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Application Note

Avoid Potential Downtime Due to Column Availability: Moving an LC Method to an Alternative Stationary Phase

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Abstract

Being able to run chromatographic assays and obtain results in a timely manner can be imperative, especially in a quality control environment or when developing critical methods. While delays in testing are not uncommon, backordered columns can become a significant problem. This was experienced during the COVID-19 pandemic, where analysts often encountered longer column order lead times due to supply chain issues. For validated methods which rely on specific column chemistries, a shortage of columns can lead to laboratory downtime and even delays in QC, and thus product release.

Moving a method from one stationary phase to another is a possible solution, particularly if a column is no longer being manufactured or if the manufacturer experiences significant supply chain issues. While the process to transfer a method across stationary phases is straightforward, some insight into column chemistries and understanding of selectivity differences is required. The differences between stationary phases can be accounted for by selecting an appropriate column with similar base particle and bonded phase attributes. The work shown here demonstrates the tools and process of moving a method found in a peer reviewed paper from the original stationary phase, an Inertsil ODS-SP, to another stationary phase, a Waters XSelect HSS T3 Column, in the event of a column shortage. The method to be transferred, a forced degradation of the oncological drug pomalidomide was published in 2015. Forced degradation studies are important in drug discovery labs as understanding how a new compound degrades ensures safety and improves the understanding of how the compound interacts with a biological system. These studies can also be used as impurity assays in QC environments.

Benefits

- Demonstrate how to move a method from one stationary phase to another
- Minimizing selectivity differences while maintaining separation performance
- Providing alternate solutions to column availability issues

Introduction

During the COVID-19 pandemic, global supply chains for many products were strained, and availability of several products decreased. These shortages had a pronounced impact, specifically in the quality control release testing of life saving medicines. Depending on the manufacturer, some LC columns have become very difficult to obtain, leading to backlogs in critical testing. Some manufacturers are less affected by supply issues due to diligent sourcing and ensuring supply chains are robust. If columns become difficult to obtain from a certain vendor, being able to move a critical method to a more reliable vendor may be a prudent way to avoid potential downtimes.

This switch to a new stationary phase can be challenging, as there are many different column chemistries available, and even materials with similar names and marketed functionalities can produce vastly different selectivity and results. While validated methods may require a certain column chemistry, selected an appropriate alternate material can still be challenging. An example of this challenge is the C_{18} stationary phases, or L1 columns, available from Waters. There is a total of eight different bonded C_{18} stationary phases available, not including the T3 phases. These eight columns are the BEH C_{18}, CSH C_{18}, HSS C_{18}, HSS C_{18} SB, CORTECS C_{18}, CORTECS C_{18}+, SunFire C_{18}, and Atlantis dC_{18}; all of which can produce slightly different results depending on the assay conditions. This list does not include the other C_{18} stationary phases made by the different vendors on the market.[1] If a validated method called for a L1 column, any of these would, in theory, be appropriate. Selecting one of these materials to use is difficult unless additional information about the chemistries is available.

The work shown here demonstrates the tools that are available when selecting a stationary phase to move a previously developed method to an alternate stationary phase, as well as an example of such a change. The method selected to demonstrate these tools is the forced degradation analysis of pomalidomide, an oncological drug used in the treatment of multiple myeloma.[2] As with many forced degradation studies for pharmaceutical compounds, the accurate quantitation and identification of degradants is important to ensure that no degradant causes significant harm to a patient. Additionally, for certain types of drugs, the degradation pathway is critical to understanding biological function. These assays can also be used in quality control for impurity testing. While all columns used in this test were readily available, that may not be the case for all assays, and the tools shown here will help migrate methods between stationary phases.

Experimental

Sample Description

Pomalidomide was purchased from Sigma-Aldrich. A 4 mL volume of a 0.5 mg/mL solution was created using a sample diluent of 30:70 Acetonitrile:Water (w/w). To induce sample degradation, 100 µL of a 1M HCl solution was added, and the solution heated at 90 °C for 30 minutes. 100 µL of a 1M NaOH solution was added to neutralize the solution. The sample was then diluted with 30:70 acetonitrile:0.1% formic acid (v/v) to an approximately 0.25 mg/mL concentration of pomalidomide. The sample was then placed on the system for analysis.

Method Conditions

LC Conditions

LC systems:	Waters Alliance e2695 with a 2489 UV/Vis Detector
Detection:	UV @ 240 nm
Vials:	LCMS Certified Clear Glass Vial 2 mL (p/n: 600000751CV)
Column(s):	XSelect HSS T3, 4.6 x 250 mm, 5 µm (p/n: 186004793) Inertsil ODS-SP, 4.6 x 250 mm, 5 µm
Column temp.:	30 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	1.0 mL/min
Mobile phase A:	90:10 (0.1% Formic acid in Water:Acetonitrile)
Mobile phase B:	Acetonitrile
Gradient:	*See Table*

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
0.00	1.0	100	0	6
5.00	1.0	100	0	6
25.00	1.0	50	50	6
30.00	1.0	20	80	6
30.01	1.0	100	0	6
40.00	1.0	100	0	6

Data Management

Chromatography software: Empower 3 Feature Release 4

Results and Discussion

To effectively move a method from one stationary phase to another, the specifics of the original material must be known. Details such as carbon load, particle type, endcapping, and bonded ligand chemistry can be used to ensure proper selection of an alternative stationary phase. Some vendors readily share the pertinent information on their website or through marketing materials, though some information like surface area or the type of endcapping is kept proprietary. Using these stationary phase characteristics, an analyst can begin looking at alternate stationary phases. However, with the sheer number of column chemistries available, it can be difficult to find a comparable material without external help. Here, the Column Coach from Waters or other tools can be used.[3, 4] The Waters Column Coach provides the user with a comparable Waters Column to a selected stationary phase from 21 different vendors. In the example presented here, the separation was originally performed with an Inertsil ODS-SP Column by GL Sciences. This separation, the acid degradation pathway for pomalidomide, is an example of an assay that could benefit from having alternate stationary phases for analysis. Having multiple stationary phases available to perform

such an assay reduces potential downtime. While the Inertsil ODS-SP Column is readily available, having an alternative column for testing is prudent in the event of unforeseen circumstances like shipping delays, or column backorders.

The Waters Column Coach recommended the XSelect HSS T3 Column as a comparable alternative to the Inertsil ODS-SP Column. Both columns use fully porous spherical silica particles, bonded with a C_{18} chemistry. Specific parameters of both materials are shown in Table 1.

Stationary phase characteristic	XSelect HSS T3 Column	Inertsil ODS-SP Column
Particle type	Fully porous spherical silica	Fully porous spherical silica
Particle surface area (m^2/g)	258	450
Pore size (Å)	100	100
Bonded chemistry	Trifunctionally Bonded C_{18}	Bonded C_{18}
Carbon load (%)	11	8.5
Ligand density ($\mu mol/m^2$)	1.6	Unlisted
Endcap style	Proprietary	Proprietary
Operational pH range	2–8	2–7.5
Temperature limit (°C)	45	60
Available particle sizes (µm)	1.8, 2.5, 3.5, 5, 10	3, 5

Table 1. Material characteristics and specifications as listed in column care and use manuals and manufacturer websites.

Notable differences between the Inertsil ODS-SP Column and the XSelect HSS T3 Column include the particle surface areas and percent carbon load. The XSelect HSS T3 Material has a slightly higher carbon load, possibly due to a higher ligand density, differences in particle endcapping, or base particle used. Unfortunately, the ligand density of the Inertsil ODS-SP Material is not listed by the manufacturer so no direct comparisons can be made. Depending on the conditions of the assay and the analytes being tested, the ligand coverage can have a significant impact on the separation. For polar analytes tested in reversed-phase, a higher ligand coverage can lead to reduced retention as the polar analytes interact less with the hydrophobic surface of the stationary phase and are more readily soluble in the mobile phase. However, for non-polar analytes, a higher ligand coverage will increase retention. The differences in surface area can also impact a separation, with higher surface area materials providing increased retention in most cases. By matching the characteristics of the particle prior to performing any experiments, an analyst can be reasonably certain that their separation will be comparable between the two stationary phases.

The degradation of pomalidomide requires a low ligand coverage C_{18} stationary phase. Pomalidomide has a cLogD value of <0 indicating it is moderately polar. When degraded, the resulting compounds are also polar, which would benefit from a low ligand coverage C_{18}. In the cited article, the major degradation products are shown, along with the pathway for their formation. In most cases, the degradation products include the breaking of an amide bond to form a carboxylic acid group and the opening of a heterocyclic ring with a terminal amine or amide group. For more specifics on the degradation pathway, please refer to the original work.[2] Figure 1 shows the chromatographic separation of an acid degraded sample of pomalidomide, obtained on the two columns using the cited method conditions on an Alliance HPLC System with UV detection.

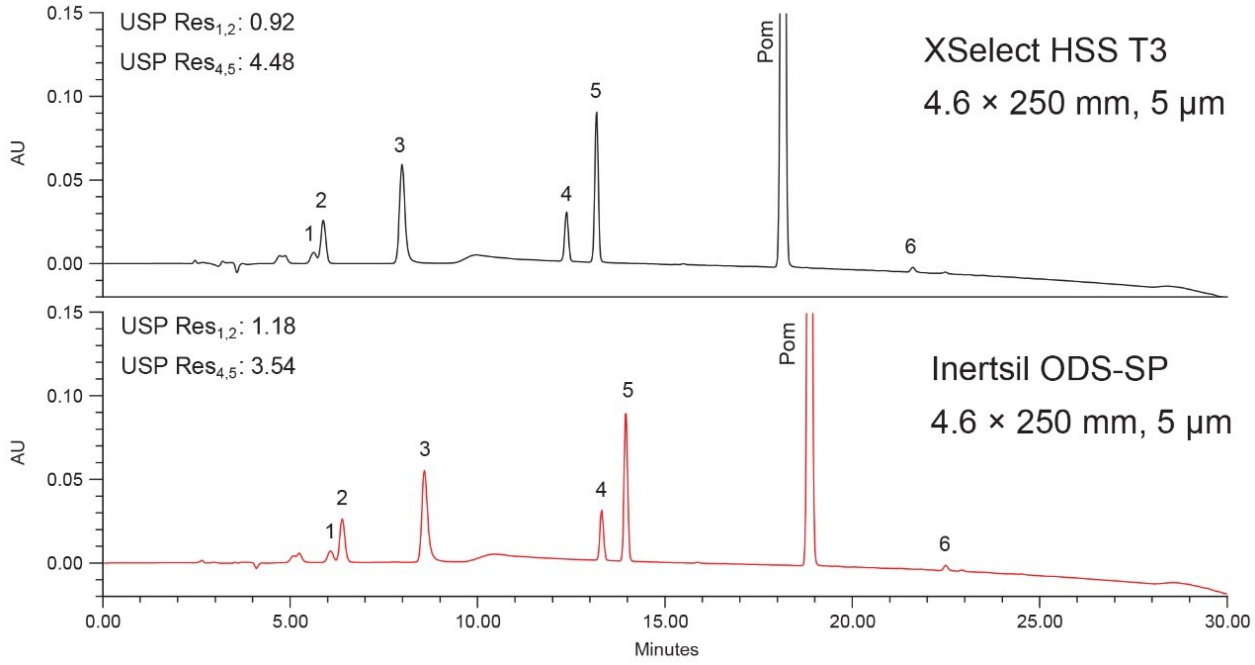

Figure 1. UV chromatographic separation of an acid degraded pomalidomide sample on two HPLC columns using an Alliance HPLC System. Numbered peaks were present both in this work as well as the original method cited. Pomalidomide peak, labeled Pom, elutes where expected on the Inertsil ODS-SP Column. The USP resolution for the critical pairs of peaks 1 and 2 as well as peaks 4 and 5 were calculated and are shown.

Here, the two columns perform similarly for the acid degraded pomalidomide sample. While the XSelect HSS T3 Column is slightly less retentive under these conditions, the chromatographic performance is similar to the Inertsil ODS-SP Column. The USP resolution for the critical pair of peaks 1 and 2 is only slightly different between the two columns, with neither achieving baseline resolution. The XSelect HSS T3 Column achieves a USP resolution for component 2 that is approximately 30% lower than the Inertsil ODS-SP Column. However, for component 5, the XSelect HSS T3 Columns has a USP resolution that is approximately 26% higher compared to the Inertsil ODS-SP

Column. Other performance measures for the two columns, notably USP plate count for pomalidomide, and peak width for pomalidomide are comparable between the two columns as shown in Table 2. For both performance measures, the two columns are very similar.

Pomalidomide peak result	XSelect HSS T3 Column	Inertsil ODS-SP Column
Peak width at 13.4% peak height (min)	0.183	0.184
USP plate count	160050	169525

Table 2. Peak width @ 13.4% peak height and USP plate count for pomalidomide for both columns tested.

Conclusion

Column availability issues can have a devastating impact on the timely analysis of samples. In extreme cases, when a column is not available, a lab may be forced to temporarily close or halt product releases while waiting for an order to arrive. While this is often mitigated via proper forecasting of column needs, unexpected delays due to supply chain issues can still occur. Using an alternate stationary phase for critical assays can prevent laboratory downtime. However, the process of selecting an appropriate stationary phase can be challenging and often requires in-depth knowledge of the material which may not always be available. Several tools exist to help guide analysts in these activities, such as the Waters Column Coach, which can suggest comparable stationary phases as a starting point for method transfer.

The analysis of an acid degradation of a small molecule oncological drug, originally performed on an Inertsil ODS-SP Column, was successfully moved to an XSelect HSS T3 Column with minimal changes in selectivity and separation performance. The XSelect HSS T3 Column was selected for this work based on the properties of the stationary phase compared to the Inertsil ODS-SP Column. This example illustrates how easily a method can be moved between stationary phases when the materials are chosen correctly.

References

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