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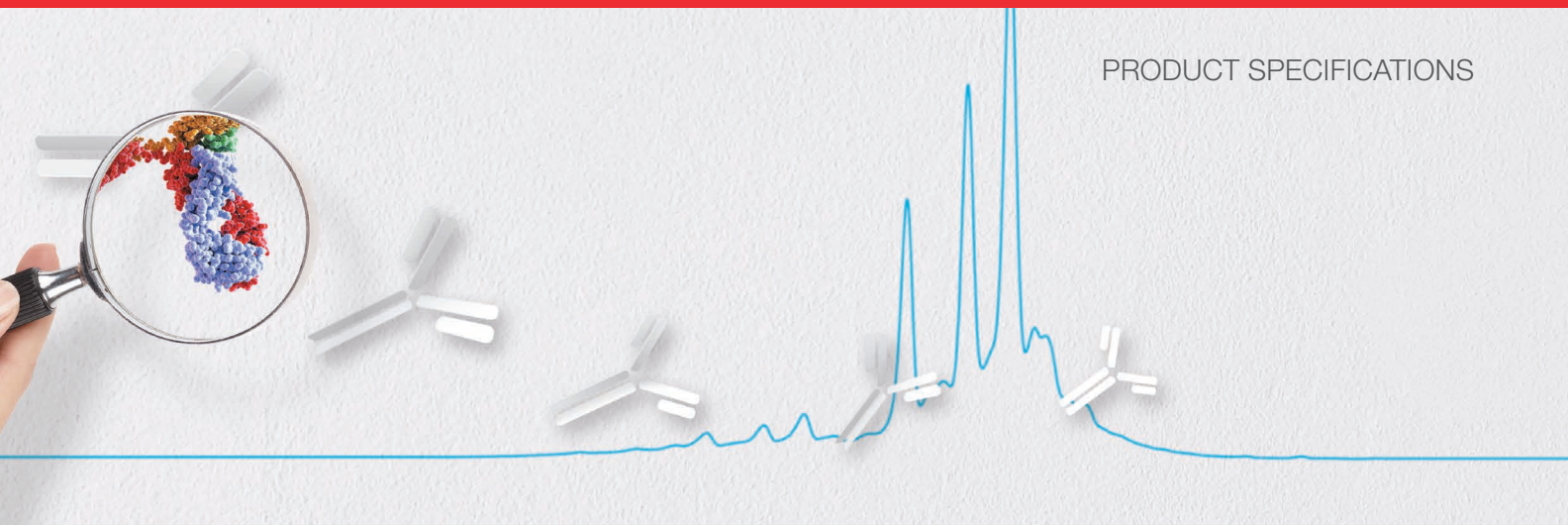

PRODUCT SPECIFICATIONS

ProPac Elite WCX Column

Chromatography column for therapeutic protein analysis

Benefits

- Superior resolution power for proteins, monoclonal antibodies, and associated charge variants
- High efficiency with reproducible separations
- High recovery with low carry-over
- Wide pH operating range: 2 – 12
- High temperature stability: up to 60 °C
- High throughput
- Compatible with CX-1 pH Gradient Buffers

Keywords
weak cation exchange (WCX), liquid chromatography (LC), protein characterization, monoclonal antibodies (mAbs), therapeutic recombinant proteins, protein change variants

The Thermo Scientific™ ProPac™ Elite WCX column is a weak cation exchange (WCX) liquid chromatography column designed for protein characterization including therapeutics such as monoclonal antibodies (mAbs). WCX chromatography is primarily used for the separation and quantitation of mAb and other protein charge variants that can arise during cellular production, downstream purification, storage, and shipping. This unique column chemistry provides excellent performance under a broad range of pH, temperature and mobile phase compositions with excellent recovery and low carryover.

Introduction

In 1980 insulin became the first recombinant protein (a protein produced by a host cell using a specific DNA sequence) to enter clinical trials and be released to market. Since then, therapeutic recombinant proteins have grown rapidly owing to their ability to perform specific biological functions. Today, monoclonal antibody (mAb) therapeutics capable of targeting specific cells and tissues comprise a significant portion of the biotherapeutics portfolio, with strong growth expected for the foreseeable future. The production of recombinant proteins, including mAbs, typically occurs in host cells (e.g., yeast, *E. coli* or mammalian cells). As a result of the cellular

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production process, these proteins are heterogeneous, typically due to post-translational modifications such as glycosylation or lysine truncation. Additional modifications such as oxidation or amino acid isomerization can be introduced during downstream processing, storage, and delivery. Since these modifications can affect efficacy and immunogenicity of the protein, comprehensive characterization of the associated variants is required for the final biopharmaceutical product prior to regulatory approval, during subsequent manufacturing, and use in patients. The increasing complexity of these proteins and use of different protein sub-classes (IgG1, IgG2, IgG4, scFv, etc.) requires the improvement of analytical methods for characterizing their structures to meet strict manufacturing and regulatory requirements.

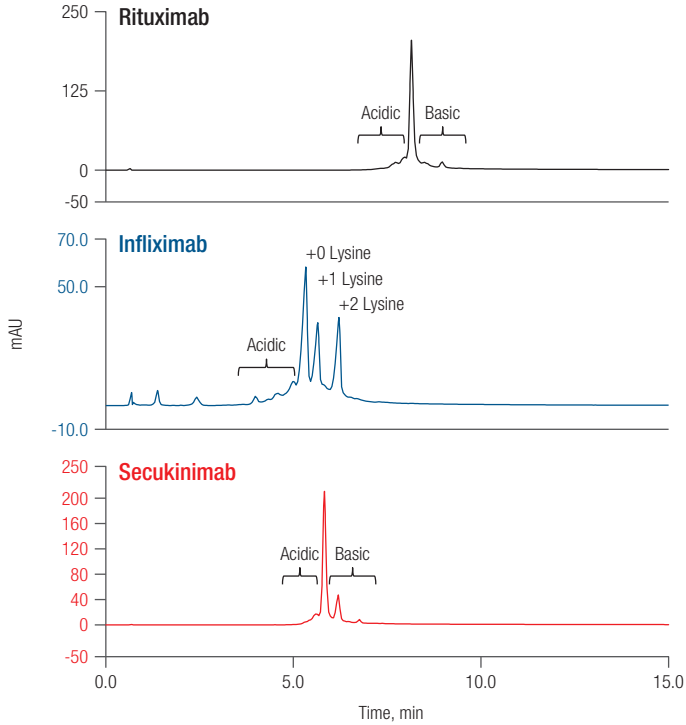
Column Technology

The ProPac Elite WCX columns are designed to provide fast, high-efficiency and high-resolution separations of proteins and glycoproteins based on their accessible surface charge. The 5 µm, non-porous particle is based on a solvent-compatible divinylbenzene resin coated with a hydrophilic polymer layer to exclude proteins from the surface of the resin, minimizing secondary interactions. Carboxylate groups grafted to this hydrophilic surface introduce anionic functionality that provides the weak cation exchange character required for promoting protein binding. 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.

Applications

Separation of intact proteins/mAbs using salt gradients

The ProPac Elite WCX column is designed for the separation of proteins and their charge variants. Figure 1 shows an example of charge variant separation for the pharmaceutical mAbs Rituximab, Infliximab and Secukinimab using a conventional salt gradient on a 4 × 150 mm column. Acidic and basic variants elute before and after the main mAb peak, respectively. For Infliximab, the lysine truncation variants are easily separated as well as associated acidic variants.

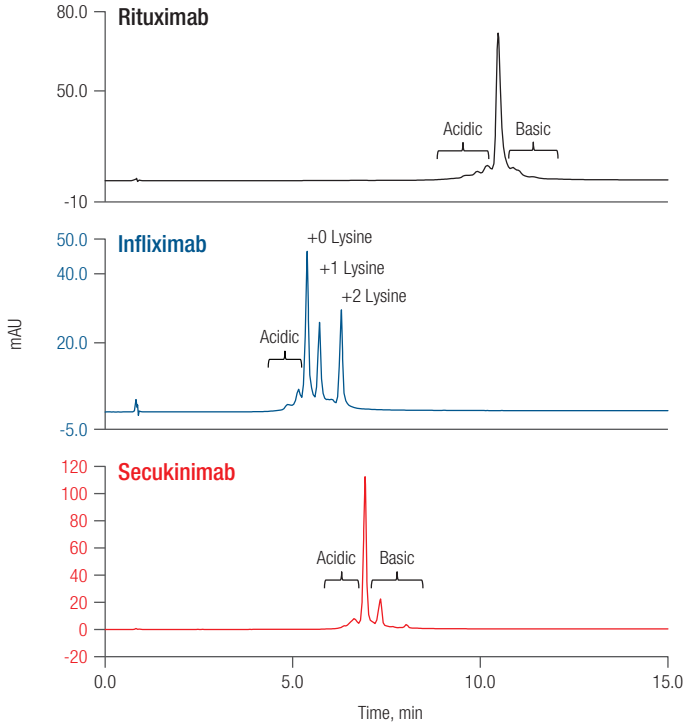


Column:	ProPac Elite WCX			Flow rate:	1.0 mL/min
Format:	4 × 150 mm			Inj. volume:	3 µL
Eluents:	A: 20 mM MES pH 6.5			Temperature:	30 °C
	B: 20 mM MES pH 6.5 + 0.5 M NaCl			Detection:	UV, 280 nm
				Sample:	Top: Rituximab, 5 mg/mL Middle: Infliximab, 5 mg/mL Bottom: Secukinimab, 5 mg/mL
Gradient:	Time (min)	%A	%B		
	0.0	95	5		
	15.0	65	35		
	15.1	60	50		
	16.0	60	50		
	16.1	95	5		
	25.0	95	5		

Figure 1. Enlarged view of the separation of Rituximab, Infliximab and Secukinimab and their associated charge variants on a 4 × 150 mm ProPac Elite WCX column using a salt gradient.

Separation of intact proteins/mAbs using pH gradients

The ProPac Elite WCX columns are compatible with CX-1 pH Gradient Buffers allowing for separations based on the isoelectric point of mAbs and their variants. Figure 2 shows the separation of charge variants for the same set of mAbs (Rituximab, Infliximab and Secukinimab) analyzed in Figure 1. As before, acidic and basic variants elute before and after the main mAb peak, respectively. Lysine variants for Infliximab are also easily separated using this method. Comparison of Figures 1 and 2 show the difference in selectivity between salt and pH gradients. The versatility of the ProPac Elite WCX column enables the chromatographer to select the optimal salt or pH gradient method without changing columns.



Column:	ProPac Elite WCX	Flow rate:	1.0 mL/min
Format:	4 × 150 mm	Inj. volume:	2 µL
Eluents:	A: CX-1 pH Gradient Buffer A (pH 5.6)	Temperature:	30 °C
	B: CX-1 pH Gradient Buffer B (pH 10.2)	Detection:	UV, 280 nm
		Sample:	Top: Rituximab, 5 mg/mL Middle: Infliximab, 5 mg/mL Bottom: Secukinimab, 5 mg/mL

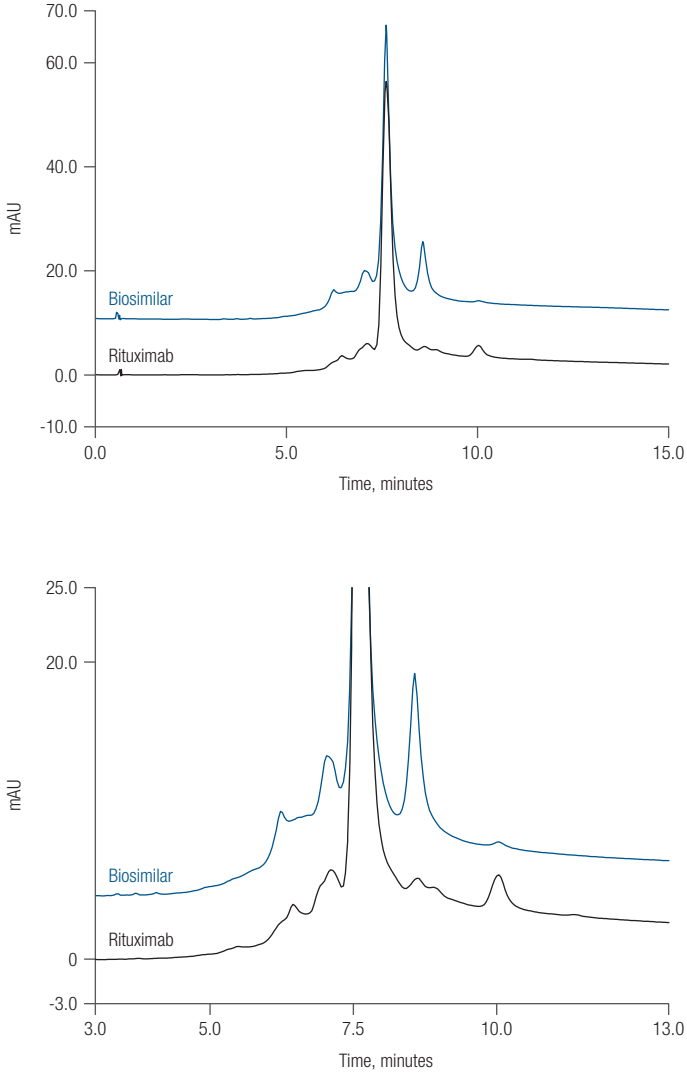
Gradient:

Time (min)	%A	%B
0.0	80	20
15.0	20	80
15.1	0	100
17.0	0	100
17.1	80	20
25.0	80	20

Figure 2. Separation of Rituximab, Infliximab and Secukinimab and their associated charge variants on a 4 × 150 mm ProPac Elite WCX column using CX-1 pH Gradient Buffers.

Innovator and biosimilar drugs

It is important for the developers and manufacturers of biosimilars to prove the similarity of the biosimilar to the original product in terms of structure, function, pharmacodynamics, pharmacokinetic properties, clinical efficacy and safety. Since charge variants could affect such parameters, cation exchange chromatography is often used to compare the innovator drug and the biosimilar. Figure 3 shows an expanded view and an enlarged view of the variant separation to compare the variant profiles of the originator Rituximab with an analogous biosimilar on a ProPac Elite WCX column using a salt gradient method. The excellent resolution of ProPac Elite WCX clearly shows differences in the acidic and basic variant profile of the originator and biosimilar.



Column:	ProPac Elite WCX	Flow rate:	1.0 mL/min
Format:	4 × 150mm	Inj. volume:	3 µL
Eluents:	A: 20 mM MES, pH 6.5	Temperature:	30 °C
	B: 20 mM MES + 0.5 M NaCl, pH 6.5	Detection:	UV, 280 nm
		Sample:	Blue: Rituximab Biosimilar, 5 mg/mL Black: Rituximab, 5 mg/mL

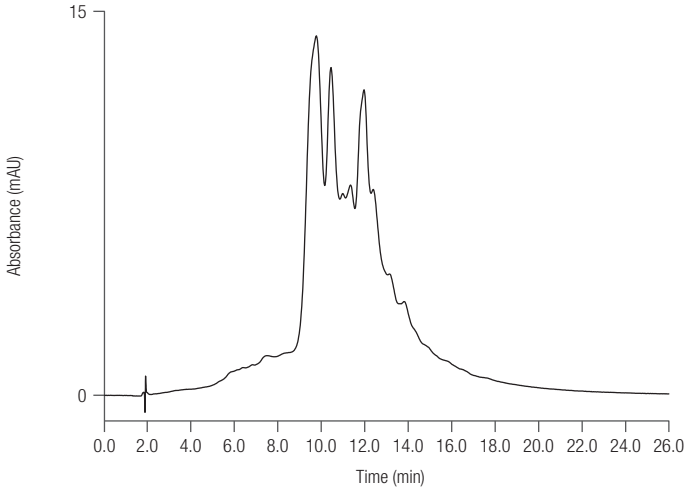
Gradient:

Time (min)	%A	%B
0.0	92	8
0.8	92	5
1.0	87	13
16.0	78	22
16.1	50	50
18.0	50	50
18.1	92	8
25.0	92	8

Figure 3. Comparison of acidic and basic variant profile for Rituximab and an analogous biosimilar using a 4 × 150 mm ProPac Elite WCX column with a salt gradient.

Analysis of pharmaceutical IgG2 and IgG4 mAbs and variants

IgG2 and IgG4 mAbs are a growing sector of the biopharmaceutical market capable of targeting specific antigenic sites, but modulating different cellular responses than IgG1. IgG2 and IgG4 are structurally distinct from IgG1 mAbs with regards to their disulfide bond linkage. As is the case for other mAbs, IgG2 and IgG4 must meet strict production and regulatory requirements. Figures 4 and 5 show the analysis of current pharmaceutical IgG2 and IgG4 mAbs using a salt gradient.



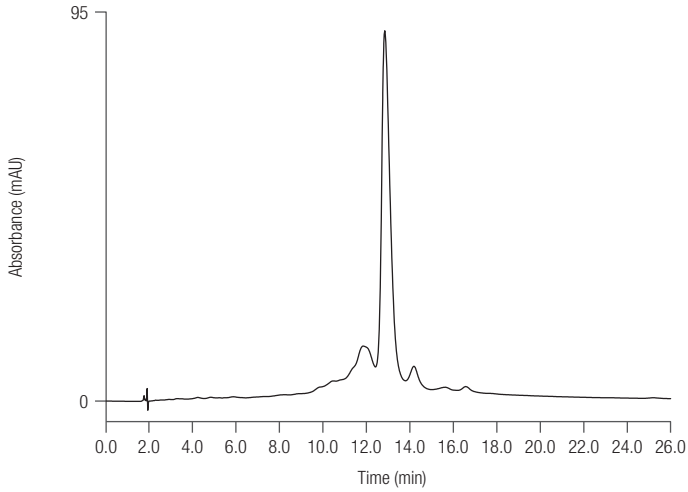
Column:	ProPac Elite WCX	Flow rate:	0.6 mL/min
Format:	4 × 250 mm	Inj. volume:	5 µL
Mobile phase A:	20 mM MES, pH 5.6	Temperature:	30 °C
Mobile phase B:	20 mM MES, 0.5 M NaCl, pH 5.6	Detection:	UV (280 nm)
		Sample:	IgG2 – Panitumumab (5 mg/mL)

Gradient:	Time (min)	%A	%B
	0.0	88	12
	1.0	88	12
	26.0	76	24
	26.1	0	100
	28.0	0	100
	28.1	88	12
	40.0	88	12

Figure 4. Analysis of IgG2 mAb Panitumumab on a 4 x 250 mm ProPac Elite WCX column using a salt gradient.

High-throughput analysis

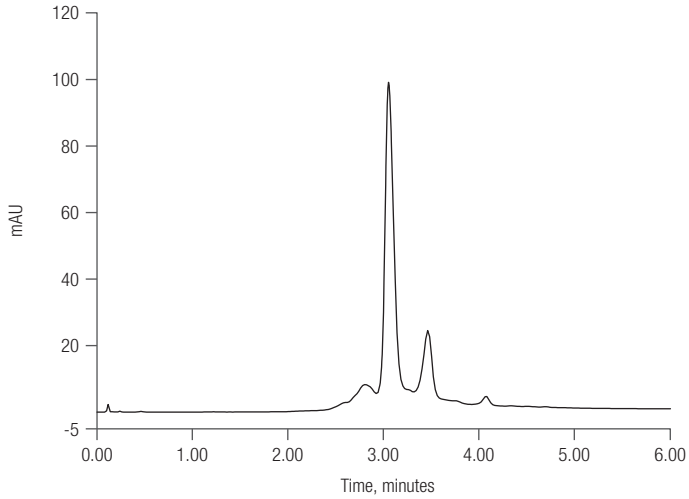
High-throughput screening methods are important during early stages of new drug development when 100's to 1000's of possible drug candidates may be produced. Short 50 mm column formats are best-suited for fast high-resolution separation of mAb variants due to their short gradient delay. Figure 6 shows the analysis of Secukinimab on a 2 x 50 mm ProPac Elite WCX column using a five-minute gradient with CX-1 pH Gradient Buffers. By using a smaller 150 µL mixer and a high flow rate of 0.8 mL/min, the total method time including column re-equilibration is reduced to only 10 minutes.



Column:	ProPac Elite WCX	Flow rate:	0.6 mL/min
Format:	4 × 250 mm	Inj. volume:	5 µL
Mobile phase A:	20 mM MES, pH 6.5	Temperature:	30 °C
Mobile phase B:	20 mM MES, 0.5 M NaCl, pH 6.5	Detection:	UV (280 nm)
		Sample:	IgG4 – Nivolumab (5 mg/mL)

Gradient:	Time (min)	%A	%B
	0.0	90	10
	1.0	90	10
	26.0	85	15
	26.1	0	100
	28.0	0	100
	28.1	90	10
	40.0	90	10

Figure 5. Analysis of IgG4 mAb Nivolumab on a 4 x 250 mm ProPac Elite WCX column using a salt gradient.



Column:	ProPac Elite WCX	Flow rate:	0.8 mL/min
Format:	2 × 50 mm	Inj. volume:	2 µL
Mobile phase A:	CX-1 pH Gradient Buffer A (pH 5.6)	Temperature:	30 °C
Mobile phase B:	CX-1 pH Gradient Buffer B (pH 10.2)	Detection:	UV (280 nm)
		Sample:	Secukinimab (5 mg/mL)

Gradient:	Time (min)	%A	%B
	-0.2	78	22
	0.0	78	22
	5.0	53	47
	6.0	53	47
	6.1	100	0
	7.0	100	0
	7.1	78	22
	10.0	78	22

Figure 6. Fast five-minute gradient separation of Secukinimab on a 2 × 50 mm ProPac Elite WCX column using CX-1 pH Gradient Buffers for a total run time of 10 minutes.

ProPac Elite WCX Column Properties

Run-to-run reproducibility for salt and pH gradient

The ProPac Elite WCX columns have excellent run-to-run reproducibility (as shown in Figure 7) for both salt (top) and pH (bottom) gradient methods. The consistent retention time, peak width and separation of variants for a Trastuzumab biosimilar demonstrates superior column ruggedness over 500 runs.

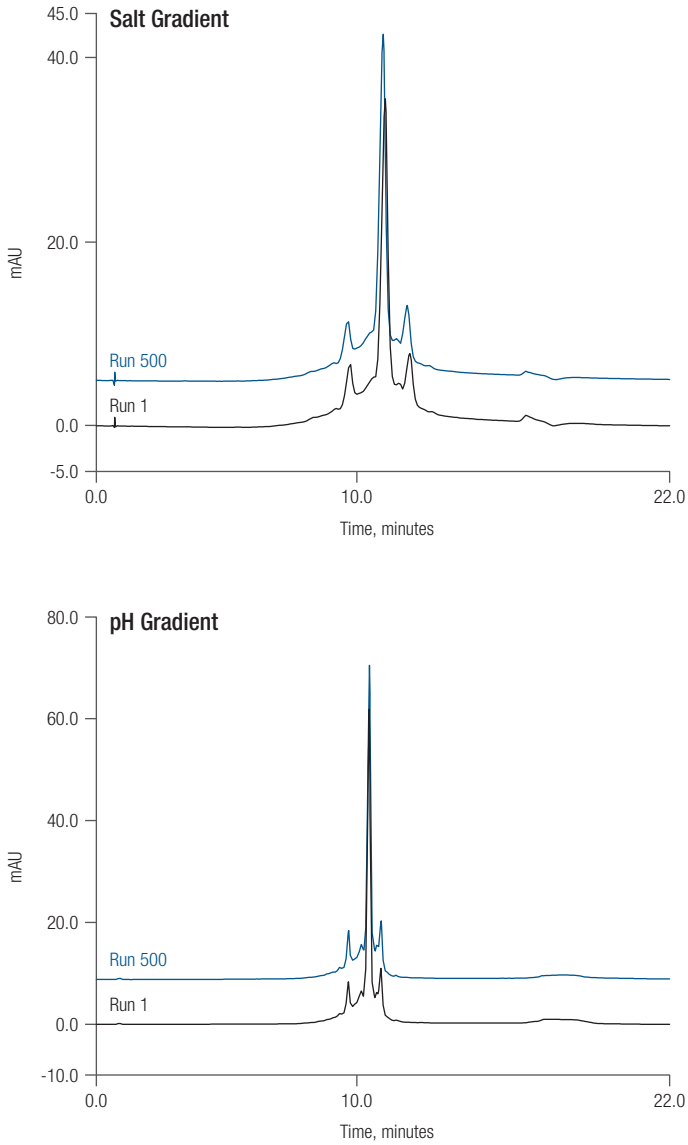
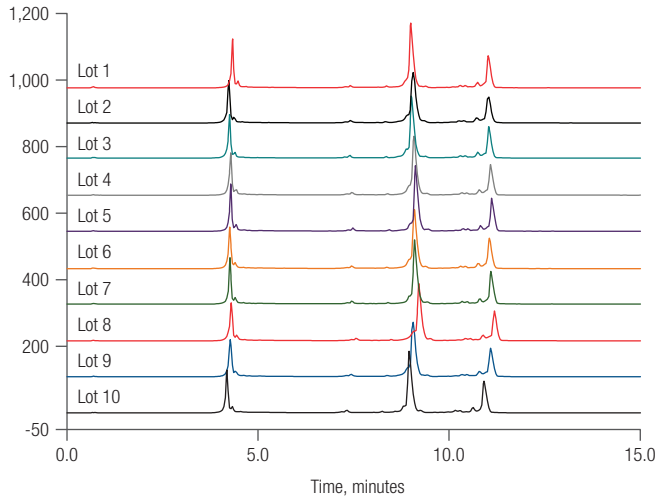


Figure 7. Run-to-run reproducibility of a 4 × 250 mm column tested using a salt gradient and a mAb (top trace) and a 4 × 150 mm column with a pH gradient and a mAb.

Lot-to-lot reproducibility

Lot-to-lot reproducibility is critical to ensure quality over time; the columns must have the same performance and provide consistent separation. The ProPac Elite WCX column has been designed to have a consistent lot-to-lot performance for protein and mAb separations. Figure 8 shows the lot-to-lot reproducibility for 10 lots of resin tested using a mixture of a monoclonal antibody and two proteins. The lots show consistent retention time and selectivity for the proteins and associated variants.

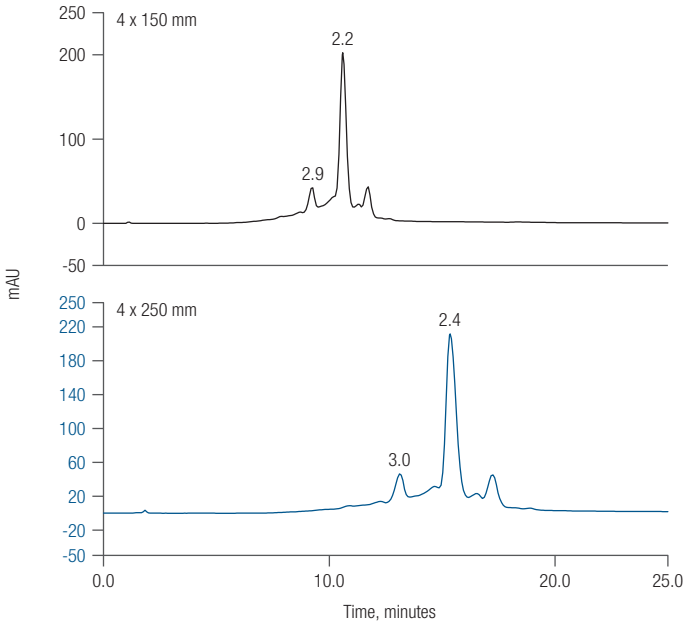


Column:	ProPac Elite WCX			Flow rate:	1.0 mL/min
Format:	4 × 150 mm			Inj. volume:	5 µL
Eluents:	A: 20 mM MES pH 6.5			Temperature:	30 °C
	B: 20 mM MES pH 6.5 + 0.5 M NaCl			Detection:	UV, 280 nm
Gradient:	Time (min)	%A	%B	Sample:	1. mAb – 2 mg/mL
	0.0	90	20		2. Cytochrome C – 4 mg/mL
	15.0	20	80		3. Ribonuclease A – 8 mg/mL
	16.0	20	80		
	16.1	90	10		
	25.0	90	10		

Figure 8. Lot-to-lot reproducibility for 10 lots of resin with the ProPac Elite WCX column.

Column formats for optimization of separation

The resolution power of a column is directly proportional to the column length. For salt gradients, longer columns will give improved resolution for a scaled gradient (e.g., a 250 mm long column should have a gradient 5/3X the length of a 150 mm column). Figure 9 compares the separation of a protein mixture on a 4 × 150 mm and 4 × 250 mm long column. Peak labels show the resolution for each peak increases when using the longer column. In compensation for the improved resolution of the longer column, the chromatographer accepts the use of a longer analysis time. For this reason, the required resolution and analysis time should be evaluated for each individual application to select the best column.

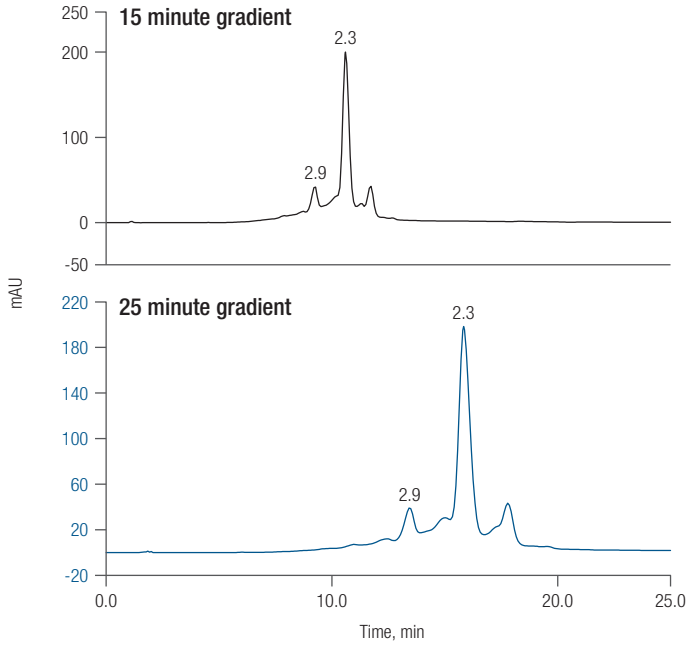


Column:	Top: ProPac Elite WCX	Flow rate:	0.6 mL/min
Format:	Top: 4 × 150 mm Bottom: 4 × 250 mm	Inj. volume:	Top: 12 µL Bottom: 20 µL
Eluents:	A: 20 mM MES pH 6.5 B: 20 mM MES pH 6.5 + 0.5 M NaCl	Temperature: Detection:	30 °C UV, 280 nm
Gradient:	Top: 12-22 %B over 15 minutes Bottom: 12-22 %B over 25 minutes	Sample: Peak label:	Trastuzumab biosimilar, 5 mg/mL Resolution

Figure 9. Comparison of a Trastuzumab biosimilar on a 4x150mm and a 4 × 250mm ProPac Elite WCX column. Gradient times were scaled to the column length.

ProPac Elite WCX 5 µm column vs. ProPac WCX-10 10 µm column

The ProPac Elite WCX 5 µm column provides a similar selectivity to the ProPac WCX-10 10 µm column; however, the smaller particle size offers improved resolution enabling faster analysis times with better resolution. This can be seen in Figure 10 showing the separation of an Trastuzumab biosimilar on a 4 × 150 mm ProPac Elite WCX 5 µm column and a 4 × 250 mm ProPac WCX-10 10 µm column using a 15 and 25 minute gradient, respectively. Comparison of the variant separation and detection demonstrates improved resolution for the ProPac Elite WCX 5 µm column compared to the 10 µm, ProPac WCX-10 column, while using a shorter gradient time.



Column:	Top: ProPac Elite WCX Bottom: ProPac WCX-10	Flow rate: Inj. volume:	0.6 mL/min Top: 12 µL Bottom: 20 µL
Format:	Top: 4 × 150 mm Bottom: 4 × 250 mm	Temperature: Detection:	30 °C UV, 280 nm
Eluents:	A: 20 mM MES pH 6.5 B: 20 mM MES pH 6.5 + 0.5 M NaCl	Sample:	Trastuzumab biosimilar, 5 mg/mL
Gradient:	12-22 %B; See chromatogram for gradient time	Peak label:	Resolution

Figure 10. Comparison of an Trastuzumab biosimilar on a 4 × 150 mm ProPac Elite WCX 5 µm column and a ProPac WCX-10 10 µm column.

Dynamic loading capacity - salt and pH gradients

Columns are commonly required to handle and separate large protein masses to enable the detection of minor charge variants. The dynamic loading capacity of a column indicates the effectiveness of the column to maintain separation power of proteins and variants with increased loading amount. Figure 11 shows the PWHH (peak width half height) for a mAb plotted against mass loaded using a salt (left) gradient and a pH (right) gradient. Maximum dynamic loading capacity is set at twice the PWHH at low loading levels, which do not result in an increase in the PWHH. This data shows that a pH gradient allows for greater protein mass loading relative to the salt gradient by a factor of ~1.5.

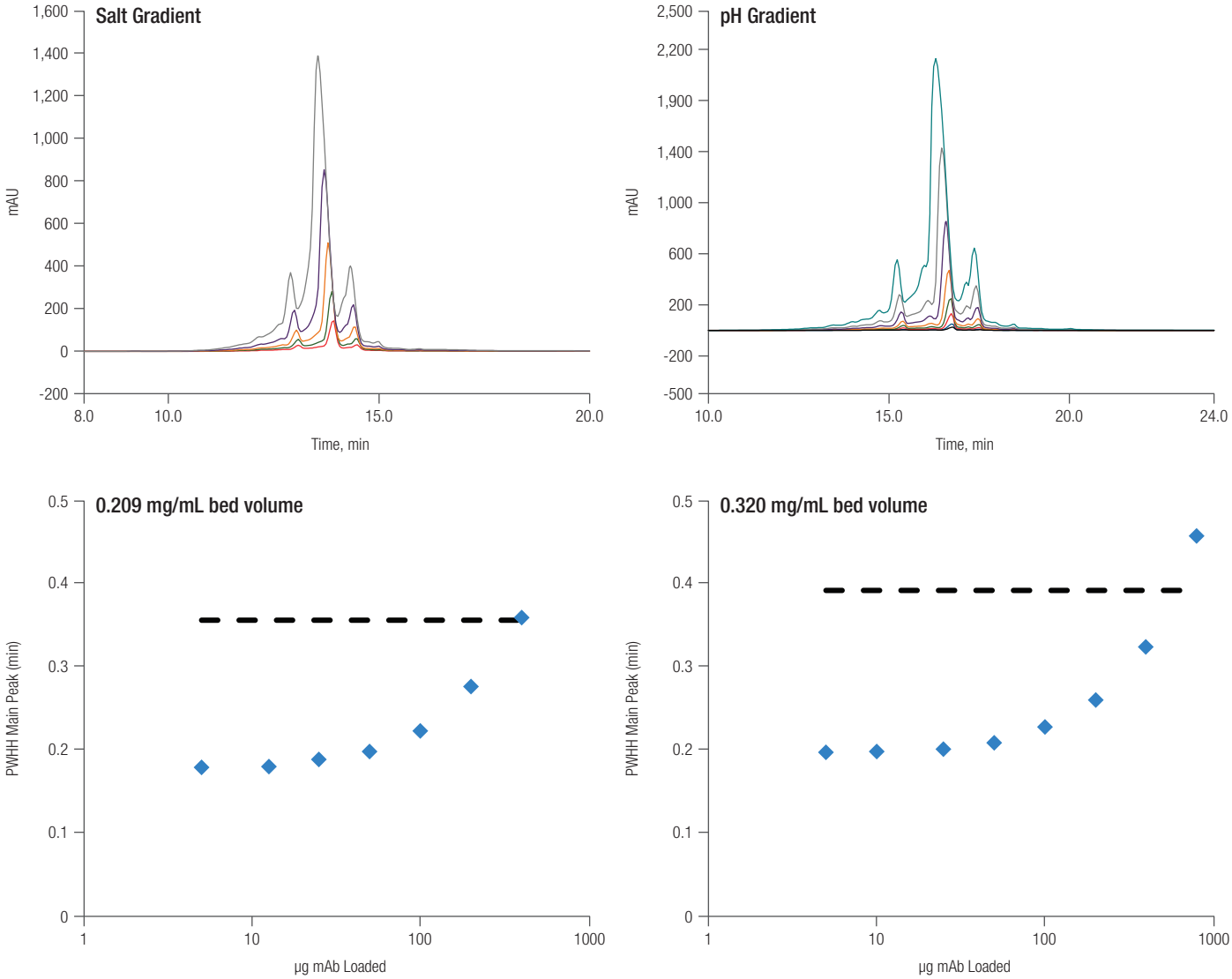


Figure 11. Dynamic loading analysis of a Trastuzumab biosimilar on a 4 × 150 mm ProPac Elite WCX column using a left salt and right pH gradient.

Carryover for salt and pH gradients

When evaluating high mass loading, the column needs to have low run-to-run carryover to prevent sample interference for subsequent injections. Figure 12 shows the carryover for salt gradient and pH gradient methods using a blank following an analytical run with a 50µg injection of a mAb. The blank runs show carryover of only 0.02% and 0.08% for the salt and pH gradient methods, respectively.

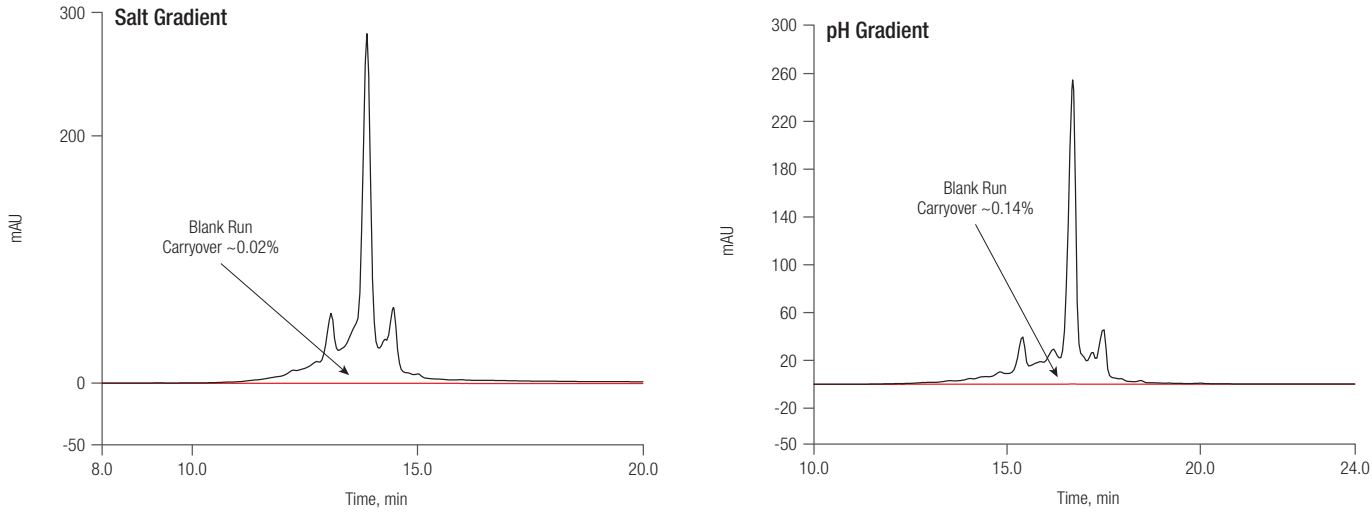


Figure 12. Demonstration of low carryover for a Trastuzumab biosimilar using a salt gradient (left) and a CX-1 pH buffer gradient (right) on a 4 x 150 mm ProPac Elite WCX column.

Consistent Manufacturing

Each ProPac Elite WCX column is manufactured according to stringent specifications to ensure column-to-column reproducibility. Each column is shipped with test chromatograms demonstrating qualification of the resin lot and qualification of the individual serialized column.

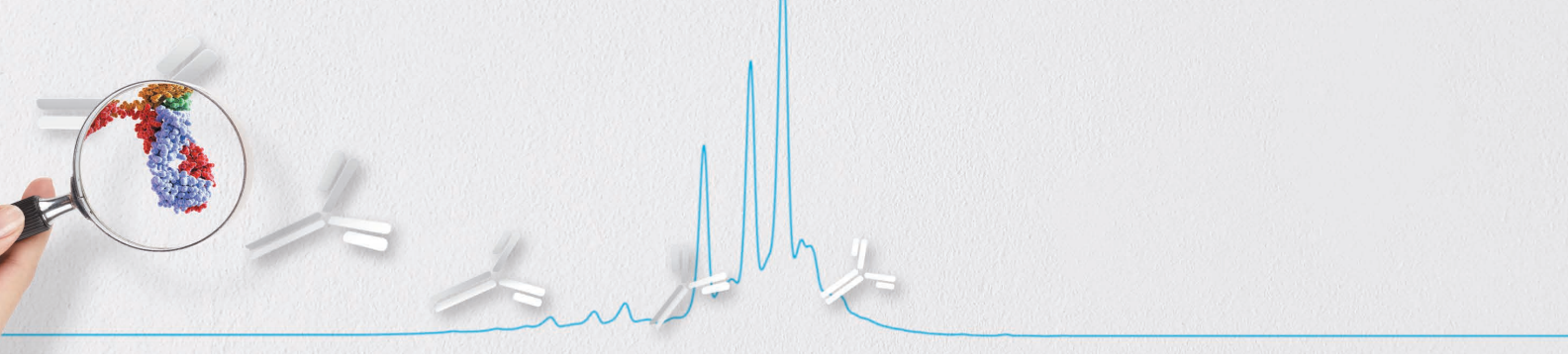
Physical Data

Chemistry	Carboxylate
Polymer Substrate	DVB particles with hydrophilic coating
Particle size	5 µm
Pore size	Non-porous
Column housing	PEEK

Operational Specifications

Column	Recommended Flow Rate mL/min	Max Column Pressure psi (bar)	Temperature °C	pH
2 × 50 mm	0.1-0.8	4500 (310)	10 – 60	2-12
2 × 150 mm	0.1-0.25			
2 × 250 mm	0.1-0.2			
4 × 50 mm 4 × 150 mm	0.4-1.0			
4 × 250 mm	0.4-0.8			

For more information, refer to the column manual.



Ordering Information

Description	Particle Size (µm)	Part Number
ProPac Elite-WCX Columns		
ProPac Elite WCX, 2 × 50 mm	5	303028
ProPac Elite WCX, 2 × 150 mm	5	303027
ProPac Elite WCX, 2 × 250 mm	5	303026
ProPac Elite WCX, 4 × 50 mm	5	302973
ProPac Elite WCX, 4 × 150 mm	5	302972
ProPac Elite WCX, 4 × 250 mm	5	303025
ProPac Elite WCX, 4 × 150 mm, 3 Columns from 1 Lot	5	302976
ProPac Elite WCX, Analytical, 4 × 150 mm, 3 Columns from 3 Lots	5	302977
ProPac Elite WCX, Analytical, 4 × 250 mm, 3 Columns from 1 Lot	5	303061
ProPac Elite WCX, Analytical, 4 × 250 mm, 3 Columns from 3 Lots	5	303062
CX-1 pH Gradient Buffers		
125 mL CX-1 pH Gradient Buffer A		083273
250 mL CX-1 pH Gradient Buffer A		085346
500 mL CX-1 pH Gradient Buffer A		302779
125 mL CX-1 pH Gradient Buffer B		083275
250 mL CX-1 pH Gradient Buffer B		085348
500 mL CX-1 pH Gradient Buffer B		302780
CX-1 pH Gradient Buffer Kit: 125 mL Buffer A + 125 mL Buffer B		083274
CX-1 pH Gradient Buffer Kit: 250 mL Buffer A + 250 mL Buffer B		085349

Find out more at **thermofisher.com/ProPac**

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