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Application News

Ultra High Performance Liquid Chromatograph Nexera™ XS inert

Analyses of Antibody Drugs Using Ultra High Performance Liquid Chromatography

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User Benefits

- Highly reproducible data can be acquired by suppressing the interaction between antibody drugs and stationary phase.
- Highly stable data can be acquired even when using a mobile phase containing high concentrations of corrosive salts.

■ Introduction

Biopharmaceuticals are being developed using biotechnology techniques such as genome editing and cell fusion. Recently, some of them have been spread worldwide due to be expected to be effective in treating various diseases including refractory diseases. Antibody drugs, such as monoclonal antibodies (mAbs) and antibody-drug conjugates (ADCs), are known to offer high therapeutic efficacy and reduced side effects due to their specificity and affinity for target molecules. However, because these biopharmaceuticals are manufactured using animal cells, the challenge is to ensure their structural homogeneity that is not encountered in small molecule pharmaceuticals manufactured by chemical synthesis. Accordingly, biopharmaceuticals require appropriate quality controls at every production step. For example, ICH-Q6B[(1),(2)], which proposes specifications and test procedures for biological products, stipulates that product-related impurities should be separated and/or determined the percentage levels in the manufacturing process. In most cases, these analyses are performed using liquid chromatography (Fig. 1).

This article introduces analyses of mAbs and ADCs for quality control using an inert UHPLC system "Nexera XS inert" that is extremely resistant to mobile phases containing high salt concentrations. The type of impurities and their amount are different depending on the type of antibody. Therefore, it is noteworthy that analytical conditions should be optimized for each sample. Thus, the optimization techniques will also be described in this article.

Table 1 shows the list of the TSKgel® column series (Tosoh Corporation), their purpose, chromatographic method for each analysis item. In this article, we determined to use an appropriate column for each analysis.

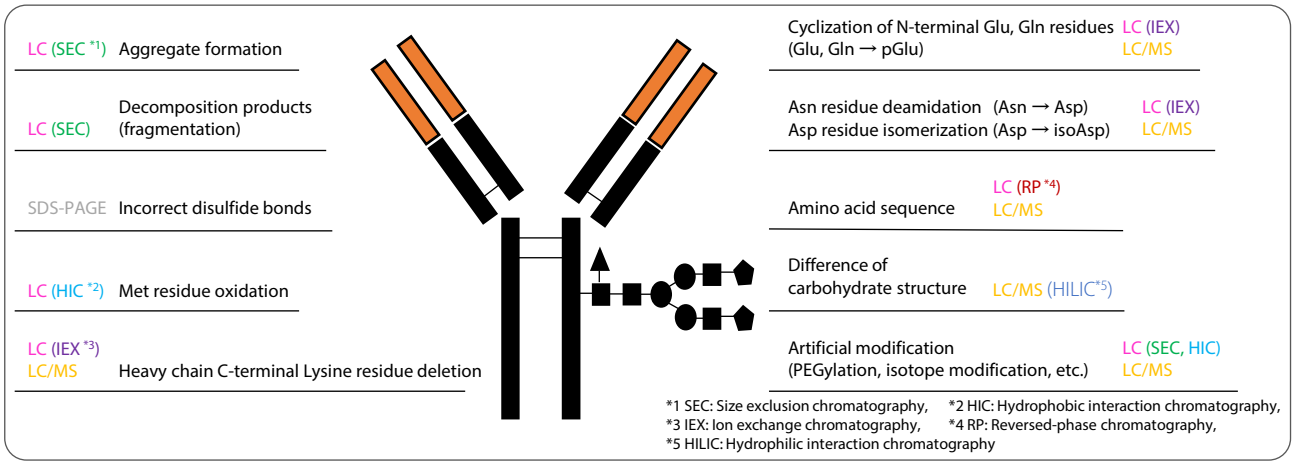

Fig. 1 Antibody Drug Impurities and Analysis Techniques

Table 1 Chromatographic Methods and Columns Used to Analyze Antibody Drug Impurities

Item	Purpose	Chromatographic Method	Column
Aggregate	Measurement of aggregates or degradates	Size-exclusion chromatography	TSKgel UP-SW3000 TSKgel UP-SW2000 TSKgel SuperSW mAb TSKgel UltraSW Aggregate TSKgel G3000 SWXL TSKgel G2000 SWXL TSKgel Super SW3000 TSKgel Super SW2000
Isomers	Determination of charge variants	Ion exchange chromatography	TSKgel CM-STAT® TSKgel SP-STAT® TSKgel Q-STAT®
Antibody-drug conjugates	Determination of isomers Calculation drug-to-antibody ratio	Hydrophobic interaction chromatography	TSKgel Butyl-NPR® TSKgel Phenyl-5PW
Structure	Evaluation of the specific characters of molecular structure or drug substance	Reversed-phase chromatography	TSKgel Protein C4-300
Sugar chain structures	Homogeneity evaluation of carbohydrate structures	Hydrophilic interaction chromatography	TSKgel Amide-80
Potency	Quantification of biological activity	Affinity chromatography	TSKgel ProteinA-5PW TSKgel Boronate-5PW TSKgel FcR-IIIA-NPR TSKgel FcR-IIIA-5PW

■ Impurities analysis of mAb and ADC by Size Exclusion Chromatography (SEC)

mAb and ADC impurities such as aggregates and degradates are important factors affecting drug efficacy, side effects, and drug stability[3] that are commonly determined by SEC. Aggregate analysis was performed with a mAb standard (commercially available) and an ADC standard (commercially available) consisting of the same mAb standard with small-molecule compounds attached. The conditions are shown in Tables 2 and 3. In the analysis of the mAb, the peaks of multimer, monomer, and degradates could be separated within 5 minutes as shown in Fig. 2. In the aggregate evaluation of ADC, hydrophobic interaction between the stationary phase and ADC often causes peak broadening and prevents a correct assessment (Fig. 3A). Therefore, in order to suppress the hydrophobic interaction between the sample and the stationary phase, 15% of 2-propanol was added to the mobile phase[4]. As a result, good separation was obtained (Fig. 3B).

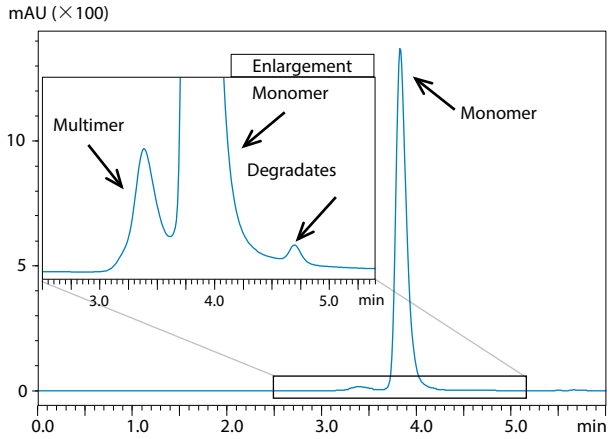

Fig. 2 Chromatogram of mAb Aggregate analysis

Table 2 Analytical Conditions for mAb Impurities Analysis (Fig. 2)

System:	Nexera XS inert
Column:	TSKgel UP-SW3000 (150 mm × 4.6 mm I.D., 2 µm)
Guard Column:	TSKgel guardcolumn UP-SW DC
Isocratic Mode	
Mobile Phase:	100 mmol/L (sodium) phosphate buffer containing 100 mmol/L Na_2SO_4
Flowrate:	0.4 mL/min
Column Temp.:	25 ˚C
Vial:	TORAST™-H Glass Vial*5
Injection Volume:	5 µL
Sample:	NIST mAb (10 mg/mL)
Detection:	280 nm (SPD-M40, UHPLC inert cell)

*5: 370-04301-01

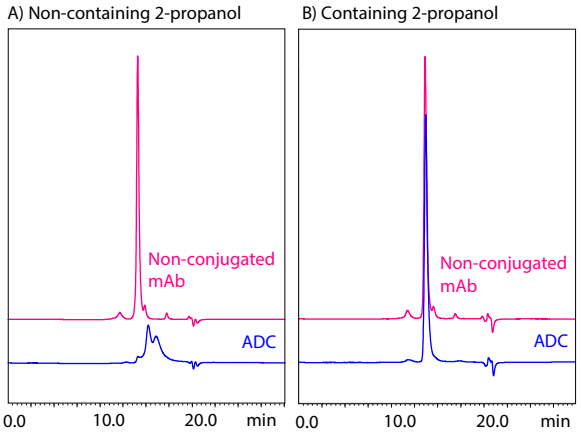

Fig. 3 mAb and ADC Chromatograms

Table 3 Analytical Conditions for mAb and ADC (Fig. 3)

System:	Nexera XS inert
Column:	TSKgel UP-SW3000 (300 mm × 4.6 mm I.D., 2 µm)
Guard Column:	TSKgel guardcolumn UP-SW DC
Isocratic Mode:	
Mobile Phase for Condition A:	200 mmol/L (potassium) phosphate containing 250 mmol/L KCl
Mobile Phase for Condition B:	200 mmol/L (potassium) phosphate containing 250 mmol/L KCl / 2-propanol = 85:15
Flowrate:	0.2 mL/min
Column Temp.:	25 ˚C˚
Vial:	TORAST-H Glass Vial*5
Injection Volume:	5 µL
Sample:	Non-conjugated mAb (0.5 mg/mL), ADC (5 mg/mL)
Detection:	280 nm (SPD-M40, UHPLC inert cell)

*5: 370-04301-01

■ Analysis of Charge Isomers (Charge Variants) by Ion Exchange Chromatography (IEX)

mAbs have complicated structures heterogeneous variants, also there are some charge variants isomers[3]. The stability and efficacy of antibody drugs are affected by their charge differences, hence charge variants must be evaluated correctly. mAb charge variants can be separated by IEX based on differences in charge strength. Evaluation of the charge variant was performed using three types of mAbs with the conditions shown in Table 4. Several peaks come from multiple charge variants were successfully detected in the respective sample (Fig. 4).

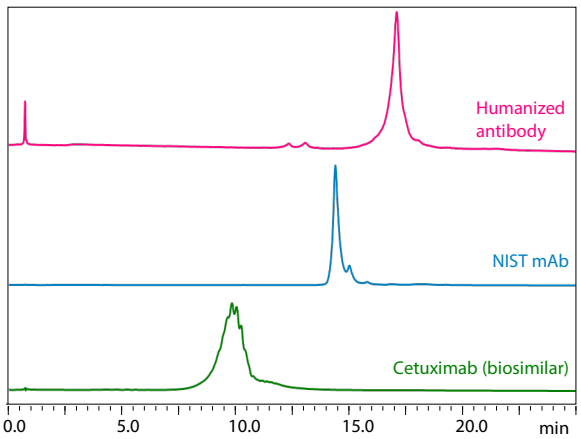

Fig. 4 Chromatograms of Three types of mAbs

Table 4 Analytical Conditions for mAb charge variants analysis (Fig. 4)

System:	Nexera XS inert
Column:	TSKgel CM-STAT (100 mm × 4.6 mm I.D., 7 µm)
Gradient Mode:	0 – 55% B (0 – 30 min)
Mobile Phase A:	20 mmol/L 2-(*N*-morpholino) ethanesulfonic acid (pH 6.0)
Mobile Phase B:	Mobile Phase A containing 500 mmol/L NaCl
Flowrate:	0.8 mL/min
Column Temp.:	25 ˚C
Vial:	TORAST -H Glass Vial*5
Injection Volume:	5 µL
Sample:	Humanized antibody (1 mg/mL), NIST mAb (5 mg/mL), Cetuximab (Biosimilar 5 mg/mL)
Detection:	280 nm (SPD-M40, UHPLC inert cell)

*5: 370-04301-01

■ Analysis of ADC drug-to-antibody ratio by hydrophobic interaction chromatography (HIC)

ADC efficacy is also affected by heterogeneity of the small-molecule drugs linked to ADCs. Analysis of mAb isomers and ADC heterogeneity by HIC is described.
A commercially available ADC mimic was analyzed without pretreatment and its drug-antibody ratio (DAR) was determined using the conditions shown in Table 5. ADCs linked to from 0 through 6 small-molecule drugs were separated successfully (Fig. 5).

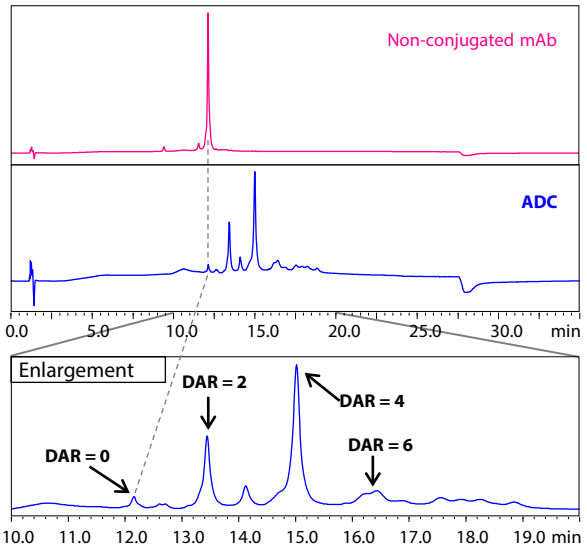

Fig. 5 Result of DAR analysis

Table 5 DAR Analytical Conditions (Fig. 5)

System	Nexera XS inert
Column	TSKgel Butyl-NPR (100 mm × 4.6 mm I.D., 2.5 µm)
Gradient Mode	0 – 100% B (0 – 20 min)
Mobile Phase A	25 mmol/L (sodium) phosphate buffer containing 1.5 mol/L sodium sulfate (pH 7.0)
Mobile Phase B	25 mmol/L (sodium) phosphate buffer (pH 7.0) / 2-propanol = 80:20
Flowrate	0.5 mL/min
Column Temp.	35 ˚C
Vial	TORAST-H Glass Vial[*5]
Injection Volume	10 µL
Sample	Non-conjugated mAb (0.5 mg/mL), ADC (5 mg/mL)
Detection	280 nm (SPD-M40, UHPLC inert cell)

*5: 370-04301-01

■ Conclusion

This article describes various analyses related to antibody drug and ADC quality control as well as optimization techniques for analytical conditions. Rapid and good mAb separation was achieved with SEC. ADC separation was also achieved with SEC by suppressing the hydrophobic interaction between the ADC and stationary phase. Acidic and basic charge variants were separated with IEX based on the difference in charge strength. A cysteine-linked ADC was separated with HIC based on a number of small-molecule drugs linked to the antibody.
The excellent corrosion resistance of the Nexera XS inert provides stable data for antibody drug analysis or any analysis that uses a mobile phase containing high concentrations of corrosive salts.

< References >
(1) ICH Q6B, Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products, International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, www.ich.org
(2) Ministry of Health, Labour and Welfare, Pharmaceutical and Food Safety Bureau, Evaluation and Licensing Division. Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. (PFSD/ELD Notification No. 571 dated May 1, 2001)
(3) Biopharmaceutical handbook, 4th Edition. Biologics production and quality control (Jiho Inc.)
(4) Tsutomu Arakawa, Daisuke Ejima, Kouhei Tsumoto, and Pete Gagnon: Solvent modulation of chromatography, Journal of Japanese Biochemical Society 80 (1) pp 45-51.

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