การพัฒนาสูตรตำรับยาผงผสมเพื่อเตรียมยาน้ำใสสำหรับรับประทาน ของคีโตโคนาโซลซึ่งเตรียมโดยการพ่นแห้งร่วมกับ ไฮดรอกซีโพรพิลเบตาไซโคลเดกซ์ทริน

นายนีรนาท โพธิยานนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชกรรม ภาควิชาเภสัชกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2550

FORMULATION OF DRY MIXTURES FOR ORAL SOLUTION OF KETOCONAZOLE PREPARED BY CO-SPRAY DRYING WITH HYDROXYPROPYL-BETA-CYCLODEXTRINS

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A Thesis Submitted Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Pharmaceutics
Department of Pharmacy
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2007

Thesis Title	**FORMULATION OF DRY MIXTURES FOR ORAL SOLUTION OF KETOCONAZOLE PREPARED BY CO-SPRAY DRYING WITH HYDROXYPROPYL-BETA-CYCLODEXTRINS**
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นีรนาท โพธิยานนท์: การพัฒนาสูตรตำรับยาผงผสม เพื่อเตรียมยาน้ำใส สำหรับรับประทาน ของคีโตโคนาโซล ซึ่ง เตรียมโดยการพ่นแห้ง ร่วมกับ ไฮดรอกซีโพรพิล เบตา ไซโคลเดกซ์ทริน. (FORMULATION OF DRY MIXTURES FOR ORAL SOLUTION OF KETOCONAZOLE PREPARED BY CO - SPRAY DRYING WITH HYDROXYPROPYL–BETA–CYCLODEXTRINS) อ. ที่ปรึกษา: รศ.ดร.สุชาดา ชุติมาวรพันธ์ 138 หน้า.

การพัฒนาสูตรตำรับคีโตโคนาโซลในรูปสารละลาย สำหรับเตรียมเป็นยารับประทาน โดยการใช้ไฮดรอกซีโพรพิลเบตาไซโคลเดกซ์ทริน (เอชพีบีซีดี) เป็นสารเพิ่มการละลายและเพิ่มความคงตัวพบว่า การละลายของคีโตโคนาโซลในสารละลายบัฟเฟอร์ที่พีเอช 5.0 เพิ่มขึ้นเป็นเชิงเส้นตรงกับความเข้มข้นของเอชพีบีซีดี ซึ่งจัดเป็นแผนภูมิเฟสชนิด A_L ค่าคงที่ความคงตัวของสารประกอบเชิงซ้อนอินคลูชันของคีโตโคนาโซล: เอชพีบีซีดีมีค่าเท่ากับ 714.76 โมล$^{-1}$ จากเฟสการละลายสามารถเตรียมสารละลายคีโตโคนาโซลความเข้มข้น 2% โดยน้ำหนักต่อปริมาตรได้ จากนั้นเตรียมผงแห้งโดยการพ่นแห้ง ศึกษาสภาวะที่เหมาะสมสำหรับการพ่นแห้งได้โดยใช้เทคนิกออพติไมเซชันโดยใช้โปรแกรมดีไซน์เอ็กซ์เปอร์ต รุ่น 7.1.3 โดยทำการศึกษา 2 ปัจจัยได้แก่อุณหภูมิลมเข้าและอัตราเร็วการป้อนสารซึ่งแต่ละปัจจัยจะทำการศึกษา 3 ระดับ จากผลการศึกษาที่ได้พบว่า สภาวะที่เหมาะสมสำหรับการพ่นแห้งคือ อุณหภูมิ120°ซ และ อัตราเร็วการป้อนสาร 3.5 มิลลิลิตร/นาที สูตรตำรับยาผงผสมเพื่อเตรียมยาน้ำใสสำหรับรับประทานได้เตรียมขึ้น โดยมีส่วนประกอบคือ ยาผงพ่นแห้งคีโตโคนาโซล แอสพาร์เทม แซคคารินโซเดียม และแซนแทนกัม ลักษณะของเพาว์เดอร์เอกซ์เรย์ดิฟแฟรกโตแกรม และดิฟเฟอเรนเชียลสแกนนิงแคลอริเมทรีเทอร์โมแกรม ของผลิตภัณฑ์สเปรย์ดรายของคีโตโคนาโซลร่วมกับเอชพีบีซีดี แสดงให้เห็นว่าคีโตโคนาโซลอาจอยู่ในรูปอสัณฐานหรือเกิดสารประกอบเชิงซ้อน อินคลูชัน ระหว่างคีโตโคนาโซลกับเอชพีบีซีดี และศึกษาความคงตัวแบบเร่งของยาผงผสมเพื่อเตรียมยาน้ำใสของ คีโตโคนาโซลที่40°ซ ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ และที่ 30 °ซ ของสารละลาย คีโตโคนาโซลที่ 40°ซ ความชื้นสัมพัทธ์ 75 เปอร์เซ็นต์ และที่ 5 และ 30 °ซ เป็นเวลา 90 วัน วิเคราะห์ปริมาณคีโตโคนาโซลที่เหลือโดยใช้ใช้ไฮเปอร์เพอร์ฟอร์มานซ์ลิควิดโครมาโตกราฟี พบว่ามียาคงเหลืออยู่ไม่น้อยกว่า 90%

ภาควิชา เภสัชกรรม ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชกรรม ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2550

4876576633 : MAJOR PHARMACEUTICS
KEY WORD: KETOCONAZOLE / HPBCD / SPRAY DRYING / DRY MIXTURES FOR ORAL SOLUTION

NEERANART POTIYANON: FORMULATION OF DRY MIXTURES FOR ORAL SOLUTION OF KETOCONAZOLE PREPARED BY CO-SPRAY DRYING WITH HYDROXYPROPYL-BETA-CYCLODEXTRINS. THESIS ADVISOR: ASSOC. PROF. SUCHADA CHUTIMAWORAPAN, Ph.D., 138 pp.

The formulation of ketoconazole as aqueous solutions for oral administration was investigated by using hydroxyproply-β-cyclodextrins (HPBCD) as a solubilizer and stabilizer. From the phase solubility study, the solubility of ketoconazole in buffer solutions of pH 5.0 increased linearly as a function of HPBCD concentrations as type A_L phase diagram. The stability constant of inclusion complexes between ketoconazole and HPBCD was 714.76M^{-1}. From the phase diagram the 2% w/v ketoconazole solution could prepared. Then dry powder was prepared by spray drying technique. To select the optimal condition for spray drying ketoconazole: solution, the optimization technique, by design expert version 7.1.3, was used and studied for 2 factors, inlet temperature and feed rate, with three levels for each. The results revealed that the optimal condition for this study is 120°C for inlet temperature and 3.5 ml/min. for feed rate. Dry mixtures for oral administration were formulated with ketoconazole spray dried powders, aspartame, saccharin sodium and xanthan gum. The x-ray diffractogram and differential scanning calorimetric thermogram of spray dried powder might be due to the existence in an amorphous state or the formation of inclusion complexes between ketoconazole with HPBCD. Accelerated stability study of ketoconazole dry mixtures for oral solution were operated at 40°C 75% RH and room temperature 30°C and ketoconazole reconstituted solutions at 40°C, 75% RH, refrigerated temperature (2-8°C) and room temperature were determined for 90 days. For comparison, ketoconazole suspension USP 29, was prepared and studied the stability. The remaining ketoconazole was analyzed by high performance liquid chromatography. Ketoconazole reconstituted solution and ketoconazole dry mixtures for oral solution had ketoconazole remained not less than 90%.

Department : Pharmacy Student's Signature: .....
Field of Study : Pharmaceutics Advisor's Signature: .....
Academic Year : 2007

ACKNOWLEDGEMENTS

This thesis has been succeeded with the great supports from several people. I shall remind in great kindness for their helps, supports and advices.

First of all, I would like to express my gratitude for the invaluable advice, guidance and enthusiastic encouragement throughout my research study to my advisor, Associate Professor Suchada Chutimaworapan, Ph.D. Her understanding, kindness and patience are honestly appreciated.

I would like to express my great gratitude to Associate Professor Parkpoom Tengamnuay, Chairman of my thesis examination committee, as well as other committee members. I would like to express deep appreciation and grateful thanks for their valuable suggestion and kindness.

My gratitude are expressed to M&H Manufacturing Co., Ltd. for contribution of ketoconazole and Silom Medical Co., Ltd. for donation of cetylpyridinium chloride used in this study. My gratefulness is given to Faculty of Pharmaceutical sciences, Chulalongkorn University for research fund that supports my thesis work.

Sincere thanks are also given to all staff members of the Department of Pharmacy for their assistance and great helpful support and other people whose names have not been mentioned here.

Ultimately, I would like to express my sincere and deepest gratitude to my family for their endless love, understanding and encouragement throughout this study.

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LISTS OF ABBREVIATIONS

ANOVA	=	analysis of variance
°C	=	degree celsius
CCD	=	Central Composite Design
CDs	=	Cyclodextrins
cps	=	centipoise
CV	=	coefficient of variation
df	=	degree of freedom
DS	=	degree of substituted
DSC	=	differentials scanning calorimetry
et al.	=	*et alii*, 'and others'
g	=	gram
hr	=	hour
HPLC	=	high performance liquid chromatography
Ks	=	equilibrium constant
M	=	Molar
mg	=	milligram
min	=	minute
ml	=	milliliter
mm	=	millimeter
MW	=	molecular weight
pH	=	the negative logarithm of the hydrogen ion concentration
R^2	=	coefficient of determination
RH	=	relative humidity
SD	=	standard deviation
µg	=	microgram
µm	=	micrometer
USP/NF	=	The United States Pharmacopoeia/National Formulary
UV	=	ultraviolet
w/v	=	weight by volume
w/w	=	weight by weight

CHAPTER I

INTRODUCTION

Candida yeasts are usually present in most people, principally kept in check by other naturally microorganisms and by the human immune system. Specifically, colonization carriage rates of Candida species are higher in the incidence of cancer patients who use chemotherapy, transplantation patients, patients who use some medicine such as steroids or some antibiotics and HIV-infected patients (Vazquez, 2000). In these patients, the rate of carriage is depended on the level of immunosuppression. In the HIV patients Candida species, especially *Candida albicans,* can cause oropharyngeal candidiasis (thrush) (อมรศรี, วาณี และ ศรีศุภลักษณ์, 2549). Thrush occurs in about 90% of patients with AIDS, making it the most frequent opportunistic infection in AIDS (Vazquez, 2000). Most patients will respond to topical therapy with azoles (clotrimazole troches) or oral polyenes (nystatin or amphotericin suspension). But topical treatment has many disadvantages such as high relapse rate, unpleasant taste, and raduced effectiveness in advanced stages of HIV-disease (Koks et al., 2002). Thrush can be treated by using some oral formulation medicine such as azoles group.

Ketoconazole, an imidazole antifungal agent, as with all azole antifungal agents, works principally by inhibition of an enzyme, cytochrome P450 14-alpha-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol. It may act by interference with the synthesis of ergosterol and therefore alter the permeability of the cell membrane of fungi. Ketoconazole is a broad spectrum antifungal, which is administered by topically or orally. The drug is classified to class 2 (BCS classification), that can be absorbed easily but poor solubility in water. Ketoconazole is weak base (pKa 6.95) that can be soluble in acidic pH, so it should not be take with antacid drug or food that can alter gastric acidity because of its bioavailability. In HIV-infected patients, especially children or geriatric, taking ketoconazole orally in tablet dosage form is very difficult.

So, crushing tablet into small particles and make them to be suspension is easier to be swallowed than tablet. However, suspensions must be shaken before use that may lead to patient compliance problem and may make the plasma concentration fluctuated (Kumer et al., 1991). To solve this problem, dosage form of solutions is very attractive. But, ketoconazole is slightly soluble in water thus using solubilizing enhancers is needed. Cyclodextrins (CDs) are one of promising solubility enhancers that are widely used in the pharmaceutical field.

CDs are cyclic oligosaccharides with hydrophobic cavity and hydrophilic outer surface. In aqueous medium, CDs are able to form inclusion-complexes with many lipophilic drugs (guest molecules) in a dynamic equilibrium with free drug molecules. CDs containing of glucopyranose unit by α-(1,4) glucosidic linkage, the three natural CDs, α-,β-, and γ-CDs consist of 6, 7 and 8 glucose units respectively, diverge in hydrophobicity cavity size and solubility property (Challa et al., 2005). To improve solubility property of CDs, many CDs derivatives are synthesis by adding substituted groups. Among CDs derivatives, hydroxypropyl-β-cyclodextrins (HPBCD) is one of the most widely use because of their solubility and low toxicity besides it can be safely used in several routes (Taraszewska and Kozbial, 2005). Drug-CDs inclusion complexes can improve physicochemical properties of guest molecules, the most commons are to enhance solubility, stability and bioavaility of guest molecules (Taraszewska and Kozbial, 2005).

Inclusion complexes can be established by many methods such as physical mixing, kneading and spray-drying method (Esclusa-Diaz et al., 1996). But spray-drying method can make complete inclusion complexes besides spray-drying can be used with thermolabile drugs because in this method drug molecules will contact high temperature in a very short period of time (Bayram, Bayram and Tekin, 2005; Mu et al., 2005). Liquid dosage forms always consist of water, so this is suitable for the growth of microorganisms and may need to add some preservatives for the formulations. (Allen, 2004) So, to formulate the drug in form of powder for reconstitution is very attractive because of its properties. Powder for reconstitution is a kind of the solid dosage forms, so it can provide longer shelf-life of drug than liquid

dosage forms and convenient for storage including transportation. Furthermore this preparation can be turned to liquid dosage form by adding purified water, so it still has good bioavailability of drug as liquid preparation.

This study is focused on development of ketoconazole dry mixtures for oral solution by spray drying with HPBCD as solubilizing enhancer.

The objectives of this study were as follows:

1. To optimize the condition of co-spray drying of ketoconazole with hydroxypropyl- β-cyclodextrin.
2. To characterize spray-dried powder of ketoconazole with hydroxypropyl-β-cyclodextrin.
3. To formulate dry mixtures for oral solution of ketoconazole.
4. To study the physical and chemical stabilities of spray-dried powder at room temperature and accelerated condition.
5. To study the physical and chemical stabilities of reconstituted solution of spray dried powder at refrigerated temperature, room temperature and accelerated condition.

CHAPTER II

LITERATURE REVIEWS

A. KETOCONAZOLE

1. Physicochemical Properties of Ketoconazole

1.1 Chemical name

The chemical name of ketoconazole is cis-1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyl]phenyl] piperazine (USP 24, 2000). Its structure is shown in Figure 1.

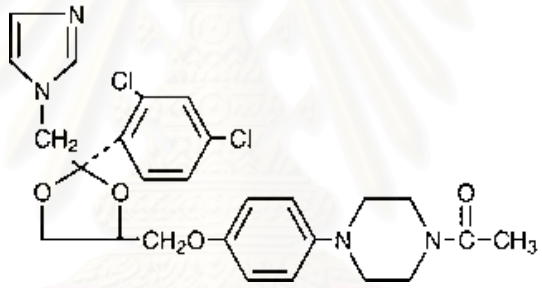


Figure 1. Chemical structure of ketoconazole (USP 24, 2000)

1.2 Chemical formula

The chemical formula of ketoconazole is $C_{26}H_{28}Cl_2N_4O_4$ (USP 29, 2006)

1.3 Molecular weight

The molecular weight of ketoconazole is 531. 43 g/mole (USP 29, 2006)

1.4 Description

Ketoconazole is a white or almost white powder (Reynolds, 2005).

1.5 Solubility

Practically insoluble in water, sparingly soluble in alcohol, freely soluble in dichloromethane, soluble in methyl alcohol (Reynolds, 2005).

1.6 Dissociation constant

Ketoconazole is a weak dibasic compound, which has pKas of 2.94 and 6.51 (Skiba et al., 2000)

1.7 Melting point

The melting point of ketoconazole is between 148-152 °C (USP 24, 2000)

1.8 Specific rotation

Ketoconazole has specific rotation between -1° and +1° (*t*=20°)**;** 40 mg/ml in methanol (USP29, 2006)

2. Synthesis of Ketoconazole (Heeres et al., 1979)

The synthesis of ketoconazole is started from 2, 4-dichloroacetaphenone and outlined in Figure 2. Ketalization of 1 with glycerine was performed in a benzene-1- butanol medium with azeotropic removal of water in the presence of a catalytic amount of *p*-toluenesulfonic acid. Without isolation, the ketal 2 was brominated at 30°C to bromo ketal 3. Benzoylation of 3 in pyridine afforded the ester as a cis/trans mixture, from which the cis form 4 could be isolated by crystallization from ethanol. The pure trans isomer could be obtained by liquid chromatography of the mother liquor. Coupling of bromo ketal 4 in dry DMA with imidazole gave the imidazole derivative 5. The ester 5 was saponified at reflux with NaOH in dioxane-water medium to the alcohol 6. This alcohol was converted to methanesulfonate 7, which was coupled with the sodium salt of 8 to give ketoconazole I.

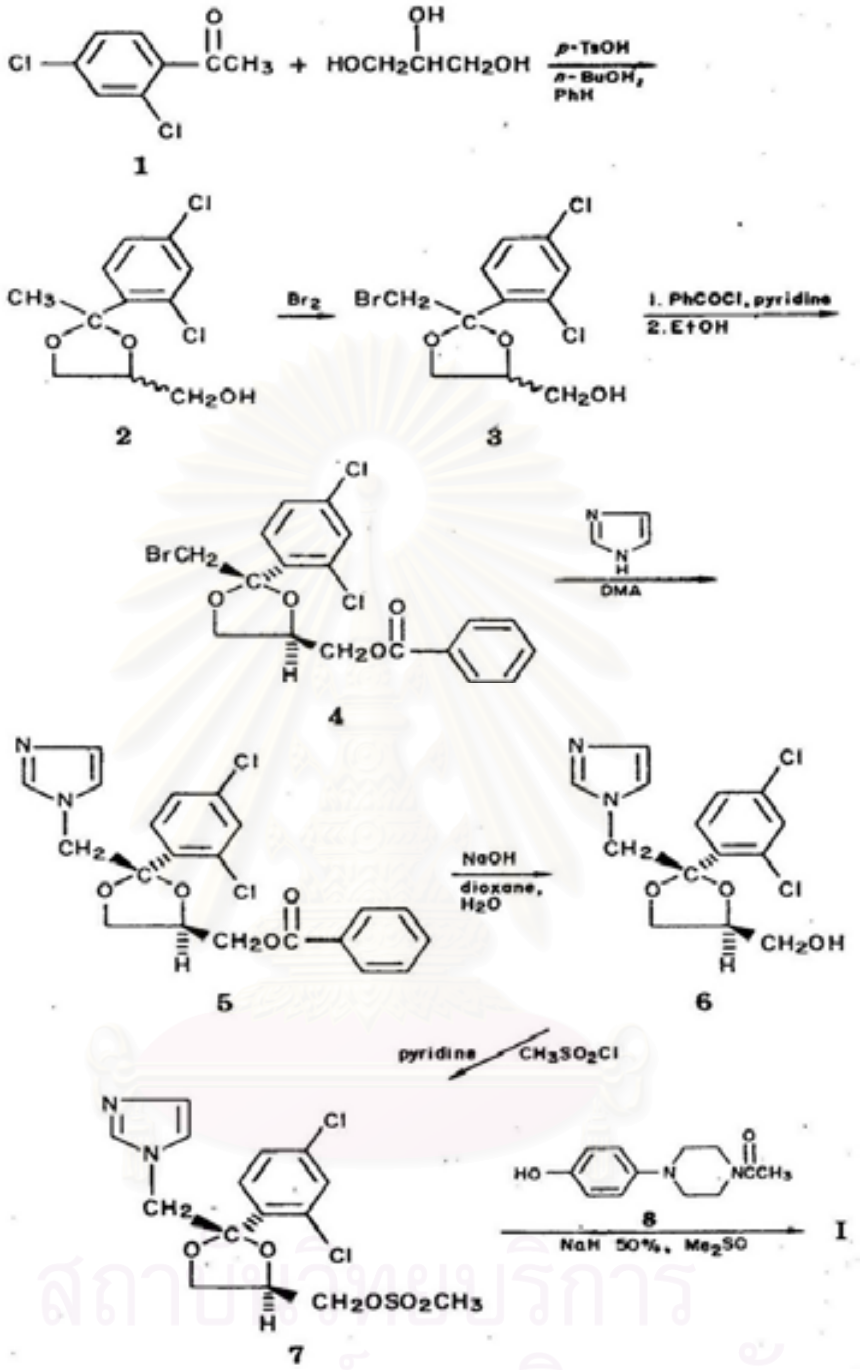


Figure 2. The synthesis pathway of ketoconazole (Heeres et al., 1979)

3. Method of Analysis of Ketoconazole

There are many methods have been used to analyze ketoconazole in pharmaceutical preparations as well as raw material.

3.1 High performance liquid chromatography (HPLC) method

There are many different methods of extraction, column types, type of mobile phase, type of detectors, detection wavelengths, types of internal standard and conditions used for each HPLC method.

3.2 Ultraviolet (UV) spectrophotometric method

Ketoconazole can be detected by Ultraviolet Spectrum in different conditions, in aqueous acidic 269 nm, aqueous alkali 287 nm, methanol 244 nm for example.

4. Pharmacology of Ketoconazole

Ketoconazole, a synthetic imidazole derivative is an azole antifungal agent. The drug was discovered in 1976 and first marketed in the United Kingdom in March 1981 by Janssen Pharmaceutical Ltd., under the trade name of Nizoral and subsequently in other countries (Koch, 1983).

4.1 Antimicrobial activity

Ketoconazole is an imidazole antifungal which has a wide spectrum of antimicrobial activity including activity against *Blastomyces dermatitidis, Candida spp., Coccidioides immitis, Epidermophyton floccosum, Histoplasma capsulatum, Malassezia spp., Microsporum canis, Paracoccidioides brasisiensis, Trichophyton mentagrophytes* and *T. rubrum*. Some strains of Aspergillus spp., *Cryptococcus neoformans*, and *Sporothrix schenckii* are sensitive. Ketoconazole has activity against some Gram-positive bacteria and some antiprotozoal activity against Leishmania spp.

4.2 Mechanism of action

Ketoconazole is structurally similar to imidazole, usually fungistatic in action, but may be fungicidal at high concentration and interferes with the fungal synthesis of ergosterol, a constituent of cell membranes, as well as certain enzymes. It is specific for fungi, as mammalian cell membranes contain no ergosterol.

As with all azole antifungal agents, ketoconazole works principally by inhibition of an enzyme, cytochrome P450 14-alpha-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol.

5. Pharmacokinetic of Ketoconazole

5.1 Absorption

Ketoconazole is classified to class II (BCS classification), so it is well absorbed from gastrointestinal tract but poor solubility in water. Absorption of ketoconazole is variable depends on the pH of the gastric content in stomach. Following oral administration, ketoconazole is dissolved in gastric secretions and turned to the hydrochloride salt before absorption from the stomach.

The bioavailability of ketoconazole depends on the pH of the gastric secretions in the stomach, increasing in the pH results in decreased absorption of the drug. Decreased bioavailability of ketoconazole has been occurred in patients with gastric hypochlorhydria. In contrast, administration of an acidic beverage may increase bioavailability of oral ketoconazole in patients with achlorhydria. Accompanying administration of drugs which can increase gastric Ph may decrease absorption of ketoconazole.

5.2 Distribution

Ketoconazole has been detected in urine, bile, saliva, sebum, cerumen, synovial fluid and celebrospinal fluid after oral administration. In human, ketoconazole is not known if the drug crosses the placenta. However, the drug crosses the placenta in rats. Ketoconazole distributes into the milk of dogs and probably distributes into human milk. Ketoconazole is 84-99% bound to plasma proteins, primarily albumin.

5.3 Elimination

Plasma concentrations of ketoconazole seem to decline with a half-life of approximately 2 hours in the initial phase and approximately 8 hours in the

terminal phase. Ketoconazole is partially metabolized, in the liver, to several inactive metabolites by oxidation and degradation of the imidazole and piperazine rings, by oxidative *O*-dealkylation, and by aromatic hydroxylation. The major route of elimination of ketoconazole and its metabolites appears to be excreted into the feces via the bile. Ketoconazole was found to excrete in adults with renal function, approximately 57% of a single 200 mg oral dose was excreted in feces within 4 days; 20-65% of this was unchanged drug, and approximately 13% of the dose was excreted in urine within 4 days; 2-4% of this was unchanged drug.

6. Adverse Reactions of Ketoconazole

The major side effects of ketoconazole in oral dosage form are gastrointestinal disturbances. Nausea and vomiting have been reported in about 3% of patients, abdominal pain about 1% and these adverse effects are dose-related and may be reduced by taking with food. Moreover, hepatitis has been reported and the risk resemble to increase when treatment with ketoconazole is continued for longer than 2 weeks and it can reversible when discontinuation of ketoconazole.

Other adverse effects include allergic reactions such as urticaria and angioedema, and rare cases of anaphylaxis have been reported. Pruritus, rash, alopecia, headache and dizziness may also occur. After topical administration of ketoconazole, irritation, dermatitis or burning sensation has occurred.

7. Drug Interaction of Ketoconazole

Use of drug that alters gastric acidity, such as antimuscarinics, antacids, histamine H_2-antagonists and proton pump inhibitors, may reduce the absorption of ketoconazole. Taking of ketoconazole with enzyme-inducing drugs such as rifampicin, isoniazid, or phenytoin may decrease plasma concentration of ketoconazole, on the other hand, plasma concentrations of isoniazid and rifampicin may be reduced by ketoconazole.

Ketoconazole inhibits certain hepatic oxidase enzymes, especially the cytochrome P450 isoenzyme CYP3A4, so it may increase the effect and the toxicity or decrease the effects of some hepatic metabolized drugs. There is a risk of cardiac arrhythmias if ketoconazole is used with astemizole, cisapride, pimozide, quinidine, or terfenadine, so combination use should be avoided.

8. Dosage and Administration of Ketoconazole

Oral ketoconazole is usually taken at a dose 200 mg once daily for treatment and prophylaxis of fungal infections. This usage may be increased to 400 or 600 mg daily if an adequate response is not obtained. Children may be given approximately 3 mg/kg daily, or 50 mg for those aged 1-4 years and 100 mg for children aged 5-12 years. The duration of treatment should be continued for 14 days and for at least one week after symptoms have been clear and mycologic test has become negative. A dose 400 mg once daily for 5 days is used for the treatment of chronic vaginal candidiasis.

9. Dosage Form of Ketoconazole

Ketoconazole is available in many dosage forms, 200 mg tablet, 2% topical cream and 2% shampoo. In particular case, for children or geriatric oral use, the tablet are often crushed to small particles or fine powder and mixed with suitable vehicle to form suspensions.

10. Stability Studies of Ketoconazole

Kumer et al. (1991) examined the stability of ketoconazole in ethanolic solution. The ethanolic solution of ketoconazole, concentration of 50 ng/20 µl and 100 ng/20 µl, were placed in clear amber glass bottles and stored at room temperature and 8°c for 29 days. The results showed no significant difference between test solutions, it was concluded that ketoconazole in ethanolic solution exhibited good stability.

The photodegradation of ketoconazole was studied by Nuntanakorn (1996). The photolysis of ketoconazole in methanol, acetone, ethylacetate and chloroform was examined the process was performed by dissolving ketoconazole in four organic solvents and irradiated with UV lamp (254 nm). It was founded that the degradation products occurred when using methanol as solvent and irradiated with UV radiation for 15 hours obtained better yields than acetone, ethylacetate and chloroform in the same condition. The photodegradation products of ketoconazole is 1-acetyl-4-[4-[(1H-imidazo[2,1-a]3,4-dihydro-7-chloro-isoquinolyl)-6-spiro-2'-(1,3-dioxan-4-yl)] methoxy] piperazine and the photolytic reaction mechanism is demonstrated in Figure 2. In addition, from the study of stability of ketoconazole solution in various pH values between 1-9. It was found that the major degradation pathway of ketoconazole solution was specific acid catalysis and the result showed that the drug was least stable in pH 1 among the pH values studied. Though, at acidic pH, ketoconazole is found unstable but its solubility is very well at this pH. Furthermore, from this study it was found that ketoconazole is stable start from pH 5 to pH 9 and there was study about thermal stability of ketoconazole between temperature 25 and 50 °C. It revealed that ketoconazole was stable at 25°C but decreased significantly at 50°C. (Skiba et al., 2000)

B. CYCLODEXTRINS

Cyclodextrins (CDs) are cyclicoligosaccharides containing at least 6 D-glucopyranose units attached by α-1,4 glucosidic linkages. CDs are produced from enzymatic hydrolysis of starch by enzyme cyclodextringlucosetransferases (CGTs) from some microorganisms (Loftsson and Masson, 2001). CDs are cone-shaped with lipophilic cavities and hydrophilic outer surfaces.

CDs as we known today, were called "cellulosine" when first discovered by Villiers in 1891. Afterthat, Schardinger characterized the three natural cyclodextrins (α-, β-and γ-CDs), these compounds were referred to "Schardinger sugars". Since 1911 to 1935, Pringsheim, in Germany, was the outstanding researcher in this field, describing that CDs were capable to form inclusion complexes with a wide variety of

guest molecules. In the middle of 1970's, each of the natural CDs had been structurally and chemically characterized and many complexes had been studied.

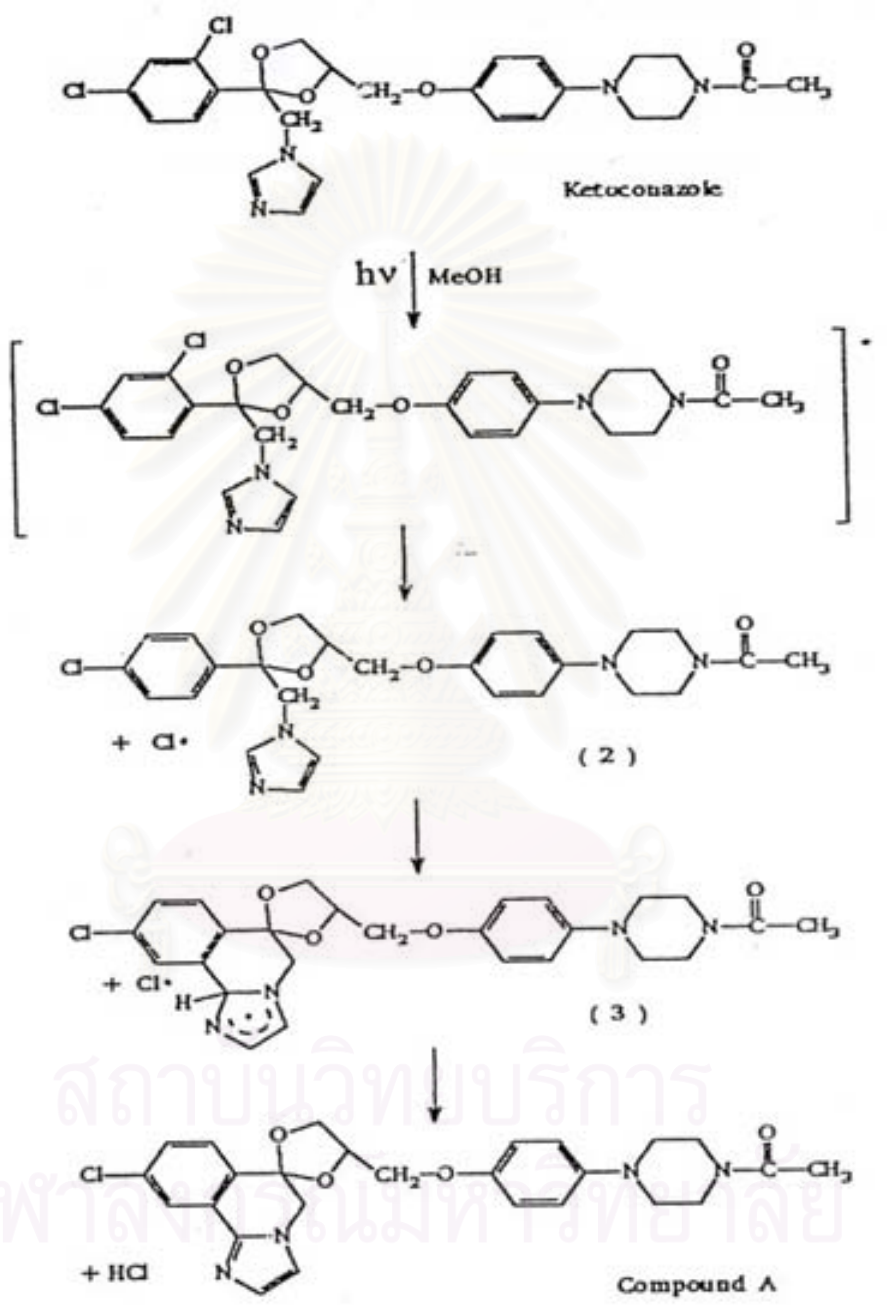


Figure 3. The photodegradation of ketoconazole in methanol (Nuntanakorn, 1996).

1. Structure and Physicochemical Properties of Cyclodextrins

1.1 Natural cyclodextrins

The three naturally occurring α-CDs (cyclohexaamylose), β-CDs (cycloheptaamylose) and γ-CDs (cyclooctaamylose) are consist of 6, 7, and 8 glucose units, respectively. These structures were found that differ in their ring sizes and solubility properties. CDs that less than 6 glucose units cannot be formed because of the steric hindrances while the molecule that larger than 9 glucose units are very difficult to purify. However, there were some studies that could isolate and purify the large ring CDs that consisting of 9 glucose units (known as δ-CDs or Cyclomaltonose) (Challa et al., 2005).

The molecular structures of three natural CDs are shown in Figure 4. These CDs molecules are shaped like a truncated cone molecules with a hydrophobic cavities and hydrophilic outer surfaces. Because these CDs consist of different numbers of glucose units, each of them showed the different properties such as solubility, capability of complex formation. Table 1 exhibits some of the physicochemical properties of natural CDs. Among these three CDs (α-, β- and γ-CDs) β-CDs seem to be the most useful in the pharmaceutical application (Loftsson and Mansson, 2001). In contrast, the natural CDs, in particular β-CDs, show limited aqueous solubility and their complex formation, inclusion complex with lipophilic substances always results in precipitation of CDs complexes. In addition, β-CDs can form intramolecular hydrogen bonding between secondary hydroxyl groups that lead to reduction of the amount of hydroxyl groups which able to forming hydrogen bonds with the molecules of surrounding water (Loftsson and Mansson, 2001).

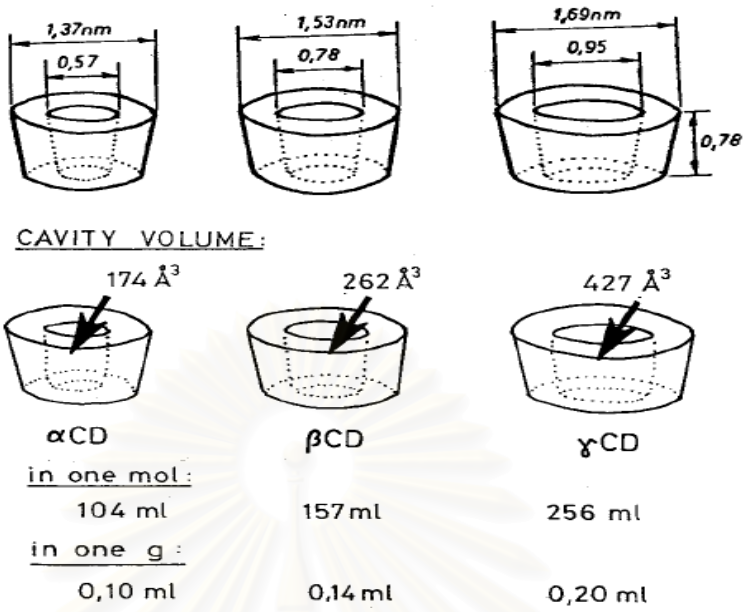


Figure 4. The molecular structure of natural CDs (Szejtli, 1998)

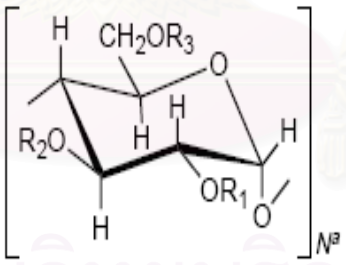
Table 1. Physicochemical properties of natural CDs (Szejtli, 1998)

	α	β	γ
No. of glucose unit	6	7	8
Unit weight	972	1135	1297
Solubility in water g100ml^{-1} at room temp	14.5	1.85	23.2
Cavity diameter A°	4.7-5.3	6.0-6.5	7.5-8.3
Approx. vol. of cavity	174	262	427
Crystal form	Hehagonal plates	monoclinic	quadratic
Crystal water wt%	10.2	13.2-14.5	8.13-17.7

1.2 Modified cyclodextrins

Natural CDs are rigid molecules and give limited capability in case of size, shape and availability of chemical functional groups. Because of these limited properties, modified CDs were synthesized to overcome these problems. Modified CDs were established for many reasons ranging from improving solubility to reducing toxicity of CDs. In fact, the solubility property of CDs is lower than that of acyclic dextrins. This is because of the relatively strong intramolecular hydrogen bonding between hydroxyl groups in the crystal lattice. It was discovered that substitution of any hydroxyl groups, even by lipophilic functions, resulted in dramatic improvement of their solubility property. To obtain these substituted compounds, methylation or hydroxyalkylation of the hydroxyl groups of CDs has been used to solve these problems (Uekama, Hirayama and Irie, 1998). The chemical structures of modified CDs are shown in Table 2.

Table 2. The chemical structures of modified CDs (Uekama, Hirayama and Irie, 1998).



Compound of side chain	R1	R2	R3
Methylated	H or CH_3	H or CH_3	H or CH_3
Hydroxyalkylated	H or CH_3CH_3OH	H or CH_3CH_3OH	H or CH_3CH_3OH
Alkylated	H or glucose, or maltose	H or glucose, or maltose	H or glucose or maltose

The random substitution of the CDs molecule transforms the crystalline CDs into amorphous mixtures of isomeric derivatives. For example, 2-hydroxypropyl-β-cyclodextrin (HPBCD) is obtained by treating a base-solubilized solution of βCD with propylene oxide, resulting in an isomeric system that has an improvement of aqueous solubility and bioavailability (Viega, 2000)

1.2.1 Hydroxylpropyl-β-cyclodextrin (HPBCD)

The most common parent CDs are α-, β-, and γ-CD with the different number of consisting glucose units (α= 6, β = 7, γ= 8). β-CDs are not very soluble in water because of the strong hydrogen bond between HO-2 and O-3. Hydroxylpropyl-β-cyclodextrin (HPBCD), a hydroxyalkyl derivative, is an alternative to α-, β-, and γ-CD, by improved water solubility (Uekama, Hirayama, and Irie, 1998) and may be slightly more toxicologically benign. As the approved CD derivatives by FDA, HPBCD have been used widely applications in food, agriculture and even in the pharmaceutical field. HPBCD are prepared by reacting β-CD with propylene oxide in alkaline aqueous solutions. The high alkali concentration prefers alkylation at O-6, while the low alkali concentration prefers alkylation at O-2. The products are always substituted randomly when it distributes among the different glucose units. Moreover, the ratio of reactants, reaction time and the temperature affect the degree of substitution (DS). It follows that the composition of the HPBCD samples show high variability, which is also reflected in the chemical and physical properties.

The effect of DS on the inclusion forming ability has been studied by different authors researching this field. Müller and Brauns (1986) reported that the DS of mixtures of HPBCD derivatives has a significant effect on the complexes formation. A low degree of substitution is preferable, since these derivatives show the highest complexation properties. Thera was a study found that the complex forming ability of HP-β-CDs first increases and then decreases with increasing DS. A clear conclusion has not been reached due to the amorphism of HP-β-CDs. Moreover, the substitution pattern affects the stability of inclusion

complexes too. It seems that both the DS and the substitution pattern influence the stereospecificity of HP-β-CDs. However, there are very few studies focusing on the effect of the substitution pattern of HP-β-CDs on the formation of inclusion complexes.

On the other hand, the stability of inclusion complexes is also influenced by the sizes and configurations of the guests. Different guest molecules have different abilities to fit into the CD cavity.

In addition, HPBCD is good tolerated in the animal species tested (rats, mice and dogs), especially when used orally, and shows only a little bit toxicity. In short duration, there were little biochemical changes while studies of a longer duration, up to three months, produced additional minor haematological changes but no histopathological changes. When administered intravenously, histopathological changes were seen in the lungs, liver and kidney but all of them were reversible and no effect levels were achieved. The carcinogenicity studies showed an increase in tumours in rats in the pancreas and intestines which are both considered to be rat-specific. There were also non-carcinogenic changes noted in the urinary tract, but these changes were also reversible and did not impair renal function. There were no effects on embryo-foetal development in either rats or rabbits. HPBCD has been shown to be well tolerated in humans, with the main adverse event being diarrhoea and there have been no adverse events on kidney function, as known at present.

1.2.2 The other modified cyclodextrins

From a wide variety of CDs, modified CDs can be classified into two types, the first one is hydrophilic or ionizable CDs and the other is hydrophobic CDs. The hydrophilic or ionizable CDs, such as 2-hydroxypropyl-β-cyclodextrin, 6-*o*-maltosyl-β-cyclodextrin, sulfobutyl-β-cyclodextrin, will improve the drug solubility and enhance the drug absorption, a promising enhancement of oral bioavailability of poorly water soluble drugs. In contrast, hydrophobic CDs, such as alkylated and acylated CDs, will be useful in controlled release preparations for water soluble drugs. In many kinds of alkylated CDs, heptakis(2,6-di-*o*-ethyl)-β-cyclodextrin and

heptakis(2,3,6-tri-*o*-ethyl)-β-cyclodextrin were first slow-release retardants to be used in combination of diltiazem and isosorbide dinitrate (Uekama, Hirayama and Irie, 1998).

2. Cyclodextrin Inclusion Complexes

CDs can form inclusion complexes with many hydrophobic drug molecules in an aqueous solution. The process of inclusion complex forming is started from the hydrophobic cavity is occupied by water molecules and then these water molecules are replaced by the whole or some part of drug molecules (guest molecule) which are less polar than water, as shown in Figure 5 (Szejtli, 1998). One, two or three molecules of CDs contain one or more guest molecules, the most frequently host:guest ratio is 1:1. However, the ratio 2:1, 1:2, 2:2 or even more complicated associations can be found (Szejtli, 1998). In forming of inclusion complex, the guest molecules must be fit or has some part of molecule that fit with hydrophobic cavity of CDs. The guest molecules as well as partially of molecules that too small cannot form stable complexes because they will slip easily from cavities of CDs. In addition, the bulky guest molecules that are larger than the cavities or have bulky functional groups, are impossible to form complexes with CDs. Moreover, the polarity of guest molecules is very important to form complex with CDs. Because of hydrophobic cavity of CDs, lipophilic molecules have more affinity to form complex than hydrophilic ones in aqueous environment. In general, ionic forms of drugs are weaker than their nonionic forms in complex formation (Chen et al., 1996; Frank, 1975).

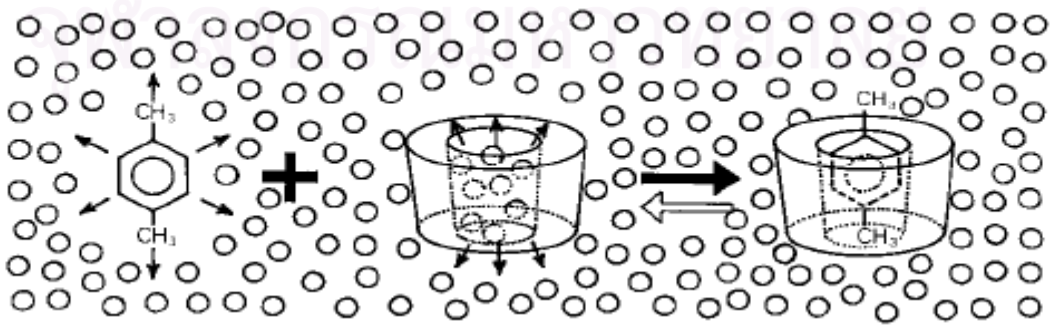


Figure 5. Schematic representation of CD inclusion complex formation. *P*-Xylene is the guest molecule; the small circles represent the water molecules (Szejtli, 1998).

The interaction force for inclusion complex formation, a variety of non-covalent forces like Van der Waals forces, hydrophobic interaction, and dipole movement are responsible.

3. The Complex Stability Constant

For all complex formation processes of CDs, the measurement and knowledge of the stability or equilibrium constant (Ks) are important since these values provide an index of changes of physicochemical properties that obtained from inclusion complexes. According to complexation, the equilibrium constant (Ks) can be written:

$$Ks = mCD + nD \leftrightarrow CD_m\ D_n \qquad \text{Equation (1)}$$

In addition, the equilibrium can be defined as:

$$Ks = \frac{[CD_mD_n]}{[CD]^m[D]^n} \qquad \text{Equation (2)}$$

As defined in Equation (2), Ks is an equilibrium constant, while m and n represent the molar ratio of the drug molecule to the CDs.

4. Preparation of Inclusion Complexes

Several methods were described for inclusion complexes formation of CDs. (Taneri et al., 2003)

4.1 Kneading

This method involves the preparing of the paste of CDs with guest molecules by using a small amount of water or ethanol to form mass. The mass of drug-CDs mixture were mixed at slightly high temperatures and then is dried.

4.2 Melting

An excess amount of guest is melted and mixed with cyclodextrin powder. After cooling, an excess quantity of guest is removed by washing with weak solvent. The method restricted to guest like menthol.

4.3 Co-evaporation

To the alcoholic solution of guest, aqueous solution of host is added and stirred for sometimes and evaporated at room temperature until obtain dried mass, pulverized and sieved and fraction is collected.

4.4 Freeze drying

The enough quantity of host and guest were added to aqueous solution of cyclodextrin and this suspension stirred magnetically for 24 hours, and resulting mixture is freeze dried at temperature -60 °C for 24 hours.

4.5 Spray drying

In this method, the enough quantity of guest were added to aqueous solution of cyclodextrin and this suspension stirred magnetically for 24 hours or until it appear to be clear solutions at room temperature and solution is spray dried by observing condition of air flow rate, inlet temperature, flow rate of solution etc.

Although, there are many ways to prepare the inclusion complexes of drug and cyclodextrins, but the promising method is spray drying because this method has many advantages.

C. SPRAY DRYING

1. The Spray Drying Process

Spray drying transforms a liquid sample into dry powder or particle form. The sample can be a solution, a suspension or a paste in the simplest form. The process involves the atomization of liquid into a spray using either rotary or nozzle

atomizers. The spray is contacted with hot air entering the drying chamber by uniformly and quickly to evaporate the water. Evaporation of moisture, particle formation, and drying proceed under controlled conditions of minimum product temperature processing is operated within a single step. The product is collected continuously from the drying chamber and fine particles entrained in the exhaust air are recovered in cyclones. Operating temperatures and spray dyer designs are selected according to the powder specification and drying characteristics (Aulton, 2002).

In general form, spray-drying consists of four process stages:

1. atomization of the feed
2. spray-air contact
3. drying
4. separation of the dried product from the drying air

The dried product can be powder, granulated or agglomerated particles that can be varied depending on the condition, type and concentration of sample, and the process involved drying, feed rate, inlet temperature, air flow rate. The flexibility of spray dryer design enables powders to be produced in the various forms. Spray-drying is often the drying method of choice because of its simple processes with relatively easy control. Spray drying has the ability to carry out heat sensitive substances with maximum retention of their content. Spray drying is an economical and continuous processing technology to produce such powder of uniform and repeatable character.

2. Parameter Effects on The Powder of Spray Drying

Kristin, Henry and Mark (2002) studied the relationship between some process and formulation variables and resulting powder characteristics using the spray drying process. In this study, both protein solutions and zinc-complex protein suspensions were spray dried. During the process, the liquid was atomized into a

chamber of heated air, producing a spray of fine droplets, forming dried particles. These particles were then separated into a collect container. Using a large-scale apparatus, the effects of various spray dry process and formulation variables on the product were examined. Process variables were inlet temperature, liquid feed rate, drying air flow rate and atomizing nitrogen pressure. It was found that protein concentration and atomizing nitrogen pressure had the greatest effects on the particle size of protein powder. To control the particle size of spray dried powder, it was important to keep the atomizing nitrogen flow rate relative high and the protein concentration relatively low. Both of these conditions produced smaller dried particles in this study. For determining product yield, the result showed that protein concentration was very variable. To maximize powder yield, protein concentration should be high. Finally, the outlet temperature was influenced by inlet temperature and liquid feed rate. A variety of particle morphologies were observed in this study ranging from smooth spheres to folded, crumpled structures, depending on the input conditions.

3. Advantages of Spray Dry Process (Aulton, 2002)

There are many ways to prepare the complexes of drug and cyclodextrins such as physical mixing, kneading, freeze drying and spray drying. The promising method is spray drying because this method has many advantages.

1. There are a large number of small droplets which provide a large surface area for heating process, so that evaporation is very quickly. The actual drying time of a droplet is only a little part of a second, and the overall time in the drier only a few seconds.

2. Because evaporation is very rapid, the droplets do not expose to a high temperature for long time. Most of the heat is used as latent heat of vaporization and so the temperature of the particles is kept low by evaporative cooling (Bayram, Bayram and Tekin, 2005).

3. The characteristic of particle form gives the product to be a high bulk density and rapid dissolution due to the large surface area.

4. Provided that a suitable atomizer or spray nozzle is used, the products will have uniform and controllable particle size.

5. The product is good free flowing, with almost spherical particles, and is especially convenient for tablet manufacture as it has excellent flow and compaction properties. Due to the good flowability of the particles, products can be easily used for mixing with other diluents for any cases.

6. Producing costs are low, the process yielding a dry, free flowing powder from a diluted solution can be operated within a single operation with no handling.

4. Application of Spray Drying Process (Aulton, 2002)

The spray drier can be used with a wide variety of substances, either solution or suspension. Especially, this method is very useful for thermolabile substances because evaporation process is very rapid, the droplets of liquid are not contacted with heat temperature for long time, so it is not degraded.

Spray drying is also enable of producing spherical particles in the controllable range that have been use for delivery of drugs from dry powder inhalers. Because spray drying can provide a good characteristic product, round shaped, uniform size, good flow and very tiny spherical particles, that can be rapidly for dissolution thus spray dried particles are suitable for preparing the powder formulations for reconstituted syrup. (Aulton, 2002)

It is possible to operate spray driers aseptically using heated filtered air to dry products such as serum hydrolysate. Also, some spray driers operate in a closed

system with an inert gas to minimize oxidation of the product. Additionally, volatile solvents can be recovered from such systems.

D. PHASE SOLUBILITY ANALYSIS

One of the most famous applications of cyclodextrins in pharmaceutical fields is to increase aqueous solubility of water-insoluble drugs by forming inclusion complexes. The ability of solubilizing of cyclodextrins can be evaluated by the phase solubility method developed by Higuchi and Connors. The phase solubility diagrams, plots of solubility of drug at maximum concentration for each concentration of cyclodextrins as series concentrations, are classified as either type A (forming of soluble complex) or type B (forming of complex by limited solubility), as shown in Figure 6. The type A can be defined in three subtypes, A_L, A_P, and A_N. The subtype A_L shows a linear increase in solubility as a series of cyclodextrins concentration, A_P profiles indicate an isotherm that the curve deviates to a positive direction from linearity (the solubilizers, cyclodextrins, is more effective at higher concentrations) and the last one is A_N systems, that indicate a negative deviation from linearity (cyclodextrins are less effective at higher concentrations). In general, the complex formation with a 1:1 stoichiometry provides the A_L type diagram, while the higher ratio complex formation which more than one cyclodextrin molecules are formed in the complexation gives the A_P type. In contrast, the interaction mechanism for the A_N-type is complicated, because of a contribution of solute-solvent interaction to the complex forming.

In the case of type B, phase–solubility profiles are indicated the formation of complexes with limited water solubility and are always occurred with naturally cyclodextrins. Type B profiles can be classified in two subtypes, B_S and B_I systems. For B_S profiles, the initial region of the solubility diagram is linear increasing, after that it is followed by a plateau region and decrease in the solubility at higher cyclodextrins concentrations, go along with some precipitation of the complexes. The B_I-type diagram is indicated the insoluble complexes formation in water, the B_I systems are similar to the B_S profiles except that the complexes formation are totally

insoluble that they do not provide rise to the initial ascending component of the solubility.

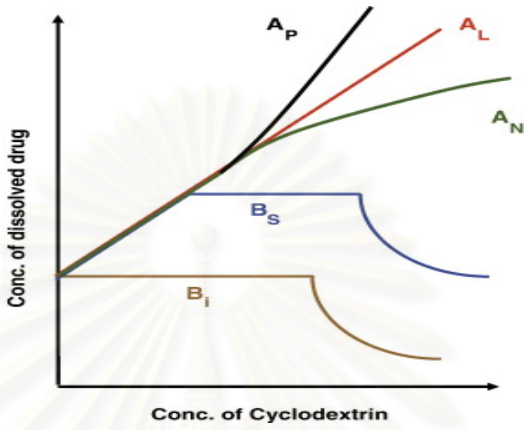


Figure 6. The diagrams of phase solubility of cyclodextrins

The stoichoimetric ratio of the complexes can be examined from the ascending and descending parts of these diagrams if exactly assumptions can be made. For the form of 1:1 complex formation, the association constant Ks can be determined from the slope of the initial linear portion of the phase solubility curve, and the intrinsic solubility, S_0, of the drug, using Equation 3.

$$K_s = \frac{\text{slope}}{S_0(1\text{-slope})} \qquad \text{Equation 3}$$

S_0 is the solubility of ketoconazole in the absence of CDs.

E. DRY SYRUP FORMULATION (Aulton, 2002)

To develop solubility of solid dosage forms, in aspect of water-insoluble drugs, there has been a big problem for pharmaceutical scientists for many years. It is known that bioavailability of drugs be limited by poor aqueous solubility because the efficacy of drugs is started with the absorption of drug across biological membrane.

Dosage forms that enter the stomach and go through the gastrointestinal tract must release the drug in form of solutions to achieve good drug bioavailability. Many drugs can be prepared to be both of liquid and solid dosage form. In general solid dosage forms seem to be more popular than liquid dosage forms due to its properties.

Although solid dosage forms, such as tablets and capsules, are more widely used than liquid dosage forms for oral administration, because dosage form is easy to transport and keep. However liquid dosage forms have many advantages. Firstly, they are easier to swallow than solids and more acceptable for pediatric and geriatric, so this can enhance patient compliance for treatment. Secondly, Drugs can be absorbed only in form of solutions, for this reason if it is administered in the solution dosage form, the drug are ready for absorption. It can be concluded that the drug in form of solution provide faster therapeutic response than solid dosage form. Thirdly, among many liquid dosage forms, solution is only one that has a homogenous system that the drug will be distributed equally throughout the preparation. For suspension and emulsion formulations, particles can be separated from medium and may be hardly to disperse. Finally, some drugs can damage gastric mucosa, especially if contacted in one area that always found in solid dosage forms. Liquid formulations can avoid the irritation of gastric mucosa because it can be immediately distributed and diluted by gastric juice.

However, there are many problems deal with solutions in term of manufacture, transport, stability of solutions. Liquids are bulky so that are inconvenient for transportation and storage. Because the container always made of fragile material, such as glass, so it can be broken during storage or transportation. The stability of liquid formulations is usually poorer than solid formulations, so that the shelf-life of liquid dosage form is often shorter than solid dosage from. Liquid dosage forms always consist of water, so this is suitable for the growth of microorganisms and may need to add some preservatives for the formulations (Allen, 2004).

From those reasons, to formulate the drug in form of powder for reconstitution is very attractive because of its properties. Powder for reconstitution is a kind of the

solid dosage forms, so it can provide longer shelf-life of drug than liquid dosage forms and convenient for storage including transportation. Furthermore this preparation can be turned to liquid dosage form by adding purified water, so it still has good bioavailability of drug as liquid preparation.

CHAPTER III

MATERIALS AND METHODS

MATERIALS

Acetonitrile, HPLC grade (Lab Scan Co., Ltd., Thailand)

Aspartame (distributed by Srichand United Dispensary)

Cetyl Pyridinium Chloride (Merck, Germany, Lot. No. 311F00345, supplied Silom Medidal)

Clotrimazole (supplied from JINTAN ZHONGXING medical and pharmaceutical factory, Lot number: 20061223)

Glacial acetic acid (Merck,Germany Lot number K33266463 422)

Hydroxypropyl-β-cyclodextrin (HPBCD) (Nutrifirst Biotech, degree substituted ~ 4.0 molecular weight ~ 1390 Lot no. 070902)

Ketoconazole (supplied by M&H manufacturing co., Ltd., Lot. KCNZ041015)

Potassium dihydrogen phosphate (Merck, Germany, Lot K24331873)

Propylene glycol (distributed by Srichand United Dispensary)

Saccharin (distributed by Srichand United Dispensary, Lot. 031127)

Sodium acetate (Asia Pacific Specialty Chemicals Limited CAN, Lot. 711053)

Sodium Hydroxide (Merck, Germany, Lot. B0035298 704)

Xanthan gum (distributed by Srichand United Dispensary)

APPARATUSES

Analytical balance (Sartorius model 1615, Germany)

Cone and plate viscometer (Model LVDV-II+, Brookfield, USA.)

Differential scanning calorimeter (NETZCH DSC 822, Mettler Toledo, Switzerland)

Disposable syringe filter nylon 13 mm, 0.45 μm (Vertical)

High performance liquid chromatography

* Automatic sample injector (SIL-10A,Shimadzu, Japan)
* Communications bus module (CBM-10A,Shimadzu, Japan)
* Column (Mightysil RP-18, 5 μm, 150mm x 4.6mm)
* Liquid chromatograph pump (LC-10AD, Shimadzu, Japan)

UV-VIS detector (SPD-10A, Shimadzu, Japan)

Magnetic stirrer (Model RCT basic, KIKA Works Guangzhou, China)

Mastersizer S (Malvern instrument, UK)

Moisture analyzer balance (Model HB43, Mettler Toledo, Switzerland)

pH meter (Orion model 420A, Orion Research Inc., USA.)

Scanning electron microscope (Model JSM-T220A, Jeol, Japan)

Sonicator (Model TO680DH, Elma, Germany)

Spray dryer (Model SD-06, Labplant, Ltd., UK)

Powder x-ray diffraction (Model JDX-3530, Jeol, Japan)

METHODS

A. QUANTITATIVE ANALYSIS OF KETOCONAZOLE BY HPLC METHOD

In this study, HPLC method was used for quantitative analysis of ketoconazole due to high sensitivity and specificity of the method.

1. Chromatographic Conditions

The chromatographic conditions were investigated and modified from the method reported by ณหทัย, กาญจนา และจุฑารัตน์, 2546. The chromatographic system was as follows:

Column	:	Mightysil RP-18 (5 µm, 150 mm x 4.6 mm)
Sample volume	:	20 µL
Internal standard	:	clotrimazole (800 µg/ml)
Mobile phase	:	acetonitrile : 0.05 M phosphate buffer pH 6.0 (65:35 v/v)
Detector	:	UV-Visible spectrophotometer 254 nm
Rate	:	1 ml/min.
Runtime	:	10 min.
Temperature	:	ambient

The 0.05 M phosphate buffer pH 6.0 was prepared by adding 0.1 N NaOH solution to 0.05 M of KH_2PO_4 solution to adjust pH to the specified pH. The pH measurement was performed by using pH meter (Orion model 420A, Orion Research Inc., USA.)

The mobile phase was prepared by mixing acetonitrile with 0.05 M phosphate buffer pH 6.0 at the ratio 65: 35 by volume. The mixtures solution was thoroughly mixed, filtered through 0.45 µm membrane filter and then degassed by sonication for 30 min before use.

2. Standard Solutions for HPLC Method

2.1 Standard solution of ketoconazole

An accurately weighed amount 20 mg of ketoconazole was transferred to a 100 ml volumetric flask. Mobile phase of HPLC was used to dissolve and adjust to volume. The obtained stock solution with the concentration of 200μg/ml was used further to prepare standard solutions

2.2 Internal standard solution

Clotrimazole was selected from the preliminary study to use as an internal standard. An accurately weighed amount 80 mg of clotrimazole was transferred to a 100 ml volumetric flask. Mobile phase of HPLC was used to dissolve and adjust to volume, these solutions were stock solution of clotrimazole with the concentration of 800 μg/ml.

Standard solutions of ketoconazole were prepared by pipetting 2, 3, 4, 5 and 6 ml of stock solution into 10 ml volumetric flasks. These solutions were added with 1 ml of clotrimazole stock solutions. The solutions were adjusted by mobile phase to volume so that the concentrations of ketoconazole of standard solutions were 40, 60, 80, 100 and 120 μg/ml, respectively.

The standard curve of ketoconazole was constructed by plotting between the ketoconazole concentrations and peak area ratio, and the regression equation was obtained as a result.

3. Sample Solutions of HPLC Method

Sample solutions of ketoconazole were prepared by pipetting 1 ml of the sample into a 100 ml volumetric flask and adjusted to volume with mobile phase. Then, 5 ml of the above solution was pipetted into a 10 ml volumetric flask with 1 ml of clotrimazole solution and adjusted to volume by mobile phase.

The peak area ratio of ketoconazole and clotrimazole was calculated and the concentration of ketoconazole was determined from the standard curve, while was freshly prepared prior to use.

For the spray dried samples of ketoconazole, it was reconstituted with an appropriate volume of purified water into a clear solution. The further procedure was as the same operation as described above.

4. Validation of HPLC Method

The analytical parameters for validation of the HPLC method were specificity, linearity, accuracy and precision (USP 24, 2000). The validation procedure was performed as follows:

4.1 Specificity

The appropriate condition used had to be the system by which the peak of ketoconazole was completely separated from the other peaks and was not interfered by any components of the sample.

4.1.1 In the presence of buffer, HPBCD, sucrose, aspartame, saccharin, xanthan gum and cetylpyridinium chloride

A 2% w/v ketoconazole in HPBCD 35% w/v solution, and 2% w/v ketoconazole in HPBCD 35% w/v solution with diluents as described above were prepared. The sample solutions were prepared by taking 1 ml of each into 100 ml volumetric flask and added mobile phase to adjust to volume, then, 5 ml of the above solution was pipetted into 10 ml volumetric flask with 1 ml of clotrimazole standard solution and adjust to volume by mobile phase. The chromatograms obtained from results of these solutions were compared with chromatogram of ketoconazole standard solution of 100 μg/ml concentration.

4.1.2 In the presence of degradation products of ketoconazole

A 2% w/v ketoconazole in HPBCD 35% w/v solution was kept in either hot air oven at 80°C for 7 days or kept in UV chamber for 3 days to accelerate the degradation of ketoconazole. The degraded sample solutions were prepared by pipetting 1 ml of each into 100 ml volumetric flask and added mobile phase to adjust to volume. Then, 5 ml of the degraded solutions were pipetted into 10 ml volumetric flask with 1 ml of clotrimazole stock solutions and adjusted to volume by mobile phase. The chromatograms of these solutions were compared with that of ketoconazole standard solution of 100 μg/ml concentration which was freshly prepared.

4.2 Linearity

Seven ketoconazole standard solutions with different concentrations were prepared and analyzed. Linear regression analysis of the peak area ratios versus their concentrations was operated. The linearity was examined from the coefficient of determination (R^2).

4.3 Accuracy

Five sets of three standard solutions of ketoconazole of low, medium and high concentrations were prepared and determined. The percentage of analysis recovery of each was examined.

4.4 Precision

4.4.1 Within run precision

The within run precision was determined by five sets of three standard solutions of ketoconazole of low, medium and high concentrations in three interval of time within one day. The peak area ratios of ketoconazole to clotrimazole were compared and the percent coefficient of variation (%CV) of each concentration was examined.

4.4.2 Between run precision

The between run precision was determined by comparing each concentration of five sets of three standard solutions of ketoconazole of low, medium and high concentrations. Each set was prepared and injected in the three different days. The peak area ratios of ketoconazole to clotrimazole were compared and the percent coefficient of variation (%CV) of each concentration was examined.

B. PREPARATION OF KETOCONAZOLE SOLUTION AND SRAY DRIED POWDERS

1. Phase Solubility Study of Ketoconazole with HPBCD at pH 5.0

Phase solubility studies were carried out in acetate buffer pH 5.0 according to the method described by Higuchi and Connors. An excessed amount of ketoconazole was added in a 10 ml of 0.1 M acetate buffer solutions (pH5.0) containing various concentrations of HPBCD, ranging from 0-25 mM, dispersed by sonication for 10min. Then, the suspensions were stirred at ambient temperature for three days and all processes were protected from light. After equilibrium, the suspensions were filtered through 0.45μm membrane filters and appropriately diluted with distilled water. Then, the total concentration of the ketoconazole in the filtrate was analyzed by HPLC, using UV-spectrophotometer detector at 254 nm.

The concentrations of dissolved ketoconazole were determined from the standard curve and the molarity of dissolved ketoconazole in each solution was calculated. The phase solubility diagram was plotted between the molar concentration of dissolved ketoconazole on the vertical axis and the molar concentration of HPBCD located on the horizontal axis.

2. Preparation of 2% Ketoconazole Solutions

From the phase solubility diagram, 2% w/v ketoconazole solution pH 5 was prepared by dissolving ketoconazole and HPBCD 35% w/v in 0.1 M acetate buffer pH 5.0. The solution was mixed by stirring for 48 hours at ambient temperature by magnetic stirrer, all processes were protected from light.

3. Experimental Design

In order to evaluate the optimal spray drying condition of the powder preparation, each sample with the same composition, was prepared under varied spray-drying conditions. All conditions were set as fast deblocker and fan speed was fixed at level 50 (300 m^3/hr.). All processes were operated with protection from light.

The spray drying variables (factors) that were varied were: feed flow rate and inlet air temperature. Three levels of feed flow rate and three levels of inlet temperature were used and classified to low, medium and high. A full factorial experiment was run and the number of experiments was 3^k i.e. 3^2 (=9).

Table 3. Parameters of spray drying process

Factor	**Level**		
inlet temperature (°C)	100	120	140
feed rate (ml/min.)	3.5	7.0	10.5

Table 4. Factorial design of spray drying conditions

Code	inlet temperature (°C)	feed rate (ml/min)
F1	100 (-)	3.5 (-)
F2	100 (-)	7.0 (0)
F3	100 (-)	10.5 (+)
F4	120 (0)	3.5 (-)
F5	120 (0)	7.0 (0)
F6	120 (0)	10.5 (+)
F7	140 (+)	3.5 (-)
F8	140 (+)	7.0 (0)
F9	140 (+)	10.5 (+)

4. Preparation of Spray Dried Products

Dry powders were prepared from solutions by spray-drying process. The powders were produced using a SD-06, Lab Plant spray-dryer (Lab Plant Limited, UK). The solution was pumped into the drying chamber at an adjusted rate and atomized through a 0.5mm nozzle. The inlet temperature was established at 100, 120 and 140 °C; the outlet temperature depended on the inlet temperature and the liquid flow rate was set at 3.5, 7.0 and 10.5 ml/min. The powders were collected and stored in a dessicator and was immediately determined. All processes were protected from light.

Figure7. Light protection during the drying process

C. EVALUATION OF CHEMICAL AND PHYSICAL PROPERTIES OF SPRAY DRY POWDERS

1. Yield from The Spray Drying

The percentage yield was calculated from the weight of spray dried powder of each condition divided by the total weight of initial dry materials and multiplied with 100. The spray dried products was collected from the collector and cyclone together.

$$\% \text{ yield} = \frac{\text{Weight of spray dried powder} \times 100}{\text{Weight of total components}} \quad \text{Equation 4.}$$

2. Moisture Content

A sample of spray dried powder was weighed on the aluminum pan of moisture analyzer (Model HB 43, Mettler Toledo, Switzerland). The sample was

exposed to a halogen lamp until a constant weight appeared and the percentage of moisture content was calculated automically.

$$\% \text{ moisture content} = \frac{\text{Weight loss} \times 100}{\text{Initial weight}} \qquad \text{Equation 5.}$$

3. Particle Morphology

Shape and surface appearances were evaluated by scanning electron microscope (SEM), (JSM-T220A, Joel, Japan) for morphological observation. Powders sample was stuck on the adhesive tape on the aluminum stubs and coated with gold prior to examination. The sample was taken a photograph under a 15 kv electron beam.

4. Particles Size and Size Distribution

Particle size was analyzed by laser light scattering. The powder sample was distributed and suspended in mineral oil, non-dissolving medium.

5. The Drug Content

The drug content in spray dried powder was evaluated by HPLC method using a chromatographic system as described before. The powder sample containing 2% w/v ketoconazole and HPBCD 35% w/v was reconstituted into solution. The 1 ml of each sample was pipetted into a 100 ml volumetric flask and added with mobile phase to adjust to volume. Then, pipetting 5 ml of sample stock solutions into volumetric flask 10 ml with added 1 ml of clotrimazole solution into a 10 ml volumetric flask and adjusted to volume by mobile phase. The result of the solution was compared with standard solution of ketoconazole with 100 μg/ml concentration.

$$\% \text{ drug content} = \frac{\text{amount of drug from HPLC} \times 100}{\text{amount of drug from calculation}} \qquad \text{Equation 6.}$$

6. Flow Property

The flowability of spray dried powder was indicated by determining angle of repose. There are numerous methods for measuring angle of repose and each produces slightly different results. Results are also sensitive to the exact methodology of the experimenter. As a result, data from different labboratories is not always comparable. One of the famous methods to determine angle of repose is fixed funnel method.

There were not specific conditions about fixed funnel method. The angle of repose of spray dried powders from each condition was determined in the same condition. A powder sample was weighed about 10 grams and let it flow continuously through the glass funnel with 1 cm diameter hole, which was fixed at 10 cm from horizontal plane. The angle of repose was determined by measuring the internal angle between the slope surface of the pile and the horizontal plane.

7. Differential Scanning Calorimetric Thermogram

To study the interaction between ketoconazole and HPBCD by differential scanning calorimetry is an effective way to examine. All different effects on changes of physicochemical properties of materials were described as a function of temperature, while heating rate was uniform. The differential scanning calorimetric thermogram of each spray dry condition was determined by differential scanning calorimeter (DSC822^{e} ,Mettler Toledo, Switzerland).

The diffractograms of ketoconazole, HPBCD, the complex of spray dried powders and physical mixture of ketoconazole and HPBCD were obtained for comparison. The sample was accurately weighed (~3 mg) into an aluminum pan (40 µl) and pierced lid. The DSC was operated on the temperature range 0-200 °C at heating rate 10 °C/min, under nitrogen gas atmosphere at flow rate 200 ml/min.

8. Powder X-ray Diffraction

The diffractogram of the complexes of ketoconazole and HPBCD spray dried powder was examined by powder x-ray diffractometry (JDX-3530, Jeol, Japan). The data was investigated over an angular range 5°-40.00817° in continuous mode, using a step angle of 0.01882° and step time: 96 seconds.

D. OPTIMIZATION OF SPRAY DRYING PROCESS

To select the best condition for spray drying, optimization was used in this experiment. All conditions were estimated by response surface methodology. A central composite design was created to obtain the response surface and to determine the optimal condition to produce maximal yield of spray drying.

The central composite design (CCD) was used in this study. CCD is composed of full factorial design and axial points by replicated the middle point at least three times. For this experiment, α was set as 1 and the middle point was triplicate operated.

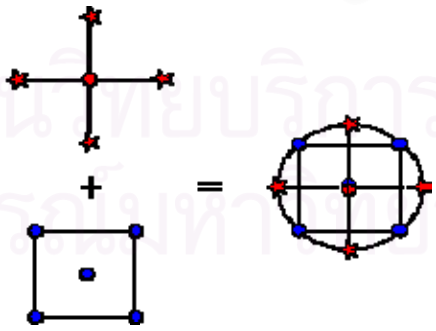


Figure 8. CCD was composed of full factorial design (dot) and axial points (star).

The response surface plots indicate the effects of factors to percentage of yielding at each level of factors. In order to evaluate the suitable condition of the

powder preparation, the statistical software program, Design-Expert version 7.1.3 was used.

E. PREPARATION OF KETOCONAZOLE DRY MIXTURES FOR ORAL SOLUTION

1. Formulation of Ketoconazole Dry Mixtures for Oral Solution

To prepare ketoconazole dry mixtures for oral solution, all ingredients were reduced to the same size as that of spray dried powders from the optimal condition. To obtain uniform size, grinding and sieving were carried out. Then, the spray dried powders and all diluents of the formulation described in the Table 5 were thoroughly mixed.

Table 5. Formulations of ketoconazole dry mixtures for oral solution

Ingredients	**Rx 1** **% w/w**	**Rx 2** **% w/w**	**Rx 3** **% w/w**	**Rx 4** **% w/w**
Ketoconazole	2	2	2	2
HPBCD	35	35	35	35
Sucrose	30	15	-	-
Aspartame	-	0.15	0.3	0.3
Sodium saccharin	-	-	0.5	0.5
Xanthan gum	-	0.15	0.15	0.3
Cetylpyridinium chloride	0.01	0.01	0.01	0.01
Purified water q.s. to	100	100	100	100

2. Characterization of Ketoconazole Dry Mixtures for Oral Solution

2.1 Flow property

The flowability of ketoconazole dry mixtures was determined by the same method as described in Topic C6.

2.2 Moisture content

The moisture content of ketoconazole dry mixtures was determined by the same method as described in Topic C2.

2.3 Physical: clarity and taste (evaluated by researcher)

2.3.1 Clarity

The clarity of ketoconazole reconstituted solution was observed by visual observation under the day light.

2.3.2 Taste

Each formulation of ketoconazole dry mixtures was reconstituted. The taste of the solution was evaluated. The interval time from each test is about 20 minutes and washes the mouth before in every testing.

2.4 Reconstitution time

A calculated amount of ketoconazole dry mixtures equivalent to 0.6 g of ketoconazole was weighed into a 100 ml dispensing bottle. The bottle was tapped for several time to loosen powder content prior to reconstitution. On addition about 20 ml of purified water, mixed well by shaking for twenty times, then adjusted volume to 30 ml. The reconstituted solution was obtained with 20 mg/ml concentration. The time, in minute scale, after shaking until the solution appeared to be clearly was recorded.

2.5 Viscosity of the reconstituted solution

Absolute viscosity can be measured directly by measuring the time required for a measured volume of liquid to flow through a capillary tube. The

experimental determination of relative viscosity is easier than the absolute viscosity. The relative viscosity of the reconstituted solution of ketoconazole dry mixtures was determined by Ostwald viscometer by using a reference fluid with known viscosity. The method was operated by determining the time for liquid flowing between two marked points of the vertical capillary tube. The result was compared with the time for purified water, as the reference, flowed in the same tube at room temperature (25 °C). For the test, the liquid and purified water were accurately measured as equal volume.

The samples which were the reconstituted solution of ketoconazole dry mixtures and purified water were measured with 2 ml and weighed. The flowing time of the liquids between two marks were recorded. The viscosity was calculated by substituting the experimental values in the equation 7 as following.

$$\eta_1 / \eta_2 = \rho_1 t_1 / \rho_2 t_2 \qquad \text{Equation 7.}$$

η_1 = viscosity of reconstituted solution at room temperature

η_2 = viscosity of purified water at room temperature

ρ_1 = density of reconstituted solution at room temperature, calculated by divided weight of the solution 2 ml. by 2ml.

ρ_2 = density of purified water at room temperature, calculated by divided weight of the purified water 2 ml. by 2ml.

t_1 = flowing time of reconstituted solution at room temperature

t_2 = flowing time of purified water at room temperature

2.6 pH

The pH of reconstituted ketoconazole solutions were measured by pH meter (Orion model 420A, USA).

From the characteristics of both ketoconazole dry mixtures and their reconstituted solutions of four formulations obtained from the above studies, the most appropriate formulation was selected for the next study.

3. Preparation of Ketoconazole Suspensions (USP 29, 2006)

The preparation of ketoconazole suspension was followed the formulation as described in USP 29. First of all, xanthan gum was dispersed into water and stirred until it was completely hydrated and swelling. Then, ketoconazole and cetylpyridinium chloride were added into the liquid and mixed thoroughly. Finally, suspension structure vehicle was added to adjust volume to 100 ml. The formulation was recommended to be kept in the refrigerator not longer than 14 days.

Table 6. Formulation of ketoconazole suspension (USP29, 2006)

Ingredients	**Amount**	
Ketoconazole	2	g
Cetylpyridinium chloride	10	mg
Xanthan gum	0.15	g
Purified water	30	ml
Suspension structured vehicle qs. to	100	ml

The preparation of the suspension structured vehicle was followed the formula by Loyd (2002). For the first step, xanthan gum was dispersed into propylene glycol, and added water to hydrate and swell the gum. Then, sodium saccharin and aspartame were added and adjust volume by syrup. The formulation of suspension structured vehicle was showed as following table, Table7. (Loyd, 2002).

Table 7. Formulation of suspension structured vehicles (Loyd, 2002)

Ingredients	**Amount**
Xanthan gum	300 mg
Sodium saccharin	100 mg
Aspartame	200 mg
Propylene glycol	5 ml
Syrup or other preserved vehicle qs.	100 ml

F. STABILITY OF KETOCONAZOLE DRY MIXTURES FOR ORAL SOLUTION AND RECONSTITUTED SOLUTION

1. Stability of Ketoconazole Dry Mixtures

The preparation of ketoconazole dry mixtures for oral solution were divided and kept in amber glass bottles. The bottles were tightly closed and sealed to protect them from humidity. The samples were kept under room temperature (30 °C) and under stress condition 40 °C 75%RH. The samples were withdrawn and investigated on day 0, 30, 60 and 90 in triplicate for each condition. Physical and chemical stabilities of the sample were evaluated as follows:

1.1 Drug remaining

The amount of drug remained in the formulation of ketoconazole dry mixtures for oral solution was determined by HPLC method. Reconstitution was made by adding water into the bottle of powders and shaken until it appeared to be clear solution. The sample of 1 ml was pipetted into a 100 ml volumetric flask and mobile phase was added to adjust to volume. Then, taking 5 ml of the above solution was pipetted into 10 ml volumetric flask with added 1 ml of clotrimazole stock solution and adjusted to volume by mobile phase. The result of the solution was compared with standard solution of ketoconazole with 100 μg/ml concentration.

$$\% \text{ drug content} = \frac{\text{amount of drug in sample}}{\text{amount of drug from calculation}} \times 100 \qquad \text{Equation 8.}$$

1.2 Flow Property

The flowability of ketoconazole dry mixtures was determined as the angle of repose by the same method as described under Topic C6

1.3 Moisture Content by Moisture analyzer balance

The moisture content of ketoconazole dry mixtures was determined by the same method as described under Topic C2

1.4 Reconstitution Time

The reconstitution time of ketoconazole dry mixtures was determined by the same method as described under the Topic E2.4.

1.5 Viscosity

The viscosity of reconstituted ketoconazole solution was determined by the same method as described under Topic E2.5

1.6 pH

The pH of the reconstituted solutions was measured by pH meter (Orion model 420A, USA). As described under the Topic E2.6.

1.7 Differential Scanning Calorimetry

The differential scanning calorimetric thermograms of ketoconazole dry mixtures were determined by differential scanning calorimeter (DSC822^e ,Mettler Toledo, Switzerland), the same method as described under Topic C7

1.8 Powder X-ray diffraction

The powder X-ray diffractometry was applied to investigated the solid property change of ketoconazole dry mixtures as the method described in topic C8.

2. Stability of Ketoconazole Reconstituted Solutions

Ketoconazole reconstituted solutions were prepared and kept in amber-glass vials. The storage conditions were under the refrigerator (5$\pm$ 3 °C), room temperature (30 °C) and under stress condition 40 °C 75% RH. Triplicate samples were withdrawn and determined on day 0, 5, 10, 15, 30, 45, 60, 75 and 90 as following (Carstensen, 1990).

2.1 Drug remaining

The amount of ketoconazole remained in the reconstituted solution was determined by HPLC method as described under Topic F 1.1.

2.2 Viscosity

The viscosity of reconstituted ketoconazole solution was determined by the same method as described under Topic E2.5

2.3 pH

The pH of the reconstituted solutions was measured by pH meter (Orion model 420A, USA). As described under the Topic E2.6.

2.4 Color change by UV-vis spectroscopy

In determination of color change of ketoconazole reconstituted solution, UV-vis spectroscopic method was used. The samples were analyzed spectrophotometrically at 410 nm (Im-erbsin, 2002), which was the range of visible light.

3. Stability of Ketoconazole Suspensions (USP 29, 2006)

The stability of ketoconazole suspensions was studied under the refrigerator (5$\pm$ 3 °C) as recommended in the USP 29. The samples were stored in amber-glass vials and the samples were measured on day 0, 5, 10, 15, 30, 45, 60, 75 and 90 as following (Carstensen, 1990).

3.1 Drug remaining

The amount of remained in the formulation of ketoconazole suspensions was determined by HPLC method. As described under the Topic F1.1.

3.2 Viscosity

The viscosity of ketoconazole suspension was determined by using cone and plate viscometer, BROOKFIELD DIGITAL VISCOMETER, Model RVTDCP.

3.3 pH

The pH of ketoconazole suspension was measured by pH meter (Orion model 420A, USA). As described under the Topic E2.6.

CHAPTER IV

RESULTS AND DISCUSSION

A. QUANTITATIVE ANALYSIS OF KETOCONAZOLE BY HPLC METHOD: VALIDATION OF HPLC

The validation of an analytical method is the process which performance characteristics of the method are established to meet the requirements for the intended analytical applications. The performance characteristics are expressed in terms of analytical parameters. The analytical parameters used for the assay validation were specificity, linearity, accuracy and precision.

1. Specificity

1.1 In the presence of buffer, HPBCD, sucrose, aspartame, saccharin, xanthan gum and cetylpyridinium chloride

The suitability of an analytical method is its ability to measure the analyte accurately and specifically in the presence of other components in the sample. The UV-visible light was used to be the detector at wave length 254 nm. This is the optimal wavelength giving the highest sensitivity without interference of any diluents of the formulations, and HPBCD showed no absorbance between 200-400 nm. The data was demonstrated in the Appendix D.

Furthermore, in this wavelength, the detection of ketoconazole and HPBCD inclusion complexes did not changed and similar to the detection of ketoconazole powders. This result concluded that HPBCD did not affect the absorption spectra of ketoconazole inclusion complexes. Therefore, this method had high specificity for analysis of ketoconazole in either its free form or inclusion complexes.

1.2 In the presence of degradation products of ketoconazole

Ketoconazole was very high sensitive to the UV light and temperature. To investigate the specificity of the HPLC system for analysis of ketoconazole and its degraded product, the ketoconazole solution was accelerated for degradation by UV light and heat (Skiba et al., 2000). The results showed that there were no interferences from the peak of degraded products. The data appear that peak area of ketoconazole was decrease, as showed in the Appendix D.

Thus, it was concluded that this method was high selectivity and sensitivity for the analysis of ketoconazole.

2. Linearity

The linearity of an analytical method is its ability to show test results accurately. Linearity should be evaluated by visual inspection of a plot of signals as a function of analyte concentration or content. In some cases, to obtain linearity between assays and sample concentrations, the data may need to be subjected to a mathematical transformation before to the regression analysis. Data from the regression line may be helpful to provide mathematical estimates of the degree of linearity. For the establishment of linearity, a minimum of 5 concentrations is recommended.

The standard curves of ketoconazole in 0.1 M acetate buffer pH 5 are shown in Figure 9. The standard curve was found to be linear with good coefficient of determination (R^2). The coefficient of determination (R^2) was 0.999. This result indicated that HPLC method was acceptable for quantitative analysis of ketoconazole in the specified range. The equations of standard curves according to Beer's Law plot were used for calculating the concentration of ketoconazole in HPBCD solution.

Table 8. Data for standard curve of ketoconazole by HPLC method.

concentration of ketoconazole	Peak area ratio			mean	SD	%CV
	set1	set2	set3			
40	5.133	4.97	5.17	5.09	0.08	1.70
60	7.23	7.35	7.41	7.33	0.07	1.02
80	9.97	10.04	9.87	9.96	0.07	0.70
100	11.98	12.03	11.87	11.96	0.07	0.56
120	14.32	14.13	14.12	14.19	0.09	0.65
140	16.83	16.56	16.73	16.71	0.11	0.68

Triplicate determination.

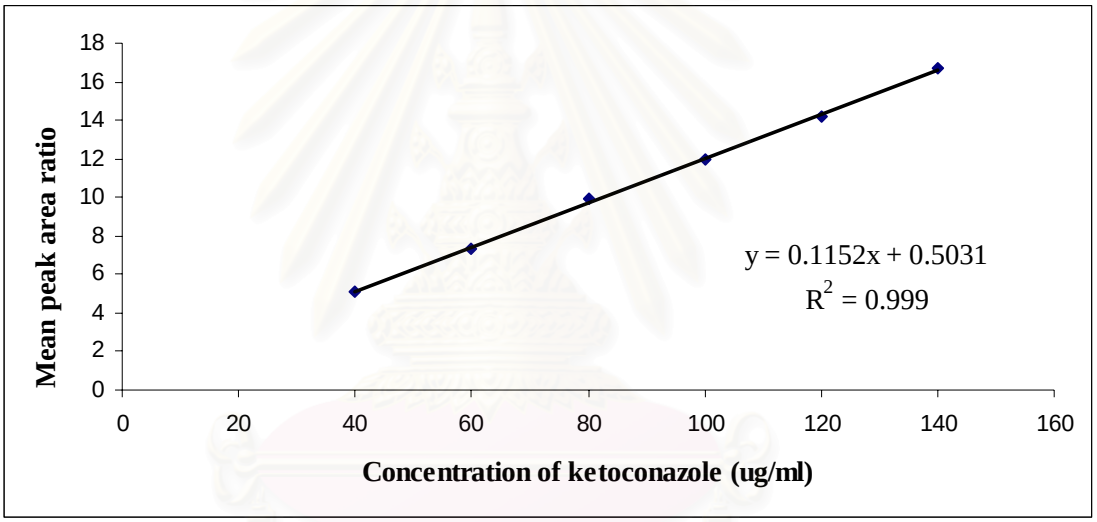


Figure 9. Standard curve of ketoconazole by HPLC method**.**

3. Accuracy

The accuracy of an analytical method describes the closeness of mean test results obtained by the method to the true value, concentration, of the analyte. Accuracy is examined by replicate analysis of samples containing known amounts of the analyte. Accuracy should be operated using a minimum of five determinations for each concentration. A minimum of three concentrations in the range of expected concentrations is determined for fives triplicates. The deviation of the mean from the true value defined as the measure of accuracy.

The percentages of analytical recovery of each ketoconazole concentration in 0.1 M acetate buffer pH 5 is shown in Table 9. All of the percentages analytical of recovery, for all drug concentrations in 0.1 M acetate buffer pH 5, were demonstrated in Table 9. The data showed percentage of confident interval less than 2 % that indicated the high accuracy of this method. Therefore, it could be used for analysis of ketoconazole on all concentration study

Table 9. The percentages of analytical recovery of ketoconazole in 0.1 M acetate buffer pH 5 by HPLC method

Conc.	Estimated concentration								%
(µg/ml)	1	2	3	4	5	mean	SD	%CV	recovery
50.00	50.504	50.739	49.923	50.975	50.371	50.502	0.397	0.787	101
90.00	90.769	90.863	89.874	91.139	90.807	90.690	0.479	0.528	101
110.00	109.821	111.286	112.007	112.095	112.045	111.451	0.970	0.870	101

Triplicate determination

4. Precision

The precision of an analytical method explains the closeness of each measurement of an analyte when the procedure is operated repeatedly to multiple aliquots of a homogeneous sample. Precision should be measured using a minimum of five determinations per concentration along with a minimum of three concentrations in the specified range. The precision determined at each concentration level should not exceed 2% of the coefficient of variation (CV). Precision is further divided into within-run which assesses precision during a single analytical run, and between-run which measures precision with different time. The precision of an analytical method is usually shown as the standard deviation or coefficient of variation.

The precision of the analysis of ketoconazole in 0.1 M acetate buffer pH 5 by HPLC method was determined both within run and between run precision as

showed in Tables 10 and 11, respectively. The values of all percentages coefficient of variation were quite low, they should not more than 2% (USP 24, 2000). Hence, the HPLC method was accurate for quantitative measurement of ketoconazole in the studied range.

Table 10. The within run precision of ketoconazole in 0.1 M acetate buffer pH 5 by HPLC method.

Ketoconazole concentration (µg/ml)	**Quantitative of ketoconazole**							
	set1	**set2**	**set3**	**set4**	**set5**	**mean**	**SD**	**%CV**
50	50.5	50.74	49.92	51	50.37	50.502	0.397	0.787
90	90.77	90.86	89.87	91	90.81	90.69	0.479	0.528
110	109.82	111.28	112.007	112	112.04	111.451	0.97	0.87

Triplicate determination.

Table 11. The between run precision of ketoconazole in 0.1 M acetate buffer pH 5 by HPLC method.

Ketoconazole concentration (µg/ml)	**Quantitative of ketoconazole**							
	set1	**set2**	**set3**	**set4**	**set5**	**mean**	**SD**	**%CV**
50	51.289	50.228	50.502	51	50.178	50.73	0.600	1.184
90	89.67	90.211	90.69	91	89.44	90.138	0.572	0.634
110	108.81	108.818	111.45	111	109.519	109.891	1.206	1.098

Triplicate determination

In conclusion, the analysis of ketoconazole by HPLC method developed in this study showed good specificity, linearity, accuracy and precision. Therefore, this method was suitable to use for determination of the quantitative of ketoconazole in the study.

B. PREPARATION OF KETOCONAZOLE SOLUTION AND SPRAY DRIED POWDERS

1. Phase Solubility Study of Ketoconazole with HPBCD at acetate buffer pH 5

The equilibration time to obtain phase solubility diagram was affected by effects of types of drug, solubilizing agents, temperatures, etc. Consequently, It was necessary to determine the exact time for equilibrium before studying the phase solubility diagram. The interaction of ketoconazole and cyclodextrins was demonstrated to need the equilibration time for 3 days (Im-erbsin, 2545). Phase Solubility data of ketoconazole with HPBCD in 0.1 M acetate buffer pH 5 at ambient temperature are shown in Table 12. The phase solubility diagram was constructed by plotting the molarity of ketoconazole solubilized in solution against the molarity of HPBCD added.

It was observed that the apparent solubility of ketoconazole increased linearly as a function of HPBCD concentration over the concentration range studied. The linearity was characteristic of A_L-type system and suggested that water soluble complex was formed in solution, as shown in Figure 9. It was obvious that HPBCD increased the solubility of ketoconazole in a great extent due to the formation of an inclusion complex in solution. This result was the same as the study reported by Diaz, Mendez et al (1996). Furthermore, the slope values were always reported lower than one, this could be indicated that the inclusion complex in the molar ratio of 1:1 between the guest (ketoconazole) and host (HPBCD) molecules was obtained.

The apparent stability constant (K_s) was calculated from the A_L type phase solubility diagram. Generally, linear diagrams were formed when each complex contained only one molecule of CD, assuming that only 1:1 (M:M) complexes were formed. Thus, the association constant was calculated based on the formation of 1:1

complexes according to the Equation 3. Consequently, the association constant (K_s) of ketoconazole: HPBCD inclusion complex in 0.1 M acetate buffer pH 5 was determined to be 714.76 M^{-1}. This constant value was different from Im-erbsin (2002) (K_s = 1263 M^{-1}), that the lower constant might be effected from the source of HPBCD, that due to the impurities and the value of degree substituted of HPBCD that was lower than that used in previous study.

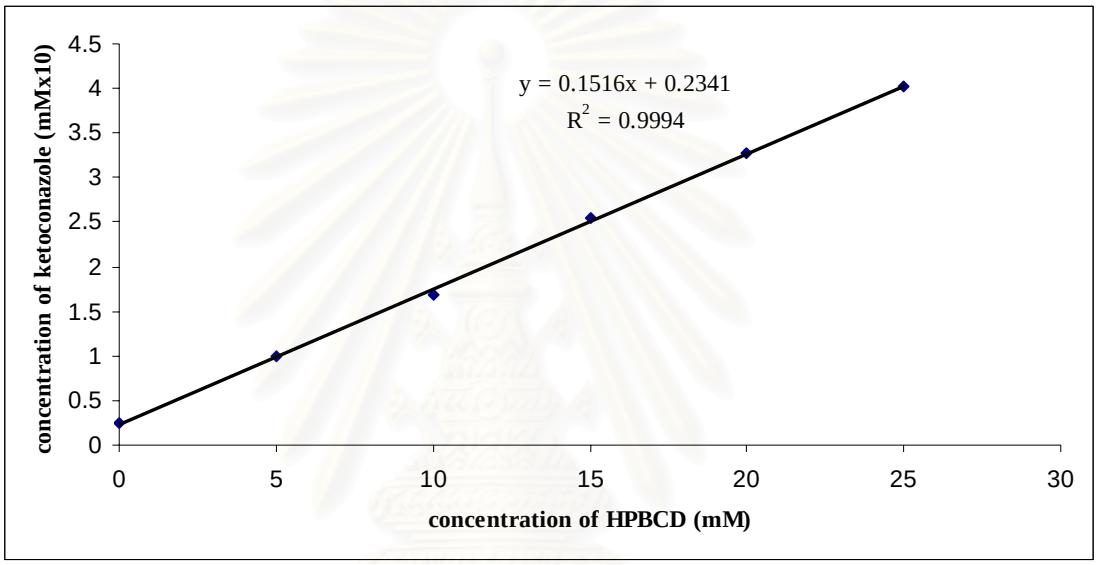


Figure 10. Phase solubility diagram of ketoconazole with HPBCD in 0.01 M acetate buffer solution at ambient temperature.

Table 12. Phase solubility data of ketoconazole with HPBCD in 0.1 M acetate buffer pH 5 at ambient temperature

Concentration of HPBCD		concentration of ketoconazole by HPLC	
(%w/v)	(mM)	(µg/ml)	(mM)(x10)
0	0	13.344	0.251
0.7	5	53.188	1.001
1.4	10	89.404	1.682
2.1	15	135.199	2.544
2.8	20	174.071	3.275
3.5	25	213.593	4.019

Triplicate determination.

From the phase solubility diagram data (Figure 10 and Table12), the intrinsic solubility of ketoconazole was demonstrated very low as 13.344 µg/ml or 0.251 x 10 M. This was similar to that reported previously as 0.131 mg/ml (Imerbsin, 2002). This demonstrated that it was impossible to prepared 2%w/v solution of ketoconazole in the absence of solubilizing agent. Thus, to prepare 2% ketoconazole solution, it have to used HPBCD. The amount of HPBCD for prepare 2% ketoconazole solution was calculated from the linearity equation of solubility diagram, that was 32.33 g of HPBCD used for 2g of ketoconazole. However, because of the impurities of HPBCD, the amount of HPBCD was used as 35 g for ketoconazole 2 g in this study, and stirred for 48 hours by magnetic stirrer.

C. PHYSICOCHEMICAL PROPERTIES OF KETOCONAZOLE SPRAY DRY POWDERS

1. Yield from Spray Drying

The percentage of yield was calculated from the weight of spray dry powder of each condition divided by the total weight of initial dry materials and multiplied with 100. The initial weight of materials for spray drying included ketoconazole, HPBCD and buffer, sodium acetate and acetic acid. From the phase solubility diagram the preparation of ketoconazole 1 g into 50 ml solution required HPBCD 17.5 g in acetate buffer solution pH 5.

Table 13. The percentages of yield from all spray dry conditions

Formulation Condition	inlet temperature (°C)	feed rate (ml/min)	%yield
F1	100	3.5	48.19
F2	100	7.0	40.76
F3	100	10.5	33.13
F4	120	3.5	58.33
F5	120	7.0	54.87
F6	120	10.5	52.12
F7	140	3.5	51.39
F8	140	7.0	47.23
F9	140	10.5	36.81

The % yield obtained was found maximal at 58.33% and minimal at 33.13 %. It was noticed that the high yield was obtained at the same inlet temperature when using a lower feed rate because at lower feed rate the spray dried products might expose to hot air much more than higher feed rate, this resulted decreasing the sticky products on chamber, and the same result could observed in higher temperature at the same feed rate for the same reason.

2. Moisture Content

The percentage of moisture content of the spray dried products was determined suddenly after calculated the % yield. This result might be varied due to the hygroscopicity of the spray dried products. In general, the humidity affected to the properties of solid substances not only the physical property but including the chemical property as well, especially the effected to the stability. Additionally, the moisture might be resulted to the aggregated of the spray dried powders that leaded to the poor of flowability of products. For this study, all conditions were given the percentage of moisture content in the spray dried products similarly. These values did not depended on neither inlet temperatures nor feed rates,so this could not be used as response in the optimization.

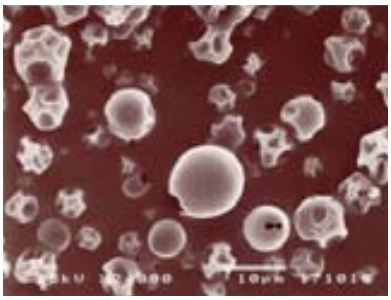
Table 14. The percentages of moisture content of all spray drying conditions.

Formulation Code	inlet temperature (°C)	feed rate (ml/min)	% moisture content
F1	100	3.5	3.54
F2	100	7.0	2.45
F3	100	10.5	4.78
F4	120	3.5	2.83
F5	120	7.0	3.32
F6	120	10.5	3.41
F7	140	3.5	3.91
F8	140	7.0	3.45
F9	140	10.5	2.23

3. Particle Morphology

The shape and surface topography of the spray dried powders were found to be affected by the formulation and processing parameters. The observation of size, shape and topography were done by scanning electron microscopy (SEM). Figure10-12 showed the scanning electron photomicrographs of nine conditions of spray dry powders of ketoconazole: HPBCD inclusion complexes. The spray dried particle from each condition was different in sizes with a rough surface and some part was loosely agglomerate together.

The sphericity of particles obtained from all spray drying conditions was good. The surfaces of the microspheres were mixed by with some part of smooth particles and some part of rough particles. However, the loose agglomeration of particles was observed in some spray dry conditions. It was observed that at higher temperature, the shrinkage of particles surfaces was found increase the result of water loss from the drying droplets at the early stage of processing. This effect was easily occurred at high temperature. This result was consistent with a previous study by Columbano, Buckton and Wikeley (2003). From the SEM, it demonstrated the agglomeration of spray dried particles. This result could observed in high temperature conditions, due to the shrinkage of spray dried powders that was the effected of quickly evaporation of spray dry particles. This effect was revealed by light scattering that showed the mean size of particle about 11-14 μm and the value of size distribution was narrow about 2. This indicated that the agglomeration from SEM technique was a loose agglomeration because it could be easily dispersed in mineral oil from light scattering technique. This agglomeration might be due to the inlet temperature that generated the shrinkage particles that could easily be aggregated.



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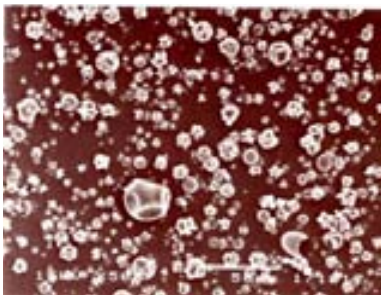
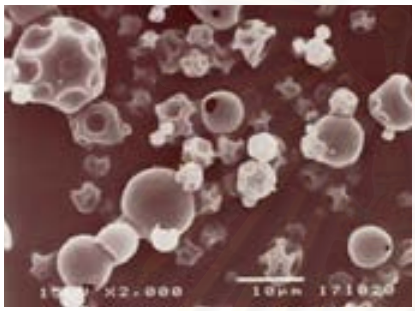


x500

Feed rate 3.5 ml/min



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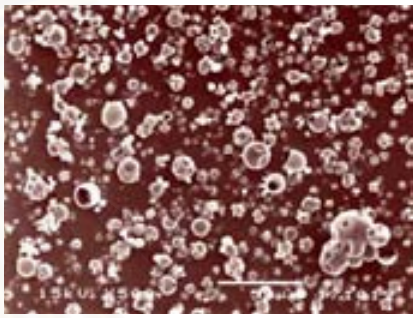
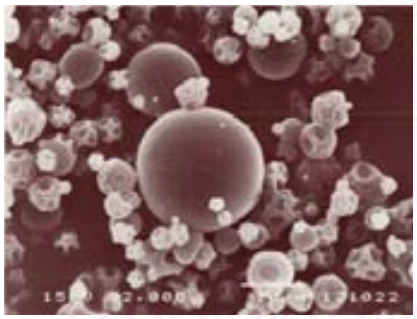


x 500

Feed rate 7.0 ml/min



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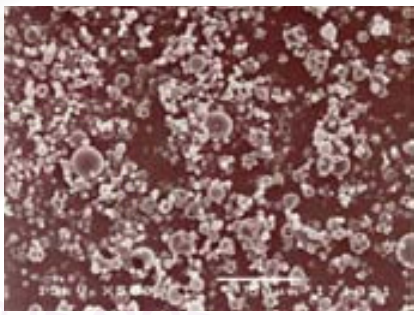
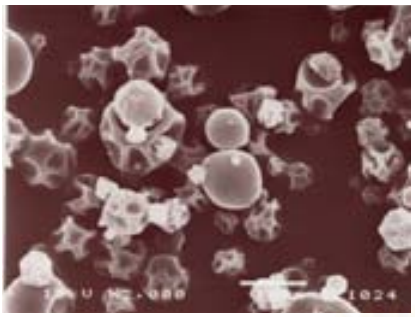


x 500

Feed rate 10.5 ml/min

Figure 11. The morphology of particles of spray dried products under 100°c and three levels of feed rate, 3.5, 7.0 and 10.5 ml/min.



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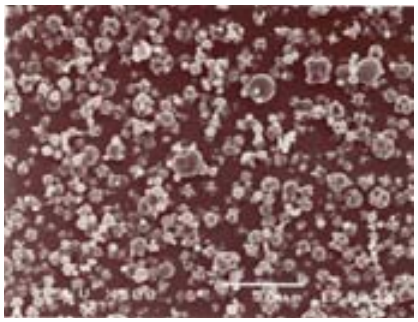
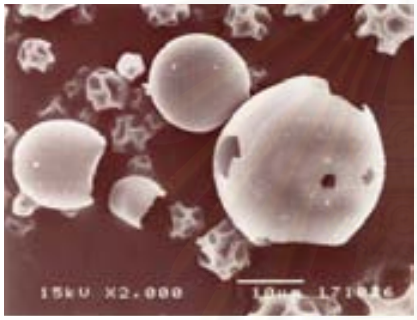


x 500

Feed rate 3.5 ml/min



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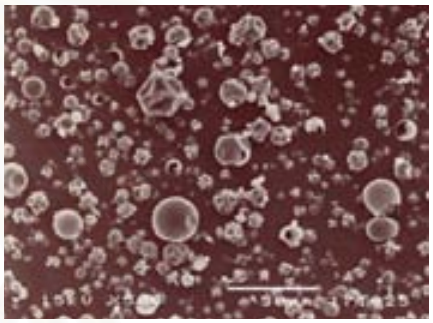
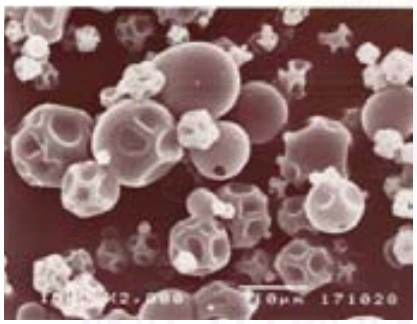


x 500

Feed rate 7.0 ml/min



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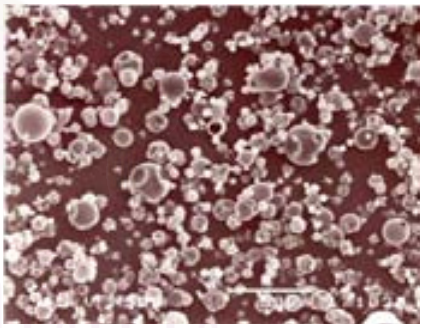
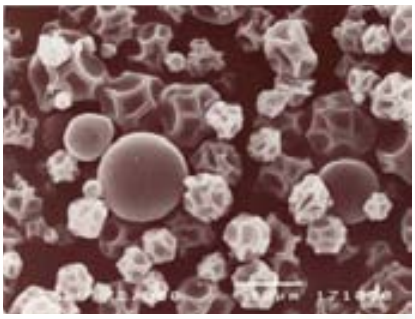


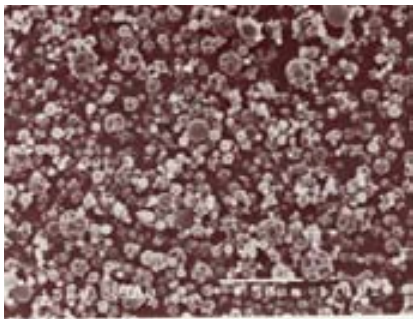
x 500

Feed rate 10.5 ml/min

Figure 12. The morphology of particles of spray dried products under 120°c and three levels of feed rate, 3.5, 7.0 and 10.5 ml/min.

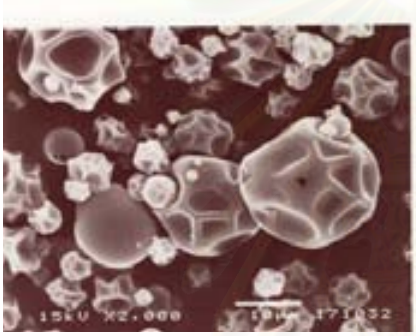


x 2000



x 500

Feed rate 3.5 ml/min



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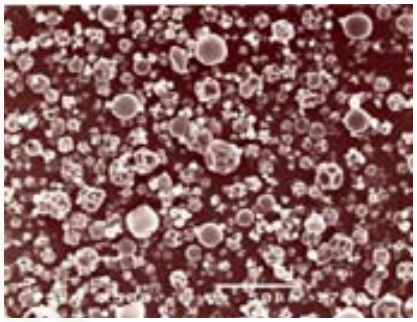
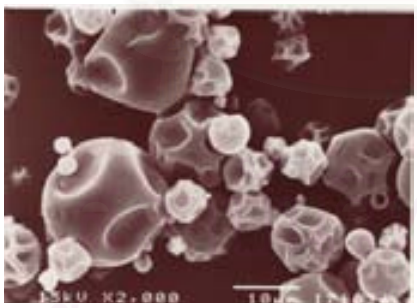


x 500

Feed rate 7.0 ml/min



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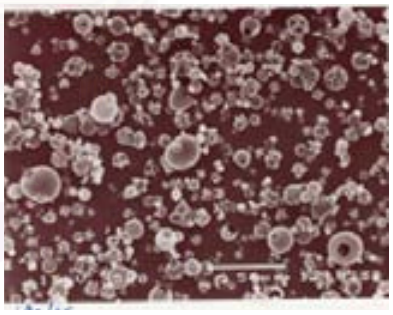

x 500

Feed rate 10.5 ml/min

Figure 13. The morphology of particles of spray dried products under 140°c and three levels of feed rate, 3.5, 7.0 and 10.5 ml/min.

4. Particle Size and Size Distribution

Usually, spray dried powders were obtained in a median diameter range between 2 and 50 μm with a narrow size distribution (Broadhead, Rouan and Rhodes 1992). In this study, the average particle size (D [4,3]) and particle size distribution (Span value) were determined and compared between different conditions of spray dried powders. The particle sizes were ranged from 11.09 to 14.33 μm and the span value ranged from 2.01 to 2.09 (Table 15).

Table 15. The analytical data of size distribution of spray dried powders

Formulation code	Average particle size D [4,3] (μm)	Span (SD)
F1	12.77 ± 0.11	2.03 ± 0.00
F2	11.09 ± 0.05	2.09 ± 0.01
F3	11.81 ± 0.10	2.09 ± 0.01
F4	11.75 ± 0.03	2.01 ± 0.00
F5	11.83 ± 0.05	2.02 ± 0.00
F6	13.32 ± 0.10	2.08 ± 0.00
F7	14.27 ± 0.06	2.04 ± 0.01
F8	14.08 ± 0.05	2.08 ± 0.01
F9	14.33 ± 0.02	2.07 ± 0.00

* formulation code was described the condition in table 14

** Triplicate determination

By the light scattering measurement, it showed the size of the microspheres ranged from 11-14 micron for all conditions. Due to the determination of particle size obtained from SEM, this method provided the same size as the average obtained from light scattering method. Thus, it was concluded that the particle size of all conditions obtained from the light scattering method and the SEM method were similar (Figure11, 12 and 13 and Table15). It implied that the loose aggregation occurred. Due to the similarly of size of particles from each condition, this could not used the value of particle size as response of the optimization.

5. Drug Content

Percentages of drug content in spray dried powders of all conditions are showed in Table 16. Each formulation had good percentage of loading drug, above 90%. This result agreed with Hascicek, Gonul and Erk (2003) that spray dry technique was provided high drug loading efficiency.

The percentage drug contents obtained showed no effect from processing parameters, inlet temperature and feed rate. Additionally, they were not related to the yield obtained. This concluded that the spray drying method was suitable for ketoconazole due to it provided high drug content for all conditions.

Table 16. The percentages of ketoconazole in spraydried for 9 conditions.

Formulation code	% content
F1	97.96
F2	98.8
F3	99.21
F4	99.4
F5	98.83
F6	99.83
F7	99.31
F8	99.54
F9	98.95

***** formulation code described in Table 14.

6. Flow Property

The flowability of spray dried powders was determined by fixed funnel method, the data was showed in Appendix F. From data observed, it concluded that spray dried powders from all conditions were poorly flowability due to the range of angle of repose about 40-50°. These results could be confirmed with SEM that revealed the shrinkage of morphology of powders. This shrinkage might be the reason that powders were poorly flow.

7. Differentials Scanning Calorimetry (DSC)

The DSC thermograms of ketoconazole, HPBCD, physical mixture of ketoconazole and HPBCD and spray dried powders of ketoconazole and HPBCD are showed in Figure 14.

The thermograms of ketoconazole demonstrated endothermic peaks, which might be attributed to melt at about 154°C. This indicated that the substance was in a crystalline form (Figure 14). This endothermic peak disappeared in the thermogram of spray dried powder of ketoconazole and HPBCD. (Figure 14). Which revealed that spray drying process promoted the appearance of inclusion complex of HPBCD and ketoconazole and might be generated amorphous form of ketoconazole or both. Similar results were reported from spray dried particle of many drugs such as paclitaxel (Mu et al., 2005).

The absence of the melting peak of ketoconazole in the DSC thermograms of spray dried products may be attributed to the transformation of ketoconazole into an amorphous form or the inclusion complex formation or to both reasons. (Diaz,Mendez et al.,1996). The effect of HPBCD on the DSC thermogram of the guest molecule could be observed as the broadening and shifting or disappearance of certain peaks. For the disappearance of melting peak, no energy absorption is observed at the melting temperature of the guest when the guest is transformed to inclusion complexes. There is no crystalline guest structure to absorb energy. The data was demonstrated in the Figure 14.

Additionally, in the DSC thermograms of ketoconazole spray dried powders, a broad endotherm ranged from 70-100 °C was observed. This might be due to the dehydration of moisture in the spray dried powder sample.

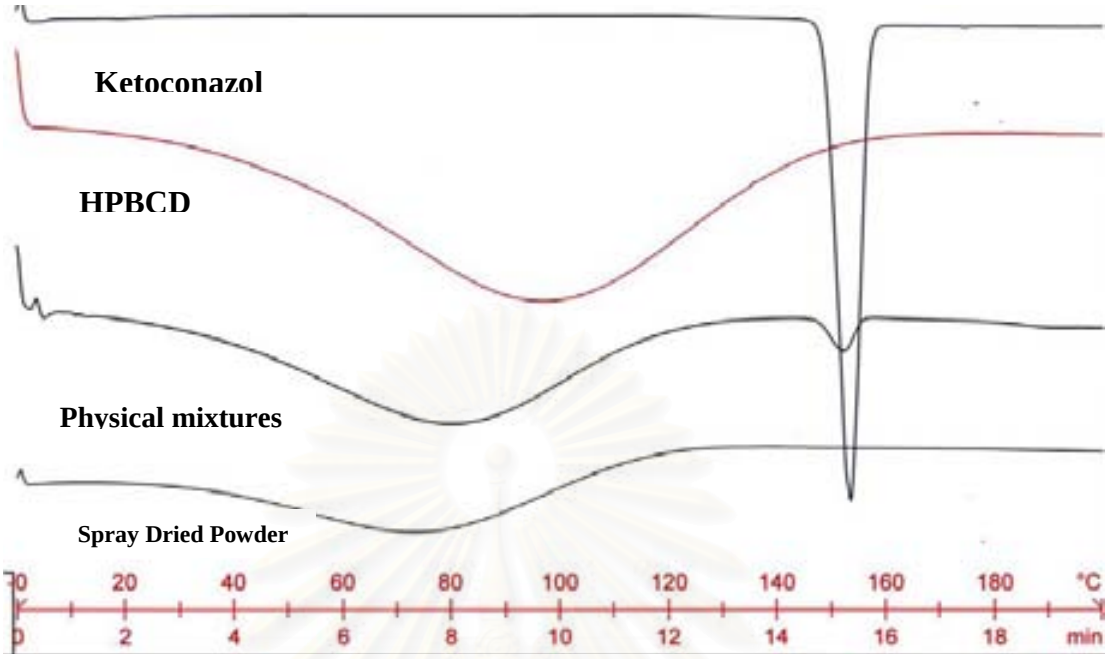

Figure 14. The thermograms of ketoconazole, HPBCD, physical mixtures of ketoconazole ans HPBCD and spray dried powder of ketoconazole with HPBCD.

8. Powder X-ray diffraction

The powder x-ray diffractograms of ketoconazole, HPBCD, ketoconazole and HPBCD spray dried powders, the physical mixture of ketoconazole with HPBCD are showed in Figure 15.

The diffractogram of ketoconazole was exhibited series of intense diffraction peaks, which indicative of their crystalline characteristic. So, the x-ray diffraction pattern of ketoconazole showed crystallinity. Ketoconazole provided a sharp and intense diffraction peaks due to its crystalline form. In contrast, HPBCD gave a diffuse diffraction pattern because of its amorphous form. For the physical mixture, the X-ray diffractogram was in combined pattern between the diffraction pattern of ketoconazole and HPBCD and gave the peak with lower intensity. Furthermore, the physical mixture of ketoconazole with HPBCD as ratio 1:17.5 w/w showed the disappearance of some peak of ketoconazole.

In contrast, the diffractograms of spray dried powder of ketoconazole and HPBCD showed the disappearance of ketoconazole diffractogram peaks, that transformed to be an amorphous pattern diffractogram. This results exhibited that ketoconazole existed in an amorphous form or inclusion complexation occurred or both. In general, formation of amorphous inclusion complexes made the disappearance of certain peak or made it turned to be less sharp than the pure compound or physical mixtures. The data was demonstrated in the Figure 15.

However, it was noticeable that there was a small peak in the diffractogram of spray dried powders. This peak might be a new one or slightly shift from 7° observed in the diffractogram of ketoconazole to 6°.

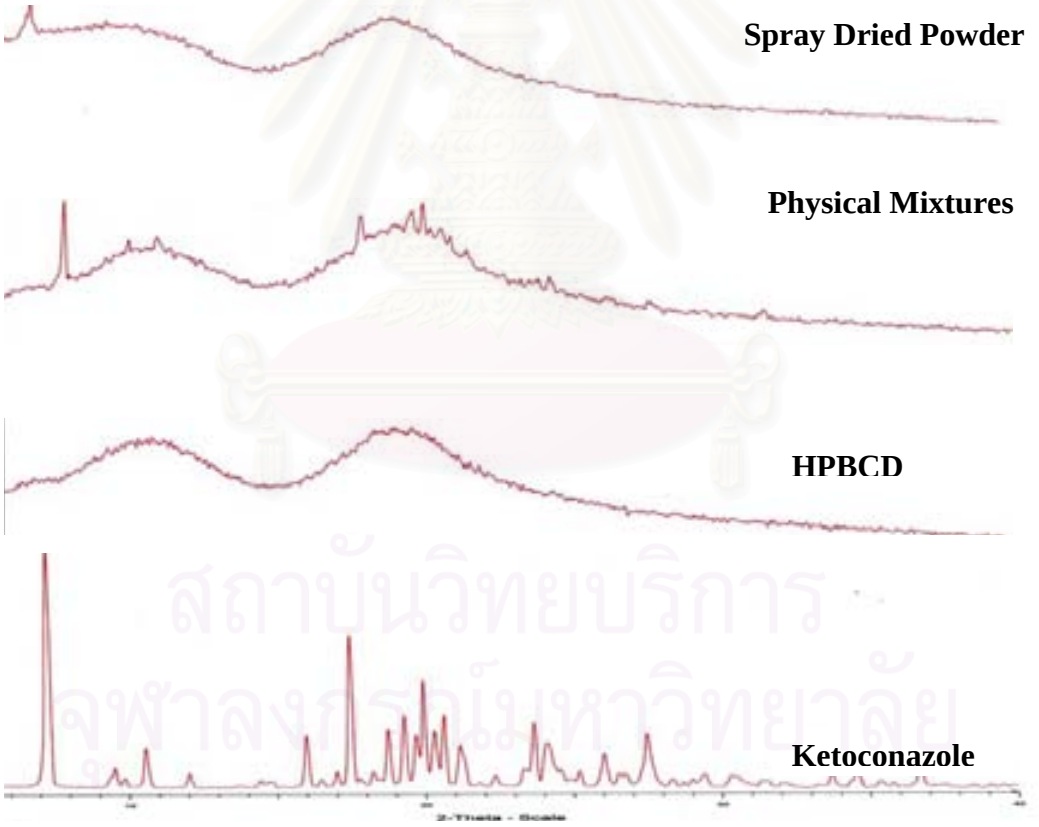

Figure 15. The diffractograms of ketoconazole, HPBCD, physical mixtures of ketoconazole ans HPBCD and spray dried powder of ketoconazole with HPBCD.

D. OPTIMIZATION OF SPRAY DRY PROCESS

Powders of ketoconazole:HPBCD complexes were prepared by two steps. Firstly, the solubilization of ketoconazole by adding ketoconazole powders to HPBCD in acetate buffer solution pH 5. Secondly, the complexes solution was dried by spray drying. A Spray Dryer, Büchi, Labortechnik AG, Flawil, 1997) equipment was performed. The instrument settings were important and affected to the product properties. Experimental design was constructed to examine the effect of factors on yield of spray dry products. In this study, two processing parameters, inlet temperature and feed rate were inveatigated. The optimal operating conditions were determined by response surface methodology. Central composite design was used to evaluate the optimal experiment parameters for producing the maximal percentage of yield.

1. Experimental Design: Full Factorial Design

Factorial design is one of the effective processes that used for determination to the response of the experiment study. This method was suitable for two or more treatment variables and it is reasonable to trust that the effect of each variable was interacted. A full factorial design declares treatment groups for all possible combinations of different treatment variables and levels. The comparison of the response variable is made between all groups, representing the various combinations of treatments.

In this study, values of nine treatments from factorial design 3^2 were observed. Two parameters, inlet temperature and feed rate, as well as three levels of each parameter were evaluated to determine the percentage of yield from spray dried products. The product, yield, were obtained from the weights of product collector and cyclone. Other than the percentage of yield, physicochemical properties of spray dried products were determined. The values of nine treatments were computed by Design-Expert version 7.1.3 statistical software.

2. Optimization Design: Central Composite Design (CCD)

In the second process, the two factors, inlet temperature and feed rate, were selected to optimize the % yield of spray dried powders. While other parameters, % solid content, % additive and fan speed, had less influence on the response, and, they were fixed at the certain value in the further study. In this study, the non-significant parameters, % solid content, % additive, were fixed at 2% ketoconazole, 35% of HPBCD and fan speed at level 50 (300 m^3/hr.) respectively.

To explore the region of the response surface of the optimum condition, an approximation to the response surface could be developed.

Table 17. Factorial design of two parameters with three levels and the response (% yield)

Treatment	Inlet temperature	Feed rate	%Yield
1	100	3.5	48.19
2	100	7.0	40.76
3	100	10.5	33.125
4	120	3.5	58.33
5	120	7.0	54.87
6	120	10.5	52.12
7	140	3.5	51.39
8	140	7.0	47.23
9	140	10.5	36.81

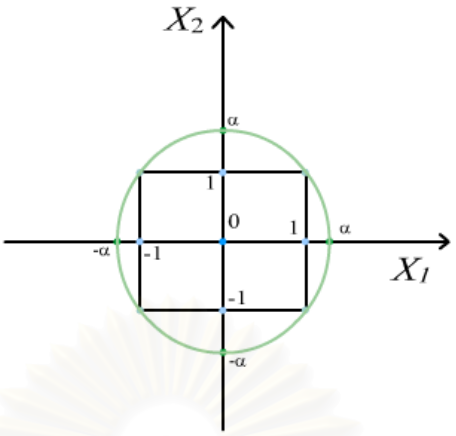

Figure 16. Central composite design for two variables.

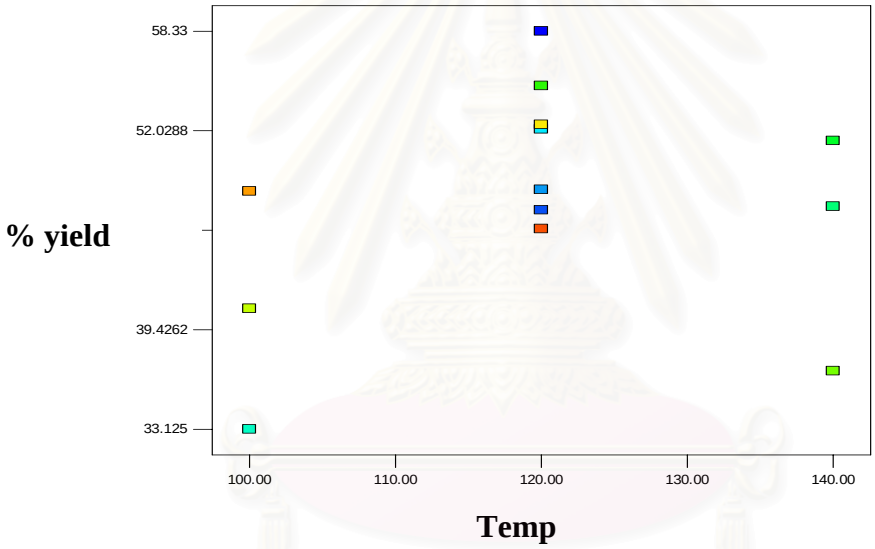

Figure 17. Scattered plot between inlet temperature and % yield on spray dried powders.

In this study, it showed that the thermal energy given by range of inlet temperatures used, was maximized effective at the sufficient energy to allow complete drying, due to the fluctuation of %yield, that was varied up to the inlet temperature. Additionally, a range of feed rates used, was varied from 3.5-10.5 ml/min. So, the higher energy was needed for high feed rate to make the spray dried products dried absolutely. In these cases, sticking occurred in the drying chamber and represent with high moisture content. However, for the high level of inlet temperature, 140 °C, the percentage of yield was not increasing as much as 120 °C, this might be concluded at

high temperature the powders were dried and they were very bulky and might be stuck the other parts of the equipment, not be collected in the collector.

The results of the response surface model fitting in the form of analysis of variance for %yield are given in Table 18. ANOVA was required to test the significance and sufficiency of the model.

Table 18. Central composite design matrix of two parameters and the observed responses

treatment	inlet temperature	feed rate	%yield
1	100	3.5	48.19
2	100	7.0	40.76
3	100	10.5	33.125
4	120	3.5	58.33
5	120	7.0	54.87
6	120	10.5	52.12
7	140	3.5	51.39
8	140	7.0	47.23
9	140	10.5	36.81
10	120	7.0	52.41
11	120	7.0	48.29
12	120	7.0	47.00
13	120	7.0	45.81

Table 19. ANOVA for response surface quadratic model of % yield.

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F
Model	481.0414	5	96.2083	5.5352	0.0222
A-temp	29.7260	1	29.7260	1.7102	0.2323
B-feed	214.2635	1	214.2635	12.3273	0.0098
AB	0.0588	1	0.0588	0.0034	0.9552
A^2	230.7328	1	230.7328	13.2749	0.0082
B^2	12.0633	1	12.0633	0.6940	0.4323
Residual	121.6681	7	17.3812		
Lack of Fit	63.1878	3	21.0626	1.4407	0.3559
Pure Error	58.4803	4	14.6201		
Cor Total	602.7095	12			

The analysis of variance (ANOVA) was used to test the significance of the model. The Fisher variance ratio, F-value, which is a statistically measure of the corrective effect of factors in explaining the variation of data, could be calculated from ANOVA by dividing the mean square due to model variance by that due to error variance ($F_{model}=S_e^2/S_r^2$). The greater, the F-value is from unity, the more certain it is that the factors explain the variation in the data, and the estimated factor effects were correct.

In this step, the analysis of variance of the regression model demonstrated that the model of % yield were significant ($P<0.05$). The response surface quadratic model was an adequate model for %yield, as were evident from the F-test ($F_{\%yield}=5.535$) and a low probability values (%yield:$P>F=0.0222$) (Table 19). Values of "Prob>F" were less than 0.05 indicated that model terms were significant ($P<0.05$) (Montgomery, 2001). Lack of fit for the model was not significant ($P>0.05$). Non significant lack of fit was good .

The goodness of fit of the models was examined by the determination coefficient (R^2). The R^2 values provided a measure how variability in the observed response values could be explained by the experimental factors and their interactions.

The R^2 values are always between 0 and 1. The closer R^2 value is to 1, the stronger the model is, and the better it predicted the response. When expressed as a percentage, R^2 is interpreted as the percent of variability in the response that explained by the statistical model (Montgomery, 2001).

The goodness of fit of the models was checked by R^2. In this step, the values of $R^2 = 0.7981$, indicated that 79.81% of variability in the response could be explained by the model for %yield.

The P-values were used as a tool to check the significance of each of the coefficients which were necessary to understand the pattern of the mutual interactions between the test variables (Montgomery, 2001). This implied that, result of %yield, the first order main effects of feed rate (B) and second order main effect of inlet temperature (A^2) were highly significant as were evident from their P-values= 0.0098 and 0.0082, respectively, so they were the most significant effect ($P<0.05$) (Table 14).

By applying multiple regression analysis on the experimental data, the experiment results of the central composite design were fitted with a second-order polynomial equation and linear equation for %yield. The coeffients of regression equation linking the response to the experimental variables and interactions are indicated in Table 20.

The coefficients of regression equations linking the responses to the experimental variables and interactions are indicated in Table 20.

Table 20. Coefficient of the regression equation linking the responses, %yield, to the experimental factors and major interactions (code unit).

Factor	Coefficient Estimate
Intercept	50.66431
A-temp	2.225833
B-feed	-5.97583
AB	0.12125
A^2	-9.14009
B^2	2.089914

Thus, the mathematical regression models for %yield fitted in the coded factors were given as following:

$$\% \text{ Yield} = 50.66 + 2.23A - 5.98B + 0.12AB - 9.14A^2 + 2.09B^2 \quad \ldots\ldots\ldots\ldots (9).$$

Where, A and B were the coded values of the test variables;

A: inlet temperature

B: feed rate

From final equation in term of coded parameters, this suggested that increasing inlet temperature (A^2) decreased the percentage of yield as well as feed rate (B) that was given the same result. The percentage yield of different value of the variables could be predicted from the response surface plot (quadratic model). Although, it may seen from the equation 9 that increasing inlet temperature (A) and feed rate (B^2) could improved %yield, but these term, A and B^2, were not significant effect on %yield (Table 19).

The normal probability plot of the residuals is an important characteristic tool to detect and declare the systemic departures from the assumptions that errors are normal distributed and are independent from each other (Montgomery, 2001). Figure 18 demonstrates normal probability plots of the results. The normal probability plot of the “Studentised” residuals specified that some few violation of the assumptions outlying the analyses.

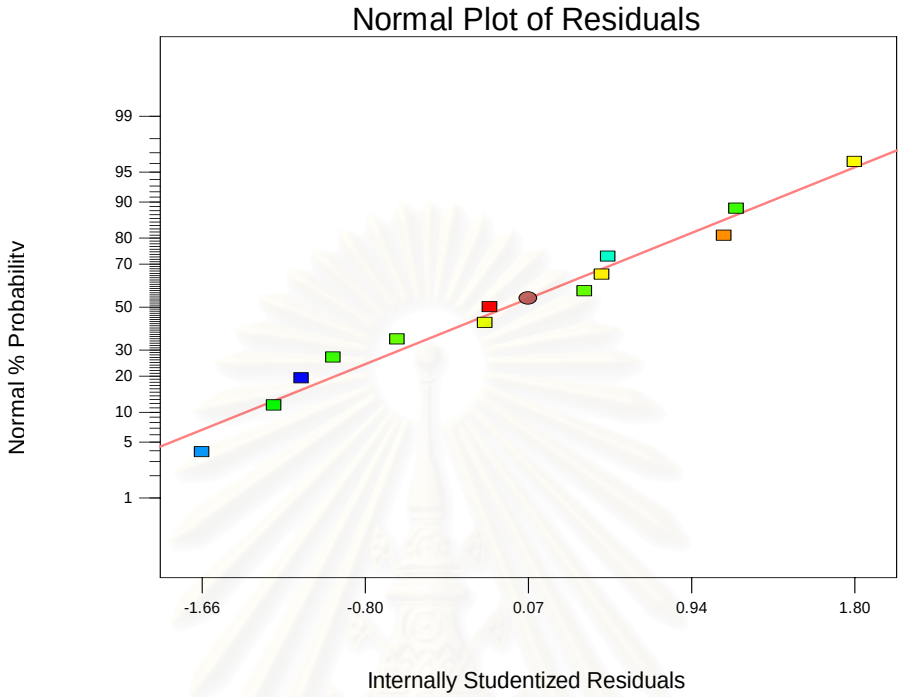

Figure 18. The normal proability plots of the % yield.

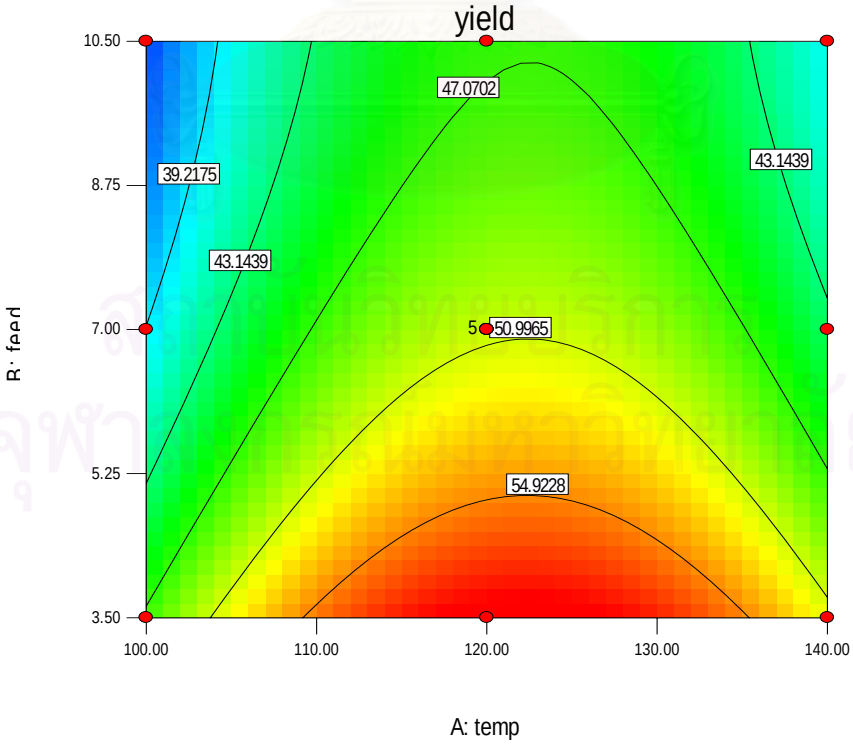

Figure 19. Contour plot of % yield and inlet temperature.

Due to the two models obtained, a graphical optimization was also conducted using the design expert version 7.1.3 statistical software. The method consisted of overlaying the curves of the models according to the criteria imposed. A comparison of the results obtained both experimentally and mathematically revealed that there was an effect to %yield. However, the main objective was to obtained %yield as high as possible. The criteria were adopted; %yield higher than 58.59% (the highest %yield in the second step in spray drying process.

As mentioned on the effect to % yield, the attained overlaying plot showed all areas that were evaluated. Thus, a point was chosen on the graph (marked by the square), accompany with the prediction value from Design Expert version 7.1.3. This point was assigned as optimum point and corresponded to 120 °C inlet temperature and feed rate 3.5 ml/min. By this condition, the model predicted 58.73 %yield (a variation = 51.78-65.66 % being possible) in the confidence range of 95%.

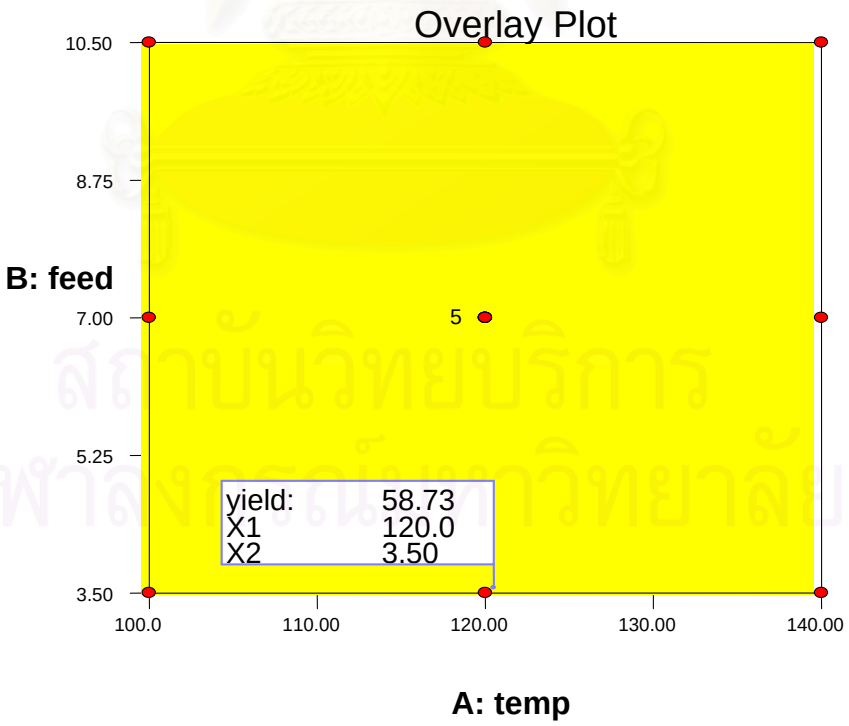

Figure 20. The optimum region by overlay plot of % yield, as response, evaluated as a function of inlet temperature and feed rate.

The statistical interpretation of the results concerning yield using the contour plot, that let us to choose optimal operating conditions. These parameters had to maximize yields.

To confirm the result, three batches were produced using the optimal conditions to validate the fabrication process (Table 21).

Table 21. The optimum region by overlay plot of two parameters and the observed response.

Code	Parameters		Response
	Inlet temperature	feed rate	(% yield)
O1	120	3.5	62.34
O2	120	3.5	52.48
O3	120	3.5	58.74

The results in Table 21 demonstrated the observed means and standard deviations of the responsed obtained for yield to be within a variation 51.78-65.66 %. They were in range of the prediction interval at 95 % confidence level. These results showed that the model fitted the experimental data well and described the region studied well.

E. PREPARATION OF KETOCONAZOLE DRY MIXTURES FOR ORAL SOLUTION

1. Formulation of Ketoconazole Dry Mixtures for Oral Solution

Due to the formulations of ketoconazole dry mixtures for oral solutions, there were many diluents for the formulations. The additives of the formulation were such as diluents, which might be sucrose as sweetener as well, viscosity enhancers, artificial sweeteners, preservatives, colors, and flavors, etc. However, the formulations were theoretically based on good properties of dry mixtures for oral solution such as good reconstitution time, stability, and good taste. For this reason, the selection for the most suitable formulations was necessary for the study.

From all formulations, there were differences from each other, such as type of diluents, quantity of diluents, that made each formulations had different properties. As, expected, all formulations could be reconstituted into clear solutions. This was certainly due to the capability of HPBCD that solubilized ketoconazole by inclusion complex formation. In addition, there were many reports showed that spray drying of water insoluble drugs improved solubility. This might be due to the existence of drugs in amorphous form during the spray drying process. (Lin and Kao, 1989). The physicochemical properties of all formulations of dry mixtures for oral solutions were evaluated and shown in Table 22.

Table 22. The evaluation of ketoconazole dry mixtures for oral solutions.

	Evaluation of formulation						
Formulation	**Reconstitution time (min.)**	**Viscosity (cps.)**	**pH**	**Flowability (angle of repose)**	**Moisture content (%)**	**Taste**	**Clarity**
Rx1	15	16.11	6.49	40	2.98	++	clear solution
Rx2	10	11.17	6.47	35	3.36	++	clear solution
Rx3	5	5.41	6.48	35	3.89	+	clear solution
Rx4	5	7.69	6.46	35	3.67	++	clear solution

* : + = degree of sweetness

** : Triplicate determination

2. Characteristization of Ketoconazole Dry Mixtures for Oral Solution

2.1 Flowability

The bulkiness of the oral mixtures was much influenced by contents of sucrose and HPBCD. In the formulation without sucrose, the solid content was reduced that resulted to be good for packaging. The flowability values that represented by angle of repose were about 35° for Rx2-4, but in Rx1 the angle was higher, about 40°, this might be the effect of sucrose that had poor flowability. In the Rx1, there was sucrose more than the others, so the flowability of Rx1 was poor than the others as well. (Table22). This might be implied that the dry mixtures had all fair

flowability. (Lin and Kao, 1989) The flowability of all dry mixtures formulations were in similar range. This might attributed to the sphericity of spray dried particles and a narrow size distribution of the mixtures.

The flowability of spray dried powder of ketoconazole was not as good as the other spray dried particle, thus it had narrow size distribution and small size about 10 μm. This result might be described from the morphology of spray dried particle that were shrinkage,so it might be made the particle not flow as good as the smooth surface particle (Figure 11, 12 and 13).

2.2 Moisture Content

The moisture contents of dry mixtures formulation were in the range 2.98-3.89 %. This was nearly the same as those of the spray dried powder of ketoconazole and HPBCD. The results confirmed that the additives in the formulation and the preparation process did not result to the increase of moisture contents.

2.3 Physical Appearances : Clarity and taste

2.3.1 Clarity

The clarity of the formulation was obviously clear for all formulations. This indicated that all ingredients including ketoconazole could dissolve completely in aqueous medium of the formulations.

2.3.2 Taste

From all of the formulations the formulation Rx4 was provided the better taste. Though Rx4 had no sucrose as sweetener in the formulation, it contained both aspartame 0.3% and saccharin sodium 0.5% instead. At this level of artificial sweeteners in the presence of xanthan gum, the taste was superior to other formulations.

2.4 Reconstitution time

From Table 22, it was obvious about the presence of sucrose as diluent or filler at 15 and 30%, gave the long reconstitution time of 10 and 15 min,

respectively. This might be the difficulty or limitation of sucrose itself to dissolve in cold water inspite of presence of present as fine particles. The reconstitution time was improved to be shorter at 5 min in the formulation without sucrose. The sweetness could be improved by using artificial sugar such as aspartame and saccharin sodium.

2.5 Viscosity

The viscosity of reconstituted solution of all formulations was in range 5.40-16.11 cps. It was noticed that the formulations with sucrose had higher viscosity than those with xanthan gum as viscosity enhancer.

2.6 pH

The pH of all reconstituted solution was in the same range of 6.46-6.49. This was the effects of all diluents used in the formulation that were rather neutral.

From the results, the suitable formulation was the Rx4 formulation. This formulation had better taste than formulation Rx3, which possessed similar properties.

F. STABILITY OF KETOCONAZOLE FORMULATIONS

1. Stability of Ketoconazole Dry Mixtures for Oral Solution

The stability study of ketoconazole dry mixtures for oral solution were performed for 3 months by triplicate samples of selected formulation (Rx4). The powders were weighed and individually filled in amber glass vials which tightly sealed with rubber closures and aluminum caps. All vials were separately stored in two conditions: room temperature (30 °C) and at 40 °C, 75% RH for three month. The mixtures were randomly sampled and assayed for remaining ketoconazole content by HPLC method and the physical properties were evaluated at corresponding time.

1.1 Physical stability study of ketoconazole dry mixtures

The physical properties of ketoconazole dry mixtures for oral solution in both conditions were evaluated as demonstrated in the Table 23 and 24. Physical properties of ketoconazole dry mixtures were evaluated as reconstitution time, viscosity, pH, flowability and % moisture content. The stability data are shown in Table 23 for storage condition at 30 °C and Table 24 for that at 40 °C, 75% RH.

Table 23. The physicochemical stability study of ketoconazole dry mixtures for oral solution at 30 °C

Test date	Reconstitution time (min)	Viscosity (cps)	pH	Flowability (angle of repose)	%Moisture content
0	5.0	11.5185	6.28	45	3.34
30	4.5	11.57567	6.00	50	2.98
60	5.0	12.04544	6.00	50	3.41
90	5.0	11.96881	6.01	50	3.12

Triplicate determination.

Table 24. The physicochemical stability study of ketoconazole dry mixtures for oral suspension at 40 °C, 75% RH.

Test date	Reconstitution time (min)	Viscosity (cps)	pH	Flowability (angle of repose)	% Moisture content
0	4.5	11.5185	6.28	45	3.34
30	5.0	12.06925	5.99	50	3.45
60	5.0	12.41617	5.98	50	3.31
90	5.0	12.19278	6.01	50	3.44

Triplicate determination.

The determination of pH showed that pH values were decreased a little bit, in the narrow range between pH 5.98-6.28. By the way, that pH was enough to keep the level of ketoconazole as stable as described by Skiba et al, 2000.

From the study, Skiba, 2000, the result indicated that the amount of ketoconazole in the aqueous formulation decreased more quickly as the pH decreases, especially for pH lower than 4.0, this was the same as this study revealed, that pH of

the formulations decreased, a small value, and the percentages of ketoconazole decreased as well.

Due to the results of the reconstitution time, that were not different from each of dates, this might be due with the percentage of moisture content of the formulations that were not changed as well. Because of low humidity of samples, the samples of ketoconazole dry mixtures had fair flowability and leaded to the unchanged of reconstitution time as long as the initial date.

The evaluations of ketoconazole dry mixtures for oral solution were similarly for both of stability at room temperature (30°C) and accelerated condition (40°C, 75% RH). The results from both conditions were declared a uniquely trend.

1.2 Differentials Scanning Calorimatry (DSC)

The DSC thermograms of ketoconazole dry mixtures for oral solution at day 0, 30, 60 and 90 at room temperature and at 40 °C, 75%RH are depicted in Figure 21 and 22.

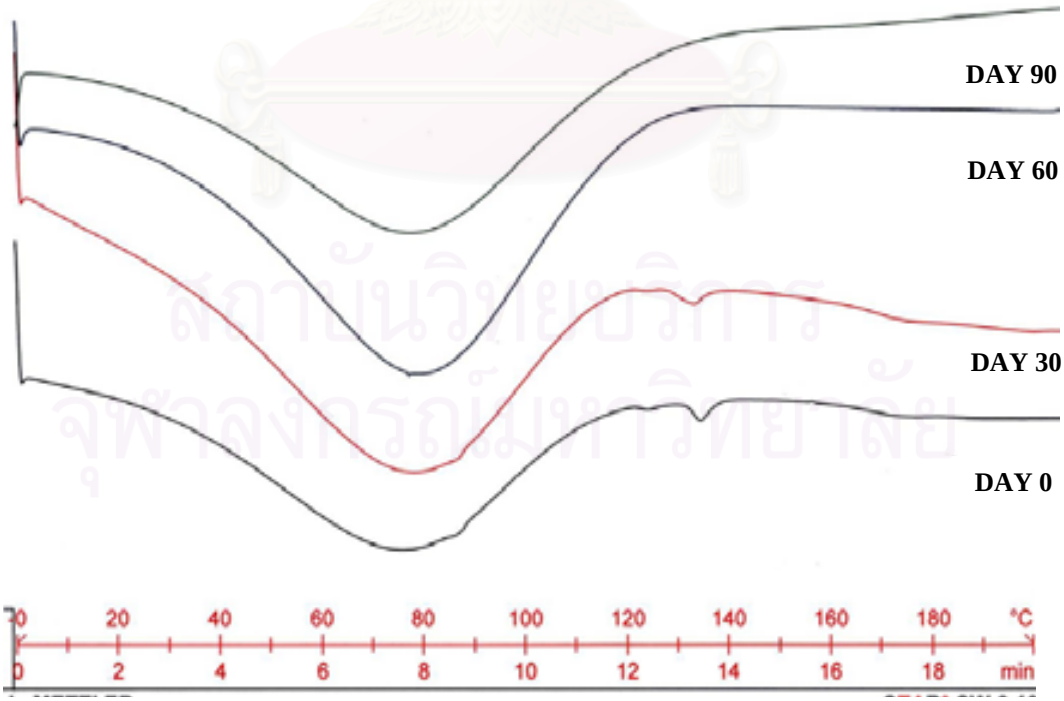

Figure 21. The thermograms of ketoconazole dry mixtures for oral solution in stability study under room temperature.

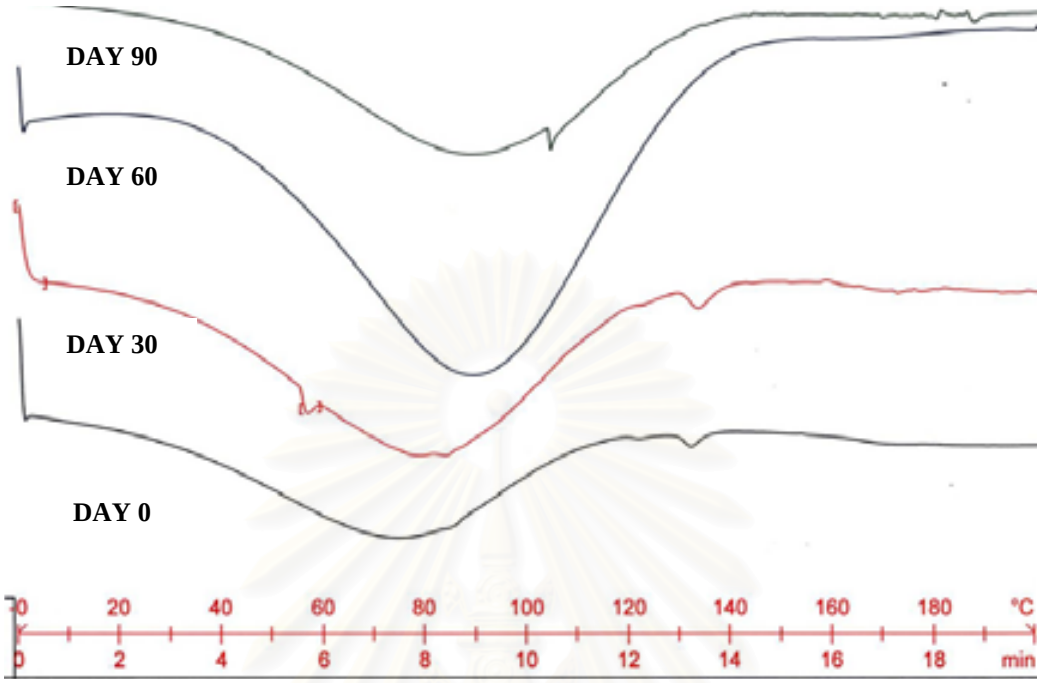

Figure 22. The thermograms of ketoconazole dry mixtures for oral solution in stability study under 40 °C, 75% RH

At room temperature, the DSC thermograms at day 0 and day 30 showed a small endothermic peak at about 135 °C. This endothermic peak might be attributed to the melting of saccharin sodium in the dry syrup formulation (Appendex G). But it was surprising that at the longer period (day 60 and 90) it disappeared. The similar results were observed in the dry mixtures under storage at 40 °C,75%RH. However, at the latter storage condition there were very small endothermic peaks at 56 and 105 °C of some other ingredients in the formulation.

However, the results revealed that ketoconazole was stable as present in an amorphous state or as in inclusion complex with HPBCD along the stability study for 90 days

1.3 Powder X-ray diffractometry

The diffractograms of ketoconazole dry mixtures for oral solution at day 0, 30, 60 and 90 at room temperature and at 40 °C, 75%RH are depicted in Figure 23 and 24.

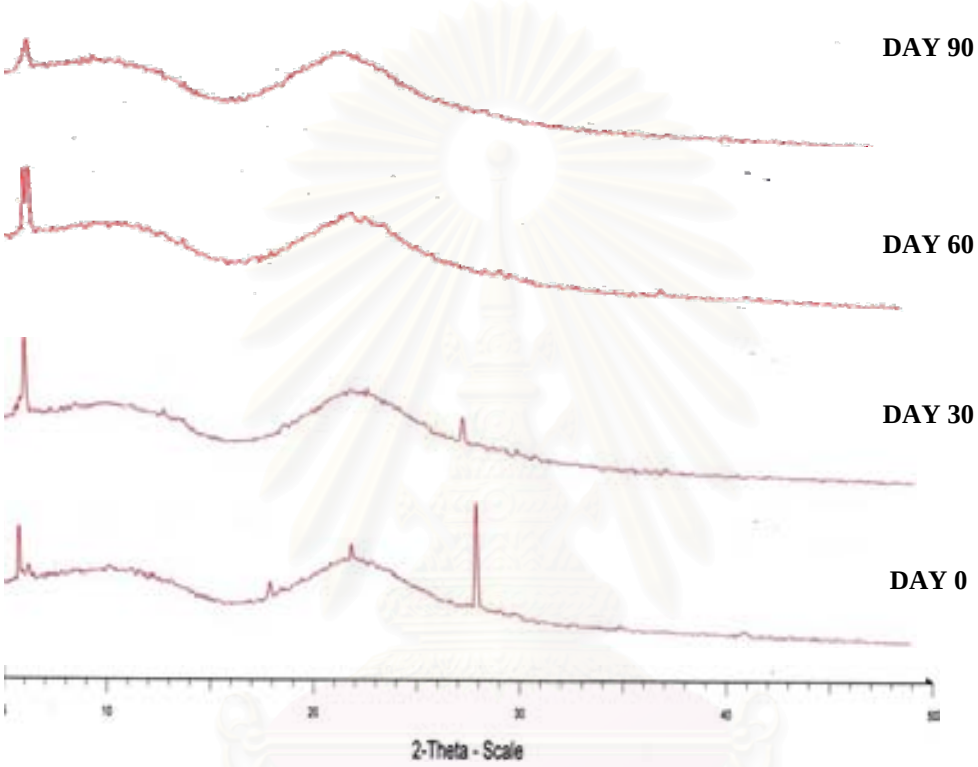

Figure 23. The diffractograms of ketoconazole dry mixtures for oral solution in stability study under room temperature (30 °C).

For the diffractograms of the stability study of ketoconazole dry mixtures, the data demonstrated in Figure 23 for under room temperature and Figure 24 for 40 °C. 75% RH. In the stability storage under room temperature, The small diffraction peak observed at 6° was also observed in the ketoconazole spray dried powder (Figure 15). This peak might be a new peak or slightly shift from 7° of ketoconazole. It could be observed in all diffractograms from day 0, 30, 60 and 90. The peak at 28° might be the diffraction peak of some ingredients in the dry syrup formulation. It was not the diffraction peak of ketoconazole.

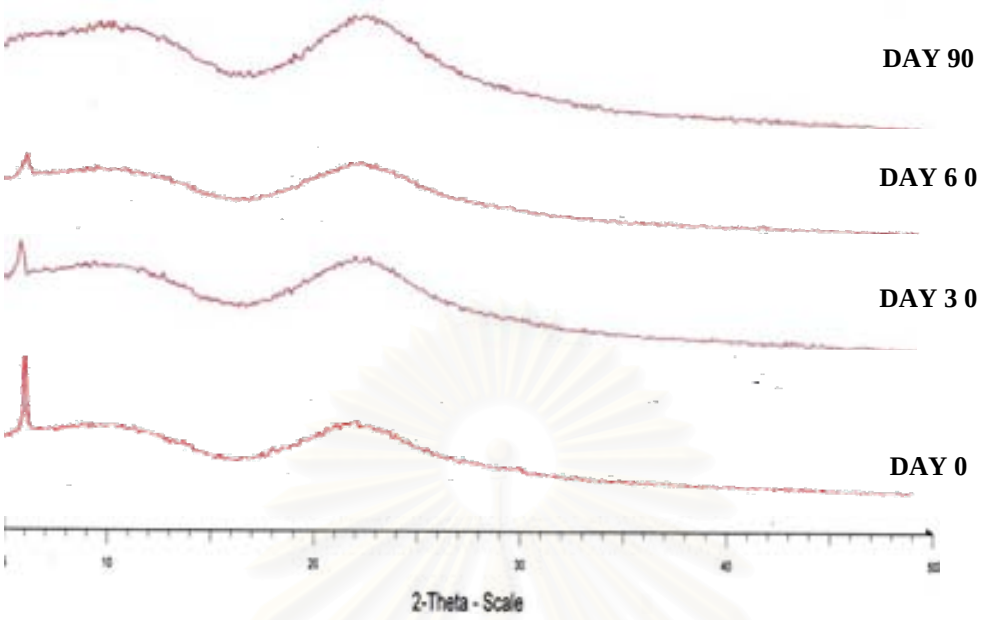

Figure 24. The diffractograms of ketoconazole dry mixtures for oral solution in stability study under 40 °C,75% RH

This result might be confirmed and agreed with the DSC thermogram that showed the melting peak at 135 °C. The diffractogram showed two small diffraction peaks on initial date at about 6° and 28°. The peak at 28° tended to be reduced on date 30 and absolutely disappeared after date 60. In contrast, the peak at 6° was appeared through out the study but it seemed to be reduced as the function of time. These results revealed that ketoconazole was stable as in amorphous state as confirmed with the DSC thermograms along the stability study at room temperature and stressed condition.

On the other hand, the diffractograms of stability study of ketoconazole dry mixtures under accelerated condition, exhibited a small peak of ketoconazole at around 6° this is as same as the DSC thermograms that showed the similarly data.

The results revealed that under both room temperature and the accelerated storage condition, 40 °C 75% RH, peak of ketoconazole was disappeared. This was implied that ketoconazole was stable as in amorphous state as in clusion complex with HPBCD along the stsbility study for 90 days.

1.4 Chemical stability of ketoconazole dry mixtures

The chemical stability of ketoconazole dry mixtures for oral solution was determined as demonstrated in the Table 25 for room temperature storage and Table 26 for 40 °C, 75% RH.

Table 25. The chemical stability study of ketoconazole dry mixtures for oral solutionon at 30°C.

	Drug remaining (%)				
Test date	**1**	**2**	**3**	**mean**	**SD**
0	102.16	103.17	104.14	103.16	0.99
30	101.39	104.90	101.94	102.74	1.89
60	95.58	97.18	95.92	96.23	0.84
90	91.88	95.44	95.59	94.30	2.10

Triplicate determination.

Table 26. The chemical stability study of ketoconazole dry mixtures for oral solutionon at 40°C, 75% RH.

	Drug remaining (%)				
Test date	**1**	**2**	**3**	**mean**	**SD**
0	102.16	103.17	104.14	103.16	0.99
30	98.95	99.69	98.83	99.16	0.47
60	98.11	98.74	98.22	98.36	0.34
90	94.71	94.58	91.70	93.66	1.70

Triplicate determination.

The results indicated that the percentage of ketoconazole remaining was decreased as the time progressed. The general pattern of stability of solid dosage forms that appeared, were described by zero-order kinetic profile. These results could be described that the constant surface areas, activated site, of solid were decomposed, and the inner surface areas, the neighboring molecules, were followed in the same rate with the constant surface areas, as described by Carstensen, 1990.

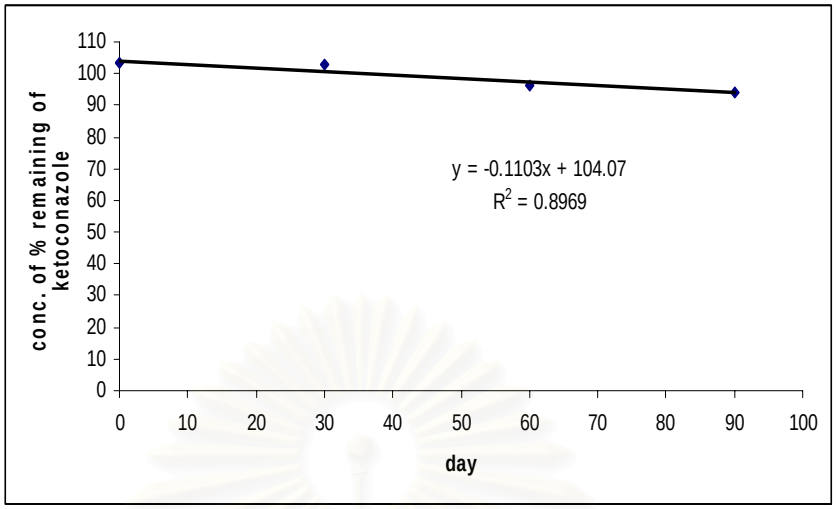

Figure 25. The chemical stability study of ketoconazole dry mixtures for oral solution at 30 °C.

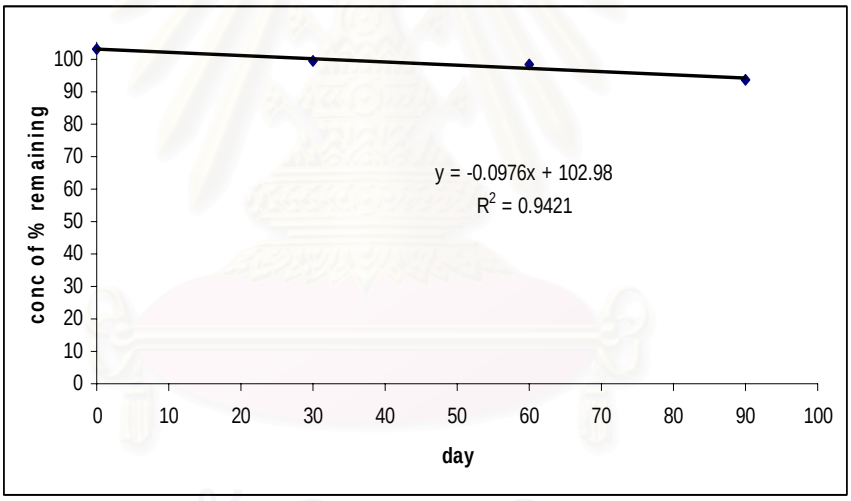

Figure 26. The chemical stability study of ketoconazole dry mixtures for oral solution at 40 °C, 75% RH.

From the results of stability studied, the data could implied that ketoconazole dry mixtures for oral solution was stable for at least three month for both of dry powders form of solution form, in all conditions of stability storage. The percentages of ketoconazole remaining were not less than 90, according to the formulation of ketoconazole suspension, USP 29.

2. Stability of Ketoconazole Reconstituted Solution

The stability study of ketoconazole oral solutions was performed for three months by triplicate samples of the selected formulation (Rx4). The solutions were prepared from the formulation of ketoconazole dry mixtures for oral solution by adding purified water in accurately to volume. The solutions were divided in equal volume and individually filled in amber glass vials which tightly sealed with rubber closures and aluminum caps. All vials were stored in three different conditions: at refrigerator temperature (2-8 °C), room temperature (30 °C) and 40 °C, 75% RH for three months. The solutions were randomly sampled and assayed for remaining ketoconazole content and the physicochemical properties were evaluated at corresponding times.

2.1 Physical stability study of ketoconazole reconstituted solutions

The evaluation of ketoconazole oral solutions were determined for viscosity, pH, clarity and color change. The effort to determine the color change quantity was performed by measuring the absorbance change at the wavelength that showed maximal absorption in the visible range. In this study the maximal absorption in the visible range reported at 410 nm (Im-erbsin, 2002). The results of evaluations demonstrated in Table 27 and 28. These results indicated there were no change of viscosity, clarity and absorbance at 410 nm, from the first date to the end of study, day 0- day 90. The visible absorbance at 410 nm was demonstrated the degradation of ketoconazole, (Im-erbsin, 2002).

The results could be described by the study of Skiba, 2000 that implied the solutions of ketoconazole were stable at pH 5 or above, which specified at pH. Those results could described in this studied that pH of ketoconazole solution was in the range 5.87-6.34 along the study time, and at this pH the degradation was slightly generated. The similar results could be found for all conditions of stability studies.

Table 27. The physicochemical properties stability study of ketoconazole reconstituted solution at refrigerator (2-8 °C).

Test date	Viscosity	pH	absorbance (410 nm)	Clarity
0	11.50	6.34	0.0453	clear solution
5	11.57	6.26	0.0467	clear solution
10	11.01	6.15	0.0451	clear solution
15	10.59	5.87	0.0471	clear solution
30	11.71	6.00	0.0496	clear solution
45	10.95	5.99	0.0468	clear solution
60	11.86	6.01	0.0457	clear solution
75	10.89	5.99	0.0463	clear solution
90	11.45	6.01	0.0471	clear solution

Triplicate determination.

Table 28. The physicochemical properties stability study of ketoconazole reconstituted solution at room temperature (30 °C).

Test date	Viscosity	pH	absorbance (410nm)	Clarity
0	11.50	6.34	0.0453	clear solution
5	12.27	6.26	0.0467	clear solution
10	11.25	6.14	0.0492	clear solution
15	10.83	6.04	0.0507	clear solution
30	10.77	6.04	0.0431	clear solution
45	11.01	5.84	0.0510	clear solution
60	10.94	5.91	0.0432	clear solution
75	11.08	5.92	0.0428	clear solution
90	11.15	5.94	0.0455	clear solution

Triplicate determination.

Table 29. The physicochemical stability study of ketoconazole reconstituted solution at 40 °C, 75% RH.

Test date	Viscosity	pH	absorbance (410 nm)	Clarity
0	11.50	6.34	0.0453	clear solution
5	13.04	6.22	0.0512	clear solution
10	13.08	6.09	0.0465	clear solution
15	13.36	6.00	0.0437	clear solution
30	12.07	5.92	0.0504	clear solution
45	12.13	5.98	0.0511	clear solution
60	12.46	5.91	0.0436	clear solution
75	12.22	5.94	0.0482	clear solution
90	12.80	5.91	0.0478	clear solution

Triplicate determination.

2.2 Chemical stability of ketoconazole reconstituted solution

The amounts of ketoconazole remaining in solutions were measured by HPLC method for all storage conditions. The analytical method, which operated in this study, was described as previous. Furthermore, the percentages of label amount of each storage conditions were calculated. The data depicted in Table 30, 31and 32.

Table 30. The percentage of ketoconazole remaining of refrigerator temperature.

	Drug remaining (%)				
Test date	1	2	3	mean	SD
0	101.22	101.55	100.24	101.00	0.68
5	98.60	97.52	98.04	98.05	0.54
10	91.45	92.42	92.28	92.05	0.52
15	91.78	90.76	93.29	91.94	1.27
30	93.23	90.78	89.73	91.25	1.79
45	90.34	88.25	93.97	90.85	2.89
60	93.09	87.01	92.07	90.72	3.26
75	93.23	87.42	90.92	90.52	2.93
90	88.50	90.64	91.87	90.34	1.71

Triplicate determination.

Table 31. The percentage of ketoconazole remaining in room temperature (30 °C).

Teat date	Drug remaining (%)				
	1	2	3	mean	SD
0	101.22	101.55	100.24	101.00	0.68
5	97.16	98.07	96.63	97.29	0.73
10	94.11	95.57	94.24	94.64	0.81
15	92.66	93.53	90.62	92.27	1.49
30	92.81	92.48	90.44	91.91	1.28
45	90.84	91.04	91.46	91.11	0.32
60	90.79	89.96	92.01	90.92	1.03
75	86.63	92.61	93.45	90.89	3.72
90	90.75	91.4	89.12	90.42	1.17

Triplicate determination.

Table 32. The percentage of ketoconazole remaining of 40 °C, 75% RH.

Test date	Drug remaining (%)				
	1	2	3	mean	SD
0	101.22	101.55	100.24	101.00	0.68
5	96.99	98.07	96.75	97.27	0.70
10	95.00	92.28	96.21	94.49	2.01
15	94.28	94.47	95.07	94.61	0.41
30	94.23	93.14	94.04	93.80	0.58
45	91.57	90.83	96.67	93.02	3.18
60	91.01	92.81	90.30	91.37	1.29
75	89.01	93.27	88.76	90.35	2.53
90	91.85	89.88	88.96	90.23	1.48

Triplicate determination

To demonstrate the stability pattern, plot between % ketoconazole remaining versus time is depicted on Figure 27, 28 and 29.

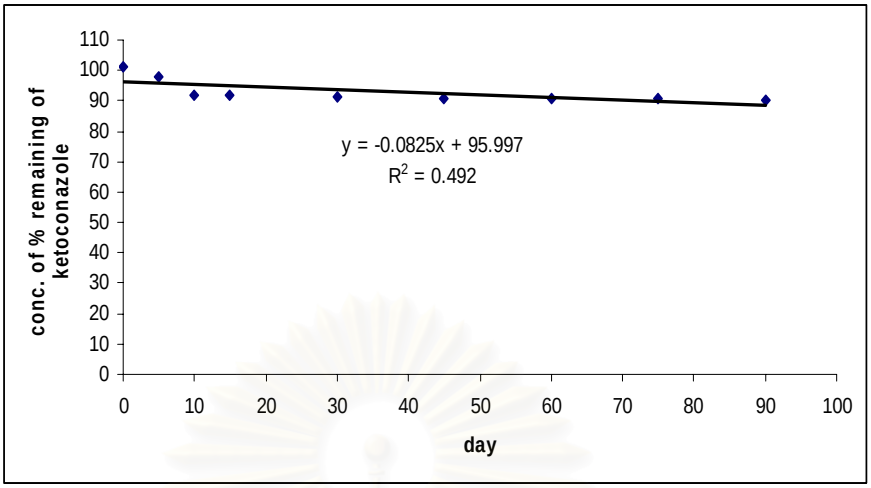

Figure 27. First order plot of solution under refrigerator condition**.**

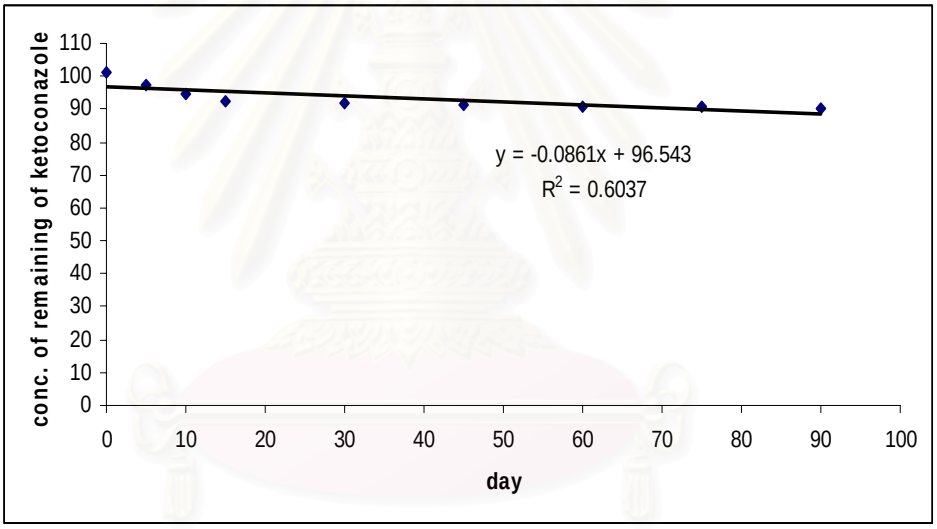

Figure 28. The percentages of label amount of ketoconazole reconstituted solutions under room temperature.

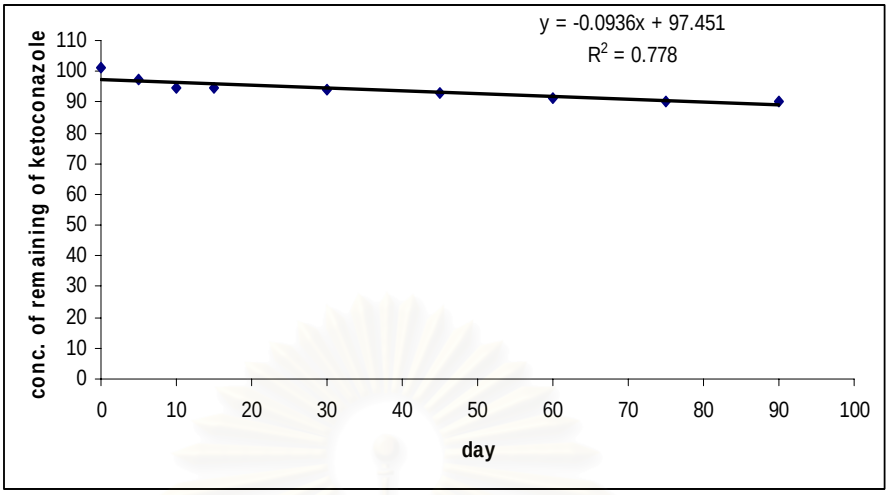

Figure 29. The percentages of labeled amount of ketoconazole reconstituted solutions under 40 °C, 75% RH.

From the plot of drug remaining of ketoconazole dry mixtures under refrigerator temperature, room temperature and accelerated condition, the R^2 of this diagram was 0.492, 0.6037 and 0.778 respectively, as showed in Figure 27, 28 and 29. That indicated the line was not linear. Thus, the plot of log concentration and time was established as depicted in Figure 30, 31 and 32. The R^2 of this diagram was 0.9003, 0.8638 and 0.9936 that demonstrated the linearity. For these results, they indicated that ketoconazole reconstituted solutions were decreased dramatically in first period then the decreasing was slightly slow down, this was observed from all storage conditions of this formulation. So, these could be concluded that the stability patterns of ketoconazole reconstituted solutions were described as first order kinetic reaction.

Due to the generally solutions, the first order kinetic was occurred with the stability studied. To describe this kinetic, there were many cases deal with this kinetic. In generally, the solutions could be decomposed and generated degradations, the processes were complicated and might be generated the degradations for one or more. At the initial stage, the reaction was interacted very rapidly up to the high concentration of initial substance for the early stage and slowly progressed along the time with the less concentration of the parent substance, as described by Carstensen, 1990.

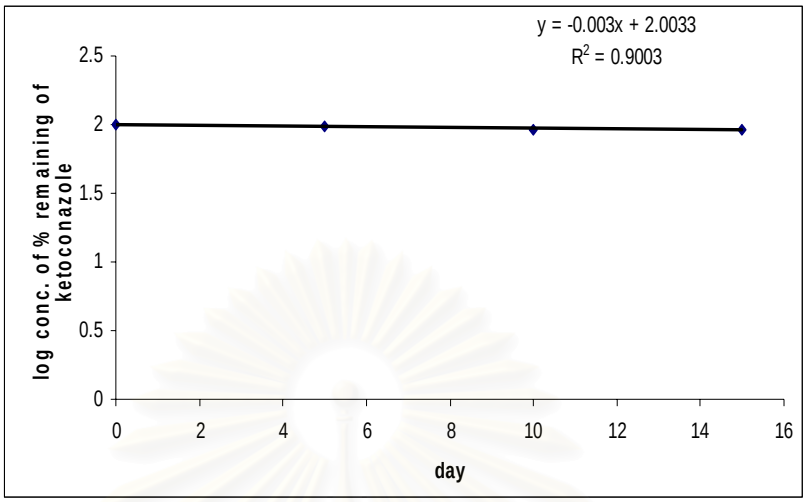

Figure 30. The plot of log concentration of ketoconazole reconstituted solution under refrigerater temperature.

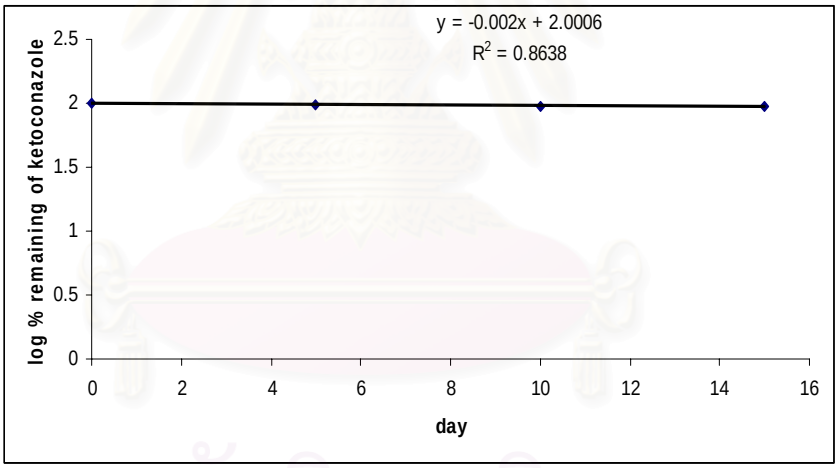

Figure 31. The plot of log concentration of ketoconazole reconstituted solution under refrigerater temperature.

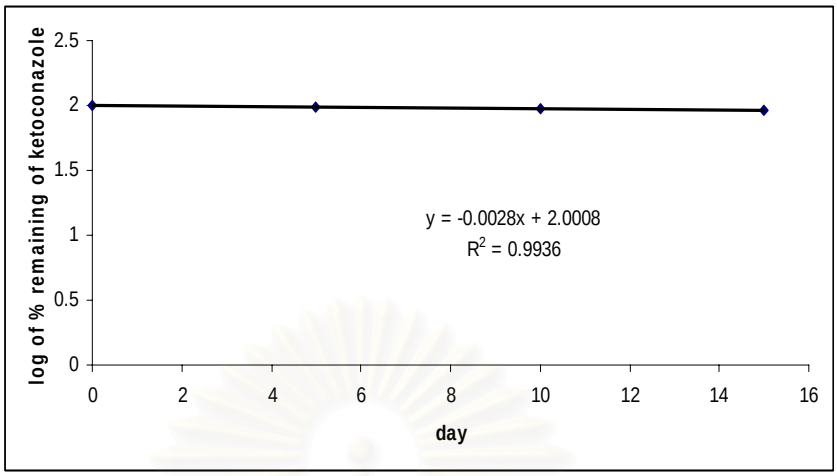

Figure 32. The plot of log concentration of ketoconazole reconstituted solution under accelerated condition, 40 °C, 75% RH

3. Stability of Ketoconazole Suspension (USP 29)

The stability study of ketoconazole oral suspension was operated for 3 month by triplicate samples of the formulation. The formulation was prepared from the monograph in USP 29, 2006. The suspensions were randomly sampled and assayed for the remaining of ketoconazole content and the physical properties were evaluated at corresponding time. The stability data are demonstrated in the Table 33.

3.1 Physical stability of ketoconazole suspension

For the ketoconazole suspensions stability study, the physical properties that meant to viscosity, pH and appearances were evaluated as demonstrated in the Table 33. The data showed that pH values were slightly decreased with the progressing of time. The values were 5.63 to 5.11, from first day to the end. In contrast, the viscosity did not changed through the study, this might be cocsistent with the study of Skiba, 2000, that discussed as at pH 5-6 the chemical stability ketoconazole was very stable. In addition to the color appearances of the ketoconazole suspensions that appeared as white powder in colorless medium showed no color change till the end of the study.

Table 33. The physicochemical stability study of ketoconazole oral suspensions at 2 °C.

Test date	Viscosity (cps.)	pH	Color change
0	6.93	5.63	no change
5	7.26	5.62	no change
10	7.26	5.51	no change
15	6.93	5.51	no change
30	6.27	5.30	no change
45	6.27	5.18	no change
60	5.94	5.06	no change
75	5.94	5.09	no change
90	6.27	5.11	no change

Triplicate determination

3.2 Chemical stability of ketoconazole suspension

The stability of ketoconazole suspensions prepared as described in USP 29, was operated at refrigerator (2-8°C), which was suggested by USP 29. According to USP 29, the percentage of labeled amount was 90-110% within 7 days after freshly prepared. The result showed that the percentage of ketoconazole remaining was decreased along with the progressing of time, as showed in the Table 34.

Table 34. The chemical stability study of ketoconazole oral suspensions at 2 °C.

	Drug remaining (%)				
Test date	**1**	**2**	**3**	**mean**	**SD**
0	103.40	100.91	103.62	102.64	1.23
5	103.70	103.28	100.21	102.39	1.56
10	102.49	102.68	102.39	102.52	0.12
15	101.84	100.92	100.82	101.19	0.46
30	103.67	93.58	106.32	101.19	5.49
45	98.78	98.63	100.82	99.41	0.99
60	98.54	98.14	96.16	97.61	1.04
75	93.45	92.70	99.27	95.14	2.94
90	95.03	93.93	95.92	94.96	0.81

Triplicate determination

It was showed that ketoconazole content decreased linearly, independent to the concentration remaining. The result might be implied to the zero-order kinetic. The plot between percentages of ketoconazole remaining versus time is depicted in Figure 30.

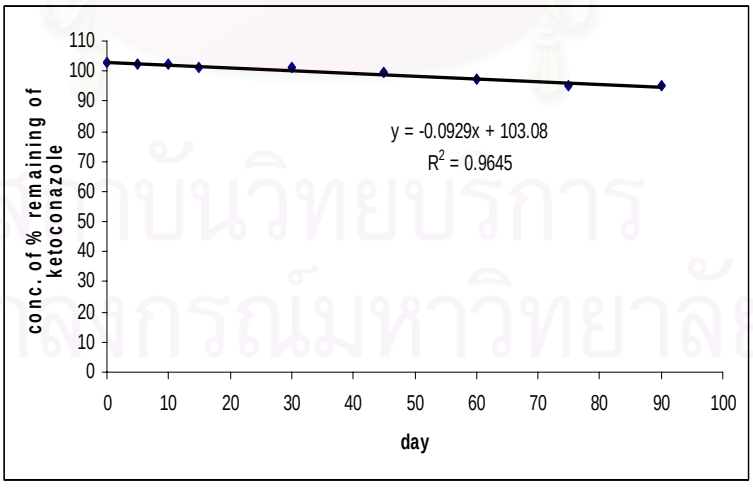

Figure 33. The percentages of label amount of ketoconazole suspension under refrigerator.

This result was consistent to that described by Carstensen, 1990 that for the suspension formulations, the amount of dissolving drugs were limited by the accurate volume of solubilizer. It was assumed that the decomposition of suspensions depended on the amount of drug dissolved in aqueous phase.

In addition, if it was assumed that each dissolved molecule was immediately replaced by a molecule which dissolved from the solid state, then the pseudo-order kinetic was assumed. The rate constant was a function of the initial concentration. This is the reason that a concentrated suspensions were always more stable than the solutions, and suitable to market.

CHAPTER V

CONCLUSIONS

The effects of cyclodextrins for oral formulations on solubility and stability of ketoconazole solutions and spray dried products were investigated. The conclusion of this study were declared as following.

1. The solubility of ketoconazole in acetate buffer solutions pH 5 increased as a function of HPBCD concentrations. The phase solubility diagram could be classified as type A_L.

2. From the phase solubility diagram, HPBCD was applied successfully to prepare the 2% w/v ketoconazole solutions and further prepared in solid form as spray dried products.

3. The optimal condition for spray drying method in the study was 120 °C for inlet temperature and feed rate 3.5 ml/min, suggest by Design Expert version 7.1.3.

4. According to the physicochemical study, X-ray diffractometry and differantial scanning calorimetry, the results indicated that the spray dried products was in an amorphous form or in inclusion complexes of HPBCD or both.

5. According to the amount of ketoconazole content of spray dried products, the results provided good labeled amount of drug content, more than 90%, this indicated that the spray dried method was suitable for generation of ketoconazole: HPBCD inclusion complexes.

6. From the stability study, ketoconazole remaining was more then 90% labeled amount for all formulations and at all conditions of stability tests. This indicated that ketoconazole dry mixtures for oral solution were stable for at least 90 days.

7. From the result of stability, in practical use, after the reconstitution of ketoconazole dry mixtures for oral solution, the reconstituted solution was stable for at least 90 days in either storage condition, refrigerated or room temperature. Ketoconazole suspension, extemporaneously prepared was stable at refrigerated temperature for more than 90 days. However, it was not now commercially available.

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THAI

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อมรศรี ชุณหรัศมิ์, วาณี วิสุทธิ์เสรีวงศ์ และ ศรีศุภลักษณ์ สิงคาลวณิช บรรณาธิการ. 2549. คู่มือ โรคผิวหนังเด็ก. พิมพ์ครั้งที่ 1. กรุงเทพมหานคร: บียอนด์ เอนเทอร์ไพรซ์

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APPENDICES

APPENDIX A

Data of weight and flowing time of

ketoconazole reconstituted solution and

suspensions: determined

with Ostwald capillary viscometer

Table A1 DATA of Flowing time of ketoconazole reconstituted solution (at refrigerator stability storage) (2-8°C) a determined with Ostwald capillary viscometer.

Test date	n1	n2	n3	mean	SD
0	11	10.5	10	10.50	0.5
5	10.5	11	10.5	10.67	0.28
10	11	11	9	10.33	1.15
15	10	10	10	10.00	0
30	10.5	12	11	11.17	0.76
45	11.5	10	10	10.50	0.87
60	11	11	12.5	11.50	0.87
75	10	11.5	10.5	10.67	0.76
90	11	12	11	11.33	0.57

Table A2 DATA of Weight for 2 ml ketoconazole reconstituted solution (at refrigerator stability storage) (2-8°C)

Test date	n1	n2	n3	mean	SD	density	Viscosity (cps)
0	2.192	2.179	2.201	2.19	0.011	1.095	11.501
5	2.171	2.169	2.168	2.17	0.001	1.084	11.569
10	2.129	2.126	2.135	2.13	0.004	1.065	11.005
15	2.117	2.112	2.125	2.12	0.006	1.059	10.590
30	2.094	2.097	2.102	2.10	0.004	1.048	11.711
45	2.079	2.086	2.092	2.09	0.006	1.042	10.949
60	2.065	2.054	2.067	2.06	0.007	1.031	11.856
75	2.034	2.045	2.047	2.04	0.007	1.021	10.8906
90	2.016	2.025	2.019	2.02	0.004	1.010	11.446

Table A3 DATA of Flowing time of ketoconazole reconstituted solution (at room stability storage) (30°C) a determined with Ostwald capillary viscometer.

Test date	n1	n2	n3	mean	SD	density	Viscosity (cps)
0	11	10.5	10	10.50	0.5	1.09	11.50
5	11	12	11	11.33	0.57	1.08	12.27
10	10.5	10	11	10.50	0.5	1.07	11.25
15	10	9.5	11	10.17	0.76	1.064	10.82
30	10	10.5	10	10.17	0.28	1.06	10.76
45	11	10	10.5	10.50	0.5	1.05	11.01
60	11	10.5	10	10.50	0.5	1.04	10.94
75	10	11	11	10.67	0.57	1.04	11.08
90	11	11.5	10.5	11.00	0.5	1.01	11.15

Table A4 DATA of Weight for 2 ml ketoconazole reconstituted solution at room temperature (30°C).

Test date	n1	n2	n3	mean	SD
0	2.192	2.179	2.201	2.19	0.011
5	2.167	2.168	2.163	2.17	0.003
10	2.134	2.149	2.148	2.14	0.008
15	2.13	2.128	2.131	2.13	0.002
30	2.1	2.14	2.114	2.12	0.020
45	2.092	2.089	2.11	2.10	0.011
60	2.083	2.078	2.091	2.08	0.006
75	2.067	2.085	2.079	2.08	0.009
90	2.034	2.029	2.017	2.03	0.008

Table A5 DATA of Flowing time of ketoconazole reconstituted solution (at stability storage 40°C 75% RH) a determined with Ostwald capillary viscometer**.**

test date	n1	n2	n3	mean	SD	density	Viscosity (cps)
0	11	10.5	10	10.50	0.50	1.09	11.50
5	12.5	12	11.5	12.00	0.50	1.08	13.03
10	13	12	11.5	12.17	0.76	1.07	13.07
15	12.5	12	13	12.50	0.50	1.06	13.36
30	12	11.5	11	11.50	0.50	1.04	12.06
45	10.5	13	11.5	11.67	1.25	1.03	12.13
60	12	11.5	12.5	12.00	0.50	1.03	12.45
75	12	12.5	11.5	12.00	0.50	1.01	12.22
90	12.5	13	12.5	12.67	0.28	1.01	12.79

Table A6 DATA of Weight for 2 ml ketoconazole reconstituted solution (at 40°C 75% RH **).**

Test date	n1	n2	n3	mean	SD
0	2.19	2.18	2.20	2.19	0.011
5	2.17	2.18	2.18	2.17	0.006
10	2.14	2.15	2.16	2.15	0.008
15	2.14	2.14	2.13	2.14	0.005
30	2.10	2.09	2.11	2.10	0.012
45	2.08	2.09	2.07	2.08	0.006
60	2.07	2.07	2.09	2.08	0.011
75	2.05	2.04	2.03	2.04	0.008
90	2.02	2.03	2.02	2.02	0.006

Table A7 DATA of Flowing time for ketoconazole dry miatures for oral solution (at room temperature stability storage) (30°C) a determined with Ostwald capillary viscometer**.**

Test date	1	2	3	mean	SD	density	Viscosity (cps)
0	10.5	11	10	10.50	0.5	1.09	11.51
30	11	11.5	10.5	11.00	0.5	1.05	11.57
60	11	11	12	11.33	0.57	1.06	12.04
90	12	10.5	11	11.17	0.76	1.07	11.96

Table A8 DATA of Weight for 2 ml ketoconazole reconstituted solution (at refrigerator stability storage) (30°C).

Test date	1	2	3	mean	SD
0	2.201	2.189	2.192	2.19	0.006
30	2.104	2.098	2.112	2.10	0.007
60	2.189	2.088	2.1	2.13	0.055
90	2.211	2.124	2.096	2.14	0.059

Table A9 DATA of Weight for 2 ml ketoconazole reconstituted solution at (40°C 75% RH) a determined with Ostwald capillary viscometer.

Test date	1	2	3	mean	SD	density	Viscosity (cps)
0	10.5	11	10	10.50	0.5	1.097	11.518
30	11	12	11.5	11.50	0.5	1.049	12.069
60	12	11	11.5	11.50	0.5	1.079	12.416
90	10.5	12	11.5	11.33	0.76	1.075	12.192

Table A10 DATA of Flowing time of ketoconazole dry miatures for oral solution at (40°C 75% RH).

Test date	1	2	3	mean	SD
0	2.201	2.189	2.192	2.19	0.006245
30	2.097	2.114	2.086	2.10	0.014107
60	2.089	2.211	2.178	2.16	0.063106
90	2.189	2.099	2.167	2.15	0.046918

APPENDIX B

Data of ketoconazole reconstituted solution and

ketoconazole suspensions in stability study

Table B1 pH data of ketoconazole reconstituted solution at 2°C

determined with pH meter (Orion model 420A, Orion Research Inc., USA.)

	pH				
Tset date	**1**	**2**	**3**	**mean**	**SD**
d0	6.43	6.32	6.28	6.34	0.077
d5	6.31	6.25	6.23	6.26	0.041
d10	6.14	6.16	6.16	6.15	0.011
d15	6.07	5.48	6.05	5.87	0.335
d30	5.96	6.02	6.01	6.00	0.032
d45	5.99	5.97	6.02	5.99	0.025
d60	6.03	6.01	5.99	6.01	0.020
d75	6.01	5.99	5.97	5.99	0.020
d90	6.05	6.0	5.99	6.01	0.032

Table B2 pH data of ketoconazole reconstituted solution (at 30°C) determined with pH meter (Orion model 420A, Orion Research Inc., USA.)

	pH				
Test date	**1**	**2**	**3**	**mean**	**SD**
d0	6.43	6.32	6.28	6.34	0.078
d5	6.26	6.28	6.25	6.26	0.015
d10	6.14	6.16	6.12	6.14	0.020
d15	6.04	6.05	6.04	6.04	0.006
d30	6.01	6.00	6.10	6.04	0.055
d45	5.84	5.88	5.79	5.84	0.045
d60	5.95	5.82	5.96	5.91	0.078
d75	5.93	5.94	5.90	5.92	0.020
d90	5.95	5.9	5.96	5.94	0.032

Table B3 pH data of ketoconazole reconstituted solution (at 40°C 75% RH) determined with pH meter (Orion model 420A, Orion Research Inc., USA.)

	pH				
Test date	**1**	**2**	**3**	**mean**	**SD**
d0	6.43	6.32	6.28	6.34	0.07
d5	6.21	6.2	6.24	6.22	0.02
d10	6.08	6.1	6.09	6.09	0.01
d15	5.97	6.0	6.02	6.00	0.02
d30	5.91	5.94	5.91	5.92	0.02
d45	5.94	5.98	6.01	5.98	0.04
d60	5.92	5.91	5.9	5.91	0.01
d75	5.96	5.98	5.89	5.94	0.05
d90	5.94	5.9	5.88	5.91	0.03

Table B4 pH data ketoconazole reconstituted solution (at 30°C) determined with pH meter (Orion model 420A, Orion Research Inc., USA.)

	pH				
Test date	**1**	**2**	**3**	**mean**	**SD**
d0	6.21	6.27	6.35	6.28	0.070
d30	6	5.99	6.02	6.00	0.015
d60	6	6.02	5.99	6.00	0.015
d90	6	6.02	6.01	6.01	0.010

Table B5 pH data of ketoconazole reconstituted solution (at 40°C 75% RH) determined with pH meter (Orion model 420A, Orion Research Inc., USA.)

	pH				
Test date	**1**	**2**	**3**	**mean**	**SD**
d0	6.21	6.27	6.35	6.28	0.07
d30	5.98	5.97	6.00	5.98	0.016
d60	5.98	5.97	6.02	5.99	0.03
d90	6.01	6.03	5.99	6.01	0.02

Table B 6 pH data of 2 ml ketoconazole suspension (at refrigerator stability storage) determined with pH meter (Orion model 420A, Orion Research Inc., USA.)

	pH				
Test date	**1**	**2**	**3**	**mean**	**SD**
d0	5.62	5.57	5.71	5.63	0.07
d5	5.52	5.69	5.65	5.62	0.01
d10	5.57	5.58	5.59	5.51	0.09
d15	5.55	5.51	5.48	5.51	0.04
d30	5.3	5.26	5.33	5.30	0.04
d45	5.13	5.19	5.22	5.18	0.04
d60	5.06	5.09	5.04	5.06	0.03
d75	5.09	5.11	5.07	5.09	0.02
d90	5.14	5.12	5.08	5.11	0.03

Table B7 Viscosity of ketoconazole suspension at 2°C determined with Cone and plate viscometer (Model LVDV-II+, Brookfield, USA.)

		Display (at 100 rpm)					
Test date	**Factor**	**1**	**2**	**3**	**mean**	**SD**	**Viscosity**
d0	0.33	20	22	21	21.00	1.00	6.93
d5	0.33	20	22	22	21.33	1.15	7.26
d10	0.33	20	22	22	21.33	1.15	7.26
d15	0.33	20	21	21	20.67	0.57	6.93
d30	0.33	20	21	19	20.00	1.00	6.27
d45	0.33	21	18	19	19.33	1.52	6.27
d60	0.33	19	19	18	18.67	0.57	5.94
d75	0.33	17	20	18	18.33	1.52	5.94
d90	0.33	21	16	19	18.67	2.51	6.27

APPENDIX C

Flow property of spray dried products and

ketoconazole dry mixtures for oral solution

(determination of angle of repose)

1. Figures demonstrated spray dried products for nine conditions

Figure C1 Spray dried products for condition, inlet temperature 100°C, feed rate 3.5 ml/min) :Angle of repose = 50°

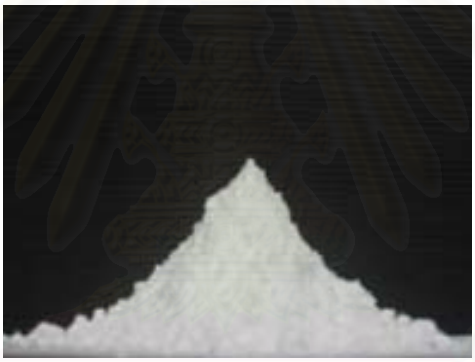

Figure C2 Spray dried products for condition, inlet temperature 100°C, feed rate 7.0 ml/min) : Angle of repose = 45°

Figure C3 Spray dried products for condition, inlet temperature 100°C, feed rate 10.5 ml/min): Angle of repose = 40°

Figure C4 Spray dried products for condition, inlet temperature 120°C, feed rate 3.5 ml/min): Angle of repose = 40°

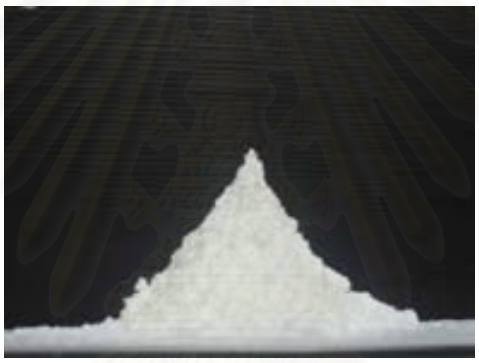

Figure C5 Spray dried products for condition, inlet temperature 120°C, feed rate 7.0 ml/min): Angle of repose = 50°

Figure C6 Spray dried products for condition, inlet temperature 120°C, feed rate 10.5 ml/min): Angle of repose = 45°

Figure C7 Spray dried products for condition, inlet temperature 140°C, feed rate 3.5 ml/min): Angle of repose = 45°

Figure C8 Spray dried products for condition, inlet temperature 140°C, feed rate 7.0 ml/min): Angle of repose = 40°

Figure C9 Spray dried products for condition, inlet temperature 140°C, feed rate 10.5 ml/min): Angle of repose = 40°

2. Figures demonstrated ketoconazole dry mixtures for oral solution for four formulations

Figure C10 Ketoconazole dry mixtures of the first formulation : Angle of repose = 40°

Figure C11 Ketoconazole dry mixtures of the second formulation : Angle of repose = 50°

Figure C12 Ketoconazole dry mixtures of the third formulation : Angle of repose = 50°

Figure C13 Ketoconazole dry mixtures of the fourth formulation : Angle of repose = 45°

3. Figures demonstrated ketoconazole dry mixtures for oral solution for stability study under room temperature

Figure C14 Ketoconazole dry mixtures on day 0 : Angle of repose = 50°

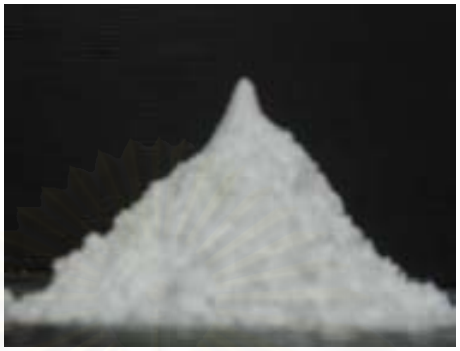

Figure C15 Ketoconazole dry mixtures under room temperature on day 30: Angle of repose = 50°

Figure C16 Ketoconazole dry mixtures under room temperature on day 60: Angle of repose = 50°

Figure C17 Ketoconazole dry mixtures under room temperature on day 90: Angle of repose = 55°

Figure C18 Ketoconazole dry mixtures under 40°, 75% RH on day 30: Angle of repose = 50°

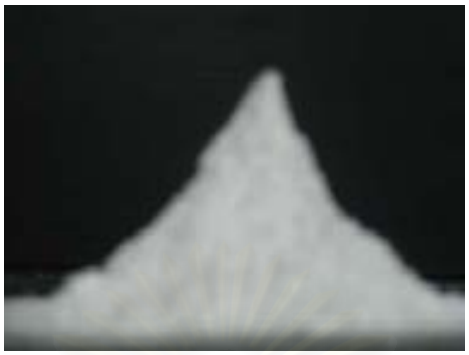

Figure C19 Ketoconazole dry mixtures under 40°, 75% RH on day 60: Angle of repose = 50°

Figure C20 Ketoconazole dry mixtures under 40°, 75% RH on day 90: Angle of repose = 55°

APPENDIX D

Validation of HPLC Method

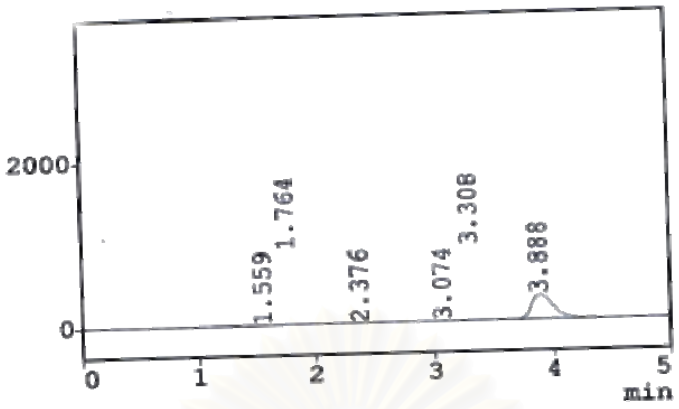

Figure D1 The peak of degraded of ketoconazole by UV spectrums

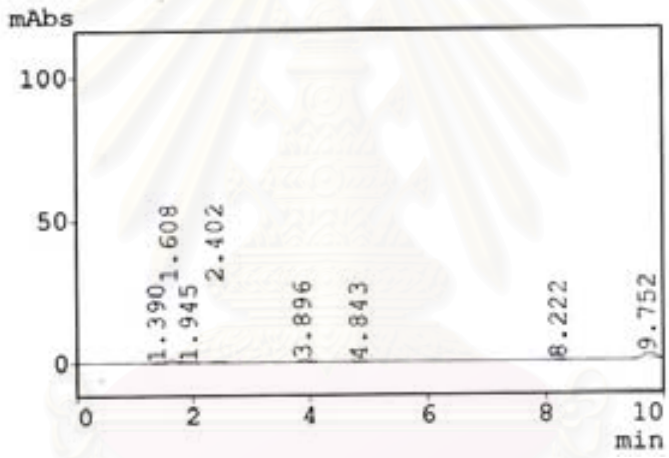

Figure D2 The peak of degraded of ketoconazole by temperature

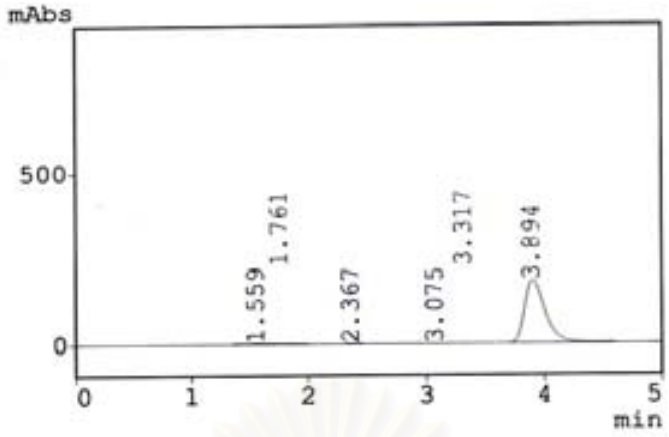

Figure D 3 The Peak of ketoconazole by the absence of any diluents of the formulations

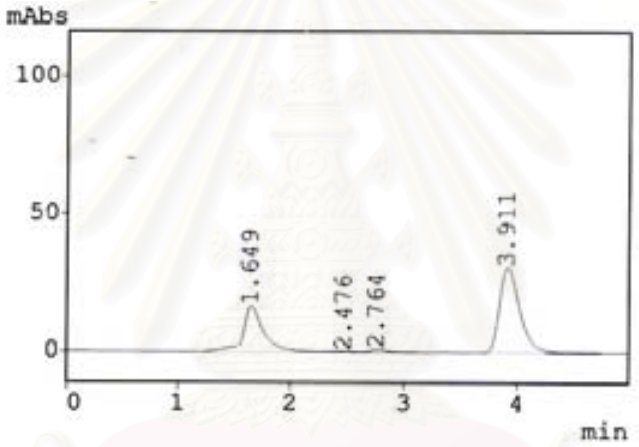

Figure D 4 The peak of ketoconazole with the presence of diluents of the formulation.

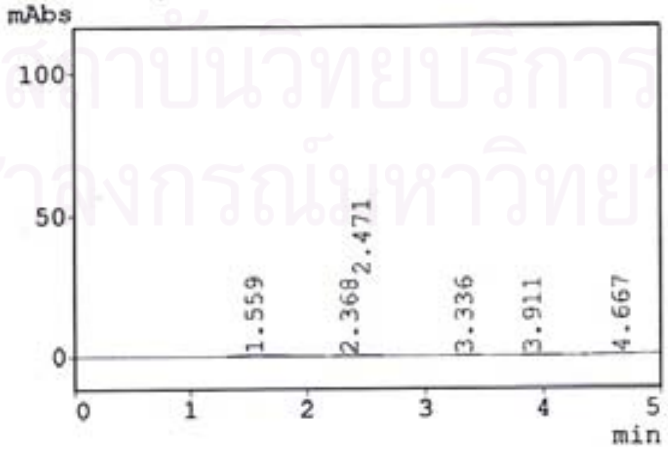

Figure D 5 : mobile phase which showed no peak

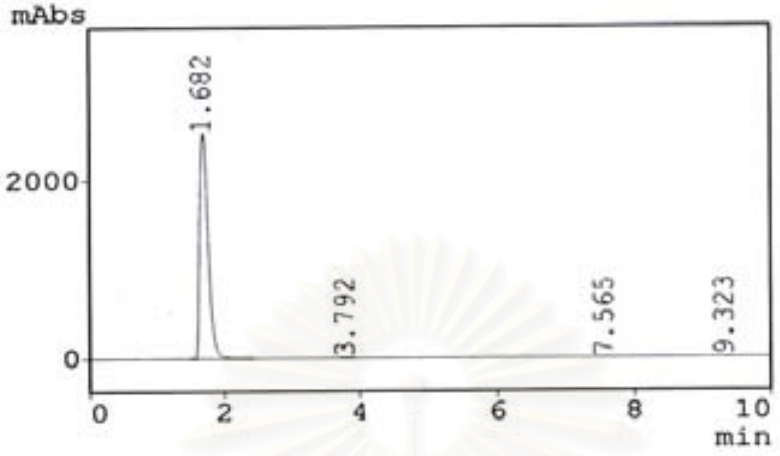

Figure D6 Formulations without ketoconazole

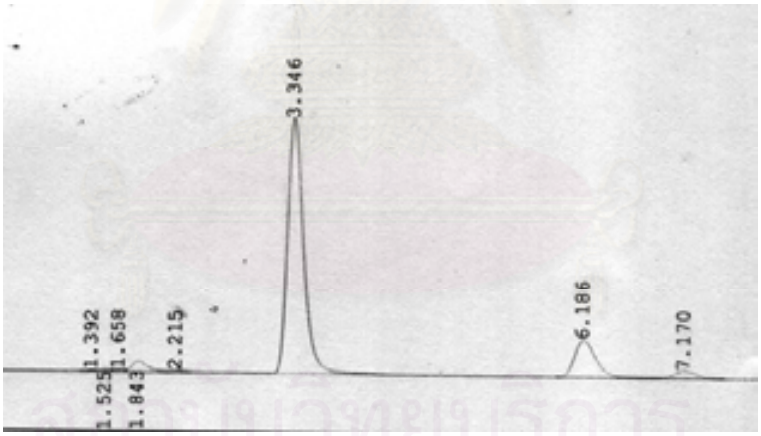

Figure D7 Peak of ketoconazole (Rt= 3.346)

Peak of Clotrimazole (Rt= 6.186) : internal standard

APPENDIX E

Size distribution of Spray dried powders

By

Laser Light Scattering

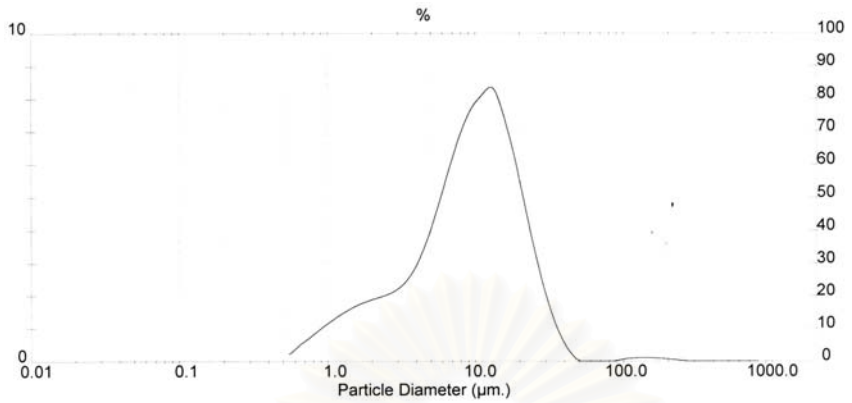

Figure E 1 Size distribution of spray dried powders by Laser light scattering

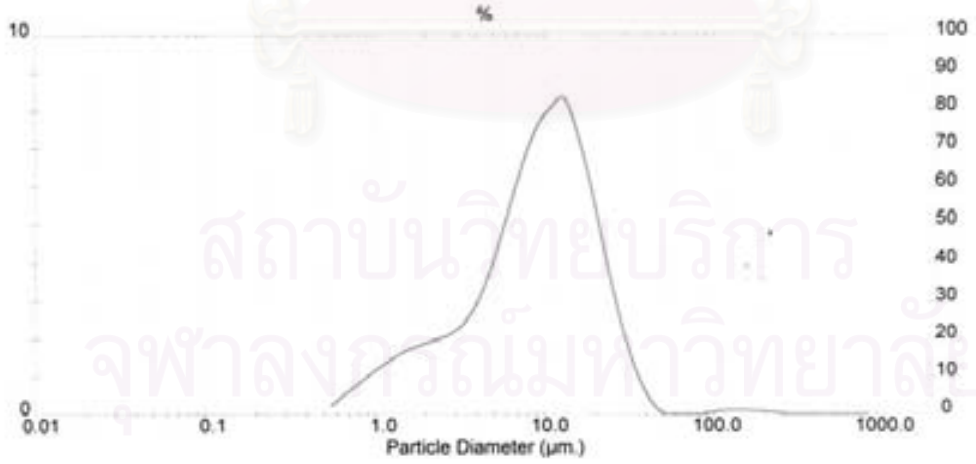

Figure E 2 Size distribution of spray dried powders by Laser light scattering

APPENDIX F

X-ray diffractometry

of spray dried powders

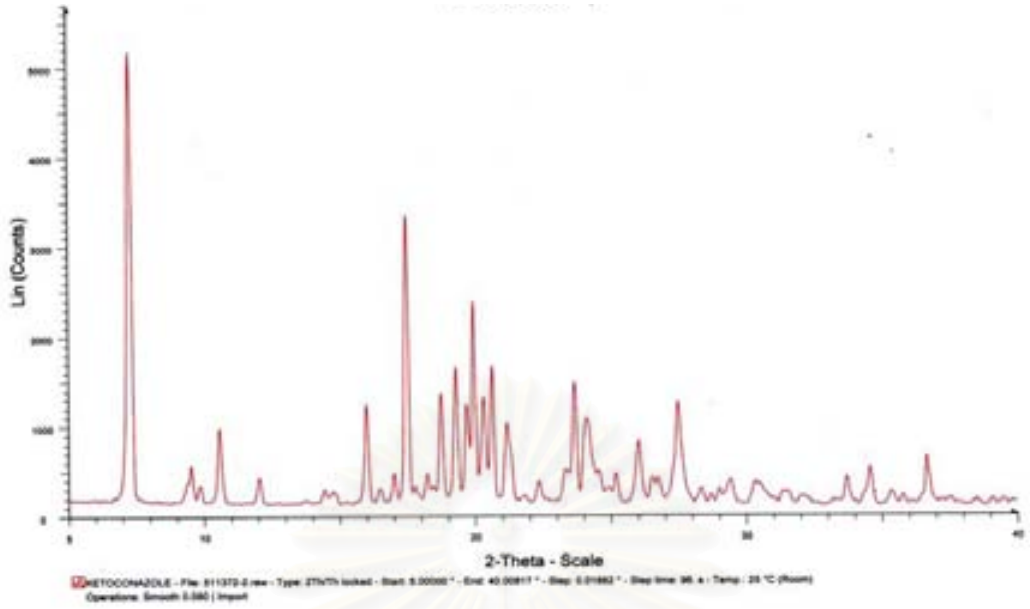

Figure F1 Diffractogram of ketoconazole

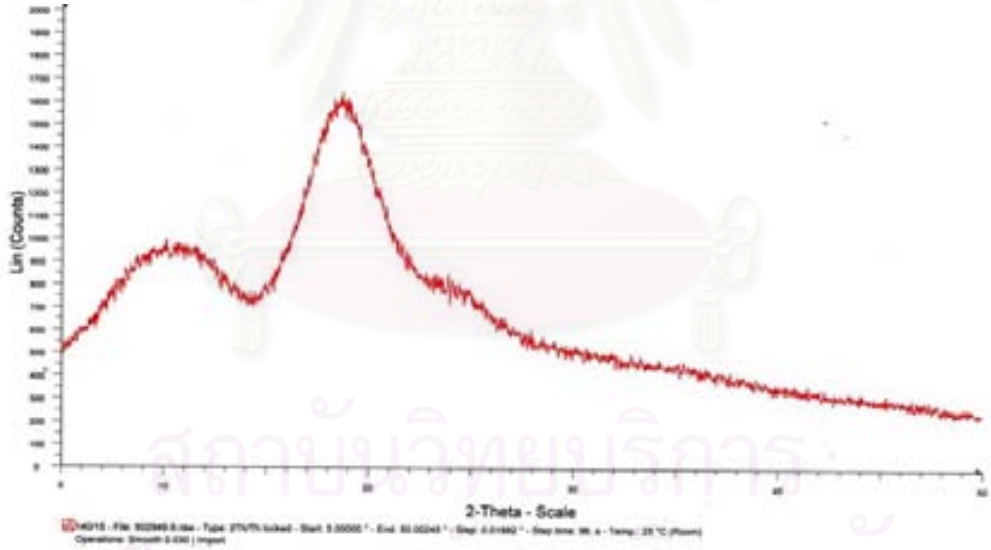

Figure F2 Diffractogram of ketoconazole inclusion complex with HPBCD

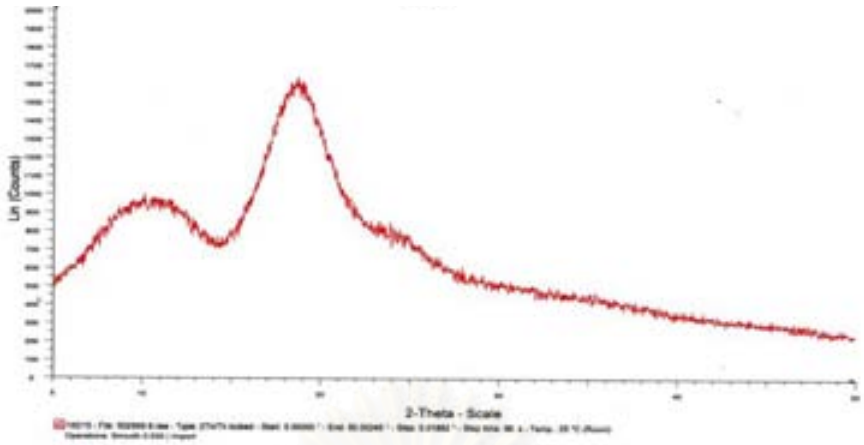

Figure F3 Diffractogram of spray dried, 100°C, 10. 5 ml/min

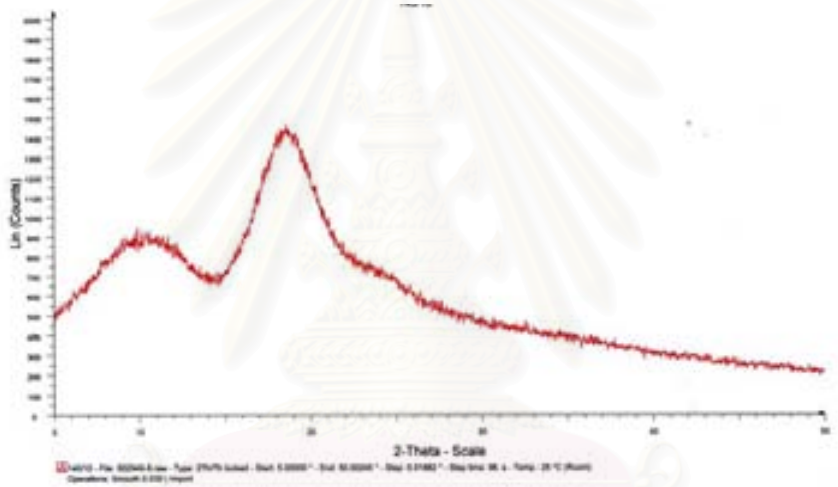

Figure F4 Diffractogram of spray dried, 100°C,7. 0 ml/min

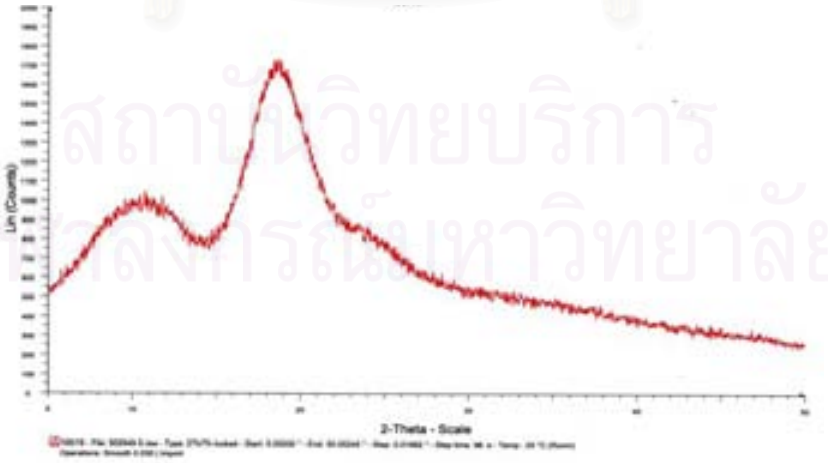

Figure F5 Diffractogram of spray dried, 100°C,3.5 ml/min

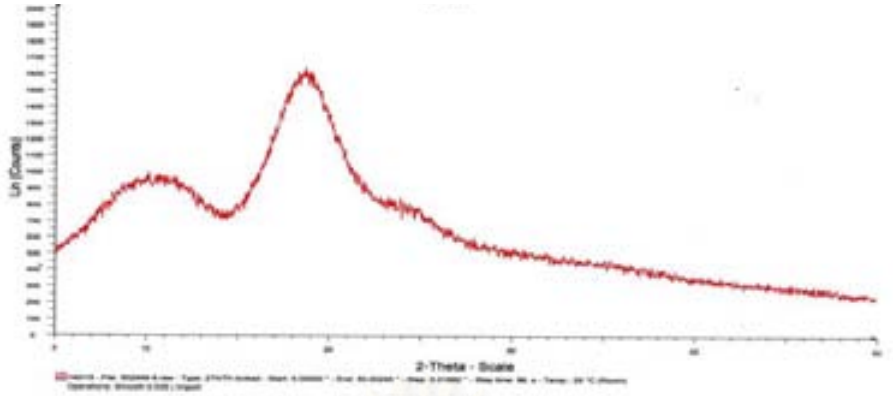

Figure F6 Diffractogram of spray dried, 140°C, 10.5 ml/min

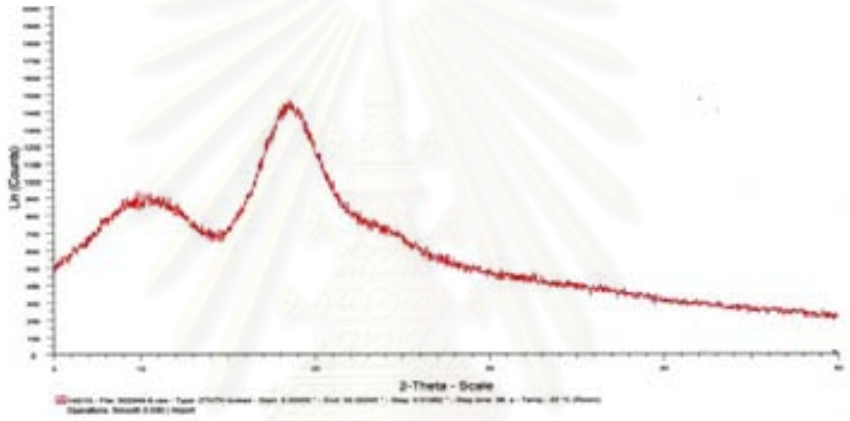

Figure F7 Diffractogram of spray dried 140°C, 7.0 ml/min

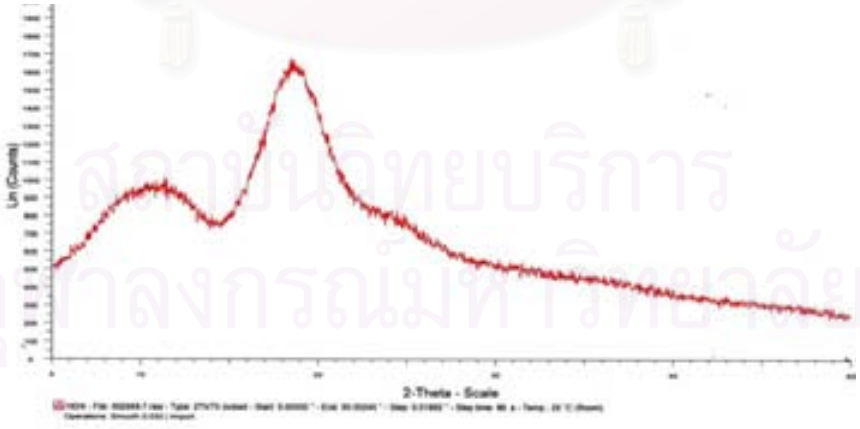

Figure F8 Diffractogram of spray dried 140°C, 3.5 ml/min

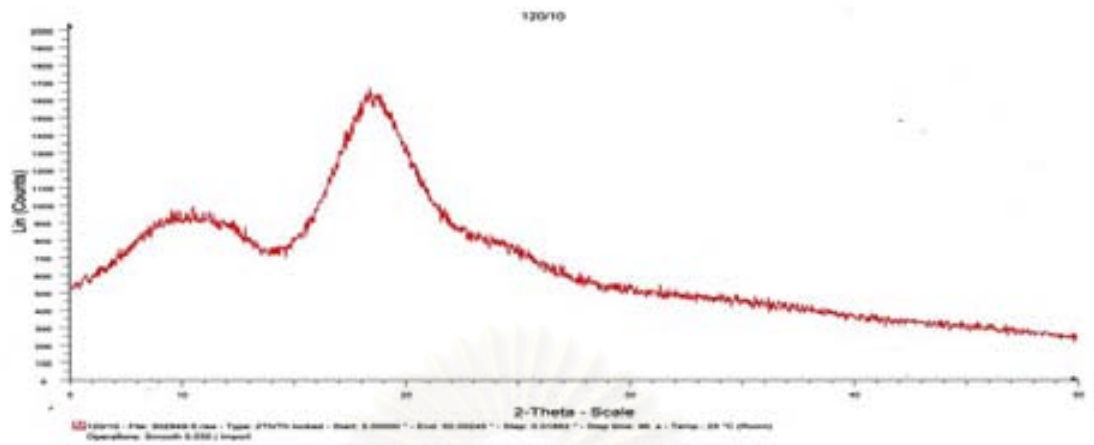

Figure F9 Diffractogram of spray dried 120°C, 3.5 ml/min

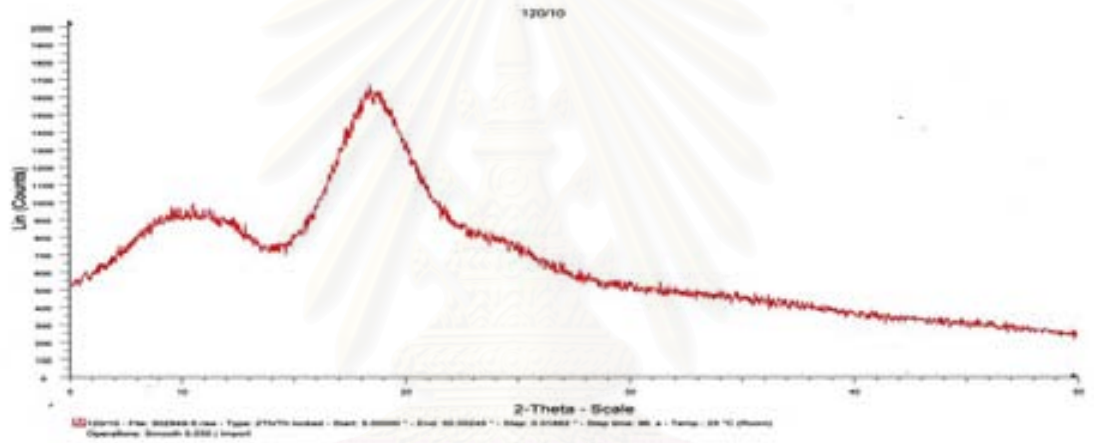

Figure F10 Diffractogram of spray dried 120°C, 7.0 ml/min

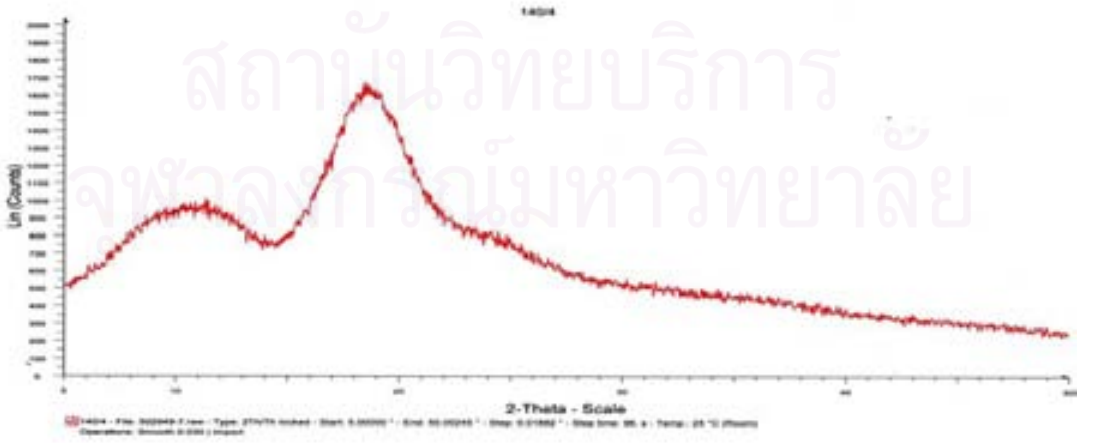

Figure F11 Diffractogram of spray dried 120°C, 7.0 ml/min

APPENDIX G

Thermograms of diluents of ketoconazole dry mixtures

by

Differential Scanning Calorimetry

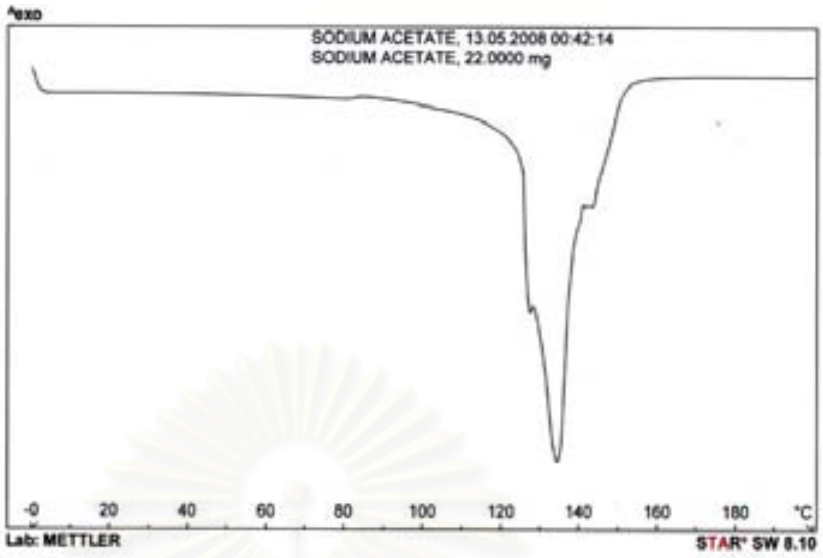

Figure G1 Thermogram of Sodium acetate

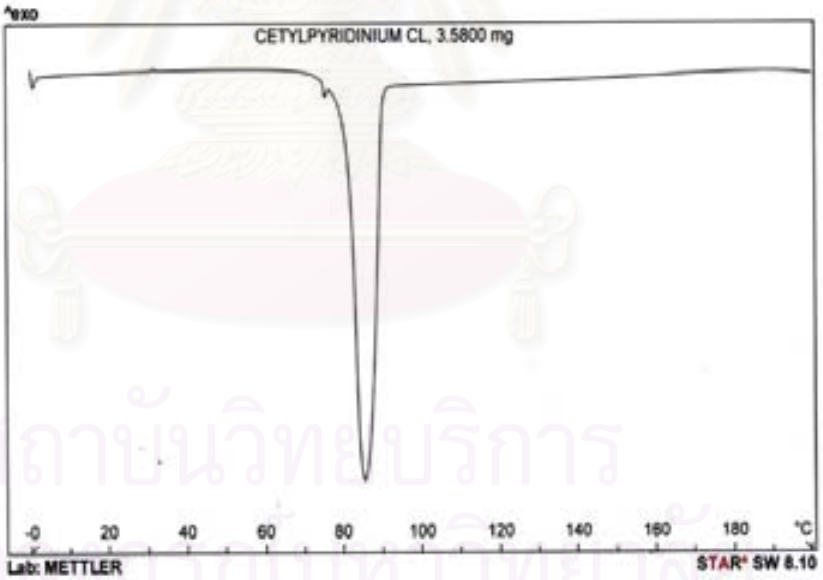

Figure G2 Thermogram of Cetylpyridinium Chloride

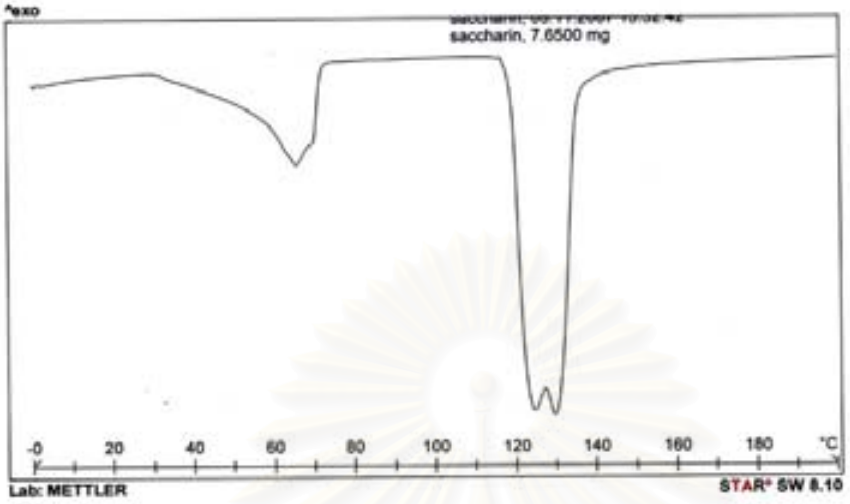

Figure G3 Thermogram of Saccharin sodium

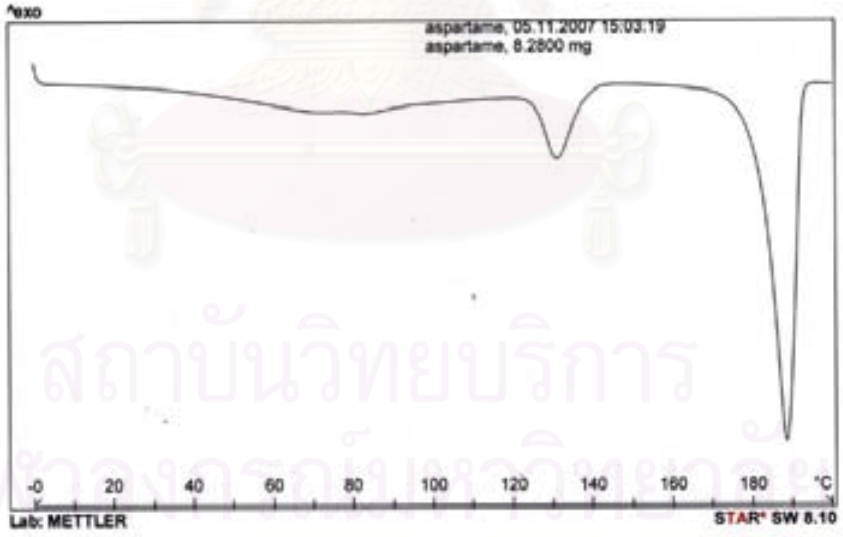

Figure G4 Thermogram of Aspartame

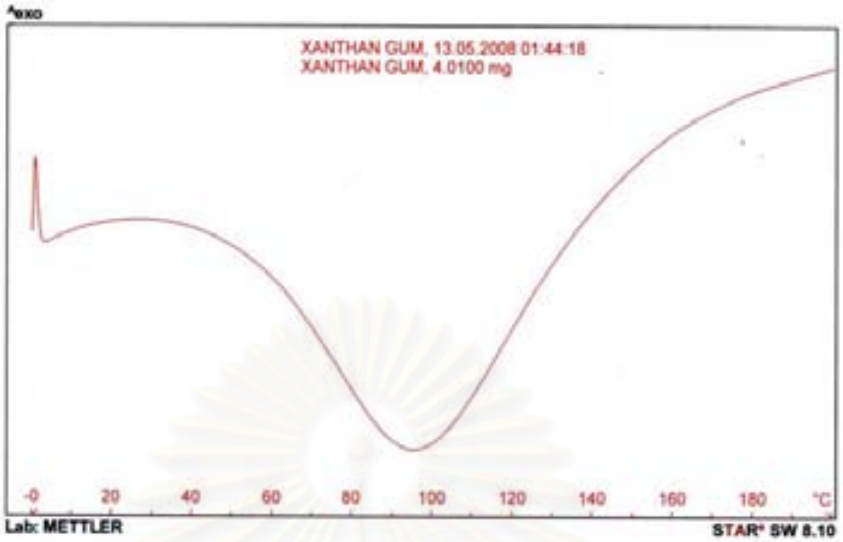

Figure G5 Thermogram of Xanthan Gum

VITA

Mister Neeranart Potiyanon was born on March 22, 1980 in Phitsanuloke, Thailand. He received his Bachelor's degree in Pharmacy from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand in 2003. Then, He was accepted to study the Master's degree program in Department of Pharmacy, the faculty of Pharmaceutical sciences at Chulalongkorn University in two years later.