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3.2.P.5.3 VALIDATION OF ANALYTICAL PROCEDURES

3.2.P.5.3.1 Verification of Compendial Analytical Test Methods

A summary for the verification of compendial test methods used for mRNA-1273 Drug Product is presented in the following section.

The verification of each compendial method is in accordance with Current USP <1226> Verification of Compendial Procedures, such that this report documents evidence of suitability under actual conditions of use. Each compendial method has been assessed as appropriate to the complexity of the procedure.

The compendial test methods have been assessed for suitability using representative sample types and deemed appropriate for testing mRNA-1273 Drug Product as summarized in the Table 1.

Table 1: Summary of Compendial Analytical Test Methods for mRNA-1273 Drug Product

Compendial Test Method	SOP #	Method Verification Report
Appearance	SOP-0278	QC-MVR-0001
pH	SOP-0288	
Osmolality	SOP-0279	
Container Content	SOP-0950	
Particulate Matter	SOP-0509	

The compendial test methods described in Table 1 have been found to be suitable for the testing of mRNA-1273 Drug Product samples. Each compendial method has been assessed as appropriate to the complexity of the procedure.

QC-MVR-0001, attached in this section, provides the method description of each compendial test method, the SOP reference, the compendial reference, the critical materials and equipment to ensure compendia suitability and the evidence of suitability under actual conditions of use.

3.2.P.5.3.2 Verification of Compendial Microbiological Test Methods

3.2.P.5.3.2.1 Bacterial Endotoxin

Bacterial endotoxin testing using a Kinetic Chromogenic method 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).

Analysis included a test for interfering factors through spike and recovery of a known amount of endotoxin from the sample matrix with an acceptance criterion of 50 – 200 % recovery after any correction for endogenous levels of endotoxin present in the sample matrix.

3.2.P.5.3.2.2 Sterility

Sterility testing for mRNA-1273 is performed as outlined in the United States Pharmacopeia, (USP) <71>, Sterility Test. These chapters are harmonized with the chapters of the same name in the European Pharmacopoeia (EP 2.6.1) and the Japanese Pharmacopeia (JP 4.06). Per compendia, a test for suitability of the method for the sample was conducted using specified micro-organisms. Bacteria, yeast and mold species were spiked at low levels (not more than 100 colony-forming units) into mRNA1273 Drug Product sample. Growth was observed in the sterility test media, meeting acceptance criteria.

3.2.P.5.3.3 Validation of Analytical Test Methods

The analytical procedures for testing of mRNA-1273 Drug Product were confirmed as suitable for their intended use through executed method validation experiments. The % purity by RP-HPLC method has been demonstrated to be stability indicating. The applicable validation parameters and method validation reports (provided as attachments) are identified in Table 2.

Table 2: Method Validation for mRNA-1273 Drug Product Analytical Methods

Attribute	Method	Method Parameter	Method Validation Report (Attached)
Protein Translation (Table 3)	In Vitro Translation/ Methionine Labelling	Specificity, accuracy, precision (repeatability and intermediate)	QC-MVR-0020
Identity (Table 4)	Reverse Transcription/ Sanger Sequencing	Specificity	QC-MVR-0018
RNA Content (Table 5)	AEX-HPLC	System Suitability, Specificity, linearity, accuracy, precision (repeatability and intermediate), range, and robustness	QC-MVR-0008
Purity (Table 6)	RP-HPLC	System Suitability, Specificity, accuracy, precision (repeatability and intermediate), linearity, range, LOQ, sample and standard stability and robustness	QC-MVR-0005
% RNA Encapsulation (Table 7)	[redacted]	System Suitability, specificity, linearity, precision (repeatability and intermediate), range, accuracy, LOQ and robustness	QC-MVR-0009
Particle Size and Polydispersity (Table 8)	Dynamic Light Scattering	System suitability, specificity, Accuracy, precision (repeatability and intermediate), range and robustness	QC-MVR-0011
Lipid Content, Lipid Identification and Lipid Impurities (Table 9)	UPLC-CAD	System suitability, Specificity, linearity, accuracy, precision (repeatability and intermediate), range, QL, DL, sample stability and robustness	QC-MVR-0010

3.2.P.5.3.3.1 In Vitro Translation/ Methionine Labelling

SOP-0937: Determination of Protein Expression from mRNA using Cell-Free In-Vitro Translation System, has been validated and shown to be suitable for the purpose of confirming protein expression of the mRNA-1273 Drug Product. The validation characteristics evaluated were Specificity, accuracy, precision (repeatability and intermediate) as described in Table 3.

Analytical test method SOP-0937 passed the acceptance criteria for validation parameters in protocol and is considered validated for testing mRNA-1273 Drug Product. Refer to QC-MVR-0020 for details of the validation results.

Table 3: Overall Validation Summary for In Vitro Translation/Methionine Labelling

Parameter	Acceptance Criteria	Pass/Fail
Specificity	[redacted]	Pass
Accuracy		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass

3.2.P.5.3.3.2 Reverse Transcription/Sanger Sequencing (Identity)

SOP-1032: Identity Confirmation of mRNA in a Lipid Nanoparticle by Sequencing Analysis, has been validated and shown to be suitable for the purpose of determining the mRNA identity of mRNA-1273 Drug Product. The validation characteristic evaluated was specificity as described in Table 4. Refer to QC-MVR-0018 for details of the validation results.

Table 4: Overall Validation Summary for RT-PCR Sanger Sequencing

Parameter	Acceptance Criteria	Pass/Fail
Specificity (QC-MVP-0018)	[redacted]	Pass
Specificity (QC-MVP-0021)		Pass

3.2.P.5.3.3.3 AEX-HPLC (RNA Content)

SOP-0999: Determination of RNA Concentration in [redacted] by IEX (Ion Exchange) Chromatography with UV Detection, has been validated and shown to be suitable for the purpose of quantitating the mRNA content in mRNA-1273 Drug Product. The validation characteristics evaluated were system suitability, specificity, linearity, accuracy, precision (repeatability, intermediate precision), range, robustness and sample stability as described in Table 5.

Analytical test method SOP-0999 passed the acceptance criteria for validation parameters outlined in the protocol and is considered validated for testing mRNA-1273 Drug Product. Refer to QC-MVR-0008 for details of the validation results.

Table 5: Overall Validation Summary for AEX HPLC

Parameter	Acceptance Criteria	Pass/Fail
Specificity	[redacted]	Pass
Linearity		Pass
Accuracy		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Robustness		Pass
Sample Stability		Pass

3.2.P.5.3.3.4 RP-HPLC (Purity)

SOP-0996: Analysis of mRNA Purity by Size Based RPIP HPLC has been validated and shown to be suitable to assess mRNA purity of mRNA containing Lipid Nanoparticles (LNPs). The validation characteristics evaluated were: repeatability precision; intermediate precision; linearity; accuracy; specificity; determination of the quantitation limit; stability of standard and sample preparation solutions; range; and robustness as described in Table 6. SOP-0996 has been validated and is suitable for testing of mRNA-1273 Drug Product test samples. Refer to QC-MVR-0005 for details of the validation results.

Table 6: Overall Validation Summary for RP-HPLC

Parameter	Acceptance Criteria	Pass/Fail
Specificity	[redacted]	Pass
Linearity		Pass
Accuracy (from Linearity)		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Robustness		Pass
Quantitation Limit (QL)		Pass
Prepared Solution Stability		Pass

a) Additional acceptance criteria added per QC-OTH-0172
Abbreviations: IG = impurity group; LNP = lipid nanoparticles

3.2.P.5.3.3.5 [redacted] (% RNA Encapsulation)

SOP-1000: % Encapsulation Efficiency by [redacted] has been validated and shown to be suitable to assess % Encapsulation efficiency of mRNA containing Lipid Nanoparticles (LNPs). The validation characteristics evaluated were: system suitability, specificity, linearity, accuracy, precision, intermediate precision, range, quantitation limit and robustness as described in Table 7. SOP-1000 is validated and suitable for testing mRNA-1273 Drug Product samples. Refer to QC-MVR-0009 for details of the validation results.

Table 7: Overall Validation Summary for [redacted]

Parameter	Acceptance Criteria	Pass/Fail
Specificity	[redacted]	Pass
Linearity		Pass
Accuracy		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Quantitation Limit (QL)		Pass
Robustness		Pass

a) Updated linearity levels and criteria per discrepancy QC-OTH-0169

3.2.P.5.3.3.6 Dynamic Light Scattering (Particle Size and Polydispersity)

SOP-0998: Determination of Particle Size Distribution and Polydispersity by Dynamic Light Scattering, has been validated and shown to be suitable for the purpose determine the hydrodynamic diameter (average size) and size distribution of Lipid Nanoparticle (LNP) formulations. The validation characteristics evaluated were accuracy, precision, specificity, range and robustness as described in Table 8.

Analytical test method SOP-0998 passed the acceptance criteria for validation parameters in protocol QC-MVP-0011: specificity, accuracy, precision (repeatability, intermediate precision), range, and robustness. Analytical test method SOP-0998 is considered validated for testing mRNA-1273 Drug Product samples. Refer to QC-MVR-0011 for details of the validation results.

Table 8: Overall Validation Summary for Dynamic Light Scattering

Parameter	Acceptance Criteria	Pass/Fail
System Suitability	[redacted]	Pass
Specificity		Pass
Accuracy		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Robustness		Pass

3.2.P.5.3.3.7 UPLC-CAD (Lipid Content, Lipid Identification and Lipid Impurities)

SOP-1001: Determination of Lipid Content, Purity, and Identity by UPLC-CAD [redacted] has been validated and shown to be suitable for the purpose of determining the Lipid Content, Purity, and Identity.

Analytical test method SOP-1001 passed the acceptance criteria for validation parameters outlined in the validation protocol and summarized in Table 9 [accuracy, precision (repeatability and intermediate), specificity, QL, DL, linearity, range, stability, and robustness] and is considered validated and suitable for testing mRNA-1273 Drug Product samples. Refer to QC-MVR-0010 for details of the validation results.

Table 9: Overall Validation Summary for UPLC-CAD

Parameter	Acceptance Criteria	Pass/Fail
Specificity	[redacted]	Pass
Linearity		Pass [a]
Accuracy		Pass
Precision (Repeatability)		Pass
Precision (Intermediate)		Pass
Range		Pass
Quantitation Limit (QL)		Pass
Detection Limit (DL)		Pass
Robustness		Pass
Solution Stability		Pass

a) Passing linearity levels:
PEG2000-DMG: [redacted]
Cholesterol: [redacted]
SM-102: [redacted]
DSPC: [redacted]