Direct Carbohydrate Analysis in Beverages and Foods Using Pulsed Amperometric Detection or Charged Aerosol Detection

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Overview

Purpose: To develop simple direct methods for carbohydrate analysis in food and beverage without requirement for sample derivatization. Two approaches are discussed for direct analysis of carbohydrates in various food matrices, high performance liquid chromatography with pulsed amperometric detection (HPLC-PAD) and high performance liquid chromatography with charged aerosol detection (HPLC-CAD).

Methods: Three HPLC-PAD methods were developed using ion exchange chromatography, including one isocratic method for simple sugar analysis in fruit juice, soda and syrups, one method for lactose and lactulose analysis in milk, and one gradient method for carbohydrate profiling of beer. Two HPLC-CAD methods using HILIC chromatography are also discussed for carbohydrate analysis in fruit juice and a sports beverage.

Results: These methods enable rapid separation and reliable determination of carbohydrates in various foods and beverages with simple sample preparation and minimal matrix interference.

Introduction

Carbohydrates are important food components affecting taste and nutrition. The determination of the types and levels of carbohydrates in foods is important for energy evaluation, nutritional labeling, and quality control and for identifying possible product adulteration.

Separation and detection of carbohydrates can be challenging. Simple carbohydrates are highly polar, uncharged, and as they lack a chromophore cannot be measured directly by UV absorbance detectors. HPLC with various detection techniques has been used for carbohydrate analysis, such as IR, mass spectrometry, UV or fluorescence following derivatization. Although methods utilize derivatization improve the chromatographic resolution and detector sensitivity, they can lead to increased assay variability. This poster presents two approaches for direct carbohydrate analysis, HPLC-PAD and HPLC-CAD, which solve the challenges for separation and detection without requirement for derivatization.

Thermo Scientific™ Dionex™ Ultimate™ U3000 electrochemical detector now with PAD capabilities when coupled with a gold working electrode provides high sensitivity and selectivity for the measurement of carbohydrates in complex food sample matrices. Thermo Scientific™ Dionex™ Corona Charged Aerosol Detector is a mass-sensitive detector that can measure all non-volatile, and many semi-volatile compounds in a sample, typically with low nanogram sensitivity. Application examples for various juice samples, milk, beer, syrup and sport beverage will be discussed.

Methods

General considerations for Pulsed Amperometric Detection
Thermo Scientific™ Dionex™ Ultimate™ U3000 with PAD platform consisted of a base-compatible HPLC system, a Thermo Scientific™ Dionex™ ECD-3000RS electrochemical detector and a gold working electrode. Carbohydrates were separated using ion exchange and determined under basic conditions using a four-pulse waveform. It is essential to make sure no titanium is in the flow path as its degradation under basic conditions can lead to deterioration of column and electrode performance.

To prevent carbonate formation, all mobile phase should be kept under helium spurge or blanketed under helium gas.

Carbohydrate Analysis by HPLC-PAD System:

Pump:	Thermo Scientific™ Dionex™ ISO-3100 SD or LPG-3400SD			
Autosampler:	Thermo Scientific™ Dionex™ WPS-3000TSL Analytical Autosampler			
Column:	Anion exchange, 4.1 x 250mm, 7µm			
Temperature:	30 °C			
EC detector:	Thermo Scientific™ Dionex™ EC-3000RS with 6041RS sensor and gold target			
EC Parameters:	E1	+200 mV	500 ms	AD 300 ms
	E2	-2000 mV	10 ms	
	E3	+600 mV	10 ms	
	E4	-100 mV	10 ms	

HPLC-PAD method for simple sugar analysis in fruit juice, cola soda and syrup

Mobile phase:	80 mM sodium hydroxide
Flow rate:	1.5 mL/min
Injection volume:	25 µL
Sample preparation:	Cola soda: 1:1000 dilution in DI water Fruit juice: 1:1000 dilution in DI water, for juice with pulp, centrifuge and filter with 0.2 µm filter before dilution Syrup: 100 µg/mL dillution in DI water

HPLC-PAD method for lactose and lactulose analysis in milk

Mobile phase A:	20 mM NaOH
Mobile phase B:	200 mM NaOH
Flow rate:	1.5 mL/min
Gradient:	20 mM NaOH for 20 min, clean the column with 200 mM NaOH at end of run for 5 min, then equilibrate with 20 mM NaOH for 10 min
Injection volume:	25 µL
Sample preparation:	Protein precipitation with 1% perchloric acid at1:10 dilution, centrifuge, then dilute in DI water

HPLC-PAD method for carbohydrate analysis in beer

Mobile phase A:	100 mM sodium hydroxide
Mobile phase B:	1 M NaOAc in 100 mM NaOH
Flow rate:	1.5 mL/min
Gradient:	100 mM NaOH for 2 mins, then 100% A to 30% B in 58min, gradient curve 6
Injection volume:	20 µL
Sample preparation:	1:10 dilution in DI water

HPLC-CAD method for carbohydrate analysis in fruit juice

Column:	Thermo Scientific™ Accucore™ 150-Amide-HILIC, 2.1 x 150 mm, 2.6 µm
Mobile phase:	6% 250 mM ammonium formate, 87% acetonitrile, 7% DI water
Flow rate:	0.8mL/min
Temperature:	75°C
Inj. volume:	3 µL
Detector:	Thermo Scientific™ Dionex™ Corona™ Veo™ SD (evaporator temperature high)
Sample Preparation:	Centrifuge with 0.2 µm centrifuge filter at 13,000 rpm, 1:100 dilution in DI water, then 1:10 dilution in acetonitrile

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HPLC-CAD method for sport beverage

Column:	Thermo Scientific™ Acclaim™ Trinity™ P2, 3 x 50mm, 3 µm
Mobile phase:	20% 100mM ammonium formate at pH 3.65, 80% acetonitrile
Flow rate:	0.6 mL/min
Temperature:	50°C
Inj. volume:	5 µL
Detector:	Thermo Scientific™ Dionex™ Corona™ Veo™ RS, evaporator 55 °C
Sample Preparation:	Decolorize with Thermo Scientific™ Dionex™ OnGuard™ II P cartridge, dilute 1:40 in mobile phase

Results and Discussion

Direct Carbohydrate Analysis with HPLC-PAD

Carbohydrates are weak acids, and they are ionized when high pH mobile phase conditions are used. This allows for their separation by anion exchange chromatography, followed by sensitive and direct detection using pulsed amperometric detection. PAD is much more sensitive than UV and refractive index and its sensitivity is comparable to fluorescence detection. It is a very selective and specific detection mode since interferences from sample matrix become invisible to the detector, making sample preparation for many applications as simple as diluting the matrix with deionized water. The reproducibility of detection is enhanced by using a four pulse waveform that keeps the electrode surface clean even when complex samples are injected on the HPLC column.

A rapid method for separation of seven simple carbohydrates commonly found in foods and beverages is shown in Figure 1. Simple sugars were completely separated with good resolution within 12 minutes. Figure 2 shows application of this method to fruit juice and soda samples. Several different types of syrup samples are shown in Figure 3 demonstrating the versatility of this HPLC-PAD method. All samples were simply diluted in DI water prior to injection.

FIGURE 1. Separation of seven common simple food carbohydrates with HPLC-PAD

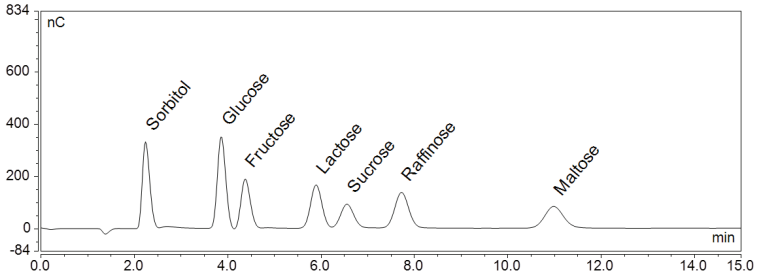


FIGURE 2. Carbohydrate analysis in fruit juice and cola soda with HPLC-PAD

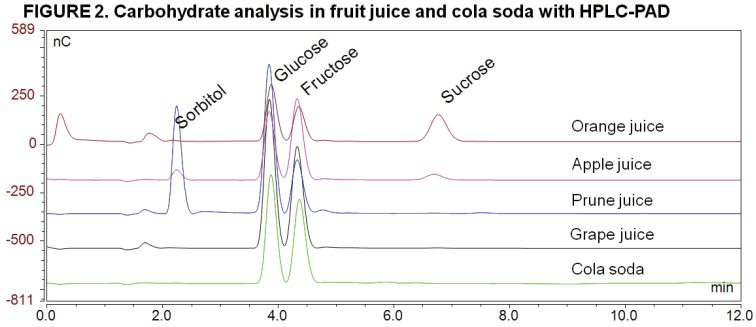
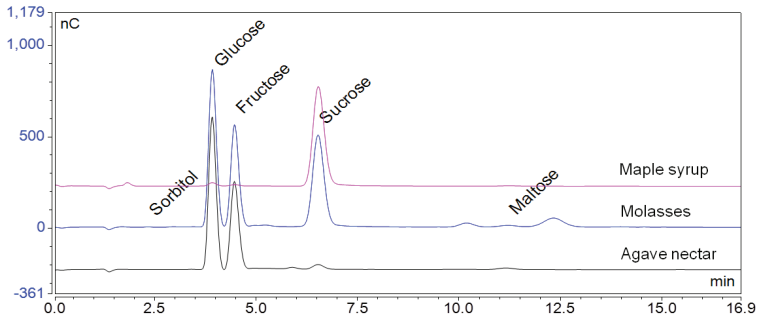


FIGURE 3. Carbohydrate analysis in syrups with HPLC-PAD



The global prevalence of lactose intolerance has created a large market for lactose-free products and the need for a simple, reliable and accurate method for determination of lactose in milk and other dairy products. The level of lactulose, which is formed by isomerization of lactose during the heat treatment of milk, can be used as an indicator to differentiate milk of different pasteurization or sterilization processes.

The AOAC enzymatic method for lactose determination is time consuming and needs extensive reagent preparation. Figure 4 shows a simple HPLC-PAD method for determination of lactose and lactulose in milk within 35 minutes cycle time and requires minimal sample preparation.

FIGURE 4. Lactose and lactulose analysis in milk with HPLC-PAD

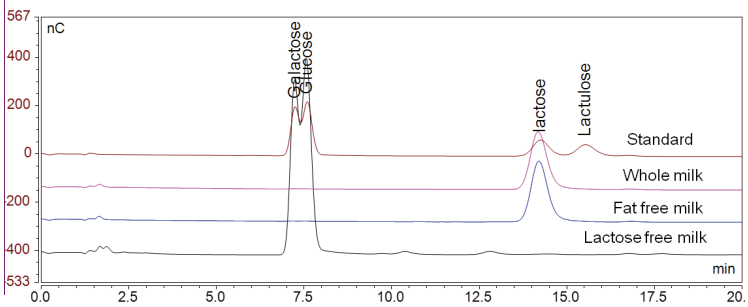
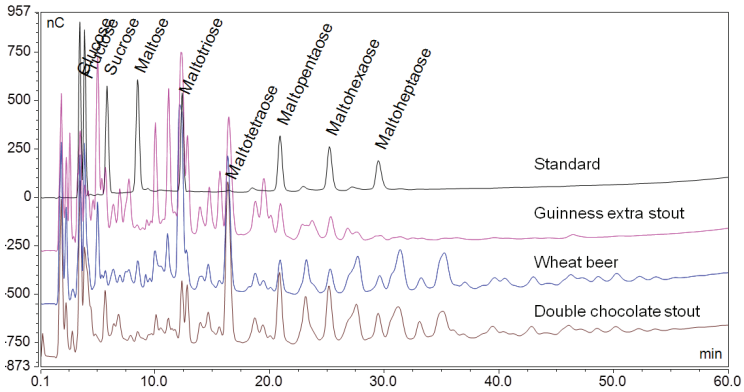


Figure 5 shows a gradient HPLC-PAD method for profiling carbohydrates in beer. This method uses sodium acetate in addition to sodium hydroxide to increase the mobile phase strength to reduce the retention of oligosaccharides. This method allows for separation of malto-oligosaccharides up to DP13-15, as well as separation of some DP≤3 simple sugars such as glucose, fructose, sucrose and maltose, which enables carbohydrate profiling of different types of beer. Three different beers were analyzed, including a double chocolate stout, a Guinness extra stout, and a wheat beer and their carbohydrate profiles are compared in Figure 5.

FIGURE 5. Carbohydrate profiling for beer with HPLC-PAD method.

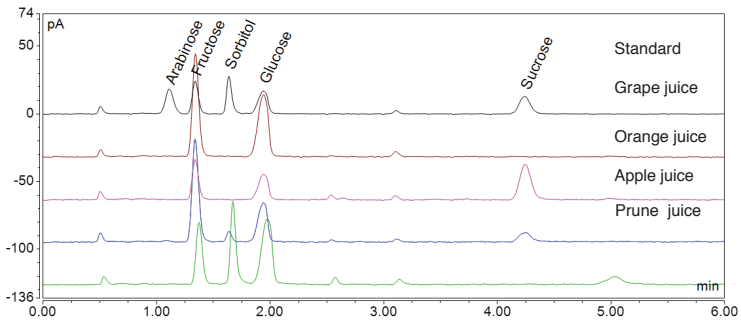


Direct Carbohydrate Analysis with HPLC-CAD

HPLC with charged aerosol detection is another direct method for carbohydrate analysis. HILIC chromatography is utilized for separation of carbohydrates prior to CAD detection. Charged aerosol detection is a universal detection technique. It allows detection of any non-volatile and many semi-volatile samples independent of analyte chemical structure.

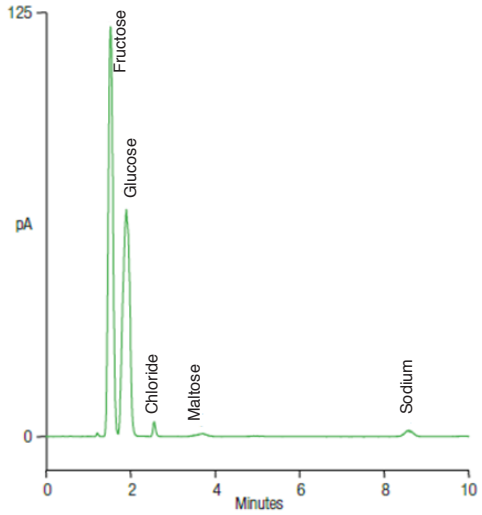
Figure 6 shows separation of carbohydrates in various fruit juices on a Thermo Scientific™ Accucore™ Amide 150 column. This column provides excellent resolution and speed of separation of the various carbohydrate species when using decreased amounts of water along with elevated column temperatures. This provides the complete analysis of carbohydrates found in various beverages with a run time of only 6 minutes. Other food products such as honey and different corn syrups (data not shown) have also been successfully analyzed using HILIC chromatography methods with CAD detection. These techniques represent good examples of detecting analytes that possess weak or no chromophores using very simple analytical conditions and sample preparation.

FIGURE 6. Analysis of carbohydrates in various fruit juice sampleswith HPLC-CAD using a Amide-HILIC column



The method for simultaneous analysis of carbohydrates and electrolytes in a sports beverage shown in Figure 7 represents a good example of the universal character of Corona Veo. An Thermo Scientific™ Dionex™ Acclaim™ Trinity™ P2 column was used in HILIC mode for separation in this method. Acclaim Trinity P2 columns offer cation-exchange, anion-exchange and HILIC retention mechanisms on the same phase. The ability of Trinity column to separate organic compounds, cations, and anions simultaneously, together with the universal detection of Corona Veo, allows for separation and detection of carbohydrates and the electrolyte chloride and sodium ions in a single run.

FIGURE 7. Carbohydrate and electrolyte analysis in sport beverage with HPLC-CAD



Conclusions

Both HPLC-PAD and HPLC-CAD provide simple, direct methods for carbohydrate analysis without the need for sample derivatization. Three HPLC-PAD methods using ion-exchange chromatography for carbohydrate analysis in fruit juice, soda, syrups and milk, as well as an gradient HPLC-PAD method for beer carbohydrate profiling were discussed. Two HPLC-CAD methods using HILIC chromatography are also discussed for carbohydrate analysis in fruit juice and a sports beverage.

Pulsed amperometric detection is highly sensitive and selective. Sample preparation can be as simple as dilution in water.

Electrochemical detection in DC mode can be used for other food components such as antioxidants, vitamins and amino acids.

Charged aerosol detector is a universal mass sensitive detector, and can be used for measurement of any nonvolatile and many semi-volatile analytes. Charged aerosol detection is applicable to many food. This includes antioxidants, amino acids, lipids, proteins, peptides, surfactants, artificial sweeteners, dyes, preservatives and emulsifiers.