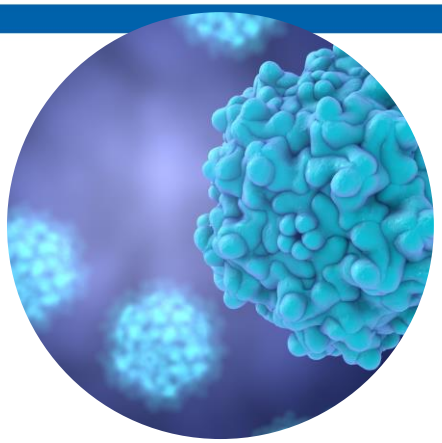

Intact Identification of Adeno-Associated Virus Capsid Proteins

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Overview

The use of Adeno-Associated Viruses (AAV) as an effective vector for the delivery of ssDNA in gene therapy continues to gain momentum. A chief attribute to this success is the broad tissue tropism enabled by a range of serotypes. To date, approximately 13 different serotypes have been identified with a range of shared amino acid sequence identity. Structurally, the viral capsid is a sixty-protein icosahedron comprised of three capsid, or viral proteins (VP), non-covalently assembled in a ratio of 1:1:10 (VP1:VP2:VP3).

These three proteins share a high degree of sequence homology, as VP2 and VP3 represent iterative, N-terminal truncations of the VP1 protein. The resulting approximate masses are VP1 – 81 kDa, VP2 – 66 kDa, and VP3 – 60 kDa. Given the inter-serotype and structural protein sequence similarities, protein identification, relative protein ratio, and post-translational modification assessments are critical. However, as the complete capsid can range in mass from 3.67 MDa, for an empty capsid, to 4.75 MDa for a capsid containing its payload (up to 4.7kb ssDNA), capsid denaturation and dissociation are required prior to interrogation.

In this application note, we profile denatured and dissociated capsid proteins from AAV5 and AAV9, via analysis with LC-MS using a Biozen™ Intact XB-C8 reversed phase column. It is notable that, although no covalent attachments are present within the capsid structure, denaturation without reduction can lead to a covalent complex of VP1 and VP2 in AAV9.

Sample Preparation

Step	Description
Sample:	AAV with stock concentration of 2E13 vg/mL (approx. 0.122 µg/µL protein)
Dilution:	5X dilution with 1X Dulbecco's Phosphate Buffered Saline
Denaturation:	10 % v/v 100 mM Acetic or Formic Acid (as indicated)
Reduction:	500 mM Tris (2-carboxyethyl) Phosphine for a 30 mM final concentration (as indicated)
Incubation:	22 °C for 15 min
Centrifugation:	9000 rpm for 5 min
Transfer:	Transfer to HPLC sample vial

LC Conditions

Column: Biozen 3.6 µm Intact XB-C8
Dimension: 150 x 2.1 mm
Part No.: 00F-4766-AN
Mobile Phase: A: 0.1 % Trifluoroacetic Acid in Water
B: 0.1 % Trifluoroacetic Acid in Acetonitrile

Gradient:

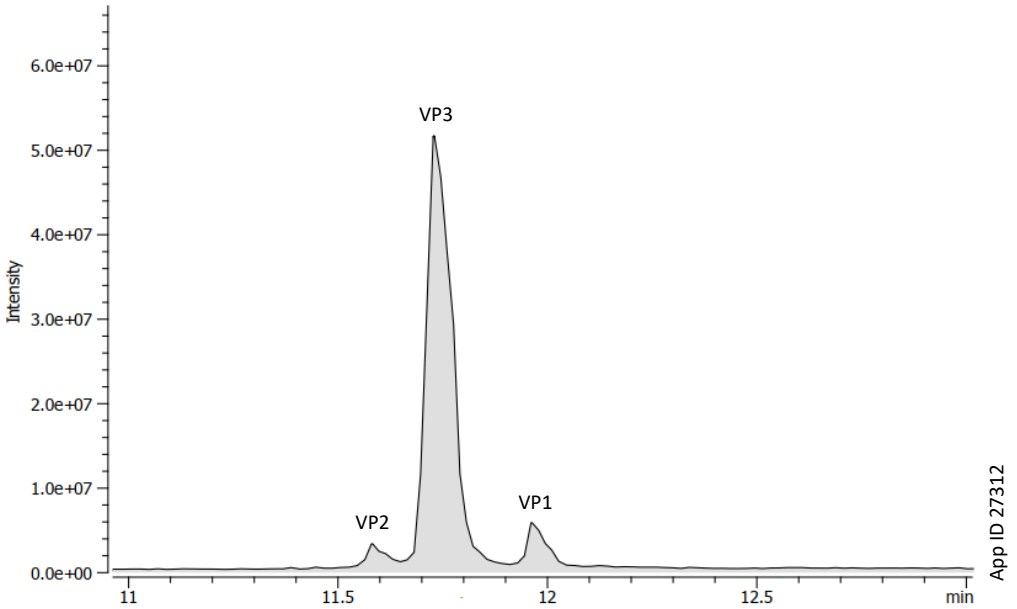
Time (min)	%B
0	10
3	10
3.1	35
7.1	35
21.94	65
22.04	90
25.04	90
25.14	10
29.14	10

Flow Rate: 0.3 mL/min
Injection Volume: 5 µL
Temperature: 60 °C
Instrument: Vanquish™ UHPLC
Detection: MS
Detector: Q Exactive™ Orbitrap™ Plus

MS Conditions

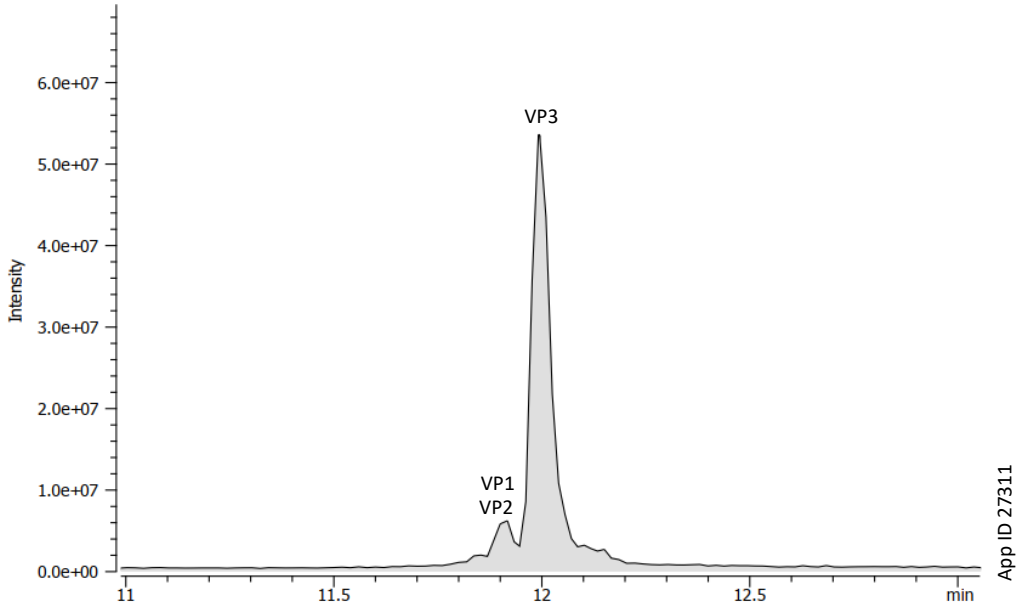
Scan Type: Full MS
Resolution: 70,000
AGC Target: 3e6 ms
Maximum IT: 100 ms
Scan Range: 500 to 3500 m/z

Figure 1. Total Ion Chromatogram of AAV5 Capsid Proteins Denatured with Acetic Acid, at a Concentration of 3.2E12 vg/mL (Approx. 98 ng Protein).



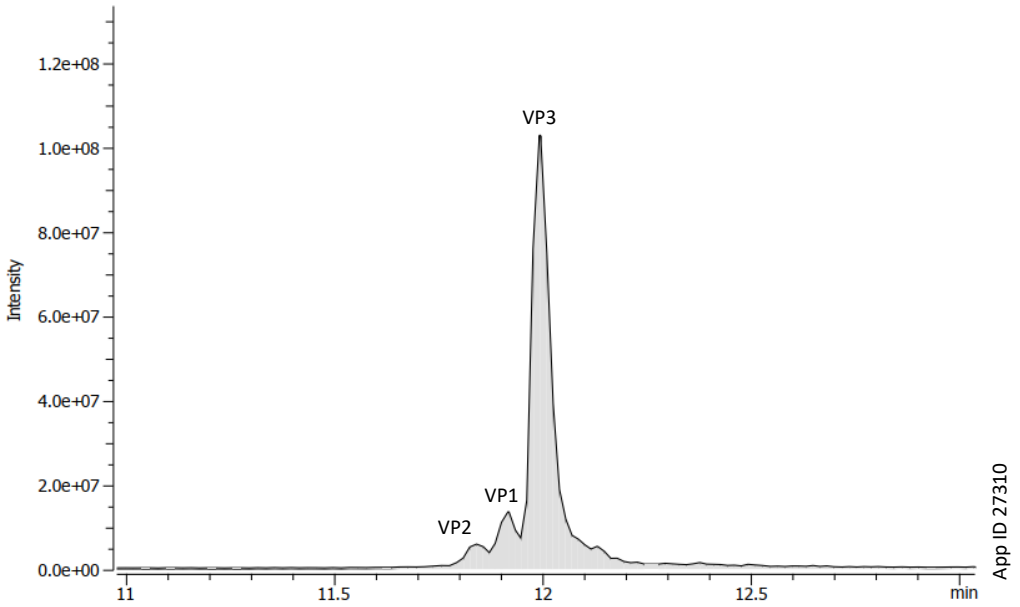
Peak	Mass	Intensity	Name	RT	Mass Area	ppm Error
1	65279.76	1.67E+03	VP2 138-724(T.APTGKRID···) , Amidation	11.58	1.96E+05	1.11
2	59462.36	7.32E+04	VP3 195-724(S.AGGGGPLG···) , +Methionine	11.73	5.92E+06	1.32
3	80341.46	3.25E+03	VP1 1-722(···IGTRYLTR.P) , + Lys	11.96	2.59E+05	16

Figure 2. Total Ion Chromatogram of AAV9 Capsid Proteins Denatured with Acetic Acid and Reduced with Tris (2-carboxyethyl) Phosphine, at a Concentration of 3.2E12 vg/mL (Approx. 92 ng Protein).



Peak	Mass	Intensity	Name	RT	Mass Area	ppm Error
1	66207.05	2.05E+03	AVV9 VP2 Deamidation	11.81	1.38E+05	5.11
2	81325.54	2.48E+03	AAV9 VP1 , Phosphorylation	11.9	4.47E+05	16
3	59729.73	1.39E+05	AV9 VP3 2-534(M.ASGGGAPV···) , Acetylation	12	7.64E+06	14

Figure 3. Total Ion Chromatogram of AAV9 Capsid Proteins Denatured with Formic Acid Acid and Reduced with Tris (2-carboxyethyl) Phosphine, at a Concentration of 3.2E12 vg/mL (Approx. 92 ng Protein).



Peak	Mass	Intensity	Name	RT	Mass Area	ppm Error
1	66207.05	9.58E+03	AV9 VP2 , Deamidation	11.84	5.23E+05	4.99
2	81279.45	8.27E+03	AAV9 VP1 1-733(···IGTRYLTR.N) , +2Met	11.92	1.19E+06	4.43
3	59685.96	5.43E+04	AAV9 VP3 203-735(M.ASGGGAPV···) , Amidation	11.99	1.10E+07	6.54

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