ThermoFisher SCIENTIFIC

HPLC columns

ProPac 3R SCX 3 µm columns

Product manual

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Introduction

Strong cation exchange technology

Thermo Scientific™ ProPac™ 3R Strong Cation Exchange (SCX) 3 µm columns are designed to provide fast, high efficiency, high resolution separations of proteins and glycoproteins based on their accessible surface charge. The 3 µm, monodisperse particle is non-porous and based on a divinylbenzene resin coated with a hydrophilic polymer layer to exclude proteins from the surface of the resin to minimize secondary interactions. Grafting of a functional polymeric layer to this hydrophilic surface introduces sulfonic acid groups that provide the strong cation exchange character for promoting protein binding using a low ionic strength mobile phase at an appropriate pH (e.g., 20 mM MES, pH 6.5). Running a gradient from low to high ionic strength mobile phase or from low to high pH disrupts the ionic protein-surface interactions resulting in protein and variant elution based on their relative strength of interaction with the surface.

SCX chromatography applications

Protein SCX column technology is widely used in biopharmaceutical development and qualification of protein-based therapeutics and their associated variants. Most commonly, they are used for the analysis of monoclonal antibodies (mAbs), which are a class of therapeutics widely applied to the treatment of cardiovascular diseases, autoimmune disorders, and cancers. MAbs are of particular interest for their ability to target specific cells for drug delivery or modulation of cellular activity with reduced side effects compared to other systemic delivery methods. Because of this specificity, mAbs have been investigated for and applied to a wide range of treatments with strong growth expected for the foreseeable future. As the complexity of these therapeutics increases, continued improvements in analytical technologies will be required to characterize these therapeutics and fulfill regulatory requirements to bring these therapeutics to market.

ProPac 3R SCX 3 µm column

The ProPac 3R SCX 3 µm column is designed to achieve high-efficiency protein separations. The packing material is based on a 3 µm, nonporous, divinylbenzene monodisperse polymer particle shown in Figure 1. Both the chemistry and size of the base monodisperse particle are tightly controlled. The chemistry consistency provides a solid platform for reproducibly producing the hydrophilic layer and sulfonic acid SCX functionality using controlled polymerization techniques. The 3 µm monodisperse particle results in shorter diffusion distances to provide reproducible mass transfer for narrow peaks. Due to increased capacity associated with smaller particles, shorter columns can also be used for faster run times with improved separation relative to larger particle media that require longer column lengths to achieve the same separation.

The uniform size of the particle enables precise control over the column packing. The ProPac 3R SCX media are packed in a PEEK (polyether ether ketone) hardware, which has well-established bioinert properties to minimize nonspecific adsorption of protein samples. The reproducible resin size and chemistry combined with controlled synthetic and packing manufacturing processes provide excellent lot-to-lot and column-to-column reproducibility.

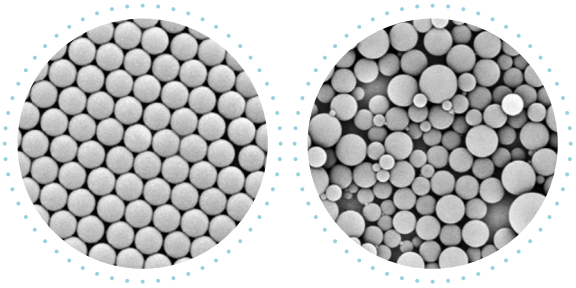


Figure 1: SEM image of 3 µm monodisperse ProPac 3R particles (left) vs. 3 µm polydisperse particles (right)

Table 1. ProPac 3R SCX 3 µm columns

Description	Dimensions	Particle size	Cat. no
Thermo Scientific ProPac 3R SCX columns	2 mm x 50 mm	3 µm	43103-052068
	2 mm x 100 mm	3 µm	43103-102068
	4 mm x 50 mm	3 µm	43103-054068
	4 mm x 100 mm	3 µm	43103-104068

Operation: System requirements and column use and care

Getting started

Prior to using the ProPac 3R SCX 3 µm column, review all the information in this section on system requirements and column operation regarding flow rate, maximum pressure, mobile phase composition, temperatures, etc. Following these specifications for your column will help to ensure the column performs as it is intended and maximize the lifetime of your column.

System requirements

ProPac 3R SCX 3 µm columns are designed to be used with a standard bore bio-inert HPLC or UHPLC system having a gradient pump module, autosampler, injection valve, and a detector appropriate for your application (UV, DAD, VWD, Fluorescence, MS). A Thermo Scientific™ Vanquish™ Flex, Thermo Scientific™ Vanquish™ Horizon, and a Thermo Scientific™ UltiMate™ 3000 BioRS totally inert UHPLC system is recommended for best results. A properly setup system is required to ensure good chromatographic performance and to extend the lifetime of your column.

A biocompatible system is highly recommended for mobile phases containing halide salts that can cause corrosion of metallic components. Metal leaching from the system components including the pump and connection tubing can contaminate the column and compromise chromatographic performance. Avoid using stainless steel tubing, ferrules, and bolt assemblies and replace them with MP35N tubing, PEEK, or other equivalent inert alternatives. For column-to-tubing connections, Thermo Scientific™ Viper™ Capillary MP35N with Thermo Scientific™ Viper™ Connection Fittings are strongly recommended. If your column has become contaminated with metals, the performance can be recovered by flushing with an EDTA (ethylenediaminetetraacetic acid) solution as described in Table 3.

System void volume

Tubing between the injection valve and detector should be ≤ 0.130 mm (0.0050" ID) tubing to minimize dispersion. Minimize the length of all liquid lines, especially the tubing between the column and the detector. The use of larger diameter and/or longer tubing may decrease peak efficiency and peak resolution

Column use and physical specifications

To ensure that you do not damage the column hardware or packed bed, take care to operate within the limits of the column. Table 2 indicates the operational limits for each column format in terms of flow rate, maximum column pressure drop from inlet to outlet, temperature, and mobile phase pH.

Table 2. Recommend column operating conditions for optimal performance and extending column lifetime

Column	Flow rate (mL/min)	Max column pressure drop[1] psi (bar)	Temp °C	pH
2 mm x 50 mm	0.1-0.3	4500[2] (310)[2]	Ambient – 60°C	2-12
2 mm x 100 mm	0.1-0.2			
4 mm x 50 mm	0.3-0.5			
4 mm x 100 mm	0.3-0.5			

[1]The column pressure drop for a given flow rate is calculated as the pressure of the system with column minus the pressure of system with union in place of column
[2]For PEEK body columns, the maximum pressure at the column inlet should not exceed 7000 psi (485 bar) to avoid damaging the column body

Additional requirements for safe column operation:

- Always set up the mobile phase flow direction as indicated on the column tag
- Avoid sharp pressure fluctuations as they may disrupt the column bed
- When starting, stopping, or changing the flow rate, a flow ramp rate (mL/min/min) of ~1/3 of the maximum flow rate for the specific column format is recommended

Operation: System requirements and column use and care Continued

Recommended buffers for salt and pH gradient separations

Salt gradient separations typically offer the best resolution possible for individual applications. Please consult Table 3 below for recommended buffer conditions to achieve optimal separations and maintain good column performance throughout its lifetime.

Table 3. Recommend buffers and mobile phase requirements

Parameter	Recommended
Buffer	• MES or other Good's buffers • Thermo Scientific™ CX-1 pH gradient buffers • LC-MS: Ammonium acetate, ammonium bicarbonate, ammonium formate and associated acids and bases for pH gradients[3]
Minimum salt concentration	• 20 mM NaCl to avoid high pressure that can damage the column stationary phase • **Caution:** Never use pure deionized water on the column as this will result in irreversible damage
Detergent additives	• Nonionic, anionic or zwitterionic detergents • **Caution:** Do not use cationic detergents as they will irreversibly bind to the column and reduce the separation power
Organic solvent compatibility[4]	• Up to 20% acetonitrile • Up to 10% methanol
Cleaning agents	• For metal contamination (Fe, Cu, etc.) removal, flush the column at 0.4× the max column flow rate for 12 hours with 10mM EDTA + 50mM NaCl adjusted to pH 8.0
Storage solution	• Short term: ≥ 20 mM NaCl and your application buffer • Long term: ≥ 20 mM NaCl and your application buffer + 0.1% sodium azide

[3]Due to the weak ionic strength of volatile pH buffers, use lower flow rates for initial method development until the column back pressure is understood. The flow rate can then be increased as needed while still observing the maximum allowed pressure for the column.
[4]Acetonitrile and methanol have viscosity maxima when mixed with water at certain ratios. This may cause unexpectedly high pressure. Always use low flow rates until the pressure behavior is understood when using these chemicals. Mixtures of ACN and MeOH should be introduced and removed gradually from the column using a gradient over 20 minutes to ensure a sharp viscosity front does not results in a rapid pressure difference in-column that may damage the packed bed.

Recommended buffers for pH gradient separations

For pH gradient separations, we recommend using Thermo Scientific CX-1 pH gradient buffers (see Table 4 for part numbers), which can be used to run a linear, highly reproducible pH gradient from pH 5.6 to 10.2. As the majority of mAbs have a pI between 6 and 10, these buffers can be used to separate the mAbs from their associated charge variants with less method development compared to salt gradients. Because of this, the CX-1 pH gradient buffers present a simple platform method for mAb analysis that can easily be tailored to the users own mAb by simple adjustment of the gradient. For examples of mAb analyses using the pH gradient buffers, please reference the applications section below.

Table 4. Thermo Scientific CX-1 pH gradient buffers

Description	Size	pH	Cat. no.
pH gradient buffer A	125 mL	pH 5.6	083273
	250 mL		085346
	500 mL		302779
	1000 mL		303274
pH gradient buffer B	125 mL	pH 10.2	083275
	250 mL		085348
	500 mL		302780
	1000 mL		303275

Operation: System requirements and column use and care Continued

Column conditioning

Your column has been designed to minimize secondary interactions and for low carryover. Depending on the nature of your sample, column conditioning may be required prior to achieving optimal performance. To quickly condition your column, we recommend performing 1-2 sample overload injections of 10× your standard sample loading and standard gradient method.

Minimum equilibration volumes for buffers

Prior to sample loading and start of the gradient, the column stationary phase must be properly equilibrated to promote binding of the analytes. Salt and pH gradient buffers require different volumes of buffer at sample loading conditions to equilibrate the column. It is strongly advised that the user evaluate the required equilibration volume by examining the UV, pH, or conductivity of the mobile phase when making a step change to their preferred loading conditions. The volume of mobile phase eluted from the time of the step change to when steady-state is observed for the mobile phase UV, pH, or conductivity trace represents the minimum volume required for equilibration of the stationary phase.

Column storage and extended care

To maintain the performance of your column between uses, always store the column filled with the recommended buffers as detailed in Table 3. Use the plugs the column was shipped with to seal the ends of the column to prevent evaporation of the buffer and drying of the stationary phase. The formation of salt crystals in a dried bed may result in column clogging or reduced column performance in subsequent uses.

Depending on the length of time between column uses, the storage buffers should be used as follows:

- Short term storage (≤1 day): Fill the column with a low ionic strength buffer (e.g., 20 mM MES + ≥20 mM NaCl)
- Long term storage (>1 day): Fill the column with a low ionic strength buffer + ≥20 mM NaCl and supplemented with 0.1% sodium azide

Sodium azide serves as a preservative to prevent bacterial growth that can damage the column phase, result in column clogging, and decrease column performance. When using the column again after storage with a sodium azide containing buffer, flush the column with ≥10 column volumes of low ionic strength buffer to remove all of the sodium azide before starting any sample runs. At least 2 blank runs with a gradient from low to high salt concentration prior to any sample runs are also recommended for best performance for the initial sample runs.

Column performance verification

Each column is shipped with two Certificates of Analysis (CoA) one verifying the resin performance and one verifying the column performance. The resin qualification CoA details a salt gradient separation of NISTmAb and variants on a 4 × 100 mm column. This test is not performed for every column but is used to qualify the resin performance prior to manufacturing columns. The column CoA test is an isocratic analysis of equine cytochrome C. Each CoA provides the test conditions used. These tests can be reproduced to check the performance of your column. The lot qualification test is performed using a 4 × 100 mm column and the gradient and flow rate should be scaled based on column length and diameter, respectively. The isocratic cytochrome C test conditions can be used as described to verify column performance. Note that differences in system configuration may result in differences in retention time and chromatographic performance. Table 5 provides purchasing information for the proteins used in the CoA tests.

Table 5. Proteins used for resin lot qualification and column performance test

Protein	Supplier	Cat. no.
NISTmAb	NIST (National Institute of Standards and Technology)	**8671**
Equine cytochrome C	Sigma Aldrich	**C2506**

SCX chromatography applications

Two approaches are typically used for protein and variant analysis on SCX columns: 1) salt gradient separation and 2) pH gradient separation. For both approaches, the protein is loaded onto the column stationary phase using a low ionic strength buffer. However, the salt gradient approach relies on increasing the solution ionic strength to elute the protein; whereas the pH gradient approach shifts the net charge of the protein from cationic to anionic to disrupt protein-surface interactions. Salt gradients typically offer optimal separation of proteins and their associated variants; however, these methods typically require considerable development time investigating different buffer components, pH, salts, gradient slopes, temperatures, etc.[1] In contrast, the pH gradient approach is a simple platform method for facile separation of proteins and their variants based on their respective isoelectric points (pI).[2]

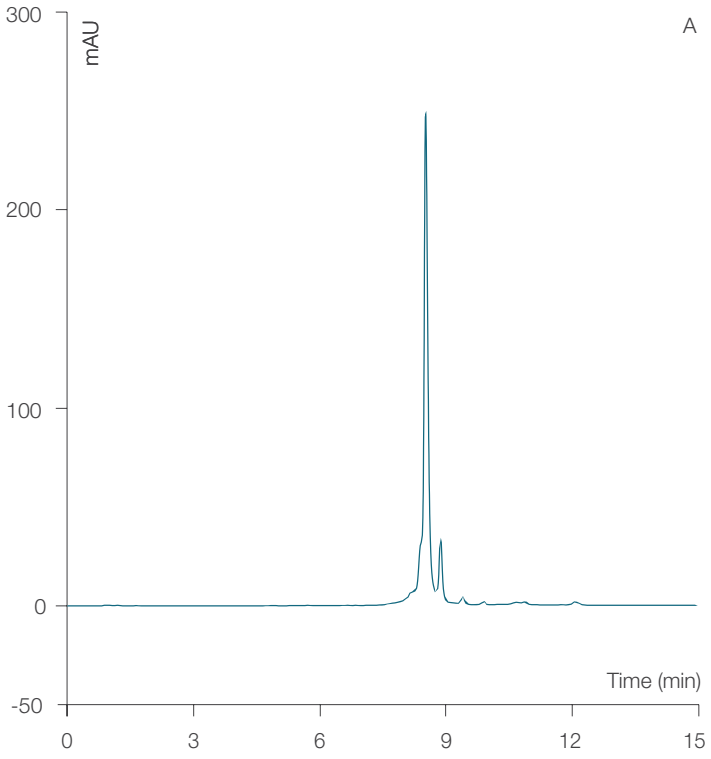
Salt gradient principles

When using a salt gradient, the ionic strength of the solution is increased over time typically using a salt such as NaCl. At higher salt concentrations, the cationic salt component will exclude the interactions of the cationic protein species from the sulfonic acid groups of the stationary phase leading to protein desorption and elution from the column. Proteins with a greater number of cationic groups and/or fewer anionic groups will tend to elute at higher salt concentrations relative to those with fewer cationic groups and/or more anionic groups. The following sections provide a few specific examples of separation using salt gradient methods. For a detailed discussion of method development using salt gradients please reference application note “Salt gradient analysis of monoclonal antibodies using a 3 µm monodisperse SCX chromatography column”.[1]

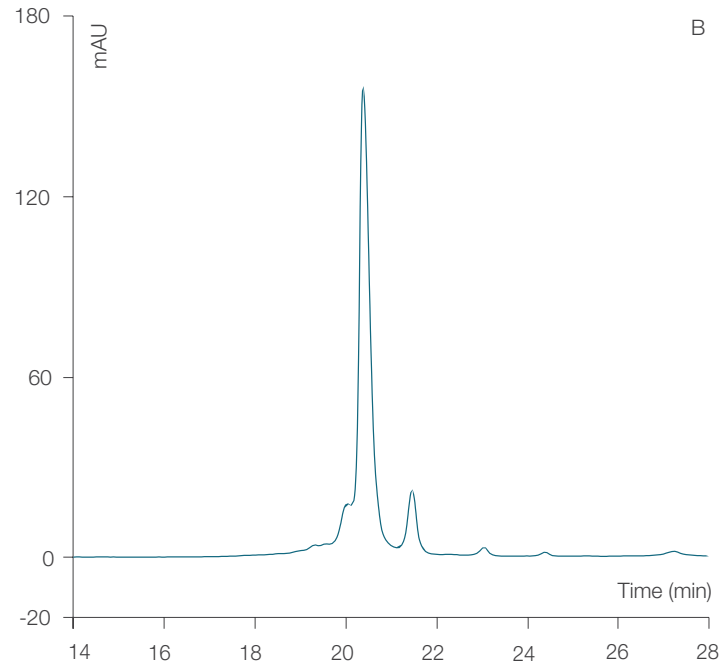
Example salt gradient methods

To effectively separate a protein and its associated variants, it is important to optimize the method used to analyze the sample. Common chromatographic parameters used to optimize methods include mobile phase pH, starting gradient conditions, gradient time, flow rate, temperature, and sample mass loading, which can be optimized to provide high resolution, high reproducibility protein separations. Figure 2 shows two methods for NISTmAb that have been optimized with (A) a fast analysis method using a 10 min gradient at 0.5 mL/min and (B) a longer high-resolution method using a 30 min gradient at 0.3 mL/min. The fast method is practical for a QC environment where rapid sample analysis is desired while the high-resolution longer gradient, provides greater separation of main peak and charge variants enabling improved separation and quantification of each variant. 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 variants associated with NISTmAb.

SCX chromatography applications Continued



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



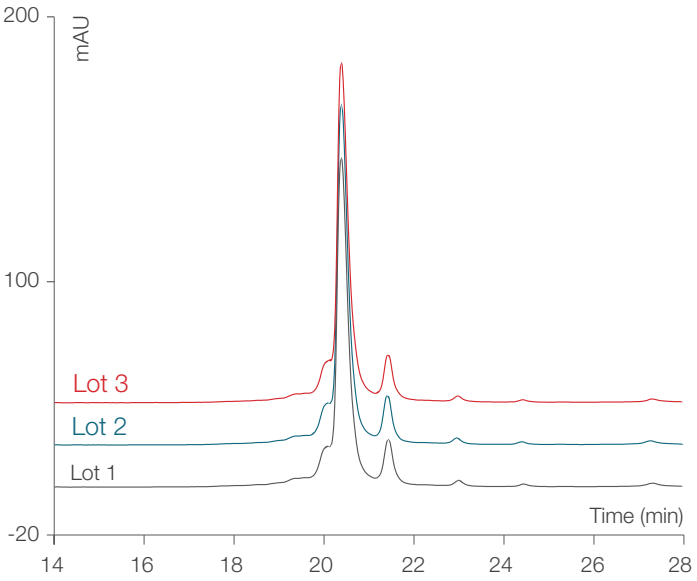
Column	ProPac 3R SCX, 3 µm		
Format	4 × 100 mm		
Mobile phase	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl		
Flow rate	0.3 mL/min		
Injection	2 µL		
Temp	30 °C		
Detection	UV, 280 nm		
Sample	NISTmAb – 10 mg/mL		
Gradient		**%A**	**%B**
Time (min)	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20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

Figure 2: NISTmAb analysis using short method with 10-minute gradient at 0.5 mL/min (A) and Long method with 30-minute gradient at 0.3 mL/min flow rate (B)

SCX chromatography applications Continued

Lot-to-lot reproducibility

Using the high resolution 30-minute gradient shown in Figure 2, 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 3 shows excellent reproducibility observed in this analysis. The ProPac 3R 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.



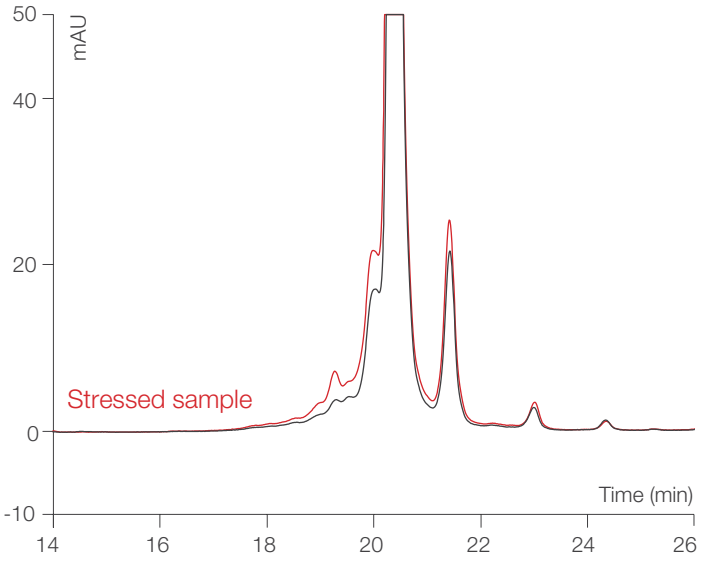
Column		ProPac 3R SCX, 3 µm	
Format		4 × 100 mm	
Mobile phase		A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl	
Flow rate		0.3 mL/min	
Injection		2 µL	
Temp		30 °C	
Detection		UV, 280 nm	
Sample		NISTmAb – 10 mg/mL	
Gradient		**%A**	**%B**
Time (min)	0.0	90	10
	30.0	70	30
	30.1	20	80
	33.0	20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

Figure 3: Chromatograms of three different lots using a long method with a 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.

SCX chromatography applications Continued

Stressed sample analysis

To illustrate the ability of the ProPac 3R SCX to differentiate samples with irregular variant profiles, we compared the separation of native forms of both NISTmAb and a sample that was 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 below in Figure 4 demonstrates 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 maintains excellent resolution of the peaks enabling easy comparison against the unstressed samples.



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

Figure 4: 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.

SCX chromatography applications Continued

Salt gradient analysis of additional mAbs

High resolution methods were also developed for four additional mAbs using a salt gradient. Each mAb was optimized using an initial non-eluting salt concentration to load the protein followed by a linear gradient to elute the sample. The general gradient used is provided here with Table 6 giving the initial and final %B gradient values for each mAb. Figure 5 demonstrates excellent mAb-variant separation for each mAb using the ProPac 3R SCX 4 × 100 mm with a simple salt gradient in MES buffer pH 6.5 at 30 °C over 30 minutes.

General mAb gradient for 4 × 100 mm		
Mobile phase	A: 20 mM MES pH 6.5 B: 20 mM MES + 500mM NaCl pH 6.5	
Flow rate	0.3 mL/min	
Temp	30 °C	
Gradient	**%B (Table 6)**	
Time (min)	0.0	Initial
	30.0	Final
	30.1	80
	33.0	80
	33.1	Initial
	40.0	Initial

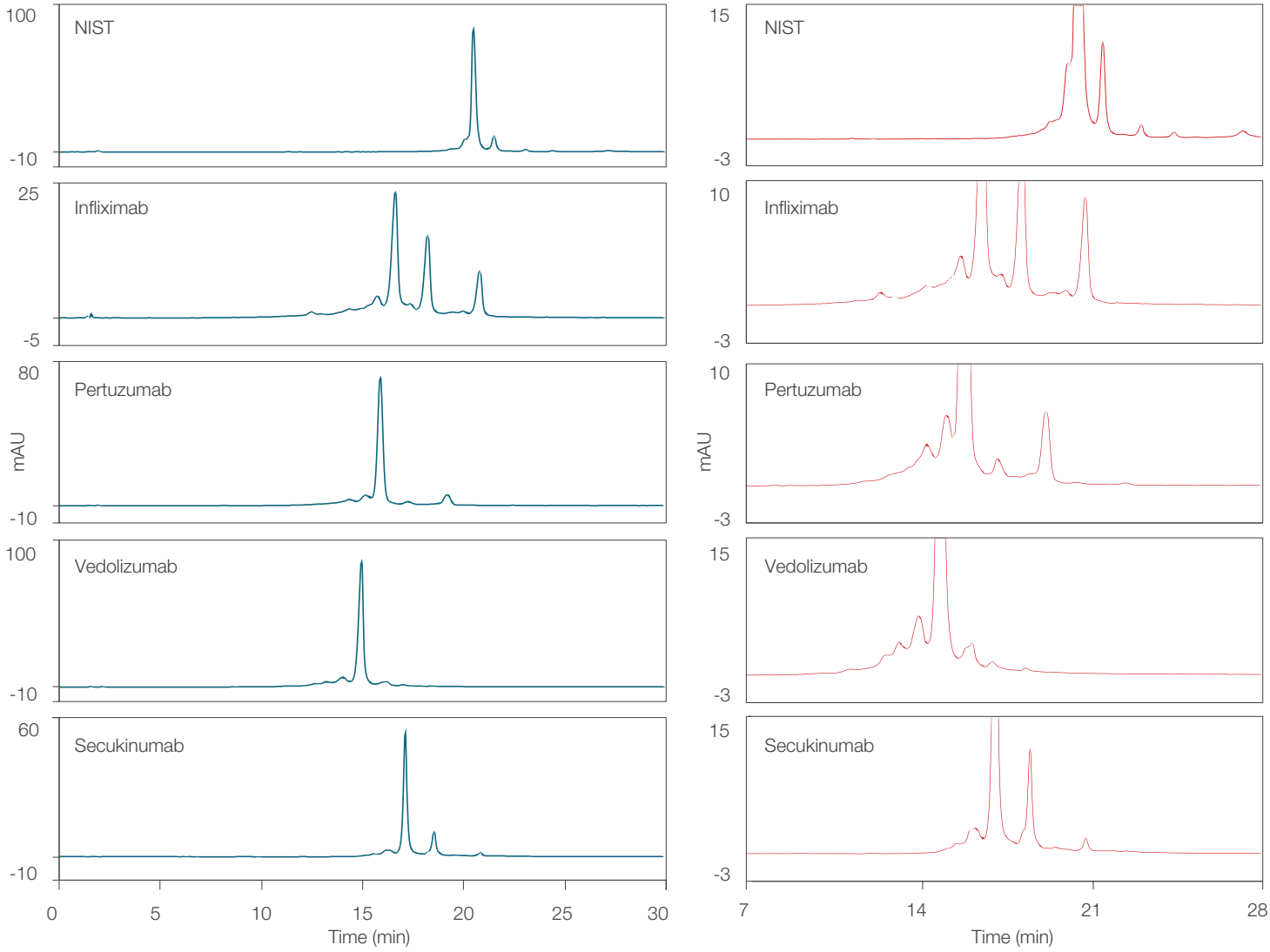


Figure 5: Analysis of selected mAbs on a 4 × 100 mm column using a general gradient with optimized initial and final %B values detailed in Table 6. Left chromatogram shows the full signal range with the right chromatogram showing the detailed view of the mAb variants.

SCX chromatography applications Continued

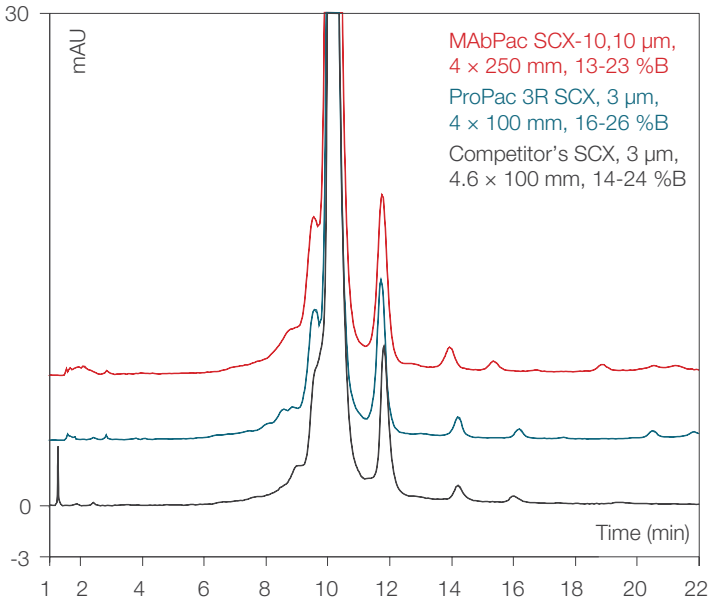
Table 6: Gradient parameters for analysis of mAbs using salt gradient

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

Competitor comparison

Figure 6 below compares the performance of the ProPac 3R SCX column against our current Thermo Scientific™ MAbPac™ SCX-10 column and a competitor's 3 µm SCX column. For the MAbPac SCX-10 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 ID. Relative to the 250 mm long MAbPac SCX-10 column, the 100 mm ProPac 3R SCX shows similar separation of the proximal acidic peak and clearly better resolution and detection of the more distal acidic peaks which elute as a single peak on the MAbPac SCX-10.

Basic peak resolution for NISTmAb is also improved with narrower peaks for improved peak detection on the ProPac 3R SCX. Compared against the competitor 3 µm SCX, the ProPac 3R SCX 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 due to its higher capacity. Overall, in both these comparisons, the ProPac 3R SCX has the advantage in variant separation to provide improved analysis and quantitation.



Column	See chromatogram for column type and format	
Mobile phase	A: 20 mM MES, pH 6.5 B: 20 mM MES, pH 6.5 + 0.5 M NaCl	
Flow rate	Red: 1.0 mL/min Blue: 0.5 mL/min Black: 0.66 mL/min	
Injection	Red: 10 µL Blue: 2 µL Black: 2.6 µL	
Temp	30 °C	
Detection	UV, 280 nm	
Gradient	See chromatogram for change in %B	
Time (min)	0.0	$B_{initial}$
	25.0	B_{final}
	25.1	50
	27.0	50
	27.1	$B_{initial}$
	37.0	$B_{initial}$

Figure 6: Comparison of NISTmAb separation on MAbPac SCX-10, 10 µm 4 × 250 mm; ProPac 3R SCX, 3 µm 4 × 100 mm; and a competitor's SCX 3 µm 4.6 × 100mm columns using a high-resolution 25-minute gradient method. Retention times are normalized for comparison of variant separation.

SCX chromatography applications Continued

pH gradient separation principles

When using a pH gradient, the cationic protein is adsorbed to the stationary phase at low pH conditions followed by a gradient of increasing mobile phase pH. As the pH of the buffer increases, the charge of the protein shifts from cationic to neutral and then anionic at higher buffer pH values. The change in protein charge results in desorption from the anionic surface and elution from the column. This mechanism is shown schematically in Figure 7 with the charge of a theoretical protein. The y-axis indicates the pH operating range from 5.6 to 10.2 for the Thermo Scientific CX-1 pH gradient buffers used in this manual. Many cationic proteins including mAbs have a pI in this range allowing them to be analyzed using this buffer system. For pH gradient methods, the ProPac 3R SCX column 2 x 50 mm is recommended for two reasons 1) the narrow ID will provide good detection sensitivity at low mass loading levels and 2) the short column length minimizes method time for analysis. The following sections provide a few specific examples of separation using pH gradient methods. For a detailed discussion of method development using pH gradients please reference application note “Method development for pH gradient analysis of monoclonal antibodies using a 3 µm monodisperse particle strong cation exchange chromatography column”.[2]

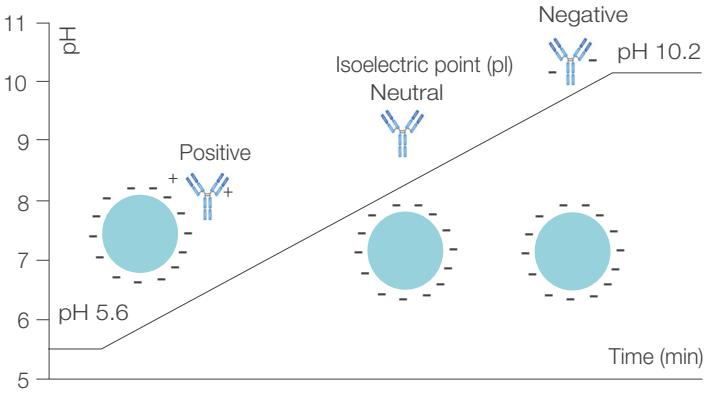
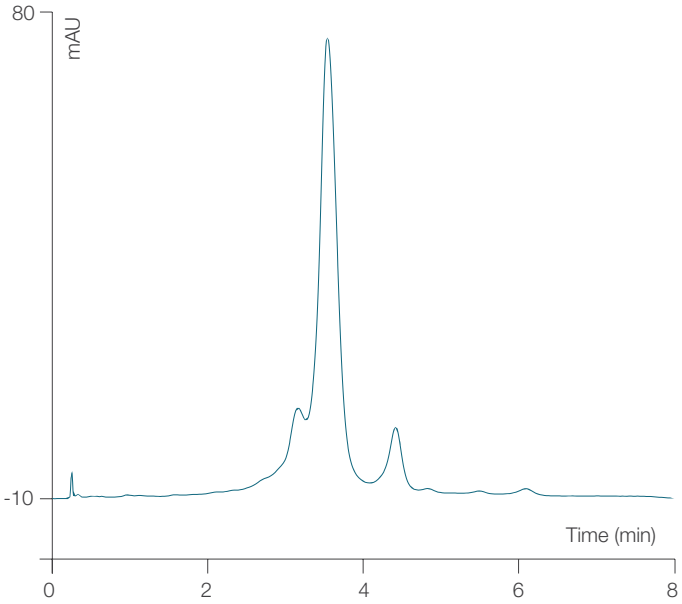


Figure 7: Schematic of adsorption and desorption mechanism of cationic proteins on anionic stationary phase using pH gradient buffer

SCX chromatography applications Continued

Optimized pH gradient method

For pH gradients, the pH range over which the separation occurs is the primary method parameter requiring optimization. The gradient time and flow rate can then adjusted to fine tune the separation and minimize run time. We provide here in Figure 8 an optimized method using a 5 min gradient at 0.3 mL/min for fast separation of NISTmAb charge variants. The high flow rate reduces gradient delay and column re-equilibration time while the fast 5-minute gradient time elutes the protein and associated variants over a short time-period. With this optimized method, the high resolution and capacity of the ProPac 3R SCX column provides narrow peaks with sufficient retention time separation to detect the variants associated with NISTmAb.



Column	ProPac 3R SCX, 3 µm		
Format	2 × 50 mm		
Mobile phase	A: 1×, CX-1 buffer A pH 5.6 B: 1×, CX-1 buffer B pH 10.2		
Flow rate	0.3 mL/min		
Injection	1 µL		
Temp	30 °C		
Detection	UV, 280 nm		
Sample	NISTmAb – 10 mg/mL		
Gradient		**%A**	**%B**
Time (min)	-0.2	50	50
	0.0	50	50
	5.0	40	60
	6.0	40	60
	6.1	100	0
	7.0	100	0
	7.1	50	50
	14.0	50	50

Figure 8: Enlarged view of chromatogram for NISTmAb analysis using an optimized 5 minute gradient at 0.3 mL/min flow rate

Using the optimized 5-minute gradient method shown in Figure 8, the performance of three different lots of media were compared to evaluate the column-to-column and lot-to-lot reproducibility of the ProPac 3R SCX columns. Figure 9 shows excellent reproducibility observed in this analysis. The ProPac 3R 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.

SCX chromatography applications Continued

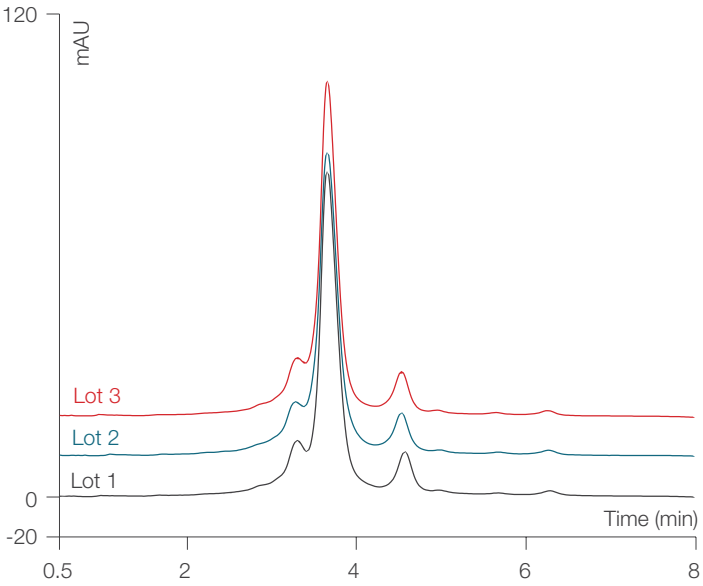


Figure 9: Zoomed-in view of chromatograms of three different lots using optimized method with a 5-minute gradient at 0.3 mL/min flow rate. Retention time of main mAb peak is normalized to aid comparison of variant separation.

pH gradient analysis on additional mAbs

The same approach of pH gradient optimization was applied to four additional mAbs. Each method was optimized for mobile phase composition and sample loading using a flow rate of 0.3 mL/min to achieve fast variant analysis with a total method time of 14 minutes using a linear pH gradient. For each mAb evaluated, initial and final gradient values for %B of CX-1 pH gradient buffers, are provided in Table 7. Figure 10 demonstrates excellent mAb-variant separation for each mAb using the ProPac 3R SCX 2 × 50 mm column with a simple pH gradient over 5 minutes using CX-1 gradient buffers at 30 °C.

Table 7: Gradient parameters for analysis of mAbs using pH gradient

mAb	Initial %B	Final %B	Concentration	Inj vol. (µL)
NISTmAb	50	60	10 mg/mL	1.0
Infliximab	24	44	5 mg/mL	3.0
Pertuzumab	36	51	5 mg/mL	1.5
Vedolizumab	23	38	5 mg/mL	1.5
Secukinumab	27	47	5 mg/mL	1.5

General mAb gradient for 2 × 50 mm		
Mobile phase	A: 1x CX-1 buffer A pH 5.6 B: 1x CX-1 buffer B pH 10.2	
Flow rate	0.3 mL/min	
Temp	30 °C	
Gradient		**%B (Table 7)**
Time (min)	-0.2	Initial
	0.0	Initial
	5.0	Final
	6.0	Final
	6.1	0
	7.0	0
	7.1	Initial
	14.0	Initial

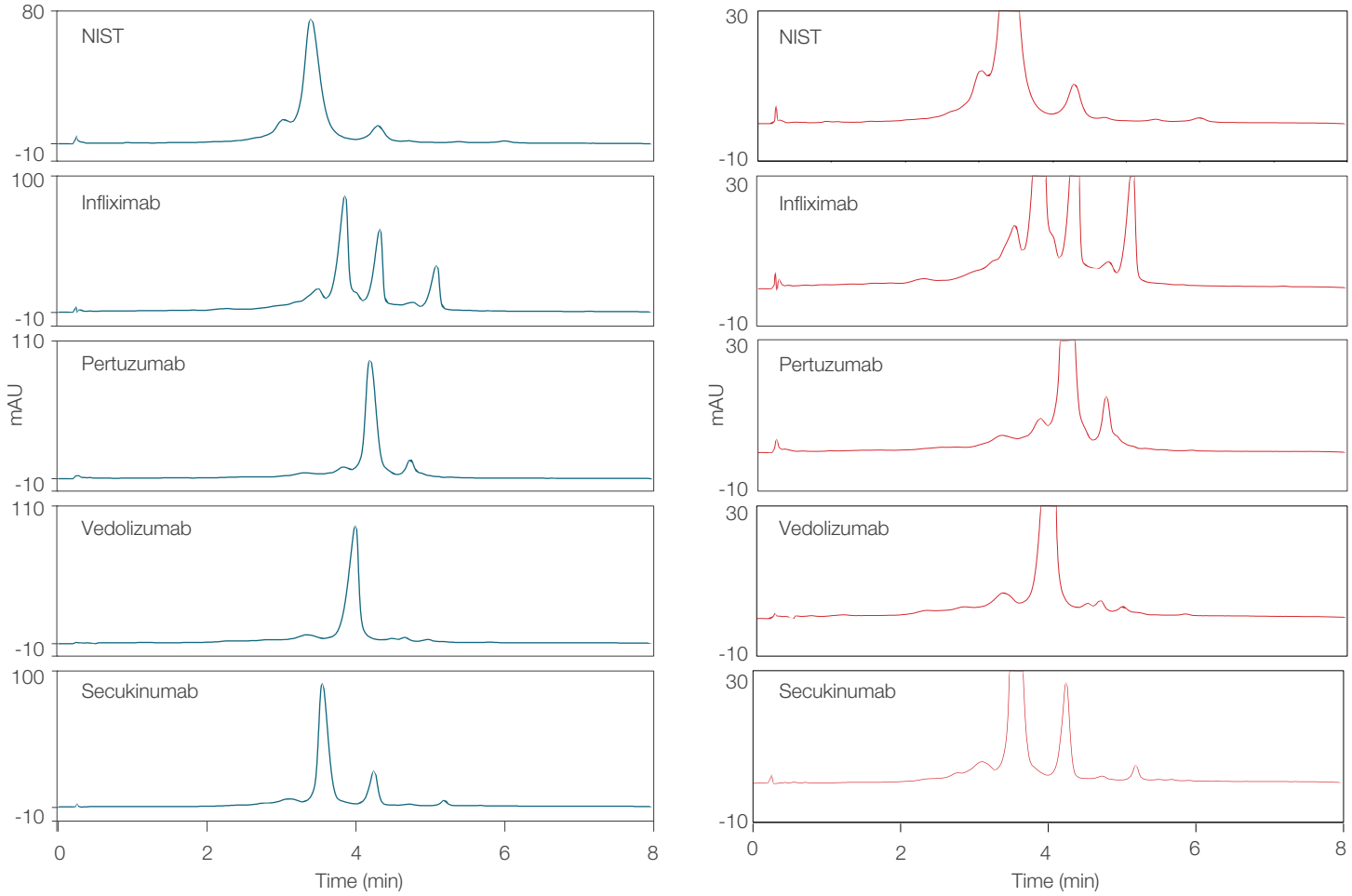


Figure 10: Analysis of mAbs on a 2 × 50 mm column using the general mAb gradient detailed in Table 7. Left chromatogram shows the full signal range with the right chromatogram showing the detailed view of the mAb variants.

Reference

1. Olivares, R., Bechler, S., Thermo Fisher Scientific Application note AN001694, Salt gradient analysis of monoclonal antibodies using a 3 µm monodisperse SCX chromatography column (2023), Thermo Fisher Scientific, Sunnyvale, CA, USA
2. Olivares, R., Bechler, S., Thermo Fisher Scientific Application note AN001814, Method development for pH gradient analysis of monoclonal antibodies using a 3 µm monodisperse particle strong cation exchange chromatography column (2023), Thermo Fisher Scientific, Sunnyvale, CA, USA

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