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Application Note

# Improved Peak Recovery and Peak Shape of Oligonucleotides Using Waters PREMIER Columns

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

## Abstract

Oligonucleotide separation using XBridge PREMIER Oligonucleotide BEH $C_{18}$ Columns yielded higher peak areas and sharper peaks compared to a competitor column based on conventional column hardware. An average of 40% peak area loss was observed on the competitor column for the five analyte separations.


### Benefits

- Improved analyte recovery
- Improved peak shape
- Out-of-box performance

## Introduction

Adsorptive losses to column hardware, commonly referred to as non-specific adsorption, is a common issue in many workflows and is most problematic for large molecule applications, like oligonucleotide separations, where multiple residues on the analyte can interact with the various surfaces. Stainless-steel and other metallic surfaces are particularly problematic in LC systems and column hardware as they introduce ionic secondary interactions and the potential for metal chelation. This can lead to reduced peak area, peak shape distortions, and in some extreme cases total peak loss. Work-arounds exist, including the use of passivation agents—like medronic acid—or even saturating the system with a similar analyte to occupy the active sites on the metal surfaces. However, these techniques are time consuming, and are often inconsistent or temporary fixes.

## Results and Discussion

By using Waters PREMIER Columns, which utilize hardware based on High Performance Surface (HPS) Technology, the undesirable interaction of oligonucleotides with column hardware can be drastically reduced. To demonstrate this, a standard LC instrument and two separate columns were used to analyze a

simple mixture of oligonucleotides. The first column to be tested was a fully porous XBridge PREMIER Oligonucleotide BEH $C_{18}$, 130 Å, 2.5 µm Column while the other was a superficially porous 2.7 µm competitor $C_{18}$ column constructed from stainless steel hardware. Both stationary phases were pH resilient and designed for use with ion pairing RPLC conditions. The analytes examined were five chains of oligodeoxythymidine (15, 20, 25, 30, and 35 nt) contained in the Waters MassPREP OST Standard (p/n: 186004135 <https://www.waters.com/nextgen/in/en/shop/standards--reagents/186004135-massprep-oligonucleotide-standard.html> ). Mobile phases containing 25 mM of hexylammonium acetate (pH 7.0) as an ion pairing agent were used along with ACN as an eluent and a column temperature of 60 °C.

| Compound | XBridge PREMIER Oligonucleotide BEH $C_{18}$ | Competitive $C_{18}$ | % Loss in peak area |
|---|---|---|---|
| 15 nt | 175616 | 112597 | 36 |
| 20 nt | 190354 | 105641 | 44 |
| 25 nt | 185748 | 109096 | 41 |
| 30 nt | 191370 | 109647 | 43 |
| 35 nt | 189961 | 114616 | 40 |

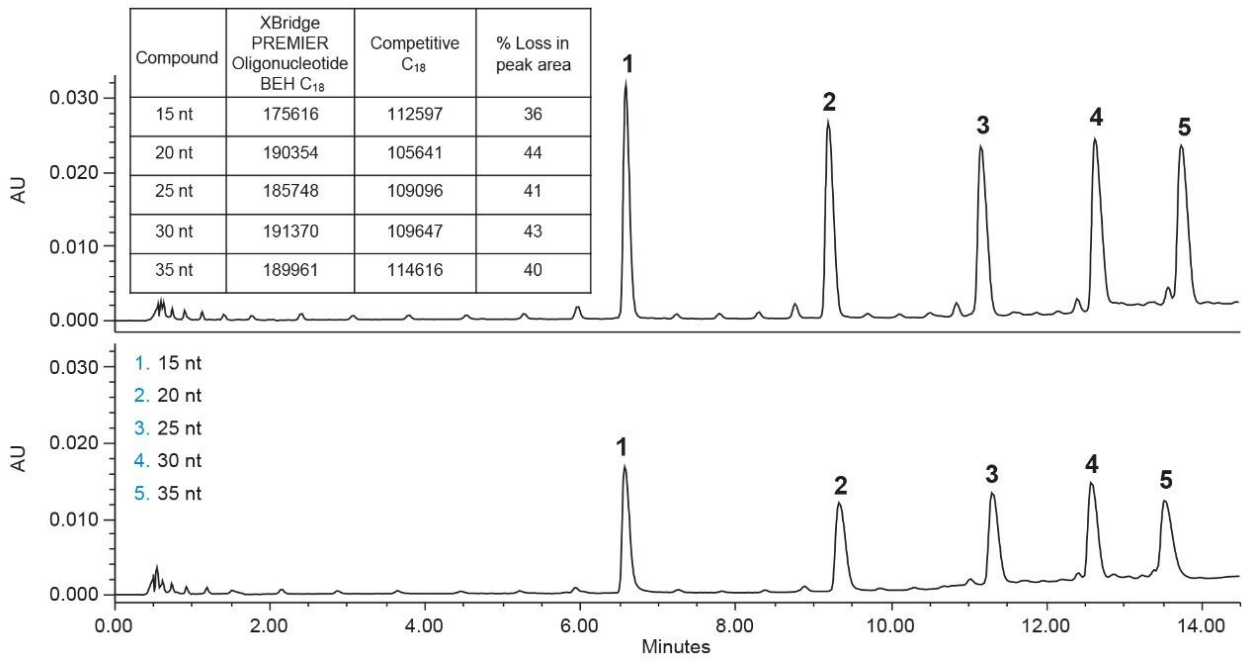


*Figure 1. Separation of five oligonucleotides on an XBridge PREMIER Oligonucleotide BEH $C_{18}$ Column (top) and a competitive $C_{18}$ column (bottom). Peak areas and % loss of peak area is shown in the table. An acetonitrile gradient was employed using 25 mM hexylammonium acetate (pH 7.0) on column. Column temperature was set at 60 °C, with UV detection at 260 nm. Method details are outlined in the MassPREP OST Standard Care and Use manual.*[1]

As shown in the figure, the competitor column showed decreased peak areas for all five of the oligos. On average, the competitor column yielded a peak area 40% lower than the XBridge PREMIER Oligonucleotide BEH $C_{18}$ Column. Additionally, peak tailing for most of the oligos was worse on the competitor column. Particularly of interest is the 35 nt which co-elutes with an impurity on the competitor column but resolves on the PREMIER column. With its HPS column hardware, the PREMIER column addresses undesired

secondary interactions. A PREMIER system was not employed for this separation, but a significant level of performance is still gained by employing the PREMIER column. Additional performance and robustness gains are likely to be found by pairing a PREMIER column with a PREMIER system.

## Conclusion

The Waters PREMIER brand of columns reduces the adsorptive interactions between analytes and column hardware surfaces. While problematic secondary interactions can occur across a wide range of applications, separation of oligonucleotides is an important and ever relevant area of separation science. Oligonucleotides are generally very sensitive to metallic surfaces as a result of their structure which can lead to significant challenges when it comes to recovery (peak area) and achieving good peak shape. In this work, an XBridge PREMIER Oligonucleotide BEH $C_{18}$ Column was compared to a competitor column based on conventional stainless-steel hardware. The PREMIER column yielded higher peak areas and sharper peaks compared to the competitor column. Switching to PREMIER columns will provide better separations and more accurate results. Even more benefits can be found by using PREMIER columns on a PREMIER LC system.

## References


1. Waters MassPREP OST Standard Care and Use Manual, 715001677EN < https://www.waters.com/webassets/cms/support/docs/715001677.pdf> .


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