

## Improving Sample Throughput of HPLC Methods Using CORTECS 5 µm Columns

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### Abstract

Validated HPLC methods are prime targets for systematic improvements like increasing throughput or reducing solvent usage. One way to attain these benefits is to use a column packed with solid-core particles. This application brief examines improving the throughput and analytical greenness of a validated HPLC method by migrating from a column packed with 5 µm fully porous particles to a CORTECS™ Premier C<sub>18</sub> 5 µm Column. Chromatographic performance, system pressure, and Analytical Method Greenness Scores (AMGS) are compared across the columns tested.

### Benefits

- Comparable chromatographic performance for abacavir impurities was achieved using a shorter CORTECS Premier C<sub>18</sub> 5 µm Column in place of a column packed with fully porous 5 µm particles
- 33% reduction in solvent usage and analysis time using a CORTECS Premier C<sub>18</sub> 5 µm Column compared to a fully porous 5 µm column
- 33% reduction in analytical method greenness score indicating improved sustainability for the new method

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## Introduction

Improving validated HPLC methods can be challenging. Two of the approaches to increase analytical throughput, increasing mobile phase flow rate or using shorter columns, have drawbacks in higher pressures or reduced chromatographic performance. Typically, using a shorter column also requires decreasing the particle size to maintain a constant length to particle size ( $L/d_p$ ) ratio to ensure similar column efficiency. However, columns packed with smaller particles generate higher back pressures. Some labs may be able to migrate methods to shorter columns packed with smaller particles by transferring to a HPLC system capable of operating at higher pressures. However, if a laboratory lacks access to such systems, then their options may be limited. In those situations, finding appropriate columns to use to improve a method is not only a matter of chromatographic performance but also system compatibility, particularly from a pressure standpoint. Older HPLC systems are limited to lower pressures compared to more modern systems.

This application brief examines the improvement of a method for testing abacavir impurities by migrating the original method from a 4.6 x 150 mm 5  $\mu$ m fully porous C<sub>18</sub> column to a CORTECS Premier C<sub>18</sub> 4.6 x 100 mm 5  $\mu$ m Column. Additionally, a fully porous 3.5  $\mu$ m C<sub>18</sub> column in 4.6 x 100 mm hardware was evaluated to determine if moving to a smaller particle size would have as much of a benefit as that provided by using a CORTECS Column. The separation conditions for method migration were obtained using the Waters Column Calculator. Along with examining throughput, solvent savings were calculated as well as analytical method greenness scores for each column tested.

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## Experimental

### Sample Description

The abacavir impurities mixture was purchased from Sigma-Aldrich and diluted to 1 mg/mL with water.

### Method Conditions

LC system:	Alliance™ HPLC System with 2489 UV/Visible
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(UV/Vis) Detector

Detection:

UV @ 254 nm

Columns:

CORTECS Premier C<sub>18</sub> Column, 4.6 x 100 mm, 5  
µm (p/n:186010791)

Fully Porous Silica C<sub>18</sub>, 4.6 x 150 mm, 5 µm

Fully Porous Silica C<sub>18</sub>, 4.6 x 100 mm, 3.5 µm

Column temperature:

30 °C

Sample temperature:

10 °C

Injection volume:

8.0 µL (150 mm column), 5.3 µL (100 mm  
columns)

Flow rate:

1.0 mL/min (5 µm columns), 1.43 mL/min (3.5  
µm column)

Mobile phase A:

0.1% TFA in Water

Mobile phase B:

Methanol:Water (85:15 v/v)

Gradient conditions:

See Table 1.

## Data Management

Chromatography software:

Empower™ Chromatography Data System  
(CDS)

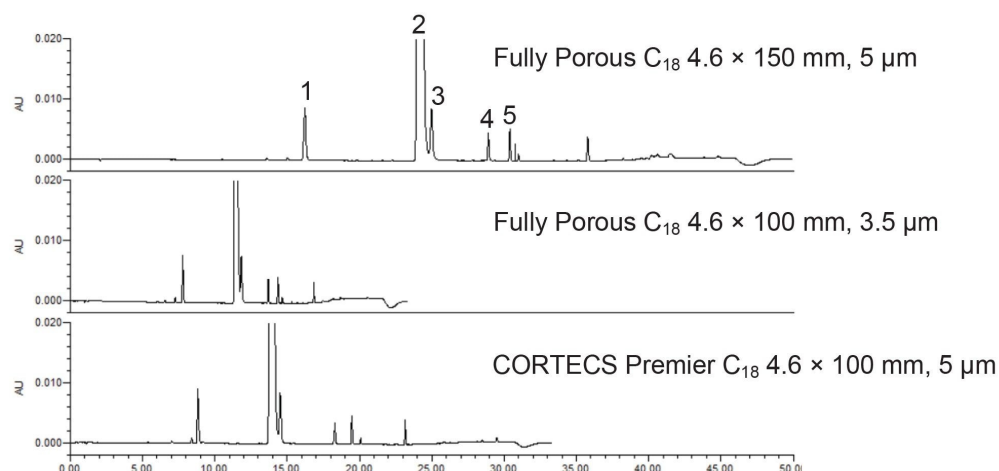
## Gradient Table

Fully Porous C <sub>18</sub> 5 µm 4.6 × 150 mm Time (min)	Fully Porous C <sub>18</sub> 3.5 µm 4.6 × 100 mm Time (min)	CORTECS Premier C <sub>18</sub> 5 µm 4.6 × 100 mm Time (min)	%A	%B
0.00	0.00	0.00	95	5
23.64	11.03	15.76	70	30
38.39	17.92	25.59	10	90
43.83	20.45	29.22	10	90
44.89	20.95	29.93	95	5
50.0	23.33	33.33	95	5

Table 1. Gradient conditions for all columns tested.

## Results and Discussion

Previously, the impurity method for abacavir was migrated to a 2.7 µm CORTECS Column.<sup>1</sup> While that work showed the benefits of scaling to a smaller particle size, not all laboratories are comfortable moving to such a small particle size. In those instances, moving from a 5 µm to a 3.5 µm particle column is a good first step. However, depending on the method, this migration might not be appropriate and could result not only in elevated system pressure but also performance loss. Figure 1 shows the chromatograms for abacavir impurities using the original method column, a 3.5 µm C<sub>18</sub> column, and the CORTECS Premier C<sub>18</sub> 5 µm Column.



*Figure 1. Separation of abacavir impurities on three different columns. 1) Descyclopropyl abacavir, 2) Abacavir glutarate, 3) Abacavir trans isomer, 4) O-pyrimidine derivative abacavir, and 5) t-Butyl derivative abacavir.*

As expected, the use of the shorter column lengths, regardless of particle size employed, leads to shorter analysis times. The fully porous 3.5 μm particle column has a run time of 23.3 minutes compared to the original 50.0 minute run time. While this improvement is certainly beneficial, the overall system pressure and chromatographic performance must be examined to determine if the separation is equivalent to the original. Table 2 shows the average (n=3) USP resolution for peaks 2 and 3 across the three columns tested as well as the total system pressure. System pressure was recorded at the apex of pressure for the gradient run.

Column	Analysis Time (min per injection)	Solvent Used Per Injection (mL)	Avg USP Resolution (pks 3,2)	Total System Pressure (psi)
4.6 × 150 mm Fully Porous 5 µm	50.00	50.00	2.28	~2100
4.6 × 100 mm Fully Porous 3.5 µm	23.33	33.36	1.64	~3800
4.6 × 100 mm CORTECS Premier C <sub>18</sub> 5 µm	33.33	33.33	2.14	~1700

*Table 2. Analysis time, solvent used per injection, USP resolution, and system pressure for the three columns tested.*

Using the 3.5 µm particle column resulted in slightly lower chromatographic performance, as measured by USP resolution for the critical pair. Additionally, the system experienced pressure near the 5,000 psi operating limit. For this sample, the use of the 3.5 µm particle column may have improved throughput and solvent usage, but at the cost of chromatographic performance. It should be noted that even with this loss of resolution the peaks are still considered baseline resolved as the USP resolution value is >1.5.

Employing a CORTECS Premier C<sub>18</sub> 5 µm Column for this separation, however, yields comparable chromatographic performance and system pressure relative to the original conditions, while also achieving higher throughput. This is possible due to the solid-core particles that are used in these columns. The benefits of solid-core particles compared to fully porous particles of the same approximate size are well documented.<sup>2-4</sup> From a practical standpoint, the increased efficiency gained with CORTECS Columns allows a shorter 100 mm column to be used to achieve approximately the same efficiency as a 150 mm column packed with fully porous 5 µm particles.

In addition to seeing an improvement in throughput and reduced solvent usage, the AMGS for this separation is greatly improved using the CORTECS Premier C<sub>18</sub> 5 µm Column. The AMGS metric was created by a group of scientists from several pharmaceutical companies to compare the environmental impacts of different separation methods so that more sustainable methods could be selected.<sup>5</sup> Previous work has shown the benefits of using AMGS values for comparing methods.<sup>6-8</sup> Table 3 shows the AMGS for the separation of abacavir impurities on each column tested using the method parameters outlined

previously. Lower AMGS values indicate a more sustainable method.

Column	Instrument Energy Score	Solvent Energy Score	Solvent EHS Score	Total Greenness Score	%Decrease in Greenness Score
4.6 × 150 mm Fully Porous 5 µm	17.17	0.80	3.18	21.15	—
4.6 × 100 mm Fully Porous 3.5 µm	8.01	0.56	2.23	10.80	48.9
4.6 × 100 mm CORTECS Premier C <sub>18</sub> 5 µm	11.45	0.56	2.23	14.23	32.7

*Table 3. AMGS values for all conditions tested, including % decrease in greenness score compared to the original method conditions.*

The CORTECS Premier C<sub>18</sub> Column method has a 32.7% lower total greenness score compared to the original method. It should be noted that the most sustainable method, i.e. lowest AMGS, used the fully porous 3.5 µm particle column. This is due to the lower instrument energy score, which factors in analysis time and the energy consumption of the instrument for the duration of that analysis. Since the method using the fully porous 3.5 µm particle column had a shorter run time, the energy needed to run the instrument is less, leading to a better total greenness score. However, a lower AMGS value does not compensate for the chromatographic performance loss seen for this method.

Using the CORTECS Premier C<sub>18</sub> 5 µm, 100 mm Column not only provided comparable separation performance as a 150 mm column packed with fully porous 5 µm particles, it did so with a shorter run time, less solvent used, lower system pressure, and a more sustainable overall method, as measured by the AMGS metric. Utilizing these columns can improve validated methods without having to change the HPLC system.

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## Conclusion

CORTECS Premier 5 µm Columns are suitable alternatives for fully porous 5 µm columns for improving validated HPLC methods. Using a CORTECS solid-core stationary phase can allow for a shorter column to be used because of their higher efficiency per unit length. To highlight this, the impurity method for

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abacavir was migrated from the original fully porous 5  $\mu\text{m}$ , 4.6 x 150 mm column to a CORTECS Premier C<sub>18</sub> 5  $\mu\text{m}$ , 4.6 x 100 mm Column, as well as a 4.6 x 100 mm 3.5  $\mu\text{m}$  fully porous column. The use of the CORTECS Column achieved comparable separation performance as the original while also exhibiting a shorter analysis time, lower solvent usage, and a better AMGS value.

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## References

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