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Reproducibility of CIMac QA HR Columns

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Abstract

This application note showcases the reproducibility of quaternary amine (QA) based analytical monolithic columns, CIMac QA HR, across multiple batches, and demonstrates the applicability of CIMac QA HR for AAV empty - full analytics. When combined with an improved anion-exchange method, CIMac QA HR enables superior separation of empty and full AAV capsids. Additionally, this application note shows the maximum expected variation in elution profiles between columns at the extreme values of the acceptance criteria.

Introduction

CIMac QA HR is a quaternary amine (QA) based analytical monolithic column (Figure 1). Quaternary amine is the most commonly used chromatographic ligand for separating empty (E) and full (F) AAV capsids. The unique properties of the CIMac monolithic column allow for better separation performance compared to alternative QA-based columns. The HR in CIMac QA HR indicates high reproducibility across different column batches.

Figure 1: *CIMac QA HR 0.1 mL Analytical Column (Quaternary Amine) (2 µm channels).*

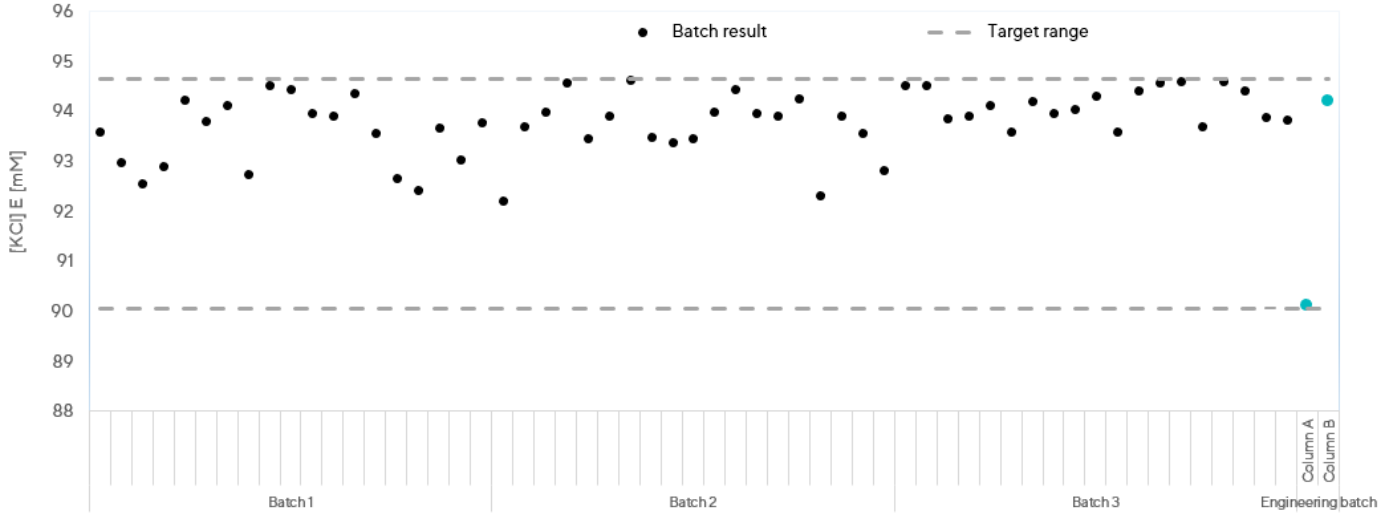
High reproducibility of CIMac QA HR columns is guaranteed with an extremely sensitive and reliable chromatographic QC (Quality Control) AAV test method, which is a critical component in QC of CIM® QA HR manufacturing process. The method separates empty and full AAV capsids of a standard sample (AAV2/8 serotype, prepared and characterized in-house) at a specific KCl concentration range within the linear ascending KCl elution gradient [1]. The KCl concentration at the elution of the empty AAV2/8 capsid peak ($[KCl]_E$) must be within the interval of 4.6 mM KCl to meet the requirements for the release of CIMac QA HR [2, 3]. This corresponds to an elution conductivity difference of less than 0.6 mS/cm in the conductivities for the elution of empty AAV capsids.

This document shows the reproducibility of CIMac QA HR within multiple batches and demonstrates the applicability of CIMac QA HR for AAV empty - full analytics.

Inter- and Intra-batch Reproducibility

To demonstrate the consistency of CIMac QA HR production, sixty different CIMac QA HR columns were evaluated from three different production batches (20 columns from each batch) and tested using the QC AAV release method (Figure 2).

Figure 2: *Batch-to-batch consistency of CIMac QA HR material demonstrated by AAV2/8 E-F separation across three different production batches and two columns from different engineering batches. The calculated RSD of [KCl]$_E$ was 0.76 % for columns from Batch no. 1, 0.72 % for Batch no. 2 and 0.37 % from Batch no. 3. Dots represent the concentration of [KCl]$_E$, tested with different CIMac QA HR columns. Grey dashed lines indicate the extreme upper and extreme lower limits of the QC acceptance criteria for the AAV test [2].*



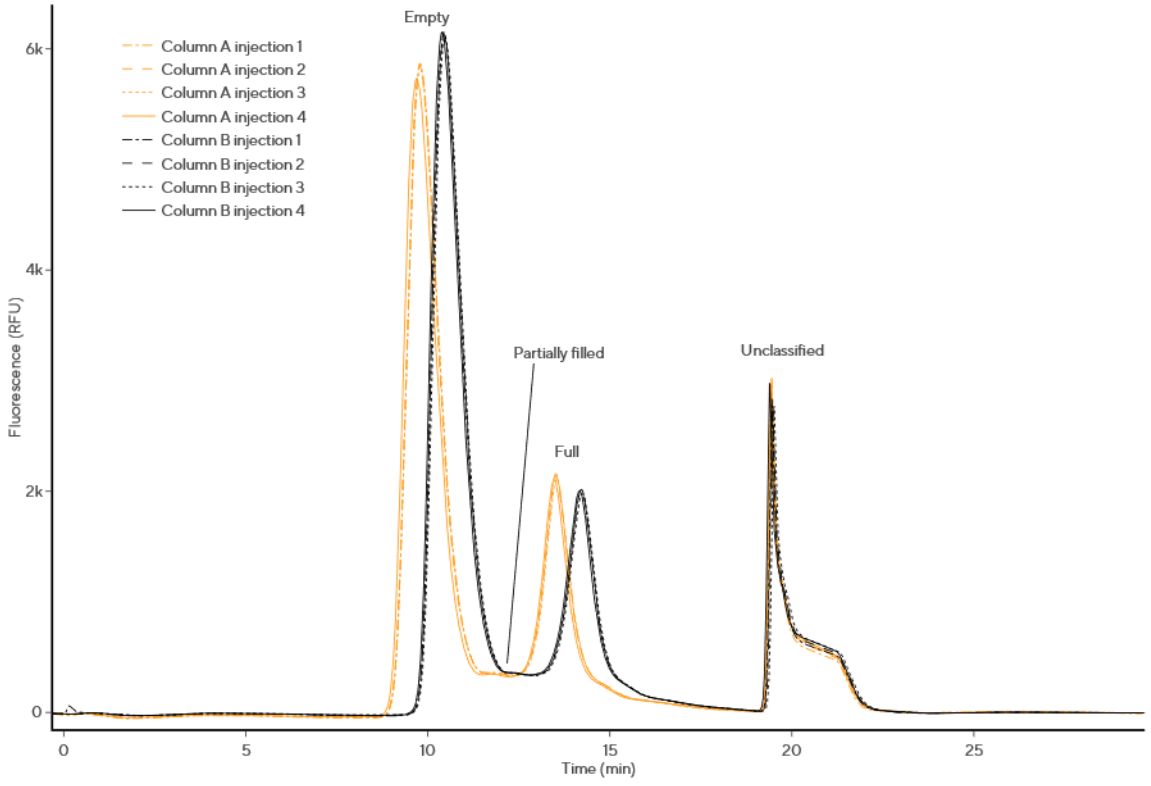
All columns met tight the release criteria for [KCl]$_E$ in the range between 90.06 and 94.64 mM. The calculated RSD of [KCl]$_E$ for 60 CIMac QA HR columns was 0.7 %. This demonstrates a highly reproducible production process of CIMac QA HR.

Inter- and Intra-batch Reproducibility

Two CIMac QA HR columns – referred to as Column A and Column B in Figure 2, were selected from different engineering QA HR batches. These columns represented the released products from extreme upper and lower limits of the QC acceptance criteria for AAV test (empty AAV capsids eluted at 90.1 mM KCl on Column A and 94.2 mM KCl on Column B, see the criteria range for [KCl]$_E$ in Figure 2). The main purpose of the experiment was to show the maximum expected difference in the elution profiles between columns at both extreme values of the acceptance criteria.

Both columns were then tested by using the improved AEX method (Figure 3) [4, 5]. The only variable in these experiments was the column, as all tests were conducted on the same day, using the same PATfix system, buffers, and the same aliquot of the AAV2/8 standard sample. Each column underwent four injections, resulting in a total of eight injections.

Figure 3: *Chromatogram overlay of tryptophan fluorescence signals for CIMac QA HR columns at upper and lower limits according to QC method tested by improved AEX method. Automatic integration was performed to determine the selected parameters for column evaluation.*



Unlike the QC method for CIMac QA HR, this improved AEX method not only distinguishes E AAV from F AAV capsids but also separates other product-related impurities from F AAV capsids. Product-related impurities could be partially filled, overfilled, or damaged AAV8 and aggregates noted as unclassified [4].

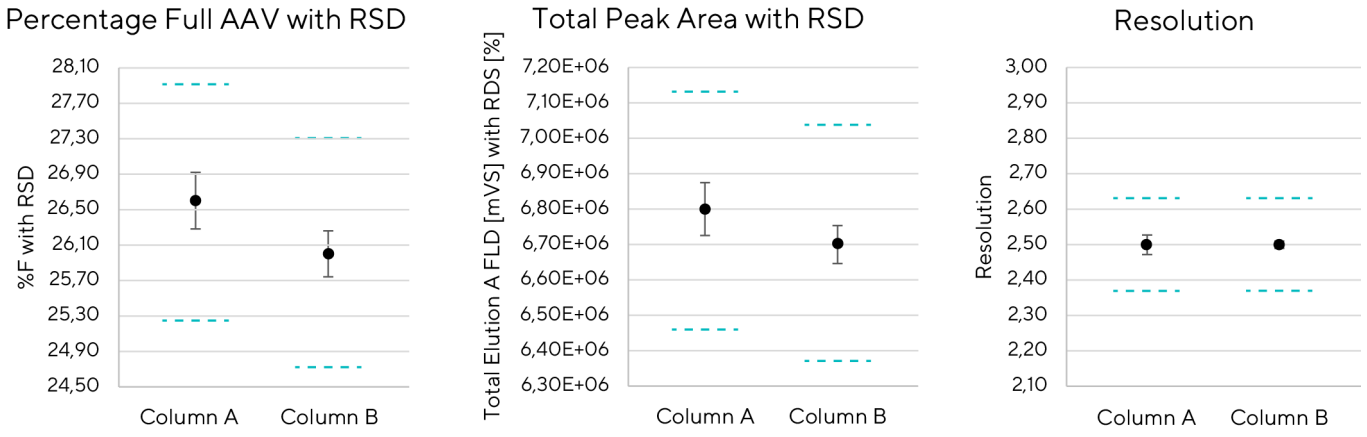
The shapes of elution profiles for both columns are comparable. Figure 3 shows high reproducibility and high-resolution separation between E and F AAV capsids of AAV2/8 standard sample when using CIMac QA HR in combination with an improved AEX method. Reproducibility was evaluated considering differences within relevant chromatographic parameters (Table 1, Figure 4).

Table 1: *Calculated values of relevant chromatographic parameters together with their relative standard deviations (RSD) for each chromatographic column. σ_E = conductivity of E elution; t_E = retention time of E AAV8 capsid; %F = % integrated tryptophan fluorescence area under full AAV8 peak using an automatic peak integration compared to all integrated peak areas; R = resolution between E and F AAV8 capsids; Total A FLD = Total integrated elution area of the fluorescent signal during elution gradient, including empty, partially filled, full capsids and unclassified peaks.*

Column Batch	σ_E (mS/cm) with RSD (%)	t_E (min) with RSD (%)	%F with RSD (%)	Total elution A FLD (mVS)with RSD (%)	R with RSD (%)
Column A	3.9 ± 0.2	9.8 ± 0.5	26.6 ± 1.2	6.8x10E+6 ± 1.1	2.5 ± 1.1
Column B	4.2 ± 0.2	10.4 ± 0.3	26.0 ± 1.0	6.7x10E+6 ± 0.8	2.5 ± 0.4

According to Table 1, the reproducibility of the chromatographic method by evaluation of the most important parameters within the same column is highly consistent. All tested parameters exhibit very low RSD (below 1.2%).

Figure 4: *Reproducibility of the three most relevant parameters for AAV analytical purposes with four injections per column. Dots represent average values, error bars represent RSD (%), and dashed lines represent maximum and minimum values of the selected interval (± 5%).*



The percentage of F AAV assessed by tryptophan fluorescence, the total area of fluorescent peaks during elution, and resolution between E and F AAV capsids remain consistent. The differences between the RSD for each parameter are negligible and fall within a commonly accepted ± 5% interval.

Conclusions

High Reproducible Analytical Performance: Each batch of CIMac QA HR column delivers the same high-resolution separation of empty and full AAV capsids, even for batches at the extreme upper and lower limits of the QC specification range.

Effective Separation: Unique properties of CIMac QA HR, combined with an improved AEX method, allow superior separation of empty and full AAV capsids.

Reliable QC Method: The chromatographic QC method is both sensitive and reliable, ensuring that any qualified CIMac QA HR columns meet tight release criteria for AAV analytics.

FAQ

Does the CIMac QA HR column already guarantee elution under exactly the same elution conditions, regardless of the width and length of the capillaries, setup of the LC analytical system, and other variables?
Tightly controlled manufacturing process of CIMac QA HR guarantees consistent quality of these chromatographic columns for the separation of large biomolecules (e.g., AAV, adenovirus, etc.), resulting in high reproducibility. While the consistent quality of chromatography columns is crucial, several additional parameters must also be well-controlled to ensure high reproducibility in elution performance:

- Sufficient column equilibration, cleaning, and regeneration
- Composition and purity of buffers & virus samples, and their preparation
- Amount of virus loaded on the column
- System setup (calibration of detectors, void volume of the system, etc.)
- Temperature

Has the improved AEX method you referred been published, and it is available for use? How can it be adapted for different AAV serotypes?
The concept of the improved AEX method has already been published [5]. The detailed methodology is available for our clients as part of Cornerstone® Biomanufacturing Services. Within the scope of these services, enhanced AEX methods for other AAV serotypes can be developed and optimized.

What are similarities and differences between CIMac QA, CIMac QA HR and CIMac AAV E/F columns?

- CIMac QA, CIMac QA HR and CIMac AAV E/F all use QA chemistry as a backbone of their functionality; however, they differ in their purpose, channel diameter and release testing.
- Release criteria of CIMac QA column are based on the separation of large proteins, while CIMac AAV E/F and CIMac QA HR release criteria are based on the separation of AAV E and F capsids. CIMac QA is appropriate for the separation of proteins, but not the best choice for the separation of viral vectors.
- CIMac AAV E/F and CIMac QA HR are both tested with AAV sample but have different QC release criteria and different channel diameter. CIMac AAV E/F has 1.3 um channels and CIMac QA HR has 2 um channels.
- CIMac QA HR delivers exceptionally reproducible chromatography results. When the reproducibility of AAV elution conditions is critical, customers should opt for CIMac QA HR rather than CIMac AAV E/F. Reproducibility is particularly important when using step elution or when the linear elution method is highly sensitive.
- CIMac AAV E/F is sufficient, if AAV elutes in the middle of the linear elution gradient and there is significant pH or conductivity difference of the elution buffers for elution in linear gradient.

References

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[2] **Specimen QA HR Method Guide** | Sartorius BIA Separations

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[5] T. Žvanut, S. Peljhan, S. Lebar, A. Štrancar, A. Gramc Livk: **Enhanced Separation of AAV Capsids Using Analytical Anion Exchange Monolithic Columns: Emphasis on Organic Modifiers** | 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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