POSTER NOTE

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Fast and Cost-effective Sugar Analysis Using HPAE-PAD

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ABSTRACT

Mono- and disaccharide sugar determinations are needed in the food and beverage industry to comply with a government mandated nutrition declaration (e.g. EU regulation No.1169/2011) or other labeling requirement. These determinations are also used to ensure the quality of a formulated product and can be used to confirm authenticity. Carbohydrates are poor chromophores so when a sensitive detection is required, a sample derivatization is needed for absorbance detection. High performance anion-exchange chromatography with pulsed amperometric detection (HPAE-PAD) can determine carbohydrates directly with high sensitivity without derivatization. However, samples with g/L carbohydrate concentrations require significant dilution to have the analytes in the linear calibration range of the method. This can impact the quality of the results. Here we demonstrate the direct determinations of mono- and disaccharides in alcoholic beverages, fruit juices, vinegars, and functional beverages by HPAE-PAD using a thicker working electrode gasket for samples with higher carbohydrate concentrations and the typical gasket for lower concentration samples. This improves the accuracy and reproducibility of the determination. These analyses are facilitated by a high pressure compact IC system that automatically produces the mobile phase necessary for HPAE-PAD from deionized water. This increases peak retention time and area reproducibilities and eliminates mobile phase preparation errors. The results show that this HPAE-PAD method allows easy and fast determinations of mono- and disaccharides in beverages

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 the increase in rates of obesity and diabetes, public awareness 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 (HPAE) coupled with pulsed amperometric detection (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™ 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.

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.

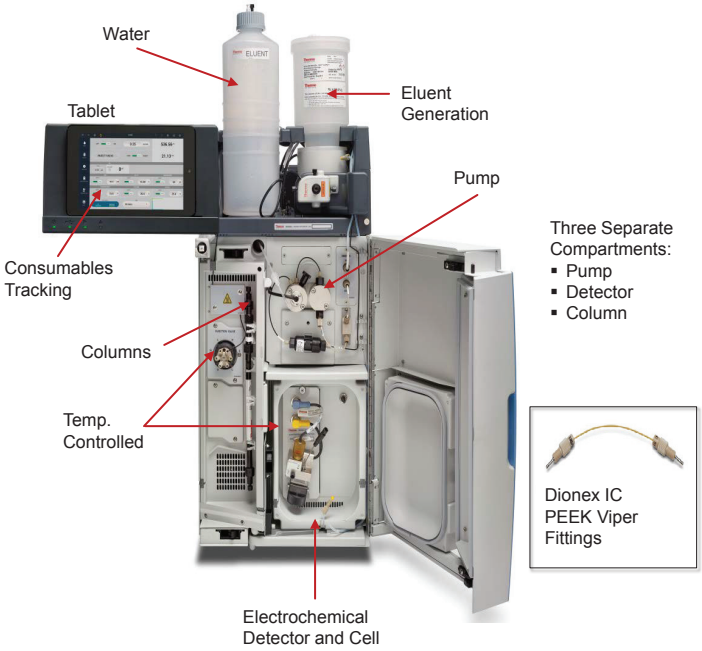

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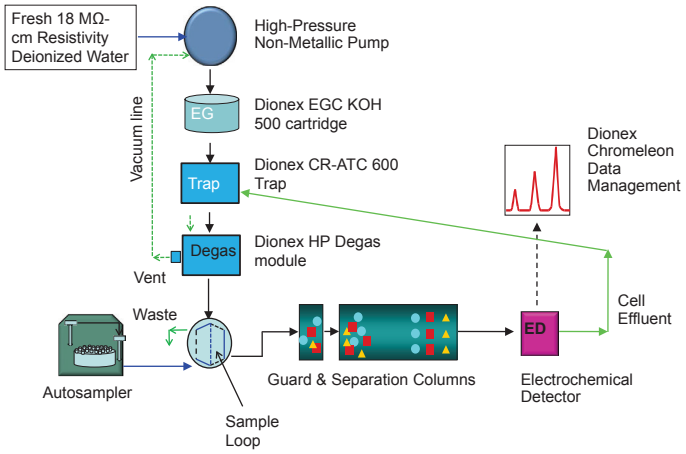
Figure 2. Flow Diagram for the Dionex Integrion HPIC Reagent-Free system configured for ED detection.



RESULTS

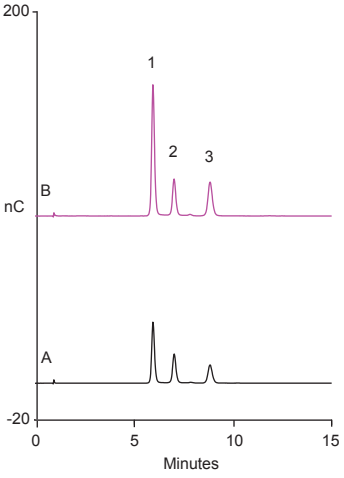
Determinations of monosaccharide and disaccharide sugars were conducted in various carbonated beverages, vinegars, and flavored distilled alcohol samples[3].

In Figures 3 and 4, 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 3). 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 4). This column was optimized for fast separations with a different selectivity, as evident by the different elution order.

Figure 5 shows the determinations of sugars in carbonated beverages. Product B contains only fructose and sucrose, implying addition of high fructose corn syrup as the sugar. Product C contains all three sugars

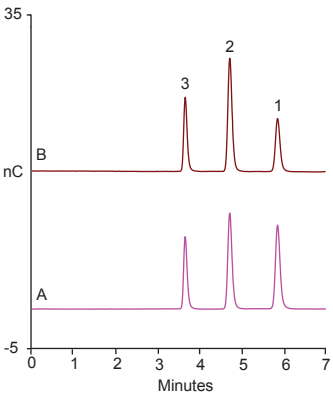
Figure 6 shows analysis of a flavored rum sample analyzed with the same High Concentration Carbohydrate Analysis kit as used in Figure 5. The Dionex CarboPac PA20 was selected over the Dionex CarboPac SA10 column because the Dionex CarboPac PA20 column selectivity fully resolves the sugars of interest from ethanol

Figure 3. Sugars in 10,000× diluted functional beverage samples using a Dionex CarboPac PA20 column.



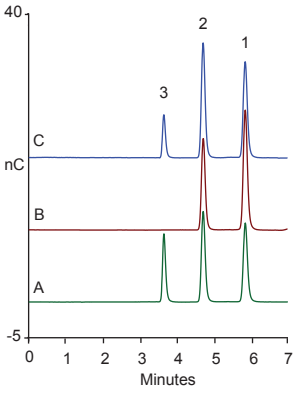
Columns:	Dionex CarboPac PA20 guard and separator, 3 mm i.d.			
Eluent:	33 mM KOH			
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser			
Flow Rate:	0.50 mL/min			
Inj. Volume:	10 µL			
Column Temp.:	30 °C			
Detection:	PAD, Carbohydrate disposable Au			
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 4. Sugars in 100× diluted functional beverage sample using a Dionex CarboPac SA10-4µm column and High Concentration Carbohydrate Analysis Kit.



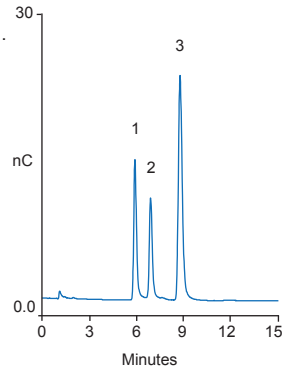
Columns:	Dionex CarboPac SA10-4µm separator and guard, 4 mm i.d.			
Eluent:	1.0 mM KOH			
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser			
Flow Rate:	1.50 mL/min			
Inj. Volume:	0.4 µL			
Column Temp.:	30 °C			
Detection:	PAD, Au on PTFE, High concentration 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 5. Sugars in 100× diluted carbonated beverage samples using a Dionex CarboPac SA10 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: 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 6. 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, thus 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.

REFERENCES

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

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