Identification of an AAV Affinity Chromatography Elution Buffer that Maximizes Product Recovery and Minimizes Product Degradation

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Abstract

Recombinant adeno-associated virus (rAAV) continues to be a promising vector for delivery of gene therapies due to its proven success in clinical trials and high potential for treatment of diseases with unmet medical needs. To meet the clinical and commercial demands while ensuring an effective product, manufacturing sufficient rAAV quantities while maintaining superior product quality is of paramount importance. Chromatography-based purification methods using AAV affinity resins are commonly used during manufacture of rAAV due to the ability to effectively remove process- and product-related impurities. However, to efficiently elute rAAV vector from these resins at acceptable recoveries, harsh buffer conditions, such as low pH, are typically employed which often result in a rapid negative impact to product quality, such as increased product aggregation or decreased product activity. Here, we discuss development work performed on an rAAV affinity chromatography step that identified modifications to the elution buffer pH, elution buffer components, and eluate collection method/neutralization resulting in a two-fold improvement in product recovery, reduced impact to product, and improved manufacturability.

Elution Buffer Composition Optimization

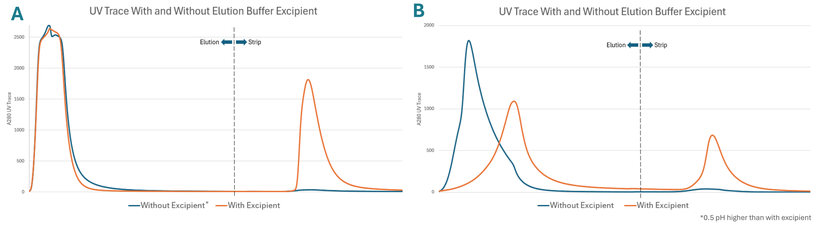


Figure 1. Affinity elution and strip UV profile differences with/without a specific excipient in the elution buffer.
A. Capsid Serotype A on POROS CaptureSelect AAVX resin
B. Capsid Serotype B on POROS CaptureSelect AAV9 resin

- Product recovery improved by about 25% for both capsid serotypes and both resins with the removal of the excipient from the elution buffer.
- There was almost no strip peak observed without the elution buffer excipient, while the runs with the excipient had strip peaks of about 40% total area under curve (AUC) of the elution peak.

Special thanks to Casey Laguna and Solid Bioscience's Analytical Development team.

Elution Buffer pH Optimization: Product Recovery

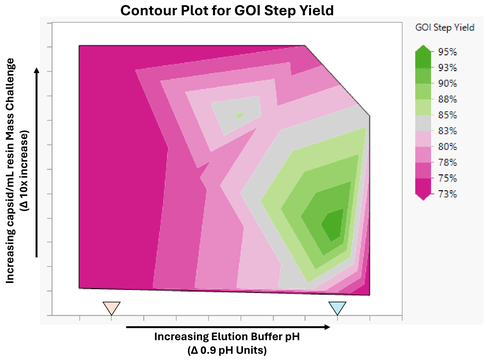


Figure 2. Contour plot of step yield versus elution buffer pH and load mass challenge.

- Recovery drops off at high and low ends of pH range tested, despite expected higher recoveries at lower pH levels.
- Mass challenge also impacts yield. Highest recovery is observed at around ½ dynamic binding capacity.
- Optimal elution buffer pH allows for ± 0.1 pH unit buffer preparation tolerance for manufacturability.

Elution Buffer pH Optimization: Product Activity

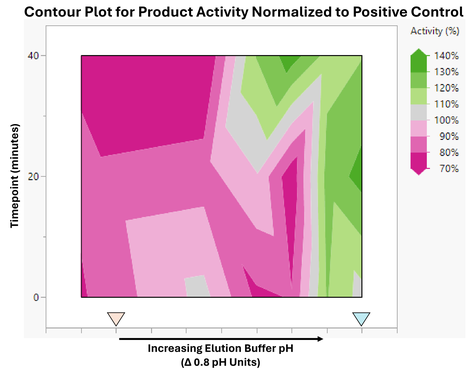


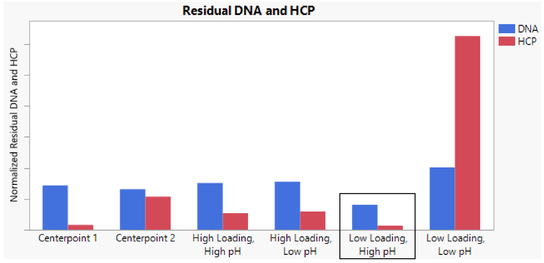
Figure 3. Contour plot of product activity versus elution buffer pH and low pH hold time. Eluate samples were neutralized at t=0min, 20min, and 40min with the same neutralization procedure.

- Product degradation is minimized with increased elution buffer pH.
- Negative impacts to product activity associated with low elution buffer pH appear immediately upon exposure, as opposed to developing slowly over time with elongated exposure to low pH.

Elution Buffer pH Optimization: Impurity Removal

Figure 4. Residual DNA and HCP levels at neutralized affinity eluate from a DOE looking at elution buffer pH and resin mass challenge.

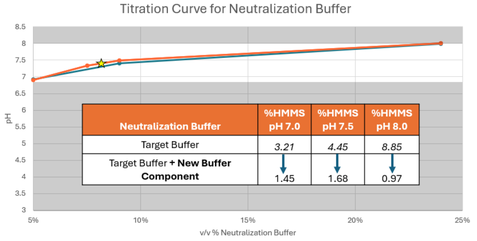
- Especially at low mass challenge, significant reductions in residual DNA and HCP were seen at the high elution buffer pH.



Elution Neutralization Optimization: Minimizing Aggregation

Figure 5. Neutralization curve and high molecular weight species.

- Affinity eluate collected in fixed volume after UV trigger into a collection vessel pre-filled with bulk (v/v%) neutralization buffer, minimizing time at low pH and reducing operating steps for improved manufacturability.
- Included a specific buffer component at 1-2mM to reduce HMW%.



Neutralization Buffer	%HMMS pH 7.0	%HMMS pH 7.5	%HMMS pH 8.0
Target Buffer	*3.21*	*4.45*	*8.85*
Target Buffer + **New Buffer Component**	↓ 1.45	↓ 1.68	↓ 0.97

Conclusions

- **Removal of a specific, commonly used buffer component from the affinity elution buffer reduced product loss to the strip by about 25%.**
- **Selection of optimal elution buffer pH resulted in a 20% increase in step yield, two-fold improvement in product activity, and improved residual DNA and HCP clearance across the affinity column.**
- **The combined pH increase and excipient removal in the elution buffer consistently results in two-fold product recovery improvements.**
- **Manufacturability was improved and the time product spends at low pH was minimized with new affinity elution collection and bulk neutralization methods.**
- **One buffer component was key to reducing neutralized eluate HMW%.**