Purity Analysis of Adeno-Associated Virus (AAV) Capsid Proteins using CE-SDS Method

Tingting Li, Mukesh Malik, Handy Yowanto, Sahana Mollah
SCIEX, Brea, CA

Introduction

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. 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 3 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.

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 PA 800 Plus Pharmaceutical Analysis system 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.

This technical note demonstrates the capability of the CE-SDS method for purity analysis of AAV viral proteins with straight forward and easy sample preparation, excellent resolving power, good repeatability and linearity of absorbance response to sample concentration.

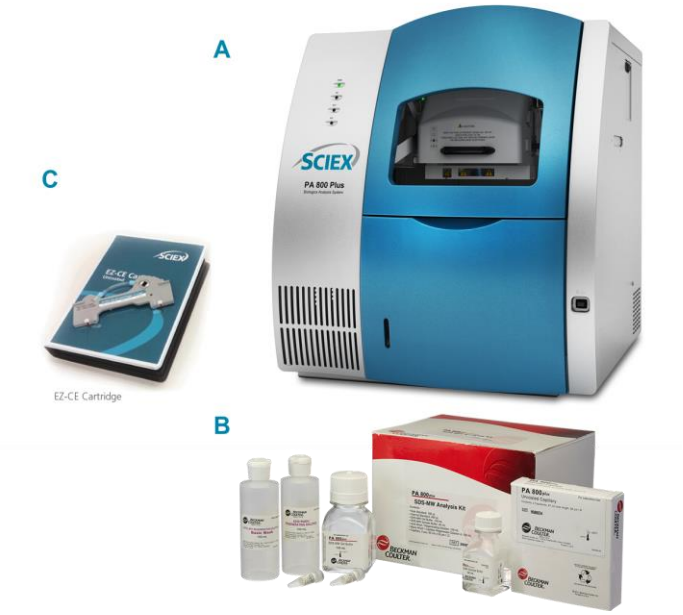


Figure 1. The PA 800 Plus Pharmaceutical Analysis System (A), the SDS-MW Kit (B) and the EZ-CE Capillary Cartridge(C).

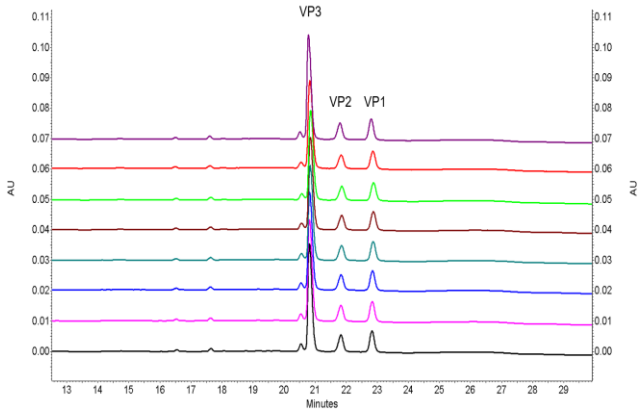


Figure 2. Eight consecutive injections of an AAV8 sample with estimated titer at 8X10^{13}GC/mL.

Key Features

- Straightforward and easy sample preparation procedure
- Excellent Resolution of VP1, VP2 and VP3
- Excellent repeatability with RSD% of CPA%(Corrected Peak Area%) of viral proteins less than 0.7%
- Good Linearity of absorbance response to sample concentration with R^2 =0.9991

Materials and Methods

Materials

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 PA 800 Plus Pharmaceutical Analysis system (SCIEX, Framingham, MA, U.S.A.) equipped with a PDA detector and 32 Karat software were used for all the experiments. 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.

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 3.99 X10^{13}GC/mL (titer as supplied by vendor) 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.

Sample Preparation

Sample Preparation Procedure. 5µL of AAV8 sample solution with final salt concentration no more than 40mM was mixed with 5µL of 1% SDS and 1.5µL of 2-mercaptoethanol in a 0.65 mL micro-centrifuge tube and incubated at 50°C for 10min. Then, 90µL of DI water was added to the mixture. The diluted mixture was transferred to the sample vial or nanoVial for analysis on the PA 800 Plus Pharmaceutical Analysis system.

Buffer exchange is necessary if salt concentration in AAV sample is higher than 40mM.

For Method Development and Optimization. In this technical note, the method is developed and optimized using AAV8 samples at 1 X 10^{13} GC/mL. Unless stated otherwise, 1.25 µL of AAV8 sample at 4 X 10^{13} GC/mL and 3.75 µL of deionized water was used in sample preparation procedure as 5 µL of AAV8 sample solution at 1X10^{13}GC/mL.

All the titer values of AAV samples used in this technical note were provided by the vendor based on results from qPCR.

For Method Evaluation. To evaluate the capability of this method for analysis of AAV8 samples with lower titer, the AAV8 sample at 4 X 10^{13} GC/mL was diluted to 1 X10^{12} GC/mL in the storage buffer provided by the vendor to represent the AAV8 samples at 1 X10^{12} GC/mL.

Buffer exchange not only can be used to exchange the buffer with slat concentration lower than 40mM, it can be also used to concentrate the AAV sample for analysis. In this technical note, four times diluted storage buffer (salt concentration lower than 40mM) was used as elution buffer in buffer exchange procedure. The use of pluronic F68 is to minimize sticking of AAV to hydrophobic surfaces of plastics.[1]

Comparison of peak profiles of the AAV8 samples with and without buffer exchange was performed and discussed in the Result and Discussion section.

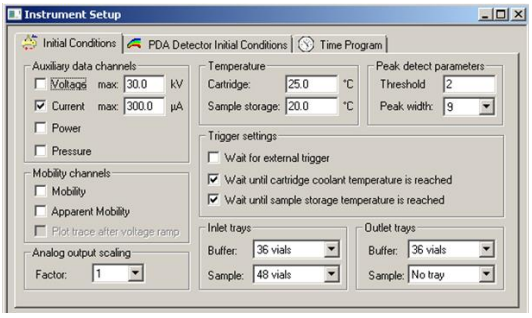


Figure 3. Initial Conditions.

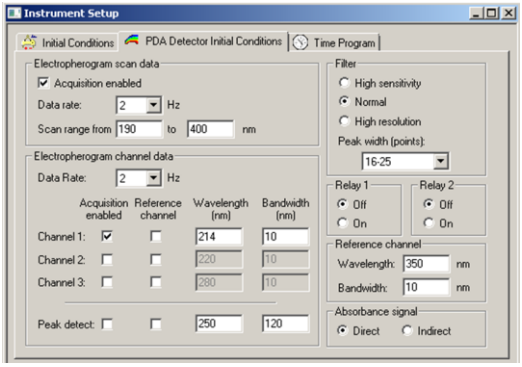


Figure 4. PDA Detector initial Conditions.

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]

The "Initial Conditions" and "PDA Detector Initial Conditions" were set up as indicated in Figure 3 and Figure 4, respectively. Same set up was used for conditioning, separation and shutdown methods.

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

Figure 8 is the configuration for buffer tray setup.

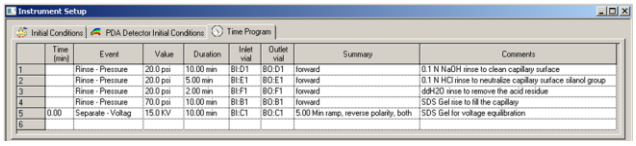


Figure 5. Time Program for Conditioning Method.

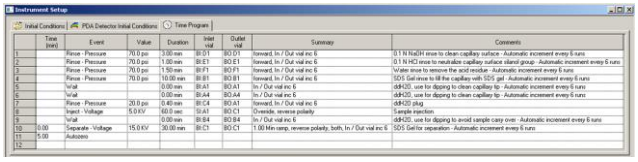


Figure 6. Time Program for Separation Method.

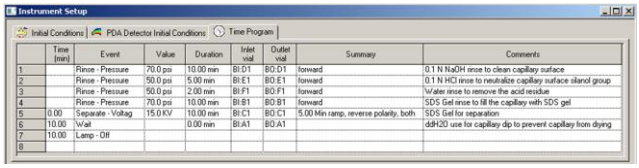
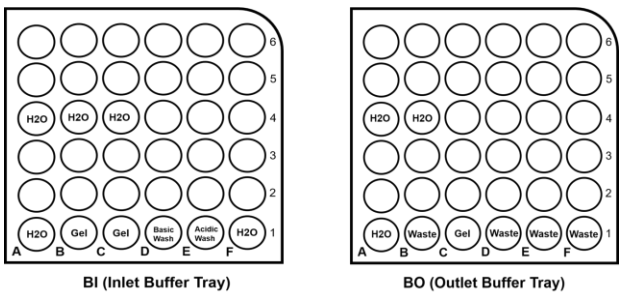


Figure 7. Time Program for Shutdown Method.



BI (Inlet Buffer Tray) **BO (Outlet Buffer Tray)**

Figure 8. Buffer Tray Configuration.

Result and Discussion

Method Development and Optimization

AAV8 samples at 1 $X10^{13}$ GC/mL were used for method development and optimization in this technical note as described in Sample Preparation section.

Different sample buffers and sample preparation procedures were evaluated to achieve the optimal analysis sensitivity. Sample Buffer from the SDS-MW kit (100 mM Tris-HCl pH 9.0, 1%SDS) was used at 1X, 2X, 4X, 5X, 8X ,10X, 20X dilutions, SDS solutions at different concentrations from 0.01% to 1% were compared. Also, the original sample preparation procedure from the SDS-MW kit instructions and modified procedures including the published samples preparation method[2] were compared for optimal analysis sensitivity. (Comparison data for sample buffers and sample preparation procedures development not shown in this technote).

Among the sample buffers and sample preparation procedures evaluated in this study, the sample preparation protocol described in Sample Preparation Procedure section provides the best sensitivity and least complexity of sample preparation steps.

Method Optimization

Figure 9 illustrates the optimization of the SDS concentration used in this procedure. 5µL of 1% SDS provides the optimal peak shape and sensitivity since it provides sufficient amounts of SDS for protein binding and minimum residual salt concentration for best efficiency of electrokinetic sample injection.

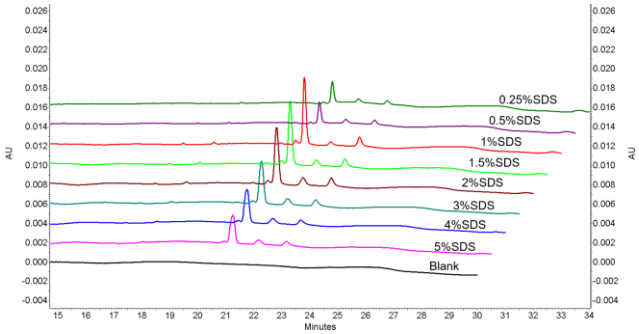


Figure 9. Optimization of SDS concentration for sample preparation.

Incubation temperature is optimized for AAV8 sample preparation as illustrated in Figure 10. With increased incubation temperature, the intensity of peaks at about 12.3min, 20.2min and 29.8min increased. This method is optimized for this specific AAV8 sample. The optimal incubation temperature may differ for AAV of different serotypes.

Other parameters such as starting sample volume for sample preparation, water plug for injections, sample injection duration, etc. were optimized for this procedure (Data not shown in this technote).

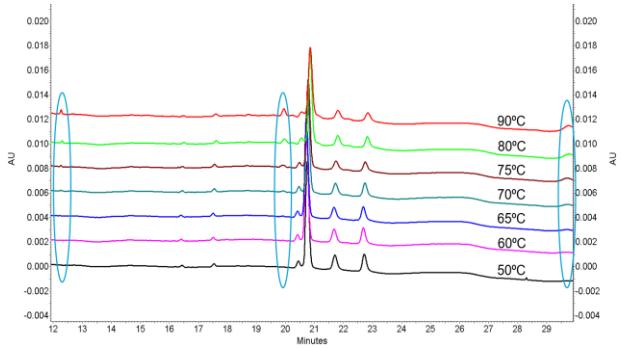


Figure 10. Optimization of incubation temperature for AAV8 sample preparation.

Method Evaluation

AAV8 sample at 1X10^{13} GC/mL. The method was developed and optimized using an AAV8 sample at 1X10^{13} GC/mL which is the nominal concentration from AAV manufacturing. Figure 11 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.

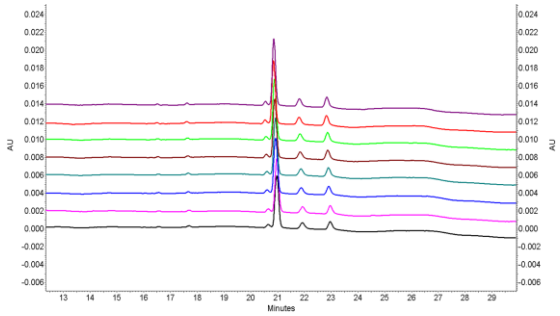


Figure 11. Consecutive injections of a 1X10^{13}GC/mL AAV8 sample.

AAV8 sample buffer exchange to 1X10^{13} GC/mL. Figure 12 shows the 6 consecutive injections of AAV8 sample buffer exchanged to 1X10^{13}GC/mL.

Comparison of Figure 11 and 12 showcase no difference in peak profile for AAV8 samples using different sample pretreatment methods (with or without buffer exchange).

AAV8 sample at 1X10^{12} GC/mL. This method was also evaluated for sample concentration as low as 1X10^{12} GC/mL. The AAV8 sample at 1X10^{12} GC/mL was prepared as described in Sample Preparation section.

The AAV8 samples with lower titer were buffer exchanged and concentrated before sample preparation procedure. Figure 13 shows 8 consecutive injections of the 1X10^{12} GC/mL AAV8 sample buffer-exchanged and concentrated to 1X10^{13}GC/mL (10 folds) for sample pretreatment.

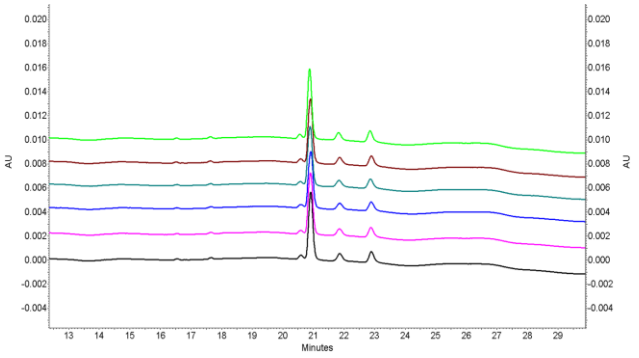


Figure 12. Consecutive injections of 1X10^{13}GC/mL AAV8 sample. AAV8 sample was buffer exchanged to 1X10^{13}GC/mL for sample pretreatment.

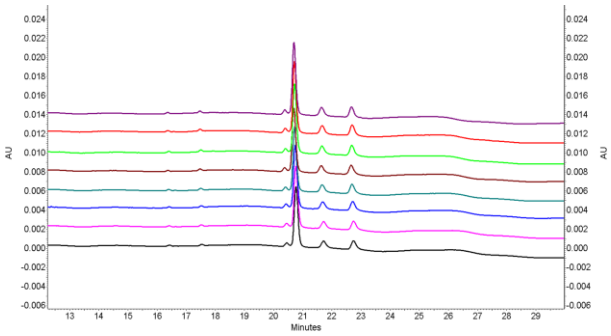


Figure 13. Consecutive injections of 1X10^{13}GC/mL AAV8. AAV8 sample was diluted to 1X10^{12}GC/mL with formulation buffer to represent samples with lower titer and then buffer exchanged to 1X10^{13} GC/mL for sample preparation.

Comparison of Figure 12 and 13 demonstrate no obvious difference in peak profile for AAV8 samples at different starting

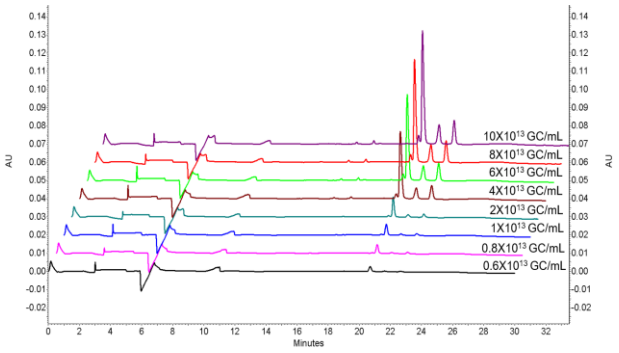


Figure 14. AAV8 sample was buffer exchanged to different concentrations (different folds). The titer values listed in the figure are a rough estimation from the folds of buffer exchange/concentration procedure.

concentration (1X10^{12}GC/mL and 4X10^{13} GC/mL) using buffer exchange pretreatment.

AAV8 samples concentrated to higher titer. Figure 2 shows the overlay of 8 consecutive injections of AAV8 sample. The sample was buffer exchanged to 8X10^{13}GC/mL (2 folds higher) before sample preparation. The concentration after buffer exchange was measured as 0.7mg/mL based on nanodrop reading at 280nm.

AAV8 samples at different titer. A similar study was done by buffer exchanging the AAV8 sample to different titers(different folds) for method evaluation as shown in Figure 14. The titer values listed in the figure are a rough estimation from the folds of buffer exchange/concentration procedure.

AAV2 samples(a different serotype). The method was applied to an AAV2 sample. Figure15 shows 8 consecutive injection of AAV2 sample buffer exchanged to 0.5 X 10^{13} GC/mL.

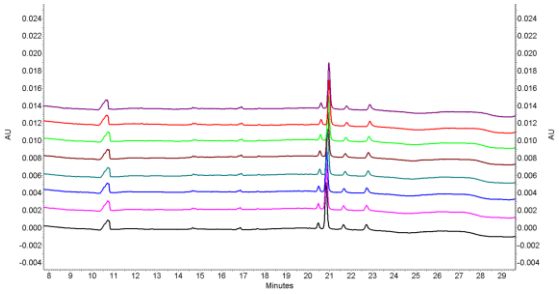


Figure 15. 8 Consecutive injections of 0.5X10^{13}GC/mL AAV2 sample.

Repeatability. All the consecutive injection studies using AAV2 and AAV8 samples at different concentration showcase good repeatability of peak profiles (Figure 2, Figure11, Figure 12, Figure 13 and Figure 15. Figures of AAV samples at other concentrations are not shown in this technical note).

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

	RSD% of Corrected Peak Area						
Viral Proteins	AAV8 _noBE_1	AAV8 _DBE_1	AAV8 _BE_8	AAV8 _BE_1	AAV8 _BE_4	AAV2 _noBE_0.5	AAV2 _BE_2
VP3	0.44	0.31	0.43	0.25	0.45	0.68	0.64
VP2	0.38	0.24	0.34	0.29	0.38	0.66	0.61
VP1	0.51	0.31	0.39	0.28	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%. AAV8_noBE_1 is the 1X10^{13} GC/mL AAV8 sample without buffer exchange treatment. AAV8_DBE_1 is the 1X10^{12} GC/mL AAV8 sample buffer exchanged to 1X10^{13} GC/mL. AAV8_BE_8 is the AAV8 sample buffer exchanged to 8X10^{13} GC/mL. AAV8_BE_1 is the AAV8 sample buffer exchanged to 1X10^{13} GC/mL. AAV8_BE_4 is the AAV8 sample buffer exchanged to 4X10^{13} GC/mL. AAV2_noBE_0.5 is the 0.5X10^{13} GC/mL AAV2 sample without buffer exchange. AAV2_BE_2 is the AAV2 sample buffer exchanged to 2X10^{13} GC/mL.

Linearity. This method demonstrates excellent linearity of analyzing AAV8 samples from 5X10^{11}GC/mL to 1X 10^{14}GC/mL by plotting absorbance response of VP3 to sample titers (Figure 16). The R^2 is 0.9991.

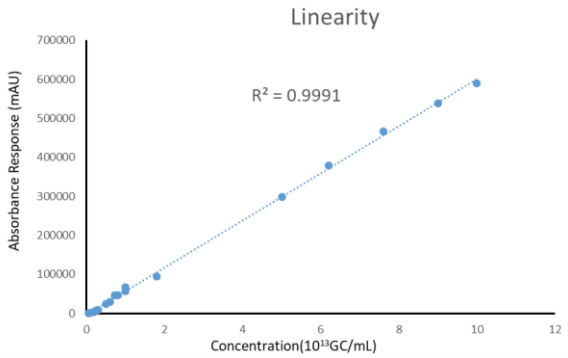


Figure 16. Linearity of Absorbance Response (mAU) to AAV 8 Sample Concentration (10^{13} GC/mL).

Conclusions

Presented in this technical note is a CE-SDS method for assessing the purity of Adeno Associated Virus (AAV) viral capsids. It uses a straight forward and easy sample preparation procedure and provides a good sensitivity for AAV samples which are of relatively lower concentration and of limited amount than monoclonal antibodies (mAbs). This method also demonstrates excellent resolution of the different viral proteins, good repeatability with less than 0.7% RSD% of CPA% of those viral proteins and good linearity of absorbance response vs sample concentration with R^2 =0.9991.

References

1. https://med.stanford.edu/gvvc/AAV.html
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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