POSTER NO 1034-A

Mass Spectrometry-based Rapid Monitoring for Sialic Acid Content in N-Glycans Released from Glycoproteins

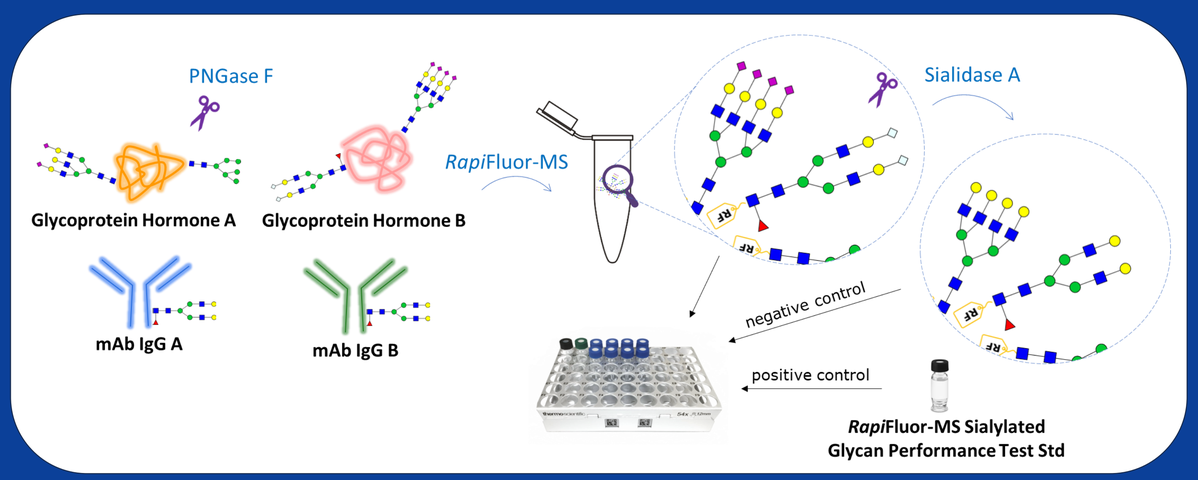
Beste Ozgumus Nitride[1], Antonio Datola[2], Maura Melchiorre[2], Patrizia Simone[2], Angela Amoresano[1], Paolo Felici[2]

[1] Department of Chemical Sciences, University of Naples Federico II, Via Cintia, 80126 Naples, Italy

[2] Merck Healthcare Business, Merck Serono S.p.A. Rome, Italy, an affiliate of Merck KGaA, Darmstadt, Germany

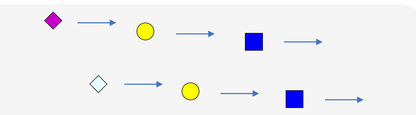
OVERVIEW

- **N-Glycosylation of therapeutic proteins is a Critical Quality Attribute (CQA). Sialylation of N-Glycans can affect circulatory half-life, bioactivity and safety.**
- **Automated rapid monitoring workflow has been developed for sialic acid content of released N-glycans from glycoproteins using mass spectrometry.**
- **Method is based on extracting signiture fragments specific to sialylated fragments.**

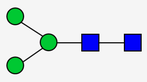


INTRODUCTION

- Increasing number of new biological entities and new manufacturing processes necessitate rapid workflows.
- N-glycans share a common core with differing number and type of capping structures. Sialic acids are added in a sequential manner as can be seen below:



Sequential addition of monosaccharides during biosynthesis of sialylated N-glycans



N-glycan core

- They are present as a mixture of similar and even isobaric structures with different bioactivity.
- LC-MS/MS workflows of released N-Glycans presumably provide the most detailed N-glycan analysis however, method development may be lengthy and may require an expert operator.
- Here, we have developed an automated rapid released N-Glycan workflow for monitoring sialylated N-glycans which can be used easily by expert/inexpert operators.

METHODS

40 min

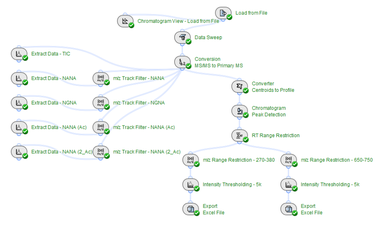
N-Glycans are enzymatically released from 2 glycoprotein hormones, 2 IgG antibodies, labelled with *Rapi*Fluor-MS™ using Andrew+ Pipetting Robot. Waters Sialylated N-Glycan Standard is used as positive control. Samples incubated with Sialidase A served as negative controls.

The samples are analyzed with LC-MS/MS method using Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer. Multiply charged precursors are fragmented continuously at previously optimized HCD energies. Acquired data can later be used for routine released N-Glycan workflows.

20 - 80 min

1 min

Rapid monitoring workflow developed on Genedata Expressionist® version 13.5 converting MS/MS signals to MS and filtering signature B ions within 15 ppm range including 3 different isotopes.



RESULTS

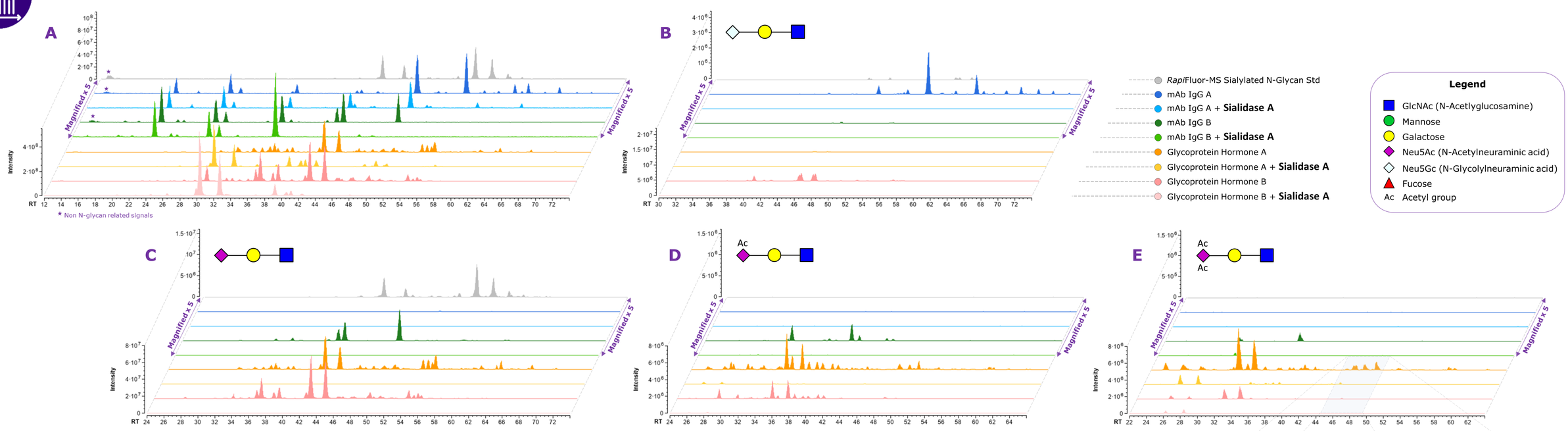
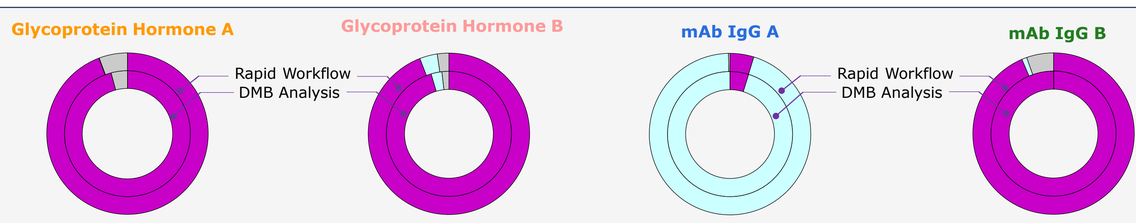


Figure 1. Extracted MS/MS ion chromatograms (EIC) of released N-glycans converted to MS signals (A) Total Ion Chromatogram (TIC) of MS/MS ions (B) EIC of B ions of Neu5Gc containing antenna (C) EIC of B ions of Neu5Ac containing antenna (D) EIC of B ions of O-acetylated Neu5Ac containing antenna (E) EIC of B ions of double O-acetylated Neu5Ac containing antenna (F) Zoomed section of EIC of B ions of double O-acetylated Neu5Ac containing antenna

- When sialic acids are monitored, peaks can be observed only in specific regions of chromatograms in clear contrast with TIC's.
- Signals disappear in samples treated with Sialidase A as expected, with exception of O-acetylated sialic acids. O-Acetyl groups on sialic acids can impede and even completely inhibit activity of the enzyme.
- High frequencies between acquisition cycles enable appearance of gaussian like peaks on EIC's even with trace amount of analytes.



- DMB analysis are provided as a means of comparison of our MS/MS based rapid workflow with an orthogonal method for sialic acids based on UPLC.
- Although rapid workflow is not quantitative still, agrees well with DMB analysis in terms of sialic acid ratios.

Figure 2. Ring charts of percent ratio of sialylated N-glycans. Outer rings are percent ratio of summed intensity of B ions normalized to all ions within ranges 270-380 m/z and 650-750 m/z. Inner ring are percent ratio of number of mols of sialic acids per mols of glycoprotein whose results are obtained by 1,2-diamino-4,5-methylenedioxybenzene dihydrochloride (DMB) labeled released sialic acid analysis performed previously by our laboratory.

CONCLUSIONS

Workflow is

- **Rapid and adaptive**
  - ✓ all process can be completed within a day
  - ✓ It can be used on different type of glycoproteins
- **Designed for inexpert operator**
  - ✓ does not require method development, preprocessing or previous knowledge of N-glycan structures
- **Provides maximum traceability**
  - ✓ automated sample preparation, online data interpretation
- **Highly sensitive**
  - ✓ species are detected under LOD of orthogonal methods
- **Easy to transfer to other structures**
  - ✓ based on signiture fragments which can be extended to other structures

Our experience showed that apart from presence/absence of certain ions, relative intensities can also be quite informative. A data interpretation platform that is able to produce EIC's based on signal ratios would be a valuable tool towards development of rapid N-glycan monitoring workflows.

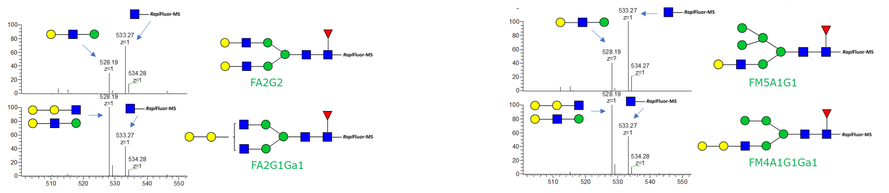


Figure 3. Sections of MS/MS spectra of *Rapi*Fluor-MS labeled released N-glycans

REFERENCES

[1] INTERNATIONAL CONFERENCE ON HARMONISATION OF TECHNICAL REQUIREMENTS FOR REGISTRATION OF PHARMACEUTICALS FOR HUMAN USE PHARMACEUTICAL DEVELOPMENT Q8(R2). https://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Quality/Q8_R1/Step4/Q8_R2_Guideline.pdf (2009).
[2] European Medicine Agency. Committee for Human Medicinal Products ICH guideline Q9 on quality risk management. 44, (2015).
[3] Mimura Y, Katoh T, Saldova R, O'Flaherty R, Izumi T, Mimura-Kimura Y, Utsunomiya T, Mizukami Y, Yamamoto K, Matsumoto T, Rudd PM. Glycosylation engineering of therapeutic IgG antibodies: challenges for the safety, functionality and efficacy. Protein Cell. 2018 Jan;9(1):47-62. doi: 10.1007/s13238-017-0433-3.
[4] Higel F, Sandl T, Kao CY, Pechinger N, Sörgel F, Friess W, Wolschin F, Seidl A. N-glycans of complex glycosylated biopharmaceuticals and their impact on protein clearance. Eur J Pharm Biopharm. 2019 Jun;139:123-131. doi: 10.1016/j.ejpb.2019.03.018.
[5] Ghaderi D, Taylor RE, Padler-Karavani V, Diaz S, Varki A. Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. Nat Biotechnol. 2010 Aug;28(8):863-7. doi: 10.1038/nbt.1651.
[6] Veillon L, Huang Y, Peng W, Dong X, Cho BG, Mechref Y. Characterization of isomeric glycan structures by LC-MS/MS. Electrophoresis. 2017 Sep;38(17):2100-2114. doi: 10.1002/elps.201700042.

Presented at the SLAS Europe 2024| 28.05.2024| Barcelona, Spain