ProtaGene
Analytical Excellence

Innovation Spotlight

mRNA-based Therapeutic Products: CMC Solutions for Enabling Development

Characterization and Quantitation of Product Quality Attributes by LC-MS

Innovation Spotlight

mRNA vaccine products have quickly advanced into the marketplace, and this type of new therapeutic molecule demands introduction of analytical methods capable of providing detailed understanding of the polynucleotide and determination of potential critical quality attributes (CQAs). Classical molecular biology tools such as polymerase chain reaction (PCR) provide a convenient way to analyze the gene sequence but cannot provide information on modifications, nonnative bases, degradants and product-related impurities that may impact therapeutic effectiveness of the mRNA product.

Overview

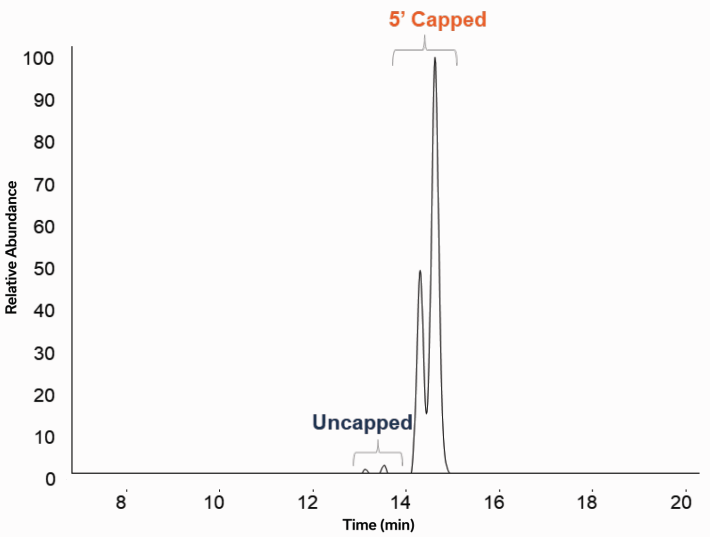
To assess consistency and achieve better understanding of mRNA products, we have developed a set of mass spectrometry (MS)-based methods to quantify the level of 5′ capping (%Cap) and to establish a quantitative PolyA tail profile. In addition, as modified bases are being used and molecular biology tools require several manipulations, including reverse transcriptase to convert the RNA strand to DNA prior to analysis, a direct approach to confirming the primary sequence of the mRNA and circular RNA molecule, including base modifications, using LC-MS was developed, creating an approach that parallels the standard approach to characterizing protein therapeutics.

Detailed Characterization Solution

- 5′ cap: Determination of %Cap by UV and MS characterization of capped and uncapped species.
- PolyA tail profiling: MS determination of PolyA tail lengths and relative proportion of individual species as well as evaluation of short (~10-40) vs long (~120-140) tail species.
- Nucleotide: LC-UV-MS approach provides >90% sequence coverage.

Determination of 5′ Capping: Quantitative Monitoring of a CQA

Figure 1.



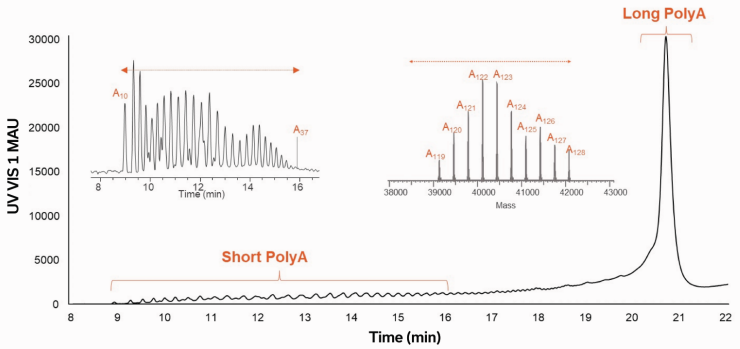
- Capping of mRNA is essential for the viability and effective translation of the therapeutic gene *in vivo*, and it is important to consistently generate drug substance (DS) with high %Cap.
- LC-MS is used to quantify the relative levels of mRNA that has been capped vs remains uncapped (Figure 1).
- Capping is a multi-step process and results in a few variants, which are detected by the high-resolution LC-MS method.

Capped	Uncapped
96.7%	3.3%

mRNA PolyA Tail Profiling: Detailed Characterization by LC-MS for Enabling Consistent Product Quality

- PolyA tail length influences translation of the mRNA to express the therapeutic protein and is an important attribute of the DS.
- Long PolyA species are productive, whereas short PolyA are quickly degraded. The PolyA tail length can be characterized and quantified through LC-MS methods (Figure 2).

Figure 2.

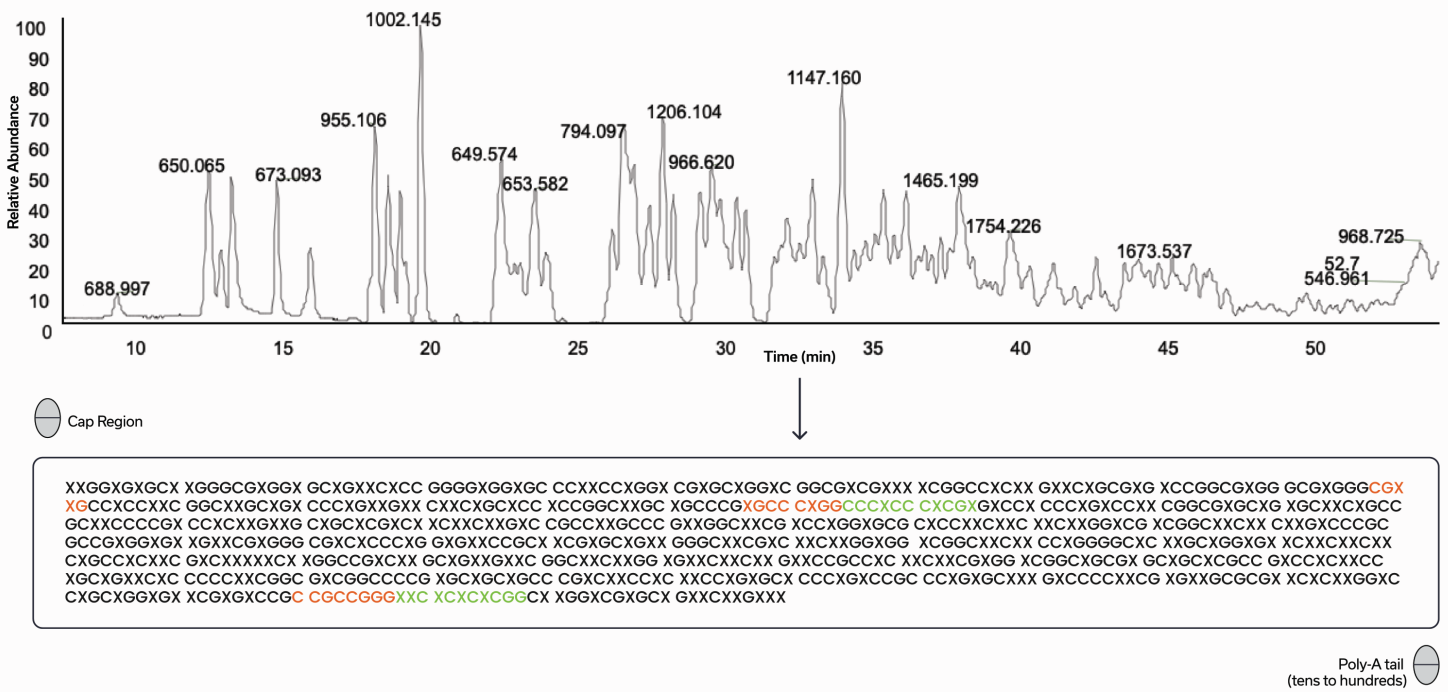


PolyA Tail	Short	Long
Relative Amount	13%	87%
PolyA Lengths Detected	10-37	119-128

mRNA Sequencing: Unambiguous identification of the sequence and modification/impurities

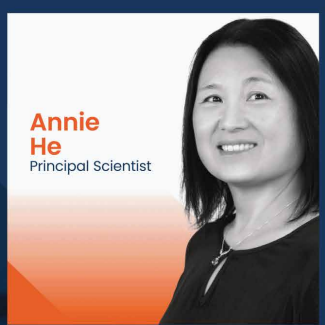
- LC-MS method was developed as a direct approach to confirming the primary sequence of RNA molecules without converting it to cDNA.
- Greater than 90% sequence coverage was achieved for one enzyme map (Figure 3).

Figure 3.



Meet the Innovator

"ProtaGene offers unique methods for deep characterization of mRNA 5' cap, 3' tail length, and sequence analysis by advanced LC-MS. The advantages of the LC-MS approaches are that the mRNA sequence can be confirmed directly without converting it to cDNA, and the cap and tail can be unambiguously identified by MS and levels quantitated by LC-UV, which make these approaches ideal choices for mRNA development process and reduce costs in the long run."



ProtaGene
Analytical Excellence

North America
4 Burlington Woods Drive
Burlington, MA 01803

Europe
Inselwiesenstraße 10
74076 Heilbronn Germany

ProtaGene.com
info@protagene.com

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