BioReliance®
Pharma & Biopharma Manufacturing & Testing Services

Technical Note

Peptide Mapping of AAV by LC-MS

Analytical Development for AAV

Challenge

The capsid of Adeno-Associated Virus (AAV) is composed of the viral proteins VP1, VP2 and VP3, which come together in a 1:1:10 ratio to form an icosahedral structure. There are at least 13 different variants (serotypes) of AAV, as well as a growing number of novel engineered serotypes. The high sequence homology between the different serotypes represents a challenge in establishing a specific test for confirming the identity of AAV products.

Peptide mapping by Liquid Chromatography – Mass Spectrometry (LC-MS) is a highly-selective technique that can be used to identify capsid proteins by confirming the protein sequence. The proteins that compose the AAV capsid are digested into peptides, separated by chromatography, and then detected by mass spectrometry. By comparing the observed and the theoretical molecular weights of the peptides, the protein sequence can be confirmed.

Furthermore, this approach can be used to identify and quantify post-translational modifications (PTMs), such as deamidation, oxidation, phosphorylation and acetylation. Certain PTMs can impact the potency of AAV and so it is important that these are characterized during development of the product.

Methodology

First, AAV samples are purified by size-exclusion chromatography to remove surfactants from the sample matrix that may interfere with the analysis. The purified samples are then evaporated to dryness, before reconstitution at the appropriate concentration for subsequent sample preparation. The samples are then denatured and digested with a protease, such as trypsin. After digestion, the resulting peptides are analyzed by reversed-phase liquid chromatography (LC) with on-line mass spectrometry (MS) detection.

The LC-MS data are processed to enable assignment of the observed peptides to those expected from the protein sequence. Peptide modifications are also considered.

Assay Details

A minimum of 55 µL AAV at 1e13 vp/mL is required. The expected primary structure (amino acid sequence) should be provided.

Case Study Results

These results were generated from an internal study where peptide mapping of two different AAV serotypes – AAV8 and AAV9 – was performed by LC-MS. The chromatograms obtained for each sample (Figure 1) show a number of common peaks due to regions of sequence homology. Overall, however, the chromatographic profiles are significantly different from each other because of peptides unique to each serotype.

High sequence coverage (>90%) was obtained when the data were processed with the correct sequence for the corresponding serotype. In contrast, low sequence coverage (<40%) was obtained when, for example, the data for AAV8 were processed with the sequence of AAV9. These results demonstrate that the method is highly specific and is therefore suitable as an identity test.

In addition, a number of PTMs were identified in each sample, including deamidation, oxidation, phosphorylation and N-terminal acetylation. For example, in the chromatogram of AAV9, a small peak at 10.6 minutes corresponding to a deamidated peptide was observed just after the unmodified peptide peak at 10.1 minutes (Table 1).

Deamidated variants of AAV can have lower potency[1], so it is important that these modifications are characterized. Peptide mapping by LC-MS is a powerful approach to identifying PTMs, determining their location in the sequence, and quantifying their relative abundance.

[1] Frederick A, Sullivan J, Liu L, et al. Hum Gene Ther. 2020; 31 (13-14): 756-774.

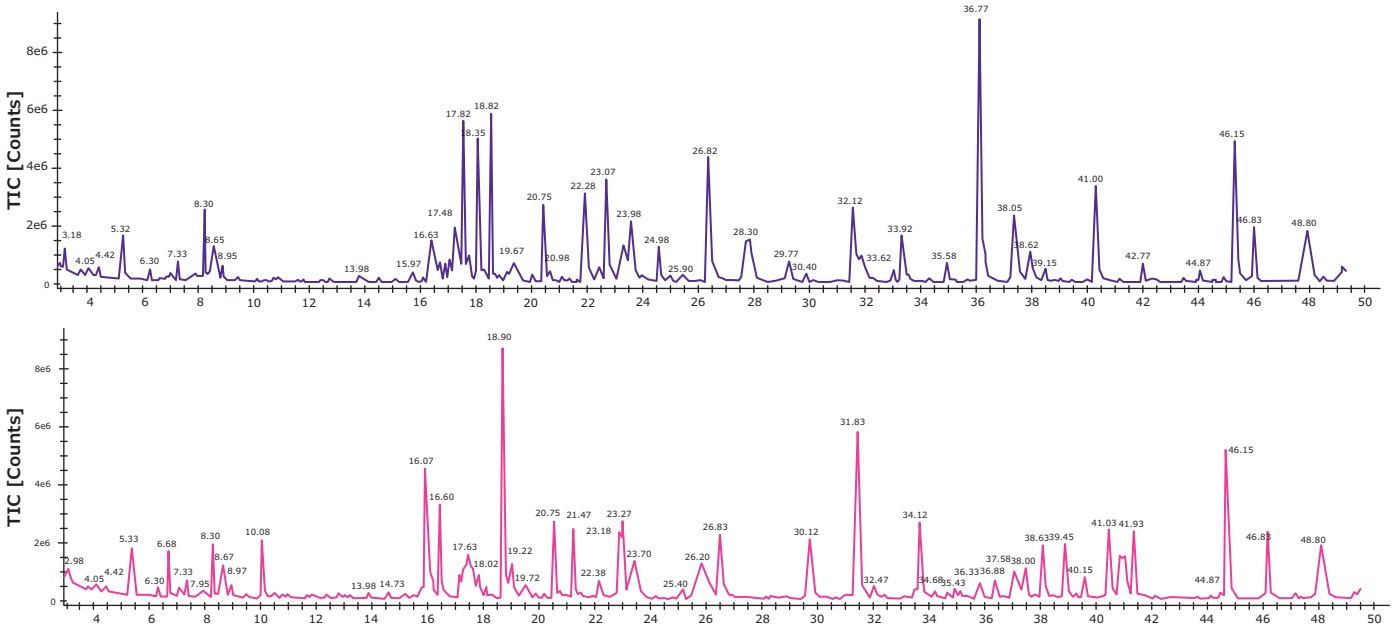

Figure 1: Zoomed LC-MS chromatograms obtained from peptide mapping of AAV8 (top) and AAV9 (bottom)

Table 1: AAV9 peptide sequence TINGSGQNQQTLK

Retention Time (min)	Modification	Relative Intensity (%)
10.10	Unmodified	90.5
10.55	Deamidated	9.5

Unveil the potential of your AAV

AAV gene therapies and any precursor starting materials must be well characterized to understand the relationship between product quality attributes, manufacturing processes, patient safety and efficacy. Our assay packages and phase-appropriate assay development and validation capabilities provide the product and process knowledge you need to develop a Quality Target Product Profile (QTPP) and identify the Critical Quality Attributes (CQAs) for your therapy.

Learn more:
SigmaAldrich.com/analyticaldevelopment

Europe:
Todd Campus
West of Scotland Science Park
Glasgow, G20 0XA,
Scotland
Tel: +44 (0)141 946 9999
info@bioreliance.com

North America:
14920 Broschart Road
Rockville, MD 20850-3349
USA
Tel: 301 738 1000
info@bioreliance.com

Singapore:
#2 Science Park Drive
#04-01/12 Ascent Building,
Tower A
Singapore 118222
info@bioreliance.com

China:
15-18F, No.3, Building C,
The New Bund World Trade
Center(Phase II) Lane 227
Dongyu Road, Pudong New
District, Shanghai,
China
info@bioreliance.com

MilliporeSigma
14920 Broschart Road
Rockville, MD 20850-3349 USA

MS_TN9609EN Ver. 1.0
43555
09/2022