Effect of ionizable lipid source on the quality attributes of siRNA lipid nanoparticle therapeutics

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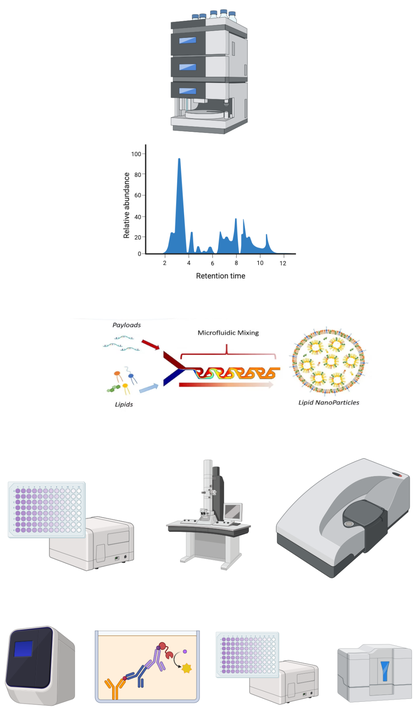
Background

The quality of lipid components is crucial for the successful development of siRNA lipid nanoparticle (siRNA-LNP) therapeutics. This study examines how the source and purity of D-Lin-MC3-DMA (MC3), a commonly used ionizable lipid, impact the critical quality attributes (CQAs) of siRNA-LNPs with a similar composition to the model drug Onpattro® (patisiran) injection. The findings offer a better scientific understanding on the effects of MC3 quality on in vitro performance of siRNA-LNPs and provide valuable insights to facilitate the development of generic formulations.

Methods



MC3 vendor screening: HPLC-CAD/ HPLC-PDA

Impurity analysis: LC-MS

Microfluidic preparation of siRNA-LNP nanoparticles

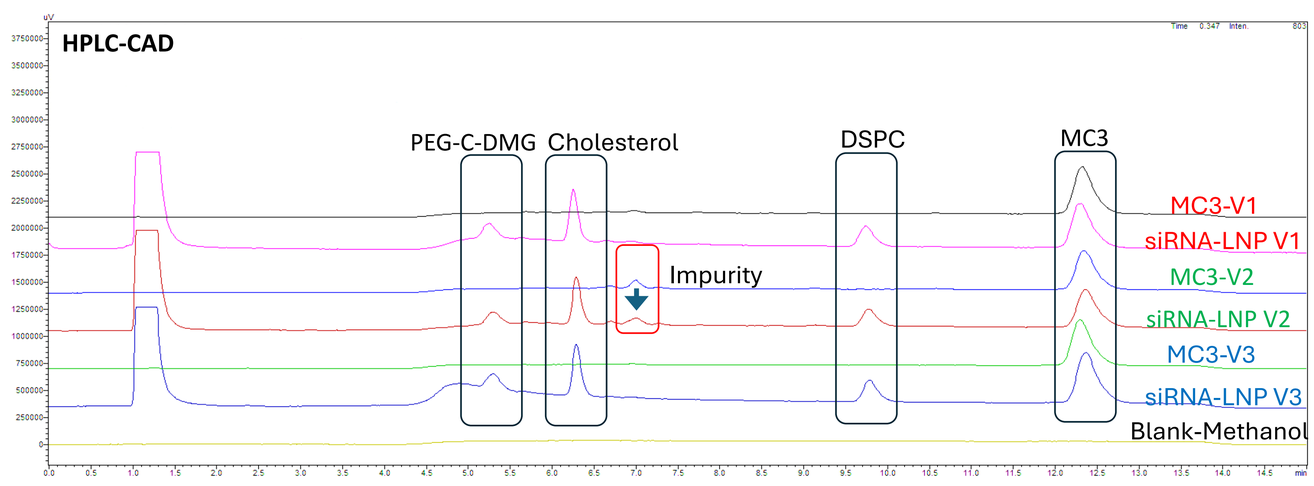
Physicochemical characterization of siRNA-LNP: size, zeta, PDI, EE%, apparent pKa, cryo-EM

In vitro efficacy and safety: mRNA knockdown, protein expression, cellular viability, immunogenicity

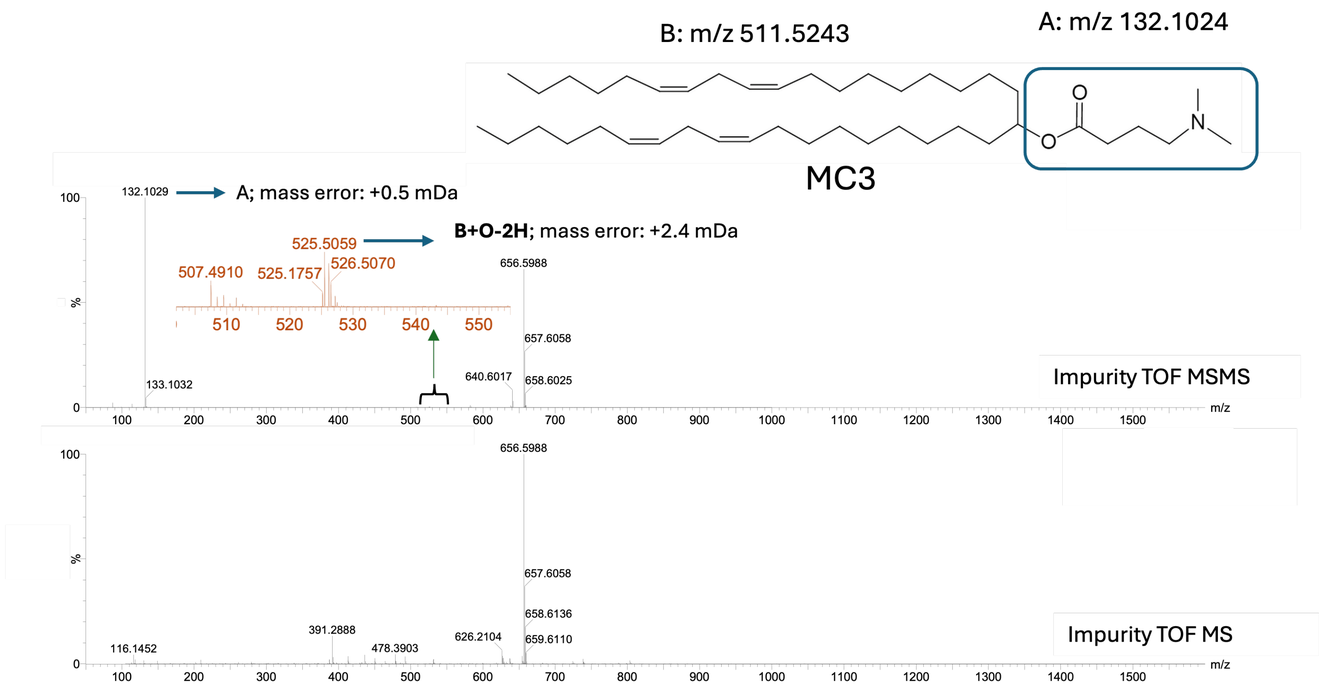
Property	Method
Size, Polydispersity index (PDI)	Dynamic Light Scattering (DLS)
Surface Charge	Electrophoretic Light Scattering
Apparent pKa	TNS Assay
Encapsulation Efficiency (EE%)	Ribogreen Assay
Internal structure	Small-angle X-ray scattering (SAXS)
Lipid Purity	LC-charged aerosol detector (CAD), LC-MS/MS
Thermal profile	Differential Scanning Calorimetry (DSC)
Cell Viability	CellTiter-Glo
TTR mRNA Knockdown	RT-qPCR
TTR Protein knockdown	ELISA
Cytokine Array Analysis	Luminex Assay

Results

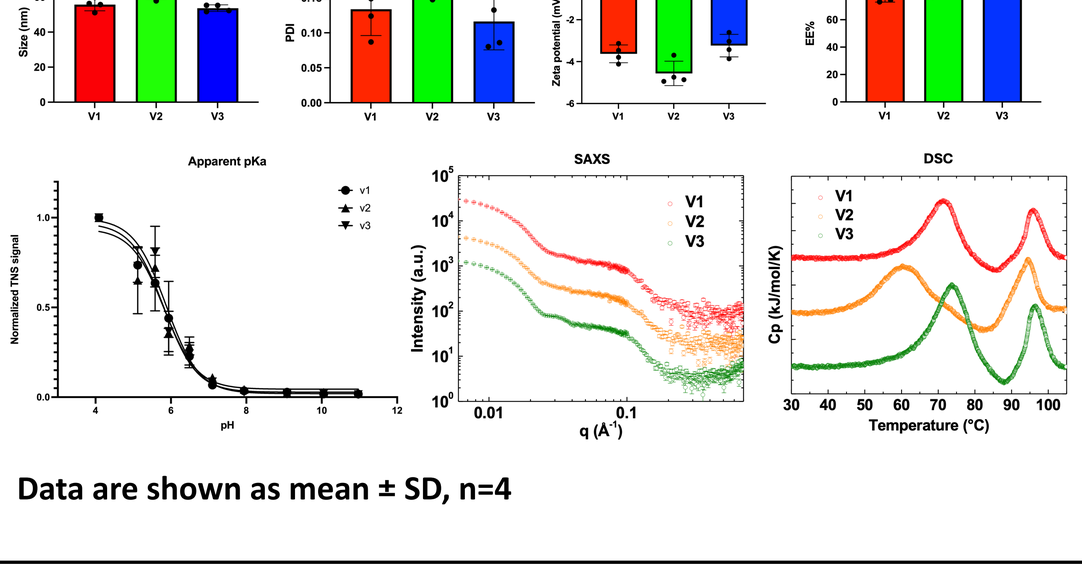
1. Chromatograms show impurities associated with the ionizable lipid MC3 are detectable in siRNA-LNP product.



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.

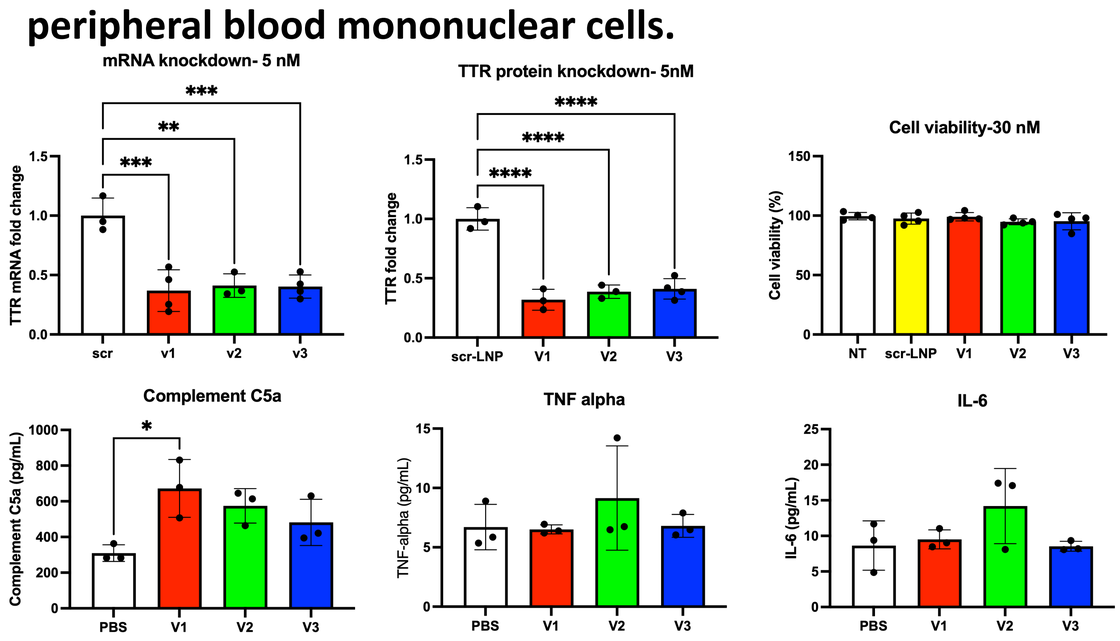


3. The impurities did not significantly affect the physicochemical properties of siRNA-LNPs such as particle size, PDI, surface charge, EE%, apparent pKa and internal structure (SAXS data). DSC showed distinct thermal behavior in siRNA-LNP vendor 2, containing highest impurity, with both peaks shift to lower temperatures indicating the participation of impurities in siRNA-LNP.



Data are shown as mean ± SD, n=4

4. The impurities did not significantly affect the cell viability or target gene/protein knockdown in HepG2 cells. The immunogenicity studies with 30 nM siRNA also show no significant difference among different MC3 vendors in complement activation, TNF-α and IL-6 secretion in peripheral blood mononuclear cells.



Data are shown as mean ± SD, n=3-4.

Conclusion

The impurities in the ionizable lipid despite being transferred to the product did not show significant effect on the physicochemical properties of siRNA-LNPs other than the thermal behavior. The main impurity is attributed to the ketone form of MC3, which did not show any significant effect on the in vitro performance of the siRNA-LNPs at levels up to 7.45% but showed minor trends in IL-6/ TNF-α elevation.

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