moderna

Method Validation Report

TITLE

Report: Validation of SOP-0996, Analysis of mRNA purity by Size-based RPIP HPLC

CX-024414 mRNA, mRNA-1273 LNP, and mRNA-1273 DP

1. Introduction

This report presents the method validation results of test method SOP-0996 for mRNA construct CX-024414, mRNA-1273 formulated Lipid Nano Particles (LNP), and mRNA-1273 Drug Product (DP). The validation was performed at the Moderna Quality Control (QC) Laboratory following method validation protocol QC-MVP-0005, Validation of SOP-0996, Analysis of mRNA purity by Size-based RPIP HPLC, and in accordance with the ICH Q2(R1) Guideline for Validation of Analytical Procedures.

CX-024414 mRNA is prepared as a [redacted] target mRNA solution in [redacted] sodium acetate, pH [redacted] buffer. The product storage temperature is -15 to -25°C. The mRNA construct is 4101 nucleotides in length.

mRNA-1273 Formulated LNP is prepared as a liquid solution with a target mRNA concentration of [redacted] in [redacted] sodium acetate, 20mM Tris, 87 g/L Sucrose, pH 7.5 buffer. The product storage temperature is -60 to -90°C.

mRNA-1273 DP is prepared as a liquid solution with a target mRNA concentration of approximately 0.2 mg/mL in [redacted] sodium acetate, 20mM Tris, 87 g/L Sucrose, pH 7.5 buffer. The product storage temperature is -15 to -25°C.

The separation and analysis of intact mRNA species by length is used as an assessment of mRNA quality by detecting loss in potency due to product degradation to inert fragments. SOP-0996 is a reversed phase (ion-pair) high performance liquid chromatography (RPIP-HPLC) method with the ability to separate intact full-length mRNA molecules and product-related impurities of different lengths. mRNA is separated based on size/length by gradient elution in a heterogeneous ion pair mobile phase system with UV detection at 260 nm. Intact mRNA elutes in the main peak, whereas impurities have shorter (Impurity Group 1) or longer (Impurity Group 2, Lipid Adduct) retention times relative to the main peak. Purity and impurities are reported as a percent of the total peak area.

Method SOP-0996 was validated according to protocol QC-MVP-0005 and validation master plan QC-VMP-0001 using the test articles described in section 4.1.

The following parameters were evaluated: system suitability; method precision; intermediate precision; linearity; accuracy; specificity; determination of the quantitation limit; stability of standard and sample preparation solutions; range; and robustness. Parameters were assessed and met the acceptance criteria listed in the protocol. Test method SOP-0994 is considered validated for testing CX-024414 mRNA, mRNA-1273 LNP, and mRNA-1273 DP.

2. Responsibilities

Table 1: 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-0996** (version 0.4) was followed for this testing. Assay information was documented on draft **FRM-0727** (version 0.3).

3.3. QC Analysts documented read and understand training on analytical method **SOP-0996** and validation protocol **QC-MVP-0005** prior to executing validation testing. Refer to Veeva documents **TR-9615, TR-9616, TR-9617, TR-9623, and TR-9624** for the training records.

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 2: Test Articles

Sample Description	Lot/Batch	RNA Concentration (mg/mL)	Summary / Certificate of Analysis Document
[redacted]Sodium Acetate, pH[redacted] (CX-024414 mRNA Formulation Buffer)			
20 mM Tris, 87 g/L Sucrose, pH 7.5 (mRNA-1273 LNP and DP Formulation Buffer)			
CX-024414 mRNA (Assay Reference Standard)	MTDS20002	[redacted]	DSAD-SOA-0254
CX-024414 mRNA	DH-03256	[redacted]	DSAD-SOA-0265
[redacted]	AMPDP-20062	[redacted]	N/A
mRNA-1273 LNP	5006820001	[redacted]	COA-0447
mRNA-1273 DP	6006820001	[redacted]	COA-0448
mRNA-1273 DP	6006920001	[redacted]	COA-0449

4.2. Materials and Equipment

Refer to the Materials and Equipment Section of draft SOP-0996 (version 0.4)

5. Validation Summary

5.1. Validation Acceptance Criteria and Results

Table 3: Summary of Parameters and Acceptance Criteria

Parameter	Acceptance Criteria	Result	Pass / Fail
System Suitability	Report system suitability results as outlined in the analytical method SOP-0996. Results were assessed during the validation and any necessary updates to draft versions of SOP-0996 and FRM-0727 were made prior to the effective version.	All system suitability criteria were met for each assay.	Pass
Specificity	**Formulation Buffer and** [redacted] No interfering peak(s) ≥ QL at the retention time of the IG1, Main, IG2, or Adduct peaks compared to the unstressed sample. **Heat Stressed Samples** An increase in the total impurities in the heat stressed sample must be observed with respect to the unstressed sample.	No interfering peaks ≥ QL present in samples at the expected RT of the IG1, Main, IG2, and IG3 peaks compared to the unstressed sample. 91.83% increase in impurities observed in the heat stressed sample with respect to the unstressed sample.	Pass
Linearity	**IG1** The coefficient of determination (r^2) of the Linear regression must be [redacted] for the IG1 peak. Report slope and y-intercept. **Main Peak** The coefficient of determination (r^2) of the Linear regression must be [redacted] for the Main peak. Report slope and y-intercept.	**IG1**: [redacted] **Main Peak:** [redacted]	Pass

<table>
<tr><th>Parameter</th><th>Acceptance Criteria</th><th>Result</th><th>Pass / Fail</th></tr>
<tr><td rowspan="2">Accuracy (from Linearity)</td><td>% Recovery of the individual and mean % Main peak at each linearity level must be [redacted] when compared to the mean % Main peak result from Precision.</td><td>% Main Peak Recovery
Level | Prep | % Recovery (Individual) | % Recovery (Mean)
[redacted]</td><td rowspan="2">Pass</td></tr>
<tr><td>% Recovery of the individual and mean main peak area at each level must be [redacted] when compared to mean main peak area from system suitability.</td><td>Main Peak Area Recovery
Level | Prep | % Recovery (Individual) | % Recovery (Mean)
[redacted]</td></tr>
</table>

Parameter	Acceptance Criteria	Result	Pass / Fail
	The RSD of the % main peak for each level must be [redacted]	Level / %RSD of Main Peak Area [redacted]	
Precision (Repeatability)	The %RSD of the % Main Peak (n=6) must be [redacted]	**Analyst 1 (n=6)** **% RSD of %Main Peak:** CX-024414 mRNA = [redacted] mRNA1273 LNP = [redacted] mRNA-1273 DP [redacted] mRNA-1273 DP [redacted]	Pass
Precision (Intermediate)	• The % RSD of the % Main Peak (n=6) must be [redacted] • The % RSD of the % Main Peak (n=12) between 2 analysts using different mobile phases, instruments, and columns must be [redacted] Absolute difference of the mean % Main Peak between analysts must be [redacted]	**Analyst 2 (n=6)** **% RSD of %Main Peak:** CX-024414 mRNA = [redacted] mRNA1273 LNP = [redacted] mRNA-1273 DP [redacted] mRNA-1273 DP [redacted] **Analyst 1 & 2 (n=12)** **% RSD of %Main Peak:** CX-024414 mRNA = [redacted] mRNA1273 LNP = [redacted] mRNA-1273 DP [redacted] mRNA-1273 DP [redacted] **Absolute Difference Mean %Main Peak:** CX-024414 mRNA = [redacted] mRNA1273 LNP = [redacted] mRNA-1273 DP [redacted] mRNA-1273 DP [redacted]	Pass
Range	Report the lowest and highest concentrations that meet the linearity, accuracy, and precision acceptance criteria.	Range: [redacted]	Pass
Robustness	Intermediate precision criteria are met.	Intermediate precision criteria were met.	Pass

Parameter	Acceptance Criteria	Result	Pass / Fail
Quantitation Limit (QL)	The lowest concentration level to meet the following acceptance criteria were set as the QL. % RSD of the Main peak area (n=3) must be [redacted] The individual and mean % Recovery of the Main peak area must be [redacted] when compared to the mean theoretical Main peak area from precision. The individual and mean Main Peak S/N ratio must be [redacted] % Main Peak Recovery of the IG1 Spike must be [redacted] of the theoretical % Peak Area[1].	QL = [redacted] % RSD = [redacted] Main Peak Area Prep / % Recovery / Mean [redacted] Prep / S/N / Mean [redacted] IG1 Spike Peak Area Prep / % Recovery / Mean [redacted]	Pass
Prepared Solution Stability	The absolute % difference for the % Main peak from T=0 and T=X days is [redacted]	**Absolute % Difference %Main Peak (T=0, T=1):** CX-024414 mRNA = [redacted] mRNA1273 LNP = [redacted] mRNA-1273 DP [redacted] mRNA-1273 DP [redacted] Reference Standard = [redacted]	Pass

[1]Additional acceptance criteria added per QC-OTH-0172

5.2. System Suitability

Experimental Design:

System suitability as outlined in SOP-0996 is evaluated each time an analysis is run.

Acceptance Criteria:

Report system suitability results as outlined in the analytical method SOP-0996. Results were assessed during the validation and any necessary updates to draft versions of SOP-0996 and FRM-0727 were made prior to the effective version based on the results.

Results:
System suitability passed the acceptance criteria listed in SOP-0996. No further updates to the system suitability criteria were made based on results of this validation.

5.3. Specificity

Experimental Design:

CX-024414 mRNA formulation buffer [redacted] Sodium Acetate, pH [redacted], mRNA-1273 DP Formulation Buffer (20 mM Tris, 87 g/L Sucrose, pH 7), and [redacted] were prepared as samples per SOP-0996. A representative sample for CX-024414 mRNA lot MTDS20002 was also prepared per SOP-0996.

Additionally, a forced degradation sample was prepared as described below:

- Aliquot [redacted] CX-024414 mRNA solution (lot MTDS20002) into a microcentrifuge tube.
- Heat stress the sample in a closed microcentrifuge tube at [redacted]
- Remove from heat and place into an HPLC vial for analysis (inject once).

Data Analysis

The chromatogram of each unstressed sample was overlaid with the associated formulation buffer and [redacted] and interference from the buffer was evaluated. The % IG1, Main Peak, IG2 and adduct of each sample was calculated by the Chromeleon software.

Acceptance Criteria:

To demonstrate specificity, the following acceptance criteria must be met:

- No interfering peak(s) shall be observed within the elution time region of IG1, Main, IG2, or adduct (LNP/DP only) peaks. Interfering peaks are defined as any peak ≥ QL.
- An increase in total impurities in the degraded sample should be observed with respect to the unstressed sample.

Results:

Test method SOP-0996 was demonstrated to be specific for CX-024414 mRNA in either nominal or heat-stressed degraded forms. The sample matrices had no peaks ≥ QL present in samples at the expected RT of the IG1, Main, IG2, and adduct peaks. There was a [redacted] increase in impurities observed in the heat stressed sample with respect to the unstressed sample. Refer to Table 4 for results. One discrepancy occurred during execution of specificity. The last injection of reference standard which bracketed the specificity samples failed to meet the system suitability area recovery acceptance criteria due to interference from lipids that were not fully extracted from one of the specificity

samples. The results for the initial specificity samples were invalidated due to the bracket failure and samples were repeated in a new assay with passing system suitability results. Refer to Discrepancy # 2 for details.

Table 4: Specificity Results

Test Article	IG1	Main Peak	IG2	% Total Impurities	Increase in Impurities (%)
Unstressed	[redacted]				
Heat Stressed					
[redacted] Sodium Acetate, pH[redacted] (CX-024414 mRNA Formulation Buffer)	No peaks ≥ QL present				
20 mM Tris, 87 g/L Sucrose, pH 7.5 (mRNA-1273 LNP and DP Formulation Buffer)	No peaks ≥ QL present				
[redacted]	No peaks ≥ QL present				

5.4. Linearity

Experimental Design:

Linearity was evaluated by spiking CX-024414 mRNA Reference Standard (lot MTDS20002) into [redacted] and mRNA-1273 LNP Formulation Buffer (20 mM Tris, 87 g/L Sucrose, pH 7.5) at 5 levels (N=3) covering [redacted] of the nominal assay concentration [redacted]. A total of 5 levels were evaluated for linearity. The target sample concentrations are [redacted] mg/mL after IPA extraction. These target concentrations are equivalent to approximately [redacted] of the nominal sample concentration of [redacted] respectively. At each target level, three preparations were independently made and each preparation was injected once for analysis.

A [redacted] mRNA stock solution and a [redacted] working lipid stock solution were prepared. The amount of mRNA utilized for sample preparation was varied to achieve the desired concentrations. IPA extraction was performed per SOP-0996 for each sample. The resulting sample concentrations were [redacted] after IPA extraction.

Data Analysis

- Plot peak the area of the Main peak versus the corrected concentration.
- Plot peak the area of the IG1 peak versus the corrected concentration.
- r^2, slope, and y-intercept for each plot was reported.

Acceptance Criteria:

- The coefficient of determination (r^2) of the Linear regression must be [redacted] for the IG1 peak. Report slope and y-intercept.

- The coefficient of determination (r^2) of the Linear regression must be [redacted] for the Main peak. Report slope and y-intercept.

Results:

Refer to Table 5 and Table 6 for the IG1 and Main Peak linearity results and Figures 1 and 2 for the linear regression plots of the IG1 and Main peaks. The coefficient of determinations (R^2) for the IG1 and Main peaks are [redacted] and [redacted] respectively which meet the acceptance criteria of [redacted] for the IG1 peak and [redacted] for the Main peak. Test method SOP-0996 was demonstrated to be linear in the range of [redacted] sample concentrations.

Table 5: IG1 Linearity Results

Level	Prep	Target Total Concentration (mg/mL)	Corrected Concentration (mg/mL)	% of Nominal	Experimental IG1 Area
1 [redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
	2				
	3				
2 [redacted]	1				
	2				
	3				
3 [redacted]	1				
	2				
	3				
4 [redacted]	1				
	2				
	3				
5 [redacted]	1				
	2				
	3				

Table 6: Main Peak Linearity Results

Level	Prep	Target Total Concentration (mg/mL)	Corrected Concentration	% of Nominal	Experimental Main Peak Area
1 [redacted]	1	[redacted]	[redacted]	[redacted]	[redacted]
	2				
	3				
2 [redacted]	1				
	2				
	3				
3 [redacted]	1				
	2				
	3				
4 [redacted]	1				
	2				
	3				
5 [redacted]	1				
	2				
	3				

Figure 1. IG1 Peak Linearity Regression

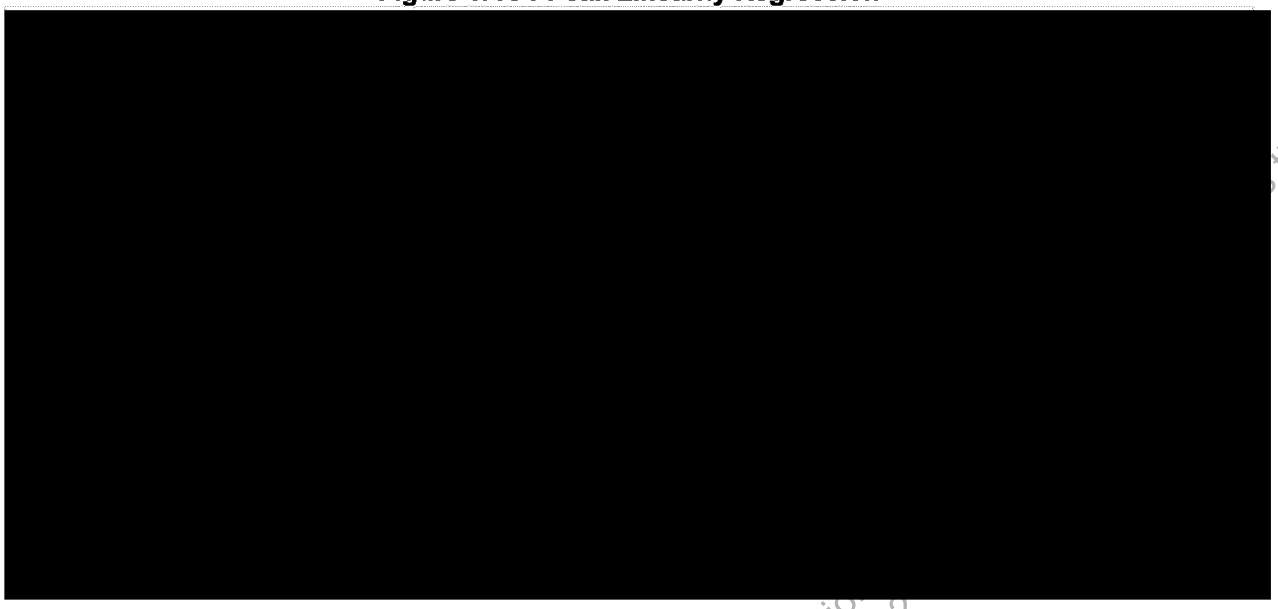

Figure 2. Main Peak Linearity Regression

5.5. Precision

Experimental Design:

Repeatability Precision was assessed for the CX-024414 mRNA, mRNA-1273 LNP, and mRNA-1273 DP test articles listed in Table 2 by preparing six independent sample preparations from the same sample to a final target concentration of [redacted] and analyzing them in one run.

Data Analysis:

The mean % area of the main peak and %RSD of the six reportable results (n=6) were determined for each applicable test article.

Acceptance Criteria

The %RSD of the % main peak area (n=6) must be [redacted]% for each test article.

Results:

The %RSD (n=6) for each lot was [redacted], passing the acceptance criteria. Results are presented in Tables 7-10.

Table 7: DH-03256 Analyst 1 Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	

Table 8: 5006820001 Analyst 1 Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	

Table 9: 6006820001 Analyst 1 Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	

Table 10: 6006920001 Analyst 1 Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	

5.6. Intermediate Precision

Experimental Design:

Intermediate precision was evaluated using the same test articles and the same preparing instructions utilized for Repeatability (section 5.5). The method variability with respect with analysts, days, Mobile Phase preparations, instruments, and column lots was assessed. A minimum of two analysts, two different vendor lots of columns, two Mobile Phase A and Mobile Phase B preparations, and two instruments were required for this study.

Data Analysis

The mean % main peak and % RSD of the six reportable results (n=6) for analyst two were determined for each test article.

The overall % RSD of the % main peak for each test article for both analysts (n=12) was calculated and analyzed.

The absolute difference of the mean % main peak between analysts was calculated and analyzed for each test article.

Acceptance Criteria (for each test article)

- The %RSD of the % main peak (n=6) must be [redacted].
- The %RSD of the % main peak (n=12) must be [redacted].
- Absolute difference of the mean % main peak between analysts must be [redacted]

Results:

The Intermediate Precision acceptance criteria was met for all test articles. The %RSD of the % main peak for Analyst 2 (n=6) was [redacted] for each test article. Overall % RSD of the % main peak results for both analysts (n=12) was [redacted] Absolute difference between sample % main peak results was [redacted] for all test articles. Results are presented in Tables 11-14.

Three discrepancies occurred during the execution of intermediate precision.

- The initial intermediate precision assay was invalidated due to high instrument pressure, refer to Discrepancy # 5 for details.
- During re-execution of Intermediate Precision / Specificity on 06AUG20, the last replicate injection for mRNA-1273 Drug Product (DP) lot 6006920001 was observed to have atypical chromatography. Pipetting error was determined to be the probable root cause. The results lot 6006920001 were invalidated and testing was repeated in a new assay. Refer to Discrepancy # 3 for details.
- The last bracket injection of reference standard in the assay performed on 06AUG20 failed to meet the system suitability area recovery acceptance criteria due to interference from lipids that were not fully extracted from one of the specificity samples. There is no impact to the intermediate precision results as all samples were within bracket injections that met the system suitability acceptance criteria. Only specificity samples were tested within the bracket that failed. Refer to Discrepancy # 2 for details.

Table 11: DH-03256 Analyst 2 Intermediate Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	
Mean (n=12)	
Std Dev (n=12)	
%RSD (n=12)	
% difference of the % Main Peak (Analyst 1&2)	

Table 12: 5006820001 Analyst 2 Intermediate Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	
Mean (n=12)	
Std Dev (n=12)	
%RSD (n=12)	
% difference of the % Main Peak (Analyst 1&2)	

Table 13: 6006820001 Analyst 2 Intermediate Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	
Mean (n=12)	
Std Dev (n=12)	
%RSD (n=12)	
% difference of the % Main Peak (Analyst 1&2)	

Table 14: 6006920001 Analyst 2 Intermediate Precision Results

Preparation	Main Peak (%Area)
100% Prep 1	[redacted]
100% Prep 2	
100% Prep 3	
100% Prep 4	
100% Prep 5	
100% Prep 6	
Mean	
Std. Dev.	
%RSD	
Mean (n=12)	
Std Dev (n=12)	
%RSD (n=12)	
% difference of the % Main Peak (Analyst 1&2)	

5.7. Accuracy

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, separate experiments were not needed to evaluate the accuracy of the test method.

Data Analysis

Accuracy was demonstrated by determination of the % recovery of the % main peak results from the linearity section. The % recovery of the % main peak was calculated using the formulas below.

% Recovery calculations from the linearity data:

$$\%\ \text{Main Peak Recovery} = \frac{\textit{Measured \% Main peak at each level}}{\textit{mean \% Main peak from system Suit.}} \times 100$$

$$\text{Main Peak Area Recovery} = \frac{\textit{Measured main peak area at each level}}{\textit{mean main peak area from system suit.}} x \frac{[redacted]}{\textit{Conc. at x level}}\ x\ 100$$

Acceptance Criteria:

- % Recovery of the individual and mean % Main peak at each level must be [redacted] when compared to the mean % Main Peak result from system suitability.
- % Recovery of the individual and mean main peak area at each level must be [redacted] when compared to mean main peak area from system suitability.
- The RSD of the % main peak (n=3) for each level must be [redacted]

Results:

Accuracy results met the acceptance criteria for % Recovery of the replicates at levels 1 – 5. Results are presented in Tables 15 and 16.

Table 15: Accuracy Results (% Main peak)

Level	Prep	% Main Peak	% Recovery vs Ref. Std. (93%)	Mean % Recovery	Mean % Main Peak	STDEV % Main Peak	% RSD % Main Peak
1	1						
	2						
	3						
2	1						
	2						
	3						
3	1						
	2						
	3						
4	1						
	2						
	3						
5	1						
	2						
	3						

Table 16: Accuracy Results (Main peak Area)

Level	Prep	Experimental Main Peak Area	Theoretical Main Peak Area	% Recovery	Mean % Recovery
1	1				
	2				
	3				
2	1				
	2				
	3				
3	1				
	2				
	3				
4	1				
	2				
	3				
5	1				
	2				
	3				

5.8. Range

Experimental Design:

Linearity, Accuracy, and Precision data were used to evaluate accuracy of the method. Separate experiments were not needed to evaluate the range of the test method.

Acceptance Criteria:

Report the lowest and highest concentrations that meet the linearity, precision, and accuracy acceptance criteria from sections 5.4, 5.5, and 5.7, respectively.

Results:

The validation target expectations for linearity, precision, and accuracy were met. This demonstrates that the range of [redacted] for sample concentrations is suitable. Refer to sections 5.4 – 5.7 for results.

5.9. Robustness

Experimental Design:

Intermediate Precision data were used to evaluate robustness of the method. Separate experiments are not needed to evaluate the robustness of the test method.

Acceptance Criteria:

Intermediate precision criteria are met.

Results:

Intermediate precision criteria were met. Refer to section 5.6 for results.

5.10. Quantitation Limit (QL)

Experimental Design:

To assess QL, surrogate fragment IG1 (CX-005128 mRNA lot MTDS17036) was spiked into CX-024414 mRNA lot MTDS20002 in the presence of [redacted] at the following concentration levels: [redacted]

At each target level, three preparations were independently prepared and each preparation was injected once for analysis. The amount of mRNA utilized for sample preparation was varied to achieve the desired concentrations. The resulting IG1 spike concentrations after IPA extraction per SOP-0996 are [redacted]

NOTE: Experimental design altered from the original design in QC-MVP-0005 to correct for insufficient mRNA present in the samples which was suspected to have impeded pellet formation. % Recovery of the IG1 spike was added to the acceptance criteria because of the revised experiment. Refer to Discrepancy #1 for further detail.

Data Analysis:

Accuracy (% recovery) of the % main peak area and precision (% RSD) of the IG1 Surrogate Spike main peak area were calculated and reported. The S/N ratio of the IG1 Surrogate Spike main peak was reported by the Chromeleon software.

$$\% \text{ Main Peak Recovery} = \frac{\text{IG1 Spike \% Main Peak Area}}{\text{Theoretical IG1 Spike \% Main Peak Area}} \times 100$$

$$\text{Peak Area Recovery} = \frac{\text{Total Main Peak Area}}{\text{Mean Ref Std Main Peak Area}} \times 100$$

The QL is the lowest level to meet the following acceptance criteria.

Acceptance Criteria

The lowest concentration to meet the following acceptance criteria was set as the QL:

- % RSD of the Main peak area (n=3) must be [redacted]
- The individual and mean % Recovery of the Main peak area must be [redacted] when compared to the mean Main peak from the system suitability standard injections.
- The individual and mean Main Peak S/N ratio must be [redacted]
- % Main Peak Recovery of the IG1 Spike must be [redacted] of the theoretical % Peak Area.

Results:

[redacted] of the nominal sample concentration of [redacted] was determined to be the QL and meets the precision, accuracy (% recovery), and S/N ratio acceptance criteria. Results are presented in Tables 17 and 18.

Table 17: Main Peak (MTDS20002) Quantitation Limit Results

Injection Name	MTDS20002 Peak Area	Total Peak Area	Total Main Peak Area Recovery	Mean % Recovery
QL [redacted] Prep 1	[redacted]			
QL [redacted] Prep 2				
QL [redacted] Prep 3				
QL [redacted] Prep 1				
QL [redacted] Prep 2				
QL [redacted] Prep 3				
QL [redacted] Prep 1				
QL [redacted] Prep 2				
QL [redacted] Prep 3				
QL [redacted] Prep 1				
QL [redacted] Prep 2				
QL [redacted] Prep 3				
QL [redacted] Prep 1				
QL [redacted] Prep 2				
QL [redacted] Prep 3				
QL [redacted] Prep 1				
QL [redacted] Prep 2				
QL [redacted] Prep 3				

Table 18: IG1 Spike (MTDS17036) Quantitation Limit Results

Injection Name	Target Conc. (mg/mL)	Peak Area	Peak Area % RSD	Peak % Area	Peak % Area Recovery	Mean % Recovery	S/N	Mean S/N
QL [redacted] Prep 1	[redacted]							
QL [redacted] Prep 2	[redacted]							
QL [redacted] Prep 3	[redacted]							
QL [redacted] Prep 1	[redacted]							
QL [redacted] Prep 2	[redacted]							
QL [redacted] Prep 3	[redacted]							
QL [redacted] Prep 1	[redacted]							
QL [redacted] Prep 2	[redacted]							
QL [redacted] Prep 3	[redacted]							
QL [redacted] Prep 1	[redacted]							
QL [redacted] Prep 2	[redacted]							
QL [redacted] Prep 3	[redacted]							
QL [redacted] Prep 1	[redacted]							
QL [redacted] Prep 2	[redacted]							
QL [redacted] Prep 3	[redacted]							
QL [redacted] Prep 1	[redacted]							
QL [redacted] Prep 2	[redacted]							
QL [redacted] Prep 3	[redacted]							

5.11. Prepared Standard and Sample Stability

<u>Experimental Design:</u>

To determine the solution stability of the test article after sample preparation, each standard / sample preparation solution (prepared in section 5.5) were tested after being stored in the following manner:

- T=0 (Injected in Repeatability Precision sequence)
- T=1 days, 5°C (in autosampler)

Each standard / sample was tested within the calendar day of the time point.

<u>Data Analysis</u>

The % area of the Main peak at each storage condition was determined and the absolute % difference from T=0 was calculated.

<u>Acceptance Criteria</u>

The absolute % difference between the % Main peak area from T=0 and T=X days is [redacted].

Results:

The results indicate that there is < 5% difference in tailed peak % area when prepared standards and samples are stored for one day at 5°C on the instrument autosampler. Prepared standards and samples are stable for up to 1 day stored at 5°C on the instrument autosampler. Stability results are presented in Table 19.

Table 19: Prepared Sample and Standard Stability Results

DH-03256	% Main Peak	Absolute % difference from T=0
T = Initial	[redacted]	
T = 1 day @ 5°C		[redacted]
6006820001	**% Main Peak**	**Absolute % difference from T=0**
T = Initial	[redacted]	
T = 1 day @ 5°C		[redacted]
5006820001	**% Main Peak**	**Absolute % difference from T=0**
T = Initial	[redacted]	
T = 1 day @ 5°C		[redacted]
6006920001	**% Main Peak**	**Absolute % difference from T=0**
T = Initial	[redacted]	
T = 1 day @ 5°C		[redacted]
Ref. Standard (MTDS20002)	**% Main Peak**	**Absolute % difference from T=0**
T = Initial	[redacted]	
T = 1 day @ 5°C		[redacted]

6. Discrepancies

Discrepancy #1:

Quantitation Limit (QL) for the method validation of SOP-0996, Analysis of mRNA purity by Size-based RPIP HPLC, was initially performed on 04AUG20 following instructions in method validation protocol QC-MVP-0005, Validation of SOP-0996, Analysis of mRNA purity by Size-based RPIP HPLC.

Analysis of the data revealed the QL to have failed to meet the recovery acceptance criteria of [redacted] when compared to the average area of the system suitability main peak. The recovery failed for all 5 levels tested [redacted].

The suspected cause of the failure is an insufficient amount mRNA present in the sample coupled with a high aqueous to organic ratio which impacted the ability of the pellet to fully precipitate during centrifugation.

The QL experiment was re-executed using the revised experimental design described in section 5.10. Spiking surrogate IG1 fragment into CX-024414 mRNA lot MTDS20002 in the presence of [redacted] ensured a sufficient amount mRNA was present and IPA extraction performed as expected.

The impact to the method validation was limited to changing the QL dilution scheme and % recovery calculation. The QL acceptance criteria remained the same as stated in the protocol, with the addition of the following criteria:

% Main Peak Recovery of the IG1 Spike must be [redacted] of the theoretical % Peak Area

The 1% QL level met the acceptance criteria in QC-MVP-0005 as well as the additional IG1 spike recovery criteria. Refer to QC-OTH-0172, "Method Validation Protocol Discrepancy for QC-MVP-0005: Discrepancy #1" for further detail.

Discrepancy #2:

During execution of Intermediate Precision and Specificity per QC-MVP-0005 on 06AUG20, the last bracketing injection of reference standard failed to meet the system suitability area recovery acceptance criteria of 95-105% (result = [redacted].

An unknown peak was observed at the IG3 retention time (RT) in the injections following the [redacted] specificity sample. The chromatograms were shared with the Analytical Development (AD) group and the probable root cause was determined to be residual lipids in the [redacted] sample which were not fully extracted during sample preparation. This was likely due to the high concentration of lipids in the sample [redacted]. All previous bracketing injections passed the area recovery acceptance criteria.

Because the starting concentration of lipids in the [redacted] is not representative of the MRNA-1273 LNP / DP samples within the scope of SOP-0996, the [redacted] sample was diluted to [redacted] total lipids to reflect the highest concentration of lipids that are present in mRNA-1273 LNP / DP samples.

The results for the initial specificity samples were invalidated due to the bracket failure and samples were repeated in a new assay. No system suitability bracketing failures occurred in the new assay and all specificity criteria were met.

There is no impact to the method validation as the specificity acceptance criteria remained the same as stated in the protocol. Refer to QC-OTH-0175, "Method Validation Protocol Discrepancy for QC-MVP-0005: Discrepancy #2" for further detail.

Discrepancy #3:

During execution of Intermediate Precision per QC-MVP-0005 on 06AUG20, the last replicate injection for mRNA-1273 Drug Product (DP) lot 6006920001 was observed to have atypical chromatography.

An unknown peak was observed at the IG1 retention time and the main peak area was significantly smaller than the previous 5 replicates. The most probable root cause was determined to be a pipetting error during sample preparation.

The results for the initial intermediate precision testing of 6006920001 were invalidated and testing was repeated in a new assay. There is no impact to the method validation as the intermediate precision acceptance criteria will remain the same as stated in the protocol. Refer to QC-OTH-0176, "Method Validation Protocol Discrepancy for QC-MVP-0005: Discrepancy #3" for further detail.

Discrepancy #4:

Protocol QC-MVP-0005 has lot number AMPDP-20053 listed as the [redacted] test article. [redacted] lot AMPDP-20062 was used for execution of the protocol. There is no impact to the validation as both lots are equivalent and have the same target lipid concentrations.

Discrepancy #5:

Assay # ARN-20-00322-023 was invalidated due to a system pressure spike. There is no impact to the method validation as it was instrument related.

7. Conclusion

Analytical test method SOP-0996 passed the acceptance criteria for validation parameters in protocol QC-MVP-0005: system suitability; method precision; intermediate precision; linearity; accuracy; specificity; determination of the quantitation limit; stability of standard and sample preparation solutions; range; and robustness.

Linearity was demonstrated over a range of [redacted] of the nominal sample concentration (0.5 mg/mL), corresponding to a validated sample concentration range of [redacted] The determined quantitation limit of the assay is [redacted]. Samples and standards are stable for 1 day when stored at 5°C on the instrument autosampler.

Analytical test method SOP-0996 is considered validated for testing CX-024414 mRNA, mRNA-1273 LNP, and mRNA-1273 DP 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.

8. Referenced Documents

Document #	Title
ICH Q2 (R1)	International Council for Harmonization, Validation of Analytical Procedures: Text and Methodology
FRM-0727	SOP-0996 Assay Performance Worksheet - Percent Poly-A Main and Main Variant mRNA by RP-HPLC
QC-MVP-0005	Validation of SOP-0996, Analysis of mRNA purity by Size-based RPIP HPLC
QC-OTH-0172	Method Validation Protocol Discrepancy for QC-MVP-0005: Discrepancy #1
QC-OTH-0175	Method Validation Protocol Discrepancy for QC-MVP-0005: Discrepancy #2
QC-OTH-0176	Method Validation Protocol Discrepancy for QC-MVP-0005: Discrepancy #3
QC-VMP-0001	Quality Control Validation Master Plan for mRNA-1273
SOP-0996	Percent Poly-A Main and Main Variant mRNA by RP-HPLC
TR-9615	[redacted] SOP-0996 v. 0.4 and QC-MVP-0005 v1.0
TR-9616	[redacted] SOP-0996 v. 0.4 and QC-MVP-0005 v1.0
TR-9617	[redacted] SOP-0996 v. 0.4 and QC-MVP-0005 v1.0
TR-9623	[redacted] SOP-0994/0996/0997/1001 drafts
TR-9624	[redacted] QC-MVP-0005/0006/0007/0010 v1.0 protocols

9. Attachments

Attachment 1: QC-MVR-0005 Data Portfolio (Veeva)

Attachment 2: QC-MVR-0005 Verified Excel Data (Veeva)

Attachment 3: QC-MVR-0005 Excel File (Veeva)

10. Revision History

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

Document Approvals

Approved Date: 08 Oct 2020

Task: Approval Task Verdict: Approve	[redacted] ([redacted]) Quality Control Approval 08-Oct-2020 02:23:50 GMT+0000

Task: QA Approval Task Verdict: Approve	[redacted] ([redacted]) Quality Assurance Approval 08-Oct-2020 19:18:58 GMT+0000