Optimization of a Protein A Chromatography Process for a Herceptin® Biosimilar (Trastuzumab)

Alpana Naresh[1], Chrissy Caroselli[2], Wiphusanee Dendamrongvit[2], Chris Yonan[2], Dan Bailey[1] and Marty Siwak[1]

1. JSR Life Sciences, Bioprocess Division, 1280 North Mathilda Avenue, Sunnyvale CA 94089
2. Oncobiologics Inc., Purification Development, 7 Clarke Drive, Cranbury, New Jersey 08512

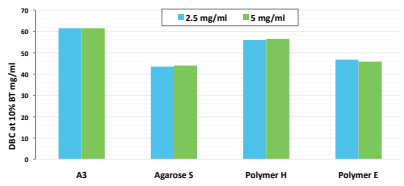
ABSTRACT

Four Protein A resins from leading industry vendors, including Amsphere™ A3, were screened for use in a capture step for a Herceptin biosimilar. Multiple parameters were assessed for their performance against the platform resin. Initially, a dynamic binding capacity (DBC) determination was made using a standard 4-minute residence time (RT) using purified protein. Performance of the resins were then screened for recovery and purity using harvested cell culture fluid (HCCF) where the mAb load was 80% of DBC at 10% breakthrough (BT). It was observed that substituting Amsphere A3 in the platform capture process led to a 40% increase in DBC relative to two of the resins tested including the platform resin and a 25% DBC increase over the third comparator resin. Additionally, a better or comparable host cell protein (HCP) impurity reduction to other resins was observed. Optimization of the capture step was done using Amsphere A3 in place of the platform resin. An optimal elution system and pH were determined that yielded similar mAb recovery, purity, pH, and eluate volume. Subsequently, the DBC was re-evaluated with Amsphere A3 using a range of residence times bracketing the previously tested 4 minutes. An optimized process utilizing a residence time of 4 minutes or higher with loads of 70-80% of DBC at 10% BT led to increased recoveries and purity of the Herceptin biosimilar. Both HCP and leached Protein A impurities were within acceptable ranges. It was further shown that filtration of the eluate after neutralization led to further reduction in HCP. A capture step has been initially developed using Amsphere A3 that provides significant improvement to the industry standard protein A resins while also demonstrating comparable or superior mAb recovery and HCP and leached protein A profiles.

1. OBJECTIVES OF THE STUDY

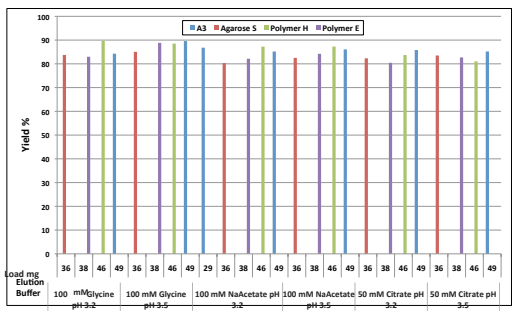
- Evaluation of DBC of four resins (Amsphere A3, platform resin referred to as Agarose S, and two other polymeric resins referred to as Polymer H and Polymer E) at 10% BT utilizing purified Herceptin biosimilar at two protein concentrations (2.5 and 5.0 mg/ml) and 1 ml columns (0.5 cm ID X 5 cm BH) at 4 min RT.
- Screening of an optimal elution matrix with all four resins using 1 ml columns (0.5 cm ID X 5 cm BH) with HCCF of Herceptin biosimilar by loading 80% of DBC determined at 10% BT at 4 min RT and utilizing six elution buffers.
  - 100 mM Glycine-HCl, pH 3.2 and pH 3.5 respectively
  - 100 mM Sodium Acetate, pH 3.2 and pH 3.5 respectively
  - 50 mM Sodium Citrate, pH 3.2 and pH 3.5 respectively
- Initial scale-up assessment
  - Perform a scale up (5 ml column, 1.13 cm ID X 5 cm BH) run with selected condition(s) including multiple residence times (i.e. 2, 4, and 6 min).
  - Evaluate resin performance with respect to product recovery and residual impurities (i.e. HCP and residual Protein A)

2. DBC AT 10% BT USING PURIFIED HERCEPTIN BIOSIMILAR



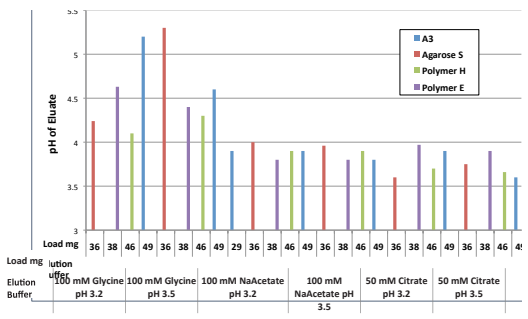
Item	Details
Column	0.5 x 5cm (1mL) with Amsphere™ A3, Agarose S, Polymer H, and Polymer E
Residence Time	4 min
Detection	UV at 280nm, Peak collection at 100 mAU – 100 mAU
Sample	Purified mAb: Trastuzumab (Herceptin) biosimilar, 2.5 mg/ml and 5.0 mg/ml
Evaluation Items	DBC at 10% BT
System	AKTA™ Avant 25

3. SIMILAR YIELDS OBTAINED AT LAB SCALE



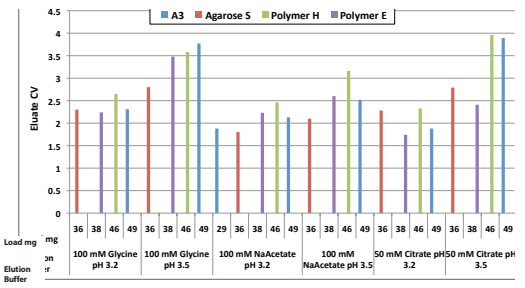
Protein concentration determined using NanoDrop 2000

4. PH OF ELUATE DEPENDENT ON ELUTION SYSTEM



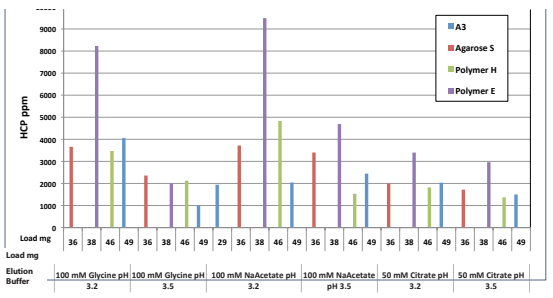
Eluate pH was in the 3.6 to 4 range when eluted with acetate and citrate buffers

5. CV OF ELUATE DEPENDENT ON ELUTION SYSTEM



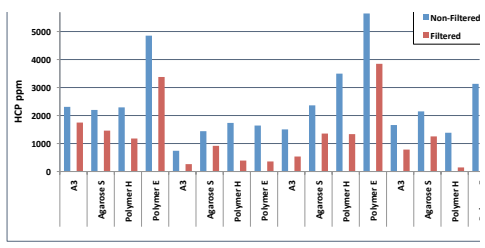
Elution with an acetate buffer showed minimal CV variation for Amsphere A3, ranging from 1.9 to 2.5 CV

6. HCP WITH AMSPHERE A3 LOWER OR SIMILAR TO OTHER RESINS



Samples were analyzed for HCP using Cygnus HCP 3G kit (#F550)

7. HCP REMOVAL IMPROVES AFTER FILTRATION*



Precipitation was observed in multiple samples after elution and neutralization with Tris to pH 7.0 (neutralization pH varied from 5.5-8.5). About 500 µl of the precipitated samples were filtered through a 0.2µm syringe filter (Whatman™ Puradisc 25mm 0.2µm PES filter). Yields were minimally impacted. Citrate elution buffer did not lead to precipitation thus not tested.

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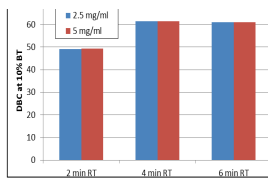
8. PROCESS OPTIMIZATION FOR HERCEPTIN BIOSIMILAR WITH A3

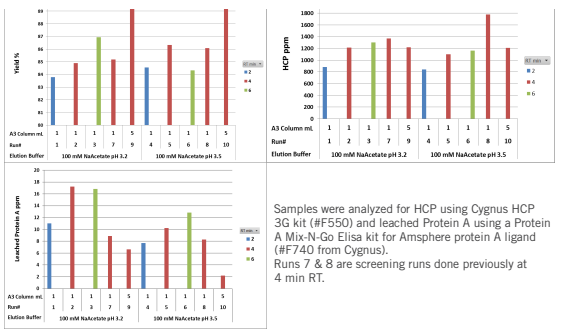
- Further process optimization with respect to RT and elution buffer
  - RT: 2, 4, and 6 min
  - Elution buffer selected based on results of screening studies:
    - 100 mM Sodium Acetate buffer, pH 3.2
    - 100 mM Sodium Acetate buffer, pH 3.5
- Analysis: Yield, HCP, leached Protein A, and samples saved for SEC
- After optimal condition is selected, perform scale-up run at 5ml scale



CONCLUSION

- DBC did not increase with 6 min RT with Oncobiologics purified Herceptin
- Process can be run at 2 min RT to a DBC of nearly 50 mg mAb/ml of resin at 10% BT, which is higher than Agarose S at 4 min RT (~ 44 mg mAb/ml resin)

9. YIELD, HCP, AND LEACHED PROTEIN A RESULTS FOR PURIFIED HERCEPTIN BIOSIMILAR USING A3



Samples were analyzed for HCP using Cygnus HCP 3G kit (#F550) and leached Protein A using a Protein A Mix-N-Go Elisa kit for Amsphere protein A ligand (#F740 from Cygnus).
Runs 7 & 8 are screening runs done previously at 4 min RT.

CONCLUSION

- Amsphere A3 has the highest DBC at 10% BT for Herceptin biosimilar at 4 min RT and performed better or similar to other resins at 2 min RT.
- Amsphere A3 performed better in terms of yield and HCP removal.
- Initial purification process using Amsphere A3 affinity resin for Herceptin biosimilar was developed
  - — 4 min RT with loading to 70-80% of 10% BT DBC led to high recovery and purity.
  - — Filtration of eluate after neutralization led to reduction in HCP.

FUTURE WORK

- Confirm at scale with HCCF
- Evaluate the defined process over several cycles to demonstrate process reproducibility.
- Assess product related impurities (e.g. aggregates, charge variants).