Exploring the selectivity of C18 phases with Phenyl and PFP functionality

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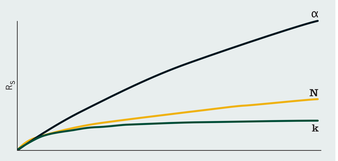
Introduction

HPLC columns packed with C18 bonded phases dominate all other types of bonded phases used for reversed-phase separations, and with good reason. C18 phases offer good retention and selectivity for a wide variety of sample types and have proved to be very rugged and reliable. However, the popularity of C18 bonded phases has obscured the advantages that other bonded phases, such as CN, phenyl, fluorinated, or polar embedded phases can offer. This is unfortunate because there are numerous cases where choosing a phase other than a typical C18 can lead to a more optimum separation—better resolution, faster analysis, higher sensitivity, etc.

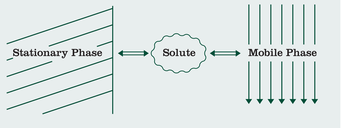
A solution to this dilemma is to add selectivity to a C18 bonded phase. This approach has been successfully applied in the development and production of C18 phases with Phenyl and pentafluorophenyl groups attached to the alkyl chain. This paper will discuss the mechanisms of separation provided by these enhanced selectivity phases and suggest how they may be used in developing reversed-phase separations.

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. Increases α (selectivity), on the other hand, does not have this problem and, therefore, becomes the most powerful of these three variable to optimize when developing a separation.

FIGURE 2A: Hydrophobic Binding Interaction



The dominant separation mechanism in reversed phase chromatography is hydrophobic binding interaction between solute molecules in the mobile phase and the stationary phase, i.e. the bonded phase, such as C18, C8, etc. Although the actual retention mechanism is not well understood, it has been found useful to describe the process as solute partitioning from the mobile phase into the bonded phase.

FIGURE 2B: π-π Interaction

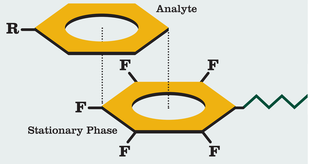


FIGURE 2C: Hydrogen Bonding Interaction

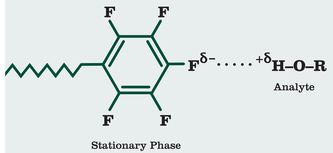


FIGURE 2D: Dipole-dipole Interaction

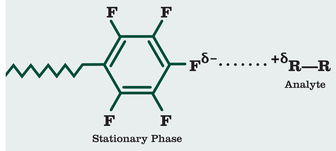


FIGURE 2E: Shape Selectivity

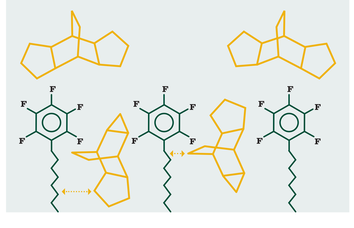
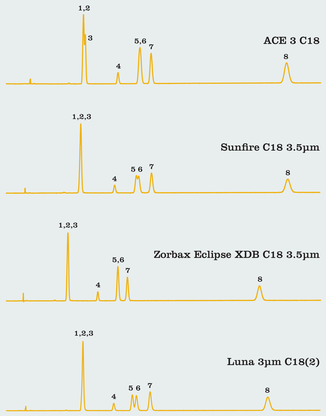


FIGURE 3: Comparison of Separation Mechanisms Offered by C18, Phenyl, and PFP Phases

Separation Mechanism	C18	Phenyl	PFP
Hydrophobicity	++++	++	++
π-π	–	++++	+++
Dipole-Dipole	–	++	++++
Hydrogen Bonding	–	+++	++++
Shape Selectivity	+	++	+++

FIGURE 4: Most "Modern" C18 Phases Provide Similar Selectivity

Conditions
Column Dimensions: 150 x 4.6 mm
Mobile Phase: 50% methanol, 50% water
Flow Rate: 1.0 ml/min Temperature: 40 °C
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. toluene

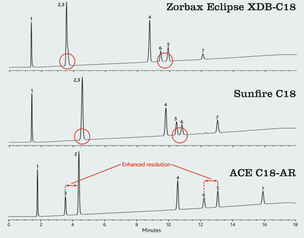


In the absence of any additional mechanisms of separation other than hydrophobic binding interaction, most modern C18 phases provide similar selectivity. Although some subtle differences in selectivity are observed in these comparison chromatograms (peaks 2,3 and 5,6), the difference is fairly insignificant. The elution order is the same for all 8 peaks on each of these C18 phases and none of the phases showed suitable selectivity to separate all 8 peaks.

FIGURE 5: Leveraging π−π Interactions to Achieve a Better Separation

Conditions:
Column Dimensions: 150 x 4.6 mm
Mobile Phase: A=20mM KH_2PO_4, pH 2.8
B= 50% Methanol, 50% 20mM KH_2PO_4, pH 2.8,
Gradient=20%B to 70%B, 15 minutes, Linear
Temperature: 40 °C
Detection: UV, 200 nm

Peak Identities:
1. pyridoxine
2. p-aminobenzoic acid
3. pantothenic acid
4. folic acid
5. cyanocobalamin
6. D-biotin
7. riboflavin

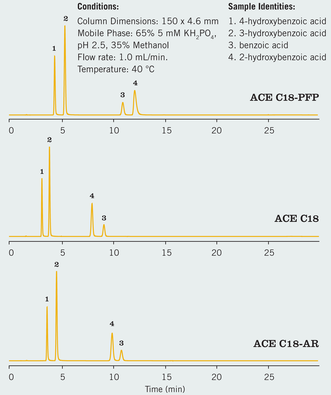


The two typical C18 phases are not able to provide a satisfactory separation of all 7 components in this mixture of water soluble vitamins. However, the phase that has additional π-π interactions from an attached phenyl group (ACE C18-AR), provides yields baseline resolution for all 7 components.

FIGURE 6: The Effect of Hydrogen Bonding Interactions

Conditions:
Column Dimensions: 150 x 4.6 mm
Mobile Phase: 65% 5 mM KH_2PO_4, pH 2.5, 35% Methanol
Flow rate: 1.0 mL/min.
Temperature: 40 °C

Sample Identities:
1. 4-hydroxybenzoic acid
2. 3-hydroxybenzoic acid
3. benzoic acid
4. 2-hydroxybenzoic acid

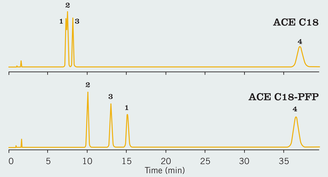


When pentafluorophenyl (PFP) groups are added to the stationary phase, an additional mechanism of separation, hydrogen bonding interaction, often plays a role in separations. In this separation of hydroxybenzoic acid isomers, hydrogen bonding interactions appear to be responsible for the peak elution order reversal on the ACE C18-PFP compared to the phases that lack hydrogen bonding interactions, i.e. the ACE C18 and ACE C18-AR.

FIGURE 7: The Effect of Dipole-Dipole Interactions

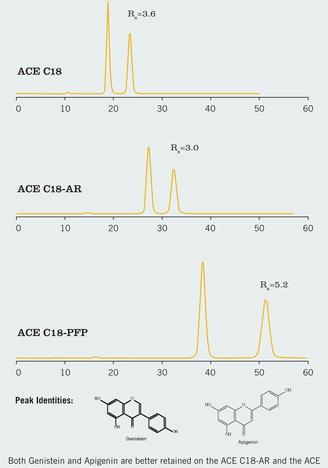
Conditions:
Column Dimensions: 150 x 4.6 mm
Mobile Phase: 60% Methanol, 40% Water
Flow Rate: 1.0 mL/min
Temperature: 40 °C
Detection: UV, 214 nm

Sample Identities:
1. 1,2-dinitrobenzene
2. 1,4-dinitrobenzene
3. 1,3-dinitrobenzene
4. Toluene



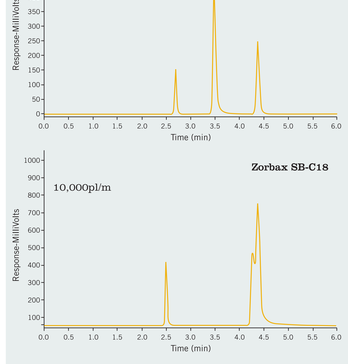
Dipole-dipole interactions are at least partially responsible for the greater retention of 1,2-dinitrobenzene on the ACE C18-PFP phase compared to ACE C18. There is evidence that π-π interactions also contribute to the greater retention of these substituted nitrobenzene isomers, but the dramatic change in elution order for 1,2-dinitrobenzene, the compound with the greatest dipole moment in this sample mixture, shows how dipole-dipole interactions can have a significant effect on selectivity.

FIGURE 8: The Effect of Shape Selectivity



Both Genistein and Apigenin are better retained on the ACE C18-AR and the ACE C18-PFP than the ACE C18 because of π-π interactions. However, significantly better selectivity, and thus Resolution, is achieved on the ACE C18-PFP due to shape selectivity. The greater rigidity of the C18-PFP phase creates conditions where Apigenin interacts more strongly with the stationary phase than Genistein due the difference in their shape in solution.

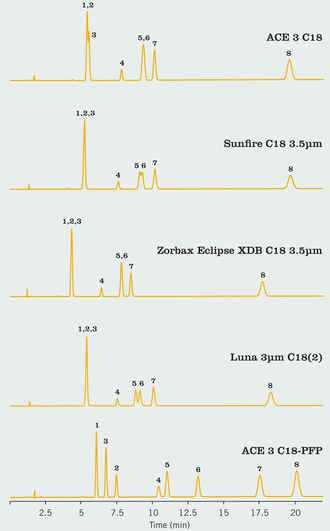
FIGURE 9: The Effect of Silanol Interactions



Pyridine is more strongly retained on the Zorbax SB-C18 compared to the ACE C18 due to the greater silanol activity on the Zorbax SB-C18 phase. In this example, the greater retention of pyridine yields poorer resolution. Silanol interactions are generally considered to be undesirable because they cause peak broadening and, unlike other retention mechanisms, are usually difficult to control, thereby leading to poor reproducibility.

FIGURE 10: Taking Advantage of Selectivity Differences in Reversed Phase HPLC

Conditions
Column Dimensions: 150 x 4.6 mm
Mobile Phase: 50% methanol, 50% water
Flow Rate: 1.0 ml/min Temperature: 40 °C
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. toluene



Popular base deactivated C18 columns are unable to provide adequate separation for all peaks in this sample of substituted methoxybenzene isomers. The additional selectivity provided by the pentafluorophenyl group in the C18-PFP phase is able to accomplish baseline separation of all 8 peaks.

Conclusion

The C18 phase remains the most popular phase for reversed phase HPLC separations because of its applicability to a wide range of sample types and because of its ruggedness and reliability. However, the lone mechanism of separation provided by a C18 phase, hydrophobic binding interactions, is sometimes not enough to achieve an acceptable separation. This poster has shown how other mechanisms of separation, i.e., π-π, hydrogen bonding, dipole-dipole, and shape selectivity, can be useful in separating peaks not well separated by hydrophobic binding interactions alone. A new generation of stationary phases that combine the benefits of C18 with the additional mechanisms of separation offered by phenyl and pentafluorophenyl can be powerful tools to use when developing new HPLC methods or improving existing methods.