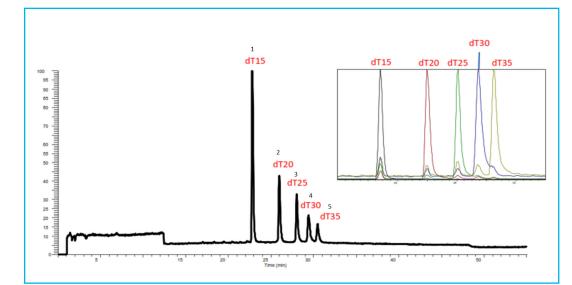
BIOPHARMACEUTICALS

HALO



HILIC Analysis of Oligonucleotides



PEAK IDENTITIES

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

TEST CONDITIONS:

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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	%В
	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

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