Quantification of total mRNA content in LNP complexes using the SoloVPE® variable pathlength system

Application Note

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Introduction

Lipid nanoparticle-encapsulated mRNA (LNP-mRNA) shows significant potential as a new approach for treating a wide variety of diseases. Being able to measure mRNA precisely in treatments helps make sure they are reliable and safe.[1] UV-Vis spectroscopy is the preferred technique for mRNA measurement due to its precision and ease of use. However, due to strong light-scattering characteristics of LNPs, conventional UV-Vis spectroscopy is unreliable, as it struggles with pathlength limitations and distinguishing between mRNA and scattering components when diluted for measurement. RiboGreen fluorescence is the favored approach for measuring mRNA encapsulated within LNPs, though it does have certain limitations and difficulties, including reduced accuracy, chemical safety concerns, poor reproducibility, concentration limitations, and significant time requirements. Here, we propose and demonstrate an alternative method for quantifying total mRNA content in an LNP complex using the CTech™ SoloVPE® System, which employs the Slope Spectroscopy® method. By employing the Dual Logarithmic (Ln) scatter correction feature within the SoloVPE control software, the light scattering effect of the LNPs can be effectively subtracted, allowing for accurate determination of the total mRNA concentration.

Improving Analytical Methods & Techniques

RiboGreen® Assay Method

Accurate measurement of total RNA content is critical for the development, scale-up, and GMP compliance of LNP therapeutic manufacturing. The RiboGreen assay is a commonly used method for quantifying mRNA in LNP formulations and is endorsed by the United States Pharmacopeia (USP) for this purpose.[2] This method utilizes a dye that, upon binding to mRNA, produces over a 1000-fold increase in fluorescence intensity, while unbound dye remains virtually non-fluorescent.[3] To enable detection, mRNA must be released from the LNPs, typically using a surfactant, most often Triton X-100. However, Triton X-100 has raised chemical safety concerns, as identified by the European Chemicals Agency (ECHA).[4] In addition, the dye only binds to free mRNA in solution and possibly to mRNA loosely associated with the surface of LNPs, but not to mRNA that is fully encapsulated within the LNPs.

While the RiboGreen assay is widely used and USP endorsed, its implementation in a GMP quality control (QC) setting presents several practical challenges. The assay relies on a drug substance reference standard to generate a calibration curve. In a GMP environment, this introduces complexity, as the reference material must be qualified, consistently available, and representative of the product being tested. During early development or process optimization, when drug substance may be limited or not yet fully characterized, this dependency can delay method validation and routine testing. Additionally, the need to prepare and verify a standard curve for each assay run increases hands-on time and introduces potential variability. When both total mRNA content and encapsulation efficiency must be assessed, multiple calibration curves may be required, further complicating the workflow. The complexity of the test method can also present challenges with the LNP manufacturing process as process steering results for mRNA content are often delayed due to the complexity of the test method.

In addition, the assay also poses technical challenges when testing concentrated LNP samples. RiboGreen fluorescence can saturate at high RNA concentrations, necessitating serial dilutions that increase the risk of pipetting errors and reduce assay precision. Furthermore, the requirement to fully disrupt LNPs using surfactants such as Triton X-100 introduces chemical handling concerns and can affect reproducibility, particularly when scaling across multiple analysts or sites. Despite these limitations, RiboGreen remains a valuable tool for assessing encapsulation efficiency, which is a key quality attribute outlined in USP guidelines.

Slope Spectroscopy Method

Unlike traditional UV-Vis methods that rely on single absorbance values, Slope Spectroscopy determines concentration using the linear relationship between multiple absorbance and pathlength data points, derived from the Beer–Lambert law:

$$m = \varepsilon c$$

where m is the change in absorbance per unit pathlength, ε is the extinction coefficient, and c is the concentration. The R^2 value from the linear regression confirms proportional changes, ensuring accuracy for each sample. The CTech SoloVPE spectrophotometer adjusts the pathlength in 5 µm steps between 5 µm and 15 mm to enable accurate measurement of high-concentration samples beyond the limits of fixed-pathlength systems.[5] It then collects absorbance data and generates a linear slope regression, with a built-in quality check of $R^2 > 0.999$. This method requires no dilution, offering speed, precision, and repeatability to streamline workflows and reduce delays in processing. Furthermore, the SoloVPE System enables a non-destructive approach for LNP-mRNA quantification, in contrast to other methods that require invasive sample manipulation.

This paper presents data demonstrating the application of the SoloVPE System for accurate, rapid, and calibration-free quantification of total mRNA content in LNP drug product, highlighting its potential as a robust analytical tool for GMP-compliant manufacturing and in-process control.

Materials

- SoloVPE Instrument #1 [IN-SOLO5-VPE]
- SoloVPE Instrument #2 [IN-SOLO5-VPE]
- Cary 60 UV-Vis Spectrophotometer #1 [IN-CARY60]
- Cary 60 UV-Vis Spectrophotometer #2 [IN-CARY60]
- Fibrette Optical Components [OF0002-P50]
- SoloVPE Sample Plastic Vessels [OC0009-P50]
- SoloVPE Vessel Holder – Small [HM0178]
- mRNA drug substance formulated in Water for Injection (used for spiking experiments and calibrator in the RiboGreen assay)
- LNP drug product (composed of 4 unique lipids) formulated with 0.1 mg/mL of standard mRNA drug substance
- mRNA encapsulated in LNP (0.10 mg/mL, 0.19 mg/mL, 0.37 mg/mL, 0.73 mg/mL, 0.91 mg/mL)
- Quant-it RiboGreen RNA Reagent and 20X TE from RiboGreen kit (ThermoFisher Scientific)
- 96-well plates
- SpectraMax M5e Multi-Mode Microplate Reader

Study Design

The SoloVPE method was performed using a wavelength of 260 nm, which is standard for mRNA quantification. Additionally, dual Ln scatter correction was applied over the 300–320 nm range. This wavelength range for scatter correction was evaluated against other options and demonstrated the highest accuracy across different concentrations.

The concentration of an mRNA sample is calculated using a modified version of the Beer-Lambert Law, expressed as

$$c = \frac{m}{\varepsilon}$$

Dual Ln scatter correction (300–320 nm) was applied to all ten absorbance data points per acquisition to remove LNP interference from the 260 nm signal. The corrected slope, representing mRNA alone, was then used with the mRNA extinction coefficient of 25 mL/(mg*cm) to calculate concentration.

The experiment evaluated the accuracy, repeatability, and linearity of the SoloVPE method by analyzing total mRNA encapsulated in an LNP complex at five different concentration levels. mRNA samples were prepared at 0.10 mg/mL, 0.19 mg/mL, 0.37 mg/mL, 0.73 mg/mL, and 0.91 mg/mL by spiking mRNA drug substance into the LNP drug product test sample. The initial total mRNA content (0.10 mg/mL) was established for the LNP drug product test sample per Aldevron's platform RiboGreen test method. The other four levels were generated by spiking mRNA drug substance into the LNP drug product test sample to generate higher mRNA content. The concentration of the mRNA drug substance was measured per Aldevron's platform SoloVPE test method. Each level was measured three times each on two different SoloVPE instruments by different analysts for a total of six measurements per concentration level.

Results

Relative accuracy (%Recovery) was calculated for each mRNA level (1–5). SoloVPE results are summarized in Table 1 and RiboGreen results are given in Table 2. Observed SoloVPE recoveries ranged from 88% to 113%, which are comparable to the observed RiboGreen recoveries (77% to 103%). All SoloVPE slope measurements had acceptable linearity ($R^2 \geq 0.999$).

SoloVPE repeatability was calculated separately for each analyst over three measurements. RiboGreen repeatability was calculated over two measurements, with the exception of concentration levels 4 and 5, where only one valid measurement was obtained. SoloVPE repeatability results are summarized in Table 3 and RiboGreen repeatability results are shown in Table 4. The observed SoloVPE RSD ranged from 0% to 13.5%, while the RiboGreen RSD ranged from 0.7% to 13.0%.

Intermediate precision was demonstrated by two analysts using two SoloVPE instruments across a total of six analytical runs. Results are summarized in Table 5. The observed RSD ranged from 1% to 10%.

Linearity was demonstrated by regression analysis of measured mRNA content against the theoretical concentration for mRNA content levels 1–5. Results of the linearity assessment are summarized in Table 6 and plotted in Figure 1 and Figure 2. The coefficient of determination was found to be 0.999 for SoloVPE and 0.991 for RiboGreen.

Table 1. SoloVPE Accuracy Results

Conc. Level	Analyst	Instrument	Theoretical mRNA Conc. (mg/mL)	SoloVPE mRNA Conc. (mg/mL)	SoloVPE Avg. Conc. (mg/mL)	%Recovery	Average %Recovery
1	1	1	0.10	0.11	0.11	110%	107%
				0.11		110%	
				0.10		100%	
1	2	2	0.10	0.10	0.11	100%	113%
				0.13		130%	
				0.11		110%	
2	1	1	0.19	0.19	0.19	100%	100%
				0.19		100%	
				0.19		100%	
2	2	2	0.19	0.19	0.19	100%	102%
				0.20		105%	
				0.19		100%	
3	1	1	0.37	0.33	0.33	89%	89%
				0.33		89%	
				0.33		89%	
3	2	2	0.37	0.33	0.34	89%	92%
				0.35		95%	
				0.34		92%	
4	1	1	0.73	0.64	0.64	88%	88%
				0.64		88%	
				0.64		88%	
4	2	2	0.73	0.64	0.65	88%	89%
				0.65		89%	
				0.65		89%	
5	1	1	0.91	0.82	0.82	90%	90%
				0.82		90%	
				0.83		91%	
5	2	2	0.91	0.83	0.83	91%	92%
				0.84		92%	
				0.83		91%	

Table 2. Ribogreen Assay Accuracy Results

Conc. Level	Analyst	Test	Theoretical mRNA Conc. (mg/mL)	Ribogreen Assay mRNA Conc. (mg/mL)	%Recovery
1	1	1	0.10	0.1010	101%
	1	2	0.10	0.1000	100%
2	1	1	0.19	0.1962	103%
	1	2	0.19	0.1885	99%
3	1	1	0.37	0.2658	72%
	1	2	0.37	0.3198	86%
4	1	1	0.73	N/A – Saturation	N/A
	1	2	0.73	0.6217	85%
5	1	1	0.91	N/A – Saturation	N/A
	1	2	0.91	0.6970	77%

Table 3. SoloVPE Repeatability Results

Conc. Level	Analyst	SoloVPE mRNA Conc. (mg/mL)	SoloVPE Average mRNA Conc. (mg/mL)	Standard Deviation (mg/mL)	%RSD
1	1	0.11	0.11	0.006	5.4%
		0.11			
		0.10			
1	2	0.10	0.11	0.015	13.5%
		0.13			
		0.11			
2	1	0.19	0.19	0.000	0.0%
		0.19			
		0.19			
2	2	0.19	0.19	0.006	3.0%
		0.20			
		0.19			
3	1	0.33	0.33	0.000	0.0%
		0.33			
		0.33			
3	2	0.33	0.34	0.010	2.9%
		0.35			
		0.34			
4	1	0.64	0.64	0.000	0.0%
		0.64			
		0.64			
4	2	0.64	0.65	0.006	0.9%
		0.65			
		0.65			
5	1	0.82	0.82	0.006	0.7%
		0.82			
		0.83			
5	2	0.83	0.83	0.006	0.7%
		0.84			
		0.83			

Table 4. Ribogreen Assay Repeatability Results

Conc. Level	Analyst	Test	Ribogreen Assay mRNA Conc. (mg/mL)	Ribogreen Assay Average mRNA Conc. (mg/mL)	Standard Deviation (mg/mL)	%RSD
1	1	1	0.1010	0.1005	0.001	0.7%
1	1	2	0.1000			
2	1	1	0.1962	0.1924	0.005	2.8%
2	1	2	0.1885			
3	1	1	0.2658	0.2928	0.038	13.0%
3	1	2	0.3198			
4	1	1	N/A – Saturation	0.6217	N/A	N/A
4	1	2	0.6217			
5	1	1	N/A – Saturation	0.6970	N/A	N/A
5	1	2	0.6970			

Table 5. SoloVPE Intermediate Precision Results

Level	Analyst	Instrument	Measured mRNA Content (mg/mL)	Average mRNA Content (mg/mL)	Standard Deviation (mg/mL)	RSD (%)
1	1	1	0.11	0.11	0.011	10.0%
			0.11			
			0.10			
	2	2	0.10			
			0.13			
			0.11			
2	1	1	0.19	0.19	0.004	2.1%
			0.19			
			0.19			
	2	2	0.19			
			0.20			
			0.19			
3	1	1	0.33	0.34	0.008	2.5%
			0.33			
			0.33			
	2	2	0.33			
			0.35			
			0.34			
4	1	1	0.64	0.64	0.005	0.8%
			0.64			
			0.64			
	2	2	0.64			
			0.65			
			0.65			
5	1	1	0.82	0.83	0.008	0.9%
			0.82			
			0.83			
	2	2	0.83			
			0.84			
			0.83			

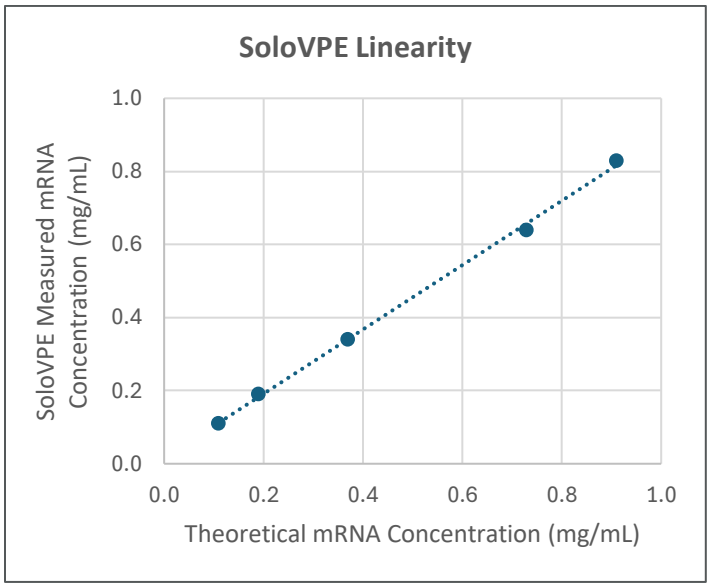

Figure 1. Linear regression analysis of theoretical mRNA content versus SoloVPE measured mRNA content.

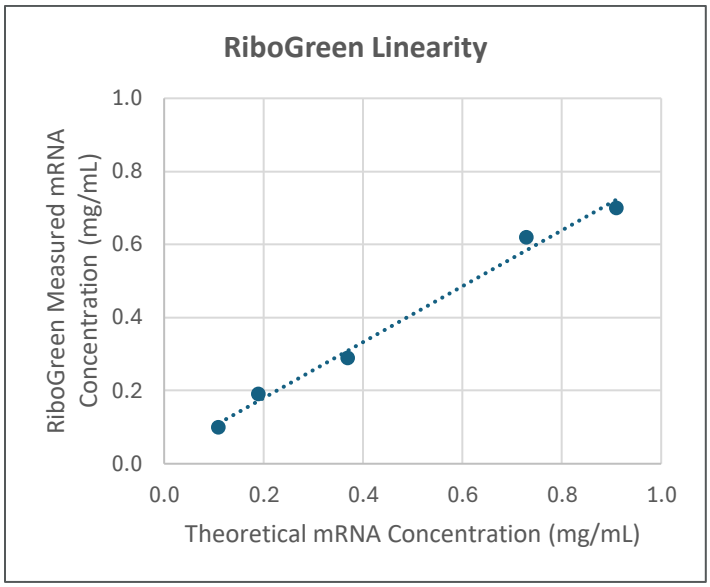

Figure 2. Linear regression analysis of theoretical mRNA content versus Ribogreen measured mRNA content.

Table 6. SoloVPE and RiboGreen Linearity results

Concentration Level	Theoretical mRNA Concentration (mg/mL)	SoloVPE Measured mRNA Concentration (mg/mL)	RiboGreen Assay Measured mRNA Concentration (mg/mL)
1	0.11	0.11	0.10
2	0.19	0.19	0.19
3	0.37	0.34	0.29
4	0.73	0.64	0.62
5	0.91	0.83	0.70
Regression Analysis			
Coefficient of determination (R^2)		0.999	0.991
Slope		0.874	0.755
Y-intercept		0.020	0.034

Conclusion

These results demonstrate that the SoloVPE variable pathlength spectrophotometer is a viable alternative to the RiboGreen assay for total mRNA quantification in an LNP-encapsulated complex. The SoloVPE method yields comparable accuracy, precision, and linearity while reducing overall analysis time, eliminating the need for hazardous chemicals, and minimizing sample handling and manipulation.

To address the limitations of the RiboGreen assay in GMP quality control workflows, the SoloVPE System offers a powerful, complementary approach for total RNA quantification in LNP formulations. Unlike fluorescence-based methods, the SoloVPE System utilizes UV absorbance at 260 nm and dynamically adjusts the optical pathlength to enable direct, dilution-free measurement of total RNA, even in highly concentrated samples. This eliminates the need for a drug substance reference standard, calibration curves, or chemical disruption of the LNPs, significantly reducing assay complexity and turnaround time.

The SoloVPE System is particularly well-suited for in-process testing, QC release, and manufacturing support, where rapid, reproducible results are essential for maintaining process control and meeting production timelines. While it does not differentiate between encapsulated and unencapsulated RNA, the SoloVPE System complements RiboGreen by providing a fast and reliable method for total RNA assessment. When used together, these methods offer a comprehensive analytical strategy, leveraging RiboGreen assays for encapsulation efficiency and SoloVPE measurements for streamlined total RNA quantification, supporting robust, scalable, and compliant production of RNA-based therapeutics.

RiboGreen and SoloVPE Method Comparison

Attribute	RiboGreen	SoloVPE
Detection Principle	Fluorescence (dye binds to free RNA)	UV absorbance at 260 nm
Requires Reference Standard	Yes	No
Sample Preparation	Requires LNP disruption with surfactants (e.g., Triton X-100)	Minimal (no disruption or dilution required)
Suitable for Concentrated Samples	No (signal saturation requires dilution)	Yes
Assesses Encapsulation Efficiency	Yes	No
Calibration Curve Required	Yes (often multiple curves needed)	No
Chemical Disruption Required	Yes	No
Turnaround Time	Moderate to Long	Rapid
GMP QC Suitability	Challenging due to complexity and variability	Highly suitable (simple, reproducible, fast)

References

1. Lowenthal M.S., Antonishek A.S., Phinney K.W. Quantification of mRNA in Lipid Nanoparticles Using Mass Spectrometry. *Analytical Chemistry* 96(3) 2024: 1214–1222; https://pubs.acs.org/doi/10.1021/acs.analchem.3c04406
2. United States Pharmacopeia Draft Guidelines: 3rd Edition "Analytical Procedures for Quality of mRNA Vaccines and Therapeutics".
3. Schultz D., Münter R.D., Cantín A.M., Kempen P.J., Jahnke N., Andresen T.L., Simonsen J.B., Urquhart A.J. Enhancing RNA encapsulation quantification in lipid nanoparticles: Sustainable alternatives to Triton X-100 in the RiboGreen assay, *European Journal of Pharmaceutics and Biopharmaceutics* Vol. 205. 2024: 114571; https://doi.org/10.1016/j.ejpb.2024.114571
4. McNamara L. Regulations in the European Union for the Use of Triton X-100 in the Pharmaceutical Industry. *Insights*, November 4, 2022. https://www.bdo.com/insights/industries/life-sciences/regulations-in-the-european-union-for-the-use-of-triton-x-100-in-the-pharmaceutical-industry
5. Duff R.J.,Gutierrez C., Schneider M., Ramirez J.G. Method Development and Evaluation of the Techniques for Oligonucleotide Concentration Determination. https://www.repligen.com/ctech-resources/pdf/Amgen-Method-Development-and-Evaluation-of-the-Techniques-for-Oligonucleotide-Concentration-Determination.pdf

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