AAPS PharmSciTech 2006; 7 (1) Article 29 (http://www.aapspharmscitech.org).

Quantitation of Drug Content in a Low Dosage Formulation by Transmission Near Infrared Spectroscopy

Submitted: July 22, 2005; Accepted: January 21, 2006; Published: March 24, 2006

Carlos Peroza Meza,[1] María A. Santos,[1] and Rodolfo J. Romañach[1]

[1]University of Puerto Rico - Mayagüez Campus, Department of Chemistry, PO Box 9019, Mayagüez, PR 00681

ABSTRACT

A transmission near infrared (NIR) spectroscopic method has been developed for the nondestructive determination of drug content in tablets with less than 1% weight of active ingredient per weight of formulation (m/m) drug content. Tablets were manufactured with drug concentrations of ~0.5%, 0.7%, and 1.0% (m/m) and ranging in drug content from 0.71 to 2.51 mg per tablet. Transmission NIR spectra were obtained for 110 tablets that constituted the training set for the calibration model developed with partial least squares regression. The reference method for the calibration model was a validated UV spectrophotometric method. Several data preprocessing methods were used to reduce the effect of scattering on the NIR spectra and base the calibration model on spectral changes related to the drug concentration changes. The final calibration model included the spectral range from 11 216 to 8662 cm^{-1}, the standard normal variate (SNV), and first derivative spectral pretreatments. This model was used to predict an independent set of 48 tablets with a root mean standard error of prediction (RMSEP) of 0.14 mg, and a bias of only −0.05 mg per tablet. The study showed that transmission NIR spectroscopy is a viable alternative for nondestructive testing of low drug content tablets, available for the analysis of large numbers of tablets during process development and as a tool to detect drug agglomeration and evaluate process improvement efforts.

KEYWORDS: near infrared spectroscopy, pharmaceutical analysis, content uniformity, chemometrics, partial least squares.

INTRODUCTION

Tablets are the most prescribed pharmaceutical formulation, and many active pharmaceutical ingredients are formulated as tablets. Tablet drug content may vary from a tenth of a percent or less to drug substances without suitable diluents.[1] The drug distribution in a pharmaceutical tablet batch is more critical in formulations where the active pharmaceutical ingredient (API) is at low concentration levels.[2] An agglomerate of 0.20 mg is not significant in a formulation where the label strength is 100 mg, but it is critical if the tablet label strength is 0.10 mg. Segregation due to particle size, or cohesiveness of an API could result in significant differences in the content uniformity of low drug content tablets.

In spite of the importance of content uniformity in formulations with low drug content, the *United States Pharmacopeia (USP)* requires the analysis of only 10 to 30 tablets for a batch that may contain as many as 3 million tablets in high volume manufacturing.[3] The analysis of 0.001% or less of the tablets manufactured is considered representative of the batch. The preferred method of analysis is high-performance liquid chromatography (HPLC), and the analysis of a greater number of tablets would certainly require additional HPLC equipment, personnel, time, and cost to the pharmaceutical industry. The use of near infrared spectroscopy (NIRS) is a viable alternative, since it does not require sample preparation and it could easily analyze 30 tablets in an hour versus the 3 to 4 days required by many HPLC methods for the analysis of 30 tablets.[4] NIR methods could also be used during the process development stage to analyze a greater number of tablets and detect any possible segregation mechanisms before the process validation studies are started. Blanco[5] recently provided an extensive list of the NIRS methods to determine the drug content of tablets, powder mixtures, liquids, gels, and coated tablets, and Table 1 provides a list of more recent publications. The vast majority of these methods have been for formulations with 20% (m/m) drug content or more. The use of NIRS for low drug content formulations with less than 1% (m/m) drug content has not been investigated, or at least the authors are not aware of any publication for a similar application.

This study presents the development of an NIR method to determine the drug content in tablets with less than 1.0% (m/m) active pharmaceutical ingredient. The use of NIRS was only explored for low drug content in one method where the drug content of a steroid tablet formulation contained 5, 10, 15, 20, and 30 mg (2.94%, 5.88%, 8.82%, 11.76%, and 17.64% m/m), respectively.[6] NIR spectra were obtained in the transmission mode since it samples a greater portion of the tablet and is less sensitive to the differences

Corresponding Author: Rodolfo J. Romañach, University of Puerto Rico - Mayagüez Campus, Department of Chemistry, PO Box 9019, Mayagüez, PR 00681-9019. Tel: (787) 832-4040, ext 3122, 2604; Fax: (787) 265-3849; E-mail: rromanac@uprm.edu

Table 1. Summary of Studies for the Determination of Drug Content in Tablets*†

Reference	Sampling Mode DR, TR	Drug Concentration % (m/m)	Description of Dosage Unit
Broad et al[6]	TR	2.9 - 17.7	Steroid tablets
Ritchie et al[7]	TR	N/A	Capsules with 150 mg and tablets with 200 mg active
Kemper et al[8]	TR	1 - 8	Translucent topical gel
Ramirez et al[9]	TR	70	Tablet thickness reduced to 3.6 mm
Laasonen et al[10]	DR	58.82	Caffeine tablets
Scheiwe et al[11]	TR	18 - 27	Diclofenac sodium, 3.2- to 3.5-mm thick tablets
Dyrby et al[12]	TR	4.8 - 9.1	Tablets 2.9- to 4.3-mm thick
Jedvert et al[13]	DR	25 - 35	Tablets

*DR indicates diffuse reflectance; TR, transmission; m/m, weight of active ingredient per weight of formulation; and N/A, not available.
†After publication of Blanco et al.[5]

in homogeneity within the tablet and the area sampled.[6] Three batches were prepared at concentrations of 0.5%, 0.7%, and 1.0% (m/m) to develop the calibration model. A commercially available product was not used because commercial products are manufactured with a tight concentration range and would have not provided the variations in concentration needed to develop a calibration model. Furthermore, for pharmaceutical products formulated at low concentrations, there are also concerns about the safety of analysts exposed to highly potent drugs. To avoid toxicity, safety, or legal concerns, ibuprofen was formulated at a concentration far below its therapeutic level.

Materials and Methods

Chemicals

Methanol HPLC grade was purchased from Fisher Scientific (Morris Plains, NJ). Microcrystalline cellulose *USP/National Formulary (NF),* EP, JP type 101 (VIVAPUR) was manufactured by Microcellulose Weissenborn GMBH (Weissenborn, Germany) and obtained from Mutchler Inc, Pharmaceutical Ingredients (Cayey, PR). Hydrous lactose *USP* grade, spray-dried for direct compression, was manufactured by DMV International (Veghel, The Netherlands). Ibuprofen *USP* 70 grade was produced by Albemarle Corp (Baton Rouge, LA) and donated by Pharmacia and Upjohn Caribe (Barceloneta, PR). Colloidal silicon dioxide and magnesium stearate *NF* powder were also donated by Pharmacia and Upjohn.

Tablet Manufacturing

Three blends of different concentrations for direct compression were manufactured. Each blend contained ibuprofen as active pharmaceutical ingredient (API) at concentrations of: 0.5%, 0.7%, and 1.0% (m/m), respectively. The formulation also included hydrous lactose spray-dried 74%-75% (m/m), microcrystalline cellulose 22%-23% (m/m), colloidal silicon dioxide 0.2% (m/m), and magnesium stearate 1.0%.

Colloidal silicon dioxide was mixed with the active ingredient into a standard testing sieve with openings of 250 μm, and then the microcrystalline cellulose was passed through the same screen. The resulting mixture was mixed in a 4-quart acrylic cross-flow shell without intensifier bar for 5 minutes. A Blend Master Lab Blender (model B, Patterson-Kelley Co, East Stroudsburg, PA) was used. The hydrous lactose was then added and mixed for 3 minutes. The last step consisted in adding the magnesium stearate and mixing for 2 minutes.

Compression of the tablets was performed using a Manesty Rotapress MKII model β3 (Thomas Engineering, Inc, Hoffman Estates, IL) with maximum load 100 kN (10 tons) with 16 compression stations. Tablets obtained were round-shaped, with average diameter surface of 10.5 mm; thickness 3.0 to 3.4 mm, and one of the faces scored with the letters "UPRM".

FT-NIR Equipment

Transmission spectra were recorded in a multipurpose analyzer (MPA) Fourier transform near infrared (FT-NIR) spectrometer (Bruker Optics, Billerica, MA) equipped with a 30-position sample wheel and a room temperature–indium gallium arsenide (RT-InGaAs) external detector positioned above the tablet. The spectra were collected with the Opus 4.0 software (Bruker Optics). Tablets were placed in custom-made holders with a 7-mm aperture in the center. Each spectrum was an average of 128 scans at a resolution of 16 cm^{-1}, over the range of 12 000 to 4000 cm^{-1}. Three spectra were collected per tablet in the calibration and validation set, to detect any possible spectral outliers or any significant differences between the spectra. The tablets remained in the same position in the sample holder for the 3 spectra.

Ibuprofen UV Reference Method

An in-house validated UV method was used to determine the drug content of the tablet. The absorbance of samples and standards was measured at 215 nm with an optical path

length of 7 mm. Each individual tablet was weighed and transferred to a 100-mL volumetric flask; then 1.0 mL of water was added to the flask and allowed to stand for 5 minutes. After this time, 75% (vol/vol) methanol was added to the flask to fill to half-volume, and the samples were gently shaken in a Lab-Rotator (Lab-Line Instrument Inc, Melrose Park, IL) for 25 minutes. Subsequently, an ultrasonic bath Aquasonic HT (model 250, VWR Scientific Products, West Chester, PA) was used for 15 minutes to complete the tablet's disintegration. The samples were allowed to reach room temperature and volumetric flasks were filled with 75% (vol/vol) methanol. Approximately 10 mL per sample were centrifuged in an Eppendorf 5804R centrifuge (Westbury, NY) at 3400 rpm for 4 minutes and the supernatant clear solution analyzed by UV detection, using a Beckman DU 650 spectrophotometer (Fullerton, CA). Triplicate readings for both standards and samples were performed. Accuracy studies were performed at the beginning, in the middle, and at the end of the analysis of all samples with an average recovery of 100% for ibuprofen (n = 21, and relative standard deviation [RSD] = 0.50%). Accuracy studies were performed each time that the assay was used to evaluate the system's and the analyst's performance. The method also showed excellent specificity at 215 nm without interferences from the sample matrix and linearity (r^2 = 0.9999).

Development of Calibration Models

The partial least squares (PLS) calibration models were obtained with the Pirouette 3.10 software (Infometrix Inc, Bothell, WA). The spectra were first mean centered in all of the calibration models developed. All first and second derivative spectra were computed using a 25-point segment size and a zero gap size.

The calibration models were developed with a training set composed of 110 tablets. As a preliminary test of the model,

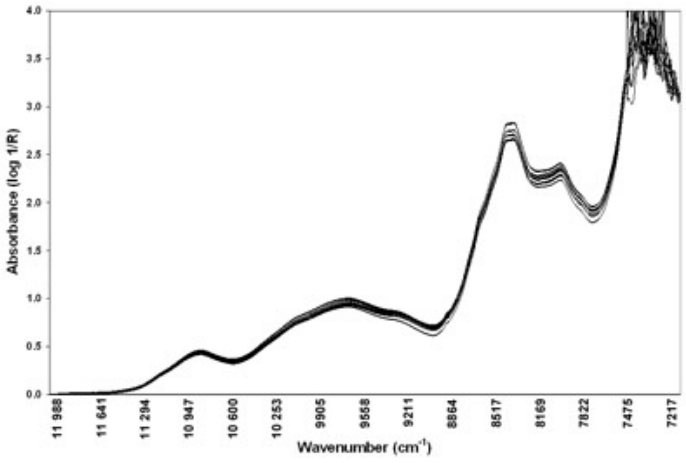

Figure 1. Transmission spectra obtained for tablets manufactured with Manesty Rotapress MK II compressing machine.

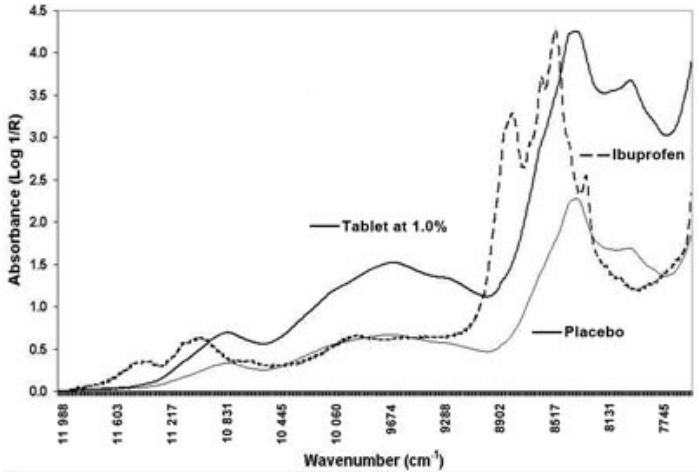

Figure 2. Transmittance spectra of ibuprofen 100% (m/m), placebo and 1.0% (m/m) ibuprofen tablets.

the software performed a leave-one-out cross-validation. This step consists in developing a calibration model with all the samples, but one. The sample left out was then predicted by the calibration model. The algorithm repeats this step until all samples have been left out once and calculated with the calibration model. Separate cross-validations were performed for the first, second, and third spectra of the tablets in the training set, excluding the replicate spectra from the cross-validation step. Any significant differences in the results obtained with the 3 cross-validations would have indicated a problem during the spectral collection step. The root mean square error of cross-validation (RMSECV) was used to decribe the results of the cross-validation and was defined as follows:

$$RMSECV = \sqrt{\frac{\sum_{i=1}^{n}(C_{REF_i} - C_{PRED_i})^2}{n_t}} \qquad (1)$$

where C_{REF} is the reference concentration; C_{PRED} is the concentration predicted by NIR; and n_t is the number of samples in the training set.

The performance of the NIR calibration models was evaluated with the prediction of an independent validation set comprised of 48 tablets. The root mean standard error of prediction (RMSEP) and the relative standard error of prediction (% RSEP) were used to describe the differences observed between the predicted drug content and the reference method value.[5]

$$RMSEP = \sqrt{\frac{\sum_{i=1}^{n}(C_{REF_i} - C_{PRED_i})}{n_v}} \qquad (2)$$

$$RSEP(\%) = \sqrt{\frac{\sum_{i=1}^{n}(C_{REF_i} - C_{PRED_i})}{\sum_{i=1}^{n} C_{REF_i}^2}} \times 100 \qquad (3)$$

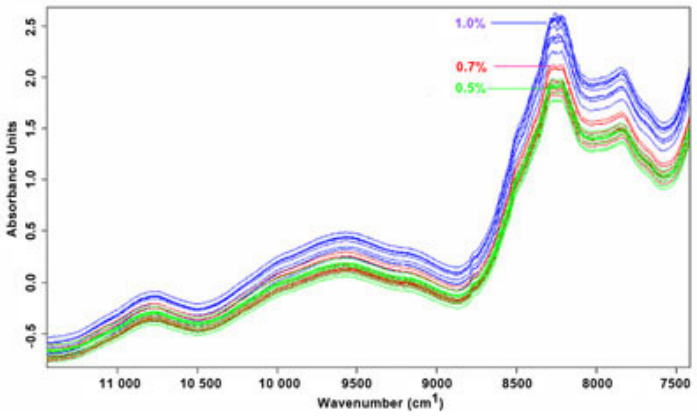

Figure 3. Absorption spectra of ibuprofen tablets at 0.5%, 0.7%, and 1.0% (m/m) with resolution of 8 cm^{-1}.

In Equation 2, n_v is the number of samples in the validation set. Although Equations 1 and 2 are very similar, the number of samples in the training set and the validation set are not necessarily the same.

Results and Discussion

The near infrared spectra of several tablets of the ibuprofen formulation compressed with a Manesty MK II press are shown in Figure 1. In the region below 7000 cm^{-1} the amount of radiation reaching the detector is low and the detector signal becomes noisy. This observation has been made in other studies involving transmission NIRS, with the consequence that calibration models for transmission have been developed with the higher frequency part of the spectrum.[9,14]

PLS is a full-spectrum method that requires that the component modeled absorb in the spectral range used to develop the calibration model.[15] Conformance to this requirement was verified by compressing a tablet with a thickness of 1.6 mm at 2000 psi in a Carver single-punch press (Wabash, IN), where ibuprofen was the sole component, then obtaining the spectrum labeled ibuprofen 100% in Figure 2. Spectra of a placebo tablet, and one of the tablets manufactured with the Manesty MK II tablet-compressing machine are also shown in Figure 2. Ibuprofen showed weak

Table 2. Standard Errors and Number of Factors for the Calibration Models Evaluated*

Data Pretreatment	Spectral Region cm^{-1}	Factors	%Variation Described	RMSECV (mg)	RMSEP (mg)	RSEP (%)
SNV	10 450-8030	4	99.4	0.20	0.14	9.5
SNV	11 216-8030	5	99.4	0.21	0.14	9.6
SNV	11 216-8662	4	99.7	0.19	0.14	9.6
SNV	8662-8030	4	99.5	0.24	0.20	13.8
SNV	9000-8000	4	99.7	0.20	0.17	11.5
SNV First derivative	10 450-8030	4	98.1	0.20	0.15	9.9
SNV First derivative	11 216-8030	4	98.0	0.20	0.14	9.2
SNV First derivative	11 216-8662	3	98.9	0.19	0.14	9.3
SNV First derivative	8662-8030	4	99.1	0.26	0.22	14.9
SNV First derivative	9000-8000	4	98.2	0.20	0.17	11.4
SNV Second derivative	10 450-8030	4	98.3	0.22	0.19	12.4
SNV Second derivative	11 216-8030	4	97.9	0.22	0.18	12.1
SNV Second derivative	11 216-8662	4	98.9	0.19	0.14	9.4
SNV Second derivative	8662-8030	4	97.3	0.26	0.18	12.2
SNV Second derivative	9000-8000	4	98.5	0.22	0.18	12.4
First derivative	10 450-8030	4	98.8	0.22	0.17	11.2
First derivative	11 216-8030	4	98.7	0.22	0.18	11.9
First derivative	11 216-8662	4	99.0	0.20	0.14	9.4
First derivative	8662-8030	4	99.4	0.26	0.19	13.2
First derivative	9000-8000	4	99.1	0.23	0.17	11.3
Second derivative	10 450-8030	4	99.2	0.21	0.16	10.2
Second derivative	11 216-8030	4	99.1	0.22	0.16	10.2
Second derivative	11 216-8662	4	98.9	0.19	0.15	9.7
Second derivative	8662-8030	4	99.4	0.25	0.20	12.8
Second derivative	9000-8000	5	99.5	0.20	0.15	9.7

*RMSECV indicates root mean square error of cross-validation; RSEP, relative standard error of prediction; and SNV, standard normal variate.

bands from 11 220 to 10 450 cm^{-1}, and strong bands between 8910 and 8100 cm^{-1}, showing less absorbance between 8100 and 7400, before its absorbance increases again. The ibuprofen bands are stronger than the excipient bands observed in the placebo tablet in the region from 8900 to 8300 cm^{-1}. The 1% (m/m) ibuprofen tablet shows a stronger absorption in the 8900 to 8300 cm^{-1} range than the placebo tablet. This evaluation of the ibuprofen spectrum indicates that the calibration model should include all or part of the spectral region from 11 220 to 8100 cm^{-1}.

Figure 3 shows spectra for tablets at 3 nominal concentrations of 0.5%, 0.7%, and 1.0% (m/m). A resolution of 8 cm^{-1} was used in this preliminary evaluation, although a resolution of 16 cm^{-1} was used to develop the calibration model. This initial evaluation showed significant differences in the absorption bands of the 3 strengths in a spectral area where ibuprofen absorbs. Although the placebo components also absorb in this region, the spectral differences were considered a first indication that the drug could be determined at levels below 1% (m/m).

The drug content was expressed in terms of milligrams per tablet since it does not require weighing the tablet and it is the term used in *USP* content uniformity methods.[14] Samples were randomly split into 2 sets: a calibration or training set comprised of 110 tablets covering the range from 0.71 mg to 2.51 mg, and a second set of 48 tablets ranging from 0.80 to 2.31 mg/tab was used as a validation or prediction set. The tablets in the calibration set were used exclusively to construct the models. The tablets in the prediction set were kept out of the calibration set and were used to challenge the calibration model.

PLS calibration models were developed with 5 different areas of the spectrum: 11 216 to 8030 cm^{-1}, 11 216 to 8662 cm^{-1}, 10 450 to 8030 cm^{-1}, 9000 to 8000 cm^{-1}, and 8662 to 8030 cm^{-1} as shown in Table 2, included all areas

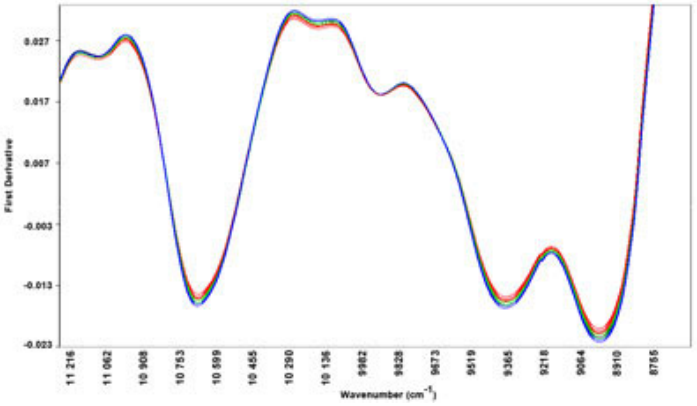

Figure 4. Spectra after SNV and first derivative from 11 216 to 8662 cm^{-1}.

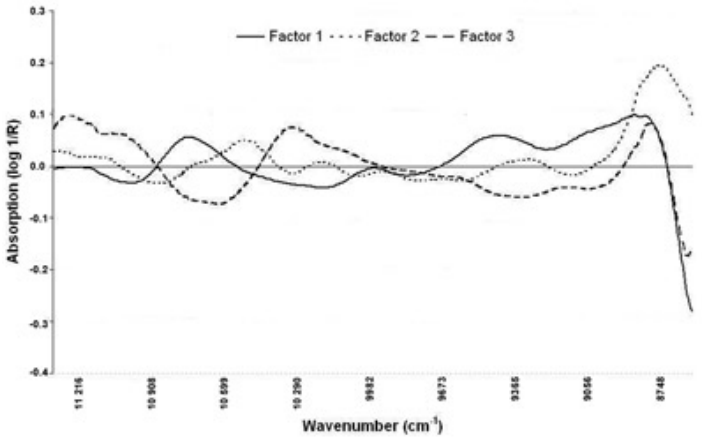

Figure 5. PLS loadings for model with mean centering, SNV, and first derivative obtained for 11 216 to 8662 cm^{-1} spectral region.

where ibuprofen absorbs. The largest RMSEP values were observed for the narrower spectral region spanning 8662 to 8030 cm^{-1}, where the RMSEP varied from 0.19 to 0.22 mg. The RMSEP obtained for the wider spectral areas varied minimally between 0.14 and 0.19 mg. The better predictions obtained with the wider spectral areas may be the result of the "multivariate advantage" from signal averaging after many nearly redundant measurements used to develop the calibration model.[15] In this case the wider spectral area provided the better prediction. Still, other studies have obtained better results by restricting the calibration model to the spectral region that shows the greatest changes in the analyte's spectral features.[6]

Table 2 shows the data pretreatments performed to remove the unwanted scattering features from the spectra. The development of a calibration model also involves the use of spectral pretreatments because NIR spectra are strongly affected by scattering that varies according to particle size and other factors that do not provide information on the chemical distribution of the analyte of interest.[16-18] The

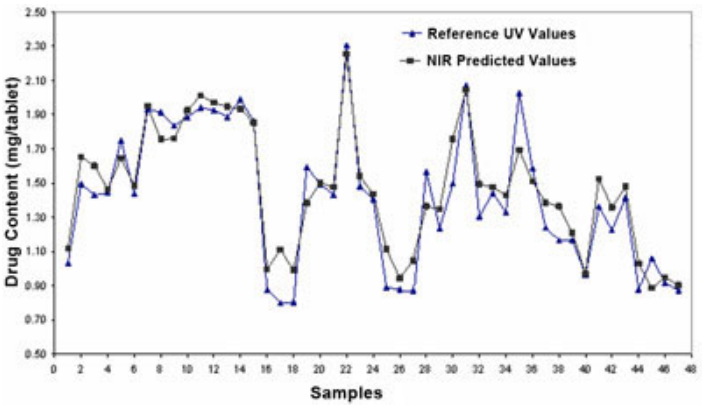

Figure 6. Plot showing the predicted NIR results and the UV results from the reference method.

Table 3. Summary of Cross-Validation and Validation Set Statistics for Chosen Calibration Model*

Statistic	Calibration Set	Validation Set
Number of samples	110	48
Range (mg/tablet)	0.71-2.51	0.80-2.31
Correlation coefficient	0.9101	0.9502
Slope	1.002	0.97
Intercept	0.63	0.30
Durbin-Watson Order Processing	1.63	1.60
Durbin-Watson Order Spectral Collection	1.76	1.53
RMSECV (mg)	0.19	N/A
RMSEP (mg)	N/A	0.14
RSEP (%)	N/A	9.3
Bias (mg)	0.0002	−0.050

*RMSECV indicates root mean square error of cross-validation; RMSEP, root mean standard error of prediction; RSEP, relative standard error of prediction; and N/A, not applicable.

scattering is caused by API particles and also by the excipient particles which are in much greater concentration in this formulation. For this application, the spectral area used in the calibration appears to be more important than the spectral pretreatment used.

All models developed explained more than 98.0% of the spectral variation with 4 or more factors, while the model in the area of 11 216 to 8662 cm^{-1} after standard normal variate (SNV) and first derivative explained 98.9% of the variation with only 3 factors, thus resulting in the simplest model. Figure 4 shows the spectra after transformation with SNV and first derivative. The differences in the spectra reflect the changes in concentration. The determination of the optimal number of factors was recognized as a key step to avoid under-fitting, where all the relevant information is not included in the model or over-fitting, where noise features are included in the model.[19,20] The choice of the number of factors is also intimately related to the spectral pretreatments used, because if the effect of scatter is not reduced by the preprocessing methods, then it will be modeled by one or more of the factors. The number of factors was first estimated with the F test developed by Haaland and Thomas,[21] which is included in the Pirouette software. This step was followed by an evaluation of spectral loadings, where only the loadings that contributed to the signal were kept in the model, and loadings that described the noise were not included. Figure 5 shows the spectral loadings for the model chosen. The 3 loadings show patterns without high frequency noise or other features indicative of noise.

As shown in Table 2, some of the models required 4 factors, while others required 5 factors. The simplest models resulted in the area from 11 216 to 8662 cm^{-1} applying the

Table 4. Individual Prediction Results for Model That Included 11 216 to 8662 cm^{-1} With Mean Centering, Standard Normal Variate, and First Derivative

Reference UV Method (mg/tab)	NIR Predicted (mg/tab)	Residuals
0.80	1.11	–0.31
0.80	1.04	–0.24
0.80	0.99	–0.19
0.81	1.05	–0.24
0.87	0.90	–0.03
0.88	1.00	–0.12
0.88	0.94	–0.07
0.88	1.03	–0.15
0.89	1.11	–0.22
0.92	0.95	–0.03
0.97	0.97	–0.01
1.03	1.12	–0.09
1.06	0.89	0.17
1.17	1.36	–0.20
1.17	1.21	–0.04
1.23	1.36	–0.13
1.24	1.35	–0.11
1.24	1.39	–0.15
1.30	1.49	–0.19
1.33	1.43	–0.10
1.36	1.52	–0.16
1.41	1.43	–0.03
1.41	1.48	–0.07
1.43	1.60	–0.17
1.43	1.48	–0.04
1.44	1.49	–0.05
1.44	1.48	–0.04
1.44	1.46	–0.02
1.48	1.54	–0.06
1.49	1.50	–0.01
1.49	1.65	–0.16
1.50	1.76	–0.26
1.57	1.37	0.20
1.59	1.51	0.08
1.60	1.38	0.21
1.75	1.65	0.11
1.84	1.76	0.08
1.86	1.85	0.01
1.89	1.92	–0.04
1.89	1.95	–0.06
1.91	1.76	0.16
1.93	1.97	–0.04
1.93	1.95	–0.02
1.94	2.01	–0.07
1.99	1.93	0.05
2.03	1.69	0.34
2.07	2.05	0.03
2.31	2.25	0.05

Table 5. Evaluation of Validation Set Results by Range of Ibuprofen Concentration*

Drug Content (mg/tab)	Tablets (No.)	Mean Drug Content by UV (mg/tablet)	Predicted Mean Drug Content by NIR (mg/tablet)	t_{crit} 1-tailed	t_{calc}	SEP (mg/tablet)	RSEP%
0.80-1.24	18	0.98	0.98	1.75	0.00028	0.16	16.3
1.30-1.60	17	1.45	1.51	1.75	0.099	0.13	9.1
1.75-2.31	13	1.95	1.90	1.80	0.13	0.12	5.9
0.80-2.31	48	1.41	1.46	1.68	0.0098	0.14	9.3

*NIR indicates near infrared; SEP, standard error of prediction; and RSEP, relative standard error of prediction.

different pretreatments. The 3-factor model in this area was considered the simplest and most suitable for prediction.[22] Cross-validation and prediction errors obtained with 4-factor models were in all cases equal or higher—never lower—than those obtained with the simplest model. The model obtained after mean centering, SNV, and first derivative for the region between 11 216 and 8662 cm^{-1} provided slightly lower results in the determination of the tablets in the calibration and validation sets.[23] The RMSEP obtained was 0.14 mg with a 9.3% RSEP, and the 3 spectral loadings for the model are shown in Figure 5. At first glance the 9.3% RSEP appears to be a high relative error, but it is expected owing to the low drug content of the tablets. The 0.14 mg RMSEP is similar to the 0.16-mg RMSEP obtained in the determination of steroid tablets with a 5-mg label claim (2.94% [m/m]), but where the RSEP was 3.25% because of the higher drug content.[6] This calibration model was selected for the subsequent validation studies where the model's performance was further evaluated.

Process Monitoring and Optimization

This NIR method could be used during the development of a new formulation as a process optimization tool to analyze a large number of tablets and detect any possible segregation mechanisms before process validation efforts start. For example, suppose that a pharmaceutical company manufactures a new product with a specified drug content of 1.30 to 1.60 mg. If the drug content were to deviate outside of this range as shown in Figure 6, the NIR method would identify the trends or changes in drug content. The NIR results shown in Figure 6 follow the same trend defined by the UV reference method.

Accuracy

Table 3 shows a summary of the cross-validation and prediction results obtained with the calibration model developed. The method's accuracy is described by the RMSEP and also by the bias.[7,24] The bias (average residual error) obtained for the prediction set was −0.051 mg per tablet. A paired *t* test between reference method UV and NIR predicted values showed no significant differences (t_{alc} = 0.0098 and t_{crit} = 1.68, 46 *df* and $\alpha = 0.05$). The correlation coefficient of 0.9502 and the regression plot in Figure 7 are evidence of good agreement between the predicted and reference values as the drug content varied. The method's accuracy was evaluated as shown in Table 4 by comparing the predicted NIR values for each of the tablets in comparison with the values obtained by the validated UV reference method. Prediction results for the best calibration model were also evaluated in terms of drug content range as shown in Table 5 for the mean drug content at the 3 concentrations used in the study. The error observed is higher at the 0.5% and 0.7% (m/m) concentrations. At the higher concentration, the relative error is slightly lower as expected.

Repeatability

As defined in the International Conference on Harmonization (ICH) guidelines repeatability expresses the precision under the same operating conditions over a short interval of time.[25] ICH suggests 2 alternatives to assess the repeatability of a method: (1) a minimum of 6 readings of a single sample at 100% of target concentration, and (2) a minimum of 3 readings on each of 3 samples, one at each of the 3 levels of concentration. In this study, 9 spectra were obtained per tablet without moving the tablet at each concentration, and

Table 6. Results for 9 Repeat Predictions at 3 Different Drug Contents*

	NIR Predicted Values (mg/tablet)		
Reading No.	Tablet 1	Tablet 2	Tablet 3
1	0.881	1.651	2.461
2	0.887	1.611	2.480
3	0.915	1.603	2.472
4	0.899	1.607	2.476
5	0.900	1.656	2.478
6	0.901	1.646	2.467
7	0.902	1.643	2.468
8	0.905	1.634	2.465
9	0.905	1.632	2.459
Average (mg/tablet)	0.899	1.632	2.470
SD (mg/tablet)	0.010	0.020	0.007
RSD	1.127	1.220	0.303

*NIR indicates near infrared; SD, standard deviation; RSD, relative standard deviation.

Table 7. Results From Intermediate Precision Study

Tablets at 0.5%	Analyst 1	Analyst 2	Rows Mean
Day 1	0.9199	1.0610	0.990
Day 2	1.0490	1.0523	1.051
Day 3	1.1275	1.1127	1.120
Column mean	1.032	1.075	N/A
Statistics	F	P value	F crit
Rows	2.315	.302	19.0
Columns	0.770	.473	18.5
Tablets at 0.7%			
Day 1	1.3577	1.3666	1.362
Day 2	1.3607	1.4578	1.409
Day 3	1.3379	1.4313	1.385
Column mean	1.352	1.419	N/A
	F	P value	F crit
Rows	0.893	0.528	19
Columns	5.324	0.147	18.5128205
Tablets at 1.00%			
Day 1	2.0316	2.0114	2.021
Day 2	2.1278	1.9965	2.062
Day 3	1.9661	2.0790	2.023
Column mean	2.042	2.029	N/A
	F	P value	F crit
Rows	0.144	0.874	19.0
Columns	0.033	0.872	18.5

then the drug content was predicted using the chosen calibration model. The approach followed fulfills the ICH requirements for either of the 2 suggested methodologies. The standard deviation was ~0.02 mg or less for all 3 strengths as shown in Table 6.

Intermediate Precision

ICH recommendation for this level of precision is to study the effect of random events during the analysis. In this precision study 2 random events were considered: the analysis of tablets on 3 different days, and 2 analysts performing the analysis on the same day. Three tablets at nominal concentration of 0.5% (m/m), 0.7% (m/m), and 1.0% (m/m) were used along with 2 analysts. Two-factor analysis of variance (ANOVA) without replication applied to data in Table 7 revealed no statistical differences between days and analysts for the 3 concentrations tested.

Linearity

In routine univariate calibration methods linearity is established within a specific range, and it is the ability of the method to respond proportionally to the changes in concentration or amount of the analyte in a sample.[25] In NIR spectroscopy the multiplicative scattering and multivariate methods usually make it worthless to evaluate linearity with "a plot of signals as a function of analyte concentration or content" as done in other analytical methods.[26] In NIR validations the linearity is usually evaluated on the basis of the predicted result versus the result obtained with the reference method. The regression line in Figure 7 shows no visible evidence of nonlinearity, which is confirmed with data in Table 3. In addition to ICH-required statistics, the Durbin-Watson (DW) statistic was calculated to test serial correlation on data.[7,24] The DW tests for serial correlation among ordered data, and its value as a statistical tool depends on how the data are ordered. In this case, serial correlation was checked for the order in which the software processed the spectra and also according to the order in which the spectra were collected. Statistical comparison ($\alpha = 0.05$) to tabulated values showed no serial correlation of the data for the 2 serial correlations evaluated.[27]

Industrial Application

The results obtained indicate that FT-NIR transmission spectroscopy could be used to determine the drug content of commercially available products of similar drug concentrations. Industrial application would require the use of tablets with a drug content spanning the range of interest. Pilot batches could be purposely manufactured outside the drug target level. Tablets from several production batches could be used to include in the calibration model other manufacturing variables such as changes in the compression forces, and variation in the particle size of excipients and the active ingredient. This variation could also be modeled following a design of experiments approach.

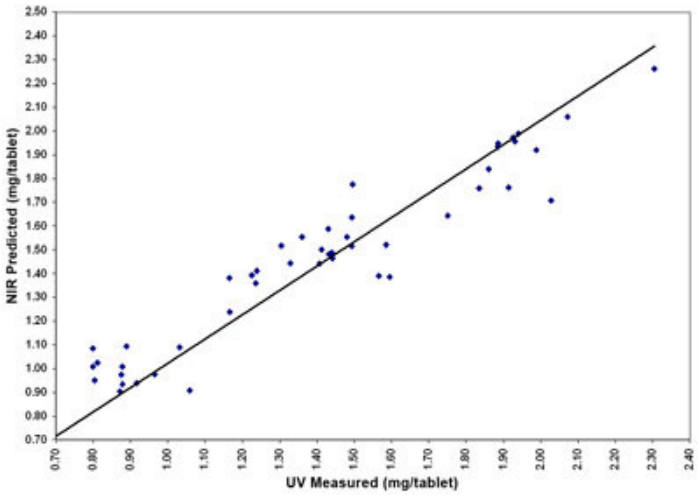

Figure 7. Results for validation samples by NIR and the UV reference method with the chosen calibration model.

CONCLUSIONS

NIRS in transmission mode can be used for quantitation of solid dosage formulations with less than 1% (m/m) drug. Because the method does not require sample preparation, it could be used to analyze a large number of tablets during process development, detect drug agglomeration problems, and facilitate process development and optimization.

ACKNOWLEDGMENTS

The authors thank the Dane O. Kildsig NSF Center for Pharmaceutical Process (West Lafayette, IN), Puerto Rico Industrial Development Co (PRIDCO, San Juan, PR), and Janssen-Ortho LLC (Gurabo, PR) for their financial support, and Angel Zapata and Alvaro Realpe for their help in operating the compressing machine.

REFERENCES

1. Rudnic EM, Schwartz JD, eds. Oral solid dosage forms. In: *Remington: The Science and Practice of Pharmacy.* Baltimore, MD: Lippincott, Williams, & Wilkins; 2000:858–893.

2. Wu LS, Pang J, Chen JG, Hussain MA. Dry blending process scale-up for a very low dose drug candidate. *AAPS PharmSciTech.* 2000; 1:E-TN2.

3. United States Pharmacopeial Convention, Inc. *USP 28-NF19. US General Chapter 905, Uniformity of Dosage Forms*. Rockville, MD: United States Pharmacopeial Convention; 2005.

4. Romañach RJ, Santos MA. Content uniformity testing with near infrared spectroscopy. *Am Pharm Rev.* 2003;6:62–67.

5. Blanco M, Coello J, Iturriaga H, Maspoch S, Pou N. Influence of the procedure used to prepare the calibration sample set on the performance of near infrared spectroscopy in quantitative pharmaceutical analyses. *Analyst.* 2001;126:1129–1134.

6. Broad N, Jee R, Moffat C, Smith M. Application of transmission near-infrared spectroscopy to uniformity of content testing of intact steroid tablets. *Analyst.* 2001;126:2207–2211.

7. Ritchie GE, Roller RW, Ciurczak EW, Mark H, Tso C, MacDonald SA. Validation of a near-infrared transmission spectroscopic procedure, part B: application to alternate content uniformity and release assay methods for pharmaceutical solid dosage forms. *J Pharm Biomed Anal.* 2002;29:159–171.

8. Kemper MS, Magnuson EJ, Lowry SR, et al. Use of FT-NIR transmission spectroscopy for the quantitative analysis of an active ingredient in a translucent pharmaceutical topical gel formulation. *AAPS PharmSci.* 2002;3:E23.

9. Ramirez JL, Bellamy MK, Romañach RJ. A novel method for analyzing thick tablets by near infrared spectroscopy. *AAPS PharmSciTech.* 2001;2:E11.

10. Laasonen M, Harmia-Pulkkinen T, Simard CR, Răsănen M, Vuorela H. Development and validation of a near-infrared method for the quantitation of caffeine in intact single tablets. *Anal Chem.* 2003;75:754–760.

11. Scheiwe MW, Schilling D, Aebi P. Near infrared analysis of intact pharmaceutical diclofenac coated tablets in transmission. *Pharm Ind.* 1999;61:179–183.

12. Dyrby M, Engelsen SB, Nørgard L, Bruhn M, Lundsberg-Nielsen L. Chemometric quantitation of the active substance (containing C≡N) in a pharmaceutical tablet using near-infrared (NIR) transmittance and NIR FT-Raman spectra. *Appl Spectrosc.* 2002;56:579–585.

13. Jedvert I, Josefson M, Langkilde FW. Quantification of an active substance in a tablet by NIR and Raman spectroscopy. *J Near Infrared Spectrosc.* 1998;6:279–289.

14. Gottfries J, Depui H, Fransson M, et al. Vibrational spectrometry for the assessment of active substance in metoprolol tablets: a comparison between transmission and diffuse reflectance near infrared spectrometry. *J Pharm Biomed Anal.* 1996; 14:1495–1503.

15. Beebe R, Pell RJ, Seasholtz MB. Multivariate Calibration and Prediction. *Chemometrics: A Practical Guide.* New York, NY: John Wiley & Sons; 1998–280.

16. Blanco M, Coello J, Iturriaga H, Maspoch S, Pezuela C. Effect of data preprocessing methods in near-infrared diffuse reflectance spectroscopy for the determination of the active compound in a pharmaceutical component. *Appl Spectrosc.* 1997; 51:240–246.

17. Olinger JM, Griffiths PR. Effects of sample dilution and particle size/morphology on diffuse reflection spectra of carbohydrate systems in the near- and mid-infrared. I. Single analytes. *Appl Spectrosc.* 1993;47:687–694.

18. Olinger JM, Griffiths PR. Effects of sample dilution and particle size/morphology on diffuse reflection spectra of carbohydrate systems in the near- and mid-infrared. II. Durum wheat. *Appl Spectrosc.* 1993;47:695–701.

19. Jouan-Rimbaud D, Walczak B, Massart DL, Last IR, Prebble KA. Comparison of multivariate methods based on latent vectors and methods based on wavelength selection for the analysis of near-infrared spectroscopic data. *Anal Chim Acta.* 1995;304:285–295.

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

21. Haaland DM, Thomas EV. Partial least-squares methods for spectral analyses. 1. Relation to other quantitative calibration methods and the extraction of qualitative information. *Anal Chem.* 1988;60:1193–1202.

22. Blanco M, Coello J, Eustaquio A, Iturriaga H, Maspoch S. Development and validation of methods for the determination of miokamycin in various pharmaceutical preparations by use of near infrared spectroscopy. *Analyst.* 1999;124:1089–1092.

23. Barnes RJ, Dhanoa MS, Lister SJ. Standard normal variate transformation and de-trending of near-infrared diffuse reflectance spectra. *Appl Spectrosc.* 1989;43:772–777.

24. 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.

25. *ICH-Q2A, Guideline for Industry: Text on the validation of Analytical Procedures, 1995*. Available at: http://www.ich.org/. Accessed: April 22, 2005.

26. *ICH-Q2B, Validation of Analytical Procedures: Methodology, November 1996.* Available at: http://www.ich.org/. Accessed: April 22, 2005.

27. Draper NR. *Applied Regression Analysis*. New York, NY: John Wiley & Sons; 1998.