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Application Note

Lentiviral Vector Characterization Made Easy with Maurice CE-SDS

Introduction

Lentiviral vectors (LVVs) are a popular choice for gene therapies and gene-modified cellular therapies for several reasons: they can transduce non-proliferating and slowly proliferating cells, maintain long-term stable expression, deliver higher amounts of the transgene, and do not trigger inflammatory responses[1,2]. Today, there are over 315 LVV-based gene therapies in clinical trials[3]. Furthermore, an increase in the gene therapy approval rate has tightened the race amongst biopharma companies to reach patients faster.

Several factors are involved in a smoother, accelerated viral vector-based gene therapy pipeline, including the adoption of robust analytical tools across various stages. The accurate characterization of viral vectors for various attributes such as purity, identity, viral titer, and safety is critical. Even today, LVVs are characterized for some of their attributes with techniques such as SDS-PAGE and ELISA. SDS-PAGE is a manual and laborious method that often comes with reproducibility issues, while ELISAs can overestimate the viral titer present.

To address the challenges presented by these analytical techniques, this application note demonstrates a robust and automated CE-SDS method on the Maurice™ instrument, which can be used as both an identity assay as well as a titer assay for LVV analysis.

About Maurice

The Maurice instrument is a fully integrated capillary electrophoresis (CE) analytical platform that enables CE-SDS for protein size analysis and icIEF for protein charge analysis. Switching between methods is as easy as plugging in the relevant cartridges without needing to worry about manual instrument setup or cleanup. You can get CE-SDS data in as little as 5.5 minutes with the Turbo CE-SDS™ Cartridge for high throughput applications, and 25 minutes with the CE-SDS PLUS™ Cartridge if characterization is the goal. Reproducible, high-quality data with 21CFR Part 11 compliant software lets you develop your methods within a day, and the instrument's ease-of-use allows seamless method transfer between labs or phases. The study described in this application note uses the CE-SDS PLUS Cartridge, which is ideal for use in QC and late-stage development.

Materials and Methods

TABLE 1 lists the all the materials used in this study, including the Maurice CE-SDS PLUS Application Kit (PN PS-MAK03-S), which contains all the materials necessary for CE-SDS analysis. LVV particles (1.1×10^{10} TU/mL) were heat-inactivated at 95°C for 2 minutes, after which the sample was kept on ice for immediate use (or at -80°C for later use). Cold acetone was added to 20 µL of the LVV sample (10X the sample volume) and briefly vortexed to precipitate the LVV proteins. The sample was then spun in a centrifuge for 10 minutes at 15000 x *g* to pellet the proteins.

The supernatant was removed, and the precipitate was allowed to dry for 5 minutes. The precipitate was then dissolved in 60 µL of 2% SDS containing 200 mM bicine (pH 5.5). For the CE-SDS analysis, all samples were prepared using a reduced protocol. The samples were denatured with β-mercaptoethanol (β-ME) at 95°C for 10 minutes and cooled on ice for 5 minutes. Then samples were vortexed briefly and spun down with a microcentrifuge. The samples were then loaded onto the Maurice instrument (PN 090-000), followed by injection for 20 seconds at 4600 V and separation for 40 minutes at 5750 V. Data were analyzed on Compass for iCE software.

Material	Vendor	Catalog #
LVV particles (Vendor 1)	Takara	0010VCT
LVV particles (Vendor 2)	Signagen	SL100269
CE-SDS PLUS Application Kit	ProteinSimple, a Bio-Techne brand	PS-MAK03-S
rHIV1Gagp24	Bio-Techne	11243-HV
β-ME	Millipore Sigma	444203
Bicine	Millipore Sigma	391336
Acetone	Millipore Sigma	100014
SDS Solution 10% (W/V)	Bio-Rad	1610416

TABLE 1. List of materials and reagents used in the study.

Results

Reproducibility

FIGURE 1A shows the results of 45 LVV sample injections from a single 20 µL sample, demonstrating excellent reproducibility despite the complex profile. The average total peak area, standard deviation, and relative standard deviation (RSD) were calculated, resulting in an RSD value of 2.91% for the method's inter-assay reproducibility. Compass for iCE also produces a lane view (**FIGURE 1B**), which is a gel-like representation of the peak profiles that can also be used to visualize large data sets.

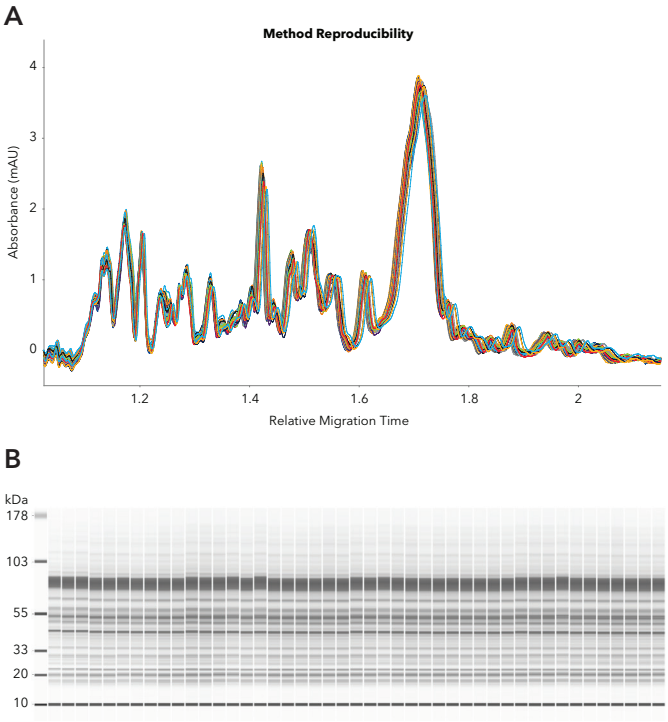

FIGURE 1. Maurice CE-SDS PLUS method reproducibility for LVV analysis. **A.** An overlay of 45 sample injections shows remarkable reproducibility of the method. **B.** The gel-like representation from the software's "Lane View" feature also establishes method reproducibility.

To assess the inter-day reproducibility of the CE-SDS PLUS method, 3 different LVV samples were prepared each day for 3 days, and 6 replicates of each sample were run on Maurice on all 3 days for a total of 54 injections. **FIGURE 2A** shows data for all 54 samples, and the reproducibility was found to be remarkable with an RSD value of 3.38%. **FIGURE 2B** shows the Lane View results of the same experiment.

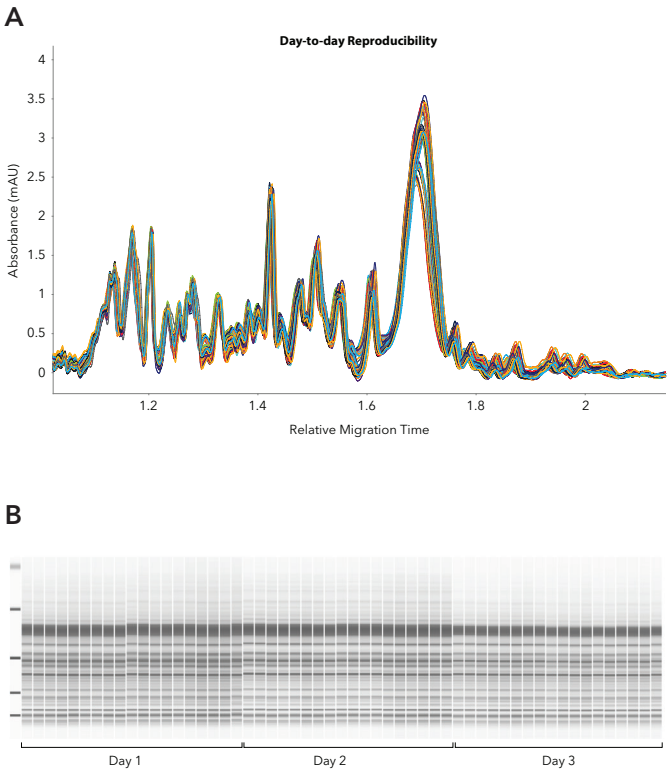

FIGURE 2. Inter-day reproducibility of the Maurice CE-SDS PLUS method. **A.** An overlay of 54 injections, taken over 3 days (18 injections per day), shows excellent inter-assay reproducibility. **B.** Lane View of the results from 54 sample injections.

Linearity and Limit of Detection

An LVV sample was serially diluted and analyzed on Maurice to establish method linearity and limit of detection. **FIGURE 3A** shows an overlay of the electropherograms obtained, where an increase in peak area is clearly seen with an increase in sample concentration. A plot of this dilution series was used to determine the limit of detection (LOD) and limit of quantitation (LOQ), as shown in **FIGURE 3B**, where an R^2 value of 0.98 was obtained. The LOD was calculated by dividing three times the standard deviation of the response by the slope of the calibration curve, and the LOQ was calculated using 10 times the standard deviation of the response divided by the slope. The LOD and LOQ values are listed in **TABLE 2.**

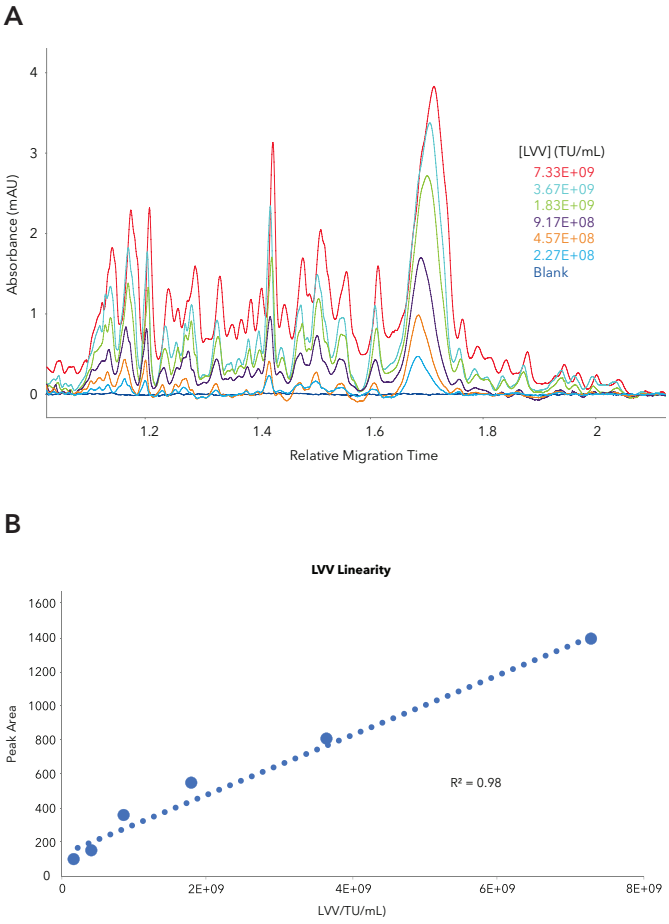

FIGURE 3. Linearity, LOD and LOQ determination of LVVs. **A.** An increase in peak area observed in the electropherograms correlate with an increase in LVV concentration. **B.** A plot of the peak area with an increase in LVV concentration resulted in an R^2 value of 0.98, showing linearity of the method.

LOD	2.56 x 10^7 TU/mL
LOQ	3.75 x 10^7 TU/mL

TABLE 2. LOD and LOQ values determined from the dilution series.

LVV Titer

Quantitating the amount of p24 is one of the main methods of determining LVV viral titer, which is used to ensure proper LVV dosing for gene delivery. In this study, LVV samples were spiked with varying concentrations of p24 and were analyzed using the CE-SDS method developed. **FIGURE 4A** shows the results of the p24-spiked LVV titration, where an increase in the p24 peak intensity is clearly seen as the concentration of p24 is increased. The peak area values from this experiment were plotted against the p24 concentration to generate a titration curve (**FIGURE 4B**). Comparing to the relationship of 1 ng of p24 = 10^5 TU established in a *Nature* publication[4], the titer concentration from this study was estimated to be approximately 8.5 µg/mL of p24 in LVV at 10^9 TU/mL, which is within 15% of the theoretical value.

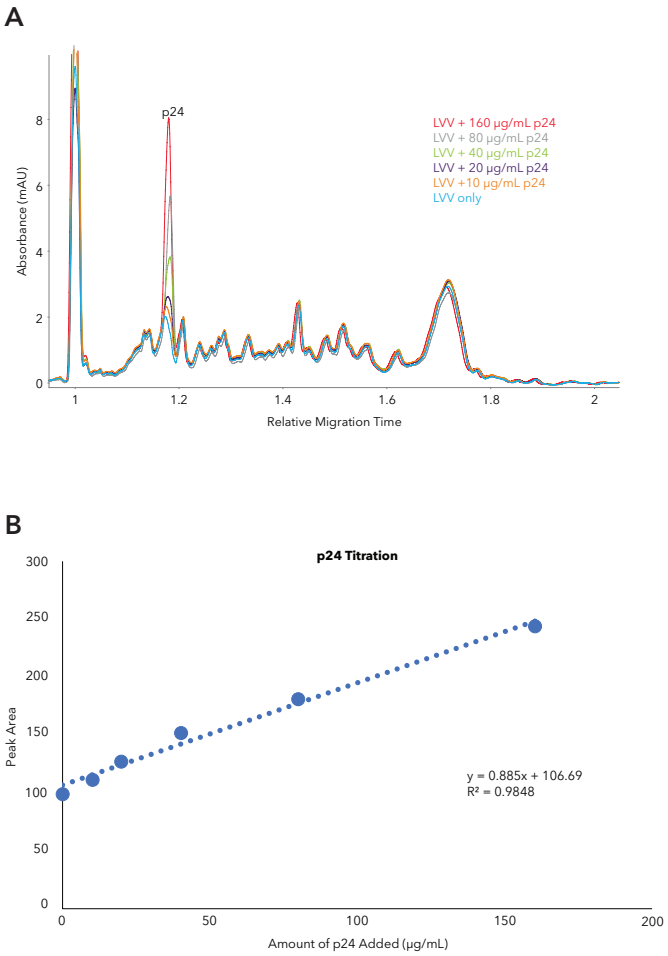

FIGURE 4. Viral titer measurement with CE-SDS PLUS through a p24 spike. **A.** An increase in peak intensity of p24 is visible as the concentration of recombinant p24 increases. **B.** Linear regression model of the data, where the straight-line equation leads to the concentration of p24 in LVV only. Titer concentration was estimated from these data.

LVV Identity

Confirming the identity of LVVs is critical for ensuring consistency. CE-SDS on Maurice offers one of the easiest methods to identify LVV particles from different vendors, as shown in **FIGURE 5**, where key similarities and differences between the profiles of different vendors are delineated.

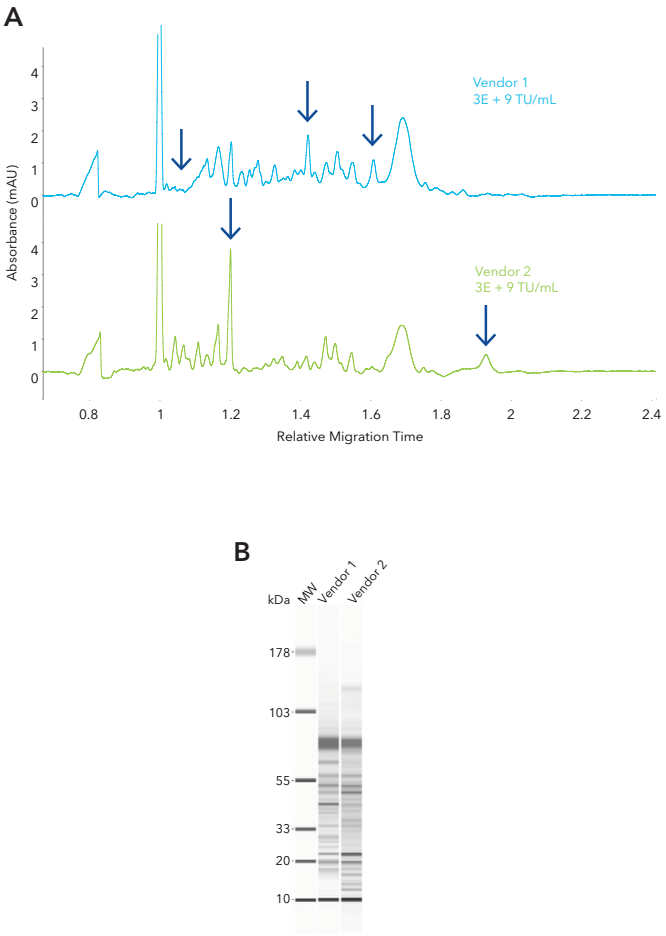

FIGURE 5. LVV identity with Maurice CE-SDS PLUS. **A.** Electropherograms of LVV samples from two different vendors highlight the similarities and differences in peaks, making this CE-SDS method a suitable identity assay. **B.** Lane View of the two electropherograms.

Conclusion

Understanding your viral vector earlier during development prevents quality issues from ensuing later in critical stages, therefore implementing the appropriate analytical tools is key. On that front, the study here described an automated CE-SDS method on the Maurice instrument to serve as LVV identity and viral titer assays. Both method reproducibility and inter-assay reproducibility were excellent, with RSD values of 2.91% and 3.38% respectively. Separation was linear, with an R^2 value of 0.98. The LOD and LOQ were determined to be 2.56×10^7 and 3.75×10^7 TU/mL, respectively. Importantly, titration with a recombinant p24 spike protein was an efficient way to deduce viral titer. Finally, as an identity assay, the CE-SDS PLUS method was easily able to distinguish between the LVV peak profiles from two different vendors.

The benefits of Maurice CE-SDS extend far beyond obtaining high-quality, reliable data. Also popular for its ease-of-use, the Maurice instrument eliminates several manual processes, simplifying sample prep and instrument setup so that you can get results fast and develop your methods in a day. Such a simplified workflow helps you confidently transfer your methods across labs, accelerating your drug development process and ultimately helping you reach patients faster. The Maurice system has also been used for the characterization of other biotherapeutic proteins including AAVs, monoclonal antibodies, biosimilars, and more. To learn more about Maurice, visit bio-techne.com/instruments/ice.

References

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