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Applikationsbericht

Transferring HPLC Gradient Methods Using CORTECS Solid-Core Particle Columns

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Abstract

This application note demonstrates a proper method transfer of a typical HPLC gradient method for abacavir-related compounds. Abacavir (Ziagen) is a nucleoside reverse-transcriptase inhibitor that is used in anti-HIV therapy.

Benefits

- ~4X decrease in analysis time resulting in faster throughput for routine sample analysis
- ~2X reduction in solvent usage and sample injected
- Operate within the <5,000 psi pressure limits of HPLC instrumentation

Introduction

Transferring HPLC gradient methods that use larger volume columns packed with larger particles to smaller volume columns packed with highly-efficient CORTECS 2.7 µm Particles is an easy way to reduce analysis time, solvent and sample consumption, and, ultimately, cost. When transferring the HPLC gradient method avoid compromising the chromatographic separation by properly adjusting the method conditions and selecting the equivalent column chemistry.

The following application note demonstrates a proper method transfer of a typical HPLC gradient method for abacavir-related compounds. Abacavir (Ziagen) is a nucleoside reverse-transcriptase inhibitor that is used in anti-HIV therapy. The sample is composed of five compounds, four of them related substances of the main component abacavir; all shown in Figure 1. A typical column for this type of assay is a fully porous C_{18}, 5 µm, 4.6 x 150 mm column. The analysis time of this gradient method can be significantly reduced by transferring to a CORTECS C_{18}, 2.7 µm Column, while maintaining the selectivity and the resolution of the peaks of interest.

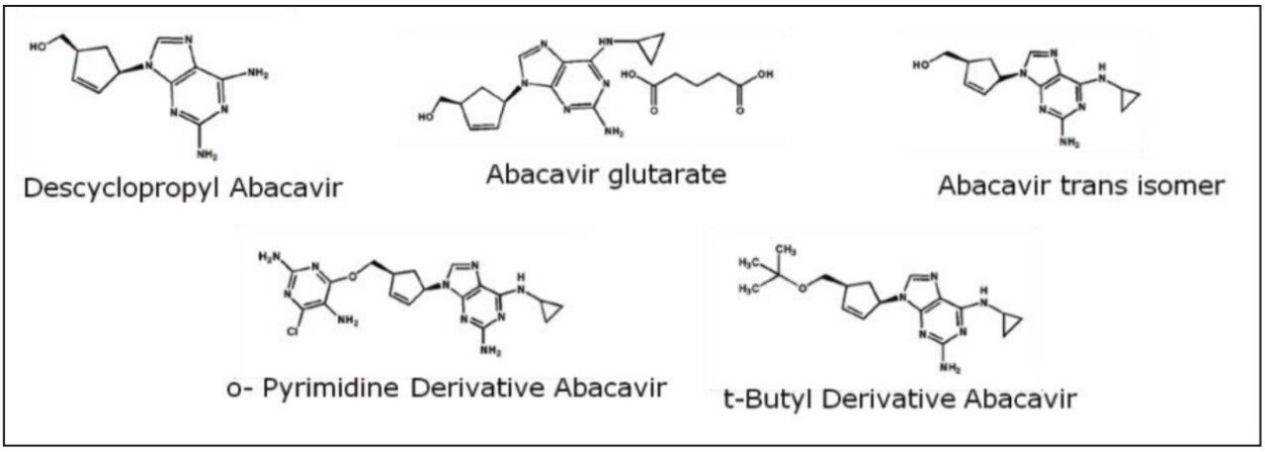

Figure 1. Abacavir and its related substances.

Experimental

Method using a fully porous C_{18}, 5 µm, 4.6 x 150 mm Column

LC conditions

System:	Alliance HPLC with 2489 TUV detector
Column:	Fully porous C_{18}, 5 µm, 4.6 x 150 mm
Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	85% methanol in water
Backpressure:	1,800 psi
Detection:	UV at 254 nm
Needle wash:	90:10 water:acetonitrile
Seal wash:	80:20 acetonitrile:water

Injection volume:	8 µL

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B
Initial	1.0	95	5
23.64	1.0	70	30
38.39	1.0	10	90
43.83	1.0	10	90
44.89	1.0	95	5
50.00	1.0	95	5

Transferred method using a CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm Column

LC conditions

System:	Alliance HPLC with 2489 TUV detector
Column:	CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm (p/n 186007376)
Mobile phase A:	0.1% trifluoroacetic acid in water
Mobile phase B:	85% methanol in water
Backpressure:	4,400 psi
Detection:	UV at 254 nm

Needle wash:	90:10 water:acetonitrile
Seal wash:	80:20 acetonitrile:water
Injection volume:	4 µL
Sample vial:	Waters LCGC certified clear glass vial with PTFE/ silicone septa (p/n 186000307C)
Data Management:	Empower 3

Gradient:

Time (min)	Flow rate (mL/min)	%A	%B
Initial	1.85	95	5
6.38	1.85	70	30
10.37	1.85	10	90
11.83	1.85	10	90
12.12	1.85	95	5
15.00	1.85	95	5

Sample Preparation

Abacavir-related compounds (USP reference standard) 1.0 mg/mL in 100% HPLC-grade water.

Results and Discussion

Method transfer equations

Maintaining efficiency when decreasing the particle size

Decreasing the particle size increases the number of theoretical plates in a given column length, therefore, shorter length columns can be 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 the following equation.

$$V_{I2} = V_{I1}\left(\frac{d_{c2}}{d_{c1}}\right)^2 \times \left(\frac{L_{c2}}{L_{c1}}\right)$$

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. The flow rates must also be adjusted in inverse proportion to the change in particle size to maintain the performance; this is done using the following equation.

$$F_{C2} = F_{C1} \times \left(\frac{d_{C2}}{d_{C1}}\right)^2 \times \left(\frac{d_{p1}}{d_{p2}}\right)$$

F_C= Flow Rate

d_C = Column Diameter

d_P = Particle Size

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.

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

F_C= Flow Rate

d_C = Column Diameter

d_P = Particle Size

The CORTECS C_{18}, 2.7 µm chemistry was chosen for the transfer; this was based on the fully porous C_{18}, 5 µm column typically used for this type of assay. Transferring to a column packed with 2.7 µm particles requires a 75 mm column length to maintain the L/d_p ratio. For this transfer, a 4.6 x 75 mm column configuration was chosen. The change in column length required that the injection volume be adjusted from 8 µL to 4 µL. Since the I.D. of the column was not changed the adjusted flow rate is based on the change in particle size only; the adjusted flow rate was calculated to be 1.85 mL/minute. Adjusting the column configuration and the flow rate requires that each time segment of the gradient also be adjusted to ensure that the separation takes place over the equivalent number of column volumes.

A comparison from the method transfer is shown in Table 1 and in Figure 2. The transferred method using the CORTECS C_{18}, 2.7 µm Column has equivalent selectivity to the method that was performed using a fully porous C_{18}, 5 µm column. Also, the resolution values for two critical peak pairs have been maintained. The 4,400 psi backpressure generated on the CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm Column is well within the 5,000 psi pressure limit of the HPLC instrument. Transferring the HPLC gradient method to the CORTECS C_{18}, 2.7 µ

m Column reduces the analysis time is by a factor of ~4X and the solvent consumption by ~2X.

Column	Column Dimension	USP Resolution Peaks 3,2	USP Resolution Peaks 4,3	Analysis Time (min)	Volume of MeCN/ Run (mL)	Backpressure (psi)
Fully Porous C_{18}, 5 µm	4.6 x 150 mm	2.7	3.3	50.0	20.8	1800
CORTECS C_{18}, 2.7 µm	4.6 x 75 mm	2.7	4.1	15.0	10.5	4400

Table 1. Results of Abacavir-related substances method transferred ssing a CORTECS C_{18}, 2.7 µm Column.

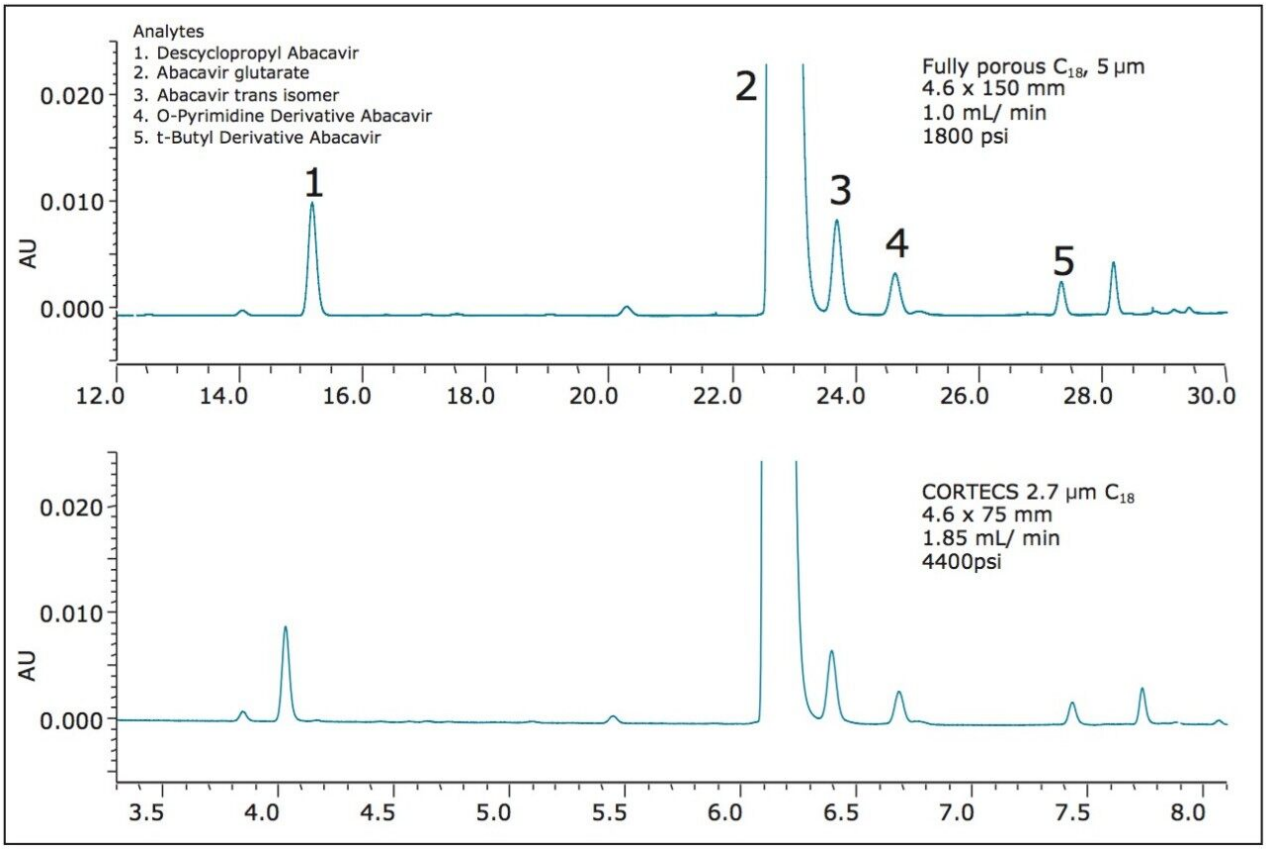

Figure 2. Chromatograms of Abacavir-related substances method transferred to a CORTECS C_{18}, 2.7 µm Column.

Conclusion

Transferring HPLC gradient methods that use larger volume columns packed with larger particles to smaller volume columns packed with highly efficient CORTECS particles can easily be achieved. A typical HPLC gradient method for the analysis of abacavir-related substances was successfully transferred to demonstrate a ~4X improvement in throughput while maintaining the chromatographic separation. In addition to the time savings, solvent consumption was reduced by ~2X. The backpressure generated when using the CORTECS C_{18}, 2.7 µm, 4.6 x 75 mm Column is well within the limits of the HPLC instrument of <5,000 psi. When transferring HPLC gradient methods to CORTECS 2.7 µm Columns the increase in throughput and the decrease in solvent consumption add up to significant cost savings.

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Alliance HPLC System <https://www.waters.com/534293>

2489 UV/Visible (UV/Vis) Detector <https://www.waters.com/515198>

Empower 3 Chromatography Data Software <https://www.waters.com/10190669>

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