ApplicationNOTE

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# 2424 EVAPORATIVE LIGHT SCATTERING DETECTOR ANALYSIS OF APPLE JUICE SUGARS

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## INTRODUCTION

The analysis of carbohydrates in fruit juice is an important assay in the food industry. In apple juice, the principle sugars of interest are Fructose, Glucose, Sucrose, and Sorbitol – all of which are typically analyzed by HPLC. The distribution of these sugars is a distinguishing characteristic of the pure juice (fructose glucose ratio for example). Separation of the various carbohydrates in apple juice can be accomplished using a variety of stationary phases including amino-propyl based, ion-exchange, and HILIC columns. As sugars have no UV chromophore, HPLC carbohydrate methods have most commonly relied on refractive index or electrochemical detection instead of UV detection. Although both of these detection techniques work well, they are not without their drawbacks, namely sensitivity and stability. In this application note, the Waters® 2424 Evaporative Light Scattering (ELS) Detector is demonstrated as an alternative technology for the high-quality, robust analysis of sugars in apple juice.

## EXPERIMENTAL

A 100 µL aliquot of a commercially-available apple juice was added to 900 µL of water and acetonitrile (15:75), and 2.5 µL of the resulting solution was loaded onto the column. Separations were performed using a 4.6 x 250 mm YMC-Pack Polyamine II column on the HPLC system configuration described below.

### LC Conditions

| | |
|---|---|
| System: | Alliance® HPLC System |
| Mobile Phase: | Water/Acetonitrile 25:75 |
| Flow Rate: | 1.4 mL/minute, 35 °C |
| Detector: | Waters 2424 ELS Detector |
| | Drift Tube Temp: 70°C, |
| | Nebulizer Gas: $N_2$ at 50 psi, |
| | Gain: 100, Nebulizer: Cooling |

The system was controlled and data were collected and analyzed using Empower™ 2 Software. A representative HPLC/ELS chromatogram of the diluted apple juice sample is shown in Figure 1.

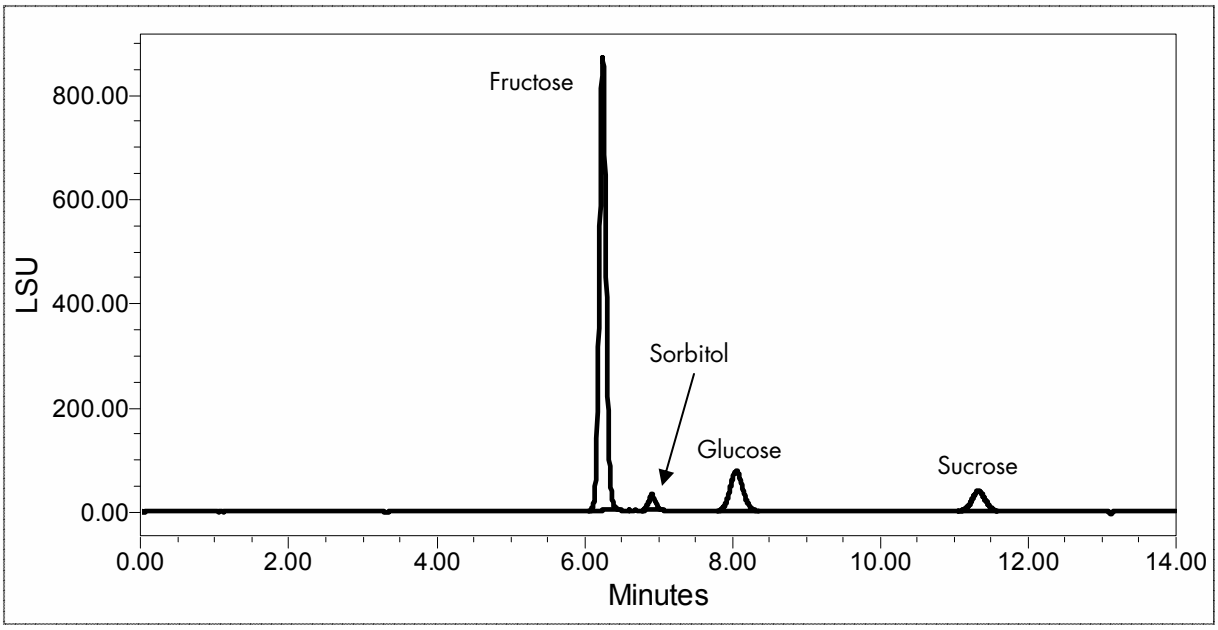


*Figure 1. Separation of diluted apple juice (2.5 µL injection volume).*

## Calibration

A standard solution of fructose, glucose, and sucrose (~5 mg/mL of each) was prepared in mobile phase. Increasing volumes of this standard were injected (1 through 5 µL). Calibration curves for all 3 compounds were generated from this data and fit to a quadratic equation and are shown in Figure 2. A calibration curve for sorbitol (~1 mg/mL) was generated in a similar manner.

## RESULTS

Retention times for fructose, sorbitol, glucose, and sucrose were 6.28, 6.94, 8.09, and 11.37 minutes, respectively, and were very reproducible (standard deviations of < 0.02 minutes for each compound), as seen in Figure 3. Quantitation was accomplished using an external standard method based on area. Sugar content of the selected apple juice sample were, on average (n=8), as follows: 139.6 g/L of fructose, 68.8 g/L of glucose, 31.1 g/L of sucrose, and 12.4 g/L of sorbitol.

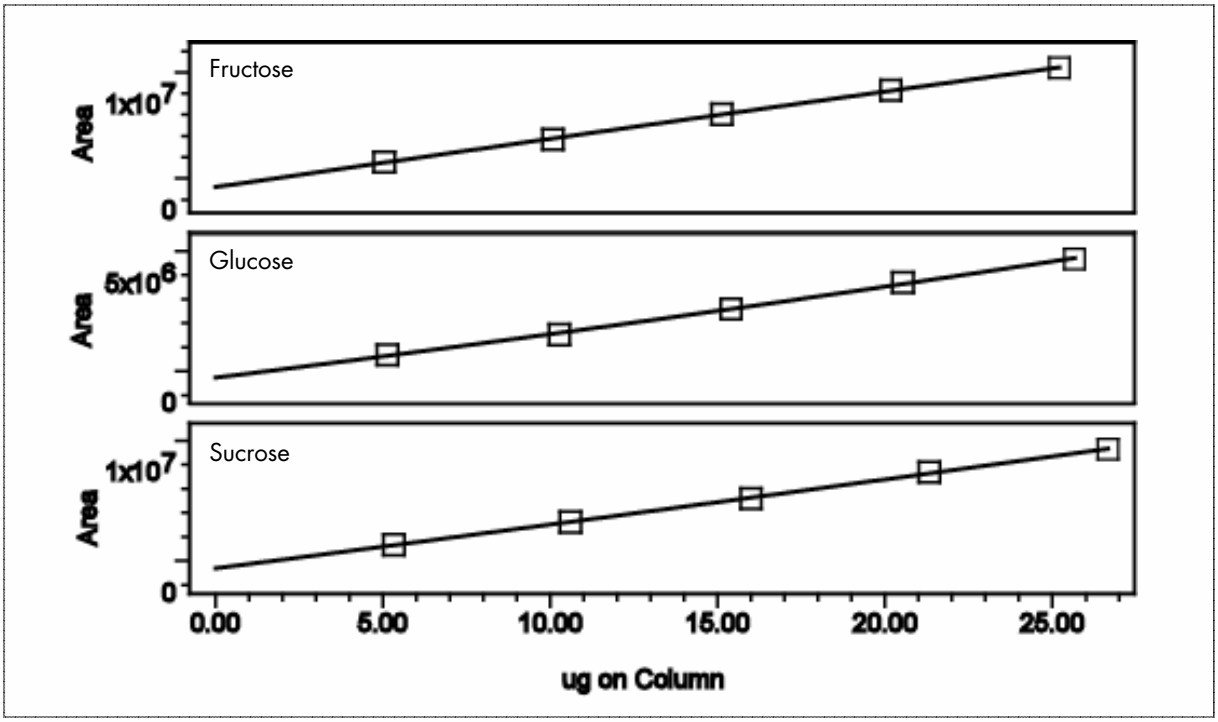


*Figure 2. Calibration curves for fructose, glucose, and sucrose.*

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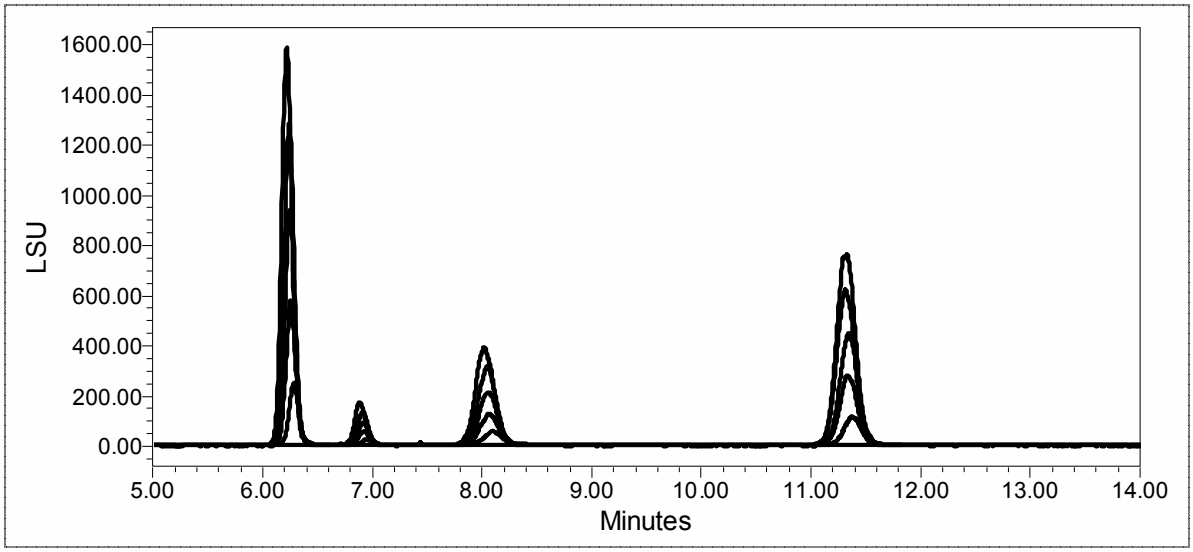


*Figure 3. Overlay of fructose, sorbitol, glucose, and sucrose standards.*

## SUMMARY

•The analysis of sugars in apple juice (and other fruit juice products) can be done reliably and reproducibly using the HPLC/ELS method described in this work.

•The Waters 2424 Evaporative Light Scattering Detector yields robust and sensitive analyses of sugars and provides an excellent alternative to traditional, less stable detection techniques.

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