



## The advantage of resolution in the FT-NIR quantification of fatty acid components in a quaternary mixture

### Keywords

Antaris, diffuse reflectance, fatty acids, FT-NIR, spectral resolution

### Abstract

Fatty acids of different chain lengths are chemically similar to one another. This similarity is quite evident when comparing their vibrational spectra. The common belief is that it is easy to discern materials when they have distinct chemical features but difficult when the materials of a mixture have similar structural moieties.

Diffuse reflectance Fourier transform near-infrared (FT-NIR) spectroscopy was investigated as a tool to quantify the concentrations of a model quaternary mixture of stearic acid, palmitic acid, myristic acid, and lauric acid. This study demonstrates the feasibility of this application as well as the advantage of the Fourier transform technique. It has been the belief that better resolution and superior wavelength repeatability allow for better quantification. This was demonstrated in this study by comparing results of data collected on a Thermo Scientific™ Antaris™ FT-NIR Analyzer at 4, 8, 16, and 32  $\text{cm}^{-1}$  optical resolution.

Calibration models using blends of these fatty acids were developed and tested using a cross-validation procedure. Errors for these predictions are good, suggesting that FT-NIR can be used as a convenient spectroscopic measurement tool for this application.

### Introduction

The use of FT-NIR for routine, quantitative analyses has increased substantially in recent years. Many users have discovered the advantages of this technique relative to other analytical procedures. The advantages of FT-NIR include:

- Reduction in technician labor for analysis
- Elimination of the use and disposal of solvents
- Elimination of worker exposure to noxious chemicals
- Quicker turnover of analytical results allowing more timely decisions for production-related analyses.

Stearic acid is a key excipient used in many pharmaceutical processes. The United States Pharmacopeia monograph for stearic acid indicates that a substantial amount of palmitic acid, and perhaps also a small amount of myristic acid, will be present in most batches of this incoming raw material. A gas chromatography test can be performed to quantify the amounts of these impurities; however, the test takes a long time to perform. An FT-NIR spectroscopic method for this type of application would provide substantial time-savings; although the feasibility of such a method is rather tenuous because there are few apparent spectroscopic differences between the compounds.

The purpose of this investigation is to determine whether FT-NIR provides advantages in the resolution that facilitate the relative quantification of four fatty acids that differ only by their carbon chain length. Stearic acid (octadecanoic acid,  $C_{18}H_{36}O_2$ ), palmitic acid (hexadecanoic acid,  $C_{16}H_{32}O_2$ ), myristic acid (tetradecanoic acid,  $C_{14}H_{28}O_2$ ), and lauric acid (dodecanoic acid,  $C_{12}H_{24}O_2$ ) were blended to produce quaternary mixtures. All of the above fatty acids have low melting points and, although solid at room temperature, have a waxy consistency. A static-electric charge is produced when they are vigorously mixed, making it hard to prepare good calibration sample sets. The mixing technique was optimized in this study, and the resulting mixtures were used to create FT-NIR calibration models to determine the feasibility of this application.

## Experimental

**Samples** – All four straight-chain fatty acids were purchased from Sigma-Aldrich and were at least 99% pure.

**Equipment** – The FT-NIR measurements were performed on an Antaris FT-NIR Method Development Sampling (MDS) system (Figure 1). This study was performed using the Integrating Sphere Module for diffuse reflectance measurements.



Figure 1. Antaris Method Development Sampling system.

**Sample preparation and measurement** – The calibration standards were prepared by weighing the appropriate amounts of materials into vials to achieve the desired weight percentages listed. The four fatty acid component samples were prepared to at least bracket 0% to 50% of each major component. Stearic acid ranged from 0–51.7%, palmitic acid from 0–54%, lauric acid from 0–59.6%, and myristic acid from 0–56.2%. The experimental design was randomized to obtain relative orthogonality for all four components. The fourth component amount, therefore, was not calculated to result in a predetermined weight but was also randomized.

The sample vials were sealed and gently heated in a boiling water bath for a few seconds and then allowed to cool to room temperature. This method produced a homogeneous opaque layer. The opacity is an important requirement for the diffuse reflectance measurement because it facilitates the scattering of sufficient light back

into the Integrating Sphere Module and, thus, to the detector. After this process, all of the samples were measured without further preparation by placing each vial directly on the integrating sphere and measuring through the bottom of the vial. Each calibration sample was measured in duplicate.

**Collection parameters** – The parameters used for data collection are tabulated below. All data were collected with Thermo Scientific™ RESULT™ Analysis Software. The instrument performance was verified prior to use with the Thermo Scientific™ ValPro™ system qualification tests. The performance test utilizes the internal validation wheel, which has NIST-traceable and serialized standards. The photometric linearity and wavelength accuracy of the Antaris system are verified by these tests. This is essential for a demanding application. The wheel also contains a NIST-traceable polystyrene standard to confirm band-position accuracy. To establish the effect of spectral resolution on the calibration performance, four resolutions were used.

- **Spectroscopic range:** 10000 to 4000  $cm^{-1}$
- **Resolution:** 4, 8, 16, 32  $cm^{-1}$
- **Co-averaged scans:** 32
- **Background:** Internal gold glag for automatic, background collection

**Calibration development** – The calibration model was developed using the Thermo Scientific™ TQ Analyst™ Software package for quantitative analysis. All spectra were mean-centered and subsequently converted to their respective second-derivative spectra prior to the development of calibration models. This was accomplished using a Norris derivative with a 3-point segment and a 5-point gap. Derivatization is commonly used to normalize the slope and offset found in NIR data. This was made necessary because each of the samples exhibited different levels of scattering as a function of the composition. Stepwise Multiple Linear Regression (SMLR) calibration models were developed for quantitative analysis.

## Results and discussion

Figure 2 shows the diffuse reflectance spectra of the fatty acid samples prior to derivatization. The baseline of the spectra varies due to the different scattering characteristics of the mixtures. Figure 3 shows the spectra following the derivative processing, and it is worth noting that the spectral features that can be used for the quantitative assay are very sharp. This indicates that higher resolution would fare better in characterizing these features.

Upon examining the spectra of the pure materials, it becomes clear that the four ingredients are very similar. After expanding a representative spectral region, the differences manifested as very small spectral shifts due to the chain length differences of the fatty acids can be observed. The shift at the band shown in Figure 4, for example, is only a few wavenumbers. Because the spectral shifts among the pure materials are small, the x-axis reproducibility from the Antaris analyzer is critical for this application.

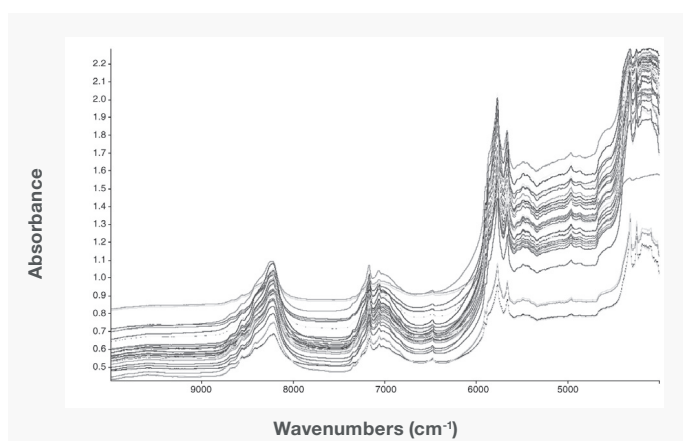


Figure 2. Unprocessed spectra for calibration samples.

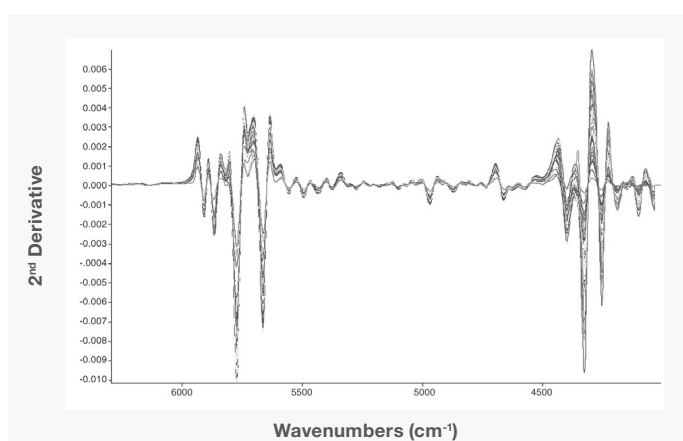


Figure 3. Second-derivative spectra of fatty acid mixtures.

The effect of the enhanced resolution is shown in Figure 5. This plot shows an expansion of the second derivative spectra of one of the calibration samples at the four resolution settings used in this study. The second derivative is shown in order to emphasize the spectral detail. The derivative functions were calculated over the same range for each resolution setting used to ensure consistency. There are several places where the loss of spectral information is evident as the resolution is degraded. This loss of information directly affected the calibration performance.

For this mixture, SMLR calibration equations were found to be adequate for the measurement of all four components. The use of simple SMLR models in a feasibility study is generally desirable because few calibration samples are typically available. A simple model that yields good correlation and good predictive ability normally portends success for an application. This is because there is little or no risk of incorporating inclusive information unique to the calibration samples, which is a danger with other calibration techniques.

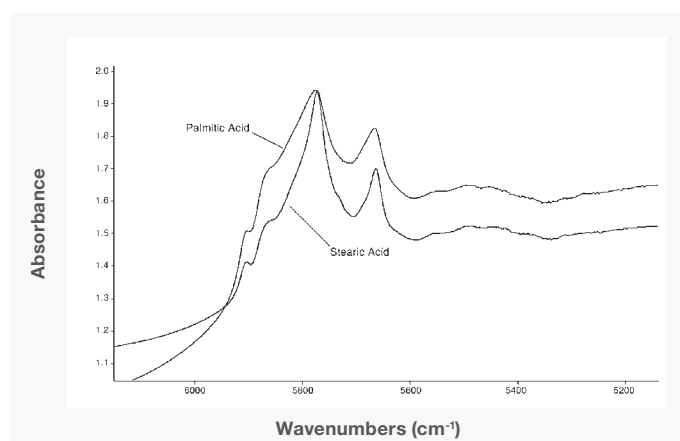


Figure 4. Small wavelength shift observed between stearic and palmitic acids.

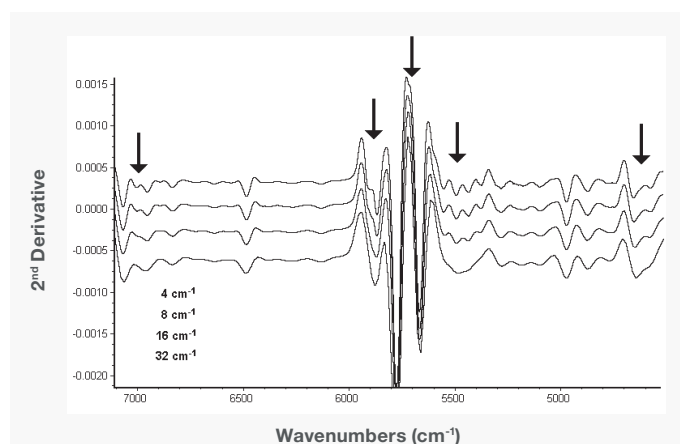


Figure 5. 2nd derivative spectra for the four resolution settings used in the study.

|               | 4 cm <sup>-1</sup> |        | 8 cm <sup>-1</sup> |        | 16 cm <sup>-1</sup> |        | 32 cm <sup>-1</sup> |        |
|---------------|--------------------|--------|--------------------|--------|---------------------|--------|---------------------|--------|
|               | R <sup>2</sup>     | RMSECV | R <sup>2</sup>     | RMSECV | R <sup>2</sup>      | RMSECV | R <sup>2</sup>      | RMSECV |
| Stearic acid  | 0.968              | 0.0581 | 0.917              | 0.0860 | 0.890               | 0.0979 | 0.780               | 0.0903 |
| Palmitic acid | 0.919              | 0.0833 | 0.824              | 0.1350 | 0.518               | 0.1970 | 0.425               | 0.1180 |
| Lauric acid   | 0.973              | 0.0336 | 0.967              | 0.0380 | 0.905               | 0.0636 | 0.827               | 0.0822 |
| Myristic acid | 0.944              | 0.0499 | 0.909              | 0.0623 | 0.820               | 0.0873 | 0.513               | 0.1210 |

**Table 1. Data showing differences in calibration results, with varying resolution. Higher resolution improved the calibrations.**

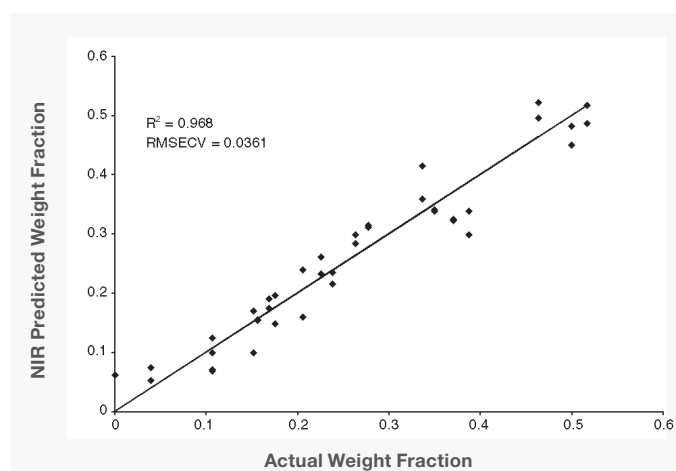
The correlation coefficients for all models were >0.9 using the spectra with 4 cm<sup>-1</sup> resolution. For example, Figure 6 shows a cross-validation prediction plot using a four-point, SMLR calibration for stearic acid. The coefficient of variation and the root mean squared error of cross-validation (RMSECV) are indicated on the plot.

The calibration and subsequent cross-validation procedures were carried out for the four resolutions and for all four components. Four-wavelength SMLR calibrations were used for each model for consistency. Table 1 reports the results for these experiments. The data in Table 1 shows that the increased resolution provides much better predictions for all of the components.

## Conclusions

The Thermo Scientific Antaris FT-NIR Analyzer is a good tool to measure the multicomponent mixtures of straight-chain fatty acids. The components are chemically very similar, but the diffuse reflectance spectra of the pure materials show 1–2 cm<sup>-1</sup> shifts in some of the sharp bands. The high resolution and superior x-axis reproducibility of the Antaris FT-NIR analyzer are substantial advantages for this type of application. These capabilities are critical for difficult applications.

The data were collected using an older model instrument Antaris FT-NIR. Currently, Thermo Scientific offers an improved model, the Antaris II FT-NIR, which offers superior speed and performance over its predecessor model.



**Figure 6. Cross-validation prediction plot for stearic acid.**

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