

# 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.



## 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.

## Highly Monodisperse 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 furthermore coated with quaternary amine functionalized nanoparticles.



SEM image of 5 µm SweetSep particles

## High Efficiency with Low Backpressure

The resin particles packed in inert PEEK columns result in exceptional column efficiencies with 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 operates with significantly lower system back pressures. The size and exchange capacity of the latex nanoparticles is optimized to enable the analysis of a wide variety of carbohydrates samples ranging from monosaccharides present in food, plants and glycoproteins up to oligosaccharides such as FOS (fructo-oligosaccharides) and N-linked glycans.

## 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 Metrohm or Thermo Fisher Scientific. 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 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 4.

#### 3. HPAEC-MS

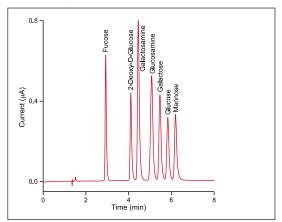
Depending on the volatility of the buffer systems used, for the on-line coupling with MS, the installation of a desalter (ion suppressor) becomes necessary. Basically, any type of (ESI)-MS can be used for detection.

#### 4. HPAEC/(PAD)-MS

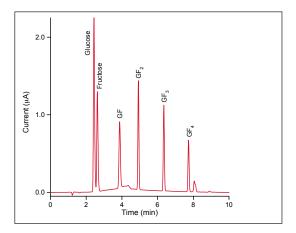
Parallel detection by PAD and MS to allow simultaneous identification and quantification of the carbohydrates can be done easily by using a simple T-piece flow split after the SweetSep column.

## Examples

#### Mono- up to Pentasaccharides

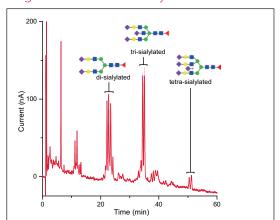


Isocratic separation of monosaccharides on a SweetSep  $^{\text{m}}$  AEX20 column, 4.0 mm ID  $\times$  200 mm. 10  $\mu$ L inj. of a 10  $\mu$ M mixtures of monosaccharides std in water (HPAEC-PAD).

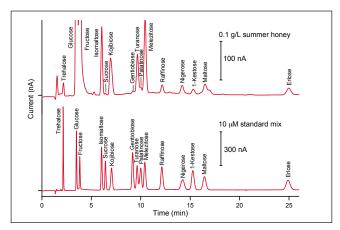


Separation of short-chain fructooligosaccharides up to DP5 by HPAEC-PAD. SweetSep<sup>™</sup> AEX200 column, 4.0 mm ID  $\times$  200 mm. 10  $\mu$ L inj. of 10 ppm mixtures GFs.

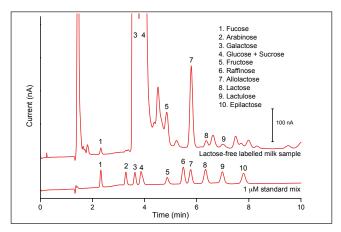
### Oligosaccharides & N-Glycans



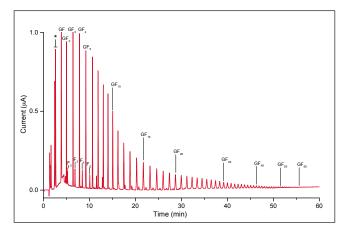
Separation of N-glycans standard containing di-, tri-, and tetra sialylated oligosaccharides by HPAEC-PAD on a SweetSep $^{\text{m}}$  AEX200 column, 4.0 mm ID  $\times$  200 mm.



HPAEC-PAD of honey on a SweetSep<sup>™</sup> AEX200 column, 4.0 mm ID  $\times$  200 mm. Top: 10  $\mu$ L inj. of a 0.1 g/L Swiss summer honey sample. Bottom: 10  $\mu$ L inj. of a 10  $\mu$ M standard of 15 sugars present in honey.



Lactose intolerance: Analysis of milk on a SweetSep  $^{\mathtt{M}}$  AEX200 column, 4.0 mm ID  $\times$  200 mm. Top: 10  $\mu L$  inj. of a 10 g/L lactose-free labelled milk. Bottom: 10  $\mu L$  inj. of a 10  $\mu M$  standard of 11 sugars commonly found in milk.



Gradient separation of inulin from chicory. 10  $\mu$ L inj, 200 ppm. SweetSep™ AEX200 column, 4.0 mm ID  $\times$  200 mm using HPAEC-PAD.

## Trap Columns

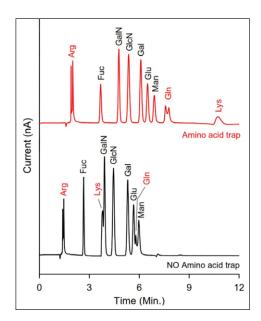
#### Amino Acid Trap (AAT)

- Efficient trapping of amino acids/small peptides
- Easy installation as precolumn
- 4 x 50 mm column, 5 μm resin

In compositional analysis of monosaccharides from glycoproteins using HPAEC-PAD, amino acids and small peptides co-elute with the carbohydrates of interest making proper qualification 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 page 5.



Analysis of monosaccharides from glycoproteins with and without Amino Acid Trap (AAT) column. Interfering peaks such as lysine and glutamine are fully removed.

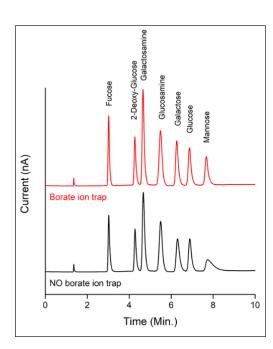
#### Borate Ion Trap (BIT)

- High borate trapping capacity
- Easy installation between pump and injector
- 4 x 50 mm column, 10 µm resin

In carbohydrate analysis, the peak shape of certain sugars, such as mannose, fructose and sugar alcohols, are 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 available as 4.0 mm ID x 50 mm column and is installed inline between pump and injector of the HPAEC-PAD system.

For specifications/ordering information, see page 5.



Analysis of carbohydrates with and without Borate Ion Trap (BIT) column. The peak of mannose shows significantly less tailing using the BIT column (upper trace).

# Specifications / Ordering Information

| Specifications SweetSep™ Anion Exchange Columns |               |  |  |  |  |
|---|---------------|--|--|--|--|
| Parameter                                       |               | AEX200                                     | AEX20                                      |  |  |
| Туре  |               | 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                                |  |  |
| Туре                        |                  | 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                    |  |   |  |  |  |
|---|--|---|--|--|--|
| Part no.                                | Description                                | Additional info   |  |  |  |
| Analytical columns (4.0 mm ID x 200 mm) |  |   |  |  |  |
| 260.0010                                | SweetSep™ AEX200, 5 μm                     | Universal column for separation of mono- to polysaccharides in F&B, plants and glycans.   |  |  |  |
| 260.0020                                | SweetSep™ AEX20, 5 μm                      | Fast, high-resolution separation of monosaccharides from food samples, incl. monosaccharides from glycoproteins, FDG, Heparin, etc. |  |  |  |
| Pre-columns (4.0 mm ID x 50 mm)         |  |   |  |  |  |
| 260.0015                                | SweetSep™ AEX200, 5 μm                     | For use with the AEX200 analytical column.  |  |  |  |
| 260.0025                                | SweetSep™ AEX20, 5 μm                      | For use with the AEX20 analytical column.   |  |  |  |
| Trap-columns (4.0 mm ID x 50mm)         |  |   |  |  |  |
| 260.0040                                | Amino acid trap, 5 μm                      | Traps amino acids present in the sample that interfere with the monosaccharide separation.  |  |  |  |
| 260.0030                                | Borate ion trap, 10 μm                     | Traps borate contaminants from mobile phase.  |  |  |  |
| Accessories                             |  |   |  |  |  |
| 260.0100                                | Pre-column inlet filter PEEK,<br>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,<br>0.5 μm, 1 pcs | Replacement filters for Pre-column inlet filter.  |  |  |  |

SweetSep columns with other chemistries and dimension are currently under development and will be released shortly. For futher information, please contact info@AntecScientific.com

Antec Scientific (USA)

info@AntecScientific.com www.AntecScientific.com T 888 572 0012

Antec Scientific (worldwide)

info@AntecScientific.com www.AntecScientific.com T +31 172 26 88 88



SweetSep, ALEXYS, and DECADE Elite are official trade marks of Antec Scientific