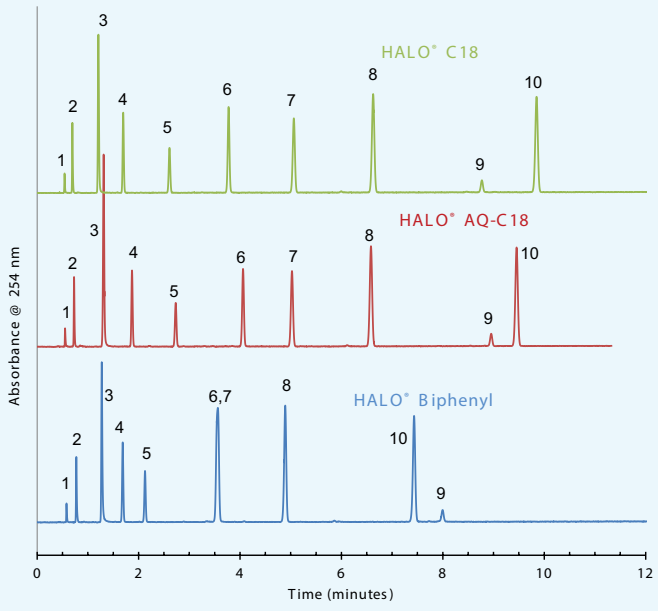
New Small Molecule Phases For Added Selectivity

HALO® AQ-C18 and HALO® Biphenyl are two new additions to the popular 90 Å HALO® line of columns for separating small molecules. Most new method development begins with a C18 column because of its high success rate, reputation for stability, and wide range of utility. If HALO® C18 doesn't work after the usual options of changing mobile phase solvents and pH have been explored, the extra polarity of the HALO® AQ-C18 or HALO® Biphenyl should be excellent options to achieve about the same solute retention while providing a mild to moderate increase in polar selectivity. Overlapping peaks may also be resolved, and some peaks can change elution order.

Figures 1A and 1B show screening gradients for HALO® AQ-C18 and Biphenyl for a selected list of aromatic standards. Note that relative retention and selectivity often vary significantly with organic solvent. This is especially true for phases containing aromatic ligands such as Biphenyl. Methanol, while not often selected as the first choice due to the higher backpressure generation, does not pose issues for fused-core® or superficially porous particles (SPPs) because it is not necessary to use sub-2-micron particles to achieve high resolution performance with SPPs. For this reason, both acetonitrile and methanol should be screened, especially with phases such as HALO® AQ-C18 and HALO® Biphenyl.

Figure 1A. Comparison of Aromatic Test Mix in Acetonitrile



PEAK IDENTITIES

1. uracil
2. resorcinol
3. 4-nitrophenol
4. benzonitrile
5. anisole
6. pterostilbene
7. valerophenone
8. biphenyl
9. impurity
10. o-terphenyl

TEST CONDITIONS

Columns: HALO 90 Å C18, 2.7 µm;
HALO 90 Å AQ-C18, 2.7 µm;
HALO 90 Å Biphenyl, 2.7 µm;
all 4.6 x 100 mm
Mobile Phase A: water
Mobile Phase B: Acetonitrile
Gradient: Time %B
0 40
15 90
Flow Rate: 1.5 mL/min
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2 µL

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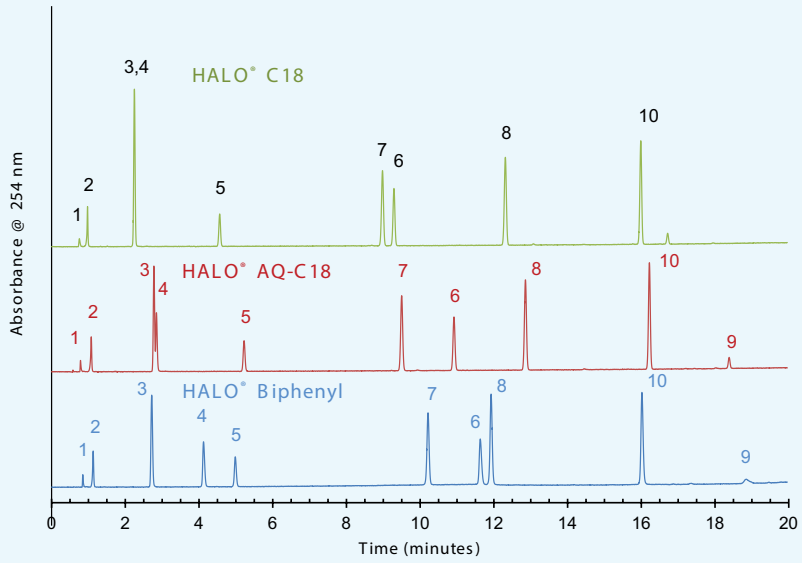
Figure 1B. Comparison of Aromatic Test Mix in Methanol



PEAK IDENTITIES

1. uracil
2. resorcinol
3. 4-nitrophenol
4. benzonitrile
5. anisole
6. pterostilbene
7. valerophenone
8. biphenyl
9. impurity
10. o-terphenyl

TEST CONDITIONS

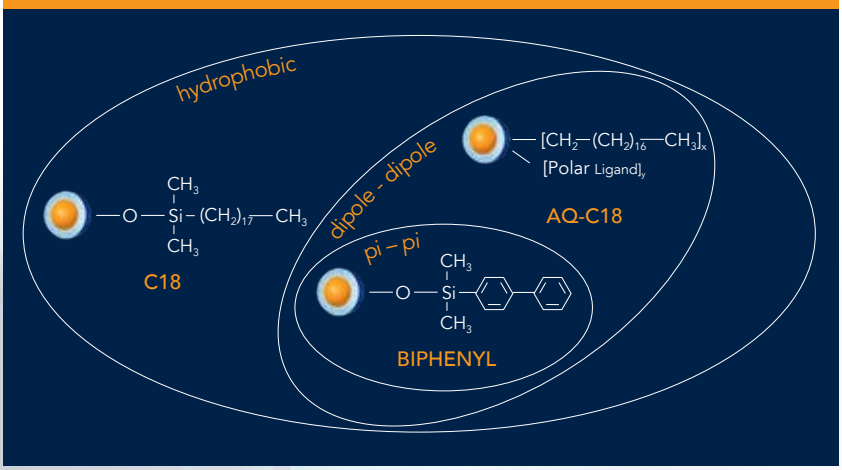
Columns: HALO 90 Å C18, 2.7 µm;
HALO 90 Å AQ-C18, 2.7 µm;
HALO 90 Å Biphenyl, 2.7 µm;
all 4.6 x 100 mm
Mobile Phase A: water
Mobile Phase B: Methanol
Gradient:

Time	%B
0	45
20	55

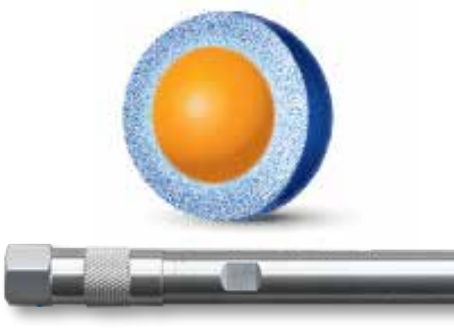
Flow Rate: 1.2 mL/min
Temperature: 30 °C
Detection: UV 254 nm, VWD
Injection Volume: 2 µL

Biphenyl often becomes equally retentive to C18 and AQ-C18 when methanol is used. The same gradient was employed for all three columns in each solvent, but a weaker gradient was employed in methanol to increase retention and resolution to resolve peaks 7 and 8. Note that the C18 and AQ-C18 columns resolve all 10 peaks in acetonitrile, while only Biphenyl resolves all 10 peaks in methanol. The Biphenyl column resolves 4-nitrophenol and benzonitrile, while these analytes are coeluted on the C18 and AQ-C18 phases. Also, Biphenyl has a greater selectivity difference compared to C18 in both organic solvents. That selectivity difference can be beneficial for closely related analytes.

Figure 2. Bonded Phase Structures and Associated Interactions

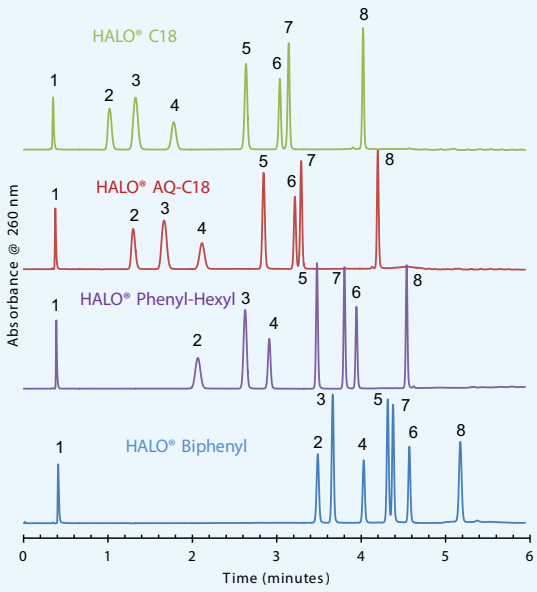


The bonded phase structures and their associated interactions are listed in Figure 2. All of the bonded phases (C18, AQ-C18, Biphenyl) participate in hydrophobic interactions. These types of interactions are useful for separations of compounds that differ by a single methyl group. Only AQ-C18 and Biphenyl participate in dipole-dipole interactions, which would be useful for separating polar analytes. Finally, Biphenyl with its two phenyl rings participates in pi-pi interactions, which is useful for compounds with aromatic moieties.



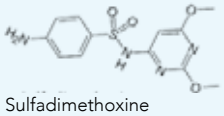
Examples of the interactions discussed above are observed in the following separation (Figure 3) which shows a dramatic difference in retention and selectivity for sulfa drugs that have a heterocyclic ring that interacts strongly with Phenyl and Biphenyl phases. The same methanol gradient was employed for all four columns. While the HALO® C18 interacts primarily by hydrophobic dispersion forces; AQ-C18 retains slightly more due to the same hydrophobic dispersion forces and a small amount of dipole interaction; Phenyl-Hexyl retains even more due to dispersion forces plus pi-pi interaction; Biphenyl has uniformly stronger selectivity from pi-pi interaction because it has minimal alkyl character. Under these conditions, the Biphenyl column shows the most retention. Note that the gradient was not optimized for any particular phase, but was run for illustrative purposes.

Figure 3. Comparison of Sulfa Drugs on Four Phases



PEAK IDENTITIES

1. uracil
2. sulfadiazine
3. sulfathiazole
4. sulfamerazine
5. sulfamethizole
6. sulfachloropyridazine
7. sulfamethoxazole
8. sulfadimethoxin



Sulfadimethoxine
Veterinary drug

TEST CONDITIONS

Columns: HALO 90 Å C18, 2.7 µm;
HALO 90 Å AQ-C18, 2.7 µm;
HALO 90 Å Phenyl-Hexyl, 2.7 µm;
HALO 90 Å Biphenyl, 2.7 µm;
all 3.0 x 100 mm
Mobile Phase A: 20 mM Ammonium Formate, pH 3
Mobile Phase B: Methanol
Gradient:

Time	%B
0	12
1.5	13
4.0	50
6.0	60

Flow Rate: 0.65 mL/min
Temperature: 30 °C
Detection: UV 260 nm, VWD
Injection Volume: 1 µL

Similar to RP-Amide and ES-CN, both HALO® AQ-C18 and Biphenyl are 100% aqueous compatible. In Figure 4, the HALO® C18 column shows typical dewetting behavior since retention is not maintained with 100% aqueous mobile phase after the pump is stopped and then restarted causing smaller hydrophobic pores to become vacated (note that column void volume has been reduced). The HALO® AQ-C18 is able to maintain retention even after the pump has been stopped and restarted. Identical results to AQ-C18 were obtained for HALO® Biphenyl. All HALO® columns are manufactured by a bonding method that has been engineered for maximum reproducibility, stability and performance.

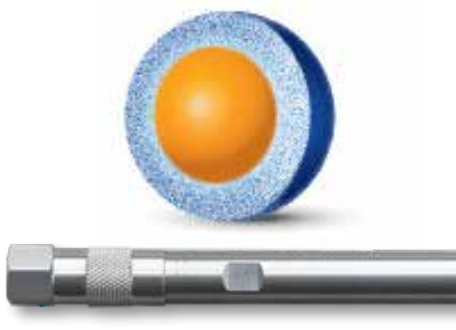
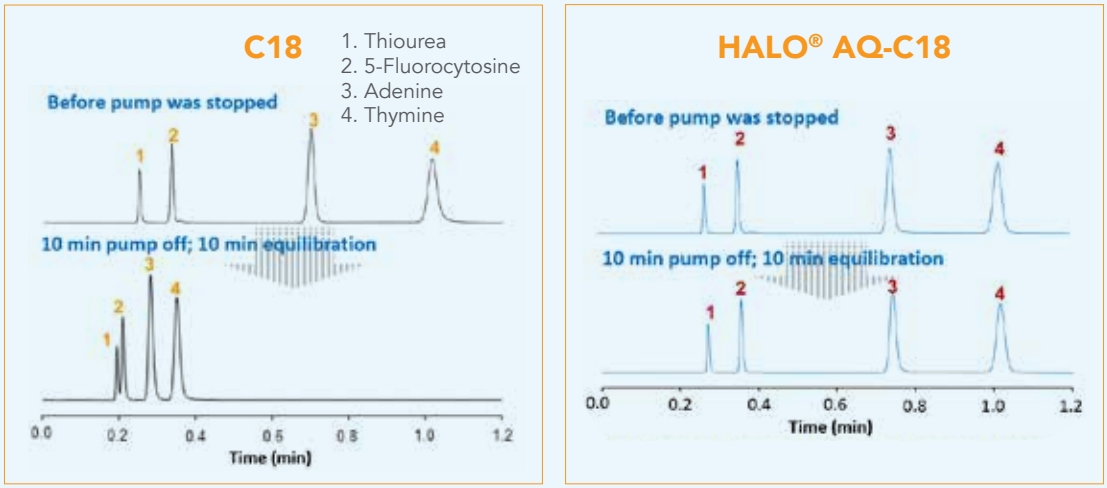


Fig. 4. Results of Dewetting Test: C18 compared to AQ-C18



Conclusions

HALO® AQ-C18 and HALO® Biphenyl offer additional selectivity options to the collection of HALO® bonded phases that are designed for small molecule analysis. These phases offer solutions for highly polar compounds that would normally not be retained very much by traditional alkyl bonded phases. Screening these phases with both acetonitrile and methanol is recommended to fully explore their selectivity advantages. They also offer the additional feature of being compatible with 100% aqueous mobile phases thereby extending their useful range for separating polar solutes that would otherwise elute at the void volume of many reversed-phase columns such as C8 or C18.