Quality and consistency of TheraPure GMP N1-methylpseudouridine triphosphate

Introduction

At Thermo Fisher Scientific, we have proven our ability to help our partners navigate obstacles on their paths to developing and commercially manufacturing mRNA therapeutics or vaccines. With the help of the Thermo Scientific™ TheraPure™ GMP* product portfolio of enzymes and nucleotides, you can expect quality, experience, and reliability on your journey to bring transformative therapeutics to the world.

When developing an mRNA therapeutic or vaccine, using materials suitable for good manufacturing practice (GMP) production is a necessity. Many suppliers of raw materials for mRNA production promote that their materials are "GMP grade" and suitable for use in manufacturing mRNA therapeutics and vaccines. However, there is no real definition of the product quality attributes for "GMP grade", so a wide range of quality attributes are found in products marketed as "GMP grade". This range of quality attributes leads to a great deal of confusion and misunderstanding about exactly what "GMP grade" materials are.

When choosing raw materials for mRNA production, many process development scientists often focus on a few familiar product quality attributes such as analytical quality, use of animal origin–free (AOF) materials, and even cost. However, there are many other product quality attributes that are just as important to consider. Many of these attributes can have a significant effect on not just regulatory approval of a drug but also the development timeline and commercial manufacture of a drug.

Presented here is an analysis of some of the key product quality attributes of a modified nucleotide product, and their impact on the development and manufacturing of a therapeutic. This study not only demonstrates the analytical quality of the product but also addresses many of the other key product quality attributes of a material that help make it suitable for current good manufacturing practice (cGMP) production of an mRNA therapeutic or vaccine.

* "TheraPure GMP" refers to the quality level of the raw, ancillary, or starting materials to be used for further manufacturing. TheraPure GMP products are manufactured in facilities with ISO 9001–certified quality management systems operating in accordance with relevant good manufacturing practice (GMP) principles as outlined in ICH Q7 or equivalent guidance documents or standards.

N1-methylpseudouridine triphosphate

One of the key building blocks of mRNA therapeutics and vaccines is N1-methylpseudouridine triphosphate (N1-Me-pUTP). This nucleotide is a modification of the naturally occurring nucleoside pseudouridine and is a substitute for uridine (U) in RNA therapeutics and vaccines. Using this modified nucleotide in an RNA therapeutic can result in increased protein expression and lower immunogenicity relative to unmodified mRNA [1].

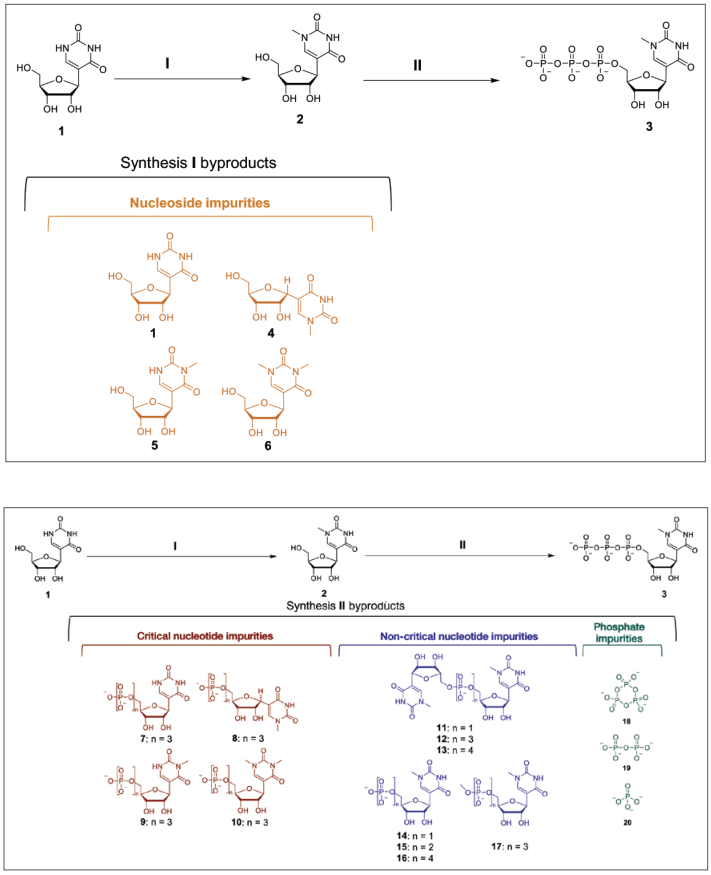
Controlling impurities in N1-methylpseudouridine triphosphate

We performed an in-depth analysis of the N1-Me-pUTP synthesis process to determine the potential impurities that could be generated and assess their potential impact on a drug synthesized from this material (Figure 1). Understanding these impurities and their origin affords a high level of control on the quality of the N1-Me-pUTP produced.

As a result of the analysis, several undesirable critical impurities were identified. These impurities may interfere in mRNA synthesis or cause undesired RNA residues to be incorporated into the mRNA sequence of the therapeutic, which could potentially affect its structure and functionality. Ideally, these critical impurities would not be found in the N1-Me-pUTP product. Consequently, a proprietary synthetic process designed to minimize the generation of these critical impurities was developed and validated.

Various nuclear magnetic resonance (NMR) techniques were used alongside high performance liquid chromatography–mass spectrometry (HPLC–MS) to examine the range of impurities discovered in the synthesis processes (Figures 2 and 3). Specific impurities were isolated or synthesized for their identification in the final product.

Analysis of the N1-Me-pUTP product generated by the chosen synthetic method indicated the method could produce N1-Me-pUTP. When the impurity profile of the N1-Me-pUTP product was analyzed, no critical impurities were found in the manufactured N1-Me-pUTP product. Several low-level, noncritical impurities were identified.



Compound	Description
1	Pseudouridine
2	N1-methylpseudouridine
3	N1-methylpseudouridine triphosphate
4	N1-methyl-alpha-pseudouridine
5	N3-methylpseudouridine
6	N1,N3-dimethylpseudouridine

Compound	Description
7	Pseudouridine triphosphate
8	N1-methyl-alpha-pseudouridine triphosphate
9	N3-methylpseudouridine triphosphate
10	N1,N3-dimethylpseudouridine triphosphate
11	Bis (N1-methylpseudouridine) monophosphate
12	Bis (N1-methylpseudouridine) triphosphate
13	Bis (N1-methylpseudouridine) tetraphosphate
14	N1-methylpseudouridine monophosphate
15	N1-methylpseudouridine diphosphate
16	N1-methylpseudouridine tetraphosphate
17	N1-methylpseudouridine methylated triphosphate
18	Cyclic triphosphate
19	Pyrophosphate
20	Orthophosphate

Figure 1. Synthesis scheme of N1-Me-pUTP and identification of potential impurities. Synthesis byproducts are classified as nucleoside impurities, critical and noncritical nucleotide impurities, and phosphate impurities. Nucleotide impurities are classified as critical or noncritical based on their ability to interfere with the activity or function of N1-Me-pUTP and its incorporation into an RNA therapeutic or vaccine.

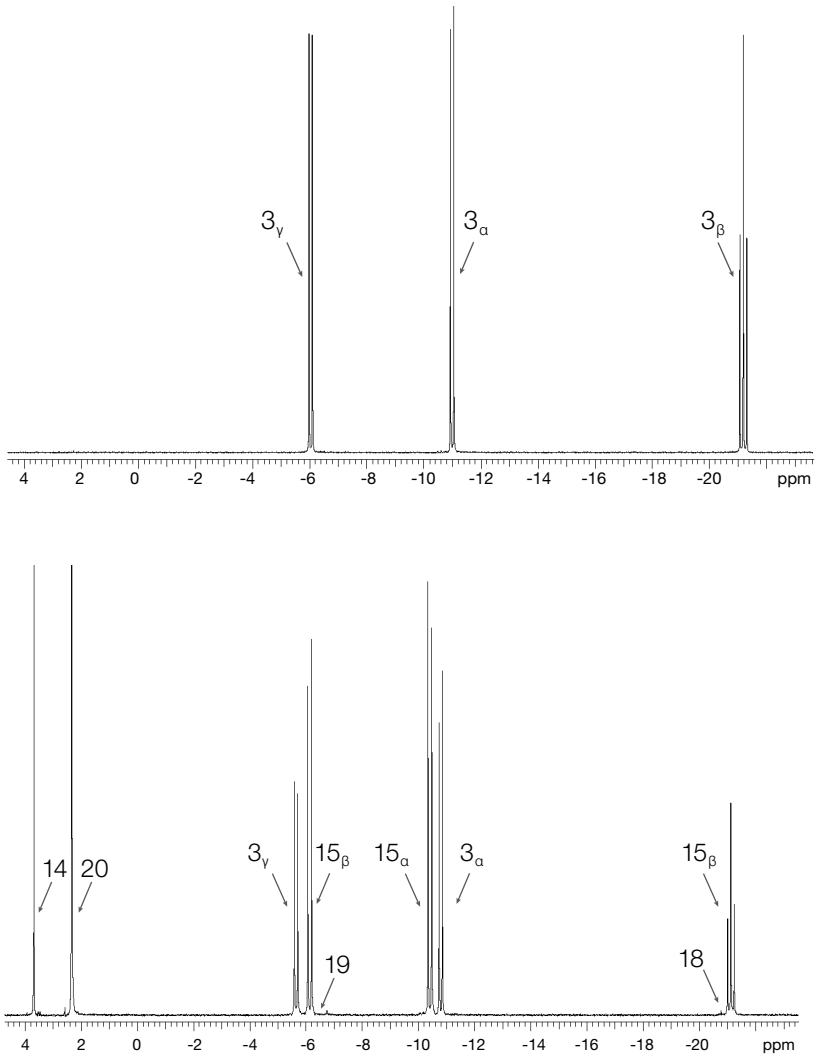


Figure 2. ^{31}P-NMR spectra of N1-Me-pUTP and phosphate impurities. The ^{31}P-NMR spectrum of N1-Me-pUTP product (3) is overlaid on the ^{31}P-NMR spectrum of a mixture of N1-Me-pUTP (3), N1-Me-pUMP (14), N1-Me-pUDP (15), cyclic triphosphate (18), pyrophosphate (19), and orthophosphate (20). Each unique ^{31}P signal is labeled.

Lot-to-lot consistency of N1-methylpseudouridine triphosphate

Another key product quality attribute of a material used for cGMP manufacture of an RNA therapeutic or vaccine is lot-to-lot consistency. This helps minimize the amount of testing on incoming material and can significantly reduce the potential for production delays caused by inconsistencies in production materials. One of the most effective ways to control the consistency of a product is to validate the manufacturing processes and analytical methods used to test it.

Figure 3 is an HPLC analysis of 4 lots of TheraPure GMP N1-Me-pUTP. The product is extremely consistent and exhibits minimal lot-to-lot variability. These data help illustrate the impact of using validated processes when manufacturing products.

In contrast, Figure 4 is an example of a nucleotide component that was not manufactured with a validated process. Two lots of material were manufactured and tested, and both were able to pass the relevant quality control metrics for release by the supplier. But, without a validated process, these materials exhibited differences in their impurity profiles. This simple difference can cause significant issues in the cGMP manufacture of a therapeutic or can result in development or production delays, as the lack of consistency needs to be resolved.

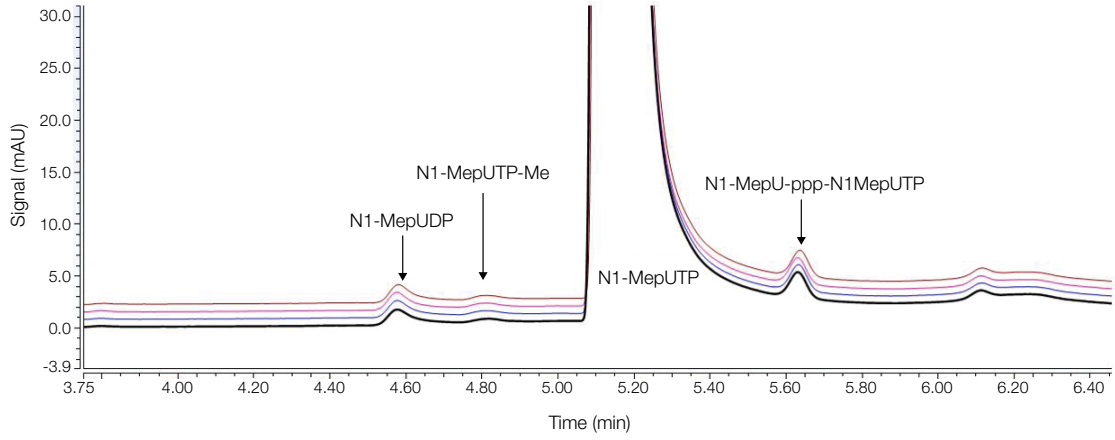


Figure 3. HPLC analysis of multiple lots of N1-Me-pUTP. Four lots of N1-Me-pUTP were analyzed and compared. Consistency of material from lot to lot was very good with little variation. All of the impurities identified here are noncritical impurities; they will not interfere with the function of N1-Me-pUTP and cannot be incorporated into an mRNA molecule.

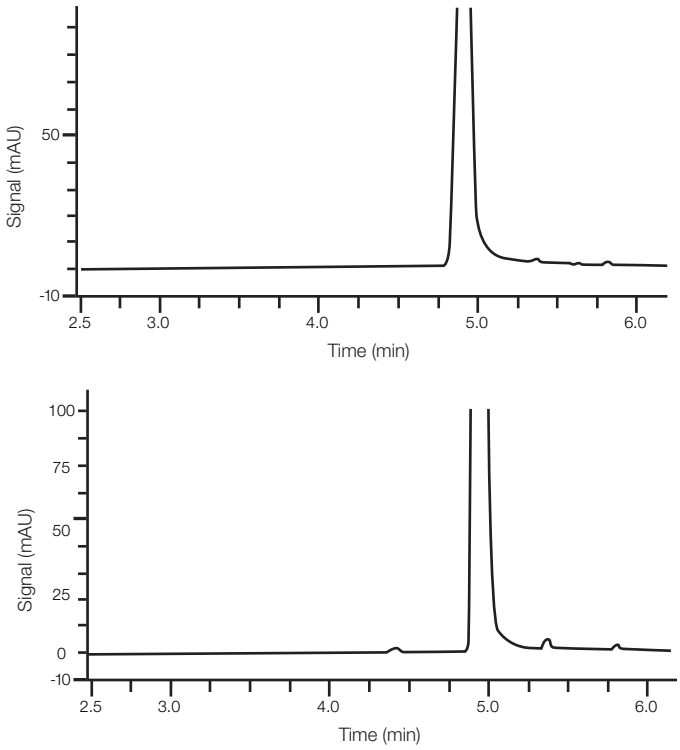


Figure 4. LC analysis of a nucleotide component. Two lots of a nucleotide component material manufactured with a nonvalidated process were analyzed by HPLC. Different impurities at different levels were found in these products.

Conclusions

Here we have demonstrated that our synthetic process for producing N1-Me-pUTP meets several of the stringent product quality attributes required for materials that will be used in cGMP manufacture of RNA therapeutics and vaccines.

We identified the source and formation of key upstream impurities in N1-Me-pUTP and employed controls for raw materials and synthesis processes to ensure minimization of impurities in the final product.

In addition, the validated manufacturing process used to produce N1-Me-pUTP has been demonstrated by HPLC analysis to be consistent and exhibit very little lot-to-lot variability.

TheraPure GMP product quality

TheraPure GMP products are manufactured to some of the most stringent quality and quality documentation requirements of any products on the market used for development and manufacture of mRNA therapeutics and vaccines (Table 1).

Table 1. Manufacturing quality and documentation requirements for TheraPure GMP products.

Product or process requirement
Manufacture follows relevant ICH Q7 GMP principles
AOF manufacturing processes and raw materials
Validated manufacturing processes and analytical methods
Product-specific stability data
Impurity profile
Verified compendial test methods, where applicable
Manufactured in β-lactam–free facilities
Drug Master File

Thermo Fisher Scientific has decades of experience in chemical synthesis, enabling industry-leading development, manufacturing, and analysis of key components for the production of RNA therapeutics and vaccines. We are dedicated to providing nucleic acid products to our customers, enabling them to make the world healthier through nucleic acid therapeutics for the treatment and prevention of diseases.

Ordering information for TheraPure GMP products

Product			Quantity	Cat. No.
Nucleotides	ATP, sodium solution	100 mM	3.5 mL	R044SKB001
			17 mL	R044SKB002
			50 mL	R0441SKB003
			500 mL	R0441SKB009
	CTP, sodium solution	100 mM	3.5 mL	R045SKB001
			17 mL	R045SKB002
			50 mL	R0451SKB003
			500 mL	R0451SKB009
	GTP, sodium solution	100 mM	3.5 mL	R046SKB001
			20.5 mL	R046SKB002
			50 mL	R0461SKB003
			500 mL	R0461SKB009
	UTP, sodium solution	100 mM	3.5 mL	R047SKB001
			20 mL	R0471SKB006
			100 mL	R0471SKB002
			500 mL	R0471SKB003
Modified nucleotides	N1-Me-pUTP, sodium solution	100 mM	900 mL	R0491SKB014
			500 mL	R0491SKB007
			225 mL	R0491SKB013
			100 mL	R0491SKB012
			50 mL	R0491SKB015
			20 mL	R0491SKB009
			5 mL	R0491SKB007

Reference

1. Cohen J (2021) What went wrong with CureVac's mRNA vaccine? *Science* 372:1381. doi: 10.1126/science.372.6549.1381.

Learn more at **thermofisher.com/therapure**