Evaluation of Intermediate Hold Stability of AAV8 for Manufacturing Suitability

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Introduction

The Downstream purification of adeno-associated virus (AAV)–based therapies typically spans several days and often involves unavoidable hold periods between unit operations. Consequently, understanding the maximum allowable hold time for in-process AAV intermediates—and the conditions under which they can be held—is critical for robust manufacturing process design.

In this study, various AAV8 downstream intermediates were held at 4˚ C for up to 14 days prior to the subsequent purification steps to evaluate the impact of hold conditions on product quality and process performance. Hold-time stability of AAV8 process intermediates were assessed by vector genome (VG) and capsid recovery, packaging efficiency, purity, aggregation, residual protein and DNA, and relative potency across multiple storage intervals (Table 1).

Methods

An AAV8 lysate encoding for Green Fluorescence Protein (GFP) from a 50L bioreactor was clarified by Depth Filtration (DF) and purified through Affinity (AF) capture, and Anion Exchange (AEX) polishing. The process intermediates obtained after each of these unit operations were aliquoted and stored at 4 °C for defined hold time periods. Once a hold time was reached, the material was frozen at -80 ºC and thawed for processing (see Table 1 for a summary of the time points and the purification steps used after the thaw).

At each time point, AAV8 attributes were characterized using a comprehensive analytical panel, including vector genome (ddPCR) titer, capsid titer (ELISA), packaging (Analytical Ultracentrifugation), aggregation (SEC), purity (CE-SDS), and relative potency (GFP Expression). Measurement of residuals included Host Cell Protein (ELISA) and Host Cell DNA (qPCR).

Figure 1: Overview of the Purification Steps used for the Hold Time Stability Study

Table 1: Summary of In-Process Hold Conditions

Stability Step	Hold Temperature (ºC)	Hold Time (Days)	Processing Method
DF Product Stability	4 ºC	0, 4, 7 and 14	Affinity and Anion Exchange Chromatography
AF product Stability	4 ºC	0, 4, 7 and 14	Anion Exchange Chromatography
AEX Product Stability	4 ºC	0, 4, 7 and 14	N/A

Depth Filtration Stability

Depth Filtration Process Intermediates have comparable product quality

The chromatography peak overlay for Affinity and Anion Exchange product peaks are comparable for depth filtration stability (Figures 2A and 2B). Peak area analysis and titers comparisons across the time points further suggests consistent product quality (Figures 3A and 3B).

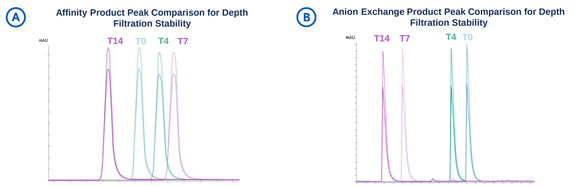

Figure 2: Chromatographic comparison of depth filtration stability. Affinity product peaks (A) and anion exchange product peaks (B) derived from the depth-filtered material across time points. Comparable peak profiles indicate stability of the depth filtration products over 14 days of storage at 4 ºC

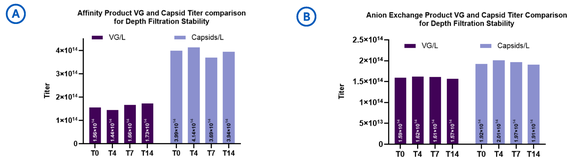

Figure 3: The VG and Capsids titer for Affinity (A) and Anion Exchange (B) products were compared to assess depth filtration stability. Results are consistent from day 0 to day 14, suggesting depth-filtered products can be stored for up to 14 days without compromising product quality

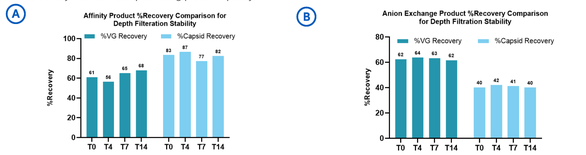

Figure 4: The VG and Capsids recovery for Affinity (A) and Anion Exchange (B) products were compared to assess depth filtration stability. Results remain consistent from day 0 through day 14, indicating that depth-filtered products can be stored for up to 14 days without compromising product quality

Table 2: Comparison of other Physicochemical Attributes for Depth Filtration Stability Intermediates

AF Product from Depth Filtration Stability					
Time Point (Days)	AUC (%E %P %F)	Purity (%)	Aggregation (%)	Host Cell Protein (ng/mL)	Host Cell DNA (ng/mL)
T0	51, 7, 42	99.7	0.6	BLOQ (<50)	46.5
T4	52, 6, 42	99.7	0.6	BLOQ (<50)	44.2
T7	52, 6, 42	99.7	0.6	BLOQ (<50)	46.4
T14	52, 6, 42	99.5	0.5	BLOQ (<50)	49.1
AEX Product from Depth Filtration Stability					
Time Point (Days)	AUC (%E %P %F)	Purity (%)	Aggregation (%)	Host Cell Protein (ng/mL)	Host Cell DNA (ng/mL)
T0	16, 7, 77	99.7	9.0	BLOQ (<50)	20.2
T4	8, 7, 85	99.8	2.4	BLOQ (<50)	17.6
T7	5, 8, 87	98.9	6.3	BLOQ (<50)	20.0
T14	7, 7, 86	99.9	8.3	BLOQ (<50)	21.1

Cumulative recovery of VG and Capsids across the depth filtration hold stability time points remained comparable through 14 days (Figures 4A and 4B). In addition, product quality attributes—including packaging, purity, aggregation, and residual protein and DNA—showed no change after a 14-day hold when subsequently processed via Affinity and AEX (Table 2). These findings indicate that AAV8 depth-filtered product can be held for up to 14 days without an expected impact on product quality.

Affinity Product Stability

Affinity Product Stability Intermediates have overall comparable product quality

The VG and Capsids titers for affinity products remain consistent across the time points, indicating stable product quality (Figure 5A). The chromatography peak overlays for Anion Exchange product peaks are also comparable, supporting the stability of the affinity products (Figure 5B).

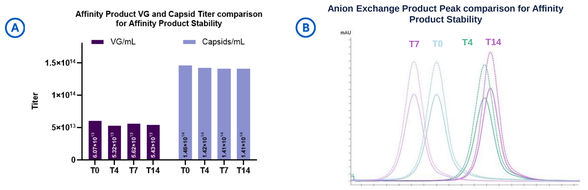

Figure 5: Comparison of affinity product stability over time. (A) VG and capsid titers for the affinity products remain consistent over the 14-day storage period. (B) Chromatographic peak overlays from anion exchange chromatography show comparable profiles from T0 to T14, indicating stable recovery and maintained product quality throughout the storage duration

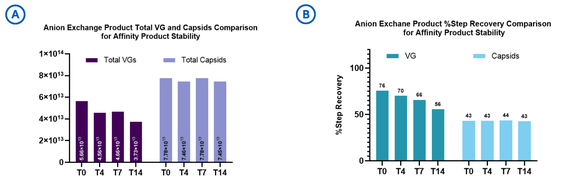

Figure 6: Anion exchange product performance for affinity product stability over time. (A) The total VGs and capsids remain comparable through 7 days of storage at 4 ºC, while lower total VGs was observed for T14. (B) Capsid step recoveries are comparable, whereas VG step recovery shows a decreasing trend, indicating reduced step yield with extended storage of the affinity product at 4 ºC.

Table 3: Comparison of other Physicochemical Attributes for Affinity Product Stability Intermediates

Affinity Product from Affinity Product Stability			
Time Point/ AD Test	AUC (%E %P %F)	Purity%	Aggregation%
T0	53, 6, 41	99.7	0.7
T4	54, 5, 41	99.7	0.7
T7	52, 6, 42	99.7	0.7
T14	43, 5, 41	99.5	0.7
Anion Exchange Product from Affinity Product Stability			
Time Point/ AD Test	AUC (%E %P %F)	Purity%	Aggregation%
T0	8, 7, 85	98.2	1.5
T4	9, 5, 86	98.3	0.8
T7	9, 7, 84	97.5	3.8
T14	7, 7, 86	98.1	1.2

Total VG and capsid levels for affinity product stability remained comparable through 7 days, while T14 showed lower total VG relative to other time points (Figure 6A). Capsid step recoveries remained consistent through 14 days; however, VG step recovery exhibited a decreasing trend from T0 to T14 (Figure 6B). In addition, product quality attributes—including packaging, purity, aggregation, residual protein, and residual DNA—showed no noticeable changes for affinity products held up to 14 days, or in the corresponding anion exchange products generated during affinity stability assessment (Table 3). These findings suggest that the affinity product remains largely stable at 4 °C for up to 7 days prior to purification by anion exchange chromatography.

Anion Exchange Product Stability

Anion Exchange Product Stability Intermediates have comparable product quality

The VG and capsids titers for anion exchange purified products remained consistent across all time points, indicating stable product recovery and quality (Figure 7). In addition, product quality attributes shown in the Table 4 exhibited no noticeable changes in samples held up to 14 days. These findings suggest that anion exchange purified products remains stable at 4 °C for up to 14 days.

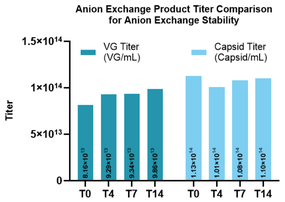

Figure 7: Comparison of anion exchange product stability over time. VG and capsid titers for anion exchange products remain consistent throughout the 14-day storage period at 4 ºC, indicating stable product recovery and quality.

Table 4: Comparison of other Physicochemical Attributes for Anion Exchange Product Stability

AEX Products held for 14 days at 4 ºC					
Time Point/ AD Test	AUC (%E %P %F)	Purity%	Aggregation%	Host Cell Protein (ng/mL)	Host Cell DNA (ng/mL)
T0	10, 7, 83	98.1	1.4	BLOQ (<50)	64.1
T4	11, 7, 82	98.8	0.9	BLOQ (<50)	62.5
T7	10, 7, 83	98.7	0.9	BLOQ (<50)	73.3
T14	11, 8, 81	98.8	0.7	BLOQ (<50)	56.5

AAV8 Intermediates Relative Potency Comparison

Depth Filtration Stability Intermediates Exhibits Reduced Relative Potency at T14

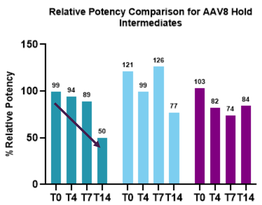

Figure 8: Comparison of percent relative potency of anion exchange products derived from AAV8 hold intermediates over the study duration. Relative potency of the depth filtration hold intermediates decreased over time. In contrast, affinity and anion exchange stability samples exhibited comparable percent relative potency from T0 to T14, although, the relative potency for T14 was slightly lower than at T0.

A relative potency assay for AAV evaluates the biological activity of an AAV product by comparing the potency of a test sample to that of a reference standard. In this study, the relative potency of the depth filtration stability samples decreased over time. In contrast, the affinity and anion exchange stability samples maintained overall comparable relative potency throughout the study, although the relative potency at T14 was slightly lower than T0 for each sample (Figure 8).

Conclusion

Although the product quality data support the stability of AAV8 intermediates stored at 4 ºC for up to 14 days, the relative potency results indicate that, prolonged hold times under these conditions lead to decreased potency of the intermediates. These findings suggest that storing AAV8 intermediates at 4 °C for no longer than 7 days prior to further processing may be optimal for maintaining robust material quality during manufacturing.

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