Application Note

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Reliable AAV Viral Protein Analysis with the Agilent ProteoAnalyzer System

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Abstract

Analysis of the viral proteins that form the adeno-associated virus (AAV) capsid is essential for gene therapy vector characterization and is a critical quality attribute (CQA). This application note demonstrates the ability of the Agilent ProteoAnalyzer system to perform high-resolution CE-SDS separation and determine the ratio between the three viral protein (VP) subunits—VP1, VP2, and VP3—across AAV samples. To confirm the results of the ProteoAnalyzer system, the VP ratios were compared with those measured by a similar CE-SDS system.

Introduction

Adeno-associated virus (AAV) vectors are widely used in gene therapy due to their favorable safety profile and efficient delivery of genetic material. The AAV capsid is a protein shell that protects the viral genome and enables its delivery. The capsid consists of three viral proteins VP1, VP2, and VP3 in varying amounts, each affecting the virality, stability, or therapeutic performance of the vector. A critical quality attribute (CQA) in AAV manufacturing is the ratio of these viral proteins normalized to VP1, which is generally recognized to be 1 : 1 : 10 (VP1 : VP2 : VP3).

Reliable separation and quantification of these viral proteins are essential for AAV capsid analysis. The Agilent ProteoAnalyzer system is a parallel capillary electrophoresis instrument that uses a gel matrix to separate proteins by size and LED-induced fluorescence detection for high-quality analysis of protein species. This enables reliable characterization and supports both process development and routine AAV quality control (QC), including automated analysis of VP1, VP2, and VP3 relative concentrations.

This application note demonstrates the capability of the ProteoAnalyzer system to deliver reliable assessments of viral protein ratios across multiple AAV samples. The results obtained from the ProteoAnalyzer were confirmed using a secondary CE-SDS platform (System B). Together, these findings highlight the suitability of the ProteoAnalyzer for integration into viral vector QC pipelines.

Experimental

The following AAV samples were used in this application note:

- Sample 1: AAV eGFP (Genecopeia, p/n AA002),
- Sample 2: AAV8 4.16x 10 (Charles River, p/n CV10008)],
- Sample 3: AAV8 CMV Luciferase 1 x 10 (Virovek, p/n 288BAVA 13-1E14-10)],
- Sample 4: AAV8 CMV GFP 1 x 10 (Virovek, p/n: 288B445-10-100).

Each sample was prepared in phosphate buffered saline (PBS) under reduced conditions, following the Agilent Protein Broad Range P240 kit (p/n 5191-6640) manual. The samples were covalently labeled by incubation with the supplied reagents at 85 °C for 10 minutes. The samples were analyzed across multiple capillaries on the Agilent ProteoAnalyzer system using the Protein Broad Range kit using the lower marker (LM)-only method. Samples were analyzed using the Agilent ProSize data analysis software and AAV viral protein ratios and precisions were calculated.

Sample preparation for System B was performed using the reagents provided in the associated protein kit. A 5 µL aliquot of each AAV sample was diluted in PBS to a final concentration of 1×10^{11} GC/mL. The diluted sample was then mixed with 5 µL of Tris sample buffer and 1 µL of 1 M DTT, followed by incubation at 60 °C for 10 minutes. Next, 0.5 µL of 1 mg/mL Chromeo P503 dye was added, and the mixture was incubated again at 60 °C for an additional 10 minutes. After cooling to room temperature, 38.5 µL of deionized water was added to the sample. Samples were loaded on System B and electrokinetically injected at –5.0 kV for 20 seconds. The samples were then separated using –15.0 kV for 30 minutes. Samples were analyzed using the System B analysis software and AAV viral ratios and precision were calculated using the corrected area % metric calculated for each peak.

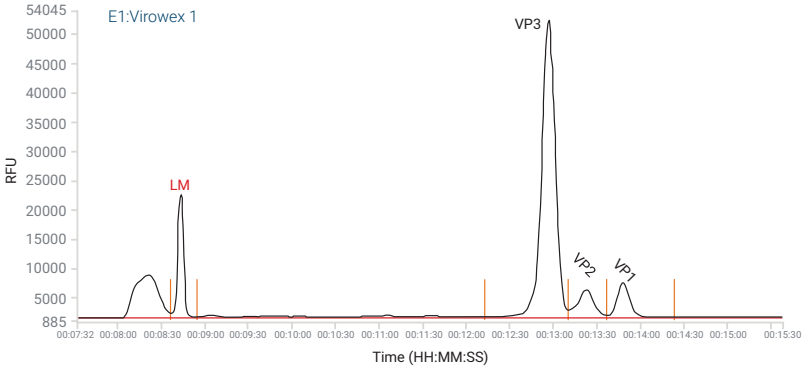
Results and discussion

To demonstrate the capability of the ProteoAnalyzer to measure the AAV CQA of viral protein ratios, four commercially available AAV samples were assessed. The electropherogram of sample 3, shown in Figure 1, provides an example of the separation achieved with the ProteoAnalyzer. VP1 of the AAV capsid has the largest size of the viral proteins and therefore migrates the slowest during electrophoresis, displaying a peak towards the right side of the electropherogram. VP3 has the smallest size of the proteins, migrating the fastest and appearing farther to the left, while VP2 falls between them. Also shown in the electropherogram are two system peaks that occur in every run. The small smear on the left is unbound dye, while the sharper peak to the right is the lower marker which is used for alignment.

The Agilent ProSize data analysis software used with the ProteoAnalyzer automatically calculates the relative concentration of each peak detected. Using the concentration of each peak, ratios of VP1, VP2, and VP3 were determined relative to VP1. For example, sample 3 showed average concentrations of 45.2 ng/µL (VP1), 49.6 ng/µL (VP2), and 466.5 ng/µL (VP3), resulting in a ratio of 1 : 1.10 : 10.32, close to the expected 1 : 1 : 10 ratio. Sample 4 also had a ratio close to expected at 1 : 1.30 : 12.57. For samples 1 and 2, VP1 and VP2 were of similar concentrations, while VP3 was lower than expected, resulting in ratios of 1 : 1.32 : 5.12 and 1 : 1.58 : 5.07, respectively. To confirm the validity of the ProteoAnalyzer values,

results were compared to those from System B which provided values similar to the ProteoAnalyzer for all samples analyzed (Table 1).

Reproducibility was assessed by calculating the coefficient of variation (%CV) for each viral protein peak's concentration assessed on the ProteoAnalyzer. The system demonstrated high precision, with consistent results across replicates as shown by the low %CVs in Table 2. These findings support the ProteoAnalyzer as an effective platform for AAV QC.



Peak table			
Viral protein	Size (kDa)	ng/µL	% (Conc.)
	6 (LM)	120.0000	
VP3	81.4	467.1128	82.9
VP2	99.3	50.4253	8.9
VP1	116.4	45.9124	8.1
	TIC:	563.4504	

Figure 1. A representative electropherogram and data table using sample 3 assessed by the Agilent ProteoAnalyzer system clearly resolves VP1, VP2, and VP3 peaks. The data table displays the automatically calculated concentrations and percent concentrations of total for the peaks in the electropherogram.

Table 1. Comparison of the ratios of AAV viral protein peaks from the Agilent ProteoAnalyzer system and System B normalized to the VP1 peak. N = 3.

Ratio of Viral Proteins to VP1								
	Sample 1		**Sample 2**		**Sample 3**		**Sample 4**	
	ProteoAnalyzer	System B	ProteoAnalyzer	System B	ProteoAnalyzer	System B	ProteoAnalyzer	System B
VP1	1	1	1	1	1	1	1	1
VP2	1.32	1.10	1.58	1.63	1.10	0.91	1.30	1.21
VP3	5.12	4.66	5.07	5.38	10.32	10.62	12.57	11.79

Table 2. Quantitative precision (%CV) of AAV capsid viral protein peak concentrations or peak area%, as measured by the Agilent ProteoAnalyzer system and System B. N = 3.

Precision (%CV)								
	Sample 1		**Sample 2**		**Sample 3**		**Sample 4**	
	ProteoAnalyzer	System B	ProteoAnalyzer	System B	ProteoAnalyzer	System B	ProteoAnalyzer	**System B**
VP1	1.57%	16.31%	10.33%	13.26%	1.40%	8.10%	5.61%	4.31%
VP2	1.05%	1.44%	9.84%	14.31%	1.52%	10.04%	1.58%	5.36%
VP3	3.25%	3.39%	1.44%	6.37%	1.44%	1.52%	2.12%	0.65%

Conclusion

The Agilent ProteoAnalyzer system provides accurate and reproducible assessment of AAV viral protein ratios, a CQA in gene therapy vector production. This application note demonstrates consistent results across multiple AAV samples and confirmed these findings using a comparable CE-SDS system. The results highlight the robustness of the ProteoAnalyzer platform and its suitability for integration into AAV QC workflows.

References

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