Poster Note 72096

Fast and Cost-effective Sugar Analysis Using High-Performance Anion Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)

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ABSTRACT

Purpose: To demonstrate applications using HPAE-PAD with a high concentration carbohydrate analysis kit to determine mg/L and g/L sugars in beverages, alcoholic samples, and vinegars

Methods: Monosaccharide and disaccharide sugars are ionized in strong base, separated by high performance anion-exchange (HPAE) chromatography, and detected by pulsed amperometric detection (PAD). Analysis is facilitated by the Thermo Scientific™ Dionex™ Integrion™ HPIC™ system.

Results: mg/L and g/L concentrations of sugars were directly determined in various diluted beverage, alcoholic, and vinegar samples.

INTRODUCTION

Sugar analysis is needed in the beverage industry to assure product quality and consistency, detect adulteration, and to meet labeling requirements. As a result of the Nutrition Labeling and Education Act of 1990 (NLEA), the U.S. Food and Drug Administration (FDA) requires all packaged foods and drinks to list the total sugar content per serving according to Code of Federal Regulations (CFR) Title 21.1 For food labeling, sugars are defined as the sum of all free mono- and disaccharides. With increased rates of obesity and diabetes, public awareness has increased and health concerns have been raised about excess consumption of sugar. As a result, the beverage industry has introduced functional beverages, such as vitamin-fortified water, energy drinks, anti-aging water, and herbal nutritional supplements.

High-performance anion-exchange chromatography coupled with pulsed amperometric detection (HPAE-PAD) is a well-established technique to accurately identify and quantify carbohydrates in food and beverage samples.2 By accurately determining the sugar concentrations, HPAE-PAD is used to identify contamination and adulteration, maintain product consistency, and to ensure regulatory compliance of raw ingredients (water, additives, and fruit) and the final product. Here we show the determination of sugars in drinks using the Thermo Scientific™ Dionex™ Integrion™ HPIC™ IC system. This system allows fast determination of sugars without manual eluent preparation or sample derivatization.

MATERIALS AND METHODS

Sample Preparation

Beverage samples were diluted with deionized water prior to analysis. Samples that were opaque were first diluted, then filtered (0.2 µm), and treated with a Thermo Scientific™ Dionex™ OnGuard™ II RP sample preparation cartridge.

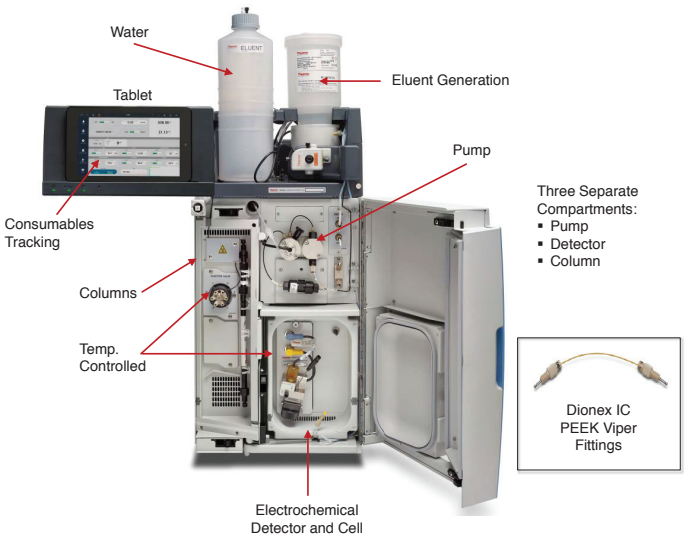
Chromatography

See chromatograms for conditions.

Instrument

Dionex Integrion HPIC system (Figure 1) configured for electrochemical detection, which includes eluent generation and Thermo Scientific™ Dionex™ IC PEEK Viper™ fittings (Figure 1 bottom left). The flow diagram is shown in Figure 2.

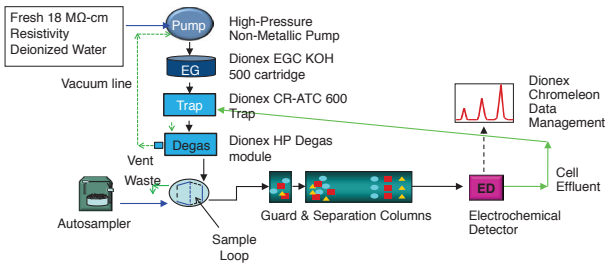
Figure 1. Dionex Integrion HPIC System Configured for Electrochemical Detection.



Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR4. This software includes a more automated instrument configuration procedure, consumables inventory, knowledgebase for troubleshooting, consumables installation guides, retention time standard processing method that predicts retention time shifts, tablet manual control, and personal phone application.

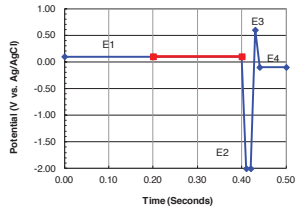
Figure 2. Flow Diagram for the Dionex Integrion HPIC Reagent-Free System Configured for ED Detection.



In PAD using the four-potential waveform, the disposable working electrode is pulsed through the different potentials at set times, completing two cycles within one second (Figure 3). This waveform is optimized to provide a clean, stable gold-oxide layer in preparation for detection of the next eluting peak.

Figure 3. Four-Potential Carbohydrate Waveform.

Time (s)	Potential (V)	Integration
0.00	+0.10	
0.20	+0.10	Start
0.40	+0.10	End
0.41	-2.0	
0.42	-2.0	
0.43	0.60	
0.44	-0.10	
0.50	-0.10	



1. Detection potential (E1)
2. Duration time of E1 (t1)
3. Integration period (Integrate)
4. Reductive cleaning potential (E2)
5. Duration time of E2 (t2)
6. Oxidative cleaning potential (E3
7. Duration time of E3 (t3)
8. Pre-detection (oxide reduction) potential (E4)
9. Duration time of E4 (t4)

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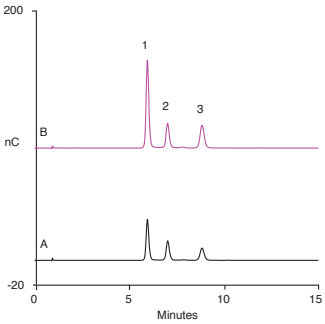
RESULTS

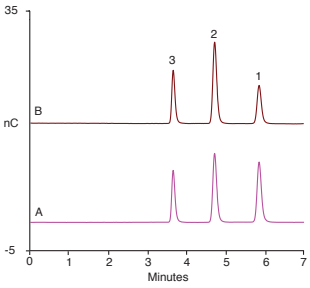
Determinations of monosaccharide and disaccharide sugars were conducted in various carbonated beverages, vinegars, and flavored distilled alcohol samples. In Figures 4 and 5, sugars were determined in the same functional beverage using the carbohydrate gold disposable working electrode. The three sugars in the 10,000-fold diluted sample were separated on the Thermo Scientific™ Dionex™ CarboPac™ PA20 column and detected using the standard 0.002-in thick gasket (Figure 4). In contrast and for confirmation, samples were diluted only 100-fold and analyzed on the Thermo Scientific™ Dionex™ CarboPac™ SA10 column using the 0.062 in thick gasket from the Thermo Scientific™ High Concentration Carbohydrate Analysis kit (Figure 5). This column was optimized for fast separations with a different selectivity, as evident by the different elution order.

Figure 4. Sugars in 10,000× Diluted Functional Beverage Samples Using a Dionex CarboPac PA20 Column.



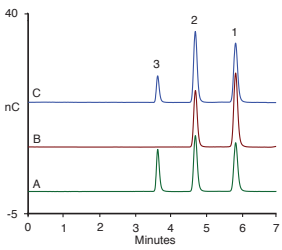
Columns:	Dionex CarboPac PA20 guard and separator, 3 mm i.d.			
Eluent:	33 mM KOH			
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser			
Flow Rate:	0.50 mL/min			
Inj. Volume:	10 µL			
Column Temp.:	30 °C			
Detection:	PAD, Carbohydrate disposable Au			
Detect. Comp.:	30 °C			
Waveform:	Four Potential Carbohydrate Vs Ag/AgCl			
Sample:	A: 5 mg/L Standard B: Energy beverage			
Sample Prep.:	10,000× dilution with deionized water, filter, 0.2 µm			
		A	B	
Peaks:	1. Glucose	5.1	4.4	mg/L
	2. Fructose	4.9	1.8	
	3. Sucrose	5.1	3.9	

Figure 5. Sugars in 100× Diluted Functional Beverage Sample Using a Dionex CarboPac SA10-4µm Column and High Concentration Carbohydrate Analysis Kit.



Columns:	Dionex CarboPac SA10-4µm separator and guard, 4 mm i.d.			
Eluent:	1.0 mM KOH			
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser			
Flow Rate:	1.50 mL/min			
Inj. Volume:	0.4 µL			
Column Temp.:	30 °C			
Detection:	PAD, Au on PTFE, High Concentration Carbohydrate Analysis kit			
Detect. Comp.:	30 °C			
Waveform:	Four Potential Carbohydrate Vs Ag/AgCl			
Sample:	A: 400 mg/L Standard B: Energy beverage			
Sample Prep.:	100× dilution with deionized water, filter, 0.2 µm			
		A	B	
Peaks:	1. Glucose	411	483	mg/L
	2. Fructose	406	254	
	3. Sucrose	406	409	

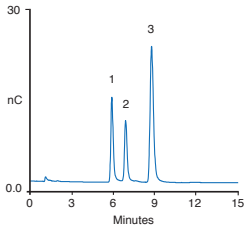
Figure 6. Sugars in 100× Diluted Carbonated Beverage Samples Using a Dionex CarboPac SA10 Column and High Concentration Carbohydrate Analysis Kit.



Column:	Dionex CarboPac SA10 guard, Dionex CarboPac SA10, 3 mm i.d.				
Eluent:	33 mM KOH				
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser				
Flow Rate:	0.50 mL/min				
Inj. Volume:	0.4 µL				
Column Temp:	30 °C				
Detection:	PAD, PAD, Au on PTFE, High Concentration Carbohydrate Analysis kit				
Waveform:	Four Potential Carbohydrate Vs Ag/AgCl				
Detect. Comp:	30 °C				
Sample:	A: 400 mg/L Standard B: Cola carbonated beverage C: Non-caffeinated carbonated beverage				
Sample Prep:	100× dilution with deionized water				
		A	B	C	
Peaks:	1. Glucose	411	414	521	mg/L
	2. Fructose	406	620	490	
	3. Sucrose	406	--	253	

Figure 7 shows analysis of a flavored rum sample analyzed with the same High Concentration Carbohydrate Analysis kit as used in Figure 6. The Dionex CarboPac PA20 was selected over the Dionex CarboPac SA10 column because the Dionex CarboPac PA20 column selectivity fully resolves the sugars of interest from ethanol.

Figure 7. Sugars in a 100× Diluted Flavored Rum Sample Using a Dionex CarboPac PA20 Column and High Concentration Carbohydrate Analysis Kit.



Column:	Dionex CarboPac PA20 guard, and separator, 3 mm i.d.		
Eluent:	35 mM KOH, 100 mM KOH wash from 15 to 25 min		
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser		
Flow Rate:	0.50 mL/min		
Inj. Volume:	0.4 µL		
Column Temp.:	30 °C		
Detection:	PAD, Au on PTFE, High Concentration Carbohydrate Analysis kit		
Detect. Comp.:	30 °C		
Waveform:	Four Potential Carbohydrate Vs Ag/AgCl		
Sample:	Flavored Rum, Brand P		
Sample Prep.:	100× dilution with deionized water,		
Peaks:	1. Glucose	232	mg/L
	2. Fructose	230	
	3. Sucrose	230	

CONCLUSIONS

- HPAE-PAD is a sensitive method to directly determine sugars in diverse samples. HPAE-PAD combined with the High Concentration Carbohydrate Analysis kit extends the analytical range to mg/L and g/L concentrations with minimal dilution of samples, thereby reducing dilution errors and improving reporting accuracy.
- This technique was demonstrated by the determination of mg/L to g/L sugars in sports drinks, colas, and alcoholic beverages.
- The Dionex Integrion HPIC system enables electrochemical detection on an integrated IC system, saving laboratory space.
- The Dionex Integrion HPIC system, reagent-free model has many features that increase ease of use:
  - Dionex IC PEEK Viper fittings—easy to install and provide consistent connections resulting in better chromatography
  - Reagent-free IC—supplies accurate and precise eluents, producing reliable peak retention times for isocratic or gradient separations

REFERENCES

1. 21 CFR 101.9 - NUTRITION LABELING OF FOOD
2. Thermo Scientific Technical Note 20.
3. Thermo Scientific™ AppsLab Library. (search word: sugars, Integrion)

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