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Quantification of Vitamin C Using FT-NIR Spectroscopy

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Key Words

- Antaris
- Ascorbic Acid
- Dietary Supplements
- FT-NIR
- Nutraceuticals
- Vitamin C

Introduction

Ascorbic acid (vitamin C) is one of the most popular dietary supplements consumed in the United States, accounting for well over $150 million in sales annually. Its use in the prevention of scurvy is well known, and it is increasingly recognized for its antioxidant properties. Ascorbic acid is also widely used in the food industry to prevent browning in canned or frozen fruits. Additionally, it is often used in plastics manufacture to aid in the polymerization process, or to neutralize iodine in chemically treated water. Traditionally, ascorbic acid is mixed with excipients such as starch that act as fillers or binders in addition to enhancing flow characteristics. Analysis and quantitation of ascorbic acid in these mixtures is difficult and time consuming, requiring complicated titrations or extractions coupled with fluorimetry. These methods necessitate the use of complicated equipment, corrosive or toxic chemicals, and highly trained analysts. Fourier transform near-infrared (FT-NIR) spectroscopy is a simpler, more rapid method of ascorbic acid analysis that avoids the disadvantages associated with these traditional techniques, while providing highly accurate, reliable data.

Near-infrared spectroscopy relies on the part of the electromagnetic spectrum between the visible and infrared regions (approximately 4000 to 10000 cm^{-1}). Nearly all complex organic molecules exhibit characteristic vibrational overtones and combination bands in this region. Broadband light from an FT-NIR analyzer interacts with and is absorbed by the sample, setting up these characteristic molecular vibrations. The remaining light is collected by the analyzer and is displayed as a spectrum. FT-NIR is unique in spectroscopic analysis in that it can be used to quantitatively determine several components of a mixture with little or no sample preparation, making the technique appropriate for use by non-technical individuals. For the current study, different mixtures of ascorbic acid in starch were analyzed using a Thermo Scientific Antaris™ II Method Development Sampling (MDS) FT-NIR analyzer.

Experimental

Ascorbic acid and starch (Sigma-Aldrich, St. Louis, Missouri) were weighed and mixed to produce ten standards. Table 1 describes the make-up of the standard mixtures as well as the subsequent validation mixtures. Samples were placed in glass vials and scanned through the bottom using the SabIR probe attached to the analyzer (Figure 1). Spectra were collected within the entire NIR range, between 4000 and 10000 cm^{-1}. Each sample was analyzed three times using 110 scans with 8 cm^{-1} resolution. Before each analysis, the contents of the bottles were mixed to ensure sample homogeneity. The three measurements of each sample were averaged into a composite spectrum that was used in the chemometric model. A representative spectrum of a typical mixture is shown in Figure 2.

	Ascorbic Acid (mg/g)	
Sample	Calibration Standards	Validation Samples
1	53.30	
2	100.90	100.9
3	149.40	150.1
4	199.75	200.45
5	249.75	249.95
6	299.65	300.05
7	349.60	350.05
8	397.05	400.2
9	449.95	450.25
10	500.00	

Table 1: Sample preparation showing the amount of ascorbic acid per gram of material. Calibration standards were used to create the chemometric model. Separate validation samples were used to test the predictive ability of the model.

Figure 1: Antaris MDS with SabIR probe (inset). Samples were placed in glass vials and sampled through the bottom using the SabIR probe.

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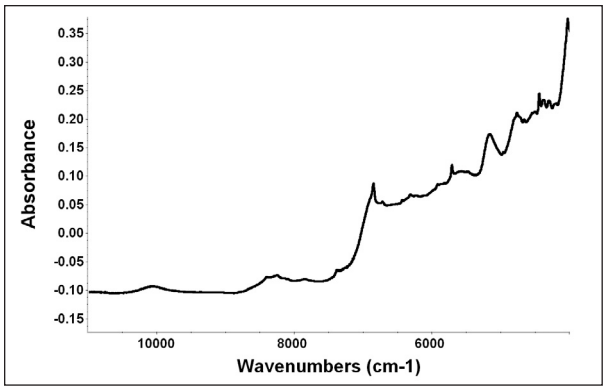

Figure 2: Representative spectrum of an ascorbic acid-starch mixture used as a standard

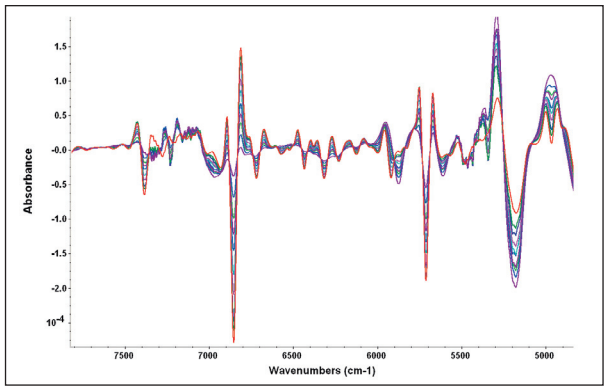

Figure 3: Second derivative spectra of the standards used to build the chemometric model. The variation between the standards is clearly shown in the range from 4800 to 7600 cm^{-1}.

Chemometric Modeling

A Partial Least-Squares (PLS) mathematical regression model was used for calibration of the second derivative spectra using Thermo Scientific TQ Analyst™ software. The region between 4800 and 7600 cm^{-1} (Figure 3) was analyzed using the Multiplicative Signal Correction (MSC) pathlength option. The Prediction Residual Error Sum of Squares (PRESS) plot suggested one factor was required for the calibration using these parameters. The correlation coefficient for this model was found to be 0.99841 with a Root Mean Square Error of Calibration (RMSEC) of 8.05. Cross-validation analysis resulted in a correlation coefficient of 0.99692 with a Root Mean Square Error of Cross Validation (RMSECV) of 11.4.

New mixtures were used to predict ascorbic acid ratios as well as validate the developed chemometric model. Eight new samples were prepared using similar methods to those of the original calibration blends. These new samples were similarly scanned with the FT-NIR analyzer and analyzed with the TQ Analyst model. Root Mean Standard Error of Prediction (RMSEP) was found to be 11.2. Figure 4 shows the Calibration and Residual plots for the calibration standards (○) and validating test samples (+) and summarizes the quality of fit for the developed chemometric model.

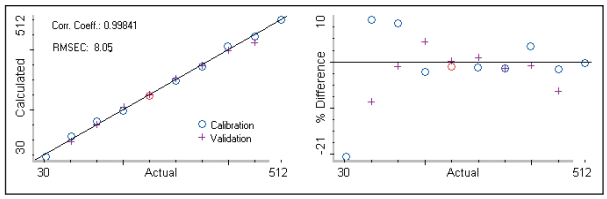

Figure 4: Calibration (left) and residual (right) plots showing excellent performance of the chemometric model. Standard samples (○) were used to develop and calibrate the model; separate samples (+) were created and analyzed for validation.

Conclusions

Accurate analysis of ascorbic acid-starch mixtures was successfully performed using FT-NIR spectroscopy. Calibration standards ranging from 53 to 500 mg/g of ascorbic acid in starch were used to develop a PLS chemometric model of analysis. The model yielded excellent statistical results with high correlation coefficients and low errors. Furthermore, this chemometric model was used to analyze validation samples and was shown to be highly predictive in determining the concentration of ascorbic acid in the starch matrix.

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