



1.0 PURPOSE

This method describes the procedure for the analysis of lipid concentration (mg/mL), lipid content (µg/vial), lipid impurities (%area) and lipid identity by retention time comparison using Ultra-High-Performance Liquid Chromatography with Charged Aerosol Detection UPLC-CAD.

2.0 SCOPE

This method applies to release and stability testing of [REDACTED] formulated LNP (fLNP) containing mRNA, and Drug Product (DP) samples containing SM-102, Cholesterol, DSPC, and PEG2000-DMG lipids in the QC cGMP laboratory.

3.0 REFERENCED DOCUMENTS

Document #	Title
FRM-0089	QC Column Tracking Form
FRM-0180	Quality Control Solution Preparation Form
FRM-0260	HPLC/UHPLC Usage and Maintenance Log
FRM-0741	SOP-1001 Assay Performance Worksheet
FRM-0743	SOP-1001 Resolution Standard Preparation Worksheet
FRM-0744	SOP-1001 Standard Preparation Worksheet
SOP-0017	Maintaining a RNase Free Work Environment
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-0144	Operation of the [REDACTED] Analytical Balance
SOP-0227	[REDACTED] Operation and Maintenance
SOP-0242	Operation and Maintenance of [REDACTED] UHPLC System
SOP-0243	Operation of [REDACTED] Chromatography Data System
SOP-0271	Quality Control HPLC/GC Column Monitoring
SOP-0403	QC [REDACTED] Operations Procedure
SOP-0409	Quality Control Invalid Assay Procedure
SOP-0820	Reconstitution of Lyophilized Drug Product

4.0 RESPONSIBILITIES

Department/ Functional Area	Responsibility
Department Manager or Designee	<ul style="list-style-type: none"> Ensuring that laboratory personnel are properly trained on this procedure. Ensuring that all procedures outlined in this document are followed.
Quality Control Personnel	<ul style="list-style-type: none"> Following safe operation and maintenance practices outlined within this document. Executing this procedure as described. Documenting daily operation and maintenance activities in the corresponding instrument logbook using FRM-0260. Documenting and monitoring column use per SOP-0271 and FRM-0089. Documenting standard preparation and assay information using FRM-0741, FRM-0743, and FRM-0744. Preparing solutions per SOP-0081 and documenting preparation using FRM-0180 or in [REDACTED] per SOP-0403. Maintaining an RNase free work environment as per SOP-0017. Reporting any instrument malfunction, safety concerns or incidents to Area Managers.

5.0 DEFINITIONS

Term	Definitions
CAD	Charged Aerosol Detection
DP	Drug Product(s)
DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
fLNP	Formulated Lipid Nanoparticle
ID	Identity
LNP	Lipid nanoparticle
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
mRNA	Messenger ribonucleic acid
PEG2000-DMG	1,2-Dimyristoyl-sn-glycerol, methoxypolyethylene glycol (2000)
QS	Quantum Sufficit: as much as suffices
RP-UHPLC	Reverse Phase Ultra-High Performance Liquid Chromatography
RT	Retention Time
SM102	Heptadecan-9-yl 8-((2-hydroxyethyl)(6-oxo-6-(undecyloxy)hexyl)amino)octanoate
UV	Ultraviolet

6.0 EQUIPMENT AND MATERIALS

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

Equipment

Equipment	Manufacturer	Part Number/ Model
[REDACTED]	[REDACTED]	[REDACTED]
Analytical Balance, capable of reading to [REDACTED] mg		
Centrifuge, capable of [REDACTED] x g		
[REDACTED]		
[REDACTED] variable adjustable pipets, capable of measuring [REDACTED] to [REDACTED] (recommended: [REDACTED])		
[REDACTED] Pipetter		
Volumetric Flasks various volumes including [REDACTED] and [REDACTED]		

Reagents

Reagents	Manufacturer	Part Number
[REDACTED]		

Reagents	Manufacturer	Part Number
[REDACTED]		

Reference Standards

Standard	Molecular Weight	Manufacturer	Part Number
Cholesterol	[REDACTED]		
DSPC			
PEG2000-DMG			
SM-102 [REDACTED]			
[REDACTED]	N/A	[REDACTED]	
[REDACTED]	N/A		

Materials and Consumables

Materials and Consumables	Manufacturer	Part Number
[REDACTED]		

Materials and Consumables	Manufacturer	Part Number
[REDACTED]		

7.0 SAFETY

- 7.1. Adhere to EPA, OSHA, and any other applicable health and safety procedures including Safety Data Sheets (SDS) when working with hazardous chemicals.
- 7.2. Wear the appropriate personal protective equipment when working in the Quality Control laboratory.

8.0 PROCEDURE

8.1. Solution Preparation:

8.1.1. General Considerations:

8.1.1.1. [REDACTED] FRM-0180 [REDACTED]

SOP-0403.

8.1.1.2. [REDACTED]

8.1.1.3. [REDACTED]

8.1.1.4. [REDACTED]

8.1.2. [REDACTED]

8.1.2.1. [REDACTED]

8.1.2.2. [REDACTED]

8.1.3. [REDACTED]

8.1.3.1. [REDACTED]

- 8.1.4. [REDACTED]
- 8.1.4.1. [REDACTED]
- 8.1.4.2. [REDACTED]
- 8.1.5. [REDACTED]
- 8.1.5.1. [REDACTED]
- 8.1.6. [REDACTED]
- 8.1.6.1. [REDACTED]
- 8.1.6.2. [REDACTED]
- 8.1.6.3. [REDACTED]
- 8.1.7. [REDACTED]
- 8.1.7.1. [REDACTED]
- 8.1.7.2. [REDACTED]
- 8.1.7.3. [REDACTED]
- 8.1.7.4. [REDACTED]
- 8.1.7.5. [REDACTED]
- 8.1.8. [REDACTED]
- 8.1.8.1. [REDACTED]
- 8.1.8.2. [REDACTED]
- 8.1.8.3. [REDACTED]
- 8.1.8.4. [REDACTED]
- 8.1.8.5. [REDACTED]
- 8.1.9. [REDACTED]
- 8.1.9.1. [REDACTED]
- 8.1.9.2. [REDACTED]
- 8.1.9.3. [REDACTED]
- 8.1.9.4. [REDACTED]
- 8.1.9.5. [REDACTED]

8.1.10 [REDACTED]

- 8.1.10.1. [REDACTED]
- 8.1.10.2. [REDACTED]
- 8.1.10.3. [REDACTED]
- 8.1.10.4. [REDACTED]
- 8.1.10.5. [REDACTED]

8.1.11 [REDACTED]

- 8.1.11.1. [REDACTED]
- 8.1.11.2. [REDACTED]
- 8.1.11.3. [REDACTED]
- 8.1.11.4. [REDACTED]

8.1.12. **Sample Diluent:** [REDACTED]

8.2. **Sample and standard preparation**

8.2.1. **General Considerations**

- 8.2.1.1. [REDACTED]
- 8.2.1.2. [REDACTED]

8.2.2. [REDACTED]

- 8.2.2.1. [REDACTED] **FRM-0741.**
- 8.2.2.2. [REDACTED]
- 8.2.2.3. [REDACTED] **SOP-0820.**
- 8.2.2.4. [REDACTED]

8.2.2.5. [REDACTED]

Sample Type	If Total Lipid Concentration is	Then dilute with ethanol to a total lipid concentration of
[REDACTED]		

8.2.2.6. [REDACTED]

8.2.2.7. [REDACTED]

8.2.2.8. [REDACTED]

8.2.2.9. [REDACTED]

8.2.3. [REDACTED]

8.2.3.1. [REDACTED]

8.2.3.2. [REDACTED]

[REDACTED] FRM-0744.

Table 1. Preparation of the [REDACTED] Standard Stock Components

Component	Weight (mg)
Cholesterol	[REDACTED]
PEG2000-DMG	
SM102	
DSPC	

*Refer to CoA for actual molecular weight of PEG2000-DMG.

8.2.3.3. [REDACTED]

8.2.3.4. [REDACTED]

8.2.3.5. [REDACTED]

8.2.3.6. [REDACTED]

8.2.4. Preparation of Calibration Standard Solutions

8.2.4.1. [REDACTED] FRM-0744.

8.2.4.2. [REDACTED]

Table 2. Preparation of Calibration Standard Solutions.

Calibration Level	STD-Stock (mL)	Final Volume (mL)	Concentration of Total Lipid (mg/mL)
[REDACTED]			

8.2.4.3. [REDACTED]

8.2.4.4. [REDACTED]

8.2.4.5. [REDACTED]

8.2.5. Check Standard Preparation [REDACTED]

8.2.5.1. [REDACTED] FRM-0741.

8.2.5.2. [REDACTED]

8.2.5.3. [REDACTED]

8.2.5.4. [REDACTED]

8.2.5.5. [REDACTED]

8.2.6. Preparation of Sensitivity Solution [REDACTED]

8.2.6.1. [REDACTED]

8.2.6.2. [REDACTED] FRM-0741.

8.2.6.3. [REDACTED]

8.2.6.4. [REDACTED]

8.2.7. Resolution Standard and Components preparation:

8.2.7.1. [REDACTED] FRM-0743.

8.2.7.2. [REDACTED]

8.2.7.2.1. [REDACTED] PEG2000-DMG [REDACTED]

8.2.7.2.2. [REDACTED]

8.2.7.2.3. [REDACTED]

8.2.7.3. [REDACTED] SM102 [REDACTED]

8.2.7.3.1. [REDACTED] SM102 [REDACTED]

8.2.7.3.2. [REDACTED]

8.2.7.3.3. [REDACTED]

8.2.7.4. SM102 [REDACTED]

8.2.7.4.1. [REDACTED] SM102 [REDACTED]

8.2.7.4.2. [REDACTED] SM102 [REDACTED]

8.2.7.4.3. [REDACTED]

8.2.7.5. [REDACTED]

8.2.7.5.1. [REDACTED] Cholesterol [REDACTED]

[REDACTED] Cholesterol [REDACTED]

8.2.7.5.2. [REDACTED] DSPC [REDACTED]

[REDACTED] DSPC [REDACTED]

8.2.7.5.3. [REDACTED]

8.2.7.5.4. [REDACTED]

8.2.7.5.5. [REDACTED]

Table 3. Resolution Standard Components

Component	Volume (mL)
[REDACTED]	[REDACTED]
SM102 [REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
PEG2000-DMG [REDACTED]	[REDACTED]
SM102 [REDACTED]	[REDACTED]
[REDACTED] Cholesterol	[REDACTED]
[REDACTED] DSPC [REDACTED]	[REDACTED]

NOTE: [REDACTED]: SM102, PEG-DMG, Cholesterol, DSPC, [REDACTED]

[REDACTED]

8.2.7.6.

8.2.7.7.

8.2.7.8.

8.3. Instrument Set-up

8.3.1.

8.3.2.

8.3.2.1.

8.3.2.2.

8.3.2.3.

8.3.2.4.

- 8.3.2.5. [REDACTED]
- 8.3.3. [REDACTED]
- 8.3.3.1. [REDACTED]
- 8.3.3.2. [REDACTED]
- 8.3.3.3. [REDACTED]
- 8.3.4. [REDACTED]
- 8.3.4.1. [REDACTED] SOP-0243 [REDACTED]
- 8.3.4.2. [REDACTED] SOP-0243 [REDACTED]
- 8.3.5. Load the [REDACTED]
- 8.3.5.1. [REDACTED]

Table 4 Instrument Parameters

Parameter	Setting
Instrument:	[REDACTED]
Column:	
Acquisition/Run Time:	
Flow Rate:	
Detection:	
Injection Volume:	
Column Temperature:	
Pre-Column Heater Temperature:	
Post-Column Cooler Temperature:	
Auto-sampler temperature	
Injection/Needle Wash	
Needle Draw and Dispense Speed	

Table 5. Mobile Phase Gradient

Time (min)	% Mobile Phase A	% Mobile Phase B
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[REDACTED]		
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8.3.6. [REDACTED]

8.3.6.1.	[REDACTED]	
8.3.6.2.	[REDACTED]	
8.3.6.3.	[REDACTED]	
8.3.6.4.	[REDACTED]	

8.3.7. [REDACTED]

8.3.7.1.	[REDACTED]	
8.3.7.2.	[REDACTED]	
8.3.7.3.	[REDACTED]	
8.3.7.4.	[REDACTED]	

8.3.7.5. [REDACTED]

Table 6. Recommended Sequence Order

Sample	Number of injections	Injection Volume (µL)
[REDACTED]		

8.3.8. [REDACTED]

8.3.8.1. [REDACTED]

Table 7. System Wash Mobile Phase Gradient

Time (min)	% Mobile Phase A	% Mobile Phase B	Flow (mL/ min)
[REDACTED]			

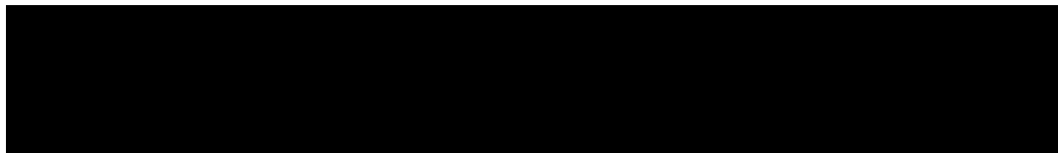
8.4. System Suitability and Sample Acceptance Criteria

8.4.1. [REDACTED]
[REDACTED] FRM-0741.

Table 8 System Suitability Acceptance Criteria

Parameter	Acceptance
[REDACTED] SM102, Cholesterol, DSPC or PEG2000-DMG.	[REDACTED]
[REDACTED]	[REDACTED]
SM102 [REDACTED]	[REDACTED]
[REDACTED] PEG2000-DMG [REDACTED]	[REDACTED]
[REDACTED] PEG2000-DMG, DSPC, SM102, and Cholesterol	[REDACTED]
[REDACTED] DSPC, SM102, and Cholesterol [REDACTED]	[REDACTED]
[REDACTED] (DSPC, SM102, Cholesterol, & PEG2000-DMG)	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

8.5. Calculations



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

$$\text{Stock Standard Concentration calculation} = \frac{\text{Weight}_{\text{lipid component}} * \text{purity}_{\text{lipid component}}}{\text{volume of diluent}}$$

$$\text{Std. Level x Concentration calculation} = \frac{\text{Stock Std. Concentration}}{\text{Level x Dilution Factor}}$$

$$\text{Response Factor} = \frac{\text{Peak Area}}{\text{Concentration}}$$

$$\text{Relative Retention Time (RRT)} = \frac{\text{Retention Time Peak}}{\text{Retention Time Cholesterol Peak}}$$

$$\% \text{ Blank Interference} = \frac{\text{Interfering Peak Area (pA * min)}}{\text{Mean response Level 3 Cal. Std. Area (pA * min)}} * 100$$

$$\% \text{ RSD Peak Area Precision} = \frac{\text{Standard deviation of Peak Area (pA * min)}}{\text{Average Peak Area (pA * min)}} * 100$$

$$\% \text{ RSD Retention Time Precision} = \frac{\text{Standard deviation of retention times}}{\text{Average retention time}} * 100$$

$$\text{Accuracy of Check Standard} = \frac{\text{Response Factor of Check Standard Inj}}{\text{Average Response Factor of Level 3 standard Injections}} * 100$$

$$\% \text{ Difference of Sample Preparations} = \frac{\left| \text{Prep. 1 Conc.} \left(\frac{\text{mg}}{\text{mL}} \right) - \text{Prep. 2 Conc.} \left(\frac{\text{mg}}{\text{mL}} \right) \right|}{\frac{(\text{Prep. 1 Conc.} \left(\frac{\text{mg}}{\text{mL}} \right) + \text{Prep. 2 Conc.} \left(\frac{\text{mg}}{\text{mL}} \right))}{2}} * 100$$

$$\% \text{ Lipid Retention Time Agreement} = \frac{\text{Retention Time of Lipid (Sample)}}{\text{Average Retention Time of Lipid (Level 3 Cal. Standard)}} * 100$$

$$\% \text{ Area of Individual Impurity} = \frac{\text{Peak Area of Impurity}}{\text{Total Area of all peaks including SM102, Cholesterol, DSPC, PEG2000 – DMG}} * 100$$

$$\% \text{ Area of Total Impurity} = \text{Sum of all individual impurities}$$

µg/Vial Calculation

$$(\text{Concentration Result} \left(\frac{\text{mg}}{\text{mL}} \right) * \text{Recon Volume (mL)}) * 1000$$

Where mL = Total reconstituted volume of the vial (Diluent Volume + Cake Volume)

8.5.1. [REDACTED]

8.6. Reporting Parameters

[REDACTED]

8.6.1. [REDACTED]

8.6.2. [REDACTED]

(SM102, PEG-DMG, Cholesterol and DSPC) [REDACTED]

8.6.3. [REDACTED]

8.6.3.1. [REDACTED] SM102, Cholesterol, DSPC and
PEG2000-DMG [REDACTED]

8.6.3.1. [REDACTED]

8.6.3.2.

8.6.3.3.

8.6.3.4.

8.6.3.5.

8.6.3.6.

[REDACTED] Cholesterol [REDACTED]

8.6.4. Follow **SOP-0082** for QC data reporting. Document sample results on **FRM-0741**.

8.6.5. [REDACTED] **SOP-0227**.

8.6.5.1.

[REDACTED] **SOP-0227** [REDACTED]

8.6.5.2.

[REDACTED] **FRM-0120**

8.6.6.

[REDACTED] **SOP-0033** [REDACTED]

8.6.7.

[REDACTED] **SOP-0409** [REDACTED]

9.0 ATTACHMENTS

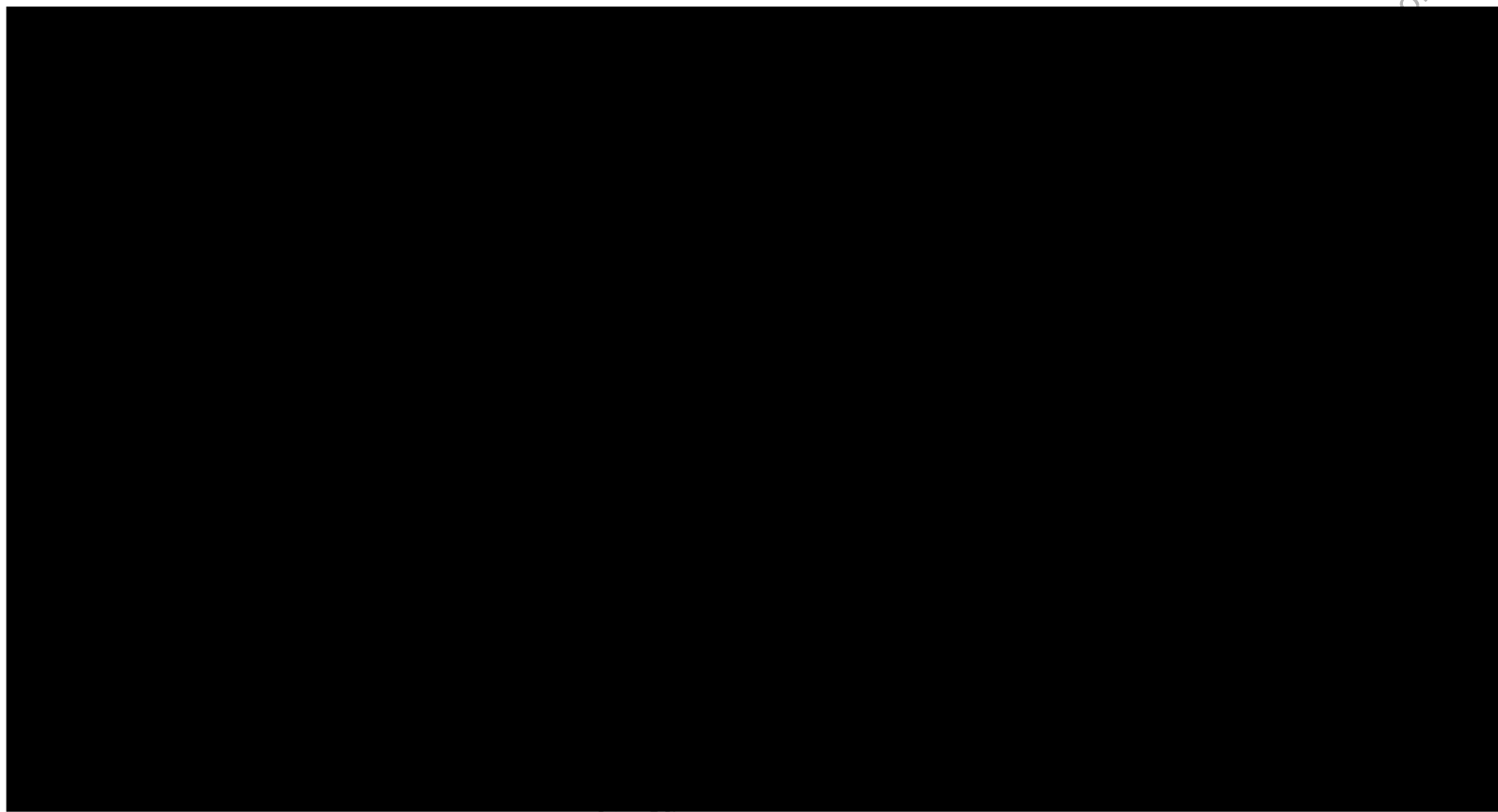
- 9.1. ATTACHMENT 1: Representative Chromatogram of Diluent
- 9.2. ATTACHMENT 2: Representative Chromatogram of Sensitivity Solution
- 9.3. ATTACHMENT 3: Representative Chromatogram of Resolution Standard Solution
- 9.4. ATTACHMENT 4: Representative Chromatogram of a Single Lipid Standard Showing the Retention Times of PEG2000-DMG, Cholesterol, DSPC and SM102 Lipid Components

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 Chromatogram of Diluent

(Page 1 of 1)

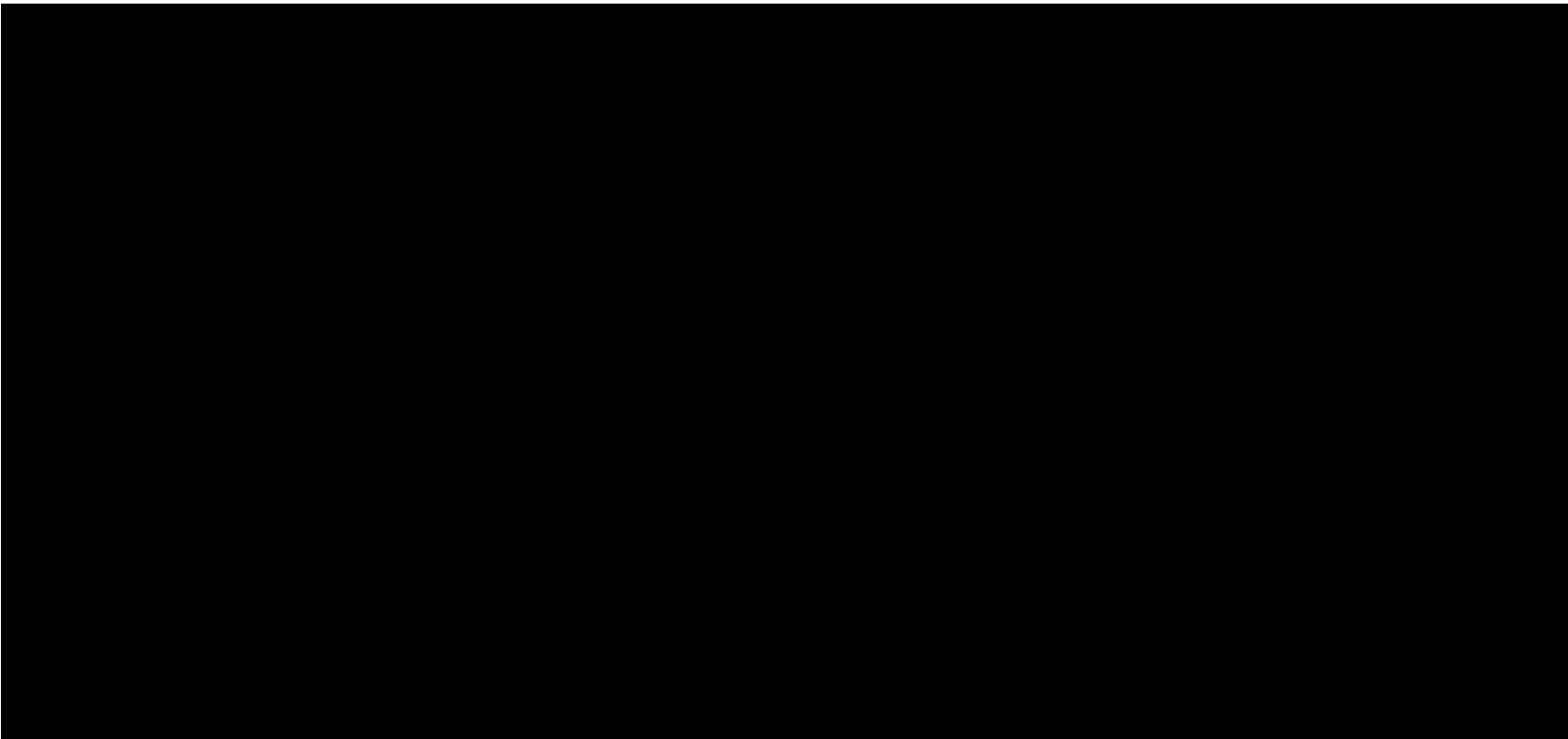


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ATTACHMENT 2: Representative Chromatogram of Sensitivity Solution

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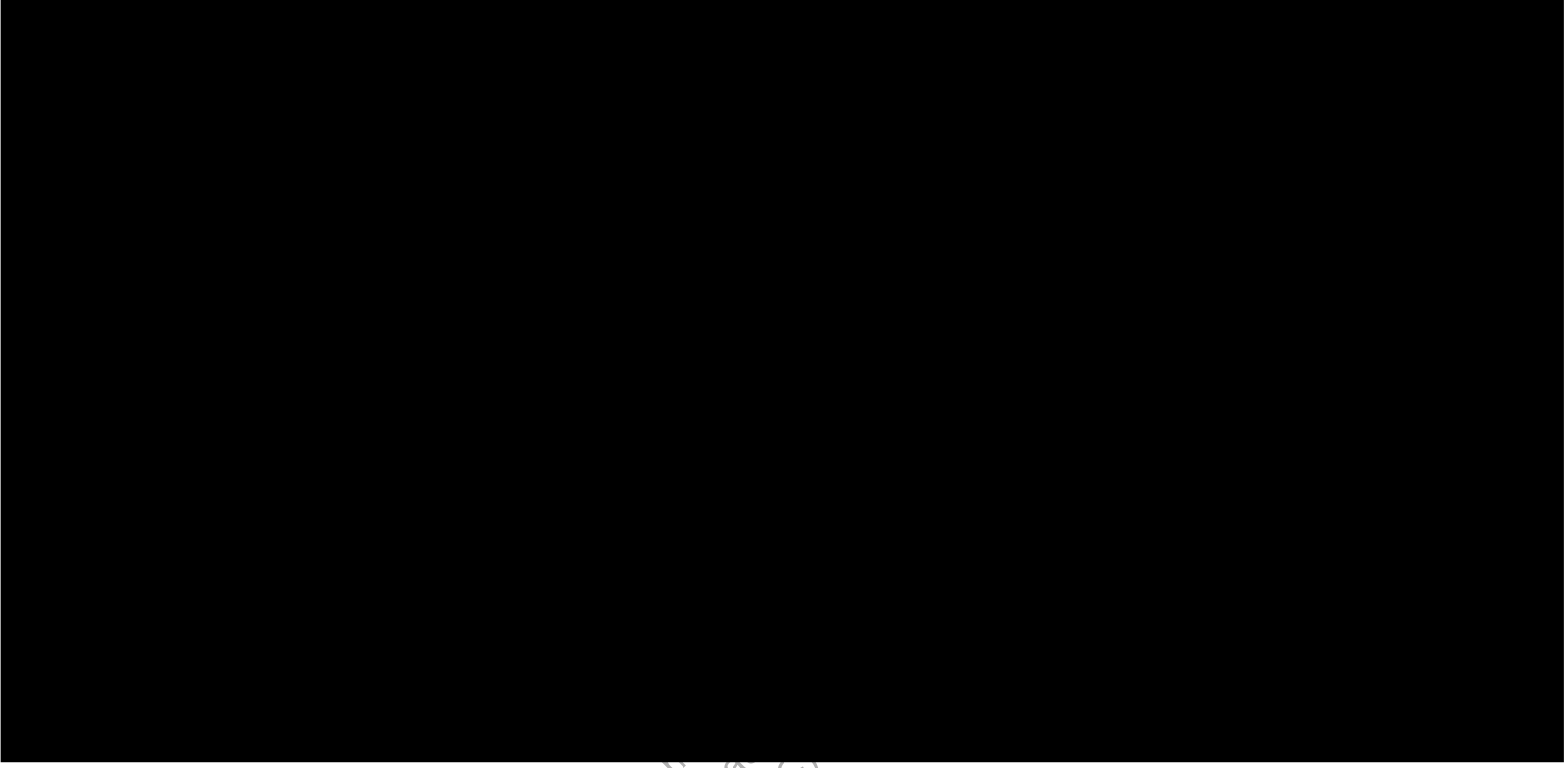


Peak No.	Peak Name	Approximate Retention Time (min)
[REDACTED]		

This document cannot be used to support
Released under [REDACTED]

ATTACHMENT 3: Representative Chromatogram of Resolution Standard Solution


(Page 1 of 1)



Peak No.	Peak Name	RRT (min)	Approximate Retention Time (min)
[REDACTED]			

ATTACHMENT 4: Representative Chromatogram of a Single Lipid Standard Showing the Retentions of PEG2000-DMG, Cholesterol, DSPC and SM102 Lipid Components

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Peak No.	Peak Name	Approximate Retention Time (min)
[REDACTED]		

Document Approvals
Approved Date: 09 Oct 2020

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