

mRNA Cap Purity Analysis on a Biozen™ Oligo Column

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Overview

In order to preserve mRNA from 5' nuclease degradation, a variety of CAPPING molecules have been created to preserve the biological stability of the precious mRNA sequence. The mRNA sequence can be administered to a host through multiple formats including liquid nanoparticles (LNPs) and adeno-associated viruses (AAVs). This emerging modality was highlighted during the COVID-19 vaccine development. Analytical characterization of the diversity and integrity of the 5' CAPPING agents is essential for molecule stability and administration safety. In this application note, mRNA oligonucleotide strands of 1250 nucleotides was enzymatically digested with Rnase H and injected into a Biozen 2.6 µm Oligo column. We present a Liquid Chromatography (LC) approach paired with high resolution mass spectrometry (HRMS) to distinguish a pool of multiple caps and their relative integrity.

LC Conditions

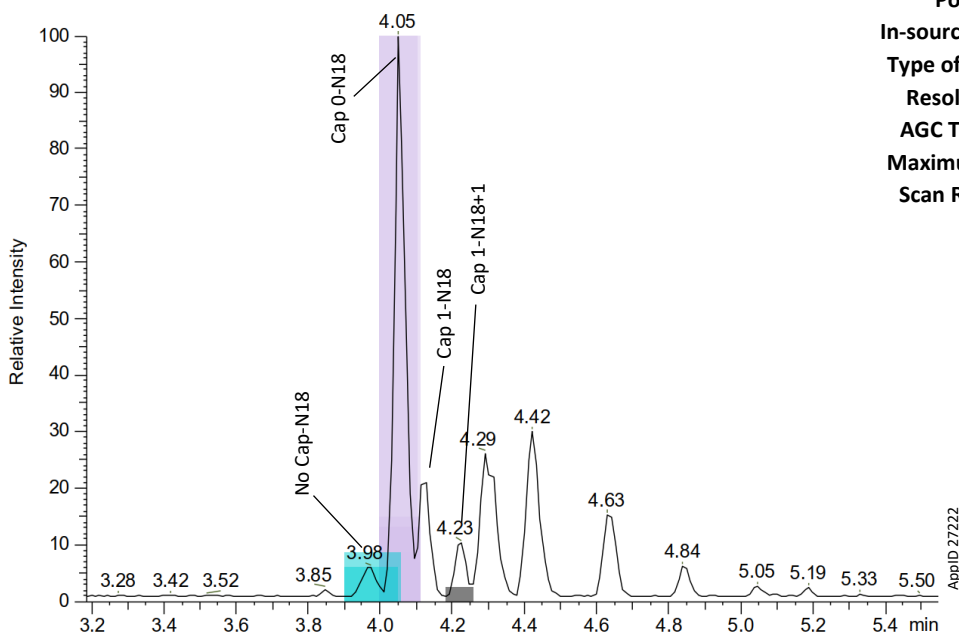
- Columns:** Biozen 2.6 µm Oligo
- Dimensions:** 50 x 2.1 mm
- Part No.:** [00B-4790-AN](#)
- Mobile Phase:** A: 35 mM Hexafluoro-2-propanol + 15 mM N,N-Diisopropylethylamine, in Water
B: 35 mM Hexafluoro-2-propanol + 15 mM N,N-Diisopropylethylamine, in Methanol / Water (90:10, v/v)
- Gradient:**

Time (min)	%B
0	3
20	35
20.5	80
25	80
25.5	3
30	3
- Flow Rate:** 0.3 mL/min
- Injection Volume:** 5 µL
- Temperature:** 70 °C
- Instrument:** Vanquish™ UHPLC
- Detection:** HRMS
- Detector:** Q Exactive™ Plus

HRMS Conditions

- Source:** HESI-II
- Polarity:** Negative
- In-source CID:** 3 eV
- Type of Scan:** MS
- Resolution:** 140,000
- AGC Target:** 3e6 ms
- Maximum IT:** 100 ms
- Scan Range:** 600 to 6000 m/z

Figure 1. mRNA Cap Purity Analysis.



Type	Delta (ppm)	% Area
No Cap	3.27	9.02
Cap 0	-0.75	79.86
Cap 1	-3.45	9.65
Cap 1,n+1	-1.04	1.47



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