# 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 10um 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.

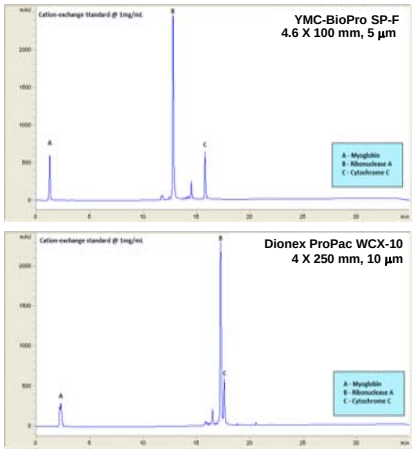
## Results and Discussion

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 um 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 um particles in either packed columns or available for purchase as bulk materials offering the possibility of larger scale prep separations.

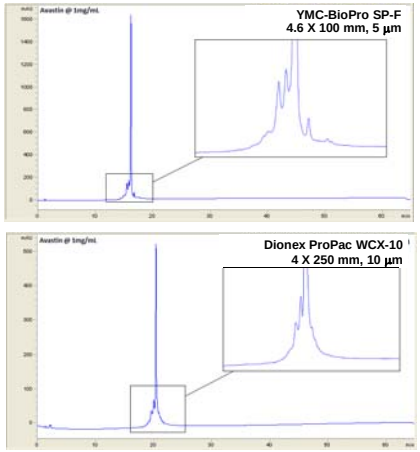
## Avastin

### 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 |

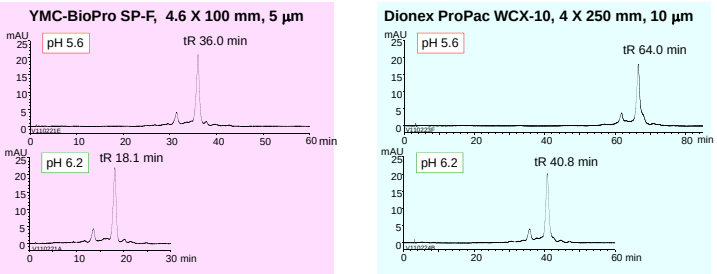
**IEX Columns:**

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

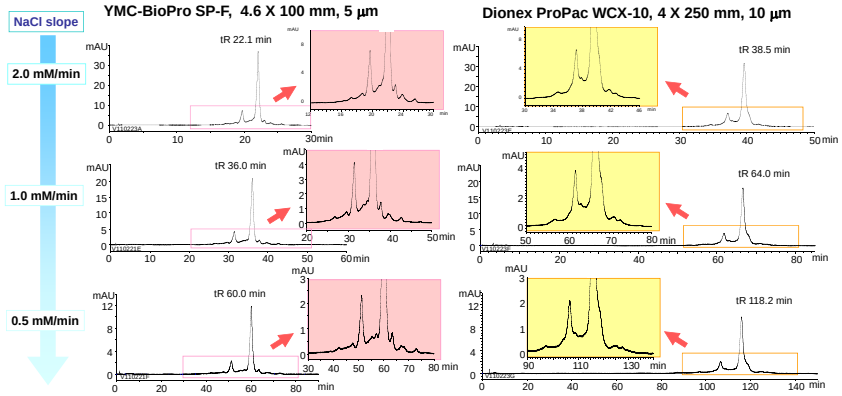
Other experimental as noted under chromatograms

## 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 mmI.D., 0.378 mL/min for 250 X 4.0 mmI.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.