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**

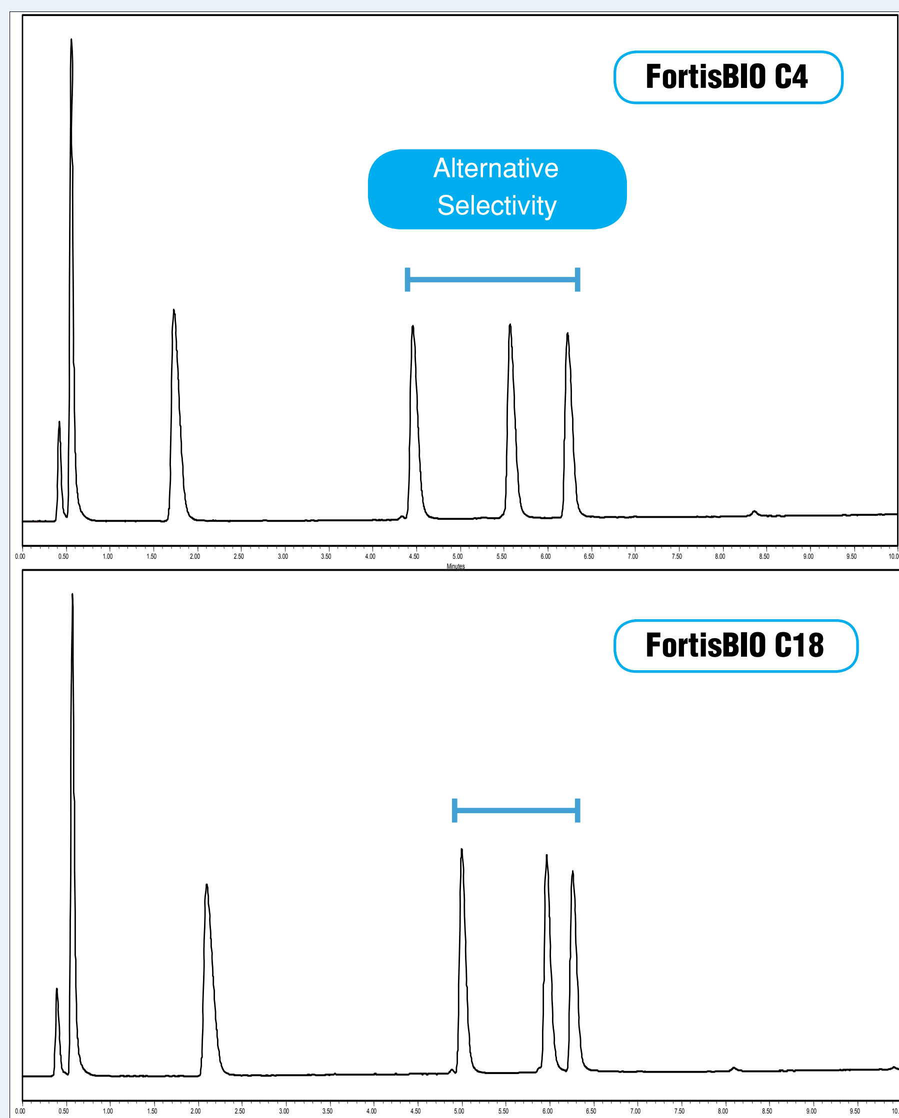
FortisBIO C4 - C4 vs C18

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



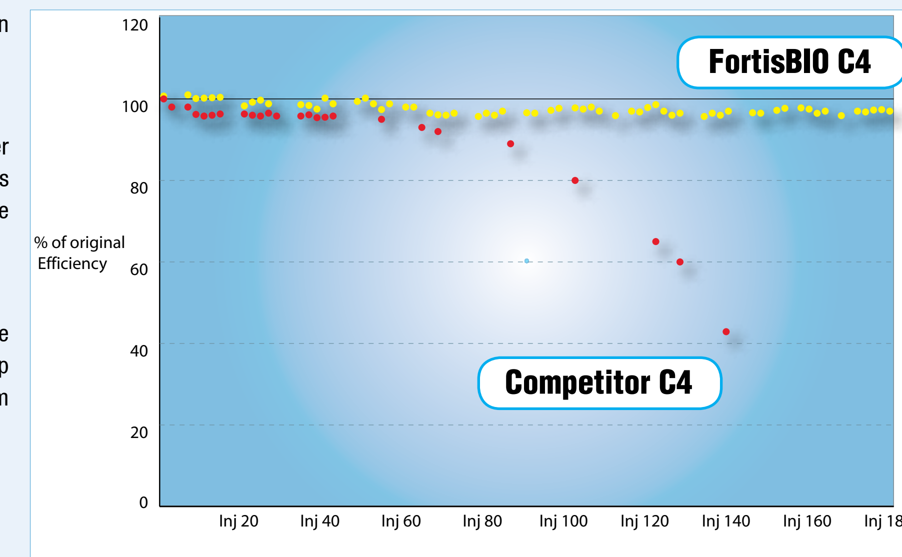
FortisBIO C4 - C4 STABILITY IN ACIDIC CONDITIONS

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



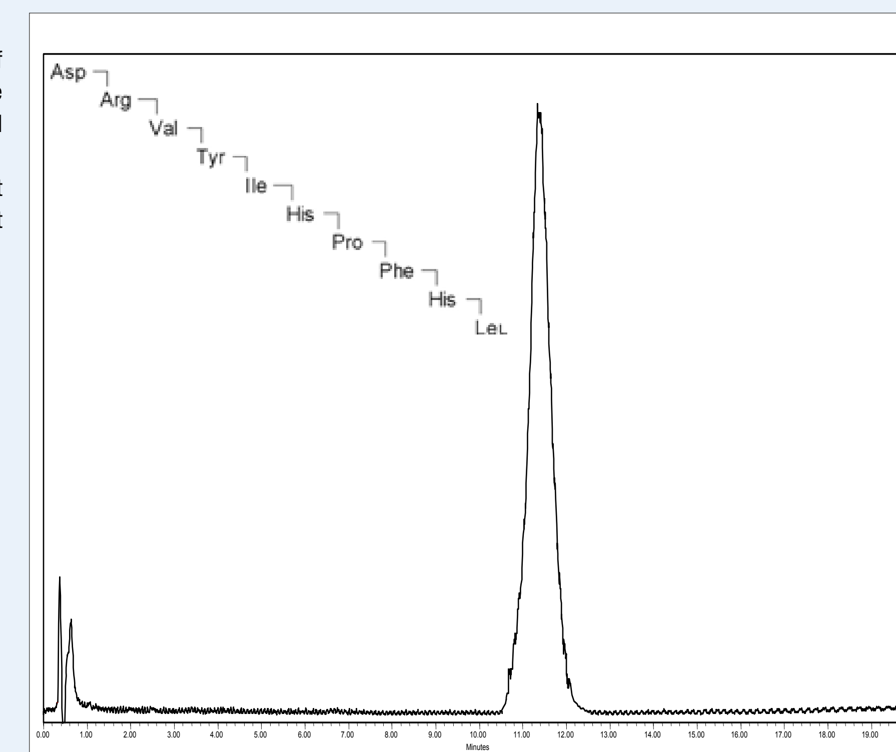
FortisBIO C4 - ANGIOTENSIN I

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

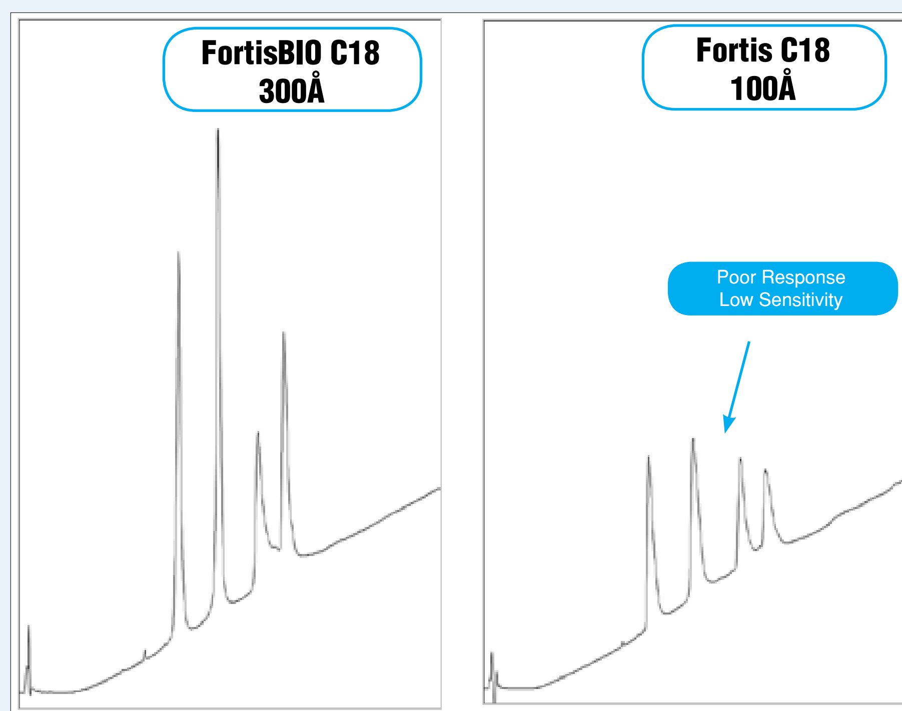
- 300Å large pore size for Peptides and Proteins
- Reproducible bonding
- Sharp efficient peak shapes for peptide mapping
- UHPLC correct fittings

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.



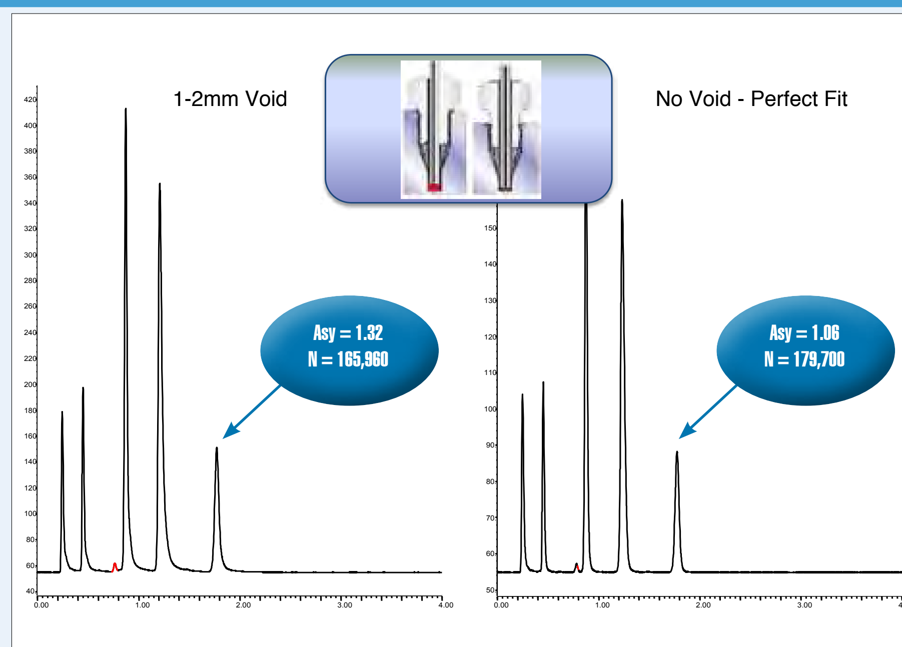
CORRECT FITTING OF UHPLC COLUMNS

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.

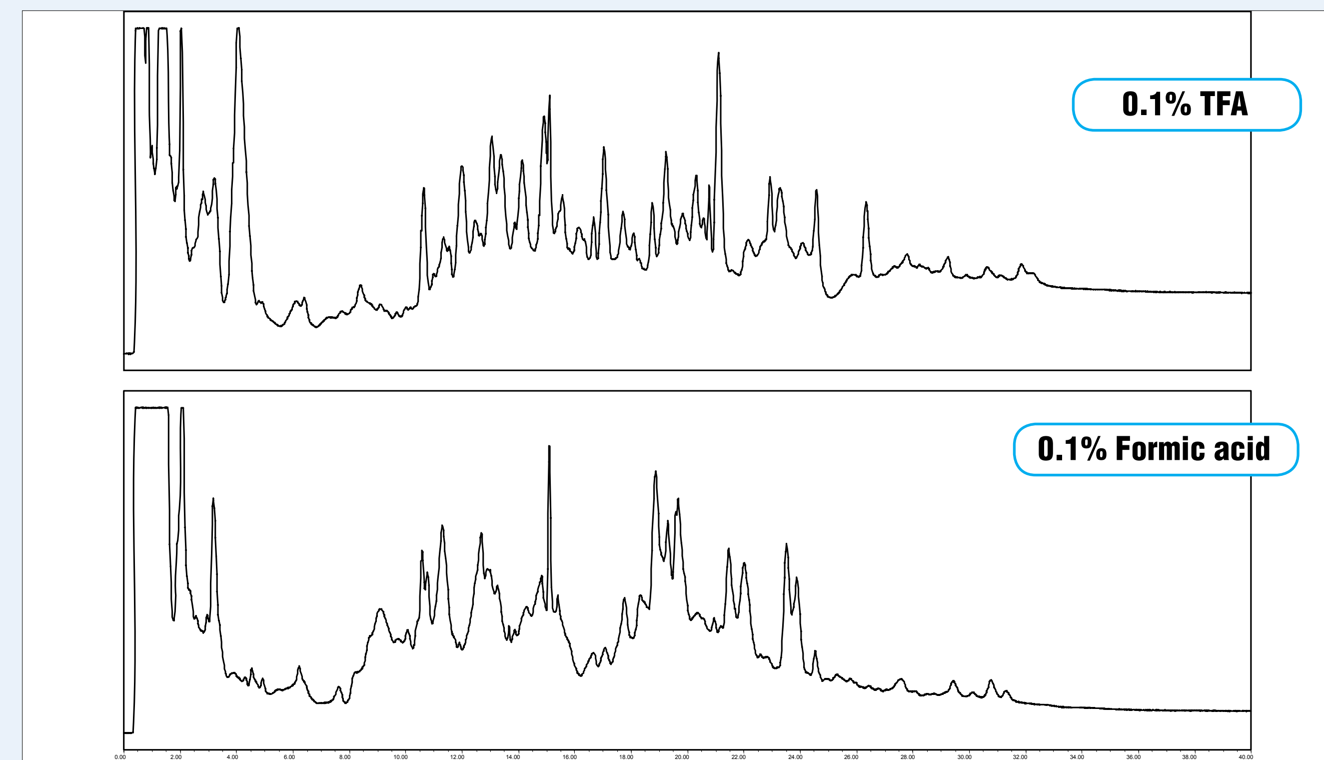
- Adjustable fittings

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.