# SweetSep™ a New HPLC Column for the Fast Analysis of Carbohydrates and Glycans by LC-MS

Antec Scientific

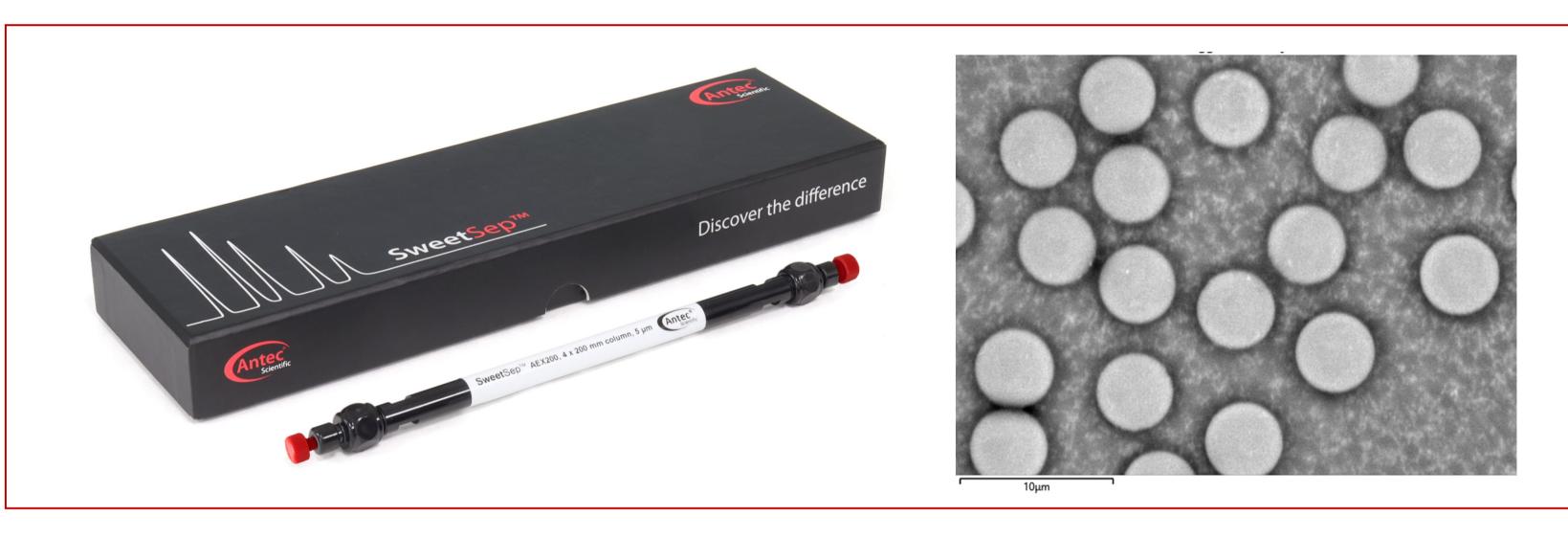
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# Introduction

High-Performance Anion Exchange Chromatography in combination with Pulsed Amperometric Detection (HPAEC-PAD) and/or MS (HPAEC/PAD-MS) is the method of choice for the analysis of carbohydrates. It combines superior selectivity with sensitive detection down to femtomole levels without the need for derivatization or complicated sample preparation. A novel anion-exchange stationary phase SweetSep™ has been developed. The stationary phase is based on a monodisperse 5 µm resin coated with quaternary amine functionalized nanoparticles. The high uniformity and monodispersity of the resin allow for rapid, high-resolution separations of carbohydrates. The size and exchange capacity of the latex nanoparticles was 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 (fructooligosaccharides) and *N*-linked glycans.



**Figure 1:** Left: SweetSep™ AEX200 column, 4.0 mm ID × 200 mm. Right: Scanning Electron Microscope (SEM) micrograph of the 5  $\mu$ m agglomerated pellicular anion-exchange resin particles.

## Instrumentation

The ALEXYS Carbohydrate Analyzer (Antec Scientific) is a dedicated HPAEC system for PAD and/or MS detection consisting of:

- ET210 Eluent Tray, for sparging and blanketing of eluent with inexpensive N2 gas.
- HPLC Pump P6.1L, quaternary pump with highest flow accuracy and minimal pulsation.
  Inert Autosampler AS6.1L, high accuracy and
- reproducibility.
   Column Oven / Thermostat CT2.1, wide range from
- -5°C and 85°C.
   DECADE Elite, highly sensitive electrochemical detector with dedicated flow cells for



Figure 2: ALEXYS Carbohydrate
Analyzer (Antec Scientific)

# Results

carbohydrates.

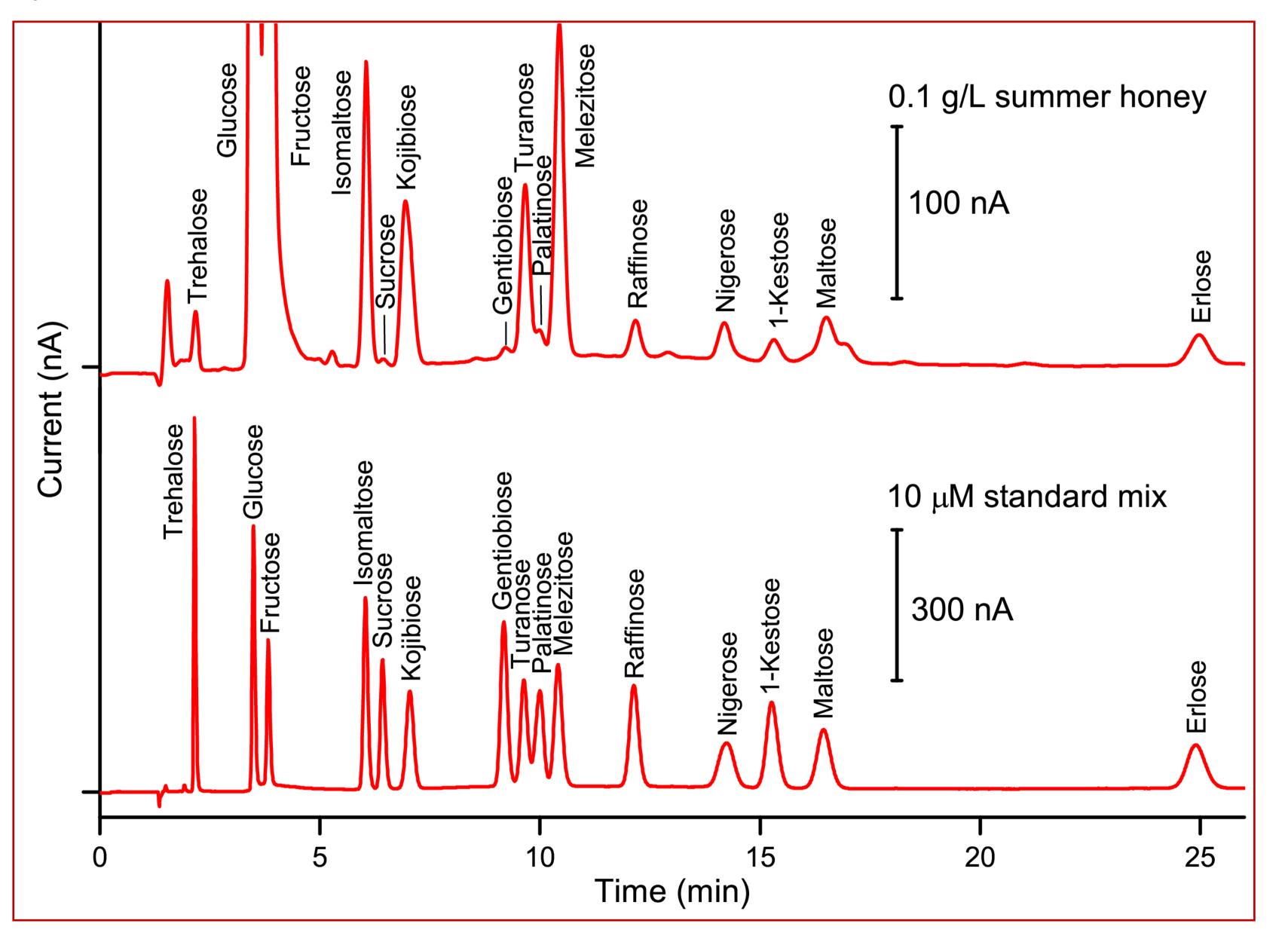
### 1.1. Analysis of Milk and Honey (Mono- up to Trisaccharides)

The amount of lactose in milk are often overestimated due to the presence of lactose isomers such as allolactose, lactulose, and epilactose. Using SweetSep™ AEX 200, all 4 lactose isomers were separated in under 8 minutes (Figure 3), allowing for fast and accurate quantification of lactose in milk samples.

Similar to milk, the amount of sugars in honey are important to quantify for fingerprinting purpose and preventing adulteration. A total of 15 sugars commonly found in honey were separated in under 25 minutes with high resolution using SweetSep™ AEX200 (Figure 4).

# 1. Fucose 2. Arabinose 3. Galactose 4. Glucose + Sucrose 5. Fructose 6. Raffinose 7. Allolactose 8. Lactose 9. Lactulose 10. Epilactose 1 μM standard mix 1 μM standard mix 1 μM standard mix 1 μ standard mix

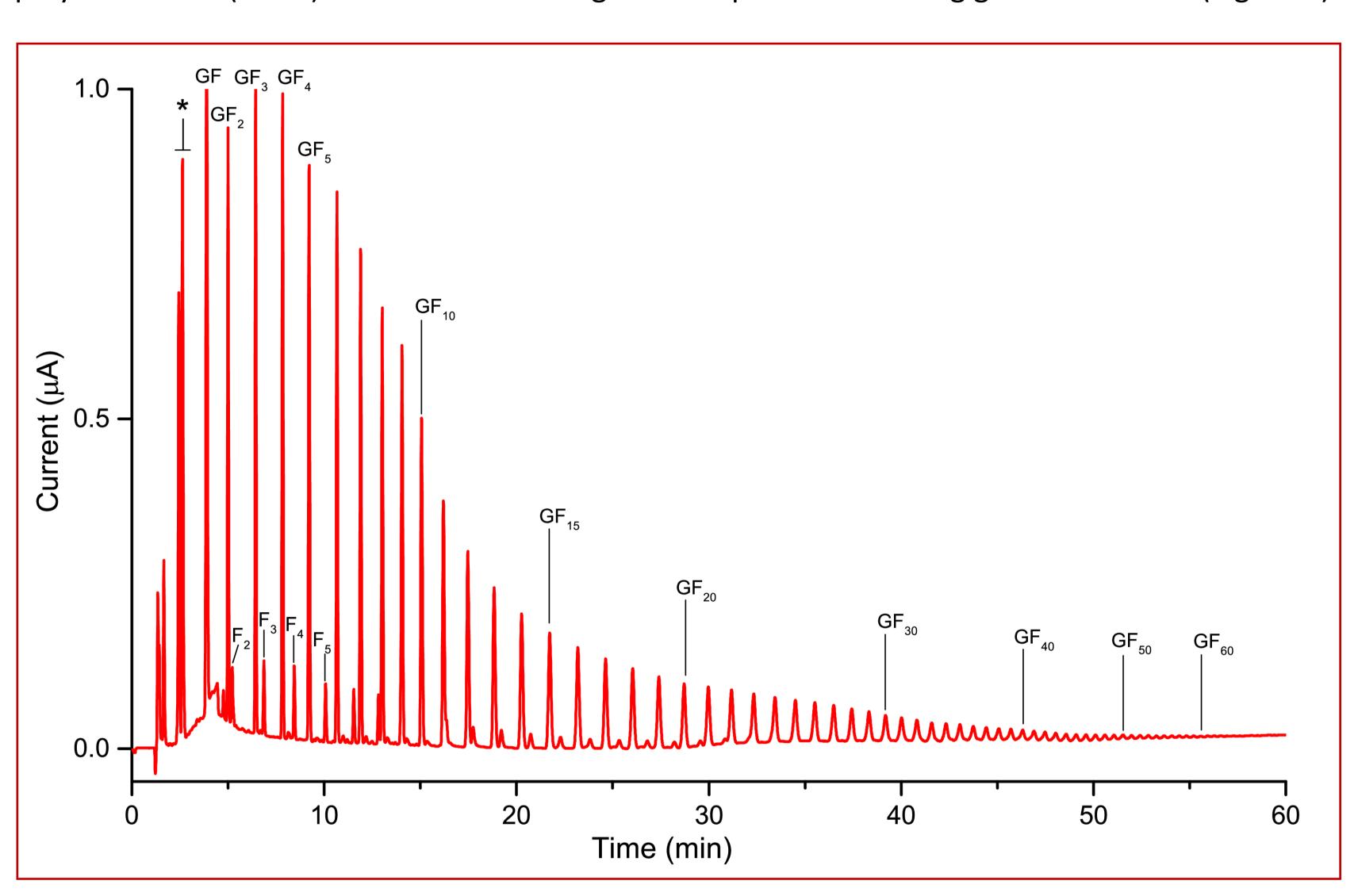
**Figure 3:** Analysis of milk on a SweetSep<sup>™</sup> AEX200 column, 4.0 mm ID × 200 mm. Top: 10  $\mu$ L injection of a lactose-free labelled milk sample. Bottom: 10  $\mu$ L injection of a 1  $\mu$ M standard of 11 sugars commonly found in milk. Isocratic elution: 12 mM NaOH + 3 mM NaOAc, 0.7 mL/min, 30°C.



**Figure 4:** Analysis of honey on a SweetSep<sup>™</sup> AEX200 column, 4.0 mm ID × 200 mm. Top: 10  $\mu$ L injection of a 0.1 g/L Swiss summer honey sample. Bottom: 10  $\mu$ L injection of a 10  $\mu$ M standard of 15 sugars commonly found in honey. Isocratic elution: 68 mM NaOH, 0.7 mL/min, 20°C.

# 1.2. Analysis of Oligosaccharides

Separation of more complex sugars such as oligosaccharides up to very high degree of polymerization (DP60) were achieved using SweetSep™ AEX200 using gradient elution (Figure 5).

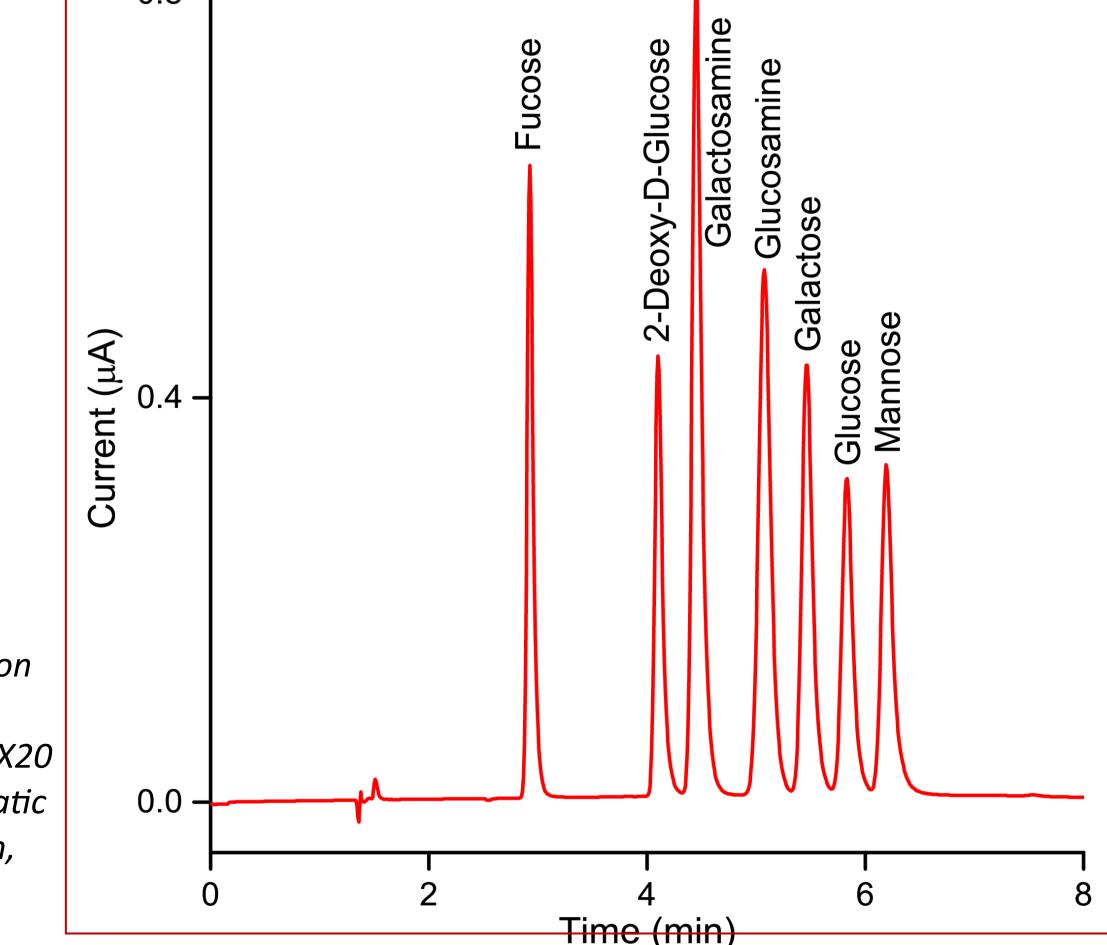


**Figure 5:** Gradient elution of 10 μL injection of a 200 ppm inulin from chicory on a SweetSep<sup>™</sup> AEX200 column, 4.0 mm ID × 200 mm. Gradient elution: 0 min: 100 mM NaOH, 12 min: 100 mM NaOH + 180 mM NaOAc, 60 min: 100 mM NaOH + 450 mM NaOAc, 0.8 mL/min, 25°C.

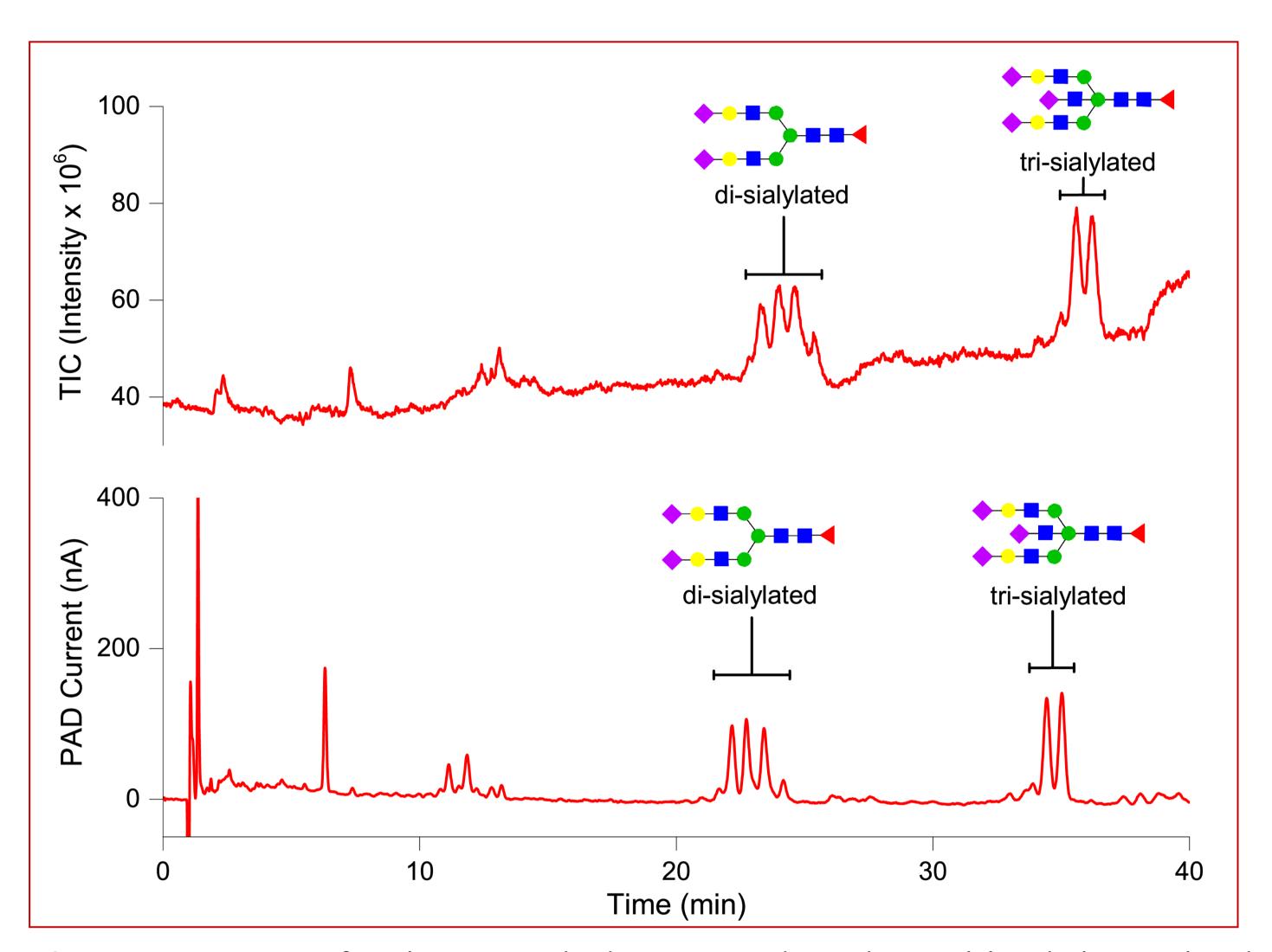
# 1.3. Analysis of Glycoprotein Monosaccharides and N-Glycans

Complete hydrolysis of glycans results in variety of monosaccharides, which are relevant for structure elucidation of the glycans. On SweetSep™ AEX20, 7 monosaccharides commonly found in glycoproteins were baseline separated in under 8 minutes (Figure 6).

Figure 6: Separation of 10 μL injection of a 10 μM mixtures of glycoprotein monosaccharides on SweetSep™ AEX20 column, 4.0 mm ID × 200 mm. Isocratic elution: 17.5 mM NaOH, 0.7 mL/min, 30°C.



Intact N-glycans can be released from glycoproteins using N-glycosidase F (PNGase F) enzyme. SweetSep<sup>TM</sup> is an ideal column for separation of intact N-glycans without sample derivatization. In Figure 7 the separation of N-glycans on a SweetSep<sup>TM</sup> AEX200 column is shown.



**Figure 7:** Separation of N-glycans standard containing di— and tri—sialylated oligosaccharides on a Sweet-Sep™ AEX 200 column, 4.0 mm ID × 200 mm. Top: Total Ion Chromatogram. MS conditions: eluent flow rate 1.0 mL/min, nebulizer gas pressure 70 psi, drying gas 12L /min, drying gas temperature 365°C. Bottom: Pulsed Amperometric Detection Chromatogram. Gradient elution: 0 min: 100 mM NaOH + 6 mM NaOAc, 70 min: 100 mM NaOH + 190 mM NaOAc, 1.0 mL/min, 30°C separation.

# Conclusions

A newly developed stationary phase based on highly monodispersed 5 µm particles coated with quaternary amine functionalized nanoparticles (SweetSep™) were utilized for analysis of carbohydrates and glycans. We found that:

- SweetSep™ **AEX200** column provide the best separation of mono— up to pentasaccharides as demonstrated by analysis of sugars in milk and honey samples.
- More complex sugars such as oligosaccharides up to very high degree of polymerization (DP<sub>60</sub>)
  can be separated using SweetSep™ AEX200 column.
- SweetSep™ AEX200 column is ideally suited for the analysis of intact N-glycans from glycoproteins by PAD or MS detection.
- SweetSep™ AEX20 is the perfect choice for detection and quantification of monosaccharides from glycoproteins.

# References

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