UHPLC Method Development

1.7µm Fortis columns will allow the transfer of methods from traditional HPLC to UHPLC, saving both time and solvent. In order to perform method transfer there are several 'method development' calculators available to help in making appropriate changes to column dimension, flow rate and gradient conditions. If done properly the overall method time will reduce but resolution and selectivity of solutes will remain constant or indeed improve. Download at : www.uhplccolumns.com/UHPLC_Calculator

Equivalent UHPLC Column - 'Separating Power'

First consideration is the ability to scale the method down in column dimension, length and diameter:

– Equivalent UHPLC column
If you can retain equivalent column plate count or 'separating power' then it is much easier to scale down effectively.

– Example
If you move from a 5µm 150x4.6mm to a 1.7µm 50x2.1mm the equivalent separation should be achieved but a several fold improvement in analysis time will be achieved

Column Length	Efficiency of 5µm	Efficiency of 3µm	Efficiency of 1.7µm
250	22,000		
150	12,700	16,800	26,460
100	8,300	10,700	21,000
50	4,000	6,000	11,200
30		3,200	7,000
20			3,000

Method Development Calculator

Fortis™ Technologies Ltd.

Method Development Calculator

Fill these boxes | Reset Calculator

Current HPLC Method

Adjust Column Length

Existing Column length	150	mm
Existing Particle Size	5	µm
Existing Column Diameter	4.6	mm

Adjust injection Volume

Existing Injection Volume	20	µl

Adjust Flow Rate

Existing flow rate	1.00	ml/min

Adjust Gradient Program

UHPLC Method

New Column Length	51	mm
New Particle Size	1.7	µm
New Column diameter	2.1	mm

New injection volume	1.42	µl

New flow rate	0.21	ml/min

DOWNLOAD AT:
www.uhplccolumns.com/UHPLC_Calculator

UHPLC Method Development

Scaling a Method - Isocratic

To scale to a UHPLC column first we change flow rate and injection volume in order to maintain the linear velocity across the method and not overload the column

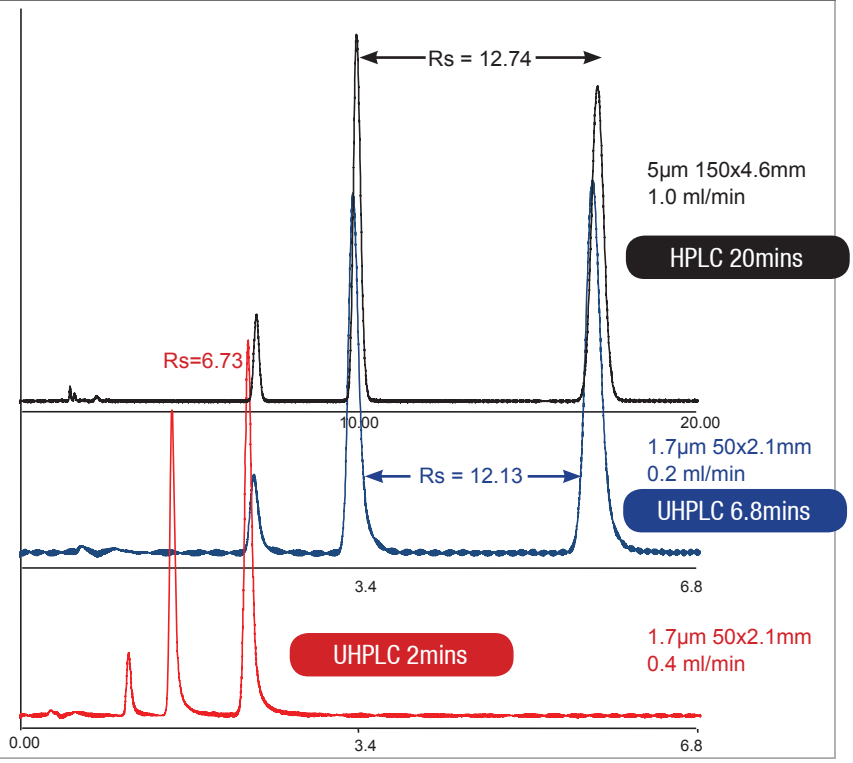
– Change Flow rate

$$F_2 = F_1 \times (Dc_2 / Dc_1)^2$$

– Change Injection Volume

$$V_2 = V_1 \times \frac{(Dc_2^2 \times L_2)}{(Dc_1^2 \times L_1)}$$

F_2 = New flow rate
F_1 = Original flow rate
Dc_2 = New column Diameter
Dc_1 = Original column Diameter
L_2 = Length of new column
L_1 = Length of original column
V_2 = New injection Volume
V_1 = Original injection Volume



Scaling a Method - Gradient

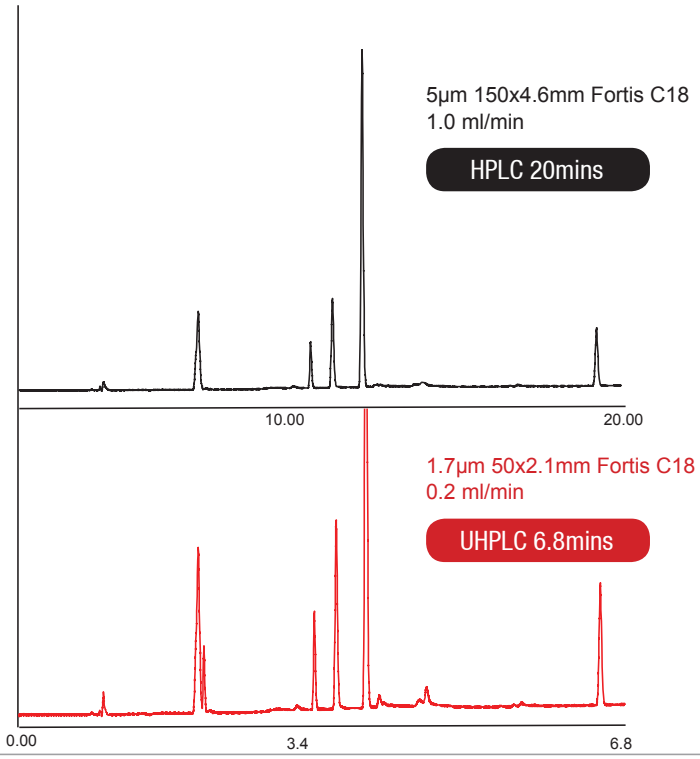
In order to change our gradient we must aim to keep the slope and the start point the same but lower the time the gradient runs in.

Altering the gradient time, retains the same linear gradient and slope, but reduces the run time:

– Change Gradient

$$tg_2 = tg_1 \times (F_1 / F_2) \times (Dc_2^2 / Dc_1^2) \times (L_1 / L_2)$$

tg_2 = New Gradient time
tg_1 = Original Gradient time



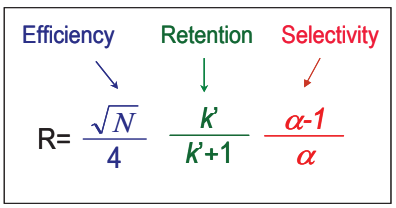
UHPLC Method Development

Resolution vs Efficiency vs Selectivity

1.7µm Fortis C18 will provide hydrophobic selectivity which is suitable for many compounds. However as the resolution equation shows us having multiple phase chemistries available is a definite advantage even in UHPLC. Selectivity can then be used in conjunction with higher efficiency.

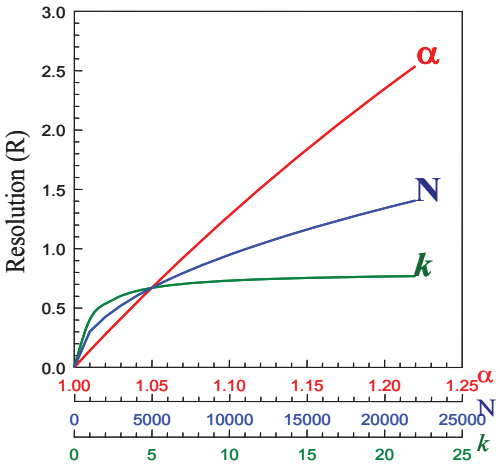
1.7µm Fortis UHPLC columns are also available as:

- **1.7µm Fortis Diphenyl**
- **1.7µm Fortis H2o (Polar C18)**
- **1.7µm Fortis C8**
- **1.7µm Fortis Cyano**
- **1.7µm Fortis HILIC**
- **1.7µm Fortis HILIC DIOL**
- **1.7µm Fortis Amino**



$$\alpha = \frac{k_2}{k_1}$$

- **Selectivity (α) has the greatest impact on improving resolution.**



Improve Selectivity

If we are scaling a method and hoping that an increase in efficiency alone will provide the necessary resolution we can be disappointed.

– Efficiency Alone

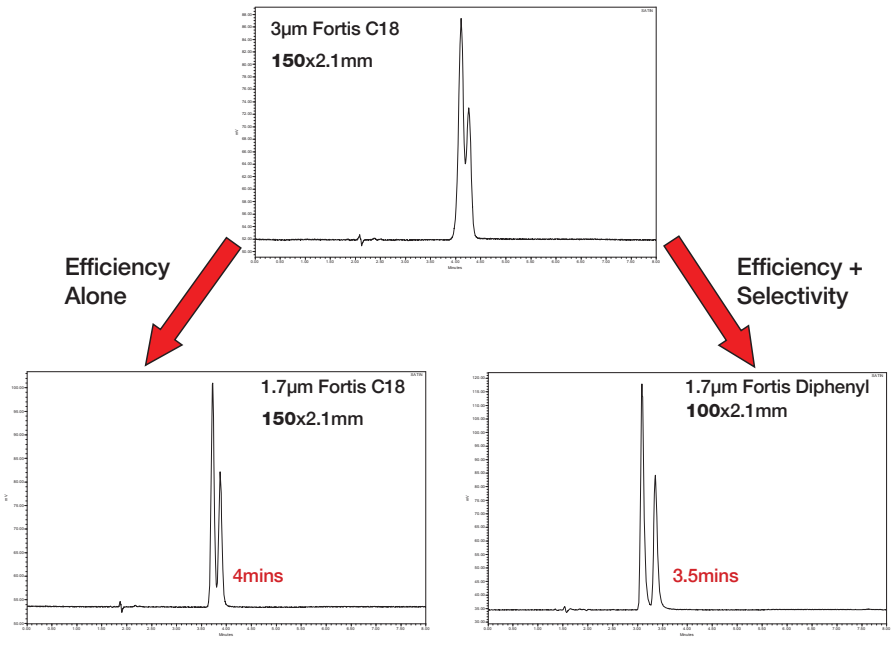
Scaling from 3µm to 1.7µm C18 has not provided baseline resolution between the compounds.

– Efficiency & Selectivity

Adding selectivity by choosing an alternative phase chemistry has allowed us to go faster on a shorter column and now achieve full baseline separation.

– Conclusion

In this instance 1.7µm Fortis Diphenyl provides more resolution than C18. This then leads to the ability to increase speed by use of shorter columns.



Isonicotinamide and Nicotinamide