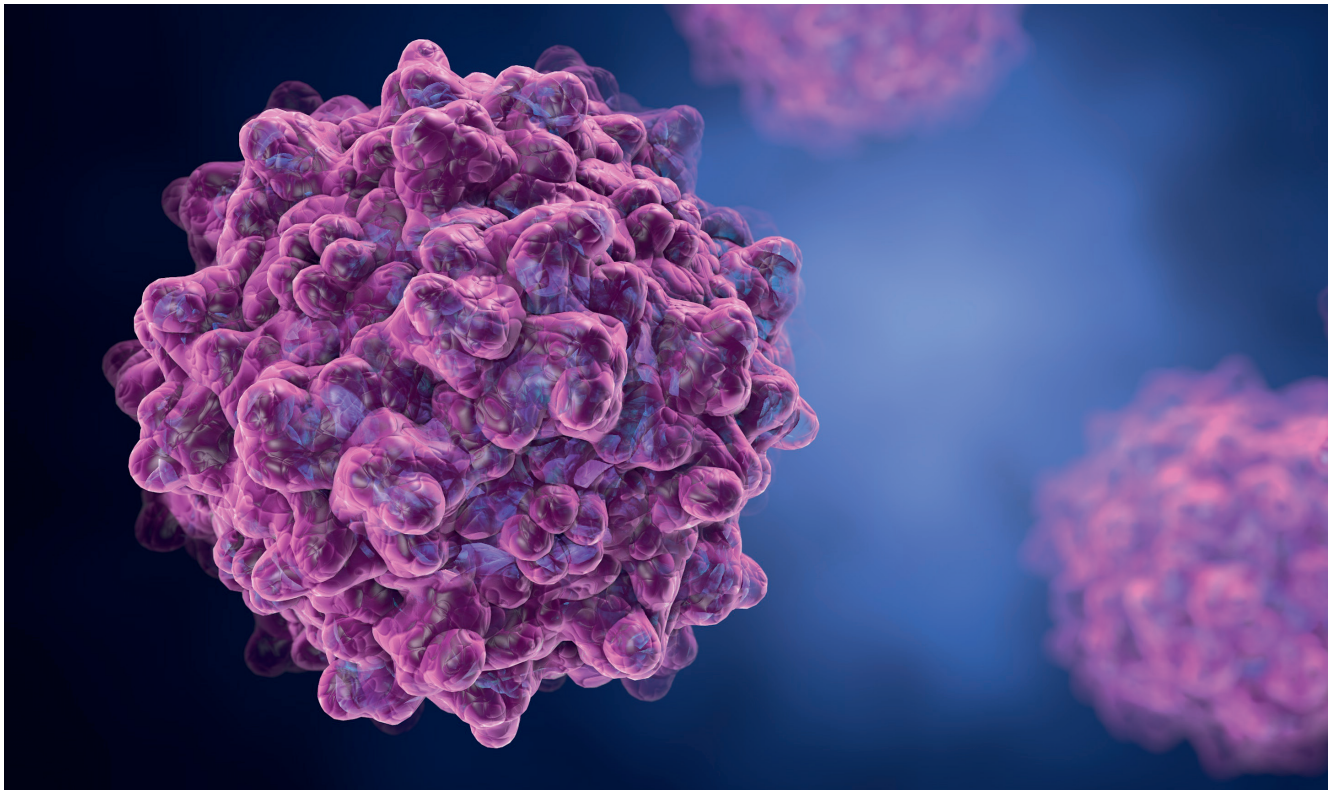
The best choice for AAV capsid protein analysis – YMC-Accura Triart Bio C4

Adeno-associated viruses (AAVs) are the leading and most effective gene delivery vehicles to treat human diseases. Three AAV-based drugs have already been approved in the US by the FDA and many more are currently being tested in clinical trials. The AAV capsid, which encloses the genes to be delivered, is composed of three viral proteins (VPs): VP1 (≈90 kDa), VP2 (≈68 kDa), VP3 (≈62 kDa).

The molar ratio of the single proteins is estimated to be 1:1:10. Altered ratios, for example due to degradation, might change the vectors potency and carry an increased risk of immunogenicity. Therefore, the quantity each of these proteins needs to be closely controlled during product development to ensure drug safety.



Several analytical methods such as enzyme-linked immunosorbent assay (ELISA), sodium dodecyl sulphate- polyacrylamide gel electrophoresis (SDS-PAGE) and Western Blot analysis have been applied to monitor capsid composition. However, these methods are often labour intensive and have low precision. Therefore, reversed phase liquid chromatography (RP-LC) coupled to mass spectrometry (MS) is a viable option for quantification and identification of VPs. In order to enhance the quality of results the use of bioinert hardware can prevent unwanted interactions of analytes with the column hardware.

The bioinert YMC-Accura column possess fully bioinert coated column body and frits, while revealing the same robustness as stainless steel columns. In this technical note, analysis of VPs of AAV2 using the bioinert YMC-Accura Triart Bio C4 was compared to the standard YMC-Triart Bio C4 column and competitor C4 column with stainless steel hardware. With its wide pores of 300 Å and its less hydrophobic butyl modification, the YMC-Triart C4 column enables the separation of large biomolecules of up to 150 kDA.

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Table 2: Chromatographic conditions.

Columns:	YMC-Accura Triart Bio C4 (1.9 µm, 30 nm) 150 x 2.1 mm ID (bioinert hardware) YMC-Triart Bio C4 (1.9 µm, 30 nm) 150 x 2.1 mm ID (standard hardware) Other C4 column (1.7 µm, 30 nm) 150 x 2.1 mm ID
Part Nos.:	TA30SP9-15Q1PTC TA30SP9-15Q1PT
Eluent:	A) water + 0.1% difluoroacetic acid B) acetonitrile + 0.1% difluoroacetic acid
Gradient	20–32%B (0–1 min), 32–36%B (1–16 min), 36–80%B (16–20 min)
Flow rate:	0.2 mL/min
Detection:	UV at 280 nm ESI-MS (positive ion mode)
Temperature:	80 °C
Injection:	50 µL
Sample:	Denatured AAV2

Both, the bioinert YMC-Accura Triart Bio C4 and the standard YMC-Triart Bio C4 column are able to separate all three VPs, whereas using the other C4 column, VP1 and VP2 elute at the same time (figure 1). This proves the higher selectivity of the YMC-Triart columns compared to the competitor column. The bioinert YMC-Accura Triart Bio C4 demonstrates the advantages of bioinert hardware by showing improved resolution of all three analytes and sharper peaks.

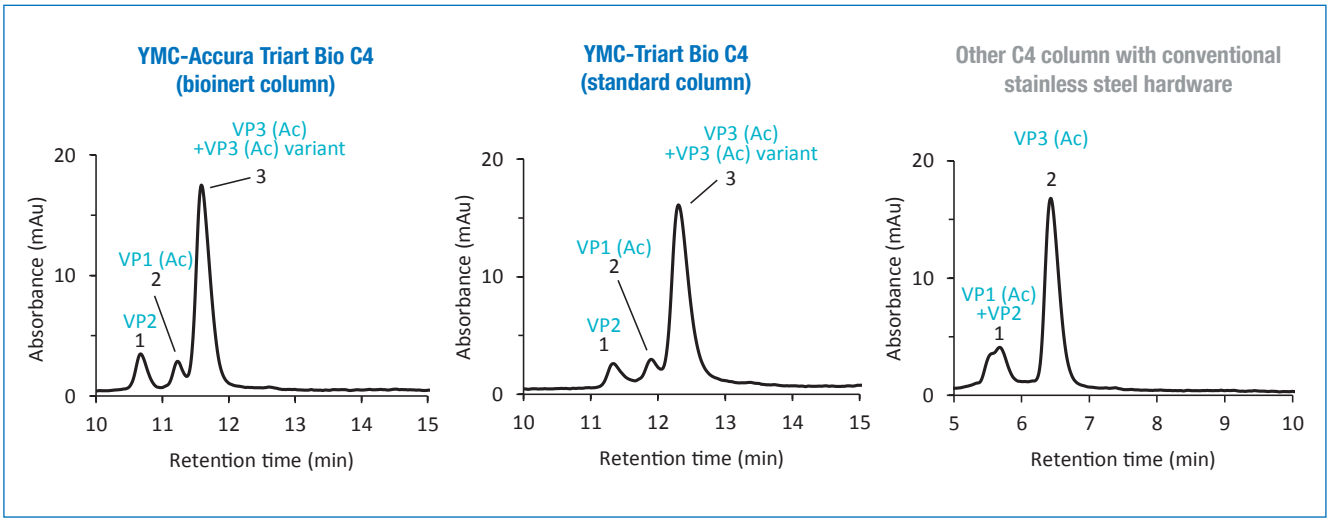

Figure 1: Comparison of VPs separation by RP-LC using the bioinert YMC-Accura Triart Bio C4, YMC-Triart C4 (standard hardware) and another C4 column with stainless steel hardware.

These results demonstrate that the wide-pore stationary phase YMC-Triart Bio C4 is highly suitable for the analysis of AAV VPs, even separating the challenging VP1 and VP2. Analysis can be further improved by use of the bioinert column YMC-Accura Triart C4, which prevents protein adsorption to column hardware.

The use of the bioinert YMC-Accura Triart Bio C4 column leads to:

- **Improved separation**
- **Better peak shapes**
- **Reduced retention time**

This makes the YMC-Accura Triart Bio C4 column an excellent choice for the analysis of AAV VPs.

Application data courtesy of Prof. S. Uchiyama, Osaka University.