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Note d'application

Improvements in the Chromatographic Performance of Antiviral Compounds Analyzed with MaxPeak HPS Technology

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Abstract

Antiviral pharmaceutical compounds are currently under investigation for their role in treating mild, moderate, and severe critical illnesses such as COVID-19.[1] It is important that routine chromatographic methods developed to analyze these pharmaceutical compounds distinguish low level-impurities and degradation products well before they reach alert/action levels. To accomplish this, chromatographic parameters such as retention, peak shape, and sensitivity are critically important.

The MaxPeak High Performance Surfaces (HPS) is a new, novel hybrid organic-inorganic surface technology that reduces sample loss due to non-specific binding of the analyte to metal-ions present within the chromatographic instrument and column.[2,3] In this application note, a panel of antiviral compounds were separated by reversed-phase HPLC. The chromatographic performance of an XBridge Premier Column with MaxPeak HPS Technology, was compared to a conventional stainless-steel column.

Benefits

Antiviral therapeutic compounds separated with the XBridge Premier Column, exhibit higher peak height

response, improved signal-to-noise (S/N), and narrower chromatographic peak widths without the need for strong ion-pairing agents, chelators, or lengthy passivation protocols.

Introduction

Pharmaceutical laboratories are routinely tasked with developing fast, sensitive, and robust chromatographic methods. The analytical method of choice to monitor quality and stability is generally High-Pressure Liquid Chromatography (HPLC) due to the high degree of both quantitative and qualitative precision.

The HPLC column surface and instrument componentry are comprised of stainless-steel, a material selected for its availability and mechanical strength. Conversely, stainless-steel is vulnerable to corrosion when exposed to harsh liquids, such as the acidic and/or halide containing mobile phases routinely employed in chromatography. Metal ions from corrosion can bind to the column stationary phase and interact with analytes through complexation, oxidation, and epimerization reactions which can result sometimes poor, unacceptable peak shape, and potentially the complete loss of the target analyte particularly at low concentrations.[2]

Mobile phase additives, such as ion-pairing agents (***e.g.*** trifluoroacetic acid (TFA)) or chelators (***e.g.*** medronic acid) are routinely employed to mitigate analyte-metal binding within the instrument and column. Use of these agents provides some measure of success, can result in instrument contamination, irreversible modifications in column chemistry, and ion suppression in LC-MS applications.[2] Passivation, performing a series of analyte injections to “condition” the instrument and column with the analyte, is also commonly utilized, although this technique may not lend overall confidence in daily precision.[3]

Columns constructed of alternative metals, such as titanium and nickel-cobalt alloys, have also been employed for some applications due to their corrosion resistance, but these metals can still deteriorate under some conditions. Additionally, columns made of Polyetheretherketone (PEEK), instead of transition metals, are an option, although solvent compatibility and pressure restraints exist.[2]

In this application note, we compare the chromatographic performance of an XBridge Premier BEH C_{18} Column to the performance of a conventional XBridge BEH C_{18} Stainless-Steel Column for the separation of twelve representative antiviral pharmaceutical compounds. These compounds are rapidly being developed and investigated for their role in the treatment of a variety of critical illnesses.[4]

Experimental

LC Conditions

LC system:	ACQUITY Arc with Quaternary Solvent Manager (rQSM), Sample Manager (rFTN), Column Manager (rCM), Empower 3 Chromatography Data Software
Detection:	ACQUITY Photodiode Array Detector (PDA), UV 245 nm
Column(s):	XBridge Premier BEH C_{18}, 2.5 µm Column, 4.6 x 150 mm, P/N 186009849 XBridge BEH C_{18} XP, 2.5 µm Column, 4.6 x 150 mm, P/N 186006711
Column temp.:	50 °C
Sample temp.:	20 °C
Injection volume:	1 µL
Flow rate:	1.5 mL/min
Mobile phase A:	10 mM Ammonium formate pH 4.0
Mobile phase B:	Acetonitrile
Gradient:	2% mobile phase B hold for 4 minutes, linear ramp to 95% B at 15 minutes, followed by a 1-minute hold, and return to 2% B

starting conditions

Results and Discussion

A mixture of antiviral compounds soluble in aqueous solvent (tenofovir, favipiravir, emtricitabine, ribavirin, abacavir, and oselatamavir) was prepared by dissolving approximately 0.5 mg of each compound in 10 mL of water. A mixture of antiviral compounds soluble in organic solvent (mastinib, darunavir, remdesivir, atazanavir, ritonavir, and lopinavir) was prepared by dissolving approximately 0.5 mg of each compound in 10 mL of methanol (Figure 1). Each mixture was filtered through a respective, solvent compatible, 0.2 µm syringe filter (Waters P/N WAT200806 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/wat200806-acrodisc-syringe-filter-pvdf-13-mm-02--m-aqueous-100-pk.html> or Waters P/N WAT200822 <https://www.waters.com/nextgen/global/shop/sample-preparation--filtration/wat200822-acrodisc-syringe-filter-ptfe-25-mm-02--m-non-polar-1000-case.html>) prior to injection. The LC instrument was flushed sequentially with 100% IPA, water, and mobile phase. New columns with no previous injections, were employed from stock inventory. To minimize potential bias due to passivation of the instrument by the analyte, injections were performed consecutively by alternating columns with the column manager switching valve for a total of 5 injections of each antiviral mixture on each column.

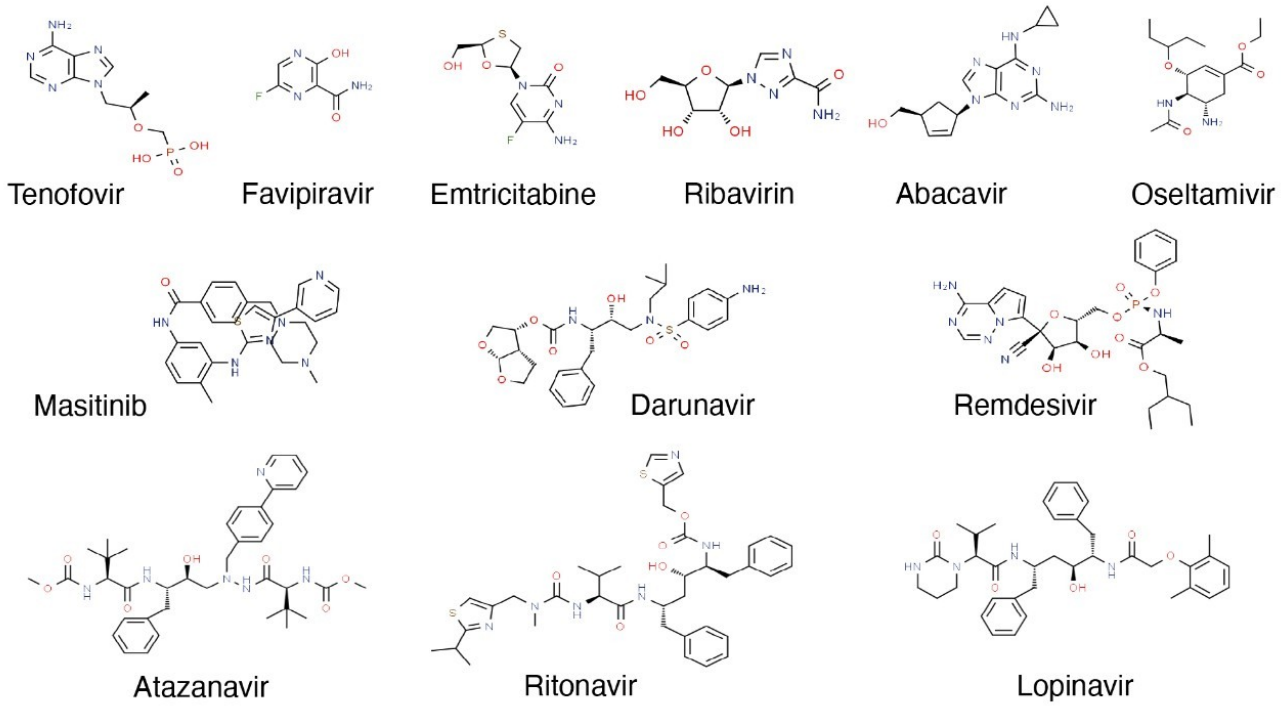

Figure 1. The panel of antiviral compounds separated by reversed phase.

The chromatographic method separated all 12 antiviral compounds. Those soluble in water were retained for less than 9 minutes, while compounds soluble in methanol, were retained for greater than 9 mins (Figure 2).

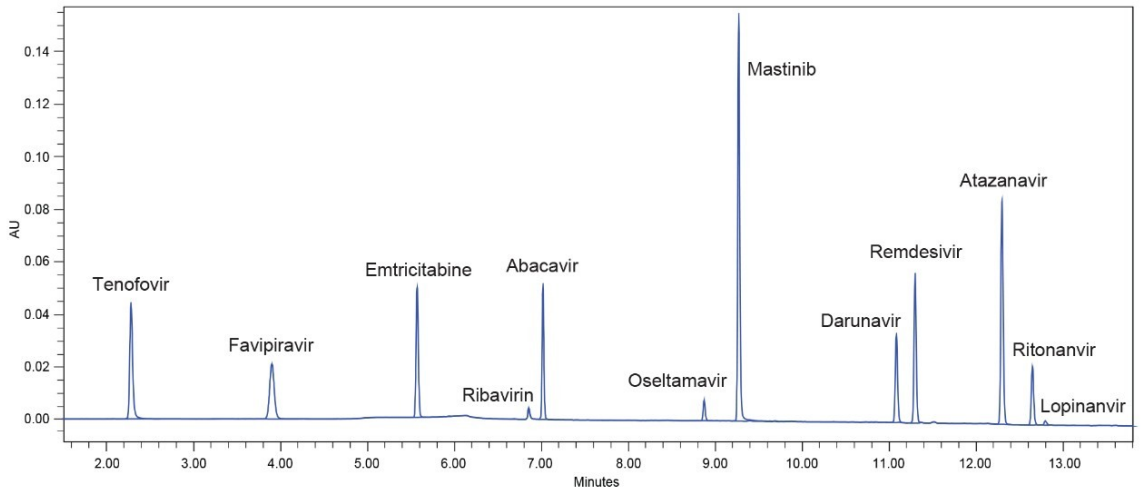

Figure 2. Separation of antiviral compounds by reversed phase.

The XBridge Premier Column yielded a greater height response for each antiviral compound in the panel resulting in higher S/N values. Additionally, by minimizing analyte interaction with metal-ions via the hybrid surface technology, peak shape improved (i.e. greater symmetry, lower tailing) for several compounds in the panel (Figure 3). Peak width at 5σ, a stringent measure of homogeneity and analyte-column interaction, is severely affected by peak asymmetry.[6] For all 12 compounds, a significant decrease in peak width at 5σ was observed when using the MaxPeak HPS Technology, which demonstrated higher column performance (Table 1).

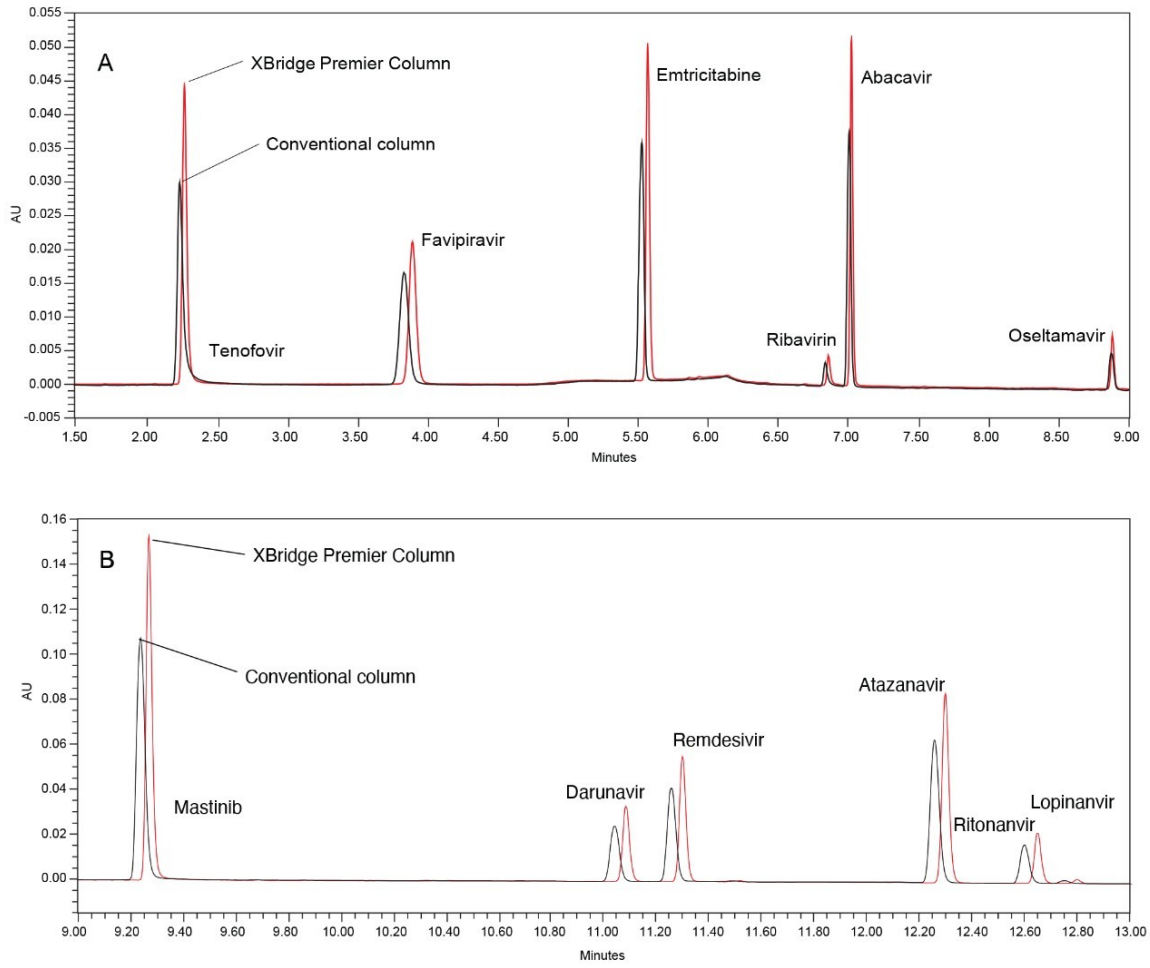

Figure 3. Overlay of the XBridge Premier Column (red) and conventional column (black) performance for antiviral compounds soluble in A) aqueous solvent and B) organic solvent.

	XBridge Premier Column	XBridge Premier Column
	Fold increase in height response and S/N	% Decrease in peak width at 5σ
Tenofovir	1.6	43
Favipiravir	1.4	13
Emtricitabine	1.5	94
Ribavirin	1.4	27
Abacavir	1.5	17
Oseltamavir	1.5	14
Mastinib	1.4	14
Darunavir	1.3	25
Remdesivir	1.3	14
Atazanavir	1.3	25
Ritonivir	1.3	25
Lopinavir	1.3	45

Table 1. Fold increase in height response, S/N and % decrease in $w_{5\sigma}$ observed with the XBridge Premier Column for the separation of antiviral compounds.

Conclusion

The work here has shown that when analyzing low-level, antiviral compounds by reversed phase, the LC column with MaxPeak High Performance Surface Technology (XBridge Premier Column) exhibits higher performance than its conventional counterpart. Increases in peak height response resulted in a higher signal-to-noise ratio (S/N), and improved peak shape ($w_{5\sigma}$) was observed with the XBridge Premier Column without the need for ion-pairing/chelator mobile phase additives or column passivation injections.

References

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720007398, October 2021