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Method Validation Report of SOP-0999: Determination of RNA concentration in [redacted] by IEX Chromatography with UV Detection

mRNA-1273 LNP, mRNA-1273 DP

1. Introduction

This report presents the method validation results of test method SOP-0999 for testing mRNA-1273 Lipid nanoparticle (LNP) and mRNA-1273 Drug product (DP). The validation was performed at the Moderna Quality Control (QC) Laboratory following method validation protocol QC-MVP-0008, Method Validation Protocol of SOP-0999: Determination of RNA concentration in [redacted] by IEX chromatography, and in accordance with the ICH Q2(R1) Guideline for Validation of Analytical Procedures.

SOP-0999, Determination of RNA concentration in [redacted] by IEX chromatography with UV detection, is used to quantitate the mRNA content in mRNA-1273 LNP and DP. [redacted]

[redacted]

Method SOP-0999 was validated according to QC-MVP-0008 using CX-024414 mRNA, mRNA-1273 LNP, mRNA-1273 DP, [redacted] and the associated formulation buffers.

The validation parameters of specificity, linearity, accuracy, precision (repeatability, intermediate precision), range, stability and robustness were evaluated, and the results are summarized in this report.

2. 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.

3. Documentation

3.1. 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.

3.2. Draft analytical method **SOP-0999** (version 0.2) was followed for this testing. Assay information was documented on draft **FRM-0731** (version 0.3) and **FRM-0732** (version 0.4).

3.3. QC Analysts documented read and understand training on analytical method **SOP-0999** and validation protocol **QC-MVP-0008** prior to executing validation testing. Refer to [redacted] documents **TR-9620, TR-9621** and **TR-9622** for the training records.

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3.4. 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. Materials and Equipment

4.1. Test Articles

Table 1: Test Articles

Sample Description	Lot/Batch	RNA Concentration (mg/mL)	Summary of Analysis Document
mRNA-1273 LNP and DP Formulation Buffers (20 mM Tris, 87 g/L Sucrose, pH 7.5)			
CX-24414 mRNA (Reference material)	MTDS20002	[redacted]	DSAD-SOA-0254
CX-024414 mRNA	DH03180.1		DSAD-SOA-0264
mRNA-1273 LNP	5006820001		COA-0447
mRNA-1273 DP	6006820001		COA-0448
mRNA-1273 DP	6006920001		COA-0449
[redacted]	AMPDP-20053		N/A

4.2. Materials and Equipment

Refer to the Materials and Equipment Section of SOP-0999 (version 0.2).

5. Validation Summary

5.1. Validation Parameters, Acceptance Criteria and Results

Table 2: Summary of Results

Parameter	Acceptance Criteria	Result	Pass/ Fail
System Suitability	Report system suitability results as outlined in analytical test method SOP-0999. Results will be assessed during the validation and any necessary updates to draft versions of SOP-0999 and FRM-0731 / FRM-0732 will be made prior to the effective versions.	All system suitability criteria were met for each assay.	Pass

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Parameter	Acceptance Criteria	Result	Pass/ Fail
Specificity	The mRNA-1273 LNP and mRNA-1273 DP formulation buffer will not produce peaks, which interfere with the mRNA-1273 LNP or mRNA-1273 DP sample peak. Interferences to main peak [redacted]	Sample: **mRNA-1273 Formulation Buffer (20mM Tris, 87g/L Sucrose, pH 7.5)** — % Interference vs. LNP: [redacted]; % Interference vs. DP: [redacted]	Pass
Linearity	Report (R^2), Slope, y-Intercept and RSS linear fittings The coefficient of determination (R^2), of the linear regression must be [redacted]	Linearity [redacted] R2 = [redacted] Slope= [redacted] Y-Intercept = [redacted]	Pass
Accuracy	The %RSD of measured concentration at each level [redacted] % Recovery for each level [redacted]	Level / Concentration (% of 0.2 mg/mL mRNA) / % RSD / % Recovery: [redacted]	Pass
Precision (Repeatability)	The %RSD of mRNA concentration results for Analyst 1 (n=[redacted]) is [redacted] for each test article.	[redacted]	Pass

Level / Concentration (% of 0.2 mg/ml mRNA) / Main Recovery (%): [redacted]

[1] Refer to discrepancy document # QC-OTH-0185

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Parameter	Acceptance Criteria	Result	Pass/ Fail
Precision (Intermediate)	% RSD of mRNA concentration results for Analyst 2 (n=[redacted] for each test article. Overall %RSD of the mRNA concentration results for (Analyst 1& 2 (n=[redacted] for each test article. % Absolute difference of the mean concentration Between analyst 1 & 2 [redacted] for each test article.	[redacted]	Pass
Range	If the validation target expectations for linearity, precision, and accuracy are met, this demonstrates that the range is suitable.	Validated Range [redacted] (corresponding to [redacted] of the nominal concentration of [redacted]	Pass
Robustness	Intermediate precision criteria are met.	Intermediate Precision criteria were met	Pass
Sample Stability	Report Absolute % difference for each stability time point. Absolute difference of all timepoints compared to its T0 reading[redacted]	[redacted]	Pass

[2] Refer to discrepancy #2 Section 6.5 of this report

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Parameter	Acceptance Criteria	Result	Pass/ Fail
		[redacted]	

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6. Validation Results

6.1. System Suitability

Experimental Design:

System suitability as outlined by SOP-0999 is evaluated each time as analysis is run.

Acceptance Criteria:

Report system suitability results as outlined in analytical test method SOP-0999. Results will be assessed and any necessary updates to draft versions of SOP-0999, FRM-0731 and FRM-0732 will be made prior to the effective version.

Results:

System suitability passed the acceptance criteria listed in SOP-0999. No further updates to the system suitability criteria will be made based on results of this validation. Refer to Attachment 1 (data portfolio) for system suitability results.

6.2. Specificity

Experimental Design:

- mRNA-1273 LNP and DP formulation buffer (20 mM Tris, 87 g/L Sucrose, pH 7.5) was prepared per SOP-0999. A starting concentration of [redacted] for the buffer was assumed.
- mRNA-1273 LNP and mRNA-1273 DP (lot 6006920001) samples were prepared per SOP-0999. These samples were used for comparison against the formulation buffer.
- Testing was performed per SOP-0999 and each sample preparation injected once.

Data Analysis:

The formulation buffer chromatographs were compared against sample chromatographs for interference.

Acceptance Criteria:

The LNP and DP formulation buffer chromatogram will not display any peaks that interfere with the integration of mRNA peaks for the LNP and DP samples.

Any interferences to main peak must be [redacted]

Results:

The test method SOP-0999 demonstrates specificity for mRNA-1273 LNP and mRNA-1273 DP; Interfering peaks from the formulation buffer [redacted] Refer to Table 3 for results.

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Table 3. Specificity Results

Sample	% Interference vs. LNP	% Interference vs. DP
mRNA-1273 Formulation Buffer (20mM Tris, 87g/L Sucrose, pH 7.5)	[redacted]	

6.3. Linearity

Experimental Design:

Linearity was evaluated by preparing spiked mRNA-1273 samples at [redacted] different levels: [redacted] of nominal concentration ([redacted]). A [redacted] sample was also prepared and injected. Samples for each linearity level were prepared in triplicate and each preparation injected once.

Data Analysis:

- The % RSD of measured concentrations for each level was calculated against the working standard as a sample [redacted].
- Individual and average % recovery at each level were calculated.
- Regression analysis of average peak area against the target load concentration was performed [redacted].
- The coefficient of determination (R^2), Slope and Y-intercept were calculated and reported.

Acceptance Criteria:

The coefficient of determination (R^2) of the linear regression must be [redacted]

Results:

The correlation coefficient (R^2) of the Linear regression was [redacted] passing the acceptance criteria and demonstrating assay linearity at the range evaluated [redacted]). Slope is [redacted] and y-intercept is [redacted] Linearity results are presented in Table 4 and Figure 1.

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Table 4: Linearity Results

Sample Name (% of Nominal Testing Conc.)	Target Sample Conc. (mg/mL)	Peak Area	Average Peak Area	Conc. (mg/mL)	Mean Conc. (mg/mL)	%RSD
[redacted]						

Figure 1. Linearity Regression

[redacted]

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6.4. Precision (Repeatability)

Experimental Design:

Repeatability Precision was assessed for each of one lot of mRNA-1273 LNP and 2 lots of mRNA-1273 DP by making [redacted] sample preparations for each sample (diluted at the nominal concentration of [redacted] and analyzing them in one run. Each preparation was injected once.

Data Analysis:

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

Acceptance Criteria

The %RSD of the mean concentration results (n=[redacted]) for each test article must be [redacted]

Results

The %RSD of the mean concentration results (n=[redacted]) for each test article was [redacted] passing the acceptance criteria.

A discrepancy occurred during the execution of repeatability precision. Initial results generated by Analyst 1 for mRNA-1273 DP lot 6006820001 failed the criteria for %RSD. The results generated were suspected to be atypical (due to low concentration) and were re-run under QC-MVP-0008 discrepancy #1 [redacted] document # QC-OTH-0185). The retested data confirmed that the original results were atypical and likely due to a preparation error. The original results were deemed invalid and not reported. A complete description of the discrepancy can be found in document # QC-OTH-0185.

The retested results for mRNA-1273 DP lot 6006820001, along with results for the other two samples, are presented in Table 5. The complete dataset, which includes all results generated, can be found in Attachments 2 and 3.

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Table 5. Repeatability Precision Analyst 1

Sample	Qualification Parameter / Variables	Preparation	mRNA Conc. (mg/mL)	Mean Conc. (mg/mL)	%RSD
[redacted] lot 6006820001 [redacted]	[redacted]				
[redacted] lot 6006920001 [redacted]					
[redacted] lot 5006820001 [redacted]					

6.5. Precision (Intermediate)

On a separate day, a second operator (Analyst 2) repeated the Precision using the same lots of mRNA-1273 LNP and DP. Analyst 2 performed the analysis using a different column lot, different preparations of mobile phases, and a different HPLC instrument than were used by Analyst 1.

Data Analysis:

The %RSD of the concentration results (n=[redacted]) for each test article for Analyst 2 was calculated.

The %RSD of the concentration results (n=[redacted] for each test article for Analyst 1 and 2 was calculated.

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

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<u>Acceptance Criteria</u>

The %RSD of the concentration results (n=[redacted]) for Analyst 2 must be [redacted] for each test article.

The overall %RSD of the concentration results (n=[redacted] for both analysts must be [redacted] foreach test article.

The absolute % difference of the mean between Analyst 1 and 2 [redacted] for each test article.

<u>Results</u>

The %RSD of Analyst 2 concentration results (n=[redacted] was [redacted] and overall %RSD of % results for both analysts (n=[redacted]) was [redacted] meeting the acceptance criteria.

The % absolute difference between analyst 1 and 2 was [redacted] for mRNA-1273 DP lot 6006820001 and met the acceptance criteria, and [redacted] for both mRNA-1273 DP lot 6006920001 and mRNA-1273 LNP lot 5006820001, both of which do not meet the pre-established acceptance criteria of [redacted] This failure is covered in is QC-MVP-0008 discrepancy #2 which is documented and discussed below.

The % difference for mRNA-1273 DP lot 6006920001 and mRNA-1273 LNP lot 5006820001 were both higher than expected [redacted] for both samples) when calculating the % difference with the raw results. The concentration difference in mean results between the analysts for mRNA-1273 DP lot 6006920001 is [redacted] while the difference in concentration for mRNA-1273 LNP lot 5006820001 is [redacted] (results in Table 7). Concentration differences in results for each of these samples, when reported per the governing product specification and associated significant figures is [redacted] respectively. This variance would be 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 [redacted] results will be considered acceptable and the inter-assay precision of the method will be reported accordingly.

Table 6. Intermediate precision Analyst 2

Sample	Qualification Parameter / Variables	Preparation	mRNA Conc. (mg/mL)	Mean Conc. (mg/mL)	%RSD
[redacted] [redacted] lot 6006820001 [redacted]	[redacted]				

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Sample	Qualification Parameter / Variables	Preparation	mRNA Conc. (mg/mL)	Mean Conc. (mg/mL)	%RSD
[redacted] lot 6006920001 [redacted]	[redacted]				
[redacted] lot 5006820001 [redacted]					

Table 7. Precision Analyst 1 and Analyst 2

Sample	Analyst	Mean Conc. (mg/mL)	Mean Conc. (mg/mL) (n=12)	%RSD (n=12)	% Absolute Difference A1 vs. A2
[redacted] lot 6006820001 [redacted]	[redacted]				
[redacted] lot 6006920001 [redacted]					
[redacted] lot 5006820001 [redacted]					

6.6. Accuracy

Experimental Design:

Linearity and Precision data was used to evaluate accuracy of the method. Since the linearity experiment has [redacted] levels with triplicate preparations at each level and precision has [redacted] preparations at 100% (nominal) concentration levels, no separate experiment was needed to evaluate the accuracy of the test method.

Data Analysis:

The mean % Recovery of each level, along with the %RSD of the replicate mean peak area were calculated.

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Acceptance Criteria:

The %RSD of measured concentration at each level[redacted]

The percent recovery of the % Recovery against precision data (n=[redacted] must be[redacted]

Results

Accuracy results met the acceptance criteria for % Recovery and %RSD of the replicates. Results are presented in Table 12.

Table 8. Accuracy Results

Sample Name (% of Nominal Testing Conc.)	Target Sample Conc. (mg/mL)	Peak Area	Average Peak Area	Conc. (mg/mL)	Mean Conc. (mg/mL)	%RSD Conc	Individual %Recovery	Mean %Recovery
[redacted]								

6.7. Range

The validation target expectations for linearity, precision, and accuracy were met. This demonstrates that the range of [redacted] (corresponding to [redacted] of the nominal sample concentration of [redacted] is suitable.

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6.8. Robustness

Robustness was assessed by varying assay conditions that may occur under normal usage in QC. The conditions were: analysts, days, HPLC 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 and 6.6 for results.

6.9. Sample stability

Experimental Design:

A single preparation of mRNA-1273 LNP, mRNA-1273 DP Lot 6006820001 and mRNA-1273 DP Lot 6006920001 were prepared per SOP-0999. Each article was analyzed per SOP-0999 at timepoints T=[redacted], T=[redacted] and T=[redacted] Days. Samples were stored in [redacted] between days.
The protocol specifies to store the samples at [redacted] in between days. This was a protocol error as the intent of this experiment is to assess sample stability after preparation and while the samples were held in the autosampler at the SOP-0999 instrument method temperature of [redacted] for a period of time (and while waiting to get injected). Thus, the temperature at which the prepared samples were held was [redacted] and not [redacted] as described in the protocol.

Acceptance Criteria:

Absolute % difference of each test article's RNA concentration result (mg/mL) as compared to its T=[redacted] reading must be [redacted] for the sample to be considered stable.

Results:

Absolute % difference for each stability timepoint was calculated and the results for all the timepoints in comparison to its T0 reading was [redacted] (Refer to table below).

Prepared samples are stable for up to [redacted] days when held in the autosampler at [redacted]

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Table 9. Sample Stability Results at [redacted]

Time Point (days)	[redacted]
Sample	
Conc. (mg/mL)	
% Difference from T[redacted]	

7. Discrepancies

Discrepancy # 1

A discrepancy was generated for the repeatability precision testing and documented in [redacted] document # QC-OTH-0185. A suspected preparation error by analyst 1 produced aberrant data compared to expected concentration results and resulted in a precision %RSD failure. The testing was repeated using freshly prepared samples and reagents and the results were within expected samples concentration values. This is also discussed in section 6.4.

Discrepancy #2

A discrepancy was generated for the intermediate precision testing and discussion in section 6.5. Higher than expected % difference values between analyst concentrations were obtained for 2 samples testing in precision. The higher % difference was accepted and reported.

8. Conclusion

Analytical test method SOP-0999 passed the acceptance criteria for validation parameters in protocol QC-MVP-0008: specificity, stability, linearity, accuracy, precision (repeatability, intermediate precision), range and robustness.

Analytical test method SOP-0999 is considered validated for testing mRNA-1273 LNP and mRNA-1273 DP samples. The validation range has been determined to be [redacted] Prepared samples are stable for up to [redacted] days when held in the autosampler at [redacted].

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

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9. Referenced Documents

Document #	Title
ICH Q2(R1)	International Council for Harmonization, Validation of Analytical Procedures
FRM-0731	SOP-0999 Assay Performance Worksheet, Standard preparation
FRM-0732	SOP-0999 Assay Performance Worksheet
SOP-0999	Determination of RNA concentration in [redacted] by IEX Chromatography with UV Detection
QC-OTH-0185	QC-MVP-0008 Discrepancy 1
TR-9620	[redacted] SOP-0999 v0.2 and QC-MVP-0008 v1.0
TR-9621	[redacted] SOP-0999 v0.2 and QC-MVP-0008 v1.0
TR-9622	[redacted] SOP-0999 v0.2 and QC-MVP-0008 v1.0
QC-MVP-0008	Method Validation Protocol of SOP-0999: Determination of RNA Concentration in [redacted] by IEX Chromatography with UV Detection

10. Attachments

Attachment 1: QC-MVP-0008 Data Portfolio ([redacted])

Attachment 2: QC-MVP-0008 Verified Excel File ([redacted])

Attachment 3: QC-MVP-0008 Excel Data ([redacted])

11. Revision History

Revision #	Effective Date	Change Details	Author
2.0	Refer to [redacted] Header for Effective Date	Added reference to data in attachment 1 for section 6.1 system suitability. Corrected typo and added sample stability discussion section 6.9 Corrected slope and y-intercept values in linearity sections. Added instrument type "HPLC" in section 6.5 for clarification. Corrected [redacted] for the concentration difference between analysts section 6.5. Corrected UHPLC to HPLC for robustness section 6.8. Added reference to discrepancy QC-OTH-0185 in referenced documents. Added "stability" in the introduction and conclusion sections.	[redacted]

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Revision #	Effective Date	Change Details	Author
		Corrected sample lot number in section 6.9 for stability and add an explanation for incorrect sample storage temperature per the protocol; clarification for tested time points was added as well. Updated Table 9 to add stability temperature of [redacted] for results.	
1.0	30Sept20	New Document	[redacted]

Document Approvals

Approved Date: 06 Nov 2020

Task: Approval Task Verdict: Approve	[redacted] Quality Control Approval 06-Nov-2020 03:23:35 GMT+0000
Task: QA Approval Task Verdict: Approve	[redacted] Quality Assurance Approval 06-Nov-2020 13:51:27 GMT+0000