Applikationsbericht

Improving Separation Efficiency with CORTECS™ Premier Columns that Feature Solid-Core Particles and MaxPeak™ Premier HPS Technology

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Abstract

High efficiency columns increase resolution by reducing peak widths. This enables easier peak integration and identification, as the peaks of interest are better separated from each other and from potential background or excipient peaks. There are several ways for an analyst to improve separation efficiency; one being to use columns packed with smaller particle size stationary phases. Another way is to use longer columns. However, a drawback

in the use of both options is that they can be limited by the system operating pressure. Another path forward is the use of solid-core or superficially porous particles (SPP), which have been proven to improve efficiency without sacrificing operating pressure.[1]

The work presented shows incremental steps on how to improve separation efficiency for a mixture of three analytes. First, the analysis was done using a column packed in stainless-steel hardware with fully porous particles (FPP) to set a separation baseline. Second, the separation efficiency was improved by running the same analysis using a stainless-steel column packed with SPP to improve peak capacity, a measure of efficiency. Third, the analysis was run using an FPP packed column that utilizes MaxPeak Premier High-Performance Surfaces (HPS) Column hardware, which includes an innovative technology that improves peak shapes and efficiency for analytes that can bind to metal ions within a stainless-steel column. Lastly, the final separation was performed using a column packed with SPP utilizing MaxPeak Premier HPS Column hardware to improve efficiency of this separation even further. All four chromatographic conditions are shown along with calculations for peak capacity. Up to a 25% increase in peak capacity was realized by employing both SPP and MaxPeak Premier HPS Technology.

Benefits

- Sharper analyte peaks using MaxPeak Premier Column hardware
- Improved efficiency of SPP
- Increased peak capacity for gradient separations using both HPS and SPP columns

Introduction

A variety of analytical workflows employ gradient methods to retain and separate analytes of interest from background and excipient peaks. Workflows ranging from metabolomics and bioanalysis to pharmaceutical impurity testing rely on high efficiency gradient methods to achieve their separation goals. Efficiency in a gradient analysis is measured by peak capacity, as outlined by Neue.[2] There are several ways to improve separation efficiency, including reducing particle size to sub-2 µm particles or using longer columns. Both possible improvements carry the same cost, however, which is higher backpressure in the analytical system. Other improvements, like using SPP, can increase separation efficiency without increasing system backpressure

eliminating the need to alter the analytical method or change the LC system. SPP contain a solid core, surrounded by a superficially porous shell. This particle morphology reduces the dispersion within the column by reducing the longitudinal diffusion and eddy diffusion of the analyte band that reduces the peak width, thereby increasing efficiency.[1]

Additionally, depending on the analytes in question, the use of MaxPeak Premier Columns may improve separation efficiency by reducing peak width. MaxPeak Premier HPS Technology was introduced in 2020 and is a unique column and system hardware designed to mitigate interactions between acidic analytes and any exposed metal surfaces.[3–5] Unlike other technologies used to mitigate analyte/surface interactions, such as peek-lined steel columns or using mobile phase additives, MaxPeak Premier HPS Technology is a controlled part of the column manufacturing process and maintains the column's performance and pressure tolerances.6 MaxPeak Premier HPS Column hardware has been shown to be particularly effective in improving the efficiency and peak shapes for phosphorylated compounds like nucleotides, phosphopeptides, and steroid phosphates.[7–9] By combining both MaxPeak Premier HPS Column hardware and SPP, the separation efficiency of a gradient method can increase dramatically.

This application note demonstrates how combining MaxPeak Premier HPS Technology and SPP, which are utilized in CORTECS branded columns, can improve separation efficiency. A mixture of analytes was first analyzed on an XBridge™ BEH C_{18} XP Column using standard hardware and fully porous BEH particles. Next, the mixture was analyzed with a CORTECS C_{18} Column, also using standard hardware, and an XBridge Premier BEH C_{18} Column. Notable improvements in separation efficiency were achieved with both technologies separately, so for the final experiment, the two technologies were combined by using a CORTECS Premier C_{18} Column. Replicate injections, n=5, were performed for each column and average peak widths were calculated. Peak capacity was then calculated for all columns and compared.

Experimental

Sample Description

A sample containing 10 µg/mL each of betamethasone, betamethasone phosphate, and fostemsavir was prepared from 1 mg/mL stock solutions in water. Final sample composition contained <1% acetonitrile.

LC Conditions

LC systems:	ACQUITY™ Premier QSM with Column Manager and PDA
Detection:	UV @ 254 nm
Columns:	XBridge Premier BEH C_{18}, 2.1 x 50 mm, 2.5µm, p/n: 186009827 XBridge BEH C_{18} XP, 2.1 x 50 mm, 2.5 µm, p/n: 186006029 CORTECS Premier C_{18}, 2.1 x 50 mm. 2.7 µm, p/n: 186010441 CORTECS C_{18}, 2.1 x 50 mm, 2.7 µm, p/n: 186007365 Competitive Solid-Core C_{18}, 2.1 x 50 mm, 2.7 µm
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	3.0 µL
Flow rate:	0.5 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase D1:	2% Formic Acid in water
Gradient conditions:	Table 1

Time (min)	%A	%B	%D1
0.00	90	5	5
0.50	90	5	5
7.00	0	95	5
7.01	90	5	5
10.00	90	5	5

Table 1. Gradient profile for both original monograph and modernized methods.

Data Management

Chromatography software: Empower™ 3 Feature Release 4

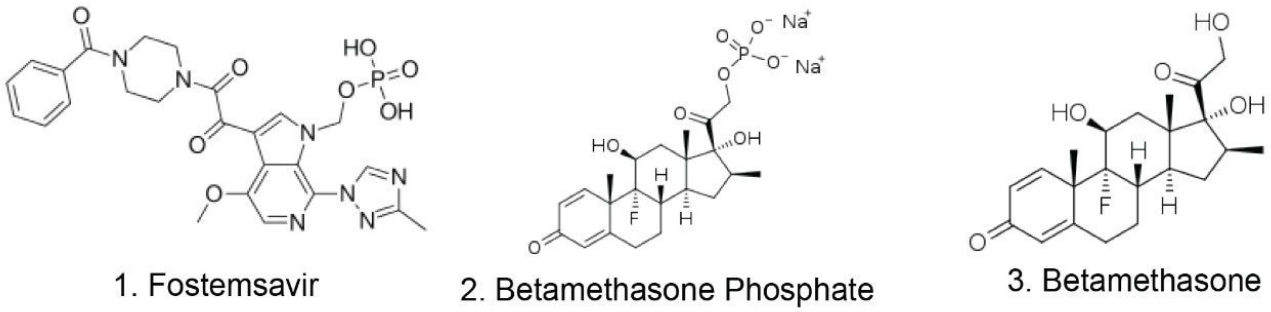

Figure 1. Chemical structures of analytes.

Results and Discussion

The XBridge BEH C_{18} XP Column is one of the most popular column chemistries. The rugged design of the base particle, capable of withstanding high pressure as well as elevated temperatures and mobile phase pH make it ideal as a starting point for method development. While this column is a good starting point, better performing columns may be more applicable depending on the test analytes and conditions. As shown in Figure 2, the chromatographic results using the XBridge BEH C_{18} XP Column are reasonable, with good UV detection. Both fostemsavir (1) and betamethasone phosphate (2) show severe tailing, with USP tailing factors of 1.67 and 2.09, respectively. Additionally, the calculated peak capacity for this separation was determined to be 201 based on the average peak widths shown in the figure.

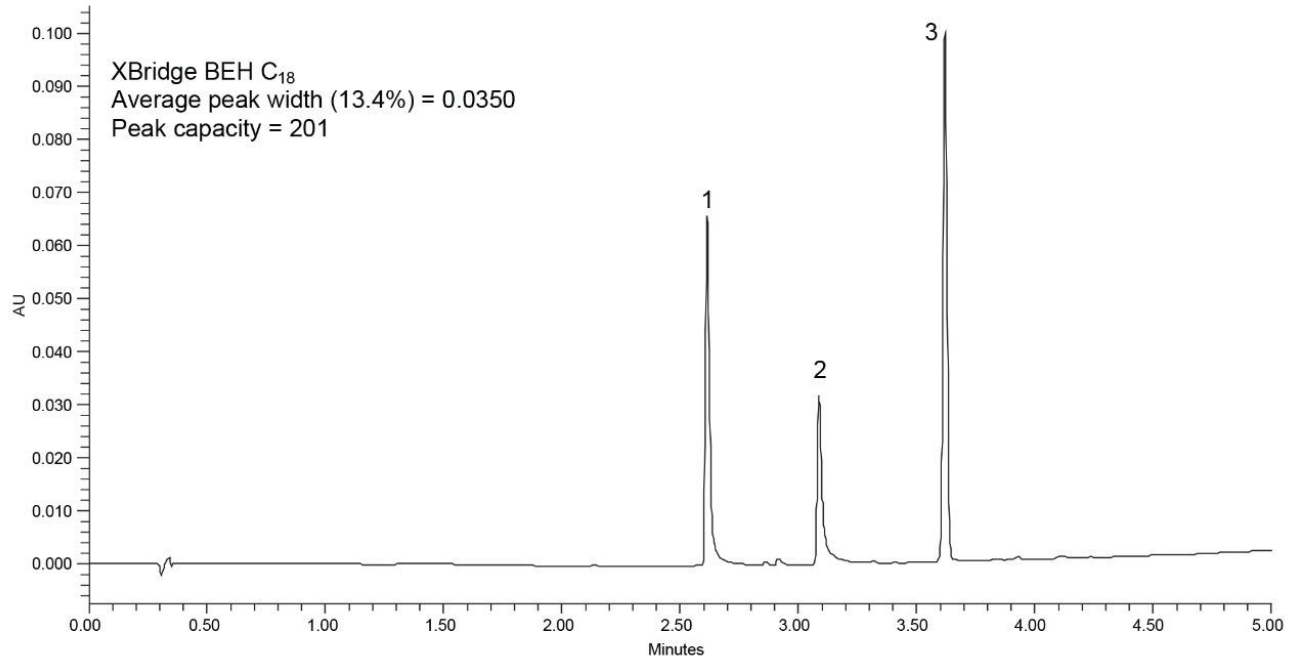

Figure 2. Separation of three analytes using the XBridge BEH C_{18} XP Column. Calculated peak capacity is shown. 1) Fostemsavir, 2) Betamethasone Phosphate, 3) Betamethasone.

Since the compounds that have the worst peak shape are both metal sensitive, there are two possible routes forward to improve the separation. The first is the use of CORTECS particle columns, which boost efficiency of the separation due to the solid-core design of the particle. The other route is to use MaxPeak HPS Column technology, which should improve separation efficiency especially for the metal sensitive compounds. Both a CORTECS 2.7 µm and an XBridge Premier BEH C_{18} Column were used next to separate these compounds. Figure

3 shows the resulting chromatograms from those tests compared to the XBridge BEH C_{18} XP Column results obtained previously.

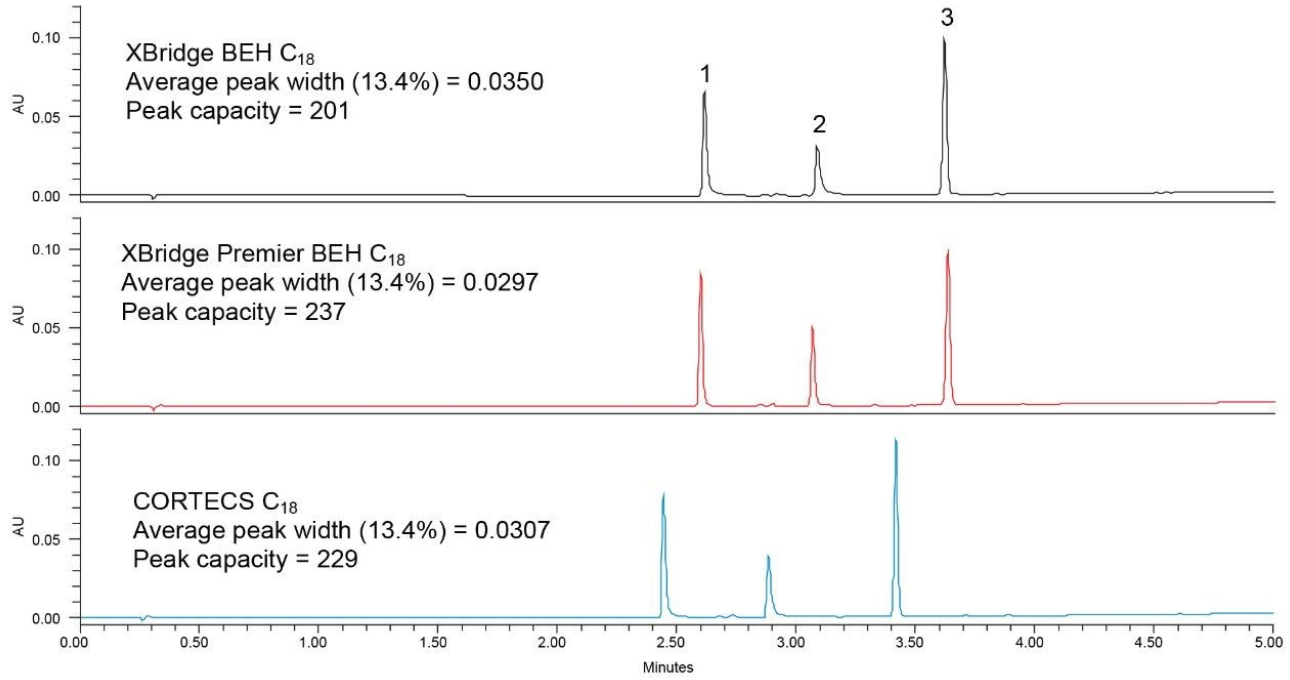

Figure 3. Separation of three analytes using XBridge BEH C_{18} XP, XBridge Premier BEH C_{18}, and CORTECS C_{18} columns. Calculated peak capacity is shown. 1) Fostemsavir, 2) Betamethasone Phosphate, 3) Betamethasone.

Examining the results obtained with the XBridge Premier BEH Column first, the peak shapes for both fostemsavir and betamethasone phosphate are greatly improved. USP tailing factors for those compounds using the HPS column were reduced to 1.23 and 1.26, respectively. This is a drastic reduction in USP tailing factor as well as average peak width. This translates to an increase in peak capacity as well. Alternatively, looking at the CORTECS C_{18} Column, which still uses stainless steel hardware, the peak shapes have also been improved. USP tailing factors for fostemsavir, and betamethasone phosphate are 1.57 and 1.91, respectively. Peak capacity of the separation using CORTECS particle columns show just over a 10% increase.

It would appear, based on the data, that the peak shape improvements are governed mostly by secondary interactions with the hardware, especially for the metal sensitive analytes. CORTECS particles improve overall performance, but don't improve peak shape for the metal sensitive analytes. Theoretically, the best results should

be obtained by combining both the HPS technology and the high efficiency CORTECS particles. A CORTECS Premier C_{18} Column was used to separate these compounds, and the results were compared to the original separation, Figure 4.

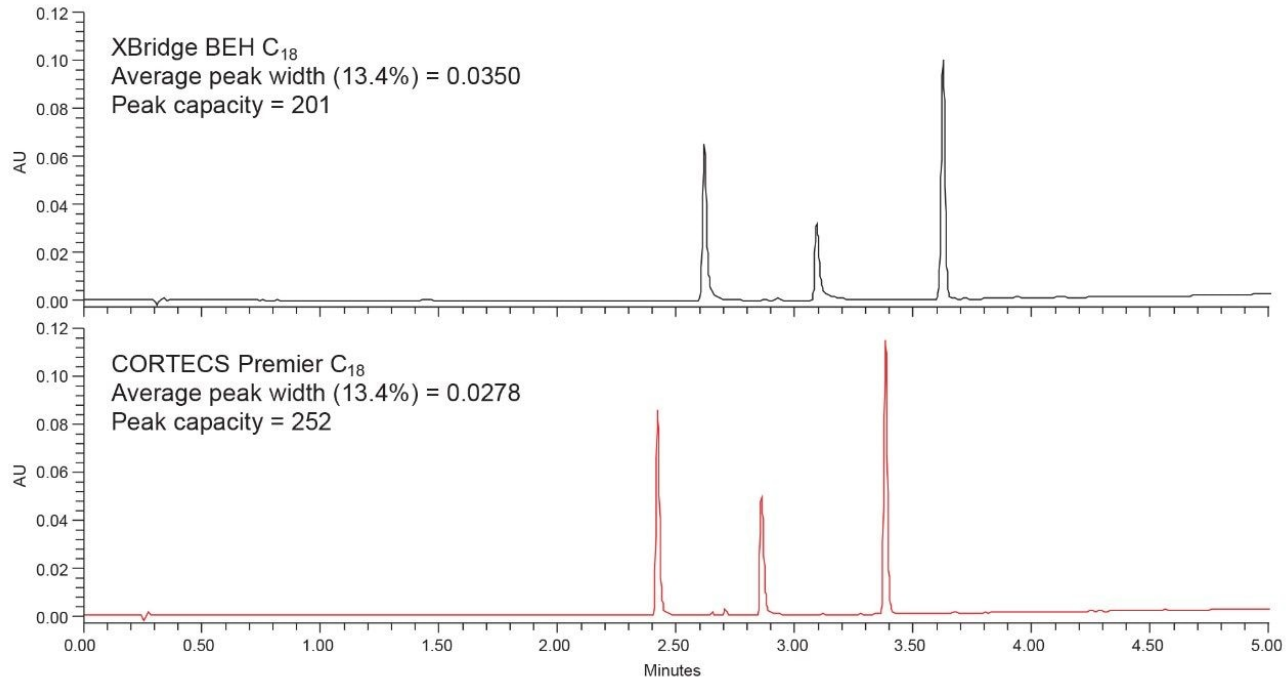

Figure 4. Separation of three analytes using XBridge BEH C_{18} XP and CORTECS Premier C_{18} Columns. Calculated peak capacity is shown. 1) Fostemsavir, 2) Betamethasone Phosphate, 3) Betamethasone.

Looking at the peak shapes, the CORTECS Premier Column produces very symmetrical peaks with USP tailing values of 1.22 and 1.20 for fostemsavir and betamethasone phosphate, which is comparable to the results seen with XBridge Premier BEH C_{18} Column. However, the peak capacity using the CORTECS Premier Column is higher than that produced by the XBridge Premier BEH C_{18} Column. The combination of the HPS technology, which improves peak shape for metal sensitive analytes, and the CORTECS particles produces the best result for this separation. High efficiency separations are advantageous for any workflow but are almost a requirement for highly complicated samples. The CORTECS particles coupled with MaxPeak Premier hardware provides the best separation efficiency, especially for metal sensitive analytes.

Conclusion

Column efficiency is a critical aspect of many analytical methods with a strong need to maximize it, as it directly correlates to overall performance and quality. Measuring efficiency in gradient separations is done by measuring the peak capacity, or how many peaks can be eluted within a gradient based on the average peak width of the analytes present. Higher peak capacity equates to a higher efficiency separation as peak widths are narrower. Certain stationary phases and column hardware can be used to improve separation performance, with minimal changes to selectivity. The use of SPP can improve separation efficiency as these particles are designed specifically to improve efficiency by minimizing column dispersion. MaxPeak HPS technology can also improve separation efficiency, especially for metal sensitive analytes, by reducing secondary interactions between analyte and column hardware leading to sharper, more symmetrical peaks. By packing SPP into MaxPeak HPS columns, a separation can be vastly improved, which is advantageous for many workflows and separations.

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