Table of Contents

List of Tables

List of Figures

3.2.P.5.6 JUSTIFICATION OF SPECIFICATIONS

Specifications are critical quality standards that are proposed and justified by the manufacturer and approved by regulatory authorities. Specifications are chosen to confirm the quality of the mRNA-1273 Drug Product rather than to establish full characterization and should focus on those characteristics found to be useful in ensuring the safety and efficacy of the mRNA-1273 Drug Product (ICH Q6B and Q11).

A Critical Quality Attribute is defined as a physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality per ICH Q8(R2) - Pharmaceutical Development.

Table 1 summarizes the critical quality attributes (CQAs) and the corresponding justifications for the mRNA-1273 Drug Product based on risk assessment performed and process and product characterization knowledge gained for mRNA-1273 and general SM-102 lipid formulated drug product with other mRNAs.

Table 1: Critical Quality Attributes for mRNA-1273 Drug Product

Quality Attribute	Classification	Justification
Appearance	CQA	• This group of attributes comprises the product characteristics that are expected by the regulatory authorities as part of product filing. This group thus consists of the **Regulatory CQAs.** • Appearance (USP <631> and Ph. Eur. 2.2) help to define the expected visible quality attributes of the mRNA-1273 DP. Changes in this attribute may indicate a potential impact to the quality of the drug product. • Identity is a CQA to ensure that the product contains the intended mRNA and Lipids and not any other. This attribute will impact the safety and efficacy of the product. • pH and Osmolality are indicators of consistent manufacturing performance. pH is an important control parameter that can impact the downstream quality of the product. The control of osmolality is an important parameter that is indicative of the correct excipient levels in the product which has a direct impact on stability. • Container Content (USP<697>; Ph.Eur. 2.9.17) ensures that each container of the product contains sufficient volume of solution to allow successful withdrawal of the labeled quantity / number of doses. [Note: This attribute is not relevant when varying portions of the drug product solution may be withdrawn for further manipulation (e.g. dilution) before administration]. • Particulate matter (USP<788>; Ph.Eur. 2.9.19) testing is performed to ensure lack of contamination (foreign particulate matter), and to monitor the product for the formation of inherent (i.e. product related) particles which may indicate product stability or quality concerns. It is also a CQA per regulatory requirements (USP<1>; Ph.Eur. 0520). • Endotoxins and Sterility are safety CQAs that ensure the product is safe to administer by the parenteral route.
Identity (mRNA)	CQA	
Identity (Lipids)	CQA	
pH	CQA	
Osmolality	CQA	
Container Content	CQA	
Particulate Matter	CQA	
Bacterial Endotoxins	CQA	
Sterility	CQA	
mRNA Content	CQA	mRNA content represents the strength of the drug substance (Assay in USP <1>). It is representative of the dose being delivered that impacts the efficacy of the product. The potency will be based on the measurement of RNA content (AEX-HPLC) and in conjunction with RNA purity (RP-HPLC).
mRNA Purity	CQA	Purity (of mRNA) is related to the mRNA activity of the product. Degraded mRNA cannot be translated into full-length protein and will not be active.
mRNA Impurities (Product-related)	CQA	Purity (of mRNA) is related to the mRNA activity of the product as degraded mRNA cannot be translated into full-length protein and will not be active. Short mRNA fragments do not represent a safety risk as these fragments are compositionally identical to full-length mRNA.
% RNA Encapsulation	CQA	mRNA that is not encapsulated in lipid nanoparticles is subject to rapid degradation by RNases in the body. Thus, RNA Encapsulation is a quality attribute that impacts the efficacy of the product.
Particle Size	CQA	The (mean) size of the LNP by DLS, is important for the cellular uptake and functionality, and thus the efficacy of the product.
Polydispersity	CQA	Polydispersity (by DLS) is a measure of the range of sizes of the particles present in the LNPs. This parameter is an indication of the process control and DP stability.
Lipids Content	CQA	Encapsulation of mRNA in LNPs is required for cellular uptake and functionality. The control of the levels of individual constituent lipids ensures that the optimal amount is present to maximize the encapsulation of mRNA during manufacture, to provide biophysical stability [redacted] to enable circulation *in vivo,* and finally to allow uptake as well as endosomal release.

Quality Attribute	Classification	Justification
Impurities (Lipid-related)	CQA	Measurement of Impurities (Product-related) is required under conditions in which the product is customarily used (see USP <1> and General Notices, 5.60 Impurities and Foreign Substances). Lipid impurities have the potential to impact both safety and activity of the product. Impurities have been qualified through toxicology studies and with clinical experience. It is proposed that these impurities will be reported, and action limits established.
Protein expression	CQA	In vitro translation is a qualitative test to confirm the expected molecular weight of the protein translated using the mRNA.

Abbreviations: CQA = critical quality attribute; DLS = dynamic light scattering; DP = drug product; LNP = lipid nanoparticle

The specifications for release of the mRNA-1273 Drug Product are presented in SPC-0948, SPC-1063 and SPC-1128, these specifications include the attributes, test methods, and acceptance criteria that together confirm the quality of mRNA-1273 Drug Product lots. Acceptance criteria were established according to ICH Q6B guidelines and reflect historical product quality data from preclinical and clinical lots, release results (utilizing methods effective at the time of testing) for manufactured mRNA-1273 Drug Product, stability study results, relevant development data and analytical method performance data. A list of the lots used to establish acceptance criteria ranges is presented in Table 2. Manufacturing experience of mRNA-1273 Drug Product is limited to two (2) development lots and eleven (11) GMP lots at the time of generation of this report, the relevant data generated to date using each of the methods listed is provided to demonstrate the current process and analytical experience. As additional data becomes available with future batch manufacture, the specifications may be re-visited. The rationale for the establishment and justification of the release specification is provided in each method section.

Table 2: mRNA-1273 Drug Product Lots Used for Establishing Specification

Lot	Scale	Status	Date of Manufacture
DHM-47516	2.2 g	Development	01 Apr 2020
DHM-47519	2.2 g	Development	01 Apr 2020
8520100101	380 mg	GMP Ph 1/2	07 Feb 2020
8520100102	380 mg	GMP Ph 1/2	19 Mar 2020
8520100103	380 mg	GMP Ph 1/2	23 Mar 2020
8520100104	380 mg	GMP Ph 1/2	02 Apr 2020
6007520001	15 g	GMP Ph 3	28 May 2020
6007520002	15 g	GMP Ph 3	02 Jun 2020
6007520003	15 g	GMP Ph 3	04 Jun 2020
6007520004	15 g	GMP Ph 3/PPQ	25 Jun 2020
6007520005	15 g	GMP Ph 3/PPQ	30 Jun 2020
6007520006	15 g	GMP Ph 3/PPQ	08 Jul 2020
6007520007	15 g	GMP Ph 3	09 Jul 2020
6007320001	15 g	PPQ, Intended for Clinical/Commercial Use	30 Jul 2020
6007320002	15 g	PPQ, Intended for Clinical/Commercial Use	06 Aug 2020
6007320003	15 g	PPQ, Intended for Clinical/Commercial Use	11 Aug 2020

3.2.P.5.6.1 Appearance

CQA Determination

Appearance is considered a CQA as it provides an overall assessment of product integrity. For example, large aggregates or precipitates may indicate incorrect storage which may impact product activity. It is to be noted that the product may demonstrate some phase-separation which is expected in LNP dispersions, these visible particulates do not impact product quality or safety. This method is also able to provide an overall assessment of visible process-related particulates that could represent a safety risk (e.g., material shed from vial or stopper), however as the mRNA-1273 Drug Product is a turbid solution, the degree to which these particles are observable may be limited.

Method Description

Appearance testing is by visual inspection of the mRNA-1273 Drug Product. The contents of a vial are observed against both black and white backgrounds under full-spectrum lighting. Color and visual appearance characteristics are reported. The method is aligned with requirements of USP <631> and Ph. Eur. 2.2 for color and clarity.

Method Development and Analytical Performance

The method has been established in the analytical development organization and has been run as a qualitative assessment on numerous batches. This information has informed the typical physical appearance of the LNP dispersion, and any product changes with degradation.

Method Validation Data

The method is run as a qualitative test and is not formally validated. The method is aligned with the requirements of USP <631> and Ph. Eur 2.2 for color and clarity as verified and detailed in Section 3.2.P.5.3.

Process Data

The data shown in Table 3 represent batches that have been assessed.

Table 3: mRNA-1273 Drug Product Lots Assessed by Visual Inspection

Lot	Result
DHM-47516	White to off-white dispersion; Essentially free of particulates
DHM-47519	White to off-white dispersion; Essentially free of particulates
8520100101	White to off-white dispersion; Essentially free of particulates
8520100102	White to off-white dispersion; Essentially free of particulates
8520100103	White to off-white dispersion; Essentially free of particulates
8520100104	White to off-white dispersion; Essentially free of particulates
6007520001	White to off-white dispersion; Essentially free of particulates
6007520002	White to off-white dispersion; Essentially free of particulates
6007520003	White to off-white dispersion; Essentially free of particulates
6007520004	White to off-white dispersion; Essentially free of particulates
6007520005	White to off-white dispersion; Essentially free of particulates
6007520006	White to off-white dispersion; Essentially free of particulates
6007520007	White to off-white dispersion; Essentially free of particulates
6007320001	White to off-white dispersion; Essentially free of particulates
6007320002	White to off-white dispersion; Essentially free of particulates
6007320003	White to off-white dispersion; Essentially free of particulates

Statistical Analysis

Statistical analysis is not applicable to this analytical method as the result is non-numerical.

Conclusion including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities, the following specification and acceptance criterion of "**White to off-white dispersion. May contain visible, white, or translucent product-related particulates"** for visual inspection of mRNA-1273 Drug Product is proposed. In the representative Drug Product lots generated to date, product-related particulates have not been observed, the proposed specification allows for this however as for similar (platform) products these have been observed to be formed upon stability. When formed and present, these product-related particulates represent a small percentage of the total product and do not impact the other product quality characteristics.

3.2.P.5.6.2 Identity: Reverse Transcription Sanger Sequencing

CQA Determination

Identity is determined as a CQA for the mRNA-1273 Drug Product. The Identity method establishes positive identity of the encapsulated CX-024414 mRNA and confirms the accuracy of the coding region of the mRNA to ensure that the correct protein is encoded. Inclusion of the incorrect sequence will impact protein expression (the correct protein required to engender the required pharmacological effect will not be included) and could also impact safety if a sequence encoding a toxic or poorly tolerated protein was included.

Method Description

mRNA is first extracted from the mRNA-1273 Drug Product using a phenol/chloroform/isoamyl alcohol methodology to yield the CX-024414 mRNA. The molecular sequence of the coding region of the CX-024414 mRNA is then determined by reverse transcription of the mRNA into a cDNA followed by sequencing using dideoxynucleotide chain elongation terminators of DNA polymerization (Sanger sequencing). Synthetic DNA oligonucleotide primers starting in the 5' and 3' untranslated regions of CX-024414 are used for PCR amplification of the cDNA template and specific sequencing primers are used to obtain 4x coverage (duplicate coverage of both the sense and antisense strands of the cDNA) [redacted] For regions where only limited data can be obtained (including but not limited to regions after homopolymers or where structure impacts sequencing quality) [redacted] is acceptable. For the mRNA-1273 Drug Product, the approach to sequencing [redacted] is considered acceptable as the methodology is required simply to determine identity of the product, for the mRNA Drug Substance [redacted] to ensure that no mutations have been introduced during the mRNA manufacturing process.

Method Development and Analytical Performance

The method has been developed to enable the unequivocal determination of sequence identity. The forward and reverse primers used in the PCR assay are designed with sufficient sequence length such that specificity of amplification is assured. Thereafter, specific primers throughout the coding region are used to perform Sanger sequencing combined with electrophoretic separation and fluorescence detection of the termination products. This analysis is based upon well-established molecular biology techniques and provides an unequivocal determination of the sequence of the coding region.

Method Validation Data

The Identity method is validated as detailed in Section 3.2.P.5.3. The validated method for mRNA-1273 Drug Product is defined in SOP-1032. Specificity was assessed by sequencing CX-024414 mRNA no-template controls per SOP-0492. Figure 1 shows a representative analysis of CX-024414 and Figure 2 shows an example result from a no-template control (NTC) sample. Note that for a positive read, clear separation of the expected terminated fragments is apparent for CX-024414 (Figure 1), whereas bases could not be discerned or identified in the no-template control (Figure 2).

Figure 1: Example of a Positive RT Sanger Sequencing Analysis using the Positive Control mRNA Molecule CX-024414

Figure 2: Example of a Negative RT Sanger Sequencing Analysis using the Assay No-template Control (NTC)

Process Data

Data shown in Table 4 represent batches that have been verified for identity using the RT/Sanger sequencing methodology.

Table 4: mRNA-1273 Drug Product Lots Verified for Identity using the RT/Sanger Sequencing

Lot	Result
8520100101	Sequence matches description
8520100102	Sequence matches description
8520100103	Sequence matches description
8520100104	Sequence matches description
6007520001	Sequence matches description
6007520002	Sequence matches description
6007520003	Sequence matches description
6007520004	Sequence matches description
6007520005	Sequence matches description
6007520006	Sequence matches description
6007520007	Sequence matches description
DHM-47516	Sequence matches description
DHM-47519	Sequence matches description
6007320001	Sequence matches description
6007320002	Sequence matches description
6007320003	Sequence matches description

Statistical Analysis

Statistical analysis is not applicable to this analytical method as the result is non-numerical.

Conclusion including Proposed Acceptance Criterion

The method is designed to confirm that the correct sequence is present based upon well-established method principles that have been developed in the field of molecular biology. Note that the only parameter used to support the release criterion is the specificity of the method which is aligned with ICH Q2(R1) expectation for an identity test. Based upon the analytical method and process capabilities, the following specification and acceptance criterion of "**Sequence matches description**" for identity for mRNA-1273 Drug Product is proposed.

3.2.P.5.6.3 RNA Content: Anion Exchange High-Performance Liquid Chromatography

CQA Determination

Total RNA content is determined as a CQA for the mRNA-1273 Drug Product. An inaccurate concentration will lead to mRNA-1273 Drug Product that may not have the required activity; therefore, the CQA assessment is based upon the impact to potency, which is based on the measurement of RNA content and in conjunction with RNA purity.

Method Description

RNA content is measured using an anion exchange ultra-high-performance liquid chromatography (AEX-UPLC) methodology with UV absorption detection [redacted] In the method, LNPs are disrupted using [redacted] and the RNA is separated using a low-salt RNA binding mobile phase and a high-salt RNA elution mobile phase. In the method all mRNA species (including degradants) co-elute into a single peak enabling the assessment of total mRNA content for the mRNA-1273 Drug Product. The UV absorption response is compared to a standard of known concentration of reference mRNA to determine the total RNA content value. The reference mRNA concentration is determined using the Total RNA content method described in Section 3.2.S.4.2 {CX-024414}.

Method Development and Analytical Performance

The method has been developed to enable accurate determination of the RNA concentration contained within the mRNA-1273 Drug Product. The methodology is essentially a "capture and elute" methodology that is agnostic to higher order structure and mRNA length, this enables a robust measurement of total RNA content irrespective of the age or degradation state of the encapsulated mRNA. Consistency with CX-024414 manufacturing and measurement is achieved by assessing the mRNA-1273 Drug Product chromatography against reference standard-derived standard curve using the CX-024414 sequence-specific extinction coefficient.

Method Validation Data

The method is validated as a quantitation method for RNA content. The validated method for determination of RNA content of the mRNA-1273 Drug Product is defined in SOP-0999. A detailed validation summary is presented in Section 3.2.P.5.3. The method was shown to be specific for mRNA-1273 Drug Product as no interference was observed from the measurement of formulation buffer. In addition, the method was demonstrated to be accurate, precise, and linear. The target sample dilution is [redacted] and the validated range is from [redacted]

Process Data

Data shown in Table 5 represent RNA concentrations measured for development and GMP lots.

Table 5: Total RNA Content for Process Development and GMP Lots

Lot	Concentration (mg/mL) (Target Range [redacted])	Concentration (mg/mL) (Target Range [redacted])	Concentration (mg/mL) (Target Range [redacted])	Concentration (mg/mL) (Target [redacted])
DHM-47516	-	-	[redacted]	-
DHM-47519	-	-	-	[redacted]
8520100101	-	-	[redacted]	-
8520100102	-	-	[redacted]	-
8520100103	-	-	[redacted]	-
8520100104	-	-	[redacted]	-
6007520001	[redacted]	-	-	-
6007520002	[redacted]	-	-	-
6007520003	[redacted]	-	-	-
6007520004	[redacted]	-	-	-
6007520005	[redacted]	-	-	-
6007520006	[redacted]	-	-	-
6007520007	[redacted]	-	-	-
6007320001	-	[redacted]	-	-
6007320002	-	[redacted]	-	-
6007320003	-	[redacted]	-	-
Mean	[redacted]		Not Applicable*	Not Applicable*
Standard deviation	[redacted]		Not Applicable*	Not Applicable*
No. of batches	[redacted]		Not Applicable*	Not Applicable*
Range	[redacted]		Not Applicable*	Not Applicable*
Tolerance interval (Upper) (99% Coverage, 95% CI)	[redacted]		Not Applicable*	Not Applicable*
Tolerance interval (Lower) (99% Coverage, 95% CI)	[redacted]		Not Applicable*	Not Applicable*

* Data analysis not undertaken as development and early phase clinical lots were generated at a different concentration range to the intended commercial product.

Statistical Analysis

The summation of relevant process data generated to date are presented in Table 5. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set, this was completed for the lots with a commercially representative concentration (6007520001 through 6007520007, and 6007320001 through 6007320003). For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied.

Conclusion Including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities, the following specification and acceptance criterion of "[redacted]" for the RNA content determination of mRNA-1273 Drug Product is proposed.

3.2.P.5.6.4 Purity and Product-Related Impurities: Reverse-Phase High-Performance Liquid chromatography

CQA Determination

RNA purity is determined to be a CQA for the mRNA-1273 Drug Product. Short mRNA fragments may be generated as part of premature termination of transcription ("abortive transcripts") or degradation of the mRNA during the manufacturing process. Full length mRNA is critical for translation of CX-024414 mRNA. Main peak purity is quantified and is representative of full-length mRNA. Pre- and post-main peaks represent shorter (abortive transcripts or degradants) or longer species (for example run-on transcripts). Impurities are reported as area percent. Impurity group 1 (pre-main peak) contains mRNA degradants and abortive transcript impurities, impurity group 2 (post-main peak) represents potential high molecular weight impurities, and impurity group 3 (mRNA-adduct species, nucleotides being modified by lipid impurities or degradants). The shelf-life of the final mRNA-1273 Drug Product will be driven largely by the degradation of the mRNA contained within the mRNA-1273 LNP; therefore, a sufficiently high purity is required to account for this degradation and align with the activity and desired shelf life of the mRNA-1273 Drug Product.

Method Description

RPIP-HPLC is used to assess mRNA integrity of mRNA contained within the mRNA-1273 Drug Product. This method utilizes a solvent gradient that enables the separation of RNA molecules based predominantly on their length. Detection is performed by UV [redacted] Note that this single method is used to determine full-length mRNA purity (main peak) and impurities, the area of each peak is quantified and reported as percentage of the total peak area.

Method Development and Analytical Performance

The method has been developed to accurately resolve and quantify CX-024414 mRNA (contained within the mRNA-1273 Drug Product) in the presence of the expected impurities generated by the process and the components of the formulation buffer. The method has been optimized over a number of rounds of analytical method development, this has included the selection of both the stationary and mobile phases, and optimization of the chromatographic gradient and ion pairing agents. Through forced degradation studies, the method has been demonstrated to be able to resolve and quantitate the main peak below a purity of 50%.

Method Validation Data

This method is validated as a quantitative purity test for mRNA-1273 Drug Product. The validated method for determination of RNA purity of the mRNA-1273 Drug Product is defined in SOP-0996. A detailed validation summary is presented in Section 3.2.P.5.3. The method was shown to be specific, accurate, precise, and linear for mRNA-1273 Drug Product. The validated range of the assay is from [redacted], the LOQ of the assay is 1% peak area.

Process Data

mRNA purity has been demonstrated to be reduced in the mRNA-1273 Drug Product as compared to the source CX-024414 mRNA. The degradation of the mRNA through formulation occurs though a stochastic mechanism of strand breakage anywhere along the length of the mRNA molecule, this continues to occur on stability. The predominant mechanism for strand scission is through a trans-esterification reaction that results in nucleophilic attack of the phosphodiester backbone.

Data shown in Table 6 and Table 7 represent the RNA purity measured for development and GMP lots.

Table 6: RNA Purity for Process Development and GMP Lots

Lot	Purity (Main Peak Area %)	Impurity Groups 1, 2, 3 (%)
DHM-47516	[redacted]	[redacted]
DHM-47519		
8520100101		
8520100102		
8520100103		
8520100104		
6007520001		
6007520002		
6007520003		
6007520004		
6007520005		
6007520006		
6007520007		
6007320001		
6007320002		
6007320003		
Mean		
Standard deviation		
No. of batches		
Range		
Tolerance interval (Upper) (99% Coverage, 95% CI)		
Tolerance interval (Lower) (99% Coverage, 95% CI)		

Table 7: RNA Purity Stability Results for Process Development and GMP Lots at -20°C and 5°C

Lot	T=0	-20°C			5°C		
		T= 1 Month	T= 2 Month	T= 3 Month	T= 1 Month	T= 2 Month	T= 3 Month
DHM-47516	[redacted]	NT	[redacted]				
DHM-47519		NT					
8520100101		NT	NT	NT		NT	NT
8520100102		NT	NT	NT		[redacted]	NT
8520100103		NT	NT	NT			[redacted]
8520100104		NT	NT	NT			
6007520001		[redacted]	NA	NA		NA	NA
6007520002			NA	NA		NA	NA
6007520003			NA	NA		NA	NA

* An additional sample was pulled at 50 days for % purity by RP-HPLC analysis. The data were used for 1M results.
Abbreviations: NT = not tested; NA = not available

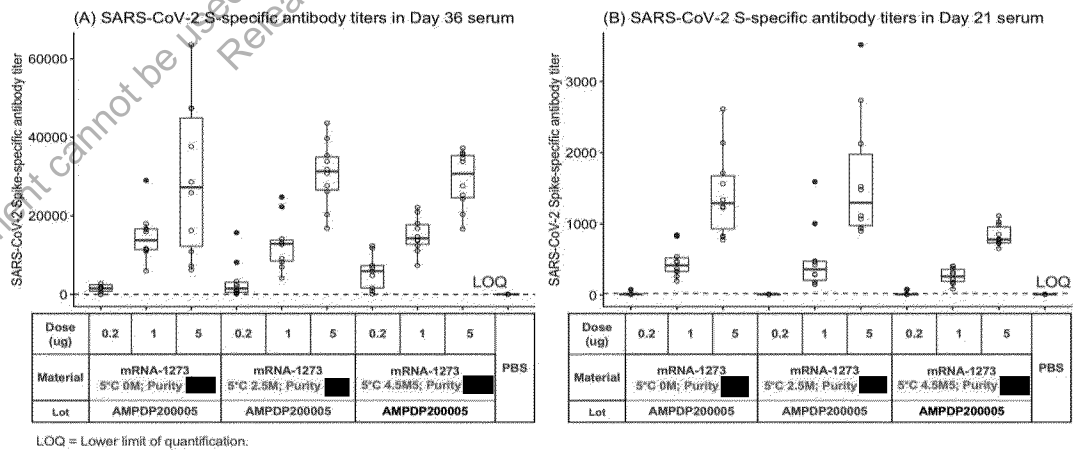
Statistical Analysis

The summation of data generated to date are presented in Table 6. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval, a 99% population coverage and 95% confidence interval were applied.

Purity and Biological activity

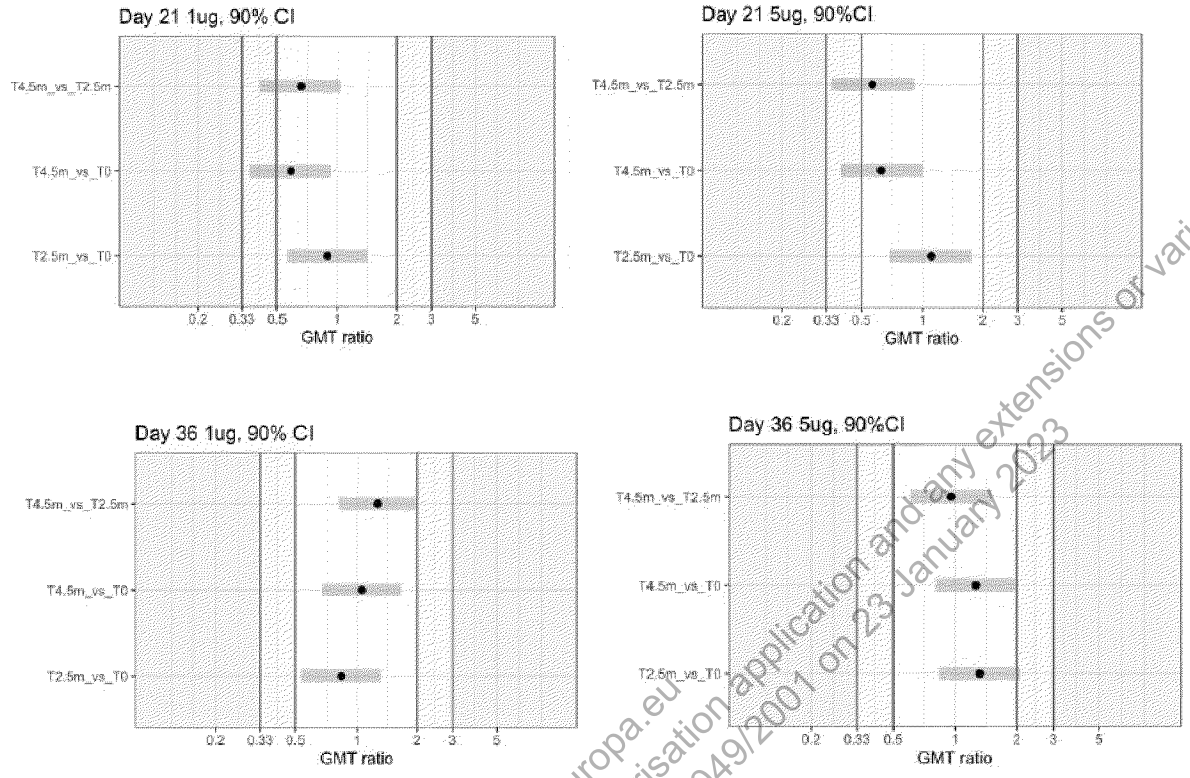
Pre-clinical studies (Study 4016) have demonstrated that the immunogenicity of mRNA-1273 Drug Product ranging from [redacted] purity (Test Articles with [redacted] purity as measured by RPIP HPLC for vials held at 5°C for 0, 2.5 and 4.5 months, respectively) were equivalent (Figure 3). The equivalence margin (90% CIs of the GMT ratios) was within approximately 2-fold margin between the three purity levels at Day 36 and within approximately 3-fold margin between the three purity levels at Day 21 in the study (Figure 4). The pre-clinical experience at therefore is supportive of the proposed 50% end of shelf-life specification.

Figure 3: SARS-CoV-2 Spike Binding Antibody Titers in Mouse Serum



Black dots correspond to the antibody titers of individual animal mouse serum samples. Boxplots display summary statistics of quartiles computed based on raw titers.

Figure 4: Comparability Analysis of Immunogenicity in Day 21 and Day 36 Samples



Black dots correspond to GMT ratios of each pair of lots, and the purple bars correspond to 90% CIs of GMTs based on the analysis of variance including 1 µg and 5 µg. The left plot shows results in 1 µg samples and the right plot shows results in 5 µg samples

Conclusion including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities, the following specification and acceptance criterion of [redacted] **main peak area; report impurity groups 1, 2, and 3 (End of Shelf-Life) and** [redacted] **main peak area; report impurity groups 1, 2, and 3 (Target for Product Release)"** for mRNA Purity for mRNA-1273 Drug Product is proposed. This criterion is within the validated range of the assay and covers the manufacturing experience to date, stability behavior and the likely analytical variability. Additionally, as mRNA purity is a key determinant of activity of the mRNA-1273 Drug Product, the specification limit proposed takes into account the pre-clinical experience with this product where activity (immunogenicity) has been demonstrated down essentially to the proposed purity specification. Furthermore, consideration for the shelf-life of the product has been made.

The manufacturing release for the product will be targeted at a level higher than the end of shelf-life specification to allow for product degradation upon storage. The shelf-life specification is recommended based upon the process and analytical capabilities; this is further supported by the statistical analysis detailed in Table 6. The proposed 100 µg dose with a [redacted] purity shelf-life specification therefore represents a suitable specification that permits sufficient shelf-life for the product.

3.2.P.5.6.5 % RNA Encapsulation: [redacted]

CQA Determination

RNA encapsulation is determined to be a CQA for the mRNA-1273 Drug Product. The LNP acts as a delivery vehicle and protectant for the mRNA Drug Product, therefore, encapsulation of the mRNA is essential for biological activity of mRNA-1273 Drug Product as well as stability of the mRNA. mRNA that is not encapsulated in an LNP will not enter cells and will not result in expression of the desired protein, therefore this assessment of encapsulation has a potential impact on protein expression. mRNA that is not encapsulated is not a safety concern, therefore this parameter is a CQA on the basis of activity.

Method Description

Historically at Moderna and in the broader scientific community, [redacted] has been used. [redacted]

ModernaTX, Inc. has developed a method [redacted]

Method Development and Analytical Performance

The [redacted] was developed to specifically quantify the amount of free and encapsulated mRNA in the presence of the components of the formulation buffer. It is particularly important to assess the impact of formulation components [redacted] (particularly salts and detergents). As a consequence of this observation, the method was refined to employ separate sample and standard curves for the determination of free mRNA and total mRNA as the liberation of mRNA for use in the determination of total mRNA utilizes a detergent/salt extraction buffer. The relative percentage of encapsulated mRNA is calculated from the determination of both free and total mRNA.

[redacted]

[redacted] Using the known total mRNA quantity in the sample (calculated using mRNA concentration by AEX- HPLC) it is thus possible to calculate the percentage of encapsulated mRNA. [redacted]

[redacted]

Table 8: Comparison of Encapsulation Values Generated Using a Range of Products [redacted]

Program	Storage Condition (°C)	LNP Formulation Buffer	[redacted]
mRNA-4157	-20	93 mM Tris, 7% PG, 1 mM DTPA, pH 7.5	
mRNA-4157	-20	93 mM Tris, 7% PG, 1 mM DTPA, pH 7.5	
mRNA-4157	-70	125 mM Tris, 16.4 mM Sodium Acetate, 8.75% Sucrose, pH 7.5	
mRNA-1893	-20	100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5	
mRNA-1647	-20	100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5	
mRNA-1653	-20	100 mM Tris, 7% PG, 1 mM DTPA, pH 7.5	
mRNA-3704	-70	20 mM Tris, 8% Sucrose, 1 mM DTPA, pH 7.5	
mRNA-1944	5	20 mM Tris, 8% Sucrose, 1 mM DTPA, 1.3% Ethanol, 60 mM NaCl, pH 7.5	
mRNA-3927	-70	20 mM Tris, 8% Sucrose, 1 mM DTPA, pH 7.5	
mRNA-3745	-70	20 mM Tris, 8% Sucrose, pH 7.5	

Abbreviations: DTPA = diethylenetriaminepentaacetic acid; LNP = lipid nanoparticle; [redacted]

Method Validation Data

[redacted] were validated as a quantitative purity test for mRNA-1273 Drug Product. A detailed validation summary is presented in Section 3.2.P.5.3,

Process Data

Data shown in Table 9 represents the mRNA encapsulation percentage measured for development and GMP lots using [redacted] (SOP-1000) assays. Stability results for development and GMP results are summarized in Table 10 and demonstrate the ability of the

method to detect changes in mRNA encapsulation at accelerated conditions using both methods. Upon storage at the intended condition (-20°C ± 5°C), no changes in encapsulation were observed, and good agreement of the 2 methods was demonstrated.

Table 9: RNA Encapsulation for Process Development and GMP Lots

Lot	[redacted]
8520100101	
8520100102	
8520100103	
8520100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	
6007520007	
DHM-47516	
DHM-47519	
6007320001	
6007320002	
6007320003	
Mean	
Standard deviation	
No. of batches	
Range	
Tolerance interval (one-sided) (99% Coverage, 95% CI)	

Abbreviations: [redacted] NA=Not Available; NT=Not Tested * Note, where a value is reported as "greater than or equal to" or "greater than", the numerical value reported is used in statistical analysis.
*The 95% confidence, 99% coverage for one-sided tolerance interval has a lower limit of 87.7. Since results of more than 100 are not possible, the upper acceptance limit will be set at 100 until more results are available.
**The 95% confidence, 99% coverage for one-sided tolerance interval has a lower limit of 95.1. Since results of more than 100 are not possible, the upper acceptance limit will be set at 100 until more results are available

Table 10: RNA Encapsulation [redacted] for Process Development and GMP Lots on Stability

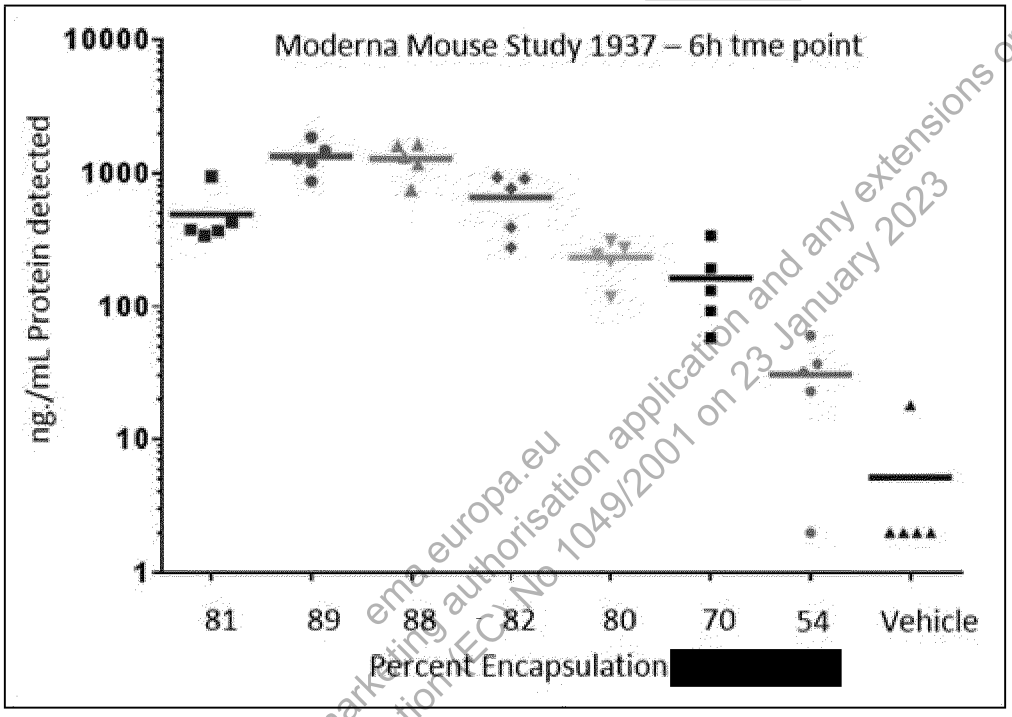
Lot	T=0	-20°C			5°C		
		T= 1 Month	T= 2 Month	T= 3 Month	T= 1 Month	T= 2 Month	T= 3 Month
DHM-47516	[redacted]	NT	[redacted]				
DHM-47519		NT					
8520100101		NT	NT	NT	NT	NT	NT
8520100102		NT	NT	NT	[redacted]		NT
8520100103		NT	NT	NT			[redacted]
8520100104		NT	NT	NT			
6007520001		[redacted]	NA	NA		NA	NA
6007520002			NA	NA		NA	NA
6007520003			NA	NA		NA	NA

Abbreviations: NT = not tested; NA = not available

Encapsulation and Immunogenicity

It has been demonstrated that low encapsulation can result in a reduction in expression, as is shown in Figure 5. mRNA that is not encapsulated is expressed to a lower level and result in a reduction of immunogenicity.

Figure 5: In Vivo Assessment of Lipid Nanoparticle Products Generated with Different Levels of Encapsulation, Measured Using [redacted]



Statistical Analysis

The summation of data generated to date are presented in Table 9. Data were not analyzed for statistical distribution or tolerance interval, all results were above 93% including upon stability. Furthermore, data for the proposed assay format [redacted] are in generation.

Conclusion Including Proposed Acceptance Criterion

Based upon the analytical method validation, historical data, and stability data the following specification and acceptance criterion of [redacted] **encapsulation"** for mRNA-1273 Drug Product are proposed. This criterion is also aligned with the historical data generated using [redacted] including preclinical evaluations of the impact of encapsulation for related products upon immunogenicity.

3.2.P.5.6.6 Particle Size and Size Distribution: Dynamic Light Scattering

CQA Determination

Average particle size is a CQA for the mRNA-1273 Drug Product. Particles of a defined size distribution are required in order to ensure delivery of the mRNA payload. A controlled particle size distribution is indicative of consistent manufacturing performance, and therefore a consistent performance of the product. Whereas polydispersity is indicative of the spread of the distribution, and is indicative of manufacturing performance, it has not been demonstrated, that this parameter impacts activity. There are no known safety concerns related to particle size or distribution within the ranges achievable in the process.

Method Description

Quantitation of particle size and distribution is achieved using Dynamic Light Scattering (DLS). DLS is a well-established methodology that utilizes fundamental, first-principles methodology in order to deconvolute light scattered by particles moving under Brownian motion in order to infer a particle size distribution and polydispersity of the sample. The determination of mean nanoparticle size (hydrodynamic diameter) is performed using batch-mode DLS in a dilute aqueous suspension. The hydrodynamic diameter is the diameter of an equivalent hard sphere that diffuses at the same rate as the analyte. The mean particle size is reported as the Z-average (diameter) and the distribution width is described by the polydispersity index.

Method Development and Analytical Performance

The method has been developed to determine the Z-average particle size and polydispersity index of mRNA-1273 Drug Product in the presence of the expected formulation buffer components. Specifically, the impact of instrumentation, scattered light gain settings and data reporting has been optimized. It is known that larger particles can impact the accuracy of the Z-average determination (larger particles disproportionately scatter more light than smaller particles) and accordingly a [redacted] filtration step was introduced to remove large interfering particulate matter and obtain a more accurate determination of the particle Z-average. The polydispersity index is reported as an indicator of the quality of the Z-average value and also reflects the breadth and heterogeneity of the particle size distribution.

Method Validation Data

This method is validated as a first-principles biophysical test, largely utilizing well-characterized analytical particle size standards, a detailed validation summary is presented in Section 3.2.P.5.3. The method was shown to be accurate, precise, and robust for the application of analysis of mRNA-1273 Drug Product. The validated method for determination of particle size and distribution of the mRNA-1273 Drug Product is defined in SOP-0998.

Process Data

Data shown in Table 11 represent the particle sizes and polydispersity measured for released development and GMP lots following SOP-0107.

Table 11: Dynamic Light Scattering Results for Process Development and GMP Lots

Lot	Particle Size (nm)	Polydispersity Index
8520100101	[redacted]	
8520100102		
8520100103		
8520100104		
6007520001		
6007520002		
6007520003		
6007520004		
6007520005		
6007520006		
6007520007		
DHM-47516		
DHM-47519		
6007320001		
6007320002		
6007320003		
Mean		
Standard deviation		
No. of batches		
Range		
Tolerance interval (Upper) (99% Coverage, 95% CI)		
Tolerance interval (Lower) (99% Coverage, 95% CI)		

*The 95% confidence, 99% coverage tolerance for one-sided interval has an upper limit of [redacted] Since negative results are not possible, the lower acceptance limit will be set at 0 until more results are available.

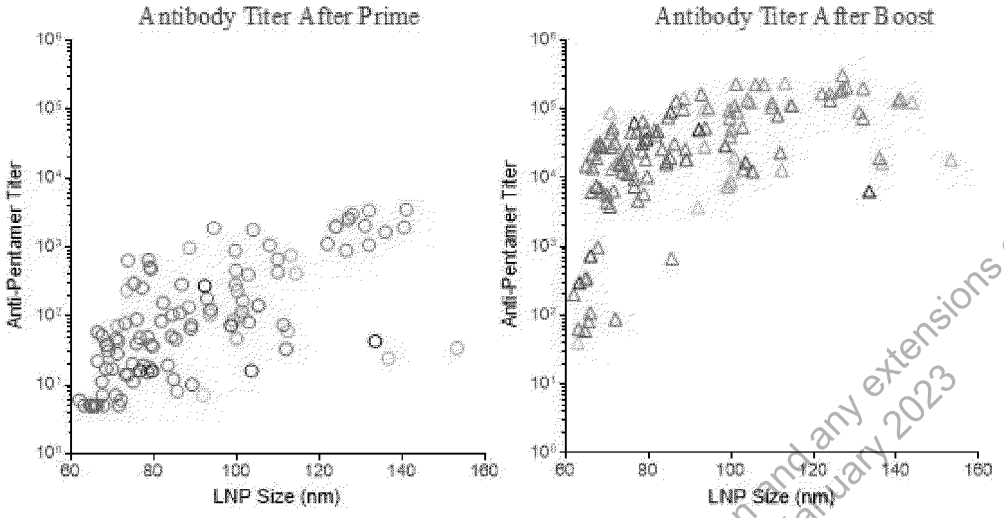
Statistical Analysis

The summation of data generated to date are presented in Table 11. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of particle size.

Particle Size and Immunogenicity

Preclinical studies conducted at ModernaTX, Inc. have previously shown that there is a measurable impact on immunogenicity in vaccines as a function of particle size as shown in Figure 6. It has been demonstrated on numerous occasions that there is lower boundary for activity of the product, particularly as demonstrated in mice. As the particle size increases, there is typically a trend towards an increase in immunogenicity.

Figure 6: Retrospective Analysis of 23 mRNA-1647 (CMV) Immunogenicity Studies Evaluating 129 LNP Formulations



Abbreviation: LNP = lipid nanoparticle
Note: Studies were conducted in Balb/C mice using an intramuscular administration and 3 µg dose.

Conclusion including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities, a statistical evaluation of the ranges expected and indicative platform preclinical data the following specifications and acceptance criteria of [redacted] for average particle size and [redacted] for polydispersity are proposed for mRNA-1273 Drug Product.

Polydispersity is indicative of manufacturing consistency, and as it has not been correlated to a CQA parameter, however the proposed specification of [redacted] is consistent with process and analytical capabilities. In addition, it has been reported that the methodology is capable of accurately determining polydispersity indices at the proposed level. Specifically, values [redacted] have been proposed as being applicable the dynamic light scattering (DLS) technique, as the various size distribution algorithms typically used work with data that falls between these two extremes (as detailed in the ISO standard document 13321:1996 E and ISO 22412:2008).

3.2.P.5.6.7 Lipid Identity

CQA Determination

Lipid identity is determined to be a CQA for the mRNA-1273 Drug Product. Inclusion of the incorrect lipids may impact protein expression (the correct lipids are ultimately required to engender the required pharmacological effect).

Method Description

The 4 lipids composing the mRNA-1273 Drug Product (SM-102, Cholesterol, DSPC and PEG2000-DMG) are identified by matching the observed retention times of each lipid in the LNP with known lipid standards. RP-UHPLC with CAD is used to separate each lipid and compare these to the unique retention times for the individual lipid reference materials.

Method Development and Analytical Performance

The method has been developed to specifically identify and quantify lipids and their impurities, in the mRNA-1273 Drug Product. The method has been refined through selection and optimization of the stationary and mobile phases, and the nebulization conditions employed for the CAD instrument. CAD was selected as a universal detection method as the lipids within the mRNA-1273 Drug Product do not possess strong chromophores that can be used to aid in detection. Additionally, CAD has analytical advantages in sensitivity and robustness as compared to comparable universal detection methods such as evaporative light scattering detection. The method solvent extraction methodology was optimized to ensure maximal recovery from the sample, with particular focus on DSPC extraction.

Method Validation Data

The method is validated, a detailed validation summary is presented in Section 3.2.P.5.3. The validated method for confirmation of lipid identity of the mRNA-1273 Drug Product is defined in SOP-1001. Specificity of the method was demonstrated by comparing the chromatographic behavior of the lipid components of the mRNA-1273 Drug Product to formulation buffer and other closely related LNPs with different lipid components. The selection of other LNPs was based on LNPs that could reasonably be expected to be present in the GMP manufacturing facility.

Process Data

Data are shown in Table 12 represent batches that have been verified for mRNA-1273 Drug Product identity using RP-UPLC with CAD detection methodology.

Table 12: RP-UPLC Identity Results for Released mRNA-1273 Drug Product Lots

Lot	Result
8520100101	Matches the retention time of reference
8520100102	Matches the retention time of reference
8520100103	Matches the retention time of reference
8520100104	Matches the retention time of reference
6007520001	Matches the retention time of reference
6007520002	Matches the retention time of reference
6007520003	Matches the retention time of reference
6007520004	Matches the retention time of reference
6007520005	Matches the retention time of reference
6007520006	Matches the retention time of reference
6007520007	Matches the retention time of reference
DHM-47516	Matches the retention time of reference
DHM-47519	Matches the retention time of reference
6007320001	Matches the retention time of reference
6007320002	Matches the retention time of reference
6007320003	Matches the retention time of reference

Abbreviations: RP-UHPLC = reverse-phase ultra-high-performance liquid chromatography

Statistical Analysis

Statistical analysis is not applicable to this analytical method as the result is non-numerical.

Conclusion including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities, the following specification and acceptance criterion of " **SM-102, cholesterol, DSPC, and PEG2000-DMG must match the retention time of reference"** for identity for mRNA-1273 Drug Product is proposed. Note that the only parameter used to support this is the specificity of the method.

3.2.P.5.6.8 Lipid Content and Impurities

Lipid content and purity are determined to be CQAs for the mRNA-1273 Drug Product. Individual and total lipid content is an important determinant of biological performance. Consequently, lipids must be present at expected levels in order to assure the correct structural organization of the mRNA containing LNP in subsequent downstream manufacturing steps and the resultant biological performance of the mRNA-1273 Drug Product. The majority of the toxicity/local tolerability profile is driven by the lipids, particularly the amino-lipid SM-102. A number of the lipid impurities have been characterized and although not representing a confirmed safety risk as identified by in silico and in vivo toxicology assessments, lipid impurities, are nonetheless controlled and reported.

Method Description

The method utilized for lipid content and impurities is the same as that used for lipid identity, when used for quantitation, chromatographic peaks corresponding to the individual lipids are compared against corresponding standard curves samples for each lipid and quantitated using a quadratic fit.

Method Development and Analytical Performance

The method utilized for lipid content and impurities is the same as that used for lipid identity. Accordingly, the same method development activities apply.

Method Validation Data

This method is validated, a detailed validation summary is presented in Section 3.2.P.5.3. The validated method utilized for lipid content and impurities of the mRNA-1273 Drug Product is defined in SOP-1001. The method was shown to be specific, accurate, precise, and linear for each of the lipid components.

Process Data

Data shown in Table 13 represent the lipid content and lipid impurity content for development and GMP lots.

Table 13: RP-UPLC Lipid Quantitation Results for Released mRNA-1273 Drug Product Lots.

Lot	Concentration (mg/mL)			
	SM-102	Cholesterol	DSPC	PEG2000-DMG
8520100101	[redacted]			
8520100102				
8520100103				
8520100104				
6007520001				
6007520002				
6007520003				
6007520004				
6007520005				
6007520006				
6007520007				
6007320001				
6007320002				
6007320003				
DHM-47516				
DHM-47519				
Mean				
Standard deviation				
No. of batches				
Range				
Tolerance interval (Upper) (99% Coverage, 95% CI)				
Tolerance interval (Lower) (99% Coverage, 95% CI)				

Abbreviations: DSPC = 1,2-distearoyl-sn-glycero-3-phosphocholine; RP-UHPLC = reverse-phase ultra-high-performance liquid chromatography.
- Values in **bold type** were used for statistical analysis as these are representative of the final commercial product concentration.

Statistical Analysis

The summation of data generated to date are presented in Table 13. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of lipids.

Conclusion including Proposed Acceptance Criteria

Based upon the analytical method and process capabilities, the following specification and acceptance criterion of [redacted] **L SM-102,** [redacted] **cholesterol,** [redacted] **DSPC,** [redacted] **PEG2000-DMG** for lipid content and "**Individual Impurities** [redacted] **area (report RRT) Total Impurities:** [redacted] **area"** for lipid impurity quantitation for mRNA-1273 Drug Product is proposed.

3.2.P.5.6.9 Particulate Matter: USP <788> Method 2

CQA Determination

Particulate matter is a CQA for the mRNA-1273 Drug Product. Particulate matter in injections and parenteral infusions consist of mobile undissolved particles, other than gas bubbles, unintentionally present in the solution. Control of particulate matter in the mRNA-1273 Drug Product is a pharmacopeial expectation.

Method Description

The particulate matter content in mRNA-1273 Drug Product is determined following USP <788> using the microscopic particle count test. Particle counts for particles ≥ 10 µm and ≥ 25 µm are reported.

Method Development and Analytical Performance

No additional method development was performed.

Method Validation Data

USP methods are validated prior to their inclusion within the USP. No additional method validation has been performed. The method has been verified as detailed in Section 3.2.P.5.3.

Process Data

Data shown in Table 14 represent the particulate matter content measured for development and GMP lots.

Table 14: Particulate Matter Results for Process Development and GMP Lots

Lot	≥ 25 µm (particles per container)	≥ 10 µm (particles per container)
8520100101	[redacted]	
8520100102		
8520100103		
8520100104		
6007520001		
6007520002		
6007520003		
6007520004		
6007520005		
6007520006		
6007520007		
6007320001		
6007320002		
6007320003		
DHM-47516		
DHM-47519		
Mean		
Standard deviation		
No. of batches		
Range		

Statistical Analysis

The summation of data generated to date are presented in Table 14. Statistical analysis was not performed upon the data set as the specifications will be aligned with the USP stipulations.

Conclusion including Proposed Acceptance Criterion

The acceptance criteria of "**For particles ≥ 25 µm, [redacted] per container; for particles ≥ 10 µm, [redacted] per container"** for this methodology is based upon the USP <788> requirements.

3.2.P.5.6.10 pH: USP <791>

CQA Determination

pH is a CQA for the mRNA-1273 Drug Product. pH control ensures stability during is shelf life, pH *per se* is not related to safety of the product, but as it may impact stability of the product it has a potential impact upon activity.

Method Description

pH is determined by potentiometric measurement following USP <791>.

Method Development and Analytical Performance

No additional method development was performed.

Method Validation Data

USP methods are validated prior to their inclusion within the USP. No additional method validation has been performed; however, method verification has been completed using the instrumentation proposed to be used for release of commercial supplies. The method has been verified as detailed in Section 3.2.P.5.3.

Process Data

Data shown in Table 15 represent the pH values measured for development and GMP lots.

Table 15: pH Results for Process Development and GMP Lots of Drug Product

Lot	[redacted]
8520100101	
8520100102	
8520100103	
8520100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	
6007520007	
6007320001	
6007320002	
6007320003	
DHM-47516	
DHM-47519	
Mean	
Standard deviation	
No. of batches	
Range	
Tolerance interval (Upper) (99% Coverage, 95% CI)	
Tolerance interval (Lower) (99% Coverage, 95% CI)	

Statistical Analysis

The summation of data generated to date are presented in Table 15. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of pH.

Conclusion including Proposed Acceptance Criterion

Based upon method performance and historical data the following specification and acceptance criterion of [redacted] for the pH of mRNA-1273 Drug Product is proposed. This pH range also provides conditions that contribute to the physicochemical stability of mRNA-1273 Drug Product during its shelf life.

3.2.P.5.6.11 Osmolality: USP <785>

CQA Determination

Osmolality is a critical not a quality attribute for the mRNA-1273 Drug Product. This parameter is however important as a verification of manufacturing control and is a regulatory expectation to be assessed.

Method Description

Osmolality is determined by freezing point depression measurement following USP <785>.

Method Development and Analytical Performance

No additional method development was performed.

Method Validation Data

USP methods are validated prior to their inclusion within the USP. No additional method validation has been performed; however, the method performance has been verified using the methodology and instrument intended to release commercial supplies. The method has been verified as detailed in Section 3.2.P.5.3.

3.2.P.5.6.11.1 Process Data

Data shown in Table 16 represent the osmolality values measured for development and GMP lots.

Table 16: Osmolality Results for Process Development and GMP Lots

Lot	Osmolality (mOsm/kg)
8520100101	[redacted]
8520100102	
8520100103	
8520100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	
6007520007	
6007320001	
6007320002	
6007320003	
DHM-47516	
DHM-47519	
Mean	
Standard deviation	
No. of batches	
Range	
Tolerance interval (Upper) (99% Coverage, 95% CI)	
Tolerance interval (Lower) (99% Coverage, 95% CI)	

Statistical Analysis

The summation of data generated to date are presented in Table 16. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of osmolality.

Conclusion including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities, the following specification and acceptance criterion of "[redacted] for the osmolality of mRNA-1273 Drug Product is proposed.

3.2.P.5.6.12 In Vitro Translation

CQA Determination

In vitro translation is a CQA for the mRNA-1273 Drug Product. Protein expression is a measure of activity for mRNA-1273 Drug Product.

Method Description

Determination of protein expression from mRNA in a Wheat Germ Cell-Free Translation System, uses a wheat germ lysate to translate encoded proteins from mRNA extracted from mRNA Drug Products, using a methionine amino acid analog that is incorporated into the translated protein, which is subsequently fluorescently labeled using Click chemistry. Protein expression is assessed via SDS-PAGE and imaging using the [redacted] instrument. In vitro translation was developed to assess protein translation of mRNA-1273 Drug Product.

Method Validation Data

This method described in SOP-0937 was validated as a quantitative method. A detailed validation summary is presented in Section 3.2.P.5.3. The method was demonstrated to be specific and accurate.

Process Data

Data shown in Table 17 represent the in vitro protein translation results for development and GMP lots. Results demonstrate an observed Molecular Weight (MW) range of [redacted] across different manufacturing batches and scales.

Table 17: In Vitro Protein Translation for Process Development and GMP Lots

Lot	Observed Molecular Weight (kDa)
8520100101	[redacted]
8520100102	
8520100103	
8520100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	
6007520007	
6007320001	
6007320002	
6007320003	
DHM-47516	
DHM-47519	
Mean	
Standard deviation	
No. of batches	
Range	
Tolerance interval (Upper) (99% Coverage, 95% CI)	
Tolerance interval (Lower) (99% Coverage, 95% CI)	

Statistical Analysis

The summation of data generated to date are presented in Table 17. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of in vitro protein translation.

Conclusion including Proposed Acceptance Criterion

Based upon method performance and historical data the following specification and acceptance criterion of [redacted]" for the in vitro translation of mRNA-1273 Drug Product is proposed. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of protein translation.

3.2.P.5.6.13 Container Content: USP <697>

CQA Determination

Container content is a CQA for the mRNA-1273 Drug Product since it ensures sufficient extractable volume to deliver the intended dose.

Method Description

Container content is determined by extractable volume measurement following USP <697>.

Method Development and Analytical Performance

No additional method development was performed.

Method Validation Data

USP methods are validated prior to their inclusion within the USP. The method has been verified as detailed in Section 3.2.P.5.3.

Process Data

Data shown in Table 18 represent the extractable volumes measured for development and GMP lots.

Table 18: Container Content for GMP Lots

Lot	Target Extractable Volume (mL)/ Target Fill Volume (mL)	Extractable Volume (mL)
8520100101	**Not Required**	**Not Tested**
8520100102	**Not Required**	**Not Tested**
8520100104	**Not Required**	**Not Tested**
6007520001	[redacted]	
6007520002		
6007520003		
6007520004		
6007520005		
6007520006		
6007520007		
6007320001		
6007320002		
6007320003		

Statistical Analysis

The summation of data generated to date are presented in Table 18. The data generated are not appropriate for statistical analysis as this parameter is limit-based, however Results demonstrate extractable volumes [redacted] from 1 vial), and [redacted] from 1 vial) for representative batches.

Conclusion including Proposed Acceptance Criterion

Based upon method performance and historical data the following specification and acceptance criterion of [redacted] **from 1 vial)"** for the container content of mRNA-1273 Drug Product is proposed.

3.2.P.5.6.14 Bacterial Endotoxins: USP <85>, Ph. Eur. 2.6.14

CQA Determination

Bacterial endotoxins are a CQA for the mRNA-1273 Drug Product. The acceptance criterion for bacterial endotoxin content in the mRNA-1273 Drug Product is based on the ability of the overall process to maintain quantities below an acceptable threshold for subsequent processing steps to meet the compendial safety limit for a parental product.

Method Description

The bacterial endotoxin content of mRNA-1273 Drug Product is determined following USP <85> and Ph. Eur. 2.6.14 by the turbidimetric technique which uses limulus amoebocyte lysate to detect and quantitate bacterial endotoxins.

Method Development and Analytical Performance

The lipid components in the mRNA-1273 Drug Product have been observed to cause interference in the assay. As such, dilution is required to overcome the interference, reducing the LOQ of the

method, thereby necessitating report of results often as below the LOQ. The diluents used and methodology for determining a valid result based on recovery of the challenge standard endotoxin is in alignment with the requirements of USP <85>.

Method Validation Data

USP methods are validated prior to their inclusion within the USP. No additional method validation has been performed; however, the method is verified for the minimum viable dilution to account for potential matrix interference effects. The method has been verified as detailed in Section 3.2.P.5.3.

Process Data

Data shown in Table 19 represent valid bacterial endotoxin content measured for development and GMP lots. Results demonstrate bacterial endotoxin content is typically less than 1 EU/mg across different manufacturing batches and scales.

Table 19: Bacterial Endotoxin Results for Process Development and GMP Lots

Lot	Bacterial Endotoxin Content (EU/mL)
8520100101	[redacted]
8520100102	
8520100103	
8520100104	
6007520001	
6007520002	
6007520003	
6007520004	
6007520005	
6007520006	
6007520007	
6007320001	
6007320002	
6007320003	
DHM-47516	
DHM-47519	
Mean	
Standard deviation	
No. of batches	
Range	
Tolerance interval (one-sided) (99% Coverage, 95% CI)	

Abbreviations: EU = endotoxin unit(s)
*The 95% confidence, 99% coverage for one-sided tolerance interval has an upper limit of 5.35. Since negative results are not possible, the lower acceptance limit will be set at 0 until more results are available.

Statistical Analysis

The summation of data generated to date are presented in Table 19. Data were analyzed to generate descriptive statistics (range, mean) as well as a standard deviation for the data set. For the tolerance interval analysis, a 99% population coverage and 95% confidence interval were applied for the analysis of endotoxin.

Conclusion including Proposed Acceptance Criterion

Based upon the analytical method and process capabilities and compendial requirements, the following specification and acceptance criterion of "[redacted]" for bacterial endotoxin of mRNA-1273 Drug Product is proposed.

3.2.P.5.6.15 Sterility: USP <71>, JP 4.06 and Ph. Eur. 2.6.1,

CQA Determination

Sterility is a CQA for the mRNA-1273 Drug Product. The acceptance criterion for sterility is based on the compendial limit for a parenteral product.

Method Description

The sterility of mRNA-1273 Drug Product is determined following USP <71>, JP 4.06 and Ph. Eur. 2.6.1 by membrane filtration methodology.

Method Development and Analytical Performance

No additional method development was performed.

Method Validation Data

USP methods are validated prior to their inclusion within the USP. The method has been verified as detailed in Section 3.2.P.5.3.

Process Data

Data shown in Table 20 represent the sterility measured for development and GMP lots following USP <71>, JP 4.06 and Ph. Eur. 2.6.1.

Table 20: Sterility for Process Development and GMP Lots

Lot	Result
8520100101	No Growth
8520100102	No Growth
8520100103	No Growth
8520100104	No Growth
6007520001	No Growth
6007520002	No Growth
6007520003	No Growth
6007520004	No Growth
6007520005	No Growth
6007520006	No Growth
6007520007	No Growth
6007320001	No Growth
6007320002	No Growth
6007320003	No Growth
DHM-47516	N/A (Low Bioburden)
DHM-47519	N/A (Low Bioburden)

Statistical Analysis

Statistical analysis is not applicable to this analytical method as the result is non-numerical. Results demonstrate each lot was sterile across different manufacturing batches and scales.

Conclusion including Proposed Acceptance Criterion

Based historical data and compendial requirements the following specification and acceptance criterion of "**No growth**" for sterility is proposed.

3.2.P.5.6.16 Specification Revisions During Development

Product specifications will be revisited as a component of product continuous improvement as additional manufacturing experience is gained.