Advances in Mixed Mode Chromatography: HCPure™ host cell protein clearance resin case studies

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Abstract

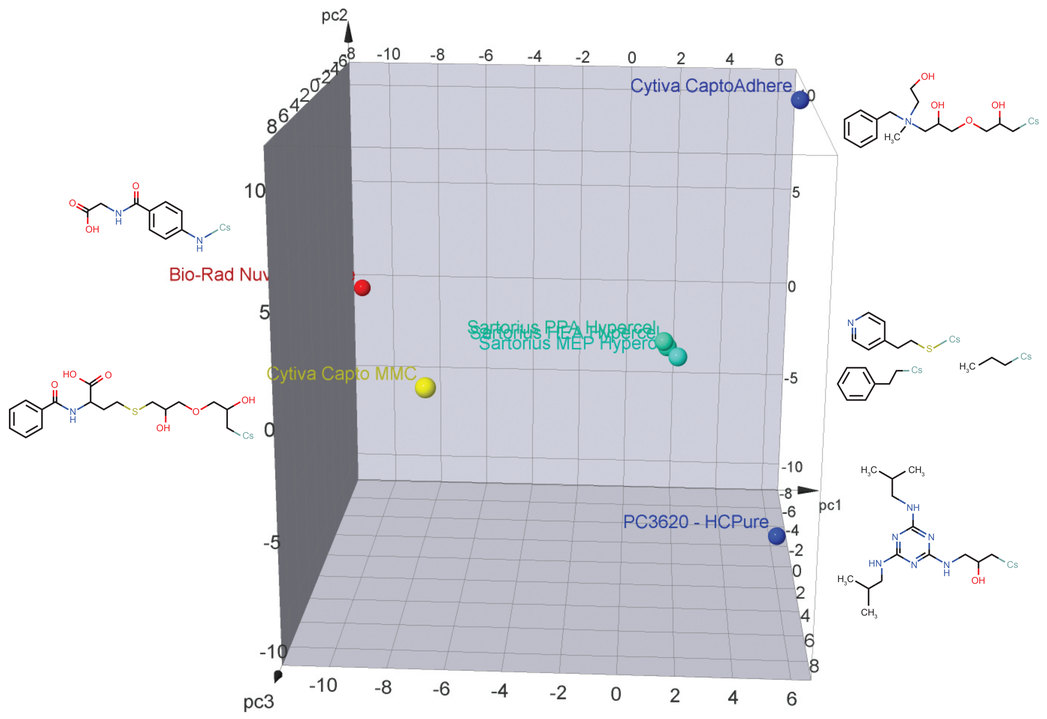
Removal of host cell proteins (HCP) and final product purity are critical to effective therapeutic outcomes. A range of negative outcomes can occur as a consequence of failing to effectively clear the final sample of HCPs, from changes in therapeutic effectiveness to immunogenic responses in the patient.

Multistep purification processes are typically followed to ensure HCPs are removed. Mixed-mode chromatography can be used to streamline the polishing process by utilizing both ionic exchange and hydrophobic interaction binding methods, resulting in significant savings in processing time, and a reduction in buffer and materials used.

HCPure™ host cell protein clearance resin from Astrea Bioseparations and its affiliates is a mixed-mode chromatography resin, designed for the removal of host cell proteins, host cell DNA, and high molecular weight aggregates.

Here we demonstrate that the unique binding profile allows for two key advantages: utilization of mixed-mode to create a highly tuneable purification platform for a variety of conditions, and the ability to purify feed streams that other resins can struggle to effectively clean.

1 Differences in structures lead to differences in binding interactions



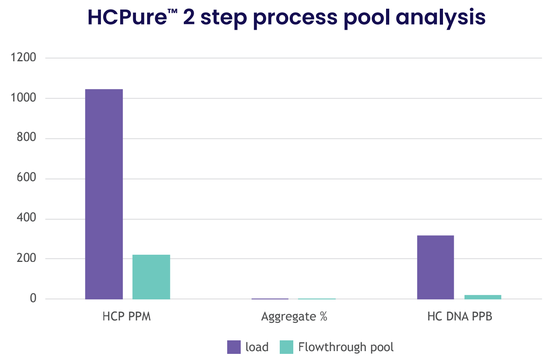
Fingerprint based comparison of several commercially available mixed mode chromatography resins, demonstrating the differences in ligand structures and the impact they will have on binding interactions with target molecules.

HCPure™ performance in IgG producing CHO feedstocks

2 Case Study 1: Removal of HCP and HCDNA from CHO feedstock

Polishing using HCPure™ as flow through step post Protein A in a 1 ml prepacked column with 2.5 cm bed height.

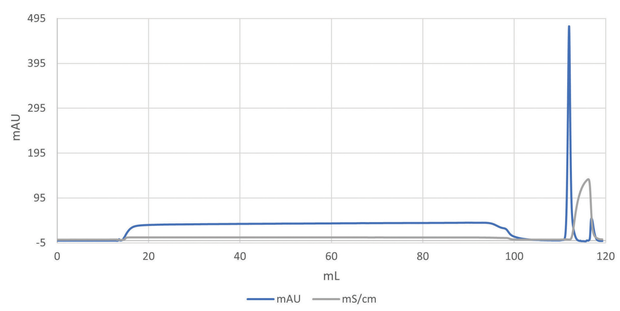
Fraction	HCP (PPM)	Aggregate (%)	HCDNA (PPB)
LOAD	1,049	0.93	319
FLOWTHROUGH	222	0.62	22



3 Increasing challenge of aggregates in CHO feed

Fraction	Aggregate (%)
LOAD	6.7
FLOWTHROUGH	2.9

After primary capture, samples were purified by CIEX, before being held at low pH and high salt to induce aggregation. Samples were then passed through HCPure™; the resulting mAb aggregates were reduced by nearly 60%



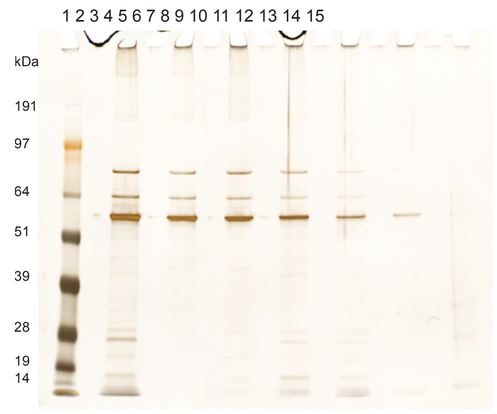
HCPure™ performance in AAV producing *HEK* feedstock

4 Repurification of AAV2 Flow Through fractions

AAV 2 from transient transfection of HEK 293 cells contained some impurities after Affinity capture using a commercially available pan serotype ligand.

The elution pool was conditioned to pH 7.2 and 350mM NaCl and a load of 5x1013 VP was passed in flowthrough mode through the HCPure™ packed in a 10cm bed height 10mm SNAP column.

Recovery of AAV 2 by ELISA was 100% with 0.9% detected in the low pH and high salt strip



Samples

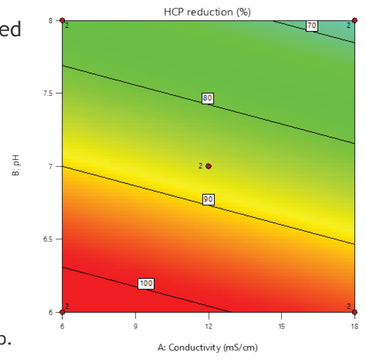
1. MW marker
2. -
3. AAV2 Load (HCPure)
4. -
5. FT1
6. -
7. FT2
8. -
9. FT3
10. -
11. FT4
12. -
13. FT5
14. -
15. Eluate

HCPure™ performance in Vκ producing *E.coli* feedstock

5 Optimization of binding conditions in *E.coli* feedstocks

Binding was performed in 50 mM Tris-citrate buffer. Elution pH was varied between pH 6.0 and pH 8.0 using conductivities of 6, 12 & 18 mS/cm.

The highest reduction of HCP was obtained at low pH and low conductivity.



Results of the condition screening of HCPure™ in E. coli feedstocks producing Vκ. Conditional screenings were conducted in 96-well plates across a range of pH and conductivities. Results showing greatest reduction in HCP were plotted on a heat map.

6 Demonstration of HCP clearance and target yield under optimized conditions

A greater than 2-log reduction in HCP concentration was seen when using HCPure™, from over 30,000 ppm in the load to approximately 200 ppm in the non-bound fraction.

Vκ recovery in the non-bound fraction was greater than 80%, indicating little loss due to binding of the target Vκ.

Fraction	HCP (ppm)	% RSD	SD	Log Clearance (from Protein L)	% PPM Reduction	HCP bound (µg)	Vκ bound (mg)	% HCP bound	% Vκ bound	% Vκ yield
Load (pH 6, 6 mS/cm)	36,283	26.2	9499	2.3	99.4	90.3	0.1	99.5	4.8	95
A1	203	11.0	22							
Load (pH 6, 6 mS/cm)	36,283	26.2	9499	2.2	99.3	90.3	0.4	99.4	15.4	85
A5	255	17.5	45							

Summary

HCPure™ from Astrea Bioseparations is a mixed mode adsorbent that can serve an effective polishing step in a wide variety of different feedstocks. For those customers manufacturing mAbs, HCPure™ has been show to reduce HCP, HCDNA and aggregates in a variety of feedstocks including CHO and E. coli. For customers performing cutting edge cell and gene therapies, HCPure™ has also been shown to remove HCP while achieving high yields of AAV in HEK feed stocks.

This ability to achieve a pure end product from a wide variety of feedstocks, allows HCPure™ to be the basis for a tunable purification platform.

Target	Expression System: CHO	*E.coli*	HEK
HCP	X	X	X
HCDNA	X		X
Aggregates	X		
IgG	X		
Vκ		X	
AAV			X

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