Near infrared spectroscopic transmittance measurements for pharmaceutical powder mixtures

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Abstract

This study describes the development of near infrared (NIR) calibration models using transmittance measurements in powder samples and compares the results obtained with those of tablet transmittance and diffuse reflectance of powders. Transmission near infrared spectroscopy is a method widely used for the analysis of tablets in the evaluation of drug concentration due to the larger sample volume analyzed, but not commonly used for the analysis of powder samples. Diffuse reflection near infrared spectroscopy is a method used in both powder and tablets for the evaluation of quality attributes. In this initial study NIR transmittance measurements were obtained using an off-line spectrometer equipped with a high intensity light source. Spectra were

obtained with three different resolutions for the analysis of powder and tablet samples of 7.50 to 22.50 % (w/w) acetaminophen. The Partial Least Squares (PLS) calibration models developed include pretreatments such as Standard Normal Variate (SNV) and first derivative in the region from 9500 - 7500 cm^{-1} . Transmittance in powder method presented low Root Mean Square Error of Prediction (RMSEP) values that varied from 0.23- 1.15 % (w/w) APAP with resolution of 64 and 16 cm^{-1} . The lowest RMSEP values (0.23-0.39 % (w/w) APAP) were obtained using a resolution of 64 cm^{-1} . The RMSEP values for powder transmittance measurements were 2.4 to 5.6 times lower than the diffuse reflectance measurements of the powder mixtures.

Keywords: pharmaceutical analysis, partial least squares (PLS), transmission, diffuse reflection, process analytical technology, multivariate analysis.

1. Introduction

Near infrared spectroscopy (NIRS) is used in the agricultural, food, oil, and pharmaceutical industries for real time analysis and to reduce costs through the elimination of sample preparation and reagents used in conventional laboratory tests. NIRS is non-destructive and provides information on both the chemical and physical properties of materials [1]. NIRS has been widely applied in the pharmaceutical industry where obtaining spectra in real time permits a better understanding of pharmaceutical processes [2-6].

40 Transmission and diffuse reflection methods are both used in pharmaceutical applications. In
41 transmission the detector is on the side opposite to the source of radiation, and in diffuse
42 reflection the two are on the same side. The term transmittance is used for the measurement of
43 light that has travelled through the sample and reflectance for the radiation that returns back to
44 the detector [7]. In recent years, the term remitted light has been used to describe the radiation
45 that is specularly reflected from a tablet surface and the radiation that undergoes diffuse
46 reflection[8]. Diffuse reflectance is frequently used to perform real time process measurements
47 [2, 3, 9, 10] and in the analysis of incoming raw materials [11]. In previous studies calibration
48 models have been developed using diffuse reflectance measurement of powder blends for
49 determination of drug concentration, humidity, and other properties [12]. Transmittance
50 measurements are used frequently to develop calibration models to determine drug concentration
51 in tablets [13-18]. In transmittance measurements the radiation crosses the entire tablet, and
52 thus a greater portion of the tablet is analyzed in comparison to diffuse reflection. In
53 transmittance of tablets multiple scattering occurs as the radiation is transmitted through the
54 particles. This multiple scattering was shown in time resolved spectroscopic measurements with
55 a 3.5 mm thick tablet where the path length for the radiation was estimated as 20 – 25 cm [19].
56 NIR transmission spectra of tablets with a thickness of 3.5 – 4.8 mm have been obtained [20].
57 More recently, spectra of tablets with a thickness of 5.6 mm were obtained using a NIR

instrument with a high intensity beam similar to the one used in this study [17] . The depth of penetration of NIR radiation in diffuse reflectance measurements is lower and is 1.7 – 2.9. mm as determined with double-layered tablets with results from [20]. A second diffuse reflectance study performed with Avicel and aspirin powders where the particle size was varied reported that the information obtained at 6086 cm^{-1} was from the top 0.8 – 1.1 mm while at 8956 cm^{-1} a depth of 2.4 mm was sampled [21]. In this study, light was transmitted through a 5.6 mm thick powder sample. The greater sample volume analyzed in transmittance measurements is an advantage but a specialized optical setup is necessary because of the few photons that reach the detector. Thus, reflectance methods are much more easily implemented than transmittance methods.

In this study powder measurements have been performed as an off line measurement. The authors understand that this is a first approach in the development of calibration models of powders using NIR transmittance spectra. The future goal is to develop a real time transmittance system for powders which would advance Process Analytical Technology and Quality by Design for pharmaceutical manufacturing. The study first discusses the necessary instrumental settings for transmittance measurements in powders. The transmittance measurements for powders were obtained with the Bruker Optics Fourier Transform Near Infrared Spectrometer (FT-NIR) which includes a high intensity light source. The authors understand that there is an absence of commercial instruments for transmittance measurements in pharmaceutical powder processes

and hope that this research will stimulate their development. The comparison between NIR transmittance spectra for powder and tablet samples and the development of calibration models with three different resolutions of 64, 32, and 16 cm^{-1} are described in this study.

The study recognized that sample deposition is important for powder transmittance measurements. Two different sample deposition methods were used to deposit powder for transmittance measurements. The first method was by manual filling with a spatula. Spatulas are conveniently used in many laboratory experiments and provide material from one or more sections of the sample that are pre-selected by the analyst. The second method consisted of a research lab developed feeder, where the powder flows from the feeder to a tablet holder. The feeder was used as an initial effort to automatically obtain a powder for transmittance measurements since the end goal is to develop a real time system. The feeder was also used to follow the Theory of Sampling (TOS), which stipulates that a correct sample is obtained when all the constituent elements of the lot have an equal probability of being sampled and when the increments and the sample are not affected in any way [22]. The feeder also provides a composite sample since as the powder flows different areas of the powder combine. TOS has also shown that composite samples are necessary to obtain representative samples [23-25]. Thus, the sampling of the powder was studied as it is essential for a future real time PAT powder transmittance system.

94

95 **2. Materials and methods**

96 2.1 Materials

97 Two pharmaceutical excipients and one active pharmaceutical ingredient (API) were used in this
98 study. The excipients were: lactose monohydrate (Granulac, Meggle Pharma), passed through a
99 U.S. Standard Sieve 60 (250 μ m opening) before mixing, and microcrystalline cellulose (MCC)
100 (Vivapur 102 provided by JRS Pharma). The API used was semi-fine acetaminophen (APAP)
101 received from Mallinckrodt Inc. (Raleigh, NC).

102

103 2.2 Preparation of powder blends.

104

105 2.2.1 Blends for powder transmittance measurements

106

107 Blends of three components (APAP, MCC and lactose) were prepared in a concentration range
108 of 7.50 % - 22.50 % (w/w) APAP as shown in Table 1. The eight calibration blends, each with a
109 weight of approximately 100 g were placed in a vibration blender for 15 min to break up
110 agglomerates in the materials and then 30 min in a tumble blender. A set of five additional

validation blends (8.99, 13.00, 15.00, 17.01, 21.01 % (w/w) APAP) were prepared with the same mixing procedure used for the calibration blends as shown in Table 1. The compositions for the calibration blends were chosen based on a D- optimal, linear model (MODDE 8.0.0.0 software from Umetrics (Umeå, Sweden)). The DOE for the calibration blends has a formulation of three components with 7.50 to 22.50 % (w/w) APAP; 0.00 to 62.50 % (w/w) lactose and 30.00 to 90.00 % (w/w) MCC.

The correlation coefficients between components are shown in Table 2. MCC and lactose show a high negative correlation indicating that if the value of a component increases, the value the other component decreases. APAP shows a weak relationship (values less than 0.5) with the other components in the blends. The low correlation coefficients between APAP and the excipients should contribute to the development of a robust calibration model.

2.2.2 Tablet preparation

Tablets were prepared using a manual model C Carver laboratory press. The compaction pressure was of 68.95 Mpa and a total of four tablets per concentration were obtained. The blends used for tablet preparation are described in section 2.2. The tablets prepared have an

average weight of 0.50 g approximately. Tablet thickness was determined using an electronic digital micrometer MARATHON CO030025 that operates from $0-25 \pm 0.001$ mm; the average thickness measured was of 2.026 mm.

2.3 Spectral acquisition and methods

2.3.1 Transmittance spectra of powders

Spectra were acquired with a Bruker Optics (Billerica, MA) Multi-Purpose Analyzer (MPA), Fourier Transform Near Infrared Spectrometer (FT-NIR), equipped with an RT-InGaAs detector. The OPUS® software package (version 7.2) Build: 7, 2, Bruker Optics, Germany) was used for control of the spectrometer. A glass cell was placed at the bottom of the tablet holder for the transmittance measurements of powder. The mass of powder placed in the holder was approximately 0.30 g, and the thickness of the powder was approximately 5.6 mm. Each spectrum was the average of 128 scans over the range of $12500-5800\text{ cm}^{-1}$, with a resolution of 64, 32 or 16 cm^{-1} . NIR reference and background single beam spectra were obtained with an NG9 filter. This is an internal filter within the Bruker system and is selected with the Opus software. Ten aliquots of each blend were evaluated and six spectra obtained for each aliquot.

2.3.1.1 Deposition methods of powder sample in tablet holder

The first method consisted in filling the tablet holder with the powder to complete a weight approximately of 0.30 g with the use of a stainless steel laboratory spatula. All calibration blends and validation blends were sampled with this method except the validation blend of 15.00 % (w/w) APAP. Figure 1 shows the second deposition method consisted in using a feeder developed by the research lab to deposit the 15.00 % (w/w) APAP validation blend in the tablet holder. The powder sample flowed through the feeder according to the movement of a screw until the last sample was collected.

2.3.2 Transmittance spectra of tablets

Spectra were acquired with the same instrument used for obtaining transmittance spectra for powders (Section 2.3.1). Tablets were placed in a holder (Bruker Optics, IN103-I1) and three spectra were obtained without moving the tablet. Each spectrum was the average of 128 scans over the range of 12500-5800 cm^{-1} , with a resolution of 16 cm^{-1} . The internal NG9 filter was used during the data collection.

2.4 Development of calibration models

Partial least squares (PLS) calibration models were developed using SIMCA v. 13.0.3. The standard normal variate (SNV) transformation was used to reduce the multiplicative and additive effects of scattering on the spectra. The SNV transformation was applied, and then the first

derivative spectra were obtained by using the Savitzky–Golay algorithm with a 15-point segment size and a second-order polynomial.

The effectiveness of the calibration was evaluated on the basis of the bias (average of residuals between NIR predictions and reference value) and the Root Mean Square Error (RMSEP) for validation samples [3].

$$RMSEP = \sqrt{\frac{\sum_{i=1}^n (\hat{y}_i - y_i)^2}{n}} \quad (1)$$

Where $\hat{y}_i$ corresponds to the predicted drug concentration and y_i is the reference drug concentration for each sample, and finally n is the number of samples predicted.

The error was also estimated in terms of relative standard error of prediction:

$$RSEP(\%) = \sqrt{\frac{\sum_{i=1}^m (\hat{y}_i - y_i)^2}{\sum_{i=1}^m y_i^2}} \times 100 \quad (2)$$

Calibration models were built from the transmittance spectra of pharmaceutical powder blends over a concentration range of 7.50 - 22.50 % (w/w) of the API. Calibration models were developed using a training set composed for eighty powder samples, ten aliquots of each of the eight calibration blends. Spectra with different resolutions (64, 32, 16 cm^{-1}) and three different spectral regions (11000-7500; 10500-7500 and 9500 – 7500 cm^{-1}) also were used for calibrations models developed.

A PLS calibration model was also built from the transmittance spectra of tablets over a concentration range from 7.50 -22.50 % (w/w) API, The calibration model was developed using a calibration set composed of 32 tablets. SNV followed by first derivative with 15 point segment size and the spectral region of 9500 – 7500 cm^{-1} was used for development of the calibration model for tablets.

3 Results and discussion

3.1 Spectral analysis

Figure 2 shows the NIR transmittance spectra for powders and tablets of 15.00 % (w/w) APAP and the spectrum of 100 % (w/w) APAP. Significant noise is observed in the region of 7000-6000 cm^{-1} because of the small amount of radiation reaching the detector; therefore this region

was not used to develop calibration models. The limited region for transmittance measurements has been discussed in several previous studies [13-17].

Figure 2 shows that transmittance spectra in powder and tablets present a baseline that varies in absorbance between -1 and -2; this condition can be attributed to the intensity of the radiation that reaches the detector when the sample and the reference are analyzed. The baseline is negative if the intensity of radiation received by the detector from the sample is higher than the intensity of radiation received when the reference is measured. The absorbance value of - 1.7 indicates that the intensity of radiation received by the detector (I_{det}) is approximately 50 times higher than the reference (I_0) as shown below:

$$A_{detected} = -\log(R_{detected}) = -\log\left(\frac{I_{det}}{I_0}\right) = -\log\left(\frac{1}{c}\right)\left(\frac{I_{refl}}{I_0}\right) = \log c + A \quad (3)$$

Figure 3 shows the near infrared transmittance spectra of a 15.00 % (w/w) APAP blend and tablet. The transmittance spectra in powders (Figure 3a) present differences in baseline for each spectrum since the particles in the blends are dispersed. The baseline difference in the transmittance spectra in tablets is minimal due to the compaction of the powder (Figure 3b). The difference in baseline can be explained by the additive effect in scattering, where the light intensity detected by the detector of the NIR spectrometer is equivalent to the fraction of the reflected radiation [9]. The change in baseline shows that the fraction of the radiation that

211 reaches the detector is not always the same way as indicated by equation (3). APAP shows
212 weak bands of 11876 - 10903 cm^{-1} , and stronger bands in the spectral region of 9193 - 8090 cm^{-1} ,
213 as shown in the top spectrum in Figure 2. The spectra of 15.00 % (w/w) APAP tablet and
214 15.00 % (w/w) APAP powder blend in Figure 2 (short dash line and dash dot line respectively)
215 presents one weak absorption band in the region from 9100 - 8500 cm^{-1} , which corresponds to
216 the absorption band of APAP. The calibration models were developed to include the spectral
217 range of API bands.

218 Figure 4 shows the SNV and first derivative transmittance spectra of powder blends in the
219 spectral region selected for the calibration model (9500 – 7500 cm^{-1}). Spectral changes were
220 observed for powder blends prepared at 7.5, 15.0 and 22.5% (w/w).

221 Figure 5 shows transmittance spectra of powder blends with 15.00 % (w/w) APAP (Fig. 5a) and
222 spectra with SNV and first derivative of powder blends with 15.00 % (w/w) APAP (Fig.5b)
223 obtained with different resolutions. Figure 5a shows significant differences between the three
224 spectra with different resolutions. Figure 5a shows that with an increase of resolution more
225 spectral features can be seen in the spectra; in addition more spectral noise is detected. The
226 evaluation of different spectral resolutions was important since a future real time transmittance

method would likely require a low resolution to obtain spectra in less time and with an improved signal to noise ratio.

Figure 5b shows that the spectrum of 16 cm^{-1} resolution presents one small APAP absorption band in the region from $9000 - 8900\text{ cm}^{-1}$; that is lost in the spectrum with 64 cm^{-1} resolution. The SNV and first derivative spectrum with 64 cm^{-1} resolution shown in Fig. 5b presents two bands defined in the region of $9500 - 7500\text{ cm}^{-1}$. In the same spectral region the spectrum with 16 cm^{-1} resolution shows a greater number of bands. The progressive increase of resolution from 64 to 16 cm^{-1} leads to a greater number of APAP bands.

3.2 Calibration models for transmittance spectra of powder blends

The calibration blends listed in Table 1 were used to develop the PLS calibration model. Different spectral regions, pretreatments, resolutions and validation sets of different levels of APAP concentration were evaluated to develop an effective calibration model. The PLS calibration models developed were then challenged with the prediction of drug concentration in validation blends. The validation blends are independent preparations and the spectra of the validation blends are not included in the calibration set. Table 3 presents the predictions of four validation sets with concentration levels of 8.99, 13.00, 17.01, and 21.01 % (w/w) APAP. A

total of nine calibration models were developed. The calibration models were developed with spectral resolutions of 64 cm^{-1} , 32 cm^{-1} and 16 cm^{-1} as outlined in Table 3. Three different spectral regions were evaluated: $9500\text{--}7500\text{ cm}^{-1}$, $10500\text{--}7500\text{ cm}^{-1}$ and $11000\text{--}7500\text{ cm}^{-1}$. The three calibration models developed with spectra of resolution 64 cm^{-1} showed the lowest RMSEP values. The next lowest RMSEP values were obtained with the three calibration models developed with spectra of resolution 16 cm^{-1} (Table 3). The best calibration models were obtained using the spectral region from $9500\text{--}7500\text{ cm}^{-1}$, which includes a number of APAP absorption bands (Table 3).

Table 4 shows the results of the three calibration models developed with the best spectral region ($9500\text{--}7500\text{ cm}^{-1}$) evaluated. Table 4 shows the individual evaluation of four different blends, each blend with ten sub-samples. The lowest RMSEP values were obtained with the validation blends evaluated in the calibration model with the spectra of 64 cm^{-1} resolution. The relative standard errors of prediction (RSEP) values were less than five percent in all validation sets with 64 cm^{-1} resolution.

Repeatability study

A repeatability study was performed taking six consecutive spectra for each sample analyzed without moving the powder holder. The spectra were then used to predict APAP concentration

with the calibration models developed, and the standard deviations of these predictions were termed repeatability. Repeatability was determined to evaluate the short-term precision using the same operational conditions [26]. The values presented correspond to the standard deviation of the predictions (Std) obtained using a validation set of 8.99 % (w/w) APAP and evaluating ten samples. Table 5 shows the repeatability results in three different resolutions evaluated (16, 32 and 64 cm^{-1}). Table 5 presents low standard deviations (high precision) from 0.001 to 0.013 for the three spectral resolutions evaluated.

3.3 Comparison between transmittance in powders and tablets

The calibration models developed with spectra of 64 cm^{-1} resolution (low resolution), have less spectral noise and are acquired in less time. The low spectral noise in the spectra contributed to development of calibration models with a good predictive capacity. The calibration models developed with spectra of 16 cm^{-1} resolution (high resolution) have more spectral information and are more robust models. In this study the calibration models development with spectra of resolution 16 cm^{-1} and SNV+1st-der (15) in the spectral regions of 9500-7500 cm^{-1} were selected to compare between transmittance in powders and tablets.

277 The blend concentration results were compared to the results of a PLS calibration model
278 developed to determine drug concentration (% (w/w) APAP) in tablets. Tablets were prepared
279 with blends of the same concentration range used for the transmittance powder spectra. The two
280 models were evaluated using one validation set of 13.00 % (w/w) APAP.

281 The validation set evaluated in the calibration model of tablets was composed of four tablets. The
282 validation set for the powder samples was composed of ten aliquots of the blend or subsample.
283 A spatula was used to deposit the powder sample in the tablet holder for subsequent analysis
284 (Deposition method 1). Table 6 shows RMSEP and RSEP values, the results of a significant test
285 for the comparison of the means of the two methods and the evaluation of significant differences
286 between both methods.

287 Table 6 present low RMSEP values for transmittance in powders and tablets and the RSEP
288 values were less than 5% for both methods. A pooled estimate of standard deviation and the
289 experimental value of t were determined (Table 6). The null hypothesis adopted in this test is
290 that there is no significant difference between the two methods. There are 12 degrees of
291 freedom, as ($P = 0.05$) the value critical of $|t|$ is 2.18. As the experimental value of $|t|$ (see
292 table 6) is smaller than the value critical of $|t|$ the difference between the two methods is not
293 significant at the 5% level, therefore the null hypothesis is retained.

3.4 Powder deposition with feeder.

The feeder deposition method (Figure 1) was used with the 15.00 % (w/w) APAP validation set. The results of transmittance in powder and tablets were compared at different resolutions. Transmittance in powders presented the lowest prediction errors for the three different resolutions evaluated. The results shown in Table 7 can be attributed to the sampling method used which is concordance with the theory of sampling (TOS). TOS explains that in a correct sampling method all the material has the same probability of being selected and the composition of the material is not affected during sampling [23-25].

Table 7 also shows the comparison between the experimental mean and the target value of the formulation (15.00 % (w/w) APAP). The statistical comparison was performed for transmittance measurements of a prediction set of 60 powder samples and a second prediction set of 60 tablets at three different resolutions. The null hypothesis adopted in this statistical evaluation considers that the analytical methods is not subject to systematic error and considering the t critical value and the t calculated value it is possible to compare the experimental mean and a known value. For this statistical comparison there are 59 degrees of freedom, as ($P = 0.05$) and the value critical of t_{critical} is 1.96. As the calculated value of t (see Table 7) is greater than the value critical of t_{critical} for transmittance in powder for all resolutions, the null hypothesis is rejected indicating

a systematic error. For tablet the calculated value of t (see Table 7) is lower than the value critical of t_{critical} for 64 and 32 cm^{-1} resolution, the null hypothesis is accepted, at 16 cm^{-1} resolution indicating a systematic error. Table 7 shows that the lowest confidence interval was observed for transmittance in powder at all resolutions evaluated and for transmittance in tablets at only one resolution (16 cm^{-1}).

3.5 Comparison between transmittance and diffuse reflectance methods in powder

Transmittance and diffuse reflectance measurements for powders were compared. The diffuse reflectance powder measurements were from a previous study with calibration models developed for blends from 7.50 to 22.50 % (w/w) APAP [27]. The diffuse reflection spectra were collected using a Bruker Optics (Billerica, MA) Matrix Fourier Transform (FT)-NIR spectrometer as the powder moved to velocity linearity of 10 mm/s, the work resolution was 8 cm^{-1} . Different validation sets were evaluated in the calibration model and RMSEP determined. Table 9 shows the comparison between transmittance and diffuse reflectance measurements. This study shows that the RMSEP values for diffuse reflectance measurements are between 2.35 to 5.61 times higher than transmittance measurements. Transmittance measurements present better predictions than diffuse reflectance because larger sample mass analyzed.

328

329 **4 Conclusion**

330 The transmittance spectra of powders and tablets are very similar; the most remarkable
331 difference is the change in baseline due to the scattering of radiation by the particles in the blend
332 and the minimum baseline differences in tablet spectra.

333 The calibration models developed for transmittance in powders present a good predictive
334 capacity, especially the calibration models developed with spectra of resolution 64 and 16 cm⁻¹.

335 The promising results obtained in this study indicate that a real time method could be developed
336 for powders in the future. However, sample deposition for transmittance measurements would
337 have to be improved or a more suitable design developed.

338

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 391 174.

392

393

394 **Tablets caption**

395 **Table 1.** Composition of calibration and validation blends used in study.

396 **Table 2.** Correlation coefficients between APAP, MCC, and lactose for the calibration model
 397 blends.

398 **Table 3.** Standard errors, PLS factors and bias values of the calibration models evaluated using
 399 different regions and pretreatments. A total of 40 validation blend samples were analyzed, ten
 400 samples at each of the four concentration levels.

401 **Table 4.** Standard errors, PLS factors, bias values of the calibration model evaluated using 64 ,
402 32 and 16 cm⁻¹ of resolution and SNV + First derivative (15) (9500 – 7500 cm⁻¹) as
403 pretreatments.

404 **Table 5.** Standard deviations observed in repeatability study at the three spectral resolutions
405 evaluated.

406 **Table 6.** Comparison between transmittance in powder and tablet using one validation set.

407 **Table 7.** Comparison between transmittance in powder after deposition of the 15.00 % (w/w)
408 APAP validation blend with the research lab developed feeder and tablets using different
409 resolutions.

410 **Table 8.** Comparison between transmittance in powder and diffuse reflectance in powder.

411

412 **Figures caption**

413 **Figure 1.** Deposition method to place the powder within the tablet holder.

414 **Figure 2.** NIR transmittance spectra for powder and tablet validation samples of 15.00 % (w/w)
415 APAP and the spectrum of 100 % (w/w) APAP.

416 **Figure 3. (a)** Transmittance spectra of 15.00 % (w/w) APAP validation blend to resolution 32
417 cm^{-1} . **(b)** Transmittance spectra of 15.00% (w/w) APAP validation tablet to resolution 16 cm^{-1} .

418 **Figure 4.** Transmittance spectra of blends after SNV and first derivative with 15 point size in
419 the spectral region of 9500 – 7500 cm^{-1} .

420 **Figure 5. (a)** Transmittance spectra of validation blends with 15.00 % (w/w) APAP, different
421 resolutions were used. **(b)** Transmittance spectra of validation blends after SNV and first
422 derivative with 15 point size in the spectral region of 9500 to 7500 to cm^{-1} with different
423 resolutions.

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428 Figure 1

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431 Figure 2

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433 Figure 3

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435 Figure 4

Figure 5

	% (w/w) APAP	% (w/w) MCC	% (w/w) LACTOSE
Cal 1	7.50	30.00	62.50
Cal 2	7.50	90.00	2.50
Cal 3	7.50	60.00	32.50
Cal 4	14.00	63.50	22.50
Cal 5	15.00	30.00	55.00
Cal 6	16.25	83.75	0.00
Cal 7	22.50	77.50	0.00
Cal 8	22.50	30.00	47.50
Val 1	8.99	44.02	73.97
Val 2	13.00	31.00	69.81
Val 3	15.00	66.67	18.33
Val 4	17.01	39.74	59.55
Val 5	21.01	37.99	53.85

Table 1. Composition of calibration and validation blends used in study.

	APAP	LACTOSE	MCC
APAP	1	-0.104	-0.096
LACTOSE	-0.104	1	-0.98
MCC	-0.096	-0.98	1

Table 2. Correlation coefficients between APAP, MCC, and lactose for the calibration model blend.

Prediction of APAP Validation Blends						
Data Pretreatment	Spectral Range (cm ⁻¹)	Resolution (Res) (cm ⁻¹)	PLS Factors	RMSEP	RSEP (%)	Bias
SNV+1 st -der (15), n=40	9500-7500	64	2	0.51	3.25	-0.09
		32	2	1.04	6.63	-0.28
		16	2	0.77	4.93	0.10
	10500 – 7500	64	3	0.40	2.54	0.10
		32	2	1.14	7.27	-0.27
		16	3	0.83	5.32	0.07
	11000 – 7500	64	2	0.63	4.05	0.06
		32	2	1.14	7.27	-0.26
		16	3	0.80	5.14	0.11

Table 3. Standard errors, PLS factors and bias values of the calibration models evaluated using different regions and pretreatments. A total of 40 validation blend samples were analyzed, ten samples at each of the four concentration levels.

Prediction of APAP Powder Blends							
Data Pretreatment	Spectra Range	Res (cm ⁻¹)	PLS Factors	Validation sets	RMSEP	RSEP (%)	Bias
SNV+1 st der (15)	9500-7500 (cm ⁻¹)	64	2	8.99	0.23	2.57	0.07
			2	13.00	0.39	2.97	0.30
			3	15.00	0.26	1.73	-0.17
			2	17.01	0.25	1.49	0.13
			2	21.01	0.32	1.52	-0.23
		32	2	8.99	1.11	12.39	1.07
			2	13.00	0.24	1.86	0.12
			2	15.00	0.49	3.27	0.46
			2	17.01	0.81	4.75	-0.77
			2	21.01	1.53	7.30	-1.52
		16	2	8.99	1.15	12.84	1.15
			2	13.00	0.30	2.29	0.25
			2	15.00	0.31	2.08	-0.15
			2	17.01	0.40	2.34	-0.37
			2	21.01	0.90	4.27	-0.64

Table 4. Standard errors, PLS factors, bias values of the calibration model evaluated using five validation set evaluated to 64 , 32 and 16 cm-1 of resolution and SNV + First derivative (15) (9500 – 7500 cm-1) as pretreatments.

Repeatability Study								
16 cm ⁻¹ Res, n = 10			32 cm ⁻¹ Res , n = 10			64 cm ⁻¹ Res, n = 10		
Std Avg	Std Max	Std Min	Std Avg	Std Max	Std Min	Std Avg	Std Max	Std Min
0.003	0.007	0.001	0.004	0.007	0.002	0.006	0.013	0.002

Table 5. Standard deviations observed in repeatability study at the three spectral resolutions evaluated.
n = 10 correspond to the number of times that the repeatability was conducted

Model 7.5-22.5 % (w/w) APAP, 16 cm ⁻¹ Res.														
Validation set.	TR powder, off-line Bruker MPA						TR tablets, off-line Bruker MPA						Significant test	
	SNV + 1 st der (15) (7500-9500 cm ⁻¹), 2 PLS factor						SNV + 1 st der (15) (7500-9500 cm ⁻¹), 2 PLS factor							
Ref														
% (w/w)	n	Avg	RMSEP	RSEP (%)	Bias	Std	n	Avg	RMSEP	RSEP (%)	Bias	Std	Spooled	t value
APAP														
13.00	10	13.25	0.30	2.29	0.25	0.16	4	13.21	0.21	1.63	0.21	0.04	0.14	0.48

Table 6. Comparison between transmission in powder and tablet using one validations set.

Transmission after powder deposition							Transmission in Tablets					
SNV + 1 st der (15) (7500-9500 cm ⁻¹)							SNV + 1 st der (15) (7500-9500 cm ⁻¹)					
Res (cm ⁻¹)	PLS Factors	Avg, (n=60)	RMSEP	RSEP (%)	t calc	Confidence interval	PLS Factors	Avg, (n=60)	RMSEP	RSEP (%)	t calc	Confidence interval
64	3	15.17	0.26	1.73	6.58	15.17 ± 0.05	2	15.06	0.53	3.55	0.88	15.06 ± 0.13
32	2	14.54	0.49	3.27	20.96	14.54 ± 0.04	2	14.91	0.58	3.85	1.22	14.91 ± 0.14
16	2	15.15	0.31	2.08	4.30	15.15 ± 0.07	2	14.66	0.48	3.17	9.08	14.66 ± 0.07

Table 7. Comparison between transmissions in powder after deposition of the 15.00 % (w/w) APAP validation blend with the research lab developed feeder and tablets using different resolutions.

Transmission vs. Diffuse Reflectance in Powders								
Description		Model 7.5-22.5 % (w/w) APAP	Test set Spectra #	Avg	Bias	RMSEP	RSEP (%)	Std
TR powder	off-line Bruker MPA	SNV + 1st der (15) (7500-9500 cm ⁻¹), 2 PLS factor	TR ; n=60	15.15	0.15	0.31	2.08	0.27
			T _S 1;n=68	15.58	0.58	0.73	4.89	0.46
DR Powder	In-line Bruker Matrix	SNV + 1st der (17) (5000-9100 cm ⁻¹), 2 PLS factor	T _S 2;n=390	14.93	-0.07	0.78	5.19	0.78
			T _S 3;n=390	16.39	1.39	2.1	14.02	1.58
			T _S 4;n=258	15.17	0.17	1.74	11.63	1.74
			All test sets ;n=1106	15.54	0.54	1.59	10.58	1.49

Table 8. Comparison between transmission in powder and diffuse reflectance in powder.

1. Methods

2. Spectral Data

3. Multivariate data analysis

PLS

METHODS	RMSEP
TR in Powder	0.31
TR in Tablet	0.48
DR in Powder	1.59

TR : transmission , DR : Diffuse reflectance ,
Res :Resolution .

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440 Graphical Abstract