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Note d'application

Mitigation of Non-Specific Adsorption on HPLC Systems Using MaxPeak™ Premier Columns

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Abstract

Non-specific adsorption can be a major problem in recovery of analytes that interact with the surfaces present in LC systems. Some compounds, such as oligos and certain metabolites interact with the metal surfaces of the LC system and column, leading to total analyte loss or peak shape disturbances. These interactions can be mitigated with a variety of approaches, but the implementation of MaxPeak High-Performance Surfaces (HPS) Technology, featured in MaxPeak Premier Columns, eliminates these issues without sacrificing column performance or lifetime. This technology has previously only been available with select column chemistry and particle sizes, limiting the applicability of the hardware.

MaxPeak Premier columns have been recently released with select 3.5 µm particle size column chemistries, allowing an analyst using HPLC systems to take advantage of the improved hardware. To demonstrate the scalability of these columns, as well as comparing the effect of NSA on HPLC systems, a previously developed method was scaled from a 1.7 µm ACQUITY™ Premier Column to a 3.5 µm XBridge™ Premier Column on two different HPLC systems. Standard stainless steel hardware columns were also used to check the benefit of the MaxPeak HPS hardware on the separation. The scaled method performs similarly on both HPLC systems compared to UPLC™ with similar improvements in peak areas for the metal sensitive compounds.

Benefits

- Improved recovery for metal sensitive analytes on two different HPLC systems
- Comparable separation performance for a scaled method from UPLC to HPLC
- Comparable improvements in peak area seen on UPLC and HPLC systems due to HPS hardware

Introduction

Non-specific adsorption (NSA) can be a troubling issue in a lot of workflows. Having different results from injection to injection, or poor recovery of an analyte during LC analysis can lead to costly rework and investigations. However, MaxPeak Premier Columns are specifically designed to alleviate that concern. This technology uses a modified surface, which incorporates a hybrid organic-inorganic chemistry, that prevents interaction between analytes and the metal surfaces in both LC columns and hardware.[1] One workflow that has been unable to take advantage of this technology is QC workflows that use HPLC instruments. QC workflows rely heavily on robust methods that produce reproducible results to process samples and release batches. Failure of an analysis can directly impact product availability and therefore sales.

MaxPeak Premier Columns, previously available with sub-2 µm and 2.x µm particles, have finally been developed using an HPLC particle size. By providing this technology in HPLC column configurations, a method developed upstream of the QC workflow can be seamlessly transferred from UPLC to HPLC instrumentation. In order to demonstrate this, a method developed on an ACQUITY Premier BEH™ C_{18}, 1.7 µm Column using an ACQUITY UPLC H-Class System was transferred to an XBridge Premier BEH C_{18} 3.5 µm Column using two different HPLC systems, an Alliance HPLC and an Arc™ HPLC System. The method was initially developed to highlight the different selectivity available in the MaxPeak Premier Column offerings.[2] Comparisons between standard hardware and MaxPeak HPS hardware were performed at each step of the transfer. To aid in transferring the method, the Waters Column Calculator was used. The Columns Calculator automatically does all of the math needed for scaling including adjustments to flow rate, injection volume, and gradient times.[3,4] The transfer was successfully completed with no change in performance between UPLC and HPLC platforms. Additionally, the use of MaxPeak Premier columns show comparable benefits compared to stainless steel columns on both UPLC and HPLC systems. For critical assays, the use of MaxPeak Premier Columns on HPLC systems can drastically improve reproducibility.[5–7]

Experimental

Sample Description

Stock solutions were created and combined to create a mixture of thiourea (4 µg/mL), metoprolol (300 µg/mL), dipropyl phthalate (40 µg/mL), amitriptyline (10 µg/mL), prednisone (10 µg/mL), hydrocortisone phosphate (10 µg/mL), and dexamethasone phosphate (10 µg/mL) using 95:5 10 mM ammonium formate pH 3.0: Acetonitrile.

LC Conditions

LC system:	ACQUITY UPLC H-Class Plus System with PDA Detector Arc HPLC System with TUV Detector Alliance HPLC System with TUV Detector
Detection:	UV @ 254 nm
Columns:	ACQUITY UPLC BEH C_{18} Column, 1.7 µm, 2.1 x 50 mm (p/n:186002350) ACQUITY Premier BEH C_{18} Column, 1.7 µm, 2.1 x 50 mm (p/n: 186009452) XBridge BEH C_{18} Column 3.5 µm, 4.6 x 100 mm (p/n: 186003033) XBridge Premier BEH C_{18} Column 3.5 µm, 4.6 x 100 mm (p/n: 186010660)
Column temperature:	30 °C
Sample tempertaure:	10 °C
Injection volume:	2.0 µL (UPLC) and 19.2 µL (HPLC)

Flow rate:	0.5 mL/min (UPLC) and 1.165 mL/min (HPLC)
Mobile phase A:	10 mM Ammonium Formate pH 3.0
Mobile phase B:	Acetonitrile
UPLC gradient conditions:	Linear ramp from 5–95% B in 5.3 minutes. Total run time 7 minutes
HPLC gradient conditions:	Isocratic hold at 5% B for 1.91 minutes. Linear ramp from 5–95% B in 21.83 minutes. Total run time 30.74 minutes.

Data Management

Chromatography software:	Empower™ 3 Feature Release 5

Results and Discussion

The previously developed method was first tested on an ACQUITY UPLC H-Class System with both stainless steel and MaxPeak Premier Columns packed with the same batch of ACQUITY UPLC BEH C_{18} 1.7 µm particles. Figure 1 shows the results obtained on both stainless steel and MaxPeak HPS hardware.

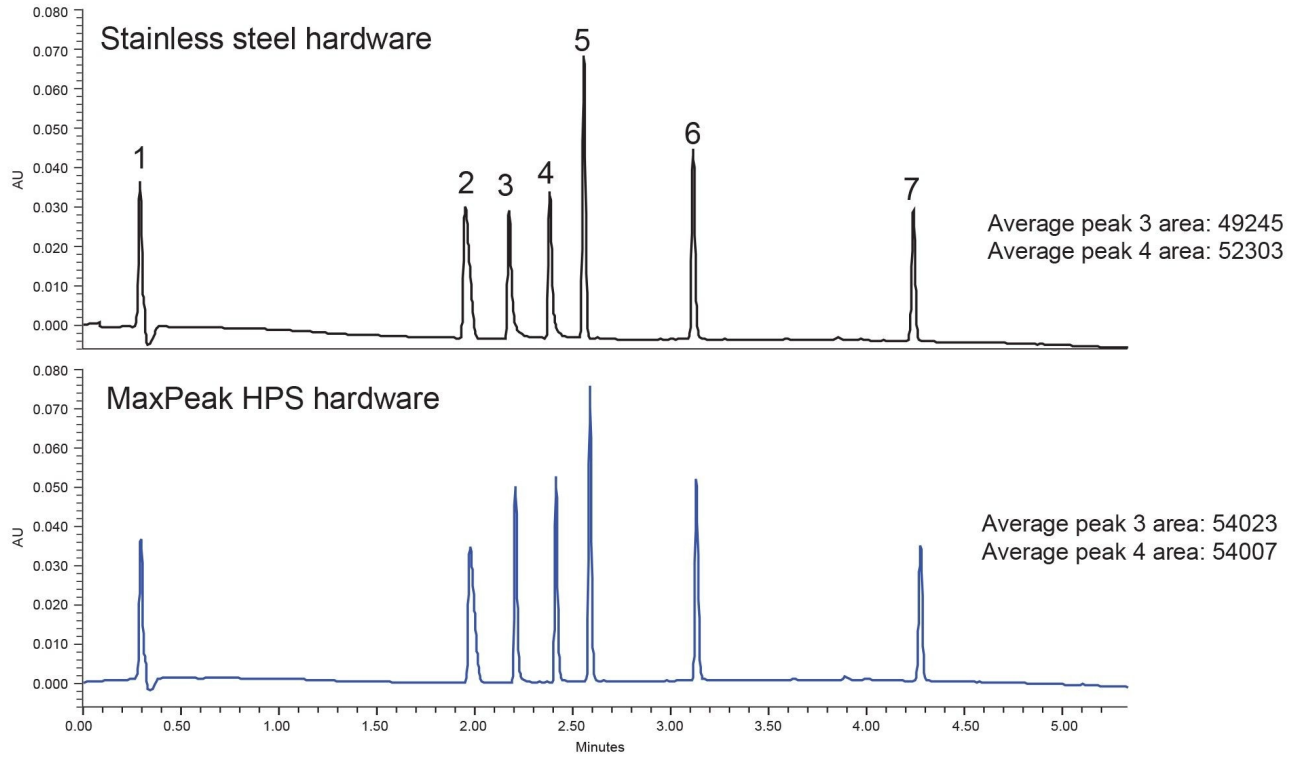

Figure 1. Separation of the test mixture on an ACQUITY UPLC H-Class Plus with PDA using two 2.1 x 50 mm, 1.7 µm particle size columns packed with the same batch of stationary phase. Column hardware varied between the tested columns as indicated. 1) Thiourea, 2) Metoprolol, 3) Hydrocortisone Phosphate, 4) Dexamethasone Phosphate, 5) Prednisone, 6) Amitriptyline, 7) Dipropyl phthalate.

The results obtained here are comparable to those obtained in the original work.[2] The MaxPeak HPS hardware shows better peak shape, higher peak areas, and taller peaks for the two metal sensitive compounds, dexamethasone phosphate and hydrocortisone phosphate. Additionally, no negative effect was seen for the other compounds present. As the results are comparable to older work, the method was next scaled to two different HPLC systems.

To ensure the method was scaled properly, the ACQUITY Columns Calculator was used. The Column Calculator generates new method conditions based on the input of the original column, original method details like gradient times, and the new column dimensions. The calculator can also recommend gradient holds based on different system dwell volumes to ensure that the new method is equivalent to the original method. Figure 2 shows the

Columns Calculator with the original method details input on the left side, and the calculated values for the new method on the right.

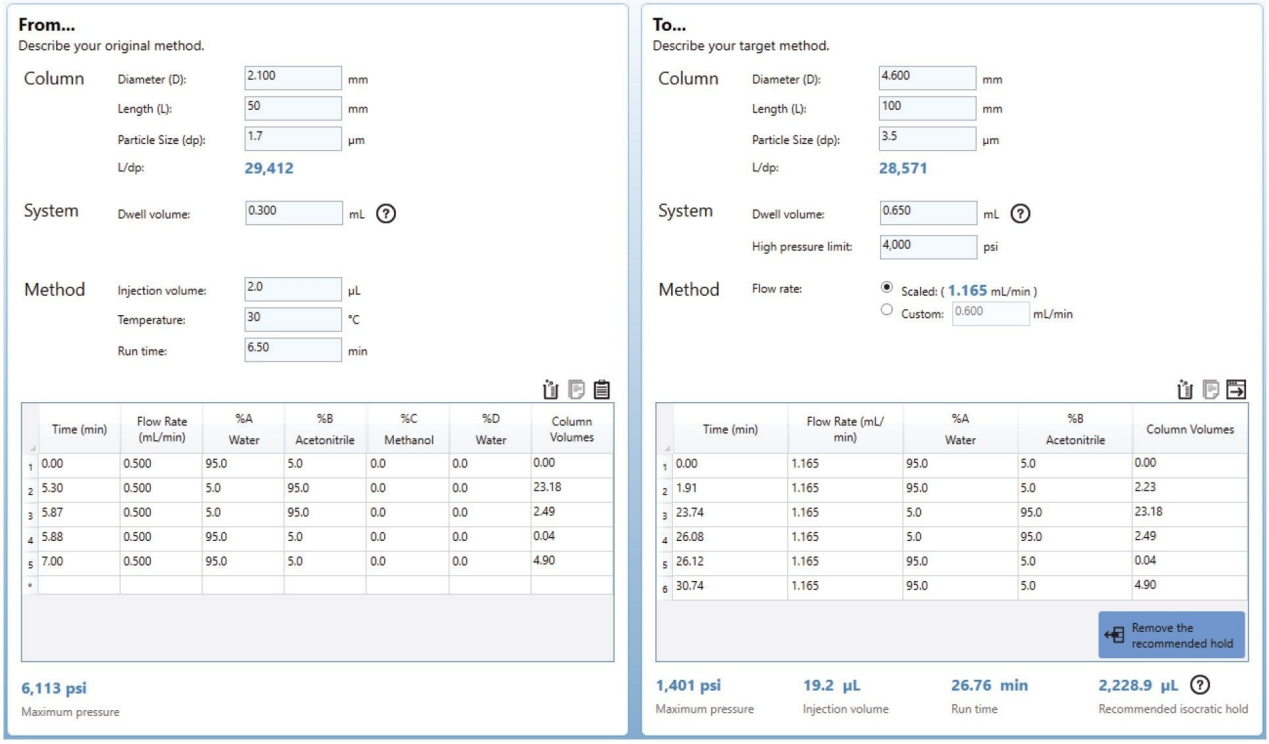

Figure 2. ACQUITY Columns Calculator. Original method details including gradient table, column dimensions, system dwell are input on the left. New column dimensions and system dwell (if applicable) are input on the right, with the new gradient table, flow rate, and injection volume calculated automatically.

System dwell volumes were not calculated for this experiment, and the values input were taken from the supporting literature of the respective systems. As shown, the new gradient table on the bottom right of Figure 2 indicates that a recommended hold of 1.91 minutes is needed. This is to ensure that the dwell volume seen on the H-Class Plus system matches what is seen with both HPLC systems. For this transfer, we are moving to 4.6 x 100 mm 3.5 µm particle size columns. This configuration was chosen as it has a comparable L/dp ratio, or length to particle size, as the original testing conditions. By matching L/dp during a method transfer, overall performance should be comparable between the two separations.

Using the new method conditions, two different HPLC systems were tested with both stainless steel and

MaxPeak HPS hardware columns. Both columns were packed in-house and used the same batch of stationary phase. First an Alliance HPLC was used. The Alliance HPLC is an older HPLC system which is still in heavy use in different industries, such as QC. Figure 3 shows the results obtained for both columns tested using the Alliance HPLC System, which included a Tunable UV Detector (TUV).

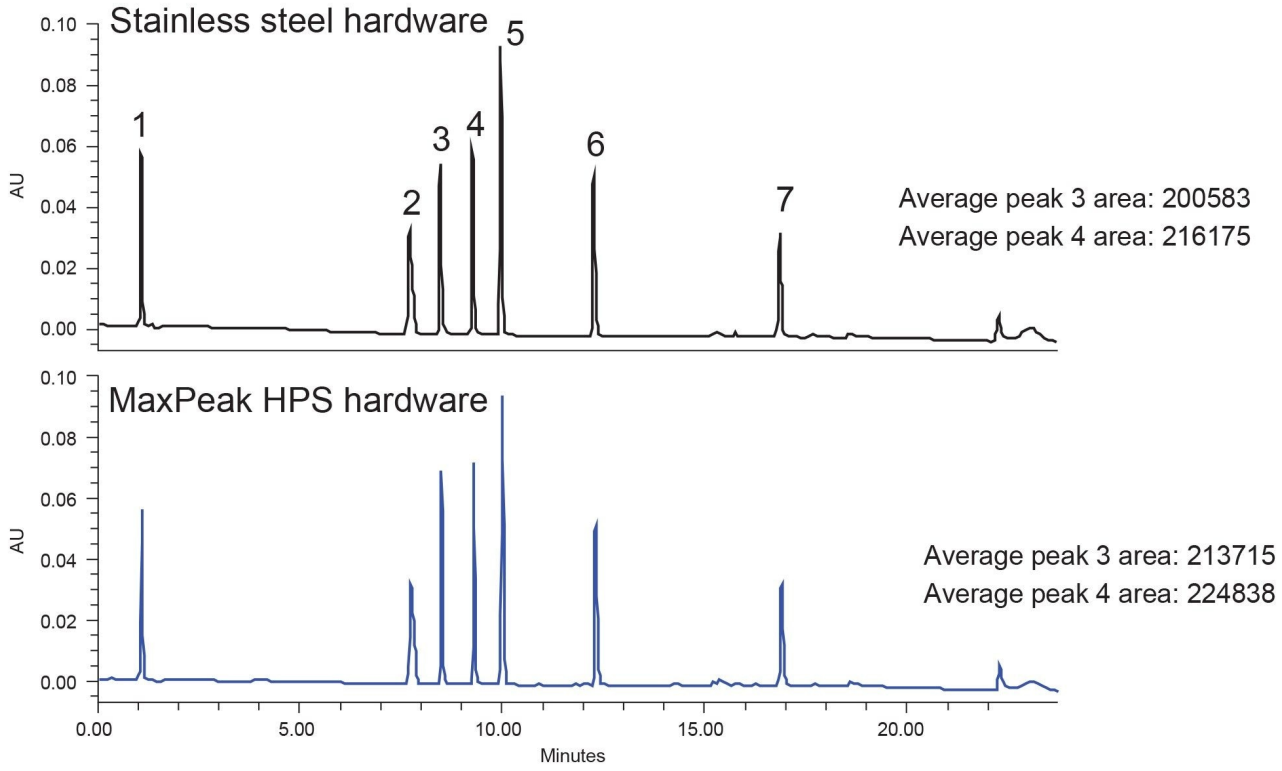

Figure 3. Separation of the test mixture on an Alliance HPLC with TUV using two 4.6 x 100 mm, 3.5 µm particle size columns packed with the same batch of stationary phase. Column hardware varied between the tested columns as indicated. 1) Thiourea, 2) Metoprolol, 3) Hydrocortisone Phosphate, 4) Dexamethasone Phosphate, 5) Prednisone, 6) Amitriptyline, 7) Dipropyl phthalate.

As shown, the overall results obtained on the Alliance HPLC are quite similar to that seen on the H-Class Plus system. Peak areas are higher, and run times are longer on the Alliance HPLC, which is expected given the increased injection volume and changes to column configuration. Comparing the stainless-steel hardware to MaxPeak HPS hardware, the results show a ~5% increase in peak area, similar to that on the H-Class Plus system. Further confirmation of the benefits of MaxPeak HPS hardware on HPLC was obtained using an Arc

HPLC System, which is a newer HPLC system. The same method conditions, injection volumes, and columns were used on both the Alliance HPLC and Arc HPLC Systems. Figure 4 shows the results obtained on the Arc HPLC System.

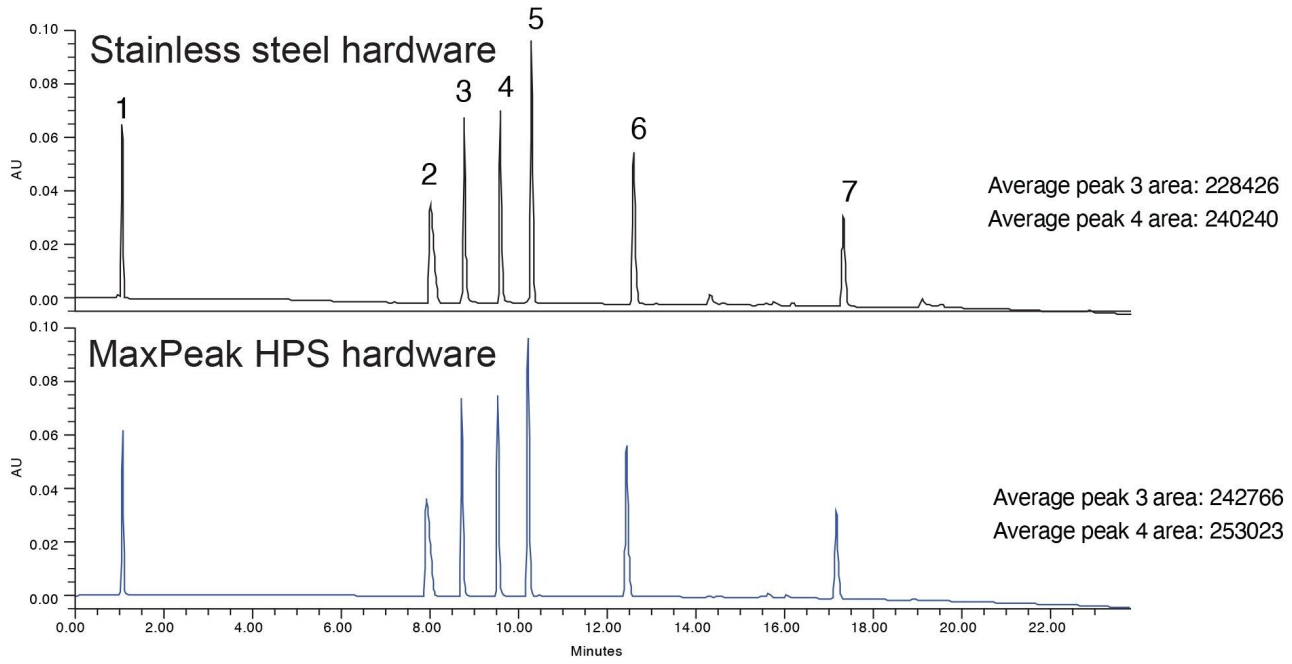

Figure 4. Separation of the test mixture on an Arc HPLC with TUV using two 4.6 x 100 mm, 3.5 µm particle size columns packed with the same batch of stationary phase. Column hardware varied between the tested columns as indicated. 1) Thiourea, 2) Metoprolol, 3) Hydrocortisone Phosphate, 4) Dexamethasone Phosphate, 5) Prednisone, 6) Amitriptyline, 7) Dipropyl phthalate.

As expected, the MaxPeak HPS hardware in the MaxPeak Premier Column shows improved peak areas for the metal sensitive compounds, while having no negative effect on the other probes in the mixture. Combined with the results seen on the Alliance HPLC System, MaxPeak HPS hardware has been proven to provide benefit for metal sensitive compounds on HPLC systems, allowing a method created using ACQUITY Premier Columns to be scaled all the way to HPLC when necessary.

Conclusion

MaxPeak High-Performance Surfaces (HPS) technology is used for both column and LC instrument hardware to mitigate unwanted interactions between analytes and exposed metal surfaces. These interactions can cause poor analyte recovery due to adsorptive loss, as well as peak shape issues and poor reproducibility. The ACQUITY Premier Column line has been successfully used in a variety of workflows to improve separation performance, however, the ability to scale these methods to HPLC columns and systems has not been possible.

Select XBridge and XSelect™ Premier Columns are available with 2.5 µm and 3.5 µm particle sizes, making them appropriate for UHPLC or HPLC analyses. This allows analysts to scale and transfer their method from UPLC columns and instruments to HPLC, a common occurrence when moving a QC test from design and development. To demonstrate this, a previously developed method was scaled from UPLC columns on an ACQUITY H-Class Plus System to HPLC columns on both an Alliance HPLC and an Arc HPLC. Regardless of system or particle size, the use of HPS technology for the column hardware improved analyte recovery for metal sensitive compounds, without changing performance for other probes. By simply changing to a MaxPeak Premier Column, HPLC analyses can gain the benefits of improved reproducibility, analyte recovery, and improved peak shape.

References

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