Comprehensive CQA analysis for AAV drug production by multiple workflows on a single CE platform

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This technical note highlights the versatility of the BioPhase 8800 system for quality assessment of adeno-associated virus (AAV). The same capillary electrophoresis (CE) system can be used for plasmid purity and isoform identification analysis, viral protein (VP) characterization, AAV purity, and genome integrity analyses. AAVs are the most commonly used viral vectors for gene therapies because they offer a safe and effective way to deliver therapeutic genes to patients, allowing for long-term expression and potentially curative treatments for various genetic diseases.[1,2] The complexity of AAV vector production requires monitoring multiple critical quality attributes (CQAs) throughout the process to ensure the safety and efficacy of AAV drugs.[3] This technical note demonstrates the capability of the BioPhase 8800 system to provide multiple workflows for assessing a range of CQAs across the AAV production process.

Key features

- **One CE platform for the entire AAV production process:** Quality assessments of starting materials, in-process samples and the final product using the BioPhase 8800 system and ready-to-use, intuitive reagent kits
- **Plasmid purity monitoring throughout the plasmid production process by CGE-LIF:** Accurate and reproducible purity analysis for purified plasmids of various sizes and in-process samples
- **High-resolution AAV purity and VP ratio analysis by CE-SDS-LIF:** High precision for AAV purity analysis with the %RSD of < 0.5% across 8 capillaries and 5 injections
- **High-resolution AAV genome integrity analysis by CGE-LIF with excellent assay reproducibility:** High-resolution separation of the intact genome peak from impurities

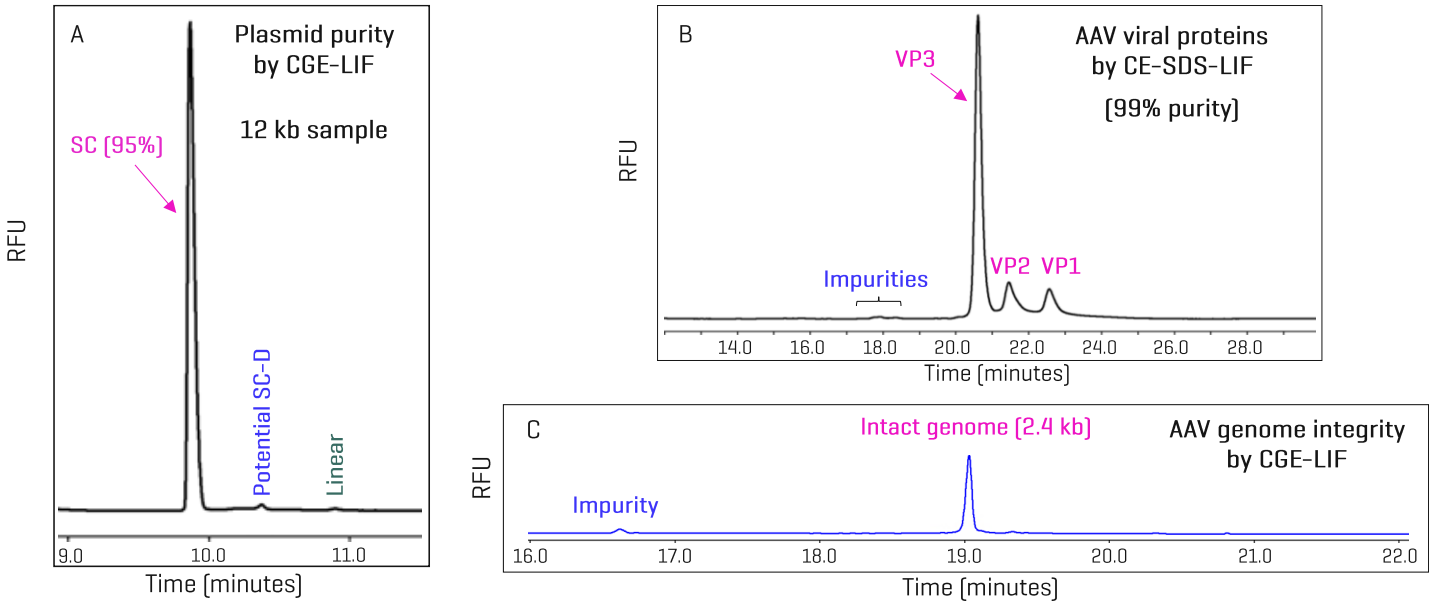

Figure 1. Analyses of plasmid purity, AAV viral proteins, and genome integrity on a single platform: the BioPhase 8800 system. A 12 kb plasmid for AAV production was analysed using the DNA 20 kb Plasmid and Linear kit (Panel A). The supercoiled (SC), the potential SC dimer (SC-D), and the linear isoforms were well resolved. The purity of this sample was determined to be 95% based on the corrected peak area percentage (%CPA) of the SC isoform. Results of viral proteins and genome integrity analyses of an AAV sample using the BioPhase CE-SDS Protein Analysis kit or the RNA 9000 Purity & Integrity kit are shown in panels B and C. Three VP proteins were resolved from each other and separated from impurities. The capsid protein purity was 99%. The intact genome of 2.4 kb in size was well-separated from the impurity peak. CGE-LIF: capillary gel electrophoresis with laser-induced fluorescence detection. RFU: relative fluorescence units.

Introduction

AAV is a small virus with a non-enveloped icosahedral protein shell and a single-stranded DNA genome of about 4.7 kilobases. Due to their excellent safety profile and high efficiency in transducing various target tissues, AAV vectors have become a popular choice for gene therapy.[1] So far, the FDA has approved 8 AAV-based drugs, with numerous others currently in clinical trials.[3,4]

The triple-transfection method is one of the most widely used techniques for producing AAV vectors.[5] This involves co-transfecting permissive cells, such as HEK293 cells, with three plasmids: a packaging plasmid with the Rep and Cap genes, a gene of interest plasmid containing the transgene flanked by AAV inverted terminal repeats (ITRs), and a helper plasmid encoding adenoviral helper genes. Chromatography and/or gradient ultracentrifugation are typically used to purify packaged AAV vectors (full capsids) from contaminants such as cellular debris, host cell DNA and RNA, and empty AAV capsids. However, some contaminants may remain in the purified viral vector product. Additionally, errors during genome encapsidation in AAV production can result in heterogeneous populations, including intact and partial genomes.[3] To ensure the safety and efficacy of the AAV drugs, assessments of multiple CQAs must be performed throughout the entire AAV production process, from the plasmid starting materials to in-process samples to the final product. Traditionally, multiple platforms have been used to accomplish this task. This technical note demonstrates streamlined quality assessments throughout the AAV production process using a single CE platform.

Methods

Materials: The DNA 20 kb Plasmid and Linear kit (P/N: 5311708), the RNA 9000 Purity & Integrity kit (P/N: C48231), the BioPhase CE-SDS Protein Analysis kit (P/N: C30085), the BioPhase BFS capillary cartridge - 8 x 30 cm (P/N: 5080121), the BioPhase Plate Pack starter kit (P/N: 5080311), and the sample loading solution (SLS) (P/N: 608082) were from SCIEX (Framingham, MA). Rainin LTS filter tips were from Mettler Toledo (Oakland, CA). Nuclease-free water (NFW) (P/N: AM9932) and N-Ethylmaleimide (NEM) (P/N: 040526) were obtained from Thermo Fisher Scientific (Cambridge, UK). The Chromeo P503 dye (P/N: 15106) was from Active Motif (Waterloo, Belgium). Benzonase (P/N: E1014-5KU) was purchased from Millipore Sigma (Dorset, UK). The QIAquick PCR purification kit (P/N: 28104) and Proteinase K (P/N: 19131) were from Qiagen (Manchester, UK).

A Rep/Cap plasmid, a gene of interest plasmid, a helper plasmid sample (ranging from 7-12 Kb), packaged AAV of two different serotypes, and an empty AAV sample were provided by Pharmaron Biologics (Liverpool, UK) or commercially sourced. The XbaI (P/N: R0145S) restriction enzyme and the Nt.AlwI nicking enzyme (P/N: R0627S) kits containing their corresponding 10x reaction buffer were from New England Biolabs (NEB) (Hertfordshire, UK).

Addition of the SYBR™ Gold Nucleic Acid gel stain to the DNA 20 kb Plasmid and Linear gel: To analyse a full plate of 96 samples, 20 mL of the gel was removed from the bottle, pre-warmed to room temperature, and transferred to a 50 mL conical tube. Then, 80 µL of SYBR™ Gold Nucleic Acid gel stain (pre-thawed) was added. The tube was capped tightly and gently inverted 20 times to mix well, avoiding air bubbles. Finally, the tube was wrapped in aluminum foil to prevent photobleaching before use.

Sample preparation for the plasmid analysis: The plasmid DNA samples at the various stages of the production process were buffer exchanged to the DNA 20 kb Plasmid and Linear sample buffer using an Amicon Ultra-0.5 Centrifugal Filter Unit (10 kDa, P/N: UFC501024) and diluted to a final concentration of 5 ng/µL. The diluted plasmid sample was then transferred at 50 µL per well to the sample plate for analysis on the BioPhase 8800 system. The purified plasmid samples and one of the in-process samples were not buffer-exchanged. Instead, they were diluted with the DNA 20 kb Plasmid and Linear sample buffer directly before CE analysis. To prepare the linearized and open circular (OC) isoforms, the 7 kb plasmid sample was digested with the XbaI restriction enzyme or the Nt.AlwI nicking enzyme following the manufacturer's instructions.

Sample preparation for AAV capsid protein analysis by CE-SDS-LIF on the BioPhase 8800 system: Sample preparation was carried out as described in a previous SCIEX technical note[6] except that the dithiothreitol was replaced with NEM. 50 µL of the prepared sample was transferred to the sample plate and analysed on the BioPhase 8800 system. After the CE separation,

the ratio of the corrected peak areas of VP1:VP2:VP3 was calculated and normalized by the lysine contents of each VP using the Empower 3 Chromatography Data System (CDS).

Sample preparation for AAV genome integrity analysis by CGE-LIF on the BioPhase 8800 system: Filter tips were used for all steps to minimize unintended nucleic acid degradation. Sample preparation was carried out as described in a previous SCIEX technical note[7] except the AAV sample at 1 x 10^{12} vg was treated with Benzonase and Proteinase K before genome extraction with the QIAquick PCR purification kit. Then, 50 µL of the sample was transferred into each well on the sample plates before CE analysis using the Nucleic Acid Extended Range Gel and the SYBR™ Green II RNA gel stain.

Instrument and software: The BioPhase 8800 system with UV/LIF (P/N: 5089278)—equipped with a laser-induced fluorescence (LIF) detector utilizing an excitation wavelength of 488 nm and an emission wavelength of 520 nm—was from SCIEX. A 600 nm bandpass emission filter (PN 5085177) from SCIEX was used for CE-LIF analysis of AAV capsid proteins. The BioPhase 8800 driver for Empower (P/N: 5306620, from SCIEX) was used to operate and acquire data from the BioPhase 8800 system utilizing the LIF detector while interfacing with Waters Empower 3 CDS. The BioPhase 8800 driver for Empower enables direct control of the BioPhase 8800 system hardware, sample setup, method design, data collection, and storage of multichannel data in the Empower 3 CDS database alongside linked sample information (sample identifier, sample set, method set, and instrument method). Post-acquisition, data processing, and analysis were performed using the Empower 3 CDS.

Instrument setup: Buffer plates and sample plates were prepared based on the experimental design and plate map generated by the BioPhase software, as described in the DNA 20 kb Plasmid and Linear kit application guide[8], the BioPhase CE-SDS Protein Analysis kit application guide[9], and the RNA 9000 Purity & Integrity kit application guide.[10]

Results and discussion

Streamlined platform assays for quality assessment throughout the AAV production process using a single CE system: Figure 1 illustrates how analyses of plasmid purity, AAV viral proteins, and genome integrity can all be performed on a single platform: the BioPhase 8800 system. In Panel A, a 12 kb plasmid that was used for AAV production was analysed using the DNA 20 kb Plasmid and Linear kit. The SC, the potential SC-D, and the linear isoforms were well resolved. The OC isoform was not detected for this particular batch of plasmid. The purity of this sample was determined to be 95% based on the %CPA of the SC isoform. Panels B and C show the results of viral proteins and genome integrity analyses of the same AAV sample using the BioPhase CE-SDS Protein Analysis kit or the RNA 9000 Purity & Integrity kit. Three VP proteins were resolved from each other and separated from impurities for an accurate determination of purity and VP ratio. The capsid protein purity of this sample was 99%. The ratio of corrected peak areas of VP1, VP2, and VP3 was calculated and normalized by the lysine contents of each VP using the custom settings in the Empower 3 CDS. The VP ratio was 1.0:2.7:17.2 for VP1:VP2:VP3 (Figure 4). The intact genome of 2.4 kb in size was well separated from the impurity peak. These platform CE assays are readily available to analyse a range of plasmid sizes and AAV serotypes, enabling efficient quality assessment throughout the AAV production process.

Plasmid purity analysis and isoform identification: Plasmid is a critical starting material for AAV drug production. Plasmid DNA has several topological isoforms, including SC, OC, linear, and multimeric. Degradation during processing and storage can convert SC to OC or linear forms. Plasmid purity is assessed by measuring the percentage of the SC isoform due to its high transfection efficiency.[11] To achieve a high AAV yield, plasmids with %SC above 90% are desired. Therefore, it is critical to achieve high-resolution separation of different plasmid isoforms. In addition, the main isoforms need to be clearly identified. Three plasmids used for AAV production with sizes of 7 kb, 10 kb, and 12 kb were analysed using the DNA 20 kb Plasmid and Linear kit and the pre-assembled bare-fused silica (BFS) cartridge on the BioPhase 8800 system. Results in panels A to F of Figure 2 demonstrate a high-resolution separation of the SC isoform from the potential SC-D, the linear, and the OC isoforms for all 3 plasmids. The purity was determined as 98% for the 7 kb plasmid, 95% for the 10 kb plasmid, and 94% for the 12 kb plasmid based on the %CPA of the SC isoform. In addition, electropherograms obtained with the 3 plasmids (panels G to I) illustrate the positive correlation between the migration time of the SC isoform and the size of the plasmids. This capability can be beneficial for fast screening of the crude plasmid samples during plasmid production.

As an example of isoform identification, panels J, K, and M of Figure 2 present a stacked view of electropherograms obtained with the undigested 7 kb plasmid sample (red trace), the linearized 7 kb plasmid (blue trace), and the nicked 7 kb plasmid (black), highlighting the identification of peaks corresponding to the SC, the linear and OC isoforms. These platform methods for plasmid purity and peak identification were also developed for plasmids ranging from 6 to 15 kb (data not shown) and can be readily applied to analysing various plasmids for AAV production.

Successful purity monitoring throughout the plasmid production process: Previous work has shown that plasmid analysis by CGE-LIF with the DNA 20 kb Plasmid and Linear kit has the potential to analyse in-process plasmid samples due to an excellent limit of detection (LOD) of < 5 pg/µL.[12] Monitoring the plasmid quality across the production process is beneficial for process control and improvement. A 12 kb plasmid was purified by ion exchange chromatography. Samples from the "Chromatography 1 Load", "Chromatography 1 Eluate", and "Purified Plasmid" stages of the plasmid production process were buffer exchanged and analysed using the DNA 20 kb Plasmid and Linear kit. As shown in Figure 3, panels A to C, the purity for the "Chromatography 1 Load" sample was 90% with the detection of the linear, OC, potential SC-D isoforms, and the unknown impurities. The purity of the "Chromatography 1 Eluate" sample was 93%, with a relatively lower amount of linear, OC, potential SC-D, and unknown impurities than in the

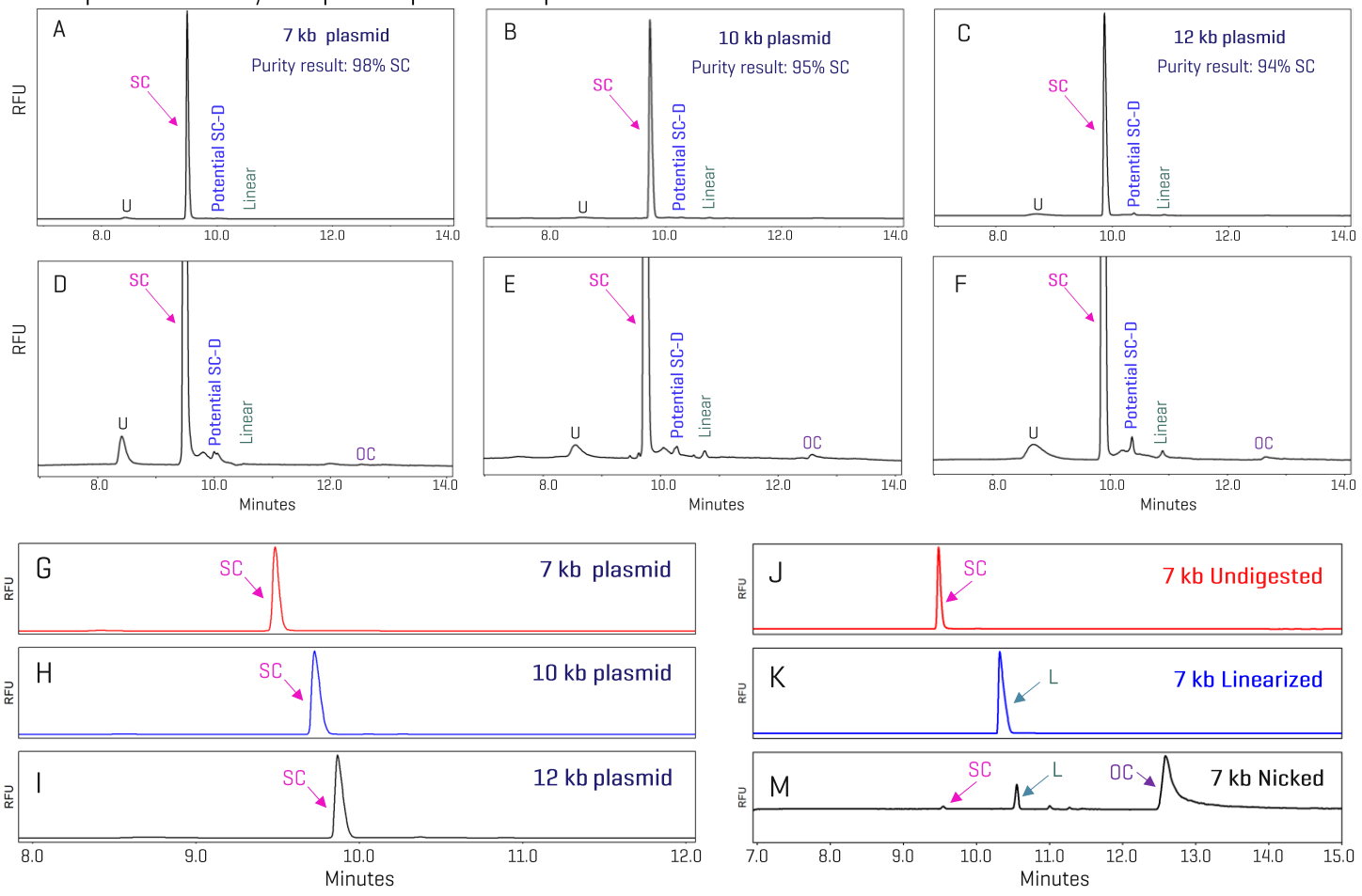

Figure 2. Plasmid purity analysis and peak identification using the DNA 20 kb Plasmid and Linear kit. Three AAV starting material plasmids: a 7 kb plasmid (panel A), a 10 kb plasmid (panel B), and a 12 kb plasmid (panel C) were analysed on the BioPhase 8800 system. Panels D, E, and F are expanded views of panels A, B, and C, showing a high-resolution separation of the SC isoform from the potential SC-D, the linear, and the OC isoforms. The purity was determined as 98% for the 7 kb plasmid, 95% for the 10 kb plasmid, and 94% for the 12 kb plasmid based on the %CPA of the SC isoform. U: unknown impurities. Panels G, H, and I show a stacked view of the electropherograms obtained with the 3 plasmids, demonstrating the positive correlation between the migration time of the SC isoform and the size of the plasmids. Panels J, K, and M show a stacked view of electropherograms obtained with the undigested 7 kb plasmid sample (red), the linearized 7 kb plasmid (blue), and the nicked 7 kb plasmid (black), identifying the peaks for the linear and OC isoforms.

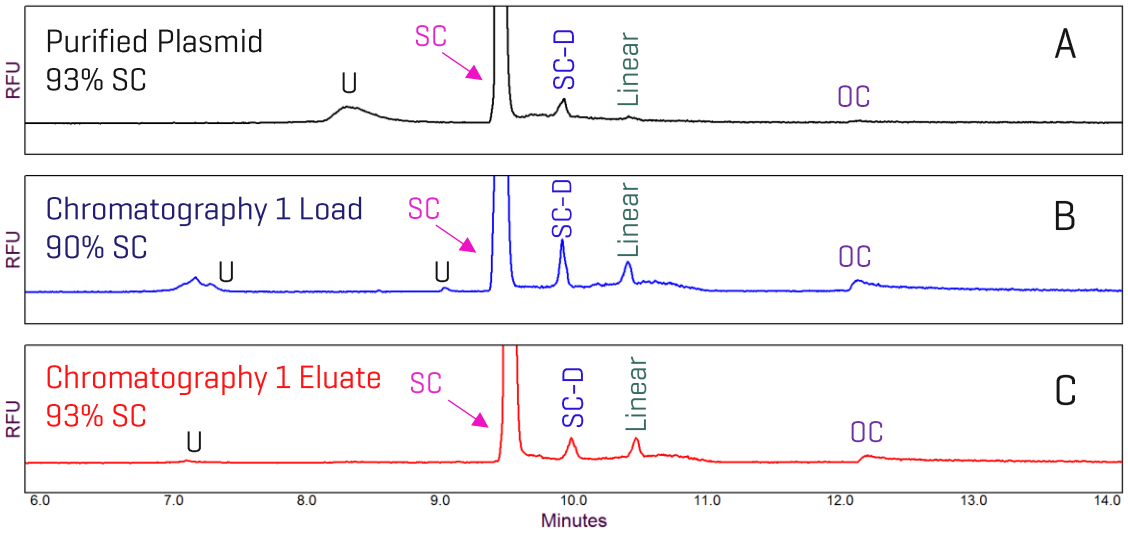

Figure 3. Successful purity monitoring throughout the plasmid production process. A 12 kb plasmid used for AAV production was purified by ion exchange chromatography. Samples from the "Chromatography 1 Load", "Chromatography 1 Eluate," and "Purified Plasmid" stages of the plasmid production process were buffer exchanged and analysed using the DNA 20 kb Plasmid and Linear kit. The purity was determined and indicated in panels A, B, and C. U: unknown impurity.

"Chromatography 1 Load" sample, indicating the improvement in purity by the chromatography process. The purified plasmid sample had a 93% purity with very low linear and no detectable OC isoform, indicating further removal of linear and OC isoforms by post-chromatography steps in the production process. Similar results were obtained with a 7 kb plasmid and a 10 kb plasmid (data not shown). These results demonstrate that CGE-LIF can successfully monitor the plasmid purity throughout the plasmid production process. Moreover, plasmid purity analysis of in-process samples without buffer exchange yielded results comparable to those with buffer exchange (data not shown), further enhancing the efficiency of the workflow.

Excellent assay reproducibility for plasmid purity analysis on the BioPhase 8800 system: Since the BioPhase 8800 system is a multi-capillary system that separates 8 samples simultaneously and can analyse up to 96 samples in a sequence, it is essential to assess the inter-capillary and the intra-capillary variations. The purified 7 kb and 12 kb plasmids were diluted in the DNA 20 kb Plasmid and Linear sample buffer to 5 ng/µL and analysed to evaluate the inter- and intra-capillary repeatability using the DNA 20 kb Plasmid and Linear kit. The results, including mean and %RSD values of the plasmid purity, are summarized in Tables 1 and 2. For the 7 kb plasmid, %RSD values of plasmid purity were within 1.5% across 8 different capillaries or between 4 different days in the same capillary. The overall average purity value of 32 injections was 98% for this sample, with an overall %RSD of 0.8%. For the 12 kb plasmid, %RSD values of plasmid purity were within 4.0% across 8 different capillaries or between 4 different days in the same capillary. The overall average purity value of 32 injections was 94% for this sample, with an overall %RSD of 2.3%. These results demonstrate high assay reproducibility for plasmid purity analysis on the BioPhase 8800 system using the DNA 20 kb Plasmid and Linear kit.

Successful AAV purity analysis through characterization of viral proteins: The AAV capsid is composed of 3 main proteins, which are approximately 87kD (VP1), 73kD (VP2), and 61kD (VP3) in size.[13] AAV purity analysis is a CQA for AAV products. In addition, the VP ratio is crucial for AAV integrity and directly impacts effective genetic payload delivery.[14] The CE-SDS-LIF workflow was used to assess AAV purity and VP ratio through the analysis of VPs. Figure 4 shows results obtained with two serotypes of AAV (panels A and B) using the BioPhase CE-SDS Protein Analysis kit and the Chromeo P503 dye on the BioPhase 8800 system. With both AAV samples, the 3 VPs were resolved from each other and well separated from the impurities. In addition, the VP3' was also detected with the AAV serotype A sample. The VP3' and VP3 peaks differ by 8 amino acids due to their difference in translation start site, as reported previously.[15] The resolution between VP3 and VP3' peaks demonstrates the outstanding resolving power of CE on the BioPhase 8800 system. The VP3' was not detected in the AAV serotype B sample, which is consistent with the published literature that covered both serotypes.[15] The purity of the two AAV samples

was determined to be 90% and 99%, respectively, indicating the high quality of the AAV samples. Furthermore, the normalized VP ratios [VP1:VP2:VP3] for the AAV serotypes A and B samples were determined as 1.0:3.4:19.0 and 1.0:2.7:17.2, respectively. The VP ratio values were previously shown to be characteristics of different AAV serotypes,[16] and of different production processes of the same serotype.[14] Therefore, assessment of the VP ratio can be useful for process development of a range of AAV serotypes. The same workflow was applied to two additional AAV serotypes with minor modifications based on their physical properties [data not shown], supporting the idea that the CE-SDS-LIF can be used as a platform method for AAV purity and VP ratio analyses.

Table 1. Assay reproducibility for purity analysis of a 7 kb plasmid using the DNA 20 kb Plasmid and Linear kit on the BioPhase 8800 system. Purified plasmid samples were diluted with the DNA 20 kb Plasmid and Linear sample buffer and transferred to the BioPhase sample plate for plasmid purity analysis. Mean and percent relative standard deviation (%RSD) values of plasmid purity were calculated across the 8 capillaries or between 4 sequences, each on a different day. The %RSD values were within 1.5% across 8 different capillaries or between 4 different days. The overall average purity value from all 32 injections was 98%, with an overall %RSD of 0.8%.

Sample sequence	Cap A	Cap B	Cap C	Cap D	Cap E	Cap F	Cap G	Cap H	Mean	% RSD (Inter-capillary)
1	98.69	97.14	98.28	97.54	97.81	97.81	98.53	98.66	98.06	0.6
2	98.63	98.22	98.79	98.14	98.36	98.34	98.36	98.42	98.41	0.2
3	97.53	97.60	98.47	96.46	97.20	97.85	98.26	98.34	97.71	0.7
4	98.52	98.13	98.67	98.21	95.39	99.23	96.68	98.43	97.91	1.3
Mean	98.34	97.77	98.55	97.59	97.19	98.31	97.96	98.46	**98.02**	
% RSD (Intra-capillary)	0.6	0.5	0.2	0.8	1.3	0.7	0.9	0.1		**0.8**

Table 2. Assay reproducibility for purity analysis of a 12 kb plasmid using the DNA 20 kb Plasmid and Linear kit on the BioPhase 8800 system. Purified 12 kb plasmid was diluted with the DNA 20 kb Plasmid and Linear sample buffer and transferred to the BioPhase sample plate for plasmid purity analysis. Mean %RSD values of plasmid purity were calculated across the 8 capillaries or between 4 sequences, each on a different day. The %RSD values were within 4.0% across 8 different capillaries or between 4 different days. The overall average purity value from all 32 injections was 94%, with an overall %RSD of 2.3%.

Sample sequence	Cap A	Cap B	Cap C	Cap D	Cap E	Cap F	Cap G	Cap H	Mean	% RSD (Inter-capillary)
1	95.42	95.29	95.55	95.11	95.10	94.89	94.76	94.91	95.13	0.3
2	95.32	94.42	95.21	95.29	95.70	93.85	94.79	94.66	94.91	0.6
3	93.64	92.94	94.59	92.40	92.33	91.91	94.09	93.64	93.19	1.0
4	96.18	88.96	87.95	88.92	95.19	89.86	94.89	95.08	92.13	3.8
Mean	95.14	92.90	93.33	92.93	94.58	92.63	94.63	94.57	**93.84**	
% RSD (Intra-capillary)	1.1	3.0	3.9	3.2	1.6	2.4	0.4	0.7		**2.3**

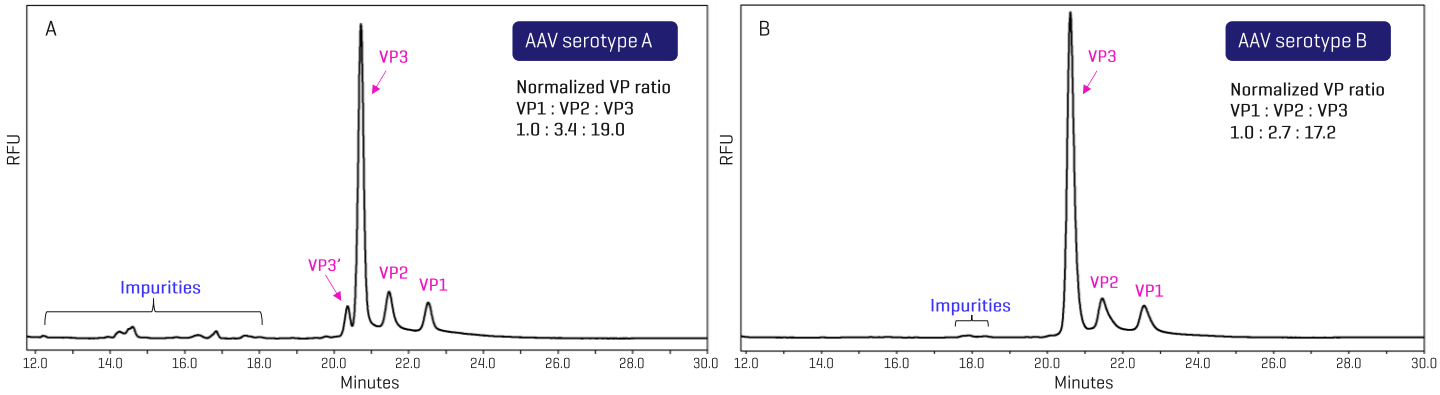

Figure 4. AAV purity and VP ratio analyses of two samples with different serotypes. Purified AAV serotype A and AAV serotype B samples were denatured and reduced in the presence of SDS and then labelled with a fluorescent dye Chromeo P503 before CE separation on the BioPhase 8800 system using the BioPhase CE-SDS Protein Analysis kit. The purity of the AAV serotype A and AAV serotype B was determined to be 90% and 99%, respectively. The normalized VP ratios (VP1:VP2:VP3) for the AAV serotype A and AAV serotype B samples were 1.0:3.4:19.0 and 1.0:2.7:17.2, respectively. VP3' is a variant of VP3 and was grouped with VP3 as a natural VP species for the purity calculation of the serotype A sample.

High precision AAV purity analysis by CE-SDS-LIF on the BioPhase 8800 system: To assess assay reproducibility for AAV purity analysis, an AAV serotype B sample was added to the BioPhase plate and injected five times. Table 3 summarizes the results obtained. The mean purity value was 99% in each column across eight capillaries or within each capillary for five injections. The mean purity value from all 40 injections was also 99%, indicating high consistency in purity measurements. The %RSD values were less than 0.5% across eight capillaries or between five injections within each capillary, demonstrating excellent assay repeatability and high precision.

Table 3. High assay repeatability of AAV purity analysis. The AAV serotype B sample was transferred to the BioPhase sample plate and injected 5 times for AAV purity analysis. The results are summarized in the table below. Mean and %RSD values for AAV purity were calculated across the 8 capillaries or between 5 injections. The %RSD was less than 0.5% across 8 different capillaries or between 5 injections.

Sample Injection	Cap A	Cap B	Cap C	Cap D	Cap E	Cap F	Cap G	Cap H	Mean	% RSD Across Capillaries
1	99	99	99	99	99	99	99	99	99	0.3
2	99	99	99	99	99	99	99	99	99	0.2
3	99	99	99	99	99	99	100	99	99	0.3
4	99	99	99	99	98	99	99	98	99	0.4
5	98	99	99	99	98	99	99	99	99	0.3
Mean	99	99	99	99	99	99	99	99	99	
% RSD Across injections	0.4	0.1	0.2	0.1	0.4	0.2	0.2	0.3		0.3

High-resolution AAV genome integrity analysis by CGE-LIF with excellent assay reproducibility: Genome integrity is a CQA for AAV drugs. Errors during the encapsidation could lead to AAV capsids containing a partial or truncated genome and negatively impact the efficacy and safety of AAV drugs.[3] Therefore, assessing the encapsidated genome's quality and size is necessary. Two different AAV serotypes consisting of full capsids were tested for genome integrity, along with an empty capsid AAV sample. The same sample-preparation procedure was used to extract AAV genomes from all samples before analysing them on the BioPhase 8800 system using the RNA 9000 Purity & Integrity kit as described in the methods section. Results are shown in Figure 5A. No peak correlating to the intact genome was observed in the empty AAV serotype A capsid, and peaks correlating with the size of the expected intact genome were observed in the full 4.6 kb AAV serotype A and 2.4 kb AAV

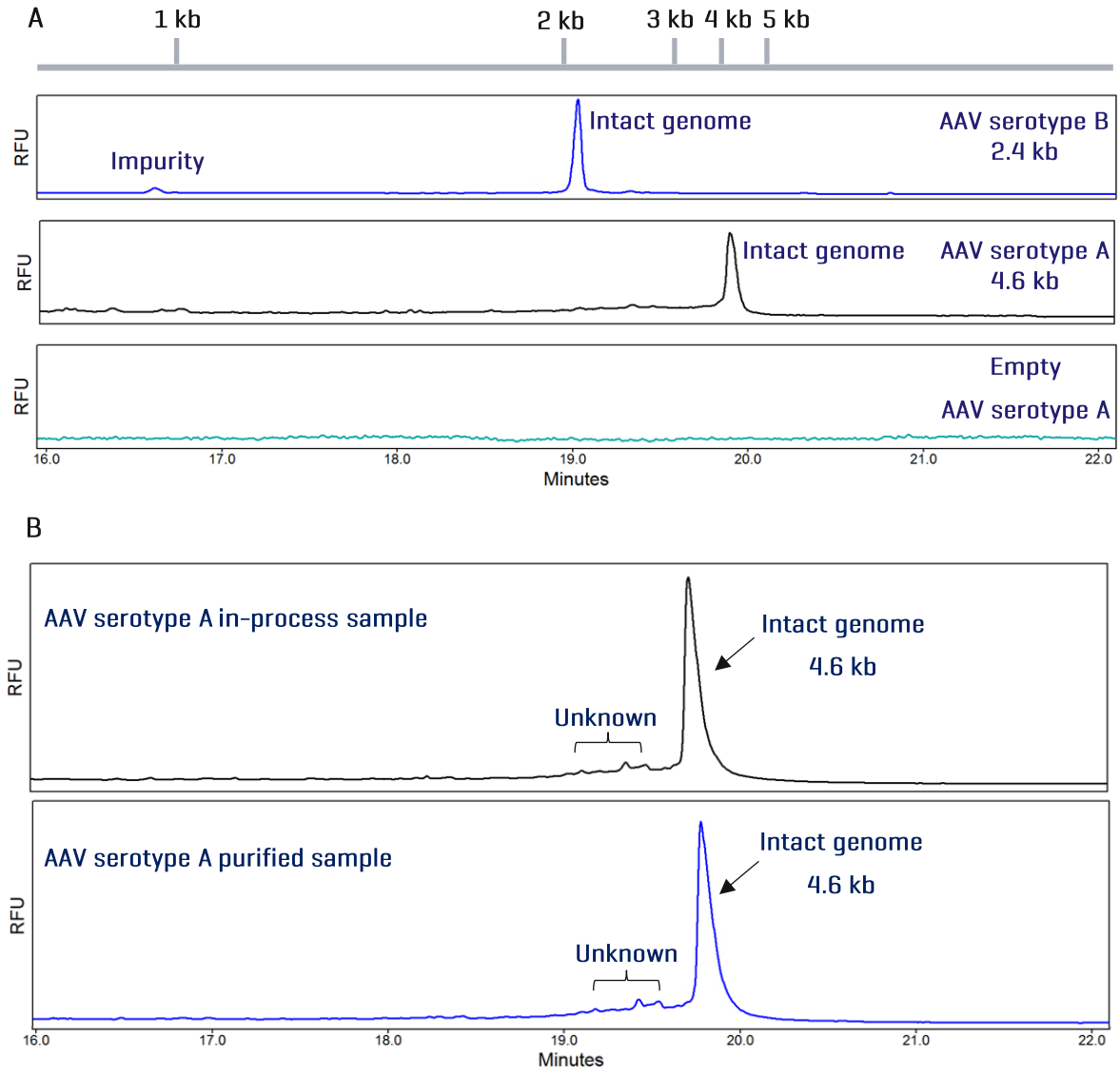

Figure 5. Genome integrity analysis of purified full capsids, empty capsids, and in-process AAV samples. The AAV genomes were extracted from the AAV samples and analysed on the BioPhase 8800 system using the RNA 9000 Purity & Integrity kit as described in the methods section. In panel A, purified AAV serotype B (blue trace) and AAV serotype A (black trace) full capsids and empty AAV serotype A (green trace) capsids were analysed. The intact genomes were well separated from impurities. The top bar shows the sizes of standard fragments from an ssDNA ladder. Panel B shows a comparison of the results obtained from an in-process (black trace) and a purified (blue trace) sample of the AAV serotype A. The AAV serotype A purified sample in panel B was a different sample from the one used in panel A.

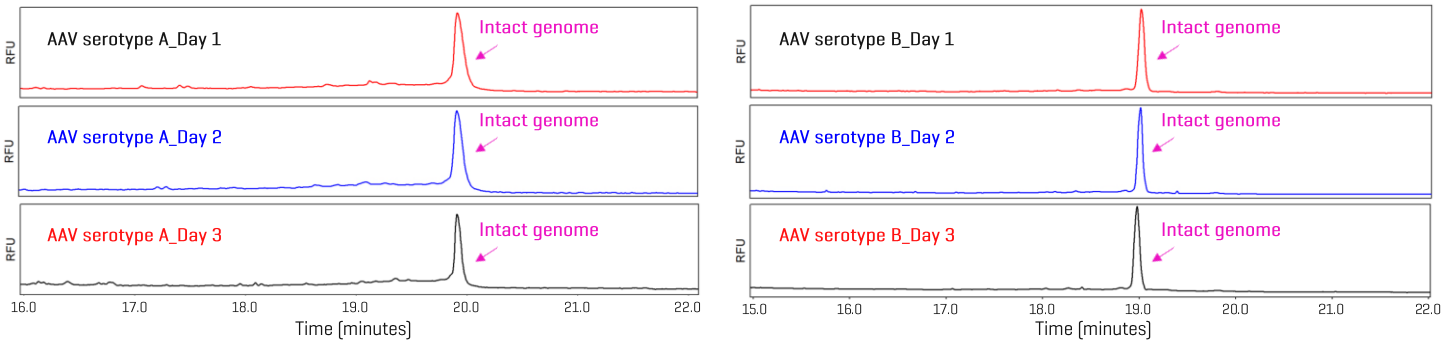

Figure 6. Excellent assay reproducibility of the genome integrity analysis. The purified genomes of the AAV serotype A and serotype B samples were analysed on three different days on the BioPhase 8800 system using the RNA 9000 Purity & Integrity kit. Profiles obtained on 3 different days were consistent.

serotype B samples. In addition, the intact genome peak was well-separated from the impurity peak for the full capsid AAV serotype B samples. The sizes of the intact genomes were verified using an ssDNA ladder. Figure 5B shows results obtained with an in-process and a purified AAV serotype A sample. A comparison of the two electropherograms indicates that consistent peak profiles were obtained. In addition, the relative migration times and sizes of the intact genomes were comparable between the two samples. These results demonstrate that the CGE-LIF method can be used as a platform method for genome integrity analysis for multiple AAV serotypes and both purified and in-process samples.

To assess the assay reproducibility of the genome integrity analysis, the purified genomes from an AAV serotype A sample and an AAV serotype B sample were analysed on three different days on the BioPhase 8800 system using the RNA 9000 Purity & Integrity kit. As shown in Figure 6, profiles in electropherograms obtained on 3 different days were consistent for both samples. With both serotypes, the relative migration times of the intact genome peaks were consistent among the runs on 3 different days, indicating excellent assay reproducibility. The profile of the AAV serotype B in Figure 6 looked slightly different from the one in Figure 5A. This is because the AAV serotype B samples in Figure 5A and in Figure 6 were from two different batches, both of which were commercially sourced. This observation indicates that the genome integrity workflow can be used to monitor batch-to-batch variations.

Conclusions

- Platform CE workflows for plasmid and AAV purity and genome integrity assessments were developed across the AAV production process
- ALL workflows were performed on one single instrument with pre-assembled cartridges and ready-to-use, user-friendly reagent kits
- The CGE-LIF workflow was validated for plasmids of various sizes with excellent repeatability and was successfully utilized to monitor plasmid purity quantitatively throughout the production process, offering a clear advantage over slab gels
- The CE-SDS-LIF workflow was successfully employed for high-precision AAV purity analysis and VP ratio assessment.
- High-resolution AAV genome integrity analysis by CGE-LIF workflow was demonstrated with excellent assay reproducibility.

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