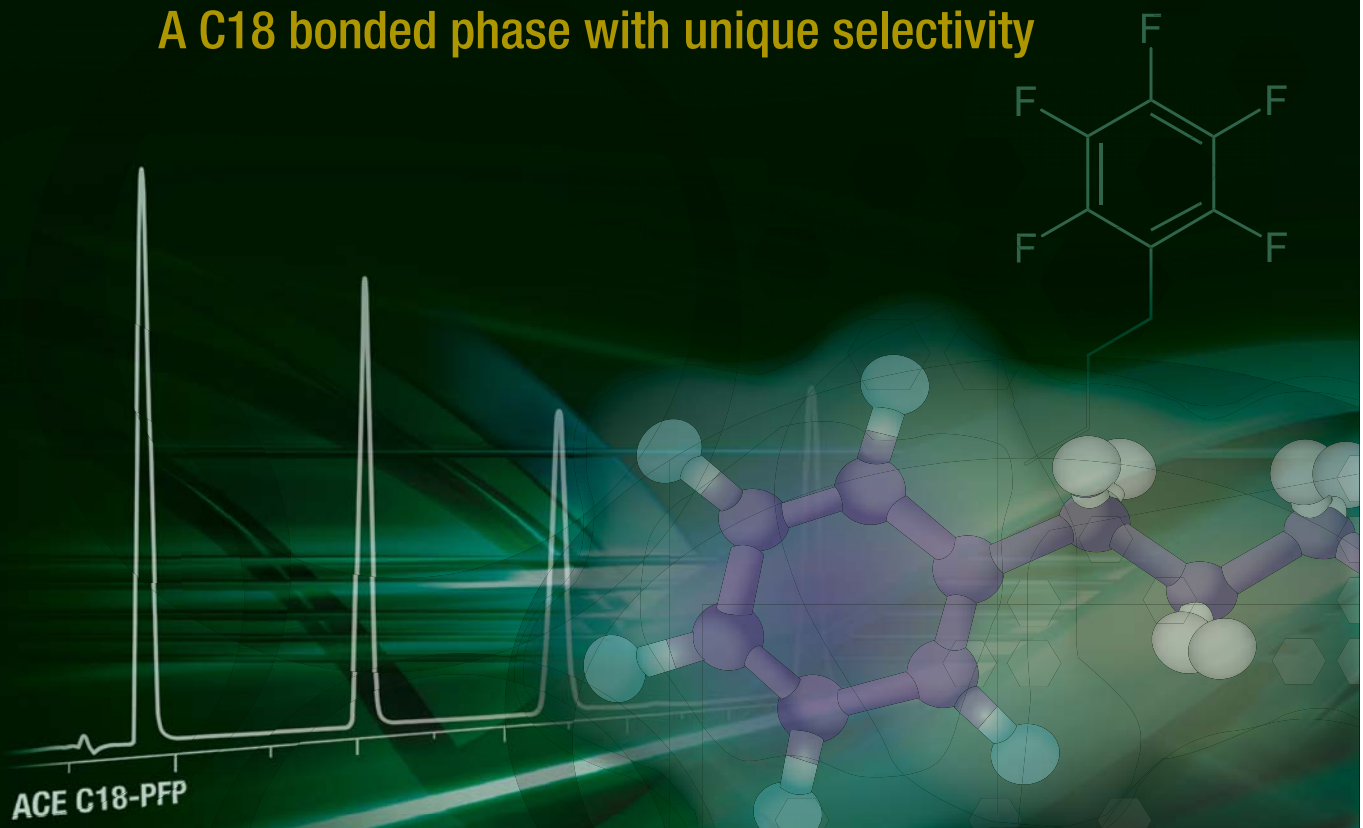
AZ Chrom s.r.o. azetchrom@hplc.sk www.azetchrom.sk

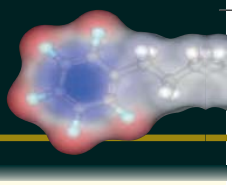
ACE® C18-PFP

A C18 bonded phase with unique selectivity



- Guaranteed reproducibility
- Exceptional bonded phase stability
- Hydrophobic and pentafluorophenyl “mixed mode” interaction

ACE®
HPLC Columns



Explore the Advantages of ACE C18-PFP

a unique C18 bonded HPLC column with the extra selectivity of a pentafluorophenyl (PFP) phase

- Combines C18 and PFP mechanisms of separation to separate mixtures not possible with either phase alone
- Improved retention of polar basic compounds for better separations
- Ultra inert, ultra high purity silica, for excellent peak shape and reproducibility
- Exceptional bonded phase stability for elevated temperature applications
- Ultra low bleed phase ensures UV and LC/MS compatibility
- Available in high throughput column dimensions

Contents **Page**

Why do I need another new C18 phase?

The use of an ultra pure, ultra inert silica has many recognised benefits including improved reproducibility, lifetime and chromatographic performance (particularly with basic molecules). However, since the ultra inert silica surface effectively no longer contributes to the separation, C18 columns manufactured with high purity silicas show near identical selectivity. It is therefore highly likely that a problem separation on one leading brand will not be significantly improved by changing to an alternate manufacturer's equivalent product.

For many years, experienced chromatographers have been seeking phases with the proven performance and reproducibility benefits shown by such leading C18 column brands, but which additionally provide the alternate selectivity required for their challenging applications.

How is ACE C18-PFP different?

C18 bonded phases currently dominate the HPLC market, with recent surveys indicating that they are still responsible for 50-60% of all HPLC columns sold. In recent years the use of **P**enta**F**luoro**P**henyl (**PFP**) bonded phases has grown significantly due to the alternate selectivity they provide.

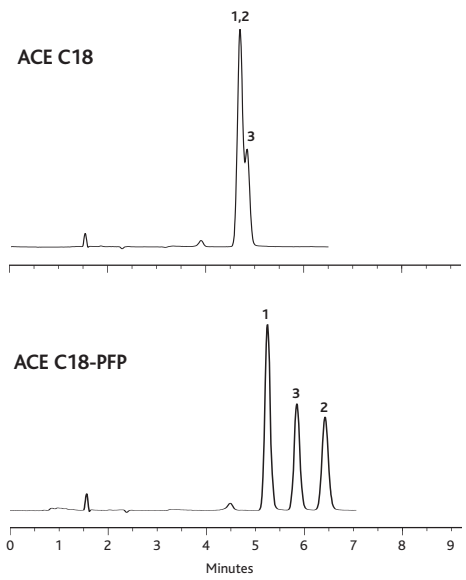
However, compared to C18 bonded phases, PFP phases have traditionally been compromised with reduced hydrophobicity, reduced stability and significant column bleed.

The ACE C18-PFP phase utilises a specially developed ligand combining a C18 chain with integral PFP functionality, resulting in a phase that maintains the hydrophobic, stability and low bleed characteristics of leading C18 phases, yet provides the multiple retention mechanisms of a PFP phase that are responsible for the unique selectivity of ACE C18-PFP (as further detailed on page 4).

"ACE C18-PFP is a valuable method development tool – a column combining C18 retention and stability with PFP selectivity"

R&D Team Leader, Leading Pharmaceutical Company

ACE C18-PFP shows alternate selectivity to C18 bonded columns



Compounds: 1) 1,2,3-trimethoxybenzene 2) 1,2,4-trimethoxybenzene 3) 1,2-dimethoxybenzene
Column: 150 x 4.6 mm, 5µm Flow Rate: 1.00 ml/min Temperature: 40°C Detection: UV, 254 nm

Mobile Phase: 50:50 v/v MeOH/H_2O

When should I use ACE C18-PFP?

Due to their similar hydrophobic characteristics, ACE C18-PFP columns may be used for applications in which "standard" C18 columns would normally be considered. However, due to its integral pentafluorophenyl functionality, ACE C18-PFP is additionally recommended for separations that involve halogenated aromatic compounds, regioisomers and those analytes with differing shape constraints.

As the applications contained within this brochure demonstrate, ACE C18-PFP can be used to improve separations that are proving problematic on C18 columns. The unique ACE C18-PFP phase provides an alternate selectivity to C18 columns, but remains a valid selection for methods in which C18 bonded columns are specified. In many instances, the same evaluation conditions that prove unsuitable for the C18 column prove suitable for the C18-PFP column, avoiding the need for lengthy method redevelopment.

Improve Chromatographic Resolution

The goal of chromatographic separation is to obtain adequate resolution (R_s) of the components of interest in the minimum time. Baseline separation is achieved with a resolution of 1.5, although for rugged, reproducible methods that can be readily transferred between laboratories, a resolution of 1.8-2.0 is desirable.

The resolution equation tells us what variables affect resolution:

$$R_s = \left(\frac{1}{4}\right) N^{0.5} \left(\frac{\alpha - 1}{\alpha}\right) \left(\frac{k}{1+k}\right)$$

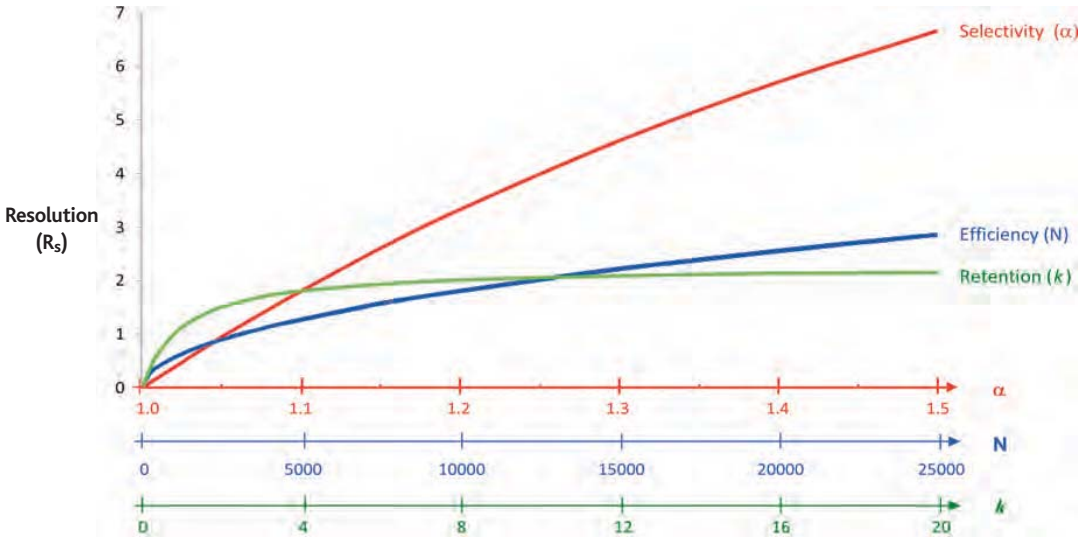
Efficiency | Selectivity | Retention Factor

R_s = Resolution between peaks of interest
N = Efficiency – measured by theoretical plates
α = Selectivity – the ratio of retention (k values) for two peaks
k = Retention factor – the number of column volumes required to elute a peak

Resolution, R_s, can be increased by increasing either N, α or k. However, increasing either N or k to improve R_s suffers from quickly diminishing returns, as can be seen graphically demonstrated in Figure 1 below. For example, R_s increases only with the square root of the increase in N. N can be increased by either adding column length or decreasing the particle size of the column packing material, or some combination of the two. Either way, the system back pressure increases with increases in N, so the "cost" of achieving a satisfactory separation by increasing N can be extremely high pressure.

Similarly, increasing retention (k values) will increase R_s, but also with quickly diminishing returns. Increasing k beyond a value of 10 is usually a poor trade-off between R_s and analysis time, as only marginal gains in R_s are achieved with increasing retention times. A graphic representation of this effect can also be seen in Figure 1 below.

Figure 1. The Effect of N, α and k on Resolution (R_s)
For a typical separation where: N = 10,000, k = 4, α = 1.1



Increasing N, α or k increases resolution (R_s). However, as can be seen from these plots, increasing either N or k suffers from quickly diminishing returns. Increasing selectivity (α) on the other hand, does not have this problem and, therefore, becomes the most powerful of these three variables to optimise when developing a separation.

Increasing α increases R_s but, unlike N and k, without the constraint of diminishing returns. Changes in α also have no effect on pressure and only negligible effects on separation time (see Figure 2). Therefore, α is the most powerful variable to change when developing a separation. Optimising α can allow you to achieve satisfactory resolution between all peaks of interest, while keeping system back pressure and separation times acceptable.

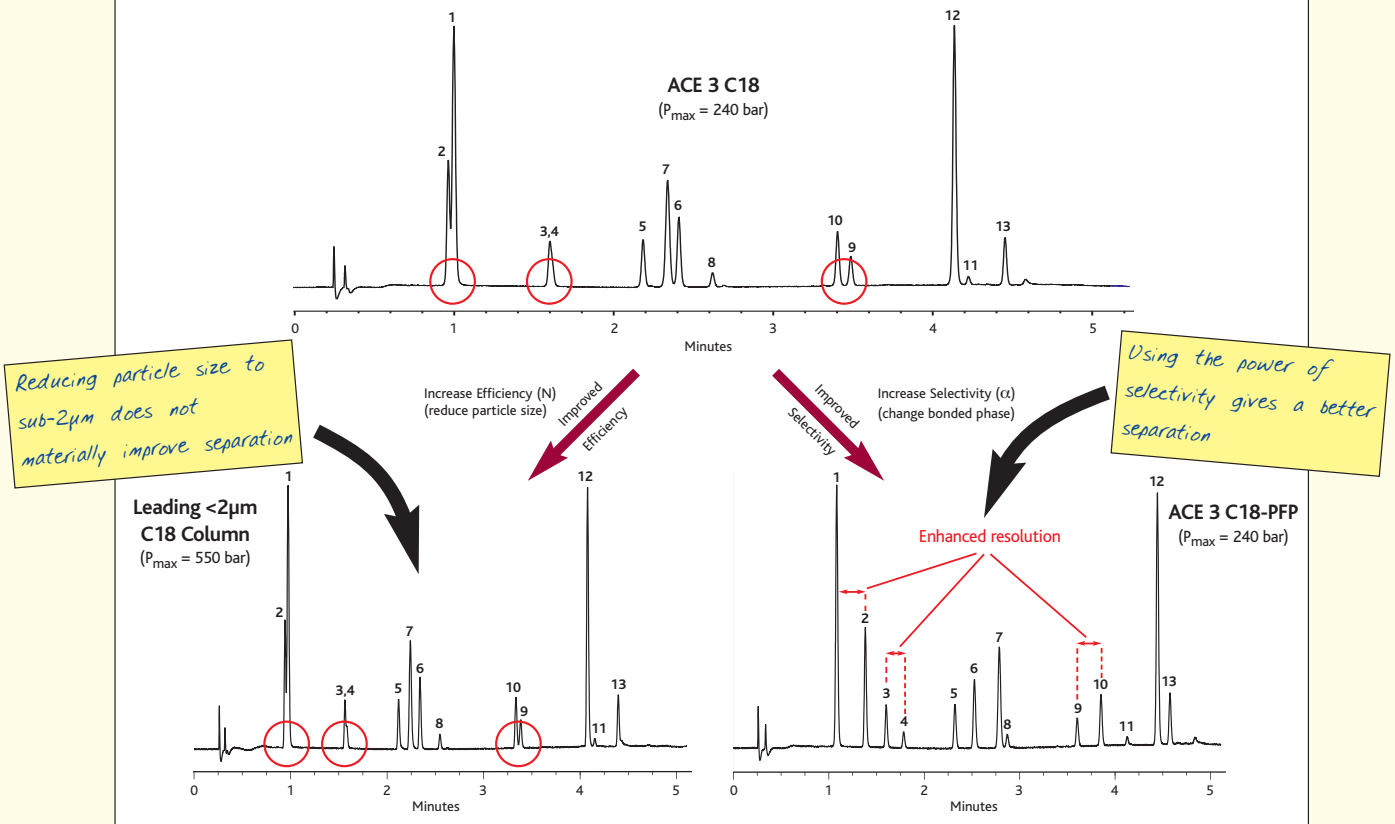
Improve Chromatographic Resolution – Selectivity or Efficiency?

Selectivity (α) is controlled by the mobile phase, temperature and stationary phase chemistry. Most method development strategies will explore all of these chromatographic variables.

When sufficient resolution is not achieved with a "standard" 3µm C18 phase, it is recommended to optimise the chromatographic selectivity of the separation rather than the separation efficiency, as highlighted in the following example.

By simply changing the stationary phase chemistry (i.e. column) to one with an alternate chromatographic selectivity, the desired resolution can be readily obtained on a standard HPLC system without the need for expensive UHPLC instrumentation. Complex mobile phase compositions, elevated temperature and aggressive pH conditions may also be avoided.

Figure 2. Leveraging Selectivity to Achieve Fast, High Resolution Separations



Sample: 1) paracetamol 2) hydrochlorothiazide 3) methylphenylsulphoxide 4) methylphenylsulphone 5) aspirin 6) phenacetin 7) 1,3-dinitrobenzene 8) 1,2,4-trimethoxybenzene 9) ethylbenzoate 10) nimesulide 11) ibuprofen 12) indomethacin 13) mefenamic acid

Column Dimensions: 50 x 2.1 mm Flow Rate: 0.60 ml/min Temperature: 40°C Detection: UV, 254 nm
Mobile Phase: A = 5 mM formic acid in H_2O and B = 5 mM formic acid in MeOH, Gradient = 3 to 100% B in 5 minutes

Reducing particle size from 3µm to sub-2µm whilst retaining the C18 bonded phase does not materially improve the separation and additionally results in a significant pressure increase.

The ACE C18-PFP column provides better selectivity (α) for the three critical pairs and therefore provides a superior separation compared to the sub-2µm C18 column, even though the sub-2µm column provides higher efficiency.

Leveraging the power of selectivity leads to a better separation than that obtained by trying to force peaks apart using a column with a high plate count and high pressure.

PFP Separation Mechanisms

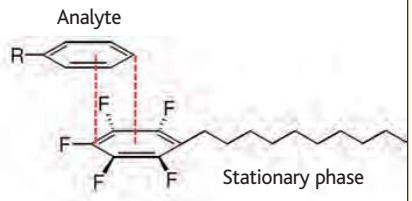
The ACE C18-PFP phase exhibits multiple retention mechanisms including hydrophobic, π-π interaction, dipole-dipole, hydrogen bonding and shape selectivity. Whilst approximations of relative strengths are provided below, the predominance of each retention mechanism is dictated by the solute's physico/chemical properties, its structure and the chromatographic conditions employed.

Separation Mechanism	Typical C18	Typical PFP	ACE C18-PFP
Hydrophobicity	++++	+ / ++	++++
π-π Interaction	-	+++	+++
Dipole-Dipole	-	++++	++++
Hydrogen Bonding	-	+++	+++
Shape Selectivity	++	+++	++++

π-π Interaction

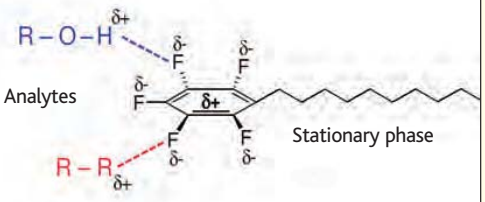
The PFP rings add aromatic character to the surface of the phase. However, PFP phases are different from phenyl phases since the electronegative fluorine atoms produce an electron deficient phenyl ring, which makes the PFP phase act as a Lewis acid. This will interact with an analyte able to donate electrons (i.e. a Lewis base).

This is the opposite of phenyl phases, which contain an electron rich aromatic ring (due to the absence of electron withdrawing groups) and which therefore act as Lewis bases.



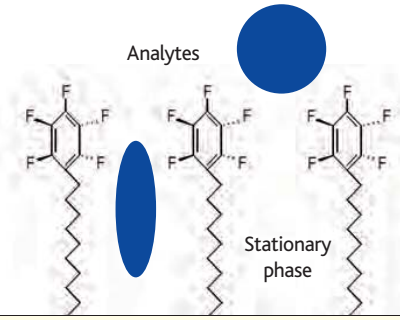
Dipole-dipole and hydrogen bonding

The carbon-fluorine bonds in the PFP ring are extremely polar. Therefore, PFP phases can additionally retain analytes by dipole-dipole or hydrogen bonding interactions that occur between the analyte and the electronegative fluorine atoms. Any such interactions will result in increased retention.



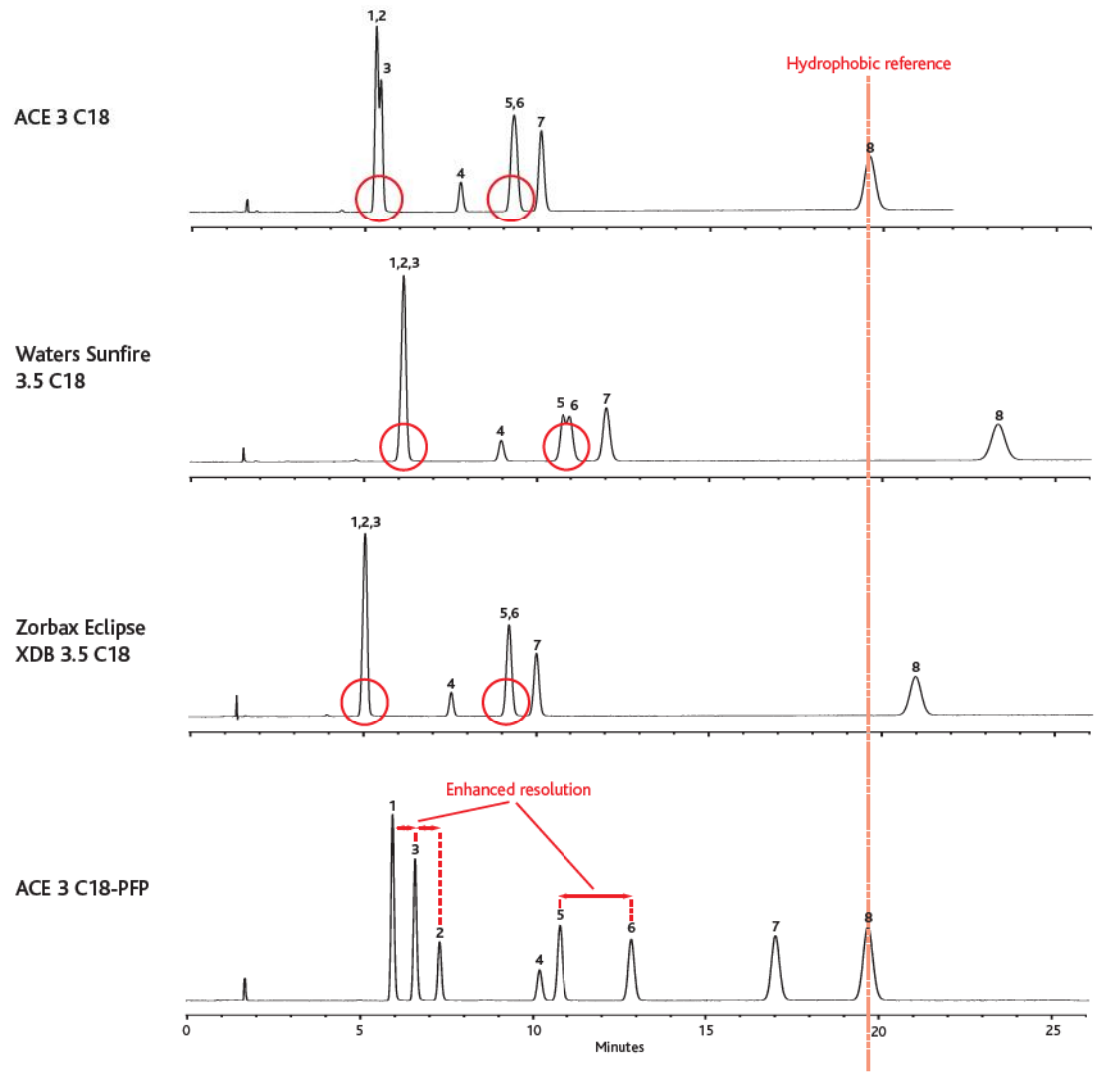
Shape selectivity

The PFP has a rigid ring structure which, when combined with the other retention mechanisms that are possible, confers outstanding shape selectivity on the PFP phase.



The ACE C18-PFP phase exhibits the multiple retention mechanisms of a PFP phase, which chromatographers may exploit in order to resolve mixtures that are difficult, if not impossible, to separate on traditional C18 phases (which rely primarily on hydrophobic retention mechanisms only).

Application #1 - Substituted Methoxybenzene Isomers - Leading C18 Columns



Sample: 1) 1,2,3-trimethoxybenzene 2) 1,2,4-trimethoxybenzene 3) 1,2-dimethoxybenzene 4) 1,4-dimethoxybenzene 5) methoxybenzene 6) 1,3-dimethoxybenzene 7) 1,3,5-trimethoxybenzene 8) neutral molecule (reference).

Column Dimensions: 150 x 4.6 mm Flow Rate: 1.00 ml/min Temperature: 40°C Detection: UV, 254 nm Mobile Phase: 50:50 v/v MeOH/H_2O

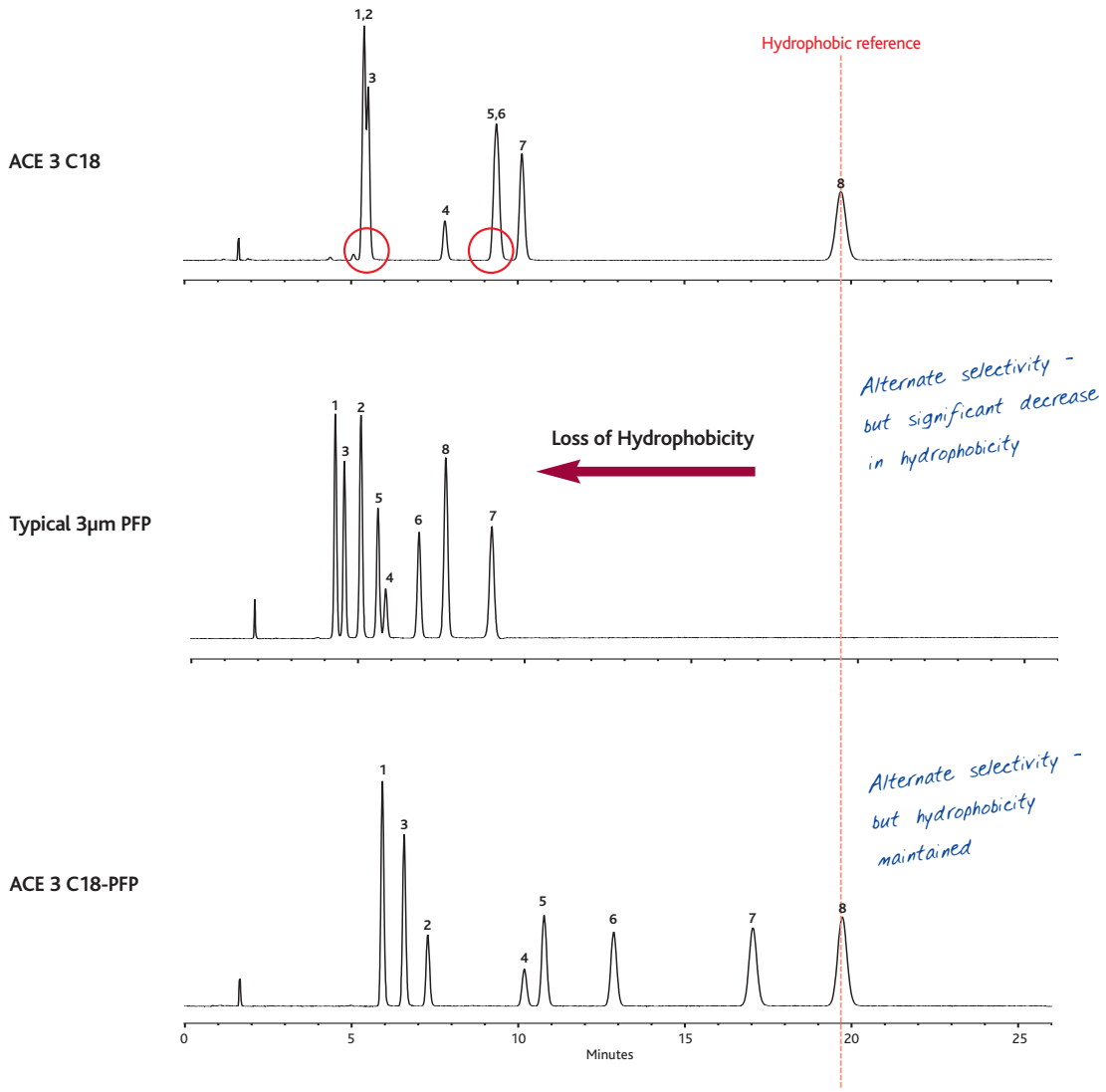
Comparative data may not be representative of all applications.

Refer to page 17 for trademarks

The above example highlights the fact that leading C18 brands provide similar selectivity and all fail to separate tri-, di- and monomethoxybenzene isomers. The differences in absolute retention (as illustrated by the neutral reference marker) are due to purely hydrophobic effects and related to parent silica characteristics (e.g. surface area).

The ACE C18-PFP exhibits excellent resolution of all components, resulting from the integral PFP functionality contained within the unique ACE C18-PFP ligand.

Application #2 - Substituted Methoxybenzene Isomers - PFP Columns



Sample: 1) 1,2,3-trimethoxybenzene 2) 1,2,4-trimethoxybenzene 3) 1,2-dimethoxybenzene 4) 1,4-dimethoxybenzene 5) methoxybenzene 6) 1,3-dimethoxybenzene 7) 1,3,5-trimethoxybenzene 8) neutral molecule (reference)

Column Dimensions: 150 x 4.6 mm Flow Rate: 1.00 ml/min Temperature: 40°C Detection: UV, 254 nm

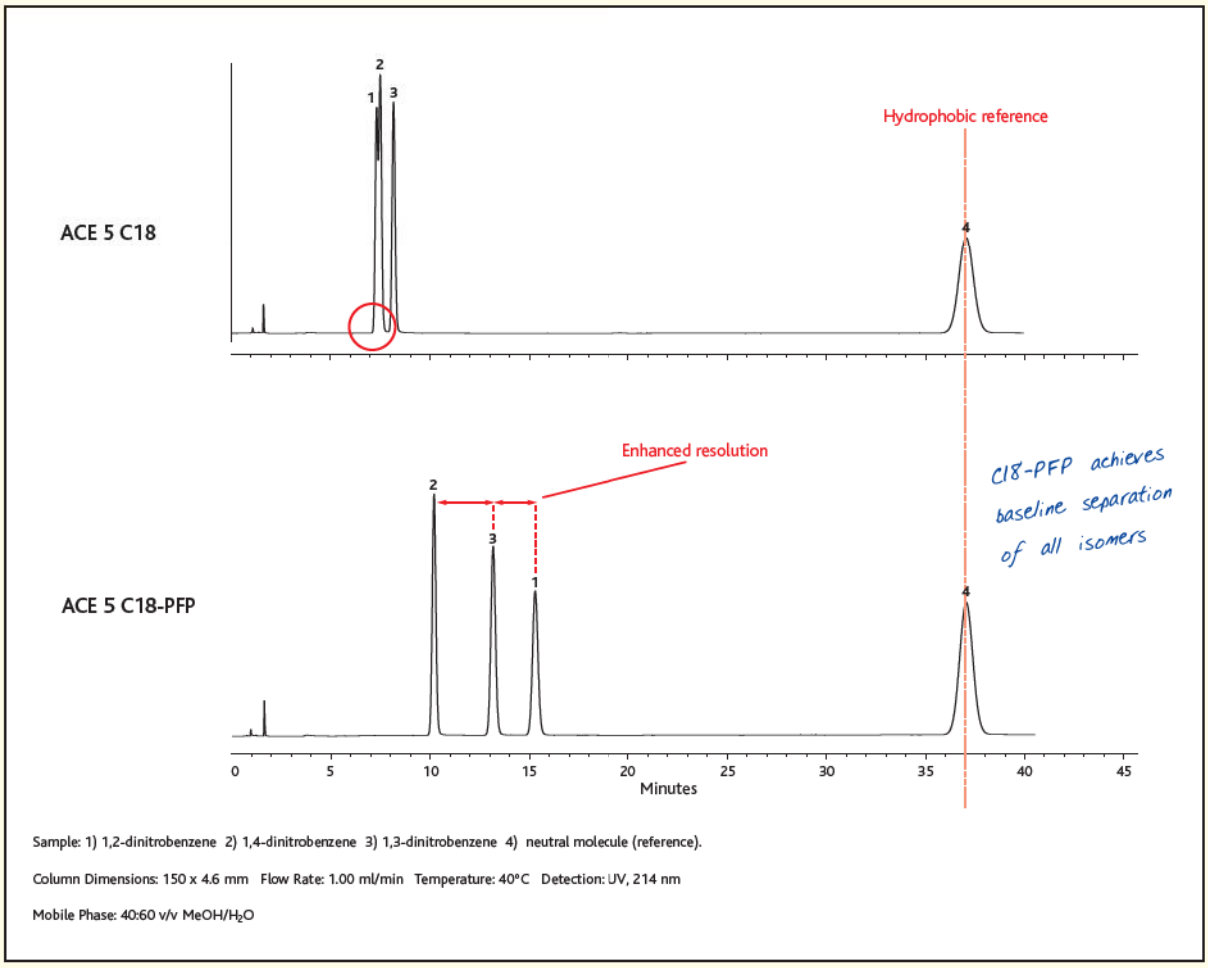
Mobile Phase: 50:50 v/v MeOH/H_2O

The above application highlights the usefulness of pentafluorophenyl phases in the separation of regioisomers. As previously shown on page 5, standard C18 phases fail to separate the tri-, di- and monomethoxybenzene isomers.

Traditional PFP phases provide an alternate selectivity but at the expense of a significant decrease in hydrophobicity, which compromises the separation.

The ACE C18-PFP maintains the hydrophobic characteristics of a C18 phase and provides a superior separation that can be further optimised to reduce analysis time.

Application #3 - Substituted Dinitrobenzene Isomers



Sample: 1) 1,2-dinitrobenzene 2) 1,4-dinitrobenzene 3) 1,3-dinitrobenzene 4) neutral molecule (reference).

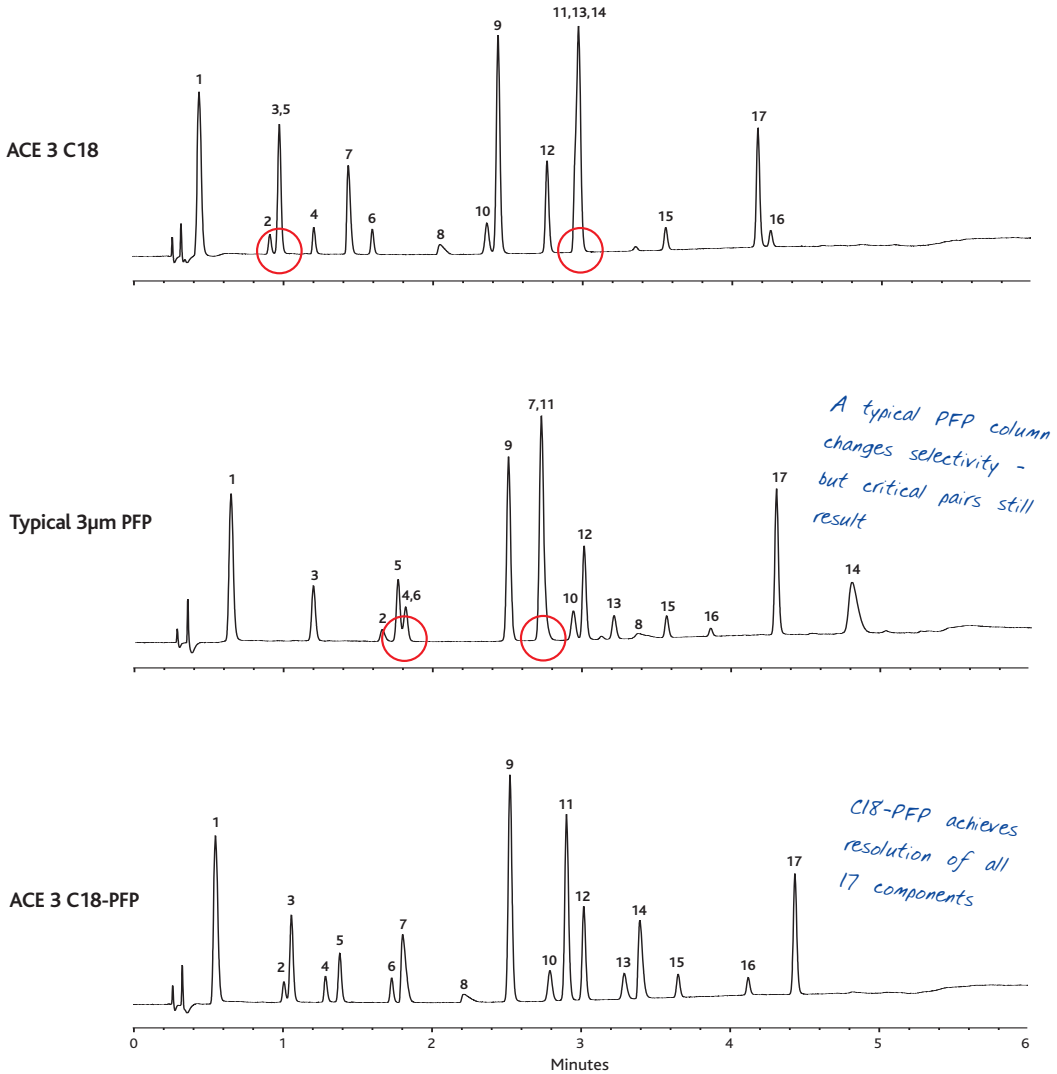
Column Dimensions: 150 x 4.6 mm Flow Rate: 1.00 ml/min Temperature: 40°C Detection: UV, 214 nm

Mobile Phase: 40:60 v/v MeOH/H_2O

This test containing aromatic dinitrobenzene isomers, performed under simple isocratic conditions, highlights that while these phases possess similar hydrophobicities, the C18-PFP phase exhibits differing chromatographic selectivity (via an enhanced dipole-dipole interaction) towards the dinitrobenzene isomers compared to C18 phases.

The C18 phase fails to separate the isomers under these conditions while the C18-PFP achieves baseline separation of all the isomers due to its integral PFP functionality providing additional retention mechanisms.

Application #4 - Pharmaceutically Relevant Analytes



Sample: 1) sulphanilamide 2) nizatidine 3) metronidazole 4) amiloride 5) hydrochlorothiazide 6) caffeine 7) pindolol 8) metoprolol 9) phenacetin 10) 1,3-dinitrobenzene 11) hexobarbitol 12) furosemide 13) piroxicam 14) carvedilol 15) ketoprofen 16) ibuprofen 17) indomethacin

Column Dimensions: 50 x 2.1 mm Flow Rate: 0.60 ml/min Temperature: 40°C Detection: UV, 254 nm

Mobile Phase: A = 0.1% v/v formic acid in H_2O and B = 0.1% v/v formic acid in MeOH

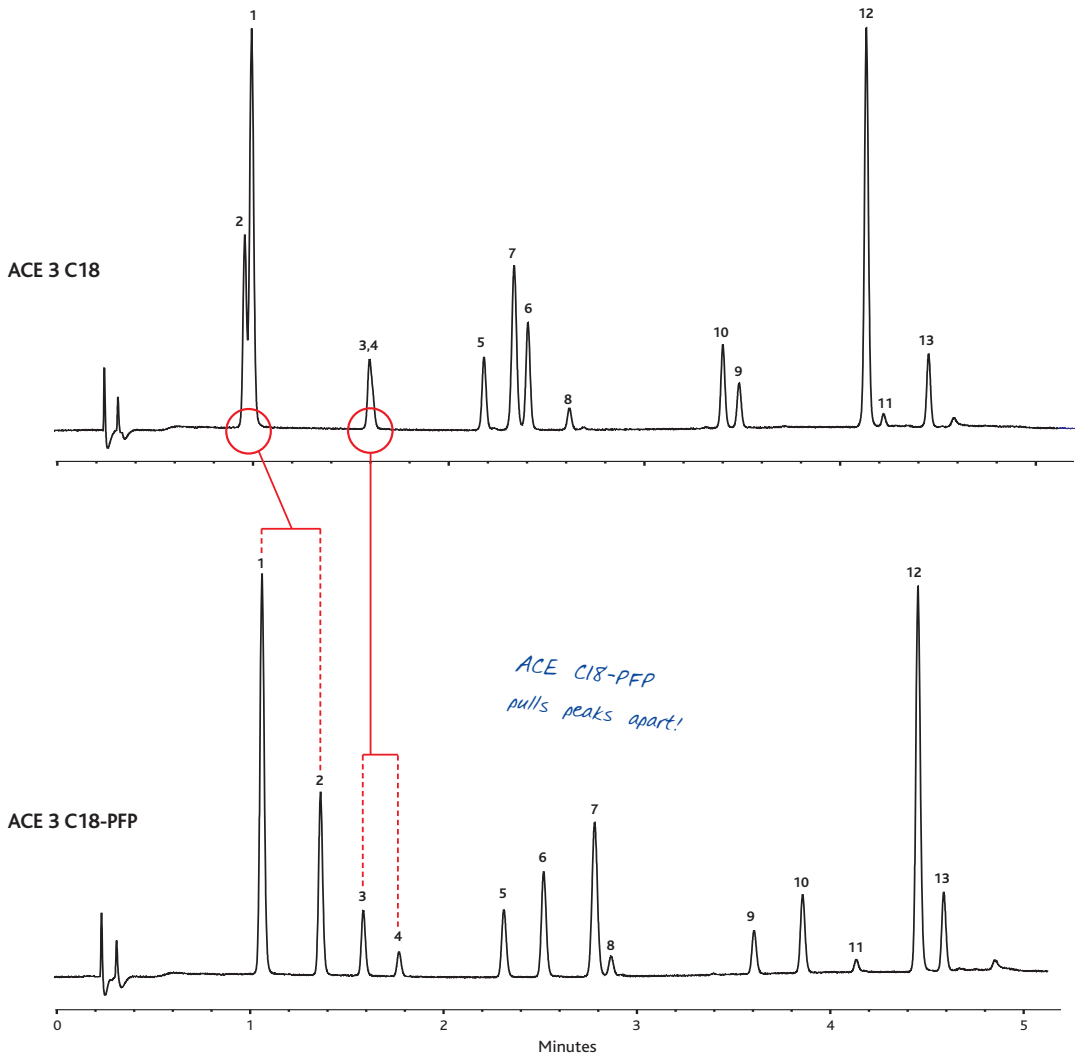
Gradient = 3 to 100% B in 5 minutes

The above application shows the separation of a range of pharmaceutically active analytes on an ACE C18 column, with 2 sets of co-eluting peaks being observed. This separation on the ACE C18 is also consistent with that expected with other leading C18 column brands, which exhibit very similar selectivity due to the same (predominantly hydrophobic) retention mechanism.

Changing to a typical PFP phase results in a change of selectivity, but different critical pairs now result.

The same evaluation conditions that proved unsuitable for the ACE C18 and a typical PFP phase, were found to be suitable for the ACE C18-PFP column, enabling resolution of all 17 components including all critical pairs previously identified.

Application #5 - Structurally Diverse Analytes



Sample: 1) paracetamol 2) hydrochlorothiazide 3) methylphenylsulphoxide 4) methylphenylsulphone 5) aspirin 6) phenacetin 7) 1,3-dinitrobenzene 8) 1,2,4-trimethoxybenzene 9) ethylbenzoate 10) nimesulide 11) ibuprofen 12) indomethacin 13) mefenamic acid

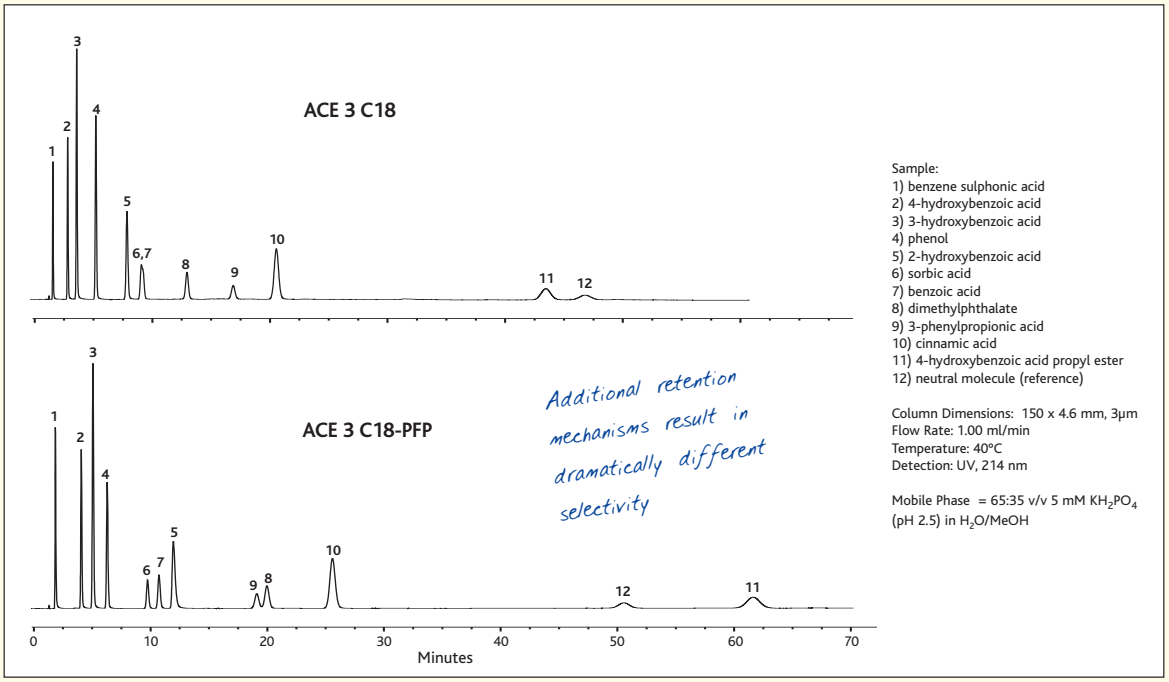
Column Dimensions: 50 x 2.1 mm Flow Rate: 0.60 ml/min Temperature: 40°C Detection: UV, 254 nm

Mobile Phase: A = 5 mM formic acid in H_2O and B = 5 mM formic acid in MeOH

Gradient = 3 to 100% B in 5 minutes

Based upon the same ultra inert, ultra high purity silica platform as ACE C18, the unique ACE C18-PFP phase again provides alternate selectivity, leading to superior resolution of all 13 structurally diverse analytes, without the need for lengthy method redevelopment.

Application #6 - Acidic Analytes



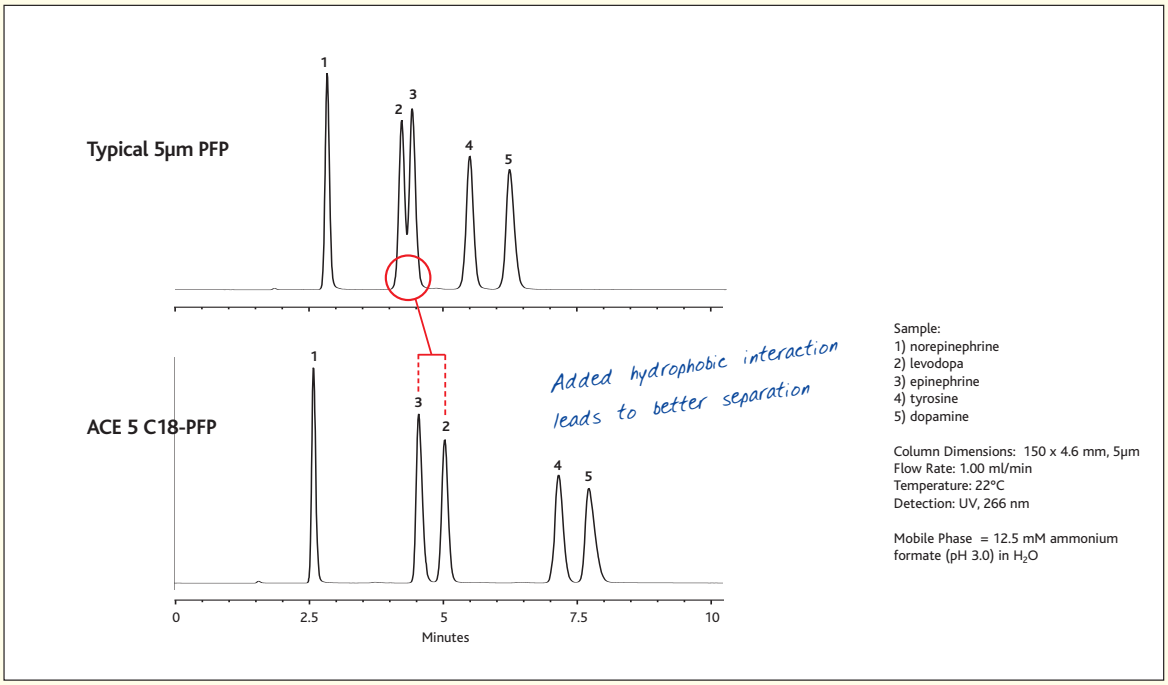
Sample:
1) benzene sulphonic acid
2) 4-hydroxybenzoic acid
3) 3-hydroxybenzoic acid
4) phenol
5) 2-hydroxybenzoic acid
6) sorbic acid
7) benzoic acid
8) dimethylphthalate
9) 3-phenylpropionic acid
10) cinnamic acid
11) 4-hydroxybenzoic acid propyl ester
12) neutral molecule (reference)

Column Dimensions: 150 x 4.6 mm, 3µm
Flow Rate: 1.00 ml/min
Temperature: 40°C
Detection: UV, 214 nm

Mobile Phase = 65:35 v/v 5 mM KH_2PO_4 (pH 2.5) in H_2O/MeOH

The additional retention mechanism provided by the ACE C18-PFP phase results in dramatically different chromatographic selectivity compared to a typical C18 phase, including many reversals in peak elution order.

Application #7 - Catecholamines



Sample:
1) norepinephrine
2) levodopa
3) epinephrine
4) tyrosine
5) dopamine

Column Dimensions: 150 x 4.6 mm, 5µm
Flow Rate: 1.00 ml/min
Temperature: 22°C
Detection: UV, 266 nm

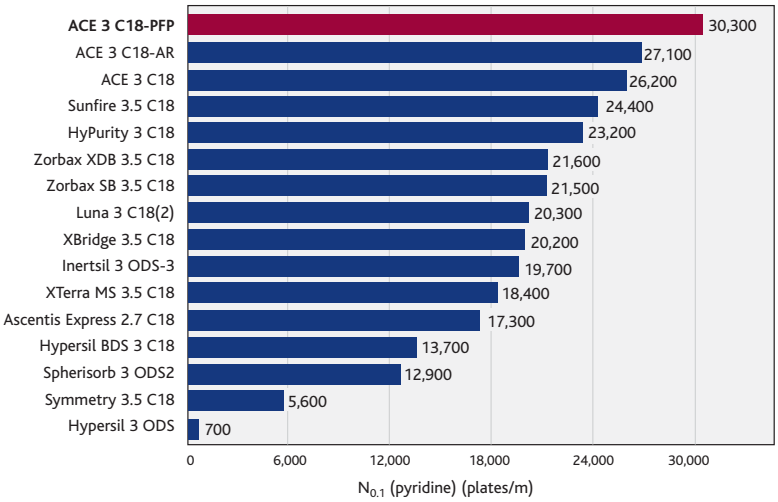
Mobile Phase = 12.5 mM ammonium formate (pH 3.0) in H_2O

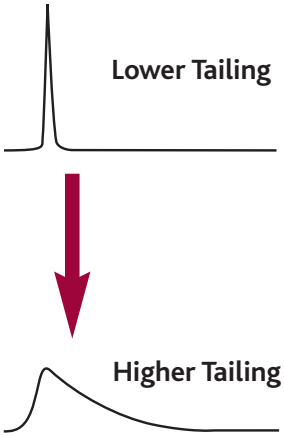
This separation of catecholamines illustrates how strong hydrophobic binding interaction on the ACE C18-PFP provides a better separation of levodopa and epinephrine than is achieved by a typical PFP with weak hydrophobic binding interaction.

Comparison of Column Inertness

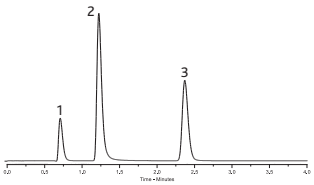
- Leading 3µm, small pore C18 column brands
- 50 x 2.1 mm i.d. LC/MS compatible dimensions
- Basic molecule inertness test
- Peak efficiency and asymmetry investigation

Peak Efficiency Comparison

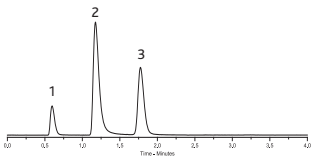




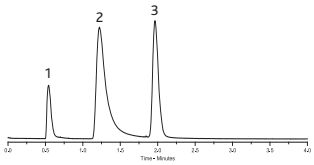
ACE 3 C18-PFP
$N_{0.1}$(pyr) = 30,300pl/m



Waters XTerra MS 3.5 C18
$N_{0.1}$(pyr) = 18,400pl/m



Waters Symmetry 3.5 C18
$N_{0.1}$(pyr) = 5,600pl/m



Column Dimensions: 50 x 2.1 mm Sample: 1) uracil 2) pyridine 3) phenol Mobile Phase: 40:60 v/v MeOH/H_2O Flow Rate: 0.20 ml/min Temperature: 22°C Wavelength: 254 nm

Conclusion

Significant differences in efficiency, peak shape and selectivity are seen when analysing pyridine – a small highly basic molecule.

Increased tailing and retention are indicative of undesirable secondary interactions between pyridine and silanol groups on the stationary phase surface. These interactions can also result in poor column reproducibility.

ACE C18 columns have been previously independently tested and found to be the highest efficiency, most inert columns available. The new ACE C18-PFP maintains this excellent performance.

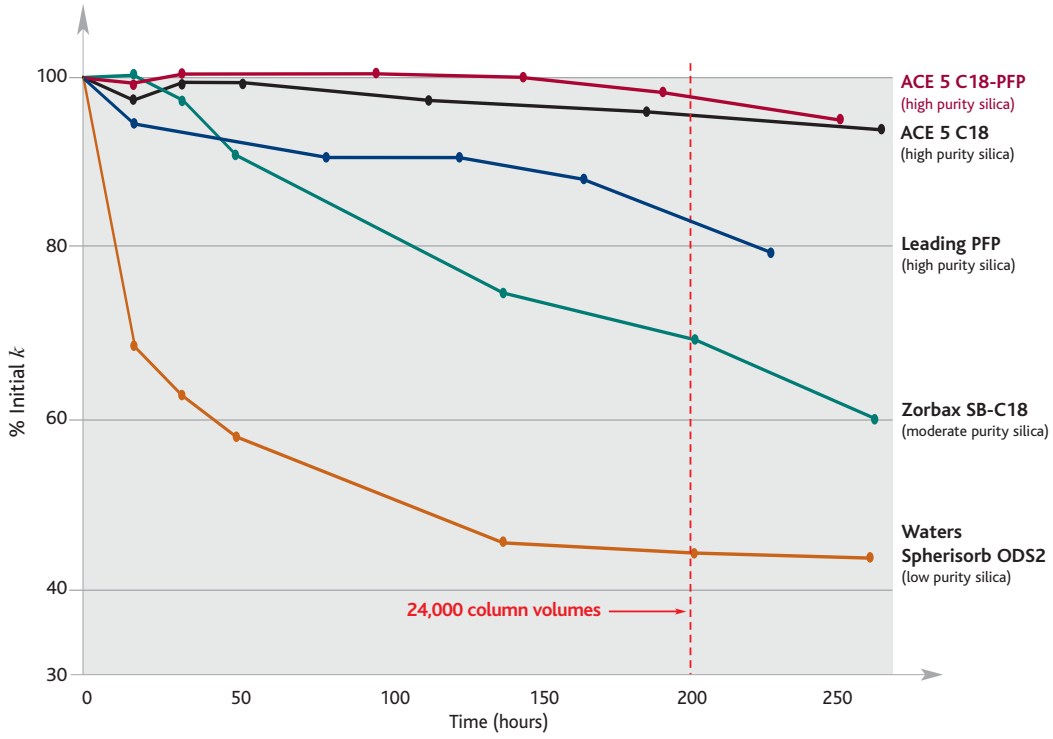
ACE® Stationary Phases Virtually Eliminate the Negative Effects of Silanols on HPLC Separations

Further inertness test data is contained within the current ACE HPLC column catalogue. Additionally, a Comparison Guide to C18 Columns is also available, detailing material characteristics for over 50 HPLC column brands and comparing performance with a number of test probes. Please contact your local distributor to request your copies.

Excellent Temperature and pH Stability

At low pH, column deterioration is caused by hydrolysis of the bonded phase, with a decrease in retention observed. The nature of the bonded phase, the purity of the silica surface and bonding density are all critical parameters. The use of a lower purity silica, a shorter ligand and a lower bonding density are all factors that will contribute to accelerated ligand cleavage and reduced column lifetime.

Accelerated Column Stability Study - 80°C at pH 1.9



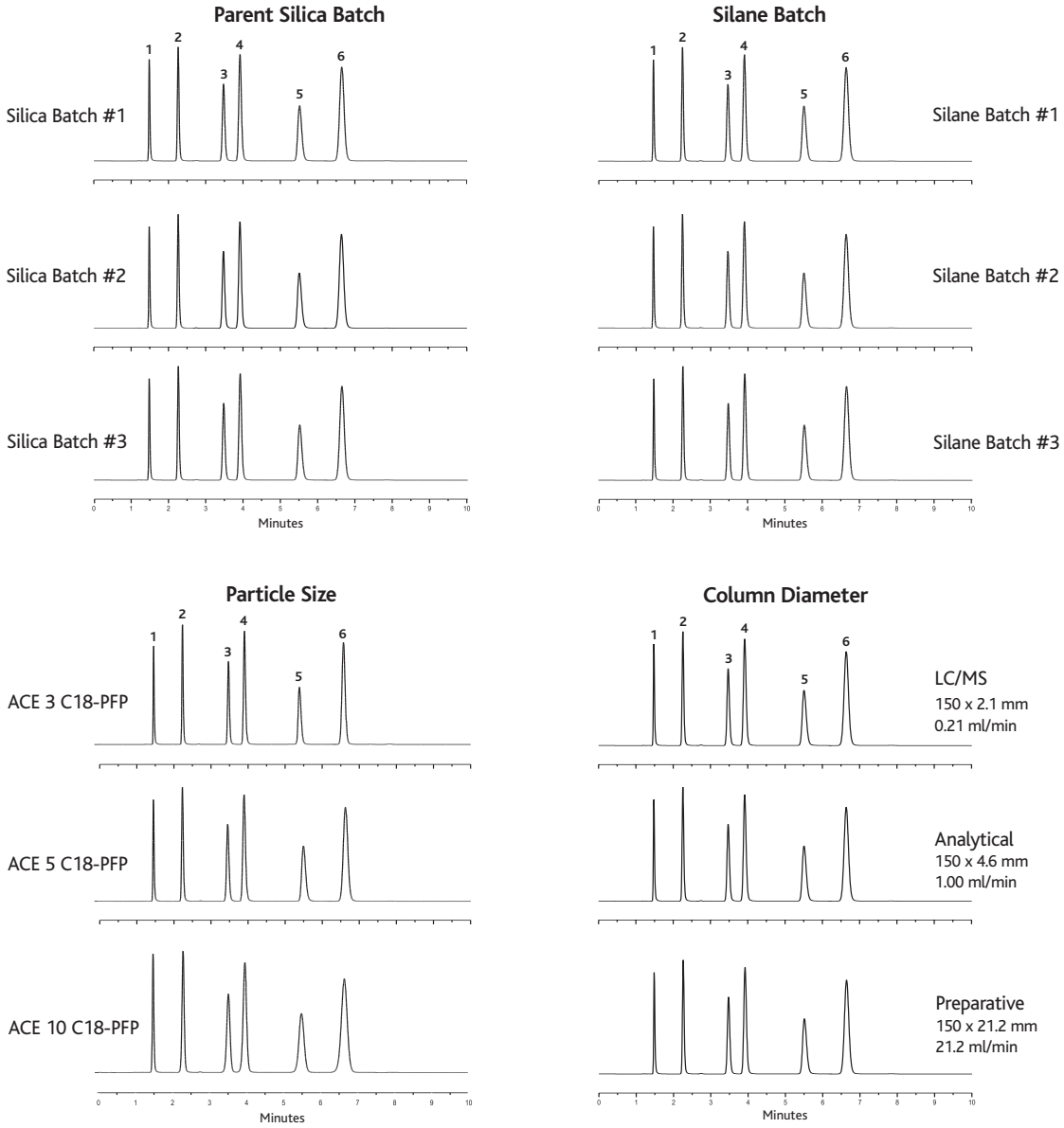
Acidic Exposure Conditions: Mobile Phase: 5:95 v/v MeOH/0.1% TFA in H_2O (pH 1.9) Column Dimensions: 50 x 2.1 mm
Flow Rate: 0.20 ml/min
Temperature: 80°C

Using conditions designed to accelerate column degradation, the ACE C18-PFP phase shows little retention loss, with lifetime equivalent to the highly robust ACE C18 phase, suggesting that the ACE C18-PFP may be suitable for applications in which PFP columns exhibit reduced lifetime.

As expected, a C18 bonded column based upon a moderate purity silica (Zorbax SB-C18 – a phase previously recognised to provide excellent stability for high temperature and low pH applications) and a low purity silica (Waters Spherisorb ODS2) show significantly reduced lifetimes.

Guaranteed Reproducibility and Fully Scalable

Of equal importance to alternate selectivity is excellent reproducibility. Variations between different batches of stationary phase are the most common cause of customer concern. ACE stationary phases virtually eliminate the unpredictable negative effects of silanols on HPLC separations by maintaining a rigid control of the complete manufacturing process and establishing tight specifications for purity, selectivity, retention, efficiency and asymmetry. Therefore, as demonstrated in the figure below, absolute batch-to-batch and column-to-column reproducibility are guaranteed for all ACE C18-PFP columns.



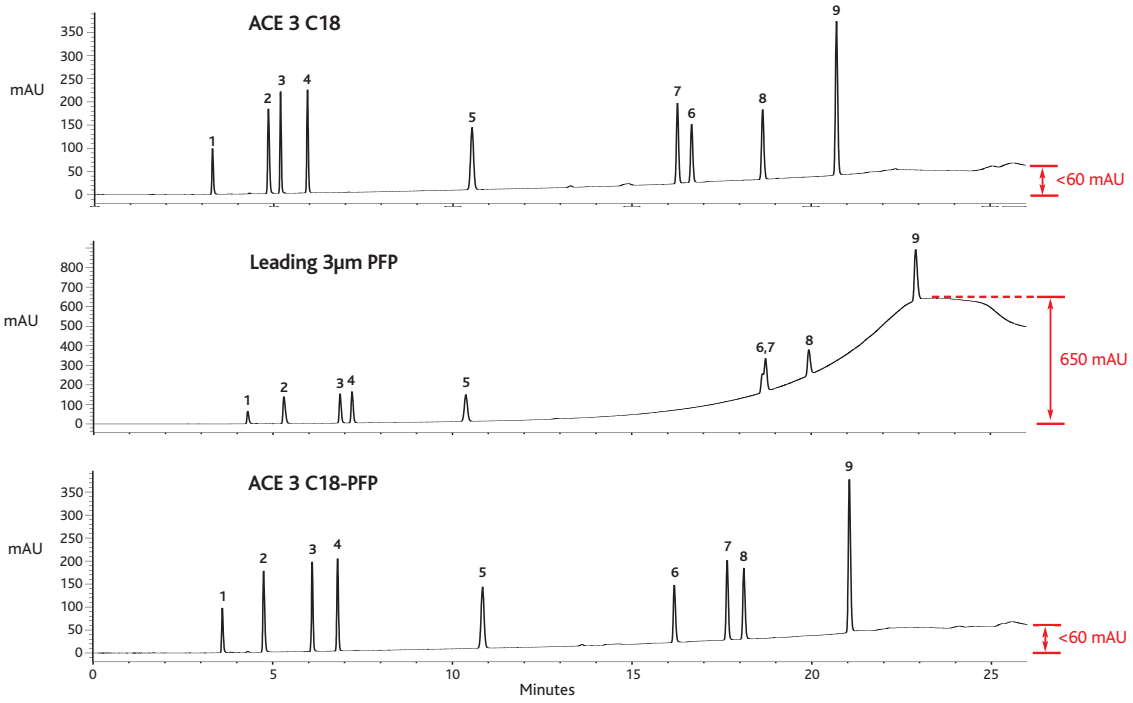
Column: ACE 5 C18-PFP, 150 x 4.6 mm (unless specified otherwise)
Sample: 1) uracil 2) 4-hydroxybenzoic acid 3) acetylsalicylic acid 4) benzoic acid 5) 2-hydroxybenzoic acid 6) ethyl paraben
Mobile Phase: 35:65 v/v MeCN/0.1% TFA in H_2O Temperature: 22°C Wavelength: 254 nm Flow Rate: 1.00 ml/min (unless specified otherwise)

The availability of 3µm, 5µm and 10µm particle sizes combined with a range of column dimensions from capillary through to preparative scale ensures that methods can be reproducibly scaled up or down. The chromatograms above demonstrate the excellent reproducibility achieved when silica batch and silane batch are changed, and the reproducible scalability obtained when changing particle size and column diameter.

Low Bleed for UV and LC/MS Compatibility

Many phases exhibit bleed of the bonded phase, which is most clearly seen under gradient conditions when baseline stability is affected. Whilst most ultra pure C18 phases can be expected to give low column bleed, careful selection of an alternate selectivity bonded phase is required to ensure that column bleed does not cause unforeseen problems when analysing at low UV wavelengths or by LC/MS. Note, absolute column bleed depends on a number of factors and may vary from day to day and system to system. Therefore it is important, when comparing column bleed, to do so under controlled conditions.

ACE C18-PFP Exhibits Low Column Bleed for UV

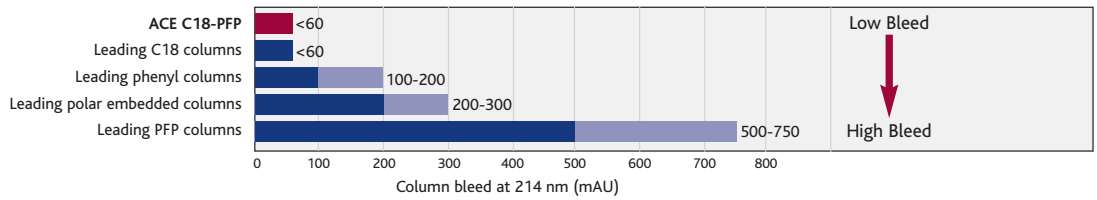


Compounds: 1) nicotine 2) benzylamine 3) procainamide 4) terbutaline 5) phenol 6) internal standard A 7) internal standard B 8) internal standard C 9) nortriptyline
Column Dimensions: 150 x 4.6 mm Flow Rate: 1.00 ml/min Temperature: 60°C Detection: UV, 214 nm
Mobile Phase: A = 20 mM KH_2PO_4 in H_2O (pH 2.7) B = 35:65 v/v 20 mM KH_2PO_4 in H_2O (pH 2.7) / MeOH Gradient: 5 to 95% B in 20 mins, hold for 5 mins at 95% B

This example compares the low bleed characteristics of an ultra pure ACE C18 column (top) with the high bleed typical of PFP columns (middle). The ACE C18-PFP column (bottom) shows bleed levels comparable to the ACE C18, despite containing an integral PFP functionality, which provides the alternate selectivity.

Comparison of UV Bleed

Evaluation of Leading C18 Brands and Popular Alternate Bonded Phases



Further analysis of a wider range of columns under the same conditions confirms that leading high purity C18 column brands show similarly low levels of column bleed. However, the evaluation of alternate bonded phases traditionally recommended to change selectivity (i.e. phenyl, polar embedded and PFP surface chemistries) reveal that all these non-C18 columns show significantly higher bleed.

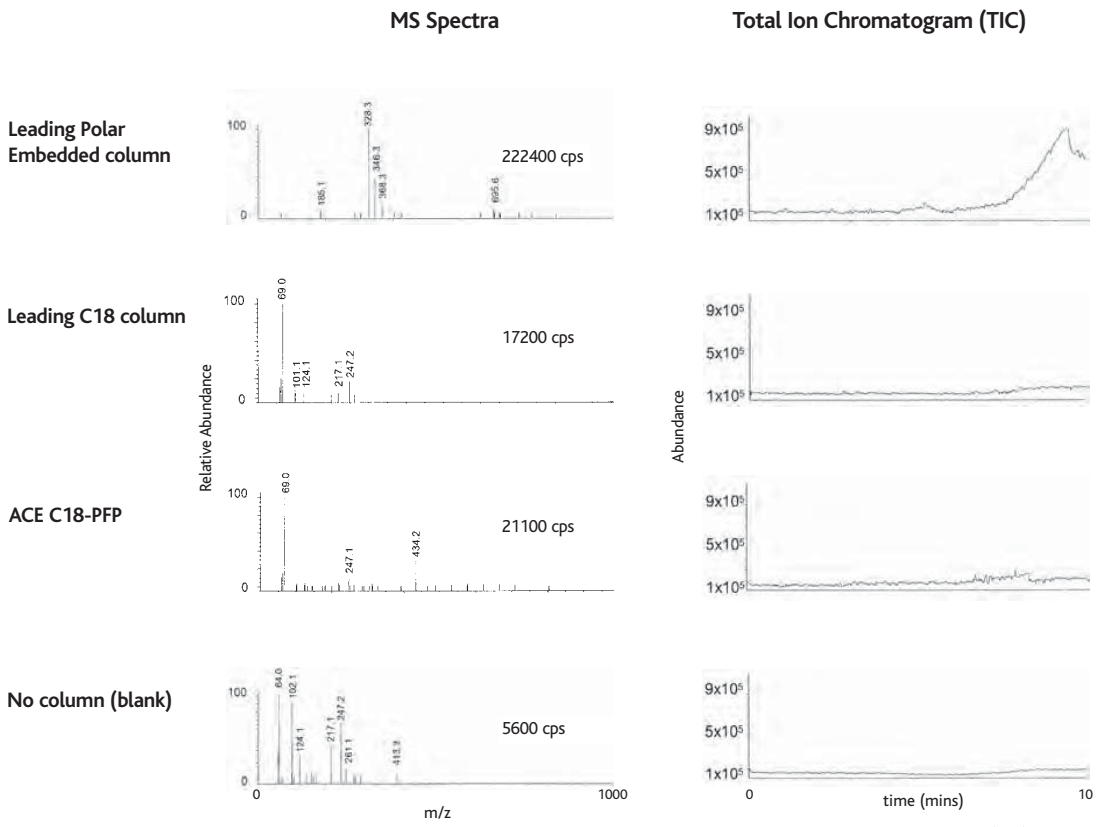
The ACE C18-PFP combines a low bleed level (typical of the leading C18 column brands) with an alternate selectivity, thus providing the analyst with a valuable method development tool.

Low Bleed for UV and LC/MS Compatibility (cont'd)

When detecting by MS, the use of non-C18 phases has traditionally presented additional challenges to the analyst. In extreme instances, column bleed may swamp the detector signal and mask the analyte of interest.

In the following example, column bleed is monitored in the 8-10 minute segment of the gradient run, at which point the % organic increases to its maximum level and column bleed is therefore also highest. The MS Spectra (left series) provides an m/z breakdown of the bleed detected during this 8-10 minute window, whereas the Total Ion Chromatogram (right series) illustrates the bleed obtained during this same 8-10 minute time period.

ACE C18-PFP Exhibits Low Column Bleed for LC/MS



Column Dimensions: 50 x 3.0 mm
Flow Rate: 0.43 ml/min
Temperature: 60°C
Detection: +ve ESI fast scan mode (Agilent 1100 MSD), full scan range 50 – 1000m/z

Mobile Phase: A = 0.1% v/v formic acid in H_2O
B = 0.1% v/v formic acid in MeCN

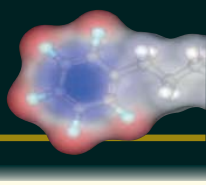
Gradient:

T(mins)	%B
0	5
0.1	5
5	95
10	95
10.5	5

The TIC trace and MS spectra for the polar embedded column (previously seen to show significant bleed by UV detection) again shows a high level of column bleed when analysing by LC/MS. The MS spectra from a blank run (performed with no column attached) enables the background system bleed to be quantified. Both the ACE C18-PFP column and leading C18 column exhibit bleed levels similar to the blank run, denoting that negligible column bleed is occurring.

Conclusion

The ACE C18-PFP phase may be used to provide a different selectivity to leading C18 columns without encountering the column bleed issues associated with many alternate (i.e. non-C18) bonded phases.



Material Characteristics

PHASE	FUNCTIONAL GROUP	ENDCAPPED	PARTICLE SIZE (µm)	PORE SIZE (Å)	SURFACE AREA (m^2/g)	CARBON LOAD (%)	MAXIMUM pH RANGE
C18-PFP	Proprietary octadecyl with embedded PFP functionality	Yes	3, 5, 10	100	300	14.3	1.5-10.0[a]
C18	Octadecyl	Yes	3, 5, 10	100	300	15.5	1.5-10.0[a]

[a] For optimum column lifetime, a pH range of 2-8 is recommended. To increase column lifetime at high pH, organic buffers, low buffer concentrations, high % organic solvent and low temperatures must be considered. Further information is contained within "A Guide to HPLC and LC-MS Buffer Selection" by John Dolan – contact your distributor to request your FREE copy.

ACE 3µm Columns (Contact your distributor for the full range of ACE 3µm phases available)

ACE 3µm C18-PFP

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	-	ACE-1110-0301	ACE-1110-3501	ACE-1110-0501	ACE-1110-7501	ACE-1110-1001	ACE-1110-1201	ACE-1110-1501	ACE-1110-2501[a]	ACE-1110-0101GD[1]
2.1mm	ACE-1110-0202[6]	ACE-1110-0302	ACE-1110-3502	ACE-1110-0502	ACE-1110-7502	ACE-1110-1002	ACE-1110-1202	ACE-1110-1502	ACE-1110-2502[a]	ACE-1110-0102GD[2]
3.0mm	ACE-1110-0203[6]	ACE-1110-0303	ACE-1110-3503	ACE-1110-0503	ACE-1110-7503	ACE-1110-1003	ACE-1110-1203	ACE-1110-1503	ACE-1110-2503[a]	ACE-1110-0103GD[3]
4.0mm	-	-	ACE-1110-3504	ACE-1110-0504	ACE-1110-7504	ACE-1110-1004	ACE-1110-1204	ACE-1110-1504	ACE-1110-2504[a]	ACE-1110-0103GD[3]
4.6mm	ACE-1110-0246[6]	ACE-1110-0346	ACE-1110-3546	ACE-1110-0546	ACE-1110-7546	ACE-1110-1046	ACE-1110-1246	ACE-1110-1546	ACE-1110-2546[a]	ACE-1110-0103GD[3]

ACE 3µm C18

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	-	ACE-111-0301	ACE-111-3501	ACE-111-0501	ACE-111-7501	ACE-111-1001	ACE-111-1201	ACE-111-1501	ACE-111-2501[a]	ACE-111-0101GD[1]
2.1mm	ACE-111-0202[6]	ACE-111-0302	ACE-111-3502	ACE-111-0502	ACE-111-7502	ACE-111-1002	ACE-111-1202	ACE-111-1502	ACE-111-2502[a]	ACE-111-0102GD[2]
3.0mm	ACE-111-0203[6]	ACE-111-0303	ACE-111-3503	ACE-111-0503	ACE-111-7503	ACE-111-1003	ACE-111-1203	ACE-111-1503	ACE-111-2503[a]	ACE-111-0103GD[3]
4.0mm	-	-	ACE-111-3504	ACE-111-0504	ACE-111-7504	ACE-111-1004	ACE-111-1204	ACE-111-1504	ACE-111-2504[a]	ACE-111-0103GD[3]
4.6mm	ACE-111-0246[6]	ACE-111-0346	ACE-111-3546	ACE-111-0546	ACE-111-7546	ACE-111-1046	ACE-111-1246	ACE-111-1546	ACE-111-2546[a]	ACE-111-0103GD[3]

[a] Consider operating pressure limitations for maximum column lifetime

ACE 5µm Columns (Contact your distributor for the full range of ACE 5µm phases available)

ACE 5µm C18-PFP

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	-	ACE-1210-0301	ACE-1210-3501	ACE-1210-0501	ACE-1210-7501	ACE-1210-1001	ACE-1210-1201	ACE-1210-1501	ACE-1210-2501	ACE-1210-0101GD[1]
2.1mm	ACE-1210-0202[6]	ACE-1210-0302	ACE-1210-3502	ACE-1210-0502	ACE-1210-7502	ACE-1210-1002	ACE-1210-1202	ACE-1210-1502	ACE-1210-2502	ACE-1210-0102GD[2]
3.0mm	ACE-1210-0203[6]	ACE-1210-0303	ACE-1210-3503	ACE-1210-0503	ACE-1210-7503	ACE-1210-1003	ACE-1210-1203	ACE-1210-1503	ACE-1210-2503	ACE-1210-0103GD[3]
4.0mm	-	-	ACE-1210-3504	ACE-1210-0504	ACE-1210-7504	ACE-1210-1004	ACE-1210-1204	ACE-1210-1504	ACE-1210-2504	ACE-1210-0103GD[3]
4.6mm	ACE-1210-0246[6]	ACE-1210-0346	ACE-1210-3546	ACE-1210-0546	ACE-1210-7546	ACE-1210-1046	ACE-1210-1246	ACE-1210-1546	ACE-1210-2546	ACE-1210-0103GD[3]
7.75mm	-	-	-	ACE-1210-0508	ACE-1210-7508	ACE-1210-1008	ACE-1210-1208	ACE-1210-1508	ACE-1210-2508	ACE-1210-0110GD[4]
10.0mm	-	-	-	ACE-1210-0510	ACE-1210-7510	ACE-1210-1010	ACE-1210-1210	ACE-1210-1510	ACE-1210-2510	ACE-1210-0110GD[4]
21.2mm	-	-	-	ACE-1210-0520	ACE-1210-7520	ACE-1210-1020	ACE-1210-1220	ACE-1210-1520	ACE-1210-2520	ACE-1210-0110GD[4]
30.0mm	-	-	-	ACE-1210-0530	ACE-1210-7530	ACE-1210-1030	-	ACE-1210-1530	ACE-1210-2530	ACE-1210-0220GD[5]

ACE 5µm C18

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
1.0mm	-	ACE-121-0301	ACE-121-3501	ACE-121-0501	ACE-121-7501	ACE-121-1001	ACE-121-1201	ACE-121-1501	ACE-121-2501	ACE-121-0101GD[1]
2.1mm	ACE-121-0202[6]	ACE-121-0302	ACE-121-3502	ACE-121-0502	ACE-121-7502	ACE-121-1002	ACE-121-1202	ACE-121-1502	ACE-121-2502	ACE-121-0102GD[2]
3.0mm	ACE-121-0203[6]	ACE-121-0303	ACE-121-3503	ACE-121-0503	ACE-121-7503	ACE-121-1003	ACE-121-1203	ACE-121-1503	ACE-121-2503	ACE-121-0103GD[3]
4.0mm	-	-	ACE-121-3504	ACE-121-0504	ACE-121-7504	ACE-121-1004	ACE-121-1204	ACE-121-1504	ACE-121-2504	ACE-121-0103GD[3]
4.6mm	ACE-121-0246[6]	ACE-121-0346	ACE-121-3546	ACE-121-0546	ACE-121-7546	ACE-121-1046	ACE-121-1246	ACE-121-1546	ACE-121-2546	ACE-121-0103GD[3]
7.75mm	-	-	-	ACE-121-0508	ACE-121-7508	ACE-121-1008	ACE-121-1208	ACE-121-1508	ACE-121-2508	ACE-121-0110GD[4]
10.0mm	-	-	-	ACE-121-0510	ACE-121-7510	ACE-121-1010	ACE-121-1210	ACE-121-1510	ACE-121-2510	ACE-121-0110GD[4]
21.2mm	-	-	-	ACE-121-0520	ACE-121-7520	ACE-121-1020	ACE-121-1220	ACE-121-1520	ACE-121-2520	ACE-121-0110GD[4]
30.0mm	-	-	-	ACE-121-0530	ACE-121-7530	ACE-121-1030	-	ACE-121-1530	ACE-121-2530	ACE-121-0220GD[5]

[1] 5 pack - use with cartridge holder H0001 and column coupler C0001
[2] 5 pack - use with integral microbore cartridge holder H0004 (not 20mm column length)
[3] 5 pack - use with integral analytical cartridge holder H0005 (not 20mm column length)
[4] 3 pack - use with semi-prep cartridge holder H0002 and column coupler C0001
[5] 1 pack - use with prep cartridge holder H0006 and column coupler C0002
[6] When using guards, cartridge holder H0001 and column coupler C0001 required

ACE 10µm Columns (Contact your distributor for the full range of ACE 10µm phases available)

ACE 10µm C18-PFP

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
4.6mm	ACE-1310-0246[6]	ACE-1310-0346	ACE-1310-3546	ACE-1310-0546	ACE-1310-7546	ACE-1310-1046	ACE-1310-1246	ACE-1310-1546	ACE-1310-2546	ACE-1310-0103GD[3]
7.75mm	-	-	-	ACE-1310-0508	ACE-1310-7508	ACE-1310-1008	ACE-1310-1208	ACE-1310-1508	ACE-1310-2508	ACE-1310-0110GD[4]
10.0mm	-	-	-	ACE-1310-0510	ACE-1310-7510	ACE-1310-1010	ACE-1310-1210	ACE-1310-1510	ACE-1310-2510	ACE-1310-0110GD[4]
21.2mm	-	-	-	ACE-1310-0520	ACE-1310-7520	ACE-1310-1020	ACE-1310-1220	ACE-1310-1520	ACE-1310-2520	ACE-1310-0110GD[4]
30.0mm	-	-	-	ACE-1310-0530	ACE-1310-7530	ACE-1310-1030	-	ACE-1310–1530	ACE-1310-2530	ACE-1310-0220GD[5]
50.0mm	-	-	-	enquire	enquire	enquire	enquire	enquire	enquire	enquire

ACE 10µm C18

COLUMN DIAMETER	COLUMN LENGTH									GUARD CARTRIDGE
	20 mm	30 mm	35 mm	50 mm	75 mm	100 mm	125 mm	150 mm	250 mm	
4.6mm	ACE-131-0246[6]	ACE-131-0346	ACE-131-3546	ACE-131-0546	ACE-131-7546	ACE-131-1046	ACE-131-1246	ACE-131-1546	ACE-131-2546	ACE-131-0103GD[3]
7.75mm	-	-	-	ACE-131-0508	ACE-131-7508	ACE-131-1008	ACE-131-1208	ACE-131-1508	ACE-131-2508	ACE-131-0110GD[4]
10.0mm	-	-	-	ACE-131-0510	ACE-131-7510	ACE-131-1010	ACE-131-1210	ACE-131-1510	ACE-131-2510	ACE-131-0110GD[4]
21.2mm	-	-	-	ACE-131-0520	ACE-131-7520	ACE-131-1020	ACE-131-1220	ACE-131-1520	ACE-131-2520	ACE-131-0110GD[4]
30.0mm	-	-	-	ACE-131-0530	ACE-131-7530	ACE-131-1030	-	ACE-131–1530	ACE-131-2530	ACE-131-0220GD[5]
50.0mm	-	-	-	enquire	enquire	enquire	enquire	enquire	enquire	enquire

[3] 5 pack - use with integral analytical cartridge holder H0005 (not 20mm column length)
[4] 3 pack - use with semi-prep cartridge holder H0002 and column coupler C0001
[5] 1 pack - use with prep cartridge holder H0006 and column coupler C0002
[6] When using guards, cartridge holder H0001 and column coupler C0001 required

Method Validation Kits

ACE phases are widely recognized to offer outstanding reproducibility. To aid method validation, a convenient kit containing three columns of the same bonded phase and dimensions, packed with three different batches of silica is available. Method Development Kits are available for all phases and column dimensions, with the most common kits shown below.

2.1mm METHOD VALIDATION KITS (three different batches of the same phase)	50 X 2.1mm	150 X 2.1mm	250 X 2.1mm
ACE 3 C18-PFP Method Validation Kit ACE 5 C18-PFP Method Validation Kit	ACE-1110-0502-MVK ACE-1210-0502-MVK	ACE-1110-1502-MVK ACE-1210-1502-MVK	ACE-1110-2502-MVK[a] ACE-1210-2502-MVK
ACE 3 C18 Method Validation Kit ACE 5 C18 Method Validation Kit	ACE-111-0502-MVK ACE-121-0502-MVK	ACE-111-1502-MVK ACE-121-1502-MVK	ACE-111-2502-MVK[a] ACE-121-2502-MVK

3.0mm METHOD VALIDATION KITS (three different batches of the same phase)	50 X 3.0mm	150 X 3.0mm	250 X 3.0mm
ACE 3 C18-PFP Method Validation Kit ACE 5 C18-PFP Method Validation Kit	ACE-1110-0503-MVK ACE-1210-0503-MVK	ACE-1110-1503-MVK ACE-1210-1503-MVK	ACE-1110-2503-MVK[a] ACE-1210-2503-MVK
ACE 3 C18 Method Validation Kit ACE 5 C18 Method Validation Kit	ACE-111-0503-MVK ACE-121-0503-MVK	ACE-111-1503-MVK ACE-121-1503-MVK	ACE-111-2503-MVK[a] ACE-121-2503-MVK

4.6mm METHOD VALIDATION KITS (three different batches of the same phase)	50 X 4.6mm	150 X 4.6mm	250 X 4.6mm
ACE 3 C18-PFP Method Validation Kit ACE 5 C18-PFP Method Validation Kit	ACE-1110-0546-MVK ACE-1210-0546-MVK	ACE-1110-1546-MVK ACE-1210-1546-MVK	ACE-1110-2546-MVK[a] ACE-1210-2546-MVK
ACE 3 C18 Method Validation Kit ACE 5 C18 Method Validation Kit	ACE-111-0546-MVK ACE-121-0546-MVK	ACE-111-1546-MVK ACE-121-1546-MVK	ACE-111-2546-MVK[a] ACE-121-2546-MVK

[a] Consider operating pressure limitations for maximum column lifetime

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