APPLICATION NOTE 73905

Easy and sensitive method for sorbitol determination in food products

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Goal

Develop an easy method for sorbitol determination in food products using eluent generation, HPIC, and HPAE-PAD technologies

Introduction

Sorbitol is a sugar alcohol, or polyol, used in pharmaceuticals and cosmetics. Sorbitol is also used as an additive in food, where it is labeled by its E-number (E420) on the ingredients list. Sorbitol is a texturizing agent or sweetener. It is 60 percent less sweet than sucrose and contains about one-third fewer calories. Sorbitol is often used in chewing gums, candies, cookies, cakes, and ice cream. Sorbitol occurs naturally in some berries and fruits: apples, peaches, cherries, apricots, or dried fruit, like dates, figs, prunes, and raisins. Sorbitol is also produced from cereal starch and is one of the most commonly used polyols. The use of polyols in food applications is regulated. A list of approved food additives and their conditions of use is established by Regulation (EU) 1129/2011.[1,2]

A rapid and accurate method is required for quality control in factories or contract testing labs. Liquid chromatography with refractive index detection remains the reference for this application. However, depending on the targeted limit of quantification, insufficient sensitivity has been reported. Also, depending on the sample, some resolution issues or coelutions can appear. The Thermo Scientific™ Dionex™ CarboPac™ MA1 macroporous anion-exchange column can resolve many carbohydrates that are poorly retained on conventional columns. This column successfully separates alditols such as glycerol, arabitol, sorbitol, dulcitol, and mannitol found in food products. This column remains the best in class solution for multiple alditols analysis.[3,4]

Unfortunately the high the hydroxide concentrations needed for the Dionex CarboPac MA1 column preclude the use of electrolytic eluent generation.

In this application note, we describe a new method involving eluent generation and high pressure ion chromatography with 4 µm particle size resin for sorbitol determination in fruits and beverages. This methodology provides faster results and improves reliability with perfect and automatic control of eluent concentration.

Experimental

Equipment

- Thermo Scientific™ Dionex™ ICS-6000™ HPIC™ system:
  - Dionex ICS-6000 DP Quat / Iso Pump module (P/N 21181.60010)
  - Dionex ICS-6000 EG Eluent Generator Dual (P/N 22181-60019)
  - Dionex ICS-6000 EG Cartridge Kit: HP Degasser and Tubing (Analytical) (P/N 075522)
  - Dionex ICS-6000 DC with Dual Temperature Zones, Two Injection Valves, Microbore (P/N 22181-60049)
  - Dionex ICS-6000 Electrochemical Detector (P/N 072042)
  - Valve Pod 0.4 µL (P/N 074699)
  - Thermo Scientific™ Dionex™ AS-AP Autosampler and sample tray cooling, 250 µL sample syringe (P/N 074925)
  - Dionex ICS-6000 ED Electrochemical Detector Cell (P/N 072044)
  - Diverter Valve Assembly, one 2-way, 6-port Valve and Mounting Hardware (P/N 074123)
  - EO Eluent Organizer Tray with two 2 Liter bottles (P/N 072057)
  - EO Regulator Accessory and Stand (P/N AAA-074423)
  - Dionex IC PEEK Viper Fitting Kit for Dionex ICS-6000 2mm systems with ED (P/N 302965)
- Thermo Scientific™ LP Vortex Mixer (P/N 15298834)
- Fisherbrand™ Analytical Balance (Model FAS224)
- Thermo Scientific™ Barnstead™ Smart2Pure™ Pro Water Purification System (model Smart2pure Pro UV/UF 16LPH, P/N 5129887)
- Thermo Scientific™ F1-ClipTip™ Variable Volume Single Channel Pipette 2–20 µL (P/N 4641180N)
- Thermo Scientific™ F1-ClipTip™ Variable Volume Single Channel Pipette 20–200 µL (P/N 4641210N)
- Thermo Scientific™ F1-ClipTip™ Variable Volume Single Channel Pipette 100–1000 µL (P/N 4641230N)

Software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3

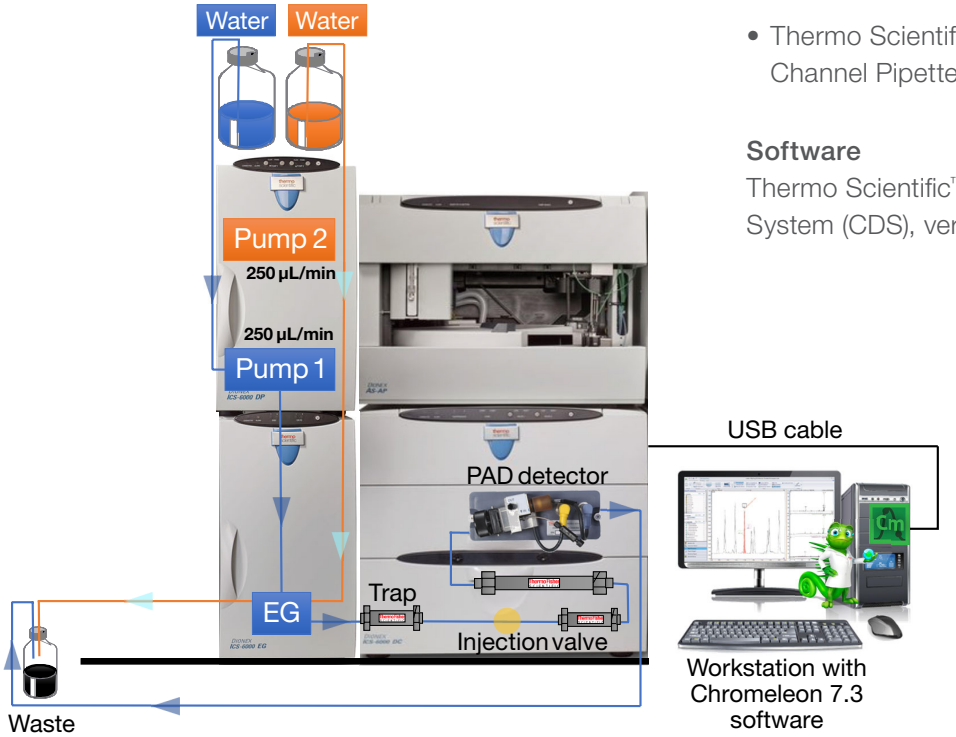

Figure 1. Instrument setup

Reagents and consumables

- Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm Analytical Column 2 × 250 mm (P/N 303346)
- Thermo Scientific™ Dionex™ CarboPac™ PA300-4µm Guard Column 2 × 50 mm (P/N 303347)
- Thermo Scientific™ Dionex™ BorateTrap Inline Trap Column (P/N 047078)
- Gold working electrode with 2 mil gaskets (1 mil = 25.4 µm) (P/N 061875)
- Reference electrode pH, Ag/AgCl (P/N 061879)
- Thermo Scientific™ Titan3™ Syringe filter 17 mm PVDF Membrane (P/N 44513-PV)
- Fisherbrand™ 1 mL Plastic Syringe PP Fisher Scientific (P/N 14955456)
- Thermo Scientific™ Vial Kit 1.5 mL Glass with Caps and Septa, 100 Each (P/N 055427)
- Thermo Scientific™ Dionex™ CR-ATC 600 (P/N 088662)
- Thermo Scientific™ Dionex™ EGC 500 KOH Cartridge (P/N 075778)
- D-Sorbitol, 98+%, ACROS Organics™ (P/N AC220521000)

Instrument method

Table 1. IC setup

Conditions	
Column	Dionex CarboPac PA300-4µm, 2 × 250 mm with guard column
Eluent	KOH gradient generated by Dionex ICS-6000 EG Eluent Generator For details see Table 2
Eluent source	Dionex EGC 500 KOH cartridge (P/N 075778), Thermo Scientific™ Dionex™ CR-ATC 600 trap column (P/N 088662), high pressure degasser module Borate trap has been installed between eluent generator and injection valve
Flow rate (eluent)	250 µL/min
Injection volume	0.4 µL
Column temperature	30 °C
Detection	Pulsed amperometric detection @ 20 °C Ref. Ag/AgCl Waveform used, see Table 4

Table 2. KOH gradient generated by the Dionex ICS-6000 EG module

Time (min)	Concentration (mM)
0	50
16	50
16	90
21	90
21	50
36	50

Table 3. PAD waveform (vs. Ag/AgCl)

Time (ms)	Voltage (V)
0	0.100
200	0.100
400	0.100
410	-2.000
420	-2.000
430	0.600
440	-0.100
500	-0.100

Sample preparation

Diluent preparation: Dissolve 100 mg of sodium azide in DI water in a 1,000 mL volumetric flask. The diluent solution, used as a bacterial preservative, does not affect the separation and quantification of sugars as already reported by Ting-Jang et al.[5]

Sorbitol, mannitol, and myo-inositol 100 ppm stock solution: Weigh 100 mg of each compound in a separate 100 mL volumetric flask and dissolve powders in the diluent solution using a magnetic stirrer at room temperature. Dilute 10 times more with fresh diluent.

The standard solution is an equal mix of sorbitol, mannitol, and myo-inositol. In this solution, the amount for each compound is 33.33 ppm. The ready-to-use stock solutions and working standard solutions are stored at -20 °C.

Crush lychees in a blender for sample homogeneization.

Depending on the sample, perform an appropriate dilution from 50 to 500 times using the diluent. Filter the diluted sample using a PVDF syringe filter directly into the injection vial.

Results and discussion

Figure 2 shows the separation of myo-inositol, sorbitol, and mannitol standard solutions. The use of the 4 µm column technology allows for baseline resolution of sorbitol and mannitol despite the short elution time. This resolution facilitates the separation of sorbitol and mannitol in complex sample matrices.

The calibration curve was established using eight concentrations of sorbitol, from 0.0333 to 33.3 ppm. The calibration range was extended by using a very low injection volume. The linear calibration mode was applied. The coefficient of determination was 0.9999, and the relative standard deviation was 1.66%.

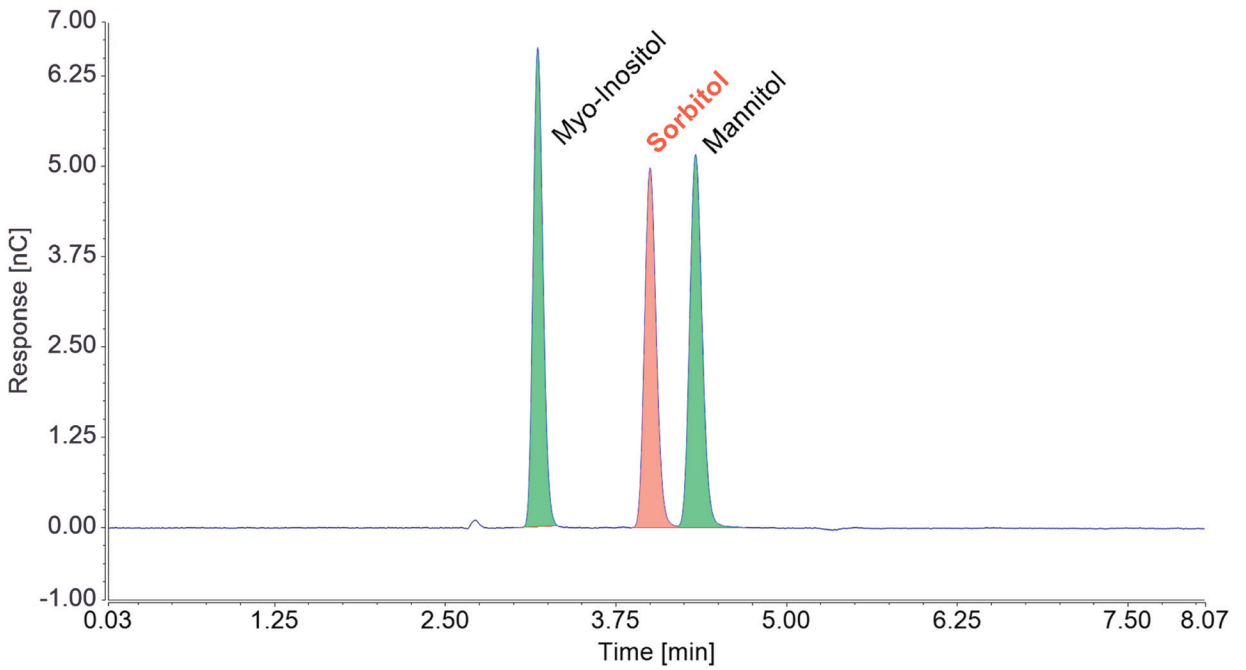

Figure 2. Chromatogram showing the separation of a 3.33 ppm carbohydrate standard using the Dionex CarboPac PA300-4µm column on a Dionex ICS-6000 system

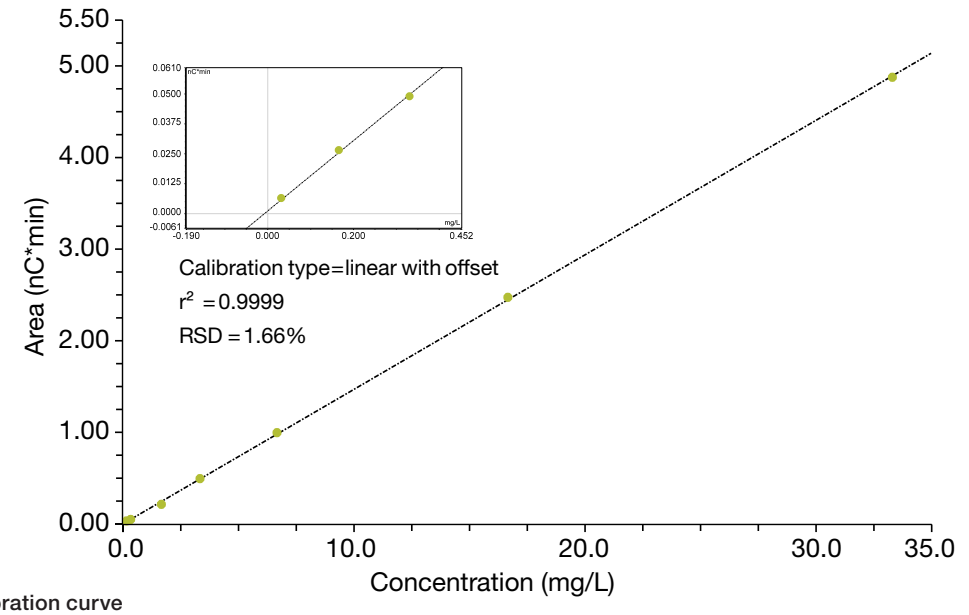

Figure 3. Sorbitol calibration curve

Figure 4 shows three different samples: lychees, mixed fruit juice, and prune juice. Depending on the amount of sorbitol present in the sample, each was diluted 50 to 500 times. All chromatograms show baseline resolution of sorbitol from other peaks. This allows faster integration and more precise quantification of sorbitol. The calculated amount of sorbitol in mashed lychees was 162 mg/L, in the mixed fruit juice 721 mg/L, and in the prune juice 179 mg/L. Prune juice is a calibrated reference material. Our result, 179 mg/L, is close to the sample calibrated value of 180 mg/L, and the relative standard deviation is lower than 1% for this matrix.

The full cycle time is 36 min. Even if samples are diluted up to 500 times, some interferents could remain visible in the chromatogram. Figure 5 shows the effect of a cleaning step that elutes contaminants at 35 min. Under these conditions, the sorbitol peak area is not impacted by sample compounds from the previous injection. The peak area follow-up was done using the control chart Chromeleon 7.3 software feature. Twenty-one consecutive injections of diluted prune juice were performed using this application. Peak areas were monitored and Figure 6 shows the response stability sample by sample. Peak area relative standard deviation is less than 1%.

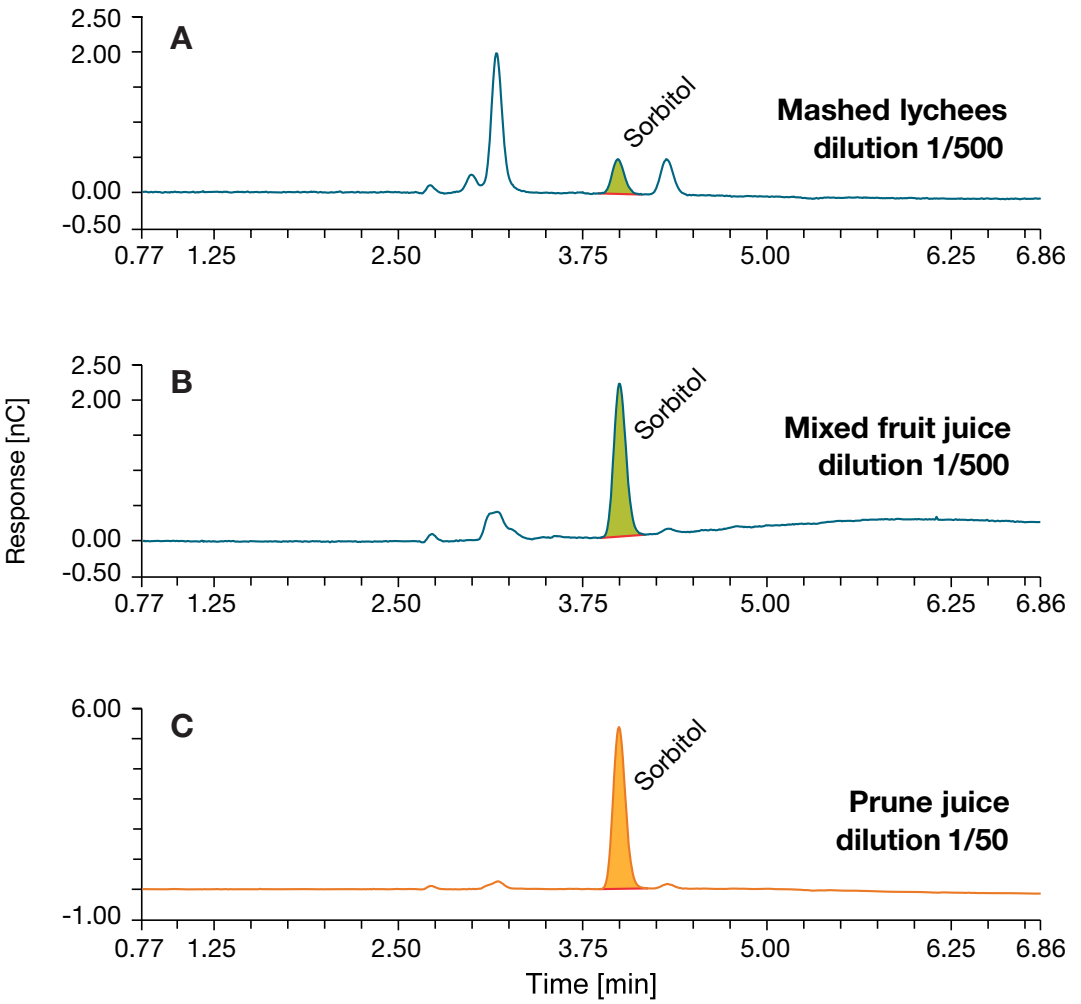

Figure 4. Chromatogram overlay obtained after a 400 nL injection of (A) 1:500 diluted mashed lychees, (B) 1:500 diluted mixed fruit juice, and (C) 1:50 diluted prune juice

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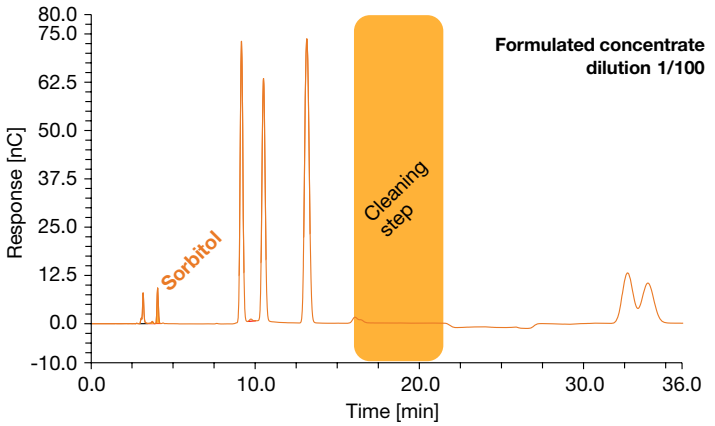

Figure 5. Chromatogram obtained after 0.4 µL injection of formulated food concentrate

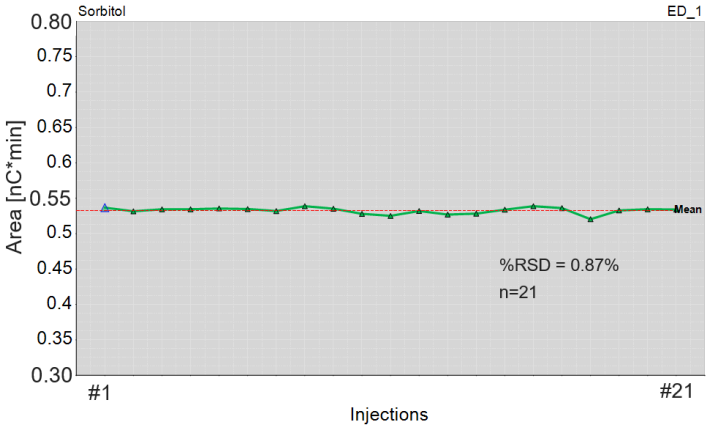

Figure 6. Trend chart of sorbitol peak area detected in diluted prune juice

Conclusion

- The new Dionex CarboPac PA300-4µm column allows simplified, high-resolution sorbitol separation and quantification in complex samples.
- The sorbitol amount could be determined in a few minutes increasing sample throughput.
- Data reprocessing with Chromeleon software is easy to manage for fast routine implementation with a high degree of automation. The in situ preparation of the eluents ensure high reproducibility of retention times and peak area. The high purity eluents facilitate the high sensitivity detection of sorbitol, reducing the necessary sample preparation to a simple dilution with DI water.

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