Fortis™
Technologies Ltd.

Strength in Technology

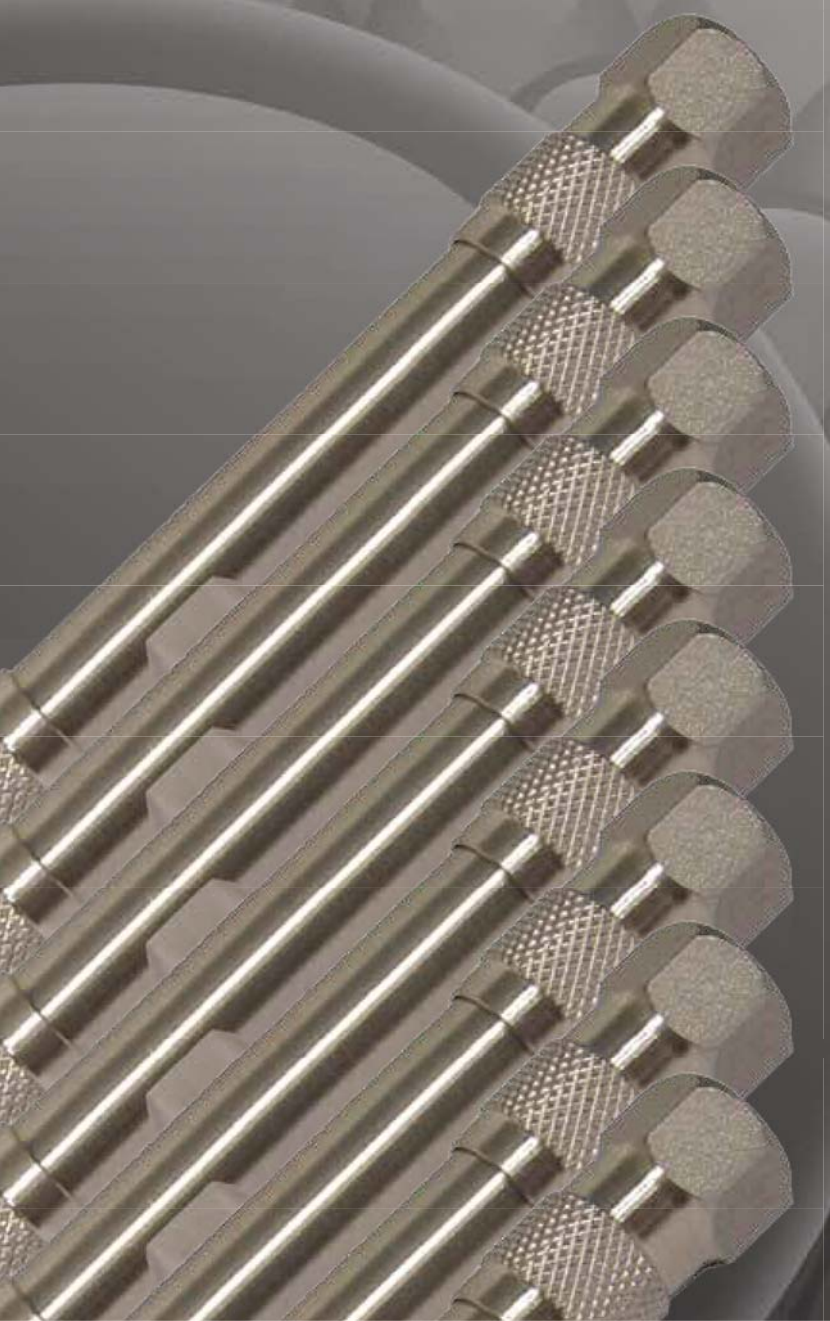
1.7 µm Fortis UHPLC Columns

Improve your UHPLC performance

- Ultra High Pressure Chromatography
- 8 Chemistry Choices
- Increase Efficiency
- Increase Speed
- Improve Resolution
- Greater Sensitivity
- Lower Backpressure

UHPLC Columns

- Optimised for Ultra High Pressure LC (UHPLC)
- Available in 8 different Selectivities
- Operate at 18,000psi
- Fully Scalable analytical to prep



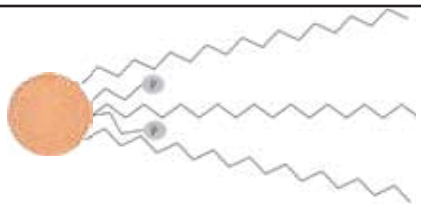
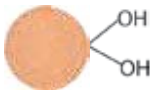
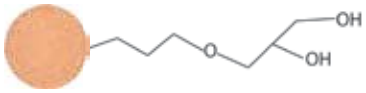
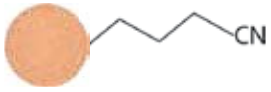
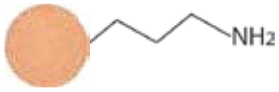
Speed

Resolution

Selectivity

Sensitivity

1.7µm Phase Chemistry Selectivity

	**1.7µm Fortis C18** – **General UHPLC use** – **Method Development from pH 1-12**	Acids Bases Neutrals
	**1.7µm Fortis H2o** – **Polar endcapped** – **Increased polar retention**	Hydrophilic analytes Organic acids Catecholamines
	**1.7µm Fortis Diphenyl** – **Unique di-phenyl structure** – **Metabolite profiling** – **Separate positional isomers**	Metabolites Positional Isomers Hydrophilic / Hydrophobic analytes
	**1.7µm Fortis C8** – **General UHPLC use** – **Method Development**	Lipids Steroids Highly Hydrophobic analytes
	**1.7µm Fortis HILIC** – **High polar retention** – **Homogenous silanol concentration** – **Improve MS sensitivity**	Carboxylic acids Nucleotides Vitamins
	**1.7µm Fortis HILIC Diol** – **Alternate selectivity to bare silica** – **Stable bonding** – **HILIC or Normal phase mode**	Steroids Proteins Metabolites
	**1.7µm Fortis Cyano** – **Cyano functionality** – **Reversed phase or Normal phase**	Explosives Pesticides Steroids
	**1.7µm Fortis Amino** – **Reproducible, Robust bonding** – **Reversed phase, Normal phase or Ion exchange mode**	Saccarides Oligonucleotides Steroids

1.7µm UHPLC Columns

- **380m²/g Surface Area Provides Increased Peak Capacity**
- **Available in 8 Phase Chemistries**
- **Operate to 18,000psi**
- **Fully Scalable to Analytical and Prep Size**

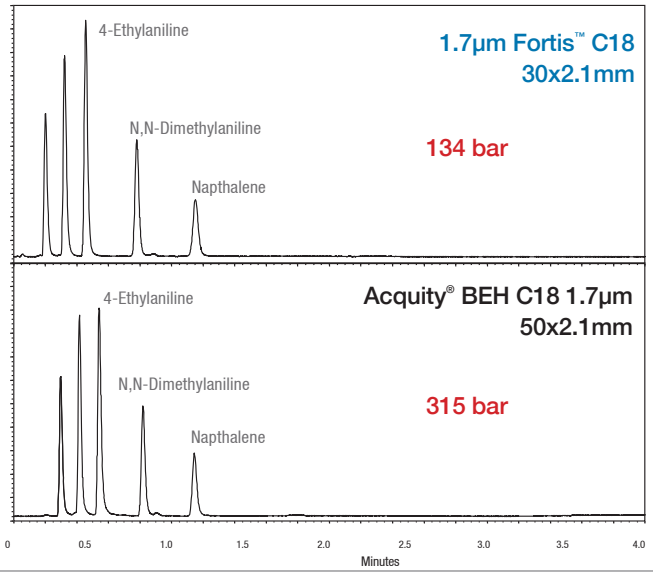
1.7µm Fortis™ particles are designed to provide characteristics, which will aid in increased productivity within ultra high pressure chromatography (UHPLC). Designed to be robust, reproducible and fully scalable with 3µm, 5µm and 10µm particles. 1.7µm Fortis particles will operate upto 1200bar providing increased efficiency at high linear velocities, whilst allowing speed and sensitivity to be achieved on all the latest UHPLC systems. By choosing a high surface area UHPLC phase the analyst can increase peak capacity using their existing column dimension, or maintain existing capacity whilst lowering backpressure on a shorter column.

High Efficiency with Lower Backpressure

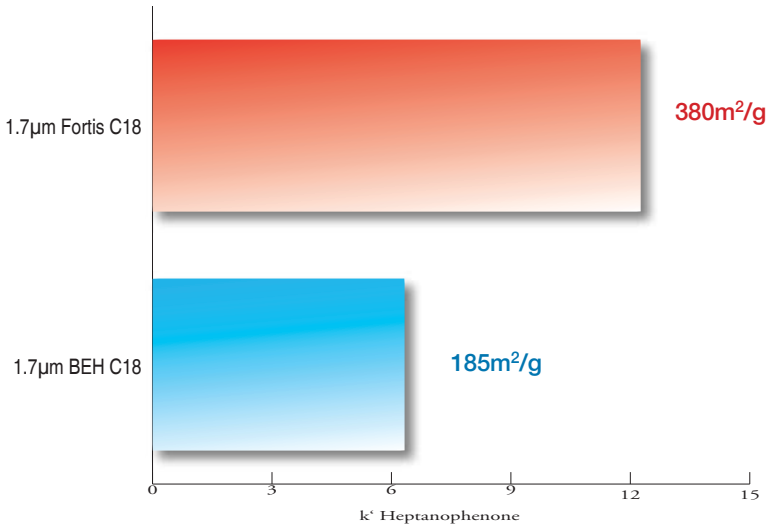
1.7µm Fortis C18 provides increased efficiency over 3µm and 5µm particles. This provides the opportunity to increase resolution or speed of analysis.

– Higher Efficiency
Compare 1.7µm Fortis C18 with your existing column to see high retention, high efficiency.

– Lower Backpressure
Use the high surface area to lower your column length and reduce the backpressure in the system.



Comparison of Hydrophobicity and Peak Shape



1.7µm Fortis™ C18 50x2.1mm	
Surface Area	380m²/g
Efficiency	191,670
Peak Shape (N,N-Dimethylaniline)	1.03
Psi - 0.4ml/min (60:40 ACN:Water)	225bar

Acquity® BEH 1.7µm C18 50x2.1mm	
Surface Area	185m²/g
Efficiency	167,400
Peak Shape (N,N-Dimethylaniline)	1.28
Psi - 0.4ml/min (60:40 ACN:Water)	292bar

Comparison

Pressure Comparison 1.7µm Fortis C18 vs 1.7µm Acquity® BEH C18

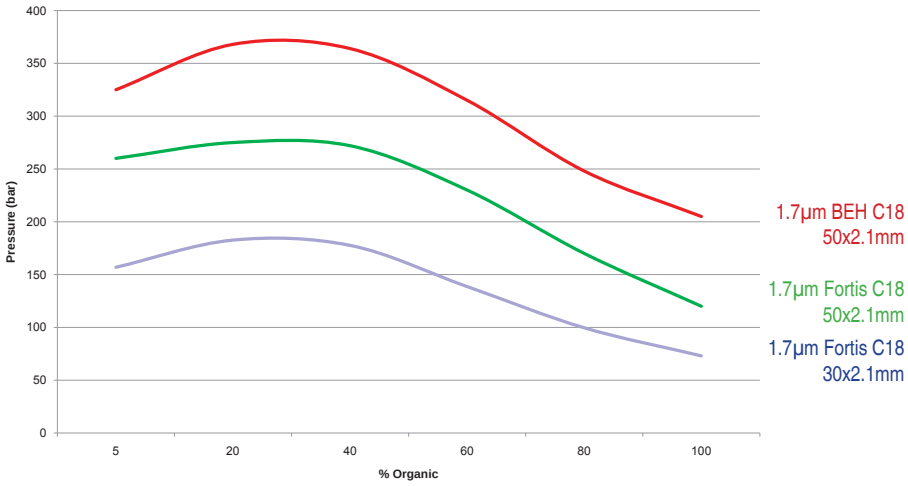
High surface area of the silica 1.7µm Fortis C18 gives you the choice to lower backpressure:

– Lower Backpressure

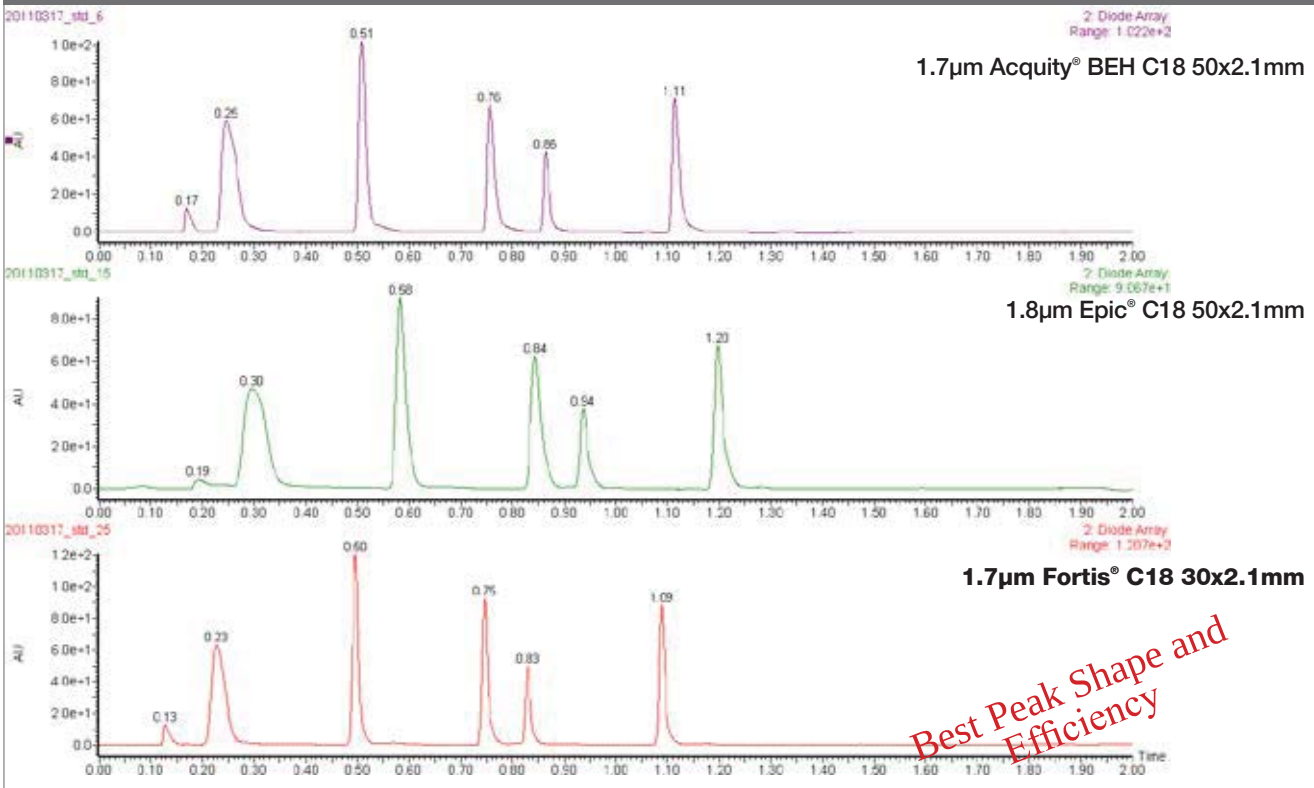
1.7µm Fortis C18 provides less backpressure then many of the other UHPLC columns available

– Shorten Column

If you can then use a shorter column and still have the same retention due to the higher surface area, you will reduce the pressure even further with no loss of separation.



Comparison of UHPLC Columns - Sharper Peak Shapes



1. Sulfanilamide 2. Sulfamerazine 3. Sulfamethoxaxole 4. Hydrocortisone 5. Ketoprofen

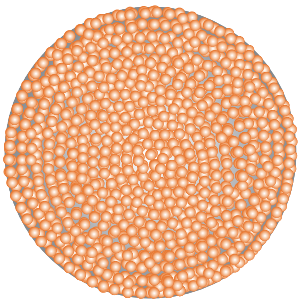
Data Courtesy of: GNF, San Diego

UHPLC

High Performance

- **Use 1.7µm Fortis particles as a traditional UHPLC column**
- **Use 1.7µm Fortis particles in place of core-shell**
- **Fully pH stable 1-12**
- **Fully Scalable to analytical and Preparative scale**
- **Can be used on traditional HPLC Instruments****

1.7µm Fortis UHPLC columns can be used in UHPLC systems or in 'standard 400-600bar systems' to produce ultra-high pressure or ultra-high performance chromatography. If you use a high surface area stationary phase (Fortis = 380m^2/g) then in comparison with smaller surface area phases, such as hybrid's and core-shell, you will gain distinct advantages:

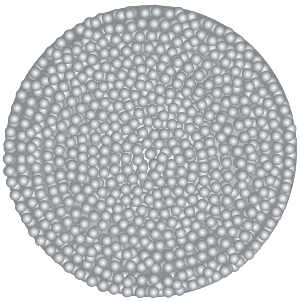


1.7µm Fortis™

+ 380m^2/g +

30x2.1mm

= 65 bar
N 182k
pH 1-12

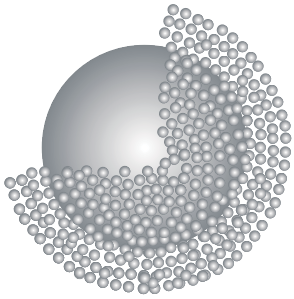


1.7um Hybrid
UHPLC particle

+ 185m^2/g +

50x2.1mm

= 160 bar
N 167k
pH 1-12



Core-Shell

+ 200m^2/g +

50x2.1mm

= 80 bar
N 178k
pH 2-8*

* pH range for gradient
** Column length and optimisation required

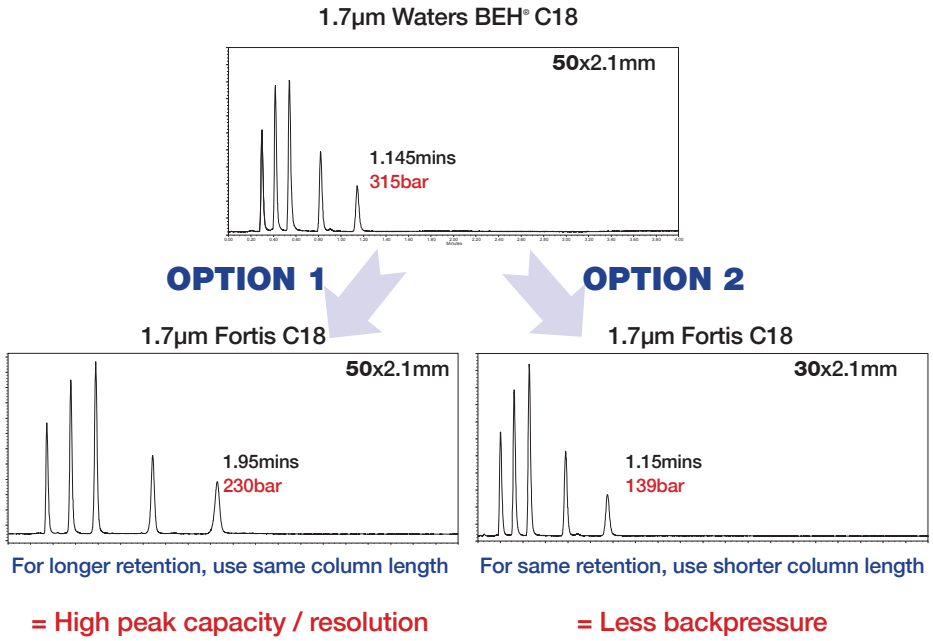
Comparison

1.7µm Fortis C18 vs 1.7µm Acquity® BEH C18

High surface area of the silica 1.7µm Fortis C18 gives you the choice to lower backpressure or increase Retention/ Resolution:

OPTION 1
– Increase Peak Capacity
1.7µm Fortis C18 high surface area will increase retention over the same length Acquity® BEH column leading to more available resolution.

OPTION 2
– Reduce Backpressure
1.7µm Fortis C18 high surface area means that you can use a shorter column to maintain the same retention as an Acquity® BEH column but reduce backpressure even further



For longer retention, use same column length

= High peak capacity / resolution

For same retention, use shorter column length

= Less backpressure

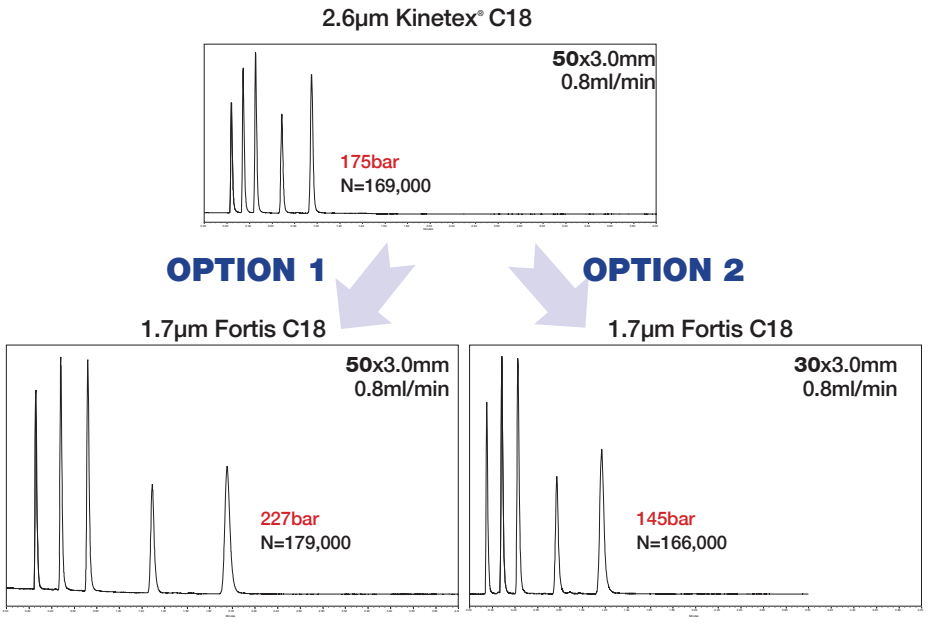
1.7µm Fortis C18 vs 2.6µm Kinetex® C18

High surface area of the silica 1.7µm Fortis C18 gives you the choice to lower backpressure or increase Retention/Resolution over core-shell particles:

OPTION 1
– Increase Peak Capacity
1.7µm Fortis C18 high surface area will increase retention over the equivalent length Kinetex® C18 column, leading to more resolution.

OPTION 2
– Reduce Backpressure
1.7µm Fortis C18 high surface area means that you can use a shorter column to maintain the same retention as a Kinetex® C18 column but reduce backpressure

– Other Gains
- 1.7µm Fortis C18 will operate at extended pH ranges over core-shell.
- 1.7µm Fortis C18 can be scaled to analytical and preparative scale unlike core-shell.



For longer retention, use same column length

= High peak capacity / resolution

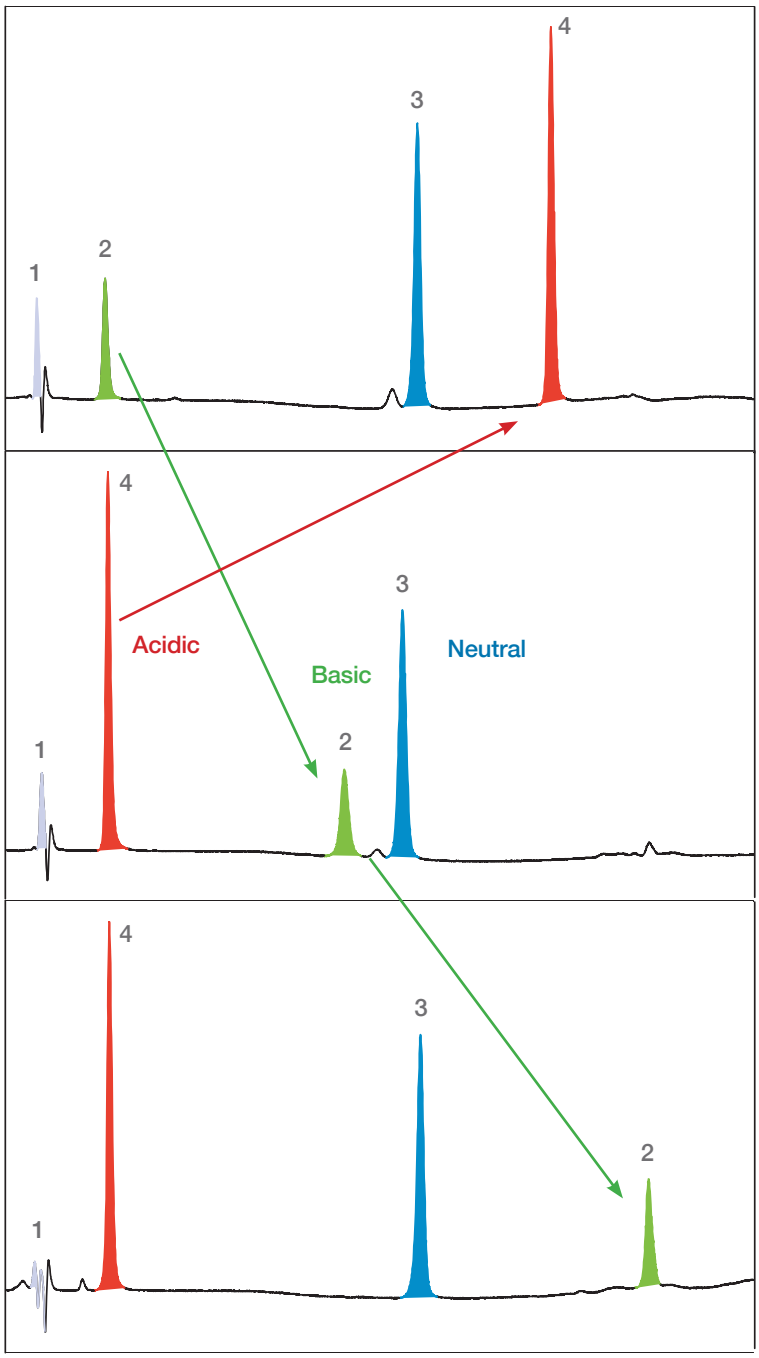
For same retention and performance, use shorter column length

= Less backpressure

1.7µm Fortis C18 pH options

- **pH selectivity for method development**
- **pH stable 1-12**
- **Gives high speed of equilibration**

1.7µm Fortis C18 will operate across the pH spectrum giving the analyst the ability to optimise the correct pH region for their separation. Quickly equilibrating from formic acid to ammonium acetate through to ammonia allows pH, as a method variable, to be rapidly evaluated. Resolution of compounds can be changed radically by altering pH to optimise separation between compound classes.



Column:	1.7µm Fortis C18 30x2.1mm
p/n:	F18-020201
Gradient:	10 - 50% in 5min
Flow:	0.4ml/min
Temp:	20°C
Wavelength:	254nm

1. Uracil
2. Procaine
3. Fenuron
4. 3-Nitrobenzoic acid

pH 2.2

pH 7.2

pH 11.2

Critical Considerations in UHPLC

Fully Scalable

All Fortis phases can be scaled from 1.7µm all the way through analytical 3µm and 5µm particles to prep size without any change in retention profile.

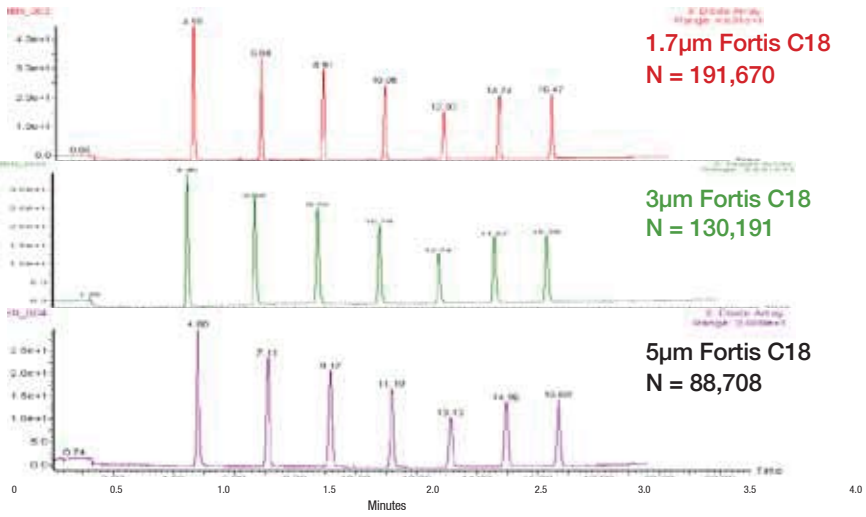
– Improve transfer of methods

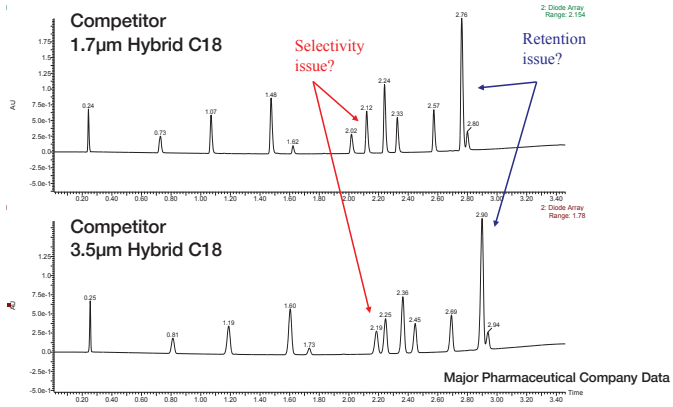
By combining the same surface area, pore size characteristics with the identical bonding you have the ability to scale up or down, also the confidence to transfer to another laboratory without change in selectivity

– Issues

If a small particle used in UHPLC is not the same as its larger 3µm and 5µm particle then changes in resolution and retention can occur, neither of which are acceptable in method validation.

1.7um Fortis C18 will alleviate all these potential issues, leaving the analyst confident in method transfer.





Sensitivity Gains

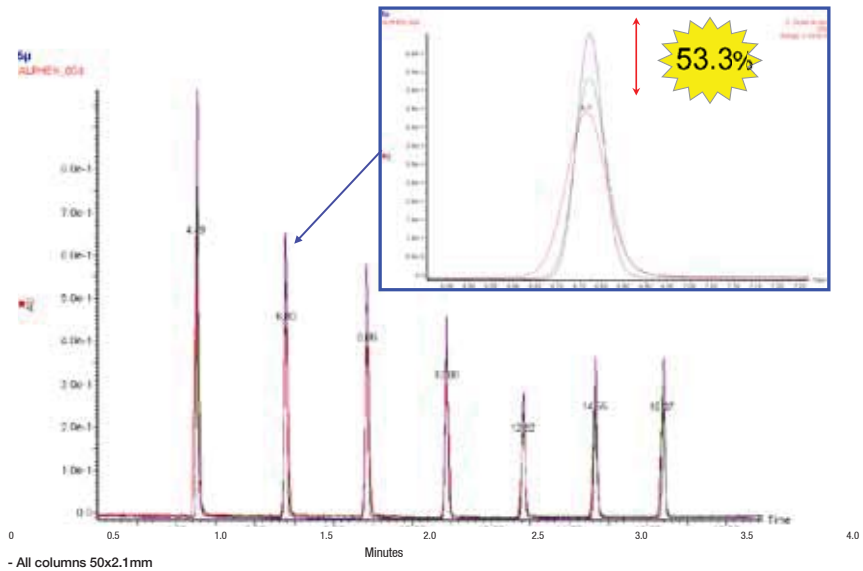
Peak height increases in UHPLC due to the rise in efficiency (N) from the smaller particle, but it is also inversely proportional to peak width, so symmetrical peaks will lead to increased sensitivity.

– Sharp Peak Shapes

All Fortis phases are designed to give the sharpest possible peak shapes.

– High Efficiency

Moving from 3µm to 1.7µm Fortis C18 gives a peak height increase of 27% in this example. The increase from 5um particles is



- All columns 50x2.1mm

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

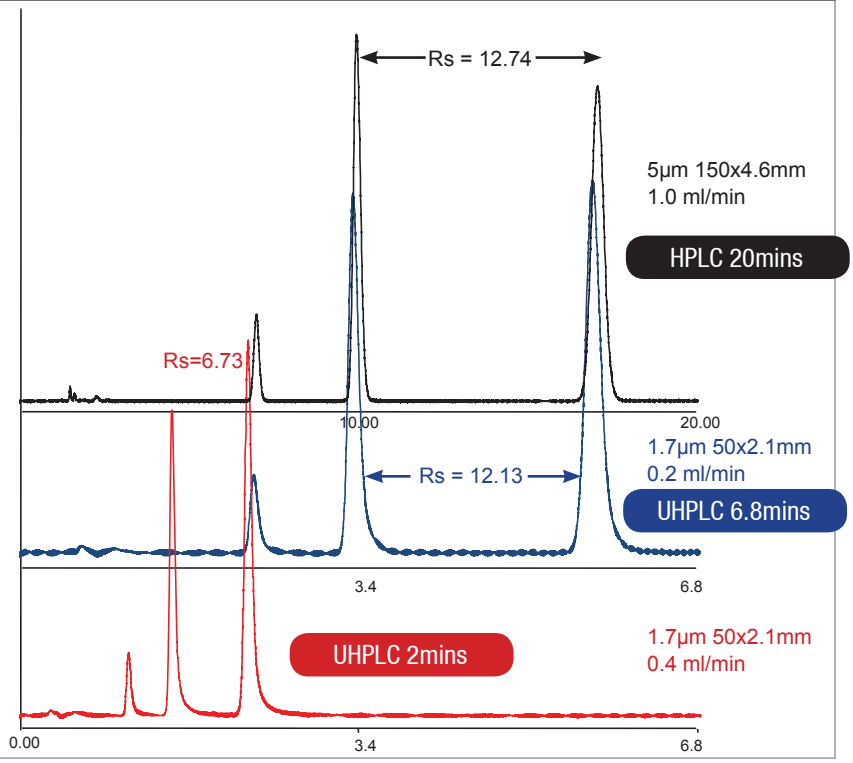
– Change Flow rate

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

– 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

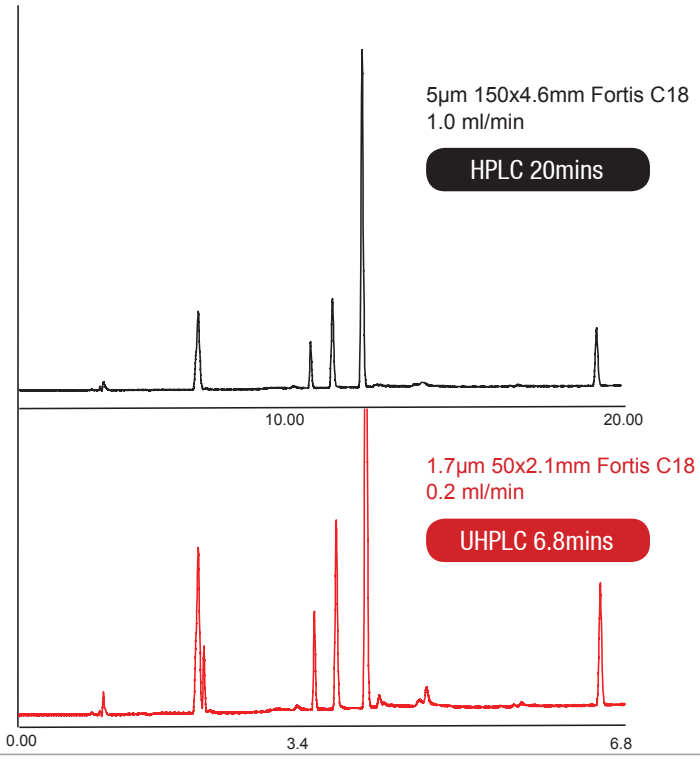
In order to change our gradient we must aim to keep the slope and the start point the same but lower the time the gradient runs in.

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



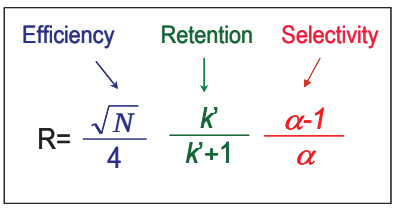
UHPLC Method Development

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.

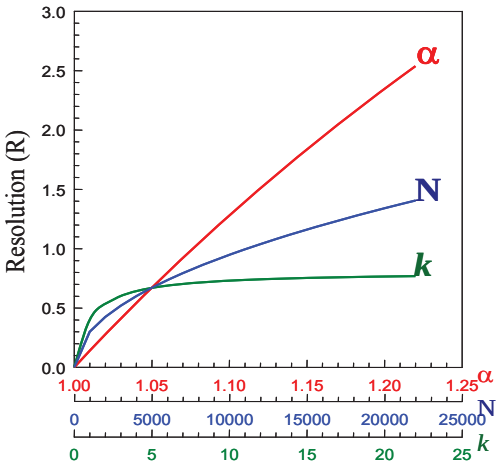
1.7µm Fortis UHPLC columns are also available as:

- **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

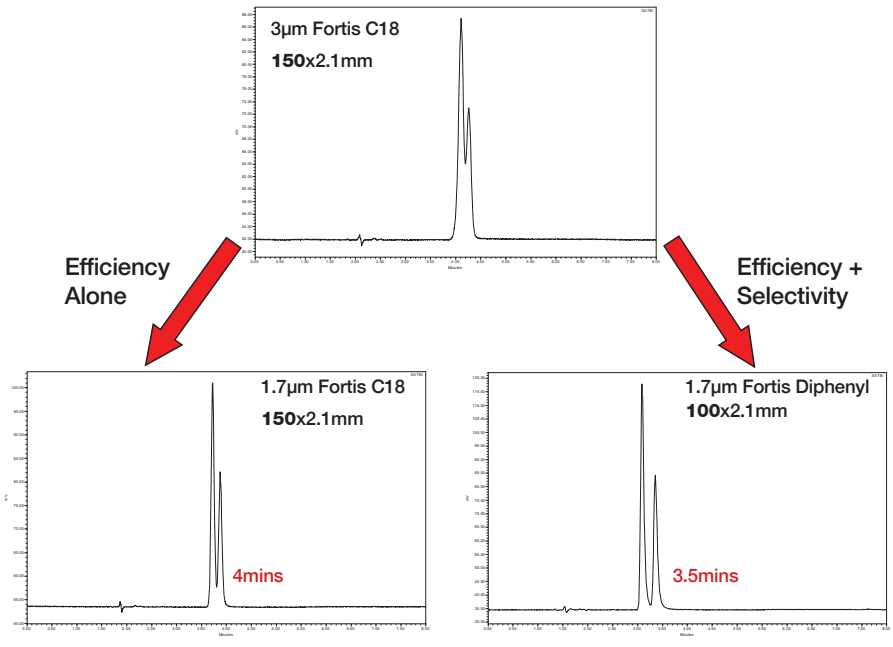
Scaling from 3µm to 1.7µm C18 has not provided baseline resolution between the compounds.

– 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

UHPLC Sample Filter

- **Low volume in-line filter for all UHPLC columns**
- **No backpressure increase**
- **Increase lifetime of UHPLC columns**
- **Change over time seconds not minutes**

Column Protection - No lose in performance

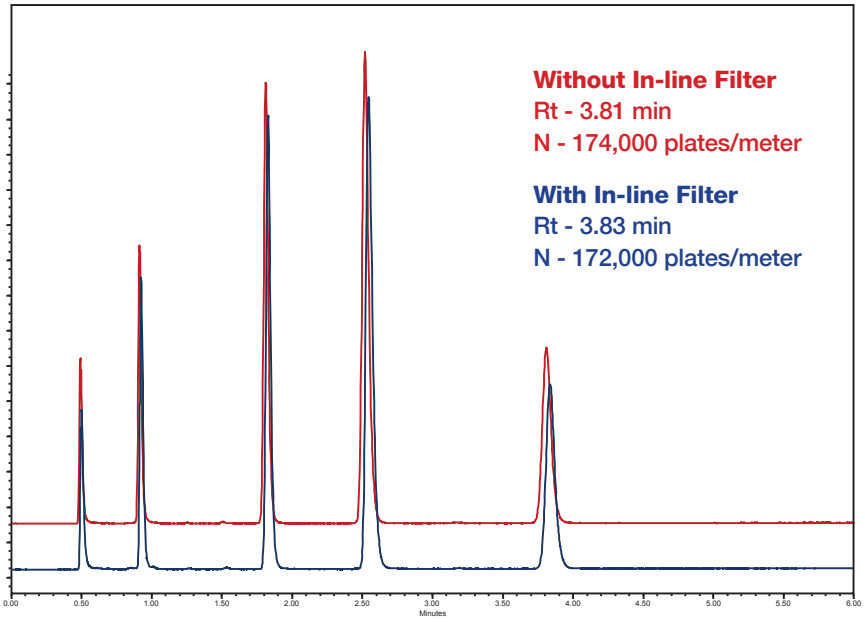
Fortis UHPLC in-line filters are direct connect design, fitting in between the UHPLC column and the conventional system fitting to filter out particulate matter.

They contain low dead volume and pressure.

In-line filters are ideal for 1.7µm Fortis UHPLC columns where extra packed bed from a guard would be detrimental.

UHPLC in-line filters are manufactured to withstand 20,000psi.

– In-line Filter or Guard cartridge
In-line filters are more suitable to many instances



Column Protection - No loss in performance

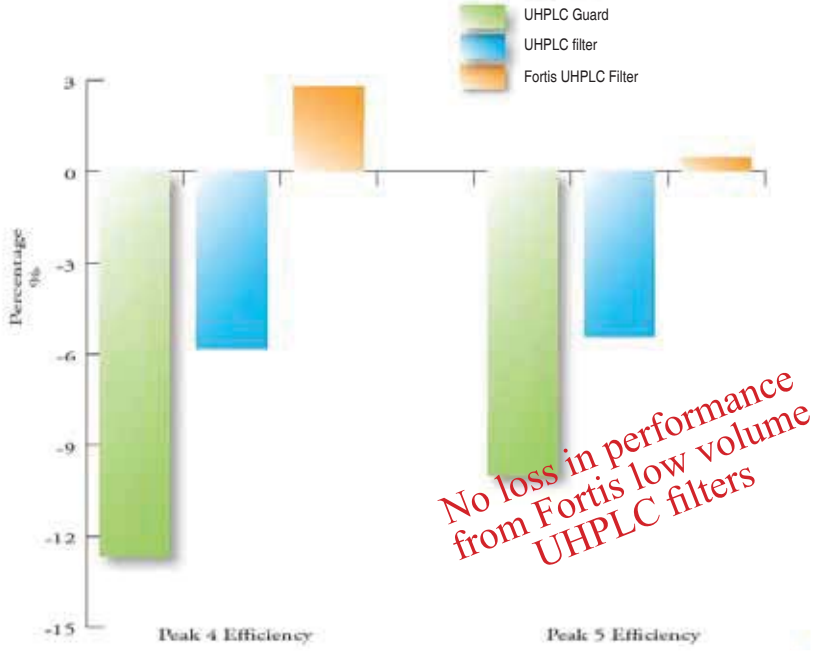
UHPLC column fitting is of crucial importance, since the addition of the smallest "dead" or void volume to these new low volume UHPLC systems will severely impact upon the performance of the column.

– In-line Filter or Guard cartridge
In-line filters are more suitable to many instances of UHPLC since with very short run times guard columns will add retention that is not required.

Guards can also reduce the efficiency of the system.

– In-line Filter comparison
In-line filters are not all the same, both efficiency and peak shape can be affected by a filter that is not optimal.

Fortis In-line filters are optimised for UHPLC.



UHPLC In-line Filters	
UHPSAV2	UHPLC In-line filter pk 2
UHPSAV4	UHPLC In-line filter pk 4

UHPLC Fittings

- **Perfect fit every time**
- **No dead volume**
- **No tools required**
- **Change over time seconds not minutes**

Fortis UHPLC fittings are designed to offer the perfect fit for all UHPLC columns. Quickly change the ferrule depth to adapt to any column. Hand-tight fitting requires no tools. Fitting is ideal for 1.7µm Fortis UHPLC columns as they are manufactured to withstand 20,000psi.

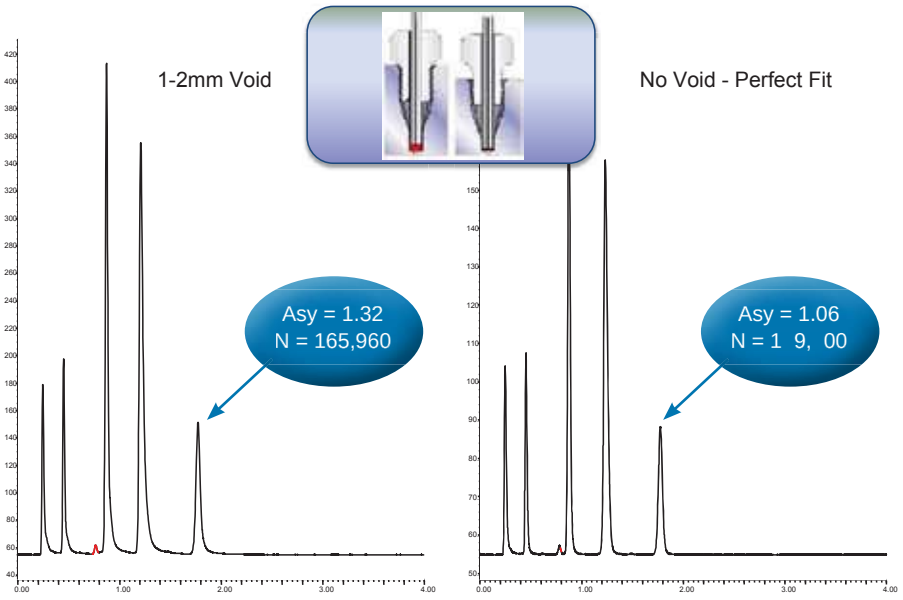
Correct Fitting

UHPLC column fitting is of crucial importance, since the addition of the smallest "dead" or void volume to these new low volume UHPLC systems will severely impact upon the performance of the column. Even the smallest 1mm void produced from fitting the column can lead to a sharp decrease in efficiency and peak shape from what should be achieved.

- Adjustable fittings

Stainless steel fittings have been widely adapted due to the high pressures involved, however if the ferrule is also stainless steel and immovable once in place then this can create a void when switching between different manufacturers columns.

A fully adjustable UHPLC fitting should always be adapted in order to ensure that the fitting of the column is perfect every single time regardless of UHPLC hardware.



UHPLC Fittings	
UHPFIT-2	UHPLC Fitting pk 2
UHPFIT-4	UHPLC Fitting pk 4

1.7µm UHPLC part numbers

Fortis C18		Column Length				
		20	30	50	100	150
Column Diameter	2.1	F18-020101	F18-020201	F18-020301	F18-020501	F18-020701
	3.0	-	F18-030201	F18-030301	F18-030501	-
	4.6	-	F18-050201	F18-050301	F18-050501	-

Fortis Diphenyl		Column Length				
		20	30	50	100	150
Column Diameter	2.1	FPH-020101	FPH-020201	FPH-020301	FPH-020501	FPH-020701
	3.0	-	FPH-030201	FPH-030301	FPH-030501	-
	4.6	-	FPH-050201	FPH-050301	FPH-050501	-

Fortis H2o (Polar Endcapped C18)		Column Length				
		20	30	50	100	150
Column Diameter	2.1	FHO-020101	FHO-020201	FHO-020301	FHO-020501	FHO-020701
	3.0	-	FHO-030201	FHO-030301	FHO-030501	-
	4.6	-	FHO-050201	FHO-050301	FHO-050501	-

Fortis C8		Column Length				
		20	30	50	100	150
Column Diameter	2.1	F08-020101	F08-020201	F08-020301	F08-020501	F08-020701
	3.0	-	F08-030201	F08-030301	F08-030501	-
	4.6	-	F08-050201	F08-050301	F08-050501	-

Fortis Cyano		Column Length				
		20	30	50	100	150
Column Diameter	2.1	FCN-020101	FCN-020201	FCN-020301	FCN-020501	FCN-020701
	3.0	-	FCN-030201	FCN-030301	FCN-030501	-
	4.6	-	FCN-050201	FCN-050301	FCN-050501	-

Fortis HILIC		Column Length				
		20	30	50	100	150
Column Diameter	2.1	FHI-020101	FHI-020201	FHI-020301	FHI-020501	FHI-020701
	3.0	-	FHI-030201	FHI-030301	FHI-030501	-
	4.6	-	FHI-050201	FHI-050301	FHI-050501	-

Fortis HILIC DIOL		Column Length				
		20	30	50	100	150
Column Diameter	2.1	FDI-020101	FDI-020201	FDI-020301	FDI-020501	FDI-020701
	3.0	-	FDI-030201	FDI-030301	FDI-030501	-
	4.6	-	FDI-050201	FDI-050301	FDI-050501	-

Fortis Amino		Column Length				
		20	30	50	100	150
Column Diameter	2.1	FNH-020101	FNH-020201	FNH-020301	FNH-020501	FNH-020701
	3.0	-	FNH-030201	FNH-030301	FNH-030501	-
	4.6	-	FNH-050201	FNH-050301	FNH-050501	-

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