Astrea Bioseparations

Technical Note

HCPURE™: A FIT FOR PURPOSE HOST CELL PROTEIN CLEARANCE SOLUTION IN *E. COLI* WORKFLOWS

Introduction

Monoclonal antibody (mAb) pharmaceutical products are one of the fastest growing segments of biologic therapeutics available, with over 100 different mAbs approved by the FDA today. These mAb biologics have been released for a wide variety of conditions including cancer treatments, rheumatology, hematology and infectious diseases. As the global population ages, and chronic illnesses become more prevalent, these mAbs and processes to develop them will only become more necessary.

Removal of host cell proteins and final product purity are critical to effective therapeutic outcomes. By failing to effectively clear the final sample of HCPs, a range of negative outcomes can occur, from changes in therapeutic effectiveness to immunogenic responses in the patient. Typically, purification is a multistep process to ensure all host cell proteins are removed. However, mixed mode chromatography can streamline the polishing process by utilizing both ionic exchange and hydrophobic interaction binding methods. This process compression can result in significant savings in both processing time and buffer and material reduction.

HCPure™ host cell protein clearance resin from Astrea Bioseparations Ltd and its affiliates (Astrea Bioseparations) is a mixed mode chromatography resin, designed for the removal of host cell proteins, host cell DNA, and high molecular weight aggregates. The unique molecular structure of the HCPure™ ligand utilises hydrophobic interaction as a primary capture mechanism and ionic exchange as a secondary. This unique binding profile allows for two key advantages: utilization of mixed mode as a single purification step to create a highly tunable purification platform for a variety of conditions, and the ability to purify feedstreams that other resins, which are primarily ion exchangers, can struggle to effectively clean up.

This application note describes both the binding properties of HCPure™ ligand as well as demonstrating that HCPure™ can act as an effective single polishing step when purifying Vκ in *E. coli* feedstocks.

Materials and Methods

Starting Materials & Primary Capture

In order to characterize the binding efficiencies and behaviors of HCPure™, feedstocks from E. coli containing Vκ were generated. These feedstocks were then passed through a column containing Protein L affinity resin as part of the primary capture step.

The eluate from this protein was adjusted to pH 8 to provide a feedstock load solution with a Vκ concentration of 2.42 mg/mL.

Polish Condition Optimization

A key step in effectively removing as much host cell protein as possible, is to determine the optimum conditions during the polishing step, including both pH and conductivity. To find the process conditions which remove the most contaminating host cell proteins, a screening assay was set up evaluating three different conductivities, 6, 12 & 18 mS/cm, at pH's ranging from 6 to 8.

Two conditions were measured, Vκ yield as % and remaining E. coli HCP as ppm in the flow through.

To measure these parameters, a 96 well micro column plate containing 0.25 mL per column of the Protein L elution fraction, Vκ enriched E. coli periplasmic extract, was equilibrated by flushing the columns with 3 x 1.0 mL aliquots of 50 mM Tris-citrate buffer (10 mM citric acid, 40 mM Tris base) at different pH's and conductivities in duplicate.

	1	2	3	4	5	6	7	8	9	10
A	pH 6 6 mS	pH 6 18 mS	pH 8 18 mS	pH 7 12 mS	pH 6 6 mS	pH 8 6 mS	pH 6 18 mS	pH 8 6 mS	pH 7 12 mS	pH 8 18 mS

Figure 1 – pH and conductivity conditions in 96 well plate

The columns were loaded with E. coli Vκ feed (2.5 mg Vκ per column). The columns were then treated with two post load washes consisting of 2 x 0.75 mL aliquots of equilibration buffer. The flow through from the load and the first post load wash were collected and pooled to give a non-bound fraction. Following post load wash, the columns were treated with 50 mM sodium citrate, pH 3.0 elution buffer and the elution fractions collected. As the resin works in flowthrough mode, only the non-bound fractions were analyzed by SDS-PAGE.

Determination of Binding Capacity

In addition to determining the optimum conditions for the polishing process, it is also necessary to ensure that the HCPure™ host cell protein clearance resin is fit for purpose in a variety of incoming concentrations, including challengingly high concentrations.

Initial samples with concentrations of over 30,000ppm of HCP were prepared and then passed through a column containing HCPure™ resin, after which both HCP concentration and % recovery of Vκ were measured.

HCP concentration in the load and non-bound sample was determined using a *E. coli* HCP ELISA kit from Cygnus Technologies.

The concentration of Vκ in the load and non-bound fractions was determined by UV-Visible spectrophotometry, measuring the absorbance of the solutions at 280 nm.

Results and Discussion

Optimal Process Conditions

Measurement of HCP removal using different elution conditions was performed in 96-well filter plates as described in Materials and Methods. Binding was performed in 50 mM Tris-citrate buffer. Elution pH was varied between 6.0 and 8.0 using conductivities of 6, 12 & 18 mS/cm. The result, **Figure 2**, shows that the highest HCP removal was obtained at low pH and low conductivity.

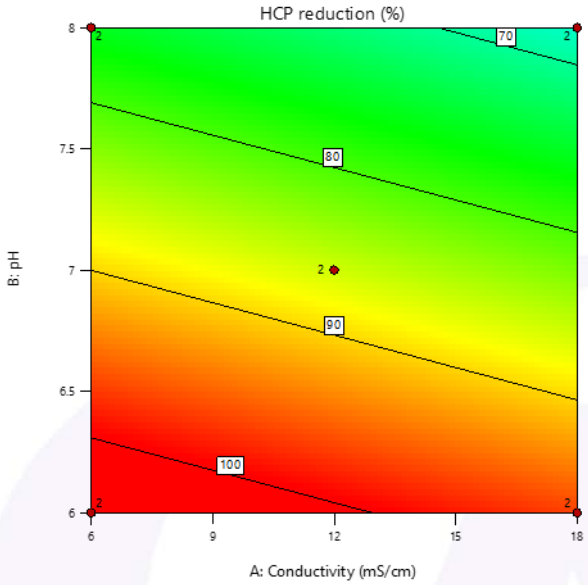

Figure 2 – Highest removal of HCP is at low pH and low conductivity (pH 6 & 6 mS/cm)

Binding Capacity

HCPure™ is designed to not only simplify the polishing process after primary capture, but also to serve as a platform that can accommodate a range of incoming HCP concentrations while still achieving high target yields. To ensure that the HCPure™ host cell protein clearance resin did not become saturated and allow contaminants to pass through and into the final product, the initial concentration levels of HCPs in the sample were far higher than what would be expected in a typical sample.

After analysis, this demonstrated a reduction in HCP concentration from over 30,000 ppm in the load to approximately 200 ppm in the non-bound fraction. This reduction of HCPs represents a greater than a 2 log clearance.

The concentration of Vκ in the non-bound fraction compared to the load concentration was greater than 80% indicating little loss due to binding of the target Vκ.

		HCP (*E. coli* ELISA)			Vκ (A280)												
Fraction	Fraction volume (mL)	Avg conc. (µg/mL)	% RSD	Mass (ng)	Conc (mg/mL)	% RSD	Mass (mg)	HCP (ppm)	% RSD	SD	Log Clearance (from Capto L)	% PPM Reduction	HCP bound (µg)	Vκ bound (mg)	% HCP bound	% Vκ bound	% Vκ yield
Load (pH 6, 6 mS/cm)	2.91	31.20	26.18	90802	0.86	0.20	2.50	36283	26.18	9499	2.25	99.44	90.32	0.12	99.47	4.84	95.16
A1	4.41	0.11	10.96	482	0.54	0.43	2.38	203	10.97	22							
Load (pH 6, 6 mS/cm)	2.91	31.20	26.18	90802	0.86	0.20	2.50	36283	26.18	9499	2.15	99.30	90.26	0.39	99.41	15.42	84.58
A5	4.41	0.12	17.46	539	0.48	0.72	2.12	255	17.48	45							

Table 1 – Removal of HCP and V_k yield

Conclusion

As demonstrated in this application note, the mixed-mode HCPure™ clearance adsorbent from Astrea Bioseparations utilizes multiple binding methods to remove HCPs while maintaining high target yields. HCPure™ does not require harsh conditions, (e.g. high salt or extremes of pH), to effectively remove HCP from samples, simplifying the process of removing HCP without having to significantly adjust sample conditions between each step in the process.

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