Phase
Analytical Technology LLC

TIP OF THE PEAK

Edition 1

Phase Analytical Technology is proud to introduce its newest phase chemistry to the ACME product line – the F5/C18.

F5/C18 is the column of choice when a standard C18 column does not provide adequate resolution. The F5/C18 provides enhanced selectivity and retention for applications when the compounds of interest contain an amide, amine (1°, 2°, 3°), methoxy (R-OCH3), alcohol (R-OH), substituted phenyl or other heteroatomic moieties.

Examples of compound classes that exhibit alternate selectivity compared to standard C18 are:

- antibiotics e.g. tetracyclines
- aromatics e.g. phenols
- benzodiazepines
- beta-blockers
- catecholamines
- explosives e.g. nitro-compounds
- halogenated aromatics
- nucleotides, nucleosides, nucleobases
- pharmaceuticals
- stimulants e.g. amphetamine
- structural isomers e.g. methoxy-isomers
- opiates & opioids
- unsaturated compounds

The F5/C18 combines octadecyl (C18) and pentafluorophenyl (PFP) functionalities to produce a novel proprietary phase that maximizes the properties of these two-phase chemistries. The result is a hybrid phase that is very orthogonal to a standard C18. The retention window matches the standard C18 very well, so during method development, exchanging columns without changing mobile phase conditions will rapidly provide an alternate selectivity for many compounds of interest.

C18 + PFP = F5/C18

The F5/C18 also functions as a 100% aqueous compatible C18 for separation of highly polar compounds and for use in gradient methods that begin at very low organic or no organic mobile phase compositions. The proprietary manufacturing process of the F5/C18 phase shows column lifetimes similar to standard C18.

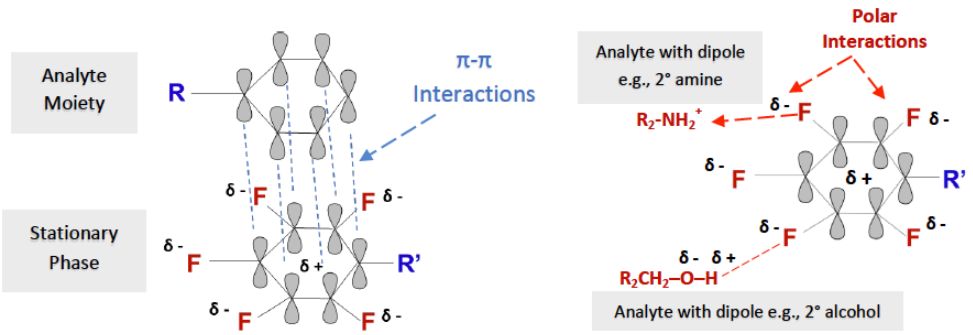
Today's C18 columns provide excellent retention, efficiency and peak symmetry but selectivity mechanisms of C18 phases are limited to hydrophobic interactions. However, many applications require the separation of compounds that vary not only in hydrophobicity but also in the number and/or arrangement of functional groups that affect the polarity of any analyte. In these cases, it is beneficial to have a column phase chemistry, such as the F5/C18, that offers selectivity mechanisms other than simple hydrophobic interactions.

The resolution equation has three parameters that impact resolution of any two peaks in a chromatogram: Efficiency, Selectivity and Capacity (Retention Factor). Of these, selectivity has the strongest effect. Selectivity is controlled by the organic mechanism of interaction between the analyte and the stationary phase.

$$R_s = \left(\frac{1}{4}\right) \bullet (\sqrt{N}) \bullet \left(\frac{\alpha - 1}{\alpha}\right) \bullet \left(\frac{k'}{k'+1}\right)$$

Resolution Efficiency Selectivity Retention

A small change in the selectivity term (α) of only 10% (e.g., from 1.1 to 1.2) will effectively double the resolution.



Reversed phase separations on F5/C18 columns are influenced by a variety of intermolecular forces. The C18 moiety itself provides the majority of the hydrophobic retention. The PFP moiety can function as a Lewis acid (π-acid, electron acceptor) or the equatorial fluorine atoms have Lewis base properties. Overall mechanisms of interactions include hydrophobic, π-π, dipole-dipole, and steric (shape selectivity) interactions.

As a result, orthogonal selectivity relative to a standard C18 column can be achieved on the F5/C18 with analytes that have electron donating groups on an aromatic ring (π-π interaction), amine or other polar moieties (dipole interactions), geometric isomers (shape selectivity) and to a lesser extent, alcohols and carboxylic acids (hydrogen bonding interactions).

The table below summarizes the interaction mechanism differences between a standard C18 and the F5/C18.

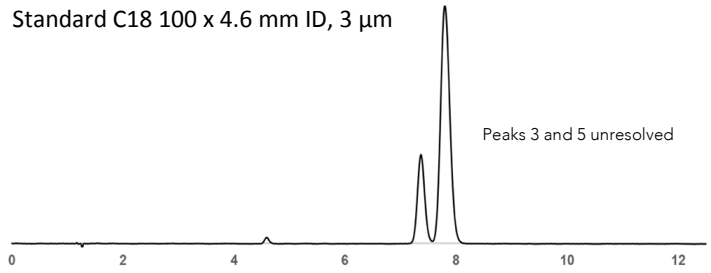
Chromatographic Interaction	Standard C18	ACME F5/C18
Hydrophobic	++++	++++
π-π interaction	–	+++
Dipole-dipole	–	++++
H-bonding	–	+++
Steric (shape selectivity)	+	++++

The F5/C18 column combines the strengths of the pentafluorophenyl (PFP) and octadecyl (C18) functionalities to enhance chromatographic selectivity. With the F5/C18 phase in the analyst's arsenal, greater flexibility is available for separation challenges of complex mixtures.

Specifications:
Ultra-high purity (99.999%) extra-treated porous spherical silica, 120 Å pore size, 260 m^2/g, 16% C, proprietary endcapping, currently available in 3 and 5 micron.

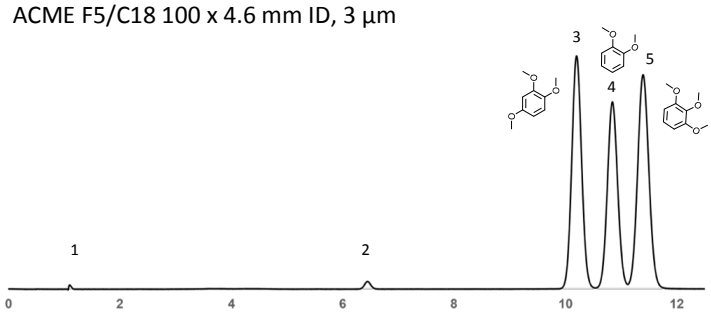
F5/C18 Selectivity Differences vs. Standard C18 – Geometric Isomers

Standard C18 100 x 4.6 mm ID, 3 µm



Mobile Phase: methanol: water, (40:60, v/v).
Flow rate: 1.5 mL / min.
Temperature: 22 °C.
Detection: UV @ 225 nm.
Injection Vol.: 3 µL

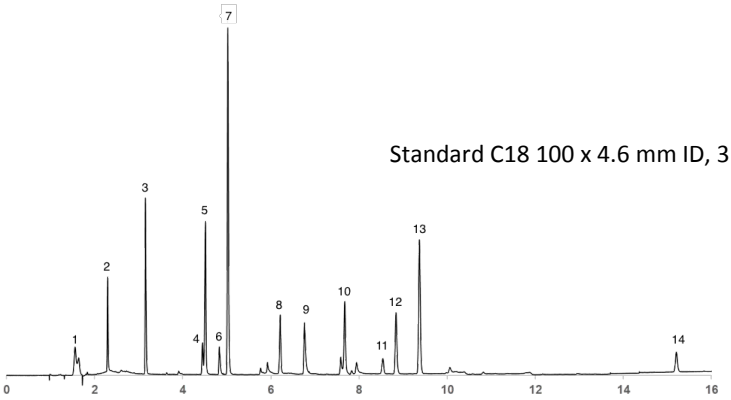
ACME F5/C18 100 x 4.6 mm ID, 3 µm



Sample:
1. Void peak
2. Impurity peak
3. 1,2,4-trimethoxybenzene
4. 1,2-dimethoxybenzene
5. 1, 2,3-trimethoxybenze

F5/C18 Selectivity Differences vs. Standard C18 -Pharmaceutical Drugs

Standard C18 100 x 4.6 mm ID, 3 µm



Sample:
1. Uracil
2. Adenine
3. Atenolol
4. Pindolol
5. Codeine
6. Norfentanyl
7. Acebutolol
8. Verapamil
9. Propranolol
10. Oxazepam
11. Fentanyl
12. Tolbutamide
13. Diazepam
14. Δ^9-Tetrahydrocannabinol

ACME F5/C18 100 x 4.6 mm ID, 3 µm

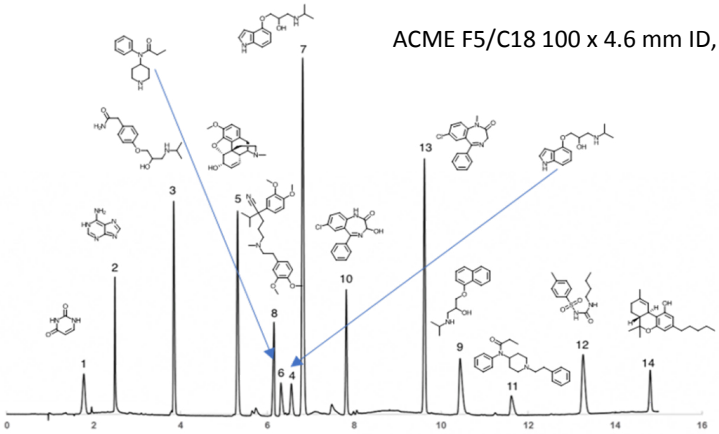


Table 1: Selectivity Change - Retention Time Shift

Large Increase	Moderate Increase	Little or No Increase
4. Pindolol	1. Uracil	8. Verapamil
6. Norfentanyl	2. Adenine	10. Oxazepam
7. Acebutolol	3. Atenolol	
9. Propranolol	5. Codeine	
11. Fentanyl	13. Diazepam	
12. Tolbutamide		

Mobile Phase: A: 25 mM Ammonium Formate in water, B: Acetonitrile. Gradient: 0%B/0min, 65%B/10 min, 90%B/11min, 90%B/15 min.Flow rate: 1.5 mL / min. Temperature: 25 °C. Detection: UV @ 245 nm. Injection Vol.: 3 µL

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ac·me
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the point at which someone or something is best, perfect, or most successful.

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