

FortisBIO™



Peptide & Protein Columns



INCLUDES
1.7 μ FORTISBIO
UHPLC
COLUMNS

HPLC/UHPLC Columns

FortisBIO™ C18 and C4 are stationary phases with optimised pore size to allow the efficient and robust separation of Biomolecules.

1.7µm FortisBIO™ phases allow ultra high performance separation of proteins and peptides.

Large Pore optimum for Peptides

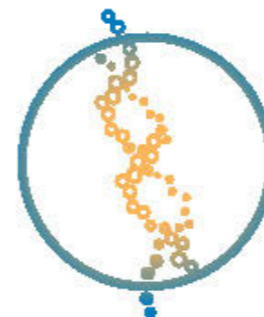
Choose from C18 or C4 ligands

1.7µm Particle for Bio UHPLC

5µm Particle for Analytical scaling

Biomolecules are a diverse range of compounds, amino acids, proteins, peptides, nucleic acids, vitamins and obtaining quality separations is paramount. FortisBIO™ C4 and C18 are 300Å optimised stationary phases providing improved selectivity, throughput, sensitivity and column robustness for biomolecules. Choose C18 for more hydrophilic proteins and C4 for more hydrophobic molecules.

Choose particle size based upon your LC system and the sensitivity of analysis that you require.



FortisBIO™ C18

- 300Å Pore size optimised for Peptides and Proteins
- 1.7µm UHPLC particle size - increases sensitivity options
- Sharp Efficient peak shapes
- Excellent for Peptide mapping

FortisBIO™ C4

- 300Å Pore size optimised for Peptides and Proteins
- 1.7µm UHPLC particle size - increases sensitivity options
- Stable, robust ligand density, low bleed.
- Analyse large hydrophobic molecules



FortisBIO™ UHPLC



- 1.7µm UHPLC particle size - increases sensitivity options
- High loading stationary phase
- Fully Scaleable 1.7µm - 5µm particle sizes for method transfer
- Sharp efficient peak shapes lead to improved resolution

FortisBIO™ C18

FortisBIO™ C18 is a 300Å material specifically optimised for the retention and resolution of peptides and proteins. Based upon our traditional silica and bonded technology, sharp peak shapes, excellent analyte recovery and high sensitivity can all be achieved whether in analytical scale or in UHPLC scale. Improvements in sensitivity and resolution of peptides, proteins, tryptic digests can all be gained with the minimum of effort

- 300Å Pore size optimised for Peptides and Proteins
- 1.7µm UHPLC particle size - increases sensitivity options
- Sharp efficient peak shapes
- Fully scaleable 1.7µm - 5µm particle sizes for method transfer

1.7µm FortisBIO C18 - INCREASE SENSITIVITY

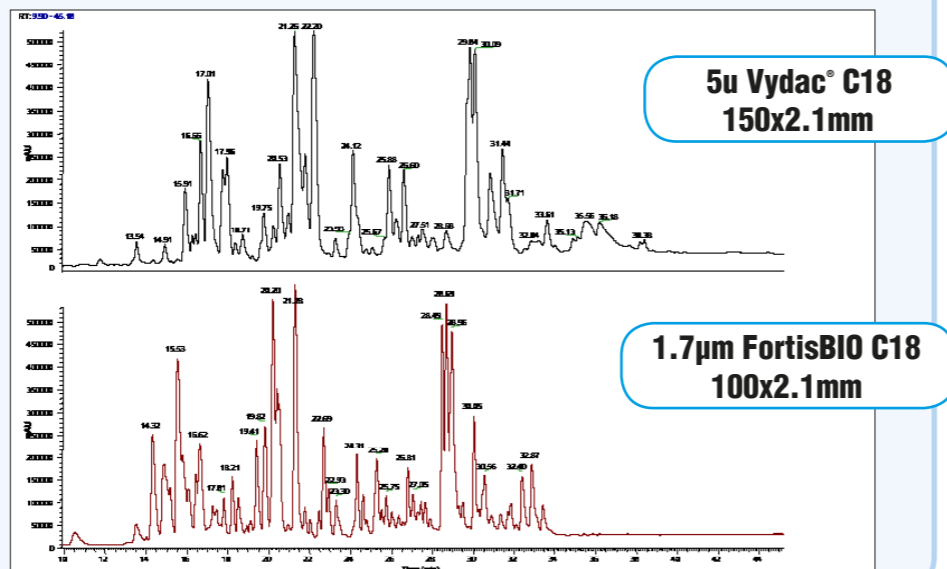
FortisBIO™ C18 is available as 1.7µm particle size for use in UHPLC, this means that sensitivity of methods and resolution of complex mixtures such as tryptic digests and closely related peptides can be improved:

– Sharp Peaks

1.7µm FortisBIO's sharp peak shapes provide a high level of resolution between compounds. Efficiency of this small particle and its ability to work at high pressure means that methods can now be developed with more speed.

– Increase Sensitivity

If you use a small 1.7µm particle then sensitivity of low abundance peptides will be enhanced.



FortisBIO C18 - FULLY SCALEABLE

FortisBIO 300Å material is available as 1.7µm for use in UHPLC or as 5µm for use in analytical LC. A pure silica porous particle and optimum bonding chemistry leads to fully scaleable methods, reducing the time spent in method development and QC/QA transfer.

– Improved Scaling

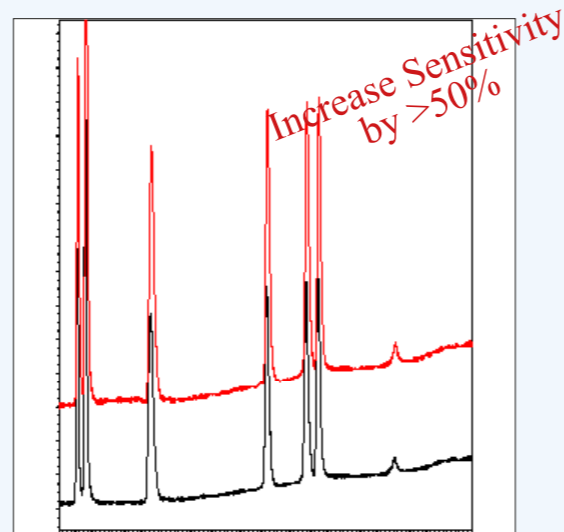
FortisBIO C18 provides the same selectivity whichever particle size is chosen.

However in the region of 50% gain in peak sensitivity can be achieved by moving to UHPLC.

– FortisBIO C18 50x2.1mm 1.7µm

– FortisBIO C18 50x2.1mm 5µm

1. GLY-TYR (238.2MW)
2. VAL-TYR-VAL (379.5 MW)
3. MET-Enkephalin (573.7)
4. LEU-Enkephalin (555.6 MW)
5. Angiotensin II (1046.2 MW)



FortisBIO C18 - PEPTIDES

The excellent surface coverage of the C18 ligand on the FortisBIO 300Å material gives multiple benefits for peptide analysis:

– Improved Peak Shape

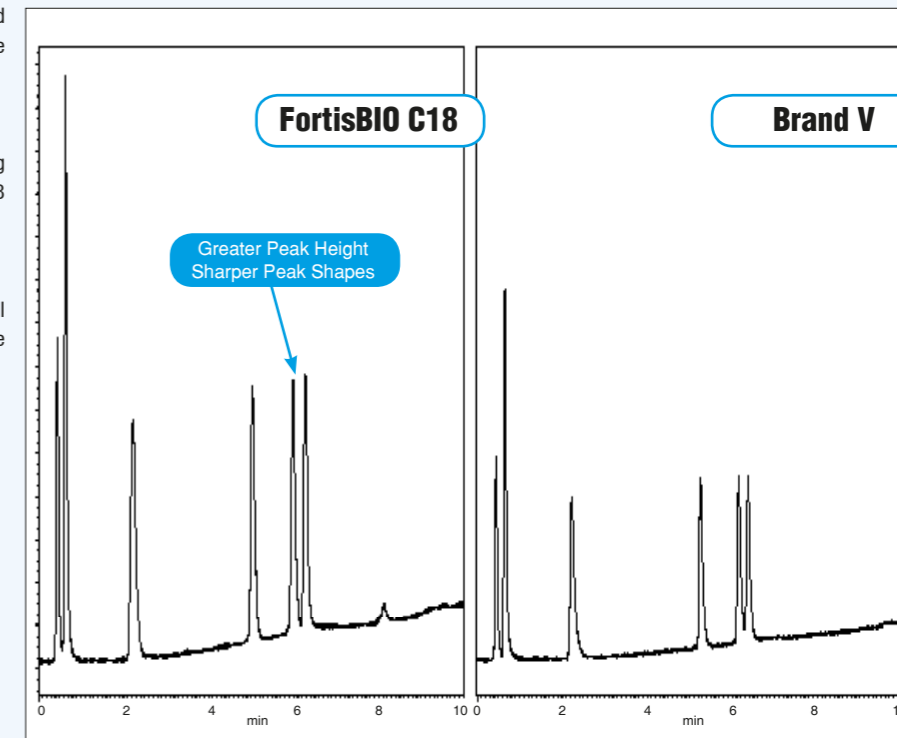
FortisBIO C18 is built around the same bonding technology as our existing small molecule C18 hence superior peak shapes are achieved.

– Improved Sensitivity

If you can improve peak shape then you will improve both resolution and sensitivity of the method.

1. GLY-TYR (238.2MW)
2. VAL-TYR-VAL (379.5 MW)
3. MET-Enkephalin (573.7)
4. LEU-Enkephalin (555.6 MW)
5. Angiotensin II (1046.2 MW)

Columns: 50x2.1mm 5µm
A: 0.1% TFA
B: 0.1% TFA in ACN
10-40% B in 10min
λ - 220nm



FortisBIO C18 - PROTEINS

The excellent surface coverage of the C18 ligand on the FortisBIO 300Å material gives multiple benefits for protein analysis:

– Improved Recovery

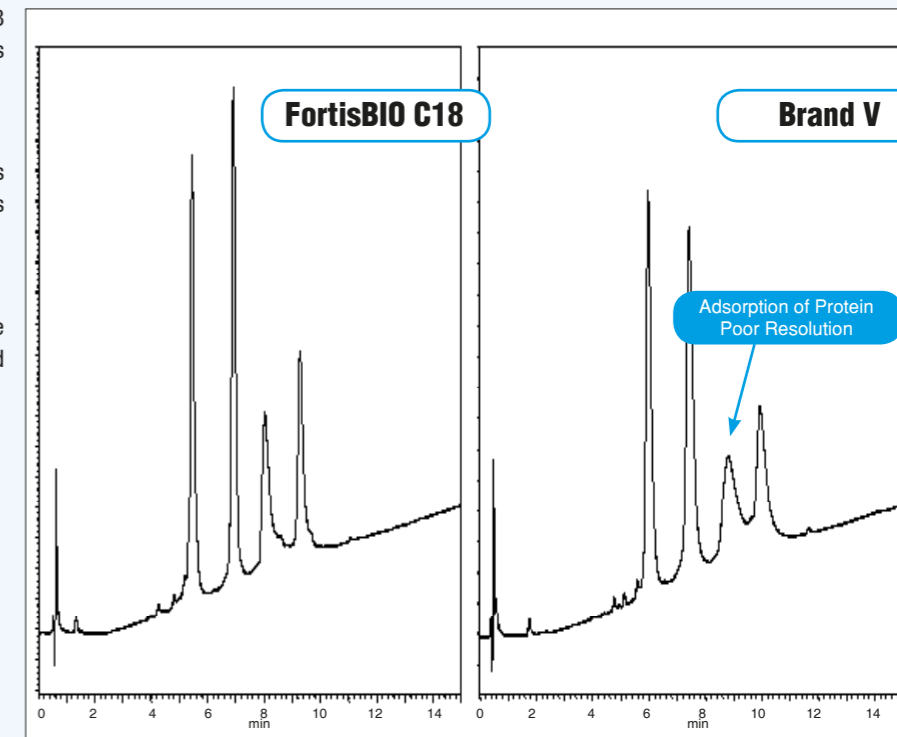
FortisBIO C18 provides sharp peak shapes due to the surface coverage, this provides increased recovery of protein samples.

– Improved Reproducibility

By having a controlled surface coverage reproducibility of the analysis is improved from sample to sample.

1. Ribonuclease A
2. Cytochrome C
3. Halo-Transferrin
4. Apomyoglobin

Columns: 50x2.1mm 5µm
A: 0.1% Formic acid
B: 0.1% Formic acid in ACN
10-60% B in 15min
λ - 220nm



Improve Recovery

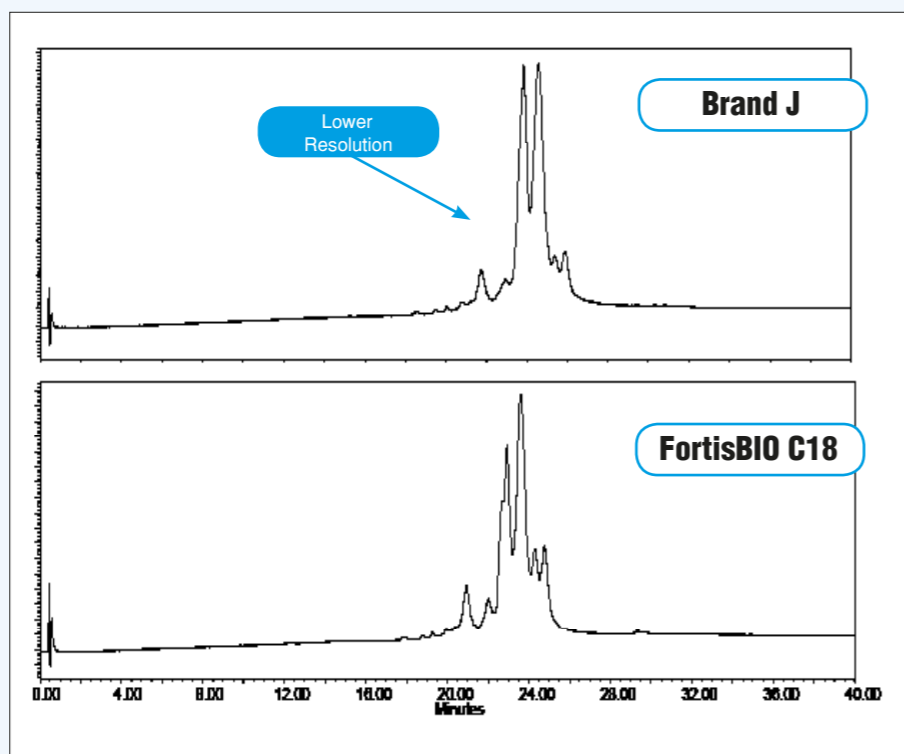
FortisBIO C18 - CASEIN COMPARISON

Recovery of proteins is of paramount importance to the analyst, FortisBIO C18 ensures good peak shape, resolution and recovery of complex protein and peptide samples.

If recovery of low abundance proteins is reduced then sensitivity will be degraded for these proteins.

5µm FortisBIO C18 50x2.1mm

A: 0.1% Formic acid in Water
B: 0.1% Formic acid in ACN
10-60% Gradient in 15mins
214nm



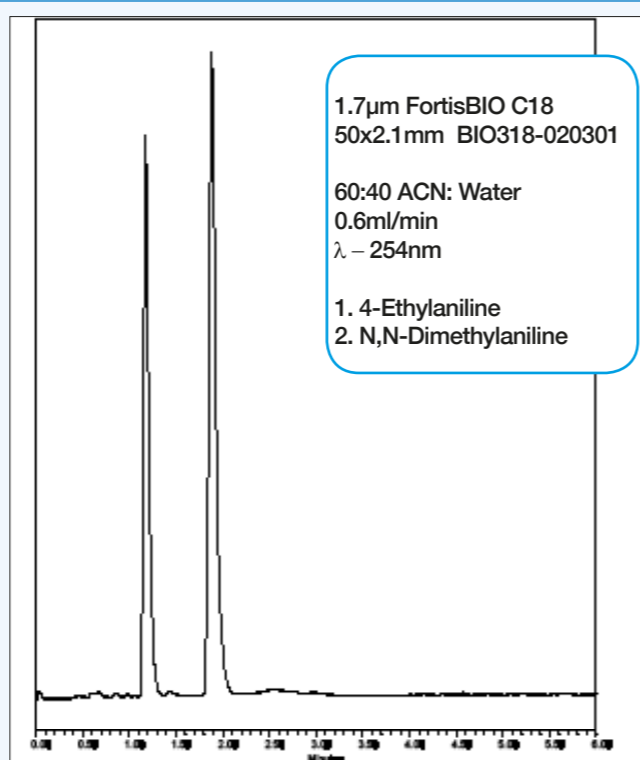
FortisBIO C18 - BASIC PEAK SHAPES

Peak Shapes are of great significance in analysis of biomolecules since both resolution of structurally similar compounds and also sensitivity of molecules will be heavily effected.

FortisBIO C18 and C4 are both manufactured to ensure that surface coverage is high, therefore reducing any secondary negative interactions which could compromise peaks shapes.

Small basic probes prove whether there are any secondary silanol interactions which would compromise peak shapes.

Basic Compound	Asymmetry Competitor	Asymmetry FortisBIO C18
4-Ethylaniline	1.58	1.30
N,N- Dimethylaniline	1.52	1.28



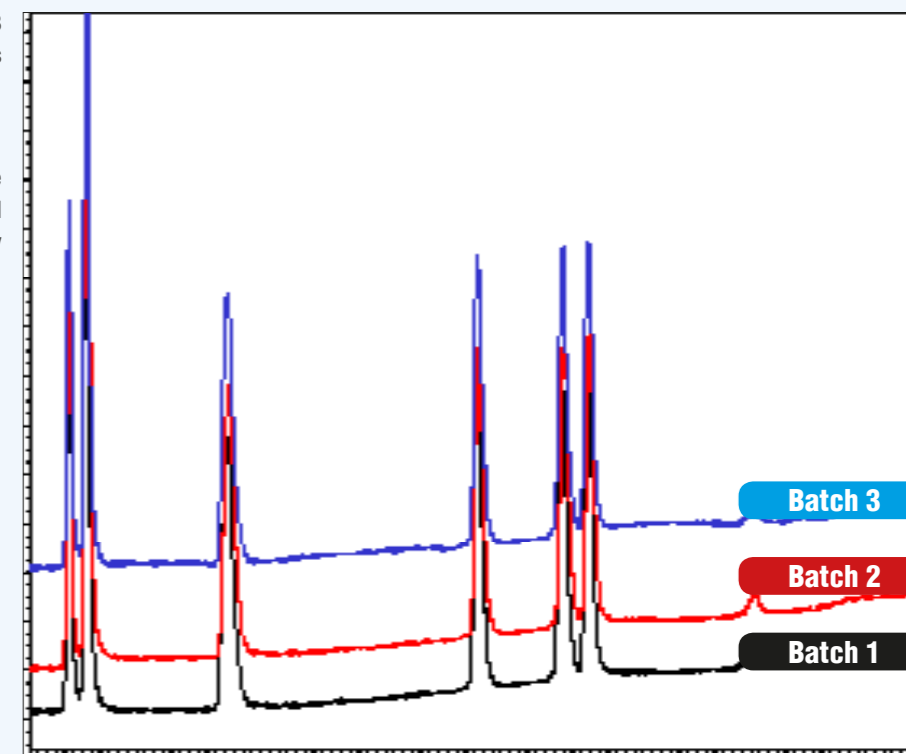
Reproducibility

REPRODUCIBILITY

The excellent surface coverage of the C18 ligand on the FortisBIO 300Å material gives multiple benefits for peptide analysis:

- **Improved Reproducibility**
FortisBIO C18 is built around the same bonding technology as our existing small molecule C18 (100Å) hence reproducibility is assured. A most important variable
- **Different Batches**
Multiple batches show reproducible results

1. GLY-TYR (238.2MW)
2. VAL-TYR-VAL (379.5 MW)
3. MET-Enkephalin (573.7)
4. LEU-Enkephalin (555.6 MW)
5. Angiotensin II (1046.2 MW)

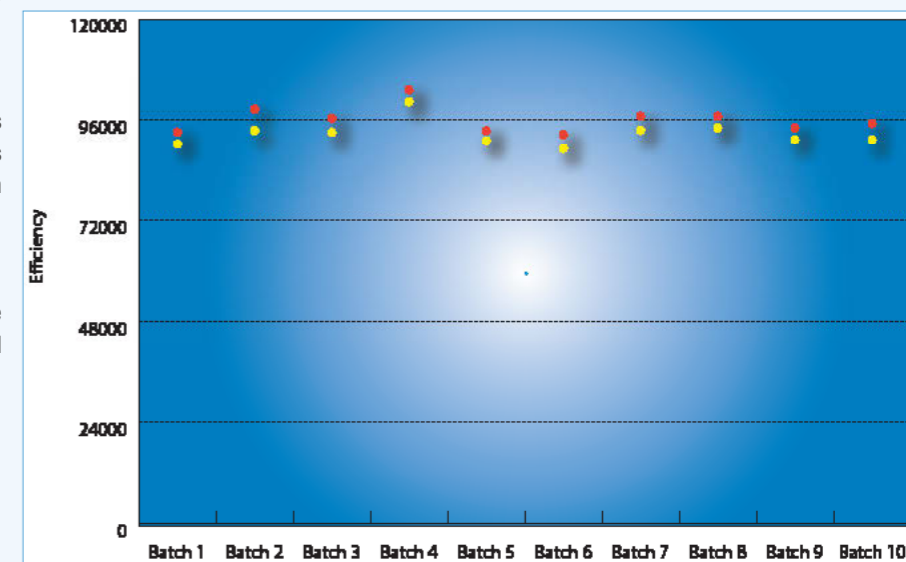


REPRODUCIBILITY

The excellent surface coverage of the C18 ligand on the FortisBIO 300Å material gives multiple benefits for protein analysis:

- **Improved Recovery**
FortisBIO C18 provides sharp peak shapes due to the surface coverage ligand, this provides increased recovery of protein samples.
- **Improved Reproducibility**
By having a controlled surface coverage reproducibility of the analysis is improved from sample to sample.

- Probe 1
- Probe 2



FortisBIO™ C4

FortisBIO™ C4 is a 300Å material specifically optimised for the retention and resolution of large peptides and proteins. Sharp peak shapes, excellent analyte recovery and high sensitivity can all be achieved whether in analytical scale or in UHPLC scale. The C4 ligand provides a high density bonding, resulting in a chemically stable, robust, low bleed phase.

- 300Å Pore size optimised for Peptides and Proteins
- 1.7µm UHPLC particle size - increases sensitivity options
- Sharp efficient peak shapes
- Fully Scaleable 1.7µm - 5µm particle sizes for method transfer

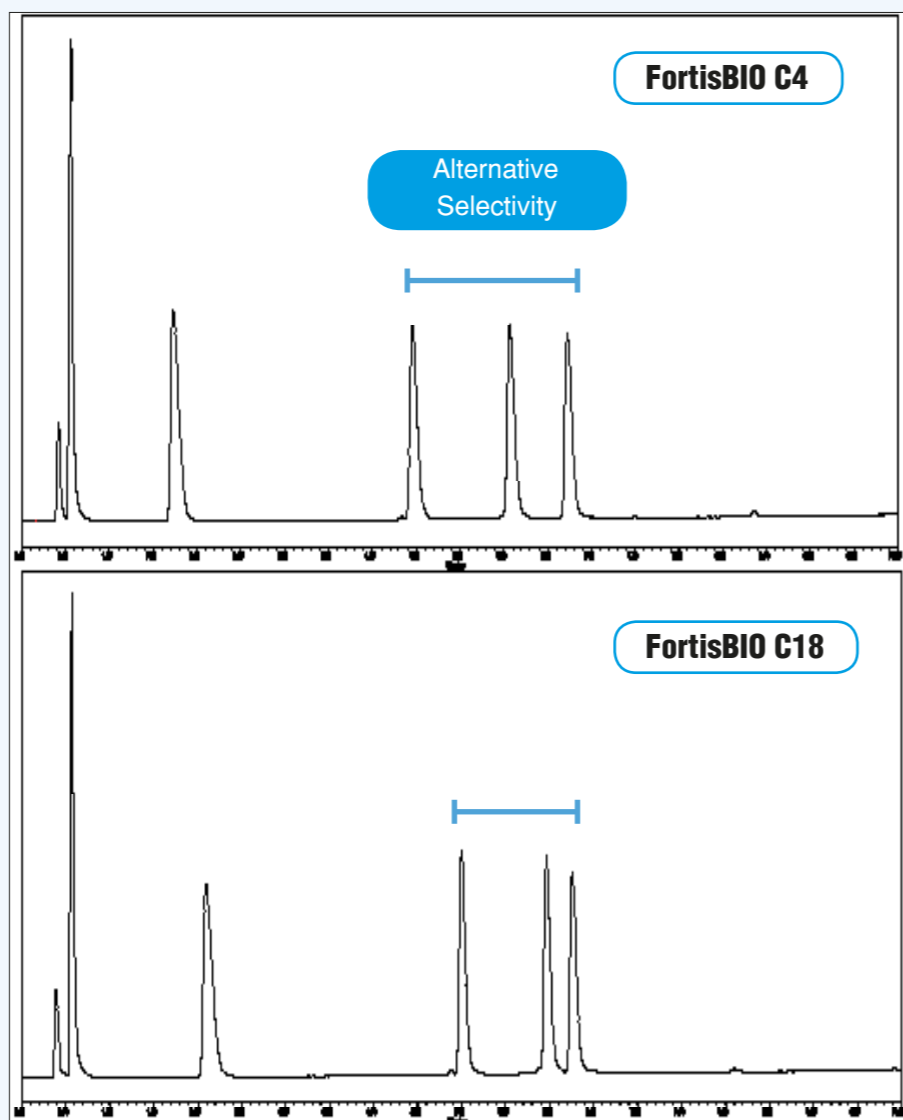
FortisBIO C4 - C4 vs C18

If proteins are analysed with a short alkyl chain phase such as a C4, improvements will often be highlighted in the separation achieved. Resolution and indeed selectivity can both be altered by the hydrophobic and steric interaction difference between C4 and long alkyl chain C18.

In this example the spacing between peaks 4-6 varies based upon the chain length of the stationary phase. C4 in this case providing increased selectivity (α)

As with the FortisBio C18 FortisBio C4 is available as a 1.7µm or 5µm particle so that method development can be scaled between UHPLC and analytical methodologies.

Columns: 50x2.1mm 5µ
 A: 0.1% TFA
 B: 0.1% TFA in ACN
 10-40% B in 10min
 λ - 220nm



FortisBIO C4 - C4 STABILITY IN ACIDIC CONDITIONS

The excellent surface coverage of the C4 ligand on the FortisBIO 300Å material gives multiple benefits for peptide and protein analysis:

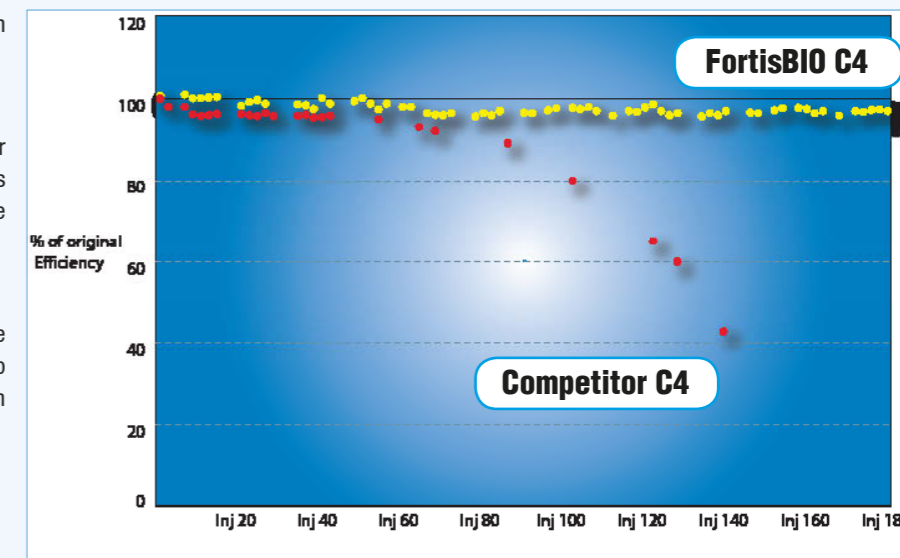
– Improved Lifetime

FortisBIO C4 has an improved lifetime over other C4 columns. The bonding process is optimised to increase the surface coverage and remove secondary interactions

– Improved Peak Shapes

Improved peak shapes occur due to the bonded phase coverage. Resulting in sharp peak shapes and increased sensitivity from the resulting peak height gain.

Stability study is 0.1% TFA : ACN gradients



FortisBIO C4 - ANGIOTENSIN I

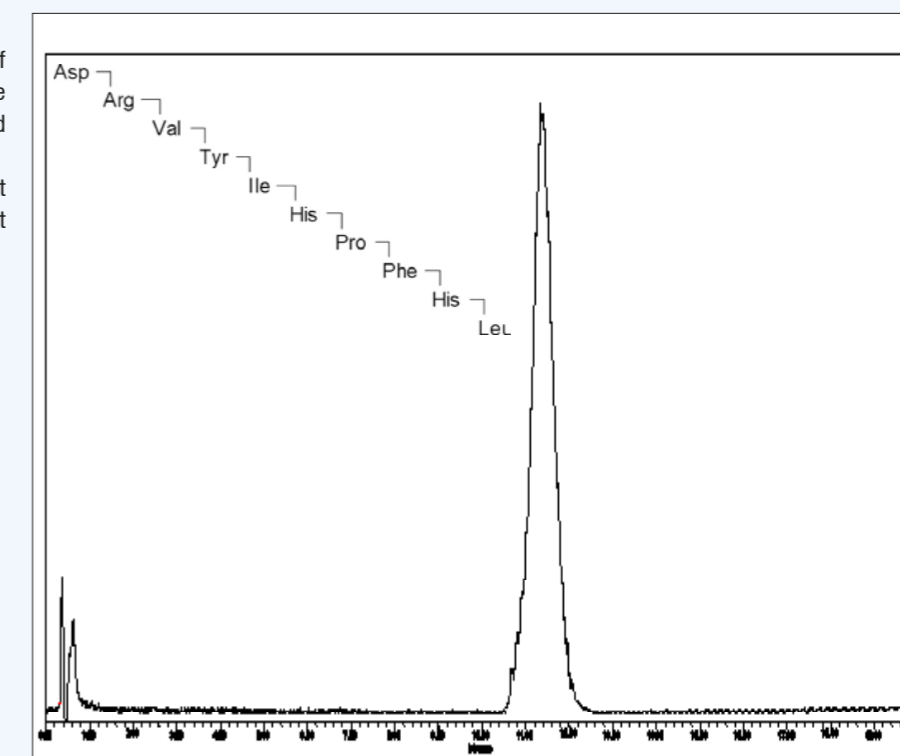
Angiotensin I is formed by the action of renin on angiotensinogen. Cleavage of the Leucine-Valine bond leaves a 10 amino-acid peptide.

Angiotensins are peptide hormones that cause vasoconstriction and a subsequent increase in blood pressure.

1.7µm FortisBIO C4 50x2.1mm
 pn: BIO304-020301

A: 0.1% formic acid
 B: ACN + 0.1% formic acid

50-90% B in 20min
 Temp: 40°C
 λ : 210nm



BIO Considerations

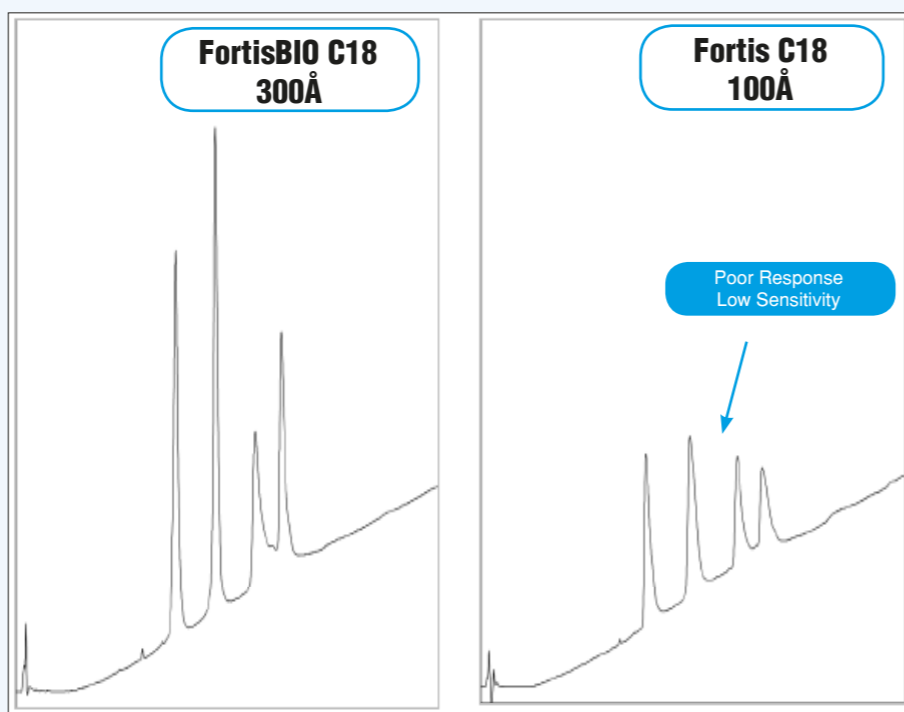
- 300Å large pore size for Peptides and Proteins
- Reproducible bonding
- Sharp efficient peak shapes for peptide mapping
- UHPLC correct fittings

PORE SIZE - 100Å vs 300Å

The importance of pore size is critical in the separation of large peptides and proteins. In 'normal' analytical small molecule work a 100Å silica template is typically used, however this can lead to poor resolution, sensitivity and recovery of biomolecules.

More common therefore for biomolecule work of analytes >2 KDa would be the use of a 300Å silica particle.

Even with this wider pore size Fortis 1.7µm columns will still operate at the higher pressures required by UHPLC systems.



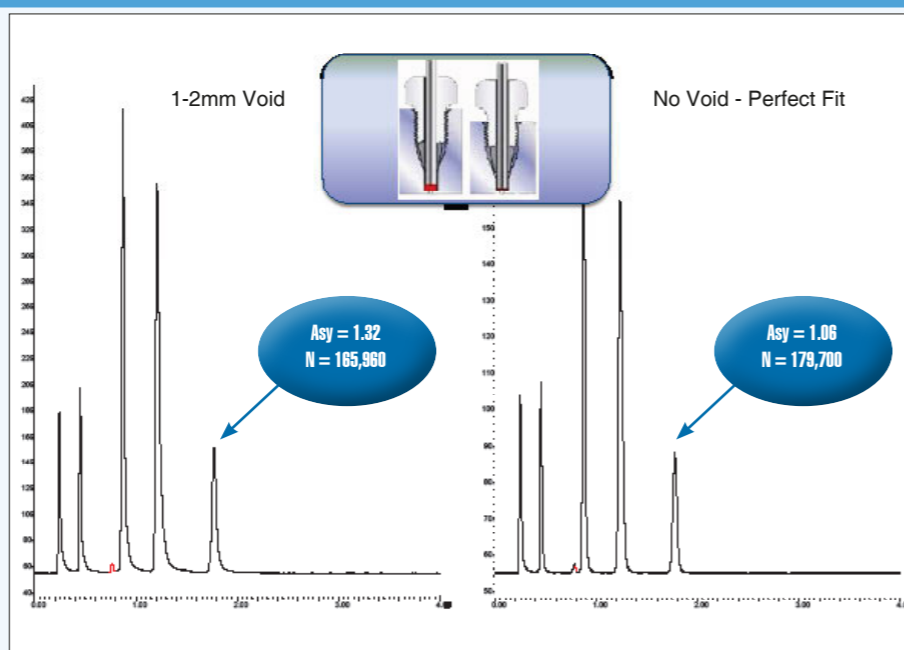
CORRECT FITTING OF UHPLC COLUMNS

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.

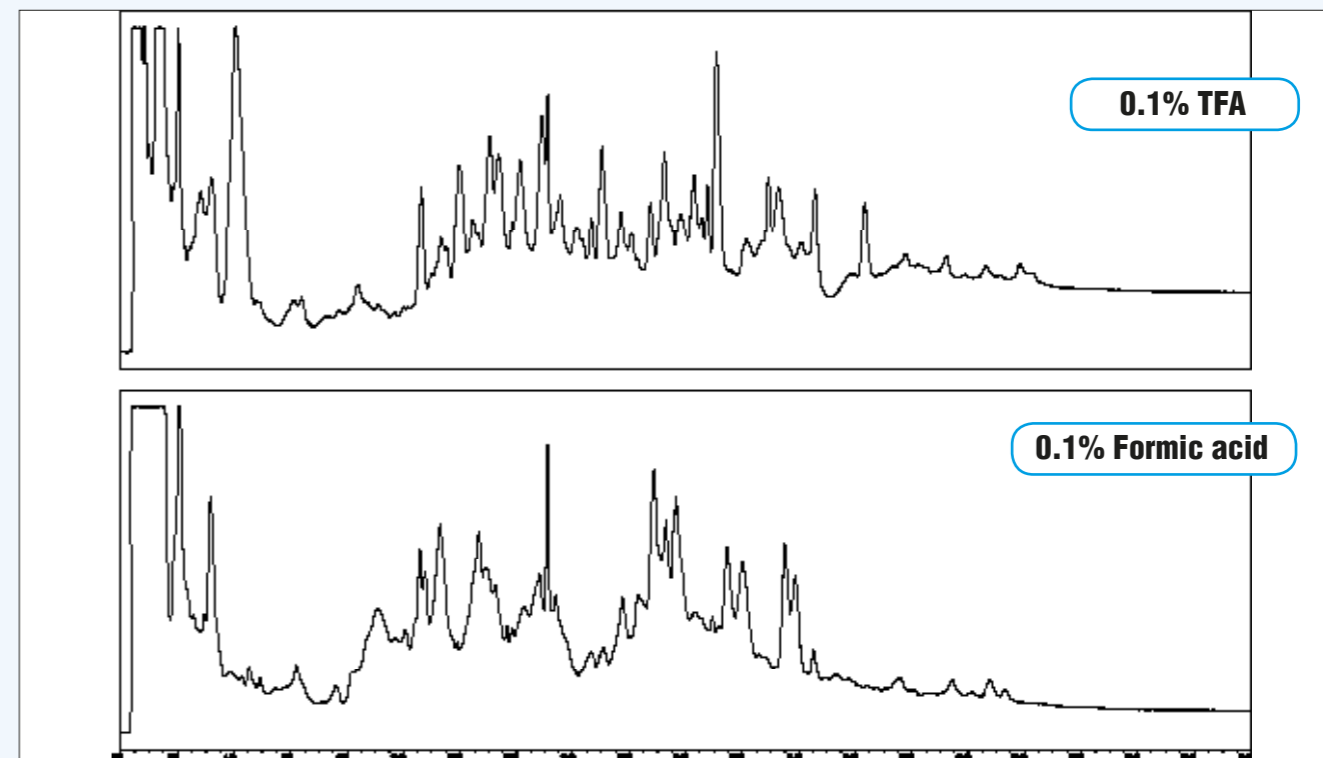
- Adjustable fittings

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.



DIFFERENT BUFFERS - TFA vs FORMIC ACID

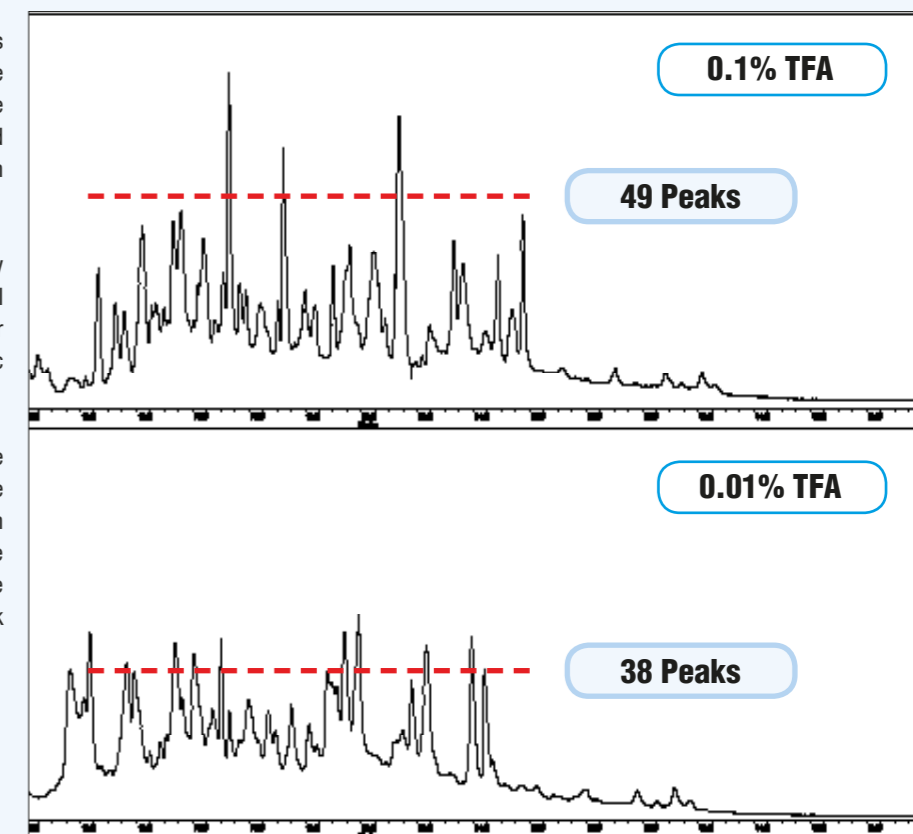


DIFFERENT CONCENTRATION OF BUFFER

TFA is a most common buffer in bioanalysis due to its ion pair effects. When peptide mapping the choice of mobile phase helps reduce or eliminate the charged functionalities of the peptides so they retain on the hydrophobic surface.

TFA usually provides the best chromatography because as an acid it protonates carboxyl groups whilst its ability as an ion-pair reagent neutralises positively charged basic functions.

The analyst has to take care that the concentration of TFA does not compromise MS detection, as it may cause ion suppression in electrospray MS. Choice of buffer concentration will therefore be a compromise between resolution, peak shapes and sensitivity in UV and MS.



Applications

Fortis H2o - AMINO ACIDS

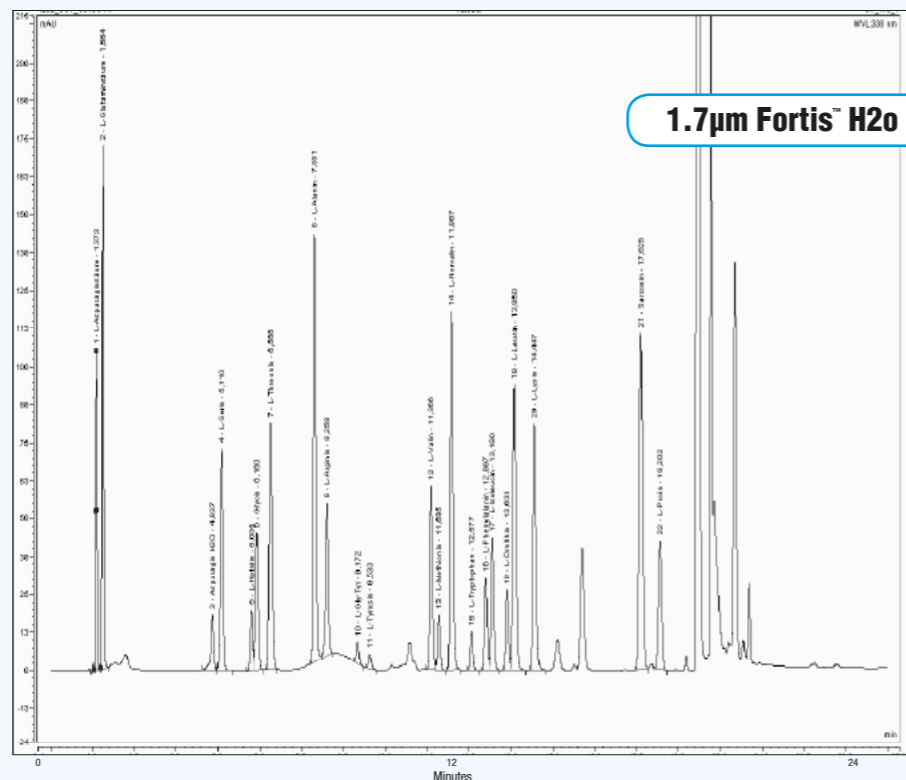
Amino acids play a fundamental role in bioanalysis.

Having many functions in metabolism critical to life and seen as the building blocks of proteins. Linear chains of amino acids can be formed in many sequences to produce a variety of proteins.

HPLC analysis is made challenging by the diversity of the various analytes involved. A variety of hydrophobicities and functionality, basic, acidic and neutral. The amine and carboxylic functional groups present allow the amino acid to have amphiprotic or zwitterionic properties, either the carboxylic acid or the amino group being charged.

Fortis H2o shows the ability to retain and resolve all of the amino acids essential to humans.

For small molecules such as amino acids a 100Å material such as the Fortis H2o is complimentary to the FortisBIO columns.



FortisBIO C18 - INSULIN

Insulin is a hormone which is central to regulating carbohydrate and fat metabolism in the body.

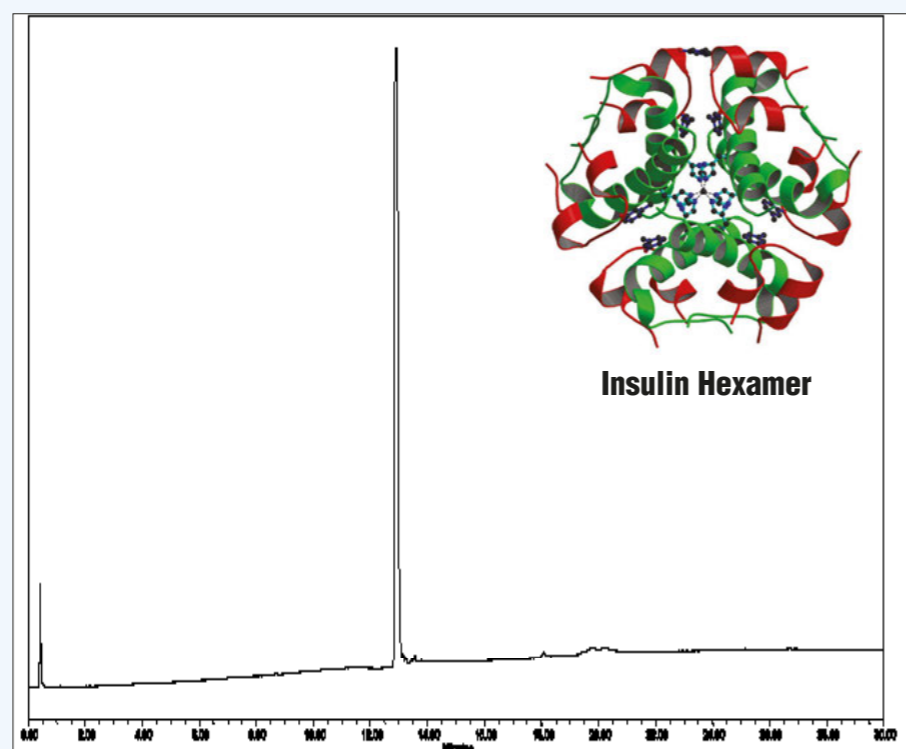
Human Insulin is a peptide hormone comprising of 51 amino acids and has a molecular weight of 5808Da.

Insulin is produced and stored in the body as a hexamer (a unit of six insulin molecules) whilst the active form is the monomer.

Insulin = (mw 5808Da)

1.7µm FortisBIO C4 50x2.1mm

A: 0.1% TFA in Water
B: 0.1% TFA in ACN
5-70% B in 25min
210nm



Applications

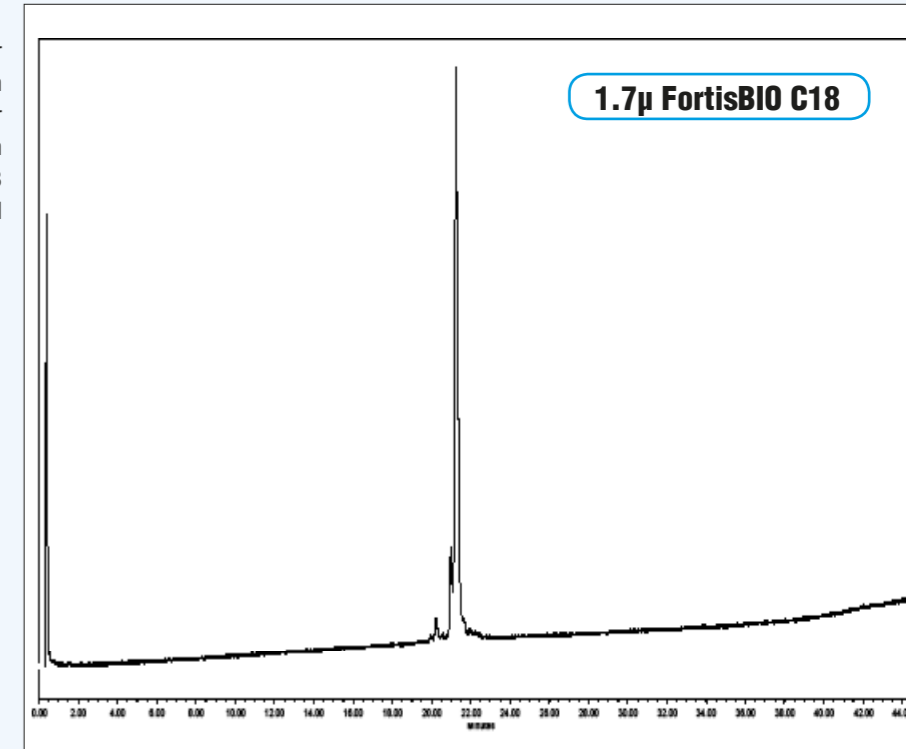
FortisBIO C18 - HUMAN GROWTH FACTOR

Epidermal Growth Factor is a growth factor that stimulates cell growth, proliferation and differentiation by binding to its receptor EGFR. Human EGF is a 6045Da protein with 53 amino acid residues. FortisBIO C18 ensures good peak shape, resolution and recovery of large complex proteins.

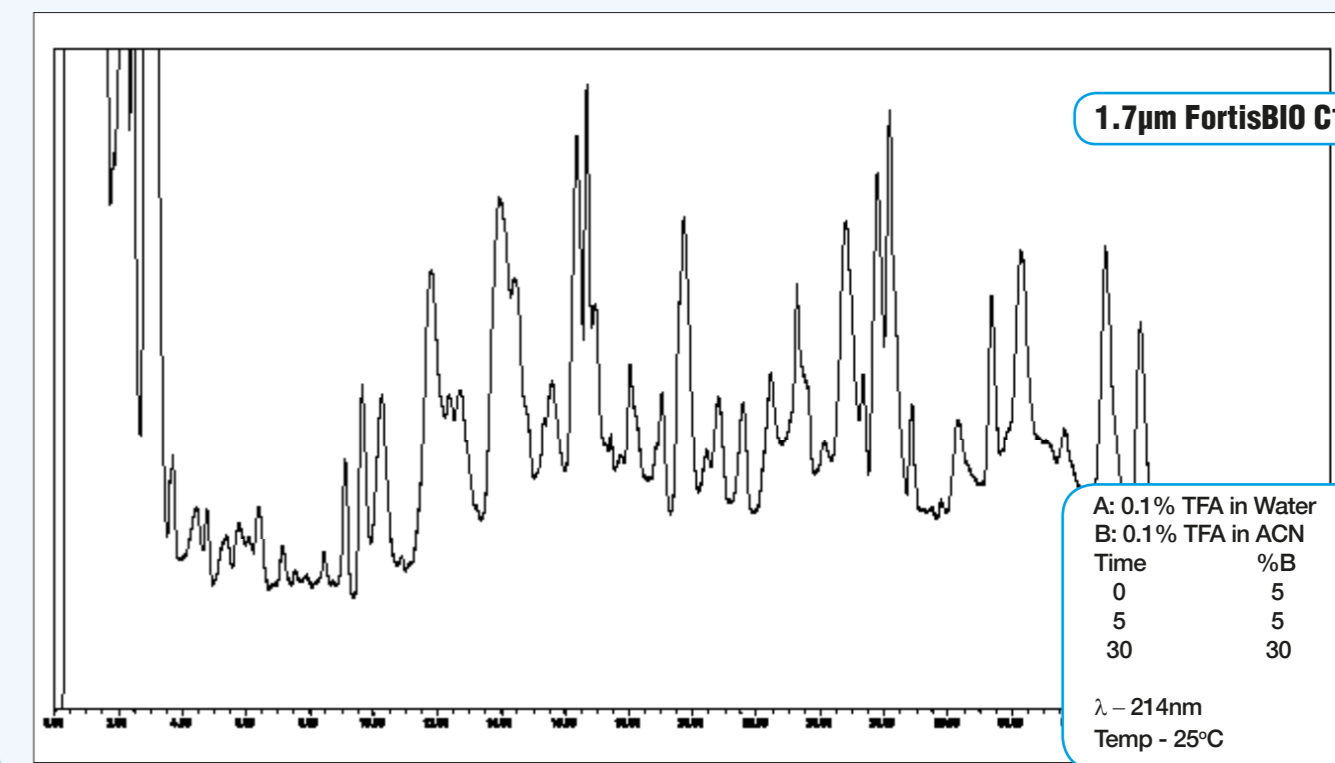
Human Growth Factor (mw = 6045Da)

1.7µm FortisBIO C18 50x2.1mm

A: 0.1% TFA in Water
B: 0.1% TFA in ACN
10-50% Gradient in 45mins
214nm



FortisBIO C18 - CASEIN TRYPTIC DIGEST



A: 0.1% TFA in Water
B: 0.1% TFA in ACN
Time %B
0 5
5 5
30 30

λ - 214nm
Temp - 25°C

BIO Column Care

- Each FortisBIO™ column is shipped with its own test certificate
- Please confirm the column performance on your system
- FortisBIO will operate between pH 2-9
- Operate upto 90°C
- 1.7µm is pressure rated to 1000bar

Operating Conditions

FortisBIO™ columns are stable between pH 2 to 9 and can be used with all common reversed phase mobile phase solvents, Acetonitrile, MeOH, Water as well as common buffers, Formic acid, TFA, Acetic acid, Ammonium acetate. They will operate at elevated temperatures to aid the separation upto 90°C.

NB: Most columns will have a reduced lifetime if operated at extremes of temperature, pressure and pH. Try and avoid excessive use or increased combinations of temperature and pH.

5µ FortisBIO columns will safely operate upto 600bar (8940 psi), whilst 1.7µm FortisBIO UHPLC columns will operate upto 1000bar (15,000psi)

All columns should be installed to minimise dead-volume, this is especially important in UHPLC operating systems. Use a UHPLC fitting designed to handle the increased pressures whilst providing a flexible connection to minimise any extra volume, such as Fortis UHPFIT-2.

Column Regeneration

Cleaning of contaminated columns can be achieved by careful flushing following the procedure below:

- Column should be flushed free from buffer to start any cleaning procedure. High concentrations of water should be used to do this
- Column should be flushed in the reverse direction to ensure minimal wash path and also to ensure no blockage of the frits (not all manufacturers columns can do this, please confirm with your manufacturer)
- 20 column volumes should be used for each wash stage
- 95:5 Water : ACN (removal of buffer)
- 100% ACN
- 100% IPA
- 100% ACN
- 50:50 Water : ACN
- Reorientate column in correct flow direction and re-equilibrate into the required mobile phase.

Column Storage

- Columns should be stored in at least 40% organic solvent with no buffers present
- Endfittings should be fitted to ensure no evaporation of solvent and consequent column "dry-out"
- Columns should be stored at room temperature free from vibration
- If a column is to be stored for any period of time, ideally column cleaning should take place as per guidelines

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

1.7µm UHPLC part numbers

1.7µm FortisBIO C18		Column Length				
		20	30	50	100	150
	2.1	BI0318-020101	BI0318-020201	BI0318-020301	BI0318-020501	BI0318-020701
Column Diameter	3.0	-	BI0318-030201	BI0318-030301	BI0318-030501	-
	4.6	-	BI0318-050201	BI0318-050301	BI0318-050501	-

1.7µm FortisBIO C4		Column Length				
		20	30	50	100	150
	2.1	BI0304-020101	BI0304-020201	BI0304-020301	BI0304-020501	BI0304-020701
Column Diameter	3.0	-	BI0304-030201	BI0304-030301	BI0304-030501	-
	4.6	-	BI0304-050201	BI0304-050301	BI0304-050501	-

Capillary pricing is available upon request

5µm part numbers

5µm FortisBIO C18		Column Length					
		20	30	50	100	150	250
	2.1	BI0318-020105	BI0318-020205	BI0318-020305	BI0318-020505	BI0318-020705	-
Column Diameter	3.0	-	BI0318-030205	BI0318-030305	BI0318-030505	BI0318-030705	-
	4.6	-	BI0318-050205	BI0318-050305	BI0318-050505	BI0318-050705	BI0318-050905

5µm FortisBIO C4		Column Length					
		20	30	50	100	150	250
	2.1	BI0304-020105	BI0304-020205	BI0304-020305	BI0304-020505	BI0304-020705	-
Column Diameter	3.0	-	BI0304-030205	BI0304-030305	BI0304-030505	BI0304-030705	-
	4.6	-	BI0304-050205	BI0304-050305	BI0304-050505	BI0304-050705	BI0304-050905

Capillary pricing is available upon request

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

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

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

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.

WORLDWIDE AVAILABILITY



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VAT No. 866 8966 43

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- Ecuador
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- Israel
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- Japan
- Korea
- Malaysia
- Mexico
- Netherlands
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- Taiwan
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- Turkey
- USA

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