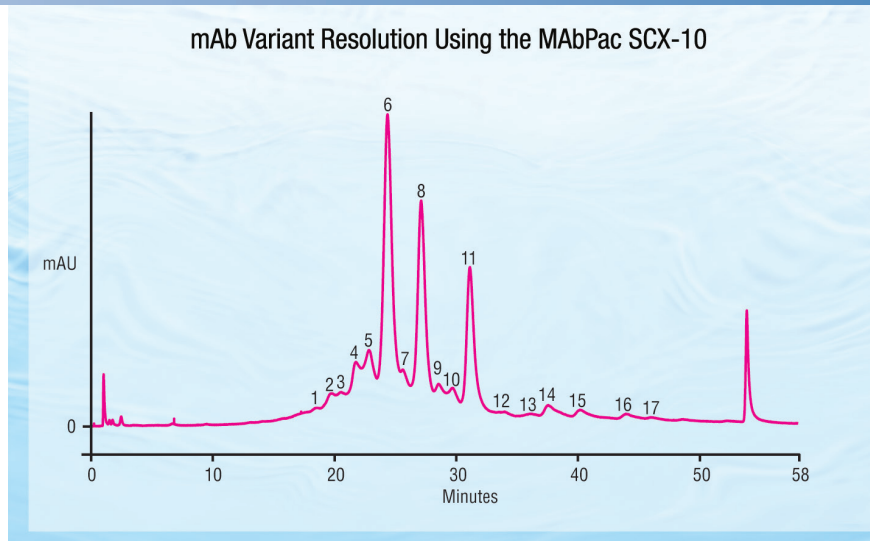


MABPac SCX-10 Column for Monoclonal Antibody Variant Analysis and Characterization

The Thermo Scientific™ MABPac™ SCX-10 columns separate closely-related monoclonal antibody variants for characterization and quality control assessment.

- High resolution of monoclonal antibody variants
- Exceptionally high efficiency
- Available in 3, 5 or 10 μm particle size
- PEEK and UHPLC column formats
- High throughput capability
- Excellent lot-to-lot and column-to-column reproducibility
- Ideal for R&D and QA/QC development



High-Resolution, High-Efficiency Analysis of Monoclonal Antibody (mAb) Variants

The MABPac SCX-10 column is a strong cation-exchange column designed specifically for the high resolution, high efficiency analysis of monoclonal antibodies and associated variants. The unique nonporous pellicular resin provides high resolving power, permitting the separation of monoclonal antibody variants that differ by as little as one charged residue.

The MABPac SCX resin technology is the basis for the superior performance of monoclonal antibody variant analysis. The nonporous core particle provides high rates of mass transfer, which results in high efficiency separations. A proprietary hydrophilic layer surrounds the polymeric beads preventing hydrophobic interactions between proteins and the core of the resin. A proprietary grafted cation-exchange surface provides an ideal separation platform for high-resolution separations. MABPac SCX-10 small particle columns are available in PEEK and PEEK-lined stainless steel (RS series of columns) housings. While PEEK columns are available

in three different particle sizes of 10 μm , 5 μm and 3 μm , PEEK-lined stainless steel columns are currently available in 5 μm particle sizes. Depending on the resolution desired and the time allotted for specific applications, 10, 5 or 3 μm columns may be used. For HPLC based applications, PEEK columns are suitable and are recommended. For UHPLC applications, PEEK-lined stainless steel RS columns are recommended as they are compatible with high pressure and can withstand pressures up to 7,000 psi. Higher flow rates may be used with Rapid Separation (RS) columns when compared to PEEK columns, for faster run time and to achieve high throughput separations. RS version of the MABPac SCX-10 columns are built with PEEK-lined stainless steel. These columns are designed to be used with bioinert UHPLC system.

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MABPac SCX-10, 3 μ m and MABPac SCX-10, 5 μ m columns are recommended for high resolution and high-throughput separations of mAbs. The main advantage being the run time is reduced when 3 or 5 μ m, shorter columns are used as compared to a longer MABPac SCX-10, 10 μ m, 4 \times 250 mm columns. Since the column length is short, chromatography runs can be completed at a faster rate, therefore increasing the throughput.

The MABPac SCX-10 column is complementary to the industry-leading Thermo Scientific™ ProPac™ WCX-10 column for monoclonal antibody variant analysis, offering an alternative selectivity and providing higher resolution and efficiency for variant analysis of monoclonal antibody samples.

Selectivity Differences Between ProPac WCX-10 and MABPac SCX-10

MABPac SCX columns exhibits different selectivity as compared with ProPac WCX for protein separations. When a protein mixture was separated on these columns under identical conditions, ribonuclease A elutes first on the MABPac SCX column, followed by cytochrome C and lysozyme. In comparison, cytochrome C elutes first on ProPac WCX column followed by lysozyme and ribonuclease A (Figure 1A and 1B), confirming the selectivity changes.

Characterization of Monoclonal Antibody Heterogeneity; Acidic and Basic Variant Analysis

Monoclonal antibodies are currently developed by pharmaceutical and biotechnology companies for various therapeutic applications. They undergo several modifications including oxidations, deamidations, glycosylation, incomplete C-terminal processing, and others (1–3). These modifications cause antibody microheterogeneity. Variations in a monoclonal antibody's composition can impact its activity and stability as a biotherapeutic. Monitoring stability of therapeutic monoclonal antibodies is regarded as essential for demonstrating safety and efficacy of a monoclonal antibody drug, and is expected by the FDA and other regulatory agencies. The MABPac SCX-10 column is useful for characterizing monoclonal heterogeneity, and for stability testing.

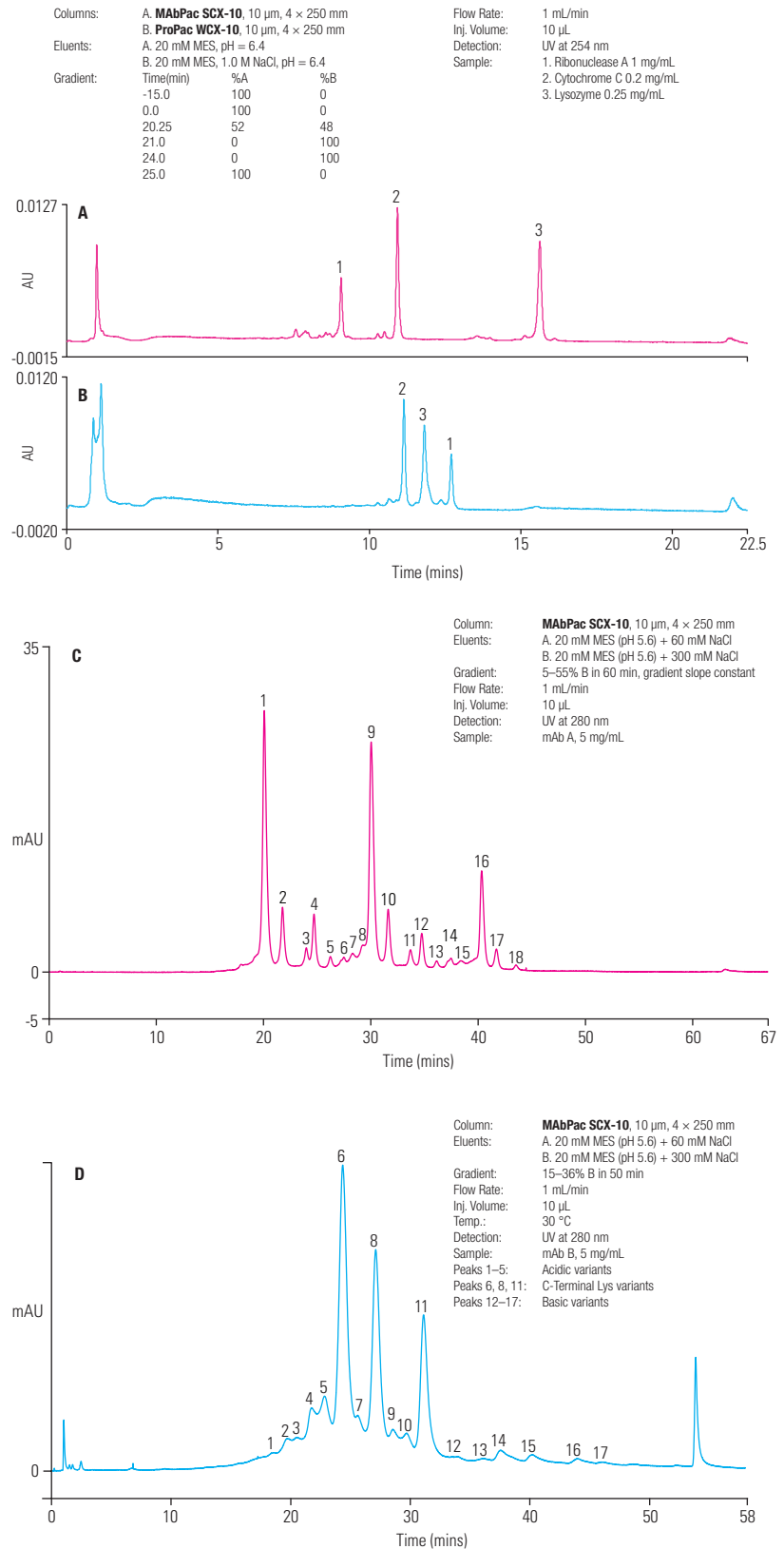


Figure 1: The MABPac SCX-10 exhibits selectivity differences from ProPac WCX-10 (Figure 1A and B) when separating proteins. Additionally, MABPac SCX-10 provides excellent peak separation and resolution for different mAbs (Figure 1C and 1D)

One of the most important and common analyses of monoclonal antibody heterogeneity is the monitoring and determination of acidic and basic variants. The MAbPac SCX-10 column provides high resolution for variant analysis of monoclonal antibodies, where two different monoclonal antibodies are separated (Figure 1C, 1D).

C-Terminal Lysine Variant Analysis

During the development and the production of therapeutic monoclonal antibodies, characterization of structural variants is a critical challenge. C-terminal processing of lysine residues on the heavy chain of monoclonal antibodies is a common structural variation that demands analysis. Incomplete monoclonal antibody processing results in charge heterogeneity, which is readily identified using the MAbPac SCX-10 column. Figure 2 illustrates this point with excellent resolution of C-terminal lysine truncation variants, and other acidic and basic variants of a monoclonal antibody. To verify that the different retention times of the three peaks were due to the different heavy chain C terminal lysine content the mAb was treated with carboxypeptidase B. This is an exopeptidase that specifically cleaves C terminal lysine residues (Figure 2, sample 2). This treatment of the mAb sample resulted in the absence of peaks 7 and 8 (containing 1 and 2 terminal lysine residues, respectively, on their heavy chains). The decreased peak areas in peaks 7 and 8 are accompanied by a corresponding increase in area of peak 6 where no lysine is present. Another minor variant with lysine truncations, Peaks 9–11) collapsed to peak 9 after carboxypeptidase B treatment.

Analysis of mAb Fragments After Digestion with Papain and Carboxypeptidase

Monoclonal antibodies treated with papain enzyme are separated into their Fab and Fc fragments. Figure 3 shows the well resolved Fab and Fc fragments after a monoclonal antibody and its variants are treated with papain alone, or with papain and carboxypeptidase together. The expected acidic, C-terminal lysine truncation-containing Fc fragments and Fab fragment peaks are determined and well resolved. Lysine truncation variant peaks collapse into one main peak with the carboxypeptidase treatment.

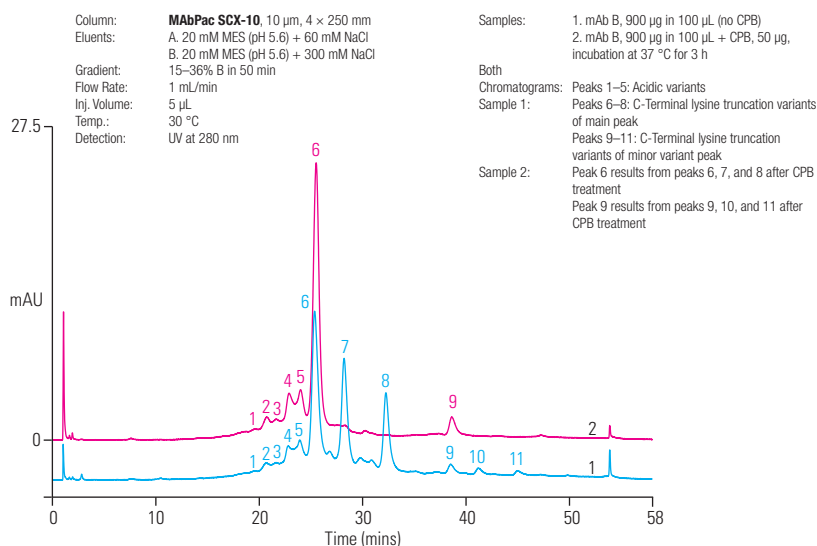


Figure 2: Baseline resolution of C-terminal lysine variants of a monoclonal antibody sample. A second chromatogram verifies that the three major peaks are due to variations in C-terminal content: after the treatment with CPB, only one major peak remains.

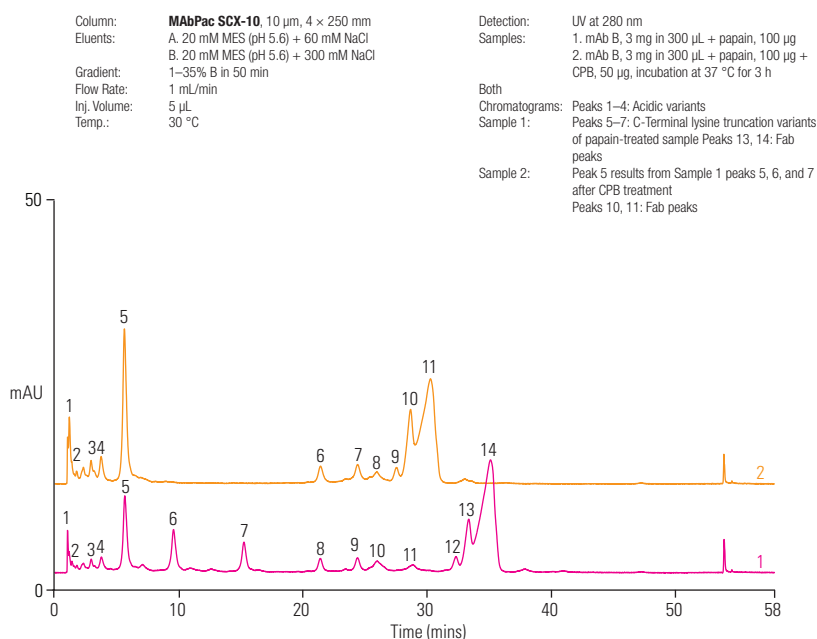


Figure 3: High resolution analysis of monoclonal antibody fragments after treatment with papain or papain and CPB enzymes. The expected acidic, C-terminal lysine variants containing Fc, and Fab fragment peaks are well resolved.

Optimizing the Separation with pH Gradients

A pH gradient can also be used for monoclonal antibody variant separations. The isoelectric point (pI) is the pH at which a particular protein carries no net charge and can no longer bind to the charged surface and therefore elutes. pH gradients are becoming increasingly popular to ease the method development process (4-6). The recently introduced Thermo Scientific™ CX-1 pH Gradient Buffers meet the fast and robust platform method requirement. This buffer system consists of a low-pH buffer A at pH 5.6 and a high-pH buffer B at pH 10.2. A linear pH gradient from pH 5.6 to pH 10.2 is generated over time by running a linear pump gradient from 100% buffer A to 100% buffer B (Figure 4A). This is followed by a shallow gradient run, to optimize the mAb separation (Figure 4B).

Fast mAb Characterization Analysis

MABPac SCX-10, 3 μ m, 50 mm columns provide high resolution monoclonal antibody separation when compared to longer MABPac SCX-10, or ProPac WCX-10, 10 μ m columns but with significantly faster analysis time (Figure 5). Total analysis time is reduced nearly by 5 fold for the 3 μ m column (Figure 5C) when compared to 10 μ m, 4 \times 250 mm columns Figure 5A and B).

Column: **MABPac SCX-10**, 10 μ m 4 \times 250 mm
 Eluents: A 1X CX-1, pH Gradient Buffer A (pH 5.6)
 B 1X CX-1, pH Gradient Buffer B (pH 10.2)
 Gradients: A 0–100% B in 30 minutes
 B 25–50% B in 30 minutes
 Flow Rate: 1.0 mL/min
 Inj. Volume: 10 μ L
 Sample: mAb, 5 mg/mL

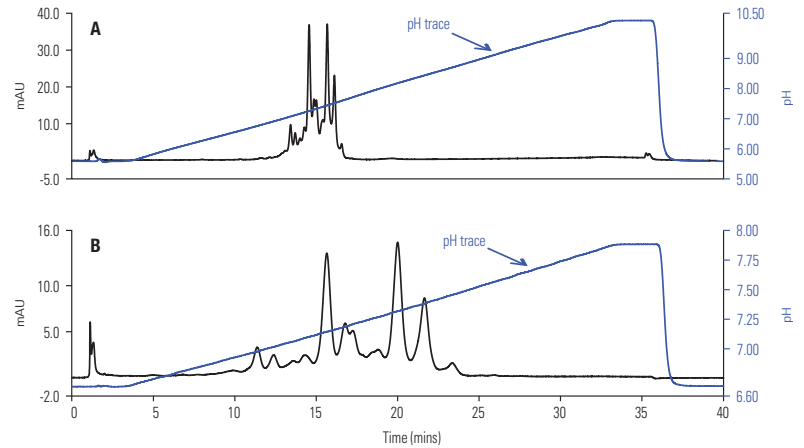


Figure 4: Separation of monoclonal antibody variants using pH gradient on a MABPac SCX-10, 10 μ m, 4 \times 250 mm column

Columns:	A) ProPac WCX-10 , 10 μ m 4 \times 250 mm B) MABPac SCX-10 , 10 μ m 4 \times 250 mm C) MABPac SCX-10 , 3 μ m, 4 \times 50 mm	Inj. Volume:	A and B: 5 μ L (50 μ g) C: 15 μ L (15 μ g)
Eluents:	A: 20 mM MES + 60 mM NaCl, pH 5.6 B: 25–46.44% B in 50 min C: 20–35% B in 10 min	Temp.:	30 °C
Gradients:	A) 25–46.44% B in 50 min B) 15–36.44% B in 50 min C) 20–35% B in 10 min	Detection:	280 nm
Flow Rate:	1 mL/min (0.6 mL/min for C)	Sample:	mAb; A and B) 10 mg/mL C) 1 mg/mL
		Peaks:	1–5: Acidic variants; 6,8,11: Lysine truncation variants 12–16: Basic variants

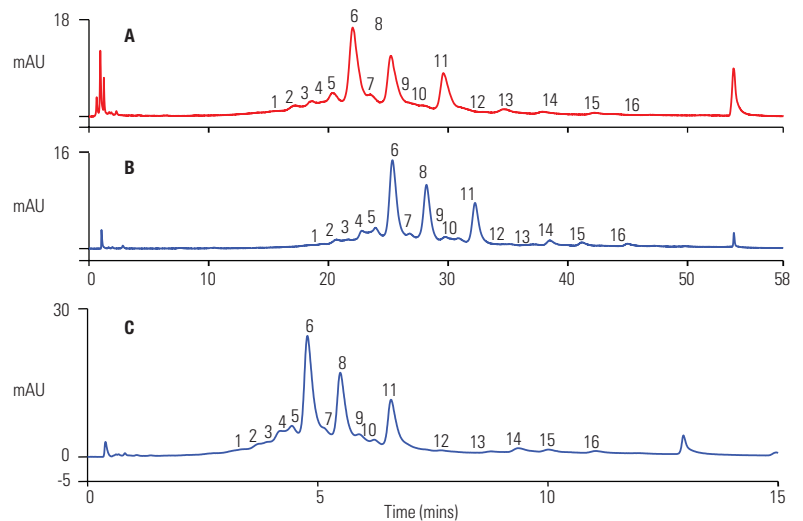


Figure 5: The 3 μ m MABPac SCX-10, 4 \times 50 mm column provides significantly faster analysis while maintaining the similar high resolution as that obtained using the MABPac SCX-10 and ProPac WCX-10, 4 \times 250 mm columns

Figure 6 demonstrates MAbPac SCX-10, 5 μm column providing fast, monoclonal antibody variant analysis using MES-based salt gradients. (Figure 6A). Higher resolution is achieved with a longer 10 minute gradient (Figure 6B) when compared to a 5 minute gradient.

A comparative lysine truncation verification analysis was performed on a 10 μm MAbPac SCX-10, 4 \times 250 mm column (Figure 7A) and a 3 μm MAbPac SCX, 4 \times 50 mm column (Figure 7B) using different flow rates and gradient conditions. In both cases, three variant forms differing by the presence of lysine at the C-terminal of the heavy chains with either no lysine, or 1 lysine, or 2 lysine residues is separated as individual peaks (Figure 7A and B; sample 1). Please note that time scales are substantially different.

To verify that the reason for the different retention times of the three peaks was the different content of heavy chain C terminal lysine, the mAb was treated with carboxypeptidase B, an exopeptidase that specifically cleaves C terminal lysine residues (Figure 7A and B, sample 2). This treatment of the mAb sample resulted in the quantitative disappearance of peaks 7 and 8 (containing 1 and 2 terminal lysine residues, respectively, on their heavy chains). The decreased peak areas in peaks 7 and 8 are accompanied by a corresponding increase in peak area of peak 6 where no lysine is present (Peak area values not shown). Similarly, another minor variant with lysine truncations shown as Peaks 9, 10 and 11 collapsed to peak 9 after carboxypeptidase B treatment.

High throughput advantage of a small particle 3 μm column for monitoring C-terminus lysine truncations is shown in B. Total analysis time is reduced nearly by 5 fold for 3 μm column when compared to 10 μm , 4 \times 250 mm column.

Columns: **MAbPac SCX-10**, 5 μm , 4 \times 50 mm
 Eluents: A: 20 mM MES + 60 mM NaCl, pH 5.6
 B: 20 mM MES + 300 mM NaCl, pH 5.6
 Gradients: A) 18–33% B in 5 min
 B) 18–33% B in 10 min
 Flow Rate: 1 mL/min
 Inj. Volume: 15 μL
 Temp.: 30 $^{\circ}\text{C}$
 Sample: mAb, 5 mg/mL
 Detection: UV at 280 nm
 Peaks: 1–4. Acidic variants
 5, 6, 8. C-Terminal lysine variants
 9–13. Other basic variants

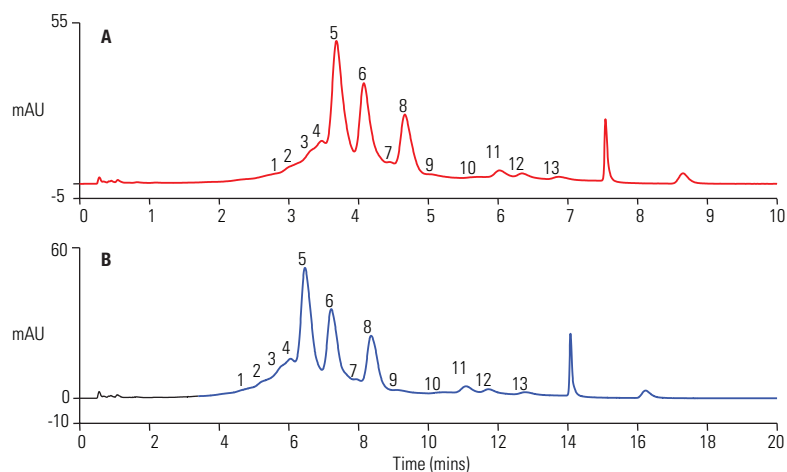


Figure 6: The 5 μm MAbPac SCX-10, 4 \times 50 mm column provides fast, high resolution monoclonal antibody variant analysis

Eluents: A: 20 mM MES (pH 5.6) + 60 mM NaCl
 B: 20 mM MES (pH 5.6) + 300 mM NaCl
 Temp: 30 $^{\circ}\text{C}$
 Detection: 280 nm
 Sample: 1. mAb
 2. mAb + Carboxypeptidase B (CPB)
 A. Column: **MAbPac SCX-10**, 10 μm , 4 \times 250 mm
 MAb: 0.9 mg/100 μL +/- 50 μg CPB in 10 μL
 Gradient: 15–36.44% B in 50 min
 Flow Rate: 1 mL/min
 Inj. Volume: 5 μL
 B. Column: **MAbPac SCX-10**, 3 μm , 4 \times 50 mm
 MAb: 0.25 mg/100 μL +/- 50 μg CPB in 10 μL
 Gradient: 22–37% B in 5 min
 Flow Rate: 0.5 mL/min
 Inj. Volume: 6 μL
 Peaks 1–5: Acidic variants; Peaks 6, 7, 8: C-terminal Lys truncation variants of main peak; Peaks 9, 10, 11: C-terminal Lys truncation variants of a minor variant peak

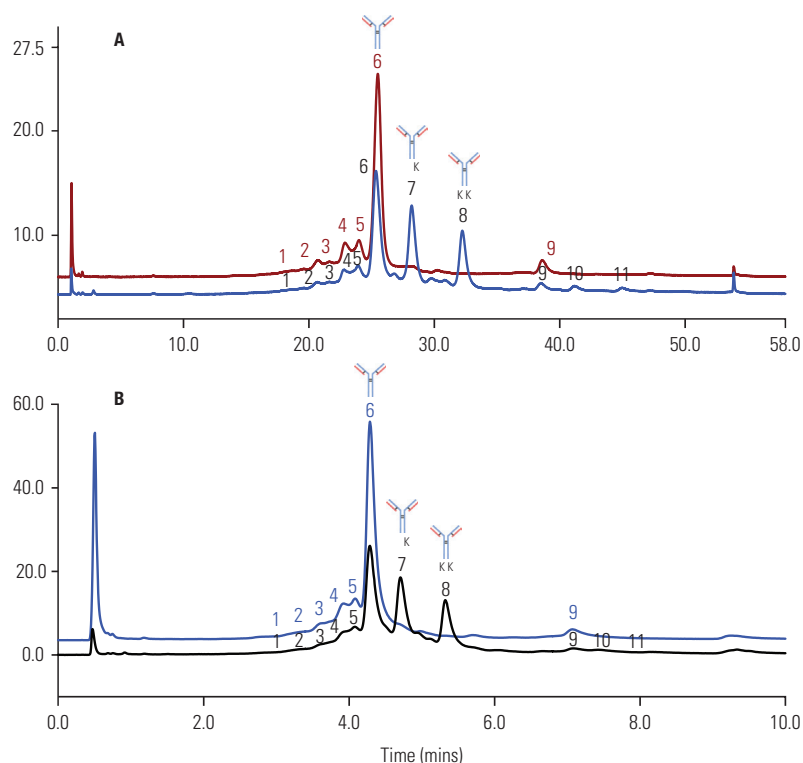


Figure 7: Characterization of mAb C-terminal lysine truncation variants using MAbPac SCX-10, 10 μm (Panel A) or 3 μm (Panel B) columns. 3 μm , 4 \times 50 mm column separation is more than 5 times faster than MAbPac SCX-10, 10 μm , 4 \times 250 mm column.

Note: timescales are different.

UHPLC mAb Separation

High resolution analysis of mAb charge variants is achieved using UHPLC based separations. Bioinert PEEK-lined stainless steel column housings are used for these Rapid Separation (RS), small particle columns. These columns take advantage of small resin size as well as longer column length to maximize the resolution of mAb variant separation. They are suitable for operation up to the higher pressure limit of 7,000 psi. MAbPac SCX-10 RS, 5 μ m columns are designed to be used with bioinert UHPLC system; Thermo Scientific™ UltiMate™ BioRS totally inert UHPLC system is recommended for best results. The RS columns are available in 4.6 mm I.D. as well as 2.1 mm I.D. to suit different applications. There are several advantages of using 2.1 mm I.D. columns including sample and eluent conservation. However, it should be noted that the same gradient method developed with the 4.6 mm column might not work with a 2.1 mm format column. This is especially evident when using lower flow rates due to influence of instrument delay volume. It is important to re-establish the optimal gradient run conditions for 2.1 mm formats.

Both salt gradients and/or pH gradients can be used for high resolution separation of mAbs.

Salt Gradients

Figure 8 shows an example of mAb separation comparing MAbPac SCX-10 RS, 5 μ m, 4.6 \times 150 mm with 4.6 \times 250 mm columns at 1.3 mL/min flow rate (A and C). Both columns resolved acidic and basic variants from the main lysine truncation peaks. Resolution values of lysine truncation peaks are shown. A longer 4.6 \times 250 mm column produces high resolution when compared to a 4.6 \times 150 mm column (Figure 8A and C). Comparison of different flow rates was made using MAbPac SCX-10 RS, 5 μ m, 4.6 \times 150 mm column (Figure 8B). High pressure compatibility of the column allows the use of high flow rates (2 mL/min) and short run times while maintaining decent resolution. This capability enables high throughput characterization of samples.

Figure 9 shows the separation of a mAb using a salt gradient on MAbPac SCX-10 RS, 5 μ m, 2.1 mm \times 250 mm column. As expected a shallow gradient (Figure 9B) produces higher resolution when compared to a steep gradient (Figure 9A).

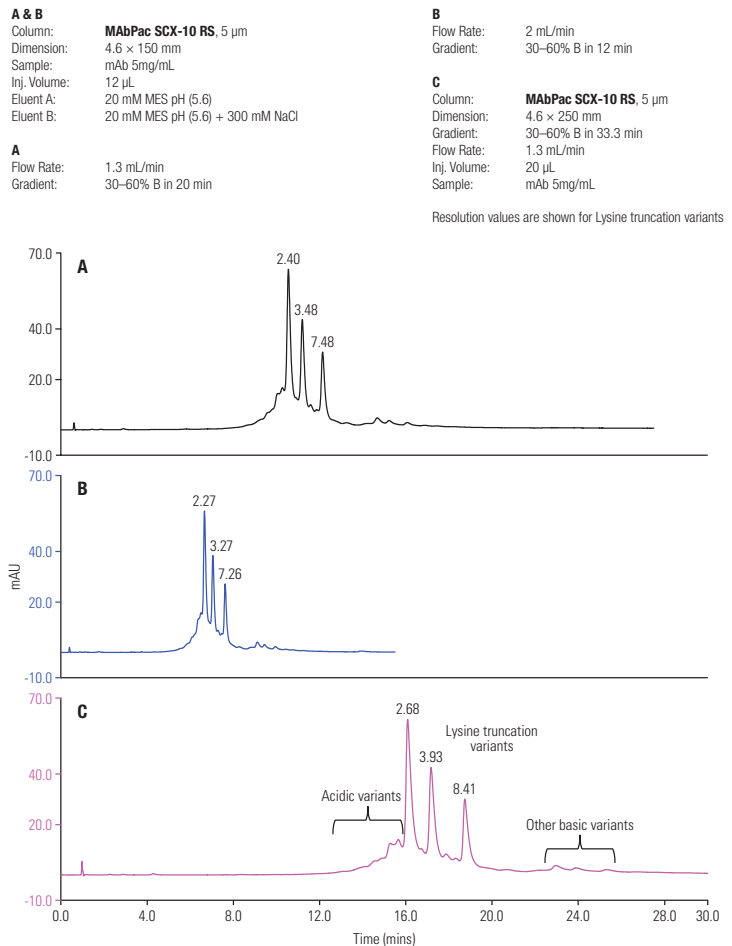


Figure 8: MAb separation on MAbPac SCX-10 RS, 5 μ m columns, comparison of different flow rates and different lengths of RS columns

Column: MAbPac SCX-10 RS, 5 μ m
 Dimension: 2.1 \times 250 mm
 Eluent A: 20 mM MES pH (5.6)
 Eluent B: 20 mM MES pH (5.6) + 300 mM NaCl
 Gradients: 30–60% B in 16.5min (Panel A)
 30–60% B in 33min (Panel B)
 Flow Rate: 0.32 mL/min
 Inj. Volume: 3.3 μ L
 Sample: mAb 5mg/mL

Resolution values are shown for Lysine truncation variants

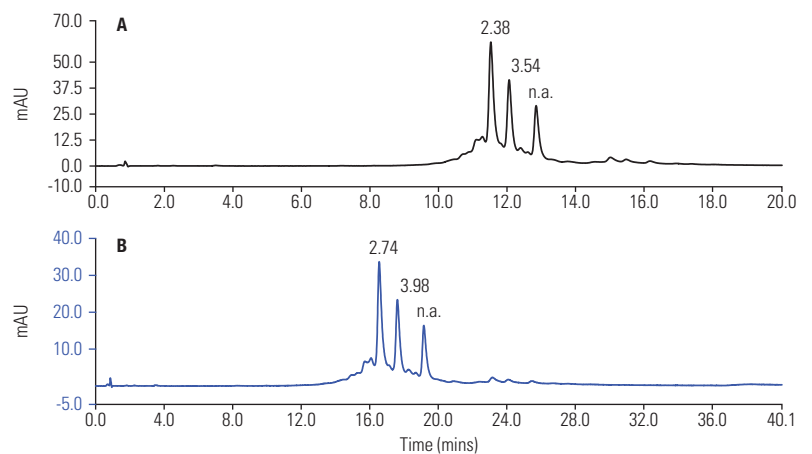


Figure 9: MAb separation on MAbPac SCX-10 RS, 5 μ m 2.1 \times 250 mm column using different gradient conditions

Faster pH Gradients

Faster pH gradient separations can be achieved using the RS format: MAbPac SCX-10 RS column. Figure 10 shows a separation using a 5 μm , 4.6 mm \times 150 mm column completed in half the time of a 10 μm column. Similar to the 10 μm separation described in Figure 4, the pH gradient can be first run with a broad gradient (Figure 10A) and then optimized for greater peak resolution with a narrow gradient (Figure 10B).

Higher sensitivity, with lower eluent consumption, can be achieved using the 2.1 mm RS format MAbPac SCX-10 RS column, as is shown in Figure 11. Panel A shows the initial broad separation, whereas Panel B shows a optimized narrow gradient.

Column: **MAbPac SCX-10 RS**, 5 μm
 4.6 \times 150 mm
 Eluent A: 1X CX-1, pH Gradient Buffer A (pH 5.6)
 Eluent B: 1X CX-1, pH Gradient Buffer B (pH 10.2)
 Gradient: 0–100% B in 20 minutes (Panel A)
 26–50% B in 20 minutes (Panel B)
 Flow Rate: 1.0 mL/min
 Inj. Volume: 12 μL
 Sample: mAb, 5 mg/mL

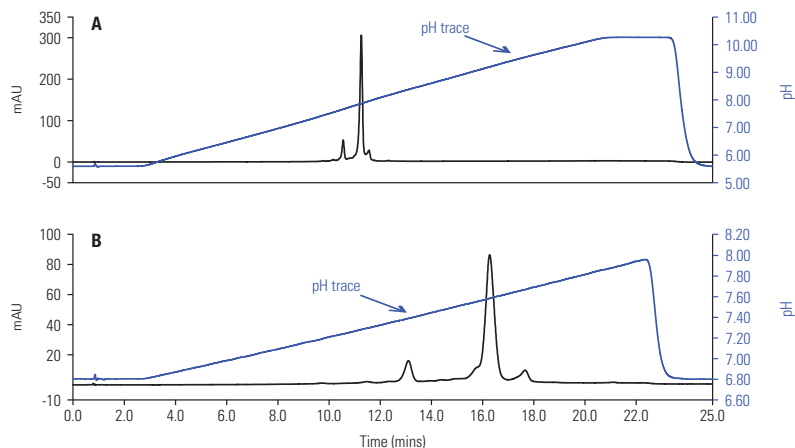


Figure 10: Faster separation of Monoclonal Antibody Variants using pH gradient on a MAbPac SCX-10 RS, 5 μm , 4.6 \times 150 mm column

Column: **MAbPac SCX-10 RS**, 5 μm
 Dimension: 2.1 \times 150 mm
 Eluent A : 1X CX-1, pH Gradient Buffer A (pH 5.6)
 Eluent B: 1X CX-1, pH Gradient Buffer B (pH 10.2)
 Gradient: 0–100% B in 12 minutes (Panel A)
 30–60% B in 12 minutes (Panel B)
 Flow Rate: 0.32 mL/min
 Inj. Volume: 2 μL
 Sample: mAb, 5 mg/mL

Peak Resolution values are shown

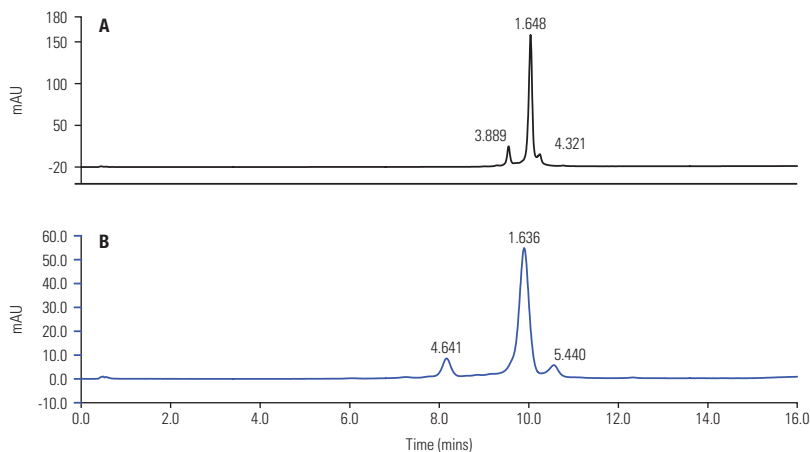


Figure 11: Higher sensitivity separation of Monoclonal Antibody Variants using pH gradient on a MAbPac SCX-10 RS, 5 μm , 2.1 \times 150 mm column

Ruggedness of MAbPac SCX-10 Columns

Ruggedness of MAbPac SCX-10 RS, 5 μ m, 4.6 \times 150 mm column was demonstrated. This was evaluated at 2 mL/min flow rate for more than 300 runs (Figure 12). Peak width data obtained for the lysine truncation peaks is shown in Table 1.

Column: **MAbPac SCX-10**, 5 μ m, 4.6 \times 150 mm
 Eluent: A: 20 mM MES (pH 5.6)
 B: 20 mM MES (pH 5.6) + 300 mM NaCl
 Gradient: 30–60 % B in 12 min
 Flow Rate: 2 mL/min
 Inj. Volume: 12 μ L
 Temp: 30 $^{\circ}$ C
 Detection: 280 nm
 Sample: mAb, 5 mg/mL
 Sample was injected intermittently

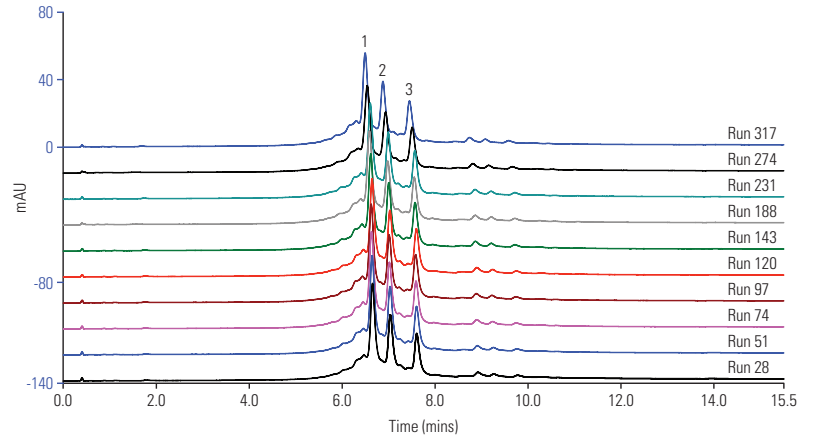


Figure 12: Ruggedness testing of MAbPac SCX-10 RS, 5 μ m, 4.6 \times 150 mm column. At flow rate of 2 mL/minutes lysine truncation peaks are identified as 1, 2, 3 peaks and their Peak Width Half Height data (in minutes) is given overpage.

Sample No	Peak 1	Peak 2	Peak 3
28	0.099	0.099	0.104
51	0.098	0.097	0.104
74	0.100	0.098	0.105
97	0.098	0.096	0.103
120	0.098	0.098	0.103
143	0.100	0.096	0.104
188	0.107	0.105	0.106
231	0.103	0.100	0.105
274	0.115	0.111	0.111
317	0.107	0.103	0.112
Average	0.103	0.100	0.106
RSD (%)	5.47	4.75	3.03

Table 1: Ruggedness testing of MAbPac SCX-10 RS, 5 μ m, 4.6 \times 150 mm column at flow rate of 2 mL/min (Figure 12). Peak Width at Half Height values in minutes are shown for Lysine truncation peaks.

References

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Specifications and Operational Parameters

Column construction	PEEK and PEEK lined stainless steel BioRS columns	
Bead size	3 µm, 5 µm, 10 µm	
Substrate	Nonporous, highly crosslinked vinyl aromatic media	
Pellicular layer	Proprietary hydrophilic	
Functional group	Sulfonic	
Pressure limit	PEEK Columns 10 µm: 3000 psi 3 µm and 5 µm: 5000 psi RS Columns 5 µm: 7000 psi	
Temperature range	Ambient to 60 °C	
pH range	2–12	
Dynamic loading capacity	Up to 100 µg, depending on the monoclonal antibody sample (10 µm, 4 × 250 mm)	
Recommended buffers	MES, or other Good's buffers, tris, Thermo Fisher Scientific pH gradient buffers CX-1(pH 5.6) and CX-1 (pH 10.2).	
Detergent compatibility	Nonionic, anionic, or zwitterionic detergents	
Dynamic loading capacity (For a typical mAb)	10 µm, 4 × 50 mm: 20 µg 10 µm, 4 × 250 mm: 100 µg 5 µm, 4 × 50 mm: 30 µg 5 µm, 4 × 150 mm: 90 µg 5 µm, 4 × 250 mm: 150 µg	RS 5 µm, 4.6 × 50 mm: 40 µg RS 5 µm, 4.6 × 150 mm: 120 µg RS 5 µm, 4.6 × 250 mm: 200 µg RS 5 µm, 2.1 × 50 mm: 8–10 µg RS 5 µm, 2.1 × 150 mm: 24 µg RS 5 µm, 2.1 × 250 mm: 40 µg

Ordering Information

MABPac SCX-10 Analytical Column	Part Number
MABPac SCX-10, 3 µm, Analytical Column (4 × 50 mm)	077907
MABPac SCX-10, 5 µm, Analytical Column (4 × 50 mm)	078656
MABPac SCX-10, 5 µm, Analytical Column (4 × 150 mm)	085198
MABPac SCX-10, 5 µm, Analytical Column (4 × 250 mm)	078655
MABPac SCX-10, 10 µm, Analytical Column (4 × 150 mm)	075602
MABPac SCX-10, 10 µm, Analytical Column (4 × 250 mm)	074625
MABPac SCX-10, 10 µm, Analytical Column SCX-10HT (4 × 50 mm)	075603
MABPac SCX-10, 10 µm, Analytical Column SCX-10 (2 × 250 mm)	075604

UHPLC, MABPac SCX-10 RS, Analytical Column	Part Number
MABPac SCX-10 RS, 5 µm, Analytical Column (4.6 × 50 mm)	082674
MABPac SCX-10 RS, 5 µm, Analytical Column (4.6 × 150 mm)	085209
MABPac SCX-10 RS, 5 µm, Analytical Column (4.6 × 250 mm)	082673
MABPac SCX-10 RS, 5 µm Analytical Column (2.1 × 50 mm)	082675
MABPac SCX-10 RS, 5 µm Analytical Column (2.1 × 150 mm)	088242
MABPac SCX-10 RS, 5 µm Analytical Column (2.1 × 250 mm)	082515

Lot Select Column Set	Part Number
Lot Select Column Set—Three columns from one resin lot (4 × 250 mm)	088782
Lot Select Column Set—One column from each of three resin lots (4 × 250 mm)	088783

MABPac SCX-10 Semipreparative Column	Part Number
MABPac SCX-10, 10 µm, Semipreparative Column (9 × 250 mm)	088784

MABPac SCX-10 Guard Column	Part Number
MABPac SCX-10 G, 10µm, Guard Column (2 × 50 mm)	075749
MABPac SCX-10 G, 10µm, Guard Column (4 × 50 mm)	074631

pH Buffer Concentrates	Part Number
CX-1 pH Gradient Buffer A (pH 5.6), 125 mL	083273
CX-1 pH Gradient Buffer B (pH 10.2), 125 mL	083275
CX-1 pH Gradient Buffer Kit (pH 5.6 to 10.2), 125 mL	083274
CX-1 pH Gradient Buffer A (pH 5.6), 250 mL	085346
CX-1 pH Gradient Buffer B (pH 10.2), 250 mL	085348
CX-1 pH Gradient Buffer Kit (pH 5.6 to 10.2), 250 mL	085349

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