307379: Rapid Analysis of Synthetic mRNA Cap Structure using Ion Pairing Reversed Phase Liquid Chromatography coupled to Mass Spectrometry

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Introduction

The 5' cap of mRNA is vital to characterize in vaccine development. Ion-pairing reversed phase liquid-chromatography mass spectrometry (LC-MS) can be used to analyze pre-defined 5' fragments of synthetic mRNA. However, accurate quantitation can be difficult to achieve due to non-specific binding with the system. Moreover, the choice of ion-pairing system can heavily influence the quality and sensitivity of the mass spectra. Here, we developed a rapid and sensitive LC-MS method applicable to synthetic mRNA capping using an inert chromatographic surface in combination with a compliance-ready UHPLC-MS system. Analysis of 5' capping of IVT mRNA preparations could be achieved in less than 5-minutes. Linearity was demonstrated to detecting product-related impurities down to less than 0.1% of the target 5' capped fragment.

Experimental

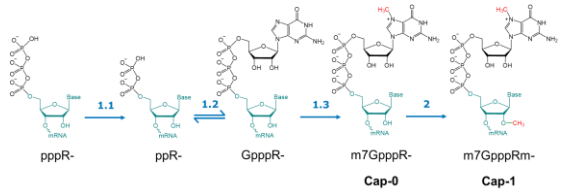

Figure 1. Enzymatic capping structures of RNA.

Synthetic RNA oligonucleotides containing the same sequence but different 5' groups (Cap-1, pppG-, pp-, GpppG, and Cap-0) were evaluated. An RNase H probe was included to mimic products of targeted RNA fragmentation. An equimolar solution of Cap-1 and probe at 2.5 pmol/µL concentrations and 1:10, 1:100, and 1:1000 dilutions of the four precursor oligonucleotides versus Cap-1 at a constant 2.5 pmol/µL concentration were prepared in water. Analyses were performed using a UHPLC system with UV detection and a quadrupole time-of-flight mass spectrometer. Columns employed were 2.1 x 50 mm columns in stainless steel or hybrid surface hardware and packed with a 130 Å, 1.7 µm hybrid silica C18 stationary phase. Diisopropylethylamine and HFIP were used as mobile phase conditions.

Results

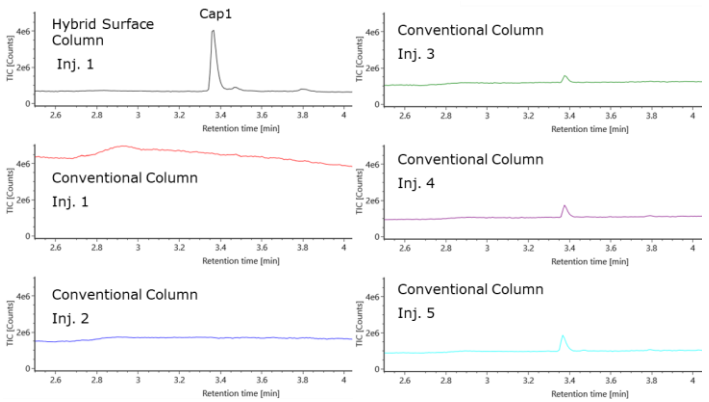

Figure 2. UV chromatograms of the first injection of Cap-1 fragment obtained using hybrid surface column and from the first to fifth injections of a conventional column

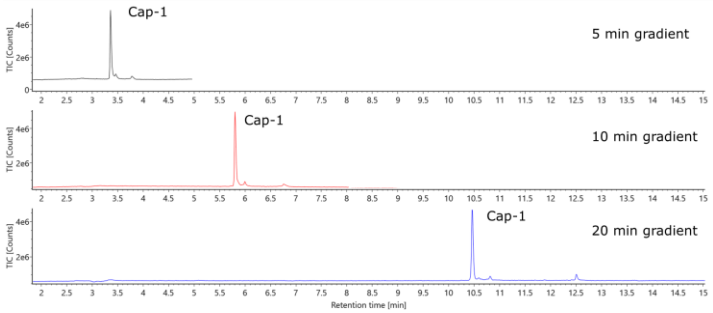

Figure 3. TIC chromatograms of Cap-1 fragment obtained using a gradient from 5–25% B in 5, 10, or 20 min.

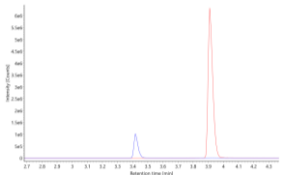

Figure 4. Overlaid XICs of Cap-1 and a representative probe from an equimolar solution.

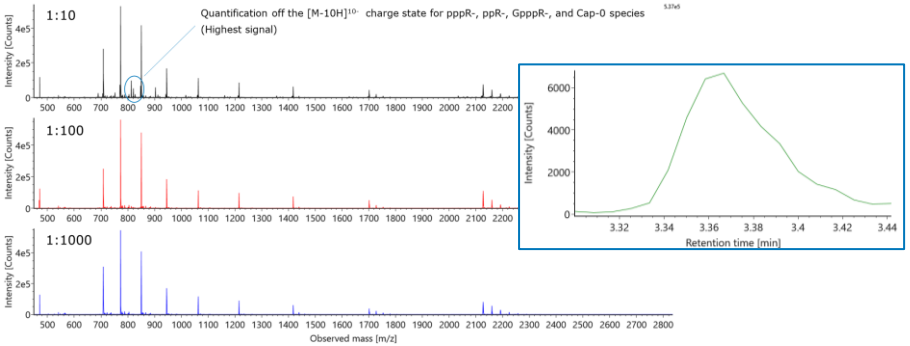

Figure 5. Mass spectra of Cap-1 fragment and its product related impurity fragments (pppG, ppG, GpppG, and m7GpppG, or Cap-0). The inset is an XIC as obtained from the 1:1000 dilution using $[M-10H]^{10-}$ charge state of the Cap-0 fragment.

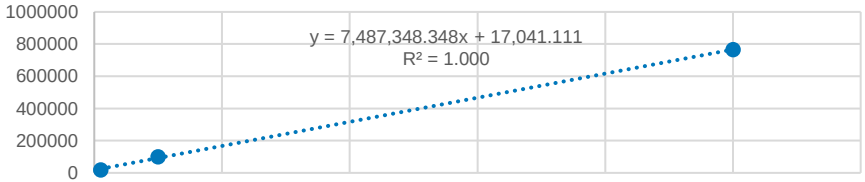

Figure 6. Calibration curves generated from a 1:10, 1:100, and 1:1000 dilution series of Cap-1 at 12.5 pmol/µL versus its product-related impurity fragments Cap-0.

Conclusion(s)

Marked improvements in recovery can be achieved when analyzing oligonucleotides with a specialized, hybrid surface column versus typical metal-based hardware. With this hybrid surface column, we demonstrate that a rapid LC-MS method applicable to evaluating the extent of 5' capping of synthetic mRNA, an important CQA for synthetic mRNAs, could be achieved in less than 5 minutes and resolved from a synthetic RNAse H probe when coupled with a low dispersion UHPLC and highly sensitive quadrupole time-of-flight mass spectrometer. Accurate quantitation, even at low limits of detection, is possible and could be applied to validate the manufacturing of Cap-1 mRNA, and potential presence of product related impurities. These precursor impurities could be detected down to 0.1% of Cap-1. In all, these results highlight the potential of MS-based quantitation for high throughput assays, which could help accelerate the development of mRNA modalities.