	2	1	.3591707	.1707888	.157	-.113951	.832292
		3	-.4518810	.2640902	.270	-1.139702	.235940
	3	1	.8110517*	.2104341	.008	.224122	1.397981
		2	.4518810	.2640902	.270	-.235940	1.139702

*. The mean difference is significant at the 0.05 level.

Conclusion (Clopidogrel Related Compound A obtained from freeze drying method)

- Storage at 30°C 30%RH and 40°C 75%RH have significant difference (P=0.008)
- Storage at 40°C 30%RH and 40°C 75%RH have not significant difference
- Storage at 30°C 30%RH and 40°C 30%RH have not significant difference

Clopidogrel Related Compound C (Spray dried samples)

Descriptives

Data1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	11	1.311741	.5353119	.1614026	.952114	1.671369	.7041	1.9419
2	11	1.295851	.5442203	.1640886	.930239	1.661463	.6675	1.9460
3	11	1.310109	.4625464	.1394630	.999366	1.620852	.7722	1.9254
Total	33	1.305900	.4990152	.0868674	1.128957	1.482843	.6675	1.9460

Test of Homogeneity of Variances

Data1

Levene Statistic	df1	df2	Sig.
1.276	2	30	.294

ANOVA

Data1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	2	.001	.003	.997
Within Groups	7.967	30	.266		
Total	7.969	32			

Conclusion (Clopidogrel Related Compound C obtained from spray drying method)

- Storage at 30°C 30%RH and 40°C 75%RH have not significant difference
- Storage at 40°C 30%RH and 40°C 75%RH have not significant difference
- Storage at 30°C 30%RH and 40°C 30%RH have not significant difference

Clopidogrel Related Compound C (Freeze dried samples)

Descriptives

Data1

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	11	1.296944	.5939246	.1790750	.897940	1.695948	.7107	2.2104
2	11	1.307939	.5796219	.1747626	.918543	1.697334	.6589	2.0857
3	11	1.317583	.5051140	.1522976	.978243	1.656923	.7737	2.0857
Total	33	1.307488	.5431628	.0945525	1.114891	1.500086	.6589	2.2104

Test of Homogeneity of Variances

Data1

Levene Statistic	df1	df2	Sig.
.815	2	30	.452

ANOVA

Data1

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.002	2	.001	.004	.996
Within Groups	9.438	30	.315		
Total	9.441	32			

Conclusion (Clopidogrel Related Compound C obtained from freeze drying method)

- Storage at 30°C 30%RH and 40°C 75%RH have not significant difference
- Storage at 40°C 30%RH and 40°C 75%RH have not significant difference
- Storage at 30°C 30%RH and 40°C 30%RH have not significant difference

VITA

Tharinchai Songrojjanawan was born in Bangkok, Thailand, on July 4th 1982. He received Bachelor of Science in Pharmacy degree in 2006 from the Faculty of Pharmaceutical sciences, Chulalongkorn University, Thailand. He presented a poster titled "Solid-state conversion of amorphous clopidogrel generated by spray drying" in The 39th Congress on Science and Technology of Thailand "Innovative Sciences for a Better Life" on October 21 - 23, 2013 at BITEC, Bangkok, Thailand.