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Method Validation Report of SOP-1001: Determination of Lipid Content, Purity, and Identity by UPLC-CAD for [redacted]

mRNA-1273 LNP, mRNA-1273 DP, [redacted]

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1.0 INTRODUCTION

This report presents the method validation results of test method SOP-1001 for mRNA-1273 LNP, mRNA-1273 DP and [redacted]. The validation was performed at the Moderna Quality Control (QC) Laboratory following method validation protocol QC-MVP-0010, Method Validation Protocol of SOP-1001: Determination of Lipid Content, purity, and Identity by UPLC-CAD for [redacted]. This was performed in accordance with the ICH Q2(R1) Guideline for Validation of Analytical Procedures.

SOP-1001, Determination of Lipid Content, Purity, and Identity by UPLC-CAD for [redacted], is a gradient-reverse phase Ultra-High-Performance Liquid Chromatography (UHPLC) with charged aerosol detection (CAD) method. The testing utilizes a calibration curve of known concentrations and the total purity and impurities are calculated as percent peak area.

Method SOP-1001 was validated according to this protocol using samples which contain [redacted] (SM102, PEG2000-DMG, Cholesterol, and DSPC lipids), mRNA-1273 DP and the associated formulation buffers. The validation parameters of specificity, linearity, accuracy, precision (repeatability, intermediate precision), range, Quantitation limit, Detection limit, robustness and solution stability will be evaluated. Test method SOP-1001 is considered suitable for testing mRNA-1273 LNP, mRNA-1273 DP and [redacted].

2.0 RESPONSIBILITIES

Department/ Functional Area	Responsibility
Quality Control	• Authors, reviews and approves validation protocols and reports. • Executes, reviews and approves executed data packages and data summaries. • Authors validation summary reports.
Quality Assurance	• Reviews and approves validation protocols, data summaries, and reports. • Ensures that validation documents are in alignment with Moderna • policies and regulatory requirements.

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3.0 DOCUMENTATION

All documentation, execution, and review of the work performed for this study was conducted under current Good Manufacturing Practices (cGMP) as required by Moderna standard operating procedures.

Draft analytical method **SOP-1001** (version 0.2) was followed for this testing. Assay information was documented on draft **FRM-0741** (version 0.2), **FRM-0743** (version 0.2) and **FRM-0744** (version 0.3).

QC Analysts documented read and understand training on analytical method **SOP-1001** and validation protocol **QC-MVP-0010** prior to executing validation testing. Refer to Veeva documents **TR-9612, TR-9613** and **TR-9614** for the training records.

All relevant data collected during validation testing and formulae used for calculating validation characteristics was peer reviewed and included as attachments to this validation report.

4.0 MATERIALS AND EQUIPMENT

4.1. Test articles

Table 1. Test Articles

Description	Lot	Total Lipid Concentration (mg/mL)	Summary / Certificate of Analysis
CX-024414 Reference Standard	MTDS20002	N/A	DSAD-SOA-0254
[redacted]	AMPDP-20063	[redacted]	N/A
mRNA-1273 LNP	5006820001		COA-0447
mRNA-1273 Drug Product	6006820001		COA-0448
mRNA-1273 Drug Product	6006920001		COA-0449
[redacted] Formulation Buffer [redacted] Sodium Acetate, [redacted] Sucrose, [redacted]	N/A		
mRNA-1273 LNP and DP Formulation Buffer (20mM Tris, 87 g/L Sucrose, pH 7.5)	N/A		

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4.2. Materials and Equipment

Refer to the Materials and Equipment Section of SOP-1001 (version 0.2).

5.0 VALIDATION SUMMARY

Validation Parameters, Acceptance Criteria and Results

Table 2. Summary of Results

Parameter	Acceptance Criteria	Results	Pass /Fail
Specificity	The formulation buffer and sample diluent will not have any peaks which interfere with the main peak [redacted] or which interfere with known degradant peaks greater or equal to QL.	No peaks were detected in any of the preparations of reference standard material or sample diluent preparations	Pass
Linearity	The coefficient of determination (R^2) of the linear regression of the calculated concentrations against the theoretical concentration must be [redacted] Report Slope and intercept.	**PEG2000-DMG** passes linearity for [redacted] with a R^2 value of [redacted] and a slope intercept equation of y=[redacted] **Cholesterol** passes linearity for [redacted] to [redacted] with a R^2 value of [redacted] and a slope intercept equation of y=[redacted] **SM102** passes linearity for [redacted] to [redacted] with a R^2 value of [redacted] and a slope intercept equation of y=[redacted] **DSPC** passes linearity for [redacted] to [redacted] with a R^2 value of [redacted] and a slope intercept equation of y=[redacted]	Pass

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Parameter	Acceptance Criteria	Results	Pass /Fail
Accuracy	The individual and average lipid concentration of each level ([redacted] of DSPC, SM102 and Cholesterol must have a percent recovery of [redacted] compared to the theoretical concentration. The individual and average lipid concentration of each level ([redacted] of PEG2000-DMG must have a percent recovery of [redacted] compared to the theoretical concentration.	**PEG2000-DMG** passes accuracy for levels [redacted] **Cholesterol** passes accuracy for levels [redacted] **SM102** passes accuracy for levels [redacted] **DSPC** passes accuracy for levels [redacted]	Pass

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Precision (Repeatability)	The lipid concentrations for DSPC, SM102, and Cholesterol must have an RSD [redacted] The %RSD for PEG2000-DMG concentration must be [redacted] The % difference of mean concentration of SM102, Cholesterol and DSPC obtained at [redacted] and [redacted] preparations is [redacted] of the preparation The % difference of mean conc of PEG obtained at [redacted] and [redacted] preparations is [redacted] of the preparation	**mRNA-1273 LNP results:** %RSD [redacted] PEG2000-DMG: [redacted] %RSD [redacted] Cholesterol: [redacted] %RSD [redacted] SM102: [redacted] %RSD [redacted] DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC% Diff: [redacted] [redacted] **Results:** %RSD [redacted] PEG2000-DMG: [redacted] %RSD [redacted] Cholesterol: [redacted] %RSD [redacted] SM102: [redacted] %RSD [redacted] DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC% Diff: [redacted] **mRNA-1273 DP 6006920001 Results:** %RSD [redacted] PEG2000-DMG: [redacted] %RSD [redacted] Cholesterol: [redacted] %RSD [redacted] SM102: [redacted] %RSD [redacted] DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted]	Pass

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Parameter	Acceptance Criteria	Results	Pass /Fail
		[redacted] SM102 % Diff: [redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC% Diff: [redacted] **mRNA-1273 DP 6006820001 Results:** %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] SM102 % Diff: [redacted] DSPC% Diff: [redacted]	

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Precision (Intermediate)	The second analyst lipid concentrations for DSPC, SM102, and Cholesterol must have an RSD [redacted] The second analyst %RSD for PEG2000-DMG concentration must be [redacted] The overall sample concentration %RSD of the DSPC, SM102, Cholesterol and Total Lipid Content in each sample for both analysts (n=12) must be [redacted] The overall concentration %RSD for PEG2000-DMG (n=12) must be [redacted] The percent difference of the mean between the two analysts is [redacted] for DSPC, SM102, and Cholesterol. The percent difference of the mean between the two analysts is [redacted] for PEG2000-DMG.	**mRNA-1273 LNP results:** %RSD [redacted] PEG2000-DMG: [redacted] %RSD [redacted] Cholesterol: [redacted] %RSD [redacted] SM102: [redacted] %RSD [redacted] DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] [redacted] Cholesterol % Diff: [redacted] [redacted] SM102 % Diff: [redacted] [redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] [redacted] Cholesterol % Diff: [redacted] [redacted] SM102 % Diff: [redacted] [redacted] DSPC% Diff [redacted] <u>%RSD between analysts at</u> [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] <u>%RSD between analysts at</u> [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] <u>%RSD between analysts at</u> [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] <u>% Difference between analysts at</u> [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted]	Pass

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

		% Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] **SM102 LNP Results:** %RSD [redacted] PEG2000-DMG: [redacted] %RSD [redacted] Cholesterol: [redacted] %RSD [redacted] SM102: [redacted] %RSD [redacted] DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] [redacted] Cholesterol % Diff: [redacted] [redacted] SM102 % Diff: [redacted] [redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] [redacted] Cholesterol % Diff: [redacted] [redacted] SM102 % Diff: [redacted] [redacted] DSPC% Diff: [redacted] %RSD between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] %RSD between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted]	

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		%RSD DSPC: [redacted] %RSD between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102 [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] **mRNA-1273 DP 6006920001 Results:** %RSD [redacted] PEG2000-DMG: [redacted] %RSD [redacted] Cholesterol: [redacted] %RSD [redacted] SM102: [redacted] %RSD [redacted] DSPC: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] Cholesterol % Diff: [redacted] [redacted] SM102 % Diff: [redacted]	

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		[redacted] DSPC % Diff: [redacted] [redacted] PEG2000-DMG % Diff: [redacted] [redacted] Cholesterol % Diff: [redacted] [redacted] SM102 % Diff: [redacted] [redacted] DSPC% Diff: [redacted] %RSD between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] %RSD between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] %RSD between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted]	

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%RSD PEG2000-DMG: [redacted]
%RSD Cholesterol: [redacted]
%RSD SM102: [redacted]
%RSD DSPC: [redacted]

mRNA-1273 DP 6006820001
Results:

%RSD [redacted] PEG2000-DMG: [redacted]
%RSD [redacted] Cholesterol: [redacted]
%RSD [redacted] SM102: [redacted]
%RSD [redacted] DSPC: [redacted]
[redacted] PEG2000-DMG % Diff: [redacted]
[redacted] Cholesterol % Diff: [redacted]
[redacted] SM102 % Diff: [redacted]
[redacted] DSPC % Diff: [redacted]
[redacted] PEG2000-DMG % Diff: [redacted]
[redacted] Cholesterol % Diff: [redacted]
[redacted] SM102 % Diff: [redacted]
[redacted] DSPC% Diff: [redacted]

%RSD between analysts at [redacted]
%RSD PEG2000-DMG: [redacted]
%RSD Cholesterol: [redacted]
%RSD SM102: [redacted]
%RSD DSPC: [redacted]

%RSD between analysts at [redacted]
%RSD PEG2000-DMG: [redacted]
%RSD Cholesterol: [redacted]
%RSD SM102: [redacted]
%RSD DSPC: [redacted]

%RSD between analysts at [redacted]
%RSD PEG2000-DMG: [redacted]
%RSD Cholesterol: [redacted]
%RSD SM102: [redacted]
%RSD DSPC: [redacted]

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Parameter	Acceptance Criteria	Results	Pass /Fail
		% Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted] % Difference between analysts at [redacted] %RSD PEG2000-DMG: [redacted] %RSD Cholesterol: [redacted] %RSD SM102: [redacted] %RSD DSPC: [redacted]	
Range	If the Validation target expectations for Linearity, Precision, and Accuracy are met, this demonstrates that the range is suitable.	Range of PEG2000-DMG is [redacted] to [redacted] based off linearity and accuracy. Range of Cholesterol is [redacted] to [redacted] based off linearity and accuracy. Range of SM102 is [redacted]) to [redacted] based off linearity and accuracy. Range of DSPC is [redacted] to [redacted] based off linearity and accuracy.	Pass

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Parameter	Acceptance Criteria	Results	Pass /Fail
Quantitation Limit (QL)	Report the lowest [redacted] that meets the following acceptance criteria: • Average signal to noise ratio [redacted] • %RSD of [redacted] peak area (n=3) ≤ [redacted] • %Recovery	Lowest [redacted] that meets acceptance criteria: [redacted] Average Signal to Noise Ratio: [redacted] %RSD of SM102 Peak area: [redacted] Average % Recovery of [redacted] Data for the QL preparations resulted in a R^2 value of [redacted] and a slope intercept equation of y= [redacted] Level | % Recovery [redacted]	Pass
Detection Limit (DL)	Report the DL (% Peak Area)	DL was calculated to be [redacted] for this method.	Pass
Robustness	Intermediate precision criteria are met.	Intermediate precision criteria are met.	Pass

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Solution Stability	Solutions showing an impurity increase of [redacted] from the T=0 result and no new impurities greater than the QL are considered stable for the respective time point. The Absolute % difference of the lipid concentrations of SM102, Cholesterol and DSPC will be [redacted] when compared to the T=0 result to be considered stable. The Absolute % difference of the lipid concentration for PEG2000-DMG will be [redacted] when compared to the T=0 result to be considered stable.	**mRNA-1273 LNP % Difference results:** PEG2000-DMG Day 1: [redacted] PEG2000-DMG Day 3: [redacted] Cholesterol Day 1: [redacted] Cholesterol Day 3: [redacted] SM102 Day 1: [redacted] SM102 Day 3: [redacted] DSPC Day 1: [redacted] DSPC Day 3: [redacted] [redacted] **% Difference Results:** PEG2000-DMG Day 1: [redacted] PEG2000-DMG Day 3: [redacted] Cholesterol Day 1: [redacted] Cholesterol Day 3: [redacted] SM102 Day 1: [redacted] SM102 Day 3: [redacted] DSPC Day 1: [redacted] DSPC Day 3: [redacted] **mRNA-1273 DP 6006820001 % Difference Results:** PEG2000-DMG Day 1: [redacted] PEG2000-DMG Day 3: [redacted] Cholesterol Day 1: [redacted] Cholesterol Day 3: [redacted] SM102 Day 1: [redacted] SM102 Day 3: [redacted] DSPC Day 1: [redacted] DSPC Day 3: [redacted] **mRNA-1273 DP 6006920001 % Difference Results:** PEG2000-DMG Day 1: [redacted] PEG2000-DMG Day 3: [redacted] Cholesterol Day 1: [redacted]	Pass

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Parameter	Acceptance Criteria	Results	Pass /Fail
		Cholesterol Day 3: [redacted] SM102 Day 1: [redacted] SM102 Day 3: [redacted] DSPC Day 1: [redacted] DSPC Day 3: [redacted] **Check Standard % Difference Results:** PEG2000-DMG Day 1: [redacted] PEG2000-DMG Day 3: [redacted] Cholesterol Day 1: [redacted] Cholesterol Day 3: [redacted] SM102 Day 1: [redacted] SM102 Day 3: [redacted] DSPC Day 1: [redacted] DSPC Day 3: [redacted] mRNA-1273 LNP, mRNA-1273 DP, [redacted], and prepared check standard are stable for 3 days at 20° C.	

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6.0 VALIDATION RESULTS

6.1. System Suitability

Experimental Design:

System suitability as outlined by SOP-1001 was evaluated each time an analysis is run.

Acceptance Criteria:

Report system suitability results as outlined in the analytical test method SOP-1001. Results will be assessed during the validation and any necessary updates to the draft versions of SOP-1001, FRM-0741, FRM-0743 and FRM-0744 will be made prior to the effective versions.

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Results:

System suitability passed the acceptance criteria listed in SOP-1001. No further updates to the system suitability criteria will be made based on results of this validation.

6.2. Specificity

Experimental Design:

The diluent and drug substance (DS) reference standard listed below were analyzed per SOP-1001 to establish that the test method is specific to the product-related components.

- Standard and Sample Diluent (Ethanol)
- DS Reference Standard lot MTDS20002 (diluted to final concentration of [redacted] mRNA in the vial).

Each of the above were prepared per SOP-1001 in triplicate (N=3) and injected once.

Data Analysis:

The diluent and reference DS standard chromatograms were compared to the average level 3 standard area for interference with the peaks of interest.

Acceptance Criteria:

The sample diluent and DS reference standard will not have any peaks which interfere with the main Lipid peaks [redacted] or which interfere with known degradant peaks greater or equal to QL.

Results:

No interfering peaks were detected in the sample diluent or in the DS reference standard in any of the preparations. Results are presented in Tables 3 and 4.

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Table 3. Sample / Standard Diluent Specificity Results

Lipid	Sample peak area		Mean std peak area	% Interference
PEG2000-DMG	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		
SM102	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		
Cholesterol	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		
DSPC	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		

Table 4. DS Reference Standard Specificity Results

Lipid	Sample peak area		Mean std peak area	% Interference
PEG2000-DMG	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		
SM102	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		
Cholesterol	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		
DSPC	Prep 1	Not Detected	N/A	N/A
	Prep 2	Not Detected		
	Prep 3	Not Detected		

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

6.3. Linearity

Experimental Design:
Linearity was determined for each individual lipid. The linearity was assessed for samples with known lipid concentration and prepared per instructions in protocol QC-MVP-0010.The lipid concentrations prepared represent the total lipid content at the [redacted] of the assay standard. Linear regression analysis was performed for each individual lipid.

Stock solutions were prepared in triplicate by weighing the lipid amounts and performing dilutions according to the plan in protocol QC-MVP-0010 and each preparation was injected once.

Data Analysis:
Linear regression analysis of the individual sample preparations was performed for the measured concentration against the theoretical concentrations (mg/mL).
The regression line for the individual lipids was plotted.
The coefficient of determination (r^2), slope, and y-intercept were calculated and reported.

Acceptance Criteria:
The coefficient of determination for each lipid's linear regression plot must be [redacted]
Report the slope and intercept for each lipid.

Results:
Linearity results are presented in Tables (5-9) and Figures (1-4). Linearity is only plotted for the levels that met the accuracy criteria. Accuracy and range are discussed in section 6.6.

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 5. PEG2000-DMG Linearity Results

Level	Target Conc. (mg/mL)	Experimental Conc. (mg/mL)	Mean %Recovery
[redacted]			

Table 6. Cholesterol Linearity Results

Level	Target Conc. (mg/mL)	Experimental Conc. (mg/mL)	Mean %Recovery
[redacted]			

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Level	Target Conc. (mg/mL)	Experimental Conc. (mg/mL)	Mean %Recovery
[redacted]			

Table 7. SM-102 Linearity Results

Level	Target Conc. (mg/mL)	Experimental Conc. (mg/mL)	Mean %Recovery
[redacted]			

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Table 8. DSPC Linearity Results

Level	Target Conc. (mg/mL)	Experimental Conc. (mg/mL)	Mean %Recovery
[redacted]			

Table 9. Linearity Results Summary

Lipid	R2	Slope	Y-Intercept	Range
PEG2000-DMG	[redacted]			
Cholesterol				
SM-102				
DSPC				

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]	

Figure 1. **PEG2000-DMG Linearity Regression**

[redacted]

Figure 2. **Cholesterol Linearity Regression**

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Figure 3. **SM-102 Linearity Regression**

[redacted]

Figure 4. **DSPC Linearity Regression**

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

6.4. Precision (Repeatability)

Experimental Design:

Precision was assessed for [redacted], mRNA-1273 LNP and mRNA-1273 DP samples.

Six preparations of each sample of [redacted] levels were made by using the formulation buffer to dilute each sample.

Table 10. Precision Preparations Final Lipid Concentrations

Test Article	[redacted] Final Conc. (mg/ml)	[redacted] Final Conc. (mg/ml)	[redacted] Final Conc. (mg/ml)
[redacted] AMPDP-20063	[redacted]		
mRNA-1273 LNP 5006820001			
mRNA-1273 DP Lot 6006820001			
mRNA-1273 DP Lot 6006920001			

Data Analysis:

The %RSD of the [redacted] level results (n=6) for each lipid of each test article was calculated.

The % Difference of the mean lipid concentration results for the [redacted] and [redacted] levels against the [redacted] level results was calculated for each test article.

Acceptance Criteria
The %RSD of the lipid concentrations for DSPC, SM102 and Cholesterol for mRNA-1273 LNP, [redacted] and DP at each level must be [redacted]

The %difference of the mean concentrations of SM102, Cholesterol and DSPC in the [redacted] and [redacted] preparations (corrected for dilution) compared to the mean concentrations at [redacted] preparations [redacted]

The %RSD of the concentration for PEG2000-DMG for mRNA-1273 LNP, [redacted] and DP at each level must be [redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]	

The %difference of the mean concentrations of PEG in the [redacted] and [redacted] preparations (corrected for dilution) compared to the mean concentrations at [redacted] preparations [redacted]

<u>Results</u>
The %RSD of the lipid concentrations for DSPC, SM102 and Cholesterol for all samples at each level met the acceptance criteria and the results were [redacted]

The %RSD of the concentration for PEG2000-DMG for all samples at each level met the acceptance criteria and the results were [redacted].

The %difference of the mean concentrations of SM102, Cholesterol and DSPC for the [redacted] and [redacted] level preparations compared to the mean concentrations at the [redacted] level preparations was [redacted] for all samples.

The %difference of the mean concentrations of PEG for the [redacted] and [redacted] level preparations compared to the mean concentrations at [redacted] level preparations was [redacted] for all samples. Results are presented in Tables 11-14.

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 11. Precision for Analyst 1 [redacted] Lot AMPD-20063

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 12. Precision for Analyst 1 mRNA-1273 LNP Lot 5006820001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 13. Precision for Analyst 1 Drug Product Lot 6006820001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 14. Precision for Analyst 1 Drug Product Lot 6006920001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

6.5. Precision (Intermediate)

Experimental Design:
On a separate day, a second operator (Analyst 2) repeated Precision (Section 6.4) using the same lot of mRNA-1273 LNP, [redacted] and mRNA-1273 DP materials. Analysis was performed with a different column lot, different instruments, and freshly prepared mobile phases.

Data Analysis:
The %RSD of the concentration results for Analyst 2 (n=6) and for Analyst 1 and 2 (n=12) for each test article were calculated and tabulated.

The % difference of the mean concentration results between analyst 1 and 2 for each test article was calculated.

Acceptance Criteria
The second analyst lipid concentrations for DSPC, SM102, and Cholesterol (n=6) must have an RSD [redacted]

The second analyst %RSD for PEG2000-DMG concentration (n=6) must be ≤ [redacted]

The overall sample concentration %RSD of the DSPC, SM102, Cholesterol and Total Lipid Content in all sample types for Analyst 1 and 2 (n=12) must be ≤ [redacted]

The overall concentration %RSD for PEG2000-DMG (n=12) must be ≤ [redacted]

The absolute % Difference of the mean between the two analysts is [redacted] for DSPC, SM102, and Cholesterol.

The absolute % Difference of the mean between the Analyst 1 and 2 ≤ [redacted] for PEG2000-DMG.

Results
The %RSD of the lipid concentrations for Analyst 2 for DSPC, SM102 and Cholesterol met the acceptance criteria for all sample types at each level and the results were [redacted]

The %RSD of the concentration for Analyst 2 for PEG2000-DMG for met the acceptance criteria for all sample types at each level and the results were [redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

The overall sample concentration for Analyst 1 and 2 (n=12) %RSD of the DSPC, SM102, Cholesterol and total lipid content in the mRNA-1273 LNP, [redacted] and DP samples for Analyst 1 and 2 (n=12) was [redacted]

The overall concentration %RSD for PEG2000-DMG and total lipid content in the mRNA-1273 LNP, [redacted] and DP samples for Analyst 1 and 2 (n=12) was [redacted].

The absolute % Difference of the mean concentrations of DSPC, SM102, and Cholesterol in the [redacted] and [redacted] level preparations for SM102 LNP, and mRNA-1273 DP samples compared to the mean concentrations at [redacted] level preparations for analyst 1 & 2 met the acceptance criteria and results were [redacted]

The absolute % Difference between analyst 1 and 2 of the mean concentrations of DSPC, SM102, and Cholesterol in the [redacted] and [redacted] level preparations for the mRNA-1273 LNP sample compared to the mean concentrations at [redacted] level preparations for analyst 1 & 2 met the acceptance criteria of [redacted] for [redacted] and [redacted] levels.

The [redacted] level, only the DSPC lipid met the % difference criteria of [redacted]. The % difference results for SM102 and Cholesterol at the [redacted] were slightly above the acceptance criteria of [redacted] for SM102 and [redacted] for Cholesterol. The % difference of [redacted] and [redacted] results will be considered acceptable and the inter-assay precision of the method will be reported accordingly.[1]

The %difference of the mean concentrations of PEG in the [redacted] and [redacted] level preparations compared to the mean concentrations at 100% level preparations for analyst 1 & 2 was [redacted]. Results are presented in Tables (15-22).

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[1] Discussion for accepted results, refer to discrepancy #1

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Table 15. Intermediate precision for Analyst 2 [redacted] Lot AMPD-20063

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 16. Intermediate precision for Analyst 2 mRNA-1273 LNP Lot 5006820001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 17. Intermediate precision for Analyst 2 Drug Product Lot 6006820001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 18. Intermediate precision for Analyst 2 Drug Product Lot 6006920001

[redacted]

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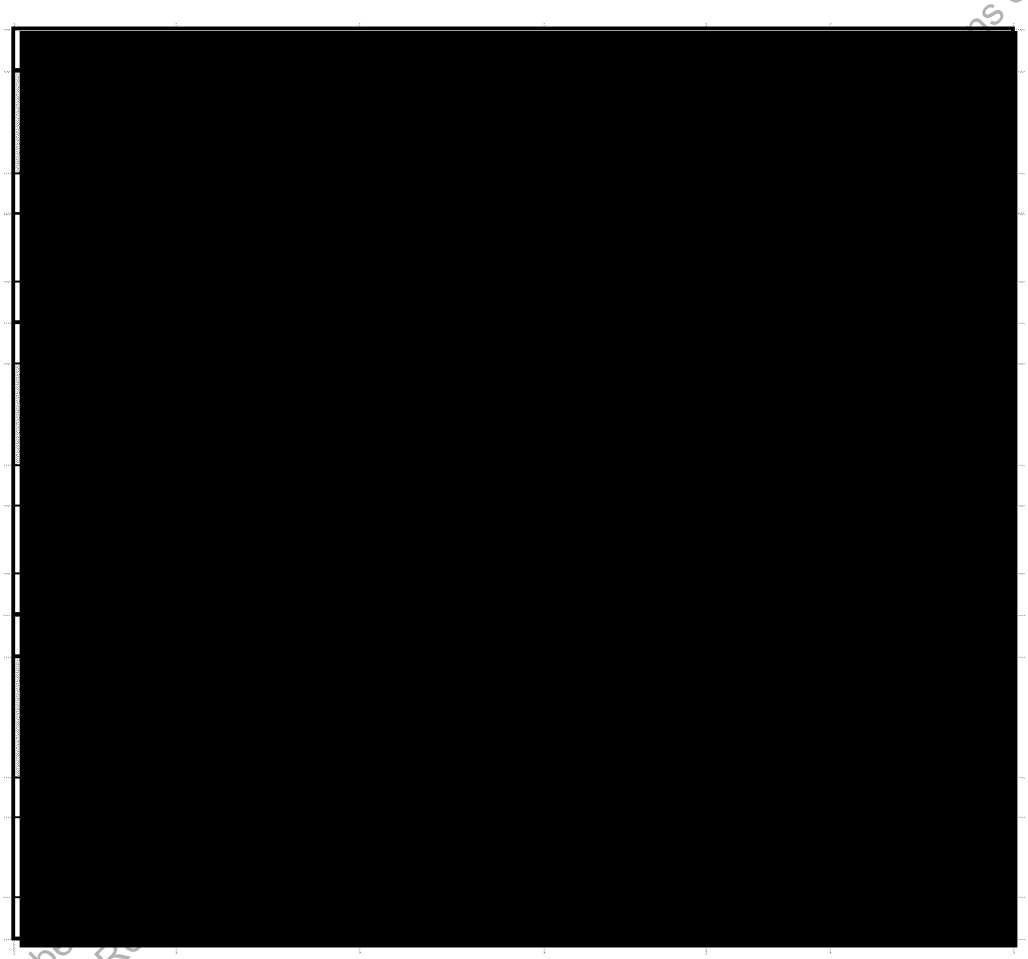
mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 19. Total %RSD for Analyst 1 & 2 [redacted] Lot AMPD-20063



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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 20. Total %RSD for Analyst 1 & 2 mRNA-1273 LNP Lot 5006820001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 21. Total %RSD for Analyst 1 & 2 Drug Product Lot 6006820001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 22. Total %RSD for Analyst 1 & 2 Drug Product Lot 6006920001

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

6.6. Accuracy and Range

Experimental Design:
Linearity data was used to evaluate accuracy of the method. Since the linearity experiment has 5 levels with triplicate preparations at each level and precision has 6 preparations at [redacted] (nominal) concentration levels, no separate experiments were needed to evaluate the accuracy of the test method.

Data Analysis:
The % Recovery of measured concentration of each lipid (DSPC, SM-102, Cholesterol, and PEG2000-DMG) for each level ([redacted]) was calculated and compared to the theoretical concentration.

Acceptance Criteria:
% Recovery of measured concentration of each lipid (DSPC, SM-102 and Cholesterol) for each level ([redacted]) compared to the theoretical concentration must be [redacted].

% Recovery of measured concentration of PEG2000-DMG for each level ([redacted]) compared to the theoretical concentration must be [redacted]

For the range: If the validation target expectations for Linearity, Precision, and Accuracy are met, this demonstrates that the range is suitable.

Results:
Accuracy results are presented in Tables 5-8 in the Linearity section.

A summary of the linearity levels and range for each lipid (based on the levels that met the acceptance criteria) is presented in Table 23.

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]	

Table 23. Table Linearity and Range Summary

Lipid	Passing Linearity Levels	Validated Range (mg/mL)
PEG2000-DMG	[redacted]	[redacted]
Cholesterol	[redacted]	[redacted]
SM-102	[redacted]	[redacted]
DSPC	[redacted]	[redacted]

The validation strategy on linearity and accuracy was designed following the worst-case sample preparation scenario and assuming total lipids of [redacted] sample extracted with [redacted] of method diluent) to bring samples to a [redacted] total lipid final concentration.

6.7. Quantitation and Detection Limits

<u>Experimental Design:</u>
The QL for the method was determined using the three lipid stock solutions prepared for linearity/accuracy samples to create the QL stock solutions (n=3). The lipid stock was further diluted by using [redacted] and diluting it in [redacted] of method diluent to prepare a total lipid solution of [redacted]

An RNA stock was also prepared for QL analysis. The DS reference standard was diluted with the formulation buffer. Then the three QL stock solutions were used to further dilute to a target [redacted] (Refer to protocol for detailed preparation and dilution scheme).

<u>Acceptance Criteria:</u>
Standard Level 3 ([redacted] of Nominal Concentration): Report total area (sum of average peak areas of [redacted] Cholesterol, DSPC, and PEG2000-DMG)

[redacted] Report the lowest concentration level with an average signal to noise ratio [redacted] and %RSD of [redacted] measured concentrations (n=3) ≤ [redacted] The results will be considered reportable if the [redacted] concentration have %Recovery of [redacted]

Plot the measured [redacted] concentration for each prep against theoretical concentration and report slope, r2 and y-intercept
Method QL: Report the theoretical level as QL in two decimal places.
Method DL: Report DL as [redacted] of the QL in two decimal places.

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Results:

The lowest [redacted] concentration level was determined to be the [redacted] level ([redacted]). Results are presented in Table 24 and Figure 5.

Table 24. QL Data Summary

Level	Average S/N	%RSD of [redacted] Conc. (mg/mL)	% Recovery
[redacted]			

The resulting detection limit is [redacted] (QL) / [redacted]

Figure 5. **Quantitation Limit Data**

[redacted]

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

6.8. Robustness

Robustness was assessed by varying assay conditions that may occur under normal usage in QC. The conditions were: analysts, days, UHPLC instrument, mobile phases, and column lot used for sample analysis.

Intermediate Precision data is used to support the robustness assessment.

Acceptance Criteria:
Intermediate precision criteria are met.

Results:
Intermediate precision criteria were met, refer to section 6.5 for results.

6.9. Solution Stability

Experimental:
The stability of [redacted], mRNA-1273 LNP and mRNA-1273 DP was evaluated over a period of 3 days. The sample and the check standard preparations from the initial run were aliquoted into two separate vials. One aliquot was injected in the initial run and the other aliquot was held in an autosampler over a period of 3 days. The autosampler temperature was held at 20°C.

The sample preparation was reanalyzed at T=1 Days and T= 3 Days, where T=days, and compared to T=0, the initial injections.

Acceptance Criteria:
Solutions showing an impurity increase of [redacted] from the T=0 result and no new impurities greater than the QL are considered stable for the respective time point.

The Absolute % difference of the lipid concentrations of SM102, Cholesterol and DSPC will be [redacted] when compared to the T=0 result to be considered stable.
The Absolute % difference of the lipid concentration for PEG2000-DMG will be [redacted] when compared to the T=0 result to be considered stable.

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Results:
The Absolute % difference of the lipid concentrations of SM102, Cholesterol and DSPC at T=1 and T=3 days was [redacted] when compared to the T=0 result. The Absolute % difference of the lipid concentration for PEG2000-DMG was [redacted] Impurities for check standard and all sample solutions T=1 and T=3 days were [redacted] as compared with T=0 results, with no new impurities greater than the quantitation limit.
mRNA-1273 LNP, mRNA-1273 DP, [redacted], and prepared check standard are stable for 3 days when stored at 20°C.

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

Table 25. Sample and Standard Stability

[redacted]

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Table 26. Impurities results

[redacted]

7.0 DISCREPANCIES

Discrepancy #1
A discrepancy occurred in the intermediate precision study. The absolute % Difference between analyst 1 and 2 of the mean concentrations of DSPC, [redacted], and Cholesterol in the [redacted] and [redacted] level preparations for the mRNA-1273 LNP sample compared to the mean concentrations at [redacted] level preparations for analyst 1 & 2 met the acceptance criteria of [redacted] for [redacted] and [redacted] levels. For the [redacted] level, only the DSPC lipid met the % difference criteria of [redacted]. % Difference results for [redacted] and Cholesterol at the [redacted] were slightly above the acceptance criteria of [redacted] for [redacted] and [redacted] for Cholesterol. Concentration differences in results for each of these lipids, when reported per the governing product specification and associated significant figures are [redacted] for SM-102 and [redacted] for DSPC. This variance is insignificant when considering the product specification ranges and does not impact the fitness of the method for its intended use. This variability is also a worst-case scenario where numerous variables are altered (analysts, days, instruments, column lot, mobile phases). The % difference of [redacted] and [redacted] results will be considered acceptable and the inter-assay precision of the method will be reported accordingly.

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mRNA-1273 LNP, mRNA-1273 DP, [redacted]

8.0 CONCLUSION

Analytical test method SOP-1001 passed the acceptance criteria for validation parameters in protocol QC-MVP-0010: specificity, linearity, accuracy, precision (repeatability, intermediate precision), range and robustness. The method is suitable for ranges [redacted] for PEG2000-DMG, [redacted] for Cholesterol, [redacted] for [redacted] and [redacted] for DSPC.

The quantitation limit of this method is based on the lowest [redacted] concentration level and was determined to be [redacted] The detection limited was calculated from the quantitation limit is [redacted] for this method.

Analytical test method SOP-1001 is considered validated for testing mRNA-1273 LNP, mRNA-1273 Drug product, and [redacted] samples.

A verified data summary for the validation experiments is attached, along with the peer-reviewed source raw data packages. Refer to Attachments 1 – 3.

9.0 REFERENCED DOCUMENTS

Document #	Title
ICH Q2(R1)	International Council for Harmonization, Validation of Analytical Procedures
FRM-0741	SOP-1001 Lipid content, purity and ID by UPLC-CAD for [redacted] Assay Parameters
FRM-0743	SOP-1001 Lipid content, purity and ID by UPLC-CAD for [redacted] Resolution Standard Preparation
FRM-0744	SOP-1001 Lipid content, purity and ID by UPLC-CAD for [redacted] Standard Preparation
SOP-1001	Determination of Lipid Content, Purity and Identity by UPLC-CAD for [redacted]
TR-9612	[redacted] SOP-1001 v0.2 and QC-MVP-0010 v1.0
TR-9613	[redacted] SOP-1001 v0.2 and QC-MVP-0010 v1.0
TR-9614	[redacted] SOP-1001 v0.2 and QC-MVP-0010 v1.0
QC-MVP-0010	Method Validation Protocol of SOP-1001: Determination of Lipid Content, Purity and Identity by UPLC-CAD for [redacted]

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TITLE	
Method Validation Report of SOP-1001: Determination of Lipid Content, Purity, and Identity by UPLC-CAD for [redacted]	
mRNA-1273 LNP, mRNA-1273 DP, [redacted]	

10.0 ATTACHMENTS

Attachment 1: QC-MVR-0010 Data Portfolio (Veeva)

Attachment 2: QC-MVR-0010 Verified Excel File (Veeva)

Attachment 3: QC-MVR-0010 Excel Data (Veeva)

11.0 REVISION HISTORY

Revision #	Effective Date	Change Details	Author
1.0	Refer to Veeva Header for Effective Date	New Document	[redacted]

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Document Approvals

Approved Date: 09 Oct 2020

Task: Approval Task Verdict: Approve	[redacted]
Task: QA Approval Task Verdict: Approve	[redacted]

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