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Stability Study of mRNA - Lipid Nanoparticles under Different Formulation and Storage Conditions

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Introduction

Messenger ribonucleic acid (mRNA) biomolecules have emerged as a new class of biopharmaceuticals. With recent advances in the nanomedicine field, lipid nanoparticles(LNPs) are becoming well-known in vivo mRNA delivery systems. Encapsulating mRNA into the LNP system protects nucleic acids from degradation. The LNP formulation usually consists of an ionizable amino lipid, phospholipid, cholesterol, and polyethylene glycol-lipid conjugate (PEG-lipid). Structural and biological properties of each lipids play an important role in LNP stability. Microfluidic technique yields robust nanoparticles in shorter periods however, it is uncertain how stable the nanoparticles will be under prolonged storage conditions. LNP stability is one of the key quality attributes since aggregation and degradation processes during storage could result in an imbalance of the lipid ratio. Therefore, lipid identification and quantification are critical to the stability and functionality of LNPs and call for robust stability-indicating analytical method. In this study, we developed LC/MS method for the simultaneous detection of a wide range of lipids used in the development and preparation of LNP formulations. The performance of the LC/MS method was demonstrated by analyzing the lipid composition in mRNA-LNP formulation. Further, the method was applied to examine the LNP stability under various formulation and storage conditions.

Sample preparation

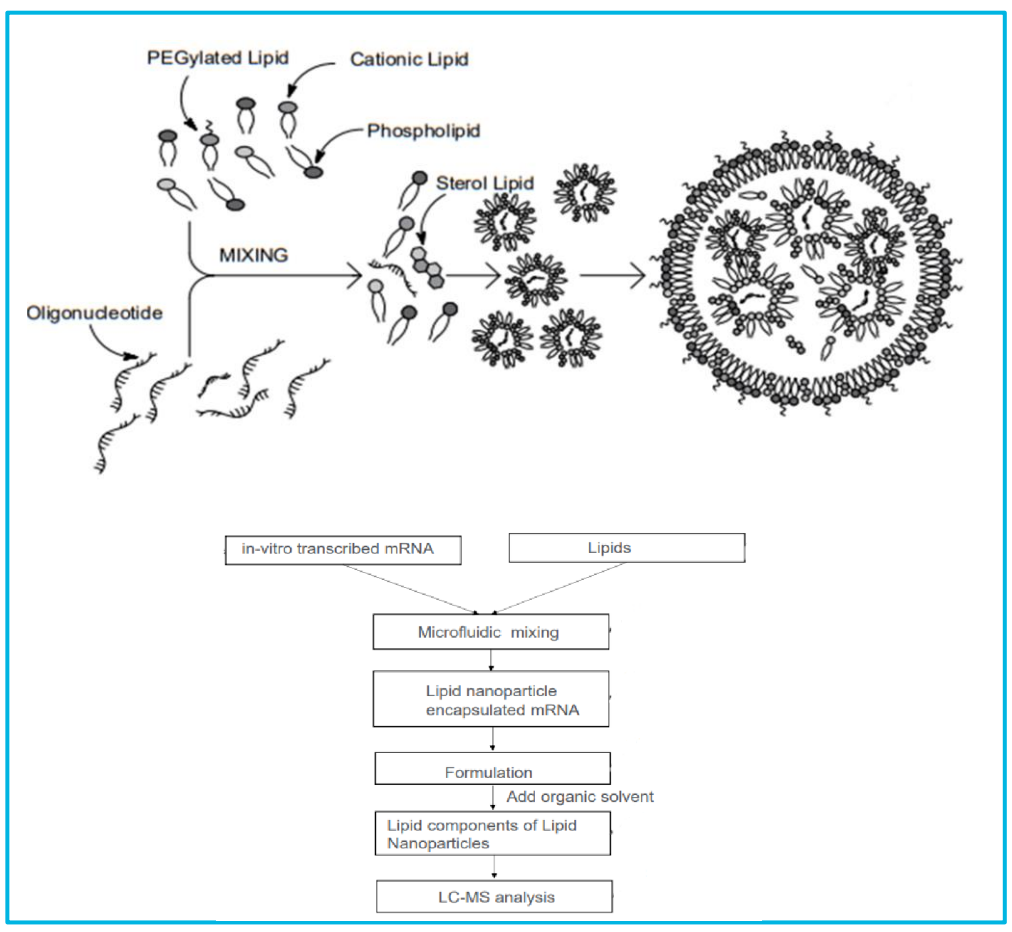


Figure 1. mRNA-LNP preparation schematics and flowchart

Experimental

Figure 2. Agilent 1290 Infinity II LC and 6545XT AdvanceBio LC/Q-TOF system

Parameter	Agilent 1290 Infinity II LC System
Column	InfinityLab Poroshell 120 phenyl hexyl 2.1 × 50 mm, 1.9 µm
Mobile phase A	90% MeOH in 10 mM ammonium acetate
Mobile phase B	90% ACN in 10 mM ammonium acetate
Gradient	Time (min) %A %B 0.00 100 0 2.00 100 0 7.00 0 100
Column temperature	55 °C
Flow rate	0.4 mL/min
	Agilent 6545XT AdvanceBio LC/Q-TOF
Ion mode	Positive ion mode, dual AJS ESI
Drying gas temp	250 °C
Drying gas flow	10 L/min
Sheath gas temp	300 °C
Sheath gas flow	12 L/min
Nebulizer	35 psi
Capillary voltage	3500 V
Nozzle voltage	500 V
Fragmentor voltage	150 V
Skimmer voltage	65 V
Oct RF Vpp	750 V
Reference mass	922.009798
Acquisition mode	Data were acquired in Extended Dynamic Range (2 GHz)
MS mass range	110–1700 *m/z*
Acquisition rate	8 spectra/sec
/MS range	350–3200 *m/z*
MS acquisition rate	2 spectra/sec

Table 1. LC and MS Acquisition parameters

LC/MS of Lipid Nanoparticles(LNPs) Components

Simultaneous measurement of seven lipids with high-resolution separation using phenyl-hexyl column. The lipids were eluted in the order of Cholesterol, DOPE, DOTAP, DSPC, SM-102, ALC-0315 and DMG-PEG 2K. The most abundant charge states observed in the mass spectrum are indicated and all spectra matched theoretical masses to within 2.5 ppm.

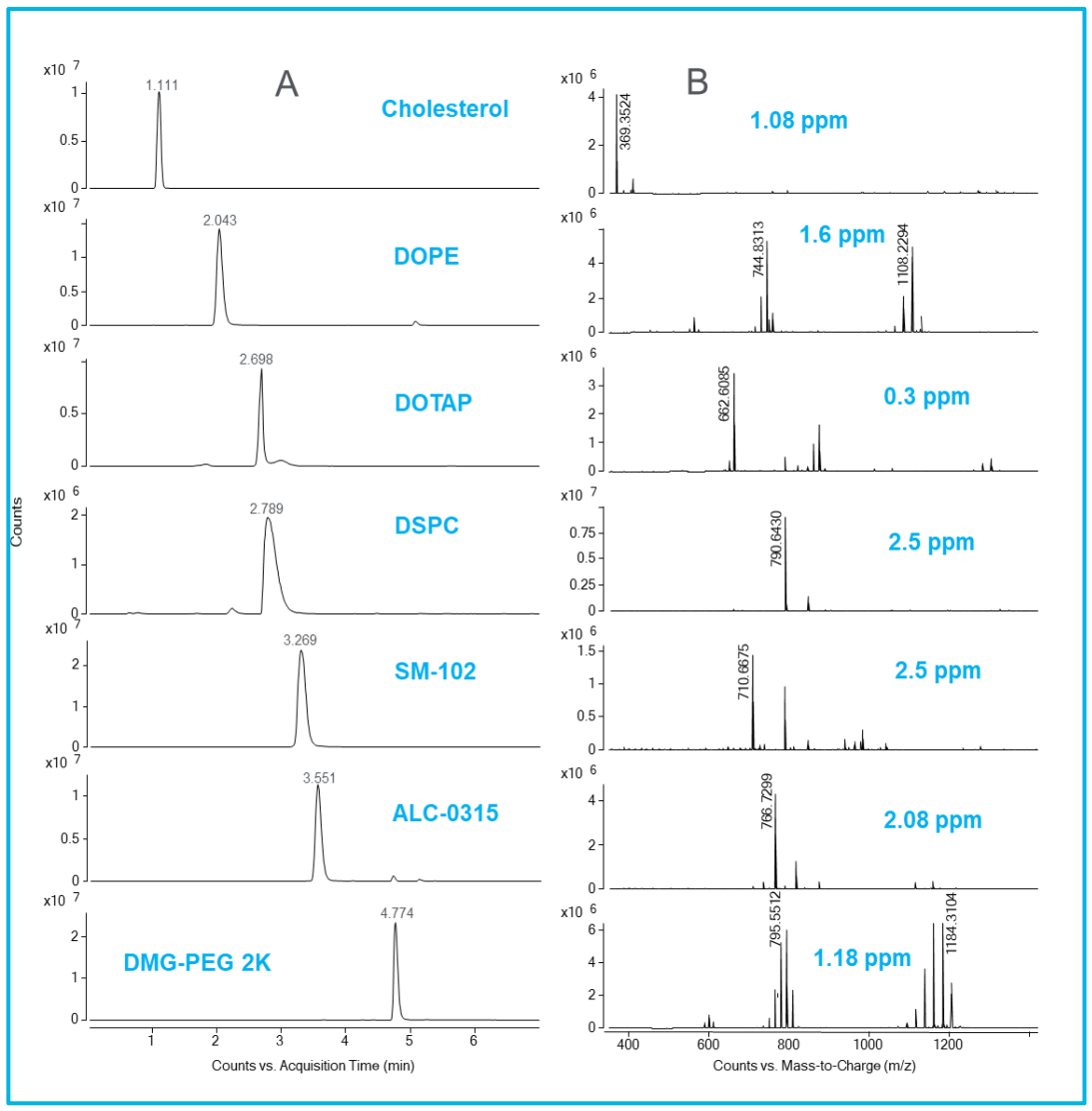
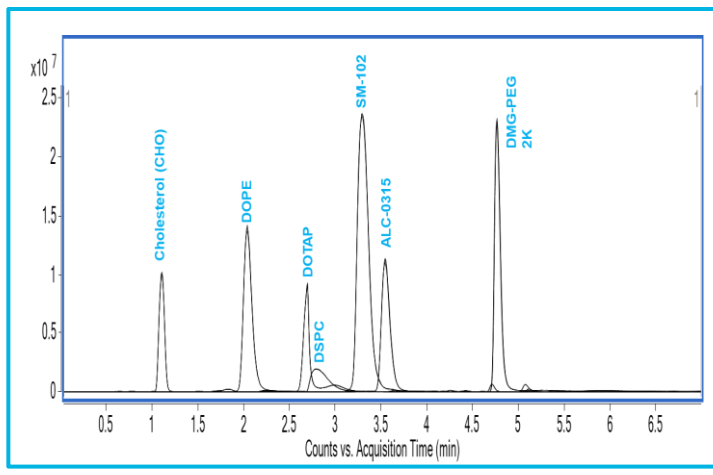


Figure 3. Excellent resolution and accurate mass identification of LNP components. Extracted ion chromatogram (A) & mass spectrum (B) of the seven lipid components

Reproducibility

Excellent lipid separation with no observable shift in the chromatographic profile between the replicate injections.



Lipids	RSD (n=3)	
	Rt	Area
CHO	0.31	1.45
DOPE	0.05	0.68
DOTAP	0.09	1.06
DSPC	0.33	0.67
SM-102	0.47	0.60
ALC-0315	0.04	0.56
DMG-PEG 2K	0.12	0.82

Figure 4. Overlay extracted ion chromatogram of the lipid standards

Linearity of Lipid Standards

A correlation coefficient >0.99 demonstrated the method is linear.

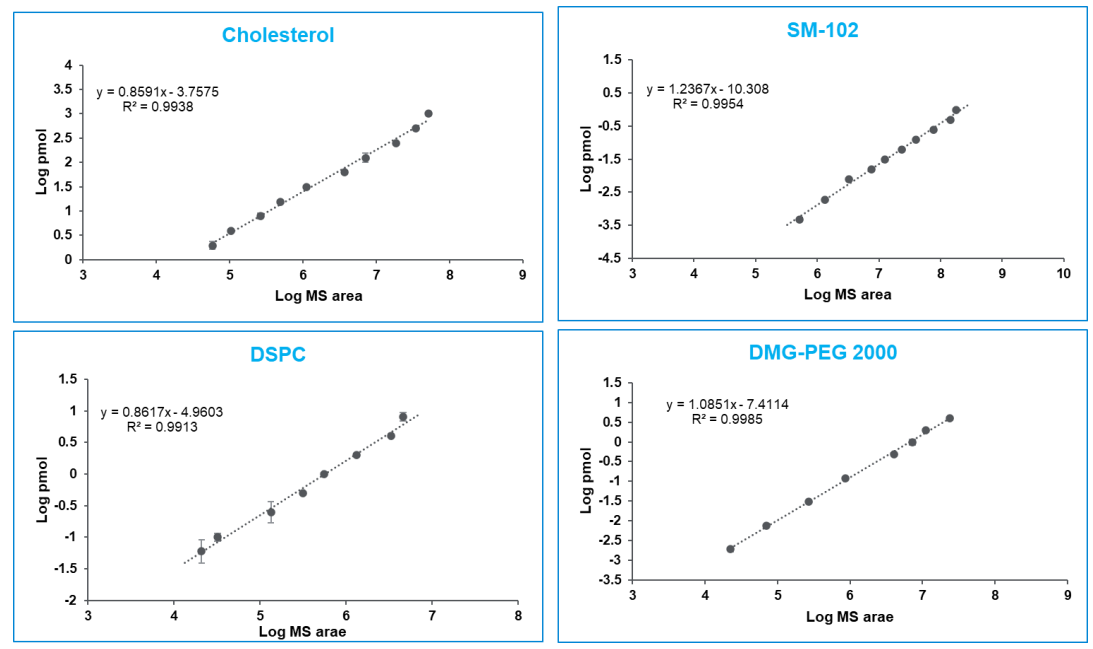


Figure 5. Calibration curve of lipid standards (n =3)

LC/MS of LNPs lipids

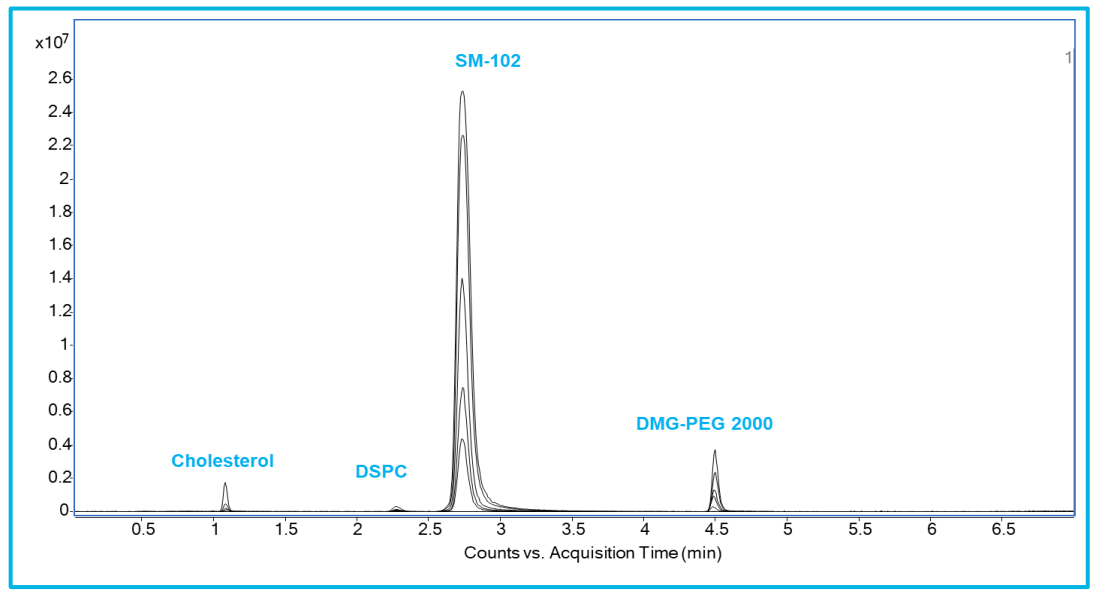


Figure 6. Extracted ion chromatogram overlay of lipid standards

Lipid composition of mRNA-LNPs

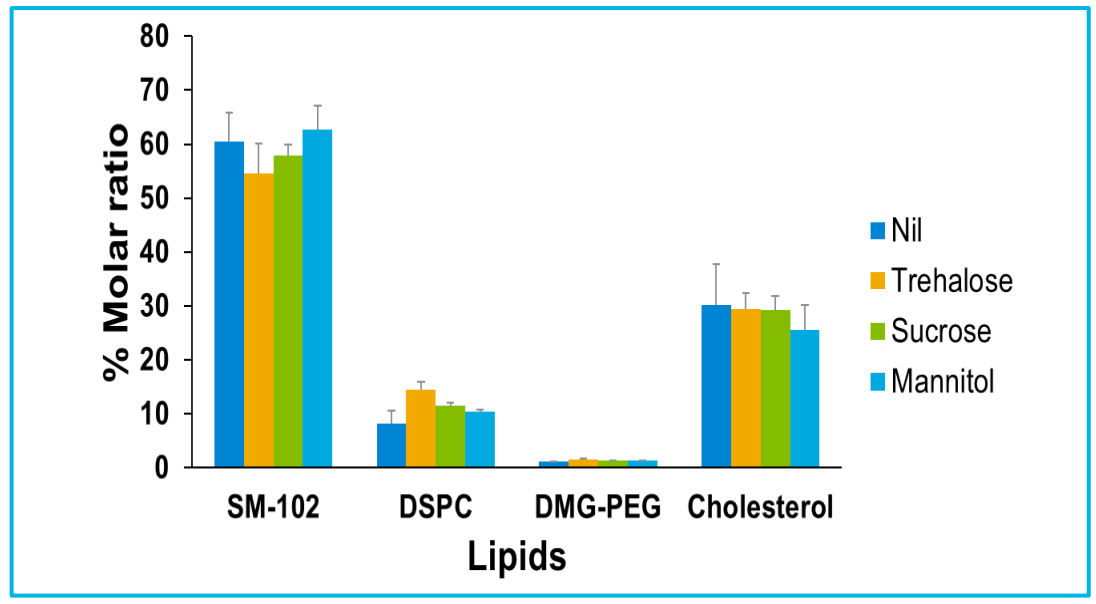


Figure 7. LC/MS measurement of lipid composition of mRNA-LNP lyophilized samples (stored at -70oC).)

Results and Discussion

Non-lyophilized LNPs stability

Figure 8 depicts the % molar ratio changes of the lipid composition of non-lyophilized mRNA-LNPs under different formulation and storage conditions. At -70°C, fresh (control) LNPs maintained their lipid % molar ratio and there were no changes in lipid composition under the conditions of different cryoprotectants. Regardless of cryoprotectants, however, significant lipid composition alterations occurred during the course of different storage conditions (both 1 week and 4 weeks at -20°C and -70°C). These findings showed that storing mRNA-LNPs in non-lyophilized conditions is not recommended for long-term storage.

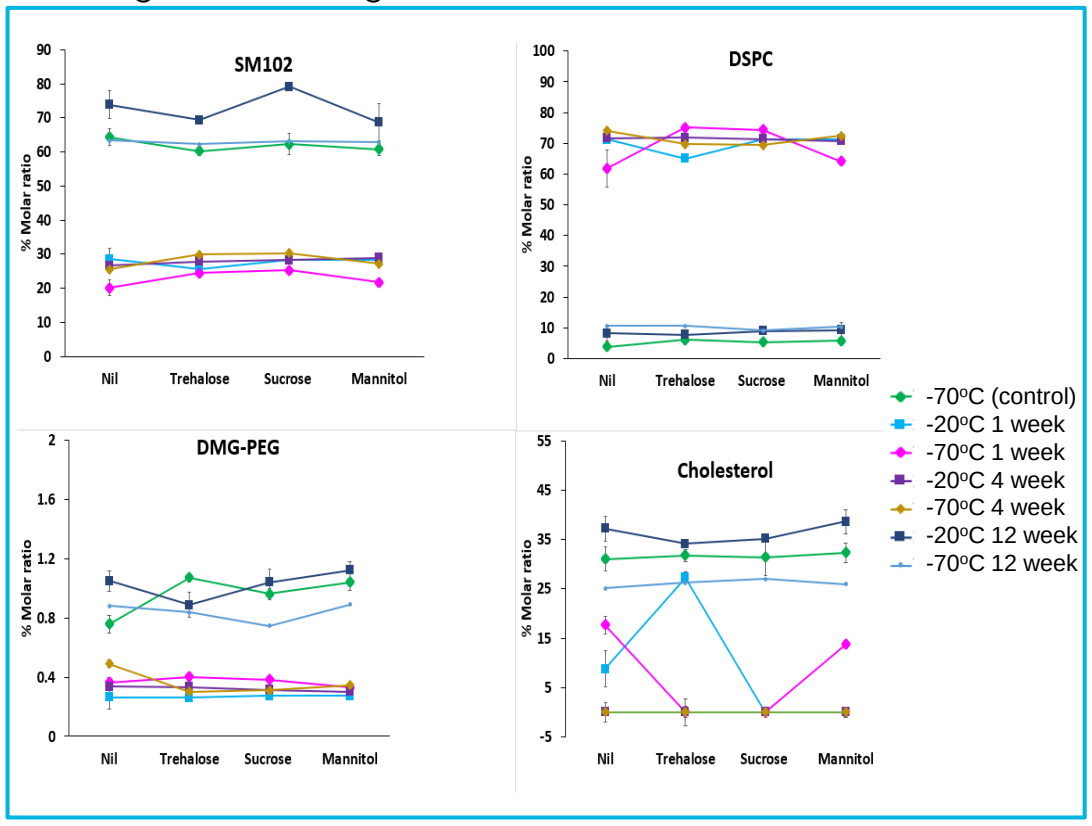


Figure 8. LC/MS measurement of non-lyophilized LNPs stability

Post-reconstitution of lyophilized LNPs stability

Figure 9 depicts the % molar ratio changes of the lipid composition of post-lyophilized mRNA-LNPs under different formulation and storage conditions. The % molar ratios of the lipid components of lyophilized mRNA-LNPs are consistent with the target molar ratio used for the preparation of LNPs at -70°C. Lipid composition is maintained in sucrose and mannitol conditions at all the storage conditions, except for DMG-PEG at 4 weeks, 4°C which shows decreased levels. Storage of lyophilized LNPs for 12 weeks tested did not result in many changes in the lipid composition demonstrating the stability and integrity of lyophilized LNPs. In general, lyophilized conditions provide improved stability.

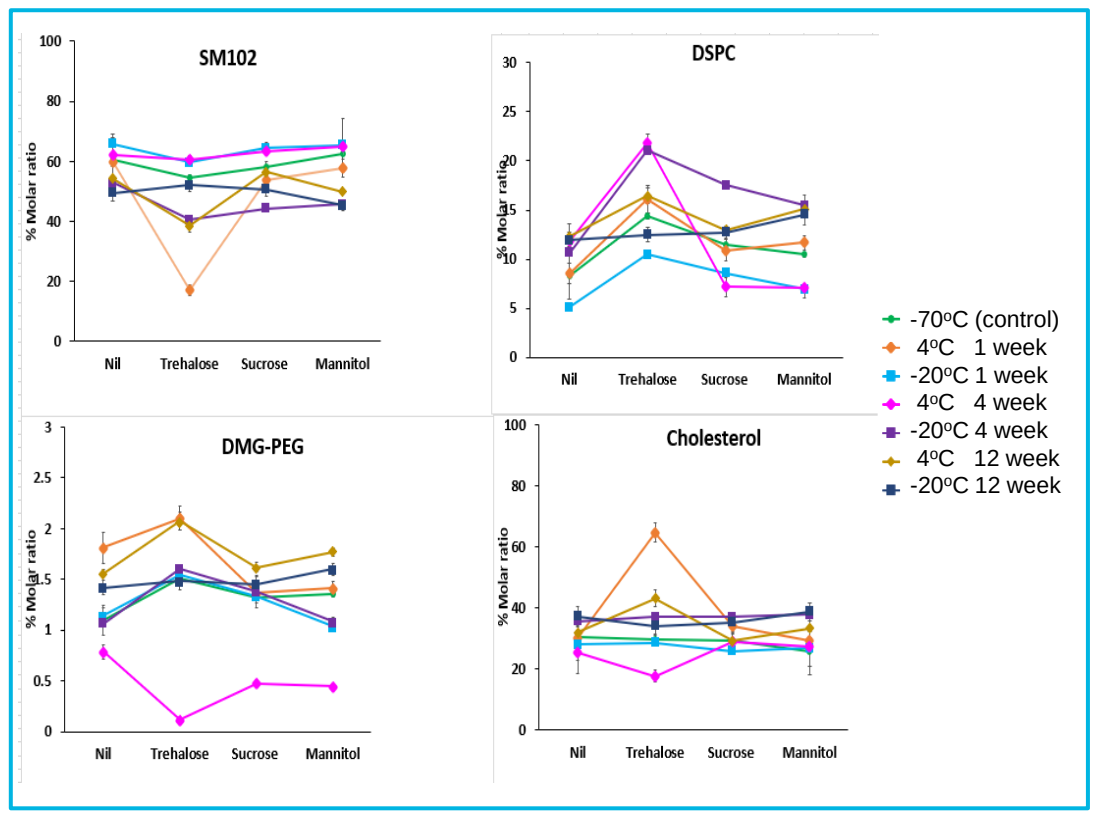


Figure 9. LC/MS measurement of post-reconstitution of lyophilized LNPs stability

Conclusions

- Development of a simple and fast LC/MS method to quantify LNP components.
- Method performance was demonstrated by analyzing the lipid composition of mRNA-LNPs.
- Application of LC/MS method for comprehensive stability study of mRNA-LNP formulation.

References

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- Varache M., Ciancone M., Couffin A.-C. Development and validation of a novel UPLC-ELSD method for the assessment of lipid composition of nanomedicine formulation. Int. J. Pharm. 566, 11-23 (2019).

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