1.7 μm Fortis

UHPLC Columns

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

IMPROVE YOUR UHPLC PERFORMANCE
# UHPLC Columns

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

## 1.7μm Phase Chemistry Selectivity

<table>
<thead>
<tr>
<th>1.7μm Fortis C18</th>
<th>Acids</th>
<th>Bases</th>
<th>Neutrals</th>
</tr>
</thead>
<tbody>
<tr>
<td>General UHPLC use</td>
<td>Method Development from pH 1-12</td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>1.7μm Fortis H2o</th>
<th>Hydrophilic analytes</th>
<th>Organic acids</th>
<th>Catecholamines</th>
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<tbody>
<tr>
<td>Polar endcapped</td>
<td>Increased polar retention</td>
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<table>
<thead>
<tr>
<th>1.7μm Fortis Diphenyl</th>
<th>Metabolites</th>
<th>Positional isomers</th>
<th>Hydrophilic / Hydrophobic analytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unique di-phenyl structure</td>
<td>Metabolite profiling</td>
<td>Separate positional isomers</td>
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<table>
<thead>
<tr>
<th>1.7μm Fortis C8</th>
<th>Lipids</th>
<th>Steroids</th>
<th>Highly Hydrophobic analytes</th>
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</thead>
<tbody>
<tr>
<td>General UHPLC use</td>
<td>Method Development</td>
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<table>
<thead>
<tr>
<th>1.7μm Fortis HILIC</th>
<th>Carboxylic acids</th>
<th>Nucleotides</th>
<th>Vitamins</th>
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</thead>
<tbody>
<tr>
<td>High polar retention</td>
<td>Homogenous silanol concentration</td>
<td>Improve MS sensitivity</td>
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<table>
<thead>
<tr>
<th>1.7μm Fortis HILIC Diol</th>
<th>Steroids</th>
<th>Proteins</th>
<th>Metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alternate selectivity to bare silica</td>
<td>Stable bonding</td>
<td>HILIC or Normal phase mode</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.7μm Fortis Cyano</th>
<th>Explosives</th>
<th>Pesticides</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyano functionality</td>
<td>Reversed phase or Normal phase</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>1.7μm Fortis Amino</th>
<th>Saccarides</th>
<th>Oligonucleotides</th>
<th>Steroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reproducible, Robust bonding</td>
<td>Reversed phase, Normal phase or Ion exchange mode</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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 up to 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

1.7µmFortis C18 provides increased efficiency over 5µm and 6µm 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
- Surface Area: 380m²/g
- Efficiency: 191,670
- Peak Shape (N,N-Dimethylaniline): 1.03
- PSI - 0.4ml/min (60:40 ACN:Water): 225bar

1.7µm BEH C18
- Surface Area: 185m²/g
- Efficiency: 147,400
- Peak Shape (N,N-Dimethylaniline): 1.28
- PSI - 0.4ml/min (60:40 ACN:Water): 220bar

Comparison of UHPLC Columns - Sharper Peak Shapes

1.7µm Acquity® BEH C18 50x2.1mm
- PSI - 0.4ml/min (60:40 ACN:Water): 292bar
- Data Courtesy of: GNF, San Diego

1.8µm Epic® C18 50x2.1mm
- PSI - 0.4ml/min (60:40 ACN:Water): 275bar

Comparison

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

- Lower Backpressure
  1.7µmFortis C18 provides less backpressure than 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.
**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 = 380m²/g) then in comparison with smaller surface area phases, such as hybrid’s and core-shell, you will gain distinct advantages:

- **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 longer retention, use shorter column length

= Less backpressure

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**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.

**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.
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

pH 2.2
- Uracil
- Procaine
- 3-Nitrobenzoic acid

pH 7.2
- Acidic
- Neutral
- Basic

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.

1.7μm 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 5μm particles is

1.7um Fortis C18  
N = 191,670

3μm Fortis C18  
N = 130,191

5μm Fortis C18  
N = 88,708

Competitor 1.7μm Hybrid C18

Solute by acid?
Residue in gmp

Competitor 3.5μm Hybrid C18

Major Pharmaceutical Company Data
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’

<table>
<thead>
<tr>
<th>Column Length</th>
<th>Efficiency of 5μm</th>
<th>Efficiency of 3μm</th>
<th>Efficiency of 1.7μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>22,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>12,700</td>
<td>16,800</td>
<td>26,460</td>
</tr>
<tr>
<td>100</td>
<td>8,300</td>
<td>10,700</td>
<td>21,000</td>
</tr>
<tr>
<td>50</td>
<td>4,000</td>
<td>6,000</td>
<td>11,200</td>
</tr>
<tr>
<td>30</td>
<td>3,200</td>
<td></td>
<td>7,000</td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td>3,000</td>
</tr>
</tbody>
</table>

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.

### Method Development Calculator

![Method Development Calculator](https://www.uhplccolumns.com/UHPLC_Calculator)

**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**
  
  \[ F_2 = F_1 \times \left( \frac{D_{c2}}{D_{c1}} \right)^2 \]

- **Change Injection Volume**
  
  \[ V_2 = V_1 \times \left( \frac{D_{c2}}{D_{c1}} \right)^2 \times \left( \frac{L_2}{L_1} \right) \]

**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**
  
  \[ t_{g2} = t_{g1} \times \left( \frac{F_1}{F_2} \right) \times \left( \frac{D_{c2}}{D_{c1}} \right)^2 \times \left( \frac{L_2}{L_1} \right) \]

**Download at:**

www.uhplccolumns.com/UHPLC_Calculator
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 Cyan
- 1.7μm Fortis HILIC
- 1.7μm Fortis HILIC DIOL
- 1.7μm Fortis Amino

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.

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 loss 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

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.
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 1.7 μm Fortis UHPLC columns as they are manufactured to withstand 20,000psi.

1.7μm UHPLC part numbers

<table>
<thead>
<tr>
<th>Column Diameter</th>
<th>Column Length</th>
<th>Column Diameter</th>
<th>Column Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.0</td>
<td>20</td>
<td>3.0</td>
<td>20</td>
</tr>
<tr>
<td>2.1</td>
<td>30</td>
<td>2.1</td>
<td>30</td>
</tr>
<tr>
<td>1.7</td>
<td>50</td>
<td>1.7</td>
<td>50</td>
</tr>
<tr>
<td>1.0</td>
<td>100</td>
<td>1.0</td>
<td>100</td>
</tr>
<tr>
<td>0.8</td>
<td>150</td>
<td>0.8</td>
<td>150</td>
</tr>
</tbody>
</table>

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 adopted 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.
Fortis products are available worldwide. For the distributor in your country, contact Fortis international Sales Office, UK by telephone, fax or email: info@fortis-technologies.com

For technical support or applications contact:
technicalsupport@fortis-technologies.com

For more information VISIT:
www.fortis-technologies.com