Development of a robust downstream strategy for high quality AAV using an all-column purification method

Byron Carper, Hannah Roodhouse, John Ketz, Matthew Janson & Samir Acharya

Andelyn Biosciences, 5185 Blazer Parkway, Dublin, OH, USA

P280

Abstract

Andelyn Biosciences has recently developed a robust scalable suspension platform for production of high yields of AAV for preclinical and all phases of clinical production. This platform is based on a quality by design approach that ensures scalability and quality at different stages of development. The standard purification strategy involves use of ultracentrifugation for enrichment of full capsids. An alternative all-column chromatography technique will be presented which replaces ultracentrifugation for downstream purification of AAV. The process flow includes concentration of the clarified harvest followed by two IEX chromatography steps – one for capture and another for enrichment of full capsids. Data will be presented showing reproducibility and robustness of the strategy, with consistency in recovery and purity at each stage. Compared to the standard ultracentrifugation approach for enriching full capsids, the all-column method results in 75% reduction in processing time. The IEX procedure results in 50% recovery which consists of 88-98% full capsids with final purity of 99-100% as evaluated using HPLC, AUC and capillary electrophoresis.

Background

Andelyn Biosciences has developed a robust purification strategy for separation of empty and full capsids of AAV in a suspension platform from 1-500L **(1)**. Due to scalability constraints, there is a need to replace the current full particle enrichment (density gradient ultracentrifugation) procedure in the current purification platform. A chromatography method was developed to replace the ultracentrifugation step.

The process will be used for AAV productions made with the newly developed high yielding clonal cell line **(2; Poster 301)** and supported by analysis of empties and fulls via HPLC **(3; Poster 331)**. The method drastically reduces process time and is suited for simple scale up with the use of step elution.

This presentation showcases typical chromatograms and purity of the process. Normally, empty/full separation required the use of gradient elution, however, use of a step elution allows for equivalent separation, purity, and recovery.

Step 1: Capture

- The process begins with concentration of clarified harvest by tangential flow filtration (TFF).
- Concentration of harvest before chromatography allows for a cleaner feed stream, faster processing, and lower volumes of buffer.
- Cation Exchange Chromatography (CEX) is performed following TFF.
- CEX is used as a capture step, creating a cleaner product before separation of empty and full capsids.

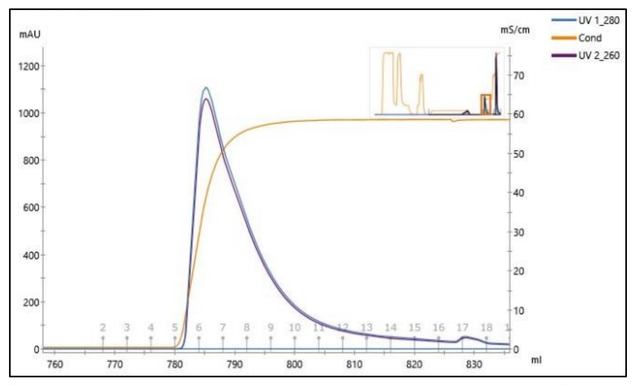

Figure 1: Chromatogram of CEX Elution. Step elution was performed on an AKTA Pure 150M to elute AAV from the column. In this step, many contaminants stay bound under acidic buffer conditions resulting in a purer product. The 260/280 ratio is typically 0.9-1.1 for this step as empty and full capsids are not yet separated.

Step 2: Separation

- Approximately 2-3.0E+14 vg/mL (full capsids) can be loaded onto the CEX column.
- Fast flow rates (10-20 CV/min) allow for decreased processing time.
- CEX eluate is loaded onto a primary amine (PA) column for anion exchange chromatography (AEX).
- Primary amines allow for binding at higher salt conditions, resulting in less manipulation of the product.
- AAV also elutes much later than traditional quaternary amine (QA) columns.

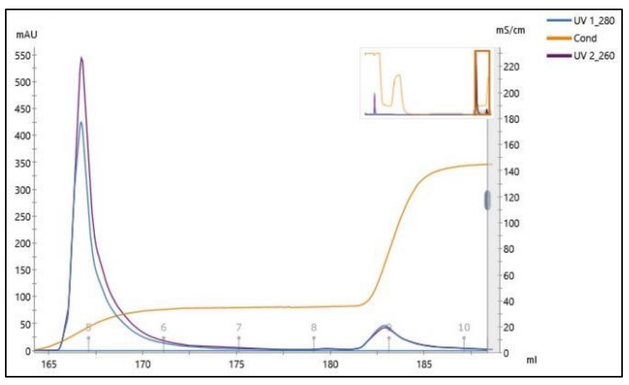

Figure 2: Chromatogram of PA Column Elution. Step elution was performed on an AKTA Pure 150M to elute AAV from the column. In this step, many contaminants stay bound under basic buffer conditions resulting in a purer product. The 260/280 ratio is typically 1.2-1.3 for this step, indicating separation of empty from full capsids. Only full capsids are eluted using this step elution method.

Previous Method

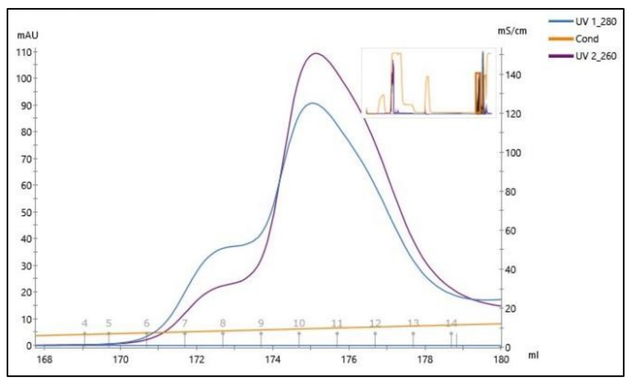

Figure 3: Chromatogram of QA Column Elution. Gradient elution was performed on an AKTA Pure 150M to elute AAV from the column. The 260/280 ratio of the first peak is typically 0.6, indicating empty capsids. The 260/280 ratio of the second peak is typically 1.2-1.3, indicating separation of empties from fulls. Using different buffer conditions, it is possible to get better resolution of the peaks, but it is difficult to scale.

- QA methods (**Figure 3**) rely on a shallow salt gradient to create separation of empty and full capsids.
- The PA column creates an easily scalable process due to its simplistic elution profile (**Figure 2**).
- Although it is comparable to ultracentrifugation as a purification method, the ease of the PA column makes it the superior choice for downstream processing.
- Typical recovery of the empty/full step is ≥50%.

Highly Pure

- Eluate is buffer exchanged via TFF before final fill.
- Purity is comparable to the ultracentrifugation process.

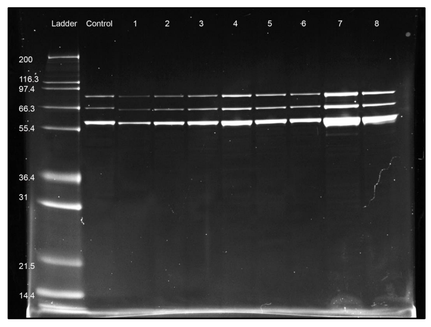

Figure 4: SDS-PAGE analysis of different separation methods. The control is AAV purified using ultracentrifugation. 2-6 are final products of various QA methods. 7-8 are final products of the PA method. All final samples are ≥97% pure.

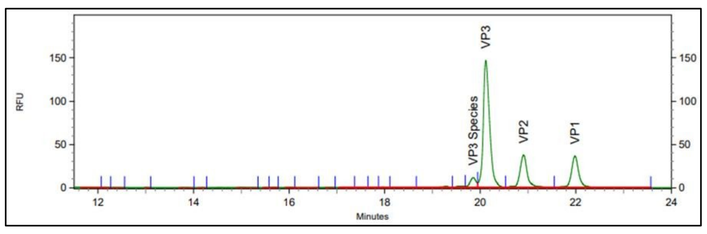

Figure 5: Purity Analysis of Final Drug Product by Capillary Electrophoresis-Laser Induced Fluorescence. AAV was purified using the all-column chromatography process. Product purity was evaluated with the SciEx PA 800 Plus Pharmaceutical Analysis System. Blue lines represent process impurities which are not detectable by SDS-PAGE. The impurities detected were less than 1%. Viral protein bands 1, 2 and 3 can be seen. The peak labelled 'VP3 Species" is seen in all purified samples of Serotype A and remains to be fully characterized. This sample was 99% pure.

Highly Enriched

- Other release criteria of AAV purified to final fill, such as empty/full capsid ratios, are also comparable those obtained by analytical ultracentrifugation.
- Empty/Full ratios were measured via HPLC **(3)** and AUC, demonstrating similar results.
- Both methods report 88-98% full capsids.

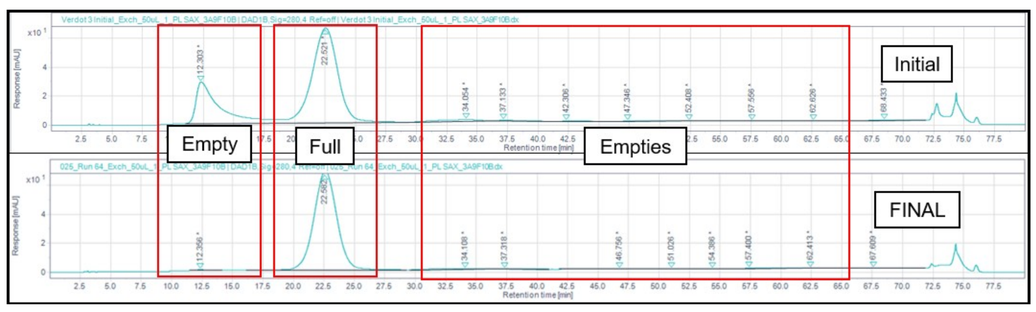

Figure 6: Chromatogram of Empty/Full Analysis by HPLC. Empty and full capsids are fully resolved using a QA method under high pressure. Multiple empty peaks are detected and have been confirmed with a control. 'Initial' represents a sample before the separation step. 'Final' shows the sample after the PA separation method. Nearly all empties are removed while maintaining most full capsids.

References

(1). Development of a robust suspension platform for viral vector manufacturing.
Samir Acharya. Talk presented**: Tools and Technology Forums 2.0**. ASGCT 25th Annual Meeting, Washington, DC, May 2022.

(2). Derivation of a clonal HEK293 suspension cell line for high yield AAV production. Poster 301.
Michael Mcilhatton, Brittany Hurley, Sarah Bergman, Hannah Roodhouse, Byron Carper, Matthew Janson, John Ketz & Samir Acharya. ESGCT 29th Congress, Edinburgh, Scotland, Oct. 2022.

(3). Novel HPLC applications for rapid in-process characterization during AAV platform development. Poster 331.
Matthew Janson, Byron Carper, Hannah Roodhouse, John Ketz & Samir Acharya. ESGCT 29th Congress, Edinburgh, Scotland, Oct. 2022.

Scale-Up

- This process has been performed at the 50 and 200L scale.
- Recoveries are presented in Table 1. Different production methods demonstrate robustness of the purification process.
- Initial analysis of Empty/Full ratios for AAV serotype A with HPLC is ≥90%. This analysis will be confirmed and correlated with AUC.
- Chromatography performed with VERDOT Ips^2 Flexipro Chromatography system, pictured below.
- Single-use flowpaths available at multiple scales with the same piece of equipment. Flowpaths are capable of flow rates from 1 to 600 LPH.
- Future work will involve purification at 500 and 2000L scales.

Table 1: ddPCR yield analysis of all-column bioreactor runs

Bioreactor	Sample	Yield (vg)	Recovery
STR50-1	TFF	4.53E+15	32.89%
	Final	1.49E+15	
STR50-2	TFF	2.12E+16	59.91%
	Final	1.27E+16	
STR200	TFF	7.23E+16	40.94%
	Final	2.96E+16	

For inquires contact:

andelynbusiness@andelynbiosciences.com