Table of Contents

List of Tables

List of Figures

3.2.P.3.4 CONTROLS OF CRITICAL STEPS AND INTERMEDIATES

This section presents the process control strategy, cumulative process duration, the microbial control strategy, and methods for critical in-process controls testing for mRNA-1273 Drug Product manufacturing process.

The control strategy for mRNA-1273 Drug Product manufacture is a science- and risk-based approach to ensure consistent process performance and quality. It has been developed with a comprehensive understanding of the process and product to ensure critical quality attributes (CQAs) consistently remain within acceptable limits. Process parameters are classified as either critical or non-critical and have target values and manufacturing limits represented by proven acceptable ranges (PARs). Definitions used in the control strategy are summarized in Table 1.

Table 1: Control Strategy Definitions

Term	Definition
Acceptance Criteria	Numerical limits, ranges, or other suitable measures for acceptance of the results of analytical procedures which the drug substance or drug product or materials at other stages of their manufacture should meet.
Control Strategy	A planned set of controls, derived from current product and process understanding, that ensures process performance and product quality. The controls can include parameters and attributes related to drug substance and drug product materials and components, facility and equipment operating conditions, in-process controls, finished product specifications, and the associated method and frequency of monitoring and control.
Critical In-process Control (CIPC)	Only those IPCs that impact forward processing or release decisions. Also known as in-process specifications.
Critical Process Parameter (CPP)	Those process parameters that significantly impact final CQAs and could lead to failed specifications for small excursions outside the manufacturing ranges.
Critical Quality Attribute (CQA)	A physical, chemical, biological, or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality.
In-process Control (IPC)	Consists of in-process tests (IPTs, measurement of product attributes of in-process intermediates) and in-process measurements (IPMs, for monitoring and control of process parameters)
Process Parameter (PP)	Directly controlled operating conditions to ensure process conditions are met. Includes process parameters that employ feedback control in manufacturing to ensure ranges are met.
Proven Acceptable Range (PAR)	A characterized range of a process parameter for which operation within this range, while keeping other parameters constant, will result in producing a material meeting relevant quality criteria (ICH Q8 R2).
Specification	A list of tests, references to analytical procedures, and appropriate acceptance criteria which are numerical limits, ranges, or other criteria for the tests described. It establishes the set of criteria to which a drug substance, drug product, or materials at other stages of its manufacture should conform to be considered acceptable for its intended use. "Conformance to specification" means that the drug substance and drug product, when tested according to the listed analytical procedures, will meet the acceptance criteria.

Per International Conference on Harmonization (ICH) Q8, critical process parameters (CPPs) were initially identified by risk assessment based on their potential to impact CQAs. Process risk assessments were conducted for each unit operation to assess impact to CQAs. These risk assessments guided process characterization activities and informed parameter and IPC criticality and process capability. The characterization results are discussed in Section 3.2.P.2.3.5. The CPPs and their associated PARs and CIPCs and their associated acceptance criteria are provided in the following subsections.

3.2.P.3.4.1 Critical Process Parameters

CPPs and their PARs for the mRNA-1273 Drug Product manufacturing process are provided in Table 2 Excursions outside of these established ranges will be reported, investigated, and assessed by the Quality Unit per established standard operating procedures.

Table 2: Critical Process Parameters

Step	Process Variable	Acceptance Criteria / PAR	CQA Impact	Rationale
Dilution	Dispensed Dilution Buffer Weight	[redacted]	RNA Content	Deviations to the buffer addition step could cause OOS mRNA concentration in final product that would fail the batch
Stoppering and Capping	Crimp Pressure		Container Integrity	Container closure integrity testing needs to be completed on the fill-finish line to ensure a properly sealed vial. If improperly sealed, the drug product is at risk to sterility breach and patient safety.
Cumulative Processing Duration	Cumulative Process Duration Time Out of Refrigeration (TOR)		mRNA Purity	Longer than specified TOR processing times are unlikely to cause a failed batch, but may significantly impact mRNA-1273 CQAs
Cumulative Processing Duration	Cumulative Process Duration at [redacted]		mRNA Purity	Longer than specified [redacted] processing times are unlikely to cause a failed batch, but may significantly impact mRNA-1273 CQAs
Vial Freezing and Storage	Vial Conditioning Temperature		Container Integrity	Too low a temperature during conditioning (below T_g of stoppers, approximately [redacted] to [redacted] could impact container closure integrity.

Abbreviations: PAR = proven acceptable range; TOR = time out of refrigeration; OOS = out of specification; CQA = critical quality attribute; Tg = glass transition temperature

3.2.P.3.4.2 Microbial Control Strategy

The mRNA-1273 Drug Product manufacturing process is carried out in a Grade C manufacturing area. Filling, stopper placement, and capping are performed in a Grade A isolator within the Grade C manufacturing area. The Dilution Buffer (20 mM Tris, 87 g/L sucrose, pH 7.5) is formulated and then filtered through a 0.2 µm filter prior to use. All assemblies, storage bags, and product contact filling components used in the manufacturing of mRNA-1273 Drug Product are single use. Manufacturing controls specifically intended for

microbial control of the mRNA-1273 Drug Product manufacturing process are listed in Table 3. Sterility of the Automated Filling line is demonstrated through media fill qualification as described in Section 3.2.P.3.5. Environmental monitoring of the manufacturing areas is performed as described in Section 3.2.P.3.5. Sterilization of the container closure system is described in Section 3.2.P.7. Microbial attributes of the container closure are discussed in Section 3.2.P.2.5. Any deviations incurred in the implementation of these microbial controls are assessed for product quality impact.

Table 3: Microbial Controls

Step	Operation / IPC	Test / Description	Limit	Environment Background Classification
Dilution Buffer Preparation	Filtration	Filtration through [redacted] filter	N/A	Grade C
	Post-Filtration Filter Integrity Test (FIT)	Minimum Bubble Point	[redacted]	
	In-Process sampling	Endotoxin (Post-filtration)		
	In-Process sampling	Bioburden (Post-filtration)		
Sterile Filtration	In-Process sampling	Prefiltration Bioburden		Closed system in Grade C
	Pre-Use Post Sterilization Filter Integrity Test	Minimum Bubble Point		
	Filtration	Redundant Filtration through [redacted] filter	N/A	
	Post- Filtration Filter Integrity Test (FIT)	Minimum Bubble Point	[redacted]	
Filling, Stopper, Capping	Filler qualified for sterile operations			Grade A Isolator in Grade C background

Abbreviations: FIT = filter integrity test; N/A = not applicable; WFI = water for injection; TAMC = total aerobic microbial count; TYMC = total yeast and molds count; EU = endotoxin unit; CFU = colony forming unit; IPA = isopropanol;

3.2.P.3.4.3 Cumulative Process Duration

Cumulative processing durations are provided in Table 4.

Table 4: Cumulative Process Duration

Step	Operation	Activity/Description	Duration
Total (Cumulative)	Cumulative process time out of refrigeration (TOR, [redacted]	Cumulative TOR across all unit operations	[redacted]
Total (Cumulative)	Cumulative processing duration at [redacted]	Cumulative processing duration at [redacted] across all unit operations	

Abbreviation: TOR = time out of refrigeration

3.2.P.3.4.4 Critical In-Process Control Testing

The critical in-process control testing and associated method references for the mRNA-1273 Drug Product manufacturing process are summarized in Table 5. In-process sample matrices were qualified and/or validated for the appropriate criteria according to ICH Q2 (see Section 3.2.P.3.4.4.1 for mRNA Content and Section 3.2.P.3.4.4.2 for USP <790>).

Table 5: Critical In-process Controls

CIPC	Step	Sample Pool	Acceptance Criteria
Post Clarification mRNA Content	Clarification Filtration	Pooled Clarified mRNA-1273 Bulk LNP	[redacted]
Filtration Pressure	Sterilizing Filtration	N/A	
Pre-sterilizing Filtration Bioburden	Sterilizing Filtration	Diluted mRNA-1273 Bulk DP	
Post-filtration Integrity Test Value	Sterilizing Filtration	N/A	
Fill Weight	Filling	In line [b]	
USP <790> Destructive Visual Inspection	Visual Inspection	Vialed mRNA-1273 Drug Product	

Abbreviations: CFU = colony-forming unit(s); FIT = filter integrity testing; NMT = not more than; TAMC = total aerobic microbial count; TYMC = total yeasts and mold count
a [redacted] is the LNP specification and the IPC should not exceed those boundaries.
b Fill weight is performed 100% during the filling process
c Based on [redacted] target dispensing volume and density of [redacted]

3.2.P.3.4.4.1 In-process Testing: mRNA Content

AM-256-100-001, Determination of RNA Concentration in 256-100s by IEX chromatography with UV detection, is used to quantitate the mRNA content in mRNA-1273 Drug Product in-process samples at Catalent. The mRNA is extracted from the lipids through a process using triton and salt buffer. The extracted mRNA is then separated using an anion exchange column and an increasing sodium perchlorate gradient. The mRNA concentration is quantitated using a reference standard (Section 3.2.S.5 {CX-024414}) and single point calibration calculation based on reference standard concentration and UV absorbance at 260 nm.

Instrument, Equipment, and Reagents

Instrumentation, equipment, and reagents for IEX HPLC analysis are provided in Table 6. Standard laboratory equipment is not listed. Equivalent instruments and reagents may be substituted where indicated. Solutions prepared for use in this method are described in Table 7.

Table 6: Instrument, Equipment, and Reagents

Instrument and Equipment
UHPLC or HPLC system with UV detection
[redacted] column, or equivalent
pH meter
Analytical Balance, capable of reading to 0.1 mg
Reagents
Water, Ultrapure (RNase free) – Milli-Q, or equivalent
Ethanol, 200 proof (100%), USP
Glycine, HPLC Grade [redacted]
Sodium Perchlorate Monohydrate, ACS Grade [redacted]
10N Sodium Hydroxide, ACS Grade
Concentrated Hydrochloric Acid [redacted], ACS Grade
100X Tris EDTA solution (Tris 10 mM, EDTA 1 mM)
Triton X-100 reduced (Density: [redacted]
Sodium Chloride [redacted]

Table 7: Solution Preparation

Solution
Stock Mobile Phase Buffer 1: [redacted]
Stock Mobile Phase Buffer 2: [redacted]
Mobile Phase A: [redacted]
Mobile Phase B: [redacted]
Method Diluent: [redacted]
Column Wash: [redacted]
Needle Wash: [redacted]
Reference Standard (Section 3.2.S.5 {CX-024414})

Sample and Standard Preparation

- Reference Standard Preparation: [redacted] reference standard in method diluent
- Check Standard Preparation: [redacted] reference standard in diluent
- Sample Preparation: [redacted] sample in diluent, acceptable range is [redacted] to [redacted]

Procedure

[redacted] The chromatographic conditions for analysis are summarized in Table 8 and an example injection sequence in presented in Table 9. A representative chromatographic profile is shown in Figure 1.

Table 8: HPLC Operating Parameters

Parameter	Condition		
Mobile phase A (MPA)	[redacted]		
Mobile phase B (MPB)			
Needle wash			
Column Wash			
Flow rate			
Column temperature			
Autosampler temperature			
Recommended Needle Drawing Speed			
Detection			
Acquisition time			
Injection volume			
Gradient	**Time (minutes)**	**% MPA**	**% MPB**
	[redacted]		

Table 9: Example Injection Sequence

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

Data Analysis and Reporting

Calculate carryover (% blank interference), check and bracketing standard (% Recovery).

Report the average concentration of the sample replicates.

$$\text{Dilution Factor} = \frac{\text{Total Volume of Sample Preparation}}{\text{Volume of Sample Added}}$$

$$\%\text{Blank Interference} = \frac{\text{Diluent Area (mAU)}}{\text{Mean Response Reference Standard Area (mAU)}} \times 100$$

Check Standard Recovery

$$Recovery = \frac{\frac{(Check\ Standard_{Peak\ Area} \times Reference\ Standard_{Conc.(mg/mL)})}{Mean\ Reference\ Standard_{Peak\ Area}}}{Nominal\ (Theoretical)\ Conc.\ \left(\frac{mg}{mL}\right)} \times 100$$

Bracketing Standard Recovery

$$\text{Recovery} = \frac{\text{Bracket Standard Area(mAU)}}{\text{Mean Response Reference Standard Area (mAU)}} \times 100$$

$$\text{Sample Conc.} = \frac{(\text{Sample}_{Peak\ Area} \times \text{Standard}_{Conc.})}{\text{Mean Standard}_{Peak\ Area}} \times \text{Dilution Factor}$$

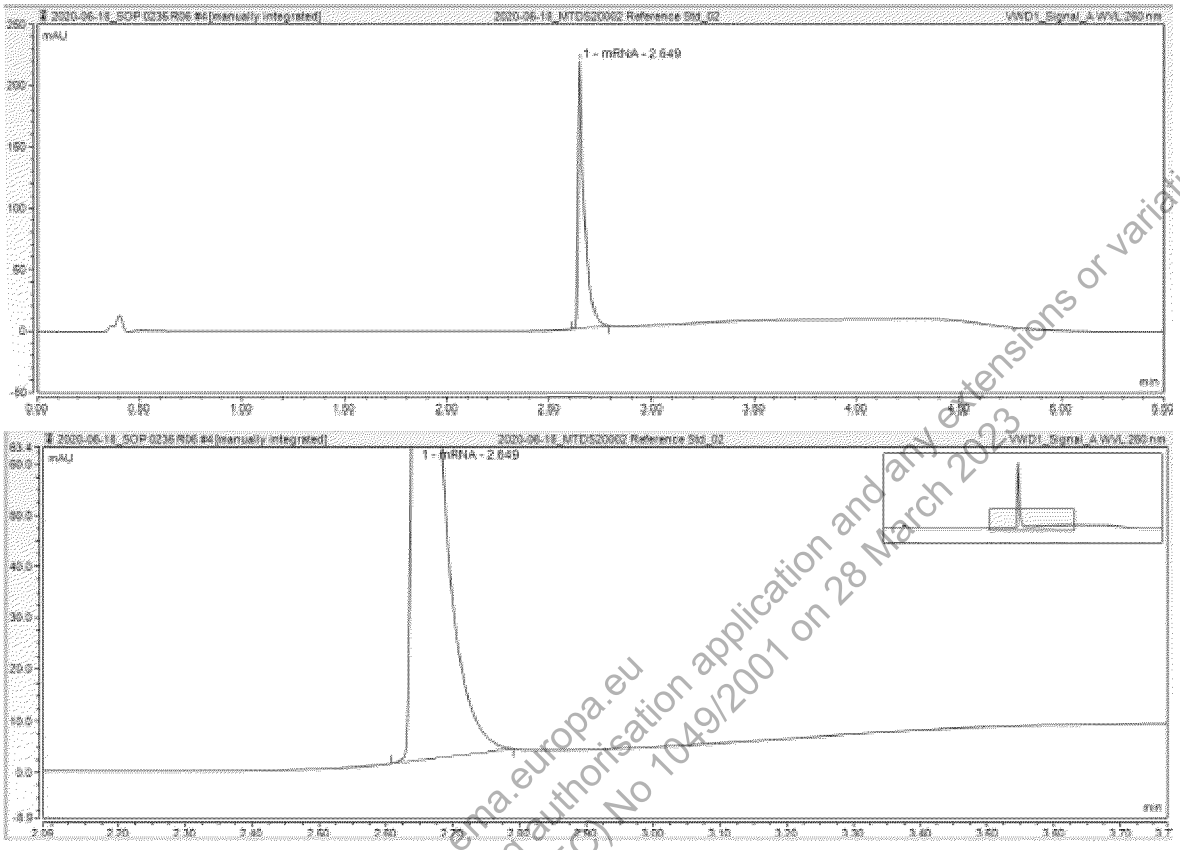
System Suitability and Test Article Acceptance Criteria

Reference standard is analyzed within each analysis to ensure the system is suitable for use on each day of analysis. System suitability and acceptance criteria are summarized in Table 10.

Table 10: System Suitability and Test Article Acceptance Criteria

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

Figure 1: Representative Reference Standard Chromatogram on HPLC System



Analytical Method Validation

AM-256-100-001, *Determination of RNA Concentration in 256-100s by IEX chromatography with UV detection*, has been validated and shown to be suitable for testing in-process samples during mRNA-1273 Drug Product manufacturing and quantitating the mRNA content in mRNA-1273 Drug Product. The validation characteristics evaluated were linearity, accuracy, specificity, precision (repeatability, intermediate precision), range, robustness and solution stability as described in Table 11.

Analytical test method AM-256-100-001 passed the acceptance criteria for validation parameters outlined in the protocol and is considered validated for testing mRNA-1273 Drug Product. Details of the validation results are captured in QMV-256-100-001-R (on file at Catalent).

Table 11: Overall Validation Summary for AEX HPLC

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

As document in QMV-256-100-003-R, *Analytical Method Reproducibility Summary Report – Determination of RNA Concentration in 256-100 by IEX Chromatography with UV Detection*, an inter-laboratory precision study was performed between Catalent and Moderna Quality Control laboratories. The reproducibility study included the evaluation of common representative test samples, [redacted] mRNA-1273 LNP and [redacted] in-process samples, using AM-256-100-001, *Determination of RNA Concentration in 256-100 by IEX Chromatography with UV Detection,* at both laboratories.

Table 12: Inter-Laboratory Precision Study for AEX HPLC

Precision – Reproducibility		
Parameter	**Acceptance Criteria**	**Meets Criteria**
Precision (Reproducibility)	[redacted]	Pass
		Pass
		Pass

3.2.P.3.4.4.2 In-process Testing: Visual Inspection

AM-256-100-002 provides the procedure for the supplementary in-process control inspection of mRNA-1273 Drug Product for visible particulate testing in compliance with United States Pharmacopoeia (USP) <790>.

As mRNA-1273 Drug Product is a non-settling opalescent suspension which could potentially limit the ability of the inspection process to detect particles by a traditional approach following USP <790> Visible Particulates in Injections and USP <1790>, Visual Inspection of Injections, a method was developed to perform supplemental testing for visible particulates to ensure compliance with USP <790>. The method was developed based on a modified USP <788>, Particulate Matter in Injections Method 2 procedure.

<u>Instrument, Equipment, and Reagents</u>

Instrumentation, equipment, and reagents for visible particulate testing are provided in Table 13. Equivalent instruments and reagents may be substituted where indicated.

Table 13: Materials and Equipment

Materials and Equipment
[redacted] filter [redacted] or equivalent)
Forceps
Plastic filtering apparatus and clamp
Filtration receiving container
Beaker capable of containing the entire pooled sample volume
Isopropyl Alcohol (IPA)
Nylon Net Filter [redacted] or equivalent)
[redacted] Microscope capable of [redacted] total magnification or equivalent
Pressure Vessel with a Hand-held pressure Nozzle
Petri dish and covers [redacted] or equivalent)
Purified water
Vacuum pump/port
20 sample vials (or per governing document)

<u>Blank Test</u>

[redacted]

Procedure

[redacted]

Analytical Method Validation

AM-256-100-002, *Inspection for Visual Particulates in Client 256-100 Drug Product*, has been validated and shown to be suitable for the purpose of performing supplemental visible particulate testing on mRNA-1273 Drug Product samples at Catalent Quality Control laboratory. The validation characteristics evaluated were limit of detection, specificity, robustness and ruggedness as described in Table 14.

Analytical test method AM-256-100-002 passed the acceptance criteria for validation parameters outlined in the protocol and is considered validated for testing mRNA-1273 Drug Product. Details of the validation results are captured in QMV-256-100-002-R (on file at Catalent).

Table 14: Overall Validation Summary for Supplemental Inspection for Visible Particulates

Parameter	Acceptance Criteria	Pass/Fail
Limit of Detection	[redacted]	Pass
Specificity	[redacted]	Pass
Robustness	[redacted]	Pass
Ruggedness	[redacted]	Pass