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Design of Experiments for Efficient HCP Removal with Mustang® Q XT Chromatography Membrane During RevEr3mAb Purification

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INTRODUCTION

RevEr3mAb is an innovative humanized monoclonal antibody (mAb) that targets HER3/ErbB3 (human epidermal growth factor receptor 3), a member of the EGFR family of receptor tyrosine kinases. As the other members of the family, HER3/ErbB3 is an important regulator of growth and cell differentiation, and has emerged as a key target for antibody-mediated tumor therapy during the past few years. In cancer cells, HER3/ErbB3 is highly expressed and forms heterodimers with other family members to activate signalling pathways. Activation of HER3/ErbB3 is often associated to cancer metastasis and therapy resistance, reducing survival in cancer patients.

RevEr3mAb binds a unique epitope on HER3/ErbB3, inhibiting dimerization of the receptor extracellular domains. This approach is effective in reverting proliferation of tumoral cells *in vitro* and *in vivo,* and strongly promotes RevEr3mAb as a suitable therapeutic agent against a wide range of cancers, in a monotherapy, or along with conventional oncologic therapies. The purification process of RevEr3mAb for preclinical and clinical phases is currently under development.

Here, we propose the screening and evaluation of polishing conditions for RevEr3mAb on a Mustang Q XT chromatography membrane (Pall Biotech), following a Design of Experiments (DoE) approach. A typical monoclonal antibody purification process includes a capture step on Protein A followed by two polishing steps on orthogonal chromatography sorbents to reduce the level of aggregates, fragments and other contaminants. Mustang Q membrane chromatography has proven to be an effective solution for host cell proteins (HCP), endotoxins and DNA removal. With respect to conventional packed bed resins, adsorptive membrane technology offers reduced processing time and reduced buffers usage, with comparable mAb recovery in flow through mode.

The screening was performed on a range of pH and conductivity values. Experiments performed for this case study demonstrated that the Mustang Q membrane was efficient for HCP removal within the selected conditions.

MATERIALS AND METHODS

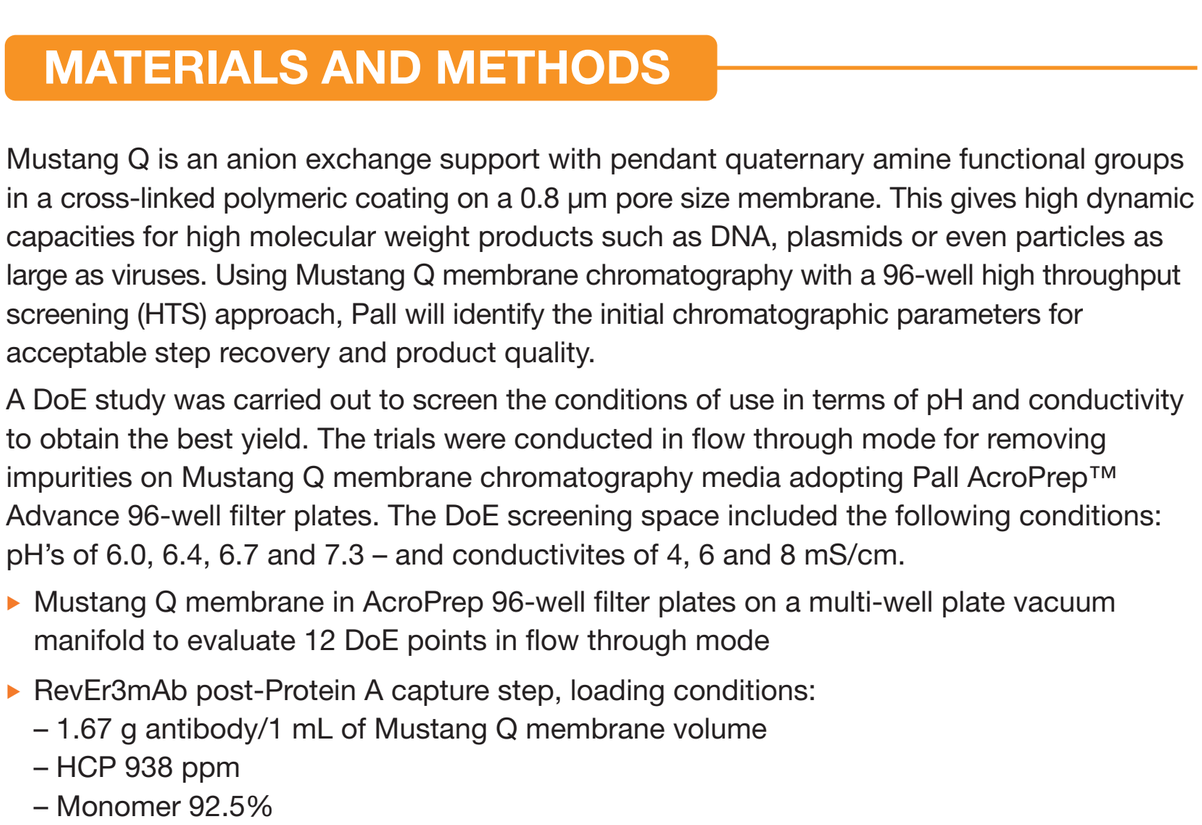
Mustang Q is an anion exchange support with pendant quaternary amine functional groups in a cross-linked polymeric coating on a 0.8 µm pore size membrane. This gives high dynamic capacities for high molecular weight products such as DNA, plasmids or even particles as large as viruses. Using Mustang Q membrane chromatography with a 96-well high throughput screening (HTS) approach, Pall will identify the initial chromatographic parameters for acceptable step recovery and product quality.

A DoE study was carried out to screen the conditions of use in terms of pH and conductivity to obtain the best yield. The trials were conducted in flow through mode for removing impurities on Mustang Q membrane chromatography media adopting Pall AcroPrep™ Advance 96-well filter plates. The DoE screening space included the following conditions: pH's of 6.0, 6.4, 6.7 and 7.3 – and conductivites of 4, 6 and 8 mS/cm.

- Mustang Q membrane in AcroPrep 96-well filter plates on a multi-well plate vacuum manifold to evaluate 12 DoE points in flow through mode
- RevEr3mAb post-Protein A capture step, loading conditions:
  – 1.67 g antibody/1 mL of Mustang Q membrane volume
  – HCP 938 ppm
  – Monomer 92.5%

Figure 1

Mustang Q membrane in AcroPrep 96-well filter plate was used to identify the design space, and accurately develops the operating space



RESULTS

HCP

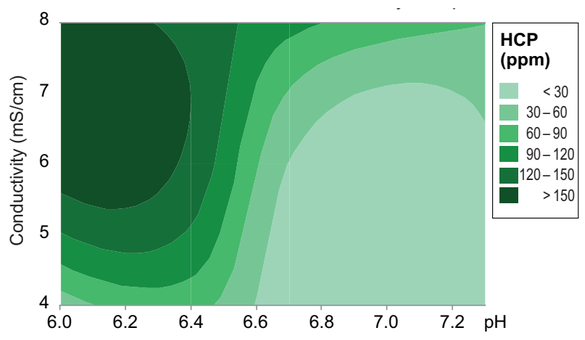
Mustang Q membrane performance on RevEr3mAb concerning the HCP reduction based on DoE results

Yield

Mustang Q membrane performance on RevEr3mAb yield based on DoE results

Figure 2

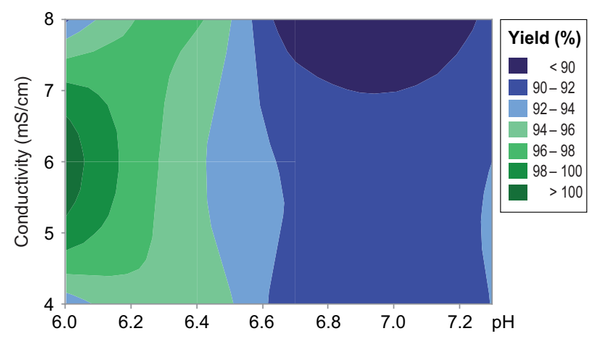
Contour plot of HCP vs. conductivity and pH



HCP reduction in ppm terms is boosted at higher pH. Lower conductivity highly increased the overall HCP reduction.

Figure 3

Contour plot of yield vs. conductivity and pH



Conductivity variation did not significantly impact the Mustang Q membrane performance on RevEr3mAb yield. A lower pH increased product recovery.

Table 1

Mustang Q membrane data and trend on RevEr3mAb concerning the HCP reduction and on RevEr3mAb yield, based on DoE results

	pH Set Point	6.0	6.4	6.7	7.3	Trend
HCP	Conductivity 8 mS/cm	176	138	100	61	
	6 mS/cm	166	142	29	22	
	4 mS/cm	42	70	12	17	
	Mean	**128.00**	**116.67**	**47.00**	**33.33**	
Yield	Conductivity 8 mS/cm	91.60	96.20	88.80	90.70	
	6 mS/cm	101.10	94.30	91.70	92.00	
	4 mS/cm	92.70	95.50	91.00	92.10	
	Mean	**95.13**	**95.33**	**90.50**	**91.60**	

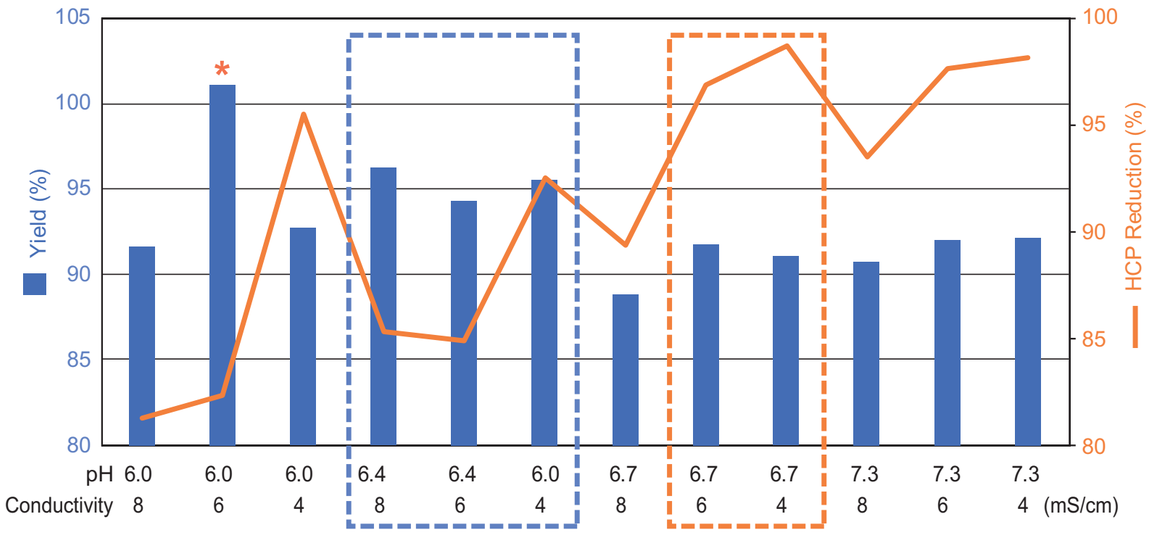
CONCLUSION

A Mustang Q membrane chromatography polishing step was successfully tested at Istituto Biochimico Italiano (IBI) in flow through mode for the removal of residual HCP in a RevEr3mAb purification process at laboratory scale, following a conventional Protein A column chromatography step.

The contribution of the Mustang Q membrane chromatography polishing step to HCP reduction was shown with >88 % product recovery.

Figure 3

Efficient HCP removal and mAb recovery based on conductivity and pH



Despite an outstanding HCP reduction at pH 6.7, the best yield was reached at pH 6.4
(pH 6.0, 6 mS/cm point resulted out of trend therefore is not considered)*

The AcroPrep 96-well plates platform is made of chemically resistant, biologically-inert polypropylene with a clear polystyrene lid and 14 µL of Mustang Q membrane per well. The maximum well volume is up to 350 µL, so every dose could have a variability that might explain the yield results over the analytical method fluctuations. The data will be re-evaluated on a Mustang Q membrane in XT Acrodisc® units to reach a higher data accuracy.

The collaboration between IBI and Pall Biotech provided an accurate and precise DoE, which estimates the effects at different conditions to demonstrate an HCP reduction of >88%.

Adopting a DoE approach ensures a thorough risk assessment and enables the generation of an appropriate mitigation strategy.

DoE results is an increased method knowledge that might be further optimized via upscaling the volume trials through Mustang Q membrane in XT Acrodisc units adopting the best conditions determined through the analytical results.

Based on the DoE results, a Mustang Q capsule of 10 mL bed volume can be used with the RevEr3mAb feed for up to a 28 L batch size, assuming a mAb concentration of up to 0.3 g/L.

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