ผลของตัวทำละลายในสารกระจายตัวสำหรับเคลือบฟิล์มต่อการเปลี่ยนแปลงในภาวะของแข็ง
ของโอแลนซาปีนในยาเม็ดเคลือบ

นางสาวอินทิรา ระงับพิศม์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชอุตสาหกรรม ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2558
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EFFECTS OF SOLVENTS IN FILM-COATING DISPERSION

ON THE SOLID STATE TRANSFORMATION OF OLANZAPINE IN COATED TABLETS

Miss Intira Rangubpis

Thesis Title	EFFECTS OF SOLVENTS IN FILM-COATING DISPERSION ON THE SOLID STATE TRANSFORMATION OF OLANZAPINE IN COATED TABLETS
By	Miss Intira Rangubpis
Field of Study	Industrial Pharmacy
Thesis Advisor	Narueporn Sutanthavibul, Ph.D.
Thesis Co-Advisor	Wanchai Chongcharoen, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

............................Dean of the Faculty of Pharmaceutical Sciences

(Assistant Professor Rungpetch Sakulbumrungsil, Ph.D.)

THESIS COMMITTEE

............................Chairman

(Associate Professor Parkpoom Tengamnuay, Ph.D.)

............................Thesis Advisor

(Narueporn Sutanthavibul, Ph.D.)

............................Thesis Co-Advisor

(Wanchai Chongcharoen, Ph.D.)

............................Examiner

(Jittima Chatchawalsaisin, Ph.D.)

............................External Examiner

(Chiravi Pavitrapok, Ph.D.)

อินทิรา ระงับพิศม์ : ผลของตัวทำละลายในสารกระจายตัวสำหรับเคลือบฟิล์มต่อการเปลี่ยนแปลงในภาวะของแข็ง ของโอแลนซาปีนในยาเม็ดเคลือบ (EFFECTS OF SOLVENTS IN FILM-COATING DISPERSION ON THE SOLID STATE TRANSFORMATION OF OLANZAPINE IN COATED TABLETS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภญ. ดร.นฤพร สุตัณฑวิบูลย์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: อ. ภก. ดร.วันชัย จงเจริญ, 137 หน้า.

ผงยาโอแลนซาปีนที่ปราศจากน้ำรูปแบบที่หนึ่งสัมผัสกับน้ำ,เอทานอล และสารผสมระหว่างน้ำและเอทานอล วิเคราะห์ผลด้วยการส่องกล้อง (polarized light microscopy) การเลี้ยวเบนรังสีเอ็กซ์ (XRPD) การวิเคราะห์ปริมาณความร้อนที่แตกต่างกัน (DSC) การวิเคราะห์การเปลี่ยนแปลงน้ำหนักของสารโดยอาศัยคุณสมบัติทางความร้อน (TGA) รามาสเปกโทรสโกปี (Raman) และการวิเคราะห์ส่วนประกอบสำคัญ (PCA) พบว่าการเปลี่ยนแปลงของโอแลนซาปีนที่ปราศจากน้ำรูปแบบที่หนึ่งเป็นโอแลนซาปีนไดไฮเดรตรูปแบบบีต้องใช้น้ำปริมาณมาก ขณะที่การเปลี่ยนเป็นโอแลนซาปีนรูปแบบโซเวทของน้ำผสมเอทานอลใช้เอทานอลปริมาณเล็กน้อย นอกจากนี้อุณหภูมิ (25°C, 40°C และ 70°C) และแรงตอกอัด (1000 psi, 2000 psi และ 3000 psi) ไม่มีผลต่อการเปลี่ยนแปลงภาวะของแข็งของโอแลนซาปีน

ยาเม็ดแกนของโอแลนซาปีนที่ปราศจากน้ำรูปแบบที่หนึ่งถูกเคลือบด้วยสารกระจายตัวสำหรับเคลือบฟิล์มโดยวิธีปืนสำหรับพ่น (spray gun) และทำให้แห้งที่อุณหภูมิที่ 25°C, 40°C และ 70°C เป็นระยะเวลา 3 ชั่วโมง การศึกษานี้แสดงให้เห็นว่ามีเพียงวิธีการ PCA ที่นำข้อมูลมาจากสเปกตรัมของ Raman ในช่วงระหว่าง 2950 ถึง 2750 cm^{-1}, 1500 ถึง 1400 cm^{-1}, 1100 ถึง 800 cm^{-1}, 700 ถึง 600 cm^{-1} และ 200 ถึง 150 cm^{-1} เท่านั้นที่แสดงให้เห็นว่าอัตราการระเหยช้าที่อุณหภูมิ 25°C และ 40°C ของน้ำหรือเอทานอลในสารกระจายตัวสำหรับเคลือบฟิล์มกับโอแลนซาปีนที่ปราศจากน้ำรูปแบบที่หนึ่ง จะเกิดการเปลี่ยนเป็นโอแลนซาปีนไดไฮเดรตรูปแบบบี หรือ โอแลนซาปีนรูปแบบโซเวทของน้ำผสมเอทานอลตามลำดับ อย่างไรก็ตามอัตราการระเหยเร็วที่อุณหภูมิ 70°C ไม่ก่อให้เกิดการเปลี่ยนแปลงดังกล่าว จึงสรุปได้ว่าการควบคุมปริมาณและอัตราการระเหยสำหรับสารกระจายตัวสำหรับเคลือบฟิล์มในระหว่างกระบวนการผลิตยาเม็ดเคลือบโอแลนซาปีนเป็นสิ่งสำคัญในการคงภาวะของแข็งตั้งต้นโอแลนซาปีนที่ปราศจากน้ำรูปแบบที่หนึ่ง

ภาควิชา	วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม	ลายมือชื่อนิสิต	....
สาขาวิชา	เภสัชอุตสาหกรรม	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2558	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

5576233333 : MAJOR INDUSTRIAL PHARMACY

KEYWORDS: OLANZAPINE / FILM-COATING DISPERSION / SOLID STATE TRANSFORMATION / SOLVENT / PRINCIPAL COMPONENT ANALYSIS

INTIRA RANGUBPIS: EFFECTS OF SOLVENTS IN FILM-COATING DISPERSION ON THE SOLID STATE TRANSFORMATION OF OLANZAPINE IN COATED TABLETS. ADVISOR: NARUEPORN SUTANTHAVIBUL, Ph.D., CO-ADVISOR: WANCHAI CHONGCHAROEN, Ph.D., 137 pp.

Anhydrous olanzapine Form I powder was exposed to water or ethanol or mixtures thereof, and analyzed by polarized light microscopy, X-ray powder diffractrometry (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), Raman spectroscopy (Raman) using Principal Component Analysis (PCA). It was found that conversion of Form I to Form B required high amount of water, while Form I can be easily transformed to EtOH-H_2O mixed solvate by using only minute amount of ethanol. In addition, temperatures of 25℃, 40℃ and 70℃ and compression forces of 1000 psi, 2000 psi and 3000 psi had no effect on olanzapine solid state transformation.

Form I core tablets were coated with film coating dispersions by spray gun and dried at 25℃, 40℃ and 70℃ for 3 hours. It was found that only PCA method, obtained from Raman spectra within ranges of 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} was able to differentiate between forms. The slow evaporation rate at 25 ℃ and 40 ℃ of water or ethanol in film-coating dispersion from Form I core tablets will induce conversion to Form B or EtOH-H_2O mixed solvate, respectively. However, no change was observed when fast evaporation rate at 70 ℃ was used. Results suggest that the control of optimal quantity and rate of evaporation for film-coating dispersion during pharmaceutical manufacturing of olanzapine coated tablets are critical in maintaining the original Form I solid state form.

ACKNOWLEDGEMENTS

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

APIs	Active Pharmaceutical Ingredients
XRPD	X-ray powder diffractrometry
DSC	Differential Scanning Calorimetry
TGA	Thermogravimetric Analysis
KF	Karl Fischer Titrametry
Raman	Raman Spectroscopy
FT-Raman	Fourier Transform Raman
5-HT	5-hydroxytryptamine or Serotonin
D	Dopamine
°C	Degree Celsius (centigrade)
%	percentage
RH	Relative Humidity
Θ	Angle
i.e.	That is
cm^{-1}	Centimeter-gram-second
PCA	Principal Component Analysis
PCs	Principal Components
PC1	The first Principal Component
PC2	The second Principal Component
MD	Mahalanobis distance
mL	Milliliter (s)
λ	Lamda
Å	Angstrom (s)
mA	Milli amp (s)
mW	Milli watt (s)
kV	Kilo Voltage (s)
min	Minute (s)
μm	Micrometre (s)

mg	Milligram (s)
g	Gram (s)
USP	United States Pharmacopoeia
psi	Pound per square inch (es)
mm	Millimetre (s)
Jg^{-1}	Joule per gram
w/w	Weight by weight
et al.	et alli, and others

CHAPTER I

INTRODUCTION

The solid state characterization of active pharmaceutical ingredients (APIs) had become a necessary requirement in drug development and pharmaceutical manufacturing because it relates to the properties of drug products such as physicochemical and biopharmaceutical properties, such as, solubility, dissolution rate, bioavailability and stability (1).

Polymorphism is a phenomenon by which a compound pure chemical may exist in many structural orientations due to distinct conformation and arrangements of drug molecules in crystal lattices. Solvates consist of molecule solvent included in crystal lattices. If the solvent is, water it is called hydrate(2). Polymorphs, solvates and hydrates have different physicochemical properties because their differences in molecular bonding and free energies (3). These variations may affect the pharmaceutical manufacturing, and properties of the drug for example stability, bioavailability, toxicity, and therapeutic efficacy. Furthermore, each solid state form can be protected by patents which are problematic for the development of generic drug products (4). Polymorphisms are shown in many APIs used in drug products. Solid state transformation can occur during manufacturing processes such as grinding, mixing, sieving, drying, tableting and coating. During coating process, the exposure to solvent (water or ethanol) presents higher risks for process-induced transformations in the solid-state of drugs and change their physiochemical properties. (5), (6). As a result, pharmaceutical manufacturing industry carefully monitor and control the solid state forms of drug substances and drug products throughout the manufacturing process. Thus, quantitative solid state characterization and evaluation of APIs and drug products are crucial (7).

Olanzapine is a benzodiazepine which belongs to the class of thienobenzodiazepines. Olanzapine is used as clinical treatment of positive and negative symptoms of schizophrenia, bipolar disorder and other psychosis. (8). Olanzapine consists of more 25 crystalline structures such as, 6 anhydrates (I-VI), 3 dihydrate (B, D, and E), a higher hydrate and mixed solvates (6, 8-11). Anhydrous olanzapine form I (Form I) was chosen for this study because it often relates to polymorphic transformation problems. Crystalline structures of olanzapine are transformed when it exposed to solvents, humidity and high temperature (10, 12). It is important to characterize, monitor and control solid state forms as early in the product development process as possible.

There are many solid state techniques for identification and quantification of solid state forms such as polarized light microscopy, X-ray powder diffractrometry (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), Karl Fischer titrametry (KF), and Raman spectroscopy (Raman). During the past two decades, the use of Raman in the pharmaceutical industry has become substantially popular with wide variety of uses. Raman is a potentially rapid method due to no need to prepare samples, it is non-destructive, and can both characterize and quantify drug substances and drug products (3). However, Raman spectroscopy alone without data treatment is not sufficient to discriminate between two suspected polymorphs that have similar Raman spectra. Thus, chemometric analysis (called Principal Component Analysis (PCA)), is more appropriate for characterizing fine spectral variation, for reducing the number of variables in a process and for extracting information from dataset (13, 14). Raman spectroscopy and PCA may be used to qualitatively and quantitatively characterize solid forms such as identification of solid state forms in pharmaceutical tablets and capsules. Consequently, the results from Raman spectra are analyzed using PCA to distinguish between groups of data in this study.

The aims of this study are to investigate whether in pharmaceutical coating process will induce solid state transformation of Form I, especially the effect of solvents in film-coating dispersion (water or ethanol). Furthermore, this study aims to explore the use of solid state analytical techniques such as Polarized light microscopy, XRPD, DSC, TGA, and KF to characterize the solid state conversion of Form I when exposed to solvents. Moreover, this study aims to evaluate the use of Raman and PCA to distinguish between groups of polymorphs. These techniques are appropriate for the solid state forms which have similar and overlapping data which cannot be clearly distinguished by other solid state analytical techniques, for example, polarized light microscopy, XRPD, DSC, TGA, and KF.

Objectives

1. To study the effect of components in film-coating dispersion on the solid state transformation of olanzapine coated tablets.

2. To evaluate the results from the solid state transformation of olanzapine after film coating by chemometrics.

CHAPTER II

LITERATURE REVIEW

1. THE MODEL DRUG

Olanzapine is an atypical antipsychotic drug which belongs to the group of thienobenzodiazepines. It blocks multiple neurotransmitter receptors such as serotonergic receptors, dopaminergic receptors, $alpha_1$ adrenergic receptor, H_1 receptor, acetylcholine at muscarinic receptors. Olanzapine is used as clinical treatment of schizophrenia, bipolar disorder and other psychosis. Olanzapine is first-line drug for the treatment of bipolar disorder for this mood-stabilizing property. Recently, olanzapine is chosen for the treatment of schizophrenia because of high clinical efficacy (8, 15-17). Olanzapine inventor product is available in both injection liquid dosage form and oral solid dosage form called Zyprexa®.
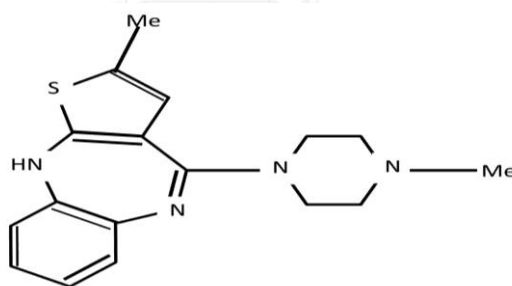
Figure 1 Molecule of olanzapine (Modified from Ayala and coworkers (8))

Olanzapine, ($C_{17}H_{20}N_4S$) (Figure 1) or scientific name called 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2,3-*b*][1,5]benzodiazepine (8) with molecular weight of 312.43, melting point at 195 °C and pKa values are 5.0 and 7.4. Olanzapine powders are yellow crystalline powder. The drug is soluble in n-propanol, sparingly soluble in acetonitrile, slightly soluble in methanol and dehydrated alcohol practically insoluble in water. Olanzapine should be stored in a tight and light resistant container at controlled room temperature because it is not stable when exposed to light and moisture (16, 18). It was reported that olanzapine had several

solid state structures. Olanzapine have more than 25 different solid forms, including 6 anhydrous polymorph (I-VI), 3 polymorphic dihydrates (B, D, and E), a higher hydrate and mixed solvates (6, 8-11). The most stable olanzapine is anhydrous olanzapine form II (Form II). Form II is selected to be used in the pharmaceutical manufacturing of olanzapine tablets. On the other hand, anhydrous olanzapine form I (Form I) is not appropriate for commercial use because Taiwari and coworkers reported that Form I is metastable as its color change during storage (19). In 2003, Reutzel-Edens and coworkers studied and characterized many crystalline structures of anhydrous and hydrate of olanzapine by X-ray diffractometry (6). Powder X-ray diffractogram of these common solid structures are shown in Figure 2.
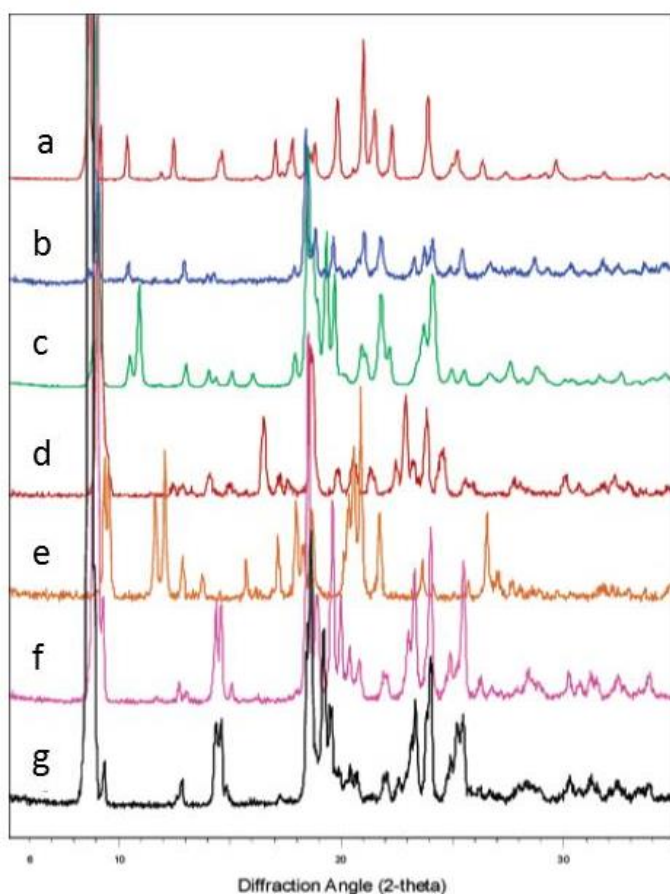
Figure 2 Powder X-ray diffractograms of olanzapine Form II (a), Form I (b), Form III (c), dihydrate B (d), dehydrate D (e), dihydrate E (f) and higher hydrate (g) (6)

Moreover, X-ray diffractogram of Form E is closely resembles that of similar to EtOH-H$_2$O mixed solvate because the crystal arrangements of two solid forms are very similar with only minor difference in peak positions (Figure 3). These are called isostructures (6).
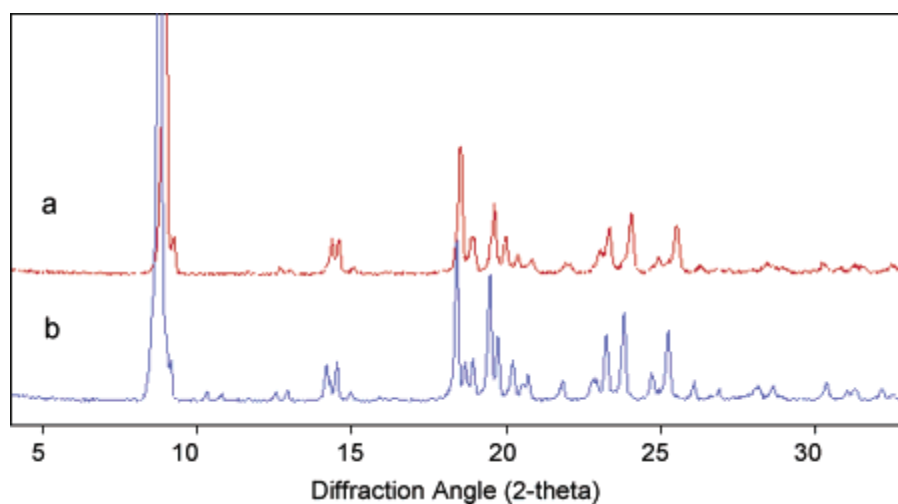
Figure 3 Powder X-ray diffractometry of Olanzapine; dihydrate E (a) and EtOH-H$_2$O mixed solvate (b) (6)

More researchers are interested in solid state transformations of olanzapine. In 2005, Polla and coworkers studied thermal behavior of olanzapine. The XRPD results exhibited the conversion of anhydrous olanzapine form I into anhydrous olanzapine form II as a function of temperatures from 60°C to 180°C (Figure 4) (10). This result confirmed that anhydrous olanzapine form II is most stable, when exposed to high temperatures, as reported by Reutzal-Edens and coworkers. (6)
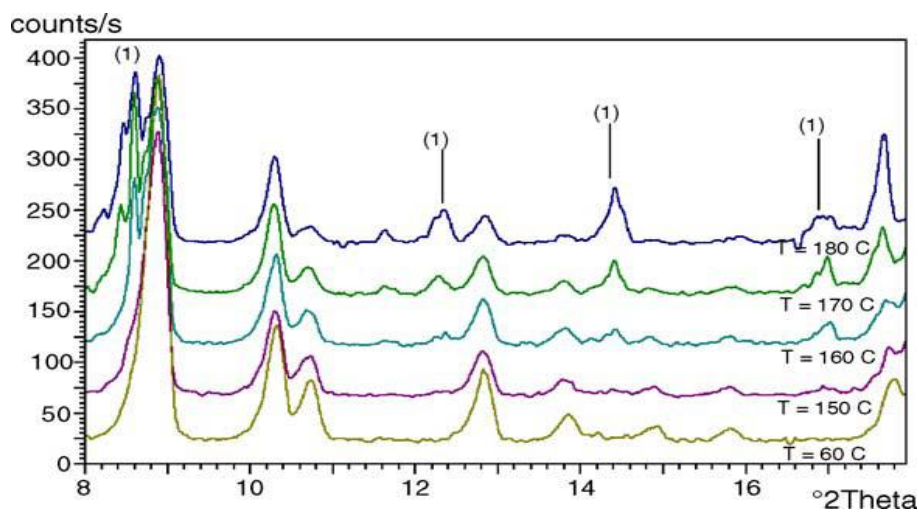
Figure 4 XRPD diffraction patterns of anhydrous olanzapine form I taken as a function of temperature from 60 to 180°C (10)

In 2011, Paisana and coworkers studied the stability of anhydrous olanzapine form I and anhydrous olanzapine form II when exposed to 3 different relative humidity environments at 11%RH, 75% RH and 93% RH and stored for 30 days before analyzed. They investigated that moisture can also affect the solid state structure of olanzapine polymorphs. The transformation of anhydrous forms into hydrated ones seems to be more pronounced in form I. The results are shown in DSC thermograms (Figure 5). It can be concluded that anhydrous olanzapine form I are less stable than anhydrous olanzapine form II under high humidity (20).
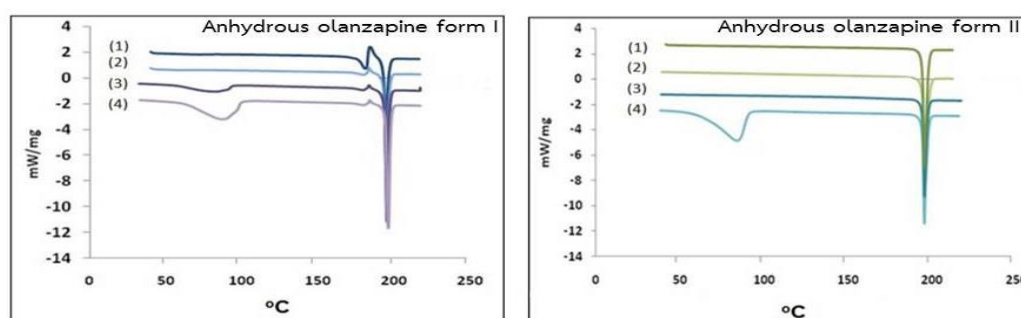
Figure 5 Anhydrous olanzapine form I (left) and anhydrous olanzapine form II (right) upon storage 30 days at different relative humidities (before storage (1); 11% RH (2); 75% RH (3) and 93% RH (4) (20)

Figure 6 shows the change in XRPD diffractrograms of anhydrous olanzapine form I and anhydrous olanzapine form II when collected at the relative humidity of 93% RH. Anhydrous olanzapine form I sample collected at 93% RH presented new peaks at 16.4° 2Θ, 19.7° 2Θ, 20.4° 2Θ, 21.3° 2Θ, 22.7° 2Θ and 23.7° 2Θ which are characteristic of olanzapine dihydrate B, whereas anhydrous olanzapine form II sample presented different peaks at 9.4° 2Θ, 11.7° 2Θ, 12.1° 2Θ, 15.7° 2Θ and 23.7° 2Θ characteristic of dihydrate D.   However, the transformation of anhydrous olanzapine form II into dihydrate form D was not complete because some characteristic peaks of anhydrous olanzapine form II, such as, 8.7° 2Θ and 22.3° 2Θ were still present.   Moreover, solid state transformation of anhydrous form I into anhydrous form II was not presented in low moisture environments.
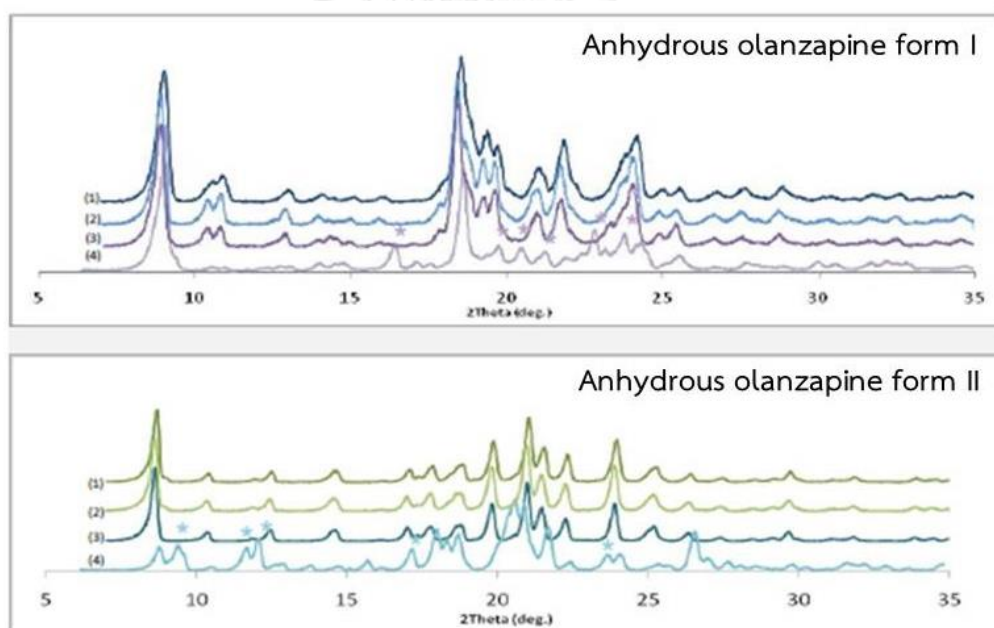
Figure 6 XRPD diffractrograms of anhydrous olanzapine form I (top) and anhydrous olanzapine form II (bottom) upon storage 30 days at different relative humidities (before storage (1); 11% RH (2); 75% RH (3) and 93% RH (4)) (20)

2. POLYMORPHISM

Polymorphism is a phenomenon that crystalline structure consists of one chemical compound but exists in more than one packing arrangement or conformation. Each solid form presents individual solid state characteristics (2). The occurrence of polymorphism can be explained for two reasons; the first is different conformers may occur in the different crystal structures molecule (conformational while polymorphism) the second has same molecular conformation in the polymorphic forms but different packing (packing polymorphism) (21). Solvates can occur by incorporation the solvent molecules in the crystal structure or crystalline lattice, drug which contains molecules of water in crystalline structure are known as hydrates (22, 23). Mixed solvate occur by the incorporation of two types of solvents in crystalline structure or crystalline lattice. A polymorph is stable at a particular condition and it can convert to other polymorphic forms (phase transformation). Thus, the stability relationship between polymorphs and metastable polymorphs can be classified as enantiotropic system or monotropic system (Figure 7). If one polymorph is stable at temperatures less than the transition temperature (T_t) while, the other polymorph is stable above the T_t, it called enantiotropic system. Enantiotropic polymorph is reversible polymorph. For a monotropic system, only one polymorph is stable at every temperature range. Monotropic polymorph is irreversible polymorph.(24)

Different polymorphs, solvates and hydrates exhibit individual physiochemical and biopharmaceuticals properties, such as, density, melting temperature, solubility, rate of dissolution, bioavailability and stability during storage (2, 22). So, Identification and characterization of solid state forms has become a necessity in the development and pharmaceutical manufacturing process due to quality monitoring of drug substances and drug products (2, 7).
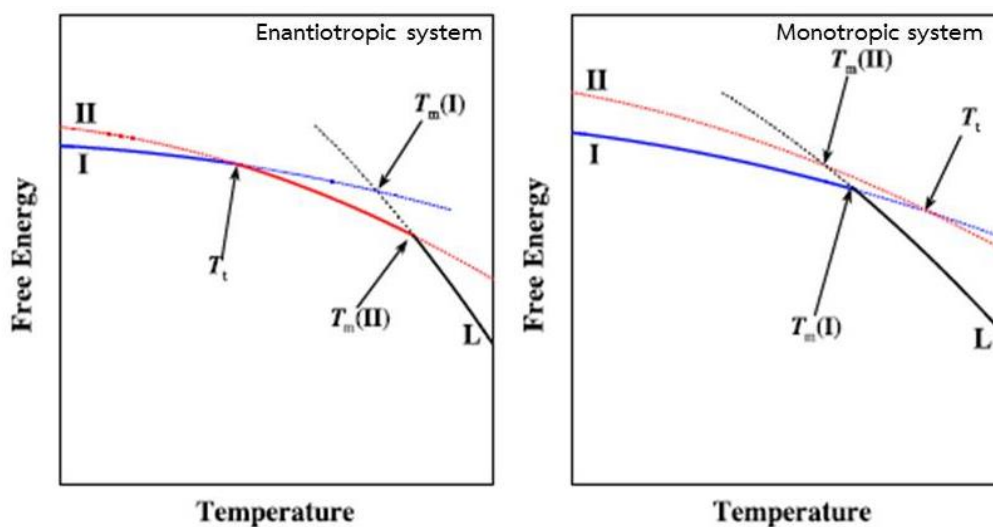
Figure 7 Free energy phase diagrams for polymorphic systems; Enantiotropic system (left) and Monotropic system (right) (24)

3. EFFECT OF SOLVENT ON SOLID STATE TRANSFORMATION

Manufacturing processes such as, grinding, mixing, sieving, drying, tableting and coating, can induce solid state transformation of APIs. Thus, the knowledge about solid state form and solid state transformation is critical for the development of pharmaceutical products (3).

During film coating process, solvents can induce solid state transformation of drug substance through the mechanisms of solubilization or solution-mediated transformation. Solubilization mechanism can occur when drugs are readily dissolved in film-coating solvents, (for example, water or ethanol), by which the transformation will only occur after solvent removal. The conversions can be from a metastable phase to stable phase or stable phase to one or more metastable phases or original phase to final phase that consist of a mixture of phases. Moreover, this mechanism regenerates solid form which is different from the original crystal form. The type of final solid form depends on the rate of solvent removal or rate of drying and the ease nucleus of formation and crystal growth under the processing conditions. The solution-mediated mechanism however, is the conversion from

metastable phase to more stable phase and this mechanism depends on the difference in solubility between the two solid phases. The solution-mediated mechanism occurs when metastable phase is exposed to solvent and dissolved of the metastable phase into the solution medium until nucleus of stable phase are formed. If nucleus formation is the rate-limiting step, solubility, the difference in solubility between the phases, temperature used contacting surface, agitation rate, and insolubility excipients/impurities can greatly this transformation. When crystal growth is the rate-limiting step, the difference in solubility, ratio of solid/solvent, agitation rate and temperature used, size of particle of the original phase, and insoluble excipients/impurities determine its transition of kinetics. Both mechanisms affect phase transition such as polymorphic transition, hydration or dehydration, and vetrification or amorphous formation (24).

In 2003, Reutzel-Edens and coworkers studied anhydrate and hydrate form of olanzapine. They discovered that anhydrous olanzapine Form II was most stable in organic solvents. Polymorphic transformation of the anhydrous olanzapine Form I, anhydrous olanzapine Form III and olanzapine dihydrate to Form II occurred at room temperature within a matter of hours. In water, anhydrous olanzapine form I is less stable (high soluble) than the olanzapine dihydrate at room temperature. Anhydrous olanzapine Form II exposed to water for several hours to a few days, it was transformed to highly stable olanzapine dihydrate D (low soubility) (6)

In 2012, Lust and coworkers studied about the water-mediated solid-state transformation of piroxicam (PRX). The transformation occurs during an aqueous drug-layer coating of pellets, identified by Raman spectroscopy. The result indicated that anhydrous PRX form I (AH) and monohydrate (MH) were found to be more stable form than amorphous PRX (SD) during aqueous drug-layer coating (Figure 8a and b). Amorphous PRX (SD) was unstable and its recrystallization to MH was identified by Raman spectroscopy within 10 min from the beginning of drug-layer

coating of pellets (Figure 8c) (5). Lust and coworkers discovered that AH and MH did not transform to any other solid-state form within 1 hour aqueous drug-layer coating of pellets. Thus, revealing that AH and MH were stable in aqueous HPMC solution and during film coating.
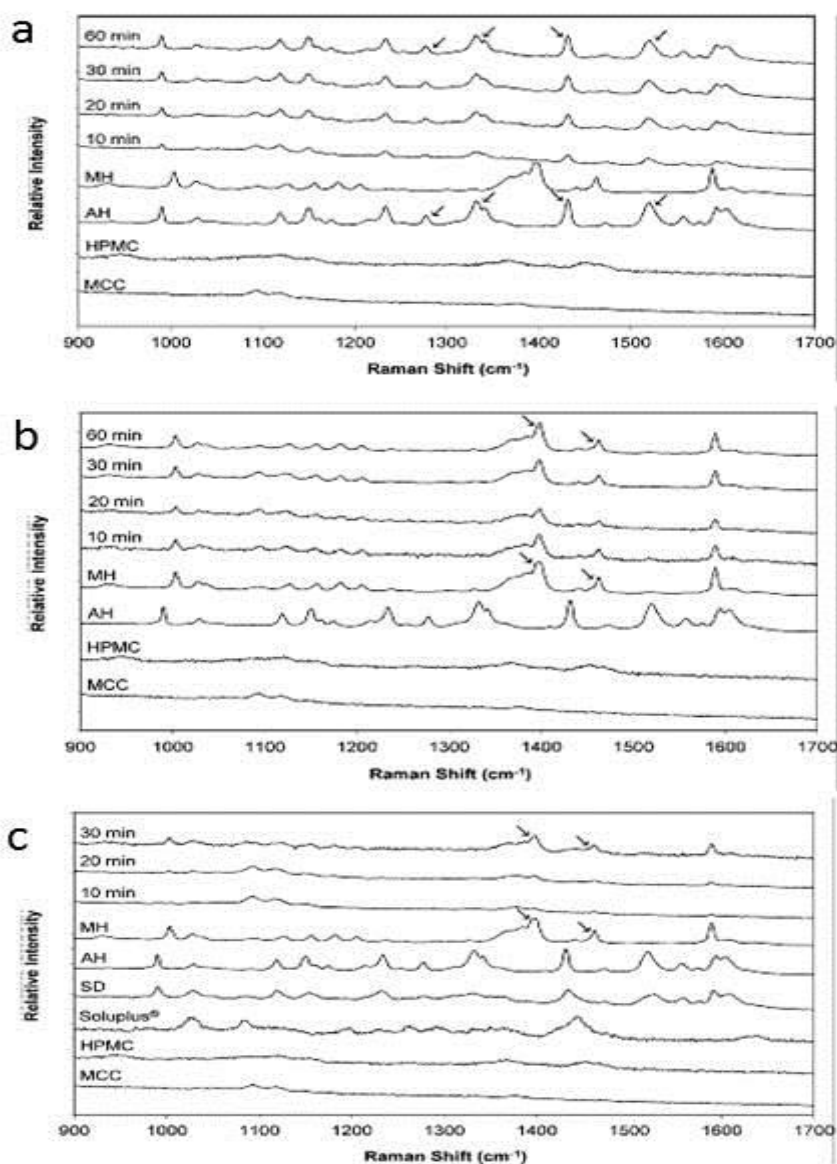
Figure 8 Raman spectra of neutral pellets with drug-layer of PRX anhydrous form I (AH) (a), PRX monohydrate (MH) (b) and amorphous PRX as a solid dispersion (SD) of PRX and Soluplus® 1:4. Multiple (c) Raman spectra are taken after 10, 20, 30 and 60 min of exposure (5)

4. SOLID-STATE CHARACTERIZATION

The correct solid form of active pharmaceutical ingredients (APIs) or drug substance is necessary for product development and manufacturing. The solid form chosen during development and manufacturing should be able to with stand manufacturing, soluble, bioavailable and stable during processing. Solid state forms consist of polymorphs, solvates and amorphous forms. For example, when a hydroscopic drug was exposed to water during wet granulation, it generates hydrate and after drying, the hydrated form converts to another form. It is of high priority to determine phase transformations during development and manufacturing due to effect on the physicochemical and biopharmaceutical properties of the final drug product (6).

There are many methods that were used to evaluate the solid state properties of drug substances and to control the solid form such as, in 2006, Ayala and coworkers investigated anhydrous olanzapine form I and anhydrous olanzapine form II by using vibrational spectroscopy (Raman, infrared and near infrared) for the discrimination of these crystalline forms (8). In 2007, Tawari and coworkers determined the quantity of anhydrous olanzapine form I in anhydrous olanzapine form II by developing a quantitative X-ray powder diffraction (XRPD) method (19). In 2013, Cavallari and coworkers analyzed anhydrous and hydrate forms of olanzapine by thermal analysis techniques (differential scanning calorimetry (DSC) and thermogravimetry (TGA)) for identification and characterization (12). These techniques are excellent methods for obtaining structural information and normally used to discriminate polymorphic forms.

4.1 POLARIZED LIGHT MICROSCOPE

The polarized light microscopy uses plane-polarized light in order to evaluate the morphology and when combined with polarized lens, one can analyze the

crystallinity (birefringence) of the solid sample. Normally, amorphous forms will not show crystallinity (birefringence). This method can be used to screen and distinguish between crystalline and amorphous solid samples. This technique is only suitable for pure chemical compounds. The disadvantages of polarized light microscopy are that it cannot provide the chemical information of the sample and cannot detect chemical degradation. (25-27).

The eyepiece, analyzer, compensator, objective, specimen, condenser, polarizer, mirror and lamp are main composition of light microscope. This microscope is adjusted by placing one polarizing filter (the polarizer) below the specimen and placing another polarizing filter (the analyzer) after the objective lens (Figure 9). The vibration direction of the electromagnetic ware which passes the polarizer is usually aligned called plane polarized light. Its plane of polarized light aligns from left to right when observed through the microscope. Moreover, the vibration direction of the analyzer is aligned 90 degree (perpendicular) to the polarizer. The polarizer is embedded in the light path at all times and settled between the specimen and the illuminator. The modern polarizer is typically incorporated into the light path. The analyzer is placed between the objective and the ocular tube. Some microscope will permit the analyzer to be rotated in association with the polarizer. Therefore, specimens can be observed between uncrossed polarizers and analyzers using specialized compensator plates for characterizing optically active specimens.
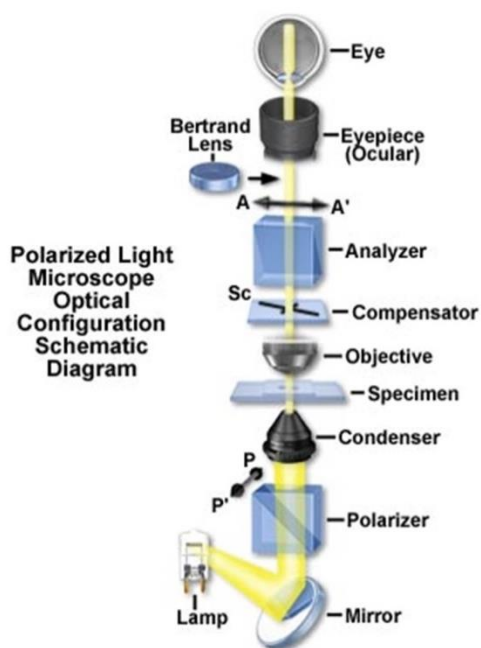
Figure 9 Schematic diagram of polarized light microscope (28)

4.2 X-RAY POWDER DIFFRACTOMETRY

X-ray Powder diffractometry (XRPD) is a primary technique for the analysis of polymorphism in solid forms. XRPD is the standard technique for identification of solid state forms. The size of particles and morphology of the sample affected the intensity of PXRD peaks. XRPD diffractograms of amorphous materials exhibit broad peak (amorphous halo). Moreover, XRPD have several uses, for example, qualification and quantitation of polymorphs of materials and powders, tablets, compacts, and identify counterfeit and determination of crystal structures from XRPD patterns (29).

The main analytical disadvantage of XRPD, is that it can be time consuming as samples must be generally analyzed off-line. When preparation of tablets particle size be reduced by grinding. This induced change in solid state forms and XRPD diffractrograms of all crystalline samples (2, 3). The misinterpretations of XRPD patterns are reasonable from low peak intensities or missing reflections.

For example, the highly crystalline excipients show XPRD peaks with overlapping with peaks of APIs. Moreover, this problem can solve by increase dose of drug product. (30).

In 2007, Tiwari and coworkers studied the ability of XRPD to quantify solid state forms of olanzapine. Olanzapine mixtures were prepared and it was shown that XRPD produced a linear calibration curve. The result presented that the relationship between predicted concentrations versus actual concentration was linear, with a slope of nearly one and an intercept of zero. The validation parameters such as accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ) were tested and found to be satisfactory. Moreover, the potential errors of XRPD in quantitative analysis were identified; including morphology, particle size of the samples and the effects of instrument reproducibility (31).

4.3 DIFFERENTIAL SCANNING CALORIMETRY

Differential scanning calorimetry (DSC) is the most often used thermal analytical method used to characterize solid state forms. DSC can characterize the solid form by its melting point and analyze the sample undergoing the change of forms, for example recrystallization of polymorphs and glass transitions of amorphous materials. When the sample recrystallizes, the sample give out energy as heat (exothermic). When the sample melts, the sample takes in energy or heat (endothermic). Exothermic or endothermic may be occur Solid–solid phase transitions. DSC is also used in the study of relative phase stability of polymorphic solids (25, 27, 29). DSC is especially useful in the calculation of the stoichiometric water/solvent content in the drug substance by weight loss of sample. DSC has its advantages in accuracy, precision, speed, simplicity and availability and no sample preparation required. On the other hand, the disadvantages of this method are the

high cost of aluminum sample pan, instrument and maintenance. In case of solid mixtures, upon heating, the samples may, decomposed and can change the DSC signals of the ingredients and APIs. (22, 30, 32-34).

DSC used in this experiment is heat-flux DSC instrument. The heat flux DSC consists of two sample holders (sample pan and an empty reference pan) placed on a constantan thermoelectric disk enclosed by furnace. The furnace is heated at a single heating rate, and the heat flows pass into both the sample and reference pans through constantan thermoelectric disk. The differential heat flow between reference and sample is measured by thermocouples, and the resulting heat flow is defined by the thermal equivalent of Ohm's law (35).

$$q = \Delta T / R$$

Where q = sample heat flow

ΔT = temperature difference between sample and reference

R = resistance of thermoelectric disk

Two ways products are introduced to temperatures, either by increasing the temperature at a specified constant rate called dynamic DSC or hold one temperature constant until the end of the experiment called Isothermal DSC. The final result interprets information obtained from the difference in heat flow between the sample and the reference.
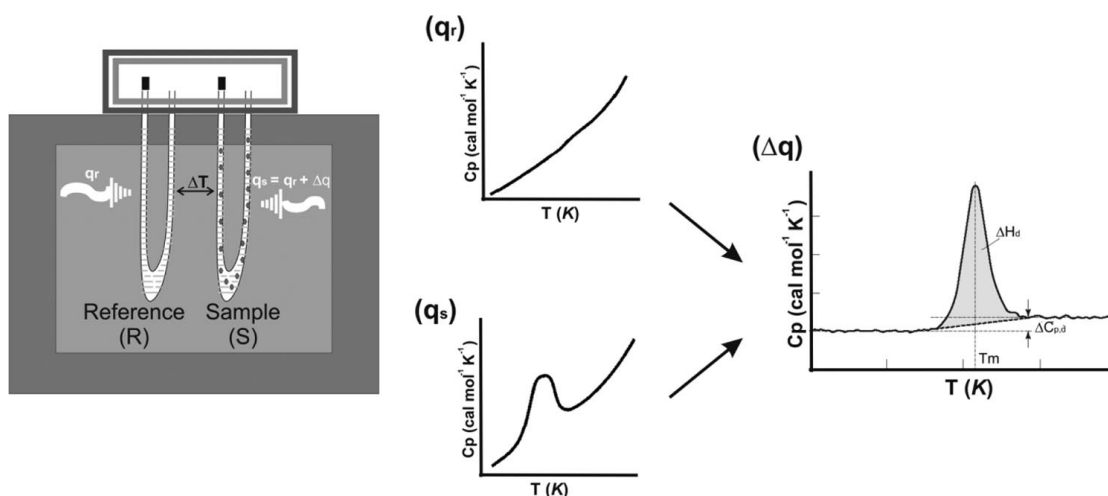
Figure 10 A schematic diagram of a heat-flux DSC and the resulting DSC thermograms with the reference subtracted from the sample resulting in excess heat changes as a function of temperature (35)

The software induced in the DSC apparatus is used to help the user analyze the onset temperature (melting points), peak temperature, end-set temperature, glass transition temperatures and heat capacity of events.

4.4 THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric analysis (TGA) is a solid state characterization technique that analyses amount and rate of weight change in a sample relative to temperature. The schematic diagram of the instrument is shown in Figure 11. TGA studies weight gain or weight loss, desolvation processes and decomposition of sample. The application of TGA is for quantitative analysis of the total volatile content of a sample. The sample with higher decomposition temperature, that sample is more stable than the other one. Moreover, it is used in combination with DSC to compare the enthalpy of the transition obtained by DSC and the weight loss or weight gain of sample obtained by TGA. A plot between mass or percentage of mass as a function of time or function of temperature is known as TGA thermogram (25, 27, 29). The advantages of TGA are that the method quantifies the solvent or water in a solid sample, ease in experimental set-up and use only small sample size

(approximately 3-10 mg). However, the disadvantages of this technique are such as destruction of sample during analysis and unsuitable for sample that degrades at low temperature (22).
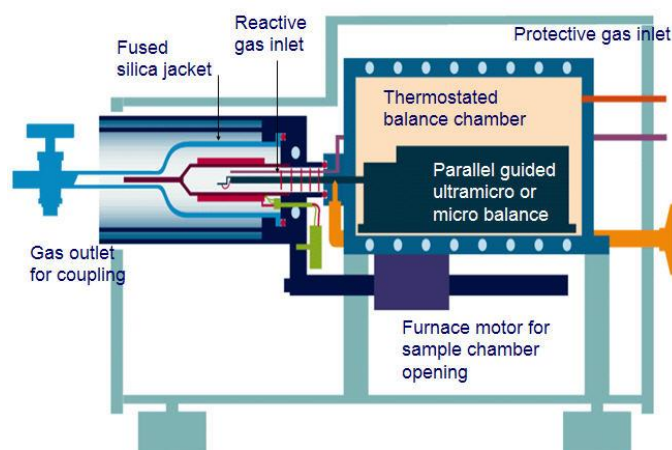
Figure 11 A diagram of the thermogravimetric analysis (TGA) instrument (36)

TGA records the mass of the sample as it is heated in a furnace (Figure 11). The weighing device is called a microbalance, which can detect weight within microgram of sample. The purge-gas system is used for providing inert gases and eliminating reaction gases. Computer system is used for device management, data acquisition and data processing. When TGA starts working, the purge-gas flows into the system and increase temperature at a specified rate which are usually adjusted during every experiment called programmable furnace. The results of TGA are dependent on instrumental setting and nature of sample. The rate of heating can affect the transition temperatures, whereas the atmosphere within the furnace can affect the thermal reactions. The nature of the sample has high impact on the resulting TGA thermograms, namely, the quantity of samples, the size of particles, the temperature of reaction, the sample packing and thermal conductivity (27).

4.5 KARL FISCHER TITRATION

The determination of water content in drug substances or drug products is usually done by Karl Fisher Titration (KF) technique. The technique quantitates the

reaction of water with iodine and sulfur dioxide in lower alcohol, such as methanol, and an organic base such as pyridine. The reactions are shown in the following chemical equations:

$$H_2O + I_2 + SO_2 + 3\ C_5H_5N \longrightarrow 2(C_5H_5N+H)I- + C_5H_5N \cdot SO_3$$

$$C_5H_5N \cdot SO_3 + CH_3OH \longrightarrow (C_5H_5N+H)O\text{-}SO_2 \cdot OCH_3.$$
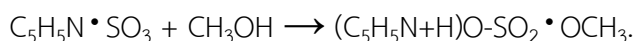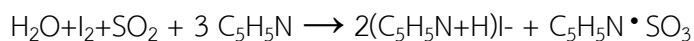
The technique was developed by a chemist named Karl Fischer. Karl Fischer Titration can be classified into two methods: the method of volumetric titration and the method of coulometric titration. This experiment uses volumetric titration method. In the method of volumetric titration, the water that dissolved is reacted with iodine and analyzed by measuring quantity of iodine used in the reaction. (37).

The advantages of KF are that, selectivity and accuracy for water, used less amount of sample for analysis, preparation of sample is not difficult, fast analysis, can analyze solids, liquids and gases. On the other hand, the disadvantages of this KF are that, many samples, such as chocolate, release its water very slowly and need further attempts to expose the total amount of water into the Karl Fischer reagents.

4.6 RAMAN SPECTROSCOPY

One of the spectroscopic technique for investigating drug substances and the production processes of drug products is Raman spectroscopy (Raman). Raman is a vibrational spectroscopic method for identification and solid state characterization of drug substances and during the production processes. Raman spectra measures the vibration of molecules, change in rotational energy and change polarizibility of the sample (38). The advantages for using Raman are that, to study solid state forms in pharmaceuticals, no sample preparation necessary, quick analysis and non-invasive measurements are possible. Bulk and final products can be tested non-destructively though packaging has the ability to analyze small volume of samples, in situ and on-line capability in production line, not interfered by water, quantitative analysis

possible by imaging method and automated high-throughput analysis with high sensitivity. Moreover, Raman can be used in with conjunction a microscope to analyze along narrow sample space such as tablet surfaces. On the other hand, the drawback of Raman are the cost of instrument for routine analysis, high level of fluorescence overlaying the Raman spectra and the high excitation intensities may thermally decompose the drug sample (2, 3, 22, 38-40).

The energy transition of Raman scattering can occur by three types of phenomenon seen in Figure 12.
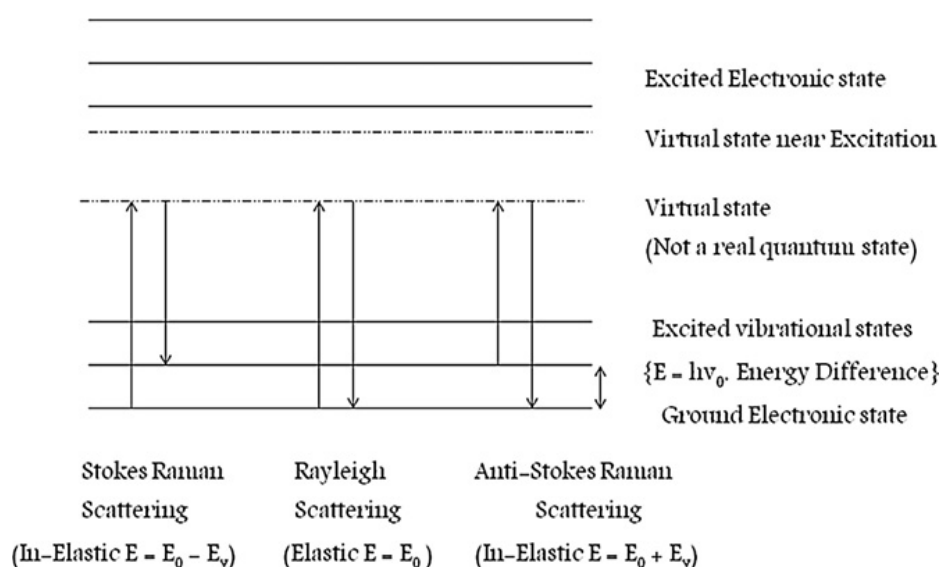
Figure 12 Schematic representation on energy transitions of Raman scattering(40)

When, the incident light interacts with the molecule, energy of elastic scatting radiation obtain from emission of a photon is the same as the energy of the photon excitation. This radiation of scattering is called Rayleigh scattering (E = Eo).

If the radiation of scattering is higher frequency than the excitation radiation, it is called anti-Stokes Raman scattering. (E = Eo + Ev)

On the other hand, if the radiation of scattering is lower frequency than the excitation radiation, it is called Stokes Raman scattering (E = Eo – Ev).

Raman spectra are collected by two technologies; Dispersive Raman and Fourier transform Raman (FT-Raman). In this experiment, Dispersive Raman is chosen for solid state characterization.

Both of these technologies are separated by the type of laser and the type of detector and the evaluation processes of Raman scatting. Each technique has individual advantages and disadvantages in utilization and applications as shown in Table 1 and Figure 13 (38, 40).

Table 1 Comparison of Fourier Transform and Dispersive Raman spectroscopy (38)

FT-Raman	Dispersive Raman
Laser NIR : 1064 nm	VIS : 785 nm, 633 nm, 532 nm, …
Spectral analysis by Interferometer	Grating

Detector
   - room temperature indium gallium arsenide
   - liquid nitrogen-cooled germanium
	Silicon CCD detector

Advantages
   - limited fluorescence
   - maximal compatibility with libraries and analysis software
	- higher sensitivity
	- higher spatial resolution for microscopy applications
	- lower laser power

Applications
   - in the pharmaceutical industry:
     - unknown identification
     - incoming raw material characterisation
     - final product quality
     - quantitative analyses
   - investigating polymorphs
   - forensic analysis through sample containers or evidence bags
	- minor component analysis
	- more sensitive for aqueous samples
	- analysis of dark samples
	- depth or cross-sectional information in samples
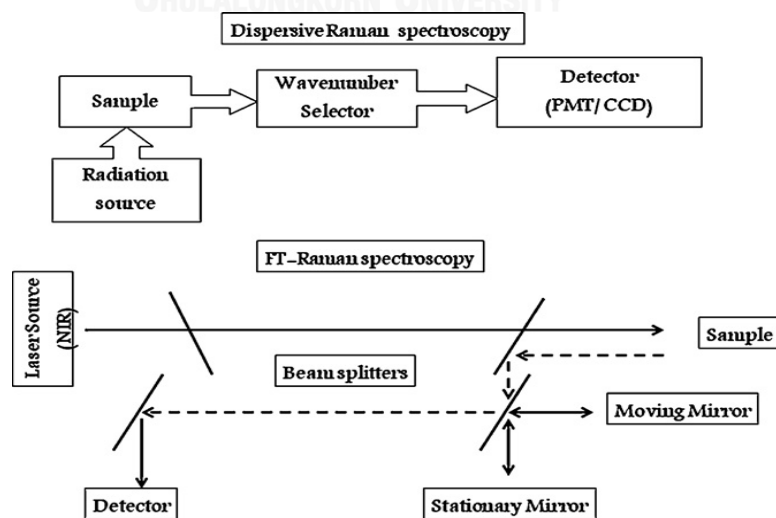
Figure 13 Schematic representations of Dispersive and Fourier Transform-Raman spectroscopy (40)

Pharmaceutical manufacturing may benefit from the advantages offered by Raman. In 2000, Taylor and coworkers investigated solid state forms of active pharmaceutical ingredient in tablets and capsules by Raman. The result indicated that Raman can detect low concentration of active ingredient (lower than 1%) and mixture of solid forms in tablets and capsules which, contained enalapril maleate, prednisolone, ranitidine theophylline, and warfarin sodium clathrate. (41).

In 2006, Ayala and coworkers characterized olanzapine polymorphs by vibrational spectroscopy method (FT-Raman, infrared and near infrared). The result indicated that a combination of infrared and Raman spectroscopy was used to characterize two polymorphs of olanzapine, with spectral assignments being deduced for all observed spectra in the two solid-state forms to obtain insight into the crystalline structures (8).
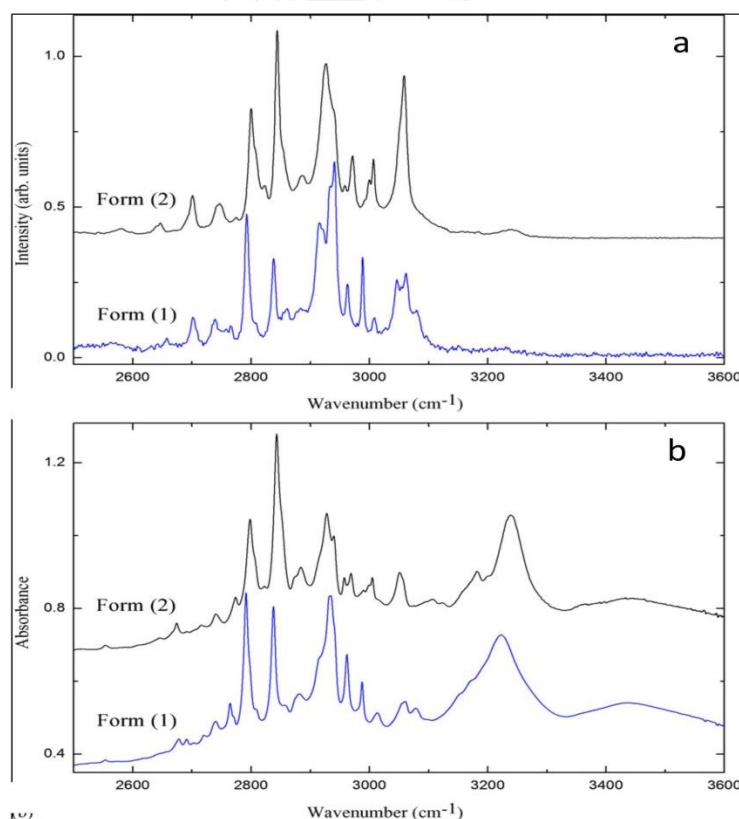
Figure 14 Raman (a) and infrared (b) spectra of the polymorphs of olanzapine above 2500 cm^{-1} (8)
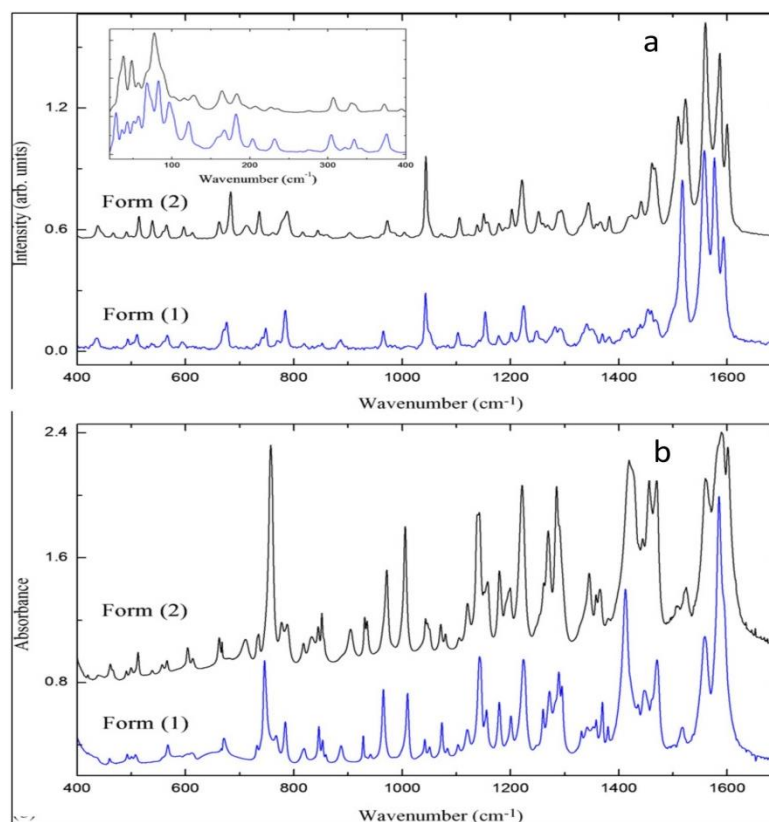
Figure 15 Raman (a) and infrared (b) spectra of polymorphs of olanzapine below 1700 cm^{-1} (8)

In 2008, Veji and coworkers detected counterfeit Viagra® tablet by Raman spectroscopy. The researchers detected eighteen sildenafil tablets which consisted of distinct excipient. The Raman spectra within ranges of 1150 and 700 cm^{-1} could be used to differentiate between genuine tablet and counterfeit tablet (42).

5. CHEMOMETRIC ANALYSIS

Chemometric is the utilization of mathematical and statistical science for extraction of data from physical and chemical events related to production processes. Chemometric is used for the collection and analysis of multivariate data, calibration process, selection of suitable process for process modeling, pattern recognition, classification of data, correction and compression the data and use

statistics to control the production processes.   Data obtained from hundreds to thousands of experiments are more than hundreds to thousands of variables. Some data have small quantity but they are very large and highly complex. Moreover, the data that generated by most recent automated instruments in biological/medical laboratory are complicated and difficult to interpret. The application of chemometric tries to build a bridge between methods and their variables. It is used in various areas such as medicine, pharmacy, food, and environmental monitoring.

Chemometric can be employed to solve descriptive and predictive issues in life sciences.   The descriptive issues are to investigate the properties that are modeled related to the system.   While, the predictive issues including properties of numerous system that are used in a complex model with the purpose of predicting the target properties, desired features, or behavior of interest (14, 43).

PRINCIPAL COMPONENTS ANALYSIS

The Principal Component Analysis (PCA) is the most famous multivariate statistical method used in chemometrics. The PCA is a dimension-reduction method that is used to reduce the large number of variables to smaller number of variable but still retained the most important features as in the large data set. PCA is used for extraction of the information from large datasets, reorganization the data and grouping the similar data into the same group.

The first step in PCA is the collection of chemical data that contains large variable. PCA will reduce large set of variables to smaller set that still maintain the crucial information as the original large dataset. The smaller set is called correlation matrix. The second step is factor extraction by finding the number of components that are able to explain the correlation of variables. The numbers of components are determined by Factor loading. After that, grouping data which are similar to form

similar group. The third step is factor rotation to reduce variables and reduce the original dataset to smaller ones to ease the interpretation.

The PCA technique transforms the large data space of all dataset into smaller X matrix which is easier to interpret shown in the equation below.

$$X = TP^T + E = t_1 p^T_1 + t_2 p^T_2 + \ldots + t_a p^T_a + E$$

Where E is a matrix that represent the irrelevant information for the model. The sample projections in this new coordinate system are called scores vectors (t_i) (related to sample information), the coefficients of linear combination are called loadings vectors (p_i) (refer to original variable information).

PCA consists of the first principal component (PC1) explaining the maximum amount of variability in the data. The second principal component (PC2) explains the orthogonal direction of the second highest variability in the data uncorrelated to the first principle component (PC1). The two-dimensional plot will be suitable if it is close to 100% of the variation described by only 2 PCs.
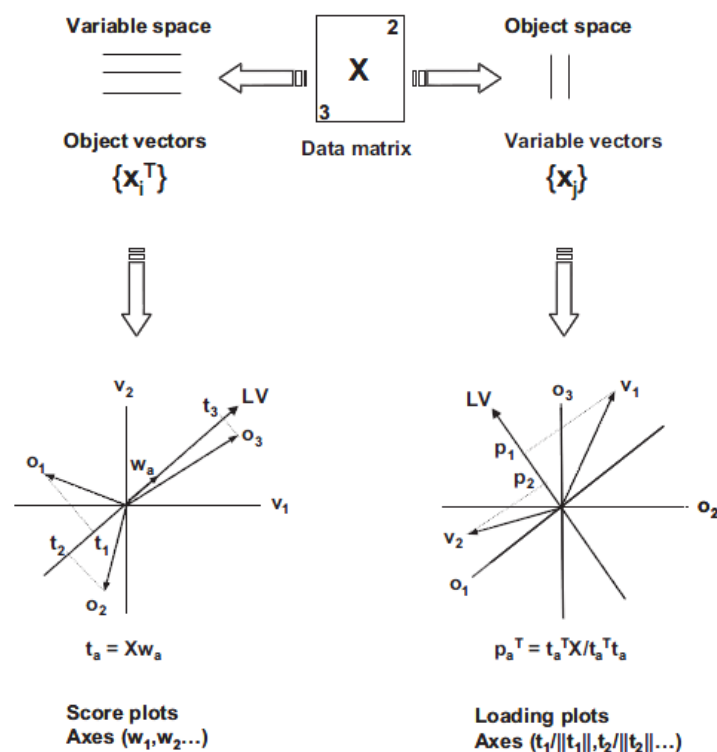
Figure 16 Schematic representations of data transformation by PCA technique (44)

The PCA presents the results by graphical presentation such as score plots and loading plots. Score plots show samples in PC space and allow finding sample similarities and group structure; loading plots determine the influence of each original variable on the principal component (14, 44).

MAHALANOBIC DISTANCES

Mahalanobis distance (MD) is a statistical method for measurement of distance between the central point of known sample and central point of unknown sample. The distance can be calculated in the original variable space or in the principal component (PC). About the original variable space, the MD takes into account the correlation in the data, since it is calculated using the inverse of the variance-covariance matrix of the data set of interest. Mahalonobis distance consists of scale and vector but in this experiment only scale is examined

The limitations of calculation of the variance-covariance matrix are the large number of variable such as NIR spectra or Raman spectra and the number of variables had to be smaller than the number of object in data set. Thus, MD is suitable in computing using a smaller number of variables (PCs) obtained from PCA.

Mahalanobis distance is found on the mean, variance of the predictor variables and the matrix of covariance of all variable (45).

Mahalanobis distances are calculated as:

$$D^2 = (x - w)^T \, C^{-1} \, (x - w)$$

Where: D^2 = Mahalanobis distance

x = Vector of data

w = Vector of mean values of independent variables

C^{-1} = Inverse Covariance matrix of independent variable

T = Indicates vector should be transposed

The quantity D is called the Mahalanobis distance from the feature vector x to the mean vector w, where C is the covariance matrix for x. It can be shown that the surfaces on which D is constant are ellipsoids that are centered about the mean w. In the special case where the features are uncorrelated and the variances in all directions are the same, these surfaces are spheres, and the Mahalanobis distance becomes equivalent to the Euclidean distance.
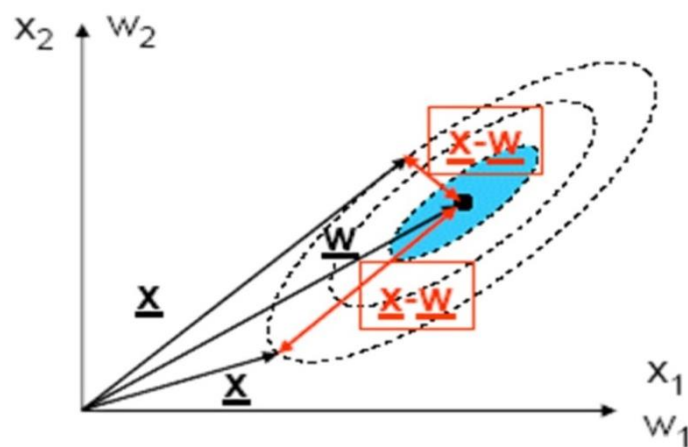
Figure 17 Schematic representations of Mahalanobis distance (46)

CHAPTER III

MATERIALS AND METHODS

Materials

Anhydrous polymorphic form I of olanzapine (Form I) (Lot. OL0291113) was obtained from Hetero Drugs Ltd., Hyderabad, India. Spray dried lactose (Lot. HR02) was purchased from DMV-Fonterra Excipients (NZ) Ltd, New Zealand. Microcrystalline cellulose PH102 (Lot. 2155) was purchased from Asahi Kasei Corporation, Japan. Sodium starch glycolate (Lot. 4111034027) was purchased from JRS Pharma LP, United States. Magnesium stearate (Lot. MGS Q0423) and hydroxypropyl methylcellulose E5 (Lot. RJ08012405) was purchased from Colorcon Asia Pacific Ple Ltd, Singapore. Polyethylene glycol 400 (Lot. 418101810) was purchased from Srichand United Dispensary Co., LTD, Thailand. Titanium dioxide (Lot. H07102298) was purchased from Saguachai Chemical Import, China. Sodium hydroxide pellets (Lot. B730298 537) was purchased from Merck, Germany. 95% Ethanol (about 95% ethanol and 5% water) was purchased from The Government Pharmaceutical Organization, Thailand. Distilled water was obtained from the laboratory. Karl Fischer Reagent (Apura® Titrant) was purchased from Merck, Germany. Methanol (dried) was purchased from Chariau, Spain.

Instruments

1. Confocal Microscopic Raman Spectrometer (DXR Raman Microscopic Spectrometer, Thermo Fisher Scientific Inc., USA)
2. X-ray Powder Diffractometer (MiniFlex II Desktop X-Ray Diffractometry, Rigaku, Japan)
3. Differential Scanning Calorimeter (D244^{e}, Mettler Toledo, Switzerland)

4. Thermogravimetric Analyzer (SDTA851[e], Mettler Toledo, Switzerland)

5. Polarized Light Microscope (Nikon Eclipse E200, Nikon, Japan)

6. Analytical balance (XP205, Mettler Toledo, Switzerland)

7. Analytical balance (ML303, Mettler Toledo, Switzerland)

8. Hydraulic press (Carver[®] Press, USA)

9. Hot air oven (Model UL 80, Memmert, Schwabach, Germany)

10. Spray gun (Thai coater FC 15", Pharmaceutical and Medical Supply LTD Part, Thailand)

11. TQ Analyst 8[®] Pro edition software (Thermo Fisher Scientific Inc., USA)

12. Homogenizer (Polytron[®] PT-MR 3100, Kinematica AG, Switzerland)

Experimental methods

1. Preparation of reference olanzapine solvate forms

Reference olanzapine solvate forms were prepared from anhydrous olanzapine form I (Form I) by mixing with appropriate solvents.

1.1 Olanzapine dihydrate form B (Form B)

Olanzapine dihydrate form B (Form B) was prepared by mixing 5 grams of anhydrous olanzapine form I in 25 mL water. Ground by mortar and pestle for one hour and dried in hot air oven at 40°C for 24 hours. The dried sample was stored in tight light resistant container at controlled room temperature and approximately 0% RH prepared from sodium hydroxide pellets.

1.2 Olanzapine ethanol-water mixed solvate (EtOH-H_2O mixed solvate)

Olanzapine ethanol-water mixed solvate (EtOH-H_2O mixed solvate) was prepared by mixing 5 grams of anhydrous olanzapine form I in 25 mL ethanol. Ground by mortar and pestle for one hour and dried in hot air oven at 40°C for 24 hours. The dried sample was stored in tight light resistant container

at controlled room temperature and approximately 0% RH prepared from sodium hydroxide pellets.

2. Identification of olanzapine polymorphs

The identity of polymorphic forms of olanzapine were charterized and confirmed by polarized light microscopy, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), Karl Fischer Tritrator (KF), X-ray powder diffractrometry (XRPD) and confocal microscopic Raman spectrometry (Raman).

2.1 Polarized light microscopy

Polarized light microscope (Nikon Eclipse E200, Nikon, Japan) equipped with 10x objective lens and 10x eyepiece lenses and polarized len were used. Polarized light produced from normal light passed through polarized lens. Crystalline samples were expected to show birefringence behavior while amorphous samples will not show this phenomenon.

2.2 X-ray Powder diffractrometry (XRPD)

X-ray Powder diffraction patterns of sample were recorded using MiniFlex II (Rigaku, Japan) equipped with CuKα (λ = 1.5406 Å), 15.0 mA, 30.0 kV and slit part 1.25°. Diffraction data were collected at 1 °2Θ/min using an angular step size of 0.01 °2Θ. The scanning range was in range of 5° - 40 °2Θ. Measurement was done in continuous scan mode using quartz sample holder. All data were analyzed using PDXL$^®$ (Version 1.8.1.0) software.

2.3 Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed using Mettler Toledo DSC244e and operated under constant nitrogen purge gas rate of 60 ml/min. The instrument was calibrated by using indium as reference standard. Each sample was weighed approximately 3-5 mg into aluminum pan 40 μL and hermetically sealed with small pinhole in the lid and was heated form 25 to 300°C at the rate of

10°C/min. However, DSC thermograms did not clearly exhibit some endotherm peak, so changed the rate of 10°C/min to 40°C/min.

2.4 Thermogravimetric analysis (TGA)

Thermogravimetric analysis was performed using Mettler Toledo SDTA851[e] operated under constant nitrogen purge gas rate of 20 ml/min. The samples were placed in alumina crucibles 70 µL with lid and were heated from 25 to 300°C at the rate of 10°C/min.

2.5 Karl fischer titration (KF)

Karl fischer tritation was performed according to the method proposed by USP 35 (Method Ia Direct Tritration), using an automatic METROHM Titrino 785 DMP titrator. Titrations were performed with Apura® Titrant (Merck) and methanol (dried) solvent (Chartau, Spain) as the solvent. The sample was weighed approximately 0.5-1 g and put directly into the titration vessel. Transferred enough dried methanol to the titration vessel and titrated with the Karl Fischer Reagent to the visual endpoint in consuming any moisture that may be present.

2.6 Solid-state characterization by Raman spectroscopy and chemometric analysis

2.6.1 Confocal microscopic Raman spectrometry (Raman)

The Raman spectra of sample were recorded using DXR Raman Microscopic Spectrometer (Thermo Fisher Scientific Inc., USA). This machine consisted of 532 nm diode laser source at 10 mW with power of 100%, using size of aperture at 25 µm pinhole and an Olympus TH4-200 microscope. Each Raman spectrum was collected with 20X objective lens using an acquisition time of 2 seconds and accumulating 16 measurements at a time. Raman shifts were collected from 70 to 3600 cm^{-1}. The system was controlled by OMNIC® 8.0 software. All Raman spectral data were collected in 10 replicates and analyzed by

Principal Component Analysis (PCA) in order to distinguish between each data component.

1.6.2 Principal Component Analysis (PCA)

All Raman spectral data of olanzapine samples were analyzed by Principal Component Analysis (PCA) to distinguish between groups of data. The data were analyzed between the ranges of 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1}, respectively, because these ranges showed most differences between all Raman spectral data of olanzapine. The discrimination of all data was achieved by Principal Component Analysis (PCA) using TQ Analyst®8 Pro edition software (Thermo Fisher Scientific Inc., USA). PCA is a multivariate statistical method for characterizing fine spectral variation, to reduce the number of variables in a process and for extracting relevant information from dataset. The maximal amount of variance in the data set and its direction are often explained by the first PC (PC1). The loading plot depicts the identification of important variables and the score plots indicate similarity or dissimilarity of samples.

3. Solid state transformation of olanzapine powder

Anhydrous olanzapine form I was exposed to varying conditions during each unit operation such as, drying, tableting and coating process.

3.1 Effect of drying temperatures

Anhydrous olanzapine form I (Form I) powder was stored at room temperature, approximately 0% RH in desiccator. Each 1 g sample was weighed and dried in hot air oven at 3 different drying temperatures (25℃, 40℃ and 70℃) for 3 hour. Samples were stored at room temperature, approximately 0% RH prepared by sodium hydroxide pellets in tight light resistant desiccator and evaluated 24 hours after preparation by solid state analytical techniques such as, polarized light

microscopy, XRPD, DSC, TGA and Raman using PCA with condition according to section 2. The results were compared with reference olanzapine solid state forms.

3.2 Effect of compaction forces

Anhydrous olanzapine form I (Form I) powders were stored at room temperature, approximately 0% RH in tight light resistant desiccator Each 200 mg sample was weighed per tablet and compressed by Carver$^®$ hydraulic press with force of 1000 psi, 2,000 psi and 3,000 psi using 6.35 mm flat-face punch-face. Compressed tablets were stored at room temperature, approximately 0%RH that was prepared by sodium hydroxide pellets in tight light resistant desiccator and evaluated 24 hours after preparation by solid state analytical techniques such as XRPD and RAMAN using PCA with condition according to section 2. The results were compared with reference olanzapine solid state forms.

3.3 Effect of solvents

3.3.1   Effect of types and quantities of solvents

Anhydrous olanzapine form I (Form I) powders were stored at room temperature, approximately 0% RH in tight light resistant desiccator. Samples of olanzapine were prepared by mixing 1 g of Form I with either water or ethanol by mortar and pestle for 15 minutes and dried in hot air oven at 40°C for 3 hours. The ratios by weight between Form I and each solvent were 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5. Samples were stored at room temperature, approximately 0% RH that was prepared by sodium hydroxide pellets in tight light resistant desiccator and evaluated 24 hours after preparation by solid state analytical techniques such as, polarized light microscopy, XRPD, DSC, TGA and RAMAN using PCA with condition according to section 2. The results were compared with reference olanzapine forms.

Table 2 The ratios between Form I and water were 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5

	$W_{3/1}$	$W_{2/1}$	$W_{1/1}$	$W_{1/2}$	$W_{1/3}$	$W_{1/4}$	$W_{1/5}$
Form I	3 g	2 g	1 g	1 g	1 g	1 g	1 g
Water	1 g	1 g	1 g	2 g	3 g	4 g	5 g

Table 3 The ratios between Form I and ethanol were 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5

	$E_{3/1}$	$E_{2/1}$	$E_{1/1}$	$E_{1/2}$	$E_{1/3}$	$E_{1/4}$	$E_{1/5}$
Form I	3 g	2 g	1 g	1 g	1 g	1 g	1 g
Ethanol	1 g	1 g	1 g	2 g	3 g	4 g	5 g

3.3.2 Effect of type and quantity of solvent mixture

Anhydrous olanzapine form I (Form I) powders were stored at room temperature, approximately 0% RH in tight light resistant desiccator. Samples of olanzapine were prepared by mixing 1 g of Form I with mixed solvents (water and ethanol) by mortar and pestle for 15 minutes. The ratios between Form I and mixed solvents were 1:1 and 1:5. The ratios of mixed solvents between water and ethanol were 0:100, 10:90, 30:70, 50:50, 70:30, 90:10 and 100:0. Then, the samples were dried in hot air oven at 40°C for 3 hours. Samples were stored at room temperature, approximately 0% RH prepared by sodium hydroxide pellets in tight light resistant desiccator in tight light resistant desiccator and evaluated 24 hours after preparation by solid state analytical techniques such as, polarized light microscopy, XRPD, DSC, TGA and RAMAN using PCA with condition according to section 2. The results were compared with reference olanzapine forms.

Table 4 The ratios between Form I and mixed solvents were 1:1, and the ratios of mixed solvents between water and ethanol were 0:100, 10:90, 30:70, 50:50, 70:30, 90:10 and 100:0.

	$1WE_{0/100}$	$1WE_{10/90}$	$1WE_{30/70}$	$1WE_{50/50}$	$1WE_{70/30}$	$1WE_{90/10}$	$1WE_{100/0}$
Form I	1 g	1 g	1 g	1 g	1 g	1 g	1 g
Water	-	0.1 g	0.3 g	0.5 g	0.7 g	0.9 g	1 g
Ethanol	1 g	0.9 g	0.7 g	0.5 g	0.3 g	0.1 g	-

Table 5 The ratios between Form I and mixed solvents were 1:5, and the ratios of mixed solvents between water and ethanol were 0:100, 10:90, 30:70, 50:50, 70:30, 90:10 and 100:0.

	$5WE_{0/100}$	$5WE_{10/90}$	$5WE_{30/70}$	$5WE_{50/50}$	$5WE_{70/30}$	$5WE_{90/10}$	$5WE_{100/0}$
Form I	1 g	1 g	1 g	1 g	1 g	1 g	1 g
Water	-	0.5 g	1.5 g	2.5 g	3.5 g	4.5 g	5 g
Ethanol	5 g	4.5 g	3.5 g	2.5 g	1.5 g	0.5 g	-

4. **Solid state transformation of olanzapine tablets**

Preparation of olanzapine coated tablets

Preparation of core tablet

The core tablets of olanzapine were prepared by conventional direct compression method. The core tablet consisted of Form I (5% w/w), spray dried lactose (43% w/w), microcrystalline cellulose PH102 (43% w/w), sodium starch glycolate (8% w/w) and magnesium stearate (1% w/w) as shown in Table 6. All ingradrient were accurately weighed and blended homogenously. Form I, spray dried lactose, microcrystalline cellulose PH102, sodium starch glycolate were first blended by mortar and pestle for 15 minutes and then blended again with magnesium stearate by blending in a plastic bag for 3 minutes. The core tablets were compressed by using Carver[®] hydraulic press with force of

1000 psi using 6.35 mm flat-face punch. The total weight of core tablet was approximately 200 mg.

Table 6 The formulation of core tablets

Ingredient	Quantity (% w/w)
Anhydrous olanzapine form I (Form I)	5
Spray dried lactose	43
Microcrystalline cellulose PH102	43
Sodium starch glycolate	8
Magnesium stearate	1
Total	100

4.1 Effect of types of solvents in film-coating dispersion

The core tablets of olanzapine were coated by spray gun with manufacturing process according to section 4.2.1 and they were further coated with film-coating dispersion. That consisted of hydroxylpropyl methylcellulose E5 (5% w/w), polyethylene glycol 400 (1.5% w/w), titanium dioxide (3.5% w/w) and varying solvents (water or ethanol). The film-coating dispersion as aqueous formula (Table 7) was prepared by heating half of the calculated amount of water at 70 °C, and mixed hydroxylpropyl methylcellulose E5 with hot water by homogenizer (Polytron® PT-MR 3100, Kinematica AG, Switzerland), and was homogenously mixed with polyethylene glycol 400 and titanium dioxide. Adjust to volume of film-coating dispersion by cold water. Then, it was stirred by magnetic stirrer overnight and filtered by passing through an 80-mesh sieve before use. The film-coating dispersion as ethanolic formula (Table 7) was prepared by mixing hydroxylpropyl methylcellulose E5 with ethanol by homogenizer, and was homogenously mixed with polyethylene glycol 400 and titanium dioxide. Adjust the volume of film-coating dispersion by the remaining ethanol. Then, it was stirred by magnetic

stirred overnight and filtered by passing through an 80-mesh sieve before use. In the film coating process, the coating was applied by spray gun (Thai coater FC 15", Pharmaceutical and medical supply LTD Part, Thailand) until the weight of the film layer reached the target value by weight gain (approximately 5%). The parameters, such as, tablet surface, temperature, spray rate and distance from the spray gun to tablets, were kept constant. In addition, other parameters, such as atomizing air pressure, drying air temperature were optimized and adjusted according to the types and ratios of solvent system of film-coating dispersion. The film coated tablets were stored at room temperature, 0%RH prepared by sodium hydroxide pellets in tight light resistant desiccator and evaluated 24 hours after coating by Raman spectroscopy (Raman) using PCA.

Table 7 The formulation of film-coating dispersion

Ingredient	$COAT_{100/0}$	$COAT_{0/100}$
Hydroxylpropyl methylcellulose E5	5%	5%
Polyethylene glycol 400	1.5%	1.5%
Titanium dioxide	3.5%	3.5%
Water	qs	-
Ethanol	-	qs
Total	100%	

Solid state analytical technique such as, DSC, TGA, XRPD must prepare sample before analysis and it induce solid state transformation of drug substance. Thus, Raman was chosen in this experiment. Before analysis, peeling the film and sampling yellow powders on surface of tablet (Figure 18). The yellow powders are Form I while, excipients are white powders. The results were compared with reference olanzapine hydrated form.
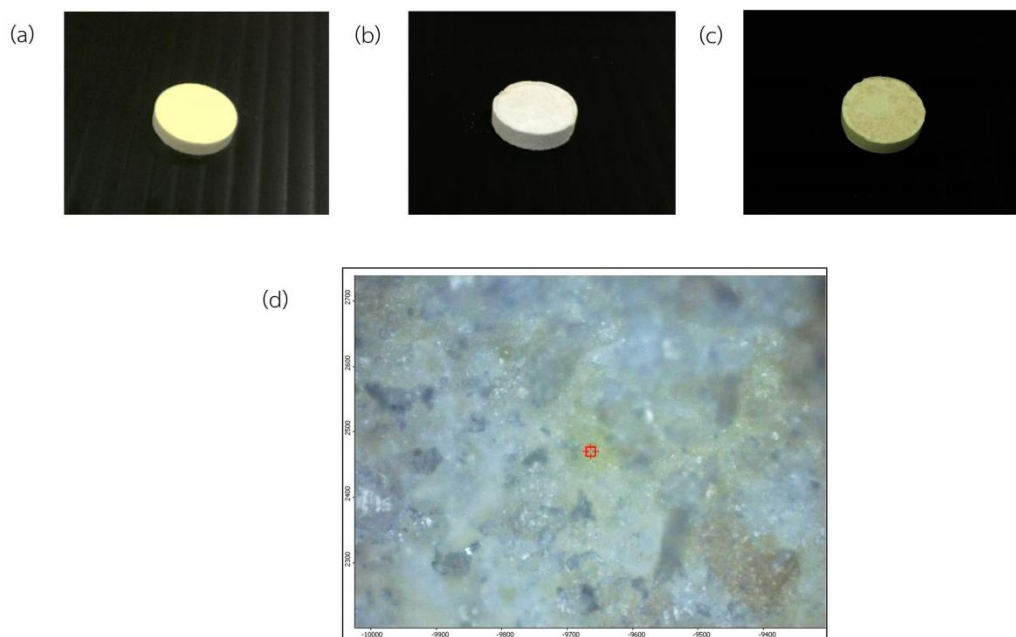
Figure 18 Olanzapine tablets, core tablet, (a) tablet after film coating process (b), tablet after peeling the film (c) and Raman laser focused on yellow powders on surface of tablets (d)

4.2 Effect of solvent evaporations on solid state transformation of film coated tablets

Core tablets of olanzapine were coated by spray gun. The film-coating dispersion used in this experiment consisted of 100% water or 100% ethanol. After film coating was completed (approximately spray time 10 minutes), the coated tablets was dried in hot air oven at 3 different drying temperatures (25°C, 40°C and 70°C) for 3 hour (Table 8). Samples were stored at room temperature, 0% RH prepared by sodium hydroxide pellets in tight light resistant desiccator and evaluated 24 hours after preparation by peeling off the film using Raman with PCA at conditions according to section 4.4.2. The results were compared with reference olanzapine forms.

Table 8 Film-coating dispersions of water and ethanol. The coated tablets were dried in hot air oven at 3 evaporation rates (25°C, 40°C and 70°C)

	$COAT^{25}_{100/0}$	$COAT^{40}_{100/0}$	$COAT^{70}_{100/0}$
Drying temperature	25 ℃	40 ℃	70 ℃
Water	100%	100%	100%
	$COAT^{25}_{0/100}$	$COAT^{40}_{0/100}$	$COAT^{70}_{0/100}$
Drying temperature	25 ℃	40 ℃	70 ℃
Ethanol	100%	100%	100%

CHAPTER IV

RESULTS AND DISCUSSION

1. Preparation of reference olanzapine hydrate forms

Reference anhydrous olanzapine Form I (Lot. OL0291113) was obtained from Hetero Drugs Ltd. Form B and EtOH-H_2O mixed solvate were prepared by in-house methods as indicated in section 1.1 and 1.2. All reference forms were identified by six different techniques and the results are shown below.

2. Identification of olanzapine polymorphs

2.1 Polarized light microscopy

Birefringence phenomena under polarized light microscope was seen in reference anhydrous olanzapine Form I (Figure 19 (a), Form B (Figure 19(b) and EtOH-H_2O mixed (Figure 19(c) solvate, respectively. Form B and EtOH-H_2O mixed solvate were fine crystals, while Form I was coarse crystals. These results initially indicate that three reference olanzapine polymorphs are crystalline. However, polarized light microscopy was not a method of choice to clearly distinguishing the olanzapine polymorphs.

(I) (II)

(a)

(b)

(c)
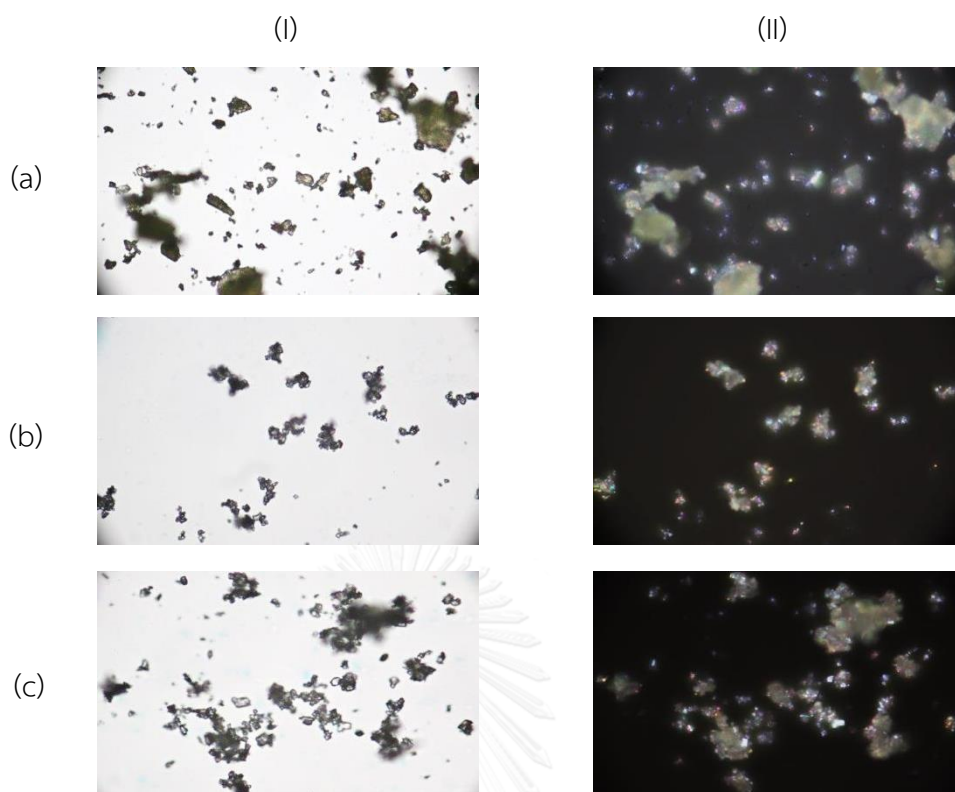
Figure 19 Non-polarized (I) and polarized light (II) photomicrograph of reference olanzapine polymorphs, reference anhydrous olanzapine Form I (a), reference olanzapine dihydrate Form B (b), and reference olanzapine EtOH-H$_2$O mixed solvate (c)

2.2 X-ray Powder diffractometry (XRPD)

Figure 20 shows X-ray powder diffraction (XRPD) profiles of reference anhydrous olanzapine Form I, Form B and EtOH-H$_2$O mixed solvate. All samples exhibit characteristic crystalline peaks. The distinct powder X-ray diffraction peaks of Form I were observed to be at 10.5 °2Θ, 19.0 °2Θ and 21.6 °2Θ (Figure 20(a)), Form B at 16.2 °2Θ, 20.4 °2Θ, 22.7 °2Θ and 24.5 °2Θ (Figure 20(b)) and EtOH-H$_2$O mixed solvate at 14.3 °2Θ, 19.8 °2Θ, 20.5 °2Θ and 25.2 °2Θ (Figure 20(c)). Three polymorphic forms exhibit peaks which were consistent with the report from other studies (6, 20). These results indicate that water and ethanol can affect the

crystalline structure of olanzapine polymorph by transforming anhydrous form into desired solvated forms.
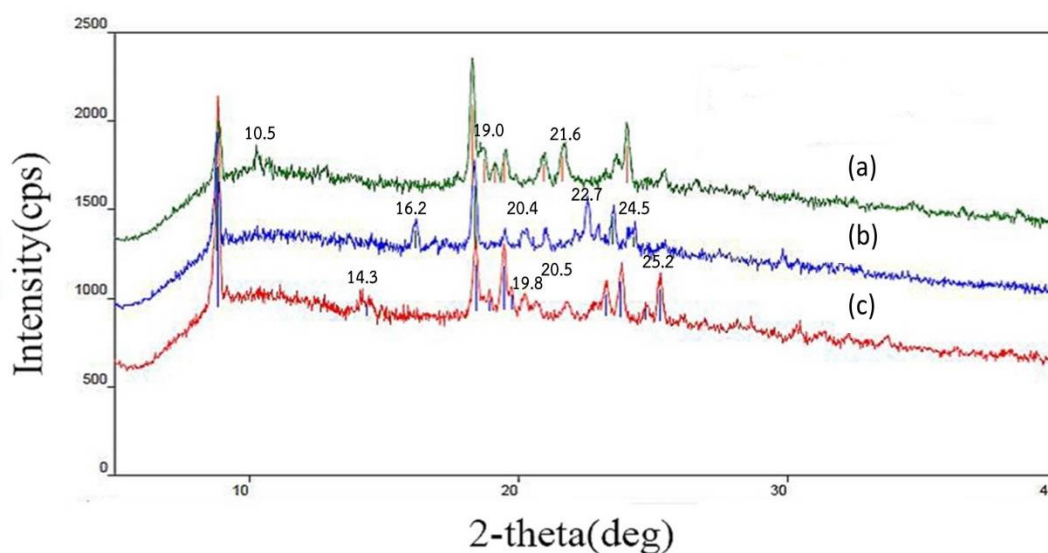
Figure 20 XRPD diffractograms of reference olanzapine polymorphs, reference anhydrous olanzapine Form I (a), reference olanzapine dihydrate Form B (b), and reference olanzapine EtOH-H_2O mixed solvate (c)

2.3 Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) thermograms of reference anhydrous olanzapine Form I, Form B and EtOH-H_2O mixed solvate showed sharp melting with degradation peaks at 196.80°C (Figure 21(a)), 196.75°C (Figure 21(b)) and 197.50°C (Figure 21(c)), respectively. The results indicate that olanzapine is crystalline form before and after exposure to solvents. The thermogram of Form I exhibit melting endotherm at 183.90°C, followed by a recrystallization exotherm and second melting endotherm at 196.80°C (Figure 21(a)). But the hydrated Form B and EtOH-H_2O mixed solvate show new endothermic at 119°C, ΔH = 180.64 Jg^{-1} (Figure 21(b)) 101°C, ΔH = 9.59 Jg^{-1} and 124.44°C,

ΔH = 151.77 Jg^{-1} (Figure 21(c)), respectively. These peaks resulted from the loss of water and ethanol molecule in samples (12, 20).
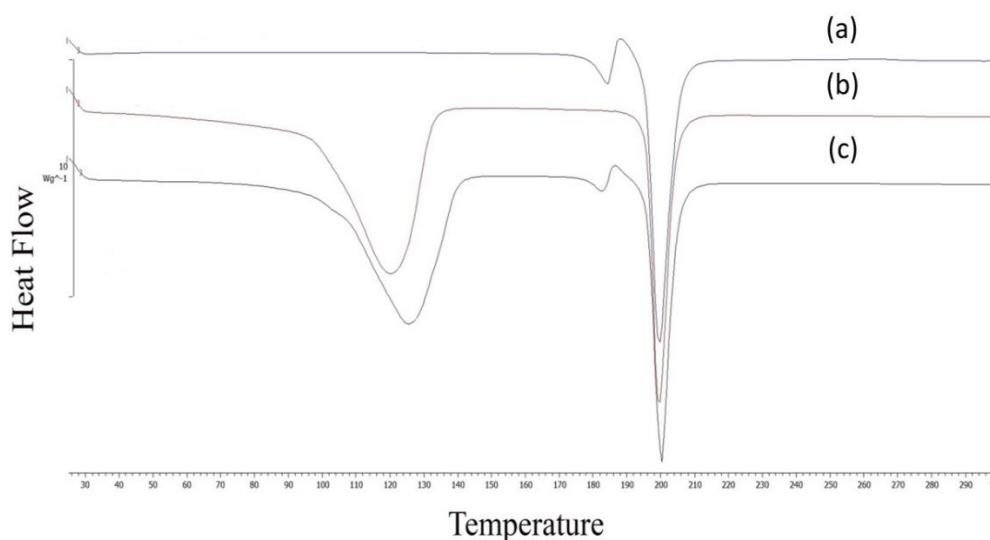
Figure 21 DSC thermograms of reference olanzapine polymorphs, reference anhydrous olanzapine Form I (a), reference olanzapine dihydrate Form B (b), and reference olanzapine EtOH-H$_2$O mixed solvate (c)

2.4 Thermogravimetric analysis (TGA)

Figure 22 shows thermogravimetric analysis (TGA) result of Form I exhibiting linearity in weight with no weight loss until degraded at 200°C. Figure 22(a) and Figure 22(b and c) show TGA thermograms of solvate forms by water and ethanol respectively. TGA thermogram of Form B indicates weight loss of 10.50% w/w between 40-120°C and degradation endotherm at 200°C, whereas TGA thermograms of EtOH-H$_2$O mixed solvate exhibits weight loss 11.76% w/w between 40-120°C and degradation endotherm at 200°C. As the weight loss were similar, solvated forms obtained by both solvents cannot be distinguished by TGA.
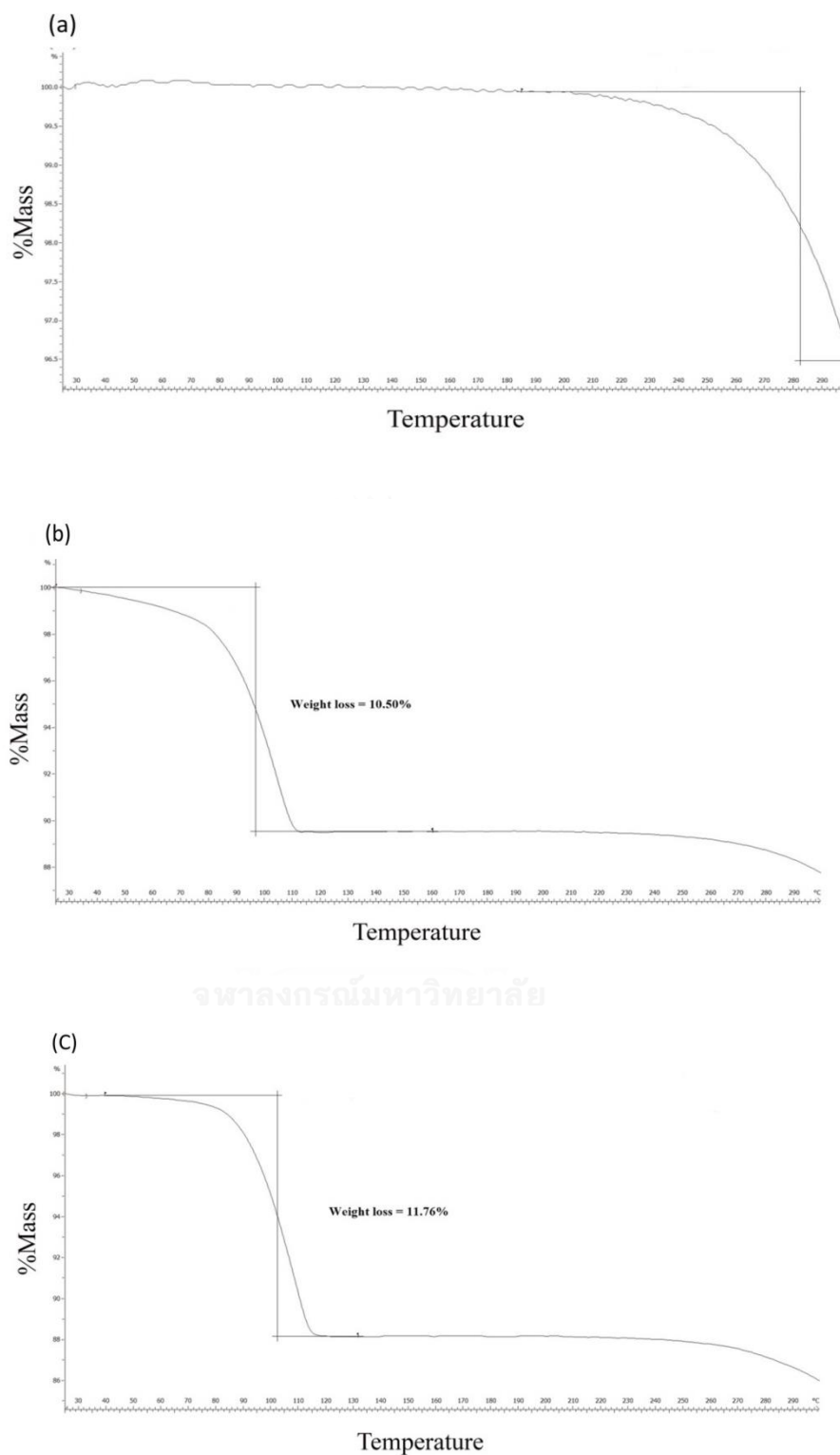
Figure 22 TGA thermograms indicating % weight loss of reference olanzapine polymorphs, reference anhydrous olanzapine Form I (a), reference olanzapine dihydrate Form B (b) and reference olanzapine EtOH-H_2O mixed solvate (c)

2.5 Karl Fischer titration (KF)

To ensure that the both solvated forms (Form B and EtOH-H$_2$O mixed solvate are different. Karl Fischer titrations (KF) of the samples were investigated due to the fact that KF only detect water molecules. The results that the water content of Form B is 10.14%, while the water content of EtOH-H$_2$O mixed solvate is only 5.17%. In addition, the water content of Form I is only 0.17%. From these results, as determined by KF method corresponded to the presence of only one water molecule per molecule of olanzapine. A stoichiometric calculation allowed the estimation of theoretical yield of water content in monohydrate form of olanzapine is 5.45% and the theoretical yield of water content of dihydrate form was 10.33% (12). So, KF was successful in distinguishing the two solvated forms.

2.6 Solid-state characterization by Raman spectroscopy

Figure 23(a) shows Raman spectrum of Form I. Figure 23(b) shows Raman spectrum of hydrate sample prepared by water (Form B) and figure 23(c) shows Raman spectrum of solvated sample prepared by ethanol (EtOH-H$_2$O mixed solvate). All of the spectra indicate characteristic shifts crystalline forms similar to the result of XRPD. Raman shifts between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to represent clear visual distinction between samples. Raman spectral results for Form B and EtOH-H$_2$O mixed solvate are visually similar to Raman spectrum of Form I. The use of visual inspection of Raman shifts alone could not successfully distinguished between Form I, Form B and EtOH-H$_2$O mixed solvate. Hence, chemometric analysis tool called Principal Component Analysis (PCA) is more appropriate for characterizing fine spectral variation which visual observation was not able to do.

Figure 24(a) and (b) indicates that Raman spectral data of Form I, Form B and EtOH-H$_2$O mixed solvate can be differentiated 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 between the ranges of 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1}. For discrimination of all data by Principal Component Analysis (PCA) with TQ Analyst[®]8 Pro edition software was used. The first PC (PC1) accounted for 70.22% of overall variance, the second PC (PC2) accounted for 25.68% of overall variance and the third PC (PC3) accounted for 2.82% of overall variance. The PCA results indicate that all samples can be clearly separated into 3 distinct groups. For this reason, confocal microscopic Raman spectroscopy and chemometric analysis (PCA) was chosen as mathematical statistics tool to successfully classify between three solid states of olanzapine in this study.

Figure 23 Raman spectra of reference olanzapine polymorphs, reference anhydrous olanzapine Form I (a), reference olanzapine dihydrate Form B (b), and reference olanzapine EtOH-H_2O mixed solvate (c)
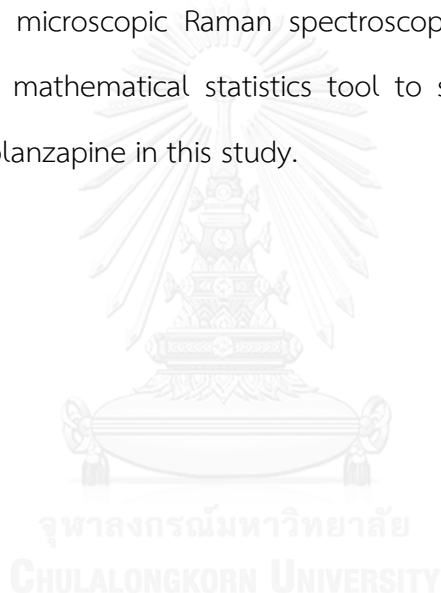
(a)
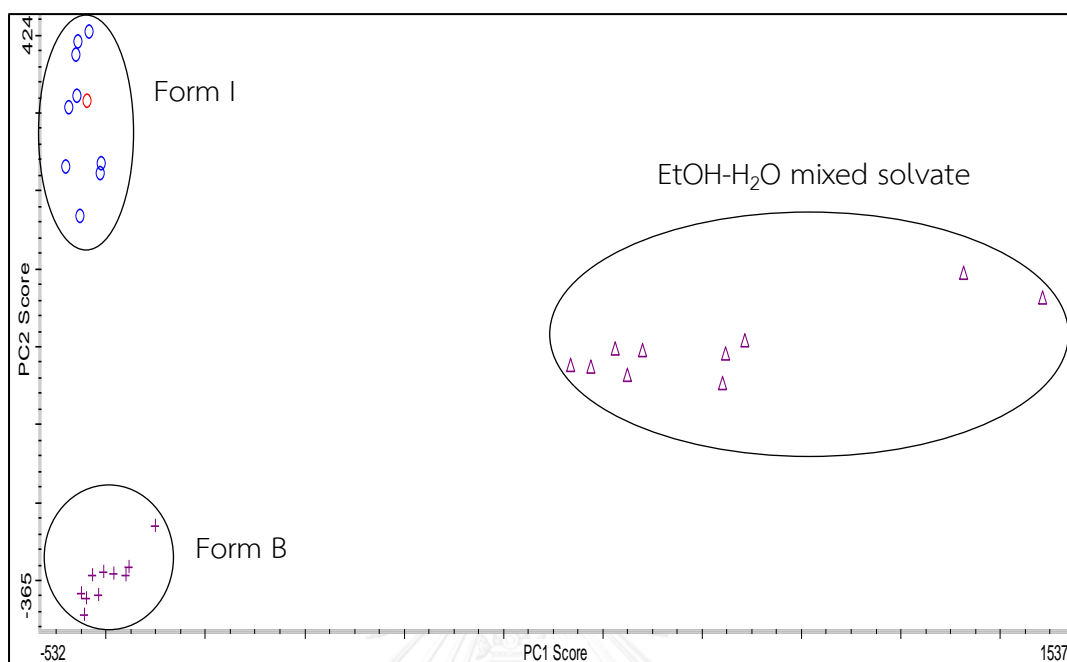
(b)
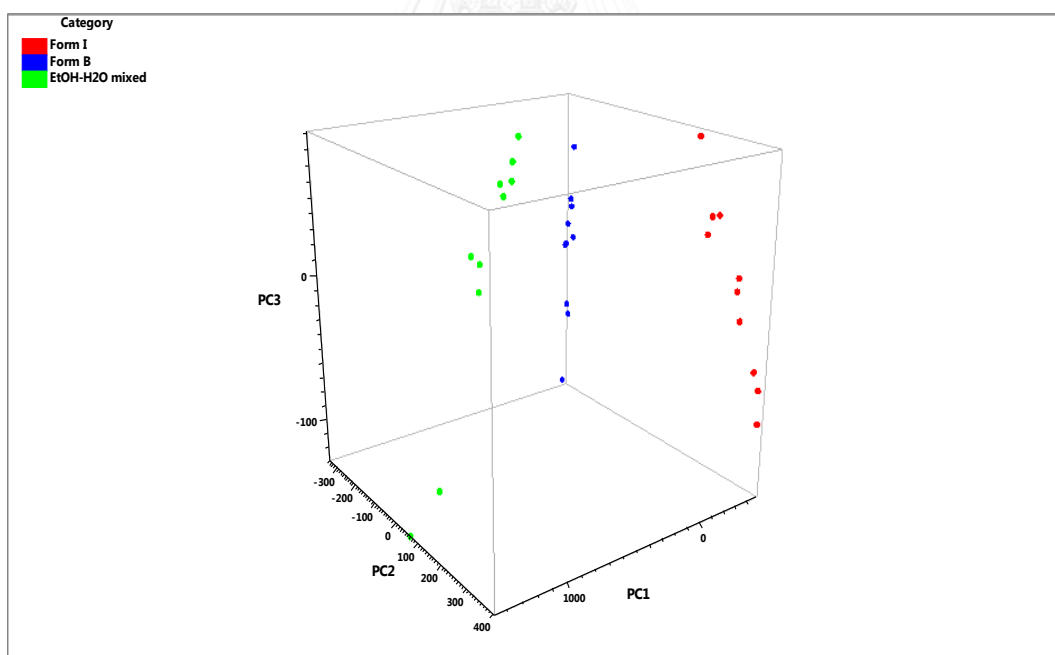
Figure 24 PCA score plots of reference olanzapine forms to distinguish between groups of data using TQ Analyst®8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensianal PCA score plot (b)

Characterization results from all six techniques suggest that these samples were pure polymorphic forms. Some techniques were able to clearly distinguish between reference olanzapine samples for example, XRPD, DSC, KF and Raman spectroscopy.

3. Solid state transformation of olanzapine powder

Factors encountered during manufacturing process of coated tablets which might induce solid state transformation, for example, drying temperature, compaction force and solvent contact. These transformations may affect the physicochemical and biopharmaceutical properties of the drug products, development protocol and regulatory requirements. Thus, solid state techniques, such as, polarized light microscopy, XRPD, DSC, TGA and Raman with PCA are selected for identification and quantitation of olanzapine polymorphic forms.

3.1 Effect of drying temperatures

Effect of drying temperatures on solid state transformation of anhydrous olanzapine Form I powder after drying in hot air oven at constant temperatures of 25℃ , 40℃ and 70℃ for 3 hours. Samples were evaluated by several solid state analytical techniques.

3.1.1 Polarized light microscopy

Figure 25(a and b), Figure 26(a and b) and Figure 27(a and b) show appearance and birefringence phenomena under polarized light microscope of olanzapine sample that dried at 25℃, 40℃ and 70℃. The olanzapine samples dried at 25℃, 40℃ and 70℃ are shown to exhibit coarse crystals and all shown to have birefringence indicating crystallinity.

All above results from polarized light microscope of olanzapine samples dried at 3 different conditions could only indicate that the samples were crystalline

form not amorphous.   Moreover, this method could not be used to indicate crystalline polymorphic transformation by exposing to various drying temperatures.
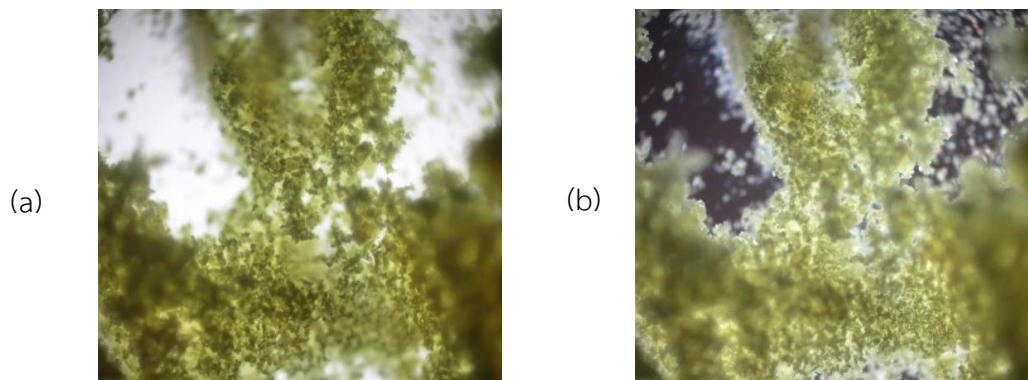
Figure 25 Polarized photomicrographs of olanzapine sample dries in hot air oven at 25 °C under non-polarized (a) and polarized lens (b)
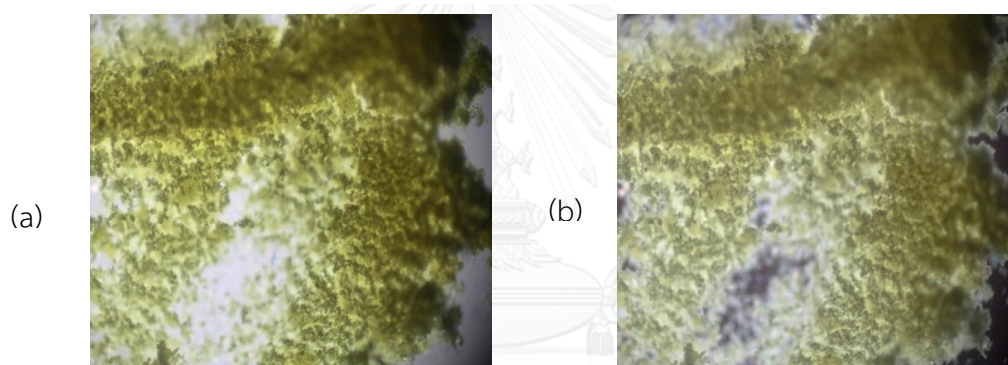
Figure 26 Polarized photomicrographs of olanzapine sample dries in hot air oven at 40 °C under non-polarized (a) and polarized lens (b)
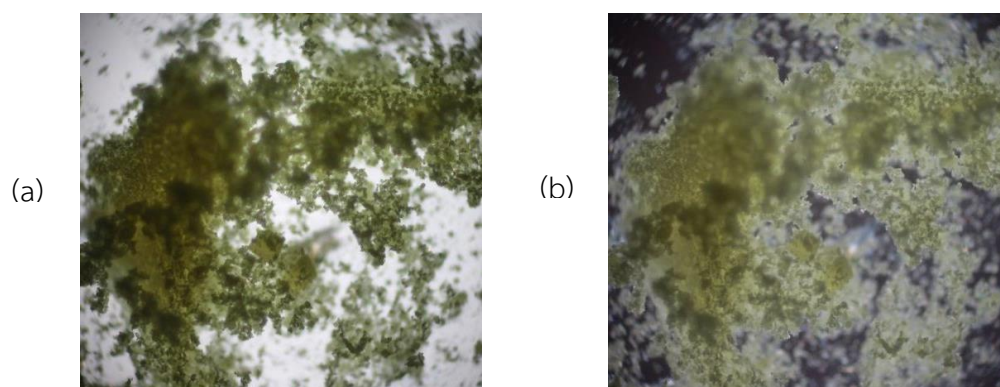
Figure 27 Polarized photomicrographs of olanzapine sample dries in hot air oven at 70 °C under non-polarized (a) and polarized lens (b)

3.1.2 X-ray Powder diffractrometry (XRPD)

Figure 28 exhibits X-ray powder diffractograms of olanzapine samples dried at 25℃, 40℃ and 70℃ for 3 hours compare to anhydrous olanzapine Form I and both olanzapine solvated forms (Form B and EtOH-water mixed solvate). The XRPD diffractograms of all olanzapine samples dried at 25℃, 40℃ and 70℃ show crystalline peaks identical to reference anhydrous olanzapine Form I.

The results indicate that drying temperatures of 25℃, 40℃ and 70℃ did not induce solid state transformation of olanzapine even at the highest drying temperature of 70℃.
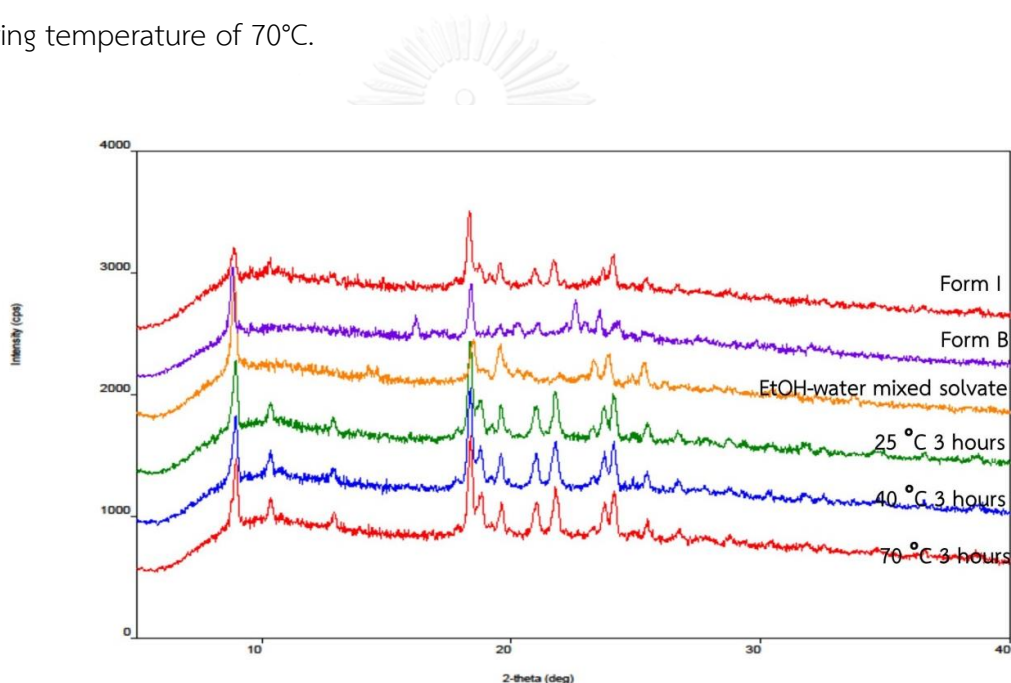
Figure 28 XRPD diffractograms of olanzapine samples dry at 25℃, 40℃ and 70℃ for 3 hours compare to reference anhydrous olanzapine Form I, Form B and EtOH-H_2O solvated forms

3.1.3 Differential scanning calorimetry (DSC)

Effect of drying temperatures on the thermal behaviors of prepared samples was studied by DSC method. Figure 29 shows DSC thermograms of olanzapine samples dried at 25℃, 40℃ and 70℃ for 3 hours. The DSC thermograms

of 3 samples exhibit endotherm peak at 183.37°C (a), 183.78°C (b) and 183.30°C (c) respectively, and followed by a recrystallization exotherm and second melting endotherm at 196.73°C (a), 196.31°C (b) and 195.51°C (c) of olanzapine samples dried at 25°C, 40°C and 70°C, respectively. From the thermograms, Form I exhibits lower melting point than Form II (melting point 195°C). Form I was converted to a more stable polymorph, at 183°C. These thermal behaviors are characteristic of monotropic polymorphs (6, 10, 20). The DSC themograms show endotherm peaks similar to reference anhydrous olanzapine Form I in accordance to section 2.3.

From the results, it can be concluded that drying temperature had no effect on the solid state transformation of olanzapine Form I.
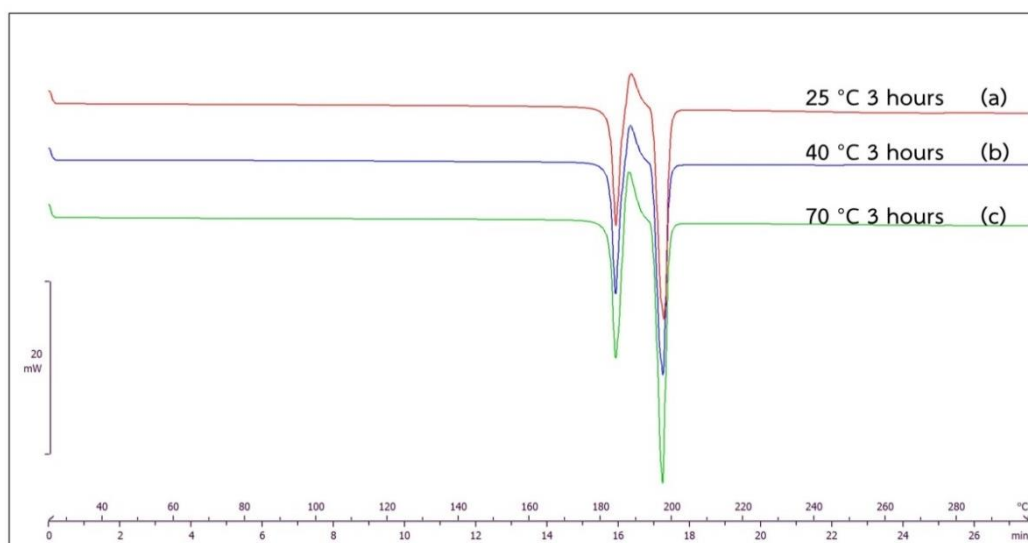
Figure 29 DSC thermograms of olanzapine samples dry at 25°C (a), 40°C (b) and 70°C (c) for 3 hours

3.1.4 Thermogravimetric analysis (TGA)

Figure 30 displays TGA thermograms of olanzapine samples dried at 3 different conditions (25°C, 40°C and 70°C) for 3 hours. TGA thermograms of all samples dried at 25, 40 and 70 °C exhibit linearity in weight until degraded at 220°C.

All samples exhibit TGA thermograms similar to TGA thermogram of reference anhydrous olanzapine Form I as depicted in section 2.4.

All the results indicated that drying olanzapine Form I at these temperatures did not affect the weight loss behavior of olanzapine Form I.
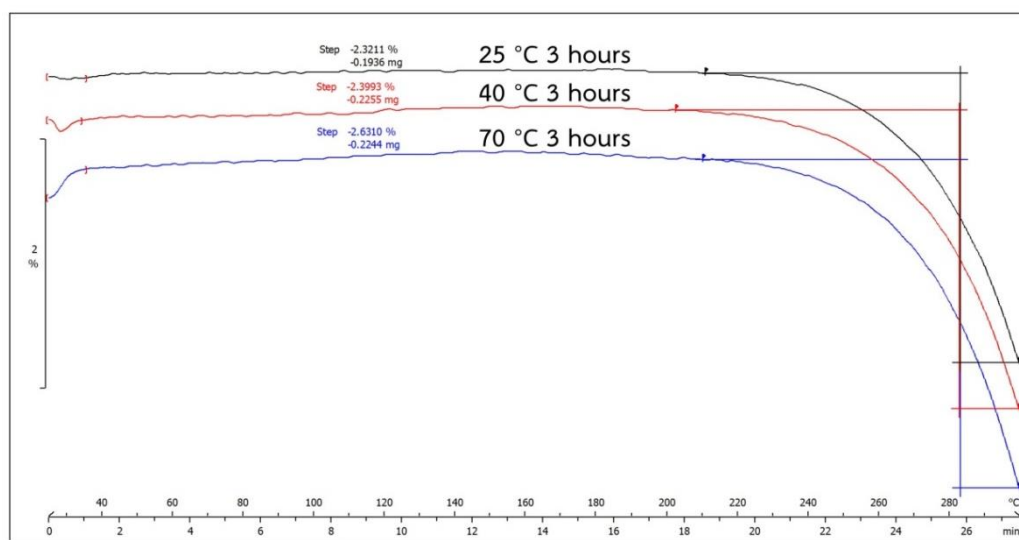
Figure 30 TGA thermograms of olanzapine samples dry at 25°C, 40°C and 70°C for 3 hours

3.1.5 Solid-state characterization by Raman spectroscopy

Figure 31 shows Raman spectra of olanzapine Form I samples dried at 25°C, 40°C and 70°C for 3 hours compare to reference anhydrous olanzapine Form I and both olanzapine solvated forms (Form B and EtOH-water mixed solvate). Raman shifts between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to exhibit clear distinction between reference forms. All Raman spectral data were collected in 10 replicates and analyzed by OMNIC[®] (Version 8.0) and discrimination of all data were done by Principal Component Analysis (PCA) using TQ Analyst[®]8 Pro edition software. Results from Raman method in Figure 31 indicate that olanzapine samples dried at 25°C, 40°C and 70°C show Raman spectra similar to Form I. However, according to a

previous section, Raman spectra alone cloud not be used to differentiate between olanzapine solid state forms visually. Thus, Chemometric method utilized PCA was used as an analytical tool to distinguish between each forms as proven in the initial section and utilized Mahalanobis distance for determination similarity of the samples and reference olanzapine forms. Mahalanobis distance is a statistical distance that measures distance between the central point of sample and central point of reference olanzapine form. Mahalonobis distances consist of scale and vector but in this study only scale were evaluated.

Figure 32(a and b) indicate Principal Component Analysis (PCA) of olanzapine samples dried at 3 drying temperatures (25°C, 40°C and 70°C for 3 hours) compare to 3 reference olanzapine forms (Form I, and Form B and EtOH-H_2O mixed solvate). Raman spectral data from dried olanzapine samples and 3 reference olanzapine forms can be distinguished by PCA as 3 distinctive groups. The first group composed of Raman spectral data obtained from olanzapine samples dried at 25°C, 40°C, 70°C and reference Form I. The second group composed of Form B. The third group composed of EtOH-H_2O mixed solvate. The first principal component (PC1) accounted for 63.00% of overall variance and the second principal component (PC2) accounted for 30.63% of overall variance, and the third PC (PC3) accounted for 3.13% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine dried samples at 25°C, 40°C, and 70°C are shown in Table 9 and was calculated from data in Appendix A.

Table 9 Mahalanobis distances between reference olanzapine Form I, Form B,

EtOH-H$_2$O mixed solvate and olanzapine samples dry at 25℃, 40℃, and 70℃

Olanzapine samples dry at	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H$_2$O mixed solvate
25℃ 3 hours	1.51	5.02	3.54
40℃ 3 hours	1.41	4.91	3.45
70℃ 3 hours	1.46	4.91	3.53

The results presented that the reference olanzapine forms and olanzapine samples can be classified by PCA and clearly distinguished as 3 distinctive solid state groups. Olanzapine Form I samples dried at 25℃, 40℃ and 70℃ for 3 hours still retained the structure as similar to Form I as indicated by the least Mahalanobis distance. In conclusion, drying temperatures had no affect on the solid state transformation of olanzapine Form I samples.
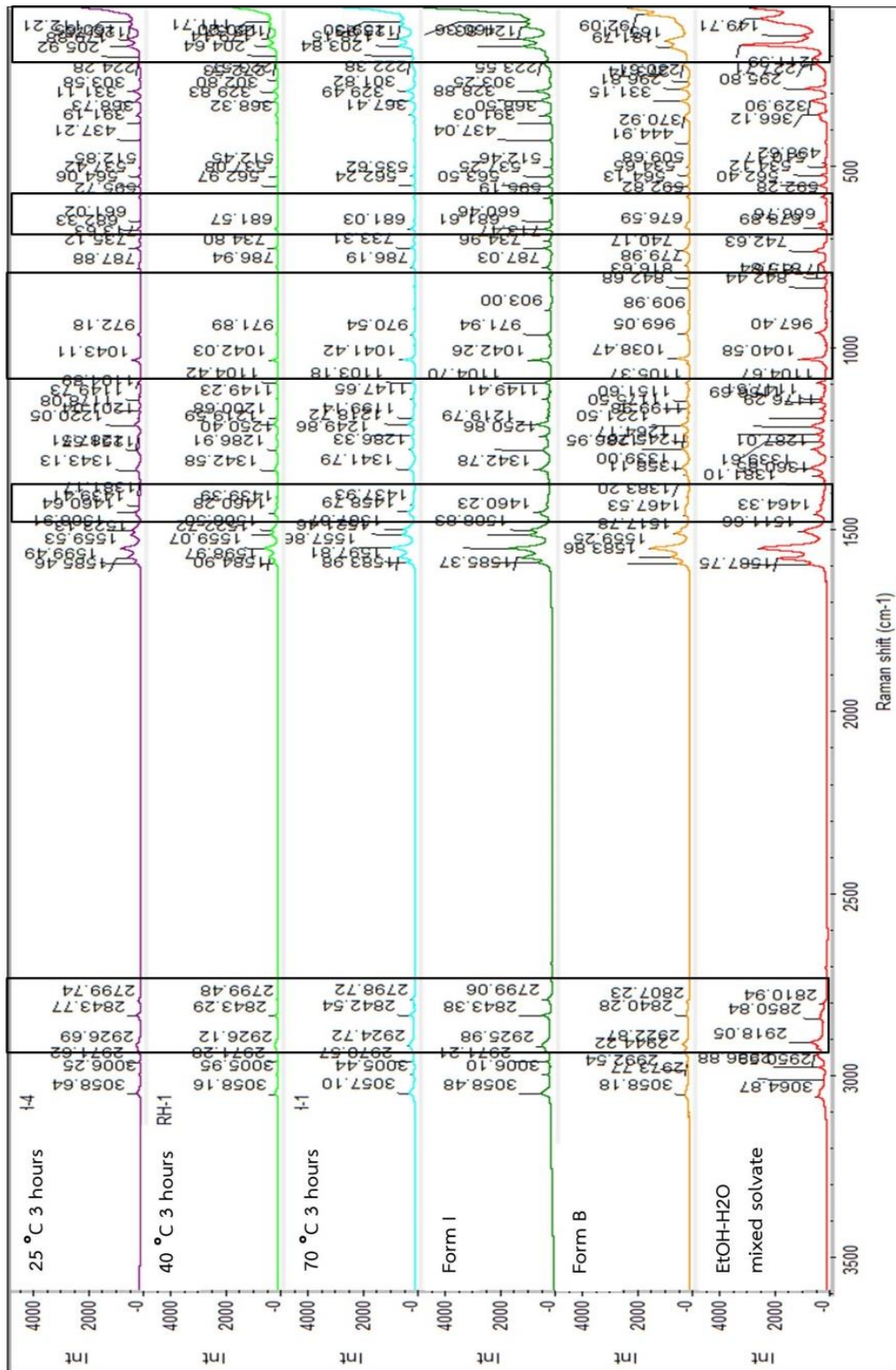
Figure 31 Raman spectra of olanzapine samples dry at 25°C, 40°C and 70°C for 3 hours compare to Form I, and Form B and EtOH-H₂O mixed solvate

a)
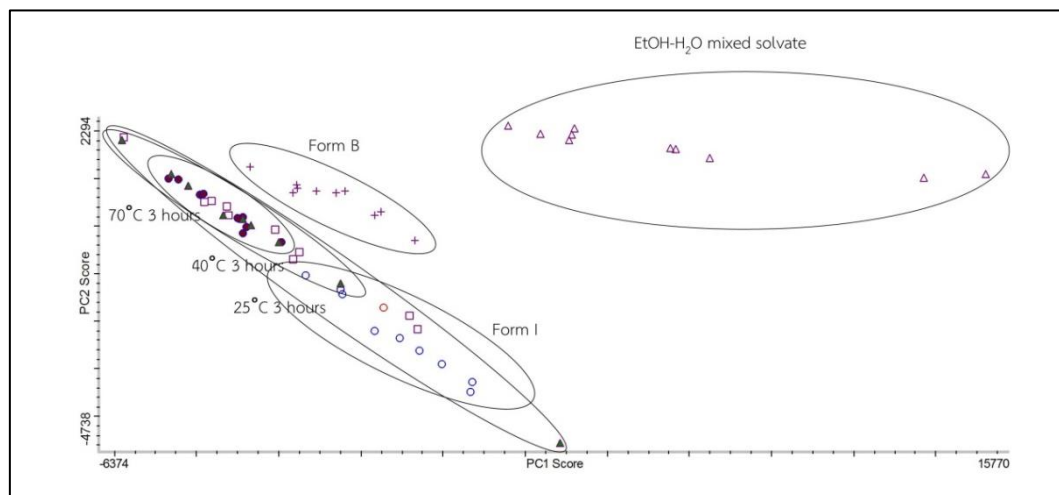
(b)
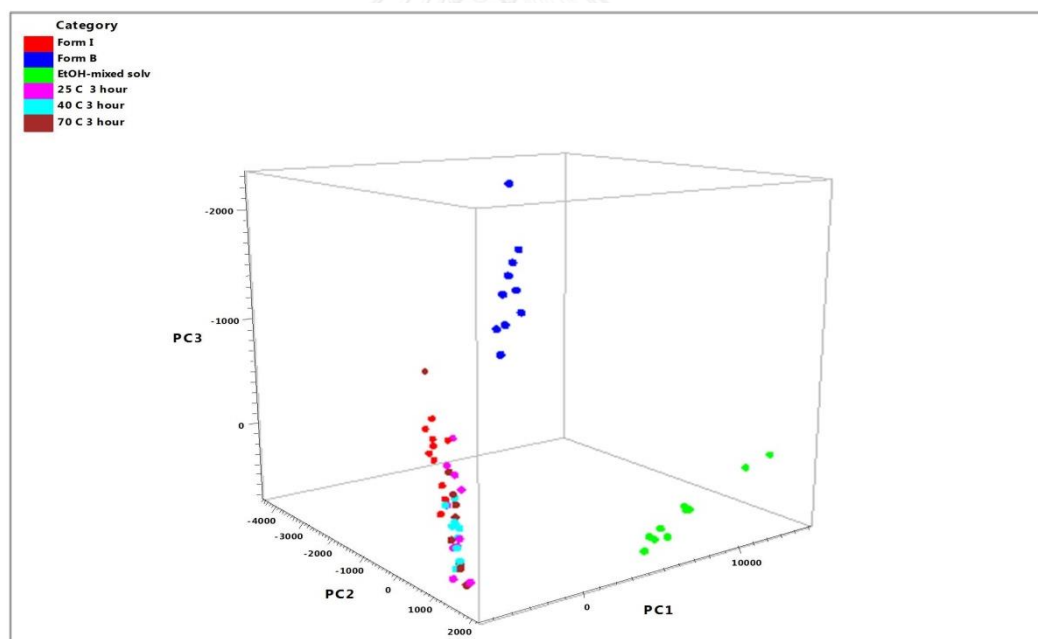
Figure 32 PCA score plots of olanzapine samples dry at 25°C, 40°C and 70°C for 3 hours compare to Form I, and Form B andEtOH-H_2O mixed solvate using TQ Analyst[®]8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

The effect of drying temperatures on solid state interconversion of olanzapine Form I was analyzed by polarized microscope (Figures 25, 26 and 27), XRPD (Figure 28), DSC (Figure 29), TGA (Figure 30) and Raman spectra with PCA (Figure 32a and b) It could be concluded from above results that the drying temperatures of 25°C, 40°C and 70°C did not induced solid state transformation of anhydrous olanzapine Form I powder.

3.2 Effect of compaction forces

Effect of compaction forces on solid state transformation of anhydrous olanzapine Form I powder that prepared by Carver® hydraulic press with forces of 1,000 psi, 2,000 psi and 3,000 psi using 6.35 mm punch-face. Samples were analyzed 24 hours after preparation by solid state analytical techniques. Figure 33 shows XRPD diffractrograms of olanzapine samples and Figure 34 and 35 (a and b) shows Raman spectra and PCA score plots of olanzapine samples respectively.

3.2.1 X-ray Powder diffractrometry (XRPD)

Figure 33 shows X-ray powder diffractograms of olanzapine samples compressed with forces of 1,000 psi, 2,000 psi and 3,000 psi compare to anhydrous olanzapine Form I, (Form B) and EtOH-H_2O mixed solvate. The XRPD diffractograms of all samples exhibited peaks at 10.5° 2Θ, 19.0° 2Θ and 21.6° 2Θ which are characteristic of Form I and did not exhibit characteristic peaks of Form B and EtOH- H_2O mixed solvate. However, as the compaction forces increased, the XRPD diffraction peaks exhibited lower intensity indicating decrease in crystallinity.

From the results indicated that low compaction forces (1,000 psi, 2,000 psi and 3,000 psi) did not exert enough energy to induce significant change for solid state transformation on the surface of olanzapine tablets. However,

compaction forces used could only partially converted crystalline Form I to amorphous structure as seen by reduced and broadened XRPD peak intensities.
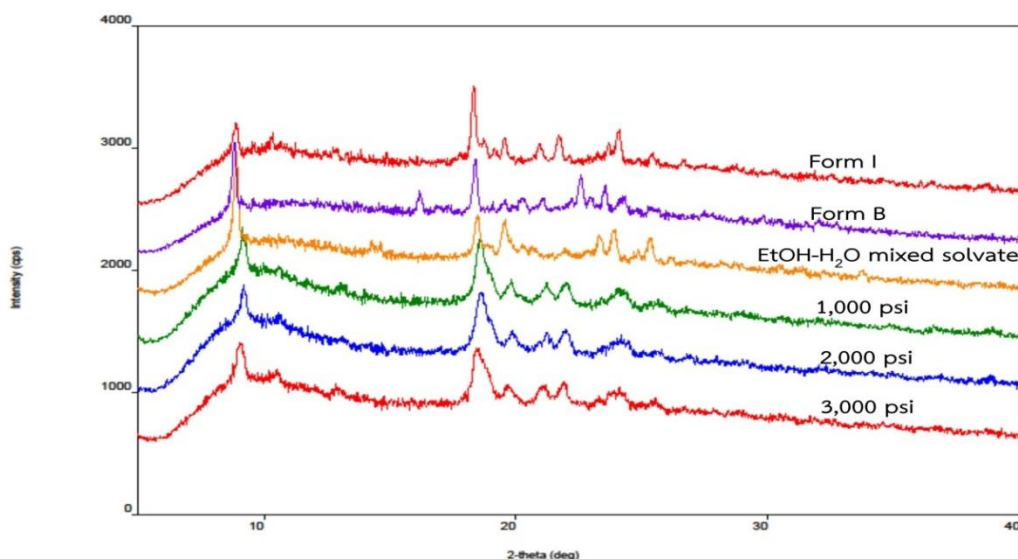
Figure 33 XRPD diffractograms of olanzapine samples compress with forces of 1,000 psi, 2,000 psi and 3,000 psi compare to anhydrous olanzapine Form I and both olanzapine solvated forms

3.2.2 Solid-state characterization by Raman spectroscopy

Figure 34 shows Raman spectra of olanzapine samples compressed with force of 1,000 psi, 2,000 psi and 3,000 psi compare to reference anhydrous olanzapine Form I, Form B and EtOH-water mixed solvate. Raman shifts between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to represent clear distinction between Form I, Form B and EtOH-water mixed solvate. All Raman spectral data were collected in 10 replicates per compressed solid and analyzed by OMNIC® (Version 8.0) software. Discrimination and correlation of all data was done by Principal Component Analysis (PCA) and Mahalanobis distance using TQ Analyst®8 Pro edition software. Raman spectral results of samples being compressed with forces of 1,000 psi, 2,000 psi and 3,000 psi are visually similar to Raman spectrum of reference anhydrous olanzapine Form I.

Thus, by visually observing Raman spectra could not be used to clearly distinguish the solid state transformation of the samples and reference olanzapine forms.

Raman spectral data from compressed olanzapine samples and reference olanzapine forms could be differentiated by PCA as 3 characteristic groups. Figure 35(a and b) exhibits PCA of compressed olanzapine samples with forces of 1,000 psi, 2,000 psi and 3,000 psi and stored at 25 °C, 0%RH for24 hours compares to Form I, Form B and EtOH-H_2O mixed solvate. The first principal component (PC1) accounted for 67.54% of overall variance, the second principal component (PC2) accounted for 26.22% of overall variance and the third principal component (PC3) accounted for 4.52% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine compressed samples at 1,000 psi, 2,000 psi and 3,000 psi was shown in Table 10 and were calculated from data in Appendix B. The first group composed of Raman spectral data obtained from compressed olanzapine Form I. The second group composes of only Form B and the third group composed of only EtOH-H_2O mixed solvate.

Table 10 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine compress samples at 1,000 psi, 2,000 psi and 3,000 psi

Olanzapine sample	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H_2O mixed solvate
1,000 psi	1.99	5.92	4.56
2,000 psi	2.62	5.41	3.94
3,000 psi	2.80	5.46	3.99

Principal Component Analysis (PCA) could be used to clearly distinguish the 3 distinctive solid states of olanzapine. Olanzapine samples compressed with forces of 1,000 psi, 2,000 psi and 3,000 psi were most similar to Form I as shown by the least Mahalanobis distance. It can be conclude that the compaction forces had no effect on solid state transformation of olanzapine Form I samples. However, compression force could induce partial conversion of Form I to amorphous structure with majority still retaining crystalline Form I.

The compaction force could induce solid state phase transition by rearrangement, fragmentation, bond formation, deformation of particles or crystals and friction at the die wall. The transformation took place only on the surface of the tablet so the surface had lower crystallinity than tablet inferior. High compaction force was found to be able to induce solid state transformation (47-50). In this study, the XRPD patterns (Figure 33) corresponded well with results obtained from PCA. It can be concluded that the compaction forces in this condition (1,000 psi, 2,000 psi and 3,000 psi) could not induced solid state transformation between olanzapine crystalline forms but could induced a minor decreased in crystallinity.

Figure 34 Raman spectra of olanzapine samples prepared by compression forces of 1,000 psi, 2,000 psi and 3,000 psi using Carver® hydraulic press

(a)
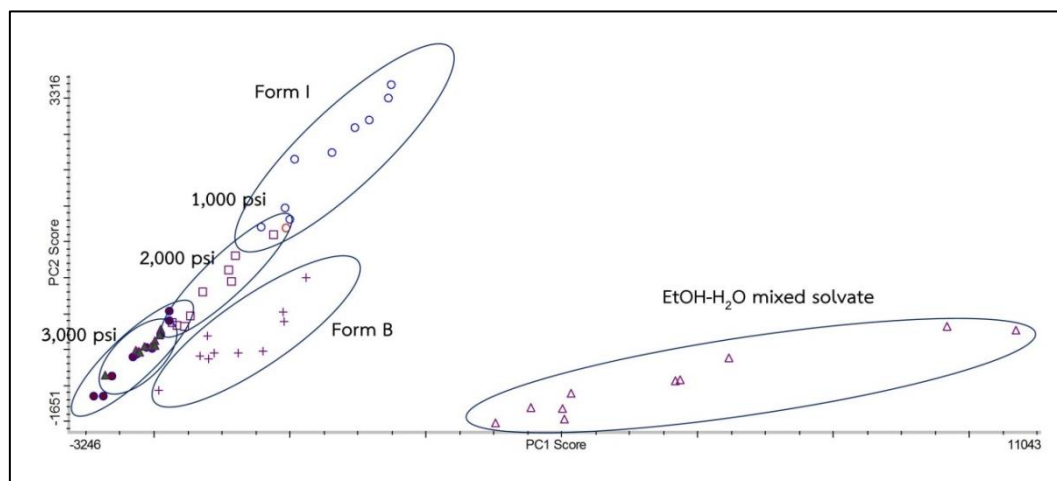
(b)
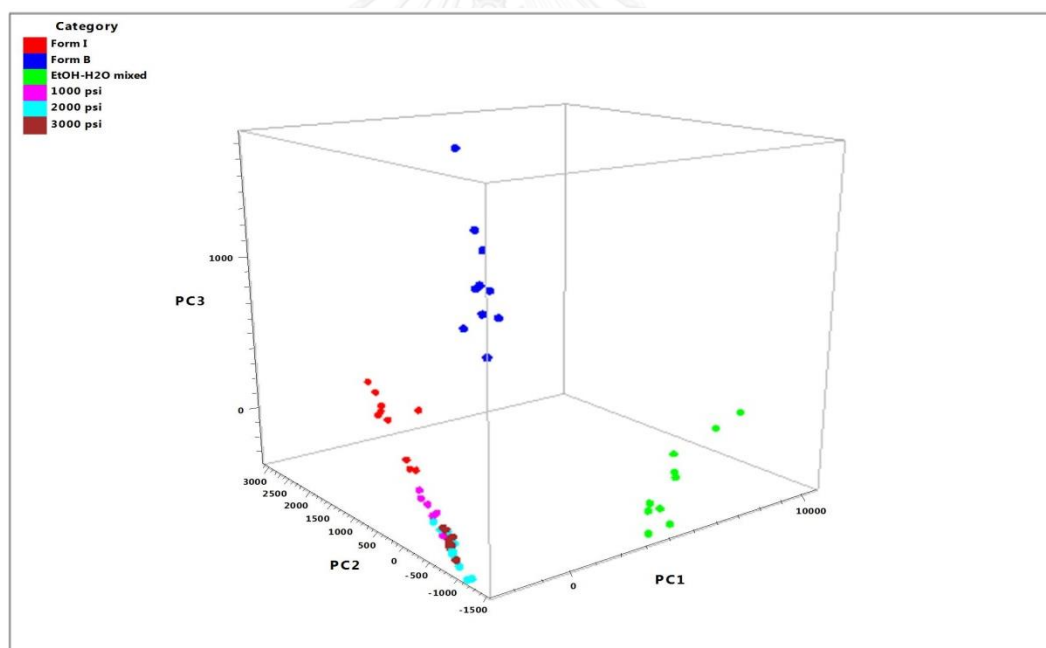
Figure 35 PCA score plots of olanzapine samples compress with forces of 1,000 psi, 2,000 psi and 3,000 psi compare to reference Form I, Form B andEtOH-H$_2$O mixed solvate using TQ Analyst$^®$8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

3.3 Effect of solvent

3.3.1 Effect of type and quantity of solvents

Effect of type and quantity of solvents on solid state transformation of anhydrous olanzapine Form I powder were studied. Sample were prepared by mixing one gram of Form I with two different solvents and dried at 40°C for 3 hours. The solvent used were either water or ethanol. The ratios between Form I and each solvent were 3:1, 2:1, 1:1, 1:2, 1:3, 1:4 and 1:5. After preparation, samples were stored at 0%RH for 24 hours and analyzed by solid state analytical techniques.

3.3.1.1 Polarized light microscopy

Figure 36(a-g) shows crystalline birefringence phenomena of Form I when mixed with water and dried. The mixing ratios between Form I and water were 3:1 ($W_{3/1}$), 2:1 ($W_{2/1}$), 1:1 ($W_{1/1}$), 1:2 ($W_{1/2}$), 1:3 ($W_{1/3}$), 1:4 ($W_{1/4}$) and 1:5 ($W_{1/5}$). Figure 37(a-g) shows crystalline birefringence phenomena of Form I when mixed with ethanol. The ratios between Form I and ethanol were 3:1 ($E_{3/1}$), 2:1 ($E_{2/1}$), 1:1 ($E_{1/1}$), 1:2 ($E_{1/2}$), 1:3 ($E_{1/3}$), 1:4 ($E_{1/4}$) and 1:5 ($E_{1/5}$). Olanzapine samples obtained after mixing with water or ethanol and dried were fine crystals and shown birefringence phenomena.

The results from polarized light microscopy of olanzapine samples mixed with both solvents at 7 ratios could only indicate that the samples were crystalline. However, it could not be concluded from these results how the type and quantity of solvents will have on the extent of solid state conversion of olanzapine

(I) (II)

(a)

(b)

(c)

(d)

(e)
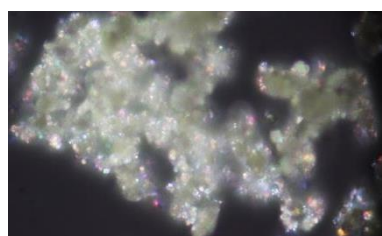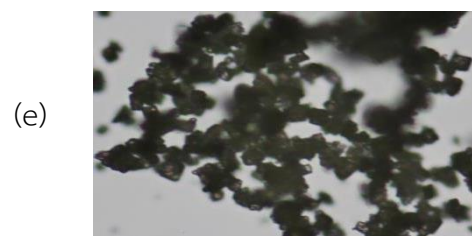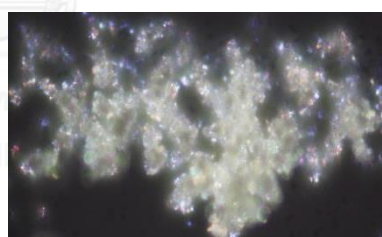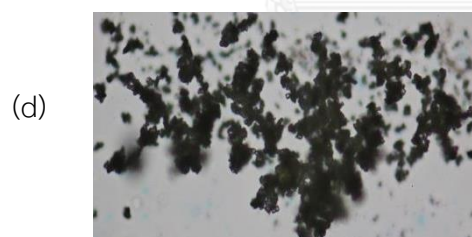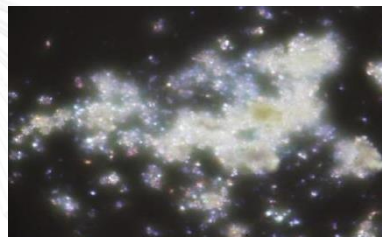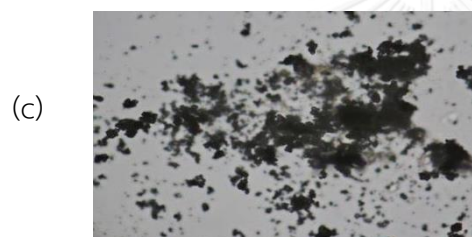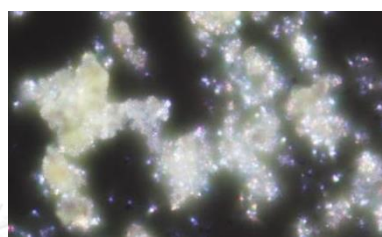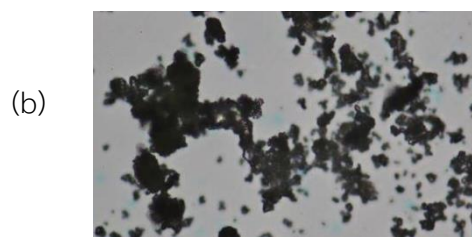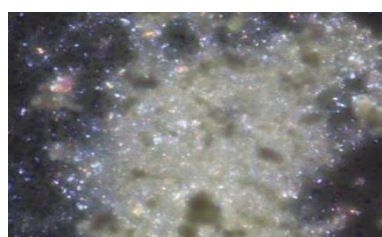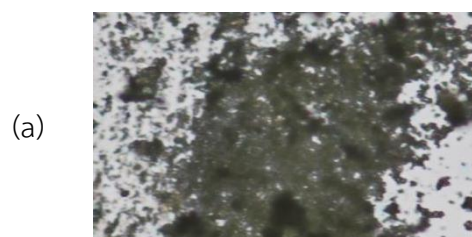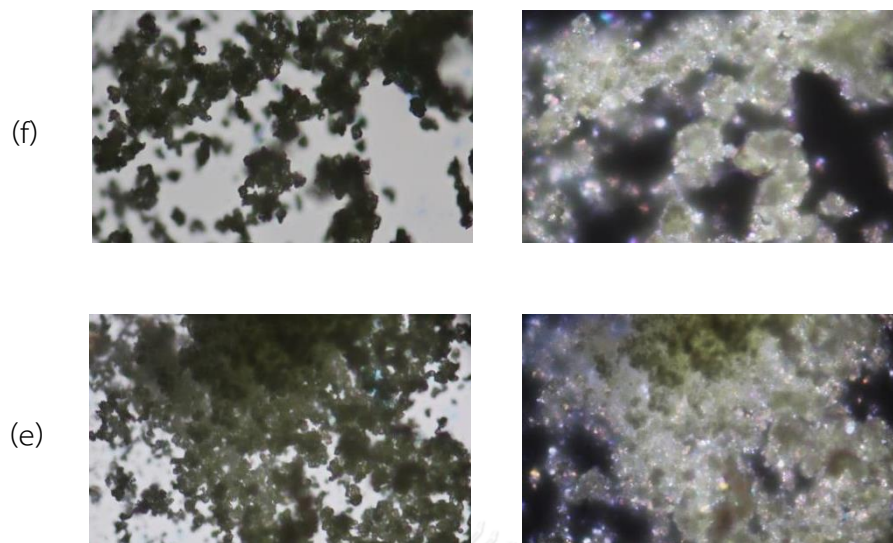
Figure 36 Non-polarized (I) and polarized (II) photomicrographs of anhydrous olanzapine Form I mixes with water and dries at 40°C for 3 hours. The ratio between Form I and water are 3:1 (a), 2:1 (b), 1:1 (c), 1:2 (d), 1:3 (e), 1:4 (f), 1:5 (g)

(I) (II)
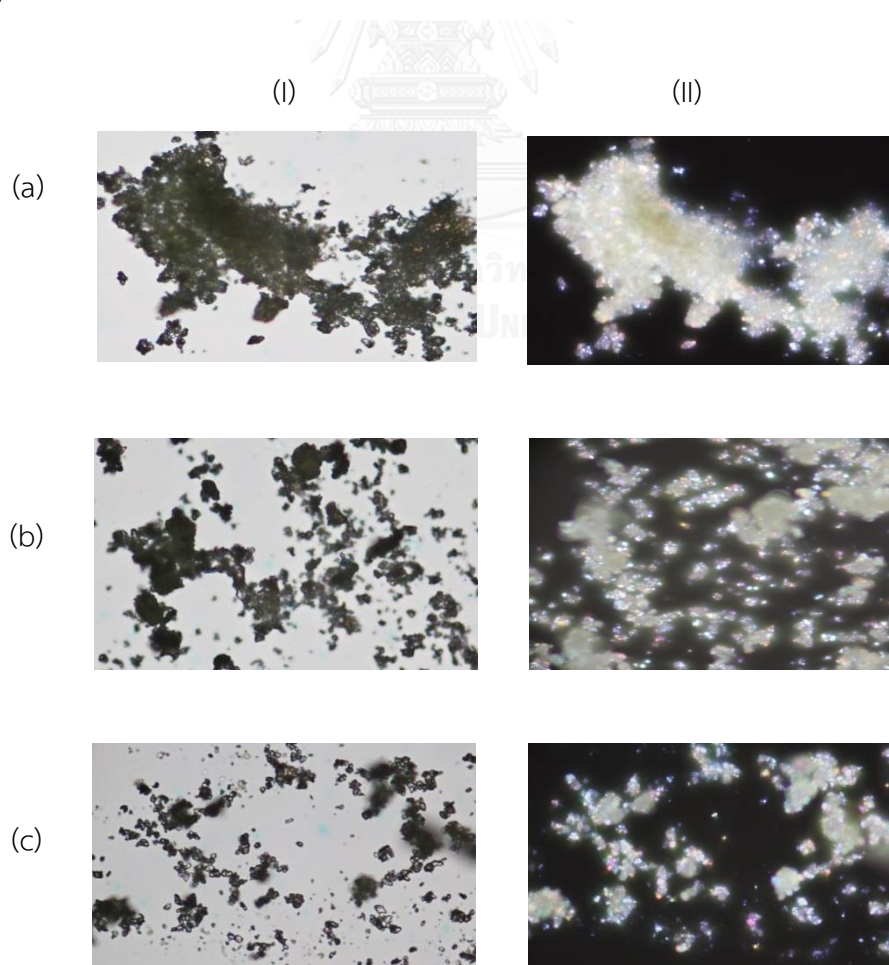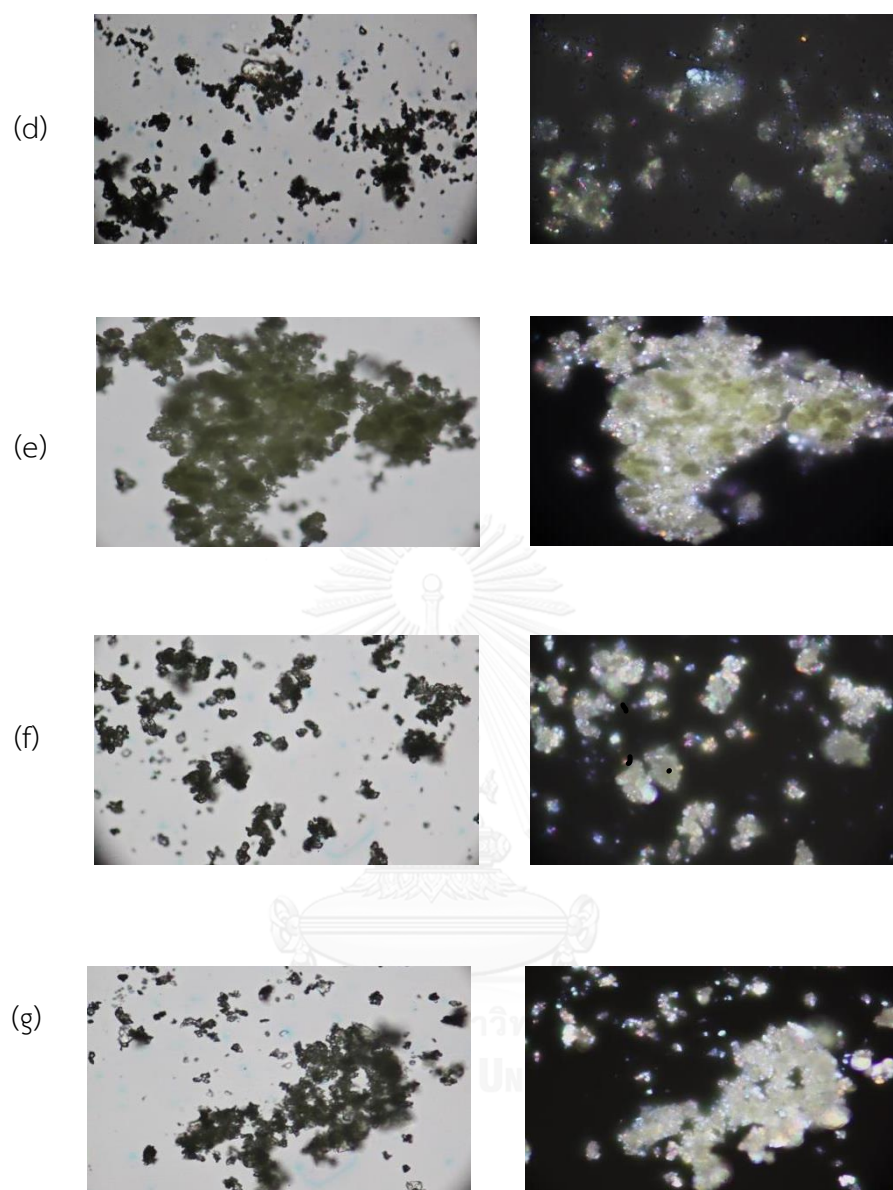
Figure 37 Non-polarized (I) and polarized (II) photomicrographs of anhydrous olanzapine Form I mixes with ethanol and dries at 40°C for 3 hours. The ratios between Form I and ethanol are 3:1 (a), 2:1 (b), 1:1 (c), 1:2 (d), 1:3 (e), 1:4 (f), 1:5 (g)

3.3.1.2   X-ray Powder diffractrometry (XRPD)

Figure 38 and Figure 39 show solid-state morphology change when Form I was exposed to water and ethanol, respectively. Exposing Form I to water at ratios of 1:4 ($W_{1/4}$) and 1:5 ($W_{1/5}$) resulted in new peak at 16.2 °2Θ, 20.4 °2Θ, 22.7 °2Θ and 24.5 °2Θ (Figure 38) which were found to be the characteristic peaks of Form B (6, 20). However, the ratio between Form I and water at 1:4 indicated that the transformation of Form I to hydrated Form B was incomplete in this experimental condition. XRPD diffractrogram still found to exhibit characteristic peaks of Form I at 19.0 °2Θ. On the other hand, exposing Form I to ethanol from the ratio of 3:1 ($E_{3/1}$), 2:1 ($E_{2/1}$), 1:1 ($E_{1/1}$), 1:2 ($E_{1/2}$), 1:3 ($E_{1/3}$), 1:4 ($E_{1/4}$) and 1:5 ($E_{1/5}$) were found to exhibit characteristic peaks of EtOH-H_2O mixed solvate at 14.3 °2Θ, 19.8 °2Θ, 20.5 °2Θ and 25.2 °2Θ (Figure 39) (6, 20). However, it was found to retain dominant peak of Form I at 19.0 °2Θ signifying incomplete transformation.

The result indicated that type and quantity of solvents can affect solid state transformation of anhydrous olanzapine Form I. When Form I was exposed to water, it should be transform to Form B. While when Form I was exposed to ethanol, it should be converted to EtOH-H_2O mixed solvate. However, both conversions of Form I to Form B or EtOH-H_2O mixed solvate were found to be incomplete under the present study conditions. It was found that for complete transformation to occur, optimal amount of solvent should be used.
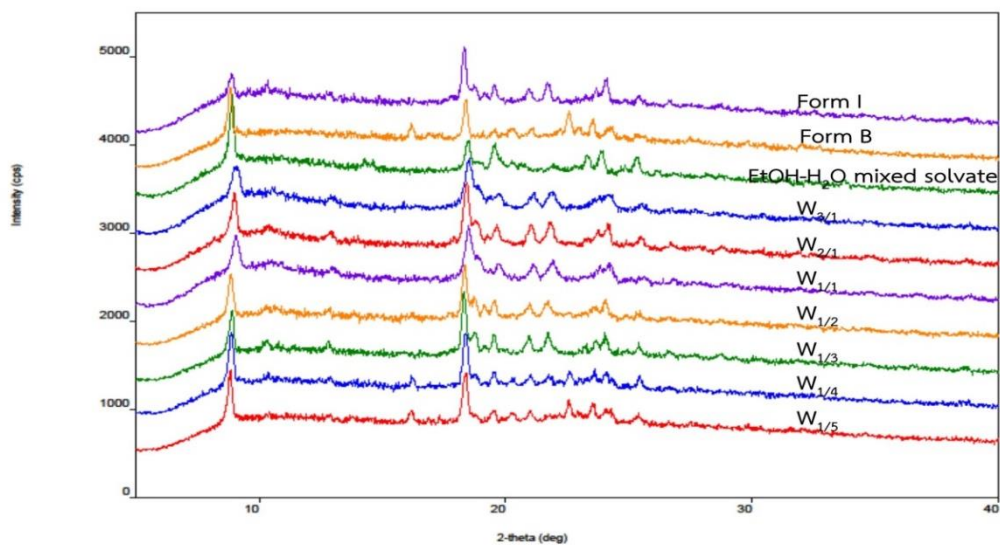
Figure 38 XRPD diffractograms of anhydrous olanzapine Form I mix with water at various drug: solvent ratios
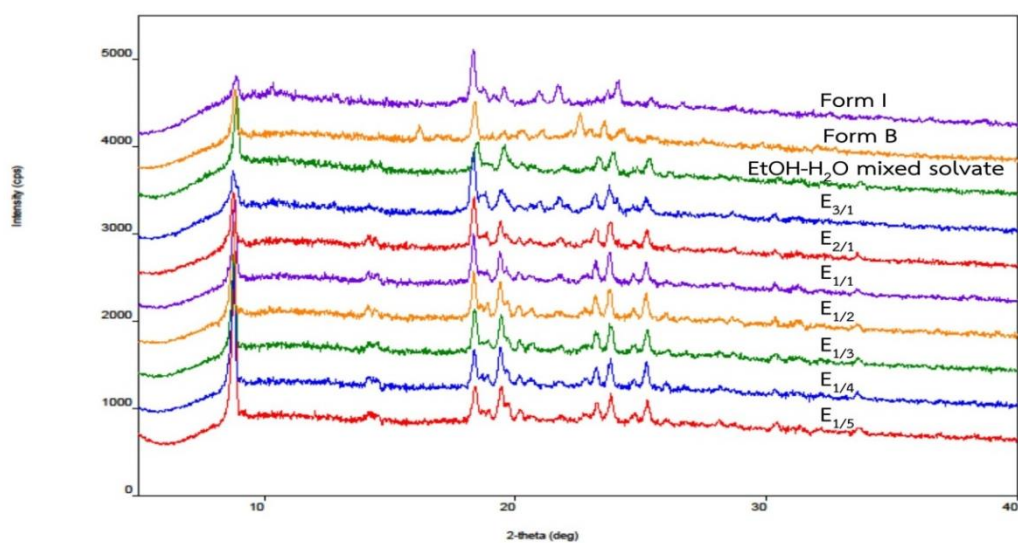
Figure 39 XRPD diffractrograms of anhydrous olanzapine Form I mix with ethanol at various drug: solvent ratios

3.3.1.3   Differential scanning calorimetry (DSC)

Figure 40 shows DSC thermograms of Form I after exposing to water. After Form I was exposed to water, DSC thermograms show new dehydration endotherm between 67-89 °C when only the ratios between Form I and water of 1:4 ($W_{1/4}$) and 1:5 ($W_{1/5}$) were utilized. In summary, transformation was evident when high water content was used. However, if Form I was exposed to ethanol, additional desolvation endotherm between 80-120 °C (Figure 41) were observed even when very low amount of ethanol was used (Form I: water ratio of 2:1). These endothermic peaks were found to be desolvation of ethanol and water in the sample (12, 20). From DSC results, it shows good correlation to XRPD results in section 3.3.1.2. XRPD indicated that transformation to Form B and EtOH-H2O mixed solvated were incomplete which DSC thermograms still retained the melting endotherm and recrystallization exotherm at specific position for Form I at 180-190°C instead of patterns characteristic of Form B (Figure 21(b)).

From these results, it can be concluded that Form I needs sufficiently high amount of water for solid state transformation to Form B. In contrast, Form I conversion by ethanol only needs small amount of the solvent to initializing the transformation to EtOH-H_2O mixed solvate.
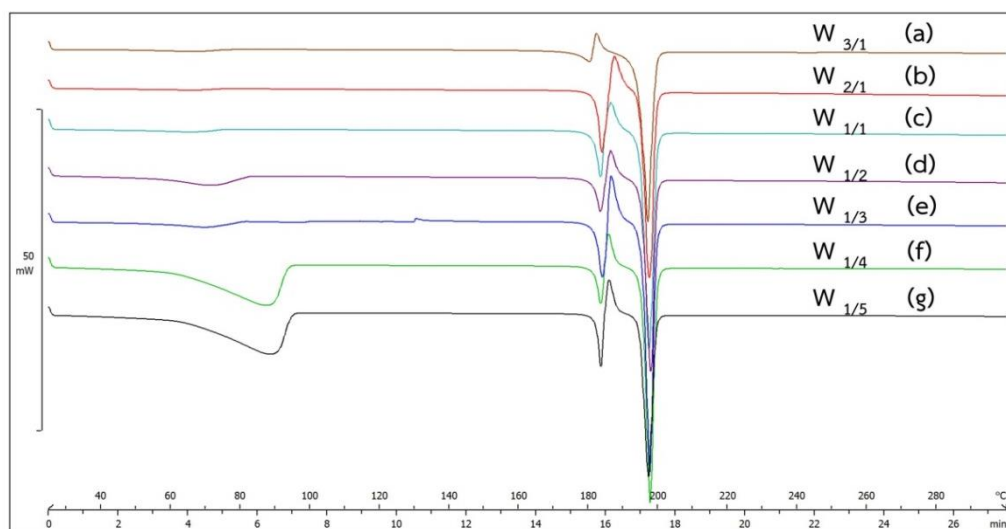
Figure 40 DSC thermograms of anhydrous olanzapine Form I mix with water at various drug: solvent ratios.
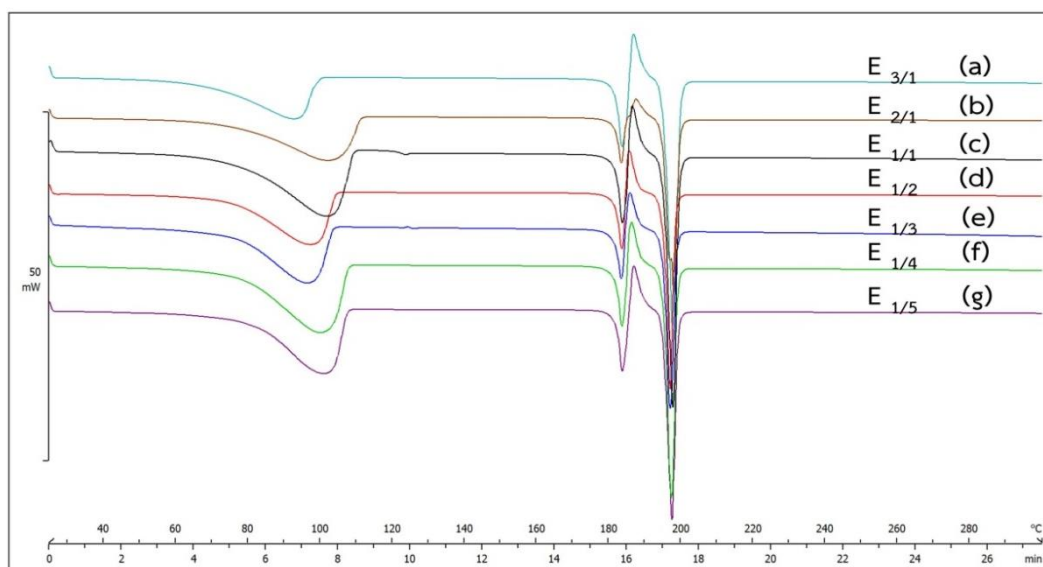
Figure 41 DSC thermograms of anhydrous olanzapine Form I mix with ethanol at various drug: solvent ratios

3.3.1.4   Thermogravimetric analysis (TGA)

Figure 42 exhibits TGA thermograms of olanzapine Form I mixed with water at 7 different ratios. TGA thermograms of olanzapine samples exposed to dimensional water at 3:1 ($W_{3/1}$), 2:1 ($W_{2/1}$), 1:1 ($W_{1/1}$), 1:2 ($W_{1/2}$), 1:3 ($W_{1/3}$), 1:4 ($W_{1/4}$) and 1:5 ($W_{1/5}$) exhibit linearity in weight between 40-180 °C until degraded at 180-220 °C. Whereas, Figure 43 shows TGA thermograms of olanzapine Form I exposed to ethanol at various ratios of 2:1 ($E_{2/1}$), 1:1 ($E_{1/1}$), 1:2 ($E_{1/2}$), 1:3 ($E_{1/3}$), 1:4 ($E_{1/4}$) and 1:5 ($E_{1/5}$) which indicated weight loss between 40-120°C of 5.42%w/w, 6.20%w/w, 11.54%w/w, 10.76%w/w, 11.19%w/w and 11.69%w/w, respectively. Final degradation endotherm occurred at 180-260 °C.

Because Thermogravimetric Analyzer (TGA) that was used in this experiment was broken. Thus, samples were evaluated at approximately 4 months after preparation. From TGA results, it was found that the conversion pathway of Form I to Form B was not stable because Form B could easily convert back to Form I. Form I was thermodynamically unstable when compare to Form B. In contrast, the transformation of EtOH-H_2O mixed solvate was more stable than Form B because EtOH-H_2O mixed solvate did not show conversion back to Form I under 25°C, 0% RH after stored 4 months. However, the weight loss of $E_{2/1}$ and $E_{1/1}$ were lower than the weight loss of reference EtOH-H_2O mixed solvate (11.76 %w/w) (Figure 22(c)) because transformation was incomplete under this experimental study. Although, the weight loss of other ratios were equivalent to reference EtOH-H_2O mixed solvate and were observed to be incomplete as shown in XRPD diffractograms (Figure 39).

The thermogravimetric profile indicated that the type and quantity of solvents could affect Form I crystal transformation to varying degrees. Form I need sufficiently high amount of water for solid state transformation. In contrast, Form I

conversion by ethanol only needs small amount of the solvent to readily transform to EtOH-H$_2$O mixed solvate.
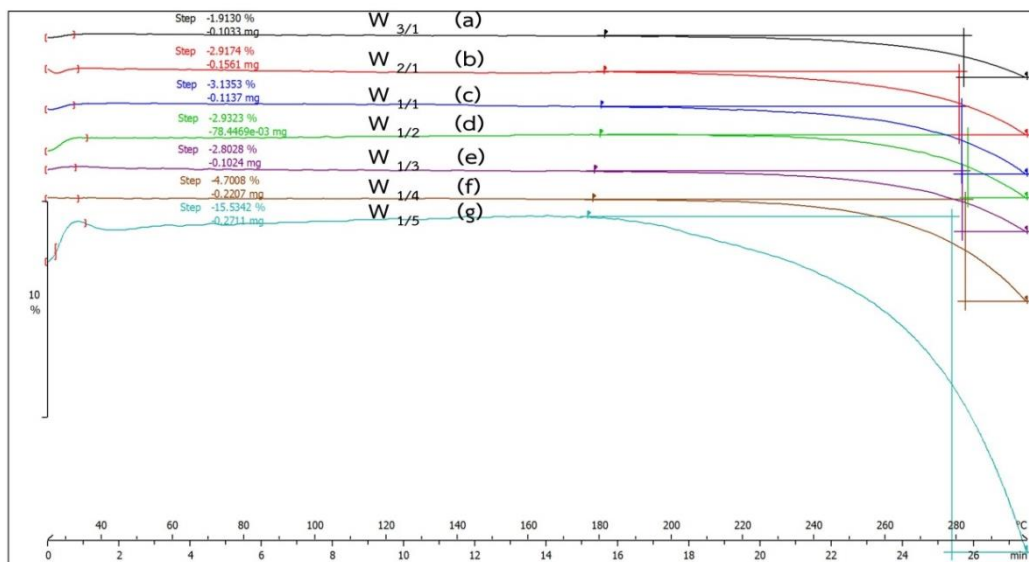
Figure 42 TGA thermograms of anhydrous olanzapine Form I mix with water at various drug: solvent ratios
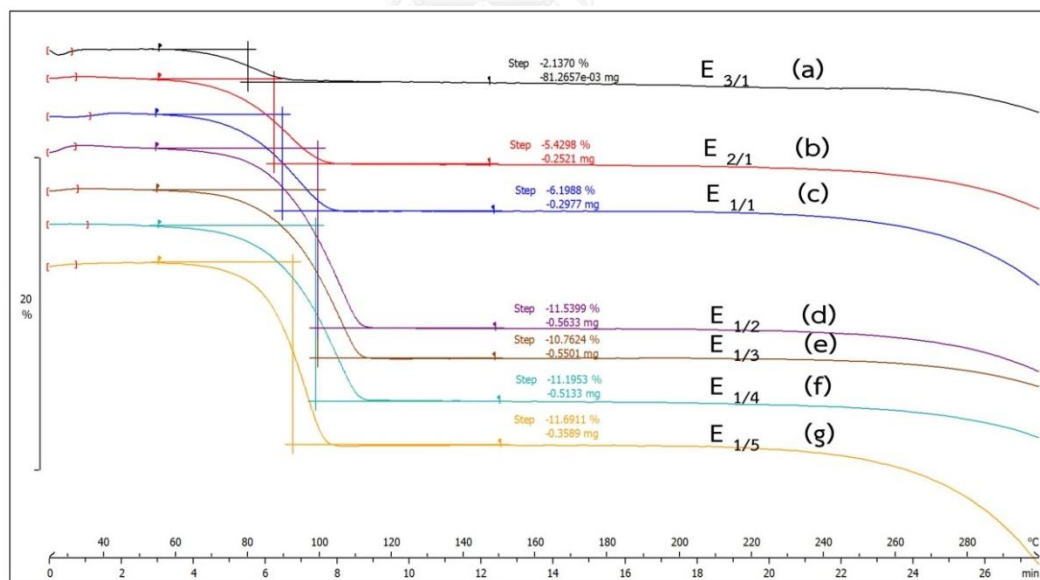
Figure 43 TGA thermograms of anhydrous olanzapine Form I mix with ethanol at various drug: solvent ratios

3.3.1.5   Solid-state characterization by Raman spectroscopy

Figure 44 presents Raman spectra of anhydrous olanzapine Form I exposed to water at 7 different ratios compare to reference anhydrous Form I and both solvated forms (Form B and EtOH-H_2O mixed solvate). Raman shifts between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to represent clear distinction between each sample. All Raman spectral data were collected in 10 replicates and analyzed by OMNIC[®] (Version 8.0). Discrimination of data were done by Principal Component Analysis (PCA) Using TQ Analyst[®]8 Pro edition software. Raman spectral result of $W_{1/5}$ sample presents characteristic peaks similar to Raman spectrum of Form B. While $W_{1/4}$ presents mixture of characteristic peaks of Form I and Form B. On the other hand, $W_{3/1}$, $W_{2/1}$, $W_{1/1}$, $W_{1/2}$, and $W_{1/3}$ retained characteristic peaks of reference Form I.

Figure 45 shows Raman spectra of anhydrous olanzapine Form I exposed to ethanol at 7 different ratios compare to Form I, Form B and EtOH-H_2O mixed solvate. Results from Figure 45 indicate that $E_{1/2}$, $E_{1/3}$, $E_{1/4}$ and $E_{1/5}$ samples show the same characteristic peaks of EtOH-H_2O mixed solvate. Whereas, Raman spectra of $E_{2/1}$, $E_{1/1}$ show characteristic peaks of both Form I and EtOH-H_2O mixed solvate. Raman spectrum of $E_{3/1}$ retained characteristic peaks as Form I.

From the above results, Raman spectroscopy indicates that amount of water and ethanol contents significant by influence the extent of solid state transformation of Form I. However, this transformation phenomenon was incomplete as could be seen by some characteristic peaks of $W_{1/4}$, $E_{2/1}$ and $E_{1/1}$ which retained patterns similar to the original Form I. The results from Raman spectroscopy of olanzapine samples correlate well with XRPD, DSC and TGA. These techniques showed clear distinctions between anhydrous Form I and both solvated forms (Form B and EtOH-H_2O mixed solvate).

Raman spectral data from olanzapine samples prepared, Form I, Form B and EtOH-H_2O mixed solvate could be differentiated by PCA as 3 distinct groups. Figure 46(a and b) indicates Principal Component Analysis (PCA) of Form I mixed with water at various drug : solvent ratios compare to Form I, Form B and EtOH-H_2O mixed solvate. The first principal component (PC1) accounted for 69.72% of overall variance, the second principal components (PC2) accounted for 21.76% of overall variance and the third PC (PC3) accounted for 6.70% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine samples of $W_{3/1}$, $W_{2/1}$, $W_{1/1}$, $W_{1/2}$, $W_{1/3}$, $W_{1/4}$ and $W_{1/5}$ are shown in Table 11 and was calculated from data in Appendix C.

Table 11 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine samples mixes with water at various ratios

Olanzapine samples (drug/water)	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H_2O mixed solvate
$W_{3/1}$	3.57	4.38	4.61
$W_{2/1}$	3.26	4.39	4.48
$W_{1/1}$	3.20	4.30	4.35
$W_{1/2}$	3.59	4.11	4.22
$W_{1/3}$	3.12	4.21	4.25
$W_{1/4}$	3.47	3.57	4.11
$W_{1/5}$	4.00	2.91	4.22

Mahalanobis distances indicated that $W_{1/5}$ sample was closest to Form B. Whereas, other ratios were classified within the group of Form I. However, this solid

state transformation was still incomplete as can be seen by non-overlapping data points of $W_{1/5}$ to Form B.

Figure 47(a and b) indicate Principal Component Analysis (PCA) of Form I mixed with ethanol at various drug : solvent ratios compare to Form I, Form B and EtOH-H_2O mixed solvate. The first principal component (PC1) accounted for 67.39% of overall variance, the second principal components (PC2) accounted for 27.11% of overall variance and the third PC (PC3) accounted for 3.13% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine samples at $E_{3/1}$, $E_{2/1}$, $E_{1/1}$, $E_{1/2}$, $E_{1/3}$, $E_{1/4}$ and $E_{1/5}$ are shown in Table 12 and was calculated from data in Appendix D.

Table 12 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine samples mixes with ethanol at various ratios

Olanzapine sample (drug/ethanol)	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H_2O mixed solvate
$E_{3/1}$	2.46	6.57	3.21
$E_{2/1}$	2.66	6.17	2.59
$E_{1/1}$	2.90	6.33	2.52
$E_{1/2}$	3.16	6.34	2.24
$E_{1/3}$	3.21	6.50	2.19
$E_{1/4}$	3.11	6.42	2.25
$E_{1/5}$	4.96	6.40	2.25

From these results, the distances between olanzapine samples with drug to ethanol ratios of $E_{2/1}$, $E_{1/1}$, $E_{1/2}$, $E_{1/3}$, $E_{1/4}$ and $E_{1/5}$ were nearest to reference EtOH-H_2O mixed solvate. Whereas $E_{3/1}$ was found to retain the structure similar to

Form I. It could be concluded from all PCA results that Form I had a tendency to transform to Form B at high level of water exposed. However, it took only very low amount of ethanol to easily changed Form I to EtOH-H_2O mixed solvate. This transformation phenomenon was incomplete as can be seen by non-overlapping data points of samples to each reference olanzapine forms. PCA results confirmed that both the type and quantity of solvents play key role in the conversion of reference anhydrous olanzapine Form I to solvated forms (Form B and EtOH-H_2O mixed solvate).

Figure 44 Raman spectra of anhydrous olanzapine Form I mix with water at various

drug: solvent ratios. The ratios between Form I and water are 3:1 (W$_{3/1}$), 2:1 (W$_{2/1}$),

1:1 (W$_{1/1}$), 1:2 (W$_{1/2}$), 1:3 (W$_{1/3}$), 1:4 (W$_{1/4}$), 1:5 (W$_{1/5}$)

Figure 45 Raman spectra of anhydrous olanzapine Form I mix with ethanol at various drug: solvent ratios. The ratios between Form I and ethanol are 3:1 ($E_{3/1}$), 2:1 ($E_{2/1}$), 1:1 ($E_{1/1}$), 1:2 ($E_{1/2}$), 1:3 ($E_{1/3}$), 1:4 ($E_{1/4}$), 1:5 ($E_{1/5}$)

(a)
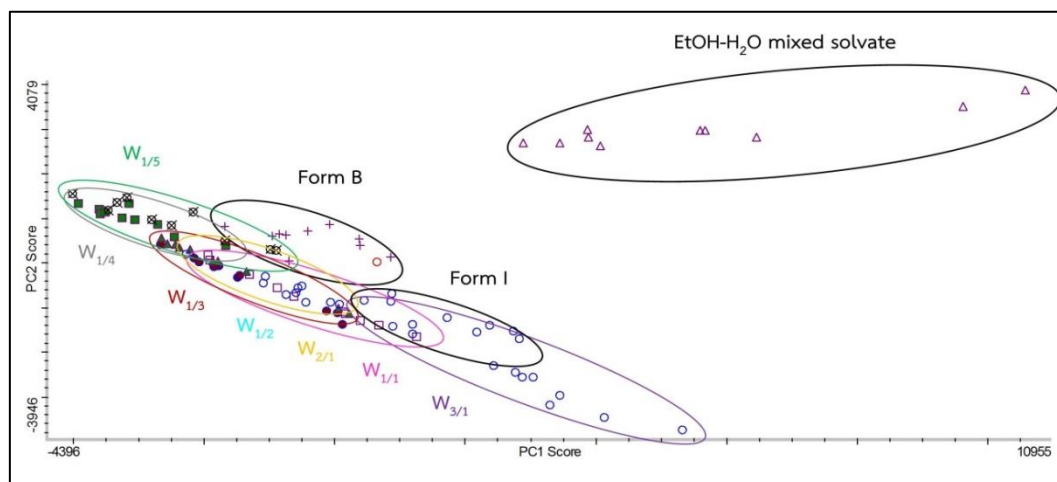
(b)
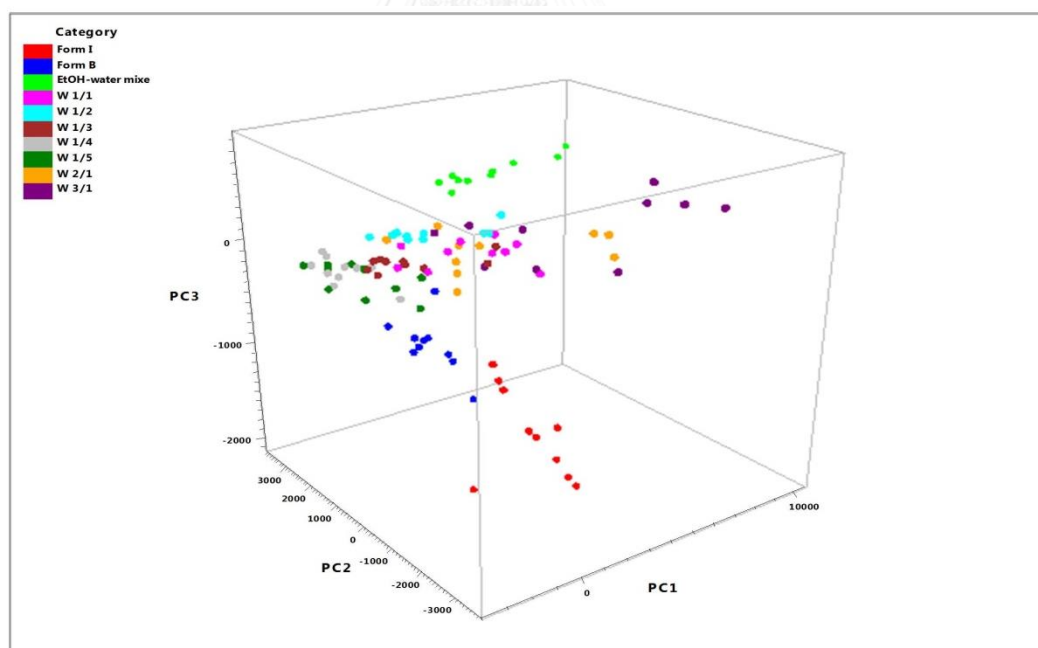
Figure 46 PCA score plots of anhydrous olanzapine Form I mix with water at various drug: solvent ratios compare to reference Form I, Form B and EtOH-H_2O mixed solvate using TQ Analyst[®]8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

(a)
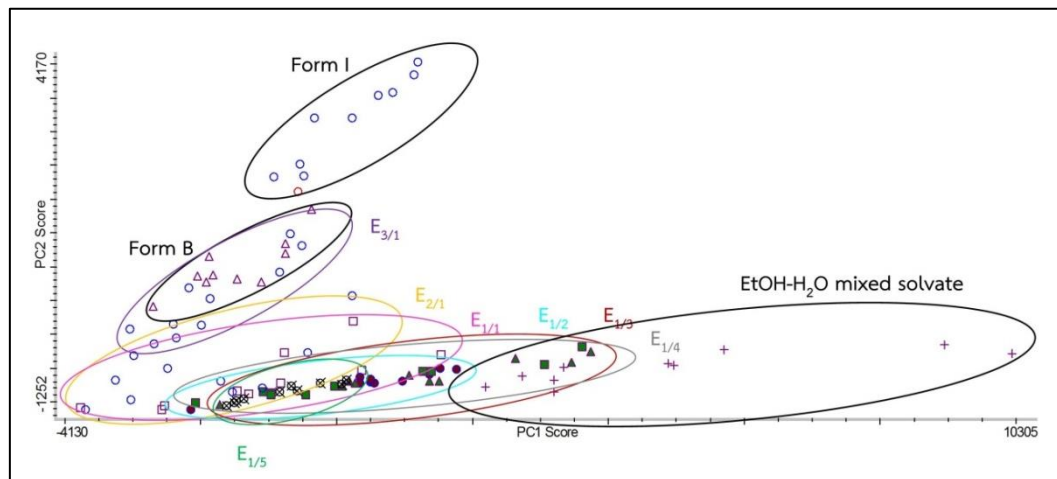
(b)
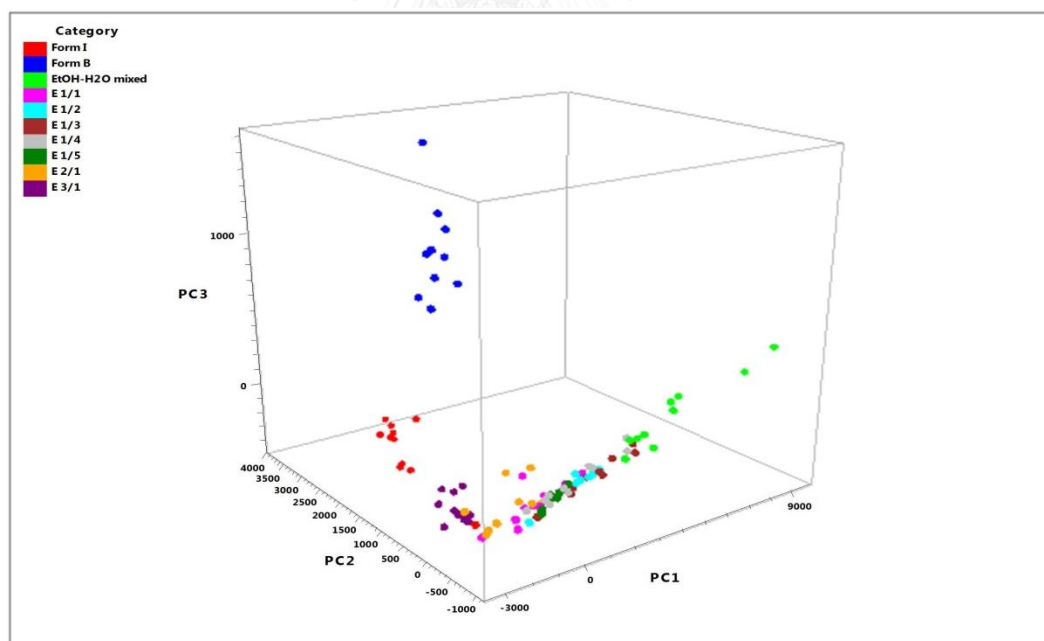
Figure 47 PCA score plots of anhydrous olanzapine Form I mix with Ethanol at various drug: solvent ratios compare to reference Form I, Form B and EtOH-H_2O mixed solvate using TQ Analyst[®]8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

3.3.2   Effect of type and quantity of solvent mixtures

Effect of type and quantity of solvent mixtures on solid state transformation of reference anhydrous olanzapine Form I were prepared by mixing one part of Form I with various solvent mixtures of different ratios and dried at 40°C for 3 hours. The solvents used were water and ethanol. This experiment controlled Form I at a constant amount. The ratios between Form I and solvent mixtures were 1:1 and 1:5. In addition, the ratios between water and ethanol were 0:100 (1WE0/100 and 5WE0/100), 10:90 (1WE10/90 and 5WE10/90), 30:70 (1WE30/70 and 5WE30/70), 50:50 (1WE50/50 and 5WE50/50), 70:30 (1WE70/30 and 5WE70/30), 90:10 (1WE90/10 and 5WE90/10) and 100:0 (1WE100/0 and 5WE100/0). After drying for 3 hours, samples were stored at 0%RH and analyzed by solid state analytical techniques.

3.3.2.1   Polarized light microscopy

Figures 48(a-g) and 49(a-g) show crystalline birefringence phenomena under polarized light microscope from all samples obtained after exposing to solvent mixtures. Olanzapine samples mixed with water and ethanol were found to exhibit fine crystals and show birefringence. These results initially indicate that olanzapine samples exposed to solvent mixtures at all 14 ratios were crystalline. However, it could not be concluded from these results how these solvent mixtures affect the extent of conversion of olanzapine solid state structures.

(I)   (II)

(a)

(b)

(c)

(d)

(e)

(f)

(g)
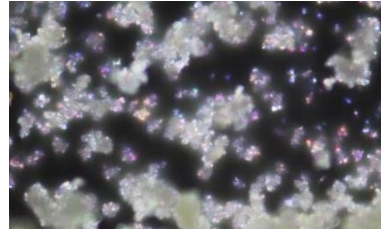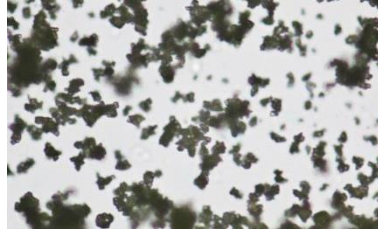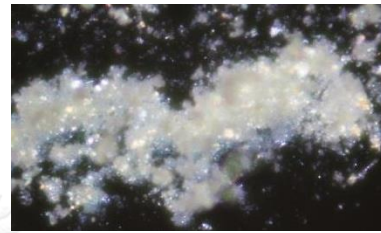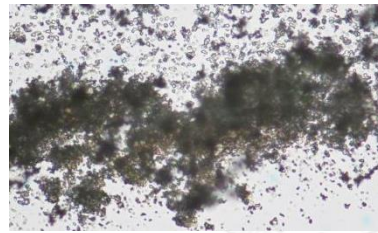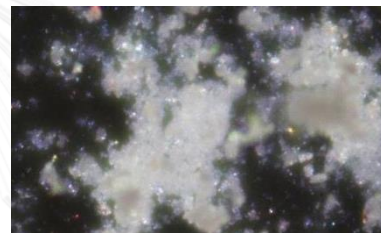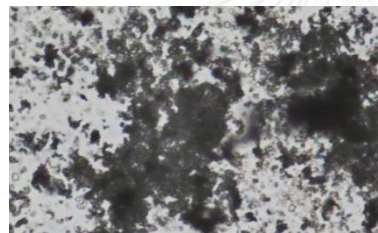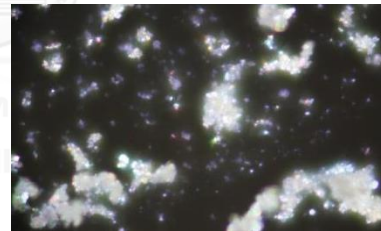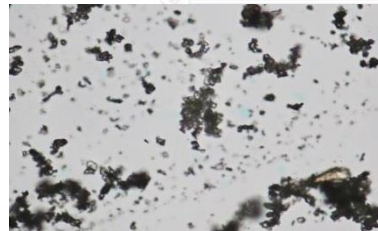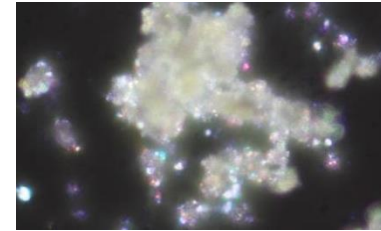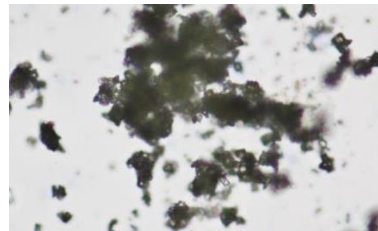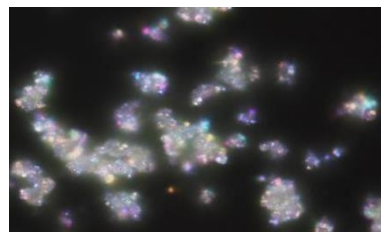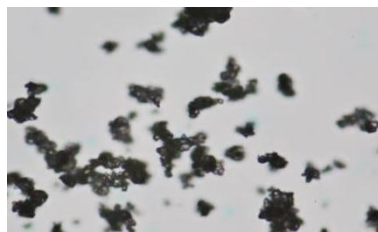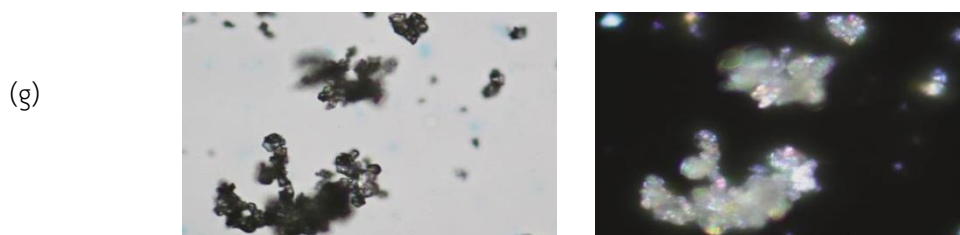
Figure 48 Non-polarized (I) and polarized (II) photomicrographs of one part of anhydrous olanzapine Form I exposed to one part of solvent mixtures. The ratios of solvent mixtures between water and ethanol are 100:0 (a), 10:90 (b), 30:70 (c), 50:50 (d), 70:30 (e), 90:10 (f), 0:100 (g)

(I)   (II)

(a)

(b)

(c)

(d)
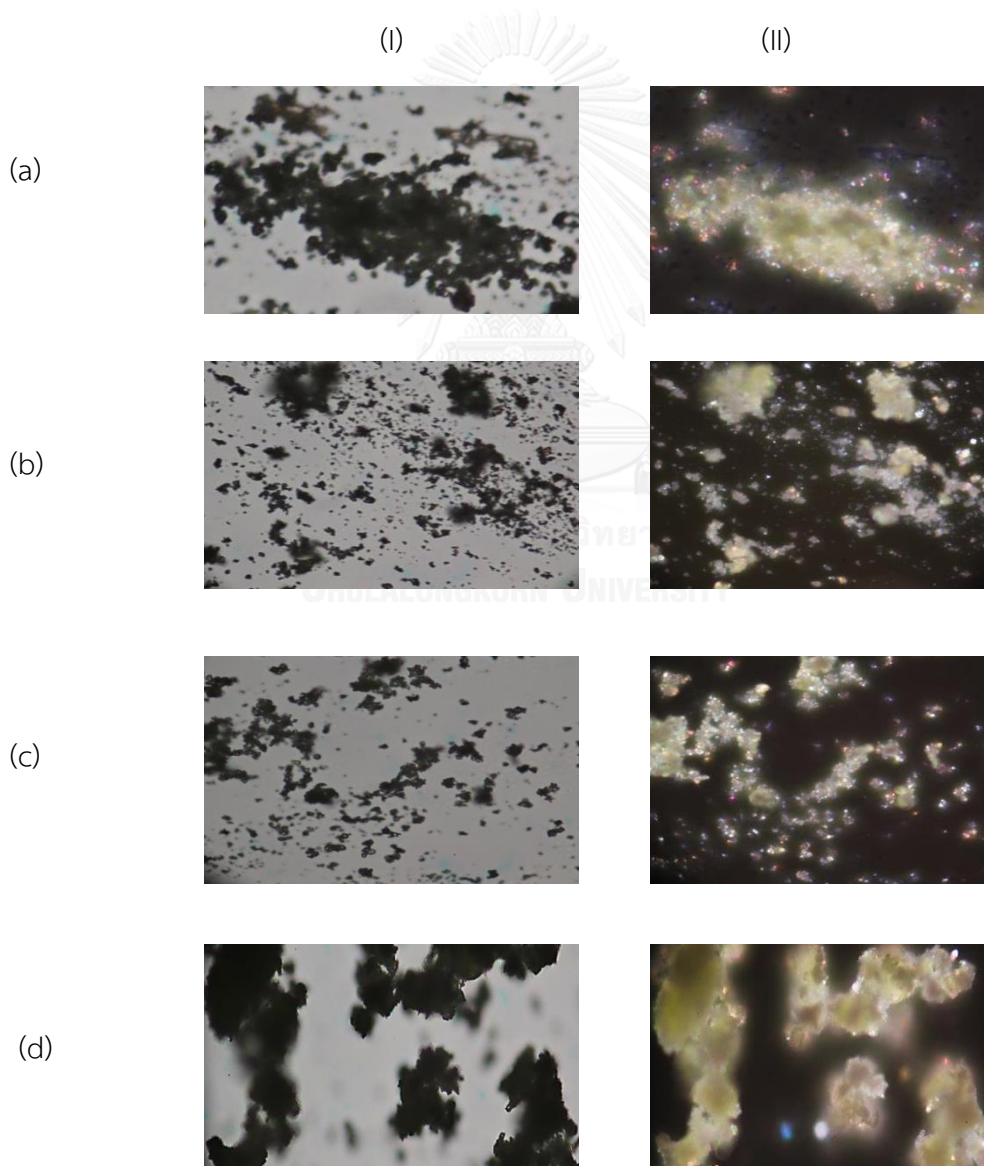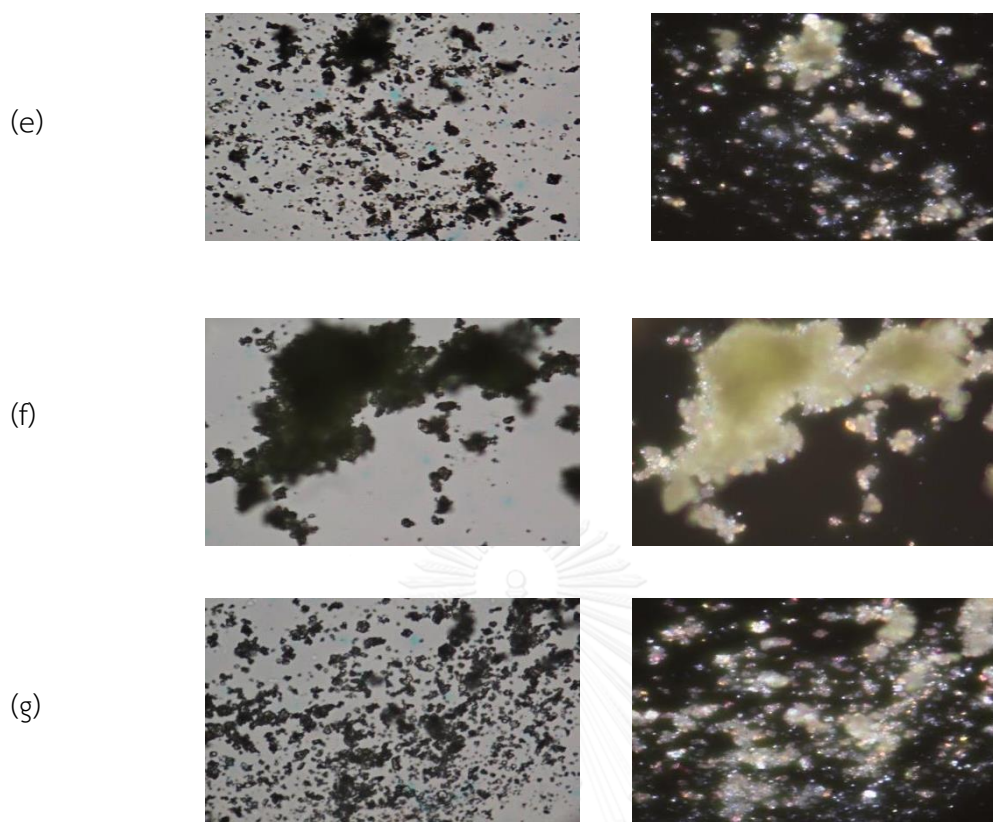
Figure 49 Non-polarized (I) and Polarized (II) photomicrographs of one part of anhydrous olanzapine Form I exposed to five parts of solvent mixtures. The ratios of solvent mixtures between water and ethanol are 100:0 (a), 10:90 (b), 30:70 (c), 50:50 (d), 70:30 (e), 90:10 (f), 0:100 (g)

3.3.2.2   X-ray powder diffractrometry (XRPD)

Figure 50 shows XRPD diffractograms of olanzapine samples at ratio between drug : solvent mixtures as 1:1. Various ratios of solvent mixtures between water and ethanol at 0:100 (1WE0/100), 10:90 (1WE10/90), 30:70 (1WE30/70), 50:50 (1WE50/50), 70:30 (1WE70/30), 90:10 (1WE90/10), 100:0 (1WE100/0) were compared to reference anhydrous olanzapine Form I and both solvated forms (Form B and EtOH-H_2O mixed solvate).   XRPD diffratrograms of 1WE0/100, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30 and 1WE90/10 exhibited characteristic peaks of EtOH-H_2O mixed solvate at 14.3° 2Θ, 19.8° 2Θ, 20.5° 2Θ and 25.2° 2Θ (6, 20).

Moreover, this transformation to EtOH-H_2O mixed solvate was not observed for 1WE100/0 sample where only Form I was dominant.

Figure 51 presents XRPD diffractograms of olanzapine samples at ratio between drug : solvent mixtures as 1:5. The ratios of solvent mixtures between water and ethanol at 0:100 (5WE0/100), 10:90 (5WE10/90), 30:70 (5WE30/70), 50:50 (5WE50/50), 70:30 (5WE70/30), 90:10 (5WE90/10), 100:0 (5WE100/0) were compared to reference anhydrous olanzapine Form I and both solvated forms (Form B and EtOH-H_2O mixed solvate). The sample at ratios of 5WE0/100, 5WE10/90, 5WE30/70, 5WE50/50, 5WE70/30 and 5WE90/10 present characteristic peaks of 14.3° 2Ө, 19.8° 2Ө, 20.5° 2Ө and 25.2° 2Ө corresponding to EtOH-H_2O mixed solvate (6, 20). However, ratios of 5WE50/50, 5WE70/30 and 5WE90/10 were also found to exhibit peak, characteristic of only Form B 20.4° 2Ө and 22.7° 2Ө. On the other hand, the sample at the ratio of 5WE100/0 present characteristic peaks of only Form B (16.2° 2Ө, 20.4° 2Ө, 22.7 ° 2Ө and 24.5° 2Ө) (6, 20).

It can be concluded from above results that quantity of solvent used had significant affect on the solid state transformation of anhydrous olanzapine Form I. Results confirmed that the transformation of Form I to Form B was possible only when Form I was exposed to both high amount and high ratio of water such as at 5WE100/0. While, transformation of Form I to EtOH-H_2O mixed solvate could occur when Form I was in contact with even very low ratio of ethanol in the solvent mixtures no matter what amount were used such as 1WE0/100, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30, 1WE90/10, 5WE0/100, 5WE10/90, 5WE30/70, 5WE50/50, 5WE70/30 and 5WE90/10.

In summary, Form I was very sensitive to the presence of ethanol in the solvent mixtures. When Form I come in contact with hydro-alcoholic mixtures, it will readily change to EtOH-H_2O mixed solvate even as low as when only 10% w/w of ethanol was used and irrespective of the volume of solvent mixtures to drug

incorporated.   However, when there was no ethanol presence in the solvent mixtures (only water was used), volume of solvent compare to drug was found to affect the resulting solid structure.   At low volume, the drug did not convert and remain as Form I. At high volume, it could be transformed to Form B.
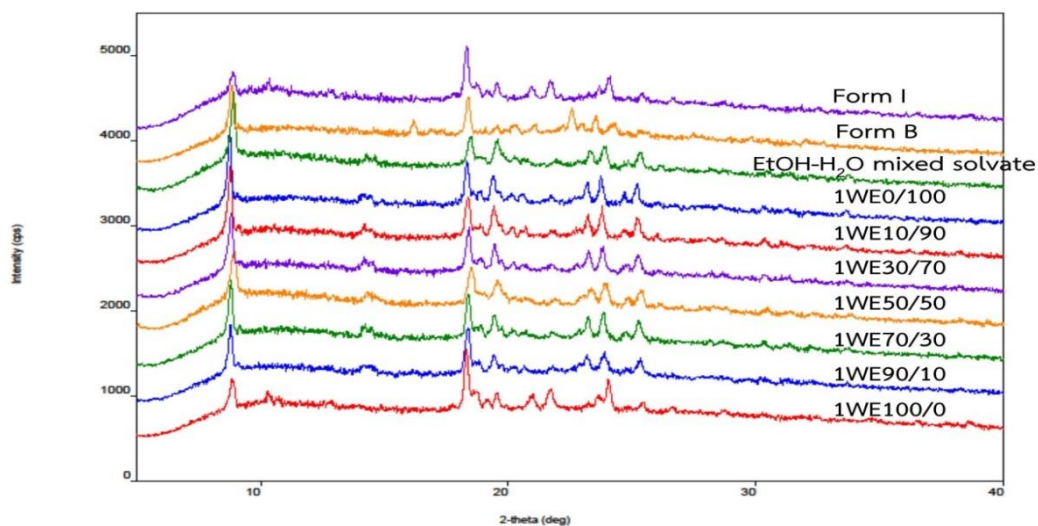
Figure 50 XRPD diffractrograms of one part of anhydrous olanzapine Form I exposes to one part of solvent mixtures at various ratios of water to ethanol as 0:100 (1WE0/100), 10:90 (1WE10/90), 30:70 (1WE30/70), 50:50 (1WE50/50), 70:30 (1WE70/30), 90:10 (1WE90/10), 100:0 (1WE100/0)
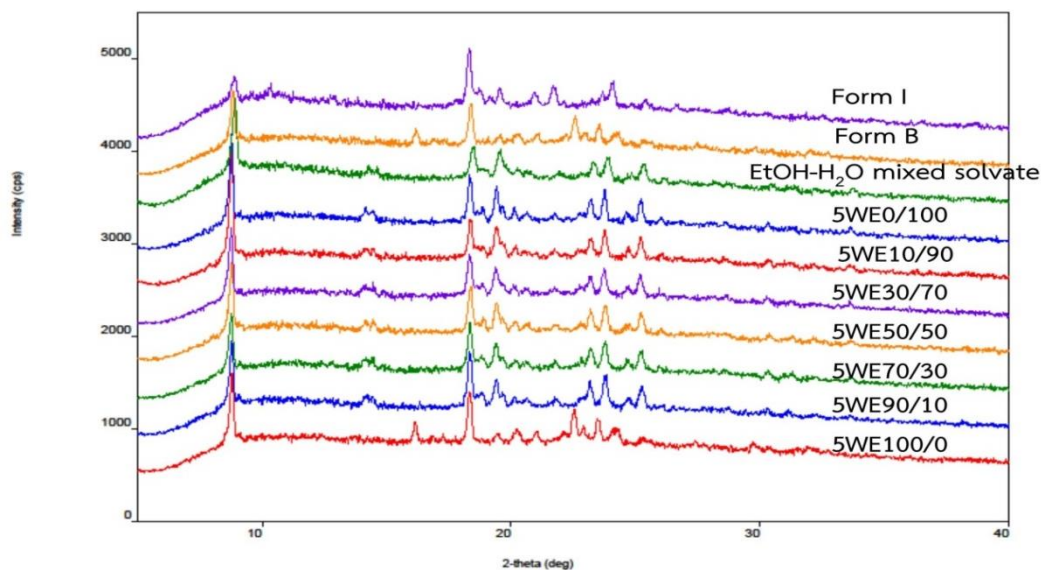
Figure 51 XRPD diffractrograms of a part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various ratios of water to ethanol as 0:100 (1WE0/100), 10:90 (1WE10/90), 30:70 (1WE30/70), 50:50 (1WE50/50), 70:30 (1WE70/30), 90:10 (1WE90/10), 100:0 (1WE100/0)

3.3.2.3   Differential scanning calorimetry (DSC)

Figure 52 shows DSC thermograms after exposing Form I to solvent mixtures at the amount of drug to solvent mixtures of 1:1. The samples at ratios of 1WE0/100, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30 and 1WE90/10 presented broad desolvation endotherms between 80-140 °C, first melting with recrystallization endotherm at 180-185 °C and final melting with degradation at 195-210 °C. These peaks are characteristic of EtOH-H_2O mixed solvate (Figure 21(c)). On the other hand, the sample at ratio of 1WE100/0 (Figure 52) represent thermogram specific to reference Form I (Figure 21(a)).

Figure 53 shows DSC thermograms of exposing Form I to various ratios of solvent mixtures at the amount of drug to solvent mixtures of 1:5. DSC thermograms of 5WE0/100, 5WE10/90, 5WE30/70, 5WE50/50, 5WE70/30, 5WE90/10 and 5WE100/0 exhibit broad desolvation endotherms between 80-140 °C. First melting endotherm

with recrystallization at 180-185 °C and final melting with degradation at 195-210 °C which are characteristics of reference EtOH-H$_2$O mixed solvate (Figure 21(c)). However, when only water was used (5WE100/0), Form I was transformed to Form B (Figure53). All results indicate that Form I needs high amount and pure water for transformation to Form B. On the other hand, Form I needs only small amount of ethanol to convert to EtOH-H$_2$O mixed solvate. All above DSC results are in correlation to the results obtained from pervious XRPD diffraction studies (Figure 50 and 51).
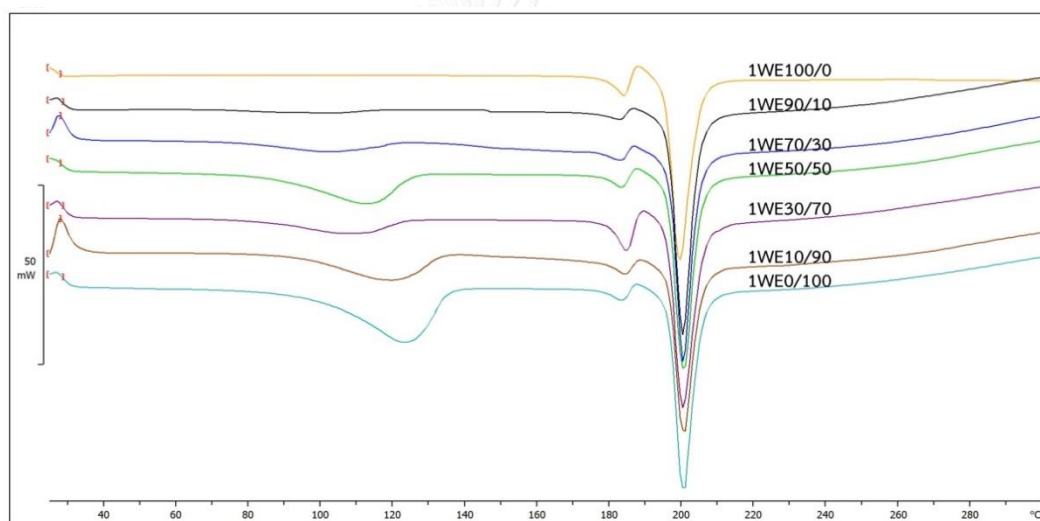
Figure 52 DSC thermograms of one part of anhydrous olanzapine Form I exposes to one part of solvent mixtures at various ratios
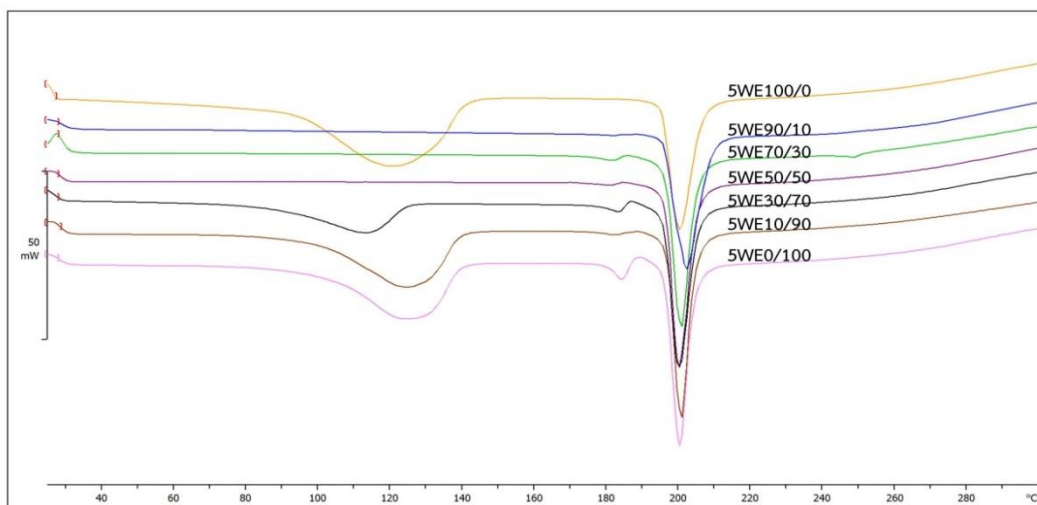
Figure 53 DSC thermograms of one part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various ratios

3.3.2.4   Thermogravimetric analysis (TGA)

Figure 54 shows TGA thermograms of one part of Form I exposed to one part of solvent mixtures at 7 ratios.   TGA thermograms of olanzapine sample exposed to solvent mixture at 1WE0/100, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30 and 1WE90/10 showed weight loss between 40-150 °C of 6.26%w/w, 11.71%w/w, 10.48%w/w, 11.43%w/w, 10.66%w/w, 8.32%w/w and 3.13%w/w, respectively. All samples degraded at 200-220 °C. No weight loss was observed for 1WE100/0 which was similar to TGA result of reference Form I (Figure 22(a)).   Whereas, the resulting weight loss of, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30 and 1WE90/10 are similar to TGA results obtained for both reference Form B (10.50%w/w) and reference EtOH-H_2O mixed solvate (11.76%w/w). Thus, Form B and EtOH-H_2O samples could not be differentiated by TGA.

Figure 55 exhibits TGA thermograms of one part of Form I exposed to five parts of solvent mixtures at 7 ratios.   TGA thermograms of olanzapine samples exposed to solvent mixtures at 5WE0/100, 5WE10/90, 5WE30/70, 5WE50/50, 5WE70/30, 5WE90/10 and 5WE100/0 indicated weight reduction between 40-120 °C

of 12.03%w/w, 12.12%w/w, 12.06%w/w, 11.83%w/w, 11.51%w/w, 10.64%w/w and 8.29%w/w, respectively. Every sample degraded at 220-260 °C. The weight reduction of all sample are similar to TGA results obtained for both reference Form B (10.50%w/w) and EtOH-H_2O mixed solvate (11.76%w/w). Thus, other analytical methods are needed to differentiate between Form B and EtOH-H_2O mixed solvate.

The results from thermogravimetric analysis (TGA) are in good correlation to the results obtained from X-ray powder diffractrometry (XRPD) and Differential Scanning Calorimetry (DSC).
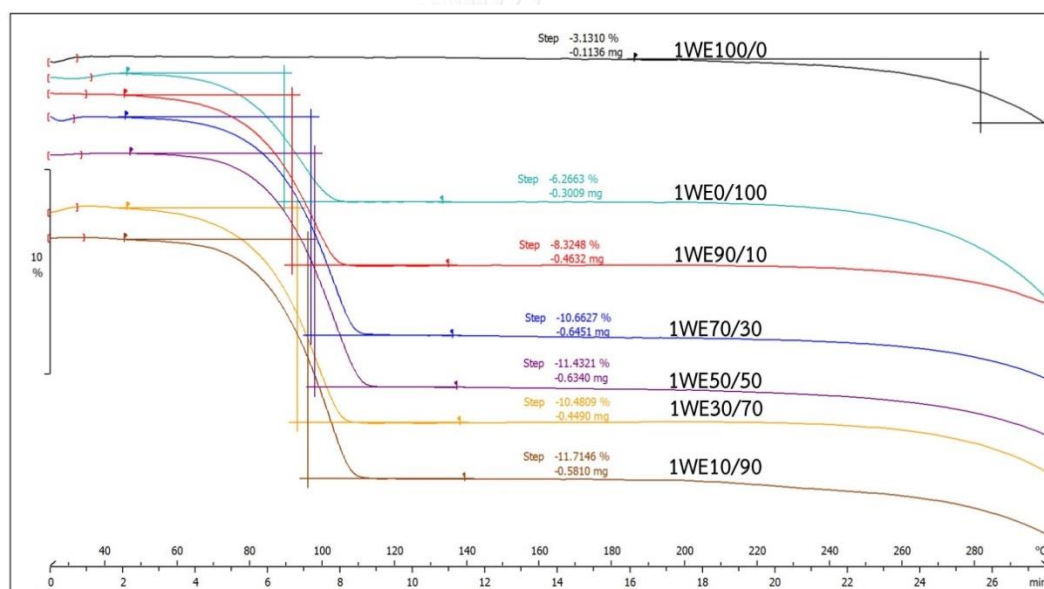
Figure 54 TGA thermograms of one part of anhydrous olanzapine Form I exposes to one part of solvent mixtures at various solvent ratios
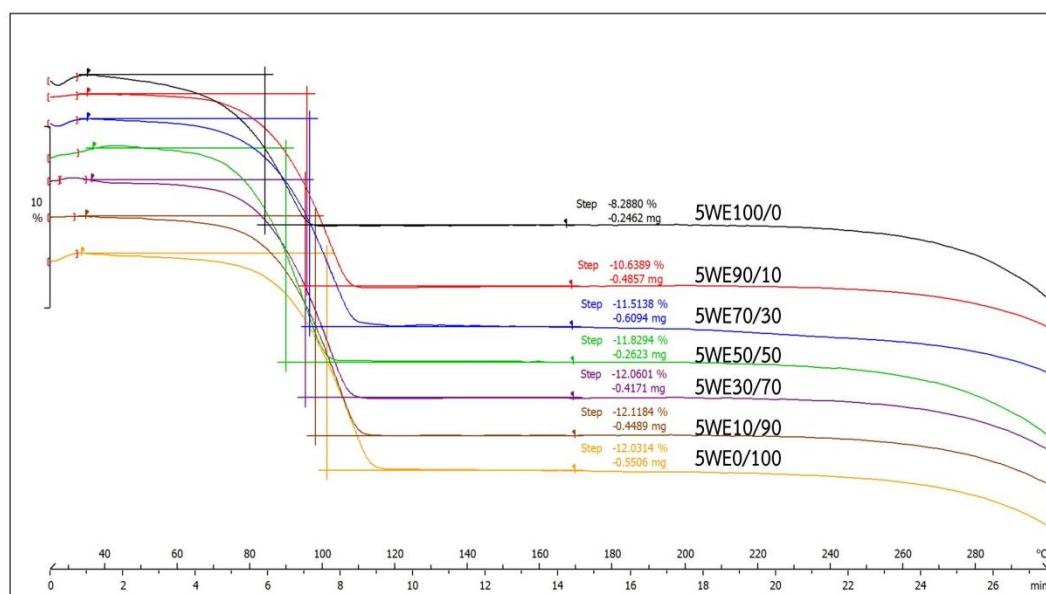
Figure 55 TGA thermograms of one part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various solvent ratios

3.3.2.5   Solid-state characterization by Raman spectroscopy

Figure 56 shows Raman spectra of samples prepared by physical mixing of one part of Form I exposed to one part of solvent mixtures at 7 ratios. Figure 57 shows Ramam spectra of sample prepared by physical mixing of one part of Form I exposed to five parts of solvent mixtures.   Raman shifts between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to represent clear distinction between olanzapine solid state forms.   All Raman spectral data were collected in 10 replicates and analyzed by OMNIC[®] (Version 8.0) and data discrimination and data correlation were done by Principal Component Analysis (PCA) and Mahalanobis distance using TQ Analyst[®]8 Pro edition software. Raman spectrum of 1WE100/0 sample was found to be similar to Raman spectrum of reference Form I while Raman spectra of 1WE0/100, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30, 1WE90/10 were observed to be similar to EtOH-H_2O mixed solvate. However, Raman spectrum of 5WE100/0 was shown to be similar to Form B while Raman spectrum result of 5WE0/100, 5WE10/90,

5WE30/70, 5WE50/50, 5WE70/30, 5WE90/10 were observed to be similar to EtOH-H$_2$O mixed solvate. However, by visual observation of Raman spectra alone one cloud not clearly distinguish the degree of transformation between each sample prepared.

Figure 58(a and b) indicate PCA of Form I physically mixed with solvent mixtures (water and ethanol) at the ratio of 1:1 compare to reference anhydrous Form I and both solvated forms (Form B and EtOH-H$_2$O mixed solvate). The first principal component (PC1) accounted for 62.83% of overall variance, the second principal components (PC2) accounted for 19.17% of overall variance and the third PC (PC3) accounted for 9.33% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and 1WE0/100, 1WE10/90, 1WE30/70, 1WE50/50, 1WE70/30, 1WE90/10 and 1WE100/0 are shown in Table 13 and was calculated from data in Appendix E.
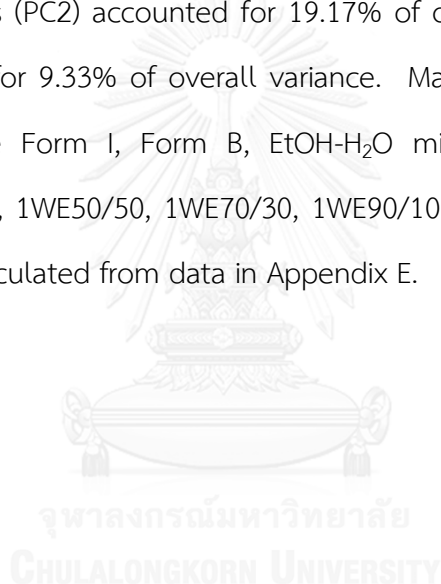
Table 13 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and one part of anhydrous olanzapine Form I exposes to one part of solvent mixtures at various drug: solvent ratios

One part of olanzapine samples to one part of solvent mixtures	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H$_2$O mixed solvate
1WE0/100	4.01	6.30	1.96
1WE10/90	4.26	6.43	2.14
1WE30/70	4.19	6.41	2.26
1WE50/50	4.27	6.60	2.60
1WE70/30	3.81	6.29	2.71
1WE90/10	3.55	5.80	3.49
1WE100/0	2.20	6.71	3.70

Mahalanobis distance indicated that 1WE100/0 sample was classified as Form I. Whereas, at other ratios are classified within group of EtOH-H$_2$O mixed solvate.

Figure 59(a and b) indicated PCA of Form I samples physically mixed with solvent mixtures (water and ethanol) at ratio of 1:5 compared to reference anhydrous olanzapine Form I and both solvated forms (Form B and EtOH-H$_2$O mixed solvate). The first principal component (PC1) accounted for 67.98% of overall variance, the second principal components (PC2) accounted for 25.61% of overall variance and and the third PC (PC3) accounted for 4.27% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and 5WE0/100, 5WE10/90, 5WE30/70, 5WE50/50, 5WE70/30,

5WE90/10 and 5WE100/0 are shown in Table 14 and was calculated from data in Appendix F.

Table 14 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and one part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various drug: solvent ratios

One part of olanzapine samples to five parts of solvent mixtures	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H_2O mixed solvate
5WE0/100	6.87	2.37	1.59
5WE10/90	6.73	2.97	1.73
5WE30/70	6.72	3.01	1.98
5WE50/50	6.73	3.08	1.95
5WE70/30	6.73	2.86	1.64
5WE90/10	6.72	3.06	1.78
5WE100/0	7.47	1.11	3.38

From Mahalanobis distance indicated that 5WE100/0 sample was classified as Form B. Other ratios are classified within group of EtOH-H_2O mixed solvate. However, this transformation phenomenon was only partial and incomplete as could be seen by non-overlapping data points, except 5WE100/0 which converted totally to Form B.

It could be concluded from PCA results that the transformation of Form I to Form B could only occur when Form I was exposed to high amount of water. While, transformation of Form I to EtOH-H_2O mixed solvate can be seen even when

Form I was exposed to only minute amount of ethanol. The results confirmed that type and quantity of solvents were important factors in controlling the conversion of anhydrous olanzapine Form I to either Form B or EtOH-H_2O mixed solvate. All above PCA results were consistent with the results obtained from XRPD, DSC, TGA and non-treated Raman spectra.
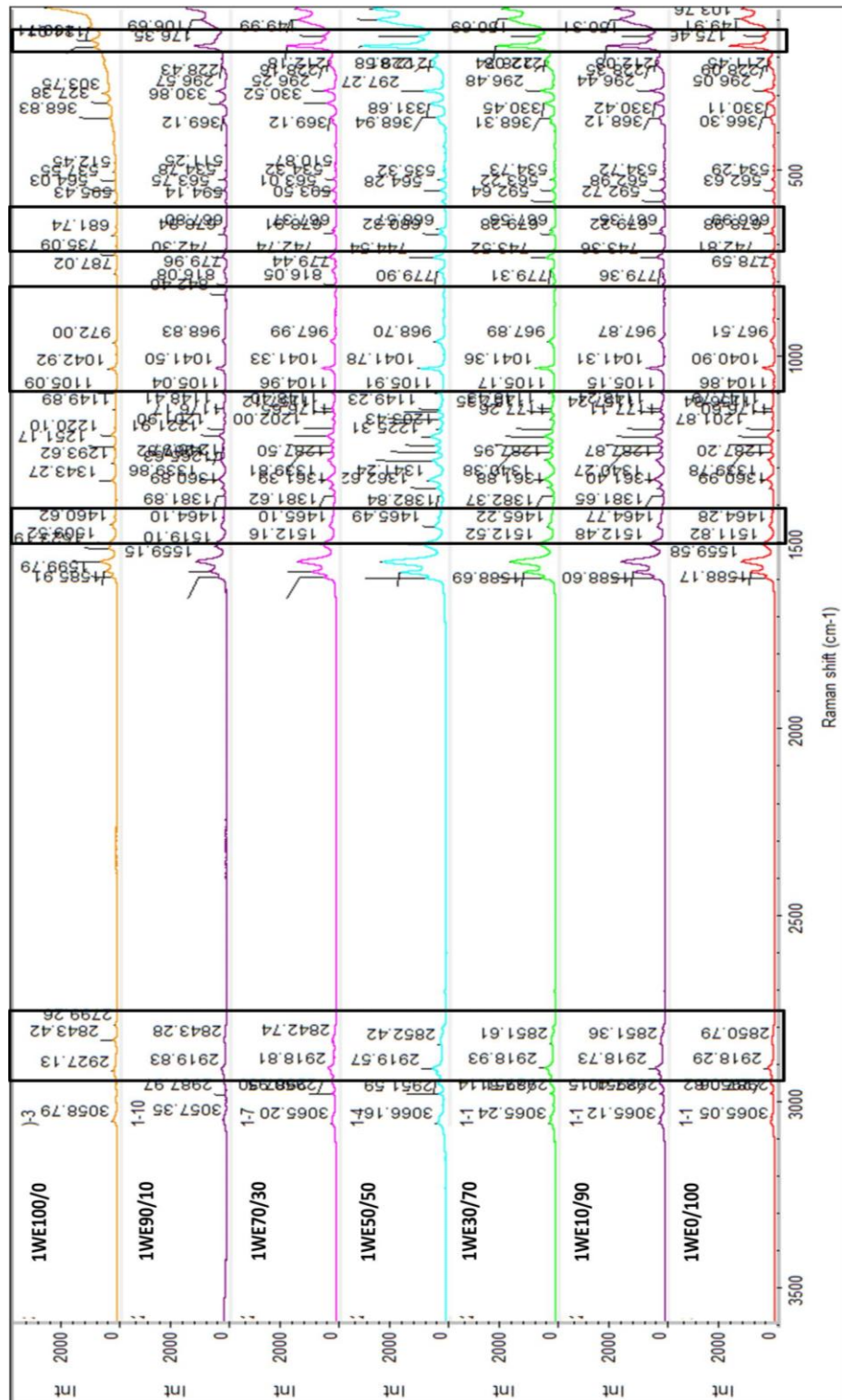
Figure 56 Raman spectra of one part of anhydrous olanzapine form I (Form I) exposed to one part of solvent mixtures at various ratios
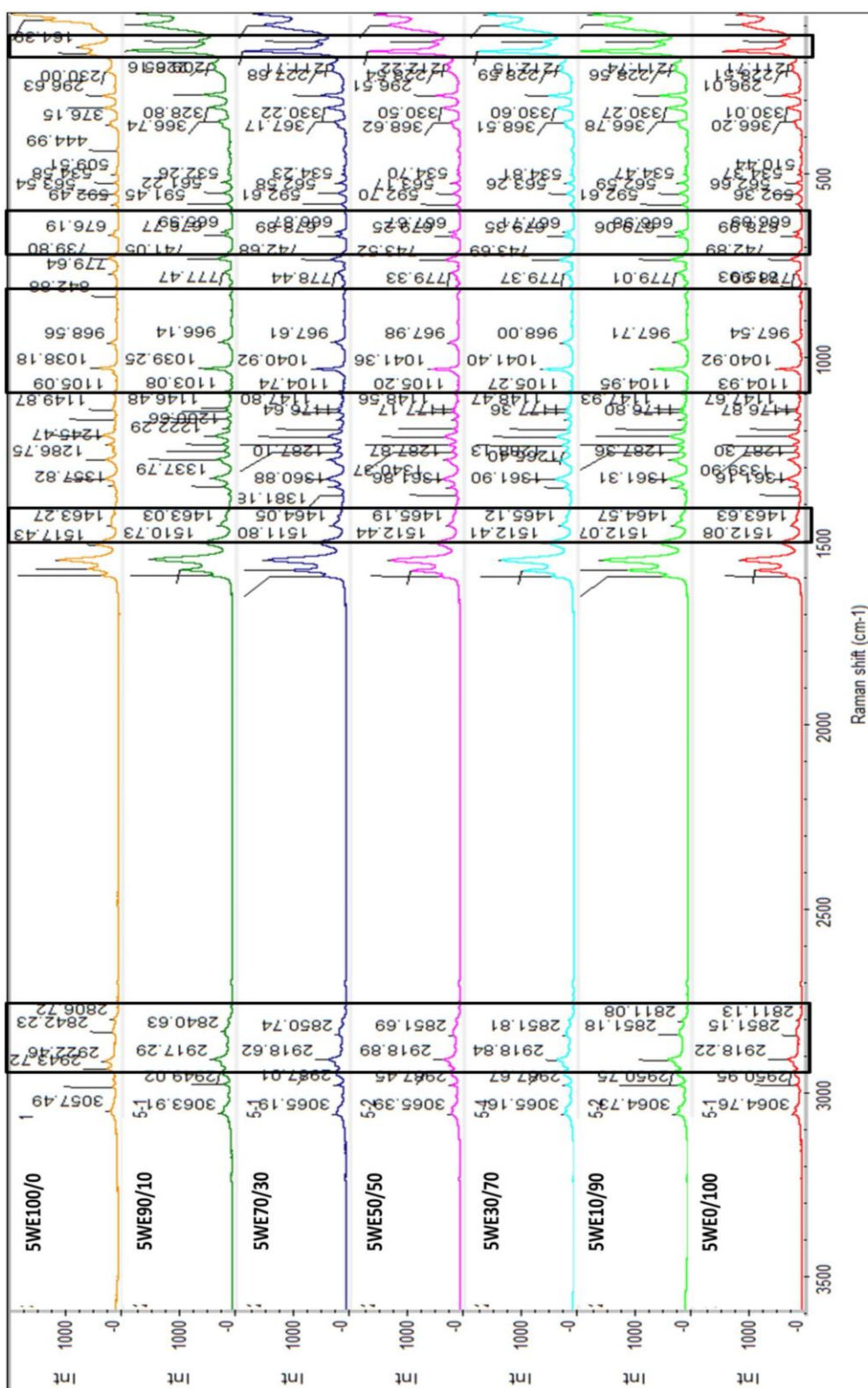
Figure 57 Raman spectra of one part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various ratios

(a)
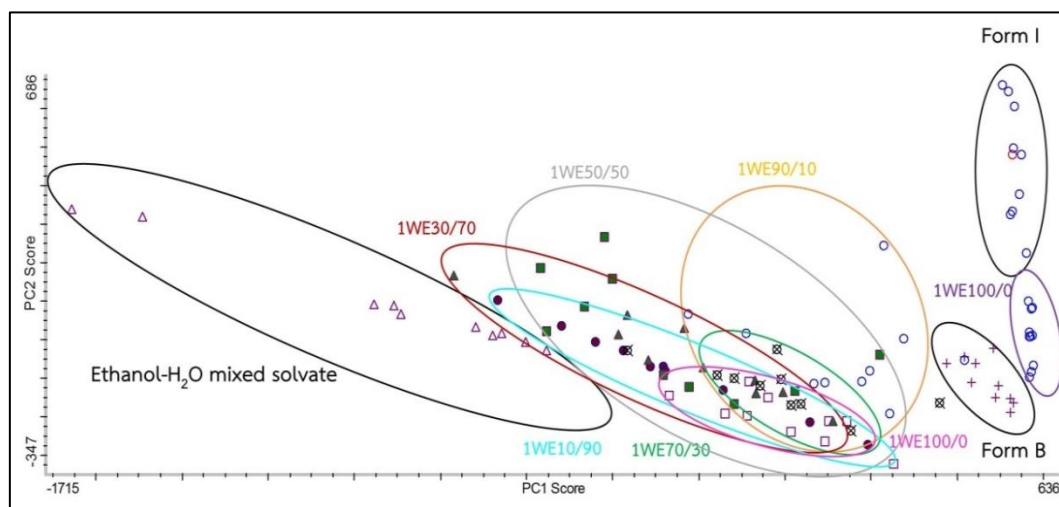
(b)
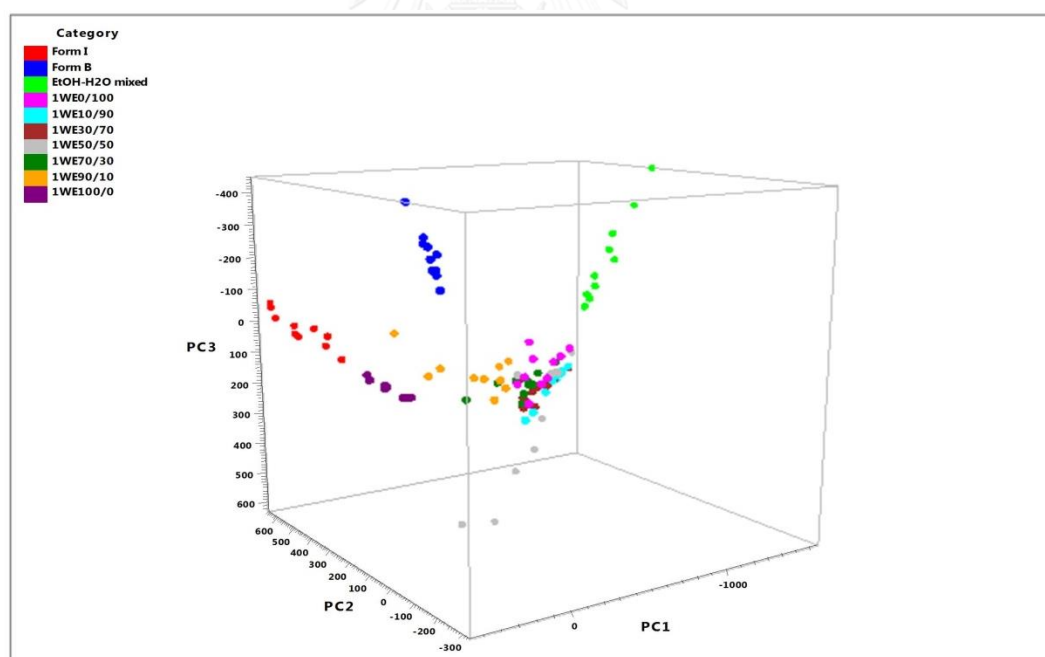
Figure 58 PCA score plots of one part of anhydrous olanzapine Form I exposes to one part of solvent mixtures at various ratios compare to reference Form I, Form B and EtOH-H$_2$O mixed solvate using TQ Analyst[®]8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

(a)
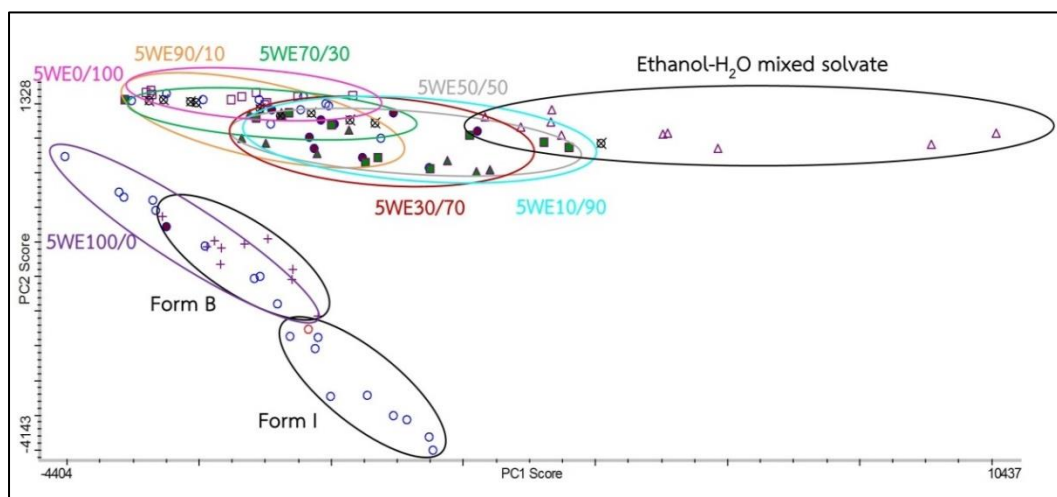
(b)
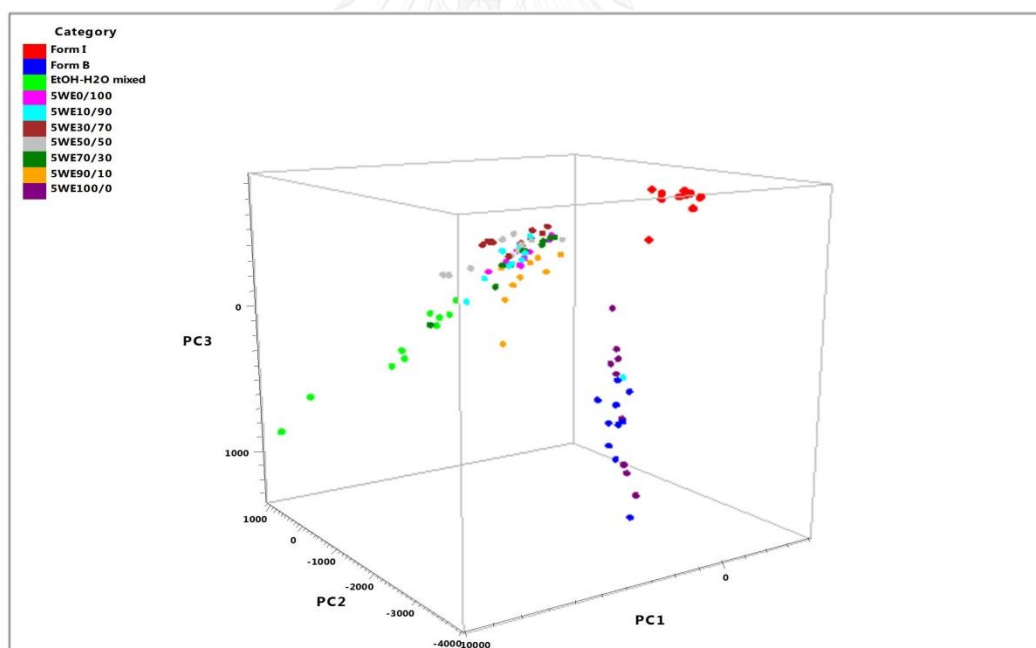
Figure 59 PCA score plots of one part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various ratios compare to Form I, Form B and EtOH-H$_2$O mixed solvate using TQ Analyst[®]8 Pro edition software of 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

All pervious results from polarized light microscopy, X-ray powder diffractrometry (XRPD), Differential Scanning Calorimetry (DSC), Thermogravimetric analysis (TGA) and Raman spectroscopy indicated that drying temperatures (25 °C, 40 °C and 70°C) and compaction forces (1,000 psi, 2,000 psi and 3,000 psi) had no significant effect on the solid state transformation of anhydrous olanzapine Form I powder. Although, high compaction force have shown to induced amorphous portion and decreased crystallinity of Form I. On the other hand, type and quantity of solvent exposure could affect Form I solid state transition. When Form I was exposed to water, it was transformed to Form B. If Form I was exposed to ethanol, it will readily change to EtOH-H_2O mixed solvate. The conversion of Form I to Form B requires significantly high amount of water. On the other hand, Form I can be transformed to EtOH-H_2O mixed solvate by presence of only minute amount of ethanol in hydroalcoholic solvents.

4. Solid state transformation of olanzapine tablets

4.1 Effect of type of solvent used in film-coating dispersion

Effect of type of solvent used in film-coating dispersion was studied by Raman spectroscopy and Chemometric analysis. Core tablet consisted of anhydrous olanzapine Form I (5% w/w), spray dried lactose (43% w/w), microcrystalline cellulose PH102 (43% w/w), sodium starch glycolate (8% w/w) and magnesium stearate (1% w/w) and the film-coating dispersion consisted of hydroxypropyl methylcellulose E5 (5% w/w), Polyethylene glycol 400 (1.5% w/w), titanium dioxide (3.5% w/w) and varying solvents. Core tablets were prepared by conventional direct compression and coating was applied to olanzapine tablets by spray gun. The solvents in film-coating dispersion used were water and ethanol. After tablets were coated, samples were stored at room temperature, 0%RH in desiccator and evaluated within 24 hours. The results were compared with

reference anhydrous olanzapine Form I and both reference solvated forms (Form B and EtOH-H$_2$O mixed solvate) using Raman spectroscopy.

4.1.1 Solid-state characterization by Raman spectroscopy

Figure 60 shows Raman spectra of olanzapine tablets coated with film-coating dispersions consist of dispersion medium of only water (COAT 100/0) or only ethanol (COAT 0/100). Samples were compared to reference anhydrous Form I and both solvated forms (Form B and EtOH-H$_2$O mixed solvate). Raman shifts between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to represent clear visual distinction between each sample. All Raman spectral data were collected in 10 replicates and analyzed by OMNIC$^{®}$ (Version 8.0) and data were sorted using Principal Component Analysis (PCA) by TQ Analyst$^{®}$8 Pro edition software. The spectral results of the surface of tablets coated with COAT 100/0 or COAT 0/100, retained characteristic peaks of Form I. These initial results indicated that film-coating dispersion consist of water or ethanol had no effect on the solid state conversion of Form I on the surface of tablets. However, the visual inspection by Raman alone could not effectively distinguish between reference olanzapine solid state forms on tablets and reference olanzapine Form I. The combination of Raman spectroscopy and chemometric method (PCA) was used to confirm solid state transformation results.

Figure 61(a and b) show PCA score plots of Raman spectral data of olanzapine tablets coated with film-coating dispersion consist of only water (COAT 100/0) and only ethanol (COAT 0/100), Form I, Form B and EtOH-H$_2$O mixed solvate. Each solid form could be classified by PCA as 3 distinctive groups. The first principal component (PC1) accounted for 84.78% of overall variance, the second principal component (PC2) accounted for 9.89% of overall variance and the third PC (PC3) accounted for 3.38% of overall variance. Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine tablets

coated with film-coating dispersion consist of water (COAT100/0) or ethanol (COAT0/100COAT 100/0 and COAT 0/100 are shown in Table 15 and was calculated from data in Appendix G.

Table 15 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine tablets coat with film-coating dispersion consist of water (COAT100/0) or ethanol (COAT0/100)

Olanzapine sample	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H$_2$O mixed solvate
COAT 100/0	2.59	4.50	3.30
COAT 0/100	2.70	4.51	3.14

These results show that olanzapine particles on the tablet surfaces obtain by COAT 100/0 and COAT 0/100 retained as Form I.

All above PCA results indicated that type of solvent (water and ethanol) in film-coating dispersion had no significant effect on the solid state transformation of olanzapine particles on surfaces of tablets in this experimental condition. This may be due to the very short contact time between "wet" coating dispersion and surface of core tablets

Figure 60 Raman spectra of olanzapine tablets coat with film-coating dispersions consist of water (COAT100/0) or ethanol (COAT0/100) compare to reference anhydrous olanzapine Form I and both solvated forms (Form B and EtOH-H$_2$O mixed solvate).

(a)
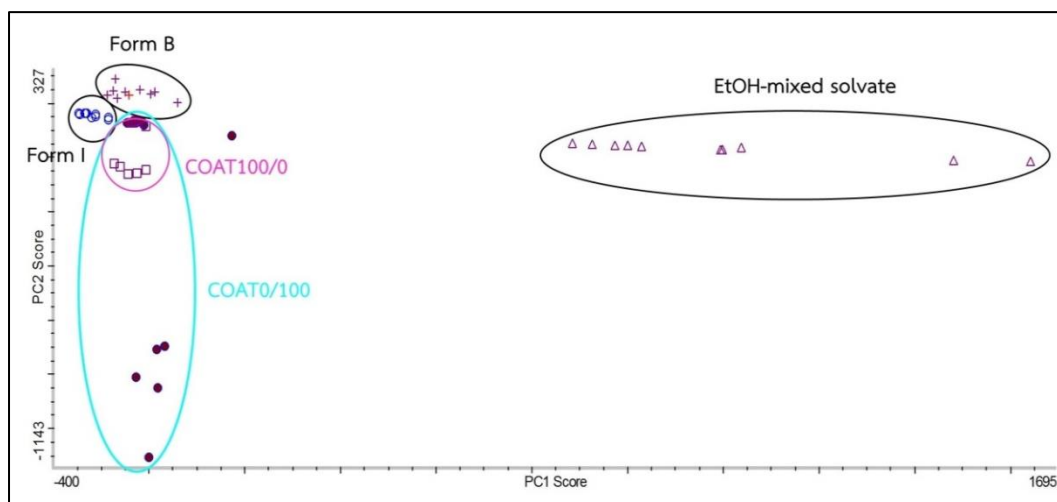
(b)
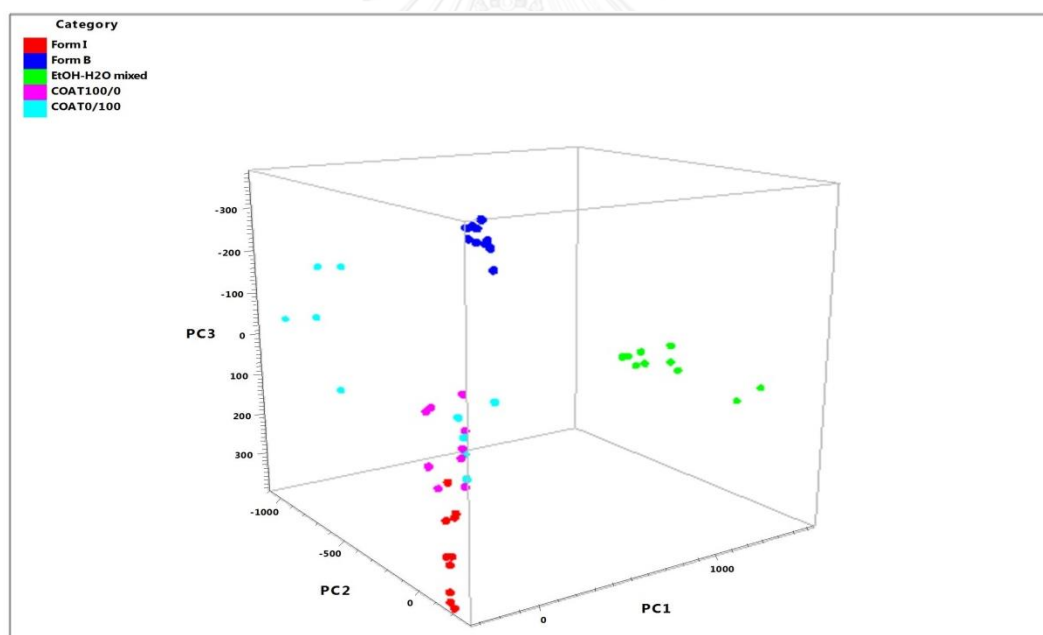
Figure 61 PCA score plots of olanzapine tablets coat with film-coating dispersions consist of water (COAT100/0) or ethanol (COAT0/100) compare to Form I, and Form B and EtOH-H$_2$O mixed solvate using TQ Analyst$^®$8 Pro edition software as 2 dimensional PCA score plot (a) and 3 dimensional PCA score plot (b)

4.2 Effect of solvent evaporations on solid state transformation of film coated tablets

The results obtained from pervious study (section 4.1) indicate that type of solvents used in film-coating dispersion had no effect on the conversion of anhydrous olanzapine Form I on the surfaces of tablets. These results were not in good correlation with results obtained using olanzapine powder (section 3.3.1 and 3.3.2). This might be due to the differences in the evaporation rates of solvents in film-coating dispersions on tablet surfaces. This experiment is the study on evaporation rates of solvent in film-coating dispersions at varying temperatures of 25°C, 40°C and 70°C. The compositions of core tablets and film-coating dispersions were the same as in section 4.1. The solvent in film-coating dispersion used were either water or ethanol. After coating, the solvents were evaporated at three evaporation rates (25°C, 40°C and 70°C) for 3 hours. These three temperatures used were previously proven to not exert any effect on the solid state transformation of olanzapine powder. However, when the drug was exposed to these temperatures while in contact with film-coating dispersion, further evaluation was needed. The three different drying temperatures indirectly correlate to the rates of solvent evaporation in film-coating dispersions on olanzapine Form I core tablets. Dried samples were stored at room temperature, 0%RH, in tight and light resistant desiccator and evaluated within 24 hours by Raman spectroscopy and chemometric analysis. The results were compared with reference olanzapine Form I, Form B and EtOH-H$_2$O mixed solvate.

4.2.1 Solid-state characterization by Raman spectroscopy

Figure 62 presents Raman spectral data of olanzapine tablets coated with film-coating dispersion consists of water and evaporated at 25°C (COAT100/0 25C), 40°C (COAT100/0 40C) and 70°C (COAT100/0 70C) compare to reference anhydrous Form I and both solvated forms (Form B and EtOH-H$_2$O mixed solvate). Raman shifts

between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were chosen to represent clear visual distinction between each sample. All Raman spectral data were collected in 10 replicates and analyzed by OMNIC$^{®}$ (Version 8.0). Data were discriminated and correlated by Principal Component Analysis (PCA) and Mahalanobis distance using TQ Analyst$^{®}$8 Pro edition software. Results from COAT100/0 25C and COAT100/0 40C exhibit some Raman shifts (2842 cm^{-1} and 970 cm^{-1}) which were characteristic of Form B. However, majority of Raman shifts of COAT100/0 25C and COAT100/0 40C still retained characteristic peaks of Form I. While, Raman shifts of COAT100/0 70C maintained all the characteristic peaks of pure Form I.

PCA score plots present discrimination between Raman spectra of reference olanzapine Form I, Form B and EtOH-H$_2$O mixed solvate and Raman spectra of samples (COAT100/0 25C, COAT100/0 40C and COAT100/0 70C) and shown in Figure 63(a and b). The spectral regions between 2950 to 2750 cm^{-1}, 1500 to 1400 cm^{-1}, 1100 to 800 cm^{-1}, 700 to 600 cm^{-1} and 200 to 150 cm^{-1} were used to explore the spectroscopic differences between these samples. The first, second and third component (PC1, PC2 and PC3) explained 38.41%, 20.32% and 12.89% of the total variance, respectively. It was found that Mahalanobis distance of COAT100/0 25C (Mahalanobis distance is 1.25) and COAT100/0 40C (Mahalanobis distances is 1.87) were closest to reference Form B. COAT100/0 70C (Mahalanobis distances is 2.36) were furthest to Form B. (Appendix H). This result indicated that evaporation rate of water in film-coating dispersion played great role in the solid state transformation of olanzapine Form I particles on the surface of tablets.

Table 16 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H_2O mixed solvate and olanzapine tablets coat with film-coating dispersion consists of water expose to solvent evaporation rates of 25 °C (COAT100/0 25C), 40 °C (COAT100/0 40C)and 70 °C (COAT100/0 70C) for 3 hour

Olanzapine coated tablets at various evaporation rates	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H_2O mixed solvate
COAT 100/0 25C	0.96	1.25	3.10
COAT 100/0 40C	1.35	1.87	3.38
COAT 100/0 70C	1.81	2.36	3.64

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY
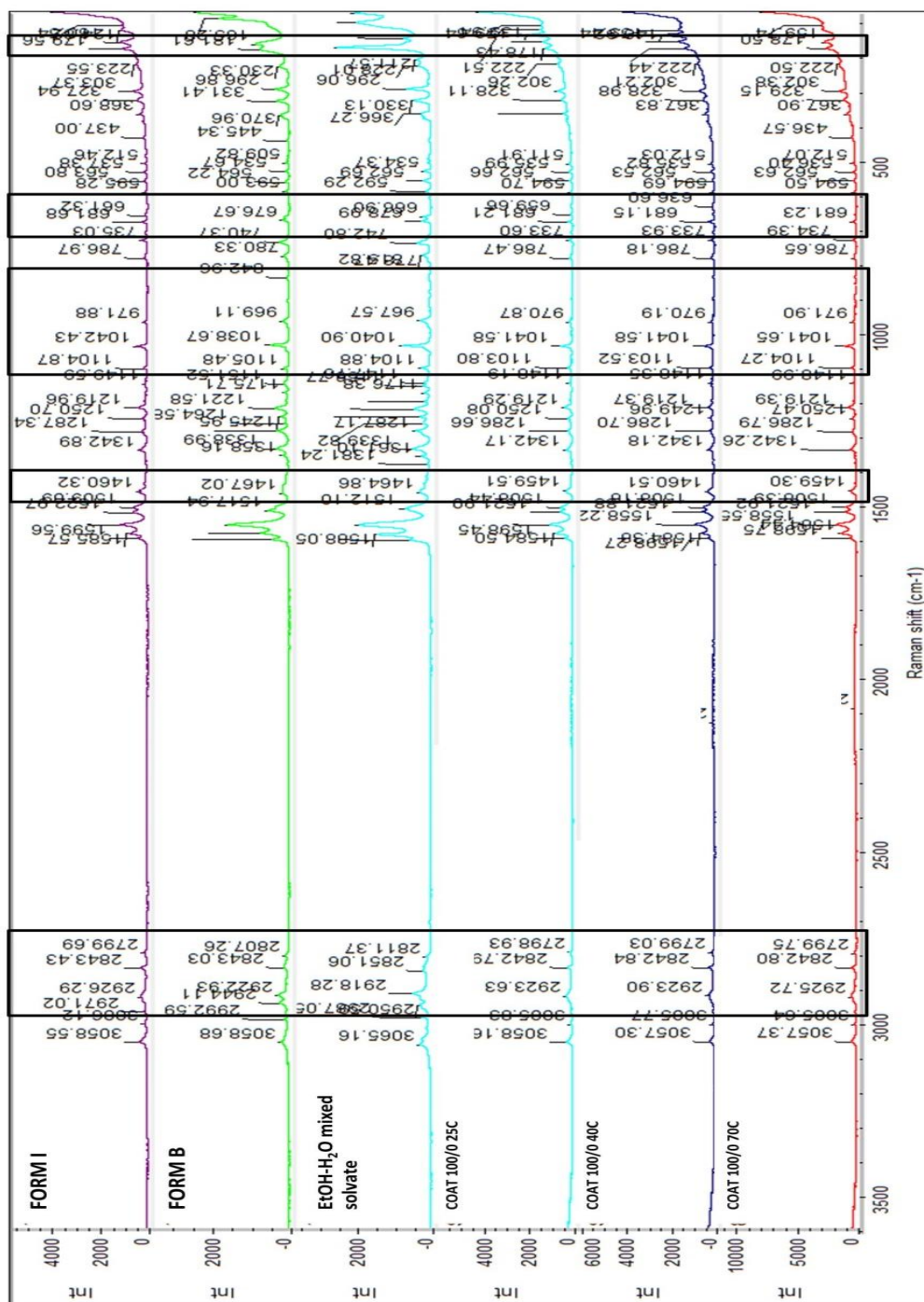
Figure 62 Raman spectra of olanzapine tablets coat with film-coating dispersion consists of water and expose to solvent evaporation rates of 25 °C (COAT100/0 25C), 40 °C (COAT100/0 40C)and 70 °C (COAT100/0 70C) for 3 hours compare to reference anhydrous olanzapine Form I, Form B and EtOH-H$_2$O mixed water

(a)
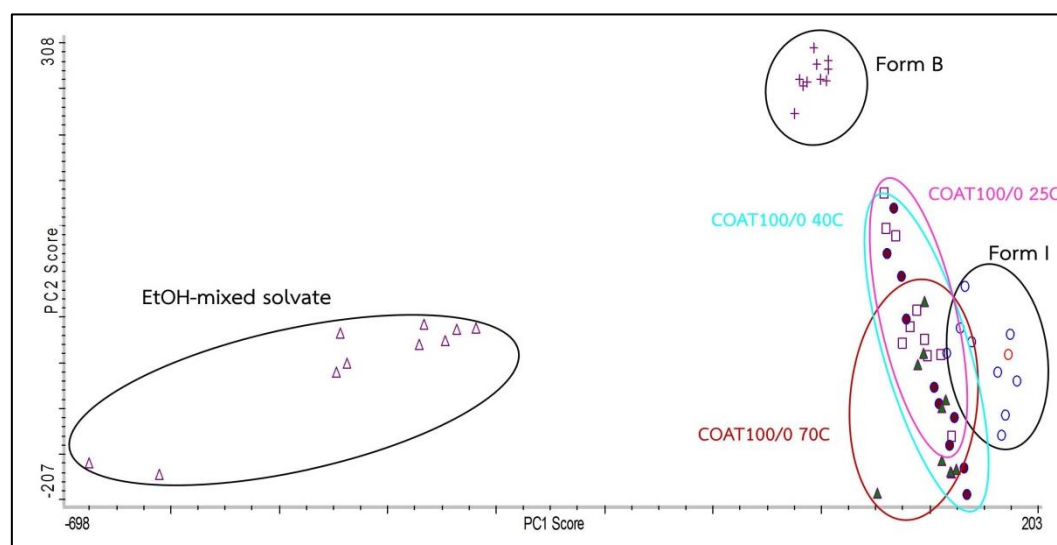
(b)
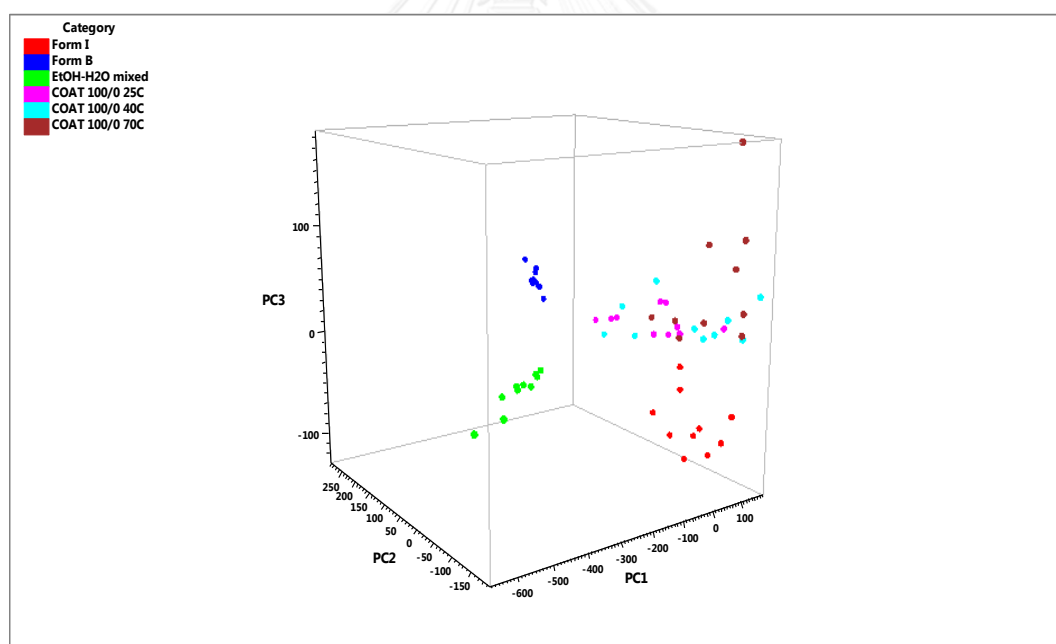
Figure 63 PCA score plot of olanzapine tablets coat with film-coating dispersion consists of water and expose to solvent evaporation rates of at 25 °C (COAT100/0 25C), 40 °C (COAT100/0 40C) and 70 °C (COAT100/0 70C) for 3 hours compare to reference anhydrous olanzapine Form I, Form B and EtOH-H$_2$O mixed solvate

Figure 64 exhibits Raman spectral data of olanzapine tablets coated with film-coating dispersion consists of ethanol and expose to solvent evaporation rates of 25°C (COAT0/100 25C), 40°C (COAT0/100 40C) and 70°C (COAT0/100 70C) compare to reference anhydrous Form I, Form B and EtOH-H_2O mixed solvate. COAT0/100 25C exhibits some specific Raman shifts at 2917 cm^{-1}, 1464 cm^{-1}, 967 cm^{-1} and 175 cm^{-1} indicating the characteristic of EtOH-H_2O mixed solvate. Raman spectra of all samples still retained some characteristic peaks of Form I. However, the visual observation of Raman spectra alone could not clearly distinguished between solid samples treated.

Figure 65(a and b) exhibit PCA score plots of olanzapine tablets coated with film-coating dispersion consists of ethanol and evaporated at the rate of 25°C (COAT0/100 25C), 40°C (COAT0/100 40C) and 70°C (COAT0/100 70C) compare to reference anhydrous Form I, Form B and EtOH-H_2O mixed solvate. Results could be classified with PCA as 3 distinctive groups. The first principal component (PC1) accounted for 53.12% of overall variance, the second principal component (PC2) accounted for 33.08% of overall variance and the third PC (PC3) accounted for 10.56% of overall variance. From the Mahalanobis distances between reference EtOH-H_2O mixed solvate and samples; COAT0/100 25C (Mahalanobis distances is 2.46) was found to have closest distance to EtOH-H_2O mixed solvate (Appendix I). COAT0/100 40C (Mahalanobis distances is 2.89) and COAT0/100 70C (Mahalanobis distances is 2.91) were further away. The results indicated that evaporation rate of ethanol in film-coating dispersion had significant effect on the conversion of Form I particles on surfaces of tablet. Fast rate of evaporation was more preferable in preventing solid state conversion.

Table 17 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine tablets coat with film-coating dispersion consists of ethanol expose to solvent evaporation rates of 25 °C (COAT0/100 25C), 40 °C (COAT0/100 40C)and 70 °C (COAT0/100 70C) for 3 hours

Olanzapine coated tablets at various evaporation rates	Mahalanobis distance		
	Distance to Reference Form I	Distance to Reference Form B	Distance to Reference EtOH-H$_2$O mixed solvate
COAT 0/100 25C	2.46	4.99	2.46
COAT 0/100 40C	2.45	5.34	2.89
COAT 0/100 70C	2.24	5.28	2.91

From above results, solid state transformation of anhydrous olanzapine Form I on tablet surfaces can occur by exposing solvent (water or ethanol) in film-coating dispersion for long duration. Form this study, the slower evaporation rate of water in coated tablets (low drying temperatures) resulted in the conversion of olanzapine from Form I to Form B. While, slower evaporation rate of ethanol in coated tablet (low drying temperatures) resulted in the conversion of Form I to EtOH-H$_2$O mixed solvate. In addition, PCA score plots also revealed the conversion pathways of anhydrous form to hydrated forms when evaporation rate was decreased by reducing the drying temperature.
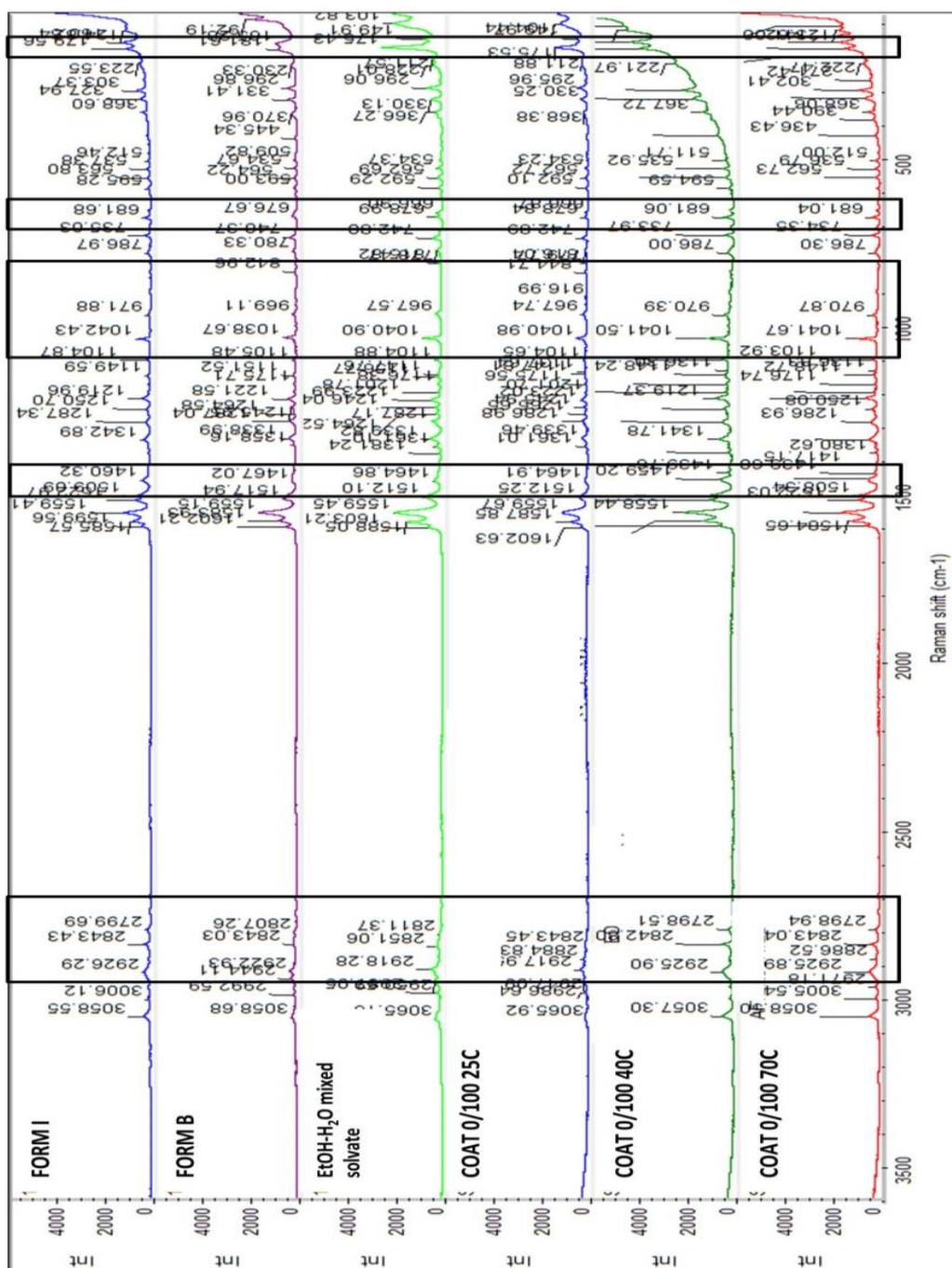
Figure 64 Raman spectra of olanzapine tablets coat with film-coating dispersion consists of ethanol expose to solvent evaporation rates of 25 °C (COAT0/100 25C), 40 °C (COAT0/100 40C) and 70 °C (COAT0/100 70C) for 3 hours compare to reference anhydrous olanzapine Form I, Form B and EtOH-H$_2$O mixed water.

(a)
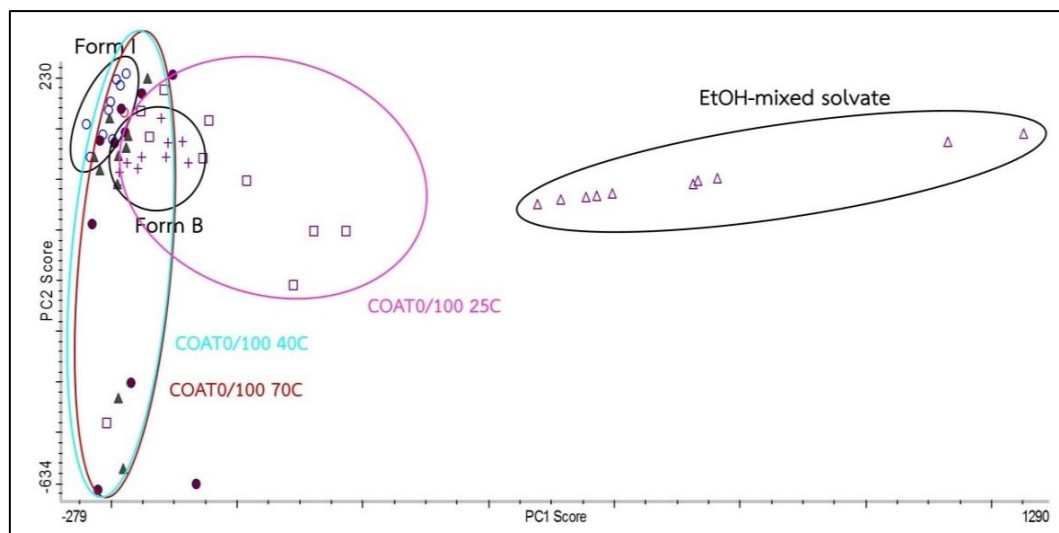
(b)
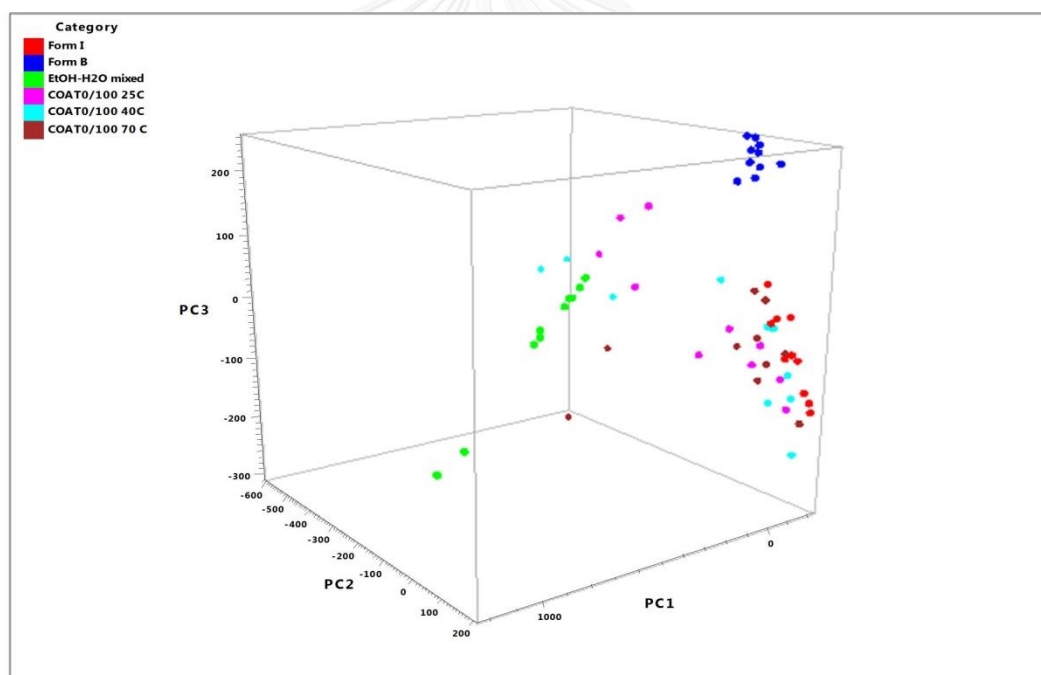
Figure 65 PCA score plot of olanzapine tablets coat with film-coating dispersion consists of ethanol expose to solvent evaporation rates of 25 °C (COAT0/100 25C), 40 °C (COAT0/100 40C) and 70 °C (COAT0/100 70C) for 3 hours compare to reference anhydrous olanzapine Form I, Form B and EtOH-H_2O mixed water

CHAPTER V

CONCLUSIONS

During manufacturing processes of olanzapine coated tablet many factors may affect solid state transformation, for example, drying temperature, compaction force, solvent used and formulation compositions. Solid state interconversion may alter the physicochemical and biopharmaceutical properties of the drug product. The conventional solid state characterization techniques are used in compliment to each other in quantify and identify solid state transformation when it occurs with percentage of usually more than 10%w/w .These techniques include polarized light microscopy, X-ray powder diffractrometry (XRPD), differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Karl Fishcer titratimetry (KF). However, if the transformation occur at less than 10% w/w a more sensitive technique and calculation must be used, i.e. Raman spectroscopy (Raman) along with Principal Component Analysis (PCA) play an important role in investigating solid state transformation in this experimental study. Moreover, this study explores the use Raman spectroscopy and Principal Component Analysis (PCA) to distinguish between groups of solid structures arising from anhydrous olanzapine Form I.

The results of this study may be concluded that no conversion occur when Form I powder dried in hot air oven at 25°C, 40°C and 70°C for 3 hours. These results are in the same direction as the effect of compaction force. When olanzapine Form I powder was compressed with forces of 1,000 psi, 2,000 psi and 3,000 psi and using 6.35 mm punch-face transformation of olanzapine powder did not occur but crystallinity was decreased slightly. Thus, drying temperatures and compaction forces used in this study did not induce transformation of olanzapine powder. Moreover, the transformation of olanzapine powder is evaluated by exposing to

different type and ratio of solvents (water and ethanol). Type, quantity and ratio of solvents can affect Form I crystal transformation to varying degrees. Conversion of Form I to olanzapine dihydrate Form B contact with requires very high amount and ratio of water. While Form I can be transformed to olanzapine EtOH-H_2O mixed solvate by mixing only minute amount a low percentage of ethanol in the dispersing liquid.

Olanzapine form I coated tablets were prepared by conventional direct compression and coating of olanzapine tablet was applied using spray gun. Assumption was made that the solid state transformation will only occur on the surface of tablets; hence, conventional solid state analytical techniques cannot be used. In this case Raman spectroscopy with PCA was the method of choice to detect the transformation extent and pathways. The solvent in film-coating dispersion consists of either water or ethanol. To study the rate of evaporation of solvent in film-coating dispersion on the tablet surfaces the coating method was modified to an indirect method by drying in hot air oven at 25°C, 40°C and 70°C for 3 hours after coating. The results indicate that the transformation of olanzapine Form I tablets occurred by slow solvent evaporation rate and allow enough time of contact. All above results reveal that type and rate of evaporation of solvent in film-coating dispersion have high impact on the conversion of anhydrous Form I to solvated forms either Form B or EtOH-H_2O mixed solvate.

REFERENCES

1. Kuminek G, Rauber GS, Riekes MK, Campos CEMd, Monti GA, Bortoluzzi AJ, et al. Single crystal structure, solid state characterization and dissolution rate of terbinafine hydrochloride. Journal of Pharmaceutical and Biomedical Analysis. 2013;78–79(0):105-111.

2. Croker DM, Hennigan MC, Maher A, Hu Y, Ryder AG, Hodnett BK. A comparative study of the use of powder X-ray diffraction, Raman and near infrared spectroscopy for quantification of binary polymorphic mixtures of piracetam. Journal of Pharmaceutical and Biomedical Analysis. 2012;63(0):80-86.

3. Hennigan MC, Ryder AG. Quantitative polymorph contaminant analysis in tablets using Raman and near infra-red spectroscopies. Journal of Pharmaceutical and Biomedical Analysis. 2013;72(0):163-171.

4. Láng P, Kiss V, Ambrus R, Farkas G, Szabó-Révész P, Aigner Z, et al. Polymorph screening of an active material. Journal of Pharmaceutical and Biomedical Analysis. 2013;84(0):177-183.

5. Lust A, Lakio S, Vintsevits J, Kozlova J, Veski P, Heinämäki J, et al. Water-mediated solid-state transformation of a polymorphic drug during aqueous-based drug-layer coating of pellets. International Journal of Pharmaceutics. 2013;456(1):41-48.

6. Reutzel-Edens SM, Bush JK, Magee PA, Stephenson GA, Byrn SR. Anhydrates and hydrates of olanzapine: crystallization, solid-state characterization, and structural relationships. Crystal growth & design. 2003;3(6):897-907.

7. Kamada K, Yoshimura S, Murata M, Murata H, Nagai H, Ushio H, et al. Characterization and monitoring of pseudo-polymorphs in manufacturing process by NIR. International Journal of Pharmaceutics. 2009;368(1–2):103-108.

8. Ayala AP, Siesler HW, Boese R, Hoffmann GG, Polla GI, Vega DR. Solid state characterization of olanzapine polymorphs using vibrational spectroscopy. International Journal of Pharmaceutics. 2006;326(1–2):69-79.

9.   Kolodziejski W, Herold J, Kuras M, Wawrzycka-Gorczyca I, Koziol AE. Kinetics of 1H⟶13C NMR cross-polarization in polymorphs and solvates of the antipsychotic drug olanzapine. Solid State Nuclear Magnetic Resonance. 2011;39(3–4):41-46.

10.   Polla GI, Vega DR, Lanza H, Tombari DG, Baggio R, Ayala AP, et al. Thermal behaviour and stability in Olanzapine. International Journal of Pharmaceutics. 2005;301(1–2):33-40.

11.   Wawrzycka-Gorczyca I, Borowski P, Osypiuk-Tomasik J, Mazur L, Koziol AE. Crystal structure of olanzapine and its solvates. Part 3. Two and three-component solvates with water, ethanol, butan-2-ol and dichloromethane. Journal of Molecular Structure. 2007;830(1–3):188-197.

12.   Cavallari C, Fini A, Pérez Artacho-Santos B. Thermal study of anhydrous and hydrated forms of olanzapine. Pharm Anal Acta. 2013;4(237):2.

13.   Gendrin C, Roggo Y, Collet C. Pharmaceutical applications of vibrational chemical imaging and chemometrics: A review. Journal of Pharmaceutical and Biomedical Analysis. 2008;48(3):533-553.

14.   Singh I, Juneja P, Kaur B, Kumar P. Pharmaceutical Applications of Chemometric Techniques. International Scholarly Research Notices. 2013;2013.

15.   Navari RM. Olanzapine for the prevention and treatment of chronic nausea and chemotherapy-induced nausea and vomiting. European Journal of Pharmacology. 2014;722:180-186.

16.   Allen LV. The Science and Practice of Remington. 22 ed. United Kingdom: Pharmceutical Press; 2013.

17.   JW D. Drug information handbook with international trade names index. 21 ed. United state: Lexi-Comp Incorperation 2012.

18.   United State Pharmacopia 35 (USP 35). Official Monographs. Olanzapine 2012. p. 4102-4103.

19.   Tiwari M, Chawla G, Bansal AK. Quantification of olanzapine polymorphs using powder X-ray diffraction technique. Journal of Pharmaceutical and Biomedical Analysis. 2007;43(3):865-872.

20.   Paisana M, Wahl M, Pinto JF, editors. Effect of moisture on stor-age of Olanzapine polymorphs. In Proceeding of the 3rd PharmSciFair; 2011 June 13-17; Prague, Czech Republic.

21.   Flood AE, Wantha L. Population balance modeling of the solution mediated transformation of polymorphs: Limitations and future trends. Journal of Crystal Growth. 2013;373(0):7-12.

22.   Chieng N, Rades T, Aaltonen J. An overview of recent studies on the analysis of pharmaceutical polymorphs. Journal of Pharmaceutical and Biomedical Analysis. 2011;55(4):618-644.

23.   Chongcharoen W, Byrn SR, Sutanthavibul N. Solid State Interconversion Between Anhydrous Norfloxacin and Its Hydrates. J Pharm Sci. 2008;97(1):473-489.

24.   Zhang GGZ, Law D, Schmitt EA, Qiu Y. Phase transformation considerations during process development and manufacture of solid oral dosage forms. Advanced Drug Delivery Reviews. 2004;56(3):371-390.

25.   Winger B, Kemp C. Characterization of Pharmaceutical Compounds and Related Substances by using Related Substances by using HPLC FTICR-MS and Tandem Mass Spectrometry. American Pharmaceutical Review. 2001;4:55-63.

26.   Brittain HG. Polymorphism in pharmaceutical solids. New York: Marcel Dekker; 1999.

27.   Brittain HG. 2 - Characterization of Pharmaceutical Compounds in the Solid State. In: Satinder A, Stephen S, editors. Separation Science and Technology. Volume 10: Academic Press; 2011. p. 11-58.

28.   Inoue S. Introduction to Biological Polarization Microscopy: Polarized Light Microscopy [Online]. Massachusetts: Marine Biological Laboratory; 2005 Jul 26 [cited 2016 June 18]. Available from: http://micro.magnet.fsu.edu/primer/techniques/ polarized/biologicalparttwo.html.

29.   Munson EJ. Chapter 3 - Analytical Techniques in Solid-state Characterization. In: Porter YQCGZZLR, editor. Developing Solid Oral Dosage Forms. San Diego: Academic Press; 2009. p. 61-74.

30. Auer ME, Griesser UJ, Sawatzki J. Qualitative and quantitative study of polymorphic forms in drug formulations by near infrared FT-Raman spectroscopy. Journal of Molecular Structure. 2003;661–662(0):307-317.

31. Sachin TV, Deodhar MN, Prakya V. Advances in analytical techniques used in predicting drug-excipient interactions. International Journal of Pharmacy and Technology. 2014;6(1):6388-6417.

32. Német Z, Kis GC, Pokol G, Demeter Á. Quantitative determination of famotidine polymorphs: X-ray powder diffractometric and Raman spectrometric study. Journal of Pharmaceutical and Biomedical Analysis. 2009;49(2):338-346.

33. Larhrib H, Zeng XM, Martin GP, Marriott C, Pritchard J. The use of different grades of lactose as a carrier for aerosolised salbutamol sulphate. International Journal of Pharmaceutics. 1999;191(1):1-14.

34. Peres-Filho MJ, Gaeti MPN, de Oliveira SR, Marreto RN, Lima EM. Thermoanalytical investigation of olanzapine compatibility with excipients used in solid oral dosage forms. Journal of Thermal Analysis and Calorimetry. 2010;104(1):255-260.

35. Gill P, Moghadam TT, Ranjbar B. Differential Scanning Calorimetry Techniques: Applications in Biology and Nanoscience. Journal of Biomolecular Techniques : JBT. 2010;21(4):167-193.

36. Sungwarl W. Thermogravimetric Analysis (TGA) [Online]. Scientific and Technological Instruments Center Mae Fah Luang University 2012 [updated 2015 Jul 22; cited 2016 Jun 18]. Available from: http://web2.mfu.ac.th/center/stic/index.php/thermal-analysis-instrument-menu/item/111-thermogravimetric-analysis-tga.html.

37. United State Pharmacopia 35 (USP 35) Physical Tests. Water Determination2012. p. 424-427.

38. Vankeirsbilck T, Vercauteren A, Baeyens W, Van der Weken G, Verpoort F, Vergote G, et al. Applications of Raman spectroscopy in pharmaceutical analysis. TrAC Trends in Analytical Chemistry. 2002;21(12):869-877.

39. Ayala AP. Polymorphism in drugs investigated by low wavenumber Raman scattering. Vibrational Spectroscopy. 2007;45(2):112-116.

40.   Das RS, Agrawal YK. Raman spectroscopy: Recent advancements, techniques and applications. Vibrational Spectroscopy. 2011;57(2):163-176.

41.   Taylor LS, Langkilde FW. Evaluation of Solid‐State Forms Present in Tablets by Raman Spectroscopy. J Pharm Sci. 2000;89(10):1342-1353.

42.   de Veij M, Deneckere A, Vandenabeele P, de Kaste D, Moens L. Detection of counterfeit Viagra® with Raman spectroscopy. Journal of Pharmaceutical and Biomedical Analysis. 2008;46(2):303-309.

43.   Braun DE, Maas SG, Zencirci N, Langes C, Urbanetz NA, Griesser UJ. Simultaneous quantitative analysis of ternary mixtures of d-mannitol polymorphs by FT-Raman spectroscopy and multivariate calibration models. International Journal of Pharmaceutics. 2010;385(1–2):29-36.

44.   Rajalahti T, Kvalheim OM. Multivariate data analysis in pharmaceutics: A tutorial review. International Journal of Pharmaceutics. 2011;417(1–2):280-290.

45.   Geladi P. Chemometrics in spectroscopy. Part 1. Classical chemometrics. Spectrochimica Acta Part B: Atomic Spectroscopy. 2003;58(5):767-782.

46.   Debes K, Koenig A and Gross H. Transfer Functions in Artificial Neural Networks A Simulation-Based Tutorial [Online]. Department of Neuroinformatics and Cognitive Robotics, Technical University 2005 [cited 2016 Jul 25]. Available from: http://www.brains-minds-media.org/archive/151/copy_of_bmm-debes-suppl-050704.html.

47.   Koivisto M, Heinanen P, Tanninen VP, Lehto VP. Depth profiling of compression-induced disorders and polymorphic transition on tablet surfaces with grazing incidence X-ray diffraction. Pharm Res. 2006;23(4):813-820.

48.   Otsuka M, Matsumoto T, Kaneniwa N. Effects of the mechanical energy of multi-tableting compression on the polymorphic transformations of chlorpropamide. J Pharm Pharmacol. 1989;41(10):665-669.

49.   Lin S-Y. Grinding and compression processes affecting the solid-state transition of famotidine polymorphs. Asian Journal of Pharmaceutical Sciences. 2007;2:211-219.

50.   Newman AW, Byrn SR. Solid-state analysis of the active pharmaceutical ingredient in drug products. Drug Discovery Today. 2003;8(19):898-905.

APPENDIX

APPENDIX A

Table 18 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine samples dry at 25℃, 40℃, and 70℃

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	25 ℃ 3 hours No.1	Form I	1.3019	4.8590	3.3624
2	25 ℃ 3 hours No.2	Form I	1.6646	4.9338	3.5852
3	25 ℃ 3 hours No.3	Form I	1.1877	5.0271	3.3879
4	25 ℃ 3 hours No.4	Form I	0.9637	5.0291	3.4499
5	25 ℃ 3 hours No.5	Form I	1.7484	4.8093	3.2942
6	25 ℃ 3 hours No.6	Form I	1.6587	5.0839	3.6264
7	25 ℃ 3 hours No.7	Form I	1.9311	5.1711	3.8621
8	25 ℃ 3 hours No.8	Form I	1.5275	5.2458	3.8911
9	25 ℃ 3 hours No.9	Form I	1.3351	5.0909	3.4738
10	25 ℃ 3 hours No.10	Form I	1.8268	4.9219	3.5053
11	40 ℃ 3 hours No.1	Form I	1.5405	4.8620	3.5835
12	40 ℃ 3 hours No.2	Form I	1.6487	4.9204	3.4499
13	40 ℃ 3 hours No.3	Form I	1.4551	5.0030	3.6355
14	40 ℃ 3 hours No.4	Form I	1.3052	4.9630	3.4194
15	40 ℃ 3 hours No.5	Form I	1.6606	5.0398	3.5839
16	40 ℃ 3 hours No.6	Form I	1.2987	4.6867	3.4163
17	40 ℃ 3 hours No.7	Form I	1.4196	4.8168	3.3339
18	40 ℃ 3 hours No.8	Form I	1.2848	5.0518	3.3131
19	40 ℃ 3 hours No.9	Form I	1.3423	4.9223	3.4001
20	40 ℃ 3 hours No.10	Form I	1.1526	4.8902	3.3687
21	70 ℃ 3 hours No.1	Form I	1.0465	5.014	3.3525
22	70 ℃ 3 hours No.2	Form I	1.6512	4.7818	3.3378
23	70 ℃ 3 hours No.3	Form I	1.2341	4.8535	3.2718
24	70 ℃ 3 hours No.4	Form I	1.3500	4.7705	3.3344
25	70 ℃ 3 hours No.5	Form I	1.4281	4.808	3.4763
26	70 ℃ 3 hours No.6	Form I	1.7063	4.637	3.665
27	70 ℃ 3 hours No.7	Form I	1.6857	4.9118	3.5942
28	70 ℃ 3 hours No.8	Form I	1.3424	4.7975	3.6169
29	70 ℃ 3 hours No.9	Form I	1.8736	5.6806	4.4133
30	70 ℃ 3 hours No.10	Form I	1.2341	4.8535	3.2718

APPENDIX B

Table 19 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine compresses samples at 1,000 psi, 2,000 psi and 3,000 psi

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	1,000 psi No.1	Form I	2.1337	6.2542	5.284
2	1,000 psi No.2	Form I	1.9016	6.0079	5.0782
3	1,000 psi No.3	Form I	1.8181	5.8588	4.7788
4	1,000 psi No.4	Form I	2.1907	5.9225	4.7808
5	1,000 psi No.5	Form I	2.1439	5.6949	4.7565
6	1,000 psi No.6	Form I	1.8782	5.7841	4.8208
7	1,000 psi No.7	Form I	2.0473	5.8132	4.7030
8	1,000 psi No.8	Form I	1.9673	5.8690	4.8288
9	1,000 psi No.9	Form I	1.8732	5.8470	4.6923
10	1,000 psi No.10	Form I	2.0273	6.0861	5.0317
11	2,000 psi No.1	Form I	2.4750	5.4109	3.9611
12	2,000 psi No.2	Form I	2.7837	5.3883	3.9080
13	2,000 psi No.3	Form I	2.2261	5.6123	4.3854
14	2,000 psi No.4	Form I	2.2571	5.6841	4.2947
15	2,000 psi No.5	Form I	2.5674	5.4576	4.1190
16	2,000 psi No.6	Form I	2.4449	5.4260	4.0636
17	2,000 psi No.7	Form I	2.9565	5.7252	4.2588
18	2,000 psi No.8	Form I	2.7012	5.2875	3.9002
19	2,000 psi No.9	Form I	2.5781	5.4969	4.1410
20	2,000 psi No.10	Form I	2.6072	5.1762	3.8710
21	3,000 psi No.1	Form I	3.0799	6.0099	4.3338
22	3,000 psi No.2	Form I	3.3795	5.1815	3.5680
23	3,000 psi No.3	Form I	2.9044	5.5861	3.9485
24	3,000 psi No.4	Form I	3.3242	6.0040	4.4124
25	3,000 psi No.5	Form I	3.1546	5.7834	4.1170
26	3,000 psi No.6	Form I	2.8286	5.6260	4.0160
27	3,000 psi No.7	Form I	3.3035	5.9952	4.4062
28	3,000 psi No.8	Form I	3.0079	5.5732	4.0455
29	3,000 psi No.9	Form I	2.9783	6.0329	4.4582
30	3,000 psi No.10	Form I	3.0620	5.7491	4.1907

APPENDIX C

Table 20 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine samples mix with water at various ratios

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	W 3/1 No.1	Form B	3.7704	3.4755	4.2147
2	W 3/1 No.2	Form I	2.8927	4.2443	4.4662
3	W 3/1 No.3	Form I	2.7687	4.4306	4.4798
4	W 3/1 No.4	Form I	3.4816	4.1875	4.2222
5	W 3/1 No.5	Form I	3.7316	4.0579	4.4569
6	W 3/1 No.6	Form I	4.1355	5.0806	5.2582
7	W 3/1 No.7	Form I	2.9242	4.5313	4.7801
8	W 3/1 No.8	Form I	3.8984	4.7874	4.8282
9	W 3/1 No.9	Form I	3.9716	4.3210	4.5939
10	W 3/1 No.10	Form I	4.1325	4.7062	4.7770
11	W 2/1 No.1	Form I	3.4144	4.1221	4.1490
12	W 2/1 No.2	Form I	3.2739	4.2224	4.2671
13	W 2/1 No.3	Form I	2.9048	4.4140	4.4213
14	W 2/1 No.4	Form I	3.5243	4.5817	4.7118
15	W 2/1 No.5	Form I	3.5828	4.2247	4.2542
16	W 2/1 No.6	Form I	3.2322	4.2196	4.3151
17	W 2/1 No.7	Form I	3.5125	4.7443	4.7786
18	W 2/1 No.8	Form I	2.9049	4.5556	4.8987
19	W 2/1 No.9	Form I	3.1752	4.6200	4.7037
20	W 2/1 No.10	Form I	3.0636	4.2356	4.3171
21	W 1/1 No.1	Form I	3.1290	4.3148	4.376
22	W 1/1 No.2	Form I	3.0498	4.1532	4.2889
23	W 1/1 No.3	Form I	3.3508	4.1341	4.1702
24	W 1/1 No.4	Form I	2.9308	4.2155	4.284
25	W 1/1 No.5	Form I	3.1643	4.4041	4.3778
26	W 1/1 No.6	Form I	3.3316	4.2824	4.3088
27	W 1/1 No.7	Form I	3.2421	4.2463	4.2824
28	W 1/1 No.8	Form I	3.4800	4.2906	4.323
29	W 1/1 No.9	Form I	3.3365	4.4345	4.4359
30	W 1/1 No.10	Form I	2.9863	4.5314	4.6563
31	W 1/2 No.1	Form I	3.4590	4.2737	4.2928
32	W 1/2 No.2	Form I	3.5829	3.9961	4.1024
33	W 1/2 No.3	Form I	3.5758	4.0197	4.1553

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H₂O mixed solvate
34	W 1/2 No.4	Form I	3.5331	3.9368	4.1708
35	W 1/2 No.5	Form I	3.8550	4.3455	4.3943
36	W 1/2 No.6	Form I	3.6612	4.0532	4.1531
37	W 1/2 No.7	Form I	3.6571	4.0318	4.1572
38	W 1/2 No.8	Form I	3.4681	4.0722	4.2283
39	W 1/2 No.9	Form I	3.5214	4.244	4.3529
40	W 1/2 No.10	Form I	3.6433	4.1153	4.2220
41	W 1/3 No.1	Form I	3.3288	4.3768	4.3140
42	W 1/3 No.2	Form I	3.0447	4.3757	4.4045
43	W 1/3 No.3	Form I	3.0950	4.1564	4.2120
44	W 1/3 No.4	Form I	3.0429	4.1331	4.2027
45	W 1/3 No.5	Form I	3.2058	4.1289	4.1746
46	W 1/3 No.6	Form I	3.1506	4.1703	4.2069
47	W 1/3 No.7	Form I	3.0376	4.1577	4.3034
48	W 1/3 No.8	Form I	3.0203	4.2654	4.2664
49	W 1/3 No.9	Form I	3.1438	4.1448	4.1458
50	W 1/3 No.10	Form I	3.1503	4.1599	4.2437
51	W 1/4 No.1	Form B	3.7559	3.4673	4.1660
52	W 1/4 No.2	Form B	3.4715	3.2846	4.4740
53	W 1/4 No.3	Form B	3.6286	3.2730	3.9282
54	W 1/4 No.4	Form I	3.2701	3.6461	4.1037
55	W 1/4 No.5	Form I	3.3046	3.7165	4.2120
56	W 1/4 No.6	Form I	3.4117	3.5734	4.1061
57	W 1/4 No.7	Form I	3.5231	3.6174	4.0140
58	W 1/4 No.8	Form I	3.1567	3.5735	4.1596
59	W 1/4 No.9	Form I	3.5891	3.9724	4.0227
60	W 1/4 No.10	Form I	3.5977	3.5828	3.9951
61	W 1/5 No.1	Form B	3.7056	3.1947	3.9511
62	W 1/5 No.2	Form B	3.6601	3.2017	4.0693
63	W 1/5 No.3	Form B	3.6832	3.4477	3.9913
64	W 1/5 No.4	Form B	4.1445	3.4951	3.9992
65	W 1/5 No.5	Form B	4.5275	1.8413	4.9522
66	W 1/5 No.6	Form B	4.0938	2.3897	4.0403
67	W 1/5 No.7	Form B	4.2462	2.7765	4.3794
68	W 1/5 No.8	Form B	4.1172	2.8922	4.2562
69	W 1/5 No.9	Form B	3.7774	3.1848	4.3321
70	W 1/5 No.10	Form B	4.0226	2.7246	4.2015

APPENDIX D

Table 21 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine samples mix with ethanol at various ratios

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	E 3/1 No.1	Form I	2.9009	7.0593	3.9339
2	E 3/1 No.2	Form I	2.3648	6.3942	2.7901
3	E 3/1 No.3	Form I	2.5690	6.6031	3.2581
4	E 3/1 No.4	Form I	2.4317	6.5392	3.0289
5	E 3/1 No.5	Form I	2.5806	6.9002	3.6086
6	E 3/1 No.6	Form I	2.3919	6.4289	2.9331
7	E 3/1 No.7	Form I	2.2199	6.4434	3.2295
8	E 3/1 No.8	Form I	2.5115	6.5623	3.3460
9	E 3/1 No.9	Form I	2.3081	6.4560	3.1556
10	E 3/1 No.10	Form I	2.3454	6.2970	2.8639
11	E 2/1 No.1	EtOH	2.5027	6.2148	2.6567
12	E 2/1 No.2	EtOH	2.7588	6.2863	2.2544
13	E 2/1 No.3	EtOH	2.6977	6.0916	2.6328
14	E 2/1 No.4	EtOH	2.8773	6.2891	2.4113
15	E 2/1 No.5	EtOH	2.6524	6.2895	2.6524
16	E 2/1 No.6	EtOH	2.6612	6.2056	2.6612
17	E 2/1 No.7	EtOH	2.6834	5.8923	2.6834
18	E 2/1 No.8	EtOH	2.6612	6.1601	2.6612
19	E 2/1 No.9	EtOH	2.6703	6.0727	2.6703
20	E 2/1 No.10	EtOH	2.5027	6.2148	2.6567
21	E 1/1 No.1	EtOH	2.6531	6.6334	2.7132
22	E 1/1 No.2	EtOH	2.6896	6.2177	2.6150
23	E 1/1 No.3	EtOH	2.7392	6.2237	2.3436
24	E 1/1 No.4	EtOH	2.9735	6.4718	2.8149
25	E 1/1 No.5	EtOH	2.9777	6.3265	2.2850
26	E 1/1 No.6	EtOH	3.3316	6.5231	2.6425
27	E 1/1 No.7	EtOH	2.8665	6.3744	2.4682
28	E 1/1 No.8	EtOH	2.8769	6.1398	2.3888
29	E 1/1 No.9	EtOH	2.8974	6.1689	2.4519
30	E 1/1 No.10	EtOH	2.9726	6.2990	2.4466
31	E 1/2 No.1	EtOH	3.3324	6.6008	2.2913
32	E 1/2 No.2	EtOH	3.0232	6.4168	2.1240
33	E 1/2 No.3	EtOH	2.8793	6.1986	2.3561

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
34	E 1/2 No.4	EtOH	3.0298	6.4476	2.0842
35	E 1/2 No.5	EtOH	3.2879	6.4799	2.3290
36	E 1/2 No.6	EtOH	3.2124	6.4540	2.4213
37	E 1/2 No.7	EtOH	3.2138	6.3792	2.1379
38	E 1/2 No.8	EtOH	3.3244	6.4026	2.3213
39	E 1/2 No.9	EtOH	3.1272	6.3488	2.1900
40	E 1/2 No.10	EtOH	3.1347	6.3387	2.1828
41	E 1/3 No.1	EtOH	3.3218	6.4937	2.4365
42	E 1/3 No.2	EtOH	3.0325	6.4832	2.0216
43	E 1/3 No.3	EtOH	2.9009	6.3744	2.1597
44	E 1/3 No.4	EtOH	3.0090	6.2144	2.2512
45	E 1/3 No.5	EtOH	3.0991	6.5447	2.0794
46	E 1/3 No.6	EtOH	3.3118	6.6285	1.8258
47	E 1/3 No.7	EtOH	3.5462	6.5972	2.1896
48	E 1/3 No.8	EtOH	3.2764	6.4257	2.0921
49	E 1/3 No.9	EtOH	3.5629	6.7413	1.9817
50	E 1/3 No.10	EtOH	3.0810	6.505	2.8310
51	E 1/4 No.1	EtOH	2.9638	6.3281	2.2375
52	E 1/4 No.2	EtOH	2.8822	6.3988	2.2169
53	E 1/4 No.3	EtOH	3.5830	6.7854	2.4852
54	E 1/4 No.4	EtOH	3.0233	6.3684	2.1079
55	E 1/4 No.5	EtOH	2.9337	6.2294	2.1812
56	E 1/4 No.6	EtOH	3.5155	6.4777	2.2605
57	E 1/4 No.7	EtOH	2.8467	6.3211	2.3463
58	E 1/4 No.8	EtOH	3.0138	6.3272	2.2144
59	E 1/4 No.9	EtOH	3.0965	6.4986	2.0481
60	E 1/4 No.10	EtOH	3.2395	6.5062	2.3738
61	E 1/5 No.1	EtOH	3.0904	6.4723	2.7969
62	E 1/5 No.2	EtOH	2.9063	6.2646	2.2424
63	E 1/5 No.3	EtOH	3.0460	6.4869	2.1321
64	E 1/5 No.4	EtOH	2.9888	6.4590	2.1217
65	E 1/5 No.5	EtOH	2.9048	6.3973	2.1890
66	E 1/5 No.6	EtOH	2.9917	6.3981	2.1333
67	E 1/5 No.7	EtOH	2.8694	6.3530	2.1920
68	E 1/5 No.8	EtOH	3.0428	6.4407	2.1908
69	E 1/5 No.9	EtOH	2.8831	6.3350	2.2006
70	E 1/5 No.10	EtOH	2.8825	6.3803	2.2265

APPENDIX E

Table 22 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and one part of anhydrous olanzapine Form I exposes to one part of solvent mixtures at various drug: solvent ratios

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	1WE0/100 No.1	EtOH	3.8423	5.8286	2.5235
2	1WE0/100 No.2	EtOH	4.1806	6.4342	1.6729
3	1WE0/100 No.3	EtOH	3.7154	6.433	1.7516
4	1WE0/100 No.4	EtOH	3.8601	6.3132	1.8245
5	1WE0/100 No.5	EtOH	4.1412	6.3363	1.9558
6	1WE0/100 No.6	EtOH	3.9591	6.1955	2.0238
7	1WE0/100 No.7	EtOH	4.0968	6.3827	1.7695
8	1WE0/100 No.8	EtOH	4.1849	6.5671	1.7107
9	1WE0/100 No.9	EtOH	4.1180	6.2599	2.0462
10	1WE0/100 No.10	EtOH	4.0787	5.8286	2.2846
11	1WE10/90 No.1	EtOH	4.1725	6.298	2.3776
12	1WE10/90 No.2	EtOH	4.1724	6.2399	2.4196
13	1WE10/90 No.3	EtOH	4.2480	6.4507	2.0237
14	1WE10/90 No.4	EtOH	4.2548	6.4441	2.0621
15	1WE10/90 No.5	EtOH	4.3361	6.507	2.0672
16	1WE10/90 No.6	EtOH	4.2987	6.3591	2.0258
17	1WE10/90 No.7	EtOH	4.3652	6.4514	2.0898
18	1WE10/90 No.8	EtOH	4.3785	6.5118	2.0317
19	1WE10/90 No.9	EtOH	4.1935	6.6269	2.1668
20	1WE10/90 No.10	EtOH	4.5212	6.3660	2.1162
21	1WE30/70 No.1	EtOH	4.2639	6.4354	2.0824
22	1WE30/70 No.2	EtOH	3.9991	6.2386	2.3569
23	1WE30/70 No.3	EtOH	4.5416	6.6846	2.3898
24	1WE30/70 No.4	EtOH	4.0568	6.3443	2.1139
25	1WE30/70 No.5	EtOH	3.9367	6.3007	2.2724
26	1WE30/70 No.6	EtOH	4.2274	6.4764	2.1975
27	1WE30/70 No.7	EtOH	4.0305	6.3438	2.3826
28	1WE30/70 No.8	EtOH	4.1296	6.3686	2.3002
29	1WE30/70 No.9	EtOH	4.9847	6.3953	2.2824
30	1WE30/70 No.10	EtOH	3.7774	6.4764	2.2704
31	1WE50/50 No.1	EtOH	4.2716	6.6057	2.6822
32	1WE50/50 No.2	EtOH	3.6367	6.3561	2.2537

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
33	1WE50/50 No.3	EtOH	4.1217	6.4213	2.0687
34	1WE50/50 No.4	EtOH	4.0751	6.3197	1.9635
35	1WE50/50 No.5	EtOH	4.2212	6.6077	2.2881
36	1WE50/50 No.6	EtOH	4.1215	6.4158	2.0719
37	1WE50/50 No.7	EtOH	4.6245	6.6960	3.1831
38	1WE50/50 No.8	EtOH	4.8393	7.0425	3.4708
39	1WE50/50 No.9	EtOH	4.4119	6.7094	2.9725
40	1WE50/50 No.10	EtOH	4.4494	6.8603	3.0542
41	1WE70/30 No.1	EtOH	3.8702	6.2688	3.1631
42	1WE70/30 No.2	EtOH	4.1150	6.3120	2.6184
43	1WE70/30 No.3	EtOH	3.8993	6.5600	2.8319
44	1WE70/30 No.4	EtOH	3.8247	6.3024	2.7225
45	1WE70/30 No.5	EtOH	3.9330	6.2938	2.6305
46	1WE70/30 No.6	EtOH	3.9018	6.2374	2.5537
47	1WE70/30 No.7	EtOH	3.7068	6.3168	2.7118
48	1WE70/30 No.8	EtOH	3.8888	6.2278	2.6373
49	1WE70/30 No.9	EtOH	3.2678	6.1731	2.5239
50	1WE70/30 No.10	EtOH	3.7661	6.2285	2.8404
51	1WE90/10 No.1	EtOH	3.7661	5.7627	3.0916
52	1WE90/10 No.2	Form I	4.3132	5.9984	4.3767
53	1WE90/10 No.3	EtOH	3.9043	5.7667	3.3959
54	1WE90/10 No.4	EtOH	3.4365	5.6338	3.1945
55	1WE90/10 No.5	Form I	2.9846	5.9164	3.2561
56	1WE90/10 No.6	EtOH	3.8437	5.6405	3.6137
57	1WE90/10 No.7	EtOH	3.9647	5.5742	3.4748
58	1WE90/10 No.8	EtOH	3.5989	5.6842	3.2086
59	1WE90/10 No.9	EtOH	2.9209	5.9168	3.2411
60	1WE90/10 No.10	Form I	2.8171	6.0977	4.0804
61	1WE100/0 No.1	Form I	2.2443	6.7272	3.7833
62	1WE100/0 No.2	Form I	2.4706	6.5133	3.2998
63	1WE100/0 No.3	Form I	1.9703	6.9557	3.9858
64	1WE100/0 No.4	Form I	2.2111	6.6363	3.7015
65	1WE100/0 No.5	Form I	2.1720	6.7131	3.7536
66	1WE100/0 No.6	Form I	2.5257	6.3489	3.2524
67	1WE100/0 No.7	Form I	2.0079	6.9646	4.0222
68	1WE100/0 No.8	Form I	2.4724	6.672	3.5273
69	1WE100/0 No.9	Form I	2.0765	6.7342	3.7318
70	1WE100/0 No.10	Form I	1.8053	6.8577	3.8992

APPENDIX F

Table 23 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and one part of anhydrous olanzapine Form I exposes to five parts of solvent mixtures at various drug: solvent ratios

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	5WE0/100 No.1	EtOH	6.8098	2.8267	1.3538
2	5WE0/100 No.2	EtOH	7.0391	2.7707	1.3567
3	5WE0/100 No.3	EtOH	6.8447	2.6902	1.8332
4	5WE0/100 No.4	EtOH	6.8621	2.7523	1.5577
5	5WE0/100 No.5	EtOH	6.9059	2.7818	1.4086
6	5WE0/100 No.6	EtOH	6.6921	2.6712	1.5028
7	5WE0/100 No.7	EtOH	6.8191	2.6839	1.8247
8	5WE0/100 No.8	EtOH	6.9642	2.6648	1.6784
9	5WE0/100 No.9	EtOH	6.8412	2.7250	1.8495
10	5WE0/100 No.10	EtOH	6.9204	2.6858	1.5112
11	5WE10/90 No.1	EtOH	6.6001	2.9004	1.9233
12	5WE10/90 No.2	EtOH	6.6591	2.9112	1.5361
13	5WE10/90 No.3	EtOH	6.5455	3.1339	1.8125
14	5WE10/90 No.4	EtOH	6.6585	3.1297	1.8483
15	5WE10/90 No.5	EtOH	7.0566	3.1656	1.5287
16	5WE10/90 No.6	EtOH	6.8155	2.9479	1.5954
17	5WE10/90 No.7	EtOH	6.6518	3.2458	1.1922
18	5WE10/90 No.8	EtOH	6.8369	3.0488	1.5123
19	5WE10/90 No.9	EtOH	6.7384	3.0372	1.4882
20	5WE10/90 No.10	Form B	6.7202	2.1887	2.8671
21	5WE30/70 No.1	EtOH	6.7822	2.8672	1.8063
22	5WE30/70 No.2	EtOH	6.7339	2.8809	1.9172
23	5WE30/70 No.3	EtOH	6.7352	3.1196	1.7470
24	5WE30/70 No.4	EtOH	6.7206	2.8680	2.0631
25	5WE30/70 No.5	EtOH	6.6100	3.0960	2.0566
26	5WE30/70 No.6	EtOH	6.8089	2.9715	1.9758
27	5WE30/70 No.7	EtOH	6.6204	3.2594	2.1596
28	5WE30/70 No.8	EtOH	6.7752	3.2034	2.2009
29	5WE30/70 No.9	EtOH	6.8628	2.8489	2.0164
30	5WE30/70 No.10	EtOH	6.5594	3.0193	1.9191
31	5WE50/50 No.1	EtOH	6.7610	3.2103	2.1742
32	5WE50/50 No.2	EtOH	6.6702	3.0501	2.1298

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
33	5WE50/50 No.3	EtOH	6.6802	2.8238	1.8681
34	5WE50/50 No.4	EtOH	6.6860	2.7128	1.7360
35	5WE50/50 No.5	EtOH	6.7475	3.0892	1.9129
36	5WE50/50 No.6	EtOH	6.5942	2.9035	1.9498
37	5WE50/50 No.7	EtOH	6.7175	3.5021	1.7837
38	5WE50/50 No.8	EtOH	6.7246	3.2987	2.1821
39	5WE50/50 No.9	EtOH	6.8795	3.2223	1.9840
40	5WE50/50 No.10	EtOH	6.8089	3.0143	1.7553
41	5WE70/30 No.1	EtOH	6.7179	2.6574	1.8816
42	5WE70/30 No.2	EtOH	6.7002	3.0656	1.3130
43	5WE70/30 No.3	EtOH	6.8705	3.3859	1.4358
44	5WE70/30 No.4	EtOH	6.7235	2.7755	1.7489
45	5WE70/30 No.5	EtOH	6.6787	2.8480	1.4635
46	5WE70/30 No.6	EtOH	6.7878	2.7731	1.6927
47	5WE70/30 No.7	EtOH	6.7191	2.7538	1.8856
48	5WE70/30 No.8	EtOH	6.6493	2.7885	1.6825
49	5WE70/30 No.9	EtOH	6.7315	2.8767	1.5127
50	5WE70/30 No.10	EtOH	6.7521	2.7124	1.8218
51	5WE90/10 No.1	EtOH	6.7501	2.9240	1.8685
52	5WE90/10 No.2	EtOH	6.6451	2.9487	1.5151
53	5WE90/10 No.3	EtOH	6.6723	2.8042	1.6003
54	5WE90/10 No.4	EtOH	6.8136	2.7755	1.4316
55	5WE90/10 No.5	EtOH	6.6940	3.8902	2.5367
56	5WE90/10 No.6	EtOH	6.6670	2.8894	1.9350
57	5WE90/10 No.7	EtOH	6.7129	2.9643	1.7512
58	5WE90/10 No.8	EtOH	6.8939	3.2759	1.9221
59	5WE90/10 No.9	EtOH	6.8555	3.0352	1.8108
60	5WE90/10 No.10	EtOH	6.5168	3.1017	1.4724
61	5WE100/0 No.1	Form B	7.7874	1.0919	3.9774
62	5WE100/0 No.2	Form B	7.8489	1.0342	4.0058
63	5WE100/0 No.3	Form B	7.1743	1.2107	2.9996
64	5WE100/0 No.4	Form B	7.0420	1.2710	2.6623
65	5WE100/0 No.5	Form B	7.2928	0.7825	3.0337
66	5WE100/0 No.6	Form B	7.5041	0.5867	3.4494
67	5WE100/0 No.7	Form B	7.8674	1.4584	3.9385
68	5WE100/0 No.8	Form B	6.9754	1.4891	2.6063
69	5WE100/0 No.9	Form B	7.4013	1.0503	3.1774
70	5WE100/0 No.10	Form B	7.7874	1.0919	3.9774

APPENDIX G

Table 24 Mahalanobis distances between reference olanzapine Form I, Form B, EtOH-H$_2$O mixed solvate and olanzapine tablets coat with film-coating dispersion consist of water (COAT100/0) or ethanol (COAT0/100)

Index	Spectrum Title	Next Class	Distance to Form I	Distance to Form B	Distance to EtOH-H$_2$O mixed solvate
1	COAT 100/0 No.1	Form I	2.0176	4.2187	3.0151
2	COAT 100/0 No.2	Form I	2.2171	4.2269	2.9404
3	COAT 100/0 No.3	Form I	2.7490	4.8403	3.6765
4	COAT 100/0 No.4	Form I	2.3106	4.6916	3.6418
5	COAT 100/0 No.5	Form I	2.3851	4.5048	3.3414
6	COAT 100/0 No.6	Form I	3.2345	4.4937	3.4759
7	COAT 100/0 No.7	Form I	2.5840	4.7121	3.4147
8	COAT 100/0 No.8	Form I	2.8031	4.6362	3.3265
9	COAT 100/0 No.9	Form I	2.8506	4.2540	2.8968
10	COAT 100/0 No.10	Form I	2.7561	4.4199	3.2677
11	COAT 0/100 No.1	Form I	2.3348	4.3181	2.9732
12	COAT 0/100 No.2	EtOH	2.8226	4.187	2.6140
13	COAT 0/100 No.3	Form I	2.3899	4.646	3.1880
14	COAT 0/100 No.4	Form I	2.6710	4.665	3.2937
15	COAT 0/100 No.5	Form I	2.7110	4.5439	3.3337
16	COAT 0/100 No.6	Form I	3.0621	4.7846	3.4848
17	COAT 0/100 No.7	Form I	2.6847	4.2305	2.8899
18	COAT 0/100 No.8	Form I	2.5326	4.6449	3.4148
19	COAT 0/100 No.9	Form I	2.9539	4.4351	2.9587
20	COAT 0/100 No.10	Form I	2.9147	4.6403	3.2749

VITA

Intira Rangubpis was born in Bangkok, Thailand, on July 30th 1985. I received Bachelor of Science in Pharmacy degree in 2010 from the Faculty of Pharmacy, Mahidol University, Thailand. I presented a poster titled "Effect of solvents on the solid-state transformation of olanzapine polymorph" in The 1st International Conference on Pharmacy Education and Research Network of ASEAN (ASEAN PharmNET I) on December 2-4, 2015 at The Landmark Bangkok, Thailand.