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Determination of High Sugar Concentrations in Distilled Spirits Samples Using a Compact Ion Chromatography System

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ABSTRACT

This poster will demonstrate the determinations of glucose, fructose, and sucrose in flavoured rum, scotch liqueur and Irish whisky by high performance anion exchange with pulsed amperometric detection (HPAE-PAD). Typically, samples with g/L concentrations require large dilutions, 1000 -10,000 fold, to remain in the linear range of the very sensitive HPAE-PAD technique. However, in these applications, the method is performed using a Thermo Scientific™ Dionex™ Integrion™ RFIC™ system equipped with a 0.4 µL internal injection loop and the Thermo Scientific™ High Concentration Carbohydrate Analysis Kit to extend the linearity from low mg/L to g/L concentrations, allowing smaller sample dilutions to be made. Having a reduced number of dilutions not only saves sample preparation time but also minimises the chance of dilution errors. This gives an improved reporting accuracy and confidence in the results.

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 characterise and confirm the source of the carbohydrates. Carbohydrates have poor chromophores and are therefore problematic to detect by UV absorption without lengthy and costly derivitisation. However, carbohydrates can be determined directly by HPAE-PAD, a well-established method that eliminates the need for derivitisation, saving time and money, including reagent costs.

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.[1] 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 Dionex Integrion HPIC IC system. This system allows fast determination of sugars without manual eluent preparation or sample derivatisation.

MATERIALS AND METHODS

Sample Preparation

The distilled spirit samples were diluted with deionised water prior to analysis. Any beverage samples exhibiting any opacity were first diluted, then filtered (0.2 µm), and treated with Dionex OnGuard II RP sample preparation cartridge.

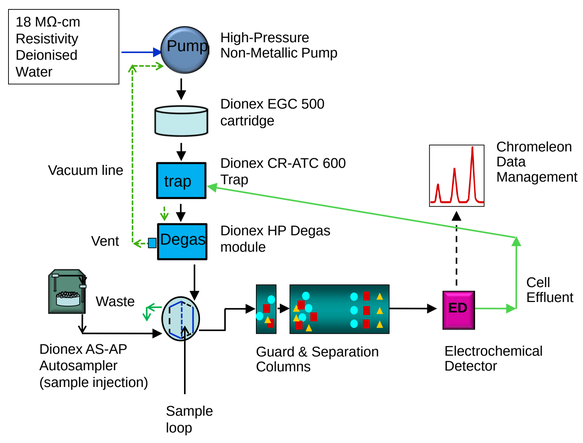
Ion Chromatography

Instrument:	Thermo Scientific™ Dionex™ Integrion™ RFIC™ System with column heater and IC PEEK Viper fittings
Columns:	Thermo Scientific™ Dionex™ CarboPac™ PA20 guard, 3x30 mm and separation 3x150 mm
Eluent:	35 mmol/L KOH with 100 mmol/L KOH wash
Eluent Source:	Thermo Scientific™ Dionex™ EGC 500 KOH cartridge, CR-ATC 500 trap column
Flow Rate:	0.50 mL/min
Injection Vol.:	0.4 µL
Temperature:	30 °C
Detection:	Pulsed Amperometric, Waveform A (Figure 4)
Working/Ref. Electrodes:	Disposable Au on PTFE/Ag/AgCl in pH mode
Gasket:	High Concentration Carbohydrate kit (0.062” thick)

Data Analysis

Thermo Scientific™ Chromeleon™ Chromatography Data Systems (CDS), Chr 7.2.4. Includes a more automated instrument configuration, consumables inventory, knowledgebase for troubleshooting, consumables installation guides, retention time standard processing method predicts retention time shifts, tablet manual control, personal phone application.

Figure 1: Flow Diagram for the Dionex Integrion HPIC Reagent-Free System Configured for ED Detection.



Discussion of Method

High Performance Anion-Exchange Chromatography (HPAE)

Carbohydrates (including sugars) are weak acids as shown in Table 1. At high pH, they are ionised (Figure 2), and thus can be separated by anion-exchange chromatography.

Table 1: Sugar pK_a Values

Sugar	pK_a
Fructose	12.03
Mannose	12.08
Xylose	12.15
Glucose	12.28
Galactose	12.39
Dulcitol	13.43
Sorbitol	13.43
α-Methyl glucoside	13.71

Figure 2: Ionisation of Sugars at High pH

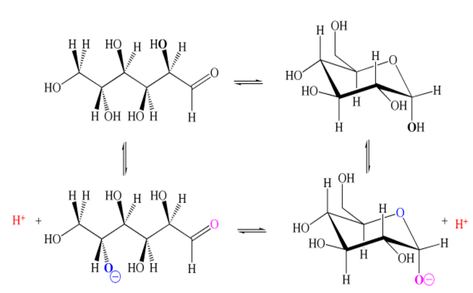


Figure 3 illustrates an anion exchange bead in a Thermo Scientific™ Dionex™ CarboPac™ column. The core polymer is polystyrene divinylbenzene, which has been surface-sulfonated, followed by latex agglomeration. This pellicular resin structure permits excellent mass transfer, resulting in high-resolution IC chromatography.

Figure 3: Dionex CarboPac PA20 Microporous bead with pellicular resin structure

Pulsed Amperometric Detection (PAD)

In PAD using the four-potential waveform specific for carbohydrates, the disposable working electrode is pulsed through the different potentials at set times, completing two cycles within one second (Figure 4). This waveform is optimised to provide a clean stable gold-oxide layer in preparation for detection of the next eluting carbohydrate peak.

Figure 4. Four-Potential Carbohydrate Waveform[2]

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	

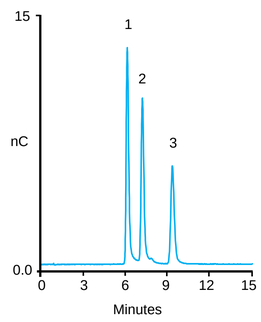
1. Detection potential (E1) and Integration period
2. Reductive cleaning potential (E2)
3. Oxidative cleaning potential (E3
4. Pre-detection (oxide reduction) potential (E4)

RESULTS

With electrochemical detection, IC PEEK Viper fittings and Reagent-Free™ IC (RFIC™), the newly introduced compact IC system (Dionex Integrion HPIC system) offers a fast and cost-effective HPAE-PAD method for sugar analysis. The determination of sugars in drinks using the Dionex Integrion HPIC system are shown in Figures 5–10.

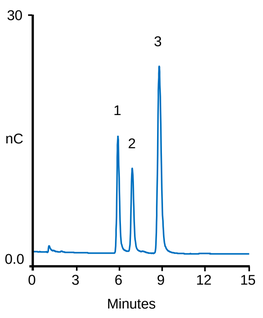
Figures 5-7 demonstrate sugar determinations in sparkling wine and flavoured rum samples by HPAE-PAD with the High Carbohydrate Concentration kit. These high sugar concentration samples typically require up to 10,000x dilution for the very sensitive HPAE-PAD analysis method. Here the samples were diluted 100-fold, thereby reducing the errors that may occur from multiple or serial dilutions.

Figure 5: Sugars in a Sparkling Wine Sample



Column:	Dionex CarboPac PA20 guard and separation column, 3 mm i.d.
Eluent:	35 mmol/L KOH, 100 mmol/L KOH wash (15-25 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 deionised water
Peaks:	1. Glucose 232 mg/L 2. Fructose 230 3. Sucrose 230

Figure 6: Sugar profile of a flavoured rum sample



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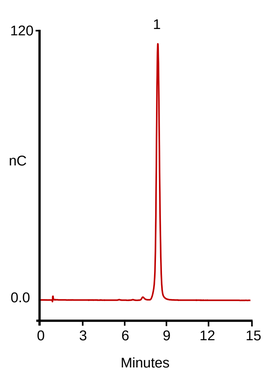
Eluent:	35 mmol/L KOH, 100 mmol/L KOH wash (15-25 min)
Eluent Source:	Dionex EGC 500 KOH cartridge, Dionex CR-ATC 600 trap column, high pressure degasser
Flow Rate:	0.50 mL/min
Inj. Volume:	0.4 µL
Column Temp.:	30 °C
Detection:	PAD, Au on PTFE, High Concentration Carbohydrate Analysis kit
Detect. Comp.:	30 °C
Waveform:	Four Potential Carbohydrate Vs Ag/AgCl
Sample:	Flavoured rum, Brand P
Sample Prep.:	100× dilution with deionised water
Peaks:	1. Glucose 0.30 g/L 2. Fructose 0.28 3. Sucrose 1.08

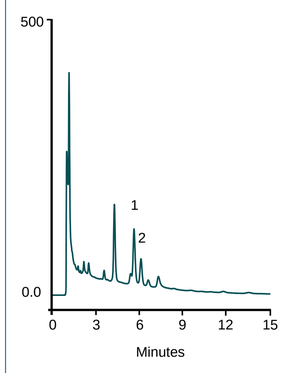
Figure 7: Sugar profile of a Scotch whisky sample



Column:	Dionex CarboPac PA20 guard and separation column, 3 mm i.d.
Eluent:	35 mmol/L KOH, 100 mmol/L KOH wash (15-25 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:	Scotch liqueur, Brand C
Sample Prep.:	5× dilution with deionised water
Peaks:	1. Sucrose 36 mg/L

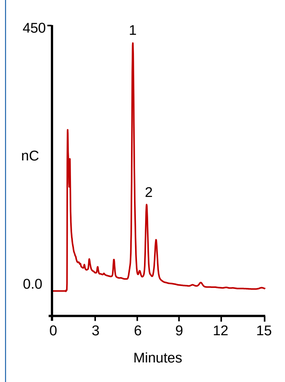
This application is also suitable for low mg/L concentrations of sugar. Figures 8 and 9 demonstrate sugar determinations in unflavored distilled spirit samples.

Figure 8: Sugar profile of an American bourbon sample



Column:	Dionex CarboPac PA20 guard and separation column, 3 mm i.d.
Eluent:	35 mmol/L KOH, 100 mmol/L KOH wash (15-25 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:	American Bourbon, Brand J
Sample Prep.:	5× dilution with deionised water
Peaks:	1. Glucose 54 mg/L 2. Fructose 27

Figure 9: Sugar profile of an Irish whisky sample



Column:	Dionex CarboPac PA20 guard and separation column, 3 mm i.d.
Eluent:	35 mmol/L KOH, 100 mmol/L KOH wash (15-25 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:	Irish whisky, Brand J
Sample Prep.:	5× dilution with deionised water
Peaks:	1. Glucose 43 mg/L 2. Fructose 28

CONCLUSIONS

- HPAE-PAD is a sensitive method for direct determination of sugars in diverse samples
- This technique was demonstrated on the determination of sugar concentrations in distilled spirits, flavored liqueur and rum samples
- The high concentration carbohydrate kit extends the analytical range to g/L, thereby minimising errors caused by dilution

REFERENCES

1. Thermo Scientific Technical Note 20: Analysis of Carbohydrate by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD), Sunnyvale, CA.
2. Thermo Scientific Technical Note 21: Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector, Sunnyvale, CA.
3. Thermo Fisher Scientific AppsLab Library application database. https://appslab.thermofisher.com/Brief

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