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Implementation of Analytical Methods for mRNA-LNPs addressing Stability Indicating Critical Quality Attributes (CQAs) and their Applications

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Introduction

Since the rapid development of mRNA-lipid nanoparticle (LNP)-based COVID-19 vaccines during the COVID-19 pandemic, these modalities became the focus of attention in the field of biotherapeutics. A rapidly increasing number of mRNA-LNP-based vaccine and therapeutic candidates are currently under development. All these clinical and commercial products based on mRNA-LNP technology share the same characteristic of limited stability of both components, the encapsulated mRNA and the lipids assembling the LNP for mRNA encapsulation, stabilization, and delivery [1]. Literature data on the stability of mRNA-LNPs are very limited and specific regulatory guidance for the development of safe, potent and stable mRNA-LNP therapeutics is under development. A draft guideline regarding analytical methods for the assessment of mRNA quality exists and an additional draft guideline regarding the methods for the assessment of LNP quality is in preparation by the USP. Leukocare implemented an analytical toolbox of stability-indicating analytical methods in accordance with the draft USP guidelines. The application of these methods address Critical Quality Attributes (CQAs) defined by USP for mRNA-LNP drug products (DPs). The methods were successfully employed in stability studies of mRNA-LNPs to get insights into the degradation pathways of mRNA encapsulated in the LNPs and of lipids assembling the LNPs.

Methods

Microfluidic mRNA-LNP Preparation

Lipids in the organic ethanolic phase and mRNA in the aqueous phase (25 mM Acetate buffer pH 4.5) were mixed using a nanoformulator NanoAssemblr Ignite from Precision Nanosystems Inc. (Vancouver, Canada) to produce mRNA-LNPs. The prepared mRNA-LNPs were formulated into 20 mM Tris pH 7.5 with 8 % sucrose.

RiboGreen Assay

For the quantification of the mRNA content inside the mRNA-LNPs (encapsulation efficiency) the Quant-iT RiboGreen RNA Quantification Assay kit and the associated protocol (Thermo Fisher Scientific, Karlsruhe, Germany) were used.

Dynamic Light Scattering (DLS)

The size and polydispersity of the mRNA-LNPs were determined using the Cuvette DLS instrument DynaPro NanoStar (Wyatt; Santa Barbara, USA). The measurement was performed at 25 °C with an acquisition time of 3 s and a number of 25 acquisitions.

Reverse Phase (RP)-Ion Pair (IP)-HPLC [2]

Adapted from [2]

Reverse Phase (RP)- HPLC-Charged Aerosol Detector (CAD) [3]

Adapted from [3]

LabChip micro Capillary Electrophorese (µCE)[4]

Adapted from [4]

Results

Stability Indicating CQAs of mRNA-LNP Drug Products and Implemented Analytical Methods at Leukocare

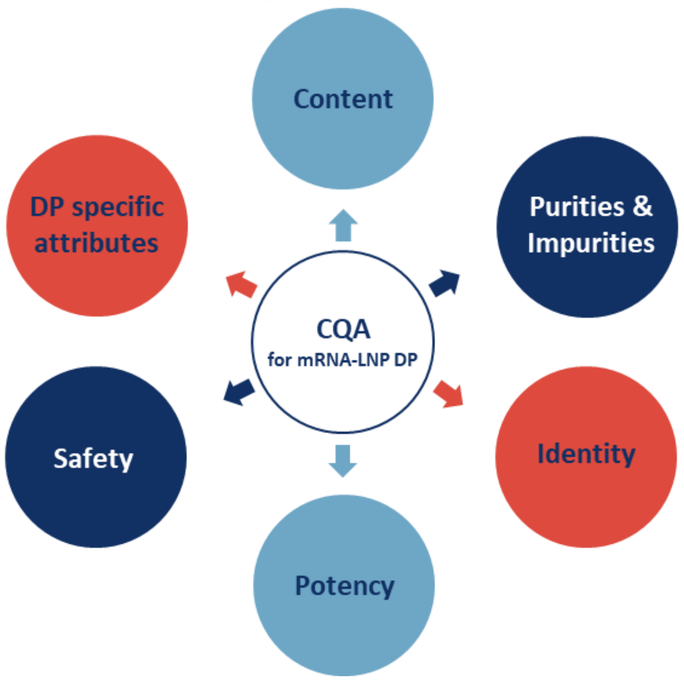

Figure 1: Stability indicating Critical Quality Attributes (CQAs) of mRNA-LNP Drug Product (DP) according to the USP guidelines.

Critical quality attributes of mRNA-LNP Drug Product can be divided in six sections (Figure 1).

Table 1: Implemented methods for analysis of stability indicating Critical Quality Attributes (CQAs) of mRNA-LNP Drug Product

Quality	Attribute	Method
Content	mRNA content (API)	RiboGreen Assay
	mRNA Encapsulation (API)	RiboGreen Assay
Purity & Impurities	% mRNA Main Peak	RP-IP-HPLC/µCE
	% Fragments	RP-IP-HPLC/µCE
	% Aggregates	RP-IP-HPLC/µCE
	mRNA-lipid adducts	RP-IP-HPLC
	Lipid impurities	RP-HPLC-CAD
Drug specific attributes	Lipid content	RP-HPLC-CAD
	mRNA-LNP size	DLS
	mRNA-LNP polydispersity	DLS
	Surface charge	ELS
Potency	*In vitro* expression	Luciferase Assay (Model)

Both components of mRNA-LNP DP, mRNA as well as the lipids assembling the LNPs are prone to several degradation reactions during the application of various types of stress. According to the USP guidelines, particularly sensitive methods are suitable to assess the stability of these pharmaceutical modalities. The assays implemented at Leukocare and the associated stability indicating CQA are summarized in Table 1.

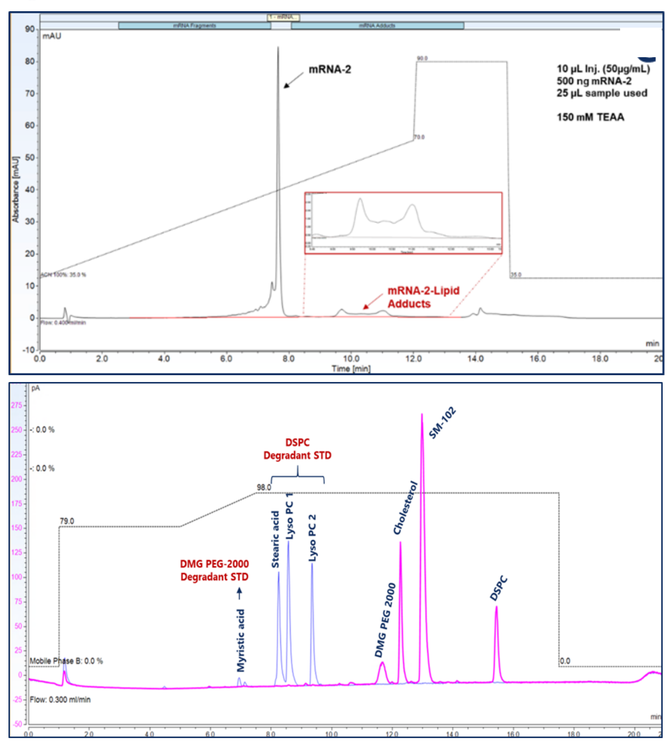

Figure 2: RP-IP-HPLC analysis of the mRNA integrity and the formation of mRNA-lipid adducts (Top). RP-HPLC-CAD analysis of the 4 lipids assembling the LNP and well-known degradation products.

Application of these Stability Indicating Methods during Exposure to Various Types of Stresses

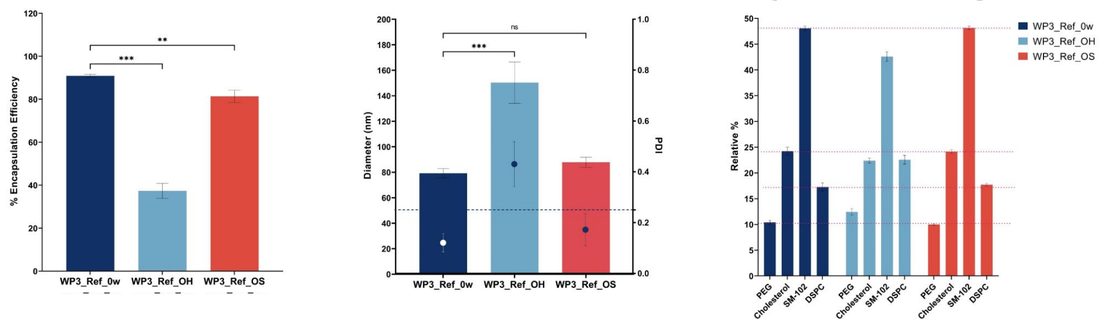

Figure 3: Percent Encapsulation Efficiency (EE) of mRNA-LNPs unstressed and after application of mechanical stress monitored by RiboGreen assay (left). Hydrodynamic diameter (D_h) and Polydispersity Index (PDI) of unstressed mRNA-LNPs and after application of mechanical stress monitored by DLS (right). Lipid recovery in mRNA-LNPs at time-point t = 0 and after application of mechanical stress by RP-HPLC-CAD analysis.

Application of overhead rotation (OH) as a mechanical stress led to the disruption of the mRNA-LNP molecular structure resulting in significant increase in size and in PDI (Figure 3, middle) as analyzed by DLS as well as an associated decrease in mRNA content (Figure 3, left) as monitored by RiboGreen assay. In contrast, orbital shaking (OS) had only a minor effect on the mRNA stability. OH resulted in a loss of the ionizable lipid as demonstrated by the changed relative % values of LNP-assembling lipids (Figure 3, right). This result is in line with the results of the RiboGreen assay and DLS, demonstrating that OH was the most devastating form of stress.

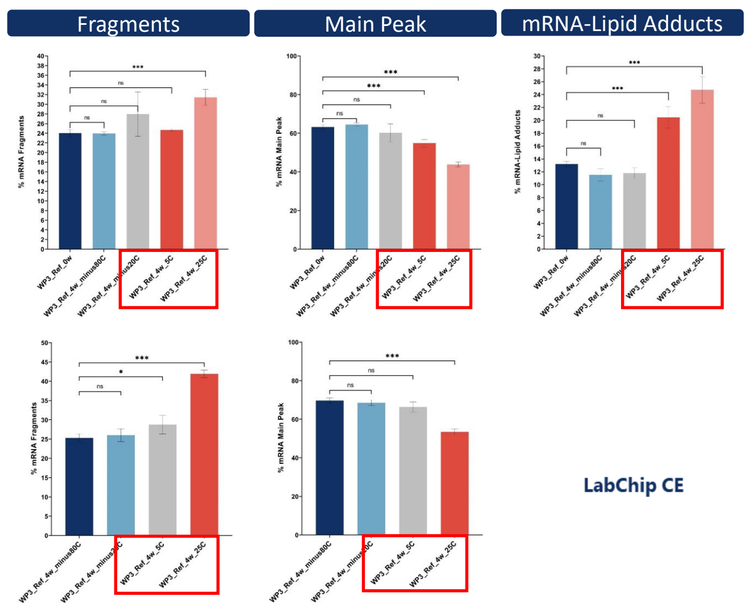

Figure 4: Quantification of mRNA integrity in mRNA-LNPs during storage for 4 weeks at different temperatures analyzed by RP-IP-HPLC and µCE. The red boxes indicate the comparison between the same samples in RP-IP-HPLC and µCE LabChip.

Comparison of RP-IP-HPLC and LabChip CE results after 4 weeks of storage at 5 °C and 25 °C revealed a high significant fragmentation of the mRNA, particularly at 25 °C and is associated with a significant decrease in % main peak. Additionally, a strong formation of mRNA-lipid adducts during storage for 4 weeks at 5 °C and 25 °C could be observed with RP-IP-HPLC analysis .

Summary

As a prerequisite for the characterization of mRNA-LNP-based biotherapeutics according to the Critical Quality Attributes (CQAs) suggested by the draft USP guideline, Leukocare established a microfluidic preparation method for mRNA-LNPs using the NanoAssemblr Ignite from Precision Nanosystems. Subsequently, a toolbox of analytical methods to characterize the manufactured mRNA-LNPs, e.g. the RiboGreen Assay, DLS, RP-IP-HPLC, RP-HPLC-CAD and LabChip CE were successfully implemented at Leukocare. The application of these stability indicating methods in a basic characterization of mRNA-LNPs provided deep insights in the degradation pathways of mRNA as well as lipids. Mechanical stress was identified as the most devastating stress for the LNPs themselves and storage for 4 weeks at ambient temperature for the mRNA.

Conclusion

Leukocare´s set of established stability indicating methods allows for further screening of mRNA-LNP formulations with improved stability.

References

[1] Blenke E. O., et al.; *Journal of Pharmaceutical Sciences*; 112(2); 2023; 386-403.
[2] Packer M., et al.; *Nature Communications*; 2021; 12:6777
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