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Application Note

Characterize Your Viral Vectors from Discovery to GMP Release with Maurice™

A Multi-Attribute Platform for Capillary Electrophoresis Analysis

As a scientist developing adeno-associated virus (AAV)- and lentivirus (LVV)-based gene and cell therapies, evaluating the critical quality attributes (CQAs) of your viral vectors is routine and required. While the criteria surrounding these CQAs aren't changing anytime soon, the techniques used for their analysis continue to evolve rapidly to increase productivity and shorten timelines. The more automated and reliable your analytical instruments are, the sooner you can get your product to market. To help simplify your workflow, this application note describes the use of a single platform for capillary electrophoresis-sodium dodecyl sulfate (CE-SDS) analysis of two AAV CQAs - identity and purity. Instead of using SDS-PAGE for AAV purity and confirming serotypes using PCR or ELISA, you can get data on different attributes of your samples with one platform. As different phases across development have their unique analytical challenges and requirements, this study demonstrates two label-free CE-SDS methods you can use on the same platform to address unique analytical requirements across different stages of development from discovery to GMP release.

CE-SDS on Maurice™ offers a faster, robust and highly reproducible alternative method to SDS-PAGE for routine analysis of proteins. You don't need to spend hours on gels. Instead, all you need to do is prepare your samples, load them onto the Maurice system, insert the appropriate CE-SDS cartridge, and hit Start. You'll get high-quality quantitative results from anywhere between 5.5-40 minutes. There are two CE-SDS cartridges for the Maurice system, Turbo CE-SDS™ and CE-SDS PLUS™. The former enables high-throughput analysis and is ideal for discovery stages, while the latter offers superior resolution and is ideal for analytical development and QC release testing. Additionally, Maurice also enables imaged cIEF (icIEF) analysis, which is the gold standard for protein charge heterogeneity characterization. Analysis of AAV identity and stability with Maurice icIEF has been reported previously[1,2]. No matter what analytical modality you need, you can develop your methods in a day, and easily transfer them across labs. All these assays are run using an easy-to-use software, Compass for iCE, which is also 21 CFR Part 11 compliant.

Materials and Methods

TABLE 1 lists the materials used in this study, including the Maurice Turbo CE-SDS and CE-SDS PLUS Size Application Kits that contain all materials needed for their respective CE-SDS assays. Cold acetone (4X the sample volume) was added to 20 µL of the AAV sample (**TABLE 2**) and briefly vortexed. The sample was kept at -20 °C for an hour, followed by centrifugation at room temperature for 10 minutes at 15000 xg to pellet the proteins. The supernatant was removed carefully, and the precipitate was allowed to dry for 5 minutes.

The precipitate was dissolved in the same volume of CE-SDS PLUS buffer as the AAV sample (20 µL) and vortexed. For denaturation, 0.7 M β-mercaptoethanol (β-ME) was added to the buffer first, and then incubated at 70 °C for 10 minutes. The sample was then cooled on ice for 5 minutes and spun down with a microcentrifuge before addition of distilled water up to either 50 µL or 100 µL final for CE-SDS PLUS and Turbo CE-SDS respectively. For analysis with Turbo CE-SDS, samples were injected for 8 seconds at 3500 V and separated for 8 minutes at 4200 V. For analysis with CE-SDS PLUS, samples were injected for 20 seconds at 4600 V and separated for 35 minutes at 5750 V. All data were analyzed with Compass for iCE software.

TABLE 1. Materials and reagents used

Material	Vendor	Catalog #
Maurice Turbo CE-SDS Size Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK01-TS
Maurice Turbo CE-SDS Cartridge	ProteinSimple	PS-MC02-TS, PS-MC01-TS
Maurice CE-SDS PLUS Size Application Kit	ProteinSimple	PS-MAK03-S
Maurice CE-SDS PLUS Cartridge	ProteinSimple	PS-MC02-SP
Maurice CE-SDS Molecular Weight Markers	ProteinSimple	046-432
β-mercaptoethanol (β-ME)	Millipore Sigma	M-3148
Acetone	Millipore Sigma	100014

TABLE 2. AAV samples (2 x 10^{13} GC/mL) used

Material	Vendor
AAV1-CMV-GFP	Virovek
AAV6-CMV-GFP	
AAV8-CMV-GFP	
AAV9-CMV-GFP	
scAAV9-CMV-GFP	
AAV9-CK8-CAS9	
scAAV9-hSyn-GFP	
AAV9-CMV-Luciferase	

Results

Identity

Four different AAV serotypes were each analyzed with the Turbo CE-SDS and CE-SDS PLUS cartridges. **FIGURES 1A** and **1B** show stacked profiles of the four serotypes (AAV1, 6, 8 and AAV9) resulting from each cartridge. A distinct profile for each serotype is clearly illustrated through both methods. The average ratios of viral proteins (VP1, VP2, and VP3) are also comparable between the two methods, as seen in **TABLE 3**. Similar relative migration times between both cartridges observed for each serotype further demonstrate the comparability of the two methods. The overlaid profiles of four serotypes from each method are shown in **FIGURES 2A** and **2B**. Overall, the results from this study show that both Turbo CE-SDS and CE-SDS PLUS methods successfully serve as identity assays for AAV vectors.

FIGURE 1. AAV serotype analysis with Maurice CE-SDS

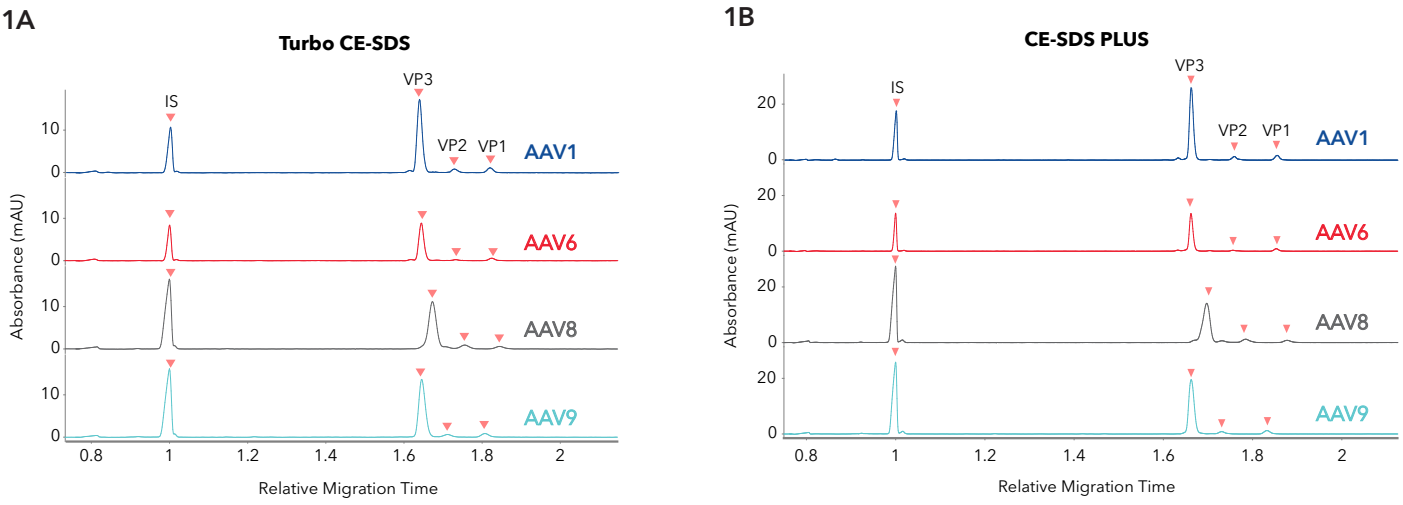


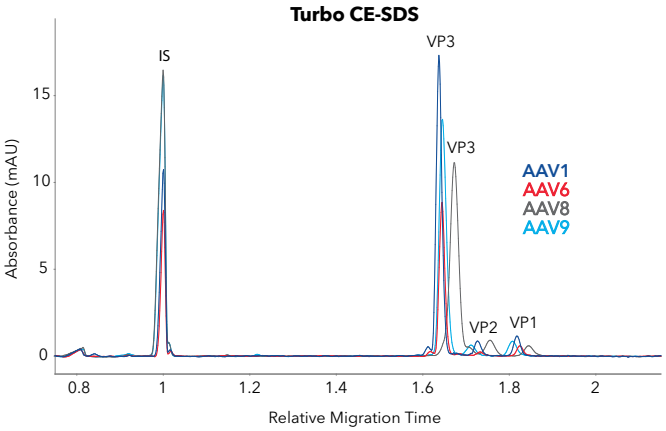
FIGURE 1. Identifying different AAV serotypes with Maurice CE-SDS. **A.** Stacked profiles of four different AAV serotypes analyzed with the Turbo CE-SDS cartridge and **B.** the Maurice CE-SDS PLUS cartridge. Both methods could easily distinguish between serotypes. The internal standard is labeled as IS.

TABLE 3. Comparable average ratios of viral proteins between the Turbo CE-SDS and CE-SDS PLUS methods

Average Ratio of Viral Proteins						
	VP3		VP2		VP1	
	Turbo	PLUS	Turbo	PLUS	Turbo	PLUS
AAV1	14.15	14.16	0.84	0.86	1.00	1.00
AAV6	13.18	14.29	0.54	0.29	1.00	1.00
AAV8	19.56	18.39	1.69	1.67	1.00	1.00
AAV9	14.33	15.03	0.88	0.84	1.00	1.00

FIGURE 2. Identifying serotype differences with Maurice CE-SDS

2A



2B

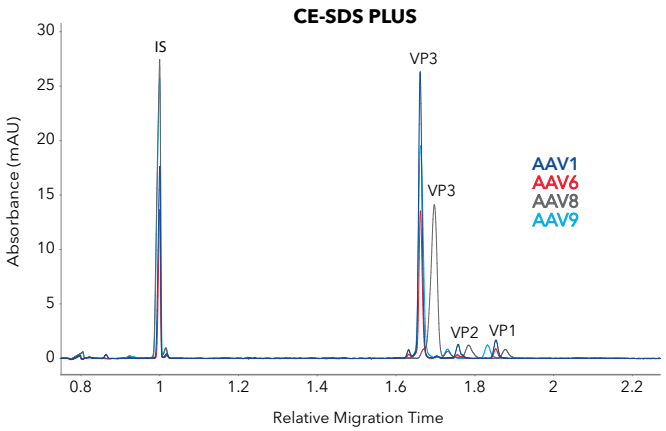


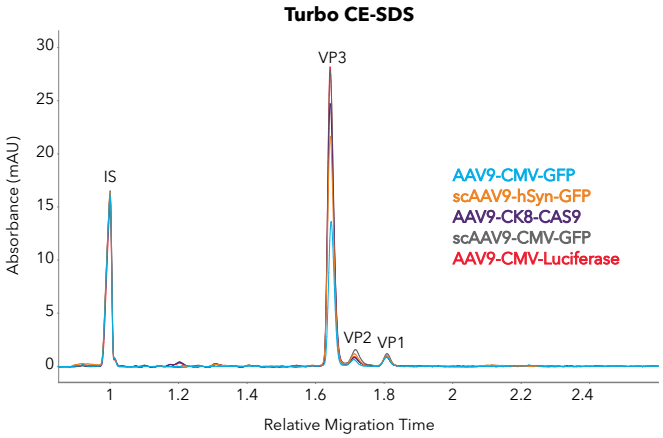
FIGURE 2. Overlaid profiles of the same four AAV serotypes analyzed with A. the Turbo CE-SDS cartridge and B. the Maurice CE-SDS PLUS cartridge show the differences between serotypes.

Capsid Protein Ratio

The stoichiometry of the three viral proteins VP1, VP2, and VP3 vary between different serotypes and production batches of the same serotype. These variations can influence the potency and transfer efficiency of AAV vectors[3]. Therefore, measuring this stoichiometry or capsid protein ratio is a critical step in quality control. In this experiment, five AAV9 samples produced in SF9 cells encapsulating five different transgenes were evaluated for their capsid protein ratios. The samples were first analyzed with the Turbo CE-SDS cartridge followed by the CE-SDS PLUS cartridge on the same instrument to better understand the data comparability. **FIGURES 3A** and **3B** show the results of from this study. As expected, there is a difference in peak intensity between the two cartridges, however, the differences between the five samples captured by each cartridge remain comparable. The viral protein ratios, also comparable between the two cartridges, are listed in **TABLE 4**.

FIGURE 3. Analyzing AAV9 with different transgenes with Maurice CE-SDS

3A



3B

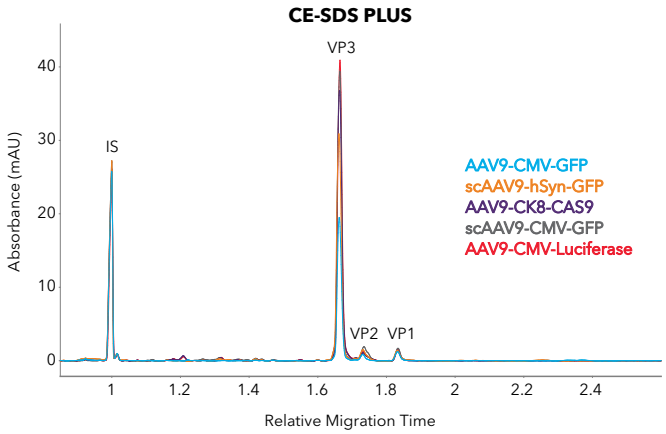


FIGURE 3. Measuring the capsid protein ratio of AAVs with Maurice CE-SDS. Five AAV samples with different transgenes were analyzed with **A.** Turbo CE-SDS and **B.** CE-SDS PLUS. Different sample profiles are clearly captured by both methods, from which the respective capsid protein ratios were measured.

TABLE 4. Capsid protein ratios for five different AAV samples determined with Maurice Turbo CE-SDS and CE-SDS PLUS. VP1 not listed as ratios presented are set with VP1 = 1

	VP3		VP2	
	PLUS	TURBO	PLUS	TURBO
AAV9-CMV-GFP	16.09	15.10	0.87	0.84
scAAV9-hSyn-GFP	20.73	17.96	1.49	1.34
AAV9-CK8-CAS9	27.31	27.28	1.40	1.36
scAAV9-CMV-GFP	21.40	21.01	1.64	1.80
AAV9-CMV-Luciferase	26.98	26.22	1.40	1.30

Impurity Measurements

The same five AAV9 samples with different transgenes were also analyzed for their impurities using both CE-SDS methods on the same Maurice instrument. **FIGURES 4A** and **4B** demonstrate how well both cartridges can detect sample impurities as well as the AAV genome. While there is some expected difference in resolution between the two, both methods were sensitive enough to detect minor impurities, as seen from the various AAV sample profiles. **TABLE 5** lists the percentage of impurities detected between the two instruments, which were similar. A single experiment was enough to provide critical information on AAVs such as capsid protein ratio (described in the previous section) and impurity levels, showing how the Maurice system is suitable for multi-attribute analysis.

TABLE 5. Impurity quantitation for AAV samples with the Turbo CE-SDS and CE-SDS PLUS methods

	Percent Impurities	
	Turbo	PLUS
AAV9-CMV-GFP	6.34	8.52
scAAV9-hSyn-GFP	7.29	7.89
AAV9-CK8-CAS9	15	13.3
scAAV9-CMV-GFP	6.5	6.79
AAV9-CMV-Luciferase	16.09	14.28

Reproducibility

FIGURE 5A demonstrates the reproducibility of the Turbo CE-SDS cartridge, resulting from 93 injections from ten different sample preparations with bracketing MW ladders. A gel-like representation of the same results is shown in **FIGURE 5B**, which was generated using the "Lane View" feature on Compass for iCE. **FIGURE 6A** shows the reproducibility of the CE-SDS PLUS method for 45 sample injections from a single well, along with the gel-like results in **FIGURE 6B**. Both methods show excellent reproducibility, and percent peak area precision is shown in **TABLE 6**.

FIGURE 4. AAV Impurity Measurements with Maurice CE-SDS

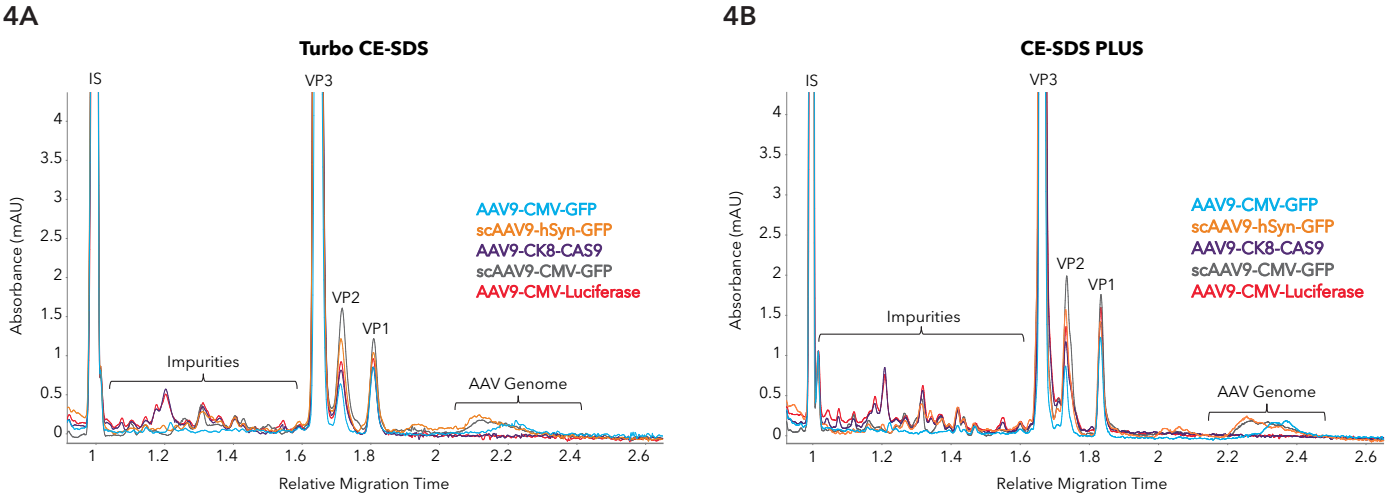
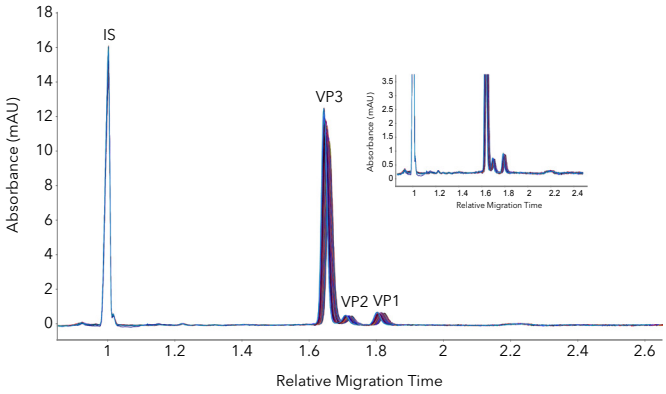


FIGURE 4. Measuring impurities in different AAV samples with Maurice CE-SDS. Five AAV samples with different inserts were analyzed with **A.** Turbo CE-SDS and **B.** CE-SDS PLUS. Both methods were able to detect and quantitate the level of impurities and the AAV genome present in the samples.

FIGURE 5. Turbo CE-SDS assay reproducibility

5A



5B

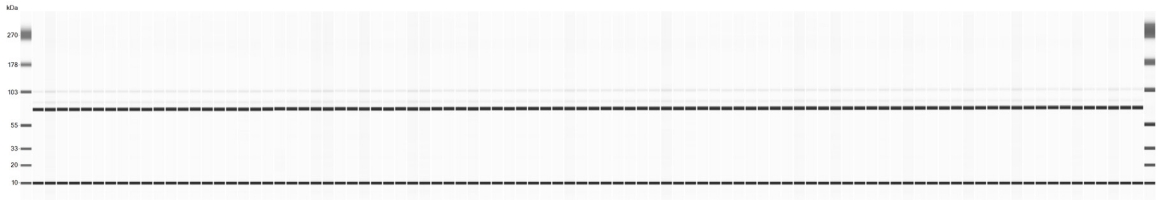
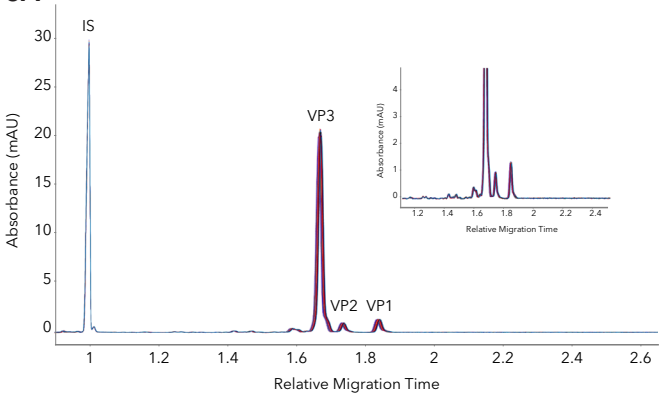


FIGURE 5. Method reproducibility of Turbo CE-SDS. A. Overlaid profiles of 93 AAV sample injections show excellent reproducibility of the method. B. Gel-like view of the same results also demonstrates that the method is highly reproducible.

FIGURE 6. CE-SDS PLUS assay reproducibility

6A



6B

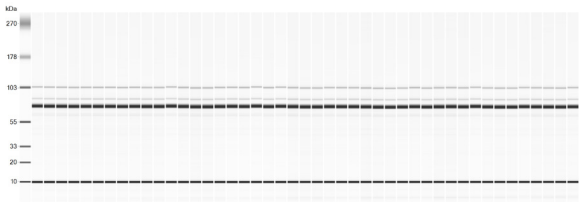


FIGURE 6. Method reproducibility of the CE-SDS PLUS assay. A. Overlaid profiles of 45 AAV sample injections demonstrate the high reproducibility of the method. B. Gel-like view of the same results.

TABLE 6. Precision of the Turbo CE-SDS and CE-SDS PLUS assays analyzing AAV samples on Maurice

Cartridge	Precision of Percent Peak Area (%RSD)		
	VP3	VP2	VP1
Turbo CE-SDS	3.47%	6.12%	5.55%
CE-SDS PLUS	2.49%	3.73%	2.89%

Intra-assay reproducibility of Turbo CE-SDS

The CE-SDS PLUS cartridge was released a few years before Turbo CE-SDS, therefore this section provides some additional information on the intra-assay reproducibility of Turbo CE-SDS for the analysis of AAV and Lentiviral (LVV) vectors. Studies on AAV and LVV analysis with CE-SDS PLUS on Maurice have been published previously[4,5]. For this study, 18 injections of AAV9 samples were analyzed from three different preparations each day for three consecutive days, resulting in a total of 54 injections. **FIGURE 7** demonstrates the intra-assay reproducibility of the Turbo CE-SDS method for all 54 injections, and the average capsid protein ratio obtained per day is shown in **TABLE 7**. **FIGURE 8** shows the intra-assay reproducibility of the Turbo CE-SDS method for LVV analysis, which resulted from nine injections of three different sample preparations (RSD = 1.92%). The intra-assay reproducibility of the Turbo CE-SDS was found to be excellent overall for both viral vectors, suggesting that it is suitable for upstream viral vector characterization

FIGURE 7. Turbo CE-SDS intra-assay reproducibility - AAV

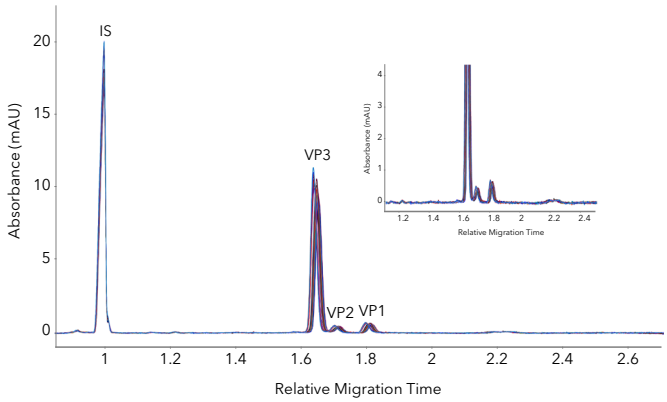


FIGURE 7. Intra-assay reproducibility of the Turbo CE-SDS cartridge analyzing AAV samples. Profiles of 54 sample injections run over a course of three days show how reproducible the assay is.

TABLE 7. Average capsid protein ratio and precision of peak area for 54 AAV sample injections analyzed with Turbo CE-SDS

Turbo CE-SDS (54 injections)	Average ratio of VPs		
	VP3	VP2	VP1
	15.1	0.8	1.0
Day 1	15.2	0.8	1.0
Day 2	14.8	0.9	1.0
Day 3	15.3	0.7	1.0
% RSD (% Peak Area)	5.98	12.57	7.05

FIGURE 8. Turbo CE-SDS intra-assay reproducibility - LVV

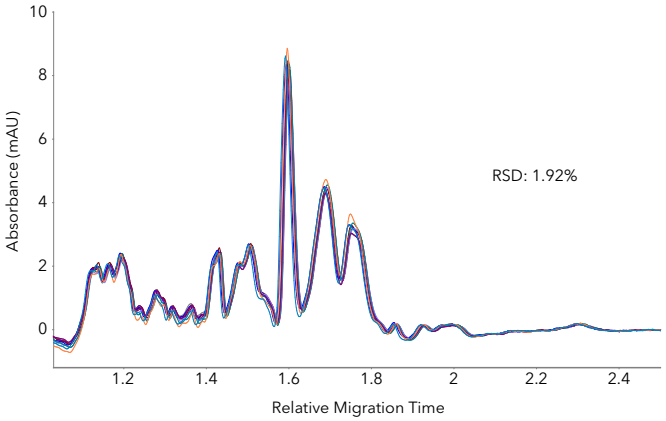


FIGURE 8. Intra-assay reproducibility of the Turbo CE-SDS cartridge analyzing LVV samples. Profiles of nine sample injections from three different preparations demonstrate high reproducibility of the method, with an RSD value of 1.92% for the total area.

Conclusion

The ideal analytical techniques are those that not only provide fast results but are also reproducible and reliable. This application note showed how a single platform offers two CE-SDS assays for the rapid analysis of AAV and LVV vectors, with each method offering its own set of advantages and therefore being best suited for specific stages of drug development. Both the Turbo CE-SDS and CE-SDS PLUS assays successfully characterized the identity and purity of AAV samples while demonstrating excellent inter- and intra-assay reproducibility. Distinct profiles of different AAV serotypes were generated with both assays, capsid protein ratio was measured, and impurities in samples were easily detected. Importantly, both assays performed comparably to one another.

There are several benefits of using Maurice CE-SDS for the analysis of viral vectors. Experiment times are considerably shortened owing to automation and making critical decisions about your samples becomes faster and easier because of the high-quality, reproducible data you get. Furthermore, you can use the Maurice system across different stages and labs—you only need to switch between cartridges—as method transfer is fast and easy. You can also analyze other CQAs of your viral vectors by leveraging the icIEF capability on the same Maurice system. The more value you derive from one instrument, the more you can save on time, capital, and bench space while confidently moving towards the finish line. To learn more about Maurice, visit our website.

References

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2. Application Note – Assessing Your AAV Product Quality? Get the Confidence You Need With Maurice.
3. Bosma, B., du Plessis, F., Ehlert, E. et al. Optimization of viral protein ratios for production of rAAV serotype 5 in the baculovirus system. Gene Ther 25, 415–424 (2018). https://doi.org/10.1038/s41434-018-0034-7.
4. Application Note - Characterization of Adeno-Associated Viral (AAV) Vector Proteins Using Maurice CE-SDS.
5. Application Note – Lentiviral Vector Characterization Made Easy with Maurice CE-SDS.