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Supplemental Information

Rapid In-Process Monitoring of Lentiviral Vector Particles by High-Performance Liquid Chromatography

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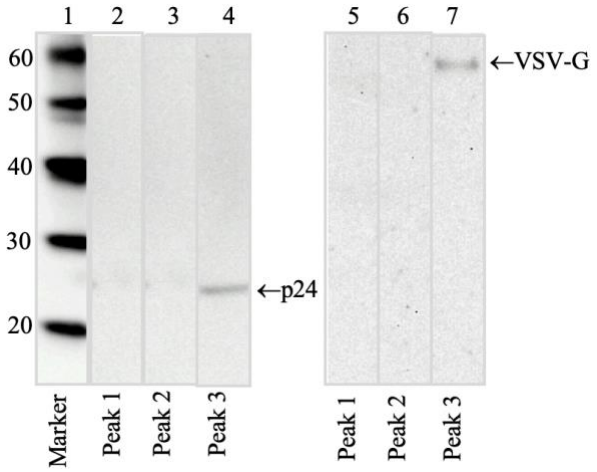
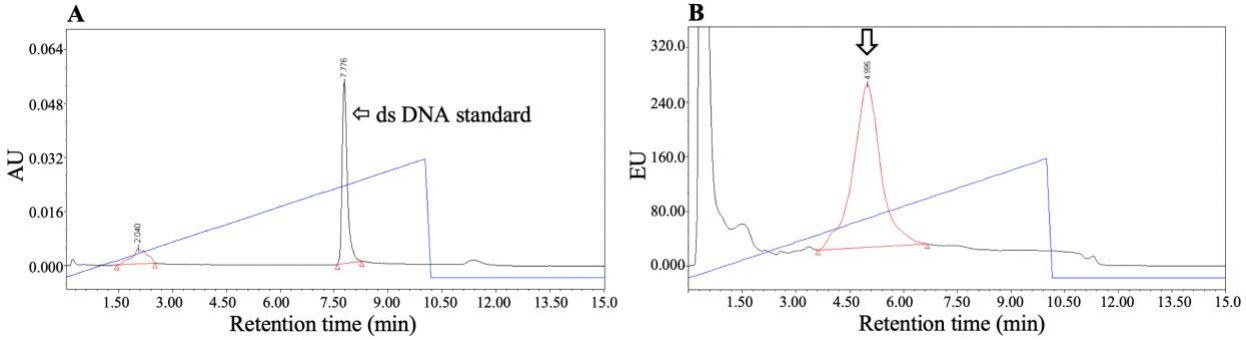
Figure S1



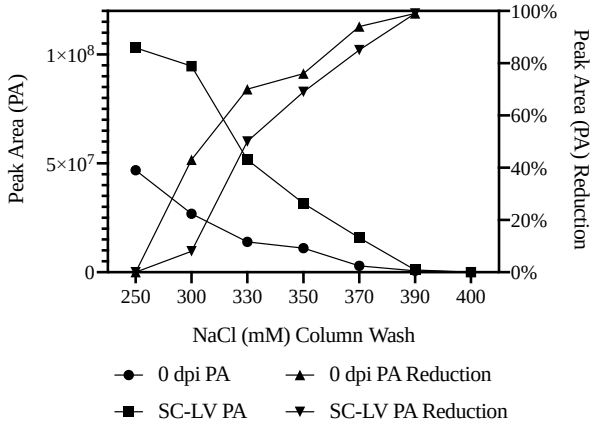
Characterization of HPLC peaks from the in-house LV standard, SC-LV, by western blot against p24 capsid and VSV-G envelope. Lane 1: protein molecular weight markers. Lanes 2, 3, and 4: peaks 1, 2, and 3, respectively, against p24 capsid. Lanes 5, 6, and 7: peaks 1, 2, and 3, respectively, against VSV-G envelope. Peak 3 is the only peak with the presence of p24 capsid and VSV-G envelope bands. Note that peak 4 was not collected and, thus, not analyzed.

Figure S2



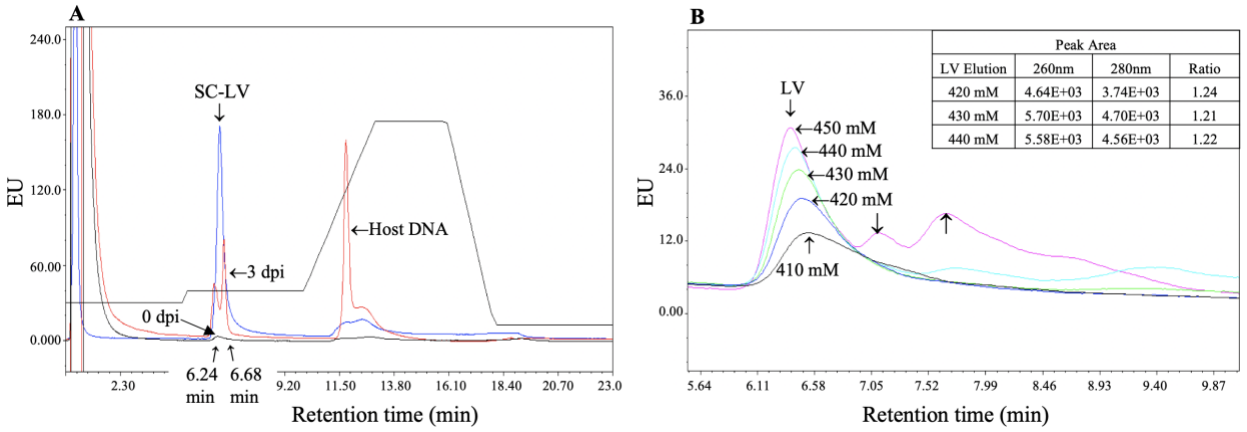
Chromatographic profiles of peaks 4 and 3, respectively, of the in-house LV standard, SC-LV, during HPLC method development. **A)** Double stranded (ds) DNA standard detected by absorbance at 260 nm (OD260). The ds DNA standard was eluted at 777 mM NaCl, which is the same salt elution as peak 4 in Figure 2A. Generally, DNA is strong negatively charged molecules that bind tightly to anion exchange columns and resins, which results in requiring high salt concentrations for elution. The small peak area of peak 4 in Figure 2A suggests that the SC-LV sample has an insignificant presence of residual DNA. **B)** Fluorescence (FL) detection of the SC-LV sample at excitation and emission wavelengths of 290 nm and 335 nm, respectively. The LV peak (indicated by an arrow) corresponds to peak 3 of the OD260 profile in Figure 2A. The FL profile shows a significantly higher LV peak than the OD260 profile; thus, chromatograms monitored by FL were used for the remainder of the HPLC method development.

Figure S3



NaCl step gradient column wash, from 250 mM to 400 mM, post sample injection to minimize co-eluting EVs. Figure S3 shows a similar gradual reduction pattern of the peak area (PA) for both SC-LV and 0dpi as the concentration of NaCl column wash post sample injection was increased, with the highest peak area reduction at 370 mM NaCl column wash of 85% for SC-LV and 94% for 0dpi samples.

Figure S4



Chromatographic profiles of SC-LV, 3dpi, and 0dpi samples as well as optimization of LV peak elution. **A)** 370 mM NaCl column wash with 500 mM NaCl elution conditions tested on SC-LV, 3dpi, 0dpi samples; chromatogram shows a split peak with retention times of 6.24 min and 6.68 min in the 3dpi sample. **B)** 410 mM to 450 mM NaCl step elution to resolve the split peak found in the LV peak shown in the 3dpi sample in A). Lowering the NaCl gradient elution to 450 mM elution resulted in the appearance of 2 peaks that were eluted later than the original LV peak (indicated by arrows). These 2 peaks gradually disappeared with 440 mM, 430 mM, and 420 mM NaCl. The 410 mM eluted peak was significantly lower than the other eluted peaks and it was assumed that this NaCl concentration was not sufficient to elute all LV particles. The OD260/280 ratio of the 420 mM, 430 mM and 440 mM elutions was determined to be 1.22, 1.21, and 1.24, respectively (inset figure of Figure S4-B). Although the ratios were similar, it was observed that the LV PA at 420 mM and 430 mM elutions was lower than at 440 mM elution. Likewise, it was assumed that not all LV particles were eluted at these concentrations; thus, 440 mM was selected for the NaCl step gradient elution.

Table S1

Sample	RFU
SC-LV	554.8
3dpi	434.7
0dpi	281.6

Acetylcholinesterase activity results for SC-LV, 3dpi, and 0dpi samples. The relative fluorescence units (RFU) is the fluorescence intensity of a thiol probe provided in the assay kit that quantifies the thiocholine produced from the hydrolysis of acetylthiocholine by acetylcholinesterase (AChE) in samples, indicating the presence of EVs. RFU as low as 5 can be detected with 20 minutes of incubation.

Table S2

NaCl Column Wash (mM)	SC-LV Sample			0dpi Sample		
	OD260 PA	OD280 PA	Ratio	OD260 PA	OD280 PA	Ratio
250	1.63E+05	1.50E+05	1.09	2.51E+04	2.96E+04	0.85
300	1.34E+05	1.30E+05	1.03	7.83E+03	1.07E+04	0.73
330	7.44E+04	7.14E+04	1.04	6.35E+03	7.56E+03	0.84
350	4.18E+04	3.82E+04	1.10	3.07E+03	4.22E+03	0.73
370	3.59E+04	2.90E+04	1.24	Not detectable		NA
390	2.19E+04	1.77E+04	1.24	Not detectable		NA
400	Not detectable		NA	Not detectable		NA

Minimization of co-eluting EVs in HPLC method development. For the SC-LV sample, the OD260/280 ratios obtained were similar for 250 mM to 350 mM NaCl washes and increased to 1.24 for 370 mM and 390 mM NaCl washes. Noticeably, for 370 mM and 390 mM NaCl washes, although the OD260/280 ratio did not change, the PA for 390 mM NaCl wash showed a slight reduction as compared to 370 mM NaCl wash with both 260 nm and 280 nm absorbances (results not shown), which is an indication that at the 390 mM NaCl concentration, some of the LV particles were eluted in the flow-through. For 0dpi, the OD260/280 ratios obtained for 250 mM to 350 mM NaCl washes were <1.0 and the peak was no longer detectable from 370 mM to 400 mM NaCl washes. Thus, 370 mM was selected for the NaCl step gradient column wash post sample injection.

Table S3

	Mean LP/mL			
	SC-LV	**UFDF Product**	**Supernatant A**	**Supernatant B**
Tris Buffer	6.69E+09	4.00E+09	1.42E+09	1.90E+09
SFM4Transfx-293	1.18E+09	5.55E+08	5.27E+09	7.41E+09
Increase in Titer	82%	86%	63%	61%

p-24 ELISA method optimization. Four LV samples were diluted in Tris buffer (20 mM Tris-HCl, pH 7.5) and SFM4Transfx-293 medium for comparison. Titers, in LV particle concentration per milliliter (LP/mL), were 82% higher for SC-LV, 86% higher for UFDF Product, 63% higher for Supernatant A, and 61% higher for Supernatant B when using Tris buffer as the diluent as compared to cell culture medium. Thus, Tris buffer was established to be used as the diluent for this assay.