Determination of Sugar Alcohols in Confections and Fruit Juices by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

Thermo Fisher Scientific Inc.

Introduction

Sugar alcohols are used in confectionary products because they impart a sweet taste without the calories associated with sugars. Sorbitol (60% as sweet as sucrose)[1] and mannitol are sugar alcohols commonly used as replacements for sucrose in dietetic candy. However, their use in foods is regulated because they exhibit laxative and diuretic properties.

Sugar alcohols, also called alditols, are the reduced forms of monosaccharide aldoses. For example, D-glucose can be reduced to glucitol (sorbitol).[2] See Figure 1.

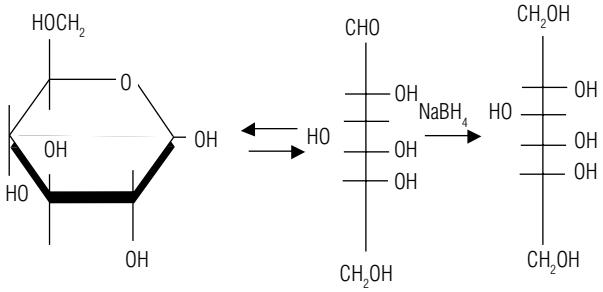

Figure 1. D-glucose reduced to glucitol (sorbitol).

The Thermo Scientific™ Dionex™ CarboPac™ MA1 column has unique selectivity for sugar alcohols. As a high-performance, high-capacity anion exchange column, the Dionex CarboPac MA1 column permits sugar alcohols to be resolved at elevated sodium hydroxide concentrations. Unlike metal-loaded columns (often used for sugar alcohol analysis), the Dionex CarboPac MA1 column operates at ambient temperature and employs sodium hydroxide eluent for high pH separations and maximum sensitivity using pulsed amperometric detection (PAD). Also unlike metal-loaded columns, which elute large saccharides first and sugar alcohols last, the elution order using the Dionex CarboPac MA1 column is determined by pK_a values. The sugar alcohols with higher pK_a values elute first, then monosaccharides and disaccharides that have lower pK_as (see Figure 2).

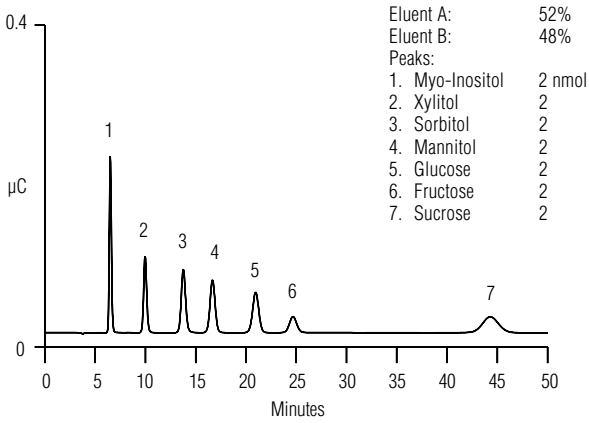

Figure 2. Sugar alcohols with high pK_a values elute first.

Thermo SCIENTIFIC

Carbohydrates (e.g., alditols and aldoses) are weak acids that ionize at pH 12 to 14. At these pH levels, carbohydrates can be separated by anion-exchange mechanisms. Sugar alcohols have higher pK_a values than mono- and disaccharides, thus higher pH eluents are required to separate them from one another by anion exchange. The Dionex CarboPac MA1 column uses a higher concentration of sodium hydroxide (typically up to 600 mM) for its eluent than the other Dionex CarboPac columns.

This application note focuses on sugar alcohols found in confections and fruit juices. However, the Dionex CarboPac MA1 column is also useful for sugars found in physiological fluids, living tissues, and for reduced carbohydrate moieties of glycoconjugates (e.g., β-elimination reaction products).

Equipment

Dionex chromatography system* consisting of:

- Gradient Pump
- Liquid Chromatography Module
- Pulsed Electrochemical Detector
- Thermo Scientific Dionex PeakNet** or Thermo Scientific Dionex AI-450 Chromatography Workstation**

**Equivalent or improved results can be achieved using the Thermo Scientific Dionex ICS-5000+ system.*

***Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) Software, version 7.2 can be used.*

Reagents and Standards

- 18 MΩ-cm Deionized water
- Sodium hydroxide solution, 50% (w/w), low carbonate
- Standards consisting of: Inositol, Xylitol, Sorbitol, Mannitol, Glucose, Fructose, Sucrose, Dulcitol, Arabinose, Mannose, Xylose, and Galactose

Conditions

Columns:	Dionex CarboPac MA1, 4 × 250 mm
Expected Operating Pressure:	5.5 to 7.6 MPa (800 to 1100 psi)
Injection Volume:	10 µL
Eluents:	A: Deionized water B: 1.0 M Sodium hydroxide
Flow Rate:	0.4 mL/min
Detection:	Pulsed amperometry, gold working electrode

Thermo Scientific Dionex ED40 Electrochemical Detector Settings as follows*:

t (ms)	E (volts)	Integration (s)
400	+0.05	0.2–0.4
200	+0.75	
400	−0.15	

** See Technical Note 21 for a discussion of pulse potentials, including settings to use with a PAD.[3]*

Preparation of Solutions and Reagents

Eluent A: Deionized Water

Vacuum degas 1 L of 18 MΩ-cm deionized water.

Eluent B: 1 M Sodium Hydroxide

Dilute 52 mL of sodium hydroxide solution in 1.0 L of degassed 18 MΩ-cm deionized water. Use a sodium hydroxide solution that is 50% (w/w) and contains low carbonate. Sodium hydroxide pellets are coated with a layer of carbonate and will not produce acceptable eluents

Sample Preparations

"Sugarless" Hard Candy

Dissolve one candy drop (weighing 3.4 g) in 10 mL of deionized water and dilute 1:1000 with 18 MΩ-cm deionized water.

Apple Juice

Dilute apple juice 1:1000 with 18 MΩ-cm deionized water.

Chewing Gum Extract

Divide one stick of chewing gum (weighing 2.7 g) into small pieces (of approximately 3 × 3 mm), and sonicate gum in 10 mL of deionized water for 10 minutes. Then pass the supernatant through an Thermo Scientific™ Dionex™ OnGuard™ A cartridge (prepare by passing 5 mL of deionized water through the cartridge at a flow rate of 2 mL/min; discard the first 3 mL of the sample), to remove anions and pass it through a 0.45 µm filter to remove particulates. Dilute the filtrate 1:1000 with 18 MΩ-cm deionized water.

Discussion and Results

The Dionex CarboPac MA1 column allows 0 to 1 M sodium hydroxide eluent concentrations. This is important for the separation of sugar alcohols because their pK_as are high and each is unique. Separation on the Dionex CarboPac MA1 column can be achieved by choosing an eluent pH near the pK_a value of a sugar alcohol. Altering an eluent's pH by varying the sodium hydroxide concentration changes the effective charge on the compounds. This in turn can change the elution order so that those sugar alcohols of interest will be resolved from one another. Figures 3A and 3B show capacity factors vs. eluent concentration for some common sugar alcohols. It is possible to predict which eluent strength will work best for a particular separation.

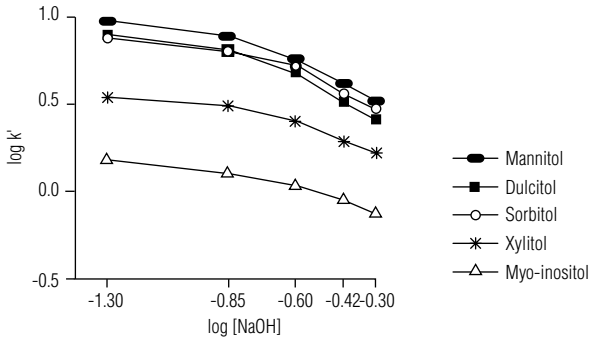

Figure 3A. Log capacity factor vs. log sodium hydroxide concentration of some sugar alcohols.

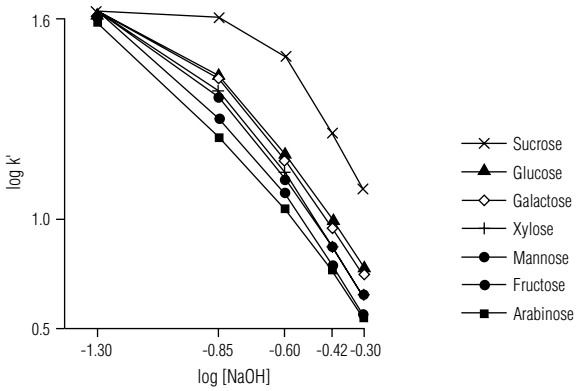

Figure 3B. Log capacity vs. log sodium hydroxide concentration of selected carbohydrates.

Figure 4 shows a chromatogram of "sugarless" hard candy. This particular candy is sold as dietetic candy. Its major PAD-active, water-soluble components are sorbitol and mannitol. The 3.4-g candy drop contained 2.70 g of sorbitol and 50.5 mg of mannitol.

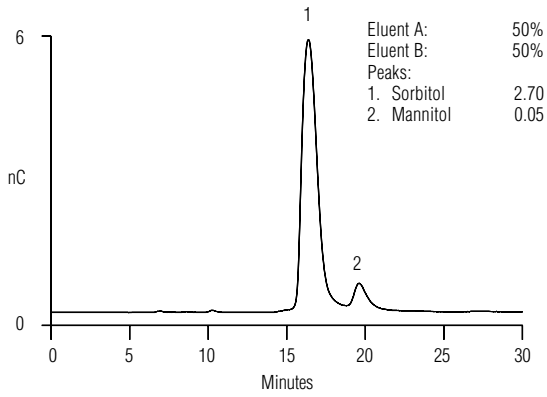

Figure 4. "Sugarless" hard candy containing sorbitol and mannitol.

Figure 5 shows a chromatogram of diluted apple juice. Glucose, fructose, and sucrose are found in all fruit juices. Sorbitol is found in apples, pears, and plums, among other fruits.[4] An 8 ounce (237 mL) serving of this apple juice contains 1.86 g of sorbitol, 8.22 g of glucose, 18.8 g of fructose, and 7.76 g of sucrose.

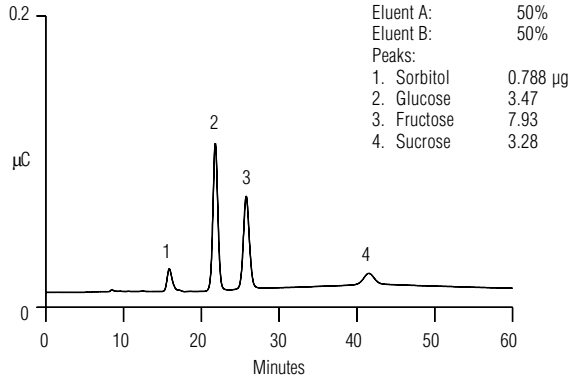

Figure 5. Diluted apple juice containing sorbitol.

Figure 6 shows a chromatogram of chewing gum extract. The peaks are glycerol, sorbitol, mannitol, and hydrogenated glucose syrup, respectively. The 2.7 g stick of chewing gum contains 218 mg of glycerol, 1140 mg of sorbitol, and 280 mg of mannitol. Hydrogenated glucose syrup was not quantitated.

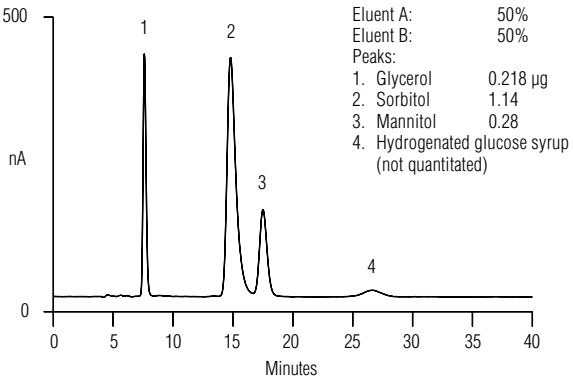

Figure 6. Chewing gum extract containing glycerol, sorbitol, and mannitol.

Operators of refractive index (RI) detection may notice the low detection limits presented in Table 1. PAD is routinely able to achieve these low levels. Pulsed amperometric detectors apply a potential at an electrode to oxidize the carbohydrate. This not only allows for low detection limits, but also increases specificity and freedom from matrix interferences.

Table 1. Detection limits and linearity data for the carbohydrates in Figure 1.

Standard	r^2 [a]	Method Detection Limit (pmol)[b]
Myo-inositol	0.9995	2
Xylitol	0.9953	4
Sorbitol	0.9951	5
Mannitol	0.9995	4
Glucose	0.9984	10
Fructose	0.9995	10
Sucrose	0.9999	10

[a] *The coefficients of determination were calculated over the range of 10 to 30,000 picomoles oglr 3 orders of magnitude.*

[b] *The method detection limits of myo-inositol, xylitol, sorbitol, and mannitol were determined at three times the noise.*

PAD utilizes a repeating sequence of three potentials. The durations of these three potentials are optimized for carbohydrates. If a single potential is used, the peak heights will steadily decrease as the electrode surface fouls. For further details concerning pulsed sequences for PAD, refer to Thermo Scientific Technical Note 21: *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector.*[3]

To drive the oxidation reaction at the working electrode of the pulsed amperometric detector, the eluent must be greater than pH 12. Dionex CarboPac columns are designed using a polymer-base for stability and durability in the 0 to 14 pH range. For further details concerning carbohydrate determination with PAD, refer to Thermo Scientific Technical Note 20: *Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection.*[5]

Conclusion

The Dionex CarboPac MA1 column is the preferred column for the analysis of sugar alcohols. It yields excellent resolution and allows for changes in carbohydrate selectivity; the column permits changes in the eluent concentration and hence pH levels. The Dionex CarboPac MA1 column is used for pulsed amperometric detection of sugar alcohols with high sensitivity and specificity without derivatization or the addition of a postcolumn reagent. This column operates at ambient temperatures to promote ease-of-use and an increased lifetime. As with other Dionex CarboPac columns, the Dionex CarboPac MA1 column demonstrates long-term reproducibility and durability.

Precautions

The Dionex CarboPac MA1 column has an operational flow rate range of 0.2–0.5 mL/min. The maximum flow rate possible without irreversible compression of the column resin is 0.8 mL/min. The maximum pressure that the column can withstand without irreversible compression of the macroporous resin is 13.8 MPa (2000 psi).

Metal should be eliminated from the flow path prior to column use. If an autosampler is used, all flow paths should be metal-free. Metal contamination of the analytical column can result in poor peak efficiency and/or symmetry, which may lead to poor reproducibility.

If a PED is used, the Ag/AgCl reference electrode is preferred over the combination pH-Ag/AgCl reference electrode. Sodium hydroxide eluents above 200 mM will cause the delicate glass bulb of the electrode to become brittle and break.

References

1. Shaw, P.E. *CRC Handbook of Sugar Separations in Foods by HPLC;* CRC Press, Boca Raton, Florida, 1988.
2. Solomons, T.W.G. *Organic Chemistry,* 2nd ed., John Wiley & Sons, New York, 1980.
3. Thermo Scientific Technical Note 21: *Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex Electrochemical Detector.* Sunnyvale, CA, 2013.
4. Coppola, E.D. *Food Technology.* **1984**, *38(4),* 88-91.
5. Thermo Scientific Technical Note 20: *Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection.* Sunnyvale, CA, 2013.

©2016 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries. This information is presented as an example of the capabilities of Thermo Fisher Scientific products. It is not intended to encourage use of these products in any manners that might infringe the intellectual property rights of others. Specifications, terms and pricing are subject to change. Not all products are available in all countries. Please consult your local sales representative for details.

Africa +43 1 333 50 34 0
Australia +61 3 9757 4300
Austria +43 810 282 206
Belgium +32 53 73 42 41
Brazil +55 11 3731 5140
Canada +1 800 530 8447
China 800 810 5118 (free call domestic)
400 650 5118
Denmark +45 70 23 62 60
Europe-Other +43 1 333 50 34 0
Finland +358 9 3291 0200
France +33 1 60 92 48 00
Germany +49 6103 408 1014
India +91 22 6742 9494
Italy +39 02 950 591
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Latin America +1 561 688 8700
Middle East +43 1 333 50 34 0
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Norway +46 8 556 468 00
Russia/CIS +43 1 333 50 34 0
Singapore +65 6289 1190
Sweden +46 8 556 468 00
Switzerland +41 61 716 77 00
Taiwan +886 2 8751 6655
UK/Ireland +44 1442 233555
USA +1 800 532 4752

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

AN71407-EN 0816M