Thermo Scientific

MabPac™ SCX-10

Product Manual

P/N: 065393-06 January 2012

Thermo SCIENTIFIC

PRODUCT MANUAL

FOR

MAbPac™ SCX-10, 10µm ANALYTICAL COLUMN

(10µm, 4 x 250 mm, P/N 074625)
(10µm, 4 x 150 mm, P/N 075602)
(10µm, 4 x 50 mm, P/N 075603)
(10µm, 2 x 250 mm, P/N 075604)

MAbPac™ SCX-10, 5µm ANALYTICAL COLUMN

(5µm, 4 x 50 mm, P/N 078656)
(5µm, 4 x 250 mm, P/N 078655)

MAbPac™ SCX-10, 3µm ANALYTICAL COLUMN

(3µm, 4 x 50 mm, P/N 077907)

MAbPac™ SCX-10, 10µm GUARD COLUMN

(4 x 50 mm, P/N 074631)
(2 X 50 mm P/N 075749)

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SECTION 1 – INTRODUCTION

1.1. Features of the MAbPac SCX-10 Strong Cation Exchange Columns

The MAbPac™ SCX-10 strong cation exchange columns are designed specifically for high-resolution, high efficiency separations of monoclonal antibodies and their variants. The nonporous pellicular resin provides high resolution, permitting the separation of monoclonal antibody variants that differ by as little as one charge. The MAbPac SCX-10 columns are a complimentary addition to the existing ProPac WCX-10 column providing complimentary selectivity for various MAb separations. The MAbPac SCX-10 columns are available in 10, 5 and 3 µm particle sizes. The MAbPac SCX-10, 10 µm particle size 4x250 mm column provides high resolution for routine separations of monoclonal antibody variants, where time of analysis is not a factor. The MAbPac SCX-10, 3 and 5µm particle size 4 x 50 mm columns are designed to provide fast separations of monoclonal antibody variants while maintaining high resolution and are ideal for high throughput needs. Since the 4x50 mm version of 3 and 5 µm columns are shorter, analysis time is reduced and therefore throughput can be increased. The longer 5 µm, 4x250 mm columns are designed for even higher resolution. The MAbPac SCX-10 columns are packed with ethylvinylbenzene-divinylbenzene copolymer non-porous beads that are uniformly coated with a layer of highly hydrophilic material. This layer prevents non-specific interactions between the hydrophobic bead core and the biopolymers/proteins. On the surface of the hydrophilic layer, sulfonic acid functional groups are attached using an ATRP-based grafting approach to control the chain length as well as the density of functional groups.

1.2. Key Applications of the MAbPac SCX-10 Columns

The MAbPac SCX-10 column is recommended for high resolution separation of monoclonal antibodies and their variants. Some typical applications are listed below. Depending on the need 10, 5 or 3 µm columns may be used. For the best resolution, if the time is not a factor, MAbPac SCX-10, 10 or 5 µm 4x250 columns are recommended. For high throughput separations and relatively high resolution separations MAbPac SCX-10, 3 and 5 um 4x50 mm columns are recommended.

1. Monoclonal antibody analysis by salt gradients; heterogeniety characterization of acidic and basic variants
2. Monoclonal antibody analysis by pH gradients; heterogeniety characterization of acidic and basic variants
3. Analysis of MAb C-terminal lysine truncation variants
4. Analysis of MAb Fab and Fc variants after carboxy peptidase and papain treatments
5. Other MAb applications involving charged variants

1.3. Key Applications of the MAbPac SCX-10, 5µm Columns

The MAbPac SCX-10, 4x50 mm, 5µm columns are recommended for high-throughput MAb separations where fast separations are required with relatively high resolution. The smaller particle size column produces high resolution as compared to the 10 µm column of the same length. Since the particle size is larger than the 3 µm column, higher flow rates can be employed to decrease the run time and to increase the throughput.

The MAbPac SCX-10, 4x250 mm, 5µm columns take advantage of the longer length as well as smaller particle size for producing very-high resolution separations.

Appropriate flow rates and gradients should be established to achieve the best resolution and efficiencies. The same gradient method and flow rate developed for 10 or 3µm columns may not be ideal when using the 5 µm particle size columns due to the differences column volume. Therefore, it is essential to optimize these parameters for the best results. Any applications developed with 10 or 3 µm columns can be performed with the MAbPac SCX-10, 5 µm columns. Please refer section 1.2 for a list of various applications.

1.4. Key Applications of the MAbPac SCX-10, 3µm Columns

The MAbPac SCX-10, 4x50 mm, 3µm columns are recommended for high-throughput MAb separations where fast separations are required with high resolution. The longer length (4x250 mm) advantage from a 10 µm particle column for producing high resolution is mostly compensated for by the smaller particle size short (4x 50 mm) columns. Since the column length is shorter, analysis time is reduced and throughput can be increased. It should be noted that small particle columns generate high back pressures. Appropriate flow rates and gradients should be established to achieve the best efficiency and resolution. The same gradient method and flow rate developed for 10 µm, 4x250 mm column might not be ideal when using the 3 µm particle size columns due to the differences in the column volumes. Therefore, it is essential to optimize these parameters for best results. Any applications developed with a 10 µm column can be performed with MAbPac SCX-10, 3 µm or 5 µm (See below) columns. Please refer section 1.2 for a list of various applications.

1.5. MAbPac SCX-10 Column – Operating Limits and Specifications

MAbPac SCX-10 columns are compatible with both aqueous mobile phases and those containing solvents, such as acetonitrile. MAbPac SCX-10 columns should be operated at recommended flow rates for each of these different particle types of columns. It is essential to keep the backpressure below the recommended value. When setting up a system for use with this column, check the special precautions listed in Section 3, “Operation”. PEEK™ (Polyetheretherketone) is used to make the column hardware. PEEK has excellent resistance to most organic solvents and inorganic solutions.

NOTE ***Although MAbPac SCX columns are compatible with acetonitrile and other solvents, since ion-exchange separations are based on salt gradients and/or pH based gradients, usage of solvents may be restricted to column cleaning procedures only to avoid precipitation of biomolecules on the column. Otherwise, the precipitate can lead to clogging the column with increased back pressures and compromised performance of the column.***

1.5.1. MAbPac SCX-10, 10µm Column Operating Conditions

PARAMETER	Column	RECOMMENDATION
Flow Rate Range: *Not to exceed the maximum column pressure limit*	10µm, 4 x 250 mm 10µm, 2 x 250 mm	0.2 to 2 mL/min 0.1 to 0.5 mL/min
	5µm, 4 x 250mm, 5µm, 4 x 50mm	4x250 mm: 0.7-0.8 mL/min 4x50 mm : up to 2 ml/min for best resolution
	3µm, 4x 50 mm	0.5 to 1 ml/min
Shipping Solution	10µm	10 mM Na_2HPO_4 (pH 6.0) + 0.1% sodium azide
	3µm and 5µm	20 mM MES (pH 6.0) + 0.1% Sodium Azide.
Short Term Storage Solution (overnight): *Note: Do not store columns in high/low pH buffers*	10µm	Your high salt concentration eluent (pH 3-10)
	5µm	Your high salt concentration eluent (pH 5 – 7.5).
	3µm	Your high salt concentration eluent (pH 3 – 10).
Long Term Storage Solution:		10 mM Na_2HPO_4 (pH 6.0) + 0.1% sodium azide
Typical buffers:		MES, ACES or other Good's buffers or Tris ***Always maintain a minimum ionic strength of at least 20 mM, to ensure optimum resolution***
Solvents:		50% acetonitrile if needed for cleaning. Never use H_2O alone for washing the column.
Detergent Compatibility		Nonionic, anionic or zwitterionic detergents**.** ***Do not use cationic detergents.***
Temperature Range:		Ambient to 60 ºC
Pressure Limit:	10µm	3,000 psi
	3µm and 5µm	5,000 psi
pH Range		2-12
Capacity*: determined as the “dynamic capacity”.	10µm , 4 x 250mm (column volume =3.14 mL)	Capacity is determined as the “dynamic capacity”. 10-100 µg MAb can be injected.
	5µm, 4 x 50mm 5µm, 4 x 250mm,	10-50 µg MAb 20-150ug
	3µm, 4x 50 mm	20-100 µg MAb.

*Dynamic Capacity”. Depending on the MAb for best resolution.

1.5.2. Physical Characteristics

Substrate Pore Size	Non-porous
Substrate Monomers	Ethylvinylbenzene-divinylbenzene
Substrate Cross-linking	55%
Mode of Interaction	Cation Exchange
Functional Group	Sulfonic Acid; SCX

1.5.3. MAbPac SCX-10 Columns

Product Description	Part Number
MAbPac SCX-10, 10µm Analytical Column (4 x 250 mm)	074625
MAbPac SCX-10, 10µm Guard Column (4 x 50 mm)	074631
MAbPac SCX-10, 10µm Analytical Column (4x 150 mm)	075602
MAbPac SCX-10HT, 10µm Analytical Column (4x 50 mm)	075603
MAbPac SCX-10, 10µm Analytical Column (2x 250 mm)	075604
MAbPac SCX-10, 10µm Guard Column (2 x 50 mm)	075749
MAbPac SCX-10, 10µm Preparative Column (9 x 250 mm)	SP6866
MAbPac SCX-10, 3 µm Analytical Column (4x50 mm	77907
MAbPac SCX-10, 5 µm Analytical Column (4x50 mm)	078656
MAbPac SCX-10, 5 µm Analytical Column (4x250 mm)	078655

WARNING ***ALL ELUENTS, SAMPLES SHOULD BE FILTERED USING A 2 µm filter unit before use. FAIL TO DO SO WILL WILL RESULT IN CLOGGING THE COLUMN WITH INCREASED BACK PRESSURE, RESULTING IN COMPROMISED PERFORMANCE. It is beneficial to include a wash procedure with a buffer with high salt solution (1M NaCl) to minimize the residue build up on the column during chromatography runs.***

NOTE ***For assistance, contact Technical Support for Dionex Products. In the U.S., call 1-800-346-6390. Outside the U.S., call the nearest Thermo Fisher Scientific office..***

SECTION 2 – SYSTEM REQUIREMENT

2.1. A Metal Free System is Strongly Recommended

The MAbPac SCX-10, columns were designed to be used with a standard bore HPLC system having a gradient pump module, injection valve, and a UV detector.

A metal-free system is recommended for halide-salt based mobile phases which are predominantly used and can cause corrosion of metallic components. Metal leaching from the system on to the column will lead to decreased performance from metal contamination. A metal-free pump is highly recommended. Use of stainless steel tubing, ferrules, and bolt assemblies is not recommended.

Typical Flow Rate:	1 mL/min (for 10 µm columns)
Injection Volume:	10–100 µL (or, other)
Autosampler:	Ultimate
System Void Volume:	Minimize the lengths of all connecting tubing and remove all unnecessary switching valves and couplers.
Pumps:	SP (single pump) or DP (dual pump)
Detectors:	VWD (Variable Wavelength Detector) with microflow cell

2.2. System Void Volume

Tubing between the injection valve and detector should be < 0.0050" ID PEEK tubing. Minimize the length of all liquid lines, but 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.

2.3. Mobile Phase Limitations

The MAbPac SCX-10 columns are compatible with typical mobile phases, such as sodium or potassium chloride salts in phosphate, MES or acetate buffers, up to the limit of their solubility. Use of organic solvents in the mobile phase is usually unnecessary. However, they may be used for cleaning the column, if necessary. If you choose to use one, test the solubility limit of the mobile phase in the presence of the chosen organic solvent. Some combinations of salts and organic solvents are not miscible.

WARNING ***Cationic detergents irreversibly bind to the MAbPac SCX-10 columns and their use should be totally avoided.***

2.4. Chemical Purity Requirements

Reliable, reproducible results require mobile phases that are free from impurities and prepared consistently.

2.4.1. Deionized Water

The deionized water used to prepare your mobile phase should be Type I reagent grade water with a specific resistance of 18.2 megohm-cm. The water should be free from ionized impurities, organics, microorganisms and particulate matter. UV treatment in the water purification unit is recommended. Follow the manufacturer's instructions regarding the replacement of ion exchange and adsorbent cartridges. All filters used for water purification must be free from UV-absorbing components. Contaminated water in the mobile phase causes high background signals, gradient artifacts, and even sample degradation.

SECTION 3 – OPERATION

3.1. Mobile Phase Selection

Monoclonal antibody samples and variants are eluted by using a gradient of either increasing ionic strength or by pH titration.

Salt based Gradient Elution:
The mobile phase for the MAbPac SCX-10 columns consists of a buffer component and a salt component. The buffer selected depends upon the pI of the proteins to be separated, and should provide minimal UV interference at the wavelength to be monitored. Although phosphate buffers are widely used for various applications, usage of MES containing buffers is becoming increasingly popular for MAb separations between the pH range of 5 - 6.5. The advantage of using MES buffer is the buffering of the stationary phase of the column effectively with improved resolution.

pH gradient Elution:
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 gets eluted. pH gradients are becoming increasingly popular to ease the method development process (1-2). For pH based separation, a set of buffers with different pKa values in the useful pH range are chosen and they constitute the buffer (See the link shown as Ref 3)

Minimum concentration of 20 mM NaCl or equivalent in buffers:
Thermo Scientific recommends a minimum concentration of 20 mM NaCl or equivalent in buffers. Failure to maintain a minimum ionic strength in buffer A will result in alteration of the stationary phase conformation resulting in an increase in the column backpressure beyond the maximum recommended value. If this occurs, remove the column from the system, flush the buffer from the system and replenish with buffer B containing your high salt concentration. Pump buffer B through the column at a low flow rate (0.1-0.2 mL/min), until the backpressure falls back to normal.

MOBILE PHASE CONSTITUENT	RECOMMENDATIONS
Buffer	Salt Gradients: MES, ACES or other Good's buffers, Tris, Phosphate or others
	pH gradients: Buffers with various pka values to cover pH range from 6 to 11
Salt	Potassium or sodium salts of chloride, acetate
pH Modifier	Phosphoric acid, HCl, or NaOH
Column Cleaning / Pretreatment	10 mM Sodium hydroxide at room temperature
Solvent	Up to 80% acetonitrile
Detergent	Non-ionic, anionic, or zwitterionic detergents
Anti-Microbial	0.1% sodium azide

WARNING

Do not operate the MAbPac SCX-10 in the absence of a minimal ionic strength of at least 20 mM. If the ionic strength is too low, the structure of the stationary phase will be affected, causing a significant increase in backpressure. This effect can be reversed by pumping a buffer containing high salt solution (≥500 mM NaCl) through the column at a low flow rate (0.1-0.2mL/min) until the backpressure is reduced. It is recommended that at least 20 mM be present in the mobile phase at all times.

3.2. Mobile Phase Preparation

3.2.1. Adjusting the pH of the Mobile Phase

The mobile phase should contain all the electrolytes before adjusting the pH. It is important to prepare buffers gravimetrically (by weight) when possible and without need to adjust the pH each time. Slight pH adjustment variations can lead to substantial differences in the reproducibility of retention times. If a pH meter is used in order to make sure that the pH reading is correct, the pH meter should be calibrated at least once a day choosing two standards, one below and one above the desired pH. Stirring as well as temperature correction should be employed. (Note that pH measurements of buffers containing Tris should not be performed with a Ross electrode as this electrode produces erroneous results with amine containing solutions.)

3.2.2. Filtering the Mobile Phase

To extend the lifetime of your column as well as your HPLC pump, all the eluents must be filtered using a 0.2 µm membrane filter to remove insoluble contaminants from the eluents. This is absolutely essential especially when using 3 and 5 µm columns.

3.2.3. Degassing the Mobile Phase

Before using buffers, they must be degassed. The degassing can be done either using the Thermo Scientific pump degas function as described in the manual, or by using a vacuum pump. Vacuum degas the solvent by placing the mobile phase reservoir in a sonicator and drawing a vacuum on the filled reservoir with a vacuum pump for 5-10 minutes while sonicating.

3.3. Validating Column Performance

Thermo Scientific recommends that you perform an efficiency test on your MAbPac SCX-10 column before your first use. The purpose of column performance validation is to make sure that no damage has been done to the column during shipping. Test the column using the conditions described on the Quality Assurance Report enclosed in the column box, and also included in the appendix of this manual. Repeat the test periodically to track the column performance over time. Note that slight variations may be obtained on two different HPLC systems due to system electronics, plumbing, operating environment, reagent quality, column conditioning, and operator technique.

Each column is shipped with a Quality Assurance Report.

3.3.1. Procedure for Validating Column Performance

1. Connect the column to the LC system.
2. Purge the column with the mobile phase listed on the QA report for 20 to 40 column volumes.
3. Inject the test mix shown in the QA report and collect the data.
4. Compare your result with the QA report provided in the column box.
5. If the chromatograms look similar, you can use the column for your application work.

3.4. Equilibrating the Column

Equilibrate the MAbPac SCX-10 column after installing it for the first time. Always re-equilibrate the MAbPac SCX-10 column prior to use following periods of storage.

Purge the column of shipping or storage solvent until the baseline is stable. Equilibrate the MAbPac SCX-10 column with at least 5-10 column volumes of mobile phase A, or until a stable baseline is achieved.

3.5. Caring for the MAbPac SCX-10 Column

To ensure the high performance of the MAbPac SCX-10 columns, the following guidelines should be followed.

1. Protect the column from contamination using a guard column, if available.
2. Make sure that solvents are miscible when changing mobile phases.
3. Always degas and filter mobile phases through a 0.22 µm membrane filter.
4. When switching to a new mobile phase, the column should be equilibrated with at least 30 column volumes before injecting the sample.
5. The recommended pH range is from pH 2 to 12. However, it is preferred that the column be used between pH 3 and pH 11 to achieve longer lifetime.
6. The column can be stored in mobile phase for short-term storage (e.g. overnight). However, it is highly recommended that the column be stored in storage buffer containing 0.1% sodium azide (more than 3 days) to prevent from bacterial growth.

WARNING

NEVER WASH THE MAbPac SCX-10 COLUMN WITH H_2O. Always maintain minimum ionic strength 20 mM buffer (MES or Sodium phosphate, or equivalent) in the eluents.

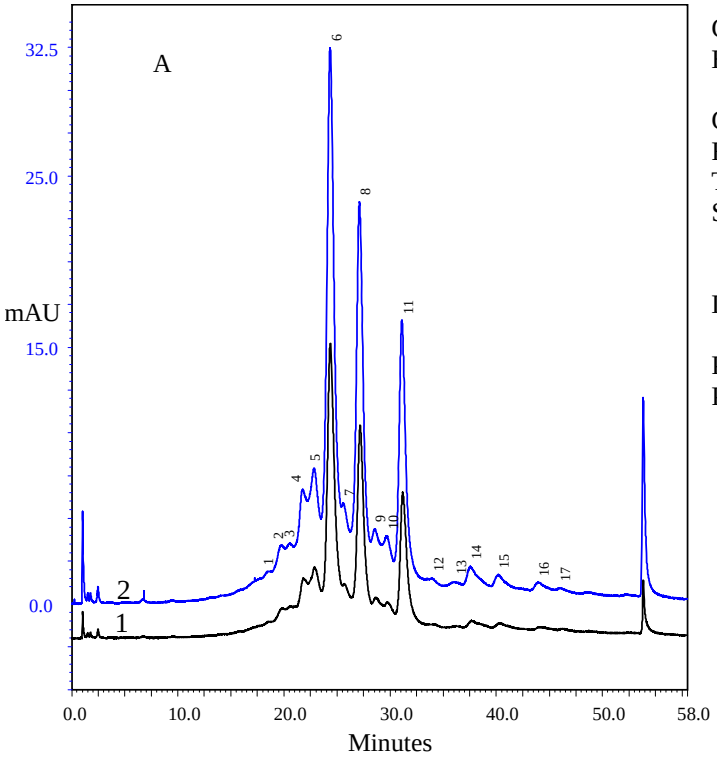
SECTION 4 – APPLICATIONS

The MAbPac SCX-10, 10 µm column is recommended for high resolution separation of monoclonal antibodies and their variants. *The MAbPac SCX-10 columns are a complimentary addition to the existing ProPac WCX-10 columns providing orthogonal selectivity for MAb heterogeneity characterization.* 3 and 5µm particle size 4 x 50 mm columns are designed to provide fast separations of monoclonal antibody variants while maintaining reasonably high resolution. Therefore, they are ideal for high throughput separation needs. The MAbPac SCX-10, 4x250 mm, 5µm columns take advantage of the longer length as well as smaller particle size for producing very-high resolution separations. Some typical application examples are listed below.

4.1. Separation of Acidic and Basic Variants of Monoclonal Antibodies using the MAbPac SCX-10, 10µm Column

Monoclonal antibodies (MAbs) are developed by pharmaceutical and biotechnology companies for various therapeutic applications. MAbs undergo several post-translational modifications including oxidations, deamidations, truncations as well as glycan modifications (4-13). Manufacturing of MAbs and subsequent stability testing procedures involve routine analysis and monitoring of the impurities resulting from asparagines deamidation, aspartic acid isomerization, disulfide interchange, peptide bond cleavage, oxidation and others. The MAbPac SCX-10 column can be used to characterize MAb heterogeneity. Different MAbs separation using salt gradient is shown in Figure 1A, 1B and 1C.

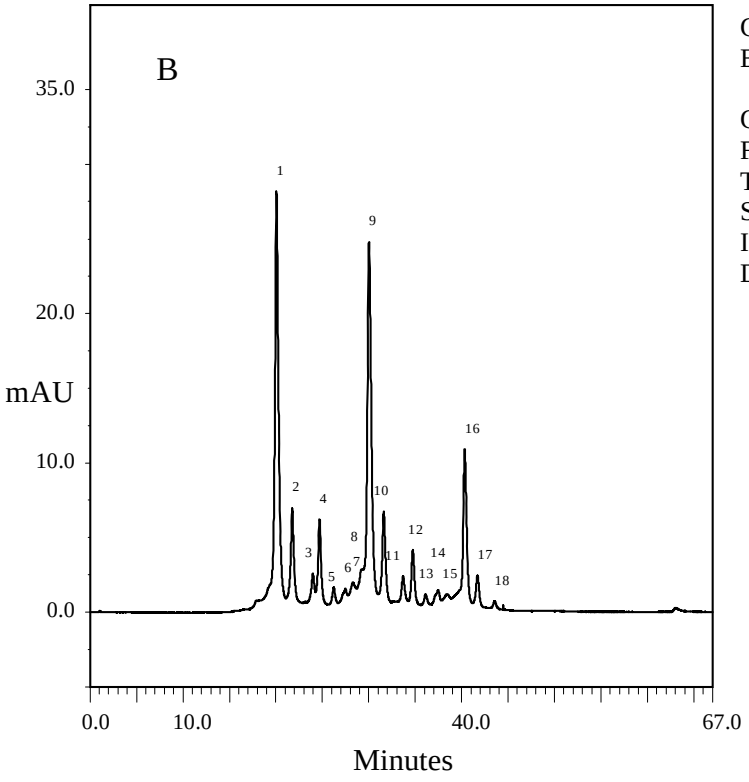
4.1.1. Separation of Monoclonal Antibody Variants using MES Eluents



Column:	MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent:	A: 20 mM MES + 60 mM NaCl pH 5.6 B: 20 mM MES + 300 mM NaCl, pH 5.6
Gradient:	15 - 36.44 % B in 50 min
Flow Rate:	1 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL 1) Sample 1: 25 µg 2) Sample 2: 50 µg
Detection:	280 nm
Peaks 1 – 5:	Acidic variants
Peaks 6,8,11:	C-terminal Lys variants

Figure 1A
Separation of Monoclonal Antibody Variants using MES Eluents
on a MAbPac SCX-10, 10µm column

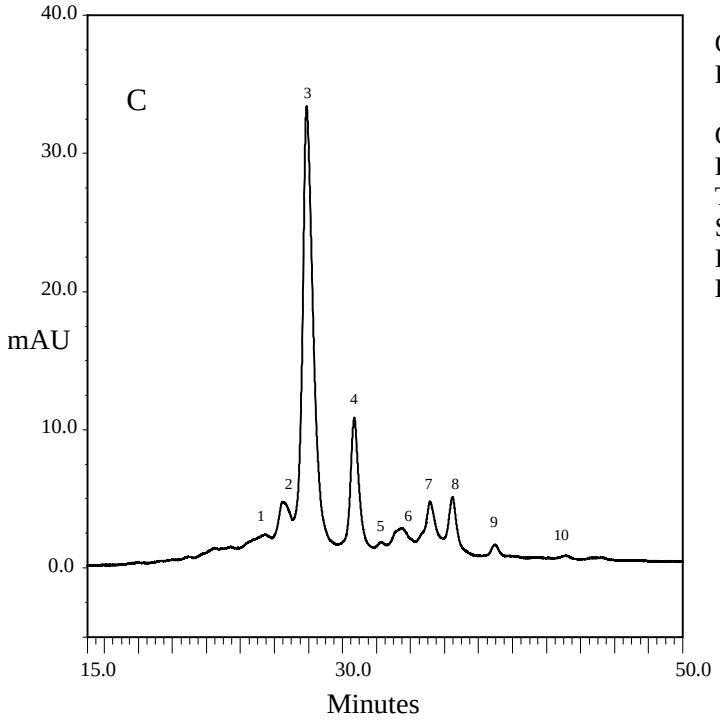
4.1.2. Separation of Monoclonal Antibody Variants using MES Eluents



Column:	MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent:	A: 20 mM MES + 60 mM NaCl pH 5.6
	B: 20 mM MES + 300 mM NaCl, pH 5.6
Gradient:	5 – 55 % B in 60 min
Flow Rate:	1 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	280 nm

Figure 1B
Separation of Monoclonal Antibody Variants using MES Eluents
on a MAbPac SCX-10, 10µm column

4.1.3. Separation of Monoclonal Antibody Variants using Tris Eluents



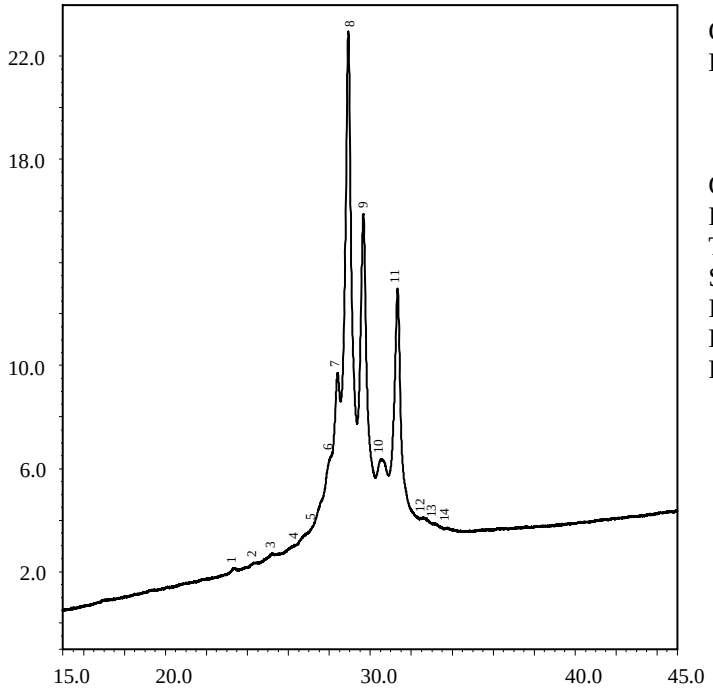
Column:	MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent:	A: 20 mM Tris pH 7.3
	B: 500 mM NaCl in Eluent A
Gradient:	5 – 15 % B in 60 min
Flow Rate:	1 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	280 nm

Figure 1C
Separation of Monoclonal Antibody Variants using Tris-based Eluents on a MAbPac SCX-10, 10µm column

4.2. pH Gradient Based MAb Separation on the MAbPac SCX-10, 10µm column

pH gradients are becoming increasingly popular to ease the method development process (1-2). For pH based separations, a set of buffers with different pKa values in the desired useful pH range are chosen. pH based gradient separation of a MAb is shown in Figure 2. In this example 2x eluent was made with three buffer components with the desired concentrations. The eluent is divided into two portions. Depending on the pH of the eluent one portion's pH is adjusted to 6.0 with HCl/NaOH (Eluent A). The second portion is titrated with NaOH to reach a pH of 10.5 (Eluent B). MAb sample is dissolved in Eluent A and is separated using a linear increase in the percentage of eluent B (pH 10.5).

The equilibration of the column with start buffer is generally achieved with 5-10 column volumes. Under these conditions, acidic and basic variants could be separated from the main peak. If you know the pI values of MAb of your interest, you can design a buffer with components covering that pI range. If the pI of the MAb is not known, a broad range of pH gradient (0-100%B) may be employed to obtain a specific pH range of interest. At this time, an appropriate pH range may be selected to obtain a desired resolution. A pH based gradient separation is shown in Figure 2 (1, 2). Please note that Eluent B is titrated to a final pH of 10.5 to facilitate the elution of a basic MAb).

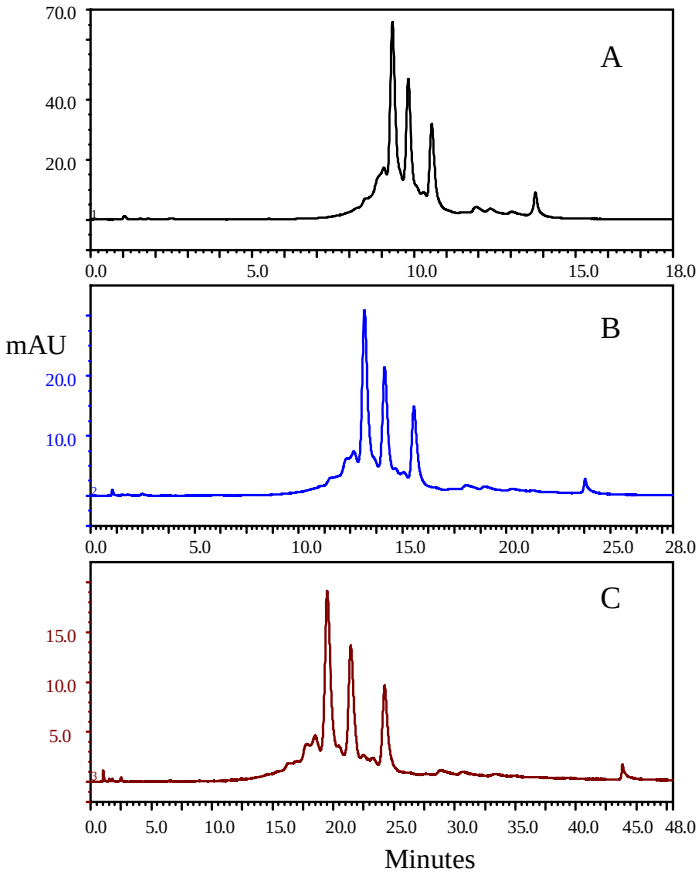


Column:	MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent:	A: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM Piperazine pH 6.0
	B: 2.4 mM Tris + 1.5 mM Imidazole + 11.6 mM Piperazine pH 10.5
Gradient:	40 – 100 % B in 60 min
Flow Rate:	1 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	280 nm
Peaks 8,9,11:	Lys truncation variants

Figure 2
Separation of Acidic and basic variants of MAbs on MAbPac SCX-10, 10 µm column using pH gradient method

4.3. Separation of MAb using Different Salt Gradients and Various Flow Rates.

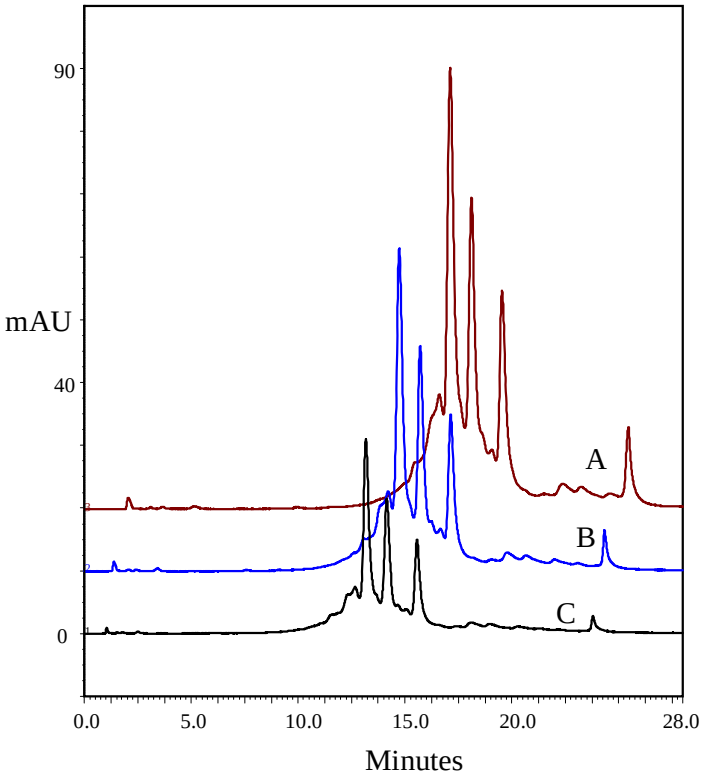
Separation of a MAb using different gradient conditions (Figure 3) and various flow rates (Figure 4) on a MAbPac SCX-10, 10 µm column is shown below. Steep gradients can be used if the desired resolution is met for faster analysis time. Different flow rates and gradients may be attempted to finalize the most appropriate flow rate and gradient for the specific analyte.



Column:	MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent:	A: 20 mM MES + 60 mM NaCl pH 5.6
	B: 20 mM MES + 300 mM NaCl pH 5.6
Gradient:	A: 15 – 40 % B in 10 min
	B: 15 – 40 % B in 20 min
	C: 15 – 40 % B in 40 min
Flow Rate:	1 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	280 nm

Figure 3
Chromatograms Obtained for MAb Sample
using different gradient conditions
on a MAbPac SCX-10, 10µm, column

4.4. MAb separation on MAbPac SCX-10, 10µm Column using different flow rates



Column:	MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent:	A: 20 mM MES + 60 mM NaCl pH 5.6
	B: 20 mM MES + 300 mM NaCl pH 5.6
Gradient:	15 – 40 % B in 20 min
Flow Rate:	A: 0.5 mL/min
	B: 0.75 mL/min
	C: 1.0 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	280 nm

Figure 4
MAb Separation using different flow rates

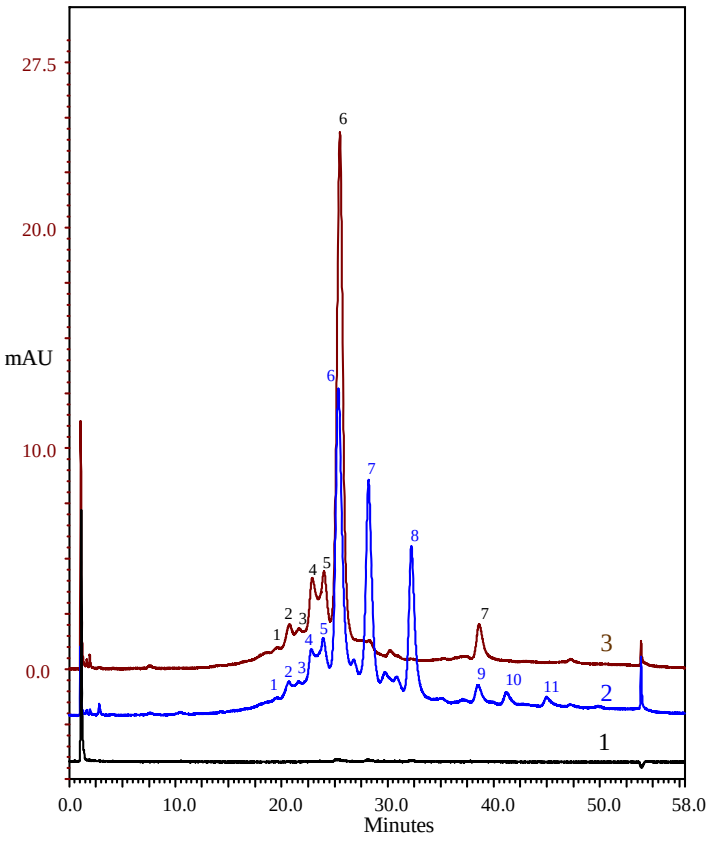
4.5. Monitoring Processing of C-Terminal Lysine Residues of Proteins

Processing of C-terminal lysine and arginine residues of proteins isolated from mammalian cell culture has been described [13]. As a result of processing techniques, the presence of C-terminal Lys or Arg residues, which could be expected based on gene sequence information, are often absent in proteins isolated from mammalian cell culture. This discrepancy, which is common in plasma derived proteins, may result from the activity of one or more basic carboxypeptidases. Charge heterogeneity can result if the processing is incomplete. The resulting charge heterogeneity of the variant forms can be identified by cation exchange chromatography. C terminal processing of lysine residues from heavy chains of monoclonal antibodies from a variety of sources has been reported [14-18].

In this example the MAbPac SCX-10 column was used to separate variants of a humanized IgG, suspected of having lysine residue variation at the C-terminal of the heavy chains. As shown in Fig. 5, a shallow NaCl gradient resolves three variant forms differing by the presence of lysine at the C-terminal of the heavy chains (with either 0, 1, or 2 lysine residues).

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 IgG preparation was treated with carboxypeptidase B, an exopeptidase that specifically cleaves C terminal lysine residues. This treatment of the IgG preparation 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 were accompanied by a corresponding quantitative increase in peak area 6 (0s Lysine present). Similarly, another minor variant with lysine truncations shown as Peaks 9, 10 and 11 collapsed to peak 7 after carboxypeptidase treatment.

4.6. Analysis of MAb Lysine Truncation Variants on the MAbPac SCX-10, 10µm, column



Column: MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent: A: 20 mM MES + 60 mM NaCl pH 5.6
B: 20 mM MES + 300 mM NaCl pH 5.6
Gradient: 36 – 44 % B in 50 min
Flow Rate: 1 mL/min
Temp: 30 °C
Inj. Volume: 5 µL
Detection: 280 nm
Total Volume: 100 µL
Sample:

1. Carboxypeptidase blank 50 µg / 100 uL (No MAb)
2. MAb 900 ug in 100 µL (No Carboxypeptidase)
3. MAb 900 ug in 100 µL+ Carboxypeptidase 50 µg

Peaks 1-5: Acidic variants

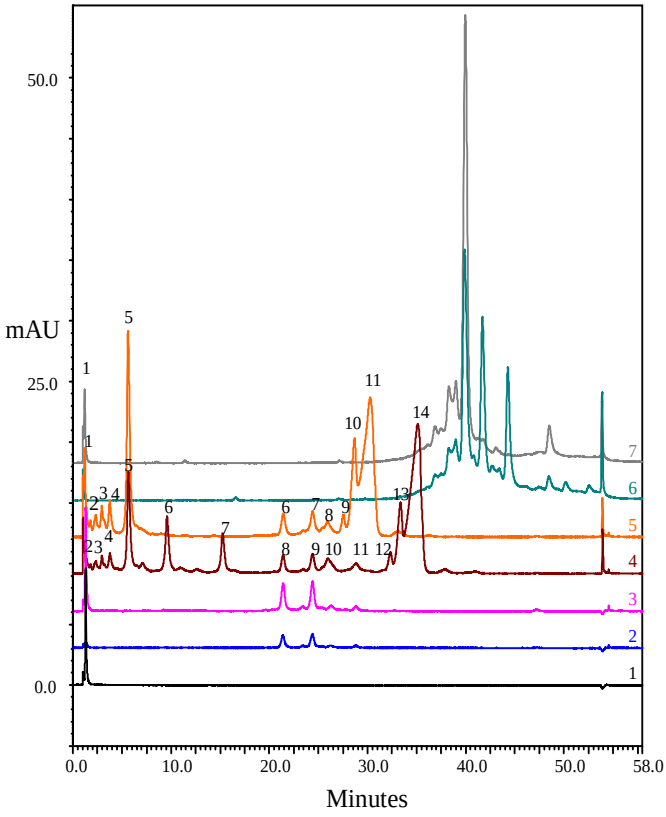
Sample 2
Peaks 6,7,8: C-terminal Lys truncation variants of Main Peak
Peaks 9,10,11: C-terminal Lys truncation variants of a minor variant peak

Sample 3
Peak 6 is resulting from 6, 7, 8 peaks after CPB treatment
Peak 7 is resulting from Peaks 9, 10 and 11 after CPB treatment

Figure 5
Characterization of Lysine truncation variants:
MAb Sample +/- Treatment with Carboxypeptidase B (Sigma) for 3 hrs at 37 ºC

4.7. Analysis of MAb Fab and Fc Fragments after Carboxy Peptidase and Papain Treatments

Figure 6 shows the analysis of MAb after papain treatment alone (See sample 4) or, papain and carboxypeptidase treatments together (Sample 5) on a MAbPac SCX-10, 10 µm column. Lysine truncations are located on the C terminus of the heavy chain. Papain is a cysteine protease and cleaves antibodies into Fab and Fc fragments.



Column: MAbPac SCX-10, 10µm, 4 x 250 mm
Eluent: A: 20 mM MES + 60 mM NaCl pH 5.6
B: 20 mM MES + 300 mM NaCl pH 5.6
Gradient: 1 – 35 % B in 50 min
Flow Rate: 1 mL/min
Temp: 30 °C
Inj. Volume: 5 µL
Detection: 280 nm
Samples: Total Volume 300 µL

1. Carboxypeptidase blank 10 uL (50 µg; No MAb)
2. Papain Blank 10 µL (100 µg; No MAb)
3. Carboxypeptidase + Papain Blank (1 and 2)
4. MAb 3mg/300 µL + Papain10 µL
5. MAb 3mg/300 µL + Papain10 µL + Carboxypeptidase10µL
6. MAb 3mg/300 µL
7. MAb 3mg/300 µL + Carboxypeptidase 10 µL

<u>Sample 4 (Papain treated):</u>
Peaks 1-4: Acidic variants
Peaks 5,6,7: C-terminal Lys truncation variants of papain treated sample.
Peaks 12-14: Fab peaks

<u>Sample 5 (Papain and Carboxypeptidase treated):</u>
Peaks 1-4: Acidic variants
Peak 5 is resulting from 5,6,7 peaks (from sample 4) after papain and CPB treatments together
Peaks 9-11: Fab peaks

Figure 6
MAb Sample +/- Treatment
with Carboxypeptidase B and/or papain for 3 hrs at 37° C

4.8. Separation of Acidic and Basic Variants in Monoclonal Antibodies using MAbPac SCX-10, 3µm Column and MAbPac SCX-10, 5 µm columns

MAbPacSCX-10, 3 µm and MAbPac SCX-10, 5 µm columns are recommended for high resolution and high-throughput separations of MAbs for characterization of MAb heterogeneity. The main advantage being the run time is reduced several fold when 3 or 5 µm, 4x50 column was used as compared to a longer MAbPac SCX-10, 10 µm, 4x250 mm column. Since the column length is short, chromatography runs can be completed at a faster rate, therefore increasing the throughput.

WARNING

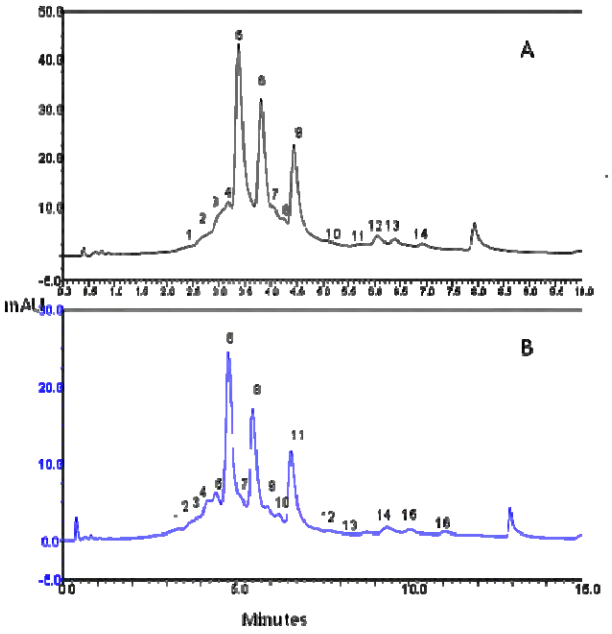
ALL ELUENTS, SAMPLES SHOULD BE FILTERED USING A 2 µm filter unit before use. FAIL TO DO SO WILL WILL RESULT IN CLOGGING THE COLUMN WITH INCREASED BACK PRESSURE THIS WILL LEAD TO COMPROMISED COLUMN PERFORMANCE.

NOTE

Include a wash procedure using a buffer with high salt solution (For eg. 1M NaCl) to minimize the sample build up on the column during chromatography runs.

4.8.1. Salt Gradient Elution: MES based eluents; MAb Separation using MAbPac SCX-10, 3 and 5 µm Columns

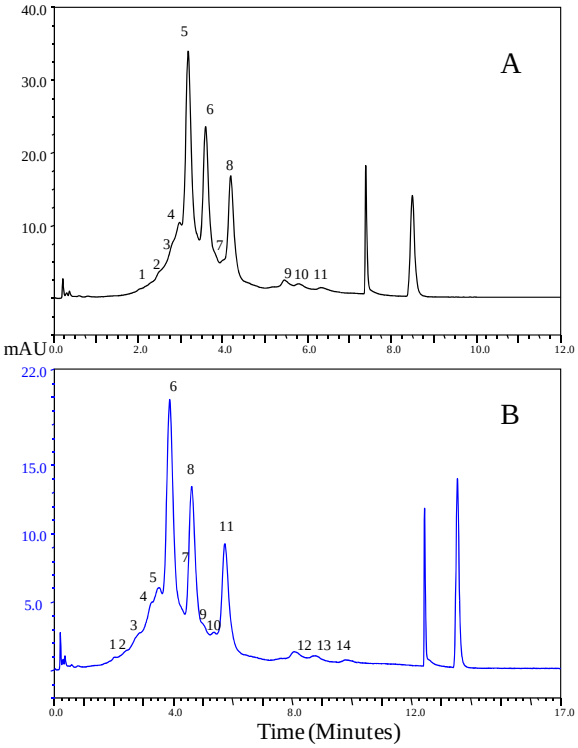
MAb separation using MES based salt gradients for the 3 µm column is shown in Figure 7. Two different gradients were used to separate the MAb. Although, both gradients, using the same flow rate resolved the variants quite well, as expected, a shallow 10 minute gradient resulted in a better resolution than the shorter 5 minute steep gradient. MAb separation on a MAbPac SCX-10, 5 µm column 4x50 mm and 4x250 mm columns are shown in Figures 8 and 9 respectively. Similar to the 3 µm, 4x50 column, a shallow 10 minute gradient resulted in a better separation with higher resolution as expected (Figure 8). Either 3 µm or 5 µm column can be used for high throughput separations. MAb variant characterization on MAbPac SCX-10, 5um, 4x250 mm column resulted in a very efficient, high resolution separation of variants with narrow peak widths. In this example, a 20 minute gradient was used (Figure 9). It should be noted that, for each of these chromatographic runs, column went through a wash procedure using a buffer with high salt solution (1M NaCl) to minimize the sample build up on the column.



Column:	MAbPac SCX-10, 3µm, 4 x 50 mm
Eluent:	A: 20 mM MES, pH 5.6 + 60 mM NaCl
	B: 20 mM MES, pH 5.6 + 300 mM NaCl
Gradient:	A: 25 – 35 % B in 5 min
	B: 20 – 35 % B in 10 min
Flow Rate:	0.6 mL/min
Temp:	30 °C
Sample:	MAb, 1 mg/mL
Inj. Volume:	15 µL
Detection:	UV at 280 nm
Peak:	A: 5 min Gradient
Peaks 1-4:	Acidic variants
Peaks 5,6,9:	C-Terminal lysine variants
Peaks 10-14:	Basic variants
	B: 10 min Gradient
Peaks 1-5:	Acidic variants
Peaks 6,8,11:	C-Terminal lysine variants
Peaks 12-17:	Basic variants

Figure 7
Separation of Acidic and basic variants of MAbs
using MES based two different salt gradients on MAbPac SCX-10, 3 µm column

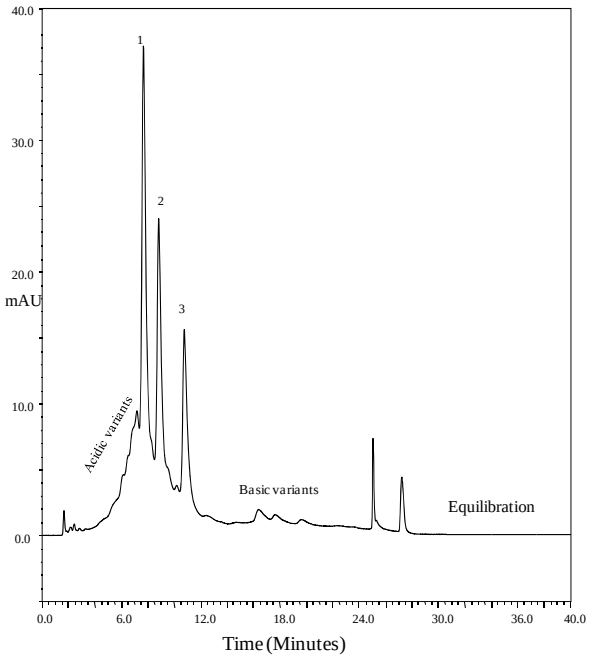
4.8.2. Separation of Acidic and basic variants of a Mab using two different salt gradients on MAbPac SCX-10, 5 µm 4x50 mm column



Column:	MAbPac SCX-10, 5 µm, 4 × 50 mm
Eluents:	A) 20 mM MES + 60 mM NaCI, pH 5.6 B) 20 mM MES + 300 mM NaCl, pH 5.6 C) 20 mM MES + 1M NaCl, pH 5.6 (For Wash)
Gradients:	A: 20–35% B in 5 min B: 20–35% B in 10 min
Eluent C wash:	1 min Eluent C wash before equilibration
Equilibration:	~5 min
Flow Rate:	1.5 mL/min
Temperature:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	UV at 280 nm
Peaks:	A: 5 min Gradient 1–4 Acidic variants 5, 6, 8 C-Terminal lysine variants 9-11 Basic variants B: 10 min Gradient 1–5 Acidic variants 6, 8, 11 C-Terminal lysine variants 12–14 Basic variants

Figure 8
Separation of Acidic and basic variants of a Mab using two different salt gradients on MAbPac SCX-10, 5 µm 4x50 mm column

4.8.3. Separation of Acidic and basic variants of a Mab using a salt gradient on MAbPac SCX-10, 5 µm 4x250 mm column

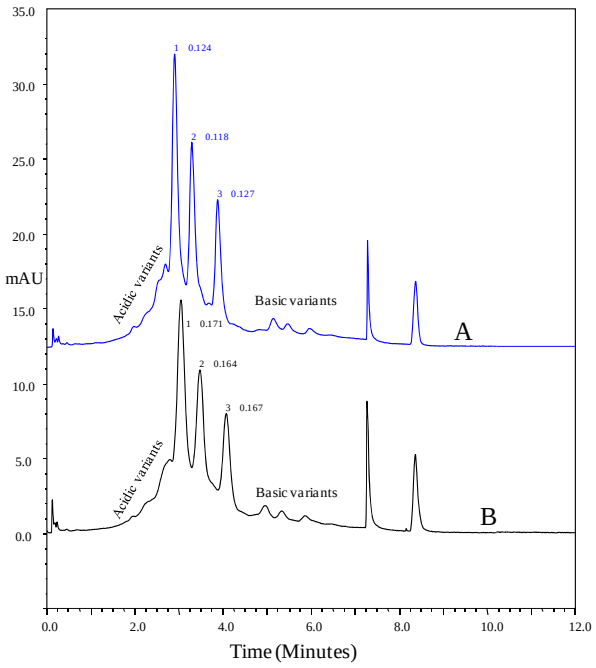


Column:	MAbPac SCX-10, 5 µm, 4 × 250 mm
Eluents:	A) 20 mM MES + 60 mM NaCI, pH 5.6 B) 20 mM MES + 300 mM NaCl, pH 5.6 C) 20 mM MES + 1M NaCl, pH 5.6 (For Wash)
Gradients:	25–40% B in 20 min
Eluent C wash:	2min Eluent C wash before equilibration
Equilibration:	15 min
Flow Rate:	0.7 mL/min
Temperature:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	10 µL
Detection:	UV at 280 nm
Peaks:	1, 2, 3 : C-terminal lysine truncation variants

Figure 9
Separation of Acidic and basic variants of a Mab
using a salt gradient on MAbPac SCX-10, 5 µm 4x250 mm column

4.8.4. Comparison of separation of MAb on MAbPac SCX-10, 5 µm, 4x50 column with a MAbPac SCX-10, 10 µm, 4x50 mm column

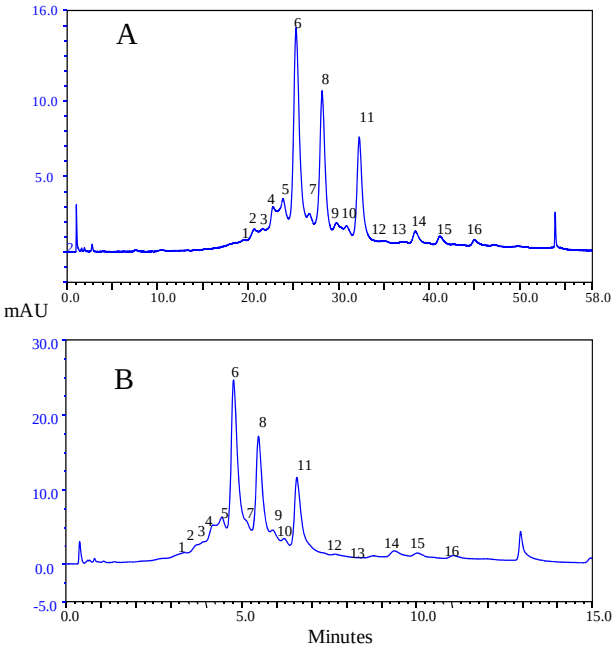
Figure 10 shows the comparison of a fast MAb variant analysis on MAbPac SCX 5 µm and 10 µm columns. As expected, MAb separation on a 5 µm column provides superior performance over the 10 µm column of the same length (4x50 mm). Both acidic and basic variants resolved better with the small particle column. MAb C-terminal lysine truncation peaks are identified as peaks 1, 2, 3. Their efficiencies are shown as peak width at half height measurements in minutes. For 5 µm column, these are as follows. peak 1: 0,124, peak 2: 0.118 and peak 3: 0.127. In comparison, for 10 µm column for the same peaks, they are noted as 0.171, 0.164 and 0.167 respectively. When 5 µm column is used, the resolution of peak1 and 2 are 1.88 and 2.83. These values are lower for 10 µm column (peak 1: 1.53 peak 2: 2.11). These MAb variant separation data clearly indicates that both peak efficiency and resolution are superior for small particle size columns as compared with the large particle columns.



Columns:	A: MAbPac SCX-10, 5 µm, 4 × 50 mm B: MAbPac SCX-10, 10 µm, 4 × 50 mm
Eluents:	A) 20 mM MES + 60 mM NaCI, pH 5.6 B) 20 mM MES + 300 mM NaCl, pH 5.6 C) 20 mM MES + 1M NaCl, pH 5.6 (For Wash)
Gradients:	20–35% B in 5 min
Eluent C wash:	1 min Eluent C wash before equilibration
Equilibration time:	4 min
Flow Rate:	2 mL/min
Temperature:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	5 µL
Detection:	UV at 280 nm
Peaks:	1, 2, 3 : C-terminal lysine truncation variants Peak width at half height is shown next to the peak number

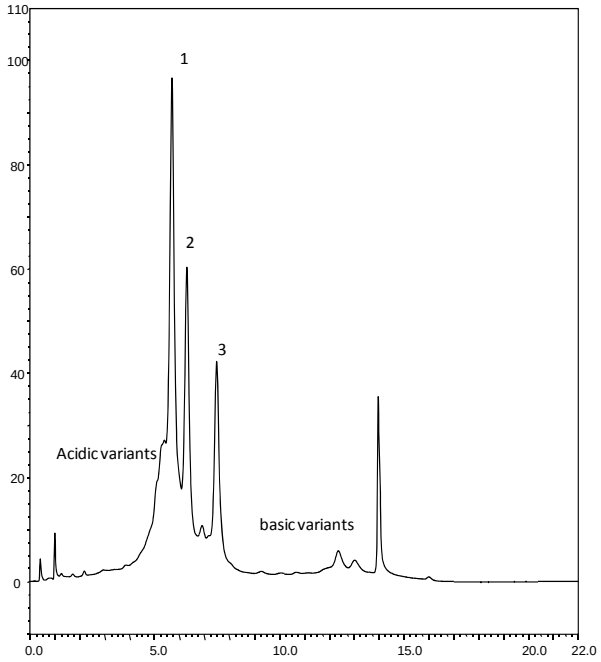
Figure 10
Comparison of separation of MAb on MAbPac SCX-10, 5 µm, 4x50 column with a MAbPac SCX-10, 10 µm, 4x50 mm column

Comparison of MAb Separation using MAbPac SCX-10, 10 µm column (4x250 mm) and 3µm Columns (4x50 mm) is shown in Figure 11. Please note the gradient time differences in this comparison. For 3 µm columns it is a 10 minute gradient vs 50 min gradient for the 10 µm column. The variant separation appears to be quite comparable. Tris based eluents separation is shown in Figure 12



Columns:	A: MAbPac SCX-10, 10 **µ**m 4 x 250 mm
	B: MAbPac SCX-10, 3 **µ**m, 4 x 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: 15 - 36.44% B in 50min
	B: 20 - 35% B in 10min
Flow Rate:	1mL/min for A and 0.6 mL/min for B
Temp:	30°C
Sample:	A: 10 mg/ mL
	B: 1mg/mL
Inj Volume:	A: 5 uL (50 ug)
	B: 15 uL (15 ug)
Detection:	280 nm

Figure 11
Comparison of Monoclonal Antibody separation using MES eluents
On MAbPac SCX-10, 10 µm, 4x250 mm, and MAbPac SCX-10, 3 µm, 4x50 mm, columns
(Please note that time scale is different)



Column:	MAbPac SCX-10, 3µm, 4 x 250 mm
Eluent:	A: 20 mM Tris pH 7.3
	B: 500 mM NaCl in Eluent A
Gradient:	11 – 16 % B in 10 min
Flow Rate:	0.6 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	15 µL
Detection:	280 nm
Peaks:	1, 2, 3: Lysine truncation variants

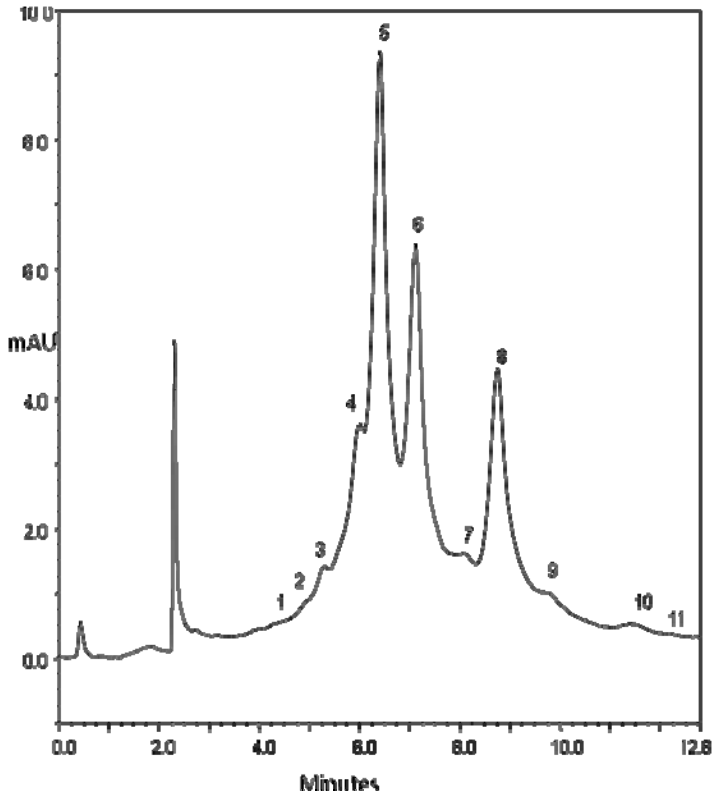
Figure 12
Separation of Acidic and basic variants of MAb
using Tris based salt gradient on MAbPac SCX-10, 3 µm, 4x50 mm column

4.8.5. pH Gradient Elution: MAb Separation using MAb SCX-10, 3µm Columns

The MAbPac SCX-10, 3µm 4 x 50 mm column provides excellent resolution and very short separation times for MAb variant analysis using pH gradients.

pH gradients are becoming increasingly popular to ease the method development process (1-2). For pH based separations, a set of buffers with different pKa values in the desired useful pH range are chosen. pH based gradient separation of a MAb is shown in Figure 13. In this example 2x eluent was made with three buffer components with the desired concentrations. The eluent is divided into two portions. Depending on the pH of the eluent one portion's pH is adjusted to 6.0 with HCl/NaOH (Eluent A). The second portion is titrated with NaOH to reach a pH of 10.5 (Eluent B). MAb sample is dissolved in Eluent A and is separated using a linear increase in the percentage of eluent B (pH 10.5).

The equilibration of the column with start buffer is generally achieved with 5-10 column volumes. Under these conditions, acidic and basic variants could be separated from the main peak. If you know the pI values of MAb of your interest, you can design a buffer with components covering that pI range. If the pI of the MAb is not known, a broad range of pH gradient (0_100%B) may be employed to obtain a specific pH range of interest. At this time, an appropriate pH range may be selected to obtain a desired resolution. An example application is shown in Figure 13.

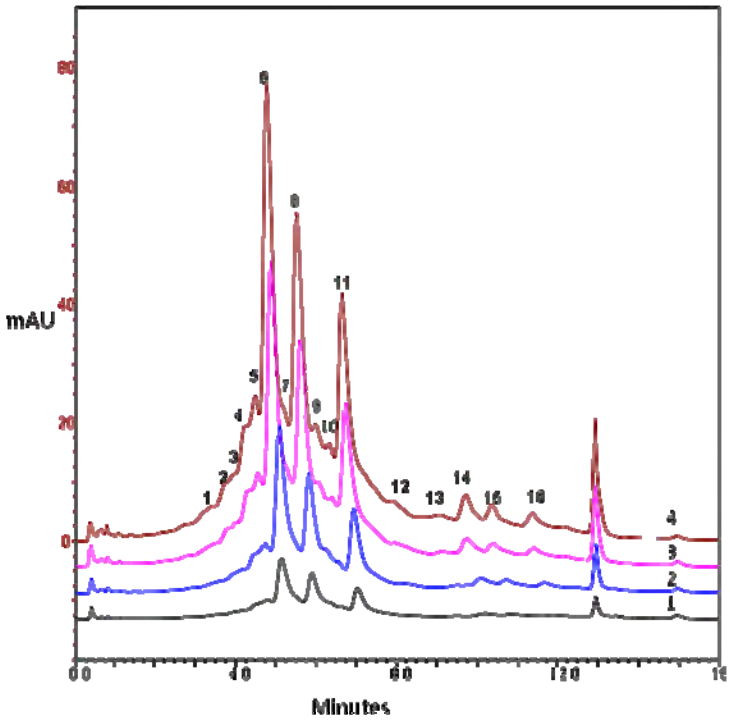


Column:	MAbPac SCX-10, 3µm, 4 x 50 mm
Eluent:	A: 4.8 mM Tris + 3 mM imidazole + 23.2 mM Piperazine, pH 6.0 B: 4.8 mM Tris + 3 mM imidazole + 23.2 mM Piperazine, pH 10.5
Gradient:	49 – 56 % B in 10 min
Flow Rate:	0.6 mL/min
Temp:	30 °C
Sample:	MAb, 1 mg/mL
Inj. Volume:	15 µL
Detection:	UV at 280 nm
Peak:	
Peaks 1-4:	Acidic variants
Peaks 5,6,7:	C-Terminal lysine truncation variants
	9-11 Basic variant

Figure 13
Separation of MAb variants using a pH gradient on MAbPac SCX-10 3 µm, 4 x 50 mm column;

4.9. Dynamic loading of MAb on MAbPac SCX-10, 4x 50 mm, 3 µm column

Figure 14 shows the loading dynamic loading of MAbPac SCX 3µm column. In this experiment, different amounts of MAb (10, 25, 50 and 75 ug) were loaded. Even at 75 µg loading of the MAb, the resolution is quite decent. It is important to verify the loading capacity for the MAb of interest. Overloading of the column will result in reduced resolution of the MAb variants.

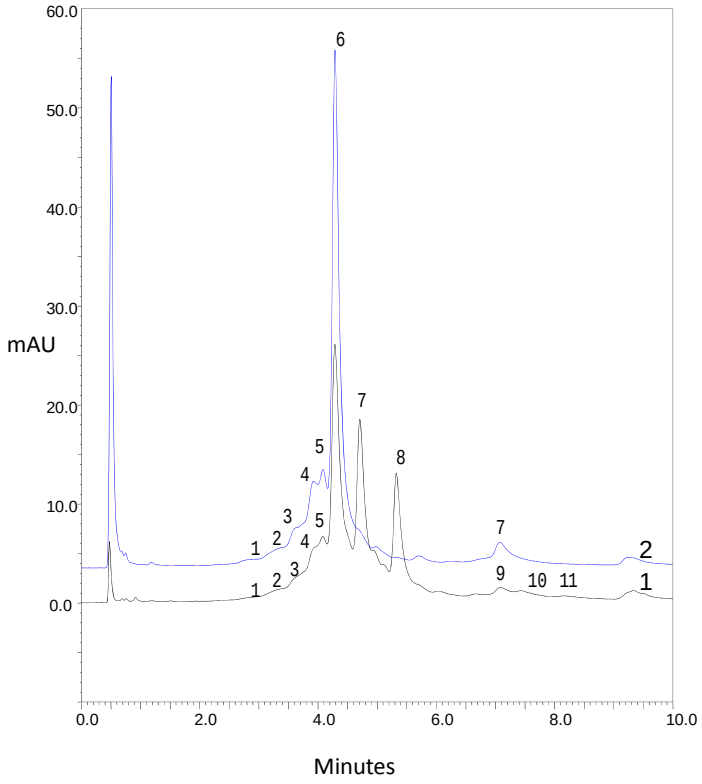


Column:	MAbPac SCX-10, 3µm, 4 x 50 mm
Eluent:	A: 20 mM MES, pH 5.6 + 60 mM NaCl
	B: 20 mM MES, pH 5.6 + 300 mM NaCl
Gradient:	20 – 35 % B in 10 min
Flow Rate:	0.6 mL/min
Temp:	30 °C
Sample:	MAb, 5 mg/mL
Inj. Volume:	1: 2 µL (10 µg); 2:5 µL (25 µg)
	3:10 µL (50 µg); 4:15 µL (75 µg)
Detection:	UV at 280 nm
Peak:	
Peaks 1-5:	Acidic variants
Peaks 6,8,11:	C-Terminal lysine variants
Peaks 12-16:	Basic variants

Figure 14
Different amounts of loading of MAb on MAbPac SCX-10, 3 µm, 4x 50 mm column

4.10. Monitoring Processing of C-Terminal Lysine Residues of Proteins using MAbPac SCX-10, 3 um columns

In this example the MAbPac SCX-10, 3 µm column was used to achieve a fast separation of variants of a humanized IgG, suspected of having lysine residue variation at the C-terminal of the heavy chains. As shown in Fig. 15, an NaCl gradient resolves three variant forms differing by the presence of lysine at the C-terminal of the heavy chains with either 0, 1, or 2 lysine residues. (Please see section 4.3 for details; and Figure 5 for comparison with 4x 250 mm, 10 µm column
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 IgG preparation was treated with carboxypeptidase B, an exopeptidase that specifically cleaves C terminal lysine residues. This treatment of the IgG preparation 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 were accompanied by a corresponding quantitative increase in peak area 6 (0s Lysine present). Similarly, another minor variant with lysine truncations shown as Peaks 9, 10 and 11 collapsed to peak 7 after carboxypeptidase treatment.



Column: MAbPac SCX-10, 3µm, 4 x 50 mm
Eluent: A: 20 mM MES + 60 mM NaCl, pH 5.6
B: 20 mM MES + 300 mM NaCl, pH 5.6
Gradient: 22 – 37 % B in 5 min
Flow Rate: 0.5 mL/min
Temp: 30 °C
Inj. Volume: 6 µL
Detection: 280 nm
Total Volume: 200 µL
Sample:
1. MAb, 2.5 mg/mL ,100 µL (No Carboxypeptidase)
2. MAb, 2.5 mg/mL, 100 µL + Carboxypeptidase (CPB) 50 µg

Peaks 1-5: Acidic variants

Sample 1:
Peaks 6,7,8: C-Terminal Lys truncation variants of main peak
Peaks 9-11 C-Terminal Lys truncation variants of a minor variant peak

Sample 2: (After CBP treatment for 3 hrs at 37C)

Peak 6 is resulting from peaks 6, 7 and 8
Peak 7 is resulting from peaks 9, 10 and 11

Figure 15
Characterization of Lysine truncation variants on MAbPac SCX-10, 3 **µ**m **4x50 mm column**
MAb Sample +/- Treatment with Carboxypeptidase B (Sigma) for 3 hrs at 37 ºC

SECTION 5 – TROUBLESHOOTING GUIDE

5.1. Finding the Source of High System Backpressure

WARNING ***NEVER WASH THE MAbPac SCX-10 COLUMN WITH H_2O. Always maintain minimum ionic strength (20 mM MES or equalent ionic strength) in the eluents.***

a. If you observe high back pressure, wash the column with an eluent containing high salt (Buffer containing 1M NaCl) at a lower flow rate (0.1 to 0.5 mL/ min) until the pressure becomes normal.

b. A significant increase in the system backpressure may be caused by a plugged inlet frit (bed support).

c. Before replacing the inlet bed support assembly of the column, make sure that the column is the cause of the excessive backpressure.

d. Check for pinched tubing or obstructed fittings from the pump outlet, throughout the eluent flow path to the detector cell outlet. To do this, disconnect the eluent line at the pump outlet and observe the backpressure at the usual flow rate. It should not exceed 50 psi (0.3 MPa). Continue adding components (injection valve, column, detector) one by one while monitoring the system backpressure. The 4 x 250 mm MAbPac SCX-10, 10µm column should add no more than 2000 psi backpressure at 1 mL/min. The 4 x 50 mm MAbPac SCX-10, 10µm columns as expected should add no more than 1/5th of the analytical column (300-350psi) back pressure at 1 mL/min. No other component should add more than 100 psi (0.7 mpa) to the system backpressure.

e. If the high backpressure is due to the column, first try cleaning the column. If the high backpressure persists, replace the column bed support at the inlet of the column.

f. NEVER USE H_2O ALONE for washing the column. This will lead to significant increase in back pressure. This abnormality can be reversed by washing the column for long periods of time with buffered high ionic strength eluents. Please make sure to start at a low flow rate to keep the pressure under control. Gradually increase the flow rate as the column pressure drops further.

5.2. Column Performance is Deteriorated

5.2.1. Peak efficiency and resolution is decreasing; loss of efficiency.

WARNING ***One of the sources of decreased performance could be metal leaching from the system. To avoid denaturation of the protein samples and corrosion of components with halide-salt mobile phases we strongly recommend a metal-free inert system, including pump, tubing, ferrules, and bolt assemblies.***

a. If changes to the system plumbing have been made, check for excess lengths of tubing, tubing diameters larger than 0.010 ID in., larger than normal tubing diameter, and for leaks.

b. Check the flow rate and the gradient profile to make sure your gradient pump is working correctly.

c. The column may be fouled. Clean the column using the recommended cleaning conditions.

d. If there seems to be a permanent loss of efficiency, check to see if headspace has developed in the column. This is usually due to improper use of the column such as submitting it to high backpressure. If the resin doesn't fill the column body all the way to the top, the resin bed has collapsed, creating a headspace. The column must be replaced.

e. If the peak shape looks good, but the efficiency number is low, check and optimize the integration parameters. If necessary, correct the integration manually, so the start-, maximum-, and end of the peak are correctly identified.

5.2.2. Unidentified peaks appear as well as the expected analyte peaks.

a. The sample may be degrading. Proteins tend to degrade faster in solutions; therefore, store your protein samples in the freezer in dry form, and prepare only a small amount of solution/mixture for analysis.

b. The eluent may be contaminated. Prepare fresh, filtered eluent.

c. Run a blank gradient to determine if the column is contaminated. If ghost peaks appear, clean the column.

5.2.3. Peak efficiency and resolution is poor.

a. Try to use different eluents (buffer, pH, concentration etc.), to make sure you are using the optimum conditions for your separation problem.
b. The column may be overloaded. Dilute the sample and/or inject smaller volumes.

5.2.4. Peak retention time varies from run-to-run.

The column may not be adequately equilibrated or washed.

a. Make sure that the equilibration time is adequate (5 to 10 column volumes) and remains constant after every gradient run. Re-equilibration should be part of the method.
b. Column washing is usually not necessary between every run, unless your sample is extremely "dirty." If you need to use a wash, a consistent and adequate method for washing and equilibrating should be part of the method.

5.2.5. Metal poisoning of columns causing reproducibility and recovery problems (See Ref 19).

a. Periodic passivation is a must for stainless steel HPLC systems to reduce rust build up.
b. If you have experienced, performance, reproducibility and recovery problems, it could be due to metal poisoning or rust from one or, several SST components of your HPLC system. Restoring the column to original metal free status is a tedious and time consuming process. Potentially restoration can be achieved by treating the column with oxalic acid dihydrate (200mM) at 0.2 mL/min for 6 hrs or longer followed by a 20 mM NaOH wash for 30 min at 0.5mL/min Please equilibrate your column thoroughly for an extended period of time (1 to 2 hrs) before testing with your sample of interest**. Please note that you need an inert device for pumping the oxalate or high pH eluents. Do not use stainless steel pumps to perform this step.**

5.3. No Peaks, Small Peaks, Noisy Baseline

5.3.1. Detection Problem

a. Make sure that you are using the correct wavelength for your sample/buffer system.
b. Adjust the selected detector range (AU) according to your injected sample amount.
c. Check your lamp: aged UV lamps tends to give noisier response. Replace the lamp if necessary.

5.3.2. Chromatographic Problem

Make sure that your sample can be eluted with the buffers and conditions you are using. Before trying a gradient separation, try isocratic elution with 100% B (high salt) buffer: the sample should elute at, or near to, t0 (void). If not, try a higher salt concentration or different pH.

5.3.3. Temperature Stability Problem of Mab/Protein

If your MAb/Protein is stable to elevated temperatures, it should be established before routinely used in the method

APPENDIX A. REFERENCES

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APPENDIX B. COLUMN CARE

B.1 New Column Equilibration

The MAbPac SCX-10, columns are shipped in a buffer containing 0.1% NaN_3. Before use, equilibrate the column with approximately 20 mL of the starting eluent (20 min at 1 mL/min).

B.2 Column Clean Up

NOTE ***When cleaning an analytical and guard column in series, move the guard column after the analytical column in the eluent flow path. Otherwise contaminants that have accumulated on the guard column will be eluted onto the analytical column.***

1. For minor contamination, use a mild cleaning protocol by running consecutive gradient runs, using a high (1-2 M) salt concentration at the end of the gradient.
2. For more severe contamination, inject 100-500 µL (or more as needed) of 0.1-1 M NaOH consecutively.
3. If necessary, the column can be washed with strong acid such as 1.0 M HCl followed by base 0.1 - 0.5 M NaOH. Usually 5 - 30 min at 1 mL/min is sufficient. Do not exceed 20x the column volume of 0.5 M NaOH (60 mL). The use of high concentrations of base and/or larger volumes of base are not recommended. For the SCX-10 columns, the above mentioned strong acid or base cleaning solutions should be used at room temperature (<30 °C). After the wash, rinse the column with at least 20 mL of the starting buffer solution. Note: Do not store the column in strong acid or base solution.

B.3 Column Storage

Short Term Storage: For short term storage, use the low salt concentration eluent (pH = 3 - 8) as the column storage solution. DO NOT STORE THE COLUMN IN HIGH pH BUFFERS (>pH 8.0) EVEN FOR SHORT TERM STORAGE

Long Term Storage: For long term storage, use storage eluent (or other low salt concentration eluent with pH=6.0-7.5) with 0.1% sodium azide added to prevent bacteria growth on the column. NEVER STORE COLUMNS IN HIGH pH (>pH 8.0) BUFFERS

Flush the column with at least 10 mL of the storage eluent. Cap both ends, securely, using the plugs supplied with the column.

B.4 Replacing Column Bed Support Assemblies

NOTE ***Replace the inlet bed support ONLY if the column is determined to be the cause of high system backpressure, AND cleaning of the column does not solve the problem.***

1. Carefully unscrew the inlet (top) column fitting. Use two open end wrenches.
2. Remove the bed support. Tap the end fitting against a hard, flat surface to remove the bed support and seal assembly. Do not scratch the wall or threads of the end fitting. Discard the old bed support assembly.
3. Removal of the bed support may permit a small amount of resin to extrude from the column. Carefully remove this with a flat surface such as a razor blade. Make sure the end of the column is clean and free of any particulate matter. Any resin on the end of the column tube will prevent a proper seal. Insert a new bed support assembly (p/n 057804) into the end fitting and carefully thread the end fitting and bed support assembly onto the supported column.
4. Tighten the end fitting finger-tight, then an additional ¼ turn (25 in x lb.). Tighten further only if leaks are observed.

WARNING ***If the end of the column tube is not clean when inserted into the end fitting, particulate matter may prevent a proper seal between the end of the column tube and the bed support assembly. If this is the case, additional tightening may not seal the column but instead damage the column tube or break the end fitting.***

