Beverages Applications Notebook

Sugars in Beverages

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Introduction to Beverages

The global beverage industry is growing each year with the introduction of new products, such as vitamin-fortified water, energy drinks, anti-aging water, and herbal nutritional supplements. With this growth, come many more analytical challenges. These challenges are compounded by the continuing and new needs to analyze classic favorites such as sodas, fruit juices, milk drinks, alcoholic beverages, and bottled water. One such example would be the melamine contamination in milk and infant milk formula.

For all beverages, the compositional quality and safety must be monitored to help track contamination, adulteration, product consistency, and to ensure regulatory compliance from raw ingredients (water, additives, and fruits) to the final product.

Thermo Fisher Scientific is a recognized leader in providing analytical solutions for sample preparation, liquid chromatography for compositional testing, and chromatography data management for compliance and quality testing of beverages. From inorganic ions, organic acids, biogenic amines, glycols and alcohols, carbohydrates and sugar alcohols, to vitamins, additives, and sugar substitutes, we are unique in our commitment to provide fast, accurate testing and labeling information for all applications in this industry.

Thermo Scientific and Dionex Integrated Systems

Dionex Products are now a part of the Thermo Scientific brand, creating exciting new possibilities for scientific analysis. Now, leading capabilities in liquid chromatography (LC), ion chromatography (IC), and sample preparation are together in one portfolio with those in mass spectrometry (MS). Combining Dionex's leadership in chromatography with Thermo Scientific's leadership position in mass spec, a new range of powerful and simplified workflow solutions now becomes possible.

For more information on how the new lineup of Thermo Scientific products can expand your capabilities and provide the tools for new possibilities, choose one of our integrated solutions:

- Ion Chromatography and Mass Spectrometry
- Liquid Chromatography and Mass Spectrometry
- Sample Preparation and Mass Spectrometry

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UltiMate 3000 UHPLC+ Systems

Best-in-class HPLC systems for all your chromatography needs

Thermo Scientific Dionex UltiMate 3000 UHPLC+ Systems provide excellent chromatographic performance while maintaining easy, reliable operation. The basic and standard analytical systems offer ultra HPLC (UHPLC) compatibility across all modules, ensuring maximum performance for all users and all laboratories. Covering flow rates from 20 nL/min to 10 mL/min with an industry-leading range of pumping, sampling, and detection modules, UltiMate™ 3000 UHPLC+ Systems provide solutions from nano to semipreparative, from conventional LC to UHPLC.

- Superior chromatographic performance
- UHPLC design philosophy throughout nano, standard analytical, and rapid separation liquid chromotography (RSLC)
- 620 bar (9,000 psi) and 100 Hz data rate set a new benchmark for basic and standard analytical systems
- RSLC systems go up to 1000 bar and data rates up to 200 Hz
- ×2 Dual System for increased productivity solutions in routine analysis
- Fully UHPLC compatible advanced chromatographic techniques
- Thermo Scientific Dionex Viper and nanoViper–the first truly universal, fingertight fitting system even at UHPLC pressures

Thermo Fisher Scientific is the only HPLC company uniquely focused on making UHPLC technology available to all users, all laboratories, and for all analytes.

Rapid Separation LC Systems: The extended flow-pressure footprint of the RSLC system provides the performance for ultrafast high-resolution and conventional LC applications.

RSLCnano Systems: The Rapid Separation nano LC System (RSLCnano) provides the power for high-resolution and fast chromatography in nano, capillary, and micro LC.

Standard LC Systems: Choose from a wide variety of standard LC systems for demanding LC applications at nano, capillary, micro, analytical, and semipreparative flow rates.

Basic LC Systems: UltiMate 3000 Basic LC Systems are UHPLC compatible and provide reliable, high-performance solutions to fit your bench space and your budget.

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IC and RFIC Systems

A complete range of ion chromatography solutions for all customer performance and price requirements

For ion analysis, nothing compares to a Thermo Fisher Scientific ion chromatography system. Whether you have just a few samples or a heavy workload, whether your analytical task is simple or challenging, we have a solution to match your needs and budget. And with your IC purchase, you get more than just an instrument—you get a complete solution based on modern technology and world-class support.

- Thermo Scientific Dionex ICS-5000: The world's first capillary IC system
- Dionex ICS-2100: Award-winning integrated Reagent-Free™ IC system
- Dionex ICS-1600: Standard integrated IC system
- Dionex ICS-1100: Basic integrated IC system
- Dionex ICS-900: Starter line IC system

Ranging from the Dionex ICS-900 to the ICS-5000, these IC systems cover the entire range of IC needs and budgets and come with superior support and service worldwide.

Dionex ICS-5000: Developed with flexibility, modularity, and ease-of-use in mind, the Dionex ICS-5000 combines the highest sensitivity with convenience

Dionex ICS-2100: An integrated Reagent-Free IC (RFIC™) system for electrolytically generated isocratic and gradient separations with conductivity detection, now with electrolytic sample preparation.

Dionex ICS-1600: The Dionex ICS-1600 combines high sensitivity with convenience. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

Dionex ICS-1100: With dual-piston pumping and electrolytic suppression. Now ready for eluent regeneration, with available dual-valve configuration for automated sample preparation.

Dionex ICS-900: Can routinely analyze multiple anions and cations in 10–15 min—fully automated with Displacement Chemical Regeneration (DCR).

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MS Instruments

Single-point control and automation for improved ease-of-use in LC/MS and IC/MS

Thermo Fisher Scientific provides advanced integrated IC/MS and LC/MS solutions with superior ease-of-use and modest price and space requirements. UltiMate 3000 System Wellness technology and automatic MS calibration allow continuous operation with minimal maintenance. The Dionex ICS-5000 instrument and the family of RFIC systems automatically remove mobile phase ions for effort-free transition to MS detection.

- Thermo Scientific MSQ Plus mass spectrometer, the smallest and most sensitive single quadrupole on the market for LC and IC
- Self-cleaning ion source for low-maintenance operation
- Thermo Scientific Dionex Chromeleon Chromatography Data System software for single-point method setup, instrument control, and data management
- Compatible with existing IC and LC methods
- The complete system includes the MSQ Plus™ mass spectrometer, PC datasystem, electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) probe inlets, and vaccum system

You no longer need two software packages to operate your LC/MS system. Chromeleon™ LC/MS software provides single-software method setup and instrument control; powerful UV, conductivity, and MS data analysis; and fully integrated reporting.

MS Systems and Modules: MSQ Plus Mass Spectrometer; MSQ18LA nitrogen gas generator; Thermo Scientific Dionex AXP-MS digital auxiliary pump

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Chromeleon 7 Chromatography Data System Software

The fastest way to get from samples to results

Discover Chromeleon software version 7, the chromatography software that streamlines your path from samples to results. Get rich, intelligent functionality and outstanding usability at the same time with Chromeleon software version 7—the Simply Intelligent™ chromatography software.

- Enjoy a modern, intuitive user interface designed around the principle of operational simplicity
- Streamline laboratory processes and eliminate errors with eWorkflows, which enable anyone to perform a complete analysis perfectly with just a few clicks
- Access your instruments, data, and eWorkflows instantly in the Chromeleon Console
- Locate and collate results quickly and easily using powerful built-in database query features
- Interpret multiple chromatograms at a glance using MiniPlots
- Find everything you need to view, analyze, and report data in the Chromatography Studio
- Accelerate analyses and learn more from your data through dynamic, interactive displays
- Deliver customized reports using the built-in Excel-compatible speadsheet

Chromeleon software version 7 is a forward-looking solution to your long-term chromatography data needs. It is developed using the most modern software tools and technologies, and innovative features will continue to be added for many years to come.

The Cobra™ integration wizard uses an advanced mathematical algorithm to define peaks. This ensures that noise and shifting baselines are no longer a challenge in difficult chromatograms. When peaks are not fully resolved, the SmartPeaks™ integration assistant visually displays integration options. Once a treatment is selected, the appropriate parameters are automatically included in the processing method.

Chromeleon software version 7 ensures data integrity and reliability with a suite of compliance tools. Compliance tools provide sophisticated user management, protected database stuctures, and a detailed interactive audit trail and versioning system.

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Process Analytical Systems and Software

Improve your process by improving your process monitoring with a Thermo Scientific Dionex on-line IC or HPLC system

Our process analytical systems provide timely results by moving liquid chromatography-based measurements on-line. Information from the Thermo Scientific Dionex Integral process analyzer can help reduce process variability, improve efficiency, and reduce downtime. These systems provide comprehensive, precise, accurate information faster than is possible with laboratory-based results. From the lab to the factory floor, your plant's performance will benefit from the information provided by on-line LC.

- Characterize your samples completely with multicomponent analysis
- Reduce sample collection time and resources with automated multipoint sampling
- Improve your process control with more timely results
- See more analytes with unique detection capabilities
- 25 years of experience providing on-line IC and HPLC capabilities to a wide range of industries
- The Thermo Scientific Integral Migration Path approach lets you choose the systems that best meets your needs

The Integral Migration Path™ approach enables on-line IC/HPLC to generate timely, high-resolution information when monitoring a small-scale reactor in a process R&D lab, in a pilot plant, or improving current manufacturing plant processes. No matter what the application, the Integral™ process analyzer has the versatility to place a solution using on-line IC/HPLC, whenever and wherever it is needed.

Integral: The Integral Migration Path approach: System solutions wherever you need them: lab, pilot plant, or manufacturing

Chromeleon Process Analytical (PA) Software: Chromeleon PA software provides unique capabilities to support on-line IC or HPLC analysis

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Automated Sample Preparation

ACCELERATED SOLVENT EXTRACTORS

Two new solvent extraction systems with pH-hardened Dionium components

We offer two solvent extraction systems. The Thermo Scientific Dionex ASE 150 Accelerated Solvent Extractor is an entry-level system with a single extraction cell, for laboratories with modest throughput. The Dionex ASE™ 350 system is a sequential extraction system capable of automated extraction of up to 24 samples. Both systems feature chemically inert Dionium components that allow the extraction of acid- or base-pretreated samples.

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SOLID-PHASE EXTRACTION SYSTEMS

Faster, more reliable solid-phase extraction while using less solvent

The Thermo Scientific Dionex AutoTrace 280 Solid-Phase Extraction (SPE) instrument unit can process six samples simultaneously with minimal intervention. The instrument uses powerful pumps and positive pressure with constant flow-rate technology. Current analytical methods that require SPE sample preparation include gas chromatography (GC), GC-MS, LC, and LC-MS, IC and IC-MS. The Dionex AutoTrace™ 280 instrument is approved or adapted for U.S. EPA clean water methods and safe drinking water methods (600 and 500 series) and can extract the following analytes:

- PCBs (polychlorinated biphenyls)
- OPPs (organophosphorus pesticides), OCPs (organochlorine pesticides), and chlorinated herbicides
- BNAs (base, neutral, acid semivolatiles)
- Dioxins and furans
- PAHs (polyaromatic hydrocarbons)
- Oil and grease or hexane extractable material

With SPE, large volumes of liquid sample are passed through the system and the compounds of interest are trapped on SPE adsorbents (cartridge or disk format), then eluted with strong solvents to generate an extract ready for analysis. Automated SPE saves time, solvent, and labor for analytical laboratories.

Dionex AutoTrace Systems: The new Dionex AutoTrace 280 system provides fast and reliable automated solid phase extraction for organic pollutants from liquid samples

Dionex AutoTrace Accessories: High-quality parts and accessories are available for Dionex AutoTrace 280 instruments

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Analysis of Sugars in Beverages

Determination of Sucralose Using HPAE-PAD

INTRODUCTION

Sucralose is used in the manufacture of nonnutritive sweetened food and beverage products. Sucralose, like most carbohydrates, lacks a good chromophore and therefore requires high concentrations to be detected by UV absorbance. Many food and beverage ingredients are chromophoric and can interfere with the direct detection of sucralose by absorbance. Refractive index detection has similar limitations.[1,2] Carbohydrates, glycols, alcohols, amines, and sulfur-containing compounds can be oxidized and therefore directly detected by amperometry. This detection method is specific for those analytes that can be oxidized at a selected potential, leaving all other compounds undetected. Pulsed amperometric detection (PAD) is a powerful detection technique with a broad linear range and very low detection limits.

High-performance anion-exchange chromatography (HPAE) is a technique capable of separating most carbohydrates and their analogues.[3,4] For complex samples containing mixtures of sucralose and biologically derived material, such as foods and beverages, the high resolving power of HPAE and the specificity of PAD allow the determination of sucralose with little interference from other ingredients.[5,6]

This application note describes the use of HPAE-PAD to determine sucralose in food and beverage samples. Red Raspberry Diet Rite® is a soft drink artificially sweetened with sucralose. This beverage also contains natural and artificial flavors, organic compounds (potassium benzoate and citrate), food dye, and another nonnutritive sweetener (acesulfame potassium). Splenda® is a solid artificial sweetener used as a sugar substitute and consists of dextrose, maltodextrin, and sucralose. These model food and beverage samples present a great challenge for most chromatographic methods. In this application note, the CarboPac™ PA20 anion-exchange column is used to separate sucralose from other ingredients in Red Raspberry Diet Rite and Splenda.

EQUIPMENT

Dionex BioLC® system consisting of:

- GP50 Gradient Pump, microbore (2 mm) with degas option.
- ED50 Electrochemical Detector with combination pH/Ag/AgCl reference electrode and either or both of the following:
  - Carbohydrate Certified Disposable Au Working Electrode (P/N 060139, 6 electrodes; P/N 060216, 4 packages of 6 electrodes)
  - *AAA-Direct*™ Certified Disposable Au Working Electrode (P/N 060082, 6 electrodes; P/N 060140, 4 packages of 6 electrodes)

AS50 Autosampler

AS50TC Thermal Compartment

EO1 Eluent Organizer, including three 2-L plastic bottles and pressure regulator

Chromeleon® Chromatography Workstation

Helium; 4.5-grade, 99.995%, <5 ppm oxygen (Praxair)

Filter unit, 0.2-µm nylon (Nalgene 90-mm Media-Plus, Nalge Nunc International, P/N 164-0020) or equivalent nylon filter

Vacuum pump (Gast Manufacturing Corp., P/N DOA-P104-AA or equivalent)

1.5-mL glass injection vials with caps (Vial Kit, Dionex P/N 055427)

REAGENTS AND STANDARDS

Reagents

Sodium hydroxide, 50% (w/w) (Fisher Scientific and J. T. Baker)

Deionized water, 18 MΩ-cm resistance or higher

Sodium acetate, anhydrous (*AAA-Direct* Certified, Dionex Corp., P/N 059326)

Standards

Glucose, D- (Dextrose; Pfanstiehl Labs, Reference Grade, Cat #RGG-116)

Sucralose (McNeil Nutritionals, Inc.)

Food and Beverage Samples

Red Raspberry Diet Rite (Dr. Pepper/Seven Up, Inc.; product 29500-85254)

Splenda, (McNeil-PPC, Inc.)

CONDITIONS

Method

Columns:	CarboPac PA20 Analytical (P/N 060142)
	CarboPac PA20 Guard (P/N 060144)
Flow Rates:	0.5 mL/min
Eluent:	A: Water
	B: 250 mM NaOH
	C: 1 M sodium acetate
Injection Volume:	10 or 25 µL
Temperature:	30 °C
Detection (ED50):	Pulsed amperometry, Carbohydrate Certified disposable gold working electrodes (P/N 0600139), or *AAA-Direct* Certified disposable gold working electrodes (P/N 060082).
Background:	11–54 nC (using the carbohydrate waveform)
Typical System Operating Backpressure:	2622–2700 psi

Isocratic Method (40 mM NaOH with 75 mM sodium acetate)

Time (min)	*%A*	*%B*	*%C*	*Comments*
Initial	76.5	16.0	7.5	
0.0	76.5	16.0	7.5	Inject
9.55	76.5	16.0	7.5	End

Gradient Method

Time (min)	*%A*	*%B*	*%C*	*Comments*
Initial	84.0	16.0	0.0	
0.0	84.0	16.0	0.0	Inject
5.0	84.0	16.0	0.0	Start acetate gradient
15.0	76.5	16.0	7.5	End acetate gradient
20.0	76.5	16.0	7.5	
20.1	4.0	16.0	80.0	Begin column wash
30.0	4.0	16.0	80.0	
30.1	4.0	16.0	0.0	Begin reequilibration
45.0	84.0	16.0	0.0	End run

Periodic Column Wash Method

Time (min)	*%A*	*%B*	*%C*	*Comments*
Initial	4.0	16.0	80.0	
0.0	4.0	16.0	80.0	No Injection
30.0	4.0	16.0	80.0	
30.1	76.5	16.0	7.5	Reequilibration
45.0	76.5	16.0	7.5	End run

Carbohydrate Waveform for the ED50 (Waveform A, Recommended)*

Time (s)	*Potential (V)*	*Integration*
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	–2.0	
0.42	–2.0	
0.43	+0.6	
0.44	–0.1	
0.50	–0.1	

Reference electrode in Ag mode.

* The waveform used for this application note appears in Technical Note 21.[7]

AAA-Direct Waveform for the ED50 (Alternative, for Increased Sensitivity)**

Time (s)	*Potential (V)*	*Integration*
0.00	+0.13	
0.04	+0.13	
0.05	+0.33	
0.21	+0.33	Begin
0.22	+0.55	
0.46	+0.55	
0.47	+0.33	
0.56	+0.33	End
0.57	–1.67	
0.58	–1.67	
0.59	+0.93	
0.60	+0.13	

Reference electrode in pH mode.

** The alternate waveform used for this application note appears in the Product Manual for the *AAA-Direct* Amino Acid Analysis System.[8]

PREPARATION OF SOLUTIONS AND REAGENTS

Eluents

250 mM Sodium Hydroxide

It is essential to use high-quality water of high resistivity (18 M -cm). Biological contamination should be absent. It is important to minimize contamination by carbonate, a strongly eluting divalent anion at high pH that causes changes in carbohydrate retention times. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should not be used. A 50% (w/w) sodium hydroxide solution is much lower in carbonate (carbonate precipitates at this pH) and is the preferred source for sodium hydroxide.

Dilute 26 mL of 50% (w/w) sodium hydroxide solution into 1974 mL of thoroughly degassed water to yield 250 mM sodium hydroxide. Immediately blanket the NaOH eluent with inert gas.

1 M Sodium Acetate

To prepare 1 L of eluent, dissolve 82 g of *AAA-Direct* Certified anhydrous sodium acetate in ~800 mL purified water. Adjust the total volume to 1000 mL with additional water. Filter the solution through a 0.2-µm nylon filter unit and place it under 4–5 psi helium or nitrogen to reduce microbial contamination and carbonate buildup. Note, a sodium acetate that is not *AAA-Direct* Certified may lead to a loss of detector response due to electrode contamination by impurities when using the recommended *AAA-Direct* Waveform. To date, no loss in detector response is known to occur when using non-*AAA-Direct* Certified sodium acetate with Waveform A, the recommended quadruple potential waveform for carbohydrate determinations.

Keep the eluents blanketed under 5–8 psi (34–55 kPa) of inert gas (helium or nitrogen) at all times. On-line degassing is necessary because amperometric detection is sensitive to oxygen in the eluent. Set the pump to degas for 30 s every 4 min. Although not investigated in this note, long-term simple sucralose determinations should be possible using only two eluent channels: (1) mixture of 40 mM NaOH and 75 mM sodium acetate for the separation, and (2) 40 mM NaOH and 800 mM sodium acetate for periodic column washes.

STOCK STANDARDS

Solid sucralose standard was dissolved in deionized water to a 10-mg/mL concentration. This solution was further diluted with water to yield the desired stock mixture concentrations. The solutions were maintained frozen at –5 °C until needed.

SAMPLE PREPARATION

Red Raspberry Diet Rite

Red Raspberry Diet Rite beverage was diluted 50-fold, or as needed, in deionized water. Diluted sample was analyzed directly.

Splenda

Splenda sugar substitute was prepared at a concentration of 100 mg/mL with deionized water. The solution was diluted to 100 µg/mL and analyzed directly.

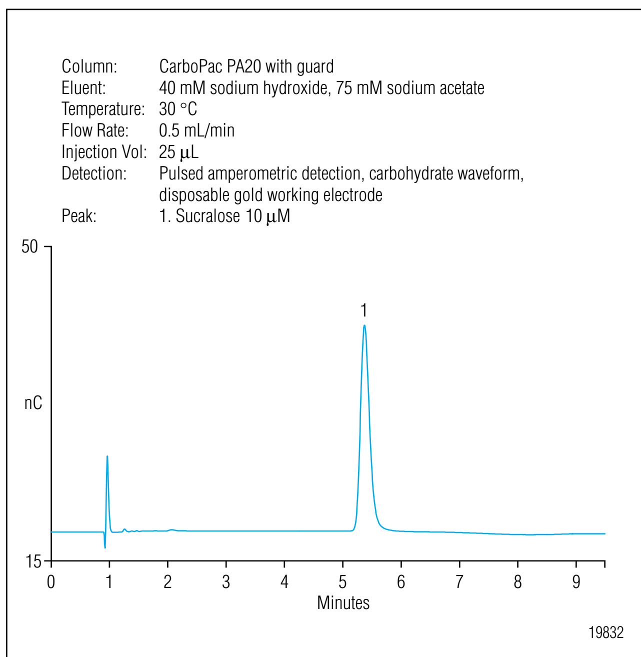

Figure 1. Separation of sucralose using a CarboPac PA20 column set.

RESULTS AND DISCUSSION

Separation

Figure 1 shows the separation of sucralose using a CarboPac PA20 analytical and guard column set with an eluent of 40 mM sodium hydroxide (NaOH) and 75 mM sodium acetate flowing at 0.5 mL/min. This isocratic method was optimized for retention time and detector response. Using this method, sugar alcohols (alditols), glycols, and mono- and disaccharides are not retained and elute near the void, whereas sucralose is retained and typically elutes at about 5.6 min. The retention time of sucralose was determined to vary slightly due to minor variations between different batches of prepared eluent (up to ~4%, including just the replacement of water in eluent A), and the use of different columns. Use of gravimetric measurement in place of volumetric measurement for sodium hydroxide eluent preparation can improve eluent-to-eluent reproducibility.

Run times were optimized on the system to account for the oxygen dip. This dip results from oxygen present in the samples and appears as a function of the column's gas permeation volume. Eluting oxygen produces less background than the eluent, so a dip appears in the baseline where oxygen elutes. The retention time of an

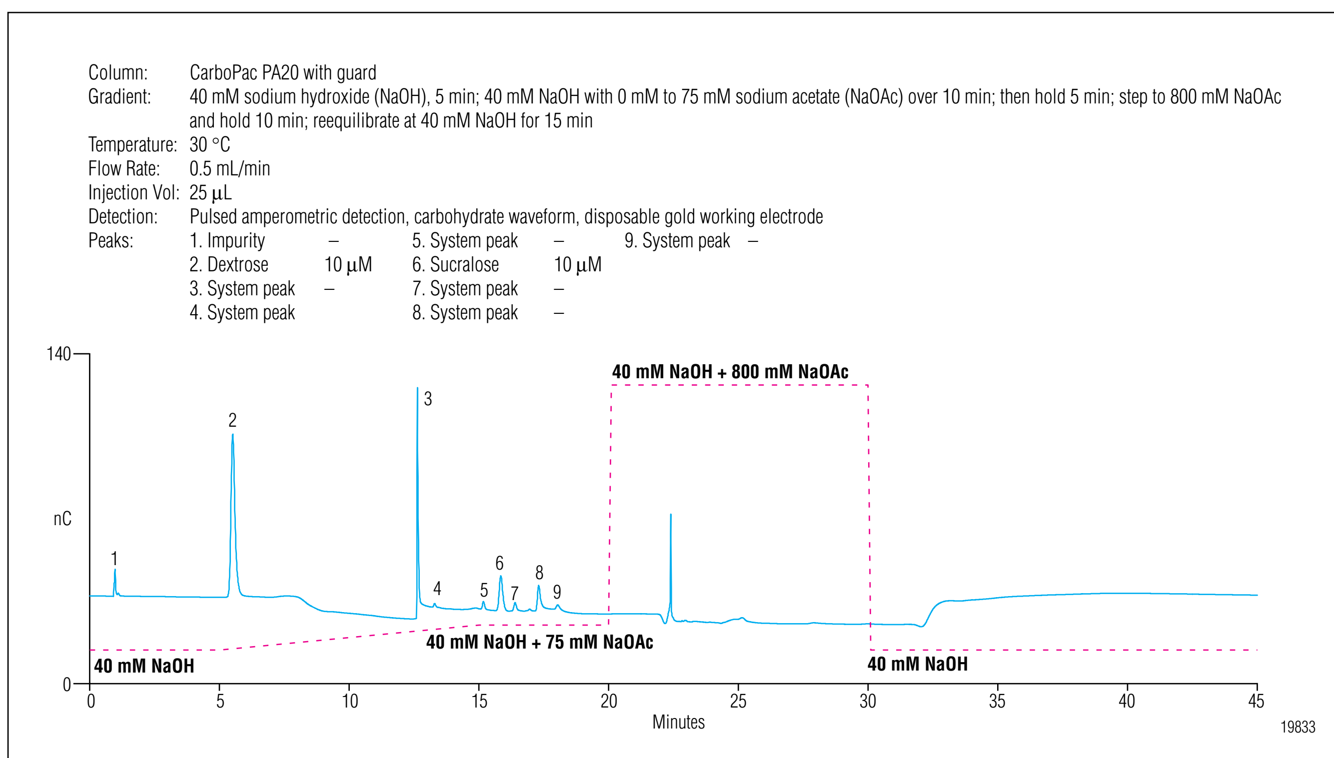

Figure 2. *Simultaneous determination of 10 µM dextrose (glucose) and sucralose using a CarboPac PA20 column set with a gradient method.*

oxygen dip varies from column to column, and depends on the flow rate but not the eluent strength. Setting the run time to allow two injections to occur before the first oxygen dip elutes doubled the sample throughput. For this system, the oxygen dip at 19.4 min resumed a stable baseline at 20.3 min, and the lag period was 1.22 min (time from the end of the first injection to the beginning of the next injection). With the run time of 9.55 min used in this application note, the oxygen dip was observed in the second and all subsequent injections of a sequence at a retention time of 8.6 min, and did not interfere with the elution of the sucralose peak at 5.6 min. A suitable run time may vary slightly from system to system, and column to column, and depends on the rinse volume selected for the autosampler, as well as whether the sample overlap feature of Chromeleon software is enabled (overlap was enabled for this application note). The oxygen dip and lag period should be determined for each system configuration. Run times longer than the oxygen dip will have no possibility of interferences, but sample throughput will be reduced.

Simultaneous determinations of monosaccharides (e.g., dextrose) and sucralose are possible using a gradient method. Figure 2 shows the separation of both dextrose (glucose) and sucralose using a gradient method where dextrose is first eluted using 40 mM NaOH for 5.0 min, and then a linear sodium acetate gradient is applied (0–75 mM) over 10 min. Dextrose elutes at about 5–6 min (peak 2), and sucralose elutes at about 16 min (peak 6). Peak 1 is the void, and Peaks 3, 4, 5, and 7–9 are unidentified peaks found in a blank (water) injection and result from the accumulation of impurities in the eluent prior to application of the acetate gradient. Peaks eluting during the acetate gradient also occur in the absence of any injection. The source and extent of the impurities can vary with the lag period prior to each run and the quality of reagents used for eluent preparation. An autosampler enables the lag period to be reproducible within a sequence, and therefore makes system peaks reproducible. Although chemical manufacturers have criteria for defining the conformity of the reagents they produce, the tests are not always specific enough to identify suitability for IC or HPLC applications with PAD or IPAD. Therefore, lot-to-lot variability can exist with respect to trace electrochemically active impurities.

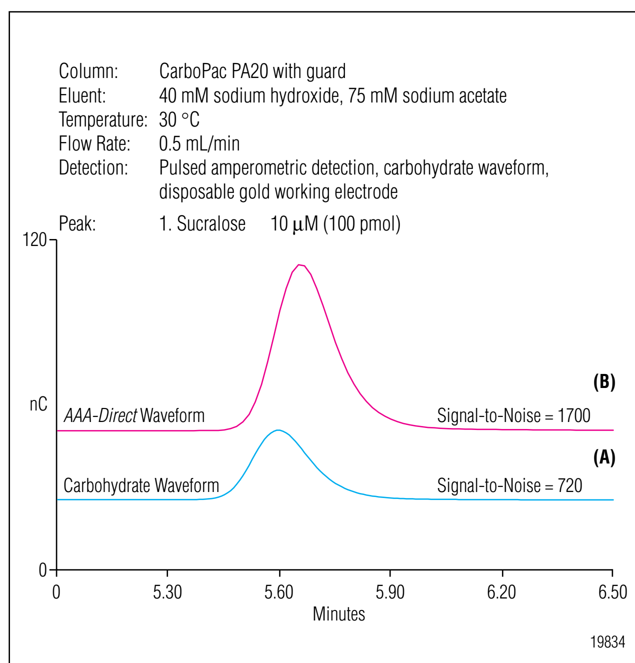

Figure 3. Comparison of 10-µM sucralose peak height (10-µL injection) using the carbohydrate waveform (A) and the AAA-Direct *waveform (B).*

Detection

Figure 3 compares the peak height for 10 µM sucralose (100 pmol, 10-µL injection) detected using (A) the carbohydrate waveform, and (B) the *AAA-Direct* waveform. The use of the *AAA-Direct* waveform increased signal-to-noise by more than two times. Although the *AAA-Direct* waveform improved sucralose sensitivity, we recommend the carbohydrate waveform because it allows longer use of each disposable Au working electrode and improves day-to-day peak area reproducibility. The *AAA-Direct* Certified disposable Au working electrode is guaranteed for 1 week when used with the *AAA-Direct* waveform, whereas the Carbohydrate Certified disposable Au working electrode is guaranteed for two weeks when used with the carbohydrate waveform. For applications where greater sensitivity is required, the *AAA-Direct* waveform should be considered.

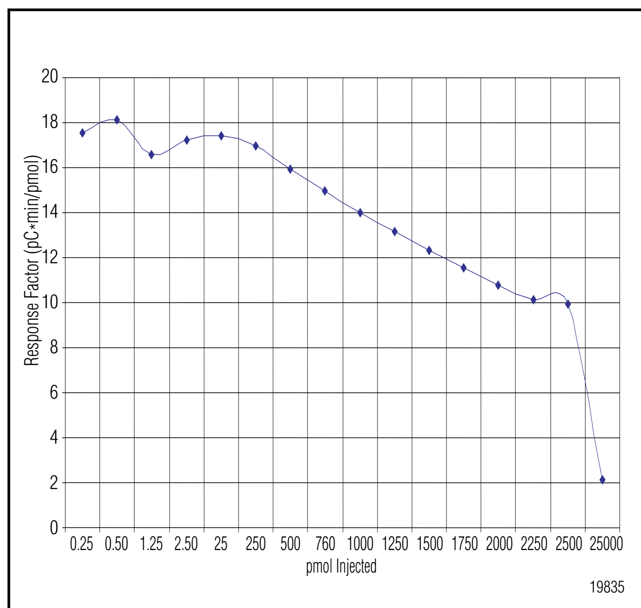

*Figure 4. Relationship of sucralose peak area response factors (pC * min/pmol injected) to sucralose concentration injected (25-µL injection) using the isocratic method (see Figure 1). Note that x-axis is not linear.*

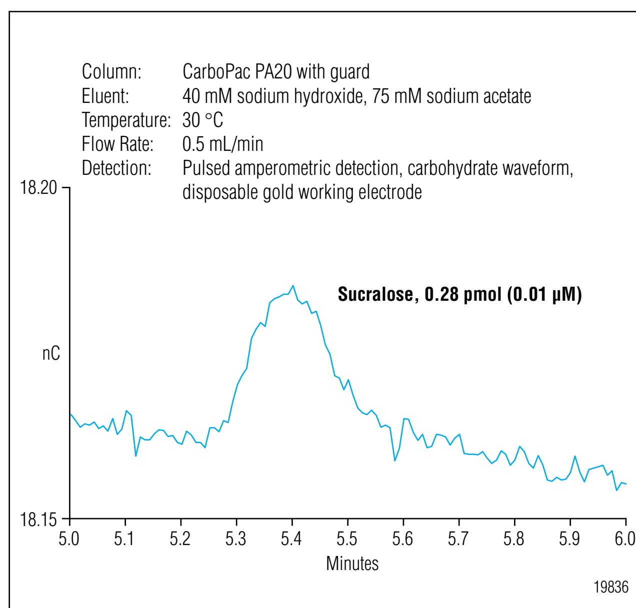

Figure 5. Sucralose at the lower limit of detection of 0.28 pmol (0.01 µM, with a 25-µL injection using the isocratic method (see Figure 1).

Linearity

Figure 4 presents the relationship of sucralose peak area response factors (pC*min/pmol injected) to concentration of the analyte injected (25 µL) using the isocratic separation. The figure shows concentration ranges where the response factor remains unchanged with increasing concentration. In this application note, we consider concentration range to be linear where the response factor remains within a 20% variance from the mean of the plateau region. The plateau region was estimated to extend from 1.24 to 250 pmol, and corresponding mean response factor for this range was 17.0 nC*min/pmol). Concentrations (pmol) of sucralose injected having response factors below 13.6 nC*min/pmol were considered outside the upper linear range. These results show peak area linearity (r^2 = 0.9958) up to 1000 pmol (40 µM for 25-µL injection). The peak height was linear (r^2 = 0.9942) to 760 pmol (30 µM for 25-µL injection).

Lower Limits of Detection and Quantification

The lower limit of detection (LOD) in this application note is based on the average measured baseline noise for 1-min intervals collected over 20 min (9.0 pC). In this study, baseline noise for the standard isocratic method ranged 5.3–112.5 pC (mean ± SD; 15.4 ± 11.3, n = 1690 injections) using the carbohydrate waveform. The concentration (or mass injected) of sucralose at LOD was calculated from three times the average peak-to-peak noise (a height value), divided by the average peak height response factor for sucralose within its linear region. Similarly, the lower limit of quantification (LOQ) is the concentration (or mass injected) calculated from ten times the average peak-to-peak noise. The estimated LOD was found to be 0.28 pmol (0.01 µM for 25-µL injection), and the LOQ was 0.93 pmol (0.04 µM for 25-µL injection). Figure 5 shows the sucralose peak at the LOD where the peak height is about three times above the noise (signal-to-noise = ~3).

Precision and Reproducibility

The peak area and retention time RSDs were determined for replicate injections of sucralose standards (10 µM for 10-µL injection) over 4.7 days (619 injections) using the isocratic method. Over this time, the 250 mM NaOH (eluent B) eluent was replaced once while the (A) water and (C) sodium acetate eluents were unchanged. The sucralose retention time ranged from 5.49 to 5.67 min (mean ± SD; 5.60 ± 0.04 min, 0.7% RSD). No upward or downward trend was observed. When a relatively pure sucralose sample (e.g., a standard) must be analyzed, the isocratic method can be used without any column regeneration for at least four days. For complex samples such as the Red Raspberry Diet Rite beverage, the same isocratic method can also be used, but periodic column washes

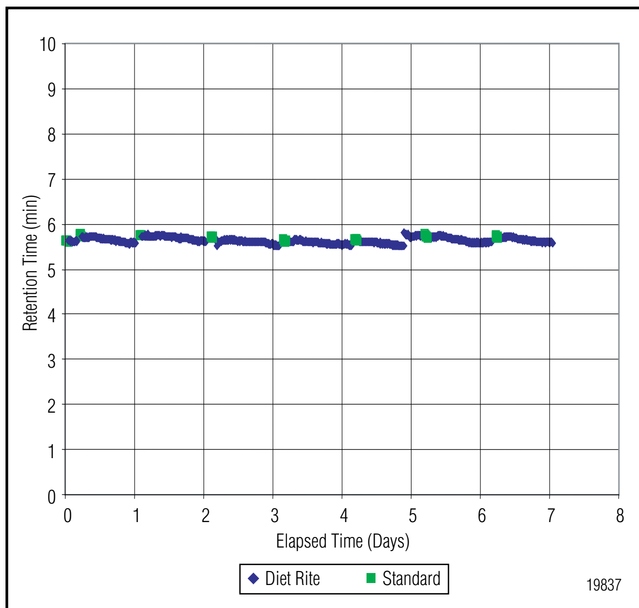

Figure 6. Reproducibility of sucralose retention times for a standard and for sucralose in 50-fold diluted Red Raspberry Diet Rite beverage over seven days (836 injections) using the isocratic method (see Figure 1), with column washes every 24 h.

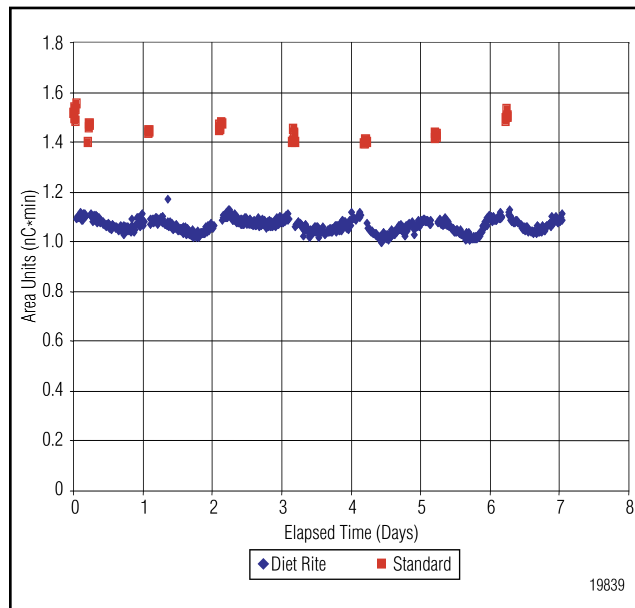

Figure 7. Reproducibility of sucralose peak area for a standard and for sucralose in 50-fold diluted Red Raspberry Diet Rite beverage over seven days (836 injections) using the isocratic method (see Figure 1), with column washes every 24 h.

(e.g., 30 min every 24 h) should be used to regenerate the column to full capacity. Figure 6 shows sucralose retention times over seven days for the following sequence of injections: six injections of sucra-lose standard (B), followed by 120 injections of a 50-fold diluted beverage (A), followed by a 30 min wash with 40 mM NaOH with 800 mM sodium acetate at 0.5 mL/min. This sequence was repeated seven times. Retention times ranged 5.51–5.83 min with a slight trend to shorter retention times within each day that were restored to original retention times after each 30 min column wash. Table 1 presents the day-to-day precision with an RSD range of 0.1–1.2% for standards within each of the seven 24-h periods (10 µM), and 0.5–1.2% for the Diet Rite beverage (7.8 µM measured concentration).

The peak area precision for a sucralose standard 10 µM for 10-µL injection) injected for 4.7 days (619 injections) using the isocratic method ranged from 1.23 to 1.43 nC*min (mean ± SD; 1.34 ± 0.04 min, 2.7% RSD). Peak height ranged from 6.493 to 7.616 nC (mean ± SD; 7.093 ± 0.222 min, 3.1% RSD. No increasing or decreasing trending was observed over the 4.7 days. Figure 7 presents the sucralose peak area measured in 50-fold diluted Red Raspberry Diet Rite over 7 days. The peak area RSD was 3.0% for the standard and 2.4% for the beverage. Daily RSDs ranged from 0.3% to 2.9% for the standard and from 1.6% to 2.6% for the sample. The high retention time and peak area reproducibilities indicate that this method is suitably rugged for this application.

Table 1. Sucralose Peak Rentention Time and Peak Area Precision Over Seven Days Using the Isocratic Method (See Figure 1)

	Sucralose in Red Raspberry Diet Rite Beverage								Sucralose Standard							
	Retention Time (min)				Peak Area * (nC min)				Retention Time (min)				Peak Area (nC * min)			
Days	Mean	SD	N	RSD	Mean	SD	N	RSD	Mean	SD	N	RSD	Mean	SD	N	RSD
1	5.65	0.05	120	0.8%	1.074	0.022	120	2.0%	5.68	0.07	11	1.2%	1.491	0.044	11	2.9%
2	5.70	0.04	120	0.8%	1.058	0.024	120	2.3%	5.75	0.00	4	0.1%	1.444	0.003	4	0.3%
3	5.62	0.03	113	0.5%	1.082	0.017	120	1.6%	5.71	0.01	6	0.2%	1.464	0.013	11	0.9%
4	5.59	0.04	118	0.7%	1.058	0.019	118	1.8%	5.63	0.02	6	0.4%	1.422	0.022	6	1.5%
5	5.59	0.07	120	1.2%	1.052	0.027	120	2.6%	5.65	0.02	6	0.3%	1.402	0.008	6	0.5%
6	5.68	0.06	120	1.0%	1.054	0.028	120	2.6%	5.73	0.03	6	0.5%	1.428	0.009	6	0.6%
7	5.64	0.05	125	0.8%	1.072	0.022	125	2.0%	5.71	0.02	46	0.3%	1.504	0.015	6	1.0%
Total 7 Days	5.64	0.06	836	1.1%	1.064	0.025	836	2.4%	5.69	0.05	45	0.9%	1.456	0.043	45	3.0%

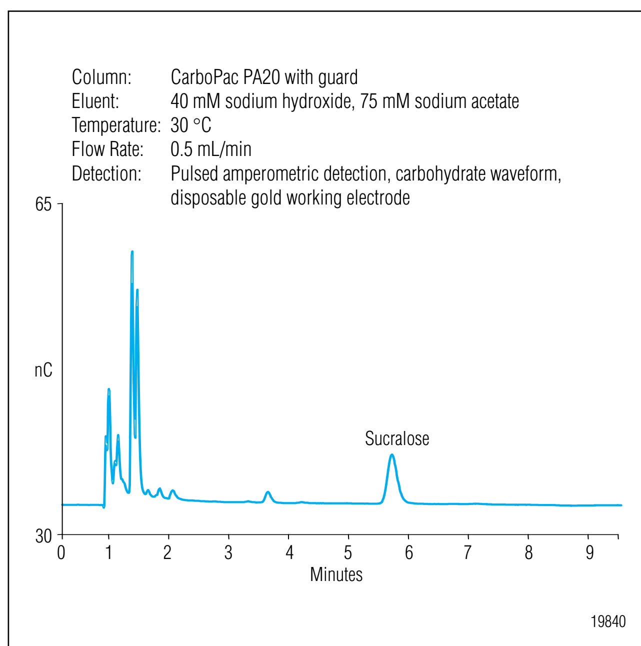

Figure 8. Determination of sucralose in 50-fold diluted Red Raspberry Diet Rite beverage (25 µL) using the isocratic method (see Figure 1).

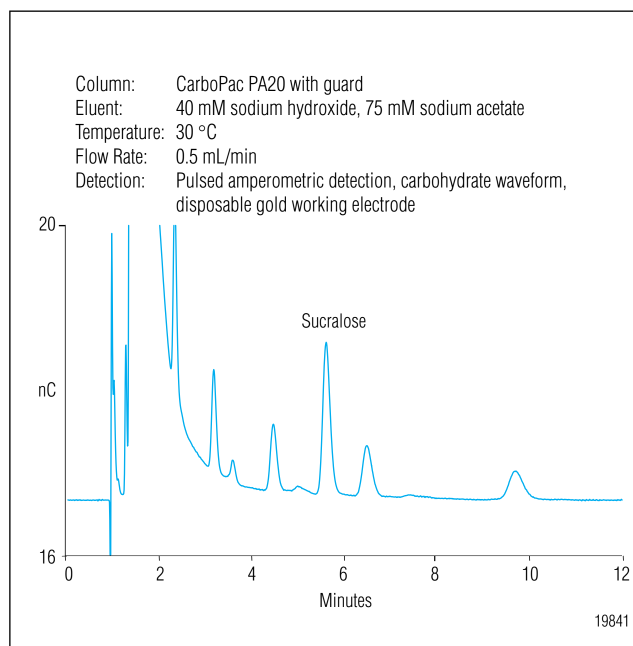

Figure 9. Determination of sucralose in 100 µg/mL Splenda (25 µL) using the isocratic method (see Figure 1).

Determination of Sucralose in Food and Beverages

Red Raspberry Diet Rite Beverage

The Red Raspberry Diet Rite beverage product contains carbonated water, citric acid, natural and artificial flavors, sucralose (Splenda brand nonnutritive sweetener), caramel color, potassium citrate, potassium benzoate, acesulfame potassium (Sunett® Brand, nonnutritive sweetener), and Red Dye #40. Figure 8 shows the isocratic separation of Diet Rite. Sucralose is well resolved from the other detected ingredients of the soda. Most ingredients, including acesulfame, will not be detected by PAD. The measured concentration of sucralose in a 50-fold dilution of this beverage was 7.8 µM for a 25-µL injection, and therefore the concentration of sucralose in the undiluted beverage was calculated to be 390 µM (155 µg/mL). Quantitative spike recovery, ranging from 92% to 96%, was observed for 10 µM sucralose in 50-, 100-, 500-, 1000-, and 10000-fold dilutions of the beverage. The recovery of sucralose can vary with different food and beverage types, and should be determined for each sample type to ensure accurate measurement.

Splenda

Splenda is a granular "no-calorie" sweetener added to food and beverages in a manner similar to granulated table sugar. The ingredients, as listed on the product label, include dextrose, maltodextrin, and sucralose. Dextrose is also known as D-glucose. Maltodextrins are partial hydrolysates of starch. Figure 9 presents the isocratic separation of 100 µg Splenda/mL (10-µL injection). The sucralose peak was resolved from other ingredients, whereas the dextrose peak eluted at the void. Maltodextrin peaks elute from the column after sucralose and during the recommended column wash with 800 mM sodium acetate.

Recognizing that a simultaneous determination of dextrose and sucralose could be advantageous, we developed an alternative gradient method that elutes dextrose at a low hydroxide concentration (40 mM), and then elutes sucralose with an acetate gradient. Figure 10 presents the chromatographic separation of both dextrose and sucralose in Splenda using the described gradient method. This figure presents a chromatogram where the dextrose peak (Peak 2) is displayed full scale; and the sucralose peak appears as a minor peak (Peak 6).

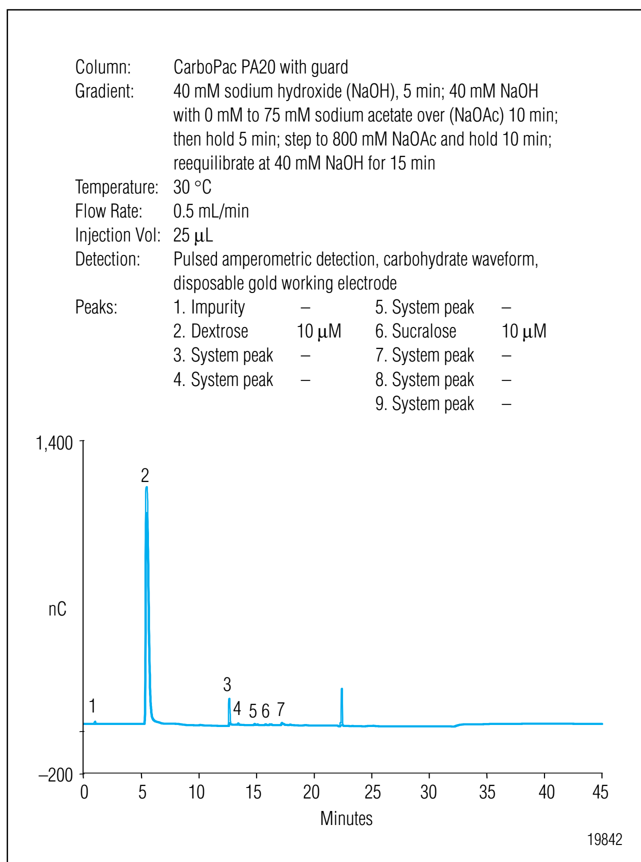

Figure 10. Determination of dextrose and sucralose in 100 µg/mL Splenda using the gradient method (see Figure 2).

Figure 11 rescales the chromatogram to better display the sucralose peak, and overlays it with a gradient without injection (system blank, Panel A), and on a mixed 10-µM dextrose and sucralose standard (Panel B). Peaks 5, 6, 9, 12, 13, 14, and 16 were observed in the system blank. The system peaks were also found in the chromatograms for the standards and Splenda (Panel C). The system blank did not have dextrose (Peak 3) or sucralose (Peak 10). Peaks 1 and 2, found near the void in the dextrose and sucralose standards, were also found in the Splenda sample. Besides dextrose (Peak 3) and sucralose (Peak 10), peaks 4, 7, 8, 11, 15, and 17 were unique peaks—probably maltrodextrins—found in Splenda. The dextrose concentration was determined to be 474 µM (85.3 µg/mL; 85% by weight) for a 100-µg/mL solution of Splenda, whereas sucralose was 3.6 µM (1.4 µg/mL; 1.4% by weight) for the same solution of Splenda.

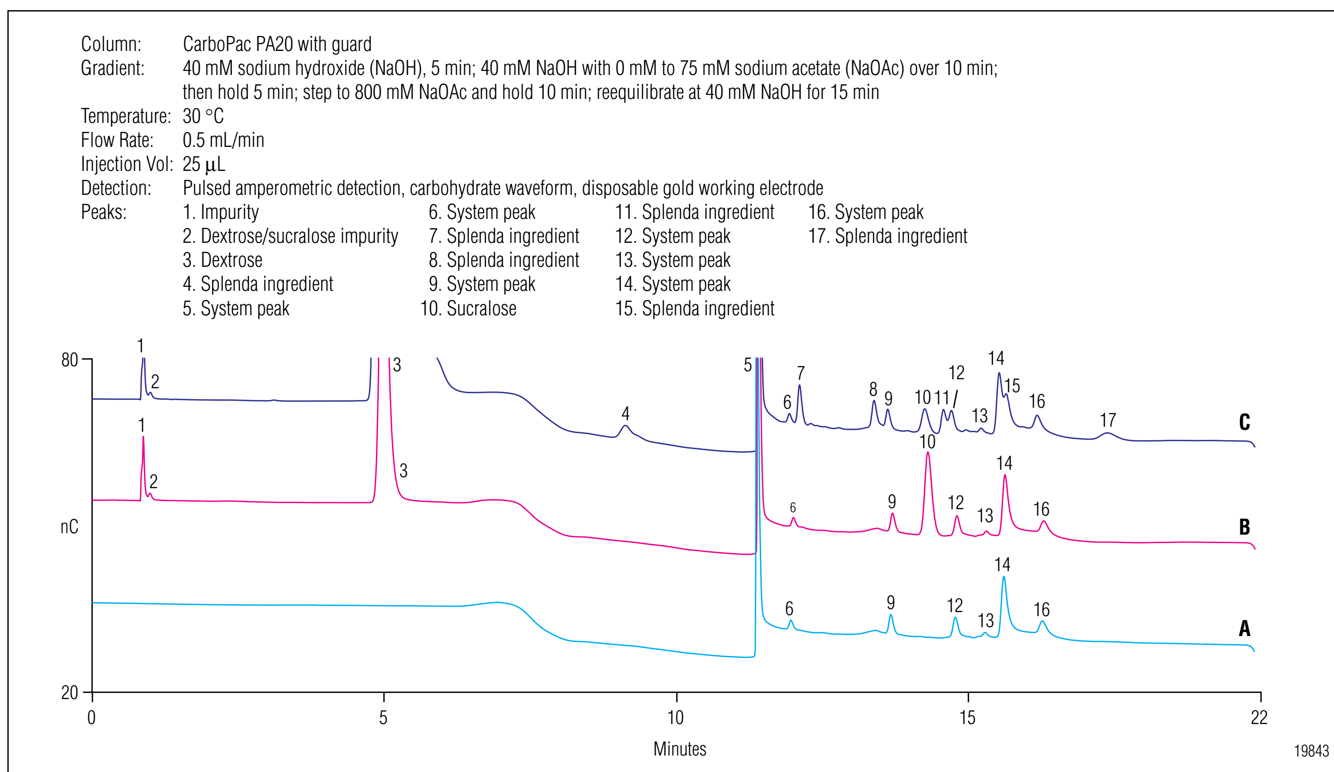

Figure 11. Expanded view of chromatograms using the gradient method (see Figure 2), comparing: (A) blank (without injection), (B) 10 µM dextrose and sucralose standards, and (C) 100 µg/mL Splenda.

CONCLUSION

HPAE-PAD can be used to determine sucralose food and beverages. The linear range of electrochemical response extended over 3 orders of magnitude, from 0.01 µM (LOD) up to 40 µM (16 µg/mL; 25-µL injection). High precision, method ruggedness, and high spike recovery are possible for these complex sample matrices. Mixtures of sucralose and other carbohydrates (e.g., dextrose) can be determined simultaneously.

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LIST OF SUPPLIERS

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Determination of Sugars in Molasses by High-Performance Anion Exchange with Pulsed Amperometric Detection

INTRODUCTION

The accurate measurement of the amount of sugar in final molasses is economically important to sugar mills, because the purchase price of the final molasses is directly related to its sugar content.

Historically, gas chromatography (GC) has been used extensively for sugar analysis. While GC results are accurate, the analytical process involves a time-consuming derivatization procedure. High-performance liquid chromatography (HPLC) was developed in the early 1970s, and sugar mill chemists had hopes of using this new technique for their sugar analysis. However, amino-bonded silica columns gave imprecise results for reducing sugars.[1] In final molasses samples, cation exchange columns gave slightly higher glucose and sucrose response and much higher fructose response when compared with gas chromatography results.[2] The discrepancies were attributed to impurities in the molasses that coeluted with the sugars. The nonspecific refractive index detection used for these analyses could not differentiate between the sugars and other substances present in the molasses.

A high-performance anion exchange method with pulsed amperometric detection (HPAE-PAD) has recently been developed to accurately determine the sugar concentrations of cane and beet final molasses samples.[3] Carbohydrates are weak acids with pK_as above 11. The use of sodium hydroxide as an eluent promotes ionization of the carbohydrates to their anionic form. The pellicular resin of the anion exchange column is stable and durable within the 1–14 pH range. PAD is highly specific when using pulsed potentials optimized for carbohydrates. An eluent pH of greater than 12 facilitates the oxidation reaction at the working electrode and the pulsed potentials keep the electrode clean.

Chemists at the Sugar Milling Research Institute have used HPAE-PAD to measure glucose, fructose, and sucrose in cane molasses samples.[4] J. Thompson refined this method with the use of internal and external standards.[5] C. Day-Lewis compared this improved method with the official GC method and found that the two methods agreed with respect to precision and accuracy.[3] An interlaboratory study using this method has been completed and approved by the International Commission of Uniform Methods for Sugar Analysis (ICUMSA). Submission to the Association of Official Analytical Chemists International (AOAC International) is also planned.

EQUIPMENT

Dionex chromatography system consisting of:

- High-Performance Pump
- Liquid Chromatography Module
- Pulsed Electrochemical Detector
- Eluent Organizer or Eluent Degas Module

Dionex PeakNet Chromatography Workstation or AI-450 Chromatography Workstation

REAGENTS AND STANDARDS

Deionized water, 18 MΩ-cm resistance

Sodium hydroxide solution, 50% w/w, low carbonate

Glucose, fructose, lactose, sucrose, and raffinose

CONDITIONS

Columns:	CarboPac™ PA1 Analytical (4 x 250 mm) and guard (4 x 50 mm)
Expected Operating Pressure:	8–10 MPa (1200–1500 psi)
Inj. Volume:	50 µL
Eluent:	150 mM Sodium hydroxide (NaOH)
Flow Rate:	1 mL/min
Detection:	Pulsed amperometry, gold working electrode standard carbohydrate settings

Note: See Dionex Technical Note 21[6] for a discussion of Pulse Potentials.

PREPARATION OF SOLUTIONS AND REAGENTS

Eluent: 150 mM Sodium Hydroxide

Dilute 7.8 mL of sodium hydroxide solution (50% w/w, low carbonate) in 1.0 L of helium sparged deionized water. Sodium hydroxide pellets are coated with a layer of carbonate, and will not produce an acceptable eluent.

CALIBRATION STANDARD

Lactose Solution (32g/L)

Lactose is used as the internal standard. In a 250-mL volumetric flask, dissolve 8 g of lactose in deionized water and dilute to the 250-mL mark with deionized water.

Raffinose Solution (7.05g/L)

Weigh 0.14 g of raffinose and 19.86 g of deionized water into a container. Weight/weight measurement is used here for precise concentration.

Molasses Calibration Standards

Dissolve and dilute each of the standard sets in Table 1 (Cane Molasses) and each of the standard sets in Table 2 (Beet Molasses) in individual 100-mL volumetric flasks, with deionized water to the 100-mL mark. Dilute 1 mL of each stock standard to the 100-mL mark with deionized water in another 100-mL volumetric flask.

SAMPLE PREPARATION

Cane

Weigh 1.0 g of cane sugar in a wide-mouth container. Add 5 mL of the lactose solution, dissolve in approximately 50 mL of deionized water, transfer it to a 100 mL volumetric flask, and dilute to the mark with deionized water. Dilute 1 mL of this stock cane sample to the mark of a 100-mL volumetric flask with deionized water. Filter this sample through a 0.45-µm filter. Make a duplicate sample in a separate container.

Beet

Weigh 0.7 g of beet sugar in a wide-mouth container. Add 5 mL of the lactose solution, dissolve in approximately 50 mL of deionized water, transfer it to a 100-mL volumetric flask, and dilute to the mark with deionized water. Dilute 1 mL of this stock beet sample to the mark of a 100-mL volumetric flask with deionized water. Filter this sample through a 0.45-µm filter. Make a duplicate sample in a separate container.

Table 1 Cane Molasses

Sugars	Std C1	Std C2	Std C3
Glucose (g)	0.02	0.06	0.10
Fructose (g)	0.03	0.07	0.11
Sucrose (g)	0.25	0.31	0.37
Lactose Solution (mL)	5.00	5.00	5.00

Table 2 Beet Molasses

Sugars	Std B1	Std B2	Std B3
Sucrose (g)	0.25	0.30	0.35
Raffinose Solution (mL)	1.00	1.50	2.00
Lactose Solution (mL)	5.00	5.00	5.00

EXPERIMENTAL SETUP

The same protocol applies for both cane and beet samples. The three calibration standards are initially run in sequence and sucrose is checked for linearity. For the sample runs, the middle calibration standard is run first, followed by the two duplicates of a molasses

EXAMPLE 1: Example of calculations for determining sucrose in final cane molasses

Calibration Mass for Sucrose in C2 run before molasses sample	0.3100 g
Calibration Mass for Sucrose in C2 run after molasses sample	0.3100 g
Calibration Height for Sucrose in C2 run before molasses sample	132444
Calibration Height for Sucrose in C2 run after molasses sample	132438
Calibration Mass for Lactose in C2 run before molasses sample	0.160 g
Calibration Mass for Lactose in C2 run after molasses sample	0.160 g
Calibration Height for Lactose in C2 run before molasses sample	161321
Calibration Height for Lactose in C2 run after molasses sample	161830
Mass of molasses sample	1.0725 g
Height for Sucrose in molasses sample	144579
Mass for Lactose in molasses sample	0.160 g
Height for Lactose in molasses sample	165183

RRF of sucrose before sample:

$$\frac{0.3100}{132444} \times \frac{161321}{0.160} = 2.36 \qquad \{1\}$$

RRF of sucrose after sample:

$$\frac{0.3100}{132438} \times \frac{161830}{0.160} = 2.37 \qquad \{1\}$$

Average of the RRFs:

$$\frac{2.36 + 2.37}{2} = 2.36 \qquad \{2\}$$

Percentage of sucrose in the molasses sample:

$$\frac{144579 \times 0.160}{165183} \times 2.36 \times \frac{100}{1.0725} = 30.8\% \qquad \{3\}$$

sample, followed by another middle calibration standard, followed by the two duplicates of a different molasses sample, etc. This pattern is repeated until all molasses samples have been run. The middle calibration standards that bracket the molasses sample runs are used to determine the relative response factors (see the "Calculations" section).

CALCULATIONS

Relative Response Factors (RRFs) are calculated for each sugar (i.e., glucose, fructose and sucrose for cane; and sucrose and raffinose for beet) of each calibration standard that is run. For example, the RRF of sucrose in a standard can be calculated by using the following equation:

$$RRF = \frac{M_{suc\text{-}std}}{H_{suc\text{-}std}} \times \frac{H_{lac\text{-}std}}{M_{lac\text{-}std}} \qquad \{1\}$$

where: RRF = Relative Response Factor
$M_{suc\text{-}std}$ = Mass of sucrose standard (g)
$H_{suc\text{-}std}$ = Peak height of sucrose standard
$M_{lac\text{-}std}$ = Mass of internal lactose standard (g)
$H_{lac\text{-}std}$ = Peak height of lactose standard

The sugar percentages in each final molasses sample can then be calculated in two steps (see Example 1).

Step 1: Determine the average of the RRFs for sucrose from the standards immediately *before* and *after* the final molasses sample by using the following equation:

$$RRF_{ave} = \frac{RRF_1 + RRF_2}{2} \qquad \{2\}$$

where: RRF_{ave} = Average RRF for sucrose
RRF_1 = RRF of sucrose *before* the cane molasses sample
RRF_2 = RRF of sucrose *after* the cane molasses sample

Step 2: Determine the percentage of sucrose in the final cane molasses sample by using the following equation:

$$\%_{suc} = \frac{H_{suc\text{-}smp} \times M_{lac\text{-}smp}}{H_{lac\text{-}smp}} \times RRF_{ave} \times \frac{100}{M_{mol\text{-}smp}} \qquad \{3\}$$

where: $\%_{suc}$ = Percentage of sucrose in the sample
$H_{suc\text{-}smp}$ = Peak height for sucrose in the sample
$H_{lac\text{-}smp}$ = Peak height for lactose in the sample
$M_{lac\text{-}smp}$ = Mass of internal lactose standard (g)
RRF_{ave} = Average RRF taken from Step 1
$M_{mol\text{-}smp}$ = Mass of molasses (g)

Relative Standard Deviation (RSD) should not be greater than 1% for duplicates of sucrose, should be less than 2% for duplicates of glucose and fructose, and should be less than 6% for duplicates of raffinose. If the RSD is not within these guidelines, the sample should be reinjected or prepared again.

RESULTS AND DISCUSSION

The CarboPac PA1 column reproducibly separates the sugar components of cane and beet final molasses. PAD, with pulse potentials optimized for carbohydrates, permits detection of the sugars in final molasses samples without interferences from coeluting components, if any.

Figure 1 shows the separation of the sugars in cane final molasses and the internal standard, lactose. The elution order is glucose, fructose, lactose, then sucrose. After performing the experiment, the percentage of each sugar is calculated (see Example 1). The expected ranges for cane final molasses are 2–10% for glucose, 3–11% for fructose, and 25–37% for sucrose. The percentages determined for this sample, as shown in Figure 1, are 4.39% for glucose, 6.67% for fructose, and 30.8% for sucrose.

Figure 2 shows the separation of the sugars in beet final molasses and the internal standard, lactose. The elution order is lactose, sucrose, then raffinose. The percentage of each sugar is calculated (as in Example 1). The expected ranges for beet final molasses are 35–50% for sucrose and 1–2% for raffinose. The percentages determined for this sample were 47.7% for sucrose and 2.10% for raffinose. Because of the low concentration of raffinose in beet molasses and the high dilution factor required for optimal sucrose analysis, the raffinose percentage is less precise than the sucrose percentage. When necessary, the raffinose can be determined separately using the first 1:100 dilution, rather than the second 1:100 dilution.

HPAE-PAD methodology uses pellicular anion exchange resin technology coupled with selective amperometric detection. The monosaccharides will elute first, followed by disaccharides and then trisaccharides. Pulsed amperometric detection uses a repeating sequence of three potentials, which are applied for specific durations. Using the pulsed conditions in Technical Note 21,[6] detection is optimized for carbohydrates.

For further details concerning pulse sequences used in pulsed amperometric detection, refer to Dionex Technical Note 21.[6] To drive the oxidation reaction at the working electrode of the detector, the eluent pH should be greater than 12. For further details concerning carbohydrate determination, refer to Dionex Technical Note 20.[7]

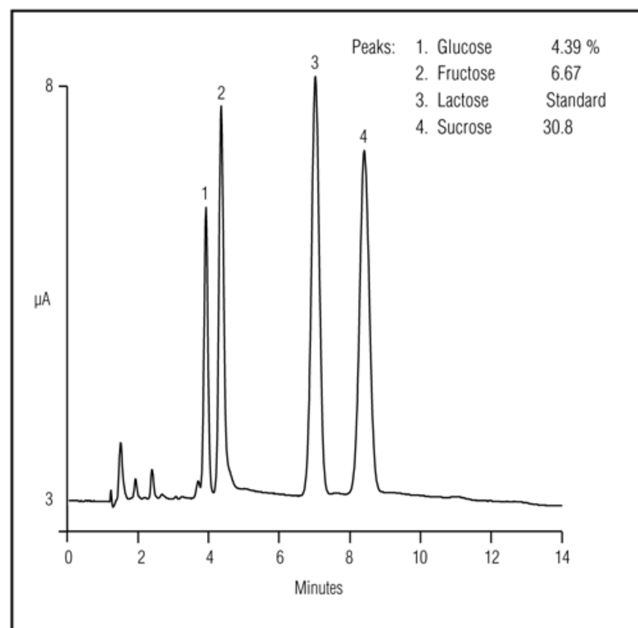

Figure 1 *Sugar cane sample prepared and run by this method.*

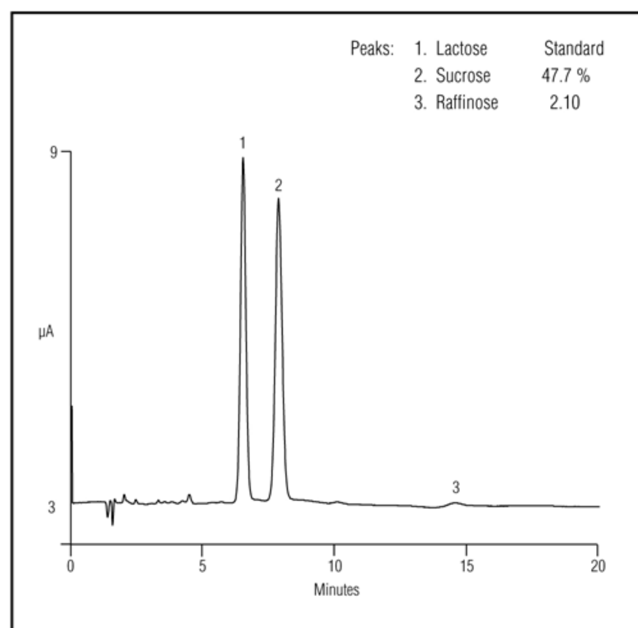

Figure 2 *Sugar beet sample prepared and run by this method.*

PRECAUTIONS

Metal should be eliminated from the eluent flow path, including the injection valve, prior to the column. Metal contamination of the analytical column can result in poor peak efficiency and/or symmetry, which may lead to poor reproducibility.

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Analysis of Carbohydrates by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAE-PAD)

INTRODUCTION

Methods for the liquid chromatographic analysis of carbohydrates have often employed silica-based amino-bonded or polymer-based, metal-loaded, cation-exchange columns, with refractive index (RI) or low-wavelength ultraviolet (UV) detection. These analytical methods require attention to sample solubility, sample concentration and, in the case of the metal-loaded cation-exchange columns, also require column heating. In addition, RI and low-wavelength UV detection methods are sensitive to eluent and sample matrix components. This usually precludes the use of gradients and often requires stringent sample cleanup prior to injection.

As a result, an improved chromatographic technique known as high-performance anion exchange (HPAE) was developed to separate carbohydrates. Coupled with pulsed amperometric detection (PAD), it permits direct quantification of nonderivatized carbohydrates at low-picomole levels with minimal sample preparation and cleanup. HPAE chromatography takes advantage of the weakly acidic nature of carbohydrates to give highly selective separations at high pH using a strong anion-exchange stationary phase. This technical note is intended as an introduction to HPAE-PAD carbohydrate analysis. The technique has been reviewed extensively,[1–4] and those articles should be consulted for more specific details.

HPAE-PAD is extremely selective and specific for carbohydrates because:

1. Pulsed amperometry detects only those compounds that contain functional groups that are oxidizable at the detection voltage employed (in this case, sensitivity for carbohydrates is orders of magnitude greater than for other classes of analytes).
2. Neutral or cationic sample components in the matrix elute in, or close to, the void volume of the column. Therefore, even if such species are oxidizable, they do not usually interfere with analysis of the carbohydrate components of interest.

ANION-EXCHANGE CHROMATOGRAPHY

I. Mechanism of Separation

Although anion-exchange chromatography has been used extensively to analyze acidic carbohydrates and glycopeptides, it has not been commonly used for analysis of neutral sugars. However, examination of the pK_a values of the neutral monosaccharides listed in Table 1 shows that carbohydrates are in fact weak acids. At high pH, they are at least partially ionized, and thus can be separated by anion-exchange mechanisms. This approach cannot be used with classical silica-based columns because these matrices dissolve at high pH. Anion exchange at high pH is, however, ideally suited to base-stable polymer anion-exchange columns.

Table 1. Dissociation Constants of Some Common Carbohydrates[5] (in water at 25 °C)

Sugar	pK_a
Fructose	12.03
Mannose	12.08
Xylose	12.15
Glucose	12.28
Galactose	12.39
Dulcitol	13.43
Sorbitol	13.60
α-Methyl glucoside	13.71

II. CarboPac™ Columns

A. CarboPac PA1 and PA-100 Columns

Dionex designed the CarboPac series of columns specifically for carbohydrate anion-exchange chromatography. These columns permit the separation and analysis of mono-, oligo-, and polysaccharides. The CarboPac PA1 and CarboPac PA100 are packed with a unique polymeric, nonporous, MicroBead™ pellicular resin. MicroBead resins exhibit rapid mass transfer, high pH stability (pH 0 – 14), and excellent mechanical stability that permits back pressures of more than 4000 psi (28 MPa). Column reequilibration after gradient analysis is fast, generally taking 10 min or less. A diagram of a typical pellicular anion-exchange resin bead is shown in Figure 1.

Both the CarboPac PA1 and the CarboPac PA100 are designed for the rapid analysis of mono- and oligosaccharides. The CarboPac PA1 is particularly well-suited to the analysis of monosaccharides and the separation of linear homopolymers, while the CarboPac PA100 is optimized for oligosaccharide resolution and separation. Several examples of separations obtained using these columns are shown in the “Applications” section of this technical note.

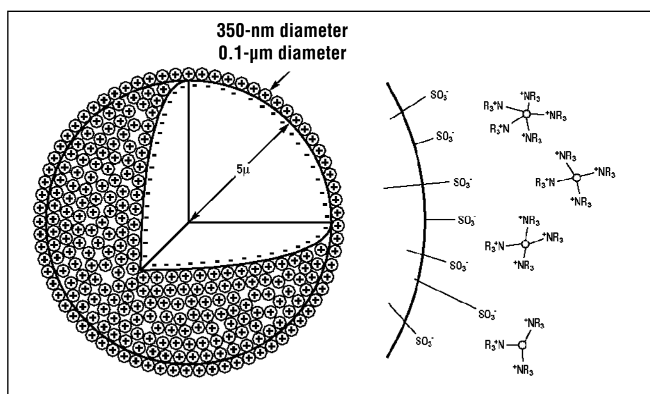

Figure 1. Pellicular anion-exchange resin bead.

B. CarboPac MA1 Column

Reduced carbohydrates (also called sugar alcohols) have traditionally been a difficult class of carbohydrates to separate by liquid chromatography. They are weaker acids than their nonreduced counterparts (compare the pK_as of glucose and sorbitol or galactose and dulcitol in Table 1), and are therefore poorly retained on the CarboPac PA1 and PA100 columns. The CarboPac MA1 was developed to address the challenge of retaining and separating extremely weak acids. This column is packed with a macroporous polymeric resin which has an ion-exchange capacity 45 times that of the CarboPac PA1. As a result, weak anions bind more strongly to the column, requiring higher sodium hydroxide concentrations for elution. The increase in hydroxide ion concentration leads to greater ionization of the sugar alcohols, with greatly improved retention and resolution on the column.

Table 2. k´ Values of Selected Analytes on the CarboPac MA1 Column[a]

Analyte	Eluent Concentration (M NaOH)				
	0.05	0.14	0.25	0.38	0.50
Glycerol	1.13	0.99	0.89	0.80	0.72
m-Inositol	1.32	1.08	0.86	0.69	0.56
s-Inositol	1.63	1.30	1.02	0.81	0.64
GlcNol	1.81	1.40	1.09	0.89	0.75
Fucitol	1.94	1.63	1.40	1.18	1.05
Erythritol	2.02	1.71	1.44	1.25	1.13
GalNol	2.29	1.81	1.39	1.13	0.95
GalNAcol	2.35	1.81	1.38	1.12	0.95
GlcNAcol	2.61	1.96	1.48	1.18	0.95
Xylitol	3.09	2.48	1.95	1.59	1.35
Arabitol	4.69	3.62	2.76	2.24	1.92
Sorbitol	6.43	4.72	3.33	2.55	2.06
Dulcitol	6.52	5.04	3.73	2.87	2.26
Adonitol	7.09	5.31	3.83	2.96	2.43
Mannitol	8.98	6.38	4.37	3.28	2.63
Fucose	10.34	4.72	2.52	1.69	1.25
Isomaltitol	12.22	8.15	4.89	3.30	2.43
Lactitol	14.97	9.61	5.49	3.57	2.43
Gp-Man	15.66	10.36	6.18	4.15	3.05
GalN	18.56	7.16	3.39	2.13	1.48
GlcN	20.88	7.71	3.61	2.24	1.55
Maltitol	31.21	17.25	8.80	5.44	3.67
Glucose		15.70	7.19	4.31	2.91
Mannose		13.55	6.15	3.72	2.53
Galactose		17.82	8.25	4.99	3.43

[a] The capacity factor, k´, is defined as: $k' = (V_A - V_0)/V_0$, where V_A is the retention volume of the analyte on the column and V_0 is the void volume.

Nonreduced neutral oligosaccharides can also be analyzed on the CarboPac MA1 column, although their analysis times are longer than on the CarboPac PA1 and PA100 columns. Retention of carbohydrates on the CarboPac MA1 can be manipulated by altering the sodium hydroxide concentration of the eluent (see Table 2). Note that the elution order of several of the compounds changes with the sodium hydroxide concentration. This can be used to design separation strategies for specific sets of analytes. Examples of separations obtained with the CarboPac MA1 column are shown in the "Applications" section of this technical note.

Table 3. Comparison of the CarboPac MA1, PA1, and PA100			
Characteristic	**CarboPac MA1**	**CarboPac PA1**	**CarboPac PA100**
Recommended applications	Mono- and disaccharide alcohol analysis in food products, physiological fluids, tissues, and reduced glycoconjugate saccharides[b]	Monosaccharide compositional analysis, linear homopolymer separations, saccharide purification	Oligosaccharide mapping and analysis
Resin composition	8.5-µm-diameter vinylbenzylchloride/divinylbenzene macroporous substrate fully functionalized with an alkyl quaternary ammonium group	10-µm-diameter polystyrene/divinylbenzene substrate agglomerated with 350-nm MicroBead quaternary amine functionalized latex	10-µm-diameter ethylvinylbenzene/divinylbenzene substrate agglomerated with 350-nm MicroBead quaternary amine functionalized latex
MicroBead latex cross-linking	N/A, no latex	5% cross-linked	6% cross-linked
Anion-exchange capacity	4500 µeq per 4 × 250-mm column	100 µeq per 4 × 250-mm column	90 µeq per 4 × 250-mm column
Recommended flow rate	0.4 mL/min (4 × 250-mm column)	1 mL/min (4 × 250-mm column)	1 mL/min (4 × 250-mm column)
pH compatibility	pH 0–14	pH 0–14	pH 0–14
Organic solvent compatibility	0%	0–2%	0–100%
Maximum back pressure	2000 psi (14 MPa)	4000 psi (28 MPa)	4000 psi (28 MPa)

[b] Note that sialylated and other acidic mono- and oligosaccharides may not be recovered from the CarboPac MA1 column. It is not recommended that this column be used with these analytes.

III. Guidelines for CarboPac Column Selection

Table 3 provides a comparison of the three CarboPac columns. The following guidelines are useful in selecting the right CarboPac column for a particular application.

A. Monosaccharides

For reducing monosaccharides, the recommended column is the CarboPac PA1, while the MA1 is recommended for sugar alcohols. The CarboPac MA1 column also generates excellent neutral monosaccharide separations, although retention times are longer than on the PA1. Amino-sugars are better resolved on the CarboPac PA1 than on the MA1, but the reverse is true for N-acetamido sugars.

B. Neutral Oligosaccharides

The CarboPac PA100 is the most appropriate column for the oligosaccharide mixtures characteristic of glycoprotein-derived oligosaccharides, although these compounds are only slightly less well-resolved on the CarboPac PA1 column than on the PA100. Neutral oligosaccharides up to nine monosaccharide units in size are separable on the CarboPac MA1. However, the CarboPac MA1 will usually have longer retention times than the PA100, and selectivities of the two columns are almost identical.

Oligosaccharides cleaved by reductive β-elimination from glycoproteins contain a reduced terminal and generally elute earlier than the same oligosaccharide with a reducing terminal. Reduced di- and trisaccharides will elute significantly earlier than their nonreduced counterparts, and may be poorly resolved on the CarboPac PA1 and PA100. These compounds are readily separated on the CarboPac MA1 column.

C. Charged Oligosaccharides

Charged oligosaccharides (for example, those that are sialylated, phosphorylated, sulfated, or contain carboxyl groups) are separated based on their composition, linkage, and the level of formal negative charge. They can be separated at both high (13) and low (4.6) pH. At low pH, the separations are largely dependent on the charge-to-mass ratio of the oligosaccharide but may also be influenced by linkage. Selectivity for sialylated oligosaccharides will change with pH as a result of oxyanion formation. The CarboPac PA100 is recommended for sialylated oligosaccharides, although in many cases the PA1 performs adequate separations.

D. Glycosaminoglycans

Oligosaccharides derived from glycosaminoglycans, such as nonsulfated chondroitin disaccharides, are separable on the CarboPac PA1.[6]

E. Linear Polysaccharides

Linear polysaccharides can be separated on the basis of length almost equally well on the CarboPac PA1 and PA100. The CarboPac PA1 has a slightly higher capacity than the PA100 and is the better column to use for linear homopolymers. The CarboPac PA100 was designed for nonlinear and heterogeneous polysaccharides. N, N – 1 resolution of linear polysaccharides has been demonstrated on the CarboPac PA1 and PA100 columns with inulin polymers to over 60 monosaccharide units. The CarboPac PA1 requires a higher sodium acetate concentration than the PA100 to elute species of the same length.

Table 4 summarizes the applications for which the three CarboPac columns are the most appropriate.

The CarboPac PA1 and PA100 are available in guard (4 × 50 mm), analytical (4 × 250 mm), semi-preparative (9 × 250 mm) and preparative sizes (22 × 250 mm). A guard column should be used in front of an analytical column to prolong the analytical column life. The CarboPac MA1 column is available in analytical and guard sizes. A partial list of column part numbers follows. Please contact your local Dionex office to order any column not listed below.

Part No.	Description
35391	CarboPac PA1 Analytical (4 × 250 mm)
43096	CarboPac PA1 Guard (4 × 50 mm)
39686	CarboPac PA1 Semipreparative (9 × 250 mm)
43055	CarboPac PA100 Analytical (4 × 250 mm)
43054	CarboPac PA100 Guard (4 × 50 mm)
44066	CarboPac MA1 Analytical (4 × 250 mm)
44067	CarboPac MA1 Guard (4 × 50 mm)

Table 4. CarboPac Columns Recommended by Application

	CarboPac PA1	CarboPac PA100	CarboPac MA1
Monosaccharides	+++	+/–	++
Sialylated branched oligosaccharides	++	+++	–
Neutral branched oligosaccharides	++	+++	+
Linear oligo- and polysaccharides	+++	+++	–
Reduced mono- and disaccharides	+	–	+++

+++ indicates most suitable
— indicates that the column is not recommended for this application.

IV. Sample Stability at High pH

Carbohydrates undergo a number of well documented reactions at high pH that can potentially interfere with chromatography. However, in most cases these reactions are slow at room temperature and do not appear to occur to any noticeable extent over the time course of the chromatography. Some of these reactions are discussed below:

A. The Lowbry de Bruyn, van Ekenstein Transformations[7] (epimerization and keto-enol tautomerization)

D-fructose elutes as a single sharp peak with no evidence of formation of D-glucose or D-mannose via the Lowbry de Bruyn, van Ekenstein transformation. In addition, when glucose is left in 150 mM sodium hydroxide for four days at room temperature, there is no evidence for the presence of any mannose or fructose.

Epimerization of *N*-acetyl glucosamine (GlcNAc) to *N*-acetyl mannosamine (ManNAc) has been demonstrated for solutions of GlcNAc in 100 mM sodium hydroxide. The equilibrium ratio of GlcNAc: ManNAc was 80:20 after 2–3 hours of exposure. This epimerization is not observed in separations using the CarboPac PA1 column, presumably because the sodium hydroxide concentration is 16 mM and the chromatography is sufficiently rapid (16 min) that exposure to alkali is minimized. Oligosaccharides are separated in 100 mM sodium hydroxide and are also retained longer on the column, particularly when sialylated. Under these conditions, oligosaccharides may exhibit 0 to 15% epimerization. As alditols do not epimerize in alkali, oligosaccharide epimerization can be eliminated if the oligosaccharide is reduced to the alditol prior to chromatography. For the same reason, monosaccharide alcohols are not epimerized in the high concentrations of alkali needed to elute them from the CarboPac MA1 column.

B. De-acetylation of N-acetylated Sugars

The hydrolysis of acylated sugars at high pH is another potential problem. Approximately 20% of a sample of *N*-acetylglucosamine is hydrolyzed to free glucosamine by exposure to 150 mM sodium hydroxide overnight at room temperature. However, chromatography of *N*-acetyl glucosamine at high pH generates a single sharp peak

with no evidence of formation of the (well resolved) free-base analog. Likewise, samples of *N*-acetyl neuraminic acid and *N*-glycolyl neuraminic acid are easily separated as sharp symmetrical peaks[8].

C. ß-Elimination or Peeling of 3-O-Substituents on Reducing Sugars

The ß-elimination of 3-O-substituents on reducing sugars is also a potentially serious side reaction that proceeds, in most cases, too slowly at room temperature to be a problem. The treatment of laminaribiose (glucopyranosyl ß-1-3 glucopyranose) with 150 mM sodium hydroxide for 4 h destroys more than 80% of the disaccharide, producing glucose and a second unidentified peak. However, laminaribiose generates a single peak during chromatography by HPAE with no evidence of glucose or other breakdown products.[8] Conversely, D-glucose-3-sulfate, which has a very good leaving group, decomposes rapidly during chromatography.

PULSED AMPEROMETRIC DETECTION

I. Theory of Operation

Pulsed amperometry permits detection of carbohydrates with excellent signal-to-noise ratios down to approximately 10 picomoles without requiring derivatization. Carbohydrates are detected by measuring the electrical current generated by their oxidation at the surface of a gold electrode. The products of this oxidation reaction also poison the surface of the electrode, which means that it has to be cleaned between measurements. This is accomplished by first raising the potential to a level sufficient to oxidize the gold surface. This causes desorption of the carbohydrate oxidation products. The electrode potential is then lowered to reduce the electrode surface back to gold. The sequence of potentials is illustrated in Figure 2.

Pulsed amperometric detection thus employs a repeating sequence of three potentials. Current from carbohydrate oxidation is measured at the first potential, E_1. The second, E_2, is a more positive potential that oxidizes the gold electrode and cleans it of products from the carbohydrate oxidation. The third potential, E_3, reduces the gold oxide on the electrode surface back to gold, thus permitting detection during the next cycle at E_1.

The three potentials are applied for fixed durations referred to as t_1, t_2, and t_3. The step from one potential to the next produces a charging current that is not part of the analyte oxidation current, so the analyte oxidation current

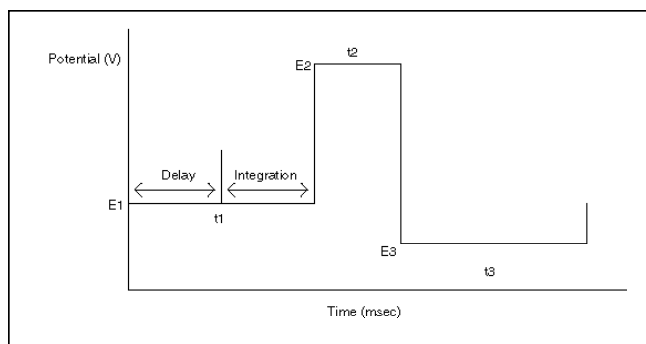

Figure 2. Diagram of the pulse sequence for carbohydrate detection.

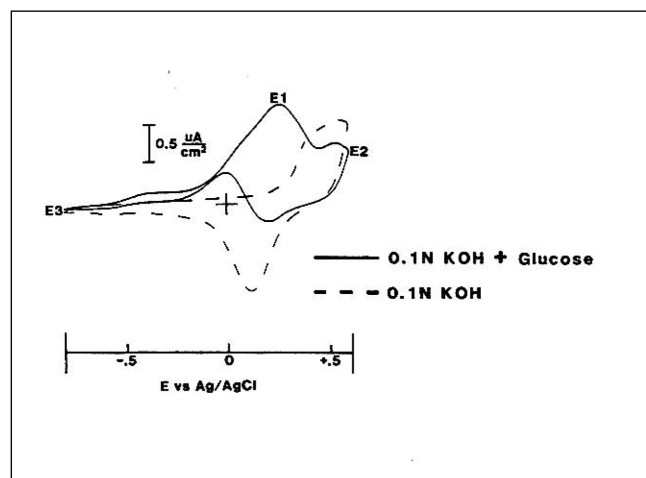

Figure 3. Cyclic voltammetry of glucose on a gold electrode.

is measured after a delay that allows the charging current to decay. The carbohydrate oxidation current is measured by integrating the cell current after the delay. Current integrated over time is charge, so the detector response is measured in coulombs. Alternatively, the average current during the integration period can be reported. In this case, the units used are amperes.

Optimal potentials can be determined by electrochemical experiments such as cyclic voltammetry, in which the applied potentials are slowly scanned back and forth between positive and negative potential limits. The resulting current is plotted on the Y-axis with oxidation (anodic) currents up and reduction (cathodic) currents down. Figure 3 shows the cyclic voltammogram of glucose in a 100 mM potassium hydroxide solution on a gold electrode. The dashed line is a background scan of a solution of 100 mM potassium hydroxide. As the potential is raised, the current starts to rise at about 0.2 V (see Figure 3, upper dashed line). This is caused by oxidation of the gold surface.

Reduction of the surface gold oxide back to gold occurs on the reverse scan (lower dashed line) with a cathodic (negative) current peak at about 0.1 V.

When glucose is present (solid line), its oxidation peaks at about 0.25 V (upper solid trace), which is also the potential at which formation of gold oxide begins. The glucose oxidation current drops as gold oxidation continues to increase, demonstrating that the formation of gold oxide inhibits oxidation of glucose. On the reverse scan, the current actually reverses from negative to positive at the onset of gold oxide reduction, further evidence of the inhibiting effect of gold oxide on the oxidation of glucose. It is thus important to use a measuring potential (E_1) below that required for gold oxidation.

All three potentials are important. However, the most important is E_1 — the potential at which the carbohydrate oxidation current is measured. A plot of detector response as a function of E_1 is shown in Figure 4. The background current is also shown. The maximum response is shown to occur at about 0.2 V for the three sugars tested, although the best signal-to-noise ratio actually occurs at a slightly lower potential. Figure 4 shows that the voltage at which the maximum response occurs is the same for three very different sugars: xylitol, a nonreducing sugar alcohol; glucose, a reducing monosaccharide; and sucrose, a nonreducing disaccharide. This is because the oxidation of the sugars at the electrode is catalyzed by the electrode surface. As a result, the amperometric response of a class of compounds is controlled primarily by the dependence of the catalytic surface state on the electrode potential and not on the redox potentials of the compounds themselves. Pulsed amperometric detection is thus a universal detection method for all carbohydrates, although derivatization of two or more hydroxyl groups will decrease (and may even abolish) detection.

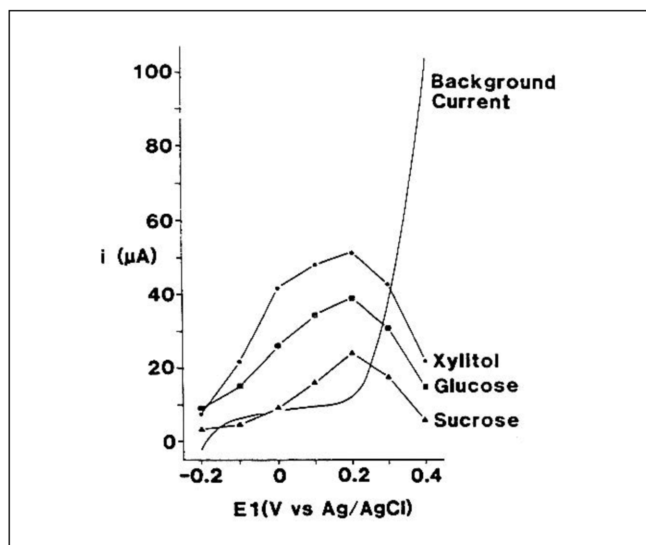

Figure 4. The oxidation current generated at different values of E^1 for three different carbohydrates.

Potential E_2 must be high enough and long enough to oxidize the electrode surface fully so that the carbohydrate oxidation products are completely removed. This potential cannot be too high, however, or excessive gold oxidation will occur and the electrode will wear too rapidly. The third potential, E_3, must be low enough to reduce the oxidized surface of the gold electrode completely without being so low that chemical reductions (for example, of oxygen to hydrogen peroxide) will occur. The results of these reactions may cause baseline disturbances during subsequent measurement at E_1.

Recommended pulse sequences for the Dionex pulsed amperometric detectors are given in Technical Note 21, which is available from your local Dionex representative.

APPLICATIONS

I. Eluent Preparation for Carbohydrate Analysis

When making eluents for carbohydrate analysis, it is important to use reagents of the grade listed:

- 50% (w/w) Sodium hydroxide solution
  Fisher Cat. No. SS254-1
- Anhydrous sodium acetate
  Fluka Cat. No. 71179

- Sodium Hydroxide: It is extremely important to minimize contamination of the eluent solutions with carbonate. Carbonate, being a divalent anion at pH ≥12, binds strongly to the columns and interferes with carbohydrate binding, causing a drastic decrease in column selectivity and a loss of resolution and efficiency. Commercially available sodium hydroxide pellets are covered with a thin layer of sodium carbonate and should *not* be used. A 50% w/w sodium hydroxide solution is much lower in carbonate. Any carbonate present will precipitate to the bottom of the container and can be avoided. The concentration of the 50% sodium hydroxide solution is approximately 19.3 M, so diluting 20.8 mL of a 50% solution into 2 L of water yields a 0.2 M sodium hydroxide solution.

- Distilled Water: It is essential to use high-quality water. It is critical that there be as little dissolved carbon dioxide as possible in the water. It should also be of high resistivity (18 MΩ) and biological contamination should be absent. The use of fresh Pyrex® glass-distilled water is recommended. The still should be fed with high-resistivity (18 MΩ) water, and the use of plastic tubing should be avoided because it often supports microbial growth. Biological contamination is often the source of unexpected glucose peaks after acid hydrolysis.

A. Guidelines to Handling the 50% (w/w) Sodium Hydroxide Solution

1. To avoid mixing of the sodium carbonate precipitate into the solution, store the 50% sodium hydroxide close to where the eluent will be prepared. *Do not shake or stir this solution.*
2. Never pour the solution from the bottle.
3. Pipet sodium hydroxide from the center of the solution, not from edges or bottom. Do not allow the pipet to stir the solution at the bottom.
4. Use only plastic pipets, as sodium hydroxide leaches borate and silicate out of glass. Borate will complex with carbohydrates and will thus alter their chromatographic behavior.
5. Never return unused liquid to the bottle.
6. Close the bottle immediately after each use and leave open for the shortest time possible to avoid carbon dioxide absorption.
7. Discard the bottle of 50% sodium hydroxide when 2 to 3 cm or less of solution remains.

B. Eluent Preparation

It is impossible to completely eliminate all carbonate from eluents. Therefore, to ensure reproducible chromatography, it is essential to use the same methods consistently in preparing the solutions. Once eluents have been prepared, they should be kept blanketed under helium (5–7 psi/34–48 kPa) at all times.

i. Distilled Water

High-quality water should be degassed by one of the following two methods:

1. Sparging with helium for 20–30 min. Degassing is complete when all of the small bubbles first formed upon degassing disappear.
2. Sonication for 30–60 s while degassing with a water vacuum aspirator, followed by a 10-min helium sparge. Degassing is again complete when all of the small bubbles first formed upon degassing disappear.

ii. Sodium Hydroxide

Degas the required volume of water, as described above. After degassing is complete, use a plastic pipet to add the appropriate amount of 50% sodium hydroxide solution to give the required concentration. Avoid bubbling air into the eluent when expelling the 50% sodium hydroxide solution from the pipet. Rinse the pipet by drawing some of the sodium hydroxide/water mixture into the pipet and expelling it back into the solution. Repeat this several times. Add a stirring bar to the mixture and stir gently without agitating the surface for about 2 min.

As an alternative, the 50% sodium hydroxide can be pipetted directly into the distilled water as it is being sparged by the Dionex Eluent Degas Module (EDM). Sparge the water for 15 min, add the sodium hydroxide, rinse the pipet, and swirl the solution in the bottle to mix. Then sparge the solution for an additional 5 min. The sparging will complete the mixing.

Both methods work well. It is most important to be consistent in the method used. Store sodium hydroxide solutions in plastic containers, as they will leach borate and silicate out of glass.

iii. Sodium Hydroxide/Sodium Acetate Solutions

Degas the required volume of water as described above, and transfer it to a graduated cylinder. Add a stir bar and start stirring, while steadily adding the anhydrous crystalline sodium acetate. After the salt dissolves, retrieve the stir bar and add the appropriate volume of 50% (w/w) sodium hydroxide to the graduated cylinder in the same manner as described previously. Bring the volume to the requisite level (e.g., 1–2 L). Vacuum filter the mixture through a 0.2-µm nylon filter. Alternatively, sparge the filtered acetate solution for 15 min, add the sodium hydroxide, swirl the solution to mix, and continue to sparge for 5 min. Once again, it is important to be consistent in the method used to make up the solution.

Sodium acetate solutions should last about one week. The most consistent chromatography has been obtained using sodium acetate purchased from Fluka.

II. Sample Preparation

It is recommended that all samples other than pure standards be passed through a 0.45-µm nylon filter prior to injection to remove particulates. Cellulose acetate and other filters should be avoided because they may leach carbohydrates. Filters of a type not previously verified as "clean" should be evaluated for contribution of "PAD-active" components before use.

Sample preparation is obviously dependent on sample matrix complexity and, as such, the recommendations that follow should be considered as guidelines only. In particular, the effect of sample pretreatment cartridges on the carbohydrate analytes themselves should be predetermined using standard solutions. It may be found that some carbohydrates have a strong affinity for particular cartridge packing materials. This is obviously of importance for quantification and in the detection of low levels of carbohydrates.

A. Samples Containing High Levels of Protein or Peptides

Physiological fluids such as plasma, urine, or other samples containing high levels of proteins should be deproteinized first. This may be achieved by standard precipitation procedures or by passing the analyte solution through a hydrophobic filter cartridge such as the Dionex OnGuard® RP Cartridge (P/N 39595).

B. Samples Containing High Levels of Humic Acids or Phenolics

To remove the phenolic fraction of humic acids, tannic acids, or lignins found in food samples (such as wine), the sample may be passed through a polyvinylpyrrolidone (PVP) filter cartridge, such as the Dionex OnGuard-P Cartridge (P/N 39597).

C. Samples Containing Halides

To remove halides, the sample may be passed through a Dionex OnGuard-Ag cartridge (P/N 39637). This cartridge selectively removes Cl^-, Br^- and I^- in preference to other anionic species. This cartridge is, however, a cation exchanger, so amino sugars will be extracted unless they are *N*-acetylated.

D. Samples Containing Sulfate and Other Anions

Sulfate may be precipitated as the barium salt by addition of barium hydroxide solution. However, it should be noted that some carbohydrates may co-precipitate with the barium sulfate in this procedure, especially carbohydrates bearing sulfate esters. The Dionex OnGuard A cartridge (P/N 42102) is designed specifically to remove anion contaminants from sample matrices. OnGuard A cartridges contain styrene-based anion-exchange resin in the bicarbonate form. They should not be used with samples that contain sialic acids, or sugars with other acid substituents.

III. Standard Chromatography Conditions for the Analysis of Carbohydrates

The conditions described in this section have been found to give reliable separations of the common classes of carbohydrates using HPAE chromatography. Samples and their matrices vary, therefore these conditions are intended to be used as guidelines only.

A. Monosaccharides—Neutral and Amino Sugars

These sugars can be successfully separated on the CarboPac PA1 column using isocratic conditions with 16 mM sodium hydroxide as the eluent. A representative chromatogram is shown in Figure 5. Because the concentration of sodium hydroxide used for the separation is only 16 mM, the column should be regenerated after each run. Otherwise, carbonate will start to contaminate the column, irrespective of the care taken to eliminate it from eluents and samples. Regenerate the column by washing it with 200 mM sodium hydroxide for 10 min at a flow rate of 1.0 mL/min. This procedure will also remove other strongly bound contaminants such as peptides and amino acids. This step is extremely important and should not be omitted. After washing, the column should be reequilibrated with 16 mM sodium hydroxide at a flow rate of 1.0 mL/min for 10 min. It is very important to keep the rinse and reequilibration times consistent from run to run.

B. Sugar Alcohols

Mono- and oligosaccharide sugar alcohols can be separated using the CarboPac MA1 column with sodium hydroxide eluents. Examples of isocratic separations are shown in Figures 6 and 7. Gradients can be used to improve separations (Figure 8) or to accelerate the elution of late-eluting components (Figure 9). Table 2 shows that the elution order of certain carbohydrates may be altered by changing the sodium hydroxide concentration.

C. Sialic Acids, Sialylated, and Phosphorylated Oligosaccharides

The elution of acidic sugars from the CarboPac PA1 or the CarboPac PA100 columns requires stronger eluents than those used with neutral sugars. This is usually accom

-plished by the addition of 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. Sodium acetate/sodium hydroxide solutions can be used isocratically (Figure 10) or in gradients (Figure 11 and 12). Sodium acetate gradients are *not recommended* for the CarboPac MA1 column because column regeneration will require several hours. The use of sodium acetate gradients is not a problem with the CarboPac PA1 and CarboPac PA100, because these columns have a lower anion- exchange capacity and thus regenerate quickly.

To maintain baseline stability, it is helpful to keep the sodium hydroxide concentration constant during the sodium acetate gradient, because acetate has no buffering capacity at high pH. This is achieved by making eluents as follows:

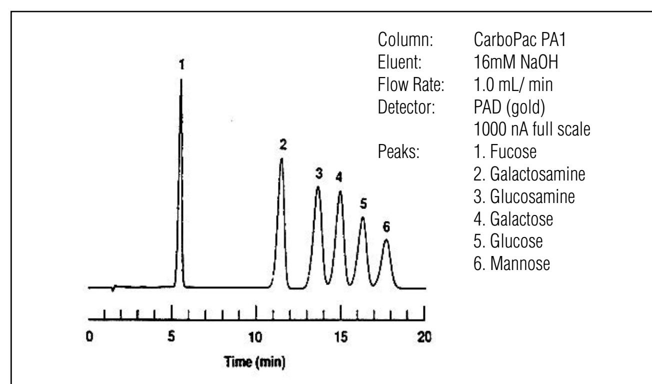

Figure 5. Separation of neutral and amino monosaccharides derived from glycoproteins.

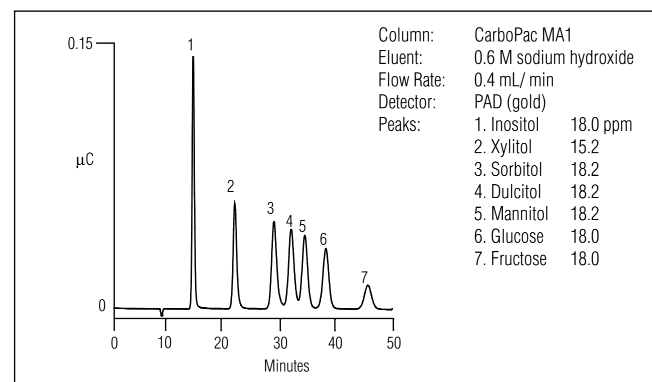

Figure 6. Isocratic separation of a group of alditols plus glucose and fructose on the CarboPac MA1 column.

Eluent A: x mM NaOH
Eluent B: x mM NaOH, y mM NaOAc

When devising gradients for the analysis of a carbohydrate sample of unknown composition, it is generally good practice to run a “scouting gradient”. This gradient consists of a rapid linear gradient ($t \leq 15$ min) from a low to a sufficiently high acetate concentration that it will elute all of the components. It is then possible to fine tune the separation, with the assurance that the gradient is sufficiently broad to include all of the sample components.

Sialic acids can also be separated at neutral pH. This is particularly useful for O-acetylated species, which are unstable at high pH [9–11].

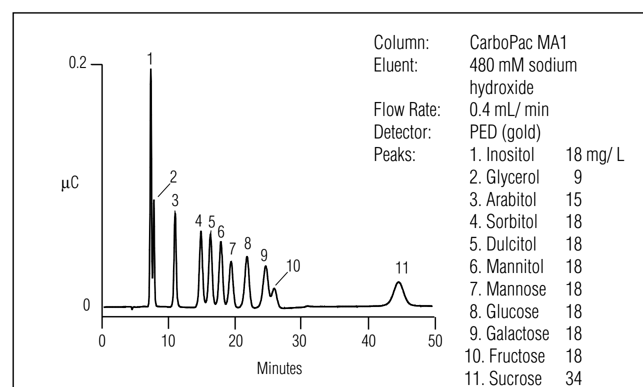

Figure 7. Separation of reducing and nonreducing carbohydrates. Food alditols and aldoses are separable under isocratic conditions on the CarboPac MA1.

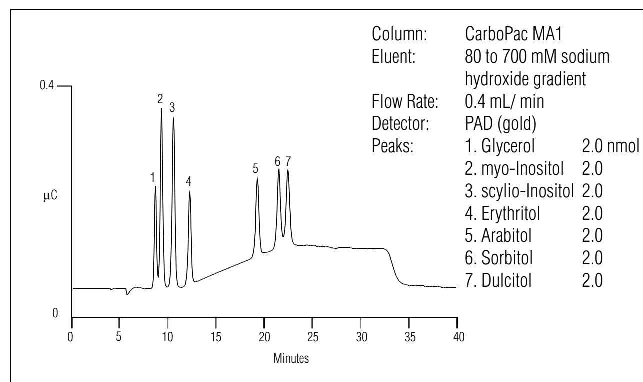

Figure 8. Separation of alditols found in biological fluids. The NaOH gradient improves the separation of sorbitol and dulcitol, which are poorly resolved at NaOH concentrations that permit resolution of glycerol from inositol.

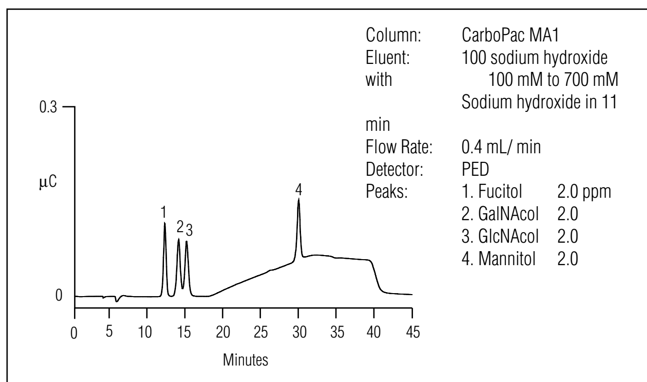

Figure 9. Separation of monosaccharide alditols released by direct ß-elimination from glycoproteins. The hydroxide gradient following the isocratic separation of the first three components accelerates the elution of mannitol as well as any oligosaccharide alcohols that may have been released during the ß-elimination process.

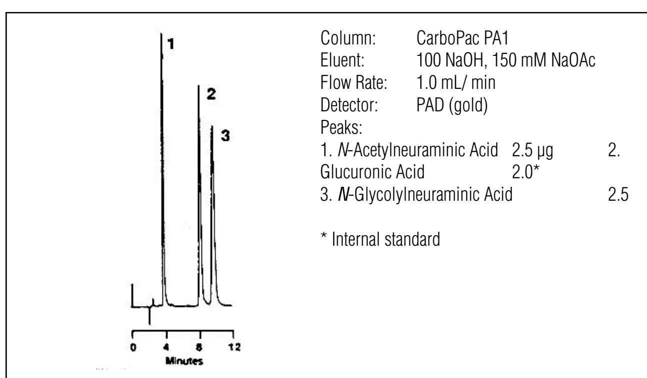

Figure 10. Isocratic separation of sialic acids using a sodium hydroxide/sodium acetate mixture.

D. Oligo- and Polysaccharides

Separations of high mannose, hybrid, and complex oligosaccharides are best accomplished using the CarboPac PA100 column. Linear homopolymers are successfully separated on the CarboPac PA1 or PA100. In all cases, separations are accelerated and improved by using sodium acetate gradients in sodium hydroxide. Sodium hydroxide gradients are also useful. Separation of several polysaccharides using either the CarboPac PA1 or the PA100 are shown in Figures 13 through 15. Figure 14 also shows the structure of inulin.

Figure 16 shows the separation of a group of neutral glycoprotein-derived oligosaccharides on the CarboPac PA100, while Figures 17 through 20 show examples of how HPAE-PAD analysis on the CarboPac PA100 column

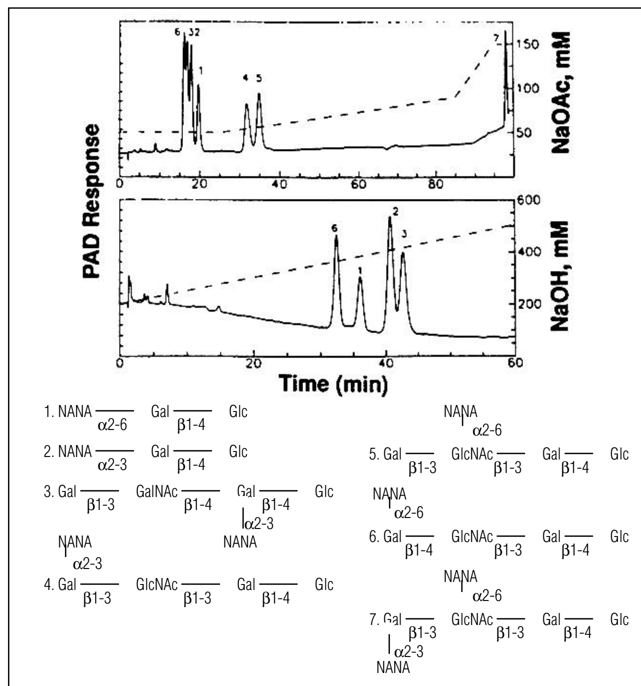

Figure 11. Gradient separation of sialylated oligosaccharides using the CarboPac PA1 column.

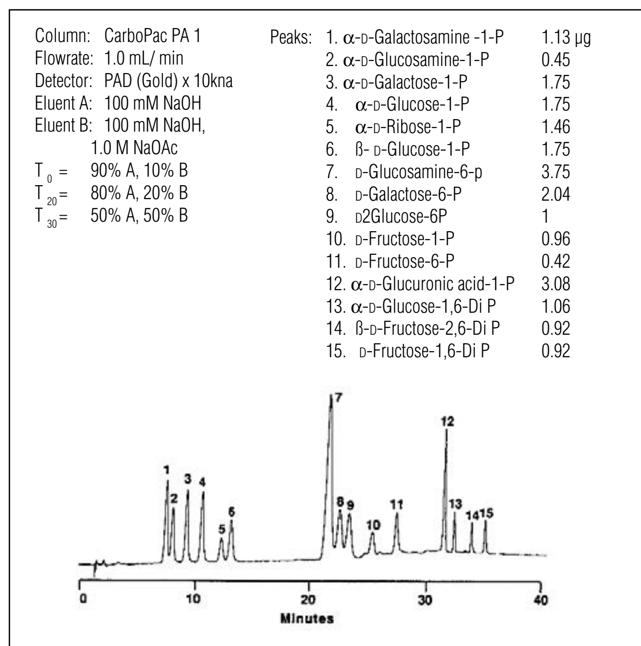

Figure 12. Analysis of mono- and diphosphorylated monosaccharides.

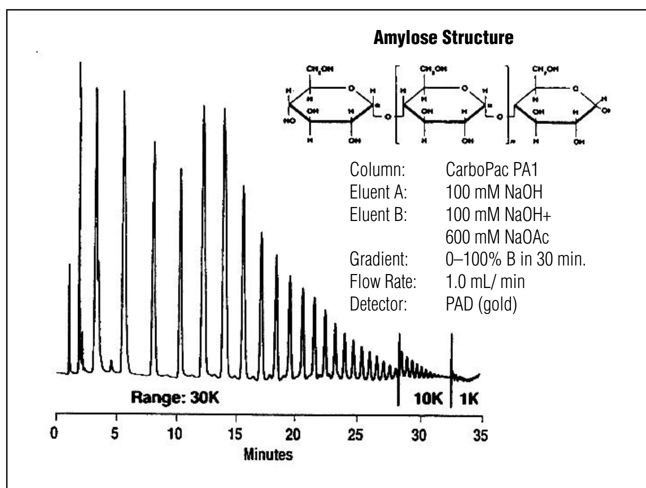

Figure 13. Analysis of "Dextrin 7" glucose polymer.

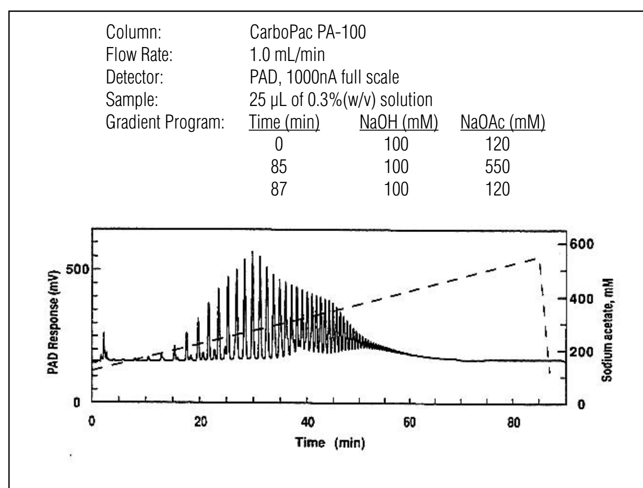

Figure 15. Gradient separation of chicory inulin using the CarboPac PA100.

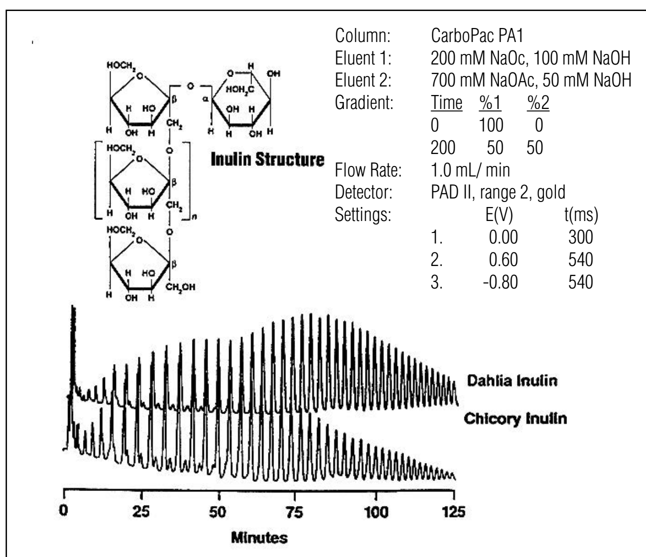

Figure 14. Comparison of water washed inulins (Cichorium intybus vs Dahlia sp.) using the CarboPac PA1.

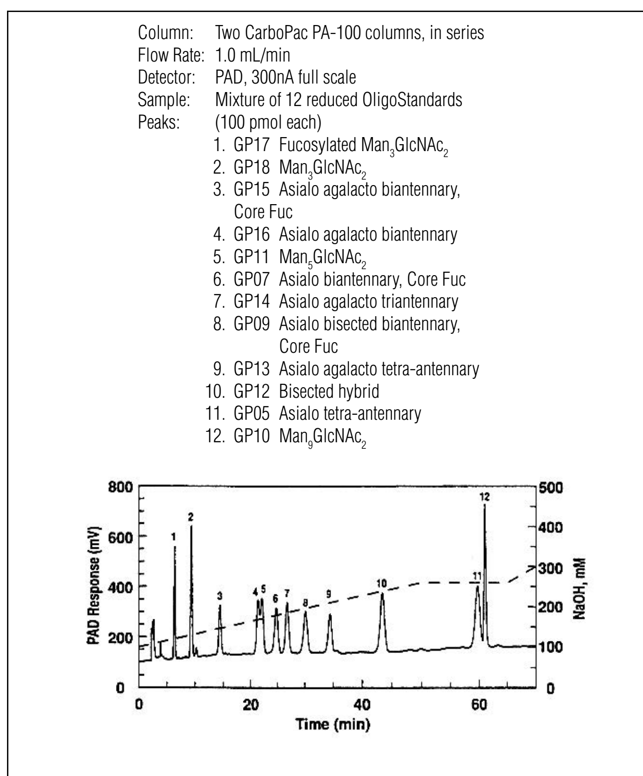

Figure 16. Separation of Dionex OligoStandard™ glycoprotein-derived oligosaccharides.

can be used to map oligosaccharides released from glycoproteins by enzyme digestion. Note that in Figure 18 the two oligosaccharide peaks marked 1 and 2 differ only in the linkage position of the sialic acid on one branch of the triantennary structure (α2→6 versus α2→3).

Neutral O-linked oligosaccharides released by reductive elimination are alditols and may be best separated on the CarboPac PA1 column (≥3 carbohy-drate units) or on the CarboPac MA1 column (<3 carbohydrate units).

V. Postcolumn Addition of Base

To optimize baseline stability and detector sensitivity, it is sometimes necessary to add strong base to the eluent stream postcolumn, particularly when using neutral pH eluents or when the eluent contains a low concentration sodium hydroxide. Postcolumn addition of base can help with quantification, in part by maintaining a more constant hydroxide concentration at the electrode. Users have found, however, that postcolumn addition of base is often unnecessary with routine isocratic and gradient separations at sodium hydroxide concentrations ≥15 mM.

A. Separations at Low pH

Sialic acids, sialylated oligosaccharides, and other carbohydrates bearing strongly acidic substituents can be separated by anion exchange at lower pH values.[10,11] This option is particularly useful when analyzing oligosaccharides that possess O-acetylated sialyl groups, because these groups are unstable at high pH. When low-pH eluents are used, sodium hydroxide must be added to the eluent after it has left the column and before it enters the detector, because carbohydrates are best detected at gold electrodes when pH ≥12.

B. Sodium Hydroxide Gradients

Changing the sodium hydroxide concentration alters the pH of the solution, which can affect the detector electrode response. While the PED and the solvent compatible PAD are affected very little by pH changes, the standard PAD-2 cell is fairly sensitive. A gradient from 10 mM sodium hydroxide to 100 mM sodium hydroxide results in an effective change of 1 pH unit during the gradient. The effect on detector response can be minimized by the use of optimized pulse sequence settings. Optimized pulse sequence settings are discussed in detail in Technical Note 21, which can be obtained from your local Dionex representative. If necessary, however, a solution of sodium hydroxide can be added postcolumn to minimize the pH shift. For example, the addition of 300 mM sodium hydroxide to the column effluent of a gradient of 10 to 100 mM sodium hydroxide would result in a total pH change of only 0.11 units (if the flow rates of the postcolumn base and eluent were equal). A pH change of this magnitude would generate a negligible baseline shift.

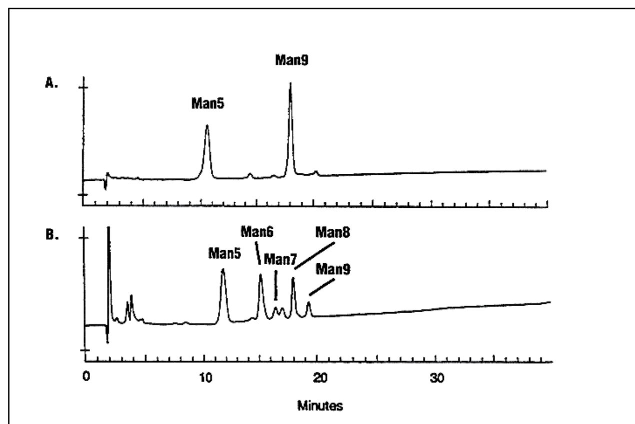

Figure 17. Separation of glycoprotein-derived mannose oligosaccharides using the CarboPac PA100 column. Panel A: Dionex OligoStandards GP11 and GP10, $Man_5GlcNAc_2$ and $Man_9GlcNAc_2$, respectively. Panel B: Endonuclease H digest of ribonuclease B.

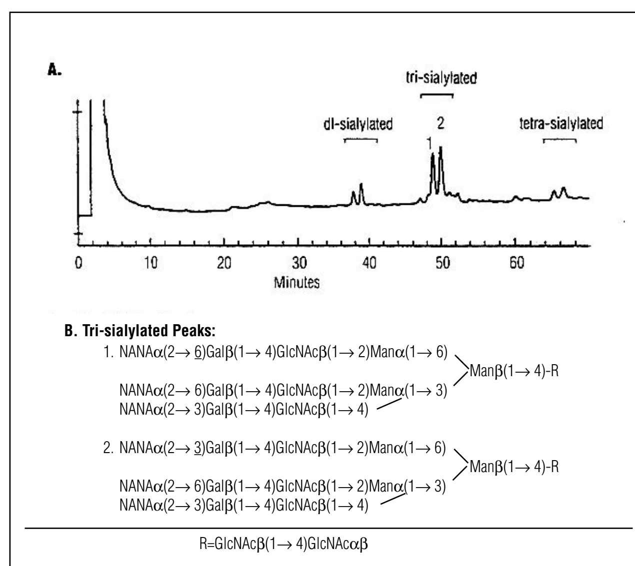

Figure 18. Separation of fetuin N-linked oligosaccharides. Panel A: HPAE-PAD analysis using a CarboPac PA100. Panel B: Structures of the trisialylated species, peaks 1 and 2.

Postcolumn base can be delivered through a mixing T using the Dionex Postcolumn Pneumatic Controller. To ensure run-to-run reproducibility, the Controller should be adjusted so that:

1. The flow rate is constant.
2. The mixture entering the detector has a pH ≈ 13.
3. The flow rate of the mixture of eluent plus postcolumn base stays the same from run to run.

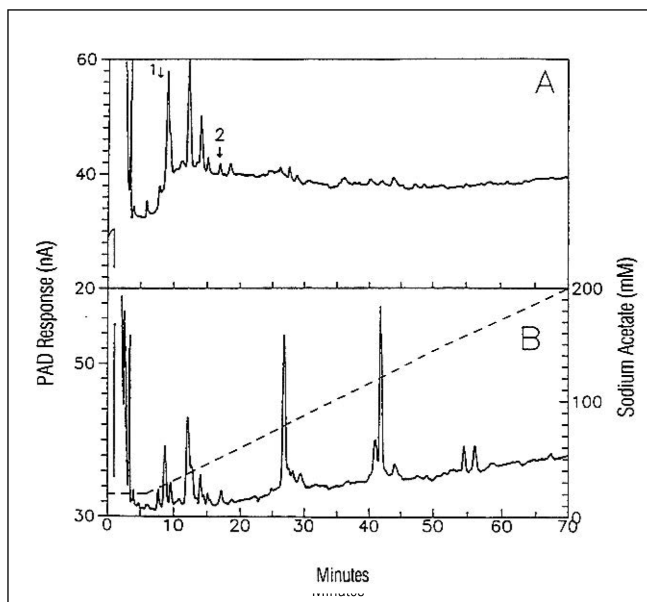

Figure 19. Gradient separation (using the CarboPac PA100 column) of oligosaccharides released by sequential enzyme digestion of recombinant tissue plasminogen activator (rtPA). Panel A: High-mannose oligosaccharides released from rtPA by digestion with endonuclease H. The elution positions of Man_5-$GlcNAc_2$ and $Man_9GlcNAc_2$ are indicated by the numbered arrows.
Panel B: Oligosaccharides released from rtPA by endonuclease F_2 (cleaves predominantly biantennary-type chains).
(From Weitzhandler et al.[12] Reproduced with permission.)

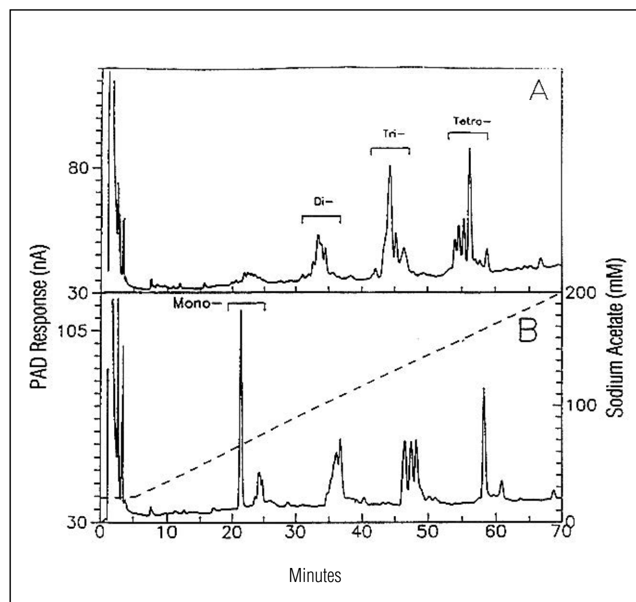

Figure 20. Gradient separation (using the CarboPac PA100 column) of oligosaccharides in recombinant erythropoietin (rEPO). Panel A: Oligosaccharides released by PNGase F digestion. Panel B: Digestion of the mixture in panel A by endo-ß-galactosidase shows that three of the four major tetrasialylated species contain polylactosamine structures.
(From Weitzhandler et al.[12] Reproduced with permission.)

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Optimal Settings for Pulsed Amperometric Detection of Carbohydrates Using the Dionex ED40 Electrochemical Detector

INTRODUCTION

Pulsed amperometry is a powerful detection method for carbohydrates that requires no sample derivatization. Pulsed amperometric detection (PAD) is particularly useful in combination with high-performance anion-exchange chromatography (HPAE). For a full description of HPAE-PAD, refer to Dionex Technical Note 20. For an introduction to amperometry refer to *Conductivity and Amperometry,* by Roy D. Rocklin, Ph. D. (P/N 34358), or Chapter 6 of the *ED40 Manual.* All are available from your Dionex representative.

At high pH, carbohydrates are electrocatalytically oxidized at the surface of the gold electrode by application of a positive potential. The current generated is proportional to the carbohydrate concentration, and therefore carbohydrates can be detected and quantified. If only a single potential is applied to the electrode, oxidation products gradually poison the electrode surface. This electrode surface poisoning causes a loss of analyte signal. To prevent signal loss, the electrode surface is cleaned by a series of potentials that are applied for fixed time periods after the detection potential. 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. The potentials of a waveform are designated E_1, E_2, E_3, etc., where E_1 is the detection potential. The remaining potentials clean and restore the electrode for subsequent detection. Potentials are maintained for time periods t_1, t_2, t_3, etc. The first time period (t_1) is subdivided into t_{del} and t_{det}. The delay period, t_{del}, is the time that is allowed for the charging current (produced when changing potentials) to decay so that only current from analyte oxidation is measured during the detection period, t_{det} (see Figure 1).

Optimal values for all waveform parameters are determined by systematic variation of one parameter while holding the other parameters constant. An excellent discussion of the optimization of pulsed amperometry waveforms was published by LaCourse and Johnson .[1]

Dionex supports three waveforms for carbohydrate analysis. This Technical Note discusses each waveform with special emphasis on their benefits and liabilities. Figure 1 shows a schematic representation of each waveform. The ED40 electrochemical cell is equipped with a combination pH-Ag/AgCl reference electrode. The potentials presented in this Technical Note require use of the Ag/AgCl half of the reference electrode.

WAVEFORM A

This Technical Note introduces Waveform A (Table 1 and Figure 1, Panel A), a new waveform for carbohydrate analysis that dramatically improves long term reproducibility. This waveform differs from Waveforms B and C in that it uses negative rather than positive potentials for electrode cleaning. When positive cleaning potentials are used there is a gradual decrease in carbohydrate peak areas over time due to working electrode wear (recession below the plastic housing). Waveform A minimizes electrode wear. This is demonstrated by comparing Figures 2 and 3. Figure 2 shows a two week repetitive analysis of monosaccharide standards (100 pmol each) using a new working electrode and Waveform B which uses a positive potential for electrode cleaning. Figure 3 shows the same analysis using Waveform A. While there is a gradual decrease in peak area response using Waveform B, response is constant using Waveform A. Waveform A provides the best reproducibility of absolute electrochemical response.

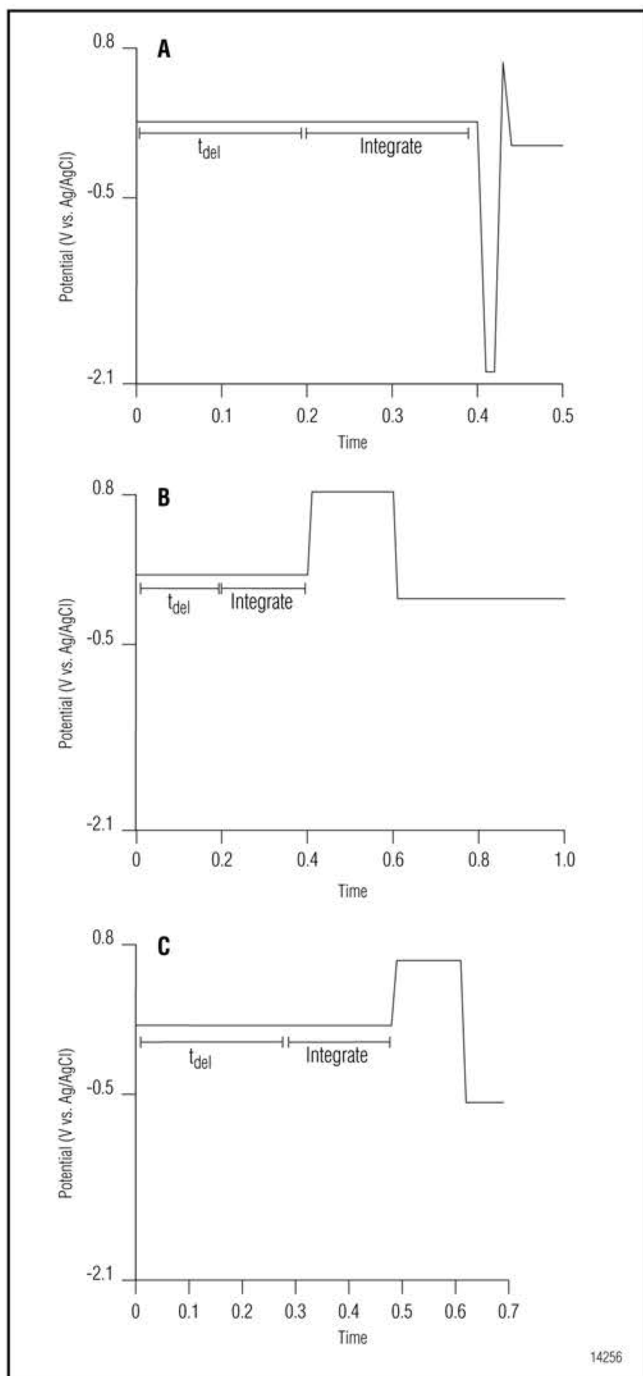

Figure 1 *Schematic representation of Waveforms A, B, and C.*

The detection potential, E_1, of Waveform A is 0.1 V. This potential is maintained for 400 ms. The first 200 ms is t_{del} and the second 200 ms is t_{det}. The working electrode is cleaned for 10 ms at -2.0 V (E_2). This is followed by a quick excursion to 0.6 V (E_3). The latter potential forms a small amount of gold oxide that was found to be necessary to maintain an active working electrode surface. These two steps, reductive cleaning and gold oxide formation, require only 40 ms. The final potential of this waveform, E_4, is -0.1V, and it is required to reduce the small amount of gold oxide formed in E_3. This potential is maintained for 60 ms and therefore this waveform requires a total of 500 ms. The development of Waveform A is described in a publication by Rocklin et al.[2] For more details on the theory of cleaning at negative potentials see the publication by Jensen and Johnson.[3]

The greatest benefit of using Waveform A is consistent long term peak area response. This is beneficial when comparing two or more systems that are analyzing monosaccharides. Because Waveform A requires only 500 ms, data can be collected at 2 Hz. This is twice the data collection rate of Waveforms B and C. Because Waveform A allows twice as many data points per peak, sharp, early eluting peaks (e.g., fucose) are detected with greater reproducibility. A further advantage of Waveform A is that it is less subject to interference from electroactive amino acids than Waveforms B and C.

There are some small disadvantages associated with Waveform A. Though the response is, in some cases, higher with Waveform A compared to Waveform B, the noise is also higher. Taken together, the minimum detection limits using Waveform A are usually not as low as those found with Waveform B. Waveform A has a greater sensitivity to dissolved oxygen and therefore higher backgrounds (16–22 nC) and higher noise. This may be apparent when using the CarboPac™ PA1, where the baseline dip, due to reduction of dissolved oxygen, is between glucosamine and mannose. The CarboPac PA10 places the baseline dip, due to dissolved oxygen, after the carbohydrate elution window.

When using Waveform A, there is occasionally a small dip observed after amino sugars (see Figure 4). This dip is largest after glucosamine, but is also observed after

Table 1 Waveform A

Time	Potential (V)	Integration
0.00	+0.1	
0.20	+0.1	Begin
0.40	+0.1	End
0.41	-2.0	
0.42	-2.0	
0.43	+0.6	
0.44	-0.1	
0.50	-0.1	

galactosamine and mannosamine. Dips are not observed after the acetylated amino sugars *N*-acetylglucosamine and *N*-acetylgalactosamine. Increasing E_1 to 0.15 V minimizes this dip, though the long term (> 1 month) effect of this change on peak area reproducibility has not been measured.

Waveform A requires a GP40 or GP50 pump with the vacuum degas option installed and a new working electrode. Polish the working electrode before installing it in the ED40 cell. The procedure for polishing the working electrode can be found in the ED40 manual. Waveform A can be programmed with PeakNet software (release 5.0 or higher) or through the detector's front panel. Set the data collection rate to 2 Hz. Set the pump to degas for 30 s every 4 min. During the first day peak areas may increase as the working electrode surface is activated. This increase may be observed anytime the electrode is polished. Only polish new working electrodes and those believed to be fouled. Evidence of fouling is visible electrode discoloration or a decrease in peak area response that occurs without working electrode wear.

WAVEFORM B

Waveform B (Table 2 and Figure 1, Panel B) has been a recommended waveform since the introduction of the ED40 in 1993. This waveform was developed to increase sensitivity, minimize the sensitivity to dissolved oxygen, and minimize baseline drift when separating oligosaccharides with sodium acetate gradients. Waveform B provides the greatest carbohydrate sensitivity, the least sensitivity to dissolved oxygen, and is equivalent to Waveform A in baseline drift using sodium acetate gradients. Because Waveform B uses oxidative cleaning (E_2 = 0.75V), there is working electrode wear and a gradual decrease in carbohydrate peak area over time (Figure 2). Even with this peak area decrease, quantitative carbohydrate analyses are possible by using internal standards and regularly spaced external standards.[4, 5]

Table 2 Waveform B

Time	Potential (V)	Integration
0.00	+0.05	
0.20	+0.05	Begin
0.40	+0.05	End
0.41	+0.75	
0.60	+0.75	
0.61	-0.15	
1.00	-0.15	

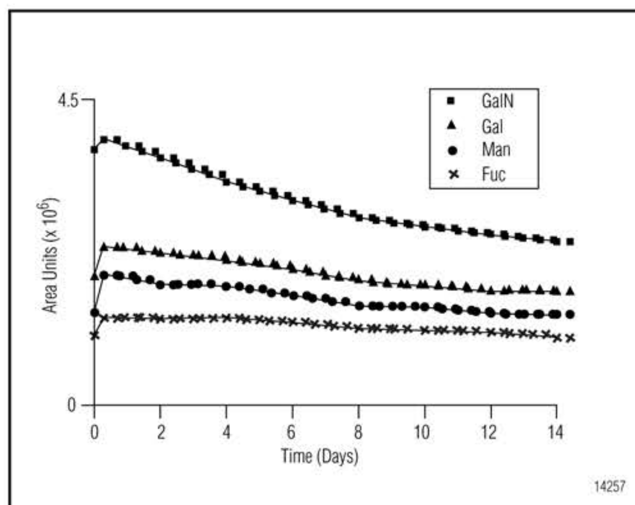

Figure 2 *Carbohydrate response as a function of time for a freshly sanded and polished electrode, using Waveform B.*

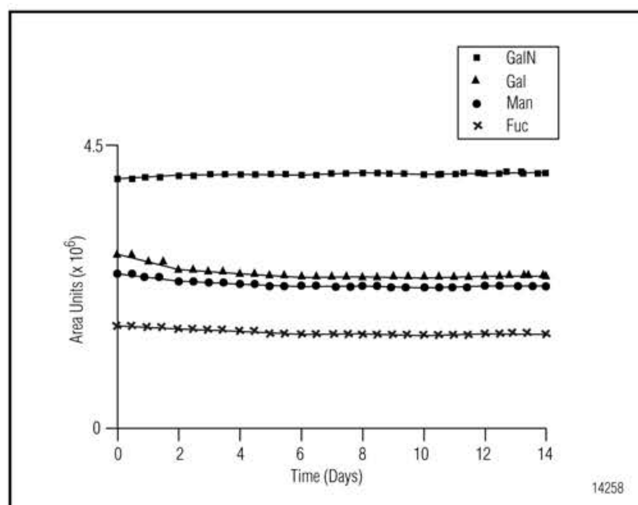

Figure 3 *Carbohydrate response as a function of time for a freshly sanded and polished electrode, using Waveform A.*

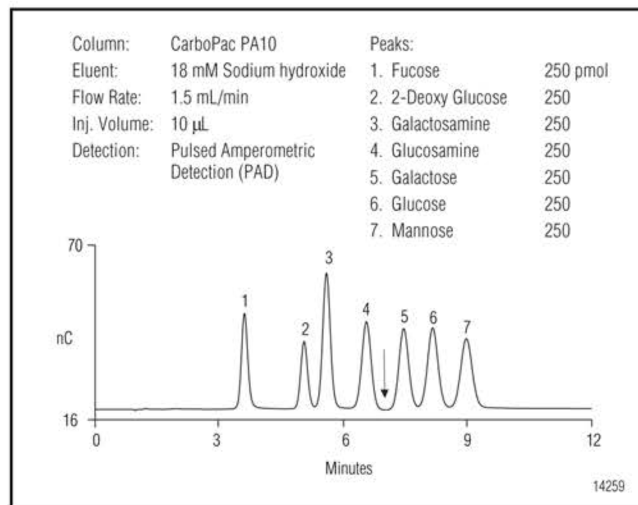

Figure 4 *A small dip is occasionally observed after amino sugars when using Waveform A.*

WAVEFORM C

Waveform C (Table 3 and Figure 1, Panel C) has been a recommended waveform since 1990. This waveform is in widespread use and gives good results when used with standard isocratic HPAE conditions and where maximum sensitivity is not needed. Because Waveform C uses oxidative cleaning (E_2 = 0.60 V), there is working electrode wear and a gradual decrease in carbohydrate peak area over time, but this does not preclude quantitative carbohydrate analyses.[4, 5] Using this waveform, the baseline dip due to dissolved oxygen is similar to that found with Waveform A.

Table 3 Waveform C

Time	Potential (V)	Integration
0.00	+0.05	
0.28	+0.05	Begin
0.48	+0.05	End
0.49	+0.60	
0.61	+0.60	
0.62	-0.60	
0.69	-0.60	

SUMMARY OF WAVEFORM CHARACTERISTICS

Long Term Peak Area Reproducibility	A >> C > B
Carbohydrate Detection Limits	B > A > C
Lack of Sensitivity to Dissolved Oxygen	B > C = A
Reproducibility for Early Eluting Peaks	A > B = C
Baseline Drift During Sodium Acetate Gradient	B ≥ A > C

OTHER CONSIDERATIONS

For best results the reference electrode should be changed every six months. When the reference electrode will not be used for a few weeks, it should be stored in its container in a saturated KCl solution.

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Thermo
SCIENTIFIC

Column Selection Guide

Silica Columns

			Reversed-Phase (RP)						Mixed-Mode			HILIC		Application-Specific					
			Acclaim 120 C18	Acclaim 120 C8	Acclaim 300 C18	Acclaim Polar Advantage (PA)	Acclaim Polar Advantage II (PA2)	Acclaim Phenyl-1	Acclaim Trinity P1	Acclaim Mixed-Mode WAX-1	Acclaim Mixed-Mode WCX-1	Acclaim Mixed-Mode HILIC-1	Acclaim HILIC-10	Acclaim Organic Acid	Acclaim Surfactant	Acclaim Explosives E1	Acclaim Explosives E2	Acclaim Carbamate	Example Applications
General Applications	Neutral Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√	√						Fat-soluble vitamins, PAHs, glycerides
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√	√	√							Steroids, phthalates, phenolics
		Low hydrophobicity	√			√	√					√	√						Acetaminophen, urea, polyethylene glycols
	Anionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							NSAIDs, phospholipids
		Intermediate hydrophobicity	√	√	√	√	√	√	√	√		√							Asprin, alkyl acids, aromatic acids
		Low hydrophobicity				√			√	√		√	√						Small organic acids, e.g. acetic acids
	Cationic Molecules	High hydrophobicity	√	√	√	√	√	√		√	√	√							Antidepressants
		Intermediate hydrophobicity	√	√	√	√	√	√	√		√	√							Beta blockers, benzidines, alkaloids
		Low hydrophobicity	√			√			√		√	√	√						Antacids, pseudoephedrine, amino sugars
	Amphoteric/ Zwitterionic Molecules	High hydrophobicity	√	√	√	√	√	√	√	√	√	√							Phospholipids
		Intermediate hydrophobicity	√	√	√	√	√	√			√								Amphoteric surfactants, peptides
		Low hydrophobicity				√	√		√	√	√	√	√						Amino acids, aspartame, small peptides
	Mixtures of Neutral, Anionic, Cationic Molecules	Neutrals and acids	√			√	√		√	√									Artificial sweeteners
		Neutrals and bases	√			√	√		√		√								Cough syrup
		Acids and bases				√			√										Drug active ingredient with counterion
		Neutrals, acids, and bases				√			√										Combination pain relievers
Specific Applications	Surfactants	Anionic	√	√	√	√	√								√				SDS, LAS, laureth sulfates
		Cationic													√				Quats, benzylalkonium in medicines
		Nonionic	√	√	√	√	√					√			√				Triton X-100 in washing tank
		Amphoteric	√	√	√	√	√								√				Cocoamidopropyl betaine
		Hydrotropes													√				Xylenesulfonates in handsoap
		Surfactant blends													√				Noionic and anionic surfactants
	Organic Acids	Hydrophobic							√	√				√					Aromatic acids, fatty acids
		Hydrophilic							√	√				√					Organic acids in soft drinks, pharmaceuticals
	Environmental Contaminants	Explosives														√	√		U.S. EPA Method 8330, 8330B
		Carbonyl compounds															√		U.S. EPA 1667, 555, OT-11; CA CARB 1004
		Phenols	√			√													Compounds regulated by U.S. EPA 604
		Chlorinated/Phenoxy acids				√													U.S. EPA Method 555
		Triazines	√			√													Compounds regulated by U.S. EPA 619
		Nitrosamines				√													Compounds regulated by U.S. EPA 8270
		Benzidines	√			√													U.S. EPA Method 605
		Perfluorinated acids				√													Dionex TN73
		Microcystins	√																ISO 20179
		Isocyanates					√					√							U.S. OSHA Methods 42, 47
		Carbamate insecticides																√	U.S. EPA Method 531.2
	Vitamins	Water-soluble vitamins				√	√		√										Vitamins in dietary supplements
		Fat-soluble vitamins	√	√	√	√	√	√		√									Vitamin pills
	Pharmaceutical Counterions	Anions							√	√									Inorgaic anions and organic acids in drugs
		Cations							√		√								Inorgaic cations and organic bases in drugs
		Mixture of Anions and Cations							√										Screening of pharmaceutical counterions
		API and counterions							√										Naproxen Na^+ salt, metformin Cl^- salt, etc.

Polymer Columns

Category	Application	IonPac AS23	IonPac AS22	IonPac AS22-Fast	IonPac AS14/A	IonPac AS12A	IonPac AS9/HC/SC	IonPac AS4A/SC	IonSwift MAX-100	IonPac AS24	IonPac AS21	IonPac AS20	IonPac AS19	IonPac AS18	IonPac AS18-Fast	IonPac AS17-C	IonPac AS16	IonPac AS15	IonPac AS11(-HC)	IonPac AS10	IonPac AS7	IonPac AS5	IonPac Fast Anion IIIA	OmniPac PAX-100	OmniPac PAX-500
ANIONS	Inorganic Anions	√	√	√	√	√	√	√	√	√		√	√	√	√	√		√	√	√					
	Oxyhalides	√				√	√			√			√												
	Bromate	√					√			√			√												
	Perchlorate										√	√					√								
	Organic Acids								√							√		√	√	√					
	Phosphoric/Citric Acids																						√		
	Poly/High-Valence Anions								√			√					√		√		√	√			
	Hydrophobic Anions								√			√					√		√						
	Hydrophobic/Halogenated Anions								√			√							√					√	
	Anionic Neutral Molecules									√	√	√	√												√
CATIONS	Inorganic Cations																								
	Sodium/Ammonium																								
	Amines/Polyvalent Amines																								
	Aliphatic/Aromatic Amines																								
	Alkanol/Ethhanolamines																								
	Biogenic Amines																								
	Transition/Lanthanide Metals																								
	Hydrophobic Cations																								
	Cationic Neutral Molecules																								
BIO-MOLECULES	Amino Acids																								
	Phosphorylated Amino Acids																								
	Amino Sugars																								
	Oligosccharides																								
	Mono-/Di-Saccharides																								
	Glycoproteins																								
	Alditols/Aldoses mono/di Saccharides																								
	ds Nucleic Acids																								
	Single-Stranded Oligonucleotides																								
	Peptides																								
	Proteins																								
	Metal-binding Proteins																								
	Monoclonal antibodies																								
ORGANIC MOLECULES	Aliphatic Organic Acids																								
	Alcohols																								
	Borate																								
	Large Molecules, Anions																								
	Small Molecules																								
	Small Molecules/LC-MS																								
	Polar/Non-Polar Small Molecules																								
	Hydrophobic/Aliphatic Organic Acids																								
	Surfactant Formulations																								
	Explosives/EPA 8330																								
MODE	Anion Exchange / Carbonate	√	√	√	√	√	√	√																	
	Anion Exchange / Hydroxide								√	√	√	√	√	√	√	√	√	√	√	√	√	√	√		
	Cation Exchange																								
	Multi-Mode																							√	√
	Affinity																								
	Ion Exclusion																								
	Reversed Phase																								
	Anion Exchange/Other																								

Category	Application	IonPac CS18	IonPac CS17	IonPac CS16	IonPac CS15	IonPac CS14	IonPac CS12A	IonPac CS11	IonPac CS10	IonPac CS5A	OmniPac PCX-100	OmniPac PCX-500	AminoPac PA10	AminoPac PA1	CarboPac PA200	CarboPac PA100	CarboPac PA20	CarboPac PA10	CarboPac PA1	CarboPac MA1	DNAPac PA200	DNAPac PA100	ProPac WAX/SAX	ProPac WCX/SCX	ProPac IMAC	ProPac HIC	ProPac PA1	ProSwift	IonPac ICE-AS6	IonPac ICE-AS1	IonPac ICE-Borate	IonPac NS1
ANIONS	Inorganic Anions																															
	Oxyhalides																															
	Bromate																															
	Perchlorate																															
	Organic Acids																															
	Phosphoric/Citric Acids																															
	Poly/High-Valence Anions																															
	Hydrophobic Anions																															
	Hydrophobic/Halogenated Anions																															
	Anionic Neutral Molecules																															
CATIONS	Inorganic Cations	√	√	√	√	√	√	√	√																							
	Sodium/Ammonium			√	√		√																									
	Amines/Polyvalent Amines	√	√																													
	Aliphatic/Aromatic Amines		√			√	√																									
	Alkanol/Ethhanolamines	√	√		√																											
	Biogenic Amines	√	√																													
	Transition/Lanthanide Metals									√																						
	Hydrophobic Cations	√	√			√					√																					
	Cationic Neutral Molecules											√																				
BIO-MOLECULES	Amino Acids												√	√																		
	Phosphorylated Amino Acids												√																			
	Amino Sugars												√		√	√	√	√														
	Oligosccharides														√	√			√	√												
	Mono-/Di-Saccharides																√	√	√	√												
	Glycoproteins																					√	√	√				√				
	Alditols/Aldoses mono/di Saccharides																√	√	√	√												
	ds Nucleic Acids																				√	√										
	Single-Stranded Oligonucleotides																				√	√						√				
	Peptides																								√	√	√	√				
	Proteins																						√	√	√	√	√	√				
	Metal-binding Proteins																								√							
	Monoclonal antibodies														√	√			√		√	√	√	√	√	√	√	√				
ORGANIC MOLECULES	Aliphatic Organic Acids																												√	√		√
	Alcohols																												√	√		
	Borate																														√	
	Large Molecules, Anions																															√
	Small Molecules																															
	Small Molecules/LC-MS																															
	Polar/Non-Polar Small Molecules																															
	Hydrophobic/Aliphatic Organic Acids																															
	Surfactant Formulations																															√
	Explosives/EPA 8330																															
MODE	Anion Exchange / Carbonate																															
	Anion Exchange / Hydroxide														√	√	√	√	√	√												
	Cation Exchange	√	√	√	√	√	√	√	√	√														√			√	√				
	Multi-Mode										√	√																				
	Affinity																								√							
	Ion Exclusion																												√	√	√	
	Reversed Phase																									√	√	√				√
	Anion Exchange/Other												√	√							√	√	√				√	√				

Column Specifications

IC Anion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac AS24	2 × 250 mm	Hydroxide	Recommended column for haloacetic acids prior to MS or MS/MS detection	7 µm	55%	-	-	140 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS23	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	6 µm	55%	-	-	80 µeq 320 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS22	2 × 250 mm 4 × 250 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	6.5 µm	55%	-	-	52.5 µeq 210 µeq	Alkyl quaternary ammonium	Ultralow
IonPac AS21	2 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to MS or MS/MS detection	7.0 µm	55%	-	-	45 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS20	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for trace perchlorate prior to suppressed conductivity detection.	7.5 µm	55%	-	-	77.5 µeq 310 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS19	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for inorganic anions and oxyhalides. Trace bromate in drinking water.	7.5 µm	55%	-	-	60 µeq 350 µeq	Alkanol quaternary ammonium	Low
IonPac AS18	2 × 250 mm 4 × 250 mm	Hydroxide	Recommended column for the analysis of common inorganic anions.	7.5 µm	55%	65 nm	8%	75 µeq 285 µeq	Alkanol quaternary ammonium	Low
IonPac AS17-C	2 × 250 mm 4 × 250 mm	Hydroxide	Trace anions in HPW matrices. Carboxylated resin, no sulfate blank. Low capacity for fast analysis of common inorganic anions using gradient elution with the Eluent Generator.	10.5 µm	55%	75 nm	6%	7.5 µeq 30 µeq	Alkanol quaternary ammonium	Low
IonPac AS16	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for hydrophobic anions including iodide, thiocyanate, thiosulfate, and perchlorate. Polyvalent anions including: polyphosphates and polycarboxylates	9 µm	55%	80 nm	1%	42.5 µeq 170 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS15	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	9 µm	55%	-	-	56.25 µeq 225 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS15-5mm	3 × 150 mm	Hydroxide	Fast run, high capacity for trace analysis of inorganic anions and low molecular weight organic acids in high purity water matrices.	5 µm	55%	-	-	70 µeq	Alkanol quaternary ammonium	Medium-High
IonPac AS14A- 5 µm	3 × 150 mm	Carbonate	Recommended column for fast analysis of common inorganic anions.	5 µm	55%	-	-	40 ueq	Alkyl quaternary ammonium	Medium
IonPac AS14A	4 × 250 mm	Carbonate	For analysis of common inorganic anions.	7 µm	55%	-	-	120 µeq	Alkyl quaternary ammonium	Medium
IonPac AS14	2 × 250 mm 4 × 250 mm	Carbonate	Moderate capacity for fast analysis of common inorganic anions.	9 µm	55%	-	-	16 µeq 65 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS12A	2 × 200 mm 4 × 200 mm	Carbonate	Moderate capacity for analysis of inorganic anions and oxyhalides. Trace chloride and sulfate in high carbonate matrices.	9 µm	55%	140 nm	0.20%	13 µeq 52 µeq	Alkyl quaternary ammonium	Medium
IonPac AS11-HC	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the determination of organic acids and inorganic anions in uncharacterized samples.	9 µm	55%	70 nm	6%	72.5 µeq 290 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac AS11	2 × 250 mm 4 × 250 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	13 µm	55%	85 nm	6%	11 µeq 45 µeq	Alkanol quaternary ammonium	Very Low
IonPac AS10	2 × 250 mm 4 × 250 mm	Hydroxide	High capacity for the analysis of inorganic anions and organic acids in high nitrate samples.	8.5 µm	55%	65 nm	5%	42.5 µeq 170 µeq	Alkyl quaternary ammonium	Low
IonPac AS9-HC	2 × 250 mm 4 × 250 mm	Carbonate	High-capacity column for inorganic anions and oxyhalides. Trace bromate in drinking water.	9 µm	55%	90 nm	18%	48 µeq 190 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS9-SC	4 × 250 mm	Carbonate	Low capacity for fast analysis of inorganic anions and oxyhalides. Specified column in US EPA Method 300.0 (B).	13 µm	55%	110 nm	20%	30-35 µeq	Alkyl quaternary ammonium	Medium-Low
IonPac AS4A-SC	2 × 250 mm 4 × 250 mm	Carbonate	Low capacity for fast analysis of common inorganic anions. Specified column in U.S. EPA Method 300.0 (A).	13 µm	55%	160 nm	0.50%	5 µeq 20 µeq	Alkanol quaternary ammonium	Medium-Low
IonPac Fast Anion IIIA	3 × 250 mm	Hydroxide	Recommended column for phosphoric and citric acids in cola soft drinks.	7.5 µm	55%	-	-	55 µeq	Alkanol quaternary ammonium	Ultralow
IonPac AS7	4 × 250 mm	Specialty Eluents	Polyvalent anions including chelating agents, polyphosphates and polyphosphonates. Cyanide, sulfide, hexavalent chromium, and arsenic speciation.	10 µm	2%	530 nm	5%	100 µeq	Alkyl quaternary ammonium	Medium-High
IonPac AS5A	4 × 150 mm	Hydroxide	Low capacity for fast profiling of organic acids and inorganic anions in well-characterized samples.	5 µm	2%	60 nm	4%	35 µeq	Alkanol quaternary ammonium	Low
IonPac AS5	4 × 250 mm	Hydroxide	Metal-EDTA complexes, metal-cyanide complexes, and oxyanions.	15 µm	2%	120 nm	1%	20 µeq	Alkanol quaternary ammonium	Low

IC Cation Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydrophobicity
IonPac CS18	2 × 250 mm	MSA	Recommended column for polar amines (alkanolamines and methylamines) and moderately hydrophobic and polyvalent amines (biogenic and diamines). Nonsuppressed mode when extended calibration linearity for ammonium and weak bases is required	6 µm	55%	-	-	0.29 µeq	Carboxylic acid	Medium
IonPac CS17	2 × 250 mm 4 × 250 mm	MSA	Recommended column for hydrophobic and polyvalent amines (biogenic amines and diamines)	7 µm	55%	-	-	0.363 µeq 1.45 µeq	Carboxylic acid	Very Low
IonPac CS16	3 × 250 mm 5 × 250 mm	MSA	Recommended column for disparate concentration ratios of adjacent-eluting cations such as sodium and ammonium. Can be used for alkylamines and alkanolamines.	5 µm	55%	-	-	3.0 µeq 8.4 µeq	Carboxylic acid	Medium
IonPac CS15	2 × 250 mm 4 × 250 mm	MSA	Disparate concentration ratios of ammonium and sodium. Trace ethanolamine in high-ammonium or high- potassium concentrations. Alkanolamines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid/ crown ether	Medium
IonPac CS14	2 × 250 mm 4 × 250 mm	MSA	Aliphatic amines, aromatic amines, and polyamines plus mono- and divalent cations.	8.5 µm	55%	-	-	0.325 µeq 1.3 µeq	Carboxylic acid	Low
IonPac CS12A-MS	2 × 100 mm	MSA	IC-MS screening column for fast elution and low flow rates required for interfacing with IC-MS	8.5 µm	55%	-	-	0.28 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A-5 µm	3 × 150 mm	MSA	Recommended column for high efficiency and fast analysis (3 min) of mono- and divalent cations.	5 µm	55%	-	-	0.94 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS12A	2 × 250 mm 4 × 250 mm	MSA	Recommended column for the separation of mono- and divalent cations. Manganese morpholine, alkylamines, and aromatic amines.	8.5 µm	55%	-	-	0.7 µeq 2.8 µeq	Carboxylic acid/ phosphonic acid	Medium
IonPac CS11	2 × 250 mm	HCl + DAP	Separation of mono- and divalent cations. Ethanolamines if divalent cations are not present.	8 µm	55%	200 nm	5%	0.035 µeq	Sulfonic acid	Medium
IonPac CS10	4 × 250 mm	HCl + DAP	Separation of mono- and divalent cations.	8.5 µm	55%	200 nm	5%	0.08 µeq	Sulfonic acid	Medium
IonPac CS5A	2 × 250 mm 4 × 250 mm	Pyridine dicarboxylic acid	Recommended column for transition and lanthanide metals analysis. Aluminum analysis.	9 µm	55%	140 nm 75 nm	10% 20%	0.02 µeq/ 0.005 µeq 0.04 µeq/ 0.01 µeq	Sulfonic acid/ alkanol quaternary ammonium	-

Ion-Exclusion Columns

Column	Format	Primary Eluent	Application	Particle Diameter	Substrate Crosslinking	Latex Diameter	Latex Crosslinking	Capacity (per column)	Functional Group	Hydro-phobicity
IonPac ICE-AS1	4 × 250 mm 9 × 250 mm	Heptafluorobutyric acid	Organic acids in high ionic strength matrices. Fast separation of organic acids.	7.5 µm	8%	-	-	5.3 µeq 27 µeq	Sulfonic acid	Ultra Low
IonPac ICE-AS6	9 × 250 mm	Heptafluorobutyric acid	Organic acids in complex or high ionic strength matrices.	8 µm	8%	-	-	27 µeq	Sulfonic and carboxylic acid	Moderate
IonPac ICE-Borate	9 × 250 mm	MSA/ Mannitol	Trace concentrations of borate	7.5 µm	8%	-	-	27 µeq	Sulfonic acid	Ultra Low

Acclaim General and Specialty Columns

Column	Bonded Phase	USP Type	Endcapped	Substrate	Particle Shape	Particle Size	Metal Impurity (ppm) Na, Fe, AL	Average Pore Diameter	Surface Area (m²/g)	Total Carbon Content
Mixed-Mode WAX	Proprietary alkyl amine	na	Proprietary	Ultrapure silica	Spherical	5 µm	<10 ppm	120 Å	300	na
Mixed-Mode HILIC	Proprietary alkyl diol	na	Proprietary			5 µm		120 Å	300	na
Mixed-Mode WCX	Proprietary alkyl carboxyl	na	Proprietary			5 µm		120 Å	300	na
Organic Acid (OA)	Proprietary	na	Yes			5 µm		120 Å	300	17%
Surfactant and Explosives E1/2	Proprietary	na	Yes			5 µm		120 Å	300	na
120 C18	C18	L1	Yes			2, 3 and 5 µm		120 Å	300	18%
120 C8	C8	L7	Yes			3 and 5 µm		120 Å	300	11%
300 C18	C18	L1	Yes			3 µm		300 Å	100	7%
Polar Advantage	Sulfamido C16	na	Yes			3 and 5 µm		120 Å	300	17%
Polar Advantage II	Amide C18	na	Yes			2, 3 and 5 µm		120 Å	300	17%
HILIC	Proprietary hydrophilic		Yes			3 µm		120 Å	300	
Phenyl-1	Proprietary alkyl phenyl		Yes			3 µm		120 Å	300	
Carbamate	Proprietary alkyl group		Yes			3 and 5 µm		120 Å	300	
Trinity			Yes					120 Å	300	

Bio Columns

Protein

Column	Phase	Target Applications	Base Matrix Material	Substrate Crosslinking	Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
MAbPac SEC-1									
MAbPac SCX-10									
ProPac WCX-10	Weak Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10-µm diameter nonporous substrate to which is grafted a polymer chain bearing carboxylate groups.	55%	6 mg/ mL lysozyme	0.2–2 mL/min	80% ACN, acetone. Incompatable with alcohols and MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-10	Strong Cation Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter nonporous substrate to which is grafted a polymer chain bearing sulfonate groups.	55%	3 mg/ mL lysozyme	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProPac SCX-20									
ProPac WAX-10	Weak Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate to which is grafted a polymer chain bearing tertiary amine groups.	55%	5 mg/ mL BSA/ mL	0.2–2.0 mL/min	80% ACN, acetone, MeOH,	3000 psi (21 MPa)	2–12.0
ProPac SAX-10	Strong Anion Exchange	High resolution and high efficiency separations of proteins and glycoproteins, pI =3-10, MW>10,000 units	10 µm diameter non-porous substrate with grafted polymer chain bearing quaternary ammonium groups.	55%	15 mg/ mL BSA	0.2–2.0 mL/min	80% ACN, acetone, MeOH	3000 psi (21 MPa)	2–12.0
ProSwift RP-1S	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Standard permeability	5.5 mg/mL Insulin	2–4 mL/min	Most common organic solvents	2800 psi (19.2 Mpa)	1–14
ProSwift RP-2H	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith High permeability	1.0 mg/mL Lysozyme	1–10 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift RP-4H									
ProSwift RP-3U	Reversed-Phase	Fast protein separation with high capacity using Reversed Phase	Monolith; polystyrene-divinylbenzene with phenyl functional group	Monolith Ultrahigh permeability	0.5 mg/mL Lysozyme	1– 16 mL/min	Most common organic solvents	2800 psi (19.3 Mpa)	1–14
ProSwift SAX-1S	Strong Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with quaternary amine functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift SCX-1S	Strong Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with sulfonic acid fuctional group	Monolith Standard permeability	30 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm)	Most common organic solvents	1000 psi (4.6 mm)	2–12.0

Column	Phase	Target Applications	Base Matrix Material	Substrate Crosslinking	Capacity	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
ProSwift WAX-1S	Weak Anion Exchange	Fast protein separation with good resolution using Anion Exchange	Monolith; polymethacrylate with tertiary amine (DEAE) functional group	Monolith Standard permeability	18 mg/mL BSA	0.5–1.5 mL/min (4.6 mm), 0.05–.25 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProSwift WCX-1S	Weak Cation Exchange	Fast protein separation with good resolution using Cation Exchange	Monolith; polymethacrylate with carboxylic acid (CM) functional group	Monolith Standard permeability	23 mg/mL Lysozyme	0.5–1.5 mL/min (4.6 mm), 0.05–.20 (1.0 mm)	Most common organic solvents	1000 psi (4.6 mm) 2000 psi (1.0 mm)	2–12.0
ProPac IMAC-10	Immobilized Metal Affinity	High resolution separation of certain metal-binding proteins and peptides	10 µm diameter non-porous polystyrene divinylbenzene substrate with poly (IDA) grafts.	55%	>60 mg lysozyme/ mL gel (4 x 250 mm)	1.0 mL/min	EtOH, urea, NaCl, non- ionic detergents, glycerol, acetic acid, guanidine HCl	3000 psi (21MPa)	2–12
ProSwift ConA-1S									
ProPac HIC-10	Reversed-Phase	Protein separation using hydrophobic interaction with salt gradient elution	Spherical 5 µm, ultrapure silica, 300 A, surface area 100 m^2/ g,	n/a	340 mg lysozyme per 7.8 x 75 mm column	1.0 mL/ min	2M Ammonium sulfate/ phosphate salts, organic solvent for cleanup	4,000 psi	2.5–7.5

Carbohydrate

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Maximum Backpressure	pH Range
CarboPac MA1	Reduced mono- and disaccharide analysis.	7.5 µm diameter macroporous substrate fully functionalized with an alkyl quaternary ammonium group	15%	No latex	1450 µeq (4 × 250 mm)	Hydroxide	0.4 mL/min	0%	2000 psi (14 MPa)	0–14
CarboPac PA1	General purpose mono-, di-, and oligosaccharide analysis	10 µm diameter nonporous substrate agglomerted with a 500 nm MicroBead quaternary ammonium functionalized latex	2%	5%	100 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–5%	4000 psi (28 MPa)	0–14
CarboPac PA10	Monosaccharide compositonal anaylysis	10 µm diameter nonporous substrate agglomerated with a 460 nm MicroBead di-functionalized latex	55%	5%	100 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–90%	3500 psi (24.5 MPa)	0–14
CarboPac PA20	Fast mono-, and disaccharide analysis	6.5 µm diameter nonporous substrate agglomerated with a 130 nm MicroBead quaternary ammonium functionalized latex	55%	5%	65 µeq (3 × 150 mm)	Hydroxide, acetate/ hydroxide	0.5 mL/min	0–100%	3000 psi (21 MPa)	0–14
CarboPac PA100	Oligosaccharide mapping and analysis	8.5 µm diameter nonporous substrate agglomerated with a 275 nm MicroBead di-functionalized latex	55%	6%	90 µeq (4 × 250 mm)	Hydroxide, acetate/ hydroxide	1.0 mL/min	0–90%	4000 psi (28 MPa)	0–14
CarboPac PA200	High resolution oligosaccharide mapping and analysis	5.5 µm diameter nonporous substrate agglomerated with a 43 nm MicroBead quaternary ammonium functionalized latex	55%	6%	35 µeq (3 × 250 mm)	Hydroxide, acetate/ hydroxide	0.5 mL/min	0–100%	4000 psi (28 MPa)	0–14

DNA

Column	Target Applications	Base Matrix Material	Substrate Crosslinking	Latex Crosslinking	Capacity	Recommended Eluents	Recommended Flow Rate	Solvent Compatibility	Max. Backpressure	pH Range
DNAPac PA100	Single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms.	13-µm diameter nonporous substrate agglomerated with a 100-nm MicroBead alkyl quaternary ammonium functionalized latex.	55%	5%	40 µeq	Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms	1.5 mL/min	0–100%	4000psi (28MPa)	2–12.5
DNAPac PA200	High resolution single stranded DNA or RNA oligonucleotides, restriction fragments, glycoprotein isoforms.	8-µm diameter nonporous substrate agglomerated with a 130-nm MicroBead alkyl quaternary ammonium functionalized latex.	55%	5%	40 µeq	Chloride, acetate, bromide, perchlorate: in lithium sodium or ammonium forms	1.2 mL/min	0–100%	4000psi (28MPa)	2–12.5
DNASwift										

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