Table of Contents

High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)

Carbohydrates Analysis Application Notebook

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Introduction

- Carbohydrates Analysis by HPAE-PAD
- High-Performance Anion-Exchange Chromatography
- Pulsed Ampermometric Detection

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Carbohydrate Analysis by HPAE-PAD

- One system determines mono-, di-, oligosaccharides, and small charged carbohydrates including sialic acids.
- Highly sensitive and selective detection
- Carbohydrate analysis without derivitization

Carbohydrates play a vital role in a variety of biological functions, including cellular communication, gene expression, immunology, organism defense mechanisms, and growth and development. They are difficult to analyze as they are very polar compounds, exhibit similar structural characteristics, and do not have a suitable chromophore. Methods for the liquid chromatographic analysis of carbohydrates have utilized silica based, amino-bonded, or polymer-based, metal-loaded, cation-exchange columns, with refractive index (RI) or low-wavelength ultraviolet (UV) detection. The low sensitivity and selectivity of RI and low-wavelength UV detection methods limit their applications in trace carbohydrate analysis. Another popular HPLC technique exists called Hydrophilic Interaction Liquid Chromatography (HILIC) which requires derivatization. HILIC also requires mobile phases of high organic content (50–80% acetonitrile) which could cause sample solubility problems at high concentrations.

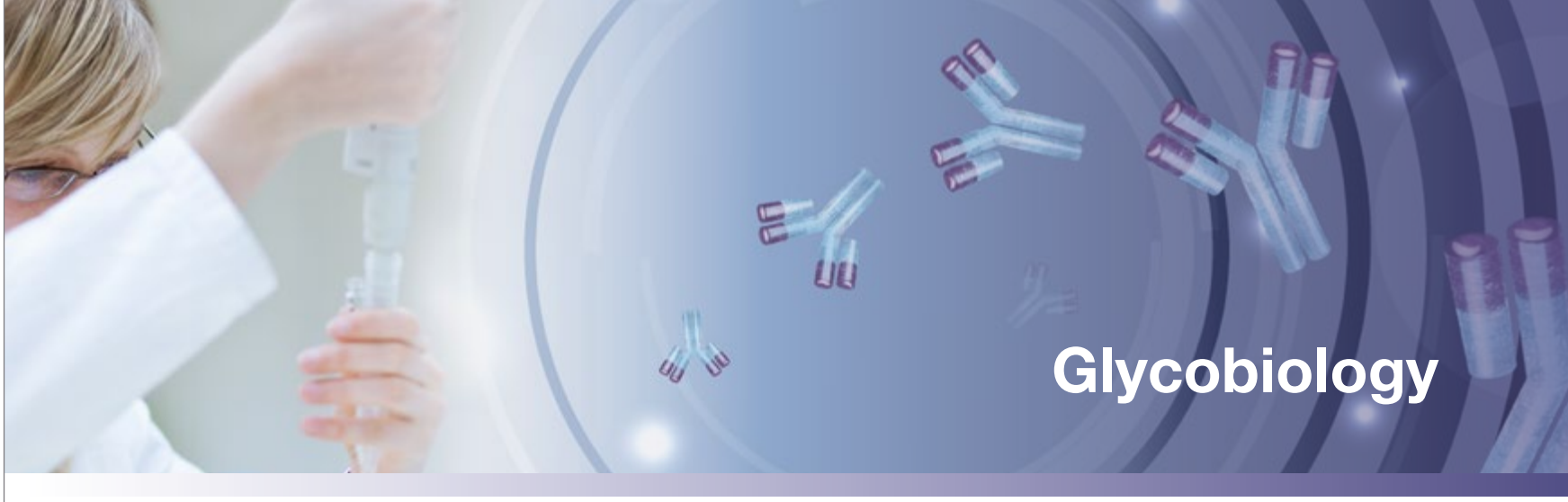
An improved chromatographic technique known as HPAE takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. Coupled with PAD, it permits direct quantification of non-derivatized carbohydrates.

High-Performance Anion-Exchange Chromatography

HPAE chromatography can be used to separate analytes that can be ionized under high pH conditions. Carbohydrates typically have pKas in the range of 12–13. Once the pH rises above the pKa of the analyte, it becomes ionized in solution. This is accomplished using hydroxide-based eluents. With the development of highly cross-linked, ethylvinyl benzene-divinyl benzene pellicular resins that have broad pH stability (0 to 14), separations at high pH conditions are feasible. The columns' nonporous resins have small anion-exchange microbeads carrying the anion-exchange functional groups. These small anion-exchange microbeads are permanently attached electrostatically to a larger cation-exchange resin particle. The nonporous nature of the resin minimizes band-broadening and imparts highly effective separation of a wide variety of carbohydrates, including branched oligosaccharides.

Pulsed Ampermometric Detection

The detection of underivatized analytes can be achieved using pulsed amperometric detection. A series of potentials known as a waveform result in oxidizing and reducing conditions on the electrode surface. This waveform causes the oxidation of analytes at the working electrode surface which automatically cleans and prepares the electrode for detection. Pulsed amperometry detects only those compounds that contain functional groups which become oxidized at the detection voltage employed. Detection is sensitive and highly selective for electroactive species, because many potentially interfering species cannot be oxidized or reduced, and are not detected. It is also important to note that neutral or cationic sample components in the matrix elute in, or close to, the void volume of the column. As a result, the carbohydrate components of interest are not impacted even if the neutral or cationic sample components are oxidized.



Glycobiology

The development of recombinant-derived glycoproteins including monoclonal antibodies for therapeutic use has led to an increased demand for methods to characterize their carbohydrate structures, especially asparagine-linked oligosaccharides that can impact the glycoprotein's function. The HPAE-PAD technique not only separates oligosaccharides according to charge, but can also resolve oligosaccharides with the same charge according to size, sugar composition, and linkage of monosaccharide units.

- Glycobiology Monsaccharide Analysis Using HPAE-PAD with Eluent Generation
- Rapid Screening of Sialic Acids in Glycoproteins by HPAE-PAD
- Evaluating Protein Glycosylation in Limited-Quantity Samples by HPAE-PAD
- HPAE-PAD Peak Area Response of Glycoprotein Oligosaccharides

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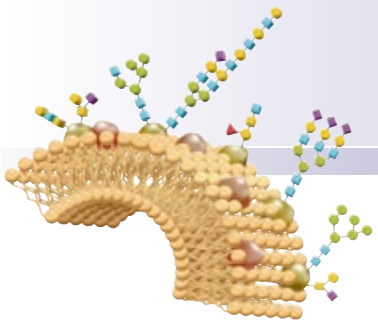
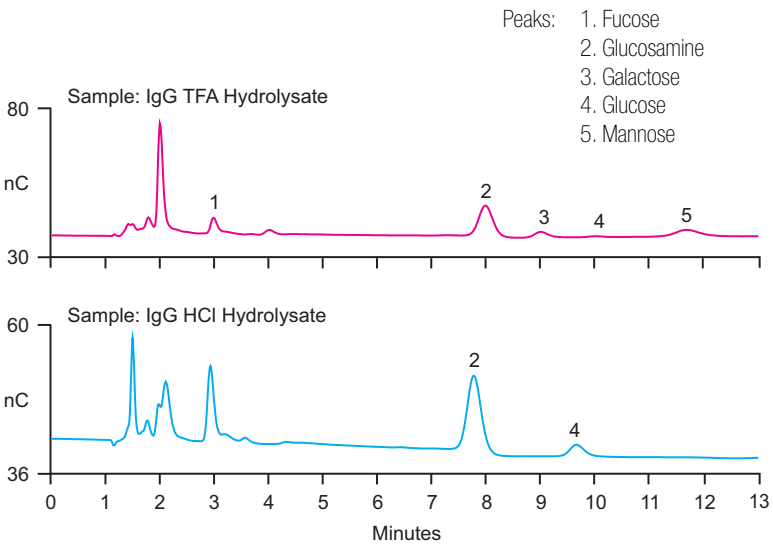
Glycoprotein Monsaccharide Analysis



Characterization of glycoproteins routinely involves carbohydrate analysis. Minor variations in glycosylation can affect the efficacy of protein therapeutics. Monosaccharide composition analysis can detect variations in glycosylation, and support quality control for process development and manufacturing procedures. There are over 30 approved glycoprotein-based biodrugs on the market and the number is increasing rapidly. Agencies such as the U.S. FDA and the European Medicines Agency have increased pressure on biopharmaceutical manufacturers to demonstrate satisfactory programs for understanding, measuring, and controlling glycosylation in glycoprotein-based drugs.

In this technical note, the HPAE-PAD-based method on a Thermo Scientific™ Dionex™ ICS-5000 chromatography system for monosaccharide composition analysis is fast and capable of providing reproducible retention time and detector response for hundreds of samples over several days. The disposable Au working electrode contributes to the reproducibility of PAD between electrodes and between laboratories. Overall, the method has high sample throughput, high precision, and performance ruggedness for glycoprotein monosaccharide analysis.

Conditions	
Method	
Columns:	Thermo Scientific™ Dionex™ CarboPac™ PA20 Analytical, 3 × 150 mm Thermo Scientific™ Dionex™ AminoTrap™, 3 × 30 mm
Eluent:	10 mM KOH
Eluent Source:	Thermo Scientific Dionex EGC III KOH Eluent Generation Cartridge Thermo Scientific Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL (partial loop injection mode with a 4 µL cut volume)
Column Temp.:	30 °C
Cell Temp.:	30 °C
Backpressure:	2200 psi
Detection:	PAD
Background:	30–50 nC
Working Electrode:	Carbohydrate PTFE Disposable Au Working Electrodes
Reference Electrode:	Mode: Ag/AgCl mode Noise: 10–30 pC



Monosaccharide composition analysis of human serum IgG.

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Download the Technical Note 40: Glycoprotein Monsaccharide Analysis Using HPAE-PAD with Eluent Generation

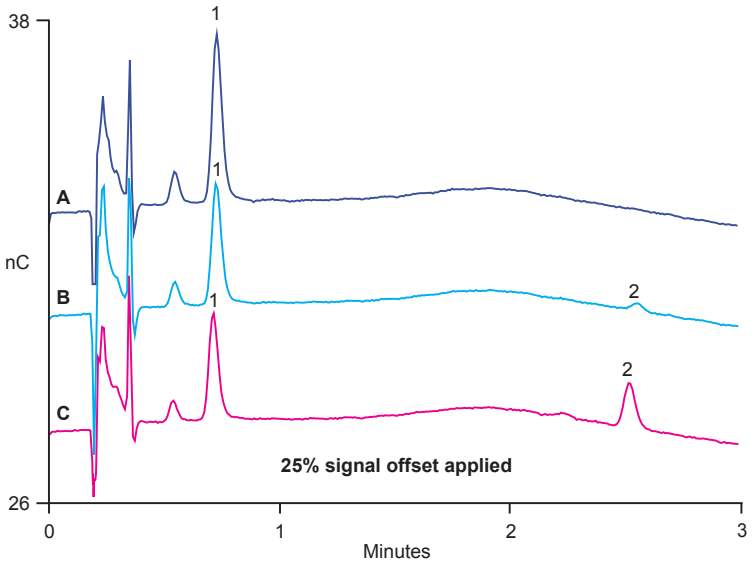
Sialic Acid in Glycoproteins

Glycoprotein sialylation has been shown to be critical to bioavailability, stability, metabolism, and immunogenicity of therapeutic proteins. As a result, such proteins are routinely analyzed to determine sialylation amount and identity. Although over 50 forms of sialic acid have been identified, two forms of this carbohydrate are routinely determined, *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc). Of these, Neu5Gc is generally not found in human proteins. Due to this lack of Neu5Gc in healthy human tissue and the natural occurrence of antibodies against Neu5Gc, this sialic acid has the potential to cause an immune response in patients when present in a glycoprotein therapeutic.

In this application update, sialic acids are determined in five representative glycoproteins by acid hydrolysis release followed by HPAE-PAD. The method is both specific and direct, eliminating the need for sample derivatization common in other chromatographic methods. Good recoveries, precision, and linear detection for Neu5Ac and Neu5Gc are demonstrated, indicating the method is appropriate for glycoprotein analysis.

Conditions	
Column:	Dionex CarboPac PA20 Fast Sialic Acid, 3 × 30 mm
Eluent Gradient:	70-300 mM acetate in 100 mM NaOH from 0–2.5 min, 300 mM acetate in 100 mM NaOH from 2.5–2.9 min, 300–70 mM acetate from 2.9–3.0 min 1.5 min of equilibration at 70 mM acetate in 100 mM NaOH
Eluents:	A: 100 mM NaOH, B: 1.0 M sodium acetate in 100 mM NaOH
Flow Rate:	0.5 mL/min
Injection Volume:	4.5 µL (full loop)
Temperature:	30 °C
Detection:	Pulsed amperometric, disposable Au on PTFE working electrode
Background:	18–25 nC (using the carbohydrate waveform)
Noise:	~15–30 pC
Backpressure:	~750 psi

Peaks:	A	B	C	
1. Neu5Ac	9.8	6.3	5.6	pmol
2. Neu5Gc	—	0.17	0.93	



Separation of fetuin, human α_1-acid glycoprotein (h. AGP), sheep α_1-acid glycoprotein (s. AGP) hydrolyzates (1:100 dilution) on the Dionex CarboPac PA20 Fast Sialic Acid column.

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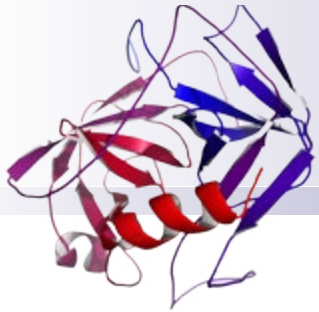
Download the Application Update 181:
Rapid Screening of Sialic Acids in Glycoproteins by HPAE-PAD



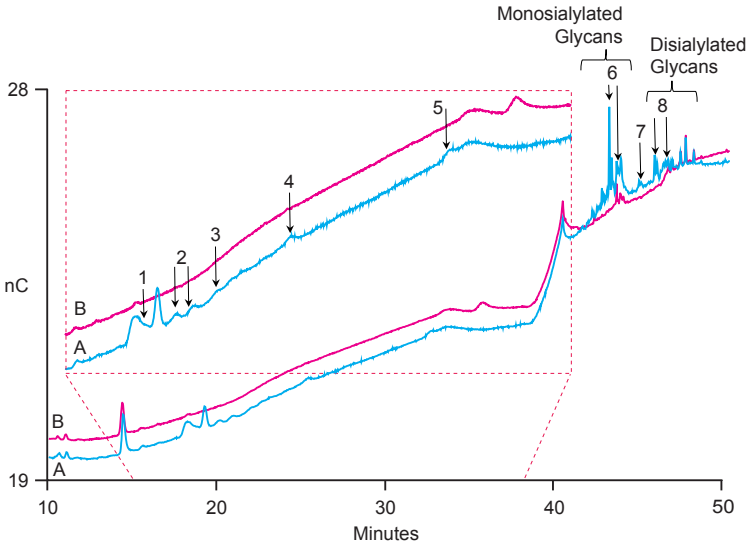
Protein Glycosylation in Limited-Quantity Samples

Changes in protein glycosylation are frequently studied in cancer research to identify potential biomarkers. Factors investigated include differences in overall oligosaccharide (glycan) content, monosaccharide content changes, differences in sialylation amount and sialylation linkages, the degree of fucosylation, and differences in glycan branching. Prostate-Specific Antigen (PSA) is an example of a well known glycoprotein cancer marker. The glycosylation of this protein is of importance as researchers seek to understand the changes that occur in this protein during carcinogenesis and tumor growth to provide a greater understanding of the disease.

In this application note, by combining HPAE-PAD analysis with acid hydrolysis and enzymatic digestion of protein glycans, information about the glycan identity as well as terminal carbohydrate linkage isomers can be determined from small amounts of protein (0.5-1.6 µg per injection). This study investigated human PSA to evaluate the potential presence of *O*-linked glycans and provided a detailed set of experiments to identify the *N*-linked glycans present on the protein.

Conditions	
Column:	Dionex CarboPac PA200 Guard, 3 × 50 mm Dionex CarboPac PA200 Analytical, 3 × 250 mm
Eluent:	A) DI water B) NaOH, 100 mM C) Sodium acetate, 200 mM, in 100 mM NaOHmM NaOH
Eluent Gradient:	50–100 mM NaOH from 0 to 30 min, 100 mM NaOH from 30 to 35 min, 0–200 mM NaOAc in 100 mM NaOH from 35 to 50 min, 200 mM NaOAc from 50 to 60 min; equilibration at 50 mM NaOH for 15 min before injection
Flow Rate:	0.5 mL/min
Injection Volume:	5 µL (partial loop)
Temperature:	30 °C
Detection:	Pulsed amperometric, disposable Au electrode, carbohydrate certified
Background:	~18 nC (using the carbohydrate 4-potential waveform)
Noise:	~50 pC
System Backpressure:	~2900 psi

Peaks:
1. G0F
2. G1F isomers
3. G1 isomers
4. G2F
5. G2bF
6. A1F isomers
7. Charged glycans
8. A2F isomers



Released PSA oligosaccharides separated on a Dionex CarboPac PA200 column. The elution region of neutral *N*-linked glycans is expanded in the inset.

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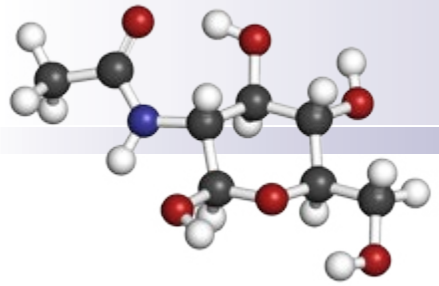
Download the Application Note 1050: Evaluating Protein Glycosylation in Limited-Quantity Samples by HPAE-PAD

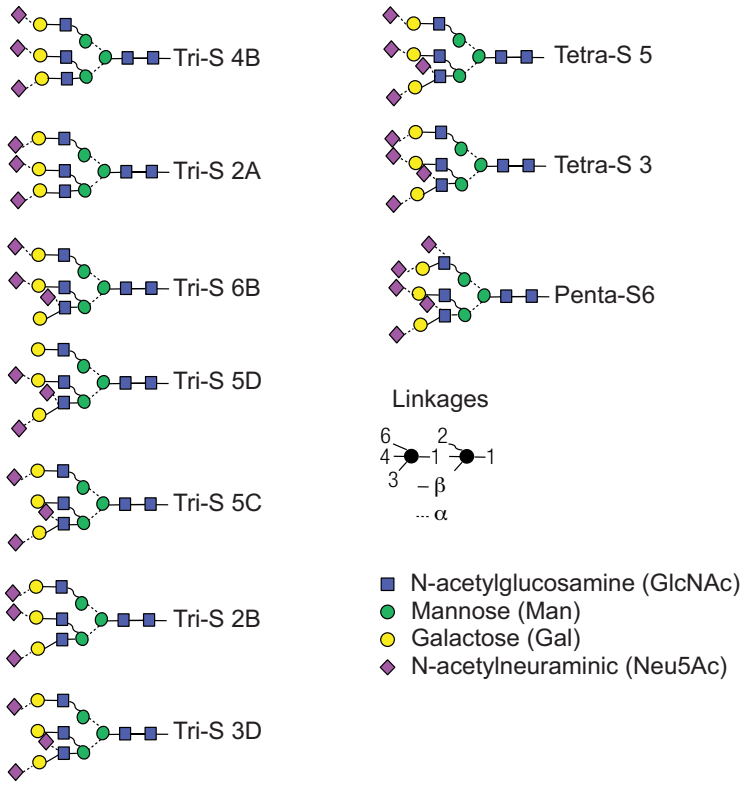


Glycoprotein Oligosaccharides

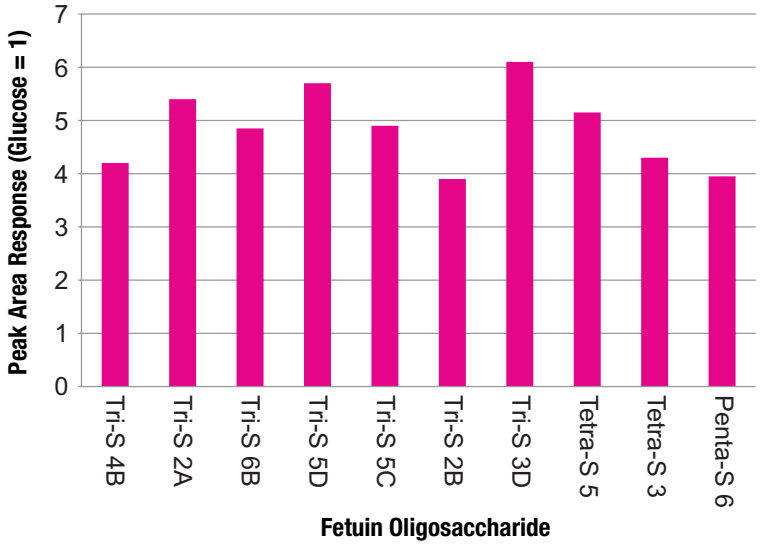
Table of Contents

HPAE-PAD was first described for separations of asparagine-linked (*N*-linked) oligosaccharides derived from glycoproteins in 1988. Shortly thereafter HPAE-PAD was cited as a key technique for characterizing recombinant therapeutic glycosylation because it can be used for monosaccharide, sialic acid, and oligosaccharide analyses (*N*-linked and Ser/Thr-linked [*O*-linked] oligosaccharides). This oligosaccharide analysis is now commonly referred to as oligosaccharide profiling.

This technical note reviews data from peer-reviewed scientific literature evaluating electrochemical response of glycoprotein-derived and related oligosaccharides to show that the changes of electrochemical response with structure are not extreme. HPAE-PAD is then compared to other common methods of oligosaccharide profiling. From one of the studies reviewed, HPAE-PAD response of *N*-linked oligosaccharides from glycoproteins used bovine fetuin.



The 10 sialylated N-linked oligosaccharides from bovine fetuin evaluated in Study 2.



Bar graph of HPAE-PAD responses of the 10 sialylated *N*-linked oligosaccharides

Download the Technical Note 133: HPAE-PAD Peak Area Response of Glycoprotein Oligosaccharides

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Food and Beverage

In the food industry, there is a significant and increasing demand for reproducible, fast, and simple methods to monitor quality, conform to labeling requirements, and check for adulteration.

- Fast Determinations of Lactose and Lactulose in Milk Products Using HPAE-PAD
- Carbohydrate in Coffee: AOAC Method 995.13 vs a New Fast Ion Chromatography Method
- Determination of Myo-Inositol (Free and Bound as Phosphatidylinositol) in Infant Formula and Adult Nutritionals
- Determinations of Monosaccharides and Disaccharides in Beverages by Capillary HPAE-PAD

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Lactose and Lactulose in Milk Products

Lactose and lactulose are important components in milk-based products. Lactose is the major milk disaccharide which is metabolized with the aid of lactase to the monosaccharides, glucose, and galactose.

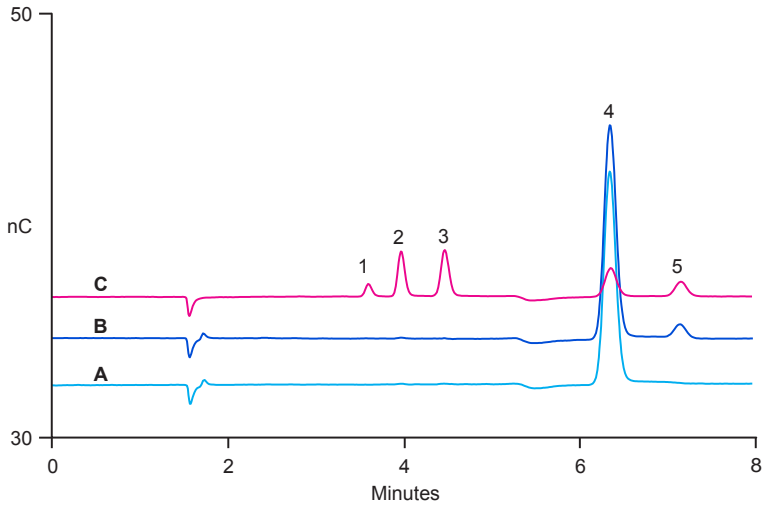
Lactose has been analyzed by many methods including photometric, polarimetry, and fluorometry, but these methods are time consuming and not specific for lactose and lactulose. The standard method by the Association of Official Analytical Chemists (AOAC) is Method 984.15 which uses enzymatic hydrolysis of lactose at pH 6.6 by β-galactosidase.

Conditions	
Column:	Dionex CarboPac SA10 guard and Dionex CarboPac SA10-4µm separation columns, 4 × 250 mm
Eluent:	4 mM KOH from -3 to 8 min*
Eluent Source:	Dionex EGC 500 KOH Cartridge
Flow Rate:	1.45 mL/min
Injection Volume:	10 µL
Column Temp.:	35 °C
Detection:	PAD, Four-Potential Carbohydrate waveform
Working Electrode:	Gold on PTFE Disposable Electrode
Reference Electrode:	pH-Ag/AgCl
Detection Temp.:	20 °C
Autosampler Temp.:	10 °C
Background:	20–40 nC
Noise:	< 20 pC
System Backpressure:	4800 psi
Sample:	A: Raw unpasteurized milk, B: U.S. Grade A Pasteurized 2% fat milk C: Lactose-free yogurt

This method is time consuming, requires extensive reagent preparations, and is not sufficiently sensitive for the determination of lactose in lactose-free samples. HPAE-PAD is a well established sensitive method that selectively and directly determines carbohydrates, such as lactose and lactulose.

Sample: A: 100-fold diluted raw, unpasteurized milk
B: Sample A + 0.5 mg/L lactulose
C: 0.5 mg/L carbohydrate standard

Peaks:	A	B	
1. Sucrose	—	—	mg/L
2. Galactose	—	—	
3. Glucose	—	—	
4. Lactose	3.75	3.77	
5. Lactulose	—	0.48	



Lactose and lactulose in raw unpasteurized milk.

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Download the Technical Note 146: Fast Determinations of Lactose and Lactulose in Milk Products Using HPAE-PAD

Carbohydrates in Coffee

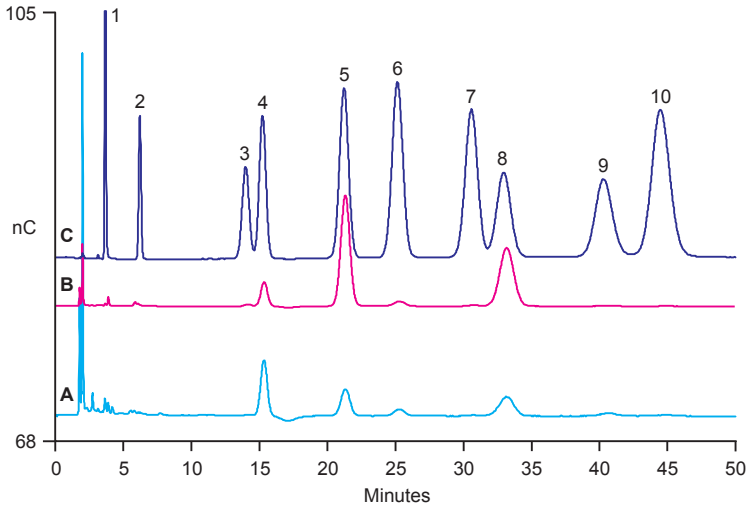
Table of Contents

Coffee carbohydrates constitute the major part (at least 50% of the dry weight) of raw coffee beans. The carbohydrates in coffee contribute to the flavor of the beverage as they undergo complex changes (react with amino acids, i.e., the Maillard reaction) during the roasting process. They act as aroma binders, foam stabilizers, and also impart viscosity of the coffee beverage. Carbohydrates are also good tracers for assessing the authenticity of soluble (instant) coffee.

Conditions	
Modified AOAC Official Method 995.13	
Columns:	Dionex CarboPac PA1 Analytical, 4 × 250 mm Dionex CarboPac PA1 Guard, 4 × 50 mm
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL (full loop)
Column Temp.:	25 °C
Detector Temp.:	30 °C
Back Pressure:	2400 psi
Eluent:	DI water from 0–50 min, 300 mM NaOH from 50–65 min DI water from 65–80 min (re-equilibration)
Postcolumn Base:	300 mM NaOH
Flow Rate for Postcolumn Base:	0.6 mL/min

In this application note, HPAE-PAD methods are demonstrated for the determination of carbohydrates in extracts from instant coffee and green coffee beans. Two methods (the AOAC Official Method 995.13 and a fast method using the Thermo Scientific™ Dionex™ CarboPac SA10 column) were compared.

Peaks:
1. Mannitol
2. Fucose
3. Rhamnose
4. Arabinose
5. Galactose
6. Glucose
7. Xylose
8. Mannose
9. Fructose
10. Ribose



Chromatograms of free carbohydrates extracted from instant coffee (A), total carbohydrates extracted from instant coffee (B), and mixed carbohydrate standards (C); using the modified AOAC Official Method 995.13 (10 mM hydroxide for 6 min, and sucrose not included in mix of standards).

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Download the Application Note 280: Carbohydrates in Coffee: AOAC Method 995.13 vs a Fast Ion Chromatography Method

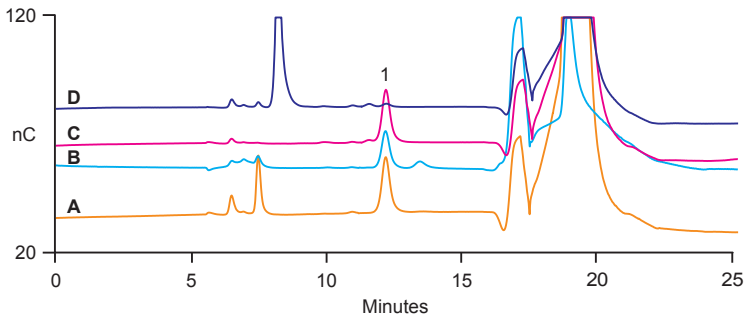
Myo-Inositol in Infant Formula and Adult Nutritionals

Myo-inositol is one of the most abundant sugars in the body, where it occurs in its free form and as a component of phosphoinositides in cell membranes. It plays an important role in various biological functions, including the regulation of cell osmolality, phosphoinositide-mediated processes of cell signaling, formation of the neural system, and pulmonary surfactant phospholipid production.

In this application note, an HPAE-PAD method (AOAC Official Method 2011.18) is demonstrated to determine free and bound myo-inositol in infant formula and adult nutritional liquid samples. A column-switching technique is used to effectively remove the strongly retained carbohydrates in the sample matrix, thereby reducing run time. PAD with a Au on PTFE disposable electrode offers high sensitivity while eliminating the need for sample derivatization and electrode polishing.

Conditions	
Dimension 1	
Column:	Dionex CarboPac PA1 Guard, 4 × 50 mm
Eluent:	750 mM Sodium Hydroxide (NaOH)
Flow Rate:	0.4 mL/min
Injection Volume:	20 µL
System Backpressure:	800–900 psi
Dimension 2	
Column:	Dionex CarboPac MA1 Guard, 4 × 50 mm Dionex CarboPac MA1 Analytical, 4 × 250 mm
Eluent:	15 mM KOH
Eluent Source:	Dionex EGC 500 KOH Cartridge with Dionex CR-ATC 500 Trap Column
Flow Rate:	0.4 mL/min
Injection Volume:	20 µL
Temperature:	30 °C
Detection:	PAD, Au on PTFE Disposable Working Electrode System
Backpressure:	2800–2900 psi
Background Conductance:	28–41 nC
Noise:	~16 pC/min peak-to-peak
Run Time:	25 min

Peak:	A	B	C	D	
1. Myo-inositol	1.00	0.904	0.878	0.0681	mg/L



Free myo-inositol in (A) SRM 1849, (B) milk-based powdered infant formula, (C) soy-based powdered infant formula, and (D) adult nutritional liquid. A 15% signal offset has been applied.

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Download the Application Note 1083: Determination of Myo-Inositol (Free and Bound as Phosphatidylinositol) in Infant Formula and Adult Nutritionals

Monsaccharides and Disaccharides in Beverages

Table of Contents

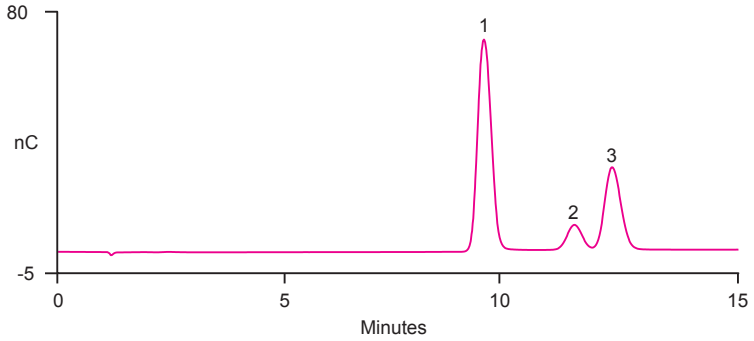
Mono- and disaccharide sugar determinations are often used in the food and beverage industry to ensure the quality of a formulated product, to maintain or select for desired sweetness, and to characterize and confirm the source of the carbohydrates. Carbohydrates have poor chromophores and are therefore problematic to detect by UV absorption without lengthy and costly derivitization. However, carbohydrates can be determined directly by HPAE-PAD, a well-established method that eliminates the need for derivitization, saving time and money including reagent costs.

This technical note demonstrates mono- and disaccharides determinations in two-fold to 10,000-fold diluted beverage samples by HPAE-PAD at capillary flow rates on the Thermo Scientific™ Dionex™ ICS-4000 HPIC™ Integrated capillary system.

Conditions	
Columns:	Dionex CarboPac PA20 column set (0.4 × 150 mm)
Eluent Source:	Dionex EGC KOH Eluent Generator Cartridge (Capillary)
Eluent:	10 mM KOH (-7 to 20 min)
Flow Rate:	0.008 mL/min
Column Temp.:	30 °C
Compartment Temp.:	27 °C
Inj. Volume:	0.4 µL
Detection:	PAD, Gold on PTFE, 0.001" or 0.015" gasket, Four-Potential Carbohydrate waveform
Reference Electrode:	pH-Ag/AgCl
Background:	10–20 nC
Noise:	< 10 pC

* Column wash/10 samples: 5 min at 100 mM KOH, 12 min equilibration at 10 mM KOH.

Peaks:	mg/L	Tota	% Ratio
1. Glucose	4.6	46 g/L	39
2. Sucrose	1.3	13	11
3. Fructose	5.9	59	50



Glucose, sucrose, and fructose in tea beverage.

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Download Technical Note 135: Determinations of Monosaccharides and Disaccharides in Beverages by Capillary HPAE-PAD

Biofuels

Feedstock used for biofuel production often requires hydrolysis to release sugars before fermentation. The Thermo Scientific™ Dionex™ ICS-5000+ HPIC™ system provides automation options for in-line matrix elimination, sample preparation, and electrochemical detection for determination of carbohydrates in feedstock. HPAE-PAD methods using the Dionex CarboPac columns are well suited for handling high concentration biofuel samples with minimal sample treatment, high precision, and acceptable recoveries. These fast, accurate, and reliable methods can be adapted for on-line monitoring of sugar levels in biomass applications.

- Carbohydrate Determination of Biofuel Samples
- Determination of Hydroxymethylfurfural in Honey and Biomass
- Determination of Carbohydrates in Acid Hydrolysates of Wood
- Determination of Uronic Acids and Wood Sugars in Wood-Based Hydrolysates

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Carbohydrate Determination of Biofuel Samples

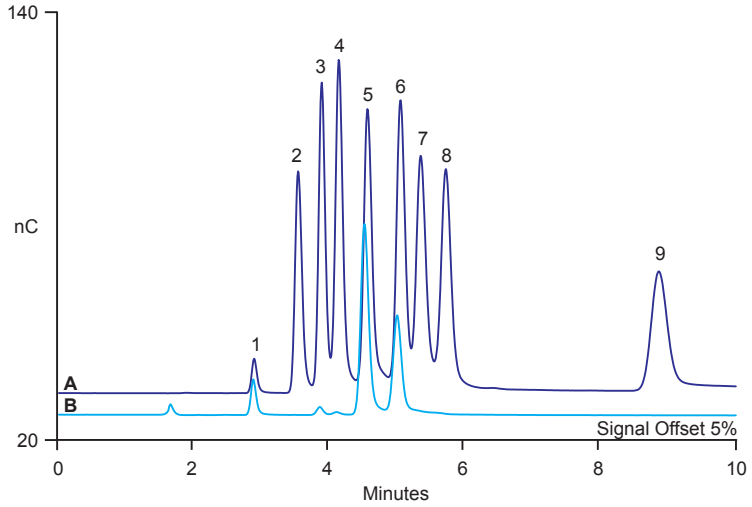
Table of Contents

Biofuels have emerged as an attractive alternative to fossil fuel. The cellulosic biomass—the starches and sugars in plant/animal matter—are typically broken down by the application of heat and/or chemicals (pretreatment) followed by enzymatic digestion and fermentation. To maximize the biofuel yield, it is critical to quantify the released carbohydrates during biofuel production.

In this application update, a rapid and robust HPAE-PAD method demonstrates the accurate determination of common sugars in acid-hydrolyzed biomass samples. The method uses the Dionex CarboPac SA10 column with electrolytically generated hydroxide eluent, reduced sample size, and a thicker gasket for the working electrode. The method is shown to have a linear range suitable for handling high-concentration biomass samples with minimal sample treatment.

Conditions	
Columns:	Dionex CarboPac SA10 Guard, 4 × 50 mm
	Dionex CarboPac SA10 Analytical, 4 × 250 mm
Eluent:	1 mM Potassium Hydroxide (KOH)
Eluent Source:	Dionex EGC III KOH Eluent Generator Cartridge with Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	1.5 mL/min
Injection Volume:	0.4 µL (full loop)
Column Temperature:	40 °C
Cell Temperature:	30 °C
Backpressure:	2500 psi
Detection:	PAD
Background:	30–70 nC
Working Electrode:	Gold on PTFE Disposable Electrode
Electrochemical Cell Gasket:	62 mil
Reference Electrode:	pH, Ag/AgCl
Mode:	Ag/AgCl mode
Noise:	30–60 pC

Peaks:
1. Fucose
2. Sucrose
3. Arabinose
4. Galactose
5. Glucose
6. Xylose
7. Mannose
8. Fructose
9. Cellobiose



Separation of biofuel sugars (A) and an acid-hydrolyzed (diluted 10-fold) corn stover sample (B) using the Dionex CarboPac SA10 column.

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Download the Application Update 192:
Carbohydrate Determination of Biofuel Samples

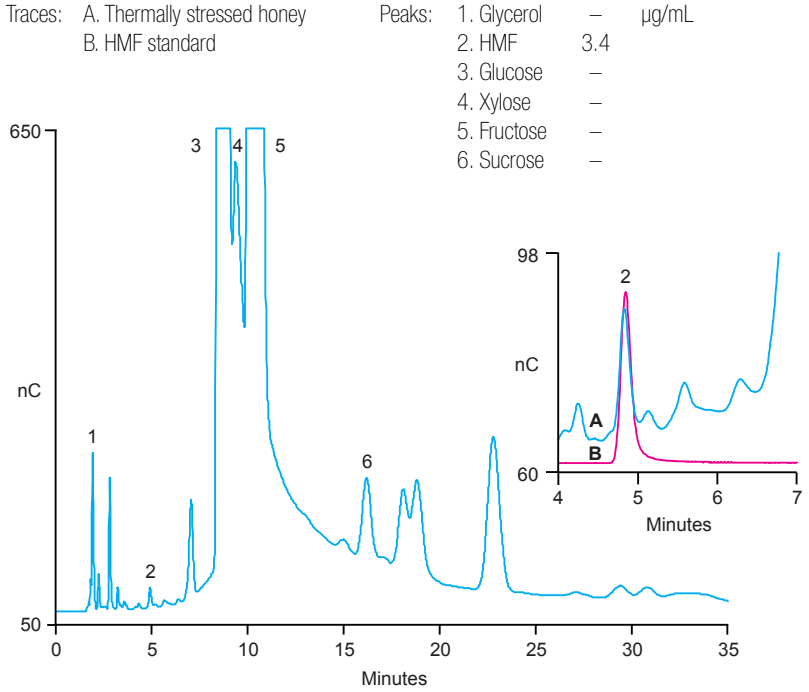
Hydroxymethylfurfural in Honey and Biomass

Table of Contents

Hydroxymethylfurfural (HMF), or 5-hydroxymethyl-2-furaldehyde, is a water-soluble heterocyclic organic compound derived from sugars. It is a derivative of furan and has both aldehyde and alcohol functional groups. Very low amounts of this compound are naturally found in fresh sugar-containing foods including milk, honey, fruit juices, spirits, and bread. Additionally, HMF is produced during food pasteurization and cooking as a result of dehydration of sugars such as glucose and fructose and in the initial stages of the Maillard reaction, a reaction between sugars and proteins responsible for changes in color and flavor of food. HMF is also formed during extended food storage under acidic conditions that favor its generation. Therefore, it is an indicator of excessive heat-treatment, spoilage, and of possible adulteration with other sugars or syrups.

In this application note, the study describes a HPAE-PAD method for the accurate determination of HMF in foods like honey and in biomass like acid-hydrolyzed corn stover. The method uses the Dionex CarboPac PA1 column with electrolytically generated hydroxide eluent. The method is shown to have a broad linear range, high precisions, and low detection limits. The disposable Au working electrode provides consistently high detector response, assuring greater instrument-to-instrument and lab-to-lab reproducibility. In summary, the described HPAE-PAD-based HMF analysis method is accurate and reliable, and should be applicable to online monitoring of HMF levels in food and biomass applications.

Conditions	
Method	
Columns:	Dionex CarboPac PA1 Analytical, 4 × 250 mm Dionex CarboPac PA1 Guard, 4 × 50 mm
Eluent:	50 mM KOH
Eluent Source:	Dionex EGC II KOH Cartridge with Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	1.0 mL/min
Inj. Volume:	10 µL (full loop)
Temperature:	30 °C
Backpressure:	2400 psi
Detection:	PAD
Background:	30–70 nC
Working Electrode:	Carbohydrate PTFE Disposable Au Working Electrode
Reference Electrode:	Mode: Ag/AgCl mode Noise: 30 pC



HMF in thermally stressed honey.

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Download the Application Note 270: Determination of Hydroxymethylfurfural in Honey and Biomass

Carbohydrates in Acid Hydrolysates of Wood

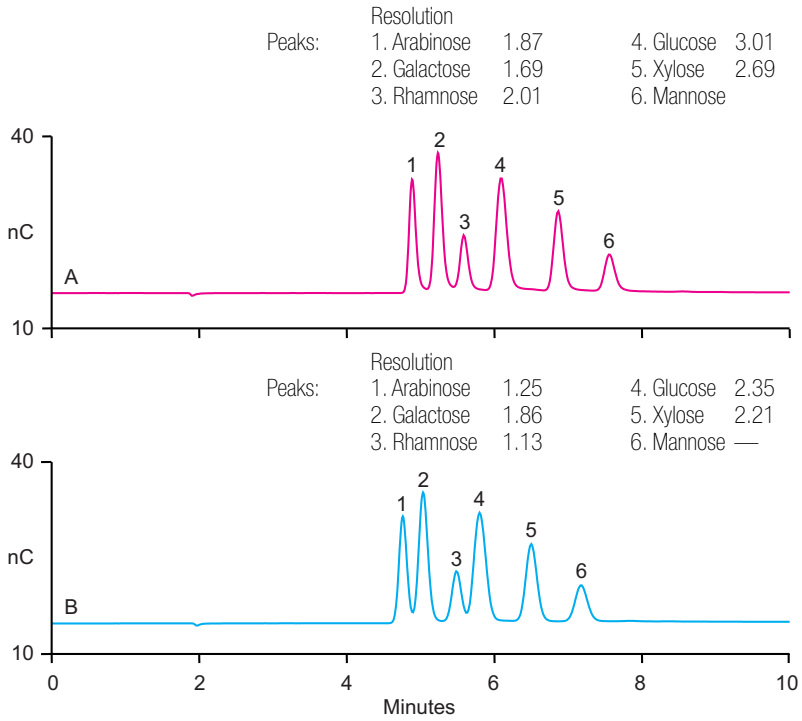
Table of Contents

The determination of carbohydrates in wood hydrolysates is of crucial importance during biofuel production. The breakdown of lignin and cellulose in wood into fermentable carbohydrates is monitored to maximize the efficiency of biomass-to-biofuel conversion, and is directly related to ethanol yield. Liquid chromatography, including HPAE-PAD, can be used to determine carbohydrates in the acid hydrolysates of wood.

Conditions	
Method	
Columns:	Dionex CarboPac SA10 Guard, 4 × 50 mm Dionex CarboPac SA10 Analytical, 4 × 250 mm Dionex CarboPac SA10-4µm Analytical, 4 × 250 mm
Eluent:	Potassium Hydroxide (KOH), 1 mM
Eluent Source:	Dionex EGC III KOH Eluent Generator Cartridge with Dionex CR-ATC Continuously Regenerated Anion Trap Column
Flow Rate:	1.5 mL/min (Method 1), 1.2 mL/min (Method 2)
Injection Volume:	0.4 µL Internal Loop
Column Temperature:	45 °C (Method 1), 30 °C (Method 2)
Cell Temperature:	20 °C*
Detection:	PAD
Background:	30–70 nC
Working Electrode:	Gold on PTFE Disposable
Electrochemical Cell Gasket:	62 mil
Reference Electrode	
Mode:	Ag/AgCl mode
Noise:	30–60 pC

*This application was run on a dual system with the detector compartment at 20 °C, which is optimal for the conductivity detector on the second system.This application can also be run with the detector compartment at 30 °C.

In this application note, two rapid and robust HPAE-PAD methods demonstrate the accurate determination of common sugars in acid-hydrolyzed wood samples. Both methods use the Dionex CarboPac SA10-4µm column with electrolytically generated hydroxide eluent, reduced sample size, and the thicker gasket for the working electrode. The smaller resin particles used in the 4 µm column allow for higher-efficiency separations compared to the 4 mm column. In particular, Method 2 allows for better resolution of galactose and arabinose and rhamnose and glucose compared to the standard column.



Comparison of the Dionex CarboPac SA10-4µm (A) and SA10 (B) columns for rhamnose determination using Method 2.

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Download Application Note 1089: Determination of Carbohydrates in Acid Hydrolysates of Wood

Uronic Acids and Wood Sugars in Wood-Based Hydrolysates

Wood hydrolysates contain a variety of hemicellulosic sugars (including glucose, xylose, mannose, arabinose, and cellobiose) and sugar acids (e.g., galacturonic and glucuronic acids). Uronic acids are a class of sugar acids with both the carbonyl and the carboxylic acid functional groups. Uronic acids are present in plant cell walls and are also formed during biofuel processing.

Accurate determination of both mono- and disaccharides and the uronic acids in biomass materials is important because compositional analysis enables evaluation of conversion yields, and carbohydrate content is directly proportional to bioalcohol yield.

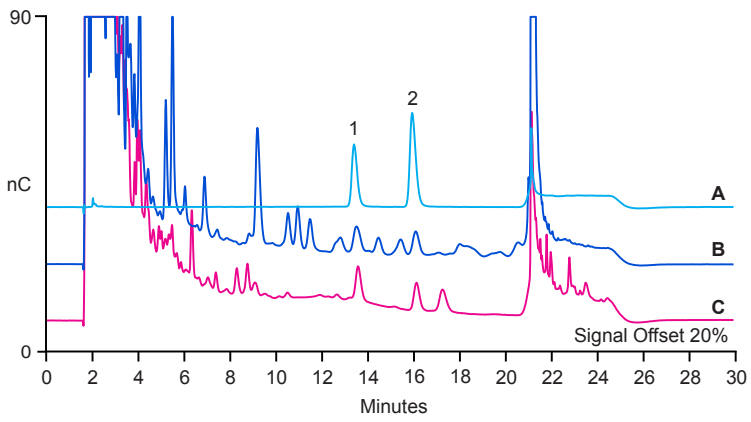
In this application note, the study describes two HPAE-PAD-based methods for the determination of uronic acids in acid-hydrolyzed wood samples. Method 2 uses the Dionex CarboPac PA200 column for accurate determination of uronic acids.

Conditions

Method 2

Columns:	Dionex CarboPac PA200 Guard, 3 × 50 mm Dionex CarboPac PA200 Analytical, 3 × 250 mm
Eluent:	A. 1 M sodium acetate, 100 mM sodium hydroxide B. 100 mM sodium hydroxide
Gradient:	Time (min) / A (%) / B (%) 0.0 / 2 / 98 18.0 / 2 / 98 18.1 / 50 / 50 22.0 / 50 / 50 22.1 / 2 / 98 30.0 / 2 / 98
Flow Rate:	0.5 mL/min
Injection Volume:	10 µL Internal Loop
Column Temperature:	30 °C (Method 1), 30 °C (Method 2)
Cell Temperature:	30 °C*
Detection:	PAD
Background:	30–70 nC
Working Electrode:	Au on PTFE Disposable
Reference Electrode	Mode: Ag/AgCl mode Noise: 30–60 pC

Peaks: 1. Galacturonic Acid
2. Glucuronic Acid



Separation of uronic acids using Method 2 on the Dionex CarboPac PA200 column in (A) a mix of standards and (B and C) wood hydrolysates.

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Download Application Note 1091: Determination of Uronic Acids and Wood Sugars in Wood-Based Hydrolysates

Other Applications

Carbohydrates play key roles in a wide range of biological recognition and regulatory functions, cellular communication, gene expression, immunology, organism defense mechanisms, and growth and development. We have developed instruments and columns that allow the separation, quantification and qualitative analyses of carbohydrates in the new and emerging field of glycomics as well as in the glycan structure elucidation and confirmation in the highly regulated biopharmaceuticals.

- Determination of Carbohydrates in Urine by HPAE-PAD

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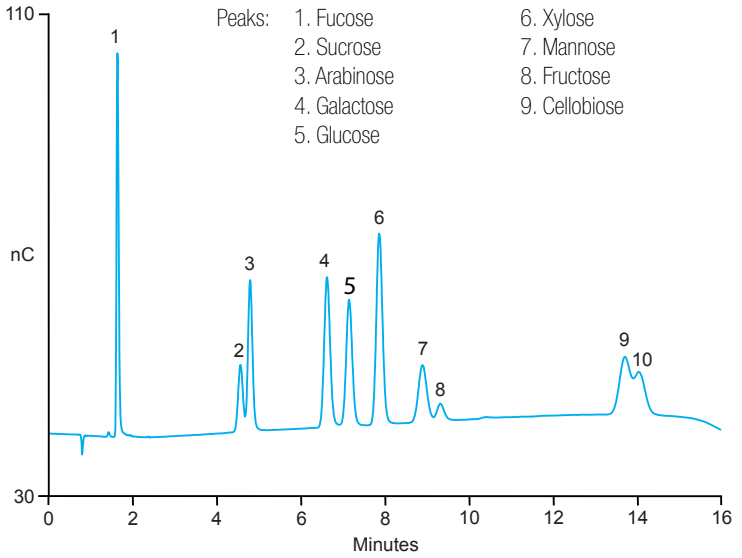
Carbohydrates in Urine

There are many methods to evaluate carbohydrates present in urine. Carbohydrates can be fluorescently labeled and then determined by HPLC or derivatized and analyzed by gas chromatography-flame ionization detection (GC-FID). However, the derivatization reaction adds reagent costs and time to the sample analysis. Another alternative is to determine the carbohydrates with direct detection by HPAE-PAD. Many HPAE-PAD methods have been published that evaluate concentrations of mannitol or rhamnose, xylose, 3-*O*-methylglucose, and lactulose in both urine and serum.

In this application note, two HPAE-PAD methods are demonstrated and compared for analysis of urine samples. The first method, using a Dionex CarboPac PA20 Analytical Column, minimizes eluent preparation with an RFIC eluent generator. The second method takes advantage of the selectivity and high capacity of the Dionex CarboPac MA1 column. Use of this column allows mannitol to be strongly retained with minimal interferences. This application can also be run on a capillary HPIC system as demonstrated in Technical Note 137: Determination of Carbohydrates in Urine by Capillary HPAE-PAD.

Conditions	
Method 1	
Columns:	Dionex CarboPac PA20 Analytical (3 × 150 mm)
Eluent Gradient:	10 mM KOH from -7 to 1 min, 10–30 mM KOH from 1–9 min, 30–35 mM KOH from 9–16 min
Eluent Source:	Dionex EGC III KOH Cartridge with CR-ATC Continuously Regenerated Anion Trap Column Or: 100 mM NaOH, manually prepared
Flow Rate:	0.5 mL/min
Inj. Volume:	10 µL (full loop)
Temperature:	30 °C (column and detector compartments)
Detection:	Pulsed amperometric, disposable Au on PTFE electrode
Background:	~45 nC (using the carbohydrate 4-potential waveform)
Noise:	~30 pC
System Backpressure:	~2400 psi



Separation of ten carbohydrates of interest using a gradient elution from the Dionex CarboPac PA20 column.

Download Application Note 1006: Determination of Carbohydrates in Urine by HPAE-PAD

Download Technical Note 137: Determination of Carbohydrates in Urine by Capillary HPAE-PAD

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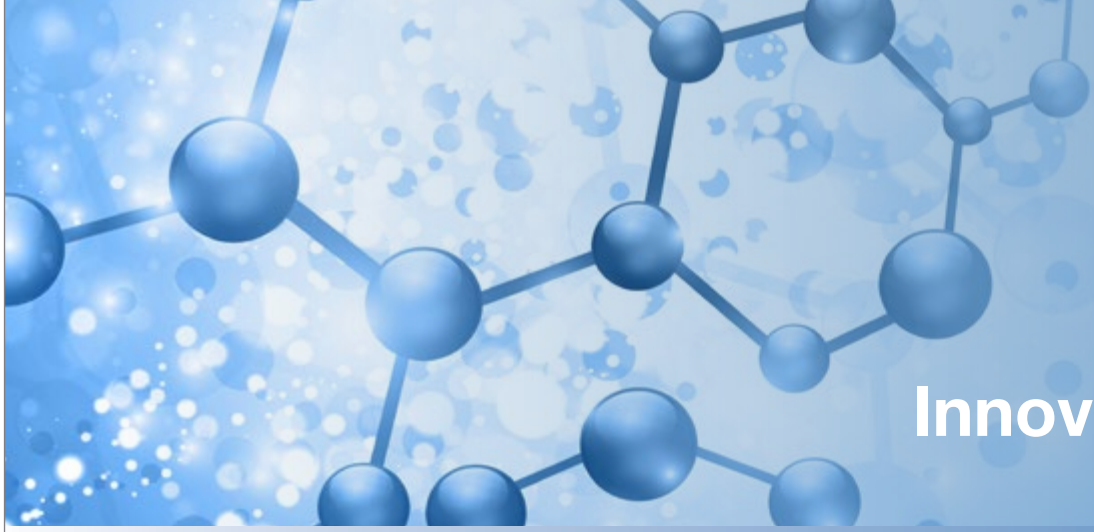

Innovative Solutions

- Technology Overview
- Carbohydrate Columns
- 3-D Amperometry
- HPAE-PAD with MS

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Technology Overview

Dionex ICS-5000$^+$ HPIC and Dionex ICS-4000 Capillary HPIC Systems

The Dionex ICS-5000$^+$ HPIC system is ideal for ion-exchange chromatography of carbohydrates using electrochemical detection. For mono- and disaccharides, the Thermo Scientific™ Dionex™ ICS-4000 Capillary HPIC™ system can also be utilized. With completely metal-free, all PEEK flowpaths, Dionex ion chromatography (IC) systems eliminate the possibility of metal contamination and improve robustness.

The analysis of mono- and disaccharides can be performed using Reagent-Free™ IC (RFIC™) systems with eluent generation that only requires deionized water to electrolytically generate the eluent. This technology provides consistent results and the highest reproducibility, day-to-day, user-to-user, and lab-to-lab. The all-PEEK pump of the Dionex ICS-5000$^+$ system is capable of performing quaternary gradients at high flow rates, for demanding application needs.

Capillary HPAE-PAD

Capillary HPAE-PAD is a technique that uses capillary columns (e.g., 0.4 mm i.d. columns) packed with ion-exchange resin, a capillary amperometric cell with a gold working electrode and a palladium hydrogen (PdH) reference electrode. The cell body is made of titanium and serves as the counter electrode. Capillary HPIC systems include an electrolytic eluent generator optimized for operation at capillary flow rates.

Dionex ICS-4000 Capillary HPIC and Dionex ICS-5000$^+$ HPIC systems.

For more information on ion chromatography systems, go to www.thermoscientific.com/icsystems

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Dionex ICS-5000+ and Dionex ICS-4000 Electrochemical Detector (ED)

The electrochemical detector cells have been redesigned to be flow and volume optimized for capillary, microbore and standard bore formats. The optional palladium hydrogen reference electrode is robust and calibration-free.

Gold working electrodes for carbohydrate analysis are available in both conventional and disposable formats. Conventional working electrodes will last for extended periods of time. However, these electrodes require periodic polishing to refinish the surface and have longer equilibration times when newly installed in a system. Disposable electrodes provide efficient, sensitive, reproducible analyses, electrode-to-electrode and lot-to-lot. After installation, rapid system re-equilibration (less than 30 min) supports quick system start-up.

Electrochemical detector cell for applications on standard bore and microbore size columns.

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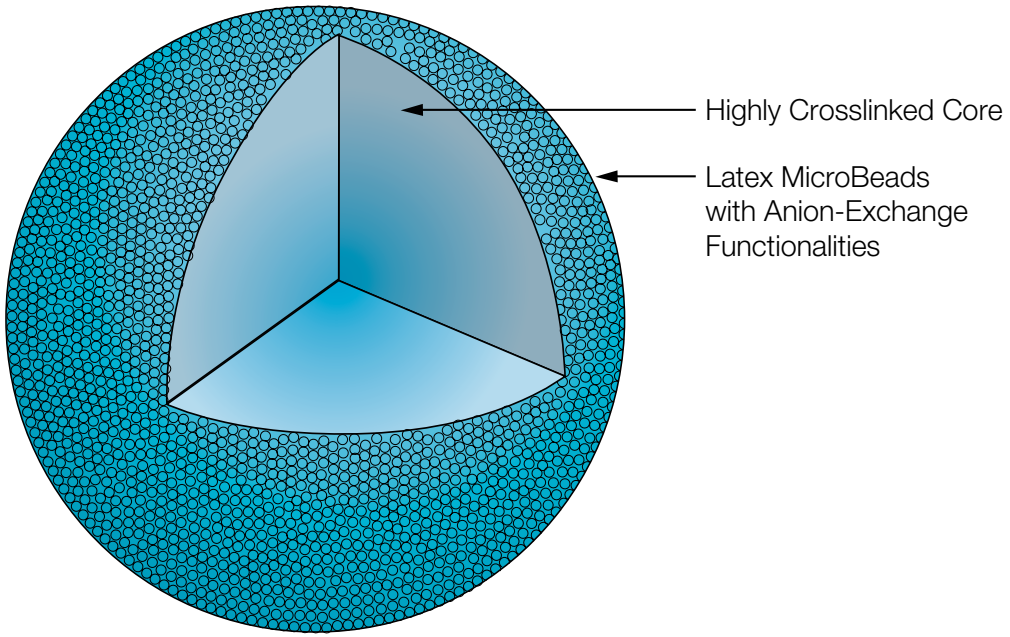
Dionex CarboPac Columns

The Dionex CarboPac family of columns offers a selection of columns, each optimized for a different class of compounds. They enable high resolution separations of closely related glycoprotein oligosaccharides, monosaccharides, sialic acids, and a wide variety of other carbohydates.

The Dionex CarboPac columns use pellicular resin technology for improved chromatographic resolution, peak shape, and efficiency. The Thermo Scientific™ Dionex™ MicroBead™ latex particle is optimized to further improve column performance by imparting a unique chromatographic selectivity. This selectivity results in a significantly improved resolution between previously problematic analytes.



Dionex MicroBead particle.

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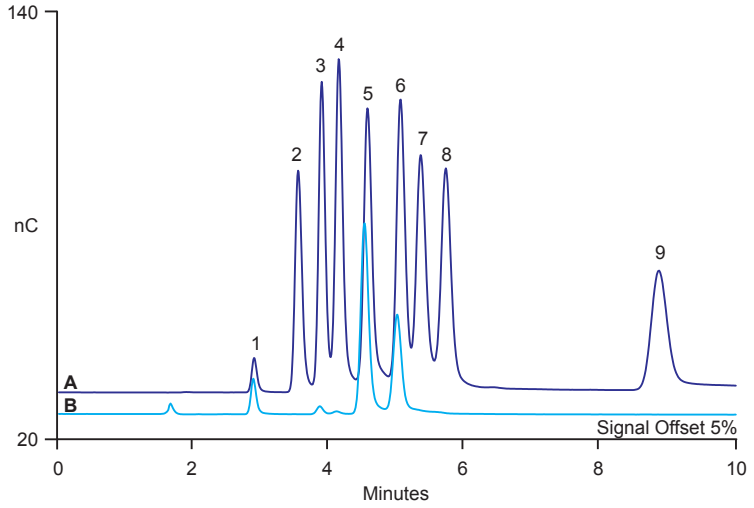
High Concentration Carbohydrate Analysis Kit

In the biofuels and food and beverage industries, it is critical to separate and quantify a large number of carbohydrate samples during the production processes. HPAE-PAD has been shown to be a highly selective and reproducible analysis method for these carbohydrates with no need for derivatization.

The Thermo Scientific High Concentration Carbohydrate Analysis Kit is specifically designed for fast and high-resolution analysis of concentrated mono- and disaccharides using the Dionex ICS-5000+ HPIC system and the Dionex CarboPac SA10 column.

Peaks:
1. Fucose
2. Sucrose
3. Arabinose
4. Galactose
5. Glucose
6. Xylose
7. Mannose
8. Fructose
9. Cellobiose



Separation of biofuel sugars (A) and an acid-hydrolyzed (diluted 10-fold) corn stover sample (B) using the High Concentration Carbohydrate Analysis Kit and the Dionex CarboPac SA10 column.

For more information, download High Concentration Carbohydrate Analysis Kit Product Specifications

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Thermo Scientific Dionex Column Selection Guide

Dionex CarboPac And Dionex AminoPac Columns

Column	Format (Capacity µeq/col)	Recommendations	Target Applications	Application Documents
Dionex CarboPac PA200	3 x 250 mm (35 µeq)	High resolution separations of charged and neutral oligosaccharides.	Separation of neutral and sialylated N-linked oligosaccharides from glycoproteins. Plant-derived oligosaccharides (e.g. maltodextrins, xylans, etc.).	AN 1050: Protein Glycosylation in Limited-Quantity Samples AN 1013: Polysialic Acid Analysis AN 215: Asparagine-Linked Oligosaccharides from Polyclonal IgG AU 150: Plant-Derived Neutral Oligo- and Polysaccharides AN 67: Determination of Plant-Derived Neutral Oligo- and Polysaccharides AN 1091: Uronic Acids and Wood Sugars in Wood Based Hydrolysates AN 202: HPAE-PAD Analysis of Mannose-6-Phosphate
Dionex CarboPac PA20	3 x 150 mm (65 µeq) 0.4 x 150 mm (1.16 µeq)	High-resolution separations of mono- and disaccharides with optimized resolution of glucosamine/galactose and glucose/mannose peak pairs. The capillary format requires high pressure IC for fastest runs.	Glycoprotein monosaccharides, sialic acids.	AN 253: Sialic Acids in Infant Formula TN 40: Glycoprotein Monosaccharide Analysis AN 1050: Protein Glycosylation in Limited-Quantity Samples AU 180: Sialic Acids in Glycoprotein Hydrolyzates by HPAE-PAD AN 1091: Uronic Acids and Wood Sugars in Wood-Based Hydrolysates AN 248: Determination of Lactose in Lactose-Free Milk Products by HPAE-PAD AN 202: HPAE-PAD Anaysis of Mannose-6-Phosphate AN 233: Determination of Galactosamine Containing Organic Impurties in Heparin by HPAE-PAD AN 197: Glucosamine in Dietary Supplements Using HAPE-PAD AU 151: Sucralose in Reduced-Carbohydrate Colas Using HPAE-PAD AN 159: Determination of Sucralose Using HPAE-PAD AU 164: Determination of Glucosamine in Chondroitin Sulfate-Containing Dietary Supplements Using HPAE-PAD
Dionex CarboPac PA20 Fast Sialic Acid	3 x 30 mm (13 µeq)	Fast separation of N-acetyl- and N-glycolylneuraminic acids.	Sialic acids.	AU 181: Rapid Screening of Sialic Acids in Glycoproteins
Dionex CarboPac MA1	4 x 250 mm (1450 µeq)	High-capacity, strong anion-exchange column for separation of small reduced sugars (sugar alcohols).	Reduced mono and disaccharides in commercial sweeteners and other food products and reduced monosaccharides from glycoproteins.	AN 267: Analysis of Amino Glycoside Antibiotics AN 246: Ethylene Glycol and Diethylene Glycol in a Sorbitol Solution AN 122: Carbohydrates, Alcohols, and Glycols in Fermentation Broths AN 117: Quantification of Carbohydrates and Glycols in Pharmaceuticals AN 87: Sugar Alcohols in Confections and Fruit Juices by HPAE-PAD
Dionex CarboPac SA10	4 x 250 mm (290 µeq) 2 x 250 mm (73 µeq)	Fast and high capacity separation of mono- and disaccharides in biofuels, foods, and beverages.	Fast analysis of monosaccharides and disaccharides in various matrices.	AN 282: Biofuel Sugars by HPAE-PAD AN 280: Carbohydrates in Coffee AU 192: Carbohydrates in Biofuel Samples

Currently recommended columns.

100% Solvent compatible with common organic solvents.

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Thermo Scientific Dionex Column Selection Guide

Dionex CarboPac And Dionex AminoPac Columns

Column	Format (Capacity µeq/col)	Recommendations	Target Applications	Application Documents
Dionex CarboPac SA10-4µm	4 x 250 mm (290 µeq) 2 x 250 mm (73 µeq)	High resolution fast and high capacity separation of mono- and disaccharides in biofuels, foods, and beverages.	High resolution fast analysis of monosaccharides and disaccharides in various matrices.	AN 1089: Carbohydrates in Acid wood Hydrolysates TN 146: Lactose and Lactulose in Milk Products
Thermo Scientific™ Dionex™ BorateTrap™ Column	4 × 50 mm	Highly recommended for optimal performance during carbohydrate analysis to remove borate contamination from eluents.	Eliminates peak tailing for mannose, fructose, and reduced monosaccharides, resulting from borate contamination in the eluent.	
Thermo Scientific™ Dionex™ AminoTrap™ Column	4 x 50 mm 3 x 30 mm 2 x 50 mm 0.4 x 35 mm	An in-line pretreatment column designed to retain amino acids present in carbohydrate samples.	Column optimized to delay the elution of amino acids and small peptides in glycoprotein hydrolysates.	TN 40: Glycoprotein Monosaccharide Analysis TN 71: Eluent Preparation for High-Performance Anion-Exchange TN 133: HPAE-PAD Peak Area Response of Glycoprotein Oligosaccharides
Thermo Scientific™ Dionex™ AminoPac™ PA10	4 x 250 mm (240 µeq) 2 x 250 mm (60 µeq) 9 x 250 mm 22 x 250 mm	Hydrophobic, polymeric, pellicular, anion-exchange resin for the separation of carbohydrates and amino acids. The capillary format requires high pressure IC for fastest runs.	Analysis of free amino acids, vitamins, amino sugars, carbohydrates, phosphorylated amino acids, and common oxidation products of sulfur-containing amino acids.	AN 179: Carbohydrate and Amino Acid Analysis AN 150: Amino Acids in Cell Cultures and Fermentation Broths AN 142: Tryptophan Using AAA-Direct TN 55: Screening of Matrices and Matrix Ingredients for AAA-Direct AN 130: Hydroxylysine-Containing Peptide Using AAA-Direct TN 50: Amino Acid Content of Peptides by AAA-Direct
Thermo Scientific Dionex Carbohydrate Removal Cartridge (CRC)	2 x 15 mm	In-line sample pretreatment cartridge for removal of carbohydrates from amino acid samples.	The Dionex CRC cartridge is an in-line pretreatment cartridge packed with cation-exchange resin to bind amino acids while carbohydrates go to waste.	

Currently recommended columns.

100% Solvent compatible with common organic solvents.

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Thermo Scientific Dionex Column Selection Guide

Dionex CarboPac And Dionex AminoPac Columns

Column	Format (Capacity µeq/col)	Recommendations	Target Applications	Application Documents
Dionex CarboPac PA100	4 x 250 mm (90 µeq) 2 x 250 mm (23 µeq) 9 x 250 mm 22 x 250 mm	Separations of oligosaccharides released from glycoproteins.	Separation of closely related oligosaccharides (isomers) and neutral and charged oligosaccharides.	AN 1070: Inositol Phosphates in Dried Distillers Grains with Solubles AN 105: Glycosylation Analysis of Human Serum Transferrin Glycoforms TN 42: Glycoprotein Oligosaccharide Analysis Using High-Performance Anion-Exchange Chromatography AN 67: Determination of Plant-Derived Neutral Oligo- and Polysaccharides AN 46: Ion Chromatography: A Versatile Technique for the Analsysis of Beer
Dionex CarboPac PA10	4 x 250 mm (100 µeq) 2 x 250 mm (25 µeq) 0.4 X 250 mm (1 µeq) 9 x 250 mm	Separation of amino, neutral, and acidic monosaccharides. The capillary format requires high pressure IC for fastest runs.	Analysis of mono- and disaccharides in foods, drugs, and plants, and separates sialic acids with the addition of sodium acetate to the eluent.	AN 117: Carbohydrates and Glycols in Pharmaceuticals TN 41: Sialic Acids Using HPAE-PAD AU 141: N-Acetylneuraminic Acid and N-Glycolylneuraminic Acid Peak Area Responses TN 53: Glycoprotein Monosaccharide Composition by HPAE-PAD Using On-Line Electrolytically Generated Eluents
Dionex CarboPac PA1	4 x 250 mm (100 µeq) 2 x 250 mm (25 µeq) 9 x 250 mm 22 x 250 mm	Rugged all-purpose column for determining monosaccharides, disaccharides and oligosaccharides.	Anion-exchange column for the separation of mono-, disaccharides, oligosaccharides, and aminoglycosides.	AN 186: Paromomycin by HPAE-PAD AN 66: Neomycin B and Impurities by HPAE-PAD AN 147: Polydextrose in Foods by AOAC Method 2000.11 AN 92: Sugars in Molasses by HPAE-PAD AN 82: Analysis of Fruit Juice Adulterated with Medium Invert Sugar from Beets AU 167: Tobramycin in Crude and In-Process Production Samples During Manufacturing Using HPAE-PAD AN 155: Trans-Galactooligosaccharides in Food by AOAC Method 2001.02 AN 61: Determination of Tobramycin and Impurities Using HPAE-PAD
Dionex AminoPac PA1	4 x 250 mm (100 µeq)	High-speed, pellicular, strong, anion-exchange column for the separation of phosphorylated, acid labile and strongly acidic amino acids.	Acidic and acid-labile amino acids and for amino acid pairs not completely resolved by cation-exchange chromatography.	

Columns currently not recommended due to the availability of better performing columns.
Columns are sold to accommodate customers using them in validated standard operating procedures.

100% Solvent compatible with common organic solvents.

Up to 90% compatible with common HPLC columns.

2-5% compatible with common HPLC columns.

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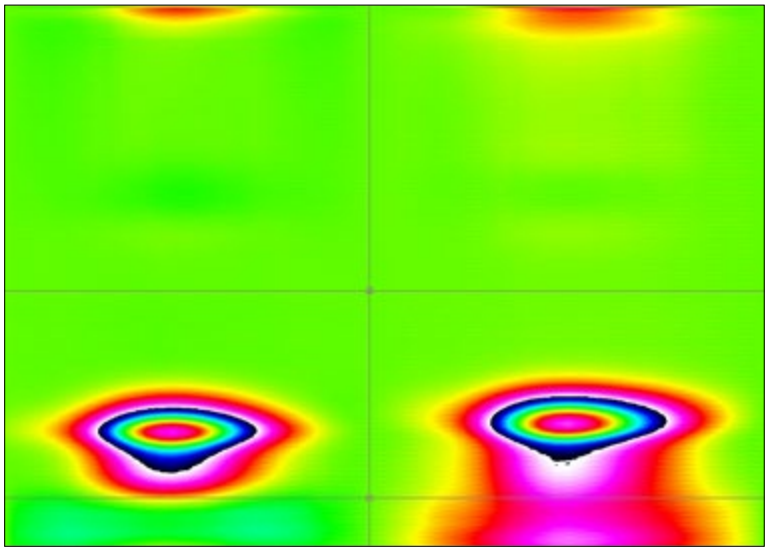
3-D Amperometry

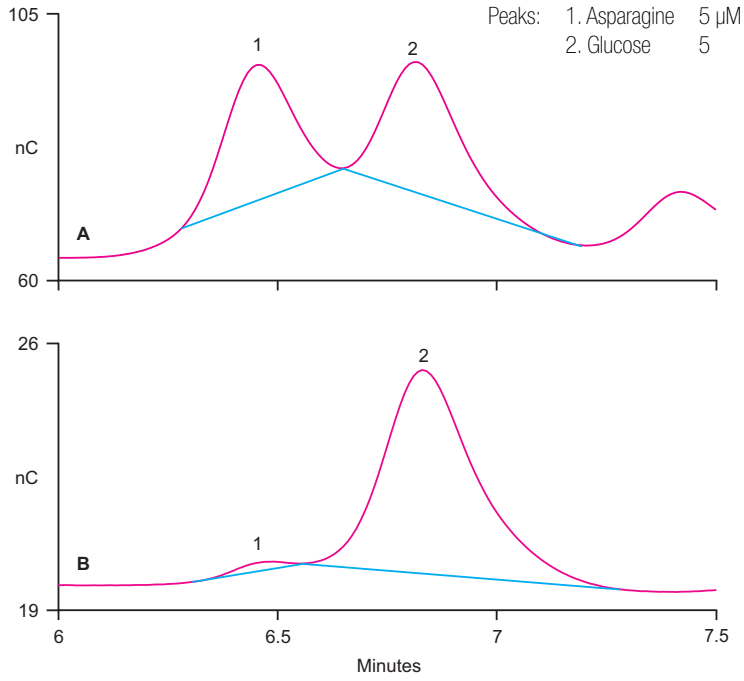
The Complete Data Set

Specific amperometric waveform integration ranges can provide a tool to selectively detect compounds of interest. 3-D amperometry enables post-chromatographic modification of waveform integration. Quantitative information in PAD is generated by current integration within a suitable time interval, called integration period, during application of a detection waveform. Different analytes may require different locations of integration periods. The capability to assign different locations within the waveform to integration periods is available post-run with the help of 3-D amperometry. This post-chromatographic modification of integration periods eliminates the need to perform multiple injections.



Iso current plot for isoleucine and leucine. Z axis magnitude is depicted with color.

This technique can enhance the detection of carbohydrates in the presence of some co-eluting amino acids. For example, resolution may be improved between asparagine and glucose through reduction of the peak area for asparagine relative to glucose. Additionally, it can minimize some baseline disturbances to improve peak integration.



Post-run re-integration allows selective reduction of size of asparagine peak, allowing better quantification of glucose.

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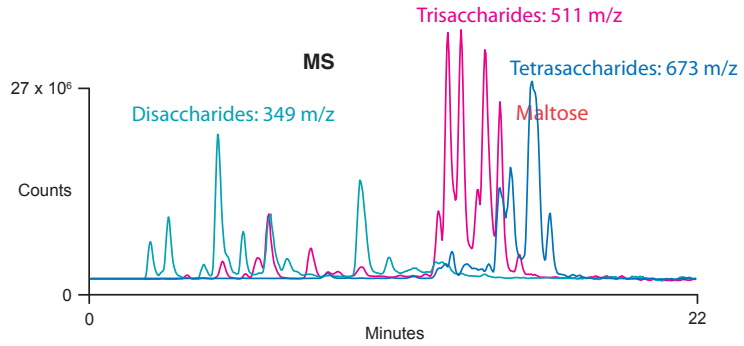
Table of Contents

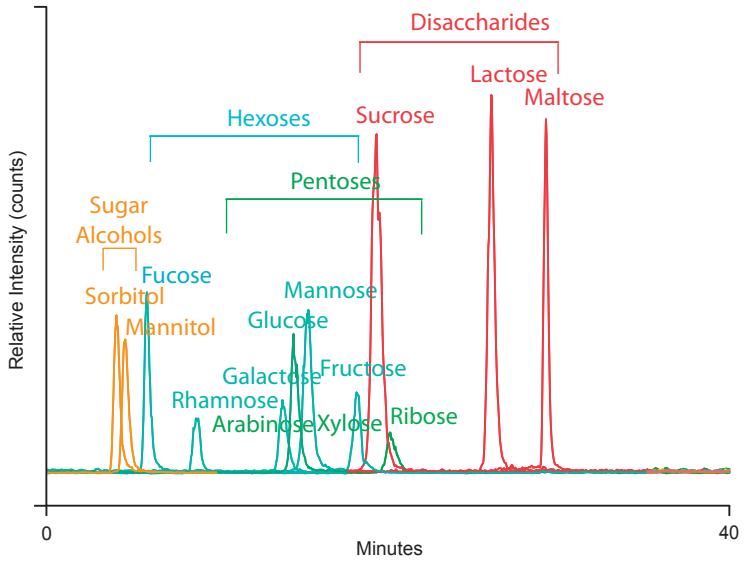
HPAE-PAD with MS

Carbohydrate Identification and Confirmation in Complex Matrices

Identification of carbohydrates in very complex matrices can be difficult using electrochemical detection alone. Mass spectrometry detection offers the advantage of faster and more reliable identification and peak confirmation by using the m/z of the saccharide classes—pentoses, hexoses, and oligosaccharides. The Thermo Scientific™ Dionex™ CMD™ 300 Carbohydrate Membrane Desalter electrolytically suppresses the effluent from the detector, reducing the pH and facilitating injection into a mass spectrometer for analysis of feedstock components, such as low molecular mass organic acids or mono-, di-, tri-, and tetrasaccharides.



Comparison of electrochemical and extracted mass chromatograms of carbohydrates in a degassed lager beer sample, separated using a Dionex CarboPac PA200 (3 × 250 mm) column.



ESI positive mass chromatograms of sugar alcohols, monosaccharides, and disaccharides in the presence of LiCl, after separation using a Dionex CarboPac PA200 (3 × 250 mm) column.

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Table of Contents

- Technical Note 20: Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)
- Technical Note 21: Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector
- Technical Note 71: Eluent Preparation for High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
- Technical Note 110: Carbohydrate Determination of HPAE-PAD with Disposable Au on PTFE Working Electrodes
- Technical Note 125: Guidelines for Successful Use of Thermo Scientific Dionex AminoTrap Columns

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