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1.0 PURPOSE

This method describes the procedure for quantitation of the total RNA concentration in mRNA samples containing [redacted] using Ion Exchange (IEX) chromatography with UV detection at 260 nm.

2.0 SCOPE

This procedure applies to cGMP release and stability testing of mRNA LNP and mRNA Drug Product (DP) samples contained in an [redacted]

3.0 REFERENCED DOCUMENTS

Document #	Title
FRM-0089	QC Column Tracking Form
FRM-0120	General Quality Control Sample Submission Form
FRM-0180	Quality Control Solution Preparation Form
FRM-0260	HPLC/UHPLC Usage and Maintenance Log
FRM-0731	SOP-0999 Standard Preparation Worksheet
FRM-0732	SOP-0999 Assay Performance Worksheet
SOP-0017	Maintaining a RNase Free Work Environment
SOP-0022	Good Documentation Practices
SOP-0033	Out of Specification (OOS)
SOP-0079	Sample Submission and Sample Tracking Procedures in the GMP Quality Control Laboratory
SOP-0081	Preparation of Solutions and Samples in the GMP Quality Control Laboratory
SOP-0082	Data Review and Reporting in the GMP Quality Control Laboratory
SOP-0111	Operation of [redacted]
SOP-0227	[redacted] Operation and Maintenance
SOP-0242	Operation and Maintenance of [redacted]
SOP-0243	Operation of [redacted]
SOP-0271	Quality Control HPLC/GC Column Monitoring
SOP-0274	Use of Quality Control Laboratory Notebook
SOP-0403	QC [redacted]
SOP-0409	Quality Control Invalid Assay Procedure

4.0 RESPONSIBILITIES

Department/ Functional Area	Responsibility
Department Manager or designee	• Ensure all procedures outlined in this document are followed.
Laboratory Personnel	• Following safe operation and maintenance practices. • Executing procedure as described. • Documenting daily operation and maintenance activities in the corresponding instrument logbook using **FRM-0260**. • Documenting standard preparation using **FRM-0731** and assay information using **FRM-0732**. • Documenting solution preparation in the corresponding logbook using **FRM-0180** or in [redacted] per **SOP-0403**. • Maintaining an RNase free work environment per **SOP-0017**.

5.0 DEFINITIONS

Term	Definition
µL	microliter
µm	micrometer
g	Gram
HCl	Hydrochloric Acid
HPLC	High Performance Liquid Chromatography
IEX / AEX	Ion-Exchange / Anion Exchange
L	Liter
LNP	Lipid Nanoparticle
mg	Milligram
mM	Milli-molar
mRNA	Messenger ribonucleic acid
N	Normal
pH	Log[H^+]
PN	Part Number
QS	Quantum Sufficit: as much as suffices; bring to volume
RNAse	An enzyme that promotes the breakdown of RNA into oligonucleotides and small molecules
RT	Retention Time
SoA	Summary of Analysis
UHPLC	Ultra-High-Performance Liquid Chromatography
UV	Ultraviolet

6.0 EQUIPMENT AND MATERIALS

Alternative vendors or part numbers may be used, provided the reagent grade or classification is maintained.

Materials

Materials/Consumables	Manufacturer	Part Number
[redacted]	N/A	N/A
	N/A	N/A
	[redacted]	

Reagents

Reagent	Manufacturer	Part Number
[redacted]		

Equipment

Equipment	Manufacturer	Part Number
[redacted]	[redacted]	
	N/A	N/A
	N/A	N/A
	[redacted]	
	N/A	N/A

7.0 SAFETY

7.1. Laboratory personnel are required to wear appropriate Personal Protective Equipment when working in the cGMP Quality Control laboratory.

7.2. Refer to chemical specific SDS for additional safety information.

8.0 PROCEDURE

8.1. Solution Preparation

8.1.1. [redacted]

8.1.2.

8.1.3.

8.1.4. [redacted] SOP-0081.

8.1.5. [redacted]

8.1.5.1. [redacted]

8.1.5.2. [redacted]

8.1.5.3. [redacted]

8.1.5.4. [redacted]

8.1.5.5. [redacted] SOP-0403.

8.1.6. [redacted]

8.1.6.1. [redacted]

8.1.6.2. [redacted]

8.1.6.3. [redacted] SOP-0403.

8.1.7. [redacted]

8.1.7.1. [redacted]

8.1.7.2. [redacted]

8.1.7.3. [redacted]

8.1.7.4. [redacted] SOP-0403.

8.1.8. [redacted]

8.1.8.1. [redacted]

8.1.8.2.

8.1.8.3.

8.1.8.4. [redacted] SOP-0403.

8.1.9. [redacted]

8.1.9.1. [redacted]

8.1.9.2.

8.1.9.3.

8.1.9.4.

8.1.9.5. [redacted] SOP-0403.

8.1.10. [redacted]

8.1.10.1. [redacted]

8.1.10.2.

8.1.10.3.

8.1.10.4. [redacted] SOP-0403.

8.1.11. [redacted]

8.1.11.1. [redacted]

8.1.11.2.

8.1.11.3.

8.1.11.4. [redacted] SOP-0403.

8.1.12. [redacted]

8.1.12.1. [redacted]

8.1.12.2.

8.1.12.3.

8.1.12.4. [redacted] SOP-0403.

8.2. Sample and Standard Preparation

[redacted]

8.2.1. [redacted]

8.2.1.1. [redacted]

8.2.1.2.

8.2.1.3.

8.2.1.4.

8.2.2. [redacted]

8.2.2.1. [redacted]

8.2.2.2.

8.2.2.3.

8.2.2.4.

8.2.3. [redacted]

8.2.3.1. [redacted]

8.2.3.2.

8.2.3.3.

8.2.3.4.

8.2.3.5.

Table 1. Sample dilutions

Sample Concentration (mg/mL)	Sample Volume (µL)	Diluent Volume (µL)	Total Volume (mL)	Theoretical Concentration (mg/mL)	Dilution Factor
[redacted]					

[redacted]

8.2.3.6. [redacted]

8.3. Instrument Operating Conditions

8.3.1. [redacted] SOP-0111 and SOP-0242 [redacted]

[redacted]

8.3.2. [redacted] SOP-0243 [redacted]

8.3.3. [redacted]

8.3.3.1. [redacted]

8.3.3.2.

8.3.3.3.

8.3.3.4.

8.3.4. [redacted]

8.3.4.1. [redacted]

8.3.5. [redacted]

8.3.5.1. [redacted]

Parameter	[redacted]
Column	
Mobile Phase A:	
Mobile Phase B:	
Needle Wash:	
Seal Wash:	
Column Wash:	
Acquisition/Run Time:	

Parameter	[redacted]
Flow Rate:	
Detection:	
Injection Volume:	
Column Temperature:	
Post-Column Cooler	
Auto sampler Temperature:	
Injection/ Needle Wash:	
Recommended Needle Drawing Speed:	
Sample Concentration:	
Calibration Settings	

8.3.6. Mobile Phase Gradient

Time (min)	Mobile Phase A (MPA) %	Mobile Phase B (MPB) %
	[redacted]	

8.3.7. [redacted]

8.3.7.1. [redacted]

8.3.8. [redacted]

8.3.8.1. [redacted]

8.3.8.2.

8.3.8.3.

Time (min)	Mobile Phase A (MPA) %	Mobile Phase B (MPB) %	Flow (mL/min)
	[redacted]		

8.3.9. [redacted]

8.3.9.1. [redacted]

8.3.9.2.

8.3.9.3.

8.3.9.4.

8.3.9.5. [redacted]

8.3.9.6.

8.3.9.7.

Sample Injections	Number of injections	Sample Type	Cal Level
[redacted]		Sample **	N/A
		Calibration	[redacted]
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A
		Sample	N/A

[redacted]

8.4. Data Processing

8.4.1. [redacted]

8.4.1.1. [redacted]

Parameter	Acceptance
[redacted]	

Parameter	Acceptance
[redacted]	

8.4.2. [redacted]

8.4.2.1. [redacted]

Parameter	Acceptance
[redacted]	[redacted]

8.4.3. [redacted]

8.4.3.1. [redacted]

8.4.3.2. [redacted]

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

8.4.3.3. [redacted]

8.4.3.4. [redacted]

8.4.3.5. [redacted]

8.4.3.6. [redacted]

8.4.3.7. [redacted]

8.5. **Reporting of Results**

8.5.1. [redacted] SOP-0082 [redacted]

8.5.2. [redacted]

8.5.3. [redacted]

8.5.3.1. [redacted]

8.5.4. [redacted] **SOP-0227.**

8.5.4.1. [redacted]

[redacted] **SOP-0082.**

8.5.4.2. [redacted]

[redacted] **SOP-0079.** [redacted]

[redacted]

8.5.5. [redacted]

[redacted] SOP-0033 [redacted]

8.5.6. [redacted]

SOP-0409 [redacted]

9.0 ATTACHMENTS

9.1. Attachment 1: Representative [redacted] Chromatogram on HPLC

9.2. Attachment 2: Representative Reference Standard Chromatogram on the [redacted] system

9.3. Attachment 3: Representative Reference Standard Chromatogram on the [redacted] system

10.0 REVISION HISTORY

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

ATTACHMENT 1 - Representative [redacted] Chromatogram on HPLC (Full Scale)

ATTACHMENT 2 - Representative Reference Standard Chromatogram on the [redacted] system (full scale and zoomed)

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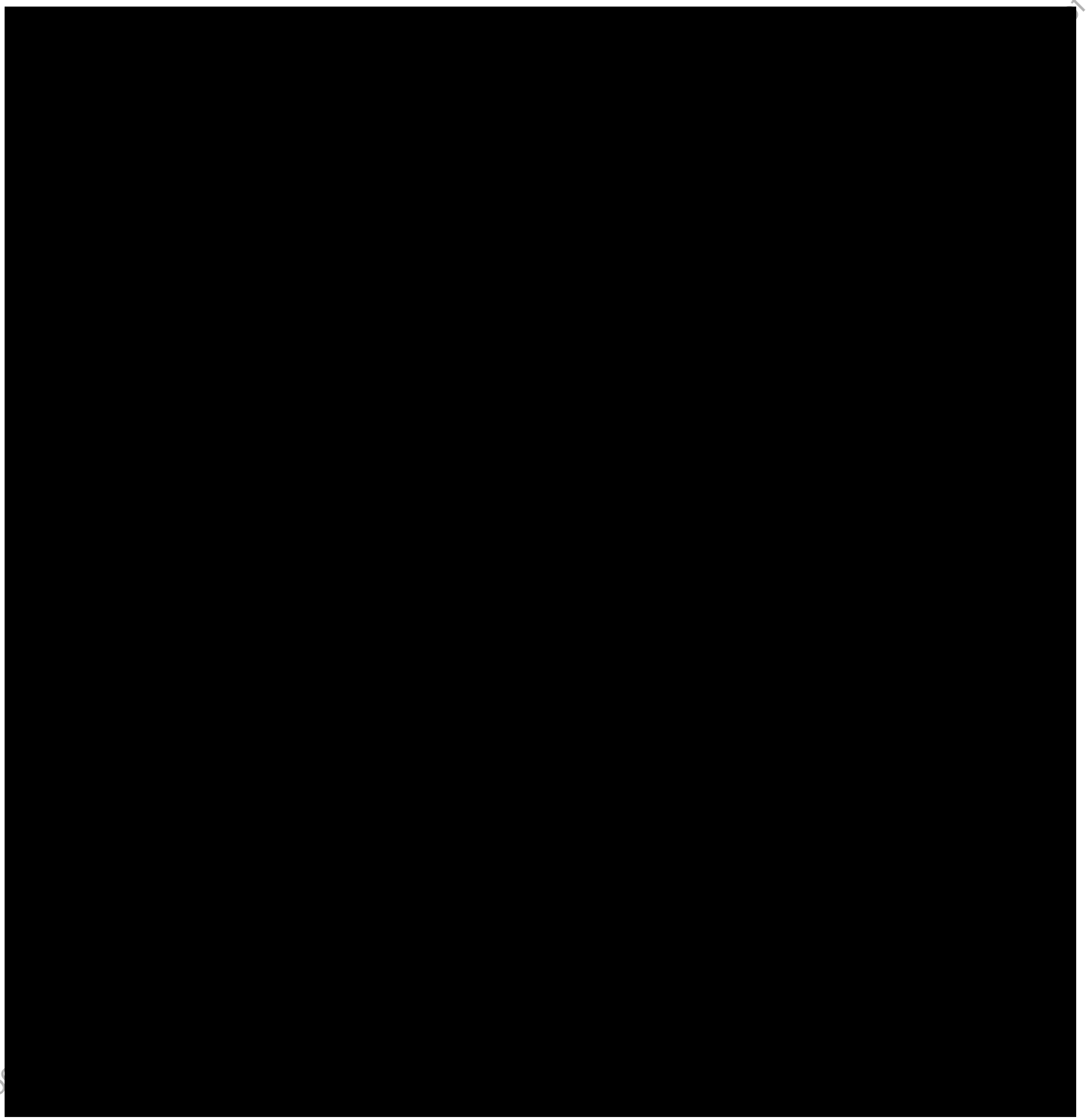
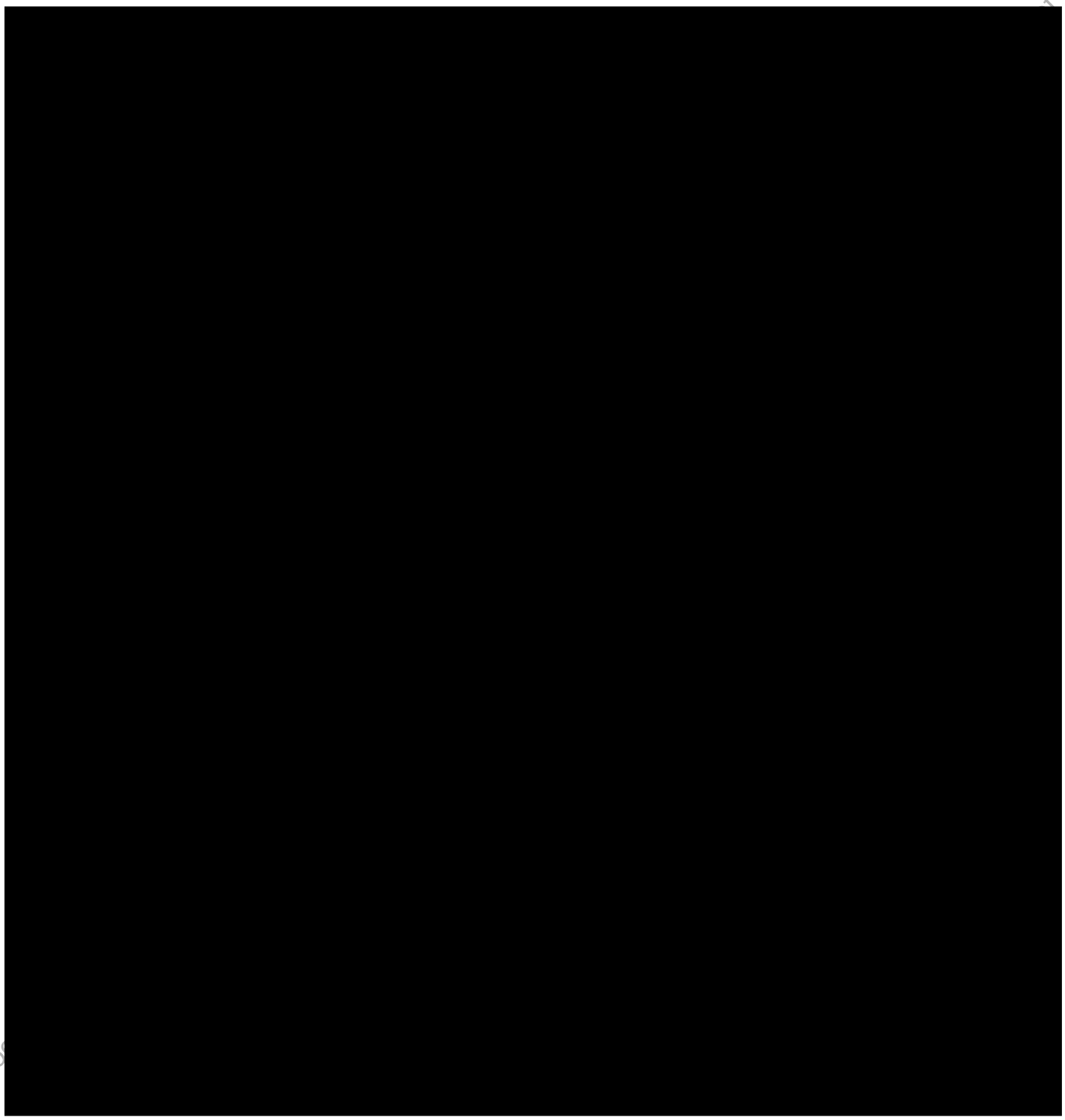


ATTACHMENT 3 - Representative Reference Standard Chromatogram on the [redacted] system (full scale and zoomed)



Document Approvals

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