Chemometric Quantitation of the Active Substance (Containing C≡N) in a Pharmaceutical Tablet Using Near-Infrared (NIR) Transmittance and NIR FT-Raman Spectra

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In this study, near-infrared (NIR) transmittance and Raman spectroscopy chemometric calibrations of the active substance content of a pharmaceutical tablet were developed using partial least-squares regression (PLS). Although the active substance contained the strongly Raman active C≡N functional group, the best results were obtained with NIR transmittance, which highlights the difference between (microscopic) surface sampling and whole tablet diffuse transmittance sampling. The tablets exist in four dosages with only two different concentrations of active substance (5 mg (5.6% w/w), and 10, 15, and 20 mg (8.0% w/w) active substance per tablet). A calibration on all four dosages resulted in a prediction error expressed as the root mean squared error of cross-validation (RMSECV) of 0.30% w/w for the NIR transmittance calibration. The corresponding error when using Raman spectra was 0.56% w/w. Specially prepared calibration batches covering the range 85–115% of the nominal content for each dosage were added to the first sample set, and NIR transmittance calibrations on this set—containing coated as well as uncoated tablets—gave a further reduction in prediction errors to 0.21–0.289% w/w. This corresponds to relative prediction errors (RMSECV/y_{nom}) of 2.6–3.7%. This is a reasonably low error when compared to the error of the chromatographic reference method, which was estimated to 3.5%.

Index Headings: **Chemometrics; Content uniformity; Cyanide; iPLS; Near-infrared transmittance; NIR; Pharmaceutical; PLS; Quantitative; Raman; Tablets.**

INTRODUCTION

Severe regulatory control through 'Good Manufacturing Practice', GMP, has given the pharmaceutical industry a need for performing large numbers of quality control analyses daily. A great effort is therefore made to find new methods that are fast and reliable. This study deals with the area of finished products control—specifically the control of Content Uniformity (the variance in active substance content) in tablets, for which the existing analysis is a chromatographic method that is time and solvent consuming and requires specifically trained personnel. Spectroscopic methods are rapid and—in combination with multivariate data analysis—have the potential to replace many existing chemical methods. Furthermore spectroscopic methods can be implemented in- or at-line, giving a better control of the entire production.

A rapidly developing spectroscopic technique is Raman spectroscopy, which, unlike infrared spectroscopy (IR), does not require special sampling techniques. All materials—fluids as well as solids—can be measured with no sample preparation and quartz can be used as optical material for measurements with fiber-optic probes. Another interesting feature of Raman spectroscopy is that the cyanide group (C≡N), present in the active substance of the tablets investigated in this study, is extremely active in Raman, while it is less active in IR and other spectroscopic techniques. Raman spectroscopy measures the weak inelastic scattering created by interaction between the incoming light and the vibration levels in molecules. The intensity of Raman signals is directly proportional to the concentration of the scattering group, described by the equation $I_s \propto I_0 \cdot \nu_s^4 \cdot c$, where I_0 is the incoming laser intensity, ν_s is the frequency of the scattered light, and c is the concentration of the scattering group. The basis for performing quantitative analyses with Raman spectroscopy has already been established,[1] but still very few examples of Raman applications for quantitation and quality control exist in the literature, and even fewer studies exist using multivariate methods in the data analysis.[2]

In recent years, near-infrared (NIR) spectroscopy has been frequently applied in the food industry[3] as well as in the pharmaceutical industry, where it has found use in many areas of production, from raw material identification[4] and process control[5] to qualitative and quantitative analyses of finished products such as tablets.[6] Heretofor, most applications have used NIR spectra recorded in diffuse reflectance mode due to the strong absorbance of a compact tablet, but the development of more sensitive detectors and more powerful light sources, as well as special sampling devices for tablets, has made the diffuse transmittance mode more widely used. The advantage of the transmittance mode, when compared to the reflectance mode, is that the spectra will contain information about the inside of the tablet, which makes this method less sensitive to sample inhomogeneity and to the use of coating materials. Recently, a number of studies have been reported on the quantitation of different active substances in tablets with NIR transmittance spectroscopy.[7–12]

The advantage of using spectroscopic techniques such as Raman and NIR spectroscopy for quality control is that they are nondestructive, noninvasive, and fast, and they do not require any special sample preparation. The rapid acquisition of spectra and the large amount of in-

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TABLE I. Tablet specifications.

Nominal content of active substance per tablet (mg)	Nominal tablet weight (mg)	Nominal weight percent (%)	Number of batches	
5.0	90	5.6	1 full scale	3 pilot scale
10.0	125	8.0	2 full scale	3 pilot scale
15.0	188	8.0	2 full scale	3 pilot scale
20.0	250	8.0	2 full scale	3 pilot scale
4.3–5.7	90	4.8–6.3	3 laboratory scale	
8.3–11.4	125	6.9–9.1	3 laboratory scale	
12.9–17.1	188	6.9–9.1	3 laboratory scale	
17.3–22.8	250	6.9–9.1	3 laboratory scale	

formation present in those spectra render quantitative interpretation practically impossible without chemometric software and powerful computers. The well-known chemometric method of partial least-squares regression (PLS) is a very powerful algorithm designed to build quantitative models calibrated to known standards, and it will be used throughout this study. This paper will present the use of NIR transmittance and Raman spectroscopy in combination with chemometrics for the prediction of active substance content in tablets.

EXPERIMENTAL

Materials. Escitalopram® tablets from the pharmaceutical company H. Lundbeck A/S, were used in this study. Four different dosage values (as shown in Table I) of this pharmaceutical drug (5, 10, 15, and 20 mg tablets) were used. The 10, 15, and 20 mg tablets had the same concentration of active substance, e.g., were dose proportional, and had a slot and print on one side. The tablets had different total weights and therefore also different shapes and sizes, with the thickness of the tablets ranging from 2.9 to 4.3 mm. Seven full scale production batches and 12 pilot plant batches were available (see Table I). Furthermore, three specially prepared batches were produced to extend the calibration range to 85–115% of the nominal content *for each dosage form,* giving 12 laboratory scale batches. In total, 31 batches were used, and from each batch 10 tablets were individually weighed and analyzed by the spectroscopic methods as well as by the reference method. The pilot plant batches were film coated, while the full scale and laboratory scale batches were uncoated. The tablets contained several excipients, the dominant one being microcrystalline cellulose (~80%), and minor excipients being magnesium stearate, talc, and—for the coated tablets only—a coating material containing titanium dioxide. All raw materials were available in pure powdered form for spectral analysis.

Raman Spectroscopy. Raman spectra of single tablets were obtained using a Perkin–Elmer System 2000 NIR FT-Raman spectrometer equipped with a diode pumped Nd:YAG laser emitting 400 mW at ν_0 = 9394.69 cm^{-1} (corresponding to 1064.4 nm) and an InGaAs detector. Raman wavenumber shifts were collected in the range 200–3600 cm^{-1} (interval, 1 cm^{-1}), with a resolution of 8 cm^{-1} and using the average of 64 scans for each sample. Tablets were placed in a sampling device for solids with the slot and print pointing away from the light source, and the Raman Stokes scatter was collected as 180° backscatter without background correction. The powdered raw materials were measured in a sampling device designed for powders, but with the same instrumental settings as mentioned above.

Near-Infrared Transmittance Spectroscopy. Near-infrared transmittance spectra were recorded in the range 4000–14 000 cm^{-1} (700–2500 nm), with a resolution of 16 cm^{-1} and using the average of 128 scans per sample. The spectrometer used was an ABB Bomem FT-NIR model MB-160 equipped with a ''Tablet Samplir'', a sampling device specially made for measuring pharmaceutical tablets using an InGaAs 1.7-μm detector. The Tablet Samplir is equipped with a universal tablet tray that can be used to measure tablets as well as capsules of varying size. The producer has not specified a range of sizes, but the device can handle samples of diameter or length from approximately 2 (beam size is 1 mm) to 15 mm, and thicknesses up to approximately 20 mm (in practice limited by the amount of transmitted light). Background transmittance spectra were recorded using the internal ceramic standard (Spectralon® 99%) and were used to convert the tablet spectra to absorbance units. Using a Spectralon® 99% standard, the level of intensity of the reference spectrum (1% of the light source intensity) and of the sample spectrum are in the same range, resulting in a narrower working range for the detector.

Tablets were measured with the score away from the light source and each tablet was measured twice (pilot plant batches were measured only once) with replacement in between measurements and the average spectrum used in the subsequent calibrations.

Reference Method, High Performance Liquid Chromatography. The content of active substance in each tablet was measured by high performance liquid chromatography (HPLC). After extraction from whole tablets, the content was determined by injecting 20 μL into a 150 × 4.6 mm 5-μm Luna C18(2) column held at 45 °C. The eluent was acetonitrile/methanol/acetate buffer, pH 5.2 (7:33:60 v/v/v), with a flow of 1.0 mL/min. The total elution time was 20–25 min, and the active substance was detected at 239 nm (UV detection) and quantitized according to standard solutions. The HPLC analysis has an estimated error of ±3.5% (total of sampling and apparatus error). The content was given in mg active substance per tablet, and from this, using the *individual* tablet weights, the weight percent (% w/w) was calculated. Both units were used in the calibrations.

Method of Data Analysis. Multivariate models were developed in order to predict the content of active substance measured by the HPLC reference method (Y variable) from the Raman or NIR transmittance spectra (X variables), which were mean-centered before calibration in all models. Models were built using PLS, and several different preprocessing methods were tested in order to obtain the best predictability. Multiplicative scatter correction (MSC), as well as first and second derivatives, was used on both Raman and NIR transmittance spectra. Furthermore, on Raman spectra the preprocessing method standard normal variate[13] (SNV), which in a previous study on tablets[2] gave excellent results in combination with the second derivative, was tested.

Partial least-squares models were initially built from the preprocessed data using the full length of the recorded

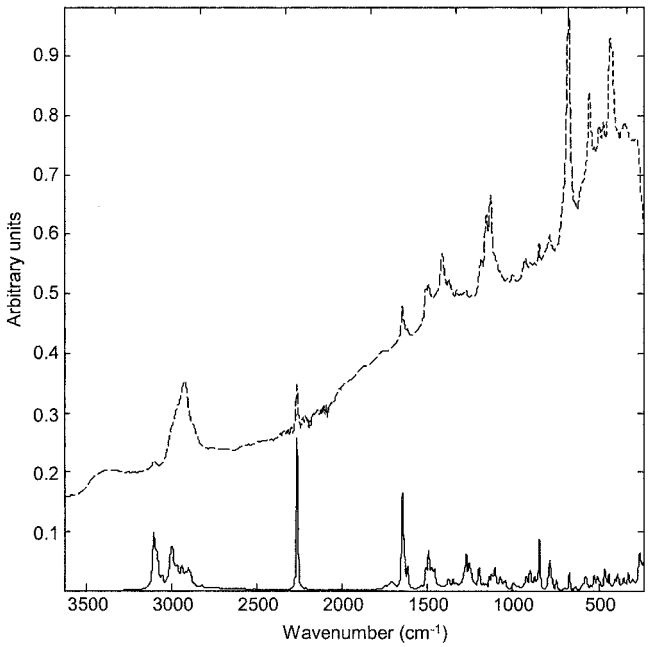

FIG. 1. Raw Raman spectra of a 20 mg tablet (dashed line) and the pure active substance (solid line).

spectra. Furthermore, different variable sets were tested both manually, selecting variable sets based on chemical knowledge about the samples, and using interval PLS (iPLS) with optimization, a systematic method for variable selection developed by Nørgaard and co-workers.[14]

In order to assure the performance of the models on future samples, validation must be performed. In this study, models were validated using cross-validation with ten segments, each batch being represented in each segment.

The predictive ability of calibrations is most often evaluated from the root mean squared error of prediction (RMSEP) or of cross-validation (RMSECV).[15] In this study the *relative prediction error* is also used, a parameter calculated as $RMSECV/y_{nom} \cdot 100\%$, where y_{nom} is the nominal active substance content for the present calibration. The advantage of using the relative prediction error is that it is directly comparable to the error of the reference method and that relative prediction errors from different calibrations with variances in nominal active substance content can be compared.

Software. Unscrambler version 7.5 (CAMO A/S, Trondheim, Norway) was used for all PLS models as well as for preprocessing with MSC and derivatives (Norris). MATLAB version 5.3 (MathWorks, Inc., Natick, MA) with PLS_Toolbox version 2.0 (Wise & Gallagher, Eigenvector Research, Manson, WA) and The Graphical User Interface iPLS Toolbox for MATLAB version 2.1 (available at www.models.kvl.dk/source/) was used for iPLS calculations. MATLAB was also used for preprocessing with SNV.

RESULTS AND DISCUSSION

Preliminary Calibration Set. Preliminary calibrations were made with a set of pilot plant batches only, with a total of 120 samples (12 batches with 10 tablets per batch) for which Raman as well as NIR transmittance

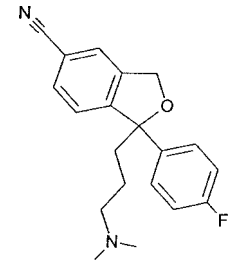

FIG. 2. Structure of the active substance.

spectra were recorded. With this sample set, a comparison was made between calibrations predicting the active substance content expressed as absolute content (mg active substance per tablet, range 4.59–21.6 mg) and as concentration (weight percent, range 5.12–8.48%). Using the absolute content yielded calibration errors that were 4–5 times higher for NIR and 5–6 times higher for Raman calibrations than the corresponding calibrations using concentration. The reason for the poor performance of the absolute content calibration, an approach that has been successfully used in other studies,[2,9,11] is that the four dosage forms have different shapes and sizes and therefore different light interactions. Last but not least, the three largest dosage forms (10, 15, and 20 mg tablets) have the same concentration of active substance, i.e., are dose proportional. Both spectroscopic methods are essentially concentration measurements since both contain information from a specific sample volume, in NIR transmittance the volume exposed to the diffusely transmitted light and in Raman the small surface volume from which the weak inelastic Stokes scatter is collected. Knowing the exact sampling volume for each tablet size in each of the two instruments, it might be possible to improve the absolute content calibration, but because the concentration approach gave markedly better results, all the following calibrations are made using concentration as the Y variable.

Raman Calibration. Figure 1 shows the raw spectra of a 20 mg tablet and of the pure active substance. In the tablet spectrum a distinct peak can be seen at 2233 cm^{-1}, which originates from the cyanide (C≡N) group in the active substance, for which the structure is shown in Fig. 2. Several other peaks from the active substance can be seen in the tablet spectrum (e.g., at 1614 and 3075 cm^{-1}), but none are as specific as the cyanide peak. The cyanide group is especially interesting because it has an extremely intense Raman signal in a spectral region where no other groups are active. The spectrum of the tablet shows several peaks from the excipients including three intense peaks from the titanium dioxide in the coating material (395, 511, and 634 cm^{-1}) and several peaks from cellulose (1095, 1121, 1380, and 2900 cm^{-1}). There is a strong fluorescence background originating from an unknown residual in the primary excipient, microcrystalline cellulose. Cellulose contains trace amounts of organic substances that can be highly fluorescent. The fluorescence will be treated as a random interference by the PLS model and should therefore not give serious problems.

Several preprocessing methods were investigated, and calibration results are shown in Table II. Several manually chosen variable sets were tested containing infor-

TABLE II. Comparison of different preprocessing methods on the performance of Raman models. All models are based on the full spectra (200–3600 cm^{-1}) as no improvement was seen using iPLS.

Preprocessing	# PC[a]	RMSECV[b]	R^2[c]
none	5	0.63	0.83
MSC	6	0.59	0.85
first derivative	5	0.56	0.87
second derivative	5	0.56	0.87
SNV	6	0.59	0.85
SNV + second derivative	5	0.65	0.82

[a] Number of PLS components.
[b] Prediction error (% w/w).
[c] Correlation coefficient.

mation about the active substance and avoiding, e.g., the peaks from titanium dioxide, but resulted in no better model than that using the full spectrum. We attempted to localize optimal intervals using iPLS as well, but no iPLS model was obtained that resulted in a better prediction error than the full spectrum model.

The best calibration model using Raman spectra was obtained using first derivative spectra, which removed some of the interference from the fluorescence background. This model has a cross-validated prediction error of 0.56% w/w using five PLS components, which is in accordance with the many variations in the tablets other than active substance, i.e., in excipients, coating thickness, fluorescence, density, etc. The relative prediction error ($RMSECV/y_{nom} \cdot 100\%$) is 10.1% for the 5 mg tablets and 7.0% for the 10, 15, and 20 mg tablets. The relatively large prediction error of this model can be explained by the fact that repeated measurements of a tablet, where the tablet was moved in between measurements, displayed large differences in intensity of the peaks. Actually, the sampling error, estimated by the prediction error of ten repeated measurements of the same tablet, was 0.51% (RMSEP) for a 20 mg tablet, which accounts for the largest part of the total prediction error for this model, 0.56%. This is mainly due to the very small volume being measured with this method in combination with the relatively high inhomogeneity of this type of sample. The coating (containing titanium dioxide, a strong Raman scatterer) is especially responsible for the variation in the intensity of the peaks because variation in coating thickness influences the volume of internal tablet material being measured. Using more than one measurement point on each tablet would most certainly lower the prediction error, but that would obviously prolong the time of analysis, which was already more than three times that of the NIR analysis. Another possibility, which would not prolong the time of analysis, would be rotating the sample during acquisition of the spectrum.[16] None of these possibilities were pursued further based on the superior results of the NIR transmittance calibration (see discussion later).

In a study by Jedvert and co-workers,[2] a model based on Raman spectra was obtained that had a relative prediction error of 1.8% using tablets with a nominal active substance content of 30%. The much higher prediction error obtained in this study is in part ascribed to the above-mentioned subsampling problems and in part to the smaller active substance content (5.6 and 8.0% w/w). The positive effect of preprocessing with SNV and second derivatives (observed by Jedvert and co-workers) was not seen in the present study, where SNV both alone and in combination with derivatives gave poor results.

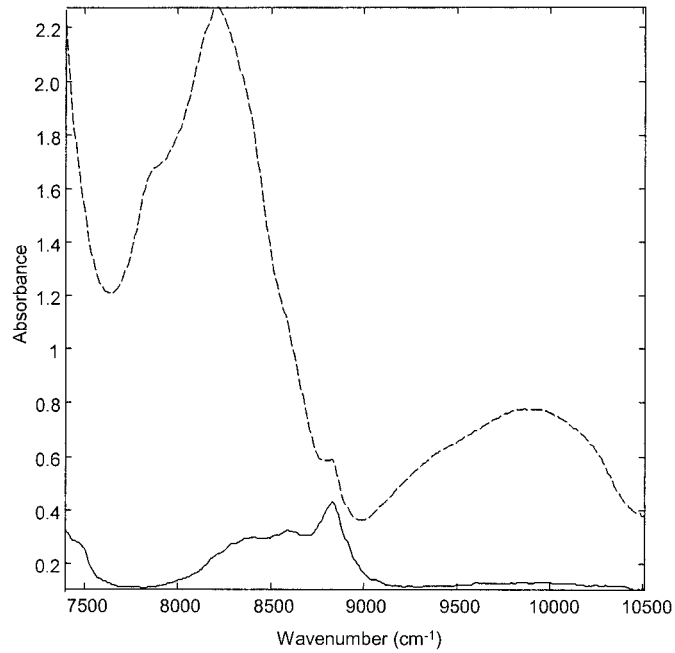

FIG. 3. NIR raw spectra of a 20 mg tablet (dashed line; transmittance spectrum) and the pure active substance (solid line; reflectance spectrum).

NIR Transmittance Calibration. Only a minor part of the recorded NIR transmittance spectra (4000–14 000 cm^{-1}) could be used in the calibrations. The range from 4000 to 7400 cm^{-1} was very noisy due to the high absorption of low-energy radiation, while the range 10 500 to 14 000 cm^{-1} contained very little information, partially due to the low detector sensitivity. Therefore, only the 7400–10 500 cm^{-1} range was used.

Raw spectra (range 7400–10 500 cm^{-1}) of a 20 mg tablet and of the pure active substance are shown in Fig. 3. The active substance has only one visually characteristic band in the tablet spectrum, which is identified as the second overtone of the aromatic C–H stretch. This peak is seen at 8830 cm^{-1} (corresponding to 1132 nm) and is partially overlapping with the peak at 8200 cm^{-1} (1220 nm) originating from the primary excipient, microcrystalline cellulose.

Calibration models were initially built from spectra ranging from 7400 to 10 500 cm^{-1} (hence referred to as 'full spectrum'). Furthermore, the informative spectral range was subsequently narrowed by iPLS, and intervals were selected that gave smaller prediction errors than the full spectrum. Several preprocessing methods were investigated and the results of some of these in combination with different variable sets (full spectrum and intervals selected by iPLS) are shown in Table III.

The best NIR transmittance model (lowest RMSECV) was obtained using second derivative spectra and an interval selected by iPLS. The prediction error of this model is 0.30%, corresponding to a relative prediction error of 5.4% for 5 mg tablets and 3.8% for the 10, 15, and 20 mg tablets. The sampling error was estimated as for the Raman model predicting the active substance content

TABLE III. Comparison of different variable sets and preprocessing methods on the performance of NIR transmittance models.

Preprocessing	Variable set (cm^{-1})	# PC[a]	RMSECV[b]	R^2[c]
None	7400–10 500	3	0.35	0.95
with iPLS	8286–8995	3	0.34	0.95
MSC	7400–10 500	3	0.32	0.96
with iPLS	8293–9119	3	0.31	0.96
first derivative	7400–10 500	2	0.36	0.95
with iPLS	8432–9273	3	0.33	0.96
second derivative	7400–10 500	2	0.39	0.94
with iPLS	8625–9173	3	0.30	0.96

[a] Number of PLS components.
[b] Prediction error (% w/w).
[c] Correlation coefficient.

from ten repeated measurements of a 20 mg tablet. The sampling error was 0.13%, which is relatively small compared to the 0.30% prediction error of the model, showing good repeatability for the NIR spectra.

The predicted vs. measured plot for the resulting NIR calibration is shown in Fig. 4. The spectral interval used in the obtained model—selected by iPLS—was centered on the peak from the active substance at 8830 cm^{-1}, as shown in Fig. 5.

Comparison of Raman and NIR Transmittance Preliminary Model Performance. The model based on NIR transmittance spectra has a prediction error of almost half that of the calibration based on Raman spectra. This is mainly ascribed to the different sampling conditions of the two methods—Raman spectroscopy employing surface sampling of a very small volume of the tablet, and NIR transmittance obtaining information about a larger volume of the tablet due to the diffuse transmittance mode. Optimizing the sampling technique, e.g., by using several spectra per tablet or spinning the sample during acquisition, could probably reduce the subsampling error of the Raman method. However, the significant difference in estimated sampling error (0.13% for the NIR and 0.51% for the Raman model) suggested that not even an optimized Raman method would be able to compete with the NIR method for this application.

Before implementation in the pharmaceutical industry, the method needs to be accepted by the authorities, and the transmittance method, containing information about a much larger volume of the tablet than a surface sampling method, would make acceptance more likely. In conclusion, the NIR transmittance method has better prediction ability due to better repeatability of the spectra, and it is better suited for at-line implementation in the pharmaceutical industry. Therefore, it was decided to continue with the NIR transmittance calibration. There are, however, two problems with the present calibration. The natural variance in active substance content is only about ±2% from the nominal values and the range of concentrations is thus very far from the ±15% acceptance limit for the Content Uniformity test. Secondly, the model contains no full scale production samples and is thus not valid for predicting future production samples. To get a valid calibration, it is necessary to add full scale batches and to expand the range of active substance content to at least ±15% of the nominal value for each dosage. This is done in the expanded calibration set.

Expanded Calibration Set. Samples covering the range 85–115% of the nominal content for each dosage form were produced in laboratory scale and—together with full scale batches—were added to the set of pilot scale batches, giving a total of 310 samples (see Table I). Calibrations on this set were made with NIR transmittance spectra only, and both global (all four dosages together) and local models (one model for each dosage), were examined. Local models were expected to give lower prediction errors, or at least yield more stable models, due to absence of variance in shape and size of the sam-

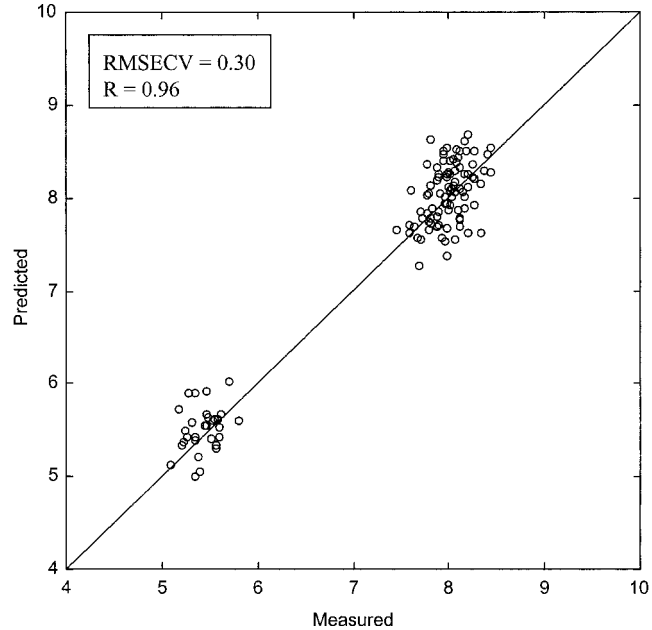

FIG. 4. Predicted vs. measured plot for the best preliminary model based on NIR transmittance spectra.

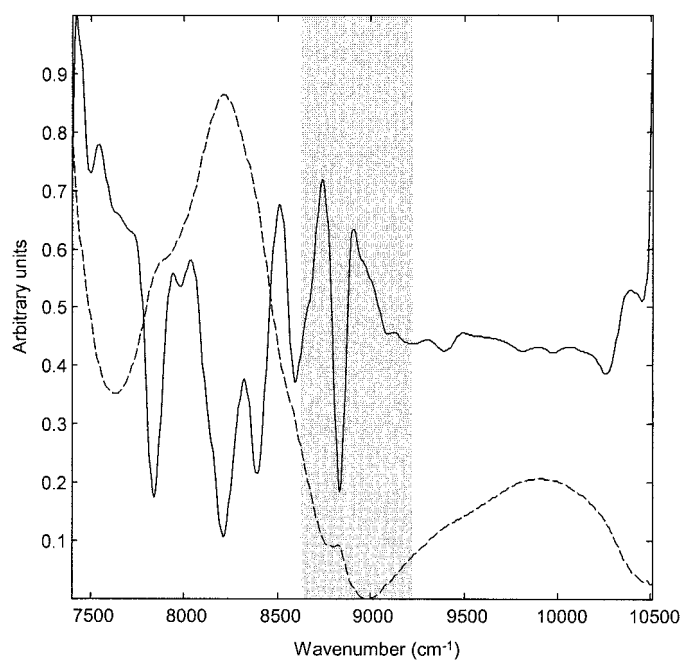

FIG. 5. The iPLS selected interval (shaded) for the best model based on NIR transmittance spectra. Shown are the raw average spectrum (dashed line) and the second derivative average spectrum (solid line).

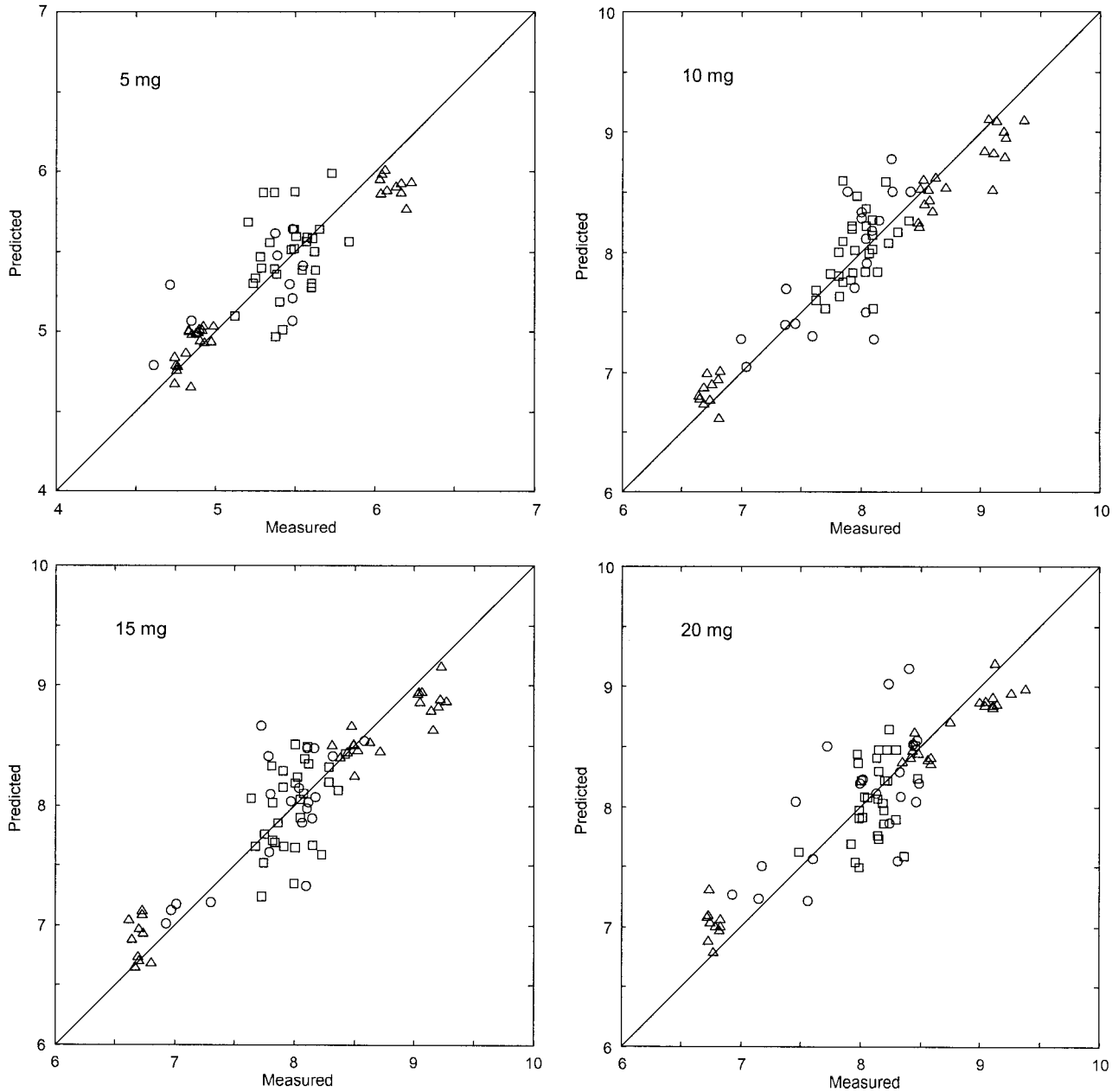

FIG. 6. Predicted vs. measured plot for the four best local models. Relative prediction errors are 3.7 (5 mg), 2.6 (10 mg), 3.5 (15 mg), and 3.5% (20 mg). Symbols refer to production scale, where ○ denotes full scale, □ denotes pilot scale, and △ denotes laboratory scale.

ples and due to narrowing the range of the individual models (thereby keeping the concentrations in a linear range). The same preprocessing methods were tested as for the preliminary sample set models, and all models were furthermore tested with iPLS to find optimal intervals (results not shown).

The best *global model* (all four dosages modeled together) used the first derivative of MSC preprocessed spectra and had a prediction error of 0.33% w/w using one PLS component, corresponding to relative prediction errors of 5.9 for 5 mg and 4.1% for 10, 15, and 20 mg tablets. The four *local models* had prediction errors before iPLS ranging from 0.22 to 0.32% w/w using two or three PLS components. With iPLS the prediction errors were slightly reduced to 0.21 to 0.28%, still with two or three PLS components, corresponding to relative prediction errors of 2.6 to 3.7%, values that are comparable to the estimated error of the reference method, HPLC, which was estimated to 3.5%. In the literature, calibrations based on NIR diffuse transmittance spectra of whole tablets show relative prediction errors between 0.8 and 4.0%.[7–12] The small relative prediction errors of some of these models are due to the markedly larger nominal active substance content being between 16 and 84% w/w, giving relatively more signal from the active substance and less from the excipients. For comparison, the absolute prediction error (RMSECV) of the 5 mg local model was only 0.21% w/w (corresponding to 0.19 mg), while the cited references have prediction errors of 0.30–0.69% w/w.

The presence of coated and uncoated tablets in the same model did not give any specific problems, since no strong signals from the coating material are present in the

NIR transmittance spectrum, but may still have contributed to the prediction error.

The lowest prediction errors were found using raw (5 mg), MSC treated (10 mg), second derivative of MSC treated (15 mg), and second derivative (20 mg) spectra, although the prediction errors did not seem to vary much for the different preprocessing methods. For all four local models, variable sets were chosen by iPLS that were centered on the 8833 cm^{-1} band of the active substance.

Figure 6 shows the predicted vs. measured plots for the four local models using the optimal preprocessing method and optimal variable set. These models clearly have a more uniform distribution of samples on the concentration axis and all samples are reasonably well predicted. However, the figure illustrates a difference in the prediction accuracy of laboratory scale as opposed to pilot and full scale samples, observed as a larger error in predictions of pilot and full scale batches (most clearly visible for the 15 mg model). A minor contribution to the error of the pilot batches is that these contain information from one spectrum only, while for the full and laboratory scale batches, the average of two spectra are used in the models, but this fact can only explain the larger error of the pilot and not the full scale batches. The observed difference is therefore mainly ascribed to the physical properties of the samples, which have a larger variation for pilot and full scale batches than for laboratory batches. This is due to the fact that all laboratory scale tablets are stamped with a tablet press using only one punch, while the pilot and full scale tablets are produced in a tablet press with a total of 40 different punches. It can be concluded that the variation in physical properties due to different punches is significant for the calibration, and it is therefore important to include the variation in physical properties due to different punches in the models in order to avoid unforeseen variances in future final full scale batches.

CONCLUSION

In this study we examined models for chemometric quantitation of the active substance in Escitalopram® tablets using Raman and NIR transmittance spectroscopy. The comparison of Raman and NIR transmittance spectroscopy showed that the sampling conditions were of utmost importance. Despite a strong baseline-separated C≡N signal in the Raman spectra from the active substance in the tablets, the inherently small sample volume measured with this technique made it inferior in quantitative performance when compared to a dedicated NIR transmittance system.

The properties of the tablets under investigation made the calibration procedure very complicated. The presence of four different dosages, of which three were dose proportional (i.e., had the same nominal concentration of active substance), different shapes and sizes of the dosages, presence of a slot in some tablets, three different scales of production, coated as well as uncoated tablets, and finally, the relatively low concentration of active substance in the tablets (5.6 to 8.0% w/w), all made the calibration procedure more complex than any calibration procedure reported in the literature. Still, successful calibrations were developed that had relative prediction errors ($RMSECV/y_{nom}$) below 3.7%, which is comparable to the error of the reference method. This error value was obtained using NIR transmittance spectra and local models, i.e., separate models for each dosage form, which solved some of the heterogeneity problems mentioned above.

Before the obtained models can finally be implemented in the production control of Escitalopram® tablets, a few modifications are required: final production batches, coated and from full scale production, must be included in the calibration in order for the models to predict future coated production batches. Furthermore, there is a need for final validation with an independent test set consisting of only final production batches in order to obtain a realistic measure of the prediction error for future samples.

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