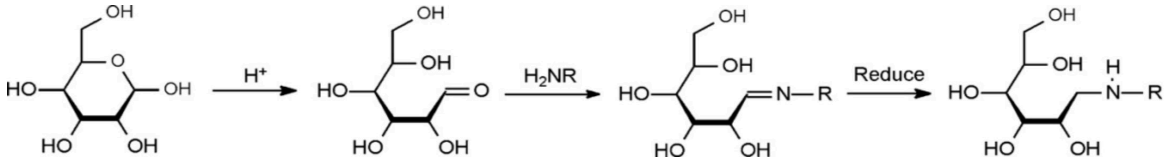
Separation of PNGase-Released and Labeled N-Glycans by HILIC Using HALO® Glycan Column

Application Note 121-GL

Digestion of N-linked proteoglycans using PNGase F releases oligosaccharides, which can be reacted with an amine via Schiff base formation. The Schiff's base derivatives (imines) can be easily reduced to form stable amine derivatives for analysis.



Many amines have been applied for labeling glycans (Harvey, 2011, J. Chromatogr. B, 879, 1196-1225). In this application brief, procainamide was chosen because of reported improvements in ESI-MS detection (Klapoetke, et. al., 2010, J. Pharm. Biomed. Anal., 53, 315-324).

Typical Labeling Conditions:

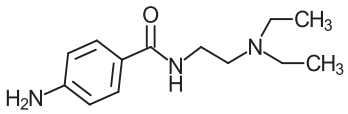
1) Glycan in water (up to 10% volume)
2) 90+% volume of:
   - 0.4 M procainamide
   - 1M sodium cyanoborohydride in 30% glacial acetic acid/70% DMSO

12-16 hr reaction at 37°C
SEC cleanup on Sephadex G-10 minicolumn
Absorbance Detection @300 nm or Fluorescence with Ex 330/Em 380 nm

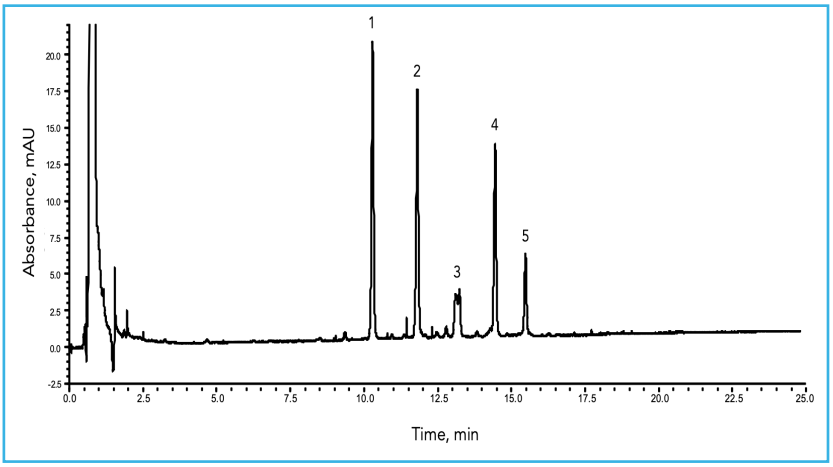
TEST CONDITIONS:

Column: HALO 90 Å Glycan, 2.7 µm, 2.1 x 150 mm
Part Number: 92922-705
Mobile Phase:
A: 50 mM Ammonium formate, pH 4.45
B: Acetonitrile
Gradient: 80% B to 55% B in 25 min
Flow Rate: 0.6 mL/min
Pressure: 190 bar
Temperature: 60 °C
Detection: UV 300 nm
Injection Volume: 3.0 µL
Sample Solvent: 70/30 ACN/water
Response Time: 0.5 sec
Data Rate: 3.3 Hz
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Nexera

STRUCTURE:



Procainamide (PAm)



A fast separation of PNGase-released and procainamide-labeled N-Glycans from Ribonuclease B is accomplished with a HALO 90 Å Glycan column.

PEAK IDENTITIES:

1. PAm-GlcNAc$_2$Man$_5$
2. PAm-GlcNAc$_2$Man$_6$
3. PAm-GlcNAc$_2$Man$_7$
4. PAm-GlcNAc$_2$Man$_8$
5. PAm-GlcNAc$_2$Man$_9$