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Improvements in Chromatographic Performance for Stability Indicating Methods of Antiviral Drugs with MaxPeak Premier Technology

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Abstract

Chromatographic methods developed to analyze pharmaceutical compounds must distinguish low-level impurities well before they reach alert/action levels. To successfully accomplish this, parameters such as retention, peak shape, and sensitivity are critically important.[1,2]

The MaxPeak Premier Technology reduces sample loss due to non-specific binding of analytes to metal-ions present within liquid chromatographic instruments and columns. Use of this new technology can have a significant impact on the ability to detect and quantify accurate concentrations of low-level analytes, such as those that are genotoxic or pharmacologically active.

Benefits

- MaxPeak Premier Technology exhibits improved chromatographic performance without the need for strong mobile phase additives, chelators, or lengthy passivation protocols

- Some low-level analytes, such as reactive pharmaceutical impurities, show a significantly improved response and peak profile when using columns and/or instruments with MaxPeak Premier

Introduction

High Pressure Liquid Chromatography (HPLC) is used throughout drug development and manufacturing to monitor the presence of impurities in active pharmaceutical ingredients (API)s. The US Food and Drug Administration (FDA) guidance for drug substances specifies that impurities be reported at a threshold of ≥0.1%, while any impurity considered to be unusually potent or toxic be reported regardless of the concentration.[3,4] Recovery at trace levels is impacted by array of both chemical and mechanical chromatographic influences.

Conventional HPLC instruments and columns are comprised of stainless-steel parts and accessories. This material, selected for its availability and mechanical strength, is vulnerable to deterioration when exposed to harsh liquids such as acidic and/or halide containing mobile phases. Metal-ions from corrosion bind inconsistently or irreversibly to the column stationary phase where they can interact with ion-sensitive analytes through complexation, oxidation, and epimerization. The outcome is sometimes poor, unacceptable chromatographic peak shape, with the potential for complete loss of the target analyte, particularly at trace concentrations.[1,2]

Prodrugs are APIs administered to improve the absorption, distribution, and metabolization of an active pharmaceutical ingredient. Prodrug Tenofovir Alafenamide Fumarate (TAF) increases bioavailability of the target molecule (Tenofovir) by masking its reactive, free hydroxyl groups. Release of the active ingredient occurs following a series of hydrolysis reactions which result in the exposure of negatively charged molecule, Tenofovir.[5] Although useful for pharmacological purposes, the same negative charge can pose challenges during chromatographic analysis due to the increased susceptibility for metal-ion interaction. In this study, we demonstrate the chromatographic performance improvements provided by the MaxPeak Premier Technology to monitor Tenofovir as a reactive, trace level impurity.

Experimental

Materials and Methods

LC system 1:	ACQUITY Arc System with Quaternary Solvent Manager (rQSM), Sample Manager (rFTN), ACQUITY Arc Column Manager (rCM), Empower 3 Chromatography Data Software
LC system 2:	Arc Premier System with Quaternary Solvent Manager (rQSM), Arc Premier Sample Manager (rFTN), Empower 3 Chromatography Data Software
Detection:	ACQUITY Photodiode Array Detector (PDA), UV 260 nm
Column(s):	XBridge *XP*, BEH C_{18}, 2.5 µm Column, 4.6 x 150 mm, p/n: 186006711 XBridge Premier, BEH C_{18}, 2.5 µm Column, 4.6 x 150 mm, p/n: 186009849
Column temp.:	43 °C
Sample temp.:	20 °C
Injection volume:	5 µL
Flow rate:	1.3 mL/min
Diluent:	(50/50) methanol/water

Mobile phase A:	10 mM ammonium formate pH 4.0
Mobile phase B:	acetonitrile
Conditions:	2 minute hold at 2% mobile phase B followed by an 8.5 minute gradient from 2–90% acetonitrile

Tenofovir, CAS No. 206184-49-8, (USP, Rockville, MD) was prepared at approximately 0.1 mg/mL in diluent to serve as a chromatographic retention time reference standard (Figure 1). A stock solution of Tenofovir Alafenamide Fumarate, CAS No. 379270-38-9 (Selleckchem, Houston, TX), was prepared in diluent at 0.5 mg/mL. The stock solution was divided into forced degradation sample preparations and exposed to a variety of conditions (6). The heated control and oxidized forced degradation sample preparations were analyzed with the instrument/column setups described in Table 1. To avoid influences from previous passivation, new columns were used, and instruments were flushed thoroughly with 100% IPA followed by HPLC grade water. In support of the proof of concept purpose of this application note, recovery results were reported by % area, rather than % w/w. The term “conventional” was used interchangeably to refer to the ACQUITY Arc System and/or XBridge XP Column, without the MaxPeak Premier Technology.

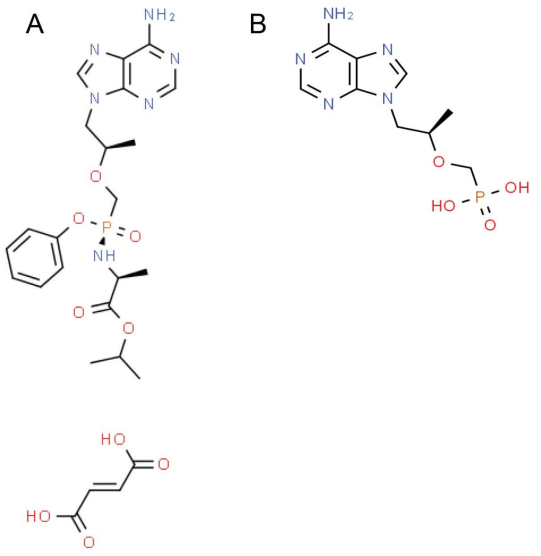

Figure 1. Structure of (A) prodrug Tenofovir Alafenamide Fumarate and (B) Tenofovir (7).

Setup	Instrument	Column type
1	ACQUITY Arc System	XBridge XP
2	ACQUITY Arc System	XBridge Premier
3	Arc Premier System	XBridge Premier

Table 1. System Setup: Conventional and MaxPeak Premier instrument and/or column.

Results and Discussion

When the Tenofovir reference standard preparation was analyzed using each of the three setups, notable chromatographic performance differences were apparent. In the conventional setup, peak height was low, and integration was very difficult due to significant peak tailing. In contrast, when the MaxPeak Premier Technology was employed, performance improvements in peak area, height, and USP signal-to-noise ratio (s/n) were

evident, while peak width at 5σ (8) and USP tailing deceased to allow straightforward integration (Figure 2, Table 2).

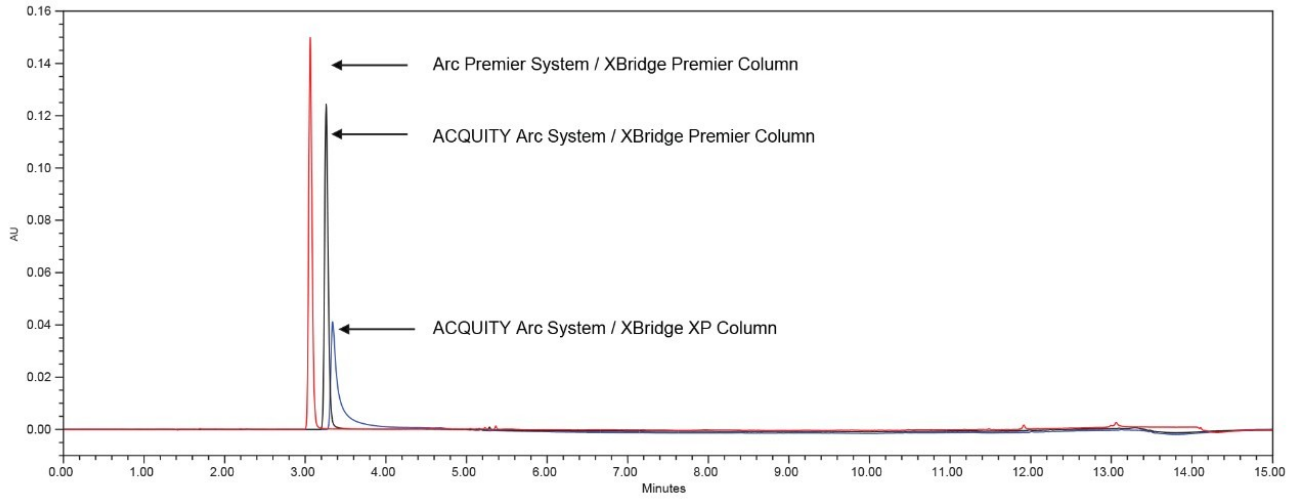

Figure 2. Overlay of peak performance for the Tenofovir reference standard preparation with each system setup.

System	Area	Height	USP s/n	USP tailing	Peak width at 5σ (8)
1	300882	41179	2612	3.85	0.40
2	391311	124377	7027	1.28	0.11
3	437073	149826	10050	1.25	0.10

Table 2. Tenofovir reference standard peak performance with conventional and MaxPeak Premier Technology.

When analyzing the forced degradation sample preparations with the stability indicating method, the parent molecule and prodrug, Tenofovir Alafenamide Fumarate, was retained until 8.4 minutes while Tenofovir, present as a degradation product, eluted at approximately 3.1 minutes (Figure 3). With conventional technology, the Tenofovir peak in the control sample preparation was challenging to distinguish from baseline noise due to the high degree of peak tailing. Manual integration of the very broad peak, performed after overlay with a blank injection, showed recovery at just above the 0.1% impurity reporting threshold. With MaxPeak Premier Technology, Tenofovir peak performance in the forced degradation sample preparations substantially improved

with recovery at nearly 4.5-fold the conventional result, to well exceed the 0.1% impurity reporting threshold (Figure 4). The oxidized forced degradation sample preparation showed a similar trend with recovery at 1.38% by conventional analysis. Recovery increased to greater than 3% with MaxPeak Premier Technology.

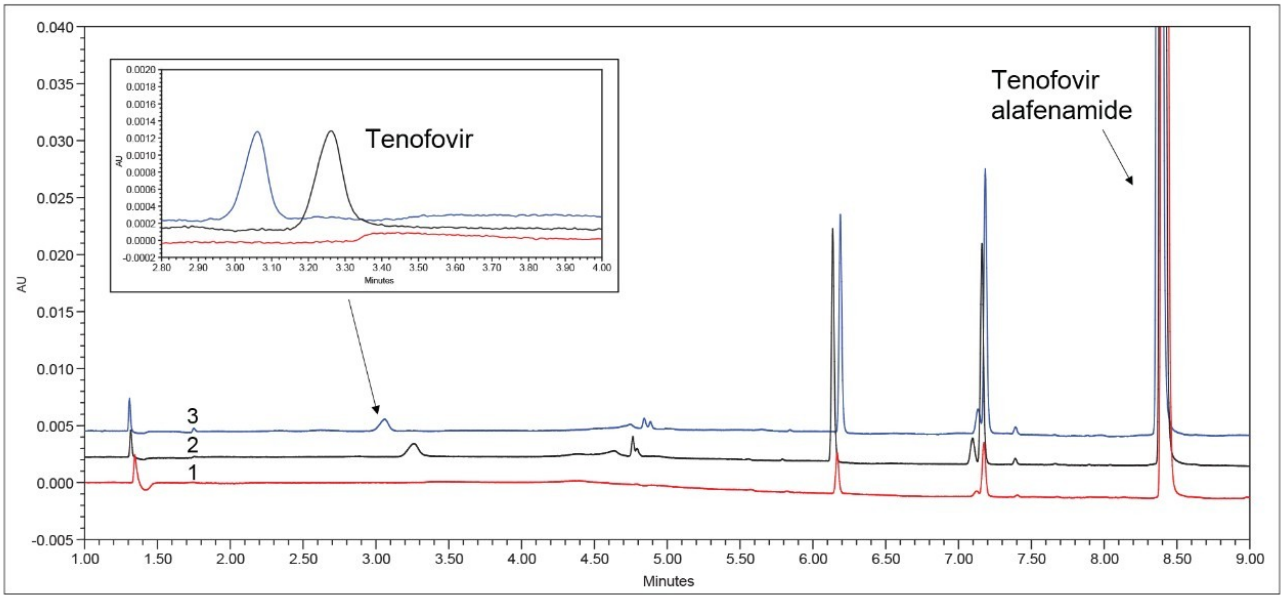

Figure 3. Comparison of chromatographic performance for stressed control sample preparations analyzed with three system set-ups: (1) ACQUITY Arc System/XBridge XP Column, (2) ACQUITY Arc System/XBridge Premier Column, and (3) Arc Premier System/XBridge Premier Column. Inset shows recovery of the low-level degradation product identified as Tenofovir.

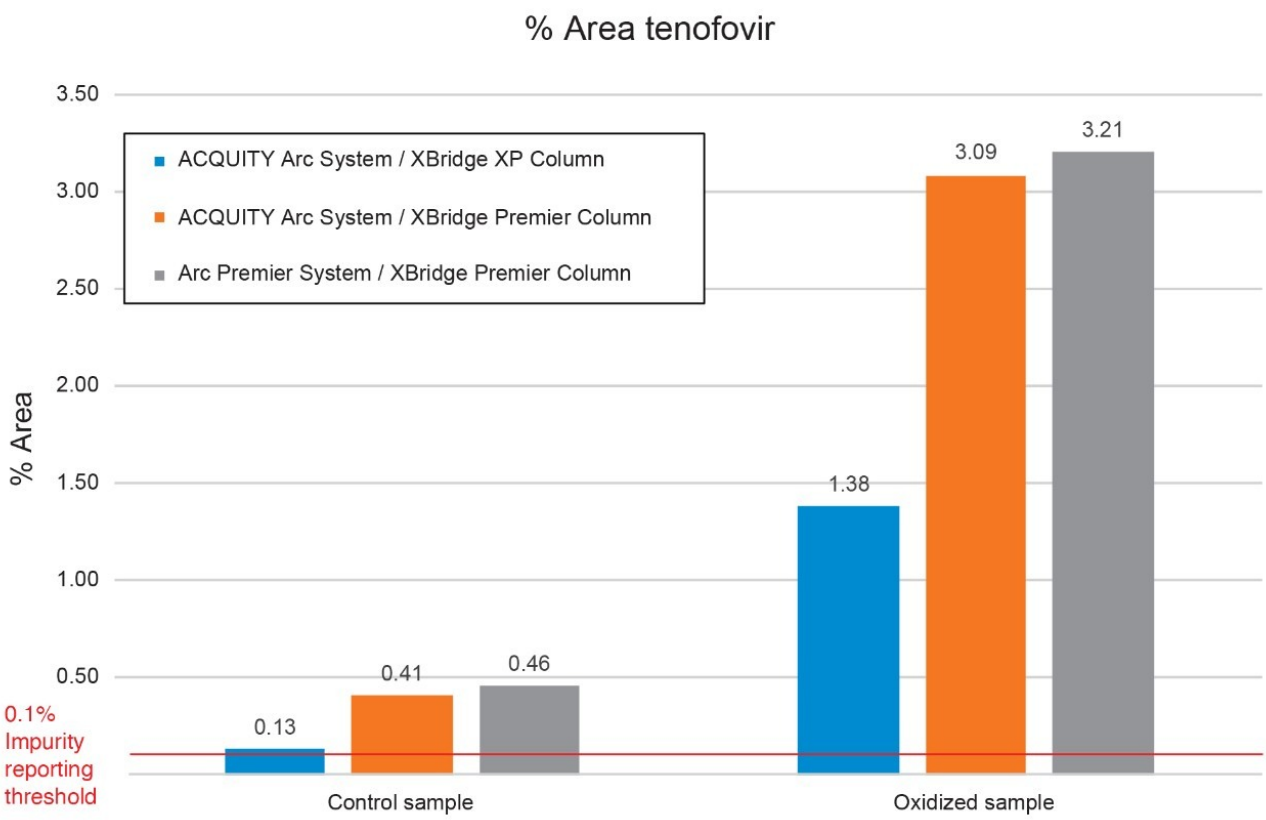

Figure 4. Tenofovir recovered from the control and oxidized forced degradation sample preparations.

Conclusion

MaxPeak Premier Technology provides a clear benefit for detection and recovery of metal-sensitive analytes. Without this new technology, Tenofovir, a highly reactive antiviral therapeutic, was nearly undetectable and required manual integration due to poor peak shape in forced degradation samples. When metal-surface interactions were reduced by employing MaxPeak Premier instrument and/or column technology, the trace level analyte was easily detectable and well exceeded FDA impurity threshold reporting limits.

References

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