Chapter IV

RESULTS AND DISCUSSION

1. Taste-masked azithromycin dry powder preparation

Eudragit® E PO was used as taste-masking agent in the pharmaceutical field, especially for bitter tasting drugs. Azithromycin dihydrate (AZD) is a model drug representing the bitter tasting drug for taste-masking preparation. Taste-masking technique in this study used granulation method to initiate physical barrier around the drug particles. Near-infrared spectroscopy (NIRs) was used as an analyzer for process analysis in at-line mode. The taste-masked azithromycin dry powder was prepared in various types of mixers, as follow:

1.1 Blending in Erweka® AR400 Universal Lab Mixer

Erweka® AR400 Universal Lab Mixer was used for the preliminary blending process studies in the lab scale. The blending capacity of this mixer is generally not more than 1 kilogram. Mixing force from spiral blade in Erweka® AR400 Universal Lab Mixer generates high shear force to blend-in taste-masking agent by physical barrier technique. Resulting taste-masked AZD dry powder was pale yellow color due to Eudragit® E PO coated on drug particles. Eudragit® E PO to AZD in the solid ratios of 0.1:1, 0.2:1, 0.3:1 and 0.4:1 were used. Other physicochemical properties of the taste-masked powder will be described in the next topic. If the solid ratio of Eudragit® E PO to AZD is high, the drug will be highly taste-masked. Other studies (Priti 2011, Alia A. Badawi 2011) had found that high levels of Eudragit® E PO, especially more than half of the drug powder, affect drug dissolution and disintegration. For this reason, Eudragit® E PO was selected in concentrations of not more than half of AZD powder. The taste-masking process using Erweka® AR400 Universal Lab Mixer was used as

proof of concept in applying Process Analytical Technology (PAT) in end-point evaluation or to determine the success parameters in this experiment. These parameters or variables can be applied in the future experiments for large-scale production or when utilizing various mixers that exhibit similar blending procedure.

1.2 Blending in PMS® MG15T high speed mixer

Compare to the 1 kilogram limitation mixing scale of Erweka® AR400 Universal Lab Mixer, high speed mixer has the capacity of blending of approximately 1-7 kilograms which is more suitable for taste-masking AZD dry powder preparation in a larger scale. The high shear force from both agitator and chopper helped in achieving better homogeneity of blending. From previous experiments by Erweka® AR400 Universal Lab Mixer, we obtained the optimal solid ratio of Eudragit® E PO and AZD powder as 0.4:1 and this ratio will be used for scale-up. Final powder mixture was pale yellow and exhibits good flow. This scale-up process utilized two batches of production where it was manufactured for building appropriate quantitative model (calibration and validation). The total number of cycles for taste-masked blending in PMS® MG15T high speed mixer was 20 cycles, with sequential addition of Eudragit® E PO alcoholic solution during each cycle of mixing which did not over-wet the mixture.

2. Physicochemical characterization

2.1 Powder X-ray Diffractometry (PXRD)

The main areas for PXRD analysis are the crystalline characterization and solid structure identification. Crystalline exhibits unique PXRD characteristic pattern that can be used for identification. PXRD is a non-destructive analytical method with unique fingerprint diffraction associated with atomic arrangements in the solid state (Gandhi 2002, Blanco 2004, Timoumi 2014).

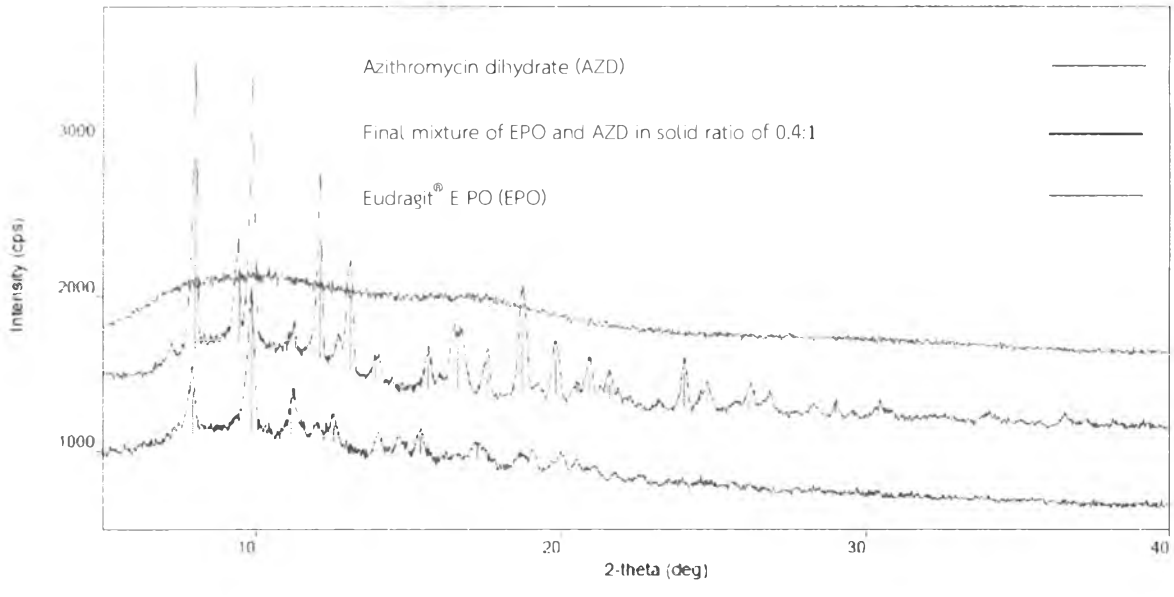

Figure 18 X-ray diffraction patterns for azithromycin dihydrate (blue), Eudragit® E PO (green) and final mixture of Eudragit® EPO and AZD in solid ratio of 0.4:1 (red).

The X-ray diffractograms for azithromycin and mixture samples is shown in Figure 18. The diffraction peaks of final mixture of Eudragit® EPO and AZD in solid ratio of 0.4:1 are at the same positions as the azithromycin dihydrate peaks but with reduction in intensity due to dilution effect of amorphous Eudragit® EPO. In observing the diffraction pattern of the crystalline azithromycin dihydrate demonstrates well-defined diffraction peaks, with emphasized diffraction peaks at 2θ 6° and 10°. The

same peak positions in the two diffractograms indicate that they correspond to the same crystalline structure.

2.2 Polarized light microscopy

Polarized light microscopy pictures of azithromycin dihydrate, Eudragit® E PO and mixture of EPO and AZD in solid ratio of 0.4:1 are shown in Figure 19. They all had different crystal habits. Azithromycin dihydrate initially exhibits birefringent crystals under polarized lens due to its crystalline nature (Figure 19a). When it was taste-masked by opaque Eudragit® E PO (Figure 19b), the mixture did not exhibit any birefringency (Figure 19c).

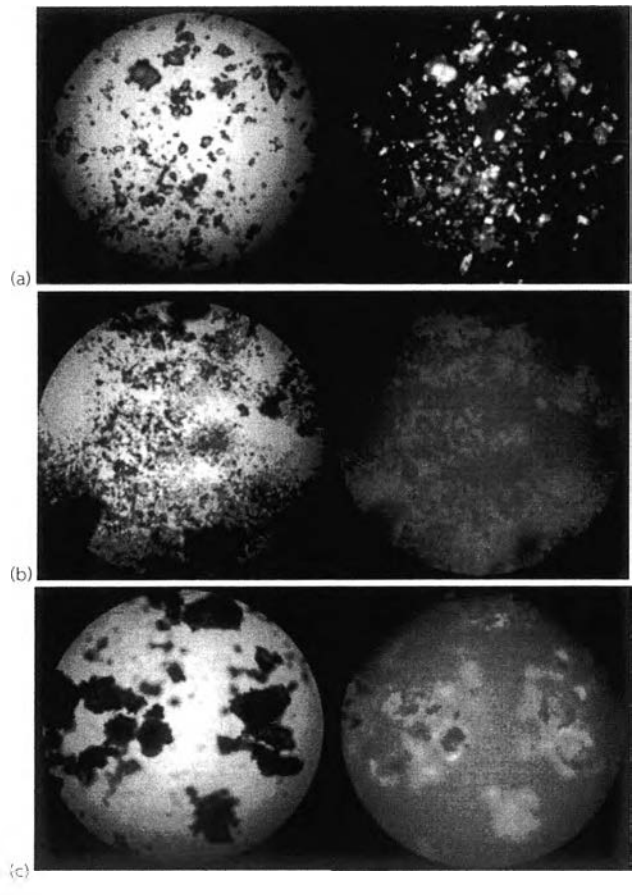

Figure 19 Polarized light photomicrographs of (a) azithromycin dihydrate (AZD), (b) Eudragit® E PO (EPO), (c) final mixture of EPO and AZD in solid ratio of 0.4:1.

Moreover, the powder mixture has the tendency to become opaque and form larger particles according to the combined interaction between taste-masking agent and model API in order to blind the bitter taste of azithromycin dihydrate as the physical barrier.

2.3 Differential Scanning Calorimetry (DSC)

DSC is used for direct enthalpy determination and confirmation of the solid-state and drug-polymer interaction obtained by FT-IR and NIRs spectra. Figure 20 demonstrates the thermograms of the model drug, taste-masking agent and final mixture of EPO and AZD.

The thermal behaviors of azithromycin and Eudragit® E PO exhibit dehydration endotherms of 86.70 and 9.29 J/g, at peak temperatures of 127°C and 55°C, respectively. The DSC profile for the final mixture of EPO and AZD consisted of two endotherms. The first endotherm is in the temperature range of 50-60°C with an enthalpy of 0.26 J/g and the second endotherm in the temperature range of 110-140°C had an enthalpy of 40.77 J/g, corresponding to the thermal energy for dehydration of both starting materials.

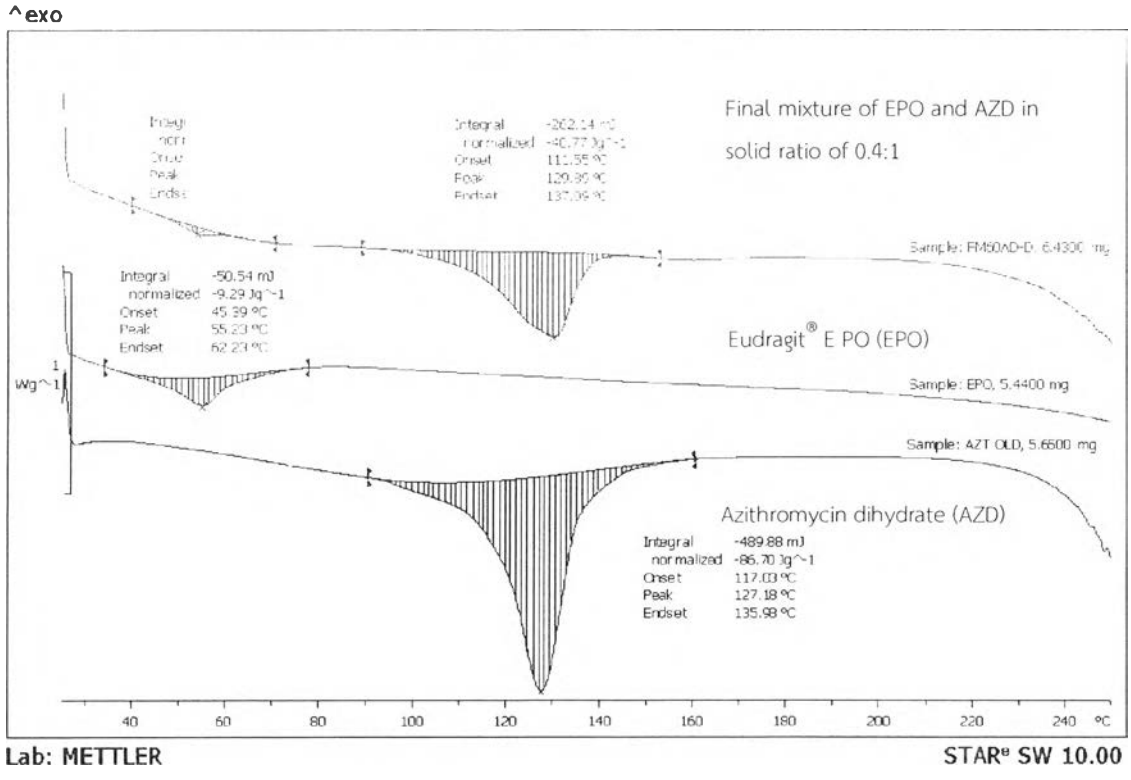

Figure 20 DSC thermograms of AZD (black), EPO (red) and final mixture of EPO and AZD in solid ratio of 0.4:1 (blue).

The property of Eudragit® E PO affected the peak area that demonstrates to reduce the amount of energy from 86.70 (AZD) to 40.77 J/g (final mixture). The reduced energy (endotherm) was about 46 J/g. The melting point temperature (T_m) of AZD and EPO are stable. T_m of AZD and Eudragit® E PO are about 127-130 °C and 53-55 °C, respectively.

2.4 Thermo Gravimetric Analysis (TGA)

TGA thermograms obtained from the final mixture of EPO and AZD in solid ratio 0.4:1 before and after drying are given in Figure 21 – 23.

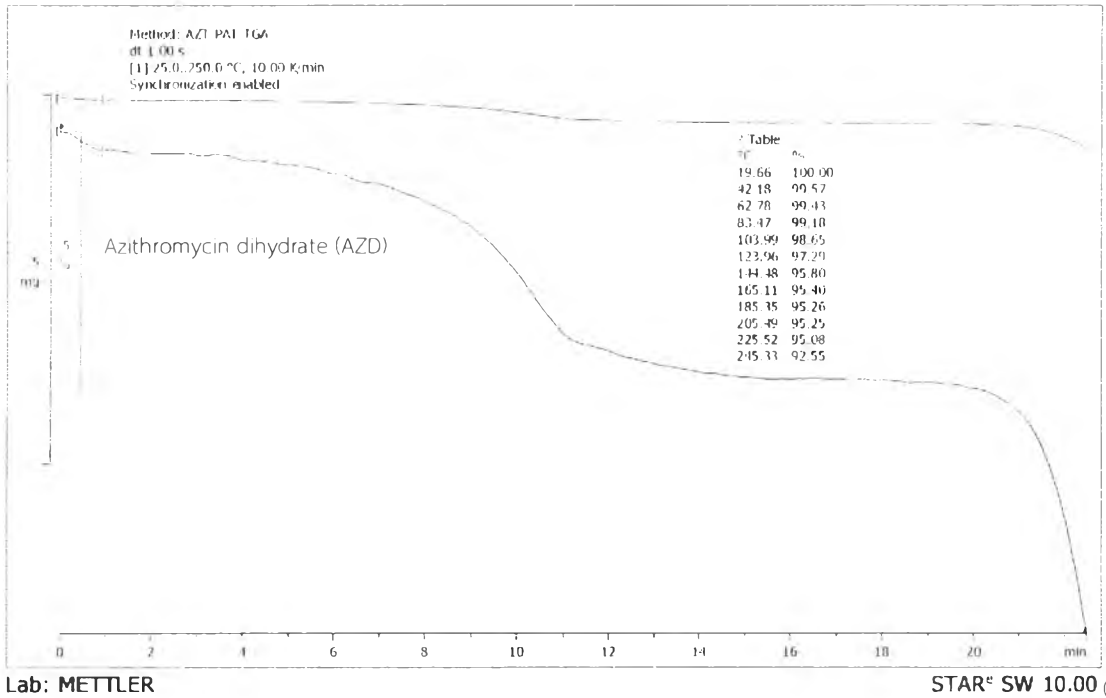

Figure 21 TGA thermogram of azithromycin dihydrate (AZD).

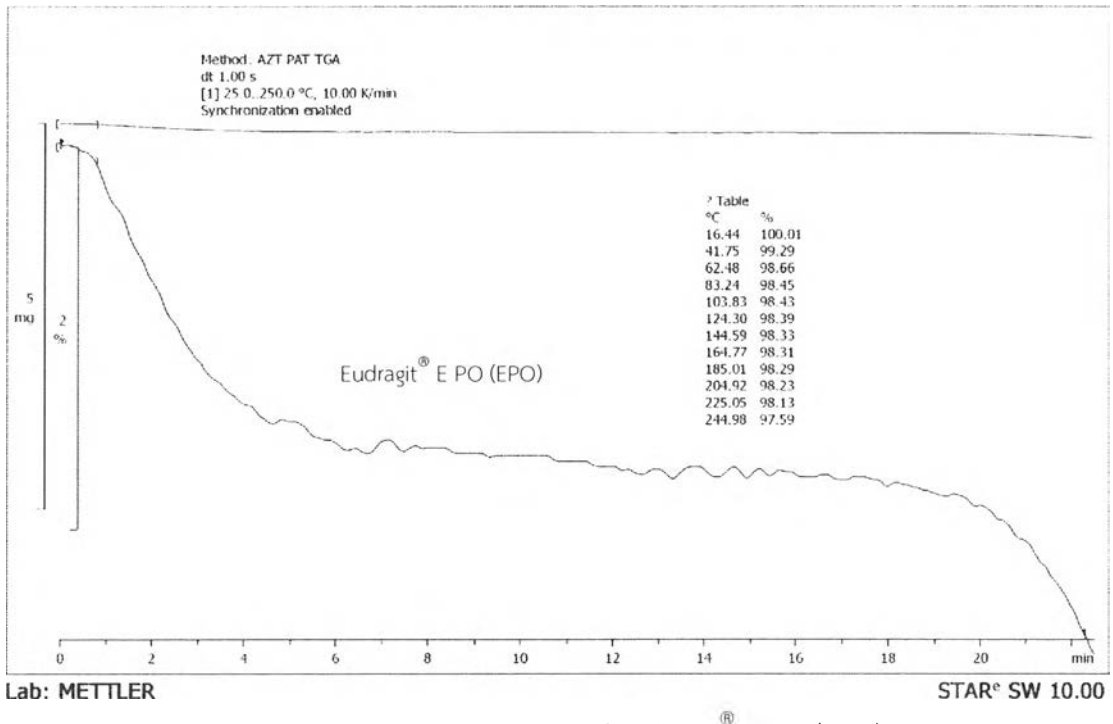

Figure 22 TGA thermogram of Eudragit® E PO (EPO).

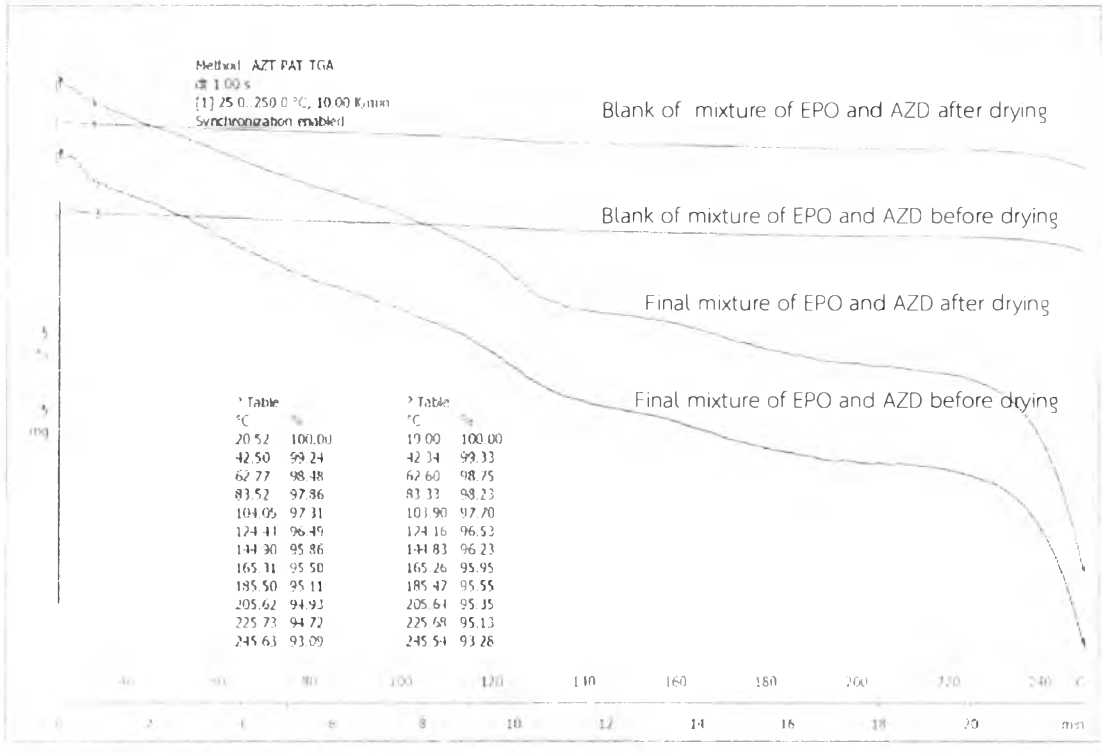

Figure 23 TGA thermograms of final mixture of Eudragit® E PO and AZD in solid ratio of 0.4:1 before and after drying.

From the Figures 21-23, TGA measures weight change associated with related thermal events obtained by DSC. The weight change of TGA measurement in AZD (Figure 21) was reduced approximately by 4.32% starting at approximately 100°C corresponded to the stoichiometric weight loss of two water molecules (4.38%). Weight change of Eudragit® E PO (Figure 22) are around 1.87% starting at 30°C. The final mixture of EPO and AZD in solid ratio of 0.4:1 before drying and after drying (Figure 23) show similar weight loss of 4.28%. The dehydration started as soon as the sample was placed under the nitrogen flow at 25°C, proceeded progressively, and was completed at 250°C. These thermograms were not significantly altered by the dehydration process using hot air oven.

2.5 Fourier Transform Infrared spectroscopy (FT-IR)

The resulting spectrum represents molecular absorption and transmission, creating a molecular fingerprint of the sample which correspond to the frequencies of vibrations between the bonds of the atoms making up the material.

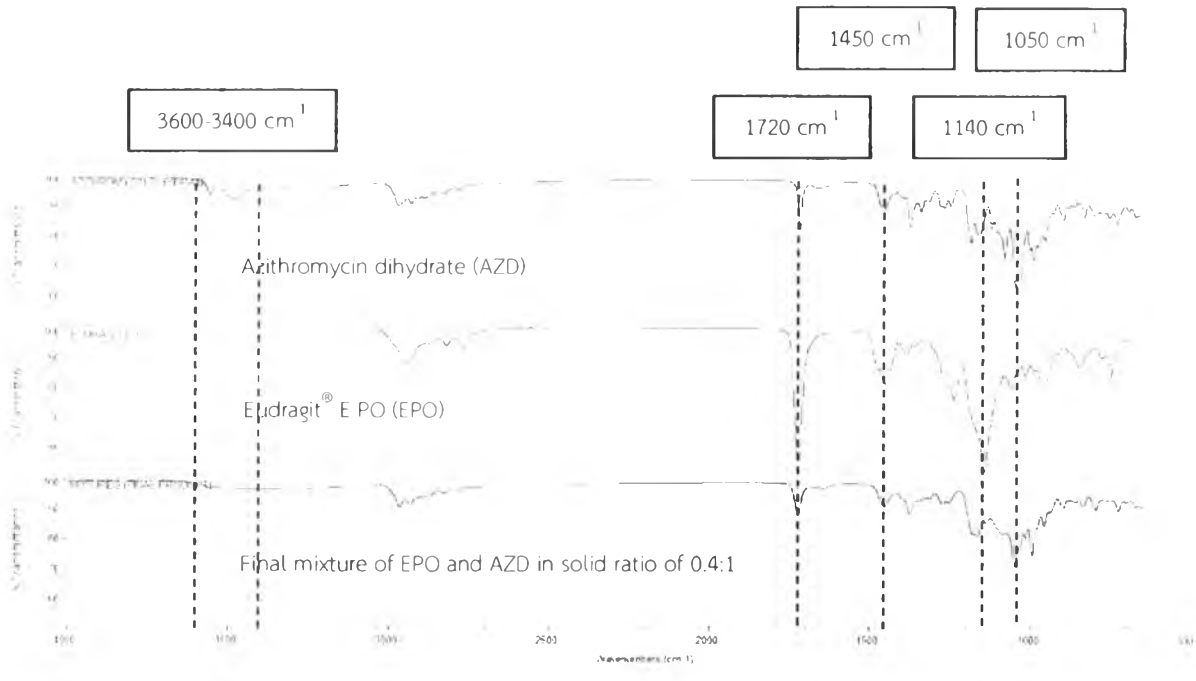

Figure 24 FT-IR spectra of AZD (purple), EPO (red) and final mixture of EPO and AZD in solid ratio of 0.4:1 (blue).

Figure 24 represents FT-IR spectra of the model API, taste-masking agent and the final mixture of Eudragit® E PO and AZD in solid ratio of 0.4:1. The AZD signals are sharp and the fingerprint areas are at wavenumbers 3600-3400 cm^{-1}, 1800-1700 cm^{-1} and 1400-1300 cm^{-1} which are O-H stretching vibrations (broad-intermolecular hydrogen bonding), C=O carbonyl ester stretch vibrations (overtone; 3400 cm^{-1}) and C-N (3^{o} amine) medium stretch vibrations, respectively. No new bands or band shifts can be seen in the mixture spectrum. The mixture of Eudragit® E PO and AZD FT-IR spectrum shows that the IR absorption band disappears in region of wavenumber

3600-3400 cm^{-1} and reduces the intensity in %transmittance in the wavenumbers of 1720, 1450, 1140 and 1050 cm^{-1} due to the association of AZD with Eudragit® E PO and form coupled vibrations. The presence of O-H stretch vibrations of alcohol groups in between wavenumber 3600-3400 cm^{-1} indicates that some independent azithromycin molecules are still present. Not all drug molecules interacted with the taste masking agent as seen by presence of peaks at wavenumbers 1800-1700 cm^{-1} and 1400-1300 cm^{-1}. According to the combined interaction between taste-masking agent and model bitter API, the bitterness of AZD is masked by the chemical interactions of carbonyl group in 15-membered ring of AZD with EPO causing steric effect to reduce the affinity to the bitterness receptor.

The chemical structure of AZD from Figure 1 in chapter 2 shows the pharmacophore and major functional group of azalide. From the studies of both Akre (2012) and Menella (2013) team, found that N-atom and carboxyl ester group in the derivative form of erythromycin A represented the bitter taste of azalide drug. Eudragit® E PO as taste-masking agent interacted with carboxyl ester group in 15-membered ring of AZD by C=O stretching in Eudragit® E PO polymer chain. From this reason, the bitter taste of AZD was decreased by combined interaction of Eudragit® E PO polymer to form a steric effect on chemical structure of AZD at the position of carbonyl group in 15-membered lactone ring. This interaction decreased the affinity of AZD to the bitter taste receptor and forming coated AZD particles. The higher range of wavenumbers are measured and described by NIR spectroscopy.

2.6 Near-infrared spectroscopy (NIRs)

In NIRs, the samples are irradiated with NIRs light. Some of NIRs light is absorbed by the molecules, leading to a higher vibrational state where a change in dipole moment of absorbed NIRs irradiated molecules. Due to the combined interactions between model API and taste-masking polymer, the spectral absorbance of final mixtures of AZD and Eudragit® E PO in Figure 25 was reduced compared to AZD spectrum. Hydrogen bonding groups have the strongest overtones as the dipole moment is high. Also O–H, C–H bonds are strong NIRs absorbers. Bending is defined as a change in bond angle. The 1^{st} and 2^{nd} C–H stretch overtone vibrations occur near 5900 cm^{-1} and 8450 cm^{-1}, respectively. An O–H combination and overtone occur near 5120 cm^{-1} and 6920 cm^{-1}, respectively.

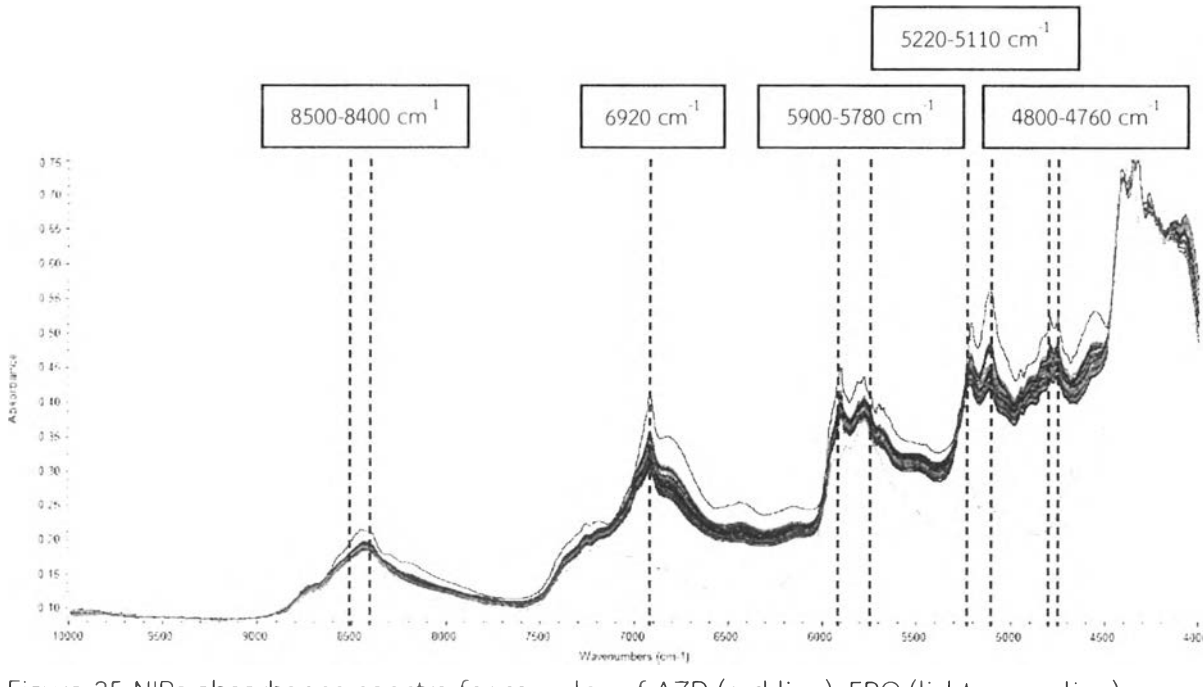

Figure 25 NIRs absorbance spectra for samples of AZD (red line), EPO (light green line) and final mixtures of AZD and EPO (other lines).

The C=O and C-O stretching around 6,900 cm^{-1} and 5,900 cm^{-1} were the part of carboxyl ester functional group in Eudragit® E PO. It interacted with carboxyl ester group in 15-membered ring of AZD. After taste-masking by Eudragit® E PO, the bitter taste of mixtures were eliminated by the reduction in absorbance about wavenumber 8400 cm^{-1} (O-H stretching), 6900 cm^{-1} (C=O stretching) and 5800 cm^{-1} (C-N stretching). The results from conventional analysis for taste-masking AZD dry powder preparation were confirmed by this NIRs secondary method.

3. Process Analytical Technology (PAT) of at-line applications in taste-masked azithromycin dry powder blending process

In the last few years, NIRs was used for quantitative and qualitative analysis due to its high efficiency (De Beer 2011, Scheibelhofer 2013, Burggraeve 2013). Many such usages have focused on the model API determination in pharmaceutical production (Peinado 2011, Rahman 2012) or physical parameters of the sample (Vanarase 2010, Martinez 2013). Others have allowed in the starting material characterization and identification or even the final product monitoring.

3.1. Qualitative analysis

Qualitative analysis refers to sample classification following NIRs spectral analysis from taste-masked azithromycin dry powder blending process using Erweka® AR400 Universal Lab Mixer. The NIRs model uses for qualitative evaluation for API or excipients identification in pharmaceutical products. In this part of qualitative analysis, attempts were made to prove the ability of this qualitative model by comparing the primary and secondary methods.

Monitoring of all steps in the process of drug production utilized NIRs technique, including starting materials analysis, in-process control and final mixture. The secondary method is another procedure to confirm and verify the primary method in a faster and easier way to analyzed and interpret the data.

3.1.1 Primary method

The homogeneity end-point for taste-masked blending process could be monitored by microscopy and infrared spectroscopy for physical and chemical characterization, respectively. The results on physical morphology and chemical spectra were already discussed in the topic of physicochemical characterization.

3.1.2 Secondary method (Principal Component Analysis: PCA)

The results are supportive for the application of PCA as a qualitative tool for determination of the homogeneity end-point of taste-mask blending process. The aim of this study was to demonstrate the potential of NIR spectroscopic technique to detect the homogeneity end-point of taste-mask blending process. This process eventually will obtain a suitable homogeneity and compatibility between bitter drug and taste-masking agent. Preprocessing of spectral data between wavenumber range 10000 cm^{-1} – 4000 cm^{-1} is by first derivative using the Norris derivative filter with a segment size of 9 and difference of 7. The first derivatives could eliminate the variations of baseline offset between samples, and improve the overlapped spectral resolution features (Rajalahti and Kvalheim 2011). The spectral transformation can be found in Figure 26. This transformation is regression procedure to reduce the impacts of varying baseline, variable path lengths and high stray lights due to scattering.

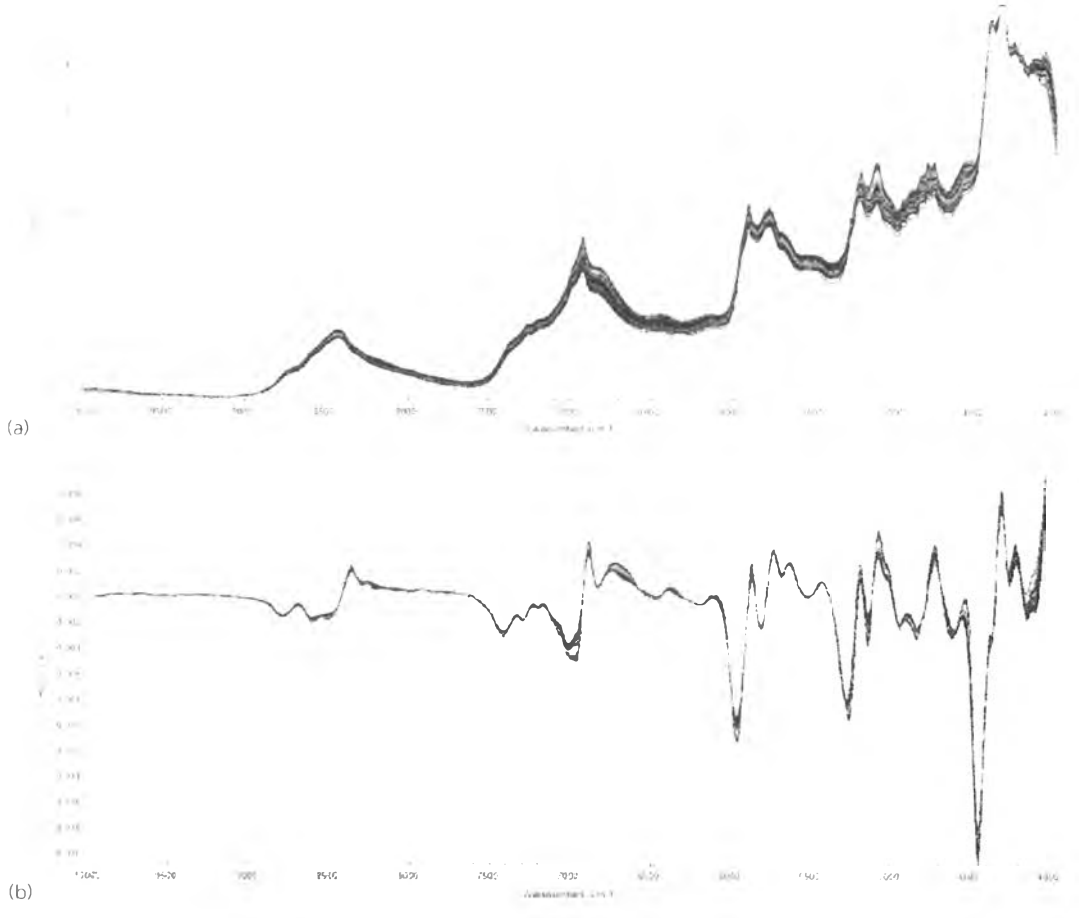

Figure 26 NIRs absorbance for mixtures of AZD and Eudragit® E PO spectral transformation from (a) conventional spectra to (b) the first derivative filtering pretreatment.

From Figure 26 shows the ability of pretreatment to remove or decrease these correlated responses, decreasing the burden on the mathematics model to construct a fit method. This spectral data were used to develop qualitative model by a chemometric Principal Components Analysis (PCA), which is a method for the visualization of complex data by dimensional reduction.

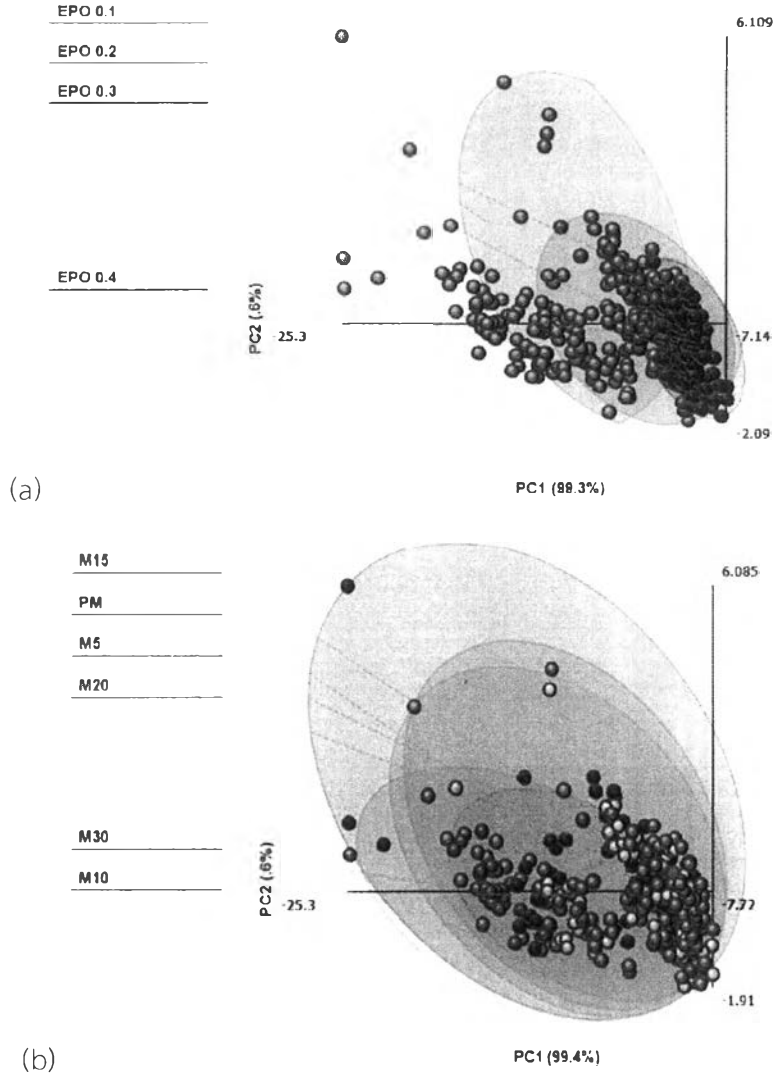

Figure 27 Principal Component Analysis (PCA) for (a) Eudragit® E PO used in AZD dry powder preparations from 0.1 to 0.4 and (b) blending time from premixing (PM) time to 30 minutes (M30).

NIRs is a non-destructive analysis for the qualification of mixture utilizing chemometric methods such as PCA. The variation of data in the NIRs analysis is a common main barrier, which may be due to multiple scattering phenomena and baseline shifts. Method to minimize these effects is derivatization.

A powerful qualitative tool was chosen as PCA for the dissimilarities identification within the experiments. We used Multibase® 2014 software, which is a process of unsupervised classification to clarify the effects of different mixer settings on the samples. Figure 27 shows the score plot for the spectral data of 4 blending trials (B_1-B_4). They are the first principal component (PC) accounts for 99.3% of the variability, showing four clusters that refer to the four different taste masking polymer ratios. The data attributions were studied by PCA application using three PCs on the raw NIRs spectra. PCA analysis for blending time shows that PC1 represents 99.4%, PC2 accounts for 0.6% and PC3 accounts for 0.1% or less for the variation in raw data. The PCs resulting from the principal component transformation are ordered in descending manner by their level of information, but still have the same dimension as the original data. Finally, the fitting of the model is evaluated with regards to the representation of the original variables in the new space and regarding potential outliers that can severely distort the results.

NIRs could be used for optimal taste masking polymer ratio identification on final mixture of Eudragit® E PO and AZD. PCA clarified similarities and trends on the information as observed on the scores plot in Figure 27. The optimal taste masking agent ratio to azithromycin and blending time were 0.4:1 and 20-30 minutes, respectively. These optimal factors were selected by calculating the Eigen values which resulted in the values nearest to the PC1 and PC2 axes. The ratio of Eudragit® E PO could be added in a ratio of more than 0.4, but it must be controlled due to concerns relating to the pharmacopoeial requirements on the dissolution profile of "azithromycin for oral suspension" when using Eudragit® E PO as taste-masking agent. The blending time could be more than 30 minutes, but in the pharmaceutical

industry, it is not preferable to be used in the manufacturing process. The results are proofs that the application of PCA can be used for constructing a qualitative multivariate model to analyze the homogeneity end-point of taste-mask blending process.

3.2 Quantitative analysis

In order to set a quantitative model between the spectral and the physicochemical parameters of the sample, the NIRs spectral characteristics required using multivariate calibration analysis. Quantitative analysis refers to the analysis of samples following NIRs spectral data were obtained from taste-masked azithromycin dry powder blending process utilizing PMS® MG15T high speed mixer.

3.2.1 Primary method

The parameter values have to be considered first by using a primary method in order to produce a calibration model. The quality of the result obtained with a multivariate analysis model can never exceed the quality of the primary method. The calibration model should be carefully evaluated as the quality obtained will affect the prediction model.

A validated method for azithromycin dihydrate analysis by HPLC, equipped with UV detector at wavelength of 210 nm, was developed. It also can be employed for the azithromycin dihydrate analyses at various concentrations and in different drug formulations as well as in the starting material.

The reason for using HPLC as primary method is for quantitative analysis of the percentage labelled amount (%LA) of AZD in the mixture. A relationship between taste masking process and percentage labelled amount analysis was

described by AZD amount in the mixture and %Relative Standard Deviation (RSD). The results are shown in Table 4.

Table 4 The proportion of AZD, in average percentage, of each of mixture from each cycle with their standard deviations (SD) and %RSD during taste masking process using PMS® MG15T high speed mixer.

% drug amount of azithromycin dihydrate in mixture of AZD and EPO							
cycle	average	SD	%RSD	cycle	average	SD	%RSD
1	97.68	8.58	8.78	11	91.58	5.10	5.57
2	96.80	7.24	7.48	12	92.86	4.07	4.38
3	96.84	7.85	8.10	13	91.91	4.76	5.18
4	97.64	6.51	6.66	14	93.20	5.10	5.48
5	96.23	7.11	7.39	15	94.31	4.28	4.54
6	96.91	6.98	7.20	16	96.22	3.54	3.67
7	92.46	5.65	6.12	17	97.33	2.95	3.03
8	93.36	4.98	5.33	18	96.83	3.37	3.48
9	93.55	5.20	5.56	19	97.51	2.77	2.84
10	93.80	3.96	4.23	20	98.55	2.36	2.40

The data in Table 4 shows the trend of average %AZD amount in mixture of Eudragit® E PO and AZD. The final solid ratio of Eudragit® E PO to AZD is 0.4:1. The data were calculated as average percentages, their standard deviations (SD) and % relative standard deviations (%RSD). The behavior of taste-mask blending process are shown during the 20 cycles used. Percent Eudragit® E PO at the range of 40-80% indicates by low drug amounts of below 95% (during cycles 7-15). It means that the

higher amount of Eudragit® E PO does not necessarily have to thoroughly interact with AZD particles in an increasing manner.

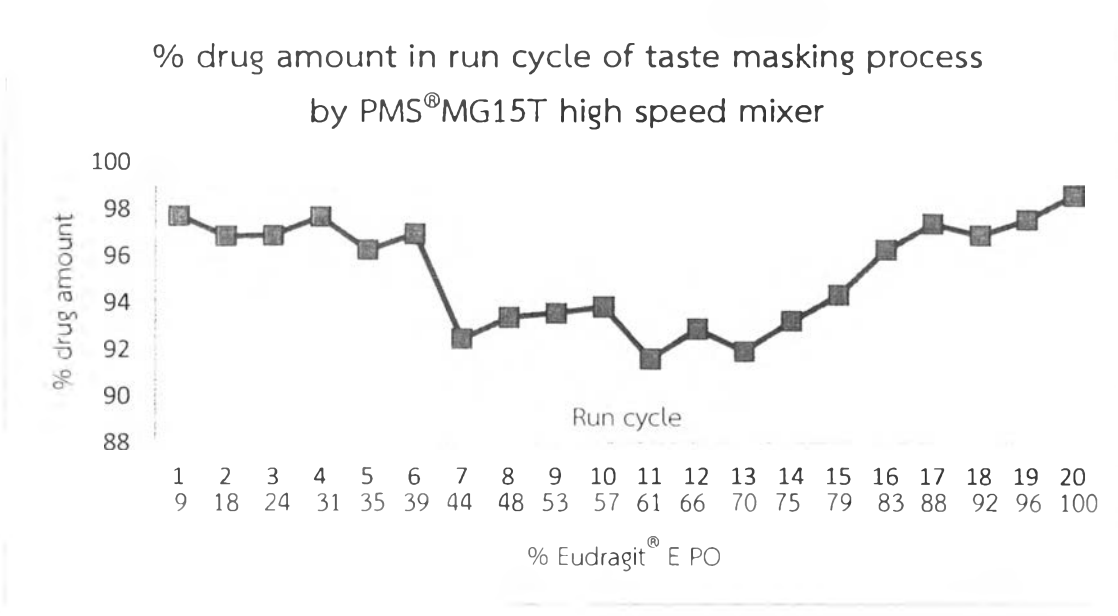

Figure 28 The average percent AZD amounts analyzed during 20 run cycles when mixed with Eudragit® E PO by PMS® MG15T.

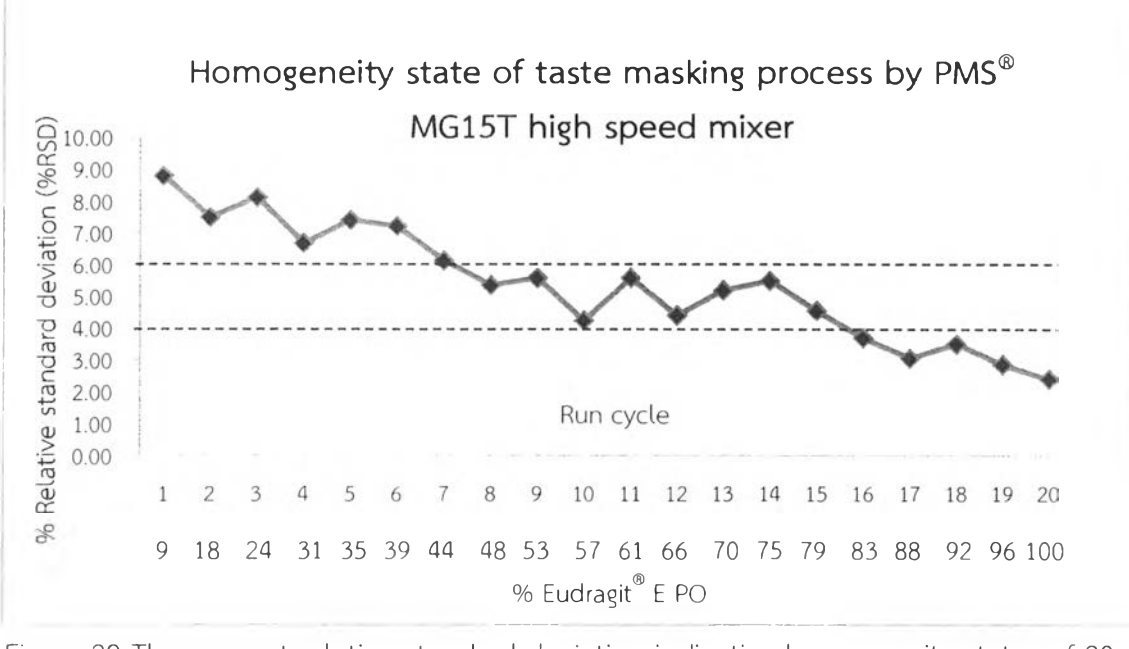

Figure 29 The percent relative standard deviation indicating homogeneity states of 20 run cycles mixing by PMS® MG15T.

Remark: criteria: the RSD value should be used to classify the testing results as either readily pass (RSD ≤ 4.0%), marginally pass (RSD ≤ 6.0%) or inappropriate for demonstration of batch homogeneity (RSD > 6.0%). (USFDA, 2003)

This method not only describes blending uniformity of AZD and EPO mixtures, but also describes the homogeneity state of mixing of this system when PMS® MG15T was used. Figures 28 and 29 show data of blending uniformity as %drug amount and %RSD as 3 homogeneous states. The first state is between run cycle 1 to 6 with %RSD of >6.0% , the second state is run cycle 7 to 15 where the % RSD between 4-6%, and the third state is run cycle 16 to 20 where %RSD falls below 4%. The model drug was sprayed by Eudragit® E PO solution of about 39% at the first state, and found that was unstable mixture due to the amount of model drug was still more than Eudragit® E PO. Then, the mixture was increasingly sprayed at the second state, which caused more stable mixture (when EPO is about 80%). At the last state, it was presumably close to steady state of mixing because Eudragit® E PO was found to be evenly coated on azithromycin dihydrate powder. From using %RSD to identify homogeneity state, it can be used to identify suitable homogeneity end-point for taste-mask blending process and was found to be at the third state by obtaining %RSD values of lower than 4.

3.2.2 Secondary method (Partial Least Squares regression: PLS)

Most common objective of quantitative multivariate analysis in PAT applications are to transform non-specific analyzer to a specific one and to enable its effective use for a specific application. The resultant quality is determined in terms of parameters, such as the Root Mean Square Error of Prediction (RMSEP) or

the Relative Standard Error of Prediction (RSEP). A set of samples not assigned in the calibration method is required for prediction in order to verify the suitable model.

The labelled amount values from HPLC analysis were used for computer calculations to generate PLS regression model. The actual and the calculated axes refer to the labelled amount of AZD from conventional analysis (primary method) and the ideal labelled amount of AZD, respectively. The primary method results from HPLC analysis are shown to be linearly related to the PLS regression model.

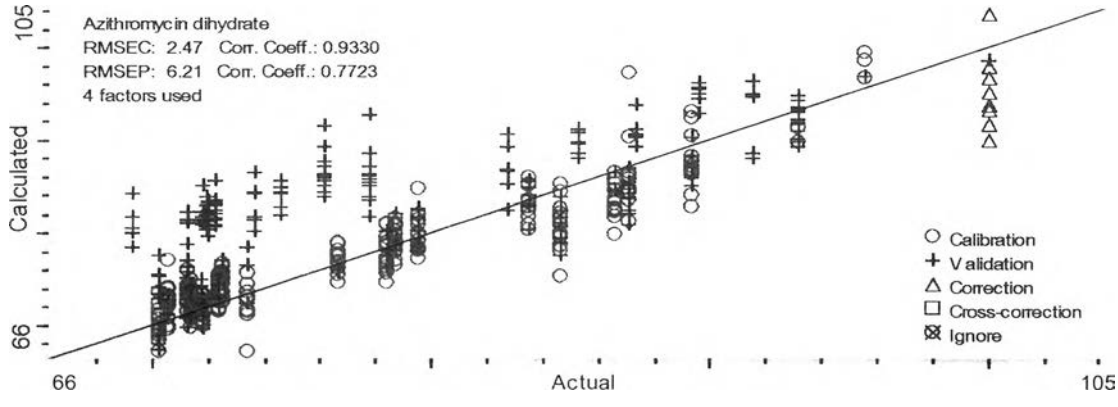

Figure 30 The linear regression model for non-pretreated NIRs calibration and validation spectra.

The NIRs multivariate calibration and validation model in Figure 30 shows that all spectra in calibration and validation sets are used for PLS model building. The r values are 0.9330, 0.7723 for calibration and validation, respectively. Root Mean Standard Error of Calibration (RMSEC) and Prediction (RMSEP) are 2.47 and 6.21, respectively. All spectral data need to reduce the possible error and variations by an appropriate pretreatment.

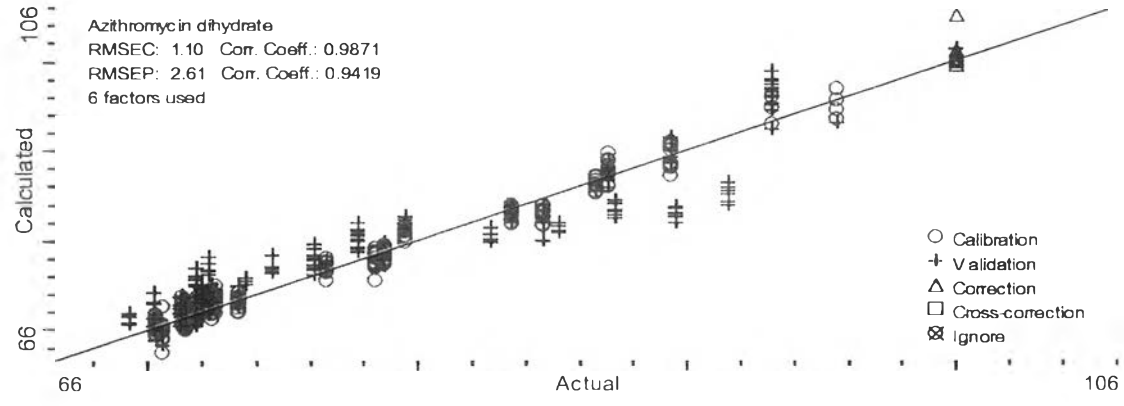

Figure 31 The linear regression model for NIRs calibration and validation by first derivative pretreatment.

The PLS models in Figures 31 and 33 are pretreated by chemometric quantitative analysis. The first derivative is mostly suitable for pre-treatment of PLS models in both calibration and validation set. The important values of first derivative show the least of error, which RMSEC, RMSEP are 1.10 and 2.61, respectively. The r value is 0.9871 for calibration set and 0.9419 for validation set.

In NIRs applications, a 1st derivative pretreatment is usually effective in eliminating the variation baseline offsets in the spectral data. The results of 2nd derivative pretreatment remove the differences in baseline offsets and baseline slopes between spectra.

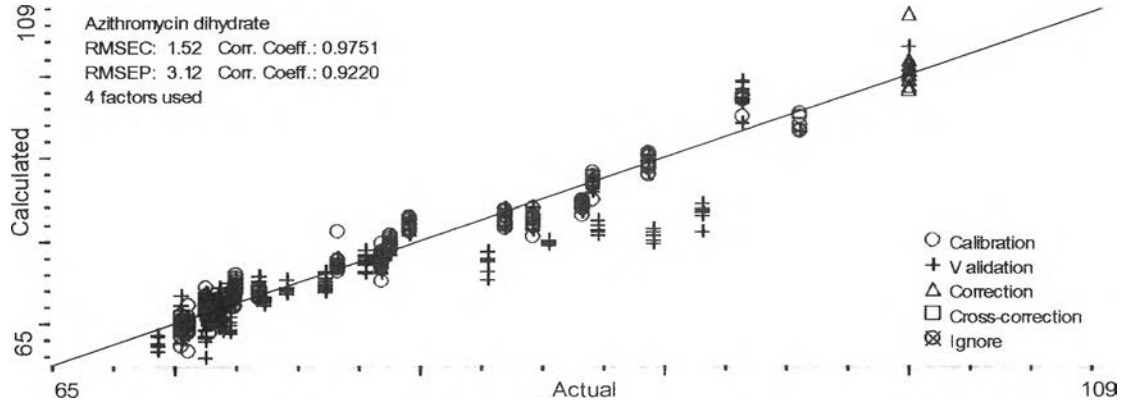

Figure 32 The linear regression model for NIRs calibration and validation by first derivative pretreatment with Savitzky-Golay filter.

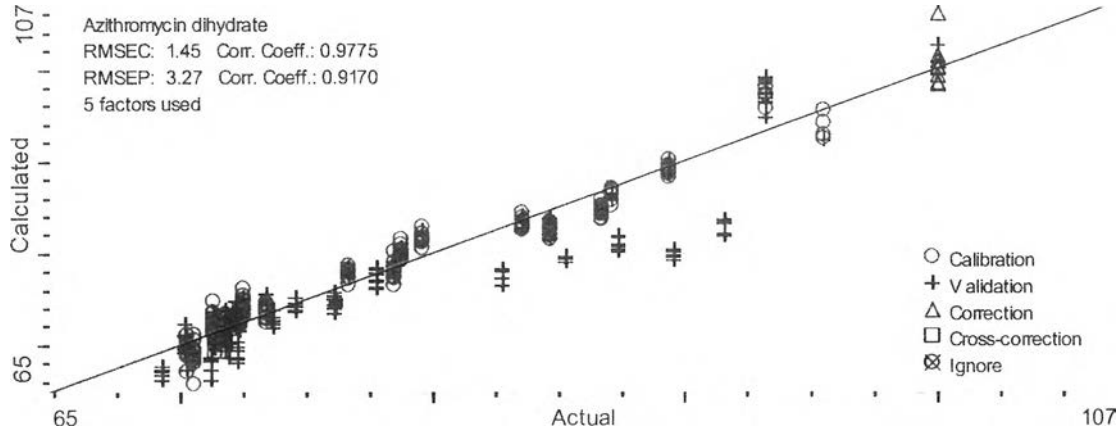

Figure 33 The linear regression model for NIRs calibration and validation by first derivative pretreatment with Norris derivative filter.

The filtering pretreatment process can be utilized whenever the variables are expressed as a physicochemical characteristic. Derivative filters can eliminate the variations of baseline offsets between samples. Therefore, it can adjust the overlapping spectral resolution patterns. Savitzky-Golay filter was modified to enable both derivative filter and smoothing computerization on discrete data.

This filter consists of a predefined coefficients data sets described by three parameters; polynomial order, window width and derivative order. Smoothing associates with derivative filter, was determined by the window width. It can be beneficial to utilize higher window width for high-noise data, although this also may improve the derivative resolution. The best fits of the windows of local data set to a 2^{nd} order polynomial was computerized based on derivative filter.

Norris derivative filter is a procedure to remove the effects of varying path lengths among samples because of scattering effects. Norris derivative optimized gap-size and smoothing segment separately, such as Norris regression. From Figures 32 and 33 demonstrate the linear regression by derivative pretreatment. In Figure 32,

Savitsky-Golay filter is calculated by data points as 7 and 3rd polynomial order. Norris derivative filter in Figure 33 is integrated by segment length of 5 and gap between segments of 5. Statistical values from above Figures are concluded in Table 5.

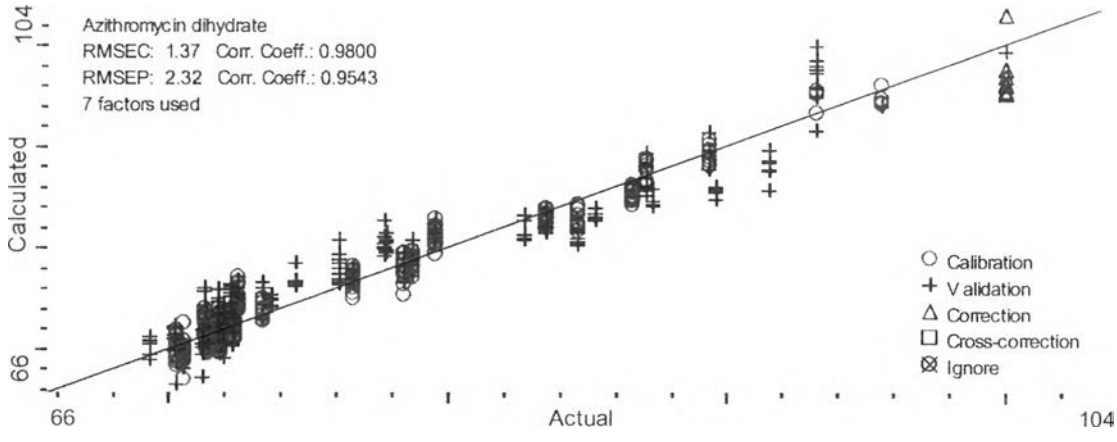

Figure 34 The linear regression model for NIRs calibration and validation by secondary derivative pretreatment with Savitsky-Golay filter.

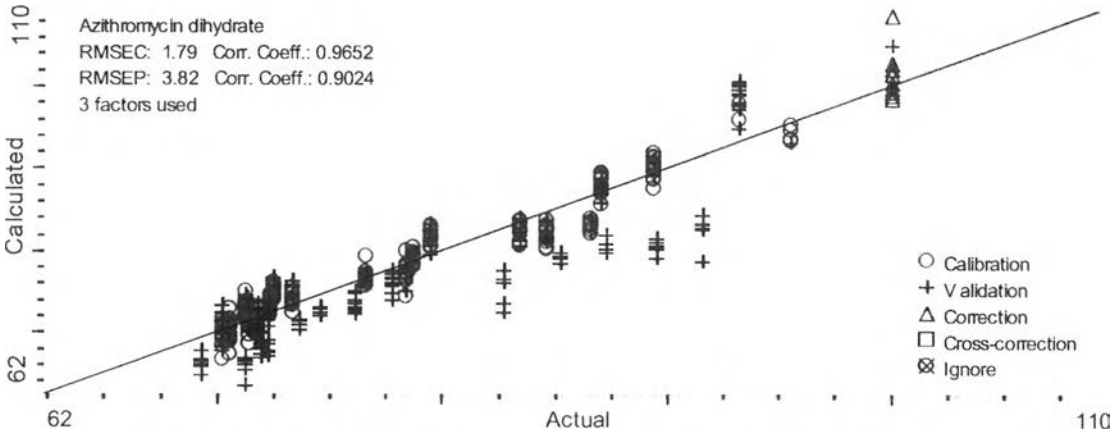

Figure 35 The linear regression model for NIRs calibration and validation by secondary derivative pretreatment with Norris derivative filter.

Data in Table 5 use centering technique and correct with calibration and correction standards. The factor is used to optimize number of factors each time the calibration is changed. Multivariate analysis by PLS is not use to multipoint baseline correction. The values from calibration and validation models prove the tool is suitable for this taste-masked blending process. This proper tool of stated method is PLS regression with first derivative pretreatment because the value of root mean square error of calibration and prediction from this tool are the lowest.

From the linear regression of PLS model in Figures 30-35 demonstrate that the possible homogeneity end-point for taste masking process is in the lower zones of actual and calculated axes. It refers to the third state of homogeneity in the primary method result. All data of this state are correlated linearly by secondary method.

The major values of PLS regression model are Root Mean Square Error of Calibration (RMSEC), Root Mean Square Error of Prediction (RMSEP) and correlation coefficient (r). These values discussed about the homogeneity end-point of taste mask process and the possibility of the PLS model application. The criteria for choosing the proper model was determine by the least of RMSEC and RMSEP with the highest r in the calibration and validation models.

Table 5 Development of statistical model by partial least square: PLS.

Data format	Calibration set		Validation set		Factor used (factor calculated:10)	Performance index
	RMSEC	r	RMSEP	r		
Spectrum *(No pre-treatment)*	2.47	0.9330	6.21	0.7723	4	75.2
1st derivative *(no smoothing)*	**1.10**	**0.9871**	**2.61**	**0.9419**	**6**	**89.6**
1st derivative *(Savitzky-Golay filter)*	1.52	0.9751	3.12	0.9220	4	87.5
1st derivative *(Norris derivative filter)*	1.45	0.9775	3.27	0.9170	5	86.9
2nd derivative *(Savitzky-Golay filter)*	1.37	0.9800	2.32	0.9543	7	90.7
2nd derivative *(Norris derivative filter)*	1.79	0.9652	3.82	0.9024	3	84.7

The spectrum data format had highest values of RMSEC and RMSEP, because spectral data was not pretreated by preprocessing method. These high values refer to high variations. It was found the lowest performance index is not appropriate to be used as quantitative model. The most suitable format for these spectral data is 1st derivative with no smoothing due to the lowest values of RMSEC and RMSEP. Moreover, the performance index of this format is at the highest value of 89.6 with 6 factors used for calculation.

The RMSE of calibration and prediction comparison shows that RMSEP values are not more than 3 times that of RMSEC. These values infer a good precision of calibration model assessment (Shenk and Westerhaus 1996). The application ability of calibration and validation model are described by correlation coefficient. From Table 5 shows the r values of calibration and validation models. The r values of calibration model by pretreatment are within the range of 0.96-0.98 which means that the ability of this model to be used for research or for quality assurance is high. In addition, the r values of validation by pretreatment are in the range of 0.91-0.95 which means that the ability of model to be used for research and general application is high.

3.3 Critical sampling points evaluation

A representative sample is a sample that resembles the total population. It is necessary to utilize a sampling method, which originates representative samples when the aim was to understand a population characteristics based on a sample study. From the literature reviews found that, the critical point of mixing were dead spot or space, central area of mixing bed, gap density area and segregation area. Moreover, the information from pharmaceutical manufacturer was determined to define sampling point for blending monitor as recommendation area. For example, the area that was near to spray gun, an axis of agitator or blade, and the center of mixture bed (De Beer, Burggraeve 2011, Martinez 2013, da Silva 2014, Hansuld and Briens 2014).

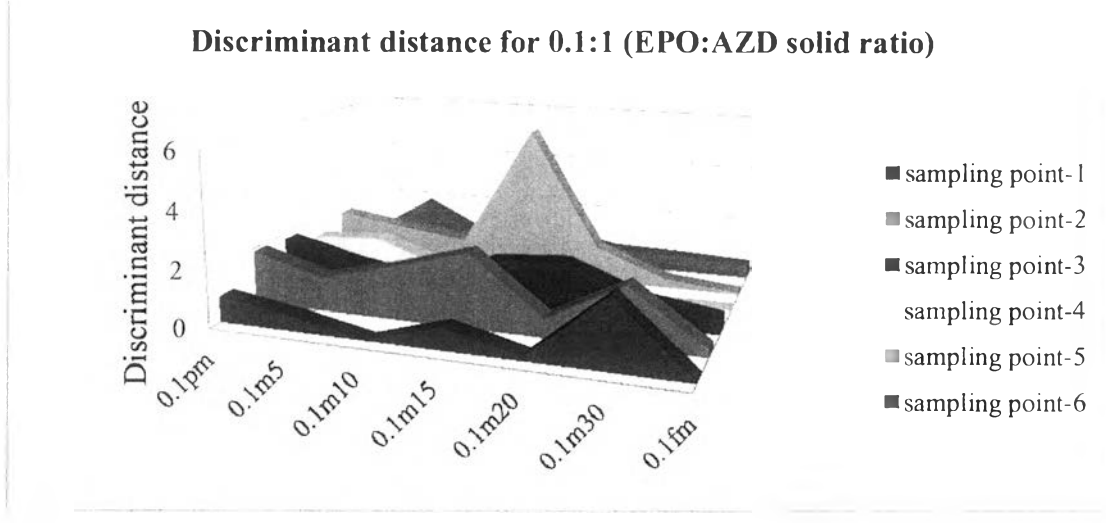

Figure 36 Discriminant distance for sampling points of Eudragit® E PO and AZD solid ratio of 0.1:1 in Erweka® AR400 Universal Lab Mixer.

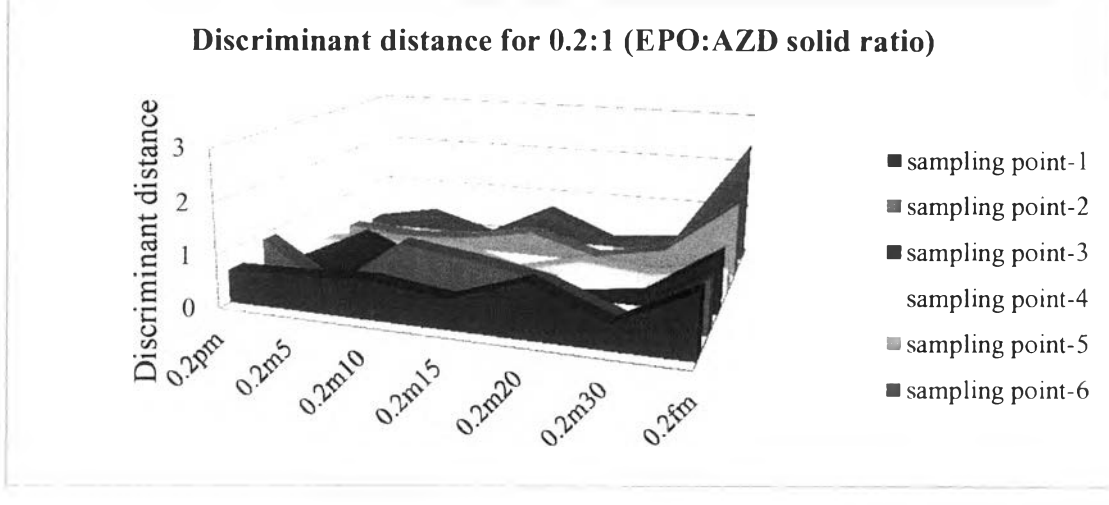

Figure 37 Discriminant distance for sampling points of Eudragit® E PO and AZD solid ratio of 0.2:1 in Erweka® AR400 Universal Lab Mixer.

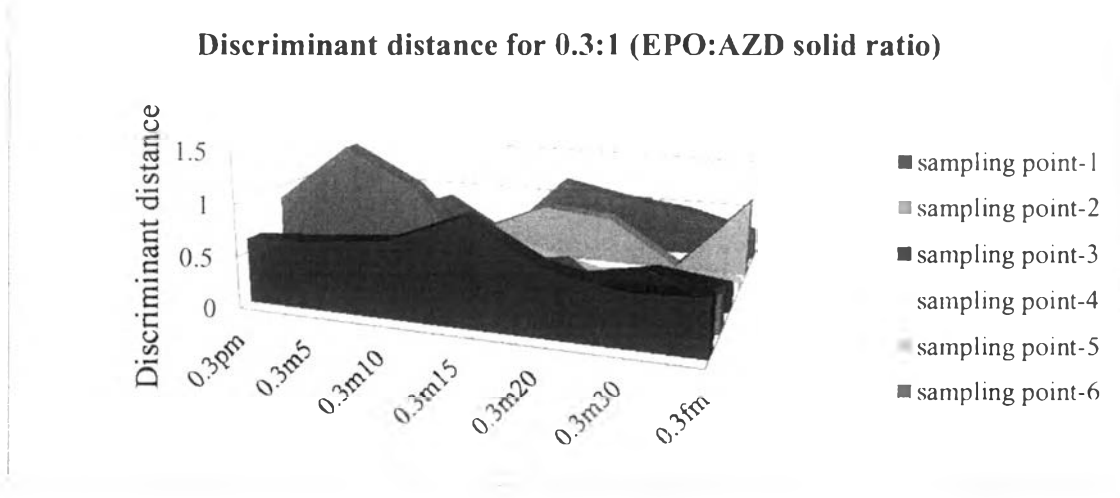

Figure 38 Discriminant distance for sampling points of Eudragit® E PO and AZD solid ratio of 0.3:1 in Erweka® AR400 Universal Lab Mixer.

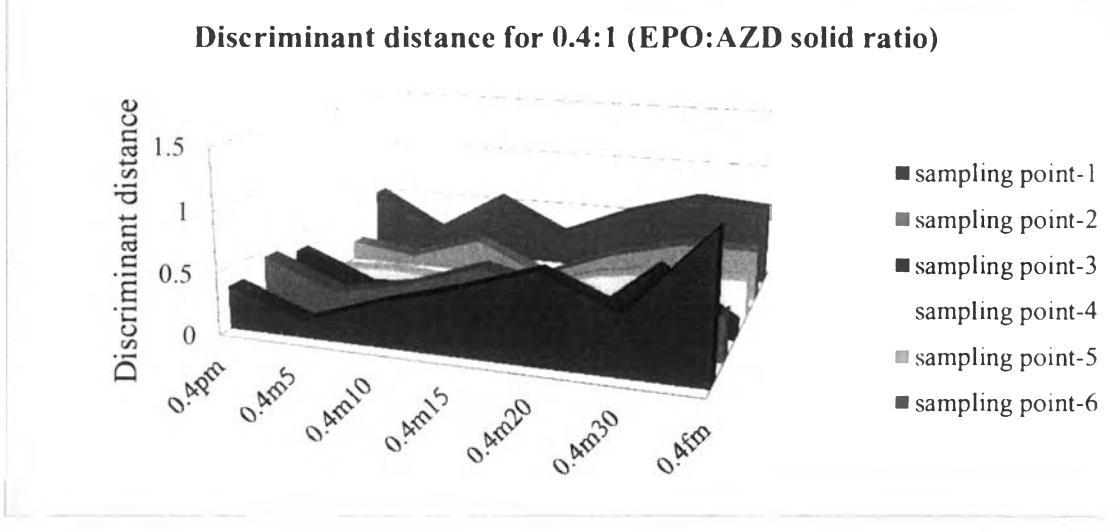

Figure 39 Discriminant distance for sampling points of Eudragit® E PO and AZD solid ratio of 0.4:1 in Erweka® AR400 Universal Lab Mixer.

From Figure 36 – 39 show the discriminant distance between sample and mean of spectral data. This discriminant distance values represent the critical sampling point or the possible area to heterogeneity in Erweka® AR400 Universal Lab Mixer. The starting of low EPO:AZD solid ratio in taste-masked blending process

found that sampling point-2 and 5 have high distance value. When mixing is higher, EPO:AZD solid ratios of 0.2:1, 0.3:1, the sampling point-1 and 2 have high distance value. The highest ratio shows that the sampling point-1 and 6 have high distance value. These values during taste-masked blending process increase to high distance values at sampling points that are near the bottom of mixing chamber. Due to the mixing force from blades in mixer tends to distribute force from the end of blades into each side direction. A possibility of heterogeneity mixing can be found in these points. The data of sampling points could be used for up-scale production in sampling process.

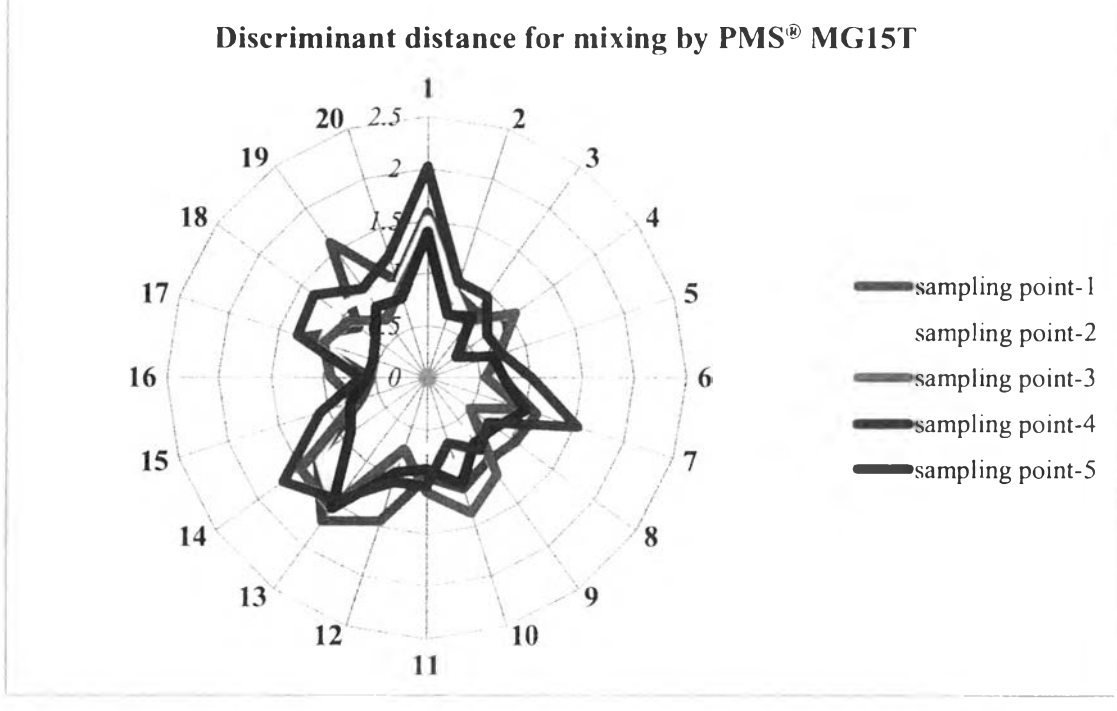

Figure 40 Discriminant distance of sampling points for mixture of Eudragit® E PO and AZD in solid ratio of 0.4:1 by PMS® MG15T high speed mixer.

The discriminant distance values from mixing by PMS® MG15T high speed mixer show in Figure 40. Sampling point-5 has generally the highest distance between the sample and the mean of spectral data. Next, the sampling point-2 and 4 have totally the lowest distance. The high distances refer to the sampling points that are near spray gun inlet and aggregation area at axis of agitator. The high speed of chopper (about 1500 rpm) is help the mixing and increasing shear force for better blending. From the Figure 30 as the homogeneity state of mixing by PMS® MG15T for 20 run cycles by percentage of relative standard deviation, the cycle phases of %RSD correlate with the area of discriminant distance plot in Figure 40. We can describe in the 1st phase as cycle 1-6, the 2nd phase as cycle 7-15 and the 3rd phase as cycle 17-20 that show the homogeneity state with the area of distance from the center of homogeneity value.

4. In Vitro of taste-masking azithromycin powder evaluation

The bitter taste of azithromycin dihydrate was masked by Eudragit® E PO. The taste-masked powder was prepared by physical barrier method. In this study, it was simulated the salivary fluid to predict the drug release in human saliva during a medicine as dry powder for suspensions is taken. The equation from standard calibration curve for azithromycin dihydrate is $y = 0.001x+0.0057$, $R^2 = 0.9992$.

After we produced the mixture from AZD and EPO by ERWEKA® AR400 and PMS® MG15T mixers, the in-vitro taste-masking evaluation by compared with commercial products in Thailand has been taken to an account. The product A is AZD dry powder for suspension that manufactured by particle coating and granulation method. The product B and C are manufactured by physical mixing and

granulation method. As a result, the AZD concentration refers to taste-masking evaluation amount of mixture is lower than product B and C. and close to product A as following in Table 6. The table is also shows the good obtained of taste-masked azithromycin powder. If the mixture was formulated to finished product as dry powder for suspensions, the AZD concentration of test sample will trend to be less.

Table 6 Comparison of AZD concentration in each products for in vitro taste-masking evaluation.

Products	AZD concentration (µg/mL) (leakage amount)
Physical mixture of EPO and AZD in solid ratio 0.4:1	91.0
AZD taste-masking powder (by ERWEKA® AR400 mixer)	18.9
AZD taste-masking powder (by PMS® MG15T mixer)	19.3
Commercial product A	18.4
Commercial product B	23.0
Commercial product C	20.4

From the Table 6, the mixture of Eudragit® E PO and AZD in solid ratio 0.4:1 was produced by physical mixing that show the highest AZD concentration value. The taste masking AZD process by particle coating and granulation by ERWEKA® AR400 Universal Lab mixer and PMS® MG15T high speed mixer exhibit the lower AZD concentration values as 18.9 µg/mL and 19.3 µg/mL, respectively. The physical mixture of Eudragit® E PO and AZD in solid ratio 0.4:1 shows the highest leakage amount of AZD. The commercial product A was manufactured by particle coating obtains the lowest AZD concentration value as 18.4 µg/mL. The commercial product B and C show the higher AZD concentration than product A and AZD taste-masking

powder from Erweka® Universal Lab mixer and PMS® MG15T high speed mixer which values about 20-23 µg/mL. The pattern of taste-masking AZD dry powder affect to the outcome of final product in aspect of the leakage amount of AZD in simulated human saliva that refer to the taste masking capacity of bitterness AZD.

Table 7 Comparison of AZD concentration products for in vitro taste-masking evaluation for three consecutive days.

Products	AZD concentration (µg/mL) (leakage amount)		
	Day 1	Day 2	Day 3
Physical mixture of EPO and AZD in solid ratio 0.4:1	91.0	111.2	130.7
AZD taste-masking powder (by ERWEKA® AR400 mixer)	18.9	20.2	20.9
AZD taste-masking powder (by PMS® MG15T mixer)	19.3	21.0	23.1
Commercial product A	18.4	18.7	19.0
Commercial product B	23.0	23.5	23.8
Commercial product C	20.4	21.6	23.1

From the Table 7, the taste masking of commercial product show the consistency of AZD concentration for in vitro taste masking evaluation in day 1, 2 and 3. The physical mixture was prepared by physical mixing exhibit the highest AZD concentrations for 3 consecutive days. It related with the procedure of taste masking. Moreover, the final product development of dry powder for suspensions of AZD taste-masking powder by ERWEKA® AR400 Universal Lab mixer and PMS® MG15T high speed mixer may be improved the bitterness of AZD better than commercial product A in Thailand.