



# An Ideal System for Xylooligosaccharide Characterization by Aqueous SEC with HPLC

## Application Note

Food Testing and Agriculture

### Authors

Graham Cleaver  
Agilent Technologies, Inc.

### Introduction

Xylooligosaccharides are naturally occurring saccharides that are found in fruit, vegetables, honey and milk. They are composed of units of xylose oligomers and can be produced at industrial scale from xylan-rich materials.

The growing commercial importance of these non-digestible oligosaccharides is based on their purported beneficial health properties, particularly their ability to stimulate the growth and activity of intestinal bacteria. Xylooligosaccharides are moderately sweet and stable over a wide range of pH and temperatures; both characteristics suitable for an ingredient in foodstuffs.

As well as the food industry, there is growing interest in xylooligosaccharides within the pharmaceutical and agricultural sectors. Consequently, there is a demand for accurate, analytical methods to characterize these compounds.

Aqueous size exclusion chromatography (SEC) is commonly employed to separate sugar oligomers, rather than conventional gradient elution HPLC because of SEC's ability to resolve oligomers of similar molecular weights.

Conventionally, detection by refractive index is used for compounds, such as xylose sugars, rather than UV because they do not possess a UV chromophore. However, a more suitable alternative is evaporative light scattering detection (ELSD), which provides better baseline stability and increased sensitivity compared to RI detection. In addition, ELSD is universal and not dependent on the optical properties of the compound, providing a more uniform response.

PL aquagel-OH 30 8µm high performance columns are an excellent match for the Agilent 380-ELSD. This column is ideal for relatively low molecular weight separations, combining a low exclusion limit, high pore volume and high column efficiency for maximum resolution. The benefits of the Agilent 380-ELSD and PL aquagel-OH columns are exemplified in the analysis of xylooligosaccharides.

Verified for Agilent  
**1260 Infinity ELSD**  
**1290 Infinity II ELSD**



**Agilent Technologies**

## Instrumentation

Columns: 2 x PL aquagel-OH 30 8 µm, 300 x 7.5 mm (p/n PL1120-6830)  
Detection: Agilent 380-ELSD (neb=50 °C, evap=90 °C, gas=1.2 SLM)

## Materials and Reagents

Eluent: Water

## Sample Preparation

Sample: Two xylooligosaccharide mixtures

## Conditions

Flow Rate: 1.0 mL/min  
Injection Volume: 20 µL

## Results and Discussion

The low dispersion of the Agilent 380-ELSD produced peak shapes comparable to those obtained by UV, and more responsive than RI detection, as shown in Figure 1.

### Peak Identification

1. Xylohexaose
2. Xylotetraose
3. Xylobiose
4. Xylose

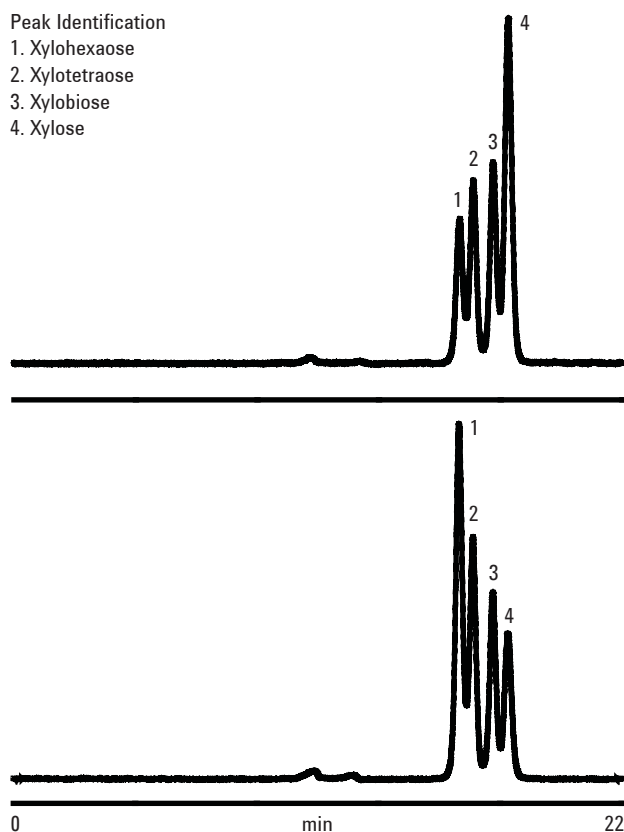


Figure 1. Comparison of two xylooligosaccharide samples from different sources using SEC-ELSD.

## Conclusion

Aqueous SEC, PL aquagel-OH columns and the Agilent 380-ELSD make up an ideal system for the characterization of xylooligosaccharides and other water-soluble sugar oligomers.

The Agilent 380-ELSD surpasses other ELSDs for low temperature HPLC applications with semi-volatile compounds. Its innovative design represents the next generation of ELSD technology, providing optimum performance across a diverse range of HPLC applications. The detector's unique gas control permits evaporation of high boiling solvents at very low temperatures. For example, 100 % water at a flow rate of 5 mL/min can be removed at 30 °C. The novel design of the Agilent 380-ELSD provides superior performance compared to detectors from other vendors for the analysis of semi-volatile compounds.

[www.agilent.com/chem](http://www.agilent.com/chem)

This information is subject to change without notice.

© Agilent Technologies, Inc. 2011-2015

Published in USA, August 1, 2015

5990-8399EN



**Agilent Technologies**