BioReliance®
Contract Testing Services

Technical Note

Lipid nanoparticle compositional analysis by UHPLC-CAD

Analytical Development for mRNA

Challenge

In vitro transcribed messenger ribonucleic acid (mRNA) has emerged as a novel therapeutic platform. Lipid nanoparticles (LNPs) facilitate the effective delivery of mRNA to specific cell types and tissues, whilst enhancing stability. The efficacy, biodistribution and potential toxicity of the therapeutic is determined in large part by the lipid composition of the LNP. Accordingly, the identity, quantity, and purity of the individual lipids are critical quality attributes of mRNA-LNP products.

Ultra-high performance liquid chromatography (UHPLC) coupled with Charged Aerosol Detection (CAD) is a powerful technique for the analysis of compounds such as lipids that do not contain chromophores.

Methodology

The LNP is dissolved in an appropriate sample diluent, resulting in disassembly of the LNP into its constituent lipids. The individual lipids are then separated by reversed-phase UHPLC and detected by CAD. Each lipid is identified based on its retention time and quantified relative to a calibration curve obtained from a series of lipid standards.

Assay Details

A minimum of 70 µL of sample is required for routine analysis. Reference solutions of all lipids should be provided with each sample set.

Case Study Results

The results were generated from an internal study using a proprietary mRNA-LNP formulation. **Figure 1** shows the obtained CAD chromatogram of the constituent lipids. Each of the individual lipids were detected and well resolved, demonstrating the utility of UHPLC-CAD for the simultaneous analysis of LNP components. The identity of each lipid in the formulation was confirmed by comparison of the observed retention time to that of the corresponding lipid standard. The purity of the formulation was indicated by the absence of other peaks in the chromatogram.

A series of dilutions were prepared for three of the lipids that were present in the sample (Cholesterol, DMG-PEG 2000 and DSPC). Data for the proprietary, cationic lipid were not obtained. As observed in **Figure 2**, the response of the assay was linear ($R2 \geq 0.995$) for each lipid over the range studied, which allowed the quantity of the individual lipids to be determined. The observed amounts were consistent with the expected quantities of each lipid in the formulation.

Thus, UHPLC-CAD can be used to confirm the identity, quantity and purity of lipids, ensuring the quality of mRNA-LNP drug products.

[See figures on the next page]

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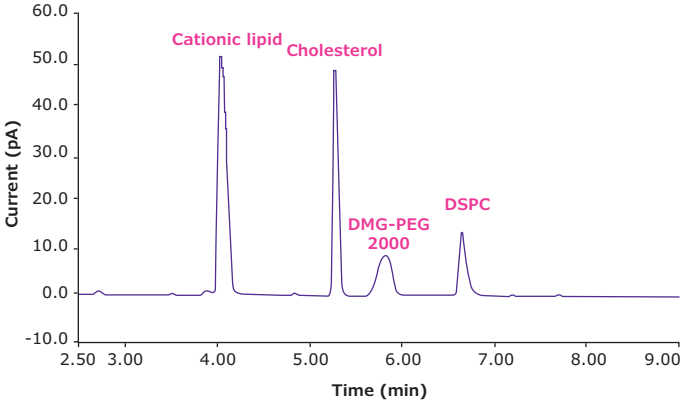

Figure 1: UHPLC-CAD chromatogram of an LNP formulation.

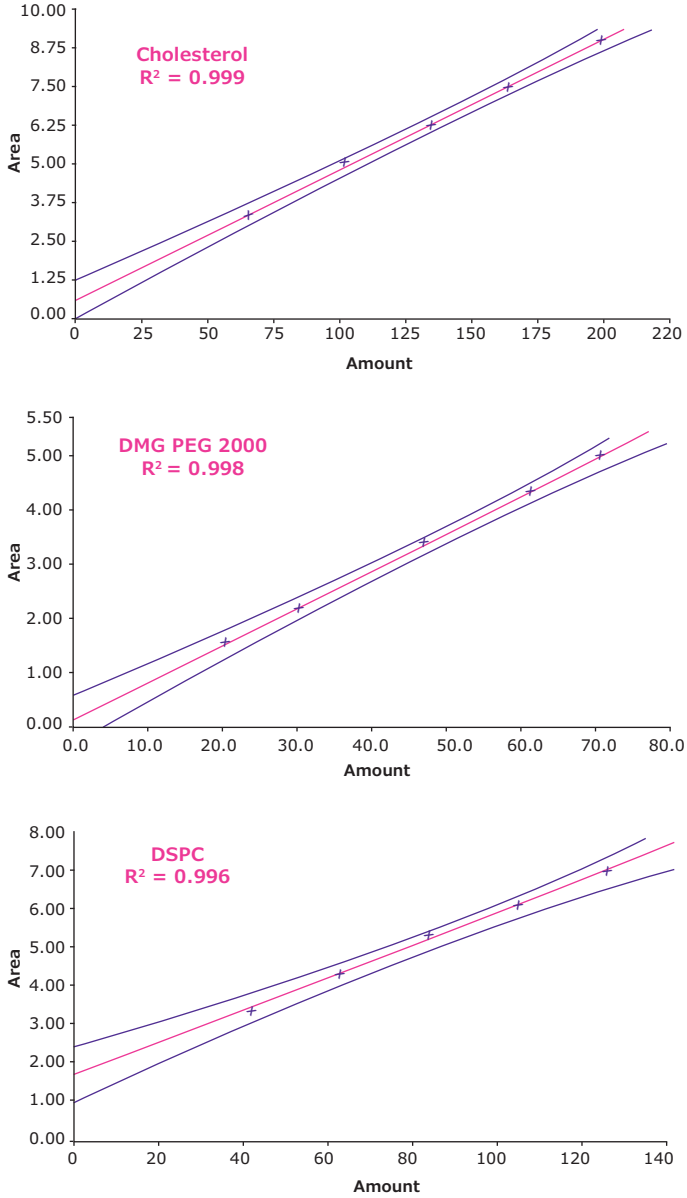

Figure 2: Calibration curves for cholesterol, DMG PEG 2000 and DSPC.

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