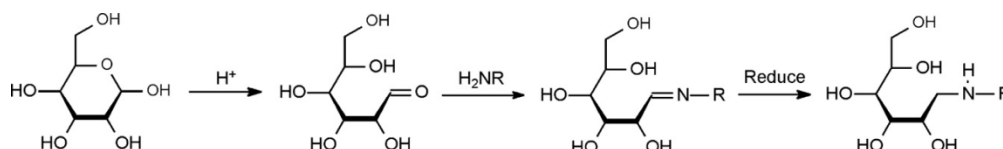


Application Note: 121-GL

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

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.

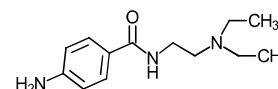


Many amines have been applied for labeling glycans (Harvey, 2011, *J. Chromatogr. B*, **879**, 1196-1225). In this application brief, procainamide was chosen because of reported improvements in ESI-MS detection (Klapoetke, et. al., 2010, *J. Pharm. Biomed. Anal.*, **53**, 315-324)

Typical Labeling Conditions

- Glycan in water (up to 10% volume)
- 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

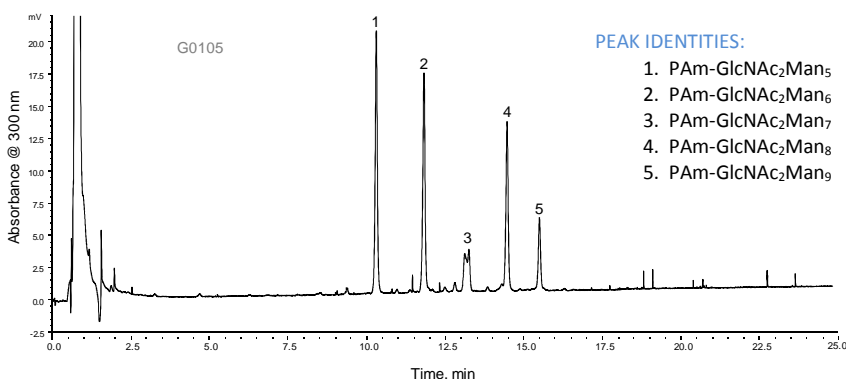


Procainamide (PAm)

TEST CONDITIONS:

Column: 2.1 x 150 mm, HALO 2.7 µm Glycan
 Part Number: 92922-705
 MP A: 50 mM Ammonium Formate, pH 4.45
 MP B: Acetonitrile
 Gradient: 80–55% B in 25 min
 Flow Rate: 0.6 mL/min.
 Temperature: 60°C
 Pressure: 190 bar
 Detection: UV 300 nm
 Injection Volume: 3 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

Ribonuclease B N-Glycans



PEAK IDENTITIES:

- PAm-GlcNAc₂Man₅
- PAm-GlcNAc₂Man₆
- PAm-GlcNAc₂Man₇
- PAm-GlcNAc₂Man₈
- PAm-GlcNAc₂Man₉

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