การประยุกต์ใช้เทคนิคสเปกโตรสโคปีรังสีใต้แดงช่วงคลื่นใกล้สำหรับ

การควบคุมคุณภาพของการผสมตัวยา

นายนิติ สันแสนดี

APPLICATIONS OF NEAR INFRARED SPECTROSCOPY TECHNIQUE FOR

QUALITY CONTROL OF PHARMACEUTICAL INGREDIENT BLENDING

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SPECTROSCOPY TECHNIQUE FOR QUALITY CONTROL OF
PHARMACEUTICAL INGREDIENT BLENDING)
อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.อนงค์นาฏ สมหวังธนโรจน์, 109หน้า.

การควบคุมคุณภาพการผสมแห้งตัวยาสำคัญกับสารประกอบตัวยามีความสำคัญมากใน
อุตสาหกรรมยาปัจจุบันการวิเคราะห์ด้วยเทคนิคสเปกโตรสโคปีรังสีใต้แดงช่วงคลื่นใกล้กำลังได้รับ
ความสนใจใช้ทดแทนเทคนิคไฮเพอร์ฟอร์แมนซ์ลิควิดโครมาโทกราฟีในการควบคุมคุณภาพการ
ผลิตยาเนื่องจากเป็นเทคนิคที่มีความรวดเร็วไม่ต้องเตรียมและทำลายตัวอย่าง

วิทยานิพนธ์นี้ทำการศึกษาการประยุกต์ใช้เทคนิคสเปกโตรสโคปีรังสีใต้แดงช่วงคลื่นใกล้
สำหรับการควบคุมคุณภาพของการผสมตัวยาให้เป็นเนื้อเดียวกัน การสร้างและตรวจสอบความ
ถูกต้องของแบบจำลองสมการมาตรฐานโดยสมการมาตรฐานคำนวณโดยใช้วิธี Partial Least
Squares (PLS) จากข้อมูลการดูดกลืนรังสีใต้แดงช่วงคลื่นใกล้กับวิธีมาตรฐานตามตำรับยา
สหรัฐอเมริกาเล่มที่ 30 ได้ค่า correlation coefficient (R^2) = 0.9980, standard error of
prediction (SEP) = 2.49 ในชุดตัวอย่างที่ไม่ได้ใส่สารหล่อลื่น และ R^2 = 0.9980, SEP = 2.24
ในชุดตัวอย่างที่ใส่สารหล่อลื่น แสดงว่าสมการมาตรฐานมีความแม่นยำและเที่ยงตรงสูง การ
ทดสอบความถูกต้องเทียบกับเทคนิคมาตรฐาน โดยใช้ตัวอย่างจากกระบวนการผลิต ไม่พบความ
แตกต่างของค่าเฉลี่ยของทั้งสองวิธีแตกต่างกันอย่างมีนัยสำคัญทางสถิติโดยใช้ค่าทางสถิติ pair t-
test ที่ความเชื่อมั่น 95% ในทุกตัวอย่างที่ทดสอบ ทำให้สามารถนำเทคนิคสเปกโตรโคปีรังสีใต้
แดงช่วงคลื่นใกล้มาวิเคราะห์หาเวลาที่เหมาะสมในการผสมแห้งผงยา พบว่าตัวยาสำคัญและสาร
ช่วยแตกกระจายตัวเม็ดยาผสมเป็นเนื้อเดียวกันในเวลา 20 นาที หลังจากนั้นใส่สารหล่อลื่นผสม
ต่ออีกอย่างน้อย 3 นาที ส่วนประกอบทั้งหมดจึงผสมเป็นเนื้อเดียวกัน

โดยสรุปเทคนิคสเปกโตรสโคปีรังสีใต้แดงช่วงคลื่นใกล้เป็นเทคนิคเทคนิคที่ให้ผลที่ถูกต้อง
แม่นยำเทียบกับวิธีมาตรฐาน ไม่ต้องเตรียมและทำลายตัวอย่างในการวิเคราะห์ ช่วยลดเวลาการ
วิเคราะห์ส่งผลทำให้เวลาและต้นทุนการผลิตลดลง เหมาะสมในการควบคุมคุณภาพการผสมแห้ง
ผงยาในอุตสาหกรรมยา

ภาควิชา	วิศวกรรมเคมี	ลายมือชื่อนิสิต	
สาขาวิชา	วิศวกรรมเคมี	ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก	
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NITI SUNSANDEE : APPLICATIONS OF NEAR INFRARED SPECTROSCOPY TECHNIQUE FOR QUALITY CONTROL OF PHARMACEUTICAL INGREDIENT BLENDING. ADVISOR : ASST.PROF. ANONGNAT SOMWANGTHANAROJ, Ph.D., 109 pp.

The blending of Active Pharmaceutical ingredient (API) and more ingredients affects the uniformity and product quality. Near-infrared spectroscopy (NIRS) has become an interesting analytical technique to control the uniformity and product quality. This technique is a particularly more powerful method than high performance liquid chromatography (HPLC) for rapid and non-invasive analysis of powder blends.

The objective of this study is to apply NIRS technique to control the quality of blend homogeneity. The NIR calibration model was performed by Partial Least Squares (PLS) model. The PLS calibration showed a strong correlation with the reference values and great accuracy were demonstrated, i.e., for API without lubricant set correlation coefficient (R^2) = 0.9980, standard error of prediction (SEP) = 2.49, and for API with lubricant set R^2 = 0.9980 SEP = 2.24. The paired t-test at the 95% confidence level did not indicate any differences between the validation results by the NIRS and the method as specified in USP30 in powder blending process. From the experiment, the suitable mixing time of API and disintegrant were determined at 20 minutes and the mixing time of lubricant was not less than 3 minute after the homogeneity of API and disintegrant.

The NIRS technique has potential to reduce the production time and cost in the future which have to be develop for on-line process control.

Department : ....Chemical Engineering....   Student's Signature _Niti Sunsandee_

Field of Study : _Chemical Engineering_...   Advisor's Signature _____

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LIST OF ABBREVAIATIONS

%L.A.	%Label Amount
%RSD	%Relative Standard Deviation
API	Active Pharmaceutical Ingredient
Bias	The average of differences between reference value and NIR value
F	The number of factors
FDA	Food and Drug Administration
FT-NIR	Fourier-Transform Near-infrared
GMP	Good Manufacturing Practice
HPLC	High Performance Liquid Chromatography
M.W.	Molecular Weight
MIN	Minute
NIR	Near Infrared
NIRS	Near Infrared Spectroscopy
P	%Purity of API working standard
P	P-value in Statistical paired t-Test
PAT	Process Analytical Technology
PCA	Principal Component Analysis
PLS	Partial Least Squares
R^2	The coefficient of multiple determination
RPD	The ratio of standard deviation of reference data in the validation set to SEP Unit: %
SD	Standard Deviation
SEE	Standard Error of Examination
SEC	Standard Error of Calibration
SEP	Standard Error of Prediction
USP	United States Pharmacopeia

CHAPTER I

INTRODUCTION

1.1. Importance and reasons for research

Process Analytical Technology (PAT) [1] is an evolving philosophy in the pharmaceutical industry to conduct strategies for controlling of manufacture processes. PAT technology which Guidance for industry has been defined by the United States Food and Drug Administration (FDA) in 2004 as a mechanism which is new and rapid analytical methodology investigating for at-line and off-line measurement. The concept of PAT [1-4] is to design and develop manufacturing processes, also consistently ensure a predefined quality during manufacturing. Fiber-optics-based spectroscopic measurement, such as near infrared spectroscopy (NIRS) is interesting alternative for a conventional at-line monitoring technique in industrial reactors, as it does not required transferring systems or sampling preparation (dilution, extraction or evaporation). NIRS technique is also a non-invasive and allow multipurpose and multipoint remote monitoring.

In the pharmaceutical industry, powder blending process is one of the most common unit operations to uniformly mix the active drug ingredient by one or more ingredients affecting in uniformity and quality of product [5-7]. Blend homogeneity ensures a uniformly distribution and reproducibility of all components in blending process, especially, for direct compression of tablets. The Current Good Manufacturing Practices (cGMPs) has described in European Pharmacopoeia in 2005 and United States Pharmacopoeia in 2007 to require the in-process controls and tests must be conducted by the appropriate samples of in-process materials in order to assure the uniformity and integrity of drug products for each batch [2,3]. The controlling procedures are required to monitor the output and validate performance of manufacturing processes which may cause variability to in-process material and drug product. The conventional validation conducts by manually analysis which collected the off-line thief samples by using the high performance liquid chromatography (HPLC).

The validity is required for an active pharmaceutical ingredient (API) [2,3], but not necessary for the non active pharmaceutical ingredient (non API) due to, there are many factors and times concerning to the analysis and also lack of overall understanding in blending process control [8-10]. To address this issue, strong analytical technologies of pharmaceutical industry has been applied for blend uniformity analysis.

Recently, NIRS technique has been employed as an alternative method for common destruction which can save time and reduced the large volume required solvent that was toxic and expensive. This NIRS method provides non-destructive physical and chemical characterizations for the active and inactive components of composite mixtures. Qualitative NIRS application depends on a pattern recognition mode to analyze the multivariate data which generated from the spectrometer [5-6]. In pharmaceutical industry, qualitative NIRS applications were used to identify raw materials for testing, to detect tablet and capsule products tamper and degradation[5,6], and to determine ointment homogeneity [6]. The advantage of NIRS in valid powder mixing process has been described [9-11] as the analytical technique which allow for rapid complex matrix analysis.

Several differential analytical methods have been developed for drug's quantitative determination for pure and pharmaceutical dosage forms. These methods included high performance liquid chromatography, high-performance thin layer chromatography, and ultraviolet-visible spectroscopy; however, their sophisticated instrumentation and high-analysis cost limited their applicability in quality control laboratories to analyze the API for pharmaceutical dosage forms.

The objective of this study is to develop NIRS technique to assess the homogeneity of direct compression pharmaceutical powder blends consisting of API, disintegrant, lubricant and other non-API. Homogeneous blend and optimal mixing times performed a qualitative analysis to control the quality control of products.

1.2. Objectives of study

The objectives of this research are as follows:

1. To develop NIRS method that uses for assessment the homogeneity of typical direct compression pharmaceutical powder blends

2. To validate quantitative analysis of the developed NIRS by manually analyzing collected thief samples off-line using HPLC

3. To optimize mixing times of blend homogeneity by using NIRS analytical technique

1.3. Scopes of Study

1. The calibration model of NIRS method was performed for assessment the homogeneity of typical direct compression pharmaceutical powder blends.

2. The validation results of NIRS method were compared with HPLC analysis by method validation division of Government Pharmaceutical Organization, Thailand.

3. The mixing times of blend homogeneity were selected to optimize by using NIRS analytical technique.

1.4. Expected Benefits

1. Using NIRS analytical technique as a rapid non-destructive technique to substitute traditional chemical technique

2. Understanding NIRS analytical technique to optimize mixing times of blend homogeneity

1.5. Methodology

1. Research and review literature relating to NIRS analytical technique

2. Study the blending process in the pharmaceutical industry

3. Make a calibration sample set and validation of the calibration models

4. Evaluate the optimal mixing times of blend homogeneity by NIRS technique

5. Compare the NIRS results with the traditional techniques such as HPLC

6. Summarize and report a document

1.6. Activity plan

Activity	Time																						
	Year 2007								Year 2008												Year 2009		
	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC	JAN	FEB	MAR
1. Research and review literature relating to NIRS analytical technique	←	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	→						
2. Study the blending process in the pharmaceutical industry			←	—	—	—	—	—	—	—	→												
3. Make a calibration sample set and validation of the calibration models								←	—	—	→												
4. Evaluate the optimal mixing times of blend homogeneity by NIRS technique											←	→											
5. Compare the NIRS result with the traditional techniques such as HPLC.											←	—	—	→									
6. Summarize and report a document															←	—	—	—	—	—	→		
7. Present the research																				←	—	—	→

4

CHAPTER II

THEORY

2.1. Blending process

Blending powders is an important unit operation in various industries, such as manufactories of chemicals, construction materials, plastics and pharmaceuticals. In these industries, especially, in pharmaceutical are restrictive quality of expensive products pose significant challenges to process quality control. Batch blending has been widely applied to many processes. Blending powders operate by made two or more powder homogeneous, if necessary, some liquid are added. The powder characteristic was poorly understood from a fundamental stand point, it might be expected that the principles behind powder blending in batch system would play an important role in continuous systems, for example, mechanisms of convective mixing, diffusive mixing and shear mixing, can also be presented in continuous blending systems as well. Convective mechanism refers to blending powders as bulk movement of powder which changed a mechanism in ribbon and rotating screw mixers. Diffusive mechanism is the random movements of powders across slip planes or failure zones and on the free exposed surface of the powder which dominate mechanism in cubic blenders [6,12,13]. Shear mechanism is considered as a combination of diffusive and shear mixing. The internal blending of elements induced shear force in particles and velocity gradients of bed, mixing of pin mills can be described of such shear mechanism.

2.2. Near-infrared spectroscopy (NIRS)

NIRS is a non destruction and rapid technique increasingly applied to evaluate the drug quality. In recent years, many handbooks and papers have described NIRS theory applied to pharmaceutical industry for quality research of pharmaceutical products. The specific technique with chemometrics has proven its effectiveness in both qualitative and quantitative analysis for pharmaceutical industry.

2.2.1. History

In 1800s, William Herschel had discovered the radiation beyond visible red light, however, prior to World War II, the near infrared (NIR) region was not considered as usefulness for spectroscopy. It was observed that near infrared bands are severe overlapping and difficulty to interpret [14-18]. Until 1950s, the early UV/Vis instruments to complement the mid-IR were done with NIRS. Initially, NIR spectroscopy was treated as an extension technique specified for other wavelengths, such as UV/Vis or mid-IR methods. The first commercial stand alone NIR system has been introduced in 1980s. Academic and industrial attentions (and commercial instrumentation) rapidly grew in 1990s; systems operation is based on optical fibers and more sensitive detectors, based on modern semiconductors.

2.2.2. Basic principles of near-infrared

The NIR signal (spectrum) is a consequence of light absorbance by molecular vibration (overtones and combinations of fundamental vibrations) of hydrogen bonds like C-H, N-H, O-H and S-H chemical bonds. The NIR spectral region includes two sub-ranges, i.e. shorter-wavelength or Herschel range which extends from approximately 750 to 1100 nm ($\sim$13,333–9000 cm^{-1}) and longer wavelengths between 1100–2500 nm which comprised of traditional NIR region [14-18]. Generally, compared to other spectrophotometers, NIRS is enable to apply in both qualitative and quantitative assessment to the chemical composition of samples. It is somehow, quite sensitive to physical properties of those samples. Measurements can be made directly on in situ samples, in addition, to standard sampling and testing procedures. Physical information as well as chemical information, both qualitative and quantitative, is available from NIR spectra. Direct comparative spectrum obtained with the substance being examined with reference spectrum of a chemical reference substance, as used in infrared absorption spectrophotometer, cannot be directly interpreted. Suitable validated mathematical treatment of data is required. Because molar absorptivities in the NIR range are low, radiation typically penetrates several millimeters into materials, including solids. Furthermore, many materials such as glass are relatively transparent in this region.

2.2.3. Instrumentation [5,6,16]

NIR spectrophotometer is an instrument operated by spectra recording in 800 nm to 2500 nm (about 12,500 cm^{-1} to 4000 cm^{-1}) regions. All NIR measurement mechanisms are to pass light through or into sample, then measure the attenuation of emergent (transmitted, scattered or reflective) beam. Efficiency NIR operation depends on selecting correct step of instrument. There are four different types, i.e., monochromaters, diode array spectrometer, filter-instrument and fourier transform instrument.

1. Monochromator is used to measure the full visible and NIR spectrums operating of transmitted or reflective mode. It generally applies to laboratory for research purpose or wide ranges of different required usages. This instrument contains silicon and lead sulfide detectors (wavelength around 400–2500 nm). Latest model restricts in measurement of powder or granular samples by diffuse reflection or transmission by fiber-optic probes. At least one model of NIR monochromator has been designed for on-line application either fiber-optics or powder sample. Another type of dispersive monochromator operated in NIR instruments is an acousto-optically tunable filter (AOTF) which comprises of TeO$_2$ transparent crystal. A plane of transferring acoustic wave is generated from the right angles through an incident light beam as shown in figure1 that made the crystal behavior as a longitudinal diffraction grating, periodicity equivalent to sound wavelength across material. Main advantages of AOTF over grating instrument are its simplicity mechanism (i.e. no moving parts) and stable wavelength.
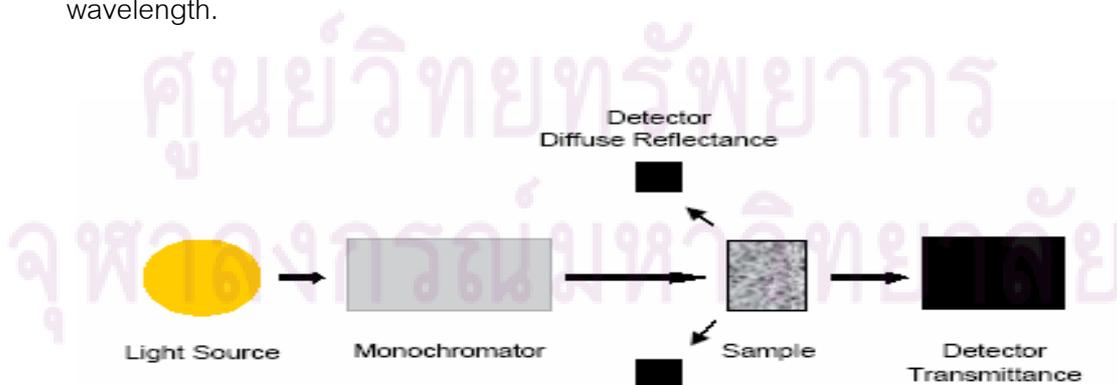
Figure 2.1   Basic NIR spectrometer configurations [14]

2. Diode array spectrometer employs array of the IR-emitting diode. This instrument functions are combined of both light source and wavelength selection mode. Diode array instrument typically covers the range of 400–1700 nm. Their advantages are very fast in measurement (e.g. one spectrum per second) and noninvasive. These features are particularly useful when a high sample throughput or ultra-rapid on-line measurements are required.

3. Filter instrument is the simplest and cheapest one of NIR instruments. Operation of an instrument is based on limited interfering filter quantity. This instrument has been chosen to represent the absorbed usages for wide types of application, e.g. moisture and solvent of powdery drug samples. Filter instrument has been designed for limited range of routine analyses, both in laboratory and on-line measurement.

4. Fourier transform instrument is new version of NIR instruments. This instrument uses an interferometer which is also common, especially above 1000 nm wavelength. Spectrum can be measured in transmittance or reflectance modes depended on sample types.

2.2.4. Sample presentation [5,6]

Wide ranges of NIRS application to pharmaceutical analysis are possible due to different in sample presentation techniques which are available for any types of liquid; slurry, powder or solid sample. There are four different types of NIR modes, i.e., diffuse transmittance, diffuse reflectance, transflectance, and on-line sampler.
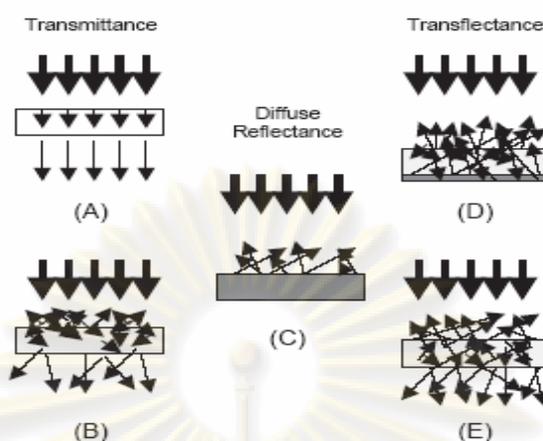
Figure 2.2   NIR measuring modes—(A/B) transmittance, (C) diffuse

reflectance and (D/E) transflectance [14]

1. For diffuse transmittance, the interaction of radiation and sample might be absorbed, transmitted or reflected as shown in figure 2.1 and 2.2. In classical spectroscopy experiment, reflection is eliminated; therefore, the proportion of attenuating radiation from sample can be measured by transmittance. Beer's law then defines a relation between transmittance and the product of concentration of the absorbing species and path length. For transparent liquid sample, path length may be fixed by means of a static or flow-through sample cuvette or a pair of fiber-optic probes and developing calibration using the known sample concentration. Diffuse transmittance usually measures in 800–1100 nm regions of the spectrum where the weak absorptions exhibit useful data to be obtained by using samples with 1–2 cm such as liquid dosage form [16].

2. For diffuse reflectance with smooth surface such as glass, most radiations is reflected from the surface regularly and no absorbed occurred. In 1100–2500 nm regions, the amount of scattering makes the path length so large that transmittance through 1 cm of most samples is negligible. This mechanism is called diffuse reflectance because most incident radiation is reflected as shown in Figure 2.1 and 2.2 [16]. Otherwise, the matted surface diffusely reflects without penetrated into the sample, like reflectance with no absorption takes place. If, however, some of the radiation

penetrates through the surface when it reaches each particle it can be reflected, absorbed or transmitted. The net result is that diffusely reflected radiation (R) can empirically be related to concentration (c) in an analogous way to Beer's law, i.e. log 1/R = kc where k is a factor which incorporates both absorbtivity and path length.

3. For transflectance, adaptation of diffuse reflectance experiment can be applied to liquid by placing ceramic tile beneath the sample as shown in figure 2. The radiation is transmitted through sample, reflected from the ceramic then transmitted back to the sample before finally reached to detector. It is thus a hybrid of transmittance and reflectance. Interactance, another hybrid of transmittance and reflectance, involves illumination and detection at laterally separated points on the sample's surface. It normally accomplishes by using a fiber-optic probe, one set of fiber-optic bundle carries an incident radiation and another carries a reflected radiation.

4. For on-line samplers [6], there are two types of NIR on-line analyzers such as remote (non contact) sensor and fiber-optic probe.

4.1. Remote (non contact) sensor is the first dedicated on-line NIR sensor. The advantages of this design are by low cost instrument and simplicity installation but impose in severe constraints by instrument design as susceptible interference from ambient light variations, dust build-up on the optical surfaces and atmospheric humidity variation. The Infrared engineering gauge is specifically to design for on-line application and to minimize such potential interferences. In this case, the instrument is inverted over open mixing bowl and continuously recorded the signal from the dough without stopping the mixer. In this way, using second derivative data at defined wavelengths assigns to moisture determination in drug powder.

4.2. Fiber-optic probe [7,12] is a new method for sample presentation. This method is the widest range applications in on-line pharmaceutical analysis. Conventionally, control is attempted to measure of input characteristics; such as moisture content, blending uniformity, screw speed and temperature. In the case of attenuation, totally internal reflection by optical fibers, the near-infrared range presents much advantage over the mid-infrared. In the NIR region, the absorption bands widely separate from its vicinity; more broadened and are dramatically reduced in intensity. In

optical fiber, light will interacted many times with absorptive species. The absorption which measured in final spectrum is in fact a sum of the absorptions. In the mid-infrared region, absorption bands are very strong, allowing only a limited number of contacts with the sample.

2.2.5. Theory and practice of chemometric data processing

1. Data pretreatment [2,3,7,12,19] usually is a vital step in the chemometric analysis of NIR spectral data, it can be defined as mathematical transformation of the NIR spectral data to enhance spectral features and remove or reduce unwanted sources of variation prior to calibration model development. Calibration is a process to construct mathematical model relating to a response from analytical instrument by each properties of samples. Many suitable chemometric algorithms of data pretreatment and calibration are existent therefore, the selection should be based on suitability of intentionally use. Any available data transformation or algorithm that can be clearly defined in exact mathematical expression and provided suitable results can be used.

2. For chemometric data processing [8], Chemometric tool is a method to establish relation between different measurements from chemical system or process by state the system through its application in mathematical or statistical methods. Typically, a chemometric problem might be defined a relation of an interested properties (e.g., difficult to measure in the laboratory) based on knowledge from other properties which is easier obtained by affecting of an interested property [5-8]. The values of these variables are generally obtained by experiment which generally, no first principle assumption is made; therefore, a set of data must be selected from process measurement, preferentially comes from designed experiment because training set should be cover the spatial span of process. The next step is to build and validate a model using either multivariate regression or multivariate classification methods depended on model purpose, much methods for model validations.

Regression method comprises of multiple linear regression (MLR), principle component regression (PCR), and partial least squares (PLS) [5,6]. Classification methods comprise of discriminant linear analysis, principal component analysis (PCA)

[19], factor analysis (FA) and cluster analysis (CA) [6,7,19]. Non-linear techniques such as neural networks calibration and Kohonen networks classification are often used but generally, their robustness is lower than linear techniques, due to lack of extrapolating capacity. Partial least squares are adopted to build calibration models and Kohonen networks are used to build classification models, details are as follows.

2.1. Calibration model is a mathematical expression relating to the response from analytical instrument for each samples properties.

2.2. Principal Component Regression (PCR) [5-7,19] is a calibrating algorithm applying for response receiving from analytical instrument related to the sample properties. This algorithm expresses a set of independent variables as linear combination of factor, it is a method related to those factors effecting to the sample properties that independent variables were obtained.

2.3. Partial Least Squares (PLS) [5-7,19] is a calibrating algorithm applying for responses receiving of sample's properties from instrument. The distinguishing feature of this algorithm is similar to PCR model; this algorithm include the data concerning to the properties of samples which is used for calibration, factor calculation are used to describe instrument responses.

2.2.6. Pharmaceutical applications

NIR spectroscopy combined with multivariate data analysis opens many interesting perspectives in pharmaceutical analysis in both qualitatively and quantitatively way. Fast and nondestructive NIR measurements without any sample pre-treatments might increase the throughput tremendously analysis. Using fiber optic probes offers an opportunity for in-line and on-line process monitoring. Special feature combining of chemical and physical information allows the assessment of spectral signature of raw materials, intermediates and final dosage forms, which in turn offers the possibility of simultaneous determination of several samples, i.e. identification, assays, process monitoring and process control.

1. For identification [5,6,15,16] in the pharmaceutical industry, before using of all incoming materials must be identified and approved suitability of their intended purpose; usually, performed by NIRS. Spectra of incoming materials have been evaluated by comparison with NIR spectra library and new compound, positively or negatively identified is one parameter which the compound has presented. Such identification can thus be considered as the part of quality control (QC) for incoming products: positive result (i.e. similar to an approved product) identify as product is conformed to quality grade requirement.

2. For assays, [5,6,15,16] NIRS can also be used to perform the quantitative analysis of specific compounds in complex matrices, for instance, a pharmaceutical preparation. Normally, an NIRS assay is not performed in traditional method, such as UV spectrometry or HPLC. After defined an analysis purpose, samples will be selected and analyzed by both NIRS and validated reference method, then data were spitted by calibration (or training) set or test set. Importantly, calibration set should cover most variability expecting for future progress; moreover, NIR spectra of calibration samples should be in mathematic relating to an interested property (e.g. concentration) by using of chemometrical tools. The relation between NIR signal and interested property is called a calibration model. In beginning of these models, one is tried to optimize the model parameters for the best fit between sample calibrating measurement and model value prediction. Once the model is optimized for the calibration set, the performance of the model can be tested on the independent test set. This step is usually called the model validation. In validation step, an interested property of test samples will be predicted according to model optimization, this is a measurement for model performance of unknown samples. The most commonly used chemometrical regression methods are principal component regression (PCR) and partial least squares regression (PLS) [18].

3. For process monitor and control [5,6,15,16], noninvasive monitor of all relevant process steps leading to a pharmaceutical drug product is an integral part of the PAT paradigm of real-time or parametric release and quality by design. Ideally, the pharmaceutical survey chain should be included incoming raw materials; all unit operations leading to intermediates and final products and packaging. The noninvasive and multivariate characters of NIRS techniques provide an interesting platform for pharmaceutical process monitoring and control. Although most of the report for NIRS applications in the pharmaceutical industry are off-line or at-line, there are also some on-line and in-line applications. In this section, the current state and future potential of NIRS techniques in pharmaceutical at-line, on-line, and in-line processes monitor and control will be reviewed and discussed, with the main focus on technological unit operations that are crucial for the manufacture of solid dosage forms such as powder blending process.
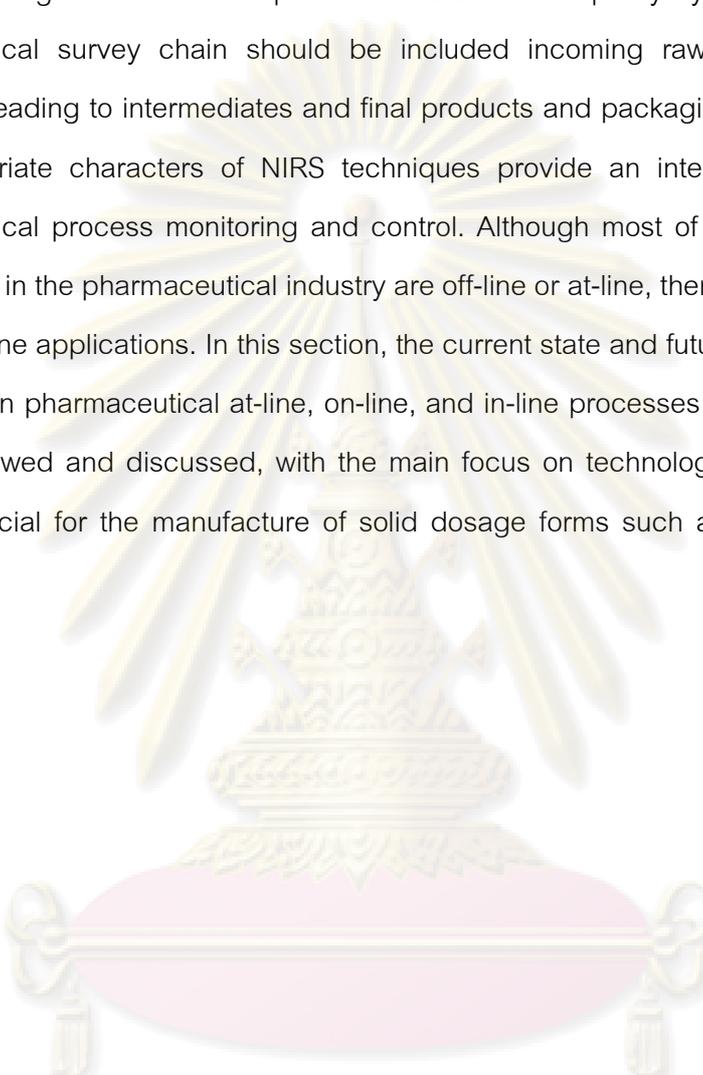
CHAPTER III

LITERATURE REVIEW

Powder blending is a basic unit operation in pharmaceutical industry. Blending uniformity is required to obtain good quality at the end of production. Homogeneous powder blend is well known that it is one of the most important steps during manufacturing of solid dosage forms in pharmaceutical industries [5]. Typically, the most consuming time of the production process is not the blending itself but analysis must be performed to validate the final homogeneity of drug substance. Usually, for the test of blending uniformity, manufacturers take some samples representing of blending product with a thief probe, then the samples are analyzed by traditional methods such as UV–visible spectrophotometer or high performance chromatography (HPLC) [5-8].

The development for more rapid, reproducible, cost-effective, and perhaps non-destructive of methods to control the products quality in pharmaceutical industry continues to be major emphasis. Near infrared spectroscopy (NIRS) is a potential method which meets those criteria [5,6]. First, NIRS is performed over a range of wavelengths which is capable in both quantitative and qualitative analyses. Second, an important feature of NIRS can be performed by the raw product without destroyed the sample property. Third, the time for sample analysis is quite less, only few minutes per sample. For many solid dosage forms, manufacturers are converted their process to NIRS analysis for their product quality control. For example, NIRS utilization in solid dosage form, the sample may be randomly taken from tablets during a process operating and quickly determine in real time. In the last few years, more quantity of NIRS techniques application in both qualitative and quantitative analysis can show their capability and efficiency. Many reports have focused on the determination of active ingredient in pharmaceutical preparations [12] or physical parameters of sample [6].

Usage of NIRS techniques both off line and on line have been reported from manufacturing factory for powder blend uniformity analysis by several authors in different blender locations at various of blending times [19-21].

Recently, NIRS has been attracted by various types of mixing systems. Ciurczak [5,7] have reported the application of NIRS for powder mixing studies, The experiment was used a spectral matching to routine and principal component to analyze the distinguishing spectra arisen from samples and various times drawn during mixing. Sekulic and team [22,23] were the first group who reported an on-line monitoring of powder blend homogeneity by NIRS in 1996, the results shown that blending has reached the homogeneous state before a typically blending period was completed. In the same year Wango and Drennen [5,7,24] reported the application of NIRS characterization to pharmaceutical industry by powder blend aspect in which the NIRS was used to qualitative assess the homogeneity of typical direct compression pharmaceutical industry. The qualitative analytical algorithms are based on Bootstrap Error-adjusted Single-sample Technique (BEST) which was proven the sensitive variations to sample homogeneity. Hailey and team [25] have developed system to monitor a homogenization of solid mixtures based on the measurements of fiber-optic probe fitting in mixer. The most advantage of these systems allows one to identify the end point of non-invasively homogeneity process in real time. The mixture homogeneity was determined by plotting the standard deviation for several replicates against the homogenization times. El-Hagrasy et al [9-12] reported the powder blend monitoring by comparing techniques between means of NIRS and NIR-imaging, using UV-spectroscopy as a reference method. The results have shown that NIRS techniques are associated with the UV reference method; moreover, NIR method reduced sampling errors and provided the possibility of on-line endpoint determination. El-Hagrasy et al [9-12] also studied the process control of pharmaceutical powder blending as Process Analytical Technology (PAT) application. The model development was successful in blend homogeneity prediction of independent blend samples under different processing conditions. The most recent study reported [12] a quantitative near-infrared calibration model for prediction of blending endpoint and mathematic and statistic models such as

PCR, PLS and MLR have been applied. Only MLR model operated by single wavelength was used to optimal calibrate and predict the results. The results were consistent with those from UV reference methods. Li and Worosila [19-21] described the NIRS technique to powder blend homogeneity, they have evaluated the quantitative analysis of powder mixtures ingredients using at line NIRS method. Their experiment has been designed to approach and to develop the calibration sample set model, in which samples were prepared by suitable both API and non-API weighing. The model was accurately generated the results of quantitative pharmaceutical active ingredient and three non active pharmaceutical ingredients. Later on, they have studied the online blend uniformity by a near infrared sensor. Quantitative calibration models were developed and validated for the results from sensor and assay obtaining in real time. The on-line and off-line data were compared and has shown the significant difference in standard deviation for pharmaceutical active ingredient and non active pharmaceutical ingredient. The data have been evaluated by real-time assay values of pharmaceutical active ingredient which was lower than other offline results. Discrepancy was the large beam size of online sensor which was important factor to quantitative online blending uniformity. Gupta [26] reported a method of real-time in-line near-infrared (NIR) monitoring for roller compaction. Multivariate analysis using partial least square projections to latent structures (PLS) was used to evaluate relation of spectral data with key compact attributes; content uniformity, moisture content, relative density, tensile strength, and Young's modulus.

Several data processing strategies for assessment of blend homogeneity and/or optimal blending times by NIR measurements have been evaluated in the literatures. Almost reports were concerned with qualitative assessments, such as dissimilarity between spectra of mixture and ideal spectrum of mixture or moving block standard deviation of NIR spectra [23]. Those mechanisms generally revealed the acceptable results. Even though, Wargo and Drennen [5] suggested that bootstrap techniques provided greater sensitivity for blend homogeneity assessment than chi-square calculations but some recent papers [27-29] still concerned quantitative analysis which the quantitative analysis was a prerequisite for completely resolved chemical and

physical properties of mixture. Non-linearity has been discovered as a feature of powder blends containing coarse and fine particles which was not any problems during cubic PLS calibration.

To summarize, it can be concluded that in- and at-line powder blend monitoring with NIR spectroscopy is feasible with the PAT paradigm for real-time release, focused on continuous process understanding and quality control of all production steps, rather than a final product control only.

In this thesis, the NIRS technique is applied to monitor and optimize the blending homogeneity of solid dosage form. The aim of study is to show an association between the NIRS technique and a conventional technique assay method.

Summary of Following Chapters

As a first step, Chapter 1 presents the necessary background theory and motivation of study. Chapter 2 deals with background theory of blending theory Near-infrared spectroscopy (NIRS) and chemometrics. Chapter 3 reviews literature. Chapter 4 describes material and methods applied to the experiments. Chapter 5 presents the results and discussion. Chapter 6 describes the conclusion.
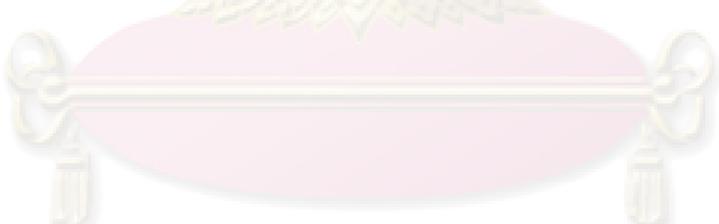
CHAPTER IV

EXPERIMENTAL PROCEDURE

4.1 Materials and reagents

API, disintegrant, lubricant and other non-API were provided by Government Pharmaceutical Organization Thailand. Methanol (HPLC grade) and Methanol (analytical reagent grade) were purchased from Lab scan. Ammonium acetate (M.W. = 77.08) was from Merck (Darmstadt, Germany). API, which was used as working standard was standardized by instrument center section of Government Pharmaceutical Organization Thailand. All materials and reagents were provided by Government Pharmaceutical Organization Thailand.

4.2 Near infrared reflectance spectroscopy (NIRS) methodology

NIRflex N400 with 2-m fiber optic probe (Buchi, Flawil, Switzerland) was used for determined all the samples. The equipment was setted at a wavelength range from 4008 to 9996 cm^{-1} with a resolution of 12 cm^{-1} in the reflectance mode was used to measure the samples. All samples were recorded by a scanning FT-NIR-spectrometer in random order for 5 locations. Each sample was scaned 5 times for one average spectrum to equilibrate in homogeneities. Chemometrical software Nircal 4.21 (Buchi, Flawil, Switzerland) was used for creating a model, i.e., selection of spectra and wavelengths, mathematical pretreatment and statistical analysis performing cluster analysis and partial least squares regression (PLS).The half of samples were used for calibration and another half of the sample sets were used for validation. The content of API was equally distributed over the whole concentration range in both the calibration set and validation set. The optimum number of factors used for the individual prediction was determined by cross-validation. The selection of the best regression model was based on the following values calculated for validation purposes:

(i) Standard error of calibration (SEC), the standard deviation of the differences between LC–UV and NIRS-results in the calibration set.

(ii) Standard error of prediction (SEP), the counterpart for the test-set samples. The parameter (i-ii) should be as small as possible.

(iii) BIAS, the average deviation between the predicted values and the actual values of the calibration set, should be close to zero.

4.2.1 Calibration set and validation set

4.2.1.1 Preparation NIR calibration set and validation set

A calibration set and validation set were prepared using a modification of a scheme published in 2005 [19]. The sample were prepared by weighing a suitable amount of API, disintegrant, lubricant and other non-APIs into a separate 20 ml bottle follow Table 4.1- 4.2 (Table 4.1 for sample without lubricant set, Table 4.2 for sample with lubricant set). An accuracy of analytical balance of ±0.01 mg was used. The total weight for each sample was approximately 7 g. The samples were mixed manually by shaking, and then visually inspected for the uniformity which later on confirmed in the validation.

Table 4.1 Designed calibration sample without lubricant set (all numbers are in % weight/ weight)

API	Disintegrant	Lubricant	Other non-API
24.88	2.6500,3.9750 5.3000,6.6250,7.9500	0.0000	to 100%
37.32	2.6500,3.9750 5.3000,6.6250,7.9500	0.0000	to 100%
49.76	2.6500,3.9750 5.3000,6.6250,7.9500	0.0000	to 100%
62.20	2.6500,3.9750 5.3000,6.6250,7.9500	0.0000	to 100%
76.64	2.6500,3.9750 5.3000,6.6250,7.9500	0.0000	to 100%

Table 4.1 Designed calibration sample with lubricant set (all numbers are in % weight by weight)

API	Disintegrant	Lubricant	Other non-API
24.88	2.6500,3.9750 5.3000,6.6250,7.9500	0.6550,0.9825 1.3100,1.6375,1.9650	to 100%
37.32	2.6500,3.9750 5.3000,6.6250,7.9500	0.6550,0.9825 1.3100,1.6375,1.9650	to 100%
49.76	2.6500,3.9750 5.3000,6.6250,7.9500	0.6550,0.9825 1.3100,1.6375,1.9650	to 100%
62.20	2.6500,3.9750 5.3000,6.6250,7.9500	0.6550,0.9825 1.3100,1.6375,1.9650	to 100%
76.64	2.6500,3.9750 5.3000,6.6250,7.9500	0.6550,0.9825 1.3100,1.6375,1.9650	to 100%

4.2.1.2 Measuring NIR calibration set and validation set

The probe was inserted through the adapter cap. The depth of probe was about 10 mm, the sample bottle was then turned upside down. The powder must cover the tip of the probe to decrease the variation of NIR scans. The equipment is as described in section 4.2, was set at reflectance mode in range of 4008 to 9996 cm^{-1} with a resolution of 12 cm^{-1}. Data selection was mahalanobis distance in principal component space with partial least squares regression.

4.2.2 Manufacturing process sample set

1. Manufacturing process (Mixing step)

A stainless steel cubic mixer with internal intensifier bar capacity was used for mixing all components. The capacity of the cubic was 420 liters. The amount of powders was 55% volume by volume of working capacity. The powder was rotated at speed 20 rpm by using drive motor with 2 Horsepower. The multi component powder system consisted of API, disintegrant, lubricant and other non-API as shown in Table 4.3.

Table 4.3 Blend composition (all numbers are in %weight by weight)

API	Disintegrant	Lubricant	Other non-API
49.76	5.30	1.31	to 100%

2. Measuring sample set

Test of blend uniformity was performed by collecting 10 samples of final blend. The total powder weight for each sample was approximately 0.6 g. The sampling locations were at top, middle and bottom level of final blend as shown in Figure 4.1 and 4.2.

Cubic mixer (side view)
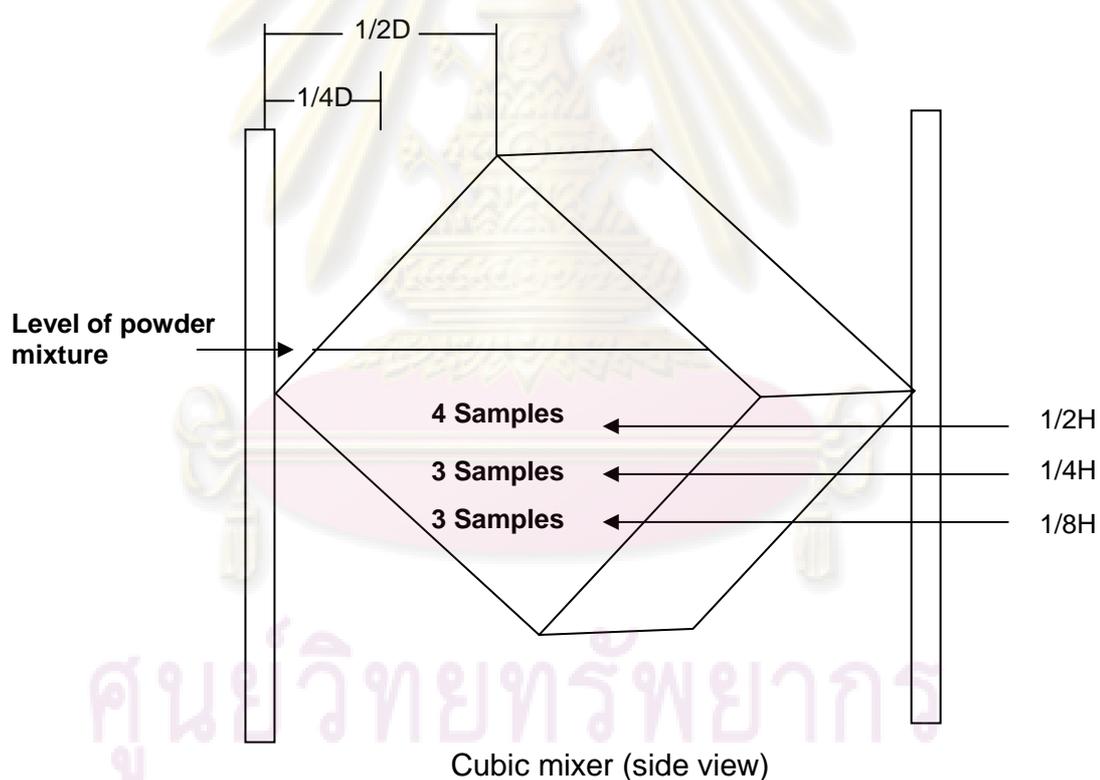
Figure 4.1 Cubic mixer (side view)
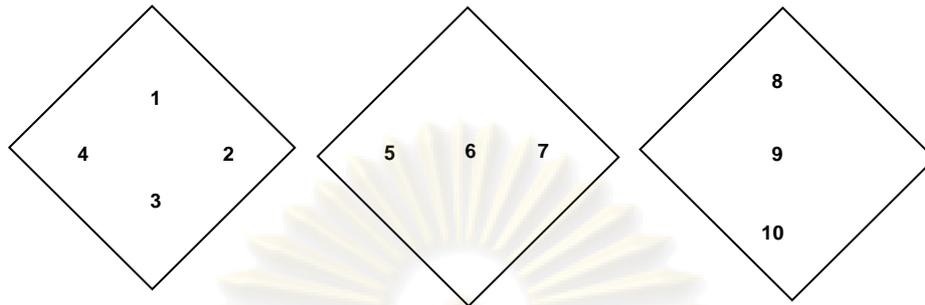
Cubic mixer (top view)

Figure 4.2 Cubic mixer (top view)

a. top level of final blend (at about a half height of the cubic mixer) the four sampling locations were all at about one –fourth diameter from the mixing wall

b. middle level of final blend (at about one –fourth height of the cubic mixer) two sampling locations were at about one –fourth diameter from the mixing wall and the other is at the center.

c. bottom level of final blend (at about one –eight height of the cubic mixer) two sampling locations were at about one –fourth diameter from the mixing wall and the other is at a half center.

The probe was inserted to measure sample through the adapter cap in the same way of measuring NIR calibration and validation set .

4.3 NIRS data analysis

All data analysis was performed by using Chemometrical software Nircal 4.21 (Buchi, Flawil, Switzerland). This software includes the partial least squares (PLS) algorithm. PLS is a spectral decomposition technique used to develop multivariate calibration model. Spectra were pre-treated by using the vector normalization algorithm in the Nircal 4.21 software. As a preliminary test of the model, the software performed a leave-one-out cross validation. This step consists in developing a calibration model with all the samples, but one. The sample left out was then predicted by the calibration

model. The algorithm repeats this step until all samples have been left out once, and calculated with the calibration model. Separate cross validations were performed for the spectra of the samples in the calibration set. Any significant differences in the results obtained with the0 cross validations would have indicated a problem during the spectral collection step.

The standard error of calibration or examination (SEC, SEE) was used to describe the results of cross validation, and was defined as:

$$SEC = \sqrt{\frac{\sum_{i=1}^{n}\left(Cref_i - Cpred_i\right)^2}{n_i - f - 1}}$$
4.1

Where : C_{REF} is the reference concentration.

C_{PRED} is the predicted concentration by NIR.

f is factors.

n_t is the number of samples in the training set.

The performance of the NIRS calibration models was evaluated with the prediction of an independent validation set. The standard error of prediction (SEP) was used to describe the differences observed between the predicted value and the reference method value.

$$SEP = \sqrt{\frac{\sum_{i=1}^{n}\left(Cref_i - Cpred_i\right)^2}{n_p - 1}}$$
4.2

In equation 4.2, n_p is the number of samples in the validation set. Although equations 4.1 and 4.2 are very similar, the number of samples in the calibration set and the validation set are not necessarily the same.

4.4. High performance liquid chromatography (HPLC) methodology

HPLC instrumentation consisted of Dionex (Germering, Germany) UltiMate® 3000 Rapid Separation LC system, analytical auto sample, binary analytical pumps and a DAD-3000RS photodiode array detector. Data analysis was carried out using Chromeleon®6.80 Chromatography Management software.

The analytical was performed follow the United States Pharmacopeia 30. The chromatographic procedure was carried out by using Inersil$^{®}$ ODS-3 column (5 μm, 4.6 x150 mm). The column was used a column heater to control temperature at 25°C. Mobile phase was a mixture of 95 % volume by volume of 0.025 M ammonium acetate buffer pH 3.8 and 5 % volume/volume of methanol. A flow rate of mobile phase was 1.0 ml/min. Injection volume was 20 μl. The retention times of API was 10.0 minutes as detected by a spectrophotometer set at UV 277 nm. The analysis time was set at 15.0 minutes per sample to eliminate potential interference from late eluting peaks. A weighted least-squares regression analysis was performed using the peak area versus the reciprocal of the squared drug concentration as weight to derive a standard curve.

The concentrations of samples were determined from the slope and intercept obtained from a daily standard curve.

1. Standard stock solution preparation (1.5mg/ml of API)

Accurately weigh and transfer 75 mg of API working standard to a 50-ml volumetric flask. Add 35 ml of 50 % volume by volume methanol and sonicate till the powder is completely dissolved. Let the solution cool to room temperature. Adjust to volume with 50 % volume by volume methanol and mix (standard stock solution).

2. Standard solution preparation

To made standard concentration curve follow below step

Standard1: Dilute 2.0 ml of standard stock solution to a 50 ml with mobile phase and mix.

Standard2: Dilute 3.0 ml of standard stock solution to a 50 ml with mobile phase and mix.

Standard3: Dilute 4.0 ml of standard stock solution to a 50 ml with mobile phase and mix.

Standard4: Dilute 5.0 ml of standard stock solution to a 50 ml with mobile phase and mix.

Standard5: Dilute 6.0 ml of standard stock solution to a 50 ml with mobile phase and mix.

3. Sample preparation.

Prepare the solution following steps. Transfer and weight of all bulk powder in sampling bottle to a 100-ml volumetric flask. Add 70 ml of 50 % volume by volume methanol and sonicate for 10 minutes. Let the solution cool to room temperature and adjust to volume with mobile phase and mix. Filter the solution through a filter no 1, discarding the first portion (10 ml). To prevent an evaporation of methanol in sample stock during the filtration, use a watch glass to cover the filter paper. Pipette 5.0 ml of sample stock solution into a 50-ml volumetric flask. Let the solution cool to room temperature. Adjust to volume with mobile phase and mix. Filter the solution through a nylon membrane filter (pore size = 0.45 μm), discarding the first portion (about 5 ml).

4. Standard and sample injection

Standards and samples were injected into the chromatographic system. All standard and sample solutions were duplicated injection for determine the variation.

4.5. HPLC data analysis

The calibration curve for data between standard concentration (mg/ml) (X) and A_{std} was used to calculation

Calculation :

standard concentration (mg/ml) =

$$\frac{\text{Weight of standard (mg)} \times P \times \text{concentration dilution}}{50 \times 50} \quad 4.3$$

$$\%w/w = \frac{(A_{sam} - C)/m \times 100 \times 100/5 \times 100}{\text{Weight of powder (mg)}} \quad 4.4$$

$$\%L.A. \quad = \quad \frac{(A_{sam} - C)/m \times 100 \times 100/5 \times 100}{150 \times \text{Weight of powder (mg) / Weight per tab (mg)}} \quad 4.5$$

Where : A_{sam} is peak area in chromatogram of assay preparation.

A_{std} is peak area in chromatogram of standard preparation.

C   is Y- intercept from standard curve.

m   is Slope from standard curve.

Weight of powder (mg) is weight in mg of bulk sample in assay

preparation.

Weight per tab (mg)   is 297.53 mg for sample without lubricant set.

Weight per tab (mg)   is 301.5 mg for sample with lubricant set.

Weight of standard (mg) is weight in mg in standard preparation.

concentration dilution is dilution factor such as 2,3,4,5,6.

P   is % purity of API working standard.

Specification limit: 90.0 – 110.0 %Label Amount, %RSD was less than 5.0%

4.6 Statistical analysis

Statistical analysis was performed with SPSS software, version 10 (SPSS, Inc., Chicago, IL). Data were presented as mean ±SD. Differences in values obtained with the two methods were compared statistically using paired t- test. A value of P <0.05 was considered to be statistically difference.

CHAPTER V

RESULTS AND DISCUSSION

5.1 NIR Spectra of Raw materials and Active Ingredient

The spectral region, which used in the experiment, was tracked where the API has the greatest absorption. The five spectra of API and other raw materials in Figure 5.1 showed the strong absorbance between 4392-4800, 5400-6600 and 7800-9996 cm^{-1} for API. This strong absorbance ranges were used as the main factor to choose the bands between 4392-4800, 5400-6600 and 7800-9996 cm^{-1} for develop the API calibration models. On the other hand the disintegrant and lubricant were used whole spectra ranges for develop the calibration models.
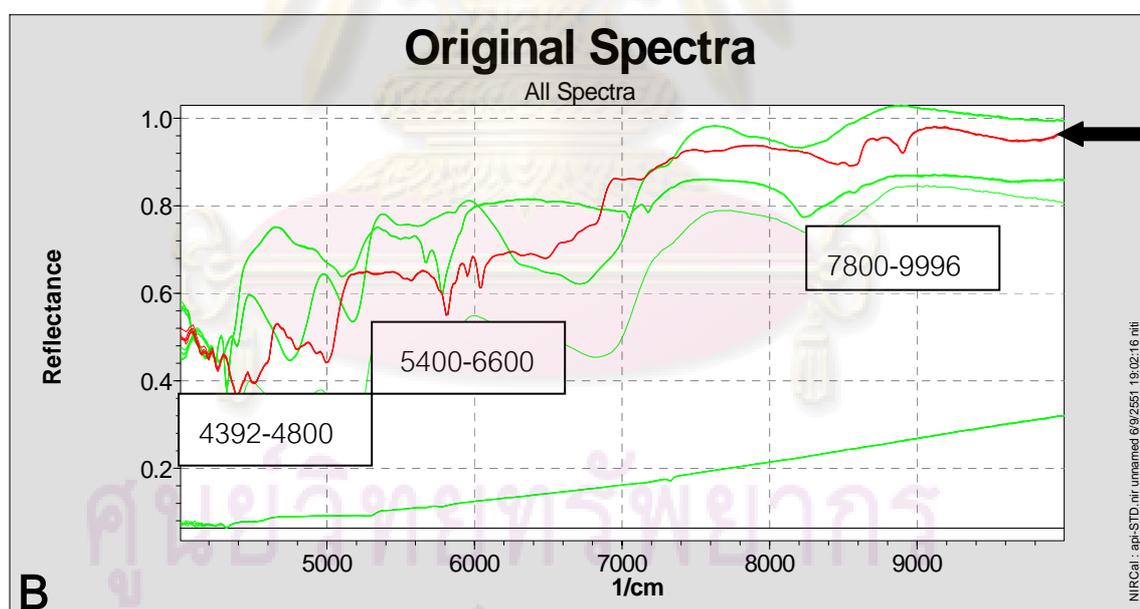
Figure 5.1   Spectra of API (pointed by ⬅) and Other Raw Materials

5.2 High performance liquid chromatography (HPLC) results

The analytical was performed follow the United States Pharmacopeia 30 by used Inersil® ODS-3 column (5 μm, 4.6 x150 mm) and the column was used a column heater to control temperature at 25°C. Mobile phase was a mixture of 95 % volume by volume of 0.025 M ammonium acetate buffer pH 3.8 and 5 % volume by volume of methanol. A flow rate of mobile phase was 1.0 ml/min. Injection volume was 20 μl. The retention time of API was 10.0 minutes as detected by a spectrophotometer set at UV 277 nm. The concentrations of samples were determined from the slope and intercept obtained from a daily standard curve (The calculation data of each sample were shown in appendix A). The chromatogram of standard API and sample were shown in Figure 5.2 and Figure 5.3, respectively.

Figure 5.2   HPLC chromatogram of analysis for API standard
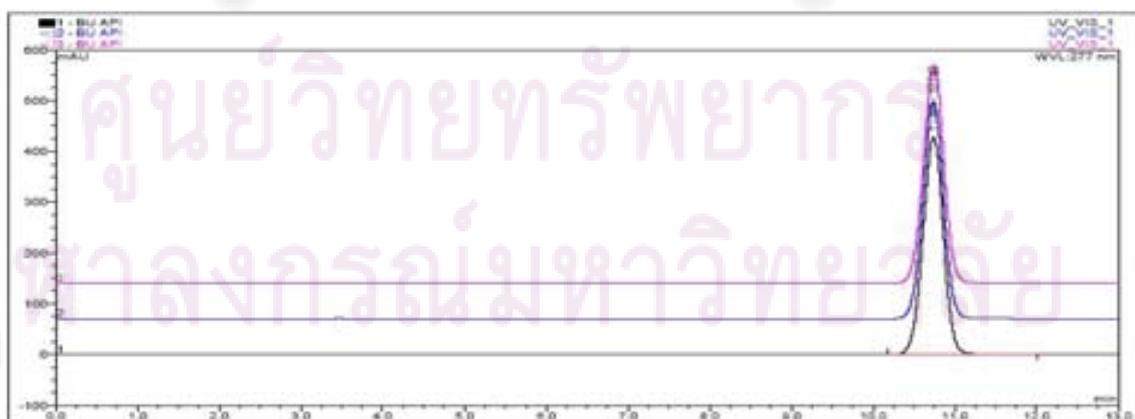
Figure 5.3   HPLC chromatograms of analysis for sample

5.3 Calibration model

The objective of this section was to build the calibration model by used partial least squares regression (PLS) algorithm to gave the best prediction of %API, %disintegrant and %lubricant. The results showed the suitability of calibration model in order to predict the amount of API and other API during blending in Table 5.1-5.2.

Table 5.1   Statistical characteristics of the calibration and the validation sample sets (Sample without lubricant set)

Items	Set	Average	Standard deviation	Minimum	Maximum	Number of samples
%API	Calibration set	99.9995%	35.50	49.9950%	150.0100%	25
	Validation set	100.0000%	35.49	49.9983%	150.0080%	25
%Disin tegrant	Calibration set	100.4430%	35.63	47.6462%	152.2360%	25
	Validation set	99.4577%	35.41	47.8349%	149.8099%	25

Table 5.2   Statistical characteristics of the calibration and the validation sample sets (Sample with lubricant set)

Items	Set	Average	Standard deviation	Minimum	Maximum	Number of samples
%API	Calibration set	100.0990%	35.44	47.5023%	151.9580%	125
	Validation set	100.1480%	35.26	48.4762%	151.9130%	125
%Disin tegrant	Calibration set	100.3894%	34.99	48.2559%	152.2546%	125
	Validation set	100.4325%	34.94	48.3436%	151.9946%	125
%Lubri cant	Calibration set	99.8243%	35.00	48.5555%	153.3391%	125
	Validation set	99.9496%	35.09	48.3566%	153.2538%	125

The statistical parameters such as Standard Error of Calibration (SEC), Standard Error of Prediction (SEP), Bias and correlation coefficient of determinant (R^2) were used to determine the suitable calibration. The detail statistical characteristics of calibration and validation were shown in Table 5.3 and 5.4.

Table 5.3   PLS calibration results of sample without lubricant set by using the second derivative NIR spectra

Variances	Wavelength region (cm^{-1})	F	R^2	SEC	SEP	Bias	RPD
%API	4392-4800, 5400-6600, 7800-9996	10	0.9980	2.21	2.49	0.00	14.25
%Disintegrant	4392-4800, 5400-6600, 7800-9996	25	0.9752	7.89	11.13	0.00	3.18

Table 5.4   PLS calibration results of sample with lubricant set by using the second derivative NIR spectra

Variances	Wavelength region (cm^{-1})	F	R^2	SEC	SEP	Bias	RPD
%API	4392-4800, 5400-6600, 7800-9996	8	0.9980	2.26	2.24	0.00	15.74
%Disintegrant	4440-9000	7	0.9792	7.10	8.40	0.00	4.16
%Lubricant	4596-9996	8	0.9561	10.25	10.17	0.00	3.45

Where F is the number of factors, R^2 is the coefficient of multiple determination, SEC is standard error of calibration, SEP is standard error of prediction, Bias is the average of differences between reference value and NIR value, RPD is the ratio of standard deviation of reference data in the validation set to SEP Unit: %.

The calibration model was developed from two spectral set, which followed experimental procedure. Selection of spectra and wavelengths, mathematical pretreatment and statistical analysis performed cluster analysis and partial least squares regression (PLS) were used as critical criteria to choose the best calibration model.

All samples were recorded by a scanning FT-NIR-spectrometer in random order for 5 locations. Each sample was scanned 5 times for one average spectrum to equilibrate in homogeneities. Chemometrical software Nircal 4.21 (Buchi, Flawil, Switzerland) was used for creating a model. Figure 5.4 and 5.5 showed the original spectra of several samples in calibration and validation set in the region range from 4008 to 9996 cm^{-1} before pretreatment spectra. Figure 5.4 and 5.5 showed the API spectrum strong bands were between 4392-4800, 5400-6600 and 7800-9996 cm^{-1} of sample in sample without lubricant set and the sample in sample with lubricant set, respectively.
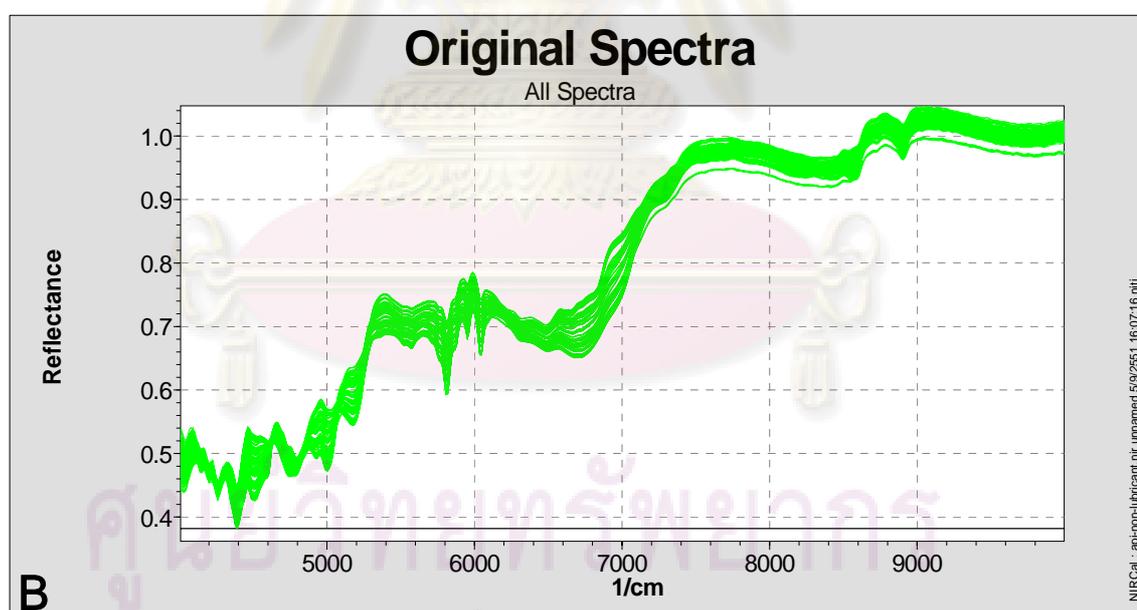
Figure 5.4   Original spectra of sample without lubricant set
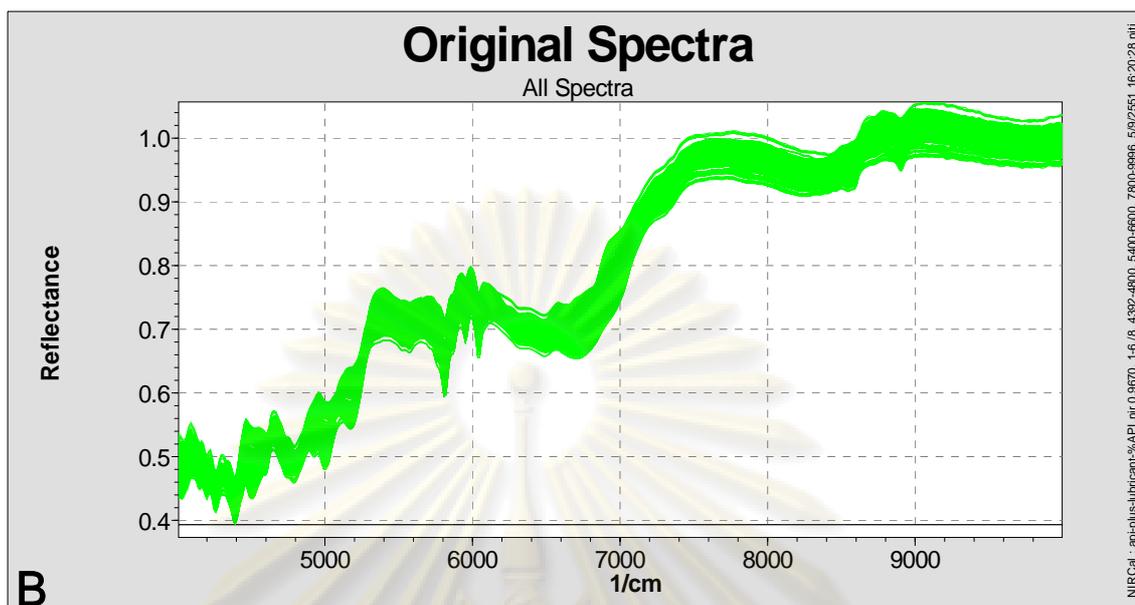
Figure 5.5   Original spectra of sample with lubricant set

The original spectra were used mathematical pretreatment and statistical analysis performing cluster analysis and PLS. Statistical parameters such as SEC, SEP and RPD were used to judge the performance of the models. SEC and SEP were small value. RPD was more than 3.0%. Another important parameter when looking for a good model is the number of factor describing the information in the calibration set. In this matter, care has to be taken to avoid under or over fitting. The calibration equation was developed on the second derivative NIR spectra. The correlation coefficient (R^2) of multiple determinate suppose to be more than 95% of the information in calibration set in model and the factors are not more than 25 factors. The Statistic parameters will give a good calibration model. The pretreated spectra were shown in Figure 5.6-5.10.
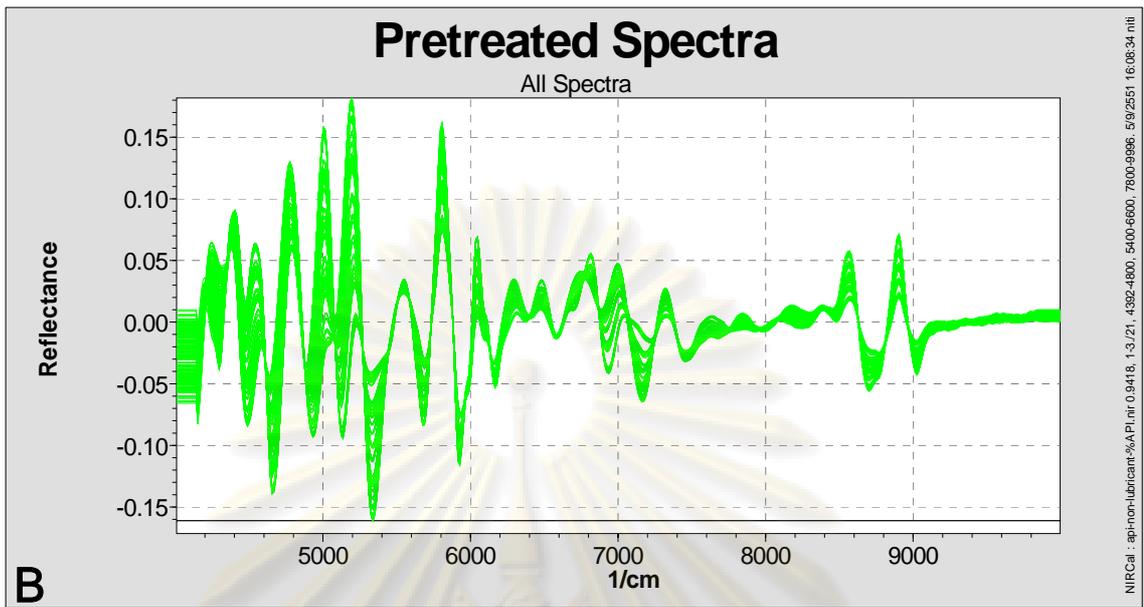
Figure 5.6   Pretreated spectra of %API in sample without lubricant set
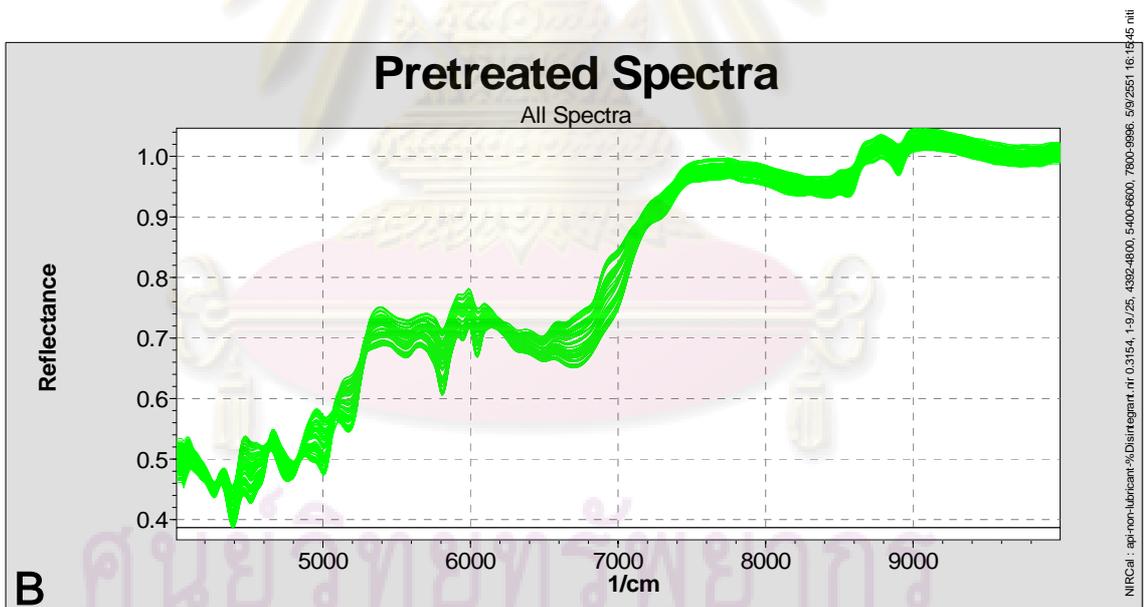
Figure 5.7   Pretreated spectra of %disintegrant in sample without lubricant set
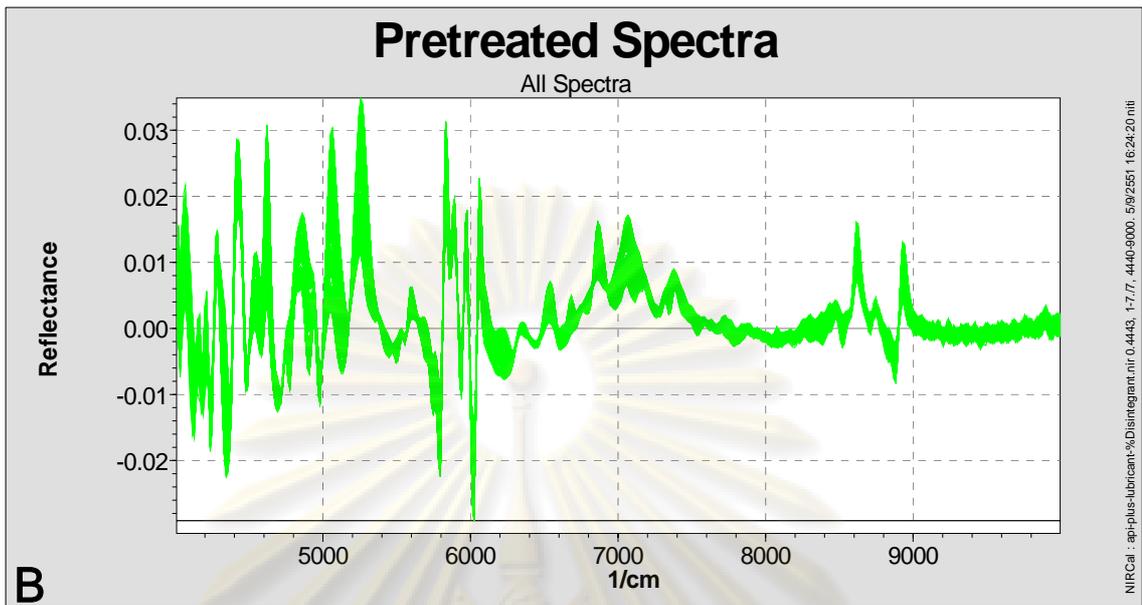
Figure 5.8   Pretreated spectra of %API in sample with lubricant set
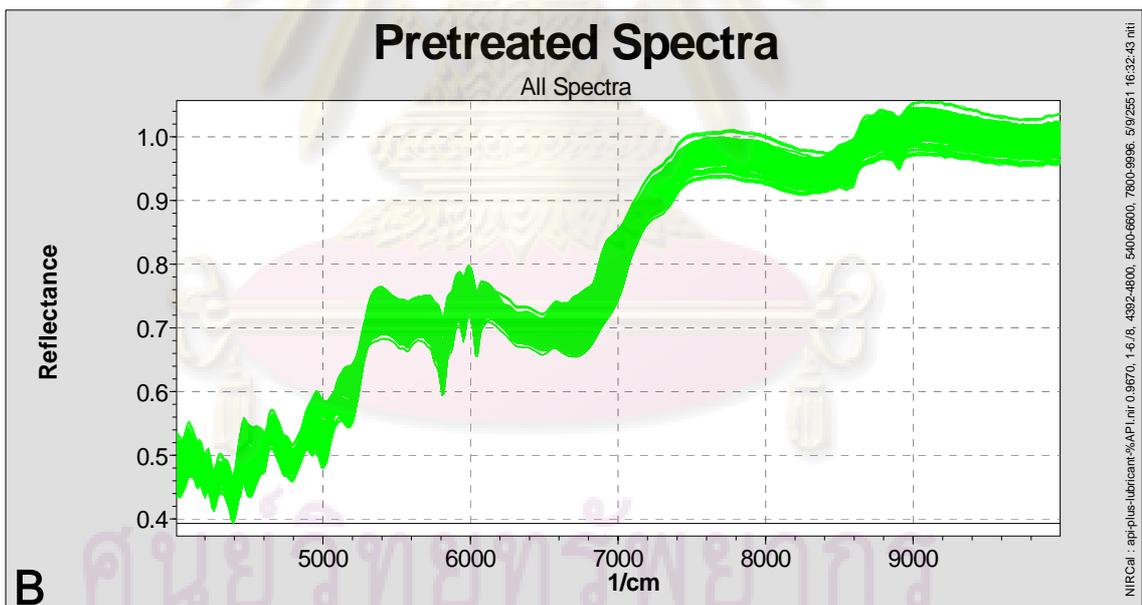
Figure 5.9   Pretreated spectra of %disintegrant in sample with lubricant set
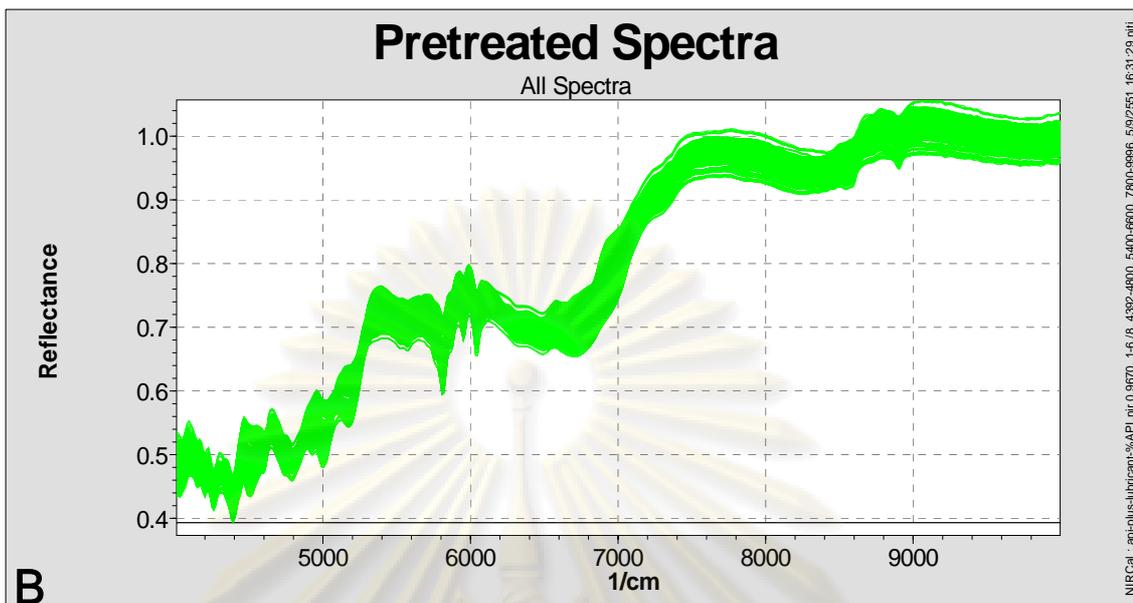
Figure 5.10   Pretreated spectra of %lubricant in sample with lubricant set

The correlation of API between true property (HPLC method) and predicted property (NIRS method) was shown in Figure 5.11-5.12. The results showed in high correlation coefficients of 0.9974 (sample without lubricant set), 0.9980 (sample with lubricant set) for calibration set and 0.9976 (sample without lubricant set), 0.9980 (sample with lubricant set) for validation set. The Bias is near zero. That expressed high accuracy. Validation showed the robustness and reproducibility of the NIRS model for the determination of %API was high. The NIRS model can be used to predict %API in blending process. Moreover, the calibration models were also accurate for the other components. The calibration model for the determination of % disintegrant also showed high correlation coefficients; 0.9752 (sample without lubricant set), 0.9792 (sample with lubricant set) for calibration set and 0.9505 (sample without lubricant set), 0.9710 (sample with lubricant set) for validation set (see Figure 5.13-5.14). The calibration model for the determination of % lubricant also showed high correlation coefficients; 0.9561 for calibration set and 0.9574 for validation set (Figure 5.15). The correlations (R^2) were more than 0.95 which proved that predicted property were not different from the true property.
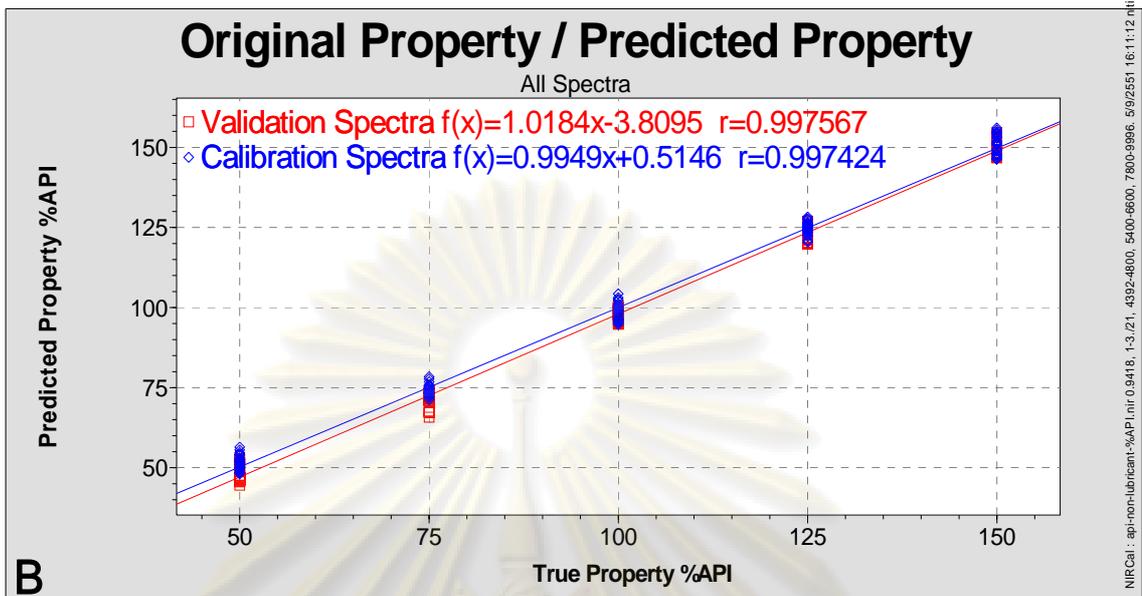
Figure 5.11   The correlation between true property and predicted property of %API in
sample without lubricant set
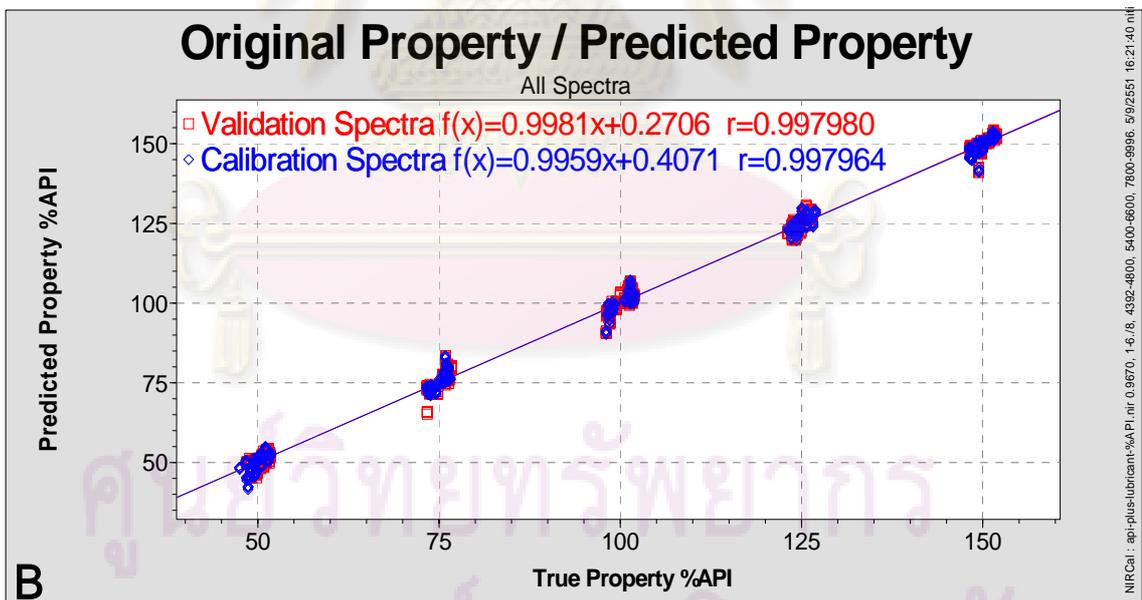
Figure 5.12   The correlation between true property and predicted property of %API in
sample with lubricant set
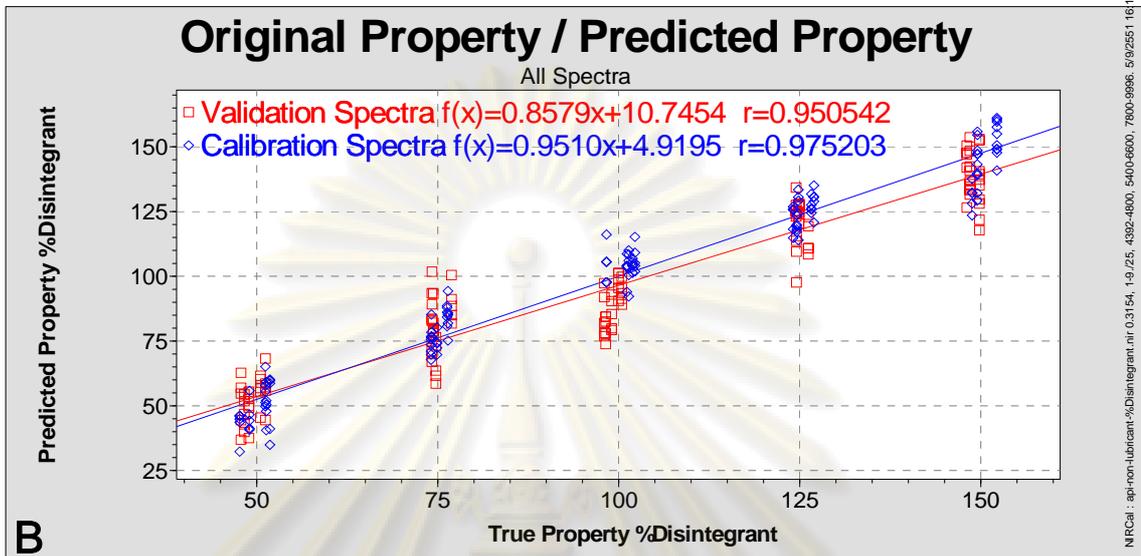
Figure 5.13 The correlation between true property and predicted property of %disintegrant in sample without lubricant set
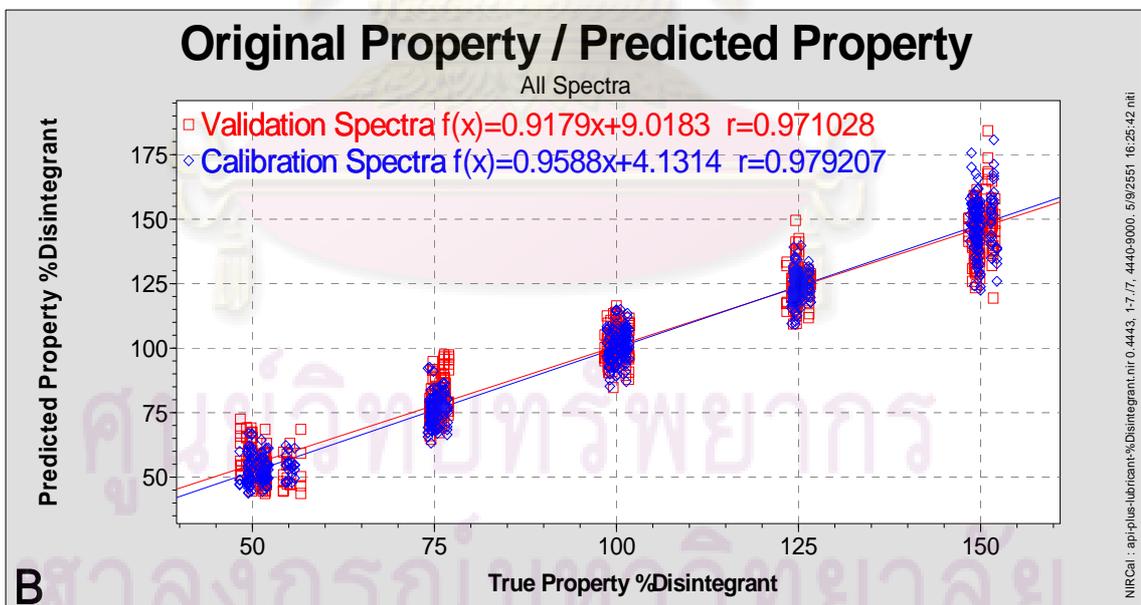
Figure 5.14 The correlation between true property and predicted property of %disintegrant in sample with lubricant set
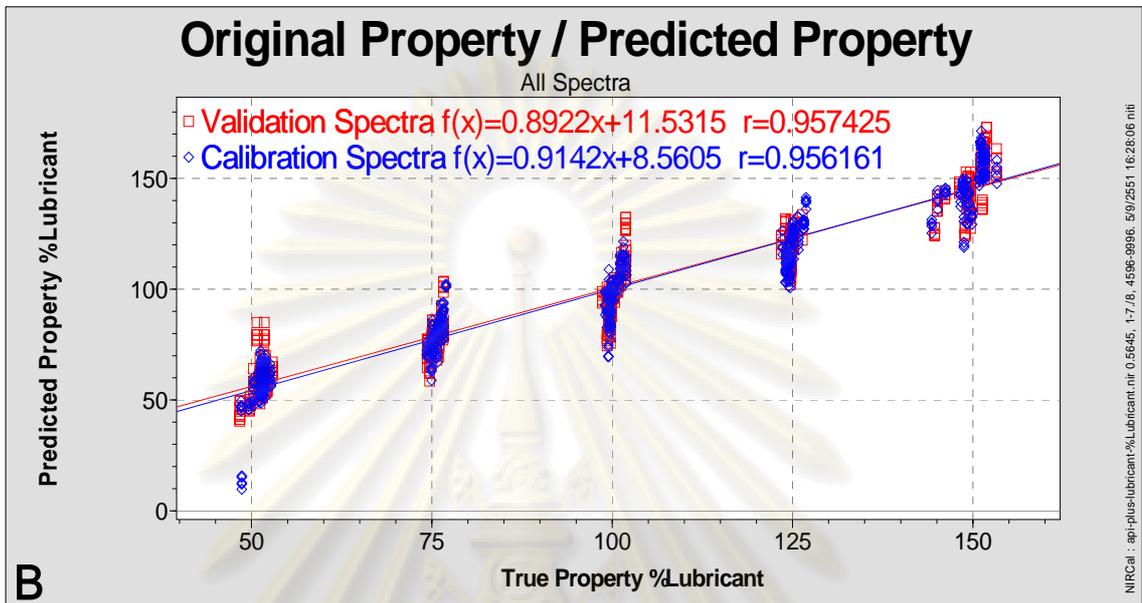
Figure 5.15 The correlation between true property and predicted property of %lubricant in sample with lubricant set

5.4. The validation results of NIRS method

The objective of this part was to validate the quantitative analysis of the developed NIRS to compare with that of HPLC. The statistic paired t-test was used to prove that the results between NIRS technique and HPLC technique were not different. The result at the 95% confidence level did not indicate any differences between the %API result via NIRS technique and that via HPLC technique powder blending process.

All samples were assayed by NIRS technique and HPLC technique for content of active ingredient in which the limitation of API was 90.0-110.0 % Label Amount and %RSD was less than 5.0%, as specified in the United States Pharmacopeia 30. On the other hand disintegrant and lubricant were assayed only by NIRS in the same specification in the United States Pharmacopeia 30. The NIRS and HPLC results for each sample were illustrated in Appendix 2. Statistical analysis was performed with SPSS software, version 10 (SPSS, Inc., Chicago, IL). Data were presented as a mean ±SD. The differences in values obtained with the two methods were compared statistically using paired t-test. A value of P <0.05 was considered to be statistically difference. In this study, the result was shown that no statistically significant difference of %API was observed between NIRS method and HPLC method. The results at the mixing time of 20th minute showed the value of P = 0.5049 (S1), P = 0.6850 (S2) and P = 0.2775 (S3). The mean values of %API at the mixing time of 20th minute were 99.71%, %RSD = 0.90 (NIRS) and 99.91%, %RSD = 1.41 (HPLC) in lot S1, 100.15%, %RSD = 0.91 (NIRS) and 100.10%, %RSD = 1.03 (HPLC) in lot S2 and 100.43%, %RSD = 0.96 (NIRS) and 100.59%, %RSD = 0.93 (HPLC) in lot S3, as showed in Table B1. According to the United States Pharmacopeia 30, this mean and %RSD of two methods were within the limits of 90.0-110.0 % Label Amount and %RSD was less than 5.0%. The summary and trend of results were shown in Figure 5.16 (S1), Figure 5.17 (S2) and Figure 5.18 (S3). This summary confirmed that results of NIRS method were not different from the HPLC result.
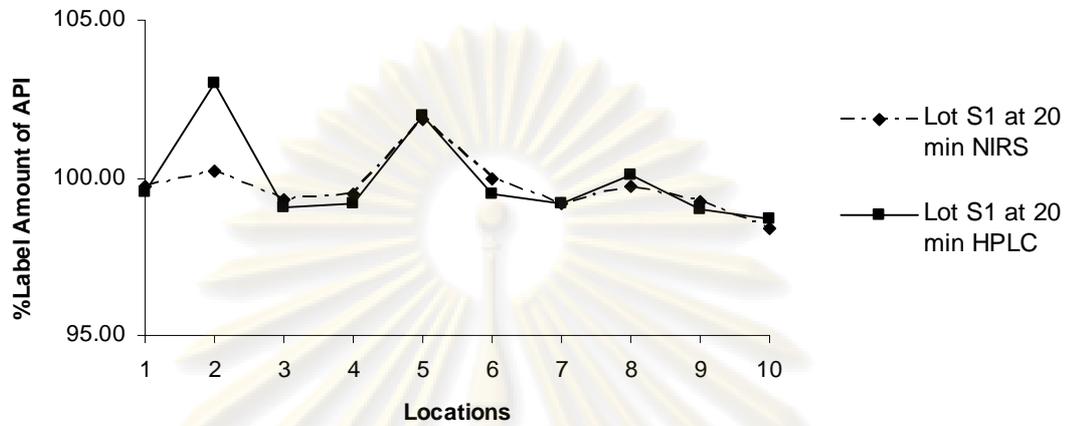
Figure 5.16   The %Label Amount of API in NIRS and HPLC results

at 20th minute in lot S1
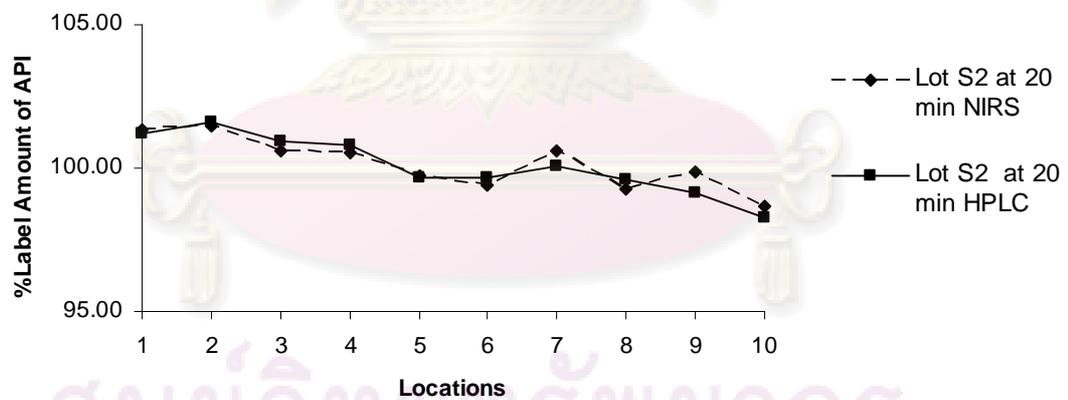
Figure 5.17   The %Label Amount of API in NIRS and HPLC results

at 20th minute in lot S2
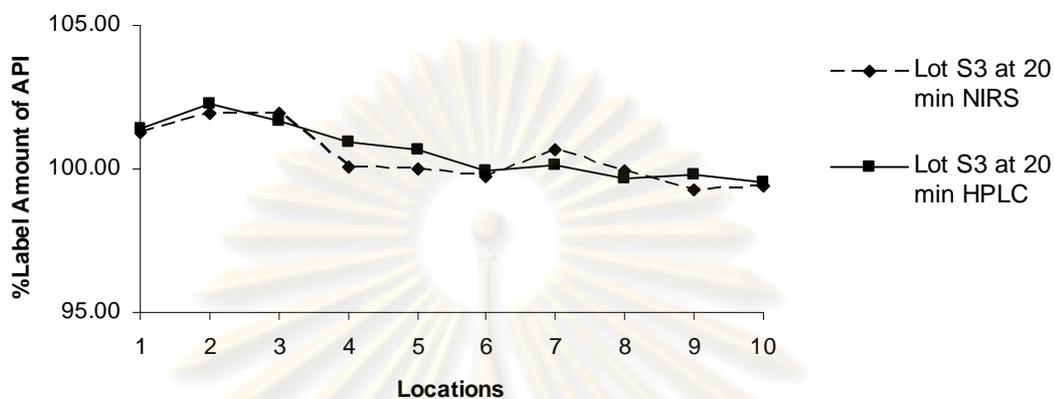
Figure 5.18   The %Label Amount of API in NIRS and HPLC results
               at 20th minute in lot S3

In this part, the validation study was continued the blending time in process at 25th minute. As exhibited in Table B2, the results showed no statistically significant difference of %API between NIRS method and HPLC method. The *P*-values at 25th minute were $P = 0.2753$ (S1), $P = 0.1192$ (S2) and $P = 0.4596$ (S3).

The mean values of %API at the mixing time of 25th minute were 101.16%, %RSD = 0.56 (NIRS) and 101.14%, %RSD = 0.57 (HPLC) in lot S1, 100.25%, %RSD = 0.77 (NIRS) and 100.20%, %RSD = 0.71 (HPLC) in lot S2 and 98.78%, %RSD = 0.69 (NIRS) and 98.75%, %RSD = 0.64 (HPLC) in lot S3, as showed in Table B2. The summary and trend of results were shown in Figure 5.19 (S1), Figure 5.20 (S2) and Figure 5.21 (S3). All of the result exhibited in Table A2.3 showed that no statistically significant difference of %Label Amount of API was observed between NIRS method and HPLC method.
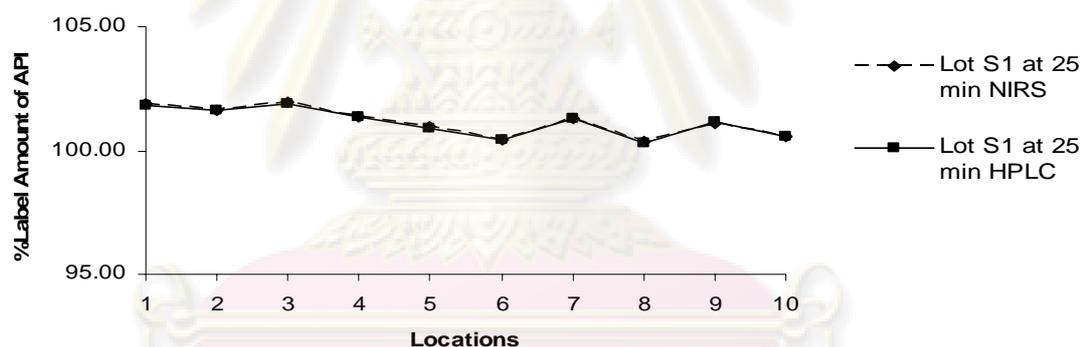
Figure 5.19   The %Label Amount of API in NIRS and HPLC results
at 25th minute in lot S1
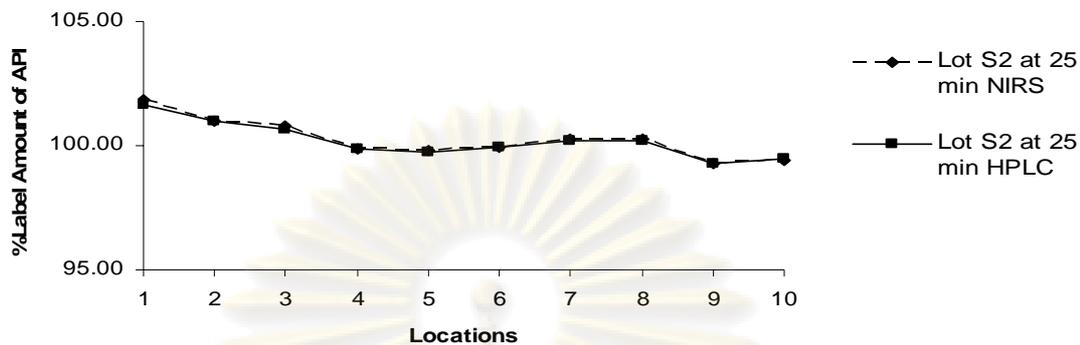
Figure 5.20   The %Label Amount of API in NIRS and HPLC results

at 25th minute in lot S2
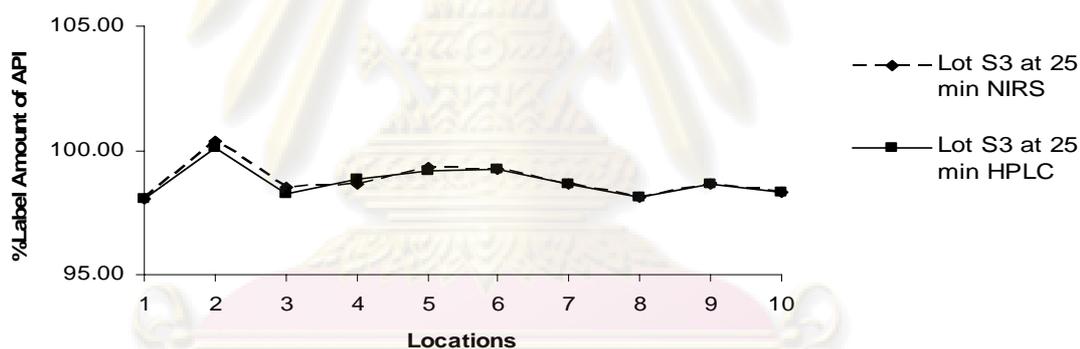
Figure 5.21   The %Label Amount of API in NIRS and HPLC results

at 25th minute in lot S3

The Figure 5.16 to Figure 5.21 showed that the %Label Amount of API results at 20th minutes in each location were not different from with %Label Amount of API results at 25th minutes. The results showed that the suitable blending time of API homogeneity in validation study was at 20th minute.

Disintegrant was assayed only by NIRS. The results compiled with the specification in the United States Pharmacopeia 30 (Table B3-B4). The summary and trend of %Label Amount of disintegrant results were shown in Figure 5.22 (20th minute) and Figure 5.23 (25th minute). This results showed that the %Label Amount of disintegrant results at 20th minute in each locations showed more variance than %Label Amount of disintegrant results at 25th minute. However, the results at 20th minute were still in the limitation and acceptability. This means the suitable blending time of disintegrant homogeneity in validation study was at 20th minute.
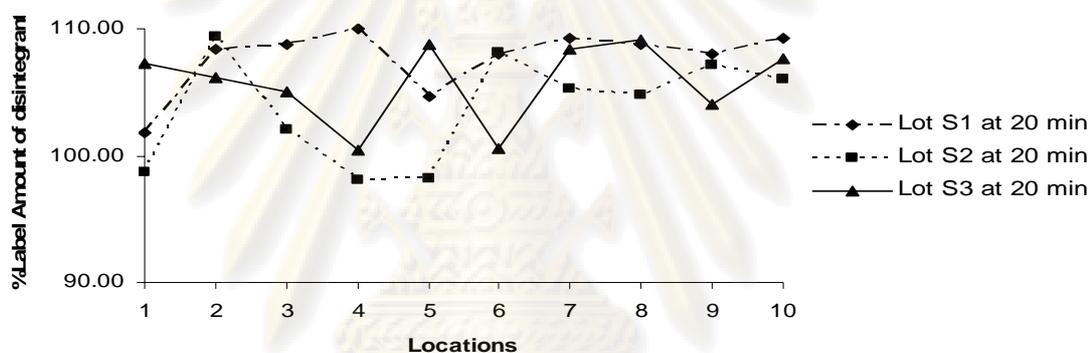
Figure 5.22   The %Label Amount of disintegrant results
at 20th minute in lot S1-S3
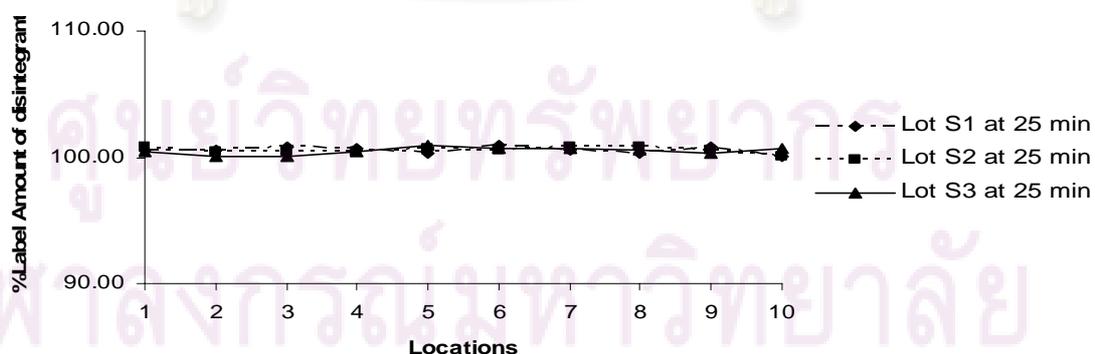
Figure 5.23   The %Label Amount of disintegrant results
at 25th minute in lot S1-S3

The results of lubricant were within the limits of 90.0-110.0 % Label Amount and %RSD was less than 5.0% (Table B5). The summary and trend of %Label amount of lubricant at 5 minutes after the homogeneity of API and disintegrant. The results were shown in Figure 5.24.
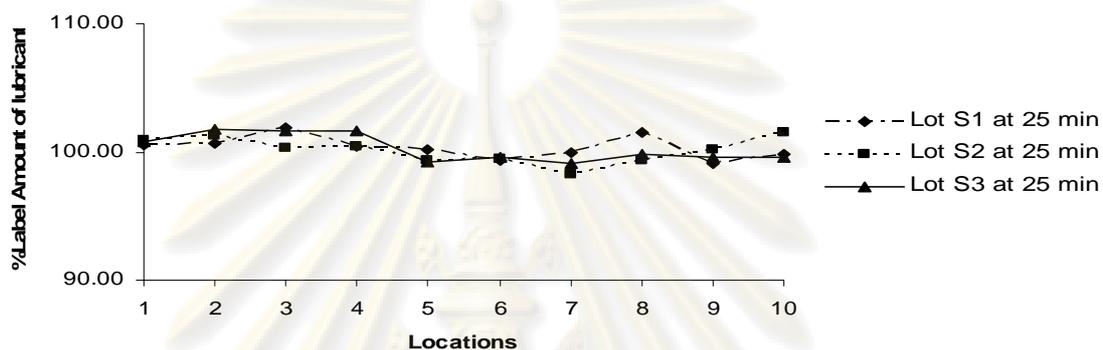
Figure 5.24   The %Label Amount of lubricant results
              at 25th minute in lot S1-S3

The results in Figure 5.24 showed that the blending time of lubricant homogeneity in validation study was 5 minutes after the homogeneity of API and disintegrant.

5.4 The mixing times of blend homogeneity

The objective of this section was to optimize mixing times of blend homogeneity by using mainly NIRS and confirm the results by using HPLC. The other 3 lots were produced to determine the homogeneity before and after adding lubricant. The calibration model was used to predict %API and % of other excipients in powder blending process. The homogeneity index is %RSD.

$$\%RSD = \left(\frac{SD}{x_{avg}} \right) \times 100 \qquad\qquad 5.1$$

$$SD = \sqrt{ \frac{ \left(n\sum_{i=1}^{n} x_i^2 - \left(\sum_{i=1}^{n} x_{avg} \right)^2 \right) }{ n(n-1) } } \qquad\qquad 5.2$$

Where : X_i is the %Label amount of each individual sample.

X_{avg} is the average %Label amount of all samples.

n is the total number of samples.

All samples were assayed in the same criteria of the validation results. The limitation of active ingredient was 90.0-110.0 %Label Amount and %RSD was less than 5.0%. The differences in values of the two methods were compared statistically by using paired t-test. A value of $P < 0.05$ was considered to be statistically difference.

In this study, the results (%Label Amount of API) showed that no statistically significant difference between NIRS method and HPLC method. The *P*-values at 15th minute are $P = 0.2335$ (W1), $P = 0.7501$ (W2) and $P = 0.8511$ (W3). The mean values of %API at the mixing time of 15th minute were 104.57%, %RSD = 9.57 (NIRS) and 104.73%, %RSD = 9.83 (HPLC) in lot W1, 104.53%, %RSD = 6.11 (NIRS) and 104.49%, %RSD = 6.10 (HPLC) in lot W2 and 110.30%, %RSD = 6.40 (NIRS) and 110.32%, %RSD = 6.45 (HPLC) in lot W3, (Table B6). The summary and trend of results were shown in Figure 5.25.
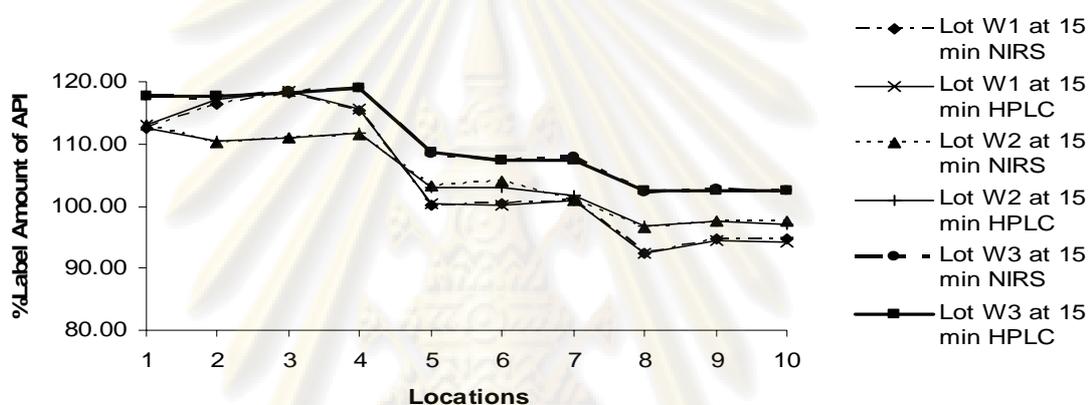
Figure 5.25   The %Label Amount of API in NIRS and HPLC results
at 15th minute in lot W1-W3

Therefore, the results (%Label Amount of API) at 15th minute were out of the acceptable limit (90.0-110.0 % Label Amount) and the homogeneity index (%RSD) was more than 5.0% in both methods (all three lots). The results showed that the mixing time at 15th minute was not suitable for mixing API in powder blending process before add lubricant for production lot W1, W2 and W3.

The mixing results (%Label Amount of API) at 20th minute of lot W1-W3, as exhibited in Table B7, showed no statistically significant difference between NIRS method and HPLC method. The P-values at 20 minute were P = 0.8229 (W1), P = 0.5330 (W2) and P = 0.3039 (W3).

The mean values of %API at the mixing time of 20th minute were 100.41%, %RSD = 1.33 (NIRS) and 100.40%, %RSD = 1.41 (HPLC) in lot W1, 100.96%, %RSD = 1.23 (NIRS) and 100.86%, %RSD = 1.28 (HPLC) in lot W2 and 100.56%, %RSD = 0.89 (NIRS) and 100.69%, %RSD = 0.99 (HPLC) in lot W3. All the results were within the acceptable limit (90.0-110.0 % Label Amount) and the homogeneity index (%RSD) was less than 5.0%. The summary and trend of results were shown in Figure 5.26.
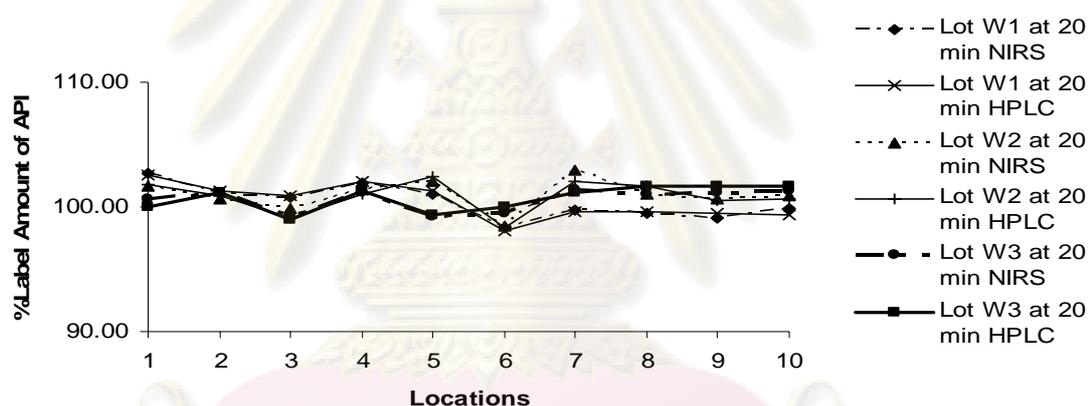
Figure 5.26   The %Label Amount of API in NIRS and HPLC results
at 20th minute in lot W1-W3

The results of all three lots showed that blending 20 minutes was the suitable time for mixing for API in powder blending process before adding lubricant.

The mixing results (%Label Amount of API) were analyzed again after added lubricant every 1 minute until 20+5 minutes to check the homogeneity. All the results still showed the homogeneity of %API in powder blending.

The mixing results at 21st, 22nd, 23rd, 24th and 25th minute of lot W1-W3 were co-response with the results at 20th minute, although the lubricant were added (Table B8, B9, B10, B11, B12, respectively). All the results (%Label Amount of API) showed no statistically significant difference between NIRS method and HPLC method. The mean values of %Label Amount of API were with in the acceptable limit and the homogeneity index (%RSD) was less than 5.0%. The summary and trend of results at the mixing time at 21st, 22nd, 23rd, 24th and 25th minute were shown in Figure 5.27, 5.28, 5.29, 5.30 and 5.31, respectively.
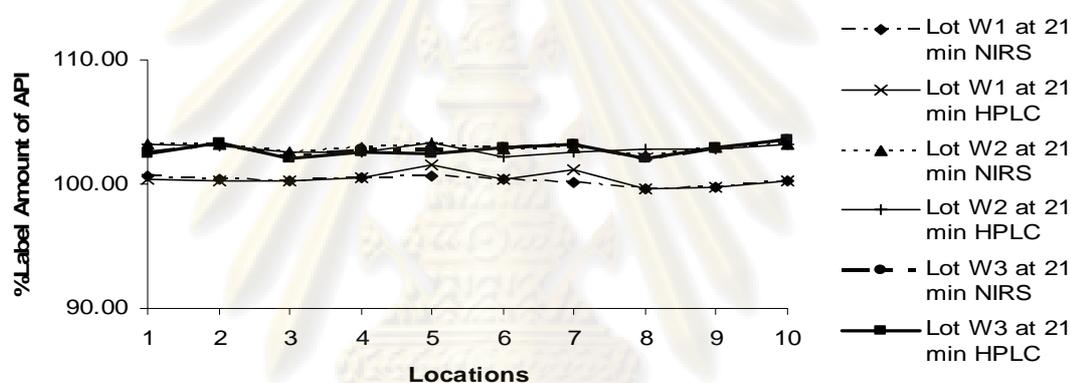
Figure 5.27   The %Label Amount of API in NIRS and HPLC results
at 21st minute in lot W1-W3
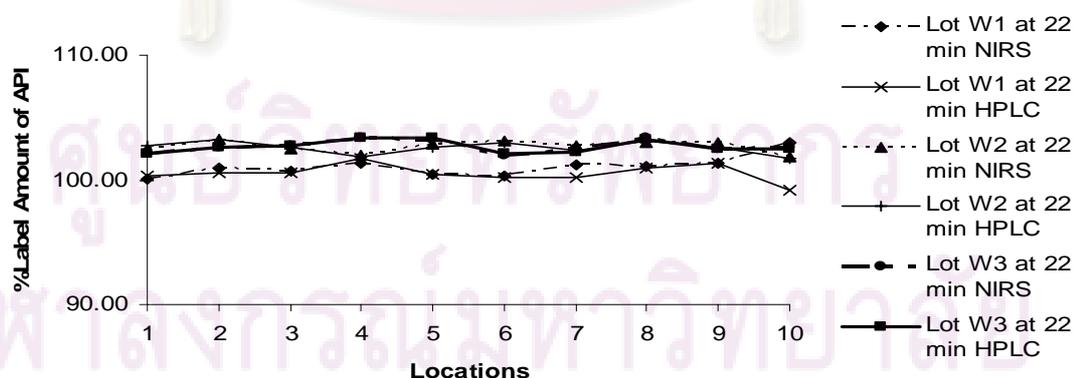
Figure 5.28   The %Label Amount of API in NIRS and HPLC results
at 22nd minute in lot W1-W3
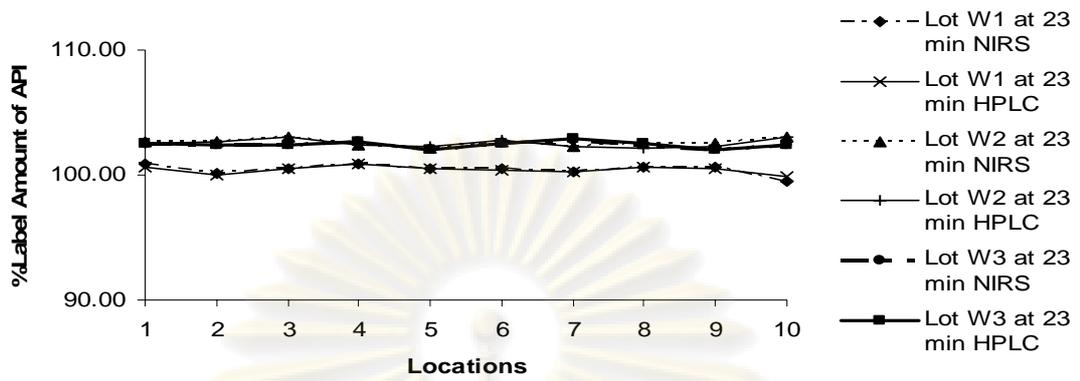
Figure 5.29   The %Label Amount of API in NIRS and HPLC results
at 23rd minute in lot W1-W3
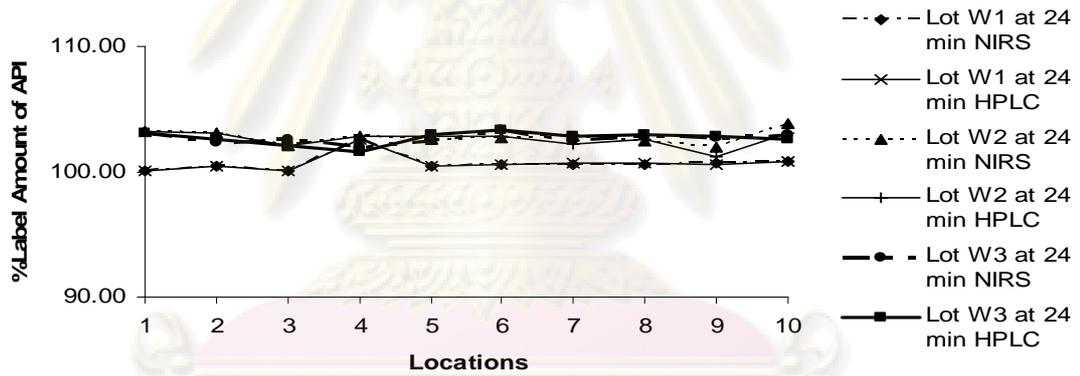
Figure 5.30   The %Label Amount of API in NIRS and HPLC results
at 24th minute in lot W1-W3
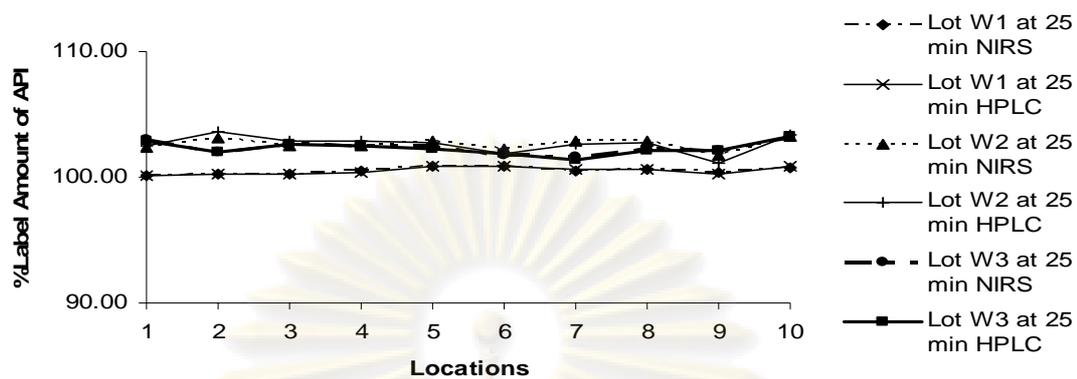
Figure 5.31   The %Label Amount of API in NIRS and HPLC results

at 25th minute in lot W1-W3

Disintegrant was assayed by only NIRS. The results after mixing for 20 minutes compiled with the specification in the United States Pharmacopeia 30 (Table B13-B19 showed %Label Amount of disintegrant). The summary and trend of %Label Amount of disintegrant results were shown in Figure 5.32 (15th minute), Figure 5.33 (20th minute), Figure 5.34 (21st minute), Figure 5.35 (22nd minute), Figure 5.36 (23rd minute), Figure 5.37 (24th minute) and Figure 5.38 (25th minute).
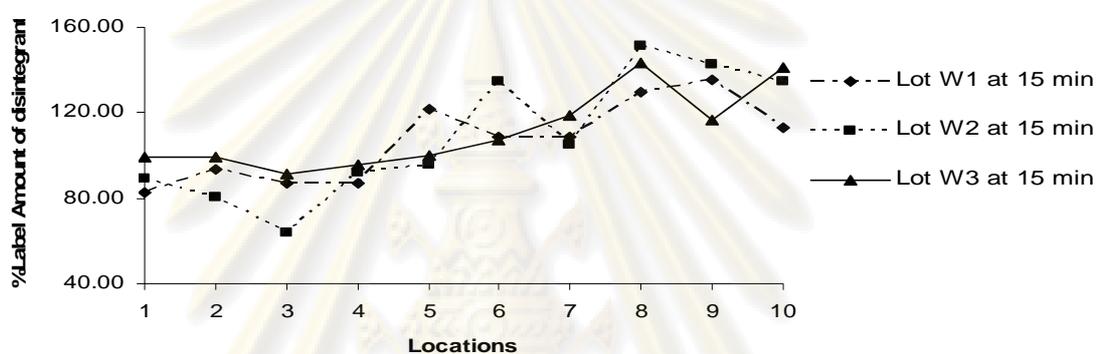
Figure 5.32   The %Label Amount of disintegrant results
at 15th minute in lot W1-W3
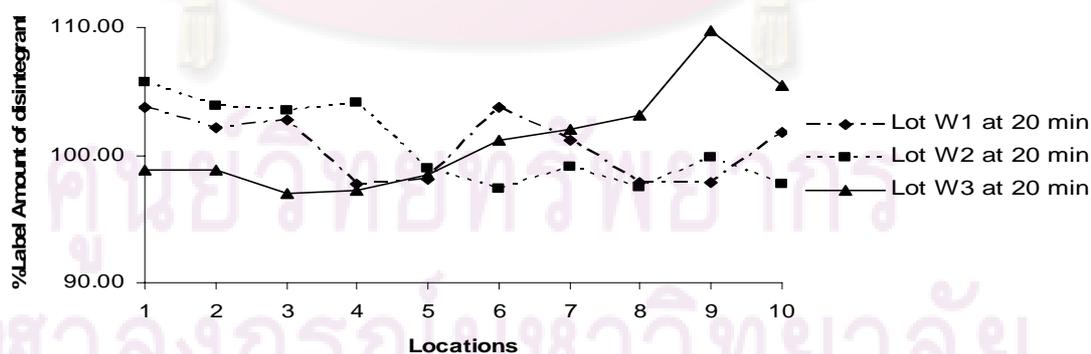
Figure 5.33   The %Label Amount of disintegrant results
at 20th minute in lot W1-W3
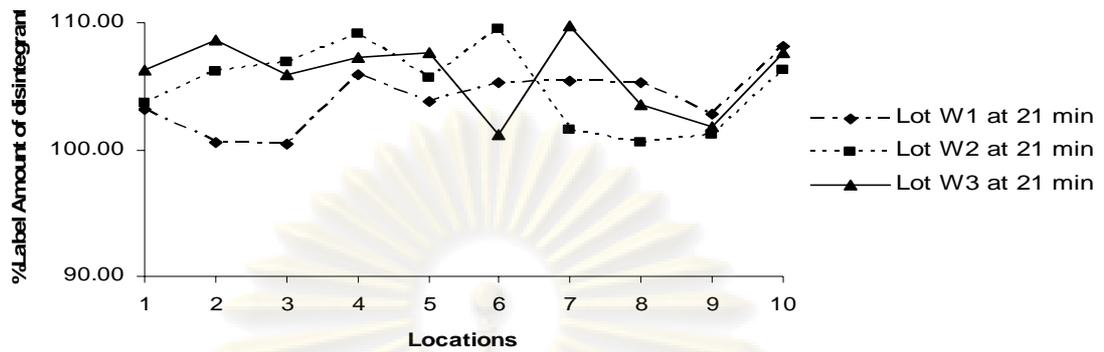
Figure 5.34   The %Label Amount of disintegrant results
at 21st minute in lot W1-W3
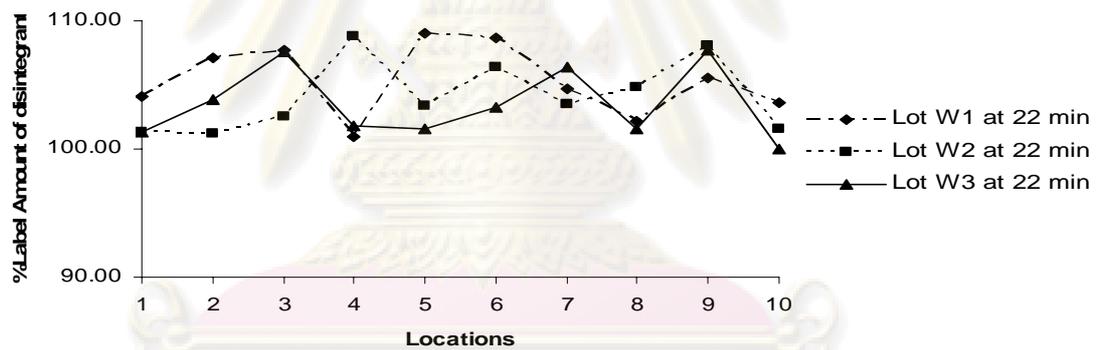
Figure 5.35   The %Label Amount of disintegrant results
at 22nd minute in lot W1-W3
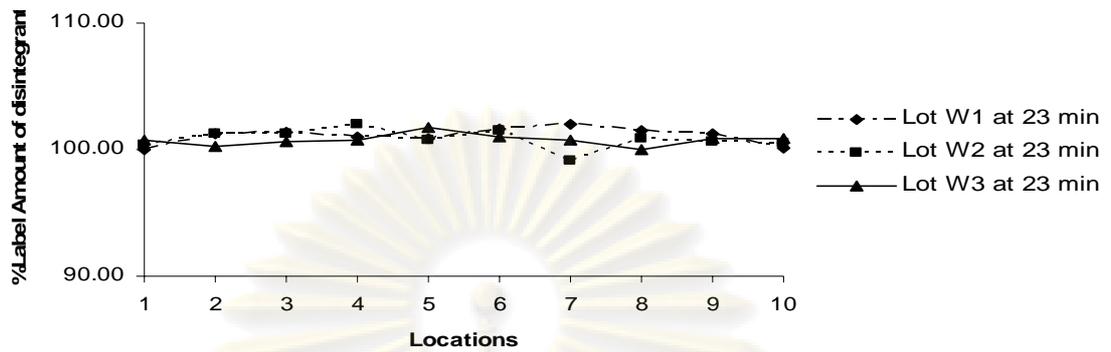
Figure 5.36   The %Label Amount of disintegrant results

at 23rd minute in lot W1-W3
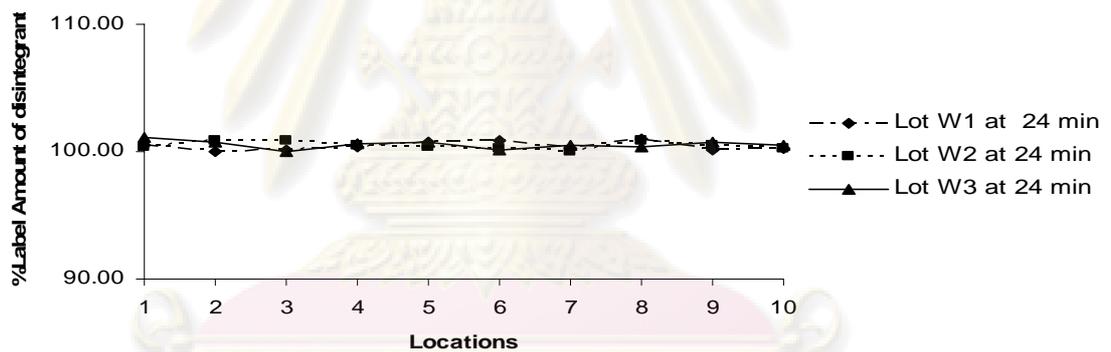
Figure 5.37   The %Label Amount of disintegrant results

at 24th minute in lot W1-W3
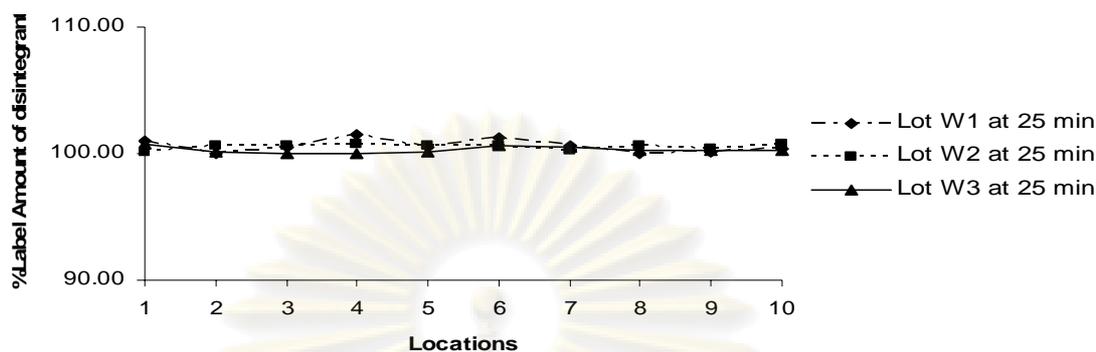
Figure 5.38   The %Label Amount of disintegrant results
at 25th minute in lot W1-W3

The results in Figure 5.32-5.38 showed that at 15th minute the %Label Amount of disintegrant results were out of the acceptable limit (90.0-110.0 % Label Amount) and the homogeneity index (%RSD) was more than 5.0% in both methods (all three lots). The results show that the mixing time at 15th minute was not suitable for mixing disintegrant, which the same trend of %Label Amount of API. The %Label Amount of disintegrant results at 25th minute in each location showed less variance. However, the results at 20th minute was acceptable because all the results still in the acceptable limit that was specified in the United States Pharmacopeia 30.

Lubricant was assayed by only NIRS similar to disintegrant (Table B20-B24 showed %Label Amount of lubricant). The summary and trend of %Label amount of lubricant results were shown in Figure 5.39 (21st minute), Figure 5.40 (22nd minute), Figure 5.41 (23rd minute), Figure 5.42 (24th minute) and Figure 5.43 (25th minute).

The results in Figure 5.39-5.43 showed that the %Label Amount of lubricant results after adding the lubricant less than 3 minutes (20+1 to 20+2 minutes) were out of the acceptable limit (90.0-110.0 % Label Amount) and the homogeneity index (%RSD) was more than 5.0% in both methods (all three lots). The %Label Amount of lubricant results at 25th minute in each location showed less variance. However, the results at 23rd minute were still in the acceptable limit. The results showed that the suitable blending time of lubricant homogeneity in this production experiment was 3 minutes or more after the homogeneity of API and disintegrant.
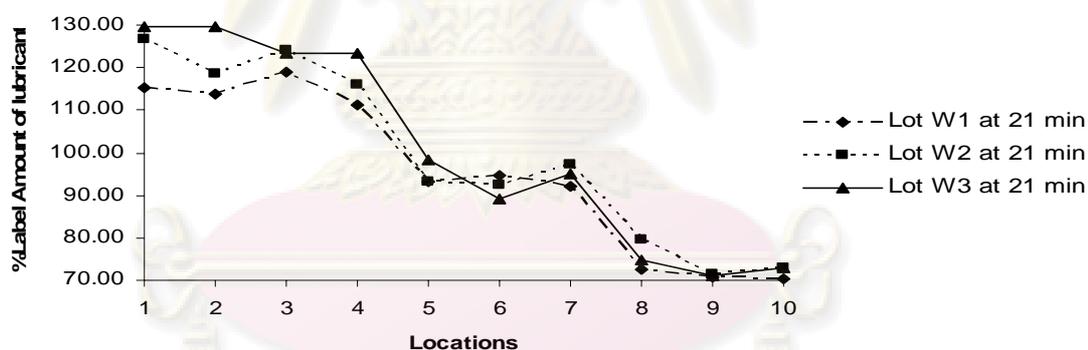
Figure 5.39   The %Label amount of lubricant results
at 21st minute in lot W1-W3
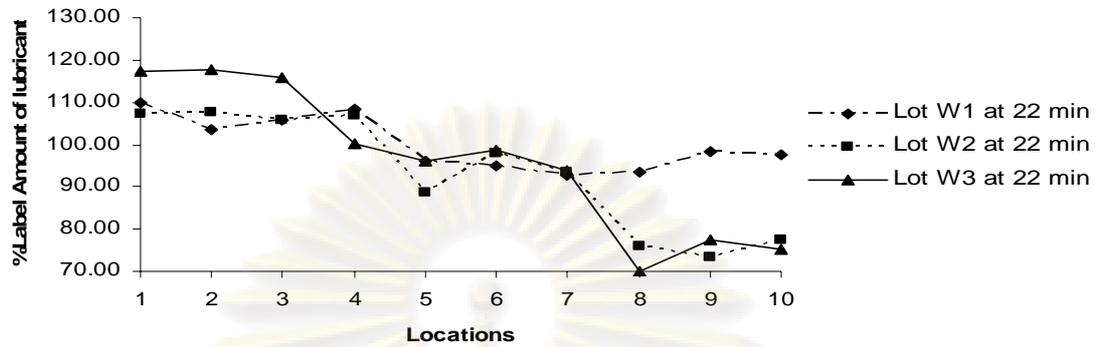
Figure 5.40   The %Label amount of lubricant results
at 22nd minute in lot W1-W3
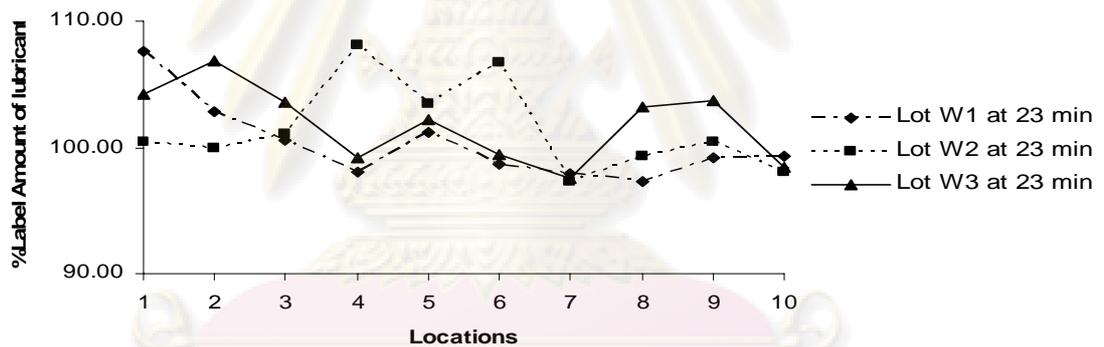
Figure 5.41   The %Label amount of lubricant results
at 23rd minute in lot W1-W3
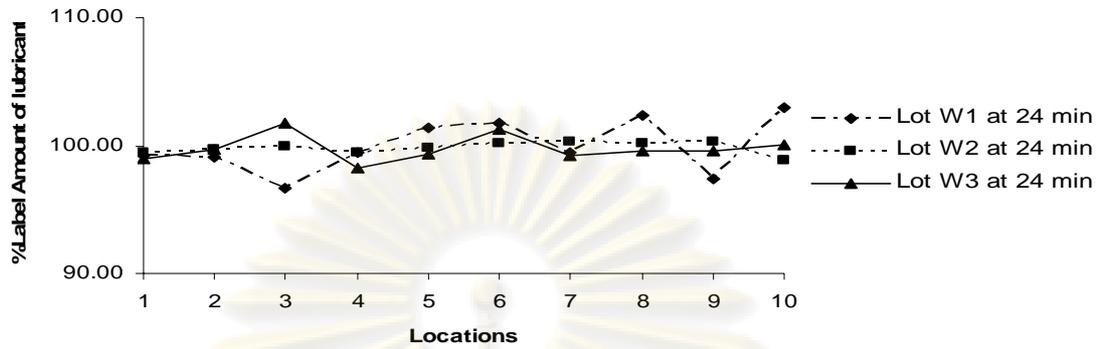
Figure 5.42   The %Label amount of lubricant results
at 24th minute in lot W1-W3
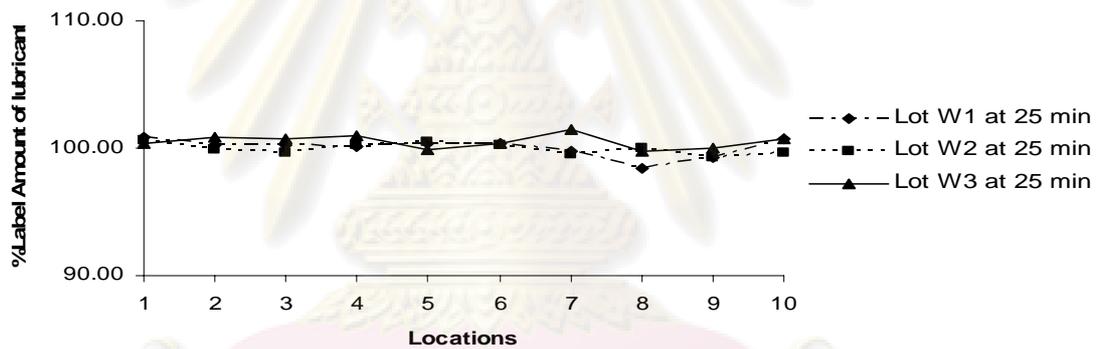
Figure 5.43   The %Label amount of lubricant results
at 25th minute in lot W1-W3

Summary of the mixing times of blend homogeneity

The Figure 5.44 was plotted from the data in Table B6-B7 to find out the optimal mixing times of blend homogeneity for API in powder blending process before adding lubricant. The optimal mixing times of blend homogeneity for API in powder blending process before adding lubricant for this production experiments (lot W1-W3) was 20th minute. The homogeneity index (%RSD) was less than 5.0% as specified in the United States Pharmacopeia 30.
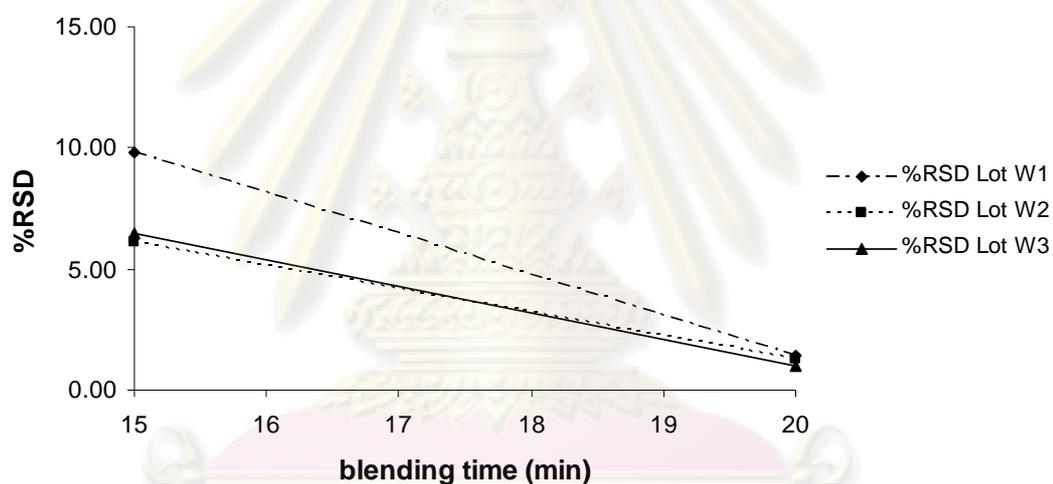
Figure 5.44   The blending profiles (%Label Amount of API) in powder blending process before adding lubricant

The Figure 5.45 was plotted from the data in Table B8-B12 to find out the optimal mixing times of blend homogeneity for API in powder blending process after adding lubricant. The optimized mixing times of blend homogeneity for API in powder blending process after adding lubricant for this production experiments (lot W1-W3) was 21st minute. The homogeneity index (%RSD) was less than 5.0% as specified in the United States Pharmacopeia 30. This homogeneity index showed API was homogenous until 25th minute (end of data collection).
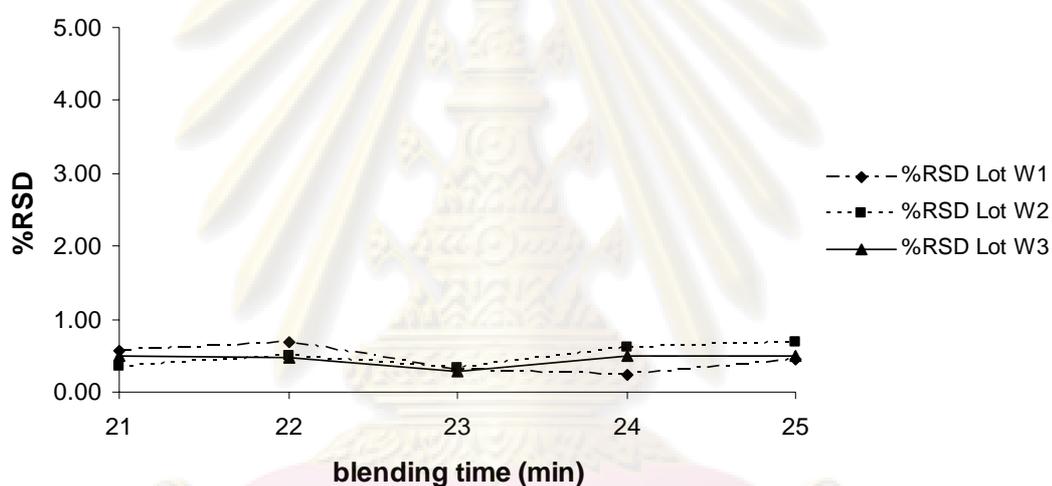
Figure 5.45   The blending profiles (%Label Amount of API) in powder blending process after adding lubricant

The Figure 5.46 was plotted from the data in Table B13-B14 to find out the optimal mixing times of blend homogeneity for disintegrant in powder blending process before adding lubricant. The optimal mixing times of blend homogeneity for disintegrant in powder blending process before added lubricant for this production experiments (lot W1-W3) was 20th minute. The homogeneity index (%RSD) was less than 5.0% as specified in the United States Pharmacopeia 30.
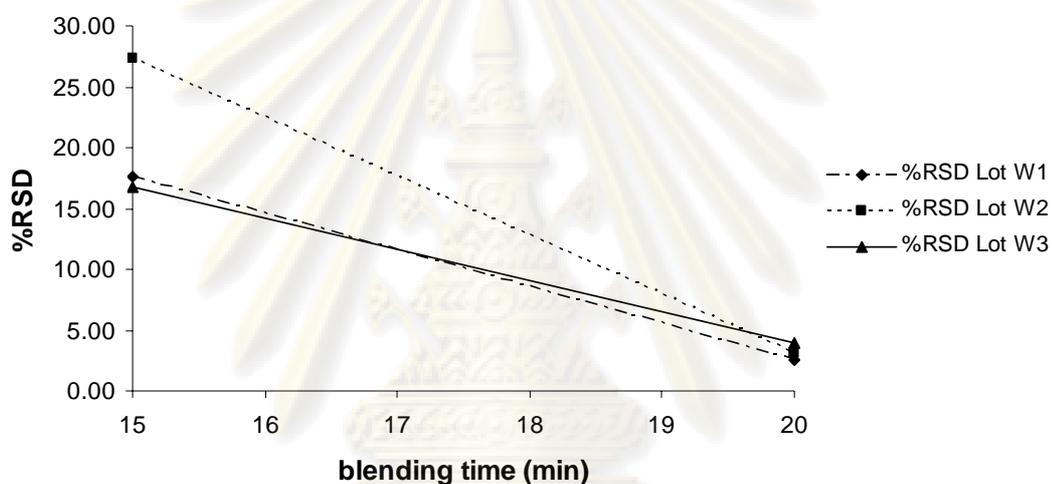
Figure 5.46   The blending profiles (%Label Amount of disintegrant) in powder blending process before adding lubricant

The Figure 5.47 was plotted from the data in Table B15-B19 to find out the optimal mixing times of blend homogeneity for disintegrant in powder blending process after adding lubricant. The optimal mixing times of blend homogeneity for disintegrant in powder blending process after adding lubricant for this production experiments (lot W1-W3) was 21st minute. The homogeneity index (%RSD) was less than 5.0% as specified in the United States Pharmacopeia 30. This homogeneity index showed disintegrant was homogenous until 25th minute.
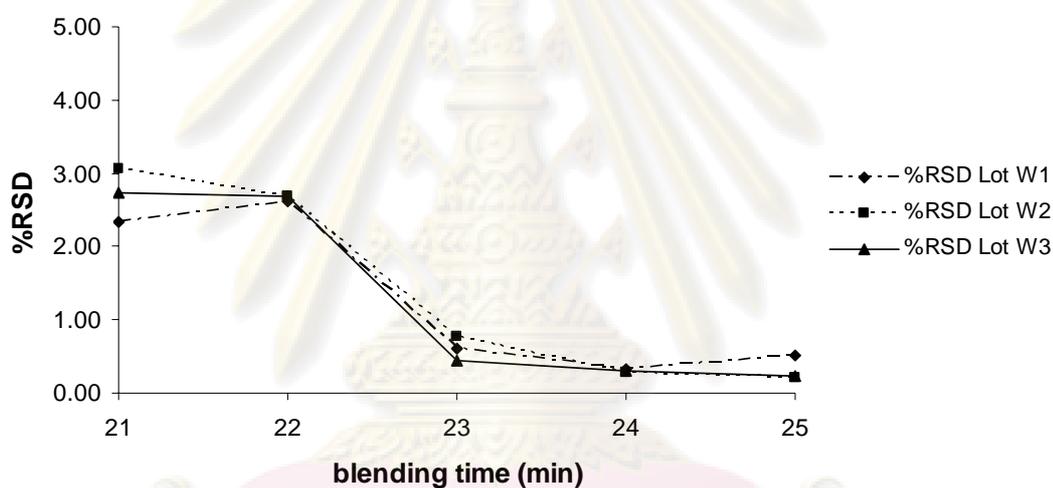
Figure 5.47   The blending profiles (%Label Amount of disintegrant) in powder blending process after adding lubricant

The Figure 5.48 was plotted from the data in Table B20-B24 to find out the optimal mixing times of blend homogeneity for lubricant in powder blending process. The minimum mixing times of blend homogeneity for lubricant in powder blending process for this production experiments (lot W1-W3) was 23rd minute. The homogeneity index (%RSD) was less than 5.0% as specified in the United States Pharmacopeia 30. However, the homogeneity index (%RSD) of lubricant results at 25th minute showed the least value. Therefore the optimal mixing times of blend homogeneity for lubricant was 25th minute for high quality control and product safety.
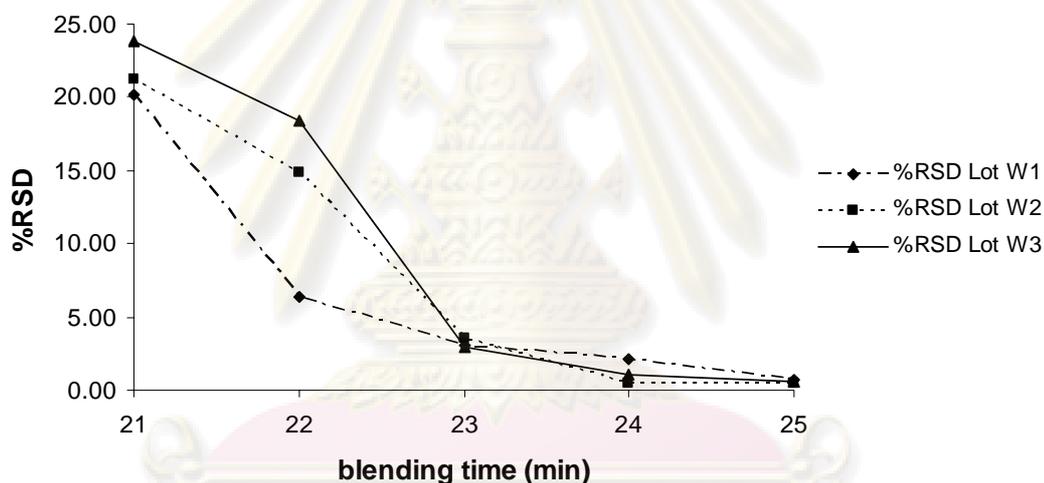
Figure 5.48   The blending profiles (%Label Amount of lubricant) in powder blending process

CHAPTER VI

CONCLUSIONS AND FUTURE WORKS

The studies are interested in multi-component blend and a lower percentage of the active pharmaceutical ingredient. These studies are directed toward facilitating the use of NIRS for the development of methods to assess blend uniformity.

The preliminary study performed show the possible to develop a relationship between NIR spectra and concentration. The internal validation studies performed show in high correlation coefficients for calibration set and validation set. Accuracy is expressed in a low bias of 0.00%. Validation show that the robustness and reproducibility of the NIRS model for the determination of %API is high. The NIRS model can be used to predict %API in blending process. Moreover, the calibration models are also accurate for the other components. The coefficient correlation is still better for prediction all ingredient in experiment.

The NIRS technique is suitable for quantitative analysis of API and exipients in pharmaceutical powder blends compare with the conventional methods (HPLC).The NIRS method can provide analytical results with minimum delay. The method predict %Label Amount of all ingredients with a low bias of 0.00%, and the mean content is not difference from the value which determined by the HPLC method. The differences in values obtained with the two methods are compared statistically using paired t-test. A value of P <0.05 is considered to be statistically different.

The NIRS technique is suitable for optimize mixing times of blend homogeneity that show in the experiment. The optimal mixing time of API and disintegrant is 20th minute and the mixing time of lubricant is not less than 3 minutes after the homogeneity of API and disintegrant (20th minute). The homogeneity index (%RSD) is less than 5.0% as specified in the United States Pharmacopeia 30.

The NIRS technique has potential for applications in product quality assurance and could benefit in process control for blending step. The technique does not require sample preparation or the use of potential environmentally harmful reagents. It could be used to analyze large number of sample during process development.

As future aspects of the study, work on larger number of additives and different compositions should be performed to widen the content of the standard database to identify more drugs. Number of samples in the sample database should be increased and multivariate statistical methods can be employed to improve the power of the technique.
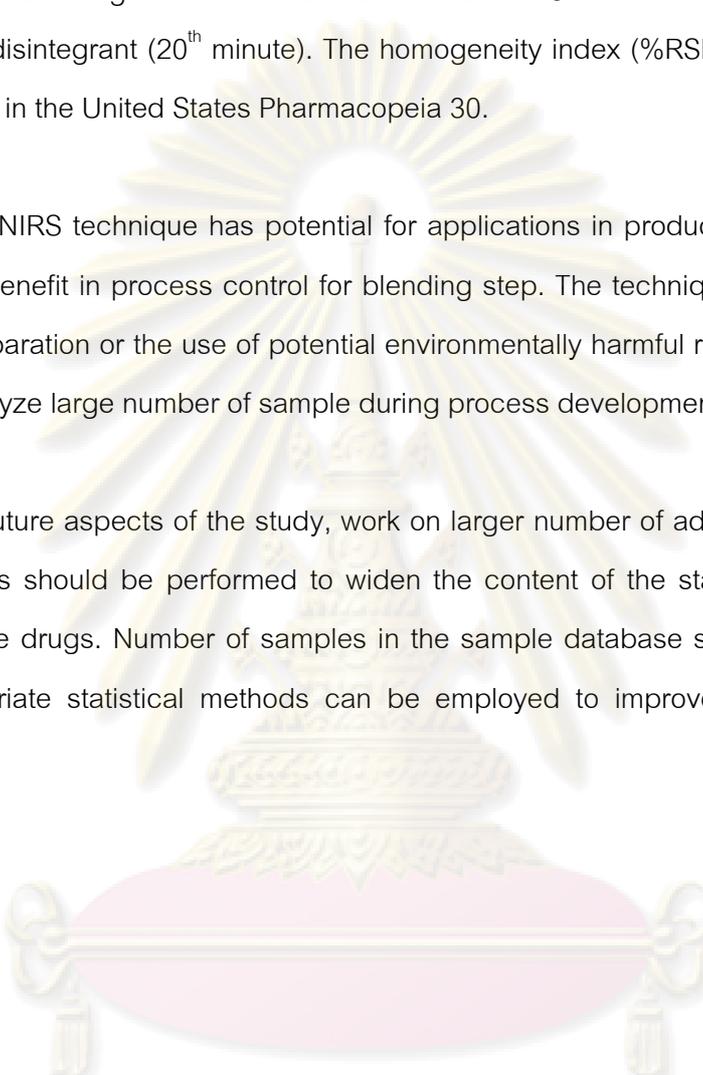
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APPENDICES

APPENDIX A

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APPENDIX A

Calculation for High Performance Liquid Chromatography data

The analytical was performed follow the United States Pharmacopeia 30. The concentrations of samples were determined from the slope and intercept obtained from a daily standard curve.

The calibration curve for data between standard concentration (mg/ml) (X) and A_{std} was used to calculation

Calculation equation :

standard concentration (mg/ml) =

$$\frac{\text{Weight of standard (mg)} \times P \times \text{concentration dilution}}{50 \times 50} \qquad 4.3$$

$$\%w/w = \frac{(A_{sam} - C)/m \times 100 \times 100/5 \times 100}{\text{Weight of powder (mg)}} \qquad 4.4$$

$$\%L.A. = \frac{(A_{sam} - C)/m \times 100 \times 100/5 \times 100}{150 \times \text{Weight of powder (mg)} / \text{Weight per tab (mg)}} \qquad 4.5$$

Where : A_{sam} is peak area in chromatogram of assay preparation.

A_{std} is peak area in chromatogram of standard preparation.

C   is Y- intercept from standard curve.

m   is Slope from standard curve.

Weight of powder (mg) is weight in mg of bulk sample in assay

preparation.

Weight per tab (mg)   is 297.53 mg for sample without lubricant set.

Weight per tab (mg)   is 301.5 mg for sample with lubricant set.

Weight of standard (mg) is weight in mg in standard preparation.

concentration dilution is dilution factor such as 2,3,4,5,6.

P   is % purity of API working standard.

Specification limit: 90.0 – 110.0 %Label Amount, %RSD was less than 5.0%

Calculation example

1. Print out the raw data from Chromeleon®6.80 Chromatography Management software.

2. Use the average of two data in the same standard from Area mAU*min column in Figure A1.1 as Y axis to calculate the daily standard curve from below equation as X axis

standard concentration (mg/ml) =

$$\frac{\text{Weight of standard (mg)} \times P \times \text{concentration dilution}}{50 \times 50} \quad 4.3$$

3. Plot the daily standard curve and build linear regression equation and check accuracy by $R^2 \geq 0.99$ that show in Figure A1.2.

1 std1

Sample Name:	std1		Injection Volume:	20
Vial Number:	BA1		Channel:	UV_VIS_1
Sample Type:	standard		Wavelength:	277
Control Program:	API		Bandwidth:	3
Quantif. Method:	API		Run Time (min):	13
Recording Time:	09.04.08 10:06		Tempurature:	25
column: Inertsil ODS-3 5 microm 4.6*150 mm C/N 5020-01731 S/N 7CI85194			Flow rate:	1

Sample No.	Sample Name	Time		Ret.Time min API UV_VIS_1	Area mAU*min API UV_VIS_1	Resolution (EP) API UV_VIS_1	Height mAU API UV_VIS_1	Asymmetry API UV_VIS_1	K' API UV_VIS_1	Plates (EP) API UV_VIS_1
1	Standard1	09.4.08	10:09	10.55	52.9902	n.a.	176.83	0.97	6.09	8700
2	Standard1	09.4.08	10:26	10.55	52.8748	n.a.	176.44	0.97	6.05	8848
3	Standard2	09.4.08	10:43	10.55	78.9324	n.a.	263.40	0.97	6.04	8833
4	Standard2	09.4.08	11:00	10.55	79.2550	n.a.	264.47	0.98	6.03	8848
5	Standard3	09.4.08	11:17	10.55	105.8554	n.a.	353.24	0.98	6.03	8848
6	Standard3	09.4.08	11:34	10.55	105.9010	n.a.	353.39	0.97	6.03	8843
7	Standard4	09.4.08	11:51	10.55	131.4389	n.a.	438.61	0.98	6.02	8838
8	Standard4	09.4.08	12:08	10.55	131.2911	n.a.	438.12	0.98	6.02	8822
9	Standard5	09.4.08	12:25	10.55	158.8079	n.a.	529.94	0.96	6.02	8822
10	Standard5	09.4.08	12:42	10.55	158.8841	n.a.	530.20	0.97	6.05	8812
11	MobilePhase	09.4.08	12:59	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
12	S1T20S1	09.4.08	13:16	10.55	104.9416	n.a.	350.19	0.97	6.08	8838
13	S1T20S1	09.4.08	13:33	10.55	105.2158	n.a.	351.11	0.98	6.08	8848
14	S1T20S2	09.4.08	13:50	10.55	96.3133	n.a.	321.40	0.99	6.08	8838
15	S1T20S2	09.4.08	14:07	10.55	96.3603	n.a.	321.55	0.97	6.08	8822

Figure A1   The chromatogram raw data lot S1

Calibration curve of API

	Area₁	Area₂	Area_avg.	API (mg/ml)
Standard0	0.0000	0.0000	0.0000	0.0000
Standard1	52.9902	52.8748	52.9325	0.0603
Standard2	78.9324	79.2550	79.0937	0.0904
Standard3	105.8554	105.9010	105.8782	0.1205
Standard4	131.4389	131.2911	131.3650	0.1507
Standard5	158.8079	158.8841	158.8460	0.1808
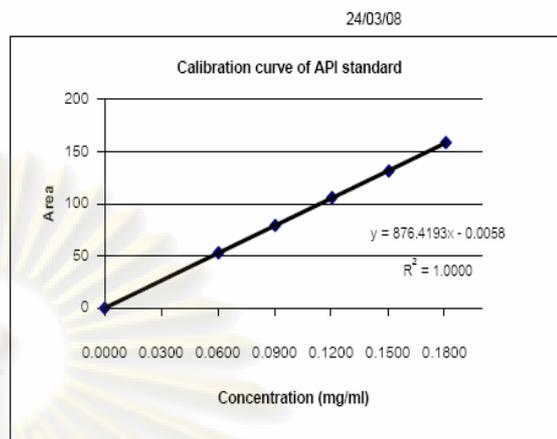
Figure A2   The daily standard curve lot S1

4. Use the average of two data in the same sample from Area mAU*min column in Figure 5.46. Calculate %Label Amount of API from below equation.

$$\%L.A. = \frac{(A_{sam} - C)/m \times 100 \times 100/5 \times 100}{150 \times \text{Weight of powder (mg)} / \text{Weight per tab (mg)}}$$ 4.5

5. The results are shown as Table A1.1

Table A1   Calculation lot S1

Sample	W_sam (mg)	API (mg)	API (mg/ml)	Inj	Area	Area_avg.	Assay (mg/ml)	Assay (mg)	%L.A.
S1T20S1	477.70	240.83	0.12	inj 1	104.9416	105.0787	0.1199	239.8042	99.57
				inj 2	105.2158				
S1T20S2	423.50	213.51	0.11	inj 1	96.3133	96.3368	0.1099	219.8550	102.97
				inj 2	96.3603				

APPENDICES B

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APPENDIX B

Data of Experiments

Table B1   The %Label Amount of API in NIRS and HPLC results for powder
blending process at 20th minute for production lot S1-S3

Locations	S1		S2		S3	
	20 MIN		20 MIN		20 MIN	
	%L.A.		%L.A.		%L.A.	
	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	99.73	99.57	101.31	101.19	101.29	101.40
2	100.19	102.97	101.46	101.61	101.90	102.25
3	99.33	99.06	100.59	100.94	101.90	101.64
4	99.49	99.20	100.56	100.77	100.08	100.92
5	101.83	101.94	99.74	99.67	100.03	100.66
6	99.96	99.47	99.42	99.65	99.76	99.96
7	99.18	99.16	100.62	100.10	100.68	100.12
8	99.75	100.07	99.28	99.61	99.93	99.64
9	99.24	99.03	99.84	99.14	99.30	99.81
10	98.37	98.67	98.64	98.28	99.43	99.51
Mean	99.71	99.91	100.15	100.10	100.43	100.59
%RSD	0.90	1.41	0.91	1.03	0.96	0.93
Min	98.37	98.67	98.64	98.28	99.30	99.51
Max	101.83	102.97	101.46	101.61	101.90	102.25
P-value	0.5049		0.6850		0.2775	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B2 The %Label Amount of API in NIRS and HPLC results for powder

blending process at 25th minute for production lot S1-S3

	S1		S2		S3	
	25 MIN		25 MIN		25 MIN	
	%L.A.		%L.A.		%L.A.	
Locations	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	101.87	101.85	101.84	101.63	98.07	98.06
2	101.65	101.60	100.97	100.98	100.35	100.13
3	101.94	101.90	100.77	100.65	98.49	98.27
4	101.39	101.38	99.86	99.89	98.63	98.86
5	100.94	100.92	99.82	99.75	99.30	99.19
6	100.45	100.44	99.96	99.94	99.21	99.21
7	101.27	101.29	100.26	100.21	98.67	98.67
8	100.38	100.28	100.28	100.21	98.13	98.14
9	101.10	101.19	99.27	99.29	98.66	98.66
10	100.59	100.56	99.44	99.49	98.30	98.31
Mean	101.16	101.14	100.25	100.20	98.78	98.75
%RSD	0.56	0.57	0.77	0.71	0.69	0.64
Min	100.38	100.28	99.27	99.29	98.07	98.06
Max	101.94	101.90	101.84	101.63	100.35	100.13
P-value	0.2753		0.1192		0.4596	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B3   The %Label Amount of disintegrant results for powder blending
process at 20th minute for production lot S1-S3

Locations	S1	S2	S3
	20 MIN	20 MIN	20 MIN
	%L.A.	%L.A.	%L.A.
1	101.83	98.67	107.31
2	108.35	109.44	106.10
3	108.73	102.05	105.01
4	109.96	98.08	100.47
5	104.72	98.19	108.80
6	108.01	108.18	100.56
7	109.31	105.31	108.33
8	108.73	104.78	109.11
9	107.96	107.16	104.03
10	109.22	106.04	107.65
Mean	107.68	103.79	105.74
%RSD	2.32	4.12	3.02
Min	101.83	98.08	100.47
Max	109.96	109.44	109.11

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Table B4 The %Label Amount of disintegrant results for powder blending process at 25th minute for production lot S1-S3

	S1	S2	S3
	25 MIN	25 MIN	25 MIN
Locations	%L.A.	%L.A.	%L.A.
1	100.51	100.85	100.45
2	100.62	100.46	100.01
3	100.87	100.45	100.07
4	100.69	100.45	100.44
5	100.28	100.63	100.96
6	100.95	100.52	100.70
7	100.57	100.83	100.74
8	100.35	100.79	100.55
9	100.88	100.62	100.35
10	100.04	100.01	100.65
Mean	100.58	100.56	100.49
%RSD	0.29	0.25	0.30
Min	100.04	100.01	100.01
Max	100.95	100.85	100.96

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Table B5   The %Label Amount of lubricant results for powder blending
process at 25th minute for production lot S1-S3

	S1	S2	S3
	25 MIN	25 MIN	25 MIN
Locations	%L.A.	%L.A.	%L.A.
1	100.49	100.90	100.80
2	100.72	101.23	101.72
3	101.92	100.35	101.62
4	100.48	100.39	101.68
5	100.18	99.35	99.25
6	99.29	99.50	99.53
7	99.89	98.29	99.09
8	101.51	99.34	99.87
9	99.05	100.22	99.59
10	99.83	101.57	99.55
Mean	100.34	100.11	100.27
%RSD	0.90	1.00	1.07
Min	99.05	98.29	99.09
Max	101.92	101.57	101.72

Table B6   The %Label Amount of API in NIRS and HPLC results

at 15th minute in lot W1-W3

Locations	W1 15 MIN %L.A.		W2 15 MIN %L.A.		W3 15 MIN %L.A.	
	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	112.62	113.14	112.94	112.59	117.73	117.58
2	116.45	117.14	110.19	110.33	117.36	117.80
3	118.12	118.50	110.89	110.91	118.34	118.30
4	115.38	115.74	111.44	111.69	118.93	118.97
5	100.08	100.48	103.32	103.09	108.26	108.76
6	100.48	100.19	103.92	103.01	107.26	107.29
7	100.82	100.79	101.02	101.72	107.78	107.23
8	92.32	92.46	96.52	96.86	102.26	102.41
9	94.72	94.51	97.58	97.51	102.66	102.49
10	94.75	94.31	97.52	97.16	102.43	102.37
Mean	104.57	104.73	104.53	104.49	110.30	110.32
%RSD	9.57	9.83	6.11	6.10	6.40	6.45
Min	92.32	92.46	96.52	96.86	102.26	102.37
Max	118.12	118.50	112.94	112.59	118.93	118.97
P-value	0.2335		0.7501		0.8511	
Statistical significance Student t-test (2-tailed) at α = 0.05	non significance		non significance		non significance	

Table B7 The %Label Amount of API in NIRS and HPLC results
at 20th minute in lot W1-W3

Locations	W1		W2		W3	
	20 MIN		20 MIN		20 MIN	
	%L.A.		%L.A.		%L.A.	
	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	102.71	102.58	101.67	101.80	100.60	100.06
2	101.06	101.27	100.63	100.95	101.13	101.15
3	100.74	100.89	99.89	99.08	99.27	99.03
4	101.98	101.99	101.47	101.01	101.12	101.29
5	101.06	101.24	102.07	102.42	99.19	99.36
6	98.37	98.02	98.42	98.38	99.44	99.97
7	99.72	99.56	102.96	102.10	101.43	101.15
8	99.53	99.57	101.05	101.69	101.07	101.61
9	99.16	99.48	100.64	100.55	101.11	101.61
10	99.81	99.35	100.84	100.64	101.24	101.68
Mean	100.41	100.40	100.96	100.86	100.56	100.69
%RSD	1.33	1.41	1.23	1.28	0.89	0.99
Min	98.37	98.02	98.42	98.38	99.19	99.03
Max	102.71	102.58	102.96	102.42	101.43	101.68
P-value	0.8229		0.5330		0.3039	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B8   The %Label Amount of API in NIRS and HPLC results
             at 21st minute in lot W1-W3

	W1		W2		W3	
	21 MIN		21 MIN		21 MIN	
	%L.A.		%L.A.		%L.A.	
Locations	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	100.61	100.35	103.20	103.20	102.51	102.39
2	100.33	100.31	103.24	103.14	103.24	103.29
3	100.25	100.25	102.53	102.51	102.16	102.10
4	100.51	100.48	102.89	102.56	102.85	102.60
5	100.58	101.48	103.24	103.28	102.78	102.38
6	100.42	100.42	102.83	102.24	102.93	102.89
7	100.14	101.16	102.93	102.62	103.15	103.17
8	99.58	99.59	102.36	102.76	102.05	102.05
9	99.80	99.80	102.95	102.86	102.76	102.89
10	100.20	100.20	103.22	103.21	103.56	103.58
Mean	100.24	100.40	102.94	102.84	102.80	102.73
%RSD	0.33	0.56	0.30	0.35	0.46	0.50
Min	99.58	99.59	102.36	102.24	102.05	102.05
Max	100.61	101.48	103.24	103.28	103.56	103.58
P-value	0.2657		0.2658		0.2228	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B9   The %Label Amount of API in NIRS and HPLC results at 22nd minute in lot W1-W3

	W1		W2		W3	
	22 MIN		22 MIN		22 MIN	
	%L.A.		%L.A.		%L.A.	
Locations	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	100.01	100.32	102.45	102.69	102.14	102.16
2	100.90	100.62	103.30	103.31	102.61	102.60
3	100.72	100.57	102.45	102.61	102.78	102.74
4	101.36	101.68	101.99	101.90	103.34	103.38
5	100.49	100.49	102.87	102.59	103.30	103.37
6	100.26	100.19	103.07	103.02	102.02	102.05
7	101.25	100.23	102.76	102.33	102.51	102.28
8	101.06	100.99	103.03	103.07	103.42	103.25
9	101.29	101.35	102.94	102.66	102.53	102.52
10	102.98	99.22	101.81	101.73	102.56	102.54
Mean	101.03	100.57	102.67	102.59	102.72	102.69
%RSD	0.81	0.68	0.47	0.49	0.48	0.48
Min	100.01	99.22	101.81	101.73	102.02	102.05
Max	102.98	101.68	103.30	103.31	103.42	103.38
P-value	0.2597		0.2843		0.3313	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B10   The %Label Amount of API in NIRS and HPLC results

at 23rd minute in lot W1-W3

Locations	W1		W2		W3	
	23 MIN		23 MIN		23 MIN	
	%L.A.		%L.A.		%L.A.	
	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	100.89	100.69	102.71	102.58	102.47	102.50
2	100.07	100.05	102.63	102.60	102.41	102.46
3	100.54	100.50	103.06	103.07	102.39	102.37
4	100.88	100.89	102.46	102.38	102.64	102.69
5	100.51	100.49	102.10	102.28	102.01	102.00
6	100.45	100.37	102.63	102.81	102.52	102.53
7	100.25	100.25	102.34	102.32	102.80	102.90
8	100.65	100.68	102.55	102.17	102.37	102.59
9	100.57	100.52	102.59	102.23	102.06	102.02
10	99.54	99.84	103.04	103.02	102.36	102.36
Mean	100.43	100.43	102.61	102.55	102.40	102.44
%RSD	0.40	0.31	0.28	0.32	0.23	0.27
Min	99.54	99.84	102.10	102.17	102.01	102.00
Max	100.89	100.89	103.06	103.07	102.80	102.90
P-value	0.8566		0.3084		0.1244	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B11   The %Label Amount of API in NIRS and HPLC results

at 24th minute in lot W1-W3

Locations	W1		W2		W3	
	24 MIN		24 MIN		24 MIN	
	%L.A.		%L.A.		%L.A.	
	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	100.10	100.10	103.15	103.16	103.07	103.04
2	100.47	100.48	103.06	103.08	102.31	102.62
3	100.08	100.02	102.03	102.02	102.54	102.04
4	102.58	102.57	102.81	102.80	101.90	101.63
5	100.44	100.45	102.62	102.89	102.43	102.95
6	100.57	100.51	102.70	102.80	103.38	103.37
7	100.62	100.63	102.85	102.14	102.51	102.82
8	100.53	100.68	102.41	102.61	102.84	102.92
9	100.69	100.58	101.93	101.22	102.68	102.81
10	100.77	100.77	103.86	103.14	102.98	102.62
Mean	100.69	100.68	102.74	102.59	102.66	102.68
%RSD	0.70	0.70	0.55	0.60	0.41	0.49
Min	100.08	100.02	101.93	101.22	101.90	101.63
Max	102.58	102.57	103.86	103.16	103.38	103.37
P-value	0.7431		0.2455		0.2455	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B12   The %Label Amount of API in NIRS and HPLC results at 25th minute in lot W1-W3

	W1		W2		W3	
	25 MIN		25 MIN		25 MIN	
	%L.A.		%L.A.		%L.A.	
Locations	NIRS	HPLC	NIRS	HPLC	NIRS	HPLC
1	100.16	100.16	102.34	102.51	102.94	102.85
2	100.29	100.25	103.11	103.63	102.05	102.03
3	100.26	100.24	102.46	102.85	102.59	102.67
4	100.49	100.38	102.55	102.85	102.56	102.47
5	100.93	100.90	102.88	102.76	102.44	102.20
6	100.88	100.88	102.31	101.93	101.80	101.84
7	100.50	100.58	102.87	102.59	101.67	101.41
8	100.57	100.57	102.89	102.74	102.26	102.17
9	100.33	100.28	101.79	101.13	102.18	102.18
10	100.77	100.85	103.30	103.34	103.16	103.20
Mean	100.52	100.51	102.65	102.63	102.36	102.30
%RSD	0.27	0.29	0.43	0.68	0.46	0.50
Min	100.16	100.16	101.79	101.13	101.67	101.41
Max	100.93	100.90	103.30	103.63	103.16	103.20
P-value	0.6286		0.8949		0.1267	
Statistical significance Student t-test (2-tailed) at α= 0.05	non significance		non significance		non significance	

Table B13   The %Label Amount of disintegrant results

at 15th minute in lot W1-W3

Locations	W1	W2	W3
	15 MIN	15 MIN	15 MIN
	%L.A.	%L.A.	%L.A.
1	82.34	89.29	99.25
2	93.39	80.52	99.34
3	87.08	63.78	90.99
4	87.07	92.07	95.66
5	121.33	95.58	100.32
6	108.54	134.36	106.93
7	108.62	105.38	118.65
8	129.51	151.19	143.59
9	135.55	142.75	116.90
10	112.71	134.99	141.36
Mean	106.61	108.99	111.30
%RSD	17.55	27.29	16.72
Min	82.34	63.78	90.99
Max	135.55	151.19	143.59

Table B14   The %Label Amount of disintegrant results

at 20th minute in lot W1-W3

	W1	W2	W3
	20 MIN	20 MIN	20 MIN
Locations	%L.A.	%L.A.	%L.A.
1	103.78	105.67	98.85
2	102.18	103.90	98.80
3	102.70	103.51	97.00
4	97.71	104.15	97.24
5	98.04	98.98	98.45
6	103.80	97.35	101.11
7	101.13	99.08	102.07
8	97.86	97.46	103.10
9	97.82	99.85	109.76
10	101.77	97.77	105.42
Mean	100.68	100.77	101.18
%RSD	2.54	3.16	4.00
Min	97.71	97.35	97.00
Max	103.80	105.67	109.76

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Table B15   The %Label Amount of disintegrant results

at 21st minute in lot W1-W3

Locations	W1	W2	W3
	21 MIN	21 MIN	21 MIN
	%L.A.	%L.A.	%L.A.
1	103.11	103.61	106.29
2	100.54	106.21	108.63
3	100.41	106.84	105.95
4	105.90	109.15	107.24
5	103.73	105.66	107.62
6	105.32	109.53	101.14
7	105.39	101.50	109.72
8	105.34	100.62	103.51
9	102.85	101.21	101.77
10	108.18	106.32	107.61
Mean	104.08	105.06	105.95
%RSD	2.34	3.05	2.73
Min	100.41	100.62	101.14
Max	108.18	109.53	109.72

Table B16   The %Label Amount of disintegrant results
at 22nd minute in lot W1-W3

Locations	W1 22 MIN %L.A.	W2 22 MIN %L.A.	W3 22 MIN %L.A.
1	104.15	101.34	101.35
2	107.12	101.20	103.83
3	107.75	102.56	107.56
4	101.00	108.83	101.76
5	109.03	103.42	101.61
6	108.73	106.44	103.25
7	104.64	103.52	106.38
8	102.16	104.84	101.55
9	105.51	108.10	107.66
10	103.61	101.52	100.05
Mean	105.37	104.18	103.50
%RSD	2.61	2.68	2.68
Min	101.00	101.20	100.05
Max	109.03	108.83	107.66

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Table B17   The %Label Amount of disintegrant results

at 23rd minute in lot W1-W3

	W1	W2	W3
	23 MIN	23 MIN	23 MIN
Locations	%L.A.	%L.A.	%L.A.
1	100.05	100.33	100.80
2	101.21	101.21	100.31
3	101.41	101.25	100.68
4	101.04	102.04	100.69
5	100.82	100.71	101.79
6	101.60	101.53	101.03
7	101.95	99.17	100.69
8	101.46	100.83	100.04
9	101.21	100.61	100.89
10	100.11	100.48	100.85
Mean	101.09	100.82	100.78
%RSD	0.60	0.77	0.46
Min	100.05	99.17	100.04
Max	101.95	102.04	101.79

Table B18   The %Label Amount of disintegrant results
at 24th minute in lot W1-W3

	W1	W2	W3
	24 MIN	24 MIN	24 MIN
Locations	%L.A.	%L.A.	%L.A.
1	100.49	100.33	101.06
2	100.03	100.86	100.76
3	100.13	100.81	100.06
4	100.37	100.45	100.63
5	100.76	100.31	100.70
6	100.83	100.30	100.15
7	100.22	100.05	100.51
8	101.00	100.88	100.31
9	100.11	100.55	100.72
10	100.26	100.26	100.545
Mean	100.42	100.48	100.54
%RSD	0.34	0.29	0.30
Min	100.03	100.05	100.06
Max	101.00	100.88	101.06

Table B19   %Label Amount of disintegrant in NIR predicted results for
powder blending process at 25 minute for production lot W1-W3

| Locations | W1 | W2 | W3 |
| | 25 MIN | 25 MIN | 25 MIN |
	%L.A.	%L.A.	%L.A.
1	100.96	100.11	100.74
2	100.03	100.64	100.08
3	100.47	100.63	100.05
4	101.49	100.79	100.06
5	100.49	100.60	100.15
6	101.27	100.57	100.59
7	100.67	100.23	100.46
8	100.06	100.61	100.19
9	100.12	100.43	100.27
10	100.33	100.76	100.27
Mean	100.59	100.54	100.28
%RSD	0.50	0.22	0.24
Min	100.03	100.11	100.05
Max	101.49	100.79	100.74

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Table B20   The %Label amount of lubricant results

at 21st minute in lot W1-W3

	W1	W2	W3
	21 MIN	21 MIN	21 MIN
Locations	%L.A.	%L.A.	%L.A.
1	115.42	126.54	129.79
2	113.77	118.76	129.67
3	118.79	124.21	123.48
4	111.23	115.95	123.23
5	93.03	93.13	98.17
6	94.72	92.31	89.19
7	92.10	97.15	95.15
8	72.59	79.45	74.91
9	70.71	71.30	71.08
10	70.34	72.95	73.12
Mean	95.27	99.17	100.78
%RSD	20.13	21.21	23.82
Min	70.34	71.30	71.08
Max	118.79	126.54	129.79

Table B21   The %Label amount of lubricant results

at 22nd minute in lot W1-W3

Locations	W1	W2	W3
	22 MIN	22 MIN	22 MIN
	%L.A.	%L.A.	%L.A.
1	109.94	107.37	117.24
2	103.62	107.66	117.78
3	105.87	105.79	115.91
4	108.29	107.05	100.18
5	96.13	88.68	96.15
6	95.03	97.88	98.74
7	92.68	93.33	93.94
8	93.48	75.98	69.97
9	98.26	73.49	77.33
10	97.61	77.55	75.21
Mean	100.09	93.48	96.24
%RSD	6.32	14.84	18.35
Min	92.68	73.49	69.97
Max	109.94	107.66	117.78

Table B22   The %Label amount of lubricant results

at 23rd minute in lot W1-W3

	W1	W2	W3
	23 MIN	23 MIN	23 MIN
Locations	%L.A.	%L.A.	%L.A.
1	107.56	100.45	104.25
2	102.80	99.90	106.91
3	100.60	101.10	103.61
4	97.99	108.07	99.14
5	101.24	103.44	102.25
6	98.68	106.68	99.40
7	97.90	97.27	97.49
8	97.24	99.30	103.18
9	99.23	100.43	103.72
10	99.27	98.05	98.37
Mean	100.25	101.47	101.83
%RSD	3.07	3.50	3.00
Min	97.24	97.27	97.49
Max	107.56	108.07	106.91

Table B23 The %Label amount of lubricant results

at 24th minute in lot W1-W3

	W1	W2	W3
	24 MIN	24 MIN	24 MIN
Locations	%L.A.	%L.A.	%L.A.
1	99.23	99.46	99.02
2	99.08	99.73	99.71
3	96.71	99.90	101.71
4	99.47	99.51	98.24
5	101.34	99.82	99.34
6	101.81	100.21	101.33
7	99.48	100.33	99.26
8	102.41	100.16	99.61
9	97.45	100.29	99.52
10	102.93	98.84	100.05
Mean	99.99	99.83	99.78
%RSD	2.08	0.47	1.04
Min	96.71	98.84	98.24
Max	102.93	100.33	101.71

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Table B24   The %Label amount of lubricant results

at 25th minute in lot W1-W3

	W1	W2	W3
	25 MIN	25 MIN	25 MIN
Locations	%L.A.	%L.A.	%L.A.
1	100.86	100.61	100.34
2	100.25	99.87	100.84
3	100.22	99.67	100.72
4	100.18	100.23	100.96
5	100.42	100.45	99.90
6	100.38	100.21	100.32
7	99.71	99.50	101.43
8	98.43	100.02	99.72
9	99.21	99.23	99.99
10	100.71	99.65	100.69
Mean	100.04	99.94	100.49
%RSD	0.73	0.44	0.53
Min	98.43	99.23	99.72
Max	100.86	100.61	101.43

APPENDICES C

APPENDIX C

List of Publications

1. Niti Sunsandee, Anongnat Somwangthanaroj and Nattakarn Navasearttavisootr. 2008. Evaluation of Pharmaceutical Blending Homogeneity by means of Near-Infrared Spectroscopy. The 1st Asian NIR Symposium and The 24th Japanese NIR Forum Tsukuba city, Japan, November, 2008, Ref. No P01A : 168-169 (Poster Presentation).

2. Niti Sunsandee and Anongnat Somwangthanaroj. 2008. Quantitative Analysis of Active Pharmaceutical Ingredient (API) and excipients in Powder blending process by Near-Infrared Spectroscopy. Proceeding of The 18th Thailand Chemical Engineering and Applied Chemistry conference (TIChE 18), Chonburi, Thailand, October, 2008, Ref. No Fundamental of Chemical Engineering and Applied Chemistry (R122-FU015-02) : 41 (Oral Presentation).

Evaluation of Pharmaceutical Blending Homogeneity by means of Near-Infrared Spectroscopy

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Powder blending process is one of the most common unit operations in the pharmaceutical industry. The blending of Active Pharmaceutical Ingredient (API) and more ingredients is for control the uniformity and product quality. Near-infrared spectroscopy (NIRS) has become an interesting analytical technique to replace high performance liquid chromatography (HPLC) in pharmaceutical process control. NIRS analytical technique is a particularly powerful method for rapid and non-invasive analysis of powder blends.

The objective of this work is to study the homogeneity of a typical direct compression pharmaceutical powder blend by means of NIRS. An experimental design approach is to use ingenerating a 5-level (%, w/w) calibration sample set; 25 samples for calibration without lubricant and 125 samples for calibration with lubricant. The calibration samples were prepared by weighing suitable amount of Active Pharmaceutical Ingredient (API) and ingredients into 20-mL bottles and were manually mixed. Reference measurements were carried out with HPLC. The data analyses were performed by partial least squares (PLS) modeling. The result of validation with an independent set of sample sets are strong correlation with the reference values and great accuracy are demonstrated, i.e., correlation coefficient (R^2) = 0.998, standard error of prediction (SEP) = 2.49422 (API without lubricant set), R^2 = 0.998, SEP = 2.24173 (API with lubricant set). Therefore, this technique has potential for applications in product quality assurance and could benefit in process control for blending step.

Keywords: near-infrared spectroscopy, blend uniformity, homogeneity, mixing, pharmaceutical process control, diffusion reflection, fiber-optic probe

Type of presentation: Poster Presentation

Evaluation of Pharmaceutical Blending Homogeneity by means of Near-Infrared Spectroscopy

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Abstract

The objective of this work is to study the homogeneity of a typical direct compression pharmaceutical powder blend by means of NIRS. The data analyses were performed by partial least squares (PLS) modeling. The result of validation with an independent set of sample sets are strong correlation with the reference values and great accuracy are demonstrated, i.e., correlation coefficient (R^2) = 0.998, standard error of prediction (SEP) = 2.49422 (API without lubricant set), R^2 = 0.998, SEP = 2.24173 (API with lubricant set). Therefore, this technique has potential for applications in product quality assurance and could benefit in process control for blending step.

Keywords: blend uniformity, homogeneity, mixing, pharmaceutical process control.

Introduction

Powder blending process is one of the most common unit operations in the pharmaceutical industry. The blending of Active Pharmaceutical Ingredient (API) and more ingredients is for control the uniformity and product quality. Near-infrared spectroscopy (NIRS) has become an interesting analytical technique to replace high performance liquid chromatography (HPLC) in pharmaceutical process control. NIRS analytical technique is a particularly powerful method for rapid and non-invasive analysis of powder blends.

Materials and methods

Sample

API, disintegrant, lubricant and other non-API were provided by Government Pharmaceutical Organization Thailand. NIR calibration models were developed and validated for the probe based on a designed 5-level (%w/w) calibration sample set; 50 samples for calibration and validation without lubricant and 250 samples for calibration and validation with lubricant.

Spectral acquisition

The NIR spectrophotometer model NIRFlex N400 (Buchi, Flawil, Switzerland) was used for spectral acquisition. The NIR spectra were measured with the Interactance fiber optics in the wavelength region of 4008 cm^{-1} to 9996 cm^{-1} with 12 cm^{-1} interval. The probe whose depth is about 10 mm was inserted through the adapter cap and the sample bottle was turned upside down. The powder must cover the tip of the probe to decrease the variation of NIR scans as seen in Figure1 and 3. All samples were recorded by a scanning FT-NIR-spectrometer in random order from 5 locations. Each sample was scanned 5 times for one average spectrum to equilibrate in homogeneities.

Data analysis

Data analysis was performed with Chemometrical NIRCal 4.21 software (Buchi, Flawil, Switzerland). First, spectral pretreatments of smoothing and second derivative were applied. Then the calibration equation was developed using partial least squares (PLS) regression. The wavelength region used for the calculation was optimized at 50 nm intervals. Validation was performed by a separated test set. Statistical characteristics of the calibration and the validation sample sets are shown in Table 1and 2.

Reference analysis

Reference API concentrations were measured with a high performance liquid chromatography Instrument model UltiMate® 3000 Rapid Separation LC system (Dionex , Germany). The reference method was performed follow the United States Pharmacopeia 30. The data was an average value calculated from duplicate measurement
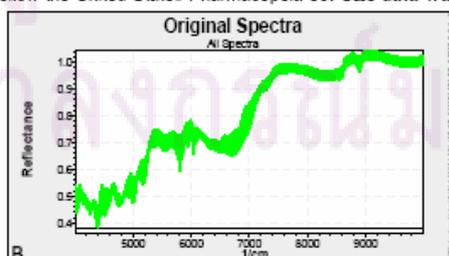
Figure 1. NIR measurement of API without lubricant set
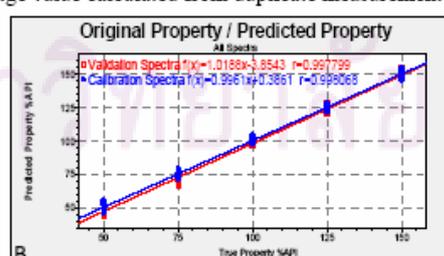
Figure 2. The correlation between true property and predicted property of API without lubricant set
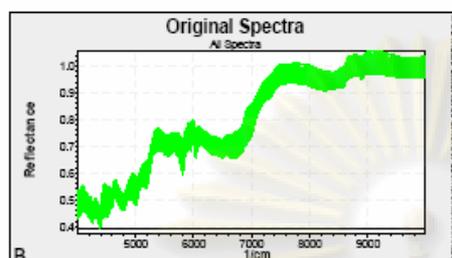
Figure 3. NIR measurement of API with lubricant set
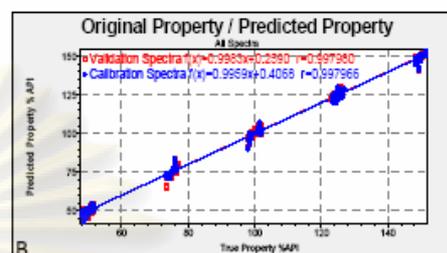
Figure 4. The correlation between true property and predicted property of API with lubricant set

Table 1-2. Statistical characteristics of the calibration and the validation sample sets.

Table 1. API without lubricant set

Items	Calibration set	Validation set
Average	99.9995 %	100.0000 %
Standard deviation	35.50	35.49
Minimum	49.9950 %	49.9983 %
Maximum	150.0100 %	150.0080 %
Number of samples	25	25

Table 2. API with lubricant set

Items	Calibration set	Validation set
Average	100.0990 %	100.1480 %
Standard deviation	35.44	35.26
Minimum	47.5023 %	48.4762 %
Maximum	151.9580 %	151.9130 %
Number of samples	125	125

Table 3-4. PLS calibration results for predicting %API in API with and without lubricant set. The calibration equation was developed on the second derivative NIR spectra.

Table 3. API without lubricant set

Wavelength region (cm^{-1})	F	R^2	SEC	SEP	Bias	RPD
4392-4800, 5400-6600, 7800-9996.	10	0.998	2.21	2.49	0.00	14.25

Table 4. API with lubricant set

Wavelength region (cm^{-1})	F	R^2	SEC	SEP	Bias	RPD
4392-4800, 5400-6600, 7800-9996.	8	0.998	2.26	2.24	0.00	15.74

F: The number of factors; R^2: the coefficient of multiple determination; SEC: standard error of calibration, SEP: standard error of prediction; Bias: the average of differences between reference value and NIR value; RPD: the ratio of standard deviation of reference data in the validation set to SEP Unit: %

Results and discussion

Statistical parameters such as standard error of prediction (SEP) and correlation coefficient of determinant demonstrate that the calibration model is suitable to predict concentration of API during blending with and without lubricant. The NIR method demonstrated variance similar to High Performance Liquid Chromatography analysis in term of API concentration.

Conclusion

The NIRS technique has potential for applications in product quality assurance and could benefit in process control for blending step.

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1. The United States Pharmacopeia Convention. *The United States Pharmacopeia 30 National Formulary 25*, Canada, Webcom Limited (2007).
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To : Niti Sunsandee, Anongnat Somwangthanaroj

Date : September 17, 2008

Dear Sir or Madam,

It is our pleasure to invite you to participate in the 18th Thailand Chemical Engineering and Applied Chemistry conference (TIChE 18) which will be held in Pattaya from October 20th to 21st, 2008 .

Your contribution entitled "Quantitative Analysis of Active Pharmaceutical Ingredient (API) and excipients in Powder blending process by Near-Infrared Spectroscopy" has been accepted as oral presentation.

Yours sincerely,

Prof. Dr. Nuttawan Yoswathana

Head of Chemical Engineering Department,

Faculty of engineering ,

Mahidol University.

Quantitative Analysis of Active Pharmaceutical Ingredient (API) and excipients in Powder blending process by Near-Infrared Spectroscopy

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Solid dosage forms are the most commonly prescribed pharmaceutical formulations. They consist of a homogeneous mixture of ingredients that provide desired characteristics. The process of blending during manufacturing processes is important to assure the quality of the product and it may be assessed by blend uniformity analysis. Recently, near-infrared spectroscopy (NIRS) has become an analytical technique of great interest for the pharmaceutical industry, particularly for the non-destructive analysis of powder blends to replace high performance liquid chromatography (HPLC) in pharmaceutical process control.

The focus of this study is to investigate qualitative tools of analysis for blend homogeneity determinations by means of NIRS. NIR calibration models were developed and validated for the probe based on a designed 5-level (%, w/w) calibration sample set; 25 samples for calibration without lubricant and 125 samples for calibration with lubricant. The calibration model was performed by partial least squares (PLS) model. The PLS calibration with a representative blend showed a strong correlation with the reference values and great accuracy. The results obtained by NIR spectroscopy were compared with a conventional HPLC method. The study showed that NIRS is suitable as an alternate analytical tool in powder blend analysis.

Keywords: pharmaceutical engineering, near-infrared spectroscopy, blend uniformity, homogeneity, pharmaceutical process control

° Oral presentation

Quantitative Analysis of Active Pharmaceutical Ingredient (API) and excipients in Powder blending process by Near-Infrared Spectroscopy

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Abstract

The focus of this study is to investigate qualitative tools of analysis for blend homogeneity determinations by means of Near-Infrared Spectroscopy (NIRS). NIR calibration models were developed and validated for the probe based on a designed 5-level (%w/w) calibration sample set; 25 samples for calibration without lubricant and 125 samples for calibration with lubricant. The calibration model was performed by Partial Least Squares (PLS) model. The PLS calibration with a representative blend showed a strong correlation with the reference values and great accuracy. The results obtained by NIRS were compared with a conventional High Performance Liquid Chromatography (HPLC) method. The study showed that NIRS is suitable as an alternate analytical tool in powder blend analysis.

1. Introduction

Solid dosage forms are the most commonly prescribed pharmaceutical formulations. They consist of a homogeneous mixture of ingredients that provide desired characteristics. The process of blending during manufacturing processes is important to assure the quality of the product and it may be assessed by blend uniformity analysis. Recently, NIRS has become an analytical technique of great interest for the pharmaceutical industry, particularly for the non-destructive analysis of powder blends to replace HPLC in pharmaceutical process control.

2. Materials and methods

API, disintegrant lubricant and other non APIs were selected as blending components. All blends were mixed in a 420-liters cubic-blender. NIR spectra were collected over the range of 4008 to 9996 cm^{-1} using a FT-NIR-spectrometer (Buchi, Flawil, Switzerland NIRflex® N400 with 2-m fiber optic probe).PLS regression was utilized to establish a calibration model for prediction of API, disintegrant, lubricant. The experimental design was optimized to generate a calibration model used weighing a suitable amount of all components into a separate 20 ml bottle mixed manually by shaking. Reference API concentrations were measured with a high performance liquid chromatography Instrument model UltiMate® 3000 LC system (Dionex, Germering, Germany). The reference method was performed follow the United States Pharmacopeia 30. The data was an average value calculated from duplicate measurements.

3. Results and discussion

3.1. Calibration model

Statistical parameters such as Standard Error of Calibration (SEC), Standard Error of Prediction (SEP), Bias and correlation coefficient of determinant (R^2) show the suitability of calibration model in order to predict %API and %other excipients during blending

Table 1 Statistical parameters results for predicting %API

Wavelength region (cm^{-1})	F	R^2	SEC	SEP	Bias	RPD
4392-4800, 5400-6600, 7800-9996.	8	0.9980	2.26	2.24	0.00	15.74

The correlation between true property (HPLC method) for %API versus predicted property (NIRS method) of %API (Figure1) resulted in high correlation coefficients of 0.9980 for calibration set and validation set. Accuracy is expressed in the Bias. Validation showed that the robustness and reproducibility of the NIRS model for the determination of %API is high. The NIRS model can be used to predict %API in blending process. Moreover, the calibration models are also accurate for the other components. The calibration model for the determination of % disintegrant show high correlation coefficients of 0.9792 for calibration set and 0.9710 for validation set from %w/w disintegrant versus predicted property (NIRS method) of %disintegrant (Figure2). The calibration model for the determination of % lubricant also show high correlation coefficients of 0.9561 for calibration set and 0.9574 for validation set from %w/w lubricant versus predicted property (NIRS method) %lubricant (Figure3). The correlations (R^2) in Figure1-3 were more than 0.95 which prove that predicted property were not differences from true property.

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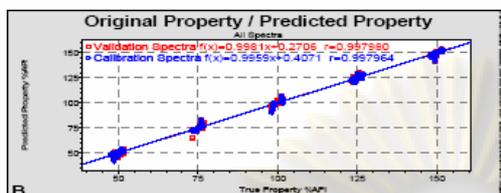
Figure1 Predicted (NIRS) vs. true property (HPLC) for determination of %API (n=250). R^2 = 0.9980.
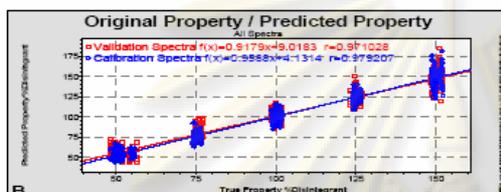
Figure2 Predicted (NIRS) vs. true property (%w/w) for determination of %disintegrant (n=250). R^2 = 0.9792.
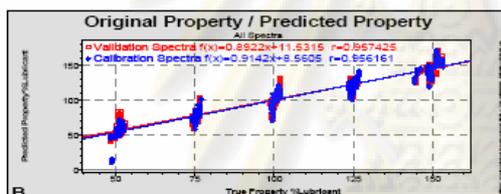
Figure3 Predicted (NIRS) vs. true property (%w/w) for determination of %lubricant (n=250). R^2 = 0.9561.

3.2. Prediction value

The calibration model was used to predict %API and %other excipients in powder blending process.

Table2 NIR predicted results for powder blending process.

Sample ID	HPLC %API	NIR predicted %API	NIR predicted %Disintegrant	NIR predicted %Lubricant	Residual
API / S1T25S1	101.85	101.67	100.51	100.49	0.00
API / S1T25S2	101.60	101.65	100.62	100.72	0.00
API / S1T25S3	101.90	101.94	100.67	101.92	0.00
API / S1T25S4	101.36	101.39	100.69	100.46	0.00
API / S1T25S5	100.92	100.94	100.26	100.16	0.00
API / S1T25OS6	100.44	100.76	100.95	99.29	0.00
API / S1T25S7	101.29	101.67	100.57	99.89	0.00
API / S1T25S8	100.28	100.38	100.35	101.51	0.00
API / S1T25S9	101.19	101.10	100.66	99.05	0.00
API / S1T25S10	100.56	100.59	100.04	99.63	0.00
Mean	101.14	101.23	100.56	100.34	
S.D	0.5764	0.5541	0.2924	0.9006	
95% CI for mean difference	(-0.0171, 0.192540)				
t Test	1.894 Sig. (2-tailed) 0.091				

The paired t-test at the 95% confidence level did not indicate any differences between the %API predicted by the NIRS method and the validated HPLC method in powder blending process.

4. **Conclusion**

The NIRS technique is suitable for quatitative analysis of API and exipients in pharmaceutical powder blends compared with the convectional methods such as HPLC, The NIRS method can provide analytical results with minimum delay. The NIRS technique has potential for applications in product quality assurance and could benefit in process control for blending step.

5. **Acknowledgement**

6. **References**

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VITA

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