Analysis of RNA nucleotides using the Bruker Fourier 80 benchtop NMR

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Introduction

Interest in the use of mRNA as a biotherapeutic has increased significantly since the success of SARS-CoV 2 mRNA vaccines. mRNA biologics are typically generated by in-vitro transcription (IVT) using RNA nucleotides, alongside a cocktail of reagents, including the linearised DNA plasmid of interest, an RNA polymerase and a 5′ capping analogue. This enzymatic reaction is then optimised for the target mRNA's sequence and length.

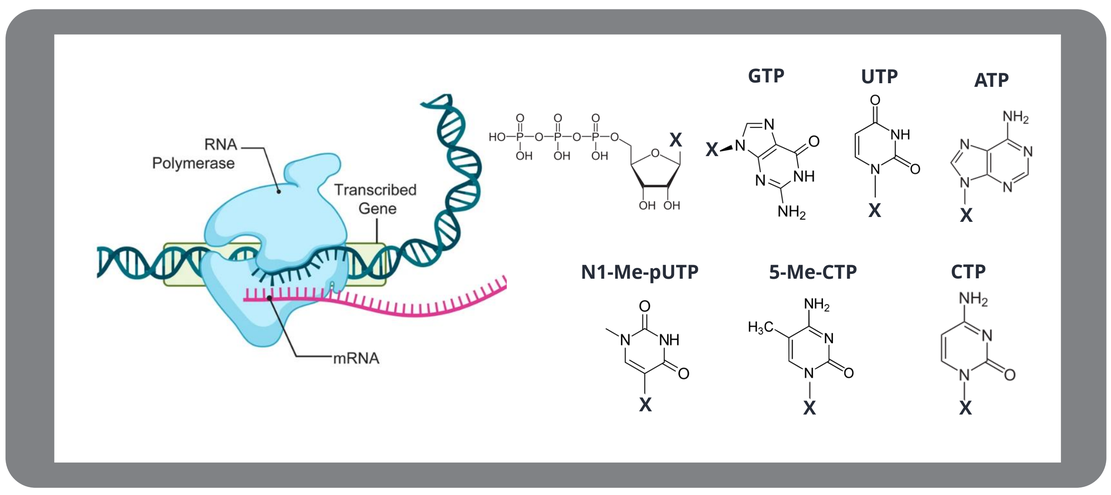

Figure 1: *Left – A schematic of mRNA IVT synthesis [1] / Right – The structure of the four RNA nucleotides and two modified RNA nucleotides*

RNA nucleotides are composed of a base, ribose sugar and triphosphate group. There are four RNA bases; specifically, i) adenine, ii) cytosine, iii) uracil and iv) guanine. These bases can also undergo modifications, such as 5-methyl-cytosine and N1-methyl-pseudo-UTP, which can increase the stability and expression of the mRNA, whilst decreasing its immunogenicity. mRNA synthesis also requires a 5′ capping agent, such as Clean Cap AG (TriLink Biotechnologies, UK), which serves as a trinucleotide primer to allow efficient translation.

Fourier 80 benchtop NMR

The Bruker Fourier 80 is a low cost, non-cryogen, benchtop NMR with an 80 MHz proton (^{1}H) base frequency using a 1.88 Tesla magnet that uses standard 5 mm o.d. NMR tubes [2].

Figure 2: *Left – Bruker Fourier 80 benchtop NMR / Right – 5mm o.d. NMR tube [2]*

Results

The NMR spectra for six RNA nucleotides and a capping agent previously described, plus a mixture of these nucleotides, were obtained on the Bruker Fourier 80 at 100 mM concentration.

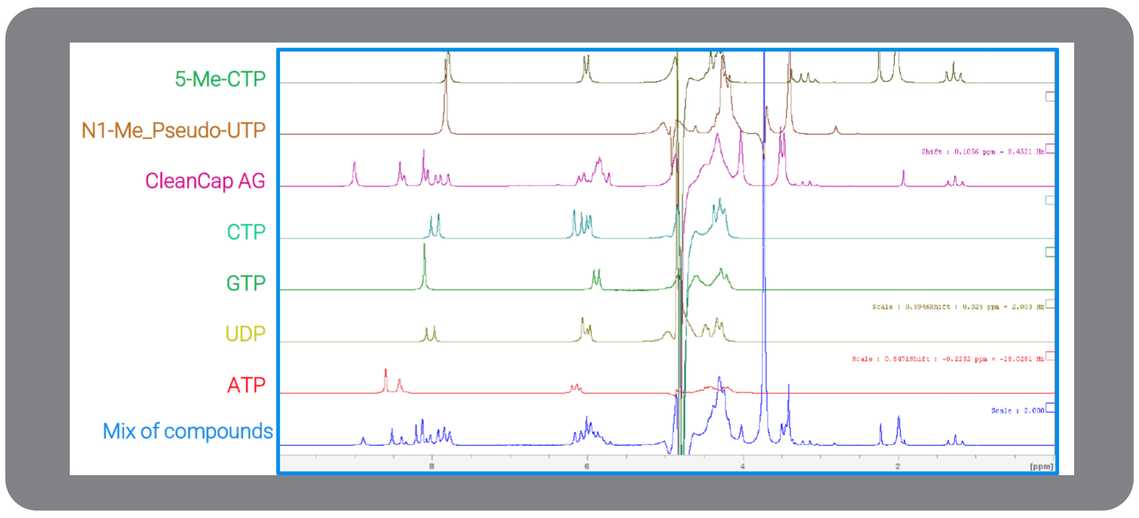

Figure 3: *NMR spectra of the eight RNA samples acquired on the Bruker Fourier 80*

The NMR spectra results show the Bruker Fourier 80 system can successfully resolve specific NMR peaks for the six RNA nucleotides, RNA cap and mixture of RNA nucleotides. This demonstrates its use in quality control (QC) for raw material compendial testing, identification and quantification using Bruker's Advanced chemical profiling (ACP) software [3].

Summary

Quality control (QC) of raw materials is critical in the GMP manufacture of biomolecules such as mRNA. Raw materials are commonly tested using chromatographic or infrared-based methods, which either require identical standards or lack quantitation capabilities. This communication demonstrates that NMR spectroscopy, using the Bruker Fourier 80, is a viable alternative method. The method shown leverages the intrinsic quantitative properties of NMR for simplified testing. With full GMP support and powerful automation capabilities the Fourier 80 is well-suited for a QC lab environment with the additional benefits of it compact size and low maintenance requirements.

References

1) Zhang, F., Wang, Y., Wang, X. *et al.* RT-IVT method allows multiplex real-time quantification of in vitro transcriptional mRNA production. *Commun Biol* 6, 453 (2023). https://doi.org/10.1038/s42003-023-04830-1

2) Bruker Fourier 80 Benchtop NMR, https://www.bruker.com/en/products-and-solutions/mr/nmr/fourier80.html

3) Bruker Advanced Chemical Profiling software, https://www.bruker.com/en/products-and-solutions/mr/nmr-epr-td-nmr-industrial-solutions/advanced-chemical-profiling.html