

## Rapid mAb Charge Variant Analysis with 20 mm Cation Exchange Columns

---

Hua Yang, Lili Macedo, Stephan M. Koza, Steve Shiner

Waters Corporation

---

### Abstract

Rapid and reliable, 6-minute, cation-exchange (CEX) charge variant analyses of NISTmAb using either a salt or a pH gradient with a BioResolve™ Premier SCX mAb, 3 µm, 2.1 x 20 mm Column are presented. Although resolution is predictably decreased on the 20 mm column in comparison to earlier results on longer columns, when increased sample throughput is needed effective separations can still often be obtained with five times faster analysis times.

### Benefits

- Rapid cation exchange methods for intact mAb profiling using 2.1 x 20 mm BioResolve Premier SCX mAb 3 µm Column
- Decreased mobile phase use and sample load requirements

---

### Introduction

High throughput methods are in demand due to the rapid growth of biopharmaceuticals such as monoclonal

---

antibodies (mAb). To meet these needs, the use of significantly reduced analytical LC column lengths to decrease analysis times, has been proposed.<sup>1-2</sup> While in these reports, the shorter format columns can noticeably compromise chromatographic detail, they may still provide adequate peak resolutions for high throughput applications. One such method is cation-exchange chromatography (CEX) which is often used to monitor native-state therapeutic protein (*e.g.* mAb) charge variants. In addition, increasing the sample throughput of CEX can greatly benefit the manufacturing and formulation development of these therapeutics. In this application note, we present rapid, 6-minute, cation-exchange charge variant analyses of NISTmAb using either salt or pH gradient cation-exchange methods with a 20 mm column length BioResolve Premier SCX mAb Column.

---

## Experimental

### Sample Description

NISTmAb RM 8671 (10 mg/mL) diluted in water to a concentration of 2.5 mg/mL.

### Method Conditions

### LC Conditions

LC system:	ACQUITY™ UPLC™ H-Class Bio
Detection:	ACQUITY TUV at 280 nm (flow cell: 5 mm, 1.5 µL)
Column:	BioResolve Premier SCX mAb 3 µm 2.1 x 20 mm, (p/n: 186011020)
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	1 µL

Flow rate: 0.3 mL/min

Mobility phases:

For salt gradient:

Mobile phase A: 20 mM MES pH 6.7

Mobile phase B: 20 mM MES + 1M NaCl, pH 6.7

For pH gradient:

Mobile phase A: 10-fold dilution of BioResolve CX  
pH concentrate A, pH 5.0 (p/n: 186009063)

Mobile phase B: 10-fold dilution of BioResolve CX  
pH concentrate B, pH 10.2 (p/n: 186009064)

## Gradient Table for salt gradient

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.0	0.3	99	1	Initial
0.5	0.3	94	6	11
1.0	0.3	94	6	6
4.0	0.3	89	11	6
4.5	0.3	50	50	6
5.0	0.3	50	50	6
5.1	0.3	99	1	6
6.0	0.3	99	1	6

## Gradient Table for pH gradient

Time (min)	Flow rate (mL/min)	%A	%B	Curve
0.00	0.3	100	0	Initial
0.20	0.3	100	0	11
4.72	0.3	0	100	6
4.92	0.3	0	100	6
5.12	0.3	100	0	6
6.00	0.3	100	0	6

## Data Management

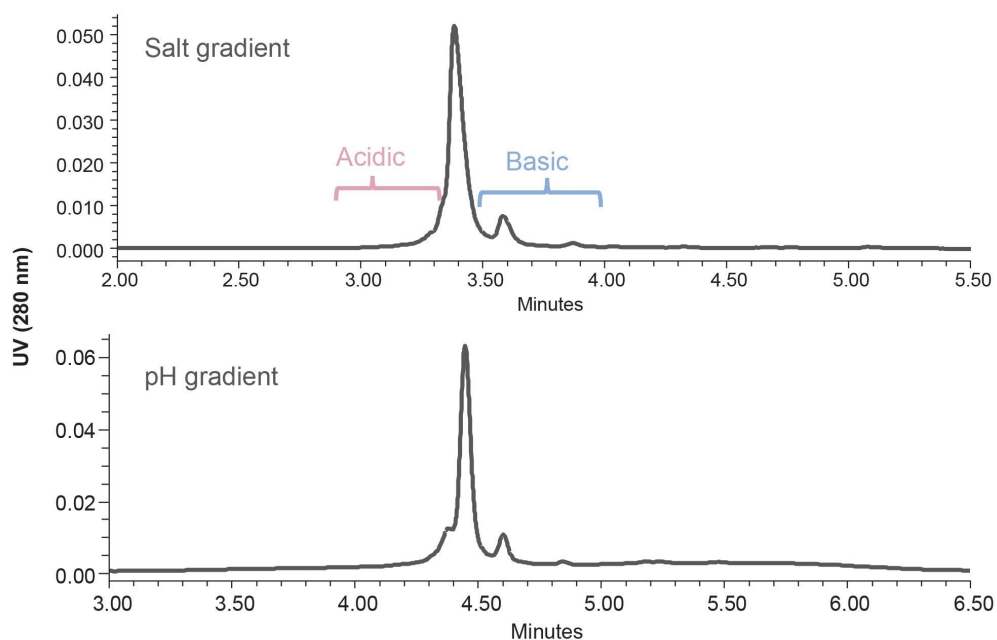
LC software:

Empower™ 3

---

## Results and Discussion

Examples of salt and pH gradient separations of intact NISTmAb on a 2.1 x 20 mm Column (3–and 4.5–min elution gradient, 2.5 µg sample load) are shown in Figure 1. In agreement with previously reported studies, the separation of the acidic charge variants is often improved for the pH gradient. We also observe that when compared to the CEX separations of NISTmAb on a longer column with 4.6 mm ID, there is a greater loss of chromatographic detail for the salt gradient separation as compared to that generated with a pH gradient on the 20 mm length column.<sup>3–4</sup> Despite these compromises, the use of a 2.1 x 20 mm CEX Column, with a low dispersion LC system may still provide more than adequate separations to enable the charge variant analyses of proteins at five times the sample throughput of the referenced higher resolution methods. As an added benefit, the sample and mobile phase volume used can be more than 10-times lower compared to a 4.6 x 50 mm CEX column.



*Figure 1. Example of salt and pH gradient separations of NISTmAb charge variants at 0.3 mL/min with a 3- and 4.5-min elution gradient using a BioResolve Premier SCX mAb 2.1 x 20 mm Column. Additional details are provided in the text.*

High throughput LC applications demand that columns be rugged and able to withstand harsh conditions. To demonstrate ruggedness, the BioResolve Premier SCX mAb 2.1 x 20 mm Column was subjected to a series of 500 rapid pressure cycles from zero to 4000 psi on-column. NISTmAb was analyzed using a salt gradient before and after pressure cycling to evaluate the change in chromatographic performance (Figure 2). Negligible changes in resolution and charge variant profiles were observed, indicating a high level of mechanical bed stability.

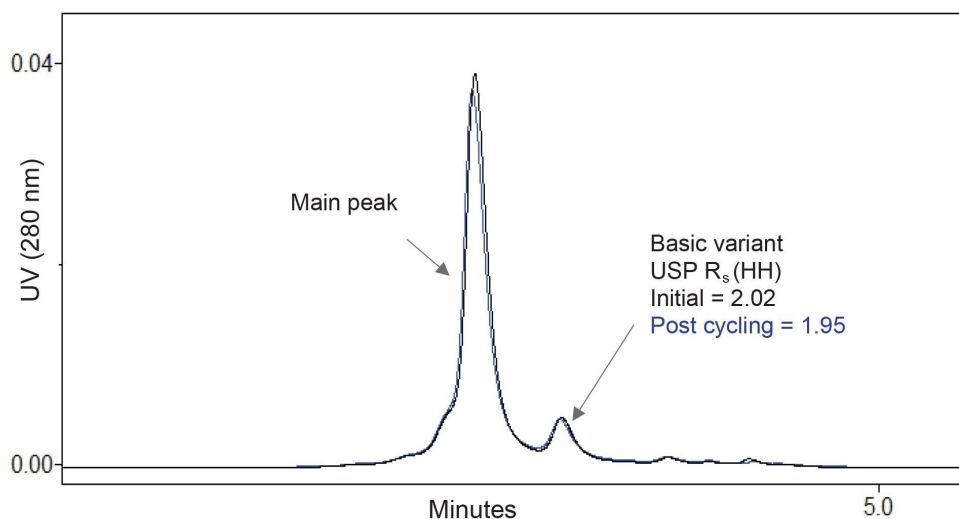


Figure 2. Example of salt gradient separations of NISTmAb charge variants obtained before and after 500 pressure cycles from 0 to 4000 PSI (275 bar) on a BioResolve Premier SCX mAb 2.1 x 20 mm Column. A minor loss of resolution (~3.5%) was observed for the basic variant. LC method was: 75 to 125 mM NaCl, 3 minute gradient, 20 mM MES pH 6.0, 0.2 mL/min, 30 °C, TUV = 280 nm.

## Conclusion

High throughput (6 minute/analysis) CEX methods run on a 2.1 x 20 mm BioResolve Premier SCX mAb 3  $\mu$ m Column may provide useful mAb charge variant separations, albeit with reduced resolutions. An elution gradient based on pH maintained more effective resolution than the salt gradient for the acidic forms of NISTmAb. In addition to higher throughput, the 2.1 x 20 mm column also enables reduced mobile phase use, instrument running time, and smaller sample volumes while providing robust performance.

## References

1. Fekete S. and Guillarme D. Ultra-short columns for the chromatographic analysis of large molecules. *Journal of Chromatography A* 1706 (2023) 464285.
2. Navarro-Huerta J.A., Murisier A., Nguyen J.M., Lauber M.A., Beck A., Guillarme D., Fekete S. Ultra-Short Ion-Exchange Columns for Fast Charge Variants Analysis of Therapeutic Proteins. *Journal of Chromatography A* 1657 (2021) 462568.
3. Yang H., Warren W., and Koza S. AutoBlend Plus Technology for Ion-Exchange Chromatography Method Development and Robustness Testing. Waters Application Note. [720006557](#), April 2019.
4. Wang Q, Rzewuski S. C., Lauber M. A., Development of pH Gradient Mobile Phase Concentrates for Robust, High Resolution mAb Charge Variant Analysis. Waters Application Note. [720006491](#), January 2019.

---

## Featured Products

ACQUITY UPLC H-Class PLUS Bio System <<https://www.waters.com/10166246>>

ACQUITY UPLC Tunable UV Detector <<https://www.waters.com/514228>>

Empower Chromatography Data System <<https://www.waters.com/10190669>>

720008295, April 2024



© 2024 Waters Corporation. All Rights Reserved.

[Conditions d'utilisation](#) [Confidentialité](#) [Marques](#) [Carrières](#) [Cookies](#) [Préférences de cookies](#)