Application Note: 50780

Determination of Moisture Content in Freeze-dried Materials by FT-NIR Spectroscopy

Key Words

- Antaris
- FT-NIR
- Integrating Sphere
- Lyophilization
- Moisture
- Pharmaceutical

Abstract

The feasibility of Fourier transform near-infrared spectroscopy (FT-NIR) for determination of moisture in lyophilized preparation of folinic acid calcium salt (Leucovorin Ca) was examined. FT-NIR spectra were collected by diffuse reflectance with the Integrating Sphere module of a Thermo Scientific Antaris™ Method Development Sampling system. Stepwise multiple linear regression (SMLR) was used for calibration. The calibration results showed that FT-NIR spectroscopy is a suitable method for quantification of moisture in freeze-dried materials.

Introduction

Many pharmaceutical formulations are produced and stored as lyophilized (freeze-dried) products. The moisture content of these freeze-dried materials must be maintained within a certain range because residual moisture can affect the chemical and physical stability of the product. Karl Fischer titration is the most common method for determining moisture content, but it requires reagents and destructive testing of several samples. Because lyophilized pharmaceutical formulations are expensive, there is a significant benefit for eliminating Karl Fischer titration. For these reasons, the pharmaceutical industry is interested in analytical techniques that are quick, reliable, and non-destructive when determining the amount of residual moisture in freeze-dried materials.

Compared with traditional analytical techniques, FT-NIR spectroscopy has many inherent advantages for moisture detection. It allows noninvasive and nondestructive analysis, and no reagents are used. Samples can be analyzed in situ through glass or plastic containers with minimal preparation. Simultaneous multi-component analysis is possible, and the total analysis time is typically less than one minute. Near-infrared absorption bands originate from overtones and combinations of the fundamental (mid-infrared) bands (mostly from C-H, N-H, and O-H bonds), making FT-NIR spectroscopy especially sensitive to hydroxyl groups as well as hydrogen bonding caused by residual moisture.

The purpose of this work was to study the feasibility of FT-NIR to accurately determine the moisture content in freeze-dried materials using a lyophilized preparation of folinic acid calcium salt (Leucovorin Ca). Leucovorin Ca is a metabolite of folic acid and is used as an antidote to prescribed folic acid antagonists (e.g. methotrexate) or as an antianemic to combat folate deficiency.

Experimental/Methodology

Standards – Eight batches of lyophilized Leucovorin Ca with different moisture content in hermetically sealed glass bottles were obtained. The moisture content of each batch was measured by gravimetry (loss on drying) and the results are listed in the table below.

Moisture content in lyophilized Leucovorin Ca

BATCH ID	WATER CONTENT %
001	8.04
002	7.31
003	7.02
004	3.54
005	3.20
006	2.99
007	2.95
008	2.65

The diffuse reflectance spectra of lyophilized Leucovorin Ca were collected using the Integrating Sphere module of an Antaris FT-NIR analyzer (see Figure 1). The spectra were acquired in the range from 12000 to 4000 cm^{-1} with a collection time of one minute and resolution of 4 cm^{-1}. Quantitative methods were developed using Thermo Scientific TQ Analyst™ method development software. Because the samples were hygroscopic, they remained sealed in the original clear glass vials, and were analyzed by placing them directly onto the window of the Integrating Sphere module. Multiple spectra were collected from each sample to account for measurement variation. The internal gold flag of the Integrating Sphere module was used as the background reference material.

Figure 1: Antaris FT-NIR analyzer

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Results and Discussion

The final moisture content of Leucovorin Ca should be at or near 8%. However, the method routinely used to indicate the end of lyophilization is not reliable and formulations with lower moisture content are often produced. The moisture content as measured by gravimetric methods can be determined after the lyophilization ends, but it takes several hours to get the results and a large batch of material can be wasted in that time. FT-NIR spectroscopy can quickly take a non-destructive measurement for at-line or near-line analysis. Rapid, accurate determination of moisture content would allow the freeze-drying process to be stopped at the optimal moisture content.

The FT-NIR spectra of lyophilized Leucovorin Ca with different moisture contents are shown in Figure 2. The spectra are significantly different in appearance. Baselines are shifted (likely due to sample morphology and differences in the vials) and there are strong changes in the bands, particularly in the OH overtone and combination regions (Figure 3).

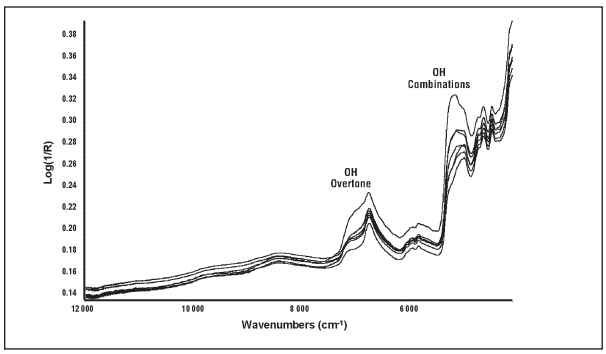

Figure 2: FT-NIR spectra of lyophilized Leucovorin Ca (common scale)

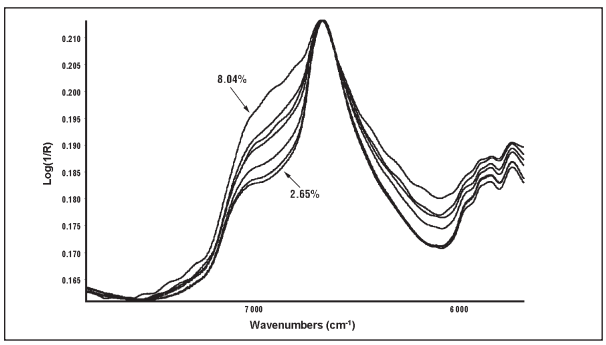

Figure 3: FT-NIR spectra of lyophilized Leucovorin Ca in detail

Stepwise multiple linear regression (SMLR) was selected for developing the calibration model. This method is one of the most common modeling approaches for quantitative analysis in the near-infrared range. Two component regions were included in the model. The stepwise algorithm of SMLR in the TQ Analyst software automatically determines the most appropriate regions.

The particle size and composition of the samples were so similar that no pathlength correction was necessary. Second derivatives of the spectra were calculated to remove baseline offset and slope.

The actual concentrations of water were plotted against the calculated concentrations. The correlation coefficient for calibration was 0.9996 and the root mean square error of calibration (RMSEC) was 0.0610% in the range that varied from 2.65-8.04% (Figure 4). The leave-one-out method of cross validation gave a correlation coefficient of 0.9989 and a RMSECV of 0.0987 (Figure 5). These results indicate that FT-NIR spectroscopy can be used for moisture content determination in freeze-dried materials.

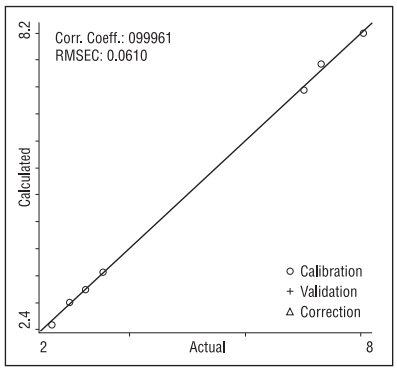

Figure 4: SMLR calibration result for lyophilized Leucovorin Ca

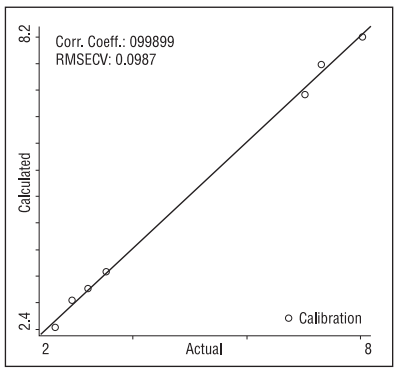

Figure 5: SMLR cross validation result for lyophilized Leucovorin Ca

Conclusion

An effective SMLR model for determination of moisture content in lyophilized preparative Leucovorin Ca was created. The results of this feasibility study show that FT-NIR analysis determined the concentration of moisture in lyophilized materials quickly and with no sample preparation or reagents. This could be a useful technique for studying different stages of the drying process. FT-NIR is of great benefit for near or at line detection of the end of the lyophilization process.

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