Advantages of Small Particle High-Resolution Separation Media for Monoclonal Antibody Analysis

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Overview

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

Methods: High-resolution separation of a MAb is achieved with the new Thermo Scientific™ Dionex™ UltiMate™ 3000 BioRS Liquid Chromatography (LC) high-pressure inert system using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software.

Results: High-pressure, biocompatible column hardware was specifically developed to achieve high throughput, high-resolution MAb analysis. Small particle separation media were used with faster flow rate to achieve this objective.

Introduction

MAbs represent a major class of biotherapeutic molecules that usually display complex 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. Therefore, quality control and stability assessment of MAbs are very challenging tasks. The increasing utilization of MAbs in the biotechnology/pharmaceutical industry is 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 variants separation. However, there is a need in the industry to have analytical columns that combine uncompromised resolution power with high flow rate compatibility.

With the availability of totally biocompatible UltiMate 3000 BioRS high-pressure system with maximum pressure of 15000 psi, we have developed new formats of the 5 µm polymeric-particle columns that are suitable for high throughput, high-resolution MAb analysis. Biocompatible column hardware is a critical component for any MAb separation to avoid metal interferences with analytes of interest. Here, we utilize PEEK™-lined, stainless steel column bodies providing a totally metal-free fluidic path. These columns take advantage of smaller resin size as well as longer column length to maximize the resolution of MAb separation.

Methods

Samples

The MAb samples are received from a local biotech company. Cytochrome C (Equine) and other chemicals were from Sigma-Aldrich®.

Columns

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

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

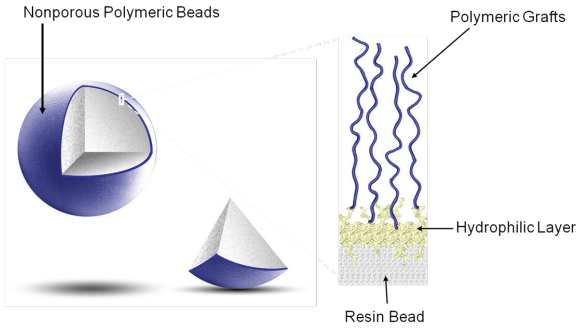
MAbPac SCX-10, 5 µm, 4 × 50 mm (PEEK), P/N 078656

High-Pressure LC (HPLC)

HPLC experiments were carried out using an UltiMate 3000 BioRS system equipped with:

- TCC-3000RS/SD Biocompatible Rapid Separation Thermostatted Column Compartment
- LPG-3400RS Biocompatible Binary Rapid Separation Pump. Flow rates of up to 8 mL/min. biocompatible materials
- Pressure of up to 1034 bar
- WPS-3000TBRS
- VWD-3400RS Rapid Separation Four Channel Variable Wavelength Detector (VWD) with micro flow cell
- Chromatography was controlled by Chromeleon Chromatography Data System software.

FIGURE 1. Separation media and mechanism of cation exchange column.



Mechanism of separation:

- Charge-charge interaction
- Based on ionic strength, or pH

Results

New MAbPac SCX columns development:

New MAbPac SCX columns are being developed. Isocratic and gradient tests were performed in the evaluation. MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm and 4.6 × 250 mm columns are packed in PEEK-lined stainless steel housings. These columns were compared with MAbPac SCX-10, 5 µm, 4.0 × 50 mm (2A). (See Figure 2 and Table 1). As expected , longer formats showed improved efficiency when compared with the shorter format.

FIGURE 2. Isocratic testing of different MAbPac SCX-10, 5 µm columns

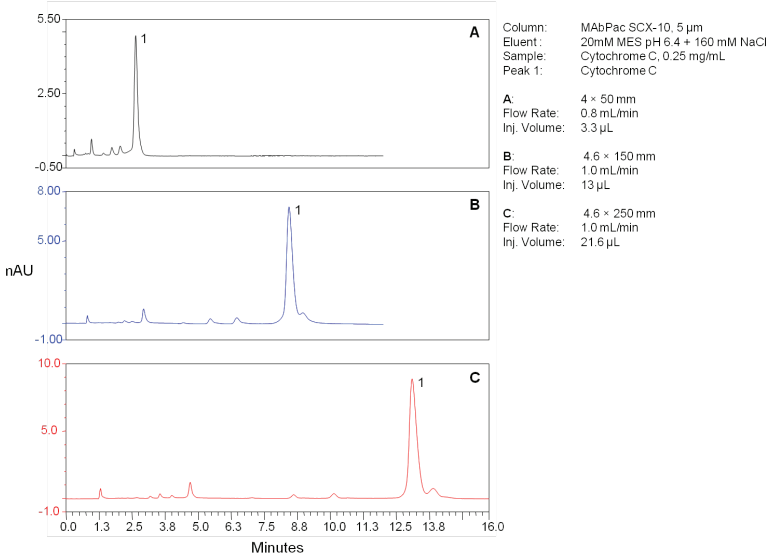
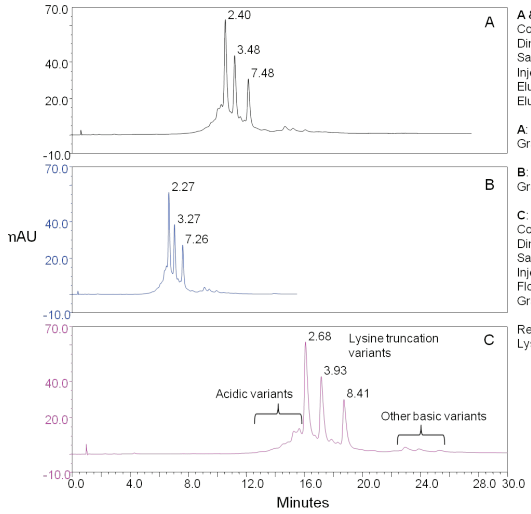


TABLE 1. Isocratic testing of different MAbPac SCX-10, 5 µm columns (See Figure 2 for chromatography details).

	Column	Flow Rate (mL/min)	Pressure (psi)	RT (minutes)	Asymmetry (AIA)	Efficiency (plates)
A	MAbPac SCX-10, 5 µm, 4 × 50 mm	0.8	1,261	2.64	1.61	2,202
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

Two monoclonal antibodies are evaluated with MAbPac SCX columns with salt gradient method (Figure 3 and 4 A) and with the newly developed pH gradient platform method (Figure 4B) . Figure 3 shows the evaluation of a MAb on 150 mm (A) as well as 250 mm length column (C). Resolution values for lysine truncation variants is shown. Also, a higher linear velocity (Flow rate 2 mL/min; Figure 3B) was used to achieve faster analysis and higher throughput. When compared to 150 mm length column, higher resolution separation was achieved with the 250 mm column. In addition, salt gradient and pH gradient was compared using 150 mm column (Figure 4). The pH Gradient was generated using the Thermo Scientific™ CX-1 pH Gradient Buffers. The pH gradient showed better resolution of charge variants (Figure 4).

FIGURE 3. Separation of MAb on MAbPac SCX-10 RS, 5 µm columns. Comparison of different dimensions of columns using salt gradients.



A &B:
Column: MAbPac SCX-10 RS, 5 µm
Dimension: 4.6 × 150 mm
Sample: MAb 5mg/mL
Injection Volume: 12 µL
Eluent A: 20 mM MES pH (5.6)
Eluent B: 20 mM MES pH (5.6) + 300 mM NaCl

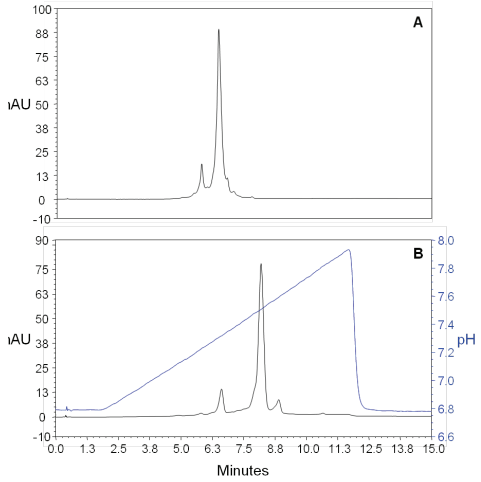
A: Flow Rate: 1.3 mL/min
Gradient: 30-60% B in 20 min

B: Flow Rate: 2 mL/min
Gradient: 30-60% B in 12 min

C:
Column: MAbPac SCX-10 RS, 5 µm
Dimension: 4.6 × 250 mm
Sample: MAb 5mg/mL
Injection Volume: 20 µL
Flow Rate: 1.3 mL/min
Gradient: 30-60% B in 33.3 min

Resolution values are shown for Lysine truncation variants

FIGURE 4. Separation Herceptin on MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm: Comparison of salt gradient and pH gradient.



Salt Gradient Separation

Column:	MAbPac SCX-10 RS, 5 µm 4.6 × 150 mm
Eluent A :	20 mM MES (pH 5.6)
Eluent B:	20 mM MES (pH 5.6) + 300 mM NaCl
Gradient:	26-50% B in 10 minutes
Sample:	Herceptin, 5 mg/mL
Inj. Volume:	12 µL
Flow Rate:	2.0 mL/min

pH Gradient Separation

Column:	MAbPac SCX-10 RS, 5 µm 4.6 × 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:	26-50% B in 10 minutes
Sample:	Herceptin, 5 mg/mL
Inj. Volume:	12 µL
Flow Rate:	2.0 mL/min

FIGURE 5. Ruggedness testing MAbPac SCX-10 RS, 5 µm, 4.6 x 150 mm column at flow rate of 2 mL/min. Two different MAb sample were injected intermittently and the ruggedness is assessed. Results of one of the MAbs is shown (See Table 2 for details).

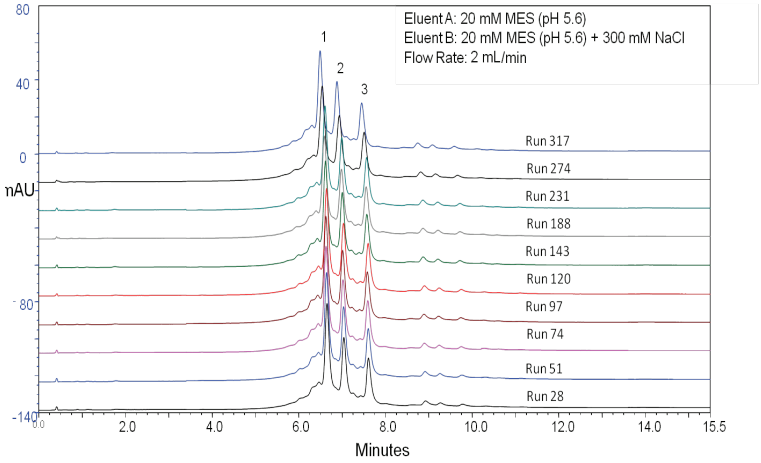


Table 2. Ruggedness testing of the MAbPac SCX-10 RS, 5 µm, 4.6 x 150 mm column (See Figure 5 for chromatography details). Peak width at half height (minutes) is shown for lysine truncation peaks 1, 2, and 3.

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

- High-pressure, biocompatible column hardware was specifically developed to achieve high throughput, high-resolution MAb analysis. Small particle separation media were used with faster flow rate to achieve this objective. A newly introduced UltiMate 3000 BioRS high-pressure inert system was used.
- PEEK-lined stainless steel columns were used to avoid any metal-related interferences with MAb/protein chromatography.
- PEEK-lined stainless steel columns are packed at higher pressure and therefore can withstand high backpressures when compared to the columns packed in PEEK. High flow rates could be used to achieve faster MAb analysis.
- Isocratic separation of cytochrome C on MAbPac SCX-10 RS, 5 µm columns showed expected increased efficiency as compared to the shorter 5 µm, 4 × 50 mm column (Figure 2 and Table 1). In these comparisons, the flow rate was adjusted according to the diameter of the column.
- MAbs separations are achieved using both salt gradients (Figure 3 and 4 A) and pH gradients (Figure 4 B).
- MAbPac SCX-10 RS, 5 µm 4.6 × 250 mm displayed highest resolution compared to other shorter lengths (Figure 3C). However, higher flow rates (2mL/min) on MAbPac SCX-10 RS, 5 µm 4.6 × 150 mm column is useful for high-throughput separation of MAbs (Figure 3 B and Figure 5).
- pH gradient gave better separation of acidic and basic variants of Herceptin as compared to salt gradients using MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm (Figure 4 B).
- The ruggedness of MAbPac SCX-10 RS, 5 µm, 4.6 × 150 mm column at 2 mL/min flow rate for over 300 runs showed no major changes in peak width measurements. This clearly demonstrates the ruggedness of the column (Figure 5 and Table 2).

Conclusion

This study demonstrates successful use of UltiMate™ 3000 BioRS high-pressure inert system along with the new PEEK-lined stainless steel column hardware for high-resolution, high-efficiency MAb/protein chromatography.

Acknowledgements

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