Application note | 001694

Biopharma

Salt gradient analysis of monoclonal antibodies using a 3 µm monodisperse SCX chromatography column

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Keywords

Monoclonal antibodies, mAb, salt gradient, strong cation exchange, monodisperse, NISTmAb, Infliximab, Pertuzumab, Vedolizumab, Secukinumab, ProPac 3R SCX 3µm column, HPLC, liquid chromatography, biopharma

Application benefits

- High-resolution separation of protein charge variants
- Easy, straightforward method development
- Consistent lot-to-lot and column-to-column performance

Goal

Demonstrate basic salt gradient method development for high-resolution analysis of monoclonal antibodies using a 4 × 100 mm, 3 µm monodisperse particle SCX column

Introduction

Proteins have been used as a major class of therapeutics for the treatment of various diseases including cancer, cardiovascular disease, and autoimmune disorders, and continue to grow as therapeutics become more complex. Proteins typically have an isoelectric point (pI) ranging from 4.0 to 12.0, based on their amino acid composition, glycosylation profile, and other post-translational modifications. Ion exchange chromatography (IEX) is a standard technique for analyzing proteins and their associated variants based on their accessible surface charge. Strong cation exchange (SCX) columns have commonly been used for the evaluation of basic proteins with a pI ≥ 7.0; however, they can also be used for lower pI proteins with a properly designed

method. As the complexity of therapeutics increases, continued improvements in analytical technologies will be required to characterize these therapeutics and fulfill regulatory requirements to bring new therapeutics to market.

The Thermo Scientific™ ProPac™ 3R SCX column is a strong cation exchange product designed to achieve high-efficiency protein separations. The packing material is based on a 3 µm, nonporous, monodisperse divinylbenzene polymer substrate to provide exceptionally high resolving power. Compared to traditional polydisperse particles (right image, Figure 1), the monodisperse particles (left image, Figure 1) have a more consistent size distribution providing better control of column fluidics and particle surface chemistry. A thin, hydrophilic layer is grafted to the particle, reducing secondary interactions of protein samples with the hydrophobic core. This paired with precisely controlled grafting of SCX functionality minimizes band broadening for maximal resolution. The sulfonate functionality grafted to the hydrophilic layer introduces permanently charged anionic sites that provide the strong cation exchange character required for promoting protein binding when using a low ionic strength mobile phase at an appropriate pH (e.g., 20 mM MES, pH 6.5). The reproducible resin chemistry and manufacturing processes eliminate column variability as a concern in method development and data analysis. ProPac 3R SCX columns are packed in a biocompatible polyether ether ketone (PEEK) hardware to minimize nonspecific adsorption of protein samples compared to metal-based hardware choices.[1] Combined, these design choices make the ProPac 3R SCX column capable of analyzing complex proteins with high resolution and excellent reproducibility.

In this application note, practical examples of method design are shown and discussed for the development of both a fast QC method and a longer high-resolution analytical method for monoclonal antibodies and associated variants. NISTmAb is used as a model protein for this case study because it has a pI value of 9 and is commercially available as a reference standard. The utility of the final method for differentiating sample quality is demonstrated by comparing unstressed NISTmAb against a temperature stressed NISTmAb. The reproducibility of the method and separation is also shown by comparing three different synthetic lots of ProPac 3R SCX media made on three different batches of 3 µm monodisperse base resin. Having established a clear protocol for creating robust chromatography methods, we apply this approach to four additional mAb therapeutics currently used for treatments. Lastly, the performance of the ProPac 3R SCX column is compared against our current Thermo Scientific™ MAbPac™ SCX-10 column and a competitor's 3 µm SCX column.

This work demonstrates that the ProPac 3R SCX column allows for easy method optimization to provide excellent sensitivity and performance under a broad range of pH, temperature, and mobile phase compositions. Users can be confident in the detection and identification of acidic or basic variants of existing and novel protein therapeutics during late-stage development, cellular production, downstream purification, storage, and shipping. The ProPac 3R SCX column provides the required performance to meet characterization and regulatory requirements of current and future therapeutics as they continue to increase in complexity.

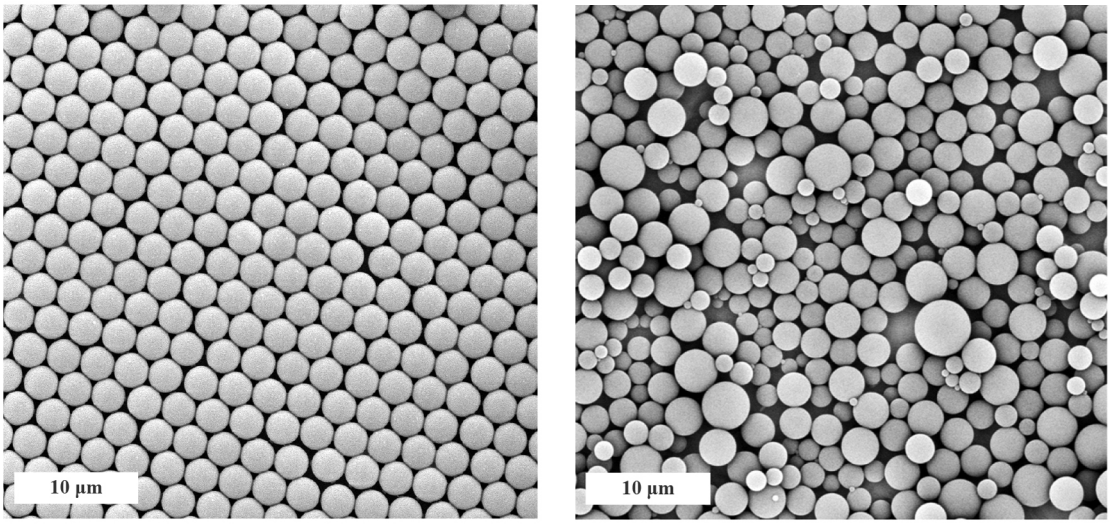


Figure 1. SEM images of monodisperse ProPac 3R (left) vs. polydisperse (right). White scale bars are 10 µm in length.

Experimental

Reagents and consumables

- Deionized water, 18.2 MΩ·cm resistivity
- MES (Sigma-Aldrich, P/N M8250)
- Sodium chloride (Sigma-Aldrich, P/N S9888)
- MOPS (Sigma-Aldrich, P/N M1254)
- NISTmAb (NIST™, 8671)
- Thermo Scientific™ SureSTART™ 2 mL Polypropylene Screw Top Microvials (P/N 6ESV9-04PP)
- Thermo Scientific™ SureSTART™ 2 mL Screw Caps (P/N 6ASC9ST1)

Sample preparation

All mAb samples except NISTmAb were diluted to 5 mg/mL using DI water. NISTmAb was used as-is from NIST at 10 mg/mL concentration.

Instrumentation

- Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system, including:
  - System Base Vanquish Flex (P/N VF-S01-A)
  - Quaternary Pump (P/N VF-P20-A)
  - Column Compartment H (P/N VH-C10-A)
  - Split Sampler FT (P/N VF-A10-A) with 25 µL (V = 50 µL) sample loop
  - Thermo Scientific™ Vanquish™ Variable Wavelength Detector (P/N VF-D40-A) with Thermo Scientific™ Vanquish™ Variable Wavelength Detector Flow cell (P/N 6077.0300)

Column

- ProPac 3R SCX 3 µm, 4 × 100 mm, P/N 43103-104068

For mobile phase compositions and gradient conditions, including flow rate, column temperature, and injection volume, reference the text and figures in the results and discussion section. Absorbance at 280 nm was used for detection of all samples.

Data processing

- Thermo Scientific™ Chromeleon™ 7.2.10 Chromatography Data System was used for data acquisition and analysis.

Results and discussion

Optimization of chromatographic parameters is critical to designing methods that provide high resolution, high reproducibility protein separations. In this section, we demonstrate a straightforward approach to develop and optimize a salt gradient method for the analysis of NISTmAb. A set of parameters in the order of importance, including mobile phase pH, sample loading conditions, gradient time and flow rate, are optimized as shown in Figure 2. A fast QC method and a longer high-resolution analytical method are shown to demonstrate the separation capability of the column. Columns from different media lots are used after method development to show the method robustness.

Mobile phase pH effect on chromatography

Mobile phase pH selection can influence the separation of proteins and their associated variants depending on the isoelectric point (pI, the pH at which the overall charge of the protein is neutral) of the protein of interest. For cation exchange chromatography, mobile phase buffer pH should be lower than the pI of the protein to promote binding of the sample to the anionic stationary phase; otherwise, the analytes will have an overall negative charge and fail to adsorb to the stationary phase.

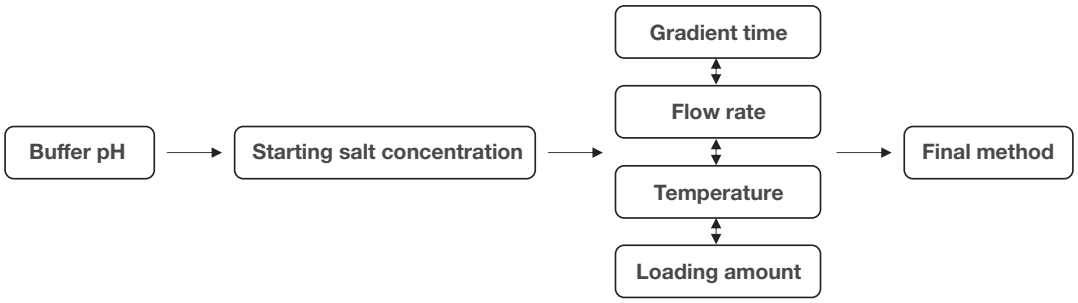


Figure 2. Method development flow chart

This effect can be observed in Figure 3 showing the analysis of NISTmAb using MES buffers at pH 6.0 and 6.5 and MOPS at pH 7.0. We recommend using Good's buffers due to their compatibility with biological molecules, good water solubility, and ability to buffer across the physiological pH range.[2] These Good's buffers were specifically chosen because they are neutral zwitterions that will not function as salts in the gradient separation. As the pH of the mobile phase increases, the proteins elute earlier in the chromatogram as basic groups are less likely to be protonated, resulting in reduced charge of the protein and/or other neutral groups becoming deprotonated, increasing the number of anionic sites. The separation of acidic variants improves from pH 6.0 to 6.5 but little improvement is visible when going to pH 7.0. Overall, the basic variant separation changes little across the pH range. Based on these results, pH 6.5 was chosen for further method development as this pH would promote stronger protein binding than pH 7.0.

Column: ProPac 3R SCX, 3 µm
Format: 4 × 100 mm
Eluents: A: 20 mM Buffer (see chromatogram)
B: 20 mM Buffer + 0.5 M NaCl

Gradient:

Time (min)	%A	%B
0.0	90	10
25.0	70	30
25.1	20	80
27.0	20	80
27.1	90	10
37.0	90	10

Flow rate: 0.5 mL/min
Inj. volume: 2 µL
Temp: 35 °C
Detection: UV, 280 nm
Sample: NISTmAb – 10 mg/mL
Peak label: Retention time

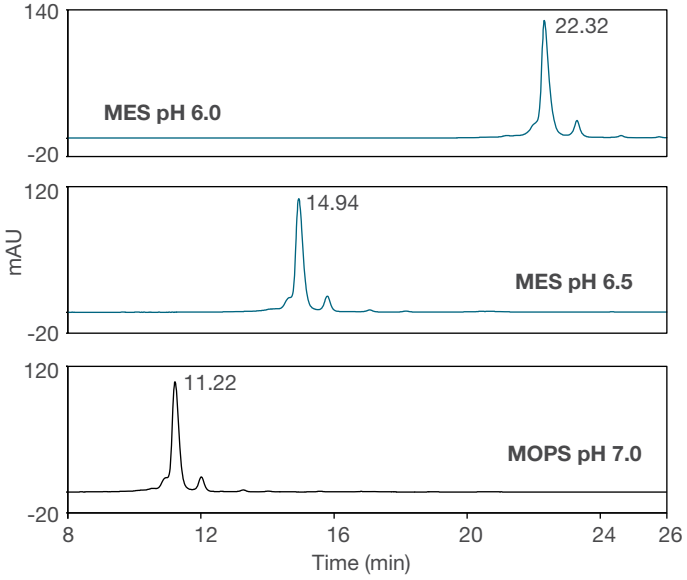


Figure 3. Zoomed in time range showing the effect of buffer pH on NISTmAb variant separation: 20 mM MES pH 6.0 (top), 20 mM MES pH 6.5 (middle), and 20 mM MOPS pH 7.0 (bottom)

Determining gradient salt concentrations for sample loading

For consistent performance, it is necessary to design the gradient method so the protein is separated by the change in salt concentration without isocratic elution occurring during loading. Isocratic elution during loading can result in reduced lot-to-lot and column-to-column reproducibility for a given method. Figure 4 compares the elution of NISTmAb using gradients at two different initial salt concentrations of 50 mM (10% B) and 80 mM (16% B) NaCl. For each starting salt concentration, the separation is evaluated with and without an isocratic hold (the change in time between the injection and start of the gradient) to determine if

Column: ProPac 3R SCX, 3 µm
Format: 4 × 100 mm
Eluents: A: 20 mM MES, pH 6.5
B: 20 mM MES, pH 6.5
+ 0.5 M NaCl

Gradient: 16–26% B, 0 min isocratic hold
16–26% B, 5 min isocratic hold
10–20% B, 0 min isocratic hold
10–20% B, 5 min isocratic hold

Gradient time: 25 min

Flow rate: 0.5 mL/min
Inj. volume: 2 µL
Temp: 35 °C
Detection: UV, 280 nm
Sample: NISTmAb – 10 mg/mL
Peak label: Retention time - PWHH

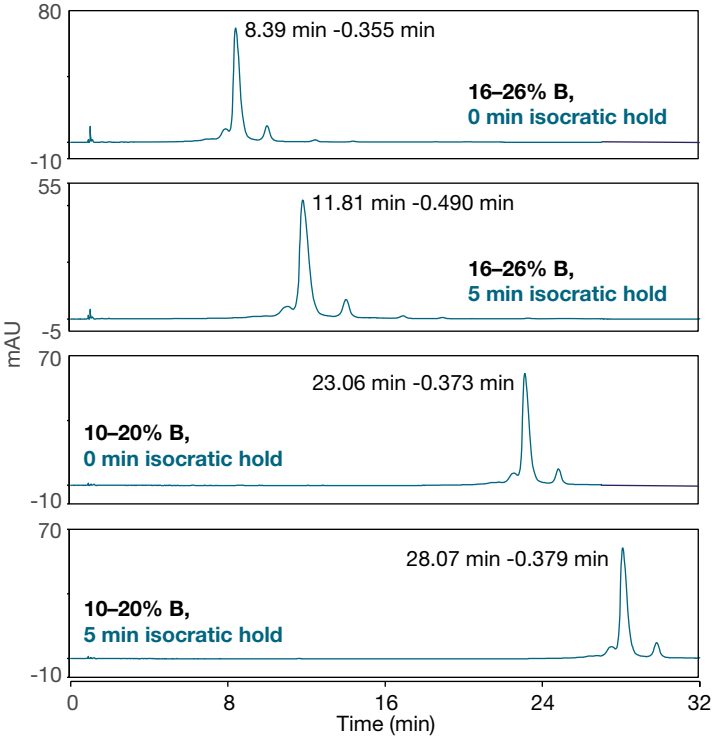


Figure 4. Effects of starting salt concentration on NISTmAb variant separation and PWHH of main mAb peak with and without a 5-minute isocratic hold: 16–26% B (top two) and 10–20% B (bottom two)

isocratic elution occurs during loading. At 50 mM NaCl loading, the separation of the variants relative to the main peak are consistent with and without the isocratic hold and the main peak PWHH is unchanged. At 80 mM NaCl loading, the separation of the variants from the main peak increases with the isocratic hold and the PWHH of the main peak increases. Based on these results, isocratic elution is observed at 80 mM NaCl loading but not at 50 mM NaCl.

Comparison of the retention times with and without the 5-minute isocratic hold is also evaluated for NISTmAb. Isocratic elution occurs when the retention time difference for the protein and variant peaks with and without the hold decreases below 5 minutes and when the PWHH ratio becomes less than 1. Based on the results in Figure 4, 50 mM NaCl would be the recommended salt loading concentration for NISTmAb with 20 mM MES pH 6.5 as it is the maximum salt concentration observed to meet both criteria. Choosing this starting condition will provide better column-to-column and lot-to-lot consistency for a given method.

Gradient slope effect on salt gradient separation

After selecting mobile phase pH and starting gradient conditions, the gradient slope (rate of salt concentration change) should be determined next as it will have the greatest effect on separation. Figure 5 shows the analysis of NISTmAb using a salt gradient from 50 to 150 mM NaCl over 10, 20, and 30 minutes. This illustrates the effect of gradient time on the retention time and separation of variant peaks from the main protein peak. Comparison of chromatograms at different gradient times shows that the gradient is primarily responsible for separation of the variant peaks from the main peak. For NISTmAb, a peak-to-valley for the proximal acidic peak eluting is only achieved at longer gradient times and in this example is maximized with the longest gradient. The gradient slope should be optimized to balance the need of analysis time with peak resolution and is dependent on the user's objectives. In a rapid QC environment where all peak identities are known, a shorter gradient could be used for fast analysis; whereas, during discovery in an R&D lab, the longer gradient maximizing peak resolution may be practical for doing further investigations of unknown peaks.

Column:	ProPac 3R SCX, 3 µm
Format:	4 × 100 mm
Eluents:	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl
Gradient:	10–30% B; see chromatogram for gradient time
Flow rate:	0.5 mL/min
Inj. volume:	2 µL
Temp:	30 °C
Detection:	UV, 280 nm
Sample:	NISTmAb – 10 mg/mL
Peak label:	Retention time

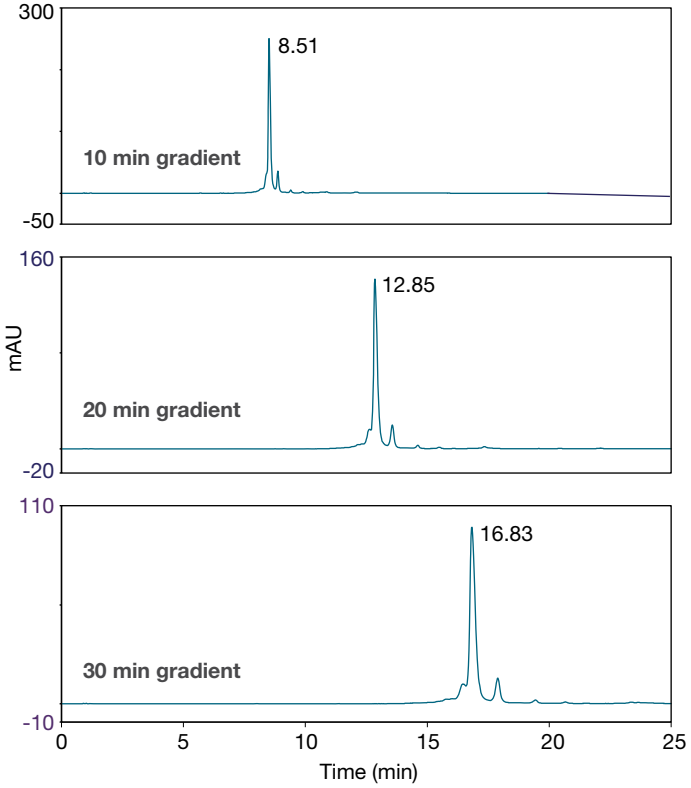


Figure 5. Effect of gradient time on the separation of NISTmAb and its charge variants. Time scale is zoomed-in to 25 minutes to aid visualization of variant peaks.

Flow rate effect on salt gradient separation

After determining an appropriate gradient, other parameters with small but important effects on the separation can be optimized. Figure 6 shows the analysis of NISTmAb using a flow rate of 0.3, 0.4, and 0.5 mL/min.

Column:	ProPac 3R SCX, 3 µm		
Format:	4 × 100 mm		
Eluents:	A: 20 mM MES, pH 6.5		
	B: 20 mM MES, pH 6.5 + 0.5 M NaCl		
Gradient:	Time (min)	%A	%B
	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20	80
	33.1	90	10
	40.0	90	10
Flow rate:	Top: 0.3 mL/min		
	Middle: 0.4 mL/min		
	Bottom: 0.5 mL/min		
Inj. volume:	2 µL		
Temp:	30 °C		
Detection:	UV, 280 nm		
Sample:	NISTmAb – 10 mg/mL		
Peak label:	Retention time		

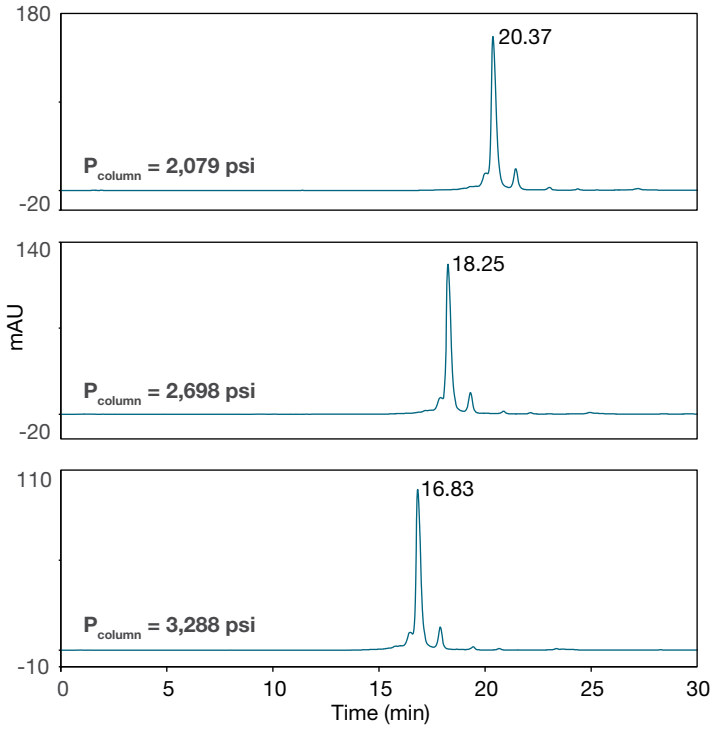


Figure 6. Effect of flow rate on the separation of NISTmAb and associated charge variants

The retention time of the main peak and associated variants decreases with increasing flow rate primarily due to a decrease in gradient delay. With increasing flow rate, the separation of the (left) acidic and (right) basic peaks from the main protein peak decreases slightly. The PWHH of the main protein peak is largely independent of flow rate under the gradient time and loading masses tested. With increasing flow rate, a small increase in the peak to valley of the proximal acidic peak is observed, which would improve the quantitation of this group. Comparison of the signal strength on the y-axis of the chromatograms shows that using lower flow rates results in increased signal strength due to a higher sample concentration in the detector at lower flow rates. Because of this, lower sample mass loading may be used with lower flow rates while still being able to detect and quantify the sample peaks. Lastly, the overall column pressure is an important consideration for some users. The total system pressure decreases from ~3,300 psi to ~2,100 psi when going from 0.5 to 0.3 mL/min. As with gradient slope, the conditions selected are dependent on the user's needs. High flow rates would benefit rapid testing QC environments; whereas R&D groups with limited sample may benefit greatly from using a lower flow rate to maximize peak signal especially for low abundance variants

Temperature effect on salt gradients

Temperature is another parameter that can be adjusted to optimize separation of proteins and their charge variants. Typically, proteins will elute later in the gradient at higher temperature due to a decrease of waters of hydration around ionic groups; however, Figure 7 shows that in this case NISTmAb elutes slightly earlier in the gradient with increasing temperature possibly due to weakened interactions between the stationary phase and the protein. While the retention time is largely unaffected, small improvements for both acidic and basic variant separations are observed with increasing temperature due to decreasing peak width and increased peak-to-valley ratios for proximal acidic and basic peaks. A secondary benefit of increasing temperature is reduced column pressure from ~3,200 psi at 30 °C to ~2,600 psi at 60 °C due to reduced viscosity of the mobile phase. However, higher temperatures may also alter the properties of the sample itself, e.g., due to on-column sample oxidation. In the absence of knowledge of how temperature may adversely affect the sample, we recommend using 30 °C. The user should consider these effects when selecting the temperature for their respective analysis.

Column:	ProPac 3R SCX, 3 µm
Format:	4 × 100 mm
Eluents:	A: 20 mM MES, pH 6.5
	B: 20 mM MES, pH 6.5 + 0.5 M NaCl

Gradient:	Time (min)	%A	%B
	0.0	90	10
	25.0	70	30
	25.1	20	80
	27.0	20	80
	27.1	90	10
	37.0	90	10

Flow rate:	0.5 mL/min
Inj. volume:	2 µL
Temp:	30 °C – 60 °C
Detection:	UV, 280 nm
Sample:	NISTmAb – 10 mg/mL
Peak label:	Retention time

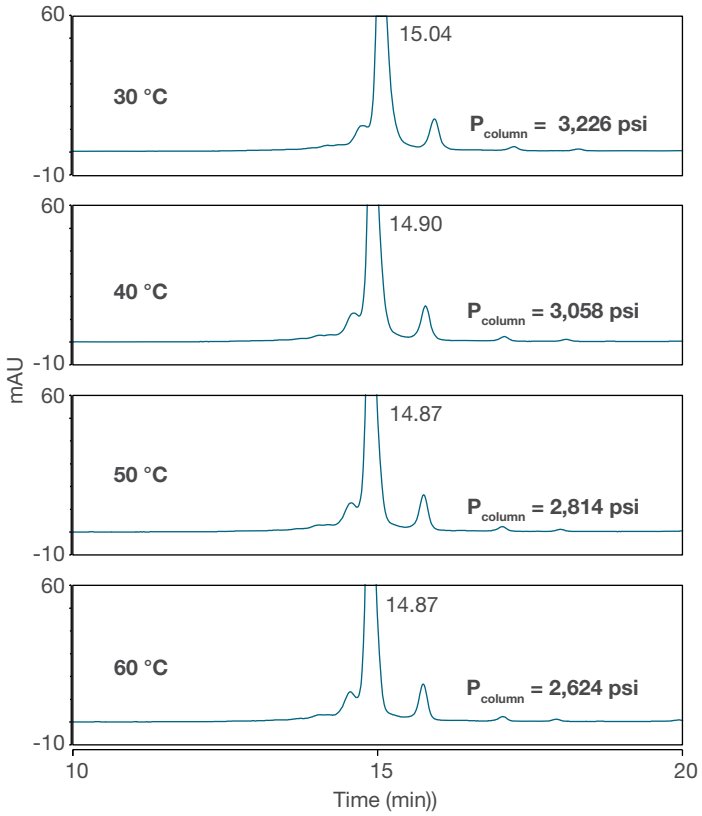
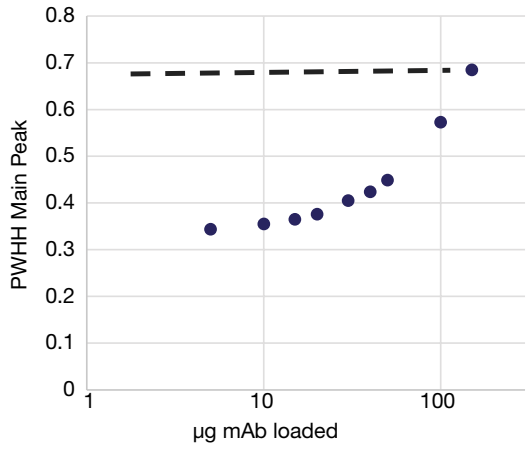


Figure 7. Zoomed in time and signal range showing the effect of temperature on NISTmAb and column pressure



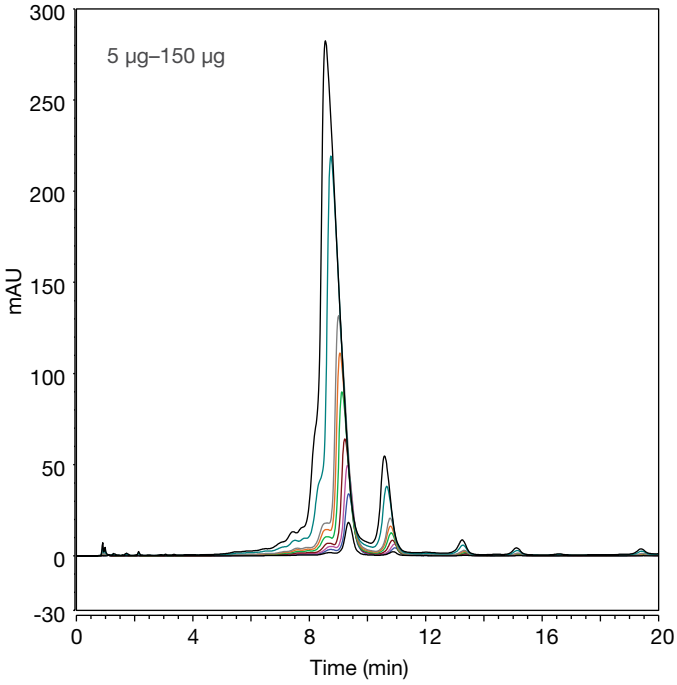


Figure 8. Chromatogram overlays showing the dynamic loading analysis of NISTmAb using a salt gradient (bottom). The plot (top) shows the corresponding PWHH of the main mAb peak against the masses of mAb loaded in the column. The dashed line indicates 2× PWHH of the lowest mass loading.

Sample loading and carryover analysis

For typical protein loading levels, sample concentration and injection volume do not significantly influence the separation of the protein and associated variants. The chromatograms in the bottom plot of Figure 8 show 5–150 µg loading of NISTmAb. As the sample loading amount increases above 50 µg shown in Figure 8, the stationary phase becomes overloaded, and the analysis of the sample begins to degrade due to peak broadening and shifting of peaks to earlier in the chromatogram as they are excluded from the stationary phase by adsorbed protein. The top plot in Figure 8 shows the PWHH of the main peak versus the mass of protein loaded. The dashed line in the plot indicates 2× the PWHH of the lowest mass loaded. This type of experiment is commonly referred to as dynamic loading capacity with overloading here defined as the loading mass of twice the PWHH of lowest mass loaded. The dynamic loading capacity is protein dependent and can vary depending on protein molecular weight and structure with lower molecular weight proteins generally having lower dynamic loading capacities due to their high surface area relative to mass. The example provided here can generally be applied to other proteins.

The ProPac 3R SCX 3 µm stationary phase is designed for very low carryover even at high mass loading levels. Figure 9 shows the overlaid chromatograms for a 50 µg injection run using 10 mg/mL NISTmAb and the following blank run with no injection. No carryover was detectable in the blank run, demonstrating the low carryover properties of the stationary phase even at high mass loading levels, which enables consecutive protein injection runs without interference due to carryover from previous injections.

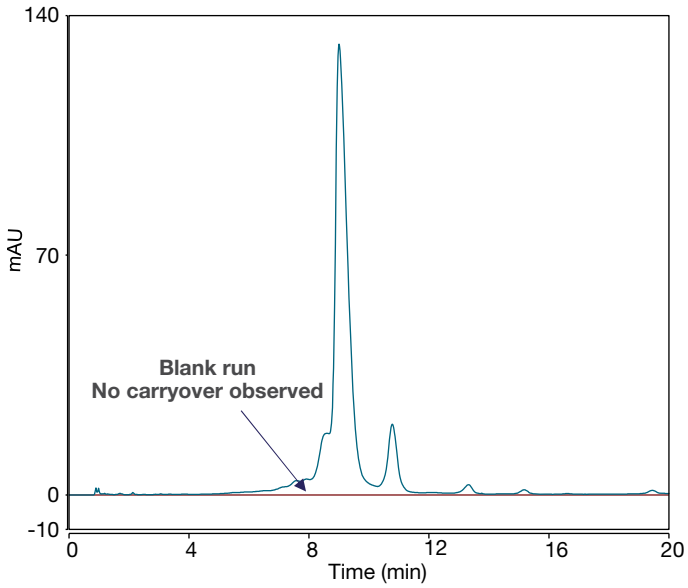


Figure 9. Overlaid chromatogram showing a 50 µg injection and elution of NISTmAb using a salt gradient and the following blank run to measure carryover

Final salt gradient method – Short vs. long

Using iterative analyses of the method development experiments described above, we provide here two optimized methods for NISTmAb analysis: 1) a fast analysis method with a 10 min gradient at 0.5 mL/min and 2) a longer high-resolution method with a 30 min gradient at 0.3 mL/min as shown in Figures 10 and 11. With a fast separation method (Figure 10), one can obtain separation between the main peak and charge variants in a short amount of time, which is suitable when time is a key factor such as in a fast analysis QC lab. For this method, a high flow rate was used to minimize method time due to gradient delay. The high-resolution longer gradient (Figure 11), provides greater separation of charge variants and main peak enabling improved separation and quantitation of each variant. A low flow rate was also used to maximize peak signal for improved detection of each variant even at low loading levels. For both methods, 30 °C was used as this is representative of physiological conditions. In both cases, the high resolution and capacity of the ProPac 3R SCX column provides narrow peaks with sufficient retention time separation to detect the different variants associated with NISTmAb. The flexibility of use and robust range of operating conditions enables the user to tailor the separation on the ProPac 3R SCX column for their specific application.

Column:	ProPac 3R SCX, 3 µm		
Format:	4 × 100 mm		
Eluents:	A: 20 mM MES, pH 6.5		
	B: 20 mM MES, pH 6.5 + 0.5 M NaCl		
Gradient:	Time (min)	%A	%B
	0.0	90	10
	10.0	70	30
	10.1	20	80
	13.0	20	80
	13.1	90	10
	20.0	90	10
Flow rate:	0.5 mL/min		
Inj. volume:	2 µL		
Temp:	30 °C		
Detection:	UV, 280 nm		
Sample:	NISTmAb – 10 mg/mL		

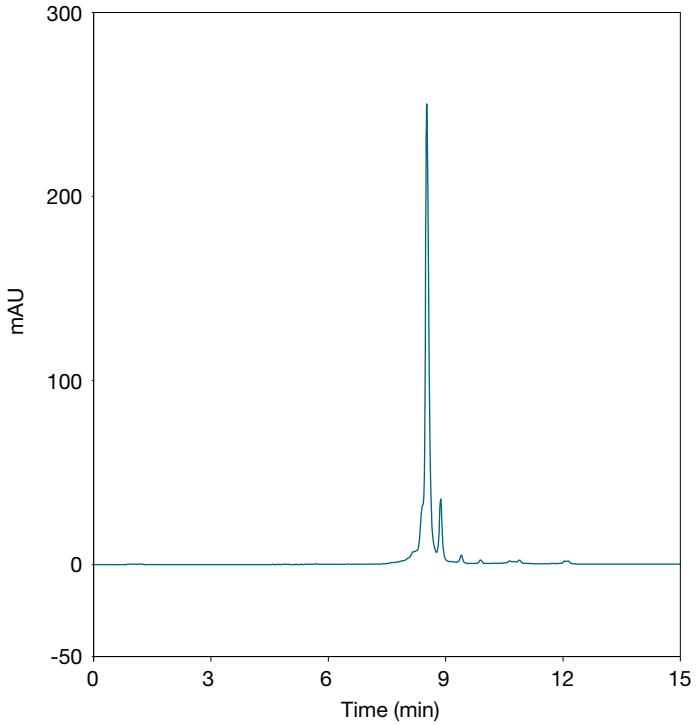


Figure 10. Chromatogram of a fast analysis method with 10-minute gradient at 0.5 mL/min flow rate

Column:	ProPac 3R SCX, 3 µm		
Format:	4 × 100 mm		
Eluents:	A: 20 mM MES, pH 6.5		
	B: 20 mM MES, pH 6.5 + 0.5 M NaCl		
Gradient:	Time (min)	%A	%B
	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20	80
	33.1	90	10
	40.0	90	10
Flow rate:	0.3 mL/min		
Inj. volume:	2 µL		
Temp:	30 °C		
Detection:	UV, 280 nm		
Sample:	NISTmAb – 10 mg/mL		

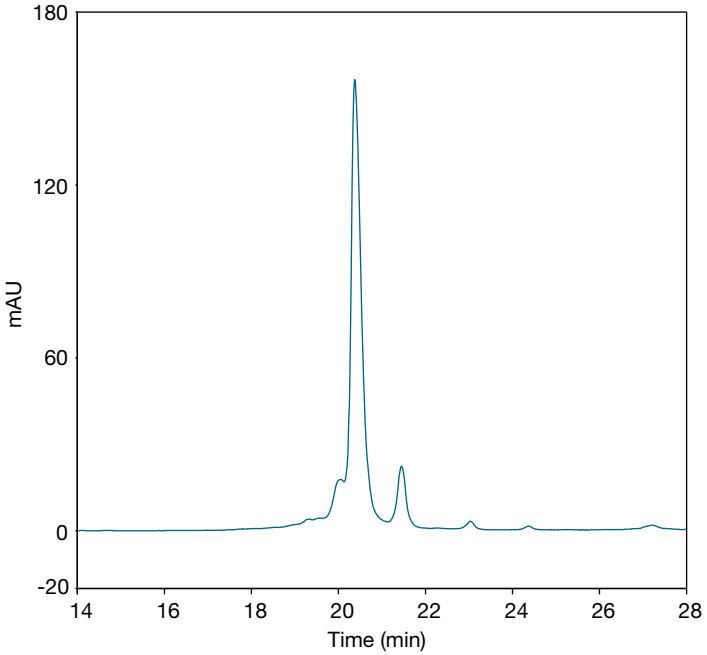


Figure 11. Chromatogram of a long method with 30-minute gradient at 0.3 mL/min flow rate

Using the high-resolution 30-minute gradient shown in Figure 11, we compared the performance of three different lots of media to evaluate the column-to-column and lot-to-lot reproducibility of the ProPac 3R SCX columns. Figure 12 shows excellent reproducibility observed in this analysis. The ProPac 3R

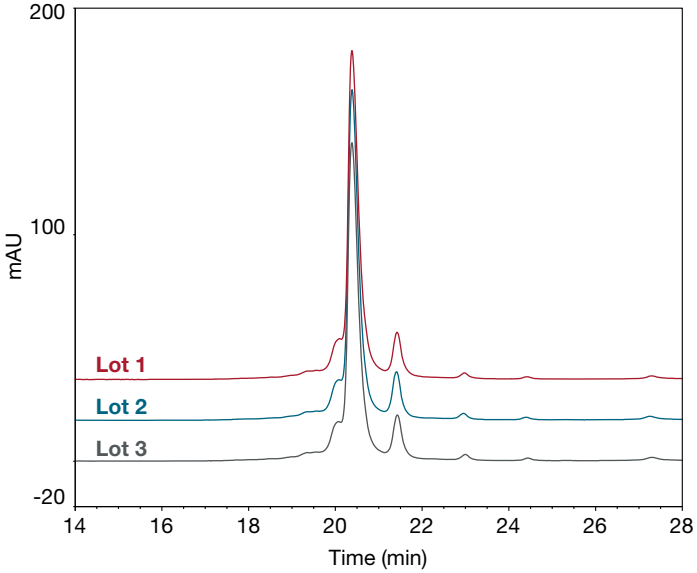
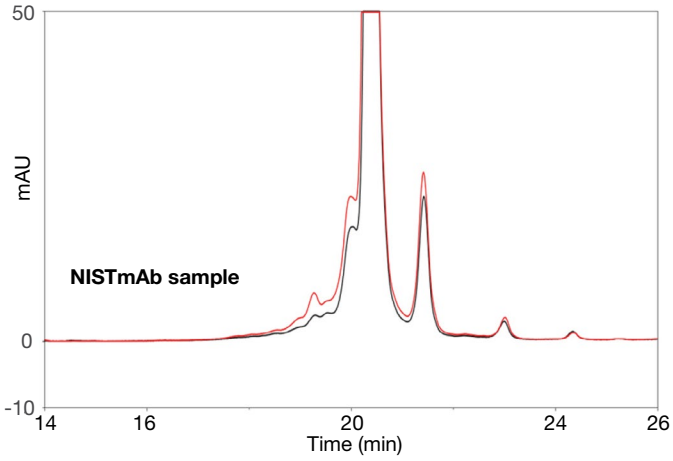


Figure 12. Chromatogram of 3 different lots using long method of 30-minute gradient at 0.3 mL/min flow rate. Retention time of main mAb peak is normalized to aid comparison of variant separation of the detailed view of the mab variants.

technology platform made using monodisperse particles and precision-controlled chemistry makes this possible, giving the user confidence in their separation with different lots of media.

Stressed sample

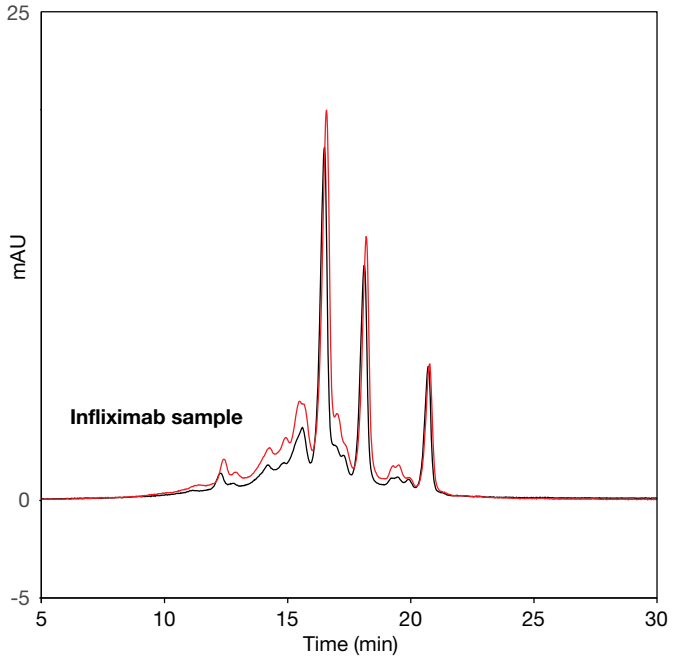
To illustrate the ability of the ProPac 3R SCX column to differentiate samples with irregular variant profiles, we compared the separation of native forms of both NISTmAb and Infliximab against respective samples that have been stressed at 40 °C for 72 hours. Thermal stressing of samples typically results in an increase in the number of acidic variants such as deamidation. The chromatograms in Figure 13 demonstrate clear differences in the peak profile due to an overall increase in the peak intensity and relative area of the variant peaks for stressed samples (red traces). The ProPac 3R SCX column maintains excellent resolution of the peaks, enabling easy comparison against the unstressed samples. This type of study can be used to determine stability conditions for user's samples and also help identify specific peaks based on how they change under thermal stress.



Column: ProPac 3R SCX, 3 µm
Format: 4 × 100 mm
Eluents: A: 20 mM MES, pH 6.5
B: 20 mM MES, pH 6.5
+ 0.5 M NaCl

Gradient:	Time (min)	%A	%B
	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20	80
	33.1	90	10
	40.0	90	10

Flow rate: 0.3 mL/min
Inj. volume: 2 µL
Temp: 30 °C
Detection: UV, 280 nm
Sample: NISTmAb – 10 mg/mL
Black - Unstressed sample
Red - Thermally stressed sample



Column: ProPac 3R SCX, 3 µm
Format: 4 × 100 mm
Eluents: A: 20 mM MES, pH 6.5
B: 20 mM MES, pH 6.5
+ 0.5 M NaCl

Gradient:	Time (min)	%A	%B
	0.0	93	7
	30.0	78	22
	30.1	20	80
	33.0	20	80
	33.1	93	7
	40.0	93	7

Flow rate: 0.3 mL/min
Inj. volume: 2 µL
Temp: 30 °C
Detection: UV, 280 nm
Sample: Infliximab – 5 mg/mL
Black - Unstressed sample
Red - Thermally stressed sample

Figure 13. Overlaid chromatograms of non-stressed mAb sample (black) against a stressed sample (red).
Top chromatogram is analysis of NISTmAb sample and bottom chromatogram is analysis of Infliximab sample.

Evaluation of method development workflows for other mAbs

The methodology discussed above has been applied to a range of mAbs. Each mAb was optimized using a non-isocratic eluting salt concentration to load the protein followed by a linear gradient to elute the sample. Figure 14 demonstrates excellent mAb-variant separation for each mAb using the ProPac 3R SCX column with a simple salt gradient in MES buffer pH 6.5 at 30 °C over 30 minutes.

Table 1. Gradient parameters for analysis of mAbs using a salt gradient

mAb	Initial %B	Final %B
NIST	10	30
Infliximab	7	22
Pertuzumab	10	25
Vedolizumab	5	22
Secukinumab	5	22

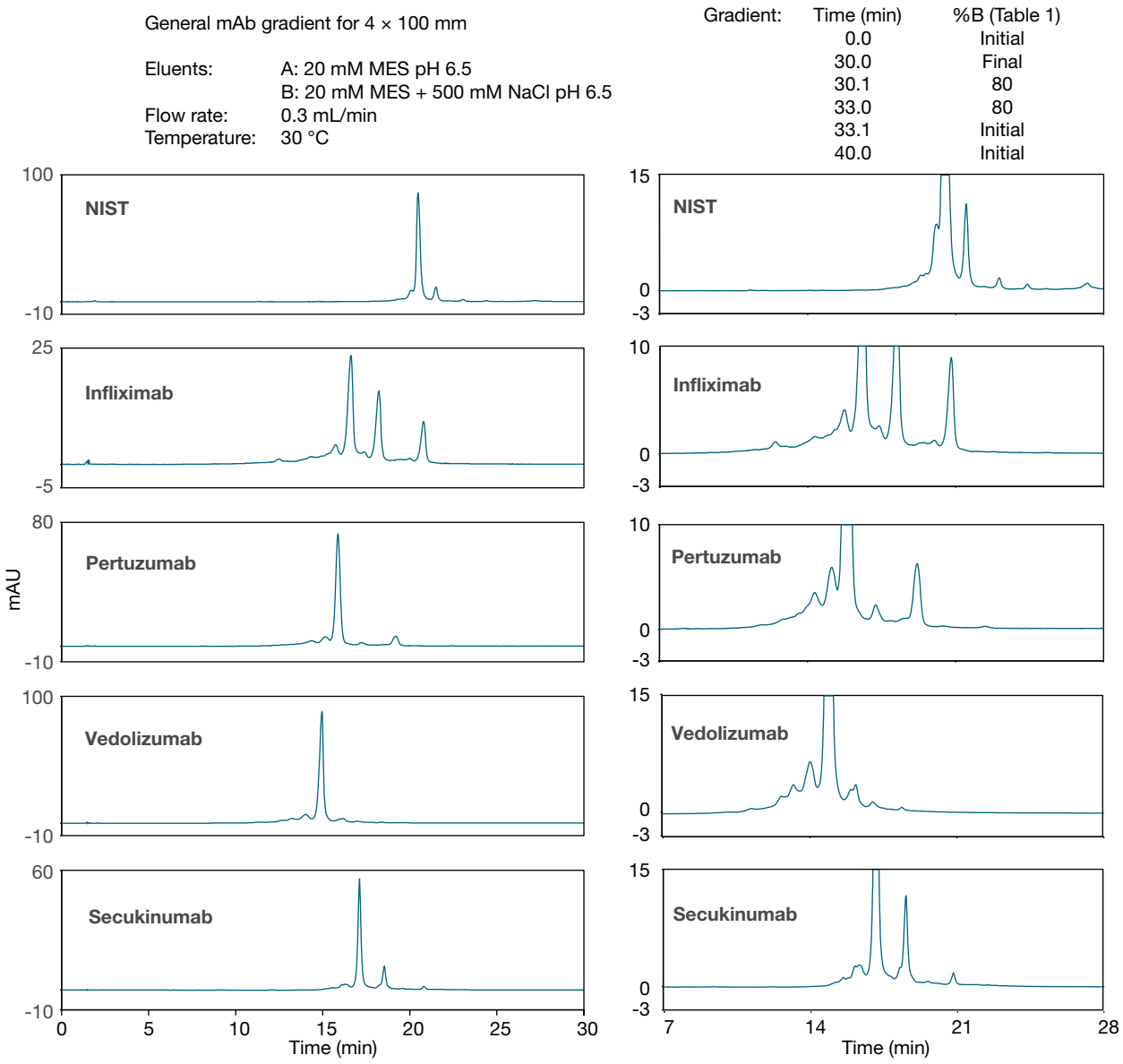


Figure 14. Analysis of mAbs using the salt gradient detailed in Table 1. Left chromatograms show the full signal strength and the ones on the right show the detailed view of the mAb variants.

Column:	See chromatogram for column type and format
Eluents:	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl

Gradient:	Time (min)	%A	%B
	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20	80
	33.1	90	10
	40.0	90	10

Flow rate:	Top - 1.0 mL/min Middle - 0.3 mL/min Bottom - 0.4 mL/min
Inj. volume:	Top - 5.0 µL Middle - 2.0 µL Bottom - 2.6 µL
Temp:	30 °C
Detection:	UV, 280 nm

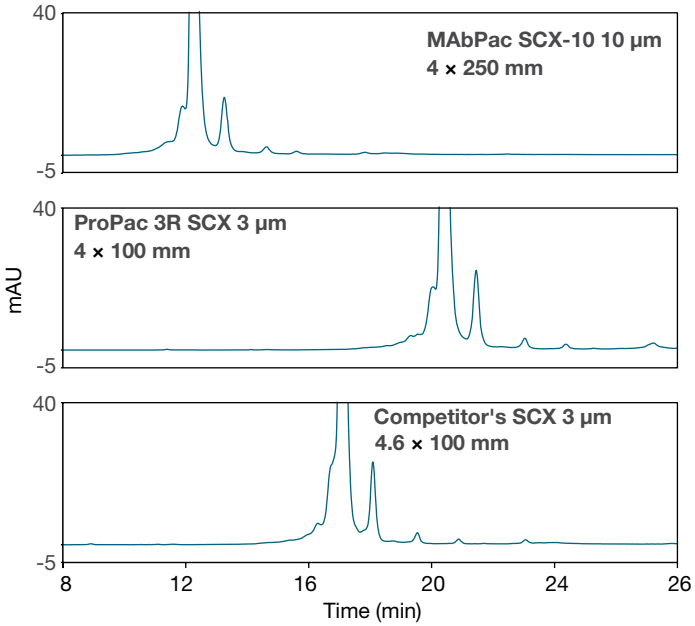


Figure 15. Chromatograms of NISTmAb using a MAbPac SCX-10, 10 µm column; a ProPac 3R SCX, 3 µm column; and a competitor's SCX 3 µm column with a long high-resolution method of 30-minute gradient

Lastly, we provide a comparison of the ProPac 3R SCX column against our current MAbPac SCX-10 column and a competitor's 3 µm SCX column in Figure 15. For the MAbPac SCX-10 column, the flow rate is increased to 1 mL/min to maximize performance. For the competitor's column, the flow rate was scaled to the larger 4.6 mm column i.d. Relative to the 250 mm long MAbPac SCX-10 column, the 100 mm ProPac 3R SCX column shows similar separation of the proximal acidic peak and clearly better resolution and detection of the more distal acidic peaks that elute as a single peak on the MAbPac SCX-10 column. Basic peak resolution for NISTmAb is also improved with narrower peaks for improved peak detection on the ProPac 3R SCX column. Compared against the competitor 3 µm SCX, the ProPac 3R SCX column shows better separation of the proximal acidic peak and has better separation of the distal acidic peaks. The basic peaks show similar performance to the competitor product but with an overall greater peak spread for the ProPac 3R SCX column due to its higher capacity. Overall, in both these comparisons, the ProPac 3R SCX column has the advantage in variant separation to provide improved analysis and quantitation.

Conclusion

- ProPac 3R SCX 3µm columns provide excellent separation of monoclonal antibodies and associated charge variants using a salt gradient to give high-resolution, robust performance and excellent reproducibility.
- Mobile phase buffer pH, starting salt concentration, flow rate, gradient time, and temperature can be optimized to provide consistent, robust, and high-resolution variant separation.

References

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