Development of a Calibration Model to Determine Drug Concentration in Pharmaceutical Mixtures

by

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Abstract

A robust calibration model was created using Near Infrared Spectroscopy (NIR) and by minimizing the correlation between the active pharmaceutical ingredient (API) and components in a pharmaceutical process. An experimental model was used to minimize correlation and create a calibration model to detect API in a mixture. A design of experiment (DoE) was prepared using six components. A total of nine placebos were prepared using different concentrations of each component.

A series of mixtures were prepared using placebo, granulation and API or using placebo and API. The correlation between components when using granulation, placebo and API was of 99% because concentrations followed a pattern: if API concentration increased, components concentrations decreased. To break that pattern and to decrease the high correlation, only placebo and API were used and the concentrations of the components were modified.

With the new approach, if the concentration of API increases, not all of the concentrations of the components will decrease. A more robust model was then created because of the change in correlation.

The predictions obtained from the calibration model show a very robust model which was achieved by reducing the high correlation presented originally.

Resumen

Se creó un modelo de calibración robusto utilizando la espectroscopia de infrarrojo cercano (NIR) y minimizando la correlación entre el ingrediente farmacéutico activo (API, por sus siglas en ingles) y los componentes que se encuentran en un proceso farmacéutico. Un modelo experimental fue utilizado para minimizar la correlación y para crear un modelo de calibración que pudiera detectar el contenido de droga en una mezcla. Se preparó un Diseño Experimental (DoE, por sus siglas en ingles) utilizando 6 componentes. Un total de nueve placebos fueron preparados utilizando diferentes concentraciones de cada componente.

Dos tipos de mezclas fueron preparadas: 1) utilizando placebo, una mezcla de API + componentes y API (con una correlación de 99%), 2) utilizando placebo y API solamente (con una correlación de 95%). La correlación del 99% fue observada debido a que las concentraciones seguían un patrón: si la concentración de API aumentaba, la concentración de todos los componentes disminuía. Para poder romper este patrón, solo se utilizo la mezcla en la que se utilizaba placebo y API y las concentraciones de los componentes fueron modificadas.

Con este nuevo acercamiento, si la concentración de API aumenta, no todas las concentraciones de los componentes disminuyen. Debido al cambio que ocurrió en la correlación, se pudo crear un modelo de calibración más robusto.

Las predicciones que se obtuvieron del modelo de calibración muestran un modelo bien robusto lo cual fue logrado al reducir la alta correlación en la mezcla presentada originalmente.

To my two angels Nefty and Gael,
Without you, I wouldn't be here…

La posibilidad de realizar un sueño es lo que hace que la vida sea interesante.
Paulo Coelho

The possibility of realizing a dream, is what makes life interesting.
Paulo Coelho

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Chapter 1

1.1 Summary

This thesis describes the development of a calibration model for a pharmaceutical formulation. A careful experimental design was followed to develop a model capable of responding to changes in the drug content. Several tests were performed to challenge the model and the results are described here.

1.2 Introduction

When developing a calibration model, it is important to make it as robust as possible. According to the International Conference on Harmonisation (ICH) guidelines for the Validation of Analytical Methods: Definitions and Terminology, robustness is the measure of the capacity of an analytical procedure to remain unaffected by small variations in method parameters[1]. The robustness of, for example, a calibration model, indicates its reliability when being used[2]. The evaluation of robustness can be done during the development of the analytical method but it could also be assessed after the method has been developed[3]. This evaluation has to demonstrate how reliable is the method developed with respect to the changes of the parameters[2]. As a consequence of the evaluation of robustness, some parameters have to be controlled to guarantee that whenever the method is used, these parameters won't affect the results[3].

One way of developing a calibration model as robust as possible is to have the least compositional correlation possible between all the components used. The use of software to design an experiment can be very helpful but additional steps may be necessary to provide the least possible correlation between components. This research describes the use of a novel approach to minimize compositional correlation.

The concentration of active pharmaceutical ingredient (API) in a pharmaceutical formulation is usually determined by high performance liquid chromatography (HPLC), which is time-consuming, sample destructive, and often requires significant volume of solvent. Near Infrared (NIR) spectroscopy allows the determination of physical and chemical parameters of API in a pharmaceutical process using a very fast, and non destructive approach without sample preparation[4,5]. NIR is a method extensively used in the pharmaceutical industry because of its ability to provide monitor important quality attributes in real time. However,

because of the wide overlapping bands of NIR spectra, the construction of multivariate models is often required[6].

A Design of Experiment (DoE) can be generated using software as one of the initial steps in developing a new NIR calibration model. Some advantages of creating a DoE are that the software will provide the concentrations needed for each component, the correlations between components, samples needed, the maximum information for a minimum number of experiments, and the identification of interactions among process parameters, thus, providing an assurance of quality[7].

Several aspects have to be considered to obtain the least possible correlation between components. For example, if a mixture of A + B is prepared, their percentages will be 100 because when one increases, the other one decreases, which will cause them to have a 100% correlation. On the contrary, when preparing a mixture of A + B + C, their percentages can be modified to break the correlations between them and make them as minimal as possible. However, if one or two components have a high concentration then the correlation cannot be completely eliminated.

The purpose of this work is to determine API concentration for a formulation with a high concentration of an active pharmaceutical ingredient (API). Six components were mixed to generate nine placebos, following a DoE. A placebo is an inactive substance or preparation that contains no API; in this case, it only contains components such as fillers, binders, disintegrants, lubricants, glidants, and/or surfactants. From these placebos, 41 mixtures were prepared in a range of concentrations from 45% to 85% of API, thus, minimizing the correlation. This innovative approach provided a decrease in the compositional correlation between components and API when working with high drug concentrations.

Chapter 2

2.1 Previous Works

Powder blending is the method most commonly used to manufacture tablets or capsules, and more than three-quarters of all pharmaceuticals are manufactured in this form[8]. Blending of powder materials is a common pharmaceutical manufacturing unit operation for many industrial and consumer products. The blending process is used with the goal of a uniform distribution of all the components in the end product[9]. To obtain a proper solid dosage, it is important to ensure a mixture that is as homogeneous as possible of the API and the components of that mixture[10]. The variation of the mixture composition must be monitored in order to characterize, optimize and control the mixing process[11].

It is difficult to accurately measure mixture composition in an efficient and non-destructive fashion. This limitation can result in the rejection of up to standard quality batches and the release of product containing incorrect amounts of active ingredients. Statistical measures and sampling protocols normally assume a random distribution within the mixture which can lead to the erroneous conclusion that the blends are accurate. Another invalid conjecture is that a sample obtained with a thief sampler is indicative of the composition of the mixture at that location[8]. Powder mixtures are typically analyzed by removing small amounts of samples from the bulk mixture and analyzing them. The most common techniques use a thief sampler[12]. These devices extract a quantity of material from the bulk mixture, and then the extracted mixture is subdivided into smaller samples and analyzed. The use of a stream sampler is an alternative of using a thief sampler. An advantage of this technique is that it collects more samples than the thief sampling. Because stream sampling uses the process of flow of the blend from a bin when the compression of the tablet occurs, it could indicate

segregation problems related to the emptying of the blender in contrast with the thief sampling which is unable to do it[13]. Some disadvantages that the use of sampling thieves has is that the insertion of a probe could disrupt the powder mixture, that the powder tends to segregate as they flow into the cavities of the sampling thief, and that it is not practical to take a lot of samples at the same time since it doesn't characterize the whole mixture[14].

NIRS has been a widely used method since it requires no reference method and because it determines, simultaneously, physical and chemical parameters in a rapid, non-destructive manner[4]. Of the physical parameters of a mixture, one of the few that has a big effect on the NIR spectra is particle size which, as a consequence, causes a change in baseline[15]. NIR also analyses samples in a non-invasive manner and requires no sample preparation[6]. A number of qualitative and quantitative methods of analysis have been developed by using NIRS in conjunction with multivariate algorithms[6]. For this reasons, NIRS has become one of the favorite methods of use to determine the active principal ingredient in pharmaceutical mixtures and tablets[16].

A Statistical Design of Experiments (DoE) is a method extensively used to help in the development of calibration models. Some advantages that this method exhibits are that it helps in the understanding of multidimensional interactions of parameters in a process[7], determines the relationships found between factors that can affect a process, and provides maximum information from a minimum number of experiments[7]. By using a DoE, a multivariate calibration model can be developed.

Multivariate calibration has been previously done using the NIR region for quantitative analysis[17]. In previous works, the API content has been determined by using PLS models constructed from calibration sets[4,6,17]. To construct a robust, accurate, and precise calibration model, physical and chemical variability have to be included[4].

The pre-treatments commonly used to construct calibration models are Standard Normal Variate (SNV), and 1st and 2nd derivatives[4,6,18,19]. The SNV correction is used to remove the effects of scattering, the 1st derivative is used to remove the background, and the 2nd derivative is used to enhance the resolution by overlapping peaks and correcting the baseline[18].

After the development of a calibration model, comparisons of prediction results have to be made to choose the best model. The optimal prediction of a calibration model developed is evaluated in terms of its Relative Standard Error of Prediction (RSEP). To verify the quality of a model, the average value of residuals (Bias) and the Standard Error of Prediction (SEP) are normally calculated[16,18].

2.2 Materials and Methods

2.2.1 Materials

All components, including the API, were received from a pharmaceutical company in Puerto Rico. The components consisted of a filler, a binder, a disintegrant, a lubricant, a glidant, and a surfactant.

2.2.2 Design of Experiments

The Design of Experiments (DoE) is a method extensively used[7,20] to develop calibration models to detect drug content in pharmaceutical mixtures. The utility of DoE is based on the fact that it helps in the determination of the relationships that exist between excipients and drug, before developing the calibration model. It also allows the selection of the most significant factors on a response and to acquire their optimal values[21]. Before choosing a specific experimental design, it is important to have in mind the quantity of factors needed, the nature of the study[7], the interactions between the components, and the resources available to create the calibration model. One of the most important things to assess is the calibration model robustness.

The objective of developing a robust model is to produce a model that cannot be affected by variations in samples. Some variations that can occur are variations in parameters, and environmental and fabrication changes. When the effect of these factors is reduced to a minimum, then a robust model is created.

2.2.3 D-Optimal Design

D-optimal design is done using computer algorithms that correlate all the components used to create it. It was used in this work because it offers the maximum information with the best quality and with the smallest number of experiments used[22]. This form of design chooses the optimal set of design runs depending on the specified model and the total number of treatment runs.

2.2.4 NIR Spectrometer

Near infrared reflectance spectroscopy (NIRS) is a fast, noninvasive, non-destructive technique that enables the analysis of complex matrices without requiring sample preparation[6,18]. NIRS has been widely used because of its ability to provide chemical and physical properties of samples[4,6,16,18,19,23]. NIR spectra depend on the physical and chemical properties of samples because it results from absorption and scattering processes. Scattering is the process in which the particles are randomly diverted after a collision. It is related to particle size, and has a multiplicative effect on the amount of light that the sample absorbs[24].

NIR spectroscopy offers the possibility of remote sampling with fiber optic probes and is also a practical analytical technique used to evaluate pharmaceutical powder blends. NIR spectroscopy is often applied as an alternative to the use of sample thieves. It is possible to obtain physical information from flowing powders because the spectra in NIRS are markedly affected by scattering. NIR radiation penetrates into the powder and NIR reflection spectroscopy thus gives information not only on the surface properties of the sample but also on the bulk composition.

Chapter 3

3.1 Development of DoE

A design of experiment was obtained using the Modde software Version 9 (from Umetrics, Umeå Sweden). The experiment was set to be designed with two different percentages (5%, 10%) for the maximum variation that could be found in the concentrations of the components in the final product. Two approaches were followed: 1) using granulation, active pharmaceutical ingredient (API), and placebo and 2) using placebo and API. The use of granulation was attempted because it had been prepared in a pharmaceutical environment with the process as it was supposed to occur for the preparation of the final mixture.

When the DoE (D-Optimal Design) was created, there was a high correlation between the components if using granulation, drug, and placebo make the mixtures as shown in Table 3.1. This high correlation occurs because the granulation mix contains the two major components that are also found in the placebo. After this analysis, it was decided to use the information when using a binder as filler at 10%, as the maximum variation found. With this information an equation was created to calculate the quantities of placebo and pure drug needed to obtain the % of drug desired in the final product. The equation is as follows:

$$\%API = \frac{WAPI}{\Sigma\left(WAPI + WPlacebo\right)} * 100 \qquad (1)$$

Where WAPI represents the weight of drug used, and WPlacebo represents the weight of the placebo used.

Table 3.1. Correlation of components using an API mixture, drug and placebo following initial D-Optimal Design.

		Placebo Components						
Correlation	**API**	**Filler**	**Binder**	**Disintegrant**	**Binder II**	**Lubricant**	**Glidant**	**Surfactant**
API %	1.00							
Filler	-0.99	1.00						
Binder	-1.00	0.99	1.00					
Disintegrant	-0.98	0.97	0.98	1.00				
Binder II	-0.56	0.47	0.53	0.58	1.00			
Lubricant	-0.96	0.93	0.95	0.98	0.62	1.00		
Glidant	-1.00	0.99	0.99	0.99	0.58	0.97	1.00	
Surfactant	-0.96	0.93	0.95	0.98	0.62	1.00	0.97	1.00

After performing all the calculations, the correlation between all the components was found to be above the 99% which shows to be a high percentage of correlation between the principal components of the mixture. The equation to obtain correlation is:

$$r = \frac{N \sum xy - (\sum x)(\sum y)}{\sqrt{[N \sum x^2 - (\sum x)^2][N \sum y^2 - (\sum y)^2]}} \quad \textbf{(2)}$$

Where N is the number of pairs of scores, $\sum xy$ is the sum of the products of paired scores, $\sum x$ is the sum of x scores, $\sum y$ is the sum of y scores, $\sum x^2$ is the sum of squared x scores, and $\sum y^2$ is the sum of squared y scores. The symbol **r** is used to stand for the correlation. Correlation (r) will always be between -1.0 and +1.0. If correlation is negative, there is a negative relationship; if it's positive, the relationship is positive.

Because this high correlation was not desired, a different approach was followed. The experimental design was then calculated using different fillers and comparing the correlation charts obtained. After analyzing all the charts, it was decided that the product would be mixed using only API and placebo, and using a binder as the filler at 10%. This was done to break the correlation between the major components of the mixture, and lead to a reduction in correlation: from 99% to 95% (Table 3.2).

Table 3.2. Correlation of components using drug and placebo

		Placebo Components					
Correlation	**API**	**Filler**	**Binder**	**Disintegrant**	**Lubricant**	**Glidant**	**Surfactant**
API	1						
Filler	-0.104	1					
Binder	-0.038	0	1				
Disintegrant	-0.958	-0.154	-0.097	1			
Lubricant	0	0	0	0	1		
Glidant	0	0	0	0	1	1	
Surfactant	0	0	0	0	1	1	1

By using placebo and API, a smaller correlation can be obtained (95%). If only one placebo is used, correlation between the components would be high because all of them would be directly related: if one increases, the other one will decrease. The use of more than one placebo is applied so that when API increases, not all of the excipients will have a decrease in concentration in the same way. This approach makes it a more sensitive method, when related to changes in drug concentration.

3.2 Sample Preparation

Nine different placebos, as shown in Table 3.3 and 3.4, were needed in order to have the minimum correlation possible. Of all the components that are in the placebos, the lubricant, glidant, and surfactant were fixed because the quantities used in each one were so small that they wouldn't make a change in concentration. Each placebo, with its corresponding components, was placed in a methacrylate eight quart V-blender for 12 minutes at 15 revolutions per minute (Figure 1). After that period, a lubricant was added and the complete mixture was blended for another 3 minutes at 15 revolutions per minute.

Table 3.3. Weight of excipients in each of the placebos prepared

Placebo	Filler (g)	Binder (g)	Disintegrant (g)	Lubricant (g)	Glidant (g)	Surfactant (g)	Total (g)
1	326.342	71.706	85.957	7.509	6.507	2.007	500.028
2	398.850	71.700	13.457	7.508	6.507	2.046	500.068
3	326.355	87.652	70.070	7.510	6.504	2.084	500.174
4	382.902	87.648	13.443	7.504	6.503	2.006	500.005
5	382.902	87.648	13.443	7.504	6.503	2.006	500.005
6	326.345	79.682	77.990	7.507	6.501	2.081	500.106
7	362.582	71.710	49.639	7.502	6.512	2.001	499.946
8	390.880	79.674	13.451	7.520	6.504	2.072	500.101
9	358.534	79.684	45.501	7.507	6.539	2.032	499.797

Table 3.4. Percentages of each of the excipients in the placebos prepared

Placebo's Percentages (%)							
Placebo	**Filler**	**Binder**	**Disintegrant**	**Lubricant**	**Glidant**	**Surfactant**	**Total**
1	65.26%	14.34%	17.19%	1.50%	1.30%	0.40%	100
2	79.77%	14.34%	2.69%	1.50%	1.30%	0.41%	100
3	65.27%	17.52%	14.01%	1.50%	1.30%	0.42%	100
4	76.58%	17.53%	2.69%	1.50%	1.30%	0.40%	100
5	76.58%	17.53%	2.69%	1.50%	1.30%	0.40%	100
6	65.27%	15.93%	15.59%	1.50%	1.30%	0.42%	100
7	72.51%	14.34%	9.93%	1.50%	1.30%	0.40%	100
8	78.17%	15.93%	2.69%	1.50%	1.30%	0.41%	100
9	71.70%	15.94%	9.10%	1.50%	1.31%	0.41%	100

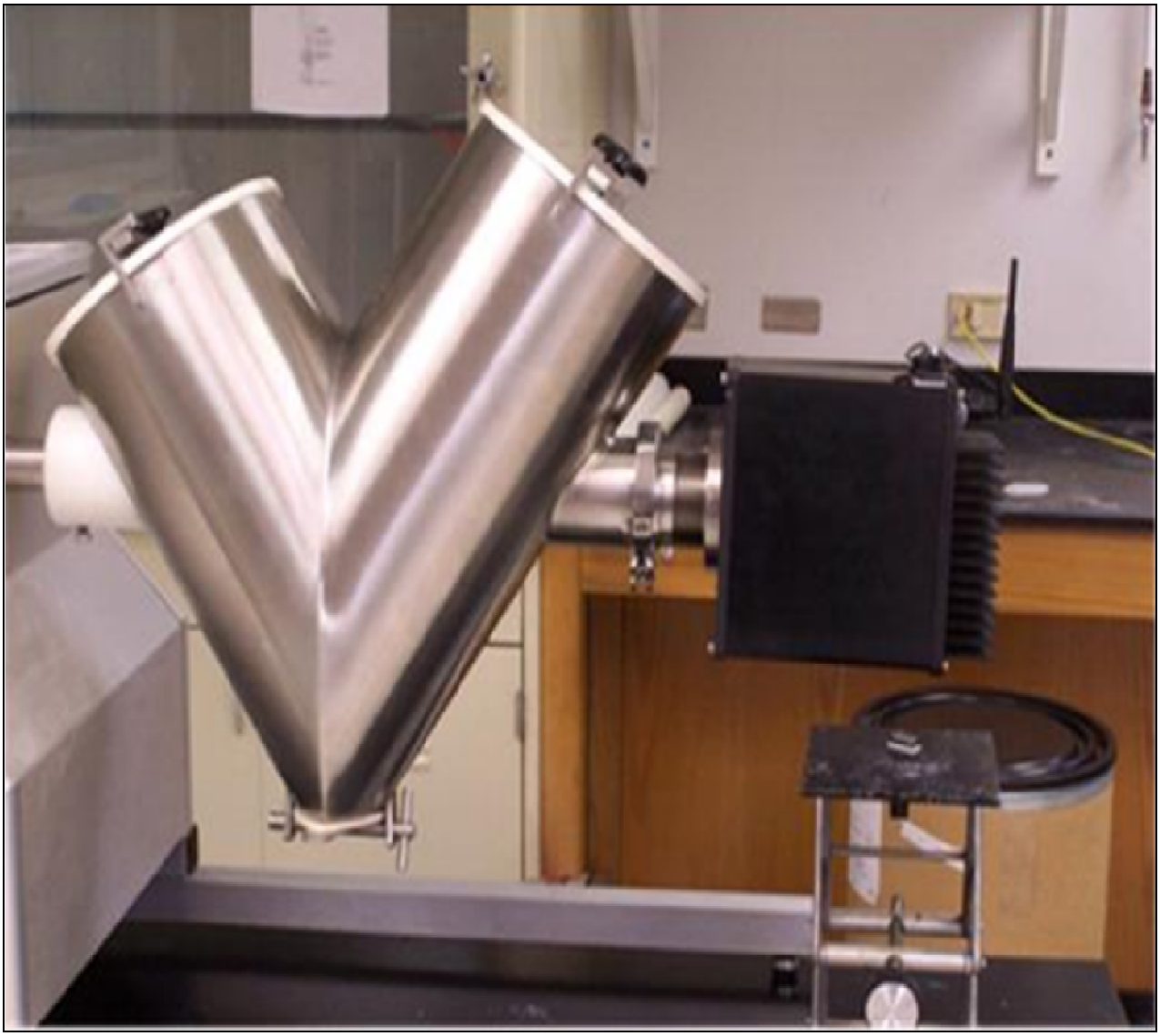

Figure 3.1. V-blender used to mix the placebos with NIR infrared spectrometer attached.

A total of 41 mixtures were prepared. These mixtures consisted of API and placebo[4,16,19] (all the components from the final mixture except the API). The matrix of concentrations of the mixtures have a design that covers from 45% to 85% w/w (±20%[25] relative over 65% w/w) and contains API and determined quantities of each of the nine placebos that were prepared. This concentration range was used to introduce the chemical composition variability found in the

samples[4,19]. With this approach, it can be ensured that the determination of the production samples will be accurate.

The final mixtures were prepared adding different quantities of API and of one of the placebos and mixing them in a 1 liter plastic bottle with a total of 10 revolutions (Table 3.5).

Table 3.5. Quantities of drug and placebo for the mixtures prepared

Placebo	Sample	Drug Concentration	API (g)	Placebo (g)	Total (g)
1	1	45	45	55	100
	10	54	172	146.5	318.5
	19	63	137	80	217
	28	72	180	70	250
	37	81	114	27	141
2	2	46	101	118	219
	11	55	89	73	162
	20	64	109	61	170
	29	73	134	50	184
3	3	47	93	105	198
	12	56	147	115.5	262.5
	21	65	173	93	266
	30	74	96	34	130
	38	82	195	43	238
4	4	48	54	58.5	112.5
	13	57	59	45	104
	22	66	98	50	148
	31	75	189	63	252
5	5	49	76	79	155
	14	58	110	80	190
	23	67	88	43	131
	32	76	143	45	188
	39	83	133	27	160
6	6	50	60	60	120
	15	59	78	54	132
	24	68	198	93	291
	33	77	159	47.5	206.5
	40	84	166	32	198
7	7	51	70	67	137
	16	60	162	108	270
	25	69	177	80	257
	34	78	127	36	163
	41	85	99	17.5	116.5
8	8	52	87	80	167
	17	61	149	95	244
	26	70	122	52	174
	35	79	191	51	242
9	9	53	55	49	104
	18	62	85	52	137
	27	71	105	43	148
	36	80	100	25	125

Of the 41 mixtures prepared with placebo and API, 29 samples were used for the calibration set (Table 3.6) and 12 for the prediction set (Table 3.7). The range of concentrations (from 45% to 85%) was chosen to differentiate the variation in drug concentration that can be found in a blending process in the pharmaceutical industry[26].The calibration set covers the whole spectra range and was chosen randomly as shown in the PCA scores plot shown in Figure 3.2.

Table 3.6. Composition of calibration set samples.

Sample Number	Concentration	Number of Placebo Used
1	45	1
3	47	3
4	48	4
5	49	5
7	51	7
8	52	8
10	54	1
11	55	2
13	57	4
14	58	5
15	59	6
17	61	8
19	63	1
20	64	2
21	65	3
22	66	4
23	67	5
25	69	7
26	70	8
27	71	9
29	73	2
31	75	4
32	76	5
33	77	6
35	79	8
37	81	1
38	82	3
40	84	6
41	85	7

Table 3.7. Composition of Prediction Set samples

Sample Number	Concentration	Number of Placebo Used
2	46	2
6	50	6
9	53	9
12	56	3
16	60	7
18	62	9
24	68	6
28	72	1
30	74	3
34	78	7
36	80	9
39	83	5

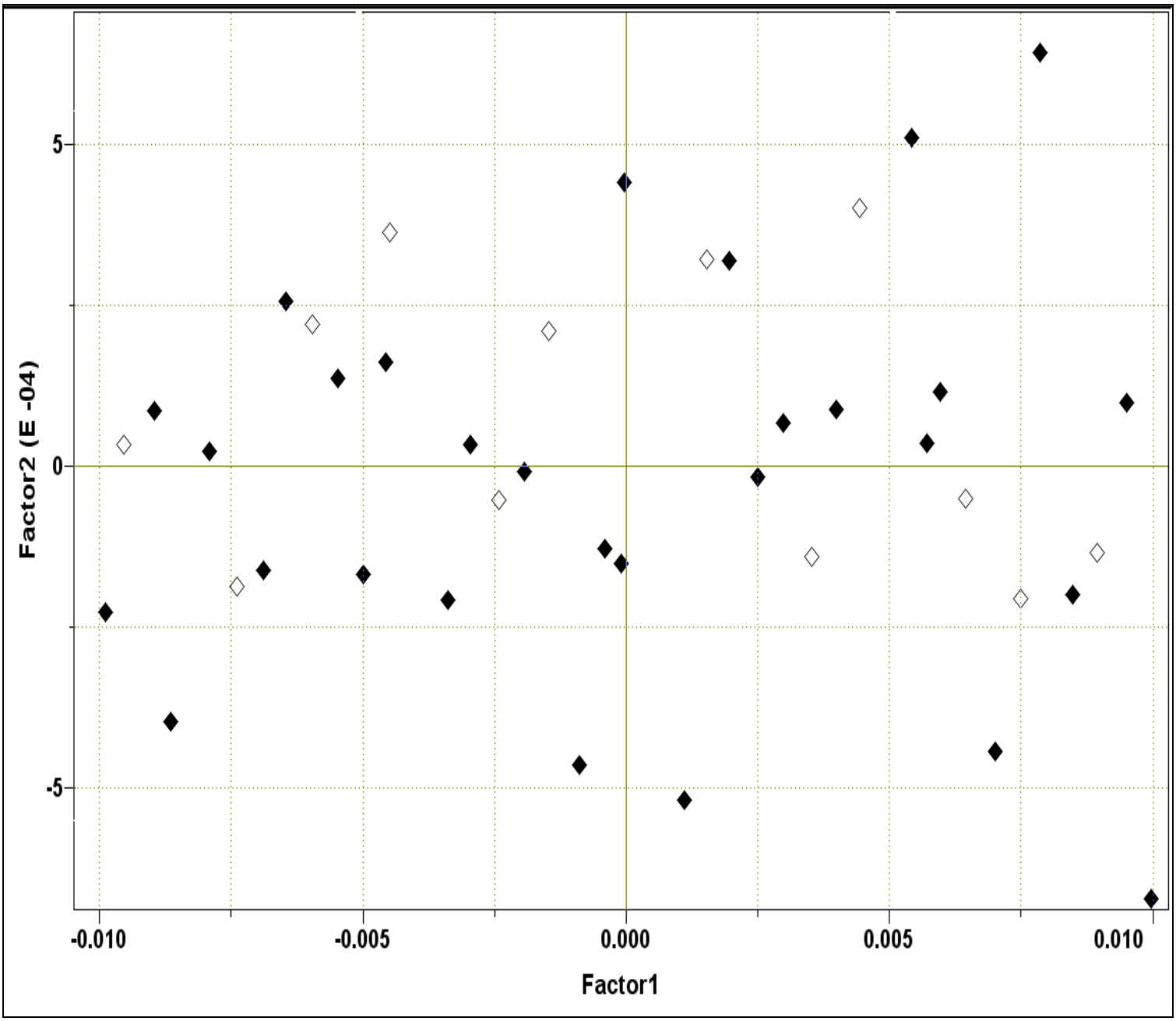

Figure 3.2. Selection of Calibration (black squares) and Prediction (white squares) Sets

Five samples (validation samples) of an API mixture + API (doped samples) were prepared using an API mixture, as obtained from a pharmaceutical industry, and different quantities of API to obtain the variation in drug concentration shown in Table 3.8. Each sample consisted of 95 grams of an API mixture plus different quantities of API. The drug concentration in these samples ranged from 64 – 68 % (w/w).

Table 3.8. Quantities of granulation and API used to prepare the Doped Samples

Sample	**Granulation (g)**	**API (g)**	**API Concentration**
Doped Sample #1	95.2377	2.79257	64.0
Doped Sample #2	95.4931	5.76268	65.0
Doped Sample #3	95.0415	8.91149	66.0
Doped Sample #4	95.0377	12.26721	67.0
Doped Sample #5	95.0959	15.83742	68.0

Eight formulation mixes were prepared to validate the calibration model created (Table 3.9). These samples consisted of an API mixture, as received from a pharmaceutical plant, and the compression mix done in the laboratory. The compression mixes were prepared mixing the API mixture, a filler, a binder, a disintegrant, a glidant, and a surfactant in a methacrylate lab scale V-blender for 12 minutes at 15 rpm. The lubricant used was then added and the mixture was left in the blender for another 3 minutes at 15rpm. Approximately, one hundred grams were prepared of each compression mix.

Table 3.9. Quantities of API and each of the excipients used to prepare the Formulation Samples

Sample #	API (g)	Filler (g)	Disintegrant (g)	Lubricant (g)	Glidant (g)	Surfactant (g)	Total (g)	Concentration (% API)
1	102.50	0.98	0.58	0.60	0.20	0.16	105.02	63.0
2	102.50	0.98	0.58	0.60	0.20	0.16	105.02	63.0
3	102.50	0.98	0.58	0.60	0.20	0.16	105.02	63.0
4	102.50	0.98	0.58	0.60	0.20	0.16	105.02	63.0
5	101.00	0.35	0.25	0.60	0.20	0.16	102.56	64.0
6	99.00	1.20	0.83	0.60	0.20	0.16	101.99	65.0
7	101.00	1.06	0.30	0.60	0.20	0.16	103.32	63.7
8	101.00	1.16	0.58	0.60	0.20	0.16	103.70	63.3

A total of 15 samples of an API mixture were prepared to be used as a validation set for the calibration model created (Table 3.10). Each sample consisted of 100 grams of an API mixture as obtained from a pharmaceutical industry.

Table 3.10. API Mixtures (granulation samples) used to validate the Calibration Model

Sample	API Concentration %(w/w)
1	65.0
2	65.0
3	65.0
4	65.0
5	65.0
6	65.0
7	65.0
8	65.0
9	65.0
10	65.0
11	65.0
12	65.0
13	65.0
14	65.0
15	65.0

A PCA comparison of the calibration model versus the three sets of samples used to validate the model is shown in Figure 3.3. The pretreatments Smooth + 1st derivative were applied because these were the pretreatments chosen for the calibration model.

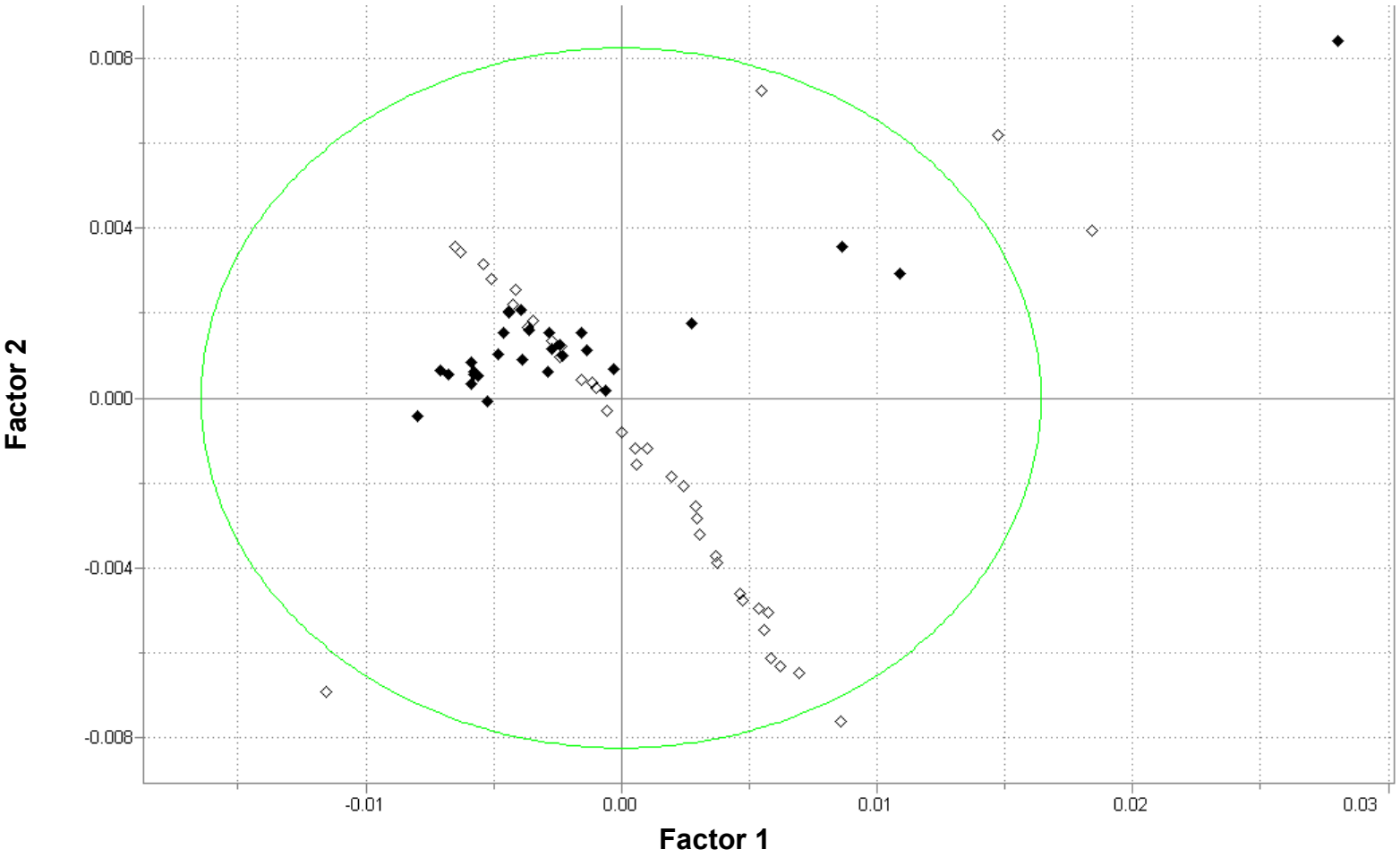

Figure 3.3. PCA comparison of the calibration model (green squares) versus the three sets of samples used to validate the calibration model (purple squares).

3.3 Acquisition of NIR Spectra

NIR spectra were acquired with a Blend Uniformity Monitor (CDI, South Bend, IN). This instrument uses an InGaAs diode array detector covering the 908 – 1687 nm spectral range. Spectra were acquired with 32 average samples and the fill buffer show average command, an integration time of about 0.003 seconds. They were stored in .spc format with the CDI Spec 32 software, and were then analyzed by using Pirouette 4.0 software (Infometrix, Bothell, WA). The spectral data was transposed using the Origin software to Excel 2003 (Microsoft Corporation, Seattle,WA) software, and graphs representing significant spectral regions were made. All spectra were mean centered for principal component analysis (PCA).

Samples were placed close to the NIR window by using 8 stainless steel plates as shown in Figure 3.4. All spectra were obtained using metal plates. The objective of this application is to predict samples in real-time during the preparation of the compression mix. The V-blender has a sapphire window through which the spectra are taken during mixing; hence, spectra were obtained in close proximity to simulate the future real-time determination of drug content.

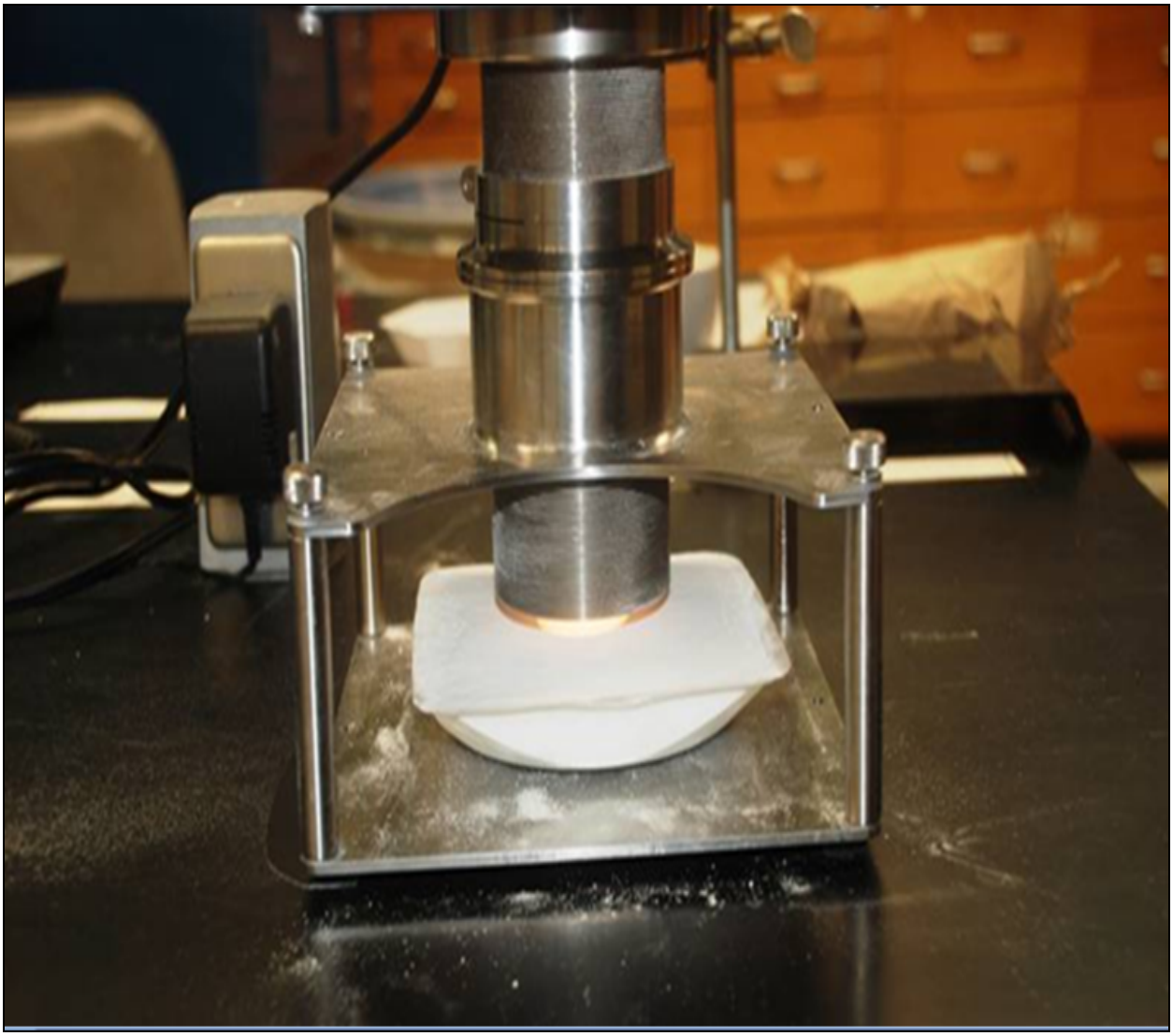

Figure 3.4. Set up used to acquire spectra for development of calibration model

Chapter 4

4.1 Calibration Model Samples

Nine placebos were prepared to achieve the minimum correlation possible. A total of 41 mixtures were prepared consisting of API and placebo in a concentration range of 45% to 85%. Of these 41 mixtures, 29 were used for the calibration set and 12 for the prediction set. An additional 5 samples were prepared containing an API mixture plus more API with a concentration range of 64% to 68%. These additional 5 samples were used to validate the calibration model prepared.

4.2 Model Development

Various pretreatments were performed to obtain an efficient calibration model. The pretreatments performed were SNV, 1st derivative, 2nd derivative[4,6,7,16,18], smooth, and some of the possible combinations that result from these pretreatments (SNV + 1st derivative, SNV + 2nd derivative, smooth + SNV, smooth + 1st derivative, smooth + 2nd derivative, smooth + SNV+ 1st derivative, smooth + SNV+ 2nd derivative). Second derivative was applied to enhance the resolution by removing the overlapping peaks and correcting the baseline[18] and the Standard Normal Variate (SNV) correction was applied to remove the major effects of light scattering[18]. SNV as well as the derivatives were applied so that the physical properties of the samples would not affect the predictions of the model[15]. These pretreatments were done using different spectral ranges. The different spectral ranges were used in order to only observe changes in spectra related to changes in concentration of API. If the whole spectra range were used to create the calibration model (Table 4.1), it would take into consideration spectral ranges that do not show changes in concentration and also areas with high noise near the detector cut-off.

After the calibration set was used to predict the prediction set, all of the samples were used to create the final calibration model. The spectral range chosen was 1123 – 1422 and the pretreatment used was smooth (5 points) + First Derivative. The same pretreatment was used to verify the model chosen when predicting the prediction set. The region 1123 – 1422 was chosen because the spectral change that takes place in those areas is directly related to changes in API concentration as is shown in Figure 4.1.

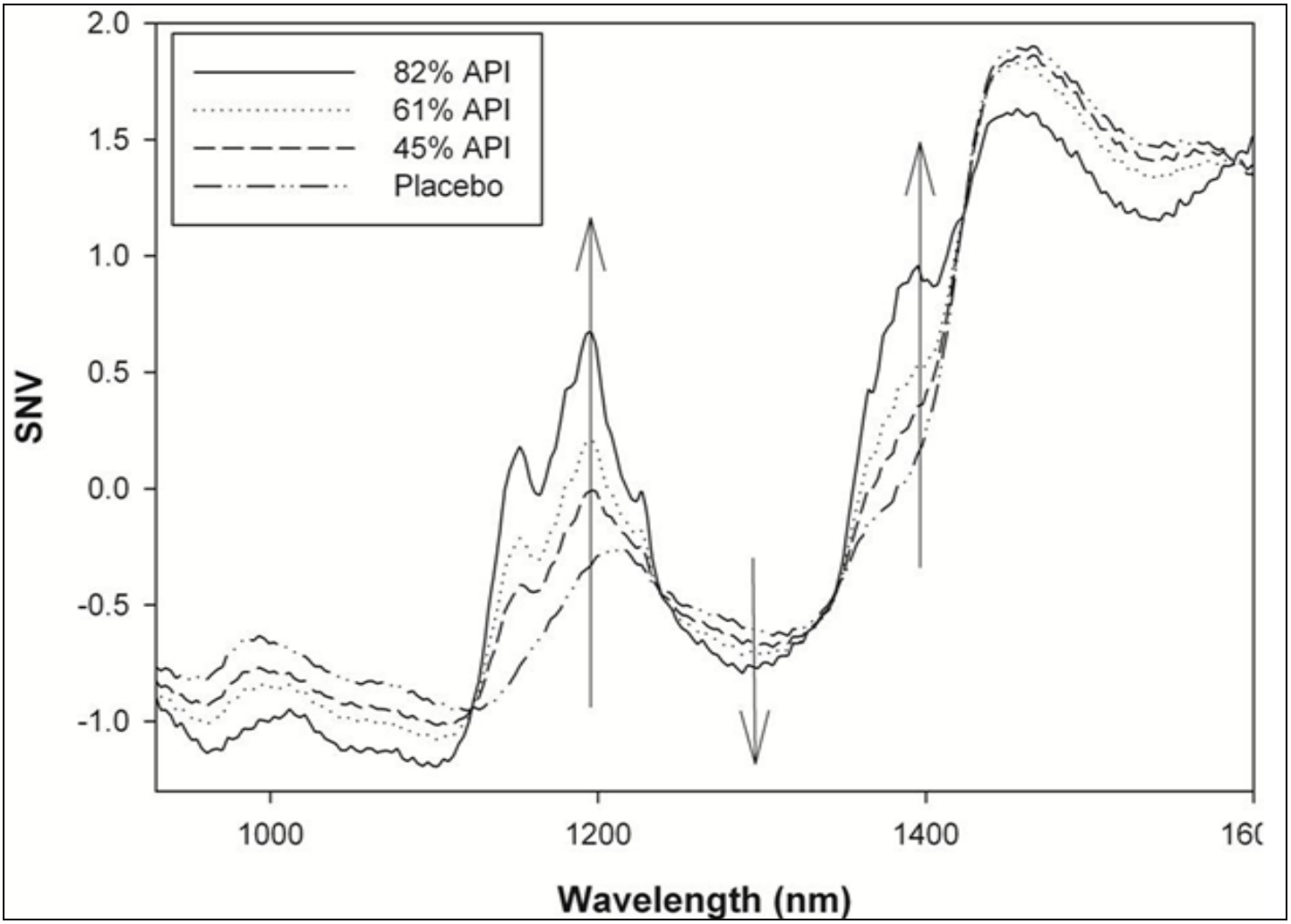

Figure 4.1. Changes in API concentration to show spectral range

Usually, the whole spectrum range is used to develop a PLS regression, but sometimes it is more useful to use smaller wavelengths. These smaller regions are usually chosen because of their high regression coefficient values, which summarize the relationship between all the predictors and a specific response[17].

Table 4.1. Samples used to create the Calibration Model

Sample Number	Concentration	Number of Placebo Used
1	45	1
2	46	2
3	47	3
4	48	4
5	49	5
6	50	6
7	51	7
8	52	8
9	53	9
10	54	1
11	55	2
12	56	3
13	57	4
14	58	5
15	59	6
16	60	7
17	61	8
18	62	9
19	63	1
20	64	2
21	65	3
22	66	4
23	67	5
24	68	6
25	69	7
26	70	8
27	71	9
28	72	1
29	73	2
30	74	3
31	75	4
32	76	5
33	77	6
34	78	7
35	79	8
36	80	9
37	81	1
38	82	3
39	83	5
41	85	7

The goodness of the predictions were assed using the Relative Standard Error of Prediction (RSEP)[6]. The equation used is as followed:

$$RSEP = \sqrt{\frac{\sum (C_{ref} - C_{pred})^2}{\sum C_{ref}^{\ 2}}} \quad \textbf{(3)}$$

Where C_{ref} is the concentration used as reference and C_{pred} is the concentration predicted.

The quality, as previously done in other works[16,18,19], was checked by calculating the residual error (Bias), PRESS, and the Standard Error of Prediction (SEP), with the following equations:

$$BIAS = \frac{\sum (C_{ref} - C_{pred})^2}{n} \quad \textbf{(4)}$$

Where C_{ref} is the concentration used as reference, C_{pred} is the concentration predicted and n is number of samples.

$$PRESS = \sum (C_{ref} - C_{pred})^2 \quad \textbf{(5)}$$

Where C_{ref} is the concentration used as reference, C_{pred} is the concentration predicted.

$$SEP = \frac{PRESS}{n} \quad \textbf{(6)}$$

Where n is number of samples.

It was found that 3 of the samples (#14 – 84% API, #33 – 64% API, #15 – 85% API) were out of range (not well predicted) with the pretreatment previously chosen as shown in Figure 4.2 in the Y-Fit Plot obtained with Pirouette.

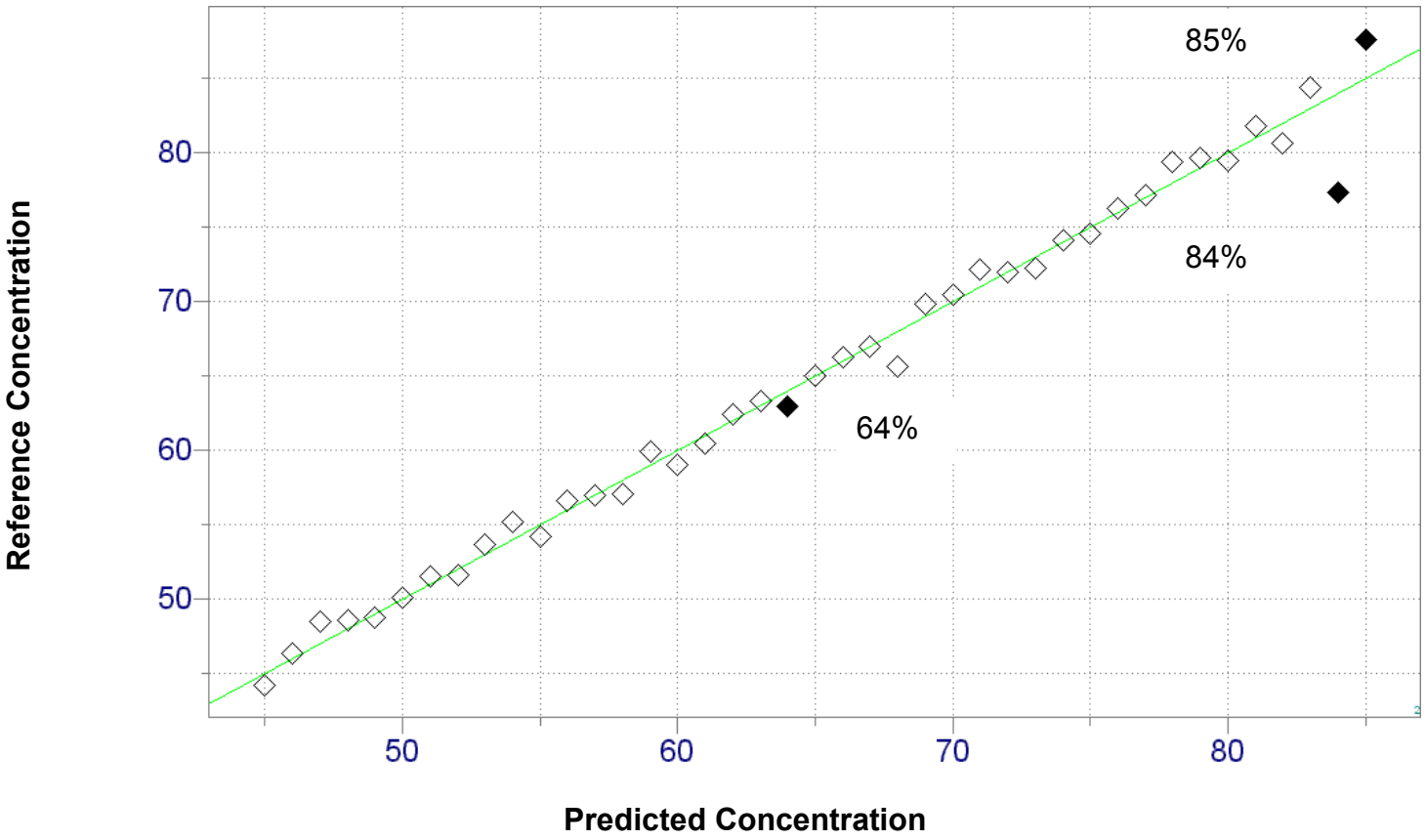

Figure 4.2. Y-Fit Plot showing the samples found that fall out of range (are not well predicted).

Several parameters were evaluated to determine whether the samples could be considered as outliers and eliminated from the model. After evaluating the Mahalanobis distance and the Studentized Residual, shown in Figure 4.3, it was decided that sample #14 (84% drug) could be eliminated because it had a small Mahalanobis distance and it was out of the studentized residual range (ranges from -2 to 2).

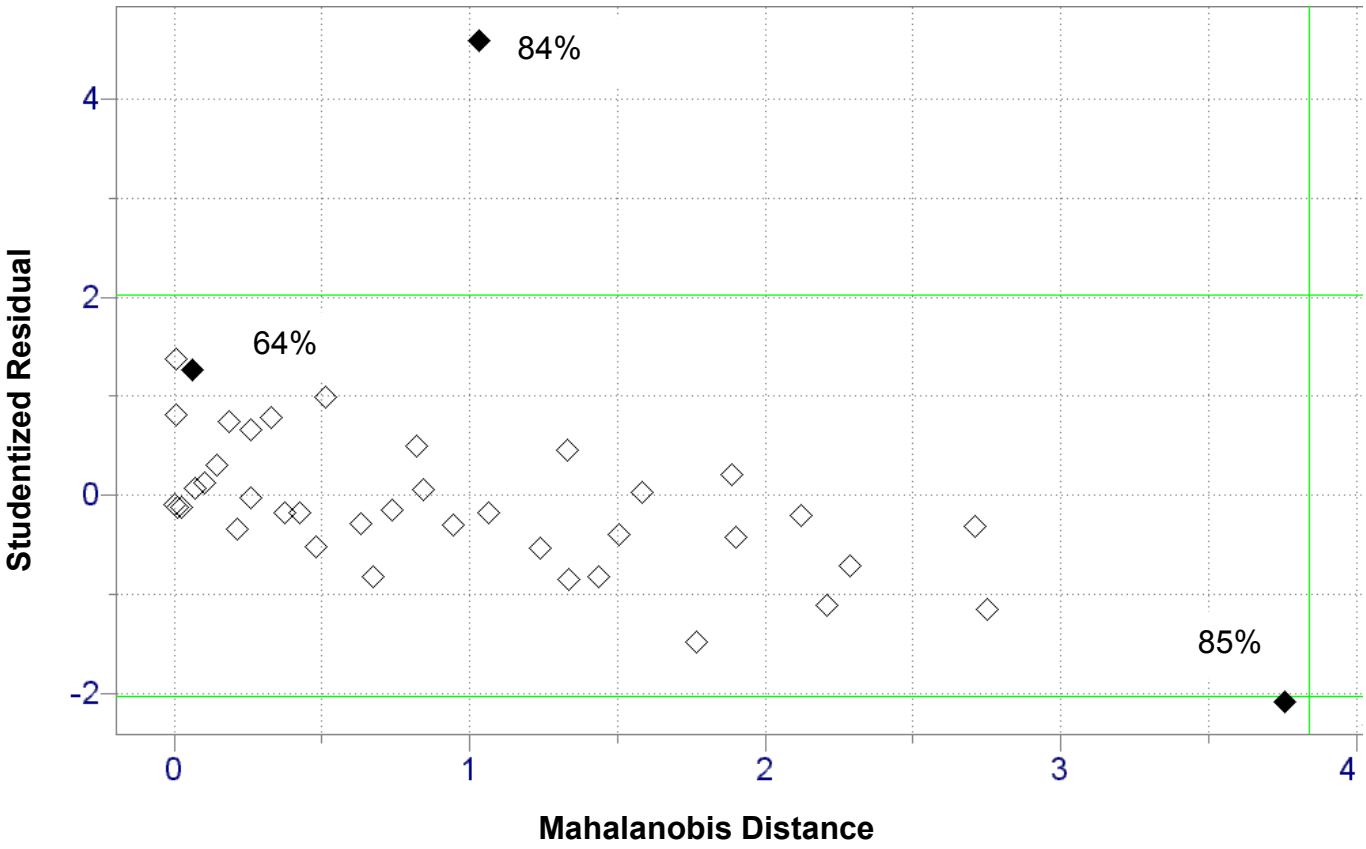

Figure 4.3. Mahalanobis Distance and Studentized Residual used to evaluate the three samples that were not well predicted

By eliminating this sample it was found that the other two samples that were not well predicted now fell into the same range and could be left in the calibration model as demonstrated in Figure 4.4 with the Y-Fit Plot obtained from Pirouette.

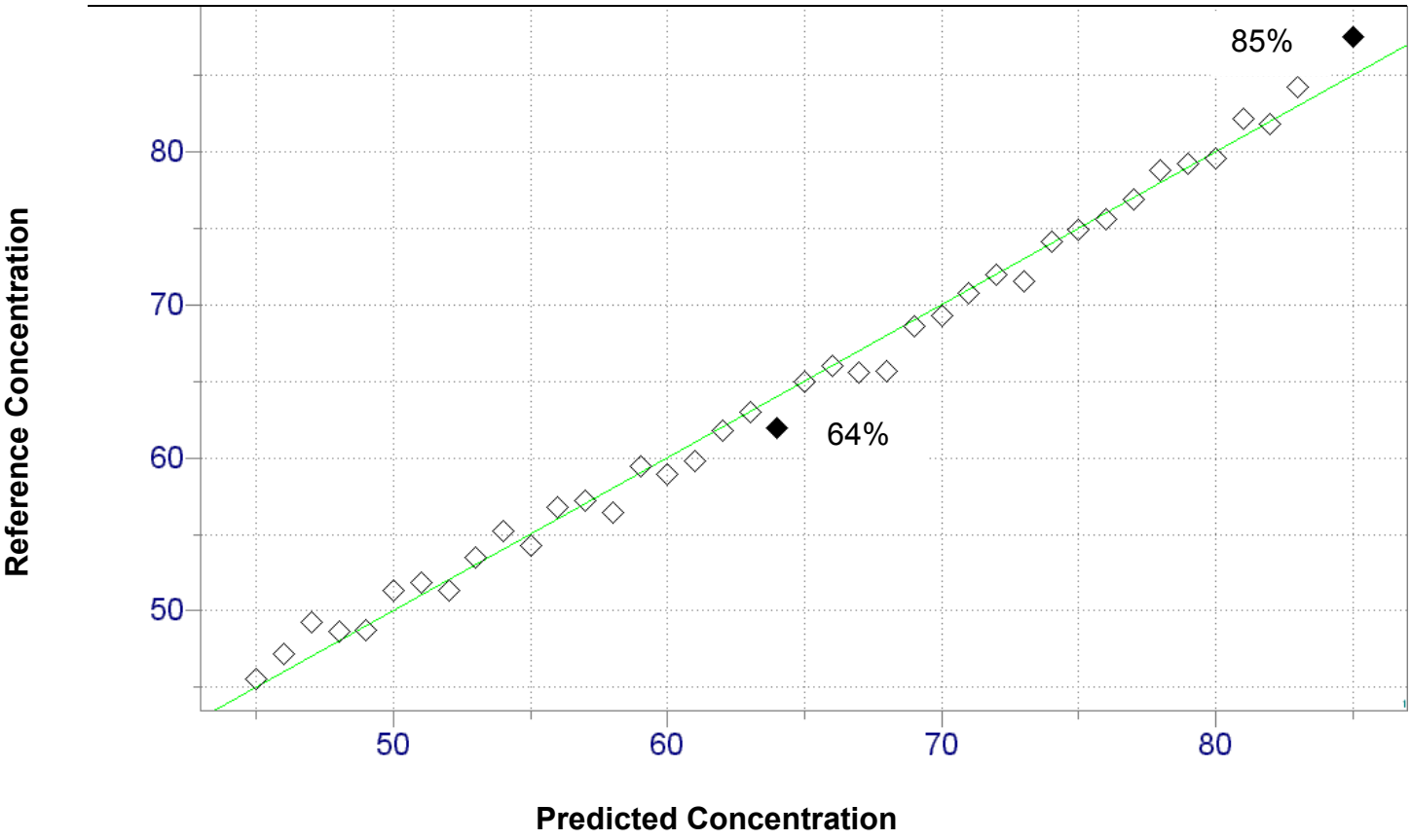

Figure 4.4. Y-Fit Plot without sample #14 (84% API). As it shows, when eliminating sample #14, the other two samples that were not well predicted before, now fall in range with the remaining samples.

None of the other two samples could be eliminated because sample #33 was inside the studentized residual and sample #15 had a big Mahalanobis distance. Experimentation also showed that none of the other two samples can be eliminated because they have a big impact on the rest of the samples used in the calibration model (Figures 4.5 and 4.6*)*. The elimination of this outlier is accepted because an approach that is based on the mean and the sample covariance matrix of the data is very sensitive to outliers and because methods based on this covariance matrix do not give good results in the presence of outlying measurements.

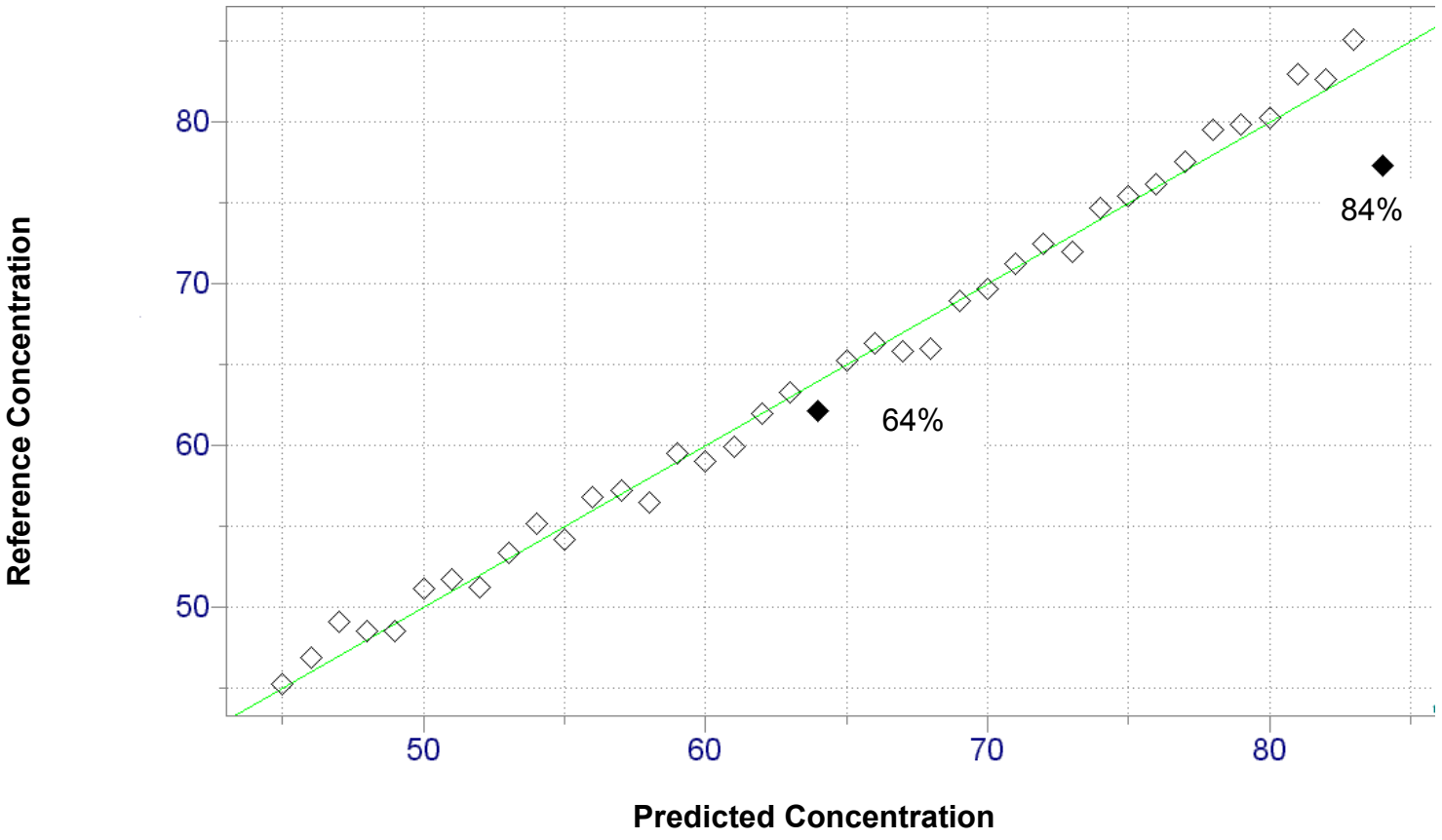

Figure 4.5. Y-Fit Plot without sample #15 (85% API)

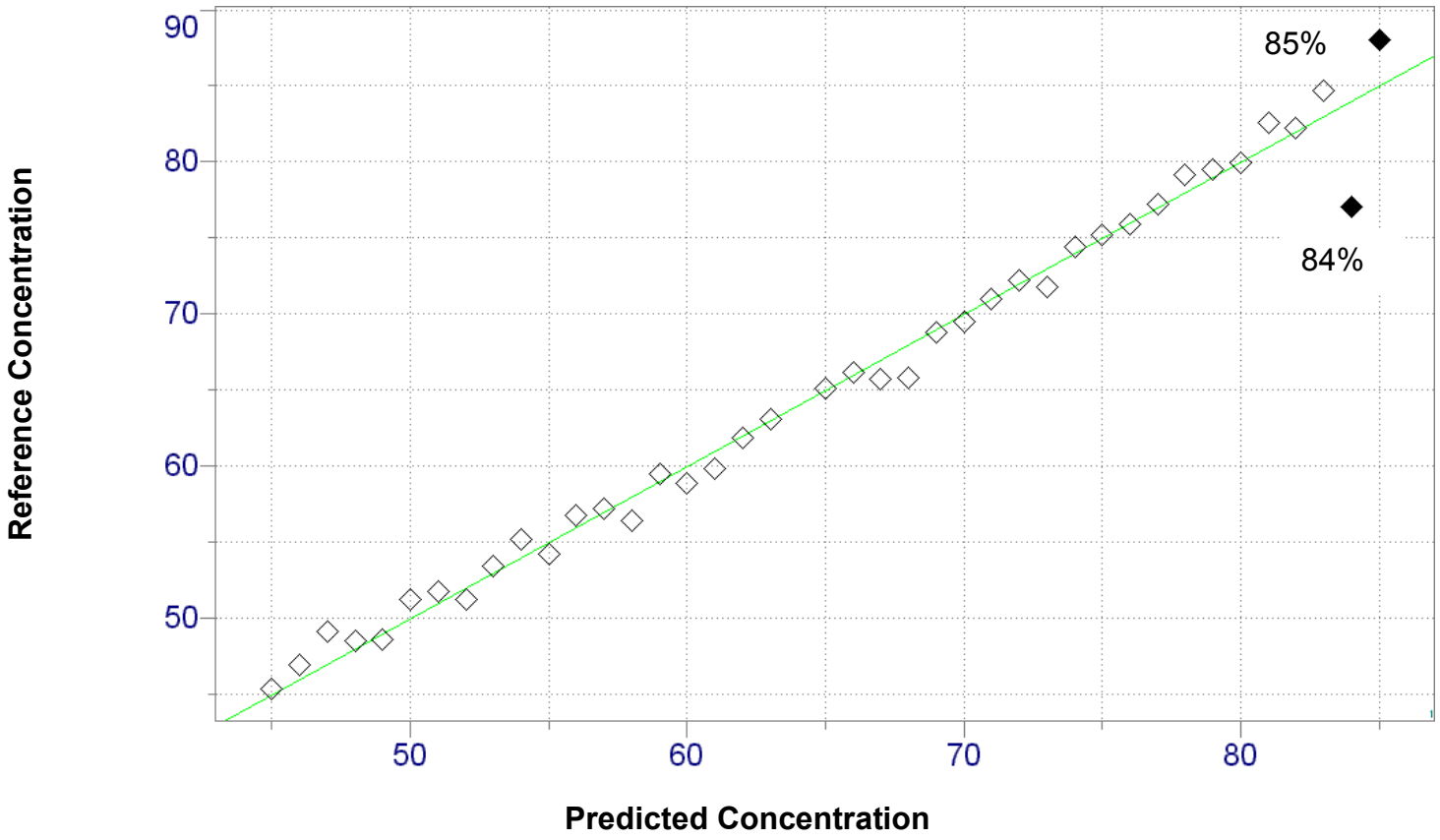

Figure 4.6. Y-Fit Plot without sample #33 (64% API)

Results on Table 4.2 show the predicted concentration for each sample in the calibration model which was developed using the leave-one-out cross validation method [4,16,18,19,27,28]. The bias (average residual value) for the predictions is -0.0065% which indicates that the predicted value is very similar to the predicted concentration for each sample used to validate the calibration model.

Table 4.2 Predictions with the calibration model (Leave-one-out Cross Validation)

Sample Concentration	Measured Y	Predicted Value	Sample Concentration	Measured Y	Predicted Value
45%	45.00	44.83	65%	65.00	64.95
46%	46.00	47.12	66%	66.00	65.86
47%	47.00	49.20	67%	67.00	66.17
48%	48.00	47.61	68%	68.00	65.46
49%	49.00	49.03	69%	69.00	69.44
50%	50.00	50.84	70%	70.00	69.93
51%	51.00	51.89	71%	71.00	71.58
52%	52.00	51.57	72%	72.00	71.82
53%	53.00	53.72	73%	73.00	71.74
54%	54.00	55.29	74%	74.00	73.89
55%	55.00	54.25	75%	75.00	74.52
56%	56.00	56.73	76%	76.00	75.91
57%	57.00	56.53	77%	77.00	76.70
58%	58.00	56.92	78%	78.00	79.10
59%	59.00	59.78	79%	79.00	79.38
60%	60.00	59.08	80%	80.00	78.92
61%	61.00	60.26	81%	81.00	81.68
62%	62.00	62.08	82%	82.00	80.33
63%	63.00	62.89	83%	83.00	84.35
64%	64.00	62.23	85%	85.00	87.68
RES VAL		**PRESS**	**RSEP**		**%RSEP**
-0.0065		42.21	1.03		1.57

4.2.1 Mahalanobis Distance

The Mahalanobis Distance is one of the most commonly used distance measures of the multivariate techniques. It can be calculated in the original variable space and in the principal component space and takes into account the correlation in the data, since it is calculated using the inverse of the variance – covariance matrix of the data set of interest.

The Mahalanobis Distance can also be calculated using a smaller number of latent variables obtained after the principal component analysis, instead of the original variables. In multivariate calibration, the Mahalanobis Distance is used to detect outliers, amongst other things, which can be observed on the regression line. Two types of outliers can be investigated before building the regression model. The first type of outliers (outliers in y), can be detected by only using the information in the y-axis. In this first case it is not necessary to use the Mahalanobis Distance to detect them. The second type (outliers in x), are identified using the information in the x-axis. The most used way of calculating outliers in the x-axis is by computing the squared Mahalanobis Distance between each point and the mean of the training set in the original variable space.

On the other hand, a studentized residual is the quotient resulting from division of a residual by an estimate of its standard deviation. Typically the standard deviations of residuals in a sample vary greatly from one data point to another even when the errors all have the same standard deviation, particularly in regression analysis; thus it does not make sense to compare residuals at different data points without first studentizing. It is a form of a Student's t-statistic, with the estimate of error varying between points. This is a very important technique used to detect outliers.

4.2.2 Validation of the Calibration Model

A total of 3 validation sets were prepared in order to validate the calibration model with different types of mixtures. One set of validation samples were prepared using doped samples. These predictions are very precise since the residual error is very small (-0.336), as shown in Table 4.3.

Table 4.3 Validation of Calibration Model Using Doped Samples

Sample	API Concentration	Prediction (% API)
Doped Sample #1	64.0	64.81
Doped Sample #2	65.0	65.50
Doped Sample #3	66.0	66.33
Doped Sample #4	67.0	66.76
Doped Sample #5	68.0	68.43
Average		66.36
Bias (residual error)		-0.366
PRESS		1.26
RMSEP		0.501
RSEP (%)		0.760

A second validation of the calibration model was done using granulation mixtures. Table 4.4 shows the predicted concentration for each sample. The results obtained are very encouraging.

Table 4.4 Validation of Calibration Model using Granulation mixtures

Sample	Concentration %(w/w) Ibuprofen	Prediction (% API)
Granulation #1	65.0	65.1
Granulation #2	65.0	62.2
Granulation #3	65.0	64.4
Granulation #4	65.0	61.6
Granulation #5	65.0	63.4
Granulation #6	65.0	62.4
Granulation #7	65.0	63.2
Granulation #8	65.0	64.3
Granulation #9	65.0	64.0
Granulation #10	65.0	62.9
Granulation #11	65.0	65.9
Granulation #12	65.0	63.7
Granulation #13	65.0	64.0
Granulation #14	65.0	62.9
Granulation #15	65.0	63.6
Average		63.6
Bias (average residual error)		1.44
PRESS		48.6
RMSEP		1.80
RSEP (%)		2.77

A third validation of the calibration model was done using formulation mixtures. These Formulation mixtures were prepared by mixing all of the components and the API, following the process that occurs in the pharmaceutical industry. Table 4.5 shows the predicted concentration for each sample.

Table 4.5 Validation of Calibration Model using Formulation mixtures

Sample	API Concentration (%)	API Prediction (%)
Formulation #1	62.0	61.85
Formulation #2	62.0	62.96
Formulation #3	62.0	61.84
Formulation #4	62.0	61.08
Formulation #5	63.0	61.99
Formulation #6	64.0	62.54
Formulation #7	62.7	61.81
Formulation #8	62.3	61.56
Average		61.95
Bias (Average Residual Error)		0.546
PRESS		6.31
RMSEP		0.888
RSEP %		1.42

As a way to verify sample with 84% (w/w) API, it was predicted with the calibration model. The results obtained were that the model predicted that this sample had a concentration of 76.74% API. The residual of this prediction is 7.26, which confirms that this sample was, in fact, compromised and can not be used in the calibration model.

Chapter 5

5.1 Comparison of Spectra inside and outside of V-Blender

This part is an additional study to validate the calibration model. These spectra include granulation, doped samples and formulation as shown in Table 5.1 with its respective concentrations. The comparison was made in order to show that the spectra used to validate the calibration model do not differ from the spectra taken inside the v-blender.

Table 5.1 Granulation, Doped Samples and Formulation samples used in the validation set

Sample	*Concentration*
Formulation #1	62.0
Formulation #2	62.0
Formulation #3	62.0
Formulation #4	62.0
Formulation #5	63.0
Formulation #6	64.0
Formulation #7	62.7
Formulation #8	62.3
Granulation #1	65.0
Granulation #2	65.0
Granulation #3	65.0
Granulation #4	65.0
Granulation #5	65.0
Granulation #6	65.0
Granulation #7	65.0
Granulation #9	65.0
Granulation #10	65.0
Granulation #11	65.0
Granulation #12	65.0
Granulation #13	65.0
Granulation #14	65.0
Granulation #15	65.0
Doped Sample #1	64.0
Doped Sample #2	65.0
Doped Sample #3	66.0
Doped Sample #4	67.0
Doped Sample #5	68.0

According to its definition, to dope is to affect with drugs or to apply or treat with dope. In this case, when doped samples are presented, they make reference to mixtures created with granulation (containing excipients and API previously prepared by the pharmaceutical that provided us with the samples) and different quantities of API, added in a specific way to achieve the concentration wanted. The concentrations of these doped samples ranged from 64 – 68 % (w/w).

Initially, all spectra were obtained in the static mode outside the v-blender but in this part, the spectra of the validation set were obtained inside the v-blender in static mode. The spectra were obtained by holding the blends against the window as shown in Figure 5.1 and against the sapphire window in interface coupling the V-blender and NIR spectrometer (Figure 5.2).

Figure 5.1 Method used to obtain spectra inside v-blender in static mode.

Figure 5.2 Sapphire window in interface coupling the V-blender and NIR spectrometer.

An initial comparison is shown in Figure 5.3. These spectra show the comparison made between the spectra recorded outside the v-blender and the spectra recorded inside the v-blender without the use of a pretreatment.

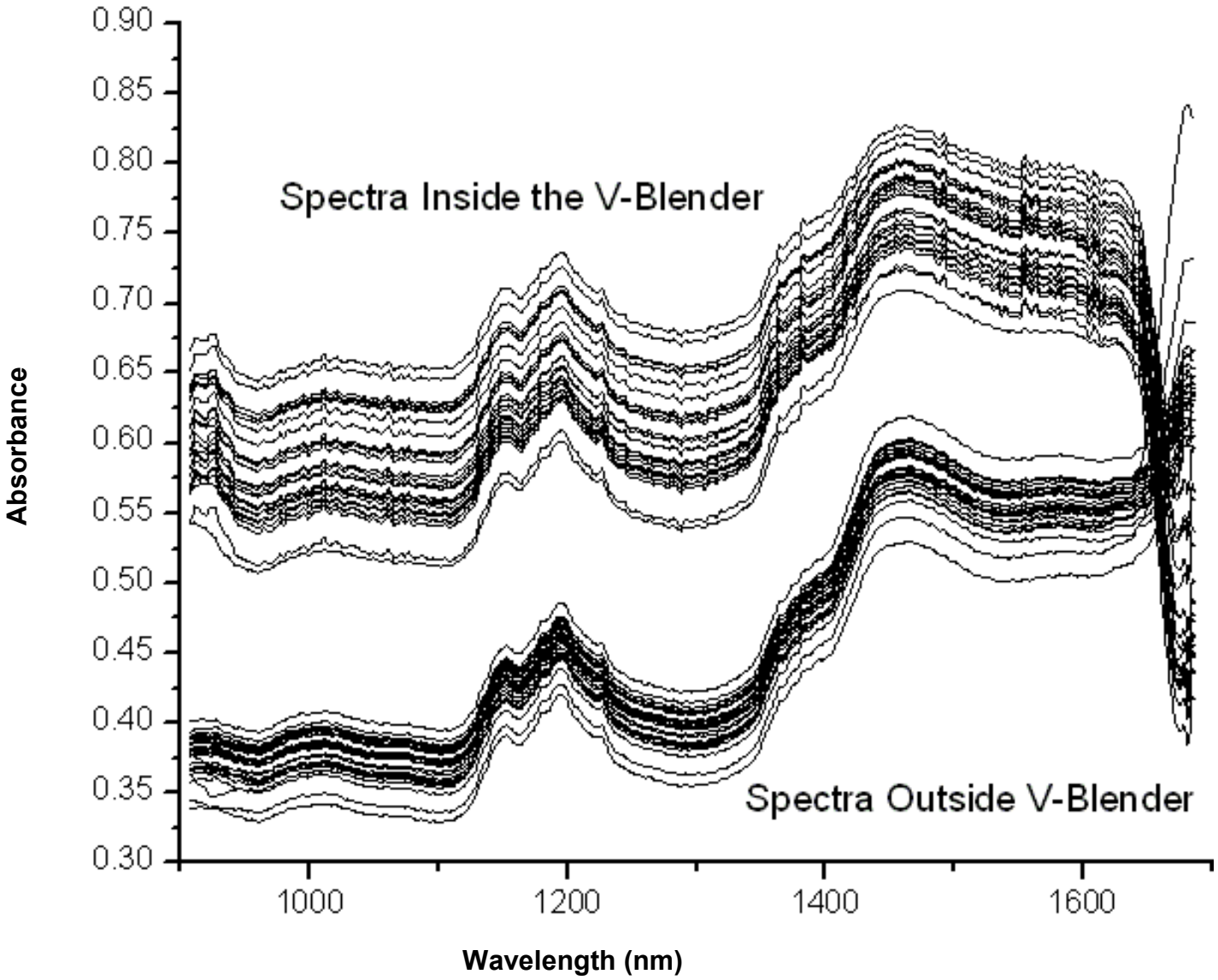

Figure 5.3 Spectra of samples taken inside and outside of v-blender in static mode

In these spectra, differences are observed between the spectra taken inside the V-blender and the spectra taken outside the v-blender when comparing the extremes. Another noticeable difference between the data can be seen in the baseline of the spectra.

To eliminate the baseline differences, a Standard Normal Variate (SNV) pretreatment was applied. The spectra taken outside of the v-blender is shown in Figure 5.4 using SNV as the pretreatment. This figure contains only the spectra taken outside the v-blender in static mode.

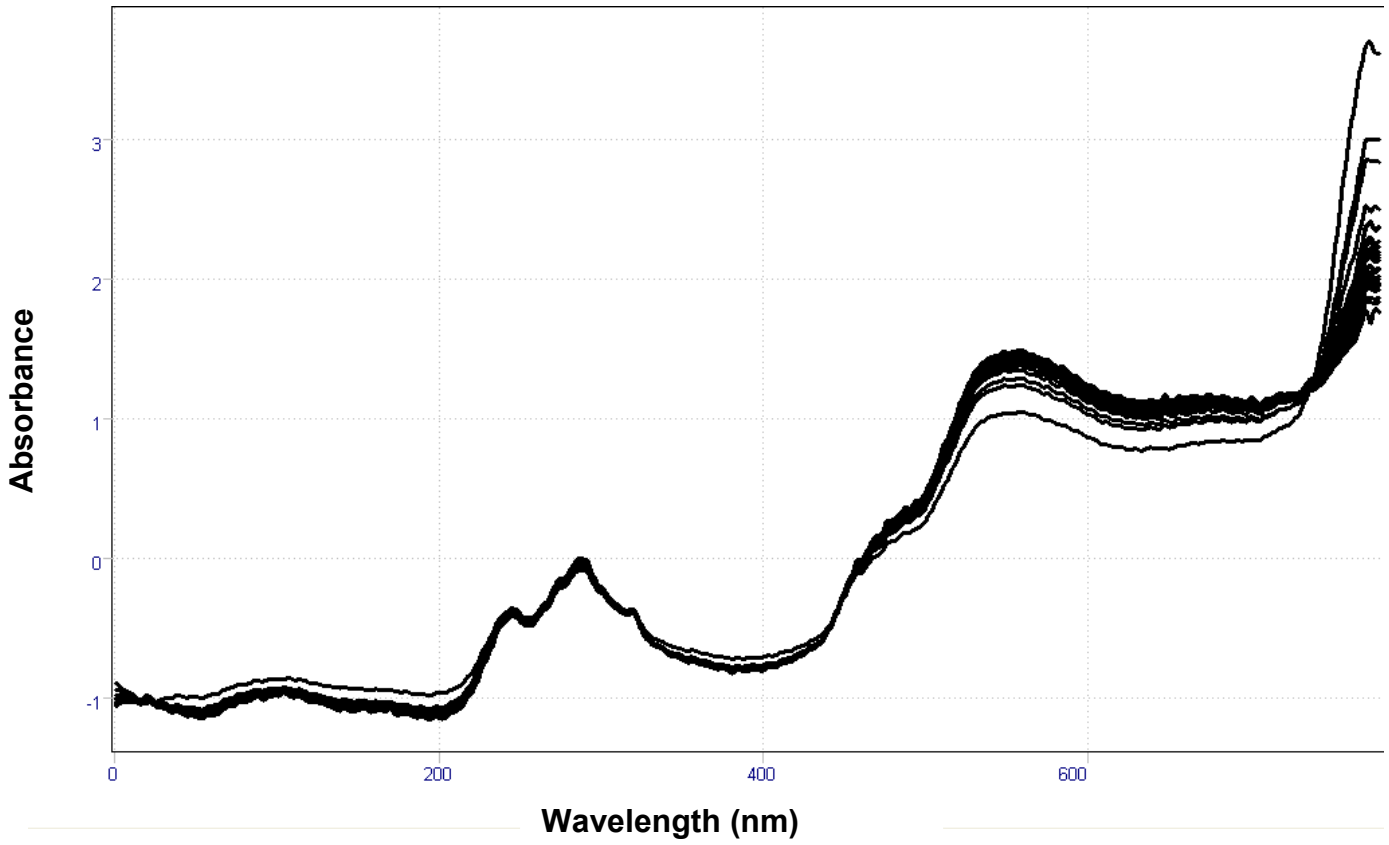

Figure 5.4 SNV Spectra of samples taken outside of v-blender in static mode

The spectra taken inside of the v-blender are shown in Figure 5.5 using SNV as the pretreatment. This figure contains only the spectra taken inside the V-blender in static mode.

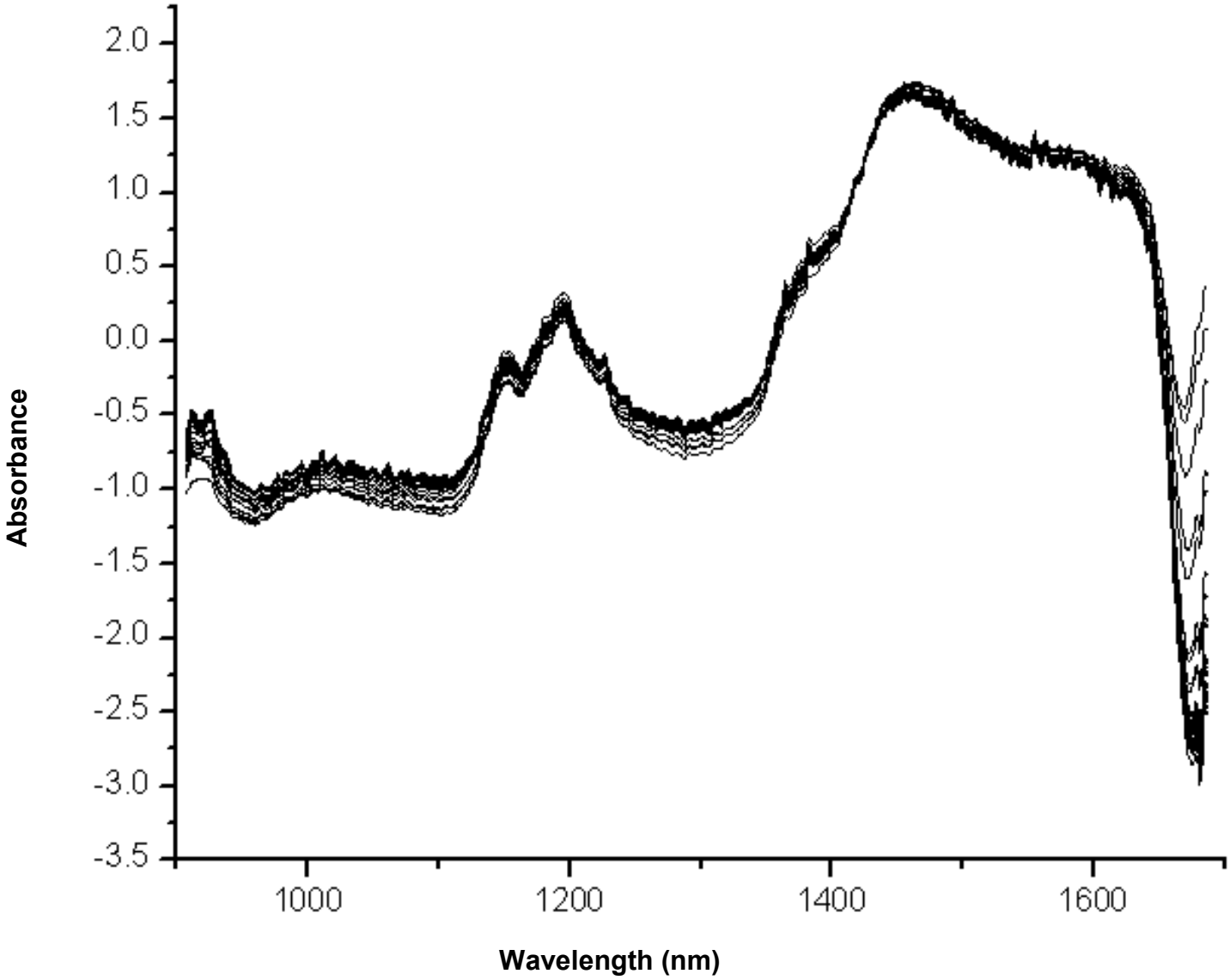

Figure 5.5 SNV Spectra of samples taken inside of v-blender in static mode

Figure 5.6 shows the spectra taken outside and the spectra taken inside the V-blender. The pretreatment used was SNV in order to eliminate their differences in baseline and be able to see the possible differences between the spectra. As can be seen, both sets of spectra are very similar. The marked differences between both set of spectra can be seen at the high wavelengths (detector cut off). The relative small differences between the rest of the spectra can be due to the sapphire crystal found inside the v-blender which goes between the NIR and the sample.

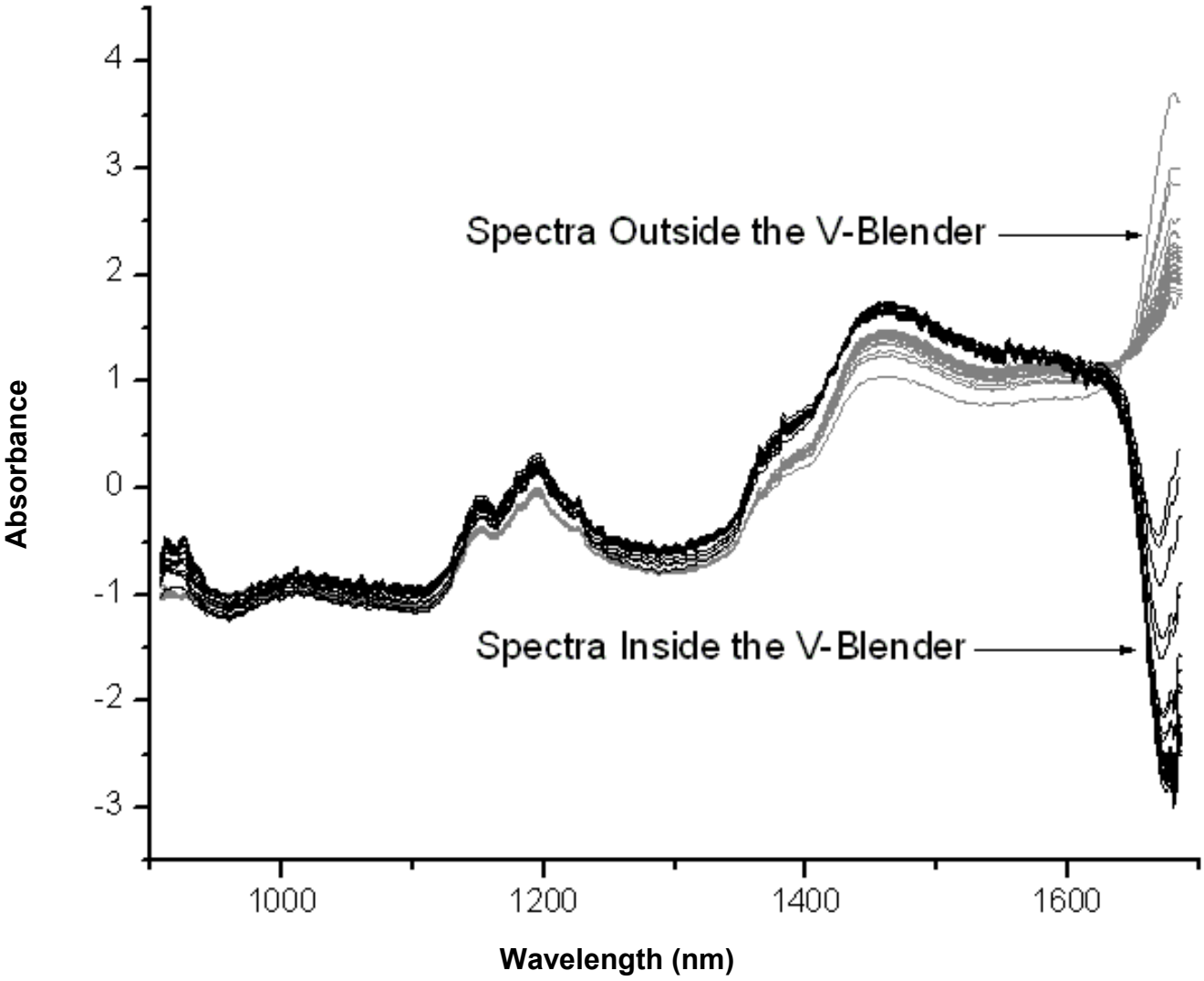

Figure 5.6 SNV Spectra of samples taken outside and inside of v-blender in static mode

If the extremes of the spectra are cut, the differences are minimized between the two sets of samples as can be seen in Figure 5.7.

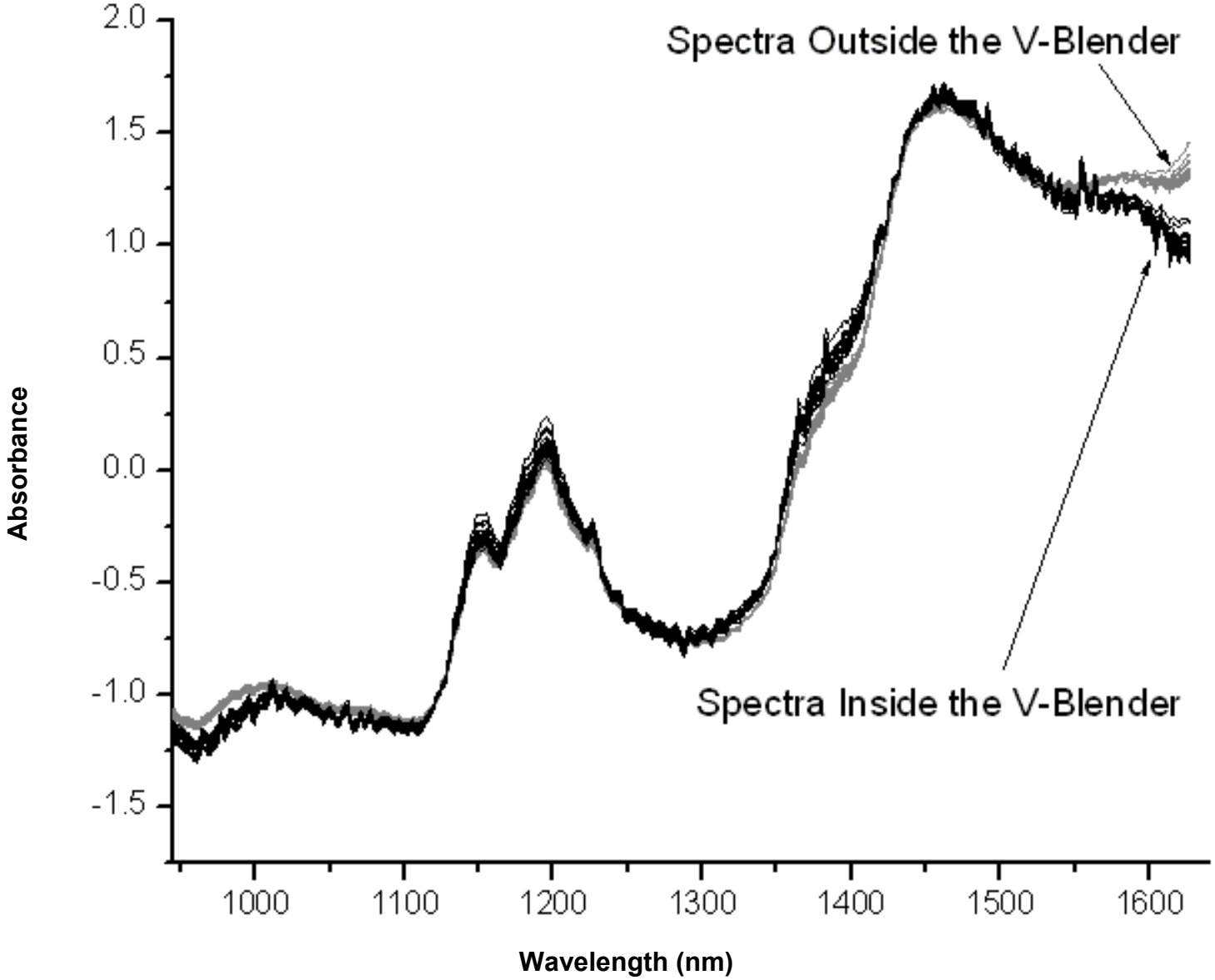

Figure 5.7 SNV Spectra outside and inside of v-blender without extremes

By comparing the spectra taken inside the V-blender and the spectra taken outside the v-blender, it can be deduced that there are no significant differences between them. This implies that the calibration model created using the spectra of the samples taken outside the v-blender can be used to predict the spectra of the samples taken inside the v-blender.

The visual comparison already indicated that the differences between the spectra taken inside the V-blender and the spectra taken outside the v-blender are not significant. To corroborate this information it is necessary to do a quantitative comparison. The spectra taken outside the v-blender was already used to validate the calibration model. After comparing the spectra inside versus the spectra outside of the blender, the spectra obtained inside the v-blender was predicted in order to compare quantitatively both sets of spectra.

5.1.1 Validation of the Calibration Model using samples inside of v-blender

The spectra taken inside the v-blender was predicted using the spectral range from 1123 – 1422 and the pretreatment used for the calibration model was PLS + Smooth + First Derivative with two factors. The samples used consisted of granulation (Table 5.1.1), doped samples (Table 5.1.2) and formulation (Table 5.1.3).

Table 5.1.1 Concentration and prediction of the Granulation Samples using the Calibration Model developed

Sample	API Concentration (% w/w)	Prediction (% API)
Granulation #1	65.0	60.2
Granulation #2	65.0	63.4
Granulation #3	65.0	60.1
Granulation #4	65.0	62.1
Granulation #5	65.0	62.7
Granulation #6	65.0	65.1
Granulation #7	65.0	65.0
Granulation #8	65.0	65.4
Granulation #9	65.0	65.4
Granulation #10	65.0	67.3
Granulation #11	65.0	66.3
Granulation #12	65.0	64.7
Granulation #13	65.0	60.7
Granulation #14	65.0	66.1
Granulation #15	65.0	66.3
Average	65.0	64.0

Table 5.1.2 Quantities of Doped Samples and their prediction using the Calibration Model developed

Sample	Granulation (g)	API (g)	API Concentration (% w/w)	Prediction (% API)
Doped Sample #1	95.2377	2.79257	64.0	60.6
Doped Sample #2	95.4931	5.76268	65.0	64.6
Doped Sample #3	95.0415	8.91149	66.0	65.1
Doped Sample #4	95.0377	12.26721	67.0	65.2
Doped Sample #5	95.0959	15.83742	68.0	70.4
Average	N/A	N/A	N/A	65.2

Table 5.1.3 Quantities used to prepare the Formulation Samples and their predictions using the Calibration Model developed

Sample	API Base Granulation (g)	Filler (g)	Disintegrant (g)	Lubricant (g)	Glidant (g)	Surfactant (g)	Total (g)	Concentration (% API)	Prediction (% API)
1	102.50	0.98	0.58	0.60	0.20	0.16	105.02	62.0	57.44
2	102.50	0.98	0.58	0.60	0.20	0.16	105.02	62.0	56.49
3	102.50	0.98	0.58	0.60	0.20	0.16	105.02	62.0	58.71
4	102.50	0.98	0.58	0.60	0.20	0.16	105.02	62.0	59.05
5	101.00	0.35	0.25	0.60	0.20	0.16	102.56	63.0	61.79
6	99.00	1.20	0.83	0.60	0.20	0.16	101.99	64.0	59.54
7	101.00	1.06	0.30	0.60	0.20	0.16	103.32	62.7	60.57
8	101.00	1.16	0.58	0.60	0.20	0.16	103.70	62.3	60.63
Average	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	59.28

A deeper analysis was made by comparing the prediction of API in each of the samples inside and outside the v-blender using the data obtained previously (Tables 5.1.4, 5.1.5, 5.1.6)

Table 5.1.4 Comparison of Prediction of Granulation Samples inside and outside of the V-Blender

Sample	Concentration % (w/w) Ibuprofen	Prediction Outside V-Blender (%API)	Prediction Inside V-Blender (%API)	Residual Between Prediction Outside and Inside V-Blender	Residual Between Concentration and Prediction Outside V-Blender	Residual Between Concentration and Prediction Inside V-Blender
Granulation #1	65.0	65.1	60.2	4.9	-0.1	4.8
Granulation #2	65.0	62.2	63.4	-1.3	2.8	1.6
Granulation #3	65.0	64.4	60.1	4.4	0.6	5.0
Granulation #4	65.0	61.6	62.1	-0.4	3.4	2.9
Granulation #5	65.0	63.4	62.7	0.7	1.6	2.3
Granulation #6	65.0	62.4	65.1	-2.7	2.7	-0.1
Granulation #7	65.0	63.2	65.0	-1.8	1.8	0.1
Granulation #8	65.0	57.1	65.4	-8.3	7.9	-0.4
Granulation #9	65.0	64.0	65.4	-1.5	1.0	-0.4
Granulation #10	65.0	62.9	67.3	-4.4	2.1	-2.3
Granulation #11	65.0	65.9	66.3	-0.4	-0.9	-1.3
Granulation #12	65.0	63.7	64.7	-0.9	1.3	0.4
Granulation #13	65.0	64.0	60.7	3.3	1.0	4.3
Granulation #14	65.0	62.9	66.1	-3.2	2.1	-1.1
Granulation #15	65.0	63.6	66.3	-2.7	1.4	-1.3
Average	65.0	63.1	64.0	-1.0	1.9	1.0
Bias					1.4	1.0
PRESS					48.6	92.1
RMSEP					1.8	2.5
RSEP %					2.8	3.8

Table 5.1.5 Comparison of Prediction of Doped Samples inside and outside of the V-Blender

Sample	API Concentration (% w/w)	Prediction Outside V-Blender (%API)	Prediction Inside V-Blender (%API)	Residual Between Prediction Outside and Inside V-Blender	Residual Between Concentration and Prediction Outside V-Blender	Residual Between Concentration and Prediction Inside V-Blender
Doped Sample #1	64.0	64.8	60.6	4.2	-0.8	3.4
Doped Sample #2	65.0	65.5	64.6	0.9	-0.5	0.4
Doped Sample #3	66.0	66.3	65.1	1.2	-0.3	0.9
Doped Sample #4	67.0	66.8	65.2	1.5	0.2	1.8
Doped Sample #5	68.0	68.4	70.4	-1.9	-0.4	-2.4
Average	----------	66.4	65.2	1.2	-0.4	0.8
Bias					-0.4	0.8
PRESS					1.3	21.2
RMSEP					0.5	2.1
RSEP %					0.8	3.1

Table 5.1.6 Comparison of Prediction of Formulation Samples inside and outside of the V-Blender

Formulation Sample	API Concentration (% w/w)	Prediction Outside V-Blender (%API)	Prediction Inside V-Blender (%API)	Residual Between Prediction Outside and Inside V-Blender	Residual Between Concentration and Prediction Outside V-Blender	Residual Between Concentration and Prediction Inside V-Blender
Sample #1	62.0	61.9	57.4	4.4	0.2	4.6
Sample #2	62.0	63.0	56.5	6.5	-1.0	5.5
Sample #3	62.0	61.8	58.7	3.1	0.2	3.3
Sample #4	62.0	61.1	59.1	2.0	0.9	3.0
Sample #5	63.0	62.0	61.8	0.2	1.0	1.2
Sample #6	64.0	62.5	59.5	3.0	1.5	4.5
Sample #7	62.7	61.8	60.6	1.2	0.9	2.1
Sample #8	62.3	61.6	60.6	0.9	0.7	1.7
Average	----------	62.0	59.3	2.7	0.6	3.4
Bias					0.5	3.2
PRESS					6.3	99.4
RMSEP					0.9	3.5
RSEP %					1.4	5.6

The quantitative comparison shows differences between the results for spectra taken inside the v-blender and the spectra taken outside the v-blender. The differences found between the predictions obtained from the spectra taken inside the v-blender and the spectra taken outside the v-blender are relatively small since the average of them is ±3. These differences could be attributed to the positioning of the samples against the sapphire crystal inside the v-blender.

Chapter 6

Conclusions

In formulations where the concentrations of the components are high, there is always going to be a high correlation between two of the components, usually the two that have the highest concentrations. Since the total sum of the percent for each sample is 100, at least one of the percentages is linearly dependent on the others.

The decrease in correlation permitted the development of a calibration model in which components were not highly related one to another. This provided the necessary tools to develop a robust model to detect drug concentrations in a pharmaceutical process.

The calibration model provided promising results for the spectra taken inside of the V-Blender.

References

1. ICH-Q2A, Guideline for Industry: Text on Validation of Analytical Procedures, **1995**.
2. ICH-Q2B Guidance for industry: Q2B Validation of Analytical Procedures: Methodology, Food and Drug Administration, Rockville, Maryland, **1996**.
3. H. Mark, G. E. Ritchie, R. W. Roller, E. W. Ciurczak, C. Tso, S. A. MacDonald, Journal of Pharmaceutical and Biomedical Analysis, **2002**, 28, 251-260.
4. M. Blanco, M. Bautista, M. Alcala, Wiley InterScience, **2008**, Vol. 97, No. 3, 1236-1245.
5. C. Peroza, M. Santos, R. Romañach, AAPS PharmSciTech, **2006**, Vol 7 (1), Art. 29, E1-E9.
6. M. Blanco, J. Cruz, M. Bautista, Anal Bioanal Chem, **2008**, 392, 1267-1372.
7. M. Shivhare, G. McCreath, BioProcess International, **2010**, 22-30.
8. F. J. Muzzio, C. L. Goodridge, A. Alexander, P. Arratia, H. Yang, O. Sudah, G. Mergen, International Journal of Pharmaceutics, **2003**, 250, 51-64.
9. R.C. Lyon, D. S. Lester, E. N. Lewis, E. Lee, L. X. Yu, E. H. Jefferson, and A. S. Hussain, AAPS PharmSciTech, **2002**, 3 (3), article 17, 1-15.
10. M. Blanco, R. Gonzalez, E. Bertran, Talanta, **2002**, 56, 203-212.
11. L. Bellamy, A. Nordon, D. Littlejohn, Analyst, **2007**, 133, 58-64.
12. T. Shinbrot, F. J. Muzzio, Encyclopedia of Pharmaceutical Technology, **2002**, 1795-1810.
13. M. Popo, S. Romero, C. Conde, R. Romañach, AAPS PharmSciTech, **2002**, 3 (3), article 24, 1-11.
14. O. Berntsson, L.-G. Danielsson, B. Lagerholm, S. Folestad, Powder Technology, **2002**, 123, 185-193.
15. M. Blanco, J. Coello, A. Eustaquio, H. Iturriaga, S. Maspoch, Journal of Pharmaceutical Sciences, **1999**, Vol. 88, No. 5, 551-556.

16. M. Blanco, M. Alcalá, Analytica Chimica Acta, **2005**.
17. L. Stordrange, D. Malthe-Sørenssen, O. M. Høyland, Journal of Process Analytical Chemistry, 6-20.
18. M. Laasonen, T. Harmia-Pulkkinene, C. Simard, M. Rasanen, H. Vuorela, Analytical Chemistry, **2003**, Vol. 75, No. 4, 754-760.
19. M. Blanco, A. Peguero, Journal of Pharmaceutical and Biomedical Analysis, **2010**, 52, 59-65.
20. S. Sjövall, L. Hansens, B. Granquist, Organic Process Research and Development, **2004**, 8, 802-807.
21. J. M. Gonzálvez, J. C. García, Journal of Chemical Education, **2006**, Vol. 83, No. 4.
22. P. van de Ven, S. Bijlsma, E. Gout, K. van der Voort Maarschalk, U. Thissen, Journal of Pharmaceutical Innovation.
23. T. Isaksson, T. Næs, Applied Spectroscopy, **1990**, Vol. 44, No. 7, 1152-1158.
24. M. Blanco, J. Coello, H. Iturriaga, S. Maspoch, C. de la Pezuela, Applied Spectroscopy, **1997**, Vol. 51, No. 2, 240-246.
25. M. Alcala, J. Leon, J. Ropero, M. Blanco, R. Romañach, Journal of Pharmaceutical Sciences, **2008**, Vol. 97, No. 12, 5318-5327.
26. A. Vanarase, M. Alcalà, J. Jerez, F. Muzzio, R. Romañach, Chemical Engineering Science, **2010**, 65, 5728-5733.
27. M. Alcalà, M. Blanco, M. Bautista, J. Gonzalez, Journal of Pharmaceutical Sciences, **2010**, Vol. 99, No. 1, 336-345.
28. A. Björk, L.-G. Danielsson, Journal of Process Analytical Chemistry, 1-5.