GTP Bioways CDMO
Continuum for biologics

Case Study

Effective removal of host cell proteins in high pI recombinant protein production

The Challenge

Develop a comprehensive purification strategy to reduce host cell protein contamination in a high molecular weight recombinant protein, optimising washing, loading, and elution conditions, and implementing effective polishing steps.

BIOLOGICS

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Context & Challenge

GTP Bioways was tasked with the production of a complex recombinant protein characterised by a high molecular weight (> 200 kDa) and a pI around 9. To achieve this, we conducted a comprehensive downstream processing (DSP) development, which involved screening chromatography media and optimizing binding and elution conditions. A three-step purification strategy was thus designed.

While no issues were anticipated prior to initiating the polishing step, the removal of host cell proteins (HCP) proved to be one of the most formidable challenges encountered during the project. Indeed, unidentified host cell proteins were systematically co-purified with the target molecule.

GTP Bioways' Solution

To decrease the level of HCP, several actions were taken:

1. Optimisation of the washing conditions during the first capture step (pH, nature of the buffering salts, additives such as detergents or glycerol),
2. Optimisation of the loading conditions for the first capture step (pH, salt concentration),
3. Optimisation of the elution strategy during each chromatography steps,
4. Screening of alternative media for polishing, and implementation of the polishing step at various steps during the purification.

Optimisation of the washing conditions

Eight washing conditions were concurrently tested to lower down the HCP level during the first capture step (Figure 1).

The initial amount of HCP in the eluate was 8000 ppm. This value was decreased significantly by different treatments. However, in some cases, it also led to a loss of material and aggregation (not shown). Taken altogether the data suggested that the condition "Wash 1" was the most appropriate for this project.

Optimisation of the loading conditions

It is often observed that some HCP can be efficiently removed by modulating the loading conditions: pH of the loaded sample, salt concentration, or loading flow rate. In this particular example, none of the conditions tested led to a significant decrease in the HCP level.

Optimisation of the elution strategy

During elution from the chromatography media, proteins can be eluted sequentially, depending on their affinity to the matrix. However, in most cases, the various proteins do not come out of the column in a discrete manner but show overlapping elution profiles. Therefore, one simple approach is to reduce the number of collected fractions at the expense of the protein of interest. A representative illustration of this is presented in Figure 2. In such a case, the HCP level can be measured in various elution fractions or pools of fractions to help determine the most appropriate elution volume, i.e. with the higher relative amount of the protein of interest and the lowest amount of host cell proteins.

	Wash 1	Wash 2	Wash 3	Wash 4	Wash 5	Wash 6	Wash 7	Wash 8
HCP (ppm)	6900	2600	4300	4900	4700	4100	5700	7500
Amount recovered (mg)	17	10	12.5	16	15	7	17	17

Figure 1: HCP levels observed after different washing conditions

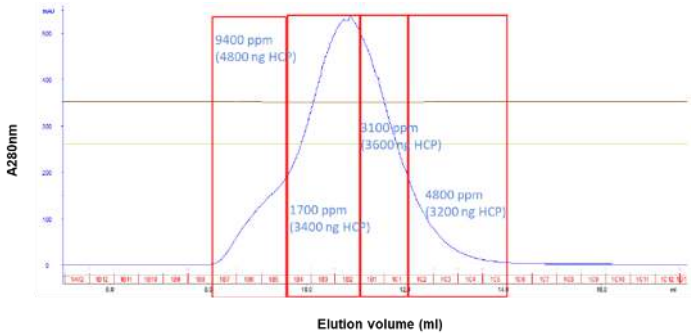

Figure 2: Quantification of HCP in various elution fractions

Screening of alternative media for polishing

Polishing is of course a key step to get rid of impurities. When facing difficult separation of HCP, a media screen can be performed. Moreover, it is often thought that a polishing step must be implemented after capture steps, at the end of the purification strategy. However, the use of a polisher upstream in the purification scheme can lead to a better resolution of the protein of interest during subsequent steps. In this case study, the screening of different polishers allowed us to identify a media that efficiently removed HCP. Moreover, the polishing was performed twice: once after the first capture step, and a second time before the nanofiltration step.

Figure 3 shows that the initial polishing step (Polishing 1) was not efficient in removing HCP. In addition, The second capture step did not allow to significantly lower down the level of HCP either. However, the implementation of an alternative polishing step (New Polishing) right after the first capture led to a decrease in HCP at this stage, and also made the second capture step more efficient in removing HCP (compare black bars Capture 1 -> Capture 2, and purple bars New Polishing -> Capture 2).

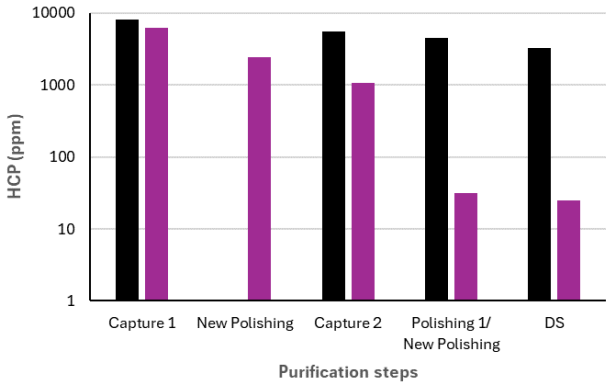

Figure 3: Evolution of the HCP level during the initial purification process (black bars) and during the optimized process (purple bars). The HCP level in the supernatant was > 100'000 ppm.

Finally, after optimization of the DSP, the HCP level in the DS batch was consistently below (>5 times below) the Customer's target of 200 ppm.