Technical Overview

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CQA Assessment of LNP-Encapsulated IVT mRNA Using Agilent Fragment Analyzer Systems

Introduction

With the development of the first IVT mRNA vaccines for COVID-19, different regulatory agencies have published recommendations and guidelines for Critical Quality Attributes (CQA) of IVT mRNA in vaccines and other biotherapeutic applications. There are specific guidelines both for the initial drug substance (the IVT mRNA), and the drug product (the lipid nanoparticle (LNP) encapsulated-IVT mRNA).

Prior to encapsulation, the IVT mRNA is thoroughly assessed for multiple CQAs. Among the many CQAs tested, Agilent Fragment Analyzer systems are used to analyze the size, integrity, and purity of the IVT mRNA drug product[1,2,3]. The Fragment Analyzer systems are automated parallel capillary electrophoresis instruments used for assessment of nucleic acids. Kits specific for both DNA and RNA analysis allow the systems to be easily integrated into established workflows, such as IVT mRNA biotherapeutics.

Encapsulation of the IVT mRNA into a delivery vehicle such as an LNP is a crucial step in IVT mRNA biotherapeutic workflows, allowing for protection and delivery of the IVT mRNA in vivo. Assessment of the drug product is thus necessary to ensure that the drug substance was not degraded during the encapsulation process. This assessment is complicated because the LNP prohibits access to the IVT mRNA for evaluation. Therefore, to accurately assess the encapsulated IVT mRNA, it must first be released from the LNP. This technical overview provides examples of sizing, purity, integrity, and quantification assessment of both drug substance and drug product using the Fragment Analyzer.

Note: HS RNA Kit specifications for IVT mRNA analysis are based upon commercially available IVT mRNA, not LNP-encapsulated IVT mRNA. LNP formulations are unique, often proprietary, and may require method optimization.

Methods

Multiple samples were used for this study, including proprietary IVT mRNAs (drug substances), the subsequent LNP-encapsulated IVT mRNAs (drug products) gifted from a collaborator (2,200, 9,400, and 11,000 nt), and LNP-encapsulated IVT mRNA encoding for Firefly Luciferase (Fluc) (mRNA-Cap1+LNP [Firefly Luciferase], PackGene Biotech, Inc.).

Each of the drug substances and products were assessed on the Agilent 5300 Fragment Analyzer system. Prior to analysis, the drug products were disrupted with varying amounts of surfactant for 20 minutes at room temperature. This disrupts the LNP and releases the IVT mRNA. Nondisrupted controls were prepared with nuclease-free water. Multiple replicates of each sample were analyzed on the Fragment Analyzer with the Agilent HS RNA kit (p/n DNF-472) and HS IVT RNA Methods A and B according to the quick guide[4]. Samples were also assessed using the Agilent RNA kit (15 nt, p/n DNF-471) with similar results (data not shown).

Results and discussion

LNP disruption for IVT mRNA analysis

The final steps of the vaccine development workflow involve encapsulation of the IVT mRNA into a delivery system, such as an LNP, to form the final drug product. A CQA for the final drug product is assessment of the integrity of the IVT mRNA after encapsulation into the LNP to ensure it was not compromised during the process. For analysis methods such as capillary gel electrophoresis, the LNP must be disrupted to release the IVT mRNA and allow for analysis of the IVT mRNA within the drug product. To demonstrate this, the drug product was analyzed on the Fragment Analyzer before and after disruption of the LNP (Figure 1).

Analysis of the sample before disruption results in a small peak at the expected size, indicating the presence of residual IVT mRNA that was not encapsulated in the LNP. After disruption of the LNP, the peak height of the IVT mRNA increases substantially, indicating that the disruption protocol successfully released the IVT mRNA from the LNP, allowing for accurate and reliable assessment of the IVT mRNA with the Fragment Analyzer.

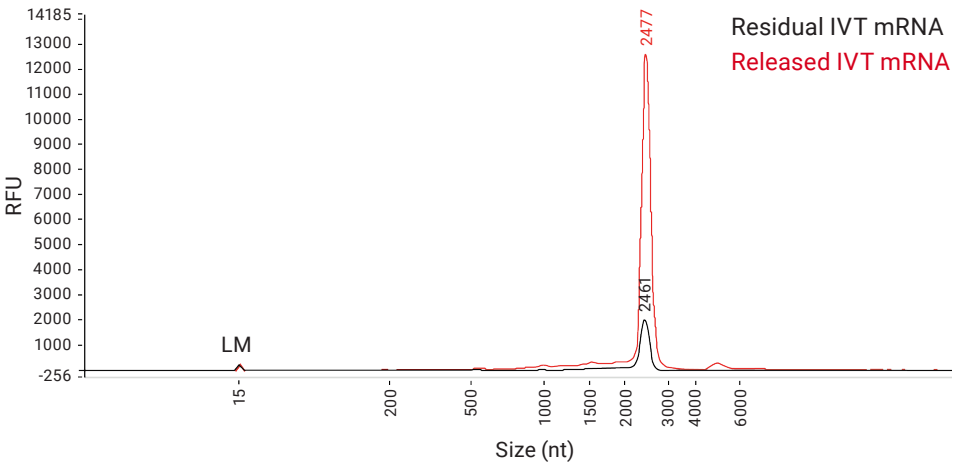


Figure 1. Analysis of an LNP-encapsulated IVT mRNA drug product using the Agilent Fragment Analyzer system, before (black) and after (red) disruption of the LNP.

To further demonstrate the assessment of IVT mRNA drug products on the Fragment Analyzer, the LNP-encapsulated IVT mRNA encoding for Fluc was analyzed before and after disruption with varying amounts of surfactant, from 0.1 to 20%, as shown in Figure 2. In this example, increasing the concentration of surfactant used to disrupt the LNP resulted in an increase in the amount of mRNA released. As more mRNA is released, the height of the peak in the electropherogram increases.

The peak height correlates to the sample concentration and is therefore indicative of the amount of IVT mRNA released from the LNP upon disruption. With this sample, the 20% surfactant displayed the largest peak height of the samples tested, indicating the release of a large amount of IVT mRNA. The peak height of the sample treated with 0.1% surfactant was only slightly larger than the residual IVT mRNA, indicating only a slight disruption of the LNP and release of very little IVT mRNA. Analysis of the surfactant by itself did not produce any signal, demonstrating that the change in peak height was not due to the surfactant alone.

Sizing of the residual and the released IVT mRNAs were consistent with each other, as shown in Figure 3A. On average, the size of the residual IVT mRNA (no surfactant) was 2,370 nt, while the released IVT mRNA was approximately 2,350 nt with each disruption condition from 0.1 to 20% surfactant, only a 1% difference between the residual and released Fluc mRNA. The percent error for sizing was less than 15% for both the residual and released IVT mRNA, demonstrating good sizing accuracy and indicating that LNP encapsulation does not affect the size of the IVT mRNA. Additionally, disruption of the LNP with 0.1 to 20% surfactant did not affect sizing of the released IVT mRNA (Figure 3).

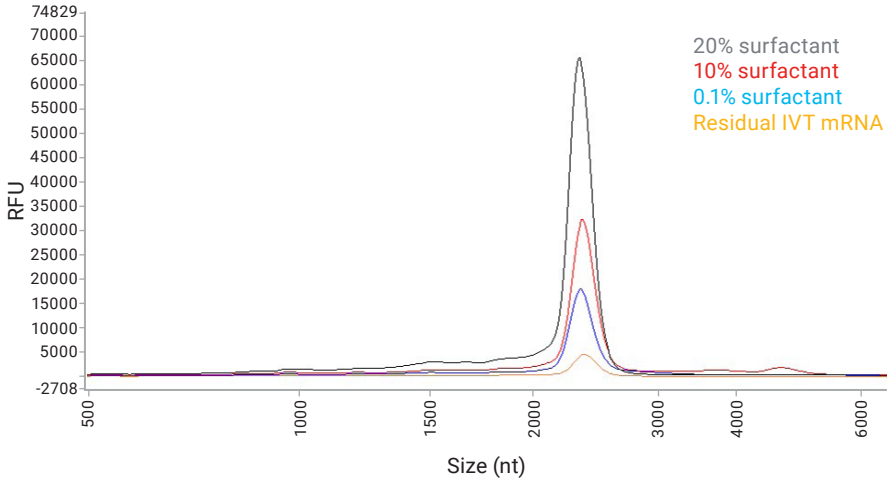


Figure 2. An LNP-encapsulated-IVT mRNA encoding for Fluc was analyzed on the Agilent Fragment Analyzer system using the Agilent HS RNA kit with IVT mRNA Method B after disruption of the LNP with decreasing amounts of surfactant.

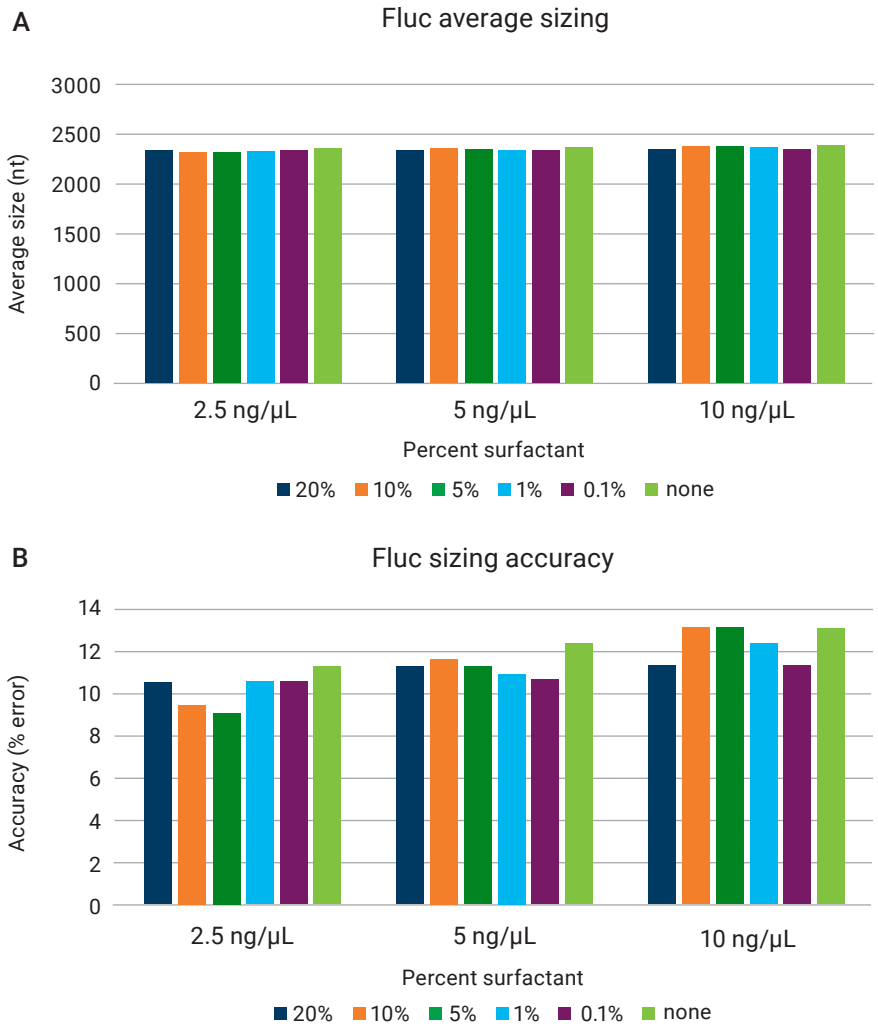


Figure 3. Fluc IVT mRNA drug product was analyzed on the Agilent Fragment Analyzer system using the Agilent HS RNA kit and IVT mRNA Method B, following disruption of the LNP with decreasing percentages of surfactant. The average size of the IVT mRNA is consistent across all disruption conditions. N=2.

Sizing accuracy and precision

The CQA for IVT mRNA integrity is assessed by confirmation of the expected size of the sample. The Fragment Analyzer systems, together with the RNA and HS RNA kits provide excellent sizing accuracy and precision of IVT mRNA[1,2,3]. To demonstrate the capabilities of the system in sizing IVT mRNA, a 2,200 nt drug substance and drug product were assessed on the Fragment Analyzer using the HS RNA kit with IVT mRNA Method B across the quantitative range of the method (2.5 to 10 ng/µL). Disruption of the LNP in the drug product was performed using a 20% surfactant prior to analysis. The average size of the IVT mRNA remained consistent across the concentration range for both the drug substance and the drug product. Each sample displayed an average sizing accuracy below 4% error, and precision of 1% CV or less, well below the assay specifications of 15% error and 5% CV (Figure 4). Samples were also assessed below the quantitative range of the method, at 1 ng/µL, and still showed excellent accuracy and precision of sizing. By confirming the size of the IVT mRNA drug product, the Fragment Analyzer is an ideal tool for CQA analysis of IVT mRNA integrity.

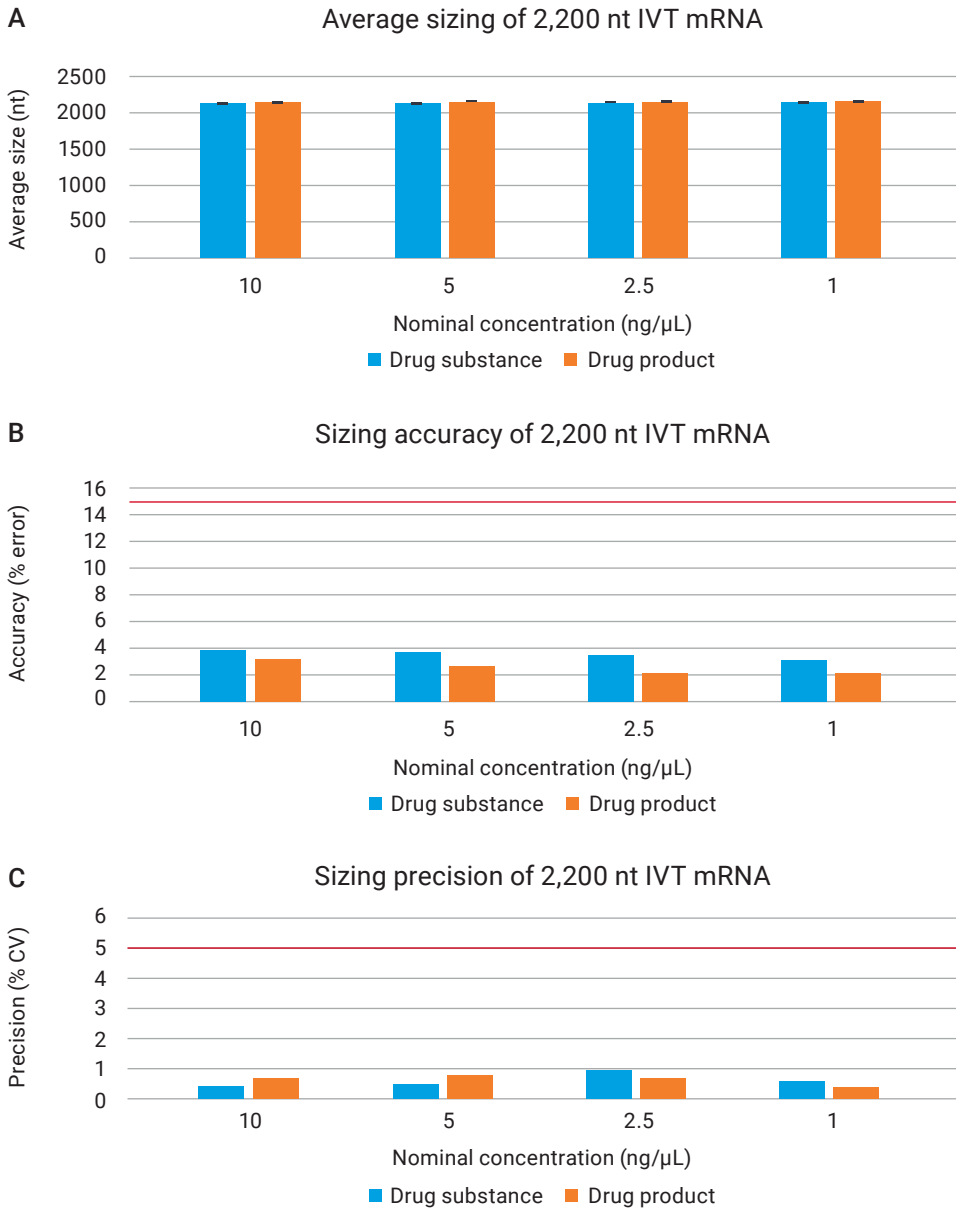
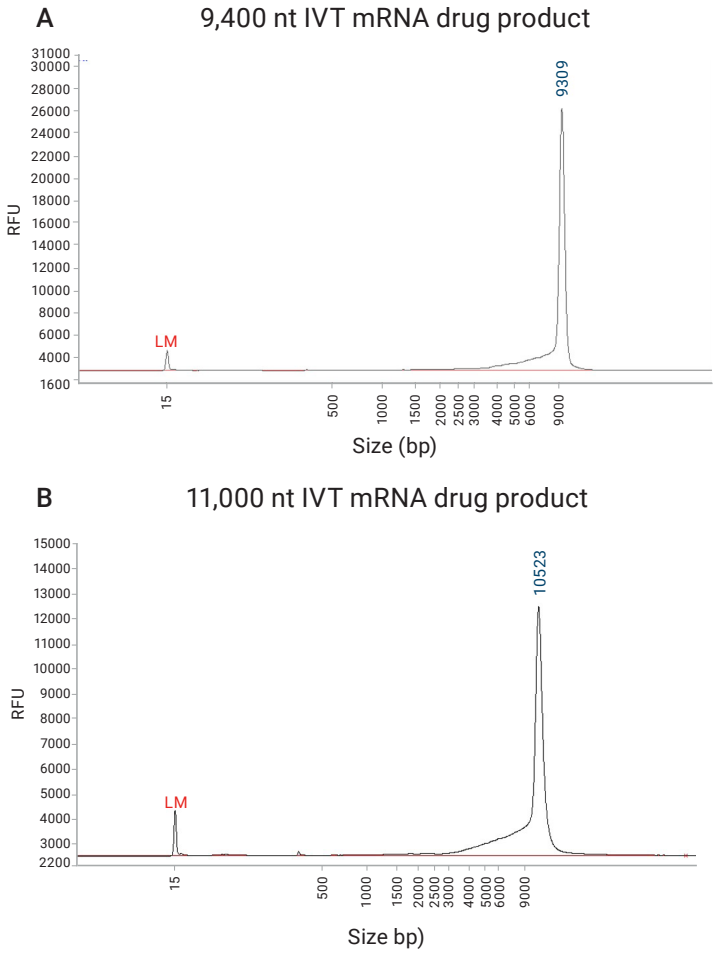


Figure 4. Agilent HS RNA Method B. Average A) size, B) accuracy, and C) precision of 2,200 nt drug substance and drug product following disruption of the LNP. Red lines indicate the kit specifications. N=9.

Sizing with extended Method BE

Extended methods for the HS RNA kit have also been developed, with optimized conditions for sizing of longer IVT RNAs. To demonstrate the capabilities of these methods, a 9,400 and an 11,000 nt drug product were each assessed using the HS RNA kit with the extended Method BE after disruption of the LNP with a 20% surfactant. Surfactant was added to the samples at a 1:1 or a 1:2 ratio to determine the optimal disruption conditions of these larger sized samples. The sizing accuracy of the 9,400 nt sample was less than 3% error with both the 1:1 and 1:2 surfactant ratio, and less than 8% error for the 11,000 sample. This indicates excellent sizing accuracy of the Fragment Analyzer for larger-sized IVT mRNAs. Both the 9,400 and 11,000 nt samples displayed accurate and consistent sizing results across the concentration range of the assay (2.5 to 10 ng/µL) with either disruption method (Figure 5). Additionally, analysis of multiple replicates across the concentration range showed excellent sizing precision, with a %CV of less than 5% across all samples tested. Together, these data indicate that the size of the IVT mRNA remains consistent with LNP encapsulation, demonstrating the reliability of the Fragment Analyzer for CQA analysis of sizing and integrity of IVT mRNA.



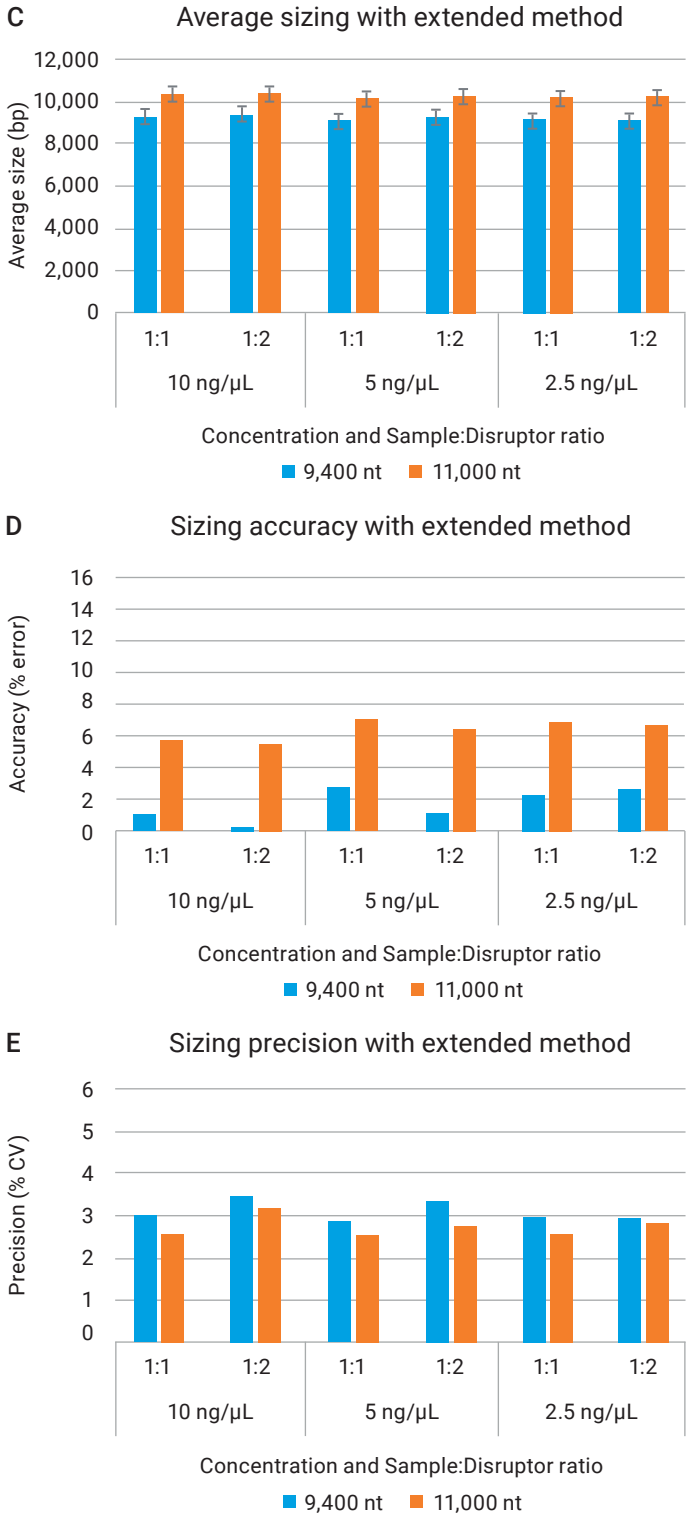


Figure 5. A) 9,400 and B) 11,000 nt IVT mRNA drug products were analyzed on the Agilent Fragment Analyzer systems using the Agilent HS RNA kit and IVT mRNA Method BE. The LNPs were disrupted, at 1:1 or 1:2 ratio of sample: surfactant, and the C) average size, D) sizing accuracy, and E) sizing precision assessed. N=18.

Quantification precision

Quantification of the IVT mRNA drug products on the Fragment Analyzer systems was also assessed. When the IVT mRNA is encapsulated within an LNP, accurate quantification of the samples is difficult. However, the Fragment Analyzer still provides precise and reliable quantification results of the drug product following disruption of the LNP, both within a single run and throughout multiple runs. To demonstrate this, the Fluc IVT mRNA drug product was analyzed in multiple wells and across three subsequent runs following disruption of the LNP. Figure 6 highlights the precision of the quantification values, with less than 6 %CV across all three runs. Assessment of multiple wells within a single run of the released IVT mRNA displayed an excellent precision at 3.3 %CV (N=11). Overall, the Fragment Analyzer provides consistent quantification data for IVT mRNA.

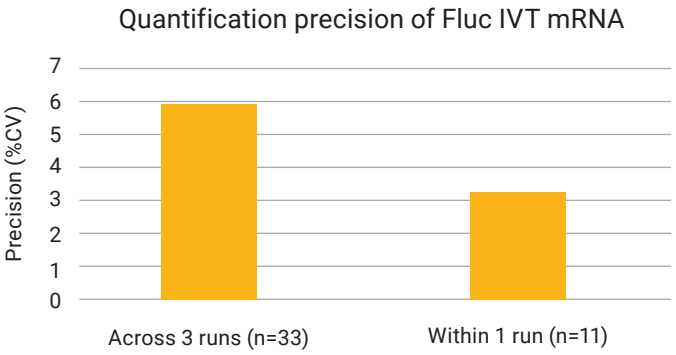


Figure 6. Precision of quantification using the Agilent Fragment Analyzer systems.

Purity and integrity

The Fragment Analyzer aids in QC of IVT mRNA drug substances and drug products by providing an analysis of the purity and integrity of the sample. A user-defined smear analysis is used to identify the area of an IVT mRNA fragment. The smear analysis reports the percent of the sample that lies within the specified area. The percent total is indicative of the purity of the IVT mRNA. In the example shown in Figure 7, a smear analysis was applied to a 2,200 nt drug substance and drug product following disruption of the LNP. The drug substance displayed a purity of approximately 78% at each concentration tested. The disrupted drug product showed a slightly lower percent total of 73% across the different concentrations. Analysis of multiple replicates showed excellent precision of the percent total calculations, with no more than 3 %CV for either sample. The Fragment Analyzer provides consistent and reliable assessment of the percent total, aiding in the CQA of purity and integrity for IVT mRNA.

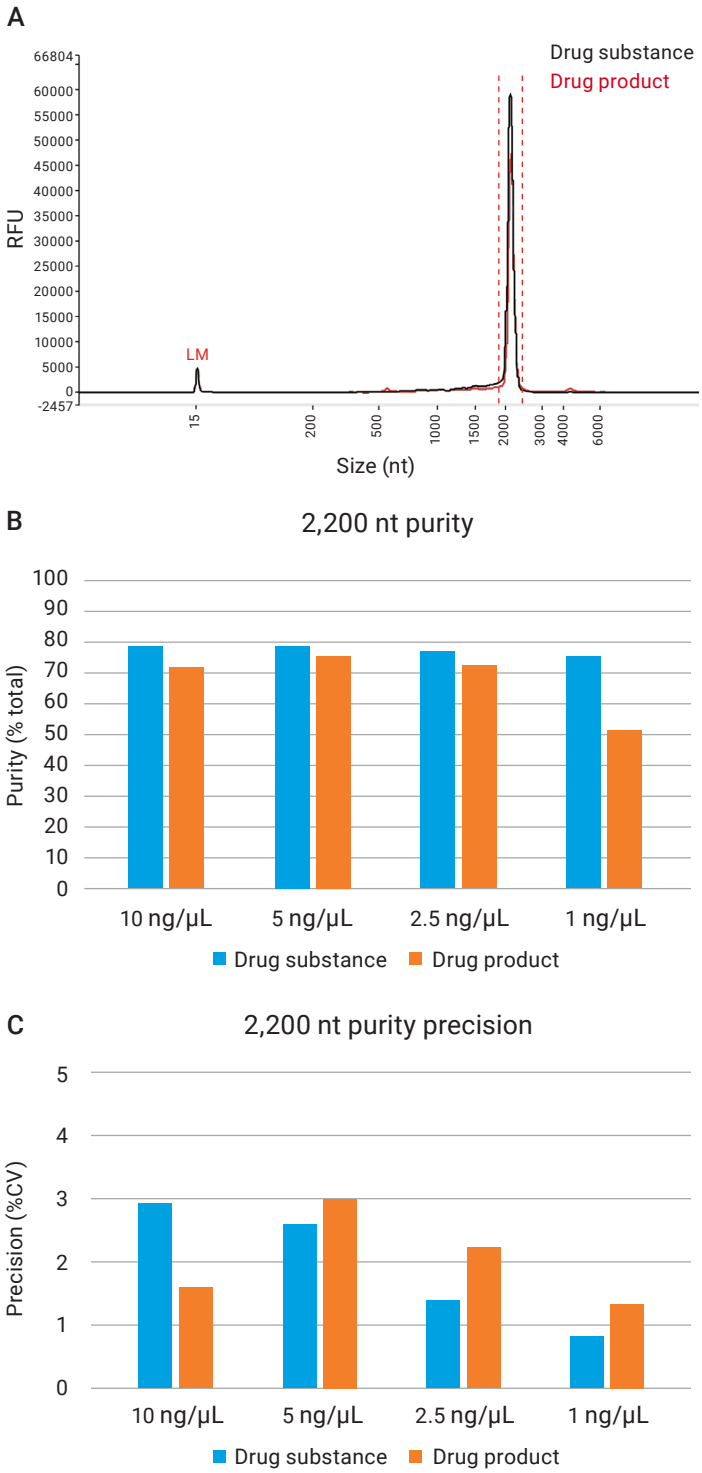


Figure 7. Purity of a 2,200 nt IVT mRNA drug substance and drug product following LNP disruption was assessed on the Agilent Fragment Analyzer system. A) A smear analysis was used to determine the percent total, or purity, of the fragment. B) The average purity and C) precision of the percent total measurements of each sample across multiple concentrations was calculated. N=9.

Conclusion

CQAs for IVT mRNA, such as size, concentration, purity, and integrity, can be addressed by the Agilent Fragment Analyzer system. This technical overview provides examples of this assessment using a variety of IVT mRNA drug substances and drug products. The Fragment Analyzer system offers accurate and reproducible sizing of IVT mRNA samples through 6,000 nt. Extended methods have been optimized for analysis of sample sizes larger than 6,000 nt. Standard and high-sensitivity RNA kits are available to cover broad concentration ranges for IVT mRNA to meet the varying needs of customers. The kits provide excellent precision for percent total measurements, used to assess the CQA of percent purity. In addition, excellent quantification precision of the drug product was provided by the Fragment Analyzer system. The Fragment Analyzer systems provide robust and reliable assessment of several CQAs throughout the IVT mRNA biotherapeutic workflow, for both the drug substance and the drug product.

References

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www.agilent.com/genomics/fragment-analyzer

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