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Note d'application

# Increased Peak Recovery and Peak Shape of Phosphorylated Compounds Using ACQUITY Premier and XBridge Premier Columns Compared to Stainless-Steel Columns

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## Abstract

The use of ACQUITY Premier LC Columns, featuring MaxPeak High Performance Surfaces, has been shown to increase the recovery of and improve the peak shape of phosphorylated compounds.[1] The work shown here was done in two parts to further demonstrate the benefits of MaxPeak Premier Columns. The first part demonstrates that ACQUITY Premier LC Columns can provide an improvement in separation quality even when using a non-Waters LC system. Four $C_{18}$ columns, installed on an Agilent 1290 LC system, were used to separate a panel of small molecules which included two phosphorylated compounds. The second part of this work shows that the improvements gained with sub-2-µm can also be achieved with 2.5 µm particles. Two $C_{18}$ columns were installed on a standard ACQUITY UPLC System and the same mixture was examined. Regardless of particle size, ACQUITY Premier Columns show significant improvement for the analysis of phosphorylated compounds compared to columns constructed with stainless-steel hardware.

## Benefits

- Higher peak area and sharper peak shape for phosphorylated compounds
- Out-of-the-box performance without the need for passivation or conditioning
- Scalable column chemistries to provide benefits across platforms

## Introduction

Adsorption onto metal surfaces, particularly the column hardware, is a common issue in small molecule analytical workflows due to interaction between anionic functional groups (carboxylates and phosphates) and the exposed metal. This phenomenon, commonly referred to as non-specific adsorption, can lead to reduced peak area and in extreme cases a complete loss of analyte. Peak shape disturbances can also occur, most notably increased tailing. There are ways to mitigate the adsorptive effect – such as using passivation additives like medronic acid[2] – however, these mitigation techniques are time consuming, come with their own compromises, and are not always effective.

## Experimental

### Sample Description

Neat standards of each probe were created and combined as described. A sample diluent of 90:10 acetonitrile:water was used to create the final sample mixture. Thiourea (5 µg/mL), metoprolol tartrate (300 µg/mL), dipropyl phthalate (40 µg/mL), amitriptyline (10 µg/mL), prednisone (10 µg/mL), hydrocortisone sodium phosphate (10 µg/mL), and dexamethasone sodium phosphate (10 µg/mL) was injected onto the system.

### LC Conditions

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|---|---|
| LC system: | Agilent 1290 Infinity I (sub-2-µm particle size columns),<br>ACQUITY UPLC H-Class (2–3 µm particle |

| | |
|---|---|
| | size column) |
| Detection: | UV detection at 254 nm,<br>DAD on Agilent 1290,<br>PDA on ACQUITY UPLC |
| Vials: | TruView LCMS Certified Clear Glass (p/n:186005668CV) |
| Column(s): | ACQUITY Premier BEH C18, 1.7 µm, 2.1 x 50 mm,<br>ACQUITY Premier HSS T3, 1.8 µm, 2.1 x 50 mm,<br>XBridge Premier BEH C18, 2.5 µm, 2.1 x 50 mm,<br>Competitor A EC-C18, 1.9 µm, 2.1 x 50 mm,<br>Competitor A HPH-C18, 1.9 µm, 2.1 x 50 mm,<br>Competitor A HPH-C18, 2.7 µm, 2.1 x 50 mm |
| Column temp.: | 30 °C |
| Sample temp.: | Off |
| Injection volume: | 2 µL |
| Flow rate: | 0.50 mL/min (sub-2-µm)<br>0.34 mL/min (2.x µm) |
| Mobile phase A: | 10 mM Ammonium formate pH 3.0 |

| | |
|---|---|
| Mobile phase B: | Acetonitrile |
| Gradient: | See Table |

## Gradient Table

| Time (min) | Flow (mL/min) | %A | %B | Curve |
|---|---|---|---|---|
| 0.00 | 0.50 or 0.34 | 95 | 5 | 6 |
| 5.30 or 7.79 | 0.50 or 0.34 | 5 | 95 | 6 |
| 6.30 or 9.26 | 0.50 or 0.34 | 5 | 95 | 6 |
| 6.40 or 9.41 | 0.50 or 0.34 | 95 | 5 | 6 |
| 8.00 or 11.80 | 0.50 or 0.34 | 95 | 5 | 6 |

## Data Management

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|---|---|
| Chromatography software: | Empower 3 Feature Release 4 |

# Results and Discussion

Waters MaxPeak Premier Columns with MaxPeak High-Performance Surface (HPS) Technology provide a robust and effective technique to avoid adsorption of metal-sensitive compounds. MaxPeak Premier Columns with HPS Technology are available across a range of Waters column chemistries, as well as in the Waters ACQUITY Premier System. While the best performance will be seen when combining MaxPeak Premier Columns with a MaxPeak Premier System, significant benefits can still be realized by pairing MaxPeak Premier Columns with any LC system, regardless of manufacturer. To demonstrate this, a panel of six probes were separated on four different $C_{18}$ columns using an Agilent 1290 chromatograph. Two ACQUITY Premier Columns and two competitor stainless-steel columns were tested in gradient mode with DAD detection. Figure 1 shows representative chromatograms of the separation, while Figure 2 shows a zoomed X-axis chromatogram to highlight peak shape

differences.

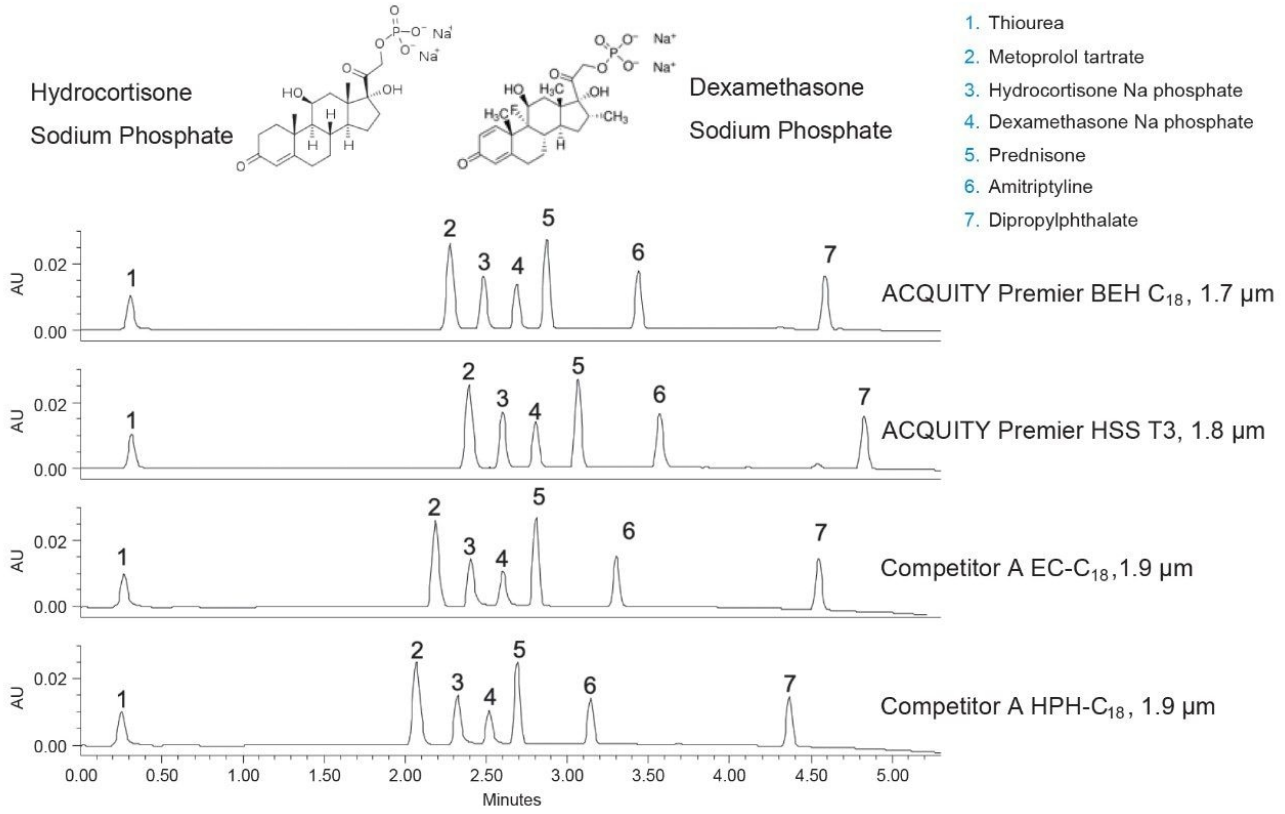


*Figure 1. Separation of six test probes using four sub-2-µm $C_{18}$ stationary phases on an Agilent 1290 chromatograph.*

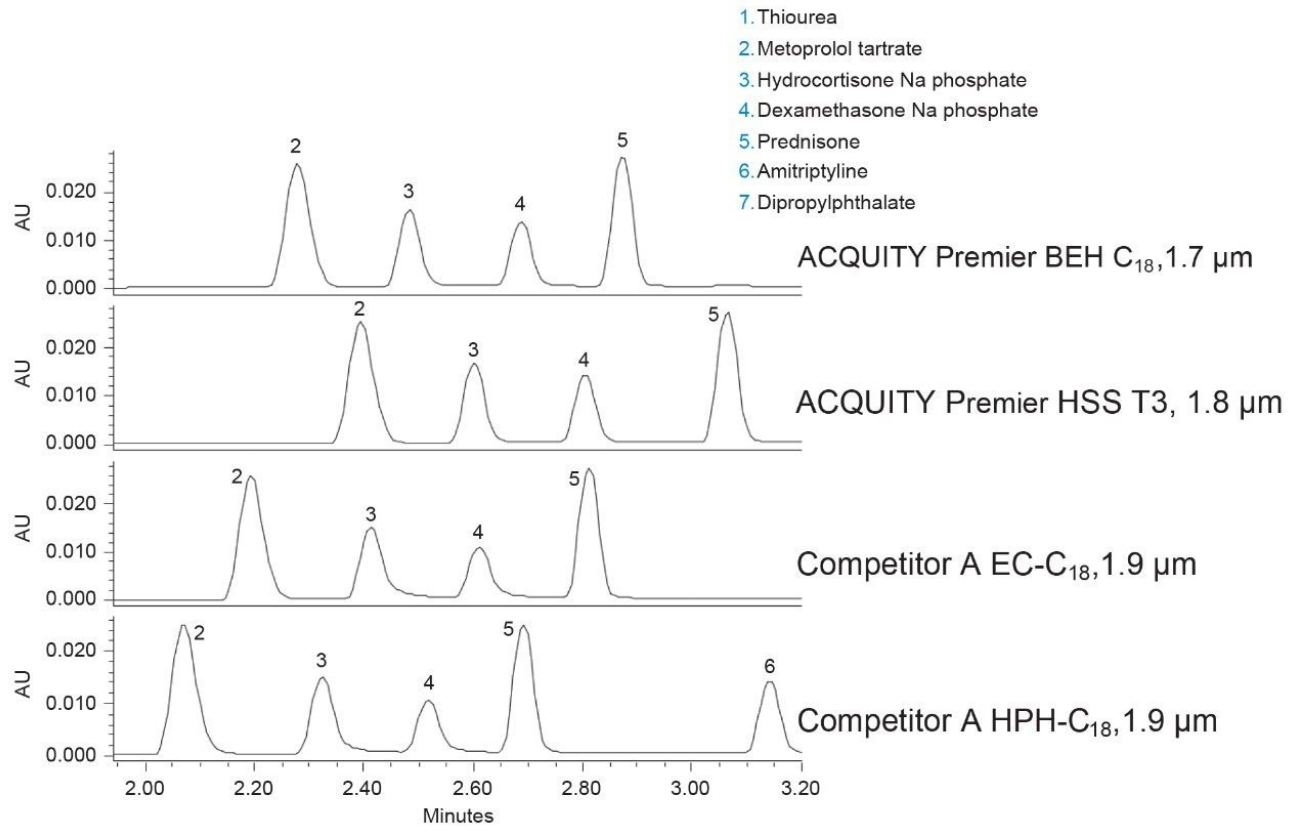


*Figure 2. Zoomed chromatogram of six compound panel analyzed on four sub-2-µm particle columns, showing the differences in peak shape for components 3 and 4, hydrocortisone and dexamethasone sodium phosphate, respectively.*

While performance was comparable for the non-metal-sensitive compounds in the test mix, analysis of the chromatography shows that for components 3 and 4 (hydrocortisone sodium phosphate and dexamethasone sodium phosphate) the MaxPeak Premier Columns drastically reduced tailing and significantly increased peak area. Compared to the competitor columns, USP tailing was reduced by approximately 0.2 units for the phosphorylated compounds, and an impressive peak area increase of ~30% was realized for dexamethasone sodium phosphate. By simply using the MaxPeak Premier Columns, a noticeable benefit can be achieved for these and other metal-sensitive analytes.

To further demonstrate the benefits of MaxPeak Premier Columns, the above separation was scaled to a larger particle size on an ACQUITY UPLC H-Class System. The method transfer did not include adjustments to gradient delay, but did incorporate changes to flow rate and gradient times consistent with a change in particle size.

Column dimensions were kept to 2.1 x 50 mm. Figure 3 shows the separation of the sample mixture on two 2–3 µm particle size columns, the XBridge Premier BEH $C_{18}$ and the competitor A HPH-$C_{18}$ columns using the ACQUITY UPLC H-Class. Figure 4 shows the same chromatogram with a zoomed baseline to further display the differences in peak shape.

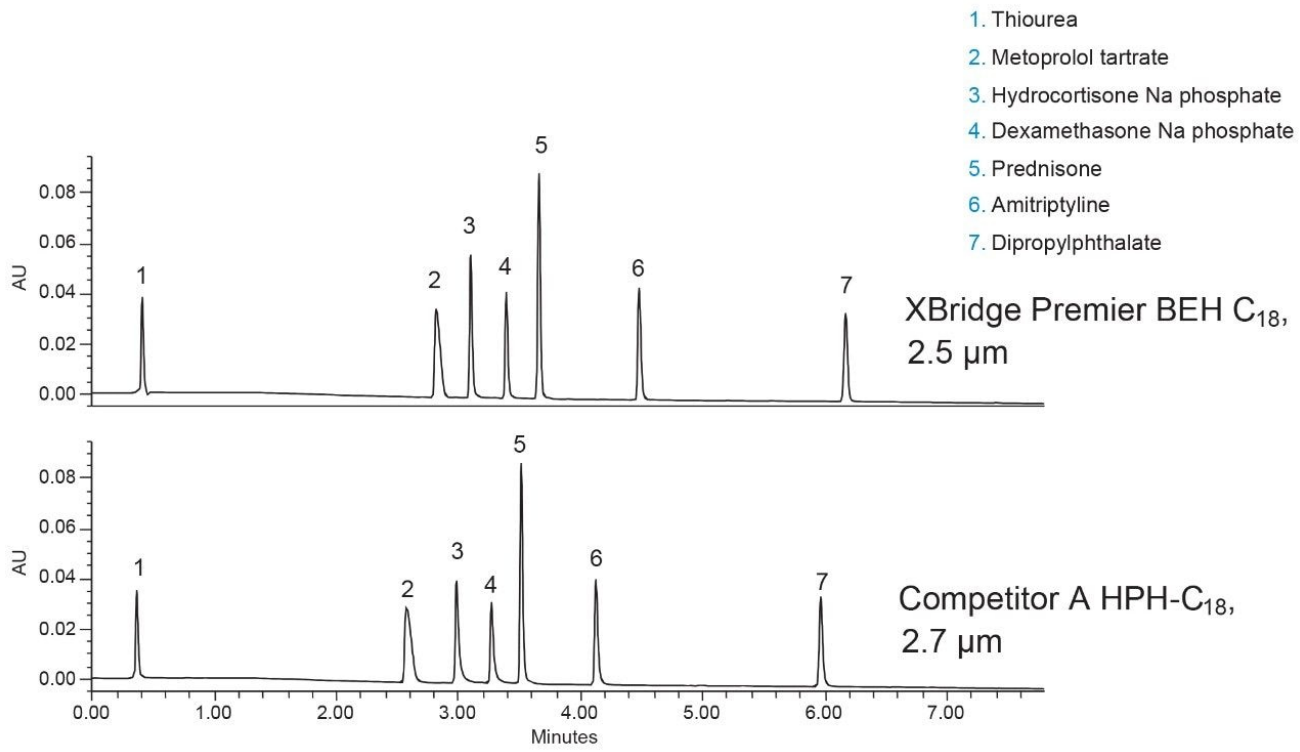


*Figure 3. Separation of six test probes using two 2–3 µm $C_{18}$ stationary phases on an ACQUITY UPLC H-Class System. Poor peak shape was observed for component 2 due to low system bandspreading of the instrument and secondary interactions between the cationic metoprolol analyte and the base particle of both materials.*

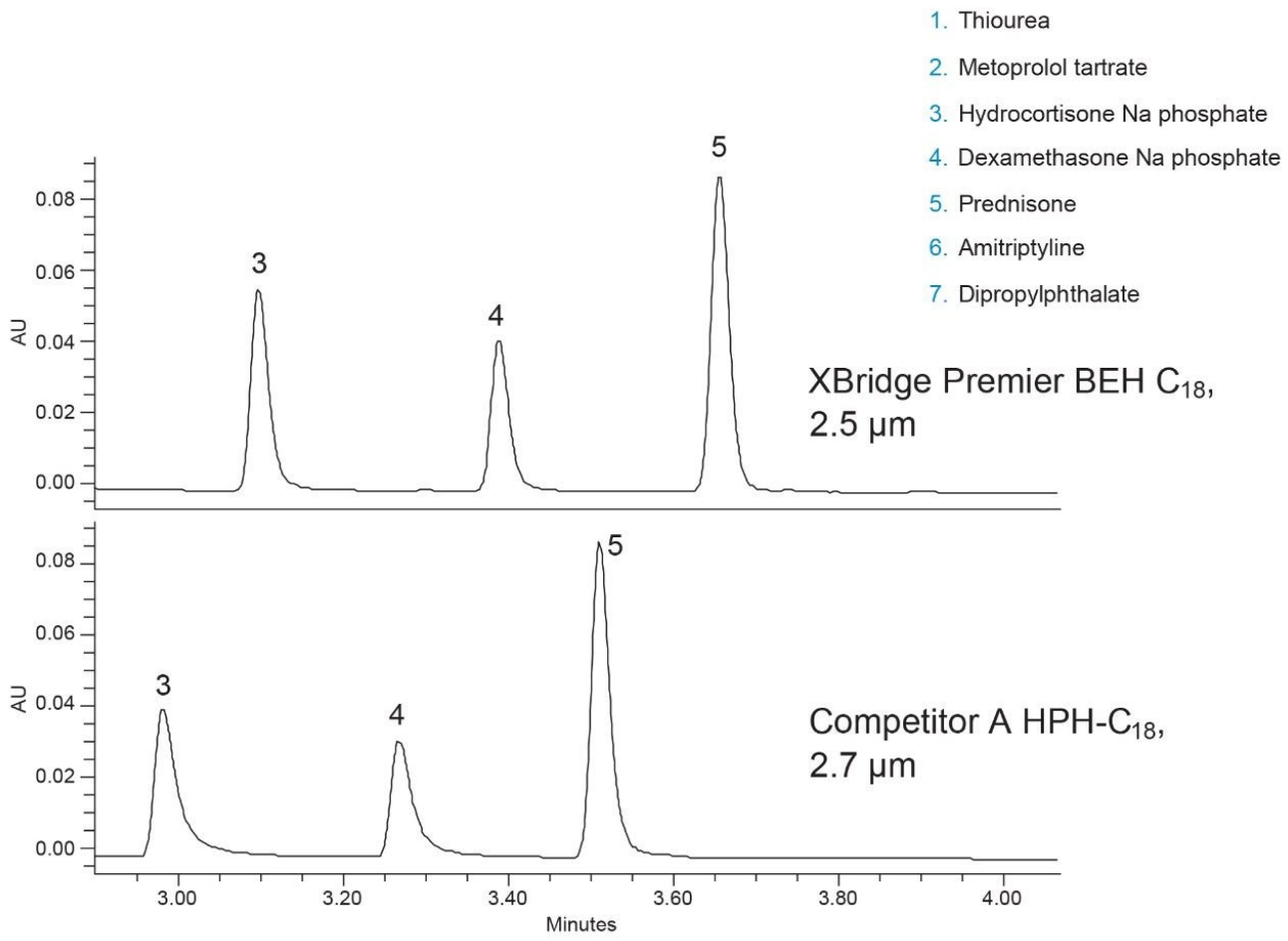


*Figure 4. Zoomed chromatogram of Figure 3 showing differences in peak shape and peak height for components 3 and 4, hydrocortisone and dexamethasone sodium phosphate, respectively.*

Even with a 2.5 µm particle size stationary phase, a MaxPeak Premier Coloumn  provides better peak shape for the phosphorylated compounds while maintaining performance for the neutral and basic probes. The basic probe metoprolol has poor peak shape on both materials due to the low system dispersion of the ACQUITY UPLC and secondary interactions with the base particles. A system with low dispersion is more susceptible to peak disturbances than systems with higher dispersion as the high dispersion system artificially widens the peaks, masking the secondary interactions. As seen earlier, the peak area for dexamethasone sodium phosphate dropped by ~30% when using the stainless-steel competitor column. The benefits of MaxPeak Premier Coloumn can be realized regardless of LC system or particle size but are most impactful when coupled with a MaxPeak Premier System.

## Conclusion

A mixture of six compounds, including two phosphorylated active pharmaceutical ingredients, was separated on a total of six columns under two test conditions. Four sub-2-µm columns, two MaxPeak Primier LC Columns and two conventional LC columns, were installed on an Agilent 1290 chromatograph. The MaxPeak Premier Columns showed decreased peak tailing, and ~30% higher peak area for the phosphorylated compounds compared to the conventional steel columns. The separation was then transferred to two 2–3 µm columns, an XBridge Premier BEH $C_{18}$ and a competitor $C_{18}$, using an ACQUITY UPLC H-Class. Similar results were seen with the larger particle size columns: MaxPeak Premier Columns improved overall separation quality. Regardless of particle size or chromatographic system, MaxPeak Premier Columns provide significant benefits for the separation and analysis of phosphorylated compounds. It is likely that benefits will also be found with their application to other acidic analytes.

## References


1. Hsiao, J.; Potter, O.; Van de Bittner, G.; Chu, T.; Yin, H. Improved LC-MS Methods for the Analysis of Anionic Analytes. Agilent Application Note, https://www.agilent.com/cs/library/posters/public/AGILENT_ASMS2018_WP526.pdf < https://www.agilent.com/cs/library/posters/public/AGILENT_ASMS2018_WP526.pdf> .
2. Boissel, C.; Walter, T. Improved Peak Shape and Wide Selectivity Range with ACQUITY Premier Columns. Waters Application Note, 720007014EN <https://www.waters.com/nextgen/us/en/library/application-notes/2020/improved-peak-shape-and-wide-selectivity-range-with-acquity-premier-columns.html> , 2020.


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MaxPeak Premier Columns <https://www.waters.com/waters/nav.htm?cid=135074078>

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