

## Rapid LC-MS Analysis of mAb Charge Variant with a 20 mm Cation Exchange Column

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This is an Application Brief and does not contain a detailed Experimental section.

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### Abstract

To demonstrate the use of a 2.1 x 20 mm BioResolve™ Premier SCX mAb 3 µm Column for rapid mAb charge variant separation and LC-MS analysis in cation exchange mode.

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### Introduction

High throughput methods can provide a benefit during biopharmaceutical product development. Cation Exchange Chromatography (CX) has been used as an effective tool for characterizing monoclonal antibody (mAb) charge variants. A 3 µm particle size cation-exchange column coupled with MS-compatible mobile phases has been shown to produce excellent results for mAb charge variant characterization.<sup>1-3</sup> Here, we show that employing a short, 2.1 x 20 mm BioResolve Premier SCX mAb 3 µm Column (p/n: [186011020](https://www.waters.com/nextgen/global/shop/columns/186011020-bioresolve-premier-scx-mab-column-3--m-) <  
<https://www.waters.com/nextgen/global/shop/columns/186011020-bioresolve-premier-scx-mab-column-3--m->

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[21-mm-x-20-mm-1-pk.html](#) ), can provide useful LC-MS mass data for intact mAb charge variant analysis with approximately four times faster analysis times compared to a 100 mm column.

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## Results and Discussion

Figure 1 shows UV chromatograms of NIST mAb (Humanized mAb mass check standard, p/n: [186009125](#) <<https://www.waters.com/nextgen/global/shop/standards--reagents/186009125-humanized-mab-mass-check-standard.html>> ) charge variant separation using MS-compatible pH gradient mobile phases (IonHance CX-MS pH concentrates, p/n: [176004498](#) <<https://www.waters.com/nextgen/global/shop/standards--reagents/176004498-ionhance-cx-ms-ph-concentrates-a--b-kit-in-ms-certified-ldpe-con.html>> ) on a 2.1 x 20 mm column and a 2.1 x 100 mm column with the same gradient slope with regard to column volumes. Despite reduction in resolution, the MS data obtained on the 20 mm column and the 100 mm column were comparable (Figure 2). Based on column length the resolution for the 100 mm column is predicted to be 2.2 times greater than that of the 20 mm column ( $R_s \propto \sqrt{L}$  column), however, in this comparison the resolution (measured at half-height) between the main and basic peaks on the 100 mm column was 1.4 times greater. The deconvoluted mass indicated that the basic peak is likely the NIST mAb with one C-terminal lysine, consistent with previous findings.<sup>1,3</sup> Notably, the analysis time is approximately four times faster using the 20 mm column than when using the 100 mm column. As an added benefit, mobile phase use is reduced 5-fold and sample volume is lowered 5-fold.

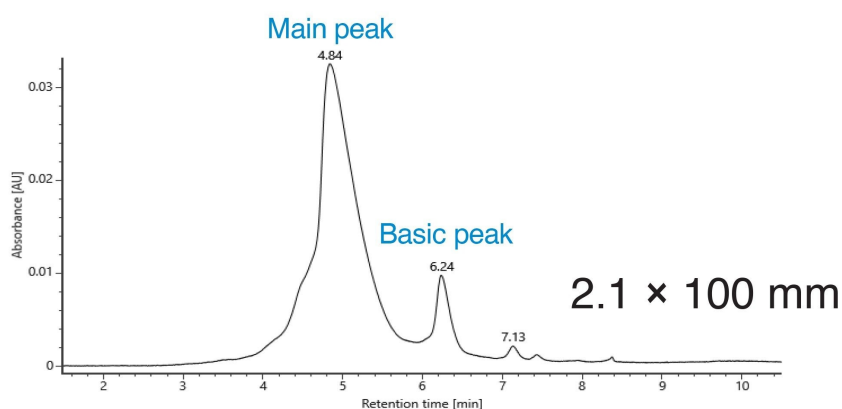
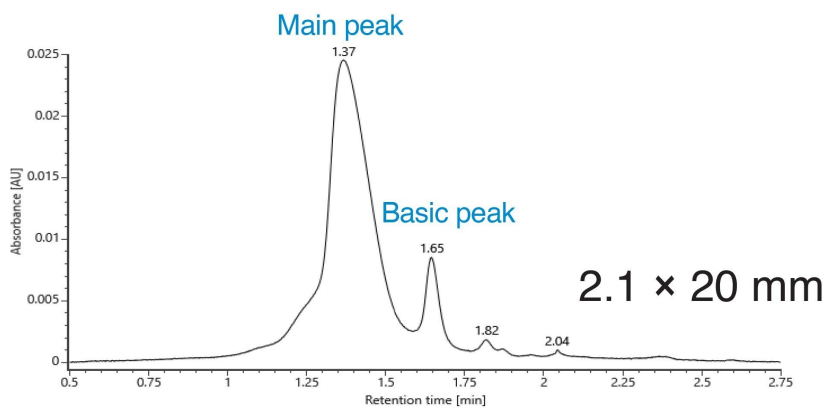


Figure 1. UV (280 nm) chromatograms of NIST mAb (Humanized mAb mass check standard, 2.5 mg/mL in water) charge variant separation on a 2.1 x 20 mm BioResolve Premier SCX mAb 3  $\mu$ m Column and a 2.1 x 100 mm BioResolve SCX mAb 3  $\mu$ m Column. Mobile phase A: 10-fold dilution of IonHance CX-MS pH concentrate A, mobile phase B: 10-fold dilution of IonHance CX-MS pH concentrate B. 57%B–80%B in 1.2 minutes (20 mm column) or 6 minutes (100 mm column). Flow rate: 0.3 mL/min. Injection volume: 1  $\mu$ L (20 mm column) or 5  $\mu$ L (100 mm column). Column temperature: 30  $^{\circ}$ C. The analysis time is approximately four times faster on the 20 mm column than on the 100 mm column, despite loss of resolution on the 20 mm column.

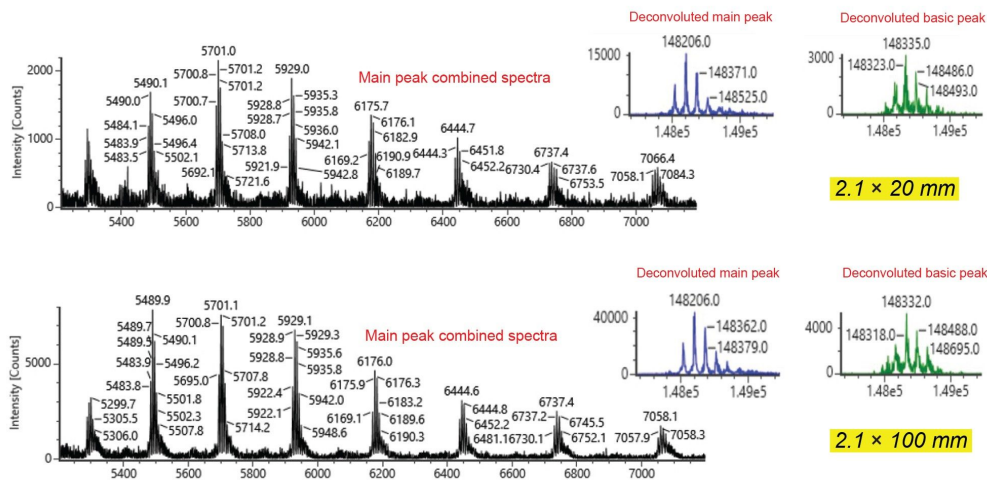


Figure 2. LC-MS analysis of NIST mAb (Humanized mAb mass check standard, 2.5 mg/mL in water) charge variants on a 2.1 x 20 mm BioResolve Premier SCX mAb 3  $\mu$ m Column and a 2.1 x 100 mm BioResolve SCX mAb 3  $\mu$ m Column. Xevo™ G2 XS QToF was used for MS detection. Data in black are combined spectra of the main peak (5200–7200 m/z window) obtained from the 20 mm column (top) and the 100 mm column (bottom); Data in blue and green are deconvoluted spectra of the main peak and the basic peak obtained from the 20 mm column (top) and the 100 mm column (bottom). The MS data were comparable using the 20 mm column and the 100 mm column.

## Conclusion

A 20 mm BioResolve Premier SCX mAb 3  $\mu$ m cation-exchange column can effectively separate intact mAb and its charge variants. The analysis time is about four times faster using the 20 mm column than using the 100 mm column and consumes significantly lower quantities of mobile phases. Although the high-throughput methods result in reduced resolution, the MS data were comparable between the 20 mm and the 100 mm columns, leading to high confidence for charge variant characterization.

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## References

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