Simplifying Progress

Comparability Study Between Ion-Exchange Monolith and Affinity Resin for Purification of AAV8

Janja Merkelj Koren[1], Rok Žigon[1], Andrej Mihevc[1], Marko Šnajder[1], Maja Leskovec[1] and Aleš Štrancar[1]

[1]Sartorius BIA Separations d.o.o, Mirce 21, SI-5270 Ajdovščina, Slovenia
Corresponding author: Maja. Leskovec@sartorius.com

Introduction

The main objective of every downstream process (DSP) for AAV is to achieve high recovery while delivering the purest, most potent product possible. The capture step in AAV gene therapy is either affinity or cation exchange chromatography, which both concentrates the product and removes impurities. Following the capture, the eluate is generally further processed to enrich for full capsids and further purification. For this enrichment and polishing of full AAV capsids, CIMmultus QA, a monolith-based anion exchange chromatography, is widely used (Rieser, 2021).

Since the polishing step relies on only small differences in charge of the AAV capsids, any process-induced heterogeneity or charge modulation of the capture eluate will diminish the separation efficiency and affect the step's robustness. Affinity elution sample is reported to contain additional impurities (Martin, 2022), which influences subsequent steps of the DSP. Processing time is critical to an efficient process since a faster process has an overall lower financial footprint.

A side-by-side comparison was performed using CIMmultus SO3-1mL (2um) column and commercially available affinity resin which binds several AAV serotypes. Both columns were evaluated for process and step recoveries, impurity reduction, product capacity and processing time. The results shown are based on two parallel experiments for each capture approach.

1. Experimental design

Two AAV8 batches of HEK293 suspension material with vector genome titer 2E+10 vg/mL (8.7E+10 vp/mL) were clarified and further processed by TFF and DNase treatment. The TFF retentate was divided and the two capture strategies were performed followed by a polishing step.

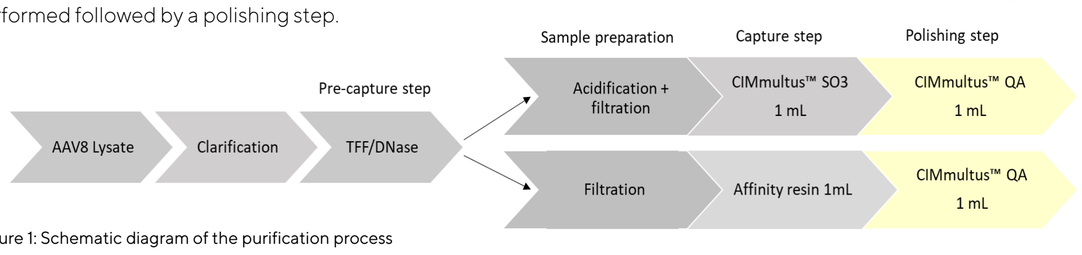


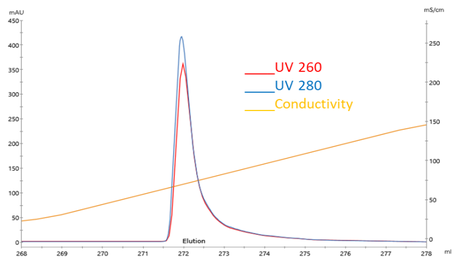
Figure 1: Schematic diagram of the purification process

Both processes were compared in terms of:

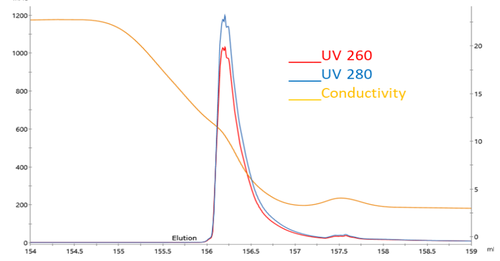
- Process and step recoveries (dPCR, PATfix biochromatography system)
- Product and process-related impurity reduction - empty capsids (PATfix biochromatography system, MP), residual hcDNA (qPCR), pDNA (dPCR), HCP (ELISA HEK293 kit), ETX (CR Endosafe), protein content (SDS-PAGE)
- Capacity (determined from TFF retentate or clarified lysate)
- Processing time

2. Results

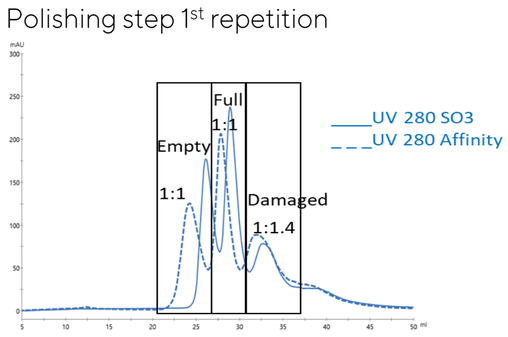
A) Capture step SO3



B) Capture step affinity



C) Polishing step 1st repetition



D) Polishing step 2nd repetition

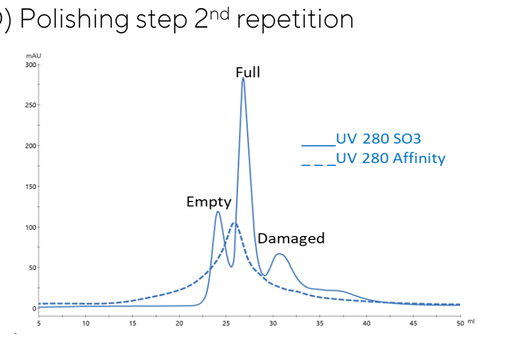
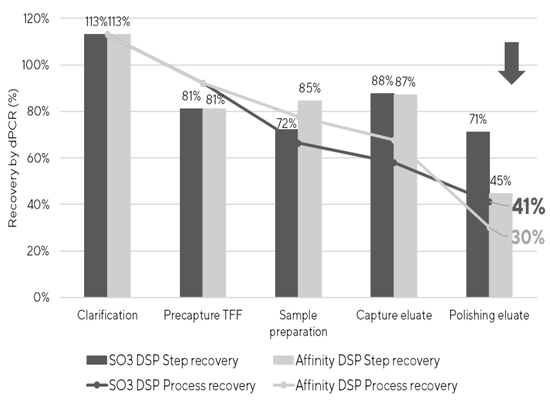


Figure 2: Chromatographic elution profiles of capture step (A and B) and their corresponding polishing QA run (C and D). Overlay of absorbance at 280 nm. Overlay of polishing step runs in 1st (C) and 2nd repetition (D).

In the first repetition of the polishing step, ratios for empty and full peak were maintained, however the 'damaged' peak was slightly more pronounced in the case of affinity purification (ratio 1:1.4, Figure 2C). Moreover, a peak migration to the left occurred in affinity approach (17.23 mS/cm in SO3 approach and 16.34 mS/cm for full in affinity process) (Figure 2C). The second repetition showed irreproducibility of the polishing step after affinity, although binding conditions were met (Figure 2D). Fractions of this run could not be included in the performed analytics. Lack of robustness of affinity process will be subject of further research.

3. Process and step recoveries

A) Process and step recoveries by dPCR



B) Process and step recoveries by PATfix biochromatography system

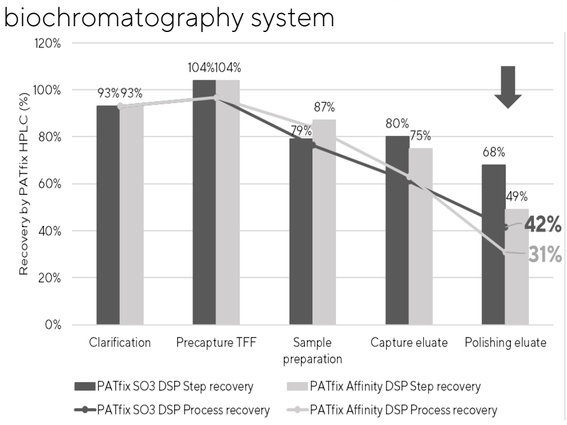


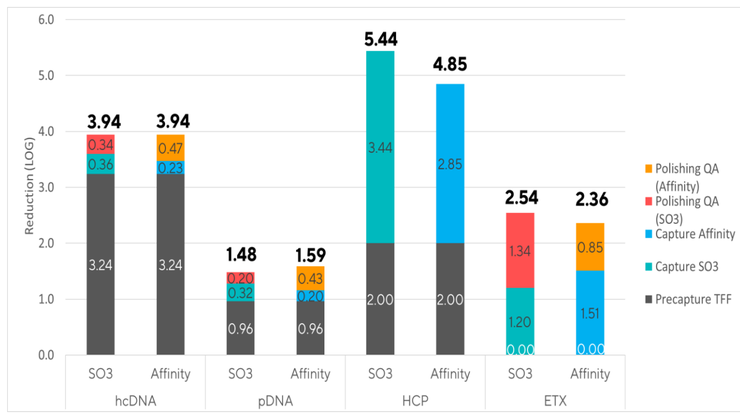
Figure 3: Process and step recoveries for both processes. (A) Analyzed by dPCR. (B) Analyzed by PATfix biochromatography system CEX-FP analytics. Results shown in step recoveries are average values of the two repetitions. Highest discrepancies between the two processes are seen on polishing step (see arrow).

The overall process recovery for the SO3 approach was 41% or 42%, in contrast to 30% or 31% for the affinity, based on orthogonal dPCR and PATfix system analytics, respectively (Figure 3). The two processes are comparable in recoveries except on final polishing step, where SO3 approach gives significantly better step recovery (arrow).

4. Impurities reduction

The two processes have comparable hcDNA, pDNA and ETX reduction efficiency, while the SO3 process has statistically significant better HCP reduction (additional 0.6 log). The majority of the hcDNA and pDNA reduction in the pre-capture step was due to DNase treatment conducted within TFF (Figure 4A). Based on mass photometry results, the full capsid enrichment step was better with the SO3 process (from 30% full in starting material to 72.3% in final fraction), compared to the affinity process (from 30% full in starting material to 56.5% in final fraction) (Figure 4C). Better results, and comparable with mass photometry, regarding purity in the final SO3 process polishing fraction, were also obtained by the PATfix biochromatography system (75.9% vs 54.6%)(Figure 4D).

A) Log removal for hcDNA, pDNA, HCP and ETX impurities



B) SDS PAGE

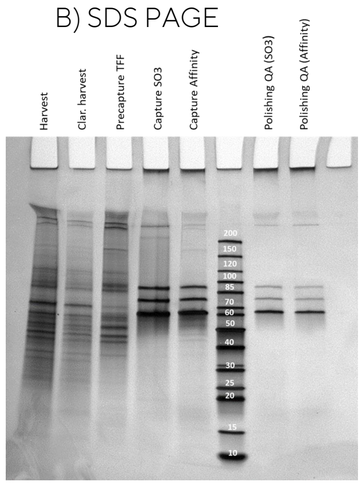
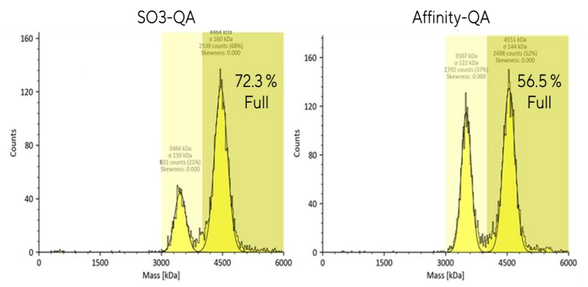


Figure 4 (A): Comparison of impurity reduction of both capture approaches. Bolded values represent total reduction - sum of individual steps. (B): Silver stained SDS page for both processes at each purification step. A total of 4E+9 vg was loaded per well for capture and polishing main elution fractions. Bands above 200 kDa present in both polishing steps correspond to vg DNA, only found in full capsids.

C) E|F ratio by Mass photometry analytics



D) E|F ratio by PATfix biochromatography system analytics

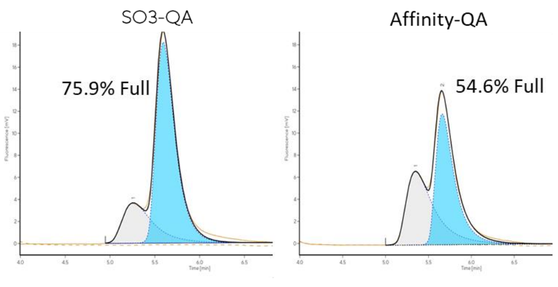


Figure 4: Percentage of full AAV capsids in the main QA elution measured by mass photometry (C) and PATfix analytics (D) Examples shown are from the 1st repetition.

5. Column capacity

Capacity was in favor for SO3 column. Implementation of a TFF step increases the capacity by 17-fold compared to direct harvest loading. For the affinity column, capacity using clarified harvest was not performed due to non-process feasible loading time duration (> 100 hrs).

A) Capacity using TFF retentate

	vg per mL column (dPCR)	vp per mL column (ELISA)	vp per mL column (PATfix AEX)
SO3	5.03E+13	2.23E+14	1.52E+14
Affinity	4.64E+13	2.06E+14	1.41E+14

B) Capacity using clarified lysate

	vg per mL column (dPCR)	vp per mL column (ELISA)	vp per mL column (PATfix AEX)
SO3	3.14E+12	1.64E+13	8.97E+12
Affinity	Loading not performed > 100 hrs		

Table 1 (A): Capacity of TFF retentate loaded for both columns calculated by three different analyses. (B): Capacity of clarified harvest loaded for both columns calculated by three different analyses.

6. Results – Process time comparison

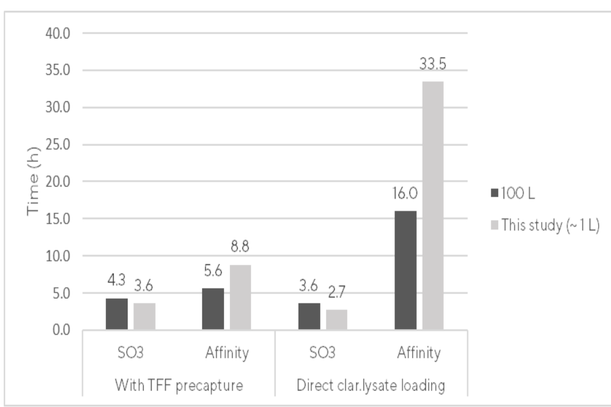


Figure 5: Actual processing time as performed in study and predicted comparison of processing time for purification of 100L batch with titer 1E+11 vg/mL (1E+16 total vg) from the initial material up to capture step eluate. Calculations for predicted comparison were based on using a 5 m² large TFF membrane and running TFF at 30 LMH permeate flow rate. For the SO3 process a 400 mL column* and flow rate of 2 CV/min was chosen and for the affinity process a 430 mL* column and 0.33 CV/min were taken into account. For the direct lysate loading, we considered a 4000 mL SO3 column* and flow rate of 1 CV/min, on the other hand, for the affinity process, the same column size and flow rate as above.

**Column size was chosen with regard to experimental capacity results.*
***For the affinity, column packaging time was not taken into consideration.*

The SO3 step performed more quickly than the affinity step with and without the TFF load preparation step. This was true for the small-scale runs and the calculated 100-L runs.

7. Conclusions

- The full process that included the SO3 capture step produced a 30% increase in doses to the clinic compared to the full process that included the affinity capture step.
- Comparable reduction of impurities were seen for the two capture steps, except HCP removal and empty capsid removal were better with the SO3 column.
- Up to 2x shorter processing times were seen when using SO3 columns than when using affinity columns.

8. References

- Martin at al., Adeno-associated virus process development: optimization & development of a scalable elution for polishing chromatography, Cell & Gene Therapy Insights 2022; 8(3), 421 – 429
- Rieser at al., Comparison of Different Liquid Chromatography-Based Purification Strategies for Adeno-Associated Virus Vectors. Pharmaceutics 2021, 13, 748.