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Application Note

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Advanced AAV Polishing Purification: Ensuring Consistency, Scalability, and Purity with CIMmultus® QA HR Columns

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Abstract

The adeno-associated virus (AAV) polishing purification step is essential for removal of product-related impurities such as empty AAV capsids. Ensuring reproducibility in this purification step is a key challenge, requiring chromatographic columns that can be reliably scaled from laboratory to industrial levels.

Quaternary amine (QA) modified CIMmultus® columns are extensively utilized in the polishing purification step of AAV vectors. To ensure reproducible product purity and recovery, a separation in an ascending linear salt gradient is preferable to minimize variability between different chromatography purification runs. While linear gradients have been commonly used with CIMmultus® QA columns (non-HR production line), step elution gradients are generally preferred for simplifying the process and reducing buffer consumption. However, reproducible separation within a step gradient for such a demanding separation as AAV separation requires extreme control over both process parameters and column-to-column repeatability.

Introduction

CIMmultus® QA HR columns provide a scalable, efficient, and cost-effective chromatographic columns, allowing reproducible full AAV step elution enrichment across the entire column scale and between different column batches.

Both CIMmultus QA and CIMmultus QA HR columns are produced using the same quaternary amine (QA) ligand. However, CIMmultus QA HR implements a specialized quality control (QC) testing procedure with extremely narrow release criteria that are based on the conductivity values of empty AAV capsid elution.

Abbreviation "HR" stands for high reproducibility, ensuring consistent performance across different column batches, which is crucial for high-precision and sensitive separations of empty and full AAV capsids.

CIMmultus QA HR columns are available in volumes from 1 mL to 8 L, supporting a seamless transition during scale-up and delivering robust, scalable, and reproducible AAV purification.

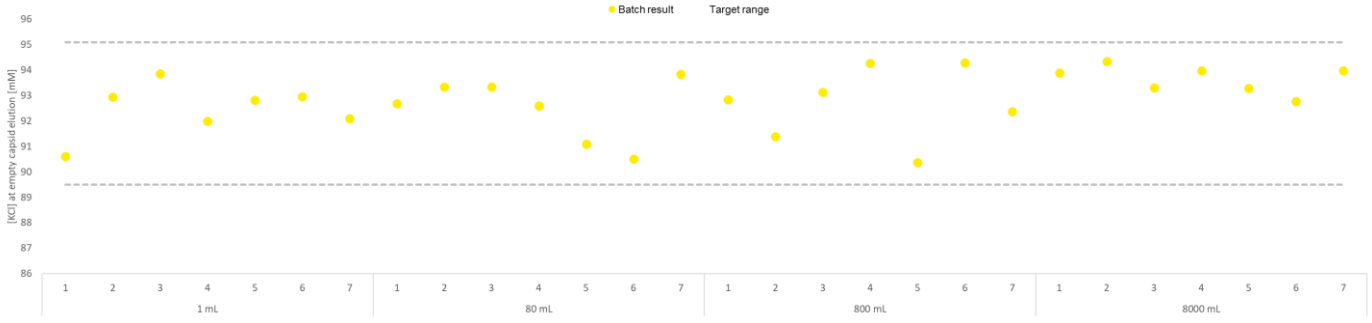
CIMmultus QA HR for AAV Empty-Full Separation

Implementation of the Column Released Test Based on the AAV Empty-Full Separation Performance

The reproducibility of AAV downstream processing is crucial when transitioning from R&D to manufacturing scale and to maintain consistent performance in AAV manufacturing. The AAV polishing purification process is sensitive not only to variations in chromatographic consumables, but also differences between characteristics of different AAV samples and purification process-derived factors. Each of them can affect the robustness and reproducibility of empty-full AAV purification.

CIMmultus QA HR columns are released based on the extremely sensitive chromatographic quality control (QC) method described in Specimen QA HR Method Guide. CIMmultus QA HR monolith batches regardless of the column scale, demonstrated excellent material consistency (Figure 1).

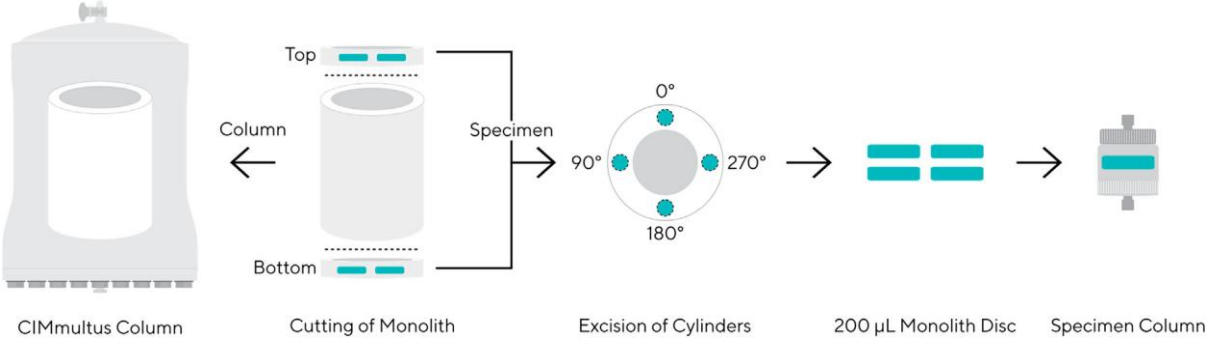
Figure 1: *Material consistency of CIMmultus QA HR monoliths, ranging from 1 mL to 8000 mL, is verified using a QC method that separates empty-full AAV capsids of an AAV standard at a specific KCl concentration within an ascending linear elution gradient. The acceptable release range for CIMmultus QA HR is the elution of empty AAV capsids at 92.3 mM KCl ± 3%.*



Specimen QA HR for Batch Testing Before Use of Parental cGMP CIMmultus QA HR

The CIMmultus QA HR cGMP column (parental column) and its Specimen QA HR are delivered in the same shipping box. Specimen QA HR is a small non-GMP unit, extracted from the same batch of bulk monolith as its parental column (Figure 2).

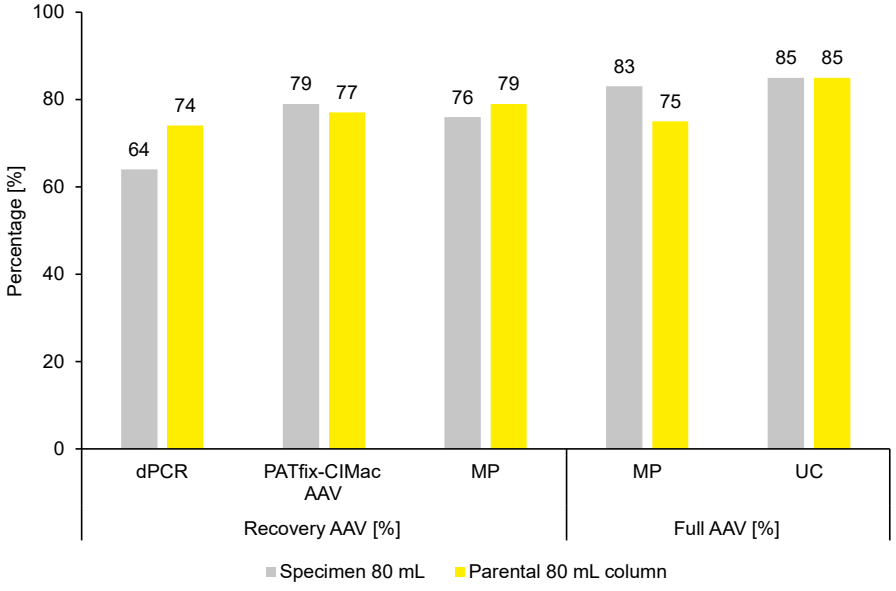
Figure 2: *Schematic representation of obtaining parental columns and specimen columns from the initial monolith. Dashed lines represent cutting or drilling.*



Specimen QA HR 0.2 mL unit matches chromatographic properties of parental column. They are commonly used for small-scale testing of monolith performance before large-scale cGMP chromatography runs, serving as a control for monolith material to prove lot-to-lot consistencies. Additionally, it can evaluate new AAV sample chromatographic characteristics and support initial process development by conserving the AAV feed stream, allowing more experiments with the same amount of AAV sample.

A comparative analysis was conducted to demonstrate that the chromatographic properties of the Specimen QA HR align with those of the large 80 mL parental column from which it was extracted. This analysis assessed the consistency of key chromatographic parameters between the 0.2 mL specimen and the parental column. The results show that the recovery and full particle percentages of the main fractions are comparable (Figure 3).

Figure 3: *Specimen and its parental column analytical comparison of recovery by dPCR, PATfix-CIMac AAV, and mass photometer (MP), and the percentage of full AAV particles by MP and ultracentrifugation (UC).*



A comprehensive explanation of the Specimen QA HR unit, including its production process and the evaluation of CIMmultus QA HR column homogeneity using the Specimen unit, is available in the scientific publication: Development and Validation of AAV Capsids Separation on Specimen Columns for Reproducibility Evaluation of Large-Scale Chromatographic Monoliths.

Scale up with CIMmultus QA HR For AAV Enrichment Step on Novel CIMmultus QA HR Line

For initial process development, linear gradient elution is recommended to identify conductivity values at elution of different AAV capsids (e.g., empty AAV, partially filled, full AAV). Once optimal elution conditions are determined on CIMmultus QA HR, it allows for a straightforward transition to step elution at the same conductivity (iso-conductivity scale-up). A case study demonstrates scaling up AAV polishing purification process with CIMmultus QA HR at different purification scales using step gradient elution, validating step gradients in bind-elute and empty flowthrough modes.

Case study: Considerations for Scaling up From CIMmultus QA HR 1 mL to 8 L Using Step Gradient Elution

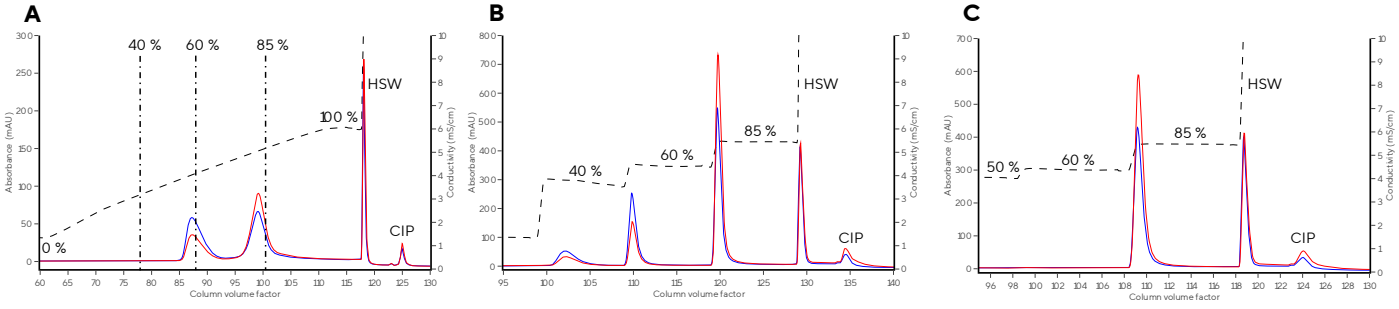
In these experiments, CIMmultus QA HR column was used in combination with the improved chromatographic method described in the document Enhanced Separation of AAV Capsids Using Analytical Anion Exchange Monolithic Columns: Emphasis on Organic Modifiers. Optimal elution conditions were identified from the linear gradient elution chromatogram (Figure 4A), by analyzing the conductivity and percentage of buffer B at which empty and full particles elute. Subsequently, two purification runs with different step gradient modes were evaluated: bind-elute (Figure 4B) and empty flowthrough (Figure 4C). Step washes were performed using individually prepared buffers based on the actual eluent concentration rather than relying on the percentage of B pump mix of a buffer set A/B. Additionally, buffer temperature was adjusted to the working range during preparation, and the exact mass of titrant at a specific temperature was determined. For all three approaches same buffers were used:

- buffer A: 20 mM Tris, 5 mM MgAc2, 1 % sorbitol, 0.1 % poloxamer 188, 2.5 % EtOH, pH 8.5
- buffer B: 20 mM Tris, 50 mM MgAc2, 1 % sorbitol, 0.1 % poloxamer 188, 2.5 % EtOH, pH 8.5
- high salt wash (HSW): 0.5 M TRIS + 1 M NaCl, pH 7.5
- cleaning in place (CIP): 0.1 M NaOH + 2 M NaCl

For a more precise assessment of conductivity values, a purification run using short incremental steps can be conducted before transferring the process directly from linear to step elution.

Figure 4: *(A) Linear gradient elution mode on a CIMmultus QA HR 1 mL column with the improved E/F method. Three optimal elution conditions were identified based on conductivity. These conditions facilitated a straightforward transition to stepwise elution at the same conductivity levels, ensuring efficient and reproducible purification.*
(B) Bind-elute mode with step wash elutions on a CIMmultus QA HR 1 mL column with the improved E/F method. Three pre-determined step washes (40 %, 60 %, and 85 % of buffer B) were conducted and sequentially eluting empty particles, intermediate species, and full particles, ensuring efficient separation and purification.
(C) Empty flowthrough mode with step wash elutions on a CIMmultus QA HR 1 mL column with the improved E/F method. Loading the sample at 50 % buffer B and displaced empty particles in flowthrough, two pre-determined step washes (60 % and 85 % of buffer B) were conducted to elute the full AAV from the column.

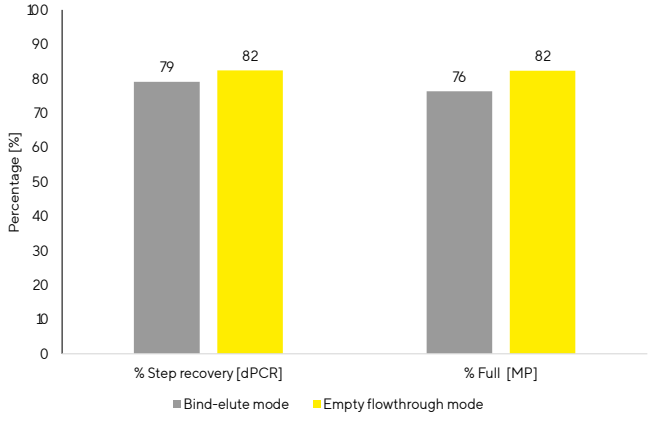
Legend: UV260 red solid line, UV280 blue solid line, conductivity black dashed line, HSW (high salt wash), CIP (cleaning in place).



The bind-elute approach offers more safety and control over loading and elution steps. However, the empty flowthrough approach opens possibilities for less buffer consumption.

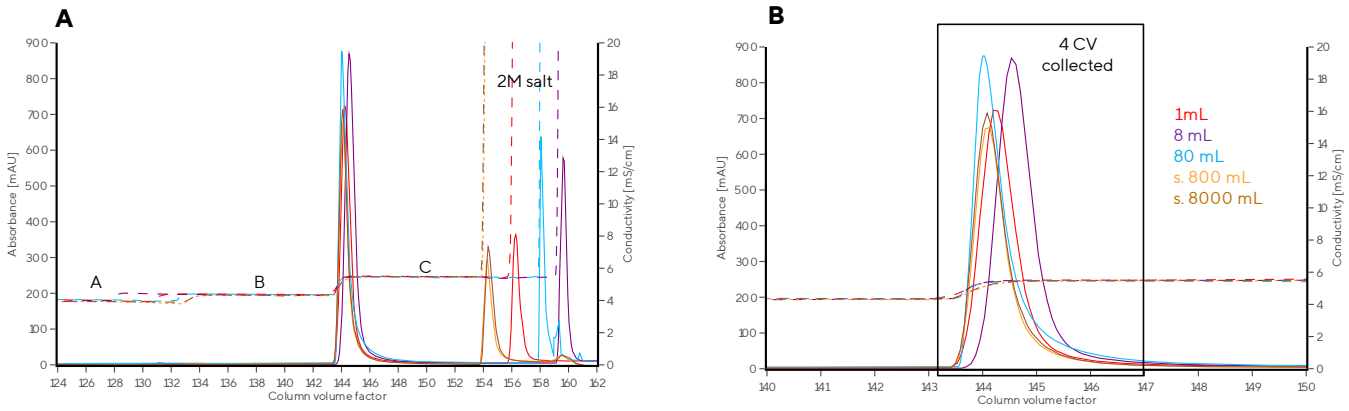
A comparison of recovery (dPCR) and the percentage of full particles (MP) for both approaches (bind-elute and flowthrough) resulted in comparable values (Figure 5).

Figure 5: *Comparison of dPCR recovery and percentage of full particles (MP) for bind-elute (grey columns) and empty flowthrough mode (yellow columns).*



The CIMmultus QA HR line was then evaluated for scalability with empty flowthrough approach across a range of column sizes: 1 mL, 8 mL, and 80 mL, specimens from 800 mL and 8000 mL columns. Consistent chromatogram elution profiles across the entire scale from 1 mL to 8000 mL columns are shown in Figure 6, using the same eluent concentration as in empty flowthrough mode (Figure 4C).

Figure 6: *(A) Overlay of elution chromatograms for scale up over most of CIMmultus QA HR column scales: CIMmultus QA HR 1 mL (red line), 8 mL (purple line), 80 mL (blue line) and QA HR specimen 800 mL (orange line) and 8000 mL (brown line) with empty flowthrough approach and zoom in of elution fractions **(B)**. The observed elution shift was attributed to the different chromatography systems used and not to the columns themselves.*



The main elution fractions of all five runs were analysed in triplicate for vector genome recovery and percentage of full AAV capsid. The data confirms excellent reproducibility across the entire scale when using empty flowthrough approach (Table 1).

Table 1*: Main elution fractions and their average values across the scale analyzed for vector genome recovery by dPCR, PATfix-CIMac AAV and MP analysis, and PATfix UC, MP, and charge-detection mass spectrometry (CD-MS) analysis to determine the percentage of full AAV capsids.*

	Vector Genome Recovery			Full AAV Capsid Percentage		
Colum volume	dPCR	PATfix-CIMac AAV	MP	PATfix UC	MP	CD-MS
1 mL	77 %	79 %	80 %	85 %	80 %	88 %
8 mL	80 %	81 %	79 %	87 %	79 %	86 %
80 mL	85 %	77 %	79 %	85 %	75 %	86 %
800 mL (specimen)	67 %	73 %	77 %	87 %	84 %	87 %
8000 mL(specimen)	66 %	84 %	73 %	86 %	82 %	87 %
Average across scale	75 %	79 %	78 %	86 %	80 %	87 %
CV	10 %	5 %	3 %	1 %	4 %	1 %

A detailed study on how the AAV polishing purification process was successfully scaled up from 1 to 8000 mL monolith using a step elution approach is available in the following publication: Optimization and Scale up Strategies for Reproducible AAV Enrichment Step on CIMmultus QA HR Line.

Conclusion

Implementation of CIMmultus QA HR columns, combined with an improved chromatographic method, has significantly elevated the purity and recovery consistency in the AAV polishing purification step at any scale. This approach achieves highly reproducible AAV binding and elution performances, even for step elution gradient. This advancement significantly enhances the efficiency and scalability of AAV vector production, meeting industry demands for robust, scalable, and reproducible AAV purification manufacturing processes.

Ordering Information

Cat No.	Product Name
BIA-311.5213-2	CIMmultus® QA HR 1 mL Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-414.5213-2	CIMmultus® QA HR 4 mL Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-411.5213-2	CIMmultus® QA HR 8 mL Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-914.5213-2	CIMmultus® QA HR 40 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-911.5213-2	CIMmultus® QA HR 80 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-924.5213-2	CIMmultus® QA HR 400 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-921.5213-2	CIMmultus® QA HR 800 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-934.5213-2	CIMmultus® QA HR 4000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)
BIA-931.5213-2	CIMmultus® QA HR 8000 mL cGMP Compliant Monolithic Column (Quaternary Amine) (2 µm channels)

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Publication

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Method guide

Improved Method for Full AAV Enrichment with CIMmultus QA HR | Sartorius BIA Separations

AAV Full Capsids Enrichment With CIMmultus QA HR | Sartorius BIA Separations

Specimen QA HR Method Guide | Sartorius BIA Separations

Release Criteria for CIM® QA HR AAV Test | Sartorius BIA Separations

Additional Literature

Library Resources for AAV

https://www.biaseparations.com/library/aav/

Library Resources for CIM HR Line

https://www.biaseparations.com/library/cim-monolith-qa-hr-line/

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