Characterization of Adeno-Associated Virus (AAV) Using Capillary Electrophoresis

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INTRODUCTION

Fast growth in cell and gene therapy industry has generated an urgent need for fast and robust analytics for characterization of both vectors and various types of nucleic acid biotherapeutics such as oligonucleotides and plasmid DNA.

Adeno-associated virus (AAV) is one of the most widely used gene delivery vehicles for gene therapy because of its non-pathogenicity, low immunogenicity and different tropism to multiple cell types. Although there are efforts to develop methods for characterization of AAV, there are still limitations and drawbacks to some of these analysis. In addition, the different workflows are done on different platforms. Purity analysis of the AAV viral proteins is important for quality assurance and safety of AAV products. Although SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) has been used for AAV capsid protein analysis in the industry, CE-SDS (Capillary Electrophoresis - Sodium Dodecyl Sulfate) method on the SCIEX PA800 Plus provides automated separation of proteins in the range of 10kD to 225kD with higher resolution, quantitation capability, better reproducibility and is less labor intensive than traditional SDS-PAGE. The CE-SDS method using UV or PDA detector and stacking injection technology could provide good results for AAV sample with titer greater than $1X10^{12}$ GC/mL. However, for in-process AAV product analysis, higher sensitivity is required for purity analysis of AAV with concentration as low as $1X10^{10}$ GC/mL.

Here we present CE based workflows for characterization of AAV viral proteins using a UV detector, and alternatively utilizing a dye for sample labeling with LIF detector when an increase in sensitivity is required. These methods provide a straight forward and easy sample preparation, excellent resolving power, good repeatability and linearity of absorbance response to sample concentration.

MATERIALS AND METHODS

Sodium dodecyl sulfate (PN L4390-100G) and 2-mercaptoethanol (PN M3148-100ML) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Amicon Ultra-0.5 Centrifugal Filters with 30,000 NMWL were purchased from EMD Millipore (Billerica, MA, U.S.A.). The SDS-MW Analysis Kit (PN 390953) was from SCIEX (Framingham, MA, U.S.A.), which includes the SDS-MW gel buffer(proprietary formulation, pH 8, 0.2% SDS), acidic wash solution (0.1 N HCl), basic wash solution (0.1 N NaOH) and the SDS-MW sample buffer(100 mM Tris-HCl pH 9.0, 1%SDS). EZ-CE Capillary Cartridge (PN A55625, SCIEX, Framingham, MA, U.S.A) pre-assembled with bare fused-silica capillary (50 µm I.D., 30 cm total length, 20 cm effective length) was used for separation. Universal vials (PN A62251), universal vial caps (PN A62250), PCR vials (PN 144709) and nanoVials (PN 5043467 from SCIEX (Framingham, MA, U.S.A.) were used for sample solution loading. A PA800 Plus Pharmaceutical Analysis CE system (SCIEX, Framingham, MA, U.S.A.) equipped with a PDA detector and 32 Karat software were used for all the experiments. For LIF work, the PA800 Plus was equipped with a Laser Induced Fluorescence (LIF) detector with a 600nm emission filter was used. For labeling of the proteins a 20 mM FQ (3-2-(furoyl quinoline-2-carboxaldehyde) dye stock solution was prepared in Methanol. This solution was further diluted in DDI water to a final concentration of 2.5 mM and was used as the FQ dye working solution. The nucleophile reagent used was potassium cyanide at a concentration of 30 mM.

Data acquisition and analysis were performed using 32 Karat™ Software 10. Packaged AAV2 of pAV-CMV-GFP with titer at $2.24x10^{13}$ GC/mL (titer as supplied by vendor) and packaged AAV8 of pAV-CMV-GFP with titer at $3.99x10^{13}$ GC/mL and $1.57x10^{14}$ GC/mL (titer as supplied by vendor) for PDA and LIF detection, respectively, was purchased from Vigene Biosciences (Rockville, MD, U.S.A.). Both samples were kept in storage solution of PBS (Phosphate Buffered Saline, pH 7.5)/0.001% pluronic F68.

RESULTS

Analysis Using CE-SDS and PDA Detector: The method was developed and optimized using an AAV8 sample at $1x10^{13}$ GC/mL which is the nominal concentration from AAV manufacturing. Figure 1 illustrates the overlay of the 8 consecutive injections of this AAV8 sample. The VP3 :VP2 :VP1 ratio of the AAV8 sample lot tested is about 8:1:1.

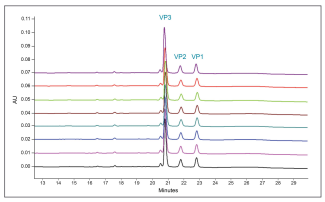

Figure 1. Eight consecutive injections of an AAV8 sample with estimated titer at $8x10^{13}$ GC/mL with a PDA detector. This shows a good baseline resolution of the 3 capsid proteins.

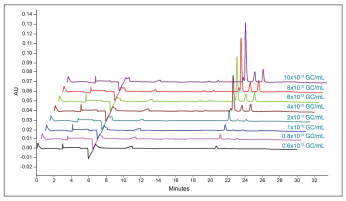

Figure 2. AAV8 sample was buffer exchanged to different concentrations (different folds) showing peak consistency over concentrations.

A similar study was done by buffer exchanging the AAV8 sample to different titers (different folds) for method evaluation as shown in Figure 2. The titer values listed in the figure are a rough estimation from the folds of buffer exchange/concentration procedure. The peaks are consistent over the various concentrations.

This method was also evaluated for different buffer treatments. Comparison of the peaks as shown in figure 3 demonstrate no obvious difference in peak profile for all conditions

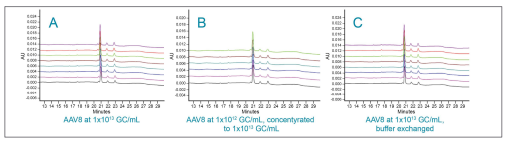

Figure 3. Consecutive injections of a) $1x10^{13}$ GC/mL AAV8 without pretreatment, b) AAV8 sample diluted to $1x10^{12}$ GC/mL and c) $1x10^{13}$ GC/mL AAV8 sample in high salt buffer was buffer exchanged to the same sample buffer as samples in figure 3a and figure 3b.

Table 1. Repeatability of AAV2 and AAV8 at different concentration.

Viral Proteins	RSD% of Corrected Peak Area%				
	AAV8 $1x10^{13}$	AAV8 $4x10^{13}$	AAV8 $8x10^{13}$	AAV2 $5x10^{13}$	AAV2 $2x10^{13}$
VP3	0.44	0.45	0.43	0.68	0.64
VP2	0.38	0.38	0.34	0.66	0.61
VP1	0.51	0.39	0.39	0.74	0.63

Table 1 demonstrates excellent repeatability of this method by evaluating the RSD% of corrected peak areas (CPA%) of 3 viral proteins of AAV2 and AAV8 at different titers and using different pretreatment methods. The calculation is based on 8 consecutive injections of each sample solution. All the RSD% of CPA% are less than 0.7%.

In addition, this method demonstrates excellent linearity of analyzing AAV8 samples from $5x10^{11}$ GC/mL to $1x10^{14}$ GC/mL by plotting absorbance response of VP3 to sample titers (Figure 4). The R2 is 0.9991.

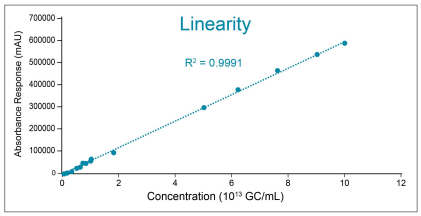

Figure 4. Linearity of Absorbance Response (mAU) to AAV 8 Sample Concentration (10^{13} GC/mL).

Analysis Using CE-SDS and LIF Detector: The CE-SDS method using UV or PDA detector and stacking injection technology could provide good results for AAV sample with titer greater than $1x10^{12}$ GC/mL.[1-3] However, for in-process AAV product analysis, higher sensitivity is required for purity analysis of AAV with concentration as low as $1x10^{10}$ GC/mL. This utilizes FQ (3-2-(furoyl quinoline-2-carboxaldehyde) dye for sample labeling and Laser induced fluorescence (LIF) detector with emission filter of 600 nm for sample detection to increase the sensitivity of AAV capsid purity analysis. Figure 5 shows the signal comparison of AAV8 using the PDA and LIF detector. Better sensitivity and baseline is seen with LIF.

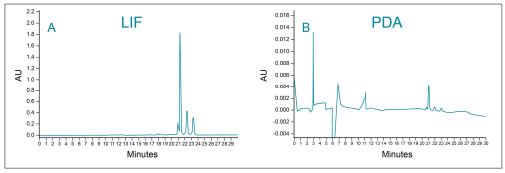

Figure 5. Comparison of capsid protein analysis using PDA detector and using FQ dye labeling with LIF detector. A is the electropherogram of $1x10^{13}$ GC/mL AAV8 capsid protein analysis using FQ dye labeling and LIF detector. B is the electropherogram of $1x10^{13}$ GC/mL AAV8 capsid protein analysis using PDA detector.

To demonstrate repeatability, multiple injections were conducted at a lower level than was conducted for PDA detection. Figure 6 shows good reproducibility from six consecutive injections of AAV8 sample at $1x10^{10}$ GC/mL with baseline separation.

Table 2 demonstrates excellent repeatability of this method by evaluating the RSD% of corrected peak areas% (CPA%) of three viral proteins of AAV2 and AAV8 at different titers. The calculation is based on six consecutive injections of each sample solution for AAV8 samples and 8 consecutive injections of each sample solution for AAV2 samples.

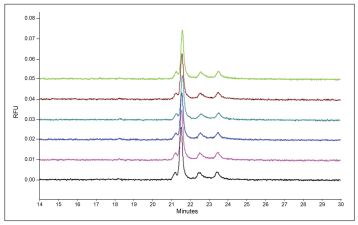

Figure 6. Six consecutive injections of an AAV8 sample with the lower titer at $1x10^{10}$ GC/mL. using LIF detector.

This LIF based method also demonstrates excellent linearity of analyzing AAV8 samples from $1x10^{10}$ GC/mL to $1.6x10^{14}$ GC/mL by plotting peak area of VP3 to sample titers (Figure 7). The R2 is 0.9989 with about 4 orders of LDR.

Table 2. Corrected Peak Area% repeatability of AAV2 and AAV8 at different concentration.

Viral Proteins	RSD% of Corrected Peak Area%					
	AAV8_ $1x10^{13}$ GC/mL	AAV8_ $1x10^{12}$ GC/mL	AAV8_ $1x10^{11}$ GC/mL	AAV8_ $1x10^{10}$ GC/mL	AAV2_ $1x10^{12}$ GC/mL	AAV2_ $1x10^{10}$ GC/mL
VP3	0.6	0.6	1.1	1.4	0.3	1.4
VP2	0.9	3.0	7.2	9.9	0.1	4.0
VP1	1.6	2.6	8.1	6.3	1.2	1.2

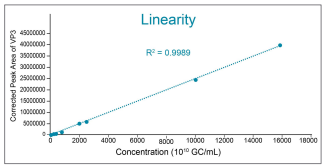

Figure 7. Linearity of corrected peak area to AAV 8 sample concentration (10^{10} GC/mL).

CONCLUSIONS

- Complete workflow solution for AAV capsid protein purity analysis including hardware, software, reagent kits and consumables
- Excellent Baseline resolution of AAV viral proteins VP1, VP2 and VP3
- Ultra-high sensitivity AAV capsid purity analysis with LIF for AAV samples down to the in-process product analysis requirement level of $1x10^{10}$ GC/mL
- Robust, good repeatability with RSD% < 0.7% of CPA%(Corrected Peak Area%) of viral proteins for PDA detection
- Good linearity of absorbance response to sample concentration with 3 and 4 orders of magnitude, respectively for PDA and LIF detection
- Suitable method for QC capsid protein purity test of AAV products

REFERENCES

1. 1.SCIEX Technical Note: Purity Analysis of Adeno-Associated Virus(AAV) Capsid Proteins using CE-SDS Method
2. Zhang, C.; Meagher, M. M. Anal. Chem. 2017, 89, 3285−3292.
3. Quirino, Joselito. (2015). Modern Injection Modes (Stacking) for CE. 10.1002/9783527678129.assep035.

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