Purity Analysis of Adeno-Associated Virus (AAV) Capsid Proteins using CE-LIF Technology

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Introduction

Adeno-associated virus (AAV) is one of the most commonly used gene delivery vehicles in gene therapeutics. It is made of a shell of protein called capsid encompassing a single-stranded DNA of about 4.8kb in size. The viral capsid is composed of three main proteins which are 87kD (VP1), 73kD (VP2) and 61kD (VP3) in size, respectively. Purity analysis of the AAV viral proteins is important for quality assurance and safety of AAV products. [1]

CE-SDS (Capillary Electrophoresis Sodium Dodecyl Sulfate) technology is popular for AAV capsid protein analysis in the cell and gene therapy industry because of its automated separation of viral proteins with higher resolution, quantitation capability, better reproducibility and is less labor-intensive than traditional SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis). 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. [2,3,4] 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 technical note utilizes FQ (3-2-(furoyl quinoline-2-carboxaldehyde) dye for sample labeling and a laser-induced fluorescence (LIF) detector with 488nm solid-state laser and an emission filter of 600nm for sample detection to increase the sensitivity of AAV capsid purity analysis. It demonstrates the capability of this CE-SDS-LIF method for purity analysis of AAV viral proteins to as low as $1X10^{10}$ GC/mL with easy sample preparation, excellent resolving power, good repeatability and linearity of absorbance response to sample concentration.

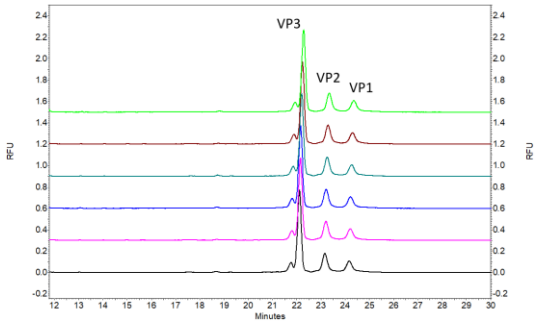


Figure 2. Six consecutive injections of an AAV8 sample with titer at $1X10^{13}$GC/mL.

Figure 1. The PA800 Plus Biologics Analysis System equipped with a LIF detector (A), the SDS-MW Kit (B) and the EZ-CE Capillary Cartridge(C).

Key Features

- Ultra-high sensitivity AAV capsid purity analysis for AAV samples down to the in-process product analysis requirement level which is $1X\ 10^{10}$ GC/mL
- Efficient labeling and simple sample preparation procedure in less than 1 hour
- Excellent baseline resolution of VP1, VP2 and VP3
- Good repeatability with RSD% of MT (Migration Time) less than 0.5% and CPA% (Corrected Peak Area%) of viral proteins less than 5% at $1X10^{12}$GC/mL and $1X10^{13}$GC/mL
- Good Linearity of absorbance response to sample concentration with R^2 =0.9989 from $1X10^{10}$GC/mL to $1.6X\ 10^{14}$GC/mL

Materials and Methods

Materials

ATTO-TAG FQ and potassium cyanide which are included in ATTO-TAG™ FQ Amine-Derivatization Kit (PN A2334) were purchased from ThermoFisher Scientific (Waltham, MA, U.S.A.). Methanol (PN 1.06018.2500) was obtained from VWR (Radnor, PA, U.S.A.). Sodium dodecyl sulfate (SDS) (PN L4390-100G) and N-Ethylmaleimide (NEM) (PN E3876-5G) were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). Amicon Ultra-0.5 Centrifugal Filters with 10,000 NMWL (PN UFC501024) were purchased from EMD Millipore (Billerica, MA, U.S.A.). The SDS-MW Analysis Kit (PN 390953) (Figure 1B) 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 (Figure 1C). Universal vials (PN A62251), universal vial caps (PN A62250) and PCR vials (PN 144709) 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 laser-induced fluorescence (LIF) detector with a 600nm emission filter (Figure 1A) was used for all the experiments.

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), packaged AAV8 of pAV-CMV-GFP with titer at 1.57 $X10^{14}$GC/mL (titer as supplied by vendor) and formulation buffer (Phosphate Buffered Saline, pH 7.5 with 0.001% pluronic F68) were purchased from Vigene Biosciences (Rockville, MD, U.S.A.). Both samples were kept in formulation buffer.

Sample Preparation

4%SDS in 150 mM NEM solution. Weigh 93.75mg NEM and 0.2g SDS in 5mL DI water. Mix to dissolve.

Labeling Solution. The 20 mM FQ 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.

1%SDS solution. Dissolve 1g of SDS in 100mL DI water.

Sample Preparation Procedure. 10µL of AAV8 sample solution was mixed with 1.2µL of 4% SDS in 150mM NEM solution in a 0.65 mL micro-centrifuge tube and incubated at 70°C for 5 minutes. Then, the sample solution was mixed with 1.5 µL of 2.5mM FQ dye working solution and 1 µL of 30mM KCN solution and incubated at 70°C for 10minutes. 28 µL of 1% SDS was added to quench the labeling reaction. The sample solution was incubated at 70°C for 5 minutes. At last, 20µL of DI water was added to the mixture. The diluted mixture was transferred to the sample vial for analysis on the PA800 Plus.[5]

Buffer exchange or desalting to lower final salt concentration is an optional step before sample solution is loaded into sample vials to improve sensitivity of the method due to less competition from the salt during electrokinetic injection. The mixture of 5% of 1%SDS, 1.25% of formulation buffer in DI water was used as buffer exchange solution in this technical note.

Instrument setup

The user guide of the SDS-MW Analysis kit was followed for instrument setup. Water plug was used in separation method for online sample concentration to improve the sensitivity of the method. [2,3,4]

The “Initial Conditions” and “LIF Detector Initial Conditions” were set up as indicated in Figure 3 and Figure 4, respectively. Same initial setup was used for conditioning, separation and shutdown methods.

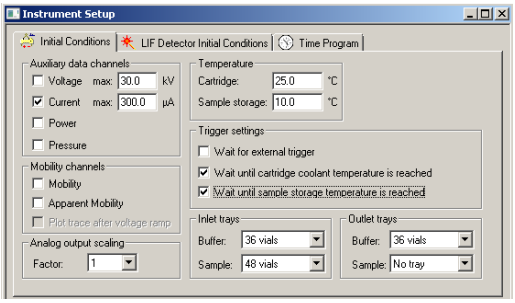


Figure 3. Initial Conditions.

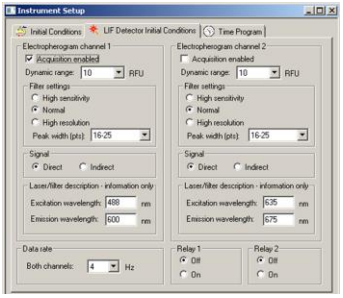
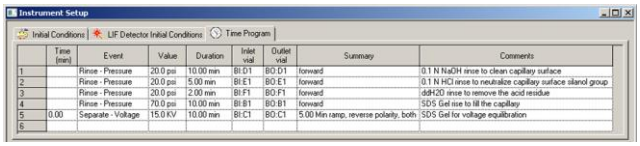
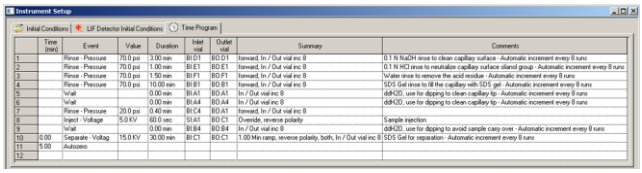


Figure 4. LIF Detector Initial Conditions.



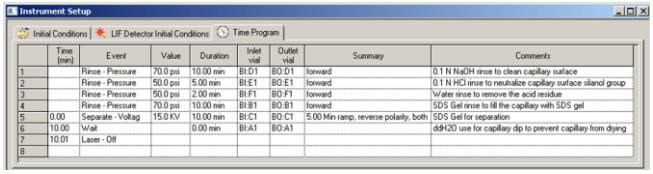
	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	20.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	20.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	20.0 psi	2.00 min	BI:F1	BO:F1	forward	ddH2O rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rise to fill the capillary
5	0.00	Separate - Voltage	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for voltage equilibration
6								

Figure 5. Time Program for Conditioning Method.



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	3.00 min	BI:D1	BO:D1	forward, In / Out vial inc 8	0.1 N NaOH rinse to clean capillary surface - Automatic increment every 8 runs
2		Rinse - Pressure	70.0 psi	1.00 min	BI:E1	BO:E1	forward, In / Out vial inc 8	0.1 N HCl rinse to neutralize capillary surface silanol group - Automatic increment every 8 runs
3		Rinse - Pressure	70.0 psi	1.50 min	BI:F1	BO:F1	forward, In / Out vial inc 8	Water rinse to remove the acid residue - Automatic increment every 8 runs
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward, In / Out vial inc 8	SDS Gel rinse to fill the capillary with SDS gel - Automatic increment every 8 runs
5		Wait		0.00 min	BI:A1	BO:A1	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
6		Wait		0.00 min	BI:A4	BO:A4	In / Out vial inc 8	ddH2O, use for dipping to clean capillary tip - Automatic increment every 8 runs
7		Rinse - Pressure	20.0 psi	0.40 min	BI:C4	BO:A1	forward, In / Out vial inc 8	
8		Inject - Voltage	5.0 KV	60.0 sec	BI:A1	BO:C1	Override, reverse polarity	Sample injection
9		Wait		0.00 min	BI:B4	BO:B4	In / Out vial inc 8	ddH2O, use for dipping to avoid sample carry-over - Automatic increment every 8 runs
10	0.00	Separate - Voltag	15.0 KV	30.00 min	BI:C1	BO:C1	1.00 Min ramp, reverse polarity, both, In / Out vial inc 8	SDS Gel for separation - Automatic increment every 8 runs
11	5.00	Autozero						
12								

Figure 6. Time Program for Separation Method.



	Time (min)	Event	Value	Duration	Inlet vial	Outlet vial	Summary	Comments
1		Rinse - Pressure	70.0 psi	10.00 min	BI:D1	BO:D1	forward	0.1 N NaOH rinse to clean capillary surface
2		Rinse - Pressure	50.0 psi	5.00 min	BI:E1	BO:E1	forward	0.1 N HCl rinse to neutralize capillary surface silanol group
3		Rinse - Pressure	50.0 psi	2.00 min	BI:F1	BO:F1	forward	Water rinse to remove the acid residue
4		Rinse - Pressure	70.0 psi	10.00 min	BI:B1	BO:B1	forward	SDS Gel rinse to fill the capillary with SDS gel
5	0.00	Separate - Voltag	15.0 KV	10.00 min	BI:C1	BO:C1	5.00 Min ramp, reverse polarity, both	SDS Gel for separation
6	10.00	Wait		0.00 min	BI:A1	BO:A1		ddH2O use for capillary dip to prevent capillary from drying
7	10.01	Laser - Off						
8								

Figure 7. Time Program for Shutdown Method.

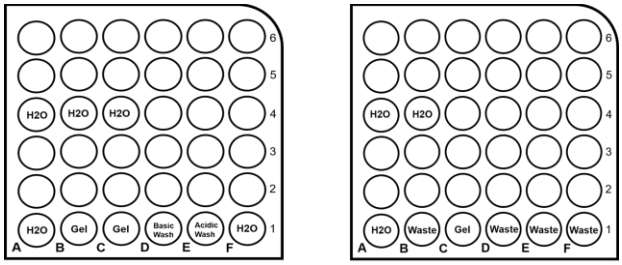


Figure 8. Buffer Tray Configuration.

The time program for conditioning method is illustrated in Figure 5. Figure 6 and Figure 7 are time programs for separation and shutdown method, respectively.

Figure 8 is the configuration for buffer tray setup.

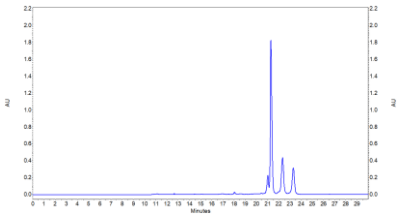
Result and Discussion

AAV8 and AAV2 sample at different titer used in this study were prepared by diluting the AAV8 sample at 1.57X 10^{14} GC/mL and AAV2 sample at 2.24X 10^{13} GC/mL by different factors in formulation buffer. If not stated otherwise, buffer exchange step was used for AAV samples at titer no more than 1X10^{12} GC/mL before loading into sample vials for PA800 plus analysis. No buffer exchange was done for samples in linearity study.

PDA and LIF detector

AAV8 sample of 1X10^{13}GC/mL was used for comparison of capsid protein analysis results using PDA detector and FQ dye labeling with LIF detector. Figure 9a is the electropherogram of 1X10^{13} GC/mL AAV8 capsid protein analysis using FQ dye labeling and LIF detector. Figure 9b is the electropherogram of 1X10^{13} GC/mL

a.



b.

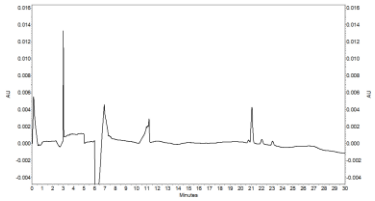


Figure 9. Comparison of capsid protein analysis using PDA detector and using FQ dye labeling with LIF detector. Figure 9a is the electropherogram of 1X10^{13} GC/mL AAV8 capsid protein analysis using FQ dye labeling and LIF detector. Figure 9b is the electropherogram of 1X10^{13} GC/mL AAV8 capsid protein analysis using PDA detector.

AAV8 capsid protein analysis using PDA detector (Sample preparation follow the procedure in Reference 2). The sample solution in Figure 9a was buffer exchanged to the same buffer as the one in Figure 9b before loading into sample vial. By comparing the electropherograms in Figure 9a and Figure 9b, the usage of FQ dye and LIF detector improves the sensitivity of CE-SDS method for AAV capsid purity analysis and provides a better and flatter baseline.

Repeatability

This method was evaluated using AAV8 sample at different titers (Figures are not shown in this technical note).

Figure 2 shows the six consecutive injections of AAV8 sample at 1X10^{13} GC/mL. Figure 10 shows the six consecutive injections of AAV8 sample at 1X10^{10} GC/mL.

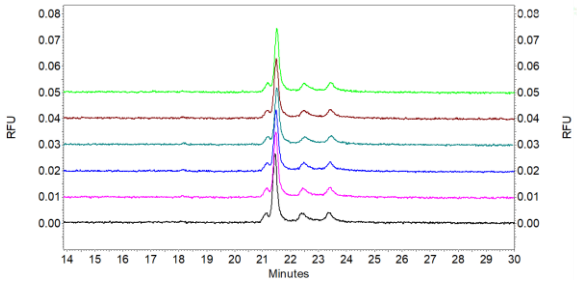


Figure 10. Six consecutive injections of an AAV8 sample with titer at 1X10^{10}GC/mL.

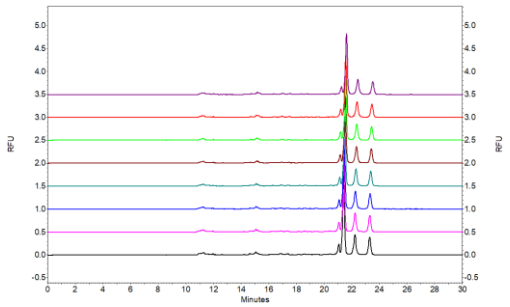


Figure 11. Eight consecutive injections of an AAV2 sample with titer at 1X10^{12}GC/mL.

This method was also evaluated using AAV serotype 2 sample at different titers. Figure 11 shows the eight consecutive injections of AAV2 sample at 1X10^{12}GC/mL.

Table 1. Migration time repeatability of AAV2 and AAV8 at different concentration.

	RSD% of Migration Time					
Viral Proteins	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	0.3	0.3	0.3	0.2	0.3	0.5
VP2	0.2	0.4	0.4	0.2	0.3	0.5
VP1	0.3	0.4	0.3	0.3	0.3	0.5

Table 2. Corrected peak area% repeatability of AAV2 and AAV8 at different concentration.

	RSD% of Corrected Peak Area%					
Viral Proteins	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

Table 1 and Table 2 demonstrates excellent repeatability of this method by evaluating the RSD% of migration time and 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. AAV8_1X10^{13} is AAV8 sample with titer at 1X10^{13} GC/mL. AAV8_1X10^{12} is AAV8 sample with titer at 1X10^{12} GC/mL. AAV8_1X10^{11} is AAV8 sample with titer at 1X10^{11} GC/mL. AAV8_1X10^{10} is AAV8 sample with titer at 1X10^{10} GC/mL. AAV2_1X10^{12} is AAV2 sample with titer at 1X10^{12} GC/mL. AAV2_1X10^{10} is AAV2 sample with titer at 1X10^{10} GC/mL. All the RSD% of MT% are no more than 0.5%. And the RSD% of CPA% are less than 10%.

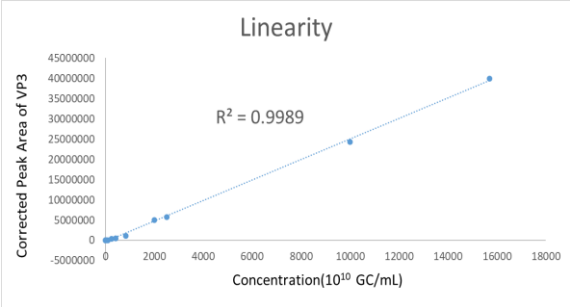


Figure 12. Linearity of corrected peak area to AAV 8 Sample Concentration (10^{10} GC/mL).

Linearity

This method demonstrates excellent linearity of analyzing AAV8 samples from 1X10^{10}GC/mL to 1.6X 10^{14}GC/mL by plotting peak area of VP3 to sample titers (Figure 12). The R^2 is 0.9989.

Conclusions

Presented in this technical note is a CE-SDS-LIF method for assessing the purity of Adeno Associated Virus (AAV) viral capsids utilizing FQ fluorescence dye labeling and LIF detector to provide an ultra-high sensitivity for in-process AAV product analysis with titer as low as 1X 10^{10} GC/mL. The sample preparation procedure is straight forward and easy. This method also demonstrates excellent resolution of the different viral proteins, good repeatability and linearity of absorbance response vs. sample concentration with R2 =0.9989.

References

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