SARTORIUS

Customer Case Study

# Downstream AAV Production: A Targeted Approach to Improve Empty | Full Capsid Separation


**Customer Profile**

Company Name:
Pharmaron Gene Therapy

Company location:
Liverpool, UK

Industry:
Cell and Gene Therapy

Company Profile:
www.pharmaron.com/services/biologics-cgt

Contact
cgt@pharmaron.com

## Customer Challenge

One of the greatest challenges for AAV production is the ability to purify full (product genome containing) from empty (non-product containing) viral capsids. Separation of empty from full capsids is challenging because they are similar in charge and size. The presence of empty capsids could affect the efficacy and safety of AAV vector products due to the risk of increased immunogenicity of the product.

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# An Introduction to Pharmaron

Pharmaron is a leading, fully integrated pharmaceutical R&D service provider with global operations and a well-established team of over 19,000 employees working across 20 different sites worldwide. Pharmaron's Gene Therapy CDMO, located in Liverpool, UK, has a strong team of around 180 people focusing on viral vector development and clinical manufacture, delivered through its MHRA IMP licensed cGMP facility. Pharmaron Gene Therapy has developed an adeno-associated virus (AAV) platform and purification toolbox to ensure the production of multiple AAV serotypes and produces the necessary critical starting materials for these products. It has world-class analytical capabilities delivered through state-of-the-art equipment and expertise and works with its customers to deliver innovative medicines to patients.

Working with Sartorius BIA Separations, Pharmaron was able to extend its toolbox of AAV purification solutions, providing multiple-modality options to achieve robust separation of genome-containing (full) from genome-free (empty) viral capsids with high purity and yield for multiple AAV serotypes.

# Solution Provider – Sartorius BIA Separations

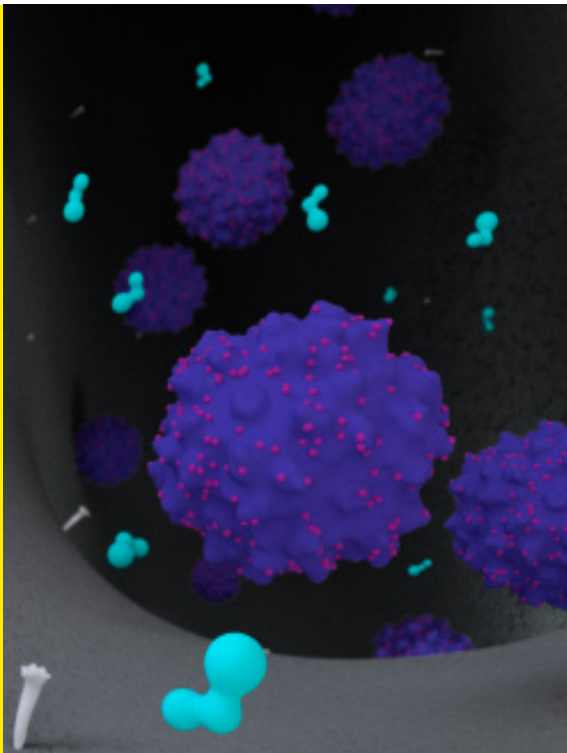
Sartorius BIA Separations is the leading developer of monolithic technology and the exclusive producer of CIM® (Convective Interaction Media) monolithic columns and PATfix™ systems for high performance liquid chromatography (HPLC) based biomolecule analysis. The CIM® monoliths are defined as single-unit structures with highly interconnected convective channels with sizes of 1.3 µm, 2 µm and 6 µm. A variety of chemistries cover several modes of AAV purification such as hydrophobic, ion exchange, metal affinity and multimodal chromatography. The monoliths are available in plug and play cGMP formats with proven scalability from 0.05 mL to 40 L covering analytical, microplate-based high throughput screening and commercial downstream processing (DSP) needs. Sartorius BIA Separations' multiuse research, production, and training facility is headquartered in Ajdovscina, Slovenia with sales and distribution offices located internationally.

**Project Key Indicators**

Molecule Type:
AAV

Sartorius BIA Separations Products:
- CIMmultus™ SO3
- CIMmultus™ QA
- PATfix™

Pharmaron's Integration of Sartorius BIA Separations Technologies
- Integrated in Pharmaron's cGMP facility (50 L to 500 L)
- Pharmaron added a monolith-based process to its purification toolbox
- Included in Pharmaron's advanced analytical toolkit for process & product characterization



# The Challenge

One of the greatest challenges for AAV production is the ability to purify full (product genome containing) from empty (non-product containing) viral capsids. The presence of empty capsids could affect the efficacy and safety of AAV vector products due to the risk of increased immunogenicity of the product (Cellular, Tissue, and Gene Therapies Advisory Committee-FDA, 2021; Committee for Advanced Therapies-EMA, 2018). Separation of empty from full capsids is challenging because they are similar in charge and size. Traditionally ultracentrifugation-based purification methods have been able to provide drug substance with a high enrichment of full capsids. However, although it may be suitable for very early-stage drug discovery and development, ultracentrifugation adaption for large scale operation is challenging. Its productivity is low, the equipment is expensive, and scaling of the process is driven by a scale-out instead of a scale-up approach – essentially the need for more and more ultracentrifuges. Although Pharmaron has the ability to deliver ultracentrifugation-based processes, Pharmaron has strategically targeted chromatography for an 'end in mind' approach. Chromatography based operations are scalable, thus more suitable for gene therapies and especially for those with high dose requirements. Pharmaron has a purification toolbox in place and ready to deliver multiple serotype AAV products, however its innovative approach includes evaluation of the latest advancements.

# The Solution

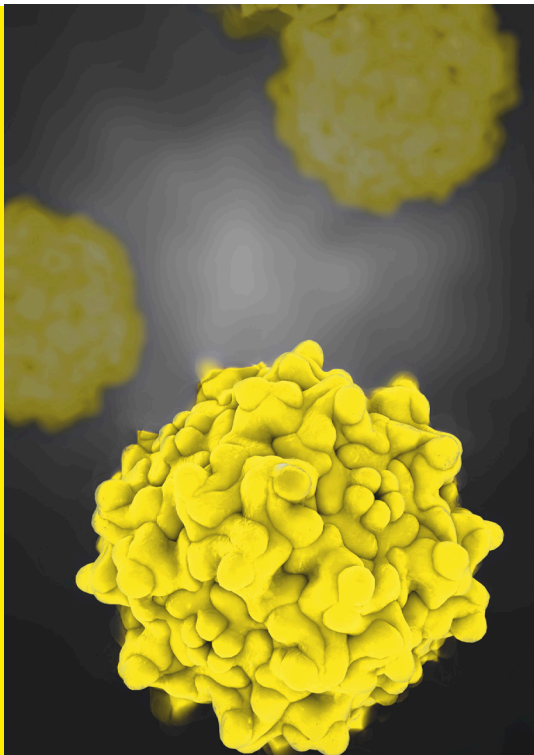
Through Pharmaron's innovation approach, a collaboration was established with Sartorius BIA Separations to explore the potential of monoliths to separate AAV full and empty capsids. Size exclusion (SEC), cation exchange (CEX) and anion exchange (AEX) high performance liquid chromatography (HPLC) analytics based on Sartorius BIA Separations' PATfix™ HPLC devices were used to estimate process recoveries with high precision and accuracy. The collaboration was established with an initial feasibility study to characterise Pharmaron's post affinity AAV material. The results of the feasibility study showed promising recovery and separation of full from empty capsids when using CIMmultus™ SO3 as a capture step followed by CIMmultus™ QA monoliths in an AEX polishing step. As a result of this successful work, the Pharmaron team in Liverpool and Sartorius BIA Separations decided to extend their efforts and optimise the separation of Pharmaron's AAV material in the AEX step. Different buffer compositions and running conditions in AEX CIMmultus™ QA monoliths were tested. High titre recoveries by capsid ELISA and ddPCR, and high full capsid content by PATfix™ AEX-HPLC were achieved.

**Key Benefits**

Downstream processing time reduced by 60%.

No dead-ends avoids entrapment of AAVs and significantly improves the process recovery.

Higher capacities than resin-based columns, due to large size channels, high flow rates with low pressure drops and high surface accessibility of binding sites for AAVs.

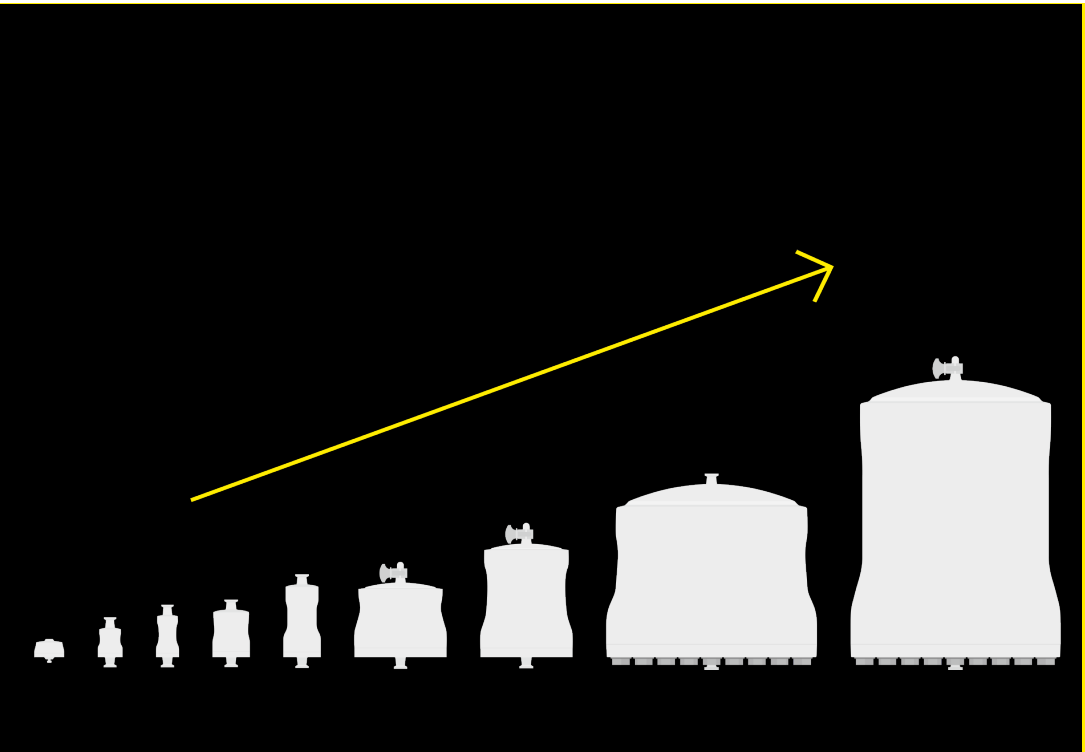


# Selection Process

Pharmaron were looking for an efficient and reproducible separation of AAV full from empty capsids with secondary benefits of reduced processing time and quicker delivery. Pharmaron selected Sartorius BIA Separations for their expertise to use monolith technology within a gene therapy process for commercial AAV production. The large size channels permit high flow rates with low pressure drops and their high surface accessibility of binding sites for AAVs allows capacities exceeding those of resin-based columns, along with rapid mass transfer based on convection. Absence of dead-ends avoids entrapment of AAVs and significantly improves the process recovery. Sartorius BIA Separations' monoliths can offer faster processing times at manufacturing scale without the need for column packing as with resins. With this approach the downstream processing time may be reduced by 60%. This cumulates to more batches manufactured per year, reduced CMC and operating timelines, and faster delivery of AAV gene therapies.

# Implementation Project

Pharmaron has a strong track record of creating effective partnerships with research organisations, universities, and industry through innovative collaboration. These partnerships develop strong relationships with both parties desiring to achieve long-term win-win benefits and innovation based on mutually desired outcomes. Pharmaron collaborated with Sartorius BIA Separations with the primary goal to identify new approaches that will position them at the forefront of industry for the purification of AAVs. Throughout collaboration between Pharmaron and Sartorius BIA Separations, effective communication and regular meetings (despite different locations on the continent) allowed both companies to develop a relationship which helped troubleshoot the many technical and scientific challenges with ease.



**CIM® Monolithic Columns**

**Key Benefits:**

- plug and play cGMP formats
- scalability from 0.05 mL to 40 L

CIM® Monolithic Columns cover analytical, microplate-based high throughput screening and commercial downstream processing (DSP) needs.

# Business Results

Pharmaron and Sartorius BIA Separations strongly value collaborations fuelled by an innovative mindset and a clear potential for success. The collaborative culture of Pharmaron and Sartorius BIA Separations helped them resolve all challenges that were encountered. Pharmaron added a monolith-based process to its toolbox which has been tested on several different AAV serotypes and transgenes with high success. During this collaboration, a strong partnership was developed leading to Sartorius BIA separations presenting the work at the second webinar of the Pharmaron Cell and Gene Therapy webinar series. The webinar was entitled "Downstream AAV Production: A Targeted Approach to Optimization" and its recording is available for viewing at https://www.pharmaron.com/webinars/cgt-webinar-series.

# References

Cellular, Tissue, and Gene Therapies Advisory Committee-FDA. (2021). Toxicity Risks of Adeno-associated Virus (AAV) Vectors for Gene Therapy (GT) (Briefing document: 151599). Food and Drug Administration, U.S. Department of Health and Human Services. https://www.fda.gov/media/151599/download

Committee for Advanced Therapies-EMA. (2018). Quality, preclinical and clinical aspects of gene therapy medicinal products (EMA/CAT/80183/2014). European Medicines Agency, European Union. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-clinical-aspects-gene-therapy-medicinal-products_en.pdf

**Germany**
Sartorius Stedim Biotech GmbH
August-Spindler-Strasse
37079 Goettingen
Phone +49 551 308 0

**Slovenia**
Sartorius BIA Separations d.o.o.
Mirce 21
5270 Ajdovščina
Phone +386 59 699 500

**For further information, visit**
www.sartorius.com
www.biaseparations.com