# Multianalyte Sugar Analysis Using Evaporative Light Scattering Detection

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## Introduction

The analysis of the simple sugars is a routine application for HPLC and Refractive Index detection, and this method is validated by the AOAC for several matrices. The separations chemistry uses a proppyl amine functionality allowing for the separation of the hexose positional isomer, such as sucrose, maltose and lactose. This chemistry allows for the use of gradient chromatography to elute the higher DP carbohydrates giving the user an enhance profile of carbohydrates.

However, the detection of carbohydrates has been complicated by the fact that these analytes have no UV active chromaphores. Hence, UV detection cannot be used. Other methods have included the use of pulsed amperometric detection.

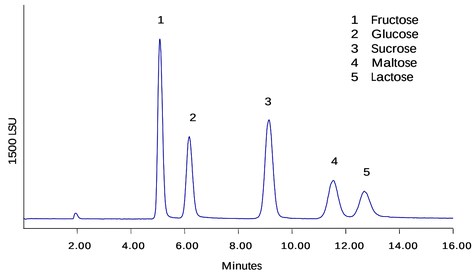
Here we shall show the feasibility of Evaporative Light Scattering as an alternative technique in analyzing these simple sugars. Included will be linearity and reproducibility studies, and applicability to various food matrices.

## The Chromatographic LC System

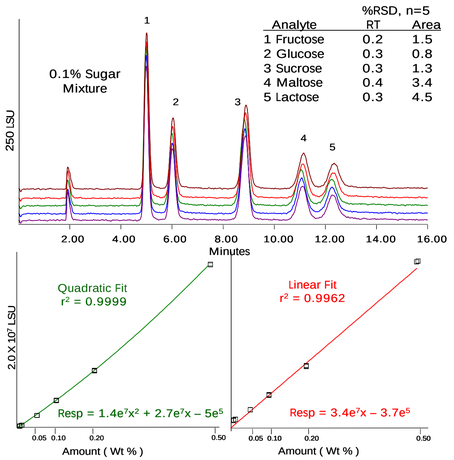
System: Waters Alliance® HPLC System
Column: Waters High Performance Carbohydrate Column 4.6 x 250 mm, 4 µm
Col Temp: 35 °C
Mobile Phase: 25% Water / 75% Acetonitrile
Flow Rate: 1.4 mL / min
Inj Volume: 20 µL

Detection: Waters 2420 Evaporative Light Scattering Detector
Nebulizer Control 30%
Drift Tube 50 °C
Gas Pressure 50 psi

## Mixed Sugar Standard 0.5% w/v




## Reproducibility of ELSD




%RSD, n=5

| Analyte | RT | Area |
|---|---|---|
| 1 Fructose | 0.2 | 1.5 |
| 2 Glucose | 0.3 | 0.8 |
| 3 Sucrose | 0.3 | 1.3 |
| 4 Maltose | 0.4 | 3.4 |
| 5 Lactose | 0.3 | 4.5 |

| Analyte | Quadratic | Linear |
|---|---|---|
| Fructose | 0.9999 | 0.9962 |
| Glucose | 0.9998 | 0.9947 |
| Sucrose | 0.9995 | 0.9945 |
| Maltose | 0.9995 | 0.9952 |
| Lactose | 0.9995 | 0.9946 |

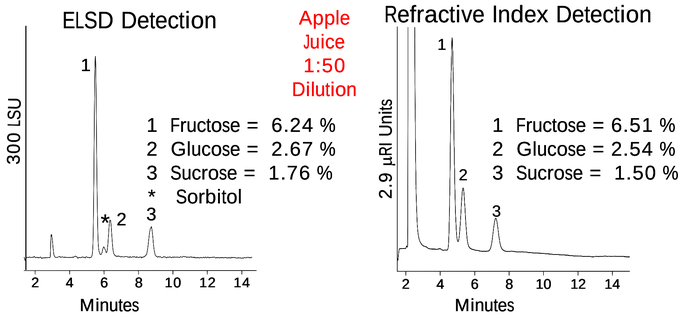
## Evaporative Light Scattering Theory

ELSD response theory says that Intensity (Response) = $km^b$
where m is the mass of the scattering particles, and k and b are variables dependant upon detector conditions and particle size.
Within the calibration range, using the recommended detector settings, the value of b is >2, suggesting a quadratic relationship is appropriate.

However, as concentration increases, the value of b increases, greater than 3, suggesting that a log /log plot would be more appropriate to provide a linear calibration curve.

For the fructose quadratic calibration curve below, the value of b is 2.8, the first derivative of the quadratic regression equation. This indicates that ELSD response is non-linear and a quadratic fit, or log/log is more appropriate than a linear regression.

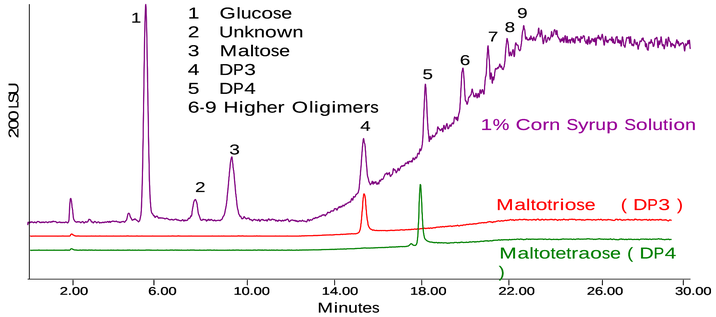
## Sugars in Apple Juice




## Higher Saccharide Profile Using Gradient Elution with ELSD

One important advantage of ELSD detection is the ability to use gradient elution. In the apple juice matrix, there may be other sugars that are strongly retained under isocratic conditions, such as the higher DP units. By increasing the water content of the mobile phase allows for the elution of the higher dextrose oligimers in the same run as mono and disaccharides. A demonstration of this capability is the carbohydrate analysis of corn syrup, a widely used food ingredient.

Column: Waters High Performance Carbohydrate Column 4.6 X 250 mm, 4 µm
Col Temp: 35°C
Mobile Phase: 25 % DI / 75% Acetonitrile for 10 minutes then a linear gradient to 50 % DI / 50 % Acetonitrile in 10 minutes hold for 10 minutes, then back to initial conditions
Detection: Waters 2420 Evaporative Light Scattering Detector
Nebulizer Control 60%
Drift Tube 50°C
Gas Pressure 50 psi




## Summary

- Evaporative Light Scattering (ELSD) detection is a suitable alternative to Refractive Index detection for the analysis of simple sugars in samples such as honey, soft drinks, and processed foods. Comparative results are achieved with both techniques.
- Response of ELSD is non-linear and is related to several detector settings. Use quadratic regression calibration, or log/log linear calibrations.
- ELSD detection is best for non-volatile analytes, whether they be UV or non-UV active. Requires the use of volatile mobile phase and buffers.
- ELSD is ideal for the gradient elution of non-UV active analytes, such as the higher dextrose oligimers.