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Applikationsbericht

Forced Degradation Study of Janus Kinase Inhibitor, Baricitinib, Using MaxPeak™ Premier HPLC Columns on an Alliance™ HPLC™ System

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Abstract

Baricitinib works as an immunomodulatory medication by inhibiting the Janus kinase subtypes, JAK1 and JAK2. This medication is used in the treatment of rheumatoid arthritis, alopecia areata, and most recently, COVID-19. Baricitinib is the first immunomodulatory medication for COVID-19, gaining approval in 2022.[1] As is the case with many novel drugs, proper analysis of process impurities and degradants is needed to ensure the safety of the medicine before it is administered.

In this work, a forced degradation study was performed using XBridge™ Premier Columns on an Alliance HPLC System. Prior to forced degradation, a comparison of MaxPeak Premier Columns to stainless-steel columns was performed to assess the benefits of the technology for the baricitinib main peak. Good benefit was seen, and MaxPeak Premier Columns were used for analysis of stressed samples.

Benefits

- Increased peak area for baricitinib and impurities using XBridge Premier Columns

- New degradant peaks obtained under stressed conditions
- Fully resolved separation of baricitinib and degradants using XBridge Premier BEH™ C_{18} Column using high pH mobile phases

Introduction

Understanding how a drug degrades is an important aspect to development and release of the therapeutic compound. Forced degradation and stability studies are required to ensure that the formulated sample is not only stable, but that any degradants formed are safe for the consumer. These studies are often done in two parts, with the first being forced degradation. For this testing, the active pharmaceutical ingredient (API) is stressed under varying conditions to show how the compound degrades under different chemical circumstances. Acidic and basic hydrolysis, peroxide, photolytic, and thermal stress is often applied to try and degrade the compound in a timely manner. The second part is stability testing, where-in the formulated sample is stored under reasonable conditions in the final packaging and tested periodically to ensure it does not degrade, or that the packaging does not affect the formulated sample. Stability and leachable testing takes much longer to set up, as the samples are not "stressed" like they are in forced degradation studies. These studies are crucial for product development and getting accurate results quickly enable faster to-market results.

Achieving the most accurate results quickly is difficult with conventional systems and column hardware. Stainless steel hardware can have deleterious effects on analyte recovery and peak shape, leading to poor results.[2–5] Additionally, stainless-steel hardware has been known to passivate over time, leading to rising peak area results for chelating or metal sensitive compounds.[2] For critical assays, getting variable peak areas over time or less reproducible results can lead to costly re-work or worse, a failed result. However, by using MaxPeak Premier Columns which incorporate MaxPeak High-Performance Surface (HPS) Technology, those issues can be mitigated leading to more accurate results with the first injection.

To demonstrate the benefits MaxPeak Premier Columns can provide, a forced degradation study of the JAK inhibitor baricitinib was performed. Prior to forced degradation testing, a comparison of stainless-steel vs. MaxPeak Premier Columns was performed to assess the impact of the MaxPeak HPS Technology. Next, the forced degradation study was performed and analyzed using a XBridge Premier BEH C_{18} Column. Degradants were detected and found to be well separated and symmetrical using an Alliance HPLC system with TUV detector. Final method conditions could be used for further study, such as identification of the degradants using

LC-MS or purification of degradants via preparatory LC.

Experimental

Sample Description

Stock solution of baricitinib created at 1 mg/mL in 50:50 (v:v) acetonitrile:water. Forced degradation studies performed by taking 1 mL of stock solution and adding 100 µL of either 1 N sodium hydroxide or 1 N hydrochloric acid. Stressed samples were heated at 70 °C for 24 hours and then combined prior to analysis.

LC Conditions

LC system:	Alliance HPLC System with TUV Detector
Detection:	UV at 260 nm
Columns:	XBridge Premier BEH C_{18} Column 3.5 µm, 4.6 x 100 mm (p/n: 186010660)
Column temperature:	30 °C
Sample temperature:	10 °C
Injection volume:	10 µL
Flow rate:	2.0 mL/min
Mobile phase A:	Water
Mobile phase B:	Acetonitrile
Mobile phase D:	200 mM Ammonium Hydroxide in Water

Gradient conditions:	5% constant D to maintain additive concentration. Linear ramp of 5–95% B/C in 16.43 minutes. Hold at 95% organic for 2.7 minutes, return to 5% organic in 0.02 minutes. Re-equilibrate column for 5.51 minutes. Total run time: 25 minutes

Data Management

Chromatography software:	Empower™ 3 Feature Release 5

Results and Discussion

Forced degradation and stability testing are essential for new drug development workflows. These studies provide valuable insights into how a compound degrades under stressed or storage conditions, and whether the degradation of the compound leads to any potentially hazardous compounds. With the ever-growing complexity of pharmaceuticals, forced degradation studies can produce a wide range of degradant peaks with varying concentration levels in the presence of the active pharmaceutical ingredient (API). These low-level compounds can then be either purified to assess their potential toxicity or are reported as related compounds and their levels monitored in batch testing. To get the most accurate results, it is important to choose the right technology for these studies. When it comes to liquid chromatography columns, the most accurate results can be obtained on MaxPeak Premier Columns, which use MaxPeak HPS Technology.

MaxPeak Premier Columns mitigate secondary interactions between analytes and any metal surfaces in the column hardware. These interactions include ionic adsorptive effects between acidic moieties and iron ions in the stainless steel, as well as other non-specific adsorption (NSA) effects. MaxPeak Premier Columns have been shown to provide higher peak recovery with better peak shape for a variety of compounds, even those without acidic moieties.[2–5] The benefit of using MaxPeak Premier Columns for the forced degradation of the Janus kinase inhibitor, baricitinib, was assessed prior to forced degradation testing. Representative chromatograms and tabular data are shown in Figure 1 and Table 1 respectively.

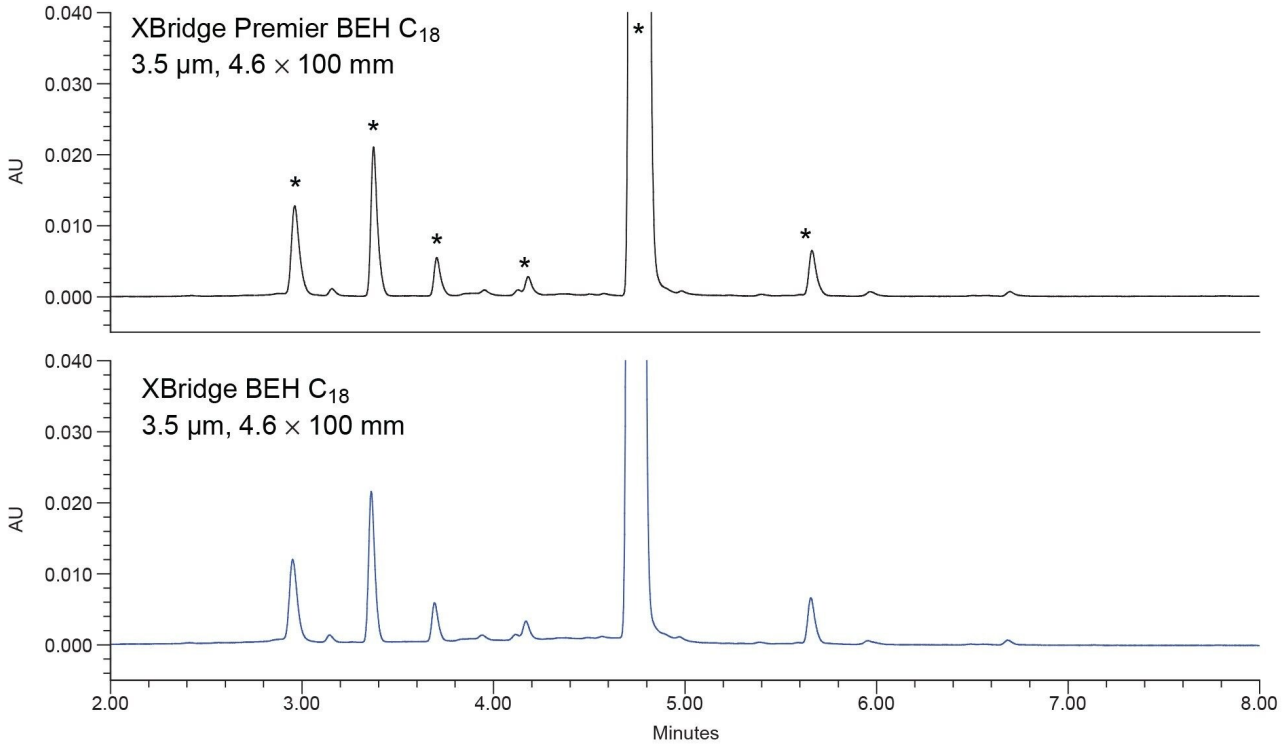

Figure 1. Comparison of XBridge Premier BEH C_{18} 3.5 µm Column and a stainless steel XBridge BEH C_{18} 3.5 µm Column for the analysis of baricitinib stock solution.

The chromatography shows little change in the results obtained. There are no obvious changes to peak shape, or peak height. All the compounds marked with asterisks in Figure 1 are present on both columns, indicating no analyte loss due to NSA or ionic interactions. However, as seen in Table 1, the peak areas for these compounds are higher using the XBridge Premier BEH C_{18} Column compared to the standard stainless-steel hardware column. The only analyte that has approximately the same peak area on both columns is the latest eluting peak.

	XBridge Premier BEH C_{18}		XBridge BEH C_{18}		
T_r	Peak area	Area %	Peak area	Area %	% Increase in peak area
2.962	38305	0.58	36222	0.58	5.44
3.374	50702	0.77	49139	0.78	3.08
3.704	13127	0.20	12699	0.20	3.26
4.181	7143	0.11	6906	0.11	3.32
Baricitinib	6431541	98.06	6152289	98.05	4.34
5.663	17783	0.27	17635	0.28	0.83

Table 1. Tabular data of the analysis of baricitinib stock solution on the XBridge Premier BEH C_{18} Column and the XBridge BEH C_{18} Column.

With modest improvements of between 3–5% increase in peak area between the two columns, it can be asserted that the use of MaxPeak Premier Columns improves the overall separation quality of the analysis. As shown in Table 1, Area % counts are approximately the same between the two columns, meaning there would be no need to re-work the method to account for the new technology used. In this instance the use of MaxPeak Premier Columns provides a more accurate result with only a change in column used.

With this improvement seen, the XBridge Premier BEH C_{18} Column was next used to analyze a forced degradation sample. Forced degradation of baricitinib stock solution was performed using acidic (1 N HCl) and basic (1 N NaOH) conditions at 70 °C for 24 hours. The two samples were then combined for analysis to not only quench the degradation but also combine the two conditions. Analysis of the forced degradation sample is shown in Figure 2.

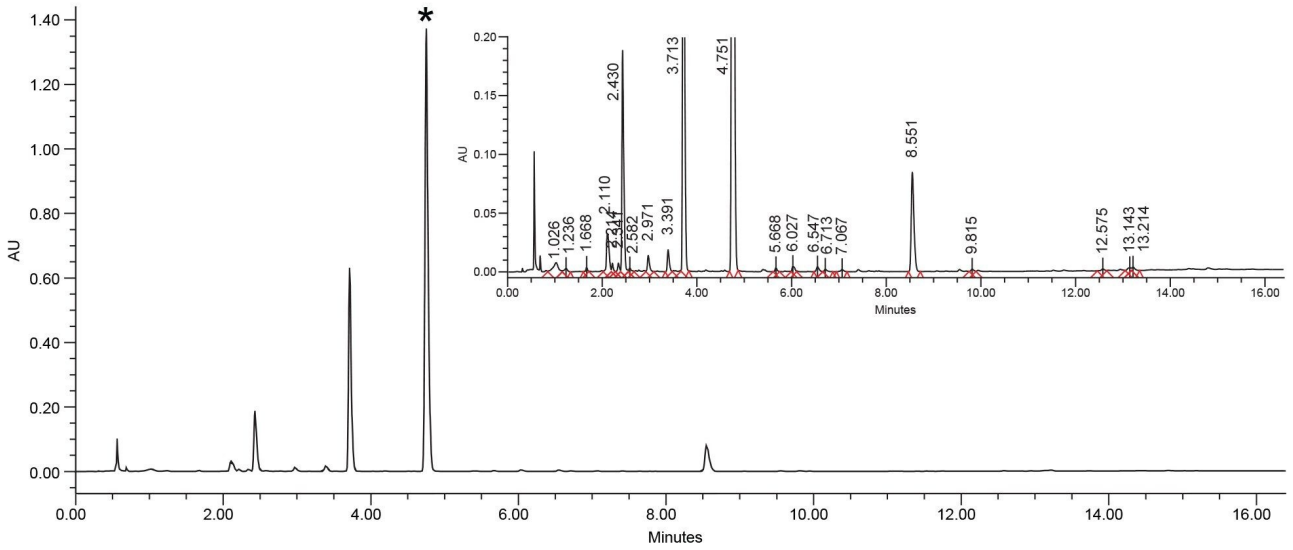

Figure 2. Analysis of baricitinib forced degradation sample using the XBridge Premier BEH C_{18}, 3.5 µm, 4.6 x 100 mm Column on an Alliance HPLC with TUV detector. Baricitinib main peak marked with asterisk. Inset is zoomed in baseline with integration applied for any impurity greater than 0.1% peak area.

As shown, the combined acid and basic hydrolysis degradation leads to a significant number of degradants that are about the typical 0.1% peak area threshold for reporting. Without the use of MaxPeak Premier Columns, some of these analytes would be below that limit, meaning they would not be reported, potentially leading to an issue if they are later found to be carcinogenic or potentially toxic. Obtaining the most accurate data possible leads to better results and ultimately less re-work. MaxPeak Premier Columns, now available in 3.5 µm particle size configurations, enables users on any system to take advantage of this technology to get the right answer fast and eliminate the doubt in their analyses.

Conclusion

MaxPeak Premier Columns, which use MaxPeak High-Performance Surface (HPS) Technology, improve LC separations by mitigating secondary interactions between analytes and the metal surface of the column hardware. By removing these interactions, retention times become more reproducible, peak recovery is improved, and in many cases peak shape is more symmetrical. Even for compounds which may not interact with metal

surfaces, MaxPeak Premier Columns have been shown to improve separation performance. An example of this is the analysis of baricitinib, a Janus kinase inhibitor. MaxPeak Premier Columns improved peak areas for not only the main peak, but also some low-level impurities. More accurate results lead to less re-work and faster turn-around of critical studies, such as forced degradation studies. By standardizing to MaxPeak Premier Columns, those benefits can be easily obtained.

References

1. "FDA Roundup: May 10, 2022". U.S. Food and Drug Administration (FDA) (Press release). 10 May 2022. Archived from the original on 10 May 2022. Retrieved 10 May 2022. https://www.fda.gov/news-events/press-announcements/fda-roundup-may-10-2022. <https://www.fda.gov/news-events/press-announcements/fda-roundup-may-10-2022>

2. Delano M, Walter TH, Lauber M, Gilar M, Jung MC, Nguyen JM, Boissel C, Patel A, Bates-Harrison A, Wyndham K. Using Hybrid Organic-Inorganic Surface Technology to Mitigate Analyte Interactions with Metal Surfaces in UHPLC. ***Anal. Chem.*** 93. 2021. 5773–5781.

3. Zabala G, Berthelette K, Gu W, Haynes K. Improved Reproducibility for Acetaminophen Assay USP Monograph Using MaxPeak Premier Columns after Modernization to 2.5 µm Particles. Waters Application Note. 720007938. July 2023.

4. Walter TH, Alden BA, Belanger J, Berthelette K, Boissel C, DeLano M, Kizekai L, Nguyen JM, Shiner S. Modifying the Metal Surfaces in HPLC Systems and Columns to Prevent Analyte Adsorption and Other Deleterious Effects. ***LCGC Supplements***. 2022. 28–34.

5. Layton C, Rainville P. Advantages of MaxPeak HPS Technology for the Analysis of Targeted Cancer Growth Inhibitor Therapies. Waters Application Note. 720007565. March 2021.

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