SweetSep™ Columns

The New Benchmark for Carbohydrate Analysis

- New columns for reliable HPAEC-PAD and MS
- Superior and fast separations
- Works for all classes of carbohydrates

High-Performance Anion Exchange (HPAE) columns for the separation of mono-, oligo- and polysaccharides using PAD or MS detection.

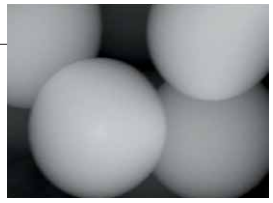
Antec® Scientific

Contents

Why HPAEC?

High Performance Anion-Exchange Chromatography (HPAEC) is the most powerful analytical technique for carbohydrate analysis due to its ability to separate all classes of alditols (polyols), aminosugars, mono-, oligo- and polysaccharides including glycans, according to structural features such as size, composition, anomericity and linkage isomerism. Moreover, HPAEC is considered an environmentally sustainable ("green") chromatographic technique, as its mobile phase utilizes non-toxic aqueous solutions of sodium hydroxide (NaOH) and sodium acetate (NaOAc), eliminating the need for environmentally restrictive solvents.

Monodisperse Particles for Highest Performance



SEM image of 5 µm SweetSep particles

Antec Scientific developed a novel pellicular anion-exchange stationary phase called SweetSep™ AEX. The phase is based on highly uniform monodisperse 5 µm resin particles of crosslinked poly(divinylbenzene-co-ethylvinylbenzene) copolymer. The particles are coated with latex nano beads, functionalized with a quaternary amine group or a bifunctional group containing a quaternary and tertiary amine.

The resin particles packed in inert PEEK columns result in exceptional column efficiencies with a typical reduced plate height close to 2.0 with only moderate column back pressure. SweetSep AEX columns allow for rapid, high-resolution separations of carbohydrates that rival the performance of existing phases based on smaller particle size but operate with significantly lower system back pressures. The size and exchange capacity of the latex nanoparticles are optimized to enable the analysis of a wide variety of carbohydrate samples, ranging from monosaccharides present in food, plants, and glycoproteins up to oligosaccharides such as FOS (fructo-oligosaccharides) and N-linked glycans.

Available Chemistries

SweetSep columns are currently available in three different chemistries, AEX18, AEX20, and AEX200. The main difference lies in the functionalization of the latex particle and the capacity. The AEX18 and 20 have a bifunctional exchange group consisting of a quaternary and tertiary amine. The AEX200, on the other hand, has a quaternary amine.

The exchange capacity for the analytical column of 4.0 mm ID × 20 cm is ca. 90 µeq for AEX 200 and ca. 160 µeq for the AEX20.

AEX18

Optimized for the separtion of Fluorodeoxyglucose ([^{18}F]FDG), according European and US Pharmacopeia.

AEX20

Ideal for fast, high-resolution separation of monosaccharides from food samples, monosaccharides from glycoproteins, sialic acids, Heparin, etc.

AEX200

Universal column for separation of mono- to polysaccharides in food & beverages, plants and glycans.

Column Types and Dimensions

The following column types and dimensions are available:

- Analytical columns
- Pre-columns
- Trap columns

All wetted column parts are made of PEEK independent of the column inner diameter (ID). The 4.0 mm ID columns are made entirely from black PEEK. All 2.1 mm ID columns are PEEK-lined stainless steel columns.

Analytical columns are available as 4.0 and 2.1 mm ID × 20 cm in length.
Pre-columns used as guard columns and/or to increase the length of the analytical column are available as 4.0 and 2.1 mm ID × 5 cm columns.
Trap columns are available in the same dimensions as the pre-columns, i.e., 4.0 and 2.1 mm ID × 5 cm.

Features 4.0 mm ID analytical columns

- Workhorse
- Maximum robustness
- Works at higher flow rates

The 4.0 mm ID × 20 cm HPAEC column is a true workhorse, designed for maximum robustness and reliability in demanding carbohydrate applications. Its optimized design allows it to operate efficiently at flow rates of typically 700 µL/min without compromising performance. Ideal for high-throughput laboratories, this column delivers consistent, reproducible results even under challenging conditions.

2.1 mm ID analytical columns - the new standard

- Up to 3 times higher sensitivity
- Green chromatography: 4 times lower mobile phase consumption and generation of waste
- No notable loss in separation efficiency using same injection volume as 4.0 mm ID column

The 2.1 mm ID × 20 cm HPAEC column is the ideal choice for high-efficiency separations with enhanced performance. It provides up to 3 times higher sensitivity, ensuring precise and accurate detection of analytes. With typical flow rates of 180 µL/min it offers 4 times lower mobile phase consumption and waste generation, making it an environmentally friendly "Green Chromatography". Additionally, the column can handle larger volume injections (up to 10 µL) with virtually no loss in separation efficiency.

Instrumental Requirements

1. HPAEC-PAD

SweetSep columns can be used with any High Performance Anion Exchange Chromatography (HPAEC) system, such as the IC systems of Thermo Fisher Scientific/Dionex, Metrohm, Shine, etc. Several bioinert HPLC systems are also suitable for use with HPAEC when equipped with a Pulsed Amperometric Detector (PAD) such as the DECADE™ Elite (Antec Scientific). For consistent results, ease of use, and the highest reproducibility, the Antec Scientific ALEXYS™ Carbohydrate Analyzer is the best choice.

2. Borate Ion Trap

The use of a Borate Ion Trap (BIT) column installed between the pump and the autosampler is highly recommended. For more information about the BIT column, see page 10.

3. HPAEC-MS

Depending on the volatility of the buffer systems used, for the online coupling with MS, the installation of a desalter (ion suppressor) becomes necessary. Basically, any type of (ESI)-MS can be used for detection.

4. HPAEC-PAD/MS

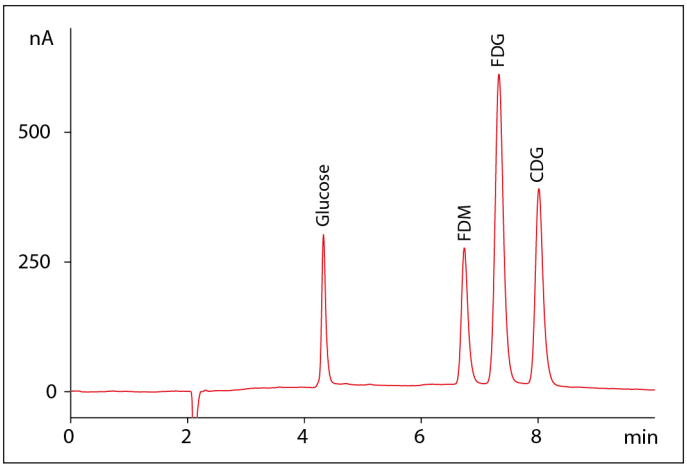
Parallel detection using PAD and MS enables simultaneous quantification and identification of carbohydrates, without the need for derivatization. This can be easily achieved by splitting the effluent from the SweetSep column into two separate flow streams. One stream is directed to the PAD detector, while the other is routed to the MS. An in-line desalter (suppressor) must be installed before the MS to remove Na^+ ions from the mobile phase.

Analytical Columns (4.0 and 2.1 mm ID × 20 cm)

1. SweetSep™ AEX18

SweetSep AEX18 columns are designed to meet the strict requirements for analyzing [^{18}F]FDG according to USP and EP standards. The SweetSep AEX18 (4 × 200 mm) is registered under the USP column code L46.

1.1 Fluorodeoxyglucose ([^{18}F]FDG), according EP & USP methods

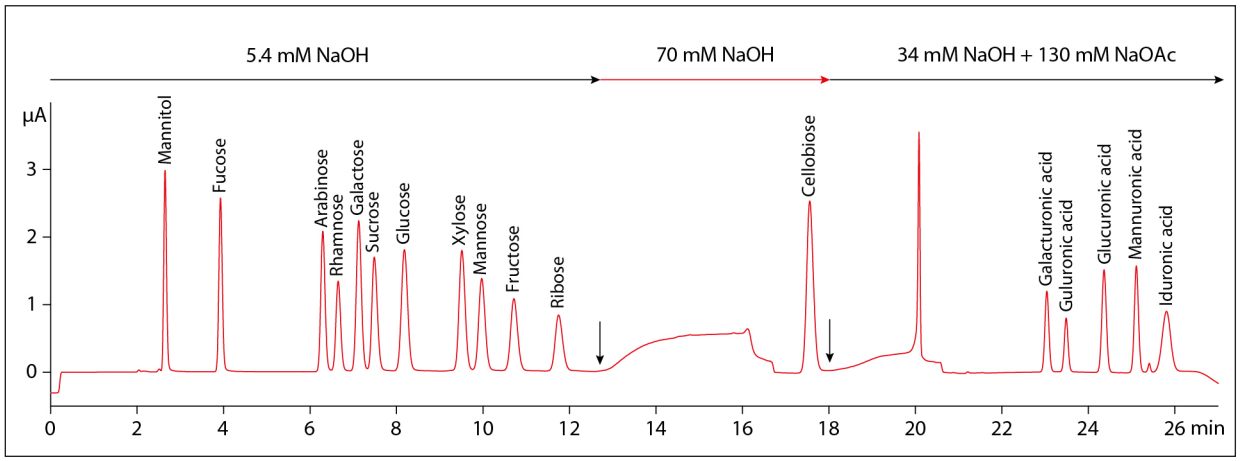


Separation of FDG and its by-products, FDM and CDG according optimized USP, SweetSep™ **AEX18** column, 4.0 mm ID × 20 cm.
10 µL inj. of a standard mix of 10 µg/mL FDG, FDM, CDG and 1 µg/mL glucose. Resolution: FDM-FDG 2.5 and FDG-CDG 2.7.

2. SweetSep™ AEX20

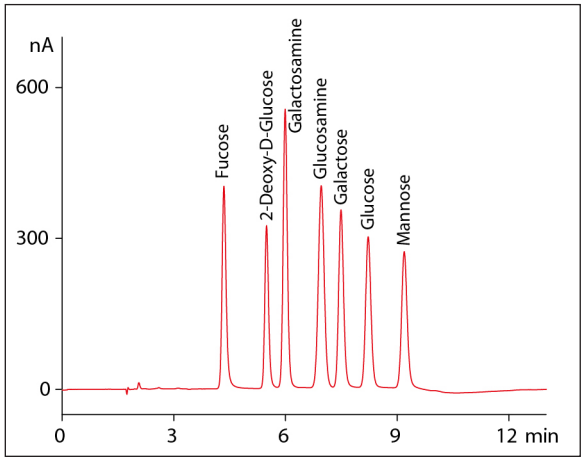
Fast, high-resolution separation of monosaccharides from food samples and biomass, including monosaccharides and sialic acids from glycoproteins, etc. For specifications, see page 11.

2.1 Monosaccharides and uronic acids from biomass

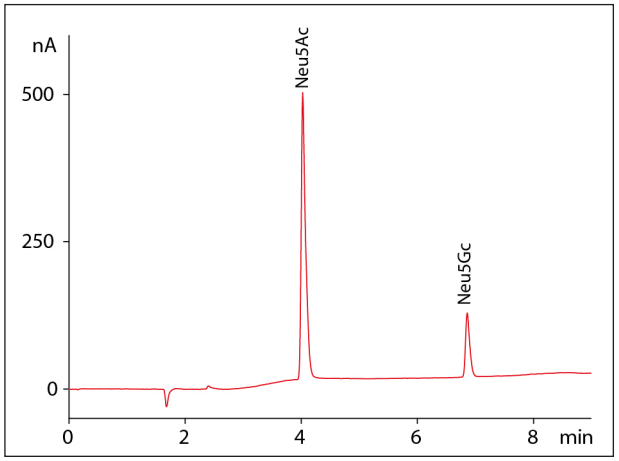


Separation of neutral sugars & uronic acids on a SweetSep™ **AEX20** column, 2.1 mm ID × 20 cm, 10 µL inj. of a 100 µM mixtures of neutral sugars & uronic acids std in water (HPAEC-PAD). Arrows indicate the start/end of the baseline disturbance due to step gradient.

2.2 Monosaccharides from glycoproteins including sialic acids



Isocratic separation of monosaccharides on a SweetSep™ **AEX20** column, 4.0 mm ID × 20 cm. 10 µL inj. of a 10 µM mixtures of monosaccharides in water (HPAEC-PAD).

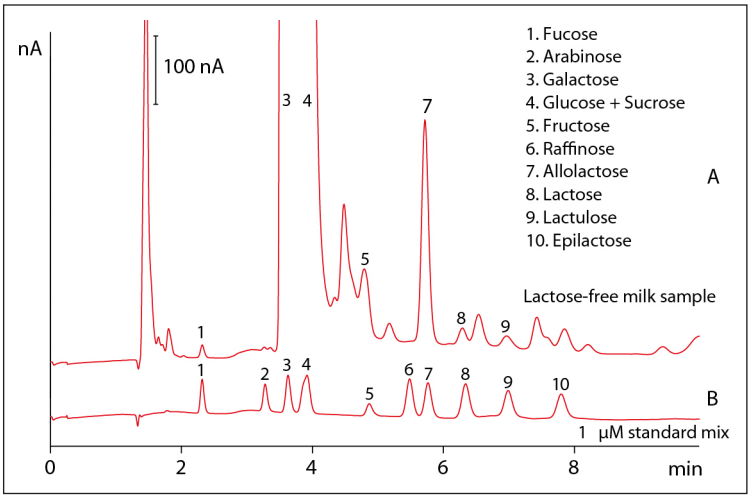


Gradient separation of sialic acids on a SweetSep™ **AEX20**, 4.0 mm ID × 20 cm. 10 µL inj. of 10 µM Neu5Ac and 1 µM of Neu5Gc in water.

3. SweetSep™ AEX200

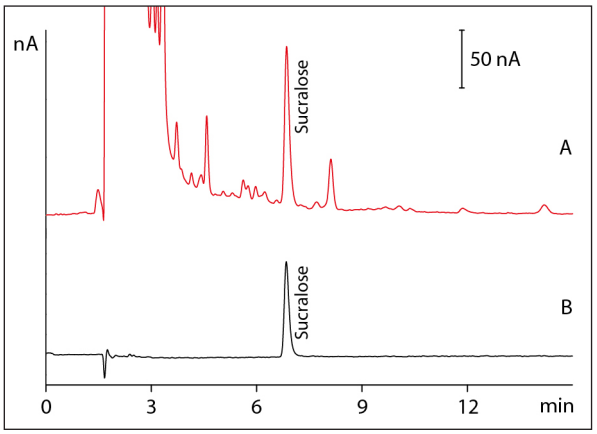
Universal column for separation of mono- to polysaccharides in F&B, artificial sweeteners, plants, glycans, etc. For specifications, see page 11.

3.1 Mono- and disaccharides in F&B: Lactose Intolerance



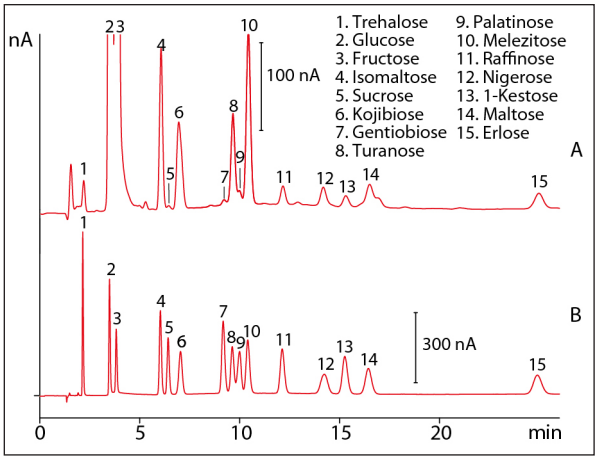
Analysis of milk on a SweetSep™ **AEX200** column, 4.0 mm ID × 20 cm. (A) 10 µL inj. of a 10 g/L lactose-free labelled milk. (B) 10 µL inj. of a 10 µM standard of 10 sugars commonly found in milk, incl. lactose (peak #8) and its isomers, allolactose (#7), lactulose (#9) and epilactose (#10).

3.2. Disaccharide: Sucralose artificial sweetener

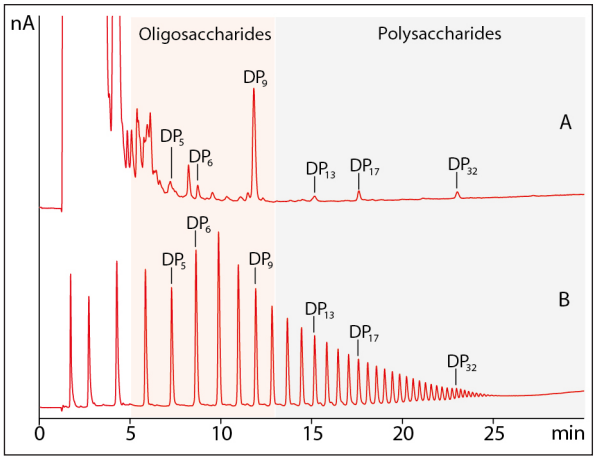


HPAEC-PAD of (A) zero sugar soft drink, diluted 1:100 and (B) 10 µM sucralose standard in DI water. SweetSep™ **AEX200** column, 2.1 mm ID × 20 cm. 2.5 µL injection.

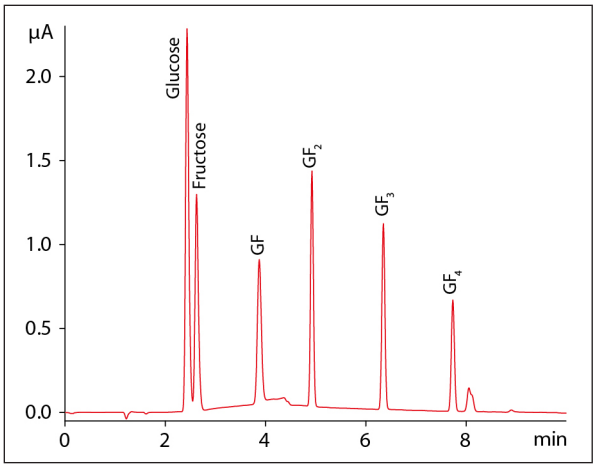
3.3. Mono-, oligo- and polysaccharides in F&B



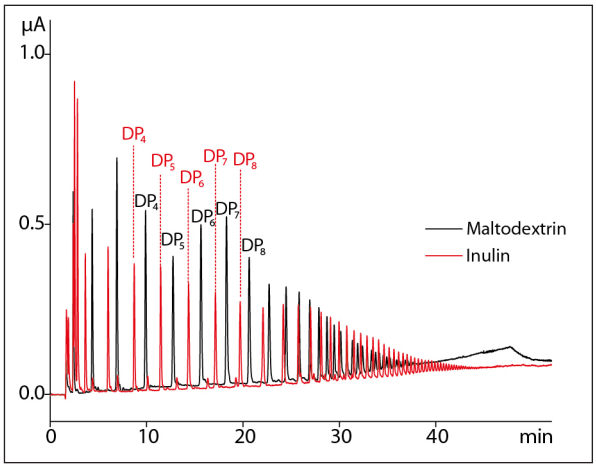
Analysis of mono-, di- and trisaccharides in honey, SweetSep™ **AEX200** column, 4.0 mm ID × 20 cm. (A) 10 µL inj. of summer honey. (B) 15 most common carbohydrates in honey.



Analysis oligo- and polysaccharides in (A) honey, (B) reference maltodextrin (DE4-7), SweetSep™ **AEX200** column, 4.0 mm ID × 20 cm.



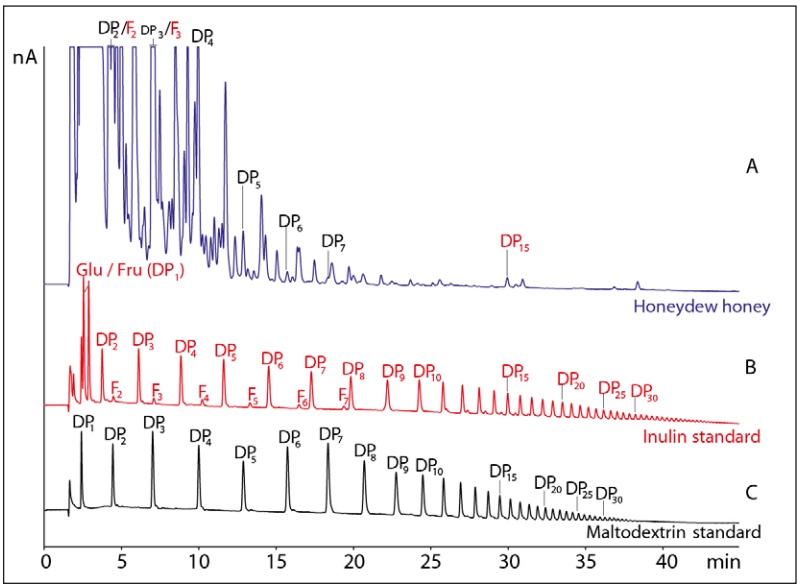
Separation of short-chain fructooligosaccharides (FOS) up to GF4 (= DP5). SweetSep™ **AEX200** column, 4.0 mm ID × 20 cm. 10 µL inj. of 10 ppm mixtures GFs



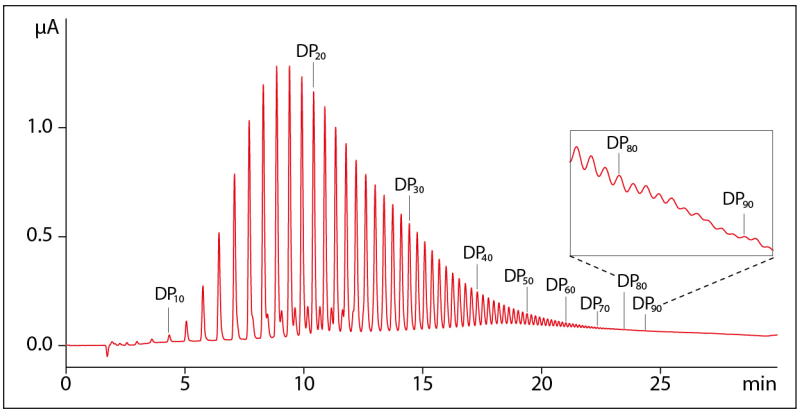
Overlay chromatogram of 10 µL injection of 500 ppm maltodextrin DE 4-7 standard (black) and 200 ppm of inulin standard (red).

Analytical Columns (4.0 and 2.1 mm ID × 20 cm)

3.4. Oligo- and polysaccharides in F&B

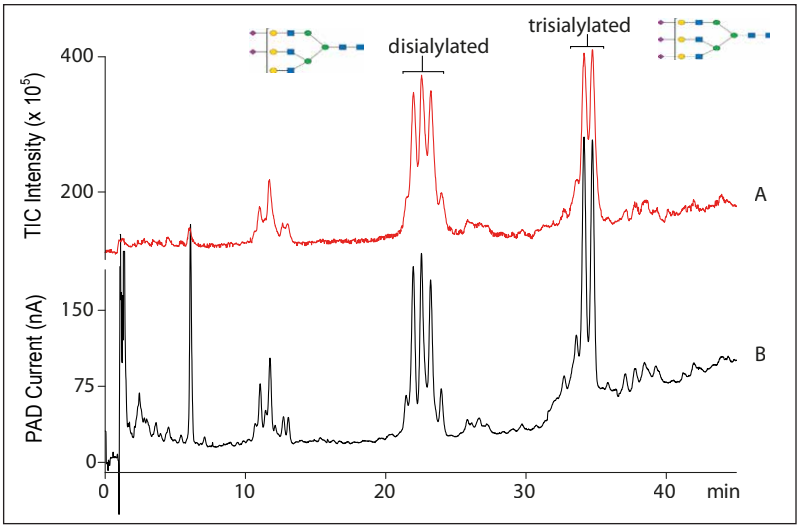


Authentic honeydew honey with natural oligosaccharides, (A) 10 µL injection of honeydew honey,, (B) 200 ppm inulin standard, and (C) 500 ppm maltodextrin standard (black line). Red peaks in (A) correspond to fructo-, black to malto-oligosaccharides. SweetSep **AEX200** column, 4.0 mm ID × 20 cm.



Separation of fructans up to DP 90. SweetSep™ **AEX200** column, 4.0 mm ID × 20 cm + Precolumn 4.0 mm ID × 5 cm

3.5. Glycans from Glycoproteins



Separation of N-glycans standard containing di-, tri-, and tetra sialylated oligosaccharides by HPAEC-PAD/MS on a SweetSep™ **AEX200** column, 4.0 mm ID × 20 cm. A) PAD for quantification, (B) MS for identification with desalter prior to MS

Trap Columns (4.0 and 2.1 mm × 5 cm)*

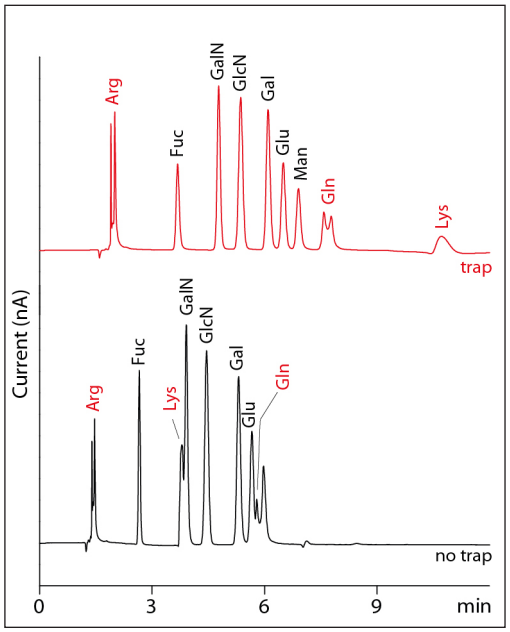
Amino Acid Trap (AAT)

- Efficient trapping of amino acids and small peptides
- Easy installation as precolumn
- Accurate quantification

In compositional analysis of monosaccharides from glycoproteins using HPAEC-PAD, amino acids and small peptides co-elute with the carbohydrates of interest, making proper quantification impossible. These amino acids and small peptides are generated during the acid hydrolysis of glycoproteins. Moreover, amino acids can contaminate the Au electrode surface, which might lead to fouling and loss of response even under PAD conditions.

To eliminate the interference of amino acids and to assure accurate quantification of the monosaccharides, the use of an Amino Acid Trap (AAT) column is highly recommended.

For specifications/ordering information, see pages 11 and 12.



Analysis of monosaccharides with and without trap column. Interfering peaks of Glutamine (Gln) and Lysine (Lys) are efficiently trapped (upper trace) and elute later during the wash step. In both cases the amino acid Arginine (Arg) elutes in the void volume (t_0) of the column.

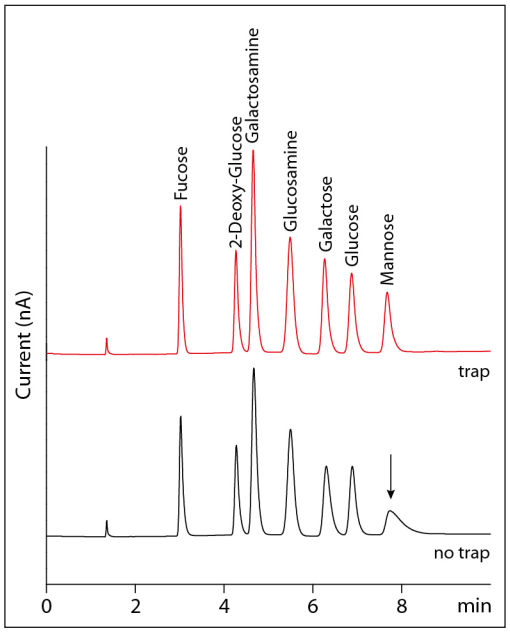
Borate Ion Trap (BIT)

- High borate trapping capacity
- Easy installation between pump and injector
- Reduce peak tailing

In carbohydrate analysis, the peak shape of certain sugars, such as mannose, fructose, and sugar alcohols, is deteriorated when traces of borate are in the solvent. These borate contaminants can come from the laboratory deionized (DI) water system.

To eliminate the presence of borate ions and to assure optimal performance Antec Scientific introduced the Borate Ion Trap (BIT) column. The trap column is installed inline between pump and injector of the HPAEC-PAD system.

For specifications/ordering information, see pages 11 and 12.



Analysis of carbohydrates with and without Borate Ion Trap (BIT) column. Without trap, the mannose peaks shows significant tailing, see arrow in lower trace.

** The 4 mm ID trap column should be used only with the 4 mm ID analytical column and the 2.1 mm trap with the 2.1 mm ID analytical, respectively.*

Pre-Columns (4.0 and 2.1 mm × 5 cm)

- Used as guard column
- Used to extend column efficiency
- Available in all chemistries

The SweetSep™ Pre-columns are high-performance columns, individually tested, and available in all chemistries (AEX 18 to 200). They are used as guard columns to extend the column lifetime of the analytical column and increase the separation path length and efficiency by 25%.

The 4 mm ID Pre-column should be used only with the 4 mm ID analytical column and the 2.1 mm Pre-column with the 2.1 mm ID analytical, respectively.

Specifications

Specifications SweetSep™ Anion Exchange Columns

Parameter		**AEX200**	**AEX18 and 20**
Type		agglomerated pellicular resin	
Particle	Material	ethylvinylbenzene-divinylbenzene copolymer	
	Diameter (µm)	5	
	Functionality	surface sulfonated	
Latex	Material	vinylbenzylchloride-divinylbenzene	
	Functionality	quaternary amine	bifunctional quaternary and tertiary amine
Organic solvent limit		0-100% ACN or MeOH for cleaning	
T operating range (°C)		10-40	
pH range		0-14	
max (psi/bar)		4350/300	

Specifications Trap columns

Parameter		**Borate ion trap**	**Amino acid trap**
Type		Chemically derivatized polymeric resin	Polymer grafted film on porous polymeric resin
Particle	Material	Polyvinylbenzyl chloride	ethylvinylbenzene-divinylbenzene copolymer
	Diameter (µm)	10	5
	Pore size (Å)	n.d.	Macro-porous, 300
	Crosslinking (%)	12%	55%
	Functionality	polyol	hydroxyethyl quaternary ammonium
Organic solvent limit		0-90% ACN or MeOH for cleaning	0-80% ACN or MeOH for cleaning
T operating range (°C)		10-40	10-40
pH range		0-14	0-14
max (psi/bar)		4350/300	4000/280

Ordering Information

Ordering Information

Part no.	Description	Additional info
Analytical columns (4.0 and 2.1 mm ID × 20 cm)		
260.0010	SweetSep™ AEX200, 4.0 mm ID × 20 cm, 5 µm	Universal column for separation of mono- to polysaccharides in F&B, plants and glycans.
260.0011	SweetSep™ AEX200, 2.1 mm ID × 20 cm, 5 µm	
260.0020	SweetSep™ AEX20, 4.0 mm ID × 20 cm, 5 µm	Fast, high-resolution separation of monosaccharides from food samples, incl. monosaccharides from glycoproteins, FDG, Heparin, etc.
260.0021	SweetSep™ AEX20, 2.1 mm ID × 20 cm, 5 µm	
Pre-columns (4.0 and 2.1 mm ID × 5 cm)		
260.0015	SweetSep™ AEX200, 4.0 mm ID × 5 cm, 5 µm	For use with the AEX200 analytical column.
260.0016	SweetSep™ AEX200, 2.1 mm ID × 5 cm, 5 µm	
260.0025	SweetSep™ AEX20, 4.0 mm ID × 5 cm, 5 µm	For use with the AEX20 analytical column.
260.0026	SweetSep™ AEX20, 2.1 mm ID × 5 cm, 5 µm	
Columns for Fluorodeoxyglucose [18F]FDG		
FDG EP		
260.0051	SweetSep™ AEX18, 2.1mm ID × 18.5 cm, 5µm	FDG column according EP
260.0056	SweetSep™ AEX18, 2.1mm ID × 3 cm, 5µm	FDG pre-column for EP method
FDG USP		
260.0050	SweetSep™ AEX18, 4.0 mm ID × 20 cm, 5µm	FDG column according USP, L46
260.0055	SweetSep™ AEX18,4.0 mm ID × 5 cm, 5µm	FDG pre-column for USP method
Trap-columns (4.0 mm and 2.1 mm ID × 5 cm)		
260.0040	Amino acid trap, 4.0 mm ID × 5 cm, 5 µm	Traps amino acids present in the sample that interfere with the monosaccharide separation.
260.0041	Amino acid trap, 2.1 mm × 5 cm, 5 µm	
260.0030	Borate ion trap, 4.0 mm ID × 5 cm, 10 µm	Traps borate contaminants from mobile phase.
260.0031	Borate ion trap, 2.1 mm ID × 5 cm, 10 µm	
Accessories		
260.0100	Pre-column inlet filter PEEK, 0.5 µm	With replaceable PEEK frits 0.5 µm porosity, for direct connection into the analytical or pre-column.
260.0110	Replacement filters PEEK, 0.5 µm, 1 pcs	Replacement filters for Pre-column inlet filter.

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