# IMPROVED TISSUE HOMOGENIZATION AND SPE-BASED SAMPLE PREPARATIONS FOR THE QUANTITATIVE LC-MS ANALYSIS OF OLIGONUCLEOTIDE THERAPEUTICS

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## INTRODUCTION

**Efficient extraction of drug analytes is a critical aspect of drug metabolism and pharmacokinetics (DMPK) studies. This has long applied to small molecules and still applies to the bioanalysis of oligonucleotides. Oligonucleotide drugs and their metabolites must be quantified within both biofluids and tissue samples. The newest of oligonucleotide drugs are both extensively modified and conjugated. These modified residues and conjugate moieties can complicate extraction recovery and reproducibility. In this work, we report several key insights on how to achieve improved extractions. Protocols for solvent-assisted proteinase K sample pre-treatments are investigated using a weak anion exchange (WAX) microplate-based solid phase extraction (SPE) device. Direct injection LC-MS quantitation is demonstrated for all three antisense oligonucleotides (ASOs).**

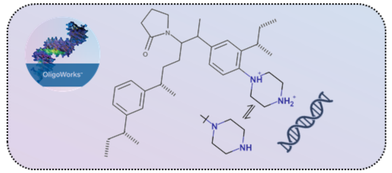



**Figure 1**: OligoWorks™ SPE ion exchanger
The polymeric base particles exhibiting hydrophilic-Lipophilic balance with weak anion exchanger are packed in both microplate, macroplate, and cartridge formats

### Tissue Homogenization

50 mg Tissue
+125 (105) µL 100mM Tris HCl pH 7
+150 µL ACN
+50 µL 0.5M TCEP HCl
+50 µL 6M GuHCl
+125 µL RapiZyme Proteinase K

Bead beating (15s@5800 rpm, 30s pause, 15s@5800 rpm)
2 h at 55°C @600 rpm
Centrifugation 5-10,000xg

## METHODS

**Four oligonucleotides (GEM132, GEM91, and a lipid- conjugated ASO) were added to bovine liver or porcine brain samples and subsequently extracted through improved sample preparation protocols using a high purity, recombinant proteinase K and WAX microplate SPE device. The impact of organic solvent and other tissue homogenization components were investigated.**

**Chromatography was performed on a UHPLC system configured with a 2.1 x 50 mm oligonucleotide batch tested and selected, low adsorption BEH™ C18 Column (130Å, 1.7 µm). The procedure is described in Figure 2. LC-MS conditions shown in Table 1. Both benchtop TOF MS and Triple Quad MS were used for testing.**

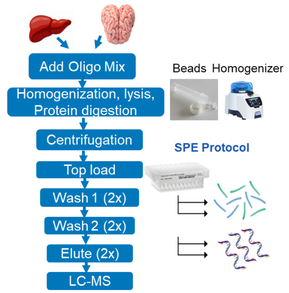



**Figure 2: Tissue Processing & SPE**

**Table 1: LC-MS conditions**

| LC System: ACQUITY™ UPLC™ I-Class PLUS System | |
|---|---|
| Mobile phase A | 0.1% *N,N*-diisopropylethylamine (DIPEA) and 1% 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) in 18.2 MΩ water |
| Mobile phase B | 0.0375% DIPEA and 0.75% HFIP in 65:35 Acetonitrile: 18.2 MΩ water |
| Column | ACQUITY Premier Oligonucleotide BEH C18 Column, 130Å, 1.7µm, 2.1 X 50mm |
| Column and Sample temperature (°C) | 55 & 15 |
| Injection volume (µL) | 10 |

| Gradient | | |
|---|---|---|
| Time (min) | Flow (mL/min) | % B |
| 0 | 0.6 | 5 |
| 3.5 | 0.6 | 30 |
| 4.25 | 0.6 | 90 |
| 4.5 | 0.6 | 90 |
| 5.5 | 0.6 | 5 |

| MS System: Xevo™ TQ-Xs MS | |
|---|---|
| Polarity | Negative |
| Cone voltage (V) | 45 |
| Capillary voltage (kV) | 0.5 |
| Desolvation temperature (°C) | 600 |
| Desolvation gas flow (L/Hr) | 1000 |
| Cone gas flow (L/Hr) | 150 |

## RESULTS

**Identified optimal conditions for tissue homogenization, protein digestion, SPE adsorption, wash, and reproducible elution conditions of oligonucleotides yielded minimal matrix interferences from tissue samples (Figure 3). Pretreatment procedures with organic solvent facilitated the extraction and solubilization of various ASOs; inclusion of NP-40 alternative, however, improved the recovery of a lipid-conjugated ASO (Figure 4). Use of a microplate SPE format made it possible to reach high concentration factors minimizing the amount of tissue sample needed to perform an analysis (Figure 5). Recoveries of ASOs is independent of tissue sample amount (Figure 6).**

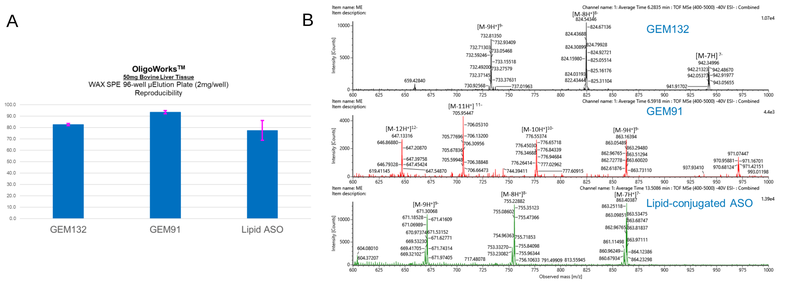



**Figure 3**: Reproducible recoveries (A), and minimal matrix interference (B) in the mass spectra following OligoWorks SPE of tissue samples containing three different Antisense oligonucleotides.

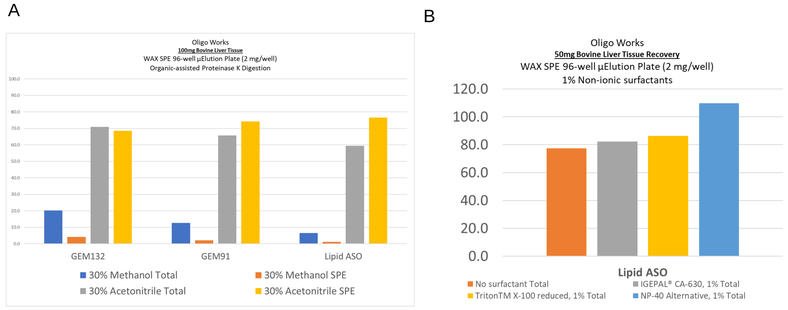
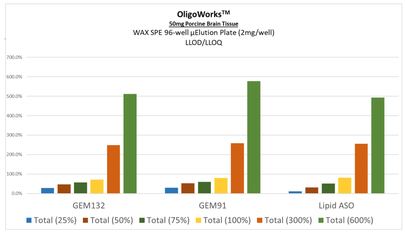



**Figure 4**: (A) Acetonitrile assisted detergent free extraction of ASOs from tissue samples.(B) Lipid-conjugated ASO benefited the presence of NP40 alternative (1%) for further improvement in recovery.




B

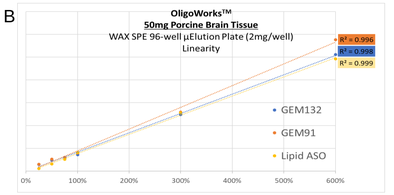



**Figure 5**: (A) Proportionate response of ASOs following SPE at various concentrations. (B) Linear regression plot.

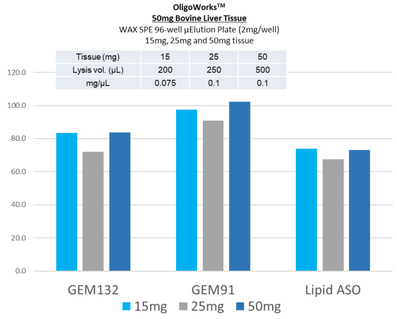



**Figure 6**: Tissue amount-independent recovery of ASOs following OligoWorks SPE

## CONCLUSIONS

- **Selective extraction and elution of ASO oligonucleotides is feasible from multiple tissue types.**
- **Extracted ASOs are amenable for either full scan mode analysis on benchtop small footprint TOF MS or MRM analysis by Triple Quad MS**
- **Reproducible and repeatable data were collected even without the application of an internal standard**
- **One novel aspect of the SPE eluate is that no further sample preparation is required for subsequent LC-MS analysis saving time and avoiding sample losses during drying and reconstitution**
- **Eluted nucleic acid components can be readily analyzed by regular ion-pairing reversed phase chromatography and UV/MS-based detection and quantitation**
- **This robust tissue extraction protocols for improved LC-MS based bioanalysis of ASO drugs was repeatable and highly reproducible across multiple batches of SPE sorbent, providing confidence in the suitability of the optimized protocols**

**References**

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