Fast Analysis of Carbohydrates in Chocolate Using Ligand-Exchange Chromatography with ELSD

Application Note

Food

Author

Stephen Bullock

Agilent Technologies, Inc.

Introduction

Methods of detection for carbohydrates are severely limited because they do not normally possess chromophores or fluorophores. Detection can sometimes be accomplished in the low UV range, 190-200 nm, but unless high-purity eluents are used and extensive sample preparation employed, excessive interference from other compounds may occur.

The refractive index (RI) detector is routinely used, but RI is relatively insensitive, relying on a refractive index difference between solute and eluent. Where increased sensitivity is required, a pulsed amperometric detector (PAD) is employed, but for uniform response, the carbohydrate must be in a high pH environment.

A better detector for the analysis of carbohydrates is the Agilent evaporative light scattering detector (ELSD). When the Agilent ELSD is used in combination with Agilent Hi-Plex ligand-exchange columns, rapid isocratic separations of mono-, di-, and oligosaccharides are achieved. The Agilent ELSD does not require the solutes of interest to have any particular 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. This is an ideal application for the Agilent ELSD because neutral carbohydrates have little UV activity. Sugars may be detected with the ELSD and a 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.

Chocolate is produced in three distinct forms: dark chocolate, milk chocolate, and white chocolate. The predominant sugar in the three varieties is the disaccharide sucrose. However, the milk sugar, lactose, will also be present in milk and white chocolate. The amount of lactose present will be indicative of the amount of milk solids used in the production process. As both sucrose and lactose are disaccharides, the Hi-Plex Pb column is the preferred choice for the analysis and quantification of these two components.

Hi-Plex resins are available in 8% crosslinked calcium 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.

Materials and Reagents

Instrumentation

Column	Agilent Hi-Plex Pb, 7.7 × 300 mm, 8 µm (p/n PL1170-6820)
Detector	Agilent ELSD

Sample Preparation

Aqueous solutions were prepared at a concentration of 100 mg chocolate/mL, and 2 µL injection volumes were used for the quantitation.

Results and Discussion

Table 1 summarizes the quantitation of the two disaccharides, sucrose and lactose. Sucrose is present in all four samples, with the plain chocolate having the highest level. Lactose, the milk sugar, can be seen in the other three samples. Differences in the sucrose and lactose content of the two milk chocolate samples from different manufacturers are evident.

The disaccharide composition of four commercial chocolate samples is shown in Figure 1.

Table 1. Disaccharide Content of Commercial Chocolate Samples Expressed as a Percentage by Weight of Chocolate

Carbohydrate	Milk sample 1	Milk sample 2	Plain	White
Lactose	7	17	nd	9
Sucrose	30	41	69	42
Total	37	58	69	51

nd - not detected

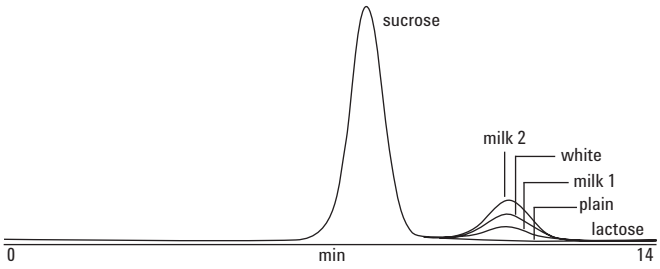

Figure 1. HPLC chromatograms of four commercial samples of chocolate, normalized to the height of the sucrose peak.

Conclusion

The composition of chocolate and levels of added milk solids in milk and white chocolate are readily achieved, using water as the mobile phase with an Agilent Hi-Plex Pb 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, the Hi-Plex columns stay active in the presence of sugar molecules. Together with fast dissolution, this benefit results in long lifetimes as compared to amino silica columns.

For More Information

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Published in USA, June 30, 2011
SI-01097

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