Astrea Bioseparations

Technical Note

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

Monoclonal antibodies can be expressed by an array of different cell types, including Chinese Hamster Ovary (CHO), *E. coli* and HEK. However, being living cells in addition to the desired mAb, these host cells produce a wide range of other proteins, known as Host Cell Proteins (HCP), which must be removed as part of the downstream purification process along with other impurities.

Removal of HCPs to increase product purity are critical to effective therapeutic outcomes. Failing to effectively clear the final sample of HCPs, a range of negative outcomes can occur, from changes to therapeutic effectiveness to immunogenic responses in the patient.

mAb Processing and HCP clearance

The most common method of producing recombinant mAbs is by expression from mammalian cell systems, most often CHO cells. After cell harvest, downstream processes for mAb purification utilize Protein A, an affinity chromatography step which involves a ligand with specific affinity to the antibody protein. The eluate from the Protein A column is then further purified using polishing steps often including multiple ion exchange and hydrophobic interaction chromatography stages. These polishing steps are necessary because of the need to purify the target antibody from upstream impurities including host cell proteins (HCPs), host cell DNA, Protein A leachates, endotoxin, high molecular weight aggregates and antibody fragments.

Anion exchange chromatography (AIEX) is often used as the step following Protein A affinity purification to achieve removal of any HCP and other impurities. Due to the conditions required for protein binding and the typically high conductivity of Protein A elution, the solution requires dilution before loading onto the

IEX column. This poses a significant issue in large scale manufacture of mAbs as the requirement to dilute the Protein A eluate before IEX creates a bottle neck due to the need for large volume hold tanks.

Ion exchange polishing is not always sufficient to remove all contaminants found in the flow through. Typically, ion exchange will only remove HCP and not high molecular weight (HMW) aggregates. In these cases, additional polishing steps incorporating hydrophobic interaction chromatography (HIC) are often required to remove HMW aggregates. As with Protein A elution, these HIC steps require the use of high concentration salts such as ammonium sulphate which can also cause precipitation of the target product. HIC chromatography also offers limited selectivity due to the generalized nature of the binding interaction, which can contribute to poor overall yields.

The profile of HCP impurities can vary significantly depending on the expression system and upstream process parameters, making removal of HCPs to the levels required by regulatory authorities particularly challenging. Therefore, the chromatography process must be 'tuneable' to allow variation in the target recombinant protein, cell expression system and other process parameters. This requirement of flexibility is not something that can be easily achieved by the utilization of both IEX and HIC and requires a different form of binding interaction.

An Astrea Bioseparations Solution

Despite considerable advances in downstream purification for mAbs and other recombinant proteins from various expression systems, there remains a need for effective removal of process and product related impurities that could impact product safety, stability and efficacy. To solve this problem, Astrea Bioseparations has designed and developed an adsorbent specifically to remove host cell proteins from mixtures using different starting materials, HCPure™. Utilizing mixed-mode purification techniques, Astrea Bioseparations delivers a solution that has a number of advantages over traditional workflows. Using a mixed mode approach to purifying the Protein A eluate, multiple sample conditions and process parameters can be considered. HCPure's mixed mode binding interactions eliminate the need to dilute the Protein A eluate, saving time and costs. Mixed mode demonstrates a higher specificity than HIC polishing, allowing for the removal of HMW aggregates without decreasing yield.

HCPure™ clearance adsorbent selectively removes HCPs and other impurities from a CHO feed stock without detrimentally affecting yield of the target protein IgG. Removing such impurities from CHO systems expressing other recombinant proteins is possible due to its highly selective nature and ability to be tuned to meet process requirements.

The Astrea Bioseparations HCPure™ clearance adsorbent may be used to remove impurities from other expression systems including but not limited to E. Coli, HEK 293, Pichia Pastoris, Saccharomyces Cerevisiae and Spodoptera frugiperda (Sf9 and Sf21).

Conclusion

The mixed-mode HCPure™ clearance adsorbent from Astrea Bioseparations utilizes multiple binding methods to remove HCP, light chain IgG and aggregates after the initial capture step. This simplifies the process of removing HCP from mAb workflows by eliminating the need for an IEX step during polishing and reducing production costs, while allowing finished products to be released quicker and easier.

- In the application of IgG purification from a CHO expression system, the product has been demonstrated to selectively bind light chain IgG as well as HCP whilst maintaining high IgG yields.
- The product retains functionality at relatively high conductivity load conditions, compared to IEX polishing steps which would require dilution. This removes potential bottlenecks in mAb manufacturing operations requiring high volume in-process hold-tanks for dilution of Protein A column elution.
- The HCP resin creates a robust purification platform that can be adjusted for variations in target analytes and upstream processes without having to requalify processes.
- Derivatized 90 µm PuraBead 6HF solid support allows for the ideal combination of fast flow and highly selective ligand density to achieve highly purified samples with minimal residence time.

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