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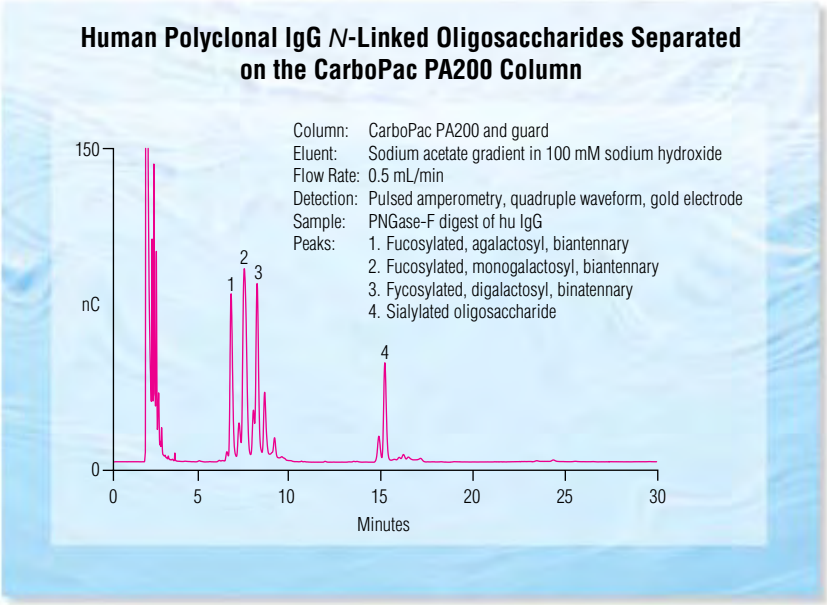
CarboPac™ PA200 Column Solutions for Oligosaccharide Analysis



CarboPac PA200 HPLC columns for high-resolution oligosaccharide mapping and analysis:

- *Predictable, high-resolution separations of oligosaccharides released from glycoproteins*
- *Neutral and sialylated* N*-linked oligosaccharides from glycoproteins*
- *Oligosaccharides with monosaccharide linkage isomerism*
- *Linear polysaccharide profiling*
- *Desalted fraction collection*

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Thermo SCIENTIFIC

Predictable, High-Resolution Separations of Oligosaccharides Released from Glycoproteins

There is a significant and increasing demand for reproducible, fast, and simple methods in the biotechnology and pharmaceutical industry for the profiling of oligosaccharides released from glycoprotein therapeutics. This demand for carbohydrate analysis is also apparent in the food industry where the need for fast, high-resolution profiling of homologous sugar series such as inulins, amylopectins, and maltooligosaccharides is also evident. Although a variety of HPLC approaches are proposed for these applications, in general most prove inadequate for separating complex mixtures and are limited by their nonspecific nature and low limits of detection.

The CarboPac PA200 is a nonporous, high-efficiency, polymeric anion-exchange column that provides the highest resolution available for oligosaccharide mapping and analysis, using a technique known as high-performance anion-exchange (HPAE) chromatography with pulsed amperometric detection (PAD). The resin consists of 5.5-µm-diameter nonporous beads covered with a fine layer of functionalized MicroBead™ latex. This pellicular resin structure permits excellent mass transfer, resulting in high-resolution chromatography and rapid reequilibration. The 3 × 250 mm format provides fast separations and the 0.5 mL/min recommended flow rate results in significant savings in eluent consumption.

DIONEX

The advent of HPAE-PAD, introduced by Dionex Corporation in the 1980s, revolutionized carbohydrate analyses by allowing separations at high pH and detection without the need for derivatization. The major advantages of HPAE-PAD are (1) fast analyses, (2) ease of use—samples are directly analyzed without the need for derivatization or the need for sample cleanup, (3) low- to sub-picomole range sensitivity, and (4) high resolution (e.g., separation of anomeric and positional isomers—HPAE separates all classes of oligosaccharides according to structural features such as size, charge, composition, anomericity, and linkage isomerism).

There are several factors affecting the elution of oligosaccharides by HPAE-PAD. The empirical relationships between oligosaccharide structure and chromatographic retention are well-documented (Rohrer, *J. Glycobiology*[1]):

- Fucosylated oligosaccharides are eluted ahead of their afucosylated analogs.
- As the number of mannose residues in a high mannose oligosaccharide increases, its retention time also increases.
- As the degree of branching increases, the retention time of the oligosaccharide increases (see Figure 1).
- Removal of the terminal galactose residues from a complex oligosac charide reduces its retention time.

Neutral and Sialylated *N*-Linked Oligosaccharides from Glycoproteins

The elution of acidic sugars from the CarboPac PA200 column requires stronger eluents than those used for the elution of neutral sugars. A stronger eluent is usually accomplished by adding sodium acetate to the sodium hydroxide eluent. Sodium acetate accelerates the elution of strongly bound species without compromising selectivity and without interfering with pulsed amperometric detection. Figures 2 and 3 show the separation of neutral oligosaccha-

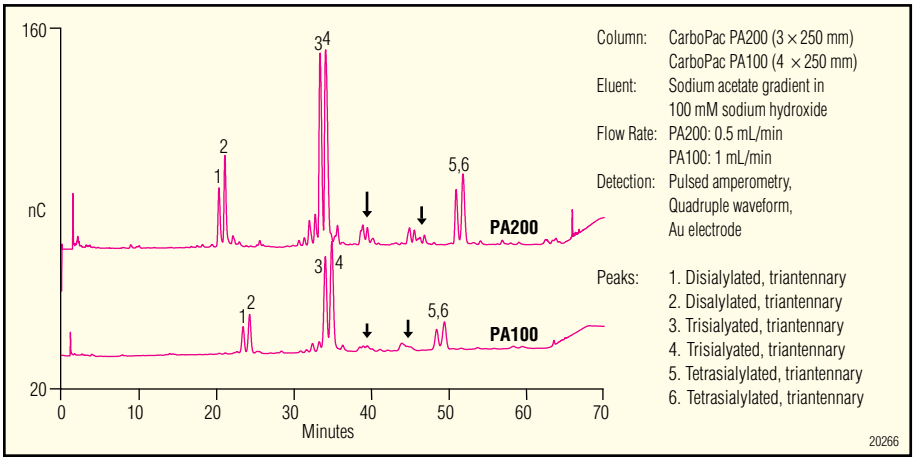

Figure 1. Fetuin oligosaccharide profile.

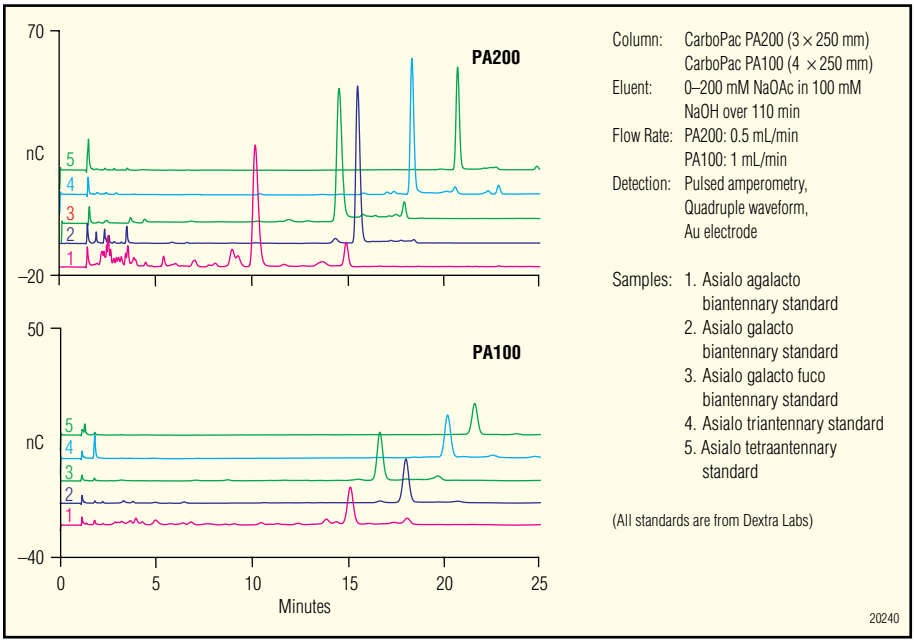

Figure 2. CarboPac PA200 vs CarboPac PA100. Separation of neutral N*-linked oligosaccharides (complex neutral structures).*

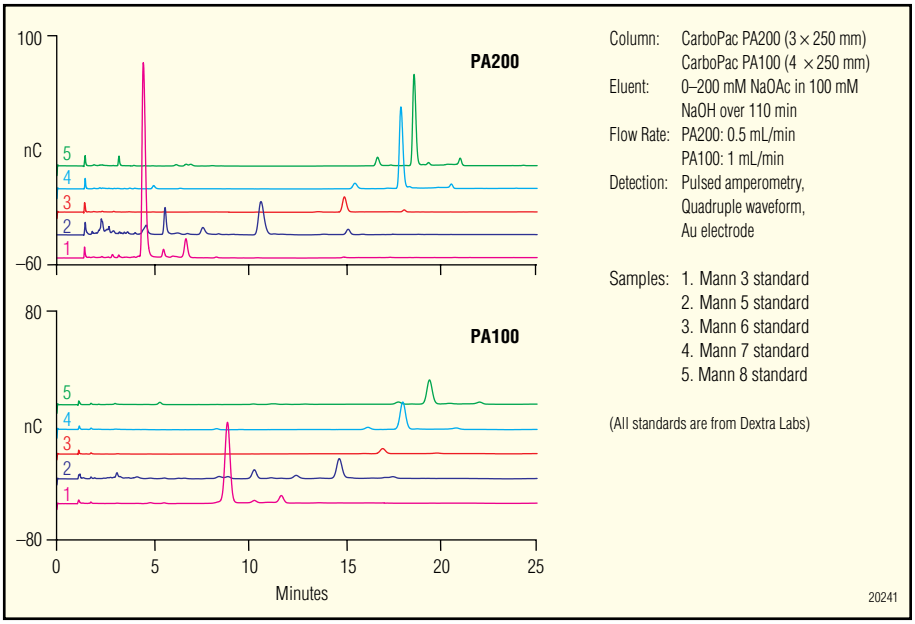

Figure 3. CarboPac PA200 vs CarboPac PA100. Separation of neutral (high mannose) N*-linked oligosaccharides.*

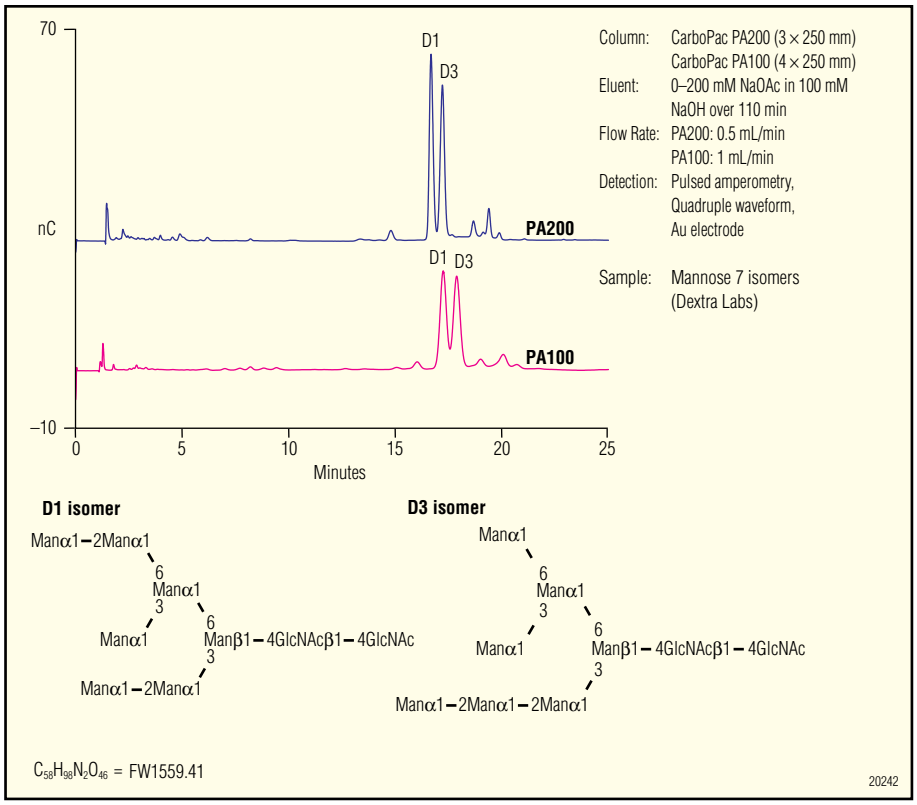

Figure 4. CarboPac PA200 vs CarboPac PA100. Separation of mannose 7 D1 and D3 isomers.

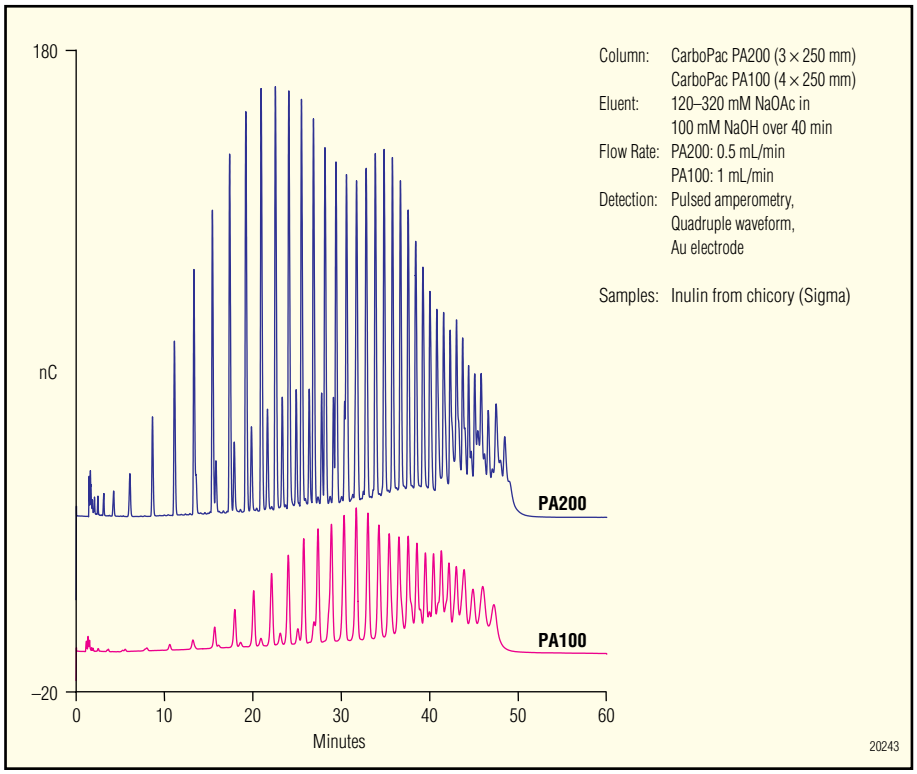

Figure 5. Inulin Profiles: CarboPac PA200 vs CarboPac PA100.

rides on both the CarboPac PA100 and PA200. The higher efficiency of the CarboPac PA200 is very apparent in these chromatograms, not only in terms of the increased peak height of the major peaks, but also in the detail of the minor peaks.

Oligosaccharides with Monosaccharide Linkage Isomerism

High-resolution separations can be obtained based on linkage isomerism, which is difficult to achieve using other chromatography technologies. Under alkaline conditions, the technique resolves these species, not only by sugar content, but also according to where the sugars are attached. Figure 4 shows the separation of the D1 and D3 mannose 7 isomers. Figure 4 also illustrates the difference in the structures. The isomers are baseline resolved on the PA200, and other minor peaks are also clearly resolved.

Linear Polysaccharide Profiling

Inulin and fructooligosaccharides (FOS) are increasingly being used as functional food ingredients. Chain-length distribution profiles of commercial products such as those derived from inulin can be determined using HPAE-PAD with gradient elution (see Figure 5). Commercial food ingredient products derived from the lower-molecular-weight fractions of inulin (DP3-20) can be determined by AOAC Method 997.08, although a more direct method has been developed[2] that allows commercially available FOS and inulin products to be identified and quantified directly in a variety of foods by HPAE-PAD.

Fraction Collection

The Carbohydrate Membrane Desalter (CMD™) is a membrane device designed for users of HPAE-PAD who need to collect and further analyze carbohydrate samples. Desalted samples are ready for lyophilization without dialysis. Greater than 99% of the sodium ions in eluents that contain up to 0.35 M sodium ions flowing at a rate of 1 mL/min will be removed by the CMD.

Placed after the electrochemical detector, the CMD exchanges sodium ions for hydronium ions. This process changes the sodium hydroxide and sodium acetate eluents to water and acetic acid immediately after leaving the detector cell. Collected fractions can then be lyophilized, leaving the pure carbohydrate sample ready for further manipulation. These samples are suitable for enzymatic and chemical digestion, NMR, mass spectrometric, or further chromatographic analysis.

Guaranteed Performance

The unique pellicular resin of the CarboPac PA200 columns offers exceptional selectivity and stability over the entire pH range. Its highly cross-linked structure ensures long column life and easy cleanup. The entire manufacturing process (resin synthesis, amination, and packing and testing of the chromatographic columns) is carefully controlled to ensure that every Dionex CarboPac PA200 column delivers reproducible performance. CarboPac PA200 columns are tested with two isomers of *N*-acetyl neuraminosyl-d-lactose to ensure lot-to-lot reproducibility.

SPECIFICATIONS

Resin Composition:
5.5-µm-diameter ethylvinylbenzene/divinylbenzene substrate (55% cross-linking) agglomerated with 43-nm MicroBead 6% cross-linked quaternary amine-functionalized latex

Anion-Exchange Capacity:
35 µeq/column

Maximum Operating Pressure:
4000 psi (28 MPa)

Chemical Compability:
pH 0–14, 100% compatible with common HPLC solvents

Temperature Limit:
60 °C

Quality Assurance Procedure:
Separation of alpha (2,6) NANLac and alpha (2,3) NANLac isomers

Typical Operating Conditions:
2,700 psi at 0.5 mL/min (guard and analytical)

Recommended Operating Temperature:
Controlled ambient (~30 °C)

Recommended Flow Rate:
0.5 mL/min

Ionic Form Eluents:
Sodium hydroxide and sodium acetate only

ORDERING INFORMATION

In the U.S., call 1-800-346-6390 or contact the Dionex regional office nearest you. Outside the U.S., order through your local Dionex office or distributor. Refer to the following part numbers.

Description	Part Number
CarboPac PA200 Analytical Column (3 × 250 mm)	062896
CarboPac PA200 Guard Column (3 × 50 mm)	062895
Carbohydrate Membrane Desalter (CMD-I)	059090
CMD-I Start-Up Package (CMD-I desalter, power supply, tubing, fitting)	059091

REFERENCES

1. Rohrer, J. *J. Glycobiology*, **1995**, *5*, 359–360.
2. Durgnat J. M., Martinez C. Determination of Fetuin Oligosaccharides in Raw Materials and Finished Products by HPAE-PAD. *Semin. Food Anal.* **1997**, *2*, 85–97.

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