IMPROVED PURIFICATION OF PROBLEMATIC COMPOUNDS USING WATERS MAXPEAK™ PREMIER OBD™ PREPARATIVE COLUMNS WITH AN INTEGRATED INERT SURFACE

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INTRODUCTION

Whether the goal is to obtain enough intermediate for the next step in a synthetic scheme or to polish a final product, preparative liquid chromatography remains a primary technique for isolating compounds from complex mixtures. Recovering the target compound at the desired purity and yield in the least amount of time is of utmost importance for meeting ambitious project timelines. Unfortunately, problematic compounds complicate the purification, often requiring extra effort or rework. Isolation of such targets contained in crude sample mixtures causes prep separations to deteriorate and thereby put the purification success at risk.

Targets that seemingly stick, linger or disappear on the column, are compounds that many consider to be 'problematic' in purification. These "sticky" compounds are metal-sensitive targets that can adsorb to metal surfaces in the LC column, causing reduced sensitivity, poor peak shape, or limited peak detection,[1] which impact fraction triggering and recovery. Although work arounds, such as column pre-conditioning, can be implemented for isolating compounds known to be interactive with metal surfaces, this option can be time-consuming and lead to sample loss and excessive solvent waste.

Previous work discussed the enhancement of LC-MS/MS analysis of B-group vitamins using MaxPeak High Performance Surfaces Technology.[2] The methodology implemented was modified in these studies to illustrate how MaxPeak Premier OBD[3] Preparative Columns were employed to isolate four B vitamins and two unknowns from a commercially available vitamin beverage. The benefits of using small particle columns with inert surfaces for preparative isolations and the positive impact on purification processes will be discussed. The purification workflow presented provides a model that mirrors the strategies that might be used by the core support scientist who isolates many different types of compounds or the process development chemist performing impurity isolation.

METHODS

Vitamin Beverage Isolation Conditions:

Analytical Columns and flow rate: ACQUITY™ Premier BEH™ C_{18} and ACQUITY UPLC™ BEH C_{18} Columns; 1.7 µm, 2.1x50 mm; 0.35 mL/min

Prep Columns and flow rate: XBridge™ BEH C_{18} OBD Prep and XBridge BEH C_{18} Premier OBD Prep Columns, 130 Å, 5 µm, 10x150 mm; XBridge BEH C_{18} Premier OBD Prep Column, 130 Å, 3.5 µm, 10x100 mm; 5.4 mL/min

Mobile phase A: 20 mM Ammonium Formate, pH 5.05

Mobile phase B: Methanol

Wavelength: 270 nm

Sample: commercially available vitamin beverage; all experiments were conducted by injecting aliquots of the neat beverage. Figure 1 shows the B vitamins identified in the sample.

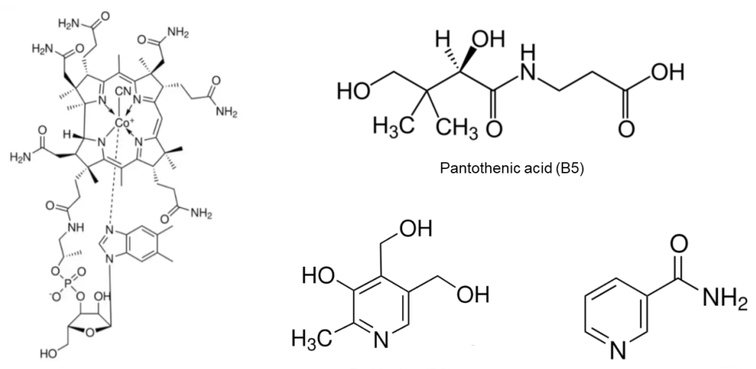

Figure 1. Chemical structures of the vitamins identified in vitamin beverage

Instrumentation

Analytical: ACQUITY UPLC H-Class System; Quaternary Solvent Manager, Sample Manager FTN-H, Column Manager, TUV Detector, ACQUITY QDa™ Detector

Prep: Waters AutoPurification™ System; 2545 Binary Gradient Module, 2767 Sample Manager, System Fluidics Organizer, 2998 Photodiode Array Detector

RESULTS AND DISCUSSION

High mechanical strength, extensive compatibility with chemicals, and ease of fabrication make stainless steel an attractive material for the manufacture of high-performance liquid chromatography columns and instruments. Despite these favorable characteristics, stainless steel can negatively impact the peak shape and recovery of some compounds.[1] Negatively charged molecules can interact ionically with the positively charged metal oxide surfaces of the column and LC flow path in neutral and acidic pH environments and lead to non-specific adsorption.[4] This non-specific adsorption (NSA) occurs with stainless steel and titanium, as well as other metal alloys. Metal sensitive compounds with strong acidic moieties like sulfates, phosphates, or carboxylates are prone to adsorption (Figure 2).

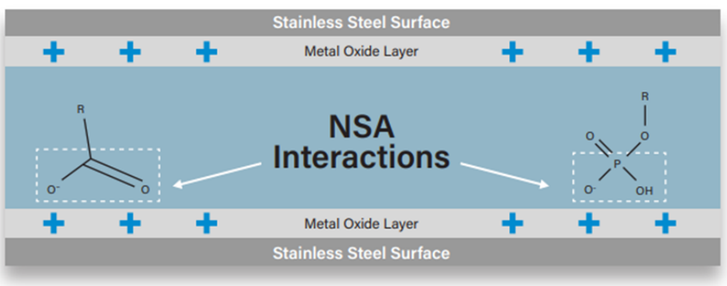

Figure 2. "Sticky" Non-Specific Adsorbers result in bad peak shapes and poor or inadequate fraction triggering, and can lead to compromised target purity and recovery.

Non-specific adsorption can cause practical problems in the purification lab, impeding efficiency and compromising both target purity and recovery. NSA may cause peak shape problems and inconsistent retention times and/or peak areas, missing peaks or low sensitivity, or increasing peak areas over time. Approaches that reduce or eliminate non-specific adsorption must be implemented for successful isolations. Preconditioning the column before use with sample or matrix, or passivating the column surfaces with acid or other metal-chelating additives in the mobile phase are time-consuming and unstable and may need to be repeated. Collecting the waste for each injection or using time-based fraction collection might also be used to minimize the risk of sample loss during isolation and purification. These strategies increase sample and waste handling, which can slow the purification process down. The easiest way to prevent non-specific adsorption on metal surfaces is use a column with an inert surface.

In these experiments, the vitamin beverage was first analyzed by UPLC using both a stainless steel ACQUITY UPLC BEH C_{18} Column and an ACQUITY Premier BEH C_{18} Column. The chromatographic profile was similar between the two columns. Peak area analysis indicated that four of the peaks had higher area counts on the Premier column than on the stainless steel column (Figure 3).

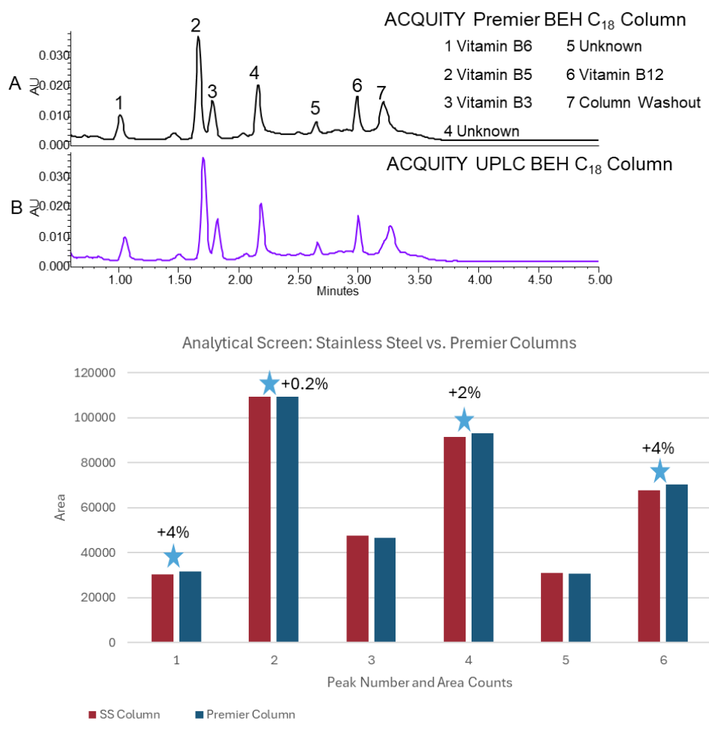

Figure 3. UPLC analysis of vitamin beverage (Inj vol 1 µL) on 2.1x50 mm, 1.7 µm ACQUITY BEH C_{18} stainless steel and Premier Columns. Gradient: hold @ 1%B for 0.25 min, 8%B in 1 min, 90%B in 1.25 min, hold for 0.5 min, 1%B in 0.05 min, re-equilibrate 1.45 min.

The separation was scaled from the 2.1x50 mm, 1.7 µm ACQUITY Premier BEH C_{18} Column to a 5 µm, 10x150 mm, XBridge Premier BEH C_{18} OBD Preparative Column. The peaks were symmetrical, eluted in the same order as on the analytical column and were sufficiently resolved, demonstrating predictable scaleup from UPLC to prep (Figure 4). The separation performed on the stainless steel 10x150 mm, XBridge BEH C_{18} OBD Preparative Column was similar (chromatography not shown) for these early injections on these new columns, but the Premier Column provided increased areas (~5-25%) as compared to the stainless steel column. Likewise, the Premier Column showed greater peak heights (~5-30%) as compared to the stainless-steel column for all the peaks (Figures 5 and 6). The stainless steel column was conditioned after a few injections, and the peak response differences between it and the Premier versions was less after several injections. In laboratories where low/high pH method switching is used for the analysis and purification of compound libraries, the high pH methods may strip the conditioning from the stainless steel column. With the first subsequent low pH injections occurring after the high pH methods, the stainless steel column peak areas and/or heights may be lower than those of the Premier column because the metal oxide sites in the column have been re-exposed. For scientists who only have enough crude sample for one prep injection, the need to have good peak shape, high peak area, and sensitivity is crucially important and can mean the difference between purification success and sample loss.

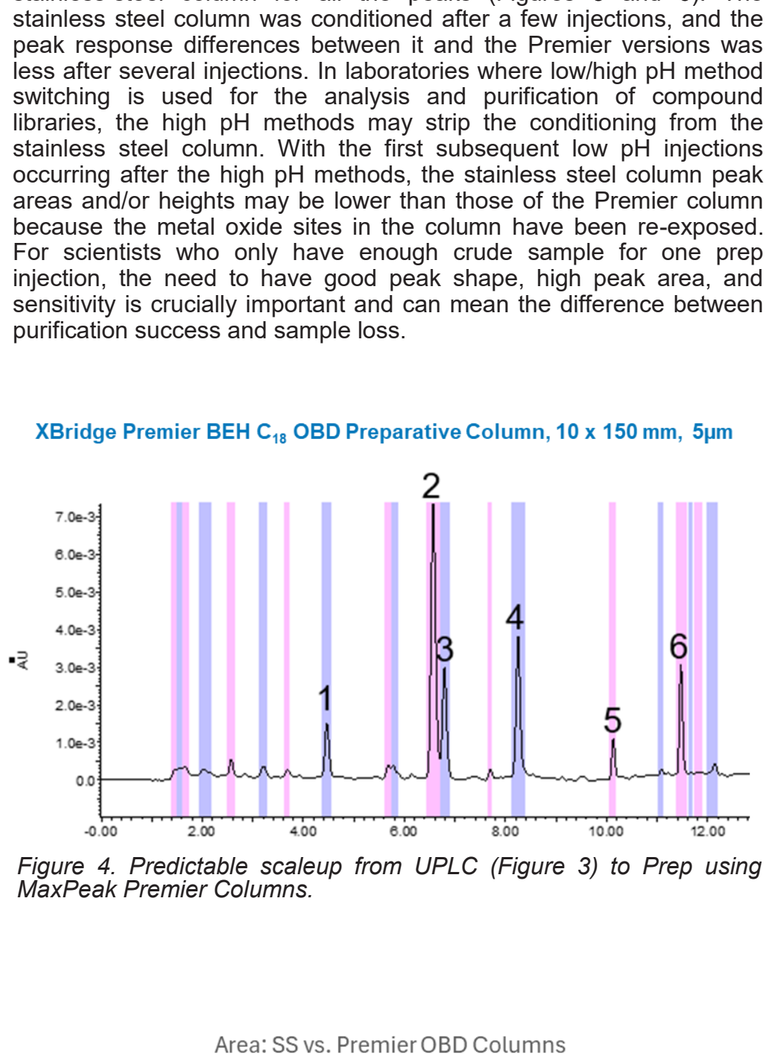

Figure 4. Predictable scaleup from UPLC (Figure 3) to Prep using MaxPeak Premier Columns.

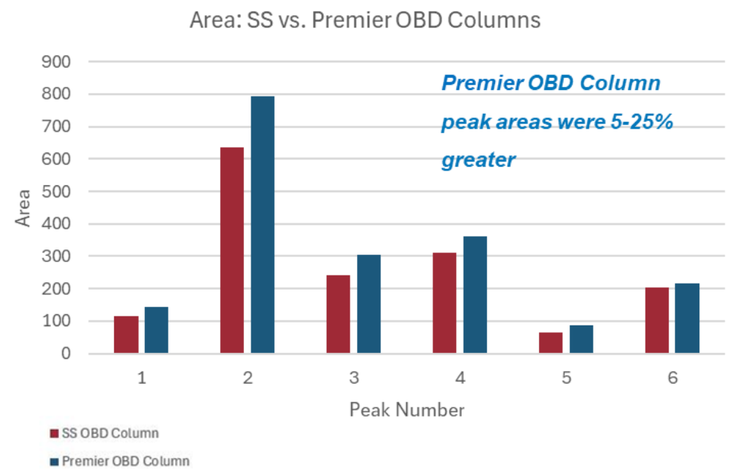

Figure 5. Areas for the peaks were greater when the Premier Column was used for the purification.

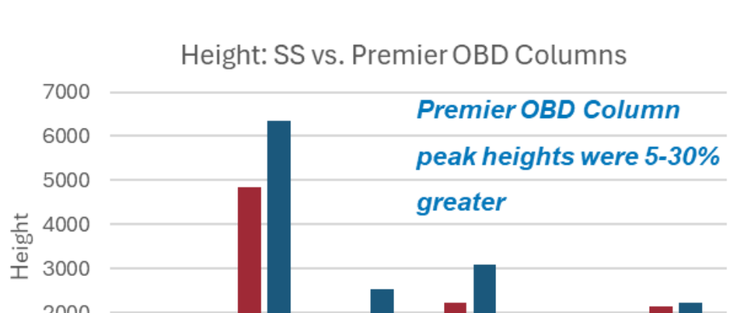

Figure 6. Peak heights were greater when the Premier Column was used for the purification.

The interest in small particle preparative separations is becoming more prevalent as pharmaceutical companies synthesize an ever-increasing number of new chemical entities for drug development. As the need grows to isolate highly pure compounds faster at increasingly reduced scale without sample loss, preparative chromatography with HPLC columns packed with smaller stationary phase particles can be critical to employ. Figure 7 shows a comparison of the chromatograms obtained using a 5 µm 10x150 mm MaxPeak Premier OBD Preparative Column (L/d_p 30,000) and a 3.5 µm 10x100 mm MaxPeak Premier OBD Preparative Column (L/d_p 28,571). Clearly, the separations are analogous since care was taken to ensure similar L/d_p (Length/particle diameter) ratios during scaleup, but the 3.5 µm column has narrower peaks (Figure 8) and a run time which is reduced by 33%. Reduced run times result in lower solvent consumption, less waste, and decreased time required for downstream sample handling. These savings translate to reduced overall process cost.

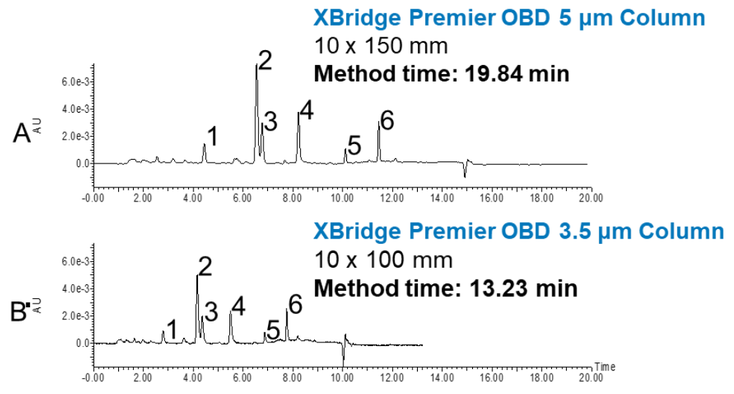

Figure 7. Comparison of the preparative separations on the 3.5 and 5 µm, 10 mm ID MaxPeak Premier OBD Preparative Columns. The 3.5 µm column shows increased speed with the separation completed in >33% less runtime. (Inj vol 45.4 & 68 µL for 5 & 3.5 µm, respectively)

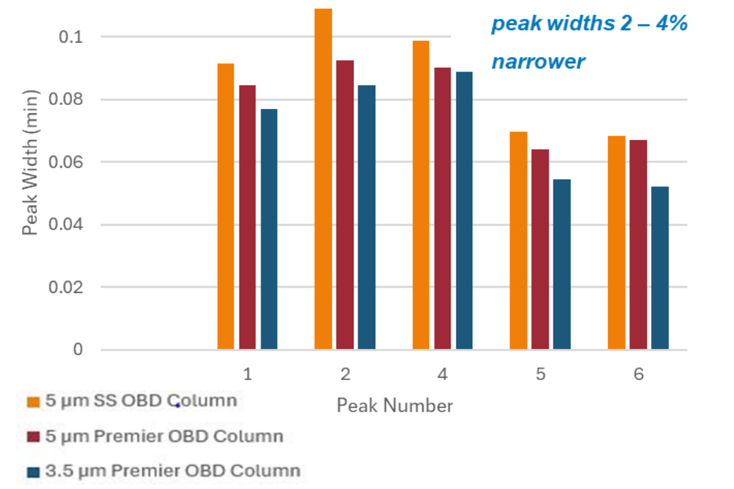

Figure 8. MaxPeak Premier OBD Preparative Columns delivered narrower peak widths than the stainless steel preparative column for all peaks collected. Narrower peak widths impact target compound purity.

Peak shape and width impact fraction collection in preparative isolations. Narrow peaks reduce fraction volumes and lead to more efficient downstream processing by decreasing the amount of time needed to dry down fractions to recover the desired product. Different compound properties and column attributes influence peak shape, but interestingly, a comparison of peaks 1, 2, 4, 5 and 6 in this separation showed that the peak widths measured at half-height on the 5 µm Premier OBD preparative column were narrower than on the 5 µm stainless steel column (Figure 8). Narrower peaks subsequently translate to lower fraction volume from collection. The differences in fraction volume between the 5 µm, 10x150 mm columns, as well as the 3.5 µm, 10x100 mm column are presented in Figure 9.

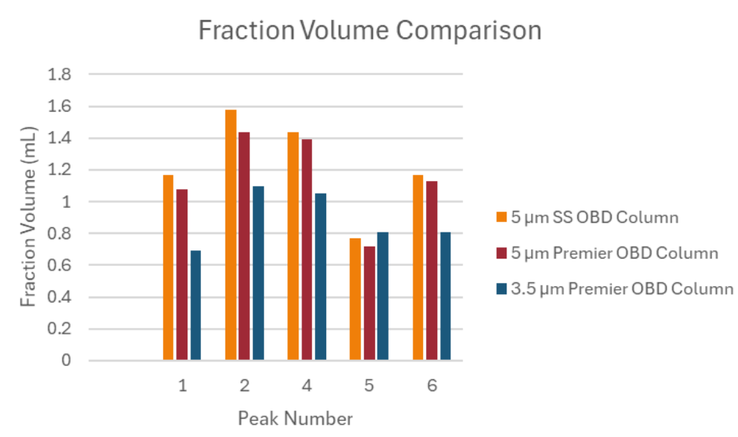

Figure 9. Comparison between the fraction volumes collected on the three preparative columns.

The inert surfaces inherent in MaxPeak Premier Columns reduce unwanted interactions between metal-sensitive compounds with strong acidic groups like sulfates, phosphates, or carboxylates from adsorbing to metal surfaces with the column. In these experiments, we demonstrated the advantages that MaxPeak Premier OBD Preparative Columns provide in the purification workflow.

CONCLUSIONS

- **MaxPeak Premier OBD Prep Columns provide full scalability for predictable target collection using Waters' highly-controlled OBD column packing process.**
- **MaxPeak Premier OBD Prep Columns reduce unwanted interactions of metal-sensitive compounds with the metal surfaces within the column and may improve peak area and sensitivity during isolation of target compounds, especially those present at low levels.**
- **MaxPeak Premier OBD Prep Columns promote enhanced target compound detection and improved peak shape for precise fraction triggering and greater confidence in target compound isolation.**
- **MaxPeak Premier OBD Prep Columns eliminate the need to pre-condition the column to reduce non-specific adsorption before starting purification, saving time and increasing process efficiency.**
- **MaxPeak Premier OBD Prep Columns in both 3.5 and 5 µm particle sizes provide smaller fraction volumes compared to the 5 µm stainless steel OBD prep column and these reduced fraction volumes minimize fraction dry-down time, solvent consumption, and purification turn-around times.**

References

1. DeLano M, Walter T, Lauber M, Gilar M, Jung M, Nguyen J, Boissel C, Patel A, Bates-Harrison A, Wyndham K, *Analytical Chemistry*, https://doi.org/10.1021/acs.analchem.0c05203 <https://doi.org/10.1021/acs.analchem.0c05203>
2. Yang J, Rainville P, Enhancing the LC-MS/MS Analysis of B-group Vitamins with MaxPeak High Performance Surfaces Technology. Waters Corporation; Waters Application Note. 720007264, 2021.
3. Waters Corporation, Topics in Liquid Chromatography, Part 2: Optimum Bed Density [OBD™] Columns: Enabling Technology for Laboratory-Scale Isolation and Purification, white Paper. 720001939.
4. Berthelette K, DeLoffi M, Collins C, Kalwood J, Walter T. Correlation Between the Adsorption of Acidic Analytes on Stainless Steel Columns and Their Ionic Charge. Waters Corporation; Waters Application Note. 720008792.

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