

## Quantitative Analysis of Fructose in Water with the Identity Raman Plate Reader

### INTRODUCTION

The Identity Raman plate reader is a Raman spectrometer system designed to quickly analyze samples in microtiter plates. This format is ideal for performing quantitative analysis of liquid solutions. The application of fructose in water is chosen as an example because a wide variety of food products and beverages, such as soda pop, incorporate high fructose sugar and water as the primary ingredients. In fact, most non-diet sodas typically contain thirty to forty grams of total sugar in a 355 mL (12 oz.) container, with the sugar content comprising approximately 10% by weight of the total. This application note will describe the preparation of standard fructose-water solutions, and the collection of their spectra, along with spectra of unknown solutions, all within a single plate scan on the Identity Raman plate reader.

### Sample Preparation

A stock solution of 20% by weight fructose was prepared by dissolving 2 grams of fructose into 8 ml of Nano-pure water. Five 1 ml calibration standards were prepared by taking aliquots of the stock solution and diluting to obtain final concentrations of 20%, 15%, 10%, and 5% by weight, with pure water as the 0% standard. In addition, blind unknown standards were prepared from the stock solution to run as samples in the method

### Procedure

Three hundred microliters of all standard and unknown solutions were pipetted into wells of clear, flat-bottom 96-well polystyrene microtiter plates. Three replicates of each standard, and 2 replicates of each unknown, were pipetted into separate wells, and Raman spectra of each sample were collected in triplicate in one plate scan method on the Identity. All spectra were acquired in the Identity Raman microplate reader, shown in Figure 1, using 70 mW of laser power at 532 nm, coadding 15 scans of 4 second integration time for a total measurement time of 60 seconds per sample. A screen shot of the method file from the Identity software is displayed in Figure 2, showing the selection of the wells, the laser and spectrometer control settings, along with the activation of the kinetics scan parameters to automatically collect three replicate scans of the samples. In total, 66 individual spectra were

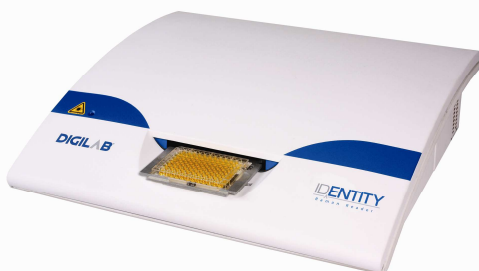


Figure 1. Identity Raman plate reader

automatically collected, with a total plate collection time of approximately 67 minutes.

## APPLICATION NOTE

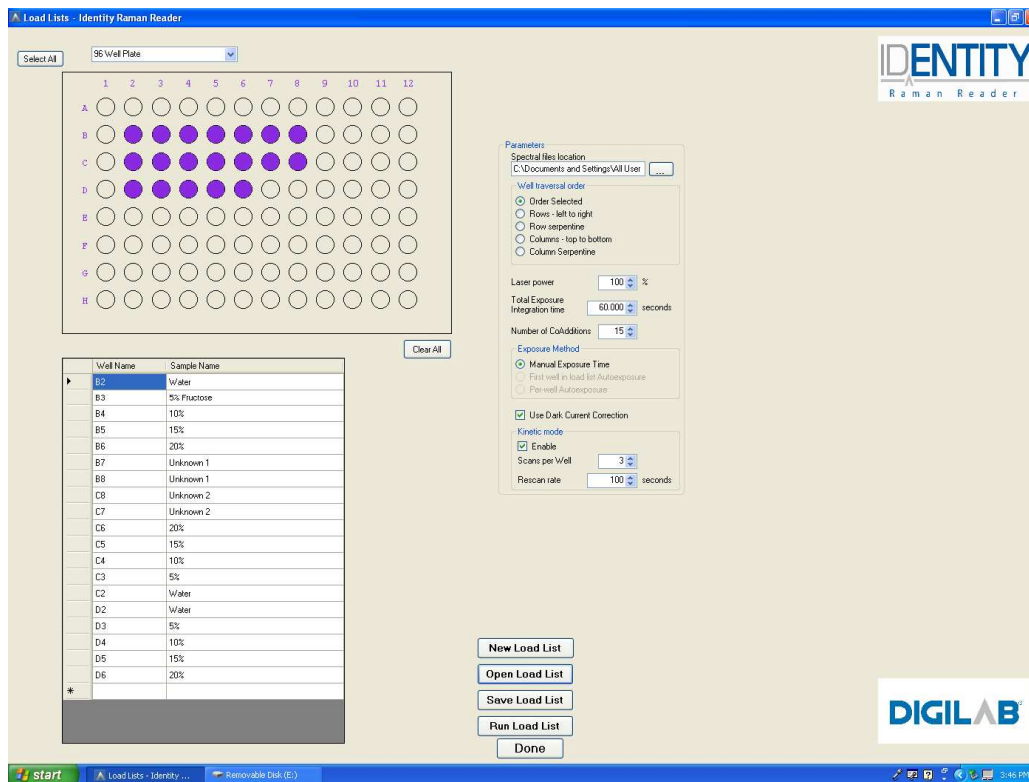


Figure 2. Load List page of the Identity software for the scan method described here. Wells are selected by clicking on individual wells or drawing a rectangular box around a group of wells with the mouse. When wells are selected a data table opens up for the operator to input textual information about the individual samples in the wells. Laser power is set as a total of total laser power (70 mw for the 532 nm laser), and integration time and coadditions can either be set manually or automatically with the autoexposure selection. Lastly, triplicate scans were collected using the kinetic mode.

Representative spectra from one row of calibration samples are displayed in Figure 3, showing the variation in the spectrum as the fructose concentration is increased from 0 to 20 weight percent fructose in water.

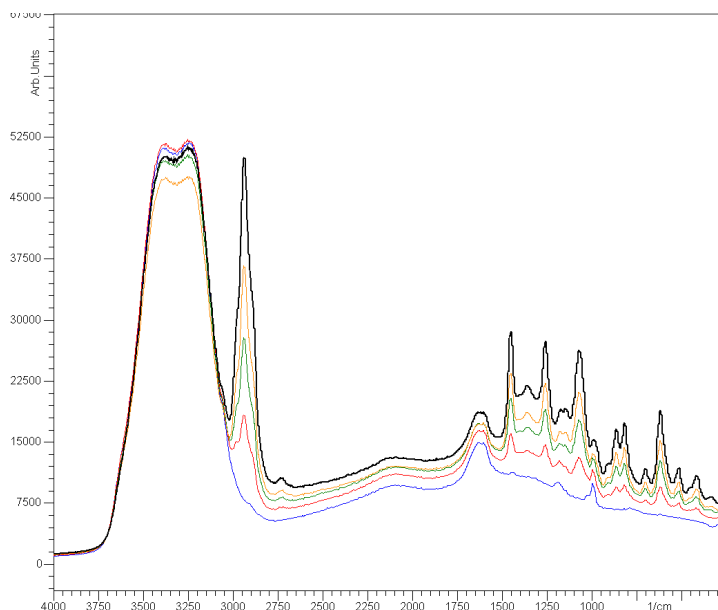


Figure 3. Calibration spectrum from Row B of the plate scan, with concentrations of 0, 5, 10, 15 and 20 weight % of fructose in water (bottom to top). Only water and weak polystyrene bands are observed in the 0% standard spectrum. The polystyrene bands from the microplate have constant intensity in all spectra and are not accounted for in the calibration methods.

## APPLICATION NOTE

**Quantitative Calibration and Results**

All calibration spectra were imported into Labcognition's (Cologne, Germany) Panorama software, and both univariate and multivariate (Partial Least Squares, PLS) calibration methods were generated. In the univariate method the integrated area for the vibration centered at  $619\text{ cm}^{-1}$ , from  $551.5$  to  $666\text{ cm}^{-1}$ , was selected for the triplicate readings of the 15 calibration spectra as shown in Figure 4. This spectral region was used for analysis because of the strength

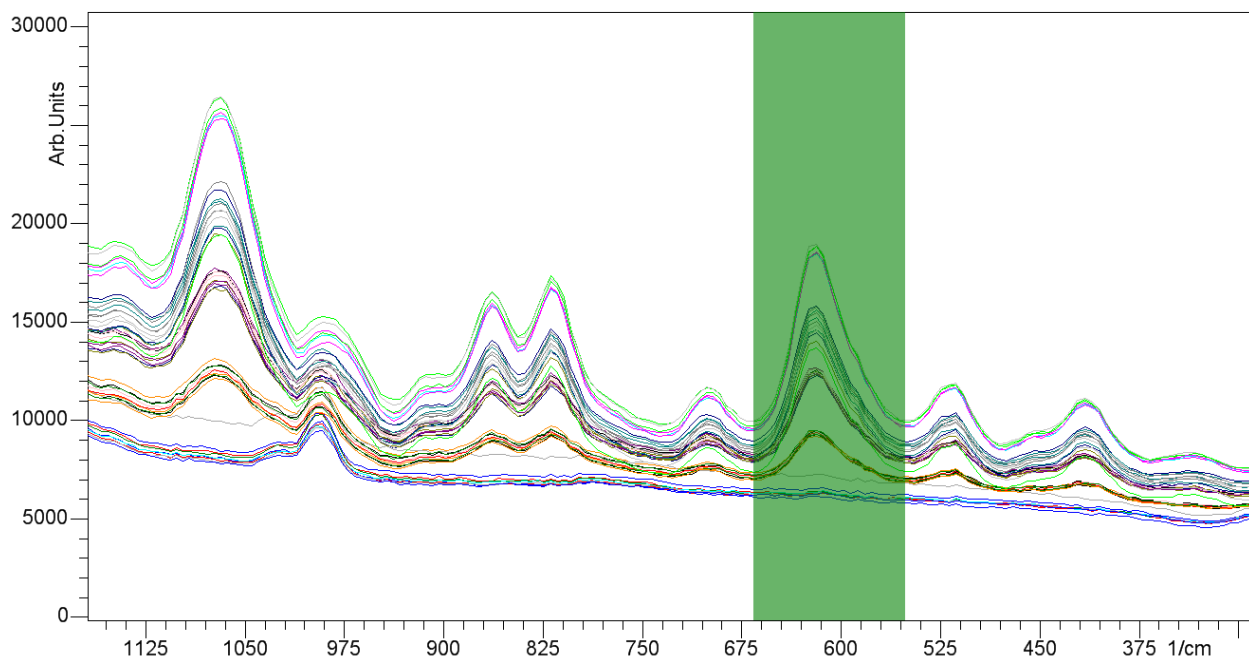


Figure 4. Integrated area region selection for univariate calibration of fructose in water calibration

of the band and it is free from overlap with water or polystyrene. Predicted results for the triplicate readings of the duplicate unknown samples are tabulated in Table 1, which were prepared as 4.0 % and 8.0% solutions. It should be noted that pipetting uncertainty can lead to an error of 0.2 wt % in the prepared concentrations of the standards and “unknown” samples (the standard deviation in the table below is related more to the precision of the measurement than the accuracy). Thus the two “unknown” samples have fructose concentrations of  $4.0 \pm 0.2\%$ , and  $8.0 \pm 0.2\%$ , and the predicted concentrations

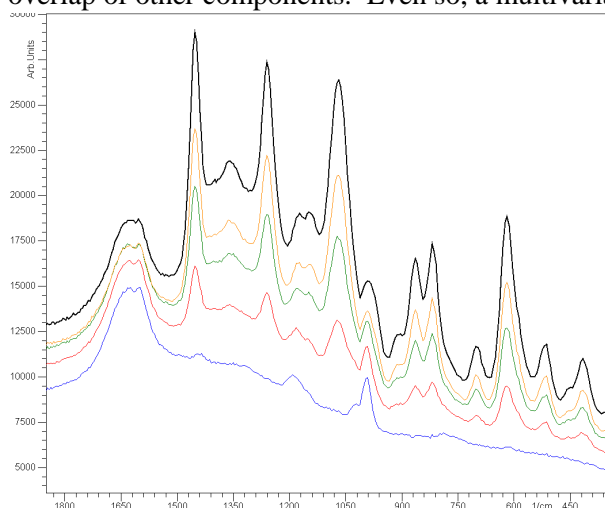
	Unknown 1 (B7)	Unknown 1 (C7)	Unknown 2 (B8)	Unknown 2 (C8)
	4.1	4.0	8.1	7.9
	4.0	3.9	8.0	7.9
	4.0	4.2	8.4	8.0
<b>Ave.</b>	4.0		8.1	
<b>St. Dev.</b>	0.1		0.2	

Table 1. Predicted fructose weight % concentrations for the triplicate readings of the two duplicate unknown samples using the univariate calibration. Unknown 1 was prepared as a 4.0 % solution, and Unknown 2 as an 8.0% solution.

agree with the prepared values within the error of the measurement.

## APPLICATION NOTE

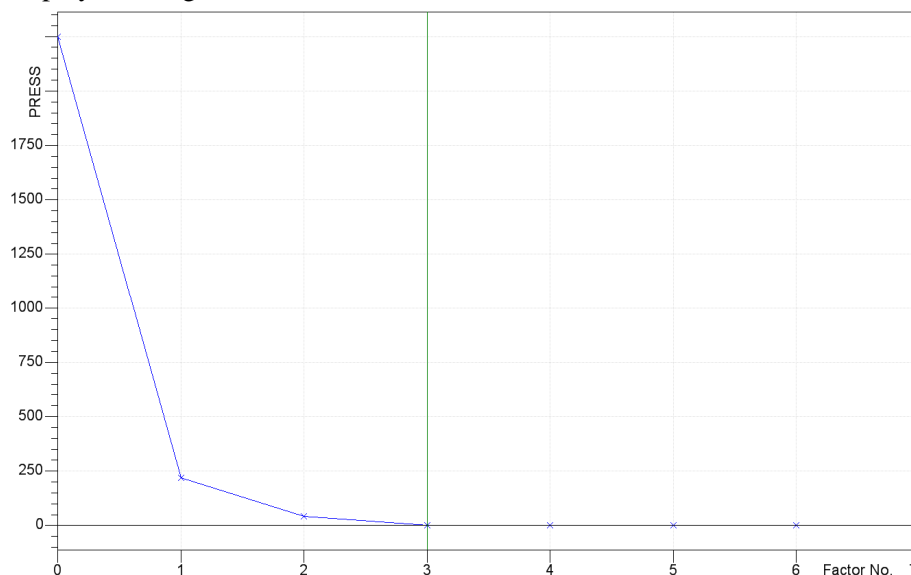
The univariate calibration works quite well in this analysis because the sample set is composed of a simple bi-component mixture, and it is possible to select a band for the analyte that is free from overlap of other components. Even so, a multivariate method was also developed to demonstrate



its applicability for such mixtures. The spectral region from 350 to 1850  $\text{cm}^{-1}$  was selected for a PLS calibration as this region contains spectral information about all components in the system (fructose, water and polystyrene), as shown for a subset of the calibration spectra in Figure 5.

*Figure 5. Spectral region selected for PLS calibration for the entire set of calibration spectra, from which a subset are displayed here showing the variation in the spectra from 0% fructose (bottom) to 20% fructose (top).*

The PLS calibration accounts for all variability in the data set, as reported in a plot of the Predicted Residual Error Sum of Squares (PRESS) versus factor number. The factors are the individual variables the calibration is fitting to the model, and an examination of the PRESS plot, displayed in Figure 6, indicates that three factors will account for all variability in this data set.



*Figure 6. PRESS values versus factor number in the PLS calibration for the fructose in water data set. The PRESS plot indicates that three factors will adequately model this system.*

The benefit of a multivariate calibration is that it will account for variability in the data set that may not be obvious from a casual inspection of the spectra, as long as that variability can be modeled in the calibration data. Such variability could be due to any number of effects that can cause variability in the spectra, such as concentration dependent chemical interactions between components, temperature changes, or variability in the measurement system. Thus the PLS method best models the calibration data set with three variables in this case when there is only one independent variable (% fructose) that was controlled by the analyst.

## APPLICATION NOTE

Concentration predictions for the “unknown” samples using the multivariate calibration are shown in Table 2. Again we conclude that the analytical method predicts the concentrations of the “unknown” samples within the error of the measurement.

	Unknown 1 (B7)	Unknown 1 (C7)	Unknown 1 (B8)	Unknown 1 (C8)
	3.8	4.0	7.7	7.9
	3.8	3.9	7.7	7.9
	3.8	4.0	7.8	7.9
<b>Ave.</b>		3.9		7.8
<b>St. Dev.</b>		0.1		0.1

Table 21. Predicted fructose weight % concentrations for the triplicate readings of the two duplicate unknown samples using the multivariate calibration. Unknown 1 was prepared as a 4.0 +/- 0.2 % solution, and Unknown 2 as an 8.0 +/- 0.2 % solution.

## Conclusions

The Identity was used to collect replicate standard spectra for generation of quantitative calibrations, and replicate “unknown” sample spectra, all within one plate scan method. In total, 66 spectra were collected automatically without any operator intervention once the plate scan method was started. The standard spectra were imported into Panorama, a third party software package in which both univariate and multivariate calibrations were generated, producing equivalently accurate results for the prediction of unknown concentrations of fructose in water at concentration levels typical for food and beverage products.

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