IDENTIFICATION AND QUANTIFICATION OF NANOPARTICLE COMPOSITION USING HPLC-UV

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Formulating and manufacturing lipid nanoparticles (LNPs) is a complex process that requires not only achieving stable structures but also the verification of their composition in order to know and control whether the nanoparticle composition is as originally planned. Thus, it is important to identify each component both individually and as part of the assembled structure.
We hypothesize that the final composition of LNPs prepared by microfluidic mixing (MFM) may not necessarily reflect the initial lipid proportions in the ethanol phase used for their preparation. This discrepancy may have important implications for calculating formulation efficiency, determining the effective availability of functional excipients, and ensuring process control, cost-effectiveness, and reproducibility.
Quantifying the lipid components is crucial to ensure reproducibility, understand formulation performance, and correlate composition with physicochemical and biological behaviour. Our objective was to develop a rapid, accessible, and reliable HPLC–UV method to identify and quantify the individual components of LNPs.

For this purpose, we evaluated LNPs prepared with DLin-MC3-DMA, DSPC, DSPE-mPEG, cholesterol, and a maleimide-functionalized lipid. The main components of the formulation contain functional groups capable of absorbing visible light. Therefore, HPLC (High Performance Liquid Chromatography) with a UV-Vis detector was selected as the analytical method. These UV active groups include double bonds and aromatic rings [1]. The HPLC-UV is a versatile and widely available analytical technique used in research laboratories for the separation, identification and quantification of components in a mixture, according to the technique's technical requirements for performance. It offers a cost-effective and robust approach that meets the technical performance requirements of both chemical and biochemical analyses. Reproducible and accurate identification depends on maintaining appropriate chromatographic conditions that ensure analyte-specific separation and response [2].
For the mobile phase preparation, various combinations of water, methanol, and isopropanol were initially tested. However, optimal component separation was achieved using a ternary mixture of isopropanol, acetonitrile, and 0.01 M ammonium acetate buffer, applied with a gradient elution profile (see table 1).
Analyses were performed using a Waters Delta 600 Chromatographic System with a Waters 2487 dual Absorbance Detector, and a C18 column (3.5 µm, 4.6 mm x 100 mm). The column temperature was 30 ºC, the flow rate of 1.0 mL/min, and the injection volume 16 µL [3]. Detection was set at 217 and 204 nm.
Each compound was analyzed separately in ethanol at 99% to determine each retention time. Aqueous suspensions of LNPs samples were lyophilized [4] and then resuspended in ethanol at 99% before analysis. Figure 1 shows the chromatogram of the whole formulation following this method. Quantification was performed by comparing the peak areas of the sample with these obtained from working standard solutions for each compound.

The developed gradient method provided baseline separation of all lipid components within a short analysis time. The method allowed reliable identification and quantification of each compound directly from the LNPs formulation. The analysis was completed within a few hours, using a simple and cost-effective setup.

Our approach provides an efficient alternative to more complex techniques such as LC–MS, offering sufficient accuracy for formulation optimization, batch-to-batch comparison, and routine quality control.

In conclusion, a robust HPLC–UV method was successfully established for the identification and quantification of major lipid components in LNP formulations. This rapid and affordable technique enables routine assessment of nanoparticle composition, supporting formulation consistency and facilitating further optimization studies.

References

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Figures

Table 1. Gradient method used for the identification and quantification of the compounds of the lipid nanoparticle.

Time (minutes)	Isopropanol (%)	Acetonitrile (%)	Regulatory solution (%)
0	30	60	10
25	30	60	10
28	70	30	0
38	70	30	0
42	30	60	10
50	30	60	10

Figure 1. Peak identification in an LNP sample using the UV-HPLC method.