Determinations of Monosaccharides and Disaccharides in Beverages by Capillary HPAE-PAD

Terri Christison, Alex Zhang, Linda Lopez
Thermo Fisher Scientific, Sunnyvale, CA, USA

Key Words
HPIC, Capillary IC, HPAE-PAD, ICS-4000, Beverage, Sugars, Electrochemical detection, Dionex CarboPac Columns

Goal

Demonstrate the determinations of glucose, sucrose, and fructose in beverage samples using electrochemical detection on the Dionex ICS-4000 HPIC system.

Introduction

Mono- and disaccharide sugar determinations are often used in the food and beverage industry to ensure the quality of a formulated product, to maintain or select for desired sweetness, and to characterize and confirm the source of the carbohydrates.[1] Sugars are added to a desired sweetness by the addition of sucrose refined from sugar beets and sugar cane or high fructose corn syrup (HFCS) where ~50% of the glucose is converted to the sweeter fructose.[2] In the U.S., corn syrup is more commonly used as a sweetener because of its availability and lower cost. Not to mention, it typically contains 55% (HFC 55) or 42% (HFC 42) fructose. In other countries, such as Mexico, sucrose is the sweetener of choice because of the availability of cane sugar. Public concerns about possible associations between total sugar consumption and the increase in obesity and diabetes have resulted in more detailed product labeling and, therefore, an increased demand for carbohydrate analysis.[3]

Carbohydrates have poor chromophores and are therefore problematic to detect by UV absorption without lengthy and costly derivitization. However, carbohydrates can be determined directly by High Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD), a well-established method that eliminates the need for derivitization, saving time and money, including reagent costs. In HPAE-PAD, neutral carbohydrates are ionized in the strong base eluent and separated by anion-exchange chromatography. The carbohydrates are detected by PAD with a gold working electrode using a four-potential waveform optimized for carbohydrates.

In this application note, glucose, fructose, and sucrose in diluted beverage samples are separated on a Thermo Scientific™ Dionex™ CarboPac™ PA20 capillary column using 30 mM KOH at 0.008 mL/min on a Thermo Scientific™ Dionex™ ICS-4000 Dedicated Capillary HPIC™ system. Here we combine the advantages of a Reagent-Free™ IC (RFIC™) system and a capillary format IC to determine sugars in diluted beverages. In an RFIC system, the hydroxide eluent is electrolytically generated inline to deliver accurate and precise concentrations for isocratic or gradient separations. Eluent generation eliminates carbonate contamination and errors from manual preparation. A capillary scale system with µL/min flow rates can run 24/7, always on and always ready for samples. Eluent consumption and waste generation are reduced to 15 mL/day and eluent generator cartridges can last 18 months. In these experiments, we compare the results using cell gaskets at two thicknesses to reduce the analyte response thereby reducing the need for dilutions. The resulting method is direct, selective, sensitive, and cost effective.

Equipment

- Thermo Scientific™ Dionex™ IC Cube™
- Thermo Scientific Dionex Electrochemical Detector (ED)
- Thermo Scientific Dionex Electrochemical Cell, reference electrode with gasket, and working electrode with gasket
- Thermo Scientific Dionex AS-AP Autosampler
- Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.1 with SR2 MUa build or later.

Reagents and Standards

- 18 MΩ-cm degassed deionized water
- ACS Grade reagents, Fisher Scientific
- Thermo Scientific™ Dionex™ MonoStandard™, Mixture of Six, 100 nmol each (P/N 043162)
- pH Buffer solutions, (pH 7/pH 10) (Fisher Scientific, P/N SB108-500 / SB115-500)

Samples

Assorted beverage samples

Conditions	
Columns:	Dionex CarboPac PA20 column set (0.4 × 150 mm)
Eluent Source:	Thermo Scientific Dionex EGC-KOH Eluent Generator Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Column Temp.:	30 °C
Compartment Temp.:	27 °C
Inj. Volume:	0.4 µL
Detection:	PAD, Gold on PTFE, 0.001" or 0.015" gasket, Four-Potential Carbohydrate waveform
Reference Electrode:	pH-Ag/AgCl
Background:	10–20 nC
Noise:	< 10 pC

* Column wash/10 samples: 5 min at 100 mM KOH, 12 min equilibration at 10 mM KOH

The consumables and accessories for this application are listed in Table 1.

Table 1. Consumables list for the Dionex ICS-4000 HPIC System with ED Detection.

Product Name	Description of High-Pressure Capillary	Part Number
Dionex EG Degas HP cartridge	High-pressure EG degas cartridge, up to 5000 psi	AAA-074459
Thermo Scientific Dionex CRD Bypass cartridge	Bypass (needed for flow path)	072056
Dionex Suppressor Bypass cartridge	Bypass (needed for flow path)	072055
Dionex high-pressure fittings (blue)	Bolts/Ferrules	074449/074373
Dionex AS-AP autosampler vials, polypropylene vials and cap kits	1.5 mL vial kit, package of 100	079812+
	0.3 mL vial kit, package of 100	055428
Columns	Dionex CarboPac PA20 Separation column	072072
	Dionex CarboPac PA20 Guard column	072073
Dionex EGC-KOH cartridge	Anion eluent generator capillary cartridge for capillary flow rates	072076
Thermo Scientific Dionex ATC-500 Anion Trap Column/ PEEK tubing	2 mm trap column between pump and Dionex EGC cartridge. PEEK tubing	079018/078497
Thermo Scientific Dionex Dionex CR-ATC Continuously Regenerated Cation Trap Column	Anion electrolytic trap column for capillary flow rates	072078
Electrochemical Detector	ED Detector module for capillary or analytical flow rates	072042
Electrochemical Cell	ED Cell body includes PEEK™ Yoke Block	072044
ED Cell Inlet Tubing kit.	Kit Includes: 9” capillary tubing for cell inlet, long neck black PEEK connector, black PEEK split cone ferrule	074221
Reference Electrode / Gasket	pH-Ag/AgCl reference electrode	061879
	Gasket for capillary applications	072162
Disposable Working Electrode/Gasket	Gold on PTFE, package of six++	066480
	0.001” thick PTFE gasket for capillary applications, Package of two	072117
	0.015” thick polypropylene gasket for mg/L concentrations	057364
Support Block	For use with 0.001”, 0.002”, 0.015” thick gaskets	062158

** Fisher Scientific P/Ns; + Previously P/N 061696

++ Kits include 0.002" gaskets intended for analytical flow rates.

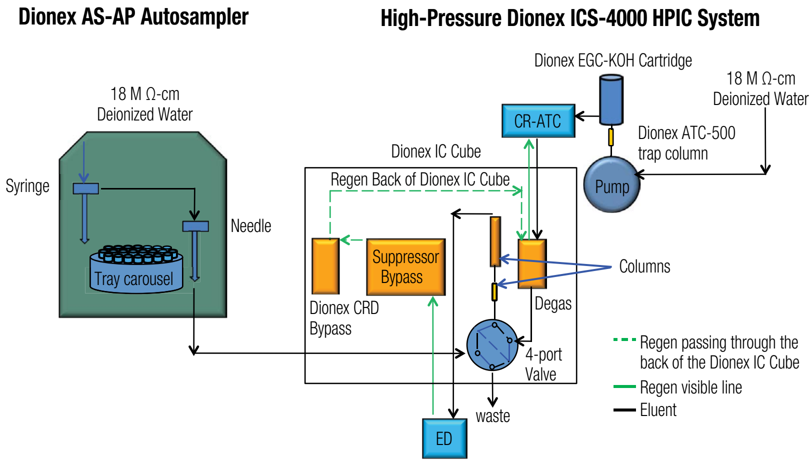

Figure 1. Flow diagram for the Dionex ICS-4000 HPIC System for HPAE-PAD.

Standard and Sample Preparation

Diluted the Dionex MonoStandard with deionized water to 10 µM. Prepared individual stock standards of 1000 mg/L glucose, sucrose, and fructose. Prepared mixed working standards from 0.1 to 200 mg/L by diluting the stock standards appropriately with deionized water.

Tip: It is important to use 18 MΩ-cm resistivity, deionized water for standards, eluent, and autosampler flush solution. It is recommended to degas the deionized water intended for eluent in anion determinations. (An appropriate degassing method is vacuum filtration.) Using deionized water with resistivity less than 18 MΩ-cm can reduce sensitivity, introduce contamination, and affect calibration, thereby resulting in inaccurate quantification. Results can vary and contamination introduced from samples can affect the chromatography.

Instrument Setup and Installation

This single-channel, Dionex ICS-4000 HPIC capillary IC system has modular detection options which allow the instrument to be dedicated to carbohydrate determinations. Although this technical note application uses only the electrochemical detector, the system has the flexibility for analyte detection using either conductivity, electrochemical, or conductivity with charge detection for different applications. The Dionex ICS-4000 HPIC system can operate at system pressures up to 5000 psi, providing a platform to use small particle size columns for sample analysis.

Install and configure the Dionex AS-AP autosampler to the Dionex ICS-4000 HPIC system according to the product manuals and "TN 136 Configuring a High-Pressure Dedicated Capillary IC System for Electrochemical Detection" .[4–6] Install the Dionex IC Cube and Dionex ED detector into the Dionex ICS-4000 system while the instrument is powered-off. To configure the system, follow the instructions in TN 136.[6] Connect the Dionex ATC-500 column to the pump with black PEEK tubing (P/N 078497) and install the Dionex EGC KOH cartridge, but temporarily leave the tubing to the Dionex EGC KOH cartridge column disconnected to flush the trap column. To flush the trap column, first initiate the priming function on the pump (1 mL/min), point the Dionex ATC-500 column upward, and flush for 30 min to allow air to escape. After 30 min, turn off the the pump prime, and connect the tubing to the Dionex EGC-KOH cartridge. To install the application described in this technical note, hydrate and condition the capillary Dionex EGC-KOH cartridge and Dionex CR-ATC device according to the Dionex ICS-4000 Operator's manual and product manuals.[4,7] Plumb the consumable products and modules of the Dionex ICS-4000 HPIC system, according to Figure 1 and Figure 2. Detailed installation instructions are described in the product manuals and TN 136.[6]

Tip: To achieve the best chromatography with high pressure IC, it is important to use high pressure connectors and ferrules (see Table 1) for all connections prior to the cell. The high pressure Dionex ICS-4000 HPIC Dedicated Reagent-Free IC system is designed to operate at pressures up to 5000 psi. Install the Dionex CRD Bypass and Suppressor Bypass cartridges in the Dionex IC Cube to complete the flow path for electrochemical detection applications (Figure 2). To achieve the best chromatography with capillary IC, it is important to minimize void volumes in all connections by using precision cut tubing, high pressure connectors and fittings (colored blue), and seating the ferrule > 2 mm above the end of the tubing. These tips are thoroughly discussed in "TN 113 Practical Guidance for Capillary IC".[8] Extra care should be used to prevent introducing air in all consumables or tubing by observing a steady flow before installing the next device in line.

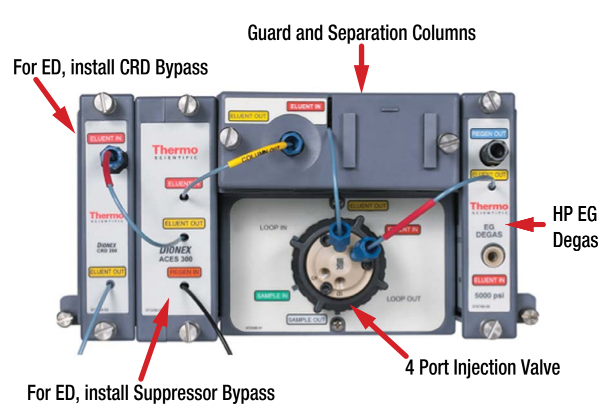

Figure 2. Dionex IC Cube.

Installing the Electrochemical Cell with a pH-Ag/AgCl Reference Electrode

The installation procedures are thoroughly described in TN 136, the Dionex ICS-4000 Operator's manual, and the User's Compendium for Electrochemical Detection.[4,6,9]

Tips: Always wear gloves when handling the electrochemical cell. If this is a new ED Cell, disassemble the cell and discard the shipping gasket. Caution: Do not touch the working electrode with any paper products, as this can contaminate the working electrode.

Tips: Remove all plugs on the cell inlet and cell out to prevent cell pressure during the installation. First condition the pH-Ag/AgCl reference electrode in a solution of pH 7 buffer. The installation procedures are thoroughly discussed in TN 136.

To prepare the cell body for capillary applications, remove the titanium inlet tube and rinse the cell body, and the wells of the reference electrode and the inlet tube thoroughly with deionized water. Then rinse the working electrode gasket (0.001" PTFE gasket) and Support Block with deionized water and dry with a lab tissue. Rinse the Gold on PTFE working electrode and shake-off the excess water. Assemble the gasket, disposable working electrode cell, Support Block, and Yoke Knob assembly according to the TN 136 and User's Compendium for Electrochemical Detection.[6,9] Avoid any wrinkles in the gasket, as this will cause a poor fit and subsequent leaks and poor detection.

Calibrate the reference electrode using pH 7 buffer and pH 10 buffer and the instructions given by the pH Calibration button on the ED Panel. Remove the o-ring gasket from the reference electrode and then install the gasket for the pH-Ag/AgCl reference electrode into the bottom of the reference electrode well and gently but firmly screw-in or rotate the reference electrode until it is finger-tight. Install the fully assembled ED cell into the Dionex ICS-4000 ED module. Immediately complete the final plumbing to the cell by installing the PEEK inlet tubing from the column outlet to the cell inlet well. Turn-on the Dionex EGC-KOH cartridge and Dionex CR-ATC capillary devices and set eluent concentration to 10 mM KOH. Connect the Suppressor Bypass tubing to the cell outlet after observing the eluent flowing out of the cell (at the cell outlet).

Creating an Instrument Method Using Chromeleon Wizard

To create a new instrument method using Chromeleon 7 CDS, select Create, Instrument Method, and specific Instrument. Table 2 describes the specific conditions for this application.

The waveforms are thoroughly discussed in the User's Compendium.[9]

Table 2. Additional conditions to create an instrument method using PAD.

Page Title	Page	Mode	Action
Sampler Options	Injection	Injection Mode	PushCap
		Capillary Overfill	50 (times) (20 µL withdrawn from the sample vial)
		Accept Recommended Values	Click on button.
	General Settings	Temperature	Specify if needed.
		Accept Recommended Values	Click on button.
		Wait for Temperature	Click box if using the temperature option.
		Injection Wash Property	Enter AfterInj (After injection).
EDet Mode Options	Mode	DC or Integrated Amperometry	Click on box for Integrated Amperometry.
Integrated Amperometry	Cell Control	On	
	Reference Electrode	Type	Select Ag/AgCl.
	Waveform	Type	Select "Gold Standard PAD" waveform with the Ag/AgCl reference electrode from pull down menu.
	Data Collection	Hz	Enter 2.0 for carbohydrates.
	pH	Lower and upper	Enter 11 and 13 for the lower and upper pH limit, respectively.
	Temperature	Column	Click on use box. Enter 30 (°C).
		Compartment	Click on use box. Enter 27 (°C).

Results and Discussion

Monosaccharides and dissacharides ratios and concentration are often used to characterize the beverage quality, and authenticity and assist in defining the plant source of the added sugar. Fruit juices have naturally occurring fructose, glucose, and sucrose in different proportions characteristic of the fruit. Cane and beet sugar are 100% sucrose but HFCs are 45/55 or 58/42 glucose/fructose. In these experiments, glucose, fructose, and sucrose were separated using electrolytically generated 10 mM KOH at 0.008 mL/min standard flow rate on the capillary Dionex CarboPac PA20 column. The analytes were detected by PAD, four-potential waveform, Gold on PTFE working electrode with 0.001" gasket, and referenced to Ag/AgCl. After 10 injections, a 100 mM KOH wash for 5 min followed by a 12 min equilibration was applied to return the column to its original state. If retention times are unstable when using a column wash, add a longer equilibration time. The 0.001" gasket is recommended for capillary applications to achieve a minimum flow path and the highest sensitivity. The Gold on PTFE working electrode was selected for this application because the electrode is more robust when using the 100 mM KOH wash and has a longer life than the standard Au working electrode used for carbohydrate determinations.[10]

Beverage Determinations Using the Capillary Gasket

Figure 3 shows the separation of 10 µM of the six standard sugars and aminosugars. The chromatography shows well-resolved, high efficiency peaks. The peak response of the three sugars was measured from 0.1 to 10 mg/L, resulting in a linear response, R^2 of > 0.99.

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.001" gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Samples:	10 µM Mixed Standard

Peaks:		
	1. Fucose	1.6 mg/L
	2. Galactosamine	1.8
	3. Glucosamine	1.8
	4. Galactose	1.8
	5. Glucose	1.8
	6. Mannose	1.8

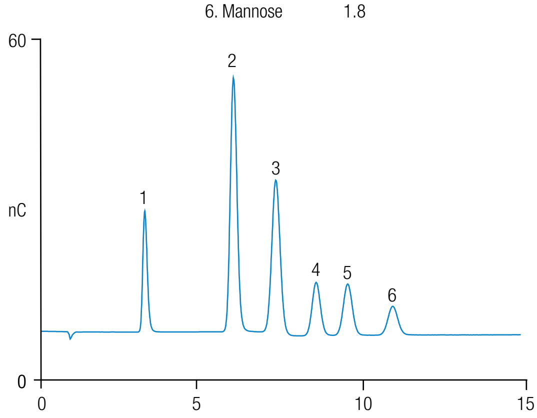

Figure 3. Carbohydrate mix of six standards.

In Figure 4, the 5000-fold diluted carbonated beverage has only glucose and fructose suggesting that HFC was the sugar added during formulation. However, the 5000-fold dilution may have introduced a dilution error.

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.001" gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Sample Prep.:	5000-fold dilution, degas

Peaks:		mg/L	Total	% Ratio
	1. Glucose	10	50 g/L	32
	2. Fructose	21	105	68

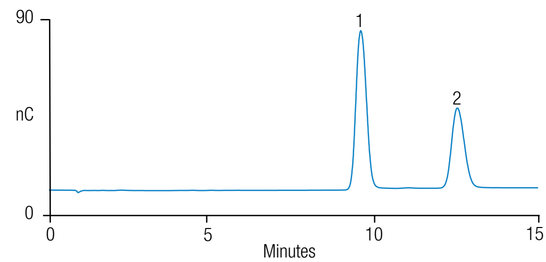

Figure 4. Glucose and fructose in a carbonated beverage.

Figure 5 shows the separation of a 10,000-fold diluted tea beverage with similar concentrations of glucose and fructose and lesser amounts of sucrose. The total sugar concentrations are only slightly lower than the carbonated beverage. The significant presence of sucrose seems to agree with the ingredient labeling that sugar cane sugar was added.

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.001" gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Sample Prep.:	10,000-fold dilution

Peaks:		mg/L	Total	% Ratio
	1. Glucose	4.6	46 g/L	39
	2. Sucrose	1.3	13	11
	3. Fructose	5.9	59	50

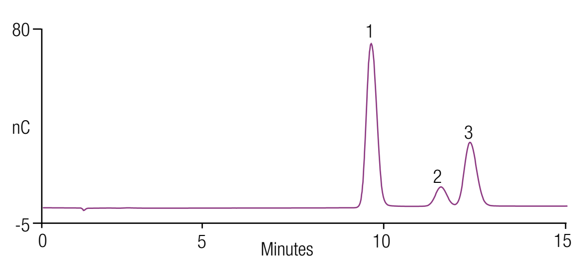

Figure 5. Glucose, sucrose, and fructose in tea beverage.

In Figure 6, the apple cider sample shows similar sugar concentrations as the carbonated beverage, primarily as fructose with lesser amounts of glucose and sucrose and negligible amounts of galactose. The sucrose concentration agrees with previous reports of apple juice analysis and therefore is in agreement with the labeling that no sugar was added to this beverage.[1]

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.001" gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Sample Prep.:	10,000-fold dilution

Peaks:	mg/L	Total	% Ratio
1. Void Volume			
2. Galactose	< 0.02	— g/L	—
3. Glucose	11	55	32
4. Mannose	0.4	2	1
5. Sucrose	3.4	17	10
6. Fructose	20	100	57

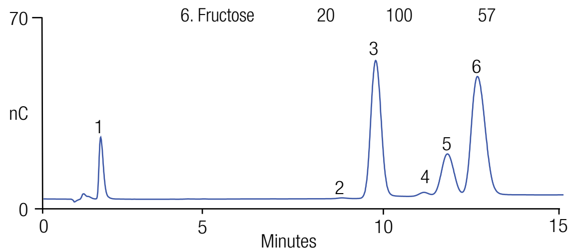

Figure 6. Diluted apple cider with native sugar only.

Beverage Determinations Using the 0.015" Gasket

In samples containing high concentrations of sugars with a sensitive detection method such as HPAE-PAD, the dilution levels required to prevent readings from being out of the linear response concentration range can introduce additional errors. Thicker gaskets such as this 0.015" thick gasket for the working electrode have been proven effective when using standard bore columns.[11] In these experiments, the coconut water and cola beverages were diluted only 500-fold prior to injection. The low sugar fruit-flavored beverage was diluted only two-fold. A column wash was used with every injection. The peak response of the three sugars was measured from 1 to 200 mg/L, resulting in a linear response, R^2 of > 0.99.

Figure 7 shows the results of a 500-fold diluted carbonated beverage. Here the glucose and fructose ratios suggest that HFC 55 was used for sweetening. The results with the 0.015" thick gasket show good chromatography for samples diluted as low as 1:2 for healthier low sugar beverages and as low as 100-fold for higher sugar beverages.

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.015" gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Sample Prep.:	500-fold dilution, degas

Peaks:	mg/L	Total	% Ratio
1. Glucose	122	61 g/L	43
2. Fructose	160	80	57

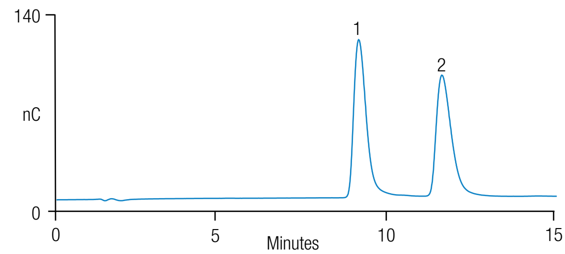

Figure 7. Sugars in carbonated beverage – 0.015" gasket.

Figure 8 shows the tea beverage diluted 500-fold. The total concentrations were similar to those calculated for the 5000-fold diluted sample in Figure 5.

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.015" gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Sample Prep.:	500-fold dilution, degas

Peaks:	mg/L	Total	% Ratio
1. Glucose	98	49 g/L	45
2. Sucrose	18	9.3	8
3. Fructose	100	50	46

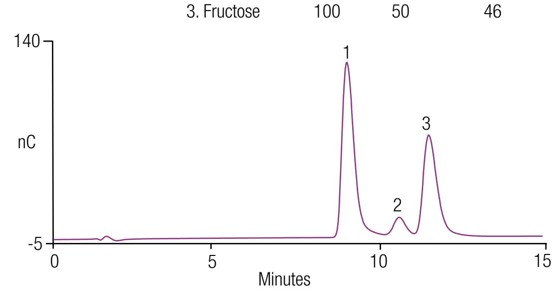

Figure 8. Glucose, sucrose, and fructose in tea beverage using 0.015" gasket.

Figure 9 shows the analysis of a low sugar fruit-flavored beverage, diluted two-fold. The sugar ratios suggest a fruit flavoring with no additions of sugar. This beverage has less than 1 g/L of total sugar.

Column:	Dionex CarboPac PA20 set, 0.4 × 150 mm
Eluent Source:	Dionex EGC-KOH Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au disposable, 0.015” gasket, 4-Potential Carbohydrate waveform
Ref. Electrode:	Ag/AgCl
Sample Prep.:	Two-fold dilution, degas

Peaks:	mg/L	Total	% Ratio
1. Void Volume			
2. Glucose	120	0.24 g/L	41
3. Sucrose	50	0.10	17
4. Fructose	125	0.25	42

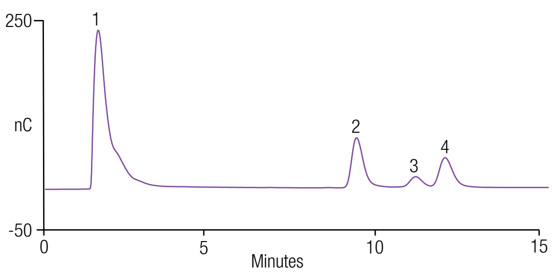

Figure 9. A Low-sugar dragon fruit beverage – 0.015” gasket.

Conclusion

This application note demonstrates mono- and disaccharides determinations in two-fold to 10,000-fold diluted beverage samples by HPAE-PAD at capillary flow rates on the Dionex ICS-4000 HPIC Integrated system. The eluent was electrolytically generated inline thereby delivering the eluent precisely and accurately while minimizing carbonate contamination and eliminating errors associated with manual preparation. The diluted beverages were analyzed using a Gold on PTFE working electrode with the capillary gasket (0.001” thick) or the thicker 0.015” gasket.

Glucose, sucrose, and fructose peaks had nearly baseline resolution (~ R_s (EP) =1.6) and similar peak responses using the capillary gasket and the 0.015” gasket as previously reported. These carbohydrate analyses of diluted beverages show separation and detection of sugars. The thicker 0.015” gasket provides more accurate results by reducing sample dilution, however more frequent column washes are needed to maintain the column. The Dionex ICS-4000 HPIC system provides all of the advantages of capillary IC on a single channel system.

For more carbohydrate applications in beverages using capillary IC separations, review Application Brief AB 127 and the Food and Beverage Capillary IC Applications at the Dionex Capillary IC Library website.[12,13] Carbohydrate determinations in beverages using standard flow rates are thoroughly discussed in Hanko, et al., AN 159, AU 151, and AN 280.[14–17]

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