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アプリケーションノート

Improving USP Monograph Analysis Time Through Scaling by N on an HPLC System Using CORTECS™ Premier Columns

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Abstract

The United States Pharmacopeia General Chapter <621> allows certain changes to monograph methods. Changes in column length and particle size are employed as part of modernization efforts from HPLC to UPLC™ instrumentation. However, some modernization activities can be performed without switching LC systems. For HPLC analyses, reducing particle size from 5 µm to 3.5 µm can improve throughput and run times, however those columns increase backpressure of the system. Additionally, depending on the monograph, modernization may not be possible due to available column configurations, and the guidelines set in General Chapter <621> for scaling by L/dp. Modernizing by plate count (N) is one path forward, especially if the column being modernized to takes advantage of highly efficient particles. This application note scales the USP impurities assay of zidovudine from the original 4.6 x 250 mm column using fully porous 5 µm particles to a 4.6 x 150 mm column using solid-core particles to improve both throughput and solvent usage.

Benefits

- USP Monograph for zidovudine impurities analyzed on various columns
- Vastly improved separation efficiency using CORTECS Premier 5 µm columns with original USP monograph column configuration
- 40% improvement in solvent usage and up to 60% improvement in analysis run time using CORTECS Premier

5 µm Column

Introduction

Monograph methods have traditionally employed the use of 5 µm particle size columns due to pressure limitations of older HPLC instrumentation. In combination with the larger particle stationary phases, longer column dimensions were needed in order to achieve appropriate column efficiency. This leads to lengthy analyses with high volumes of solvent being consumed. Monograph methods can be modernized however, and the United States Pharmacopeia (USP) outlines two routes for modernization as outlined in USP General Chapter <621>.[1] The first is by changing particle size and column length (L/dp) which can be done but may require the use of more modern LC technology like UPLC. The second route to modernization involves scaling a column based on the plate count (N) for the assay. Changes can be made to either the L/dp or N by -25% and up to 50%. Modernizing by N allows a method to be changed to any configuration as long as the plate count for the new conditions falls within the limits outlined in General Chapter <621>.

For some assays, modernizing by N can be possible by using higher efficiency stationary phases, like solid-core particles. Solid-core particle columns, like CORTECS columns, have been shown in the past to boost separation efficiency due to particle morphology.[2–4] By taking advantage of the solid-core particles, a shorter column may be used while still achieving acceptable plate counts. Thus, improvements to run time and solvent usage could be possible without changing particle size, therefore avoiding the potential increases to system pressure accompanied with smaller particle sizes. In this application note, the assay and impurities monographs for zidovudine were scaled from a 250 mm column down to a 150 mm column using the modernize by N approach. Using the new CORTECS Premier 5 µm columns results in a 40% decrease in solvent usage with up to a 60% reduction in analysis time per sample.

Experimental

Sample Descriptions

All samples were created in accordance with United States Pharmacopeia (USP) monograph for zidovudine.[5] Assay standard solution contained 0.2 mg/mL zidovudine in sample diluent. Impurities standard solution made at 1 µg/mL zidovudine in diluent. Lastly, the impurities system suitability standard made to contain 2 µg/mL related compound B, and 1 mg/mL zidovudine in diluent.

LC Conditions

LC system:	Arc™ HPLC System with PDA detector
Detection:	UV @ 265 nm
Columns (Scaling):	CORTECS Premier C_{18}, 4.6 x 150 mm, 5 µm (p/n: 186010792) CORTECS Premier C_{18}, 4.6 x 250 mm, 5 µm (p/n: 186010793) XBridge™ BEH™ C_{18}, 4.6 x 250 mm, 5 µm (p/n: 186003117)
Column temperature:	Ambient
Sample temperature:	10 °C
Injection volume:	20 µL
Flow rate:	1.50 mL/min (monograph and scaled) 2.25 mL/min (high throughput)
Mobile phase A:	2 g/L ammonium acetate in water pH 6.8 (~26mM)
Mobile phase B:	Acetonitrile
Gradient (Monograph):	Initial conditions of 5% B. Hold at 5% B for 3.00 minutes. Linear gradient to 15% B in 15 minutes. Linear gradient to 70% B in 10 minutes. Hold at 70% B for 15 minutes. Return to starting conditions and re-equilibrate at 5% B for 2 minutes. Total run time: 45 minutes
Gradient (150 mm Scaled):	Initial conditions of 5% B. Hold at 5% B for 1.8 minutes. Linear gradient to 15% B in 9.0 minutes.

	Linear gradient to 70% B in 6.0 minutes. Hold at 70% B for 9.0 minutes. Return to starting conditions and re-equilibrate at 5% B for 1.2 minutes. Total run time: 27 minutes
Gradient (150 mm High Throughput):	Initial conditions of 5% B. Hold at 5% B for 1.20 minutes. Linear gradient to 15% B in 6.0 minutes. Linear gradient to 70% B in 4.0 minutes. Hold at 70% B for 6.0 minutes. Return to starting conditions and re-equilibrate at 5% B for 1.8 minutes. Total run time: 19 minutes
Sample diluent:	Acetonitrile:Methanol:Mobile Phase A (4:20:76)

Data Management

Chromatography software:	Empower™ 3 Service Release 5

Results and Discussion

Prior to any modernization attempts the original monograph conditions were tested using a 4.6 x 250 mm 5 µm XBridge BEH C18 Column in order to get initial Plate Count (N) values and to ensure the monograph would pass system suitability criteria. Figure 1 shows the assay standard solution, impurities test standard solution and impurities test system suitability standards using the monograph column configuration.

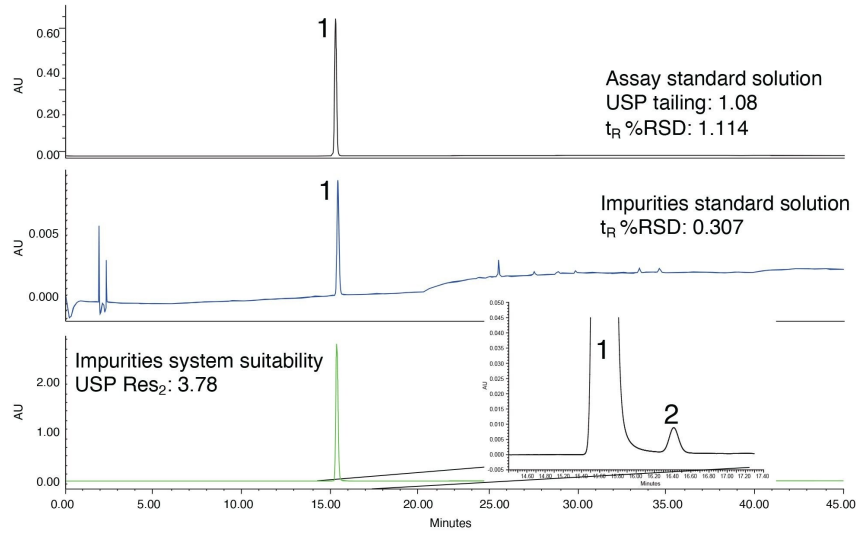

Figure 1. Assay and Impurities standards on an XBridge BEH C_{18}, 4.6 x 250 mm, 5 µm Column. 1) Zidovudine, 2) Related Compound B.

As expected, both the assay and impurities test system suitability criteria are met, Table 1. However, given the lengthy run times of 45 minutes per injection at 1.5 mL/min analyzing a sample of zidovudine for purity and impurities requires at least 720 minutes, or 12 hours. This is due to the need for replicate injections to accurately calculate results. Specifically, USP General Chapter <621> indicates that for criteria which specifies a criterion of 2.0 then five replicates are needed. Criteria that specify a higher value, for instance 5.0, requires at least six injections. In looking at the criteria for zidovudine assay and impurities, the assay standard solution must be injected five times, along with the impurities system suitability standard. All of the criteria that reference those standards have values of 2.0. However, the impurity standard solution has a criteria of 5.0, which means six injections are required for that particular sample. Sixteen total injections are needed, at 45 minutes per injection, leading to the very long analysis time. This does not include actual sample analysis and is only for system suitability testing prior to sample analysis. This testing also requires a total of 1.08 L of solvent in total, a significant amount of solvent for a single analysis.

Column	%RSD (assay)	USP tailing (assay)	%RSD (impurity)	USP resolution (impurity)	USP plate count (assay)	Range of acceptable N values for scaling
Monograph acceptance criteria	NMT 2.0%	NMT 2.0	NMT 5.0%	NLT 2.0	None	N/A
XBridge BEH C_{18}, 4.6 × 250 mm 5 µm	1.114	1.08	0.307	3.78	79631	59,723–119,447

Table 1. Tabular results for assay and impurities tests using the XBridge BEH C_{18} Column. No more than (NMT) and no less than (NLT) criteria were compared to the results of replicate injections (n=6) to satisfy USP General Chapter <621>.[1]

As shown in the table, the USP Plate Count (N) for zidovudine in the assay was just below 80,000. Applying the -25% to 50% range that' s allowable under USP General Chapter <621>, any modernized or scaled methods must have an N value between 59,723–119,447. These are the target values for the modernization of this assay using the CORTECS Premier 5 µm Columns. In order to improve analysis time and solvent usage for this assay, the CORTECS Premier C_{18}, 4.6 x 150 mm, 5 µm Column was chosen. This column uses solid-core base particles which have been shown to improve efficiency of a separation without sacrificing overall performance. Using the L/dp method of modernization this column would not be acceptable as the column length has been shortened considerably, while the particle size has remained constant. However, scaling by N allows for such a change as long as the values obtained on the new column are within the acceptable range. Adjustments to gradient profile were determined using the ACQUITY™ Columns Calculator, which has been shown to greatly improve the accuracy of modernization activities compared to manual determinations.[6] Figure 2 shows the three standards for zidovudine assay and impurity testing on the CORTECS Premier C_{18}, 4.6 x 150 mm, 5 µm Column.

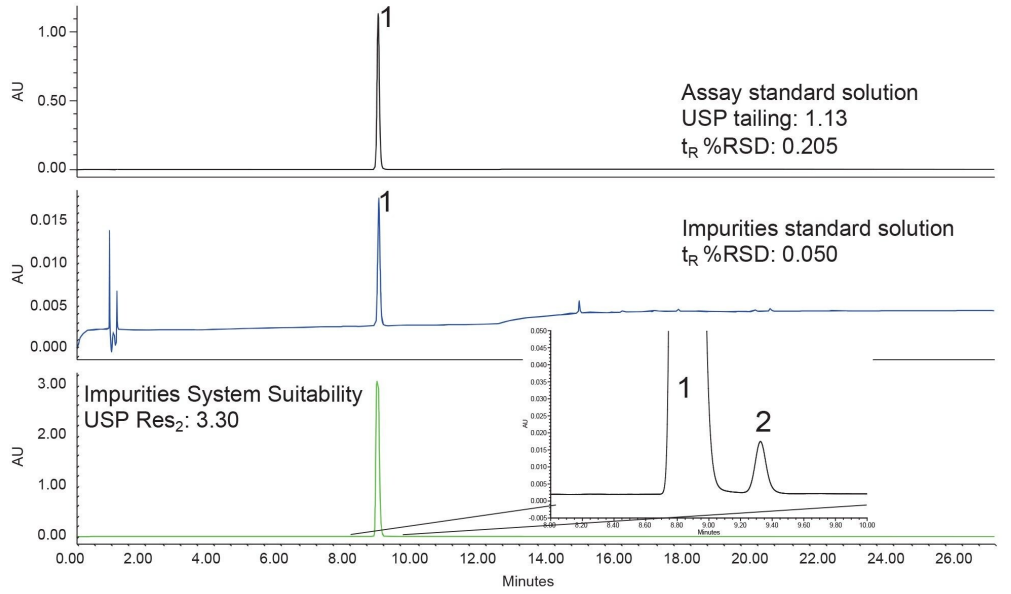

Figure 2. Assay and Impurities standards on a CORTECS Premier C_{18}, 4.6 x 150 mm, 5 µm Column. 1) Zidovudine, 2) Related Compound B.

The resulting chromatograms show comparable results to those obtained on the monograph column, with the notable difference in run time. Where previously a single injection required 45 minutes, now the standards are run in 27 minutes per injection, a 40% reduction in run time. This directly translates to higher throughput as now full testing requires only 432 minutes or 7.2 hours compared to the original 12 hours. Additionally, by scaling to the smaller column, even though the flow rate did not change, a 40% reduction in solvent usage is also realized. Using the 150 mm column requires 648 mL of total solvent compared to the 1.08 L of solvent needed on the original column. In order to ensure this modernization is allowable by USP guidelines, the Plate Count for zidovudine must be recorded. Table 2 shows the tabular results for the scaled conditions compared to original.

Column	%RSD (assay)	USP tailing (assay)	%RSD (impurity)	USP resolution (impurity)	USP plate count (assay)	Range of acceptable N values for scaling
Monograph acceptance criteria	NMT 2.0%	NMT 2.0	NMT 5.0%	NLT 2.0	None	N/A
XBridge BEH C_{18}, 4.6 × 250 mm 5 µm	1.114	1.08	0.307	3.78	79631	59,723–119,447
CORTECS Premier C_{18}, 4.6 × 150 mm 5 µm (1.5 mL/min)	0.205	1.13	0.050	3.30	71,899	
CORTECS Premier C_{18}, 4.6 × 150 mm 5 µm (2.25 mL/min)	0.097	1.12	0.546	3.07	61,467	

Table 2. Tabular results for assay and impurities tests using various testing conditions. No more than (NMT) and no less than (NLT) criteria were compared to the results of replicate injections (n=6) to satisfy USP General Chapter <621>.

When using the CORTECS Premier C_{18}, 4.6 x 150 mm Column with scaled flow rate of 1.5 mL/min, not only are all criteria met, but the USP Plate Count value for zidovudine is within the acceptable ranges for N. This means that the assay and impurity tests can be performed on the shorter column to improve throughput and reduce solvent usage.

Further improvement in throughput can be obtained by increasing the flow rate of the analysis. Increasing the flow rate of the assay is, in this case, outside of the allowable changes outlined in USP <621>, however increasing the flow rate would drastically increase the throughput of the analysis. The scaled conditions using the 150 mm conditions were adjusted to a higher flow rate, 2.25 mL/min, a 50% increase compared to the scaled flow rate of 1.5 mL/min. The higher flow rate chromatograms are shown in Figure 3.

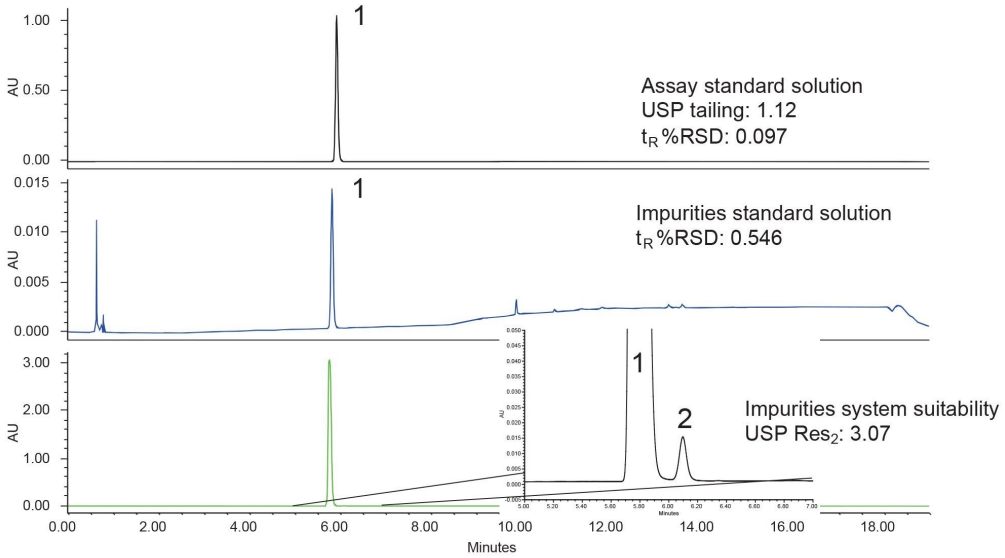

Figure 3. Assay and Impurities standards on a CORTECS Premier C_{18}, 4.6 x 150 mm, 5 µm Column at a flow rate of 2.25 mL/min. 1) Zidovudine, 2) Related Compound B.

Unsurprisingly, the higher flow rate reduced run times from 27 minutes down to 19 minutes without any significant changes in results. This brings the total time needed to analyze all of the standards to 304 minutes, or 5.07 hours. Further reduction in solvent usage was not seen, however. While the increased flow rate may not be allowable by USP <621> the throughput can be improved. Table 2 shows the tabular data for these conditions. Although the increased flow rate excludes this testing from being acceptable, the USP Plate Count for zidovudine under these conditions is still within the acceptable range. This is a testament to the highly efficient CORTECS 5 µm particles which do not lose efficiency at higher flow rates compared to fully porous particles, demonstrating the capabilities of CORTECS Premier Columns in improving throughput for validated methods.

Conclusion

Validated monograph methods often employ HPLC systems with larger particle size columns due to pressure limitations of the system. Modernization of these monograph methods can be performed under USP General Chapter <621> using either the length to particle size ratio (L/dp) or Plate Count (N). In either case, the new column configuration must be within -25% to 50% of the original conditions. Once a new column is selected,

changes to gradient profile and flow rate are calculated. This application note focused on the assay and impurity test for zidovudine, which calls for a 250 mm column in the original monograph.

By using CORTECS Premier 5 µm Column the assay and impurities test for zidovudine was successfully scaled down to a 150 mm column while maintaining the particle size. This greatly improved sample throughput with a 40% reduction in run time and solvent usage. Further improvements to throughput were realized by increasing flow rate from 1.5 mL/min to 2.25 mL/min. The increased flow rate, while not acceptable under USP General Chapter <621>, is still able to be run due to the lower system pressure generated by the 150 mm column. A 60% reduction in run time is realized with the higher flow rate. CORTECS Premier 5 µm Columns offer highly efficient solid-core particles combined with MaxPeak Premier High Performance Surface Technology hardware.

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