

BENEFITS OF USING HYBRID SURFACE TECHNOLOGY IN LC ANALYSIS OF OLIGOSACCHARIDES

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INTRODUCTION

Recently, a novel hybrid surface technology, the MaxPeak™ High Performance Surfaces (HPS), has been developed and incorporated into LC instruments and columns to mitigate metal analyte adsorption in liquid chromatography (LC). The MaxPeak HPS provides a hybrid organic-inorganic layer (or barrier) on the metal surfaces to mitigate the interaction between analytes and metals.⁽¹⁻⁴⁾

Significantly improved LC performance, such as increased peak intensity, reduced peak tailing, and more consistent results have been demonstrated for various analytes, such as peptides, oligonucleotides, phosphoglycans, sugar phosphates, organic acids, and phospholipids, by using MaxPeak HPS incorporated LC systems and columns.

The scope of this work is to investigate the impacts of MaxPeak HPS to the analysis of galactooligosaccharides (GOS) under the conditions of AOAC Official Method 2021.01 (First Action).

EXPERIMENTAL

LC conditions:

LC System/Column Setup:

- Setup without MaxPeak HPS: ACQUITY™ H-Class Plus with ACQUITY FLR Detector/ACQUITY UPLC™ Glycan BEH™ Amide Column (1.7 μm 2.1 x 150mm).
- Setup with MaxPeak HPS: Arc™ Premier System (BSM) with ACQUITY FLR Detector/XBridge™ Premier Glycan BEH Amide Column (2.5 μm 2.1 x 150mm).

Col. temp.: 25 °C
 Inj. Vol.: 2 μL
 Run time: 60 min.
 Mobile phase A: Acetonitrile
 Mobile Phase B: 100 mM ammonium formate, pH 4.4
 FLR Detector: Excitation λ = 330 nm; Emission λ = 420 nm
 Elution program: (see table below)
 Software: Empower™ 3 CDS

Time (min)	Flow (mL/min)	%A (Acetonitrile)	%B (Ammonium formate 100 mM, pH 4.4)
0	0.6	88.0	12.0
7.0	0.6	88.0	12.0
17.0	0.6	85.0	15.0
21.0	0.6	85.0	15.0
36.0	0.6	72.6	27.4
44.0	0.6	54.0	46.0
44.1	0.3	54.0	46.0
44.5	0.3	30.0	70.0
49.5	0.3	30.0	70.0
52.0	0.3	88.0	12.0
54.0	0.6	88.0	12.0
60.0	0.6	88.0	12.0

Standards and sample preparation:

The standard and sample preparation procedures specified in AOAC Official Method 2021.01 were followed. A schematic of the preparation of dextran calibration ladder, standard and samples is shown in Figure 1.

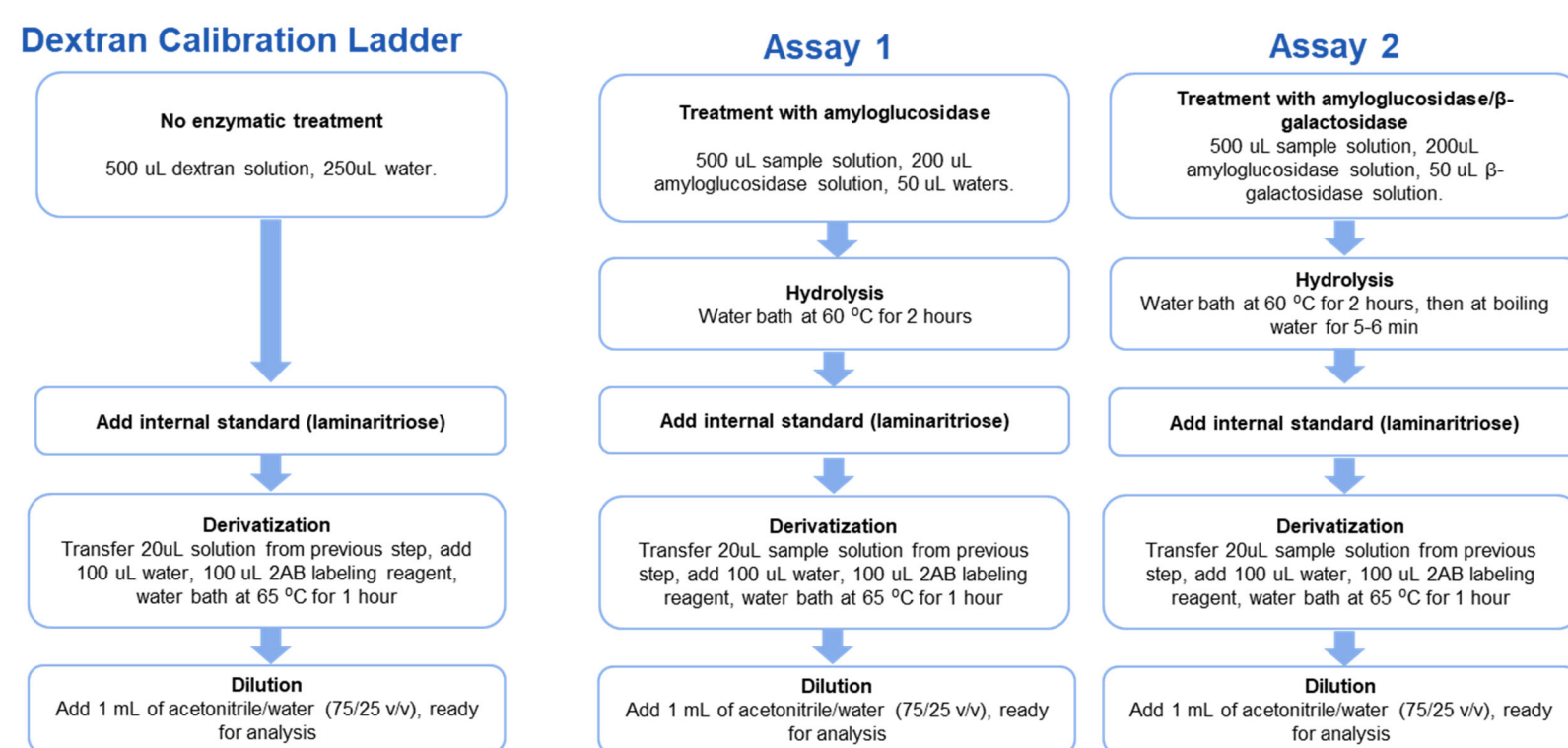


Figure 1. Schematic of the standard and sample preparation.

RESULTS

1) Comparison of oligosaccharides LC-FLR responses with and without MaxPeak HPS

The dextran calibration ladder was derivatized with 2-AB labeling reagent, then separated on different system/column setups with and without MaxPeak HPS. The system/column setup without MaxPeak HPS consisted of an ACQUITY H-Class System with an ACQUITY FLR Detector and an XBridge Glycan BEH Amide Column (2.5 μm, 2.1 x 150mm). The system/column setup incorporated with the MaxPeak HPS consisted of an Arc Premier (BSM) System with an ACQUITY FLR Detector and an XBridge Premier Glycan BEH Amide Column (2.5 μm, 2.1 x 150mm).

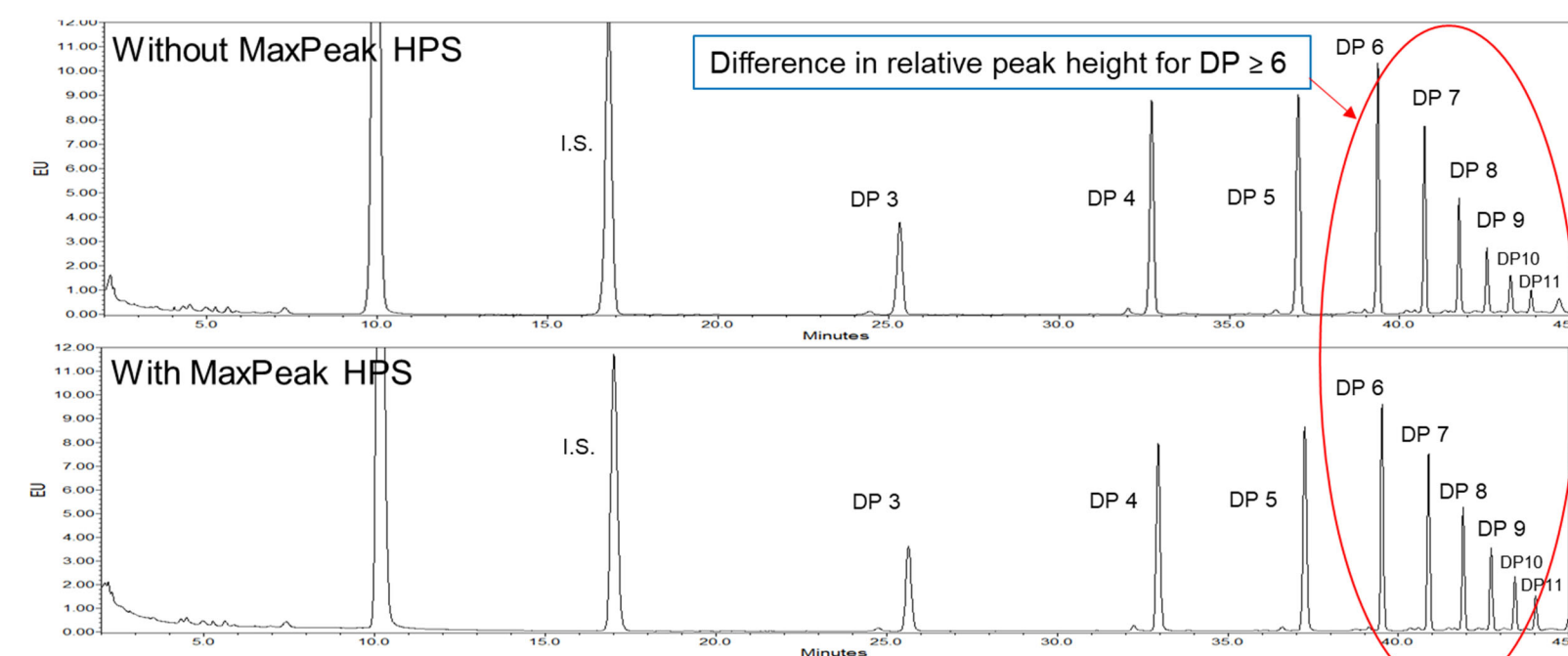


Figure 2. Comparison of oligosaccharides (2AB labeled Dextran) LC-FLR responses obtained in system/column setups with and without MaxPeak HPS.

2) Effects of the length of oligosaccharide

Figure 3 shows that only at degree of polymerization (DP) 6 and higher, dextran exhibited different results on two different system/column setups. On the setup with MaxPeak HPS, the responses were higher and consistent from injection to injection. On the setup without MaxPeak HPS, the response were lower and inconsistent from injection to injection.

On setup without MaxPeak HPS, the more injections, the higher the response. However, even after repeated injections (20 injections), the responses (for DP ≥ 6) were still not as high as those from the MaxPeak HPS system/column setup.

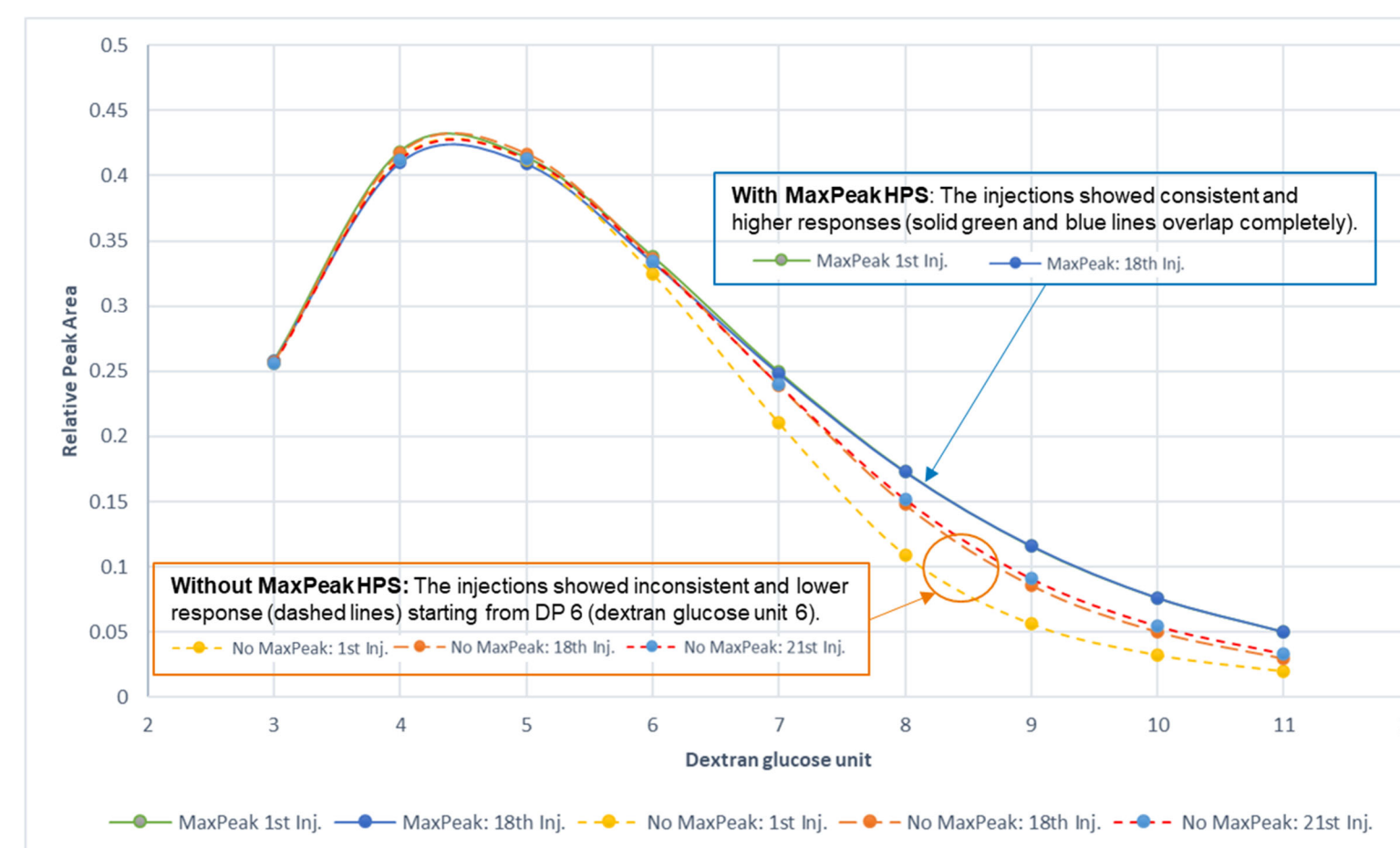


Figure 3. Plot of dextran relative peak area (relative to an internal standard) from various injections vs glucose unit obtained on different system/column setups with and without MaxPeak HPS.

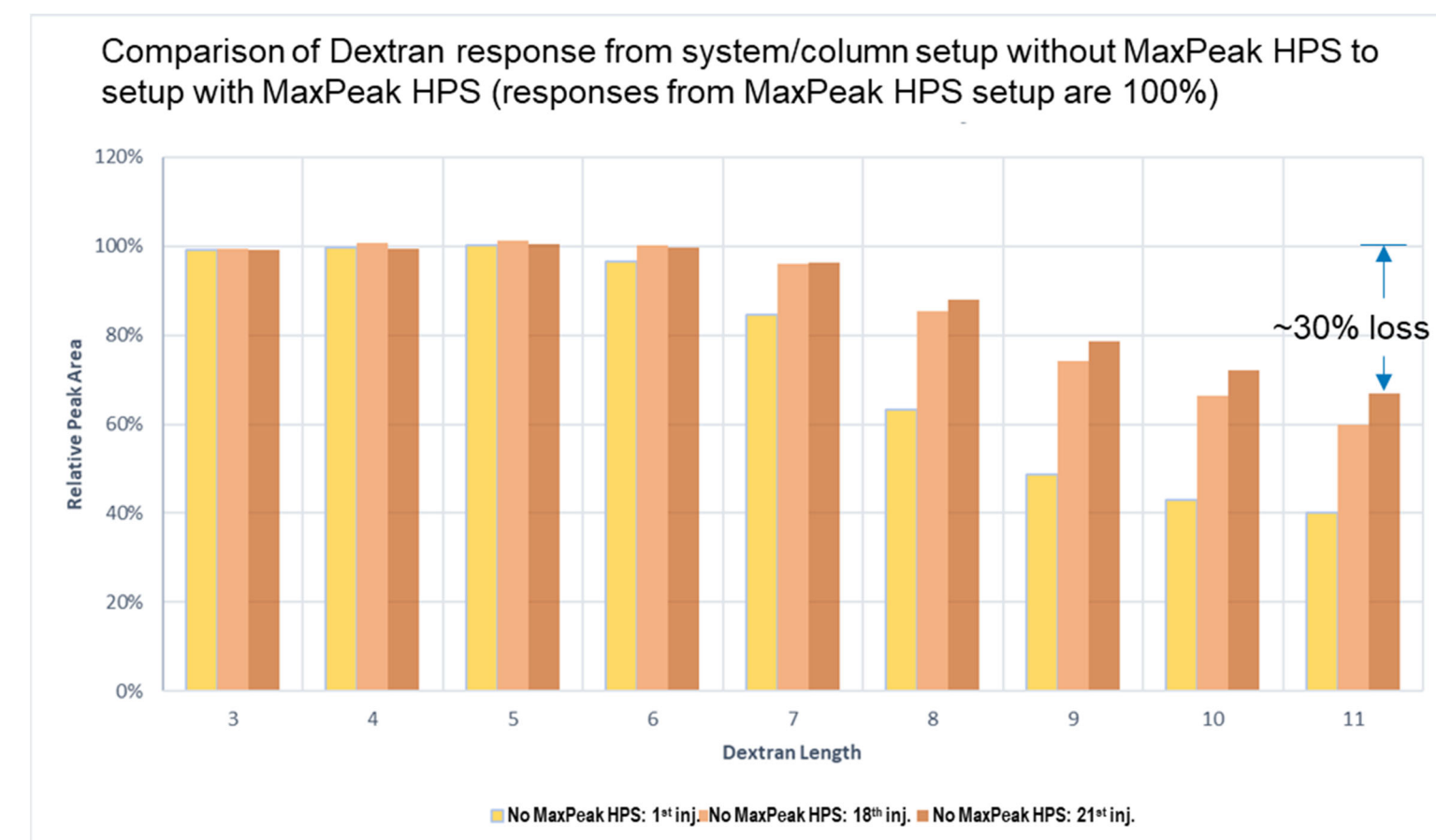


Figure 4. Plot of relative peak areas (relative to corresponding peak area from MaxPeak HPS setup) with Dextran length (DP). Inconsistent and less peak area obtained from system/column setup without MaxPeak HPS. The higher the DP, the more loss of analyte.

3) Carry-over investigation

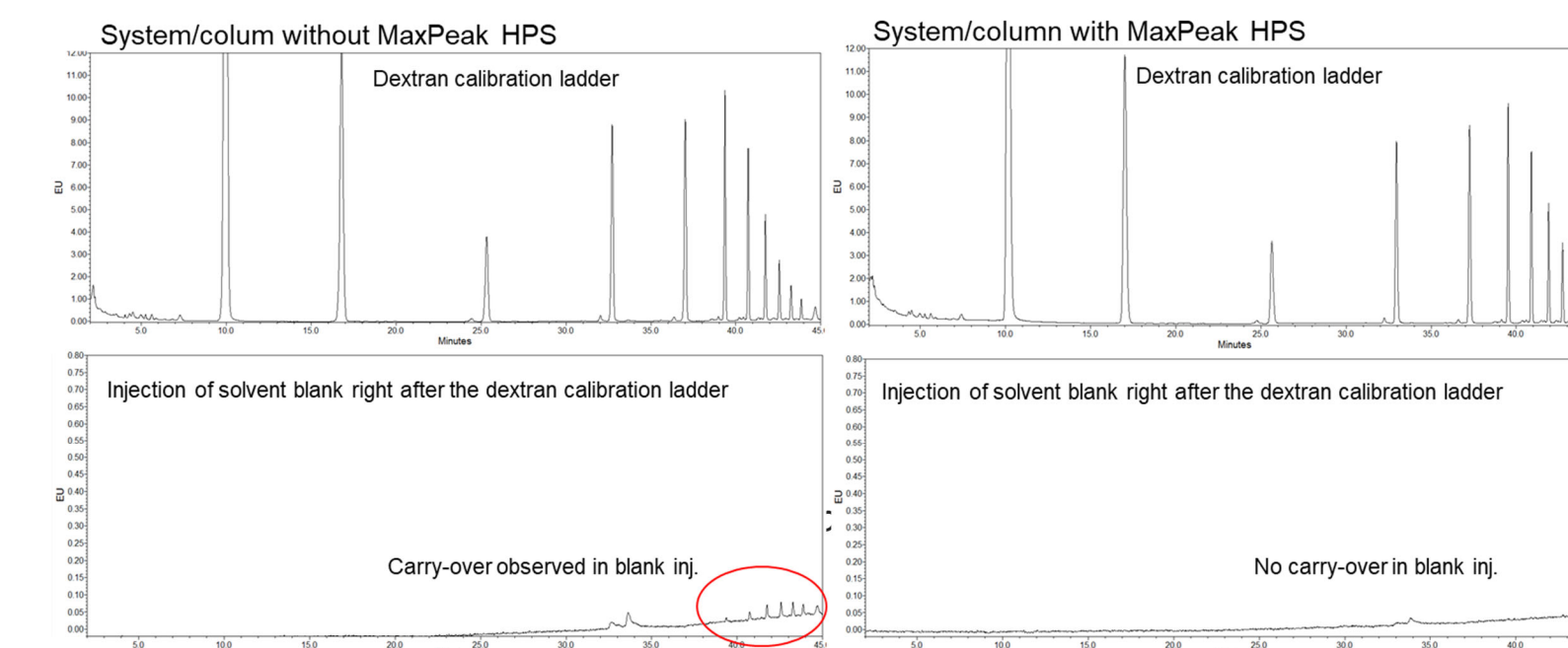


Figure 5. Comparison of the carry-over investigation on system/column setups with and without MaxPeak HPS. Carry-over was eliminated on setup with MaxPeak HPS.

4) The quantification results of GOS was not impacted by the use of MaxPeak HPS

- There was no difference in GOS determination results was observed between setups with and without MaxPeak HPS. This was probably because that majority of GOS chains were found smaller than DP 6 after sample treatment (enzymatic hydrolysis).
- The dextran calibration ladder was used for a qualitative purpose (as molecular weight markers). The loss of dextran and the carry-over did not directly affect the GOS quantitation.

CONCLUSION

- The LC of dextran oligosaccharides (2AB labeled, DP ≥ 6) under the conditions of AOAC Method 2021.01 has been improved by using MaxPeak Premier Technology (Arc Premier System and XBridge Premier Glycan BEH Amide Column) that incorporated MaxPeak HPS.
- The improvements include higher and more consistent responses and no carry-over for 2AB labeled dextran oligosaccharides with DP 6 and higher.
- This study reveals that MaxPeak HPS can benefit the LC of oligosaccharides and its related analysis.

References:

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