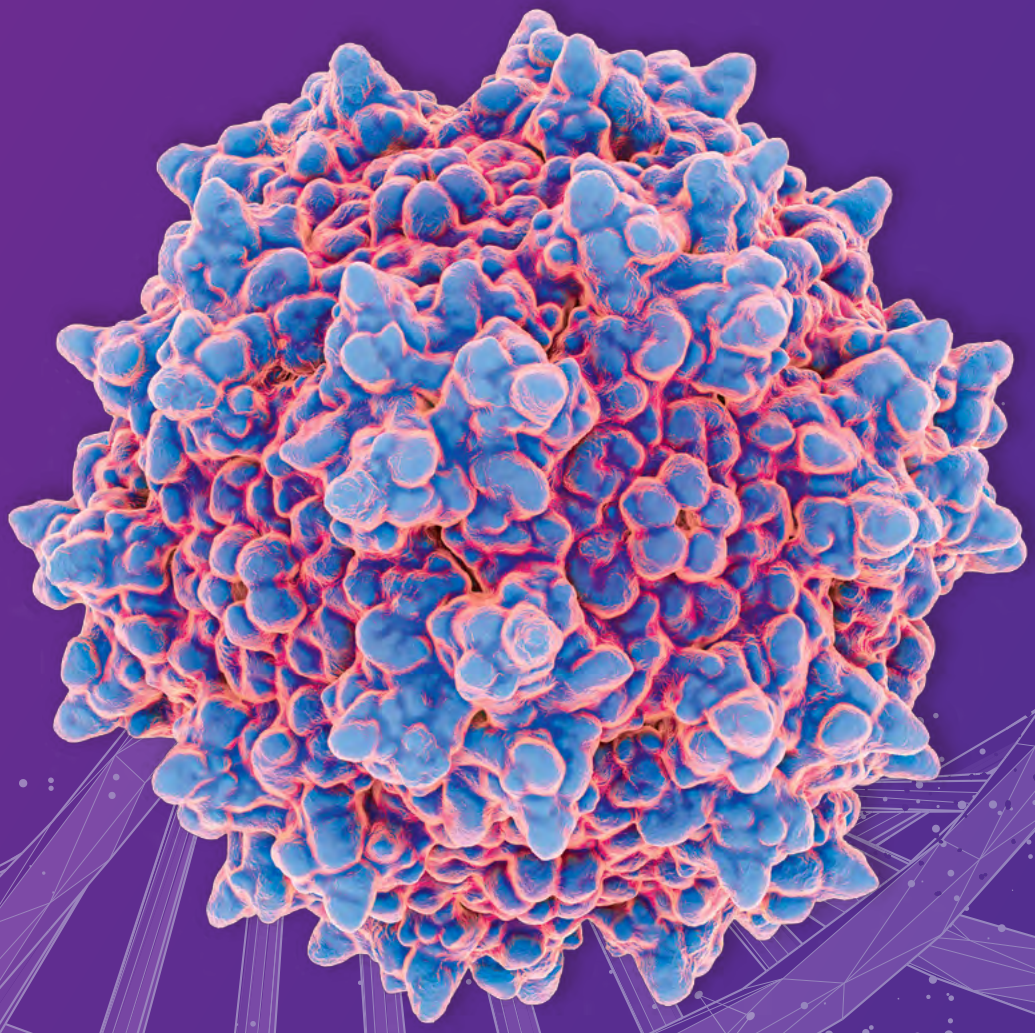
CONSUMABLES GUIDE BOOK

Characterizing AAVs

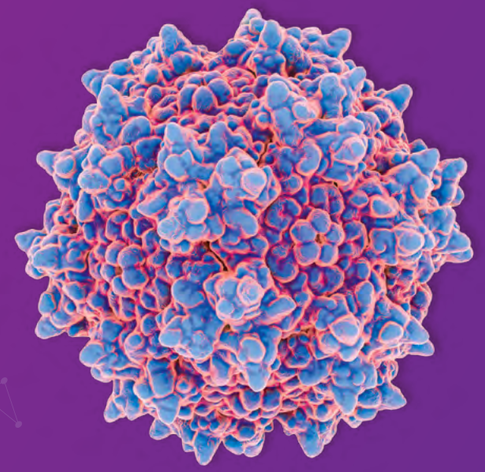
Chromatographic methods to measure heterogeneity and critical quality attributes



Waters™

A Changing Industry

The advent of FDA approved gene therapies brings to reality a decades long promise to cure chronic diseases, ranging from inherited forms of blindness to muscular dystrophy. Viral vectors, such as a small 25 nm, non-enveloped adeno-associated virus (AAV), can serve as the delivery vehicle for bringing a new gene to patient cells. Different serotypes and various engineered forms of AAV exist, and they each exhibit distinct cellular tropism to deliver their 4.8 kilobase transgene. As a non-covalent ~5 MDa complex containing both protein and nucleic acid content, this type of drug product must be extensively characterized during development and to proceed with an investigational new drug filing or biological license application. Moreover, a number of tests must be performed upon batch release to ensure safe and efficacious treatments are provided to patients. Chromatography plays an important role.



Structure of an adeno-associated viral vector

Chromatography and Method Options

Size Exclusion

Anion Exchange

VP Protein Analysis

VP Peptide Mapping

Waters Application Notes and Peer Reviewed Articles

Waters scientists and collaborators are publishing on this subject. Make sure to frequent the Resource Tab on our **waters.com/GTx** website to keep up to date on the literature.

Attribute Testing

An AAV vectored therapy contains multiple types of biomolecules compromised of more than 200,000 atoms. The complex nature and manufacturing process of an AAV necessitates a variety of tests to ensure the safety and efficacy of every batch of drug product.

IDENTITY

The identity of an AAV must be confirmed, which includes confirmation of serotype, VP protein identities, and the transgene.

PURITY

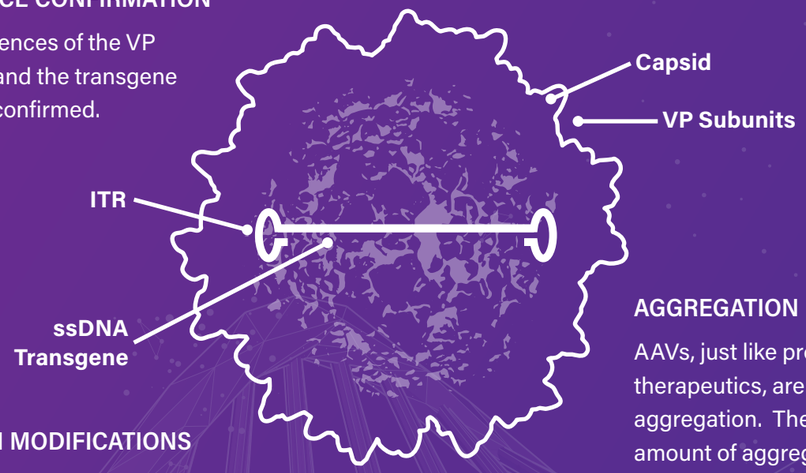
An AAV material can contain a number of different process and product related impurities. Empty and aggregated capsids are examples of several important impurity related measurements.

ENCAPSIDATION

The relative amount of full versus empty capsid matters. In addition, it is important to assess the abundance of partially filled and overfilled capsid species.

SEQUENCE CONFIRMATION

The sequences of the VP proteins and the transgene must be confirmed.



PROTEIN MODIFICATIONS

Monitoring of post-translational modifications and presence of engineered sequences is critical to AAV activity.

VP PROTEIN RATIO

The relative amounts of the AAV viral proteins (VP 1, 2, and 3) can be diagnostic of its capsid morphology and directly tied to transduction efficiency and stability.

POTENCY

An AAV's potency will depend on its titer and purity.

TITER

The concentration of capsid protein, genome, and infectious units per volume of drug substances must be determined. The titer of the drug is important to administering a safe and efficacious dose.

AGGREGATION

AAVs, just like protein therapeutics, are prone aggregation. The relative amount of aggregate should be determined to make sure a high purity monomeric AAV injection is provided to patients.

GENOME INTEGRITY

The AAV genome is the transgene that serves to replace or add an important gene for additive expression from a cell. The size of the genome should be checked for truncations and concatenated multimers.

SEC for Aggregates and Multi-Attribute Monitoring

Size exclusion chromatography is one of the most important methods for measuring critical quality attributes that are tied to the safety and efficacy of viral vectored gene therapies, including larger aggregates and impurities. Combined with mulitiangle light scattering (MALS), SEC assays can provide valuable multi-attribute measurements on size-distribution, molar mass, aggregate conformations, and absolute titer. For this purpose, a high efficiency XBridge™ Premier GTx BEH SEC column constructed with MaxPeak High Performance Surface hardware provides 2x faster analyses, 50% higher efficiency and 3x less sample consumption without sacrificing sensitivity.

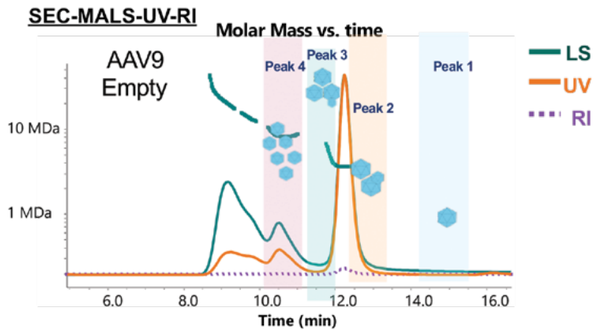


Figure 1. Aggregate & Particle Measurement by SEC-MALS with an XBridge Premier GTx BEH™ SEC 450Å 2.5 µm Column as reported in Waters Application Note 720007969.

AEX to Measure Encapsidation

Empty viral particles present in a drug product may compromise its potency and add to the overall antigen load that a patient will experience. Assays to measure empty/full ratios are thus very important. Anion exchange (AEX) is a preferred technique for the separation of empty and full capsids due to presence/absence of negatively charged genomic DNA. A non-porous, high efficiency stationary phase such as that found in a Protein-Pak™ Hi Res Q Column can be combined with an optimized salt gradient to provide a QC-friendly technique for empty versus full capsids measurements.

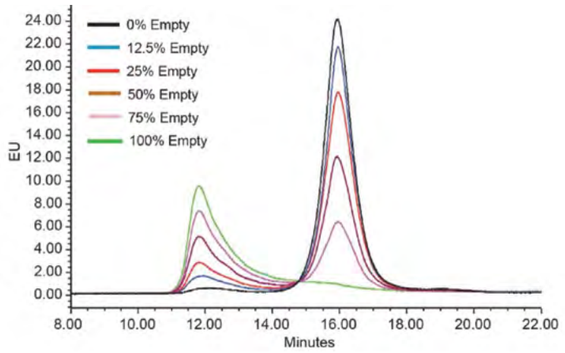


Figure 2. Quantification of % empty capsids in various AAV8 full and empty capsid mix using an optimized salt gradient and Protein-Pak Hi Res Q column as reported in Waters Application Note 720006825.

VP Protein Analysis by RPLC and HILIC

An AAV capsid is constructed from three viral proteins, VP1, VP2 and VP3. Each share a common sequence but differ in size. Their 1:1:10 ratio correlates with structure and function. In addition, the VP proteins can be post-translationally modified and subject to degradation, like deamidation. Reversed phase chromatography can be applied to identify and measure the relative abundance of the capsid components. A BEH C_4 column is recommended along with DFA ion pairing, because of its utility in balancing both LC and MS performance. Hydrophilic interaction chromatography (HILIC) is also useful, where a polar stationary phase and gradient of increasing water is applied. It is recommended to use an ion pairing acid, such as TFA or DFA, along with a BEH 300Å Amide particle, MaxPeak HPS column to minimize analyte to hardware adsorptive interactions. With a well optimized HILIC separation, it is possible to resolve phosphorylated isoforms.

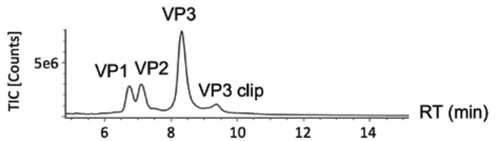


Figure 3. RPLC of AAV8 capsid proteins using DFA modified mobile phases and a Protein BEH C_4 Column as reported in Human Gene Therapy, 2021, 1501-1511.

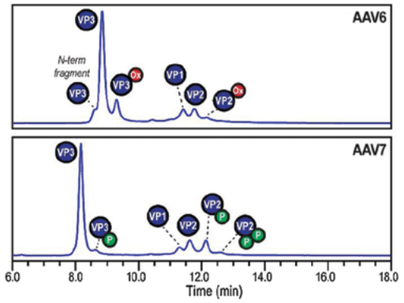


Figure 4. VP proteins separated by HILIC as reported in Journal of Pharmaceutical and Biomedical Analysis, 2020, 189, 113481. Printed with permission from Elsevier.

VP Peptide Mapping

Peptide mapping has long been applied to protein therapeutics. It is now also being applied to confirm the sequence, post translational modifications and degradation of VP proteins. Paramount to acquiring quality data is the use of high purity, autolysis resistant trypsin, like RapiZyme™ Trypsin, that can be applied to ensure quick digestions with minimal enzyme interference peaks and sample prep artifacts. Chromatography with BEH or CSH™ particle columns serves as a reliable starting point for obtaining the highest peak capacity analyses.

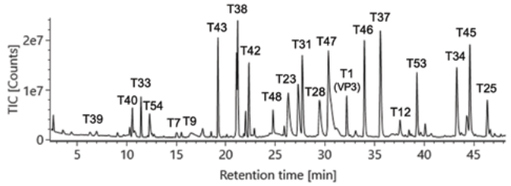


Figure 5. Peptide map of an AAV5 sample as obtained with a BEH 300Å C_{18} particle column and as reported in Human Gene Therapy, 2021, 1501-1511.

Ordering Information

The tools you need to comprehensively characterize an AAV gene therapy can be found here.

SEC

XBridge™ Premier GTx BEH SEC 2.5 µm 450Å Columns

	Dimension	150 mm	300 mm	Guard
Standard Column	4.6 mm	186010584	186010585	186010583
	7.8 mm	186010586	186010587	186010583

AEX

Anion Exchange Columns

	Dimension	P/N
Protein-Pak HiRes Q Column	4.6 x 100 mm	186004931
Gen-Pak FAX Column	4.6 x 100 mm	WAT015490

STANDARDS AND REAGENTS

Standards and Reagents

	P/N
dsDNA 50 to 1350 Ladder	186010778
BEH450 SEC Protein Standard Mix	186006842
MassPREP Peptide Mixture	186002337
RapiZyme Trypsin	186010106
IonHance DFA	186009201

PROTEIN HILIC

ACQUITY™ Premier Glycoprotein BEH Amide 300Å 1.7 µm Columns

	Diameter	50 mm	100 mm	150 mm
Standard Column	2.1 mm	186009547	186009548	186009549

PROTEIN RPLC

ACQUITY Premier Protein BEH C_4 300Å 1.7 µm Columns

	Diameter	50 mm	100 mm	150 mm
Standard Column	2.1 mm	186010326	186010327	186010328

PEPTIDE RPLC

ACQUITY Premier Peptide CSH C_{18} 130Å 1.7 µm Columns

	Diameter	50 mm	100 mm	150 mm
Standard Column	2.1 mm	186009487	186009488	186009489
VanGuard FIT Column	2.1 mm	186010709	186010710	186010711

XSelect™ Premier Peptide CSH C_{18} 130Å 1.7 µm Columns

	Diameter	50 mm	100 mm	150 mm
Standard Column	2.1 mm	186009904	186009905	186009906
	4.6 mm	186009907	186009908	186009909
VanGuard FIT Column	2.1 mm	186010712	186010713	186010714
	4.6 mm	186010715	186010716	186010717

waters.com/GTx

Waters™

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
waters.com