Advantages of High-Resolution Separation Media for Monoclonal Antibody Analysis

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Overview

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

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

Results: High-pressure, bioinert column hardware was specifically developed to achieve high-resolution MAb analysis. Longer columns with small particle separation media were used to achieve this objective.

Introduction

MAbs represent a major class of biotherapeutic molecules that usually display complex microheterogeneity 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 pharmaceutical industry is driving a growing demand for improved high-resolution stationary phases for characterization of MAbs.

Previously introduced Thermo Fisher™ 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 for high-resolution, high-throughput variant analysis on the same column. However, there is a need in the industry to have analytical columns that combine uncompromised resolution power with high flow rate compatibility.

With the launch of a new, totally bioinert UltiMate 3000 BioRS high-pressure system with maximum pressure of 15000 psi, we have developed a longer format of the 3 µm and 5 µm polymeric-particle columns that are suitable for high-resolution MAb analysis. Bioinert column hardware is a critical component for any MAb separation to avoid metal interferences with analytes of interest. In order to achieve this objective, we utilize a PEEK™-lined, stainless steel column bodies suitable for operation up to 1,000 bar, 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.

This work describes the development and applications of 3 µm and 5 µm small particle columns for high-resolution MAb analysis.

Methods

Samples

The MAb sample is a gift from a local biotech company. Cytochrome C (equine) and other chemicals were from Sigma-Aldrich®.

Columns

Prototype MAbPac SCX-10, 3 µm, 4.6 × 150 mm (PEEK-lined stainless steel)
Prototype: MAbPac SCX-10, 5 µm, 4.6 × 250 mm (PEEK-lined stainless steel)
MAbPac SCX-10, 3 µm, 4 × 50 mm (PEEK), P/N 077907
MAbPac SCX-10, 5 µm, 4 × 50 mm (PEEK), P/N 078656
MAbPac SCX-10, 10 µm, 4 × 250 mm (PEEK), P/N 074625

High-Pressure LC (HPLC)

HPLC experiments were carried out using an UltiMate 3000 BioRS system (Figure 1) equipped with:

- TCC-3000RS/SD Biocompatible Rapid Separation Thermostatted Column Compartment
- HPG-3400RS Biocompatible Binary Rapid Separation Pump
- WPS-3000TBRS Biocompatible Rapid Separation Wellplate Sampler, Thermostatted (in-line, split-loop)
- VWD-3400RS Rapid Separation Four Channel Variable Wavelength Detector with Flow Cell

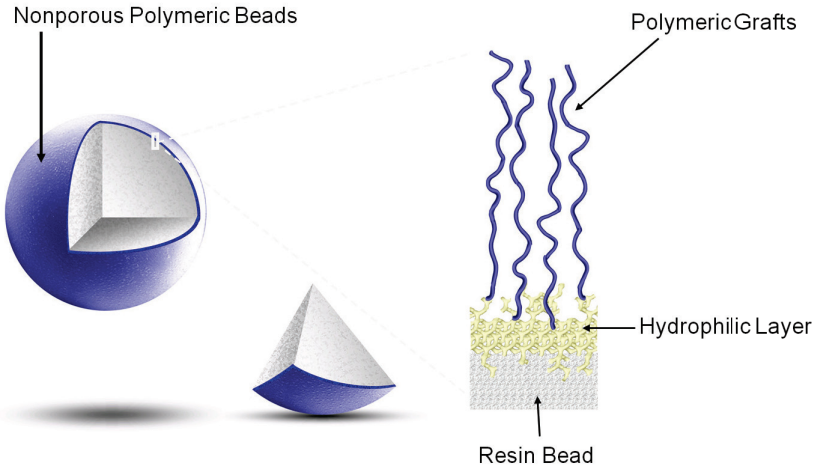
Chromatography was controlled by Chromeleon Chromatography Data System software.

FIGURE 1. The UltiMate 3000 BioRS high-pressure, bioinert system.

The UltiMate 3000 BioRS system delivers optimal separations at ultrahigh speed while maintaining resolution by combining:

- Bioinert materials
- Pressure of up to 1034 bar
- Flow rates of up to 8 mL/min
- Short sampler cycle times
- High column temperatures
- Ultrafast data collection and processing

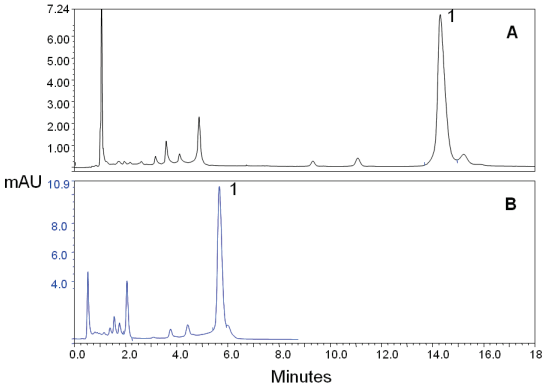
FIGURE 2. Separation media and mechanism of cation exchange column.



Mechanism of separation:

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

FIGURE 3. Isocratic testing of the prototype MAbPac SCX, 3 µm, 4.6 ×150 mm column and comparison with the MAbPac SCX, 3 µm, 4 ×50 mm column.



A
Column: MAbPac SCX, 3 µm prototype, 4.6 × 150 mm
Eluent: 20 mM MES pH 6.4 + 160 mM NaCl
Flow Rate: 0.66 mL/min
Inj. Volume: 10 µL
Sample: Cytochrome C 0.13 mg/mL

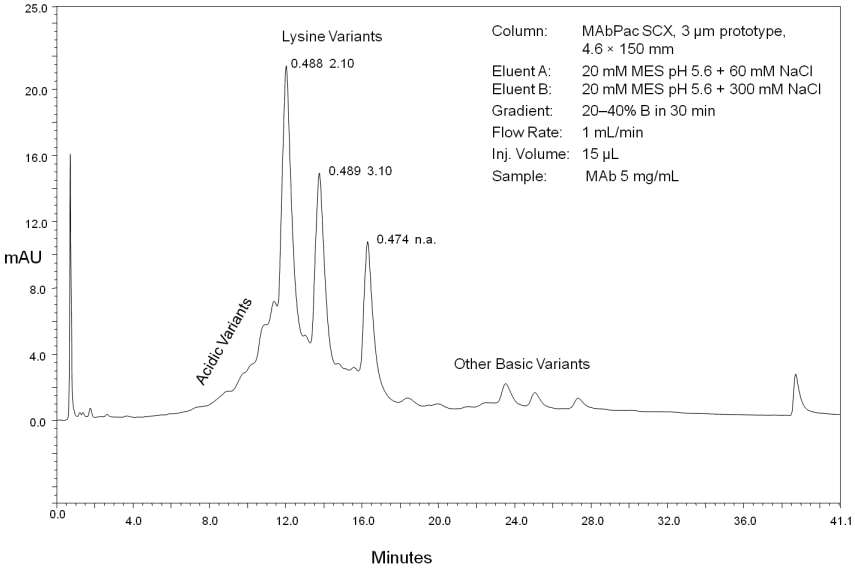
B
Column: MAbPac SCX, 3 µm, 4 × 50 mm
Eluent: 20 mM MES pH 6.4 + 160 mM NaCl
Flow Rate: 0.5 mL/min
Inj. Volume: 10 µL
Sample: Cytochrome C 0.13 mg/mL

Peak 1: Cytochrome C

TABLE 1. Comparison of prototype MAbPac SCX, 3 µm, 4.6 ×150 mm column with the MAbPac SCX, 3 µm, 4 ×50 mm column (see Figure 3 for chromatography details).

Column	Flow Rate (mL/min)	RT (min)	Asymmetry	Efficiency
A. MAbPac SCX, 3 µm prototype, 4.6 × 150 mm	0.66	14.3	1.22	13152
B. MAbPac SCX, 3 µm, 4 × 50 mm	0.5	5.67	1.21	5371

FIGURE 4. MAb separation on the prototype MAbPac SCX, 3 µm, 4.6 x 150 mm column. Peak width at half height (min) and peak resolution are shown for lysine truncation peaks, respectively.



Column: MAbPac SCX, 3 µm prototype, 4.6 × 150 mm
Eluent A: 20 mM MES pH 5.6 + 60 mM NaCl
Eluent B: 20 mM MES pH 5.6 + 300 mM NaCl
Gradient: 20–40% B in 30 min
Flow Rate: 1 mL/min
Inj. Volume: 15 µL
Sample: MAb 5 mg/mL

FIGURE 5. Isocratic testing of the prototype MAbPac SCX, 5 µm, 4.6 × 250 mm column and comparison with the MAbPac SCX 5 µm, 4 × 50 mm column.

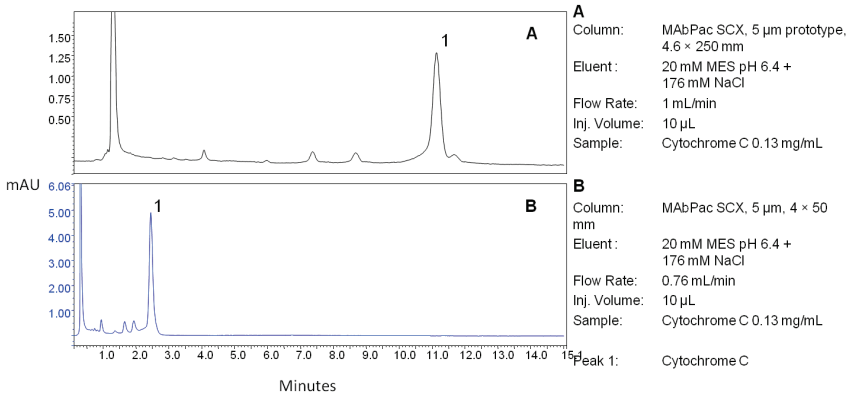


TABLE 2. Comparison of prototype MAbPac SCX, 5 µm, 4.6 ×250 mm column with the MAbPac SCX 5 µm, 4 × 50 mm column (see Figure 5 for chromatography details).

Column	Flow Rate (mL/min)	RT (min)	Asymmetry	Efficiency
A. MAbPac SCX, 5 µm prototype, 4.6 × 250 mm	1	11.14	1.02	9635
B. MAbPac SCX, 5 µm, 4.0 × 50 mm	0.76	2.45	1.21	2465

FIGURE 6. MAb separation comparison of the prototype MAbPac SCX, 5 µm, 4.6 ×250 mm column with the MAbPac SCX, 10 µm, 4 ×250 mm column. Peak resolution is shown for lysine truncation peaks. The same linear velocity and equivalent sample load were used.

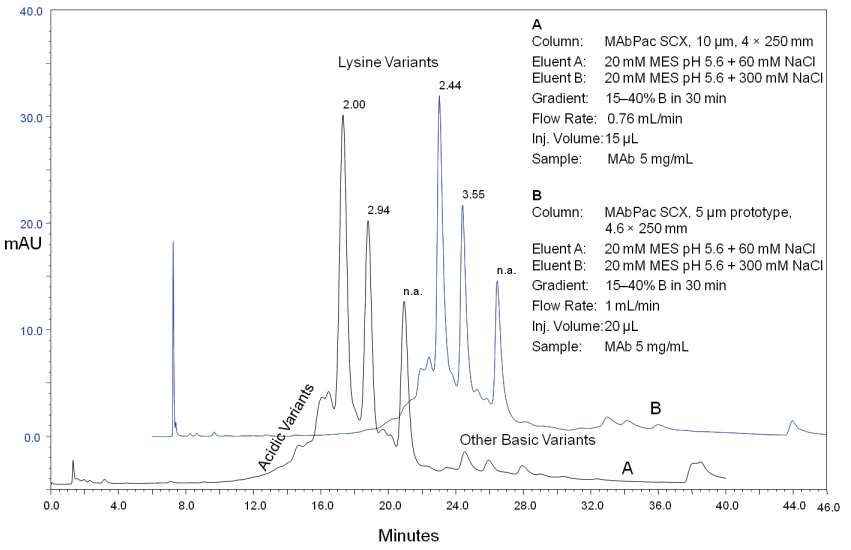


FIGURE 7. Ruggedness testing of the prototype MAbPac SCX, 5 µm, 4.6 x 250 mm column. The MAb sample was injected intermittently and the ruggedness assessed. Peak width at half height (min) is shown in Table 3 for lysine truncation peaks 1. 2. and 3.

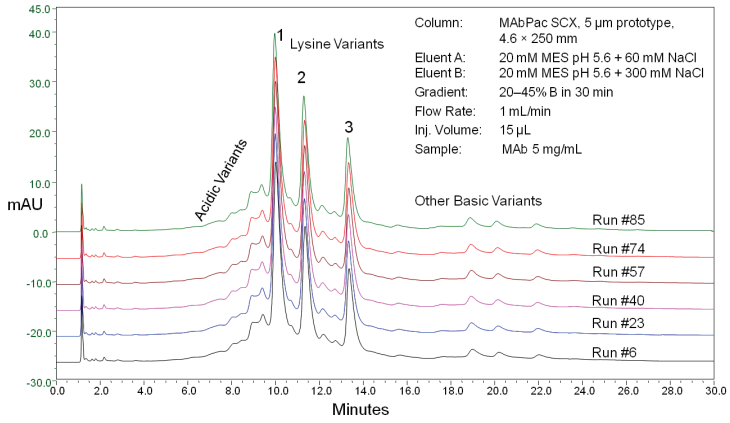


TABLE 3.

Run #	1	2	3
6	0.308	0.306	0.316
23	0.307	0.305	0.313
40	0.306	0.303	0.311
57	0.306	0.302	0.310
74	0.306	0.301	0.309
85	0.305	0.300	0.308

Results

- A new UltiMate 3000 BioRS high-pressure, totally inert system was developed. PEEK-lined stainless steel columns were used to avoid any metal-related interferences with MAb/protein chromatography.
- Isocratic separation of cytochrome c on the prototype MAbPac SCX-10, 3 µm, 4.6 × 150 mm column clearly shows better efficiency when compared to the shorter 3 µm, 4 × 50 mm column (Figure 3).
- Isocratic separation of cytochrome C on the prototype MAbPac SCX-10, 5 µm, 4.6 × 250 mm column showed several-fold increase in efficiency when compared to the shorter 5 µm, 4 × 50 mm column (Figure 5). In both of these comparisons, the same linear velocity was used.
- MAb separation at a 1 mL flow rate was possible on the new high-pressure HPLC system with the prototype MAbPac SCX-10, 3 µm, 4.6 × 150 mm column for faster analysis. At 1 mL flow, back pressure reached around 13000 Psi. An example separation of a MAb at 1 mL flow rate is shown in Figure 4.
- Improved chromatography with improved resolution was seen for MAb separations with the prototype MAbPac SCX-10, 5 µm, 4.6 × 250 column when compared to the MAbPac SCX-10,10 µm, 4 × 250 mm column (Figure 6). This improvement was due to decreased particle size.
- The ruggedness of the prototype MAbPac SCX, 5 µm, 4.6 × 250 mm column for over 85 runs without any major changes in peak width measurements. This clearly supports the view that the column is quite rugged (Figure 7 and Table 3).
- Currently, packing conditions are being optimized for both the 3 µm and 5 µm prototype columns to obtain increased efficiency and ruggedness for MAb separations.

Conclusion

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

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