of the process validation master plan, manufacturing processes conducted PPQ activities with CX-024414 mRNA at a 10 L IVT scale, [redacted] and mRNA-1273 LNP at a [redacted] mRNA input scale. The goal is to establish a continuous process validation strategy for the mRNA-1273 manufacturing processes. Phase 3 clinical supplies have been and are being manufactured with representative processes. PPQ activities will be carried out such that the understanding of critical process parameters for mRNA-1273 manufacturing processes, including CX-024414, [redacted], mRNA-1273 LNP and mRNA-1273 Drug Product will be gained.

3.2.P.2.3.7.1 Analytical Assessment for Clinical Trial Material (Phase I through [redacted] Vial Process Performance Qualification)

Given the manufacturing process changes that have occurred for mRNA-1273 Drug Product through the course of development (see manufacturing history discussion in Section 3.2.P.2.3.4), analytical data have been collected and assessed together in order to gain assurance that the process continues to be robust and consistently produce high-quality material. Release, stability, extended characterization, and impurity characterization data sets have been evaluated and were found to demonstrate that quality attributes of the material are highly similar. Lot release and stability data generated and compared across processes are discussed in Section 3.2.P.2.3.7.6, whereas this subsection focuses on the extended characterization, and impurity characterization. Forced degradation data will be included when available. These data were generated with representative lots from different manufacturing processes:

- Phase 1/2 clinical lots 8520100101, 8520100102, 8520100103, and 8520100104 [redacted]
- Phase 3 clinical lots 6007520001, 6007520002, and 6007520003 (0.20 mg/mL, 5.0 mL fill volume, Ompi 10R vial, [redacted])
- PPQ lots 6007520004, 6007520005, and 6007520006 (0.20 mg/mL, 5.0 mL fill volume, Ompi 10R vial, [redacted])

Samples were analyzed in a side-by-side format whenever possible and were evaluated for mRNA-1273 Drug Product physico-chemical properties, particle size, and impurities. A study of the Phase 1/2 and Phase 3 lots was initially executed to evaluate product similarity irrespective of process and scale changes. PPQ lots were later executed to evaluate process consistency at the [redacted] vial (0.20 mg/mL mRNA-1273) scale. In addition to assessing the various clinical lots against product release criteria, the lots were also examined by a set of extended characterization assays listed in Table 30. The table also provides a summary of the attributes assessed by the characterization assays. There are no pre-defined acceptance criteria for these assays, but the results show that the lots analyzed are similar irrespective of the manufacturing process and scale. The data generated will be used to inform and establish appropriate acceptance criteria for future process consistency and product comparability studies.

In its entirety, the evaluation of analytical data across release, stability, and characterization of mRNA-1273 Drug Product GMP and PPQ lots demonstrate a high degree of product quality similarity for the manufacturing process and scale changes that occurred throughout process development. The 3 PPQ lots are also highly similar and demonstrate process consistency at the 3,000 vial (0.20 mg/mL mRNA-1273) scale, as summarized in Section 3.2.P.2.3.7.3.

Table 30: Attribute Assessment

A. Testing panel for exploratory characterization of mRNA-1273 Drug Product from PPQ Lots 6007520004, 6007520005, and 6007520006

Product Attribute	Method	Document #	Description
LNP size distribution	Nanoparticle tracking analysis	DPTM-0039	High-resolution LNP size distribution
LNP size distribution	Asymmetric flow field-flow fractionation	DPTM-0103	Fractionation coupled with in-line MALS detection for size determination
Sub-visible particle counts	Coulter counter	DPTM-0035	Measurement of sub-visible particle counts in the [redacted] range
Sub-visible particle counts and morphology	Flow microscopy	DPTM-0115	Measurement of sub-visible particle counts and morphology in the [redacted] range
LNP surface characterization	Isothermal titration calorimetry	DPTM-0119	[redacted] to LNP surface
LNP charge	Zeta potential	DPTM-0118	Average LNP charge [redacted]
LNP charge distribution	Capillary isoelectric focusing	DPTM-0068	LNP pI distribution and polydispersity
LNP structure	Dye permeation kinetics	DPTM-0127	Qualitative characterization of LNP surface and encapsulation state based on thionine permeation kinetics
LNP density	Density gradient ultracentrifugation	DPTM-0104	Qualitative assessment of LNP density heterogeneity
LNP surface characterization	Hydrophobic interaction chromatography	DPTM-0096	Qualitative characterization of LNP surface hydrophobicity
LNP surface characterization	Esterase kinetics	DPTM-0130	Qualitative method for [redacted] distribution
mRNA encapsulation	RiboGreen fluorescence [a]	SOP-0298	Fluorescence based method for mRNA encapsulation

B. Testing panel for characterization of mRNA-1273 DP from Phase 1/2 and Phase 3 Lots 8520100101, 8520100102, 8520100103, 8520100104, 6007520001, 6007520002, and 6007520003

Product Attribute	Method	Document #	Description
mRNA encapsulation	[redacted]	DPTM-0073	[redacted]
LNP size distribution	Nanoparticle tracking analysis	DPTM-0039	High-resolution LNP size distribution
LNP size distribution	Asymmetric flow field flow fractionation	DPTM-0103	Fractionation coupled with in-line MALS detection for size determination
Sub-visible particles	Coulter counter	DPTM-0035	Sub-visible particle counts [redacted]
SVP counts and morphology	Flow microscopy	DPTM-0115	Measurement of SVP counts and morphology in the [redacted] range
LNP surface characterization	Isothermal titration calorimetry	DPTM-0119	[redacted] to LNP surface
LNP charge	Zeta potential	DPTM-0118	Average LNP charge [redacted]
LNP charge distribution	Capillary isoelectric focusing	DPTM-0068	LNP pI distribution

Abbreviations: LNP = lipid nanoparticle; MALS = multi-angle light scattering; pI = isoelectric point(s); [redacted]
PPQ = process performance qualification

a) The RiboGreen method for RNA encapsulation measurement has been used as a release method to support early development and initial PPQ lots of the mRNA-1273 vaccine. [redacted]

3.2.P.2.3.7.1.1 Nanoparticle Tracking Analysis

Nanoparticle tracking analysis is a technique designed to measure sub-micron particle distributions in liquid solutions. This analysis characterizes nanoparticles from 10 – 1000 nm in solution. Each particle is individually and simultaneously analyzed by direct observation and measurement of diffusion events. This particle-by-particle methodology produces high-resolution results for nanoparticle size distribution and concentration. Size distribution plots for each mRNA-1273 Drug Product lot are shown in Figure 5. The mode of the size distributions ranged from [redacted] (Table 31). The breadth of the size distributions, as estimated by full width at half maximum (span), ranged from [redacted] with an LNP size range broadly covering [redacted]. These results show similar size distributions across lots and processes.

Figure 5: Nanoparticle Tracking Analysis Size Distribution

[redacted]

Table 31: Nanoparticle Tracking Analysis Results

Lot	[redacted]
85201100101	
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

3.2.P.2.3.7.1.2 Asymmetric Flow Field-Flow Fractionation Analysis

Asymmetric flow field-flow fractionation (aF4) with multi-angle light scattering was employed as an orthogonal measurement of the mRNA-1273 size distributions. aF4 is a one-phase separation that uses a perpendicular flow against a membrane (cross-flow) in conjunction with a channel flow parallel to the membrane to fractionate samples based on their diffusion behavior. The channel flow gives a parabolic profile and the perpendicular flow drives macromolecules toward the boundary layer of the membrane. Diffusion related to Brownian motion moves smaller particles with higher diffusion rates in the channel where longitudinal flow is faster, eluting smaller particles more quickly. Multi-angle light scattering detection enables the particle radius of gyration, which is related to particle mass, to be determined for peaks in the aF4 separation. Results for each mRNA-1273 Drug Product lot are presented in Table 32. The radius of gyration ranged from [redacted] with polydispersity ranging from [redacted]. All lots demonstrated similar size distribution and polydispersity across processes and scales.

Table 32: Asymmetric Flow Field-Flow Fractionation Results

Lot	[redacted]
85201100101	
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

Abbreviations: Mn = number average molecular weight; Mw = weight average molecular weight; R_g = radius of gyration

3.2.P.2.3.7.1.3 Coulter Counter

Coulter counter analysis was used to assess sub-visible particle (SVP) content in the range of [redacted]. This technique utilizes electro-zone sensing to determine the size and concentration of particles in the stated size range. Sample in an electrolyte is drawn through an aperture connecting 2 fluid chambers. Particles passing through the aperture are detected by a change in impedance. Since impedance is proportional to the volume of the particle, the size of each particle can be calculated. SVP counts for each mRNA-1273 Drug Product lot are shown in Table 33. SVP counts ranged from [redacted] particles/mg for all lots, demonstrating consistency across processes.

Table 33: Coulter Counter Results

Lot	[redacted]
85201100101	
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

Abbreviations: RSD = relative standard deviation; SVP = sub-visible particle

3.2.P.2.3.7.1.4 Flow Microscopy

Flow microscopy is a characterization technique based on the [redacted] and has been developed as an orthogonal method for the assessment of concentration and morphology of SVPs. This technique takes images of the magnified particles in LNP samples and characterizes and counts the sizes of particles observed. A population of micron-sized particles is anticipated when forming nanoparticles using solvent-drop precipitation. Characterization of the level of SVPs, from approximately [redacted], provides confirmation that the nanoprecipitation reaction used to form mRNA-1273 is well-controlled. The size distribution results of each lot are presented in Table 34. A consistent and expected level of micron-size particles in the expected range for mRNA-1273 ([redacted]) were observed. The SVP counts [redacted] were low for each lot.

Table 34: Flow Microscopy Particle Size Distribution Results

Lot	[redacted]
	(Particles/mg)
85201100101	[redacted]
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

3.2.P.2.3.7.1.5 Isothermal Titration Calorimetry

Isothermal titration calorimetry is a calorimetric technique for thermodynamic analysis of binding reactions. When binding occurs, heat is either absorbed or released and this is measured by a sensitive calorimeter during gradual titration of the ligand into the sample cell containing the species of interest. This methodology is used to measure the interaction between [redacted]. The binding affinities, described by the dissociation constant (Kd), and the binding stoichiometries [redacted] are reported for each lot in Table 35. The observed binding affinities and binding stoichiometries were similar across lots.

Table 35: Isothermal Calorimetry Results

Lot	[redacted]
85201100101	
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

Abbreviations: Kd = dissociation constant; M = binding stoichiometry (number of [redacted])

3.2.P.2.3.7.1.6 Zeta Potential

Zeta potential is an indirect measurement of LNP surface charge calculated from the electrophoretic mobility of the particle. The zeta potential of mRNA-1273 was measured using a [redacted] instrument equipped with [redacted]. Zeta potential results shown in Table 36 demonstrate similar surface charge and all mRNA-1273 Drug Product lots are considered approximately neutral.

Table 36: Zeta Potential Results

Lot	[redacted]
85201100101	
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

3.2.P.2.3.7.1.7 Capillary Isoelectric Focusing

Imaged capillary isoelectric focusing was used to determine the apparent isoelectric point (pI) of [redacted], which is a direct measure of its surface charge. A mixture of [redacted] and ampholytes is introduced to a capillary and a voltage is applied across the capillary. A linear pH gradient is formed and LNPs migrate based on their pI, where their net charge is zero. [redacted] The charge profiles, presented in Figure 6, are generally consistent across all lots. The majority of the LNPs migrate with a pI between [redacted]. The average pI and polydispersities were consistently observed as shown in Table 37.

Figure 6: Imaged Capillary Isoelectric Focusing Electropherograms

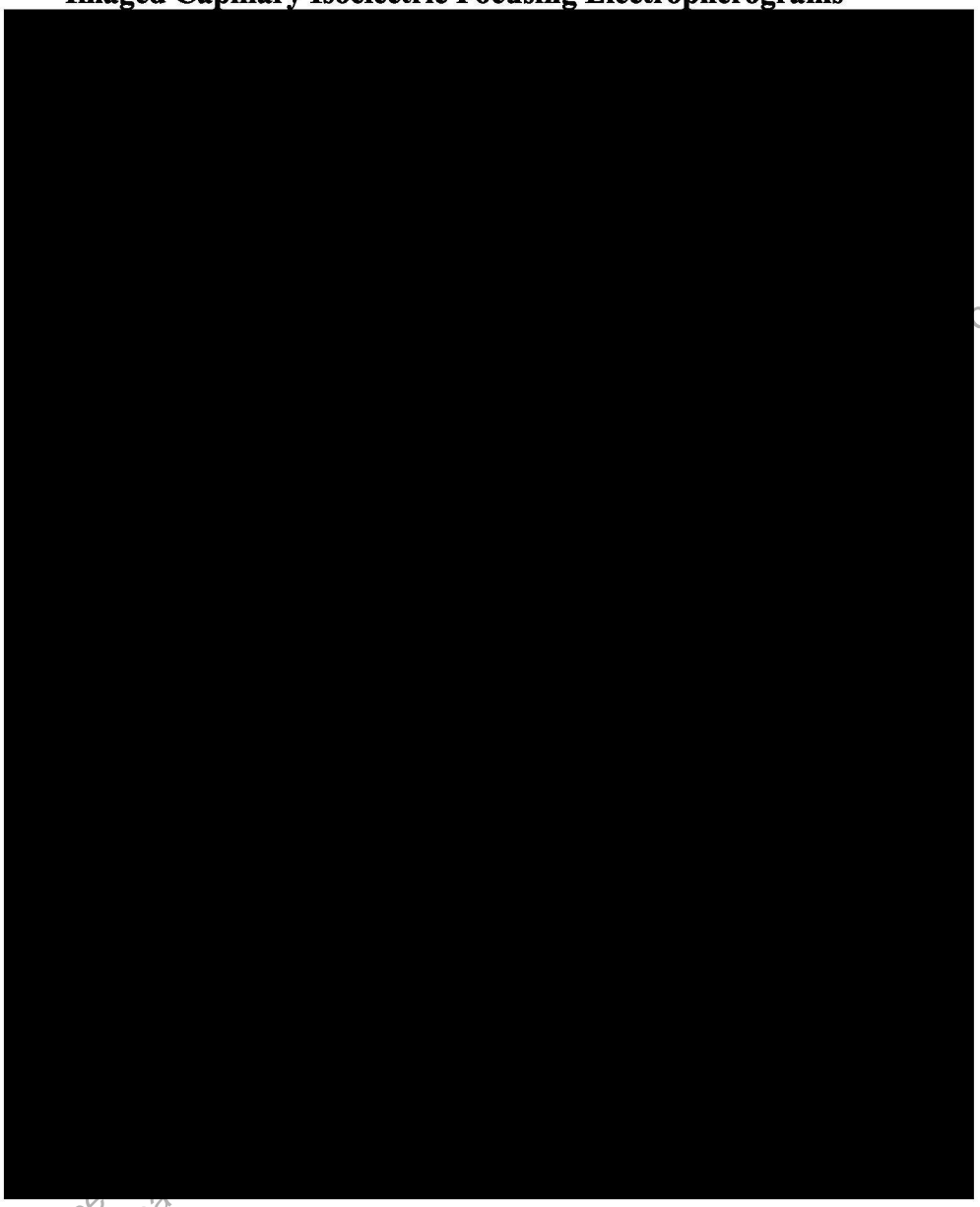


Table 37: Capillary Isoelectric Focusing Results

Lot	[redacted]
85201100101	
85201100102	
85201100103	
85201100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	

Abbreviations: pI = isoelectric point

3.2.P.2.3.7.1.8 Dye Permeation Kinetics

Dye permeation assay was used to measure the rate at which the cationic phenothiazinium dye, thionine, permeates the LNP from PPQ batches. This is an optical spectroscopic method that monitors with time the change in visible absorption that occurs due to thionine binding to encapsulated mRNA. The kinetic profile of this assay represents a biophysical signature that reports on LNP surface physical properties and mRNA encapsulation state, reported as a first order rate constant. Results shown for each PPQ lot in Table 38 demonstrate similar permeation kinetics.

Table 38: Dye Permeation Kinetic Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	

Abbreviation: CV = coefficient of variation

3.2.P.2.3.7.1.9 Density Gradient Ultracentrifugation

Separation using [redacted] works on the principle of isopycnic separation: an LNP particle of a density will sink during centrifugation until a position is reached where the density of the surrounding solution is exactly the same as the density of the particle. [redacted] was used as density gradient medium, consisting of [redacted]. Density gradient ultracentrifugation was used to characterize the density distribution of mRNA-1273 Drug Product PPQ batches. A sample tube was placed in an [redacted] box, to which a [redacted] was attached. The resulting images (Figure 7) were converted into chromatograms based on pixel intensity and the slope change was monitored. The slope change results shown in Table 39 demonstrate consistent density profiles for these three PPQ batches.

Figure 7: Density Gradient Chromatograms

Table 39: Density Gradient Ultracentrifugation Results

Lot	
6007520004	
6007520005	
6007520006	

3.2.P.2.3.7.1.10 Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC) was used to characterize mRNA-1273 Drug Product PPQ batches. It uses the retention mechanism of "salting out" biologics onto a hydrophobic stationary phase. Salt is then removed, allowing for the elution of the biologic based on its hydrophobicity. LNP samples were loaded onto a column at a flow rate of with UV absorbance monitored at 260 nm. Elution was obtained by . Three chromatographic regions are reported as relative percent areas: . HIC results generated for these three mRNA-1273 Drug Product PPQ batches are reported in Table 40. The relative percent peak areas for each chromatographic region were consistent among these PPQ lots which demonstrate similar hydrophobic profiles.

Table 40: Hydrophobic Interaction Chromatography Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	

3.2.P.2.3.7.1.11 Esterase Kinetics

Esterase kinetics was used to determine [redacted] in mRNA-1273 Drug Product PPQ batches. [redacted] across the time course for each PPQ lot is presented in Figure 8 and Table 41. Results demonstrate consistent degradation behavior across lots.

Figure 8: [redacted]

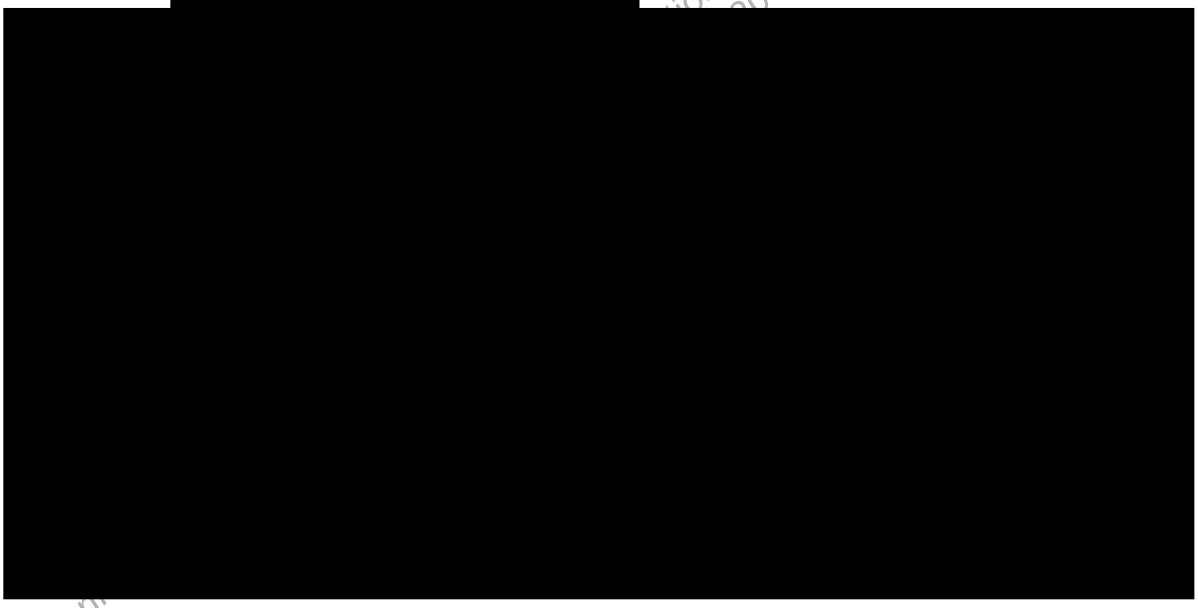


Table 41: Esterase Kinetics Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	

3.2.P.2.3.7.1.12 [redacted]

[redacted] Results for each lot are presented in Table 42. These results demonstrate high mRNA encapsulation [redacted] in all the mRNA-1273 Drug Product batches.

Table 42: [redacted] Encapsulation Results

Lot	Encapsulation (%)	CV (%)
85201100101	[redacted]	
85201100102		
85201100103		
85201100104		
6007520001		
6007520002		
6007520003		

Abbreviations: CV = coefficient of variation

3.2.P.2.3.7.1.13 RiboGreen Encapsulation

A fluorescence-based RiboGreen assay was used as a release assay to determine the encapsulation efficiency of mRNA in mRNA-1273 LNP. RiboGreen is a sensitive RNA quantitation reagent with a wide linear dynamic range. RiboGreen can bind to free RNA in solution but is not able to bind to mRNA encapsulated in an LNP. Upon binding to RNA, the Ribogreen dye undergoes fluorescent enhancement and an increase in quantum yield, which results in an emitted quantifiable fluorescent signal that is linearly dependent on available RNA. Concentration determinations of the free mRNA and total mRNA content are measured for each sample to calculate encapsulation efficiency. RNA encapsulation results for the PPQ lots are shown in Table 43. These results agree with the [redacted] and demonstrate similarly high and consistent mRNA encapsulation [redacted] for the PPQ lots. The Sponsor is intended to validate [redacted] as a release assay due to the assay robustness, simplicity, and the usage of a USP-certified assay reagent. The Sponsor will continue to determine encapsulation efficiency of mRNA in mRNA-1273 LNP by RiboGreen assay as an orthogonal characterization assay.

Table 43: RiboGreen RNA Encapsulation Results

Lot	Encapsulation (%)
6007520004	[redacted]
6007520005	
6007520006	

3.2.P.2.3.7.2 Analytical Assessment Across Process Performance Qualifications ([redacted] Vial and [redacted] Vial)

Given the manufacturing site and scale changes that have occurred for mRNA-1273 Drug Product (DP) through the course of development (see manufacturing history discussion in Section 3.2.P.2.3.4), analytical data have been collected and assessed together in order to gain assurance that the process continues to be robust and consistently produce high-quality material. Release, stability, extended characterization, and impurity characterization data sets have been evaluated and were found to demonstrate that quality attributes of the material are highly similar. Lot release and stability data generated and compared across processes are discussed in Section 3.2.P.2.3.7.6, whereas this subsection focuses on the extended characterization, and impurity characterization. Forced degradation data will be included when available. These data were generated with representative lots from different manufacturing processes:

- ModernaTX, Inc. (Norwood, MA) PPQ lots 6007520004, 6007520005, and 6007520006 (0.20 mg/mL, 5.0 mL fill volume, Ompi 10R vial, [redacted] vials)
- Catalent Biologics, LLC (Bloomington, IN) PPQ lots 057G20 (Moderna Lot 6007320001), 062G20 (Moderna Lot 6007320002), 001H20 (Moderna Lot 6007320003) (0.20 mg/mL, 6.3 mL fill volume, Ompi 10R vial, [redacted] vials)

Samples were analyzed and evaluated for mRNA-1273 Drug Product physico-chemical properties, particle size, and impurities. Initial PPQ lots were executed to evaluate process consistency at the [redacted] vial (0.20 mg/mL mRNA-1273) scale. Subsequent PPQ lots were executed at the commercial manufacturing facility (Catalent Biologics, LLC, Bloomington, IN) at a [redacted] vial (0.20 mg/mL mRNA-1273) scale. In addition to assessing all PPQ lots against product release criteria, the lots were also examined by a set of extended characterization assays listed in Table 44. The table also provides a summary of the attributes assessed by the characterization assays. There were no pre-defined acceptance criteria for the characterization assays, but the results show that the lots analyzed are similar irrespective of the manufacturing site and scale.

In its entirety, the evaluation of analytical data across release, stability, and characterization of mRNA-1273 Injection DP PPQ lots demonstrate a high degree of product quality similarity for the manufacturing site and scale changes that occurred. The PPQ lots are also highly similar and demonstrate process consistency at the [redacted] vial (0.20 mg/mL mRNA-1273) and [redacted] vial (0.20 mg/mL mRNA-1273) scales, as summarized in Section 3.2.P.2.3.7.3.

Table 44: Attribute Assessment for PPQ Lots

Product Attribute	Method	Document #	Description
LNP size distribution	Nanoparticle tracking analysis	DPTM-0039	High-resolution LNP size distribution
LNP size distribution	Asymmetric flow field-flow fractionation	DPTM-0103	Fractionation coupled with in-line MALS detection for size determination
Sub-visible particle counts	Coulter counter	DPTM-0035	Measurement of sub-visible particle counts in the [redacted] range
Sub-visible particle counts and morphology	Flow microscopy	DPTM-0115	Measurement of sub-visible particle counts and morphology in the [redacted] range
LNP surface characterization	Isothermal titration calorimetry	DPTM-0119	[redacted] to LNP surface
LNP charge	Zeta potential	DPTM-0118	Average LNP charge [redacted]
LNP charge distribution	Capillary isoelectric focusing	DPTM-0068	LNP pI distribution and polydispersity
LNP structure	Dye permeation kinetics	DPTM-0127	Qualitative characterization of LNP surface and encapsulation state based on thionine permeation kinetics
LNP density	Density gradient ultracentrifugation	DPTM-0104	Qualitative assessment of LNP density heterogeneity
LNP surface characterization	Hydrophobic interaction chromatography	DPTM-0096	Qualitative characterization of LNP surface hydrophobicity
LNP surface characterization	Esterase kinetics	DPTM-0130	Qualitative method for [redacted] distribution
mRNA encapsulation	RiboGreen fluorescence [a]	SOP-0298	Fluorescence based method for mRNA encapsulation

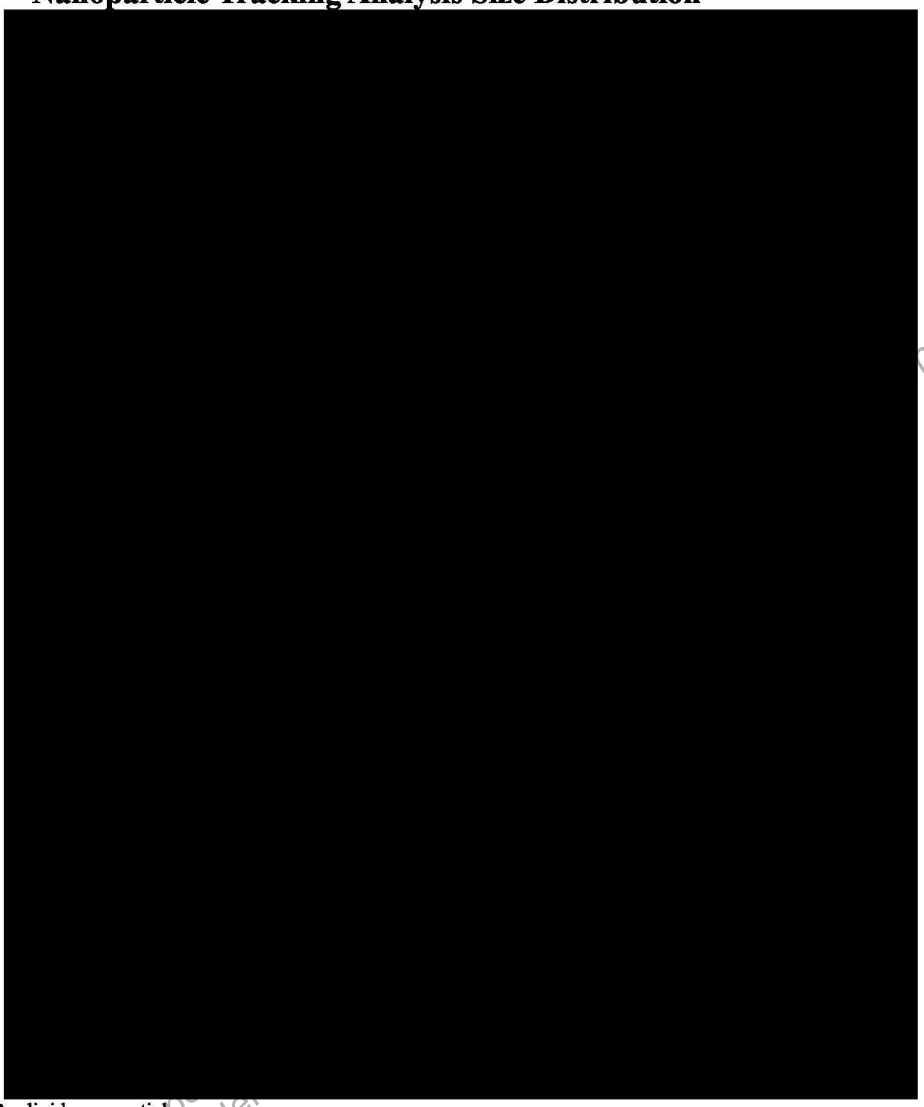
Abbreviations: LNP = lipid nanoparticle; MALS = multi-angle light scattering; pI = isoelectric point(s); [redacted]; PPQ = process performance qualification

a) The RiboGreen method for RNA encapsulation measurement had been used as a release method to support early development and initial PPQ lots of the mRNA-1273 vaccine. [redacted]

3.2.P.2.3.7.2.1 Nanoparticle Tracking Analysis

Nanoparticle tracking analysis is a technique designed to measure sub-micron particle distributions in liquid solutions. This analysis characterizes nanoparticles from 10 – 1000 nm in solution. Size distribution plots for each mRNA-1273 Drug Product PPQ lot are shown in Figure 9 . The mode of the size distributions ranged from [redacted] (Table 45). The breadth of the size distributions, as estimated by full width at half maximum, ranged from [redacted] nm with an LNP size range broadly covering [redacted]. These results show similar size distributions across lots and processes.

Figure 9: Nanoparticle Tracking Analysis Size Distribution



Abbreviation: LNP = lipid nanoparticle

Table 45: Nanoparticle Tracking Analysis Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.2 Asymmetric Flow Field-Flow Fractionation Analysis

Asymmetric flow field-flow fractionation (aF4) with multi-angle light scattering was employed as an orthogonal measurement of the mRNA-1273 size distributions. Results for each mRNA-1273 Drug Product PPQ lot are presented in Table 46. The radius of gyration ranged from [redacted] with polydispersity ranging from [redacted]. All lots demonstrated similar size distribution and polydispersity across processes and scales.

Table 46: Asymmetric Flow Field-Flow Fractionation Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

Abbreviations: Mn = number average molecular weight; Mw = weight average molecular weight; R_g or R_z = radius of gyration,

3.2.P.2.3.7.2.3 Coulter Counter

Coulter counter analysis was used to assess sub-visible particle (SVP) content in the range of [redacted]. Sub-visible particle (SVP) counts for each mRNA-1273 Drug Product lot are shown in Table 47. SVP counts ranged from [redacted] for all lots, demonstrating consistency across processes.

Table 47: Coulter Counter Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

Abbreviations: RSD = relative standard deviation; SVP = sub-visible particle

3.2.P.2.3.7.2.4 Flow Microscopy

Flow microscopy is technique that takes images of the magnified particles in LNP samples and characterizes and counts the sizes of particles observed. The size distribution results of each PPQ lot are presented in Table 48. A consistent and expected level of micron-size particles in the expected range for mRNA-1273 ([redacted]) were observed. The SVP counts [redacted] were low for each lot.

Table 48: Flow Microscopy Particle Size Distribution Results

Lot	[redacted]
	(Particles/mg)
6007520004	[redacted]
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.5 Isothermal Titration Calorimetry

Isothermal titration calorimetry is a calorimetric technique used to measure the interaction between [redacted] with the mRNA-1273 LNP. The binding affinities, described by the dissociation constant (Kd), and the binding stoichiometries (number of [redacted]) are reported for each lot in Table 49. The observed binding affinities and binding stoichiometries were similar across lots.

Table 49: Isothermal Calorimetry Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

Abbreviations: Kd = dissociation constant; M = binding stoichiometry (number of [redacted])

3.2.P.2.3.7.2.6 Zeta Potential

Zeta potential is an indirect measurement of LNP surface charge calculated from the electrophoretic mobility of the particle. Zeta potential results shown in Table 50 demonstrate similar surface charge and all PPQ lots are considered approximately neutral.

Table 50: Zeta Potential Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.7 Capillary Isoelectric Focusing

Imaged capillary isoelectric focusing was used to determine the apparent isoelectric point (pI) of [redacted]. The charge profiles, presented in Figure 10, are generally consistent across all lots. The majority of the LNPs migrate with a pI between [redacted]. The average pI and polydispersities were consistently observed as shown in Table 51.

Figure 10: Imaged Capillary Isoelectric Focusing Electropherograms

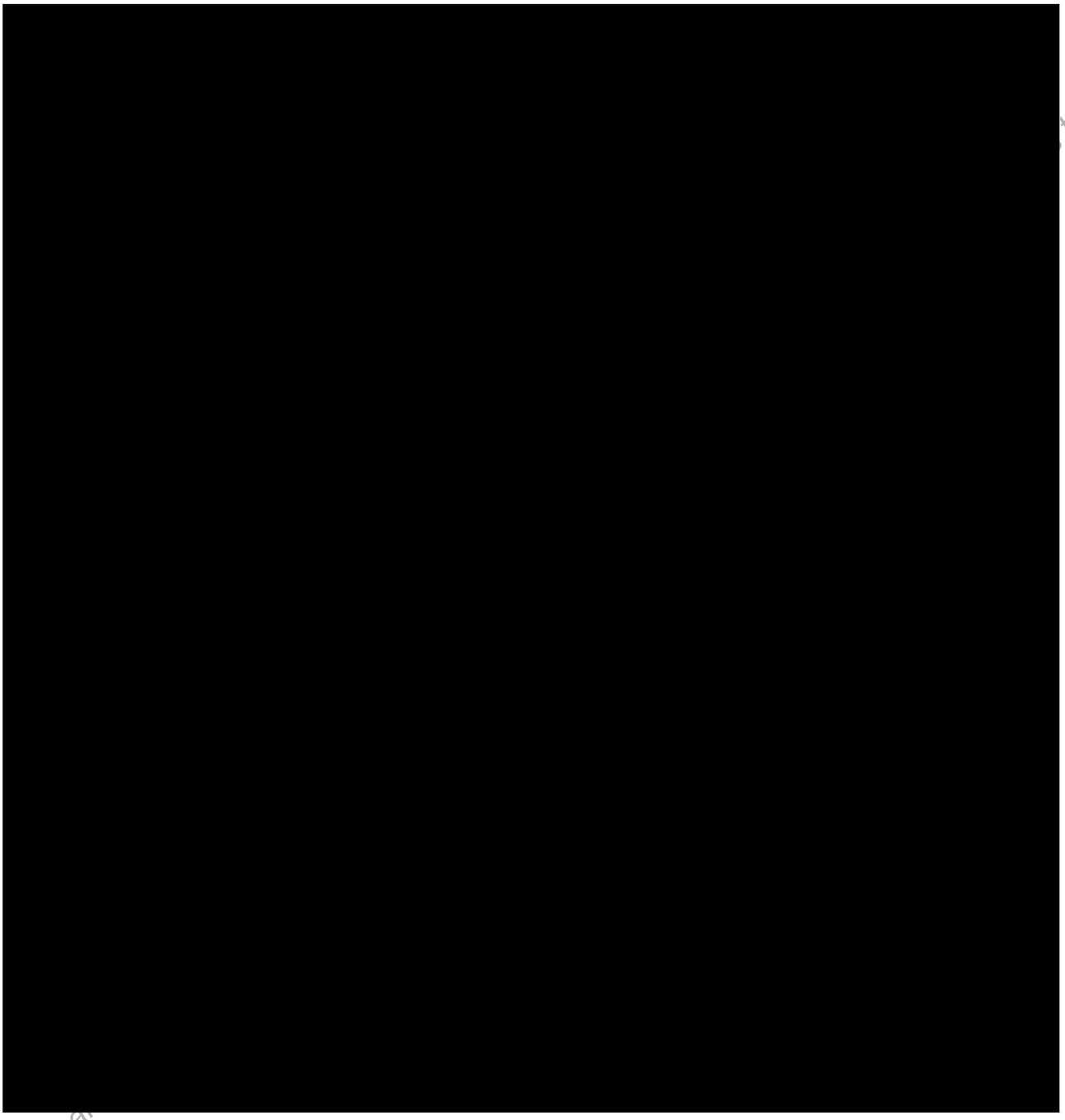


Table 51: Capillary Isoelectric Focusing Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

Abbreviations: pI = isoelectric point

3.2.P.2.3.7.2.8 Dye Permeation Kinetics

Dye permeation assay was used to measure the rate at which the cationic phenothiazinium dye, thionine, permeates the LNP from PPQ batches. The kinetic profile of this assay represents a biophysical signature that reports on LNP surface physical properties and mRNA encapsulation state, reported as a first order rate constant. Results shown for each PPQ lot in Table 52 demonstrate similar permeation kinetics.

Table 52: Dye Permeation Kinetic Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.9 Density Gradient Ultracentrifugation

Density gradient ultracentrifugation was used to characterize the density distribution of mRNA-1273 Drug Product PPQ batches. The density gradient ultracentrifugation images (Figure 11) were converted into chromatograms based on pixel intensity and the slope change was monitored. The slope change results shown in Table 53 demonstrate consistent density profiles for each of the six PPQ batches.

Figure 11: Density Gradient Chromatograms

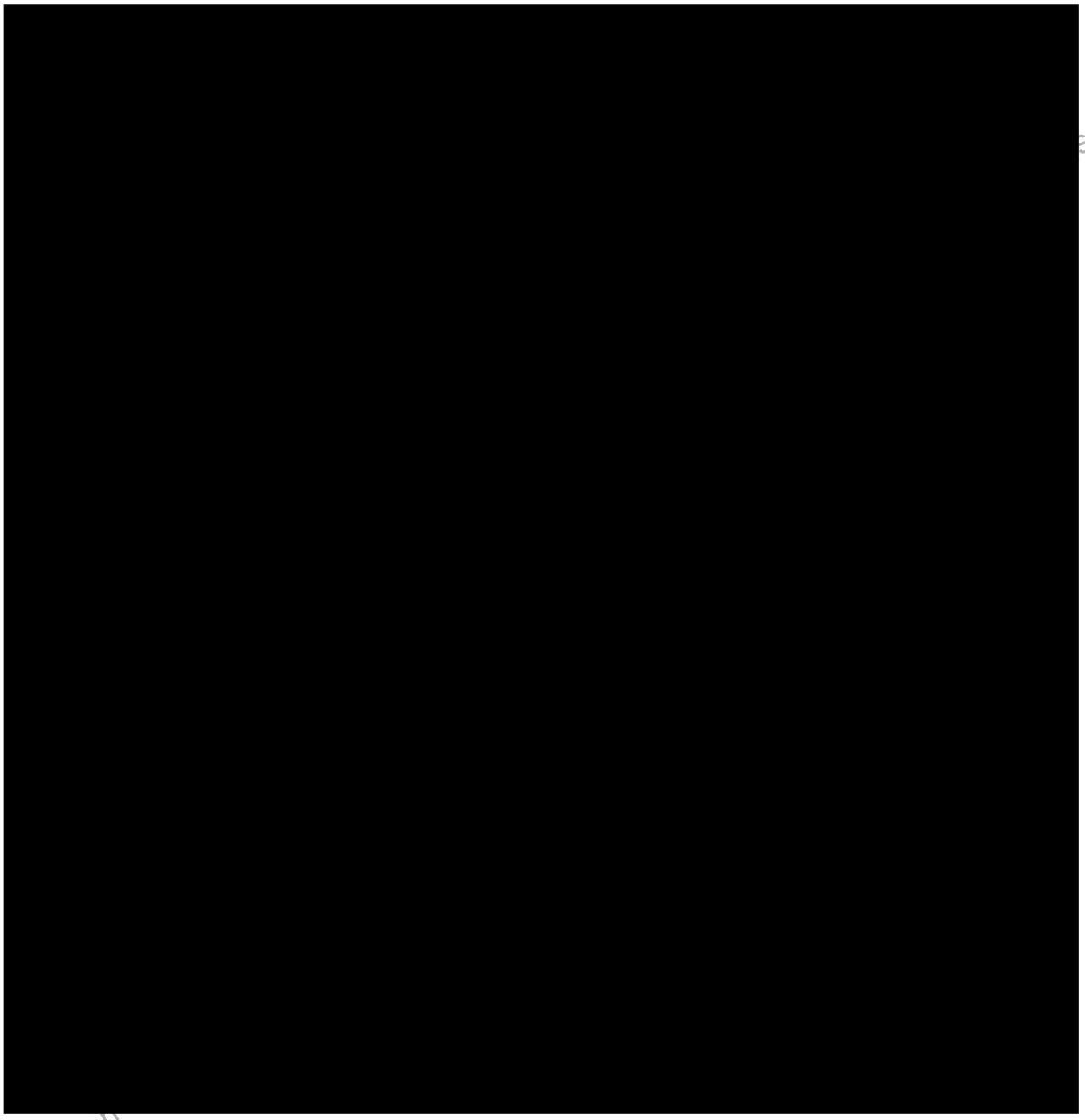


Table 53: Density Gradient Ultracentrifugation Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.10 Hydrophobic Interaction Chromatography

Hydrophobic interaction chromatography (HIC) was used to characterize mRNA-1273 Drug Product PPQ batches. Three chromatographic regions are reported as relative percent areas: [redacted]. HIC results generated for the mRNA-1273 Drug Product PPQ batches are reported in Table 54. The relative percent peak areas for each chromatographic region were consistent among these PPQ lots which demonstrate similar hydrophobic profiles.

Table 54: Hydrophobic Interaction Chromatography Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.11 Esterase Kinetics

Esterase kinetics was used to determine the [redacted] in mRNA-1273 Drug Product PPQ batches. The [redacted] for each PPQ lot is presented in Figure 12 and Table 55. Results demonstrate consistent [redacted] behavior across lots.

Figure 12: [redacted]

Figure 12 (Continued): [redacted]

Table 55: Esterase Kinetics Results

Lot	[redacted]
6007520004	
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.2.12 RiboGreen Encapsulation

A fluorescence-based RiboGreen assay was used as a release assay to determine the encapsulation efficiency of mRNA in mRNA-1273 LNP. RNA encapsulation results for the PPQ lots are shown in Table 56. These results demonstrate similarly high and consistent mRNA encapsulation [redacted] for the PPQ lots. The Sponsor intends to validate [redacted] as a release assay due to the assay robustness, simplicity, and the usage of a USP-certified assay reagent. The Sponsor will continue to determine encapsulation efficiency of mRNA in mRNA-1273 LNP by RiboGreen assay as an orthogonal characterization assay.

Table 56: RiboGreen RNA Encapsulation Results

Lot	Encapsulation (%)
6007520004	[redacted]
6007520005	
6007520006	
057G20	
062G20	
001H20	

3.2.P.2.3.7.3 Analytical Assessment Summary

The evaluation of mRNA-1273 Drug Product GMP and PPQ lots demonstrates that product quality is highly similar for the manufacturing process and scale changes that occurred throughout process development, as shown in Table 57. The 6 PPQ lots are also highly similar in terms of product quality and demonstrate process consistency at the [redacted] vial (Norwood PPQ, 0.20 mg/mL mRNA-1273) and [redacted] vial (Bloomington PPQ, 0.20 mg/mL mRNA-1273) scale, as shown in Table 58. Extended characterization results were similar between lots and continue to provide additional understanding of process control together with in-process and release testing.

Table 57: Attribute Assessment Results Summary for Clinical Trial Material

Product Attribute	Method	Document No.	Description	Results									
				85201100101	85201100102	85201100103	85201100104	6007520001	6007520002	6007520003	6007520004	6007520005	6007520006
LNP size distribution	Nanoparticle tracking analysis	DPTM-0039	High-resolution LNP size distribution	[redacted]									
LNP size distribution	Asymmetric flow field-flow fractionation	DPTM-0103	Fractionation coupled with in-line MALS detection for size determination										
Sub-visible particle counts	Coulter counter	DPTM-0035	Measurement of sub-visible particle counts in the [redacted] range										
Sub-visible particle counts and morphology	Flow microscopy	DPTM-0115	Measurement of sub-visible particle counts and morphology in the [redacted] range										
LNP surface characterization	Isothermal titration calorimetry	DPTM-0119	[redacted] to LNP surface										
LNP charge	Zeta potential	DPTM-0118	Average LNP charge [redacted]										
LNP charge distribution	Capillary isoelectric focusing	DPTM-0068	LNP pI distribution and polydispersity										
LNP structure	Dye permeation kinetics	Research-grade assay	Qualitative characterization of LNP surface and encapsulation state based on thionine permeation kinetics	NT	NT	NT	NT	NT	NT	NT	[redacted]		
LNP density	Density gradient ultracentrifugation	DPTM-0104	Qualitative assessment of LNP density heterogeneity	NT	NT	NT	NT	NT	NT	NT			
LNP surface characterization	Hydrophobic interaction chromatography	SOP-0096	Qualitative characterization of LNP surface hydrophobicity	NT	NT	NT	NT	NT	NT	NT			
LNP surface characterization	Esterase kinetics	Research-grade assay	Qualitative method for [redacted] distribution	NT	NT	NT	NT	NT	NT	NT			
mRNA encapsulation	Ribogreen	SOP-0298	Fluorescence based method for mRNA encapsulation	NT	NT	NT	NT	NT	NT	NT			
mRNA encapsulation	[redacted]	DPTM-0073	[redacted]	[redacted]							NT	NT	NT

Abbreviations: LNP = lipid nanoparticle; MALS = multi-angle light scattering; NT = not tested; pI = isoelectric point(s); PEG = polyethylene glycol; PPQ = process performance qualification; SVP = sub-visible particle

Table 58: Attribute Assessment Results Summary for Process Performance Qualifications (vial and vial scales)

Product Attribute	Method	Document No.	Description	Results					
				6007520004	6007520005	6007520006	6007320001 (057G20)	6007320002 (062G20)	6007320003 (001H20)
LNP size distribution	Nanoparticle tracking analysis	DPTM-0039	High-resolution LNP size distribution						
LNP size distribution	Asymmetric flow field-flow fractionation	DPTM-0103	Fractionation coupled with in-line MALS detection for size determination						
Sub-visible particle counts	Coulter counter	DPTM-0035	Measurement of sub-visible particle counts in the range						
Sub-visible particle counts and morphology	Flow microscopy	DPTM-0115	Measurement of sub-visible particle counts and morphology in the range						
LNP surface characterization	Isothermal titration calorimetry	DPTM-0119	to LNP surface						
LNP charge	Zeta potential	DPTM-0118	Average LNP charge						
LNP charge distribution	Capillary isoelectric focusing	DPTM-0068	LNP pI distribution and polydispersity						
LNP structure	Dye permeation kinetics	Research-grade assay	Qualitative characterization of LNP surface and encapsulation state based on thionine permeation kinetics						
LNP density	Density gradient ultracentrifugation	DPTM-0104	Qualitative assessment of LNP density heterogeneity						
LNP surface characterization	Hydrophobic interaction chromatography	SOP-0096	Qualitative characterization of LNP surface hydrophobicity						
LNP surface characterization	Esterase kinetics	Research-grade assay	Qualitative method for distribution						
mRNA encapsulation	Ribogreen	SOP-0298	Fluorescence based method for mRNA encapsulation						

Abbreviations: LNP = lipid nanoparticle; MALS = multi-angle light scattering; NT = not tested; pI = isoelectric point(s); ; PPQ = process performance qualification; SVP = sub-visible particle

3.2.P.2.3.7.4 Summary of Specification Changes – mRNA-1273 Drug Product

Table 59 and Table 60 summarize the specification changes implemented to date.

Table 59: Summary of Specification Revisions – mRNA-1273 Drug Product – PVU Process to Norwood Scale A

Test Parameter		Analytical Procedure	Acceptance Criteria			Rationale for Change	
			PVU Process (PN 85201) (SPC-0948) / (Version 2.0/3.0)	**Initial Scale A (PN 60075) (SPC-1063) / (Version 1.0)**	**Scale A PPQ (PN 60075) (SPC-1063) / (Version 2.0)**	**PVU to Initial Scale A**	**Initial Scale A to Scale A PPQ**
Appearance		Visual Inspection	White to off-white dispersion. May contain visible, white or translucent product-related particulates	White to off-white dispersion. May contain visible, white or translucent product-related particulates	White to off-white dispersion. May contain visible, white or translucent product-related particulates	No Change	No Change
RNA Content		Anion Exchange-HPLC	[redacted]			Revised based on revised dilution step. Target added for reference.	No Change
Identity		Reverse Transcription Sanger Sequencing	Sequence matches 100% of the coding region	Sequence matches description	Sequence matches description	No Change	No Change
Purity		RP-HPLC	[redacted]			No Change	Tightening acceptance criteria to ensure product remains within specification for the duration of shelf-life
Product-related Impurities		RP-HPLC	Report % area for each impurity group: Impurity Group 1 (pre-main peak area) Impurity Group 2 (post-main peak area) Impurity Group 3 (mRNA-adduct species)	Report % area for each impurity group: Impurity Group 1 Impurity Group 2 Impurity Group 3	Report % area for each impurity group: Impurity Group 1 Impurity Group 2 Impurity Group 3	No Change	No Change
% RNA Encapsulation		Fluorescence	[redacted]			No Change	No Change
Potency		In Vitro Translation Methionine Labelling				Addition of test parameter to ensure SISPQ	No Change
pH		USP <791>				No Change	No Change
Osmolality		USP <785>				Revised based on available data	No Change
Particle Size		Dynamic Light Scattering				No Change	No Change
Polydispersity		Dynamic Light Scattering	Report result	Report result	[redacted]	No Change	Initial establishment of polydispersity acceptance criteria based on analytical capability and process and stability experience.
Lipid Identification	SM-102	UPLC-CAD	Matches RT of reference	Matches RT of reference	Matches RT of reference	No Change	No Change
	Cholesterol		Matches RT of reference	Matches RT of reference	Matches RT of reference		
	DSPC		Matches RT of reference	Matches RT of reference	Matches RT of reference		
	PEG2000-DMG		Matches RT of reference	Matches RT of reference	Matches RT of reference		

Test Parameter		Analytical Procedure	Acceptance Criteria						Rationale for Change	
			PVU Process (PN 85201) (SPC-0948) / (Version 2.0/3.0)		Initial Scale A (PN 60075) (SPC-1063) / (Version 1.0)		Scale A PPQ (PN 60075) (SPC-1063) / (Version 2.0)		PVU to Initial Scale A	Initial Scale A to Scale A PPQ
Lipid Content	SM-102		[redacted]						Revised based on revised dilution step.	Changes in the LSS concentration (refer to Section 3.2.S.2.6 [redacted]) impact lipid proportions within the [redacted]
	Cholesterol									
	DSPC									
	PEG2000-DMG									
	Lipid Impurities		Individual Impurities	Report % area and RRT	Individual Impurities	Report % area and RRT	Individual Impurities	Report % area and RRT	No Change	No Change
			Total Impurities	Report % area	Total Impurities	Report % area	Total Impurities	Report % area		
Particulate Matter	[redacted]	USP <788> Method 2	[redacted]						No Change	No Change
Container Content		USP <697>	N/A [a]		[redacted]				Addition of test parameter to ensure SISPQ	No Change
Bacterial Endotoxins		USP <85> Ph. Eur. 2.6.14	[redacted]						No Change	No Change
Sterility		USP <71> Ph. Eur. 2.6.1	No Growth		No Growth		No Growth		No Change	No Change

Abbreviations: CAD = charged aerosol detector; DSPC = 1,2-Distearoyl-sn-glycero-3-phosphatidylcholine; EU = endotoxin unit(s); HPLC = high-performance liquid chromatography; LNP = lipid nanoparticle; LSS = lipid stock solution; N/A = not applicable; RT = retention time; RRT = relative retention time ; SISPQ = safety, identity, strength, purity, and quality; UPLC = ultra-high-performance liquid chromatography

a) Test parameters not present on specification at time of testing.

Table 60: Summary of Specification Revisions – mRNA 1273 Drug Product –Norwood Scale A to Catalent Scale A/Scale B

Test Parameter		Analytical Procedure	Acceptance Criteria		Rationale for Change
			Norwood Scale A PPQ (PN 60075) (SPC-1063) / (Version 2.0)	Catalent Scale A PPQ/Scale B (PN 60073) (SPC-1128) / (Version 1.0/2.0)	
Appearance		Visual Inspection	White to off-white dispersion. May contain visible, white or translucent product-related particulates	White to off-white dispersion. May contain visible, white or translucent product-related particulates	No Change
RNA Content		Anion Exchange-HPLC	[redacted]		Tightened acceptance criteria based on manufacturing and analytical performance results from Norwood Scale A batches
Identity		Reverse Transcription Sanger Sequencing	Sequence matches description	Sequence matches description	No Change
Purity		RP-HPLC	[redacted]		Establishment of purity release acceptance criteria of [redacted] for increased scale with a shelf life acceptance criteria of [redacted]
Product-related Impurities			Report % area for each impurity group: Impurity Group 1 (pre-main peak area) Impurity Group 2 (post-main peak area) Impurity Group 3 (mRNA-adduct species)	Report % area for each impurity group: Impurity Group 1 (pre-main peak area) Impurity Group 2 (post-main peak area) Impurity Group 3 (mRNA-adduct species)	No Change
% RNA Encapsulation		Fluorescence	[redacted]		No Change
In Vitro Translation		In Vitro Translation Methionine Labelling			No Change
pH		USP <791>			No Change
Osmolality		USP <785>			No Change
Particle Size		Dynamic Light Scattering			No Change
Polydispersity					No Change
Lipid Identification	SM-102	UPLC-CAD	Matches RT of reference	Matches RT of reference	No Change
	Cholesterol		Matches RT of reference	Matches RT of reference	
	DSPC		Matches RT of reference	Matches RT of reference	
	PEG2000-DMG		Matches RT of reference	Matches RT of reference	
Lipid Content	SM-102		[redacted]		No Change
	Cholesterol				
	DSPC				
	PEG2000-DMG				
Lipid Impurities			Individual Impurities: Report % area and RRT	Individual Impurities: [redacted]	Establishing acceptance criteria based on ICH M7 guidance and manufacturing experience.
			Total Impurities: Report % area	Total Impurities: [redacted]	
Particulate Matter	[redacted]	USP <788> Method 2	[redacted]		No Change
Container Content		USP <697>			To enable a 10-dose multiple-dose vial
Bacterial Endotoxins		USP <85> Ph. Eur. 2.6.14			No Change
Sterility		USP <71> Ph. Eur. 2.6.1	No Growth	No Growth	No Change