Expanding the Capabilities of a C18 Bonded Phase by the Addition of a Pentafluorophenyl (PFP) Group

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Abstract

A common goal of RPLC method development is to achieve baseline resolution, or better, of all analytes in a sample in the shortest time possible. A C18 bonded phase has been chosen, historically, because it was one of the first phases manufactured, and was the most stable. A large percentage of simple separation problems (for example, 2-6 analytes) can often be solved using a C18 bonded phase under isocratic conditions. For more complex samples with 8 or more analytes, however, gradient conditions are usually necessary. However, for some simple and most complex samples, achieving complete resolution of all analytes is frequently challenging. In those situations, the chromatographer has several options to explore to improve resolution.

From an understanding of the resolution equation, it is well known that changing selectivity (α) improves resolution much more effectively than increasing retention (k) or increasing column efficiency (N).

$$R_s = \left(\frac{1}{4}\right)\sqrt{N}\,(\alpha - 1)\left(\frac{k_2}{1 + k}\right)$$

Where

$$\bar{k} = \frac{(k_1 + k_2)}{2}$$

The experimental factors that have the greatest effect on selectivity for reversed-phase separations are: (1) column phase, (2) organic modifier choice, (3) organic modifier strength/gradient steepness, (4) pH (for ionizable compounds), and, to a lesser degree, (5) column temperature. Changing the type of bonded phase is as easy as changing these other factors. However, some chromatographers have been reluctant to use any phase other than a C18, because of their comfort and familiarity with those phases and those phases' advantages of stability, reproducibility, and hydrophobic retention. If the original C18 phase has not provided the necessary resolution, some chromatographers will then choose another C18 phase from a different column manufacturer. This latter approach often fails because modern C18 columns, based on high purity, low acidity silicas, are not sufficiently different in selectivity from each other.

In this poster we demonstrate the improved resolution that one can achieve using a C18 phase modified by the addition of a pentafluorophenyl (PFP) group. This column (commercially available as ACE® C18-PFP) not only possesses all of the retention and stability advantages of a classic C18 phase, but also provides dramatically different selectivity from the presence of several additional types of molecular interactions. These include hydrogen bonding, dipole-dipole, π–π, charge transfer, electrostatic, and steric (shape-selective) interactions. For non-polar compounds, the C18-PFP performs very similar to a C18 phase; for polar compounds, this PFP-modified C18 provides increased retention, 100% aqueous compatibility, and significant changes in selectivity.

Introduction

In spite of the fact that there are numerous bonded phases available for reversed phase chromatography (C30, C8, C4, CN, PHE, polar embedded to name a few) C18 is by far the most popular, accounting for 50 – 60% of all reversed phase separations. Much of this popularity is historic, as C18 was the first reliable bonded phase. But C18 is also a very versatile bonded phase exhibiting the following benefits:

- Excellent stability. C18 columns have good stability over the pH range 2 – 9.
- Inertness. Due to the high coverage of the silica surface, C18 phases give the best peak shapes for basic compounds.
- Retention. C18 columns have excellent retention characteristics for hydrophobic compounds.

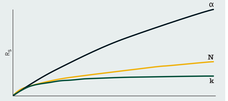
Due to its high hydrobicity however, C18 has limitations which can impact the analysis of polar compounds such as those analyzed in the pharmaceutical industry. Some problems encountered with C18 are:

- Poor retention of very polar compounds.
- Incompatibility with high or 100% aqueous mobile phases.
- C18 lacks a selectivity "handle" and often fails to resolve similar polar compounds.

As this poster will demonstrate, modification of the classic C18 silane with a PFP (pentafluorophenyl) group results in a reversed phase column which maintains all of the desirable properties of C18, while correcting its shortcomings such as inadequate polar retention and high aqueous compatibility. ACE C18-PFP maintains the stability, inertness and hydrophobic retention of C18, while providing numerous additional retention mechanisms for improved selectivity of polar compounds.

Selectivity vs. Efficiency

FIGURE 1: The effect of N, α and k on resolution (R_s)

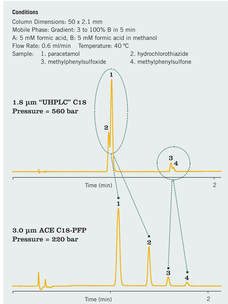


Increasing N, α or k increases Resolution (R_s). However, as can be seen from these plots, increasing either N or k suffers from quickly diminishing returns. Increasing selectivity (α), on the other hand, does not have this problem and, therefore, becomes the most powerful of these three variables to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

Figure 1 illustrates how resolution changes with changes in k, N and α. Note how a small increase in α results in a significant increase in resolution, R_s. Chromatographic selectivity is controlled by mobile phase, temperature and bonded phase chemistry.

Two ways of improving resolution are illustrated in Figure 2. Although the current trend is toward columns of smaller particle size and achieving resolution and speed through increased efficiency, this application clearly shows the benefits of increased selectivity over increased efficiency. Not only does ACE C18-AR provide the needed resolution, it does so in the same time at significantly lower back pressure.

FIGURE 2: Leveraging selectivity to achieve fast, high resolution separations

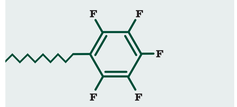


The ACE 3 µm C18-PFP column provides better selectivity for peak pairs 1, 2 and 3, 4 and, therefore, is able to provide a superior separation compared to the C18 sub-2 µm column, even though the sub-2 µm column has 50% more theoretical plates. Note: In addition to the better separation of all 4 peaks, the separation time on the ACE C18-PFP column is similarly as fast as the sub-2 µm column, but the back pressure is 60% lower.

Augmenting C18 Selectivity

With the addition of the PFP group, ACE C18-PFP offers additional retention mechanisms to a conventional C18 column.

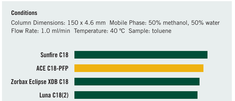
FIGURE 3: Schematic of C18-PFP Phase



Hydrophobic Binding Interactions

ACE C18-PFP offers strong hydrophobic retention for compounds with alkyl chains. Figure 4 shows the relative hydrophobicity of some popular C18 phases and a conventional PFP phase. ACE C18-PFP will retain non-polar compounds similar to a conventional C18 phase.

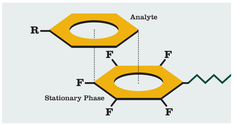
FIGURE 4: Comparison of relative hydrophobicity of different bonded phases



π – π Interactions

Bonded phases containing phenyl rings can interact with analytes containing aromatic and conjugated moieties through overlap of aromatic π-electron clouds, and p-orbitals in p-conjugated systems. These interactions are quite complex, but there is evidence that the presence of electron withdrawing groups such as fluorine in the case of ACE C18-PFP, actually enhance these interactions.

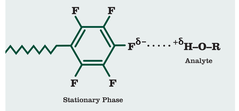
FIGURE 5: Schematic of π-π interactions



Hydrogen Bonding

Hydrogen bonding is a type of dipole-dipole interaction that occurs when an analyte containing a hydrogen bonded to a heteroatom such as oxygen, nitrogen or sulfur, interacts with the electronegative fluorine on the PFP group. This is illustrated in Figure 6.

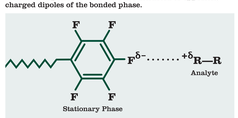
FIGURE 6: Hydrogen bonding occurs between a hydrogen atom of an analyte and the electronegative fluorine atoms of the pentafluoro group.



Dipole – Dipole Interactions

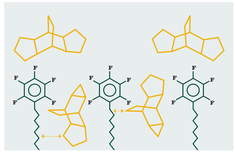
Polar molecules have permanent electric dipole moments due to the presence of partial charges on their atoms. Dipole-dipole interaction occurs when a partial charge (dipole) on the analyte is attracted to an opposite charge on the stationary phase. Although weaker than hydrogen bonding interactions, dipole-dipole interactions with ACE C18-PFP can significantly improve selectivity over a standard C18 phase.

FIGURE 7: Dipole-dipole interaction occurs when electric moments (dipoles) of molecules are attracted to oppositely charged dipoles of the bonded phase.



Shape Selectivity

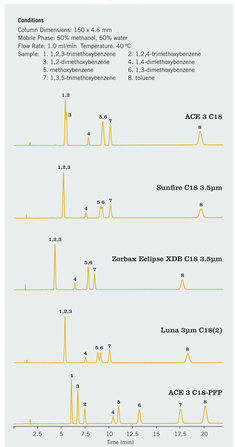
FIGURE 8: Fluorine adds significant structural rigidity to the bonded phase, thereby hindering interaction between the bonded phase and some molecules and permitting better interaction with other molecules, depending on their shape in solution. This selective interaction based on shape provides a useful mechanism for separations.



Augmenting C18 Selectivity

As Figure 9 illustrates, many popular C18 columns show similar selectivity in the separation of these substituted methoxybenzenes isomers. Due to the additional bonded phase – analyte interactions available with ACE C18-PFP however, selectivity is sufficient to resolve all isomers. Hydrophobic retention, measured by toluene, is similar for all columns.

FIGURE 9: Achieving a better separation by applying additional mechanisms of separation

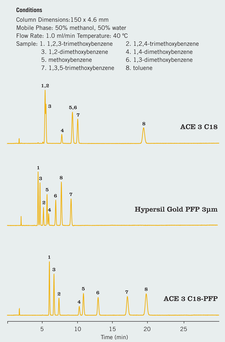


C18 columns from several manufacturers are unable to provide a satisfactory separation for all analytes in this mixture of substituted methoxybenzene isomers. By utilizing the additional separation mechanisms provided by the PFP group a complete separation is achieved.

Comparison to Typical PFP Phases

Although other PFP bonded phases are commercially available, ACE C18-PFP is unique in that it maintains hydrophobic retention similar to a conventional C18, yet adds additional selectivity due to the presence of the PFP group. This is illustrated in Figure 10.

FIGURE 10: ACE C18-PFP columns offer greater retention and dramatic differences in selectivity compared to typical PFP columns.

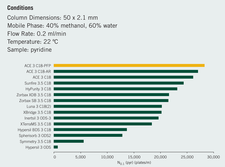


Because of the greater hydrophobicity of the C18-PFP bonded phase, greater retention and differences in selectivity can be expected from ACE C18-PFP columns when compared to typical PFP columns. The selectivities for peaks 2, 3 and 5, 6 are characteristic of the additional mechanisms of separation offered by PFP and not present in a typical C18 phase. But the ACE C18-PFP also has the retention characteristics of a C18 phase (retention of toluene, peak 8) and selectivity for some peak pairs (4, 5 and 7, 8) that is more like a C18 column than a typical PFP column.

Measuring Column Inertness

Active silanol groups (Si – OH) on the silica surface can cause undesirable secondary interactions with an analyte evidenced by decreased efficiency and peak tailing. ACE C18-PFP, manufactured with ultra-pure silica, minimizes these interactions. Figure 11 illustrates relative peak tailing for ACE C18-PFP versus other high performance bonded phases.

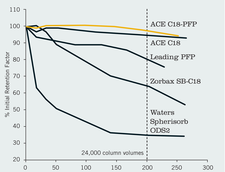
FIGURE 11: Comparison of peak tailing



Column plate count is reported for a basic compound (pyridine) on several popular C18 columns. Plate count was measured at 10% peak height so that peak tailing is included in the measurement. The ACE C18-PFP measured the highest plate count due to its ultra-inert nature.

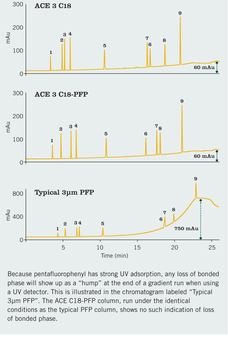
Column Stability

FIGURE 12: Accelerated column aging study proves ACE C18-PFP stability at low pH



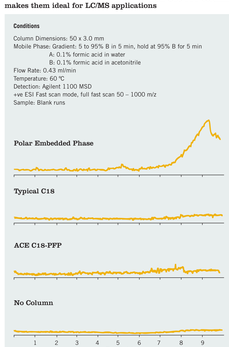
An accelerated column aging study was done to compare the stability of several reversed phase columns. Changes in retention under conditions designed to accelerate the loss of bonded phase (low pH, high temperature) were recorded versus operating time. The ACE C18 phases, including the ACE C18-PFP showed superior stability in this study, likely due to the high purity of the silica stationary phase support and the greater density of the bonded phase.

FIGURE 13: ACE C18-PFP exhibits low column bleed expected from a C18 bonded phase



Because pentafluorophenyl has strong UV adsorption, any loss of bonded phase will show up as a "hump" at the end of a gradient run when using a UV detector. This is illustrated in the chromatogram labeled "Typical 3µm PFP". The ACE C18-PFP column, run under the identical conditions as the typical PFP column, shows no such indication of loss of bonded phase.

FIGURE 14: The low column bleed for ACE C18-PFP columns makes them ideal for LC/MS applications

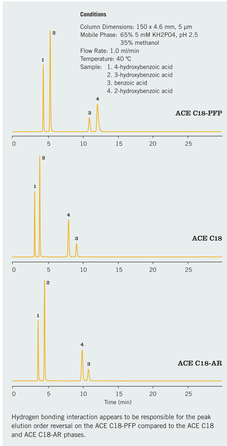


Column bleed can interfere with detection and measurement of analytes of interest in LC/MS applications. The first (top) total ion chromatogram shows how column bleed, in this case from a column packed with a polar embedded phase, can interfere with mass spec detection. The C18 column and the ACE C18-PFP column show no column bleed. The low bleed characteristics of ACE C18-PFP columns make them well suited for LC/MS applications.

Applications

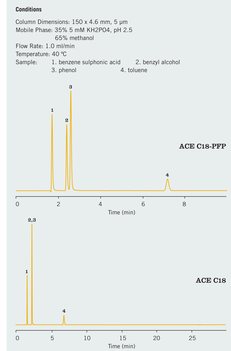
A strong argument can be made that the ACE C18-PFP should be the first column to try when starting any method development project. ACE C18-PFP offers hydrophobic retention similar to a conventional C18 column. As the following applications illustrate, the additional separation mechanisms available due to the PFP group offer improved resolution over a conventional C18.

FIGURE 15: Hydroxybenzoic acid isomers



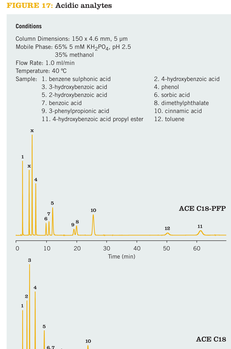
Hydrogen bonding interaction appears to be responsible for the peak elution order reversal on the ACE C18-PFP compared to the ACE C18 and ACE C18-AR phases.

FIGURE 16: Phenolic selectivity



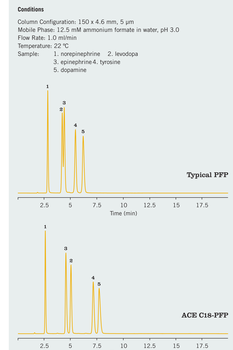
Hydrogen bonding interaction appears to be stronger for phenol than benzyl alcohol leading to greater retention for phenol on the ACE C18-PFP phase.

FIGURE 17: Acidic analytes



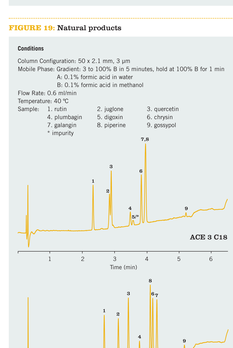
The additional mechanisms of separation provided by the ACE C18-PFP phase provide dramatically different selectivity compared to a typical C18 phase, including several reversals in peak elution order.

FIGURE 18: Catecholamines



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.

FIGURE 19: Natural products



ACE C18-PFP exhibits the same retention characteristics of a typical C18 phase, but the additional selectivity of the PFP group yield an improved separation for these plant extracts.

Conclusion

Resolution can be improved by increasing efficiency, N; retention, k; or selectivity, α. From the resolution equation shown earlier, the most effective way to improve resolution is through increased selectivity. ACE C18-PFP, a reversed phase column made by incorporating a pentafluorophenyl group onto a C18 bonded phase has proved to be a very versatile bonded phase as it augments the hydrophobic selectivity of a C18 with additional polar interactions made available by a PFP group. The column shows stability and inertness similar to other high performance C18 phases.