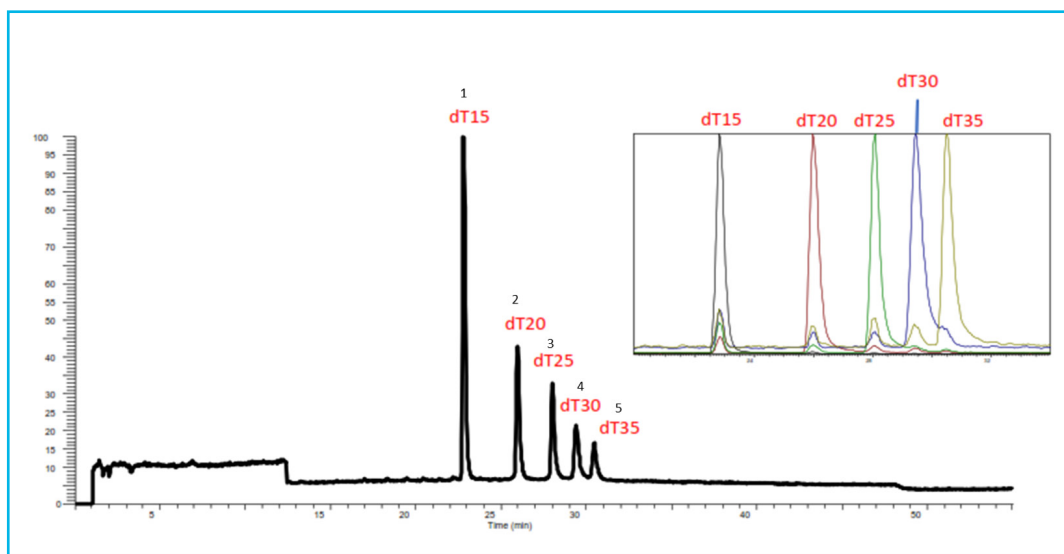




HILIC Analysis of Oligonucleotides

391



PEAK IDENTITIES

1. dT, 15 mer
2. dT, 20 mer
3. dT, 25 mer
4. dT, 30 mer
5. dT, 35 mer

TEST CONDITIONS:

Column: HALO 90 Å Penta-HILIC, 2.7 μm , 2.1 x 150 mm
 Part Number: 92812-705
 Mobile Phase A: 15 mM ammonium acetate in 30:70 water:ACN prepared from a stock solution of 50 mM aqueous ammonium acetate
 Mobile phase B: 35 mM ammonium acetate in 70:30 water:ACN prepared from a stock solution of 50 mM aqueous ammonium acetate

Gradient:	Time	%B
	0.0	5
	5.0	5
	40.0	85
	45.0	85
	46.0	5
	61.0	5

Flow Rate: 0.22 mL/min

Temperature: 60 °C

Injection: 4.0 μL

Sample Solvent: 50:50 mixture of MPA:MPB

LC System: Dionex Ultimate 3000 (Thermo Scientific) UHPLC

MS System: LTQ-XL linear ion trap mass spectrometer (Thermo Fisher Scientific)

MS CONDITIONS:

Detection: Negative
 Spray Voltage: 3.7 kV
 Capillary voltage: -30 V
 Sheath gas: 35
 Aux gas: 20
 Sweep gas: 20
 Capillary temp: 375 °C
 Max ion time: 250 ms

Using the HALO® Penta-HILIC column, 5 different poly dT oligomers in sizes ranging from 15-35 in length are separated using LCMS under HILIC conditions without the addition of an ion pairing agent. This mode of separation can be complementary to running analyses using ion-pair reversed-phase conditions. HILIC mobile phases that are ion-pair free are less expensive and less toxic compared to the typical HFIP-containing mobile phases used for LCMS ion-pair reversed-phase conditions.

Data courtesy of Asif Rayhan, Scott Abernathy, and Patrick A. Limbach

Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, PO Box 210172, Cincinnati, Ohio 45221-0172, United States.



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

