High Performance Separations Using a Novel Bonded Phase Compatible With 100% Aqueous Mobile Phases

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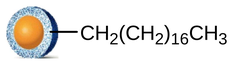
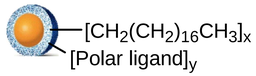
Introduction

It was estimated that more than 80% 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 limits the applications of RPLC. 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

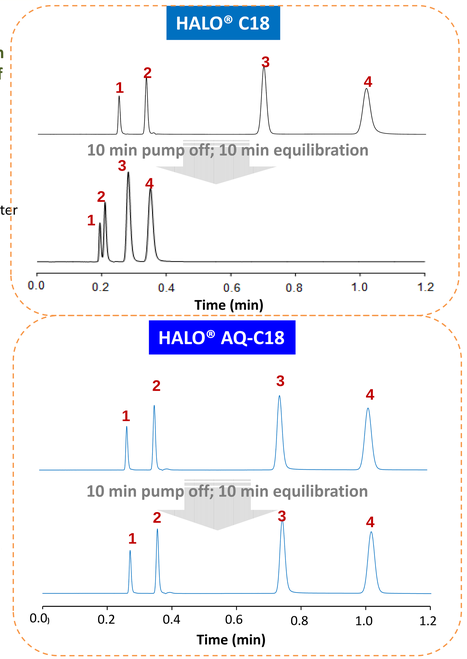


Dewetting Studies of HALO® C18 Compared to HALO® AQ-C18

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

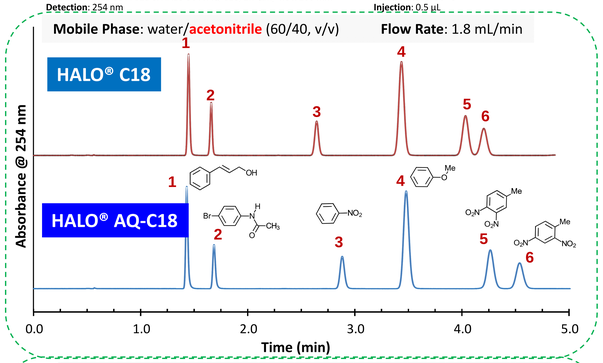
Columns: HALO 90 Å C18 and HALO 90 Å AQ-C18, 2.7 µm 4.6 x 50 mm
Mobile phase: 0.1% TFA in water
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 °C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL

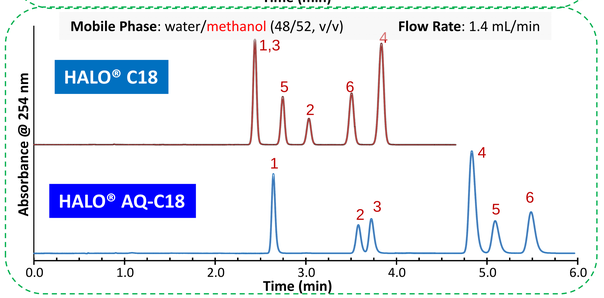
Alternatively HALO® AQ-C18 maintains identical retention.



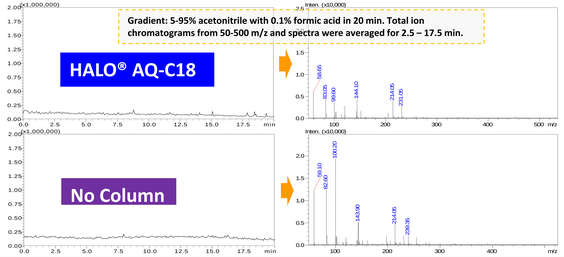
Retention and Selectivity in Different Mobile Phases

Columns: HALO 90 Å C18 & HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 100 mm
Detection: 254 nm
Temperature: 30 °C
Injection: 0.5 µL



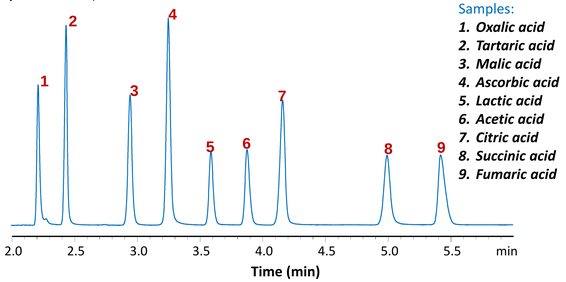


Robustness of HALO® AQ-C18 – No Bleeding



Organic Acids in 100% Aqueous Mobile Phase

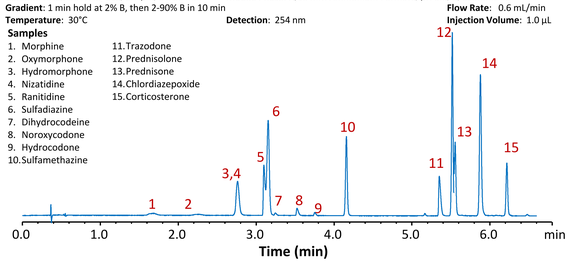
Column: HALO 90 Å AQ-C18, 2.7 µm, 4.6 x 250 mm
Flow rate: 1.0 mL/min
Injection volume: 20 µL
Mobile phase: 20mM Potassium Phosphate buffer, pH: 2.7
Temperature: 40 °C
Detection: 214 nm



Complex Drug Mix Starting with High % of Aqueous Mobile Phase

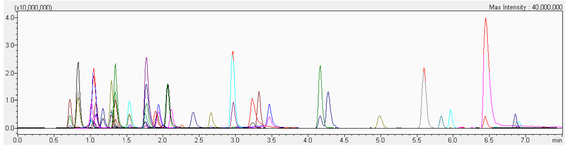
Column: HALO 90 Å AQ-C18, 2.7 µm, 3 x 50 mm
Gradient: 1 min hold at 2% B, then 2-90% B in 10 min
Temperature: 30°C
Mobile Phase: A,10 mM Ammonium Formate, pH 3.8; B, acetonitrile
Flow Rate: 0.6 mL/min
Injection Volume: 1.0 µL
Detection: 254 nm

Samples
1. Morphine
2. Oxymorphone
3. Hydromorphone
4. Nizatidine
5. Ranitidine
6. Sulfadiazine
7. Dihydrocodeine
8. Noroxycodone
9. Hydrocodone
10. Sulfamethazine
11. Trazodone
12. Prednisolone
13. Prednisone
14. Chlordiazepoxide
15. Corticosterone



52 Veterinary Drug Mix Detected on an AQ-C18 Column

Column: HALO 90 Å AQ-C18, 2.7 µm, 2.1 x 100 mm
Gradient: 10-30%B in 4.5min, 30-100%B from 4.5 to 7.5 min
Temperature: 40 °C
Mobile phase: A, 0.1% Formic acid in water; B, 0.1% Formic acid in acetonitrile
Flow rate: 0.5 mL/min
Detection: Shimadzu 8060 LC-MS-MS
Injection volume: 1 µL



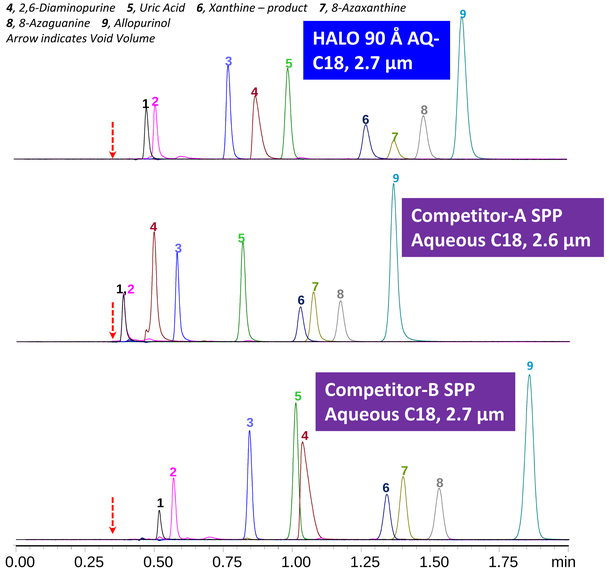
- Sample contains a mix of sulfonamides, quinolones, hormones, macrolides, chloramphenicols, nitroimidazoles, tetracyclines, and lincosamides.

Samples: *(details available upon request)*

Highly Polar Purines Using in 100% Aqueous Mobile Phase

Columns: HALO 90 Å AQ-C18, 2.7 µm, 2.1 x 100 mm
Temperature: 35 °C
Mobile phase: 0.1% Formic Acid
Detection: 254 nm
Flow rate: 0.5 mL/min
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 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 eluted using acetonitrile. This similarity may change under methanol conditions. 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 and purines.

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