Application Note

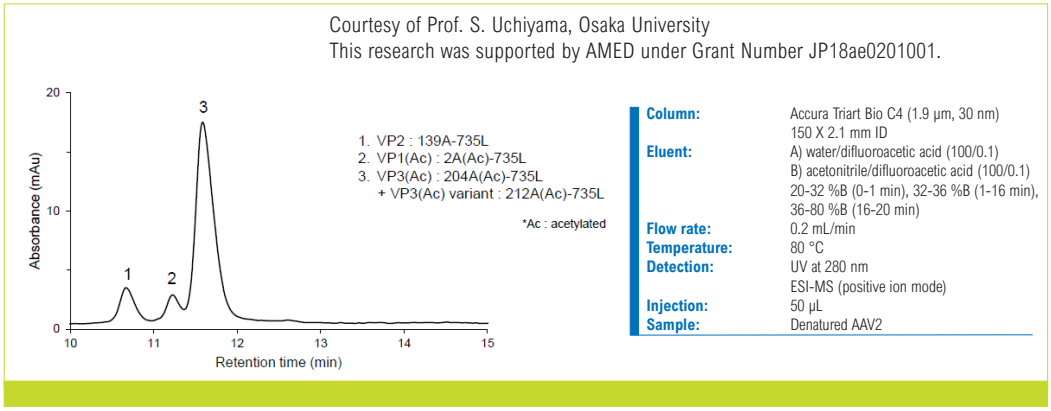
YMC offers alternatives for the analysis of adeno-associated viruses (AAVs)

Adeno-associated Virus (AAVs) technologies are studied as tools for genetic therapy, serving as viral vectors. Among the main challenges related to their use as delivery vectors, it can be said that quality control is a key aspect to ensure a reproducible and safe product.

Viral vectors are an important tool in gene therapies. Their role is to deliver therapeutic genes into target cells associated with genetic diseases, such as metabolic, hematological, neuromuscular diseases, cancer, among others. The choice of AAVs is based on several attributes, including their relative safety, with data classifying them as non-pathogenic and determining their inability to replicate, the capacity to modify their capsids, adaptability for load transport, among others (WANG et al. 2024).

Adeno-associated viruses exhibit high variability between serotypes, batches, and even within the same batch. As a result, a quality control system is needed to ensure reproducibility in achieving the biotherapeutic effect sought with the finished product. In other words, a laboratory working with AAVs must perform analyses that ensure the safety and efficacy of the product. To achieve this, instruments such as mass spectrometers and chromatographs, along with other analytical tools, are used (KONTOGIANNIS et al. 2024).

The RP-MS technique is necessary for determining viral proteins that make up the capsid. Typically, these proteins are found in a VP1:VP2:VP3 ratio of 1:1:10. The separation of viral proteins at an analytical level provides data such as their exact masses and confirmation of stoichiometries, which in turn provides information on the AAV serotype and pathways for controlling its production. The Accura Triart Bio C4 column can be used for this type of application.



It is extremely important to note that this method requires the use of 0.1% of a haloacetic acid at a temperature of 80 ºC in a high-sensitivity detector. The risks in this case involve potential deterioration of the column or its ligands due to chemical and thermal actions, or degradation of the analytical signal due to the release of artifacts from an unstable stationary phase. The Triart line is presented by YMC specifically as a next-generation hybrid silica alternative that offers high chemical, thermal, and mechanical resistance, capable of operating even under extreme and high-demand conditions.

The mentioned column carries the term ***bio***, indicating that it is a 300 Å model, ideal for working with macromolecules (up to a 150 kDa limit in a protein calibration curve for the Triart Bio C4) and the term ***Accura***, referring to the internal coating of the column walls and frits with a thin layer of ceramic material that prevents any contact between the analyte of interest and the stainless steel surface, eliminating any negative effect on the chromatogram due to undesired secondary intermolecular interactions. This ensures a result with the highest possible efficiency.

AAVs can have impurities such as empty capsids or even partially filled ones as process by-products. One alternative at an analytical or even preparative level is to explore the variation in the surface charge of this viral structure as a function of its content. In other words, distinguishing full and empty capsids through ion-exchange chromatography (KONTOGIANNIS et al. 2024). YMC offers a high-productivity alternative for this need.

The **BioPro IEX QF** is a stationary phase that uses rigid polymethacrylate polymer particles functionalized with quaternary ammonium groups. Its rigid structure allows for faster, high-efficiency runs, making this column the perfect choice for developing high-productivity quality control methods.

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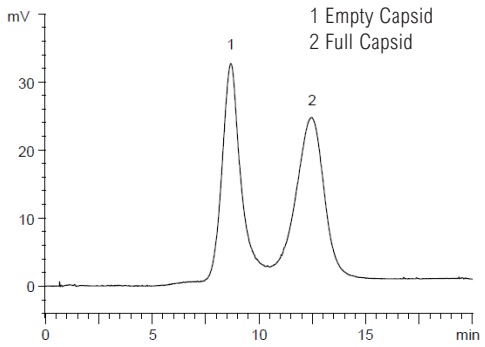
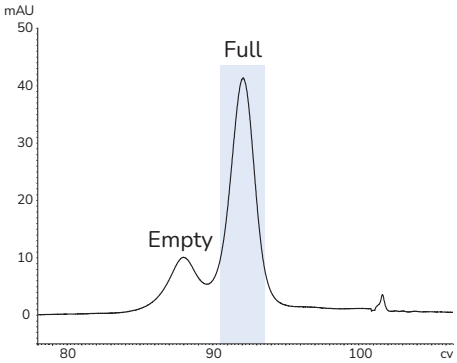
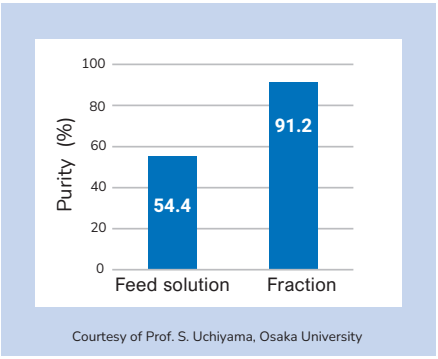


Column:	Accura BioPro IEX QF (5 µm) 50 x 4.6 mm ID
Eluent:	A) 20 mM Bis-tris propane-HCl (pH 9.0) B) 20 mM Bis-tris propane-HCl containing 0.5 M TMAC* (pH 9.0) 5 %B (0-0.25 min), 20-45 %B (0.25-15.25 min)
Flow rate:	0.5 mL/min
Temperature:	25 °C
Detection:	FLS at Ex. 280 nm, Em. 348 nm
Injection:	2 µL
Sample:	AAV2 (2.59 x 10^{12} vg/mL)

*Tetramethylammonium chloride

In this same context, YMC also offers stationary phases for the preparative-scale purification of AAVs. The **Macrosep IEX Q** models separate full and empty capsids based on the same principle as the analytical versions, with the difference being that they feature a stationary phase made of porous polymer particles with pore sizes of 900 nm. The larger pore size plays a key role in accommodating the analytes of interest, resulting in improved resolution between the target object and impurities.





Courtesy of Prof. S. Uchiyama, Osaka University

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Reference:

KONTOGIANNIS et al. Characterization of AAV vectors: A review of analytical techniques and critical quality attributes. Molecular Therapy: Methods & Clinical Development. 32:3. 2024. https://doi.org/10.1016/j.omtm.2024.101309

WANG et al. Adeno-associated virus as a delivery vector for gene therapy of human diseases. Signal Transduction and Targeted Therapy. 9:78.2024. https://doi.org/10.1038/s41392-024-01780-w

YMC AMERICA
8 Charlestown Street, Devens, MA 01434
United States
Tel: 978.487.1100
sales@ymcamerica.com • www.ymcamerica.com

YMC
YMC Co., Ltd Headquarters
Kyoto, Japan
Tel: +81.75.342.4515
sales@ymc.co.jp • www.ymc.co.jp/en