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Blend Uniformity Analysis Using Stream Sampling and Near Infrared Spectroscopy

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ABSTRACT A near infrared spectroscopic method was developed to determine drug content in a 20% (wt/wt) ibuprofen and spray-dried hydrous lactose blend. A blending profile was obtained after blending for 0.5, 1, 3, 5, 10, and 20 minutes. Stream sampling was used to collect about 20 blend samples at each of the blending times from a laboratory scale V-blender. The samples collected were used to develop a near infrared calibration model. The calibration model was then used to determine the drug content of unknown samples from 2 validation blends. The validation blends were not included in the calibration model; they were used to evaluate the effectiveness of the calibration model. A total of 45 samples from the 2 validation blends were predicted by the near infrared calibration model and then analyzed by a validated UV spectrophotometric method. The root mean square error of prediction for the first validation blend was 5.69 mg/g and 3.30 mg/g for the samples from the second blend. A paired t test at the 95% confidence level did not indicate any differences between the drug content predicted by the near infrared spectroscopy (NIRS) method and the validated UV method for the 2 blends. The results show that the NIRS method could be developed while the blending profile is generated and used to thoroughly characterize a new formulation during development by analyzing a large number of samples. The new formulation could be transferred to a manufacturing plant with an NIRS method to facilitate blend uniformity analysis.

KEYWORDS: near infrared spectroscopy, blend uniformity analysis, stream sampling, pharmaceutical analysis, powder technology.

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INTRODUCTION The Current Good Manufacturing Practices (CGMPs) as described in 21CFR 211.110 require in-process controls and tests to be conducted on appropriate samples of in-process materials to assure the uniformity and integrity of each batch of drug products. The control procedures are required to monitor the output and validate the performance of the manufacturing processes that may cause variability in the in-process material and drug product. The required in-process control procedures include testing to evaluate the adequacy of mixing to assure uniformity and homogeneity. In 1999 the US Food and Drug Administration (FDA) published a draft guidance to provide recommendations on establishing in-process acceptance criteria for blend uniformity analysis to applicants with ANDA (Abbreviated New Drug Application) products [1]. Industrial feedback to this draft guidance eventually led to industry, academia, and the FDA working together in the Blend Uniformity Working Group (BUWG) of the Product Quality Research Institute. The proposal developed by the BUWG is under review at the FDA, and the FDA recently withdrew its draft guidance [2].

Even before the FDA draft guidance, a number of researchers investigated the sampling of pharmaceutical powder mixtures and the problems associated with their analysis [3-10]. The majority of these efforts have involved the use of sample collection devices known as sample thieves. A recent survey conducted by the BUWG showed that sample thieves remain the preferred method for powder sampling, with 25 of the 28 respondents indicating that they used a side-compartment thief device [11].

Near infrared spectroscopy (NIRS) is a viable analytical technique for the evaluation of pharmaceutical powder blends [12-19]. On-line monitoring systems using NIRS are an alternative to the use of sample thieves [12-17]. NIRS is quite attractive for this type of application because it is a nondestructive method that usually does not require sample preparation, is noninvasive, and offers the possibility of remote sampling with fiber optic probes. The principal means of judging blend uniformity with on-

line measurements is on the basis of the standard deviation of the spectra obtained, even though other chemometric methods have been explored. The mixture is termed homogeneous when the standard deviation of the near infrared spectrum reaches a minimum value. An inherent advantage of these methods is that blend uniformity is judged on the basis of the distribution of both the active pharmaceutical ingredient and the excipients. The development of on-line noninvasive methods continues, and recently a system that used NIRS for remote and noninvasive monitoring of 6 sites on a V-blender was reported [16]. This report showed the necessity for sampling multiple points for the determination of the mixing end point and also described NIRS imaging experiments that allow evaluation of a larger mass of sample [16].

Stream sampling is also an alternative to the use of sample thieves. This report describes the implementation of stream sampling by capturing the blend in stainless steel cups as it flowed from the bottom of the blender. The stream sampling was performed following Allen's 2 "golden rules" of powder sampling, which state that a powder should always be sampled when in motion, and that sampling should be done in small increments of time throughout the entire powder stream rather than at the same preselected sites at all times [20]. An advantage of stream sampling is that more samples can be obtained than by thief sampling, which is limited by the difficulty of obtaining the samples and possible changes in the powder distribution as the thief is inserted. Stream sampling takes advantage of a process that has to occur, as tablet compression requires the flow of the blend from a hopper or bin located over the compressing machine. Therefore, stream sampling could indicate segregation problems related to the emptying of the blender -”problems that thief sampling is unable to pinpoint. Even though Allen's golden rules of powder sampling are widely known and sample thieves violate both rules, sample thieves have been used in the majority of the reports on pharmaceutical powder samples [11,21-23]. The preferential sampling or segregation of the powder blend by sample thieves has been reported, and as a result stream sampling could find wider use in the future [4,23].

An additional advantage of stream sampling is that its implementation does not require a significant financial investment. Small pharmaceutical companies may be unable to invest in on-line monitoring systems and the specialized personnel needed to implement and validate them. Stream sampling also complements the on-line monitoring systems by evaluating the blend 1 step beyond the blending process as a hopper or bin is emptied. Stream sampling may also be advantageous in the validation of blending processes in larger-scale equipment. As blenders increase in size, the thief handling operation becomes more difficult, since it is necessary to collect samples several feet below the powder bed surface.

The limitations of stream sampling must also be considered. Stream sampling is not able to target locations that are suspected of providing poor blending. The goal of stream sampling is to obtain representative samples and not to target specific locations, as the stratified sampling approach recommended by the BUWG proposes. However, if poor blending was to occur at a location different from those targeted by the stratified sampling approach, then there is a greater possibility of detecting the poor blending with stream sampling. The application of stream sampling is also limited in the final formulation scale up and optimization process. Thief sampling has the advantage that it allows for the collection of samples in large-scale blenders and subsequent blending until the optimum blending time is achieved.

Stream sampling was used in the development of a blending profile. Blending profiles are obtained during formulation development and scale up [24]. The blend is considered homogeneous when the active pharmaceutical ingredient is found to be within specification in the blend. The results obtained are usually reported in milligrams of active ingredient per gram of the pharmaceutical blend, and in terms of standard deviation or relative standard deviation of the drug content.

The blending profile efforts provide samples blended under very different conditions, and with variation in the concentration of the active pharmaceutical ingredient and excipients. The development of a calibration model for an NIRS method also requires samples with similar variation spanning the concentrations and conditions of interest [25]. Thus, an excellent opportunity is present during the development and optimization of a formulation to develop and optimize an NIRS method for blend uniformity. A new product could be transferred to its manufacturing site along with its near infrared method for drug content in the blending stages. This report explores the development of an NIRS method for blend uniformity of a 2-component blend.

MATERIALS AND METHODS

Materials

The materials used were spray-dried hydrous lactose (Pharmtose DCL-11, DMV International, Veghel-The Netherlands) and ibuprofen (70 grade, Albemarle, Baton Rouge, LA), both donated by Pharmacia & Upjohn Caribe (Arecibo, PR). The lactose used passed through a 250-μm sieve and was retained in a 125-μm sieve screen. The ibuprofen used passed through a 125-μm sieve screen and was retained on a 75-μm sieve screen.

The methanol used was HPLC grade (Fischer Scientific, Pittsburgh, PA).

Mixing Method

A V-blender, Blend Master Lab Blender Model "B" from Patterson-Kelley Co (East Stroudsburg, PA), was used for blending. A 4-quart acrylic cross-flow shell without intensifier bar was used for all experiments and was filled to slightly over 50% of its volume. A 20% (wt/wt) ibuprofen-in-lactose blend was prepared by weighing 200 g of ibuprofen and 800 g of lactose. The 2 materials were placed in layers: 300 g of lactose, then 100 g of ibuprofen, topped with 300 g of lactose, 100 g of ibuprofen, and finally 200 g of lactose.

The blend was collected after the mixing period by opening the acrylic shell's bottom sleeve. A different blend was prepared for each mixing time. The first blend samples were collected after only 30 seconds of blending, and the sleeve was opened and a flow of blend established. The entire contents of the P-K Blend Master were emptied, and more than 20 samples were collected from the flowing stream. The blend was captured in the stainless steel cups, manufactured by Pike Technologies (Madison, WI), capable of sampling about 100 mg of the blend. This minimum amount of sample was chosen in an effort to find areas of sample nonhomogeneity. The rest of the blend was discarded. Thus, the samples captured after 0.5, 1, 3, 5, 10, and 20 minutes of blending were not from the same blend, and each blending time required a different weighing and blending of the lactose and the ibuprofen.

Ibuprofen UV Assay

Samples were analyzed by a validated UV spectrophotometric method. After weighing, the samples were transferred to 100-mL volumetric flasks, and 75 mL of 75% (vol/vol) methanol were added. The mixture was shaken for 30 minutes on a laboratory planetary shaker and sonicated for 3 minutes. The mixture was then completed to volume with 75% (vol/vol) methanol, and the contents of the flask were mixed manually to ensure complete mixing. An aliquot was centrifuged at 3500 rpm, and the absorbance of the clear solution was obtained at 259 nm with a Beckman DU 640 spectrophotometer (Fullerton, CA) using a cuvette of 10-mm optical pathlength. The calibration curve for the ibuprofen method was linear over the range of 0.10 to 0.70 mg/mL (r^2 = 0.999). The assay's accuracy was evaluated by spiking lactose with ibuprofen, where an average recovery for the ibuprofen was 100% (n = 30, SD = 1.49%). An accuracy study was performed each time that the assay was used to evaluate the system's and the analyst's performance.

FT-NIR Equipment

A Vector 33 Fourier Transform Near-Infrared (FT-NIR) spectrometer (Bruker Optics, Billerica, MA) was used to obtain spectra of all samples described in this report. The instrument was equipped with a germanium detector and included a fiber optic probe model N262 that was composed of a bifurcated (Y-shaped) low-OH silica fiber bundle to illuminate the sample and to collect diffuse reflectance spectra from the powder samples.

The spectra were collected after the fiber optic probe was placed directly over the sample in the cup. Each spectrum was the result of averaging 32 scans of 8 cm^{-1} resolution in the 12 000 to 5000 cm^{-1} region. For each sample, triplicate spectra were obtained by rotating the sample about 120 degrees. The 3 spectra were averaged for use in the calibration model. The Norton Beer apodization function was applied to all spectra [26].

Development of Calibration Models

The calibration models were developed with the aid of OPUS NT Version 2.06 (Bruker Optics). This software includes the partial least squares (PLS) algorithm. PLS is a spectral decomposition technique used to develop multivariate calibration models [27]. Spectra were pretreated using the vector normalization algorithm in the OPUS software.

The spectra and ibuprofen concentrations of 116 samples collected by stream sampling after the 6 blending times were used to build the training set necessary for the calibration model. The first step in the development of the calibration model was a leave-one-out cross validation. During the leave-one-out cross validation, a PLS calibration model developed with all the samples except one is used to predict the sample left out [27]. The algorithm repeats this step until all samples have been left out once and calculated with the calibration model. The difference between the results predicted by the model and the actual values are summarized by the prediction residual error sum of squares (PRESS):

$$\text{PRESS} = \sum_{i=1}^{n} (C_{VAL} - C_{PRED})^2 \quad (1)$$

where C_{VAL} is the drug concentration in the blend as determined by the validated UV spectrophotometric method and C_{PRED} is the concentration predicted by the NIRS method. At this time the algorithm also indicates the number of factors or eigenvectors that provide the minimum PRESS values.

The validation set was also used to determine the number of factors to include in the calibration model. The validation set was composed of samples from the 2 validation blends, and none of the samples in this set were included in the training set. The determination of the number of factors to keep in the calibration model is recognized as one of the most challenging tasks in this process [27]. Several methods have been developed to evaluate the number of factors to include in a model, but the use of a validation set is often recognized as giving the best estimate of the model's performance [27]. The differences observed between the results predicted by the NIRS method for the validation samples and their ibuprofen concentration as determined by the UV

method were described by the root mean square error of prediction (RMSEP).

$$RMSEP = \sqrt{\frac{PRESS}{n}} \quad (2)$$

The same equation was used to describe the differences between the concentrations predicted by the calibration model and the UV spectrophotometric method in the leave-one-out cross validation. In this case it was called the root mean square error of cross validation (RMSECV).

RESULTS

For the analysis of blend samples it is important to establish a sample size. The FDA Draft Guidance document stated that the blend sample size should be 1 to 3 dosage units, and the BUWG proposed that sample quantities larger than 3X be allowed if they can be scientifically justified [1,2]. The sample size requirement was met in this study by using a cup that limited the sample captured to about 100 mg. In both the FDA Draft Guidance and the BUWG proposal, blend uniformity is measured on the basis of drug distribution. The approach followed in this study also permitted the determination of drug content in the powder samples. Spectra of the samples captured in the cups were obtained, and then the samples were weighed and their drug content determined by a validated UV spectrophotometric method. The drug concentration and the near infrared spectra were then used to develop an NIRS calibration model for the blends.

A primary concern in the development of this calibration model was whether the near infrared radiation contacted the majority of the powder blend [28]. Diffuse reflectance can be affected by many factors, such as the sample packing, the particle size, and the crystallinity of the material [28,29]. On the other hand, the entire sample is analyzed by the UV spectrophotometric method. If the NIRS radiation contacts only a small part of the sample, it would be difficult to develop a reliable calibration model if the 2 methods (NIRS and UV) evaluate different samples. To reduce this problem, 3 spectra of the blend were obtained, each 120 degrees apart, and then averaged. The sampling depth of the NIRS radiation was also investigated by placing a fine layer of talc at the bottom of the cup. The talc had 2 strong characteristic bands in the region of 7200 to 7100 cm $^{-1}$, as shown in the top spectrum in **Figure 1**. The bottom spectrum in **Figure 1** shows a very weak band for the talc superimposed over the blend spectrum, showing that the near infrared radiation reaches the talc. Therefore, using sample cups with a depth of about 2 mm limited the sample to an area that could be reached by the near infrared radiation. It is not possible to determine whether the entire blend was sampled by the near infrared radiation, but it is shown that the blend near the bottom of the cup was sampled by the near infrared radiation.

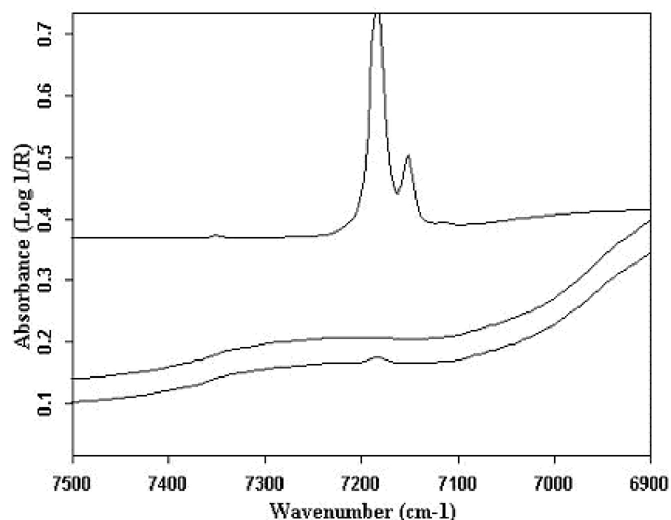

Figure 1. (Top) Spectrum of talc powder; (middle) spectrum of ibuprofen and lactose powder mixture; (bottom) spectrum of ibuprofen and lactose powder mixture over thin layer of talc. The talc was estimated to be 1.8 to 2.0 mm below the surface of the powder mixture.

The calibration model was developed with samples from the various mixing times. About 20 samples were obtained at each of the 6 blending times, and the training set was formed with 116 samples. The concentration of the samples in the training or calibration set ranged from 157.2 to 257.7 mg/g. The leave-one-out cross validation showed a correlation coefficient of 0.9242 between the results predicted by the NIRS method and those determined by the validated UV method, as shown in **Figure 2** . The RMSECV for the results obtained was 7.74 mg/g, and the model required 7 factors. The combination of 2 spectral regions, 9750 to 7497 cm $^{-1}$ and 6101 to 5773 cm $^{-1}$, provided the best correlation between the near infrared spectra and the drug concentration following pretreatment by vector normalization. These regions include features of both the ibuprofen and lactose spectra, as shown in **Figure 3** .

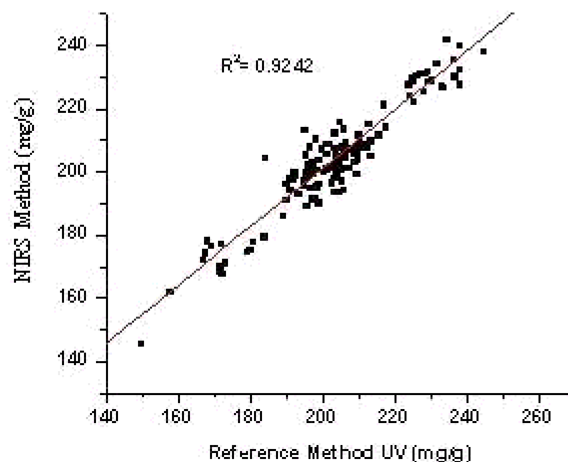

Figure 2. Cross-validation plot for the blends in the training or calibration set. The results predicted by NIRS are plotted versus the values obtained by the reference method (UV).

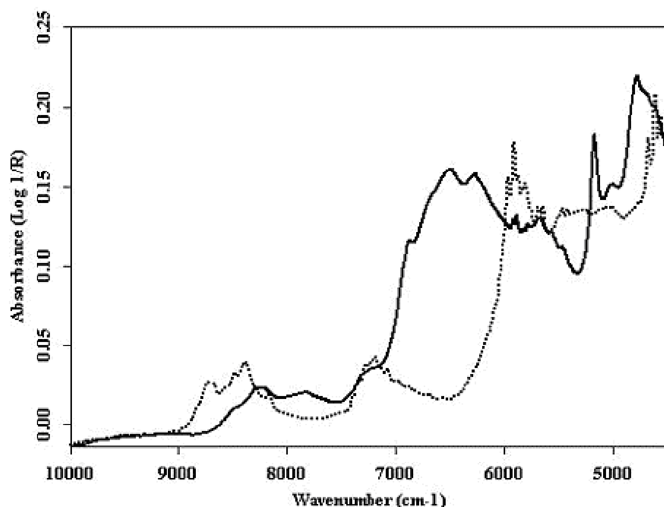

Figure 3. NIRS diffuse reflectance spectra of ibuprofen (dashed lines) and lactose.

The calibration model was used to calculate the active pharmaceutical ingredient in 2 validation blends. Validation blends were defined as blends that are not included in the calibration model. In other words, none of the spectra for the samples collected from these 2 blends are included in the calibration model. The results predicted by the NIRS method and then checked by the UV method are shown in **Table 1**. For the first validation blend, the correlation coefficient was 0.9021 between the drug content determined by the NIRS method, and the UV or reference method. The RMSEP was 5.69 mg/g, with a 7-factor model. The largest difference observed between the 2 analytical methods was about 12.5 mg/g. A paired t test statistical comparison at the 95 % confidence level did not indicate any differences between the results obtained with the 2 methods.

Two of the results observed on **Table 1** deviate significantly from the target level of 200 mg/g. The UV method confirmed the results of 143.9 mg/g and 235.4 mg/g predicted by the NIRS calibration model. The 143.9 mg/g result is outside the range established for the NIRS calibration model (157.5 to 257.7 mg/g), but the model still does a good job of predicting the sample and flagging this result that significantly differs from the target level of 200 mg/g.

The second validation blend showed a drug concentration that was higher but still within the range of the calibration model. The calibration model was able to predict the drug content of the samples with a correlation coefficient of 0.925 between the NIRS and reference method results. The RMSEP obtained was 3.30 mg/g, with 7 factors. The results obtained are shown in **Table 2** .

The correlation coefficients obtained for the 2 validation blends should not be confused with the correlation coefficients calculated in high-performance liquid chromatography (HPLC) linearity studies. The 0.9021 and 0.925 correlation coefficients refer to the correlation between the values predicted by the NIRS method and the values determined by the UV method. In HPLC linearity studies conducted at a specific wavelength, the instrument's response is evaluated versus the different concentrations of the analyte with the linear regression algorithm, and a correlation coefficient greater than 0.99 is usually expected. Future regulatory evaluations of NIRS methods will require that reviewers understand how the correlation coefficient is used and that it does not provide the entire picture. A complete evaluation will require evaluation of the method's ability to predict unknown samples (as described by the RMSEP), and statistical comparisons as shown in this report.

This blending process would not meet the requirements of the former ANDA Draft Guidance since the relative standard deviation (RSD) of the drug concentration in the validation blends (**Tables 1** and **2**) is greater than 5%. This study, however, was not directed at developing a formulation that would meet the former ANDA Draft Guidance requirements [1]. Instead, the objective was to obtain blend samples with a wide range of concentrations around the target concentration of 200 mg/g to facilitate the development of the calibration model. The short blending times required for the development of the blending profile provided most of the samples with concentrations away from the target concentration of 200 mg/g, making it possible to develop the calibration model from 157.5 to 257.7 mg/g. Some samples outside of this range were also obtained at the short blending times, but this appeared to be an optimum range for the calibration model.

Figure 4 shows the blending profile obtained after sampling the blend at 0.5, 1, 3, 5, 10, and 20 minutes. The validated UV spectrophotometric method was used to determine the drug content of the samples collected at each of the blending times, and their RSD was plotted versus the blending time. Each mixing time corresponds to a different blend since the blender was completely emptied. The RSD of the drug content showed very little variation after 3 minutes of blending time. The samples collected at 0.5 minutes and 1 minute showed significant variation in their drug content. The greater RSD observed at 1 minute is a result of the lack of homogeneity of the blend at this point. As indicated by Muzzio, a greater number of samples is required to evaluate the blend when it is not homogeneous [22]. In addition, it is not possible to compare among close blending times since for each blending time a different weighing of ibuprofen and lactose was made. The results of the samples captured at the 6 blending times are shown in **Table 3** .

Table 1. Results of Samples From the First Validation Blend*

Sample	UV Reference Method (mg/g)	Near Infrared Predicted (mg/g)	Residual (mg/g)
1	202.1	202.3	–0.2
2	205.9	213.1	–7.2
3	210.9	205.6	5.3
4	198.0	195.2	2.8
5	210.0	208.1	1.9
6	205.8	213.7	–7.9
7	231.9	235.4	–3.5
8	203.4	197.9	5.5
9	150.0	143.9	6.2
10	204.4	201.9	2.5
11	203.9	201.4	2.5
12	210.0	202.9	7.1
13	205.9	195.6	10.3
14	204.0	198.0	6.0
15	203.7	205.7	–2.0
16	209.3	212.0	–2.8
17	206.7	209.0	–2.3
18	207.3	204.5	2.8
19	204.8	217.3	–12.5
20	201.1	206.3	–5.2
Average	204.0	203.5	
SD	14.35	16.71	
RSD	7.037	8.212	
Paired *t* test			
t calculated		0.3585	
t critical		2.1009	

*RSD indicates relative standard deviation.

Table 2. Results of Samples From the Second Validation Blend*

Sample	UV Reference Method (mg/g)	Near Infrared Predicted (mg/g)	Residual (mg/g)
1	216.4	217.2	−0.8
2	215.3	215.4	−0.1
3	202.1	207.7	−5.6
4	212.7	206.3	6.4
5	201.8	203.9	−2.1
6	207.5	210.3	−2.8
7	197.6	200.0	−2.4
8	226.9	226.6	0.2
9	216.6	209.1	7.5
10	227.5	223.7	3.8
11	202.2	204.5	−2.3
12	195.6	196.5	−0.9
13	219.4	219.0	0.3
14	232.0	231.7	0.3
15	230.1	226.3	3.7
16	228.2	230.5	−2.3
17	230.4	229.8	0.6
18	199.7	200.9	−1.2
19	227.4	221.1	6.3
20	196.9	198.8	−2.0
21	223.2	222.1	1.1
22	208.0	212.3	−4.3
23	224.2	228.0	−3.8
24	209.8	208.1	1.7
25	204.3	202.8	1.5
Average	214.2	214.1	
SD	12.145	11.161	
RSD	5.669	5.213	
Paired *t* test			
t calculated		0.237	
t critical		2.068	

*RSD indicates relative standard deviation.

Table 3. Results of Samples From the 6 Experiments Used to Establish the Calibration Model and Blending Profile*

	Ibuprofen Concentration (mg/g)					
	0.5 Mins	**1 Min**	**3 Mins**	**5 Mins**	**10 Mins**	**20 Mins**
	198.4	168.4	206.5	197.4	203.6	192.3
	195.8	169.7	200.6	202.7	198.2	196.9
	196.8	172.9	200.1	205.1	195.3	190.0
	189.1	205.3	208.6	212.4	215.6	192.4
	257.7	172.9	201.7	199.1	211.5	194.9
	244.0	167.8	207.4	213.6	195.8	193.4
	192.5	171.8	200.4	225.4	204.4	195.0
	192.9	167.2	205.9	212.7	205.5	200.3
	191.0	172.2	205.7	217.6	213.2	209.1
	209.9	171.2	207.6	210.1	196.7	202.7
	197.0	181.5	199.8	203.0	194.8	197.7
	196.7	187.2	210.0	206.1	199.0	205.0
	191.0	179.7	204.9	205.7	207.9	198.4
	199.4	172.1	210.4	205.3	195.5	208.7
	197.3	471.7	212.4	201.1	195.5	197.2
	327.7	157.7	202.6	210.1	195.8	201.6
	231.3	194.6	197.8	200.0	206.1	198.3
	226.7	298.1	208.4	205.4	203.6	199.1
	194.4	275.2	212.2	217.0	203.8	204.0
	205.4	184.2	210.3	197.6	201.2	203.2
			207.2	212.9	191.1	201.4
			209.5	203.6	180.2	204.8
			193.5			
			209.1			
			200.8			
Average	211.8	202.1	205.5	207.4	200.6	199.4
SD	33.34	72.79	4.917	7.201	7.984	5.240
RSD	15.74	36.02	2.393	3.471	3.979	2.628

*The results were obtained by analyzing the entire sample with the validated UV spectrophotometric method. Each column presents results from different experiments. RSD indicates relative standard deviation.

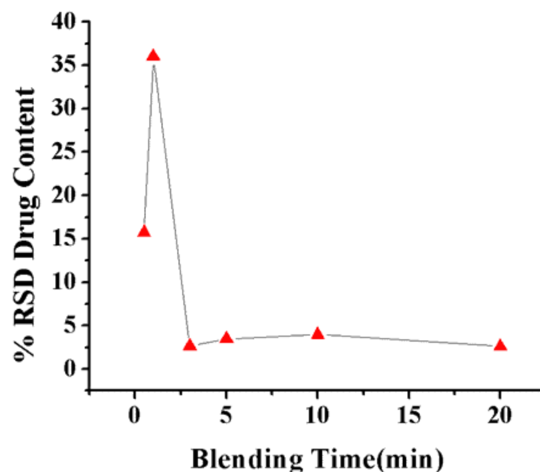

Figure 4. Blending profile for the 2-component mixture obtained after calculating the drug content with the UV spectrophotometric method. The RSD of the drug content of all the samples collected at 0.5, 1, 3, 5, 10, and 20 minutes is shown versus the blending time.

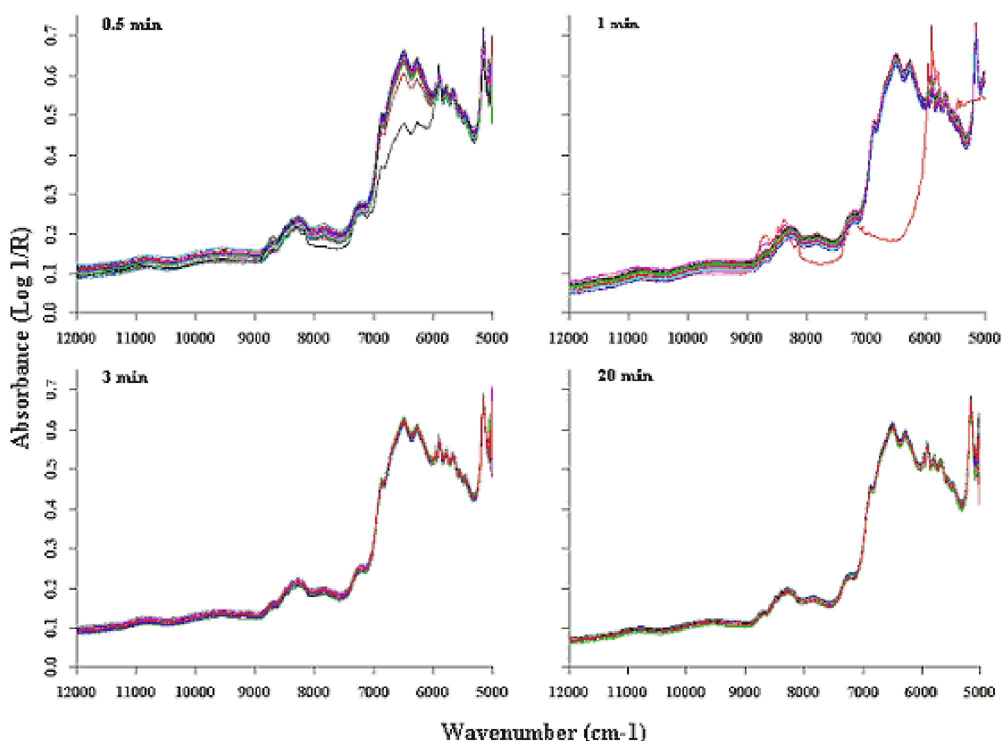

Figure 5. Spectra of samples collected after various blending times.

Figure 5 shows the spectra of the samples obtained at 0.5, 1, 3, and 20 minutes. The spectra at 0.5 and 1 minutes also reflect the high variation observed in the drug content samples. The spectra at 5 and 10 minutes are not shown since they are very similar to the spectra observed at 3 and 20 minutes.

Figure 6 shows the blending profiles obtained based on the average standard deviation of the spectra at each of the blending times. A blending profile was first obtained by calculating the standard deviation for each point of the spectra, and then averaged over the entire spectral range. The shape of this profile is very similar to that shown on **Figure 4** . However, it is not necessary to take the standard deviation of the entire spectral region; it is possible to focus on wavelengths or parts of the spectrum that relate to the components of interest [16]. **Figure 6** also shows the blending profile based on the average standard deviation over the spectral regions used to quantify the ibuprofen in the blend (from 9750 to 7497 cm $^{-1}$ and from 6101 to 5773 cm $^{-1}$). The blending profile is now somewhat different from the profile observed in **Figure 4** . These experiments show that NIRS could be used to define blend uniformity on the basis of the distribution of the excipients and the drug by taking the standard deviation of the entire spectrum, and also on the basis of only the active ingredient.

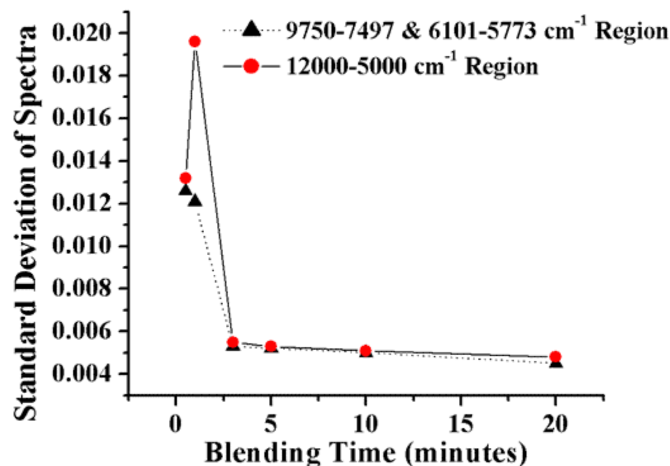

Figure 6. Blending profile obtained from the entire spectral range (▲) and over the spectral range used in developing the calibration model (•). In each case the standard deviation of the spectra at each wavelength of the spectral range was first calculated. The average standard deviation over these wavelengths was then calculated and plotted versus blending time.

A repeatability study was used to assess the precision of the NIRS method. The fiber optic probe was placed over a blend sample, and 6 spectra were obtained at the same position. Each spectrum consisted of 32 averaged scans. The drug content of the blend was determined with the spectrum obtained and the calibration model.

Table 4. Repeatability Study for 2 Different Blend Samples Analyzed by NIRS*

	Concentration Predicted (mg/g)	Concentration Predicted (mg/g)
	205.0	205.7
	207.7	202.7
	206.9	199.6
	204.6	201.3
	205.9	200.9
	206.8	203.9
Average	206.2	202.4
SD	1.195	2.199
RSD	0.580	1.087

*NIRS, near infrared spectroscopy; RSD, relative standard deviation.

Table 4 shows results for 2 samples analyzed in this manner and indicates an RSD of about 1%.

The method described in this report could be further validated for use in an industrial environment. The accuracy, repeatability, and range of the method have been addressed, and future precision and robustness studies would be required. At least 3 recent reports have addressed the validation of NIRS methods in light of the International Conference on Harmonization (ICH) guidelines [30-32]. These authors emphasize that ICH Q2A and Q2B address traditional method validations such as HPLC validations but do not address the statistics and chemometrics issues related to NIRS. These reports describe approaches for validating NIRS methods in a manner consistent with the ICH guidelines, while taking into consideration the particular aspects related to NIRS.

CONCLUSION Sample thieves are not the only means for sampling pharmaceutical powder mixtures. The use of stream sampling has been shown to be another possible option. This option is consistent with the golden rules of powder sampling that have been suggested by Allen in the field of powder technology. Stream sampling is also relatively easy to implement, as it does not require the modification of blenders with ports for near infrared radiation or other sensors. In addition, there is practically no limitation on the number of samples that may be obtained. Even if a sample thief is chosen for the evaluation of blending, stream sampling may be used as a complementary method. Stream sampling could become a useful tool in the sampling of pharmaceutical powder blends. The approval of the BUWG would not eliminate the need for sampling the powder blend, since it would still be required during formulation development and in the process validation batches [2].

The NIRS calibration model developed in this study correctly determined samples from 2 validation blends. The approach described in this study makes the development of the blending profile a more productive process, since this profile can be used to develop an NIRS calibration model. The NIRS method makes it easier to analyze blend samples and could be used to thoroughly characterize a new formulation during the development process by analyzing a large number of samples.

In pharmaceutical production, blend uniformity is usually assessed only in terms of the distribution of the active ingredient. The use of NIRS opens the door to a more thorough evaluation of blend uniformity where the distribution of the excipients is also considered.

Studies are currently under way using a 5-component blend and a lower percentage of the active pharmaceutical ingredient. These studies are directed toward facilitating the use of NIRS for the development of methods to assess blend uniformity.

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REFERENCES

1. FDA Draft Guidance for Industry ANDAs: Blend Uniformity Analysis. August 1999. Food and Drug Administration, Center for Drug Evaluation and Research (CDER).

2. Blend Uniformity Recommendations Final Report: The Use of Stratified Sampling of Blend and Dosage Units to Demonstrate Adequacy of Mix for Powder Blends. March 28, 2002. Available at: www.pqri.org. Accessed September 23, 2002.

3. Berman J, Planchard JA. Blend uniformity and unit dose sampling. Drug Dev Ind Pharm. 1995;21(11):1257-1283.

4. Berman J, Schoeneman A, Shelton JT. Unit dose sampling: a tale of two thieves. Drug Dev Ind Pharm. 1996;22(11):1121-1132.

5. Mahan S, Rankell A, Rehm C, Bhalani V, Kulkarni A. Unit-dose sampling and blend content uniformity testing. Pharm Tech. 1997;21:116-125.

6. Carstensen JT, Dali M. Blending validation and content uniformity of low-content, non-cohesive powder blends. Drug Dev Ind Pharm. 1996;22(4):285-290.

7. Garcia T, Elsheimer B, Tarczynski F. Examination of components of variance for a production scale, low dose powder blend and resulting tablets. Drug Dev Ind Pharm. 1995;21(18):2035-2045.

8. Pastor R, Gomez R, Ramirez JL, Rodriguez O, Mortensen N, Bellamy MK. A novel sample thief designed to avoid biased data. Tableting & Granulation Yearbook 1999, a supplement to Pharm Technol. 1999;23(10):47-53.

9. Tanino T, Yoshida T, Sumi Y, Ohtani S, Mizuta T. Evaluation of blend uniformity: effect of granulation sample size. J Jpn Soc Pharm Mach Eng. 1999;8:5-14.

10. Carstensen JT, Rhodes CT. Sampling in blending validation. Drug Dev Ind Pharm. 1993;19(20):2699-2708.

11. Boehm G. Report on the industry blend uniformity practices survey. Pharm Tech. 2001;25(8):20-26.

12. Hailey PA, Doherty P, Tapsell P, Oliver T, Aldridge PK. Automated system for the on-line monitoring of powder blending processes using near-infrared spectroscopy part I. System development and control. J Pharm Biomed Anal. 1996;14: 551-559.

13. Sekulic S, Ward HW, Brannegan DR, et al. On-line monitoring of powder blend homogeneity by near-infrared spectroscopy. Anal Chem. 1996;68:509-513.

14. Maesschalck RD, Sánchez FC, Massart DL, Doherty P, Hailey P. On-line monitoring of powder blending with near-infrared spectroscopy. Appl Spectrosc. 1998;52(5):725-732.

15. Ufret C, Morris KR. Modeling of powder blending using on-line near-infrared measurements. Drug Dev Ind Pharm. 2001;27(7):719-729.

16. El-Hagrasy AS, Morris HR, D'Amico F, Lodder RA, Drennen JK. Near-infrared spectroscopy and imaging for the monitoring of powder blend homogeneity. J Pharm Sci. 2001;90(9):1298-1307.

17. Berntsson O, Danielsson LG, Lagerholm B, Folestad S. Quantitative in-line monitoring of powder blending by near infrared reflection spectroscopy. Powder Technol. 2002;123(2-3):185-193.

18. Wargo DJ, Drennen JK. Near-infrared spectroscopic characterization of pharmaceutical powder blends. J Pharm Biomed Anal. 1996;14:1415-1423.

19. Miller RW. The use of near infrared technology to map roller compaction processing applications. Tableting & Granulation Yearbook 1999, a supplement to Pharm Technol. 1999;23(10):30-39.

20. Allen T. Particle Size Measurement Powder Sampling and Particle Size Measurement. Vol 1, 5th ed. London, UK: Chapman & Hall; 1997:1-4.

21. Prescott JK, Garcia TP. A solid dosage and blend content uniformity troubleshooting diagram. Pharm Tech. 2001;25(3):68-88.

22. Muzzio FJ, Robinson P, Wightman C, Brone D. Sampling practices in powder blending. Int J Pharm. 1997;155:153-178.

23. Garcia TP, Wilkinson SJ, Scott JF. The development of a blend-sampling technique to assess the uniformity of a powder mixture. Drug Dev Ind Pharm. 2001;27(4):297-307.

24. Hwang R, Gemoules MK, Ramlose DS. A systematic approach for optimizing the blending process of direct-compression tablet formulation. Pharm Tech. 1998;22(3):158-170.

25. Kramer R. Chemometric Techniques for Quantitative Analysis. New York, NY: Marcel Dekker; 1998:13.

26. Griffiths PR, de Haseth JA. Fourier Transform Infrared Spectrometry. New York, NY: Wiley-Interscience; 1986:15.

27. Duckworth J. Spectroscopic quantitative analysis. In: Workman J, Springsteen A, eds. Applied Spectroscopy: A Compact Reference for Practitioners. San Diego, CA: Academic Press; 1998:93-163.

28. Berntsson O, Burger T, Folestad S, Danielssson LG, Kuhn J, Fricke J. Effective sample size in diffuse reflectance near-IR spectrometry. Anal Chem. 1999;71(3):617-623.

29. Yeboah SA, Wang S-H, Griffiths PR. Effect of pressure on diffuse reflectance infrared spectra of compressed powders. Appl Spec. 1984;38:259-264.

30. Moffat AC, Trafford AD, Jee RD, Graham P. Meeting the International Conference on Harmonisation's guidelines on validation of analytical procedures: quantification as exemplified by a near-infrared reflectance assay of paracetamol in intact tablets. Analyst. 2000;125:1341-1351.

31. Mark H, Ritchie GE, Roller RW, Ciurczak EW, Tso C, MacDonald SA. Validation of a near-infrared transmission spectroscopic procedure, part A: validation protocols. J Pharm Biomed Anal. 2002;28:251-260.

32. Broad N, Graham P, Hailey P, Hardy A, Holland S. Guidelines for the Development and Validation of Near-Infrared Spectroscopic Methods in the Pharmaceutical Industry. Available at: www.pasg.org.uk/NIR/Home.html. Accessed September 23, 2002.