# CHAPTER IV

## RESULTS AND DISCUSSION

### 1. Preparation polyamorphous samples of clopidogrel

Polyamorphous samples of clopidogrel were prepared from clopidogrel bisulfate polymorph Form II using spray drying and freeze drying methods which are shown in Figures 47 and 48, respectively.

Figure 47 Spray dryer (Buchi Mini Spray Dryer B-290, Buchi, Switzerland)

Figure 48 Freeze dryer (Lyophilizer) (LYO LAB, Lyophilization Systems, Inc., USA)



## 2. Solid-state characterization

### 2.1. Physical Appearance

Amorphous sample prepared by spray drying method is white, fine granules (Figure 49a), while amorphous sample prepared by freeze drying method is white, fine bulky flakes (Figure 49b).

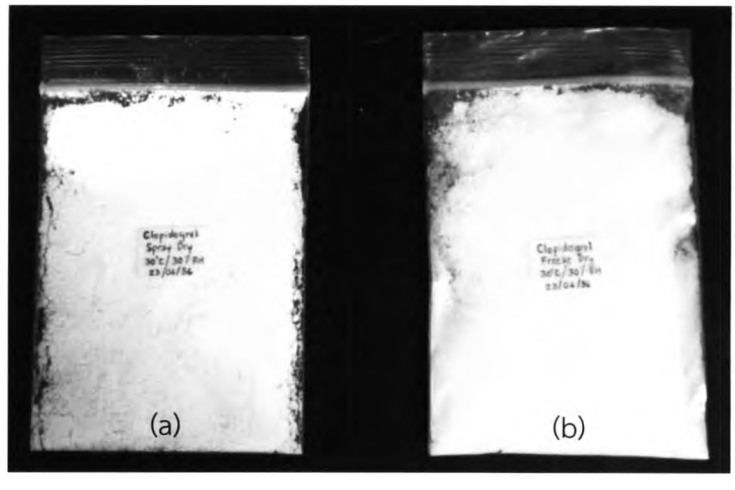



Figure 49 Polyamorphous samples obtained by spray drying method (a) and by freeze drying method (b)

### 2.2. Polarized light microscopy

Figures 50a and 50b do not show birefringence phenomena under polarized light microscope in both samples obtained by spray drying and freeze drying methods, respectively. On the other hand, Figure 50c shows birefringence of clopidogrel raw material (RM) observed under polarized light microscope. These results initially indicate that clopidogrel RM is crystalline while the prepared samples are amorphous forms.

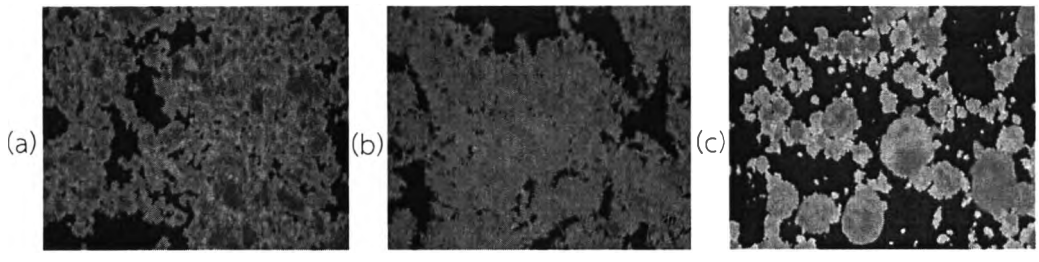



Figure 50 Polarized photomicrogragphs of samples prepared by spray drying method (a) freeze drying method (b) and clopidogrel RM (c)



### 2.3. Powder X-ray diffractometry (PXRD)

Figure 51 shows powder X-ray diffraction profiles of samples prepared by spray drying and freeze drying methods compare to clopidogrel raw material (RM) for the first batch. The powder X-ray diffraction (PXRD) patterns of both samples prepared by spray drying and freeze drying show amorphous halo and do not exhibit any crystalline diffraction peaks. On the other hand, clopidogrel RM exhibits characteristic crystalline peaks at $8.9^{\circ}$ $2\theta$ and $12.5^{\circ}$ $2\theta$ in accordance to the report from previous study (11). These results indicate that the initially prepared samples by both methods are amorphous form of the clopidogrel, whereas, clopidogrel RM is confirmed to be crystalline form. However, PXRD is unsuccessful in reliably

distinguishing the two amorphous samples. PXRD profiles of the second batch samples prepared by spray drying and freeze drying methods compare to clopidogrel raw material (RM) show repeatability in producing the same amorphous phase by both methods and are shown in Appendix A.

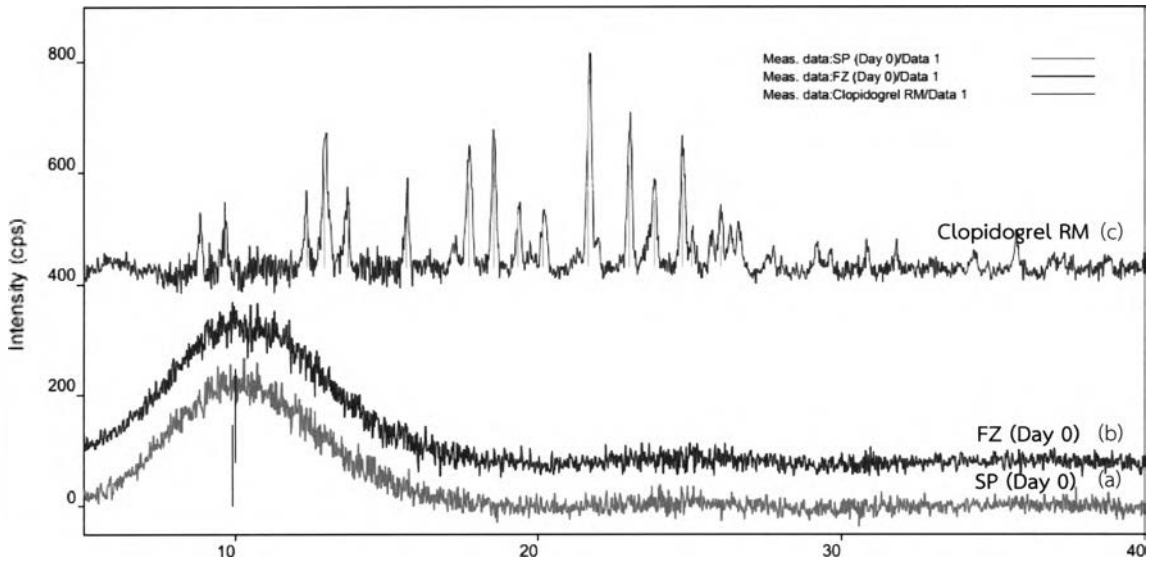



Figure 51 PXRD diffractograms of amorphous clopidogrel 1st batch prepared by spray drying method (a) and freeze drying method (b) compare to clopidogrel RM (c)

**2.4.Differential scanning calorimetry (DSC)**

Figure 52 shows differential scanning calorimetry (DSC) thermogram of clopidogrel raw material (RM) exhibiting melting endotherm at 183°C and degradation endotherm at 210°C. This result indicates that clopidogrel RM is crystalline form. Figures 53a and 53b show DSC thermograms of first batch samples prepared by spray drying method and freeze drying method, respectively. Moreover, DSC thermograms of the second batch samples prepared by spray drying and freeze drying methods show repeatability in producing the same amorphous phase by both methods and are shown in Appendix B. DSC thermogram of sample prepared by spray drying

method shows broad endotherm at 70°C due to dehydration, possible $T_g$ at 140°C and shows degradation endotherm at 220°C. DSC thermogram of sample prepared by freeze drying method also shows broad endotherm at 70°C due to dehydration, possible $T_g$ at 140°C and exhibits degradation endotherm at 220°C. These results indicate that both of the initially prepared samples are amorphous form of clopidogrel. Moreover, all results from DSC study are in good agreement with the results obtained by PXRD and both methods (DSC and PXRD) cannot distinguish the differences between the amorphous samples prepared by both methods (freeze drying and spray drying).



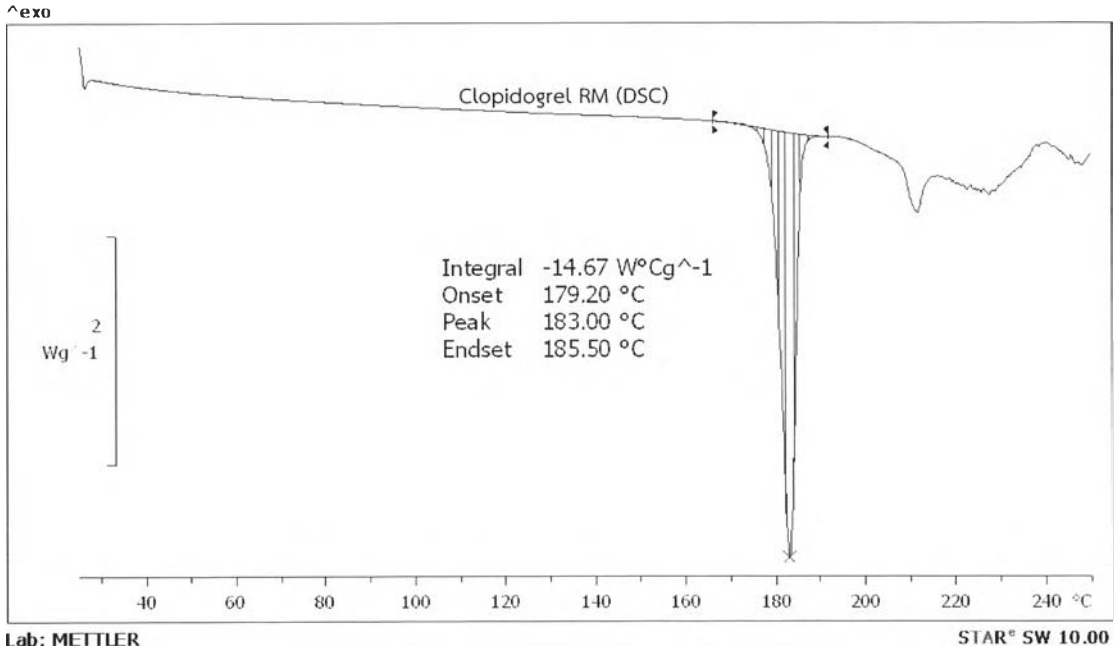



Figure 52 DSC thermogram of clopidogrel raw material (RM)

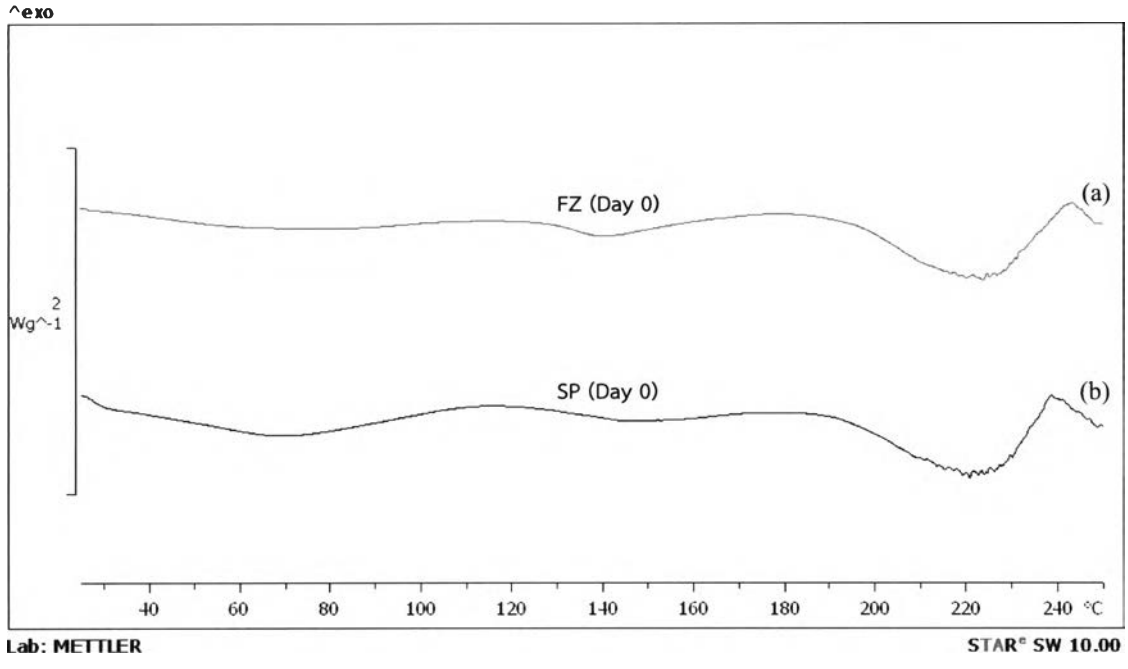



Figure 53 DSC thermograms of initially prepared spray dried (a) and freeze dried clopidogrel (b) (1st batch)



**2.5.Thermogravimetric analysis (TGA)**

Figure 54 shows thermogravimetric analysis (TGA) result of clopidogrel raw material (RM) exhibiting linearity in weight until degraded at 200°C. Figures 55a and 55b show TGA thermograms of samples prepared by spray drying method and freeze drying method, respectively. TGA thermogram of initial sample prepared by spray drying method indicates weight reduction of 3.738 %w/w between 40-100°C and indicates degradation weight loss at 200°C. Whereas, TGA thermogram of sample prepared by freeze drying method exhibits weight loss of 3.054 %w/w between 40-100°C and exhibits degradation endotherm at 200°C. As they are so similar, samples obtained by both methods cannot be distinguished by TGA.

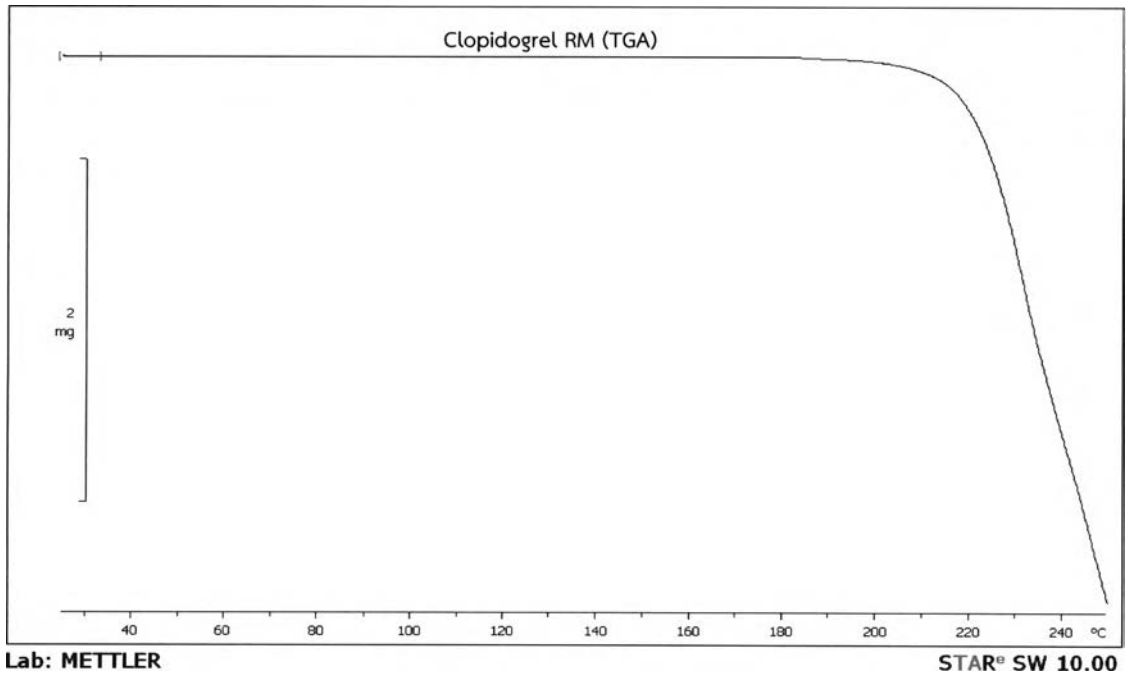



Figure 54 TGA thermogram of clopidogrel raw material (RM)

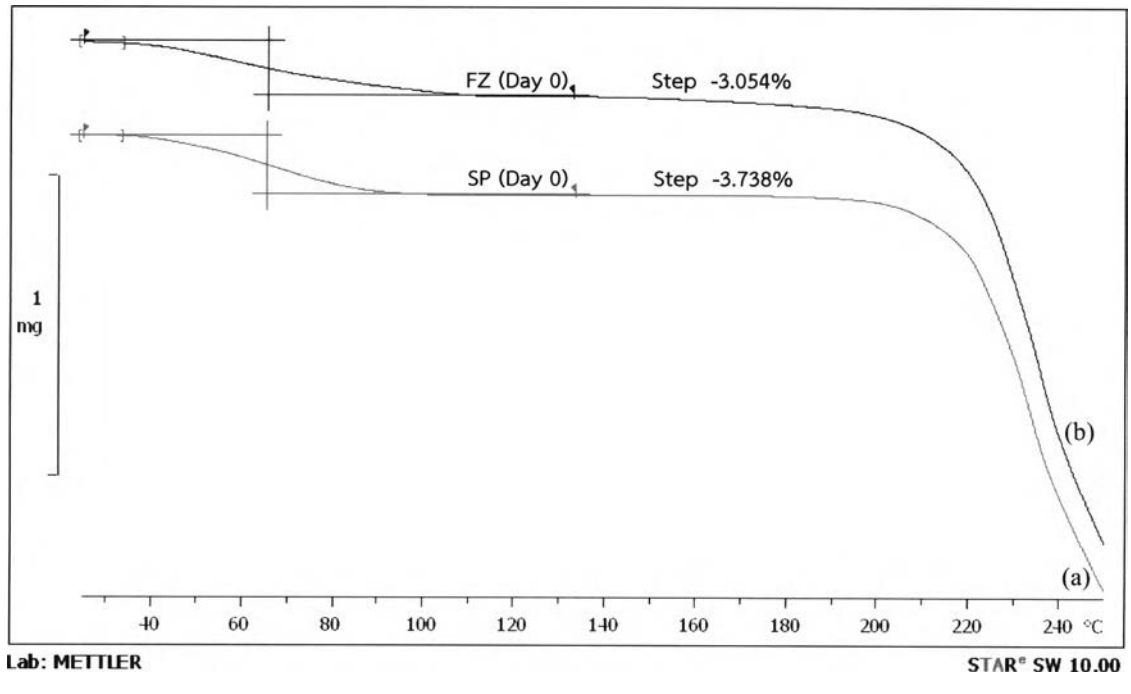



Figure 55 TGA thermograms indicating %weight loss of initial clopidogrel samples prepared by spray drying (a) and freeze drying methods (b)

### 2.6. Dynamic Vapor Sorption (DVS)

Figure 56 shows the sorption/desorption isotherm of clopidogrel raw material (RM). Figure 57 shows the sorption/desorption isotherm of clopidogrel sample prepared by spray drying method and Figure 58 shows the sorption/desorption isotherm of clopidogrel sample prepared by freeze drying method. Water sorption of spray dried sample increased sharply since the humidity reached 60 %RH and continue to adsorb upto 30 %w/w moisture when the equilibrium relative humidity reached 90 %RH and retained approximately 5 %w/w moisture when the relative humidity was reduced to 0 %RH due to hygroscopic nature of this form (Figure 57). The water sorption of sample prepared by freeze drying method also increased sharply from 60 %RH and continue adsorb upto 28 %w/w moisture when the equilibrium relative humidity reached 90 %RH and also retained at approximately 5 %w/w moisture when reduced to 0%RH because of hygroscopicity of the substance (Figure 58). On the other hand, the water sorption of clopidogrel RM increased to only 0.2 %w/w at 90 %RH and did not retain any moisture when the relative humidity was reduced to 0 %RH. These results indicate that amorphous clopidogrel readily take-up moisture at higher level than its crystalline RM. However, DVS cannot be used to successfully distinguish between the two amorphous clopidogrel prepared by two methods.

Figure 59 shows the sorption/desorption isotherms of initial spray dried and freeze dried sample. The isotherm indicates that water sorption of initial freeze dried sample is a bit higher than initial spray dried sample. Moreover, water desorption indicates that the remaining moisture of initial freeze dried sample is a bit higher than initial spray dried sample when the relative humidity reached 0 %RH. This result is caused by the difference in surface area of both samples.

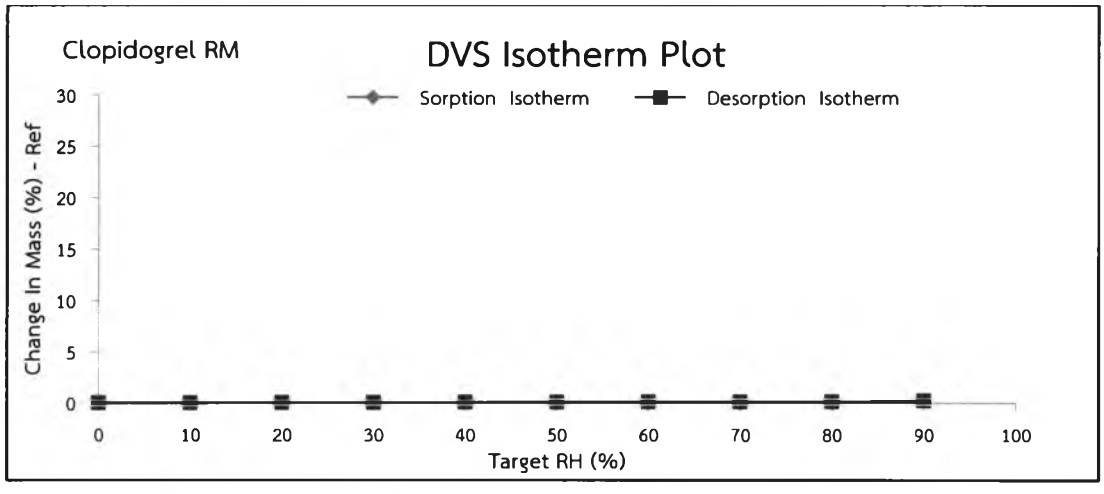



Figure 56 Sorption/desorption isotherms of clopidogrel RM

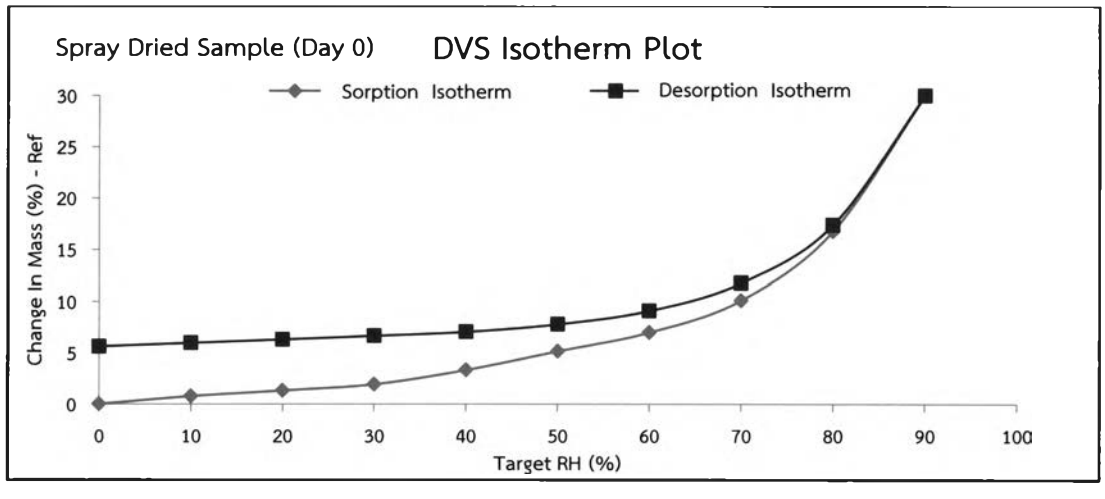



Figure 57 Sorption/desorption isotherms of initial spray dried sample

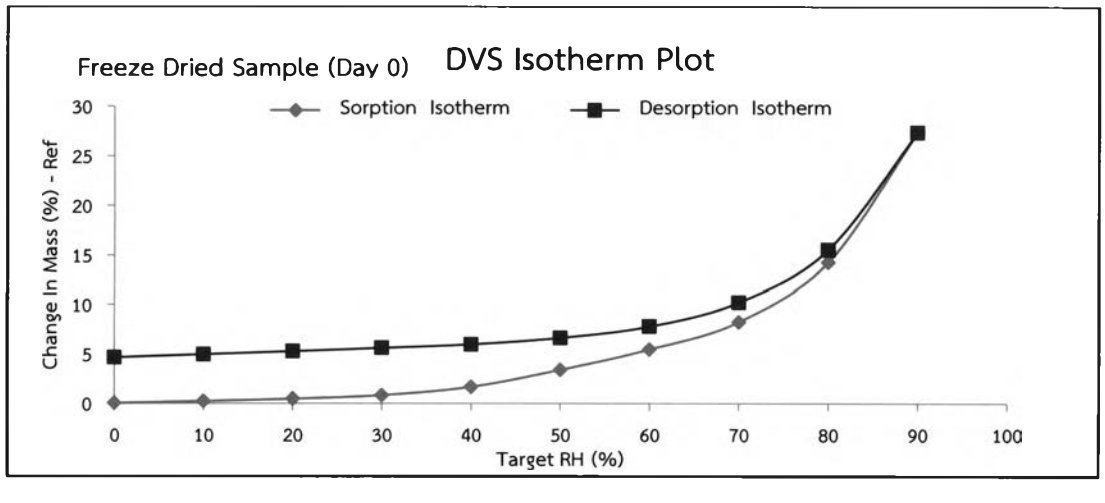



Figure 58 Sorption/desorption isotherms of initial freeze dried sample

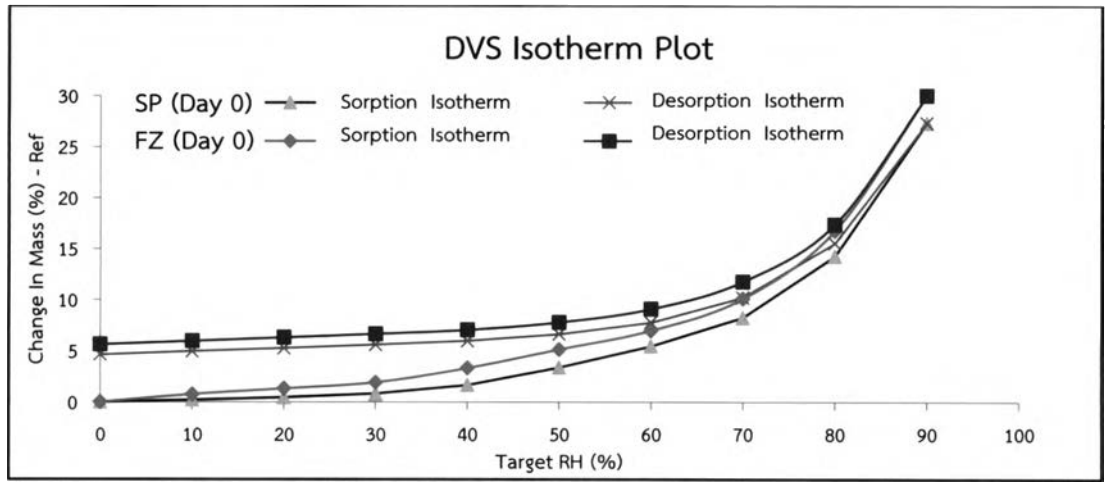



Figure 59 Comparison of sorption/desorption isotherms of initial spray dried and freeze dried sample



**2.7. Confocal microscopic Raman spectrometry (Raman)**

Figure 60a shows Raman spectrum of sample prepared by spray drying method and Figure 60b shows Raman spectrum of sample prepared by freeze drying method. Figure 60c shows Raman spectrum of crystalline clopidogrel raw material (RM). Raman shifts between 3200 to 2900 $cm^{-1}$ and 1100 to 1000 $cm^{-1}$ are chosen to represent clear visual distinction between forms. Raman spectral result of sample prepared by freeze drying method is visually similar to Raman spectrum of initial sample prepared by spray drying method but different from Raman spectrum of crystalline clopidogrel RM. These results from the prepared samples indicate characteristic shifts of amorphous form, while Raman spectra of clopidogrel RM indicates characteristic shifts of crystalline form. However, the use of Raman alone cannot successfully distinguish between amorphous samples prepared by the two methods.

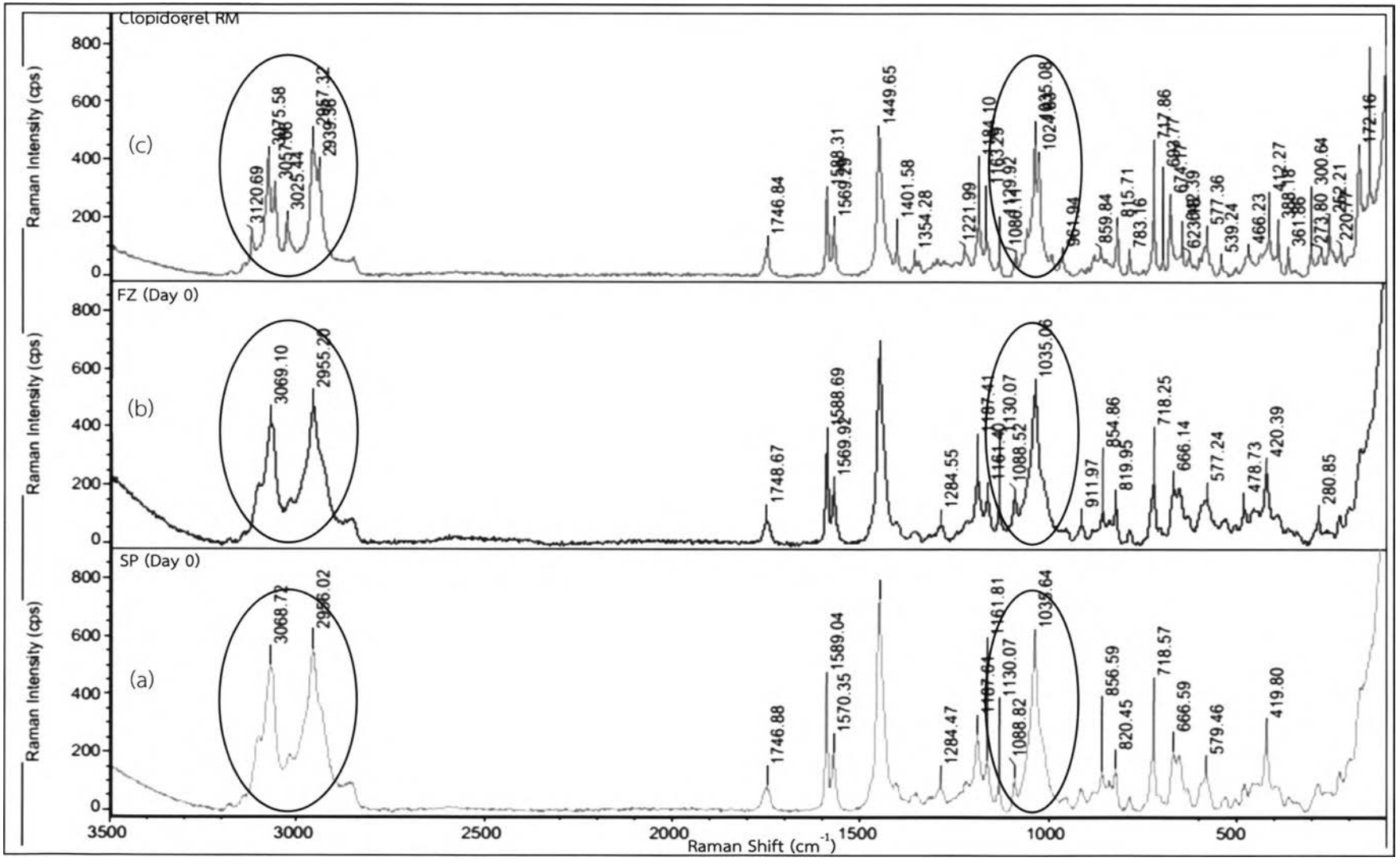
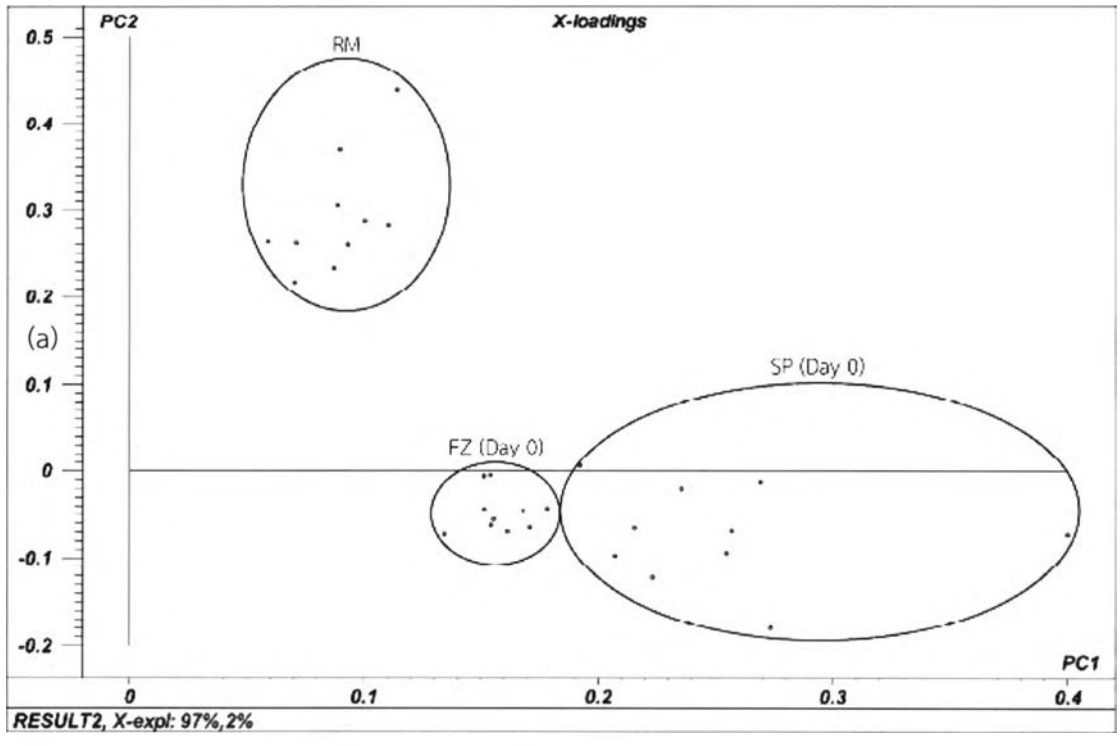



Figure 60 Raman spectrum of sample prepared by spray drying (a) Raman spectrum of sample prepared by freeze drying (b) and Raman spectrum of clopidogrel RM (c)

## 3. Principal Component Analysis (PCA)

Figure 61a and 61b indicate that Raman spectral data of initial samples prepared by spray drying method and freeze drying method and Raman spectral data of clopidogrel raw material (RM) in the first time can be classified with Principal component analysis (PCA) as 3 distinctive groups. All Raman spectral data were collected in 10 replicates and were analyzed by OMNIC® (Version 8.0) software in range of 3200 to 2800 $cm^{-1}$ and 1800 to 100 $cm^{-1}$ for discrimination of all data by Principal Component Analysis (PCA) using UNSCRAMBLER® (Version 9.8) and Multibase (2014) software. The first PC (PC1) accounted for 97.7% of overall variance and the second PC (PC2) accounted for 1.2% of overall variance. DBI value between $SP^0$ and $FZ^0$ is 0.4285. This PCA result indicates that all samples can be clearly separated into 3 groups. On the contrary, PXRD (Figure 51) patterns, DSC thermograms (Figure 53), TGA thermograms (Figure 55) and Raman spectral data (Figure 60) evaluated in previous sections cannot distinguish between amorphous forms produced initially by spray drying and freeze drying. For this reason, PCA is used as mathematical statistics tool to successfully classify two amorphous form of clopidogrel obtained by spray drying and freeze drying methods. These two different amorphous structures of the same drug substance, clopidogrel, can be differentiated by Raman spectroscopy and now defined as "polyamorphous" samples.

Repeatability of Raman spectral data of samples prepared by spray drying method and freeze drying method and Raman spectral data of clopidogrel RM for the second batch can also be classified with Principal component analysis (PCA) as 3 distinctive groups. The PC1 accounted for 98.0% of overall variance and the PC2 accounted for 1.7% of overall variance as shown in Appendix C.



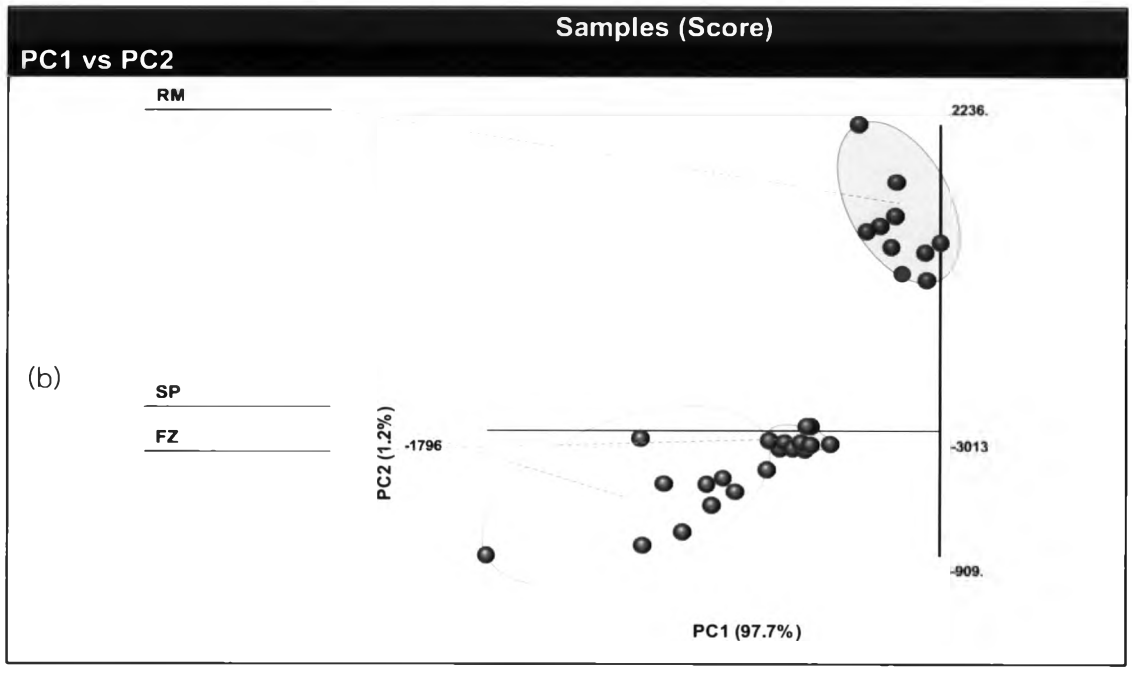




Figure 61 PCA of initial samples prepare by spray drying and freeze drying compare to clopidogrel RM obtained by Unscrambler® (a) and Multibase (b) program (1st time)

## 4. Physicochemical evaluation of polyamorphous samples

### 4.1. Appearance and size

Figure 62 shows surface appearances and approximate sizes under scanning electron microscope (SEM) at 100x (Figure 62a) and 500x (Figure 62b) magnifications of clopidogrel raw material (RM). Figures 63 and 64 show surface appearances and approximate sizes under scanning electron microscope (SEM) at 100x (Figure 63a and 64a) and 500x (Figure 63b and 64b) magnifications of initial spray dried and freeze dried samples. Figure 62b shows clopidogrel RM with small irregular particles of approximately 10 microns. Figure 63b shows sample prepared by spray drying exhibiting small spherical particle with particle size of 5 microns. Figure 64b shows prepared freeze dried sample as flakes of solids which particle size cannot be determined.



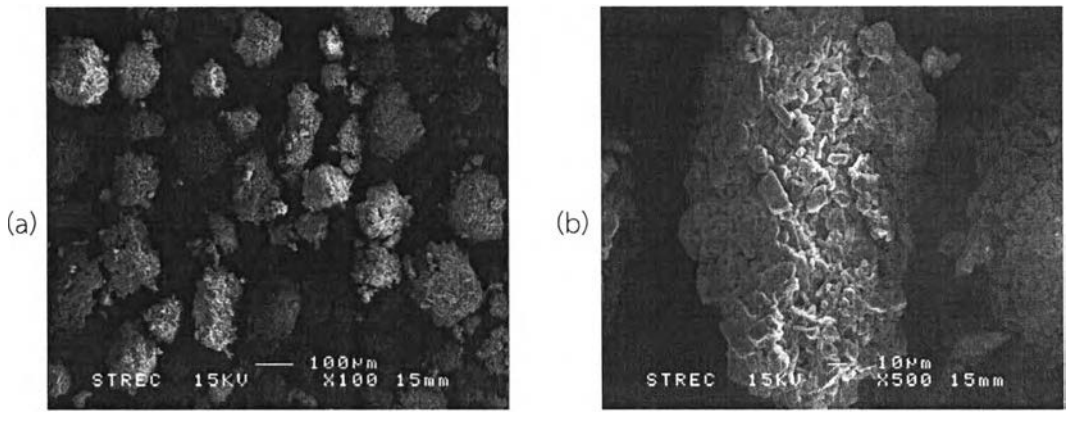



Figure 62 SEM photomicrographs of clopidogrel raw material (RM) at 100x (a) and 500x (b) magnifications

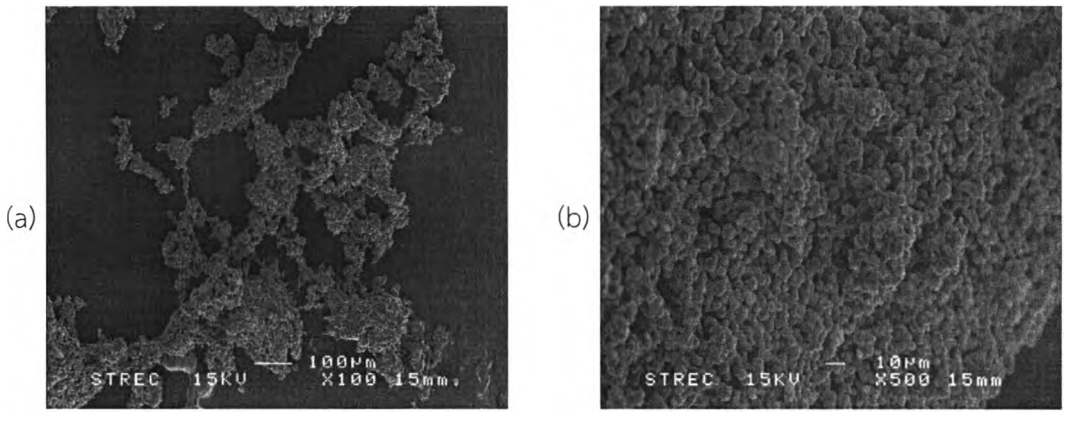



Figure 63 SEM photomicrographs of initially prepared spray dried sample at 100x (a) and 500x (b) magnifications

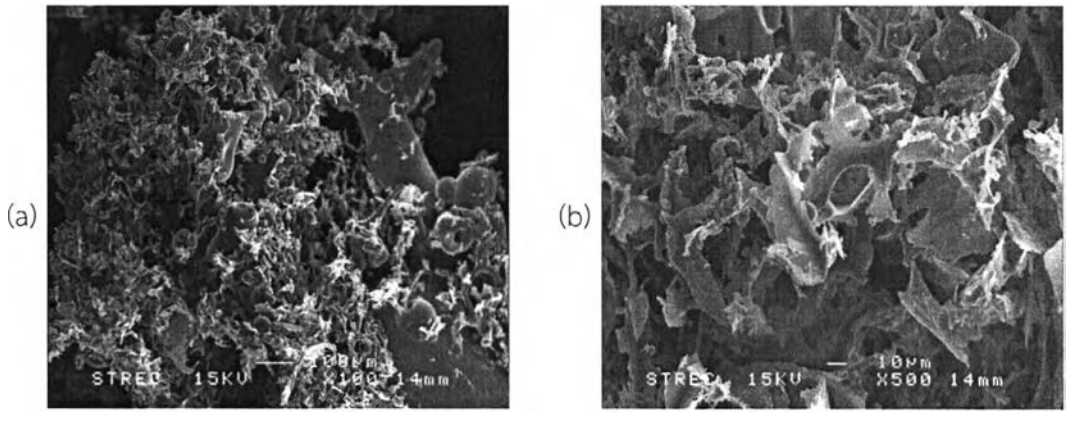



Figure 64 SEM photomicrographs of initially prepared freeze dried sample at 100x (a) and 500x (b) magnifications



### 4.2. Solubility

Solubility of clopidogrel raw material (RM) is shown to be 0.0634 g/L in water at 30°C. Solubility of initial sample prepared by spray drying method is 760 g/L in water at 30°C and solubility of initial sample prepared by freeze drying method is 877 g/L in water at 30°C. According to USP35 (Table 5), solubility of clopidogrel RM from experimental study can be classified as "practically insoluble", whereas, solubility of initial spray dried and freeze dried samples prepared can be classified as

"freely soluble". Solubility data of clopidogrel bisulfate as clopidogrel RM can be classified as "practically insoluble" in water at our neutral experimental pH but freely soluble at pH 1 (58). These different results may be due to different amorphous forms solubilized for an average of 3 days until saturated solution was reached. However, the difference in two surface areas of the two samples prepared will only increase the rate of solubility.

Table 5 Descriptive terms of approximate solubility of substances according to USP35 (13)

| Descriptive Term | Parts of Solvent Required for 1 Part of Solute |
|---|---|
| Very soluble | Less than 1 |
| Freely soluble | From 1 to 10 |
| Soluble | From 10 to 30 |
| Sparingly soluble | From 30 to 100 |
| Slightly soluble | From 100 to 1,000 |
| Very slightly soluble | From 1,000 to 10,000 |
| Practically insoluble, or Insoluble | Greater than or equal to 10,000 |



### 4.3.Related substances

Figure 65 shows HPLC chromatrogram of standards reference solution exhibiting retention time of all standard related substances. Standard clopidogrel related compound A has retention time of 3.892 min. Standard clopidogrel related compound B (First enantiomer and second enantiomer) have retention times of 8.333 and 11.467 min, respectively. Standard clopidogrel has retention time of 9.950 min and standard clopidogrel related compound C has retention time of 17.350 min. Figure 66 shows HPLC system suitability chromatrogram that exhibits the resolution between first enantiomer of related compound B and standard clopidogrel. The method verification and the system suitability are shown in Appendix D.

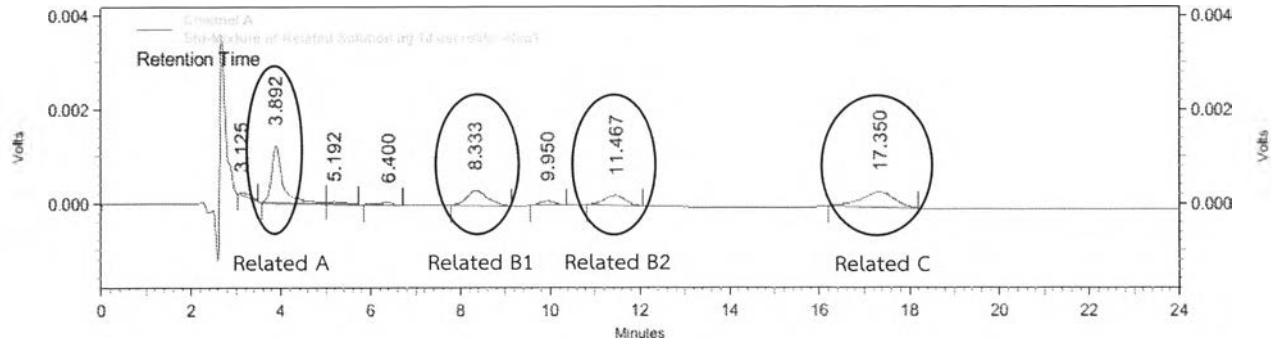



Figure 65 HPLC chromatogram of reference standards related substances solution

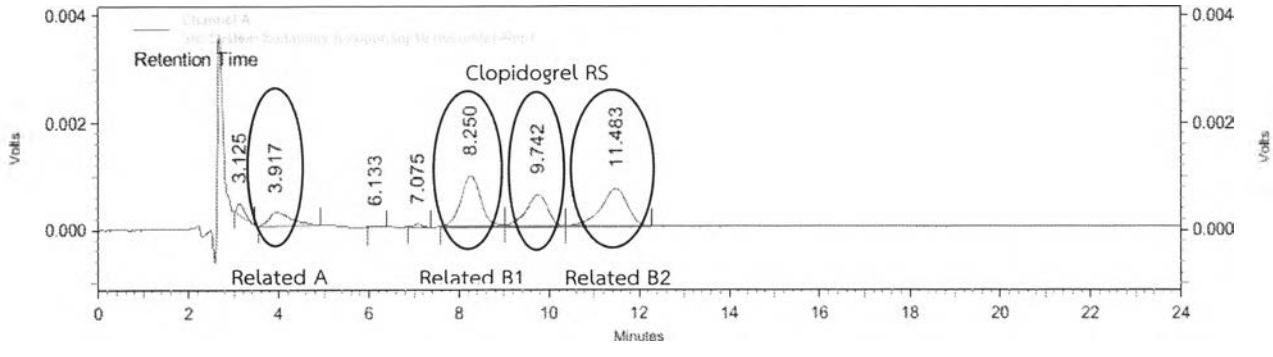



Figure 66 HPLC chromatogram of system suitability testing

Figure 67 shows HPLC chromatograms of clopidogrel RM. Figure 68 and 69 show HPLC chromatograms of initial spray dried and freeze dried sample prepared, respectively. Table 6 exhibits amount of clopidogrel related compound A and C found in clopidogrel RM and spray drying and freeze drying samples prepared. Clopidogrel related compound A and C are found in clopidogrel RM with values of 0.112% (or 0.264 area%) and 1.331% (or 1.133 area%), respectively. Furthermore, clopidogrel related compound A and C are found in initial spray dried sample with values of 0.207% (or 0.503 area%) and 1.925% (or 1.697 area%), respectively. Moreover, clopidogrel related compound A and C are found in initial freeze dried sample with values of 0.237% (or 0.534 area%) and 2.086% (or 1.700 area%), respectively. These results indicate that clopidogrel related compound A and C of both prepared samples are a slightly higher than the required specification, according

to clopidogrel bisulfate monograph in USP35 (Specification of clopidogrel related compound A and C values not more than 0.2% and 1.0%, respectively). However, it should not affect the solid-state morphology and stability of the resulting polyamorphous solids.

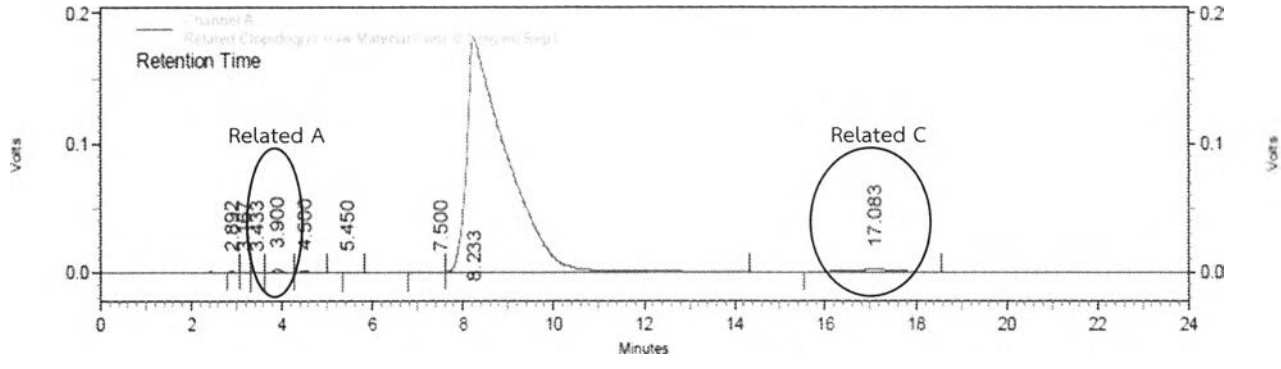



Figure 67 HPLC chromatogram of clopidogrel raw material (RM)

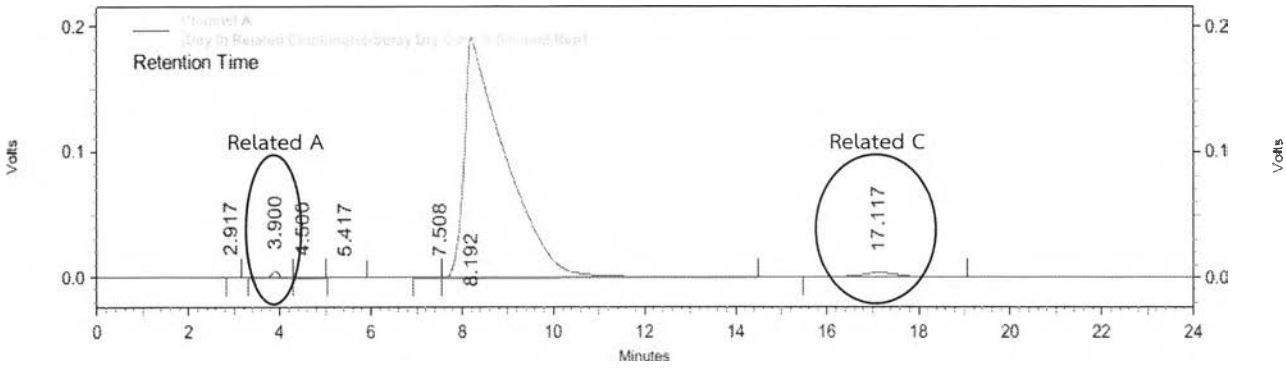



Figure 68 HPLC chromatogram of initially prepared spray dried sample

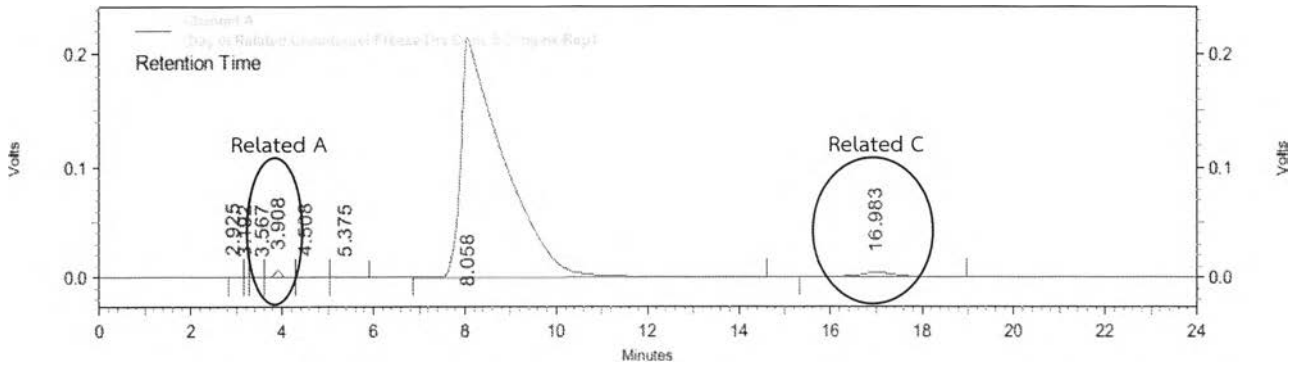



Figure 69 HPLC chromatogram of initially prepared freeze dried sample

Table 6 Clopidogrel related compound A and C are found in initially spray dried and freeze dried samples

| | Clopidogrel raw material (RM) | | | |
|---|---|---|---|---|
| | Related A (%) | Area% | Related C (%) | Area% |
| Day 0 | 0.112 | 0.264 | 1.331 | 1.133 |
| | Spray dried sample | | | |
| | Related A (%) | Area% | Related C (%) | Area% |
| Day 0 | 0.207 | 0.503 | 1.925 | 1.697 |
| | Freeze dried sample | | | |
| | Related A (%) | Area% | Related C (%) | Area% |
| Day 0 | 0.237 | 0.534 | 2.086 | 1.700 |

(Specification USP35 : Clopidogrel related compound A : not more than 0.2%)

(Specification USP35 : Clopidogrel related compound C : not more than 1.0%)

### 4.4. Dynamic Vapour Sorption

Results obtained are in accordance with the topic 2.6 indicating the sorption/desorption isotherms of clopidogrel raw material (RM), initial spray dried and initial freeze dried sample. Effect of humidity on recrystallization of both prepared samples was evaluated by DVS method according to section 4.4. Results from sorption/desorption isotherms indicate that both samples readily increased moisture uptake from 60%RH onwards reaching approximately 30%w/w of moisture when the equilibrium relative humidity exposed became 90%RH and remained constant at approximately 5%w/w of moisture when the relative humidity was reduced to 0%RH. The uptake of moisture for both samples from higher than 60%RH caused an

increase in mobility of the molecules and rearrangement of molecule occurred leading to another stable solid state form which retained approximately 5%w/w moisture in the structures.

## 5. Effect of temperature and humidity on recrystallization of polyamorphous samples

### 5.1. Effect of temperature

Effect of temperatures on recrystallization of both prepared samples was studied by isothermal DSC method using constant temperatures exposure of 40°C, 60°C and 80°C for 24 hours (1,440 min). Figures 70a, 70b and 70c show DSC thermograms of spray dried samples placed at constant 40°C, 60°C and 80°C, respectively. Figures 71a, 71b and 71c show DSC thermograms of freeze dried samples placed at constant 40°C, 60°C and 80°C, respectively. Results from isothermal DSC of both samples indicate that the 3 temperatures selected do not induce any recrystallization of both polyamorphous samples prepared within 24 hours exposure.

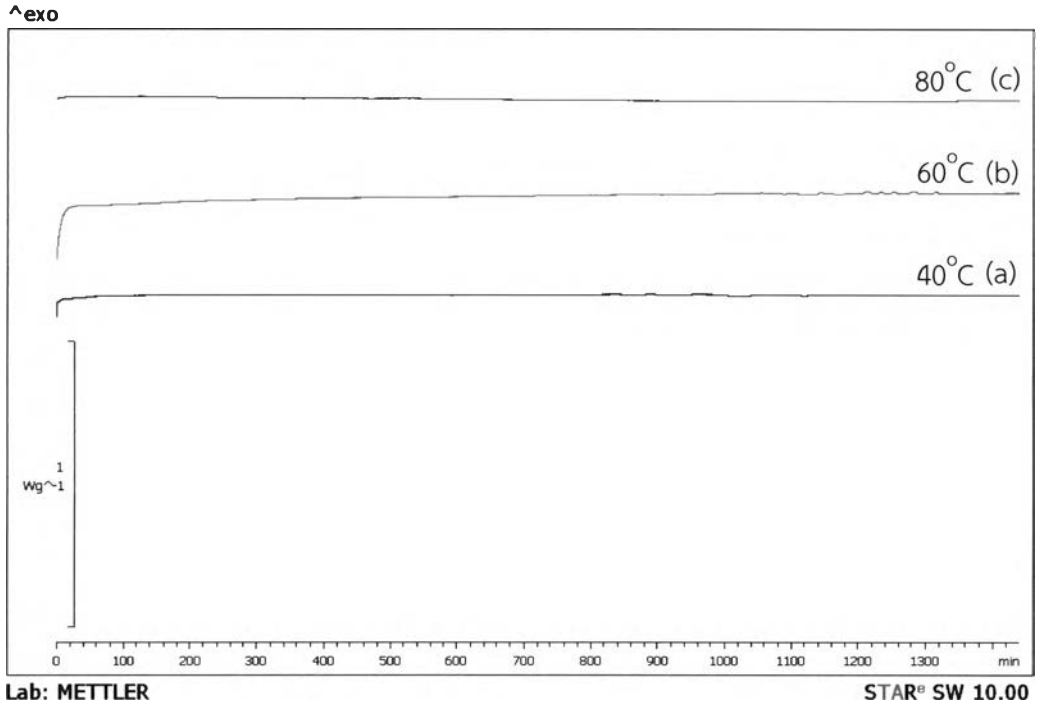



Figure 70 Isothermal DSC thermograms of spray dried samples exposed to 40°C (a), 60°C (b) and 80°C (c) for 24 hours

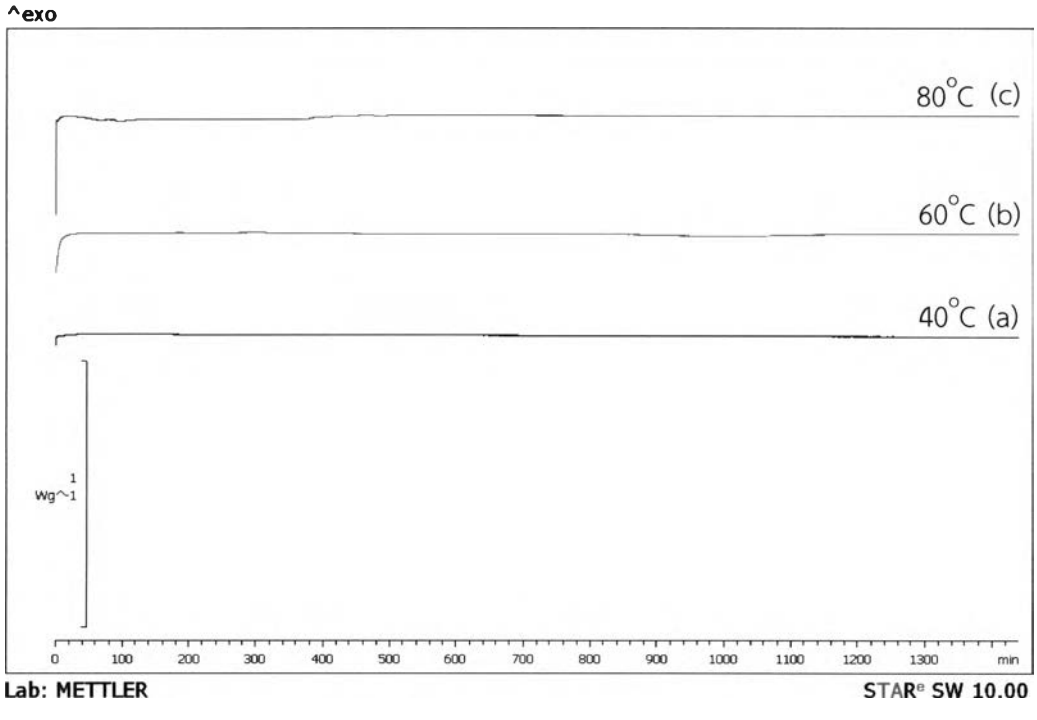



Figure 71 Isothermal DSC thermograms of freeze dried samples exposed to 40°C (a), 60°C (b) and 80°C (c) for 24 hours

### 5.2. Effect of humidity

Effect of humidity on recrystallization of both prepared samples was evaluated by DVS method according to section 4.4. Results from sorption/desorption isotherms indicate that both samples readily increased moisture uptake from 60%RH onwards reaching approximately 30%w/w of moisture when the equilibrium relative humidity exposed became 90%RH and remained constant at approximately 5%w/w of moisture when the relative humidity was reduced to 0%RH. The uptake of moisture for both samples from higher than 60%RH caused an increase in mobility of the molecules and rearrangement of molecule occurred leading to another stable solid state form which retained approximately 5%w/w moisture in the structures.



## 6. Stability evaluation

Stability study of amorphous samples prepared and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 0, 1, 2, 3, 5, 7, 10, 20, 30, 60 and 90 days were evaluated for their changes in solid-state properties and physicochemical characteristics using polarized light microscopy, DSC, TGA, PXRD, SEM, HPLC, Raman spectroscopy and PCA.

### 6.1. Physical appearance

Amorphous sample prepared by spray drying method and stored at 30°C 30%RH for 7 days ($SP^{7}_{30/30}$) and 40°C 30%RH for 7 days ($SP^{7}_{40/30}$) are white, fine granules shown in Figures 72a and 72b, respectively. Spray dried sample stored at 40°C 75%RH for 7 days ($SP^{7}_{40/75}$) resulted in slightly yellowish, irregular crystal shown in Figure 72c.

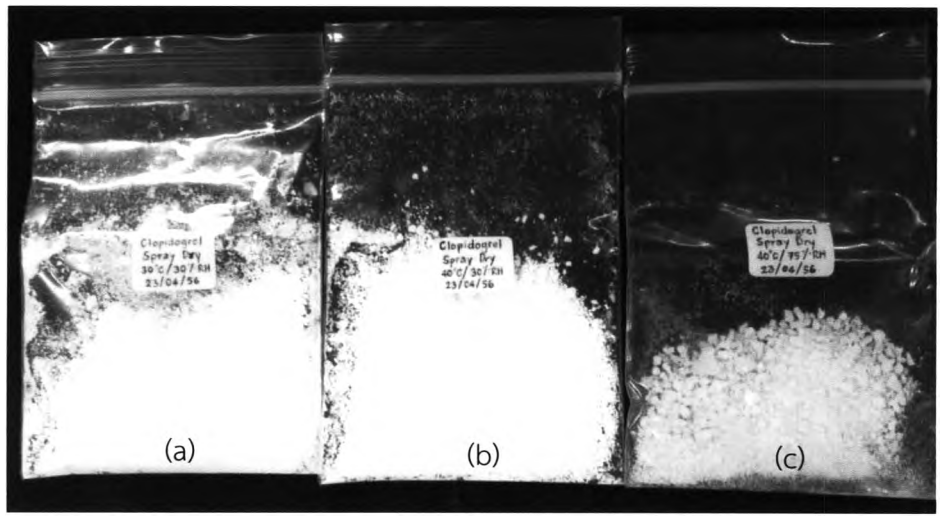



Figure 72 Polyamorphous samples obtained by spray drying method and stored for 7 days at 30°C 30%RH (a) 40°C 30%RH (b) and 40°C 75%RH (c)



Amorphous sample prepared by freeze drying method and stored at 30°C 30%RH for 7 days ($FZ^{7}_{30/30}$) and 40°C 30%RH for 7 days ($FZ^{7}_{40/30}$) are white, fine bulky flakes shown in Figures 73a and 73b, respectively. But freeze dried sample stored at 40°C 75%RH for 7 days ($FZ^{7}_{40/75}$) transforms to slightly yellowish, irregular crystal shown in Figure 73c similar to $SP^{7}_{40/75}$ sample.


Figure 73 Polyamorphous samples obtained by freeze drying method and stored for 7 days at 30°C 30%RH (a) 40°C 30%RH (b) and 40°C 75%RH (c)

### 6.2. Polarized light microscopy

Figure 74a and 74b do not show crystalline birefringence of spray dried samples prepared and after storage at 30°C 30%RH for 90 days ($SP^{90}_{30/30}$) and 40°C 30%RH for 90 days ($SP^{90}_{40/30}$), respectively. These stability results seen under microscope are similar to the result obtained from freshly prepared spray dried sample (Figure 50a). Figure 74c shows birefringence of spray dried sample which is stored at 40°C 75%RH for 90 days ($SP^{90}_{40/75}$). The result corresponds to the birefringence phenomenon of clopidogrel raw material (RM) seen under polarized light microscope shown in Figure 50c. These results indicate that $SP^{90}_{30/30}$ and $SP^{90}_{40/30}$ samples are amorphous form. In contrast, clopidogrel RM and $SP^{90}_{40/75}$ sample are crystalline structure.



Figure 75a and 75b do not exhibit birefringence phenomena of freeze dried samples prepared and after storage at 30°C 30%RH for 90 days ($FZ^{90}_{30/30}$) and 40°C 30%RH for 90 days ($FZ^{90}_{40/30}$), respectively. These stability results correlated well with the microscopic result obtained from freshly prepared freeze dried sample (Figure 50b). Figure 75c shows birefringence in sample obtained by freeze drying and stored at 40°C 75%RH for 90 days ($FZ^{90}_{40/75}$). The result is parallel to the birefringence phenomenon of crystalline clopidogrel RM seen under polarized light microscope shown in Figure 50c. These results present that $FZ^{90}_{30/30}$ and $FZ^{90}_{40/30}$ samples are amorphous form. On the contrary, clopidogrel RM and $FZ^{90}_{40/75}$ sample are crystalline structure.

All above results from polarized light microscope of spray dried samples stored at 3 different conditions for 90 days ($SP^{90}_{30/30}$, $SP^{90}_{40/30}$ and $SP^{90}_{40/75}$) show the same solid-state transformation trends as freeze dried sample stored under similar

conditions ($FZ^{90}_{30/30}$, $FZ^{90}_{40/30}$ and $FZ^{90}_{40/75}$). Moreover, it can be concluded from the above results that humidity plays greater role than temperature on the conversion of amorphous sample.

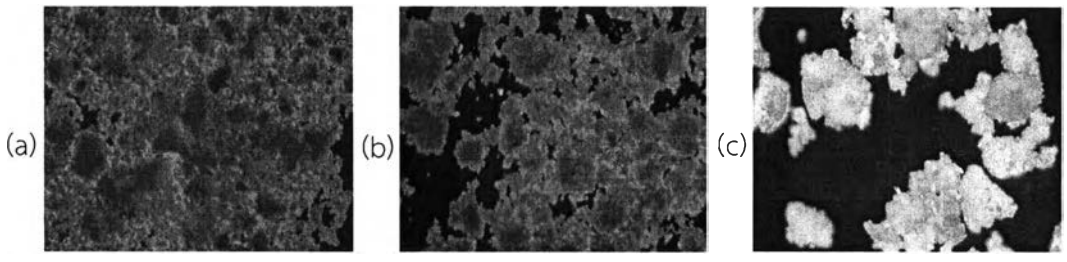



Figure 74 Polarized photomicrograph of spray dried samples stored for 90 days at 30°C 30%RH (a) at 40°C 30%RH (b) at 40°C 75%RH (c)



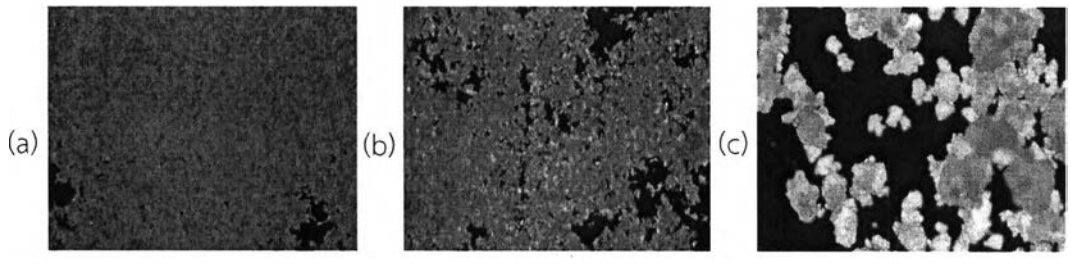



Figure 75 Polarized photomicrograph of freeze dried samples stored for 90 days at 30°C 30%RH (a) at 40°C 30%RH (b) at 40°C 75%RH (c)

### 6.3. Powder X-ray diffractometry (PXRD)

Figure 76 displays powder X-ray (PXRD) diffractograms of spray dried and freeze dried samples stored at 30°C 30%RH and 40°C 30%RH for 7 days ($SP^{7}_{30/30}$, $SP^{7}_{40/30}$, $FZ^{7}_{30/30}$ and $FZ^{7}_{40/30}$) compare to clopidogrel raw material (RM). The PXRD diffractograms of $SP^{7}_{30/30}$, $SP^{7}_{40/30}$, $FZ^{7}_{30/30}$ and $FZ^{7}_{40/30}$ samples exhibit amorphous halo pattern similar to initial spray dried and freeze dried samples shown in Figures 51a ($SP^{0}$) and 51b ($FZ^{0}$), respectively. However, PXRD diffractogram of clopidogrel RM demonstrates characteristic crystalline peaks in accordance to topic 2.3. These results indicate that clopidogrel RM is crystalline form. On the other hand, both

samples prepared and stored at 2 different conditions for 7 days remained amorphous form.

Figure 77 shows PXRD diffractograms of spray dried samples stored at 40°C 75%RH for 0, 1, 2, 3, 5 and 7 days ($SP^0$, $SP^1_{40/75}$, $SP^2_{40/75}$, $SP^3_{40/75}$, $SP^5_{40/75}$ and $SP^7_{40/75}$) compare to clopidogrel RM. From bottom to top, PXRD diffractograms of $SP^1_{40/75}$, $SP^2_{40/75}$, $SP^3_{40/75}$, $SP^5_{40/75}$ samples remained amorphous and show halo patterns corresponding to PXRD pattern of $SP^0$ sample. However, instantaneous transformation occurred on day 7 where PXRD pattern became similar to crystalline clopidogrel RM. Moreover, this transformation was not observed in $SP^7_{30/30}$ and $SP^7_{40/30}$ samples (Figure 76).

Figure 78 presents PXRD diffractograms of freeze dried samples stored at 40°C 75%RH for 0, 1, 2, 3, 5 and 7 days ($FZ^0$, $FZ^1_{40/75}$, $FZ^2_{40/75}$, $FZ^3_{40/75}$, $FZ^5_{40/75}$ and $FZ^7_{40/75}$) compare to clopidogrel RM. From bottom to top, PXRD diffractograms of $FZ^1_{40/75}$, $FZ^2_{40/75}$, $FZ^3_{40/75}$ and $FZ^5_{40/75}$ samples remained amorphous and show halo patterns corresponding to PXRD pattern of $FZ^0$ sample. However, instantaneous change occurred on day 7 where PXRD became similar to crystalline clopidogrel RM. Furthermore, this transformation was not observed in $FZ^7_{30/30}$ and $FZ^7_{40/30}$ samples (Figure 76). It can be concluded from above results that humidity plays greater role than temperature on the conversion of these polyamorphous samples similar to results obtained from polarized light microscopy in topic 6.2.

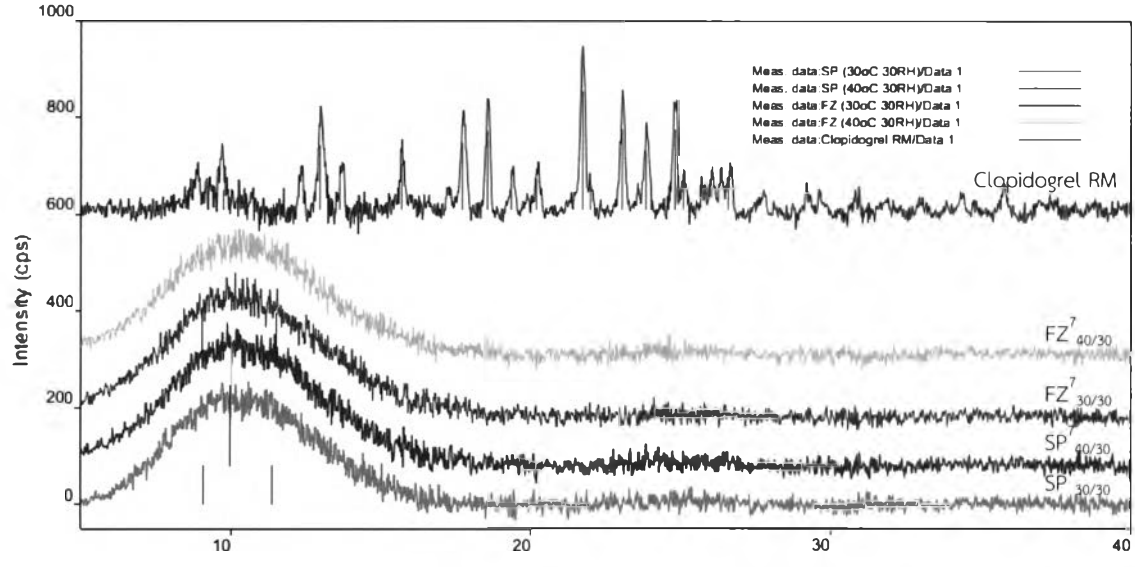



Figure 76 PXRD diffractograms of spray dried (SP) and freeze dried (FZ) samples stored at 30°C 30%RH and 40°C 30%RH for 7 days compare to crystalline clopidogrel RM

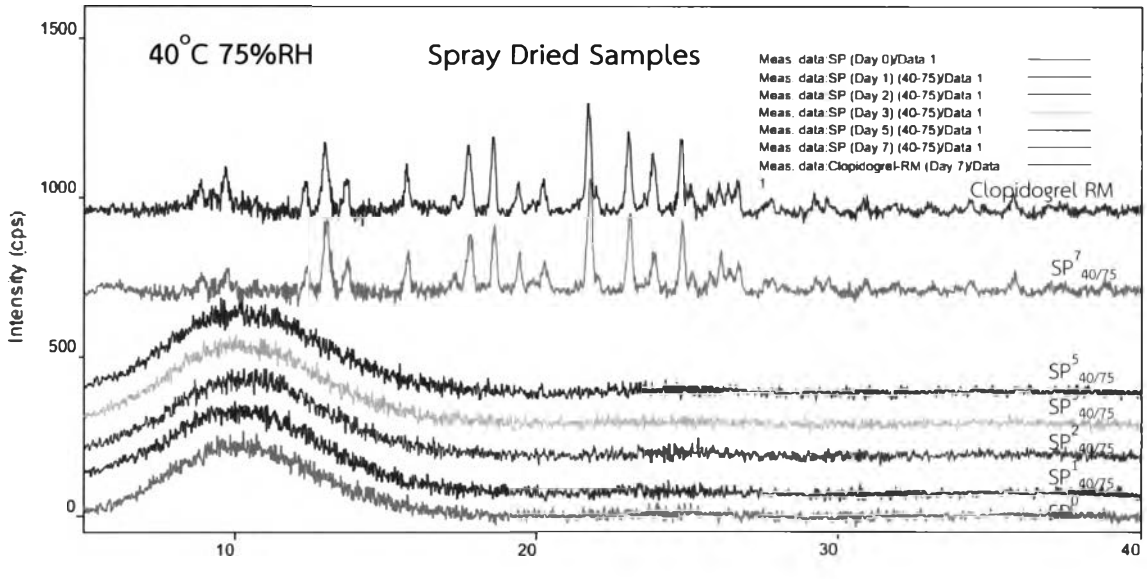



Figure 77 PXRD diffractograms of spray dried samples stored at 40°C 75%RH for 0, 1, 2, 3, 5 and 7 days compare to crystalline clopidogrel RM

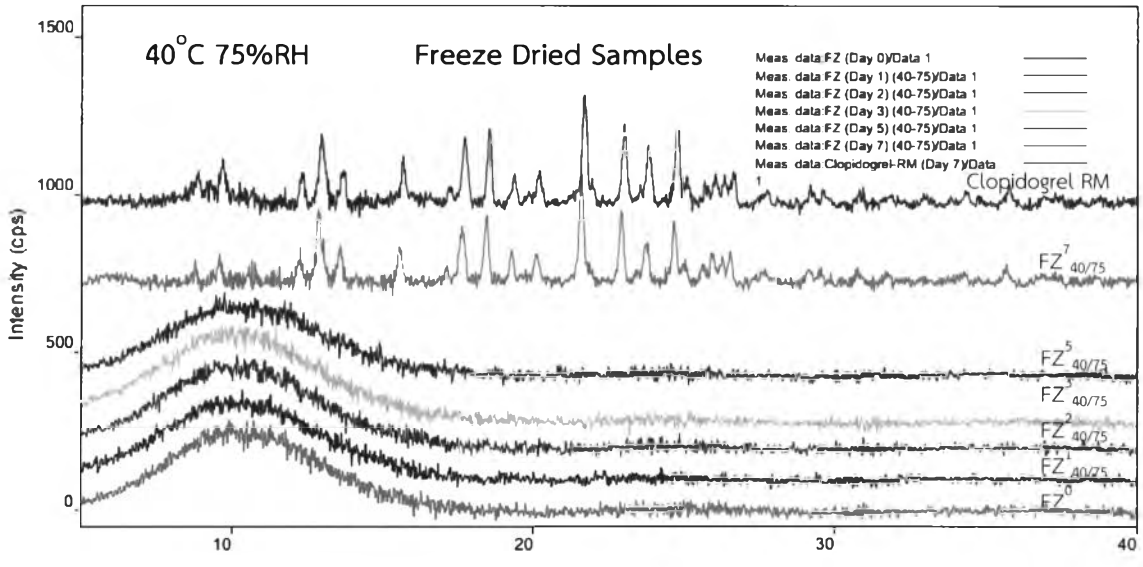



Figure 78 PXRD diffractograms of freeze dried samples stored at 40°C 75%RH for 0, 1, 2, 3, 5 and 7 days compare to crystalline clopidogrel RM



### 6.4. Differential scanning calorimetry (DSC)

Figure 79 shows differential scanning calorimetry (DSC) thermograms of spray dried samples stored at 3 various conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for duration of 7 days ($SP^{7}_{30/30}$, $SP^{7}_{40/30}$ and $SP^{7}_{40/75}$) compare to clopidogrel RM. DSC thermogram of crystalline clopidogrel RM exhibit melting endotherm at 183°C and degradation endotherm at 220°C. Furthermore, DSC thermogram of $SP^{7}_{40/75}$ sample shows melting endotherm at 180°C and degradation endotherm at 220°C corresponding to DSC thermogram of clopidogrel RM which is crystalline form. This transformation was not observed in spray dried samples stored at 30°C 30%RH ($SP^{\leq 7}_{30/30}$) and 40°C 30%RH ($SP^{\leq 7}_{40/30}$) upto 7 days and remained in amorphous form.

Figure 80 shows DSC thermograms of freeze dried samples stored under 3 various conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) on day 7 ($FZ^{7}_{30/30}$, $FZ^{7}_{40/30}$ and $FZ^{7}_{40/75}$) compare to clopidogrel RM. DSC thermogram of crystalline

clopidogrel RM exhibit melting endotherm at 183°C and degradation endotherm at 220°C. Moreover, DSC thermogram of $FZ^{7}_{40/75}$ sample observes melting endotherm 180°C and degradation endotherm at 220°C similar to DSC thermogram of clopidogrel RM and $SP^{7}_{40/75}$ which is crystalline form. This transformation was not observed in freeze dried samples stored at 30°C 30%RH ($FZ^{\leq 7}_{30/30}$) and 40°C 30%RH ($FZ^{\leq 7}_{40/30}$) upto 7 days and remained in amorphous form.

All above DSC results of spray dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) are similar to the results obtained from freeze dried sample stored at the same conditions and in good agreement with the results obtained from PXRD.



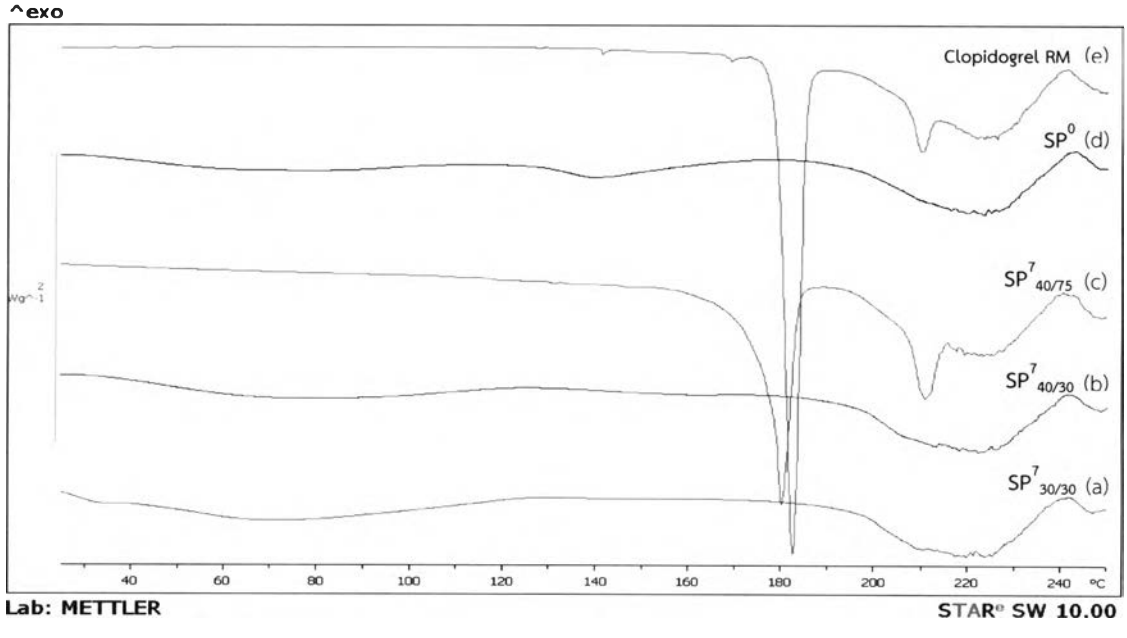



Figure 79 DSC thermograms of spray dried samples initially prepared (Day 0) and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH, 40°C 75%RH) on day 7 compare to clopidogrel RM

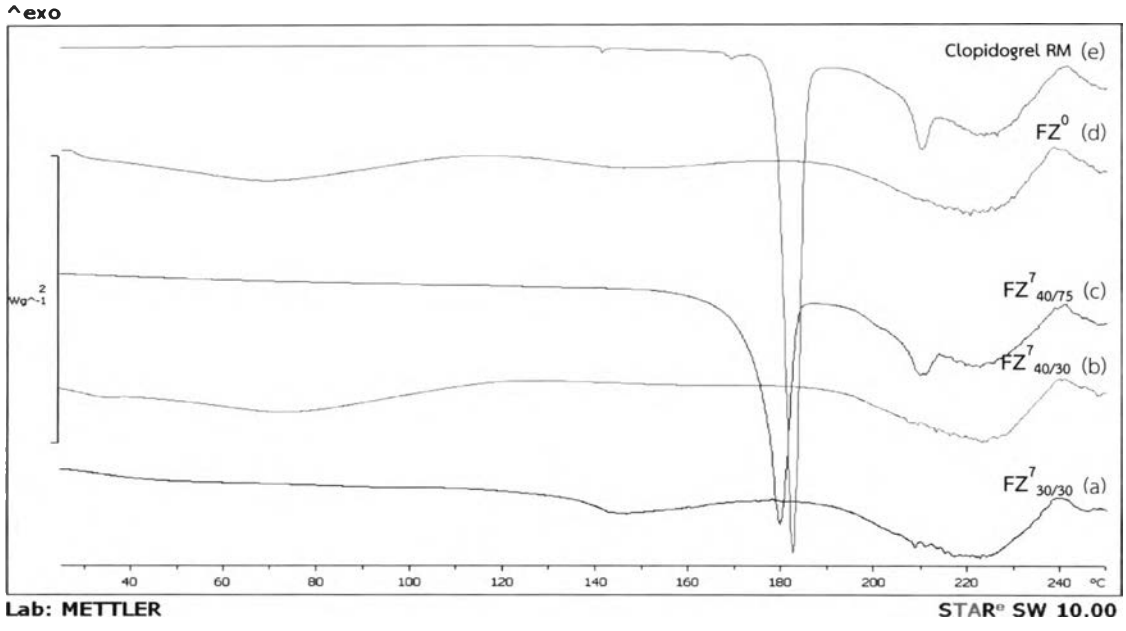



Figure 80 DSC thermograms of freeze dried samples initially prepared (Day 0) and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH, 40°C 75%RH) on day 7 compare to clopidogrel RM



### 6.5. Thermogravimetric analysis (TGA)

Figure 81 shows thermogravimetric analysis (TGA) thermograms of spray dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 7 days ($SP^{7}_{30/30}$, $SP^{7}_{40/30}$ and $SP^{7}_{40/75}$) compare to clopidogrel raw material (RM). TGA thermogram of clopidogrel RM exhibits horizontal linearity until degraded at 200°C similar to $SP^{7}_{40/75}$ sample. TGA thermogram of $SP^{7}_{40/75}$ sample presents weight loss of 0.945%w/w at 50°C. However, TGA thermograms of spray dried samples stored at 30°C 30%RH ($SP^{\leq 7}_{30/30}$) and 40°C 30%RH ($SP^{\leq 7}_{40/30}$) upto day 7 present weight loss of 1.713%w/w and 1.783%w/w at 50°C, respectively and present degradation endotherm at 200°C similar to TGA thermogram of $SP^{0}$ sample.

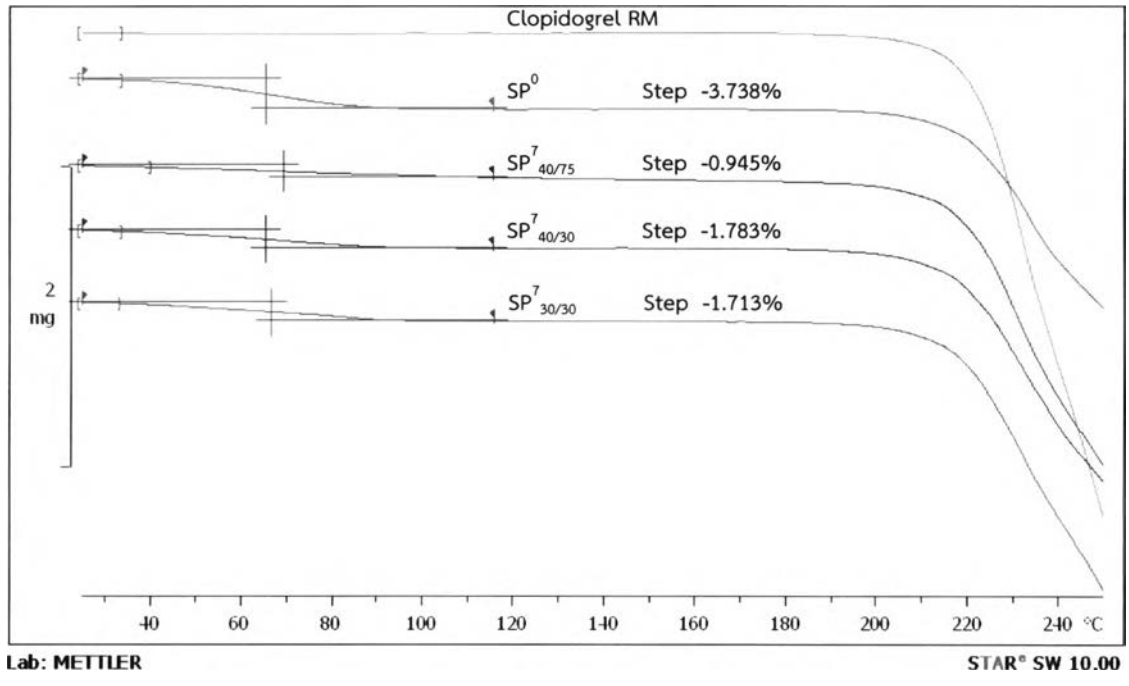





Figure 81 TGA thermograms of spray dried samples initially prepared and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH, 40°C 75%RH) for 7 days compare to clopidogrel RM

Figure 82 exhibits TGA thermograms of freeze dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) on day 7 ($FZ^{7}_{30/30}$, $FZ^{7}_{40/30}$ and $FZ^{7}_{40/75}$) compare to clopidogrel RM. TGA thermogram of clopidogrel RM shows horizontal linearity until degraded at 200°C. TGA thermogram of $FZ^{7}_{40/75}$ sample shows only degradation at 200°C and shows weight loss of 0.563%w/w at 60°C. However, TGA thermograms of freeze dried samples stored at 30°C 30%RH and 40°C 30%RH from initial until day 7 ($FZ^{\leq 7}_{30/30}$ and $FZ^{\leq 7}_{40/30}$) present weight loss of 1.092%w/w and 1.843%w/w at 60°C, respectively and present degradation endotherm at 200°C similar to TGA thermogram of $FZ^{0}$ sample.

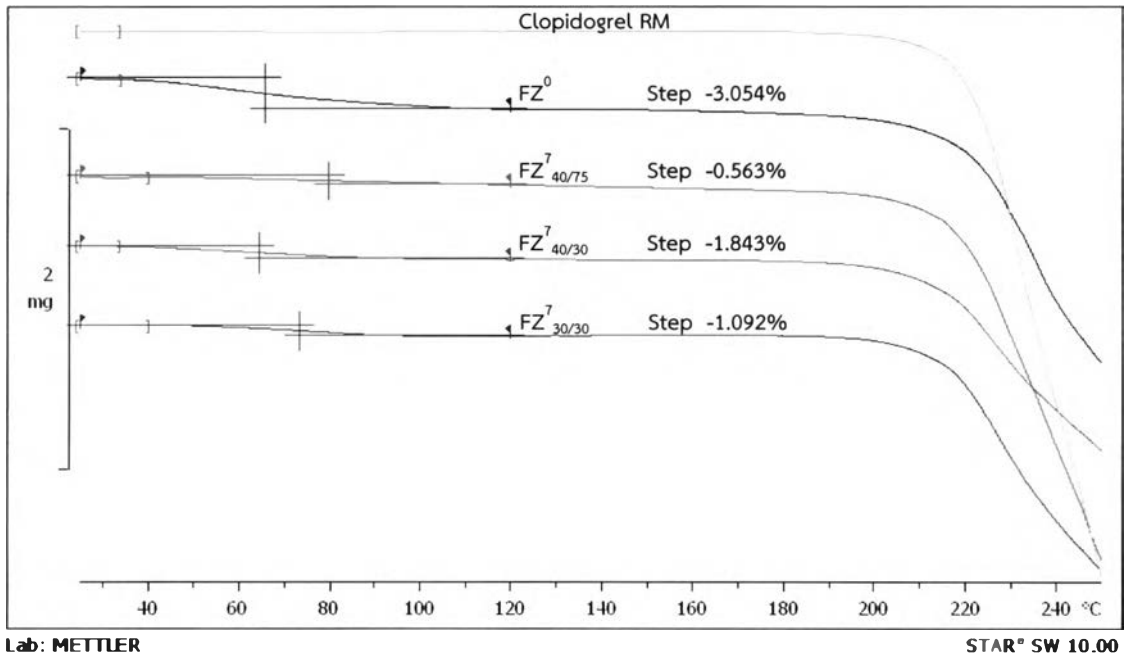





Figure 82 TGA thermograms of freeze dried samples initially prepared and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH, 40°C 75%RH) for 7 days compare to clopidogrel RM

All results indicate that at higher humidity condition (40°C 75%RH) the amorphous form of spray dried and freeze dried samples absorb moisture and molecular rearrangements to crystalline form occur with least observable water lost in TGA thermogram after storage for 7 days.

### 6.6. Confocal microscopic Raman spectroscopy (Raman)

Figure 83 shows Raman spectra of spray dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) on day 7 ($SP^7_{30/30}$, $SP^7_{40/30}$ and $SP^7_{40/75}$) compare to clopidogrel RM. Raman shifts between 3200 to 2900 $cm^{-1}$ and 1100 to 1000 $cm^{-1}$ are chosen to represent clear distinction between forms. All Raman spectral data are collected in 10 replicates and analyzed by OMNIC® (Version

8.0) software within the range of 3200 to 2800 $cm^{-1}$ and 1800 to 100 $cm^{-1}$ for discrimination of all data by Principal Component Analysis (PCA) using UNSCRAMBLER® (Version 9.8) software and Multibase (2014) program add-in for Microsoft excel. Results from Raman spectra in Figure 77 indicate that $SP^{\leq 7}_{30/30}$ and $SP^{\leq 7}_{40/30}$ samples show characteristic amorphous peaks similar to Raman spectrum of $SP^{0}$ sample. While Raman spectrum of $SP^{7}_{40/75}$, shows characteristic crystalline peaks similar to Raman spectrum of clopidogrel RM.

Figure 84 present Raman spectra of freeze dried samples stored at 3 various conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) on day 7 ($FZ^{7}_{30/30}$, $FZ^{7}_{40/30}$ and $FZ^{7}_{40/75}$) compare to clopidogrel RM. Raman spectral results of $FZ^{7}_{30/30}$ and $FZ^{7}_{40/30}$ samples present characteristic amorphous peaks similar to Raman spectra of $FZ^{0}$ sample. While Raman spectrum of $FZ^{7}_{40/75}$ sample shows characteristic crystalline peaks similar to clopidogrel RM.

All results obtained by Raman spectroscopy analysis of spray dried samples are similar to freeze dried samples and correlate well with the results obtained from PXRD, DSC and TGA. All above results observed from Raman spectroscopy, PXRD, DSC and TGA show clear distinctions between amorphous form and crystalline form. However, these conventional methods cannot clearly distinguish between the two amorphous forms obtained by spray drying method and freeze drying method.

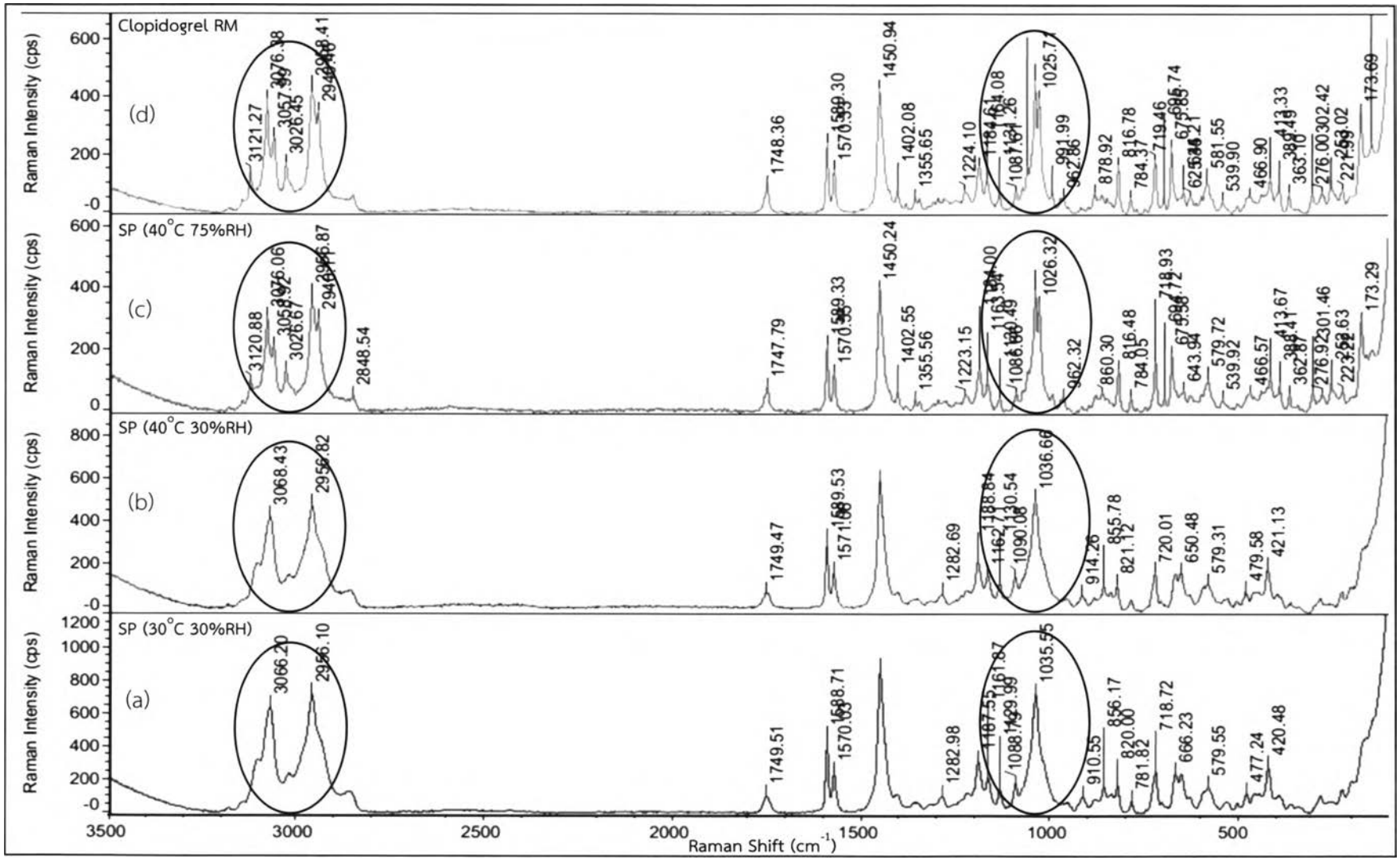



Figure 83 Raman spectra of spray dried samples stored on day 7 at 30°C 30%RH (a) 40°C 30%RH (b) and 40°C 75%RH (c) compare to clopidogrel RM (d)
(Circles show areas where there are distinctions between patterns)

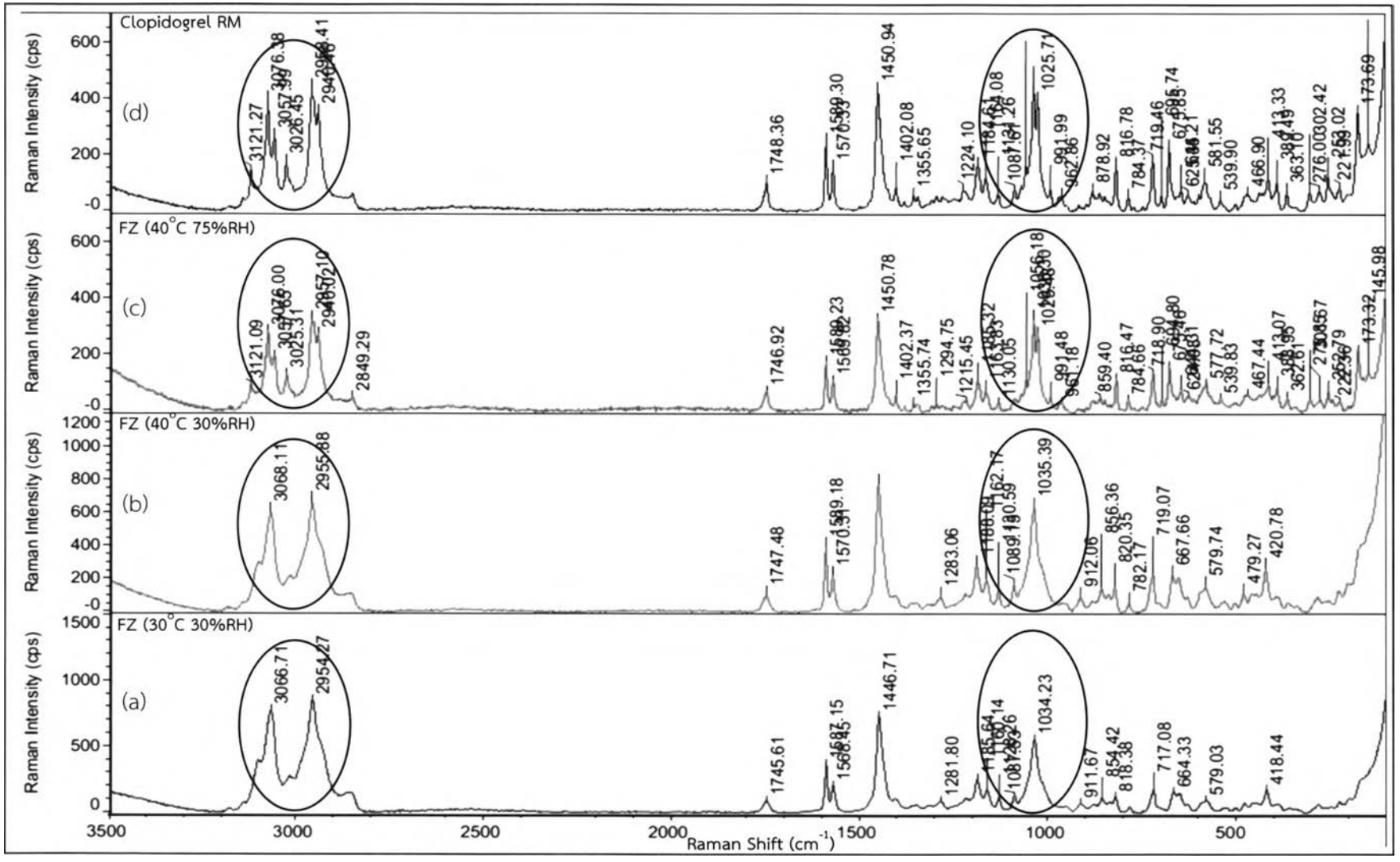



Figure 84 Raman spectra of freeze dried samples stored on day 7 at 30°C 30%RH (a) 40°C 30%RH (b) and 40°C 75%RH (c) compare to clopidogrel RM (d)
(Circles show areas where there are distinctions between patterns)

### 6.7. Principal Component Analysis (PCA)

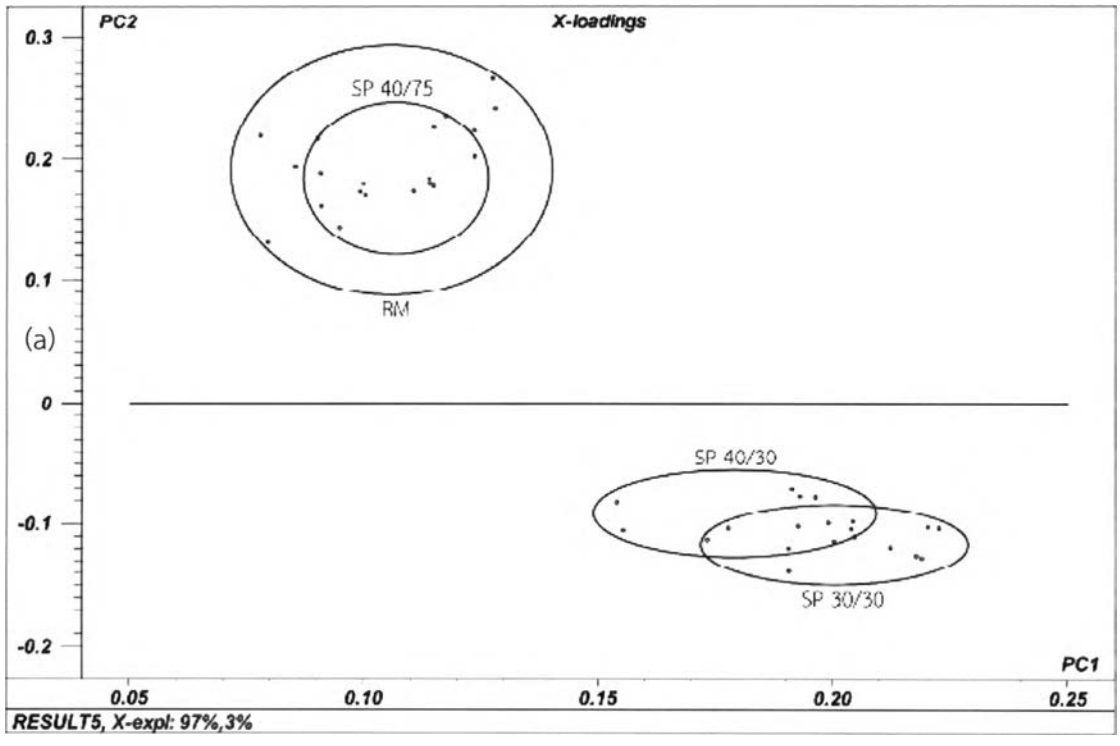
Raman spectral data from spray dried samples prepared and clopidogrel RM can be differentiated by PCA as 2 distinctive groups. Figures 85a and 85b indicate Principal Component Analysis (PCA) of spray dried samples prepared and stored at 3 various conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 7 days ($SP^{7}_{30/30}$, $SP^{7}_{40/30}$ and $SP^{7}_{40/75}$) compare to clopidogrel RM. The first principal component (PC1) accounted for 97.7% of overall variance and the second principal components (PC2) accounted for 2.0% of overall variance. DBI value between $SP^{7}_{30/30}$ and $SP^{7}_{40/30}$ sample is 1.3665. One group composes of Raman spectral data from $SP^{7}_{30/30}$ and $SP^{7}_{40/30}$ samples. Another group composes of Raman spectral data from $SP^{7}_{40/75}$ samples and clopidogrel RM. These results presented that $SP^{7}_{40/75}$ sample belongs to crystalline morphology similar to clopidogrel RM. Whereas, $SP^{7}_{30/30}$ and $SP^{7}_{40/30}$ sample is classified within amorphous group similar to $SP^{0}$.

Raman spectral data from freeze dried samples prepared and clopidogrel RM can be classified by PCA as 2 distinctive groups. Figure 86a and 86b show PCA of freeze dried samples prepared and stored at 3 various conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 7 days ($FZ^{7}_{30/30}$, $FZ^{7}_{40/30}$ and $FZ^{7}_{40/75}$) compare to clopidogrel RM. The first principal component (PC1) accounted for 98.2% of overall variance and the second principal components (PC2) accounted for 1.3% of overall variance. DBI value between $FZ^{7}_{30/30}$ and $FZ^{7}_{40/30}$ sample is 0.6593. One group composes of Raman spectral data from $FZ^{7}_{30/30}$ and $FZ^{7}_{40/30}$ samples are amorphous. Another group composes of Raman spectral data arising from $FZ^{7}_{40/75}$ samples and clopidogrel RM. These results present that $FZ^{7}_{40/75}$ sample belongs within crystalline

clopidogrel form II structure group related to clopidogrel RM. In contrast, $FZ^{7}_{30/30}$ sample have be classified by PCA within amorphous group similar to $FZ^{7}_{40/30}$ sample.

All above PCA results from freeze dried samples are associated with PCA results from spray dried samples and confirm that humidity plays greater role in the conversion of amorphous samples than temperature.

Figures 87a and 87b show PCA of first batch spray dried and freeze dried samples stored at 30°C 30%RH for 7 days ($SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$) compare to clopidogrel RM. Raman spectral data from $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples and clopidogrel RM can be classified by PCA as 3 distinctive groups similar to PCA of $SP^{0}$ and $FZ^{0}$ samples and clopidogrel RM (Figure 61). The first principal component (PC1) accounted for 98.6% of overall variance and the second principal components (PC2) accounted for 0.9% of overall variance. DBI value between $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ sample is 0.5302. These results present that clopidogrel RM can be classified by PCA as crystalline. On the contrary, $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples can be classified by PCA to be within amorphous group and can be clearly distinguished as 2 distinctive amorphous groups.



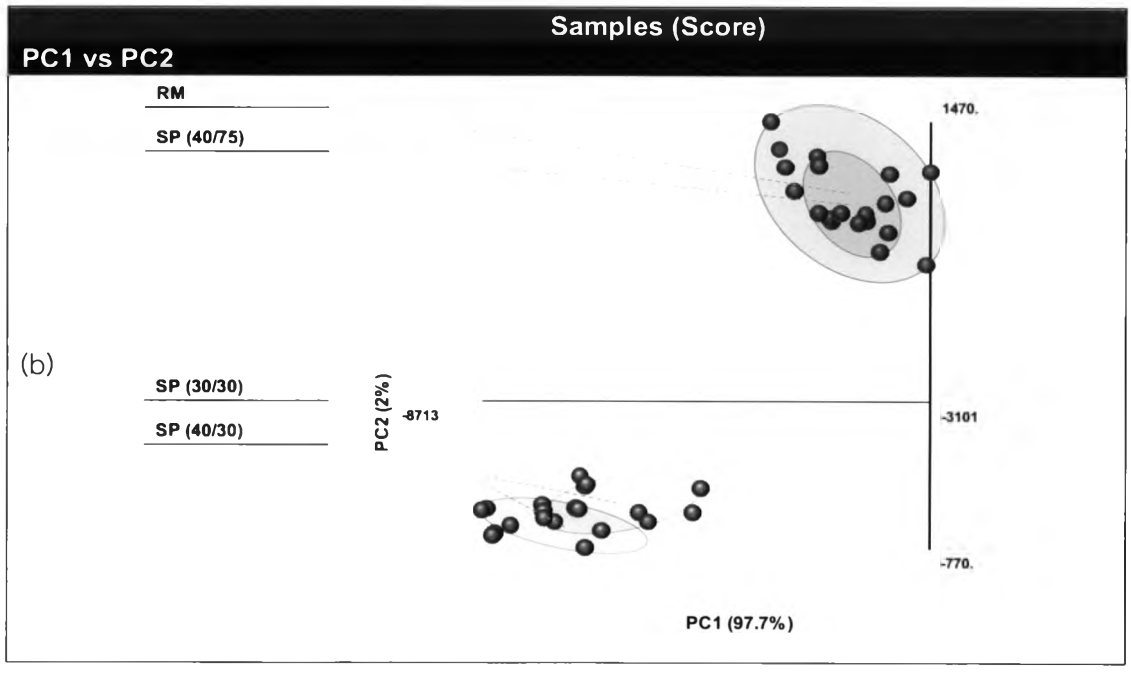
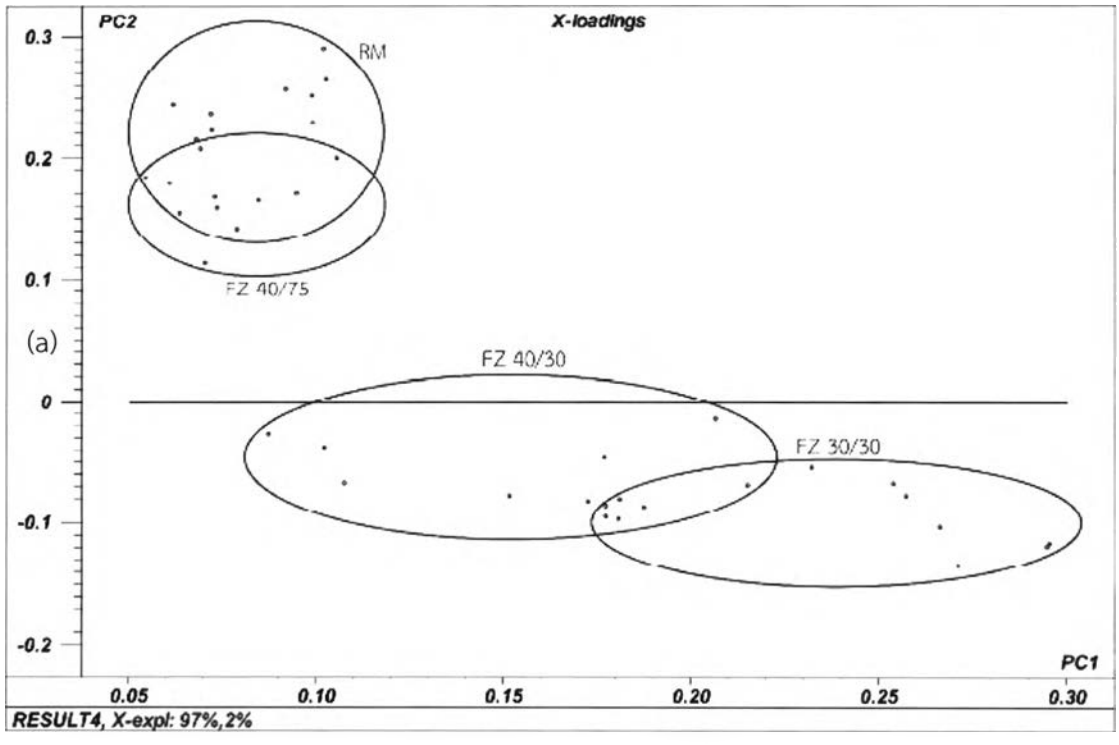




Figure 85 PCA of spray dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 7 days compare to clopidogrel RM obtained by Unscrambler® (a) and Multibase (b) program



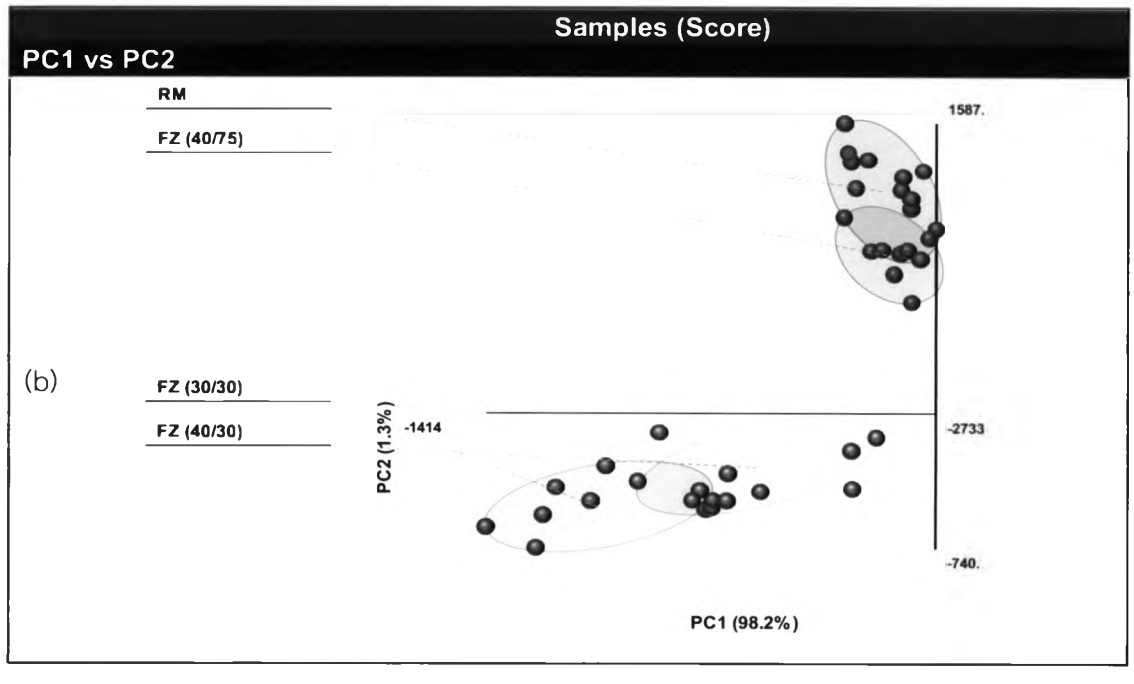
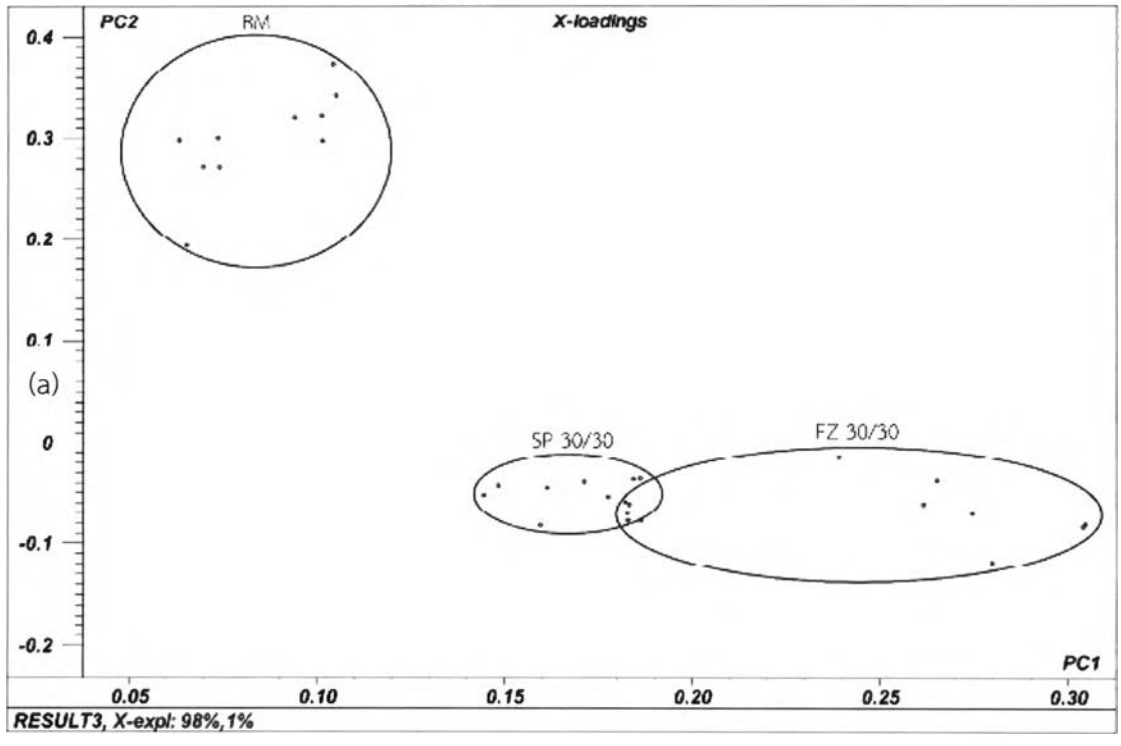




Figure 86 PCA of freeze dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 7 days compare to clopidogrel RM obtained by Unscrambler® (a) and Multibase (b) program



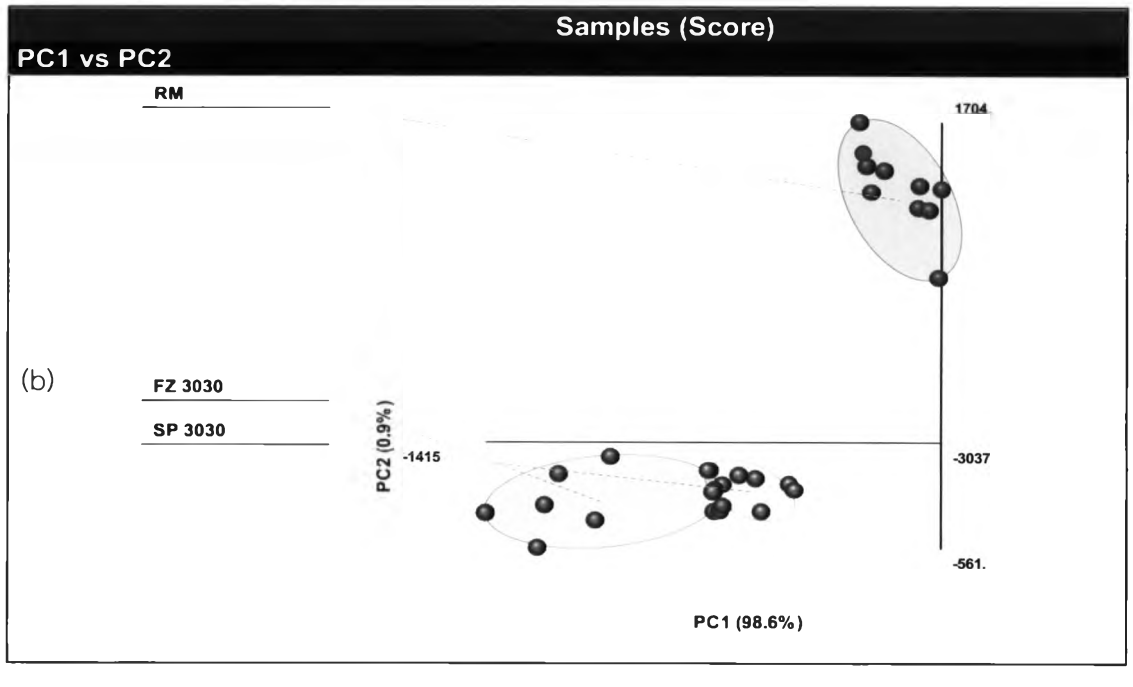




Figure 87 PCA of spray dried and freeze dried samples stored at 30°C 30%RH for 7 days compare to clopidogrel RM obtained by Unscrambler® (a) and Multibase (b) program (1st batch)

Data from DSC thermograms (Figures 79 and 80), TGA thermograms (Figures 81 and 82), PXRD patterns (Figures 76, 77 and 78) and Raman spectra (Figures 83 and 84) cannot be used to distinguish between $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples. However, PCA results in Figures 87a and 87b show that $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples and clopidogrel RM are clearly different. Furthermore, two amorphous forms of clopidogrel prepared by spray drying and freeze drying methods can be effectively classified as two different amorphous forms of clopidogrel by PCA method and referred to as "polyamorphous".

PCA of the second batch of $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples compare to clopidogrel RM show repeatability of the transformation under this specific condition and are shown in Appendix E. Raman spectral data from $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples and clopidogrel RM can be classified by PCA as 3 distinctive groups similar to PCA of $SP^{0}$ and $FZ^{0}$ samples and clopidogrel RM (Figure 61) and PCA of first batch of $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples and clopidogrel RM (Figure 87). The PC1 accounted for 93.7% of overall variance and the PC2 accounted for 4.9% of overall variance. Moreover, these results present that clopidogrel RM can be classified by PCA as crystalline. On the contrary, $SP^{7}_{30/30}$ and $FZ^{7}_{30/30}$ samples can be classified by PCA to be within amorphous group and can be clearly distinguished as 2 distinctive amorphous groups similar to the $1^{st}$ batch produced. The PCA obtained from samples prepared and stored under 3 different conditions (Figure 85, 86 and 87) compare to initial amorphous samples shown in Appendix F.

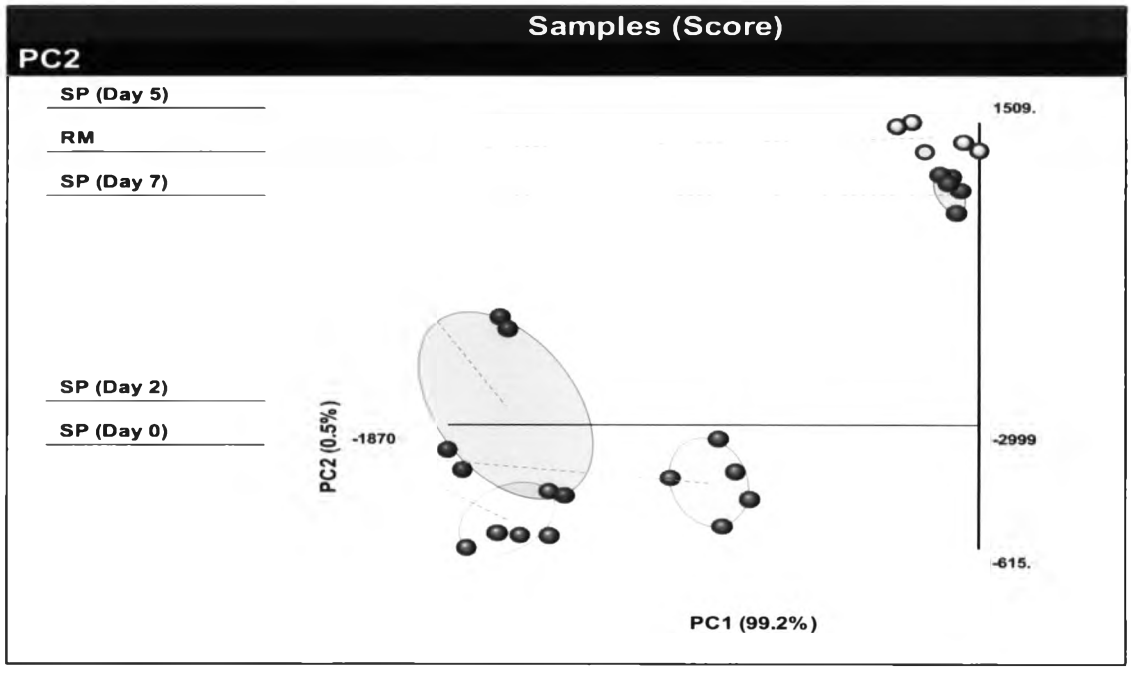



Figure 88 PCA of spray dried samples stored at 40°C 75%RH for 0, 2, 5 and 7 days compare to clopidogrel RM obtained by Multibase program

Figure 88 shows spray dried samples stored at 40°C 75%RH for 0, 2, 5 and 7 days ($SP^0$, $SP^2_{40/75}$, $SP^4_{40/75}$ and $SP^7_{40/75}$) compare to clopidogrel RM. Transformation pathway of the spray dried sample back to crystalline clopidogrel RM have characteristic pattern as clockwise rotation. Figure 89 shows freeze dried samples stored at 40°C 75%RH for 0, 2, 5 and 7 days ($FZ^0$, $FZ^2_{40/75}$, $FZ^4_{40/75}$ and $FZ^7_{40/75}$) compare to clopidogrel RM. Transformation pathway of the freeze dried samples also shows characteristic direction as clockwise rotation similar to transformation pathways obtained from the spray dried samples. Figure 90 shows spray dried and freeze dried samples stored at 40°C 75%RH for 0, 2, 5 and 7 days compare to clopidogrel RM (sum of Figures 88 and 89). This PCA also revealed that the transformation pathways of the two polyamorphous forms back to crystalline clopidogrel transformed in the same "direction" but the "location" are distinctively different.

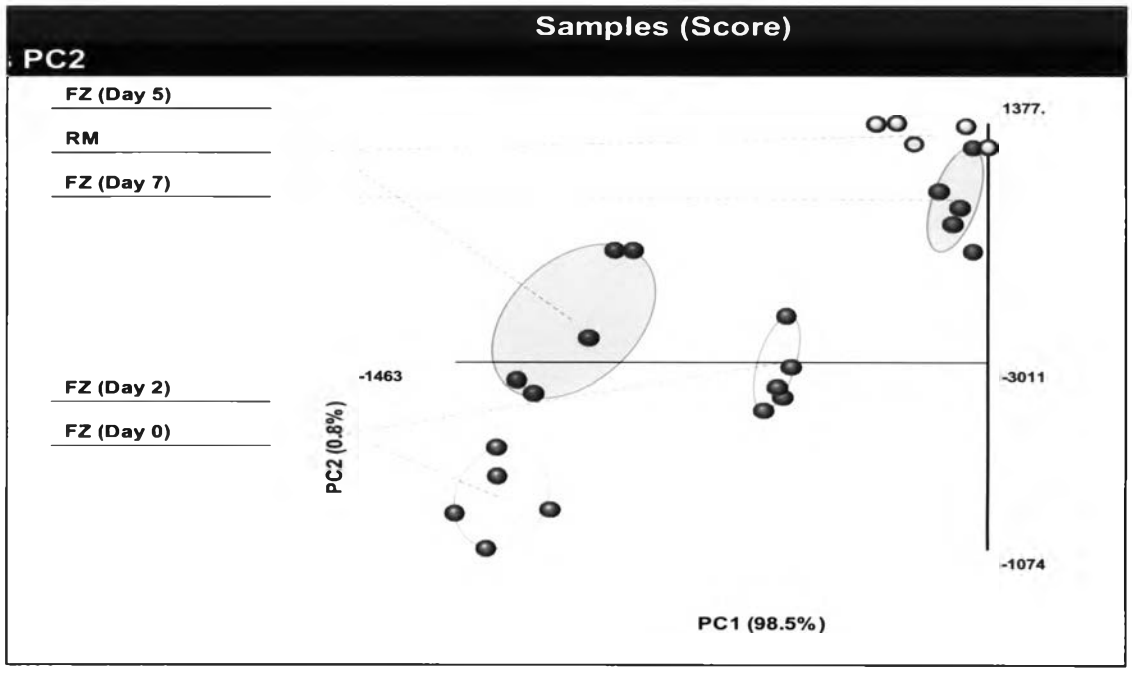



Figure 89 PCA of freeze dried samples stored at 40°C 75%RH for 0, 2, 5 and 7 days compare to clopidogrel RM obtained by Multibase program

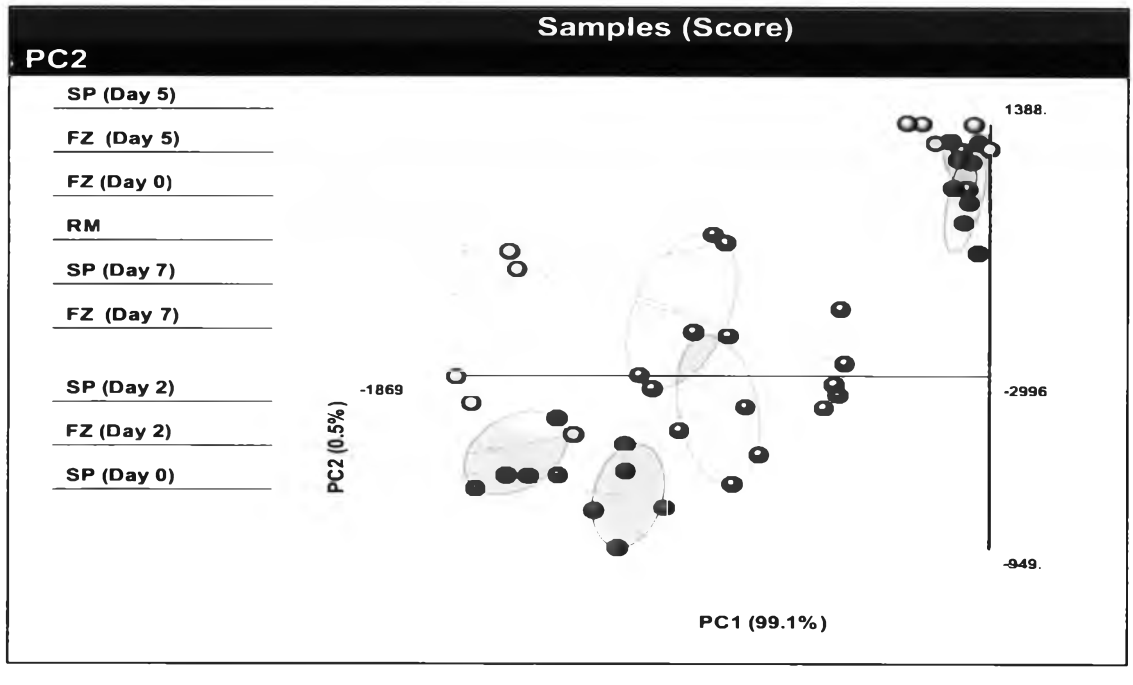



Figure 90 PCA of spray dried and freeze dried samples stored at 40°C 75%RH for 0, 2, 5 and 7 days compare to clopidogrel RM from Multibase program

### 6.8. Related substance

Figure 91a shows HPLC chromatrogram of spray dried sample prepared at day 0 ($SP^0$). Figures 91b, 91c and 91d show HPLC chromatrograms of spray dried samples prepared and stored at 30°C 30%RH, 40°C 30%RH and 40°C 75%RH for 7 days ($SP^7_{30/30}$, $SP^7_{40/30}$ and $SP^7_{40/75}$), respectively. These results show that clopidogrel related compound A clearly increased when the samples were stored at high temperature and humidity conditions. On the contrary, peaks of clopidogrel related compound C do not change when the samples were stored at high temperature and high humidity. In addition, peak of clopidogrel related compound $B_1$ is not found and peak of clopidogrel related compound $B_2$ cannot be detected due to very large tailing of the main clopidogrel peak.

Figure 92a shows HPLC chromatrogram of freeze dried sample prepared at day 0 ($FZ^0$). Figures 92b, 92c and 92d show HPLC chromatrograms of freeze dried samples prepared and stored at 30°C 30%RH, 40°C 30%RH and 40°C 75%RH for 7 days ($FZ^7_{30/30}$, $FZ^7_{40/30}$ and $FZ^7_{40/75}$), respectively. These results also demonstrate that clopidogrel related compound A clearly increased when samples were stored at high temperature and humidity conditions similar to results obtained from $SP^7_{30/30}$, $SP^7_{40/30}$ and $SP^7_{40/75}$ samples. On the other hand, peaks of clopidogrel related compound C do not change when the samples were stored at high temperature and high humidity. Moreover, peak of clopidogrel related compound $B_1$ is not found, while clopidogrel related compound $B_2$ cannot be detected due to very large tailing of the main clopidogrel peak similar to HPLC chromatrogram obtained from spray dried sample.

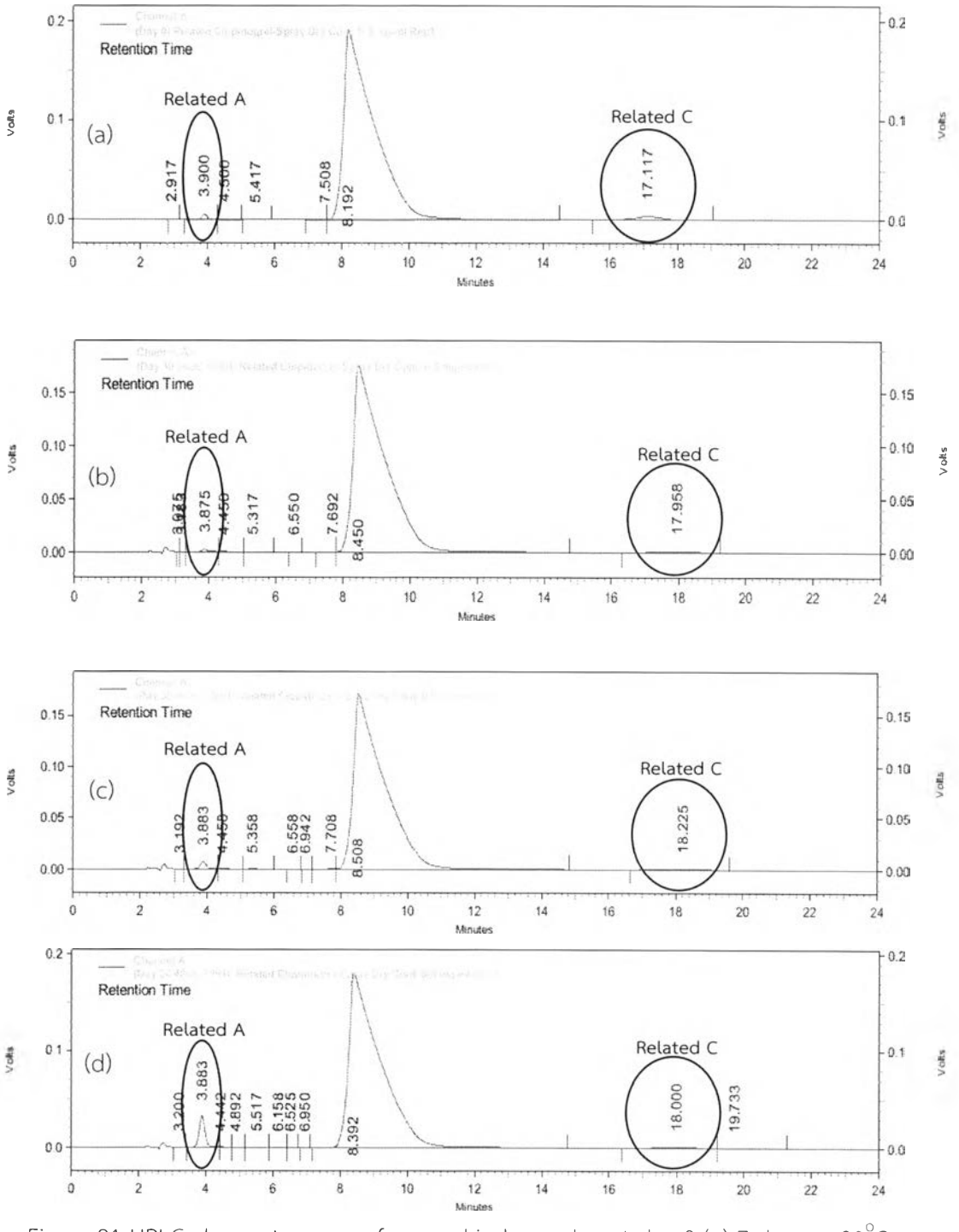



Figure 91 HPLC chromatograms of spray dried samples at day 0 (a) 7 days at 30°C 30%RH (b) at 40°C 30%RH (c) and at 40°C 75%RH (d)

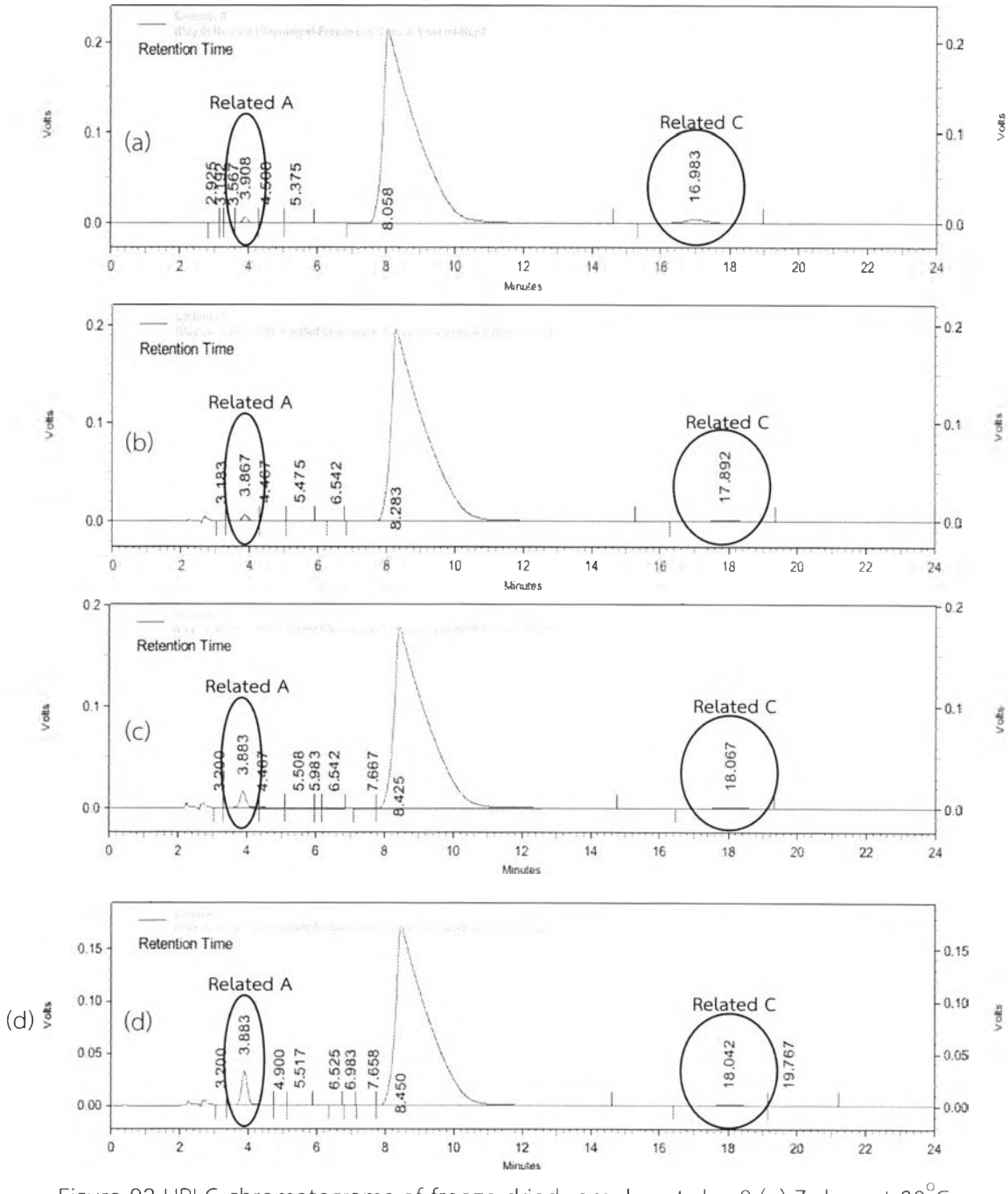



Figure 92 HPLC chromatograms of freeze dried samples at day 0 (a) 7 days at 30°C 30%RH (b) at 40°C 30%RH (c) and at 40°C 75%RH (d)

Table 7 The quantity of clopidogrel related compound A and C of spray dried and freeze dried samples prepared and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 0, 1, 2, 3, 5, 7, 10, 20, 30, 60 and 90 days

| | | Spray Dry | | | | Freeze Dry | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Related A | Area % | Related C | Area % | Related A | Area % | Related C | Area % |
| Day 0 | Condition | 0.2066 | 0.503 | 1.9254 | 1.697 | 0.2370 | 0.534 | 2.0857 | 1.700 |
| Day 1 | 30°C 30%RH | 0.2117 | 0.488 | 1.9398 | 1.619 | 0.2074 | 0.520 | 1.9240 | 1.747 |
| | 40°C 30%RH | 0.2317 | 0.564 | 1.8764 | 1.647 | 0.2493 | 0.572 | 1.9934 | 1.656 |
| | 40°C 75%RH | 0.2517 | 0.666 | 1.7859 | 1.708 | 0.2878 | 0.704 | 1.8502 | 1.638 |
| Day 2 | 30°C 30%RH | 0.2066 | 0.486 | 1.9419 | 1.657 | 0.2082 | 0.596 | 1.6648 | 1.719 |
| | 40°C 30%RH | 0.2558 | 0.596 | 1.9460 | 1.642 | 0.3233 | 0.750 | 1.9421 | 1.634 |
| | 40°C 75%RH | 0.3631 | 0.846 | 1.8455 | 1.558 | 0.3983 | 0.962 | 1.7893 | 1.567 |
| Day 3 | 30°C 30%RH | 0.2022 | 0.469 | 1.8959 | 1.594 | 0.2687 | 0.614 | 2.2104 | 1.833 |
| | 40°C 30%RH | 0.2426 | 0.572 | 1.9047 | 1.629 | 0.3278 | 0.805 | 1.8051 | 1.603 |
| | 40°C 75%RH | 0.4263 | 1.022 | 1.7960 | 1.561 | 0.5516 | 1.329 | 1.8718 | 1.634 |
| Day 5 | 30°C 30%RH | 0.0840 | 0.199 | 0.8079 | 0.692 | 0.1234 | 0.293 | 0.8520 | 0.732 |
| | 40°C 30%RH | 0.1596 | 0.362 | 0.9022 | 0.741 | 0.3002 | 0.698 | 0.8615 | 0.726 |
| | 40°C 75%RH | 0.8306 | 1.872 | 0.8992 | 0.735 | 0.8598 | 2.069 | 0.8332 | 0.727 |
| Day 7 | 30°C 30%RH | 0.1085 | 0.244 | 0.9090 | 0.741 | 0.1911 | 0.423 | 0.9029 | 0.724 |
| | 40°C 30%RH | 0.1840 | 0.414 | 0.9041 | 0.737 | 0.4348 | 1.003 | 0.8704 | 0.728 |
| | 40°C 75%RH | 1.0842 | 2.459 | 0.9577 | 0.787 | 1.1689 | 2.687 | 0.9075 | 0.756 |
| Day 10 | 30°C 30%RH | 0.1155 | 0.257 | 0.9231 | 0.743 | 0.2558 | 0.619 | 0.8206 | 0.719 |
| | 40°C 30%RH | 0.2324 | 0.502 | 0.9499 | 0.744 | 0.4061 | 0.914 | 0.9413 | 0.768 |
| | 40°C 75%RH | 1.1311 | 2.576 | 0.9398 | 0.776 | 1.4777 | 3.269 | 0.9363 | 0.751 |
| Day 20 | 30°C 30%RH | 0.2278 | 0.536 | 1.5852 | 1.352 | 0.3265 | 0.804 | 1.5486 | 1.380 |
| | 40°C 30%RH | 0.3902 | 0.929 | 1.5954 | 1.375 | 0.7152 | 1.641 | 1.6644 | 1.384 |
| | 40°C 75%RH | 1.3262 | 3.141 | 1.5402 | 1.322 | 1.4659 | 3.538 | 1.5236 | 1.333 |
| Day 30 | 30°C 30%RH | 0.1455 | 0.335 | 0.8584 | 0.715 | 0.2798 | 0.658 | 0.8314 | 0.709 |
| | 40°C 30%RH | 0.3865 | 0.875 | 0.8729 | 0.716 | 0.7864 | 1.859 | 0.8505 | 0.729 |
| | 40°C 75%RH | 1.4492 | 3.308 | 0.8905 | 0.737 | 1.5408 | 3.606 | 0.9004 | 0.764 |
| Day 60 | 30°C 30%RH | 0.1473 | 0.392 | 0.7041 | 0.597 | 0.3942 | 1.029 | 0.7107 | 0.593 |
| | 40°C 30%RH | 0.5256 | 1.381 | 0.7099 | 0.595 | 1.3711 | 4.099 | 0.6589 | 0.629 |
| | 40°C 75%RH | 1.5210 | 3.961 | 0.7722 | 0.642 | 1.7598 | 4.587 | 0.7737 | 0.644 |
| Day 90 | 30°C 30%RH | 0.2586 | 0.679 | 0.9383 | 0.887 | 0.6592 | 1.691 | 0.7152 | 0.655 |
| | 40°C 30%RH | 0.9914 | 2.812 | 0.6675 | 0.676 | 1.9509 | 5.058 | 0.7141 | 0.662 |
| | 40°C 75%RH | 2.5136 | 6.222 | 1.0587 | 0.938 | 2.3252 | 5.744 | 1.0216 | 0.913 |

Table 7 present the quantity of clopidogrel related compound A and clopidogrel related compound C from spray dried and freeze dried samples prepared and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 0, 1, 2, 3, 5, 7, 10, 20, 30, 60 and 90 days. These results indicate that clopidogrel samples prepared by freeze drying and spray drying methods, if stored at 30°C 30%RH and 40°C 30%RH, do not significantly increased the amount of related compound A. In contrast, samples prepared and stored at 40°C 75%RH shows a clear increase in the amount of clopidogrel related compound A. Furthermore, these results indicate that clopidogrel related compound C of both samples prepared and stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) do not change significantly until 90 days were reached. All statistical results obtained from ANOVA are shown in Appendix G.



### 6.9. Appearance and size

Figures 93a, 93b and 93c demonstrate surface appearances and approximate sizes when observed under scanning electron microscope (SEM) at 100x (left) and 500x (right) magnifications of spray dried samples stored at 3 different conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 60 days ($SP^{60}_{30/30}$, $SP^{60}_{40/30}$ and $SP^{60}_{40/75}$). SEM of $SP^{60}_{30/30}$ and $SP^{60}_{40/30}$ samples (Figure 93a and 93b) exhibit small spherical particles with particle size of approximately 5 microns correlated with appearances and sizes under observation using SEM of $SP^{0}$ sample (Figure 63). In contrast, SEM of $SP^{60}_{40/75}$ sample (Figure 93c) presents small irregular particles of approximate size of 10 microns or more similar to SEM of clopidogrel RM (Figure 62).

Figure 94a, 94b and 94c indicate surface appearances and approximate sizes when observe under SEM at 100x (left) and 500x (right) magnifications of freeze dried samples stored at 3 various conditions (30°C 30%RH, 40°C 30%RH and 40°C 75%RH) for 60 days ($FZ^{60}_{30/30}$, $FZ^{60}_{40/30}$ and $FZ^{60}_{40/75}$). SEM of $FZ^{60}_{30/30}$ and $FZ^{60}_{40/30}$ samples (Figure 94a and 94b) show continuous cake and when sampling, it became flakes and sizes cannot be evaluated using SEM, including $FZ^{0}$ sample (Figure 64). Whereas, SEM of $FZ^{60}_{40/75}$ sample (Figure 94c) presents small irregular particles of approximately 10 microns or more similar to the particles of clopidogrel RM (Figure 62) and $SP^{60}_{40/75}$ sample.



All above results from SEM indicate that amorphous samples prepared by both methods have all been converted to particles similar to the crystalline form after storage at 40°C 75%RH for 60 days. On the contrary, this interconversion did not occur when these amorphous samples are stored at 30°C 30%RH and 40°C 30%RH. These studies show that humidity have higher impact than temperature on the solid state conversion, appearance and size stability of amorphous samples prepared by both spray drying and freeze drying methods.

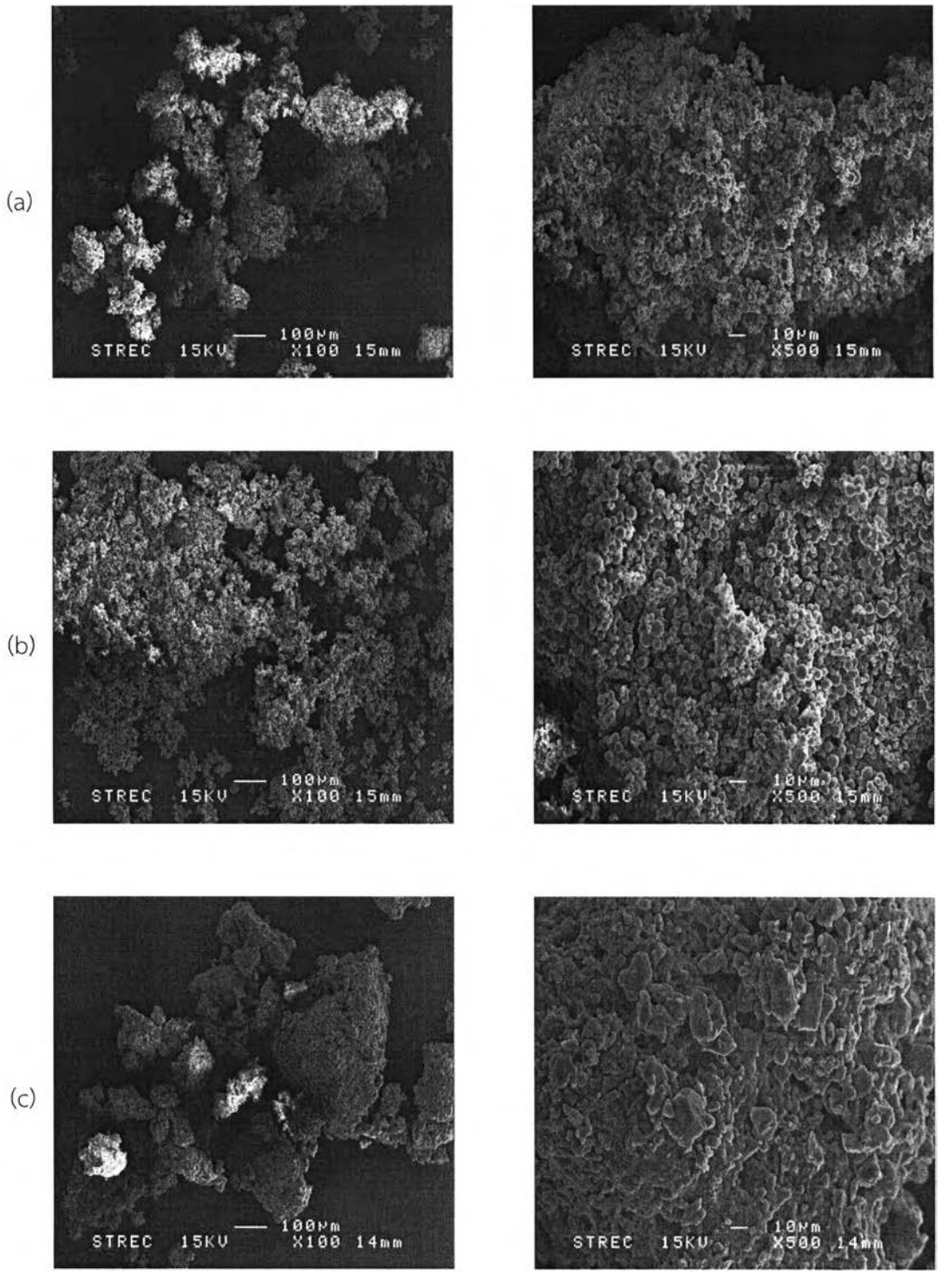



Figure 93 SEM photomicrographs of spray dried samples at 100x (left) and 500x (right) magnifications stored under 3 conditions for 60 days at 30°C 30%RH (a) 40°C 30%RH (b) 40°C 75%RH (c)

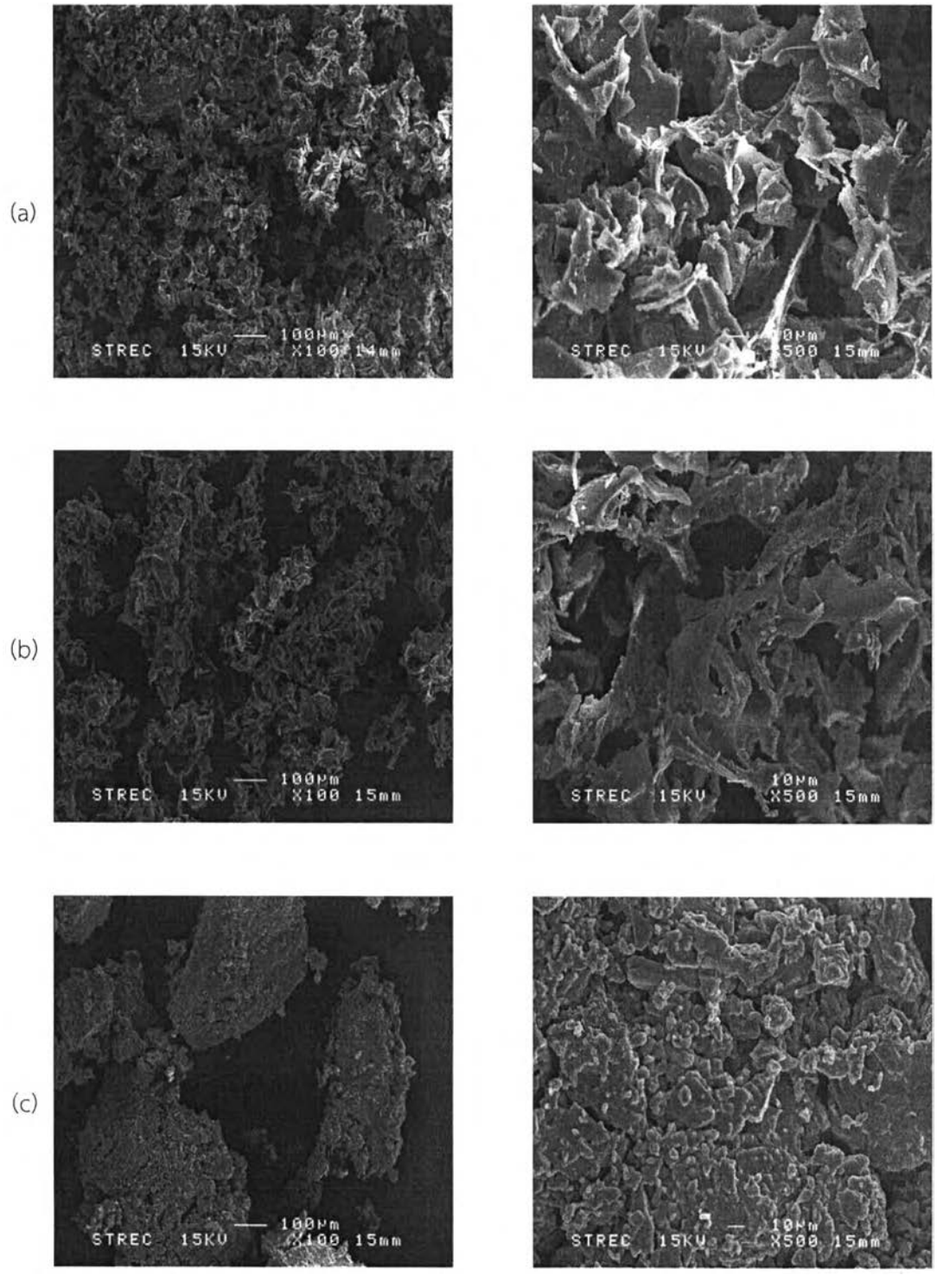





Figure 94 SEM photomicrographs of freeze dried samples at 100x (left) and 500x (right) magnifications stored under 3 conditions for 60 days at 30°C 30%RH (a) 40°C 30%RH (b) 40°C 75%RH (c)

## 7. Solid-state characterization in physical mixtures

Physical mixtures were prepared and analyzed using PXRD, DSC and Raman to evaluate their solid-state identity. Figure 95 shows PXRD diffractograms of spray dried sample physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to PXRD diffractograms of individual spray dried clopidogrel, clopidogrel RM and lactose. The PXRD pattern of spray dried sample indicate amorphous halo and do not exhibit any diffraction peaks as seen in Figure 95d. The PXRD pattern of crystalline clopidogrel RM is shown in Figure 95e. The PXRD pattern of lactose shows characteristic peaks at 12.5°, 19.1°, 19.6° and 20.0° 2θ in accordance with other studies as seen in Figure 95f (59). Therefore, PXRD patterns of physically mixed samples between spray dried samples and lactose in various ratios, present only characteristic peaks of lactose with underlying amorphous clopidogrel halo pattern (Figure 95a, 95b and 95c). As amount of lactose increased, the higher intensity of PXRD peaks of lactose is observed.



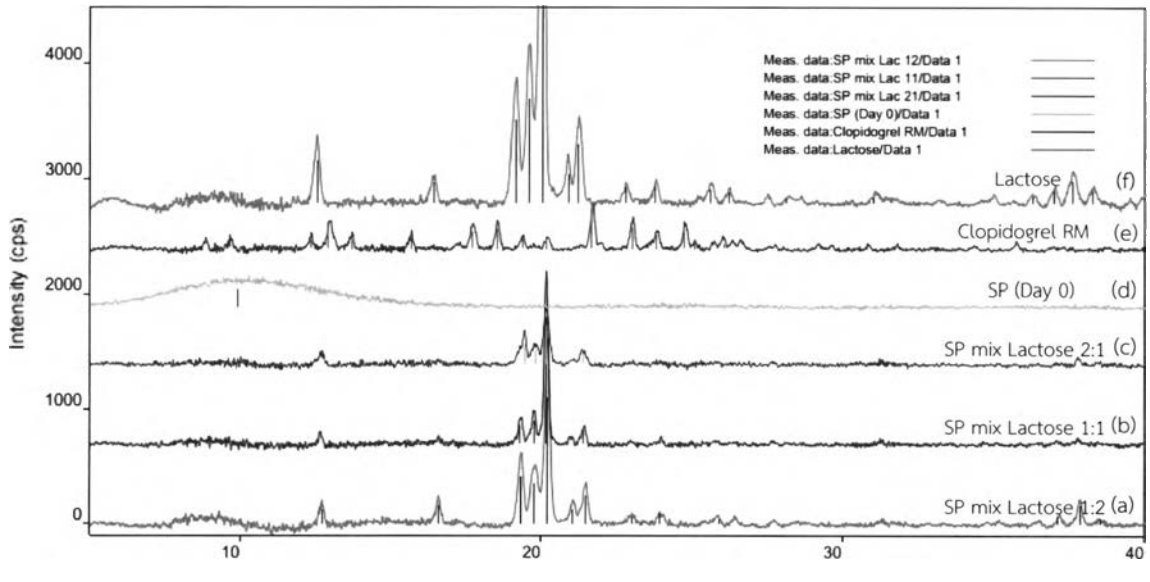



Figure 95 PXRD diffractograms of spray dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual spray dried sample, clopidogrel RM and lactose

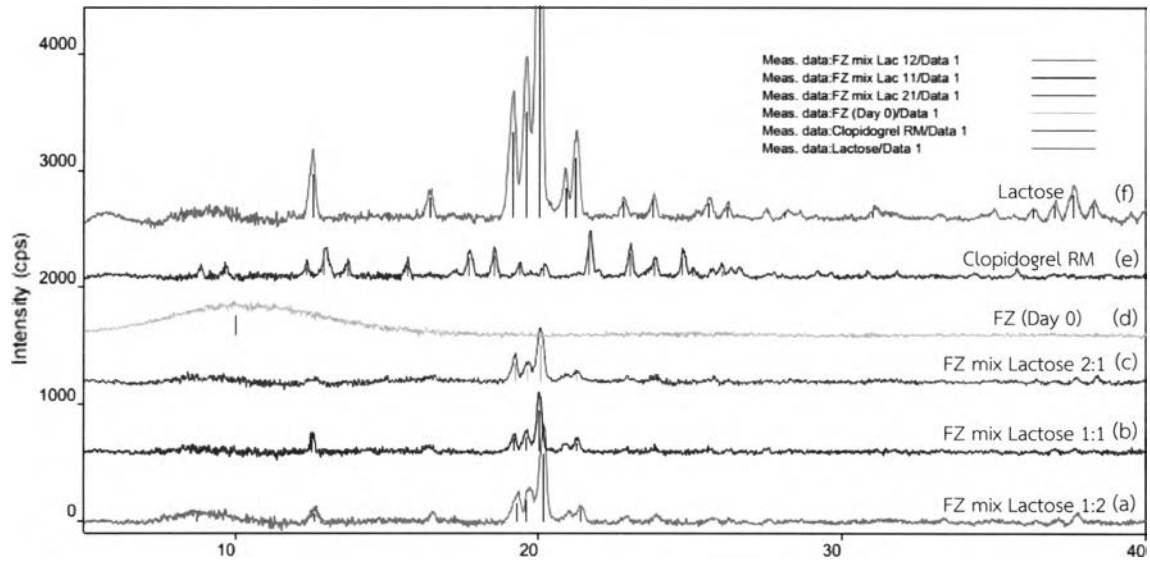



Figure 96 PXRD diffractograms of freeze dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual freeze dried sample, clopidogrel RM and lactose

Figure 96 indicate PXRD diffractograms of freeze dried samples mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to PXRD diffractograms of individual freeze dried sample prepared, clopidogrel RM and lactose. The PXRD pattern of freeze dried sample indicate amorphous halo and do not exhibit any diffraction peaks as seen in Figure 96d similar to the PXRD pattern of spray dried sample. The PXRD pattern of crystalline clopidogrel RM is shown in Figure 96e. The PXRD pattern of lactose shows characteristic peaks as seen in Figure 96f. Whereas, PXRD patterns of samples physically mixed between freeze dried sample and lactose in several proportions are shown in Figure 96a, 96b and 96c. Characteristic peaks of lactose are prominent which was due to PXRD peaks of amorphous clopidogrel show only halo pattern and do not exhibit any diffraction peaks. As amount of lactose increased, the higher intensity of PXRD peaks of lactose becomes. PXRD is clearly not appropriate in

quantitatively amorphous content in physical mixtures and cannot differentiate the two polyamorphous forms.

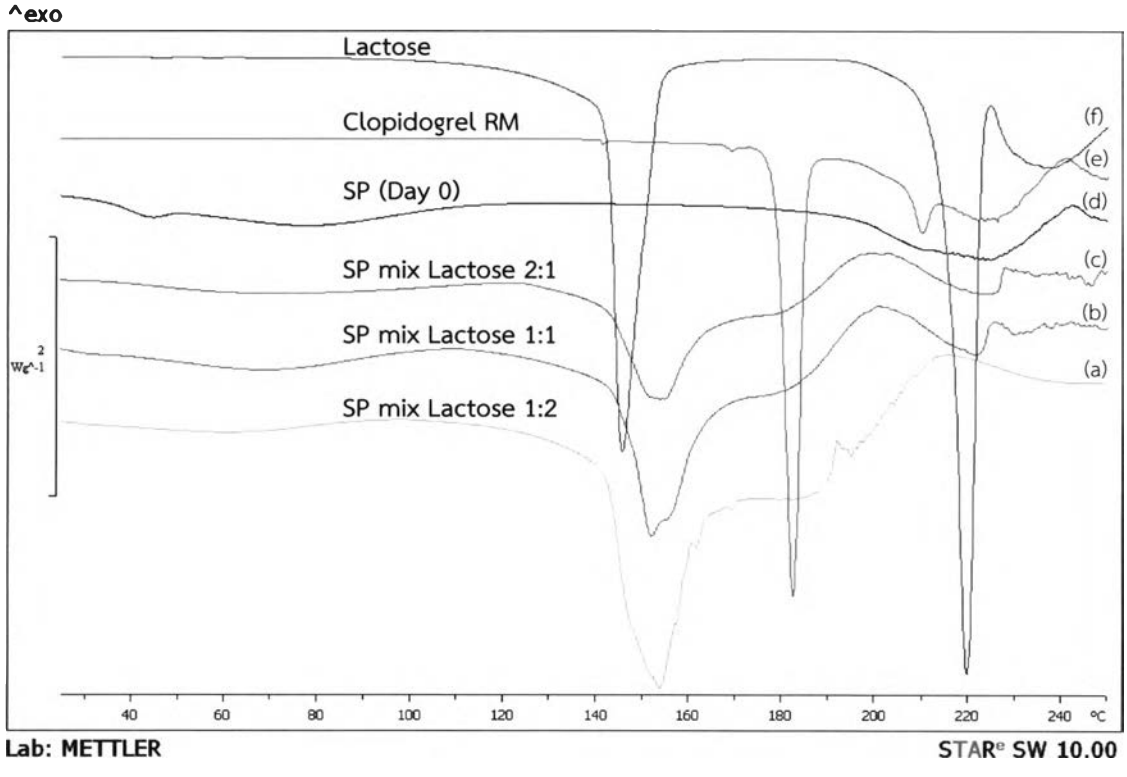



Figure 97 DSC thermograms of spray dried samples mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to initial spray dried samples, clopidogrel RM and lactose

Figure 97 shows DSC thermograms of spray dried samples mixed with lactose in ratios of 1:2, 1:1 and 2:1 compare to DSC thermograms of lactose, clopidogrel RM and spray dried. DSC thermogram of clopidogrel RM exhibits melting endotherm at 183°C and degradation endotherm at 210°C. Moreover, DSC thermogram of initial spray dried sample shows broad endotherm at 70 and 200°C. Furthermore, the DSC thermogram of lactose presents two melting endotherms at 145 and 220°C. In contrast, DSC patterns of mixed samples between spray dried samples and lactose at various ratios present only broad melting endotherms at 150°C and 220°C. These

results are similar to DSC thermogram of pure lactose due to DSC pattern of spray dried sample ($SP^0$) show only broad endotherm without any major observable peaks.

Figure 98 shows DSC thermograms of freeze dried samples mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to pure lactose, clopidogrel RM and freeze dried samples. DSC patterns of mixed samples between freeze dried samples and lactose in various ratios present only broad melting endotherms at 150°C and 220°C similar to characteristic DSC thermogram of lactose. As can be seen DSC is not a method of choice to distinguish amorphous from crystalline structure or to differentiate the two polyamorphous forms.



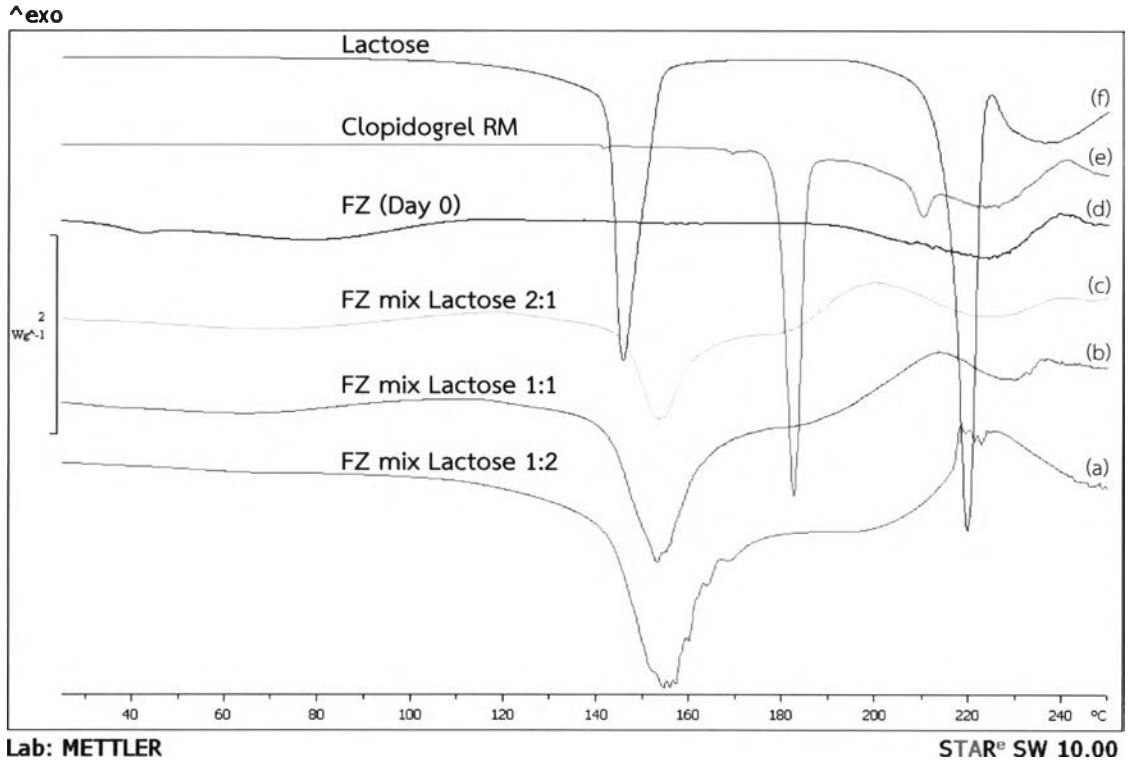



Figure 98 DSC thermograms of freeze dried samples mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to initial freeze dried samples, clopidogrel RM and lactose

Amorphous spray dried clopidogrel do not convert to its crystalline structure even under higher temperature condition. This may be due to the fact that molten lactose acted as a solvent to readily dissolve amorphous clopidogrel, thus, recrystallization was not observed or polyamorphous clopidogrel are stable at high temperatures as can be seen with $SP^0$ and $FZ^0$ where there are no recrystallizations.

Figures 99 and 100 indicate Raman spectra of spray dried and freeze dried samples mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to initial spray dried sample or initial freeze dried sample, clopidogrel RM and lactose, respectively. Raman shifts between 3200 to 2900 $cm^{-1}$, 1800 to 1500 $cm^{-1}$ and 1100 to 1000 $cm^{-1}$ are chosen to represent clear distinction of each sample. Raman shifts between 3200 to 2900 $cm^{-1}$ and 1100 to 1000 $cm^{-1}$ are used to distinguish between amorphous form and crystalline form of clopidogrel. Raman shifts between 3200 to 2900 $cm^{-1}$ and 1800 to 1500 $cm^{-1}$ are used to distinguish between physically mixed samples of spray dried and freeze dried clopidogrel and diluent (lactose) and each single sample. Raman spectra of mixed samples in Figures 99 and 100 show combined peaks between amorphous spray dried or freeze dried clopidogrel and lactose. These results demonstrated that physically mixed samples between amorphous clopidogrel and lactose can be differentiated from crystalline clopidogrel and lactose by observing Raman shifts between at 3100 $cm^{-1}$ and 1100 to 1000 $cm^{-1}$.

From the above reasons, Raman spectroscopic method is more sensitive than PXRD and DSC in qualitatively evaluating the mixed samples between amorphous clopidogrel and lactose.

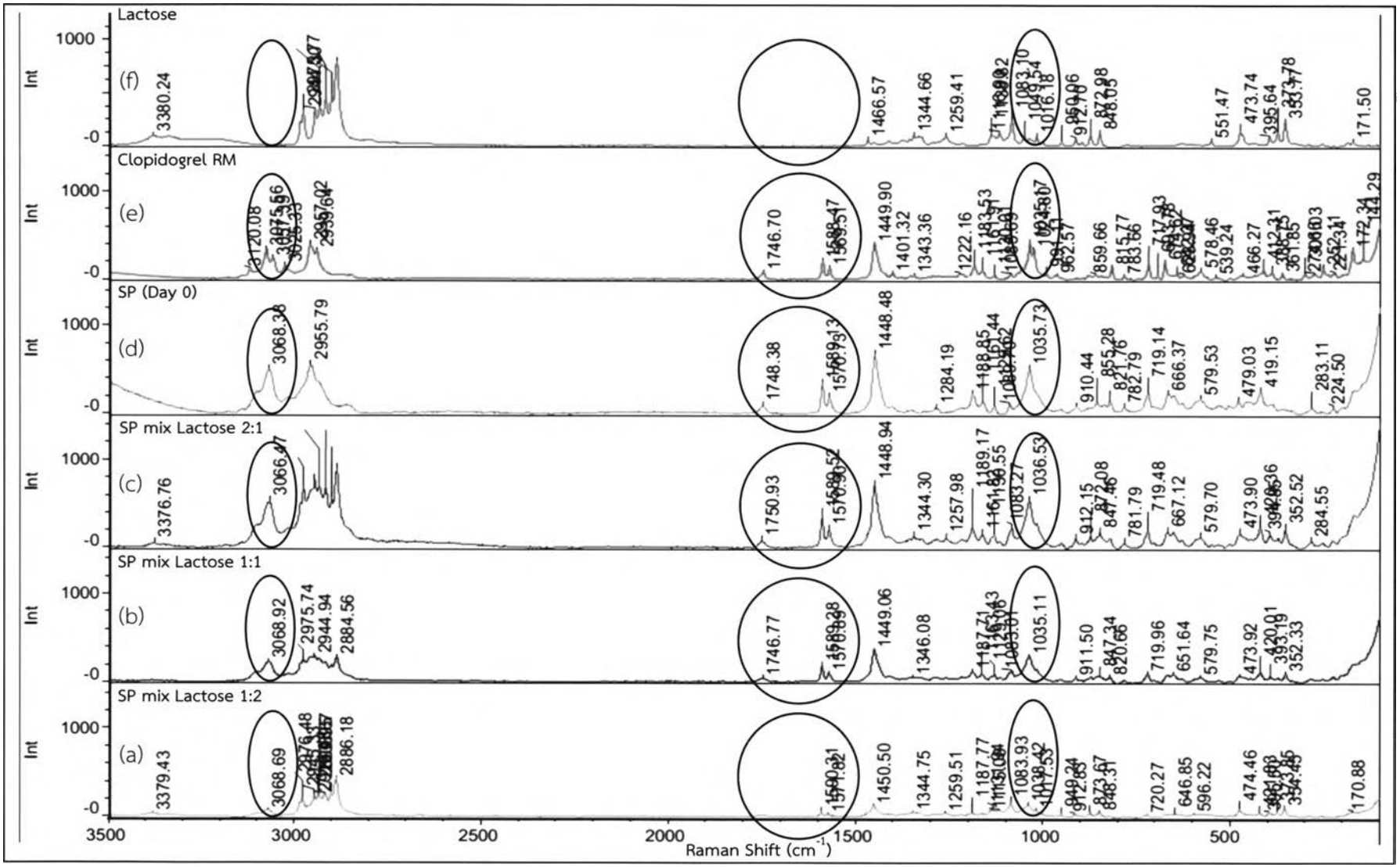



Figure 99 Raman spectra of spray dried samples mixed with lactose at various ratios of 1:2 (a) 1:1 (b) 2:1 (c) compare to initial spray dried samples (d) clopidogrel RM (e) and lactose (f)

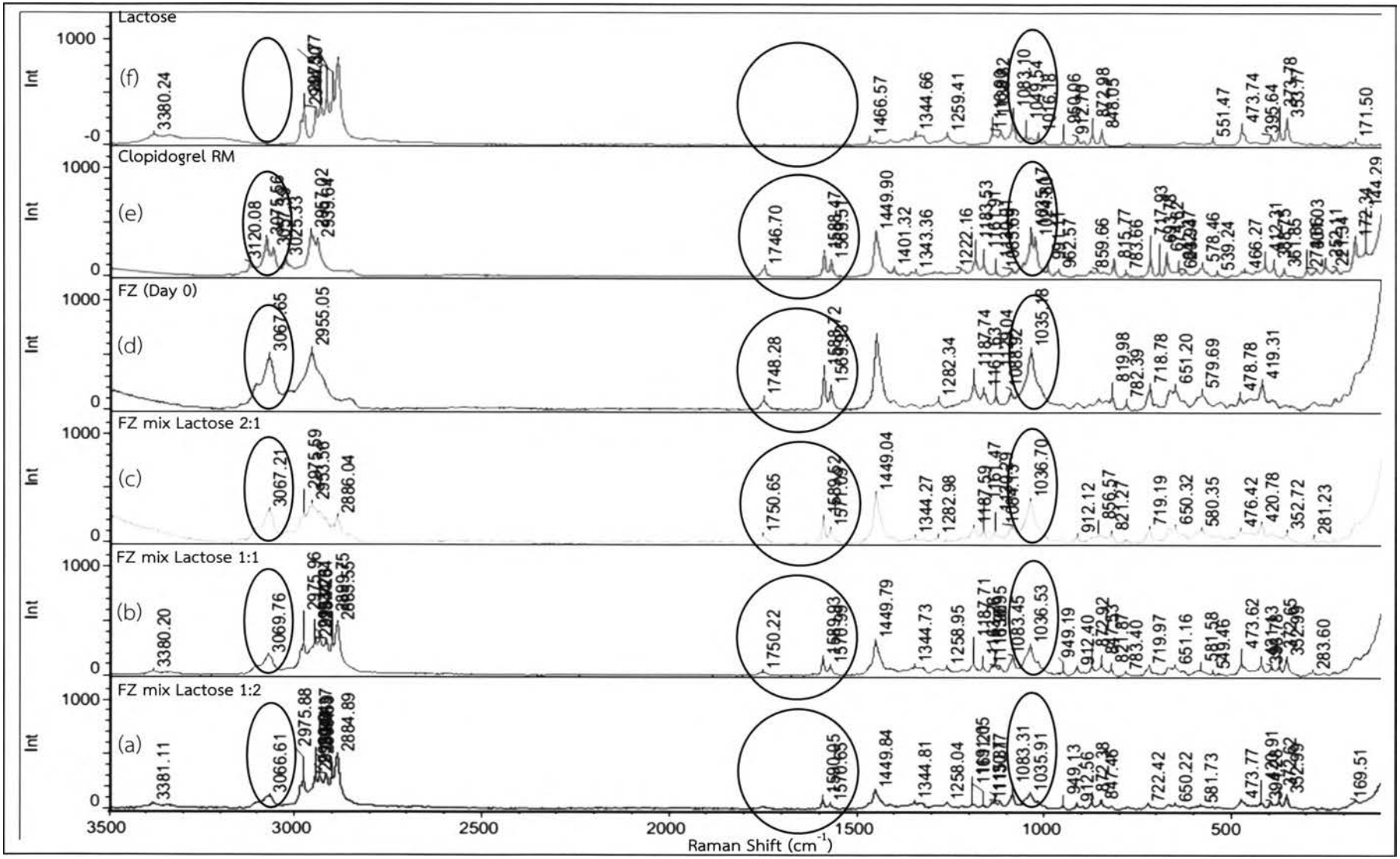



Figure 100 Raman spectra of freeze dried samples mixed with lactose at various ratios of 1:2 (a) 1:1 (b) 2:1 (c) compare to initial freeze dried samples (d) clopidogrel RM (e) and lactose (f)

Figure 101 and 102 indicate PCA of spray dried and freeze dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual spray dried sample, clopidogrel RM and lactose. PCA obtained from Figure 101 can be classified as 3 distinct groups between crystalline clopidogrel RM, non-crystalline clopidogrel RM and pure lactose monohydrate. The PC1 accounted for 96.8% of overall variance and the PC3 accounted for 1.2% of overall variance. Moreover, PCA obtained from Figure 102 can also be classified as 3 distinct groups between crystalline clopidogrel RM, non-crystalline clopidogrel RM and pure lactose monohydrate similar to PCA obtained from Figure 101. The PC1 accounted for 96.5% of overall variance and the PC2 accounted for 2.5% of overall variance. The results from Figures 101 and 102 indicate that clopidogrel RM can be classified by PCA as crystalline. On the contrary, amorphous samples physically mixed with increased ratios of lactose are expressed as groups initiating closely to pure amorphous clopidogrel and gradually move towards pure lactose as the proportion of lactose is increasing.

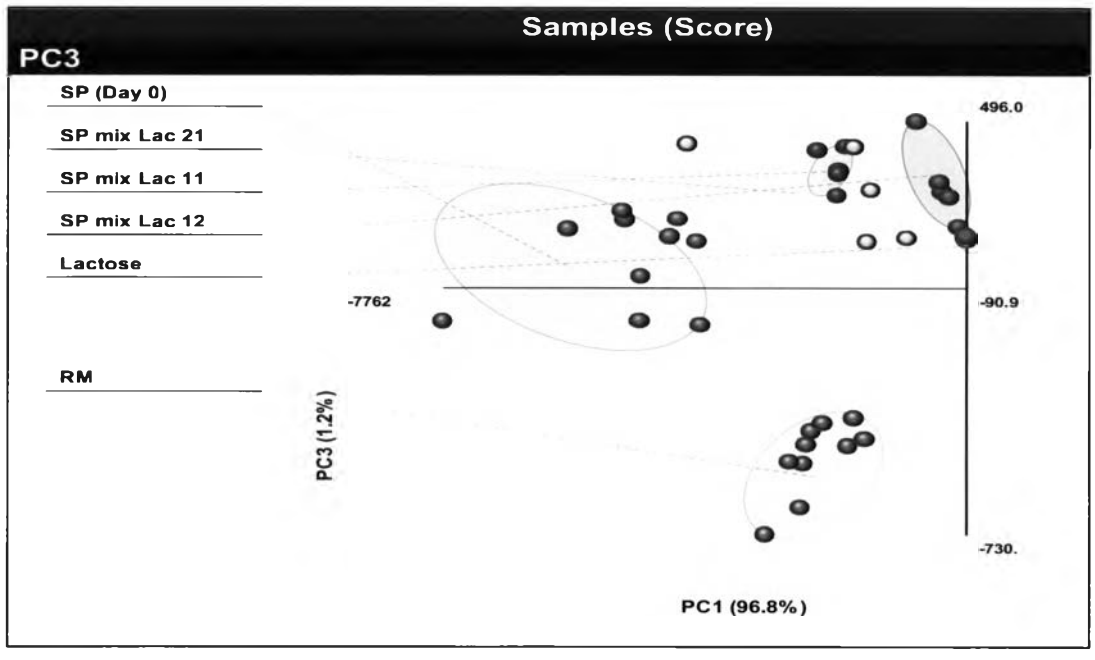



Figure 101 PCA of spray dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual spray dried sample, clopidogrel RM and lactose using Multibase program

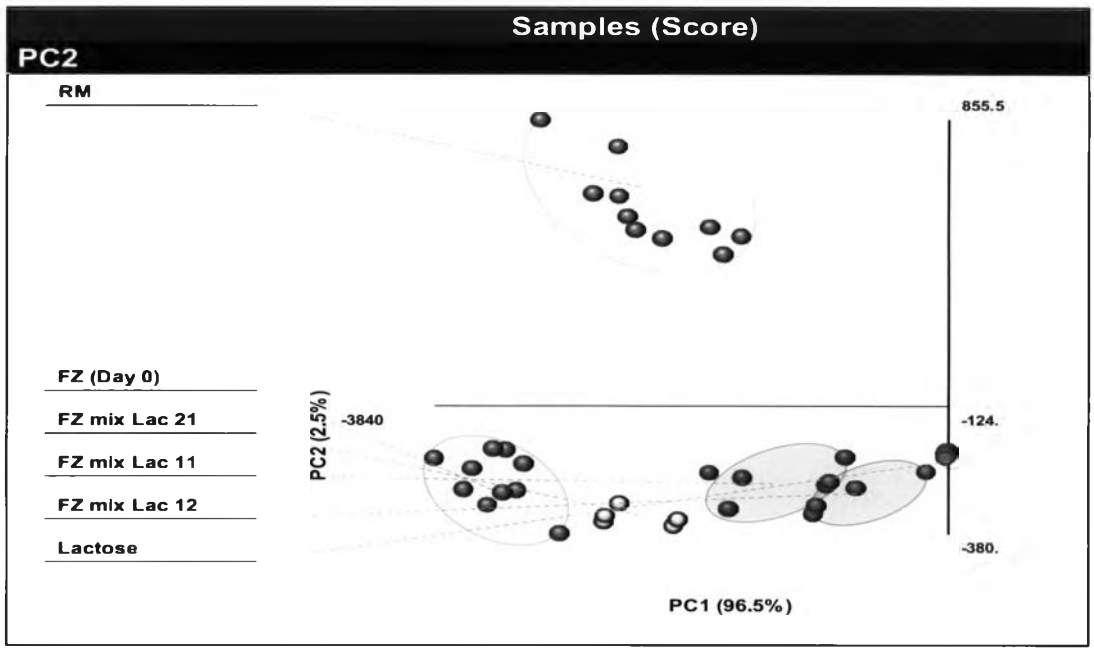



Figure 102 PCA of freeze dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual freeze dried sample, clopidogrel RM and lactose using Multibase program

Figure 103 indicates PCA of spray dried and freeze dried samples physically mixed with lactose at ratio of 2:1 compare to individual spray dried and freeze dried samples, crystalline clopidogrel RM and pure lactose monohydrate. Figure 104 indicates PCA of spray dried and freeze dried samples physically mixed with lactose at ratio of 1:1 compare to individual spray dried and freeze dried samples, crystalline clopidogrel RM and pure lactose monohydrate. Figure 105 indicates PCA of spray dried and freeze dried samples physically mixed with lactose at ratio of 1:2 compare to individual spray dried and freeze dried samples, crystalline clopidogrel RM and pure lactose monohydrate. Results from Figures 103, 104 and 105 show that lactose has high influence on where the location of the physical mixtures are located. In addition, the distance between various ratios of physically mixed samples of amorphous clopidogrel and lactose as compare to amorphous spray dried and freeze dried clopidogrel and pure lactose monohydrate are highly dependent on the lactose ratios. In case of amount of amorphous sample is higher than lactose (Figure 103), a longer distance will be observed from PCA between the sample and lactose. In contrast, the shorter distance is obtained from PCA when the amount of amorphous sample is lower than lactose (Figure 105). Figure 106 indicates PCA of spray dried and freeze dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual spray dried and freeze dried sample, clopidogrel RM and lactose (sum of Figures 103, 104 and 105). This PCA also reveals that the movement of the two polyamorphous physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 are highly dependent on the ratios of lactose in the mixtures.

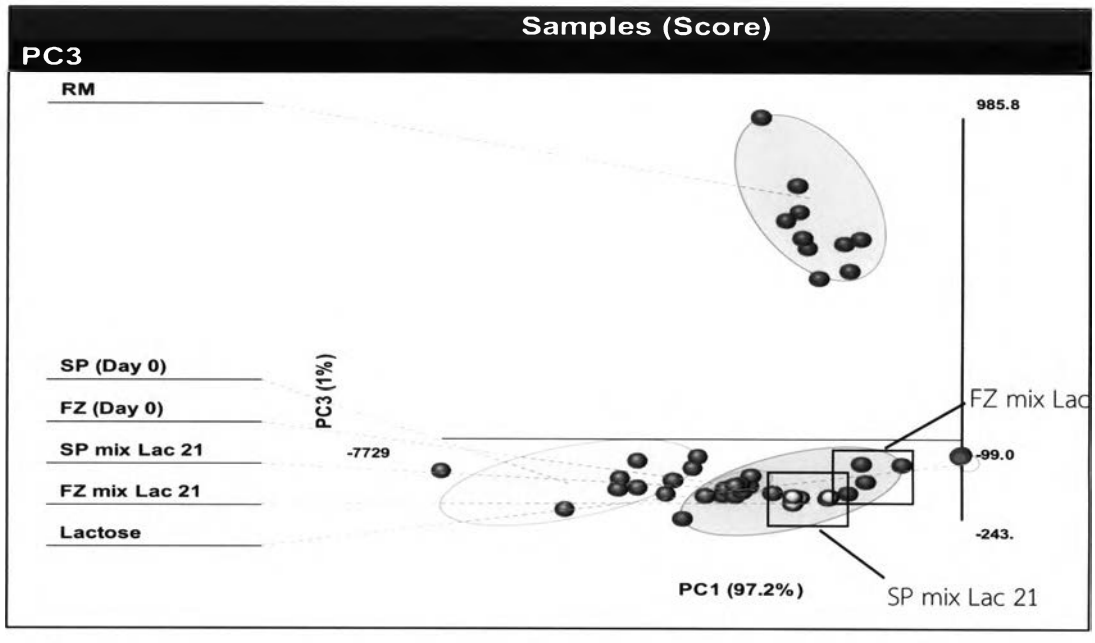



Figure 103 PCA of spray dried and freeze dried samples physically mixed with lactose at ratio of 2:1 compare to individual spray dried and freeze dried samples, clopidogrel RM and lactose using Multibase program

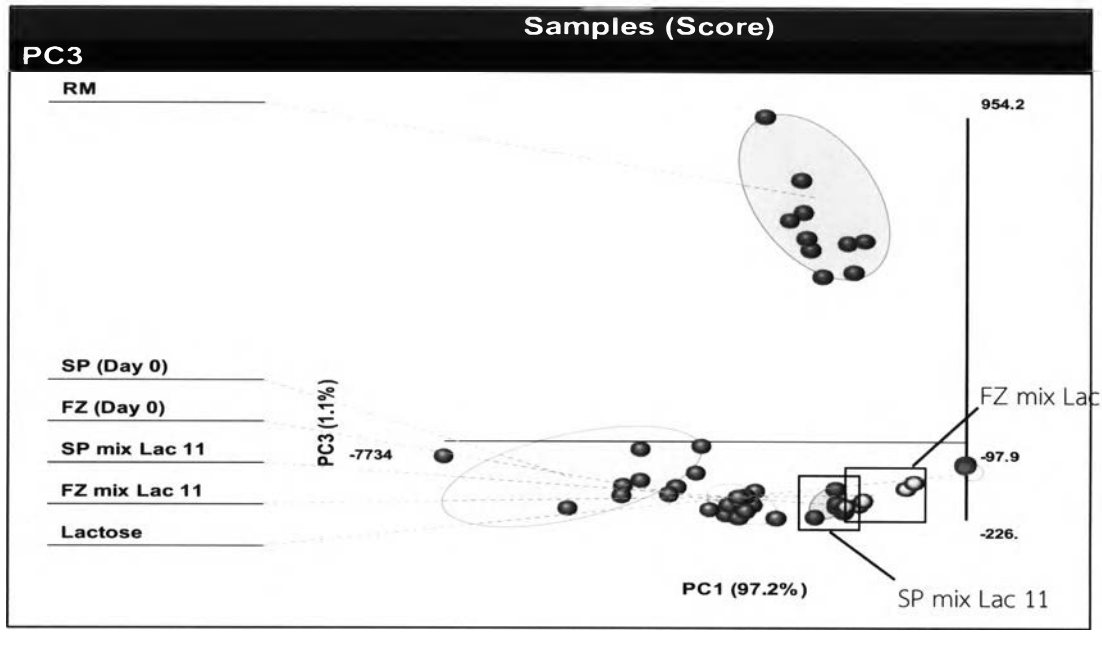



Figure 104 PCA of spray dried and freeze dried samples physically mixed with lactose at ratio of 1:1 compare to individual spray dried and freeze dried samples, clopidogrel RM and lactose using Multibase program

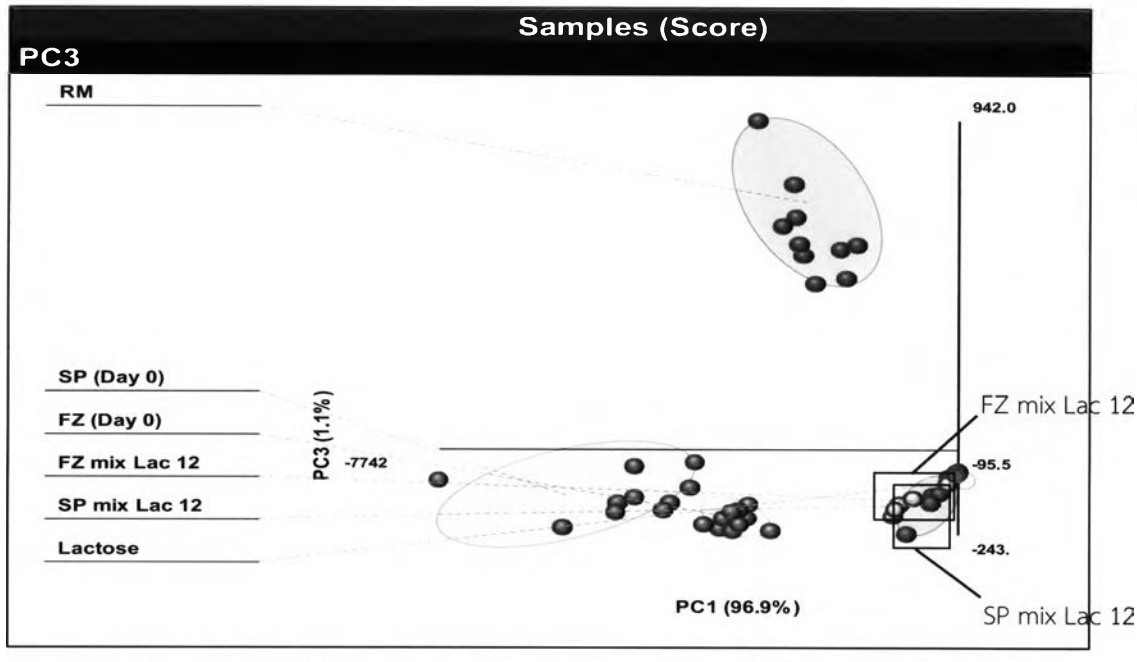



Figure 105 PCA of spray dried and freeze dried samples physically mixed with lactose at ratio of 1:2 compare to individual spray dried and freeze dried samples, clopidogrel RM and lactose using Multibase program

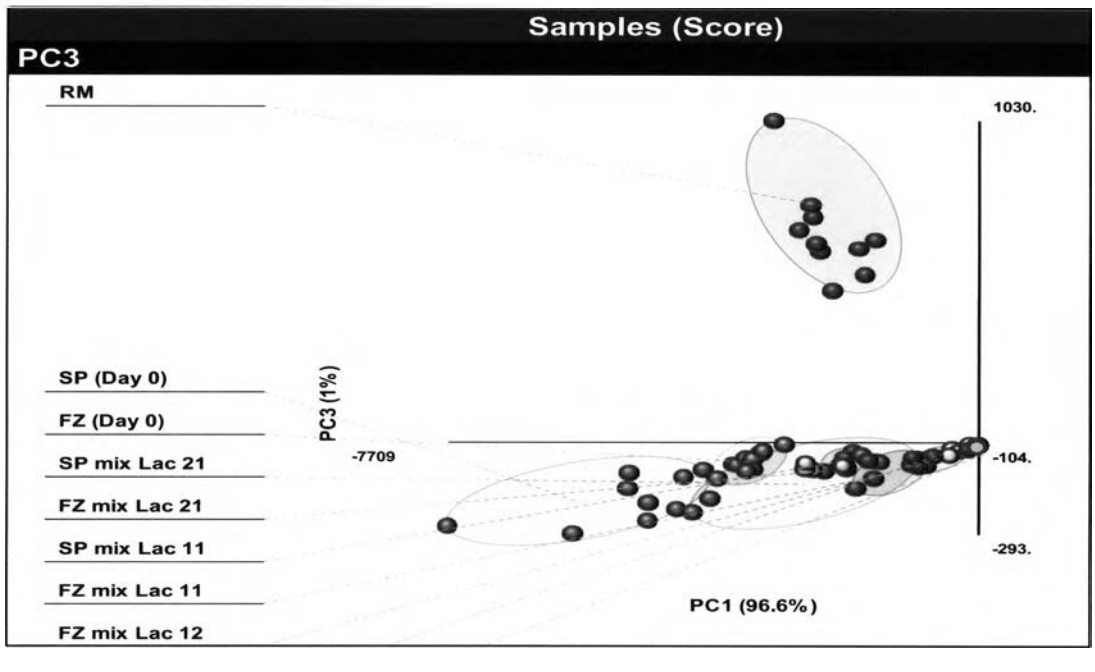



Figure 106 PCA of spray dried and freeze dried samples physically mixed with lactose at ratios of 1:2, 1:1 and 2:1 compare to individual spray dried and freeze dried samples, clopidogrel RM and lactose using Multibase program