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Taking Advantage of MaxPeak™ High Performance Surfaces Technology (HPS) in Multi-Dimensional Liquid Chromatography for Enhanced Recovery of a Phosphorothioated Oligonucleotide

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

Abstract

Liquid Chromatography (LC) systems and column hardware designed with stainless-steel or other alloys have been shown to lead to diminished performance of metal sensitive analytes due to nonspecific adsorption. Compromised performance can be exhibited through distorted peak shape, poor recovery, and greater variability in data interpretation. ACQUITY™ Premier Columns and systems with MaxPeak HPS Technology minimize analyte-surface interactions and have been shown to improve sensitivity and enable more reliable quantitation for a host of analytes that are otherwise difficult to analyze. Although these benefits have been observed with analytes such as peptides, released glycans, and oligonucleotides within the biopharmaceutical landscape, current literature has been focused on traditional one-dimensional separations. In this proof-of-concept study,

two different analytes are evaluated to show the advantages that MaxPeak Premier Solutions can provide when analytical methods are extended to a Multi-Dimensional separation space.

Benefits

- Performance of a non-metal sensitive analyte is consistent between Premier and non-Premier MDLC platforms
- Recovery of a metal sensitive analyte is conserved between the first and second dimensions of an ACQUITY Premier Multi-Dimensional System

Introduction

ACQUITY Premier Columns and systems featuring MaxPeak High Performance Surfaces (HPS) Technology have been shown to improve data quality for analytes containing electron-rich functional groups, which can otherwise be difficult to analyze due to their high affinity for metal surfaces. While many traditional LC systems and column hardware are designed with stainless-steel or other alloys, MaxPeak HPS Technology provides an organic/inorganic barrier between the analyte and metal surface that works to mitigate nonspecific adsorption. While this technology has proven benefits across many different analytes and application areas, current literature has been focused on one-dimensional (1D) analytical methods. Because of the known advantages of MaxPeak Premier Solutions, it stands to reason that if this technology was extended to Multi-Dimensional Liquid Chromatography (MDLC), that performance gains could be further enhanced in comparison to traditional columns and systems.

This work compares two MDLC configurations, one system designed with MP35N, a nickel cobalt alloy, and a second system comprised of MaxPeak HPS Technology. Proof-of-concept RPLC - RPLC experiments were designed so that the same reversed-phase separation was run in 1D and 2D, which allowed peak area to be readily compared within a single system as well as between instrument platforms. After confirming similar results between the two MDLC platforms with a non-metal sensitive analyte, a metal sensitive analyte was then used to demonstrate that MaxPeak HPS Technology could enhance recovery in a scenario where the analyte could not be recovered when using stainless-steel columns and an MDLC system having a metal flow path.

The ACQUITY Premier Multi-Dimensional System

Results and Discussion

Two MDLC systems were configured, one containing MP35N (ACQUITY UPLC™ H-Class PLUS Bio System with 2D Technology) and one containing MaxPeak HPS Technology (ACQUITY Premier Multi-Dimensional System). Both systems were designed using the same LC components: a QSM in the first dimension, a BSM in the second dimension, and optical detectors placed inline in both dimensions. To facilitate running the same reversed-phase separation in both dimensions, a single heart-cut was used to transfer the analyte of interest to the second dimension while at-column-dilution (ACD) was used to dilute the high organic content required for 1D elution and to allow the analyte to re-focus on the head of the 2D column. Stainless-steel ACQUITY UPLC BEH™ C_{18} Columns, 130 Å, 1.7 µm, 2.1 x 50 mm (p/n: 186002350 < https://www.waters.com/nextgen/global/shop/columns/186002350-acquity-uplc-beh-c18-column-130a-17--m-21-mm-x-50-mm-1-pk.html>) were used with the ACQUITY UPLC H-Class PLUS Bio System with 2D technology while ACQUITY Premier oligonucleotide BEH C_{18} Columns, 130 Å, 1.7 µm, 2.1 x 50 mm (p/n: 186009484 < https://www.waters.com/nextgen/global/shop/columns/186009484-acquity-premier-oligonucleotide-c18-column-130a-17--m-21-x-50-mm.html>) were used in combination with the ACQUITY™ Premier Multi-Dimensional System. All columns were conditioned through high sample loading prior to use in MDLC studies. A single instrument set-up was used to evaluate recovery of both a non-metal sensitive analyte, sulfadimethoxine, and a metal sensitive analyte, a fully phosphorothioated antisense oligonucleotide, Gem 91.

Sulfadimethoxine was used to first confirm that the MDLC systems performed similarly under routine conditions. Because sulfadimethoxine does not have a high affinity for metal surfaces, the expectation is that analyte recovery, or peak area, should be conserved between each dimension of an individual system as well as between the two instrument platforms. A standard mixture containing sulfadimethoxine supplied at 1 mg/mL in acetonitrile (ACQUITY/Quattro micro or Quattro Premier MS Start-up Solution Kit, p/n: 700002741 < https://www.waters.com/nextgen/global/shop/standards--reagents/700002741-acquity---quattro-micro-or-quattro-premier-ms-start-up-solution-.html>) was diluted to 0.01, 0.001, and 0.0001 mg/mL in water. In Figure 1, TUV peak area is reported in both the first and second dimension of each MDLC system over a triplicate injection series. Peak area is repeatable within each system, suggesting that a negligible amount of the analyte is lost through nonspecific adsorption, even at low concentrations. Furthermore, reported peak area values are comparable between instrument platforms and are within the standard deviation of the independent separations.

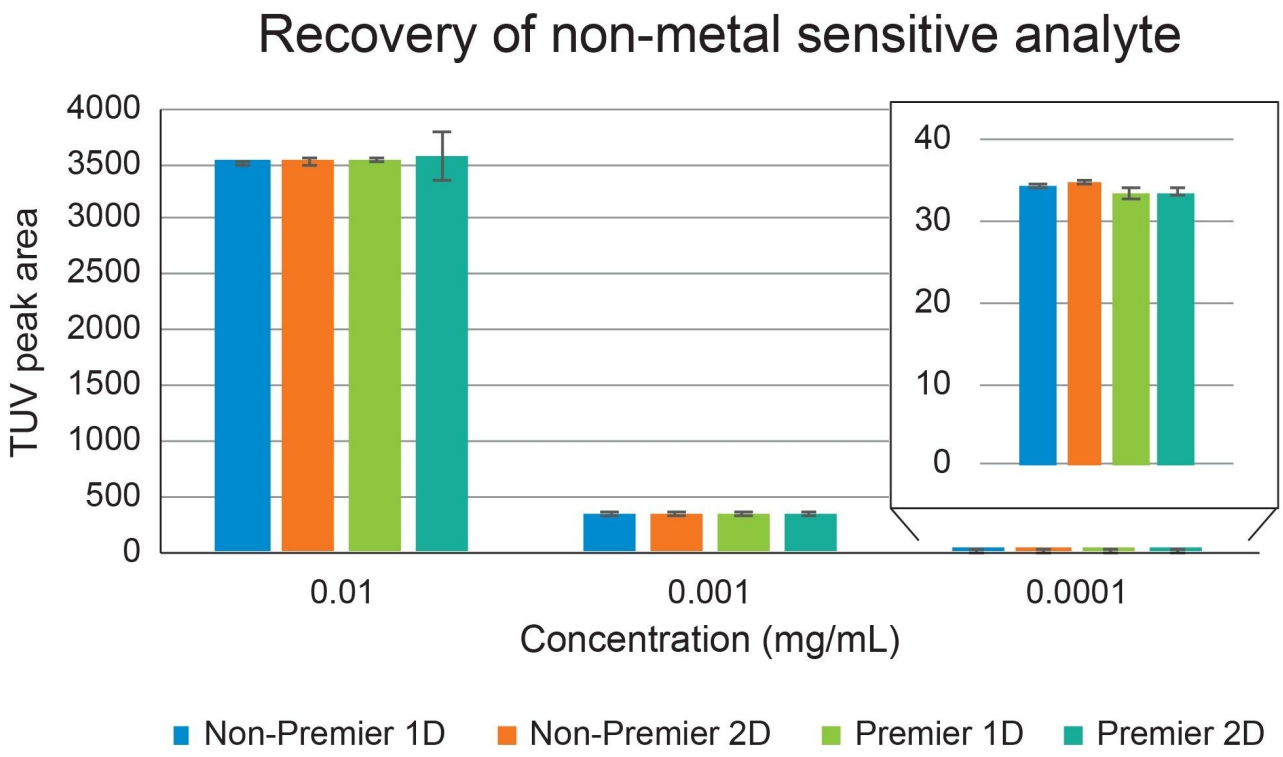

Figure 1. Recovery of sulfadimethoxine, a non-metal sensitive analyte in 2D (N=3). A reversed-phase gradient from 5 to 95% B over five minutes where MPA is 0.1% v/v FA in water and MPB is 0.1% v/v FA in acetonitrile was run in the first dimension. A heart-cut was used to transfer sulfadimethoxine to the second dimension where ACD (100% MPA) was used to reduce the organic percentage required for elution. The same reversed-phase gradient was then used for the second-dimension separation.

After confirming that each of the MDLC systems performed similarly, recovery was further evaluated using a triplicate injection series of Gem 91. Our previous work has shown that MaxPeak HPS Technology can be used to improve recovery and extend the dynamic range of Gem 91 when evaluated using 1D analytical methods.[1] Leveraging this prior knowledge, a three-point dilution series of Gem 91 (10.4 pmol, 1.04 pmol, and 0.104 pmol) was used to illustrate the benefits of MaxPeak Premier solutions for a metal sensitive analyte (Figure 2). When analyzing the Gem 91 dilution series first in 1D, the stainless-steel column and MP35N MDLC system showed a 25% (10.4 pmol) and 75% (1.04 pmol) reduction in peak area when compared to the MaxPeak Premier solution. At the lowest concentration evaluated, Gem 91 could not be detected without MaxPeak HPS Technology. When analyzing 2D peak area, approximately 70% of the analyte was lost to nonspecific adsorption between the first

and second dimension when using stainless-steel columns and a MP35N MDLC system. This is in comparison to the MaxPeak Premier solution where sample loss is negligible between the first and second dimension, regardless of sample load.

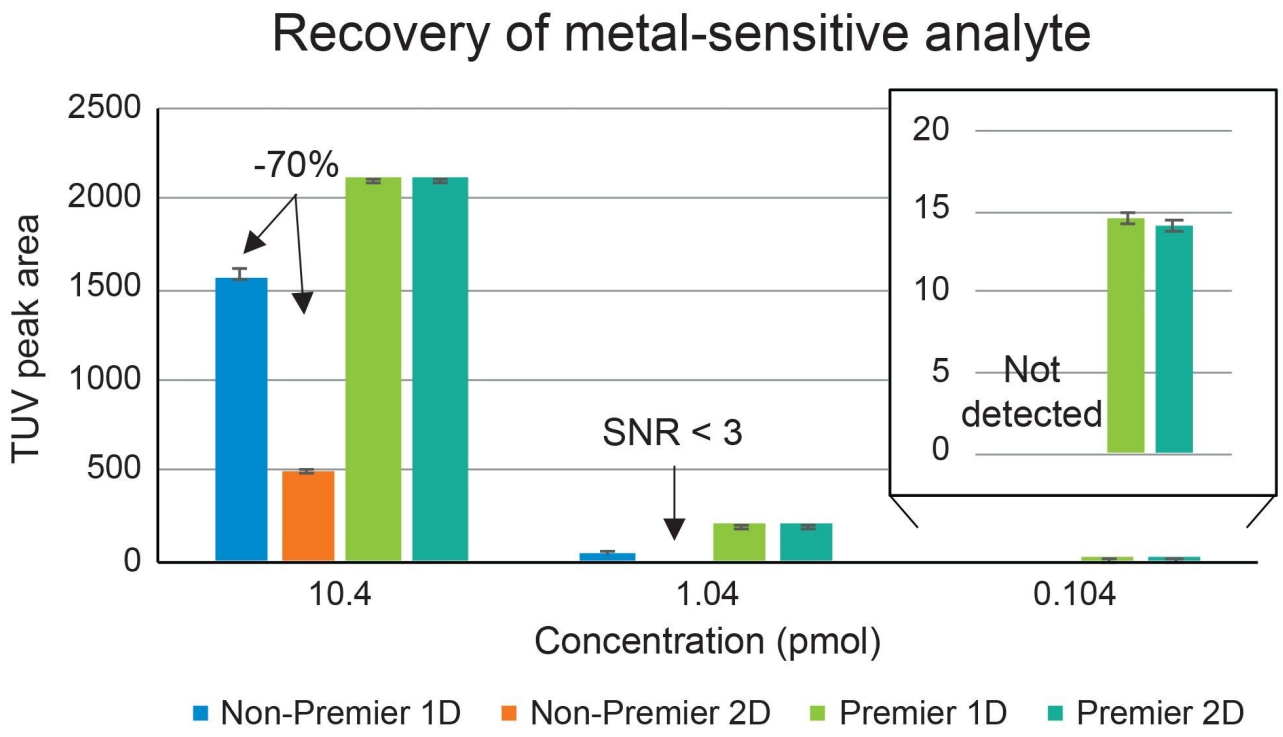

Figure 2. Recovery of Gem 91, a metal sensitive analyte in 2D (N=3). A reversed-phase gradient from 30 to 40% B over ten minutes where MPA is 8.6 mM TEA, 100 mM HFIP, pH ~8.25, and MPB is 50–50 MPA in MeOH was run in the first dimension. A heart-cut was used to transfer Gem 91 to the second dimension where ACD (100% MPA) was used to reduce the organic percentage required for elution. The same reversed-phase gradient was then used for the second-dimension separation.

Conclusion

MDLC has been used throughout the biopharmaceutical industry and is useful for applications that require

additional selectivity, speed, or sensitivity where 1D separations fall short. The decisive benefits of MaxPeak HPS Technology have been extended to MDLC to enhance recovery where stainless-steel columns and an MDLC system having a metal flow path further exacerbate analyte loss due to nonspecific adsorption. MaxPeak HPS Technology effectively mitigated analyte-surface interactions to show peak area could be conserved between the 1D and 2D analyses of a metal sensitive analyte while also maintaining performance of a non-metal sensitive analyte.

References

1. Koshel BM, Birdsall RE, Yu YQ. Improving Recovery and Quantitation of Oligonucleotide Impurities Using ACQUITY Premier with MaxPeak HPS Technology. Waters Application Note 720007238. April 2021.

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ACQUITY Premier System with Multi-Dimensional Technology <https://www.waters.com/waters/global/nav.htm?cid=135093306>

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720007602, April 2022