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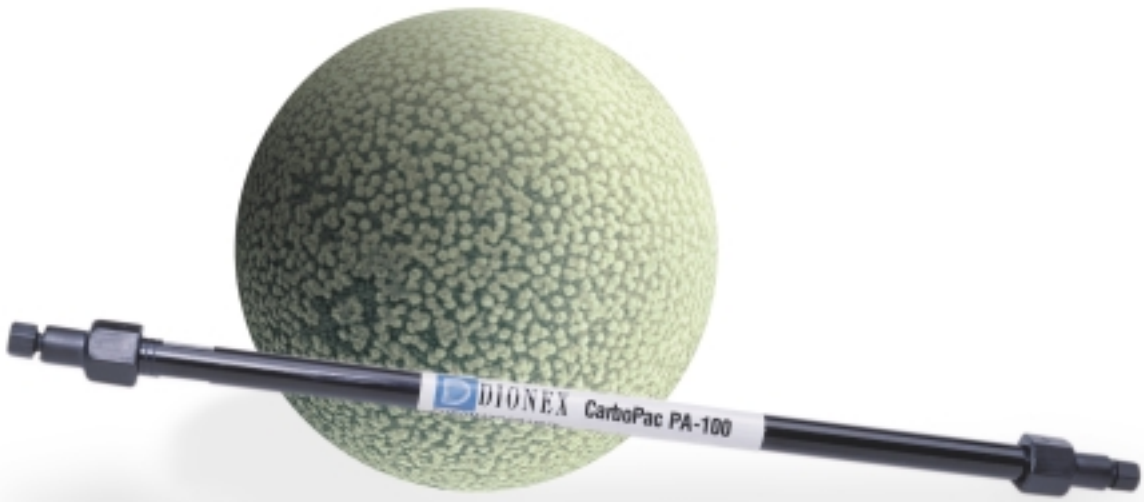
CarboPac® PA-100 Column for Oligosaccharide Analysis



HPLC Columns for Oligosaccharide Mapping and Purification

- *Predictable, high resolution separations of oligosaccharides released from glycoproteins*
- *Neutral and sialylated N-linked oligosaccharides from glycoproteins*
- *Oligosaccharides with monosaccharide linkage isomerism*
- *Oligosaccharides in food products*
- *Linear polysaccharide profiling*
- *CMD™ Carbohydrate Membrane Desalter for fraction collection*

Predictable, High Resolution Separations of Oligosaccharides Released from Glycoproteins

Characterization of oligosaccharides released from glycoproteins is an important but nontrivial task for the biotechnology and pharmaceutical industries. The CarboPac PA-100 column provides the resolution needed for routine analysis of these oligosaccharides. Neutral and charged oligosaccharides are separated in their anionic forms by high-performance anion-exchange chromatography (HPAE) using the CarboPac PA-100 column. Detection of these compounds at low picomole levels has been optimized using pulsed amperometric detection (PAD). No sample derivatization is required for detection.

Separations of oligosaccharides are based on their fine structural differences, such as the composition and the sequence of the oligosaccharides, linkage isomerism, degree of sialylation, and degree of branching.

There are several factors affecting the elution of oligosaccharides using the CarboPac PA-100 column. Twelve empirical relationships between oligosaccharide structure and chromatographic retention are documented by Rohrer (Rohrer, J. *Glycobiology*, **1995**, 5, 359-360). Some of these are illustrated in Figure 1 and include:

- Fucosylated oligosaccharides are eluted ahead of their afucosylated analogs (peaks 1 and 2).
- Retention times of high mannose oligosaccharides increase as the number of mannose residues increases (peaks 2, 5, 9).

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- As the degree of branching increases, the retention time of the oligosaccharide increases (peaks 8, 9, 11).
- Removal of the terminal galactose residues from a complex oligosaccharide reduces its retention time (peaks 7–9, 4–8) .

Neutral and Sialylated *N*-linked Oligosaccharides from Glycoproteins

The elution of acidic sugars from the CarboPac PA-100 column requires stronger eluents than those used for the elution of neutral sugars. This is usually accomplished with the addition of sodium acetate to the sodium hydroxide eluent. Sodium acetate accelerates the elution of strongly bound species and offers further selectivity control, without interfering with pulsed amperometric detection. Figure 2 shows the separation of neutral and sialylated oligosaccharides in a single run. The neutral sugars are eluted in a group at the beginning of the profile followed by the disialylated, the trisialylated, and finally the tetrasialylated oligosaccharides.

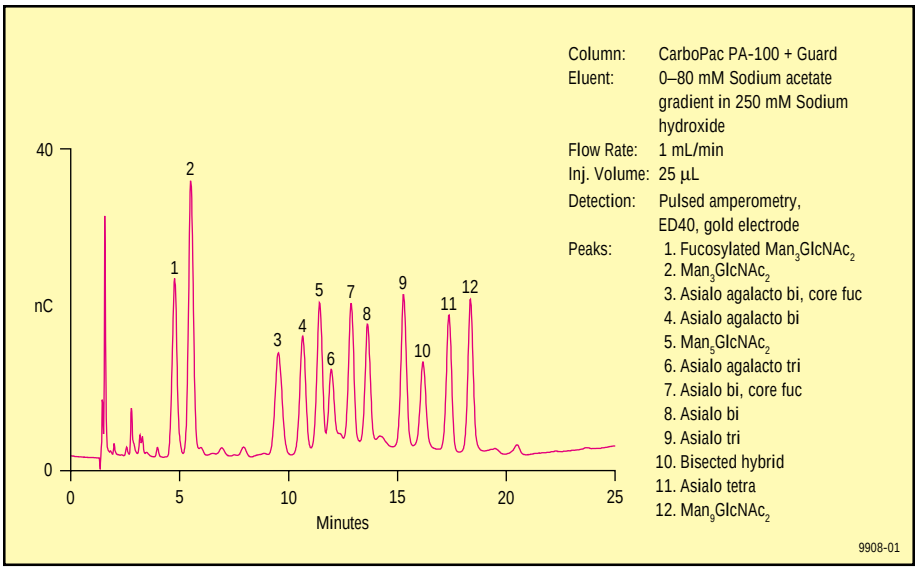

Figure 1. Separation of neutral oligosaccharide standards. Twelve commonly occurring N-linked neutral oligosaccharides are easily resolved within 20 minutes.

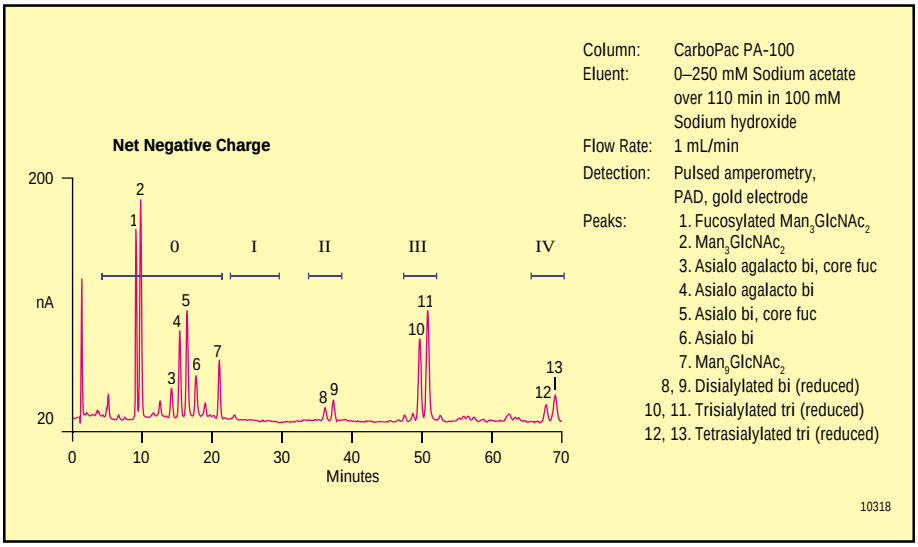

Figure 2. Separation of neutral and sialylated oligosaccharide standards. This is a commonly used method to obtain an overall profile of neutral and sialylated N-linked oligosaccharides released from glycoproteins. Oligosaccharides are separated into broad classes depending on their degree of sialylation. Based on the retention time windows of the oligosaccharide peaks, the degree of sialylation of the oligosaccharides can be predicted.

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 sialic acid content, but also according to the combination of α(2,3)- and α(2,6)-linked sialic acids within each charge class. Oligosaccharides with the greatest proportion of α(2,6)- to α(2,3)-linked sialic acids are the least retained (Figure 3). The neutral component of the oligosaccharides also influences separation: those containing a Galβ(1,3)GlcNAc sequence are retained longer than those with Galβ(1,4)GlcNAc.

Oligosaccharides in Food Products

Oligosaccharides are routinely determined in food and beverage products for a variety of purposes, including quality control, verifying food-labeling claims, establishing product authenticity, and monitoring fermentation processes. Oligosaccharide profiles obtained using HPAE-PAD with the CarboPac PA-100 can be used to establish the "fingerprint" of food samples. Suspect samples can be analyzed and compared to the known profiles. This technique is useful in detecting adulteration and in quality control. For example, oligosaccharide profiles are used to detect adulteration of natural fruit juices (Figure 4), establish the geographic origin of molasses, and analyze polysaccharides in hydrolyzed glucose syrup.

The CarboPac PA-100 column easily separates mono-, di-, and trisaccharides in citrus and sunflower honey (Figure 5). These profiles are used as "fingerprints" for quality control and labeling verification purposes.

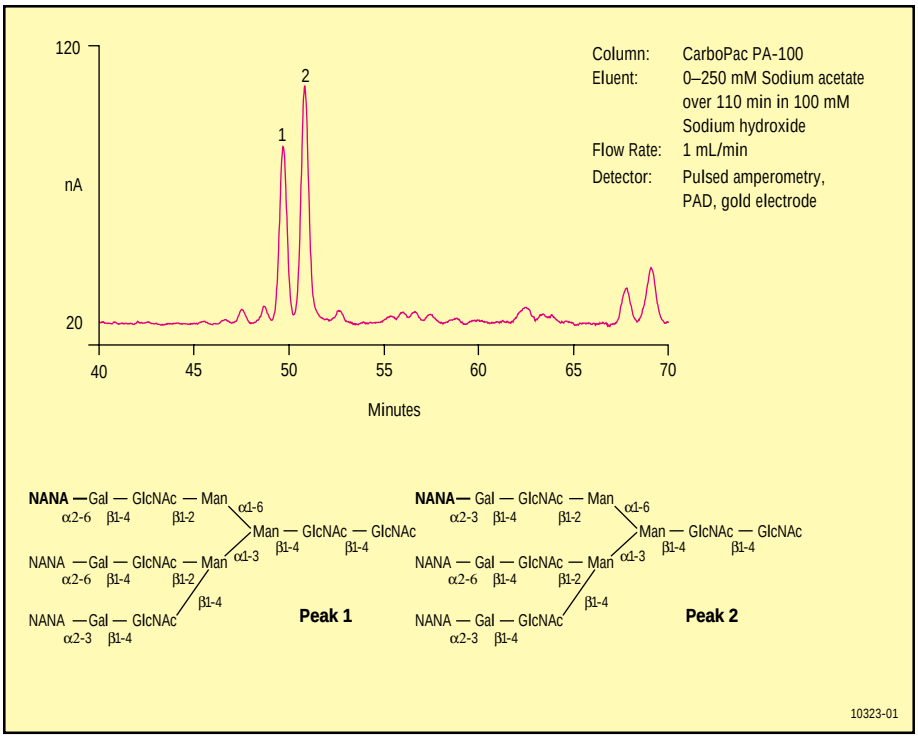

Figure 3. Separation of trisialylated oligosaccharides. The high resolving power of the CarboPac PA-100 column is demonstrated by the resolution of these two oligosaccharides, which differ by only a single linkage—alpha 2-6 vs. alpha 2-3.

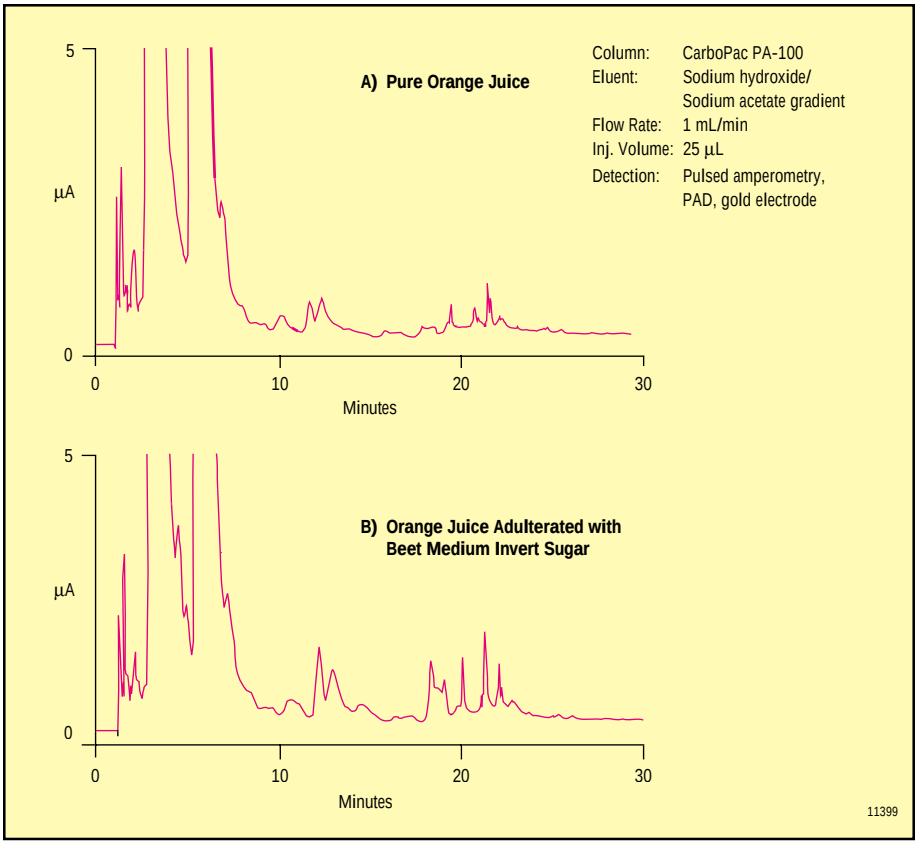

Figure 4. Oligosaccharide profiles of pure and adulterated orange juice. Oligosaccharide composition profiles can be used to detect the adulteration of natural fruit juices by inexpensive sweeteners such as beet sugar.

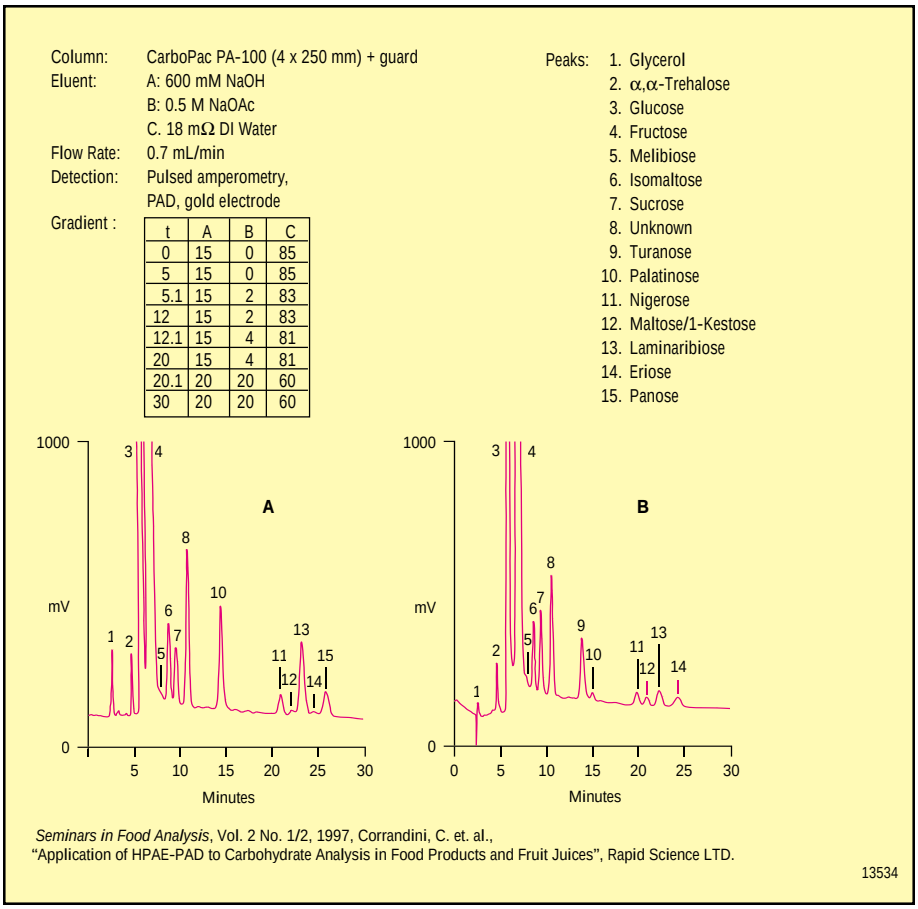

Figure 5. Chromatograms of di- and trisaccharides in honey from (A) citrus and (B) sunflower.

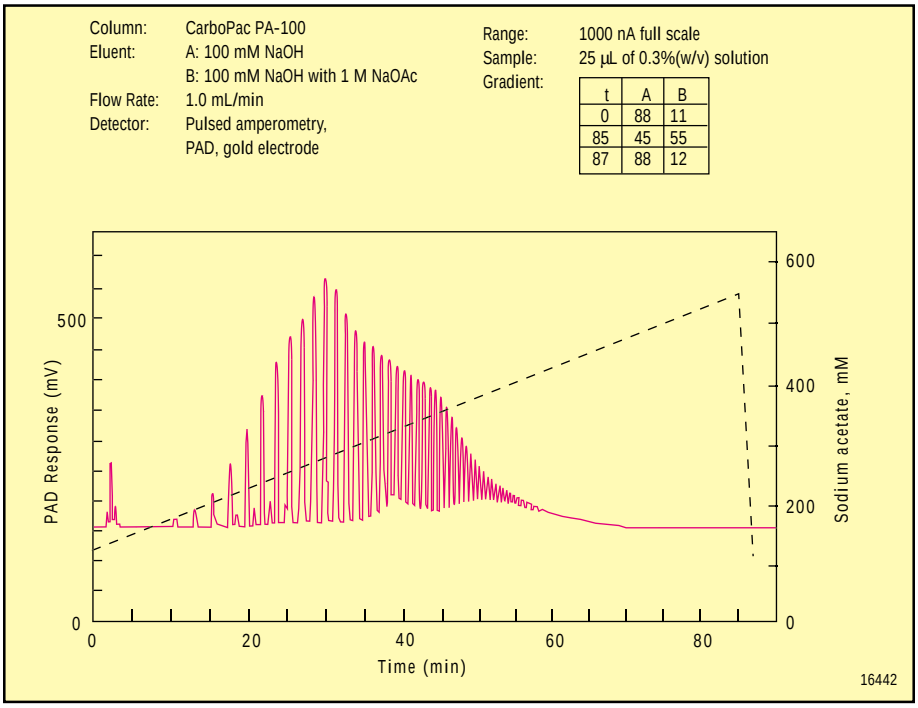

Figure 6. Gradient separation of chicory inulin using the CarboPac PA-100.

Linear Polysaccharide Profiling

Separations of high mannose, hybrid, and complex oligosaccharides are best accomplished using the CarboPac PA-100 column. Separations are accelerated and improved by using sodium acetate gradients in sodium hydroxide. Figure 6 shows the gradient separation of chicory inulin, illustrating the high resolution possible with this column.

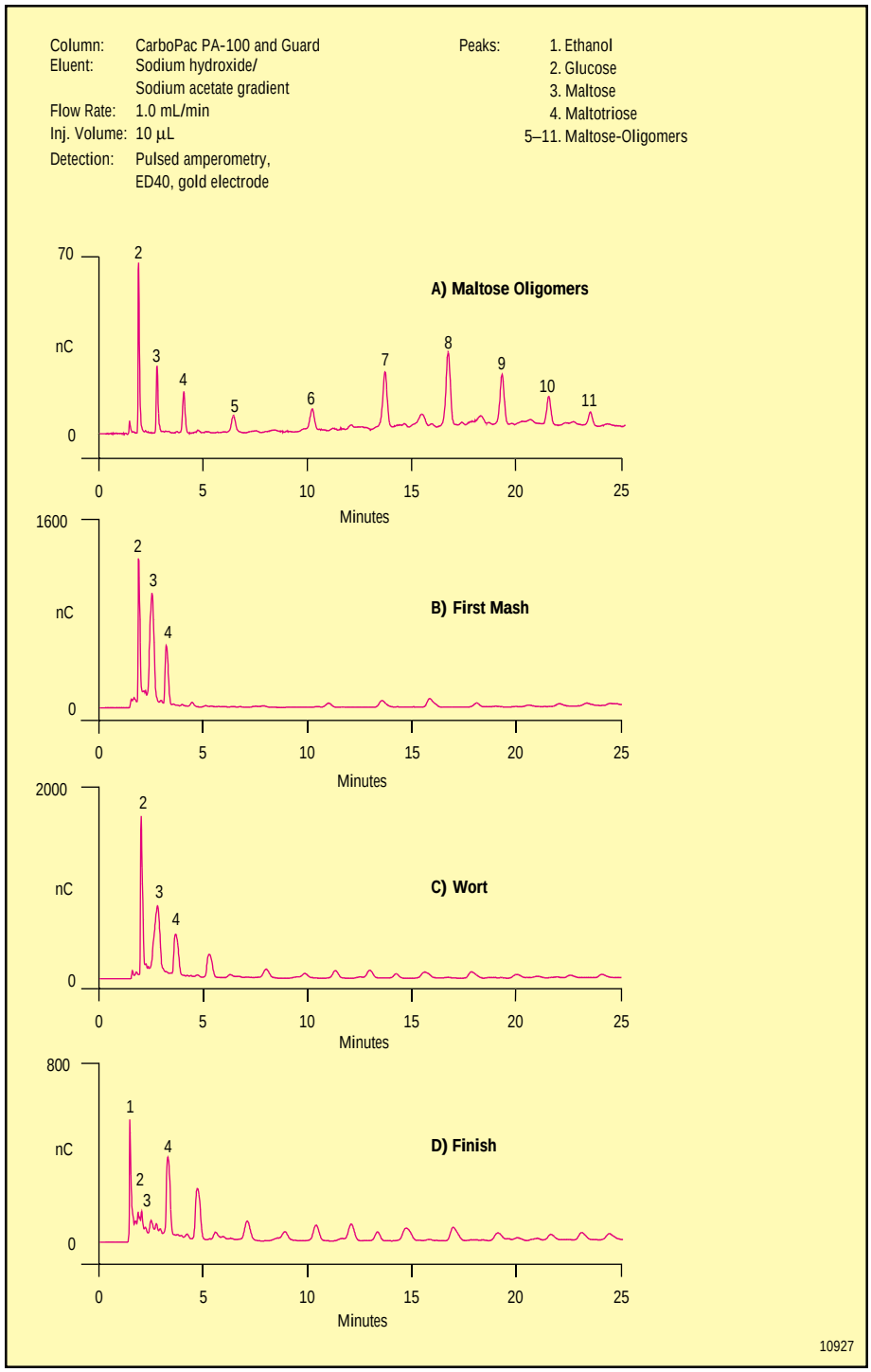

Figure 7. Sugar and oligosaccharide profiles during beer production. In panel A, maltose oligomers are baseline-separated. Chromatograms B, C, and D illustrate sugar and oligosaccharide profiles at different stages of the brewing process.

Determining the levels of fermentable and nonfermentable sugars at every stage of beer production is important because fermentable sugars determine the final alcohol content, and nonfermentable sugars contribute to the flavor and "body" of the final product. A separation of maltose oligomers up to degree of polymerization (DP) 10 is shown in Figure 7, panel A; panels B, C, and D show sugar and polysaccharide profiles at various stages of the brewing process. All samples were diluted 1:10.

Fraction Collection

The CMD Carbohydrate Membrane Desalter is a membrane device designed to desalt and reduce the pH of the samples following HPAE-PAD when the user wishes to collect and further analyze the carbohydrate samples. Desalted samples are then ready for lyophilization without dialysis.

More 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, are removed by the CMD. An advantage of the CarboPac PA-100 over the CarboPac PA1 column is that a lower salt concentration is required to elute the same oligosaccharide, making the CarboPac PA-100 column the best choice if fraction collection with subsequent desalting is required.

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 dilute 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 or mass spectrometric analysis, or further chromatographic analysis. Figure 8 shows the effect of the added volume of the CMD between detection and collection points. Loss of resolution is measurable (~6%), but chromatographic resolution is sufficient to allow acceptable purification.

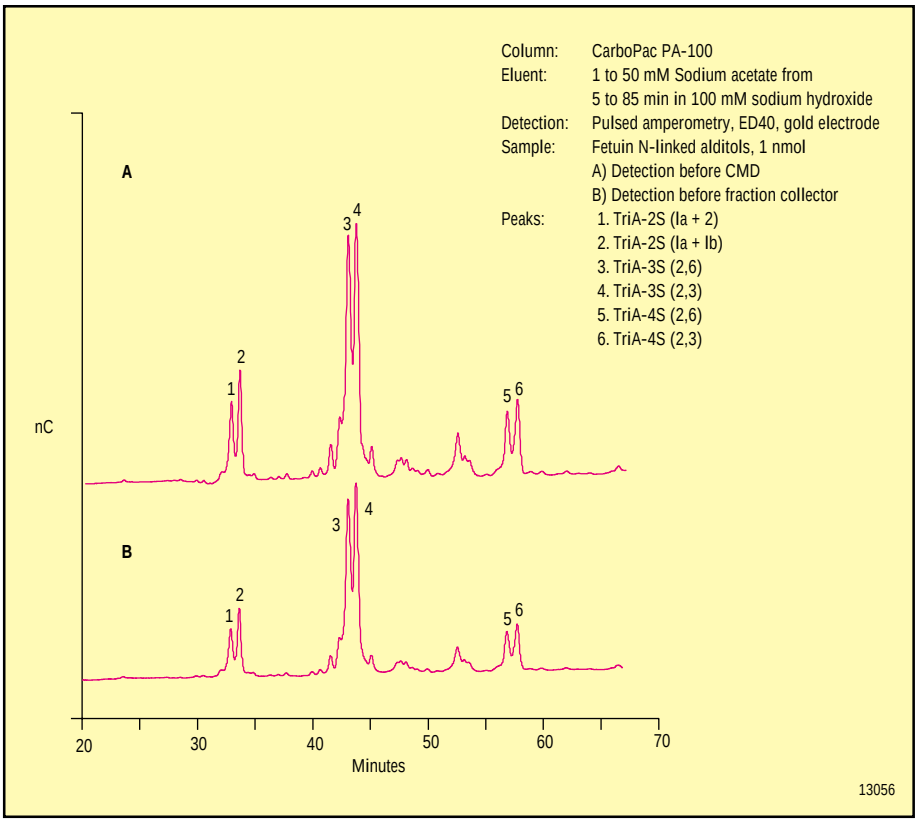

Figure 8. Elution of 1 nmol fetuin N*-linked alditols.*

SPECIFICATIONS

Resin Composition:
8.5 µm diameter ethylvinylbenzene/divinylbenzene substrate (55% crosslinking) agglomerated with 275 nm MicroBead™ 6% crosslinked quaternary amine-functionalized latex.

Anion-Exchange Capacity:
Approximately 90 µeq/column (4 x 250 mm)

Maximum Operating Pressure:
4000 psi (28 MPa)

Chemical Compatibility:
pH 0–14, 100% compatible with common organic solvents

Temperature Range:
4–90 ºC

Test Procedure:
Separation of a sialylated *N*-linked fetuin alditol test mixture (P/N 043064)

Recommended Operating Temperature:
Ambient

Recommended Flow Rate:
1.0 mL/min

Ionic Form Eluents:
Sodium acetate and sodium hydroxide only

Rugged, Reliable Separation with Guaranteed Performance

The unique pellicular resin of the CarboPac PA-100 columns offers exceptional selectivity and stability over the entire pH range. Its highly crosslinked structure ensures long column life and easy clean-up.

The entire manufacturing process (resin synthesis, amination, and packing and testing of the chromatographic columns) is carefully controlled to ensure that every Dionex CarboPac PA-100 delivers reproducible performance. CarboPac PA-100 columns are tested with two isomers of *N*-acetyl neura-minosyl-D-lactose to ensure lot-to-lot reproducibility.

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. Dionex can also make special-order CarboPac columns to your specifications; call for more information.

Description	Part Number
CarboPac PA-100 Columns	
CarboPac PA-100 Analytical Column (4 x 250 mm)	043055
CarboPac PA-100 Guard Column (4 x 50 mm)	043054
CarboPac PA-100 Microbore Column (2 x 250 mm)	SP4418
CarboPac PA-100 Microbore Guard Column (2 x 50 mm)	SP4420
CarboPac PA-100 Semipreparative Column (9 x 50 mm)	SP2089
CarboPac PA-100 Preparative Column (22 x 250 mm)	SP2667
Carbohydrate Membrane Desalter	
CMD-I Carbohydrate Membrane Desalter	059090
CMD-I Start-Up Package (CMD-I desalter, power supply, tubing, fittings)	059091

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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

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