High-resolution fractionation and analysis of monoclonal antibody charge variants with Capto HiRes S column

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Introduction

To ensure the high quality of therapeutic monoclonal antibodies (mAbs), it is critical to identify and monitor the various modifications that these mAbs can be subjected to during production, purification, and storage. The modifications can include deamidation, C-terminal lysine truncation, and other post-translational modifications.

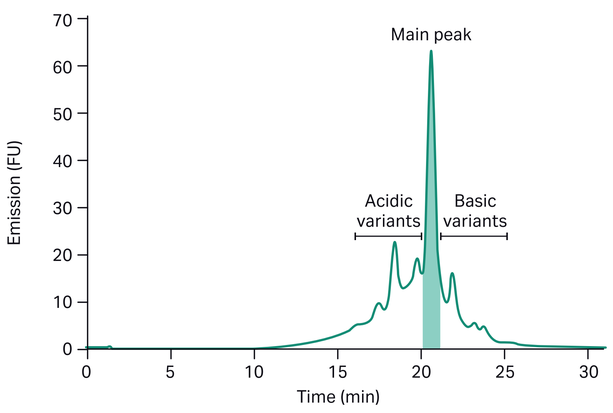

Fig 1. Chromatogram from analytical cation exchange chromatography showing the profile of a mAb after the Protein A capture step. Variants eluting early are defined as acidic, whereas variants eluting later are defined as basic.

High-resolution ion exchange chromatography (IEX) is a powerful tool to separate charge variants of a mAb, with the charge variants being the result of various modifications.

Fig 2. Capto™ HiRes S 5/50 column.

Capto HiRes S 5/50 is a high-resolution cation exchange chromatography column based on spherical, highly cross-linked agarose with a mean particle size of 9 µm. It is well-suited for analysis of mAb charge variants. With high protein binding capacity it is also capable of running preparative IEX, where a suitable amount of protein can be collected in each fraction during a salt or pH gradient. Each fraction can then be analyzed in greater detail. Here we show analytical IEX on two mAbs and preparative IEX on one mAb where the collected fractions were analyzed using Multiple Attribute Monitoring by Mass Spectrometry (MAM-MS) for identification of specific mAb modifications.

Analytical IEX

Analytical IEX was carried out on two mAbs using the same buffers and gradient. The analyses of each mAb, in triplicate, were carried out to determine reproducibility and charge profile.

Experimental

Column:	Capto HiRes S 5/50 cation exchanger
Sample:	Purified mAb1 and mAb2
Sample volume:	50 µL
Buffer A:	2.5 mM Tris, 10 mM NaH_2PO_4 pH 6.5
Buffer B:	2.5 mM Tris, 10 mM NaH_2PO_4 pH 9.5
Gradient:	0% B during sample application and column wash, step to 85% B, gradient 85%–100% B, 30 min
Flow rate:	0.3 mL/min during elution
Detection:	280 nm
System:	HPLC

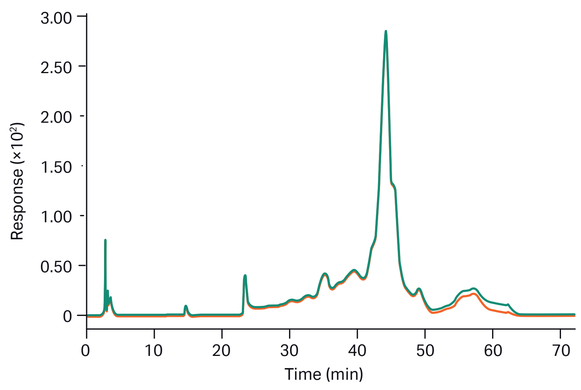

Fig 4. Analytical run of mAb1 in triplicate shows very good reproducibility, with acidic (36%) and basic (31%) variants of the mAb well-separated from the main component.

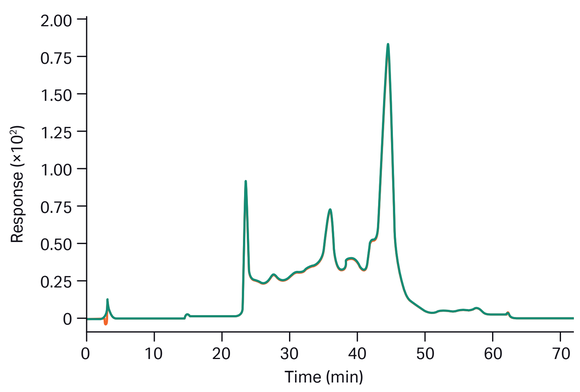

Fig 5. Analytical run of mAb2 in triplicate shows very good reproducibility with acidic variants (60%) and basic (11%) variants.

- Acidic and basic mAb variants well separated from main peak
- Good repeatability
- mAb1 and mAb2 show different charge profiles, possibly due to different cell-lines and different harvest and storage conditions.

Preparative IEX

High-resolution fractionation of charge variants of a mAb on Capto HiRes S 5/50. 30 fractions were collected and further analyzed using Multi attribute monitoring by mass spectrometry (MAM-MS). Peptides with deamidation and remaining C-terminal Lysine were identified in the fractions (Figure 6).

Experimental

Column:	Capto HiRes S 5/50 cation exchanger
Sample volume:	200 µL
Sample:	Purified mAb1, 4.7 mg/mL
Buffer A:	2.5 mM Tris, 10 mM NaH_2PO_4 pH 6.5
Buffer B:	2.5 mM Tris, 10 mM NaH_2PO_4 pH 9.5
Gradient:	0% B during sample application and column wash, step to 85% B, gradient 85%-100% B in 9 CV
Flow rate:	2 mL/min during equilibration, 0.3 mL/min during elution
Detection:	280 nm
Fraction volume:	0.5 mL
System:	ÄKTA™ pure

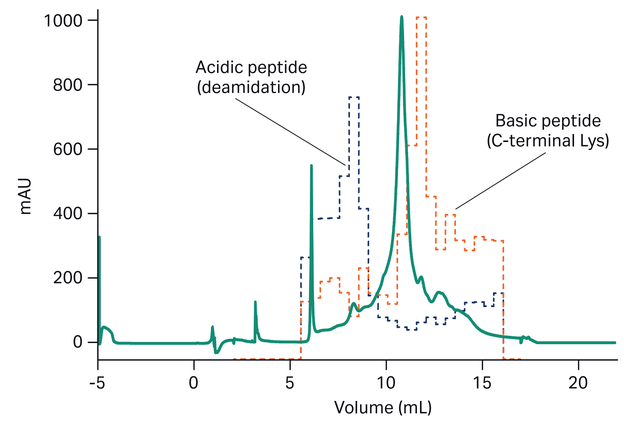

Fig 6. High-resolution fractionation of charge variants of mAb1.

The fractionation enabled detection of the modified peptides, as they are in low abundance compared to the unmodified peptides in the main peak. Figure 7 show the ratio of the deamidation in the fractions compared to the ratio of deamidation in the corresponding non-fractionated mAb standard.

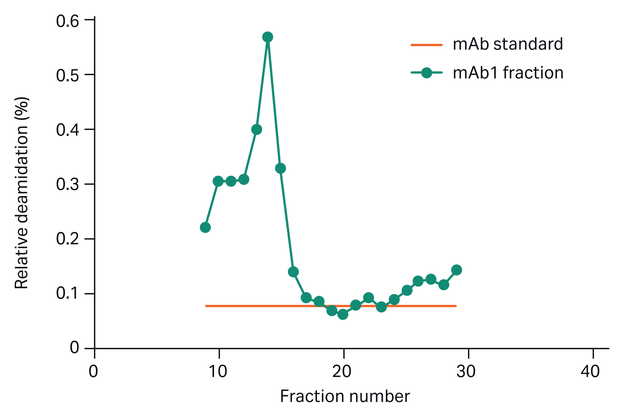

Fig 7. Relative ratio of deamidated peptide in the fractions compared to baseline of un-fractionated mAb1 standard.

- Analyses show the fractions containing acidic variants have a higher degree of deamidation of a peptide sequence.
- Analyses also show the fractions containing basic variants have a higher degree of C-terminal lysine truncation.

Workflow scheme Multiple Attribute Monitoring by Mass Spectrometry

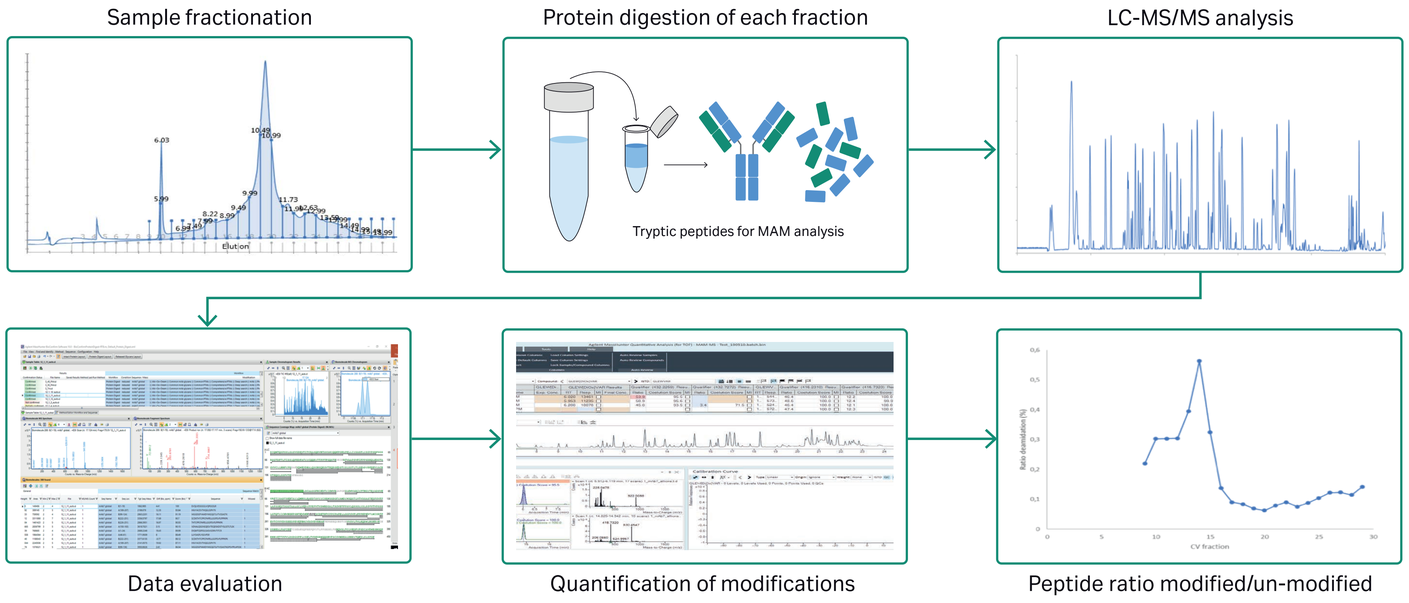

Fig 3. A typical workflow scheme for MAM-MS analysis.

Conclusions

- Capto HiRes S 5/50 column is a versatile, high-resolution IEX column, capable of separating charge variants of a purified mAb in both analytic and preparative scales.
- Capto HiRes S 5/50 preparative fractionation enables in-depth characterization of separated and collected mAb.
- Using an MAM-MS approach on Capto HiRes S fractions facilitates identification of specific mAb modifications.

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