Application note

Multi-component analysis of fructose syrup using the Antaris FT-NIR Analyzer

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Keywords

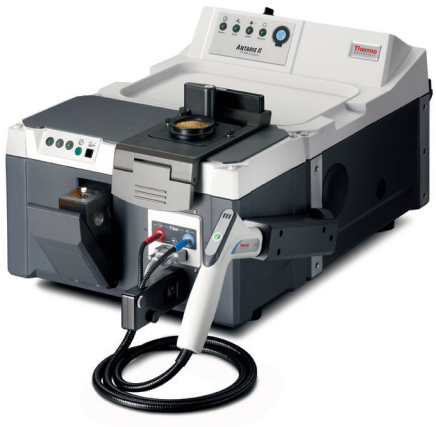
Antaris, fructose corn syrup, HFCS, multi-component, near-infrared

Introduction

Fructose corn syrup is produced in many parts of the world especially where corn supplies are abundant such as the United States. High fructose corn syrup (HFCS) is used in the soft drink and food industries as a direct replacement for sucrose (table sugar) as it has a similar sweetness. The most common grades of HFCS contain 42% and 55% fructose. High fructose corn syrup has many advantages over sucrose including longer shelf life, ease of transportation, ease of blending a liquid vs. a powder, and lower cost in areas where corn is plentiful. High fructose corn syrup is produced at large milling facilities that can process over 100,000 bushels of corn per day. If the process for making HFCS is disrupted, even for a short period of time, thousands of pounds of out-of-specification product can be produced.

The process for making HFCS starts with separation of corn into its components, the main component of which is starch. Starch is a polysaccharide $(C_6H_{10}O_5)n$ consisting of a large number of glucose monosaccharide units joined together by glycosidic bonds. The corn starch is converted to individual glucose molecules by adding enzymes, heat, and by adjusting the pH in a process called saccharification. The saccharification product contains a very high percentage of glucose along with small percentages of maltose, triose and higher sugars due to incomplete conversion of the starch. Glucose and fructose are constitutional isomers (Figure 1) meaning they have the same molecular formula but different chemical structure. An isomerization enzyme (glucose isomerase) is added to the high glucose saccharification material to yield liquid containing 42% fructose. Further processing of this stream acts to increase the fructose percentage above 70%. The high fructose stream is then blended with a lower fructose stream to yield 55% HFCS. These two streams vary in fructose % over time due to the continuous flow nature of the milling process.

Analyzing the real time carbohydrate profile, especially with respect to fructose and glucose %, at various stages of the process will lower reprocess costs as well as ensure final product quality. The ability of the Thermo Scientific™ Antaris II Fourier transform near-infrared (FT-NIR) analyzer to measure samples quickly inline, without sample preparation, using fiber optic probes makes trending and closed loop control strategies easy to implement. FT-NIR can replace time-consuming HPLC analysis which requires samples be brought to the lab frequently with a significant delay in results for a dynamically changing process.



Thermo Scientific™ Antaris™ II Method Development Sampling (MDS) FT-NIR analyzer

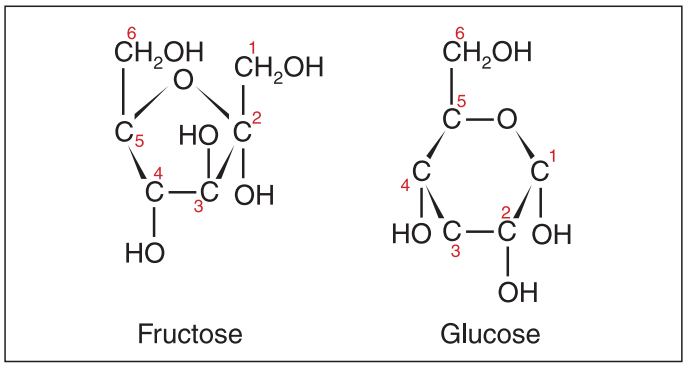


Figure 1. Chemical structure for fructose and glucose isomers.

Experiment

The standards used to develop the method for analysis of fructose syrup were clear to slightly colored liquids. The Antaris Method Development Sampling (MDS) FT-NIR analyzer was used to collect spectra using a heated transmission cell at 60 °C with a 1 mm quartz cuvette. Samples were pre-heated to 60 °C using a lab bench incubator to further decrease the sample analysis time. When quantifying components in liquid samples, it is important to collect spectra at constant temperature to decrease variability across samples. The Antaris FT-NIR family of instruments has been developed with a common optical path, standard reference materials, and precision engineering to ensure transfer of methods from lab to process analysis.

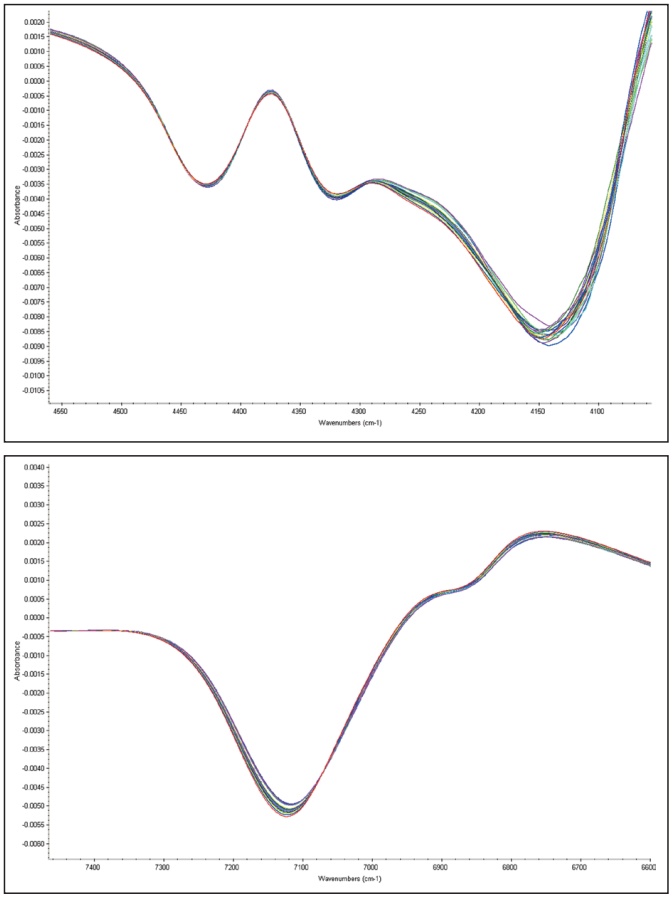


Figure 2. Regions of interest for °Bx method development.

Standard spectra were collected with 64 scans at 8 cm^{-1} resolution in less than 30 seconds. A 1 mm cuvette was used for this method to maximize signal to noise while allowing for easy sample handling. Reference values for fructose and glucose were obtained using HPLC results adjusted for Degrees Brix (°Bx). Degrees Brix is a measurement of the mass ratio of dissolved sugar to water in a liquid. It is commonly measured using an instrument called a refractometer that measures refractive index which is correlated to °Bx. A Partial Least Squares (PLS) model was developed using 18 standards for the 3 components of interest in the fructose syrup samples. The standards in this method had absolute fructose concentration that varied from 1–23%. The PLS chemometric algorithm is very effective for multicomponent quantification even when component peaks overlap due to chemically similar compounds being present in complex samples. For this application, we have a pair of chemically similar compounds (glucose and fructose are isomers). All component calibrations were developed using a 1st derivative to enhance spectral features prior to model development. The carbohydrate components calibration used a Norris derivative filter with segment length = 15 and gap = 0 while the °Bx calibration used segment length = 5 and gap = 5. The carbohydrate components used spectral regions 4850–4115 cm^{-1} and 7350–5600 cm^{-1} while the °Bx used spectral regions 7300–6770 cm^{-1} and 4500–4125 cm^{-1} (Figure 2).

Results and discussion

The calibrations developed for components in fructose syrup showed low root mean square error of calibration (RMSEC) and high correlation coefficients (Table 1) demonstrating that FT-NIR can accurately quantify similar chemical components in complex samples. The PLS models developed in this study used very few factors to produce root mean square errors of cross validation (RMSECV) that were very comparable to the RMSEC. A test of model robustness, the ability of the model to accurately predict samples not in the calibration, is how closely the RMSECV (Table 1) is to the RMSEC. For all three components in this method, the RMSECV are two times the RMSEC or less which means there is very little loss in accuracy when the calibration models are applied to unknown samples. The Predicted Residual Error Sum of Squares (PRESS) plot showed the minimum RMSECV was achieved with only 3 and 4 factors for °Bx (Figure 3) and fructose (Figure 4), respectively.

Component	Factors	Correlation RMSEC	Coefficient	RMSECV
Fructose	4	0.052	0.9992	0.090
Dextrose	5	0.056	0.9998	0.126
°Bx	3	0.126	0.9971	0.181

Table 1. Summary of calibration results for fructose syrup.

For the best PLS calibration models, the first couple of factors will explain the majority of spectral information in the standards which is related to the components of interest and not variation in the spectra caused by other physical or chemical variables not of interest to the method. The PRESS plots in Figures 3 and 4 are perfect examples of how the first few factors in the PLS model correlate the spectral and concentration information very well with each successive factor lowering the RMSECV only slightly.

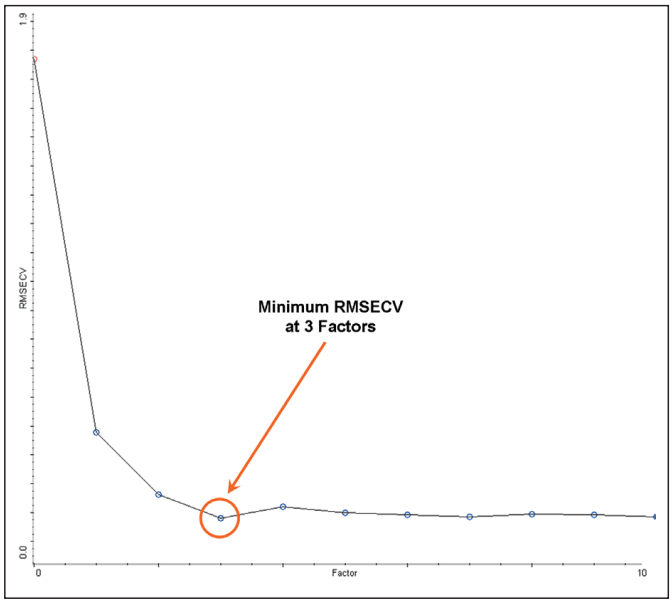


Figure 3. PRESS plot for °Bx.

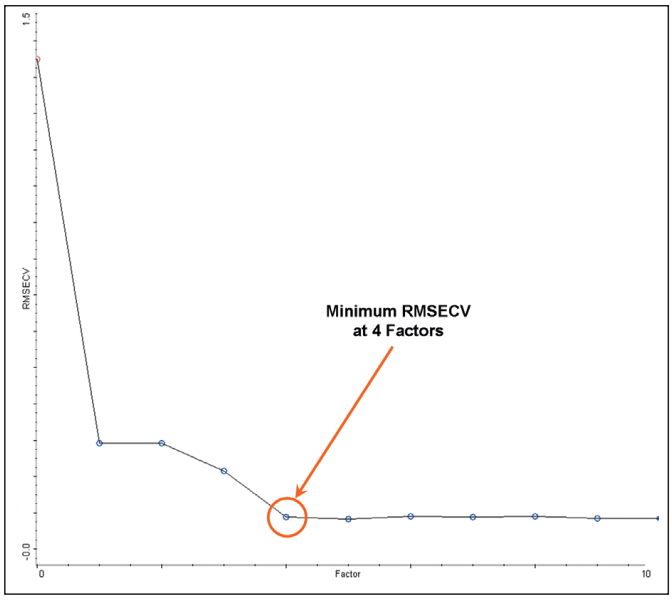


Figure 4. PRESS plot for fructose.

The most important analyses in high fructose corn syrup production are the fructose % after isomerization, adsorption separation, and blending. These 3 process points will determine if the 42% and 55% HFCS will be in specification. If the analysis is performed by chromatography, there is a delay in getting results to the operators because of the time it takes to walk a sample into the laboratory combined with the time it takes to run the sample, typically ten to twenty minutes.

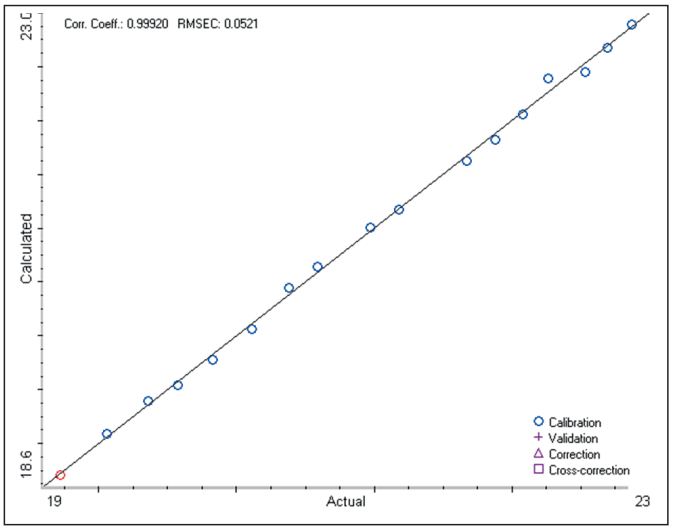


Figure 5. Calibration plot of fructose.

This problem can be overcome using FT-NIR since results are achieved much more quickly than by HPLC (less than a minute for FT-NIR vs. 15 minutes or more by HPLC) with accuracy that approaches that of the HPLC method.

The calibration plot for fructose (Figure 5) displays a low RMSEC of 0.052 and excellent correlation to the HPLC data for all standards in the method. The residual plot (Figure 6) can be used as a tool for verifying that the prediction errors are equal across all the standards used in producing the calibration. The % residual or % difference plot (Figure 6) shows that the accuracy of the FT-NIR method expressed as a percentage of the actual fructose concentration is close to 0.5% which is very similar to the HPLC accuracy for fructose. The NIR method also serves to replace two laboratory methods, HPLC for carbohydrate profile and refractometer for °Bx. These two laboratory methods require training and are susceptible to operator error. The cross-validation plot for fructose (Figure 7) demonstrates that the method does not lose prediction power based on the similarity between correlation coefficients and root mean square errors as shown in the calibration plot (Figure 5).

Conclusions

The precision, accuracy, and speed of FT-NIR spectroscopy combined with fiber optic probes for in-line analysis allows for real-time trending and closed-loop control of dynamically changing processes such as the blending of two process streams. The use of a multiplexing FT-NIR instrument capable of monitoring multiple process streams simultaneously, such as the Antaris MX FT-NIR process analyzer, would allow a production facility to monitor their entire process from reaction to purification to final product blending. For fructose syrup analysis, the Antaris FT-NIR analyzers are capable of monitoring the isomerization, adsorption separation, and fructose blending process steps due to its ability to accurately predict chemically similar carbohydrate components. The

application of FT-NIR spectroscopy for carbohydrate profile will eliminate the use of HPLC in the QA lab and result in savings in disposable lab items such as eluent, columns, vials, syringes and filters. The ability of FT-NIR spectroscopy to perform real-time analysis on process streams allows for process optimization resulting in lower reprocess cost, higher plant production capacity, and an increase in the percentage of in-specification product.

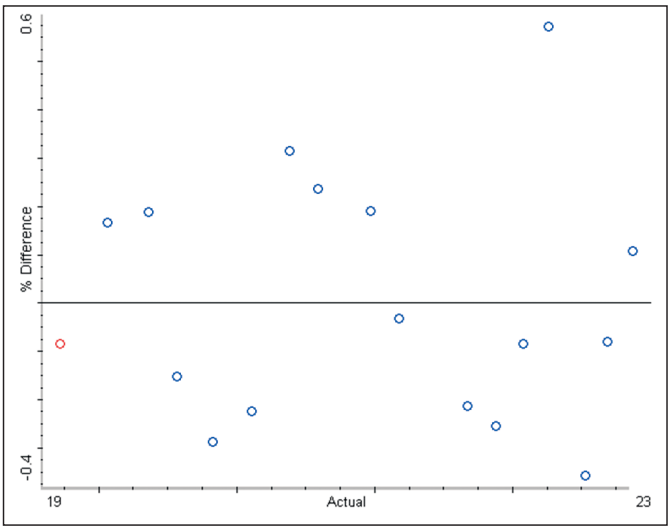


Figure 6. % Difference (Actual-Predicted)/Actual.

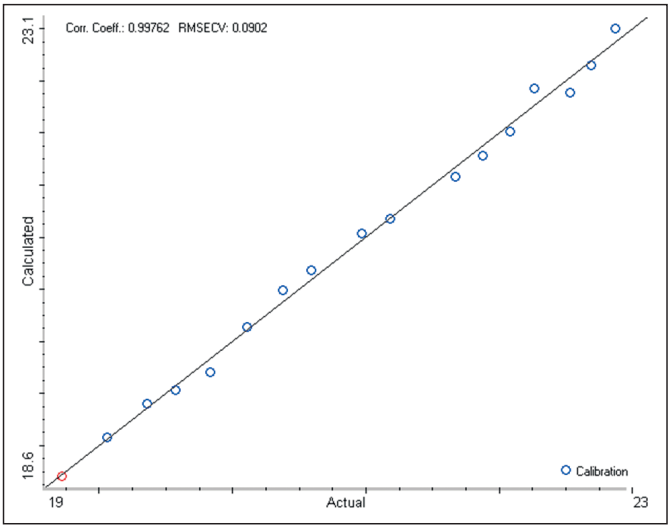


Figure 7. Cross-validation plot for fructose.

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