Small Particle Media for High Throughput, High Resolution Monoclonal Antibody Analysis

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Overview

Purpose: Demonstrate advantages of high resolution media for high-throughput monoclonal antibody (MAb) analysis.

Methods: High throughput, high resolution separation of MAb is achieved with the Thermo Scientific™ Dionex™ UltiMate™ 3000 Biocompatible Rapid Separation (BioRS) system using the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software.

Results: High pressure bio-inert column hardware was specifically used to achieve high flow rates without compromising the resolution of MAb analysis. By employing small particle resin packed into longer columns with higher flow rates are used to achieve fast, high resolution separation of MAbs.

Introduction

MAbs represent a major class of bio-therapeutic molecules that usually display complex micro-heterogeneity with several post-translational modifications including oxidation, isomerization, deamidation, glycation and others. Primary structure alterations such as lysine truncations are also known to occur in the C-terminus region of MAbs. Due to these possibilities, quality control and stability assessment of MAbs are very challenging tasks. The increasing utilization of MAbs in the pharmaceutical industry is also driving a growing demand for improved high resolution stationary phases for characterization of MAbs.

Previously introduced Thermo Scientific™ MAbPac™ strong cation-exchange phases are based on particle sizes of 10 µm, 5 µm and 3 µm resins for MAb charge variant separations. These small particle size phases were developed specifically to address the requirement of high resolution variant analysis of MAbs. However, there is a need in the industry to have analytical columns that combine uncompromised resolution power with high flow rate compatibility to achieve high-throughput separation of MAbs.

With the launch of a new, totally bio-inert high pressure UltiMate BioRS system with maximum pressure of 15000 psi, we have developed 5 µm polymeric particle size columns that are suitable for high throughput high resolution MAb analysis. Bio-inert column hardware is a critical component for any MAb separation to avoid metal interferences with analytes of interest. Here, we utilized a PEEK-lined stainless steel column bodies that are suitable for high pressure operations, providing a metal-free fluidic path.

This work describes the development and applications of 5 µm small particle columns in two different internal diameters (I.D; 4.6 and 2.1 mm) with various lengths for high-throughput, high-resolution MAb analysis. Isocratic and gradient analysis are performed to evaluate the asymmetry, efficiency, resolution and ruggedness of these different format columns.

Experimental

Samples

Monoclonal antibody samples are received from local biotechnology companies. Cytochrome C (Equine) and other chemicals are obtained from Sigma/Aldrich®.

Columns (PEEK-lined Stainless Steel Columns)

MAbPac SCX-10 RS, 5 µm, 4.6 × 50 mm (P/N 082674)

MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm (P/N 085209)

MAbPac SCX-10 RS, 5 µm, 4.6 × 250 mm (P/N 082673)

MAbPac SCX-10 RS, 5 µm, 2.1 × 50 mm (P/N 082675)

MAbPac SCX-10 RS, 5 µm, 2.1 × 150 mm (P/N 088242)

MAbPac SCX-10 RS, 5 µm, 2.1 × 250 mm (P/N 082515)

Methods

Salt Gradients are performed using MES buffers. pH gradients are performed using Thermo Scientific™ pH platform using CX-1 buffer kits (P/N: 083274; P/N: 085349)

Eluent and gradient details are given within the figures

High Pressure Liquid Chromatography (HPLC)

HPLC experiments were carried out using a New UltiMate 3000 BioRS high pressure totally inert system equipped with:
- Gradient Pump System; TCC-3000RS Thermostatted Column Compartment
- WPS-3000 TBRS Auto sampler;
- VWD-3400RS UV Detector equipped with a Micro Flow Cell

Chromatography was controlled by Chromeleon Chromatography Data System.

BioRS HPLC Instrument Specifications:
- Bio-inert materials
- Pressure of up to 1034 bar (~15000 Psi)
- Flow rates of up to 8 mL/min
- Short sampler cycle times
- High column temperatures
- Ultrafast data collection and processing

Separation media and mechanism of cation exchange column

Substrate Monomers:	Ethylvinylbenzene-divinylbenzene
Substrate Pore Size:	Non-porous
Cross-linking:	55%
Mode of Interaction:	Cation Exchange
Functional Group:	Sulfonic Acid; SCX
Separation Mechanism:	Charge-Charge Interaction; By increasing ionic strength, or by pH

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Results

Isocratic testing with Cytochrome C

Figure 1. Isocratic testing of MAbPac SCX-10 RS, 5 µm, 4.6 mm columns; Comparison of different lengths of columns. (See Table 1 for chromatography data)

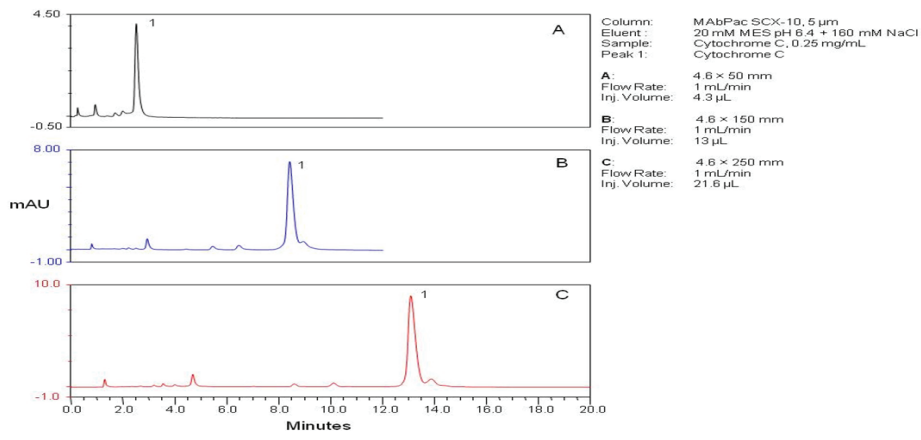


FIGURE 1: Isocratic testing of MAbPac SCX-10 RS, 5 µm, 4.6 mm columns; Comparison of different lengths of columns (From Figure 1).

	Column	Flow Rate (mL/min)	Pressure (psi)	RT (minutes)	Asymmetry (AIA)	Efficiency (plates)
A	MAbPac SCX-10, 5 µm, 4.6 × 50 mm	1.0	1,858	2.52	1.92	1,851
B	MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm	1.0	3,216	8.43	1.69	6,285
C	MAbPac SCX-10 RS, 5 µm, 4.6 × 250 mm	1.0	4,798	13.10	1.71	10,147

FIGURE 2. Isocratic testing of MAbPac SCX-10 RS, 5 µm, 2.1 mm columns; Comparison of different lengths of columns. (See Table 2 for chromatography data)

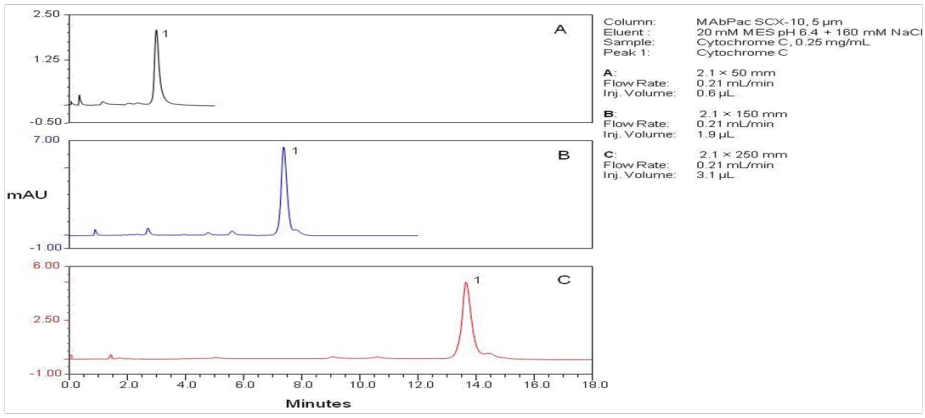
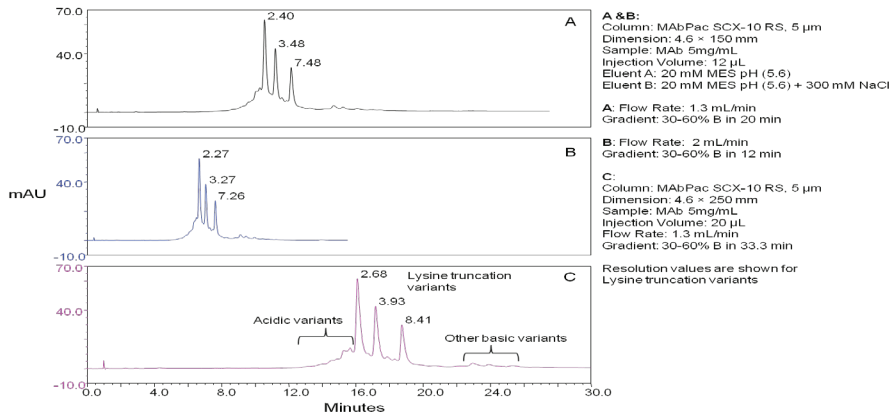


TABLE 2. Isocratic testing of MAbPac SCX-10 RS, 5 µm, 2.1 mm columns: Comparison of different lengths of columns. (From Figure 2).

	Column	Flow Rate (mL/min)	Pressure (psi)	RT (minutes)	Asymmetry (AIA)	Efficiency (plates)
A	MAbPac SCX-10RS, 5 µm, 2.1 × 50 mm	0.21	1,148	3.00	2.00	1,812
B	MAbPac SCX-10RS, 5 µm, 2.1 × 150 mm	0.21	2,360	7.38	1.33	6,436
C	MAbPac SCX-10RS, 5 µm, 2.1 × 250 mm	0.21	3,805	13.67	1.27	9,211

Gradient testing with monoclonal antibodies

FIGURE 3. MAb separation on MAbPac SCX-10 RS, 5 µm 4.6 × 150 and 4.6 × 250 mm; Resolution is maintained even at higher flow rates.



MAb Analysis on a 4.6 × 150 mm column at a flow rate of 2.0 mL/ min (12 minute gradient; Figure 3B) resolution values are slightly diminished as compared to 1.3 mL/mL flow rate (20 minute gradient; Figure 3A). Highest resolution for MAb analysis is achieved with 4.6 × 250 mm column Figure 3C).

FIGURE 4. MAb separation using either salt, or pH gradients

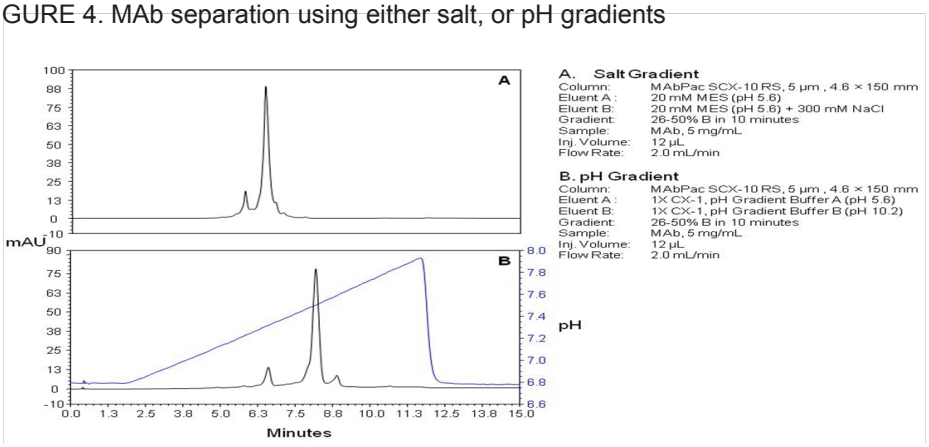
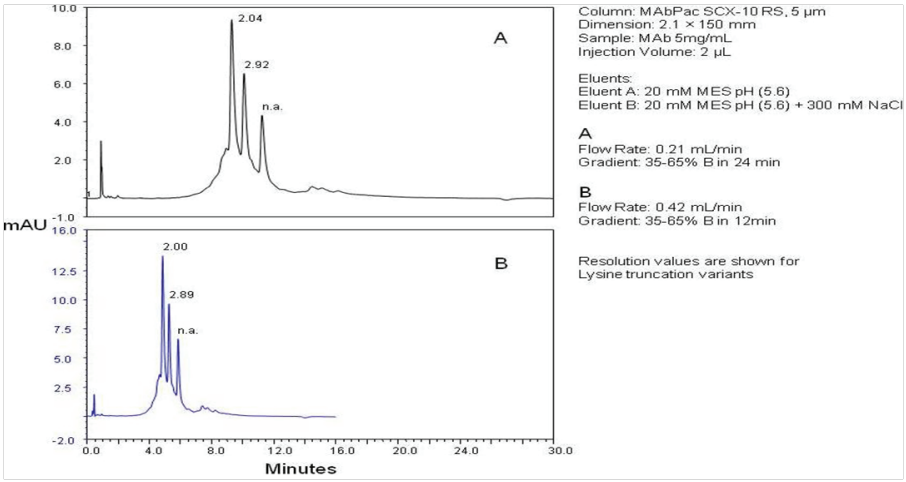
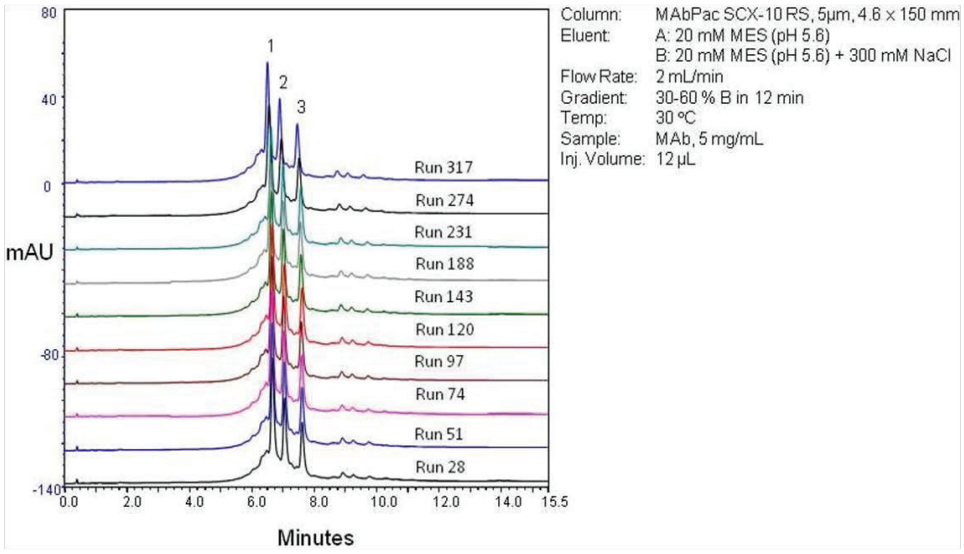


FIGURE 5. MAb separation on MAbPac SCX-10 RS, 5 µm, 2.1 × 150 mm column.



Two different flow rates and gradient conditions are used. Even at 0.42 mL/ min flow rate (Panel B), resolution values are comparable to 0.21 mL/flow rate (Panel A). At high flow rates analysis is faster and improves throughput.

FIGURE 6. Ruggedness testing of MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column.



MAb sample is injected intermittently. Peak width at half height (Minutes) is shown in; Table 3 for lysine truncation peaks 1,2 and 3. More than 300 runs are performed indicating that the column is quite rugged.

TABLE 3: Ruggedness testing of MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column.

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**

Summary

- A New UltiMate 3000 BioRS high pressure totally inert system was used. PEEK-lined stainless steel columns that are suitable for high pressure operations were used to avoid any metal related interferences with MAb/protein chromatography.
- MAbPac SCX-10 RS, 5 µm columns are developed in 2.1 mm and 4.6 mm I.D formats Three different length columns (50 mm,150 mm and 250 mm) are made available to offer various method development requirements. While both formats offer similar resolution and throughput, the larger I.D 4.6 mm columns are specifically useful for high sample loadability and the smaller I.D 2.1 mm columns are for conserving sample and eluent usage.
- A comparison of Isocratic separation of Cytochrome C on MAbPac SCX-10, 5 µm, 4.6 mm and 2.1 mm I.D columns with different lengths is shown in Figure 1 and Table 1 and Figure 2 and Table 2 respectively. As expected, the highest plate number for Cytochrome C separation is achieved with the longest column as compared to other shorter columns.
- Higher pressure compatibility of the column hardware allows the use of high flow rates 2 mL/min for 4.6 × 150 mm (Figure 3; Panel B); 0.42 mL/min for 2.1 × 150 mm (Figure 5; Panel B), while maintaining decent resolution. This results in faster analysis and improves throughput.
- MAb analysis is routinely performed by using either salt gradient, or pH gradient. pH gradient offers ease in method development process as well as better selectivity than salt gradients for a majority of MAbs (Figure 4; Panel B).
- Ruggedness of MAbPac SCX, 5 µm, 4.6 × 150 mm column for over 300 runs without any major changes in peak width at half height clearly supports the view that the column is quite rugged (Figure 6 and Table 3).

Conclusion

This study demonstrates successful development of MAbPac SCX-10 RS, 5 µm columns in different formats for high throughput and high resolution MAb analysis. This study also, shows successful usage of UltiMate 3000 BioRS high pressure inert system along with the PEEK lined stainless steel column hardware for high pressure separation applications.

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