columns

# CarboPac PA20 Column


*Anion-exchange columns for the analysis of mono- and disaccharides in glycoprotein therapeutics, foods, and beverages:*

- *Simple, direct approach using pulsed electrochemical detection*
- *Disposable gold electrodes*
- *Glycoprotein monosaccharide compositional analysis*
- *No derivatization required*
- *Specialized traps for interference-free quantification*
- *Rapid and rugged methods*

Now sold under the Thermo Scientific brand

Thermo SCIENTIFIC

## Simple, Direct Approach Using Pulsed Electrochemical Detection

The CarboPac® PA20 column has been developed to give fast, efficient separations of simple sugars without compromising resolution. Carbohydrates, without derivatization, are separated by anion-exchange chromatography at high pH and detected by pulsed electrochemical detection.

Electrochemical detection is used to measure the current resulting from oxidation or reduction of analyte molecules at the surface of a working electrode. During oxidation reactions, electrons are transferred from molecules of electroactive analytes, such as carbohydrates, to the working electrode in the electrochemical cell. Detection is sensitive and highly selective for electroactive species, because many potentially interfering species cannot be oxidized or reduced and are not detected.

When a single potential is applied to the working electrode, the detection method is dc amperometry. Pulsed amperometry, which uses a repeating sequence of potentials, is the technique employed for carbohydrate analysis, and is a reproducible and sensitive method for the detection of all carbohydrates of molecular weight up to 10,000.

DIONEX

**Passion. Power. Productivity.**

## Rapid and Rugged Methods

The CarboPac PA20 column has been designed to provide excellent resolution between the six underivatized monosaccharides commonly found in mammalian glycoproteins under a variety of sodium hydroxide concentrations. These conditions can be optimized depending on the goal of the separation. Figure 1 illustrates the effect of increasing the hydroxide concentration on the elution time.

## Innovative Resin Technology

The CarboPac PA20 column uses 6.5 µm pellicullar resin technology to provide high resolution and efficiencies for the six common monosaccharides found in glycoprotein hydrolysates. The smaller resin particle is agglomerated with an optimized latex that further improves the column performance by imparting a unique selectivity. This selectivity results in excellent resolution between galactose and glucosamine, analytes whose separation has historically been problematic. This optimized anion-exchange material is packed in 3 × 150 mm and 0.4 × 150 mm PEEK™ hardware formats which provide fast separations (mannose elutes in 9 min). The recommended low flow rates reduce eluent consumption and enable system operation over extended periods without user intervention. The CarboPac 0.4 × 150 mm capillary column offers the additional advantages of higher mass sensitivity, lower sample amounts, and significantly lower solvent use.

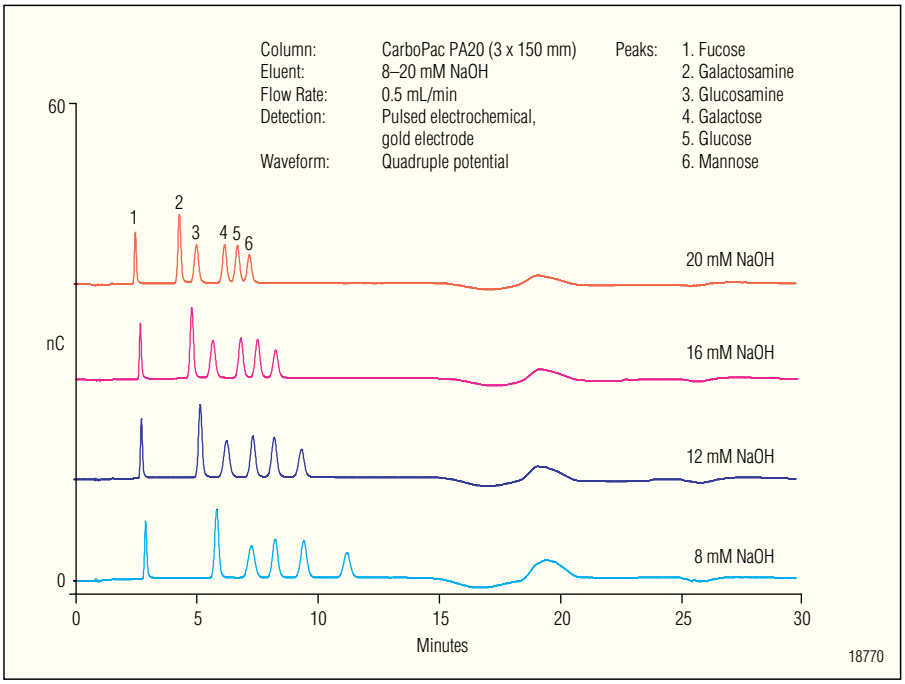


*Figure 1. Effect of hydroxide concentration on elution times.*

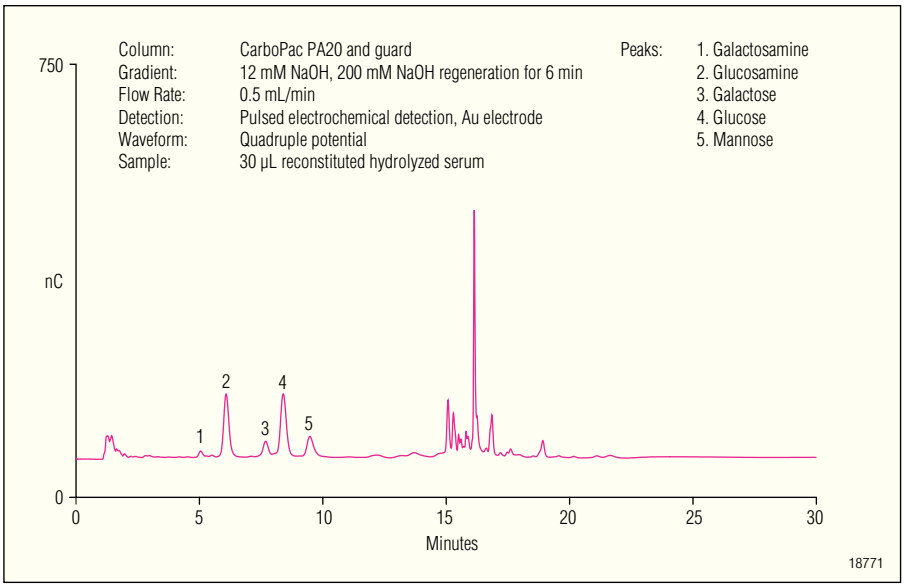


*Figure 2. Analysis of monosaccharides from hydrolyzed rabbit serum.*

## Glycoprotein Monosaccharide Compositional Analysis

Many mammalian proteins have carbohydrates attached to them. In many cases, the presence of the carbohydrate controls the biological activity of the protein or the rate at which it is cleared from the system. For example, certain glycosylated forms of tissue plasminogen activator (tPA) have more enzymatic activity than others. Thus, the study of protein glycosylation is important to many scientists, including those making a recombinant protein for therapeutic use.

Figure 2 shows the analysis of underivatized monosaccharides from hydrolyzed rabbit serum using the CarboPac PA20 column.

## Sialic Acid Analysis

Sialic acids are a family of *N*- and *O*-substituted neuraminic acids that play an important role in physiology. They occupy terminal positions on many glycoproteins and serve as markers for protein removal from blood circulation. The amino group of neuraminic acid is linked to either an *N*-acetyl or an *N*-glycolyl group. These yield *N*-acetylneuraminic acid (NANA) or *N*-glycolylneuraminic acid (NGNA), respectively. Figure 3 shows the separation of NANA and NGNA from bovine fetuin hydrolyzed with 0.1 M HCl. Using a sodium acetate gradient, NANA and NGNA are well resolved in 12 min.

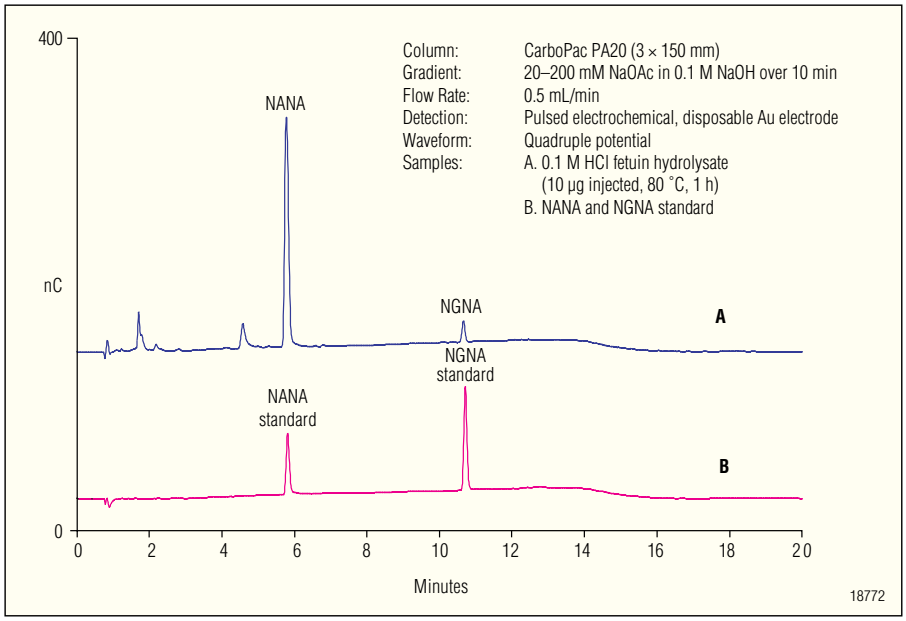


*Figure 3. Sialic acid analysis of bovine fetuin.*

## Food Applications

Monosaccharides important in food analysis are typically separated at eluent concentrations lower than those used for glycoprotein monosaccharides. Coffee sugars, such as mannitol, arabinose, galactose, glucose, xylose, mannose, and fructose, can be separated using 2 mM sodium hydroxide. High hydroxide concentrations can be used to acclerate the analyis of well-resolved sugars. Figure 4 shows the separation of glucose, fructose, and sucrose from chocolate syrup in less than 6 min using 50 mM hydroxide.

Monosaccharides important in dietary fiber analysis require higher concentrations of sodium hydroxide for timely elution and are readily eluted in < 12 min with 52 mM sodium hydroxide. Figure 5 shows the separation of monosaccharides, important in dietary fiber analysis, with good resolution between the sugar alcohols and sugars in a single isocratic run.

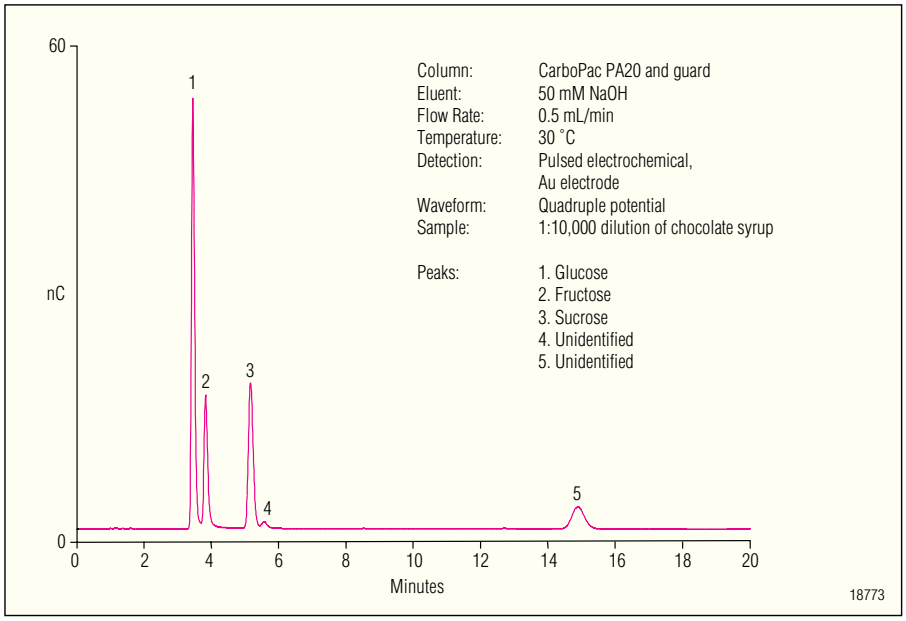


*Figure 4. Analysis of chocolate syrup using the CarboPac PA20 column.*

## Pharmaceuticals

The United States Food and Drug Administration (U.S. FDA) and regulatory agencies in other countries require that pharmaceutical products be tested for composition to verify their identity, strength, quality, and purity. Recently, attention has been given to inactive ingredients as well as active ingredients. Many of these ingredients are nonchromophoric and cannot be visualized by absorbance detection. However, carbohydrates, glycols, sugar alcohols, and sulfur-containing compounds can be oxidized and therefore detected by electrochemical detection.

Figure 6 shows the analysis of a pediatric acetominophen elixir using the CarboPac PA20 column. Using these conditions, glycols, sugar alcohols, and carbohydrates are all separated in a single run. Figure 7 shows the analysis of a pediatric oral decongestant under the same conditions.

## Fermentation Broths

Fermentation broth cultures have the best chance of producing optimum yield when the concentration of nutrients and amino acids are determined on-line and continuously monitored. The CarboPac PA20 column can be used on-line to monitor fermentation broths and cell cultures. Alternatively, if monitoring the amino acid content is of interest, the AminoPac® PA10 column should be considered for direct detection on-line. Figure 8 shows an analysis of a yeast extract-peptone-dextrose (YPD) broth supernatant. As illustrated, a large concentration of glucose is present with low concentrations of other sugars.

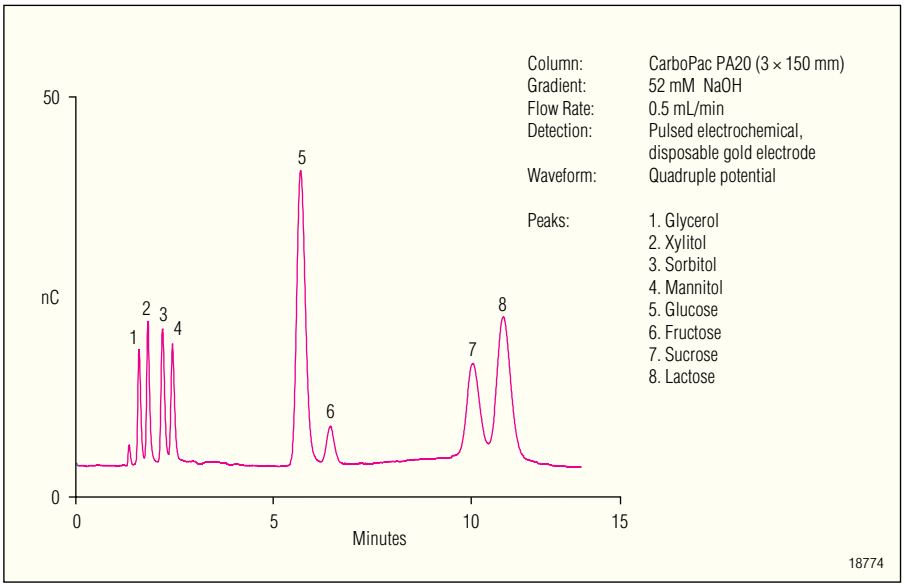


*Figure 5. Separation of monosaccharides important in dietary fiber analysis.*

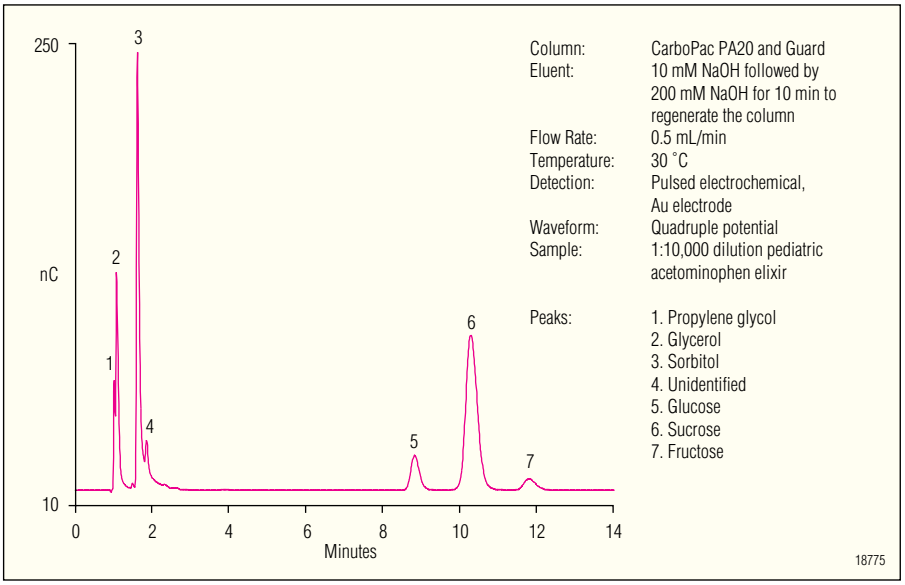


*Figure 6. Analysis of a pediatric acetominophen elixir.*

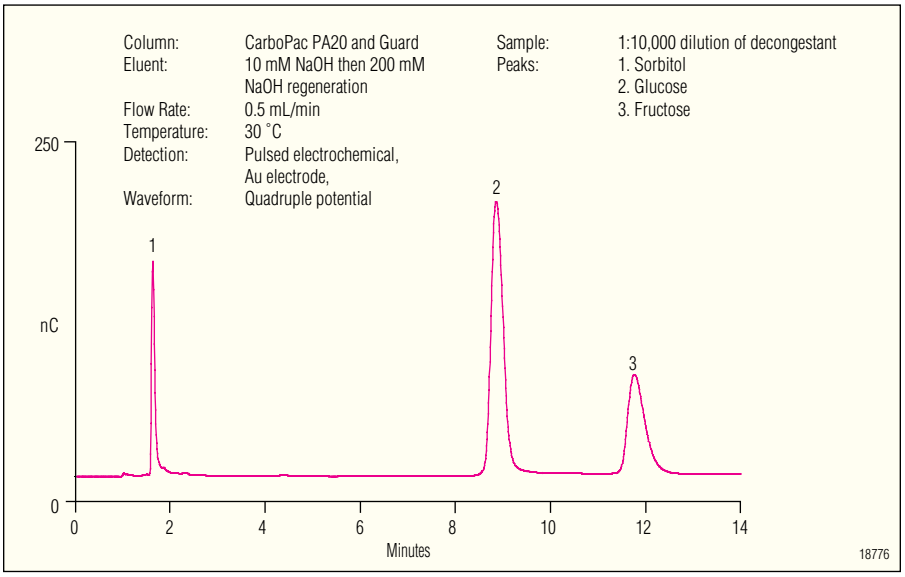


*Figure 7. Analysis of a pediatric oral decongestant.*

## Specialized Borate Trap for Interference-Free Quantification

Borate is one of the first ions to break through a water deionization system. Its presence in the carbohydrate eluents can cause a significant loss of peak efficiency, especially for mannose and reduced monosaccharides. Borate can affect monosaccharide peak symmetry, even when present in the low-µg/L concentration range. Figure 9 illustrates the use of the BorateTrap™ column with a CarboPac PA10 column. This product can also be used with CarboPac PA20 and CarboPac PA1 columns. The BorateTrap column, installed immediately before the injection valve, removes borate from the eluent.

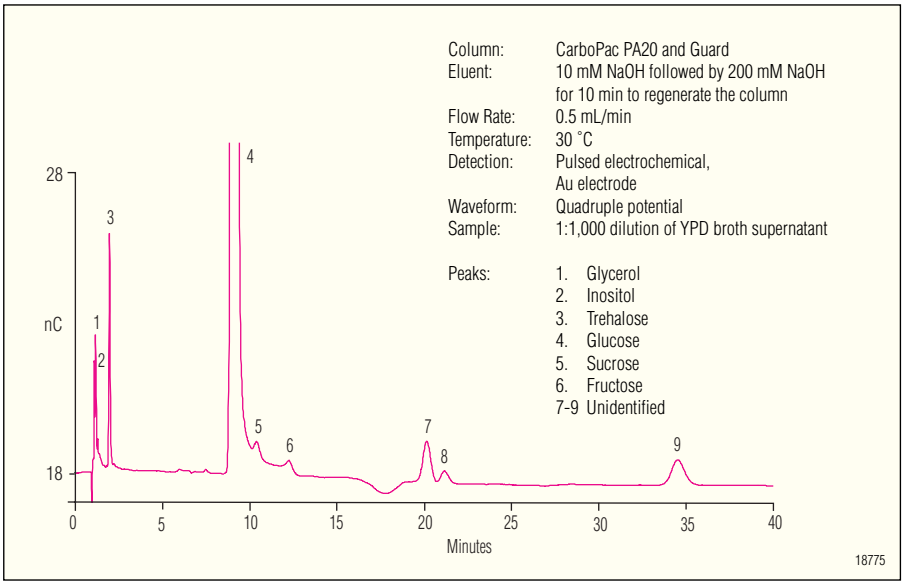


*Figure 8. Analysis of yeast extract-peptone-dextrose (YPD) broth supernatant.*

## Specialized Amino Acid Trap for Interference-Free Quantification

If the samples are glycoprotein hydrolysates with a high ratio of amino acids to carbohydrates, the AminoTrap™ column is the optimal guard column and replaces the standard guard column. Some amino acids, such as lysine, foul the working electrode. They elute as a severely tailing peak and affect the symmetry of later eluting carbohydrates. As illustrated in Figure 10, the AminoTrap column delays amino acids out of the carbohydrate elution window. This displacement of amino acid peaks results in cleaner chromatography of monosaccharides.

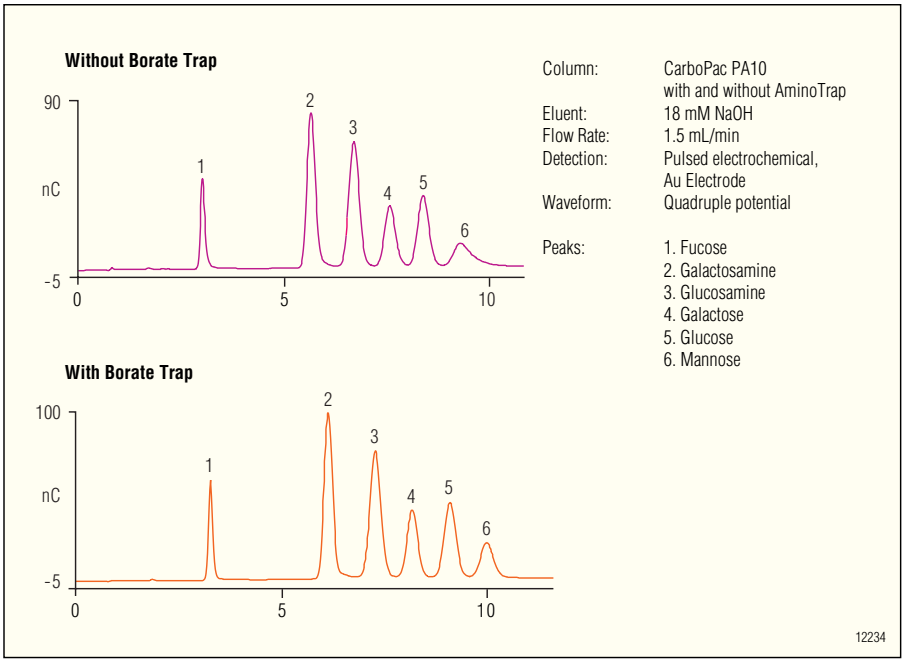


*Figure 9. Effect of borate and the BorateTrap column on monosaccharide peak symmetry.*

## Disposable Gold Electrodes

Although carbohydrates can be oxidized at a gold working electrode, the products of the oxidation reaction foul the surface of the electrode, inhibiting further analyte oxidation. Electrode fouling with oxidation byproducts or sample components may reduce response, requiring polishing to restore the surface.

Dionex disposable gold electrodes (Figure 11) eliminate the need for electrode reconditioning. Disposable electrodes are economical, and thus can be replaced frequently.

Frequent replacement of working electrodes renders electrochemical detection more predictable and reproducible. The peak area reproducibility for five disposable electrodes is shown in Table 1.

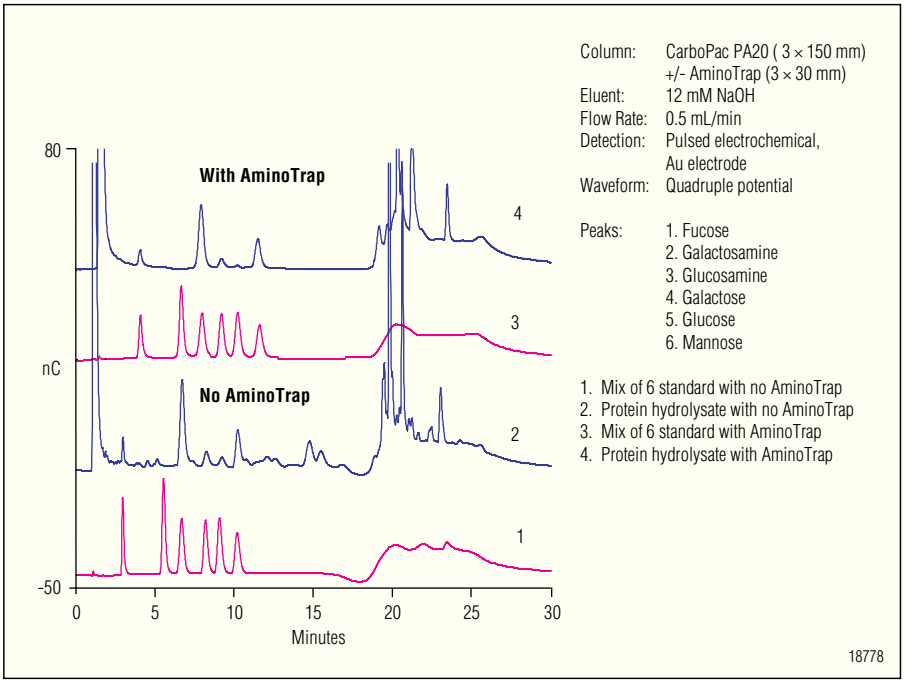


*Figure 10. Profiling MAb hydrolysate on the CarboPac PA20 column, with and without an AminoTrap column.*

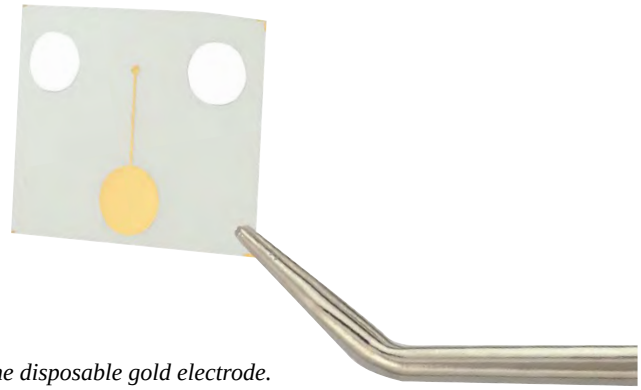

*Figure 11. Illustration of the disposable gold electrode.*

**Table 1. Area Reproducibility: Electrode-to-Electrode for Five Disposable Electrodes**

| Electrode | Average | Fuc | GalN | GlcN | Gal | Glc | Man |
|---|---|---|---|---|---|---|---|
| 1 | n = 12 | 3.09 | 7.05 | 5.58 | 4.92 | 5.82 | 4.22 |
| 2 | n = 12 | 3.13 | 7.49 | 5.95 | 4.68 | 6.24 | 4.25 |
| 3 | n = 12 | 3.35 | 7.65 | 6.04 | 5.32 | 6.23 | 4.52 |
| 4 | n = 12 | 3.12 | 6.76 | 5.33 | 4.70 | 5.52 | 4.18 |
| 5 | n = 12 | 3.12 | 6.93 | 5.46 | 4.83 | 5.54 | 4.20 |
| | **Average** | **3.16** | **7.18** | **5.67** | **4.89** | **5.87** | **4.27** |
| | S.D | 0.11 | 0.38 | 0.31 | 0.26 | 0.36 | 0.14 |
| **Elec-to-elec** | **RSD** | **3.41** | **5.30** | **5.44** | **4.35** | **6.05** | **3.28** |
| **Perm. Elec** | **n = 12** | **2.91** | **5.79** | **4.36** | **4.84** | **4.51** | **3.64** |

## ORDERING INFORMATION

To order in the U.S., call (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.

### CarboPac PA20 Fast Monosaccharide Columns

| | |
|---|---|
| CarboPac PA20 Analytical Column (3 × 150 mm) | 060142 |
| CarboPac PA20 Guard Column (3 × 30 mm) | 060144 |
| CarboPac PA20 Capillary Column (0.4 × 150 mm) | 072072 |
| CarboPac PA20 Guard Column (0.4 × 35 mm) | 072073 |
| AminoTrap (3 × 30 mm) | 060146 |
| BorateTrap (4 × 50 mm) | 047078 |

### Electrochemical Cells and Electrodes

| | |
|---|---|
| Electrochemical Detector (ED) without Cell | 079830 |
| ED with Reference Electrode and Spacer Block (no working electrode) | AAA-061756 |
| Disposable Gold Electrodes, 6 Electrodes (6 pack) | 060139 |
| Disposable Gold Electrodes, 24 Electrodes (4 bundled 6 pack) | 060216 |
| *AAA-Direct*™ Disposable Gold Working Electrode (6 pack) | 060082 |
| Gold on PTFE Disposable Electrode (6 pack) | 066480 |
| Gold Conventional Working Electrode | 079850 |
| *AAA-Certified*™ Conventional Gold Working Electrode | 063722 |
| Palladium Hydrogen Reference Electrode (PdH) | 072075 |
| pH-Ag/AgCl Reference Electrode | 061879 |

## CARBOPAC PA20 COLUMN SPECIFICATIONS

*Resin Composition:*
6.5 µm diameter substrate (ethylvinylbenzene 55% cross-linked with divinylbenzene; agglomerated with 130 nm microbead latex with difunctional quaternary ammonium ion, 5% cross-linked)

*Anion-Exchange Capacity:*
65 µeq/column (3 × 150 mm column)

1.16 µeq/column (0.4 × 150 mm column)

*Maximum operating pressure:*
3500 psi (21 MPa)

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

*Temperature Range:*
4–60 ˚C

*Test Procedure:*
Baseline resolution of fucose, galactosamine, glucosamine, galactose, glucose, mannose

*Recommended Operating Temperature:*
30 ˚C

*Recommended Flow Rate:*
0.5 mL/min for 3 × 150 mm column

0.01 mL/min for 0.4 × 150 mm column

*Ionic Form Eluents:*
Sodium hydroxide, sodium acetate

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DIONEX

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LPN 1501-01 PDF 02/10