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MASS SPECTROMETRY OF GLYCOPEPTIDES: OPTIMIZING DIFFICULT GLYCOFORM SEPARATIONS TO IMPROVE MASS SPECTRAL QUALITY

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OVERVIEW

Chromatographic separation of peptide glycoforms using <2 micron particles of C_{18} chemistries produces structurally relevant MS and MSMS spectra. Chromatographic resolution was enhanced by addition of TFA mobile phase modifier, low linear velocity and shallow gradient slope. Under optimal conditions, many glycoforms can be baseline separated, enhancing mass spectral information.

INTRODUCTION

Highest resolution of oligosaccharide species can be accomplished by capillary electrophoresis or anion-exchange chromatography. These analyses require isolating the oligosaccharides released from the glycoprotein, thus losing the site-specific information retained in a peptide map experiment. Chromatographic separations combined with electrospray mass spectrometric detection provide structural information on attached glycans with the peptide molecular weight. The fragmentation of glycopeptides reflect the glycan composition and branching pattern. Higher resolution peptide mapping will facilitate the interpretation such complicated MS/MS spectra, particularly for isobaric glycoforms.

Previous studies have shown that glycoforms can separate on reversed phase chromatoraphy, but their elution order and resolution are unpredictable.[2]

METHODS

CHROMATOGRAPHY

Pumping System: ACQUITY UPLC™ Solvent Delivery System
Operating Pressures from 5000– 13000 psi
ACQUITY UPLC™ Sample Manager
Dual-plate Autosampler

.Detection Systems: ACQUITY UPLC™ UV Detector
Wavelength 214 λ

Buffers:

"Low TFA" A = 0.02% Trifluoroacetic Acid in Water
B = 0.016% Trifluoroacetic Acid in Acetonitrile

"Formic" A = 0.1% Formic Acid in Water
B = 0.1% Formic Acid in Acetonitrile

Columns:
A BEH™ (Bridged-Ethyl-Hybrid) $C_{18,}$ (Waters) 1.7 mm particles, 120 Å pores, 2.1 x 100 mm
B BEH™ (Bridged-Ethyl-Hybrid) $C_{18,}$ (Waters) 1.7 mm particles, 120 Å pores, 2.1 x 50 mm

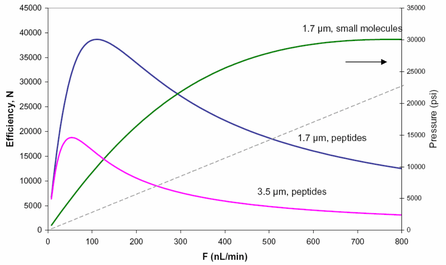


*Figure 1. Peptides and their larger glycopeptide counterparts are affected by slower diffusion compared to small molecules. When moving to 1.7 µm particle packing material one expects and increase in efficiency (**N**) with a con-*

Pure glycoproetien digests were prepared or obtained as reduced/alkylated proteins digested with modified porcine trypsin (Promega, Madison). Samples were diluted with aqueous 0.1% trifluoroacetic acid/2% acetonitrile to an appropriate concentration prior to analysis. Typically 15-30 pmol were loaded.

MASS SPECTROMETRY

Q-Tof *micro*™ Mass Spectrometer was operated in positive ion electrospray mode with a cone voltage between 30 and 35V. Under these conditions peptides did not fragment, but glycopeptides produced characteristic fragment ions from the oligosaccharide termini.[1]

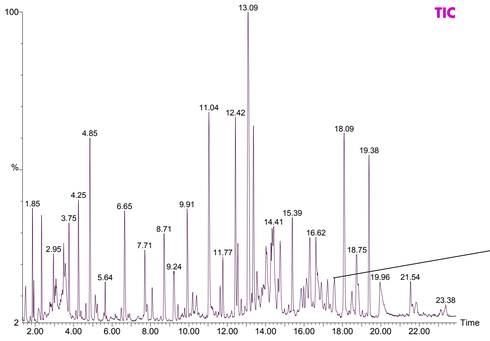
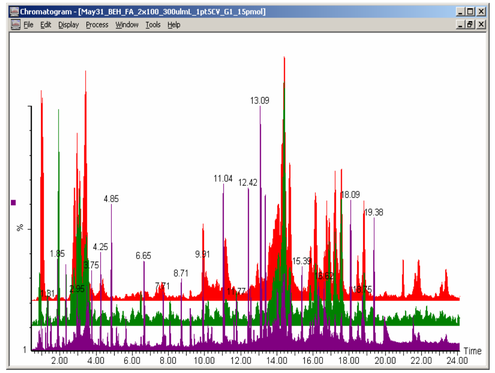


Figure 2. Total Ion Chromatogram (TIC) of a human a-1 acid glycoprotein digest (Michrom Bioresources, Auburn, CA) on an ACQUITY UPLC™ BEH (bridged-ethyl hybrid silica) C_{18} column, 2.1 x 100 mm, 1.7 µm packing.

RESULTS



Total Ion Chromatogram in purple
m/z 292 (N-acetyl neuraminic acid, NeuAc) in green
m/z 657 (Hex-HexNAc-NeuAc) in red.

Figure 3. Chromatography of a human a-1 acid glycoprotein digest (Michrom Bioresources, Auburn, CA) on an ACQUITY UPLC™ BEH (bridged-ethyl hybrid silica) C_{18} column, 2.1 x 100 mm, 1.7 µm packing.

Ultraperformance chromatography of complex glycoprotein digests have been analyzed by LCMS. Note that the peak widths for peptides are more narrow

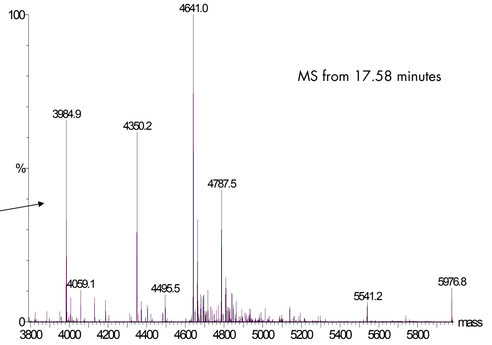


Figure 4. Molecular weight deconvoluted spectrum of glycopeptides from tryptic digest of human a-1 acid glycoprotein separated on a 1.7µm UPLC column (Figure 2.)

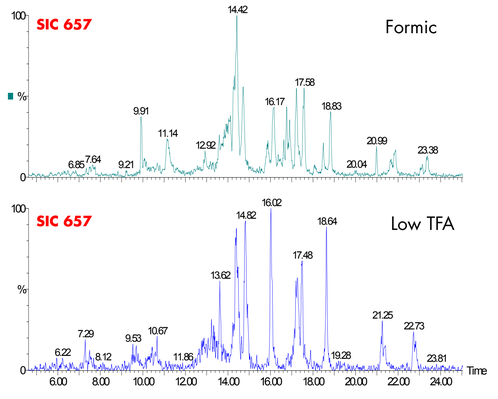


Figure 4. Selected Ion Chromatograms (SIC) m/z 657.3 of human a-1 acid glycoprotein. Column ACQUITY UPLC™ BEH (bridged-ethyl hybrid silica) C18, 2.1 x 100 mm with 1.7µm particle packing. Mobile phase modifier comparison. Note changes in selectivity, signal-to-noise ratio, an peak widths.

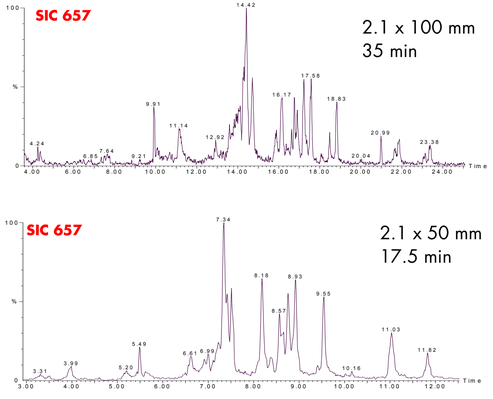
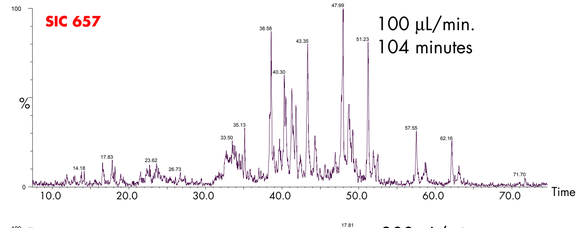


Figure 6. Selected Ion Chromatograms (SIC) m/z 657.3 of human a-1 acid glycoprotein. Column ACQUITY UPLC™ BEH (bridged-ethyl hybrid silica) C18, 2.1 x 100 mm or 2.1 x 50mm, a 1.7µm particle packing.



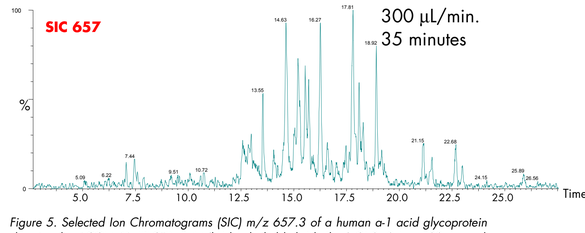


Figure 5. Selected Ion Chromatograms (SIC) m/z 657.3 of a human a-1 acid glycoprotein digest. The ACQUITY UPLC™ BEH (bridged-ethyl hybrid silica) C18, 2.1 x 100 mm column with 1.7µm particle packing was operated at backpressures of >13,000psi at 300 µL/min and > 4000psi at 100 µL/min with a restrictor in the flow path.

CONCLUSIONS

- The data presented shows the feasibility of peptide mapping using 1.7 µm particle packing material for the resolution of glycopeptides.
- The selectivity and resolution of the glycopeptide separation was dependent on the mobile phase modifier. TFA enhanced resolution and changed selectivity.
- Resolution was enhanced by lowering the flow rate and gradient slope

REFERENCES

1. Huddleston, M.J., Bean, M.F., and Carr, S.A. (1993) Anal. Chem **65**, 877-884
2. Medzihradszky, K.F., Maltby, D.A., Hall, S.C., Settineri, C.A., and Burlingame, A.L. (1994) J. Amer. Soc. Mass Spectrom. **5**, 350-358

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