Application Note

Integrated Rapifluor-MS Labeling and Bio-MAGHILIC Purification for High-Sensitivity N-Glycan Analysis: NIST mAb N-glycan Profiling

Introduction

The structural characterization of N-linked glycans from monoclonal antibodies (mAbs) is a critical step in biopharmaceutical development and quality control. Conventional workflows often involve multiple purification steps and lengthy protocols. In this application note, we demonstrate a streamlined approach for **N-glycan isolation, labeling, and purification** using NIST monoclonal antibody (NIST mAb) as a model. The workflow incorporates **Rapifluor-MS labeling** for enhanced sensitivity in fluorescence and mass spectrometry detection, and **Bio-MAGHILIC magnetic particles**, which enable rapid, reproducible, and cost-effective glycan purification without centrifugation or vacuum systems.

Materials and Methods

1. Sample Preparation

- 4 µL NIST mAb stock solution (10 µg/µL; 40 µg total)
- Dilute with 6 µL deionized water to a final volume of 10 µL.

2. Denaturation

- Prepare 3% (w/v) RapiGest: dissolve 3 mg RapiGest in 60 µL 5X PBS + 40 µL MQ water.
- Add 10 µL of RapiGest solution to the sample.
- Vortex and incubate at 90 °C for 5 minutes.

3. N-Glycan Release

- Dilute PNGase F (Promega) to 0.1 U/µL in 1X PBS.
- Add 10 µL PNGase F solution to the denatured sample.
- Incubate overnight at 37 °C (~16 h).

4. Glycan Labeling

- Dissolve 9 mg Rapifluor-MS in 110 µL DMF.
- Add 10 µL Rapifluor-MS solution to the released glycan sample.

- Incubate for 20 minutes at room temperature.

5. Glycan Purification with Bio-MAGHILIC

- Add 360 µL acetonitrile (ACN) to each sample.
- Prepare 30 µL Bio-MAGHILIC particles (20 mg/mL).
- Wash particles sequentially:
  - 2× with 200 µL MQ water,
  - 2× with 200 µL 10% water / 90% ACN.
- Load the sample onto particles and incubate with vortex mixing for 20 minutes (600 rpm, RT).
- Wash particles at least 4× with 200 µL 10:90 water/ACN (v/v).
- Elute glycans with 35 µL pure water.
- Incubate 5 minutes at RT (600 rpm).

6. Preparation for LC-HILIC-FLD-MS Analysis

- Dilute 25 µL of eluate with 75 µL ACN.
- Inject 15–25 µL into LC-MS system for analysis.

Results and Discussion

The integration of **Rapifluor-MS labeling** with **Bio-MAGHILIC magnetic HILIC purification** provides:

- Strong fluorescence (FLD) and MS signals with excellent reproducibility.
- Minimal sample loss due to magnetic handling, avoiding centrifugation/vacuum.
- Rapid workflow (~1 day total including overnight digestion).
- Compatibility with **HILIC-FLD-MS/MS** platform.

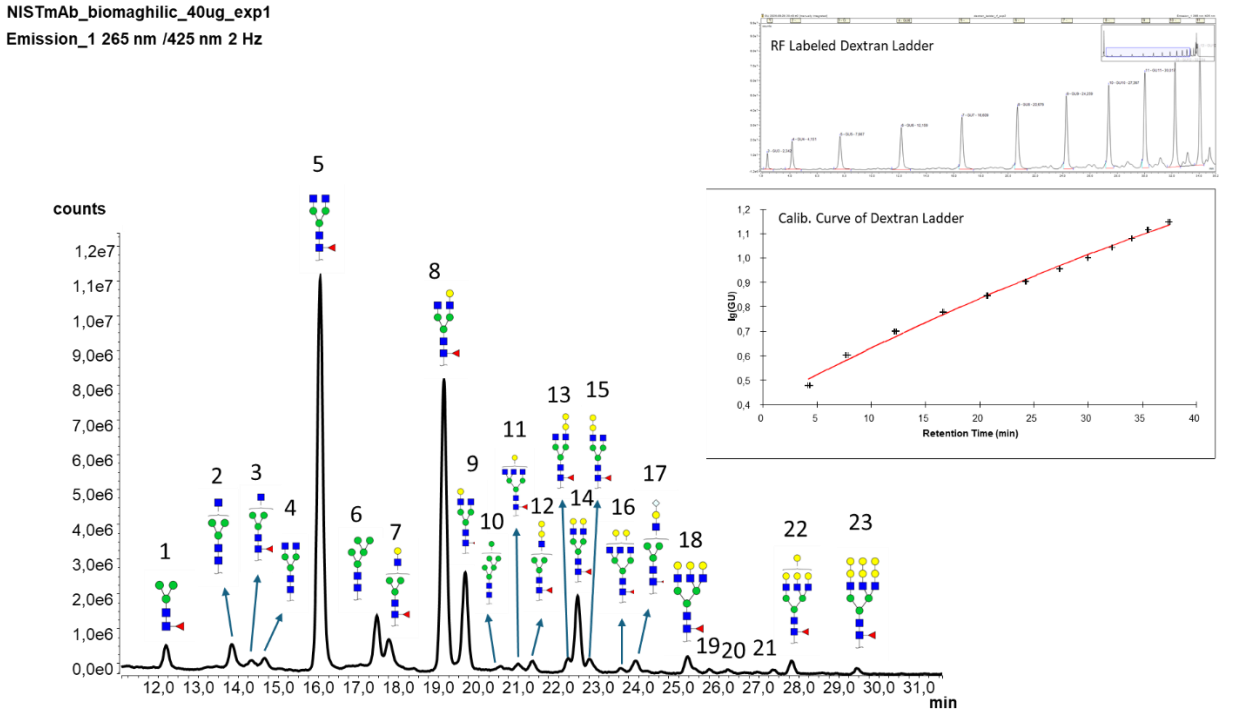


Figure 1. Glycan profile of N-glycans released from the analyzed glycoprotein following Bio-MAGHILIC purification. Individual glycan structures were assigned based on their retention times and confirmed by glucose unit (GU) values using an RF-labeled dextran ladder (upper panel). The calibration curve of the dextran ladder (lower panel) demonstrates a strong linear relationship between retention time and log(GU), ensuring accurate peak annotation.

Figure 1 presents representative chromatograms of N-glycans purified using the Bio-MAGHILIC method. The results clearly demonstrate highly resolved glycan peaks with enhanced signal-to-noise ratios when compared to conventional solid-phase extraction (SPE) approaches. The sharper peak shapes and reduced background noise observed highlight the efficiency of magnetic HILIC separation in preserving glycan integrity and improving analytical sensitivity. Furthermore, the consistency of peak intensities across replicates underlines the robustness and reproducibility of the method, which is essential for reliable glycan quantification. Taken together, these findings validate the suitability of Bio-MAGHILIC for biopharmaceutical glycan profiling, offering a practical and scalable solution for routine quality control (QC) applications

in both academic and industrial laboratories.

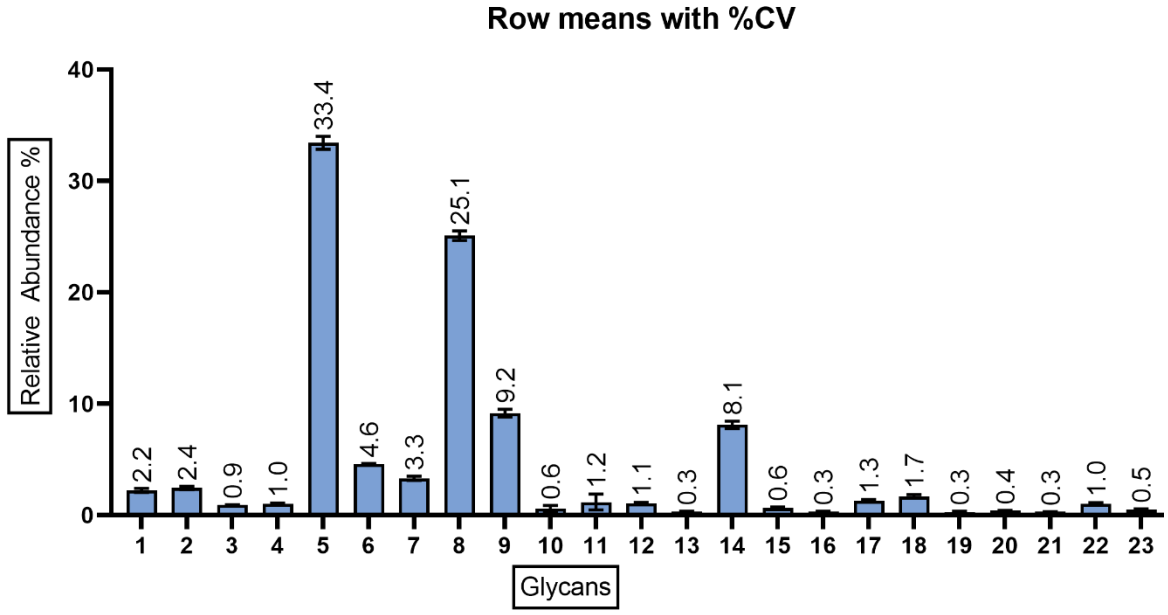


Figure 2. Glycan profile obtained using Bio-MAGHILIC purification. Major glycan peaks show strong reproducibility across replicates, with minimal variation in peak abundances, demonstrating the method's reliability for consistent glycan analysis.

Figure 2 illustrates the reproducibility of Bio-MAGHILIC purification, highlighting its fast, reliable, and cost-effective performance in the isolation of glycans from glycoproteins and monoclonal antibodies. The bar graph demonstrates consistent peak intensities across replicates, confirming the method's high specificity and reproducibility. By leveraging magnetic hydrophilic interaction chromatography (HILIC) technology, Bio-MAGHILIC eliminates the need for centrifugation or vacuum systems, instead enabling simple and efficient magnetic separation. This streamlined approach not only reduces dependence on expensive consumables but also ensures compatibility with both fluorescence (FLD) and mass spectrometry (MS) detection. Collectively, the results validate Bio-MAGHILIC as a robust and scalable tool for glycan analysis in both research and biopharmaceutical quality control applications.

Conclusion

This application note describes an efficient, reproducible, and cost-effective workflow for **N-glycan isolation and labeling from monoclonal antibodies**. By combining **Rapifluor-MS labeling** with **Bio-MAGHILIC purification**, researchers and QC laboratories can achieve high sensitivity and throughput in glycomics analysis. The protocol is readily scalable for both research and industrial applications.

Key Advantages

- Magnetic handling: **no centrifugation or vacuum** required.
- High sensitivity: **Rapifluor-MS** ensures strong FLD/MS signals.
- Scalability: suitable for both **academic and industrial QC workflows**.
- Cost-effectiveness: reduces reliance on expensive consumables.