A diffuse reflectance NIR spectroscopy method for determine drug concentration in pharmaceutical production tablets based on a PLS calibration model from manufactured tablets

by

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ABSTRACT

A diffuse reflectance near-infrared (NIR) spectroscopy method was developed to determine the active pharmacological ingredient (API) in pharmaceutical production tablets from batch and continuous manufacturing processes.

Tablets of six different API concentration manufactured by batch processes were used as the calibration set. Forty tablets were used for each concentration level. Partial Least Squares (PLS) regression was used to develop the calibration models. The NIR calibration model was challenged using two independent test sets of pharmaceutical production tablets with the same API concentration: one of tablets from a batch process and other tablets from a continuous process. The tablet NIR spectra were acquired using the integrating sphere from the FT-NIR spectrometer at a resolution of 32 cm^{-1} and 32 scans at both sides of the tablets. The Root Mean Square Error of Prediction (RMSEP), the Relative Standard Error of Prediction (RSEP), and bias were calculated to evaluate the predictive capability of the calibration models.

The NIR model predict the API concentration of the tablets test set from a batch process with acceptable values. RMSEP of 0.338% (w/w) and a RSEP of 0.611% were obtained. For the continuous process tablets test set, a difference in the API concentration was found between each side of the tablets. Material segregation and the level of shear exposure in the continuous manufacturing line are possible explanations for these differences in API concentration between the tablets sides. RMSEP of 1.441 (%w/w) was obtained for the side of the tablet with the correct API concentration. RMSEP of 1.971

(%w/w) was obtained for the side with a higher API concentration than the correct amount. An overall RMSEP of 2.526 (%w/w) was obtained evaluating both sides simultaneously of the continuous process tablets.

The NIR calibration model developed with a calibration test from a batch process has the ability to predict the API concentration of tablets manufactured batch and continuous process.

RESUMEN

Un método de espectroscopia de infrarrojo cercano (NIR, por sus siglas en inglés) de reflectancia difusa fue desarrollado para determinar el ingrediente farmacológico activo (API, por sus siglas en inglés) en tabletas de producción farmacéutica manufacturadas por un proceso por lote y por un proceso continuo.

Tabletas de seis concentraciones diferentes del API fabricadas mediante un proceso por lote fueron utilizadas como el conjunto de muestras de calibración. Cuarenta tabletas se usaron para cada nivel de concentración. La regresión de mínimos cuadrados parciales (PLS) se utilizó para desarrollar los modelos de calibración. El modelo de calibración de NIR se retó utilizando dos conjuntos de muestras de prueba independientes de tabletas de producción farmacéutica con la misma concentración del API: uno de tabletas producidas mediante un proceso por lote y otro de tabletas producidas mediante de un proceso continuo. Los espectros de NIR de las tabletas fueron adquiridos utilizando la esfera integrante del espectrómetro FT-NIR a una resolución de 32 cm^{-1} y 32 barridos en ambos lados de las tabletas. Se calculó el error de predicción cuadrático medio (RMSEP, por sus siglas en inglés), el error estándar relativo de predicción (RSEP, por sus siglas en inglés) y el sesgo para evaluar la capacidad predictiva de los modelos de calibración.

El modelo de NIR predice la concentración del API con valores aceptables en tabletas producidas mediante un proceso por lote. Se obtuvo un RMSEP de 0.338% (p / p) y un RSEP de 0.611%. Para el conjunto de muestras de prueba de tabletas producidas mediante un proceso continuo, se encontró una diferencia en la concentración del API entre cada

lado de las tabletas. Segregación del material y el nivel de exposición a la fuerza cortante en la línea de manufactura continua son posibles explicaciones a esta diferencia en la concentración del API entre ambos lados de las tabletas. Se obtuvo un RMSEP de 1.441 (% p / p) para el lado de la tableta que contiene la concentración correcta del API. Se obtuvo un RMSEP de 1.971 (% p / p) para el lado de la tableta que contiene una concentración mayor del API que la cantidad correcta. Se obtuvo un RMSEP total de 2.526 (% p / p) al evaluar simultáneamente ambos lados de las tabletas producidas mediante un proceso continuo.

El modelo de calibración de NIR desarrollado mediante un conjunto de muestras de calibración de tabletas producidas mediante un proceso por lote tiene la capacidad de predecir la concentración del API en tabletas manufacturadas mediante un proceso por lotes y tabletas manufacturadas mediante un proceso continuo.

To God,

and to my beloved parents.

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GLOSSARY OF ABREVIATIONS

API	Active Pharmaceutical Ingredients
EMA	European Medicine Agency
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization
PbS	Lead (II) sulfide
FDA	Food and Drug Administration
MIR	Mid-Infrared
MPA FT-NIR	Multi-Purpose Analyzer Fourier Transform Near Infrared
NIR	Near-Infrared
NS	Noise spectrum
PAT	Process Analytical Technology
PCA	Principal Component Analysis
PLS	Partial Least Squares
PQ	Performance qualification
RMSEP	Root Mean Square Error of Predictions
RSEP	Relative standard errors of predictions
SNV	Standard normal variate
STD	Standard deviation

1 INTRODUCTION

1.1 Justification

It is extremely important to determine the active pharmaceutical ingredient (API) content in tablets. A higher drug amount than what is necessary can cause serious secondary effects, including death. Near-infrared (NIR) spectroscopy has been shown to be a fast, sustainable, and non-destructive method to determine drug concentration in tablets [1]. Drug content is usually determined by High Performance Liquid Chromatography (HPLC). The disadvantages of the HPLC are the requirements of high cost equipment and maintenance, sample preparation, and that it takes 3 to 4 days to analyze 30 tablets for a batch of approximately 3 million tablets. NIR spectroscopy (NIR) allows faster analysis time of 30 tablets or more in one hour[1–3]. NIR spectroscopic methods, compared to the HPLC method, help to contribute with the green chemistry[4]. NIR spectroscopy does not require the use of solvents, requires less energy consumption, and is a real-time analysis that prevents pollution and enhances operational safety. Previous studies (**Figure 1.1**) demonstrate the advantages of NIR over HPLC as a green chemistry tool[5].

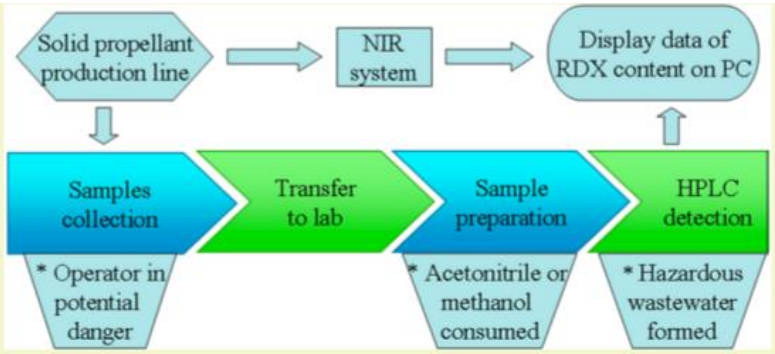

Figure 1.1 NIR spectroscopy over HPLC as a green chemistry tool in the determination of the hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) content of a solid propellant.
(Image obtained from: A Green Analytical Tool for In-Process Determination of RDX Content of Propellant Using the NIR System. *ACS Sustain. Chem. Eng.* **2013)**

Previous studies developed NIR spectroscopic calibration models to quantify the drug concentration in tablets preparing laboratory samples[1,6–8]. The preparation of laboratory samples is required when pharmaceutical production tablets are not available in a wide range of the API concentration. For example, ranitidine tablets are usually available only at concentrations of 75, 150, and 300 mg. The laboratory samples could be prepared adding excipients or API to the production samples.

The aim of this study is to develop a calibration model using diffuse reflectance NIR spectroscopy to quantify the drug content of tablets produced in a pharmaceutical industry by a batch process without the need to prepare laboratory samples. Another aim was to evaluate the calibration model using the International Conference on Harmonization (ICH) Q2(R1) guidelines[9] with tablets of the same formulation produced by continuous manufacturing process from a pharmaceutical company. These aims were possible by studying and understanding the interaction between the radiation and the tablets and how the drug content, and the embossing of tablets affects this interaction. For the

pharmaceutical company that funded this study, it will be useful to ease the transition of the batch process to the continuous manufacturing process of this drug tablet.

1.2 Intellectual merits

The intellectual merit of this study is the development of a novel reliable method to determine the drug concentration in tablets formulated with two active pharmaceutical ingredient (APIs) in its granulation form. This will be possible by studying the interaction between the radiation and the tablet. The radiation interacts with tablet in different ways when tablets are formed with material particles that vary particle size, compaction force, embossing, and other factors. In diffuse reflectance, once the NIR radiation irradiates the tablet surface, a portion of the radiation penetrates the sample and is absorbed. The radiation will refract through each of the tablet particles, part of the radiation is scattered in all directions[10]. Diffuse reflectance is the incident radiation emerging from the tablet surface.

1.3 Broader Impact

The broader impact of this study is that NIR does not require the waste of solvents, since is a non-destructive analytical technique. High Performance Liquid Chromatography (HPLC) is a destructive technique that requires dissolution of the solid sample, requires large amounts of solvents, and generates great amounts of waste. NIR is a green chemistry

technique and previous studies demonstrate the advantages of NIR over HPLC[5]. NIR requires less energy, enhances operational safety and provides a real-time analysis. The quantification of the drug content in the tablet guarantees a safe final product to the patients. This study will be useful for the pharmaceutical industry that support this study to ease the transition from a batch process to the continuous manufacturing process of this tablet drug. The results of this study will contribute in the implementation of a new pharmaceutical continuous manufacturing plant in Puerto Rico helping to retain manufacturing jobs in the Island.

2 LITERATURE REVIEW

2.1 NIR Spectroscopy

For historical reasons, the 780-2,500nm region in the electromagnetic spectrum is considered the NIR region[11]. The NIR region in terms of wavenumbers is 12,800-4,000 cm^{-1}. The bands observed in the NIR region are the overtones and the combination bands that arise from the fundamental or normal modes of vibrations in the mid-infrared spectrum. NIR has the advantage that it does not require sample preparation because the absorption bands are 10-100 times weaker than those observed in mid-infrared spectroscopy (MIR). It can be used to analyze the content uniformity, blend uniformity, particle size, hardness, moisture content, dissolution rate, and particle size distribution[8]. NIR depends on the dipole moment and specially in the anharmonicity of the bonds. The NIR spectra can be acquired using diffuse reflectance and transmittance measurements.

2.1.1 Diffuse Reflectance

Diffuse reflectance is the measurement of the absorption and scattering behavior of the sample. It is used for heterogeneous samples, powders, polymers, and solids with a rough surfaces. Diffuse reflectance spectra can be acquired using the integrating sphere window or with a fiber probe in the FT-NIR spectrometer. The source of light and the detector are in the same direction. Once the NIR radiation irradiates the tablet surface (**Figure 2.1)**, a portion of the radiation penetrates the sample and some it loss to absorption

by the particles, scatter by the particles or transmitted[10,12–14]. Other portion of the radiation will refract, where the radiation entering the tablet particles hits the surface with a bend in angle. The rest of the radiation will be reflected, where the radiation leaves the particles in the scattered backward direction. It could be specular reflection, where the incident and the reflected angles are equal; or diffuse reflected, where the radiation is scattered at all angles. The radiation interaction with the tablet particles depends on the particle size, tablet porosity, tablet compression force, embossing, and refractive index[15,16]

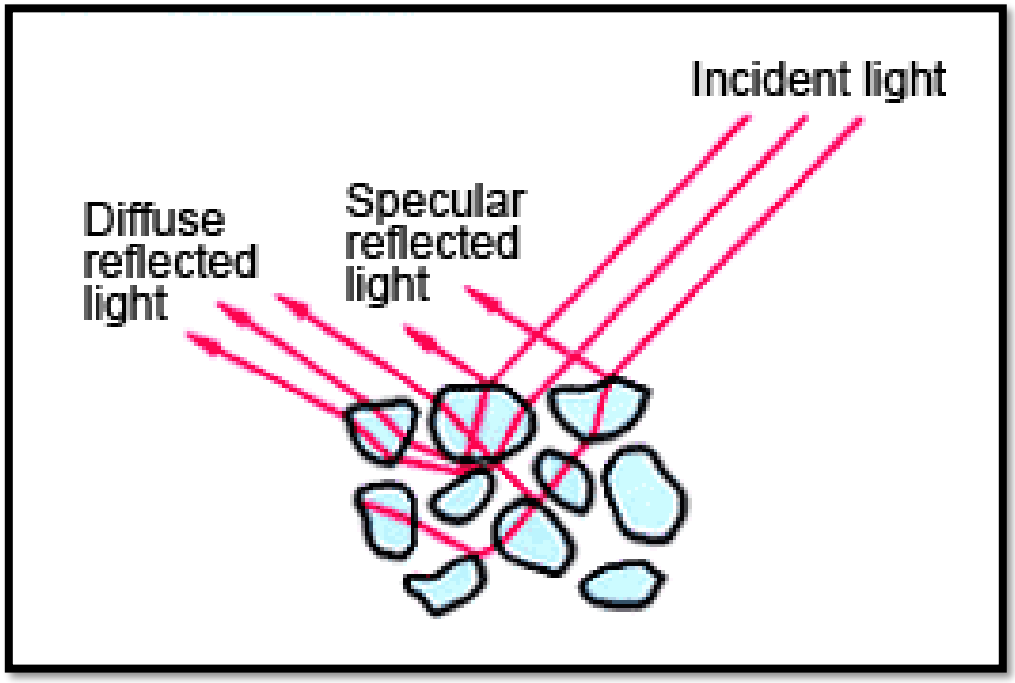

Figure 2.1 Interaction of the NIR radiation with the tablets particles arise radiation in form of specular reflection and diffuse reflectance. (Image obtained from http://www.shimadzu.com/an/ftir/support/ftirtalk/talk1/intro.html)

2.1.2 Transmittance

In NIR transmission, the radiation interacts with the tablet particles, the beam crosses the tablet and is scattered into many directions, and part of the scattered radiation reaches

the detector. **Figure 2.2** shows the radiation interacting with many particles throughout the tablet before reaching the detector. The tablet volume analyzed is higher in transmission because the detector is on the opposite side of the tablet[17]. Transmittance measurement is more complex than diffuse reflectance, and requires a complex optical configuration that implies the use of the correct filters. The spectral region is limited in transmission in comparison with diffuse reflectance where the entire spectral range is obtained.

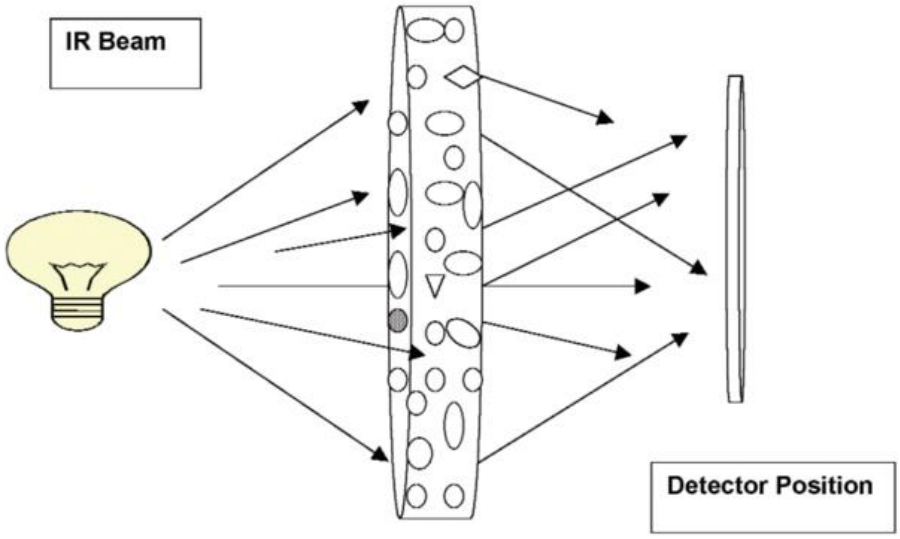

Figure 2.2 Show the transmittance measurements in a tablet.
(Image obtained from: R.J. Romañach and M.A. Santos, "Content Uniformity Testing with Near Infrared Spectroscopy", *American Pharmaceutical Review*, **2003,** 6(2), 62 – 67.)

2.2 Process Analytical Technology

NIR is one of the most used Process Analytical Technology (PAT) tools[8].The Food and Drug Administration (FDA) defines PAT *as a system for designing, analyzing, and*

controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality[18]. PAT projects have the purpose of enhancing understanding and control of the manufacturing process, and developing processes that ensure a predefined quality at the end of the manufacturing process. PAT procedures could reduce risk of quality and regulations concerns by improving efficiency. PAT also allows the use of multivariate tools for data evaluation, process analyzers, and process control tools such as NIR combined with chemometrics. NIR is now accepted in the pharmacopeias and several guidelines are recommended to develop and validate a NIR method[6]. Those guidelines are essential for the approval of the developed method by the regulatory agencies. Previous studies established recommendations at the time of developing a NIR method for quantitative purposes[6,19].

2.3 Development of a multivariate calibration model

The first step to develop a calibration consists in evaluating the spectrum of the analyte of interest in a mixture and characterize the spectral changes when the compound concentration increases or decreases[6,7]. Those spectral changes imply information and allow the determination of a specific parameter. Calibration model development will include spectra from calibration set (known concentrations) with a wider range of the API concentration[2,3]. Calibration sets need to be from different batches to confirm that they cover physical properties, moisture content, variation in concentrations, among other

factors. Concentrations in production samples are strictly established, making the available concentration ranges small. This limitation requires the preparation of samples in a laboratory that vary in the excipients and API amounts in relation to the production sample API. In this study, the analyzed productions tablets were available in six different API concentration providing an exceptional opportunity to develop a calibration model without the need to prepare tablets of different concentrations in the laboratory. Once the calibration model has been developed, it needs to be validated.

2.3.1 Validation of a calibration model

The validation of a NIR calibration model requires the use of independent production samples with a range that does not exceed the extremes of the calibration model[6]. The studies must use the validation parameters of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human (ICH) : precision, accuracy, repeatability, intermediate precision, specificity, and linearity range [1,6,9]. ICH "*make recommendations towards achieving greater harmonization in the interpretation and application of technical guidelines and requirements for pharmaceutical product registration, thereby reducing or obviating duplication of testing carried out during the research and development of new human medicines*"[20]. Two previous studies applied the ICH guidelines to validate the calibration model using diffuse reflectance NIR spectroscopy to determine the concentration of paracetamol[19] and ranitidine[6], respectively, in tablets with no significant difference between the NIR and the reference method (UV and HPLC). These studies developed the calibration model preparing laboratory samples.

2.4 Pharmaceutical manufacturing processes

2.4.1 Batch processes

Batch processes are predominant in the pharmaceutical industry. In this process, each unit operation occurs separately from each other and in most cases are performed in different rooms or buildings. Continuous operations such as dry granulation, continuous mixing, milling, feeding, and tablet compression are run in batch mode with a wall that separated each operation equipment from the next unit operation[21]. In batch process, the material is stored in a container when one unit operation finishes and transported to a different room. Incorrect handling and transportation of the material in the containers can cause segregation of the powder blend[21]. The containers in batch process need a rigorous cleaning before the next production to prevent cross contamination in accordance with the Good Manufacturing Practices [22]. By contrast, continuous manufacturing process involve an interconnection between all the unit operations.

2.4.2 Continuous manufacturing process

Continuous manufacturing is widely used in petrochemical, food, household goods, and microelectronics. Pharmaceutical industries moving from batch to continuous manufacturing process need to invest a great amount of money in research and production equipment[22]. Continuous processes do not require storage intermediate material in containers between each unit operation as in batch manufacturing, because they are

interconnected[23]. Continuous processes allow to manufacture the desired drug quantity without a performing scale up. The high quality of the drug product is assured in this process with a constant control and monitoring of each unit operation. The control and monitoring at real time for the entire process is possible to the integration of PAT tools such as NIR spectroscopy [22,23] .

Continuous manufacturing may also be applied to the organic synthesis of the API and is called continuous flow chemistry. A study from the Massachusetts Institute of Technology [24] developed a continuous flow system to produced diphenhydramine, lidocaine, diazepam, and fluoxetine. Despite the advantages of continuous manufacturing, pharmaceutical companies has been lagging in the batch process [23,25]. They are transitioning to the continuous manufacturing. In this transition, the industry is using the PAT guidance approved by the FDA and the guidance of the International Conference on Harmonization (ICH).

2.5 Tablet compression process

Pharmaceutical tablets are made by a compression process that consist of four stages: filling, metering or dosing, compression, and finally, ejection[26,27]. During the filling process, the powder blend is in the feeder. The feeder has mixing paddles that ensure a consistent flow of the powder blend into the dies. The fill chamber drags down the lower punch on the die when the punch passes under the feeder. This action causes a vacuum that pulls down the powder into the die. Following, the metering stage is the most important

stage in the tablet compression process. The tablet weight is determined volumetrically by the depth of the die cavity when the lower punch is still under the feeder. This step is known as dosing. The depth of the lower punch is controlled by the metering cam. Any powder excess is removed from the die surface. Then, compression step is follows.

The compression stage begins when the upper punch enters the die. The upper and lower punches are moved between two pressure rolls wheels. The pressure rolls push both punches at a determined pressure to form the tablet. The tablet thickness is determined in this stage by the distance between the upper and lower punch. Finally, in the ejection stage the upper punch is lifted out of the die and the lower punch is pushed up by the ejection cam. Then, the tablet is pushed out of the die cavity.

During the industrial tableting process, the powder's flow properties, the paddle's speed, and other factors cause variations in the amount of powder in the dies. This contributes to variations in the weight of the tablets and consequently the drug content diverges. Different forces will be needed to compress to an equal thickness when the tablets weight varies. Those different compression forces affect the tablet hardness, porosity, and drug release[28]. The manual tableting compression have only a minimum weight variation because the powder is weighed with accuracy in the analytical scale. However, manual tableting has more variations in the applied force rate, the dwell time, and the compression speed due to the ability and physical condition of the handler[28–30]. In this study, only production tablets were used to develop and validate the calibration model.

2.6 Segregation

Tablet properties are affected by powder material properties and powder behavior during the entire die filling process. Segregation can occur during the die filling process due to the particle's physical differences such as density, shape, or size. In particle size segregation, coarse particles come together in one area of the powder blend, while finer particles are in another area of the powder blend. This segregation causes an inhomogeneous powder blend. Computational Discrete element method (DEM) and experimental studies have shown that particle size segregation can occur due to the shoe speed[31], paddle wheel speed in the feed frame[32], and, consequently in the die filling. Mateo-Ortiz *et al*[32] study, found that smaller particles conglomerated in the bottom of the feed frame and dies, while the larger particles conglomerated in the top at a low paddle wheel speed (24 rpm). These results demonstrate particle size segregation in the feed frame and consequently in the dies. The particle size segregation was less at higher feed frame paddle wheel speed.

2.7 Shear

Shear occurs when the surface of one material slips over another causing a displacement in the direction of the surface in movement. Before tablet compaction, the powder blend experience various levels of shear intensities affected by the speed of the mixer blades and the feed frame rotation rate[33,34]. When the powder blend has a lubricant material such as magnesium stearate (MgSt), an excessive shear leads to a lubricant coating

of other particles. This effect is known as over-lubrication and affect the tablet compaction, and porosity and lead a decreased on tablet hardness and dissolution rate[33,34]

3 MATERIALS AND METHODS

3.1 Materials

The tablets used for this study were manufactured and provided by a pharmaceutical company. These tablets contain two APIs (API 1 and API 2) in its granulated form. The APIs' names are not mention in this study due to the pharmaceutical company confidentiality policy. Tablets with six different APIs concentration levels (%w/w) were provided.

3.2 Spectral Acquisition

Diffuse reflectance measurements of the tablets were performed using the integrating sphere of the Bruker MPA FT-NIR. **Figure 3.1** shows the MPA FT-NIR spectrometer. The integrating sphere within the Bruker Optics MPA FT-NIR spectrometer was used according to the spectral acquisition parameters detailed in **Table 3.1** , which describes the method as per EMA guidelines[35]. This guideline state "the key elements, principally within the NIR apparatus, which enables NIR measurement of the analyte of interest". A performance qualification test (PQ) was run before and after the spectral acquisition. The PQ test include the fallowing tests: signal to noise, 100% line, interferogram pick, energy, wavenumber accuracy, photometric reproducibility test. If the spectrometer passed the PQ test before and after the analysis, then the instrument work should have worked adequately during the analysis. Three spectra were collected at the same point (without moving the

tablet) on each side of the tablet. All tablets are placed one at a time in the center of the integrating sphere window for spectra acquisition as shown on **Figure 3.2**. **Figure 3.3a** show the 611 embossing side of the tablets and **Figure 3.3b** show the CM embossing side of the tablets. Tablet spectra were acquired at 16,32,64 cm^{-1} of resolution with 16 and 32 scans for each tablet side to determine the best resolution with a minimum time of analysis ($32cm^{-1}$ with 32 scans).

Figure 3.1 Bruker Optics MPA FT-NIR spectrometer

Table 3.1 - Spectra Acquisition Parameters	
Advance	
Resolution	32 cm^{-1}
Sample scan time	32 scans
Background scan time	254 scans
Save data from	12000 cm^{-1} – 3600 cm^{-1}
Result Spectrum	Absorbance
Data block to be saved	
Absorbance	
Single Channel	
Sample Interferogram	
Background	
Background Interferogram	
Acquisition Parameters	
Acquisition Mode	Double Sided, Forward-Backward
Correlation Mode	ON
Wanted High Frequency Limit	15000
Wanted Low Frequency Limit	0
Optic Parameters	
Aperture Setting	Open
Beamsplitter Setting	Quartz
Measurement Channel	Sphere Macrosample
Detector Setting	RT-PbS[External]
High Pass Filter	Open
Low Pass Filter	Automatic
Source Setting	High intensity NIR
Scanner Velocity	10 KHz
External Synchronization	Off
Apodization Function	Blackman-Harris 3-Term
Phase Resolution	32
Phase Correction Mode	Power Spectrum
Zerofilling Factor	2

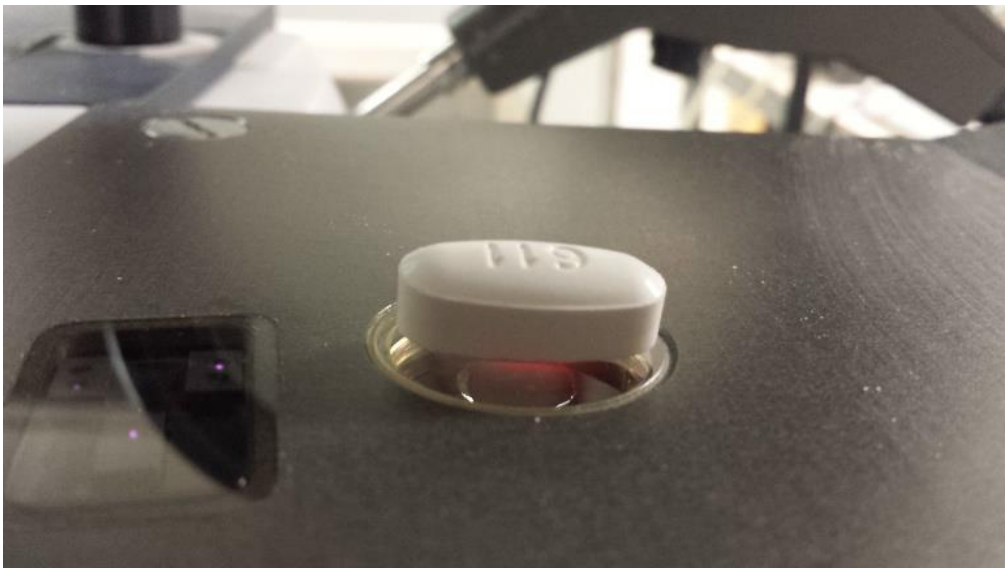

Figure 3.2 Tablet spectra acquisition illustration using the integrating sphere.

A) 

B) 

Figure 3.3 A: 611 embossing side of the tablet. B: CM embossing side of the tablets.

3.3 Calibration Set

The calibration set consisted of tablets with six API concentration levels. These tablets contain two APIs (API 1 and API 2) in its granulated form and were manufactured

by a batch process in a pharmaceutical industry. The API 2 was the only API analyzed in this study. Forty tablets were used per concentration level ranging from 54.57 %w/w to 68.81%w/w of the API 2 to be quantified. **Table 3.2** shows the composition of batch process tablets calibration set. **Table 3.3** shows the differences of the tablets in weight, width, thickness, and length between the six concentration levels. Other tablets properties such as hardness were not possible to obtain from the pharmaceutical company for all the six concentration levels. **Table 3.4** shows the API content by formulation, and the number of spectra along with the number of tablets from each formulation used in the calibration set. **Table 3.4** also shows the number of spectra used in the calibration set for the calibration model development. The calibration model was developed with this information of the batch process tablets calibration set.

Table 3.2 Composition of tablets from the batch processes used for the calibration set.

Batch Process Tablets Calibration Set		
API 1(mg) / API 2 (mg)	**API 2 % (w/w)**	**Number of tablets**
150/500	54.57	40
150/850	60.76	
150/1000	62.27	
50/500	65.48	
50/850	68.20	
50/1000	68.81	

Table 3.3 Physical differences between the six concentration levels.

Batch Process Tablets Calibration Set Physical Differences				
API 1 (mg)/API 2 (mg)	**Weight (g)**	**Thickness (mm)**	**Length (mm)**	**Width (mm)**
150/500	0.943	6.977	18.296	9.158
150/850	1.435	8.227	21.088	10.533
150/1000	1.650	8.682	22.084	11.028
50/500	0.789	6.681	17.205	8.642
50/850	1.280	7.946	18.597	10.130
50/1000	1.489	8.397	21.407	10.701

Table 3.4 Description of tablets used in calibration set

Calibration Set					
Tablet Formulation API 1(mg)/API 2(mg)	**API 2 %(w/w)**	**Number of tablets**	**Number of spectra per tablet**	**Number of spectra**	**Total number of spectra**
150/500	54.57	40	6	240	1,440
150/850	60.76				
150/1000	62.27				
50/500	65.48				
50/850	68.20				
50/1000	68.81				

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3.4 Test Set

3.4.1 Test Set #1 - Batch process tablets

Test Set #1 was composed of batch process tablets. These tablets are not included in the calibration set, but they are obtained from the same lot of batch process tablets used for calibration. This test set provides the first "test" or check of the validity of the models.

The formulation of 150/1000 (mg/mg) was selected as the test set because the API concentration is centrally located between the other API concentrations in the formulations. This formulation is also the most frequently produced formulation of this drug. Sixty tablets were used for the test set #1. Six spectra per tablet were acquired for a total number of 360 spectra for the test set #1. A large number of tablets in the test set provides a good estimate of the drug concentration even if they have variations in the drug concentration.

3.4.2 Test Set #2 - Continuous process tablets

This second test set consists of tablets manufactured in a continuous process by the same pharmaceutical industry. These continuous process tablets were manufactured for 150mg/1000mg strength. The calibration set contains tablets of this strength, but manufactured with a batch process. Fifteen tablets were used for the test set #2. Six spectra per tablet were acquired for a total number of ninety spectra for the test set #2. This test set was used to evaluate the NIR calibration model developed with the batch process tablets calibration set.

3.5 Chemometric multivariate data analysis and spectral pre-treatments

The software SIMCA-P (Umetrics Multivariate Data Analysis Software, version 14.1) was used to develop the NIR calibration model and analyze the NIR tablets spectral

data. Principal component analysis (PCA) was used to decompose the original tablets spectral data (matrix **X**) to detect the maximum variation between the tablets spectra. Furthermore, Partial least squares regression (PLS) was used to develop the NIR calibration model. PLS establishes a linear relation between the spectral data of the tablets (matrix **X**) and the reference drug content (%w/w) values (matrix **Y**). Mathematical data pre-treatments were applied to the tablets spectral data. Mathematical data pre-treatments help to reduce radiation path lengths, light scattering, instrumental noise, and other interferences in the NIR spectral data. Standard normal variate (SNV) reduce the scattering multiplicative effects. The first derivative reduce baseline differences and the second derivative reduce slope differences and the physical information. SNV, first and second derivative were used in this study.

3.6 Evaluation of NIR calibration model

The Root Mean Square Error of Prediction (RMSEP) (%w/w), the Relative Standard Error of Prediction (RSEP) (%), and Bias (%w/w) were calculated to evaluate the predictive capability of the calibration models. The equations of RMSEP, RSEP (%), and Bias (%w/w) are the following:

$$\boldsymbol{RMSEP} = \sqrt{\sum_{i=1}^{N_p}(\hat{y}_i - y_i)^2 / N_p} \qquad \textbf{Equation 3.1}$$

$$\boldsymbol{RSEP}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(\hat{y}_i - y_i)^2}{\sum_{i=1}^{n}(y_i)^2}} * \mathbf{100} \qquad \textbf{Equation 3.2}$$

$$\boldsymbol{Bias} = \frac{\sum_{i=1}^{n}(\hat{y}-y)}{N} \qquad \textbf{Equation 3.3}$$

Where $\hat{y}_i$ is the predicted concentration by the NIR; y_i is the reference concentration; and n_p is the number of samples in the test set. These measurements evaluated the accuracy of the PLS calibration model.

Precision of the PLS calibration model is one of the factor used to evaluate the model according to the ICH guidelines. ICH Q2(R1)[9] describes three (3) different types of precision studies: repeatability, intermediate precision, and reproducibility. Repeatability studies were designed to evaluate short-term instrument precision. Repeatability expresses the precision under the same operating conditions over a short interval of time (ICH Q2(R1)[9]). Three tablets were analyzed. A total of six (6) consecutive spectra were collected on both sides of the tablet, for a total of twelve (12) spectra for each tablet. The standard deviation was calculated for each side of the tablet individually.

According to ICH Q2(R1)[9], intermediate precision portrays within these laboratories variations: different days, analysts, equipment, etc. In this study, intermediate precision refers to spectra obtained from the same tablet on both sides. One spectrum was acquired on the CM side. Then, the tablet was removed from the sphere and placed again to acquire another one spectrum for the CM side. This was repeated six times for a total of six spectra of the tablet. The same procedure was then performed on the 611 side of the tablets. The standard deviation was calculated for each side of the tablets.

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage (ICH Q2(R1)[9]). Robustness was evaluated using spectral data varying different spectral acquisition settings. The noise spectrum (NS) was obtained in the absence of sample and added to the test set to incorporate this variation. The NS was multiplied by 2 and 3 to increase the noise, and both resulting spectra were added to the test set to introduce small variations and tested with the proposed model.

The linearity of the model was evaluated through leave-class-out-cross-validation. During leave class-out cross validation the model will leave out one of the six concentrations of the calibration set and then predict it with the others 5 concentrations of the calibration set. Leave class-out cross validation was required since the test set only includes one concentration. The R^2 was calculated to evaluate the linearity of the model. Furthermore, According to ICH Q2(R1)[9], specificity is the ability to assess unequivocally the analyte in the presence of other components. The specificity was determined using linearity studies which show that the calibration model responds to changes when API concentration is increased or decreased.

4 RESULTS AND DISCUSSION

4.1 Spectral resolution and acquisition time

The pharmaceutical company that sponsored this study has a method for obtaining transmission spectra at three locations of the tablets with a special holder for each side. Thick tablets are analyzed by transmission and a small amount of radiation reaches the detector and a high noise in observed in the spectra as shown in **Figure 4.1** The transmission mode required approximately eight minutes to acquire spectra at the three locations of the tablets. The diffuse reflectance method in this study requires approximately one minute. Diffuse reflectance was selected for this study using the established acquisition parameters to obtain all sample spectra. Tablet spectra resolution was evaluated and acquisition time was measured at different resolutions and scans.

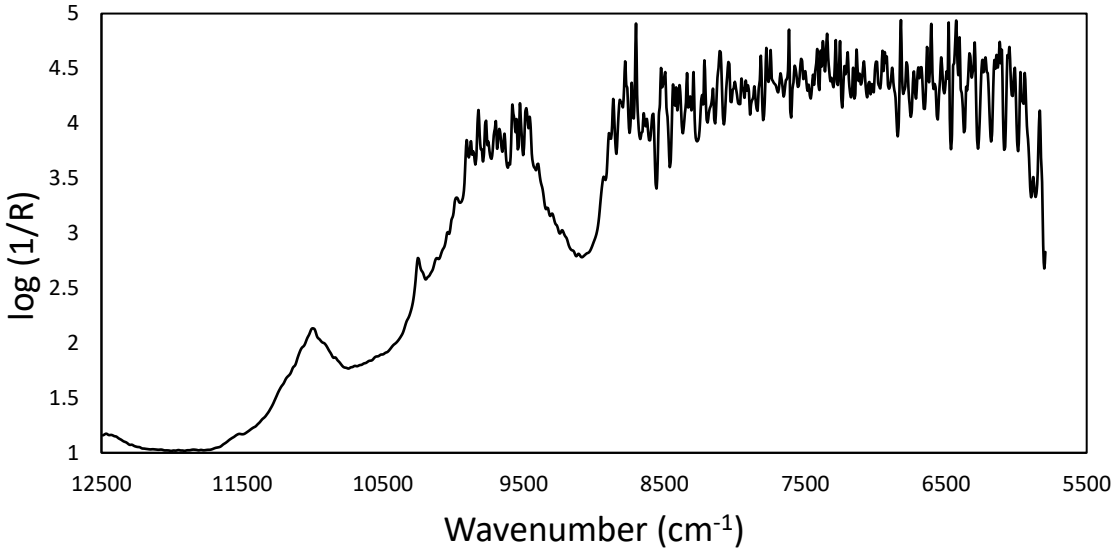

Figure 4.1 Transmission spectrum of 150/1000 mg tablet.

The tablet spectra were acquired at 16, 32, and 64 cm^{-1} of resolution with 16 and 32 scans. The highest resolution used was 8 cm^{-1} but more spectral noise was observed, and the time needed to obtain a spectrum increased. At a resolution of 16 cm^{-1}, the spectra do not show a significant difference when compared to the spectra obtained with the resolution of 8 cm^{-1}. Each spectrum, using a resolution of 8 cm^{-1} took 10.24 seconds with 32 scans. A resolution of 32 cm^{-1} shows well defined spectra bands and each spectrum took 7.45 seconds with 32 scans. When lowering the resolution to 64 cm^{-1} (5.82 seconds at 32 scans) some of the bands started to disappear. For this reason, the optimum resolution chosen is 32cm^{-1}. **Figure 4.2** shows spectra at resolutions of 8, 16, 32, and 64 cm^{-1}. **Table 4.1** shows these results. A larger number of scans improve the signal to noise ratio in the spectra, but it requires more time to acquire the spectra. The signal to noise ratio in a FT-spectrometer increases of about $\sqrt{N}$, where N is the numbers of scans[36]. The spectral acquisition time was measured to determine the analysis time for each resolution taking 16 and 32 scans. The spectral acquisition time was measured because the pharmaceutical company desired a faster method than transmission. It was determined, that having a better signal to noise ratio with 32 scans takes only 3.79 seconds more than 16 scans. A resolution of 32 cm^{-1} with 32 scans were selected as the optimum spectral acquisition parameters. The spectral acquisition times are much lower than those which would be required for a transmission study.

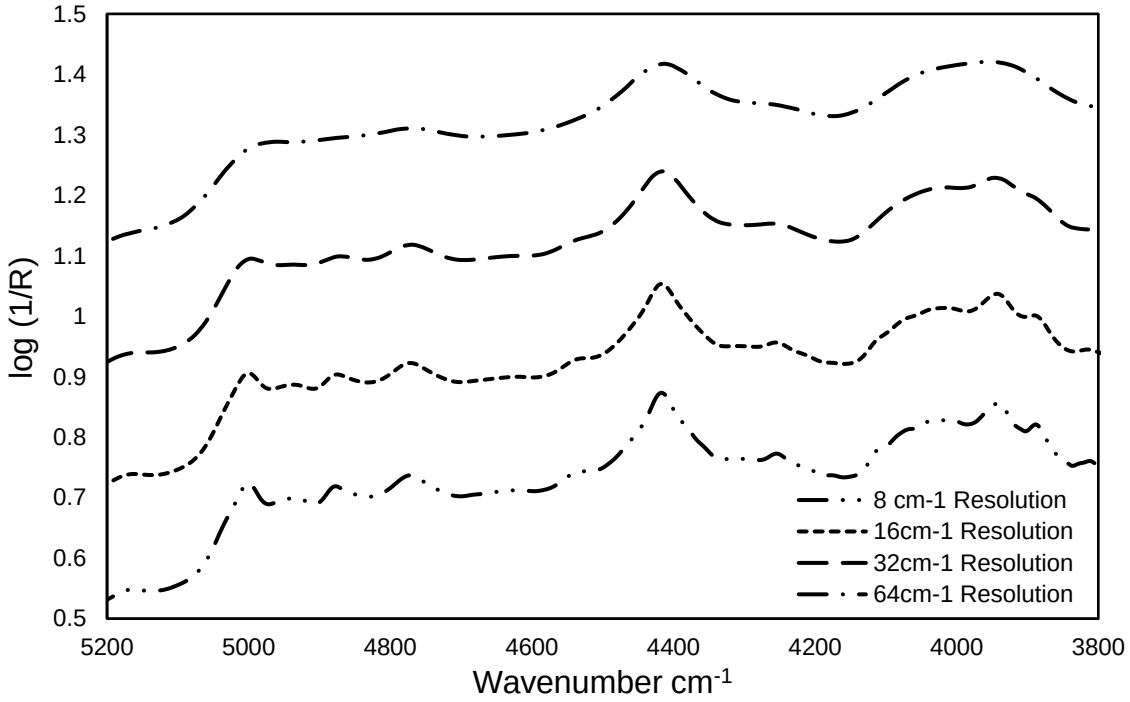

Figure 4.2 . A comparison between spectra of the same tablet taken at spectral resolution at 8, 16, 32, and 64 cm^{-1} at 32 scans.

Table 4.1 Spectral time acquisition between different resolutions (16cm^{-1}, 32cm^{-1}, and 64 cm^{-1}) with 16 and 32 scans.

Resolution	Tablet	Acquisition time (s)			
		611 embossing		CM embossing	
		16 scans	32 scans	16 scans	32 scans
16 cm-1	1	5.20	10.07	5.23	10.09
	2	4.83	10.44	5.15	10.21
	3	5.15	10.27	4.73	10.37
	Average	5.06	10.26	5.04	10.22
Resolution	**Tablet**	**611 embossing**		**CM embossing**	
		16 scans	**32 scans**	**16 scans**	**32 scans**
32 cm^{-1}	1	3.71	7.56	3.71	7.38
	2	3.69	7.32	3.70	7.38
	3	3.57	7.39	3.56	7.69
	Average	3.66	7.42	3.66	7.48
Resolution	**Tablet**	**611 embossing**		**CM embossing**	
		16 scans	**32 scans**	**16 scans**	**32 scans**
64 cm^{-1}	1	3.11	5.52	3.15	5.79
	2	3.04	6.00	3.06	5.89
	3	3.28	6.00	3.33	5.71
	Average	3.14	5.84	3.18	5.80

4.2 Development of Calibration Set – Evaluation of Spectral Changes

The spectra of tablets of different concentrations were carefully observed before developing the calibration model. **Figure 4.3** shows the tablet spectra with three different concentrations of API 54.57, 62.27, and 68.81 (%w/w). **Figure 4.4** shows the pure API spectrum and the tablet spectrum with the lower API concentration. The spectral region 10,028.7-5,508.04 cm^{-1} correspond to the API and granules components, and it was used

to develop the PLS calibration models. This selection was based in the spectral bands that are present in both: the pure API and the tablet spectra with the lower API concentration.

Note in the **Figure 4.3** that the tablet spectrum with a higher baseline does not have the higher API concentration and the one with lower baseline does not have the higher concentration. The baseline changed every time that the tablet was placed over the integrating sphere, since the tablet never was placed again in the exactly position, causing difference in the baseline. Also, the radiation does not interact with the particles of the tablets in the same manner as the first time due to the light scattering.

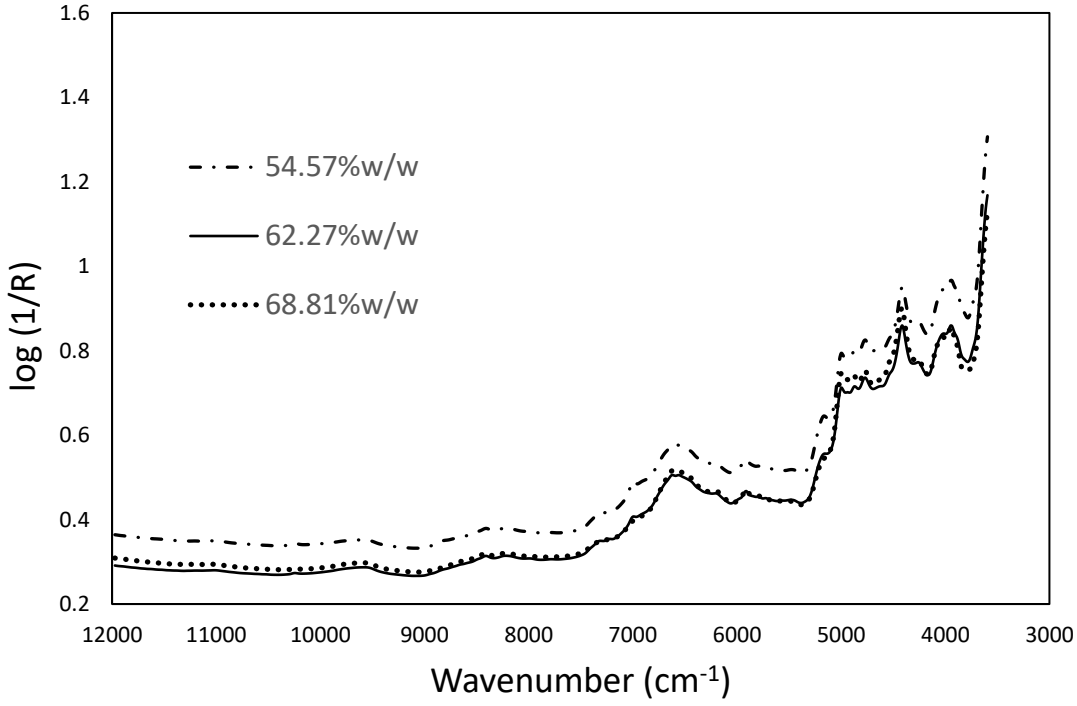

Figure 4.3 Tablet spectra with three different concentrations of API 54.57, 62.27, and 68.81%w/w.

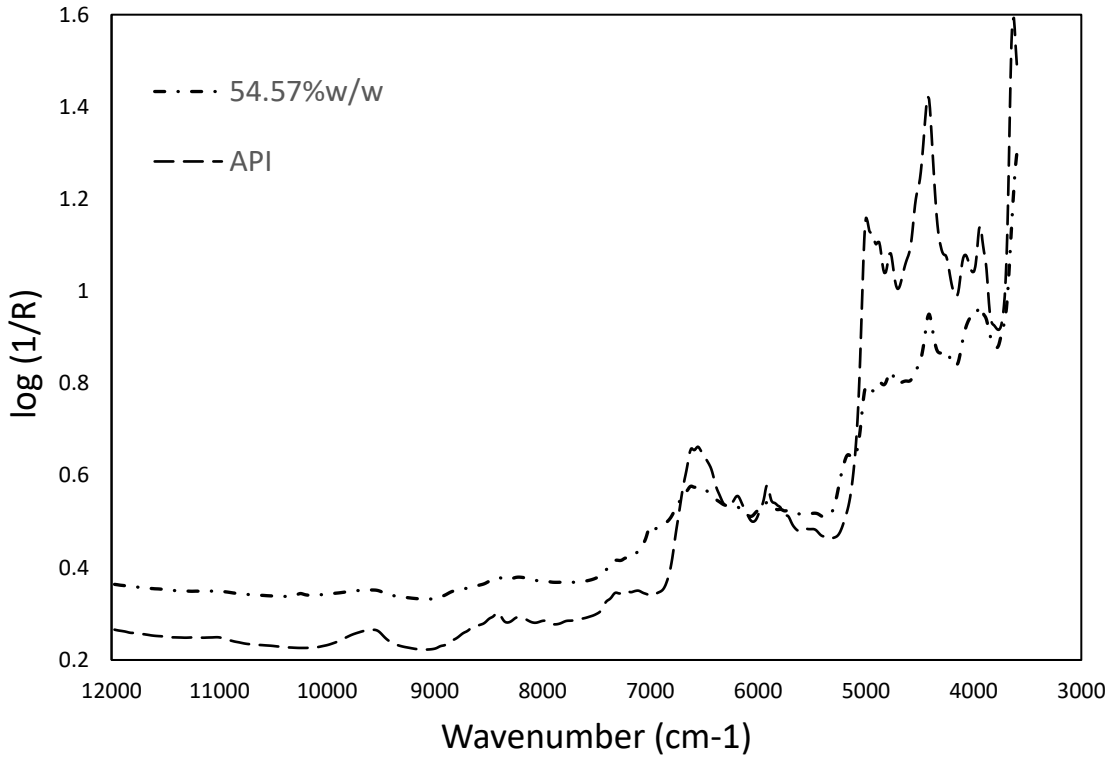

Figure 4.4. Pure API spectrum and the tablet spectrum with the lower API concentration

4.3 Development of the NIR calibration model

Different pretreatments were used to develop the three PLS calibration models: Standard Normal Variate (SNV) with first derivative (15points), SNV with second derivative (15points), and only SNV. These data pre-treatment were evaluated using Principal Components Analysis. The pre-treatment that explain the maximum variance in the batch process tablets calibration set was selected. **Table 4.2** shows the results for these pre-treatments. SNV +2 Derivative (15 points) was the pre-treatment that best explain the variance. The PC-1 explains 94% of the total variance and is related with the API

concentration in the tablets. **Figure 4.5** show the PCA scores plots for the SNV + 2 Derivative (15 points) pre-treatments. The tablet scores are grouped in ascending order of API concentration from the left to the right. **Figure 4.6** shows the SNV + 2 Derivative (15 points) tablet spectra with three different concentrations of API 54.57, 62.27, and 68.81%w/w.

Table 4.2 Explained variance of the first and second principal component (PC) for each used pre-treatment in the calibration set data.

Pre-treatment at 10,028cm^{-1} to 5,508 cm^{-1}	**Explained variance**	
	PC-1	**PC-2**
SNV	80%	9%
SNV + 1st Derivative (15 points)	91%	4%
SNV + 2nd Derivative (15 points)	94%	3%

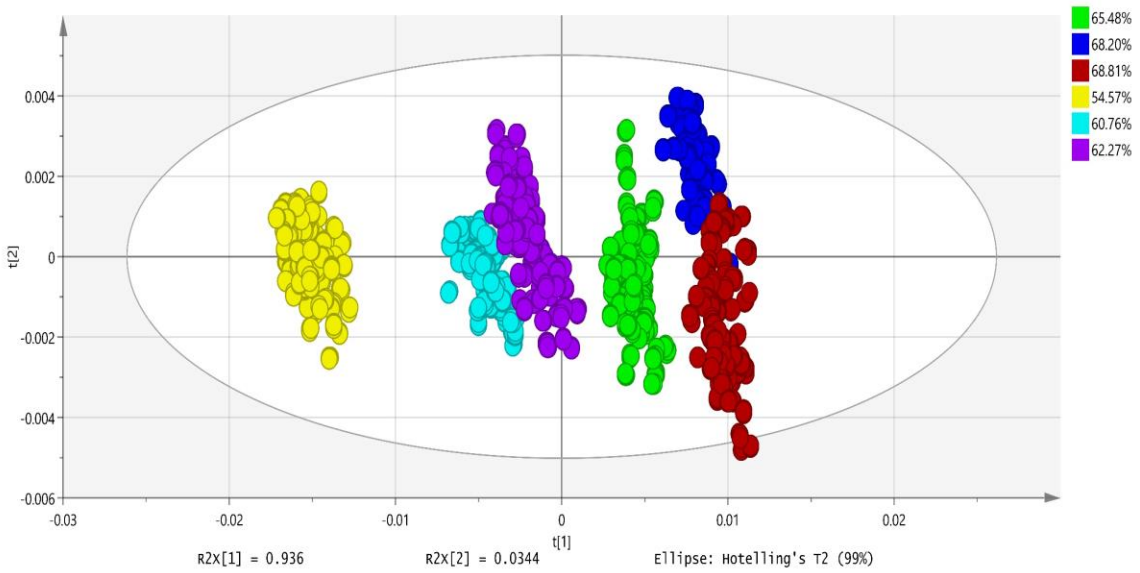

Figure 4.5 PCA scores plot for the tablets calibration set. PC-1 explain 94% variation in the data and grouped according to ascending API concentration.

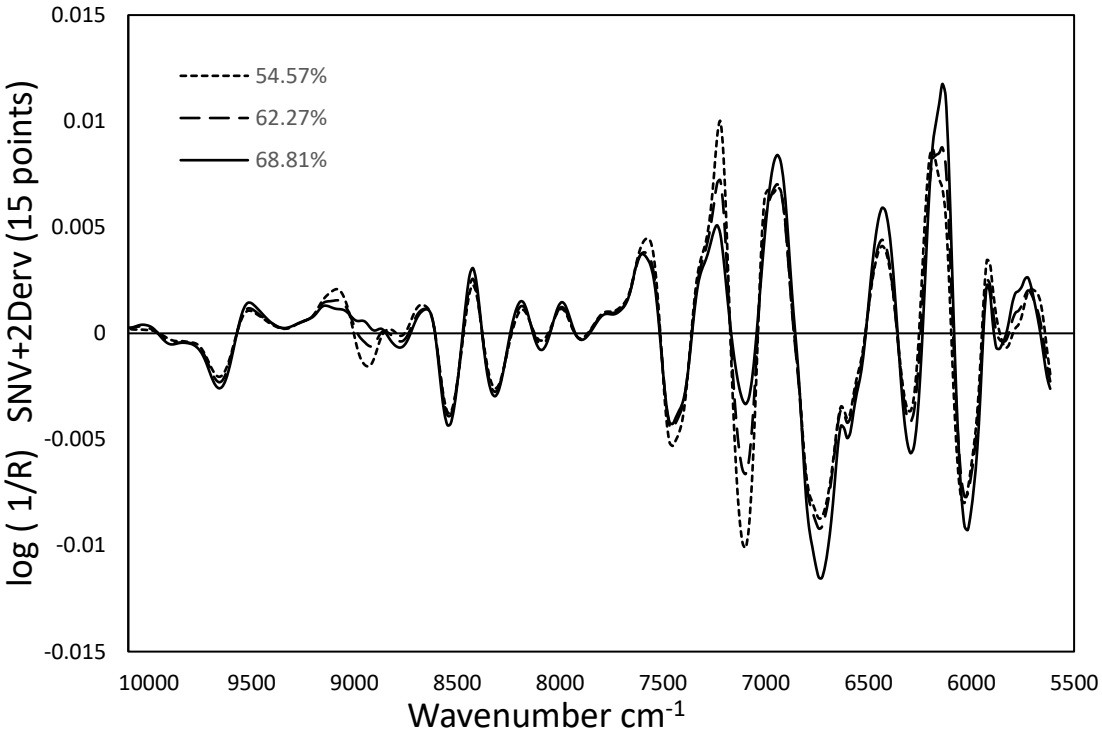

Figure 4.6 The SNV + 2 Derivative (15 points) tablet spectra with three different concentrations of API 54.57, 62.27, and 68.81%w/w.

To check the validity of these models, the test set #1 (batch process tablets) with a 62.27%(w/w) of API was used. **Table 4.3** shows the overall evaluation of models' results based on the tablets test set #1 and describes the prediction of the entire test set (n = 360). One of the novel aspects of this research is that the tablets are not been analyzed by HPLC and the nominal values were used. This approach could be very useful in the industry during development efforts for multiple formulations with different API concentration and when the time is limited. The Root Mean Square Error of Prediction (%w/w) (RMSEP), the Relative Standard Error of Prediction (%) (RSEP), and Bias (%w/w) were calculated to evaluate the predictions error of those calibration model .

The lowest overall RMSEP was obtained with Model M5 using SNV+ 2^{nd} Derivative (15points) pretreatment in a spectral range of 10,028.7-5,508.04 cm^{-1} using three PLS factors which provided the best predictions of the test set #1 (Refer to **Table 4.3**). The selected model SNV + 2nd Derivative (M5) with three PLS factors was chosen to determine the API concentration in tablets. The model M5 was evaluated using the ICH guidelines in terms of accuracy and range, precision (repeatability and intermediate), and linearity of prediction.

RMSEP and bias were used to evaluate accuracy based on the determination of sixty tablets that constitute the test set #1. The bias obtained for the sixty test set predicted tablets is 0.120% (w/w)**.** The absence of systematic error would provide a bias of zero (0). However, there is always some systematic error in analytical methods due to sampling errors. The relative higher bias obtained in the models is due to the differences in

compression forces, hardness, weight, width, thickness, length and embossing number between each of the six concentration levels of the tablets (Refer **Table 3.3** to see some of these differences). The RMSEP (%w/w) and RSEP (%) indicate how the individual predictions vary from the concentration level (reference) values. Despite the relative higher bias due to the complex tablet physical variation between the six concentration levels, the model shows good prediction capability with a low prediction error. The RMSEP is 0.380 % (w/w) and the RSEP is 0.611%. The model M5 shows good performance in terms of accuracy. The prediction error is low (0.380 %w/w) and the bias, close to zero (0.120 %w/w). The precision of the PLS calibration model is another factor used to evaluate the model according to the ICH guidelines.

Table 4.3 Prediction of Test Set 1 by the NIR calibration models developed with different pre-treatments.

Model ID	Pre-treatment	PLS Factors	RMSEP (% w/w)	RSEP (%)	Bias (% w/w)	Repeatability Std Dev. % (w/w)
M2	SNV + 1st Derivative (15points)	1	1.136	1.824	0.621	0.010
		2	1.061	1.704	1.005	0.013
		3	0.596	0.956	0.486	0.014
		4	0.527	0.846	0.394	0.014
		5	0.528	0.848	0.420	0.016
		6	0.318	0.511	0.061	0.023
M3	SNV	1	1.872	3.007	1.640	0.014
		2	1.281	2.058	1.028	0.011
		3	0.881	1.415	0.798	0.014
		4	0.444	0.713	0.259	0.010
		5	0.565	0.907	0.449	0.010
		6	0.582	0.935	0.485	0.011

Model ID	Pre-treatment	PLS Factors	RMSEP (% w/w)	RSEP (%)	Bias (% w/w)	Repeatability Std Dev. % (w/w)
M5	SNV + 2nd Derivative (15points)	1	0.757	1.215	0.304	0.016
		2	0.644	1.034	0.515	0.019
		3	0.380	0.611	0.120	0.019
		4	0.394	0.632	0.222	0.020
		5	0.305	0.490	-0.073	0.045
		6	0.296	0.475	-0.006	0.050

4.4 Validation of the NIR calibration model

4.4.1 Validation of the NIR calibration model using test set #1-batch process tablets

Repeatability, intermediate precision, and reproducibility were performed since these precision studies are described by the ICH Q2(R1)[9] guidelines. Repeatability studies evaluate short-term instrument precision. Repeatability is also termed intra-assay precision. Six consecutive spectra were acquired for each side of a tablet for a total of twelve spectra per tablet. **Table 4.4** summarizes the repeatability results for each side of the tablet individually; the two different sides of the tablet were embossed with 611 and CM. The repeatability studies varied from 0.014%(w/w), at the lowest, to 0.021%(w/w), at the highest, demonstrating the high precision of the model. The repeatability study was followed by intermediate precision studies.

Table 4.4 Precision: NIR Calibration model repeatability predictions for test set #1.

Precision: Repeatability				
Tablet	**Tablet Side**	**Reference Values (%w/w)**	**Predicted Values (%w/w)**	**Repeatability Standard Deviation (%w/w)**
1	611	62.270	62.338	0.021
	611	62.270	62.325	
	611	62.270	62.301	
	611	62.270	62.317	
	611	62.270	62.314	
	611	62.270	62.279	
1	CM	62.270	62.546	0.018
	CM	62.270	62.569	
	CM	62.270	62.573	
	CM	62.270	62.592	
	CM	62.270	62.575	
	CM	62.270	62.546	
2	611	62.270	62.778	0.021
	611	62.270	62.794	
	611	62.270	62.799	
	611	62.270	62.782	
	611	62.270	62.778	
	611	62.270	62.740	
2	CM	62.270	62.391	0.020
	CM	62.270	62.383	
	CM	62.270	62.379	
	CM	62.270	62.355	
	CM	62.270	62.349	
	CM	62.270	62.344	
3	611	62.270	62.314	0.014
	611	62.270	62.315	
	611	62.270	62.290	
	611	62.270	62.281	
	611	62.270	62.305	
	611	62.270	62.300	

Precision: Repeatability				
Tablet	**Tablet Side**	**Reference Values (%w/w)**	**Predicted Values (%w/w)**	**Repeatability Standard Deviation (%w/w)**
3	CM	62.270	62.220	0.021
	CM	62.270	62.192	
	CM	62.270	62.173	
	CM	62.270	62.214	
	CM	62.270	62.170	
	CM	62.270	62.177	

The intermediate precision studies take into consideration the heterogeneity of the tablets produced. During the intermediate precision studies, one side of the tablet was placed over the integrating sphere to acquire one spectrum. Then, the tablet was removed and placed again over the sphere to acquire one spectrum on the same side of the tablet. This procedure was repeated six times for a total of six spectra for that side of the tablet. The same procedure was then performed on the other tablet side. The standard deviation of the spectra was calculated from each tablet side. **Table 4.5** shows the intermediate precision results for the test set #1**.** Intermediate precision results show some variation of the API concentration throughout the tablets. The variation in the prediction of a tablet ranges from 0.008 to 0.045 % (w/w) and is not considered significant. Furthermore, the calibration model robustness was evaluated.

Table 4.5 . Intermediate Precision of the NIR Calibration model for the prediction of test set #1.

Intermediate Precision: Standard Deviation per concentration level			
Tablet	**Tablet Side**	**Predicted Values (%w/w)**	**Repeatability Standard Deviation (%w/w)**
1	611	62.259	0.018
	611	62.221	
	611	62.209	
	611	62.231	
	611	62.212	
	611	62.221	
1	CM	62.146	0.008
	CM	62.130	
	CM	62.121	
	CM	62.133	
	CM	62.130	
	CM	62.127	
2	611	62.444	0.044
	611	62.518	
	611	62.447	
	611	62.419	
	611	62.427	
	611	62.514	
2	CM	62.371	0.045
	CM	62.354	
	CM	62.350	
	CM	62.441	
	CM	62.310	
	CM	62.330	
3	611	62.069	0.030
	611	62.045	
	611	62.058	
	611	62.014	
	611	62.007	
	611	61.995	

Intermediate Precision: Standard Deviation per concentration level			
Tablet	**Tablet Side**	**Predicted Values (%w/w)**	**Repeatability Standard Deviation (%w/w)**
3	CM	62.080	0.034
	CM	62.171	
	CM	62.108	
	CM	62.077	
	CM	62.101	
	CM	62.117	

For the robustness study, the noise spectrum (NS) was obtained in the absence of sample with the integrating sphere aperture closed by the instrument. This sample spectrum only contains the noise of the MPA instrument because the radiation does not escape from the inside of the instrument. Thus, a flat line at 0 would be observed if there was no noise. However, there is always some spectral noise. This NS was added to the batch process test set to incorporate this variation. The NS was multiplied by 2 and 3 to increase the noise, and both resulting spectra were added to the test set to introduce small variations and tested with the proposed model. **Figure 4.7** show the measurement of instrument noise spectra. **Table 4.6** shows that the model maintains a good predictive ability after the addition of noise. Spectral noise added to the proposed model indicates robustness in this circumstance. The model is robust because it has a good prediction capability despite variations in compression forces, hardness, weight, width, thickness, length and embossing between each of the six concentration levels of the calibration set. The linearity was also evaluated for the calibration model.

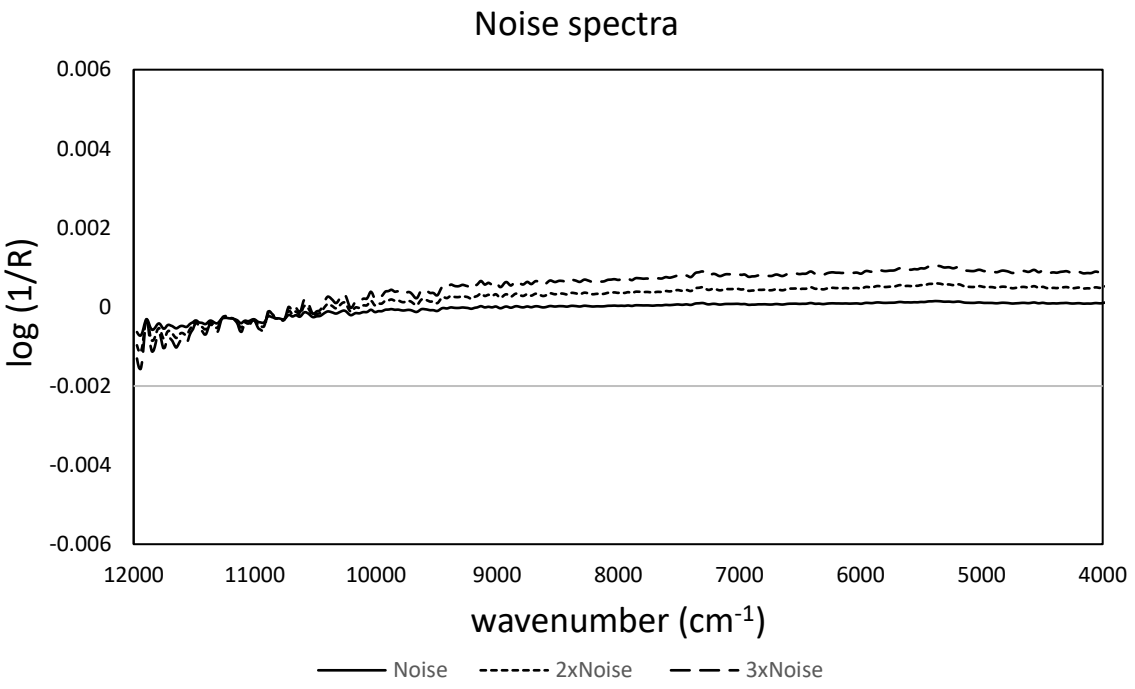

Figure 4.7 Instrument Noise Spectra

Table 4.6 Predicted drug concentration values after addition of noise spectra.

Predicted Conc. %(w/w)	Noise (%w/w)	Residual (%w/w)	2 x Noise (%w/w)	Residual (%w/w)	3 x Noise (%w/w)	Residual (%w/w)
62.624	62.615	0.009	62.606	0.018	62.597	0.027
62.648	62.639	0.009	62.630	0.018	62.620	0.028
62.193	62.184	0.009	62.175	0.018	62.166	0.027

The linearity of the model was evaluated through leave-class-out-cross-validation. Leave class-out cross validation will leave out one of the six concentrations of the calibration set and then predict it with the others five concentrations of the calibration set.

Leave class-out cross validation was required since the test set only includes one concentration. Linearity plots are illustrated for average values predicted for the API for the model developed SNV + 2nd Derivative (model M5). **Figure 4.8** shows good linearity of prediction for the API (R^2 =0.999). Also, **Figure 4.8** shows the calibration model specificity where the model responds linearly as the API concentration increases.

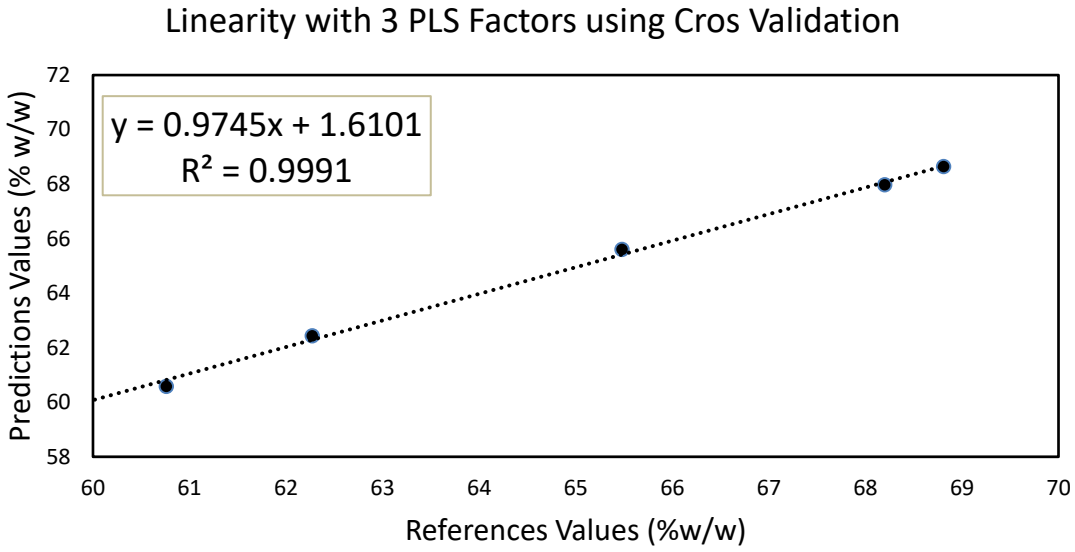

Figure 4.8 Linearity of predictions with 3 PLS Factors using Crossvalidation

The PLS calibration model M5 developed using production tablets manufactured by batch process with six different formulations demonstrate an excellent ability to predict one particular API concentration in production tablets that contains two APIs without the need of prepare laboratory samples. The M5 model results accomplish all the evaluation parameters according with the ICH Q2(R1)[9] guidelines.

4.4.2 Prediction the test set #2-continuous process tablets

This is the first study that quantifies drug content of continuous manufacturing production tablets from a pharmaceutical industry continuous line using a PLS model developed from batch production tablets. Test set #2 consists of tablets manufactured by a continuous process with the same formulation (150mg/1000mg) of the test set #1. The NIR spectral acquisition parameters for the test set #2 are the same used for the batch process tablet calibration set and test set #1. The pre-treatments SNV, SNV+1 derivative (15 points), and SNV+2 derivative (15 points) were evaluated as with the batch process tablets. Principal Components Analysis (PCA) was performed with the test set #2 and the pretreatment that explained the maximum variance in the PCA was selected. **Table 4.7** shows the results for these pre-treatments. PCA with SNV+2 derivative (15 points) pre-treatment explain the maximum variance, as observed with the batch process tablets.

Table 4.7 Explained variance of the first and second principal component (PC) for each used pre-treatment in the test set #2 data.

Pre-treatment at 10,028cm^{-1} to 5,508 cm^{-1}	**Explained variance**	
	PC-1	**PC-2**
SNV	72%	21%
SNV + 1st Derivative (15 points)	82%	16%
SNV + 2nd Derivative (15 points)	86%	13%

The first principal component (PC1) explains 86% (**Figure 4.9**) of the total variance. PCA scores are grouped by the tablet sides embossing (see **Figure 3.3**). The 611 side scores are grouped separately from the CM side scores. This indicates differences in the spectra of the side with the embossing 611 and the side with the CM embossing. The 611 side

spectra of the continuous process tablets (test set #2) is very similar to the spectra of batch process tablets (**Figure 4.10**). The CM side spectra of the test set #2 show a slightly higher base line from the spectra of the batch process tablets and the 611 side spectra of the test set #2. The spectra of the CM side show a more intensive band corresponding to the API at approx. 6,500cm^{-1} compared with the other tablet side spectra. This difference could be possible to process variation such as material segregation and shear stress (Refer to **section 2.6-2.7**).

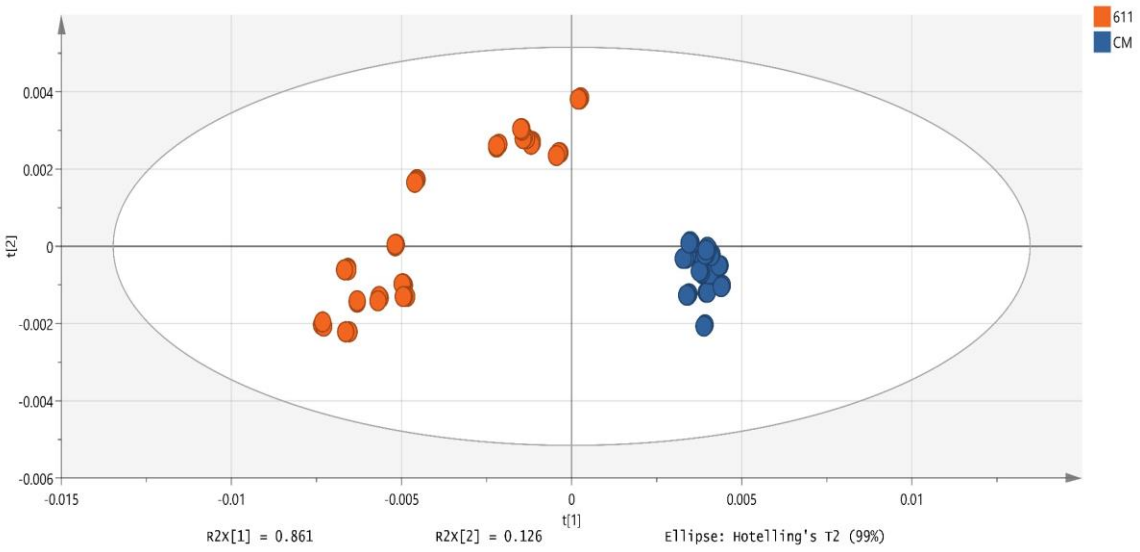

Figure 4.9 Principal Component Analysis scores plot of test set #2. The scores are grouped by the tablet sides embossing. The orange scores correspond to the 611 side of the tablets and the blue scores correspond to the CM side of the tablets.

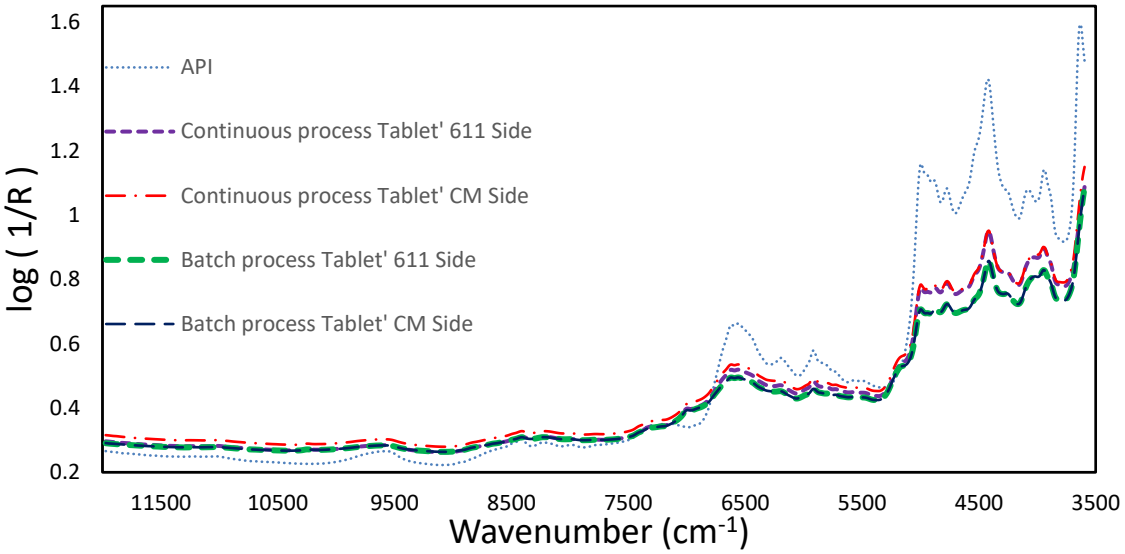

Figure 4.10 NIR diffuse reflectance spectra of pure API and spectra of the CM and 611 sides of the batch and continuous process tablets (test set #2).

The continuous and batch process tablets spectra were projected into the PCA model developed with the calibration set tablets (**Figure 4.11 A**). PC-1 explains 93% of the maximum variance and the score plots are grouped from the lower to the highest API concentration (left to right). The scores of the test set #2 tablets are divided in two groups, the CM side and the 611 side. The 611 side scores of test set #2 tablets are grouped with the batch tablets scores of the same 62.27% (w/w) drug content. The CM side scores of the test set #2 tablets are grouped with the batch tablets scores of 65.48% (w/w) drug content. CM side scores of the test set #2 tablets has higher API content that the 611 side. This indicate that a variation could occurred in one or more unit operation during the continuous manufacturing process of this tablets. This explains the higher intensity band in the spectra at approx. 6500cm^{-1}. **Figure 4.12** shows that CM side spectra of the test set #2 tablets is similar with the batch process tablet with 65.48%(w/w) of the API that is the concentration

that follows the 62.27%(w/w) batch process tablets. The batch process tablets of 65.48%(w/w) do not have the same API concentration that the tablets (62.27%w/w) and are different in weight, thickness, width, and length (Refer to **Table 3.2-3.4**).

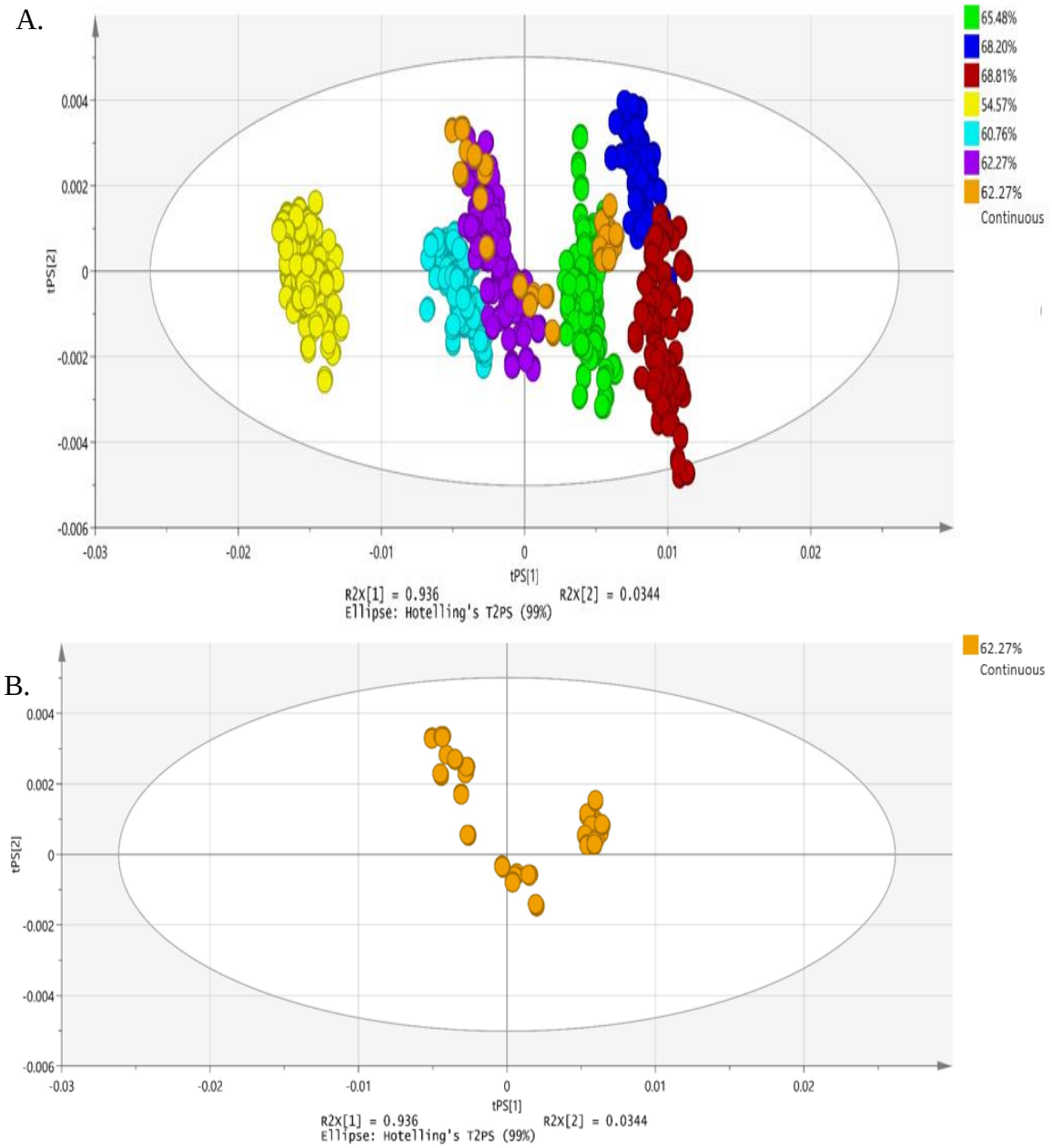

Figure 4.11 A. PCA model projecting the scores plot of the continuous tablets (test set #2) (brown color) and the scores plot of the batch process tablets. PC-1 correlated with the API content in tablets. The 611 scores of the test set #2 are grouped with the batch scores of 62.27%(w/w) API (violet color). The CM side score of the test set #2 are grouped with the batch scores of 65.48% (w/w) API (green color) **B**. The same PCA model projecting only the scores plot of the test set #2 (brown color).

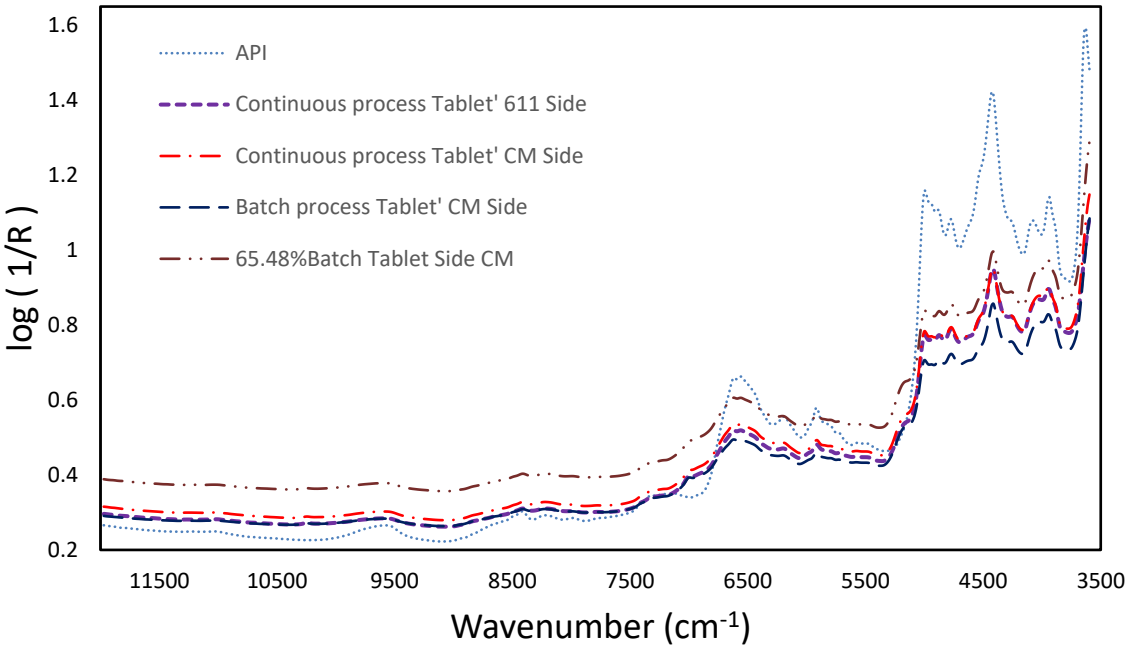

Figure 4.12 NIR diffuse reflectance spectra of the tablets sides. The spectra of batch tablet with 65.48%(w/w) of API is has a similar API band intensity at 6,500cm^{-1} with the CM side of the continuous process (test set #2) tablet.

The PLS calibration model developed from batch process tablets was used to quantify the API content of the continuous process tablets in test set #2. Five PLS factors were selected based on the evaluation the RMSEP(%w/w), RSEP(%), bias (%w/w) and repeatability results (%w/w) (**Table 4.8)**. This two additional PLS factors of the test set #2 compared with test set #1, where 3 PLS factors were selected, were necessary due to the differences in the continuous process and the differences between the tablets sides. Five PLS factors ,compared with 3 PLS factors, low the RMSEP from 2.551 to 2.054%(w/w), and the RSEP from 4.097 to 3.229%. The PLS 611 side scores of the test set #2 tablets were grouped with the corresponding PLS scores of the same batch 62.27% (w/w) API

content tablets and the CM side scores were grouped with the PLS scores of 65.48%(w/w) batch process tablets (**Figure 4.13**). Note in the **Figure 4.13** some test set #2 scores (in brown) are outside of the Hotelling's T^2 because the model, developed with tablets from a batch process, does not include some variations that are present in the continuous process tablets. The model shows acceptable prediction capability with a slightly high prediction error and a higher bias considering the complex tablet physical variation between the six batch concentration levels, the difference in process variation between batch and continuous process, and spectral differences between the two sides of the continuous process tablet. The RMSEP is 2.054 % (w/w), the RSEP is 3.229%, and the bias is -0.101% (w/w). Model M5 developed with a calibration set from batch production tablets shows acceptable performance in terms of accuracy evaluating the test set #2. The PLS model was also evaluated in terms of repeatability study.

Table 4.8 PLS model evaluation of the test set #2.

Model ID	Pre-treatment	PLS Factors	RMSEP (% w/w)	RSEP (%)	Bias (% w/w)	Repeatability Std Dev. % (w/w)	%Y Explain
M5	SNV + 2nd Derivative (15points) 10,028cm-1 to 5,508cm-1	1	3.208	5.152	2.152	0.014	98.691
		2	2.759	4.431	1.514	0.015	99.295
		3	2.551	4.097	0.972	0.015	99.382
		4	2.526	4.057	0.983	0.017	99.471
		5	2.054	3.299	-0.101	0.039	99.562
		6	1.962	3.150	-0.209	0.047	99.579

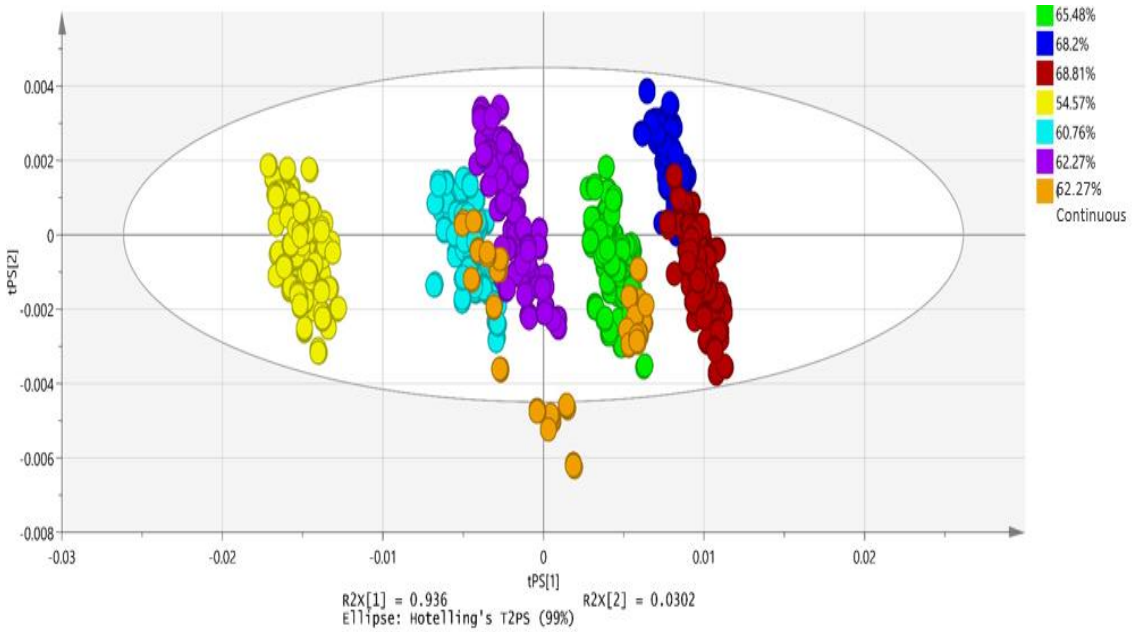

Figure 4.13 PLS score plot. The 611 side from the test set #2 scores (in brown at the left) are grouped with the batch scores (in violet) of 62.27%(w/w) API and the test set #2 CM sides scores (in brown at the rigth) are grouped with the scores of tablets produced by the batch process (in green) of 65.48% (w/w) API.

The repeatability study for the test set #2 tablets was performed in same way as with the batch process tablet calibration set to accomplish the ICH Q2(R1) guidelines. **Table 4.9** summarizes the standard deviation results for each side of the tablet. The highest value for standard deviation in the repeatability study was 0.050% (w/w) compared with 0.021% (w/w) in the batch process tablets calibration set. The repeatability studies varied from 0.028% (w/w), at the lowest, to 0.050% (w/w), at the highest, demonstrating the precision of the model. Intermediate precision results show some variation of the API concentration throughout the tablets. The variation in the prediction of a tablet ranges from 0.076 to 0.169

% (w/w), and is not considered significant. In terms of RSD, The variation in the prediction of a tablet ranges from 0.12% to 0.27%. **Table 4.10** show the intermediate precision results.

The model is still robust due to provides acceptable predictions values despite the differences between the batch and the continuous process, the differences between the sides of continuous process tablets, and variations in compression forces, hardness, weight, width, thickness, length and embossing between each of the six concentration levels of the continuous process tablets calibration set.

Table 4.9 . Precision: NIR Calibration model repeatability predictions for the test set #2.

Precision: Repeatability of tablets test set #2				
Tablet	**Tablet Side**	**Reference Values (%w/w)**	**Predicted Values (%w/w)**	**Repeatability Standard Deviation (%w/w)**
1	611	62.27	59.882	0.033
	611	62.27	59.891	
	611	62.27	59.936	
	611	62.27	59.871	
	611	62.27	59.849	
	611	62.27	59.845	
1	CM	62.27	64.533	0.044
	CM	62.27	64.619	
	CM	62.27	64.532	
	CM	62.27	64.631	
	CM	62.27	64.587	
	CM	62.27	64.544	
15	611	62.27	61.373	0.050
	611	62.27	61.294	
	611	62.27	61.420	
	611	62.27	61.427	
	611	62.27	61.355	
	611	62.27	61.339	
15	CM	62.27	64.741	0.028
	CM	62.27	64.805	
	CM	62.27	64.808	
	CM	62.27	64.800	
	CM	62.27	64.759	
	CM	62.27	64.762	

Table 4.10 Intermediate Precision of the NIR Calibration model using the test set #2.

Intermediate Precision: Standard Deviation per concentration level of the test set #2				
Tablet	**Tablet Side**	**Reference Value (%w/w)**	**Predicted Values (%w/w)**	**Standard Deviation (%w/w)**
8	611	62.27	60.728	0.160
	611	62.27	60.975	
	611	62.27	60.924	
	611	62.27	60.719	
	611	62.27	61.124	
	611	62.27	61.000	
8	CM	62.27	65.174	0.106
	CM	62.27	65.276	
	CM	62.27	65.132	
	CM	62.27	65.161	
	CM	62.27	65.182	
	CM	62.27	64.954	
16	611	62.27	62.716	0.169
	611	62.27	62.351	
	611	62.27	62.344	
	611	62.27	62.255	
	611	62.27	62.564	
	611	62.27	62.464	
16	CM	62.27	65.290	0.076
	CM	62.27	65.239	
	CM	62.27	65.396	
	CM	62.27	65.300	
	CM	62.27	65.203	
	CM	62.27	65.190	

The PLS calibration model was evaluated in terms of RMSEP(%w/w), RSEP(%), and bias (%w/w) by each of the sides 611 and CM separately of the test set #2 tablets due

to the differences found between each side. First, the side with the CM embossing was evaluated because it presents the most differences compared with the batch tables of the same 62.27% API content. The PLS model using five PLS factors has the lower RMSEP(%w/w), RSEP(%), and bias (%w/w) (**Table 4.11**) for the evaluation of the CM side of the test set #2. As a result, a total of five PLS factors were selected.

The CM side PLS scores of test set #2 tablets were grouped whith the 65.48% (w/w) PLS batch tablets scores (**Figure 4.14**). The RMSEP is 1.930 % (w/w), the RSEP is 3.099%, and the bias is 1.901% (w/w) (**Table 4.11**). These values are lower than the values of the PLS model evaluating both sides together of the test set #2 tablets because the CM side PLS scores were grouped perfectly with the 65.48% (w/w) PLS batch tablets scores. This results prove that the CM side of the test set #2 tablets has a higher amount of API than the correct amount that is 62.27% (w/w). Certain processes and physical information about the continuous process tablets (test set #2), to know with assurance the reason of these results, were not available due to the pharmaceutical company's confiecendiality policy. In the tablet compression process, the punch with the CM letters embossing was the lower punch and the the punch with the 611 embossing the upper (**Figure 4.15**). A result of material segregation and the level of shear exposure in the continuous manufracting line could be a possible explanation of this differences in API concentration between the tablets sides. Finally, the side with the 611 embossing of the test set #2 tablets test set was evaluated.

Table 4.11 PLS model evaluation of only the CM side of the test set #2.

Model ID	Pre-treatment	PLS Factors	RMSEP (% w/w)	RSEP (%)	Bias (% w/w)	Repeatability Standard Deviation (%w/w)	%Y Explain
M5	SNV + 2nd Derivative (15points) 10,028cm-1 to 5,508cm-1	1	4.348	6.983	4.345	0.015	98.691
		2	3.756	6.031	3.748	0.018	99.295
		3	3.280	5.267	3.269	0.017	99.382
		4	3.269	5.250	3.257	0.019	99.471
		5	1.930	3.099	1.901	0.036	99.562
		6	1.720	2.762	1.683	0.044	99.579

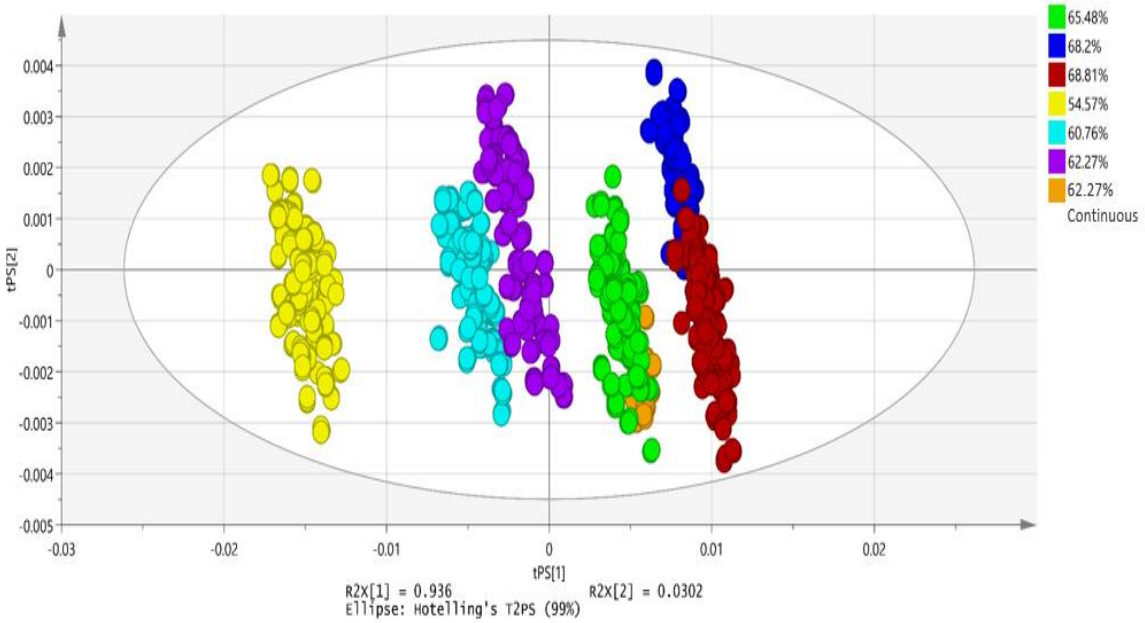

Figure 4.14 PLS scores plot evaluating only the CM side of the test set #2 tablets (scores in brown)**.** These CM side scores are grouping with the batch process scores of 65.48% (w/w) API (in green) and not with their corresponding batch process scores of 62.27%(w/w) API (in violet).

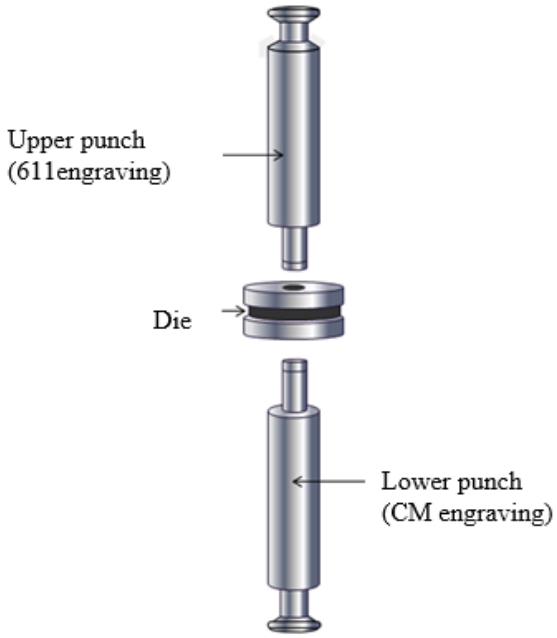

Figure 4.15 Tablet press upper and lower punches

The PLS model using two PLS factors has the lower RMSEP(%w/w), RSEP(%), and bias (%w/w) in the evaluation of the 611 side of the continuous process tablets test set #2. The 611 side PLS scores of the test set #2 tablets were grouped perfectly with the batch process tablets PLS scores that has the same formulation with 62.27% (w/w) of API content (**Figure 4.16**). The RMSEP is 1.059 % (w/w), the RSEP is 1.701%, and the bias is -0.719% (w/w) (**Table 4.12**). Those values are much lower than the PLS model values evaluating both sides together of the test set #2 tablets and the values of the CM side alone. The 611 side of the test set #2 tablets has the correct amount of API in contrast with the CM side of this tablets. The RSEP is still high with a value of 1.059 % due to the differences between the continuous and batch manufacturing process. This PLS model developed with batch process tablets calibration set has a good predictive capacity of the API content in the

continuous process test set #2 tablets despite variations between the two differences manufacturing process. This is most important contribution of this study to the analytical pharmaceutical chemistry.

Table 4.12 PLS model evaluation of only the 611 side of the test set #2.

Model ID	Pre-treatment	PLS Factors	RMSEP (% w/w)	RSEP (%)	Bias (% w/w)	Repeatability Standard Deviation (%w/w)	%Y Explain
M5	SNV + 2nd Derivative (15points) 10,028cm-1 to 5,508cm-1	1	1.295	2.080	-0.041	0.013	98.691
		2	1.059	1.701	-0.719	0.012	99.295
		3	1.503	2.414	-1.324	0.013	99.382
		4	1.441	2.313	-1.291	0.014	99.471
		5	2.171	3.487	-2.102	0.042	99.562
		6	2.176	3.495	-2.101	0.050	99.579

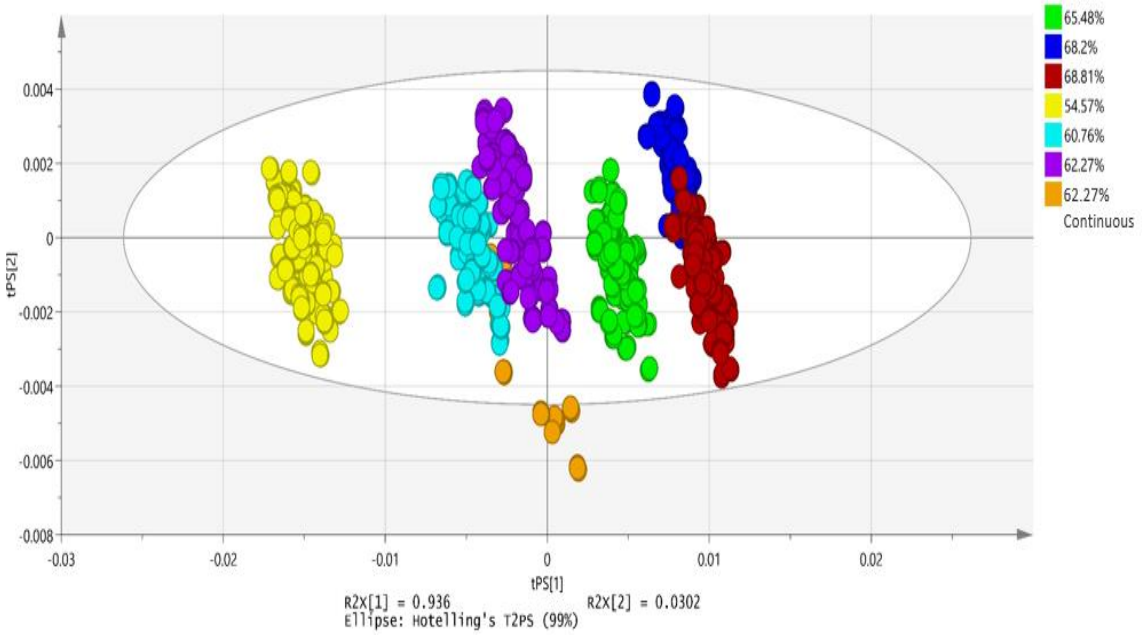

Figure 4.16 PLS plot scores evaluating only the 611 side of the test set #2 (scores in brown). These 611 side scores are grouping with their corresponding batch process scores of 62.27%(w/w) API (in violet).

5 CONCLUSION

A NIR calibration model was developed with a calibration set of production tablets manufactured by a batch process in a pharmaceutical company. The analyzed productions tablets were available in six different API concentration providing an exceptional opportunity to develop a calibration model without the need to prepare laboratory samples of different concentrations. This NIR calibration model predicted with acceptable values the API concentration in production tablets from a batch process. The precision of the NIR model was evaluated using the ICH guidelines. The novelty of this study was that the NIR model, developed with tablet from a batch process, had the ability to predict production tablets manufactured by a continuous process. A difference in the API concentration was found on each side of the continuous process tablets using diffuse reflectance measurements. This difference could be due to a variation in one or more unit operations during continuous manufacturing.

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