N-glycan analysis: combining the power of a novel glycan label and customized scientific library for confident glycan assignment

○Reiko Iizuka[1], Kenji Hirose[1], Mark Hilliard[2], Niaobh McLoughlin[2], Ying Qing Yu[3], Pauline Rudd[2]
[1]Nihon Waters K.K., [2]NIBRT, [3]Waters Corporation

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

nibrt
National Institute for Bioprocessing Research and Training

INTRODUCTION

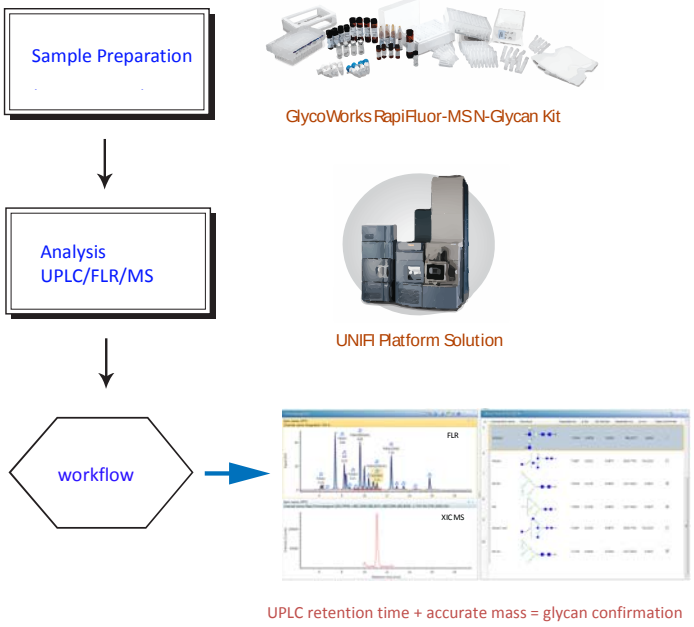
A new glycan fluorescent label, *Rapi*Fluor-MS™, is used to label N-linked glycans. This innovative label improves FLR and MS signals for glycan characterization and profiling analysis.

Waters and NIBRT are co-developing a new scientific library for *Rapi*Fluor-MS labeled N-glycans that identifies glycans based on HILIC-UPLC retention time (in Glucose Unit, GU) and accurate mass information (Ref.1).

• Each glycan contained in the scientific library is fully characterized structurally using a combination of exoglycosidase array and MS analysis. The "unknown" glycan is confirmed by matching its retention time (in GU value) and its accurate mass with the experimental data composites inside the scientific library. Glycan assignment is based on the best matched GU value and exact mass.

• NIST mAb reference standard (candidate NIST RM 8670 mAb lot #3F1b) is being used as a proof of concept sample to kick start this new scientific library development.

METHODS



RESULTS

Figure 1

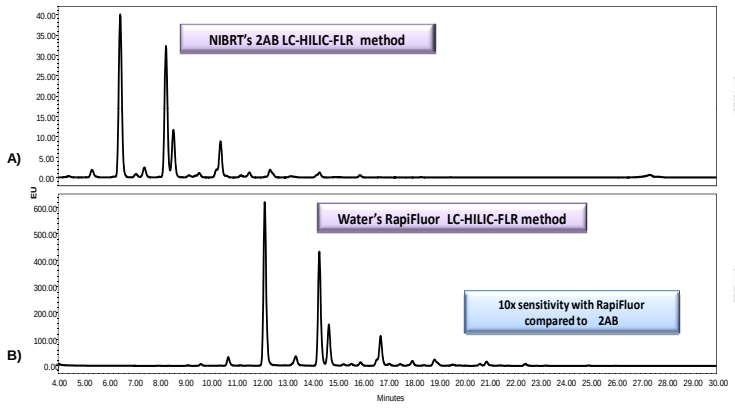


Figure 1) Comparison of N-glycans from NIST mAb via UPLC-HILIC-FLR analysis A) 2AB labeled with the NIBRT method (Ref.2) B) RapiFluor-MS labeled sample using GlycoWork sample preparation kit. We observed x10 improvement in FLR sensitivity for the RapiFluor-MS labeled glycans.

Figure 2

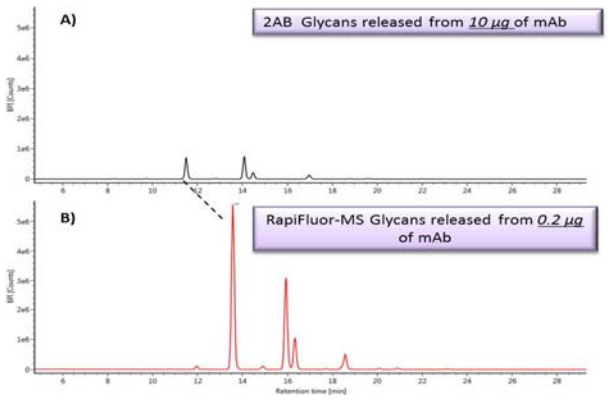


Figure 2) BPI MS chromatogram of A) 2AB-labeled N-glycans B) RapiFluor-MS labeled N-glycans from NIST mAb reference standard. Greater than x100 improvement in signal is observed for RapiFluor-MS labeled glycans.

Figure 3

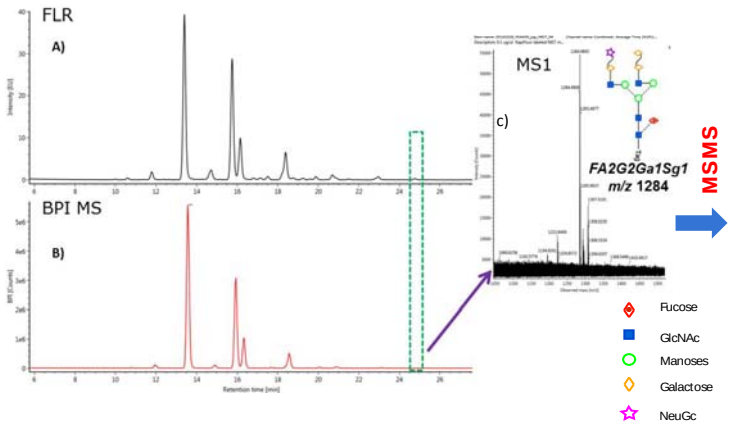


Figure 3) Characterization of low abundant RapiFluor-MS labeled glycans: UPLC-FLR-MS analysis of N-glycans from NIST mAb. A) FLR chromatogram and B) BPI MS chromatogram. C) Spectrum insert shows good S/N for a low abundant glycan, FA2G2Ga1Sg1.

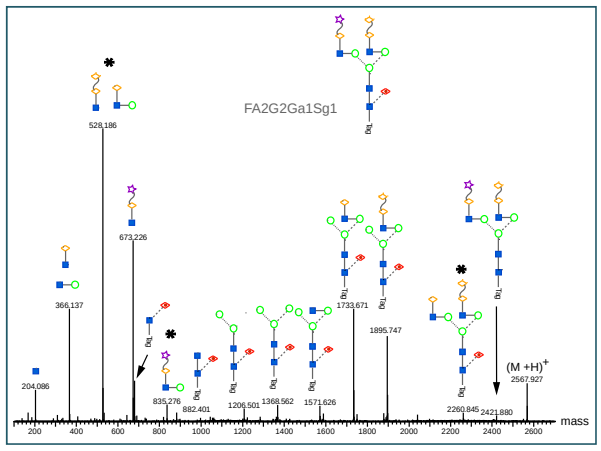


Figure 4) Collision induced fragmentation of the ion (from Fig.3c) shows that the fragmentation mechanism of RapiFluor-MS labeled glycans is very similar to those of 2AB-labeled glycans in that glycosidic bond cleavage is observed as the predominant fragmentation pathways. Structurally informative fragments (with asterisks) are observed for this low abundant ion (< 0.1% relative abundance). This fragmentation data suggests that this glycan contains alpha-gal and NeuGC which are potentially immunogenic glycan epitopes.

Figure 5 Dextran Ladder with RapiFluor-MS label

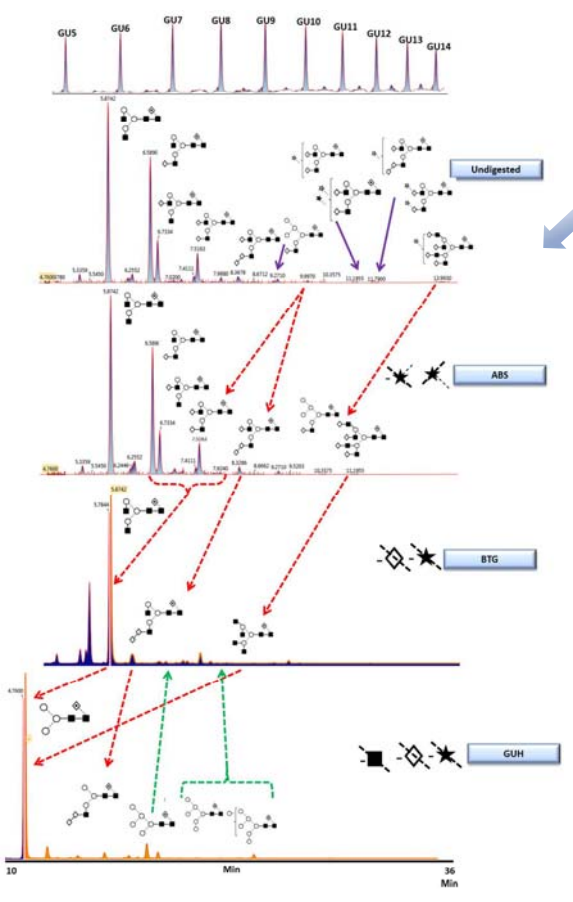


Figure 5) Exoglycosidase digestion array of RapiFluor-MS labeled NIST mAb. The exoglycosidase array is used to fully characterize the glycans to provide information on the composition, linkage and branching of the glycans present. The experimental retention time (in GU) from the exoglycosidase array and the accurate mass for each glycan are the key glycan attributes to be included in the scientific library.

Figure 6

Figure 6) Generation of the scientific library using RapiFluor-MS labeling chemistry. The key glycan features such as structure, MW (with or without the label), and the experimental GU value associated with each glycan are shown in the screen capture above. The GU value and the mass or LC/MS/MS are used to assign and confirm the structure of an unknown glycan.

CONCLUSIONS

•*Rapi*Fluor-MS™ labeling chemistry enhances both FLR and MS signals for N-Glycan analysis: 10x for FLR, and 100x for MS, compared to the 2AB label.

•Waters and NIBRT are developing a new scientific library specifically for *Rapi*Fluor-MS™ labeled glycans. This new library will be used for automated glycan assignment based on the HILIC UPLC retention time (in GU) and accurate mass measurements. We will work on generating GU values for RapiFluor tagged N-glycans from variety of therapeutic proteins.

References:

1.Waters Application note: 720004845en. Y.Q. Yu, "N-linked Glycan Characterization and Profiling: Combining the Power of Accurate Mass, Reference Glucose Units, and UNIFI Software for Confident Glycan Assignments".

2. Royle, L; Radcliffe, C. M.; Dwek, R. A.; Rudd, P. M. Methods Mol. Biol. 2006, 347, 125–43.