



mRNA Poly A Tail Degradation Detection on a Biozen™ Oligo Column

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Overview

The poly A tail of a messenger RNA (mRNA) is a repeat sequence of adenosine (A) that is an emerging popular modality among vaccine and gene therapy developers. Grown at the 3' end, the increasing length of this tail provides additional stability to the "core" sequence that invokes the generation of a protein and subsequent immune response from the host. Given the inherent RNA stability challenges as well as current delivery challenges, the ability to understand the degradation of the poly A tail is an essential tool to improving delivery of therapeutics. In this application note, mRNA oligonucleotides strands of 1250 nucleotides were enzymatically digested using RNase H followed by injection into a Biozen 2.6 µm Oligo column. We demonstrate the ability to distinguish a naturally degraded poly A tail with single nucleotide resolution through Liquid Chromatography (LC) and High-Resolution Mass Spectrometry (HRMS).

LC Conditions

Columns: Biozen 2.6 µm Oligo

Dimensions: 50 x 2.1 mm

Part No.: [OOB-4790-AN](#)

Mobile Phase: A: 95 mM Hexafluoro-2-propanol, 5.7 mM N,N-Diisopropylethylamine in Water

B: 7.1 mM Hexafluoro-2-propanol, 2.1 mM N,N-Diisopropylethylamine in 80 % Methanol

Gradient:	Time (min)	%B
	0	3
	45	35
	45.6	80
	50	80
	50.5	3
	60	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

Scan Type: Full MS

Polarity: Negative

Resolution: 140,000

AGC Target: 3e6 ms

Maximum IT: 200 ms

Scan Range: 450 to 3500 m/z

Scan Type: MS2

Resolution: 35,000

AGC Target: 1e5 ms

Maximum IT: 50 ms

Source: HESI

Temperature: 320 °C

Sheath Gas: 30 psi

Aux Gas: 8 psi

Spray Voltage: 3000 V

S-lens RF Level: 50

Aux Gas Temperature: 30 °C



Figure 1. Extracted Ion Chromatograms of Single Nucleotide Resolution for Poly A Tail <5 ppm.

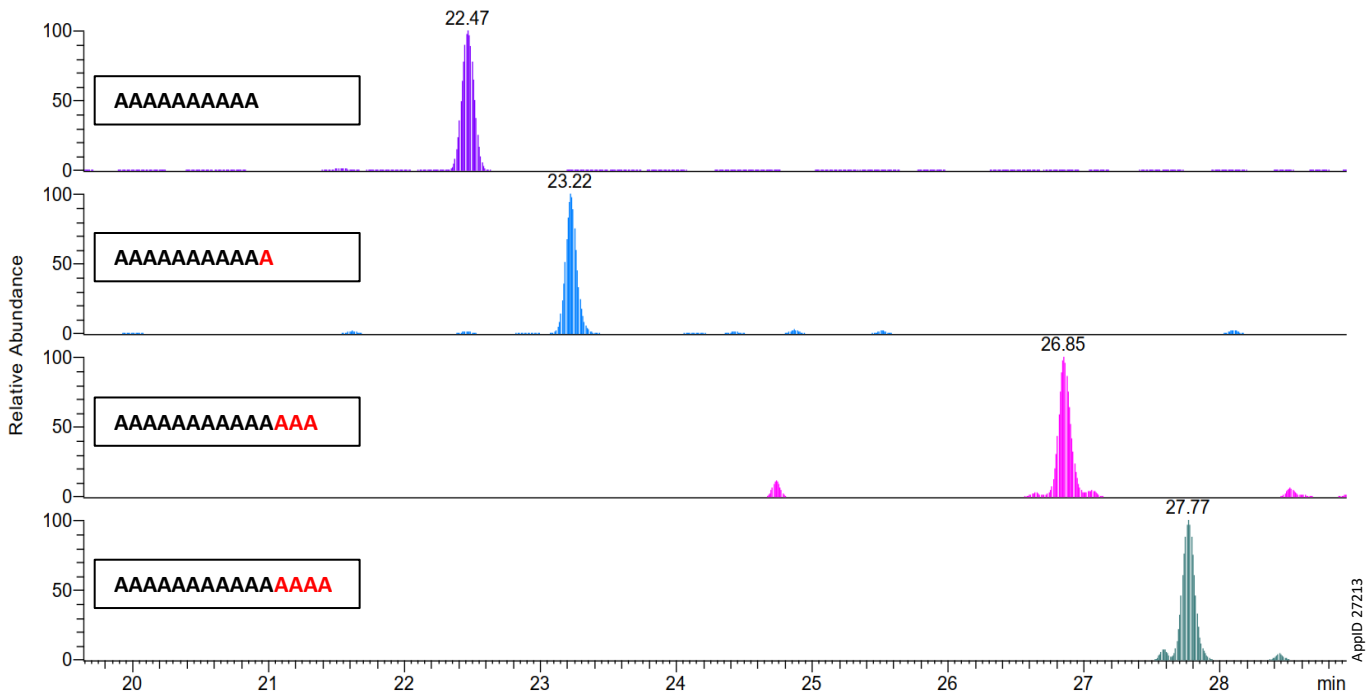
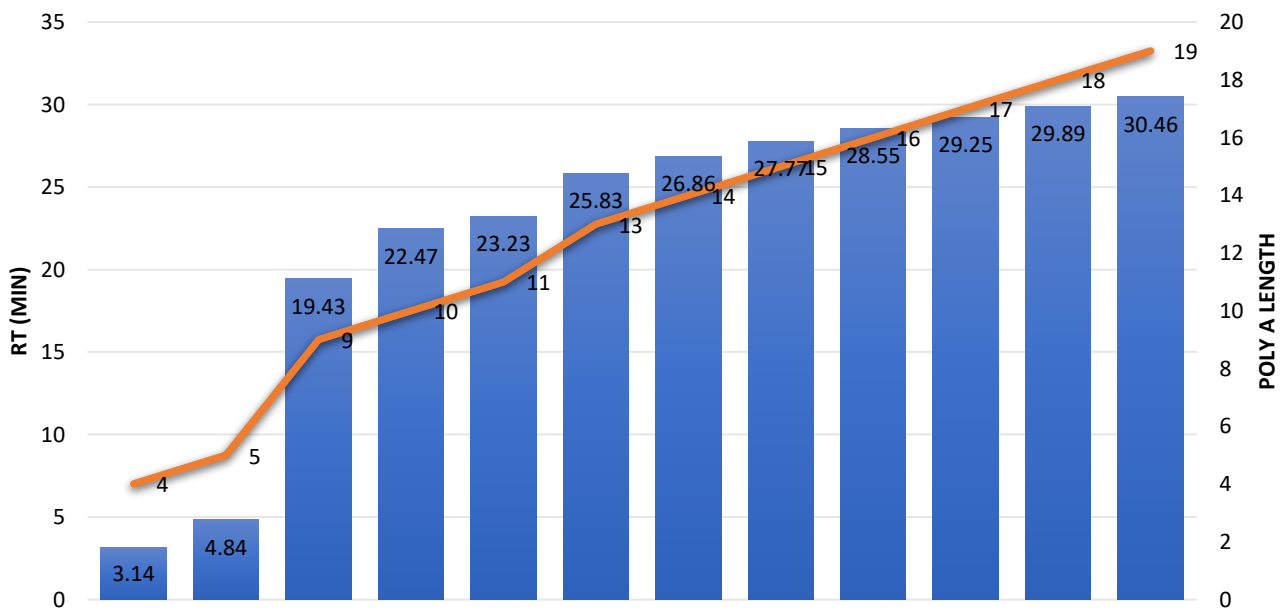


Figure 2. mRNA Poly A Tail Length with Respect to Retention Time.



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