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アプリケーションノート

Bioanalytical HILIC LC-MS Compatibility Using the GTxResolve™ Premier Amide Column and OligoWorks™ SPE Sample Preparation Solution

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Application Brief

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Hydrophilic interaction liquid chromatography (HILIC) and ion pairing reversed phase liquid chromatography (IP-RP-LC) are both commonly employed for MS-based oligonucleotide analysis. HILIC, however, is gaining traction given its ability to separate oligonucleotides with ion-pairing free mobile phases - typically ammonium-based buffers - that are less toxic and more cost-effective when compared to IP-RP-LC methods. Additionally, HILIC offers enhanced compatibility with post organic extracted bioanalytical samples, as its mobile phase LC gradient begins with higher organic compositions, reducing issues related to oligonucleotide solubility and non-specific adsorption. This study highlights the suitability of the OligoWorks SPE Bioanalytical Sample Preparation Solution for HILIC LC-MS, utilizing the GTxResolve Premier Amide Column with injection volumes ranging from 1–10 µL.

Experimental

Oligonucleotide Abbreviation	Oligonucleotide Description	Molecular Mass (g/mol)	Source
OST 15-35 ODT	**MassPREP™ Oligonucleotide Separation Technology (OST) Standard** 15 to 35-mer oligodeoxythymidine ladder	4499, 6020, 7541, 9062, 10584	Waters Corporation p/n: 186004135
GEM-132	20-mer phosphorothioated Antisense Oligonucelotide (ASO)	6600	Integrated DNA Technologies, Inc. (IDT)
GEM-91	25-mer phosphorothioated ASO	7771	IDT
GalNAc 1	21-mer GalNAc conjugated sense single strand small interfering RNA (siRNA)	8590	Alnylam
GalNAc 2	16-mer GalNAc conjugated ASO with phosphorothioate backbone and 2'-methoxy ethyl and methyl modifications	7284	LGC Biosearch Technologies
Lipid ASO	**Lipid Conjugated ASO LC-MS Standard** 16-mer lipid-conjugated antisense oligonucleotide with 5′ palmitate modification, a phosphorothioated backbone and terminal methoxy ethyl ASO	5726	Waters Corporation P/N 186010747

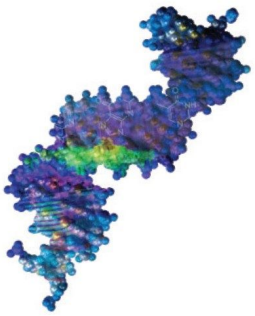


Table 1. Oligonucleotides used to assess the compatibility of the OligoWorks SPE Sample Preparation Solution with HILIC-based LC separations for quantitative LC-MS analyses.

Mobile Phase

HILIC LC mobile phase A was 25 mM ammonium acetate in water that was prepared using IonHance™ Ammonium Acetate pH 6.8 Concentrate buffer diluted using 18.2 MΩ*cm water. HILIC mobile phase B was 100% MS grade acetonitrile. IP-RP-LC mobile phase A was 0.1% N,N-diisopropylethylamine (DIPEA) and 1% 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in 100% 18.2 MΩ*cm water, and IP-RP-LC mobile phase B was 0.0375% DIPEA and 0.75% HFIP in 65:35 % MS grade acetonitrile: 18.2 MΩ*cm water. LC gradient conditions and specific LC columns used for HILIC and IP-RP-LC comparison are provided in Figures 1 and 2.

Oligonucleotide standards were prepared in RNAse-free water and/or OligoWorks SPE Eluent diluted with acetonitrile.

Results and Discussion

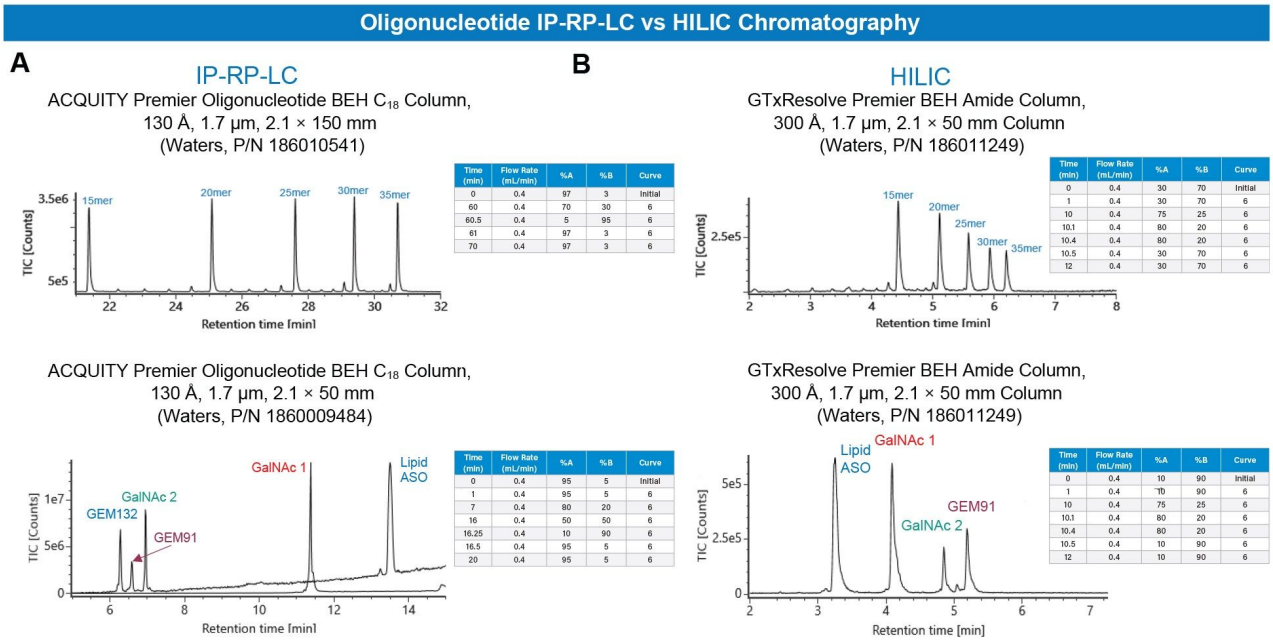


Figure 1. LC-MS chromatographic comparison of IP-RP-LC (A) and HILIC (B) for separation of the MassPREP OST 15–35 mer Oligodeoxythymidine Standard (1 µL injection; 10 pmol column load for both IP-RP-LC and HILIC), along with various conjugated oligonucleotides: GEM91, GEM132, GalNAc 21-mer siRNA (GalNAc 1), GalNAc 16-mer ASO containing 2′-MOE and 5′-MeC modifications (GalNAc 2), and a 16-mer lipid-conjugated ASO. For IP-RP-LC, the column load for each conjugated oligonucleotide was 0.02 µg, except for GalNAc 1, which was loaded at 0.1 µg. All conjugated oligonucleotides were loaded at 0.1 µg on the HILIC column.

As shown, IP-RP-LC separation relies primarily on hydrophobic interactions, and retention increases with the hydrophobicity of the oligonucleotide, driven by ion-pairing agents interacting with the negatively charged phosphate backbone to enhance retention on the hydrophobic stationary phase. In contrast, HILIC separation is governed by hydrophilicity and interactions with the polar stationary phase. Therefore, unmodified MassPREP OST Oligonucleotides exhibit stronger retention under HILIC conditions, while the more hydrophobic, highly conjugated oligonucleotides show reduced retention due to their lower polarity.

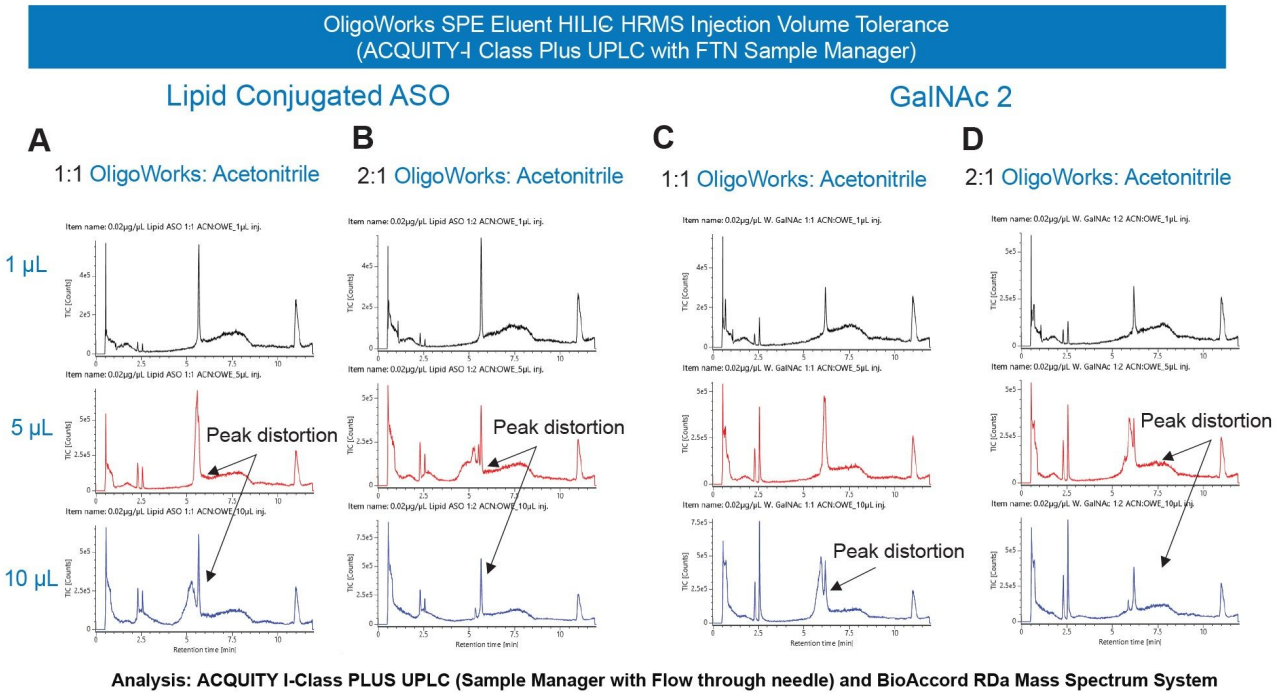
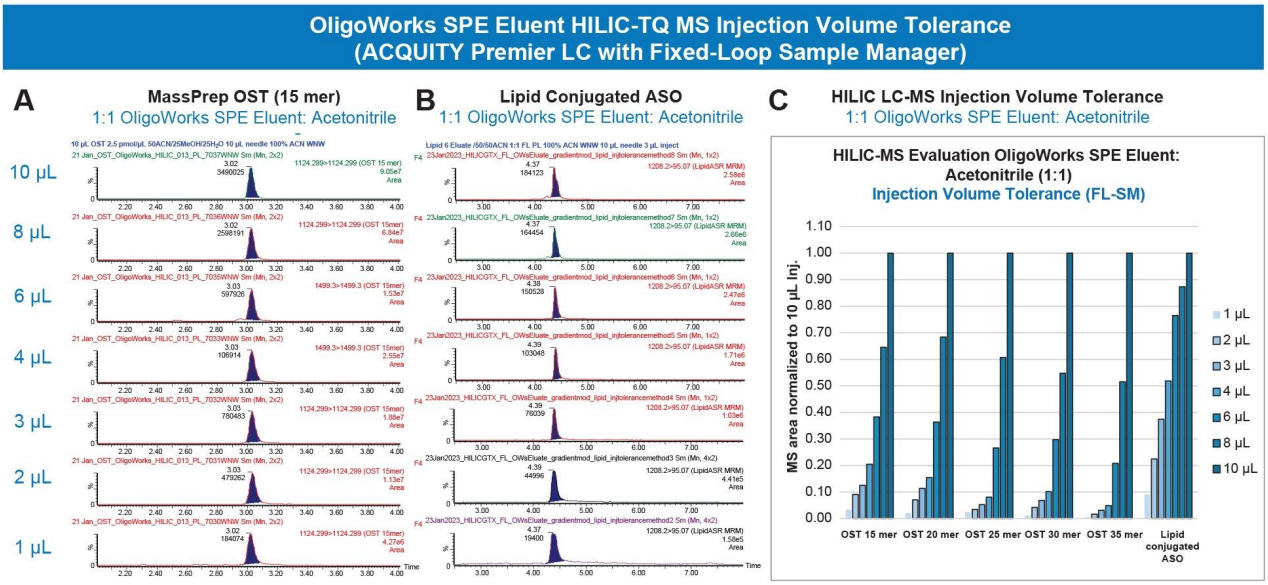


Figure 2: Illustration of injection volume of the OligoWorks SPE Eluent diluted with 1:1 or 1:2 acetonitrile and injected on an ACQUITY™ UPLC I-Class PLUS System configured with a Flow Through Needle (FTN) Sample Manager, the GTxResolve Premier BEH™ Amide Column, 300 Å, 1.7 µm, 2.1 x 50 mm, and detection by the BioAccord™ high resolution mass spectrometer (HRMS) with the ACQUITY RDa Detector. For the lipid conjugated ASO and the GalNAc 2 oligonucleotides, which are the earliest eluting in HILIC separation, a 1:2 dilution of the OligoWorks SPE Eluent with acetonitrile shows the least peak distortion with the 5 and 10 µL injections. Note: For improved injection volume tolerance, a larger needle and extension loop can be installed on the ACQUITY Premier FTN Sample Manager.



Analysis :ACQUITY Premier (Fixed-Loop Sample Manager 10 µL Loop with partial loop pressure assist ,Injection mode)-TQ Absolute MS Systems

Figure 3: Evaluation of oligonucleotide injection volume tolerance using Mass PREP OST 15–35 mer (10 pmol/µL) and a lipid-conjugated ASO (5 pmol/µL), both diluted 1:1 in OligoWorks SPE Eluent and acetonitrile. Samples were injected on an ACQUITY Premier LC System configured with a Fixed-Loop sample manager, equipped with a GTxResolve Premier BEH Amide Column (300 Å, 1.7 µm, 2.1 × 50 mm), and detected using the TQ Absolute MS System. For both the MassPREP OST 15 mer (A) and lipid-conjugated ASO (B), the most polar species showed enhanced MS response (C) across injection volumes from 1 to 10 µL, without any peak distortion. Note: Using the partial loop pressure assist injection mode with a 20 µL Loop and WNW of 100% acetonitrile as the weak needle wash (WNW) on the FL-sample manager, facilitated larger injection volumes of the 1:1 diluted OligoWorks SPE Eluent/acetonitrile samples.

Conclusion

This study demonstrates the compatibility of the OligoWorks SPE bioanalytical sample preparation solution with HILIC-MS, using the GTxResolve Premier Amide Column on both the ACQUITY UPLC I-Class PLUS System

(FTN Sample Manager) and the ACQUITY Premier System (FL Sample Manager). With minor adjustments to the gradient and injection parameters, it was feasible to inject up to 10 µL of the OligoWorks SPE Eluent diluted 1:1 and 1:2 with acetonitrile.

Ordering Information

Description	p/n
GTxResolve Premier BEH Amide Column, 300 Å, 1.7 µm, 2.1 x 50 mm	186011249
OligoWorks SPE Eluent, 25 mL	186010610
MassPREP Oligonucleotide Separation Technology Standard	186010610
Lipid Conjugated ASO LC-MS Standard	186010747
IonHance Ammonium Acetate pH 6.8 Concentrate	186009705
QuanRecovery™ with MaxPeak™ HPS 12x32 mm Screw Neck Vial, 300 µL	186009186

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