Application Note: 51595

Investigation of Different Sampling Techniques for the Analysis of Capsule Contents by Fourier Transform Near-Infrared Spectroscopy

Key Words

- Capsule Analysis
- FT-NIR
- Antaris FT-NIR Analyzer

Abstract

Fourier transform near-infrared (FT-NIR) spectroscopy was investigated as a means for qualitative and quantitative analysis of conventional hard gelatin capsules. Analyses were performed with minimal or no sample preparation. Two sampling techniques were explored: intact whole capsule reflectance and reflectance analysis of the capsule contents removed from the shell. The viability of each sampling option was investigated and the effectiveness of FT-NIR for capsule analysis was assessed.

Introduction

Solid dosage forms comprise approximately 70% of all marketed pharmaceutical formulations. Capsules represent a significant fraction of these products. Conventional hard gelatin capsules are the most common among this genre of formulations.

Hard gelatin capsules are challenging for analytical chemists because they are relatively difficult to analyze. The most challenging aspect of capsule analysis is sample preparation. Samples are generally prepared by dissolving the dosage unit in order to extract the contents. This can be difficult as special conditions such as sample heating are often required to melt the gelatin in order to facilitate the content extraction. Alternatively, the contents can be removed from the capsules prior to sample dissolution and dilution. This approach is also challenging, especially for content uniformity analysis, as it is often difficult to completely remove the contents. Since capsules are generally analyzed on the basis of the amount of material in the whole capsule, this approach can lead to results with a low bias.

Many pharmaceutical applications of FT-NIR spectroscopy have been reported in recent years. FT-NIR offers many advantages over conventional analysis methods, such as chromatographic techniques. FT-NIR analyses are rapid, precise and easy to perform. The equipment can be used in areas away from the lab. For this case, the most relevant advantage is that minimal or no sample preparation is required.

There are many sampling options for FT-NIR. This paper investigates some of the possibilities for the analysis of capsules. Three sets of samples were evaluated for this study. For one set, the objective was to qualitatively distinguish four different formulations with a common active ingredient from one another. For the other two sets of capsules, a quantitative model was required. In each case, two different sampling options were investigated. Whole capsule reflectance analysis represents the easiest sampling option. Data from this alternative was compared with data from the analysis of the powder contents. The relative accuracy and precision of the two possibilities were assessed.

Experimental

Samples

The capsules described in Table 1 were obtained from a proprietary source. All formulations are standard, hard gelatin capsule formulations filled with powders.

Sample Set	Appearance	Number Formulations	Description of Samples
1	Blue and Gray	4	300 mg active 120 mg active 120 mg active SR* 60 mg active SR*
2	Blue and White	4	1.25 mg active 2.5 mg active 5 mg active 10 mg active
3	Red and White	7	4.5 mg active 4.75 mg active 4.875 mg active 5.0 mg active 5.125 mg active 5.25 mg active 5.5 mg active

* SR indicates sustained release formulation

Table 1: Description of capsules used in study

The first sample set contained a common active ingredient but the formulations were different. It was desired to qualitatively distinguish these four formulations in order to preserve the integrity of clinical studies. The second sample set also represented a set of the clinical samples. However, these samples were common formulations allowing the use of a quantitative approach to distinguish them. Sample set three was constructed to develop a quantitative calibration for assay of the active ingredient in the marketed formulation, which contains 5 mg of the active component. These three sets represent applications that are progressively more demanding. Collectively, they comprise a good set of test cases.

The samples were analyzed in two ways. For intact capsule reflectance analysis, the individual capsules were placed directly on the window of the integrating sphere and analyzed as is. For the analysis of the capsule contents, the powder fills were emptied into either a micro sample cup with a glass window or into a small glass vial. In either case, the containers were placed on the integrating

sphere and analyzed through the glass. The sample cups are designed for analysis of powders and facilitate the minimization of any problematic sampling issues, such as packing and orientation.

Instrumentation

A Thermo Scientific Antaris™ Method Development Sampling (MDS) System FT-NIR analyzer (Figure 1) was used to generate all of the data in this study. The Antaris is equipped with a transmission compartment, an integrating sphere for reflectance analyses, a fiber optic probe for remote sampling and transmission tablet analyzers for solid sample transmittance. Data were collected with RESULT™ software. Instrument performance verification was performed with the ValPro™ system qualification package. An internal gold flag was used as the background reference for all of these measurements. This approach presents significant advantages. Because the flag is internal, the integrity of the background measurements will be preserved over time. Also, because gold has no spectral features, there is no interference with the sample measurement.

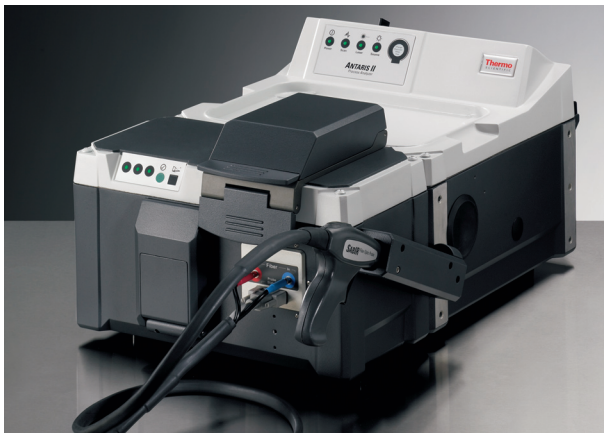

Figure 1: Antaris FT-NIR Method Development Sampling System

Data Analysis

After collection, the data were analyzed with TQ Analyst™ software. A Discriminant Analysis (DA) model was constructed for sample set one, which required the qualitative distinction of the four different dosage forms. The Mahalanobis distance unit was used as the metric to indicate the match quality. For the second sample set, a Partial Least Squares (PLS) calibration model was developed based on the nominal active content of the capsules. In the case of the final sample set, a PLS model was constructed based on the active content of the seven respective formulations, which spanned 90-110% of the nominal concentration for the marketed formulation. Root mean squared errors of calibration (RMSEC) and root mean squared errors of cross validation (RMSECV) were used to evaluate the quality of all of the quantitative calibrations.

Results and Discussion

Sampling

The focus of this investigation was to determine 1) if the Antaris FT-NIR analyzer can be a viable tool for capsule analysis and 2) the best means of sampling for the conventional hard gelatin capsules used in this study. Using these sample sets as models for typical hard gelatin capsules, we can draw general conclusions concerning the viability of FT-NIR for capsule analysis. We can also gain insight and make recommendations for specific means of sampling hard gelatin capsules.

It should be noted that although whole capsule transmittance analysis was investigated as a sampling method, the sample set used for this study was not amenable to transmittance analysis. However, in many instances, whole tablet transmittance analysis is a viable sampling technique.

Sample Set 1

For the first sample set, the goal was to demonstrate that the four different formulations (300 mg, 120 mg, 120 mg SR and 60 mg SR) could be distinguished from one another. For this purpose, intact capsule analysis was compared to the analysis of the powder fills after they were transferred to micro sample cups.

Based on comparison between the two sampling techniques, it was determined that both the data from the whole capsule reflectance sampling and the sampling of the powder contents could be used to accomplish the distinction of the dosage forms. Because the whole capsule reflectance technique represents an easy, nondestructive means of sampling, this approach was chosen for this analysis. The spectra for the samples are shown in Figure 2. For this analysis, it was determined that a derivative was not necessary to achieve the distinction. However, a view of the derivative spectra (Figure 3) gives an indication of the spectral differences that made the distinction possible.

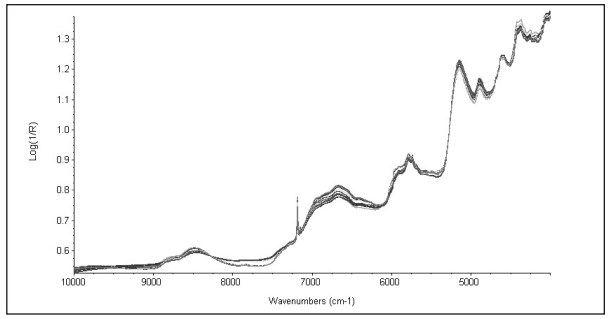

Figure 2: Untreated spectra for sample set one

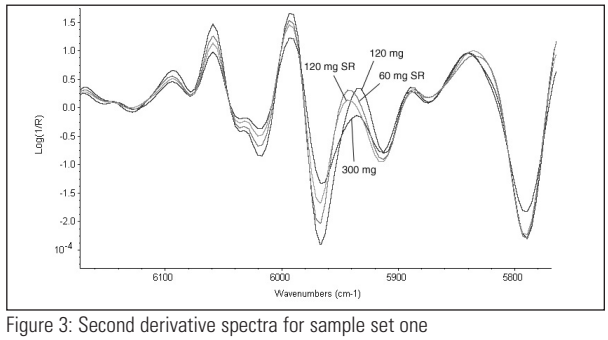

Figure 3: Second derivative spectra for sample set one

Table 2 summarizes the results for the DA model using data from the entire spectral region (4000 - 10,000 cm^{-1}). For this type of analysis, 3.0 Mahalanobis distance units is considered to be a reasonable threshold. A lower distance score represents a better match to the sample class of interest. From the data in the table, it is evident that each capsule is correctly identified using the model. In other words, there are no mismatches. It is also evident that the distance score for the next best match for each capsule is sufficiently large to allow easy distinction in each case. In other words, there are no ambiguities among the calibration set.

Spectrum	Best Class	Distance	Next Class	Next Distance
120 mg - 1	120	0.852	SR60	3.79
120 mg - 2	120	0.438	SR60	3.84
120 mg - 3	120	0.932	SR60	4.81
120 mg - 4	120	0.477	SR60	4.53
120 mg - 5	120	0.588	SR60	4.40
300 mg - 1	300	1.10	120	14.6
300 mg - 2	300	1.08	120	14.8
300 mg - 3	300	1.24	120	13.7
300 mg - 4	300	1.25	120	14.8
300 mg - 5	300	0.810	SR120	14.9
120 mg SR - 1	SR120	0.628	SR60	5.93
120 mg SR - 2	SR120	0.550	SR60	5.61
120 mg SR - 3	SR120	0.981	SR60	5.63
120 mg SR - 4	SR120	0.684	SR60	5.68
120 mg SR - 5	SR120	0.866	SR60	5.16
60 mg SR - 1	SR60	1.352	120	4.72
60 mg SR - 2	SR60	1.020	120	3.79
60 mg SR - 3	SR60	0.847	120	4.60
60 mg SR - 4	SR60	0.764	120	4.56
60 mg SR - 5	SR60	0.683	120	3.99

Table 2: Results of distinction of capsule formulations in sample set one

Sample Set 2

The desired goal was the distinction of different clinical supplies for sample set 2. However, because these samples were common formulations, the distinction could be done quantitatively. Both whole capsule reflectance and reflectance analysis of the powder contents provided data that could be used for quantification. For both sets of data, Norris second derivative pre-treatments (segment 11, gap 0) were employed. Figure 4 shows the 2nd derivative spectra for the analysis of the powder contents. The spectra are shown in a region that was particularly well-correlated to the active ingredient.

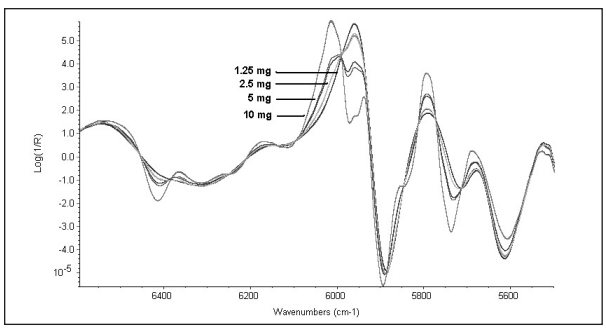

Figure 4: Second derivative spectra for sample set two

Table 3 summarizes the results for the calibrations constructed from these two sets of data. The data in Table 3 indicate that reasonable calibrations can be constructed from data collected in either manner. However, the cross-validation results suggest that better accuracy is obtained from the analysis of the powder contents. An RMSECV/RMSEC ratio of slightly more than one is desirable. Both the whole capsule analysis model and the powder analysis model are adequate for the qualitative distinction of the four formulations from one another. If non-destructive testing is mandatory, the whole capsule analysis model is a good alternative to the conventional analysis of the powder contents.

	Technique	
	Whole Capsule	Powder
Type	PLS – 3 factor	PLS – 2 factor
Corr. Coeff.	0.9991	0.9993
RMSEC*	0.143	0.126
RMSECV*	0.742	0.140

* Calibration error units are mg/capsule

Table 3: Data from calibrations constructed for sample set two

Sample Set 3

The goal for the analysis of sample set 3 was to derive a calibration in order to quantify the amount of active present in individual capsules. The standards were synthetically prepared for that purpose to encompass the range of 90-110% of the nominal drug content (5 mg /capsule). The whole capsule sampling approach, as well as the use of the powder contents provided data that could be used for quantification. Table 4 summarizes the results for the calibrations constructed from these two sets of data.

	Technique	
	Whole Capsule	Powder
Type	PLS – 2 factor	PLS – 2 factor
Corr. Coeff.	0.8686	0.9960
RMSEC*	0.152	0.0274
RMSECV*	0.302	0.151

* Calibration error units are mg/capsule

Table 4: Data from Calibrations Constructed for sample set three

A Norris second derivative pretreatment was used again in this case (segment 11, gap 0). Figure 5 shows the 2nd derivative spectra for the powder reflectance analyses. In contrast to the data for sample set 2, the spectral variance was difficult to visualize. However, small differences can still be used for quantitative analysis if they are consistent. Figure 6 shows the calibration plot using the powder reflectance data. The data points represent the average of three data-collection events at each level.

In this case, the destructive approach was the only way to accurately analyze the active content for each capsule. This calibration can be optimized with additional samples. The sample sizes were small and the use of a vial smaller than the 2-dram vials used in this study might also enhance the results. This case represents a difficult analysis because the active content is only about 4% of the formulation. Even with such challenging circumstances, the use of FT-NIR for this application still provides good results.

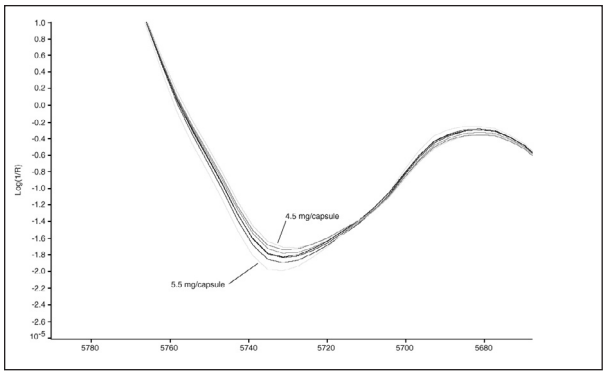

Figure 5: Expanded second derivative spectra for sample set three

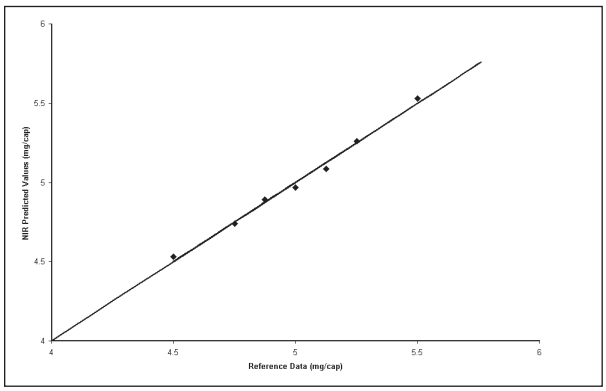

Figure 6: Calibration plot for sample set three

Conclusions

From the three sets of data presented, it is evident that FT-NIR can be used for both quantitative and qualitative analysis of capsules. In the case of the distinction of clinical formulations by qualitative and quantitative analyses, non-destructive sampling was possible. In the case of the quantitative analysis of a low percentage of active ingredient in a small capsule, the destructive analysis of the capsule contents was the only possible means of achieving reasonable results. Based on these model cases, it can be concluded that non-destructive testing for capsules is possible in many cases if such an approach presents significant logistical advantages. Not surprisingly, however, the capsule shell presents a significant source of spectral interference. This fact makes the destructive approach using only the powder contents for analysis the means for achieving better accuracy in almost every case. However, even this approach involves no extractions, no solvent disposal and no exposure of hazardous solvents to workers. The preparation time is minimal and the analysis time is very rapid (less than one minute). Hence, the destructive analysis approach still presents attractive improvements compared to conventional analysis techniques such as HPLC.

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