Praesto Jetted A50 HipH Resin Applications for Bispecific Antibody Purification

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Abstract:

Bispecific antibodies (bsAbs) can become unstable under typical Protein-A elution conditions (pH 3—3.5), causing aggregation and fragmentation. Novel Purolite Praesto Jetted A50 HipH resin is designed to elute antibodies in a higher pH range (up to pH 5). Two bsAb molecules were evaluated by Praesto Jetted A50 HipH and a Standard Protein A resin to maximize monomer purity. Praesto Jetted A50 HipH resin demonstrated increased separation and monomeric recovery when compared to the standard resin.

Keywords: Bispecific Antibody, Affinity Chromatography, Gradient Elution, CHO

Methods

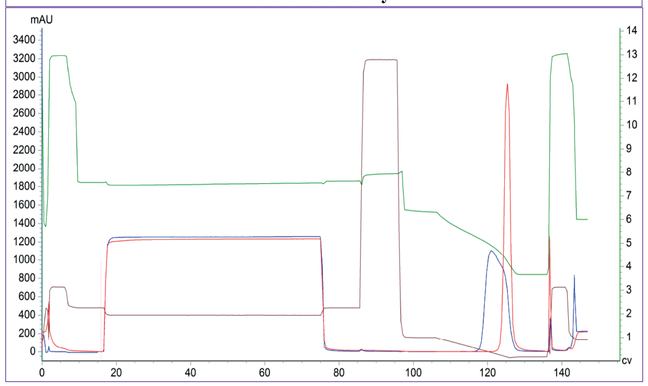
Two bsAbs produced in CHO cells, designated bsAb X and bsAb Y, were evaluated across equivalent columns (0.8 x 10 cm, 5 mL) of Praesto Jetted A50 HipH and a Standard Pro A resin at a load density of 20 mg antibody/mL packed bed resin. Runs were executed on an ÄKTA Avant 25.

Protein-A Affinity Method		
Step	CVs	Buffer/Solution
Regen	5	0.1N NaOH
Equil	10	PBS, pH 7.4
Load	—	HCCF
Wash 1	10	PBS, pH 7.4
Wash 2	10	pH 7.5, σ ~ 90 mS/cm
Wash 3	10	pH 5.8
Linear Gradient Elution	20 (0-100%)	pH 5.8 → pH 3.3
Regen	5	0.1N NaOH
Storage	7	2% BnOH

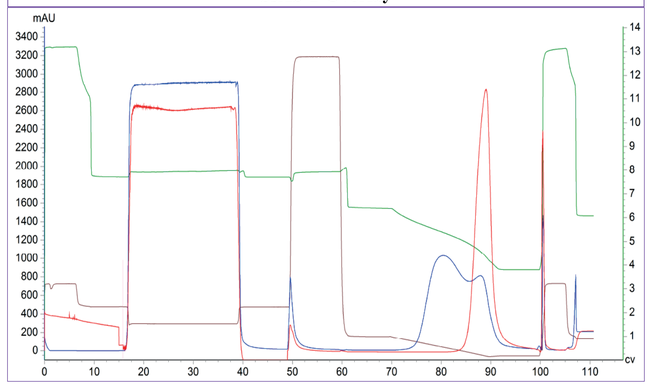
Visual separation of species is apparent in the UV trace for both bsAbs by Praesto Jetted A50 HipH resin.

No visual separation of species is observed in the Standard Pro A resin UV trace.

bsAb X Overlay



bsAb Y Overlay



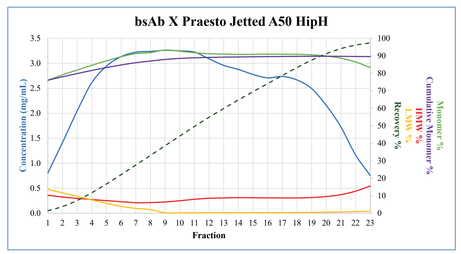
Legend

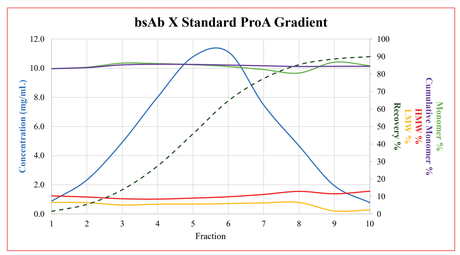
UV, Praesto Jetted A50 HipH Protein A
UV, Standard Protein A
pH
Conductivity (mS/cm)

Data Analysis

Fractions were collected into 96-deep well plate at 1/3 CV increments and analyzed for concentration and SEC.

bsAb X

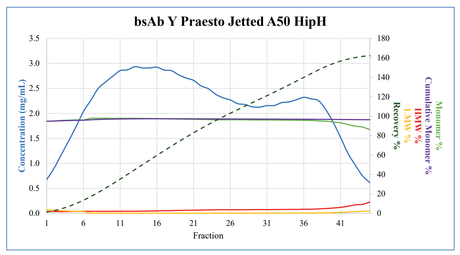


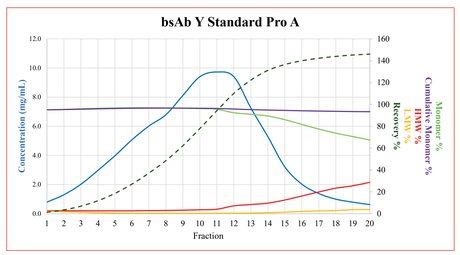


Praesto Jetted A50 HipH
- Overall cumulative monomer is ~90%
  - Fraction 9 had highest monomer at ~93%
- Most LMW species eluted first
- HMW % increased towards end of gradient

Standard Pro A
- Overall cumulative monomer is ~84%
  - No fraction was above 87% purity
- All species co-elute at consistent ratios

bsAb Y





Praesto Jetted A50 HipH
- Overall cumulative monomer is ~96%
- Slight increase in elution of HMW species at a more acidic pH
- More fractions, greater separation

Standard Pro A
- Overall cumulative monomer is ~89%
- HMW species co-eluted with other species
- Indicates formation of HMW with decreasing pH

- Recovery was unusually high for bsAb Y
  - Likely a higher titer than reported

Conclusion:

When processing bsAbs, undesirable species are frequently observed in correlation with lower pH elution buffers. Protein-A elution at higher pH improves recovery of monomer and decreases aggregation and fragmentation.