Impact of Ionizable Lipid Source on the *in Vitro* Performance of siRNA- Loaded Lipid Nanoparticles

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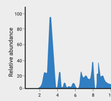
PURPOSE

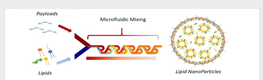
The quality of lipid components is crucial for the successful development of siRNA lipid nanoparticle (siRNA-LNP) therapeutics. Investigating the effects of lipid purity on the critical quality attributes (CQAs) of siRNA-LNPs provides valuable insights that can guide the development of both novel and generic formulations.

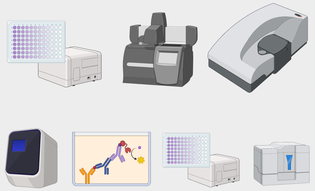
OBJECTIVE

This study examines the impact of the source and purity of D-Lin-MC3-DMA (MC3), a commonly used ionizable lipid, on the *in vitro* performance and stability of siRNA-LNPs with a similar composition to the model drug Onpattro® (Patisiran) injection.

METHODS







- MC3 vendor screening: HPLC-CAD/ HPLC-PDA
- Impurity analysis: LC-MS
- Microfluidic preparation of siRNA-LNP nanoparticles
- Physicochemical characterization of siRNA-LNP: size, zeta, PDI, EE%, apparent pKa, SAXS, DSC
- In vitro efficacy and safety: mRNA knockdown, protein expression, cellular viability, immunogenicity

Figure 1. Methodology for MC3 impurity analysis, LNP formulation using three MC3 sources, and subsequent physicochemical and in vitro assessments.

Abbreviations

HPLC: High-performance liquid chromatography, CAD: Charged aerosol detector, PDA: Photodiode array, LC-MS: Liquid chromatography-mass spectrometry, PDI: Polydispersity index, EE: Encapsulation efficiency, SAXS: Small angle X-ray scattering, DSC: Differential scanning calorimetry.

RESULTS

1. Chromatograms show impurities associated with the ionizable lipid MC3 are detectable in the siRNA-LNP product.

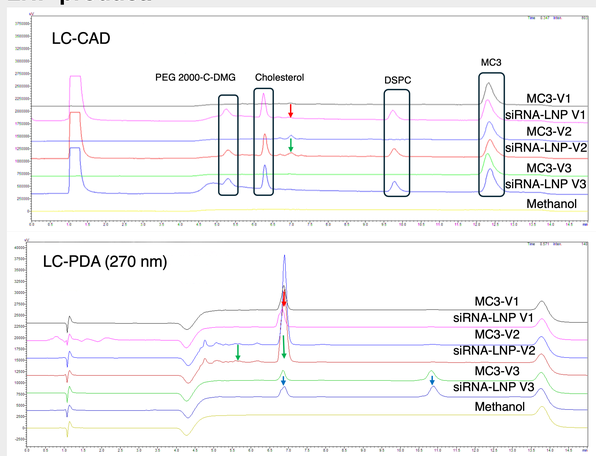


Figure 2. LC-CAD and LC-PDA (270 nm) chromatograms of MC3 samples from three vendors and their corresponding siRNA-LNP formulations, adjusted to comparable MC3 concentrations. As indicated by red, green, and blue arrows, impurity peaks detected in the raw MC3 lipids from Vendors 1, 2, and 3, respectively, were also observed in the corresponding siRNA-LNP formulations in both LC-CAD and LC-PDA chromatograms.

2. The impurity peak at retention time ~7 min is likely attributed to the ketone form of MC3 based on LC-MS/MS data, which accounts for 2.02, 7.45, and 0.8% in vendors 1,2, and 3, respectively based on LC-CAD.

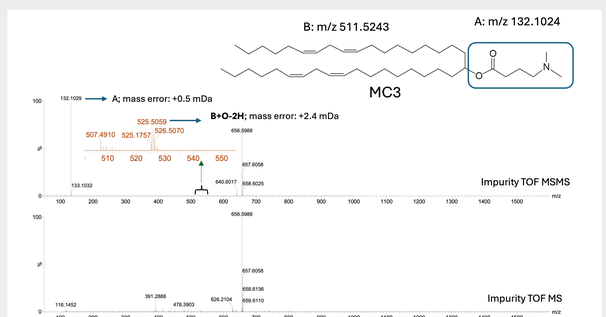


Figure 3. MS and MS/MS spectra of collected MC3 impurity (m/z= 656.5988) eluted at ~ 7 min.

3. The impurities do not significantly affect the physicochemical properties of siRNA-LNPs. DSC shows distinct thermal behavior in MC3 vendor 2 (V2), with highest impurity, both at lipid and nanoparticle level with both peaks shift to lower temperatures in V2 LNP indicating participation of impurity in formulation.

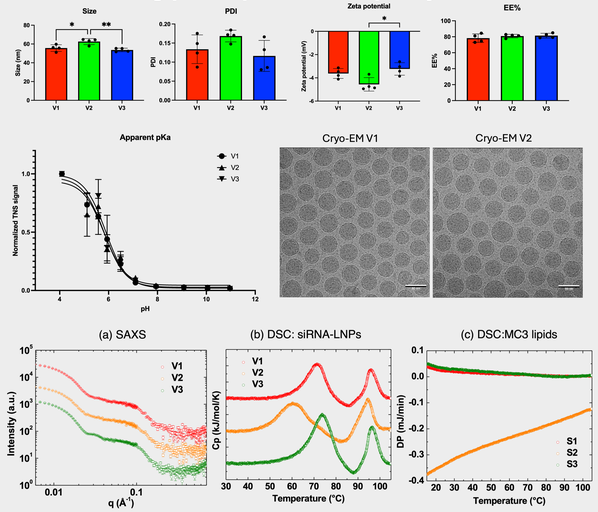


Figure 4. Physicochemical properties of siRNA-LNPs prepared by three MC3 sources. Data are shown as mean ± SD, n=3-4. P < 0.05 (*), P < 0.01 (**).

4. The impurities do not significantly affect the *in vitro* performance of siRNA-LNPs. The immunogenicity also show no significant difference among different MC3 vendors in cytokine secretion in PBMCs.

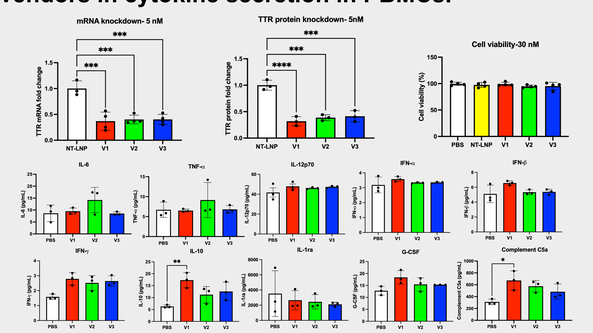


Figure 5. In vitro performance and cytokine array analysis of siRNA-LNPs prepared by three MC3 sources. Data are shown as mean ± SD, n=3-4. P < 0.05 (*), P < 0.01 (**), P < 0.001 (***), P < 0.0001 (****).

5. Despite physicochemical changes in formulations stored at 4 °C and 25 °C for 70 days, no significant effect on mRNA knockdown efficiency or cell viability is observed. No effect of vendor source is detected on the stability of formulations.

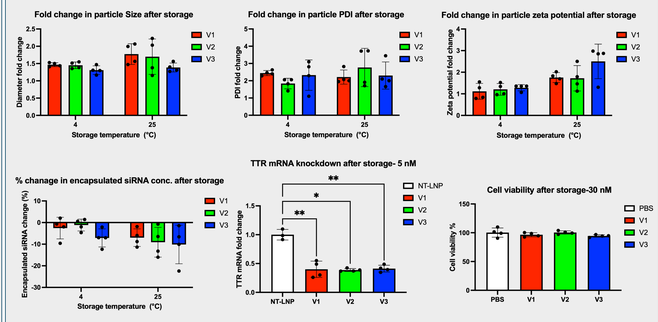


Figure 6. Physicochemical properties and in vitro performance of siRNA-LNPs prepared by three MC3 sources and stored for 70 days at 4 °C and 25 °C. Data are shown as mean ± SD, n=3-4 . P < 0.05 (*), P < 0.01 (**).

CONCLUSIONS

- Complementary analytical techniques were used to assess the impurity of MC3 obtained from different sources
- Comprehensive structural analysis was established to correlate impurity-related changes with LNP internal structure.
- The impurities in the ionizable lipid, although transferred to the final product, did not show a significant effect on the physicochemical properties other than the thermal behavior.
- The main impurity was likely the ketone form of MC3, which did not exhibit a notable impact on the in vitro performance or 70-day stability of the siRNA-LNPs at levels up to 7.45%.

FUNDING

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