ELEMENTS OF ROBUST MODIFIED OLIGONUCLEOTIDE EXTRACTION THROUGH SPE

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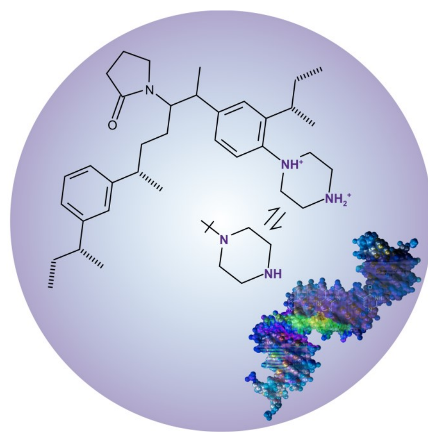
INTRODUCTION

Extraction and Quantification of Therapeutic Oligonucleotides (OTx) during Bioanalysis

Robust extraction of modified and unmodified oligonucleotides is essential for understanding their DMPK properties. Optimized sample preparation and carefully chosen ion exchange sorbent allow selective binding and purification of OTx.



Ion Exchange sorbent contains mixed-mode (reversed -phase and a weak anion-exchange) functionalities suitable for various applica-

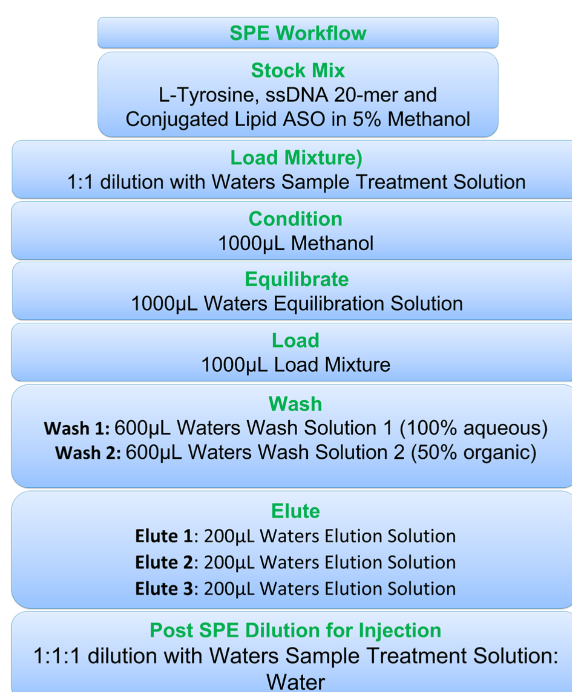
METHODS

Materials – Sample Preparation

Ion exchange sorbents were screened with a mixture L-Tyrosine as (Sigma P/N 93829), ssDNA 20-mer (Waters P/N 186009451) and Lipid Conjugated ASO (Waters P/N 186010774) using 1cc cartridge for SPE.

Rat plasma was subjected to RapiZyme™ Proteinase K Digestion Module (Waters, P/N 186009450) under denaturing conditions.

Anion-exchange sorbent and SPE workflow



IP-RP-LC-UV assay for sorbent selection

Analyte mixture before and after SPE was subjected to ion pairing reversed phase liquid chromatography (IP-RP-LC-UV) analysis using ACQUITY™ UPLCTM and ACQUITY Premier Oligonucleotide BEH™ C18, 130Å , 1.7µm, 2.1x50mm (Waters, P/N 186009484) Column at 60°C. Analyte signal at 260 nm was monitored using 0.1M 1:1 Triethylamine: Acetic Acid (TEAA) as Mobile phase A and 0.1M TEAA in 50% Acetonitrile as mobile phase B with a gradient of 0.1% -100% B in 15 min at 0.6 mL/min flow rate.

IP-RP-LC-UV assay for sorbent selection

Lipid conjugated ASO reference material was analyzed by IP-RP-LC-UV-MS using BioAccord™ System involving ACQUITY UPLC System and RDa detector. About 15 pmol of Lipid Conjugated ASO was analyzed by using mobile phase A (0.1% DIPEA, 1% HFIP in 18.2 MΩ water) and mobile phase B (0.0375% DIPEA, 0.075% HFIP in 65:35 ACN:18.2 MΩ water). After 10 µL injection at 35% B, elution performed in 9 min to get 50%B followed by 90%B in 11 min and equilibration (35%B) for 8 min at 0.4 mL/min flowrate. Mass spectra were acquired in negative ion mode at 2 Hz scan rate, 40 V cone voltage and 0.8 kV capillary voltage.

Size Exclusion Chromatography of plasma samples, following treatment with Rapizyme Proteinase K under denaturing conditions, was done using ACQUITY Premier SEC 250Å 1.7µm 4.6 x 150 mm Column (Waters P/ N186009963) and 2X PBS buffer at 0.2 mL/min flowrate.

RESULTS AND DISCUSSION

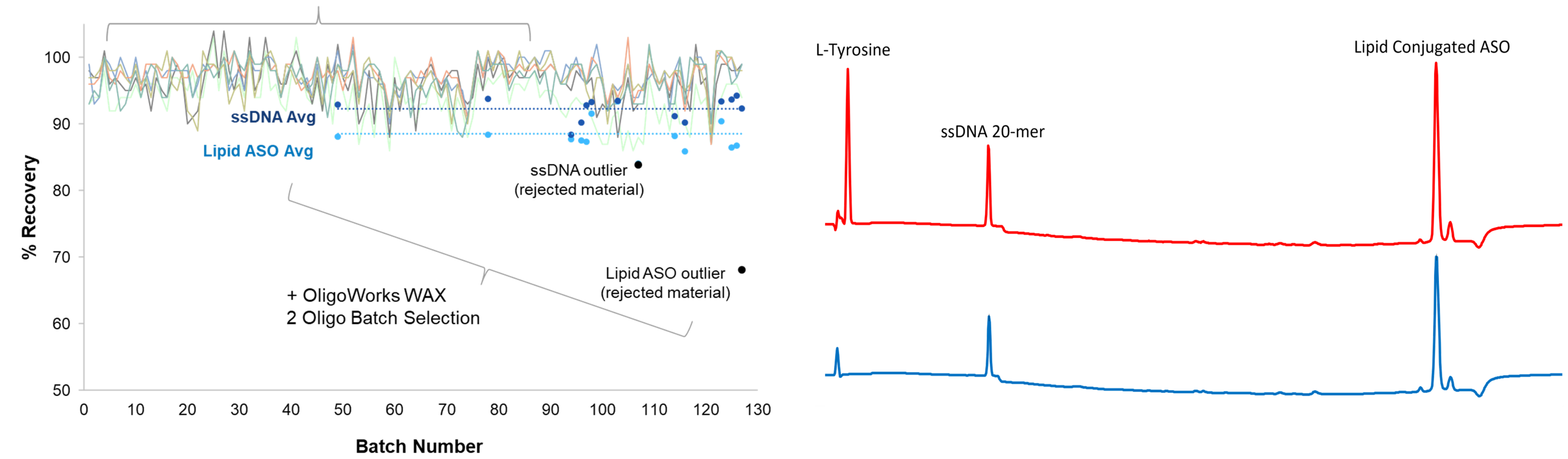


Figure 1: OligoWorks SPE Kit Sorbent selection. (A) Batch testing and Analyte recoveries. Oasis™ WAX Sorbent (30 µm) batches manufactured for 15 years and screened for 6-analyte recovery test were further screened against the recovery of two oligos in batch testing and selection. Batches that achieved precisely controlled and certified performance of oligo extraction are chosen for OligoWorks SPE Kit. (B) LC-UV profile of OligoWorks SPE Kit analytes before and after SPE.

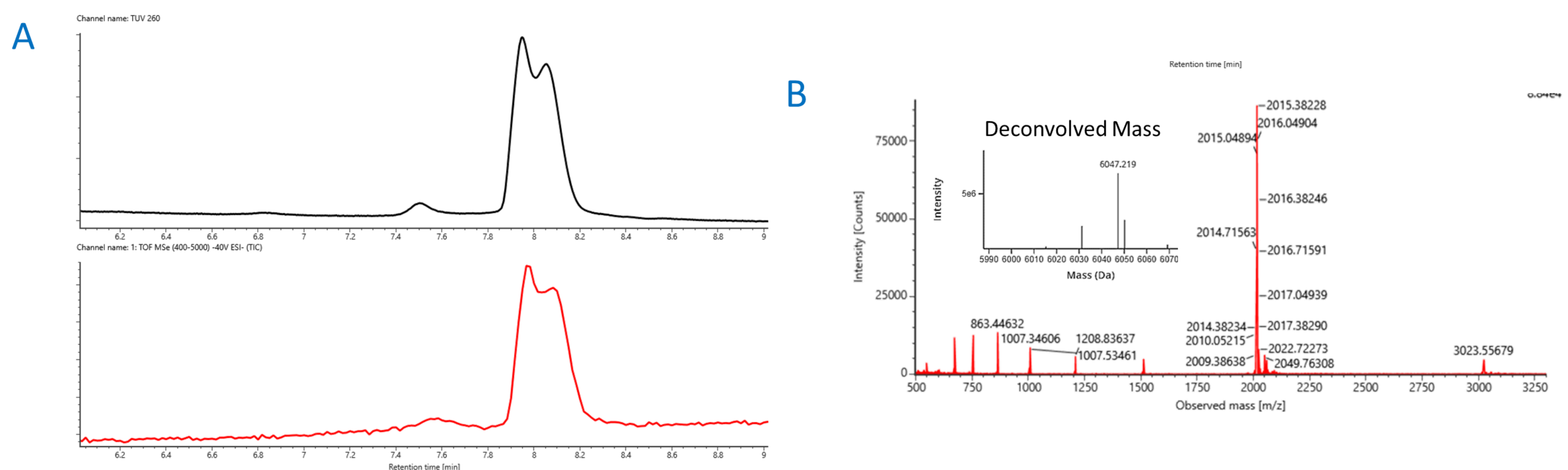


Figure 2: IP-RP-LC-UV-MS analysis of Lipid Conjugated ASO. (A) UV trace and low energy total ion chromatogram are shown. (B) Mass spectrum of the Lipid Conjugated ASO exhibiting identical mass information for both chromatographic peaks. Deconvolution of mass spectra showing the neutral mass values of 6047 Da for the intact oligomer and a low amount of oxygenated version (oxygen replacing sulfur species) with a mass of 6031 Da are shown in the inset.

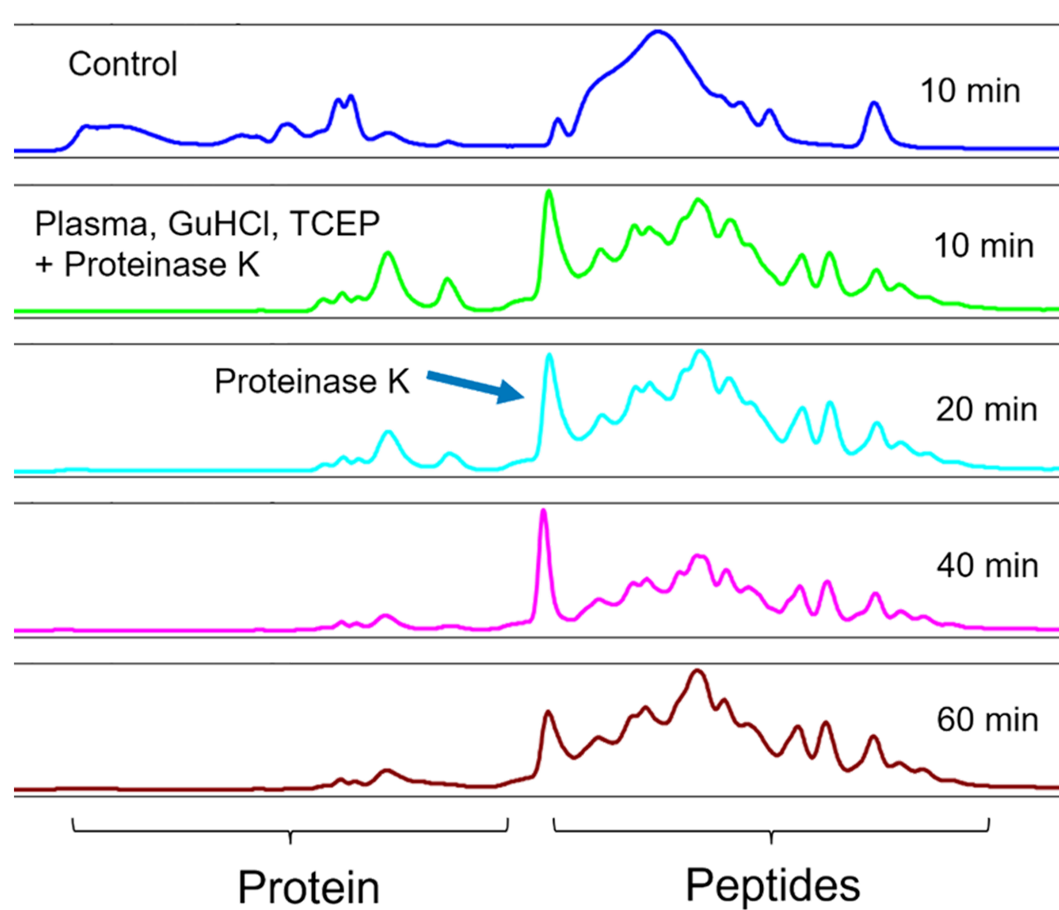


Figure 3: SEC profiles of plasma proteins following digestion with RapiZyme Proteinase K for various time intervals. Digestion reaction is completed within 40 minutes of incubation. Control: Untreated Plasma

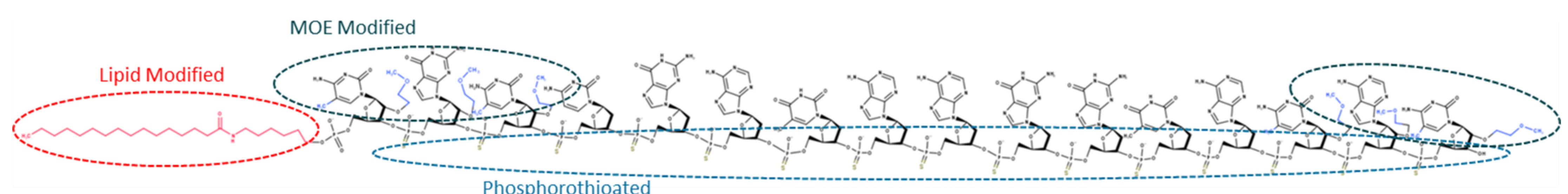
Figure 4: Sequence and molecular information for the 20-mer ssDNA LC-MS Standard (PN 186009451) and the Lipid Conjugated ASO LC-MS Standard (PN 186010774)

ssDNA 20-mer LC-MS Standard

Sequence	TAA TAC GAC TCA CTA TAG GG
Elemental Composition	$C_{196}H_{246}N_{77}O_{116}P_{19}$
Residue Composition	C:4 T:5 A:7 G:4
Monoisotopic Mass (Da)	6122.0732
Average Mass (Da)	6124.9982

5' d 5-Pal-*-MOE-MeC-*-MOE-G-*-MOE-MeC-*C*G*A*T*A*A*G*G*T*A*-MOE-MeC-*-MOE-A-*-MOE-MeC 3'

16mer	$C_{199}H_{283}N_{65}O_{91}P_{16}S_{16}$	6046.08484 (M)	6050.37740 (A)



CONCLUSION

•Usage of unmodified and Lipid Conjugated ASO oligomers is sufficient to identify the Oasis WAX Sorbent for OligoWorks SPE Kit

•Precisely controlled and Certified performance for oligonucleotide extraction is feasible with carefully selected Oasis WAX Sorbent batches.

•20-mer ssDNA and Lipid Conjugated ASO can serve as an excellent reference materials for robust and reproducible extraction while using OligoWorks SPE Kit

•LC-MS analysis of Lipid conjugated ASO require higher organic solvent content in the mobile phase. Diastereomers of Lipid conjugated ASO are partially resolved by certain mobile phase conditions

•Use of RapiZyme Proteinase K can digest the proteins in biological matrices efficiently even under denaturing conditions.

OligoWorks SPE Protocol

40–60 min. OligoWorks™ Sample Pretreatment* with RapiZyme Proteinase K Digestion Protocol

<30 min. OligoWorks SPE Protocol Using 96-well Microplate (2 mg/well)

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