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Non-destructive quantitative analysis of moving tablets by Near Infrared Spectroscopy (NIRS)

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**NON-DESTRUCTIVE QUANTITATIVE ANALYSIS
OF MOVING TABLETS BY
NEAR INFRARED SPECTROSCOPY (NIRS)**

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

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ABSTRACT

In recent years, Near Infrared Spectroscopy (NIRS) has gained wide acceptance in pharmaceutical industry. The growing interest in NIR is due to all the advantages that this powerful analytical technique presents. NIR spectroscopy is non-destructive, requires little or no sample preparation, does not require chemicals, solvents and generate little or no waste. The technique is also, simple, fast, and allows for multivariate analysis.

NIRS have become a useful analytical tool to develop a system capable of performing non-destructive analysis of drug content for an on-line testing of compressed tablets from a pharmaceutical batch. Analyzing just a fraction of the sample, thus eliminating the need to analyze instead of the entire sample, as in case of HPLC or UV analysis is it best attractive.

To explore the merits of NIRS for process control, an on-line drug system was constructed and NIR diffuse reflectance spectra were obtained with a diode array spectrometer that monitors the transport of up to 200 tablets per minute, carried in a stabilized conveyor belt. Ninety tablets with prepared at levels of 1% - 30% (w/w) of Active Pharmaceutical Ingredient (API). The wavelength used in the calibration models was 1645-1863 nm, corresponding to the API of the tablets, although others spectral regions associated with the majority excipients ingredients were also evaluated. Calibration models were constructed using several types of pretreatments to the data for the determination of drug

content of the moving tablets, obtaining better results with PLS / SNV using two factors describing a 98.5% of variation with a RSEC of 7.3% and a RSEP of 6.5%.

RESUMEN

En años recientes, la espectroscopia de infrarrojo cercano (NIRS) ha ganado aceptación en la industria farmacéutica. El creciente interés en el NIR es debido a todas las ventajas que esta poderosa técnica analítica presenta. La espectroscopia de infrarrojo cercano es no- destructiva, requiere poca o ninguna preparación de muestra, no usa químicos, solventes y genera pocos o ningún desperdicio químico. Es una técnica sencilla, rápida, portátil y permite el análisis multivariable.

NIRS se ha convertido en una herramienta analítica útil para llevar a cabo análisis no destructivo del contenido de droga provenientes de un lote farmacéutico. Analizar solamente una fracción de la muestra, en lugar de la muestra completa como en el caso de análisis por HPLC o por UV es su mejor atractivo.

Para explorar los meritos del NIRS para un proceso de control, un sistema en línea fue construido y espectros de reflectancia difusa fueron obtenidos con un espectrómetro de arreglo de diodos a razón de 200 tabletas por minuto, en una banda transportadora que reduce la vibración de la superficie al moverse las tabletas. Noventa tabletas fueron preparadas a niveles de 1% (p/p) – 30% (p/p) de ingrediente activo farmacéutico de las tabletas. El largo de onda utilizado para los modelos de calibración fue de 1645- 1863 nm, correspondiente a la banda de absorción del ingrediente activo de las tabletas, aunque otras regiones espectrales asociadas al ingrediente inactivo mayoritario de las tabletas fueron también evaluadas.

Modelos de calibración fueron construidos utilizando diferentes tipos de pre tratamientos a la data para la determinación de contenido de droga de tabletas en movimiento. Se obtuvo mejor resultados con el modelo de PLS/SNV usando dos factores el cual describe un 98.5% de la variación con un RSEC de 7.3% y un RSEP con un 6.5%.

**To Gabriela Zoé,
Soul so sweat and pure. . .**

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“Thanks for the motivation, inspiration and support I received. I am completely grateful”

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1 INTRODUCTION

Drug substances are frequently administered orally by means of solid dosage forms such as tablets, capsules or powders. The most commonly used dosage form for the pharmaceutical preparations is currently the tablet, available in various forms.¹ Tablets provide a number of advantages such as: cost effective manufacture, easy to administer, easy to store and maintains drug stability. Conventional pharmaceutical manufacturing is generally accomplished using batch processing with laboratory testing conducted on collected samples to evaluate quality. This conventional approach has been successful in providing quality pharmaceuticals to the public. The pharmaceutical industry analyzes only a small quantity of the entire batch produced. Drug content of 10 tablets is considered representative of a batch that might include millions of tablets.² Pharmaceutical Laboratories are moving from remote "off-line" laboratories to "the in process" era.

According to The American Society of Testing and Materials (ASTM), the Near Infrared Region (NIR) of the electromagnetic spectrum encompass the wavelength range of 780–2526 nm. NIR absorption bands are typically broad, overlapping and 10–100 times weaker than their corresponding fundamental mid-IR absorption bands. These characteristics severely restrict sensitivity in the classical spectroscopic sense and call for chemometrics data processing to relate spectral information to sample properties.³

Therefore, interpretation of NIR spectra is difficult. This is because the molecular overtone and combination bands seen in the near-IR are typically very broad, leading to complex spectra; it can be difficult to assign specific features to specific chemical

components. Multivariate (multiple wavelength) calibration techniques (e.g., principal components regression or partial least squares) are often employed to extract the desired chemical information.⁴ Careful development of a set of calibration samples and application of multivariate calibration techniques is essential for near infrared analytical methods. There are several spectral pretreatment methods used to reduce these effects⁴. The best method is usually the one that minimizes the prediction error for an independent test set and often is a “trial and error” process.

In recent years, NIR spectroscopy has gained wide use in pharmaceutical industry. The growing interest in NIR is due to all the advantages that this powerful analytical technique presents. NIR spectroscopy is non-destructive, requires little or no sample preparation, does not use chemicals, or generate chemical wastes requiring disposal. The technique is rapid, can be portable, and simultaneously determines numerous constituents or parameters.

1.1 Motivation

There is wide interest in developing analytical techniques that provide valuable information and in the shortest possible time. This idea is applicable in any industrial process, but it gains wide relevance in the pharmaceutical industry. The methods used for the analysis in laboratories are specially designed for each analyte. In most cases, these methods officially supervised by a regulatory agency have been proven to be accurate, but require sample presentation that is expensive and time consuming. The main objective of this research is the

application of “real time” analytical methods based on the combination of near infrared spectroscopy with multivariate methods of analysis.

Chemometrics and multivariate techniques, allows for the simultaneous study several parameters. For example, the parameters that are mostly analyzed consist of: raw material testing, product quality control and process monitoring. In addition, spectral acquisitions are fast and there is no need for sample preparation, an advantage that simplifies the implementation of this technique for “in line” monitoring.

The innovative aspect of this project is the use of NIR spectroscopy as an analytical tool to develop a system capable of performing a non-destructive analysis of drug content for in-line testing of compressed tablets from a pharmaceutical batch.. Minimization of waste and reagents, real fast analysis and a non destructive method are only a few advantages that have this technique. Near Infrared Spectroscopy accounts for all the advantages mentioned before, it can represent an alternative tool in today’s pharmaceutical industry.

Tonglei Li and Morris, 2002, proved that sampling smaller but constant parts of the dosage form gives a predictably larger error (as coefficient of variation) for content uniformity relative to that observed when sampling the whole unit. The tablets used contained two active ingredients at different levels. Entire tablets were analyzed, as well as half, and quarter tablets. Twenty two solutions of the tablets were analyzed using HPLC. The authors developed a statistical proof to show if the coefficient of variance (CV) of sampling portions is greater than or equal to the CV of sampling whole units. Assuming that the distribution of the active ingredient between tablets is not dependent on the distribution of the active drug within each tablet, it was calculated the standard deviation and mean drug

concentration analyzed, respectively for each tablet. The results confirmed that the coefficient of variance when analyzing a constant part of tablets is greater than or equal to that obtained from a full tablet analysis. Coefficients of variance (CV) from sampling half or quarter tablets are larger than the CV values from sampling whole tablets. This lays the theoretical basis for justifying using spectroscopic techniques such as NIR, where the radiation does not interact with the entire sample, to monitor content uniformity (CU).⁴ The authors indicate that if the portion of the tablet meets homogeneity requirements then the entire tablet will also meet CU.

1.2 Literature Review

Vuorela et al., 2003, validated a NIR method to determine the caffeine concentration of pharmaceutical tablets. HPLC was used as the reference method. Two types of caffeine tablets were used. The caffeine concentration of the tablets covered a range: from 0, only excipients and 100% (m/m), that means pure caffeine tablets. A FT-NIR MB160 (ABB Bomen, Inc, Quebec, Canada) was used with a spectral range: 3800 - 14000 cm^{-1} and a spectral resolution: max. 1 cm^{-1} . The analysis of six tablets per batch only took 12 minutes. In conclusion, the comparison of NIR vs. HPLC, resulted very similar, although NIR results seem to become nonlinear at very high concentrations of caffeine (>80%) but to remain linear over the lower range (0-30%).⁵

Iyer, Morris and Drennen, 2002, developed an empirical approach to evaluate the effective sampling depth and effective sample mass for reflectance and transmittance measurements using Near Infrared Spectroscopy.⁶ The authors stated that diffuse reflectance

measurements will not provide chemical information from the entire volume of a typical pharmaceutical tablet and that sample inhomogeneity may give rise to inaccurate reflectance measurements due to a limited sampling volume. In order to determine the effective sampling depth in compacted pharmaceutical mixtures, tablets containing cimetidine, Microcrystalline Cellulose and Magnesium Stearate were compressed. For studying the effect of cimetidine concentration on the effective sampling depth, four concentrations of cimetidine (1%, 4%, 7% and 10%) were used. Two-layered tablets consisting of two layers were included in the study involving determination of effective sampling depth. The first layer contained 10% cimetidine and the second layer consisted of 90% Acetaminophen. Performing reflectance measurements showed that the effective sampling depth was between 1.9 and 2.7 mm. Properties of the sample affect the effective sampling depth: diameter of the sample, particle size, etc. If the tablet thickness was between 3.4 mm to 4.9 mm for transmittance measurements, the signal was minor. The use of two layered tablets was helpful for the determination of inhomogeneity. In reflectance measurements, inhomogeneity cause significant errors, for transmittance, inhomogeneity does not affect the results.⁶.

Romañach and Colon, 2005, developed an on-line system to determine the drug content of tablets as they come out of a tablet press. A home built conveyor belt was used to present the moving tablets to the near infrared spectrometer. The concentrations of the tablets were of 15% (w/w), 20% (w/w) and 25% (w/w). Near infrared diffuse reflectance spectra were obtained with a diode array spectrometer at speeds of 50 and 110 tablets per minute, respectively. Using chemometrics evaluation, calibration models were created obtaining

good accuracy in the determination of the drug content with a bias of about 3.3 mg at 52 tablets per minute (tpm) and 1.52 mg at 111 (tpm).⁷

The research conducted by Romañach (et. al.) also,described a spectroscopic method of a non destructive analysis for the determination of drug content in tablets with less than 1% weight of active ingredient per weight of formulation (m/m) drug content. The drug concentrations of the tablets were manufactured with drug concentrations of ~0.5%, 0.7%, and 1.0% (m/m) and ranging in drug content from 0.71 to 2.51 mg per tablet. Transmission NIR spectra were obtained for 110 tablets that constituted the training set for the calibration model developed with partial least squares regression. The reference method for the calibration model was the analytical balance. Several data preprocessing methods were used to reduce the effect of scattering on the NIR spectra and base the calibration model on spectral changes related to the drug concentration changes. The final calibration model included the spectral range from 11,216 to 8662 cm^{-1} , the standard normal variate (SNV), and first derivative spectral pretreatments. This model was used to predict an independent set of 48 tablets with a root mean standard error of prediction (RMSEP) of 0.14 mg, and a bias of only -0.05 mg per tablet. NIR spectroscopy showed that is an alternative for nondestructive testing of low drug content tablets.

1.3 Summary of Following Chapters

Chapter 1 deals with the principal objectives and the justification for this thesis. Chapter 2 is an introduction of the theory of NIR followed by a description of chemometrics and the pretreatments used for the construction of the calibration and prediction models in Chapter 3. The Chapter 4 presents the materials and methods performed. Results and validation tests

performed are presented in Chapter 5 and Chapter 6, respectively. Finally conclusions are included in chapter 7.

2 THEORETICAL BACKGROUND

2.1 Near Infrared Spectroscopy (NIRS)

2.1.1 Principles of NIR

Two conditions have to be satisfied for a molecule to absorb infrared radiation. The first one is that a determinate frequency of infrared radiation corresponds exactly to the frequency of vibration for certain molecule. That vibration induces a change in dipole momentum that can be described by observing that when a molecule absorbs infrared radiation, its bonds vibrate very similar to the diatomic oscillator. This is explained by Hook's Law who established that an atom move of its position of balance with a proportional force to the displacement. The frequency of vibration for a diatomic molecule is similar that of a harmonic oscillator and therefore it can be expressed by the following equation.

$$\nu = \frac{1}{2} \pi \left(\frac{\kappa}{\mu} \right)^{1/2} \quad \text{Equation 2-1}$$

Where:

ν = vibrational frequency

κ = classic force constant

μ = reduced mass of the two atoms

Experimental observations give evidence that molecules are not ideal oscillators. When two atoms are closer, columbic repulsion between the nuclei's causes an increase most rapid of the potential energy which the harmonic approach predicts and when the interatomic distance comes near to the distance in which the dissociation takes place, the potential level of energy becomes stabilized. Thus we can say that the real molecules approach more the behavior of an inharmonic oscillator. The molecules have a single harmonic behavior when the potential energy is low, near its ground state (Figure 2-1).

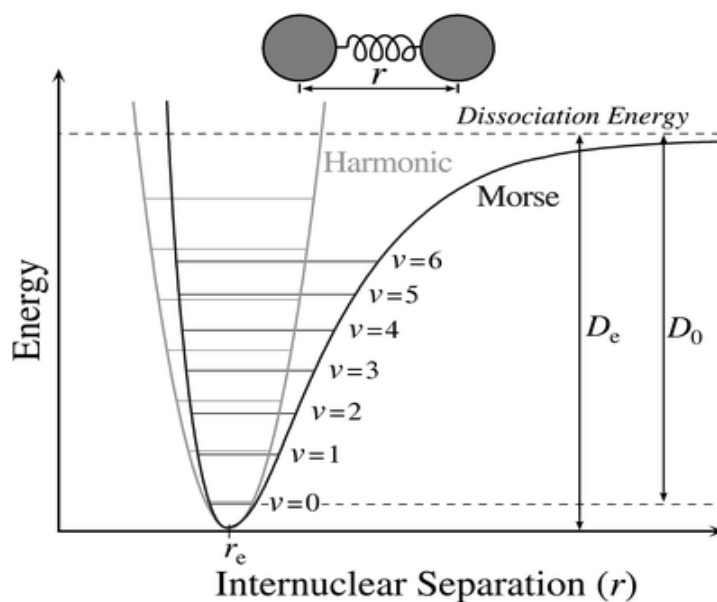


Figure 2-1 Harmonic and anharmonic potential functions for diatomic oscillators

2.1.2 Origin of absorption in NIR

In the anharmonic oscillator is possible to observe fundamental bands ($\Delta = \pm 1$) and bands corresponding to transitions states ($\Delta = \pm 2, \pm 3 \dots$) called overtones. Another consequence due anharmonicity is that the energetic levels are not equal spaced. Transition states in which the quantic vibrational number is more than 1, are less probable to occur, that is the reason that the bands observed in the NIR typically broad, overlapping and 10-100x weaker than its fundamental bands. The bands observed in the NIR are related to overtones and combination of fundamental vibrations of $-\text{CH}$, $-\text{NH}$, $-\text{OH}$, and $-\text{SH}$ functional groups. Aside of overtones, in the NIR region is observed combination bands, which are caused by simultaneous change of energy of two or more vibrational modes. The information that NIRS provides is difficult for interpretation and the intensity of the combination bands and overtones depends in the anarmonicity grade of the bond. The absorption bands of NIR contain both chemical and physical information of all the sample components. For this reason, mathematical and statistical methods are required to extract the relevant information.

2.2 NIR Registry Mode

When light interacts a surface of a sample, is either be reflected, absorbed, or transmitted. The most important are diffuse transmittance and diffuse reflectance (Figure 2-2). The appropriate measured will depend by the optical properties of the samples. Transmittance measurements are often used for transparent materials and diffuse reflectance is used for solid, semi-solids and turbid liquids.¹¹ The radiation interacting with the sample can traverse

and emerge on the opposite side as attenuated transmitted or reflected light as sketched. In diffuse transmittance, light interacts with the sample, transverse it and then is recollected by the detector. In diffuse reflectance the angle of reflected light is independent of the incident angle. Only the part of the beam that is scattered within a sample and returned to the surface is considered to be diffuse reflection.

Incident Light

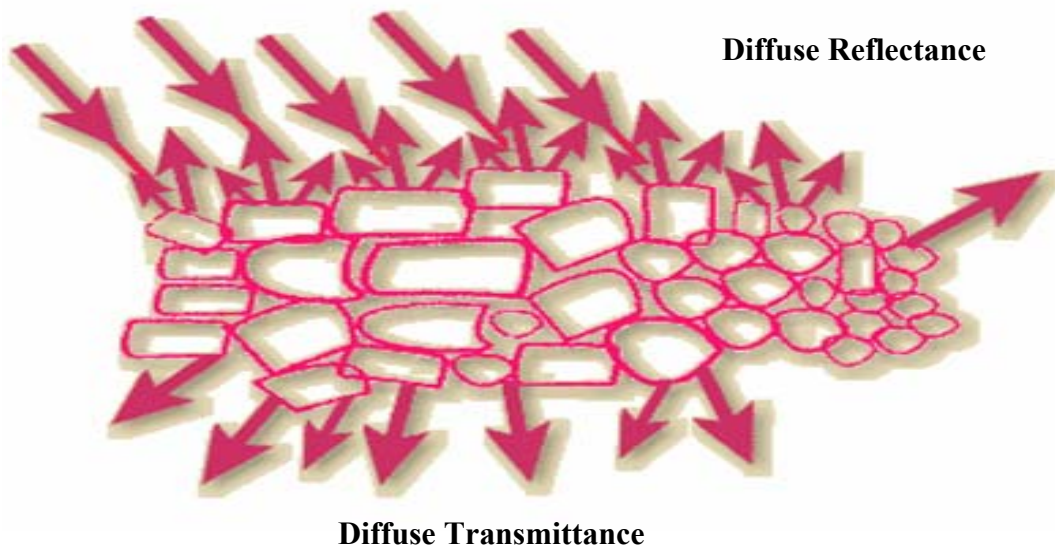


Figure 2-2 Diffuse Reflectance and Diffuse Transmittance measurements

3 Chemometrics

The Handbook of Chemometrics and Qualimetrics, defined the concept of chemometric as a chemical discipline that uses mathematics, statistics and formal logic to design or select optimal performance experimental procedures and provide maximum relevant chemical information by analyzing chemical data and obtain knowledge about chemical systems.”

3.1 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a chemometric technique used to reduce multidimensional data sets to lower dimensions for analysis. Matrix \mathbf{R} (Figure 3-1) represents the responses of I samples analyzed at J different wavelength. Because J has a high number of variables that characterized each sample, it represents a simple analysis and graphic representation of the samples.

$$R = \begin{matrix} & \hat{i} & \hat{j} \\ \begin{bmatrix} \mathbf{n}_1, \mathbf{n}_2 \\ \lambda_1, \lambda_2 \end{bmatrix} \end{matrix}$$

Figure 3-1

PCA is very useful because it allows representing the variability presents in \mathbf{R} in a few factors or principal components that are lineal combinations of the original variables. The analysis in principal component analysis provides and approximation to the matrix \mathbf{R} as a product of two matrix. One of the matrixes is named *Scores* and the other one is named

Loadings. Scores provide the structure of the lines, the relation between the samples and the loadings maintain the relation between the variables.

$$R = TP^T + E \quad \text{Equation 3-1}$$

The first principal component describes the major quantity of information contained in R . This technique is very useful for the interpretation of multivariable data. The scores establish relation between samples allowing the detection of differences of samples and groups.

3.2 Partial Least Square (PLS)

As PCA Analysis, Partial Least Square makes factor decomposition, but instead uses the concentration variable for the decomposition of the matrix of answers; R . the first factor describes the direction of the maxim variance in R , which is relationated with the concentration. Partial least square (PLS) is used to construct calibration models for the quantitative determination of drug content in tablets. NIR spectra contain chemical and physical information of all sample components; the analytical information is multivariate in nature. The use of Multivariate Analysis with the Partial Least Squares algorithm (PLS) is a very powerful alternative in the development of pharmaceutical applications. The objective in developing these calibration models using PLS is to predict the concentration of the analyte under study. A calibration model can be constructed using a set of tablets based in the leave-one-out Cross Validation (LoO-CV) method. LoOV-CV consists in constructing a model with all samples except one that is left out. Then this sample is predicted with the model. In order to evaluate the error of the model constructed, statistical test are performed.

Root Mean Square of Calibration (RMSEC) is an empirical estimate of prediction error by testing the calibration equation directly on the calibration data.

$$\text{RSEC} = \sqrt{\sum_{i=1}^n \frac{(C_{\text{REF } i} - C_{\text{NIR } i})^2}{n_i}} \quad \text{Equation 3-2}$$

Where C_{REF} = concentration by the reference method

C_{NIR} = concentration predicted by NIR

n_i = number of samples used in the calibration set

The calibration model developed is used to predict a second set of tablets often called prediction set (RSEP). This set is used to corroborate the accuracy of the model. These samples contained the same characteristics of the samples used for the calibration set. Then the calibration model is used to predict this prediction set, and the error of prediction is calculated by:

$$\text{RSEP} = \sqrt{\frac{\sum_{i=1}^n (C_{\text{REF } i} - C_{\text{PRED } i})^2}{\sum_{i=1}^n C_{\text{REF}}^2}} \quad \text{Equation 3-3}$$

3.3 Data Pretreatment

The spectral information that NIR provides are difficult for visual interpretation. These characteristics severely restrict sensitivity in the classical spectroscopic sense and call for chemometric data processing to relate spectral information to sample properties. Derivatives can be applied to improve the resolution of overlapping bands. The first derivative reduces the correlation between variables and the effect of the dispersion due to the particle size.

Derivatives are often used as a method to reduce scatter effects and can be applied to improve the resolution of overlapping bands.¹³ Derivatives from superior order are obtained applying successively first derivatives, although usually they are not used with an order greater than 2 since the signal to noise decreases. The first derivative reduces the correlation between variables, the effect of dispersion due to the particle size and removes an additive baseline. Second derivatives remove a linear baseline.

$$dA = \frac{A_{\lambda_1} - A_{\lambda_1 + \delta}}{\Delta\lambda} \quad \text{Equation 3-4}$$

Standard Normal Variate (SNV), which corrects light scattering in the diffuse reflectance mode due to difference in particle size is another pretreatment often used.¹⁴ This method centers and scales individual spectrum.

$$X_i^{SNV} = \frac{X_{ij} - \bar{X}}{S_i} \quad \text{Equation 3-5}$$

Where:

j = Wavelength

X_{ij} = Absorbance at wavelength j

s = standard deviation

The average X value of the spectrum is subtracted from all wavelengths

4 MATERIAL AND METHODS

4.1 Chemicals

The designed formulation contained Ibuprofen produced by Albemarle Corp (Baton Rouge, LA) and donated by Pharmacia and Upjohn Caribe (Barceloneta, PR) as active pharmaceutical ingredient (API) at concentrations of: 1% (w/w) 5 % (w/w) , 10% (w/w), 15 % (w/w), 17 % (w/w), 20 % (w/w), 22 % (w/w), 25 % (w/w) and 30 % (w/w). As excipients ingredients was used: Hydrous lactose spray dried USP DMV International (Veghel, The Netherlands) used as filler 45-80 % (w/w); Microcrystalline cellulose (VIVAPUR®) by Microcellulose Weissenborn, MBH + COKG; Colloidal silicon dioxide, Mutchler INC 0.2% (w/w) as binder, and magnesium stearate 0.5 % (w/w) from Mallinckrodt as lubricant.

4.2 Experimental procedure

The ingredients were accurately weighed as seen in Table 4-1 in an OHAUS ANALYTICAL Plus[®] balance. Using a standard sieve with openings of 250 μm (Figure 4-1), the first powder mixed was colloidal silicon dioxide with ibuprofen. Microcrystalline Cellulose was added using the same sieve. Then the mix was blended in a 4 quart PK Blend Master[®] from Patterson- Kelley (Model B Lab Blender) for five minutes (Figure 4-2). The hydrous lactose was added to the blend and mixed for three minutes. The last step consisted in adding the magnesium stearate to the blend for two minutes.

Table 4-1 Raw Materials used in tablets

API Concentration (w/w)	API	Lactose	Microcrystalline cellulose	SiO₂	Magnesium stearate
1%	2.5 g	210.0 g	37.5 g	0.5 g	1.25 g
5%	12.5 g	200.0 g	37.5 g	0.5 g	1.25 g
10%	25 g	187.5 g	37.5 g	0.5 g	1.25 g
15%	37.5 g	175.0 g	37.5 g	0.5 g	1.25 g
17%	42.5 g	170.0 g	37.5 g	0.5 g	1.25 g
20%	50.0 g	162.5 g	37.5 g	0.5 g	1.25 g
22%	55.0 g	157.5 g	37.5 g	0.5 g	1.25 g
25%	62.5 g	150.0 g	37.5 g	0.5 g	1.25 g
30%	75.0 g	137.5 g	37.5 g	0.5 g	1.25 g

The tablets were manually prepared, in different days at the laboratory using a manual Carver Co. single punch press machine (Figure 4.3). The compression force was at 3000 pounds and a total of 10 tablets were prepared of each concentration. The tablet weight variations were controlled to be within the range from 240-250 mg per tablets.



Figure 4-1 Standard sieves with openings of 250 µm



Figure 4-2 4 quart PK Blend Master®



Figure 4-3 Manual Carver Co. single punch press machine



Figure 4-4 Upper punch, lower punch and dies

4.3 NIR Spectral Acquisition

The on-line drug system was constructed and Near Infrared diffuse reflectance spectra was obtained with a diode array spectrometer NIR CDI 256 element thermoelectrically extended cooled Indium Gallium Arsenide (InGaAs). The system includes a buffered conveyor belt that reduces the surface vibration as the tablets move (Figure 4-5). Several experiments was performed in order to improve the acquisition of the spectra and this minimize errors like : position of tablets in the conveyor belt, velocity of the system to acquire spectra of the moving tablets The spectra obtained covered the wavelength region from 1200 to 2200 nm with a 10 nm resolution. The velocity of the conveyor belt was set in 40 rpm obtaining approximately 200 tpm.

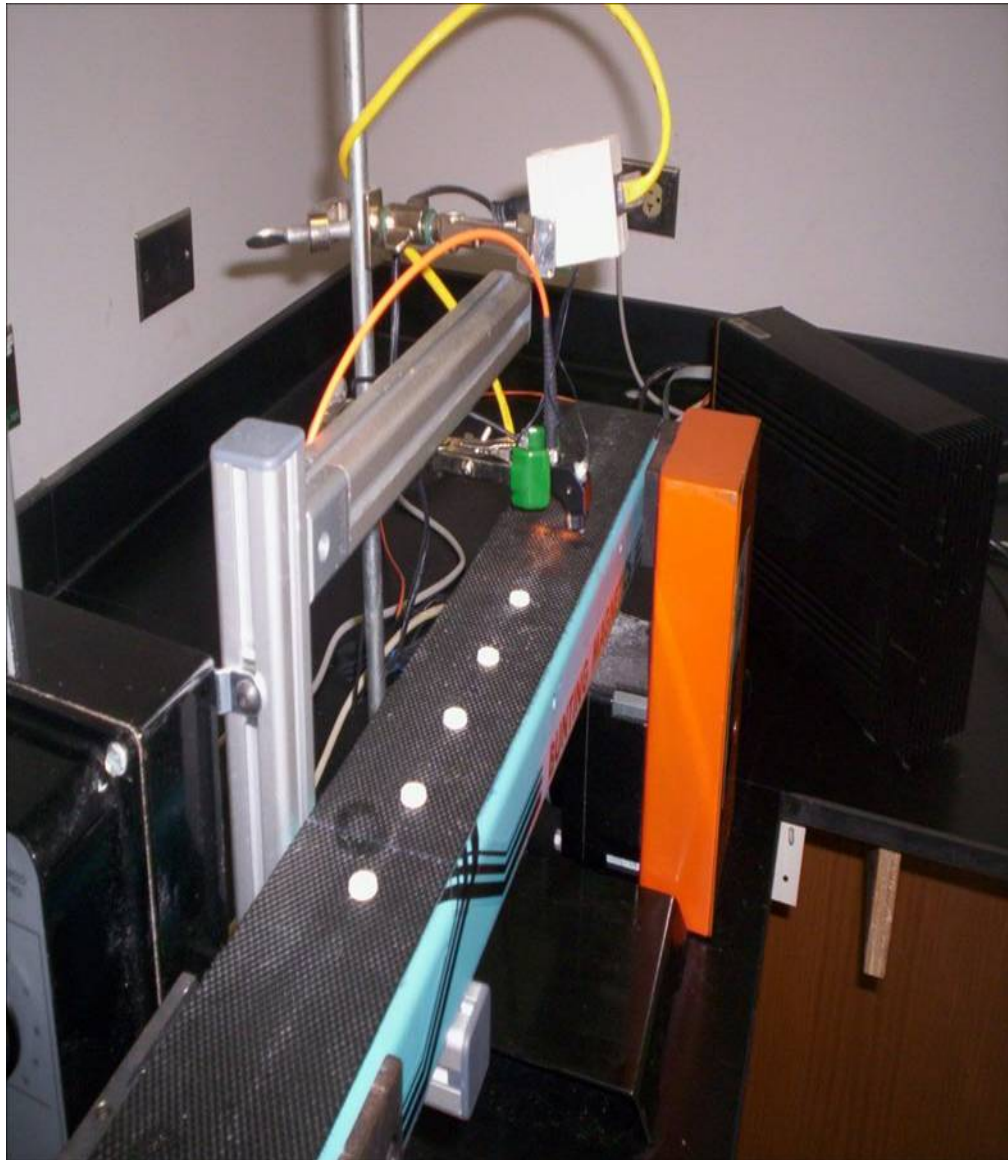


Figure 4-5 Conveyor belt system used for tablet presentation

The spectra of the moving tablets were acquired using the Spec32 Software, Version 1.5.5.0 from Control Development Inc, South Bend, IN. To optimize the collection of the spectra, the following parameters and features were kept constantly in each experiment:

1. Integration time : 5 ms
2. Sample Average : Fill Buffer/ Show All
3. Wavelength Interpolation: Revert to Non-Linear Calibration / Apply to all traces
4. Trace logging : Automatically save every “nth” file to the root path name
5. Automatically redraw the screen for every sample acquired was deactivated.

Prior to every spectral acquisition, background and reference were always taken in the modality of Amplitude Calibration – Raw A/D counts, and no compensation, using an organic microcrystalline fluorinated polymer that provides a strong reflectance. 32 spectra of each tablet were obtained. A spectrum of a static tablet was used for comparison and to eliminate those spectra associated with noise. Then the selected spectra were averaged.

4.4 Reference method

Working with absolute measurements allows the use the OHAUS ANALYTICAL PLUS as reference method. This is a high-grade analytical balance. The balance is sited in a vibration-free area and disturbance. It carries out internal calibration but prior every use it was double checked with certified check weights

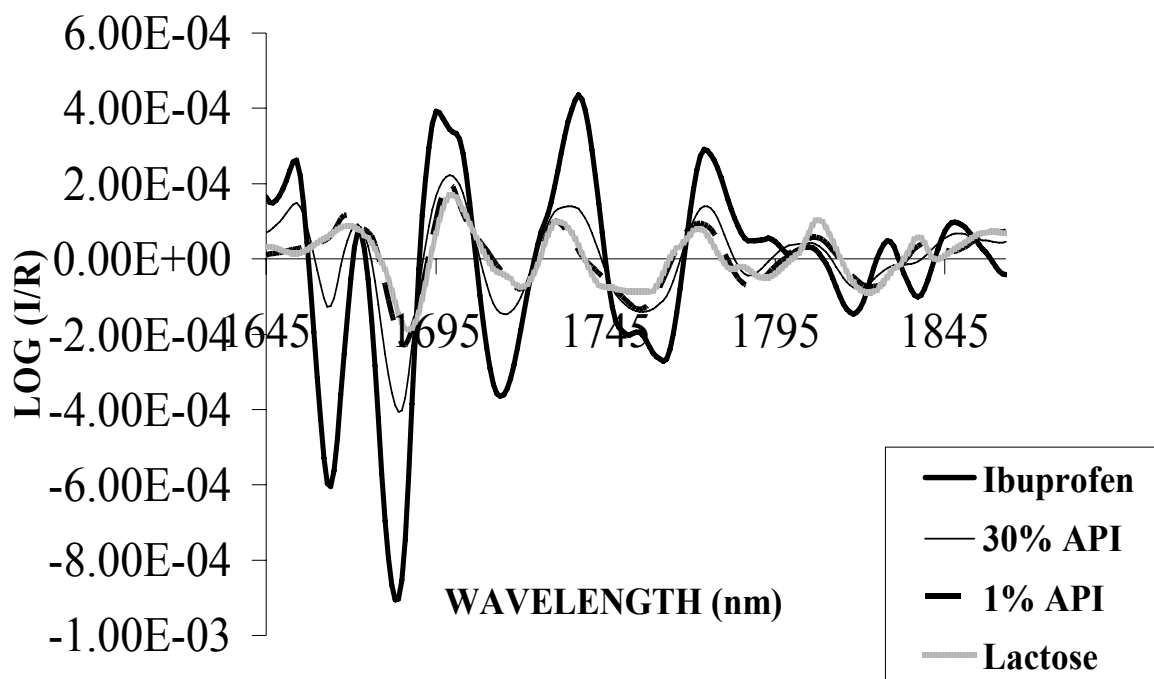
5 RESULTS

5.1 Spectral area evaluated

The selection of the spectral region of interest instead of using the whole spectra is an important aspect for development of the calibration and prediction models. This reduce spectral region associated with noise and with information that is not important to the project objectives. To construct simple models and to have an easier interpretation, it is important to select wavelengths of interest

Four standard tablets were prepared: Ibuprofen, 1% (w/w) of Active Pharmaceutical Ingredient (API), lactose, and 30% (w/w) of API, respectively. Spectra of the four tablets were obtained in order to select the wavelength of interest and its corresponding 2nd derivative (Figure 5.1) and SNV (Figure 5.2) were performed.

***EVALUATION OF SPECTRAL REGION
AFTER 2nd DERIVATIVE TRANSFORM***



**Figure 5-1 Evaluation of spectral region after 2nd derivative transform (25)
Ibuprofen (API); lactose (major excipients ingredient)**

***EVALUATION OF SPECTRAL REGION
AFTER SNV TRANSFORM***

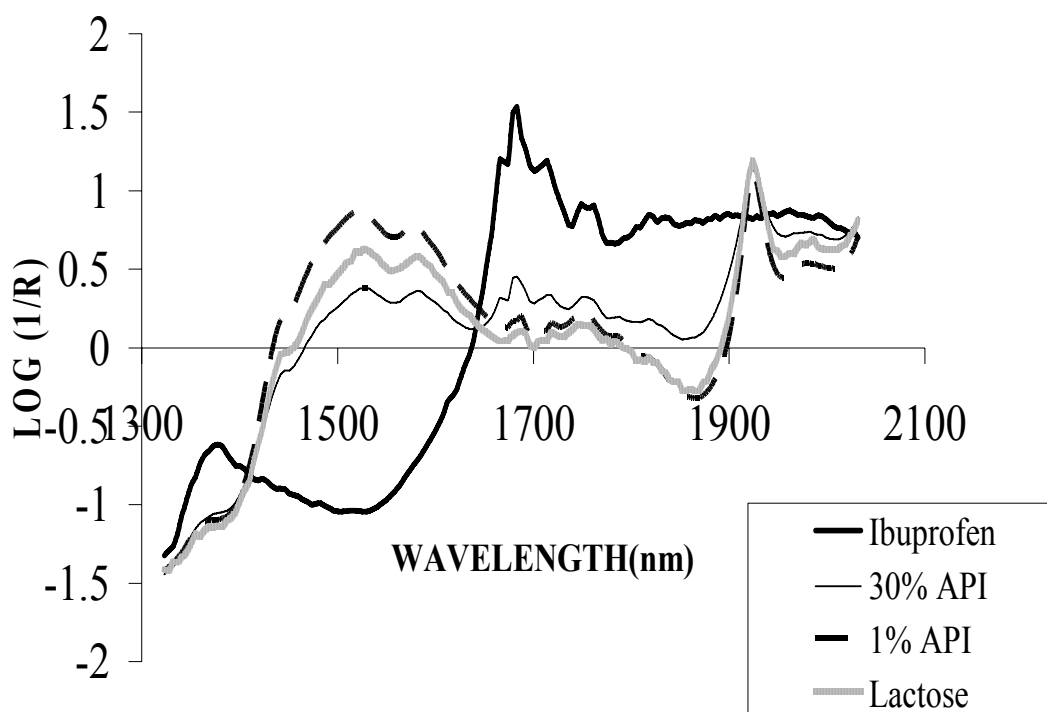


Figure 5-2 Evaluation of spectral region after SNV transform, Ibuprofen (API); lactose (major excipients ingredient)

The region of 1645-1863 nm (Figure 5-3) was the spectral region where most differences in spectra were observed and Ibuprofen has the greatest absorption. Other areas were evaluated: 1395-1649 nm in which Lactose (Figure5-4), the greater excipient ingredient has the biggest t absorption and 1870-2000 nm where appears water band (Figure 5-5). After selecting the wavelength of 1645-1863 nm as the spectral region evaluated for the quantification of the API, spectra of moving tablets were obtained .

***SPECTRA OF MOVING TABLETS AFTER
SNV TRANSFORM
IN SPECTRAL REGION 1645-1863 nm***

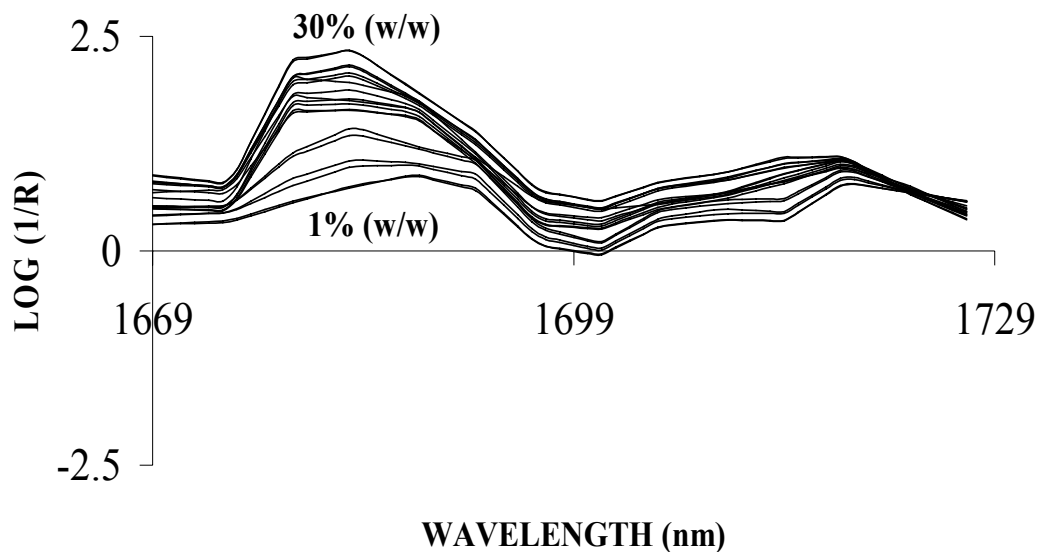


Figure 5-3 Spectra of moving tablets in spectral region of 1645-1863 nm

***SPECTRA OF MOVING TABLETS AFTER
SNV TRANSFORM
IN SPECTRAL REGION 1395-1863 nm***

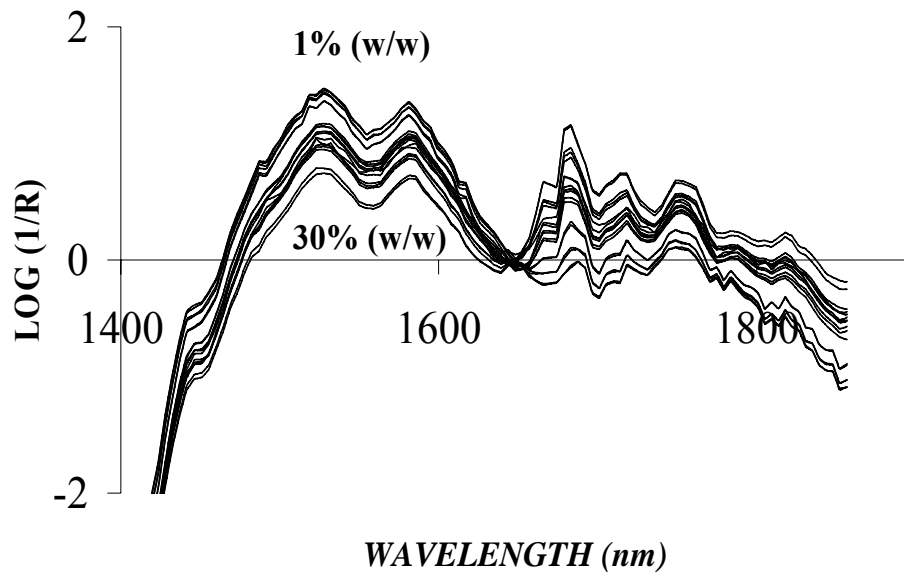


Figure 5-4 Spectra of moving tablets in spectral region of 1395-1863 nm

***SPECTRA OF MOVING TABLETS AFTER
SNV TRANSFORM
IN SPECTRAL REGION 1395-2000 nm***

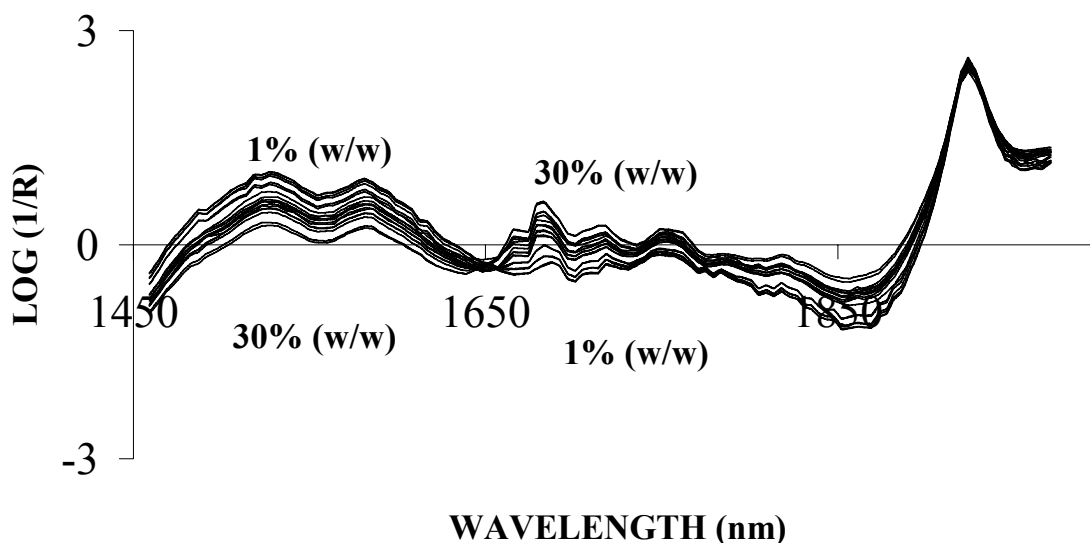


Figure 5-5 Spectra of moving tablets in spectral region of 1395-2000 nm

5.2 Development of calibration models

Several steps were performed in order to develop calibration and prediction set models. First the spectra were evaluated performing a PCA which allowed seeing if differences exist in the samples. The PCA model was constructed for a set of 90 tablets, 10 tablets for each concentration, using the area of 1645-1863 nm in which the API absorbed. Tablets for the calibration set and prediction set were selected.

The second step was the selection of the samples for each model. The calibration set must contain the greatest variability source of all the tablets. It was necessary to select those

tablets which described the most variation for each concentration. The tablets with the highest and lowest drug content were included in both sets of models. Six tablets of each concentration were used for the calibration set and four tablets were used for the prediction set. A plot of the scores of a PC vs. another PC was constructed (Figure 5-6), after selecting the tablets used for the calibration set and for the prediction set. Both tablets, for calibration and prediction, were grouped, which explains the fact that they share the same properties. Using 2 factors, the first pc explained the greater source of variation, 91.7% and factor 2 explained 4.8% of the variation.

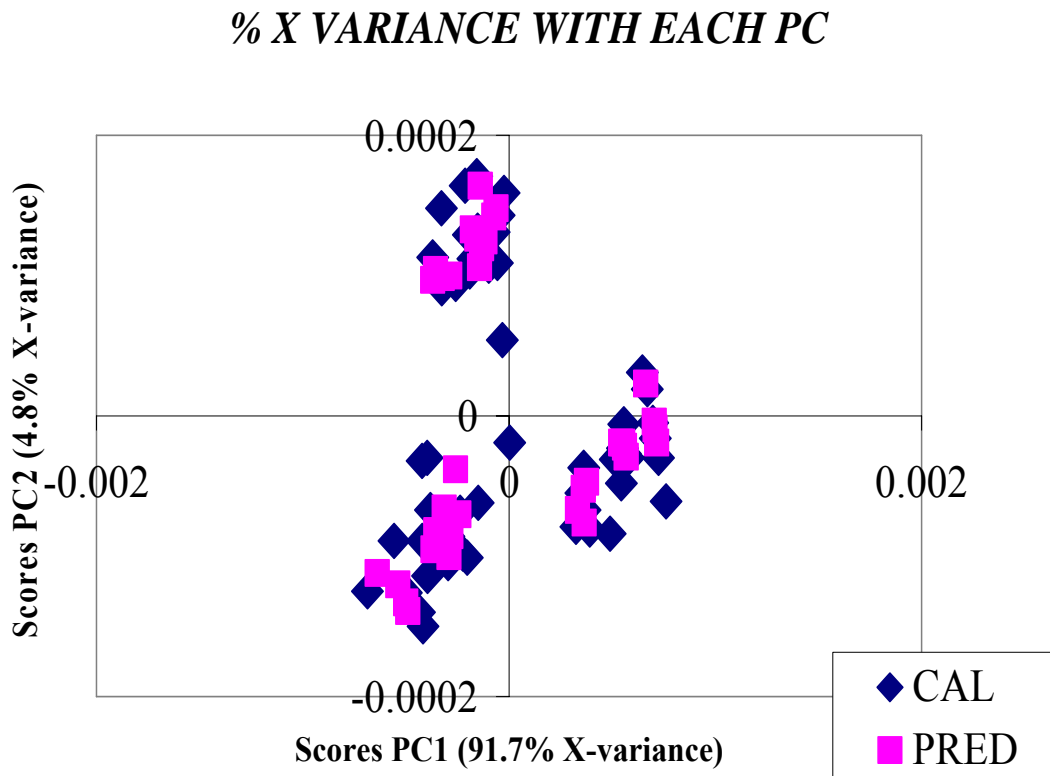


Figure 5-6 X- Variance with each PC

After performing PCA analysis, PLS models were created to evaluate differences due to drug content. Several pretreatments were performed for the areas selected: SNV, 1st derivative (25), 2nd derivative (25) and a combination of SNV with each derivative. PLS/SNV using 2 factors presented the most spectral variation. The development of these models included the evaluation of several spectral regions.

Calibration models were constructed by cross-validation, using the leave-one-out method and different spectral pretreatments were performed in order to minimize the effects of the light scattering and variability of spectra. The region of interest in which our API absorbs, 1645-1863 nm, was evaluated (Table 5.1). The third step consisted in the selection of the optimal factors to include in the model and evaluated the RSEC% and RSEP%.

Table 5-1 Pretreatments for models developed in 1645-1863 nm spectral region

Pretreatments performed	Spectral region (nm)	Factors	%Variation described	RSEC %	RSEP %	SEC	SEP
SNV	1645-1863	2	98.5	7.3	6.5	1.4	1.2
SNV	1645-1864	3	99.4	6.8	6.3	1.3	1.2
SNV	1645-1865	4	99.5	6.4	6.6	1.2	1.2
SNV 1st der	1645-1863	2	99.5	7.6	6.8	2.3	1.3
SNV 1st der	1645-1863	3	99.6	6.4	6.7	1.5	1.2
SNV 1st der	1645-1863	4	99.7	5.9	6.6	1.3	1.2
SNV 2nd der	1645-1863	2	97.2	7.8	6.6	1.7	1.2
SNV 2nd der	1645-1863	3	98.3	7.4	6.8	1.5	1.3
SNV 2nd der	1645-1863	4	98.7	6.5	6.2	1.4	1.1
1st derivative	1645-1863	2	98.1	7.7	6.7	1.5	1.2
1st derivative	1645-1863	3	98.7	7.2	6.6	1.4	1.2
1st derivative	1645-1863	4	99.7	6.6	6.8	1.3	1.3
2nd derivative	1645-1863	2	95.1	7.8	6.6	1.5	1.2
2nd derivative	1645-1863	3	97.9	7.5	6.6	1.4	1.2
2nd derivative	1645-1863	4	98.7	6.8	6.6	1.3	1.2

Table 5-2 Calibration set values predicted by PLS/SNV calibration model

Reference Values	NIR Predicted (% w/w)	Reference Values	NIR Predicted (% w/w)
1%	1.1	17%	16.3
1%	1.2	17%	17.6
1%	1.8	17%	14.1
1%	1.8	20%	20.9
1%	2.0	20%	19.4
1%	1.9	20%	20.5
5%	5.6	20%	21.4
5%	4.5	20%	18.3
5%	5.0	20%	20.1
5%	4.9	22%	17.4
5%	5.7	22%	24.7
5%	5.1	22%	22.9
10%	9.5	22%	22.0
10%	10.4	22%	25.1
10%	10.1	22%	24.8
10%	8.8	25%	23.9
10%	10.4	25%	25.8
10%	10.1	25%	23.3
15%	14.5	25%	24.3
15%	15.6	25%	22.6
15%	15.0	25%	26.0
15%	16.3	30%	30.6
15%	14.1	30%	30.6
15%	13.6	30%	28.5
17%	16.9	30%	30.1
17%	18.4	30%	27.7
17%	16.9	30%	30.4

Table 5-3 Prediction set values predicted by calibration model using SNV with 2 factors

Reference Values	NIR Predicted (% w/w)
1%	1.6
1%	1.5
1%	1.6
1%	2.5
5%	4.9
5%	4.9
5%	4.9
5%	5.0
10%	10.0
10%	10.5
10%	9.3
10%	9.1
15%	15.4
15%	14.0
15%	14.5
15%	14.2
17%	16.6
17%	16.8
17%	17.9
17%	16.5
20%	20.7
20%	20.9
20%	22.0
20%	20.5
22%	25.0
22%	22.4
22%	25.1
22%	25.5
25%	25.8
25%	23.2
25%	23.7
25%	24.2
30%	30.0
30%	29.6
30%	30.4

Another region evaluated was 1395-1863 nm. Increasing the number of the PLS factors is need because this spectral area include both active ingredient and one of the major inactive ingredients, lactose.

Table 5-4 Pretreatments for models developed in 1395-1863 nm spectral area

Data Pretreatment	Spectral region (nm)	Factors	%Variation described	RSEC %	RSEP %	SEC	SEP
SNV	1395-1863	2	98.9	7.0	6.9	1.3	1.3
SNV	1395-1863	3	99.7	6.5	6.5	1.2	1.2
SNV	1395-1863	4	99.8	6.3	6.2	1.2	1.1
SNV 1st der	1395-1863	2	94.4	7.3	6.6	1.3	1.2
SNV 1st der	1395-1863	3	98.5	6.3	6.4	1.3	1.2
SNV 1st der	1395-1863	4	98.9	6.1	6.4	1.2	1.2
SNV 2nd der	1395-1863	2	94.9	8.1	6.4	1.8	1.2
SNV 2nd der	1395-1863	3	96.2	7.2	6.5	1.5	1.2
SNV 2nd der	1395-1863	4	96.8	6.3	5.9	1.4	1.1
1st der	1395-1863	2	93.4	7.6	6.7	1.4	1.2
1st der	1395-1863	3	98.3	7.1	6.7	1.4	1.2
1st der	1395-1863	4	99.5	6.3	6.6	1.2	1.2
2nd der	1395-1863	2	91.1	7.9	6.8	1.5	1.3
2 nd derivative	1395-1863	3	92.4	7.4	6.6	1.4	1.2
2 nd derivative	1395-1863	4	96.9	6.6	6.2	1.3	1.2

The last spectral region evaluated was 1395-2000 included ibuprofen, lactose and the water band. This area was evaluated in case of having variations in humidity during the preparation of the tablets, and in the posterior analysis in the conveyor belt. Water presence causes the appearance of characteristic bands that affect the totality of the spectra due to the variation that they induced.¹⁵

Table 5-5 Pretreatments for models developed in 1395-2000 nm spectral region

Data Pretreatment	Spectral region (nm)	Factors	%Variation described	RMSEC %	RMSEP %	SEC	SEP
SNV	1395-2000	2	98.3	6.9	6.8	1.7	1.3
SNV	1395-2000	3	99.3	6.6	6.6	1.3	1.2
SNV	1395-2000	4	99.8	6.3	6.4	1.3	1.2
SNV 1st der	1395-2000	2	94.8	6.3	6.5	1.3	1.2
SNV 1st der	1395-2000	3	97.9	6.2	6.4	1.1	1.2
SNV 1st der	1395-2000	4	98.3	5.9	6.1	1.2	1.1
SNV 2nd der	1395-2000	2	91.6	7.8	6.6	1.9	1.2
SNV 2nd der	1395-2000	3	94.9	6.9	6.8	1.5	1.2
SNV 2nd der	1395-2000	4	96.6	6.2	6.2	1.3	1.1
1st derivative	1395-2000	2	94.8	7.8	6.8	1.5	1.3
1st derivative	1395-2000	3	97.5	6.4	6.4	1.2	1.2
1st derivative	1395-2000	4	99.3	6.2	6.4	1.2	1.2
2nd der	1395-2000	2	74.9	8.9	7.3	1.7	1.3
2nd der	1395-2000	3	95.5	7.6	6.7	1.4	1.2
2nd der	1395-2000	4	96.9	6.2	6.6	1.2	1.2

After selecting SNV using two factors in the area of 1645-1863, as the pretreatment used for the construction for the calibration model, statistical analysis were performed. To evaluate the selection of to factors instead of more, relative standard errors of calibration RMSEC % and prediction RMSEP % were analyzed (Figure 5-7). Both values obtained showed minima using 2 factors.

Table 5-6 Statistics summary of PLS- SNV model

STATISTICS SUMMARY OF PLS - SNV						
FACTORS	% VARIATION		RSEC %	RSEP%	SEC	SEP
	DESCRIBED					
Factor 1	93.4		10.3	9.1	1.9	1.7
Factor 2	98.5		7.3	6.5	1.4	1.2
Factor 3	99.4		6.8	6.3	1.3	1.2
Factor 4	99.5		6.4	6.6	1.2	1.2
Factor 5	99.7		6.0	6.5	1.2	1.2

RSEC% AND RSEP% vs. PLS FACTORS

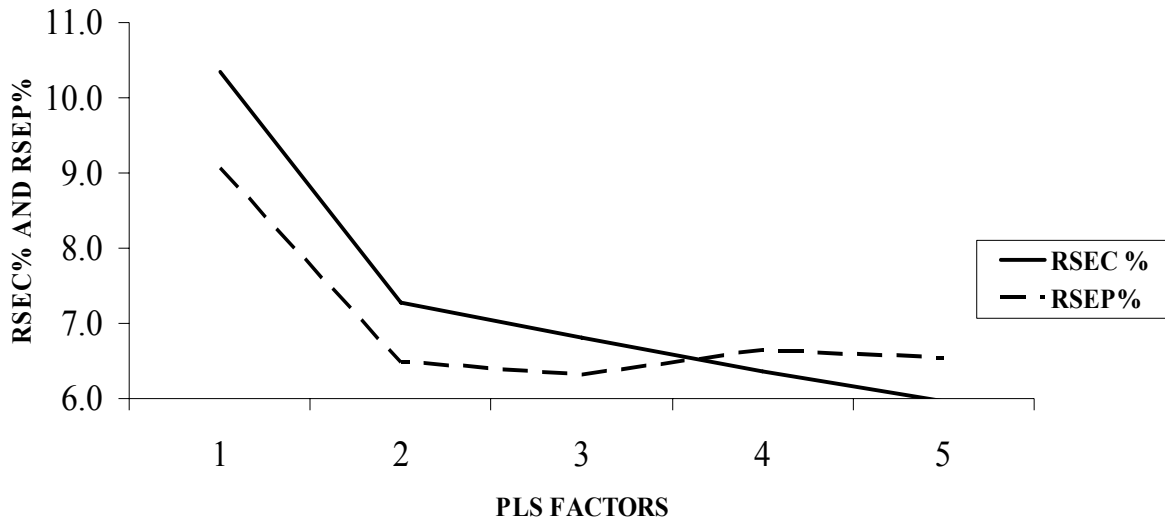


Figure 5-7 RSEC % and RSEP% vs. PLS Factors

Loadings were also evaluated for factor 1, factor 2 and factor 3. Evaluating the three factors at the same time seems to be difficult to distinguish between signal of the sample and random events (Figure 5-8)

**LOADINGS FOR SPECTRAL REGION OF
1645-1863 nm USING
FACTOR 1 AND FACTOR 2 AND FACTOR 3**

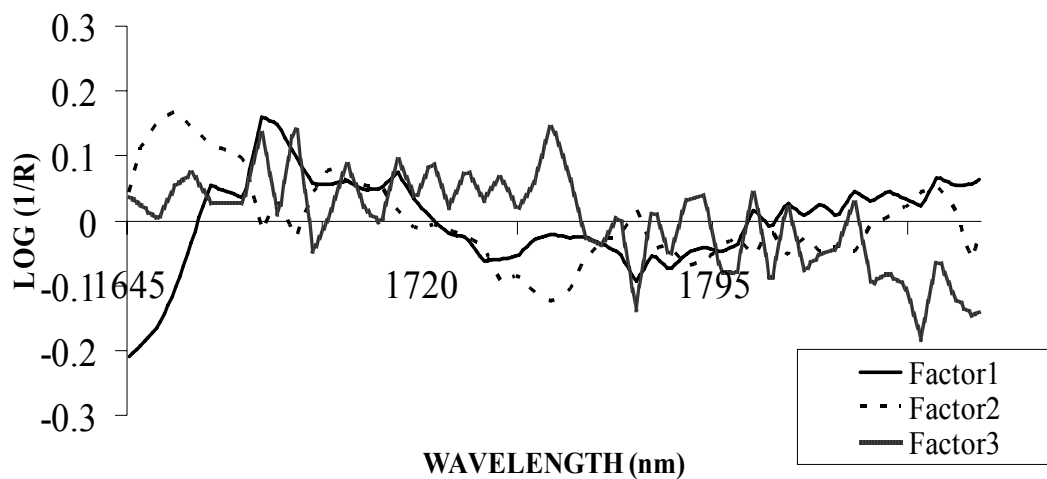


Figure 5-8 Loadings for spectral region of 1645 – 1863 nm using 3 factors

Comparing the loadings for factor 1 and factor 2 clearly showed that adding this third factor to the calibration model, will include some type of variation that will affect the performance of the models.

**LOADINGS FOR SPECTRAL REGION OF
1645-1863 nm
USING FACTOR 1 AND FACTOR 2**

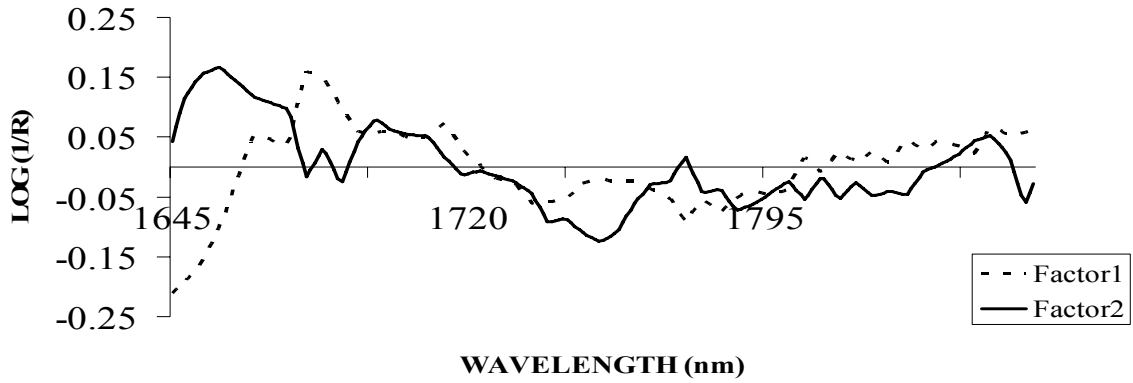


Figure 5-9 Loadings for spectral region of 1645-1863 nm using factor 1 and 2

**LOADINGS FOR SPECTRAL REGION OF 1645-1863
nm USING
FACTOR 1 AND FACTOR 3**

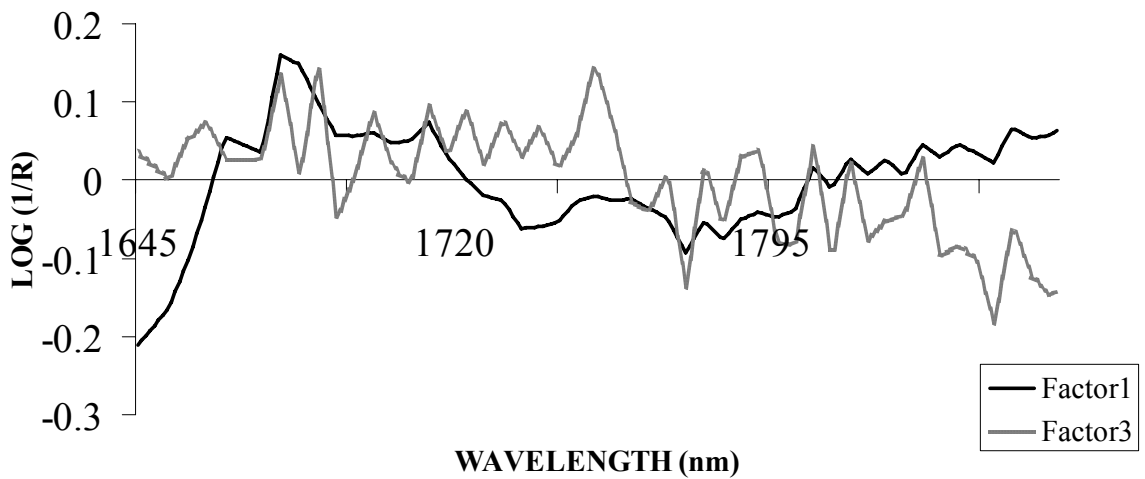


Figure 5-10 Loadings for spectral region of 1645-1863 nm using factor 1 and 3

The linearity between the proposed NIR method and the Analytical balance reference method was evaluated. Linearity studies performed proved the linearity of the method within the tested range (Figure 5-11).

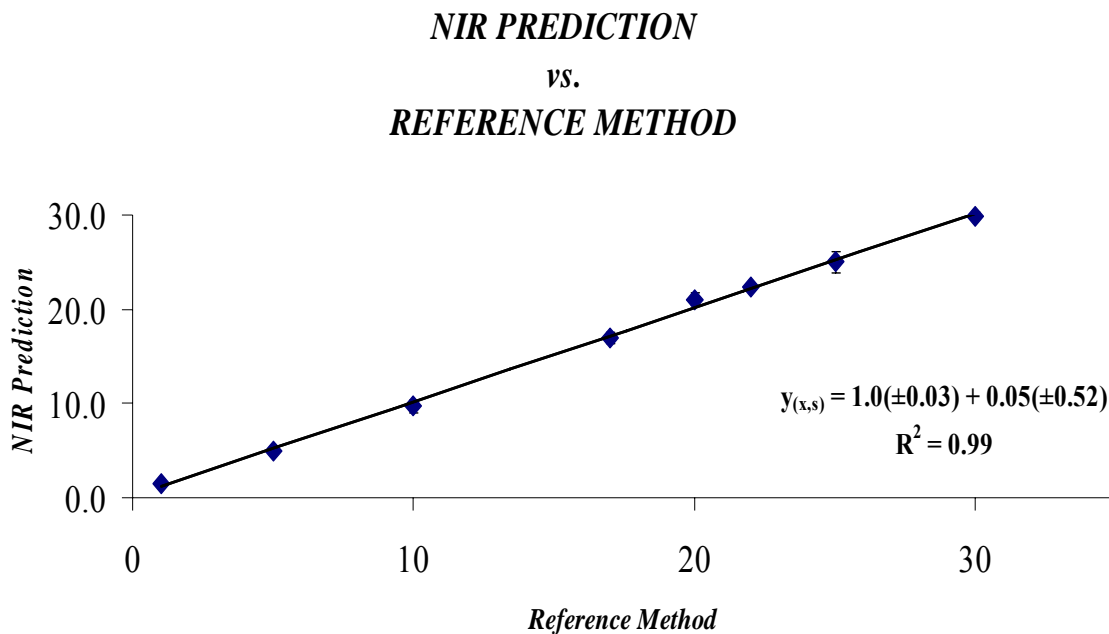


Figure 5-11 Proposed NIR method vs. Analytical balance reference method

A residual plot for the prediction values using the PLS/SNV with 2 factors as calibration model was constructed (Figure 5.12). It is noted on the graphic that there is not an established behavior of the residuals, which indicate the absence of systematic error during the analysis of the data.

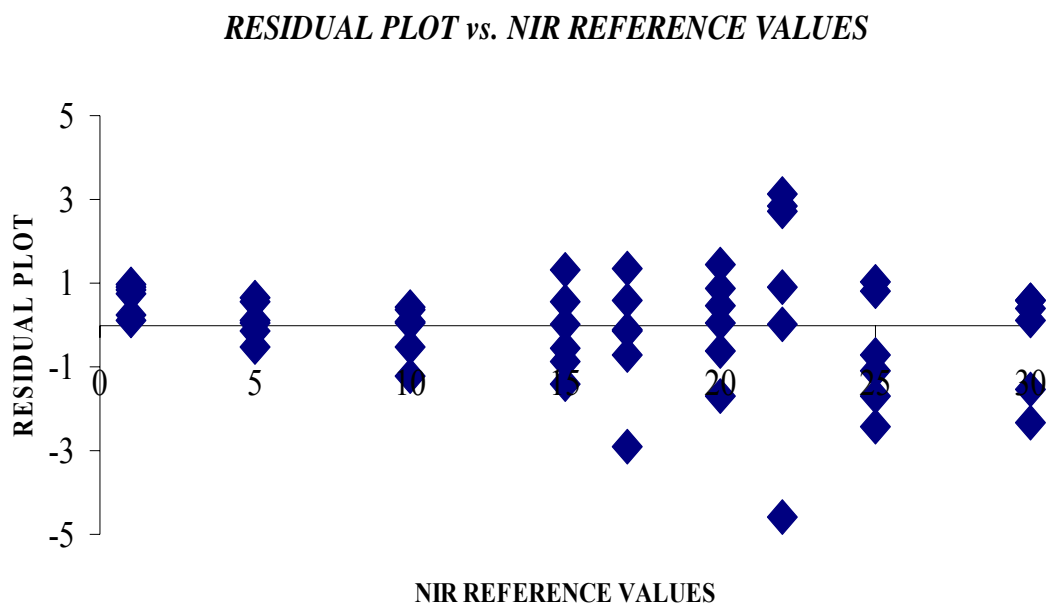


Figure 5-12 Residual plot vs. NIR reference values

6 Validation

A new set of tablets were prepared to perform validation test. Three tablets of 1% (w/w), 5% (w/w), 20% (w/w), 25% (w/w) and 30% (w/w) were predicted with the PLS/ SNV using 2 factors calibration model.

6.1 Intermediate precision

Three tablets of the target value, 20% (w/w) were used in this study. For each tablet, three spectra were obtained in three consecutive days by two analysts. The spectra were then predicted by the selected model. Two random events were considered: tablets prepared in three different days; and two analysts performing the analysis in three different days. The results revealed that there are no statistical differences between days and analyst. Were these the same tablets from the prediction set, please indicate and describe. A two factor ANOVA with replication applied to data revealed no statistical differences in between days and analyst.

6.2 Repeatability

The International Conference on Harmonization (ICH) guidelines defines the concept of repeatability as the precision under the same operating conditions over a short interval of time.¹⁶ To evaluate the repeatability of the system, five concentrations covering the range of drug content were evaluated. Three tablets per concentration with three replicates of each one were obtained. The drug content was then predicted using the chosen calibration model. The proposed method fulfills the ICH requirements.

6.3 Linearity

ICH defines linearity as the ability of the analytical procedure to obtain results directly proportional to the amount of analyte in the sample. Three tablets per concentration with three replicates of each one of five concentrations covering the range were predicted by the selected model. The presence of systematic error was evaluated by lineal regression between real concentrations and the NIR predicted values. Linearity studies performed proved the linearity of the method within the tested range. After this, an equation with slope equals 1 and an intercept which includes the 0 was obtained, which indicates that there is not systematic error in the process.

Regression test showed only random influence of any of the factor in the performance of the methods. There is not an established behavior in the data, which also indicates absence of systematic error.

Table 6-1 Evaluation of Regression for linearity test

R	0.99	
Adjusted R Square	0.99	
F	2995.55	
Regression	SS = 5690.04	MS= 5690.04
Residual	SS= 77.88	MS=1.90

***NIR PREDICTED VALUES vs.
REFERENCE VALUES***

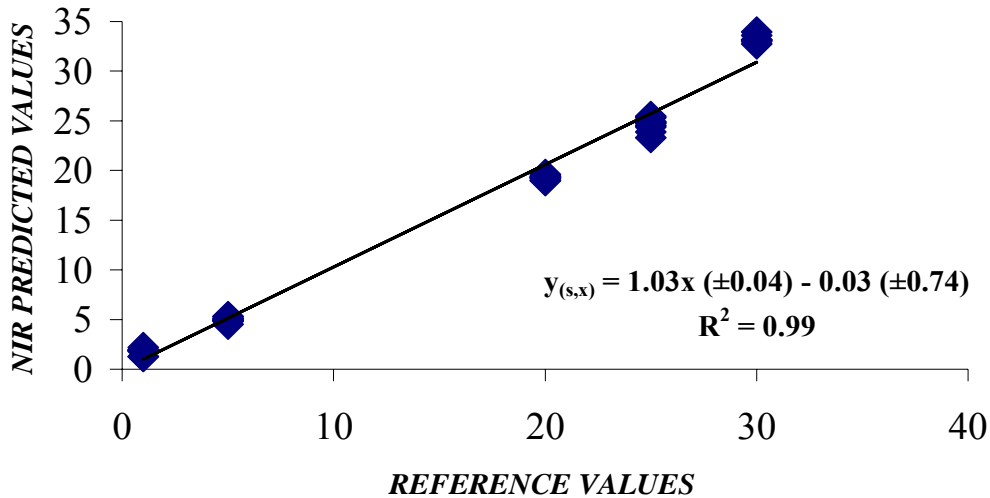


Figure 6-1 NIR Predicted Values vs. Reference Values for linearity test

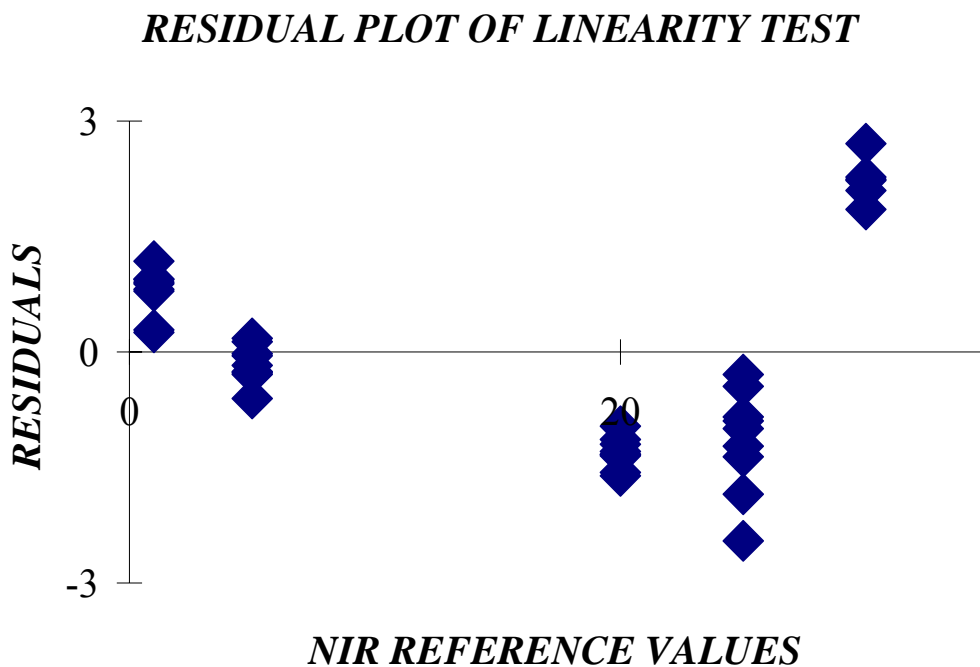


Figure 6-2 Residual plot of linearity test

6.4 Accuracy

Five concentrations covering the range were evaluated. Three tablets per concentration with three replicates of each one was obtained. Paired t-test between analytical balance reference method, and NIR predicted values were evaluated. The accuracy studies performed demonstrated that the method used was accurate was applied correctly and that the equipment worked well during the analysis.

Table 6-2 Statistical Summary of validation methods

RESULTS OBTAINED IN THE VALIDATION OF PLS/SNV MODEL						
PARAMETER	COMPONENT	IBUPROFEN				
Linearity	n	3 replicates of				
	Concentration range (%)	3 tables for each concentration				
	Intercept	1% (w/w)	5% (w/w)	20% (w/w)	25% (w/w)	30% (w/w)
	Slope	0.0 ± 0.7				
	R	1.03 ± 0.04				
Accuracy	S.D	0.3	0.2	0.2	0.7	0.4
	t Experimental	0.8				
	t Tables	2.8				
	RMSEP (%)	7.6				
Repeatability	Mean NIR (%)	1.8	5.0	19.3	24.6	33.3
	RSD (%)	17.4	4.8	1.0	2.7	1.2
Intermediate precision	Days of analysis	3				
	F experimental	2.3				
	F tables	18.5				
	Analyst	2				
	F experimental	6.5				
	F tables	19.0				

7 CONCLUSIONS

Near Infrared spectroscopy has been proven to be a fast and powerful analytical technique for the determination of drug content in moving tablets. In combination with multivariate analysis, is excellent for qualitative and quantitative analysis.

The calibration models developed presented good results for the determination of drug content in pharmaceutical tablets. The validation studies performed showed that the method developed was precise and exact for the determination of drug content in moving tablets. The pretreatments performed were selected based in the objectives of our project. Evaluation of different pretreatments is highly recommended.

In general, NIR is fast, non destructive and increase the analytical throughput which results in a reduction of costs, reagents and time of analysis.

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