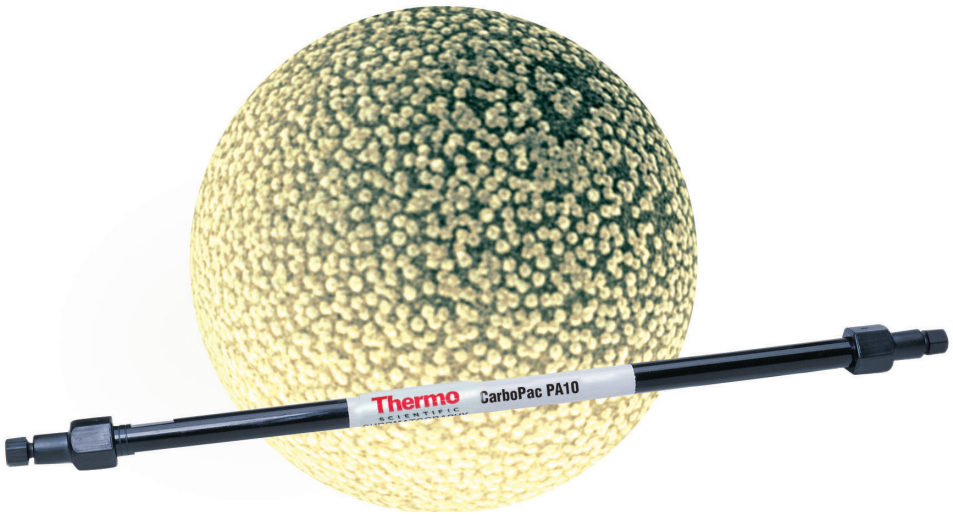
Ion chromatography

Thermo Scientific Dionex CarboPac PA10 Column for Mono- and Disaccharide Analysis

Thermo Scientific™ Dionex™ CarboPac™ PA10 Columns are ideal for the separations of mono- and disaccharides found in mammalian glycoproteins

- Simple, isocratic separations
- Gradient compatible
- No derivatization required



Unique column chemistry for optimal performance

The Dionex CarboPac PA10 column is a specialized anion-exchange column designed to be used with pulsed amperometric detection to deliver high resolution separations of mono- and disaccharides. The resins consists 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 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 microbore and standard bore formats.

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 column 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 Thermo Scientific™ 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.

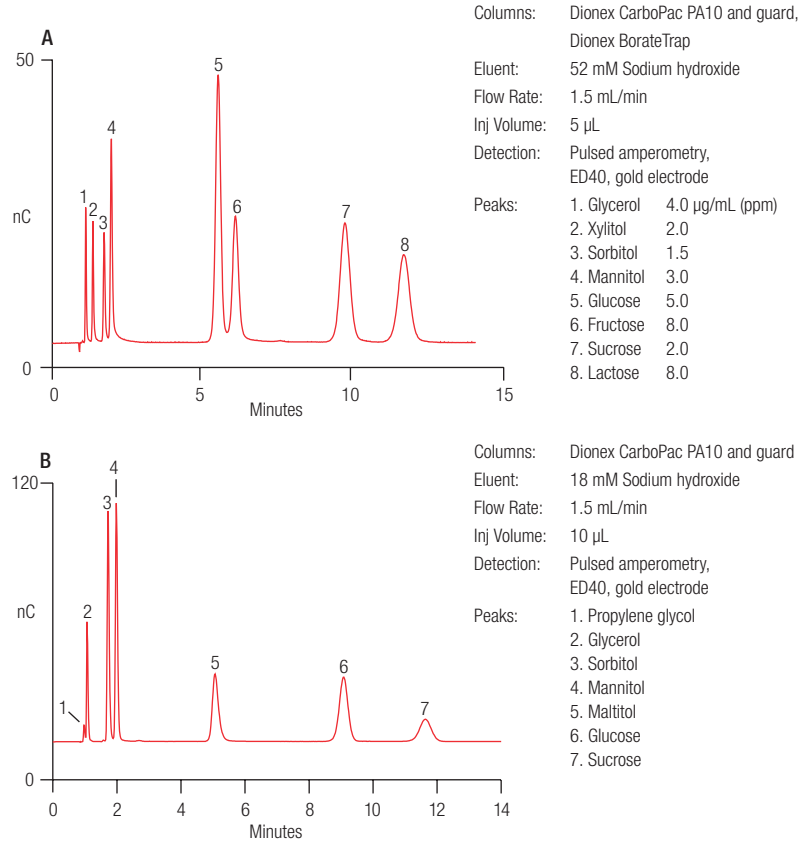


Figure 1. The Dionex CarboPac PA10 column resolves mixtures of sugar alcohols. A) Sugar alcohols and other carbohydrates found in foods. B) Glycols, sugar alcohols, and carbohydrates in a pharmaceutical formulation.

Glycoprotein monosaccharide compositional analysis

The Dionex CarboPac PA10 column is 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 Thermo Scientific™ 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.

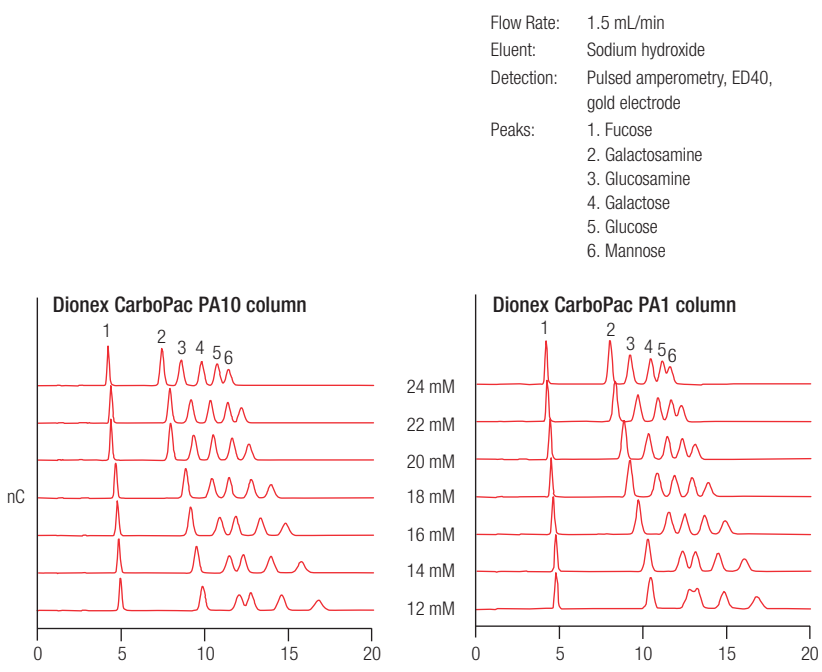


Figure 2. Comparison of the Dionex CarboPac PA1 and PA10 columns for glycoprotein monosaccharides.

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.

The elution of acidic sugars from the Dionex CarboPac PA1 or Dionex CarboPac PA10 column requires stronger eluents than those used for neutral sugars (Figure 3). 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.

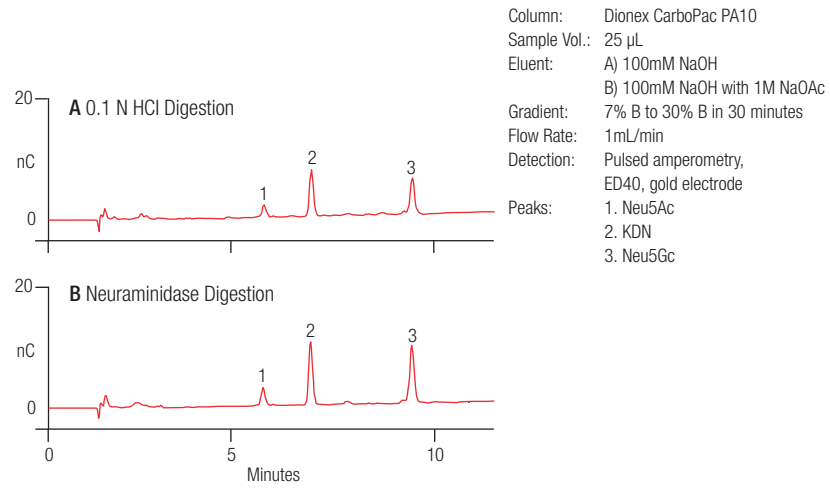


Figure 3. Sialic acid analysis of bovine transferrin on the Dionex CarboPac PA10 column.

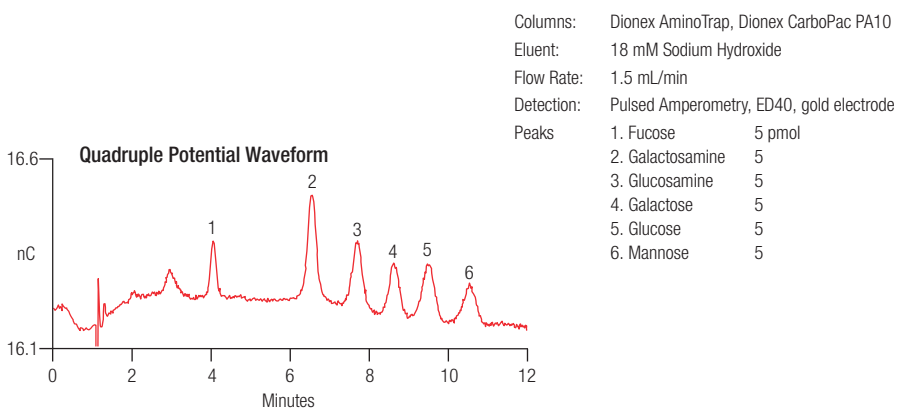


Figure 4. High sensitivity analysis using the Dionex CarboPac PA10 column.

High throughput, high sensitivity analyses

Figure 4 shows typical resolution for the separation of 5 pmol each of six monosaccharides using the Dionex CarboPac PA10 column. The Thermo Scientific™ Dionex™ 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 Dionex EGC cartridge is used in conjunction with the Dionex CarboPac PA10 and the AminoTrap columns for reproducible, high throughput, high sensitivity carbohydrate applications (Figure 5). The advantages of the use of the Dionex EGC cartridge for carbohydrate analysis include simplified eluent

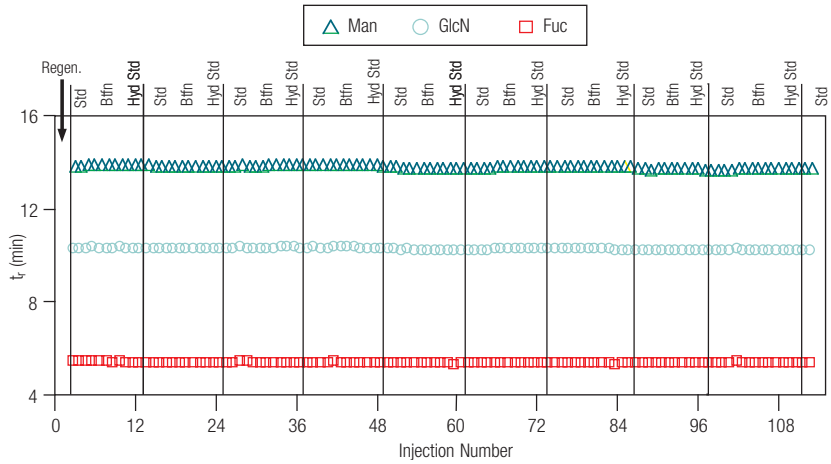


Figure 5. Monosaccharide retention time stability: Isocratic on-line eluent generation (30 min/sample plus 5 min regeneration at 100 mM sodium hydroxide to ensure amino acids are eluted).

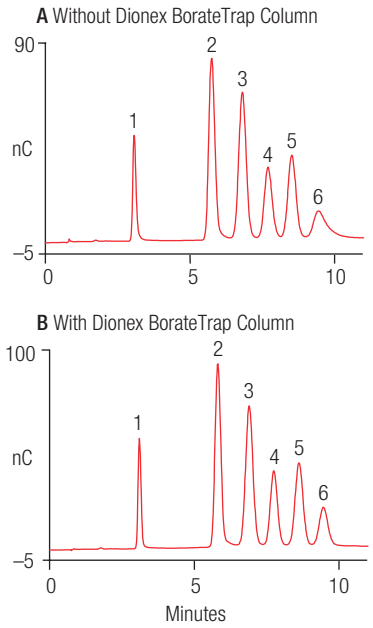
generation, because only degassed deionized water is required. Problems caused by minor differences in prepared eluents are avoided, as are salt deposits in the pump.

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

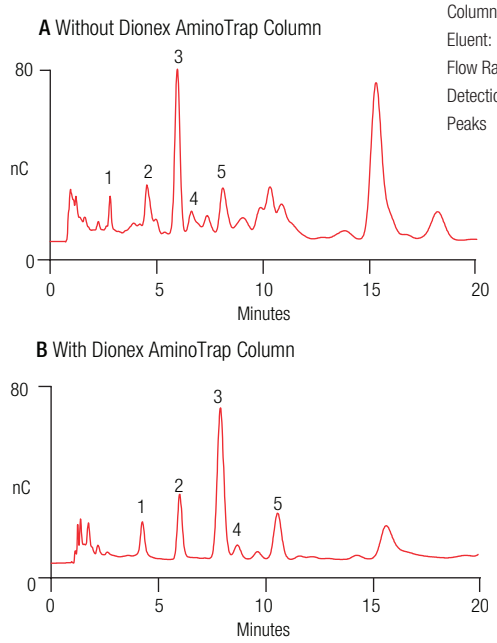
Borate can affect monosaccharide peak symmetry, even when present in the low part-per-billion concentration range. Borate is one of the first ions to break through a water deionization system. Its presence in water used to make eluents causes a significant loss of peak efficiency, especially for mannose 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 6).

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 7).



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

Figure 6. Effect of borate and the Dionex BorateTrap column on monosaccharide peak symmetry.



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

Rugged, reliable analyses with guaranteed performance

The polymeric Dionex CarboPac PA10 column has a crosslinked structure to ensure long column life and stability from pH 0–14 at all concentrations of buffer salts (Figure 8). The Dionex CarboPac PA10 column is 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 PA10 column is available in 2 × 250 mm and 4 × 250 mm PEEK hardware formats.

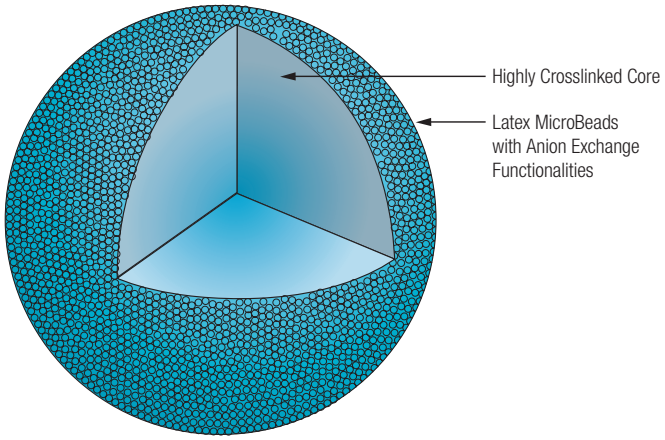


Figure 8. Pellicular anion-exchange resin bead.

Specifications	
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)
Maximum operating pressure	4,000 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	4,000 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	4,000 psi (27.9 MPa)
Chemical compatibility	pH 0–14, up to 90% of common HPLC solvents

Ordering information

To order in the U.S., call 1-800-346-6390, or contact the Thermo Fisher Scientific office nearest you. Outside the U.S., order through your local Thermo Fisher Scientific office or distributor. Refer to the following part numbers.

Description	Part number
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
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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