Astrea Bioseparations

Technical Note

HCPURE™: HOST CELL IMPURITY REMOVAL IN CHO WORKFLOWS

Introduction

Monoclonal antibody (mAb) pharmaceutical products are one of the fastest growing segments of biologic therapeutics available, with over 100 different mAb products approved by the FDA to date. Since mAbs are safer and more precise than chemotherapy treatments, more selective than antibiotics, and can be an effective treatment when vaccination is not possible, they have become a critically important biologic therapeutic. As the global population ages and chronic illnesses become more prevalent, these mAbs and processes to develop them will only become more necessary.

Almost all therapeutic antibodies are produced in mammalian cell lines to reduce the risk of immunogenicity, however further purification steps are required before the antibodies can be administered. Typically, purification is a multistep process to ensure all host cell proteins are removed. Polishing steps may be carried out in bind/elute or flow through mode to remove any remaining process-related impurities such as host cell proteins and residual host cell DNA, or product-related impurities such as high molecular weight aggregates. Mixed mode chromatography can streamline the polishing process by utilizing both ionic exchange and hydrophobic interaction binding methods to remove contamination in a single step. 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 tuneable 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 technical note describes the binding properties of HCPure™ as well as demonstrating that HCPure™ can act as an effective polisher when purifying IgG in CHO feedstocks.

Materials and Methods

Starting Materials & Primary Capture

To characterize the performance of HCPure™ in a typical feedstock and workflow, CHO cells were used to prepare feedstocks expressing two different forms of IgG. Protein A affinity resin was used as the primary capture step. Protein A eluate was used in both single and multistep polishing processes to determine the efficacy of HCPure™ in removing HCP, HCDNA and aggregates while maintaining high IgG yields.

Concentrations of HCP, HCDNA and aggregates were determined using ELISA, Picogreen assay, and SEC, respectively, shown in Table 1 below.

Feed	mAb post titration titre (mg/mL)	HCP (PPM)	Aggregate %	HCDNA (PPB)
mAb A	2.34	1,863	0.96	3,278
mAb B	3.06	40,924	0.05	1,663

Table 1 - Concentrations of target mAbs, HCP, aggregates and HCDNA post-Protein A

Using HCPure™ as a Single Step Polish to remove HCP and HCDNA

HCPure™ allows for increased separation power and selectivity by utilizing both hydrophobic and hydrogen bonding interactions simultaneously. Unlike many mixed mode resins available, the primary mode of interaction with HCPure™ is through hydrophobic interaction with hydrogen bonding being the secondary interaction. This puts HCPure™ at an advantage over other mixed mode resins and traditional ion exchangers because it retains functionality at relatively high conductivity load conditions compared to ion exchange polishing steps which would require dilution.

Once the starting concentrations had been determined, the Protein A eluate was loaded onto HCPure™ in flow through mode at a of pH 8.0 and a low conductivity of 3 mS/cm. Fractions collected in the flow through pool were measured for HCP and HCDNA.

HCP concentrations were determined via ELISA, and host cell DNA was measured using a Picogreen assay kit. Results showed a 79% reduction in HCP as well as a 93% reduction in HCDNA.

Fraction	HCP PPM	HCDNA PPB
Load	1,049	319
Flowthrough pool	222	22

Table 2 - Concentrations of HCP and HCDNA before and after HCPure™

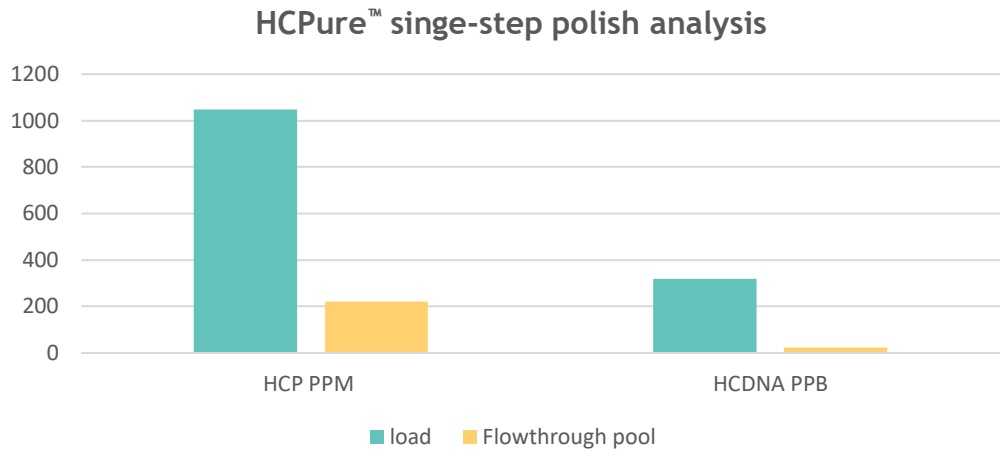

Figure 1 - Analysis of HCPure™ elution pool, showing 79% reduction in HCP and 93% reduction in HCDNA

HCPure™ is effective at removing high molecular weight aggregates

Product aggregation remains a crucial issue and results not only in product loss but also affects product safety. During manufacturing of protein therapeutics, the protein is exposed to various stresses which can result in the formation of aggregates. Aggregates are typically large, tangled clusters of denatured antibody molecules that are formed during product purification in downstream processing.

The unique properties of the mixed-mode mechanism of HCPure™ make it effective at removing these aggregates, because high molecular weight aggregates bind more tightly due to the exposed hydrophobic groups in the denatured chains of aggregate.

In order to demonstrate the effectiveness of HCPure™ in removal of aggregates, samples of Protein A eluate were purified through a strong cation exchanger and then held at low pH and high salt to encourage aggregation to occur, before being passed through a HCPure™ column in flow through mode.

The data in Table 3 below shows 6.7% aggregation in the load sample, which is higher than most processes would produce. After this highly aggregated sample was passed through the HCPure™ column, the final concentration of aggregates was 2.9%, which is a nearly 60% reduction in the overall aggregate concentration.

Fraction	Aggregate %
Load	6.7
Flow through Pool	2.9

Table 3- Reduction of high molecular weight aggregates using HCPure™

HCPure™ as part of a multistep polishing process

To achieve the maximum amount of contaminant removal possible, some users still prefer a multistep polishing approach. In addition to the ability to remove HCP, HCDNA and aggregates when used as a single polishing step, the unique binding interactions of HCPure™ allow it to compliment ion exchange when used as part of a multistep polishing process.

After Protein A, anion exchange chromatography is used because it is effective at removing HCP. However, it is not effective at removing high molecular weight aggregates which can also be present. To remove these aggregates, hydrophobic interaction chromatography is required.

Including HCPure™ after an ion exchange step gives multiple advantages over a traditional hydrophobic interaction step. The secondary binding interaction of ion exchange acts as a second pass through the ion exchange column, effectively removing any remaining HCPs present after the initial ion exchange elution.

In these situations, HCPure™ can work as an effective solution in a multistep polishing process. When combined with a traditional cation ion exchanger operated in a bind/elute mode, a reduction in all impurities, including removing HCP down to below the limit of quantitation; see Table 4.

The ability to be equally effective in both single and multistep polishing processes allows HCPure™ to be an important part of different purification strategies.

Fraction	HCP PPM	HCDNA PPB
Load	274	94
Flowthrough pool	LOQ	36

Table 4 - HCP contamination is reduced below limit of assay quantitation after being combined with cation exchange chromatography.

Conclusion

Due to the criticality of mAbs as a therapeutic product, stringent manufacturing and processing guidelines are required to ensure that therapeutics are both safe and effective. To achieve these goals, therapeutic manufacturers work to develop purification processes that can effectively remove HCP, residual HCDNA and high molecular weight aggregates, typically involving both ion exchange and hydrophobic interaction chromatography steps. This process can be compressed by using HCPure™, a mixed mode host cell protein clearance resin, to remove contaminating host cell proteins, HCDNA and high molecular weight aggregates in a single step. HCPure™ is also an effective compliment to cation exchange for customers who require an orthogonal method for challenging contaminant removal using multiple polishing steps for maximal contaminant removal. Regardless of which polishing process is used, HCPure™ is a critical part to achieving safe and effective therapeutics, free from impurities.

+44 (0) 1223 433 800 | astreabioseparations.com

sales@astrea-bio.com | techsupport@astrea-bio.com | quality@astrea-bio.com

Global bases in North America, Canada and Cambridge UK HQ:
Horizon Park, Barton Road, Comberton, Cambridge, CB23 7AJ, UK

Astrea Bioseparations