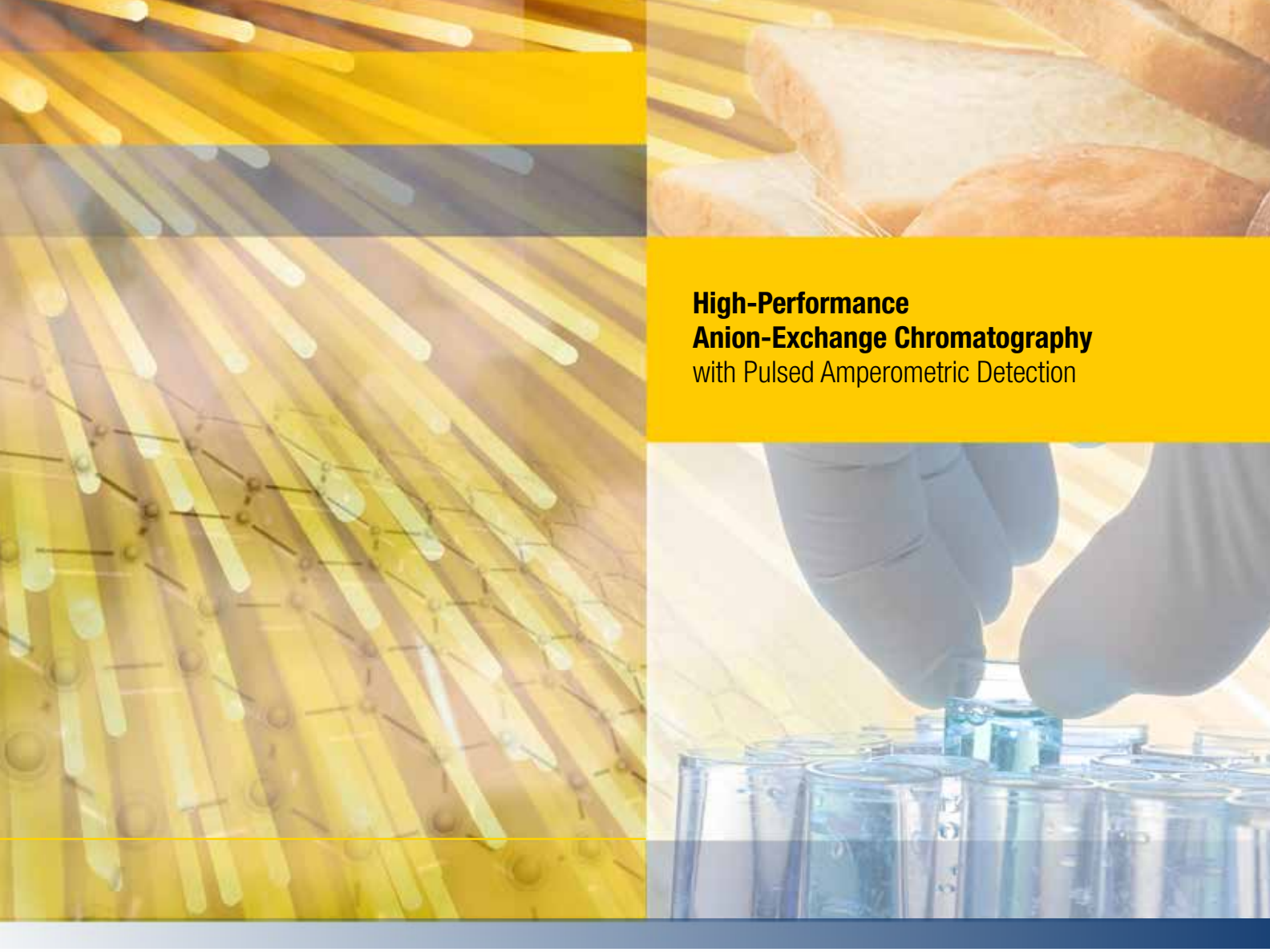

High-Performance
Anion-Exchange Chromatography
with Pulsed Amperometric Detection

Carbohydrate analysis with HPAE-PAD

Thermo SCIENTIFIC

Carbohydrate Analysis

by HPAE-PAD

Carbohydrates play vital roles in a variety of biological functions, including cellular communication, gene expression, immunology, organism defense mechanisms, and growth and development. They are difficult to analyze using common chromatography and detection methods as they are very polar compounds, exhibit similar structural characteristics, and lack a suitable chromophore.

High-Performance Anion-Exchange chromatography with Pulsed Amperometric Detection (HPAE-PAD) is widely used for determination of carbohydrates, including simple monosaccharides, oligosaccharides, sugar acids, such as sialic acids, sugar alcohols, sugar phosphates, and sugar nucleotides.

HPAE chromatography takes advantage of the weakly acidic nature of carbohydrates for highly selective separations at high pH using strong anion-exchange stationary phases. Neutral and cationic sample components elute in the void volume of the column, and do not usually interfere with the carbohydrate analysis.

Carbohydrates are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. Pulsed amperometry permits detection of carbohydrates with excellent signal-to-noise ratios and sensitivities down to sub-picomole levels for standard bore and microbore systems and femtomole levels for capillary systems, respectively, without requiring derivatization.
A series of potentials applied for defined time periods is referred to as a waveform. Repeated application of a waveform is the basis of pulsed amperometry. Waveform A (Figure 1) was introduced to improve the long-term reproducibility for carbohydrate analysis.

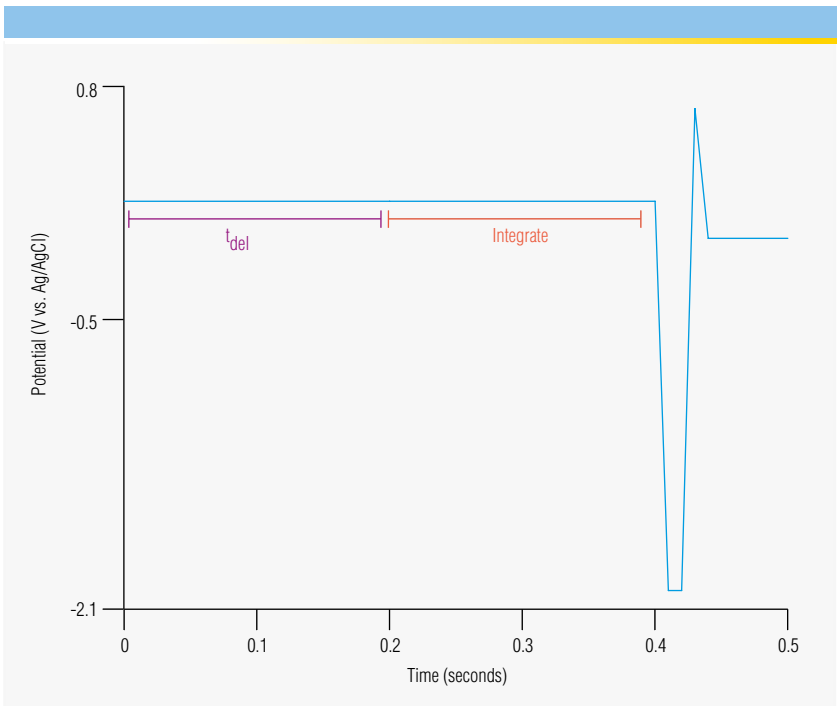

Figure 1. Waveform A for carbohydrate analysis.

Innovative Technology

enabling selective and versatile analysis

Thermo Scientific™ Dionex™ ICS-5000⁺ and ICS-4000 HPIC™ Systems

The Thermo Scientific Dionex ICS-5000⁺ and ICS-4000 HPIC™ systems are ideal for ion-exchange chromatography of carbohydrates using electrochemical detection. With completely metal-free, all PEEK™ flowpaths, Dionex IC systems eliminate the possibility of metal contamination and improve robustness.

The analysis of mono-, di-, and polysaccharides can be performed using Reagent-Free™ IC (RFIC™) systems with eluent generation that only require deionized water to electrolytically generate the eluent. This technology provides consistent results and the highest reproducibility, day-to-day, user-to-user, and lab-to-lab. The all-PEEK pump of the Dionex ICS-5000⁺ is capable of performing quaternary gradients at high flow rates, for demanding application needs.

Dionex ICS-5000⁺ and Dionex ICS-4000 Capillary HPIC systems use 0.4 mm i.d. columns with 1/100th the cross-sectional volume of 4 mm columns, requiring only 1/100th the sample size and using only 1/100th the eluent (Figure 2). Reduced eluent usage means reduced labor, reduced waste, and reduced operating costs

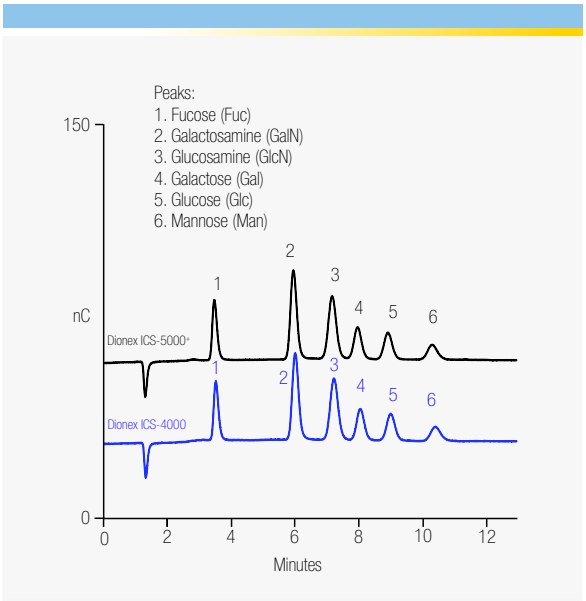

Figure 2. Monosaccharide chromatograms obtained with Dionex-ICS 4000 and Dionex-ICS 5000⁺ systems using a Thermo Scientific™ Dionex™ CarboPac™ PA 20 capillary column.

Dionex ICS-5000+ and ICS-4000

Electrochemical Detector (ED)

The electrochemical detector cells have been redesigned to be flow- and volume optimized for capillary, microbore and standard bore formats. The optional palladium hydrogen reference electrode is robust and calibration-free.

Gold working electrodes for carbohydrate analysis are available in both conventional and disposable formats. Conventional working electrodes will last for extended periods of time. However, these electrodes require periodic polishing to refinish the surface and have longer equilibration times when newly installed in a system. Disposable electrodes provide efficient, sensitive, reproducible analyses, electrode-to-electrode and lot-to-lot. After installation, rapid system re-equilibration (less than 30 min) supports quick system start-up.

Figure 3. Electrochemical detector cell for applications on standard bore and microbore size columns.

Dionex CarboPac Columns

Innovative column technology

The Dionex CarboPac family of columns offers a selection of columns, each optimized for a different class of compounds. They enable high resolution separations of closely related glycoprotein oligosaccharides, monosaccharides, and sialic acids, and a wide variety of other carbohydates.

The Dionex CarboPac columns use pellicular resin technology for improved chromatographic resolution, peak shape, and efficiency. The Thermo Scientific™ Dionex™ MicroBead™ latex particle was optimized to further improve column performance by imparting a unique chromatographic selectivity. This selectivity results in a significantly improved resolution between previously problematic analytes.

Application Focus:
Glycoprotein Oligosaccharides

AN 215 Separation of Asparagine-Linked (N-Linked) Oligosaccharides from Human Polyclonal IgG Using the CarboPac PA200 Column

AN 1050 Evaluating Protein Glycosylation in limited-Quantity Samples by HPAE-PAD

AU 176 Preparation of Peptide N-Glycosidase F Digests for HPAE-PAD Analysis

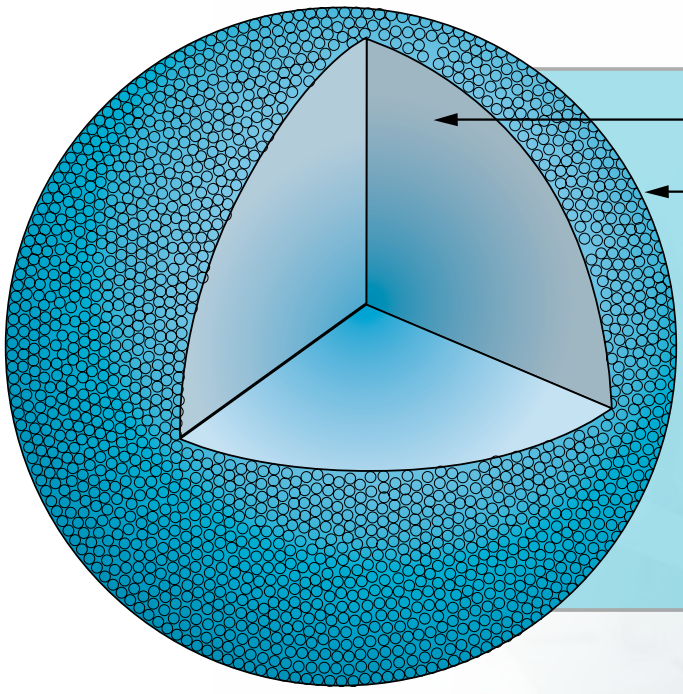

Figure 4. Dionex MicroBead particle

Profiling Carbohydrates

in food and beverages

Mono-and Disaccharide Separations

Mono- and disaccharides are important in food analysis. They are typically separated using a Dionex CarboPac PA20 column at eluent concentrations lower than 100 mmol/L NaOH or KOH. For outstanding inter-run consistency, this analysis can be run using electrolytically generated potassium hydroxide eluent by Eluent Generation (RFIC-EG™) on and RFIC system. The monosacchrides, disaccharides, and sugar alcohols can be well resolved in a single isocratic run (Figure 5).

High-Resolution Separation of Oligosaccharides

In the food industry, there is a significant and increasing demand for reproducible, fast, and simple methods to profile oligosaccharides and homologous sugar series such as inulins, amylopectins, and maltooligosaccharides, to monitor quality, conform to labeling requirements, and check for adulteration. Most HPLC approaches proposed for these applications are limited by insufficient specificity and high detection limits.

The Dionex CarboPac PA200 column is a nonporous, high-efficiency, polymeric anion-exchange column that provides the highest resolution available. The resin consists of 5.5 µm nonporous beads covered with a fine layer of functionalized Dionex MicroBead latex particles. This pellicular resin structure permits excellent mass transfer, resulting in high-resolution chromatography (Figure 6) and rapid re-equilibration after gradient elution. The recommended flow rate of 0.5 mL/min for the 3 × 250 mm column format results in significant reduction in eluent consumption compared to standard 4 mm i.d. columns.

Application Focus:
Carbohydrates in Food and Beverages

AN 253 HPAE-PAD Determination of Infant Formula Sialic Acids

AN 270 Determination of Hydroxymethylfurfural in Honey and Biomass

AN 280 Carbohydrate in Coffee: AOAC Method 995.13 vs a New Fast Ion Chromatography Method

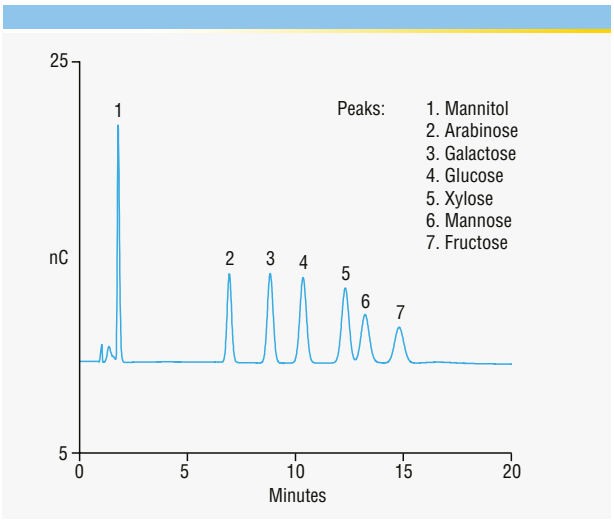

Figure 5. Separation of a sugar standard mix using the Dionex CarboPac PA20 0.4 mm capillary column.

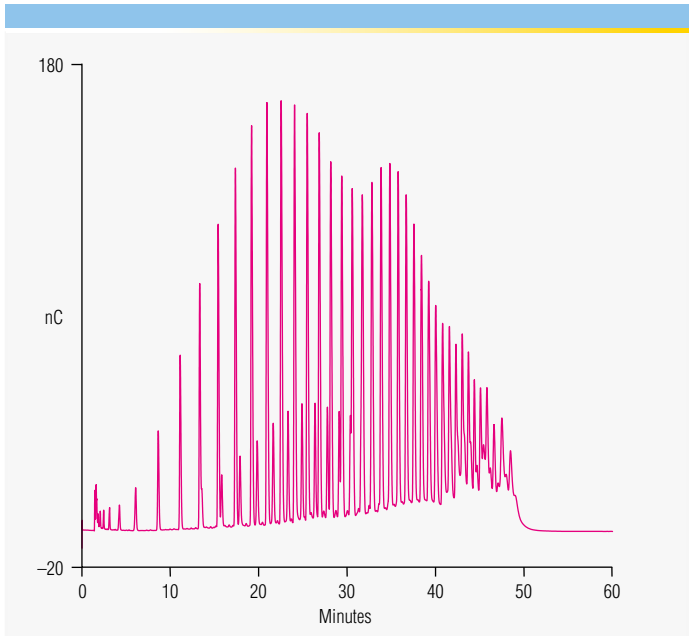

Figure 6. Inulin profile using the Dionex CarboPac PA200 column.

Carbohydrate analysis

in glycobiology

The development of recombinant-derived glycoproteins and monoclonal antibodies for therapeutic use has led to an increasing demand for methods to characterize their carbohydrate structures, especially asparagine-linked oligosaccharides that can impact the glycoprotein's function. The HPAE-PAD technique not only separates oligosaccharides according to charge, but can also resolve oligosaccharides with the same charge according to size, sugar composition, and linkage of monosaccharide units.

Analysis of sialic acids released during enzymatic digests of glycoproteins can reveal important information about their oligosaccharide structures (Figure 7). Using a Dionex CarboPac PA20 Fast Sialic Acid column, users can perform fast sialic acid determinations in less time than UHPLC methods due to the fast run times and because there is no derivatization necessary before analysis (Figure 8).

Application Focus: Glycobiology

AN 202 High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD) Analysis of Mannose-6-Phosphate

AU 180 Direct Determination of Sialic Acids in Glycoprotein Hydrolyzates by HPAE-PAD

AU 181 Rapid Screening of Sialic Acids in Glycoproteins by HPAE-PAD

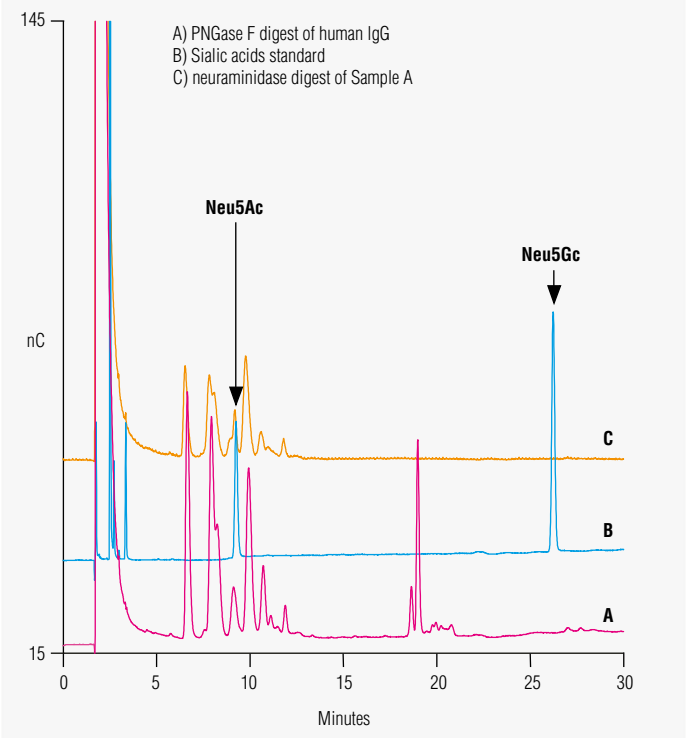

Figure 7. Monitoring release of sialic acids from human polyclonal IgG *N*-linked oligosaccharides by HPAE-PAD (CarboPac PA200 and guard column).

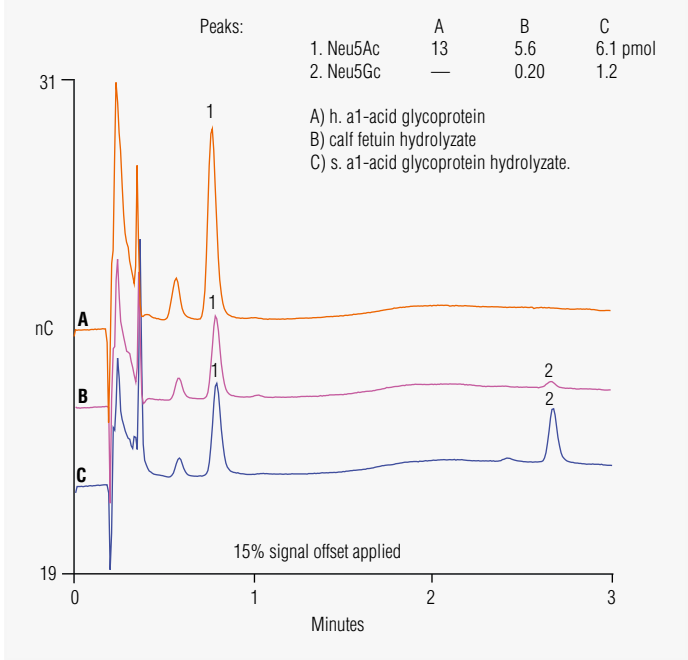

Figure 8: Separation of fetuin and alpha 1 acid glycoprotein acid hydrolyzates on the Dionex CarboPac PA20 Fast Sialic Acid column.

Carbohydrate Analysis

in pharmaceuticals

Manufacturing biological products in the pharmaceutical industry often requires fermentation processes. The Dionex ICS-5000+ and ICS-4000 systems provides automation for matrix elimination, sample preparation and sensitive electrochemical detection to help identify the wide range of analytes and concentrations found in in-process samples and final products. Dionex IC systems and Dionex CarboPac columns are suitable for many US and European Pharmacopeia analytical methods. For example, the galactosamine-containing impurities in heparin are determined by HPAE-PAD using the Dionex CarboPac PA20 column following the USP monograph method (Figure 9).

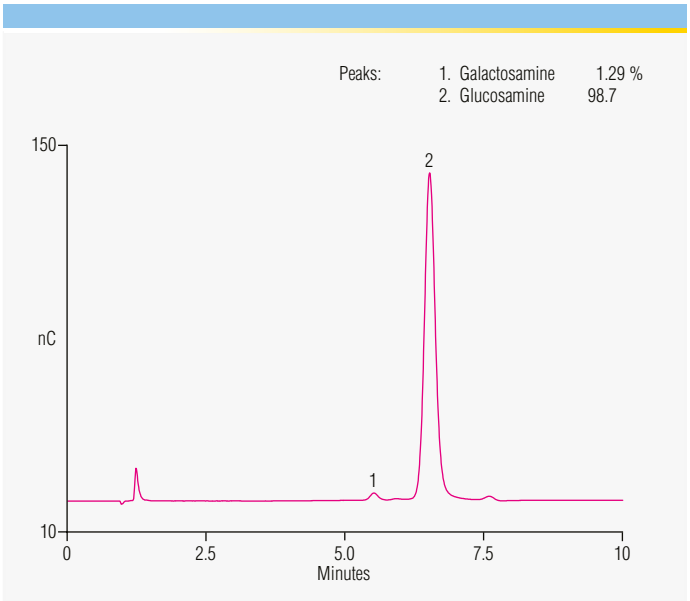

Figure 9. Separation of acid-hydrolyzed heparin spiked with 1% dermatan sulfate on the Dionex CarboPac PA20 column.

Application Focus:
Carbohydrate in Pharmaceuticals

AN 61 Determination of Tobramycin and Impurities Using HPAE-PAD

AN 66 Determination of Neomycin B and Impurities Using HPAE-PAD

AN 122 The Determination of Carbohydrates, Alcohols, and Glycols in Fermentation Broths

AN 181 Determination of Streptomycin and Impurities Using HPAE-PAD

AN 233 Determination of Galactosamine Containing Organic Impurities in Heparin by HPAE-PAD Using the Dionex CarboPac PA20 Column

AN 267 Analysis of the Aminoglycoside Antibiotics Kanamycin and Amikacin Matches USP Requirements

Carbohydrate Analysis

in biofuels

Feedstock used for biofuel production often requires hydrolysis to release sugars before fermentation. The Dionex ICS-5000⁺ provides automation options for in-line matrix elimination, sample preparation, and electrochemical detection for determination of carbohydrates in feedstock (Figure 10). HPAE-PAD methods using the Dionex CarboPac columns are well suited for handling high concentration biofuel samples with minimal sample treatment, high precision, and acceptable recoveries. These fast, accurate and reliable methods can be adapted for on-line monitoring of sugar levels in biomass applications.

Application Focus:
Carbohydrates in Biofuels

AN 225 Rapid Method for the Estimation of Total Free Monosaccharide Content of Corn Stover Hydrolysate Using HPAE-PAD

AN 255 Determination of Free and Total Glycerol in Biodiesel Samples by HPAE-PAD Chromatography

AN 270 Determination of Hydroxymethylfurfural in Honey and Biomass

AN 282 Rapid and Sensitive Determination of Biofuel Sugars by Ion Chromatography

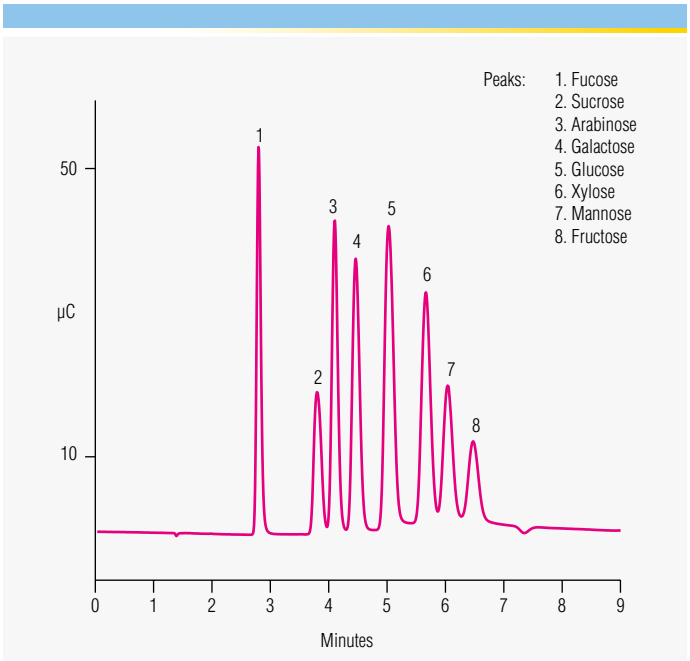

Figure 10. Fast 7 min separation of biofuel sugars on the Dionex CarboPac SA10 column.

3-D Amperometry

the complete data set

Application Focus:
3-D Amperometry

AN 179 Carbohydrate and Amino Acid Analysis Using 3-D Amperometry

Specific amperometric waveform integration ranges can provide a tool to selectively detect compounds of interest. 3-D amperometry enables post-chromatographic modification of waveform integration Quantitative information in PAD is generated by current integration within a suitable time interval, called integration period, during application of a detection waveform. Different analytes may require different locations of integration periods. The capability to assign different locations within the waveform to integration periods is available post-run with the help of 3-D amperometry. This postchromatographic modification of integration periods eliminates the need to perform multiple injections.

This technique can enhance the detection of carbohydrates in the presence of some co-eluting amino acids. For example, resolution may be improved between asparagine and glucose through reduction of the peak area for asparagine relative to glucose. Additionally, it can minimize some baseline disturbances to improve peak integration.

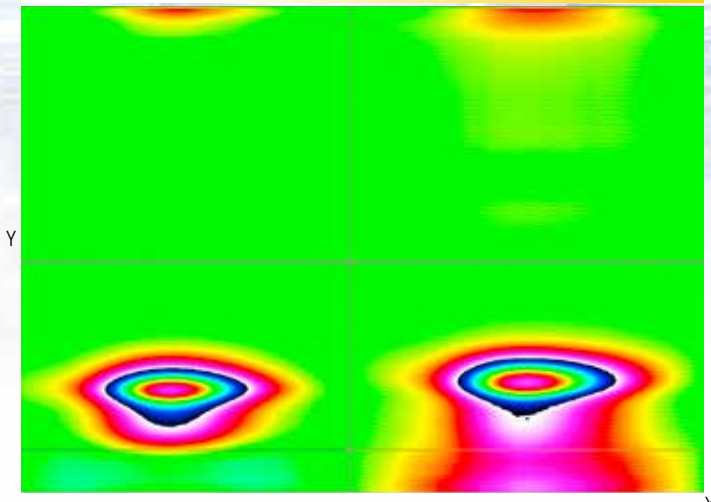

Figure 11. Iso current plot for isoleucine and leucine. Z axis magnitude is depicted with color.

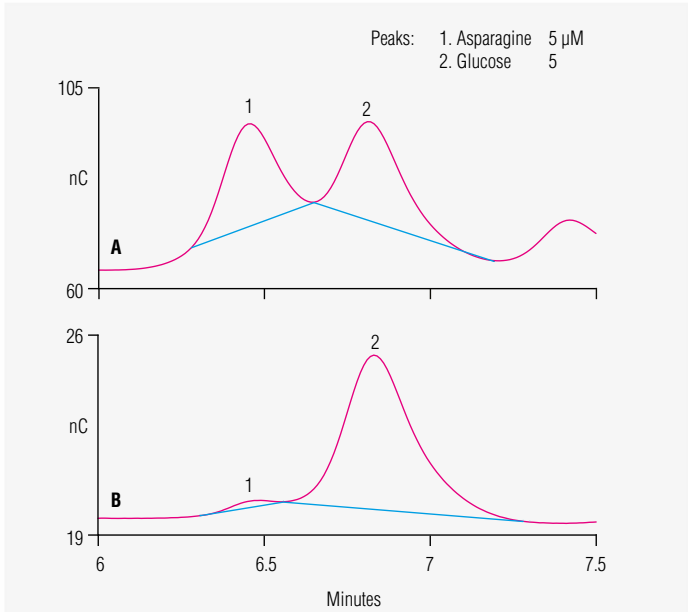

Figure 12. Post-run re-integration allows selective reduction of size of asparagine peak, allowing better quantification of glucose.

HPAE-PAD with MS

carbohydrate identification and confirmation in complex matrices

Identification of carbohydrates in very complex matrices can be difficult using electrochemical detection alone. Mass Spectrometry detection offers the advantage of faster and more reliable identification and peak confirmation by using the m/z of the saccharide classes—pentoses, hexoses, and oligosaccharides (Figures 13 and 14). The Thermo Scientific™ Dionex™ CMD™ 300 Carbohydrate Membrane Desalter electrolytically suppresses the effluent from the detector, reducing the pH and facilitating injection into a mass spectrometer for analysis of feedstock components, such as low molecular mass organic acids or mono-, di-, tri- and tetrasaccharides.

Application Focus:
Identification and Confirmation in Complex Matrices

Application Index 70420
Liquid Chromatography Techniques with Mass Spectrometric Detection

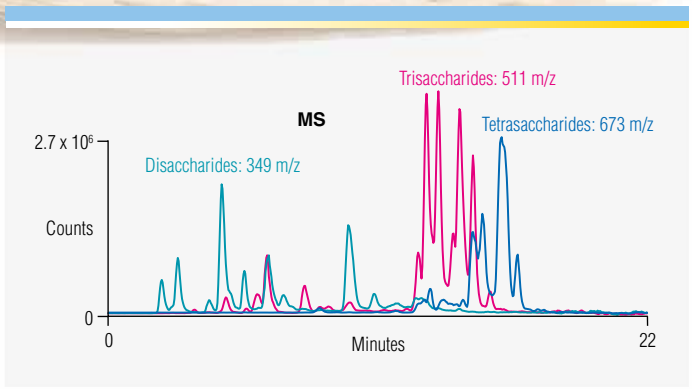

Figure 13. Comparison of electrochemical and extracted mass chromatograms of carbohydrates in a degassed lager beer sample, separated using a Dionex CarboPac PA200 (3 × 250 mm) column.

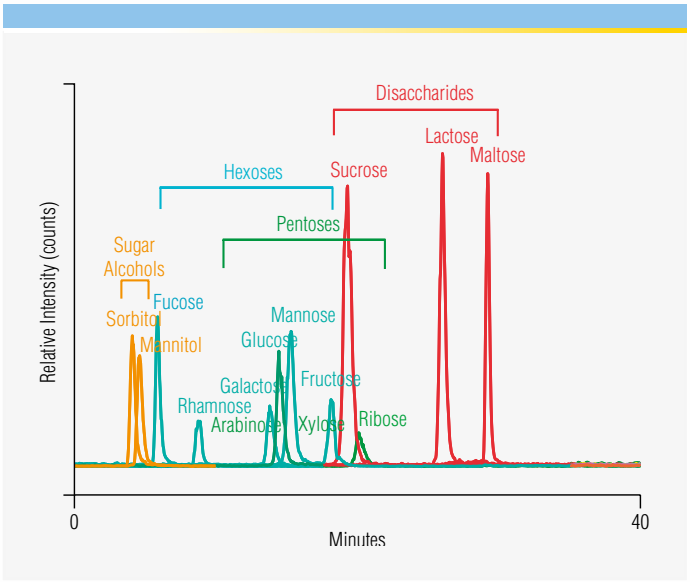

Figure 14. ESI positive mass chromatograms of sugar alcohols, monosaccharides, and disaccharides in the presence of LiCl, after separation using a Dionex CarboPac PA200 (3 × 250 mm) column.

Ordering Information

Dionex ICS-5000+ Standard System for Carbohydrate Analysis	
Part Number	Description
072244	Carbohydrates Analysis Bundle Standard Bore (Single Channel)
072245	Carbohydrates Analysis Bundle Capillary (Single Channel)
072252	Bio IC System (ED) for Carbohydrates (SB) and AAA-Direct (MB)
072253	Dual Channel Hybrid Bio IC (ED) for Carbohydrates (Cap) and AAA-Direct (MB)

Dionex ICS-4000 Standard System for Carbohydrate Analysis	
Part Number	Description
075152	ICS-4000 System with RFIC-EG and Electrochemical Detection

Part Numbers for Electrochemical Detection Parts	
Part Number	Description
072042	ED-5000 Electrochemical Detector (without cell)
075121	ED-4000 Electrochemical Detector (without cell)
072044	ED Cell (no reference or working electrode)
061879	pH, Ag/AgCl Reference Electrode
072075	Palladium Hydrogen Reference Electrode
079850	ED Electrode, Au, with gasket and polishing kit
060139	Carbohydrate Disposable Gold Working Electrodes, 1 mm, (6 pack with 2 gaskets 3 mm, 4 mm columns only)
066480	Gold on PTFE Disposable Electrodes, 1 mm, (6 pack with 2 gaskets 3 mm, 4 mm columns only)
074221	ED Cell Inlet Tubing kit. Kit Includes 9" capillary tubing for cell inlet, long neck black PEEK connector, black PEEK split cone ferrule
072117	0.001" PTFE gaskets for capillary applications with analytes diluted to µM concentrations

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Germany +49 6126 991 0
India +91 22 67429494s
Italy +39 02 51 62 1267
Japan +81 6 6885 1213
Korea +82 2 3420 8600
Netherlands +31 76 579 55 55
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