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Corresponding author: Austin T. Raper (medinfo@sarepta.com)

Step Gradient Development for Full Particle Enrichment of AAV on Anion-Exchange Monoliths is Hindered by Inconsistent Column Lots

AT Raper,* AJ Reed, S Hartbarger, KA Barber, J Zhang, Y Oh

Sarepta Genetic Therapies Center of Excellence, Columbus, OH

*Austin T. Raper is presenting on behalf of the author group

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Objective

To develop an AEX chromatography method that uses a step gradient/isocratic elution strategy for full particle enrichment of affinity-purified AAV preparations that is robust and simple to execute at scale.

Key Takeaways

Isocratic step gradient elution is limited by lot-to-lot inconsistency between monolith columns as seen at small (1 mL) and large scale (80 mL).

We recommend scale up of the monolith using a linear gradient method or using a packed resin bed for isocratic methods.

BACKGROUND

- Empty capsids are by-products of AAV production and are generally accepted as undesirable in drug product preparations due to complications surrounding immunogenicity and dose-related toxicity.
- Recent proposed draft guidance for FDA consideration recommends minimizing empty capsids in AAV-based gene therapy products.[1,2]
- AEX chromatography is often used to enrich for full AAV particles. Development of isocratic methods is ideal as they are easier to execute at scale, promising less user interaction, less buffer consumption, and less eluate volume.

METHODS

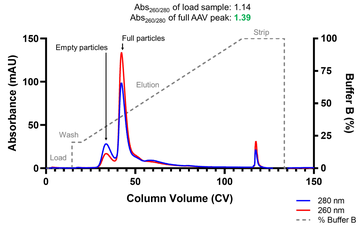
- Triple transfection of suspension-adapted HEK293 cells was used to produce AAV containing a model transgene. Cultures were processed following a downstream process and AAV was purified by affinity chromatography. Affinity column eluate from multiple production lots served as loading material for AEX chromatography process development.
- Vector genome titers were measured by qPCR and percentage of full AAV particles was measured by SEC-MALS.

CONCLUSIONS

- CIMmultus QA monolith AEX columns from Sartorius show great potential for full particle enrichment of AAV preparations via linear gradient elution.
- Efforts to develop and scale an isocratic step gradient elution method resulted in several failed chromatography runs showing significant breakthrough of full particles in the empty particle wash step.
- Run failures could be simulated at small scale; testing of different sample material, buffer lots and column lots revealed that inconsistencies among column lots resulted in the run failures.
- Given the inconsistencies across column lots, we recommend scale up of these monoliths using a linear gradient elution strategy or development of isocratic methods on packed resin beds.

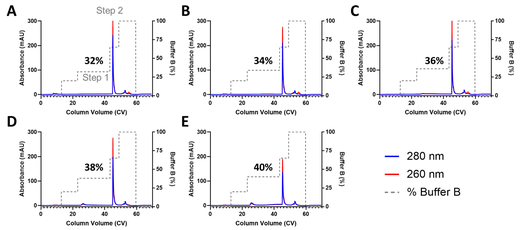
RESULTS

Full AAV particle enrichment on the CIMmultus QA monolith by linear gradient elution



- Vector genome recovery of >90% and full particle enrichment of ≥90% (from ~40%).

Development of isocratic step gradient elution for AAV on the CIMmultus QA monolith

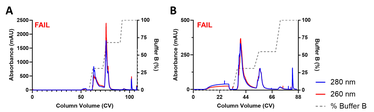


- 38% Buffer B in Step 1 results in similar recovery and enrichment relative to the linear gradient method (Table 1).

Table 1. Summary of Isocratic Step Gradient Development Runs at the 1 mL CIMmultus QA Monolith Scale

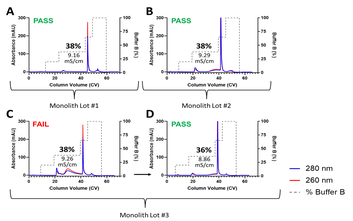
Percentage B in Step 1 wash, %	Vector genomes injected	Vector genomes in Step 1 wash, %	$Abs_{260/280}$ of Step 2 elution
32	1.4E13	0	1.30
34	1.4E13	0	1.30
36	1.4E13	0.2	1.34
38	1.4E13	2.0	1.39
40	1.4E13	18.1	1.42

Isocratic step gradient runs at the 80-mL CIMmultus QA monolith scale



- Significant breakthrough of full particles observed in Step 1.

Root cause identification for CIMmultus QA monolith run failures

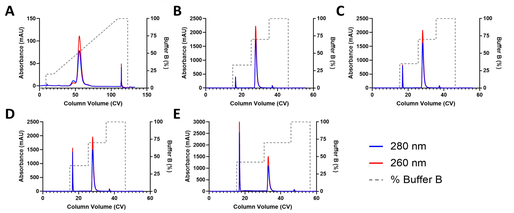


- Run failures were simulated at small scale (1 mL monoliths).
- Testing revealed that run failures occurred across different column lots (Table 2).

Table 2. Summary of root cause identification for run failures at the 1-mL CIMmultus QA monolith scale

Run ID	Vector genomes injected	Monolith lot	Sample lot	Buffer lot	Percentage Buffer B Step 1, %	Step 1 conductivity (mS/cm)	Full particles in Step 1?
4A	1.4E13	1	1	1	38	9.16	Minimal
4B	4.7E13	2	2	2	38	9.29	Minimal
4C	4.7E13	3	2	2	38	9.24	Yes
4D	4.7E13	3	2	2	36	8.86	Minimal

Development of isocratic step gradient elution for AAV on a packed-bed AEX column



- Resolution between empty and full AAV particles is smaller compared to CIMmultus QA.
- Isocratic step gradient is possible, but there is strong anti-correlation between yield and enrichment (Table 3).

Table 3. Summary of isocratic step gradient development runs on a 1-mL packed-bed AEX column

Run ID	Percentage B in Step 1 wash, %	Vector genomes injected	Vector genomes in empty particle eluate or Step 1 wash, %	Full particle enrichment of eluate, %
5A	Linear gradient	5.4E14	0	90
5B	33	5.4E14	12	66
5C	35	5.4E14	20	70
5D	37	5.4E14	22	75
5E	42	5.4E14	40	96

REFERENCES

1. Dark Horse Consulting. Proposed draft guidance for FDA consideration. darkhorseconsultingroup.com/wp-content/uploads/2022/05/DHC_Proposed-DRAFT-Guidance-for-FDA-Consideration.pdf (Accessed May 2023).
2. U.S. Food and Drug Administration. Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC) Meeting #70. https://www.fda.gov/media/151599/download (Accessed May 2023).

ABBREVIATIONS

AAV, adeno-associated virus; AEX, anion-exchange; FDA, Food and Drug Administration; qPCR, quantitative polymerase chain reaction; SEC-MALS, size exclusion chromatography with multiangle light scattering.

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