Rapid, Efficient Separations of Glycoprotein Monosaccharides Using a New Anion-Exchange Separator Column and Electrochemical Detection with Disposable Gold Electrodes

Low picomole concentrations of glycoprotein monosaccharides are separated in less than 10 min by means of a new anion-exchange carbohydrate column. Pulsed electrochemical detection is simplified by means of disposable gold electrodes.

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High performance anion-exchange chromatography (HPAEC) with pulsed amperometric detection (PAD) is a relatively new separation technique for carbohydrates. This detection technique is direct (no derivatization is required), highly sensitive and selective, and compatible with gradient elution techniques. HPAEC–PAD has been applied successfully to food, dietary fiber, and complex carbohydrate analysis and is also widely used for glycoprotein research. Increasingly, it is being applied to a variety of routine monitoring and research applications and has been approved for use in several official methods (1–3).

Experimental Conditions

Dionex Bio-LC consisting of a GP40 gradient pump with on-line degasser, an ED40 electrochemical detector, and an AS3500 autosampler. The working electrode is a disposable polyester-gold working electrode (P/N with a silver–silver chloride reference electrode. The column is a new CarboPac™ PA20 anion-exchange column (3 mm × 150 mm, P/N 060142), operated at 0.5 mL/min. All data acquisition was performed using Dionex PeakNet 6 chromatography software.

Results

Figure 1 shows 400 consecutive runs for the separation of a mix of

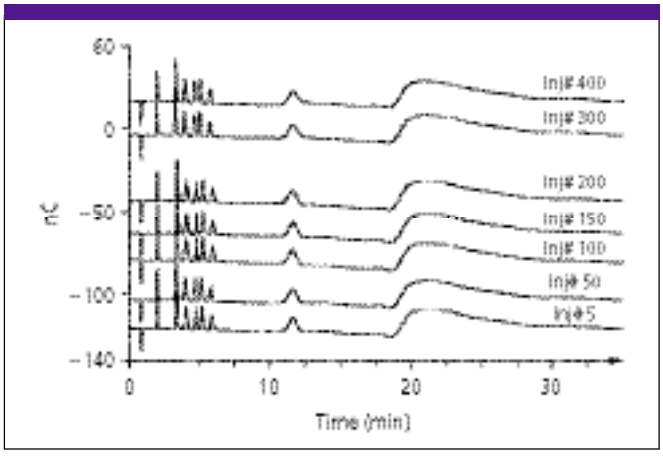

Figure 1: 400 consecutive injections of mix of six monosaccharides using the gold disposable electrode. Column: 3 mm × 150 mm CarboPac PA20, eluent: 12 mM sodium hydroxide; flow rate: 0.5 mL/min; detection: pulsed amperometry, disposable gold electrode; sample: mix of six monosaccharides standard (50 pmol).

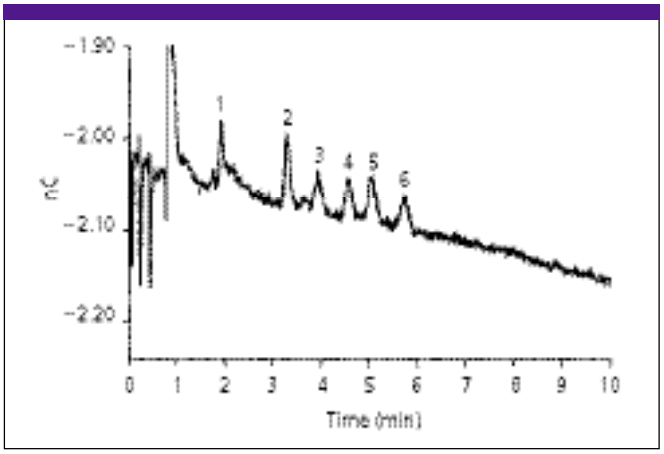

Figure 2: Low-level determinations (200 fmol). Column: 3 mm × 150 mm CarboPac PA20; eluent: 12 mM sodium hydroxide; flow rate: 0.5 mL/min; detection: pulsed amperometry, disposable gold electrode; sample: mix of six monosaccharides (200 fmol; 10 µL); Peaks: 1 = fucose, 2 = galactosamine, 3 = glucosamine, 4 = galactose, 5 = glucose, 6 = mannose.

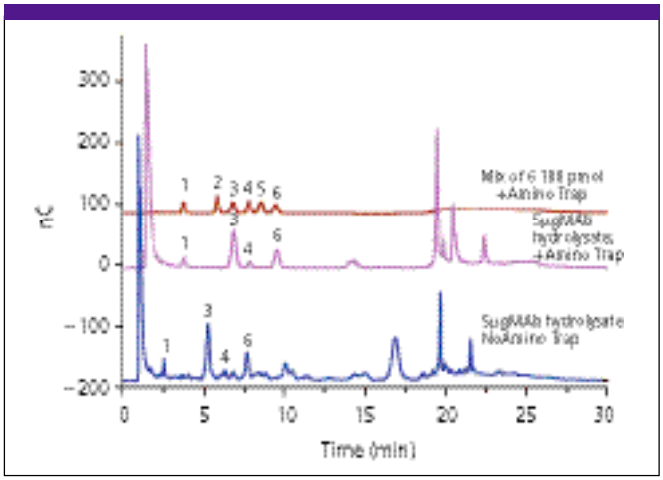

Figure 3: Monosaccharide analysis of MAb hydrolysates. Column: 3 mm × 150 mm CarboPac PA20; eluent: 16 mM sodium hydroxide; flow rate: 0.5 mL/min;, detection: pulsed amperometry, gold electrode; sample: mix of six monosaccharides standard–2 M TFA hu MAb hydrolysate (5 µg injection). Peaks: 1 = fucose, 2 = galactosamine, 3 = glucosamine, 4 = galactose, 5 = glucose, 6 = mannose.

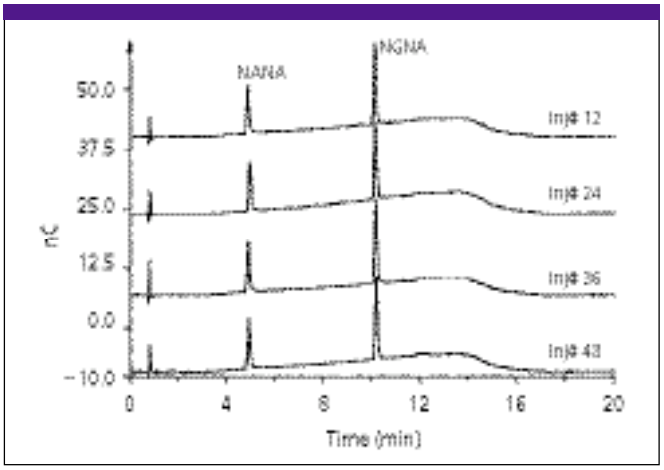

Figure 4: Sialic acids. Column: 3 mm × 150 mm CarboPac PA20; eluent: 20–200 mM NaOAc in 100 mM sodium hydroxide over 10 min; flow rate: 0.5 mL/min; detection: pulsed amperometry, disposable gold electrode; sample: NANA, NGNA.

six monosaccharides standard with electrochemical detection using a disposable gold electrode. Better sensitivity is obtained with the new column than was possible with previous columns. Figure 2 shows the detection of 200 fmol of the mix of six monosaccharide standards (P/N 043162). Figures 3 and 4 are representative chromatograms of typical carbohydrate applications for this column.

Conclusions

Faster, more efficient separations of glycoprotein monosaccharides, with better spacing, were achieved across a range of isocratic sodium hydroxide concentrations. Sensitivity of monosaccharide detection was improved as a consequence of the higher efficiency resin and narrower column diameter. The use of disposable gold electrodes significantly reduces system start up equilibration time as well as cell–cell detector peak area response variability.

References

(1) Method for the Analysis of Sucrose, Glucose and Fructose in Cane Molasses, officially adopted at the 21st Session of the International Commission for Uniform Methods of Sugar Analysis, Havana, Cuba, 1994 (first action approval of AOAC Method 996.04).

(2) ISO Method 11292:1995 Instant Coffee Determination of Free and Total Carbohydrates Method by High Performance Anion-Exchange Chromatography (first action approval AOAC Method 995.13).

(3) Determination of Fructans in Food Products (first action approval AOAC Method 997.08).

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