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Fast Sugar Analysis for Food and Beverage Using HPAE-PAD on a Compact IC System

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OVERVIEW

Purpose: To demonstrate applications using HPAE-PAD with a High Concentration Carbohydrate Analysis kit to determine mg/L and g/L sugars in beverages, alcohol and vinegar samples.

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, alcohol, and vinegar samples.[3]

INTRODUCTION

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 sugar content per serving according to Code of Federal Regulations (CFR) Title 21.[1] This information must appear on a product's nutrition facts label, along with things like calorie, fat, sodium, and fiber content. Sugars are defined as the sum of all free mono- and disaccharides (such as glucose, fructose, lactose, and sucrose). Excess sugar consumption is associated with obesity and poor health. Although labeling is not required for alcoholic beverages, consumers may need to know sugar concentrations.

As consumers' interest in health-conscious diets has grown, so has the global beverage industry – with the introduction of vitamin fortified water, energy drinks, anti-aging water, and herbal nutritional supplements. As a result, more analytical methods are needed.

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.[3]

METHODS

See chromatograms

Instrument:

Dionex Integrion HPIC system (Figure 1) configured for electrochemical detection, includes: eluent generation, 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.

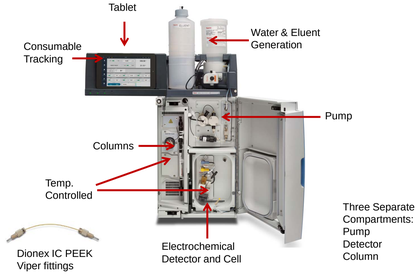
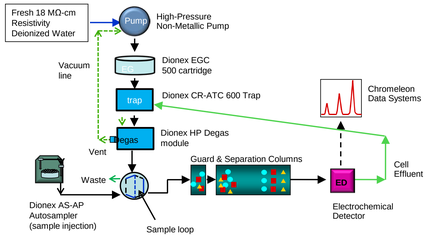


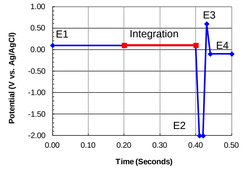
Figure 2. Flow diagram for the Dionex Integrion HPIC system configured for electrochemical detection.



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)

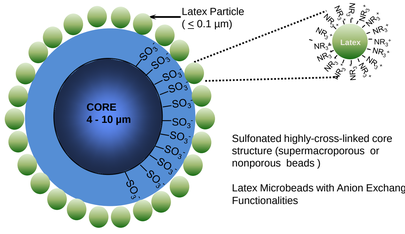
DATA ANALYSIS

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System (CDS) software, version 7.2 SR4.

RESULTS

The determinations of sugars in drinks using the Dionex Integrion HPIC system are shown here. Carbohydrates, including sugars, are weak acids. At high pH, they are ionized, and thus can be separated by anion-exchange chromatography. Figures 4 illustrates an anion exchange bead in a Thermo Scientific™ Dionex™ CarboPac™ column.The core polymer is polystyrene divinylbenzene which has been surface sulfonated, followed by latex agglomeration. This pellicular resin structure permits excellent mass transfer, resulting in high-resolution IC chromatography. With PAD, a simple electrochemical detection, HPAE-PAD directly quantifies carbohydrates with high sensitivity and selectivity and without derivatisation. With electrochemical detection, Dionex IC PEEK Viper fittings and Reagent-Free™ IC (RFIC), the newly introduced Dionex Integrion HPIC system offers a fast and cost-effective HPAE-PAD method for sugar analysis.

Figure 4. Polymeric anion exchange particles in Dionex CarboPac columns.

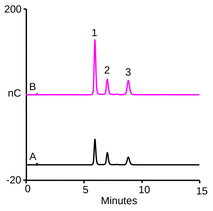


Sulfonated highly-cross-linked core structure (supermacroporous or nonporous beads)

Latex Microbeads with Anion Exchange Functionalities

Determinations of monosaccharide and disaccharide sugars were conducted in various beverages, vinegars, and flavored distilled alcohol samples. In Figures 5 and 6, sugars were determined in the same functional beverages using the Carbohydrate gold disposable working electrode. The three sugars in the 10,000-fold diluted samples were separated on the Thermo Scientific™ Dionex™ CarboPac™ PA20 column, and detected using the standard 0.003-in thick gasket (Figure 5). In contrast, and for confirmation, the 100-fold diluted samples were analyzed on the Thermo Scientific™ Dionex™ CarboPac™ SA10-4µm column using the 0.062-in thick gasket from the High Concentration Carbohydrate Analysis kit. This column was optimized for fast separations with a different selectivity as evident by the different elution order. The difference in concentration is attributed to dilution error.

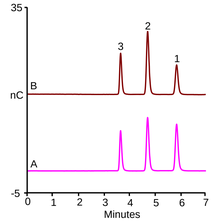
Figure 5. Sugars in 10,000× diluted functional beverage samples



Column:	Dionex CarboPac PA20 guard, Dionex CarboPac PA20, 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
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

Peaks:		A	B	
	1. Glucose	5.1	4.4	mg/L
	2. Fructose	4.9	1.8	
	3. Sucrose	5.1	3.9	

Figure 6: Sugars in 100× diluted functional beverage samples using the Dionex CarboPac SA10-4µm column and High Concentration Carbohydrate Analysis kit.

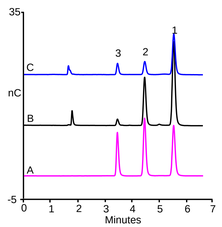


Column:	Dionex CarboPac SA10-4µm and guard, 4 mm i.d.
Eluent:	1.0 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser
Flow Rate:	1.50 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au on PTFE, High Concentration Carbohydrate Analysis 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

Peaks:		A	B	
	1. Glucose	411	483	mg/L
	2. Fructose	406	254	
	3. Sucrose	406	409	

In Figures 7 and 8, the same analysis is repeated with fruit juices and carbonated beverages on the Dionex CarboPac SA10-4µm column optimized for fast separations.

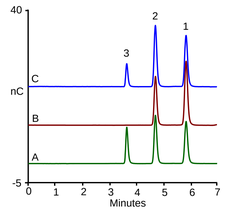
Figure 7. Sugars in 100× diluted fruit juice samples using the Dionex CarboPac SA10-4µm column and High Concentration Carbohydrate Analysis kit.



Column:	Dionex CarboPac SA10-4µm and guard, 4 mm i.d.
Eluent:	1.0 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 500 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: Orange juice C: Apple juice
Sample Prep.:	100× dilution with deionized water

Peaks:		A	B	C	
	1. Glucose	411	340	89	mg/L
	2. Fructose	406	675	321	
	3. Sucrose	406	58	99	

Figure 8. Sugars in 100× diluted carbonated beverage samples using a Dionex CarboPac SA10-4µm Column and High Concentration Carbohydrate Analysis kit.

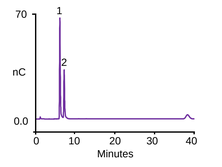


Column:	Dionex CarboPac SA10-4µm and guard, 4 mm i.d.
Eluent:	1.0 mM KOH
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser
Flow Rate:	1.50 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au on PTFE, High Concentration Carbohydrate Analysis 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

Peaks:		A	B	C	
	1. Glucose	411	414	521	mg/L
	2. Fructose	406	620	490	
	3. Sucrose	406	--	253	

In Figures 9–11, sugars were determined in fermented and flavored distilled alcohol samples. In these examples, the Au on PTFE disposable working electrode was combined with the High Concentration Carbohydrate Analysis kit. These samples were diluted only 100-fold. For the alcoholic samples, the Dionex CarboPac PA20 column was selected because ethanol and sucrose nearly co-elute on the CarboPac SA10-4µm column. The results show good chromatography as shown by the narrow peak widths and strong response. The balsamic vinegar sample can be analyzed on either column.

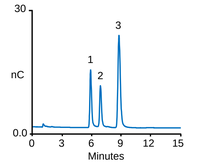
Figure 9. Sugars in a balsamic vinegar sample.



Column:	Dionex CarboPac PA20 guard, Dionex CarboPac PA20, 3 mm i.d.
Eluent:	35 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, Au on PTFE, High Concentration Carbohydrate Analysis kit
Detect. Comp.:	30 °C
Waveform:	Four Potential Carbohydrate Vs Ag/AgCl
Sample:	Balsamic vinegar
Sample Prep.:	100× dilution with deionized water

Peaks:			
	1. Glucose	1.2	g/L
	2. Fructose	0.73	

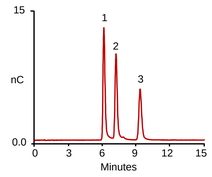
Figure 10. Sugars in a flavored rum sample.



Column:	Dionex CarboPac PA20 guard, Dionex CarboPac PA20, 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	0.30	g/L
	2. Fructose	0.28	
	3. Sucrose	1.08	

Figure 11. Sugars in a sparkling wine sample.



Column:	Dionex CarboPac PA20 guard, Dionex CarboPac PA20, 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:	Sparkling wine, Brand C
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 word: sugars, Integrion)

TRADEMARKS/LICENSING

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