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3.2.P.5.2 ANALYTICAL PROCEDURES

Analytical procedures, including compendial and non-compendial methods, will be used to assess the strength, identity, purity, safety and stability of mRNA-1273 Drug Product. Descriptions of the analytical procedures are provided in the following section.

3.2.P.5.2.1 SOP-0278: Appearance

SOP-0278 is a method to evaluate the appearance of samples (color, clarity, visible particulates) by visual inspection of mRNA-1273 Drug Product in accordance with current USP <631>, EP 2.2.1, and EP 2.9.20.

Procedure

mRNA-1273 Drug Product is assessed in a portable manual inspection hood consisting of an appropriate light source and vertical, non-glare white and matte black panel backgrounds. The light source is capable of maintaining an intensity of illumination, at the viewing point, between 2000 and 3750 lux. The product is observed against both black and white backgrounds under full-spectrum lighting. The product is examined for the presence of visible particulates. The results of the color, and visible particulates assessments are reported as required per the associated specifications Section 3.2.P.5.1.

3.2.P.5.2.2 SOP-1032: Identity

SOP-1032 is used to assess mRNA identity of mRNA-1273 Drug Product by extracting the mRNA from the mRNA-1273 Drug Product, using RT-PCR (Reverse Transcription-Polymerase Chain Reaction) to create an amplified double-stranded cDNA product, then Sanger sequence the product using an ABI genetic analyzer. Sample electropherograms are then assembled and compared to the reference sequence to confirm the sequence of the mRNA.

[redacted]

Instrument, Equipment, and Reagents

Instrumentation, equipment, and reagents for RT-PCR and Sanger Sequencing analysis are provided in Table 1. Standard laboratory equipment is not listed. Equivalent instruments and reagents may be substituted where indicated. Solutions prepared for use in this method are described in Table 2 and Table 3.

Table 1: SOP-1032: Instrument, Equipment, and Reagents

Instrument and Equipment	
Genetic Analyzer	
Thermocycler	
Thermomixer	
Electrophoresis Device	
Gel Imager	
Biosafety Cabinets (BSC)	
Reagents	
[redacted]	

Table 2: SOP-1032: mRNA-1273 [redacted] Primers

Primer Use	Primer Name	5'-Sequence-3'
RT-PCR	[redacted]	[redacted]
RT-PCR/ Sequencing		
Sequencing		
Sequencing		

Table 3: SOP-1032: Solution Preparation

Solution	Composition
Stock Primers ([redacted] primers in water)	Dilute the lyophilized primers to [redacted] with [redacted] ([redacted] water per 1 nmol primer).
RT-PCR Working Primers ([redacted] primers in water)	Dilute the [redacted] stock primers to [redacted] with [redacted]
Sequencing Working Primers ([redacted] primers in water)	Dilute the [redacted] stock primers to [redacted] with [redacted]

Sample and Control Preparation

The mRNA-1273 Drug Product samples undergo an mRNA extraction using [redacted]

The positive control is diluted to 100 ng/µL with [redacted] into a microcentrifuge tube.

Procedure

The extracted samples, the diluted positive control, and negative control undergo an RT-PCR reaction using the RT-PCR master mix containing [redacted] and RT-PCR primers. Using a thermocycler, cDNA is made from the mRNA, then PCR amplified using the following program settings (Table 4).

Table 4: SOP-1032: RT-PCR Thermocycler Program

Step	Temperature	Time
cDNA Synthesis	[redacted]	[redacted]
Initial denature		
Extension (Cycle 30 times)		
Final Extension		
Hold		

The cDNA products of the RT-PCR samples and controls then undergo gel electrophoresis to confirm cDNA synthesis and amplification, and to confirm that the primers produced a band that is the expected size. The RT-PCR product samples and controls are purified using the [redacted] reagent is added to the RT-PCR products and purified in a thermocycler using an [redacted] thermocycling program (Table 5).

Table 5: SOP-1032: [redacted] Thermocycler Program

Step	Temperature	Time
PCR Clean-up	[redacted]	[redacted]
Heat inactivation		
Hold		

The purified RT-PCR reactions are then pooled and undergo a Sanger Sequencing reaction. The samples and controls are mixed with the sequencing reaction master mix containing [redacted] 5x buffer, and the [redacted] The reaction mixtures of the samples and controls are plated into a PCR plate the corresponding sequencing primer is added per a predetermined plate map. Each sequencing reaction is prepared [redacted] The plate is then loaded into a thermocycler and the sequencing thermocycling program (Table 6) is run.

Table 6: SOP-1032: Sequence Thermocycler Program

Step	Temperature	Time
Initial denature	[redacted]	[redacted]
Extension (Cycle [redacted] times)		
Hold		

The sequencing reactions are then purified using the [redacted] purification kit. [redacted] solution is added to each reaction well of the sequencing plate and is then mixed at [redacted] using a thermomixer. The purified sequencing reactions are loaded into the genetic analyzer and are run using the [redacted] assay (instrument protocol: [redacted] Base calling protocol: [redacted].

Data Analysis and Reporting

Utilizing the [redacted] software, the sequencing data of the samples and positive control are assembled and compared to their respective reference sequence.

System Suitability and Test Article Acceptance Criteria

System suitability and acceptance criteria are summarized in Table 7.

Table 7: SOP-1032: System Suitability and Test Article Acceptance Criteria

Category	Parameter	Acceptance Criteria
System suitability	RT-PCR reaction success and specificity.	The RT-PCR band for both the samples and positive control is within [redacted] the expected size of the RNA. No band is visible in the negative control.
System suitability	No Template Control (NTC)	The KB basecaller of NTC samples generates no more than 5Ns or visual confirmation of no clear electropherogram signal.
System suitability	Sequencing Positive Control	[redacted] of the positive control must match the reference sequence 2x in the forward direction.
Sample Suitability	Sample Sequencing Coverage	The [redacted] of the sample is sequenced [redacted] with [redacted] trimming algorithm. For regions where only limited data can be obtained (including but not limited to regions after homopolymers or where structure impacts sequencing quality) a [redacted] will be acceptable; document justification for limited coverage.
Sample acceptance	Sample Identity	Evaluate the alignment of the consensus sequence to the reference sequence. If the consensus sequence matches the reference sequence with 100% homology, the test article nucleotide sequence conforms to the identity test specification.

3.2.P.5.2.3 SOP-0999: Total RNA Content

SOP-0999, Determination of RNA concentration in SM-102 LNPs by IEX chromatography with UV detection, is used to quantitate the mRNA content in mRNA-1273 Drug Product. [redacted] The mRNA concentration is quantitated using a reference standard (Section 3.2.S.5 {CX-024414}) and single point calibration calculation.

Instrument, Equipment, and Reagents

Instrumentation, equipment, and reagents for IEX HPLC analysis are provided in Table 8. Standard laboratory equipment is not listed. Equivalent instruments and reagents may be substituted where indicated. Solutions prepared for use in this method are described in Table 9.

Table 8: SOP-0999: Instrument, Equipment, and Reagents

Instrument and Equipment
UHPLC or HPLC system with UV detection
[redacted]column, or equivalent
pH meter
Analytical Balance, capable of reading to 0.1 mg
Reagents
[redacted]

Table 9: SOP-0999: Solution Preparation

Solution	
Stock Mobile Phase Buffer 1:	[redacted]
Stock Mobile Phase Buffer 2:	[redacted]
Mobile Phase A:	[redacted]
Mobile Phase B:	[redacted]
Method Diluent:	[redacted]
Column Wash:	[redacted]
Needle Wash:	[redacted]
Seal Wash (as applicable):	[redacted]

Sample and Standard Preparation

- Working (Reference) Standard Preparation: [redacted] reference standard in method diluent
- Check Standard Preparation: [redacted] reference standard in diluent
- Sample Preparation: [redacted] sample in diluent, [redacted]

Procedure

Blank diluent is [redacted] of reference standard, then blank diluent injection followed by a check standard injection at the beginning of each analysis on an HPLC system [redacted]

[redacted] Each sample is prepared [redacted] Reference standard is injected to bracket [redacted] and each sequence ends [redacted] Samples are stable in the autosampler, before injection, for [redacted] The chromatographic conditions for analysis are summarized in Table 10 and an example injection sequence in presented in Table 11. A representative chromatographic profile is shown in Figure 1.

Table 10: SOP-0999: HPLC Operating Parameters

Parameter	Condition		
Mobile phase A (MPA)	[redacted]		
Mobile phase B (MPB)			
Needle wash			
Seal wash (for [redacted] HPLC system)			
Column Wash			
Flow rate			
Column temperature			
Post-Column Cooler			
Autosampler temperature			
Injection/ Needle Wash			
Recommended Needle Drawing Speed			
Detection			
Calibration Settings			
Acquisition time			
Injection volume			
Gradient	**Time (minutes)**	**% MPA**	**% MPB**
	[redacted]	[redacted]	[redacted]

Table 11: SOP-0999: Example Injection Sequence

Sample Name	Number of Injections
[redacted]	[redacted]
Reference Standard [redacted]	
[redacted]	
Check Standard [redacted]	
[redacted]	
[redacted]	
[redacted]	
Bracketing Standard (Reference Standard)	
[redacted]	
[redacted]	
[redacted]	
Bracketing Standard (Reference Standard)	
[redacted]	

Data Analysis and Reporting

Calculate carryover, precision, % recovery of standards and sample concentration.

Report the average concentration of the sample replicates.

- $Dilution\ Factor = \frac{Total\ Volume\ of\ Sample\ Preparation}{Volume\ of\ Sample\ Added}$
- $\%\ Blank\ Interference = \frac{Diluent\ Area\ (mAU)}{Mean\ Response\ Reference\ Standard\ Area\ (mAU)} * 100$
- $Check\ Standard\ Recovery = \frac{Result\ (\frac{mg}{mL})}{Nominal\ Concentration\ (\frac{mg}{mL})} * 100$
- $Bracketing\ Standard\ Recovery = \frac{Bracket\ Standard\ Area\ (response)}{Mean\ response\ Reference\ Standard\ Area\ (mAU)} * 100$
- $Sample\ Concentration = \frac{(Sample\ Peak\ Area * Standard\ Conc.)}{Mean\ Standard\ Peak\ Area} * Dilution\ Factor$
- $\frac{ug}{vial} = \left(Concentration\ Result\ \left(\frac{mg}{mL}\right) * 1000\right) * Total\ Reconstituted\ Volume\ (mL)$

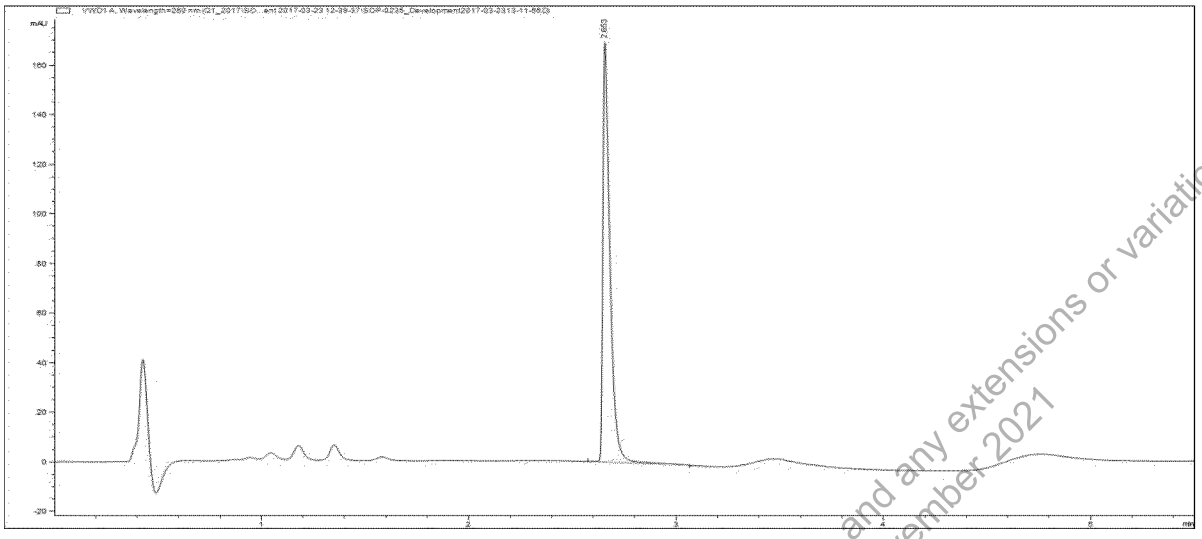
System Suitability and Test Article Acceptance Criteria

Reference standard is analyzed within each analysis to ensure the system is suitable for use on each day of analysis. System suitability and acceptance criteria are summarized in Table 12.

Table 12: SOP-0999: System Suitability and Test Article Acceptance Criteria

Category	Parameter	Acceptance Criteria
System suitability	[redacted]	[redacted]
System suitability		
System suitability		
System suitability		
System suitability		
Sample acceptance		

Figure 1: Representative Reference Standard Chromatogram on HPLC System



3.2.P.5.2.4 SOP-0996: Purity and Product-related Impurities

SOP-0996 is used to assess mRNA purity of mRNA-1273 Drug Product. The method separates mRNA species by size, using reverse phase ion-pair high performance liquid chromatography (RPIP HPLC) and gradient elution. Detection is performed by [redacted] Total purity and impurities are calculated as percent peak area. The reference material for this method is described in Section 3.2.S.5 {CX-024414}.

Instrument, Equipment, and Reagents

Instrumentation, equipment, and reagents for RPIP HPLC analysis are provided in Table 13. Standard laboratory equipment is not listed. Equivalent instruments and reagents may be substituted where indicated. Solutions prepared for use in this method are described in Table 14.

Table 13: SOP-0996: Instrument, Equipment, and Reagents

Instrument and Equipment
HPLC system with UV detector,
Refrigerated Centrifuge
[redacted]
Reagents
[redacted]

Table 14: SOP-0996: Solution Preparation

Solution	Composition
Mobile phase A	[redacted]
Mobile phase B	
Needle wash	
Seal wash	
Sample Diluent	
Reference standard solution	
Sensitivity Solution [redacted] mRNA in water)	

Standard Preparation

Dilute Reference material (Section 3.2.P.6) to [redacted] in HPLC Grade water.

mRNA-1273 Drug Product Sample Preparation

Prepare samples in duplicate.

Dilute mRNA-1273 Drug Product material to [redacted] in water. Combine [redacted] diluted mRNA-1273 Drug Product, [redacted] and [redacted] Mix sample by inversion and vortex. Centrifuge the sample [redacted]

Verify formation of a blue pellet, pour off supernatant. Blot excess supernatant, as necessary. Air dry the resulting pellet [redacted] Resuspend the sample in [redacted] Allow [redacted] o resolubilize the pellet, vortexing intermittently to reconstitute. Verify the pellet is dissolved prior to transfer to HPLC vial.

HPLC Analysis and Operating Conditions

Samples of Reference material are injected in duplicate, mRNA-1273 Drug Product sample preparations are injected in singlet, with 2 preparations per sample.

Table 15: SOP-0996: Example Injection Sequence

Sample Name	Number of Injections
[redacted]	

Table 16: SOP-0996: HPLC Operating Conditions

Parameter	Condition		
[redacted]	[redacted]		
Flow rate			
Column temperature			
Pre-Column Heater Temperature			
Post-Column Cooler			
Draw Speed			
Dispense Speed			
Needle Wash Settings			
Autosampler temperature			
[redacted]			
Acquisition time			
Injection volume			
Gradient	**Time (minutes)**	**% MPA**	**% MPB**
	[redacted]	[redacted]	[redacted]

Data Analysis, System Suitability and Data Reporting

Data Analysis

- Using the [redacted] baseline subtract all chromatograms.
- Peaks are integrated and labeled for each reference standard and sample chromatogram as depicted in the representative chromatographic profiles (Figure 2).
  - Reference Standard Profiles will have 3 peaks areas identified and integrated: Pre-Main Peak, Main Peak, Post-Main Peak.
  - mRNA-1273 Drug Product will have 4 Peaks identified and integrated: [redacted] Main Peak, [redacted]
- The signal-to-noise ratio (S/N) of the main peak in the detectability standard injection is calculated using the S/N calculation.
- Calculate the % carryover by comparing the peak areas in the [redacted] prior to the sensitivity standard and all carryover [redacted] to the average total peak area of the [redacted]
- The percent recovery of the main peak area for each bracketing standard is calculated with respect to the average main peak area from [redacted]

- The percent agreement of the main peak retention time for each bracketing standard is calculated with respect to the average main peak retention time from [redacted]
- The % relative standard deviation (% RSD) of the main peak area and retention time for [redacted] will be calculated.
- The relative percent peak area of the mRNA peak, or percent purity, is calculated as follows: $\%\ Purity = \frac{Peak\ area\ of\ mRNA\ peak}{Total\ chromatographic\ peak\ area} \times 100\%$
- The relative percent peak area of the total impurities is calculated as follows:

$$\%\ Total\ Impurities = \frac{Total\ peak\ area\ of\ impurities}{Total\ chromatographic\ peak\ area} \times 100\%$$

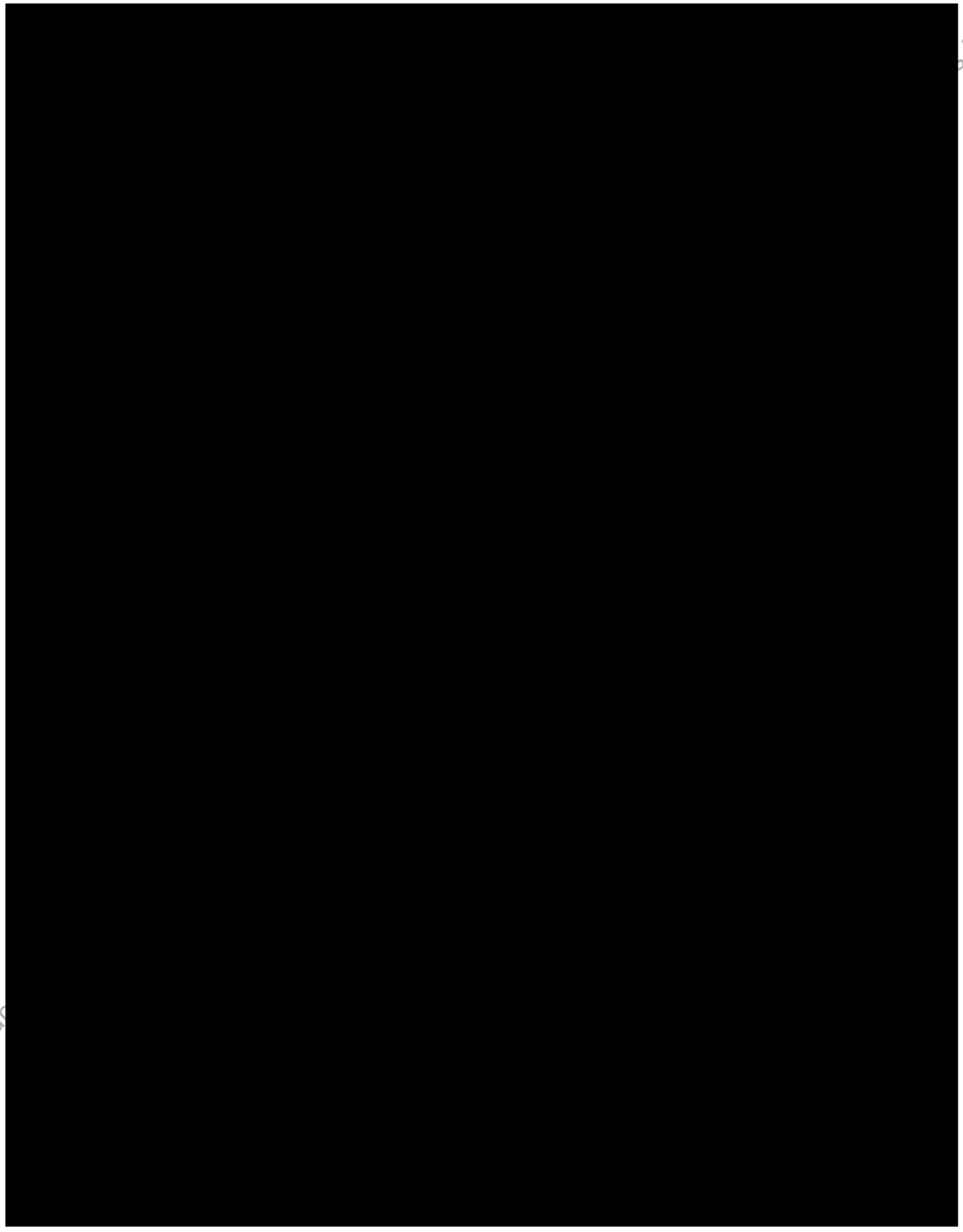
- The total peak area is the sum of the peaks noted above, not to include artifact and diluent peaks.
- Calculate the Absolute Difference between the main peak % area in duplicate injections of each sample.
- Calculate the percent recovery of the total peak area for each sample replicate with respect to the average total peak area from [redacted]

System Suitability and Test Article Acceptance Criteria

Table 17: SOP-0996: System Suitability and Test Article Acceptance Criteria

Category	Parameter	Acceptance Criteria
System suitability	[redacted]	
System suitability		
System suitability		
System suitability		
System suitability		
System suitability		
Sample acceptance		
Sample acceptance		

Figure 2: Representative Chromatograms (Top Traces – Full Scale Reference Standard and mRNA-1273 Drug Product, Bottom Traces – Peak Detail, Reference Standard and mRNA-1273 Drug Product)



3.2.P.5.2.5 SOP-1000: % RNA Encapsulation

SOP 1000 describes the absorbance-based assay for the detection of free mRNA, [redacted] This method is used to assess the percentage of total mRNA present that is encapsulated within the LNP [redacted] The % Encapsulation Efficiency is reported.

Instrument, Equipment, and Reagents

Equipment and Materials for [redacted] are provided in Table 18, reagents are provided in Table 19 and Materials and consumables are provided in Table 20. Equivalent equipment, materials, reagents and consumables may be substituted unless otherwise indicated. Solutions prepared for use in this method are described in Table 21.

Table 18: SOP-1000: Equipment and Materials

Equipment/ Materials
[redacted]

Table 19: SOP-1000: Reagents

Reagents
[redacted]

Table 20: SOP-1000: Materials/Consumables

Materials/ Consumables
PCR Clean Eppendorf pipette tips
[redacted]
Disposable Cuvettes
[redacted]
Magnetic stir bar
[redacted]

Table 21: SOP-1000: Solution Preparation

Materials/ Consumables
[redacted]

Sample Preparation

-

Procedure

System Suitability

The plate reader is set to the system suitability software parameters summarized in Table 22.

Table 22: SOP-1000 System Suitability Plate Reader Software Parameters

Parameter	Setting
Read Mode	ABS
Read Type	Endpoint
Read at Wavelength	

The absorbance of the NIST reference standard is determined a The result must be Certificate of Analysis (CoA) value +/- Absorbance units.

Sample Testing

The plate reader software is set to the parameters as indicated in Table 23.

Table 23: SOP-1000: Sample Testing Plate Reader Software Parameters

Parameter	Setting
Read Mode	ABS
Read Type	Endpoint
Read at Wavelength	

Prior to sample analysis, 3 mL of Formulation Buffer is added to a cuvette and absorbance is read on the plate reader as the buffer blank.

Data Analysis and Data Reporting

Table 24: SOP-1000: Formulas to Calculate %EE

[redacted]

System Suitability and Test Article Acceptance Criteria

Table 25: SOP-1000: System Suitability Acceptance Criteria

Parameter	Acceptance Criterion
The absorbance of the NIST reference standard [redacted]	CoA +/- [redacted] Absorbance units

Table 26: SOP-1000: Sample Acceptance Criteria

Parameter	Acceptance Criterion
% RSD of the mean % EE results for triplicate sample preparations	[redacted]

3.2.P.5.2.6 SOP-0937: In Vitro Translation

SOP-0937 describes the procedure used to confirm the ability of mRNA-1273 Drug Product to translate a polypeptide of expected molecular weight [redacted] mRNA-1273 Drug Product [redacted] and [redacted] The sample is subsequently reacted [redacted] to fluorescently label the translated protein. The protein is separated using a [redacted] and labeled protein is visualized using a [redacted] imaging system and compared against a protein molecular weight ladder standard. The actual protein molecular weight is compared to the expected value. The expected molecular weight is determined from the amino acid sequence encoded from the mRNA (CX-024414) nucleotide sequence.

Instrument, Equipment, and Reagents

Instrumentation, equipment, and reagents for in vitro translation analysis are provided in Table 27. Standard laboratory equipment is not listed. Equivalent instruments and reagents may be substituted unless where indicated.

Table 27: In Vitro Translation Assay: Materials, Equipment, and Reagents

Materials and Equipment
Hard-Shell, 96-well, thin wall PCR plates
[redacted] pipette tips
[redacted] Tips, standard, round
Adhesive PCR Plate Foils
[redacted] Microcentrifuge tubes
Plate sealer roller
[redacted] Serological Pipettes [redacted]
Corning UV plate, 96-well with UV transparent flat bottom
[redacted] pipettes
Calibrated Timer
Thermomixer C
Mastercycler Pro S
Thermomixer Heat Block Adapter 96 well half/full skirted PCR
Electrophoresis Power Supply
[redacted] Imaging System [redacted]
Microcentrifuge
[redacted]
Pipet-Aid
[redacted]
[redacted] Plate Reader
Reagents
[redacted]

Control and Sample Preparation

Extraction

[redacted]

[redacted]

Concentration Determination

The extracted mRNA concentration is determined via UV directly or stored [redacted] RNA concentration is determined with a single sample dilution using [redacted] and CX-024414 as a positive control, reading [redacted]

Denature the RNA

The negative control [redacted], the Positive control (CX-024414) and [redacted] samples are added to a 96-well plate and sealed with an aluminum foil seal. A thermomixer or thermocycler is used to denature the mRNA/negative control plate [redacted]

Reagent Preparation

[redacted]

Procedure

Enough Master Mix is prepared for the samples, positive control and negative control per Table 28.

Table 28: In Vitro Translation: Preparation of Master Mix

Reagent	A For 1 Reaction volume (µl)	B Multiplier	C Actual Volume (µl)
[redacted]	[redacted]	(n samples + 3)	(Columns A x B)
			(Columns A x B)
			(Columns A x B)
			(Columns A x B)
Total Volume			(Columns A x B)

[redacted]

Data Analysis and Reporting

Data Analysis

[redacted]

Table 29: In Vitro Translation: Example Well assignments

Lane	Contents
1	*Running Buffer Alone*
2	*Running Buffer Alone*
3	*Running Buffer Alone*
4	Molecular Weight Ladder Standard
5	Negative Control
6	Positive Control (CX-024414)
7	Sample 1 (a)
8	Sample 1 (b)
9	Molecular Weight Ladder Standard
10	*Running Buffer Alone*
11	*Running Buffer Alone*
12	*Running Buffer Alone*

The gel is run [redacted] until the dye front runs out of the gel. The gel is washed in [redacted] and then scanned [redacted] on the medium (or higher) scan setting.

Calculation and System Suitability

The positive control and sample approximate molecular weights (MW) are calculated using the scanner software. The mean MW of the duplicate samples is reported rounded to the nearest whole number. The % difference for each substance is calculated according to the following formula and rounded to the nearest whole number.

$$\%\,Difference = \frac{Molecular\ Weight\ (MW)\ actual - MW\ expected}{MW\ expected} x\ 100$$

System suitability is determined by the following conditions:

1. Clearly separate bands are visible for the Protein Ladder Standard.
2. MW band observed in the samples and positive controls are within 35% of their respective MWs.
3. The Negative Control does not have bands that correlate with the expected MW bands.

Data Reporting

The mean MW rounded to the nearest whole number for the predominant protein band expressed from the sample and the % difference from the mean are reported. The presence/absence of the MW bands for the Negative control, the Positive control and Drug Product samples are also reported.

3.2.P.5.2.7 SOP-0998: Particle Size and Polydispersity

SOP-0998 is used to determine the particle size distribution of mRNA-1273 Drug Product using Dynamic Light Scattering (DLS). [redacted]

Instrument, Equipment, and Reagents

Instrumentation, equipment, and reagents for DLS analysis are provided in Table 30. Standard laboratory equipment is not listed. Equivalent instruments and reagents may be substituted where indicated. Solutions prepared for use in this method are described in Table 31.

Table 30: SOP-0998: Instrument, Equipment, and Reagents

Instrument and Equipment
[redacted]
Sonicator Bath
Electronic repeater positive displacement pipette
Variable adjustable pipettes, capable of measuring [redacted]
Disposable low volume cuvette for size measurement, minimum volume [redacted]
[redacted]
Syringes, Luer Lok Tip, [redacted]
[redacted] clear target snap-IT ID™ vial
Reagents
[redacted]

Table 31: SOP-0998: Solution Preparation

Solution
Sample and Standard Dispersant (Diluent) [redacted] stock Solution
Standard Dispersant – [redacted] Working Solution (WS)

Sample and Standard Preparation

Standard Preparation

Three polymer standards of different nanosphere sizes [redacted] are used to verify the performance of the instrument. Each standard must be diluted prior to analysis. Ensure that the size standard being utilized is at room temperature. Vortex each bulk size standard [redacted] just prior to use. [redacted]

[redacted]

Sample Preparation

Sample preparation can be modified as long as the final sample concentration of [redacted] is maintained.

Prepare each sample in duplicate. Dilute the sample to a nominal concentration of [redacted] mRNA using 1x PBS. Prepare [redacted] solution for analysis using the equation below.

[redacted]

Procedure

Operate the [redacted] instrument, or equivalent, according to the parameters below to align with sample type; for mRNA-1273 Drug Product Table 32.

Table 32: SOP-0998: [redacted] Instrument Method for mRNA-1273 Drug Product

[redacted] Instrument Method Parameters			
Measurement type: Size			
Parameter	**Property**	**Specification**	**Comment**
Sample	Material	[redacted]	
	Dispersant		
	General Options		
	Temperature		
	Cell		
Measurement	Measurement angle		
	Measurement duration		
	Advanced		
Data Processing	Analysis model		

Suitability Verification should be performed on the DLS instrument each time the instrument is used for sample analysis. Two polymer standards of different nanosphere sizes [redacted] are used to verify the performance of the instrument prior to sample analysis.

Transfer [redacted] Sample Dispersant blank (1x PBS) to clean disposable cuvette, load cuvette into the instrument and measure particle size. Proceed to sample analysis once sample dispersant blank results shows absence of interfering particles. Transfer [redacted] filtered sample to disposable cuvette, load cuvette into the instrument and measure particle size. Once sample analysis is complete, perform an end standard verification with the [redacted] nanosphere standard to assure proper instrument performance over the timespan of the assay. Each standard must be diluted prior to analysis.

Data Analysis and Reporting

- Report the Z-average (Z-Av) and the Polydispersity Index (PDI) values found in the Intensity PSD (M) tab of the [redacted] software.
- Report the diameter as the average of the two preparations for each sample.

System Suitability and Test Article Acceptance Criteria

Table 33: SOP-0998: System Suitability and Test Article Acceptance Summary

Category	Parameter	Acceptance Criteria		
System suitability	Particle Size Z-Average (diameter, nm)	**Size Standard**	**Acceptable Range**	**Polydispersity Index (PDI)**
		[redacted]		
Sample acceptance	Sample Dispersant blank	Shows absence of interfering particles (0 nm and "refer to quality report" error)		
	Result Quality	Indicated as "good" for each sample tested		
	Polydispersity Index (PdI)	[redacted]		

3.2.P.5.2.8 SOP-1001: Lipid Identification, Lipid Content, Lipid Impurities

SOP-1001 describes the procedure for the analysis of lipid content, lipid impurities (%area) and lipid identity for mRNA-1273 Drug Product by retention time comparison using Ultra-High-Performance Liquid Chromatography with Charged Aerosol Detection UHPLC-CAD. Lipid reference materials used for this procedure are described in Section 3.2.S.5 {SM-102 LNP}.

Instrument, Equipment, and Reagents

Instrument, equipment and reagents for UHPLC-CAD analysis are provided in Table 34. Equivalent instruments, equipment and reagents may be substituted where indicated or provided they are of equivalent grade. Solutions prepared for use in this method are described in Table 35.

Table 34: SOP-1001: Instrument, Equipment and Reagents

Instrument and Equipment
UHPLC system with [redacted]
Analytical Balance, capable of reading to 0.1 mg, Mettler Toledo, XPE205
Centrifuge, capable of [redacted] Thermo Fisher Scientific, Legend Micro 17
Sonication Bath, Branson, 3800
Eppendorf variable adjustable pipettes, capable of measuring [redacted] Eppendorf, [redacted]
Eppendorf Repeater, Xstream Pipetter, Eppendorf, [redacted]
Reagents
[redacted]

Table 35: SOP-1001: Solution Preparation

Solution
[redacted]

Sample and Standard Preparation

Table 36: SOP-1001: Standard Preparation

Standard	Preparation
[redacted]	

QS: Quantum Suifficit

Table 37: SOP-1001: Sample Preparation

Sample	Preparation
[redacted]	

Procedure

[redacted] resolution standard, each level of calibration standards (once per calibration standard except for Level 3 [redacted], check standard [redacted] are performed at the beginning of each analysis on an UHPLC-CAD system equipped with a [redacted] Reverse Phase (RP) column. Each sample is prepared [redacted] and [redacted] Check standard and [redacted] and at the end of each analysis. The chromatographic conditions for analysis are summarized in Table 38 and an example injection sequence in presented in Table 39. A representative chromatographic profile is shown in Figure 3.

Table 38: SOP-1001: HPLC Operating Parameters

Parameter	Condition		
Mobile phase A (MPA)	[redacted]		
Mobile phase B (MPB)			
Wash Solvent A			
Wash Solvent B			
Needle/Seal wash			
Flow rate			
Column temperature			
Pre-Column Heater Temperature			
Post-Column Heater Temperature			
Autosampler temperature			
Detection			
Acquisition time			
Injection volume			
Gradient	Time (minutes)	% MPA	% MPB
	[redacted]		

Table 39: SOP-1001: Example Injection Sequence

Sample	Number of injections	Injection Volume (µL)
Sample Diluent	[redacted]	
Sensitivity Solution		
Resolution standard		
Standard Level 1		
Standard Level 2		
Standard Level 3		
Standard Level 4		
Standard Level 5		
Standard Level 6		
Check Standard		
Sample Diluent		
Sample 1 Prep 1		
Sample 1 Prep 2		
Sample 2 Prep 1		
Sample 2 Prep 2		
Sample 3 Prep 1		
Sample 3 Prep 2		
Check Standard		
Sample Diluent		
Samples 4 - 6		
Check Standard		
Sample Diluent		
End (Column Wash)		

*Inject additional diluent injections as needed until a stable baseline is achieved.

Data Analysis and Reporting

- **Lipid Identity**
  Use the results of the two replicate samples to calculate the average % retention time (%RT) for each of the major lipids. The retention time for each lipid sample conforms to the retention time of the corresponding lipid in the standard if % RT is 100 ± 3 %.
  Report results as "conforms to" or "does not conform".
- **Total Lipid Content**
  Calculate and report the average amount in mg/mL or µg/vial [refer to the specification (Section 3.2.P.5.1) for appropriate reporting units] for all 4 main lipids (SM-102, PEG-DMG, Cholesterol and DSPC) in each sample.
  Report mg/mL results to one decimal place and µg/vial results to a whole number.
- **Lipid Impurities (% Area)**
  - All integrated peaks including SM-102, Cholesterol, DSPC and PEG2000-DMG are used to calculate % area of individual peaks.
  - Only report impurity peaks that are [redacted] relative percent area.
  - Report % area for individual degradant/impurity [redacted] to two decimal places.

- Report % area of individual degradant/impurity [redacted] to one decimal place.
- Report the total % area for all impurities to 1 decimal place (e.g. 1.0%).
- Identify and label each known impurity as shown in Figure 3b, and unknown impurities by their relative retention time (RRT).
- Calculate the RRT of each impurity/degradant relative to Cholesterol and report to 2 decimal places.

Carryover, precision, accuracy of standards and sample concentration can be calculated and recorded manually or by the chromatography data system. Example calculations are described below.

- $\textbf{Dilution Factor} = \frac{Total\ Volume\ of\ Sample\ Preparation}{Volume\ of\ the\ Sample\ Added}$
- $\textbf{Stock Standard Concentration calculation} = \frac{Weight_{lipid\ component} * purity_{lipid\ component}}{volume\ of\ diluent}$
- $\textbf{Std. Level x Concentration calculation} = \frac{Stock\ Std.\ Concentration}{Level\ x\ Dilution\ Factor}$
- $\textbf{Response Factor} = \frac{Peak\ Area}{Concentration}$
- $\textbf{Relative Retention Time (RRT)} = \frac{Retention\ Time\ Peak}{Retetntion\ Time\ Cholesterol\ Peak}$
- $\%\ \textbf{Blank Interference} = \frac{Interfering\ Peak\ Area\ (pA*min)}{Mean\ response\ Level\ 3\ Cal.Std.Area\ (pA*min)} * 100$
- $\%\ \textbf{RSD Peak Area Precision} = \frac{Standard\ deviation\ of\ Peak\ Area\ (pA*min)}{Average\ Peak\ Area\ (pA*min)} * 100$
- $\%\ \textbf{RSD Retention Time Precision} = \frac{Standard\ deviation\ of\ retention\ times}{Average\ retention\ time} * 100$
- $\textbf{Accuracy of Check Standard} = \frac{Response\ Factor\ of\ Check\ Standard\ Inj}{Average\ Response\ Factor\ of\ Level\ 3\ standard\ Injections} * 100$
- $\%\ \textbf{Difference of Sample Preparations} = \frac{\left|Prep.1\ Conc.\left(\frac{mg}{mL}\right) - Prep.2\ Conc.\left(\frac{mg}{mL}\right)\right|}{\frac{(Prep.1\ Conc.\ \left(\frac{mg}{mL}\right) + Prep.2\ Conc.\ \left(\frac{mg}{mL}\right)}{2}} * 100$
- $\%\ \textbf{Lipid Retention Time Agreement} = \frac{Retention\ Time\ of\ Lipid\ (Sample)}{Average\ Retention\ Time\ of\ Lipid\ (Level\ 3\ Cal.Standard)} * 100$
- $\%\ \textbf{Area of Individual Impurity} = \frac{Peak\ Area\ of\ Impurity}{Total\ Area\ of\ all\ peaks\ including\ SM102,Cholesterol,DSPC,PEG2000-DMG} * 100$
- $\%\ \textbf{Area of Total Impurity} = Sum\ of\ all\ individual\ impurities$

System Suitability and Test Article Acceptance Criteria

Table 40: SOP-1001: System Suitability Acceptance Criteria

Parameter	Acceptance
Chromatographic Non-Interference: No significant interference peaks in the last diluent injection prior to calibration standards which interfere with SM-102, Cholesterol, DSPC or PEG2000-DMG. (≤ 1% interference when compared to average peak area of the 1st five 100% Level 3 standards)	[redacted]
Sensitivity Solution: SM-102 peak Signal-to-Noise Ratio (S/N)	
Resolution Standard: Resolution between PEG2000-DMG and Stearic Acid peaks in the resolution standard	
Linearity: Coefficient of Determination (R^2) of the standard curve for PEG2000-DMG, DSPC, SM-102, and Cholesterol	
Standard Precision: (Peak Area) % RSD of peak area of DSPC, SM-102, Cholesterol for the level 3 standard injections	
% RSD of peak area of PEG2000-DMG for the level 3 standard injections	
Standard Precision: (Retention Time) % RSD of the RT of PEG2000-DMG, DSPC, SM-102, and Cholesterol for the level 3 standard injections	
Accuracy: The % Recovery of the RT of the check standard for each lipid vs. average level 3 calibration standard RT	
The % area recovery of the check standard for each lipid vs. average level 3 calibration standard area	
Sample Acceptance: The % Difference Between the total concentrations of sample replicates	

Figure 3: SOP-1001: Representative Chromatograms
A: Diluent Chromatogram

[redacted]

B: Sensitivity solution Chromatogram

[redacted]

C: Resolution Standard Solution Chromatogram

[redacted]

D: Single Lipid Standard Chromatogram (Showing the Retentions of PEG2000-DMG, Cholesterol, DSPC and SM-102 Lipid Components)

[redacted]

E: mRNA-1273 Drug Product Chromatogram

3.2.P.5.2.9 SOP-0288: pH

SOP-0288 is the method used to determine the pH of mRNA-1273 Drug Product in accordance with current USP <791>. pH is a numerical scale used to specify the acidity or basicity of an aqueous solution. It is defined as the decimal logarithm of the reciprocal of the hydrogen ion activity, aH+ , in a solution

Procedure

A suitable pH meter, such as the Mettler Toledo S320 SevenExcellence Meter or the Sartorius PB11 pH Meter, is utilized. These pH meters, including their associated electrodes, are operated in accordance with manufacturer recommendations and meet the instrument requirements listed in USP <791> including pH measurement resolution and ability to compensate for temperature. The pH meter is calibrated (standardized) using commercially prepared, NIST traceable, standardization solutions each day of use using either a 2- or 3-point calibration, as appropriate to bracket the expected pH of samples to be tested. The standardization buffers will span no more than 4 pH units. The calibration must meet slope and offset acceptance criteria. The calibration is also verified using commercially prepared, NIST traceable, standardization solutions before measuring samples. The temperature adjusted pH value of the verification buffer reading must be ± 0.05 when compared to the label claim. The calibration, verification, and sample measurements are performed at room temperature.

3.2.P.5.2.10 SOP-0279: Osmolality

SOP-0279 is the method used to determine the osmolality of mRNA-1273 Drug Product in accordance with current USP <785> using freezing point depression. The Osmolality of a solution corresponds to the molality of an ideal solution containing non-dissociating solutes and is expressed in osmoles or milliosmoles per kilogram of solvent (Osm per Kg or mOsm per kg, respectively), a unit that is similar to the molality of the solution. Thus, osmolality is a measure of the osmotic pressure exerted by a solution across a semipermeable membrane. Like osmotic pressure, other colligative properties of a solution, such as vapor pressure lowering, boiling point elevation, and freezing point depression, are also directly related to the osmolality of the solution. Indeed, the osmolality of a solution is typically determined most accurately and conveniently by measuring freezing point depression (DTf): DTf = kf m where kf is the molal cryoscopic constant, which is a property of the solvent. For water, the value of kf is 1.860° per Osmol. That is, 1 Osmol of a solute added to 1 kg of water lowers the freezing point by 1.860°.

Procedure

Osmolality is determined by measuring the freezing point depression using a calibrated osmometer, such as the Advanced Instruments Model 3320 Micro-Osmometer. The osmometer is calibrated by the manufacturer's instructions, in accordance with USP <785>. A 2- or 3- point calibration is performed each day of use, prior to testing samples, as appropriate to the range of samples to be analyzed. The calibration is verified with at least one calibration standard solution such that the osmolality of the standard solution lies within 50 mOsm/kg of the expected value of the sample to be analyzed or the center of the expected range of osmolality of the sample to be analyzed, with additional calibration standards verified for additional samples to be analyzed. Each calibration check must meet acceptance criteria. Samples are tested in triplicate and the average of the three readings reported as the final osmolality of the sample

3.2.P.5.2.11 SOP-0509: Particulate Matter

SOP-0509 is performed in accordance with USP <788> (Method 2) "Particulate Matter in Injections, Method 2 Microscopic Particle Count Test" to assesses sub-visible (≥ 10 µm and ≥ 25 µm) particulate matter in mRNA-1273 Drug Product. Particulate matter in injections and parenteral infusions consists of extraneous mobile undissolved particles, other than gas bubbles, unintentionally present in the solutions.

Procedure

Particulate matter is quantified by filtering samples and counting the particulates left on the filter membrane using a microscope in a particulate-free environment. The Fein Microscope model M40, or equivalent, is utilized and meets the requirements of USP <788> including 100X magnification, two suitable illuminators (one episcopic brightfield illuminator internal

to the microscope and one focusable auxiliary illuminator adjusted to give reflected oblique illumination at an angle of 10° - 20°), and an ocular micrometer (circular diameter graticule integrated with the microscope). The filters utilized meet the requirements of USP <788> including nominal pore size. The particle sizes are estimated by comparing with the 10 µm and 25 µm reference circles on the graticule.

3.2.P.5.2.12 SOP-0950: Container Content

SOP-0950 is the method used to determine the container content volume and labeled vialed fill size injections in multi-use containers of mRNA-1273 Drug Product in accordance with current USP <697>. The scope of this procedure is to determine if sufficient excess volume is available for withdrawal according to the labeled vial quantity and dose(s) for mRNA-1273 Drug Product in multi-dose containers.

Procedure

Using an appropriately sized needle and syringe, the contents of the mRNA-1273 Drug Product container are drawn up into a dry syringe at a volume to be measured as per the actual dose volume (Section 3.2.P.5.1). The volume measured in an appropriately sized graduated cylinder is such that each syringe delivers not less than (NLT) the stated dose. This is repeated using separate syringe assemblies for as many full deliverable doses available.

3.2.P.5.2.13 SOP M-CTS-CS-0929: Bacterial Endotoxin

Associates of Cape Cod, Inc. SOP: M-CTS-CS-0929 is used for the detection and quantitation of bacterial endotoxin for mRNA-1273 Drug Product using a Kinetic Chromogenic method utilizing a Pyros Kinetix® Flex Tube Reader. Testing is performed as outlined in the United States Pharmacopeia, (USP) <85>, Bacterial Endotoxin Test. These chapters are harmonized with the chapters of the same name in the European Pharmacopoeia (EP 2.6.14) and the Japanese Pharmacopeia (JP 4.01). The endotoxin level, measured as endotoxin units (EU), in the sample is divided by mRNA-1273 Drug Product volume and reported as EU/mL.

3.2.P.5.2.14 Sterility

mRNA-1273 Drug Product is tested for sterility at Catalent Indiana, LLC in accordance with the European Pharmacopoeia procedure (EP 2.6.1); the current USP General Chapter (USP <71>) and the current Japanese Pharmacopoeia procedure (JP 4.06). [redacted]

[redacted]