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Application Note

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An Efficient End-to-End AAV8 Platform Process at 50 L Scale Using Sartorius Technology

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Abstract

The growing use of adeno-associated virus (AAV) vectors in advanced therapies highlights the need for reliable and flexible production platforms that address key manufacturing challenges, including scalability, low recovery rates, and the separation of full and empty capsids.

This application note presents an end-to-end AAV production platform covering upstream and downstream processing exclusively using Sartorius technologies. In upstream processing, HEK293 cells were seeded from a 10 L rocking motion bioreactor into a 50 L stirred-tank reactor. The downstream process employed a range of purification techniques, such as clarification by filtration, tangential flow filtration, and a serotype-independent capture and polishing chromatography step.

The platform achieved recoveries of 23% for viral genomes. Importantly, the proportion of full capsids increased from 21% in the harvest to 68% in the final product. Furthermore, host cell DNA was reduced by 98.5% (1.82 $\log_{10}$ reduction) and host cell protein was reduced to a non-detectable level (> 99.99%, 8.1 $\log_{10}$ reduction).

For further information, visit
sartorius.com/en/applications/cell-and-gene-therapy/gene-therapy/aav-gene-therapy

Introduction

The global gene therapy market is expanding rapidly, driven by the increasing demand for personalized medicine and innovative treatments for genetic disorders. Adeno-associated virus (AAV) vectors are a cornerstone in this field owing to their low immunogenicity, favorable safety profile, and capacity for long-term gene expression. AAV-based therapies are already in use for various genetic conditions, including hemophilia, Leber's congenital amaurosis (LCA), and spinal muscular atrophy (SMA), with numerous additional therapies in development.

This application note details an end-to-end production platform, developed in collaboration with Matica Biotechnology, a contract development and manufacturing organization (CDMO). The process, using AAV8 (AAV8-GFP) as an example, was carried out exclusively with Sartorius technologies across both upstream and downstream phases.

Materials and Methods

In the upstream process, AAV8 production was demonstrated in a 50 L stirred-tank bioreactor. Downstream purification of AAV8 from the 50 L culture included steps from harvest clarification post-endonuclease treatment through to final sterile filtration of the formulated product. Figure 1 provides an overview of the products used within the process, and Table 1 provides an overview of all processing steps.

The process was monitored with step-appropriate methods to assess critical quality attributes (CQAs), including viral genome (vg) and capsid | particle (vp) recoveries, and the removal of host cell DNA (hcDNA) and host cell proteins (HCP). The analyzed CQAs and technologies used are detailed in Table 2.

Figure 1: *Product Overview for the End-to-End AAV8 Production Process*

1 Flexsafe® RM 20 Basic Bag

2 Biostat® RM 20

3 Flexsafe STR® 50 L

4 Biostat STR® 50 L

5 FectoVIR®-AAV

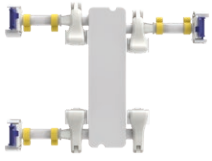
6 Sartoclear® DL75

7 Sartopore® 2 (XLG)

8 Sartocon® Self-Contained Cassette | Hydrosart®

9 Sartoflow® Expert SU

10 CIMmultus® SO3



11 Sartocon® Slice

12 CIMmultus PrimaS®

13 Sartoflow® Smart

14 Sartopore Evo®

Table 1: *Steps of the End-to-End AAV8 Production Process Using Sartorius Processing Units and Technologies*

Upstream Processing

Process Step	Description		Sartorius Solution
Expansion	Cell culture expansion in rocking motion bioreactor	1	Flexsafe® RM 20 Basic Bag
		2	Biostat® RM 20 (N-1)
Inoculation	Transfer of the expanded cells to the 50 L stirred-tank bioreactor and cell growth prior to transfection	3	Flexsafe STR® 50 L
		4	Biostat STR® 50 L
Transfection and Production	Transient plasmid transfection of the 50 L culture, followed by AAV8 expression phase	5	FectoVIR®-AAV
		4	Biostat STR® 50 L

Downstream Processing

Process Step	Description		Sartorius Solution
Harvest Clarification	Removal of cell debris and larger particles, followed by bioburden reduction through sequential filtration	6	Sartoclear® DL75 depth filter cassette (10 µm | 2 µm, EFA 0.4 m^2)
		7	Sartopore® 2 XLG Midicaps® Size 8 (0.8 µm | 0.2 µm, EFA 0.13 m^2)
UF | DF (TFF 1)	Concentration, buffer exchange, and further removal of impurities by TFF	8	Sartocon® Self-Contained Cassette | Hydrosart® | 300 kDa | 1.4 m^2 | ECO-Screen
		9	Sartoflow® Expert SU
Acidification 0.45 µm Filtration	Equilibration of AAV8 to capture chromatography load buffer and removal of precipitated impurities by bioburden filtration	7	Sartopore® 2 Midicaps® Size 9 (0.8 | 0.45 µm, EFA 0.2 m^2)
Capture	Capture of AAV8 and removal of hcDNA and HCP by CEX	10	CIMmultus® SO3 400 mL (2 µm)
UF | DF (TFF 2) 0.45 µm Filtration	Concentration, buffer exchange to an AEX loading buffer	11	Sartocon® Slice | Hydrosart® | 100 kDa | 0.14 m^2 | ECO-Screen
		7	Sartopore® 2 Midicaps® Size 5 (0.8 | 0.45 µm, EFA 0.03 m^2)
Polishing	Removal of empty capsids, remaining hcDNA, and HCP by AEX and H-bond interactions	12	CIMmultus PrimaS® 80 mL (2 µm)
UF | DF (TFF 3)	Final formulation and concentration of AAV8 by TFF	11	Sartocon® Slice 200 | Hydrosart® | 100 kDa | 0.018 m^2 | ECO-Screen
		13	Sartoflow® Smart
Sterile Filtration	Final sterile filtration of the formulated AAV8	14	Sartopore Evo® Sartoscale 47 (0.8 µm | 0.2 µm, EFA 17.3 cm^2)

Note. AEX = anion exchange chromatography CEX = cation exchange chromatography, EFA = effective filtration area, hcDNA = host cell DNA, HCP = host cell protein, TFF = tangential flow filtration, UF \| DF = ultrafiltration \| diafiltration

Table 2: *Overview of Process Monitoring Analytics, Including CQAs, During Up- and Downstream Processing*

CQA	Readout	Technology
Cell Health Assessment	Viable cell density [viable cells/mL]	Vi-CELL BLU Cell Viability Analyzer
	Cell viability [%]	
Turbidity	Nephelometric turbidity units [NTU]	Nephelometer
AAV8 Titer	Viral genome [vg/mL] titer	ddPCR
	Viral particle | capsid titer [vp/mL]	AAV8 capsid ELISA
Host Cell Impurities	HCP [ng/mL]	ELISA
	hcDNA [ng/µL]	ddPCR
Empty | Full Capsid Ratio	Empty capsids [%]	PATfix® HPLC-based analytical platform and CIMac™ AAV full/empty Analytical Column
	Full capsids [%]	

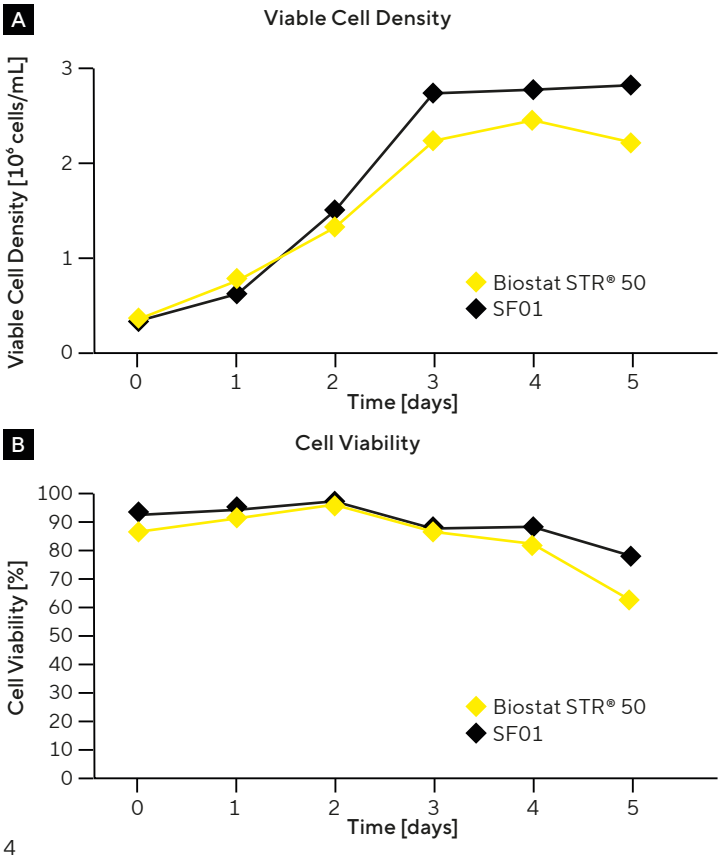
Results

Upstream Processing

Upstream AAV8 Production at 50 L Clinical Scale
AAV8 vectors were produced through transient transfection of HEK293 cells using FectoVIR®-AAV (Sartorius). For the cell expansion process (N-1 stage), cells were cultured in a Flexsafe® RM bag at 10 L working volume utilizing a Biostat® RM 20 rocking motion bioreactor. Subsequently, the cells were used to seed a 50 L culture at a density of 0.3×10^6 vc/mL and cultivated in a Biostat STR® 50 L bioreactor. Two days after inoculation, the cells were transfected with a two-plasmid system. Three days post-transfection, AAV8 expression was terminated by cell lysis and nuclease treatment for 1.5 h, followed by high-salt treatment (0.5 M NaCl) for 30 min. To assess successful AAV8 expression, a reference shake flask was transfected and cultured.

Monitoring cell growth and cell viability throughout the cultivation period, from inoculation to harvest of the AAV8-producing HEK293 cells, revealed comparable growth and viability profiles between the 50 L Biostat STR® culture and the reference culture (SF01; Figures 2A and 2B).

Figure 2: *Viable Cell Density* ***(A)*** *and Cell Viability* ***(B)*** *of the 50 L Biostat STR® Culture (Yellow Line) and the Reference Shake Flask Culture (Black Line) From Inoculation (Day 0) to Transfection (Day 2) and AAV8 Harvest (Day 5)*



Besides exhibiting similar cell growth and viability profiles, measurements of viral capsid and viral genome titers using ELISA and ddPCR, respectively, indicate comparable AAV8 expression in both the 50 L and the reference culture (Table 3).

Table 3: *Viral Capsid and Genome Titers in the 50 L Biostat STR® Culture and the Shake Flask Reference Culture*

	Capsid Titer [vp/mL]	Genomic Titer [vg/mL]
50 L Biostat STR®	2.8×10^{11}	3.92×10^{10}
Shake flask	6.11×10^{11}	7.89×10^{10}

Downstream Processing

Clarification
The clarification of the harvested and lysed cells was achieved through sequential filtration of the lysate. First, the lysate was filtered through a Sartoclear® DL75 double-layer depth filter cassette mounted on a Sartoclear® Pilot Holder with an effective flux rate of 100 LMH. Subsequently, the filtrate was passed through a Sartopore® 2 XLG Midicaps® Size 8 double-layer filter (0.8 µm | 0.2 μm^2) with a flux rate of 400 LMH.

The turbidity assessment of the filtrates indicated effective clarification of the lysate, with values reduced from 165 NTU to 2.1 NTU using the Sartoclear® DL75 depth filter. In the second filtration step, the Sartopore® 2 XLG further reduced the turbidity to 1.95 NTU (Table 4).

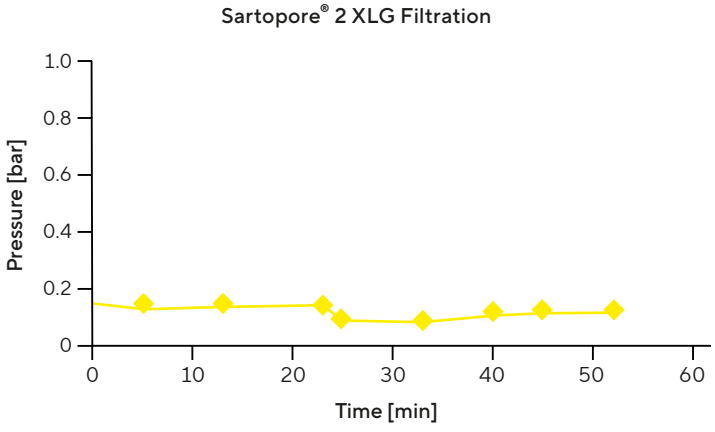
Table 4: *Turbidity Reduction (NTU) and Recovery (%) of AAV8 Genomes and Capsids After Sequential Filtration of the Lysed 50 L Cell Culture*

	Sartoclear® DL 75	Sartopore® 2 XLG
Turbidity [NTU]	2.1 (from 165)	1.95
Viral Genome Recovery [%]	102	91
Viral Capsid Recovery [%]	90	101

The strong clarification performance of the Sartoclear® depth filter was also evident in the consistently low filtration pressure during the filtration with the Sartopore® 2 XLG, which remained below 0.2 bar (Figure 3). Alongside the significant lysate clearance, both filtration steps achieved considerable AAV8 recovery.

Filtration with the Sartoclear® DL75 depth filter resulted in 102% viral genome and 90% capsid recovery. Subsequent filtration with the Sartopore® 2 XLG filter achieved recoveries of 91% for viral genomes and 101% for capsids, indicating minimal loss of the AAV product during harvest clarification (Table 4).

Figure 3: *Pressure Profile During Sartopore® 2 XLG Filtration of AAV8 Following Sartoclear® Depth Filtration*

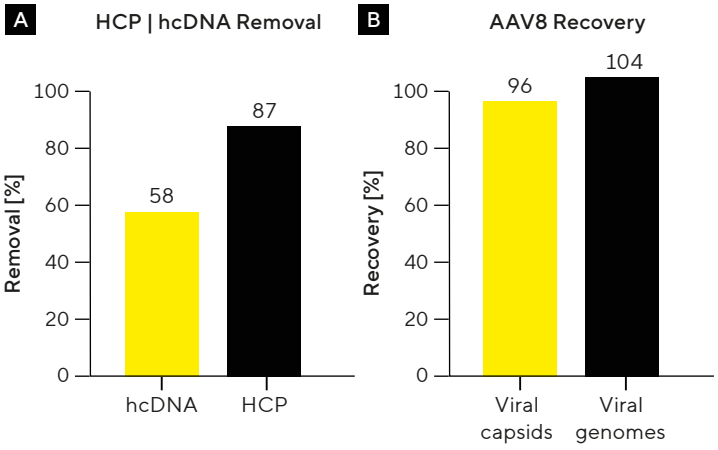


Ultrafiltration | Diafiltration 1 (Tangential Flow Filtration 1)
The filtrate obtained from harvest clarification was subjected to ultrafiltration (UF) and diafiltration (DF) through tangential flow filtration (TFF). TFF was used to concentrate the filtrate, exchange the AAV8 feed to a buffer with less conductivity, and further remove impurities. The process was performed with the Sartocon® SC Hydrosart® ECO-Screen Cassette with a membrane cut-off of 300 kDa on the single-use Sartoflow® SU TFF system.

The process parameters were controlled by the fully automated, closed-loop Sartoflow® Expert SU TFF system. In the TFF run described here, an inlet pressure (P1) of 1.25 bar and a transmembrane pressure (TMP) of 0.43 bar were applied, and facilitating rapid processing.

The filtrate was concentrated by a factor of 9.2 before it was diafiltrated 5 times. After TFF, hcDNA and HCP were significantly reduced by 58% and 87%, respectively (Figure 4A), indicating that the 300 kDa cut-off was appropriately selected. The process achieved high AAV8 yields, retaining 96% of virus capsids and 104% of genomes (Figure 4B).

Figure 4: *Removal of hcDNA and HCP **(A)** and Recovery of AAV8 Capsids and Genomes **(B)** During UF | DF 1*



Capture

Prior to the capture step, the retentate was acidified and filtered through a Sartopore® 2 0.45 µm filter. AAV8 capture and removal of hcDNA and HCP were performed by cation exchange chromatography (CEX) using a CIMmultus® SO3 monolithic column (400 mL, 2 µm channel size).

The chromatographic run was carried out at a flow rate of 0.35 column volumes (CV)/mL. The load buffer (A) and the elution buffer (B) were composed as follows:

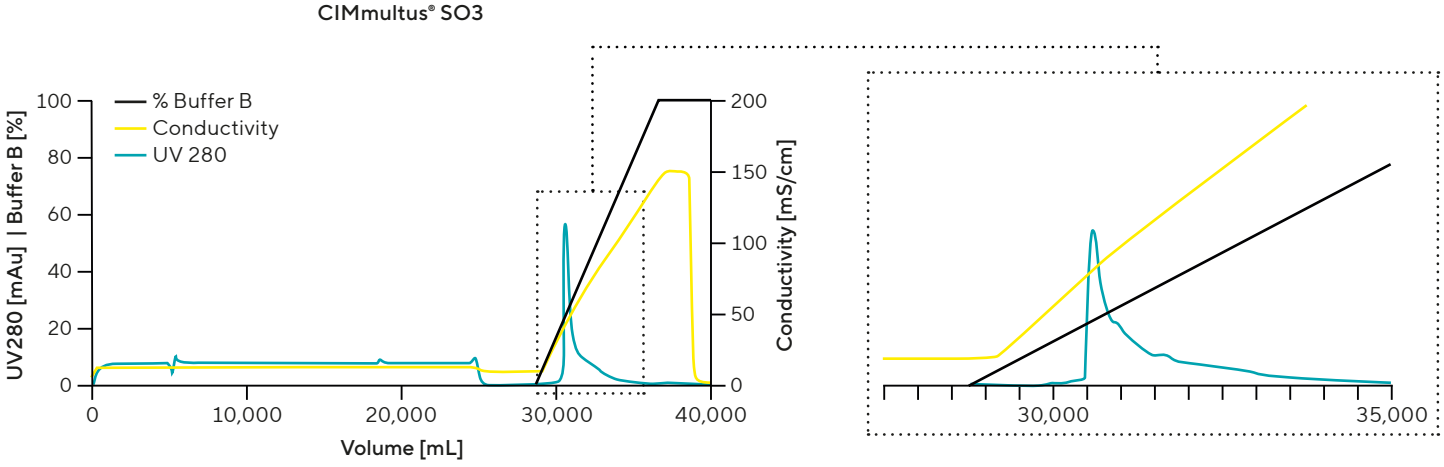
- Load buffer (A): 50 mM acetate buffer, 50 mM NaCl, 0.1% (w/v) Poloxamer 188, 2 mM $MgCl_2$, pH 4
- Elution buffer (B): 50 mM acetate buffer, 2.0 M NaCl, 0.1% (w/v) Poloxamer 188, 2 mM $MgCl_2$, pH 4

The eluted fractions were neutralized by adding 20% of 1 M Tris base, pH 9.

The CEX chromatogram is shown in Figure 5. The elution buffer curve (% Buffer B), along with conductivity and UV 280 values, exhibited characteristic patterns. The sharp elution peak of the AAV8-containing fraction suggested effective separation of the AAV product from impurities. The measurement of HCP revealed a removal of 74%, while hcDNA removal could not be determined because the level of hcDNA in the starting material was below the assay detection limit after dilution and adjustment to the CEX column binding buffer.

AAV8 recovery was determined to be 64% for the viral genomes and 63% for capsids.

Figure 5: *CEX Chromatogram of the AAV8 Capture Step Using a CIMmultus® SO3 Monolithic Column, Including a Zoom-in on the Elution Phase*



Polishing

Before loading the AAV8 to the polishing column, the viruses were further concentrated and diafiltered into polishing chromatography load buffer (TFF 2).

Following the AAV8 capture step, the separation of AAV8 full capsids from empty capsids, along with further removal of host cell impurities, was accomplished using a combination of hydrogen bonding and anion exchange chromatography (AEX) using a CIMmultus PrimaS® column.

The flow rate was 1 CV/min throughout the entire run, with the following buffers used for pH gradient-dependent elution:

- Load buffer (A): 10 mM Tris, 10 mM Bis Tris Propane, 0.1% (w/v) Poloxamer 188, 1% Sucrose, 2 mM $MgCl_2$, pH 7
- Elution buffer (B): 10 mM Tris, 10 mM Bis Tris Propane, 0.1% (w/v) Poloxamer 188, 1% Sucrose, 10 mM NaCl, 2 mM $MgCl_2$, pH 9.5

Figure 6 illustrates the chromatogram. With increasing concentration of the elution buffer (% Buffer B), empty capsids eluted first (empty peak fraction, E), followed by full capsids (full peak fraction, F). The separation of the peaks demonstrated clear distinction between full and empty capsids, resulting in enrichment of the full particle fraction.

Figure 6: *Chromatogram of Separation of Full AAV8 Capsids From Empty Capsids Using CIMmultus PrimaS® With a Zoom-in on the Full and Empty Peak Fractions in the Elution Phase (**E** = Empty Particles Peak, **F** = Full Particles Peak)*

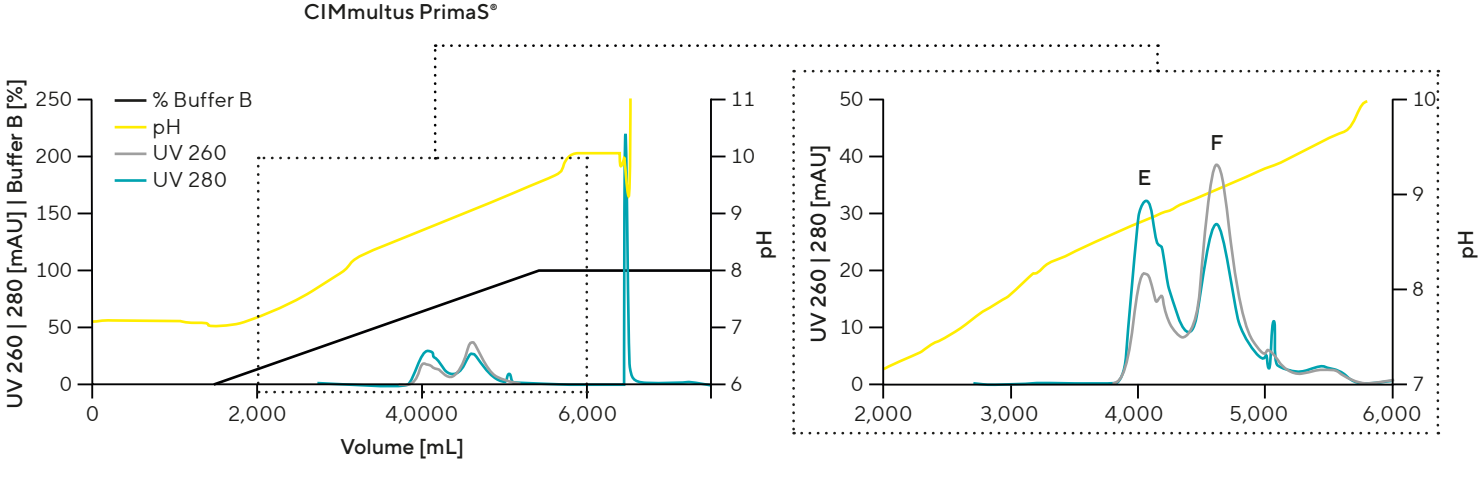
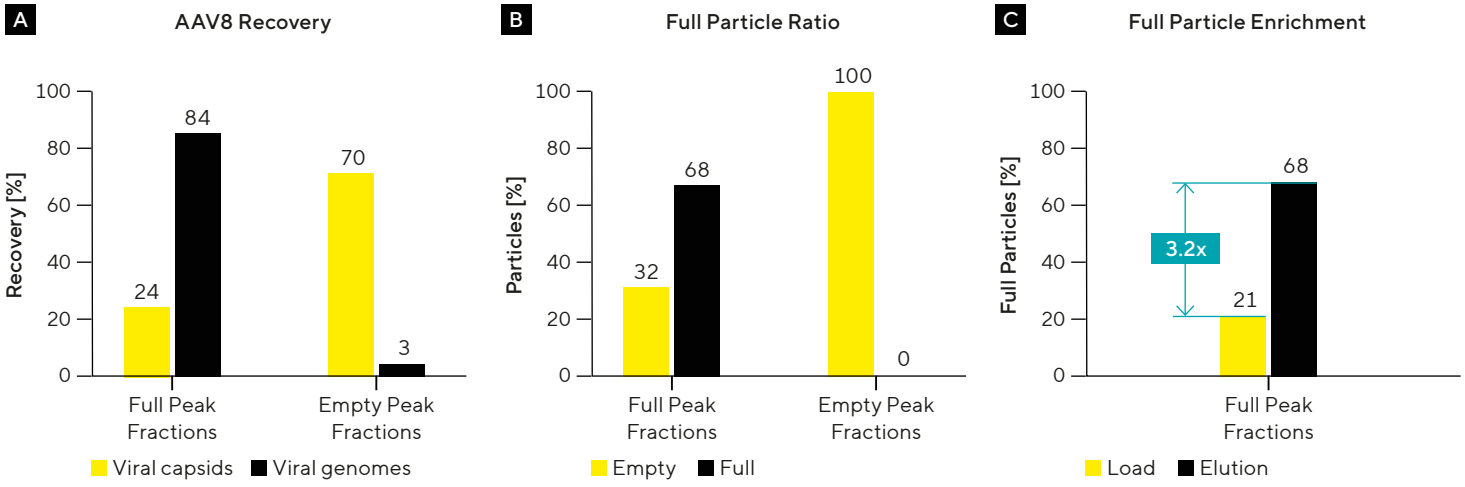


Figure 7: *Recovery of AAV8 Capsids and Genomes in the Full and Empty Peak Fractions **(A)**, Ratio of Full to Empty Capsids in These Fractions **(B)**, and Enrichment of Full Capsids During Polishing **(C)***



The analysis of the eluates confirmed clear separation during the polishing process, with recoveries of 84% for viral genomes in the full particle fraction compared to just 3% in the empty particle fraction (Figure 7A).

Furthermore, the PATfix® system, in combination with CIMac™ AAV full/empty Analytical Columns, was applied for anion AEX-based chromatographic determination of the ratio of full to empty capsids of the different elution fractions. The presence of 68% full AAV8 capsids in the full peak eluate fractions indicates an effective enrichment of more than 3-fold compared to the 21% found in the feed (Figure 7B and 7C). Additionally, the polishing step achieved further removal of 88% of HCP and 59% of hcDNA.

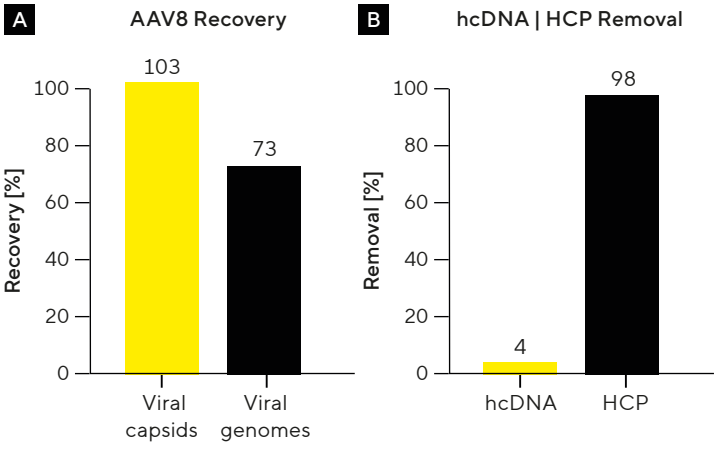
UF | DF 3 (TFF 3)

The full capsid fraction obtained during AEX-based polishing further underwent final formulation and concentration to the target titer using UF | DF through TFF employing a Sartocon® Slice 200 Hydrosart ECO-Screen cassette (100 kDa) on a Sartoflow® Smart TFF system. During diafiltration, AAV8 capsids were reformulated to the final formulation buffer containing the following ingredients: 20 mM Tris, 200 mM NaCl, 0.005% (w/w) Poloxamer 188, 1 mM $MgCl_2$, pH 8.0.

The Sartoflow® Smart system was used to control the process parameters. With the P1 set at 1.25 bar and the TMP at 0.43 bar, a permeate flux of 116.6 LMH was achieved. The feed was concentrated by a factor of 7 to the desired final product titer of 1×10^{13} vg/mL .

The analysis of the permeate showed AAV8 recoveries of 103% and 73% for virus capsids and genomes, respectively. hcDNA was determined to be removed by 4% while HCP clearance reached 98% (Figure 8).

Figure 8: *Recovery of AAV8 Capsids and Genomes After Concentration and Final Formulation Using UF | DF **(A)**, as well as hcDNA and HCP removal during the process **(B)***



Sterile Filtration

The end-to-end AAV8 processing was completed with the sterilization filtration of the formulated AAV8. Sterile filtration was conducted with the Sartopore Evo® Sartoscale 47 (0.8 µm | 0.2 µm).

Recoveries of 89% for virus capsids and 88% for viral genomes highlighted the effective performance of the Sartopore Evo® filter in final sterile filtration of the formulated AAV8 (Figure 9). With HCP almost completely removed during the previous process steps, levels fell below the limit of detection in the final filtrate. hcDNA was further reduced by 19% in this final step of the downstream purification process. In particular, the capacity limit of the Sartopore Evo® filter was not reached during the filtration (data on file).

Figure 9: *Recovery of AAV8 Capsids and Genomes After Final Sterile Filtration* **(A)***, and Removal of hcDNA and HCP During This Step* **(B)**

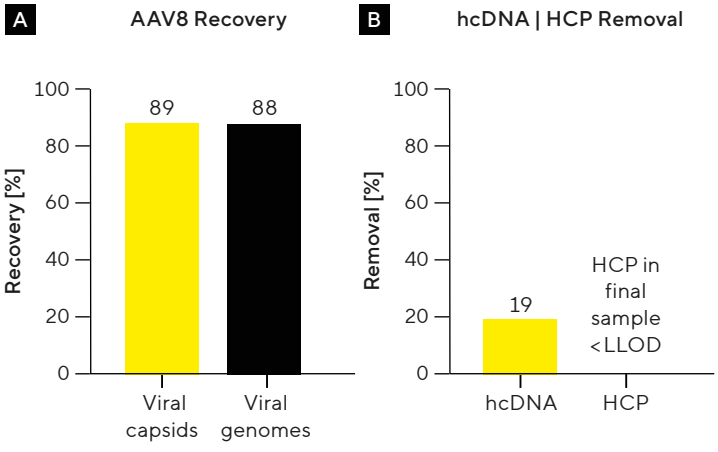


Table 5: *Key Attributes of the Final AAV8 Product*

Parameter	Value
pH	7.9
Conductivity [mS/cm]	19.4
Volume [mL]	120
Viral genome titer [vg/mL]	3.6×10^{12}
Viral capsid tier [vp/mL]	7.2×10^{12}
Full capsids [%]	70
Concentration hcDNA [ng/µL]	224
Concentration HCP [ng/mL]	< LLOD
hcDNA [ng per 1×10^{13} vg]	624
HCP [ng per 1×10^{13} vg]	n.d.

Overview of AAV8 Step and Process Recovery During the Downstream Purification Process

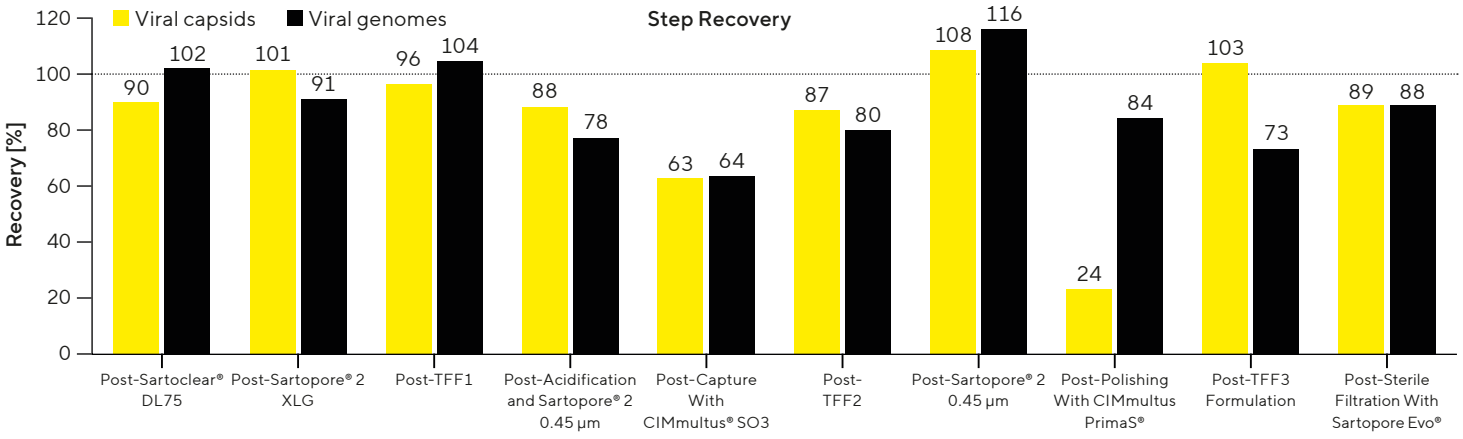
Overall, consistent step recoveries relative to the step feed—ranging from 80% to 100%—were achieved for both AAV8 genomes and capsids, highlighting the excellent performance of the entire end-to-end purification process. Minor deviations from this high performance were observed at certain steps, as illustrated in Figure 10, which provides a comprehensive overview of step recovery rates across all downstream purification steps.

The recovery of viral genomes and capsids after each individual step, relative to the starting material, indicated good overall process efficiency. Harvest clarification and UF | DF of the clarified lysate only slightly reduced AAV genomes and capsids compared to the bioreactor supernatant. In subsequent steps, the main loss occurred during AAV8 capture, which could be attributed to some issues during the execution of this step.

Despite the losses, recoveries of 23% for viral genomes and 9% for viral capsids after the final sterile filtration of the AAV8 bulk product highlighted the remarkable robustness of the overall downstream purification process (Figure 11). As expected, the capsid recovery rate is relatively low, indicating a successful enrichment of filled AAV8 capsids.

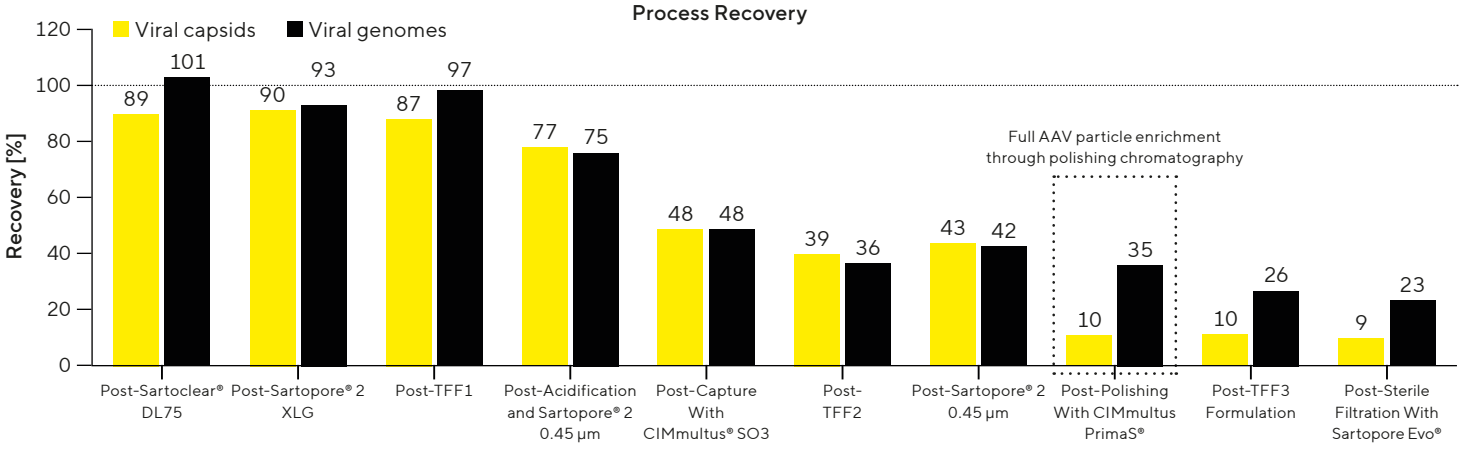
The excellent impurity removal of the downstream AAV purification process, particularly in removing hcDNA and HCP, is illustrated in Figure 12. The $\log_{10}$ reduction of residual hcDNA and HCP—relative to the initial amount measured in the 50 L upstream harvest—increases significantly after each individual purification step, indicating a gradual increase in AAV8 purity through the reduction of these impurities. Overall, hcDNA was reduced by 98.5% (1.82 $\log_{10}$ reduction) and HCP was reduced to a non-detectable level (> 99.99%, 8.1 $\log_{10}$ reduction).

Figure 10: *Step Recoveries of AAV8 Genomes and Capsids Throughout the Entire End-to-End Downstream Purification Process*



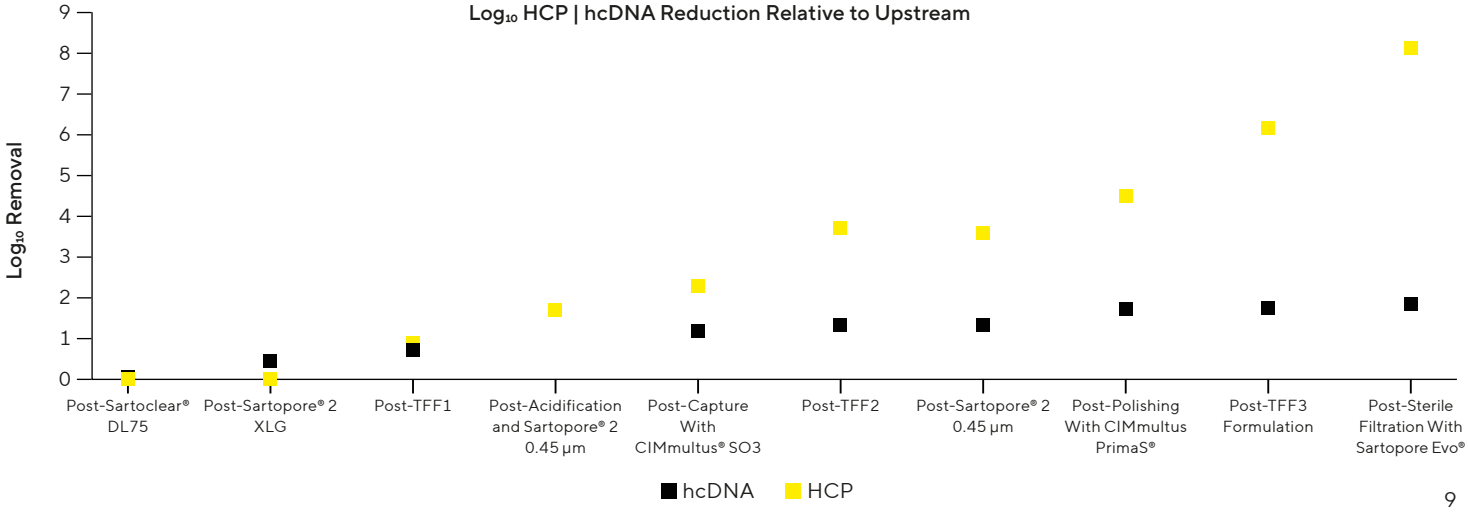
Note. Capsid recovery at polishing chromatography step is low due to full AAV particle enrichment.

Figure 11: *Process Recoveries of AAV8 Genomes and Capsids From Individual Unit Operations Across the End-to-End Purification Process Relative to the Upstream Titer*



Note. Capsid recovery at polishing chromatography step is low due to full AAV particle enrichment.

Figure 12: *Log_{10} Reduction of Residual hcDNA and HCP Across the End-to-End Downstream Purification Process Relative to the Upstream Amount*



Discussion

The biopharmaceutical production of AAVs is challenging due to issues including scalability, low recovery, and the need to separate empty and full capsids. In addition, different AAV serotypes require individual purification methods due to their unique physical and chemical properties. Robust production platforms that are easily adjustable for individual process needs (such as serotype and process scale) are currently lacking — a gap that contrasts with the growing demand for AAV-based therapies.

Here, we demonstrated effective upstream and downstream processing of AAV8, fulfilling the demand for high-quality AAV products with minimal impurities. Advanced filtration and chromatography techniques from Sartorius, achieved robust recoveries and excellent separation of full and empty capsids. Moreover, product impurities (HCP and hcDNA) were substantially removed through optimized UF | DF and chromatography steps, with HCP being below the limit of detection in the final formulated bulk.

In the upstream process, efficient AAV8 production at 50 L clinical scale was confirmed by cell growth and viability comparable to the reference shake flask culture. The downstream purification of AAV8 from the 50 L culture achieved robust recovery rates of 23% viral genomes in the final product. The crucial polishing step, which removes empty capsids that adversely affect product safety and efficacy, achieved robust results: 68% filled virus particles were detected in the full peak fraction, while 0% were found in the empty peak fraction, indicating highly efficient separation. Using the CIMmultus PrimaS® column, full capsids that represent the active product were enriched > 3-fold, increasing from 21% in the feed to 68% in the final product.

Conclusion

Sartorius technologies enable the flexible construction of serotype-independent AAV production platforms as exemplified in this application note for AAV8. The advanced filtration and chromatography technologies ensure efficient impurity removal and high recovery rates, leading to high-quality products and enhanced production efficiency. Using high-resolution monoliths enabled a 3.2-fold effective enrichment of filled AAV capsids, addressing a common challenge in AAV manufacturing.

The all-from-one-source approach not only simplifies procurement and supply chain but ultimately facilitates the development of customized production platforms tailored to individual process requirements. Application specialists with comprehensive knowledge of all platform components can provide significant support to biomanufacturers during process implementation and adaptation. Universally applicable technologies provide the flexibility to adjust the platform to any AAV serotype with minimal effort. Integrated data analytics and innovative screening tools support rapid optimization of process parameters, thereby enhancing overall efficiency and product quality. Notably, all processing units employ sterile single-use components, further ensuring flexibility and rapid changeover while reducing contamination risks for operators and patients and allowing for robust process scaling and flexible adjustments.

Ultimately, our streamlined AAV production platform reduces costs and accelerates time to market for high-quality, high-yield AAVs, giving companies a competitive advantage in the biopharmaceutical AAV landscape.

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