Utilization of Novel Low Adsorption Chromatographic Surface Technology for Improved Bioanalytical Quantitation of Oligonucleotides

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Introduction

Over the past decade, there has been increased research of oligonucleotides therapies (ONTs), increasing demand for LC-MS bioanalytical assays in support of their research and development.

Developing robust and sensitive LC-MS methods for ONTs remains quite challenging, due to their size, poly-anionic nature, physiochemical diversity, stability, non-specific binding, protein binding and poor RP chromatographic retention, resulting in limited recovery, selectivity, and sensitivity.

The work presented herein, demonstrates the improved LC-MS oligonucleotide bioanalytical quantitative performance using a novel sub-2µm analytical column and low dispersion UPLC ™ chromatography system, treated with novel MaxPeak ™ High Performance Surfaces (HPS) technology, specifically design to mitigate issues with adsorption of metal-sensitive analytes.

Use of a UPLC system and column with HPS, improved oligonucleotide recovery and facilitated low level ng/mL quantification for the fully phosphorothioated antisense oligonucleotide therapy, GEM91 was achieved.

Experimental

Neat standard solutions and plasma samples were spiked at multiple concentrations of GEM91. A 2-step LLE-SPE sample extraction was used for sample purification and concentration. Solid-phase extraction was performed on an Oasis ™ WAX 96-well µElution plate. The sample extraction protocol is listed in **Figure 1.** LC-MS/MS analysis was using a Waters Xevo ™ TQ-XS tandem quadrupole MS (ESI-). Chromatographic separation was achieved using an ACQUITY Premier System and ACQUITY Premier Oligonucleotide BEH ™ C_{18}, 1.7 µm, 2.1 x 100 mm Column. A flow rate of 0.5 mL/min and shallow gradient was employed using 150 mM hexafluoroisopraponol containing 5mM hexylamine in water or methanol. Total analysis time was 5 minutes. Injection volume of the extracted samples was 10-20 µL.

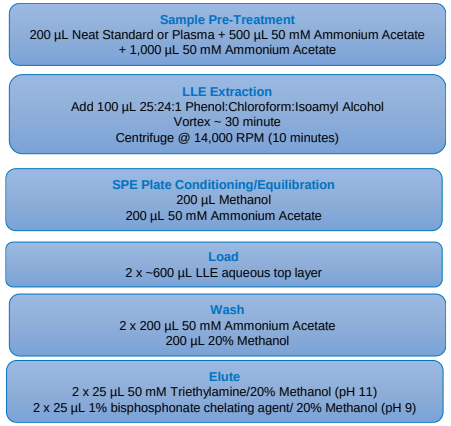

Figure 1. *Sample pretreatment and LLE-SPE oligonucleotide extraction protocol using phenol:choloroform and Oasis WAX µElution SPE in the 96-well format, effectively disrupts protein binding ensures high oligonucleotide recovery, aids in selectivity and facilitates sample concentration.*

Results

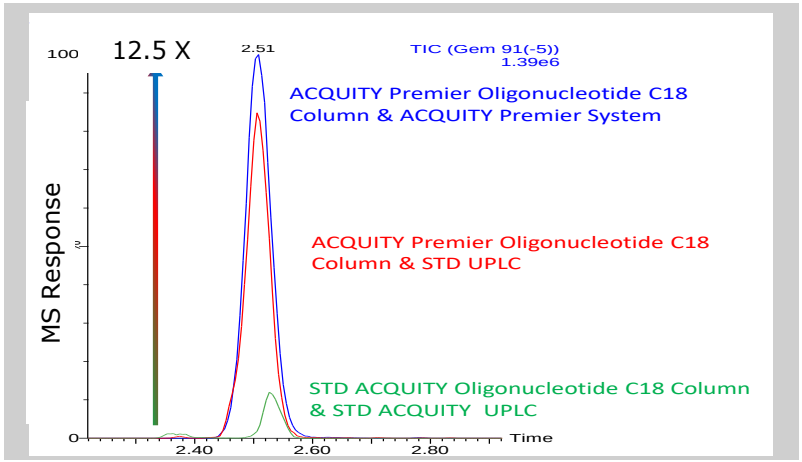

Figure 2*. Demonstration of improved chromatographic performance using ACQUITY Premier Column and System, with* MaxPeak HPS technology *which mitigates oligonucleotide loss, as compared to the standard ACQUITY UPLC System and Column.*

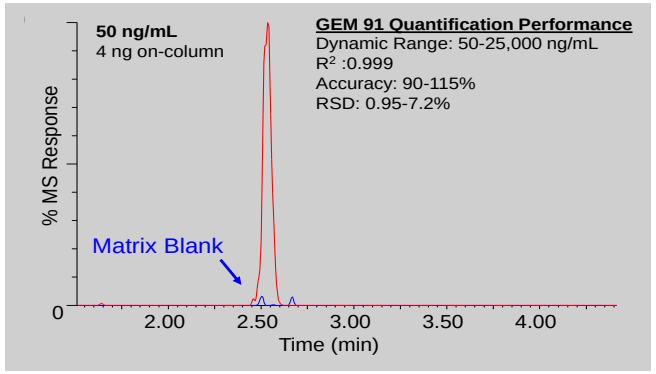

Figure 3*. High Sensitivity detection and quantification of GEM91, achieving 50 ng/mL in LLE-SPE extracted plasma.*

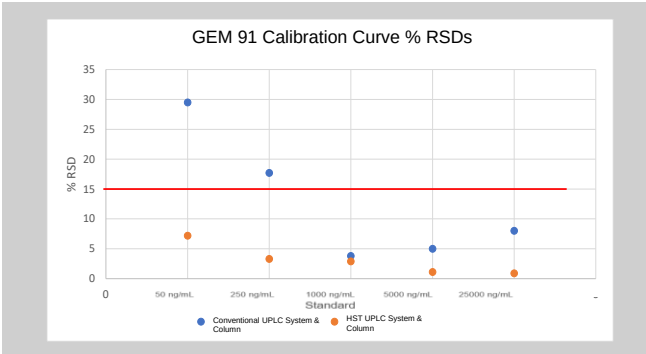

Figure 4. Improved repeatability for GEM91 calibration curve standards extracted from plasma using the ACQUITY Premier UPLC System and Column as compared to the conventional ACUITY UPLC System and Column.

Conclusions

- A LLE-mixed-mode SPE extraction effectively disrupted protein binding and ensured oligonucleotide recovery
- Use of the low adsorption ACQUITY System and Column, with HST technology, improved oligonucleotide chromatographic recovery and improved LLOQs.
- Quantification limits of GEM91, following LLE-WAX SPE extraction, was 50 ng/mL.