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ProPac Elite WCX Column

It's time to see your protein separations in higher resolution

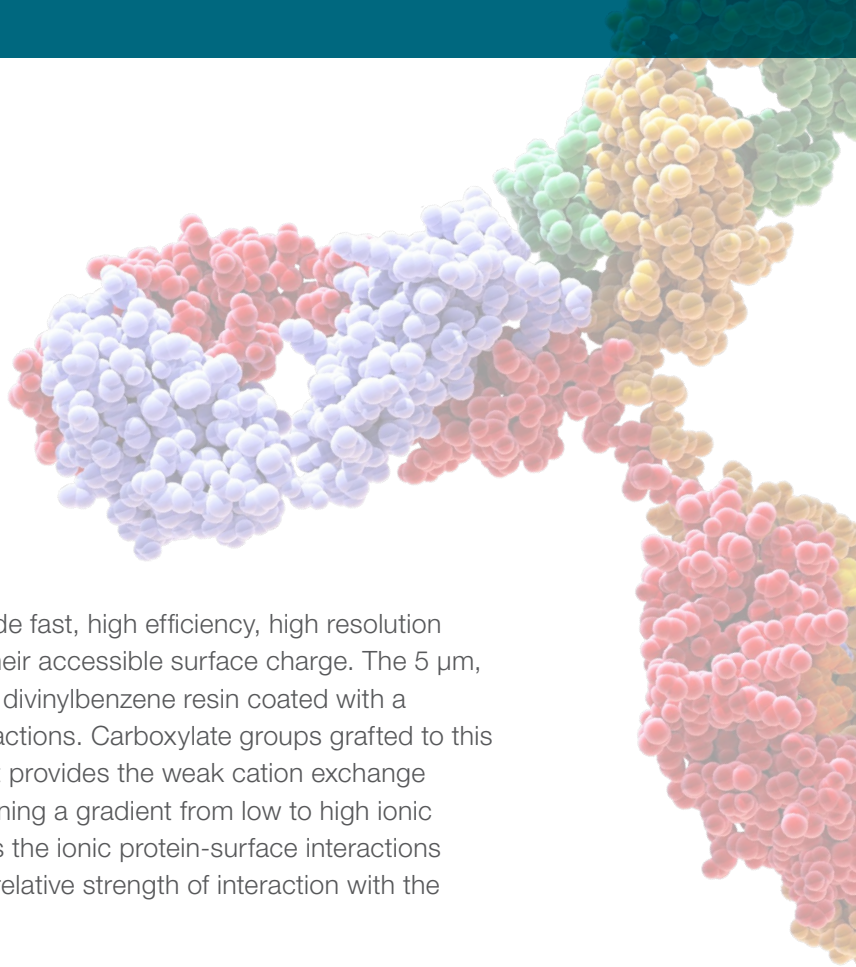
ThermoFisher SCIENTIFIC

High performance weak cation exchange chromatography column for protein and monoclonal antibody analysis

The Thermo Scientific™ ProPac™ Elite WCX 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.

Product Highlights

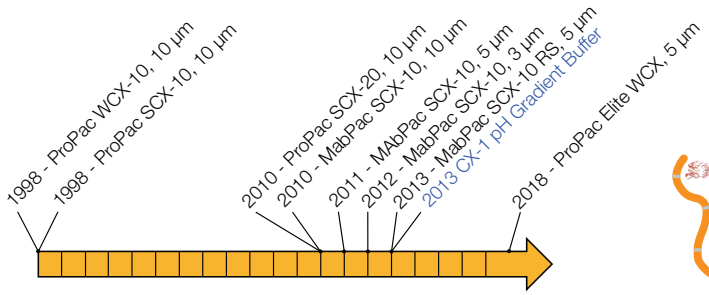
- Superior resolution power for proteins, monoclonal antibodies and associated charge variants
- High efficiency with reproducible separations
- High recovery with low carryover
- Wide pH operating range: 2 – 12
- High temperature stability: up to 60 °C
- High throughput
- Compatible with CX-1 pH Gradient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



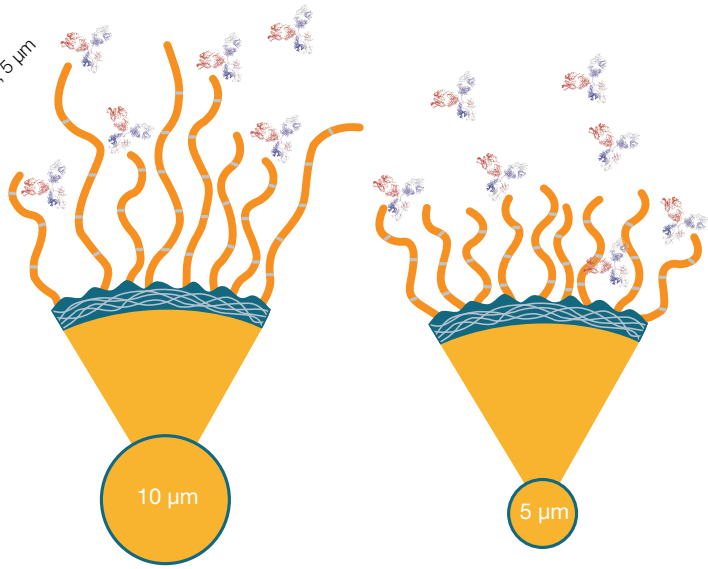
Column Technology

The ProPac Elite WCX columns are designed to provide fast, high efficiency, 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 minimize 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.

Thermo Scientific Cation Exchange Chromatography Column Timelime

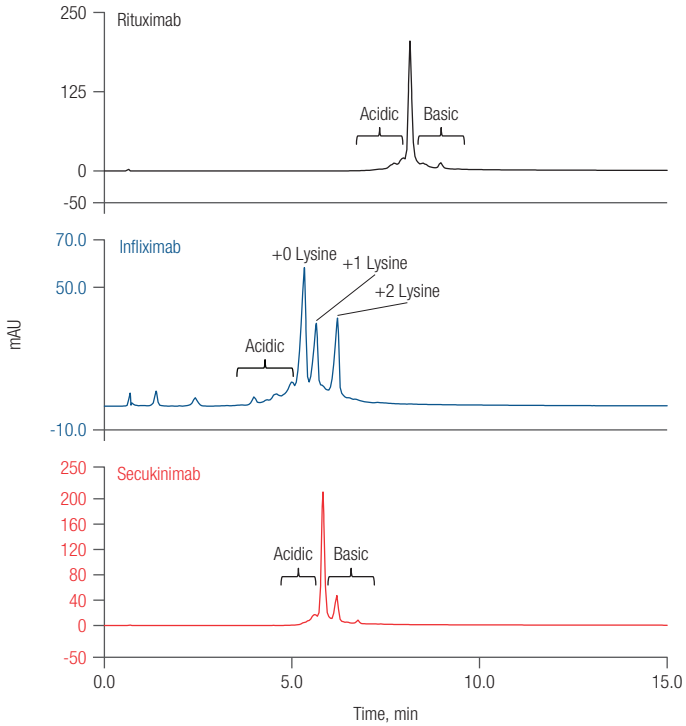


- 20 year history of cation exchange columns for protein analysis
- Weak and strong cation exchange technologies
- Standard technology for variant analysis



Separation of intact proteins/mAbs using salt gradients

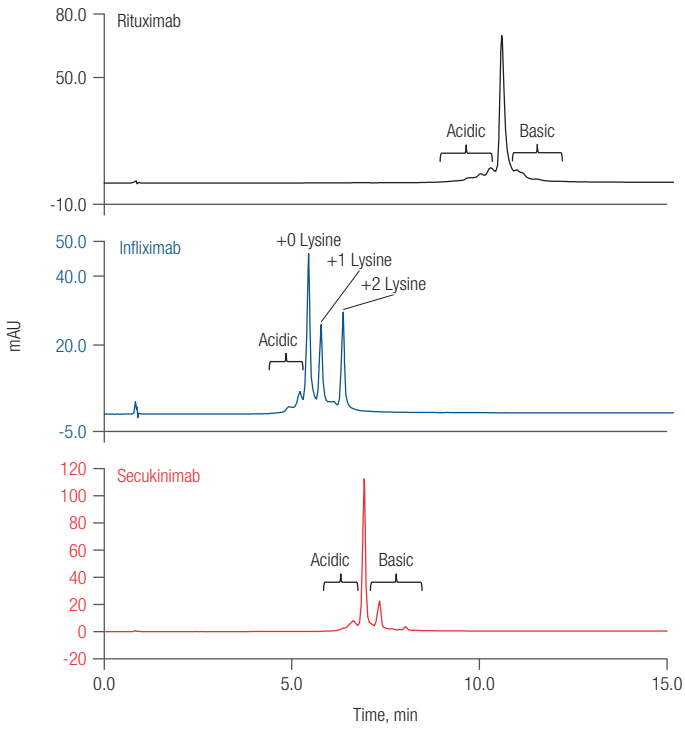
The ProPac Elite WCX column is designed for the separation of proteins and their charge variants. Figure 1 shows an example of charge variant separation for the pharmaceutical mAbs Rituximab, Infliximab and Secukinimab using a conventional salt gradient on a 4 × 150 mm column. Acidic and basic variants 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 5 µm		
Format:	4 × 150 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	95	5
	15.0	65	35
	15.1	60	50
	16.0	60	50
	16.1	95	5
	25.0	95	5
Flow rate:	1.0 mL/min		
Inj. volume:	3 µL		
Temperature:	30 °C		
Detection:	UV, 280 nm		
Sample:	Top:	Rituximab, 5 mg/mL	
	Middle:	Infliximab, 5 mg/mL	
	Bottom:	Secukinimab, 5 mg/mL	

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.

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. 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.

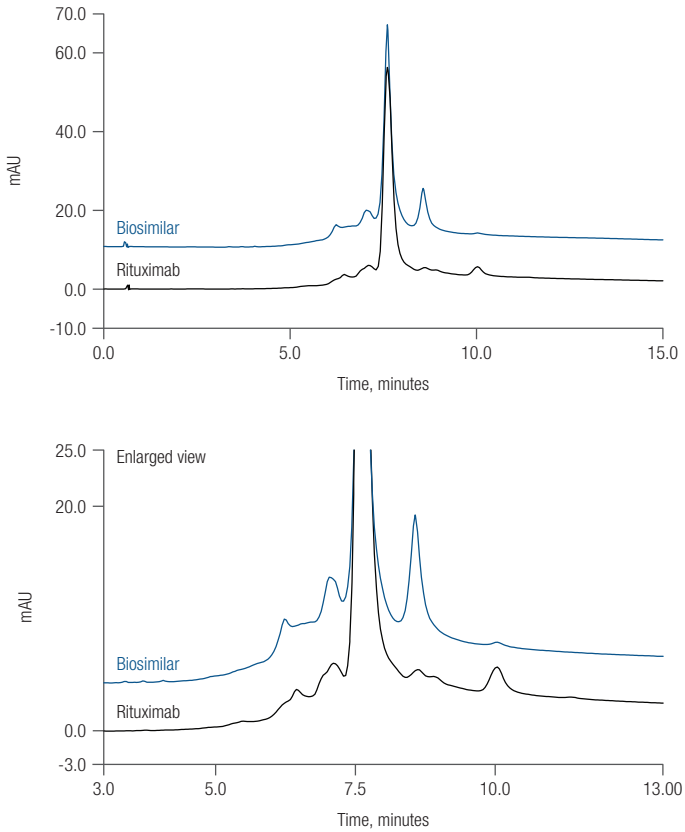


Column:	ProPac Elite WCX		
Format:	4 × 150 mm		
Eluents:	A: CX-1 pH Gradient Buffer A		
	B: CX-1 pH Gradient Buffer B		
Gradient:	Time (min)	%A	%B
	0.0	80	20
	15.0	20	80
	15.1	0	100
	17.0	0	100
	17.1	80	20
	25.0	80	20
Flow rate:	1.0 mL/min		
Inj. volume:	2 µL		
Temperature:	30 °C		
Detection:	UV, 280 nm		
Sample:	Top:	Rituximab, 5 mg/mL	
	Middle:	Infliximab, 5 mg/mL	
	Bottom:	Secukinimab, 5 mg/mL	

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 column clearly shows differences in the acidic and basic variant profile of the originator and biosimilar.

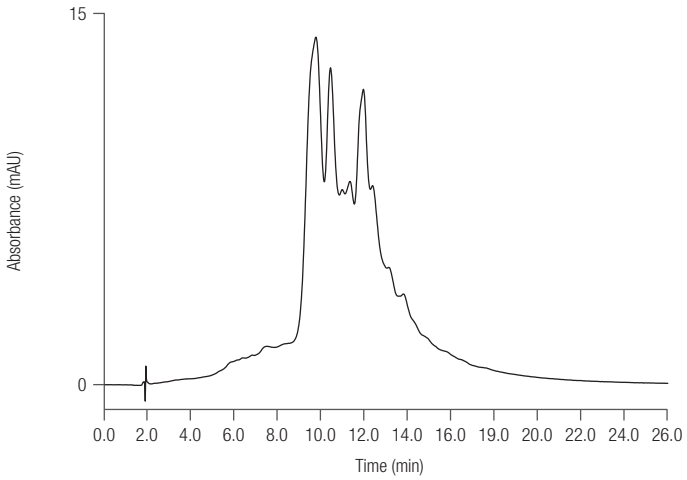


Column:	ProPac Elite WCX		
Format:	4 × 150 mm		
Eluents:	A: 20 mM MES, pH 6.5		
	B: 20 mM MES + 0.5 M NaCl, pH 6.5		
Gradient:	Time (min)	%A	%B
	0.0	92	8
	0.8	92	5
	1.0	87	13
	16.0	78	22
	16.1	50	50
	18.0	50	50
	18.1	92	8
	25.0	92	8
Flow rate:	1.0 mL/min		
Inj. volume:	3 µL		
Temperature:	30 °C		
Detection:	UV, 280 nm		
Sample:	Blue: Rituximab Biosimilar, 5 mg/mL		
	Black: Rituximab, 5 mg/mL		

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.

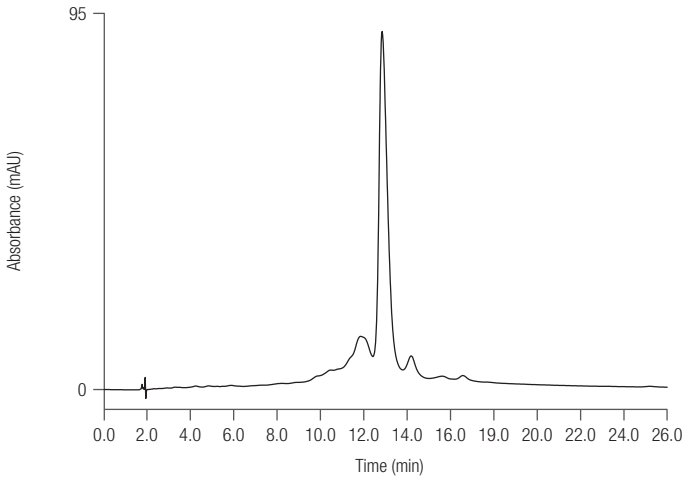
Analysis of pharmaceutical IgG2 and IgG4 mAbs and variants

IgG2 and IgG4 mAbs are a growing sector of the biopharmaceutical market capable of targeting specific antigenic sites while 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		
Format:	4 × 250 mm		
Mobile phase A:	20 mM MES, pH 5.6		
Mobile phase B:	20 mM MES, 0.5 M NaCl, pH 5.6		
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
Flow rate:	0.6 mL/min		
Inj. volume:	5 µL		
Temperature:	30 °C		
Detection:	UV (280 nm)		
Sample:	IgG2 – Panitumumab (5 mg/mL)		

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

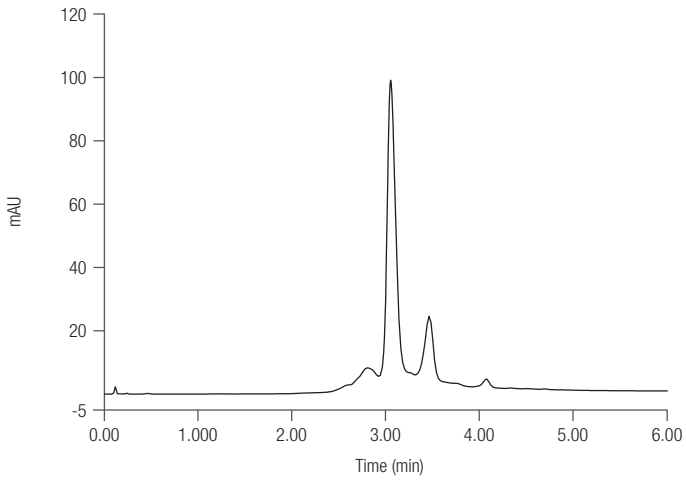


Column:	ProPac Elite WCX		
Format:	4 × 250 mm		
Mobile phase A:	20 mM MES, pH 6.5		
Mobile phase B:	20 mM MES, 0.5 M NaCl, pH 6.5		
Gradient:	Time (min)	%A	%B
	0.0	90	10
	1.0	90	10
	26.0	85	15
	26.1	0	100
	28.0	0	100
	28.1	90	10
	40.0	90	10
Flow rate:	0.6 mL/min		
Inj. volume:	5 µL		
Temperature:	30 °C		
Detection:	UV (280 nm)		
Sample:	IgG4 – Nivolumab (5 mg/mL)		

Figure 5. Analysis of IgG4 mAb Nivolumab 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 × 50 mm ProPac Elite WCX column using a 5 minute gradient with 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		
Format:	2 × 50 mm		
Mobile phase A:	CX-1 pH Gradient Buffer A		
Mobile phase B:	CX-1 pH Gradient Buffer B		
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
Flow rate:	0.8 mL/min		
Inj. volume:	2 µL		
Temperature:	30 ºC		
Detection:	UV (280 nm)		
Sample:	Secukinimab (5 mg/mL)		

Figure 6. Fast 5 minute 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 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 (left) salt and (right) pH gradient methods. The consistent retention time, peak width, and separation of variants demonstrates superior column ruggedness and reproducibility in 500 runs. The run-to-run reproducibility ensures the injection results are directly comparable over the course of the column lifetime.

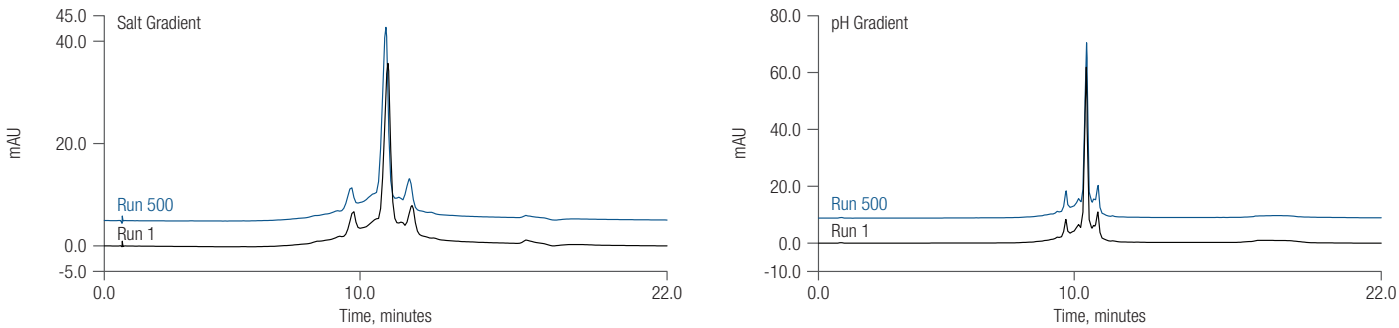
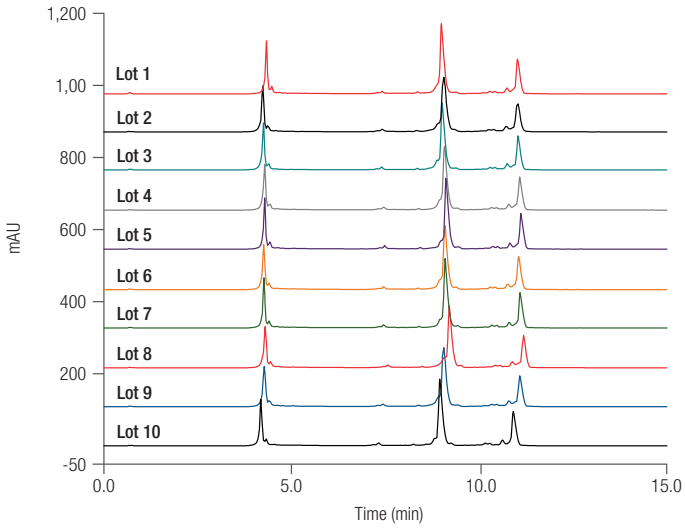


Figure 7. Run-to-run reproducibility overlay for a salt gradient and a pH gradient separation of a monoclonal antibody.

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 has been designed to have 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		
Format:	4 × 150 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	20
	15.0	20	80
	16.0	20	80
	16.1	90	10
	25.0	90	10
Flow rate:	1.0 mL/min		
Inj. volume:	5 µL		
Temperature:	30 °C		
Detection:	UV, 280 nm		
Sample:	1. mAb – 2 mg/mL		
	2. Cytochrome C – 4 mg/mL		
	3. Ribonuclease A – 8 mg/mL		

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

Complementary selectivity between WCX and SCX

The complexity of the protein 3-D structure and influence of hydrophobic, polar and charged groups proximal to cationic sites make prediction of protein and variant elution for cation exchange chromatography difficult. The relative affinities of cationic sites such as lysine, histidine and arginine differ for the anionic functional weak cation exchange carboxylate groups and strong cation exchange sulfonate groups. This difference in affinity makes the ProPac Elite WCX and MAbPac SCX-10 phases complementary. Figure 9 shows a comparison of the ProPac Elite WCX and the MAbPac SCX-10, 5 µm columns for the separations of Pertuzumab and Trastuzumab. It is important to investigate both WCX and SCX phases for their relative variant separation, so that the desired analysis is achieved.

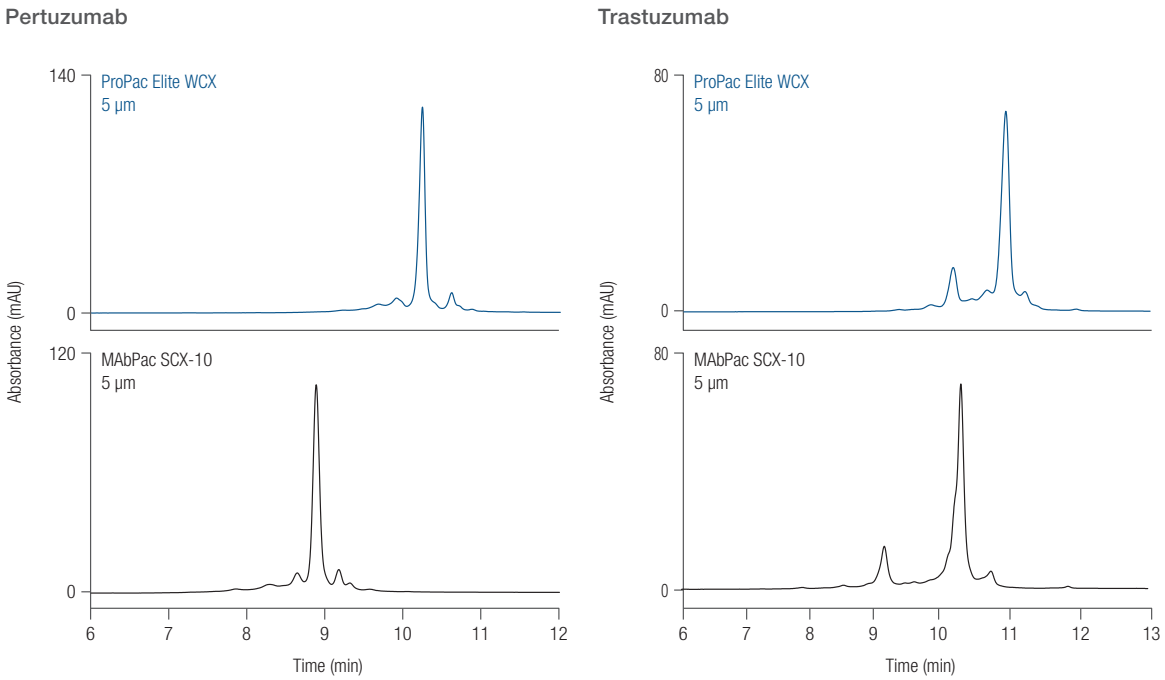
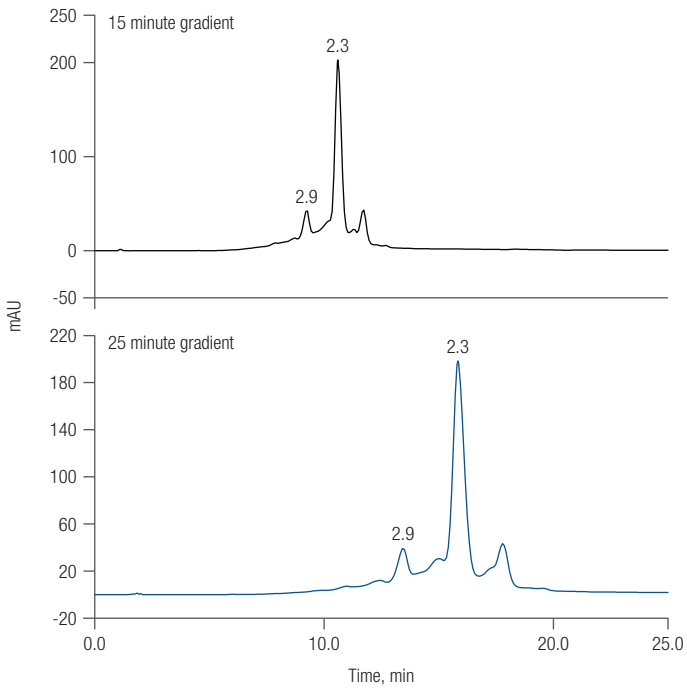


Figure 9. Comparison of a Pertuzumab and Trastuzumab separated on a 4 × 150 mm ProPac Elite WCX (top) and a 4 × 150 mm MAbPac SCX-10, 5 µm column (bottom) illustrating the selectivity differences of the two phases between mAb samples (Pertuzumab - left and Trastuzumab - right).

ProPac Elite WCX 5 µm versus ProPac WCX-10, 10 µm

The ProPac Elite WCX 5 µm columns provide a similar selectivity to the ProPac WCX-10, 10 µm columns; however, the smaller particle size offers improved resolution enabling faster analysis times with equal or better resolution. This can be seen in Figure 10 showing the separation of a Herceptin 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 equivalent resolution for the ProPac Elite WCX 5 µm compared to the 10µm ProPac WCX-10 while using a shorter gradient time.



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

Figure 10. Comparison of a Herceptin biosimilar on a 4 x 150 mm ProPac Elite WCX 5 µm column and a 4 × 250 mm ProPac WCX-10, 10 µm 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			

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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