Examining the Selectivities of Several C18 and Modified C18 Phases: Advantages of Phenyl and Pentafluorophenyl (PFP) Phases

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Abstract

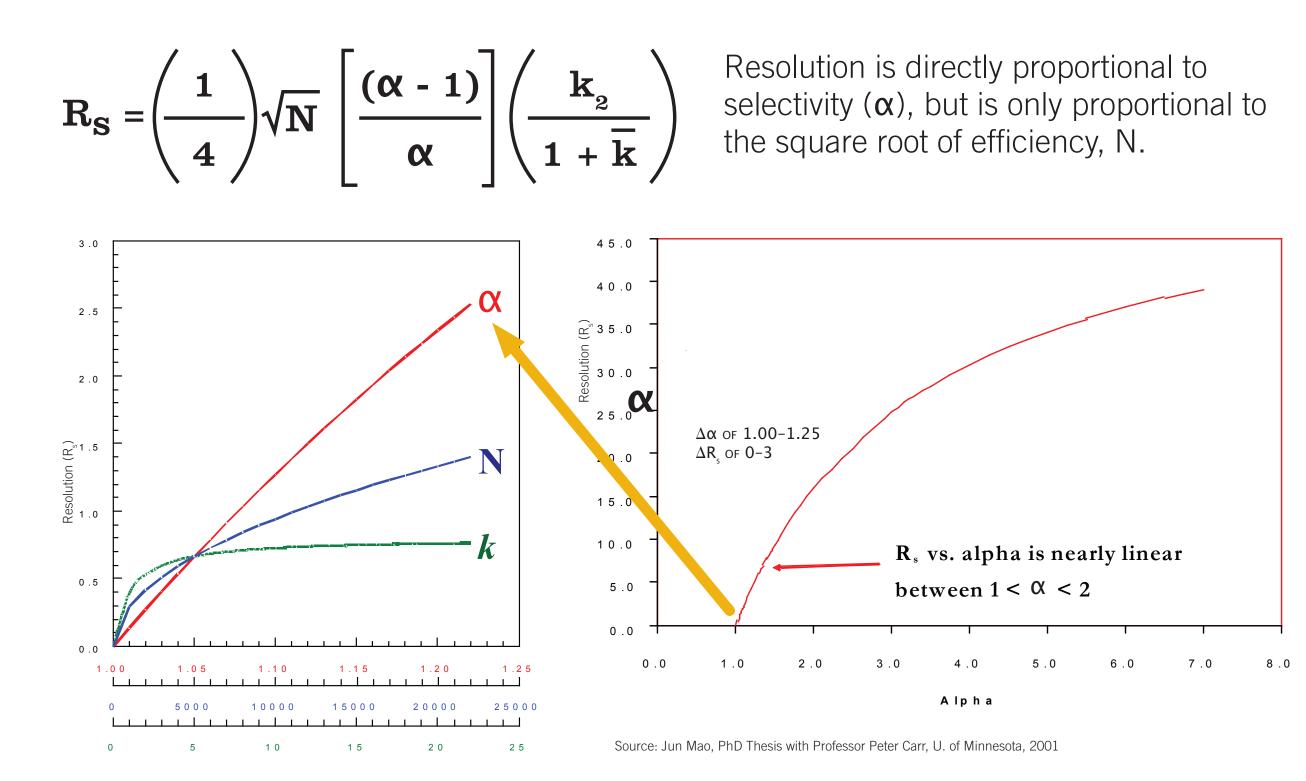
Selectivity is, by far, the most powerful parameter for improving resolution in chromatographic separations. Column stationary phase in reversedphase liquid chromatography is one of the most effective and easiest-tochange parameters for altering selectivity and achieving resolution.

A variety of different stationary phases have been developed during the course of the history of HPLC, including alkyl chains of various lengths, phenyl and cyano phases, embedded polar phases having amide and carbamate groups, and many more.

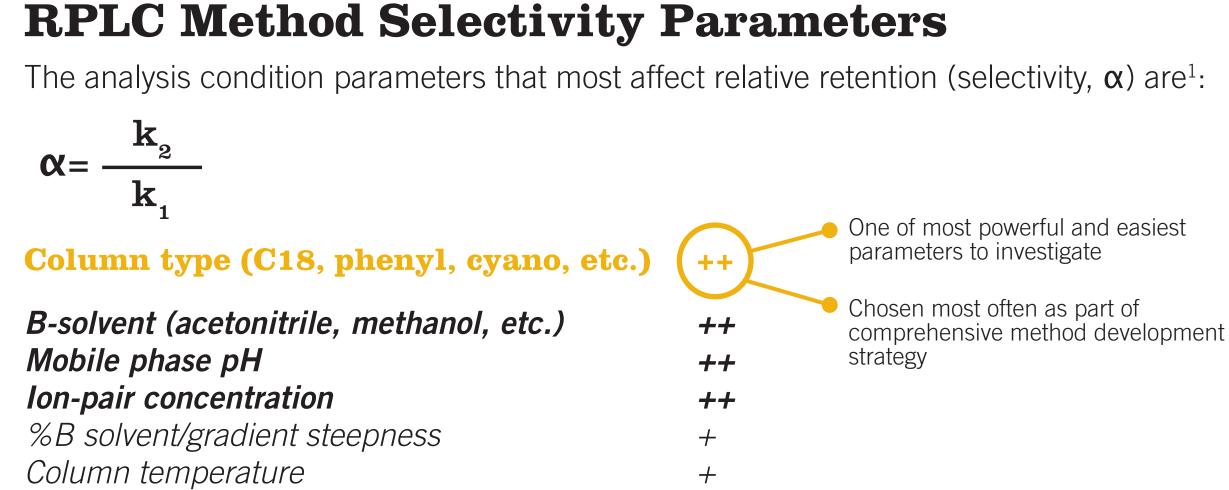
Recently, two new phases have been developed using phenylalkyl and pentafluorophenyl alkyl groups attached to ultrapure, low metal content silica. These phases not only afford selectivity differences versus each other and versus their predecessor C18 phase, but they've also been shown to provide selectivities different from many (most) other commercial

In this poster we will demonstrate the selectivity differences among the C18, C18-AR and C18-PFP phases, and will show their usefulness in developing new methods—especially for difficult-to-separate analytes. We will also attempt to show examples of the different separation mechanisms, which the various phases can exploit.

Resolution Equation Shows that Selectivity, α, is the Most Effective Parameter to Change R_s



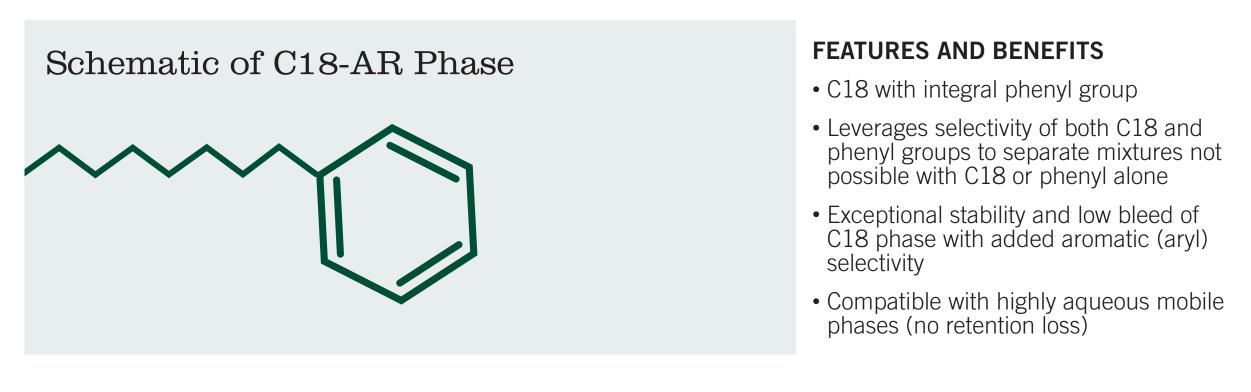
The analysis condition parameters that most affect relative retention (selectivity, α) are 1:

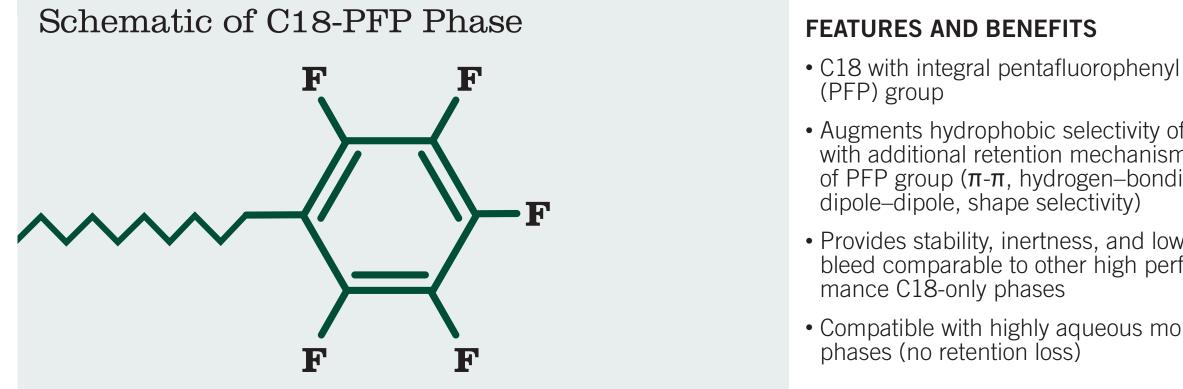


1 Adapted from "Introduction to Modern Liquid Chromatography", 3rd Edition, L. R. Snyder, J. J. Kirkland, J. W. Dolan; p. 29, 2010, John Wiley & Sons, Inc.

Buffer concentration

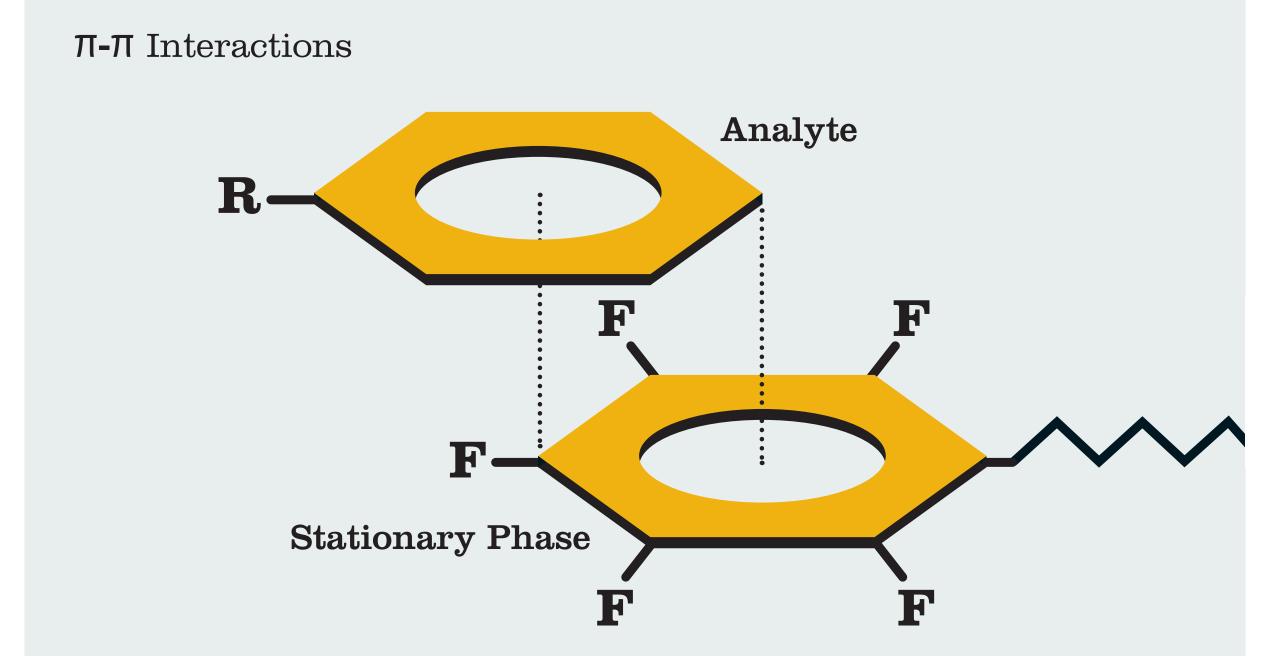
ACE C18-AR and ACE C18-PFP Phases

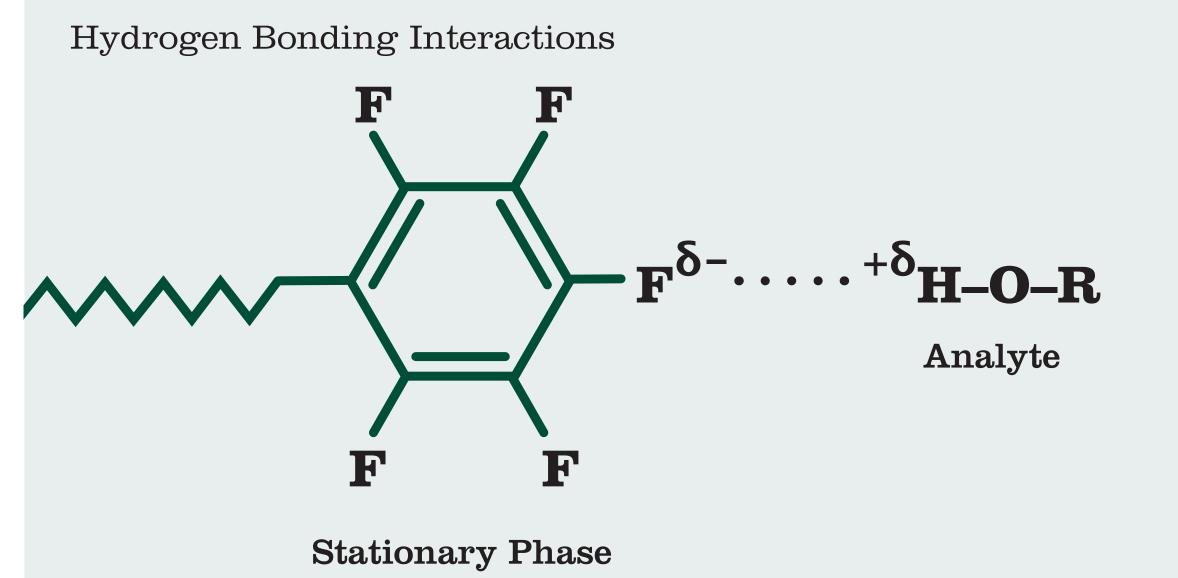


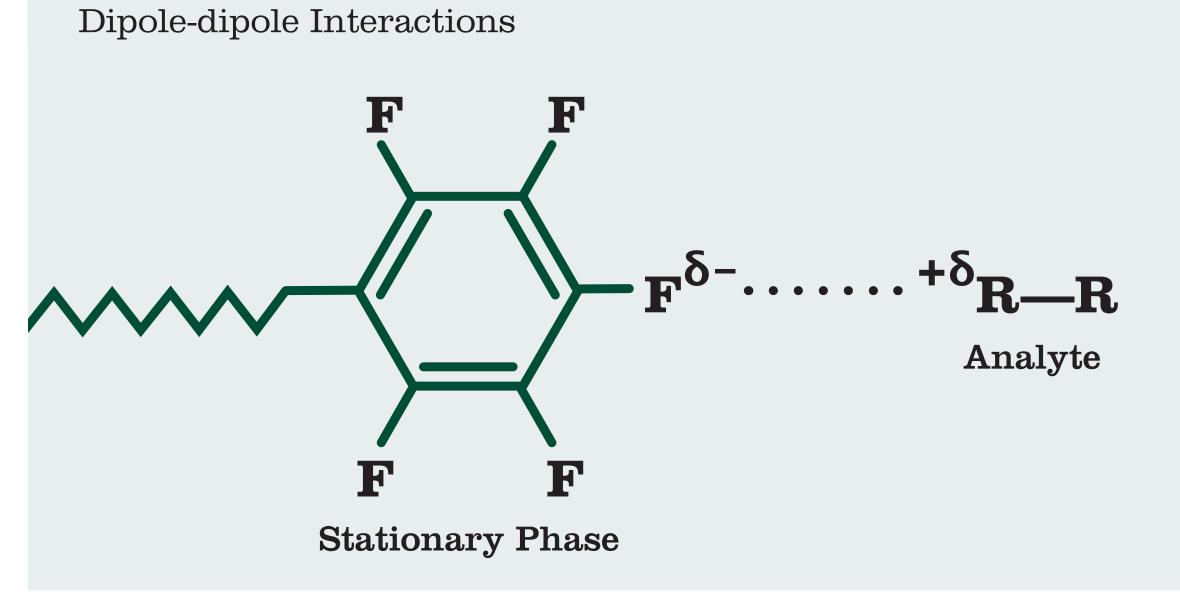


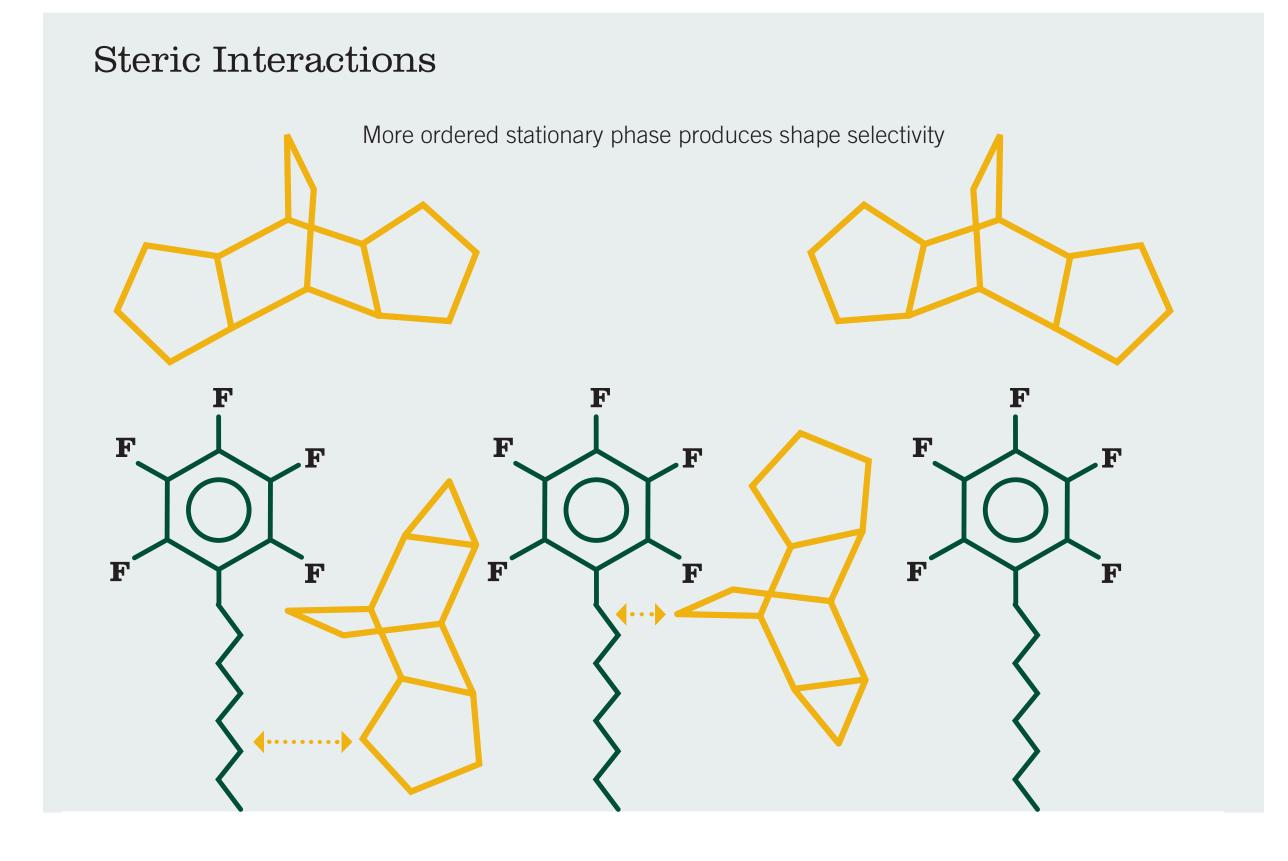
Augments hydrophobic selectivity of C18 with additional retention mechanisms of PFP group (π - π , hydrogen—bonding, dipole—dipole, shape selectivity) Provides stability, inertness, and low bleed comparable to other high performance C18-only phases Compatible with highly aqueous mobile phases (no retention loss)

Additional Selectivity for C18-AR and C18-PFP from Other **Analyte-Stationary Phase Interactions**







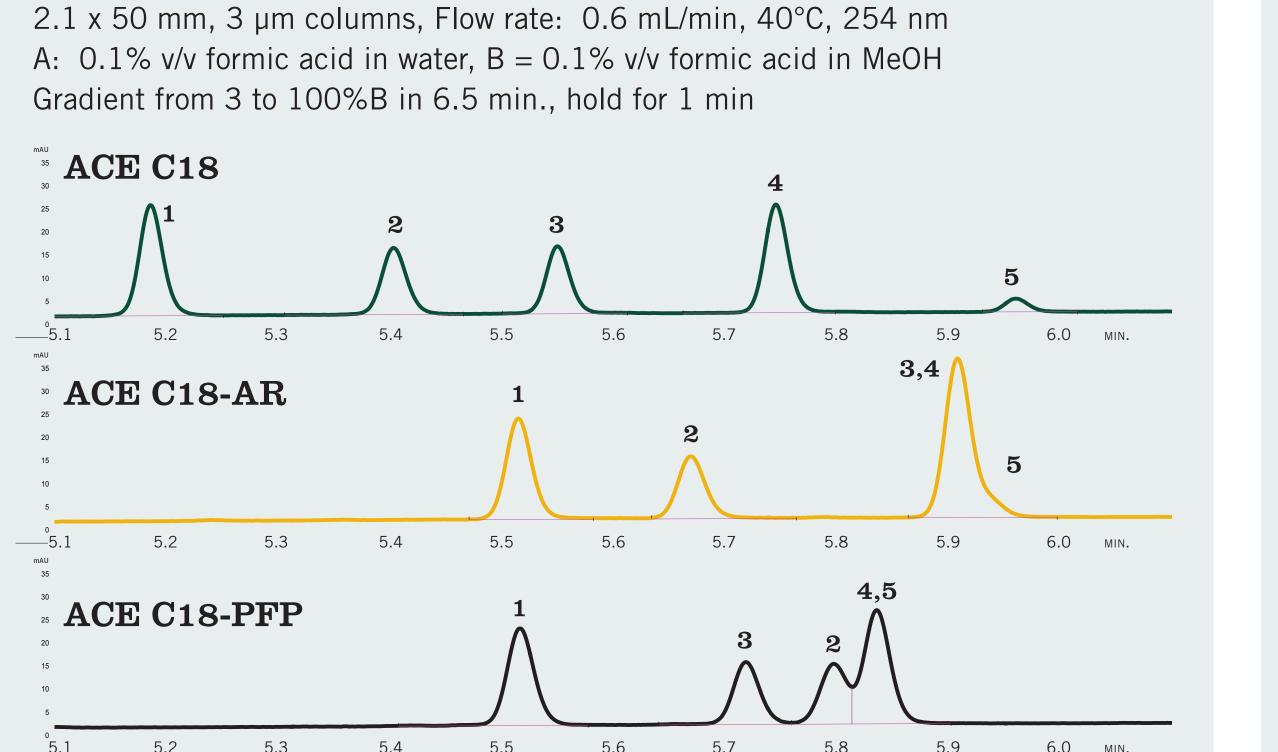


Types and Strengths of Analyte Interactions

Strengths of Interactions	ACE C18	ACE C18-AR	ACE C18-PFP
Hydrophobic	++++	++++	++++
π-π	_	++++ (electron-poor analytes)	++++ (electron-rich analytes)
Dipole–Dipole	_	++	++++
Hydrogen Bonding	_	++	+++
Shape Selectivity	+	++	+++

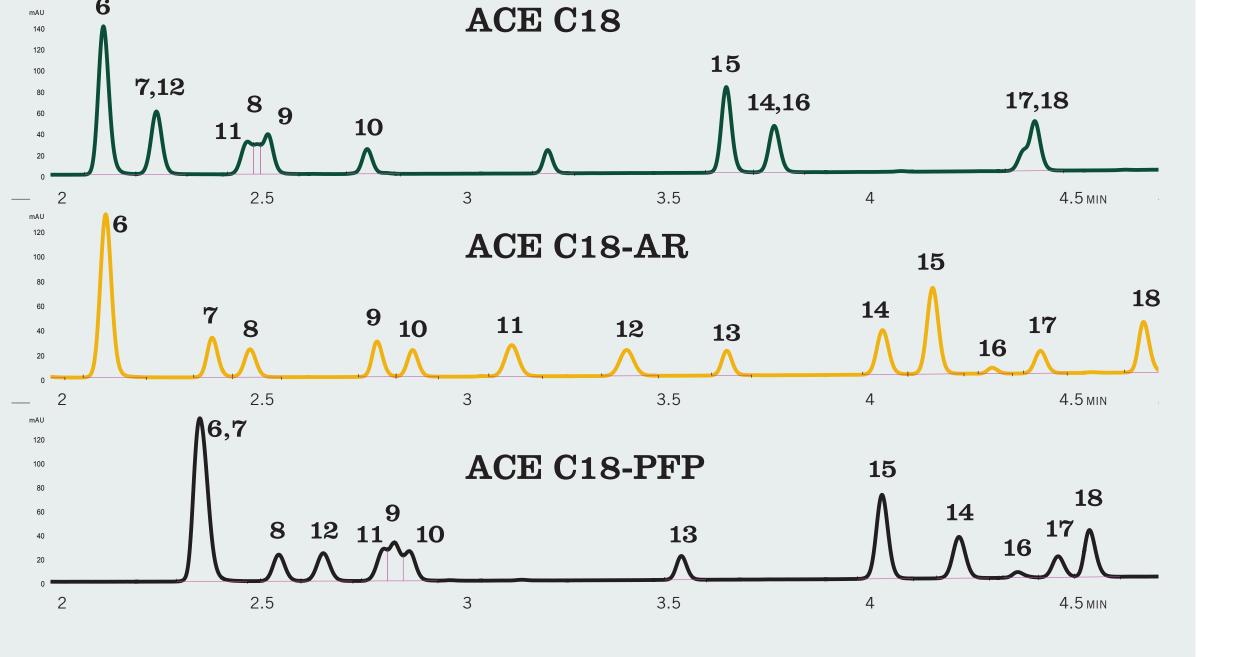
* C18-AR tends to have stronger retention for electron-poor analytes ** C18-PFP tends to have stronger retention for electron-rich analytes

Complementary Selectivities Mean No Analyte Elutes at Same Time on All 3 Phases!



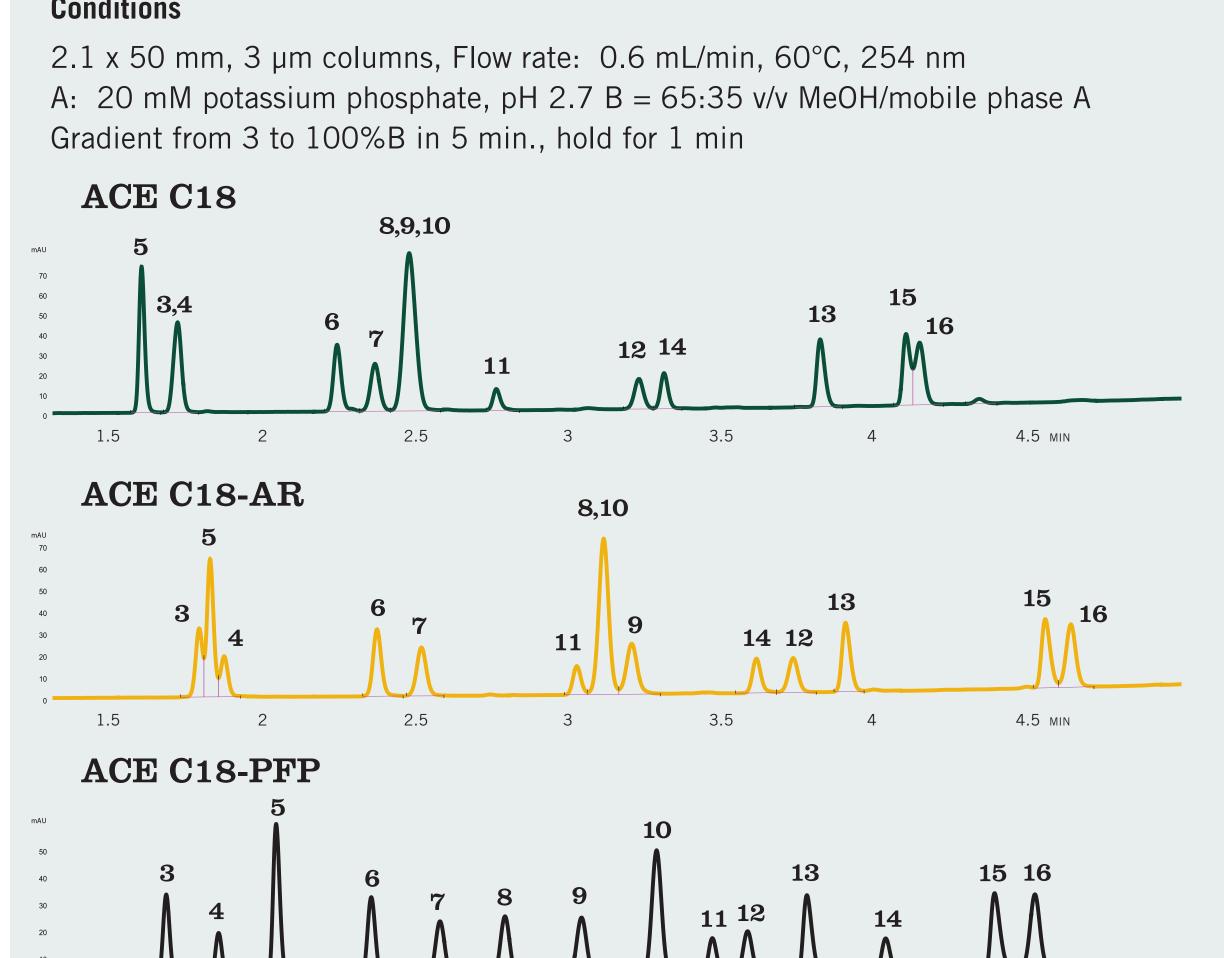
1) sulindac, 2) chrysin, 3) piperine, 4) flurbiprofen, 5) ibuprofen

2.1 x 50 mm, 3 µm columns, Flow rate: 0.6 mL/min, 40°C, 254 nm A: 20 mM potassium phosphate, pH 2.7, B = 65:35 v/v MeOH/mobile phase A Gradient from 3 to 100%B in 5 min., hold for 1 min



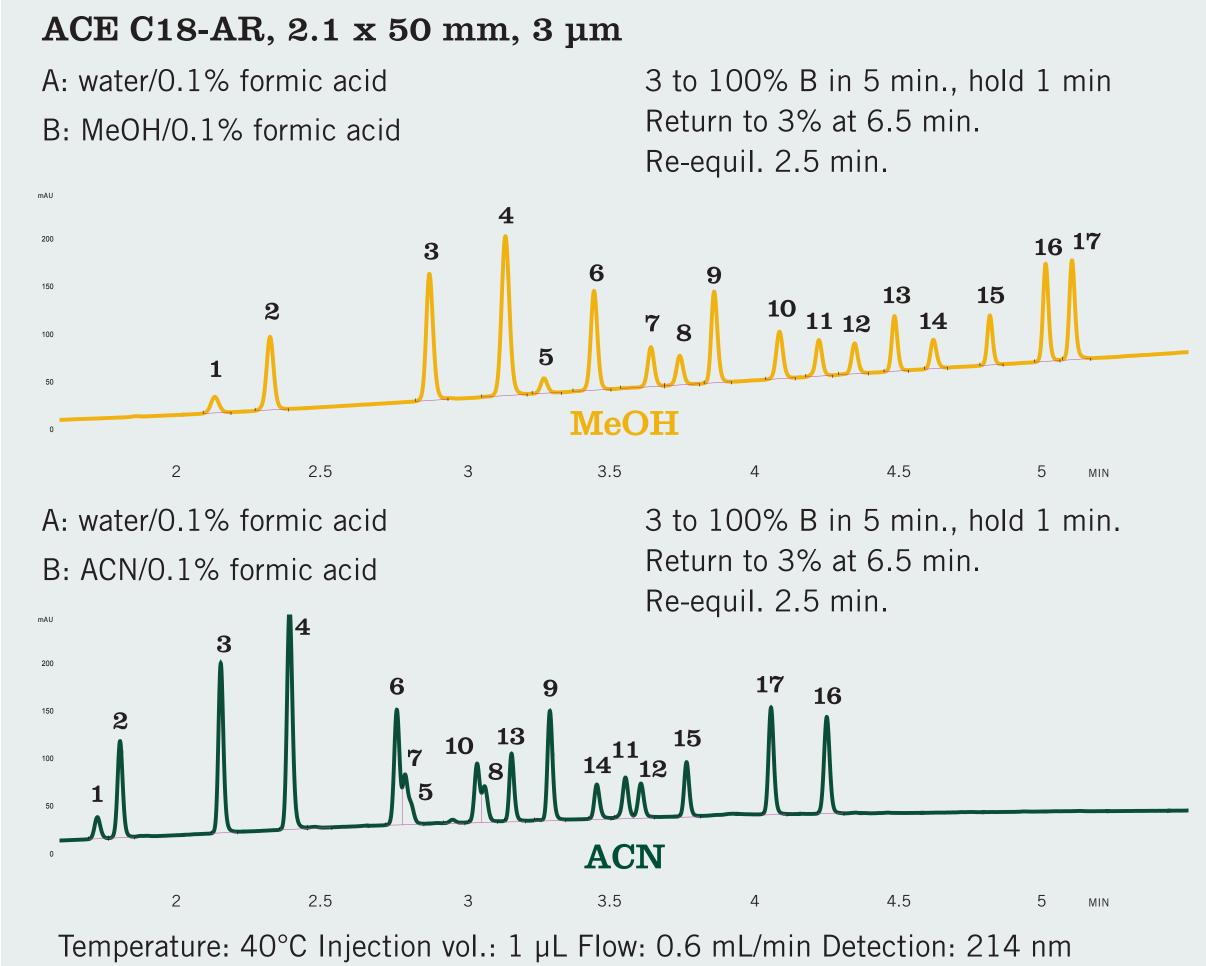
6) salicylic acid, 7) quinoxaline, 8) benzoic acid, 9) quinine, 10) phenacetin, 11) 1,4-dinitrobenzene, 12) 1,3,5-trinitrobenzene, 13) furosemide, 14) 1,3,5-trimethoxybenzene, 15) piroxicam, 16) carvedilol, 17) ethyl benzoate,

Changing Phases May Improve Resolution or just Change



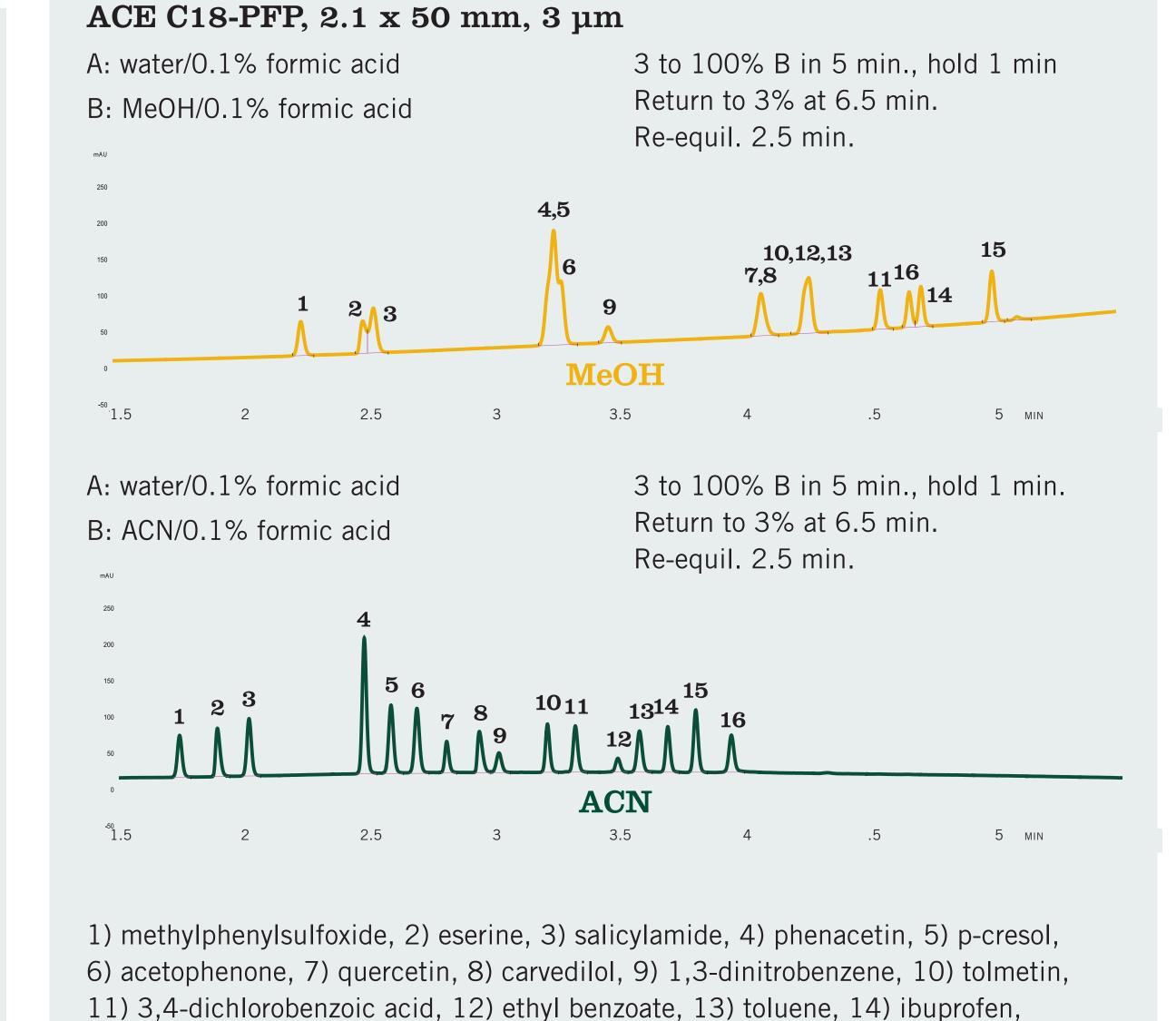
7) salicylaldehyde,8) 1,4-dinitrobenzene, 9) 1,3-dinitrobenzene 10) 1,2-dinitrobenzene, 11) myrecetin, 12) juglone, 13) remacemide, 14) quercetin, 15) methdilazine, 16) plumbagin

Large & Changes Often Occur Switching from ACN to MeOH



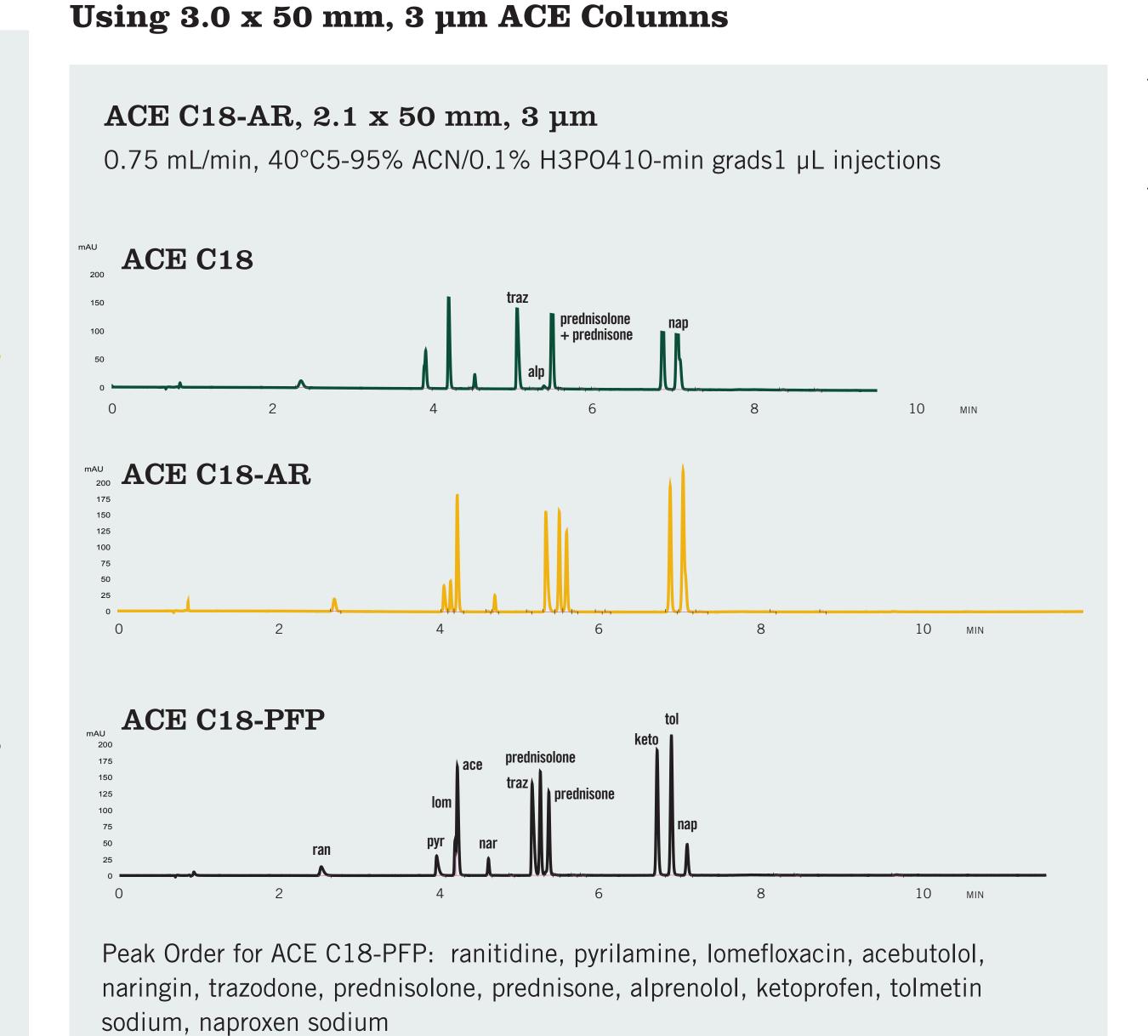
1) 3-hydroxybenzoic acid, 2) methylphenylsulfoxide, 3) quinoxaline, 4) salicylic acid, 5) benzonitrile, 6) 1,2-dimethoxybenzene, 7) ethyl paraben, 8) 1,4-dimethox benzene, 9) bendroflumethazide, 10) piroxicam, 11) benzyl chloride, 12) thioanisole, 13) sulindac, 14) chrysin, 15) ibuprofen, 16) 1,2,3-trichlorobenzene, 17) meclofenamic acid

Large & Changes Also Occur Switching from MeOH to ACN

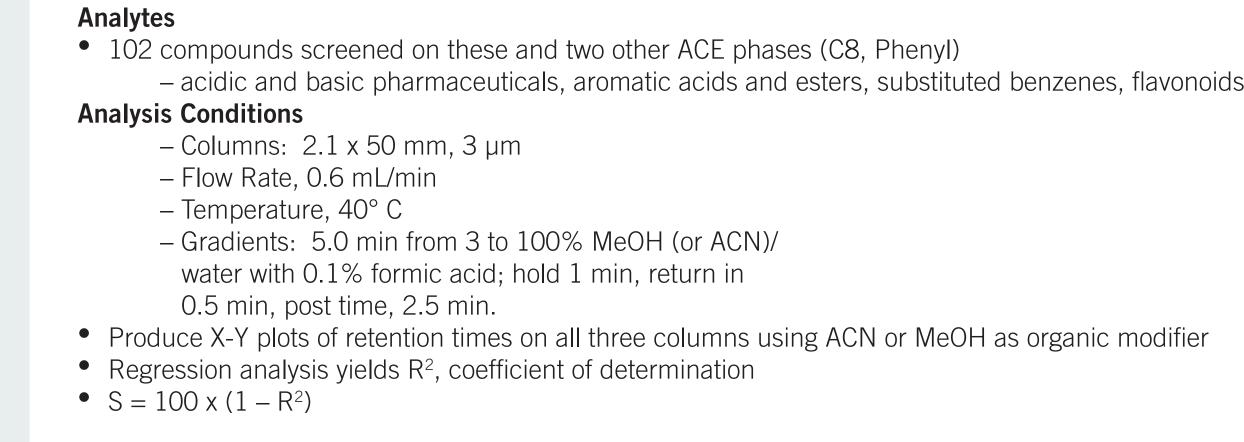


Fast Column Screening Gradients for Method Development

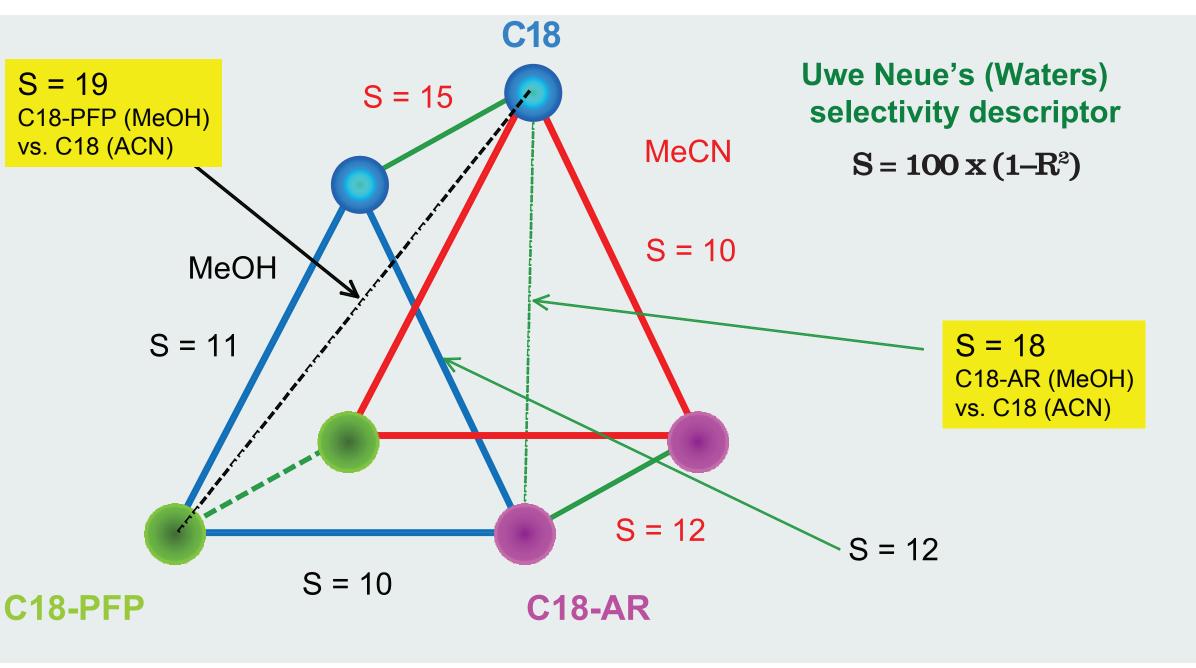
15) indomethacin, 16) 1,2-dichlorobenzene



Orthogonality of ACE C18-AR and ACE C18-PFP vs. ACE C18



Orthogonality Prism Comparing ACE C18, C18-AR, C18-PFP with ACN and MeOH Mobile Phases



ACE Bonded Phase Characteristics

	ACE Phase	Retention Mechanism	Most Commonly Used For	Best Application
5	C18	Hydrophobic interactions	Diverse analytes of differing polarities, acids, bases and neutrals at low to mid pH, strong acids or bases in ionized form using ion-pairing mode	Analytes differing in hydrophobicity, homologous compounds differing by - CH ₂ groups
	C18-AR	Hydrophobic and π – π interactions	Electron-poor compounds, analytes with electron-withdrawing groups (halogens, nitro groups, ketones)	When selectivity different from C18 is needed; Basic analytes, heterocycles, highly aqueous conditions
	C18-PFP	Hydrophobic,π–π, hydrogen bonding, dipole-dipole, and steric interactions	Electron-rich compounds, analytes with electron-donating groups, (phenols, aromatic ethers, amines), analytes with π bonds or electron delocalization, proton donors, analytes with different dipole moments	When selectivity different from C18 is needed; Positional isomers, substituted aromatics, steroids, highly aqueous conditions, proton donors

Using ACE C18, C18-AR and C18-PFP in RPLC Method Development

- ACE C18, C18-AR, and C18-PFP can be used both in simple and comprehensive, QBD method - Carry out column screening using multiple organic modifiers and blend(s) (ACN, MeOH,
- ACN/MeOH) at one or more pHs ACE C18-AR and C18-PFP can be also exploited to:
- Change selectivity or cause retention order reversals for a given separation on C18, C18-AR

ACE C18-AR and C18-PFP Summary

- Possess retention and stability advantages of ultrapure, ultra inert ACE C18 phase Excellent peak shape Excellent stability
- Superior column-to-column and batch reproducibility Possess complementary selectivities from additional types of molecular interactions
- $-\pi$ - π (charge transfer), hydrogen bonding, dipole-dipole, and steric (shape-selective) Provide a powerful toolset with ACE C18 for new method development or for method improvement and adjustment