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Application News

# An Easy and Fast Approach for Monoclonal Antibody N-linked Glycan Analysis from Sample Preparation to Data Analysis

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## User Benefits

- A simple and straightforward sample preparation workflow for N-glycan analysis
- Less time-consuming for sample preparation
- Improved peak resolution for better quantification

## ■ Introduction

Therapeutic monoclonal antibodies (mAbs) and their derivatives are emerging as one of the fastest-growing categories of biologic drugs with a wide range of applications. N-linked glycosylation plays a critical role in many biological processes, and may affect the therapeutics' bioactivity, stability and immunogenicity. To maintain consistent glycosylation profiles of mAbs during manufacturing, effective glycan characterization are required, usually relying on techniques such as fluorescence-tagging coupled with HPLC analysis. The traditional method of N-glycan analysis is time-consuming, and involves multiple steps, starting with releasing of glycan, followed by glycan purification, labeling with a fluorescence tag (e.g., 2-aminobenzamide, 2-AB), and finally cleanup of labeled glycans prior to LC analysis. This study demonstrates a simplified workflow using the S-Bio EZGlyco™ mAb-N kit, potentially reducing sample preparation time for N-glycan characterization. The solution incorporates the Shimadzu Nexera-i MT system and highly sensitive fluorescence detector (RF-20A).

## ■ Experimental

### mAb Sample:

The bevacizumab biosimilar was used in this study. It was diluted with Milli-Q water to 1 mg/mL prior to sample preparation using S-Bio EZGlyco™ mAb-N kit.

### Fast Sample Preparation (< 3h):

S-Bio EZGlyco™ mAb-N kit provides all the necessary reagents required for sample preparation except certain common reagents such as acetic acid, acetonitrile, and dimethylsulfoxide (DMSO). The kit composed of three components that perform the steps of N-glycan release, labeling with 2-AB, and labeled N-glycan cleanup. A detailed procedure for sample preparation is described in the kit instruction manual (S-Bio Cat. No. BS-X4410Z). After the final cleanup step, the eluted labeled N-glycans were diluted with equal volume acetonitrile for analysis by HPLC.

### LC-Fluorescence Detection:

The sample analyses were conducted on a Shimadzu Nexera-i MT system equipped with a highly sensitive fluorescence detector, RF-20A. Table 1 lists the LC-fluorescence conditions used in detail. Data were analyzed using LabSolutions software.

### Glycan Characterization:

Identity of glycan peaks were confirmed using Shimadzu LCMS-9030 (Q-TOF) similar to that reported in our previous application news, AD-0191.

**Table 1. LC/Fluorescence conditions**

| | |
|---|---|
| **LC conditions** | |
| LC system: | Shimadzu Nexera-i MT |
| Column: | Shim-pack GIST-HP Amide 1.9 µm, 150 × 2.1 mm |
| Column Temp.: | 50 °C |
| Flow rate: | 0.4 mL/min |
| Mobile phase A: | 50 mM Ammonium formate |
| Mobile phase B: | Acetonitrile |
| Gradient program: | 0 min, 78% B, 27.8 min, 70% B, 28.5 min, 20% B, 33.5 min, 20% B, 34.5 min, 78% B |
| Injection volume: | 5 µL |
| **Fluorescence conditions** | |
| Fluorescence detector: | Shimadzu RF-20A |
| Excitation: | 330 nm |
| Emission: | 420 nm |

## ■ Results and Discussion

LC-fluorescence analysis of released, 2-AB labeled N-glycans is one of the most popular approach to determining mAb glycosylation. We have previously published an application news AD-0191, showing an optimized separation of N-glycan profiles from bevacizumab biosimilar, including Man3, G0F-2GN, G0-GN, G0F-GN, G0, Man5, G0F, G1Fa and G1Fb (Figure 1). The separation method used in this study represents the best overall performance with maximum peak resolution (Figure 2), in which the isomers G1Fa and G1Fb were well separated. This increased resolution enables accurate quantitation of target glycans and any changes to the mAb glycosylation profile. All nine N-glycan peaks were detected in the chromatogram. Importantly, the relative abundance of these nine N-glycans was comparable between AD-0191 and this study (Figure 3). Additionally, injection-to- injection variability of the LC-fluorescence system was evaluated. Variations in peak area and retention time were less than 4% and 1% RSD respectively for all peaks (Table 2).

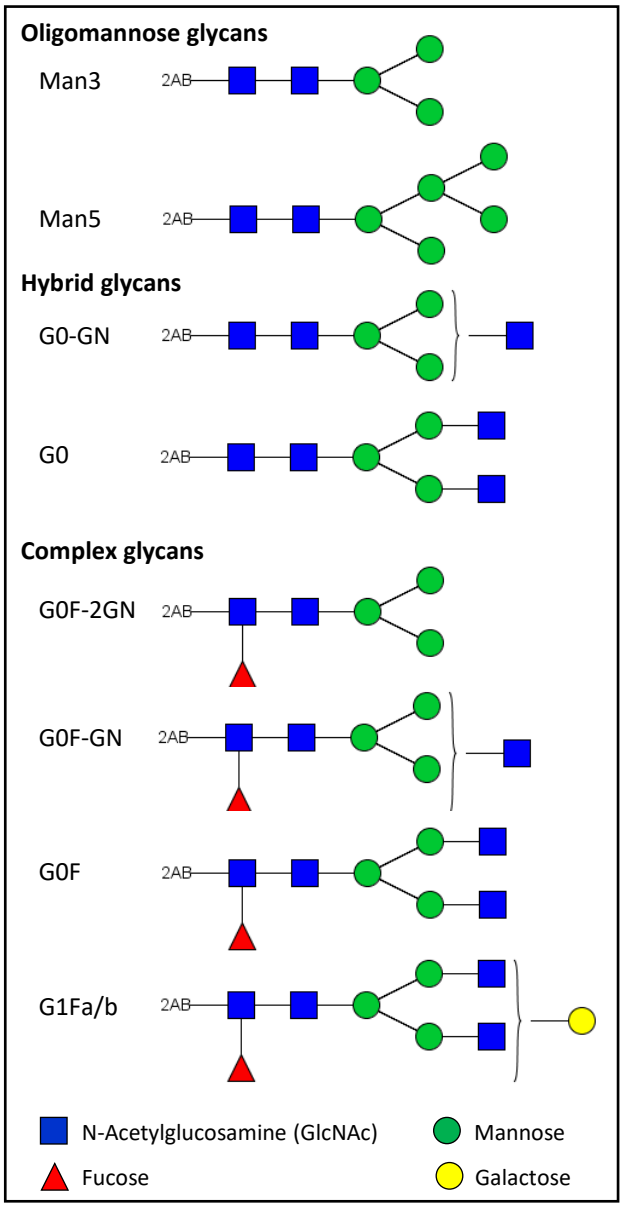


Figure 1. N-glycans from bevacizumab biosimilar.
GN = GlcNAc

## ■ Conclusion

This study demonstrated an easy and fast solution for monoclonal antibody N-linked glycan analysis, taking less than 3h in sample preparation. Meanwhile, this solution dramatically improves the peak resolutions, allowing more accurate quantitation for target glycans.

Table 2. Injection-to-injection repeatability peak area and retention time ($n$ = 6) of N-glycans

| Glycans | Peak area | RSD (%) | RT (min) | RSD (%) |
|---|---|---|---|---|
| Man3 | 0.07% | 3.69 | 10.569 | 0.60 |
| G0F-2GN | 0.31% | 1.27 | 13.483 | 0.70 |
| G0-GN | 0.39% | 2.23 | 14.468 | 0.71 |
| G0F-GN | 1.72% | 1.54 | 17.566 | 0.55 |
| G0 | 1.29% | 1.19 | 18.599 | 0.51 |
| G0F | 87.16% | 0.24 | 21.383 | 0.41 |
| Man5 | 2.05% | 0.29 | 23.076 | 0.34 |
| G1Fa | 4.51% | 0.27 | 26.412 | 0.27 |
| G1Fb | 2.50% | 0.64 | 27.098 | 0.27 |

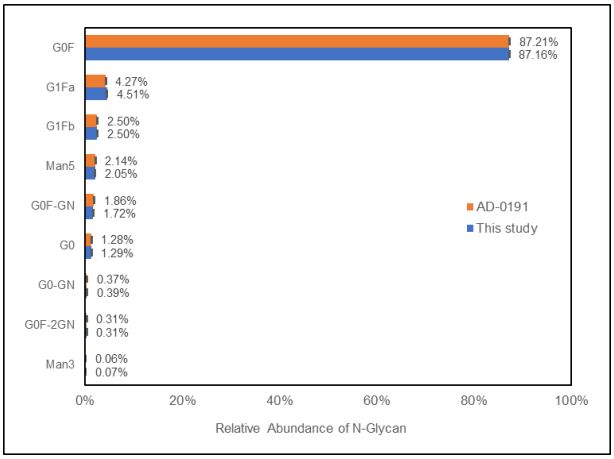


Figure 3. Relative abundance of nine N-glycans from bevacizumab biosimilar.

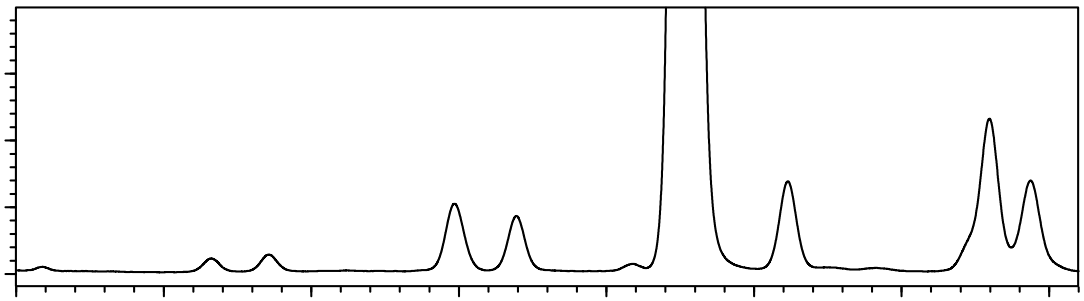

Figure 2. LC-fluorescence chromatogram of labeled N-glycans from bevacizumab biosimilar.



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