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ELEMENTS OF ROBUST MODIFIED OLIGONUCLEOTIDE EXTRACTION THROUGH SPE

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PURPOSE

Extraction and Quantification of Therapeutic Oligonucleotides (OTx) during Bioanalysis

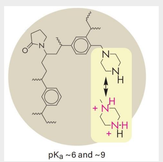
Robust extraction of modified and unmodified oligonucleotides is essential for understanding their DMPK properties. Optimized sample preparation and carefully chosen ion exchange sorbent allow selective binding and purification of OTx.

METHODS

Materials – Sample Preparation

Ion exchange sorbents were screened with a mixture L-Tyrosine (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



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

SPE Workflow

Stock Mix
L-Tyrosine, ssDNA 20-mer and Conjugated Lipid ASO in 5% Methanol

Load Mixture
1:1 dilution with Waters Sample Treatment Solution

Condition
1000µL Methanol

Equilibrate
1000µL Waters Equilibration Solution

Load
1000µL Load Mixture

Wash
Wash 1: 600µL Waters Wash Solution 1 (100% aqueous)
Wash 2: 600µL Waters Wash Solution 2 (50% organic)

Elute
Elute 1: 200µL Waters Elution Solution
Elute 2: 200µL Waters Elution Solution
Elute 3: 50µL Waters Elution Solution

Post SPE Dilution for Injection
1:1:1 dilution with Waters Sample Treatment Solution: Water

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™ UPLC™ 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-MS 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

Creating OligoWorks™ SPE Kit

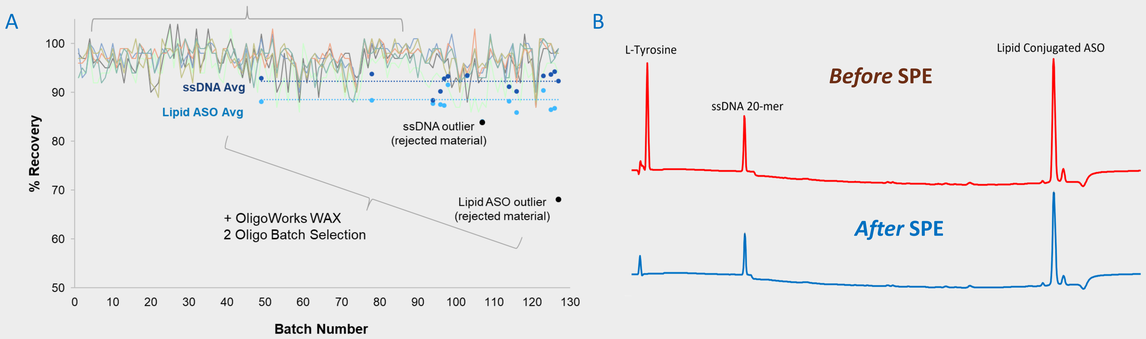


Figure 1: OligoWorks SPE Kit Sorbent selection. (A) Batch testing and Analyte recoveries. Oasis™ WAX Sorbent (30 µm) batches manufactured for 15 years and screened for 6-analyte recovery test were further screened against the recovery of two oligos in batch testing and selection. Batches that achieved 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.

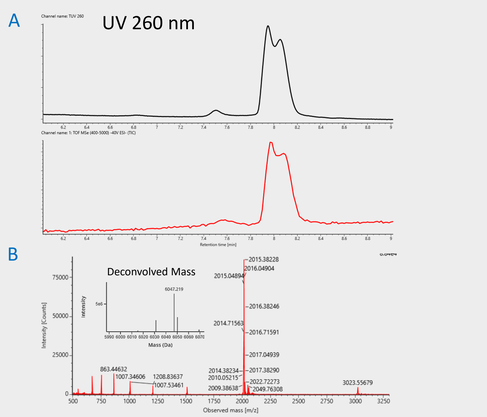


Figure 2: IP-RP-LC-UV-MS analysis of Lipid Conjugated ASO. (A) UV trace and low energy total ion chromatogram are shown. (B) Mass spectrum of the Lipid Conjugated ASO exhibiting identical mass information for both chromatographic peaks. Deconvolution of mass spectra showing the neutral mass values of 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.

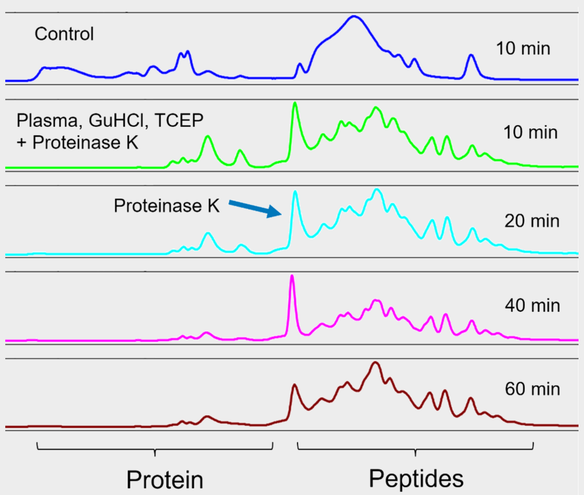
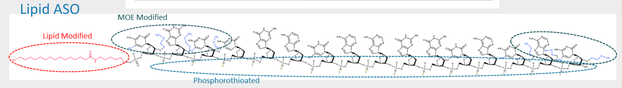


Figure 3: SEC profiles of plasma proteins following digestion with RapiZyme Proteinase K for various time intervals. Digestion reaction is completed within 40 minutes of incubation. Control: Untreated Plasma

ssDNA 20-mer LC-MS Standard	
Sequence	TAA TAC GAC TCA CTA TAG GG
Elemental Composition	$C_{196}H_{240}N_{77}O_{116}P_{19}$
Residue Composition	C:4 T:5 A:7 G:4
Monoisotopic Mass (Da)	6122.0732
Average Mass (Da)	6124.9982

Lipid ASO



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)

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)

CONCLUSIONS

- 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 material 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

REFERENCES

1. www.waters.com/oligoworks

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