Hydrophobic Interaction Process Scale Separation of Protein, MAb and Antibody Drug Conjugate

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INTRODUCTION

Hydrophobic interaction chromatographic (HIC) separation applies to all stages of the biological sample purification process, include capturing and polishing. Different forms of proteins, aggregates, and fragments can be isolated due to the hydrophobic interaction between the sample and the resin solid support. This hydrophobic interaction can be controlled by a number of factors such as protein structure, salt concentration, pH, temperature and organic solvent additives. HIC separation is a great addition to the native protein purification process including ion exchange, size exclusion, and affinity chromatography.

Generik & Polar MC-HIC resins are introduced for protein, mAb and antibody drug conjugate process scale purification. The polymeric resins are composed of a methacrylate polymer bead (also referred to as PMA) with high mechanical and chemical stability. The resins have mean particle size of 30 and 60 µm respectively, and pore size of 800 Å. On the resin surface, alkyl groups or aryl groups are attached via proprietary chemistry, so the resin selectively interacts with bio-molecules by utilizing the variations in hydrophobicity.

The characteristics for the 30 µm particle size are discussed for the Generik & Polar MC-HIC resins. Protein separation examples are presented for the resin applications. Sepax Generik MC30-HIC Butyl, Polar MC30-HIC Butyl and Polar MC30-HIC Ether resins provide excellent high efficiency and recovery separation of bio-molecules. Monoclonal antibody, ADC (antibody drug conjugate), DNA, and other oligonucleotides are all separated via the hydrophobic interaction. The HIC media also tolerates high-pressure operation up to 100 bars. All three phases are applicable at different stages from laboratory discovery, pilot-scale purification to industrial process chromatography.

EXPERIMENTAL

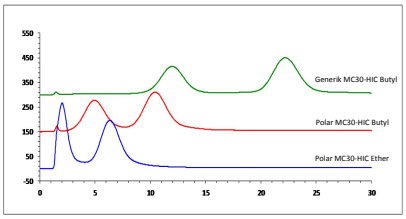
Media Type	Generik MC30-HIC Butyl Polar MC30-HIC Butyl Polar MC30-HIC Ether	
Packing	70% (v/v) slurry in 20% ethanol	
Matrix	Hydrophilic polymethacrylate	
Particle Size	20 ~ 45 µm (mean 30 µm)	
Pore Size	800 Å	
Dynamic Binding Capacity*	Generik MC30-HIC Butyl	45 ± 5 mg lys. / mL
	Polar MC30-HIC Butyl	35 ± 5 mg lys. / mL
	Polar MC30-HIC Ether	15 ± 5 mg lys. / mL
pH Stability	2-14	
Operating Temperature	Up to 40 °C	
Operating Pressure	Up to 100 bar	
Mobile Phase Compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, Tris, & acetate.	
Linear Flow Rate	Up to 1800 cm/hour	

ORDER INFORMATION

Product	PN#
Polar MC30-HIC Ether (30 µm, 800 Å, 4.6 x 50 mm)	258130-4605
Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)	258030-4605
Generik MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)	248030-4605

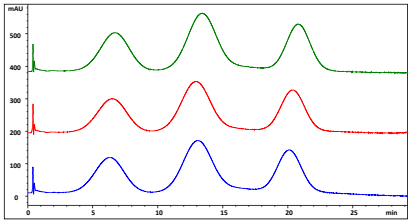
RIBONUCLEASE A AND LYSOZYME ANALYSIS ON GENERIK & POLAR MC30-HIC

Column: Polar MC30-HIC Ether (ID 10 mm, volume 5 mL)
Polar MC30-HIC Butyl (ID 10 mm, volume 5 mL)
Generik MC30-HIC Butyl (ID 10 mm, volume 5 mL)
Flow rate: 4 mL/min; Detection: UV 214 nm;
Mobile Phase A: 25 mM Phosphate + 2M $(NH_4)_2SO_4$, pH 7.0;
Mobile Phase B: 25 mM Phosphate pH 7.0;
Injection Volume: 100 µL; Gradient: 0-30 min 0-100%B;
Sample: ribonuclease A, lysozyme (5 mg/ml); Instrument: FPLC



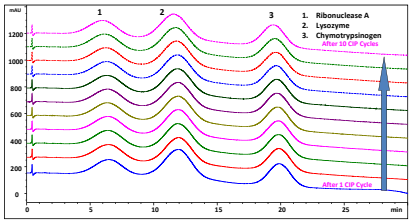
POLAR MC30-HIC BUTYL LOT TO LOT CONSISTENCY

Column : Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)
Flow rate: 1.5 mL/min; Detection: UV 214 nm;
Mobile Phase A: 25mM Phosphate + 2M $(NH_4)_2SO_4$, pH 7.0
Mobile Phase B: 25mM Phosphate pH 7.0;
Gradient: 0-30min 0-100%B; Injection Volume: 20 µL;
Sample: ribonuclease A, lysozyme, chymotrypsinogen (2 mg/ml)



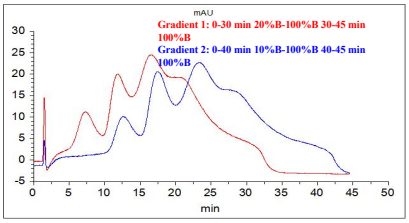
POLAR MC30-HIC BUTYL CIP TEST 10 CYCLES

Column: Polar MC30-HIC Butyl (30 µm, 800 Å, 4.6 x 50 mm)
CIP: 1 M NaOH, Flow rate: 0.2 mL/min, 15 min;
Wash: 25mM Phosphate + 2M $(NH_4)_2SO_4$, pH 7.0
Flow rate: 1.5 mL/min, 10min;
QC: Mobile Phase A: B + 2M $(NH_4)_2SO_4$, pH 7.0
Mobile Phase B: 25mM Phosphate pH 7.0
Gradient: 0-30min 0-100%B; Injection Volume: 20 µL
Sample: ribonuclease A, lysozyme, chymotrypsinogen (2 mg/ml);



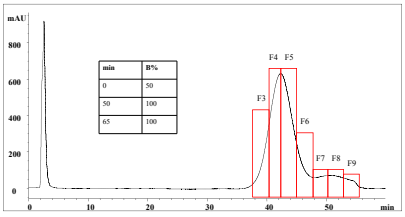
ADC ANALYSIS ON POLAR MC30-HIC

Column: Polar MC30-HIC Ether (ID 10 mm, volume 5 mL)
Flow rate: 4 mL/min; Detection: UV 214 nm;
Mobile Phase A: 25 mM Phosphate + 2M $(NH_4)_2SO_4$, pH 7.0
Mobile Phase B: 25 mM Phosphate pH 7.0;
Injection Volume: 80 µL;
Sample: ADC (6 mg/mL);
Instrument: FPLC
Pressure: 0.3-0.23 MPa



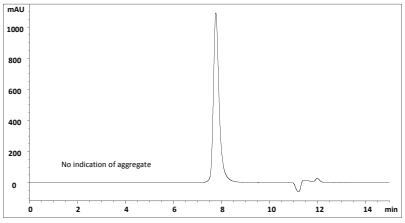
MAB321 ANALYSIS ON GENERIK MC30-HIC BUTYL

Column: Generic MC 30-HIC Butyl (1mL)
Start buffer (A): 20 mM Na_2HPO_4 , 1.7 M $(NH_4)_2SO_4$, pH 7.0
Elution buffer (B): 20 mM Na_2HPO_4 , pH 7.0
Flow rate: 0.4 mL/min, Gradient: 50-100% Elution buffer in 50 min,
Detector: UV 214 nm, Column temperature: Ambient
Injection volume: 50 µL,
Sample: mAb321 (5 mg/mL) in start buffer
Pressure: 13.7 bar, Instrument: HPLC



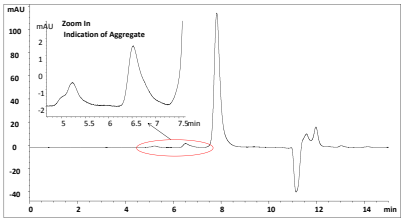
MAB321 FRACTIONS ANALYSIS ON ZENIX SEC-300

Column: Zenix SEC-300 (3 µm, 7.8 x 300 mm)
Mobile phase: 150 mM sodium phosphate buffer (pH7.0)
Flow rate: 1.0 mL/min, Detector: UV 214 nm,
Column temperature: Ambient
Injection volume: 100 µL, Sample: F3 - F5
Pressure: 94 bar, Instrument: HPLC



MAB321 FRACTIONS ANALYSIS ON ZENIX SEC-300

Column: Zenix SEC-300 (3 µm, 7.8 x 300 mm)
Mobile phase: 150 mM sodium phosphate buffer (pH7.0)
Flow rate: 1.0 mL/min, Detector: UV 214 nm,
Column temperature: Ambient
Injection volume: 100 µL, Sample: F7 - F9
Pressure: 94 bar, Instrument: HPLC



CONCLUSION

- Sepax Generik & Polar MC-HIC resins offer stable and consistent performance.
- Sepax Generik & Polar MC-HIC resins can tolerate high pressure, which allows faster flow rate and shorter running time.
- Sepax Generik & Polar MC-HIC resins provide excellent high recovery and resolution in separating proteins.
- Sepax Generik & Polar MC-HIC resins offer a potential solution for mAb separation and ADC purification.