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應用手冊

Comprehending COVID-19: Using the Atlantis PREMIER BEH C_{18} AX Mixed-Mode Column for the Analysis of Umifenovir

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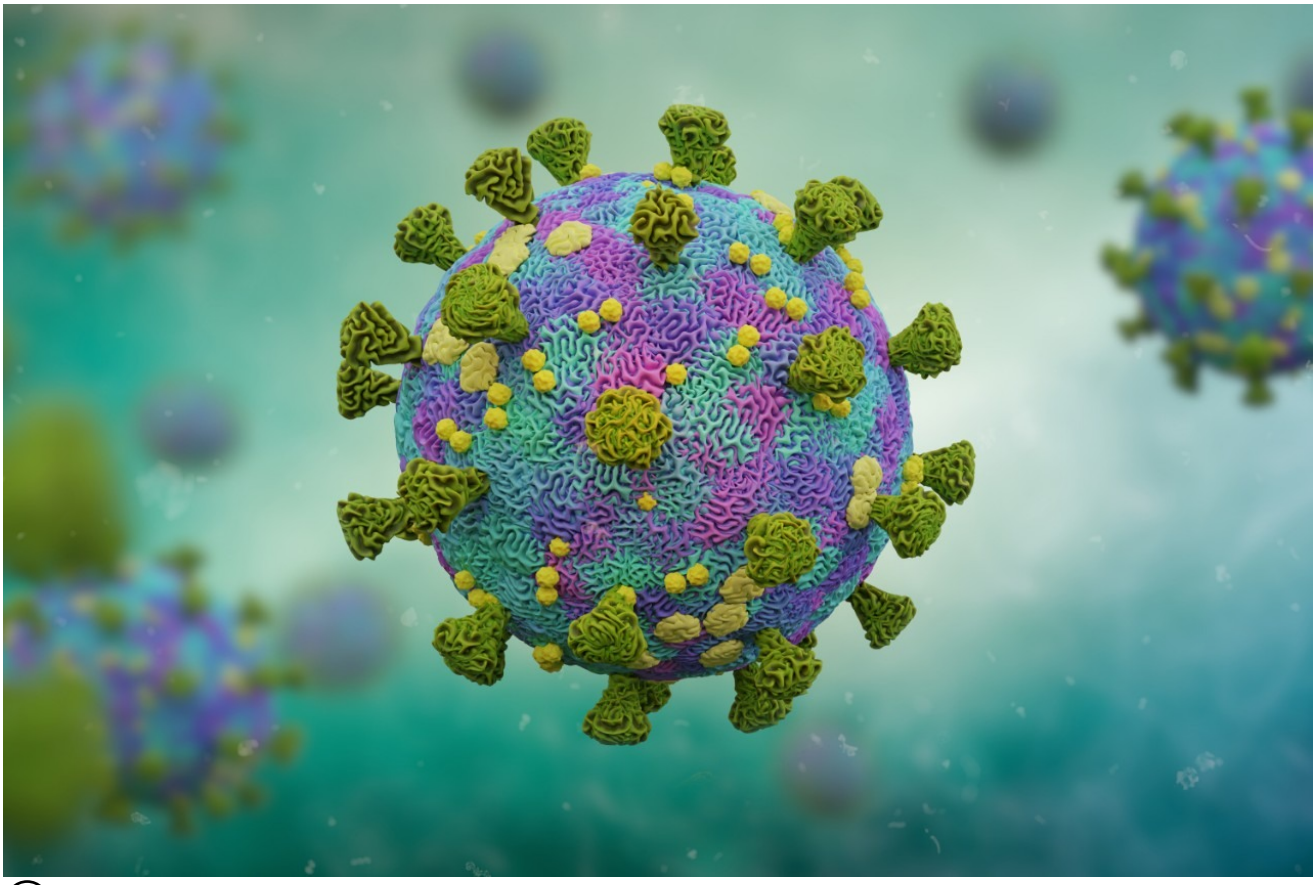

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Abstract

The global COVID-19 pandemic has given rise to extensive research focusing on repurposing small molecule drugs for the treatment of SARS-CoV-2 infections. Antiviral medications developed for the treatment and prevention of influenza are of increasing interest to those working on the novel coronavirus. Umifenovir, sold under the trade name of Arbidol®, is one of the antiviral agents currently being investigated, in combination with other drugs, in a clinical trial for COVID-19.[1] Regardless of the clinical outcome for treating the novel coronavirus, Waters demonstrates the benefits of using mixed-mode chromatography for umifenovir, which has two basic groups. This work demonstrates that sharper peaks can be achieved for umifenovir through the use of a mixed-mode stationary phase versus a more conventional reversed-phase material.

Benefits

Using the Atlantis PREMIER BEH C_{18} AX Column instead of a C_{18} column, it is possible to obtain:

- Significantly sharper and more symmetrical peaks for umifenovir
- Faster elution of umifenovir

Introduction

Mixed-mode chromatography (MMC) is a technique that can be successfully applied to create a separation with multiple retention mechanisms. Atlantis PREMIER BEH C_{18} AX Columns contain a mixed-mode reversed-phase/anion-exchange stationary phase based on bridged-ethyl hybrid particles.[2] The stationary phase contains not only C_{18} groups, but also tertiary alkylamine moieties, creating a strongly positive surface charge below approximately pH 8. Relative to conventional reversed-phase materials, this positive surface charge gives increased retention of anions, such as ionized acids, and decreased retention of cations, such as protonated bases. For basic analytes such as umifenovir, the positive surface charge can also provide improvements in peak shape and loadability, as has been previously demonstrated with Charged Surface Hybrid (CSH) stationary phases.[3] In this application brief, we compare the peak shape and peak width obtained for umifenovir using an Atlantis PREMIER BEH C_{18} AX Column versus a conventional C_{18} reversed-phase column, ACQUITY UPLC HSS

T3.

Experimental

The following experimental conditions were used to analyze umifenovir.

LC Conditions

LC system:	ACQUITY UPLC H-Class Bio
Detection:	ACQUITY UPLC PDA Detector
Vials:	Polypropylene plastic, 700 µL
Column(s):	Atlantis PREMIER BEH C_{18} AX, 1.7 µm, 95 Å, 2.1 x 50 mm ACQUITY UPLC HSS T3, 1.8 µm, 100 Å, 2.1 x 50 mm
Column temp.:	30 °C
Sample:	100 µg/mL umifenovir in 0.1% formic acid in 50% acetonitrile
Sample temp.:	12 °C
Injection volume:	1 µL
Flow rate:	0.3 mL/min
Mobile phase:	10 mM ammonium formate, pH 3.0 in 30% acetonitrile

Results and Discussion

In mixed-mode chromatography, the selectivity for ionizable analytes may be varied over a wide range by adjusting the pH and buffer concentration of the mobile phase. This is one of the most important tools to consider when developing a method using MMC. In our example using a pH 3 mobile phase, the Atlantis BEH C_{18} AX stationary phase has a positive surface charge. Relative to a conventional reversed-phase material, this positive surface charge decreases the retention of cations, such as the protonated form of umifenovir.

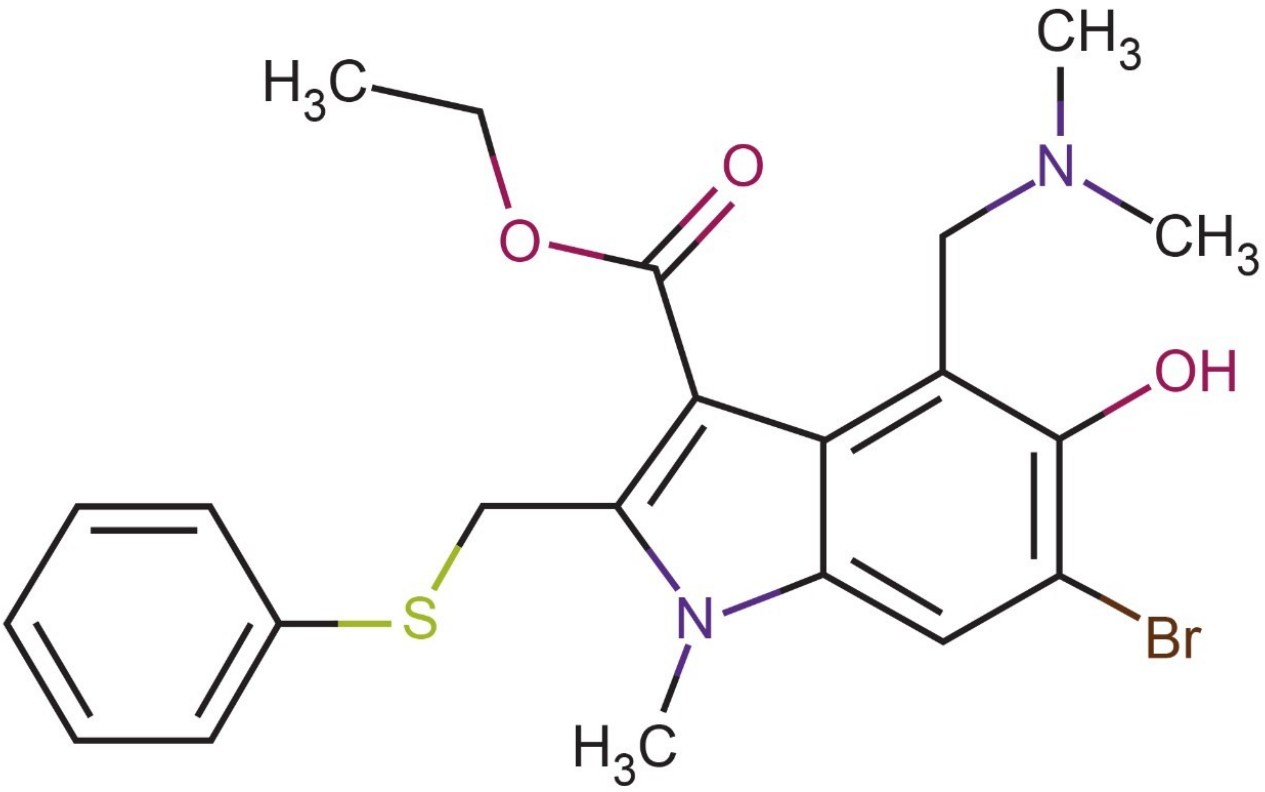

Figure 1. Umifenovir structure. https://www.drugbank.ca/drugs/DB13609

Using the same isocratic chromatographic conditions with both columns, the positively charged umifenovir was found to be less retained on the Atlantis PREMIER BEH C_{18} AX Column, as a result of ionic repulsion. At a mass load of 0.1 µg on-column, this mixed-mode column also gave a much narrower and more symmetric peak compared to that obtained using the ACQUITY UPLC HSS T3 Column. These differences are readily observed in the chromatograms shown in Figure 2. The umifenovir peak was 6.9 seconds wide on the Atlantis PREMIER BEH C_{18} AX Column versus 59 seconds wide on the ACQUITY UPLC HSS T3 Column.

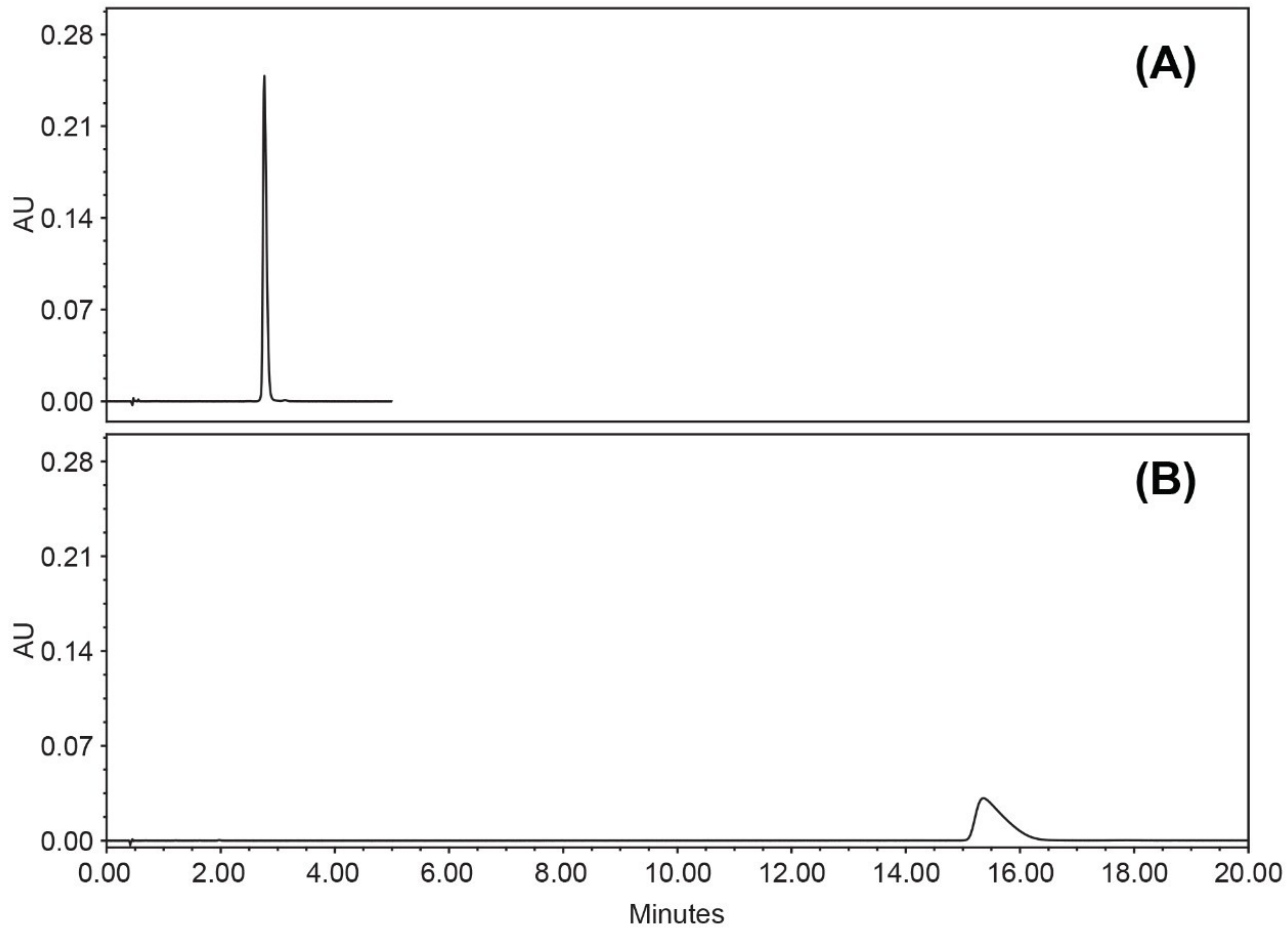

Figure 2. Comparison of the umifenovir peak shape using equivalent chromatographic conditions of 10 mM ammonium formate, pH 3.0 in 30% acetonitrile on (A) an Atlantis PREMIER BEH C_{18} AX Column and (B) an ACQUITY UPLC HSS T3 Column.

With this test condition, a high retention factor was obtained with the ACQUITY UPLC HSS T3 Column, *ca* 39. To make the retention factor more comparable to what was observed on the Atlantis PREMIER BEH C_{18} AX Column, the acetonitrile content of the mobile phase was increased to 40% (while maintaining the same buffer strength and pH). The resulting chromatogram is shown in Figure 3B. While the peak width for umifenovir decreased significantly, it was still *ca* 50% wider than the peak produced by the Atlantis PREMIER BEH C_{18} AX Column.

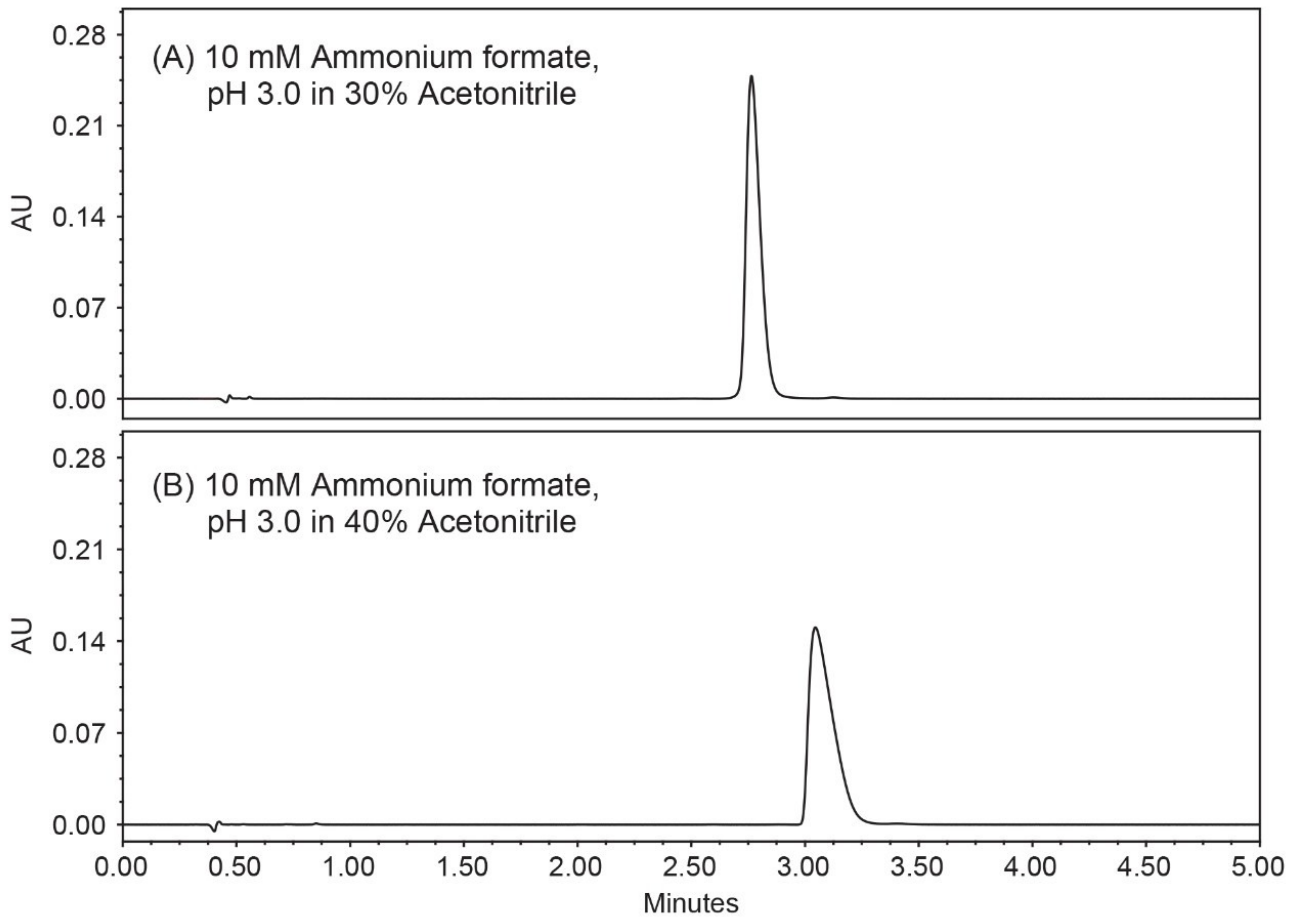

Figure 3. Comparison of the umifenovir peak shape at comparable retention factor using (A) an Atlantis PREMIER BEH C_{18} AX Column and (B) an ACQUITY UPLC HSS T3 Column.

The Atlantis PREMIER BEH C_{18} AX Column produced a narrow, symmetrical peak for umifenovir. The USP tailing factor was measured to be 1.5, while the USP tailing factor remained over 2 whenever using the ACQUITY UPLC HSS T3 Column, regardless of mobile phase composition.

Conclusion

Mixed-mode stationary phases such as Atlantis BEH C_{18} AX offer unique selectivity and are complementary to existing RP stationary phases making them valuable for column screening during method development. In addition to the ability to retain ionized acids, Atlantis PREMIER BEH C_{18} AX Columns can also produce sharp, symmetrical peaks for protonated bases and should therefore be considered when developing chromatographic separations for numerous types of compounds, whether they be polar acids or hydrophobic bases, like

umifenovir.

References

1. Liu, C. *et al. Research and Development on Therapeutic Agents and Vaccines for COVID-19 and Related Human Coronavirus Diseases*. *ACS Central Science* 2020, *6* (3), 315-331.
2. Walter, T.H. *et al. A New Mixed-Mode Reversed-Phase/Anion-Exchange Stationary Phase Based on Hybrid Particles* Waters Application Note 720006742EN < https://www.waters.com/nextgen/us/en/library/application-notes/2020/a-new-mixed-mode-reversed-phase-anion-exchange-stationary-phase-based-on-hybrid-particles.html>
3. Iraneta, P. C.; Wyndham, K. D.; McCabe, D. R.; Walter, T. H. *A Review of Waters Hybrid Particle Technology. Part 3. Charged Surface Hybrid (CSH) Technology and its Use in Liquid Chromatography.* Waters White Paper 720003929EN <https://www.waters.com/waters/library.htm?cid=511436&lid=10167251&locale=en_US> , 2011.

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