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# Application News

# No.L483

High Performance Liquid Chromatography

## High-Sensitivity Analysis of 2-AB Glycans by RF-20Axs Florescence Detector

Glycans, present in antibody-drug products have an effect on their safety and efficacy, therefore requiring that the types and quantities of the glycans present be investigated. Due to the culture conditions, the diversity and heterogeneity of the glycan structures cannot be avoided so their management must be implemented during the production process.
In Application News No. L452, the analysis of a pyridylamino (PA)-glycan using a fluorescence detector was introduced. Here, the analysis of a 2-aminobenzamide-labelled glycan (2-AB glycan) is introduced. As in Application News No. L452, the world's highest sensitivity fluorescence detector, the RF-20Axs, was used for detection.

### Analysis of Low Concentration Standard Solution

In this study, the fluorescent-labeled glycans that were used include 2-AB Man-5, 2-AB G2, and 2-AB G2FS1 (Prozyme). Their structures are shown in Fig. 1.
The analytical conditions that were used are shown in Table 1. The glycans were separated using hydrophilic interaction chromatography (HILIC). Fig. 2 shows the results of analysis of a 0.5 nmol/L standard solution using a 2 µL (1 fmol) injection. As can be confirmed from the obtained data, sufficient sensitivity is achieved even using an ultralow amount injection. The limits of detection (S/N=10) and quantitation (S/N=3.3), respectively, are shown in Table 2.

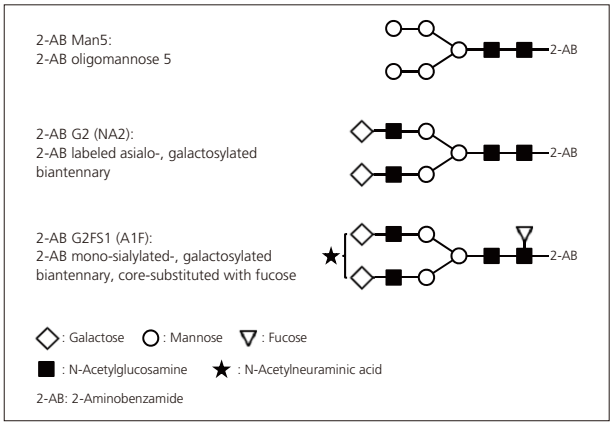


**Fig. 1 Structures of 2-AB Glycans Used in This Study**

**Table 1 Analytical Conditions**

| | |
|---|---|
| System | : Prominence |
| Column | : TSKgel Amide-80 (150 mm L. × 2.0 mm I.D., 3 µm) |
| Mobile Phase | : A: 50 mmol/L Ammonium formate pH 4.4<br>B: Acetonitrile |
| Time Program | : B.Conc. 73 % (0 min) → 60 % (48 min) → 0 % (49 - 53 min) → 73 % (54 - 80 min) |
| Flowrate | : 0.4 mL/min (0 - 48 min, 58.01 - 80 min)<br>0.2 mL/min (48.01 - 58 min) |
| Column Temp. | : 40 °C |
| Injection Vol. | : 2 µL |
| Detection | : Ex 330 nm, Em 420 nm |
| Flow Cell | : Conventional cell |

*Preparation of Mobile Phase A
After dissolving 3.15 g (50 mmol) ammonium formate in 1 L distilled water, about 340 µL formic acid was added to obtain a pH of 4.4.

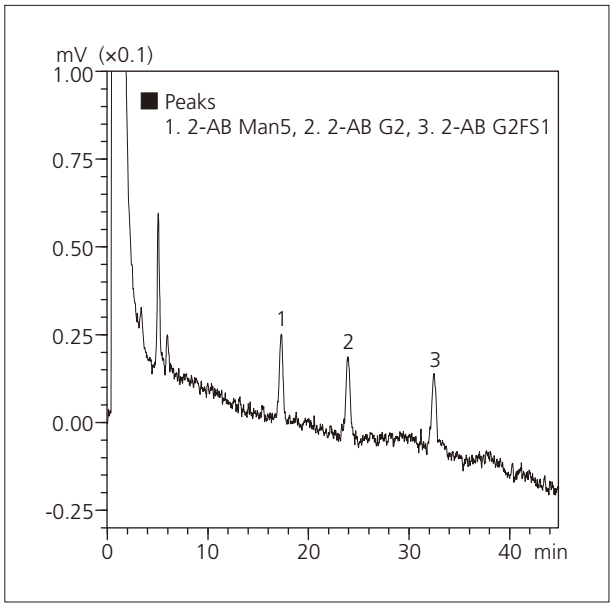


**Fig. 2 Chromatogram of 1 fmol Each of 2-AB-Labeled Glycans (0.5 nmol/L each, 2 µL injection)**

**Table 2 Limits of Detection and Quantitation**

| Glycan standard | LOD (fmol) | LOQ (fmol) |
|---|---|---|
| 2-AB Man5 | 0.44 | 1.33 |
| 2-AB G2 | 0.45 | 1.36 |
| 2-AB G2FS1 | 0.50 | 1.48 |

## Repeatability and Linearity

Fig. 3 shows the results of six repeat measurements of a 20 nmol/L standard solution, and Table 3 shows the respective retention time and peak area repeatability values obtained (n=6). As indicated by the results, good repeatability was obtained. Fig. 4 shows the results of linearity evaluation using standard solution concentrations from 1 nmol/L to 100 nmol/L. Excellent linearity was obtained with a coefficient of determination ($R^2$) value greater than 0.999.

## Analysis of 2-AB Glycan Mixture

We conducted analysis of 2-AB Human IgG N-Linked Glycan Library (Prozyme) as a mixed glycan sample. Fig. 5 shows the results of analysis in which 2 µL (160 fmol) of 80 nmol/L 2-AB Human IgG N-Linked Glycan Library was injected.

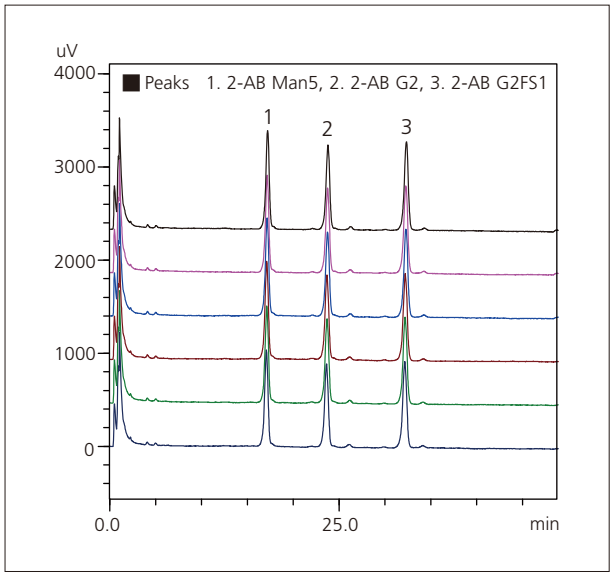


**Fig. 3 Chromatogram of 40 fmol Each of 2-AB-Labeled Glycans (20 nmol/L each, 2 µL injection)**

**Table 3 Repeatability**

| Glycan standard | R.T. %RSD | Area %RSD |
|---|---|---|
| 2-AB Man5 | 0.273 | 0.743 |
| 2-AB G2 | 0.245 | 0.684 |
| 2-AB G2FS1 | 0.196 | 0.589 |

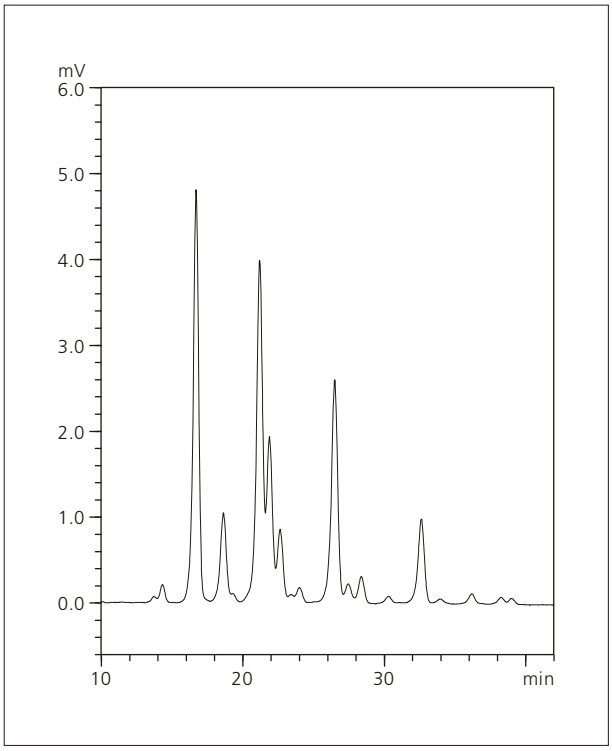


**Fig. 5 Chromatogram of 160 fmol of 2-AB Human IgG N-Linked Glycan Library (80 nmol/L, 2 µL injection)**

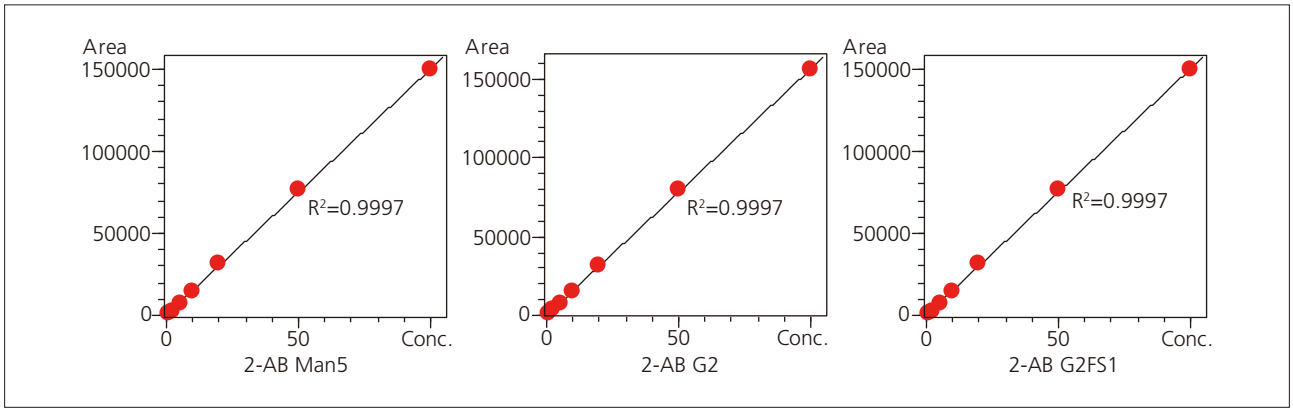


**Fig. 4 Linearity from 2 to 200 fmol (1 to 100 nmol/L, 2 µL injection)**

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