Enabling Large Scale Implementation of Anion Exchange Chromatography for Full Capsid Enrichment of a Novel Adeno-Associated Viral Vector

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EXECUTIVE SUMMARY

During production of adeno-associated virus (AAV) gene therapies, a significant percentage of capsids generated do not contain the packaged transgene of interest and must be removed to the extent possible. Anion exchange (AEX) chromatography has emerged as a scalable approach for separating empty and full capsids in downstream AAV purification. Here, we present case study data demonstrating successful scale-up and tech transfer of AEX chromatography from Process Development to Pilot and CDMO facilities.

Strategies for consistent scale-up of AAV linear gradient elution chromatography across sites, systems, and scales include:

- Correlating UV peak area with PCR titer for rapid estimation of in-process concentrations.
- Using chromatogram peak width, UV magnitude, and UV ratios to identify an "elution peak signature" for product collection.
- Understanding product stability when re-using an AEX column for multiple cycles within a batch.

DEVELOPMENT DATASET ANALYZED FOR CORRELATIONS & TRENDS

Table 1. **AEX Enrichment Method Summary**

Step	Mobile Phase Conditions
Equilibration	Low conductivity and alkaline pH
Load	AAV load material at low conductivity and alkaline pH
Wash	Low conductivity and alkaline pH
Gradient Elution	Linear increasing conductivity gradient at alkaline pH
Strip	High conductivity and alkaline pH
Clean In Place (CIP)	High conductivity and caustic pH

Materials & Methods: Thirty-two purification runs were performed with Sartorius BIA CIMmultus QA AEX monoliths using procedures described by Table 1. The runs were performed in different laboratories using a range of column volumes, systems, load challenge levels, and flowrates (Table 2). Each run utilized the same novel AAV9-derived TRACER™ capsid and packaged transgene. AAV was produced in HEK293 cells via triple transfection, lysed, clarified, and purified by affinity chromatography prior to AEX experiments. Separate batches of AAV material were generated at the scales and laboratories described in Table 2. Eluate pools from AEX experiments were assayed for step recovery by ddPCR titer and for full capsid enrichment by analytical ultracentrifugation (AUC) or SEC-MALS.

Figure 1. **Example Gradient Elution Chromatograms Performed with Different Batches, Scales, & Laboratories**

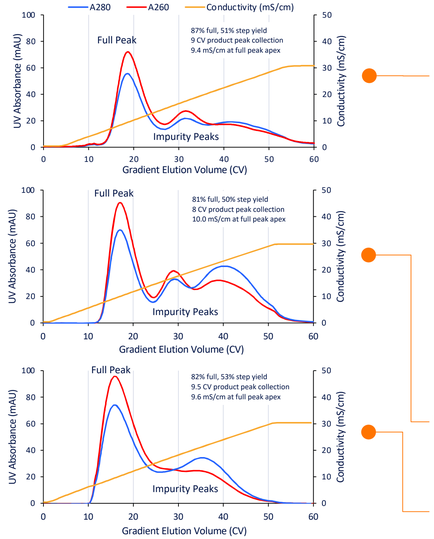
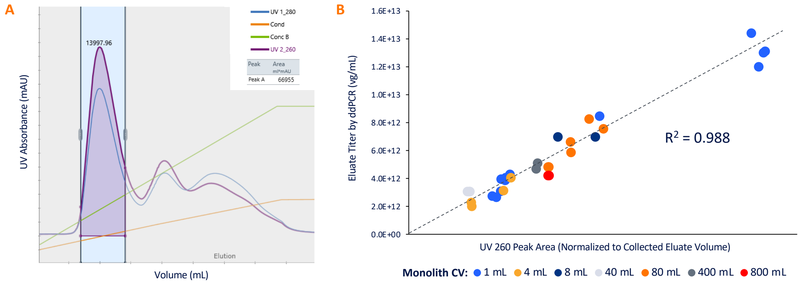


Table 2. **Summary of runs performed at Process Development, Pilot, & CDMO laboratories**

Run	Laboratory	Monolith Column Volume (mL)	AKTA System	Load Challenge	Flowrate (CV/min)
1	Process Development	1	Avant	High	10
2		1		Mid	10
3		1		Mid	10
4		1		Mid	10
5		1		Mid	10
6		1		Mid	10
7		1		Low	10
8		1		Low	10
9		1		Mid	10
10		1		High	10
11		1		High	10
12		1		High	10
13		1		High	10
14		4		Mid	10
15		4		Low	10
16		4		Low	10
17		4		Low	10
18	Pilot	40	Pilot	Low	5
19		40	Pilot	Low	5
20		40	Pilot	Low	5
21		80	Avant	High	1.5
22		80	Avant	High	1.5
23		80	Pilot	High	1.5
24		80	Pilot	High	1.5
25		400	Pilot	Mid	1.5
26		400	Pilot	Mid	1.5
27	CDMO	8	Pure	Mid	5
28		8	Pure	Mid	5
29		80	Pure	Mid	1
30		80	Pure	Mid	1
31		800	Pilot	Mid	0.75
32		800	Pilot	Mid	0.75

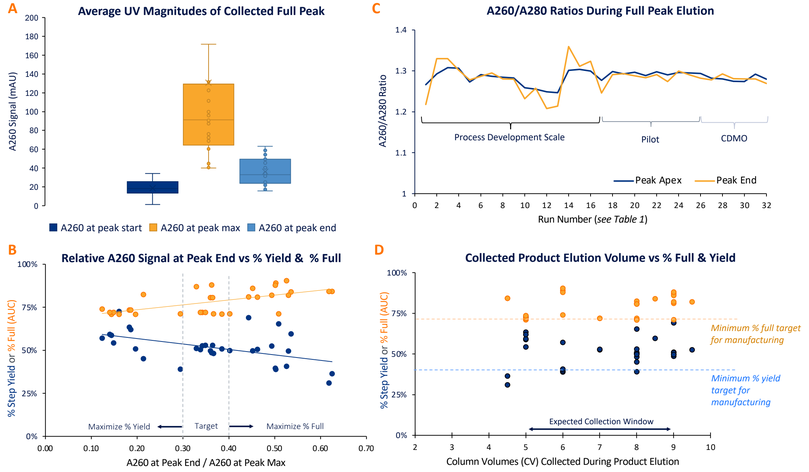
OPERATIONAL INSIGHTS TO SUPPORT LARGE SCALE IMPLEMENTATION

Figure 2. **UV Peak Area Correlates with PCR Titer for Rapid Assessment of In-process Concentrations**



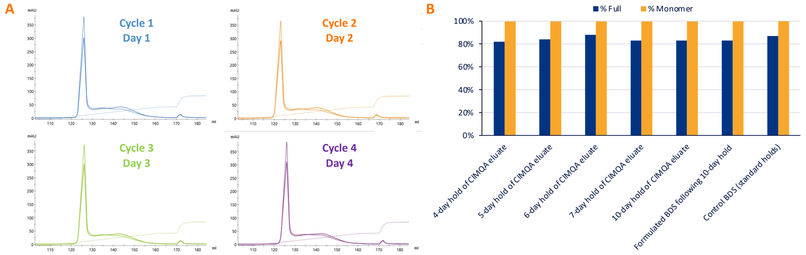
(**A**) Industry standard chromatography software enables calculation of UV peak area (mAU * mL), which is proportional to total VG eluted. (**B**) Normalizing peak area to collected eluate volume provides a well correlated analog to PCR based titer measurement. This simple linear correlation is maintained despite differences in column volume, system, or laboratory site. The peak area strategy quickly provides an in-process titer estimation for design of subsequent unit operations, for example drug substance formulation by UF/DF.

Figure 3. **Development Scale Elution Trends Reveal a Product Specific "Elution Peak Signature" to Guide Peak Collection Decisions at Scale**



Key characteristics of the product peak "signature" during linear gradient elution. (**A**) The A260 magnitude is heavily dependent on load challenge level but follows similar relative trends at peak start and peak end. (**B**) The ratio of A260 signal at Peak End / A260 signal at Peak Max is a useful metric to tune purity and yield. For the tested product, ending product collection at 30-40% of full peak maximum UV signal is ideal. (**C**) The average A260/A280 ratio at peak end is only < 1% lower than the ratio at peak apex. (**D**) Data indicates a 5 – 9 CV elution peak width maintains full capsid and step yield criteria above manufacturing targets. For large scale systems with limited fractionation capabilities, multiple outlet lines can bracket the expected collection window. The desired product pool can then be identified by UV ratio and magnitude data.

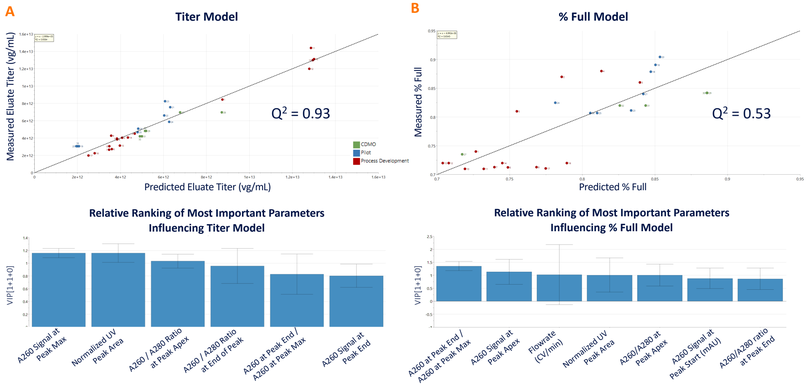
Figure 4. **Column Re-use Allows for Device Sizing Flexibility, but Product Stability Must be Considered**



A single AEX monolith column can be cycled within a batch for device sizing flexibility. (**A**) Data supports up to four re-use cycles with consistent elution profiles when applying a caustic CIP strategy (Table 1). In addition to cycle-to-cycle consistency, a development team should verify stability of AEX pools to accommodate hold times during cycling. (**B**) SEC-MALS data for full capsid and monomer content in CIMQA AEX elution pools following a 2-8°C hold at various timepoints. Extended timepoints (up to 10 days) were selected to understand impact of a manufacturing delay scenario.

MULTIVARIATE STATISTICAL ANALYSIS

Figure 5. **Multivariate Model Provides Excellent Prediction of Eluate Titer and Moderate Prediction of % Full**



(**A**) Multivariate modeling confirms high predictability of titer using chromatogram UV signal and peak area values. Importantly, the prediction model is scale-independent, as factors such as laboratory site, system, column volume, and flowrate did not strongly correlate with elution peak titer. (**B**) Multivariate modeling shows moderate predictability of full capsid enrichment using peak signal collection criteria and UV ratios. The model identified some correlation between flow rate and full capsid enrichment, with slower flowrates showing potential for improved purity.

CONCLUSIONS

- Scale-up of linear gradient elution chromatography for AAV enrichment is improved by correlating product-specific chromatogram trends to unlock real-time process monitoring capabilities.
- Column cycling provides sizing flexibility, but product stability should be monitored during in-process holds.
- Multivariate modeling confirms high predictability of in-process titers (Q^2 = 0.93) using chromatogram UV signals. System flowrate does not impact eluate titer, but slower flowrates may increase full capsid enrichment.