

An Evaluation of YMC's Strong Cation-Exchange Stationary Phase for the Analysis of Charged Isoforms of a Commercially Available Antibody Drug

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Introduction

Ion-exchange chromatography (IEC) has seen an increased role in recent years for the analysis of charged isoforms in large molecule pharmaceutical and antibody drugs. During this time, weak cation-exchange (WCX) columns in 10µm particle size have been considered the gold standard for these types of analyses. This work details an analytical comparison between a strong cation-exchange resin versus a conventional WCX stationary phase for the analysis of a commercially available antibody drug.

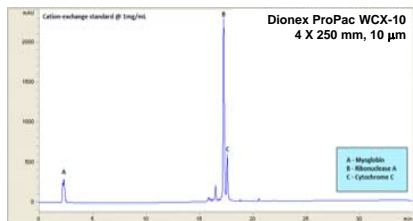
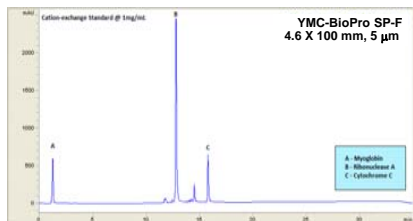
Results and Discussion

Since the 2008 introduction of analytical YMC BioPro series ion exchange columns, the BioPro SF (non-porous SP version) methacrylate based SCX ion exchange columns have allowed many scientists to improve the speed and resolution of separations of mAb isoforms. Thousands of runs of many different mAb's using a variety of mobile phase conditions (buffers, salt gradient conditions, etc.) have shown short length (30 - 100 mm), 5 µm non-porous BioPro SF columns to offer improved resolution with shorter run times than older larger particle non-porous polystyrene based WCX resins.

The BioPro family of ion exchangers is also available in scalable higher capacity porous 5, 30, and 75 µm particles in either packed columns or available for purchase as bulk materials offering the possibility of larger scale prep separations.

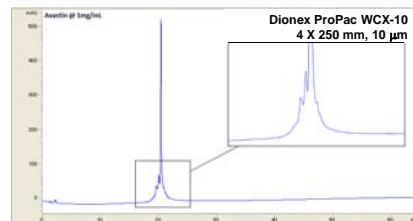
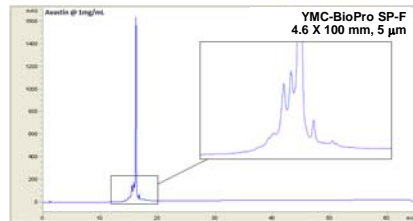
Avastin

Typical Chromatography of Protein Standard



Improved resolution on YMC BioPro SP-F column

Chromatography of Avastin Sample



Significant improvement in resolution for acidic and basic variants and approximate 20% improvement in throughput for BioPro SP-F column.

Experimental: Avastin & protein standard

Sample Preparation

Samples were made by diluting a 25mg/mL stock solution of Avastin (Genentech Corp.) to 1mg/mL using a 1X solution of phosphate buffered saline (PBS) as the diluent. Samples were mixed well and kept refrigerated (4°C) when not in use.

Mobile Phase:

The dial-a-mix method was used via quaternary pump. Mobile phases were as follows:

Mobile Phase A:	0.1M Sodium Phosphate Monobasic
Mobile Phase B:	0.1M Sodium Phosphate Dibasic
Mobile Phase C:	1.0M Sodium Chloride
Mobile Phase D:	100% Water

Antibody analyses (Avastin) were run using a pH=6.5 phosphate buffer. Analyses of a commercially available cation-exchange standard (BioRad) containing myoglobin, cytochrome C, and ribonuclease A were run using a pH=7.2 phosphate buffer. Both analyses used a linear salt gradient of 0-70% spanning 60 minutes.

All columns were equilibrated with a minimum 10 column volumes of mobile phase prior to 1st injection.

Instrument Parameters

HPLC System:	Agilent 1100
Flow rate:	0.5mL/min
Column Temperature:	25°C
Detection λ:	215 nm
Injection Volume:	10µL

ICX Columns:

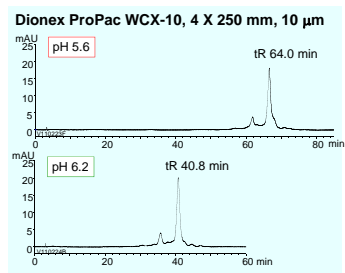
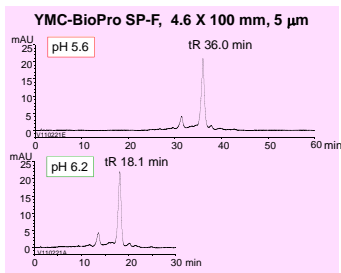
BioPro SP-F, 5µm	4.6 x 100 mm
Dionex WCX 10, 10 µm,	4.0 x 250 mm

Other experimental as noted under chromatograms

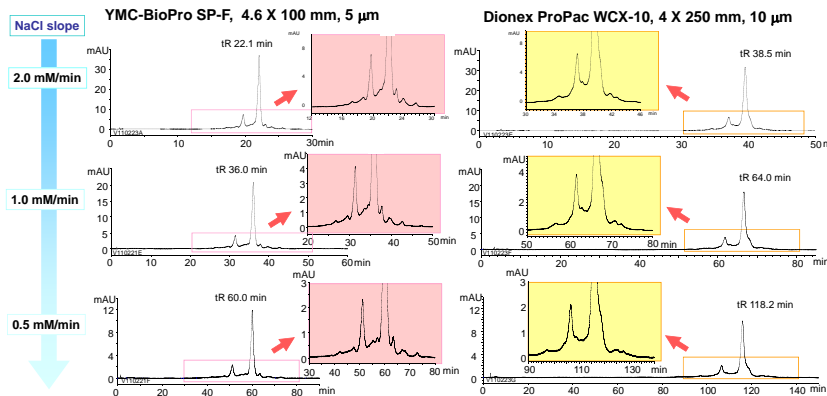
Humanized monoclonal IgG1

MAb analysis on non-porous type cation-exchange columns

Comparison of SCX (SP-F) and WCX under the different pH condition



Comparison of SCX (SP-F) and WCX under the same gradient condition



The separation of MAb is compared on SCX (YMC-BioPro SP-F) and WCX under the same gradient condition at pH 5.6. The lower NaCl slope results in better resolution of minor peaks of MAB. YMC-BioPro SP-F can achieve higher resolution of MAB than the WCX column under any condition.

Experimental: Humanized monoclonal IgG1

Eluent : A) 20 mM MES-NaOH (pH 5.6) B) 20 mM MES-NaOH (pH 5.6) containing 0.2 M NaCl
 Initial gradient conc.: 35% B (70 mM NaCl) Gradient slope: 1%B/min (2 mM NaCl/min), 0.5%B (1 mM NaCl/min), 0.25%B (0.5 mM NaCl/min)
 Flow rate: 180 cm/hr (0.5 mL/min for 100 X 4.6 mm I.D., 0.378 mL/min for 250 X 4.0 mm I.D.)
 Temperature: 30°C
 Detection: UV at 280 nm
 Sample : MAb (Humanized monoclonal IgG 1) (1 mg / mL)
 Injection: 10 µL

Conclusion:

YMC BioPro SP-F: gives superior resolution with higher throughput under all chromatographic conditions.