High Performance Separations using 100% Aqueous Mobile Phase Compatible Superficially Porous Particle Columns Coupled with Mass Spectrometry

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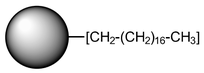
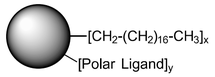
Introduction

It is estimated that 80-90% of HPLC applications are performed using reversed-phase liquid chromatography (RPLC). RPLC typically implements a robust and versatile C18 phase. However, this hydrophobic phase has a strong tendency to dewet in 100% aqueous phases. This phenomenon reduces interaction between analytes and stationary phase and manifests as complete loss of retention and elution of analytes at the void volume. To address this, we developed a HALO AQ-C18 phase that has the properties of a traditional C18 column with the addition of a polar ligand to minimize dewetting.

HALO C18



HALO AQ-C18



Dewetting Studies of HALO C18 Compared to HALO AQ-C18

HALO C18 shows a dramatic loss of retention after the pump is shut off for 10 minutes in 100% aqueous mobile phases.

Columns: HALO 90 Å C18 and HALO 90 Å AQ-C18, 2.7 µm 4.6 x 50 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

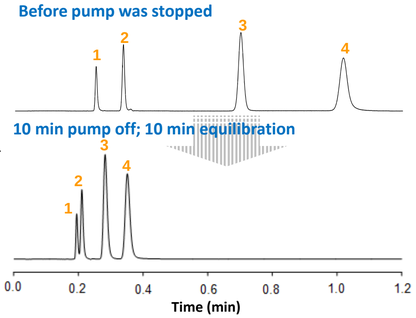
Thiourea

5-Fluorocytosine

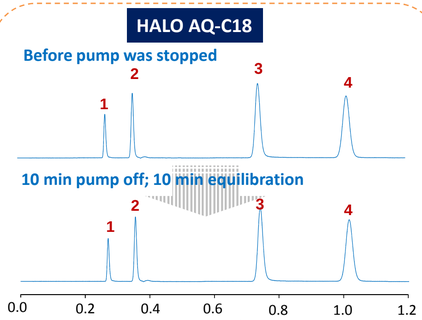
Adenine

Thymine

HALO C18

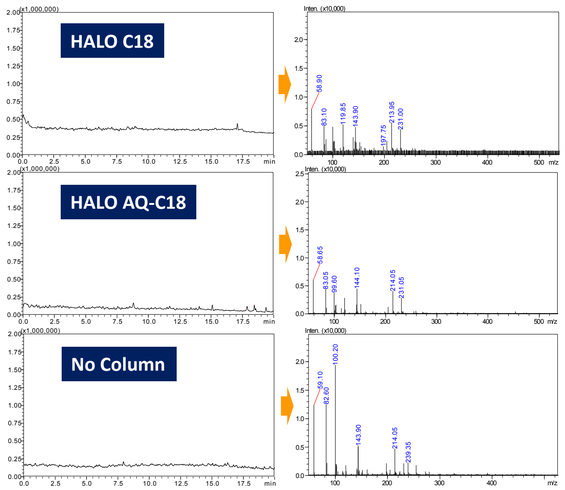


HALO AQ-C18



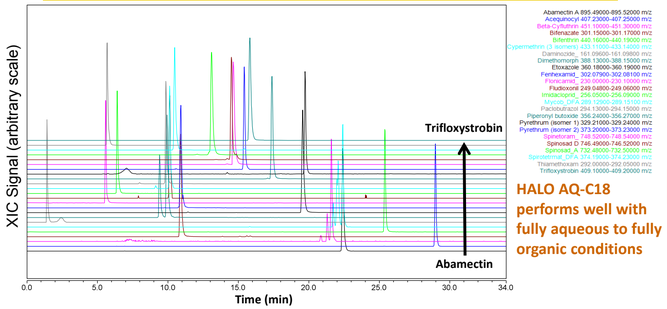
Alternatively HALO AQ-C18 maintains identical retention.

HALO AQ-C18 Coupled with Mass Spectrometry



The HALO AQ-C18 shows negligible differences in background ions. No MS signal is observed that can be ascribed to silane bleed from the surface. Gradient conditions were 5-95% acetonitrile with 0.1% formic acid in 20 min. Total ion chromatograms from 50-500 m/z and spectra were averaged for 2.5 – 17.5 minutes.

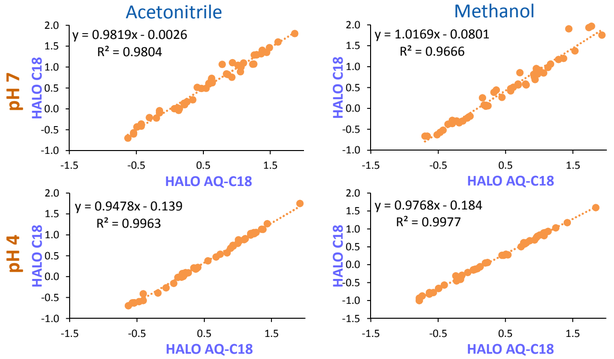
Analysis of Nevada Pesticide Mix by LC/MS



HALO AQ-C18 performs well with fully aqueous to fully organic conditions

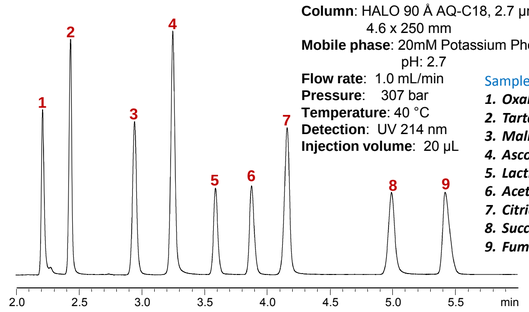
Column: HALO 90 Å AQ-C18, 2.7 µm, 2.1 x 100 mm **Flow rate**: 0.2 mL/min **Temperature**: 40°C
Mobile phase: (A), 4 mM Ammonium Formate/0.1% Formic acid in Water; (B), acetonitrile.
Gradient: 0 to 1 min: 0% B; 1.01 min: 15% B; 4 min: 35% B; 5 min: 62% B; 30 min: 100% B
Sample: The Nevada pesticide mix was dissolved to 2µg/mL in acetonitrile
MS: Orbitrap Velos Pro; 15,000 Res; XIC signals

Retention Mapping



Selectivity comparisons between the HALO C18 and HALO AQ-C18 phases were made. Importantly little difference in hydrophobic retention was shown for these columns in acetonitrile and methanol at pH 4 and 7 (Data courtesy of MilliporeSigma)

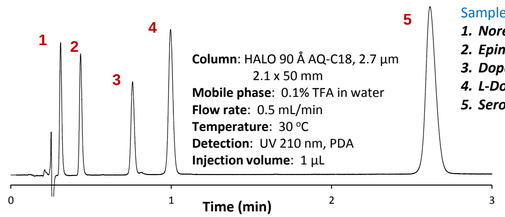
Organic Acids in 100% Aqueous Mobile Phase



Column: HALO 90 Å AQ-C18, 2.7 µm 4.6 x 250 mm
Mobile phase: 20mM Potassium Phosphate buffer pH: 2.7
Flow rate: 1.0 mL/min
Pressure: 307 bar
Temperature: 40 °C
Detection: UV 214 nm
Injection volume: 20 µL

Samples:
1. *Oxalic acid*
2. *Tartaric acid*
3. *Malic acid*
4. *Ascorbic acid*
5. *Lactic acid*
6. *Acetic acid*
7. *Citric acid*
8. *Succinic acid*
9. *Fumaric acid*

Brain Neurotransmitters in 100% Aqueous Mobile Phase



Column: HALO 90 Å AQ-C18, 2.7 µm 2.1 x 50 mm
Mobile phase: 0.1% TFA in water
Flow rate: 0.5 mL/min
Temperature: 30 °C
Detection: UV 210 nm, PDA
Injection volume: 1 µL

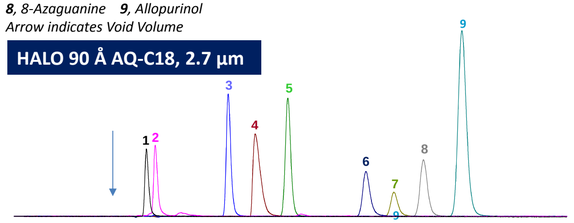
Samples:
1. *Norepinephrine*
2. *Epinephrine*
3. *Dopamine*
4. *L-Dopamine*
5. *Serotonin*

Highly Polar Purines Using in 100% Aqueous Mobile Phase

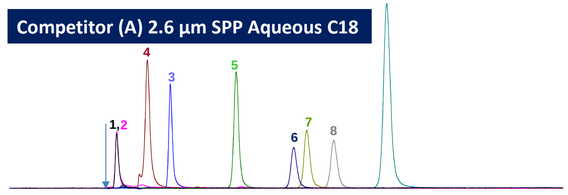
Columns: HALO 90 Å AQ-C18, 2.7 µm, 2.1 x 100 mm **Mobile phase**: 0.1% Formic Acid
Flow rate: 0.5 mL/min **Temperature**: 35 °C
Detection: UV 254 nm **Injection**: 1 µL

Samples: ***1***, *5-Amino-imidazole-4-carboxamide* ***2***, *Azepinomycin* ***3***, *Guanine - substrate* ***4***, *2,6-Diaminopurine* ***5***, *Uric Acid* ***6***, *Xanthine – product* ***7***, *8-Azaxanthine* ***8***, *8-Azaguanine* ***9***, *Allopurinol*
Arrow indicates Void Volume

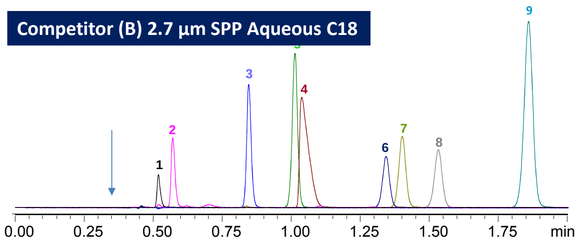
HALO 90 Å AQ-C18, 2.7 µm



Competitor (A) 2.6 µm SPP Aqueous C18



Competitor (B) 2.7 µm SPP Aqueous C18



HALO 90 Å AQ-C18, 2.7 µm column stands out in its ability to resolve this set of purines. These molecules are highly polar and are not retained under reversed-phase conditions that include an organic modifier.

Conclusion

HALO AQ-C18 is stable in 100% aqueous mobile phase and exhibits similar selectivity for many analytes in comparison to HALO C18. This phase, with an incorporated polar ligand, has minimal background ions and works well with gradients beginning at 0% organic. We have demonstrated this phase's effectiveness with a selection of very polar compounds under 100% aqueous conditions including organic acids, neurotransmitters, and purines.