LAB NOTES

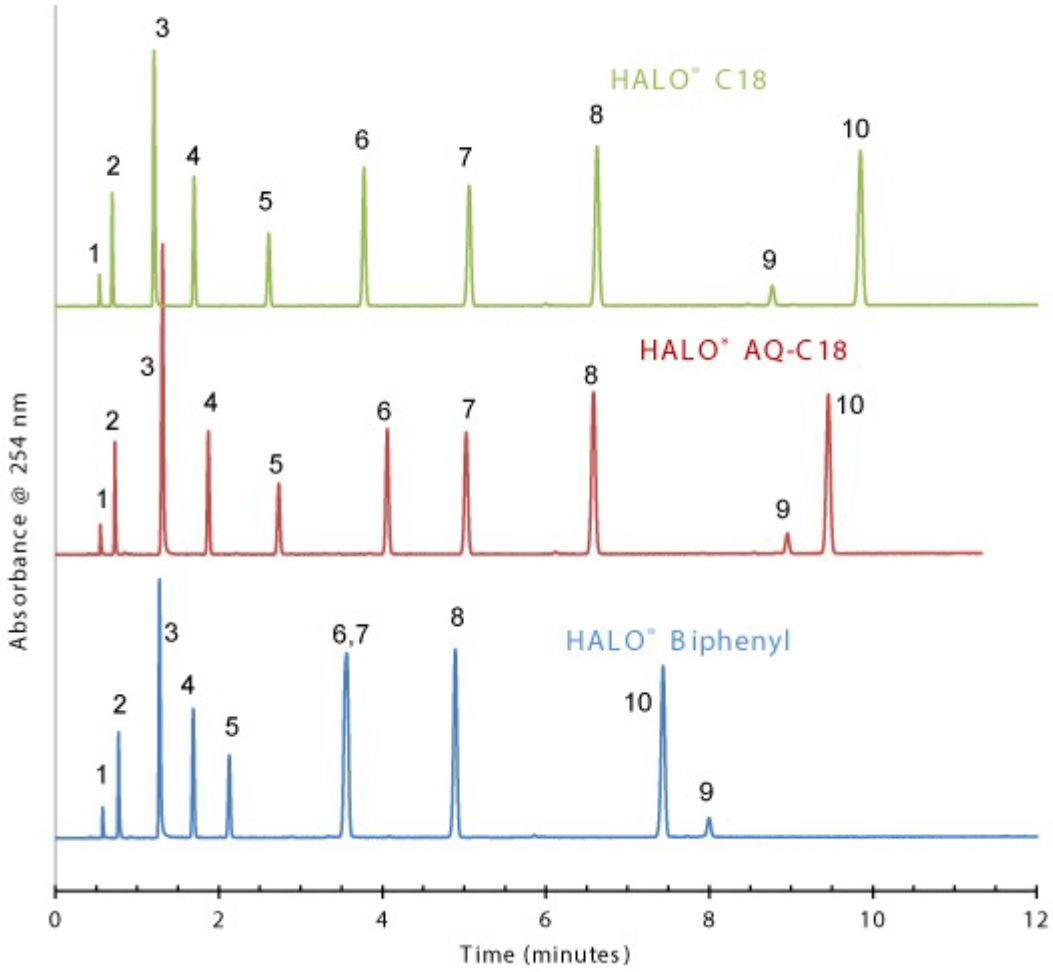
New HALO® Phases with Additional Selectivity for Method Development and the Analysis of Small Molecules

HALO® AQ-C18 and HALO® Biphenyl are two new additions to the popular 90 Å HALO® line of columns for separating small molecules. New method development often 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 organic modifiers and pH have been explored, the extra polarity of the HALO® AQ-C18 or HALO® Biphenyl phases should provide similar solute retention while providing an increase in polar selectivity. Overlapping peaks may also be resolved, and some analytes may also change elution order.

Figures 1A and 1B show screening gradients for HALO® AQ-C18 and Biphenyl for a selected list of aromatic compounds. Note that relative retention and selectivity often vary significantly with organic modifier choice. This is especially true for phases containing aromatic ligands such as Biphenyl. Methanol is often not the first choice as an organic modifier because of its higher viscosity and associated backpressure. However, it is practical and effective to use both acetonitrile and methanol as organic modifiers for column and conditions screening with HALO® Fused-Core® columns because of the lower pressures afforded by 2, 2.7, and 5 µm SPP particles compared to those generated by sub-2 µm particle columns.

Moreover, the HALO® Biphenyl phase is often comparably retentive to C18 and AQ-C18 when methanol is used as the organic modifier. In Figures 1A and 1B, the same gradient profile was used for all three phases using each of the two organic modifiers, but a shallower gradient was employed with methanol to increase retention and resolution. Note that the HALO® C18 and AQ-C18 columns resolve all 10 peaks using acetonitrile, while only the HALO® Biphenyl resolves all 10 peaks using methanol. With methanol as the modifier, the HALO® Biphenyl column resolves 4-nitrophenol and benzonitrile, while these two analytes co-elute using the HALO® C18 and AQ-C18 phases.

Figure 1A. Comparison of Aromatic Test Mix in Acetonitrile



CONDITIONS:

HALO® 90 Å C18, 2.7 µm
HALO® 90 Å AQ-C18, 2.7 µm
HALO® 90 Å Biphenyl 2.7 µm
All Columns: 4.6 x 100 mm
Mobile Phase A: Water
Mobile Phase B: Acetonitrile
Gradient:

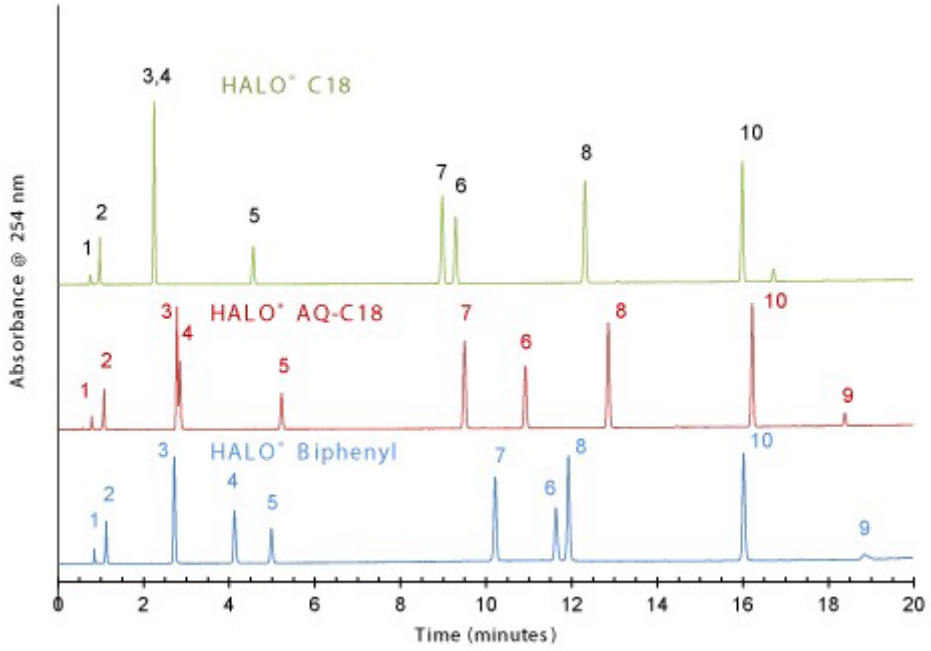
Time	%B
0	40
15	90

Flow Rate: 1.5 mL/min
Temperature: 30 °C
Detection: UV 254 nm, VWO
Injection Volume: 2 µL
.

PEAK IDENTITIES:

1. Uracil
2. Resorcinol
3. 4-Nitrophenol
4. Benzonitrile
5. Anisole
6. Pterostilbene
7. Valerophenone
8. Biphenyl
9. Impurity
10. o-Terphenyl

Figure 1B. Comparison of Aromatic Test Mix in Methanol



CONDITIONS:

HALO® 90 Å C18, 2.7 µm
HALO® 90 Å AQ-C18, 2.7 µm
HALO® 90 Å Biphenyl 2.7 µm
All Columns: 4.6 x 100 mm
Mobile Phase A: Water
Mobile Phase B: Methanol
Gradient:

Time	%B
0	45
20	35

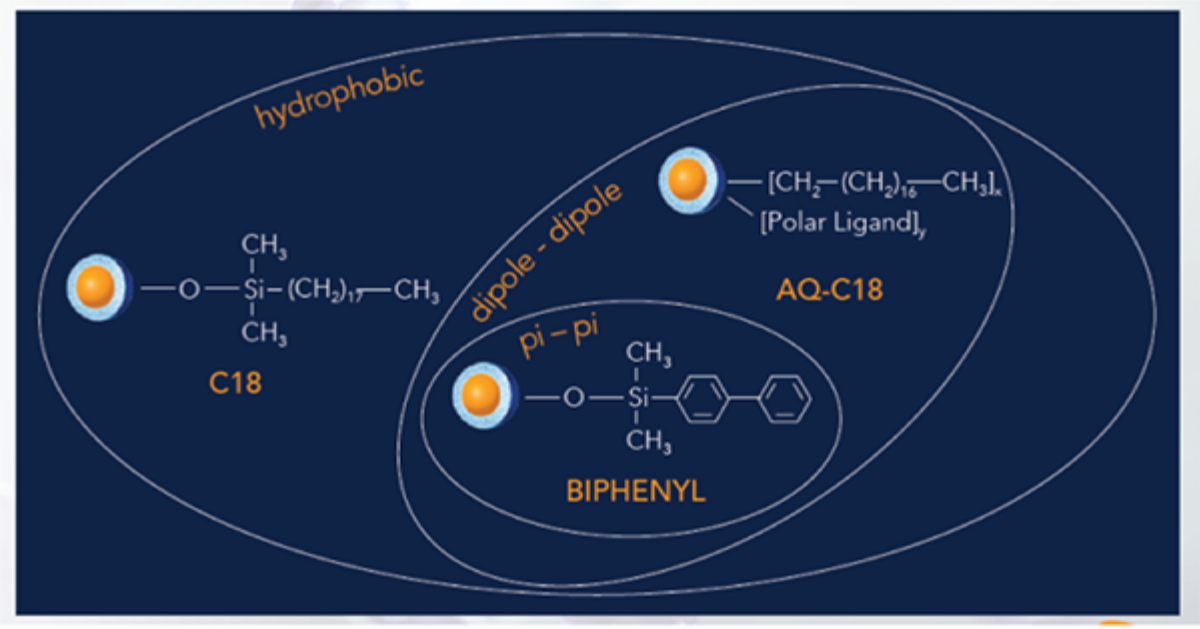
Flow Rate: 1.2 mL/min
Temperature: 30 °C
Detection: UV 254 nm, VWO
Injection Volume: 2 µL

PEAK IDENTITIES:

1. Uracil
2. Resorcinol
3. 4-Nitrophenol
4. Benzonitrile
5. Anisole
6. Pterostilbene
7. Valerophenone
8. Biphenyl
9. Impurity
10. o-Terphenyl

The bonded phase structures and their associated types of interactions are listed in Figure 2. All of the bonded phases (C18, AQ-C18, Biphenyl) retain and separate analytes via hydrophobic interactions. These types of interactions are useful for separations of compounds that differ in hydrophobicity (e.g., log P). HALO® AQ-C18 and Biphenyl also offer dipole-dipole interactions, which are useful for separating polar analytes. Finally, Biphenyl, with its two phenyl rings, can also retain analytes via π-π interactions, which are useful for compounds with aromatic and unsaturated groups (double and triple bonds).

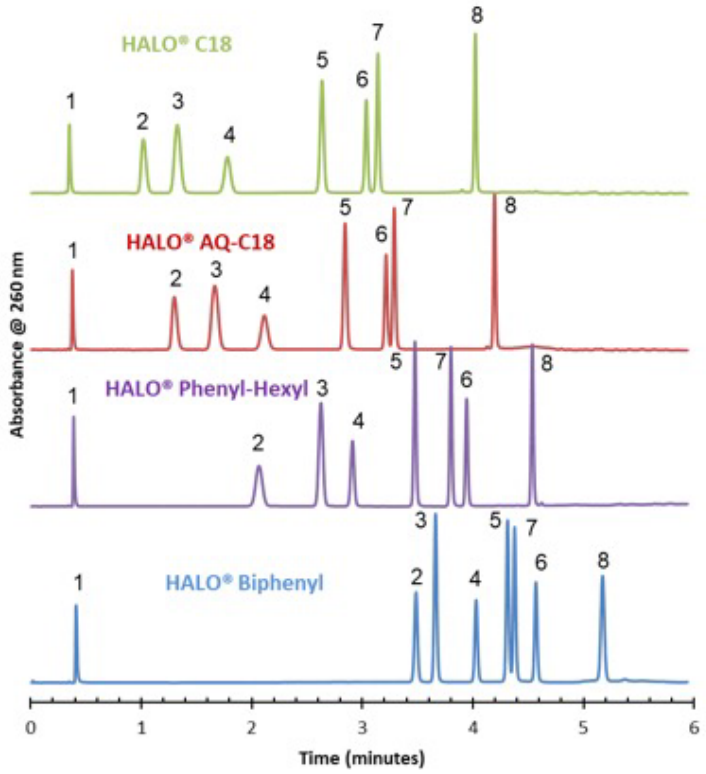
Figure 2. Bonded Phase Structures and Associated Interactions



Examples of the interactions discussed above are observed in the following separation seen in Figure 3, which shows a dramatic difference in retention and selectivity for sulfa drugs, which have different heterocyclic rings that interact strongly with the HALO® Phenyl-Hexyl and Biphenyl phases.

While the HALO® C18 interacts primarily by hydrophobic interactions, the HALO® AQ-C18 retains these analytes slightly more because of the additional dipole-dipole interactions. The HALO® Phenyl-Hexyl phase provides greater retention as a result of both hydrophobic and π-π interactions. The HALO® Biphenyl phase provides even stronger π-π interactions, because of its minimal hydrophobic interactions, and delivers the most retention of the four phases under these conditions.

Figure 3: Comparison of Sulfa Drugs on Four Phases



CONDITIONS:

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 Columns: 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

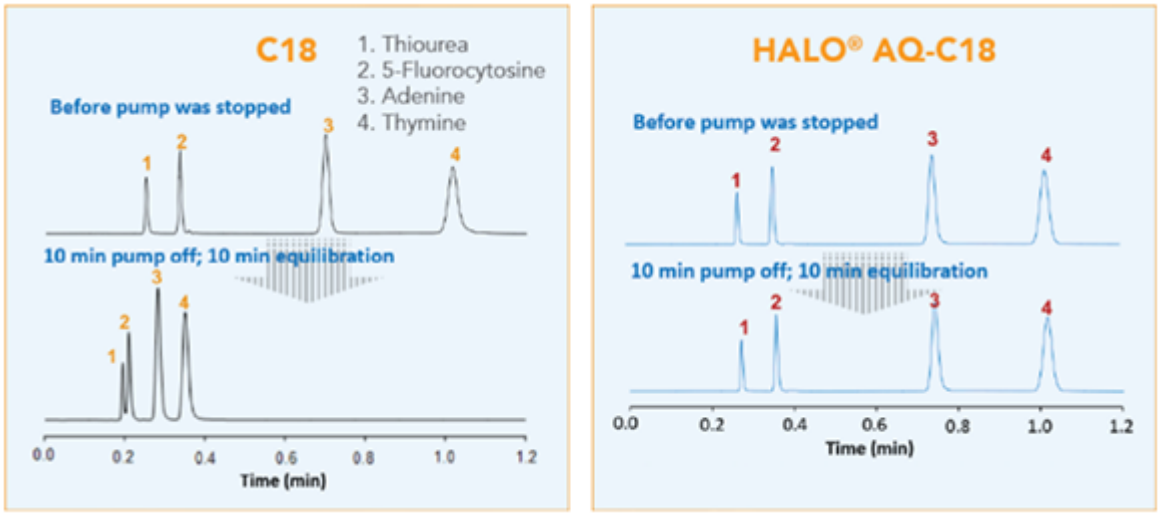
Flow Rate: 0.65 mL/min

PEAK IDENTITIES:

1. Uracil
2. Sulfadiazine
3. Sulfathiazole
4. Sulfamerazine
5. Sulfamethizole
6. Sulfachloropyridazine
7. Sulfamethoxazole
8. Sulfadimethoxin

Similar to HALO® Phenyl-Hexyl, RP-Amide, and ES-CN phases, both HALO® AQ-C18 and Biphenyl are 100% aqueous compatible in isocratic and gradient modes. In Figure 4, the HALO® C18 column shows typical pore dewetting behavior, because retention was not maintained with 100% aqueous mobile phase after the pump was stopped and then restarted. Conversely, the HALO® AQ-C18 column is able to maintain retention with low or no organic modifier, even after the pump has been stopped and then restarted. Comparable results from dewetting experiments were also obtained for HALO® Biphenyl.

Figure 4. Results of Dewetting Test: C18 Compared to AQ-C18



CONDITIONS:
HALO® 90 Å C18, 2.7 µm
HALO® 90 Å AQ-C18, 2.7 µm
All Columns: 4.6 x 100 mm
Mobile Phase: 0.1% TFA in water
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL

PEAK IDENTITIES:
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine

Conclusion:
The HALO® AQ-C18 and HALO® Biphenyl phases are two new additions to the HALO® column family, which provide additional selectivity options for the method developer and analyst for small molecule separations. These phases will provide increased retention for polar analytes, and offer unique selectivities that can be used with both acetonitrile- and methanol-containing mobile phases. They are also fully compatible with 100% aqueous mobile phases in isocratic and gradient modes, which makes them useful for very polar analytes that may show little or no retention with traditional C8 and C18 phases.

Learn More about the HALO AQ-C18 and Biphenyl Phases