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Application News

Software for Efficient Method Development Based on AQbD

Efficient Method Development for Separation of Antibody Charge Variants by Ion-Exchange Chromatography

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User Benefits

- Shim-pack™ Bio IEX ion-exchange chromatography column enables analysis of charge variants of antibodies.
- LabSolutions™ MD can automate the entire workflow for method development, including the generation of an analysis schedule, mobile phase preparation, and data processing, thanks to specific functionalities, such as for ranking chromatograms by criteria values and for design space evaluation.

■ Introduction

Monoclonal antibodies and antibody-drug conjugates (ADCs), like other antibody pharmaceuticals, are produced using animal cells, which results in structural heterogeneity and impurities. Charge variants are impurities generated from the heterogeneity of C-terminal lysine, deamidation, oxidation, and other factors, that can affect the stability and efficacy of antibody pharmaceuticals. Therefore, it is important to appropriately separate, detect, and monitor charge variants for quality control purposes.

This article describes how to efficiently achieve the optimal peak separation for charge variants of a monoclonal antibody using pH gradient ion-exchange chromatography and LabSolutions MD, which is dedicated software for supporting method development.

■ Screening of Mobile Phases and Columns

In this study, Trastuzumab diluted to a concentration of 5 mg/mL in ultrapure water was used for optimizing separation of charge variants. For screening (analytical conditions in Table 1), parameters such as the concentration of the MES, HEPES, and sodium acetate in mobile phase and two different columns (porous/non-porous type) have a large effect on separation. Mobile phases adjusted to pH 5.0 and pH 10.0 were prepared by mixing an equimolar aqueous solution of HEPES, MES, and sodium acetate with acetic acid or sodium hydroxide. Then, ten MES-HEPES-sodium acetate concentration levels (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mmol/L), and two different types of column (porous Shim-pack Bio IEX SP and non-porous Shim-pack Bio IEX NP-SP) were evaluated, for a total of 20 configurations (10 concentrations × 2 columns), with the aim of finding the combination that results in the optimal separation.

Table 1 Analytical Conditions for Screening

System:	Nexera™ lite inert (Method Scouting System)
Column-1:	Shim-pack Bio IEX SP (50 mm × 4.6 mm I.D., 5 µm) *1
-2:	Shim-pack Bio IEX SP-NP (50 mm × 4.6 mm I.D., 5 µm) *2
Temperature:	30 °C
Injection Volume:	1 µL
Mobile Phases:	
Pump A – Line A:	100 mmol/L MES-HEPES-sodium acetate in water, pH 5.0
– Line B:	Water
Pump B – Line A:	100 mmol/L MES-HEPES-sodium acetate in water, pH 10.0
– Line B:	Water
Flowrate:	0.5 mL/min (Shim-pack Bio IEX SP), 1.0 mL/min (Shim-pack Bio IEX SP-NP)
Time Program (%B):	10 % (0 min) →100 % (10-17.5 min) →10 % (17.51-22.5 min)
Detection:	280 nm (SPD-40, UHPLC inert cell)

*1: P/N 227-31006-02

*2: P/N 227-31004-02

LabSolutions MD can quickly and easily generate analysis schedules for a wide variety of parameter settings, such as different types of mobile phases and columns (by following steps (1) to (5) in Fig. 1). In addition, mobile phase blending functionality can automatically prepare mobile phases with different concentrations of the MES-HEPES-sodium acetate. Column switching values were used to automatically switch between columns. By simply clicking on the mobile phases or columns to use for automated screening (step (1) in Fig. 1), the mobile phases are automatically prepared to the selected spike concentrations and the column automatically switches to the selected columns. This significantly reduces the amount of work and human errors involved in manual preparation.

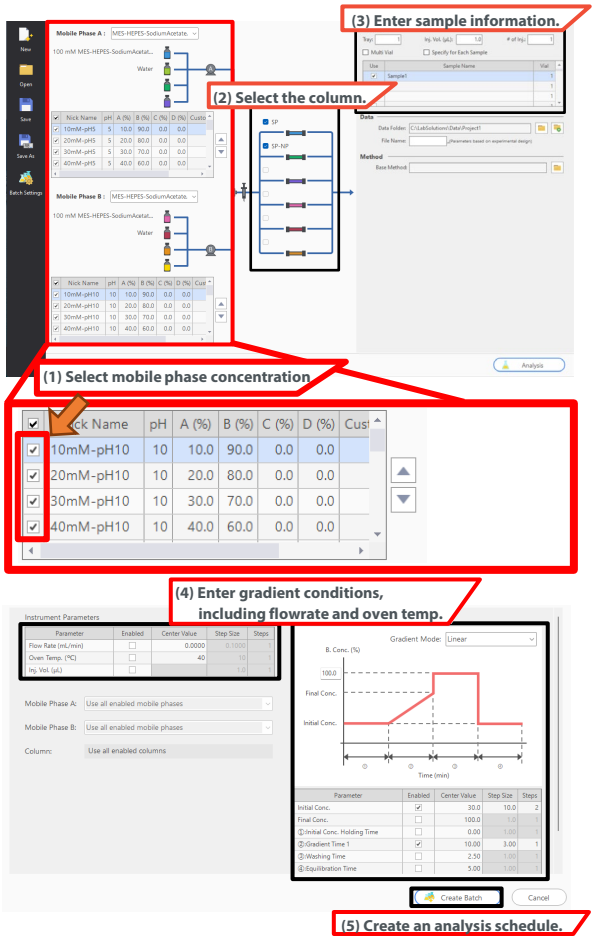

Fig. 1 Steps for Creating Analysis Schedule

■ Results of Screening

Chromatograms from the screening, measured under different MES-HEPES-sodium acetate concentration conditions in mobile phases, are shown in Fig. 2. Separation improved more using Shim-pack Bio IEX SP (porous type) than using Shim-pack Bio IEX SP-NP (non-porous type). It was also found that the MES-HEPES-sodium acetate concentration in mobile phases effected the separation of charge variants.

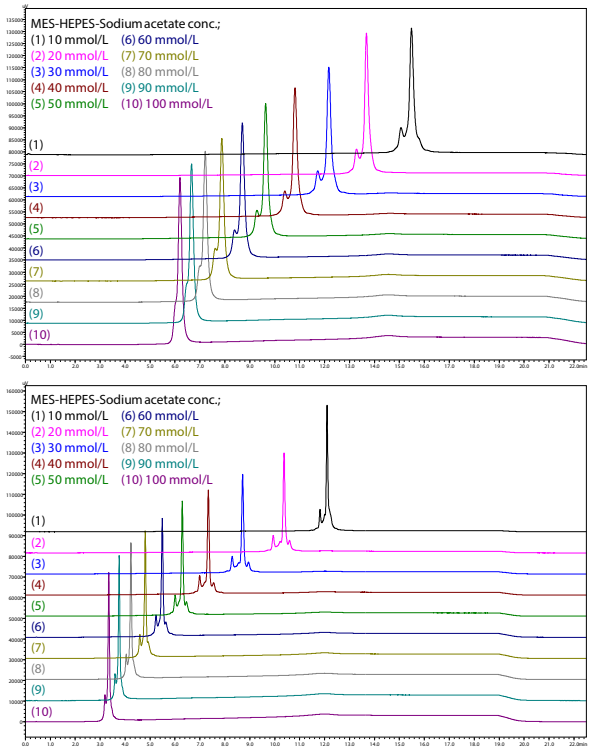

Fig. 2 Chromatograms Obtained from the Screening
Top: Shim-pack Bio IEX SP; Bottom: Shim-pack Bio IEX SP-NP

■ Quickly Determine Optimal Conditions

Because screening generates as many chromatograms as the number of analyses scheduled, they must be evaluated to determine which one is optimal. Checking all chromatograms manually is tedious and time-consuming. However, LabSolutions MD can quickly and easily determine the optimal condition setting using equation (Eq. 1) below to quantitatively evaluate the chromatographic separation.

$$\text{Evaluation Value} = P \times (Rs_1 + Rs_2 + \ldots + Rs_{P-1}) \qquad \text{(Eq. 1)}$$

The evaluation value is calculated as the number of peaks detected (P) multiplied by the sum of the resolution factor (Rs) for all peaks.

Fig. 3 shows the evaluation values obtained from mobile phase and column screening, listed in order from the highest to the lowest. It indicates that the 30 mmol/L MES-HEPES-sodium acetate mobile phase and the Shim-pack Bio IEX SP-NP (non-porous type) column provide the highest value as the optimal condition settings (chromatogram (3) of the bottom figure in Fig. 2, shown enlarged in Fig. 4).

Next, for the optimization phase, the column oven temperature and gradient program parameters are considered to further improve the separation.

			Response
MPA Nick Name	MPB Nick Name	Column Nick Name	Evaluation Val
30mM-pH5	30mM-pH10	SP-NP	21.342
20mM-pH5	20mM-pH10	SP-NP	20.568
40mM-pH5	40mM-pH10	SP-NP	19.602
50mM-pH5	50mM-pH10	SP-NP	18.989
10mM-pH5	10mM-pH10	SP-NP	17.637

Fig. 3 Condition Settings Ranked by Evaluation Value (Top 5)

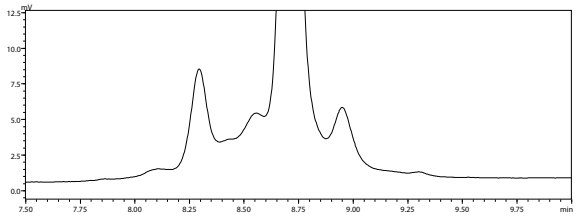

Fig. 4 Chromatogram with Highest Evaluation Value
(Enlargement of Chromatogram in Fig. 2 (3))

■ Optimization Phase

Based on the optimal mobile phase and column conditions determined from the screening phase, analytical conditions were further optimized for separation by changing the gradient time (7, 10, and 13 min), initial concentration of the gradient program (10, 20, 30, 40, and 50 %), and column oven temperature (20, 30, and 40 °C). The chromatograms obtained are shown in Figs. 5 to 7. The results show that the longer the gradient time, the higher the initial concentration, and the lower the column oven temperature, the better the resolution of the main peak and the peak after the main peak (Peak A).

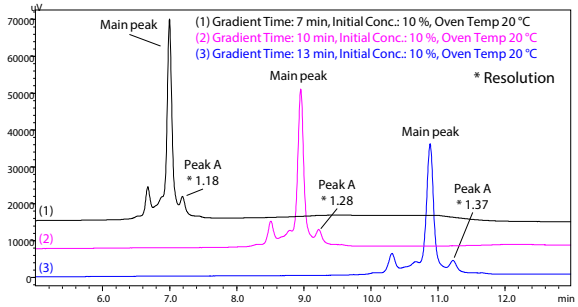

Fig. 5 Chromatograms with Different Gradient Times

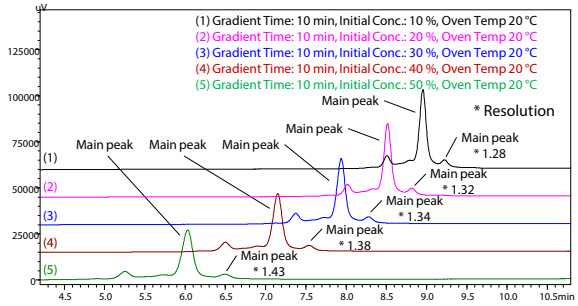

Fig. 6 Chromatograms with Different Initial Concentrations

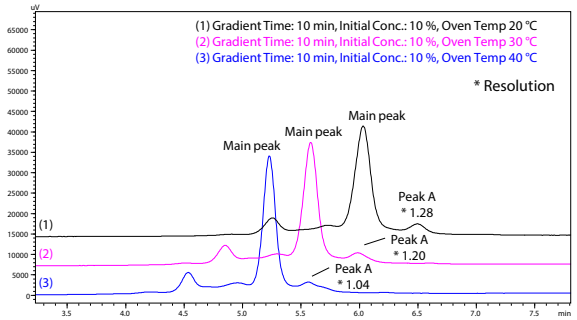

Fig. 7 Chromatograms with Different Column Oven Temperatures

■ Determining Optimal Conditions by Design Space Evaluation

To visualize the optimal conditions, a design space was created based on results from evaluating various LC parameters. The resolution of the main peak and Peak A were visualized in Fig. 8, with gradient time or initial concentration on the vertical axis and column oven temperature on the horizontal axis. The red region indicates higher resolution and the blue region indicates lower resolution. By visualizing resolution with a design space, it became evident that the longer the gradient time, the higher the initial concentration, and the lower the column oven temperature, the better the separation between the main peak and Peak A.

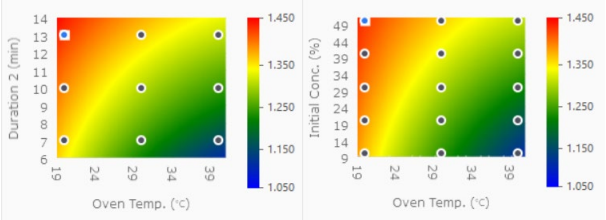

Fig. 8 Design Space for Resolution of Main Peak and Peak A
(Left: With 50 % Initial Concentration; Right: With 13 min Gradient Time)

LabSolutions MD can automatically search for the optimal condition that meets several criteria by overlaying design spaces. For example, Fig. 9 shows the area where the resolution of the main peak and Peak A is >1.3 when the retention time of the last eluting peak is <7 min. The orange region is where the resolution of the main peak and Peak A is <1.3, whereas the blue region is where the retention time of the last eluting peak is >7 min. The red point in the remaining white region indicates the condition settings determined by automatic searching that provides the maximum resolution of the main peak and Peak A (a gradient time of 11 minutes, initial gradient concentration of 50 %, and a column oven temperature of 20 °C). By overlaying design spaces for resolution and RT of the last eluting peak, optimal condition settings that provide enough resolution and a shorter analysis time can be easily determined.

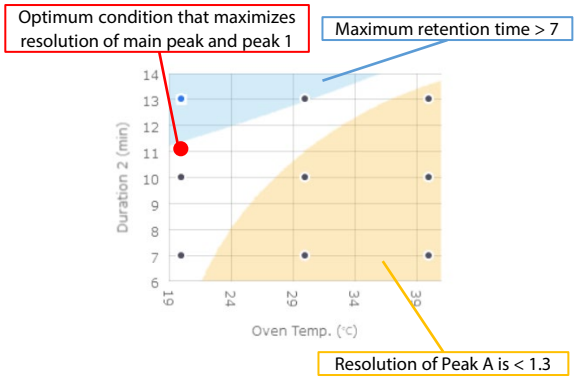

Fig. 9 Overlay of Design Spaces for Resolution and Retention Time of Last Eluting Peak

■ Chromatogram at Optimal Condition

The chromatogram obtained at optimal condition is shown in Fig. 10. It shows that the resolution of main peak and Peak A > 1.3, and retention time of last eluting peak < 7 min, which successfully satisfies the criteria for optimization.

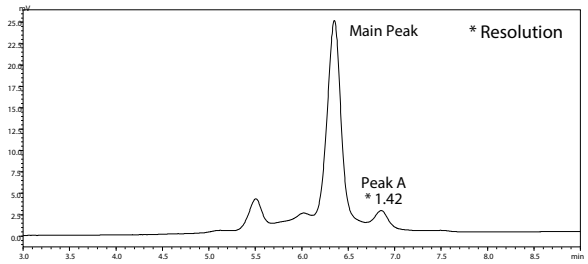

Fig. 10 Chromatogram at Optimal Condition

■ Conclusion

The separation pattern of charge variants of antibody differ depending on the concentration of reagents in the mobile phase and column pore type (porous/non-porous), in addition to column oven temperature and the gradient program. The separation behavior can differ depending on the kinds of impurities involves and the presence of modifications. Therefore, separation must be individually optimized for each antibody. On the other hand, the number of analyses and amount of data processing needed for the optimization of analytical conditions is a time-consuming challenge. However, LabSolutions MD can automate the entire workflow, including the generation of an analysis schedule, mobile phase preparation, and data processing thanks to specific functionalities, such as for ranking chromatograms by criteria values and for design space evaluation.

Related Applications

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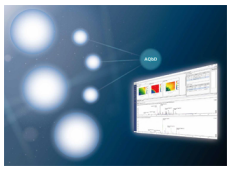

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