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Application News

LabSolutions™ MD: Software for Efficient Method Development based on Analytical Quality by Design

Efficient Method Development of Monoclonal Antibody Size Variants by Size Exclusion Chromatography

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User Benefits

- The separation between monoclonal antibody aggregates, monomers, and fragments can be visualized by design space to efficiently find the optimal condition that provides enough resolution as well as robustness.
- Nexera™ XS inert (UHPLC system) offers high resistance to corrosion for stable data acquisition even when using mobile phases with high salt concentration.
- pHM-40 not only provides real-time monitoring of the mobile phase pH but also visualizes the pH profile together with the chromatogram.

■ Introduction

Antibody drugs using monoclonal antibodies (mAbs) pose concerns about forming aggregates during production and storage in terms of their impact on safety and efficacy. ICH-Q6B requires the separation of impurities such as aggregates and fragments in antibody drugs and to determine their content. Therefore, monitoring these impurities by size-exclusion chromatography (SEC) is one of the most important analyses during the production of mAb.

For SEC analysis, It is required to optimize the separation between aggregate, monomer, and fragments by changing salt concentration and mobile phase pH while considering the interaction between mAbs and column packing materials. On the other hand, using the mobile phases with high salt concentration can cause damage on LC systems. This article describes an example of efficient method optimization for providing enough separation and robustness for mAb size variants by using Nexera XS inert that is highly corrosion-resistant, pH monitor (pHM-40), and LabSolutions MD (a dedicated software for supporting method development).

■ Analytical Conditions

The analytical conditions used in the optimization for the separation between mAb aggregate, monomer, and fragments are shown in Table 1. First, the concentration of sodium chloride in phosphate buffer was varied to evaluate its optimal level for the separation. Then, by varying the mobile phase pH, flowrate, and oven temperature, the separation was further examined to find the optimal condition.

Table 1 Analytical Conditions

System: Nexera XS inert (Method Scouting System)	
Mobile Phase:	
Pump A:	200 mmol/L disodium hydrogen phosphate in water
Pump B:	200 mmol/L sodium dihydrogen phosphate in water
Pump C:	1 mol/L sodium chloride in water
Pump D:	Water
Sample:	Monoclonal Antibody Standard (0.5 mg/mL)
Column:	TSKgel UP-SW3000 (150 mm × 4.6 mm I.D., 2 µm)
Vial:	TORAST-H Glass Vial (Shimadzu GLC)*1
Analytical Conditions (Isocratic)	
Oven Temp.:	20, 25, 30 °C
Flowrate:	0.15, 0.2, 0.25 mL/min
Injection Vol.:	5 µL
Detection:	280 nm (SPD-M40, UHPLC inert cell)

*1 P/N 370-04301-01

■ Evaluation of Concentration of Sodium Chloride in Mobile Phase

To evaluate the effect of the salt concentration in the mobile phase on the separation, mobile phase blending function (refer to C190-0563[1]) was used to automatically prepare the mobile phases that have different salt concentration. Specifically, six different concentration levels of sodium chloride in phosphate buffer from 50 mmol/L to 300 mmol/L in increments of 50 mmol/L were evaluated to optimize the separation between aggregate, monomer, and fragments. Fig. 1 shows the mAb chromatograms obtained at 50 mmol/L and 150 mmol/L. When the concentration of sodium chloride is 50 mmol/L, electrostatic interactions between the stationary phase and mAb resulted in lower resolution between aggregate (A in Fig. 1) and monomer (B in Fig. 1) and also lower peak-to-valley ratio between monomer (B in Fig. 1) and fragment (C in Fig. 1). In contrast, these values were improved at 150 mmol/L (Fig. 2). Based on this result, the concentration of sodium chloride is decided at 150 mmol/L for the subsequent optimization of mobile phase pH, flowrate, and oven temperature.

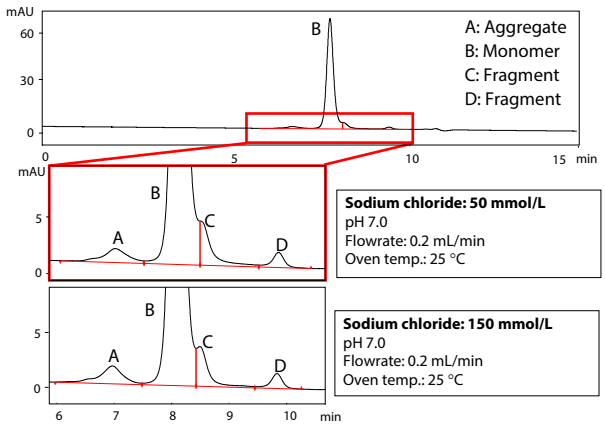

Fig. 1 Chromatograms at 50 and 150 mml/L Sodium Chloride Concentration

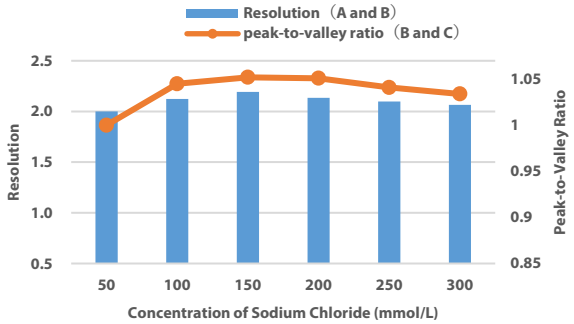

Fig. 2 Resolution and Peak-to-Valley Ratio at each Sodium Chloride Concentration

■ Evaluation of Mobile Phase pH, Flowrate, and Oven Temperature

Due to a large number of dissociable groups, the charge state of proteins generally changes depending on pH levels, which can affect peak shapes as a secondary interaction between the stationary phase and mAb. Also, optimal flowrate and oven temperature depend on the column particle size of packing material, pore size, and chemical properties of molecules. Therefore, by varying the mobile phase pH, flowrate, and oven temperature, the separation between aggregate, monomer, and fragments was optimized. The chromatograms obtained at the pH levels of 6.1, 6.7, and 7.3, the flowrate of 0.15, 0.20, and 0.25 mL/min, and the oven temperature of 20, 25, and 30 °C are shown in Fig. 4 to 6. Design spaces of the resolution between aggregate and monomer as well as the peak-to-valley ratio between monomer and fragment were also shown in Fig. 3. The horizontal axis shows flowrate and the vertical axis shows pH. The red region indicates higher resolution and peak-to-valley ratio, on the other hand, the blue region indicates lower value of them. The design spaces automatically found that the optimal condition for the separation between aggregate, monomer, and fragments is pH 7.3, flowrate 0.15 mL/min, and oven temperature 30 °C (the blue point in the upper-left area of Fig. 3). By utilizing design spaces, the optimal condition can be obtained regardless of the user experience. In terms of pH (Fig. 4), as the pH increased from 6.1 to 7.3, the separation between aggregate, monomer, and fragments was improved. Since the isoelectric point (pI) value of the target mAb is around 7, the impact on the retention deriving from the electrical-charge interaction could be more significant at pH 6.1. In terms of flowrate and oven temperature (Fig. 5 and 6), optimal chromatographic separation between aggregate, monomer and fragments was obtained at lower flowrate and higher oven temperature. The pH value monitored and overlaid on each chromatogram (blue lines) shows that the pH was stable during the analysis. The monitored pH value can also be used as a parameter for checking the analytical stability, in case of an anomalous separation behavior for example.

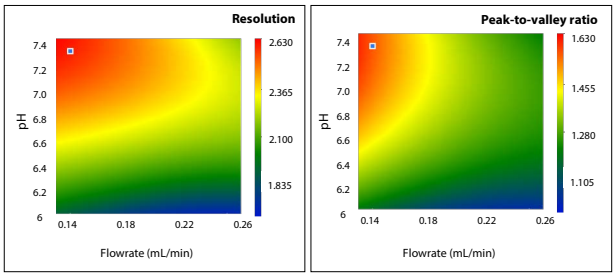

Fig. 3 Design Space of Resolution between Aggregate (A) and Monomer (B) (Left)
Design Space of Peak-to-Valley Ratio between Monomer (B)
and Fragment (C) (Right)
(Oven temp.: 30 °C)

■ Conclusion

This article describes how to efficiently optimize the separation between mAb aggregate, monomer, and fragments using Nexera XS inert, pHM-40 (pH monitor), and LabSolutions MD. Nexera XS inert offers high resistance to corrosion for reliable data acquisition with the mobile phases which have high salt concentration. In addition, design space efficiently supports the method optimization without depending on user's experience.

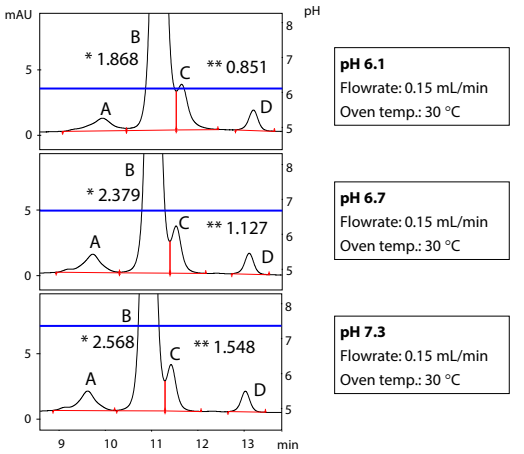

Fig. 4 Chromatograms with Different pH
(blue lines: monitored pH)
A: Aggregate; B: Monomer; C and D: Fragment;
* Resolution between A and B, ** Peak-to-valley ratio between B and C

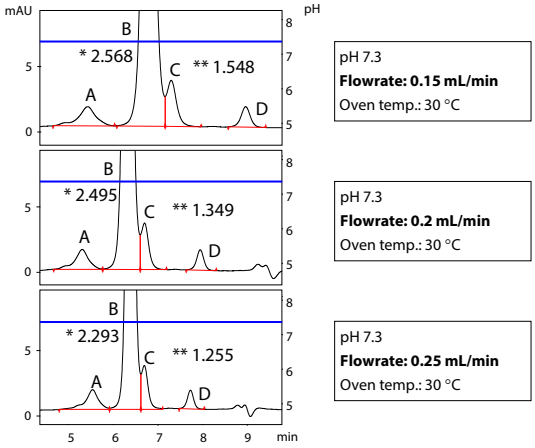

Fig. 5 Chromatograms with Different Flowrate
(blue lines: monitored pH)
A: Aggregate; B: Monomer; C and D: Fragment;
* Resolution between A and B, ** Peak-to-valley ratio between B and C

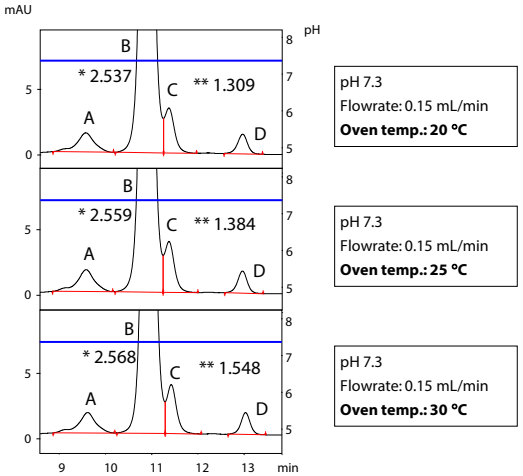

Fig. 6 Chromatograms with Different Oven Temp.
(blue lines: monitored pH)
A: Aggregate; B: Monomer; C and D: Fragment;
* Resolution between A and B, ** Peak-to-valley ratio between B and C

References:

1) Efficient Method Development by automated pH Screening with LabSolutions MD (C190-0563)

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