SHIMADZU
Excellence in Science

# Application News

# No.L452A

High Performance Liquid Chromatography

## High Sensitivity Profiling of Glycans in Antibody Drugs Using RF-20Axs

Glycans, or sugar chains, in antibody drugs play roles in the antigenicity, pharmacokinetics, and stability of higher-order structure, which could adversely affect their safety and effectiveness. Further, there is also concern about the non-uniformity of these glycans due to instability of antibody drug culture conditions, which has heightened the necessity to manage their production process. However, while there is currently no glycan test method specified in the Japanese Pharmacopoeia, there is wide demand for an assessment method.

Here, we introduce an example of analysis of glycans in antibody drugs using the Nexera X2 ultra high performance liquid chromatograph with the RF-20Axs high-sensitivity fluorescence detector. For the analysis, the Phenomenex core-shell, high-speed analytical Aeris™ PEPTIDE XB-C18 column was used. Since the permeability of the packing material is optimized for analysis of high-molecular compounds such as peptides, the column is useful for separation of glycans and impurities in antibody drugs.

### Sensitivity and Linearity of Detectors in PA-Glycan Analysis

The sensitivity and linearity of the RF-20Axs fluorescence detector was evaluated using a pyridylamino (PA)-glycan (PA-Sugar Chain 009, Takara Bio Inc.). Table 1 shows the analytical conditions.

Fig. 1 shows a comparison of the sensitivity obtained in analysis of a PA-glycan at 10 fmol (5 nmol/L, 2 µL injected) using the fluorescence detectors RF-20Axs and the previous model RF-10Axl connected in series. Excellent results were obtained with the RF-20Axs, with a good S/N ratio and low noise. Fig. 2 shows the calibration curve results obtained with the RF-20Axs fluorescence detector over a concentration range of 1 – 100 fmol (0.5 – 50 nmol/L, 2 µL injected).

There is significant improvement in performance compared to the previous model, and these results demonstrate that the RF-20Axs fluorescence detector is suitable for verification not only of the main peak, but of the trace level impurities as well.

**Table 1 Analytical Conditions**

| | |
|---|---|
| Instrument | : Nexera X2 |
| Column | : Shim-pack XR-ODS Ⅲ (50 mm L. × 2.0 mm I.D., 1.6 µm) |
| Mobile Phase* | : A) 20 mmol/L Ammonium Formate 0.0095% (v/v) Formic Acid-Water (pH 4.5) |
| | B) 20 mmol/L Ammonium Formate 0.0095% (v/v) Formic Acid-Methanol |
| | A/B=95/5 (v/v) |
| Flowrate | : 0.5 mL/min |
| Column Temp. | : 40 ˚C |
| Detection | : RF-20Axs (Ex = 320 nm, Em = 400 nm) |
| Injection Vol. | : 2 µL |

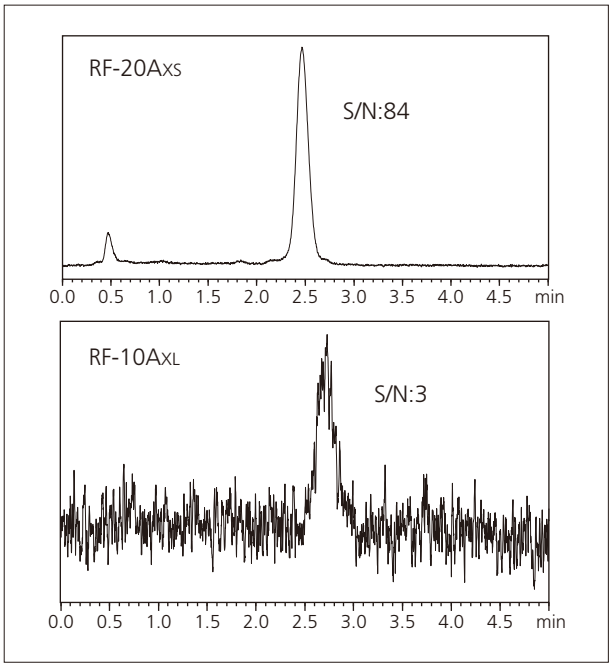


**Fig. 1 Chromatograms of 10 fmol PA-Glycan**

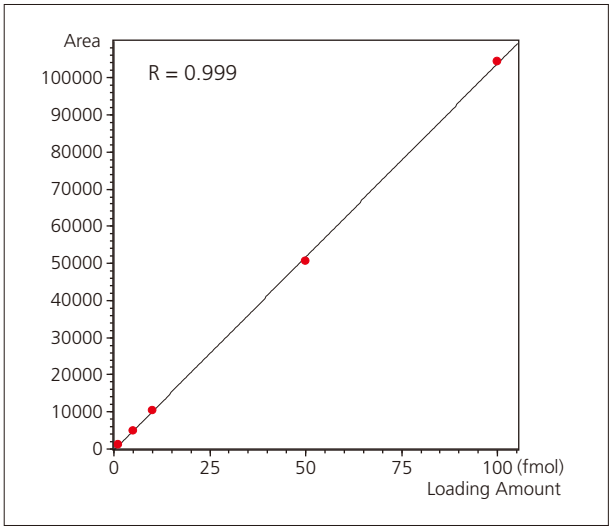


**Fig. 2 Calibration Curve (1 – 100 fmol Injected)**

*Mobile Phase Preparation
1.26 g (20 mmol) ammonium formate (M.W.: 63.026) was dissolved in 1 L of distilled water or methanol, and 95 µL of formic acid was added.

## Analysis of Glycans in Antibody Drugs

According to the pretreatment procedure of Fig. 3, the glycans were extracted from 2 types of antibody drugs, and following purification, were subjected to fluorescent derivatization by PA (pyridylamination).
Fig. 4 shows the chromatograms of PA-glycans from antibody drugs, and Table 2 shows the analytical conditions used. Comparing the peaks in the vicinity of 50 minutes for the drugs A and B, respectively, the quantity of glycans associated with that peak in antibody drug A is much greater than that in drug B. The peak response is quite different for many other peaks, which illustrates the formulation differences between drug manufacturers.

**Table 2 Analytical Conditions**

| | |
|---|---|
| Instrument | : Nexera X2 |
| Column | : Aeris™ PEPTIDE XB-C18 (150 mm L. × 2.1 mm I.D., 1.7 µm) |
| Mobile Phases | : A) 20 mmol/L Ammonium Formate 0.0095 % (v/v) Formic Acid-Water (pH 4.5) |
| | B) 20 mmol/L Ammonium Formate 0.0095 % (v/v) Formic Acid-Methanol |
| Time Program | : B Conc. 0 % (0 min) → 5 % (60 min) → 10 % (70 min) → 100 % (70.01 min → 80 min) → 0 % (80.01 min → 90 min) |
| Flowrate | : 0.4 mL/min |
| Column Temp. | : 40 ˚C |
| Detection | : RF-20Axs (Ex = 320 nm, Em = 400 nm) |
| Injection Vol. | : 3 µL |

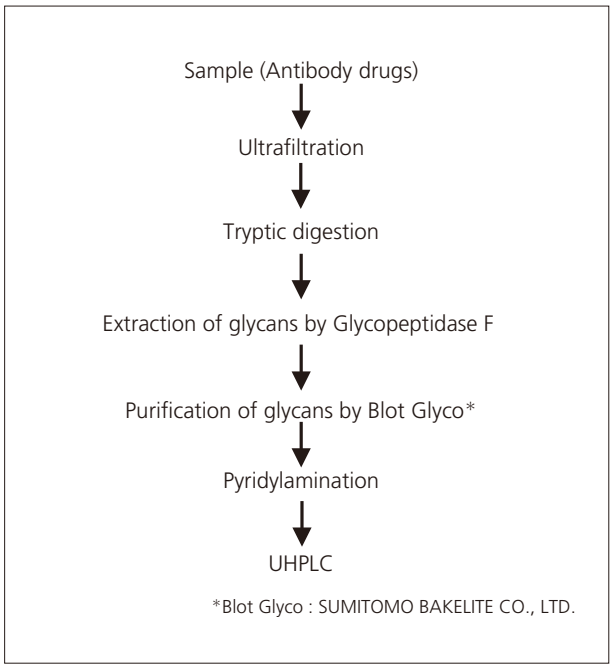


**Fig. 3 Sample Preparation**

Analysis of the glycans in the antibody drugs was conducted with the kind cooperation of Kenichiro Todoroki, Ph.D. of the Laboratory of Analytical and Bio-Analytical Chemistry, School of Pharmaceutical Sciences, University of Shizuoka.

For further information regarding the Aeris™ column, please contact
Shimadzu GLC Ltd.
TEL: +81-3-5835-0126, gsupport@glc.shimadzu.co.jp

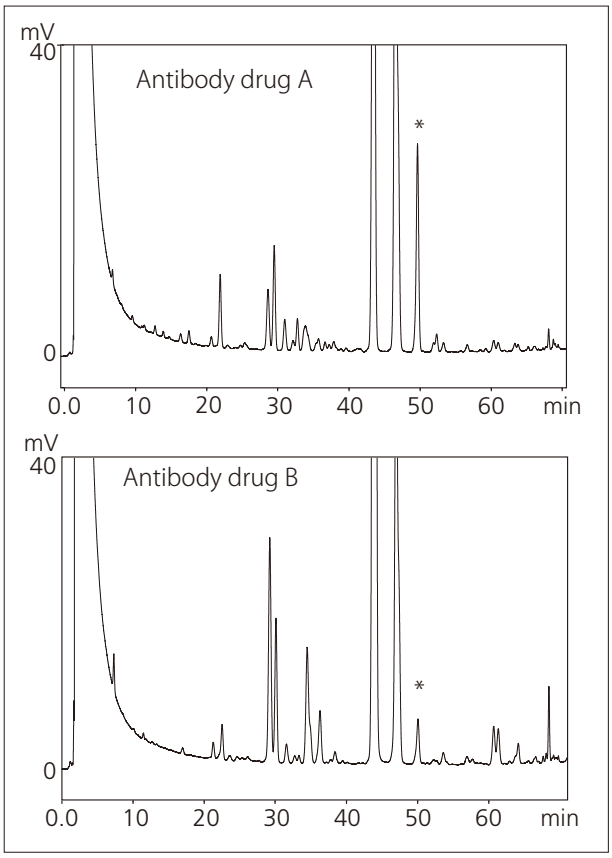


**Fig. 4 Chromatograms of PA-Glycans from Antibody Drugs**

First Edition: Jan. 2014

SHIMADZU

Shimadzu Corporation
www.shimadzu.com/an/

## Related Solutions

- Price Inquiry
- Product Inquiry
- Technical Service / Support Inquiry
- Other Inquiry