

Determination of Carbohydrates in Beverages and Milk Products by HPAE-PAD and Capillary HPAE-PAD

*Terri Christison, Monika Verma, Aaron Kettle, Carl Fisher, Linda Lopez;
Thermo Fisher Scientific, Sunnyvale, CA, USA*



Overview

- Carbohydrates in beverage samples and milk products were directly determined using capillary format high performance anion-exchange with pulsed amperometric detection (HPAE-PAD) on a capillary reagent-free, high-pressure system.^{1,2}
- This method eliminates the costly and labor-intensive derivitization used in other methods.
- Lactose and lactulose determinations are also demonstrated on the new 4 µm resin particle, high-capacity Thermo Scientific™ Dionex™ CarboPac™ SA20-4µm 4 mm column optimized for fast separations of mono- and di- saccharide sugars.²

Introduction

Monosaccharide and disaccharide determinations are important to the food industry to ensure product formulation and product quality and to report ingredients to immune- and allergy-sensitive individuals. Because carbohydrates are poor chromophores, chemical derivitization is needed for absorption. However, derivitization is costly, labor-intensive and may cause changes in molecular configuration.

High Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) is a proven sensitive method to directly and selectively determine carbohydrates. In HPAE-PAD, carbohydrates are ionized in strong base and separated by anion-exchange chromatography. The carbohydrates are detected by PAD with a gold working electrode using a four-potential waveform selective and sensitive for carbohydrates. This sensitivity allows carbohydrate analysis down to pmole concentrations or when the samples are limited. This sensitivity is moderated in beverage samples which contain g/L concentrations by minimizing the flow path combined with moderate dilution.

Here we demonstrate the determinations of the different sugars used to sweeten beverages and the fast and easy determination of lactose and lactulose in milk products. This work further demonstrates the versatility of HPAE-PAD, easily optimized for low or high carbohydrate concentrations, and separations on different carbohydrate columns, and High-Pressure™ IC (HPIC™) and capillary HPIC systems.

Experimental

Sample Preparation

The beverage samples were prepared by dilution and filtration, as appropriate.

The milk products (1 g/10 mL water) were treated with 200 µL each of Carrez I (potassium hexacyanoferrate(III)) and Carrez II (zinc sulfate) solutions for deproteinization according to AOAC Method 984.15¹ and as described in AN 248². The samples were mixed, diluted to 100 mL, centrifuged, and the supernatant filtered and treated with a Thermo Scientific™ Dionex™ OnGuard™ IIA sample preparation cartridge to remove anionic contaminants and neutralize the sample.

Method

Thermo Scientific Dionex HPIC Ion Chromatography systems used:

- Thermo Scientific Dionex ICS-5000+ ion chromatography (IC) system, standard and capillary format
 - Dionex ICS-5000+ IC modules: EG Eluent Generator, DC Detector Chromatography, and DP Dual Pump
- Thermo Scientific Dionex ICS-4000 Capillary HPIC system

Detection:

- Electrochemical Detector and Electrochemical cell
- Gold on PTFE Disposable Electrode and pH,-Ag/AgCl Reference Electrode
- PTFE Gaskets: 0.001", 0.002", or 0.015"

Autosampler: Thermo Scientific Dionex AS-AP Autosampler

Software: Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System

Figure 1 shows the instrument flow diagram for a capillary HPAE-PAD system. The Dionex ICS-5000+ system configured for 4 mm or 2 mm format columns has the same flow path but without the CRD and suppressor bypass modules.

Figures 2 and 3 show four-potential waveform for detection of carbohydrates (Figure 2) using a gold disposable working electrode (Figure 3). This waveform is optimized to create a stable gold oxide layer resulting in highly reproducible responses.

FIGURE 1. Flow diagram for a capillary HPAE-PAD system.

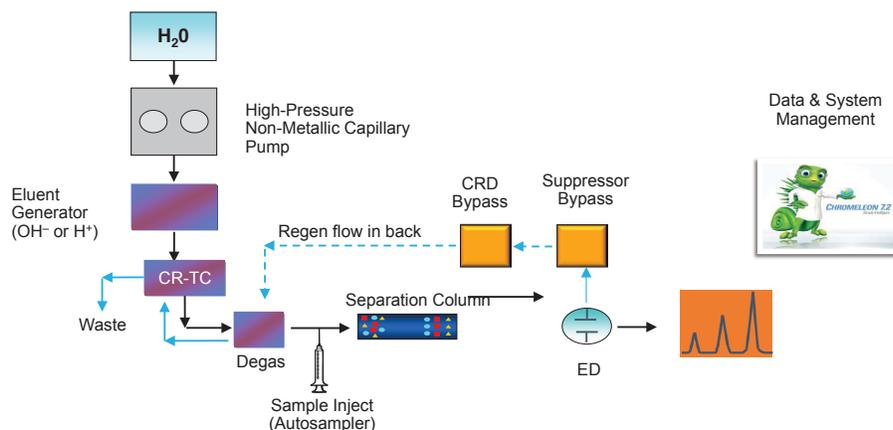


FIGURE 2. Four Potential Waveform for carbohydrate determinations.

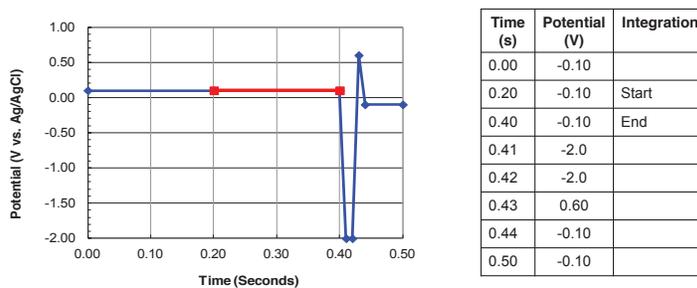
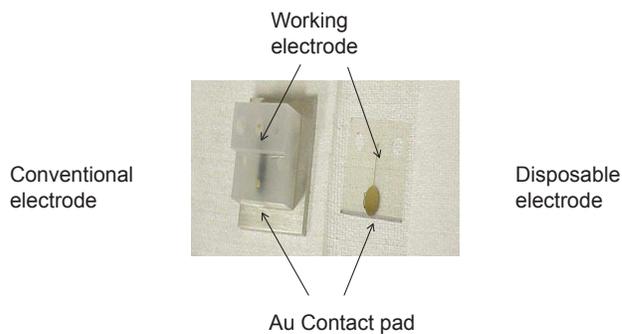


FIGURE 3. Conventional and disposable gold working electrodes.



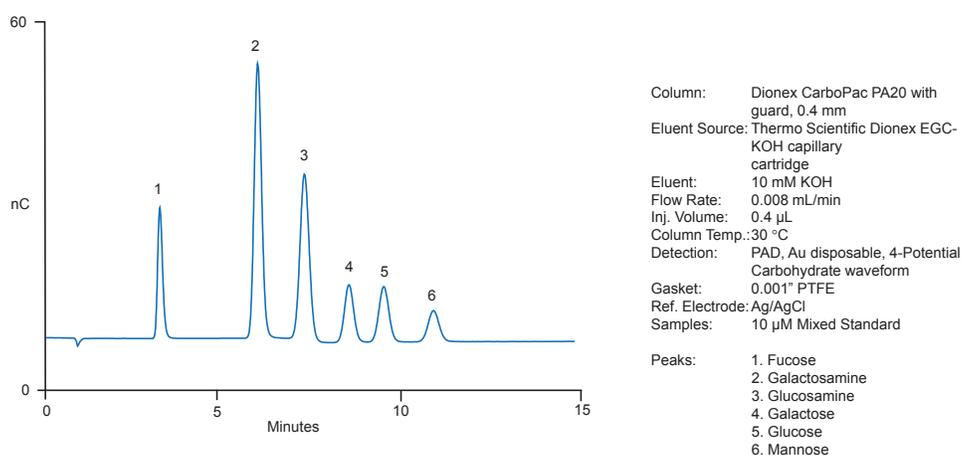
Results

Sugars in Beverages

Beverages are generally sweetened with the most available and the lowest cost sugar available in the manufacturing region. Native sugars from fruit may be used for sweetening. The manufacturer often selects the sugar for its product based on availability or lowest cost. These sugars are sourced from either corn, sugar cane or sugar beets. Corn syrup, which is primarily glucose, is enzymatically hydrolyzed to create fructose for increased sweetness. Beet sugar and cane sugar, which are sucrose, are partially hydrolyzed to glucose and fructose to increase sweetness and to minimize crystallization which can cause storage problems.

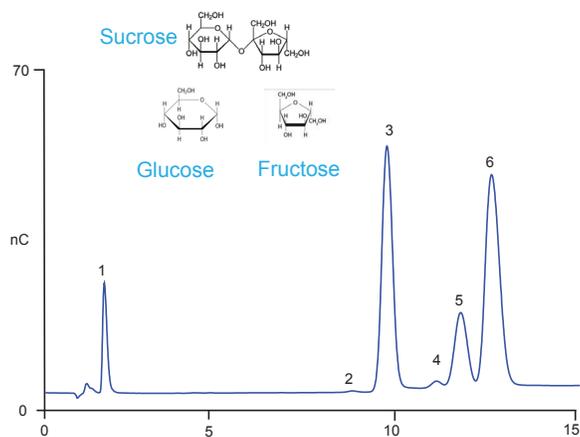
Figure 4 show the separation of 6 carbohydrates using capillary format HPAE-PAD, 0.4 μ L sample separated on 0.4 mm i.d. columns at 8 μ L/min flow rates.

FIGURE 4. Six carbohydrate standard.



Figures 5 to 7 show the analysis of beverage samples using capillary HPAE-PAD with a typical capillary gasket of 0.001" thick. In Figure 5, the sample had a mixture of sugars, characteristic of native sugars. In Figure 6, only glucose and fructose are present indicating high fructose corn syrup (HFC) for sweetening. Figure 7 shows a typical profile of product sweetened with cane sugar (predominantly sucrose with equal parts of fructose and glucose).

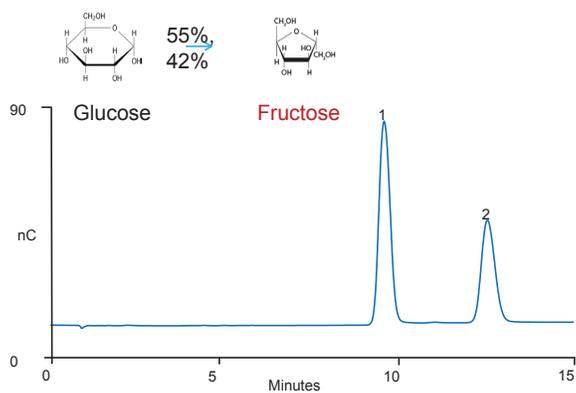
FIGURE 5. Native sugars in apple cider.



Column: Dionex CarboPac PA20 with guard, 0.4 mm
 Eluent Source: Dionex EGC-KOH capillary cartridge
 Eluent: 10 mM KOH
 Flow Rate: 0.008 mL/min
 Inj. Volume: 0.4 μ L
 Column Temp.: 30 $^{\circ}$ C
 Detection: PAD, Au disposable, 4-Potential Carbohydrate waveform
 Gasket: 0.001" PTFE
 Ref. Electrode: Ag/AgCl
 Sample Prep.: 5000-fold dilution

Peaks:	Retention Time (min)	Concentration (μ M)
1. Void Volume	--	--
2. Galactose	0.1	0.1
3. Glucose	60	60
4. Mannose	2	2
5. Sucrose	20	20
6. Fructose	110	110

FIGURE 6. HFC in a carbonated beverage.



Column: Dionex CarboPac PA20 with guard, 0.4 mm
 Eluent Source: Dionex EGC-KOH capillary cartridge
 Eluent: 10 mM KOH
 Flow Rate: 0.008 mL/min
 Inj. Volume: 0.4 μ L
 Column Temp.: 30 $^{\circ}$ C
 Detection: PAD, Au disposable, 4-Potential Carbohydrate waveform
 Gasket: 0.001" PTFE
 Ref. Electrode: Ag/AgCl
 Sample Prep.: 5000-fold dilution, degas

Peaks:	Retention Time (min)	Concentration (μ M)
1. Glucose	98	98
2. Fructose	95	95

FIGURE 7. Cane sugar in coconut water flavored beverage

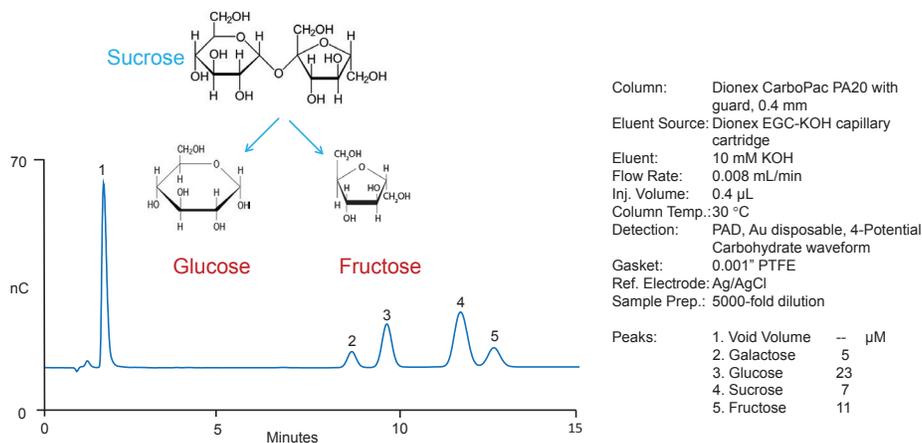


Figure 8 shows that using a 0.015" gasket versus the 0.001" capillary gasket reduces sensitivity and requires less dilution. The resulting analysis suggests the use of HFC 42 for sweetening.

FIGURE 8. HFC in a carbonated beverage -- 0.015" Gasket.

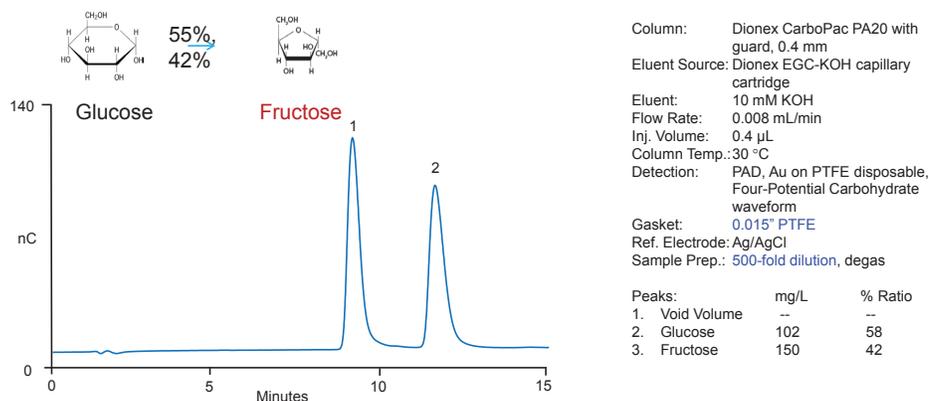
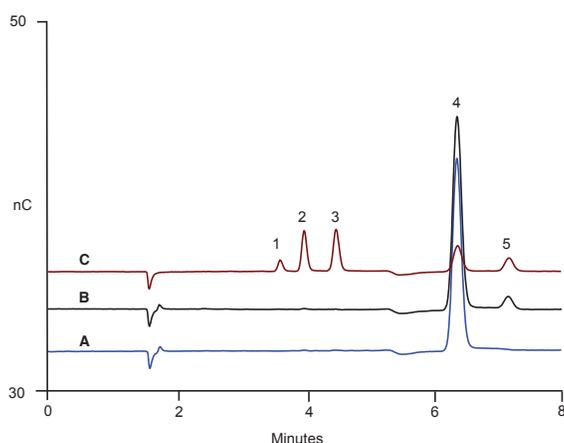


Figure 9 shows the 8 min separation of lactose and lactulose on the Dionex CarboPac SA10-4μm column using only 4 mM KOH. This method utilizes the standardbore format (4 mm) and the standard 0.002" thickness gasket. A high-pressure capable system facilitates the separation.

FIGURE 9. Lactose and lactulose in raw unpasteurized milk.



Instrument: Dionex ICS-5000+ HPLC system
 Column: Dionex CarboPac SA10-4µm and guard, 4 mm
 Eluent Source: Dionex EGC 500 KOH
 Eluent: 4 mM KOH
 Flow Rate: 1.45 mL/min
 Inj. Volume: 10 µL
 Column Temp.: 35 °C
 Detection: PAD, Au on PTFE disposable, Four-potential Carbohydrate waveform
 Gasket: 0.002" thick PTFE
 Ref. Electrode: pH-Ag/AgCl
 Sample Prep.: Carrez digestion, centrifuge, filter, Dionex OnGuard IIA cartridge
 Sample: A: 100-fold diluted raw, unpasteurized milk
 B: Sample A + 0.5 mg/L lactulose
 C: 0.5 mg/L carbohydrate standard

Peaks:	A	B	mg/L
1. Sucrose	--	--	--
2. Galactose	--	--	--
3. Glucose	--	--	--
4. Lactose	3.75	3.77	
5. Lactulose	--	0.48	

Conclusion

- Capillary HPAE-PAD and HPAE-PAD are direct, selective and sensitive methods without costly and labor intensive derivitization
- Modifying the gasket type affects the sensitivity which can be advantageous when analyzing high carbohydrate concentrations
- An RFIC system with electrolytically generated eluent requires only adding water, thereby eliminating eluent preparation, increasing reliability, stability, and ease-of-use
- Fast determinations of lactose and lactulose in milk products were demonstrated using a simply 8-min isocratic separation on the Dionex CarboPac SA10 column

References

1. Thermo Fisher Scientific Technical Note 135: Determinations of Monosaccharides and Disaccharides in Beverages by Capillary HPAE-PAD. Sunnyvale, CA, 2013. [Online] <http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Application%20&%20Technical%20Notes/Chromatography/Ion%20Chromatography/IC%20and%20RFIC%20Systems/TN-135-Determination-Monosaccharides-Disaccharides-Beverages-TN70646-E.pdf> (accessed April 7, 2014).
2. Thermo Fisher Scientific Technical Note 146: Fast Determinations of Lactose and Lactulose in Milk Products Using HPAE-PAD. Sunnyvale, CA, 2014 [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/CMD%20Documents/Product%20Manuals%20&%20Specifications/Chromatography/Ion%20Chromatography/TN146_70891_Rev3.pdf (accessed April 7, 2014).

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