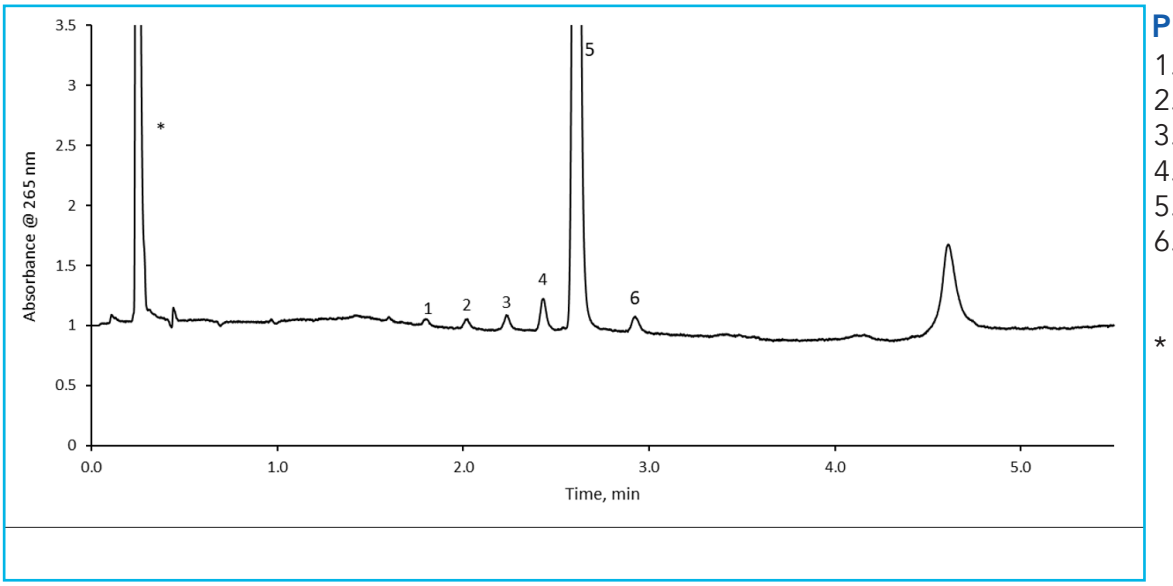
HALO®

BIOPHARMACEUTICALS

Impurity Analysis of Poly dT Oligonucleotides

389



PEAK IDENTITIES

1. n-4 (12 mer)
2. n-3 (13 mer)
3. n-2 (14 mer)
4. n-1 (15 mer)
5. 16 mer
6. 16 mer + Protecting Group

* Tris HCl

TEST CONDITIONS:

Column: HALO 120 Å OLIGO C18, 2.7 µm, 2.1 x 50 mm
Part Number: P2A62-402
Mobile Phase A: 5mM TEA/50mM HFIP @ pH 8.3
Mobile Phase B: 25/75 ACN/MeOH
Gradient:

Time	%B
0.0	5
0.5	8
3.5	13
5.0	20
5.4	20
5.5	5
9.0	5

Flow Rate: 0.4 mL/min
Back Pressure: 219 bar
Temperature: 60 °C
Injection: 1µL Primer (8µg/mL)
Sample Solvent: 10mM Tris HCl/ 1mM EDTA
Wavelength: PDA, 265 nm
Flow Cell: 1 µL
Data Rate: 40 Hz
Response Time: 0.05 sec.
LC System: Shimadzu Nexera X2

MS CONDITIONS:

Polarity: Negative mode
Sheath Gas Flow: 25
Auz Gas Flow: 10
Sweep Gas: 1
Spray Voltage: 2.5kV
Capillary Temperature: 325 °C
Aux Gas Heater Temp: 300 °C
S-Lens RF Level: 60
m/z Scan Range: 450-2000
Resolution: 120,000 MS1

A 16 mer ssDNA poly dT oligonucleotide was analyzed under UV conditions on a HALO® OLIGO C18 column. The resulting chromatograms revealed smaller peaks before and after the primary peak of interest, which required further evaluation via LC/MS. The oligonucleotide was then analyzed using a Thermo QE-HF mass spectrometer. The mass spectrometry (MS) data confirmed that the smaller, earlier-eluting peaks corresponded to n-1, n-2, n-3, n-4, n-5, n-6, poly dT sequences—truncated products from the synthesis process. Additionally, the MS data identified the later-eluting peak as a 16-mer poly dT oligonucleotide with a cyanoethyl protecting group, which typically remains attached during synthesis and is removed during the final deprotection step. These findings highlight the utility of the HALO® OLIGO C18 column for detailed oligonucleotide impurity analysis, offering insights into the synthesis and purification process.

advancedmaterialstechnology

Made in the USA | halocolumns.com

INNOVATION YOU CAN TRUST,
PERFORMANCE YOU CAN RELY ON

Expected masses of Main Product (16-mer) and truncated products

Peak of Interest	Theoretical Monoisotopic Mass	[M-H]	2-[M-H]	3-[M-H]	Calculated	Calculated Monoisotopic Mass	PPM
10-mer Poly dT	2978.501	2977.493	1488.243	991.8261	1488.2572	2978.529	9.4
11-mer Poly dT	3282.546	3281.538	1640.265	1093.174	1093.1841	3282.5742	8.59
12-mer Poly dT	3586.592	3585.584	1792.288	1194.523	1194.5339	3586.6236	8.81
13-mer Poly dT	3890.638	3889.63	1944.311	1295.872	1295.8834	3890.6721	8.76
14-mer Poly dT	4194.683	4193.675	2096.334	1397.22	1397.2326	4194.7197	8.75
15-mer Poly dT	4498.729	4497.721	2248.357	1498.569	1498.5821	4498.7682	8.71
16-mer Poly dT	4802.775	4801.767	2400.38	1599.917	1599.9272	4802.8035	5.93
16+ Cyano Group	4855.8094	4854.802	2426.897	1617.596	1617.6072	4855.8435	7.02

Actual masses of Main Product (16-mer) and truncated products