Novel HPLC Applications for Rapid In-process Characterization During AAV Platform Development

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Abstract: Andelyn Biosciences has developed a robust scalable suspension platform for Adeno Associated Virus (AAV) production suitable for all phases. It relies on utilizing a quality-by-design (QbD) approach to ensure both scalability and quality of AAV. Development of in-process analytics for tracking of AAV purity and quality at different stages of production is an integral part of the platform. HPLC can allow for rapid and accurate analysis which are critical to enable fast, robust development cycles. Various HPLC methods will be presented supporting the characterization of in-process AAV development samples. One of the described HPLC methods uses SEC/UV that provides capsid quantitation information in addition to protein impurity profiling. This method can analyze crude up through final samples. In a second method, anion exchange (AEX) chromatography is used to resolve and quantitate empty and full capsids. AEX data at final stages of the suspension process showing the separation of empty and full capsids will be presented. Additionally, process characterization AEX data will be presented that correlate with outsourced AUC data but requiring only a day for data turnaround. Thirdly, multiple in-process impurities are monitored using a single reversed-phase HPLC method. Data that tracks these impurities throughout the downstream purification process will be presented.

SEC/UV HPLC Method

- The SEC/UV method is serotype-independent and simultaneously determines:
  - Impurity profiles
    - High MW species (> capsid MW)
    - Protein impurities
  - Capsid titer estimation
- Robust method with a 30-minute injection time per sample
- Impurity profiling and capsid titer estimation can be carried out from clarification though final samples
- Method development work indicates this analytical method does not influence/skew results

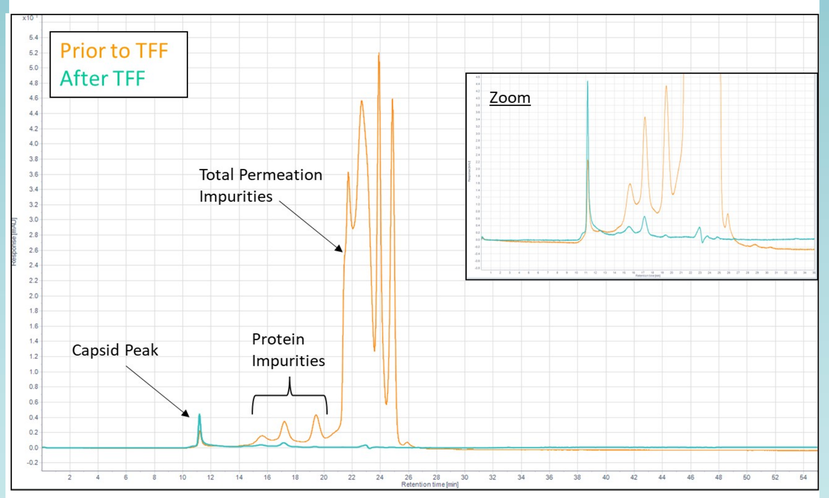

Figure 1. Overlay of before and after TFF processed sample on the SEC/UV HPLC method

- This SEC/UV method can demonstrate impurity clearance throughout various process steps
- Capsid titer can also be measured to determine losses and yields for each step

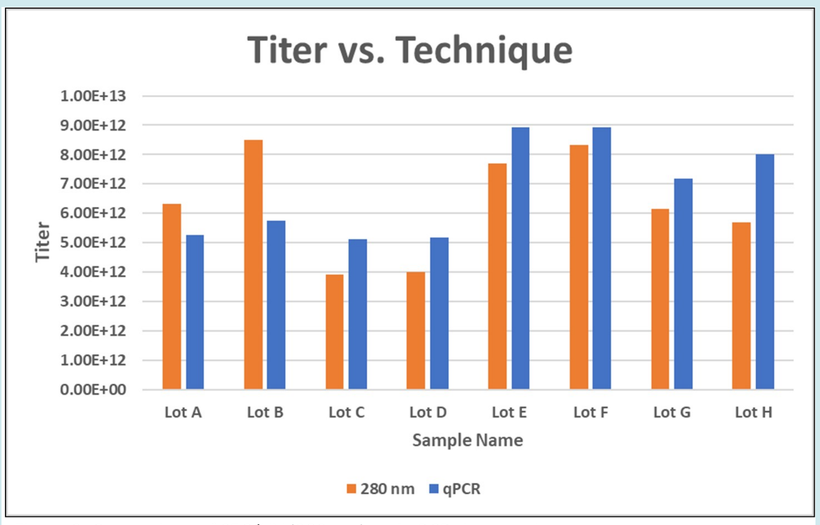

Figure 2. Comparison of SEC/UV (280 nm) and qPCR titer results

- Rapid capsid titer read outs are provided by this SEC/UV method
- SEC/UV titers are similar to PCR results but with improved precision and sensitivity
- This method was used to support the work in **Poster 280 (2)**

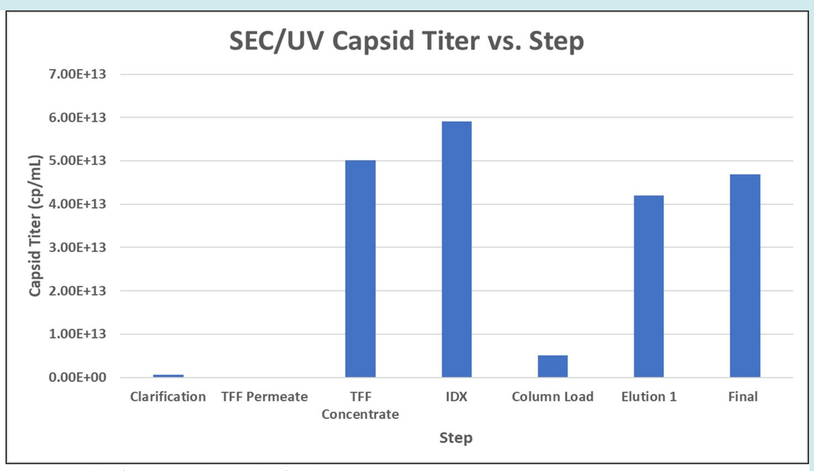

Figure 3. SEC/UV capsid titer (cp/mL) versus process step

- SEC/UV can track capsid titers from clarification up through final

Empty/Full HPLC Method

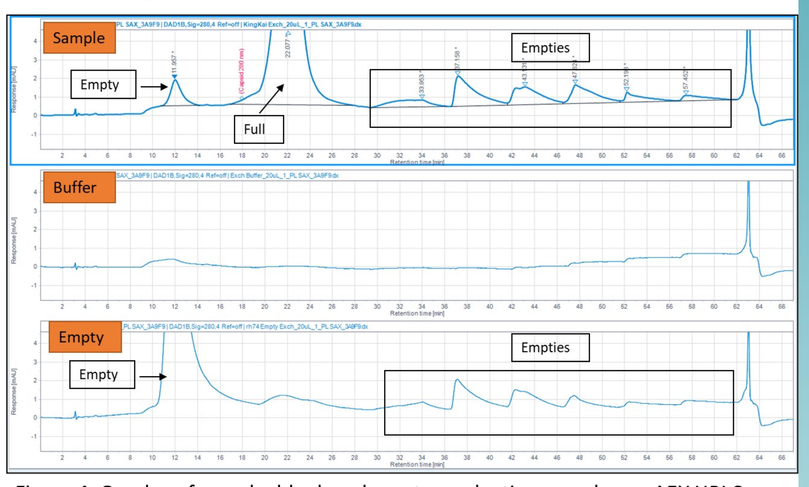

Figure 4. Overlay of sample, blank and empty production samples on AEX HPLC method

- Good resolution of empty and full peaks
- Data turn around for AEX method is < 1 day

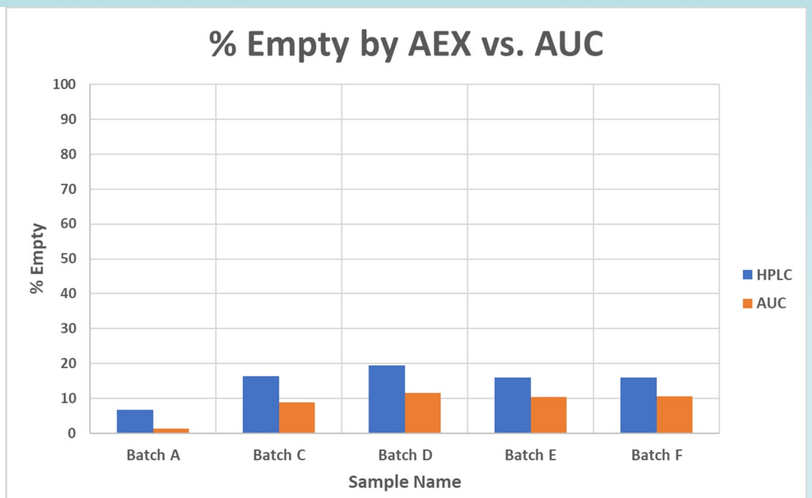

Figure 5. Comparison of AEX and AUC % empty values for different productions

- AEX data is typically higher than AUC values
- AEX data is trending with AUC values

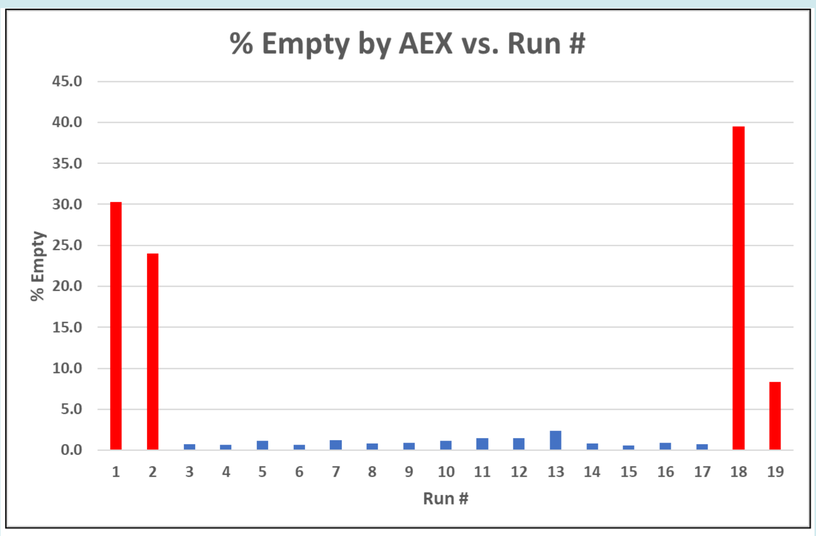

Figure 6. AEX % empty results for various production runs. The red bars are samples prior to empty capsid separation and the blue bars are sample after empty separation.

- AEX method can discern various amounts of % empty capsids across multiple productions
- This AEX method can quickly read out % empty capsids for ~ 20 samples in one day
- This method was used to support the work in **Poster 280 (2)** and **Poster 301 (3)**

Conclusion

- Three HPLC methods were presented here which enable rapid process development characterization:
  - SEC/UV method for impurity profiling and capsid titer estimation
  - AEX method for the determination of % empty capsids
  - Simultaneous quantitation of chloroquine, IDX and phenol red

Chloroquine, IDX and Phenol Red HPLC Method

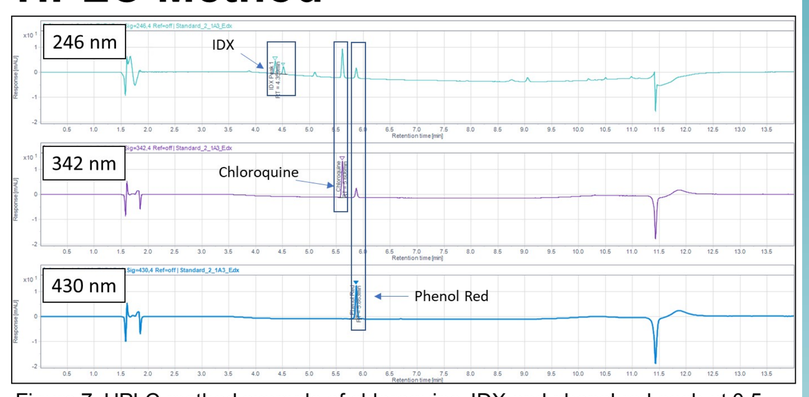

Figure 7. HPLC method example of chloroquine, IDX and phenol red each at 0.5 µg/mL

- Method LOQ currently at 0.05 µg/mL
- Single method to resolve all 3 impurities in 14 minutes
- Can likely supplement with additional compounds for enhanced tracking

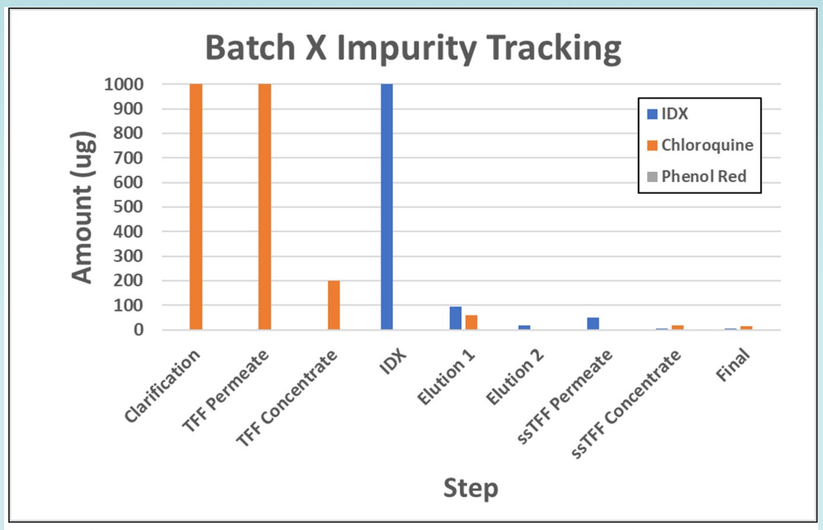

Figure 8. Impurity tracking from clarification through final in Batch X

Step	IDX Conc (ug/mL)	IDX Conc (ug)	Chloroquine Conc (ug/mL)	Chloroquine Conc (ug)	Phenol Red Conc (ug/mL)	Phenol Red Conc (ug)
Clarification	ND	ND	1.56	78749	ND	ND
TFF Permeate	ND	ND	1.14	82343	ND	ND
TFF Concentrate	ND	ND	0.23	200	ND	ND
IDX	11438	29281264	< 0.05	< LOQ	ND	ND
Elution 1	0.63	95	0.39	59	ND	ND
Elution 2	1.67	17	< 0.05	< LOQ	ND	ND
ssTFF Permeate (100 kDa)	0.13	51	< 0.05	< LOQ	ND	ND
ssTFF Concentrate (100 kDa)	0.06	6	0.17	18	ND	ND
Final (100 kDa)	0.05	5	0.13	13	ND	ND

Table 1. Chloroquine, IDX and phenol red tracking from clarification up through final for Batch X

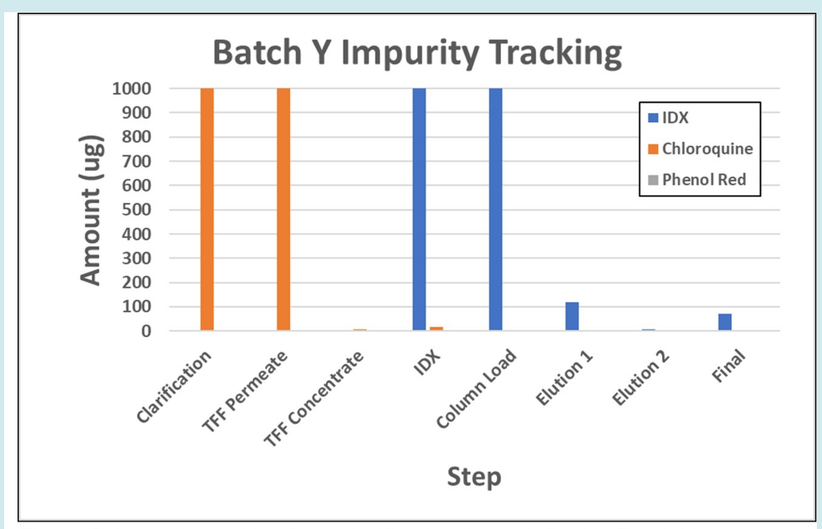

Figure 9. Impurity tracking from clarification through final in Batch Y

Step	IDX Conc (ug/mL)	IDX Conc (ug)	Chloroquine Conc (ug/mL)	Chloroquine Conc (ug)	Phenol Red Conc (ug/mL)	Phenol Red Conc (ug)
Clarification	ND	ND	3.6	9119	ND	ND
Permeate	ND	ND	1.8	5506	ND	ND
TFF Concentrate	ND	ND	0.1	6	ND	ND
IDX	>> 5	Overloaded	1.1	18	ND	ND
Column Load	>> 5	Overloaded	< 0.05	< LOQ	ND	ND
FT&W	>> 5	Overloaded	< 0.05	< LOQ	ND	ND
Elution 1	8.5	119	0.2	2	ND	ND
Elution 2	4.2	8	< 0.05	< LOQ	ND	ND
Final	5.4	71	0.2	2	ND	ND

Table 2. Chloroquine, IDX and phenol red tracking from clarification up through final for Batch Y

- Method shows clearance and low/no levels of chloroquine, IDX and phenol red in the final product though purification

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). A robust downstream strategy for high quality AAV using an all-column purification method. Poster 280. Byron Carper, Hannah Roodhouse, John Ketz, Matthew Janson & Samir Acharya. ESGCT 29th Congress, Edinburgh, Scotland, Oct. 2022.

(3). 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

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