Easy Transfer of HPLC Methods to UHPLC

Using Fully Scalable Pinnacle™ DB Columns

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- Methods on Pinnacle™ DB columns are easily transferred from 3 and 5µm to 1.9µm, allowing faster analysis without losing separation quality.
- Pinnacle™ DB columns are 100% Restek manufactured–from base silica to final packed column.
- Restek offers the widest selection of stationary phases for UHPLC—more choices mean better selectivity for your analytes.

Ultra High Pressure Liquid Chromatography (UHPLC) is a rapidly growing technique that produces significantly faster analysis times compared to conventional HPLC. While transferring HPLC methods to UHPLC can increase sample throughput, comparable method parameters must be used to maintain equivalent separations. Here we review which column properties and operating conditions should remain consistent and which need to be optimized in order to maintain selectivity.

In this example, we will perform a scale-down method transfer for sulfonamides (Figure 1). For optimal selectivity and faster analysis times, we used a Pinnacle™ DB Biphenyl stationary phase for this application (Figure 2). When performing a scale-down procedure, column pore size, carbon load, and support material must remain the same. Changes to other parameters can be made using a few simple calculations. Let's go through them sequentially.

Adjusting Column Size

The first calculation determines the appropriate column length. Keeping the same column length while decreasing the particle size increases the number of theoretical plates. Therefore, column length can be shortened without losing resolution. By adjusting the column length properly, using Equation 1, we can maintain the same separation.

Adjusting Injection Volume

Once we have determined the proper column length, we can calculate injection volume. Decreasing the column internal diameter and length decreases the overall column volume and sample capacity. Therefore, we must alter the injection volume as described in Equation 2. Note that since overall column volume has decreased, it is important to match the sample solvent to the starting mobile phase composition. Mismatched sample solvents can cause irreproducible retention times, efficiencies, and even changes in selectivity.

Adjusting Flow rate

Next, flow rate must be adjusted to maintain comparable linear velocity through a column with smaller internal diameter. To maintain the same linear velocity (which is important in maintaining

Figure 1 Chemical structures for example sulfonamides.

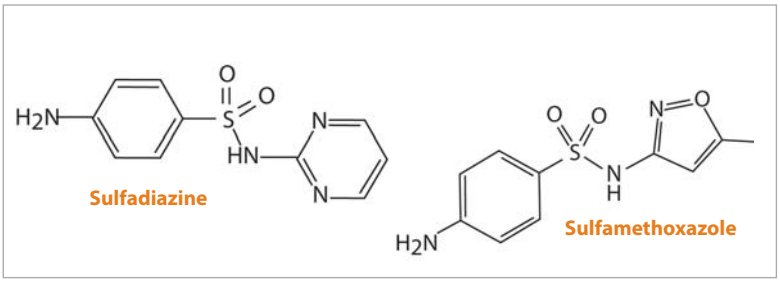
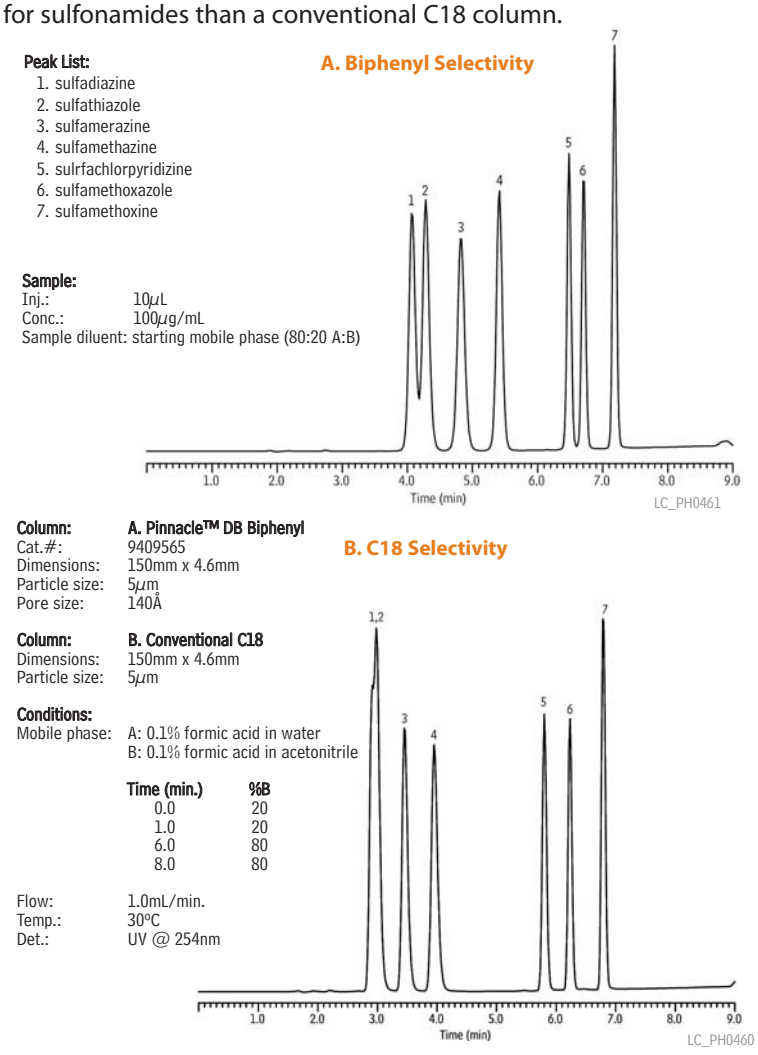


Figure 2 A 1.9µm Pinnacle™ DB Biphenyl column is more selective for sulfonamides than a conventional C18 column.



Equation 1 Adjusted column length can easily be calculated when scaling from HPLC to UHPLC.

$$L_{C2} = \frac{L_{C1} \cdot dp_2}{dp_1}$$

Example:

$$L_{C2} = \frac{150\text{mm} \cdot 1.9\mu\text{m}}{5\mu\text{m}}$$

$$L_{C2} = 57\text{mm}$$

L_C = Column Length
dp = Particle Size

Equation 2 Changing column dimensions requires an adjusted injection volume.

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

Example:

$$V_{I2} = 10\mu\text{l} \cdot \left(\frac{2.1\text{mm}^2 \cdot 50\text{mm}}{4.6\text{mm}^2 \cdot 150\text{mm}} \right)$$

$$V_{I2} = 0.69\mu\text{l}$$

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

Equation 3 Changing column internal diameter requires using an adjusted flow rate.

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

Example:

$$F_{C2} = \left(\frac{2.1\text{mm}}{4.6\text{mm}} \right)^2 \cdot 1.0\ \text{ml/min}$$

$$F_{C2} = 0.208\ \text{ml/min}$$

F_C = Column Flow
d_C = Column Diameter

Equation 4 When scaling down a gradient method, the time program needs to be adjusted.

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

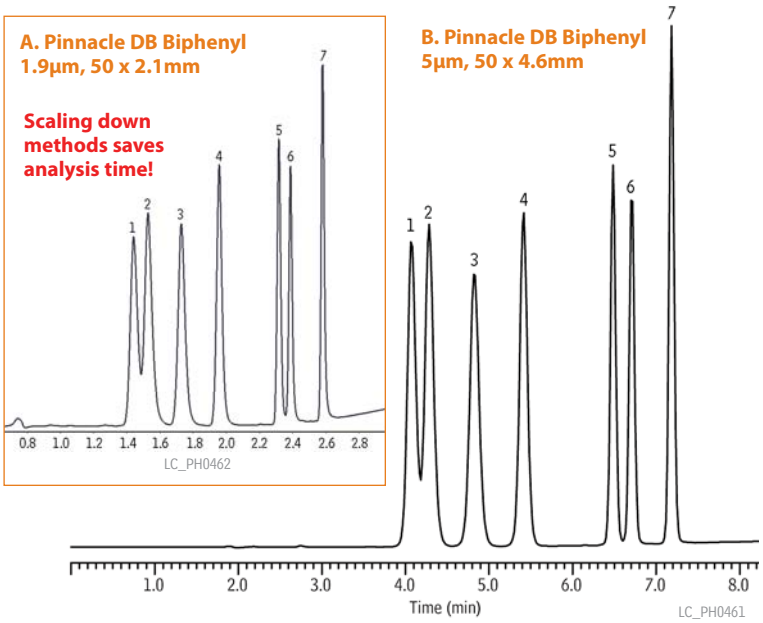
Example:

$$t_{g2} = 5\text{min.} \cdot \left(\frac{1.0\text{ml/min.}}{0.2\text{ml/min}} \right) \cdot \left(\frac{2.1\text{mm}^2}{4.6\text{mm}^2} \right) \cdot \left(\frac{50\text{mm}}{150\text{mm}} \right)$$

$$t_{g2} = 1.7\ \text{min}$$

t_g = Gradient Time
F = Column Flow
L_C = Column Length
d_C = Column Diameter

Figure 3 Restek's Pinnacle™ DB 1.9µm columns can easily be scaled from HPLC to UHPLC and vice versa.



Peak List:
1. sulfadiazine
2. sulfathiazole
3. sulfamerazine
4. sulfamethazine
5. sulrfachlorpyridizine
6. sulfamethoxazole
7. sulfamethoxine

Sample:
Inj.: 10µL
Conc.: 100µg/mL
Sample diluent: starting mobile phase (80:20 A:B)

Column: A. 1.9µm Pinnacle™ DB Biphenyl
Cat.#: 9409252
Dimensions: 50mm x 2.1mm
Particle size: 1.9µm

Column: B. Pinnacle™ DB Biphenyl
Cat.#: 9409565
Dimensions: 150mm x 4.6mm
Particle size: 5µm
Pore size: 140Å

Conditions:
Mobile phase: A: 0.1% formic acid in water
B: 0.1% formic acid in acetonitrile

Time(min.)	%B
0.0	20
1.0	20
6.0	80
8.0	80

Flow: 1.0mL/min.
Temp.: 30°C
Det.: UV @ 254nm

efficiencies), flow rates must be decreased. Also, since smaller particle sizes give rise to higher optimal linear velocities, isocratic flow rates should be calculated with particle size taken into account. In this example, a gradient elution was used and, therefore, particle size was not included in the equation. Equation 3 can be used to estimate the adjusted flow rate needed for equivalent chromatography. Also, note that since <2µm particle sizes are less affected by flow rate, faster flow rates can be used in isocratic systems without detrimental effects on peak efficiency.

Adjusting Time Program

After determining the proper column length, injection volume, and flow rate, we can calculate the time needed for gradient or step elutions. As an analytical method is scaled down, the time program also needs to be scaled down to keep the phase interactions the same. Time can be adjusted using Equation 4.

Conclusion

After determining the equivalent conditions for scaling-down the analysis of sulfonamides, we can see that the separations are equivalent, while the analysis time was greatly reduced (Figure 3). By following the procedure described here to ensure that the columns are equivalent, scaling analytical procedures from HPLC to UHPLC can easily be accomplished using Pinnacle™ DB columns.

Pinnacle™ DB Biphenyl Columns (USP L11)

Physical Characteristics:

particle size:
1.9µm, & 5µm, spherical
pore size: 140Å
carbon load: 8%

endcap: yes
pH range: 2.5 to 7.5
temperature limit: 80°C

1.9µm Column, 2.1mm	cat. #
50mm	9409252
5µm Column, 4.6mm	**cat. #**
150mm	9409565

For a full product listing, including guard cartridges for these columns, visit our website at **www.restek.com**.