[TECHNOLOGY BRIEF]

Improving Resolution Using e*X*tended *P*erformance (*XP*) Columns

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GOAL

To demonstrate improved resolution of ***XP*** 2.5 µm Columns over traditional HPLC particle size columns for challenging separations.

BACKGROUND

It is widely accepted that transferring methods to smaller particle sizes can result in faster analysis time. By transferring a method directly to a smaller particle size, there may also be improvements in resolution. As the particle size gets smaller, however, the back pressure across the column will increase. While the use of sub-2-µm columns may necessitate the use of a UPLC® System, HPLC users can still realize significant benefits in resolution by transferring their HPLC methods to a e***X***tended ***P***erformance (***XP***) 2.5 µm Column. This may be particularly beneficial for the separation of complex mixtures, where the added resolution from a smaller particle size column may help to identify impurities or target compounds without resorting to increasing column length and run times.

Improving the resolution of a related-compounds separation using e*X*tended *P*erformance (*XP*) columns.

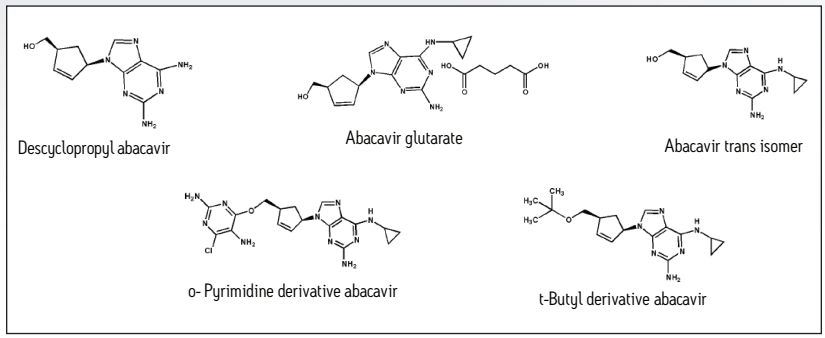

Figure 1. Abacavir components in the USP related compounds mixture.

An example of improved resolution using an ***XP*** 2.5 µm Column is demonstrated using a related compounds mixture of abacavir. Abacavir is a nucleoside reverse-transcriptase inhibitor that is used in anti-HIV therapy. The mixture of related compounds contains five compounds, including the main component, abacavir, shown here in glutarate form (Figure 1). The separation of abacavir from its trans-isomer is particularly challenging. Here, the overall improved separation of abacavir from its related compounds is demonstrated, comparing the use of a 3.5-µm column to a high efficiency ***XP*** 2.5 µm Column.

THE SOLUTION

To properly separate related compounds while minimizing extensive method development in HPLC, a highly efficient column with higher resolving power should be used. ***XP*** Columns contain 2.5-µm particles packed at high pressures in UltraPerformance hardware. The back pressure allowances of the ***XP*** 2.5 µm particle column still allow for use on an HPLC system.

To demonstrate the improvement in performance using ***XP*** Columns, the related compounds mixture for abacavir was tested on a 100-mm 3.5-µm, XSelect® CSH™ C_{18} Column. The same method was run using the same column chemistry and dimensions with ***XP*** 2.5 µm Columns. The comparative separations are shown in Figure 2.

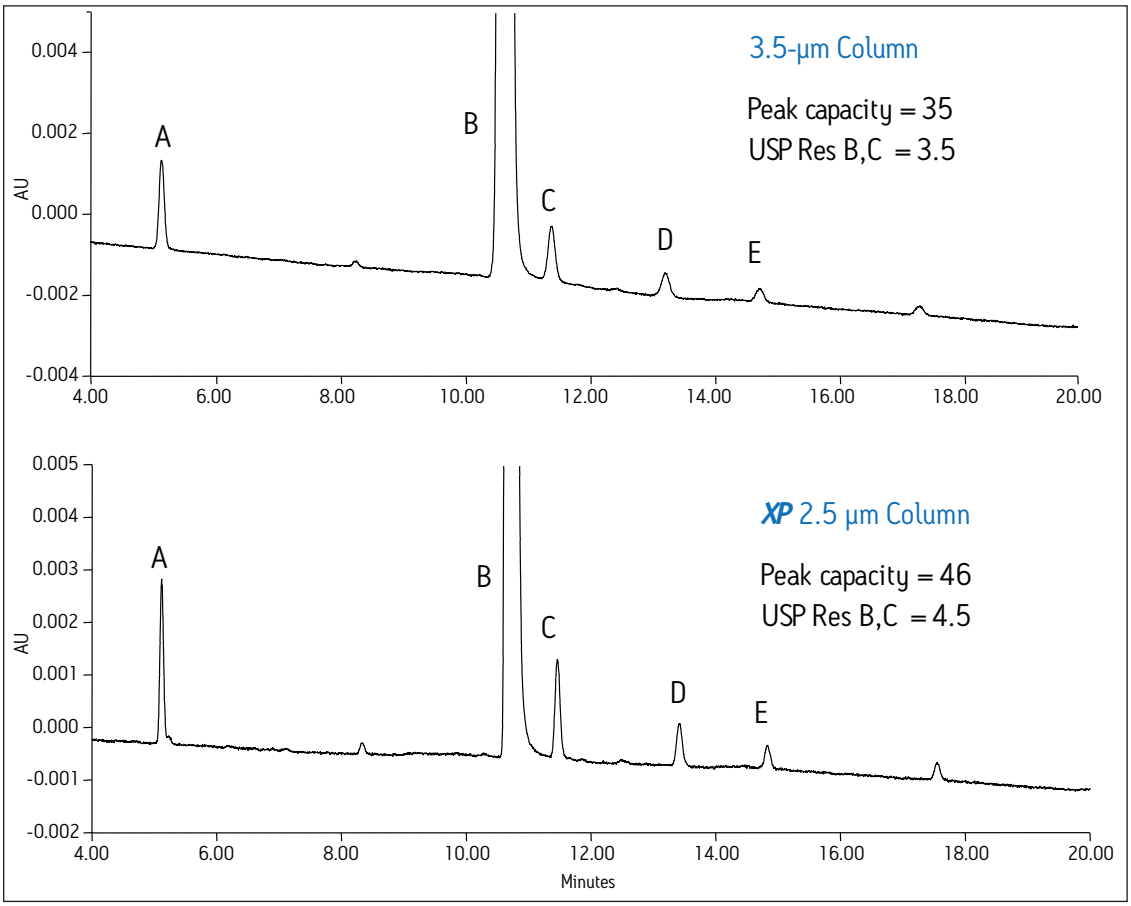

*Figure 2. Separation of abacavir related compounds, demonstrating improved assay performance using an e**X**tended **P**erformance (**XP**) Column. A) Descyclopropyl abacavir, B) Abacavir glutarate, C) 1R,4R Trans abacavir, D) o-Pyrimidine abacavir, E) t-Butyl abacavir.*

In this case, by simply changing the 3.5-µm column to an ***XP*** 2.5 µm Column, significant performance improvements are seen as the overall peak capacity for the separation increases 31%, and the peak heights increase up to 42%. A 28% increase in resolution demonstrates the improved separation between closely eluting compounds abacavir and trans-abacavir. This example illustrates the capability to increase sensitivity and resolution by using an ***XP*** Column, which can result in more accurate identification and quantification of target compounds such as impurities.

SUMMARY

By transferring HPLC methods to an ***XP*** 2.5 µm Column, improvements in resolution and sensitivity can be achieved. This was demonstrated with a related compounds method for abacavir, in which an overall improvement of 31% in peak capacity, up to 42% in peak height, and 29% in resolution were observed by changing the 3.5-µm column to an ***XP*** 2.5 µm Column. The use of ***XP*** Columns allows HPLC users to maximize the separation performance on their HPLC systems, lessening the need for further method development, and promoting cost-effective asset utilization.

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