THE APPLICABILITY OF 2.7 µm SOLID-CORE COLUMNS FOR HIGH THROUGHPUT ANALYSIS

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CORTECS™ COLUMNS

INTRODUCTION

In method development the one of the most critical choices the analyst can make is the column selection. His primary concern is the selectivity, which is the starting point for determining the success of the separation. Once the chromatographic conditions have been optimized, his next goal is often higher throughput or faster analysis times. A common strategy to reduce analysis time is using shorter columns packed with smaller particles. HPLC methods run on 150 mm or 250 mm length columns packed with 5 µm particles can easily be transferred to shorter columns packed with CORTECS™ 2.7 µm solid-core particles, reducing the time of analysis while maintaining the resolution of the separation.

CORTECS 2.7 µm columns using solid-core particle technology generate lower than expected backpressures when compared to fully porous particles of comparable particle size. The reduced backpressures them an ideal choice for the analysis of biological samples that have minimal sample preparation techniques like protein precipitation.

SCALING

Maintaining Efficiency
Decreasing the particle size, increases the number of theoretical plates in a given column length, therefore, shorter length columns can used and the separation can be maintained. The following equation is used to determine the appropriate column length when changing particle size.

$$L_{C2} = \frac{L_{C1} \times d_{P2}}{d_{P1}}$$

L_C = Column Length
d_P = Particle Size

Scaling Injection Volume
Decreasing column volume requires that the injection volume be adjusted accordingly as described in equation below.

$$V_{I2} = V_{I1}\left(\frac{d_{C2}}{d_{C1}}\right)^2 \times \left(\frac{L_{C2}}{L_{C1}}\right)$$

V_I = Injection Volume
L_C = Column Length
d_C = Column diameter

Scaling Flow Rate
Flow rates must be adjusted as column internal diameter changes to maintain the same linear velocity. This is described in the equation below.

$$F_{C2} = F_{C1} \times \left(\frac{d_{C2}}{d_{C1}}\right)^2$$

F_C = Flow Rate
d_C = Column diameter

Scaling Gradient Duration
To maintain the same number of column volumes on both columns, the gradient time must be altered to maintain the gradient slope. The gradient time can be adjusted using the following equation.

$$t_{g2} = t_{g1} \times \left(\frac{F_{C1}}{F_{C2}}\right) \times \left(\frac{d_{C2}}{d_{C1}}\right)^2 \times \left(\frac{L_{C2}}{L_{C1}}\right)$$

t_g = Gradient Time
F_C = Flow Rate
d_C = Column diameter
L_C = Column length

INCREASING THROUGHPUT

When using CORTECS 2.7 µm columns, higher flow rates can be used without significantly reducing the efficiency, and faster separations can be achieved. Flow rate scales in inverse proportion to the change in particle size, however, even higher flow rates can be used depending on the pressure limitations of the instrument. The backpressure generated by increasing the flow rate can be calculated using the equation below.

$$P_{C2} = P_{C1} \times \left(\frac{L_{C2}}{L_{C1}}\right) \times \left(\frac{d_{C1}}{d_{C2}}\right)^2 \times \left(\frac{d_{P1}}{d_{P2}}\right)^2 \times \left(\frac{F_{C2}}{F_{C1}}\right)$$

P_C = Column Pressure
L_C = Column length
d_C = Column diameter
d_P = Particle Size
F_C = Flow Rate

METHODS

The method for scaling abacavir (Ziagen®) and its four known related substances is shown below. All data was generated using a Waters® Alliance HPLC. The sample was prepared in water at a concentration of 1 mg/mL.

Parameters held constant:
Mobile Phase A: 0.1% TFA in Water
Mobile Phase B: 85% Methanol in Water
Detection: UV at 254 nm

Parameters that were changed:
Original Column: Fully porous C_{18} 5 µm, 4.6 x 150 mm
Scaled Column: CORTECS 2.7 µm C_{18}, 4.6 x 75 mm
Original Flow Rate: 1.00 mL/ minute
Scaled Flow Rate: 1.85 mL/ minute
Original Injection Volume: 8 µL
Scaled Injection Volume: 4 µL

Gradient Tables:

Time (min) 4.6 x 150 mm 5 µm	Time (min) 4.6 x 75 mm 2.7 µm	%A	%B
Initial	Initial	95	5
23.64	6.38	70	30
38.39	10.37	10	90
43.83	11.83	10	90
44.89	12.12	95	5

RESULTS

The results in ***Table 1*** below and ***Figure 1*** show the equivalent results from the scaled separation. The resolution of two critical peak pairs from the assay were maintained. Also highlighted in the table is the ~4x reduction in analysis time.

Table 1. *USP resolution results and run times for the separation of abacavir and its known related substances*

Column Dimension	Resolution USP Peaks 3,2	Resolution USP Peaks 4,3	Run Time (min)
4.6 x 150 mm	2.7	3.3	44.9
4.6 x 75 mm	2.7	4.1	12.1

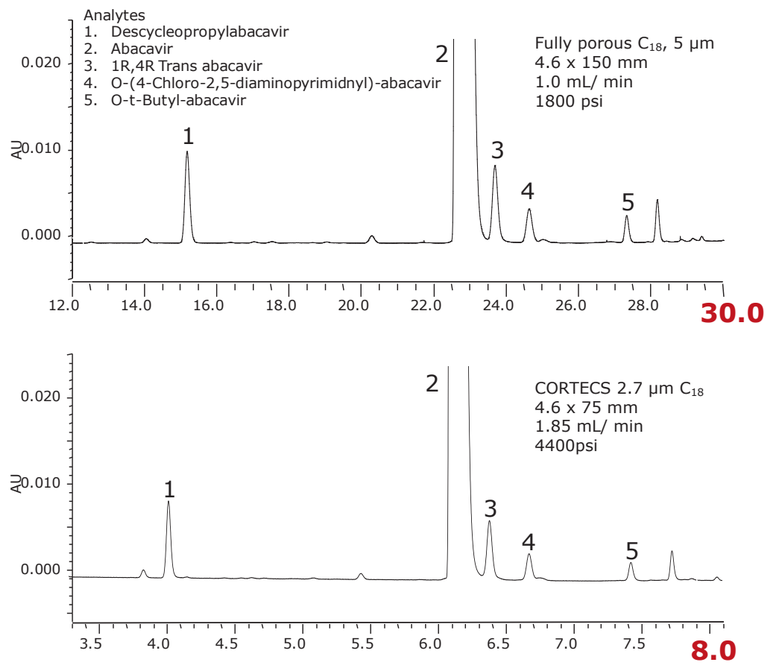

Figure 1. *The scaled separation of abacavir and its known related substances from a fully porous 4.6 x 150 mm C_{18} 5 µm column to a 4.6 x 75 mm CORTECS 2.7 µm C_{18} column.*

Column Lifetime
Complex sample matrices can be a challenge when using columns packed with smaller particles. Often sample preparation techniques are minimal or inadequate, resulting in column failures due to high backpressure. ***Figure 2*** shows the backpressure on a CORTECS 2.7 µm C_{18} after repeated injections of precipitated plasma. On this column the backpressure did not increase significantly during the evaluation.

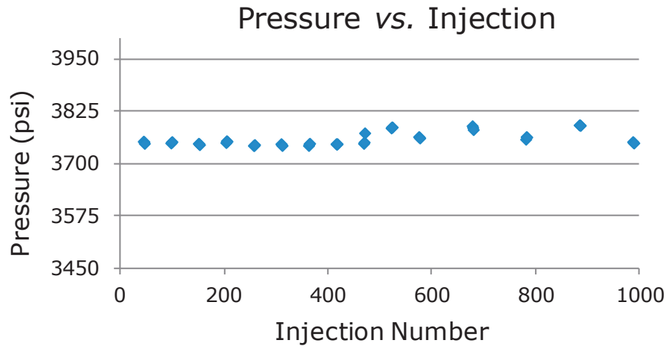

Figure 2. *Pressure vs. injection for 1000 injections of precipitated plasma using a CORTECS 2.7 µm C_{18} 2.1 x 100 mm column.*

CONCLUSION

- **CORTECS 2.7 µm columns can be used to transfer from existing methods using fully porous 5 µm particles. Some benefits are:**
  - → **Increased throughput without sacrificing performance.**
  - → **Faster separations can be achieved using higher flow rates.**
- **CORTECS 2.7 µm columns can be used for applications where the sample preparation may be minimal.**

References
1. J.W. Dolan, LCGC North Am. 32(2), 98-102 (2014)
2. J.W. Dolan, LCGC, North Am. 32(3), 188-193 (2014)

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