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Application News

Software for Efficient Method Development Based on AQbD

Method Development for Separating Charge Variants of Antibody-Drug Conjugates by Ion-Exchange Chromatography

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User Benefits

- Shim-pack™ Bio IEX ion-exchange chromatography column enables analysis of charge variants of antibody-drug conjugates (ADCs).
- LabSolutions™ MD can automate the entire workflow for method development, including the generation of an analysis schedule, mobile phase preparation.

■ 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, which are impurities generated from the heterogeneity of C-terminal lysine, deamidation, oxidation, and other causes, can impact the stability and efficacy of antibody pharmaceuticals. Therefore, it is important to appropriately separate, detect, and monitor charge variant peaks for quality control purposes.

This article describes using LabSolutions MD, which is dedicated software for supporting method development, to efficiently optimize peak separation between ADC charge variants when using pH gradient ion-exchange chromatography.

■ Screening of Mobile Phases

In this study, trastuzumab deruxtecan (T-DXd) diluted to a concentration of 5 mg/mL in ultrapure water was used for optimizing separation of charge variant peaks. For screening (analytical conditions in Table 1), the parameters that have a large effect on separation, such as the concentration of the MES, HEPES, and sodium acetate in the mobile phase and the ratio of acetonitrile or methanol in the mobile phase, were considered . 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. To determine the condition settings that result in optimal separation, a schedule was prepared with ten MES-HEPES-sodium acetate concentration levels (10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mmol/L) and six different acetonitrile or methanol ratio levels (5, 10, and 15 % in mobile phase).

Table 1 Analytical Conditions for Screening

System:	Nexera™ lite inert (method scouting system)
Column:	Shim-pack Bio IEX SP-NP (50 mm × 4.6 mm I.D., 5 µm)*1
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
– Line C:	40 % acetonitrile
– Line D:	40 % methanol
Pump B – Line A:	100 mmol/L MES-HEPES-sodium acetate in water, pH 10.0
– Line B:	Water
– Line C:	40 % acetonitrile
– Line D:	40 % methanol
Flowrate:	1.0 mL/min
Time Program (%B):	2 % (0 min) → 100 % (10 to 17.5 min) → 2 % (17.51 to 22.5 min)
Detection:	280 nm (SPD-40, UHPLC inert cell)

*1: P/N 227-31004-02

LabSolutions MD can quickly and easily generate analysis schedules for specified parameter settings, such as for several types of mobile phases and column oven temperatures (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, as well as different ratios of acetonitrile or methanol, by simply clicking the mobile phases to use for automated screening (step (1) in Fig. 1). That significantly reduces the amount of work and human errors involved in manual preparation.

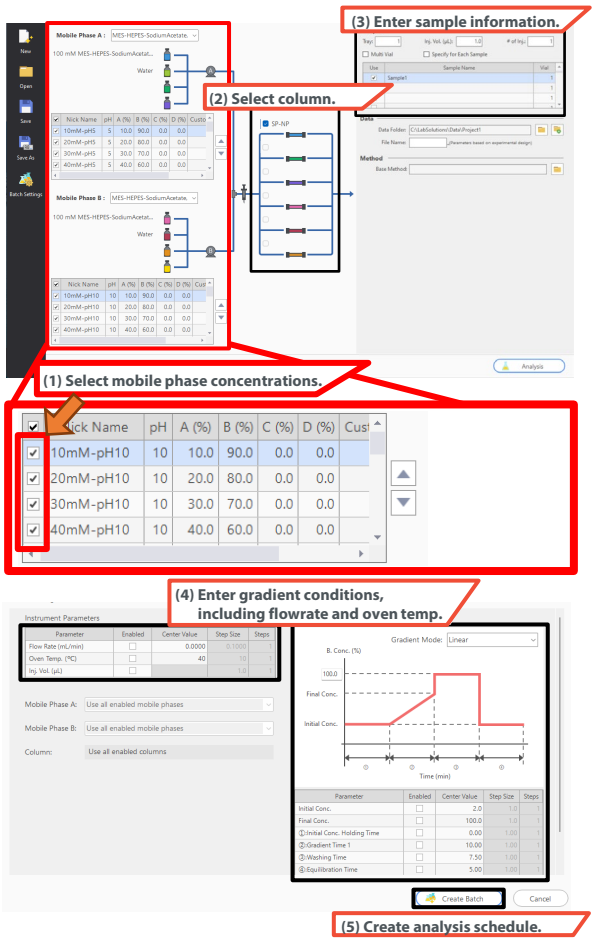

Fig. 1 Steps for Creating Analysis Schedule

■ Results of Screening Mobile Phases

Chromatograms from the screening process, measured under different MES-HEPES-sodium acetate concentration conditions in mobile phases, are shown in Fig. 2. That determined that the MES-HEPES-sodium acetate concentration in the mobile phase affected separation of charge variants.

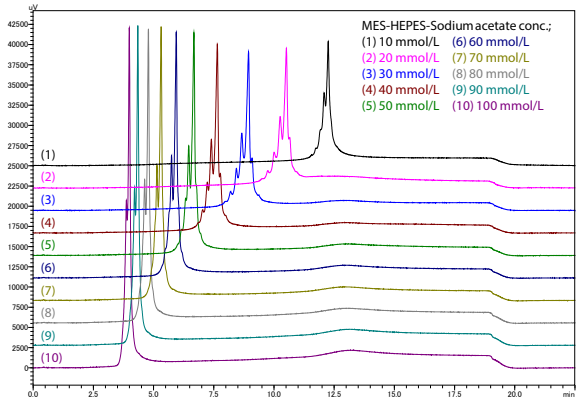

Fig. 2 Chromatograms Obtained from the Screening

■ Quickly Determines Optimal Condition Settings

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 settings, using the equation below (Eq. 1) to quantitatively evaluate chromatographic separation.

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

Evaluation values are calculated as the number of peaks detected (P) multiplied by the sum of resolution factors (Rs) for all peaks. Fig. 3 shows the evaluation values obtained from mobile phase and column screening, listed from highest to lowest. It indicates that 30 mmol/L MES-HEPES-sodium acetate is the optimal mobile phase concentration and provides the highest value (chromatogram (3) in Fig. 2, shown enlarged in Fig. 4).

		Response
MPA Nick Name	MPB Nick Name	Evaluation Val
30mM-pH5	30mM-pH10	19.321
20mM-pH5	20mM-pH10	17.719
40mM-pH5	40mM-pH10	15.339
50mM-pH5	50mM-pH10	13.746
60mM-pH5	60mM-pH10	13.313

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

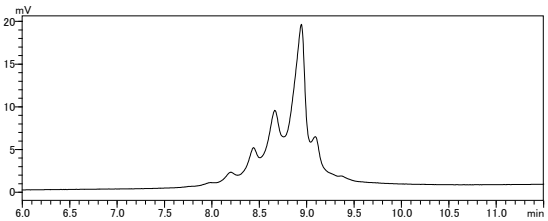

Fig. 4 Chromatogram with Highest Evaluation Value
(Enlargement of Chromatogram in Fig. 2 (3))

■ Optimization of the Ratio of Organic Solvent in Mobile Phases

Based on the optimal MES-HEPES-sodium acetate concentration in the mobile phase determined from the screening phase, analytical conditions were further optimized for the organic solvent ratio in the mobile phase by varying the acetonitrile or methanol ratio (5, 10, and 15 %). The resulting chromatograms in Fig. 5 show that the organic solvent ratio in the mobile phase affected the separation of charge variants, where the higher the acetonitrile ratio, the sharper the detected peaks. However, at 10 % and 15 % acetonitrile ratios, baseline fluctuations were observed around a retention time of 12.5 minutes (indicated by the gray arrows in Fig. 5), which seemed to occur due to the denaturation of the ADCs caused by the addition of acetonitrile.

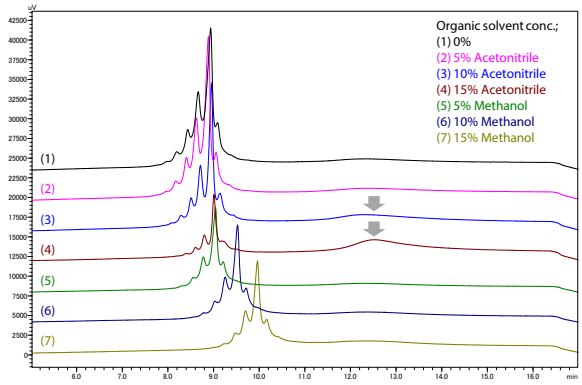

Fig. 5 Chromatograms with Different Ratios of Acetonitrile or Methanol in the Mobile Phase

The highest peak-to-valley ratio of the main peak or the peaks before and after the main peak was for a 5 % acetonitrile ratio in the mobile phase (chromatogram (2) in Fig. 5, which is shown enlarged in Fig. 6), whereas 10 % and 15 % acetonitrile ratios may have caused denaturation. Furthermore, Fig. 7 shows the peak-to-valley ratios of the main peak and the peaks before and after the main peak, listed in order from the highest to the lowest. Thus, LabSolutions MD enables evaluation of chromatograms by focusing on peaks of interest.

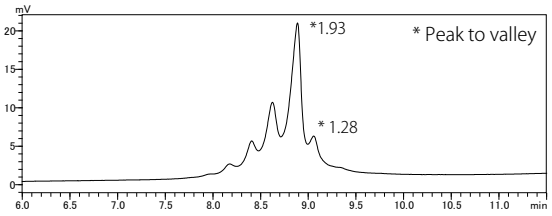

Fig. 6 Chromatogram of Highest Peak-to-Valley Ratios of the Main Peak and Peaks Before/After Main Peak
(Enlargement of Chromatogram in Fig. 5 (2))

		Compound#1 (Main Peak)	Compound#2 (Peak after Main Peak)
MPA Nick Name	MPB Nick Name	Peak Valley Ratio	Peak Valley Ratio
30mM-pH5, 10% ACN	30mM-pH10, 10% ACN	2.276	1.292
30mM-pH5, 15% ACN	30mM-pH10, 15% ACN	1.992	1.052
30mM-pH5, 5% ACN	30mM-pH10, 5% ACN	1.925	1.276
30mM-pH5	30mM-pH10	1.69	1.118
30mM-pH5, 10% MeOH	30mM-pH10, 10% MeOH	1.512	1.188
30mM-pH5, 15% MeOH	30mM-pH10, 15% MeOH	1.474	1.196
30mM-pH5, 5% MeOH	30mM-pH10, 5% MeOH	1.427	1.151

Fig. 7 Condition Settings Ranked by Peak-to-Valley Ratio

■ Conclusion

The separation patterns of ADC charge variants differ depending on the concentration of reagents in the mobile phase and the ratio of acetonitrile or methanol in the mobile phase. LabSolutions MD can automate the method development workflow, including the generation of analysis schedules, mobile phase preparation, and data processing thanks to specific functionalities, such as for ranking chromatograms by criteria values.

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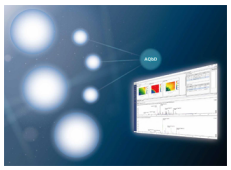

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