

## Utilization of the ACQUITY PREMIER System and Column for Improved Oligonucleotide Bioanalytical Chromatographic Performance

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

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### Abstract

LC-MS/MS bioanalytical quantification of oligonucleotide therapeutics requires sensitive and robust analytical methods for their accurate quantification. This work highlights the increased chromatographic performance for oligodeoxythymidines (ODTs) and the oligonucleotide antisense therapy, GEM91, using the ACQUITY PREMIER chromatographic system.

### Benefits

- Use of the ACQUITY PREMIER System and ACQUITY PREMIER Oligonucleotide C<sub>18</sub> Column yielded improved oligonucleotide recovery and peak shape, ultimately improving method detection limits, and reproducibility
- Incorporation of the ACQUITY PREMIER technology eliminated need for lengthy system and column passivation, saving costly ion-pairing mobile phase reagents, and maximized system up-time

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## Introduction

Amongst the many LC-MS analytical method development challenges for oligonucleotides, their well-known propensity to adsorb to metals, due to their polyanionic nature, is often the most problematic. This metal interaction negatively impacts chromatographic performance, often resulting in poor peak shape and issues with analyte recovery and reproducibility, ultimately limiting overall performance of the analytical method.

Conditioning or passivation of LC systems and columns, using high concentrations of the oligonucleotide to block sites of adsorption is often used. While effective, this passivation is not permanent. As an alternative, use of chelating reagents in mobile phases, such as EDTA, is often used. While also effective, use of chelating additives often negatively impacts LC-MS assays, suppressing MS signal and limiting sensitivity.

The work presented herein, demonstrates the improved oligonucleotide bioanalytical quantitative performance using the ACQUITY PREMIER chromatographic system and ACQUITY PREMIER Oligonucleotide C<sub>18</sub> Column. The ACQUITY PREMIER technology with MaxPeak High Performance Surfaces was specifically designed to prevent non-specific adsorption due to ionic interactions, significantly improving oligonucleotide peak shape, recovery, and overall reproducibility of the analytical method without the need for system or column passivation.

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## Results and Discussion

ACQUITY PREMIER System performance was compared to an ACQUITY UPLC I-Class PLUS System (ACQUITY UPLC). MS detection and quantification were performed using a Xevo TQ-XS Tandem Quadrupole Mass Spectrometer. Figure 1 highlights the Waters systems and products used for the LLE-SPE sample preparation and LC-MS analysis used for this evaluation with full details are described in the application note [720007019EN <https://www.waters.com/nextgen/us/en/library/application-notes/2020/improved-oligonucleotide-spe-lc-ms-analysis-using-maxpeak-high-performance-technology.html>](https://www.waters.com/nextgen/us/en/library/application-notes/2020/improved-oligonucleotide-spe-lc-ms-analysis-using-maxpeak-high-performance-technology.html) .

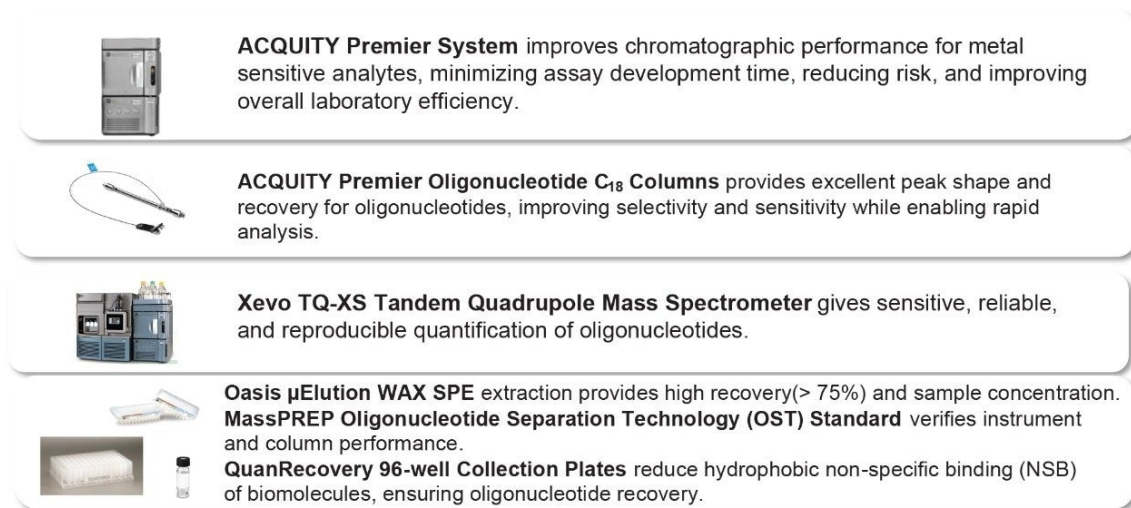


Figure 1. Bioanalytical Oligonucleotide Quantification: Waters Tools.

When comparing the standard ACQUITY Oligonucleotide C<sub>18</sub> Column and standard ACQUITY UPLC System to the ACQUITY PREMIER Column and ACQUITY PREMIER System, incremental improvements for oligonucleotide recoveries (peak area/height) were observed. These results are illustrated in Figures 2 and 3 for GEM91 and the 20mer oligodeoxythymidine, respectively. The largest cumulative recovery benefit (>12x) was seen for the fully phosphorothioated oligonucleotide, GEM91. While reduced peak tailing was seen for all oligonucleotides evaluated, when incorporating the ACQUITY PREMIER Column, the most notable improvement was seen for the 35mer oligodeoxythymidine when the ACQUITY PREMIER System was used in conjunction with the ACQUITY PREMIER Column (Figure 4).

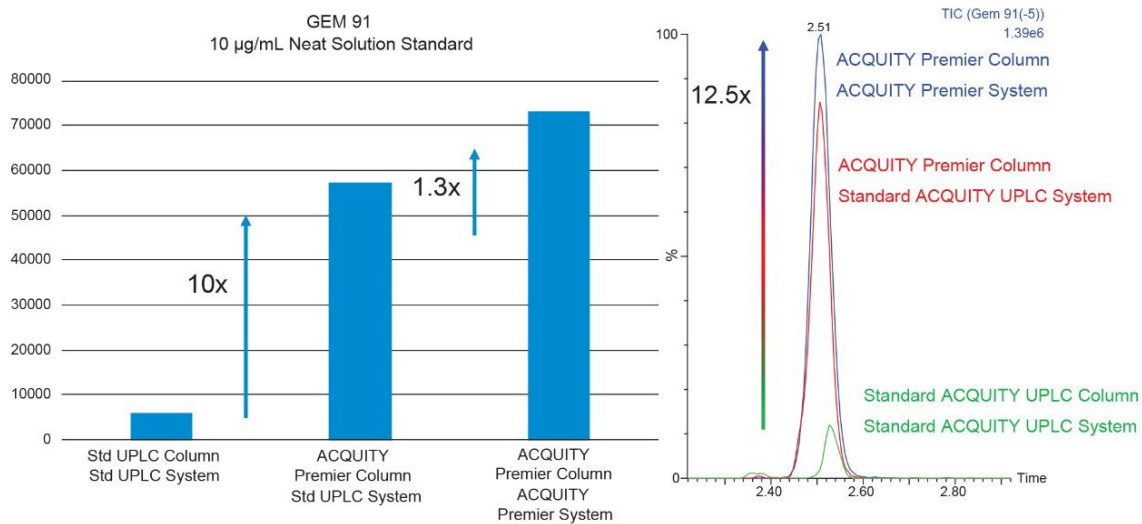


Figure 2. Demonstration of improved chromatographic performance for GEM91 using the ACQUITY PREMIER System and ACQUITY PREMIER Column as compared to the standard ACQUITY UPLC I-Class System and standard UPLC column.

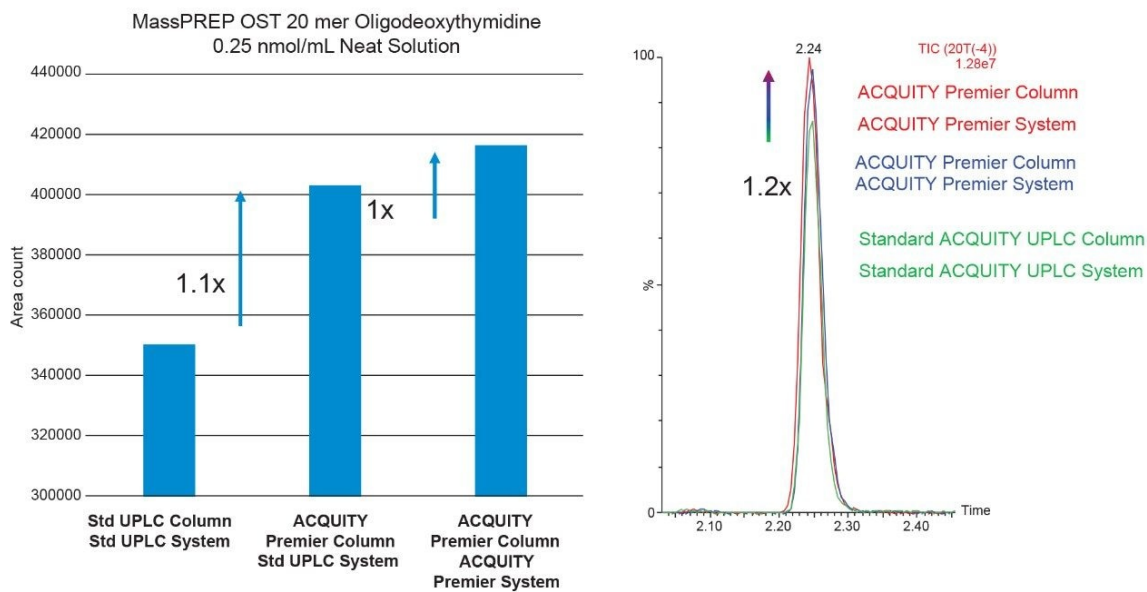


Figure 3. Demonstration of improved chromatographic performance for the 20mer oligodeoxythymidine using the ACQUITY PREMIER System and ACQUITY PREMIER Column as compared to the standard ACQUITY UPLC I-Class System and standard UPLC column.

## Oligodeoxythymidine 35 mer

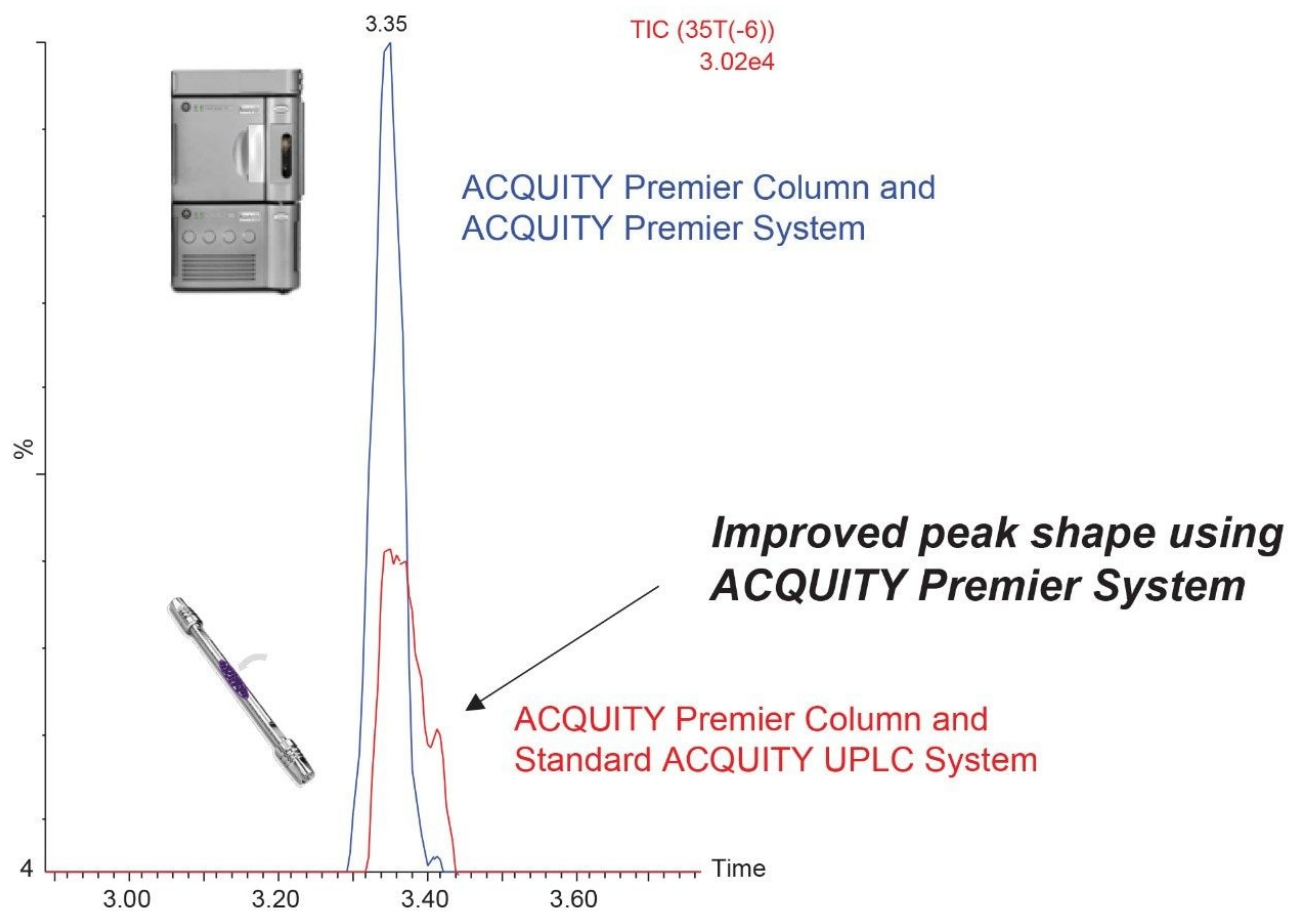


Figure 4. Improved peak shape for the oligodeoxythymidine 35mer using the ACQUITY PREMIER System vs a standard ACQUITY UPLC I-Class System.

In accordance with small molecule bioanalytical method development guidances,<sup>1,2</sup> a developed assay must be able to demonstrate linearity (correlation coefficient or  $R^2 \geq 0.98$ ), accuracy ( $\pm 15\%$ ), and precision ( $\pm 15\%$ ). These criteria were achieved using the ACQUITY PREMIER Column with the standard ACQUITY UPLC System (Figure 5A) and the assay achieved a lower limit of quantification (LLOQ) of 50 ng/mL in post-spiked extracted plasma (Figure 6). Further improvements in GEM91 quantification performance were seen when the ACQUITY PREMIER System was used in conjunction with the ACQUITY PREMIER Column (Figure 5B). Linearity ( $R^2$ ) improved from 0.996 to 0.999 and reproducibility (RSDs) ranges improved from 3.8–29.5% using the standard ACQUITY UPLC

System to 0.95–7.2% using the ACQUITY PREMIER System across the standard curve levels. Further demonstration of improved GEM91 quantification performance (accuracy and precision), using the ACQUITY PREMIER System and ACQUITY PREMIER Column, is shown for the individual calibration standards in Figure 7.

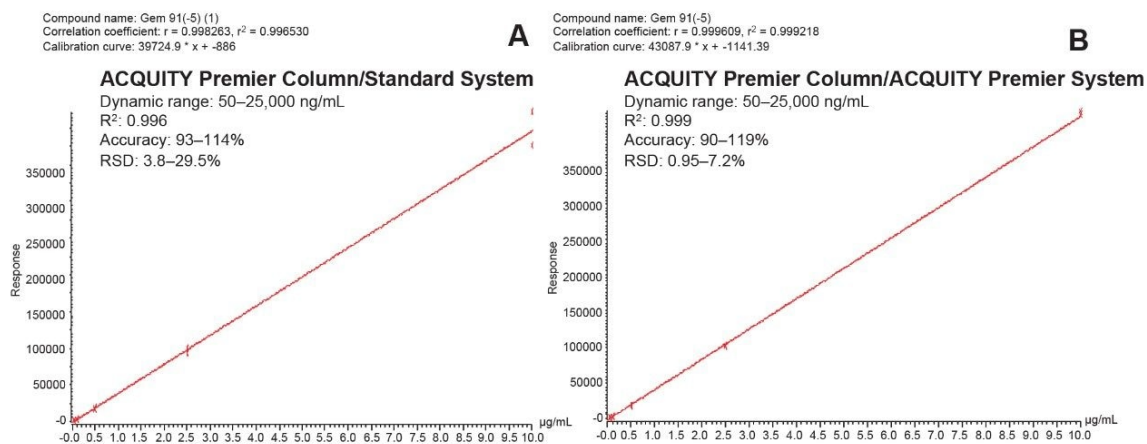


Figure 5. Improved quantification performance. Comparison of ACQUITY UPLC I-Class (A) and ACQUITY PREMIER (B) Systems for the quantification of GEM91 from post-spiked extracted plasma.

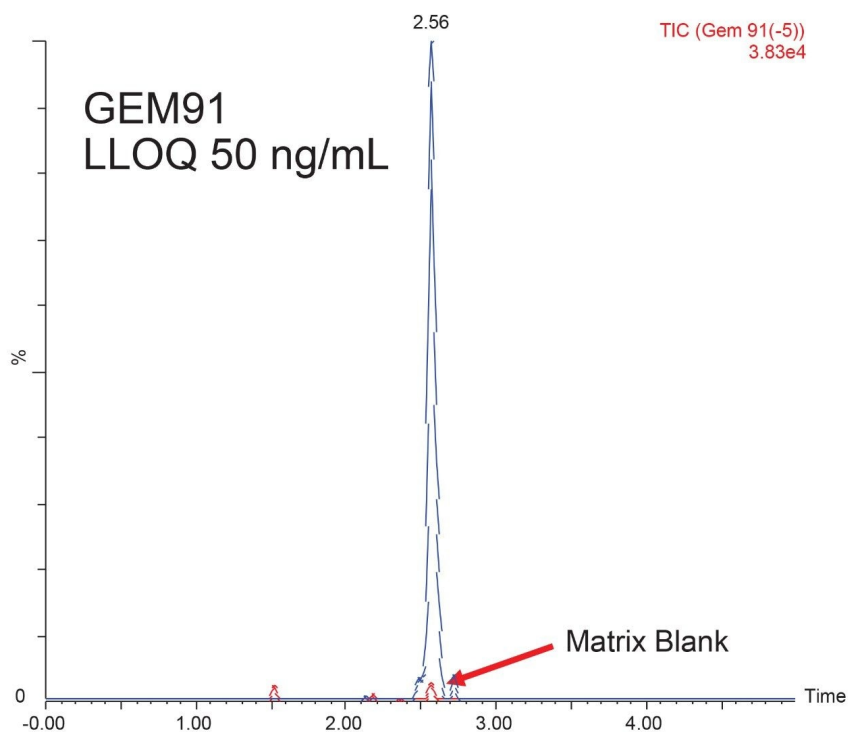
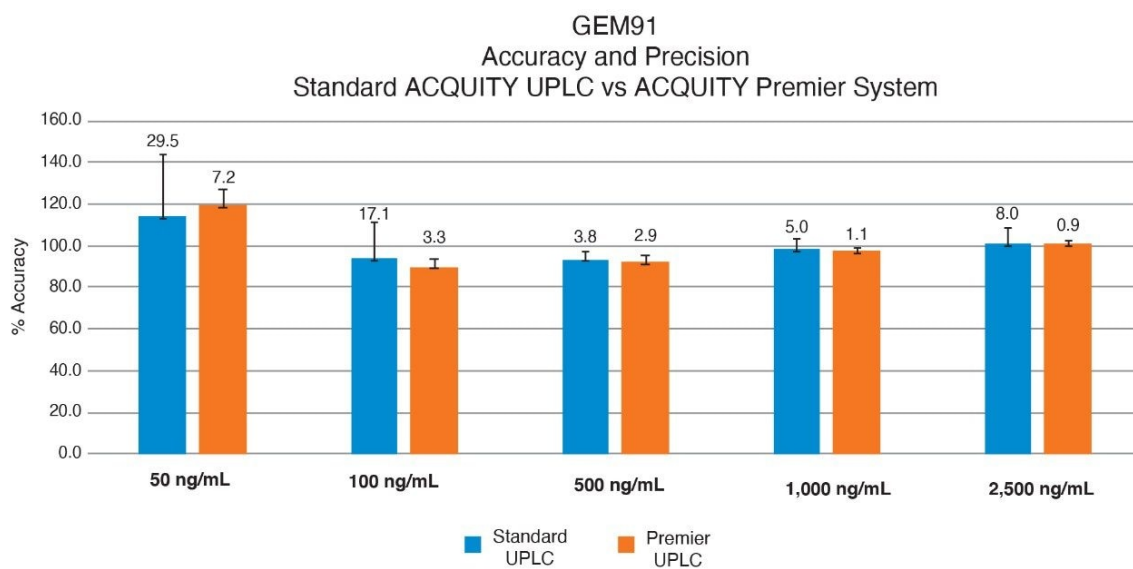


Figure 6. High sensitivity quantification and detection for GEM91, achieving 50 ng/mL in post-spiked extracted plasma using the ACQUITY PREMIER System and ACQUITY PREMIER Column.





*Figure 7. Improved GEM91 standard curve accuracy and precision in post-spiked extracted plasma using the ACQUITY PREMIER System and ACQUITY PREMIER Column.*

## Conclusion

The use of the ACQUITY PREMIER System and ACQUITY PREMIER Column enabled the development of a sensitive quantitative MRM method for oligodeoxythymidines and GEM91 oligonucleotides, achieving LLOQs of 0.025 nmol/mL and 50 ng/mL in extracted plasma, respectively. The ACQUITY PREMIER technology greatly improved quantification performance, with improved sensitivity, linearity ( $R^2 \geq 0.999$ ), accuracy ranges from 90–119%, and mean RSDs across the dynamic range from 0.95–7.2%, indicating a highly accurate, precise, and reproducible method. This proof of concept method shows great promise for accurate quantification of oligonucleotides in support of drug discovery and research.

## References

1. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG, Weiner R. Quantitative Bioanalytical Methods Validation and Implementation: Best Practices for Chromatographic and Ligand Binding Assays. *Pharm. Res.* 2007, 24, 1962–1973.
2. Bansal S, DeStefano A. Key Elements of Bioanalytical Method Validation for Small Molecules. *AAPS J.* 2007, 9, E109–114.

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[ACQUITY PREMIER System <https://www.waters.com/waters/nav.htm?cid=135077739>](https://www.waters.com/waters/nav.htm?cid=135077739)

[ACQUITY UPLC System <https://www.waters.com/514207>](https://www.waters.com/514207)

[Xevo TQ-XS Triple Quadrupole Mass Spectrometry <https://www.waters.com/134889751>](https://www.waters.com/134889751)

[MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)

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