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Application News

Charge variant analysis of mAb biotherapeutics by Nexera XS with a Shim-pack Bio IEX Column

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User Benefits

◆ Shimadzu Nexera UHPLC system provides a robust platform for charge variant analysis in biopharma industry.
◆ Shim-pack™ Bio IEX columns offers excellent reproducibility and resolution between charge variants of mAbs biotherapeutics.

■ Introduction

Overview: Monoclonal antibodies (mAbs) are the most approved biopharmaceuticals used to treat severe and chronic diseases such as cancer, autoimmune, cardiovascular, respiratory, hematology, and several infections. They have an enormous therapeutic and commercial value which makes them among the top 10 best sellers of pharmaceuticals for several past years. In recent years, the use of mAbs has been expanded due to significant advances in design thus decreasing immunogenicity in humans, improving their bioavailability, and specific affinity for antigen-binding.

The complexity of mAb with about 150 kDa molecular weight implements the use of quality by design (QbD) as an unavoidable strategy for the development and manufacturing of these molecules. QbD defines the critical quality attributes and a control strategy to assure stable and consistent quality during the manufacturing process. Different post-translational modifications during the upstream process such as amino or carboxy-terminal processing and glycosylation or during downstream processes or storage, such as deamidation, oxidation, and fragmentation will end to different charge variants which could affect the safety, quality, and efficacy of mAbs. Therefore, characterization and quantification of mAbs charge variants are required for assessing consistent product quality.

Methods of mAb Charge variant analysis: Analytical methods with the capability to separate differently charged molecules are used to characterize the variants of mAbs. Charge-based variants have been categorized as acidic, main, and basic species. Chromatographic and electrophoretic tools include ion-exchange chromatography (IEX) and isoelectric focusing (IEF), which are the most common and simple analytical methods for the analysis of mAbs charge heterogeneities. IEF may be used for visualizing the charge isoforms, but chromatographic tools are more appropriate for precise quantification.

Cation exchange chromatography (CEX) has been a standard method for the characterization of mAbs charge heterogeneity and is routinely used as a fingerprint of the distribution of posttranslational modifications present on the mAb. Also, CEX analysis is necessary for mAbs quality control analysis and is requested by regulatory agencies.

There are two major mechanisms involved in CEX of mAbs charge variants namely salt gradient and pH gradient. In salt gradient elution, mAbs charge variants are pushed down the column from exchange site by competing with the salt ions in mobile phase. With pH gradient elution, mAbs charge

variants will elute from the column when the pH reaches the point where they have little to no charge

The most common method for charge variant analysis still use a salt elution mechanism. Because of this, we investigated the robustness of this method. The control of pH during chromatography is of high importance to keep the retention times stable and the method robust. The ion exchange column itself will have an inherent buffering capacity which controls the pH during analysis. The degree of buffering a column is related to the exact column capacity and the type of charged group bound to the resin.

In this study, we describe a salt-gradient method having common gradient program for separating the charge variants of all the four mAbs namely trastuzumab, omalizumab, rituximab and cetuximab using Shimadzu Nexera XS (UHPLC system shown in Fig. 1) and a Shim-pack Bio IEX column.

Nexera™ series

Key features- ᐧᐧᐧᐧᐧnalytical Intelligence

- Automated support functions utilizing digital technology such as machine-to-machine communication (M2M), Internet of things (IoT), and Artificial Intelligence (AI) enables higher productivity and maximum reliability.

- Allows a system to monitor and diagnose itself, handle any issues during data acquisition without user input, and automatically behave as if it were operated by an expert.

- Supports the acquisition of high quality, reproducible data regardless of an operator's skill level for both routine and demanding applications.

Fig. 1 Nexera™ XS system

Shim-pack Bio IEX: Ion exchange chromatography columns

Shim-pack Bio IEX Columns are available in Q (quaternary ammonium) and SP (sulfopropyl) chemistries and are based on porous (Q and SP columns) and non-porous (Q-NP and SP-NP columns) hydrophilic polymers with low nonspecific adsorption. The columns offer excellent binding capacity with exceptionally high efficiency and resolution.

The SP groups attached to the stationary phase are strong cation exchangers. These are less prone to ionization changes with different pH during salt gradient elution and maintains consistency in the retention times of charge variants during analysis.

Fig. 2 The charge variant profile of trastuzumab

■ Experimental

The mAbs solutions namely trastuzumab, omalizumab, rituximab and cetuximab were prepared in tris buffer with concentration of 5 mg/mL.

The samples were directly injected and analyzed on Nexera XS with UV detector. The elution was monitored at 280 nm. Relative peak area (%) was used to quantify the charge variants of all the four mAbs. The gradient program was optimized to separate the charge variants of all the four mAbs.

Table 1. shows the analytical conditions for salt-gradient IEX.

Table 1. LC analytical conditions

Column	: Shim pack™ Bio IEX SP-NP, (100 mm × 4.6 mmI.D., 3 µm (P/N: 227-31005-03)
Oven temperature	: 25°C
Mobile phase	:Mobile phase A: 20 mmol/L (sodium) phosphate buffer, pH-6 Mobile phase B: 20 mmol/L (sodium) phosphate buffer with 250 mmol/L NaCl, pH-6
Gradient program (B %)	: 0-1.50 min (0 %); 1.50-15.00 min (0-100 %); 15.00-18.00 min (100 %); 18.10-20.00 (0 %)
Flow rate	: 0.5 mL/min
Total run time	: 20.0 min
Injection volume	: 2 µL
Autosampler temperature	: 15°C
Detector wavelength	: 280 nm

Fig. 3 The charge variant profile of omalizumab

■ Results and Discussion

In this study, salt-gradient IEX methods were performed for trastuzumab, omalizumab, rituximab and cetuximab charge variant analysis. Fig. 2 to Fig. 5 show the charge variant profile of all the four mAbs on a Shim-pack Bio IEX SP-NP column with a salt-gradient elution program, demonstrating good separation of charge variants in 20 minutes. The tallest peak is the main component. The early and late-eluting peaks were called acidic variants and basic variants, respectively.

The relative peak area percent of charge variants and main component for all the four mAbs based on six consecutive analyses is summarized in Table 2.

The injection-to-injection repeatability of the UHPLC-UV system was evaluated based on the six consecutive analyses. As shown in Fig. 6 to Fig. 9, the overlay of six replicates indicates an excellent separation reproducibility.

Variations in the area counts of main component peak as well as charge variant peaks of all 4 mAbs for six replicate injections were found to be less than 5 % RSD as shown in the Table 3.
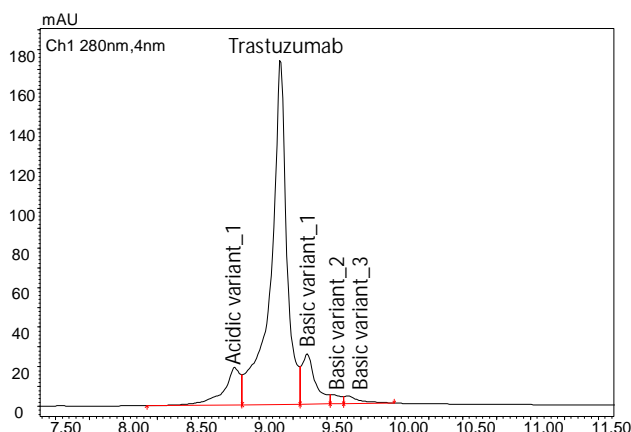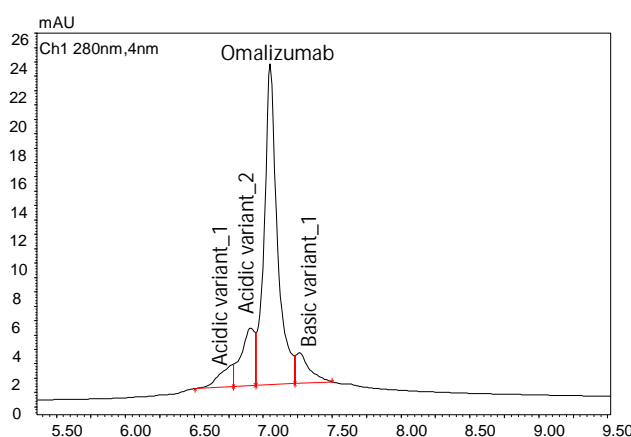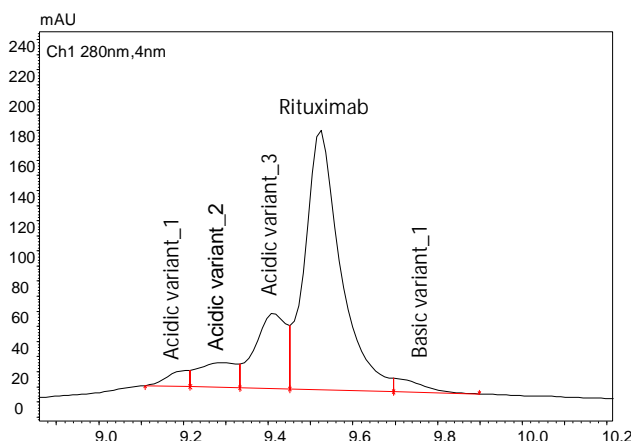

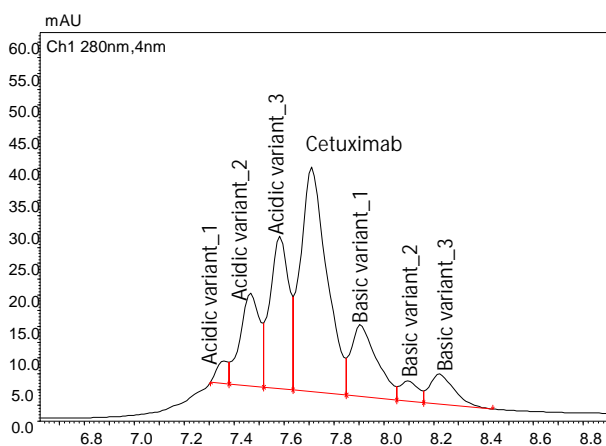Fig. 4 The charge variant profile of rituximab

Fig. 5 The charge variant profile of cetuximab

Table 2. Relative area percent of main component and charge variants (n=6)

Relative Area percent (%)	Trastuzumab	Omalizumab	Rituximab	Cetuximab
Acidic variant 1	8.94	5.65	2.46	1.97
Acidic variant 2	NA	14.26	7.36	13.02
Acidic variant 3	NA	NA	17.46	20.99
Main component	77.41	73.17	69.86	42.38
Basic variant 1	9.91	6.89	2.85	13.64
Basic variant 2	1.30	NA	NA	2.64
Basic variant 3	1.72	NA	NA	5.32

Key : NA = Not Applicable. The charge variants were not observed
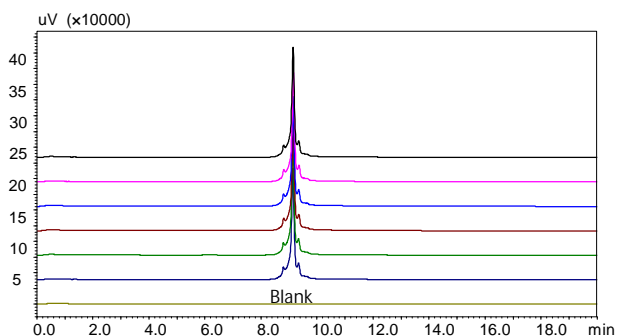
Fig. 6   Overlay chromatograms of six replicate injections for trastuzumab
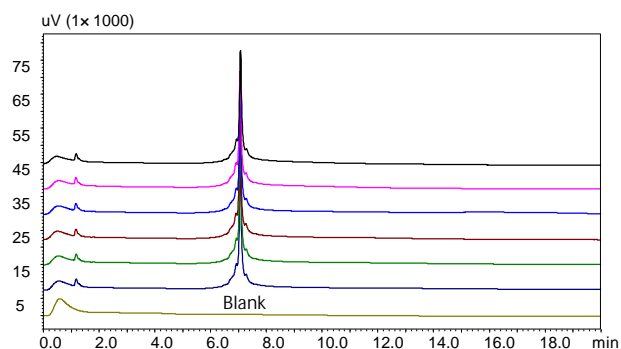
Fig. 7   Overlay chromatograms of six replicate injections for omalizuzumab
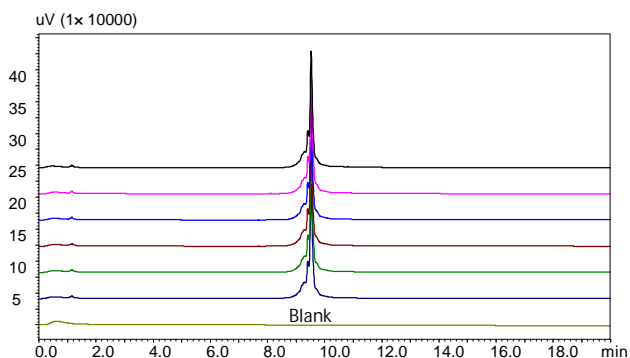
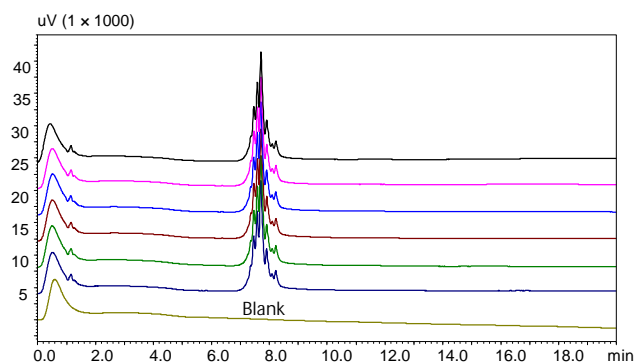Fig. 8 Overlay chromatograms of six replicate injections for rituximab

Fig. 9   Overlay chromatograms of six replicate injections for cetuximab

Table 3. : Injection-to-injection repeatability (%RSD) of peak area counts (n = 6) for charge variants and main component

Area percent (%)	Trastuzumab	Omalizumab	Rituximab	Cetuximab
Acidic variant_1	0.83	1.97	4.87	4.80
Acidic variant_2	NA	1.81	4.11	0.93
Acidic variant_3	NA	NA	1.68	4.07
Main component	2.60	1.65	0.55	1.29
Basic variant_1	1.29	2.97	3.96	3.05
Basic variant_2	0.70	NA	NA	3.26
Basic variant_3	2.38	NA	NA	2.51

■ Conclusion

- Shimadzu Nexera XS system provides a robust platform for charge variant analysis of mAbs.
- Method development and optimization have been performed on the Shim-pack Bio IEX SP-NP cation exchange column.
- The salt gradient method for charge variant analysis of four mAbs gave consistent results.
- The repeatability (n=6) results in terms of peak area counts for all the acidic and basic charge variants as well as the main component of four mAbs were found to be less than 5 %RSD.

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