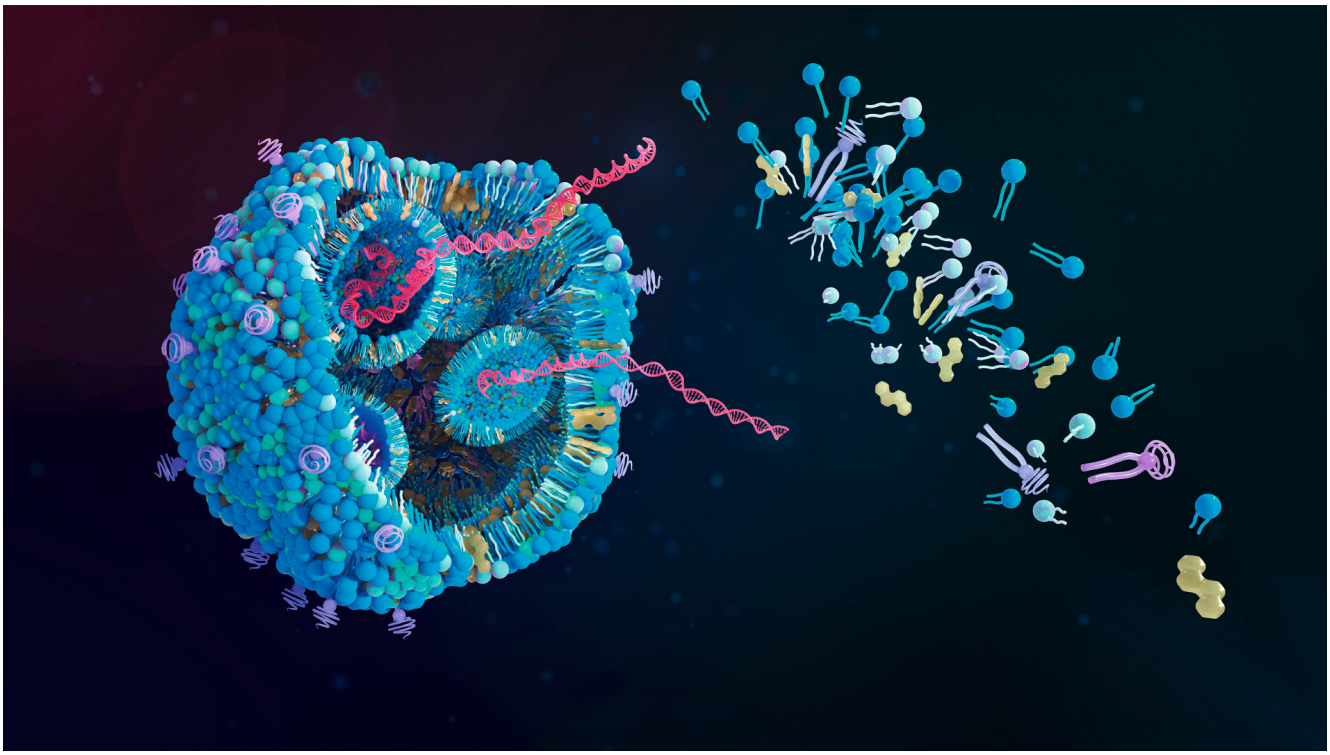
APPLICATION NOTE

Easy encapsulation efficiency determination of mRNA in LNPs via AEX

To enhance intracellular delivery and preserve mRNA integrity against RNase-mediated degradation, therapeutic mRNA can be encapsulated in lipid nanoparticles (LNPs). These LNPs transport the payload directly into the cytoplasm, enabling efficient translation. Particle size, composition, and reproducibility are now tightly controlled. However, defining the critical quality attributes of mRNA and LNPs remains challenging. The encapsulation efficiency (EE) of the mRNA in the LNP is one key element of the quality control. The encapsulation efficiency defines the proportion of mRNA that will eventually be delivered into the cells and is therefore directly linked to the therapeutic potential of the drug product. Current workflows often rely on spectrofluorimetric detection using fluorescent dyes such as RiboGreen (Thermo Fisher Scientific), which bind to mRNA. However, this method has several drawbacks such as it is prone to matrix effects, the binding of the dye to mRNA is highly sensitive to environmental factors or the structure of the LNP can be affected by dilution. While it works well at low mRNA concentrations it becomes more inaccurate at high concentrations because of optical saturation effects. As LNPs exhibit neutral charge, anion exchange chromatography (AEX) offers a targeted strategy to separate free mRNA from unretained drug product. Moreover, AEX provides native conditions, which suit the physicochemical fragility of LNPs.



This Application Note based on the publication and data of the University of Geneva and Sanofi's mRNA Center of Excellence in France presents an AEX method for determining the encapsulation efficiency as a refined and more robust alternative to commonly applied analytical workflows [1].

Table 1: Chromatographic conditions [1].

Column:	YMC Accura BioPro IEX QF (3µm) 100 x 4.6 mm ID (bioinert coated SUS)
Eluents:	A) 25 mM glycine (pH 10.1) B) 25 mM glycine + 1.5 M NaCl (pH 10.1) C) 25 mM glycine + 3 M NaCl + 0.05 % Triton X-100 reduced (pH 11.0)
Gradient:	50 %B (0–2 min), 50–53 %B (2–2.25 min), 53 %B (2.25–4 min), 50–100 %B (4–6 min), 100 %B (6–8 min) Washing step: 100 %B (8.01–12 min) Re-equilibration: 50 %B (12.0–22 min)
Flow rate:	0.2 mL/min
Temperature:	25°C
Injection:	2 µL
Detection:	UV at 230, 260 nm
Sample:	mRNA (in-house) LNP (in-house) Disrupted LNP with 4 % reduced Triton X-100 (T4TE20x)

The principle of EE determination

To determine the EE the sample has to be analysed twice. One time undiluted and under native conditions to determine the concentration of free mRNA. And for the second determination the sample has to be disrupted with a surfactant to evaluate the total mRNA concentration. The EE can be calculated with the following equation, considering the dilution factor of 10 of the second analysis. The peak area can be representatively used for the concentration.

$$EE = 1 - \frac{free\ mRNA}{total\ mRNA} = 1 - \frac{area\ undiluted\ sample}{area\ disrupted\ sample\ \times 10}$$

Figure 1 shows the chromatograms of these two analyses demonstrating how the amount of mRNA is increased for the disrupted LNP (green).

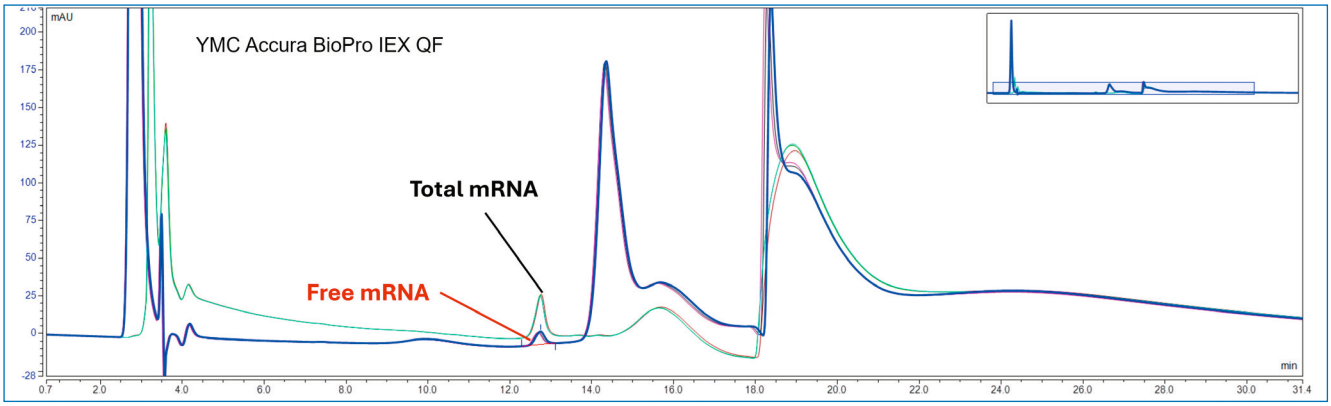


*Figure 1: Analysis of the intact drug product (blue) and the disrupted LNP (green) to determine the amount of free mRNA vs. the total amount of mRNA included in the LNP.**

**Courtesy of University of Geneva, Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO)*

The comparison of both analytical approaches reveals consistent results in many cases, however the RiboGreen method occasionally reports a notably lower EE. Figure 2 illustrates the correlation between the RiboGreen and the AEX results across selected samples. The blue square marks a sample with aligned outcomes, whereas the orange star indicates a data point where both methods diverge significantly.

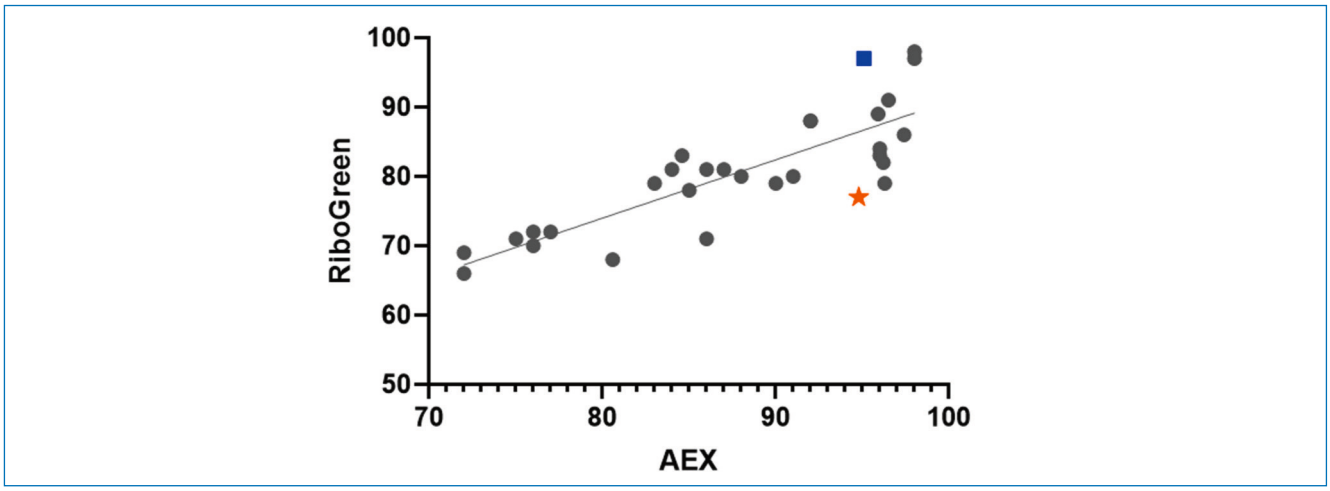


Figure 2: Correlation between EE values obtained with the RiboGreen assay and the AEX method on 30 samples [1].

Figure 3 displays the corresponding chromatograms. The blue sample produces a peak exclusively for free mRNA, while the orange sample additionally reveals a pre-mRNA signal, which is assumed to be some kind of surface-associated mRNA. The data suggest that the RiboGreen dye binds to surface-associated mRNA, which falsely contributes to the free mRNA signal and results in a reduced EE.

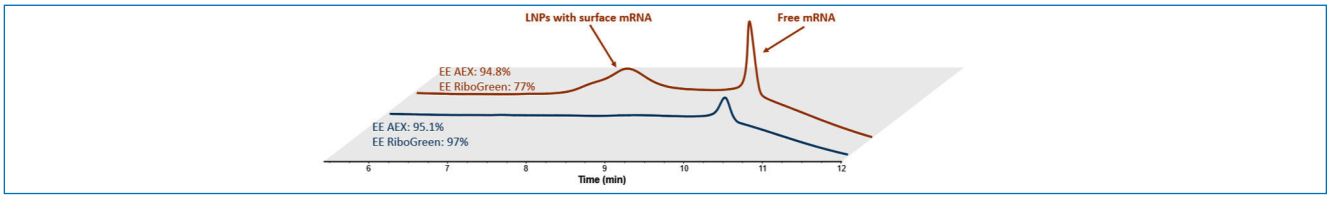


Figure 3: Chromatograms obtained for the samples highlighted in Figure 5, with the corresponding colours [1].

Conclusion

This AEX method compared to commonly used spectrofluorometric approaches provides:

- Fast analyses
- Cost efficiency
- Additional structural insights of the LNP

Making it a viable alternative for determining the encapsulation efficiency.

References

[1] Athanasios Tsalmpouris, Sofiane Mahjoubi, Camille Malburet, Chamsan Daher-Hassan, Marc François-Heude, Jean-François Cotte, Davy Guillarme, and Jonathan Maurer Analytical Chemistry 2025 97 (35), 19275-19282;
DOI: 10.1021/acs.analchem.5c03299