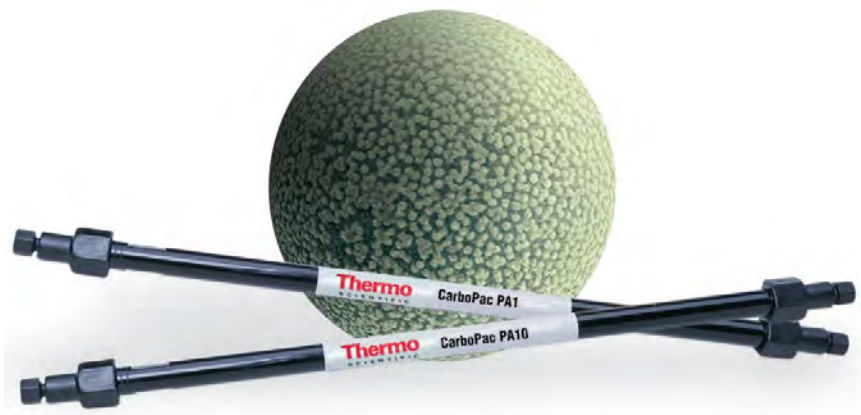
Thermo Scientific Dionex CarboPac PA1 and PA10 Columns for Mono- and Disaccharide Analysis

HPLC Columns for Quantitative Analysis of Mono- and Disaccharides Over an Extensive Range of Sample Matrices

- Reduced mono- and disaccharides
- Glycoprotein monosaccharide compositional analysis
- Linear polysaccharides
- Acidic oligosaccharides
- Exceptionally high sensitivity
- Specialized traps for interference-free quantification



Unique Column Chemistry for Optimal Performance

The Thermo Scientific™ Dionex™ CarboPac™ PA1 and PA10 are specialized anion-exchange columns designed to be used with pulsed amperometric detection to deliver high resolution separations of mono- and disaccharides. Both resins consist of 10-µm diameter nonporous beads covered with a fine latex of functionalized Thermo Scientific™ Dionex™ MicroBead™ resin. This pellicular resin structure permits excellent mass transfer, resulting in high resolution chromatography and rapid reequilibration.

The Dionex CarboPac PA1 is a rugged all-purpose column suitable for determining monosaccharides and disaccharides in a variety of matrices. It is also the column of choice for high resolution separations of linear polysaccharides. The Dionex CarboPac PA1 column is available in microbore, standard bore and semi-preparative formats.

The Dionex CarboPac PA10 column is optimized to determine the amino, neutral, and acidic monosaccharides that are found in the carbohydrate moieties of mammalian glycoproteins. It is the column of choice for high sensitivity monosaccharide analyses, in conjunction with Eluent Generation and the Thermo Scientific™ Dionex™ AminoTrap™ column. The Dionex CarboPac PA10 column is available in capillary, microbore and standard bore formats.

Thermo SCIENTIFIC

Reduced Mono- and Disaccharides

The separation of alditols and sugars is of interest to both the food and pharmaceutical industries, in matrices such as fruits and vegetables as well as drug formulations. Figure 1 shows the use of the Dionex CarboPac PA10 column for the separation of mixtures of sugar alcohol monosaccharides and disaccharides. The Dionex CarboPac PA10 is the column of choice for the separation of food sugars and food alcohols in a single run. However, if complex mixtures of sugar alcohols are present, the high capacity Dionex CarboPac MA1 column should be used.

Because alditol retention is only slightly affected by the sodium hydroxide concentration between 18 mM and 50 mM, monosaccharide and disaccharide separations can be optimized by increasing the hydroxide concentration while still maintaining adequate alditol retention.

As discussed in the "Specialized Traps for Interference-Free Quantification" section, use of the Thermo Scientific™ Dionex™ BorateTrap™ column greatly improves the peak shape of the alditols and is highly recommended for quantitative analyses.

Glycoprotein Monosaccharide Compositional Analysis

The Dionex CarboPac PA1 and PA10 columns are ideal for the quantification of amino, acidic, and neutral monosaccharides, especially those derived from glycoconjugates. The Dionex CarboPac PA10 column gives a baseline separation of mammalian monosaccharides over a broader range of isocratic sodium hydroxide concentrations than the Dionex CarboPac PA1 column (Figure 2). With the Dionex AminoTrap column in-line, the Dionex CarboPac PA10 column offers lowered detection limits, elimination of interference from oxygen, and faster run times.

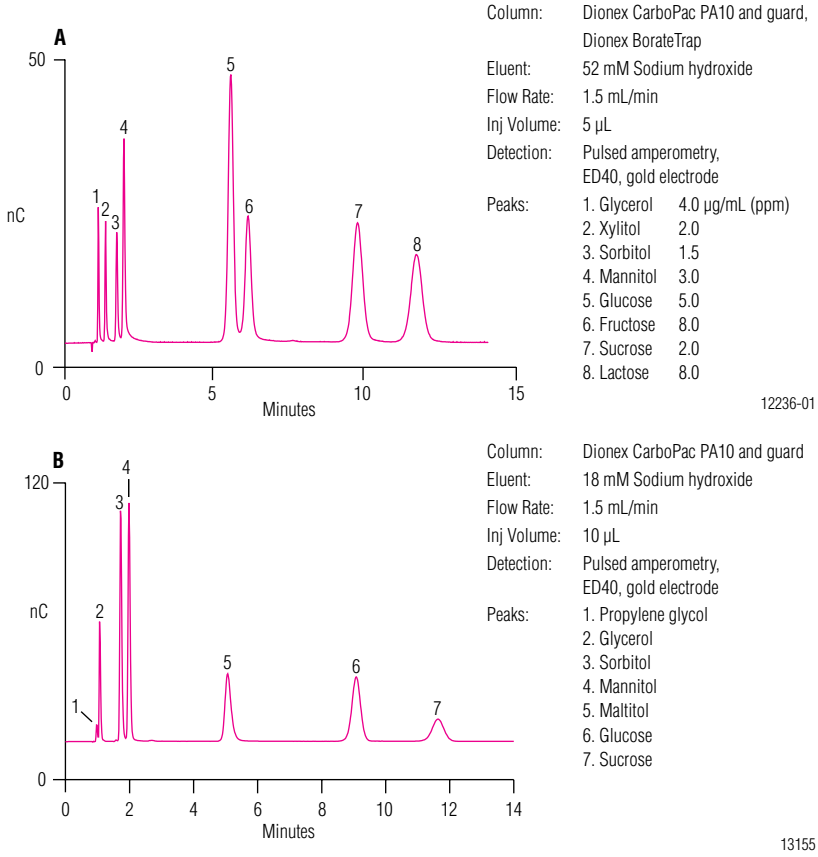


Figure 1. The Dionex CarboPac PA10 column resolves mixtures of sugar alcohols. A) Sugar alcohols and other carbohydrates found in foods. B) Glycols, sugar alcohols, and carbohydrates in a pharmaceutical formulation.

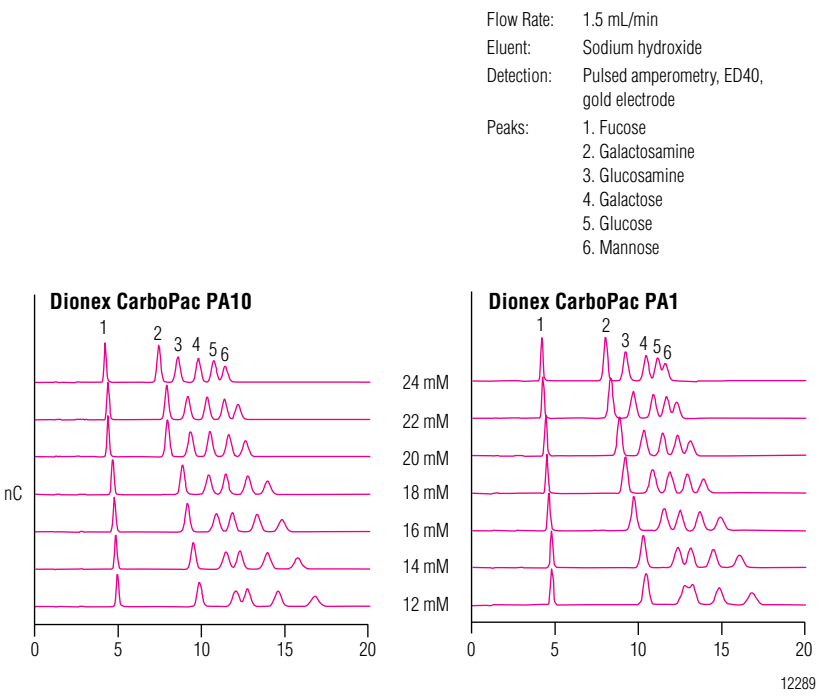
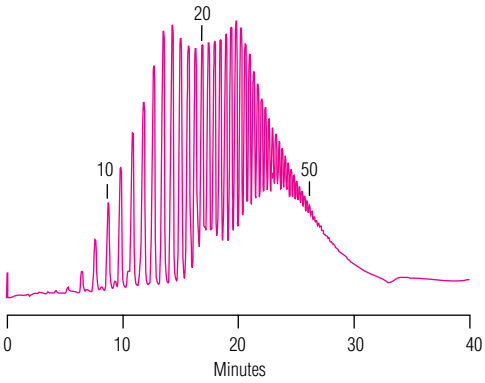


Figure 2. Comparison of the Dionex CarboPac PA1 and PA10 columns for glycoprotein monosaccharides.

Linear Polysaccharides

Chain length distribution is an important parameter in starch characterization. HPAE-PAD technology featuring the Dionex CarboPac PA1 column delivers single-residue resolution of linear polysaccharides up to a degree of polymerization (DP) of at least 60. Figure 3 shows the chain length distribution of a sample of purified inulin using the Dionex CarboPac PA1 column. Commercial inulin products have DPs that have been tailored for a particular end use; therefore, it is important to determine the chain length distribution during product development and production and for quality control of the end product. Figure 3 illustrates the excellent resolution that can be acheived for DP values up to 50 or more for purified inulin.



Column:	Dionex CarboPac PA1
Eluent:	A) 0.1 M NaOH B) 0.1 M NaOH, 1.0 M NaOH
Gradient:	20–60% B in 40 min
Flow Rate:	1 mL/min
Detection:	Pulsed amperometry, PAD, gold electrode
Range:	3000 nA
Sample:	0.3% Water Washed Inulin (Polyfructose) in 0.1 M NaOH

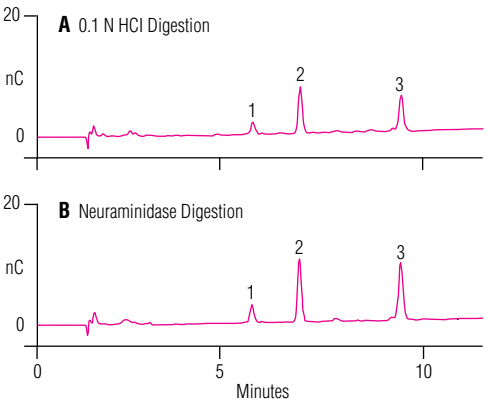
Sample Courtesy of Dr. C. Mitchell
California Natural Products, Manteca, CA

3073-01

Figure 3. Chromatographic analysis of purified inulin.

Acidic Oligosaccharides

Sialic acids comprise a large family of N- and O-substituted neuraminic acids. They occupy terminal positions on many mammalian glycoproteins and glycolipid oligosaccharides. When a glycoprotein loses sialic acid residues, it has a reduced serum half-life and in some cases reduced activity. Therefore it is important to know the sialic acid content of a glycoprotein when assaying its function or its efficacy as a pharmaceutical therapeutic. HPAE-PAD is an effective way to determine Neu5Ac and Neu5Gc without derivatization, and can be performed with either the Dionex CarboPac PA1 or Dionex CarboPac PA10 column.



Column:	Dionex CarboPac PA10
Sample Vol.:	25 µL
Eluent:	A) 100mM NaOH B) 100mM NaOH with 1M NaOAc
Gradient:	7% B to 30% B in 30 minutes
Flow Rate:	1mL/min
Detection:	Pulsed amperometry, ED40, gold electrode
Peaks:	1. Neu5Ac 2. KDN 3. Neu5Gc

13097

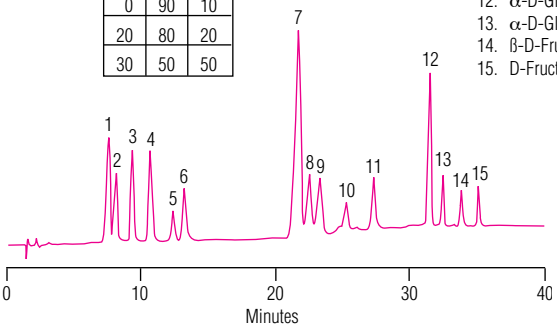
Figure 4. Sialic acid analysis of bovine transferrin on the Dionex CarboPac PA10 column.

The elution of acidic sugars from the Dionex CarboPac PA1 or Dionex CarboPac PA10 column requires stronger eluents than those used for neutral sugars (Figures 4 and 5). This is usually accomplished by the addition of sodium acetate to the sodium hydroxide eluent. Sodium acetate accelerates the elution of strongly bound species without interfering with pulsed amperometric detection.

Column:	Dionex CarboPac PA1
Flow Rate:	1.0 mL/min
Detector:	Pulsed amperometry, PAD, gold electrode
Range:	10,000 nA
Eluent A:	100 mM NaOH
Eluent B:	100 mM NaOH, 1.0 M NaOAc

Gradient:

t	%A	%B
0	90	10
20	80	20
30	50	50



Peaks:	1. α-D-Galactosamine -1-P	1.13 µg
	2. α-D-Glucosamine-1-P	0.45
	3. α-D-Galactose-1-P	1.75
	4. α-D-Glucose-1-P	1.75
	5. α-D-Ribose-1-P	1.46
	6. ß- D-Glucose-1-P	1.75
	7. D-Glucosamine-6-p	3.75
	8. D-Galactose-6-P	2.04
	9. D-Glucose-6-P	1.25
	10. D-Fructose-1-P	0.96
	11. D-Fructose-6-P	0.42
	12. α-D-Glucuronic acid-1-P	3.08
	13. α-D-Glucose-1,6-Di P	1.06
	14. ß-D-Fructose-2,6-Di P	0.92
	15. D-Fructose-1,6-Di P	0.92

3169

Figure 5. Analysis of mono- and diphosphorylated monosaccharides on the Dionex CarboPac PA1 column.

High Throughput, High Sensitivity Analyses

Figure 6 shows typical resolution for the separation of 5 pmol each of six monosaccharides using the Dionex CarboPac PA10 column. The Eluent Generator Cartridge (EGC) can be used to ensure reproducible separations by producing carbonate-free hydroxide eluent. Carbonate in the eluent results in decreased retention times and diminished resolution.

The EGC is used in conjunction with the Dionex CarboPac PA10 and the AminoTrap columns for reproducible, high throughput, high sensitivity carbohydrate applications (Figure 7). The advantages of the use of the EGC for carbohydrate analysis include simplified eluent generation, because only degassed deionized water is required. Problems caused by minor differences in prepared eluents are avoided, as are salt deposits in the pump.

The Dionex AminoTrap column is used to move the amino acids beyond the retention time of the analytes. This allows for quantitative analysis since the amino acids no longer interfere with the separation of the monosaccharides.

Specialized Traps for Interference-Free Quantification

Borate can affect monosaccharide peak symmetry, even when present in the low part-per-billion concentration range. Borate is one of the first ions to break through a water deionization system. Its presence in water used to make eluents causes a significant loss of peak efficiency, especially for mannose and reduced monosaccharides. The Dionex BorateTrap column is used immediately before the injection valve to remove borate from the eluent prior to sample injection (Figure 8).

Column:	Dionex AminoTrap, Dionex CarboPac PA10
Eluent:	18 mM Sodium Hydroxide
Flow Rate:	1.5 mL/min
Detection:	Pulsed Amperometry, ED40, gold electrode
Peaks	1. Fucose 5 pmol
	2. Galactosamine 5
	3. Glucosamine 5
	4. Galactose 5
	5. Glucose 5
	6. Mannose 5

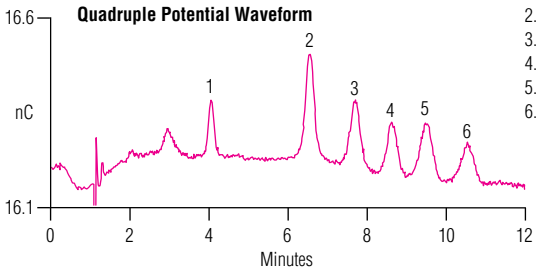


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Figure 6. High sensitivity analysis using the Dionex CarboPac PA10 column.

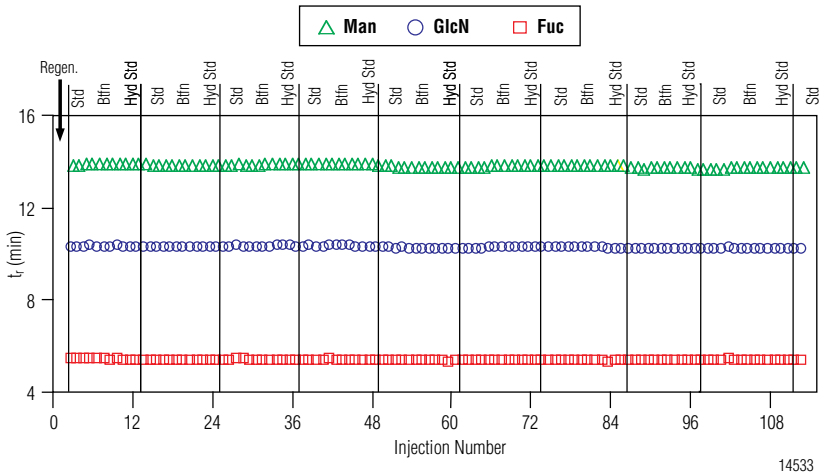

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Figure 7. Monosaccharide retention time stability: Isocratic on-line eluent generation (30 min/sample plus 5 min regeneration at 100 mM sodium hydroxide to ensure amino acids are eluted).

Column:	Dionex CarboPac PA10
Eluent:	18 mM Sodium NaOH, 10 ng/mL borate
Flow Rate:	1.5 mL/min
Detection:	Pulsed Amperometry, ED40, gold electrode
Peaks	1. Fucose
	2. Galactosamine
	3. Glucosamine
	4. Galactose
	5. Glucose
	6. Mannose

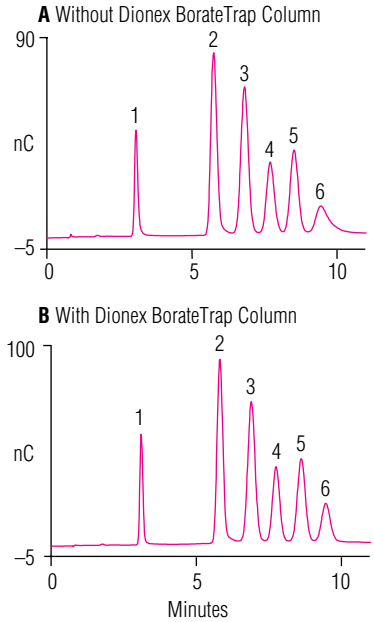

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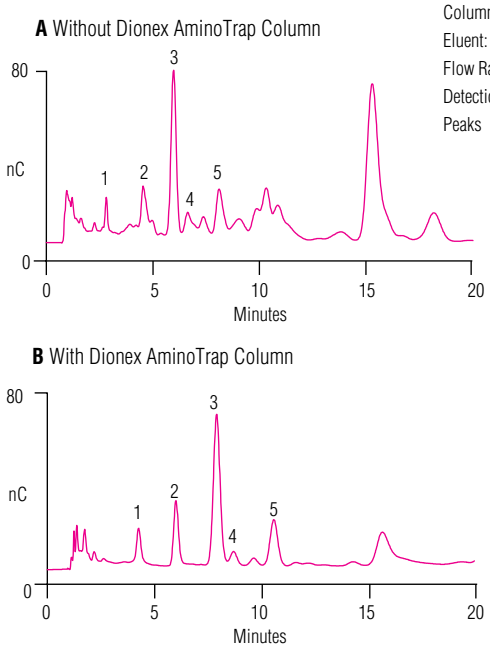
Figure 8. Effect of borate and the Dionex BorateTrap column on monosaccharide peak symmetry.

The detection of monosaccharides and amine-containing glycoconjugates with low levels of glycosylation can be compromised from fouling of the working electrode by amino acids. Specifically designed to retain amino acids, the Dionex AminoTrap column allows monosaccharides to be eluted well before interfering amino acids such as lysine. The Dionex AminoTrap is a 4 × 50 mm in-line pretreatment column that is used before the Dionex CarboPac PA10 analytical column instead of the Dionex CarboPac PA10 guard column (Figure 9).

Rugged, Reliable Analyses with Guaranteed Performance

The polymeric Dionex CarboPac PA1 and PA10 columns have a crosslinked structure to ensure long column life and stability from pH 0–14 at all concentrations of buffer salts (Figure 10). The Dionex CarboPac PA10 column is more highly crosslinked, making it more solvent compatible. The entire manufacturing process is carefully controlled in the Thermo Scientific ISO 9001-registered facility to ensure that every Dionex CarboPac column delivers reproducible performance. All Dionex CarboPac column products are tested with a set of carbohydrate standards to ensure lot-to-lot reproducibility. The Dionex CarboPac PA1 column is available in 2 × 250 mm, 4 × 250 mm and 9 × 250 mm PEEK hardware formats, while the Dionex CarboPac PA10 column is available in 0.4 × 250 mm capillary, 2 × 250 mm and 4 × 250 mm PEEK hardware formats. The Dionex CarboPac PA10 0.4 × 150 mm capillary column offers the additional advantage of higher mass sensitivity, lower sample amounts and significantly lower solvent use. The Dionex CarboPac PA1 9 × 250 mm column is designed for semi-preparative configurations.



Column:	Dionex CarboPac PA10
Eluent:	18 mM Sodium hydroxide
Flow Rate:	1.5 mL/min
Detection:	Pulsed amperometry, ED40, gold electrode
Peaks	1. Fucose 2. Deoxyglucose (internal standard) 3. Glucosamine 4. Galactose 5. Mannose

Figure 9. Effect of the Dionex AminoTrap column on monosaccharide composition analysis of IgG hydrolysate.

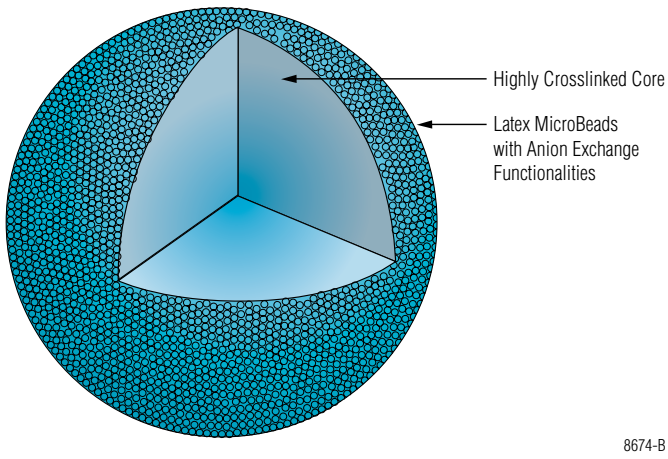
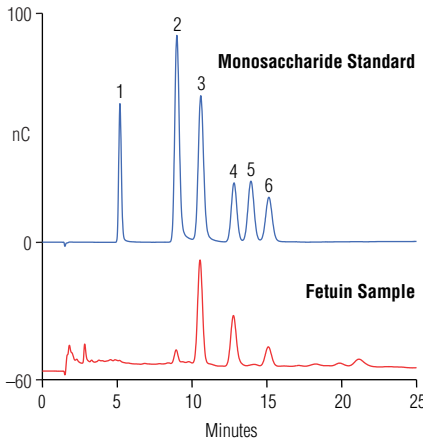


Figure 10. Pellicular anion-exchange resin bead.



Column:	Dionex CarboPac PA10G (0.4 × 50 mm) Dionex CarboPac PA10 (0.4 × 250 mm)
Eluent:	Cleaning: 100 mM KOH Elution: 18 mM KOH
Eluent Source:	Thermo Scientific™ Dionex™ EGC-KOH (Capillary) Cartridge
Temperature:	30 °C
Flow Rate:	0.01 mL/min
Inj. Volume:	0.4 µL
Detection:	Integrated Amperometry, quadruple pulse waveform
Working Electrode:	PTFE Gold, disposable electrode
Reference Electrode:	Silver/Silver Chloride
Sample:	0.4 nmol/mL Fetuin Hydrosylate
Standard:	50 nmol/mL each (with DI water)
Peaks:	1. Fucose 4. Galactose 2. Galactosamine 5. Glucose 3. Glucosamine 6. Mannose

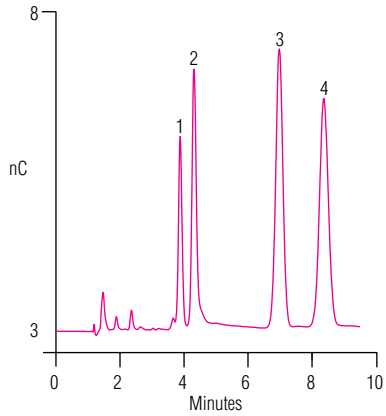
Eluent Program:

Time	KOH Concentration in mM	Comments
–45	100	Strong Wash
–25	100	
–24.9	18	Equilibration
0	18	Load/Inject
25	18	

Figure 11. Monosaccharide analysis of fetuin hydrolysate sample using Dionex CarboPac PA10 capillary column.

Official Methods Using the CarboPac PA1

The Dionex CarboPac PA1 column has been approved for use in a number of official methods, including ISO/DIS 11292 for coffee authenticity, International Committee of Uniform Methods of Sugar Analysis (ICUMSA) for sugars in molasses, and AOAC Methods 996.04 and 2000.11. Figure 12 shows an example of an isocratic method to determine sugars in molasses. Table 1 lists official food methods using Thermo Scientific technology.



Column: Dionex CarboPac PA1 and guard
Eluent: 150 mM Sodium hydroxide
Flow Rate: 1 mL/min
Inj. Volume: 50 µL
Detection: Pulsed amperometry
Peaks 1. Glucose 4.39%
2. Fructose 6.67
3. Lactose (internal standard)
4. Sucrose 30.8

10930

Figure 12. Isocratic separation of sugars found in sugarcane molasses. Official method of the International Commission for Uniform Methods of Sugar Analysis.

Table 1. Official food methods using Thermo Scientific technology.

Analysis	Dionex Technology	Official Method
Sugars in molasses	HPAE-PAD/CarboPac PA1	AOAC 996.04 ICUMSA (1994)
Carbohydrates in soluble coffee	HPAE-PAD/CarboPac PA1	AOAC 995.13 ISO 11292
Fructans in food	HPAE-PAD/CarboPac PA1	AOAC 997.08
Anions in beer by IC	IC with suppressed conductivity	Analytica-EBC International Method
Polydextrose	HPAE-PAD/CarboPac PA1	AOAC 2000.11
Transgalacto-oligosaccharides	HPAE-PAD/CarboPac PA1	AOAC method validation study in progress

SPECIFICATIONS

Dionex CarboPac PA1 Column	
Resin Composition:	10-µm diameter substrate (polystyrene 2% crosslinked with divinylbenzene) agglomerated with 500-nm MicroBead quaternary ammonium functionalized latex (5% crosslinked)
Anion Exchange Capacity:	Approximately 100 µeq/column (4 × 250 mm analytical column)
Maximum Operating Pressure:	4000 psi (27.9 MPa)
Chemical Compatibility:	pH 0–14, up to 2% of common HPLC solvents
Dionex CarboPac PA10 Column	
Resin Composition:	10-µm diameter substrate (ethylvinylbenzene 55% crosslinked with divinylbenzene) agglomerated with 460-nm MicroBead difunctional quaternary ammonium ion (5% crosslinked)
Anion Exchange Capacity:	Approximately 100 µeq/column (4 × 250 mm analytical column), or 1 µeq/column (0.4 × 250 mm capillary column)
Maximum Operating Pressure:	4000 psi (27.9 MPa)
Chemical Compatibility:	pH 0–14, up to 90% of common HPLC solvents
Dionex AminoTrap Column	
Resin Composition:	10-µm diameter substrate (ethylvinylbenzene 55% crosslinked with divinylbenzene) grafted with difunctional quaternary ammonium anion exchange sites
Maximum Operating Pressure:	4000 psi (27.9 MPa)
Chemical Compatibility:	pH 0–14, up to 90% of common HPLC solvents
Dionex BorateTrap Column	
Resin Composition:	20-µm diameter high capacity resin with very high selectivity for borate
Maximum Operating Pressure:	4000 psi (27.9 MPa)
Chemical Compatibility:	pH 0–14, up to 90% of common HPLC solvents

Ordering Information

In the U.S., call (800) 346-6390 or contact the Thermo Fisher Scientific Regional Office nearest you. Outside the U.S., order through your local Thermo Fisher Scientific office or distributor. Refer to the following part numbers. Thermo Fisher Scientific can also make special order Dionex CarboPac columns to your specifications; call for more information.

Description	Part Number
Dionex CarboPac PA1 Analytical Columns	
Analytical Column (4 × 250 mm)	035391
Guard Column (4 × 50 mm)	043096
Analytical Column (2 × 250 mm)	057178
Guard Column (2 × 50 mm)	057179
Dionex CarboPac PA10 Analytical Columns	
Analytical Column (4 × 250 mm)	046110
Guard Column (4 × 50 mm)	046115
Analytical Column (2 × 250 mm)	057180
Guard Column (2 × 50 mm)	057181
Capillary Column (0.4 × 250 mm)	082320
Capillary Guard Column (0.4 × 50 mm)	082321
Dionex AminoTrap Column (4 × 50 mm)	046122
Dionex BorateTrap Column (4 × 50 mm)	047078
MonoSaccharide Standard	
Thermo Scientific™ Dionex™ MonoStandards™ Mixture of Six, 100 nmol each. Contains fucose, galactosamine HCl, glucosamine HCl, galactose, glucose and mannose	043162

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France +33 1 39 30 01 10
Germany +49 6126 991 0
India +91 22 2764 2735
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