Simple Analysis of Carbohydrates by HPLC Using Evaporative Light Scattering Detection

Application Note

Food

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Introduction

The separation, identification, and quantification of simple sugars can be readily achieved using chromatography. High-performance liquid chromatography (HPLC) is perhaps the simplest technique, often requiring little in the way of sample preparation, particularly with liquids.

Sugars may be detected with the Agilent evaporative light scattering detector (ELSD) and an Agilent Hi-Plex column that has strong cation-exchange resins available in differing ionic forms. The sulfonated column resin gives a fundamental improvement in performance and overcomes the problems of low efficiencies and high backpressures encountered with soft gels. The separation mechanism is achieved initially by size exclusion, with larger oligosaccharides eluting before smaller monosaccharides, and then by ligand-exchange interaction of the numerous hydroxyl groups on the sugar molecules with the metal ion associated with the resin. Hi-Plex columns are used at elevated temperature with isocratic eluents.

As neutral carbohydrates have limited UV activity, the most commonly used detector with these columns is refractive index (RI). However, there are a number of issues related to the use of RI detectors, including baseline stability and sensitivity. A better method of detection is provided by evaporative light scattering detection. The Agilent ELSD does not require the solutes of interest to have any optical properties. The principle of operation is a three-stage process. The first stage involves the nebulization of the eluent; the second, the evaporation of the solvent to leave solute particles; and the third, the detection of the light scattered by the solid solute particles as they pass through the light beam. The only requirement for using the Agilent ELSD is that the eluent be more volatile than the solutes.

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When using Agilent Hi-Plex columns for the analysis of carbohydrates, water (with no buffer or added salt) is used as the eluent, making this an ideal application for the Agilent ELSD because neutral carbohydrates have little UV activity.

Hi-Plex resins are available in 8% crosslinked calcium and lead forms for the analysis of mono- and disaccharides and in hydrogen (acid) forms for the analysis of sugar alcohols and organic acids. Also available is a 4% crosslinked sodium form for the separation of high molecular weight oligosaccharides, such as corn syrups, to Dp 9.

Instrumentation

Column	Agilent Hi-Plex Ca, 7.7 × 300 mm, 8 µm (p/n PL1170-6810)
Detector	Agilent ELSD

Materials and Reagents

Mobile phase 100% DI H_2O

Results and Discussion

A separation of standard sugars – raffinose, lactose, glucose, galactose, and fructose – was obtained using the detection system (Figure 1). Calibration curves were produced for the six solutes in the test mixtures, as shown in Figure 2.

Conclusion

The separation and detection of raffinose, lactose, glucose, galactose, and fructose are readily achieved using water as the mobile phase with an Agilent Hi-Plex Ca column and the Agilent ELSD. This system avoids the use, high cost, and disposal implications of toxic acetonitrile when separations are performed on amino silica columns. In addition, Hi-Plex stays active in the presence of sugar molecules. Together with fast dissolution, this benefit results in long lifetimes compared to amino silica columns.

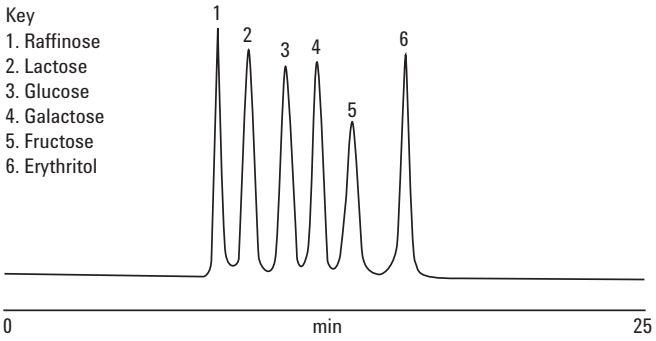

Figure 1. Good separation of six simple sugars using the Agilent ELSD and an Agilent Hi-Plex Ca column.

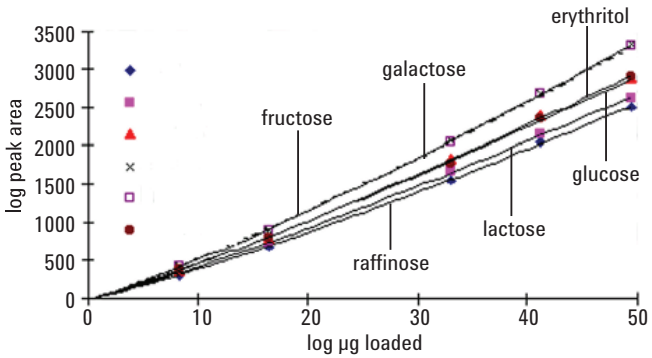

Figure 2. Calibration curves of six sugars using the Agilent ELSD and an Agilent Hi-Plex Ca system.

For More Information

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