New Superficially Porous Particle Column Selectivity Choices for Improved Separations

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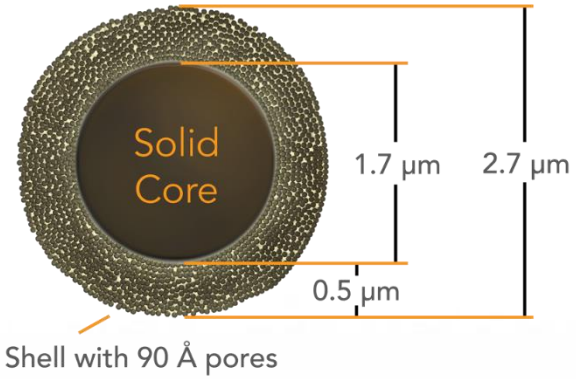
Overview

- HALO® AQ-C18
  - Objectives
    - Same C18 stability and ease of use
    - Same C18 reproducibility
    - Extended range to 100% aqueous and low organic mobile phases
    - Equally useful in both methanol and acetonitrile mobile phases
  - Applications
    - Brain Neurotransmitters
    - Nucleic Acid Bases
    - Purines
    - Organic Acids
    - Pyrethrins
    - Triazine Pesticides
    - Drugs and Metabolites

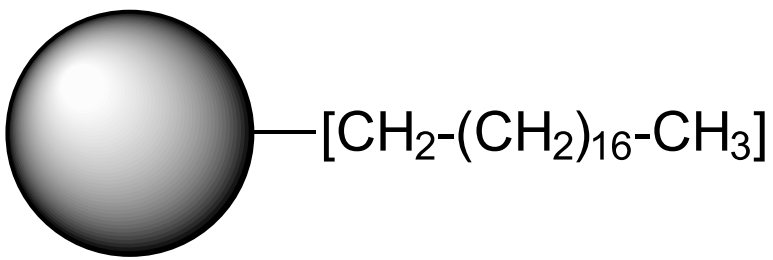
HALO® AQ-C18

- Particle type:
  - L1 USP Classification
  - Type B superficially porous silica
  - Available in 2, 2.7, and 5 µm total size
  - 0.5 µm porous shell
  - 90 Å pores

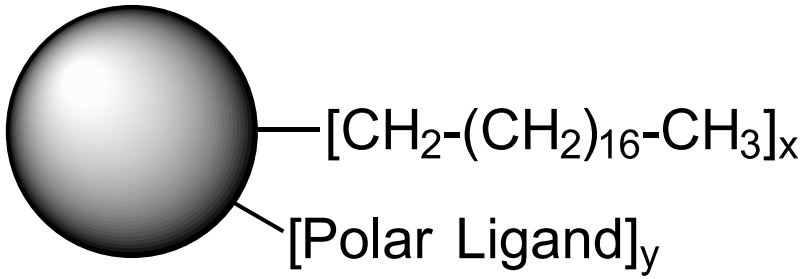


- Column Properties:
  - 60 °C maximum temperature
  - pH 2 – 9
  - 600 bar maximum operating pressure
  - Minimal MS bleed like Halo C18
  - Phase chemistry: C18 with novel addition of dipole character (proprietary treatment to increase wetting and versatility)

HALO AQ-C18 Bonded Phase



C18



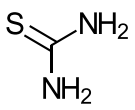
AQ-C18

- The combination of a C18 ligand and a ligand with polar characteristics increases the wettability of HALO AQ-C18 and provides small changes in selectivity when desired.

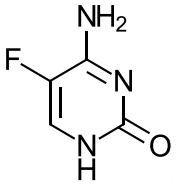
HALO AQ-C18: DESIGNED TO RESIST DE-WETTING

pH 2 De-wetting results: Nucleic Acid Bases

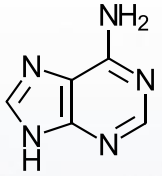
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



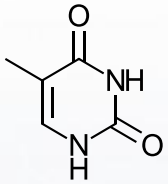
Thiourea



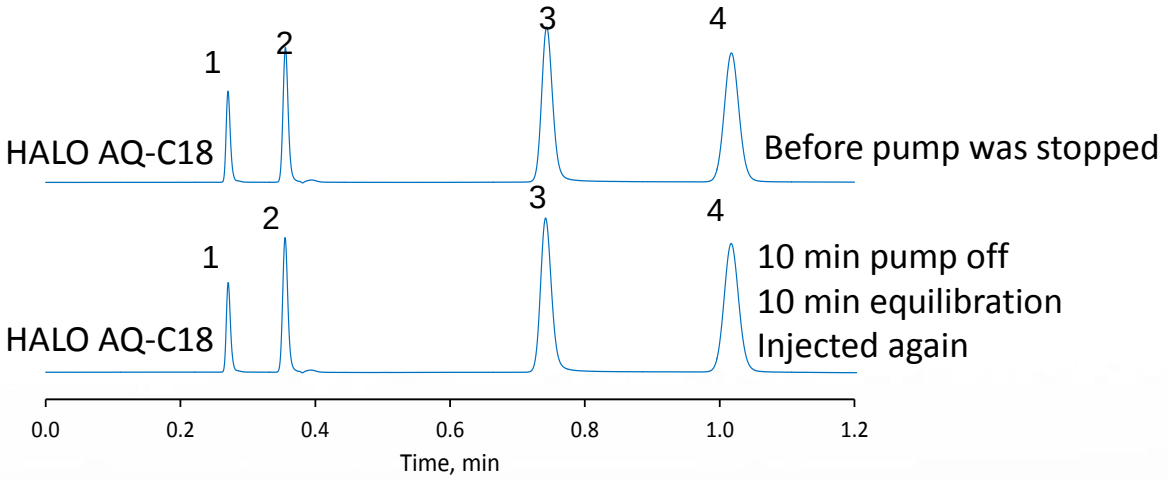
5-Fluorocytosine

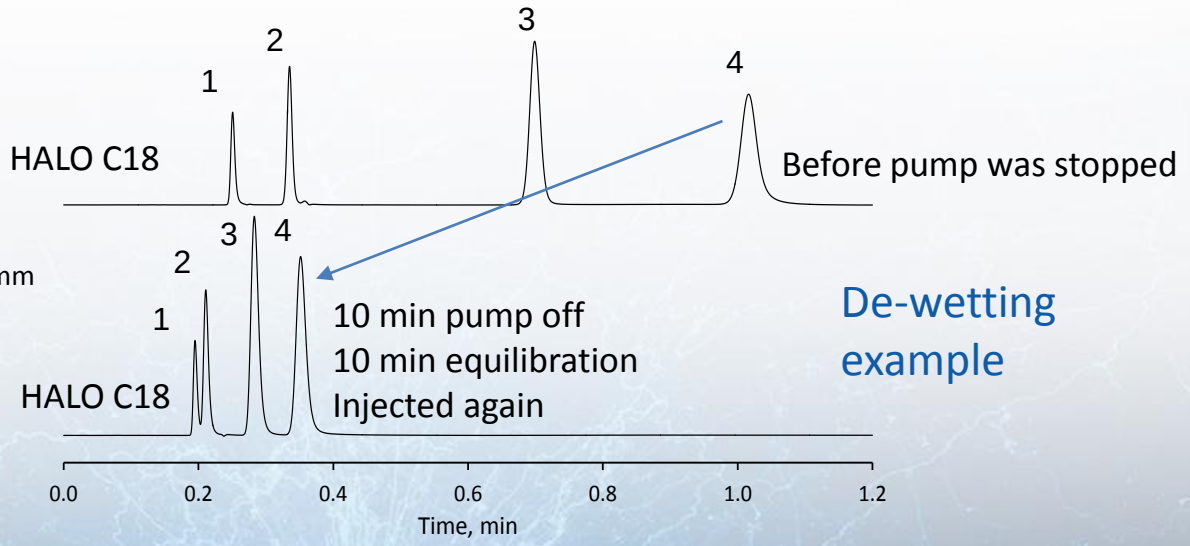


Adenine



Thymine



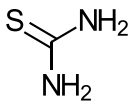


De-wetting example

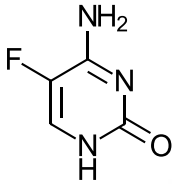
Columns: HALO 90Å, AQ-C18, 2.7 µm, 4.6 x 50 mm
HALO 90Å, C18, 2.7 µm, 4.6 x 50 mm
Isocratic: Water/0.1% TFA
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 ° C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL

pH 7 De-wetting results: Nucleic Acid Bases

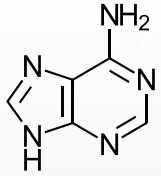
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



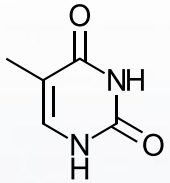
Thiourea



5-Fluorocytosine

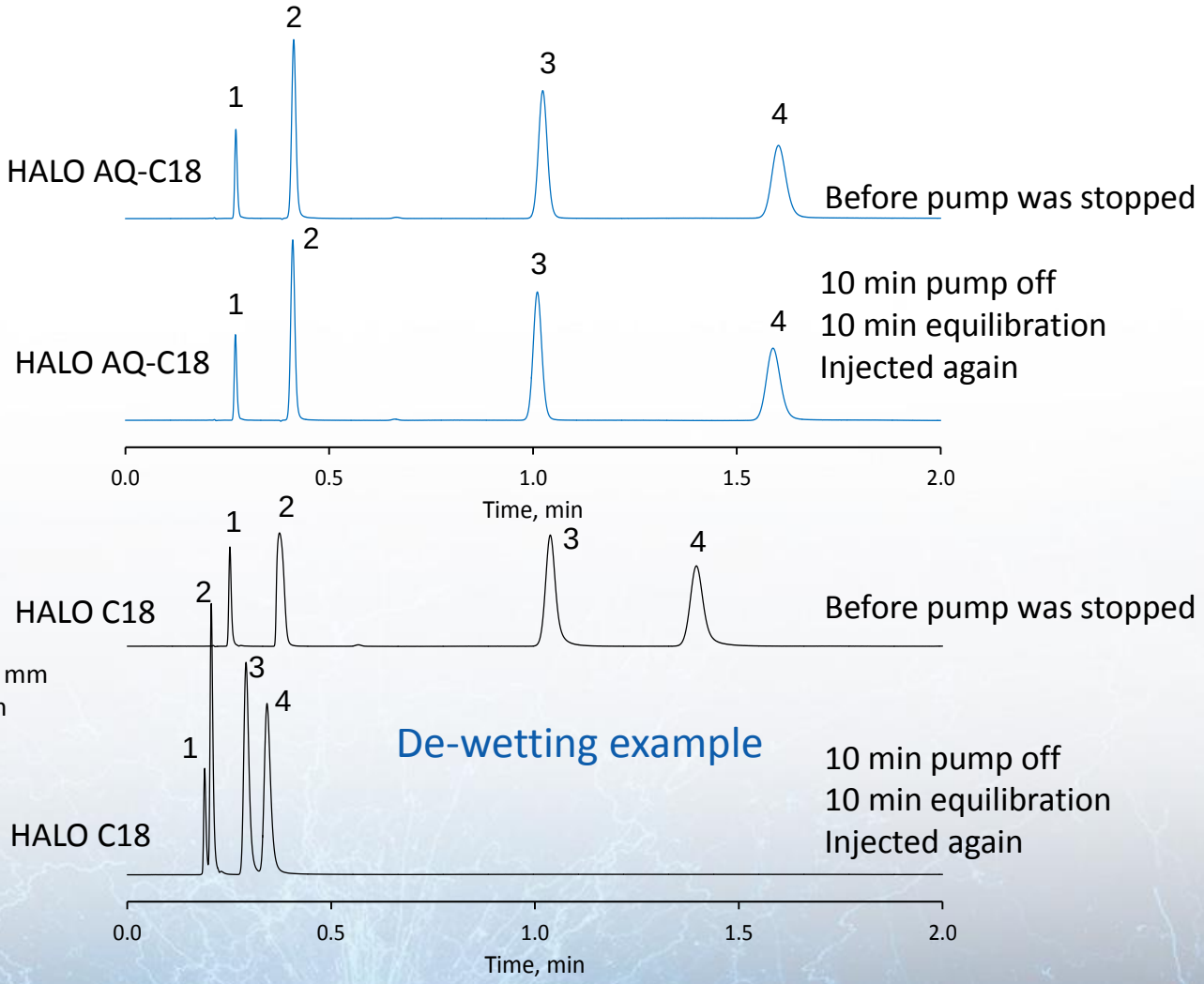


Adenine



Thymine

Columns: HALO 90Å, AQ-C18, 2.7 µm, 4.6 x 50 mm
HALO 90Å, C18, 2.7 µm, 4.6 x 50 mm
Isocratic: 20 mM Phosphate buffer, pH 7
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 ° C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL



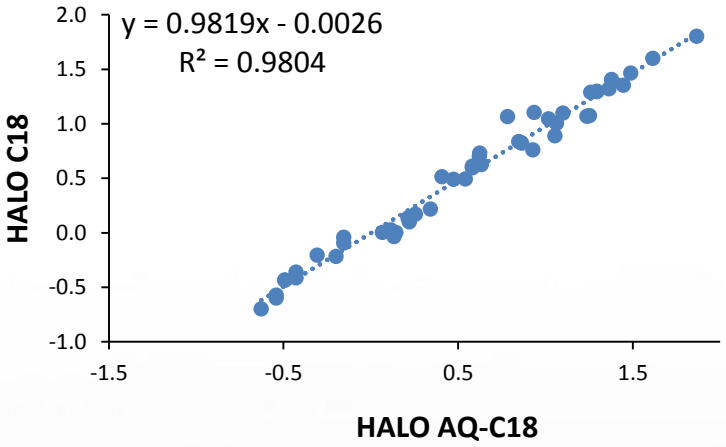
HALO AQ-C18 : DESIGNED TO BE MORE SELECTIVE FOR POLAR AND AROMATIC COMPOUNDS

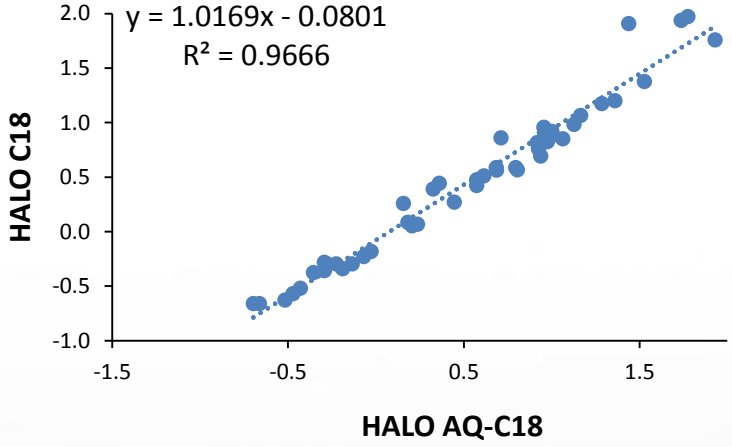
Selectivity of HALO AQ-C18 *vs* HALO C18

Acetonitrile

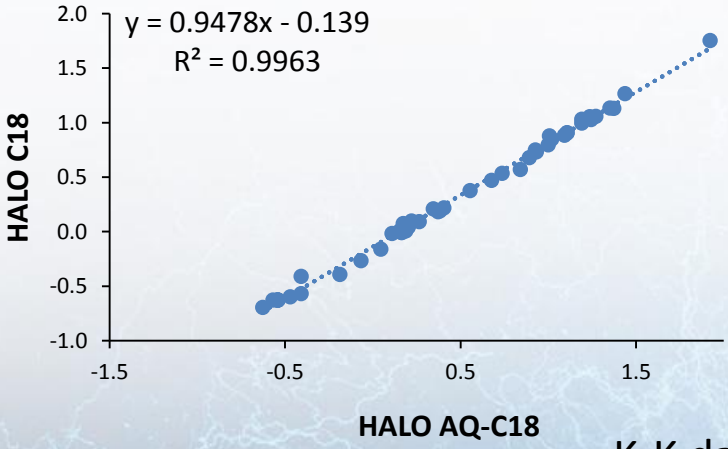
Methanol

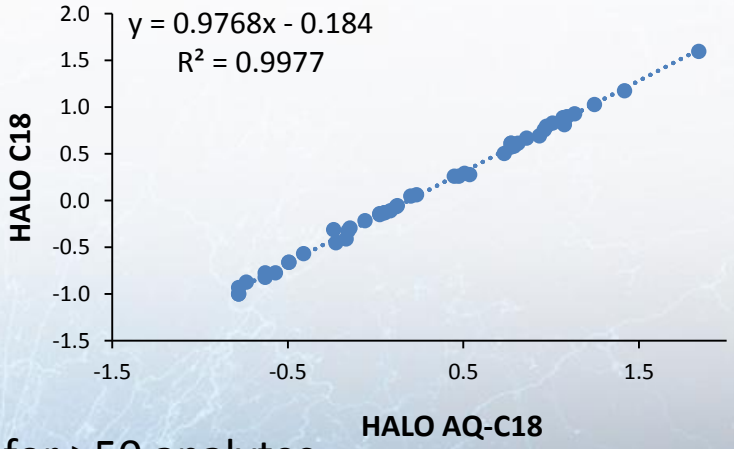
pH 7





pH 4





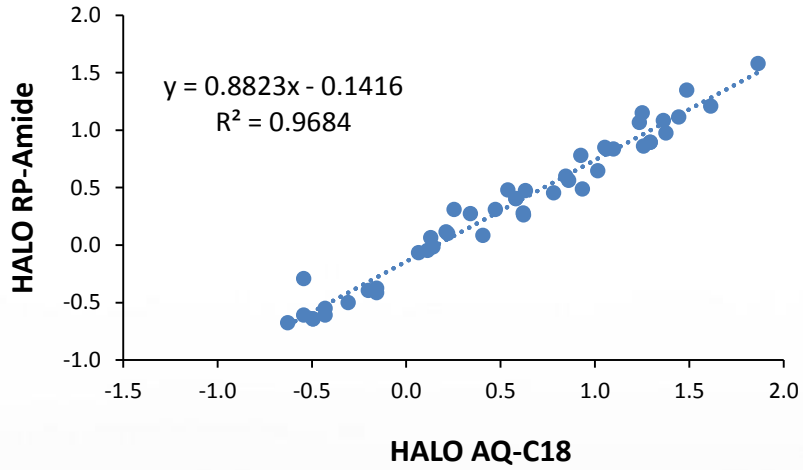
K-K data for >50 analytes provided by Millipore-Sigma

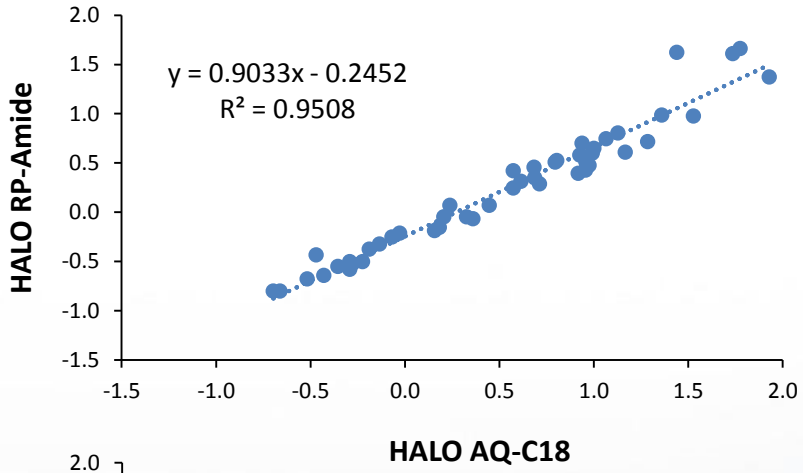
Selectivity of HALO AQ-C18 *vs* HALO RP-Amide

Acetonitrile

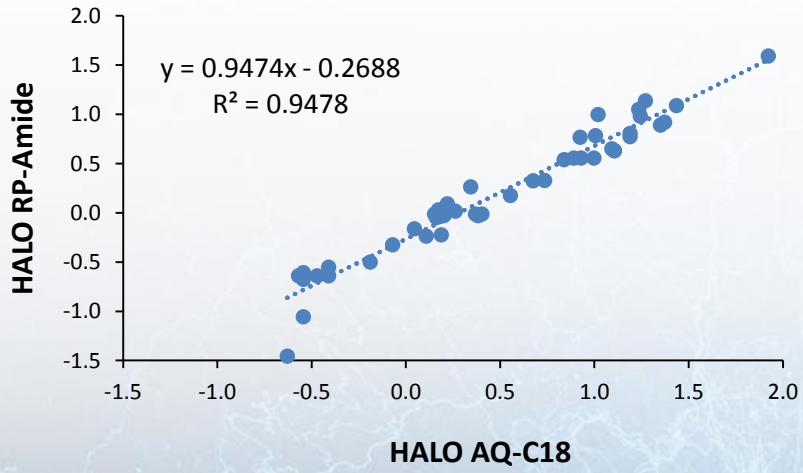
Methanol

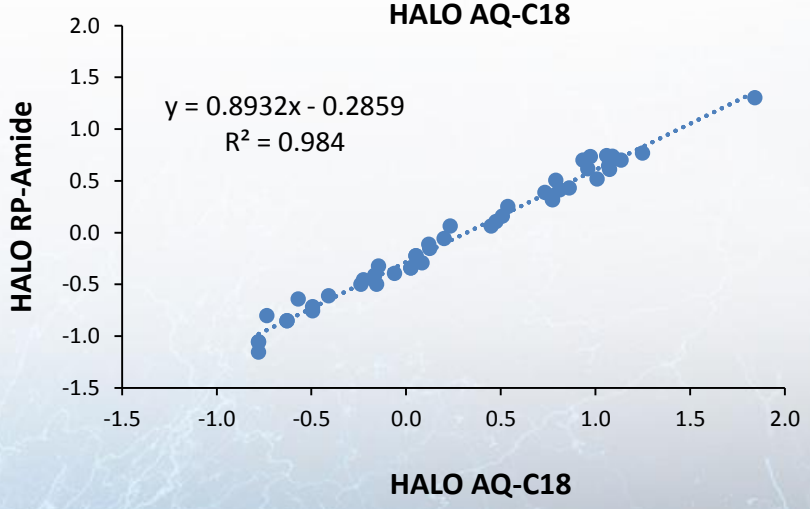
pH 7





pH 4





K-K data provided by Millipore-Sigma

Hydrophobic Subtraction Model Parameters*

F_s	Name	H	S*	A	B	C (pH 2.8)	C (pH 7.0)
0.00	HALO C18	1.10	0.04	0.00	-0.05	0.05	0.04
12.07	HALO AQ-C18	1.00	-0.04	0.10	-0.05	0.16	pending
52.83	HALO RP-Amide	0.85	0.08	-0.38	0.19	-0.41	0.31

- $F_s \leq 3$: columns are equivalent for most separations
- $3 < F_s < \sim 10$: columns are likely to be equivalent, especially for samples that contain only a few solutes with resolution, $R_s >> 2$
- $F_s \geq 35$: columns are orthogonal when the sample comprises predominantly nonionized analytes and when values of C are suppressed

Halo AQ-C18 has very similar retention characteristics to Halo C18 and clearly qualifies to be placed in the USP L1 category with other C18 columns.

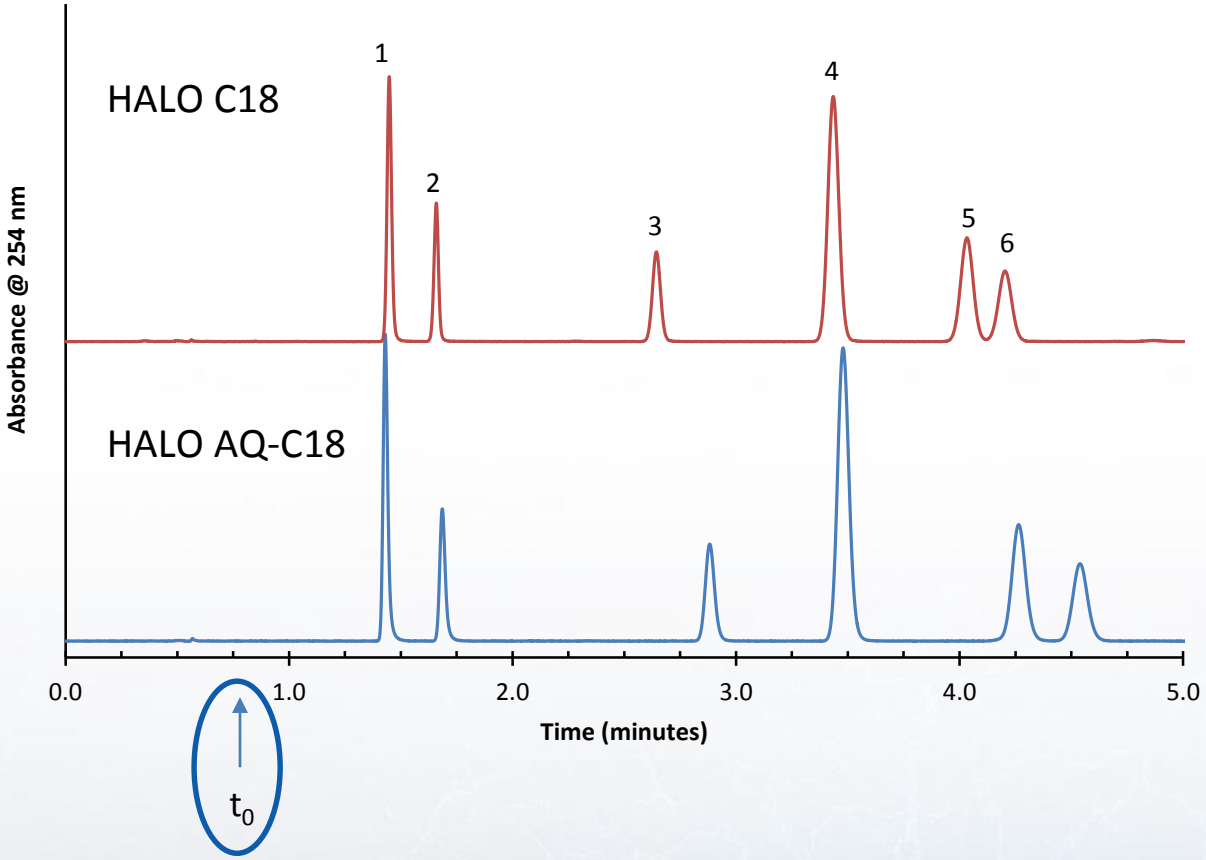
Data provided by the Stoll group at Gustavus Adolphus College. www.hplccolumns.org

*LCGC North America, Dolan and Snyder, Vol. 34, Issue 9, 730-741

Selectivity Conclusions: K-K Plots and HSM Parameters

- Choose Halo AQ-C18 to explore the solvent range of low organic or even no organic (100% aqueous) without concern for stationary phase de-wetting or major changes in C18 selectivity or retention.
- Both MeCN and MeOH should be evaluated if Halo AQ-C18 is selected to optimize a separation made on Halo C18; poorly resolved analytes may increase in resolution and in some cases may even change elution order.
- While Halo RP-Amide is also resistant to de-wetting, it is more orthogonal to both Halo C18 and Halo AQ-C18; orthogonality increases for samples that interact by hydrogen-bonding (especially strong H-bond donors).

Nearly Equivalent Selectivity in Acetonitrile



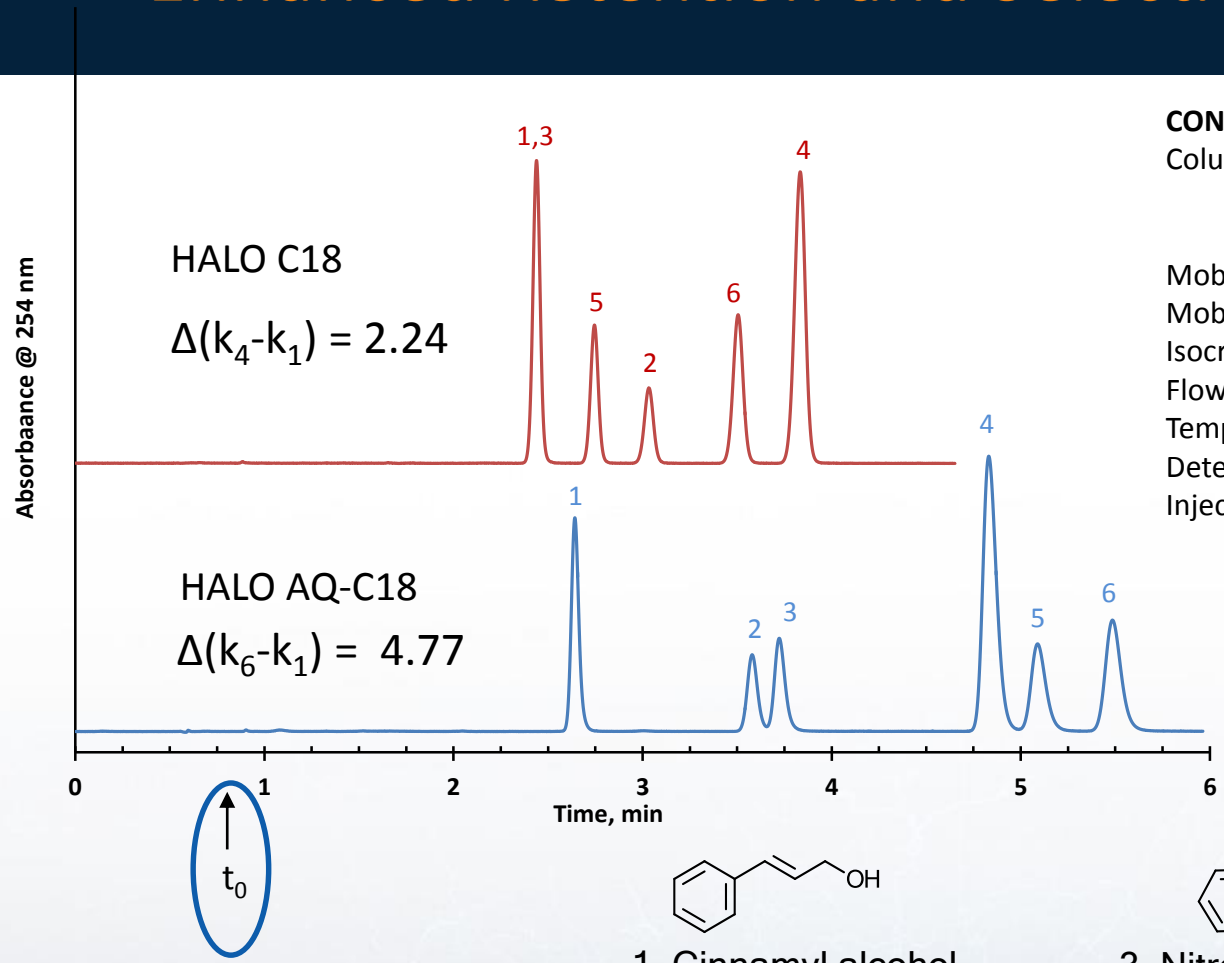
CONDITIONS:

Columns:	4.6 x 100 mm
	HALO 90Å C18, 2.7 µm (top)
	HALO 90Å AQ-C18, 2.7 µm (bottom
Mobile Phase A:	water
Mobile Phase B:	acetonitrile
Isocratic:	60/40 A/B instrument mixed
Flow Rate:	1.8 mL/min
Temperature:	30 °C
Detection:	254 nm
Injection:	0.5 µL

PEAK IDENTITIES

1. Cinnamyl alcohol
2. 4'-bromoacetanilide
3. Nitrobenzene
4. Anisole
5. 2,4-dinitrotoluene
6. 3,4-dinitrotoluene

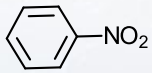
Enhanced Retention and Selectivity in Methanol



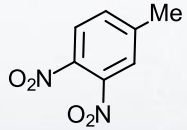
CONDITIONS:

Columns:	4.6 x 100 mm
	HALO 90Å C18, 2.7 µm (top)
	HALO 90Å AQ-C18, 2.7 µm (bottom)
Mobile Phase A:	water
Mobile Phase B:	methanol
Isocratic:	48/52 A/B instrument mixed
Flow Rate:	1.4 mL/min
Temperature:	30 °C
Detection:	254 nm
Injection:	0.5 µL

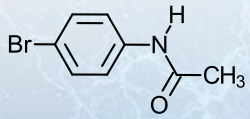
1. Cinnamyl alcohol



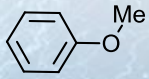
3. Nitrobenzene



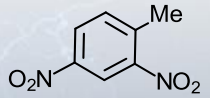
5. 3,4-Dinitrotoluene



2. 4-Bromoacetanilide



4 . Anisole



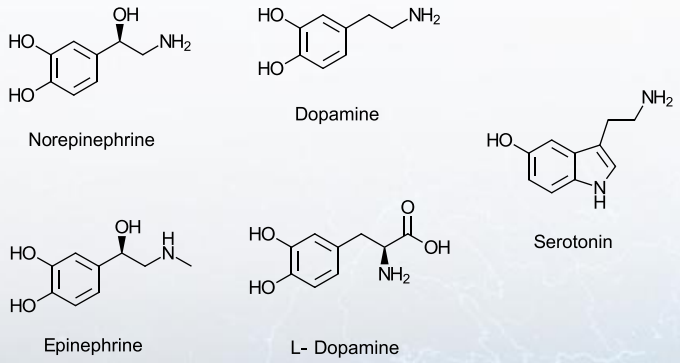
6. 2,4-Dinitrotoluene

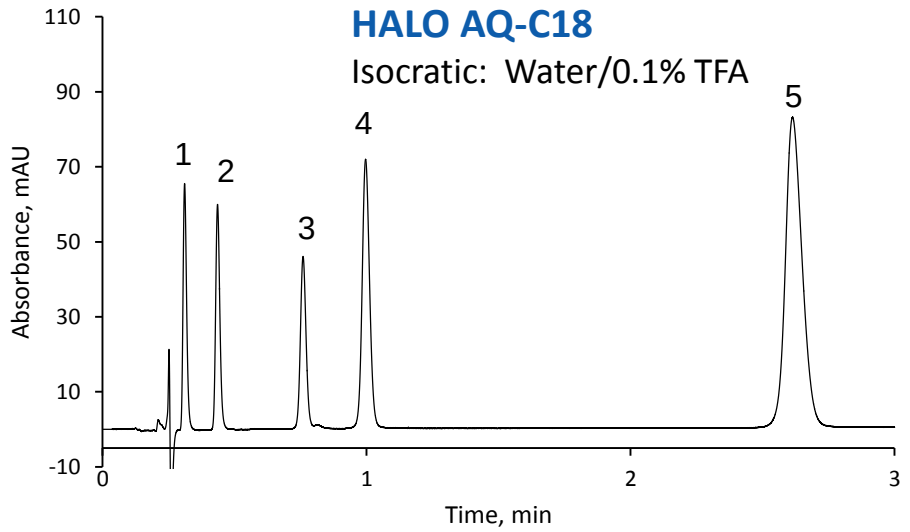
HALO AQ-C18: APPLICATIONS

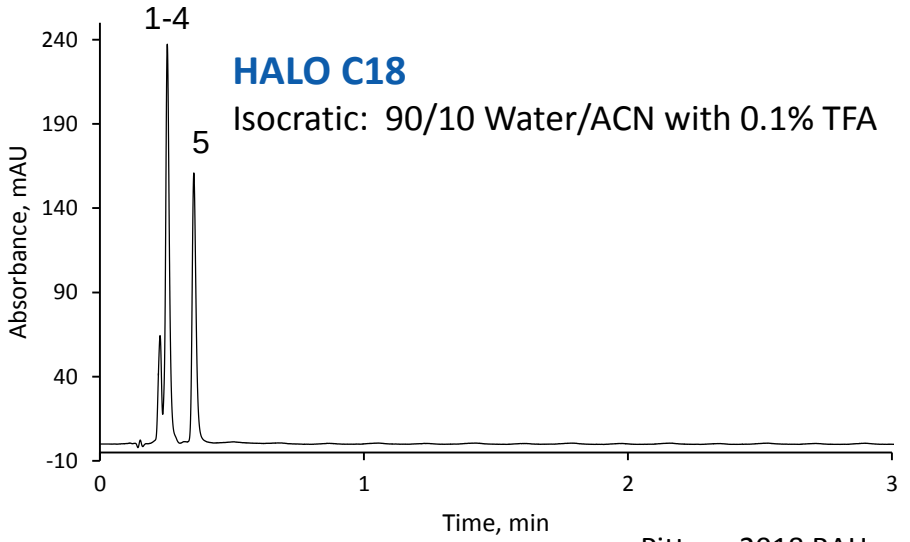
Brain Neurotransmitters

Columns: HALO 90Å, 2.7 µm, 2.1 x 50 mm
Flow Rate: 0.5 mL/min
Temperature: 30 °C
Detection: UV 210 nm, PDA
Injection Volume: 1 µL

1. Norepinephrine
2. Epinephrine
3. Dopamine
4. L-Dopa
5. Serotonin







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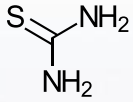
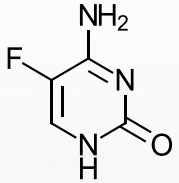
Nucleic Acid Bases

1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine

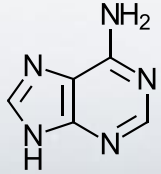
Columns: HALO 90Å, AQ-C18, 2.7 µm, 4.6 x 50 mm
HALO 90Å, RP-Amide, 2.7 µm, 4.6 x 50 mm
Isocratic: Water/0.1% TFA, pH 2
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 ° C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL



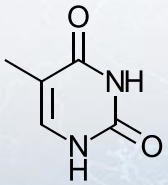
Thiourea



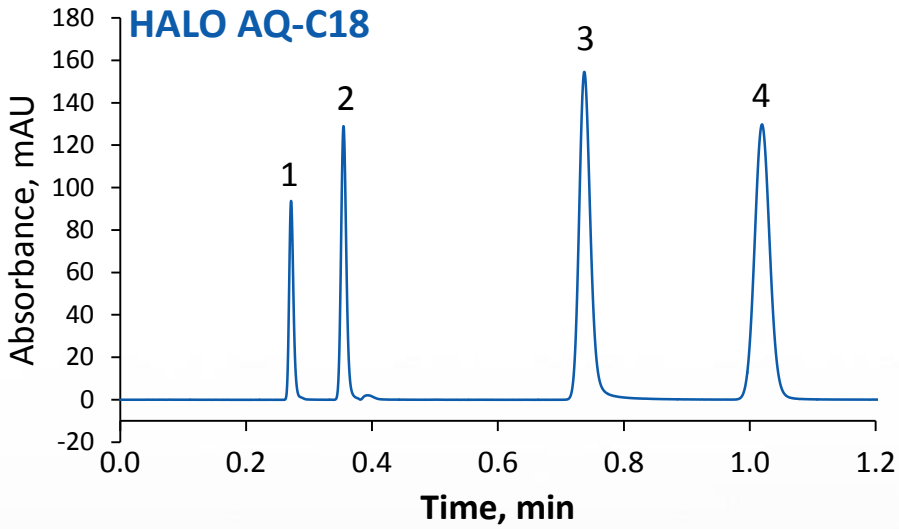
5-Fluorocytosine

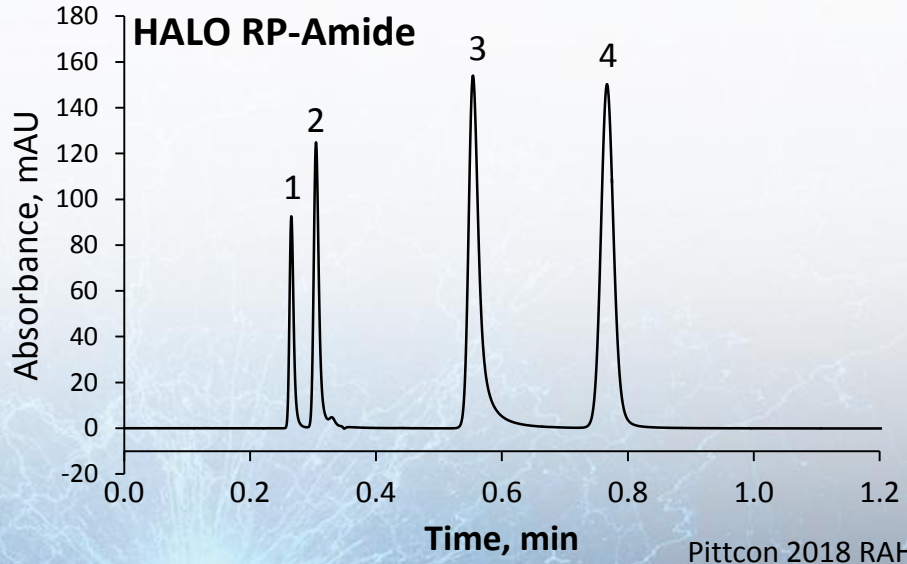


Adenine



Thymine



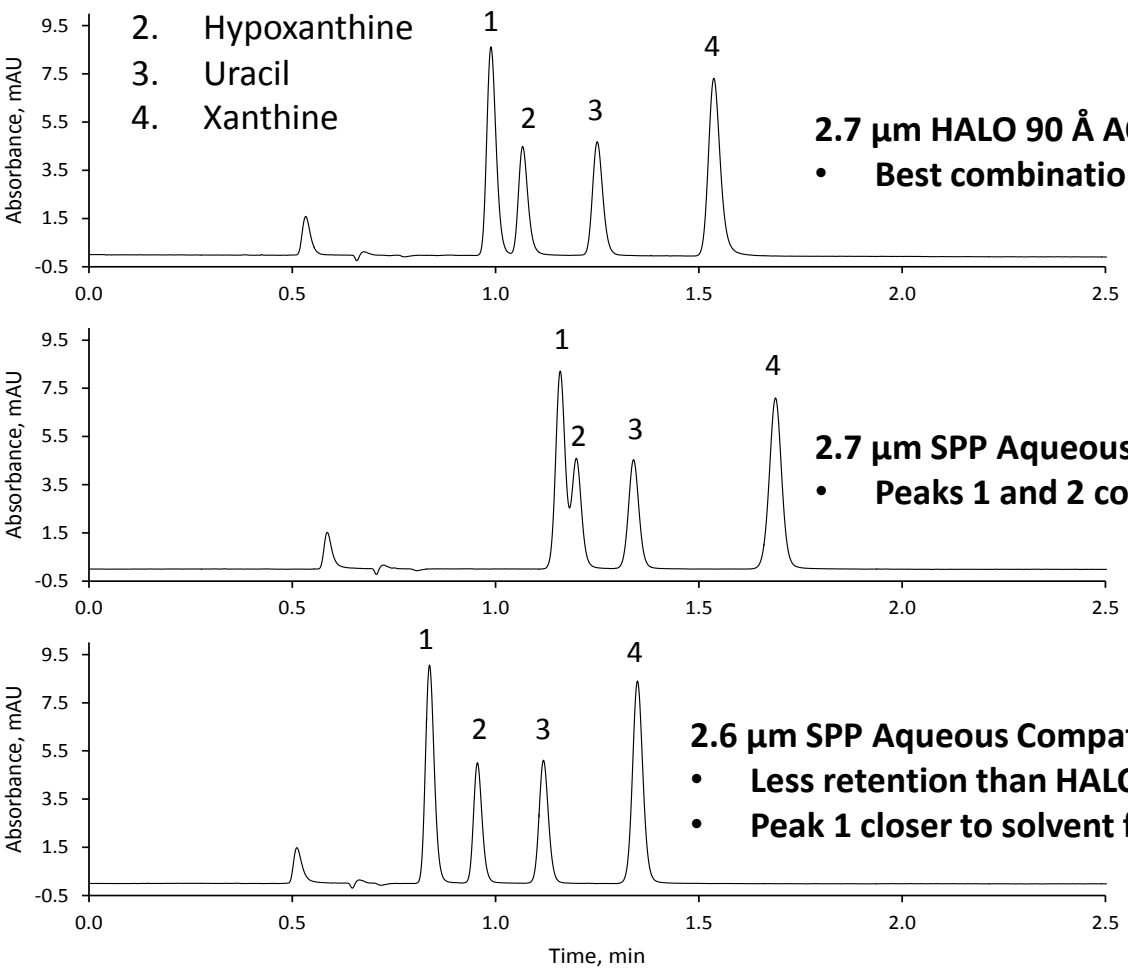


advancedmaterialstechnology

Comparison of Purines with Other 100% Aqueous-Compatible SPP Phases

2.1 x 100 mm, 0.1% DFA in water, 0.35 mL/min, 35 °C, 1 µl @ 10 mM each in 0.1% DFA, UV detection @ 265 nm

1. Guanine
2. Hypoxanthine
3. Uracil
4. Xanthine

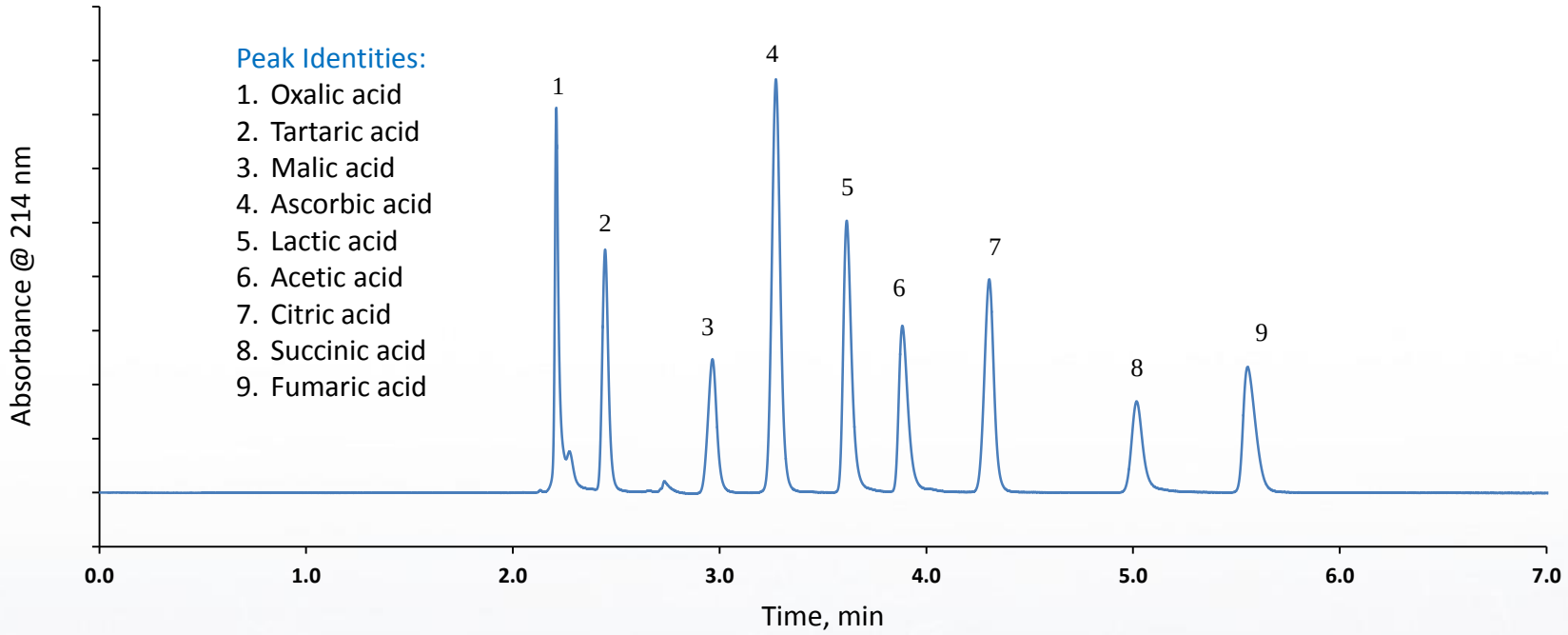


2.7 µm HALO 90 Å AQ-C18
- **Best combination of resolution and retention**

2.7 µm SPP Aqueous Compatible Phase
- **Peaks 1 and 2 coeluted**

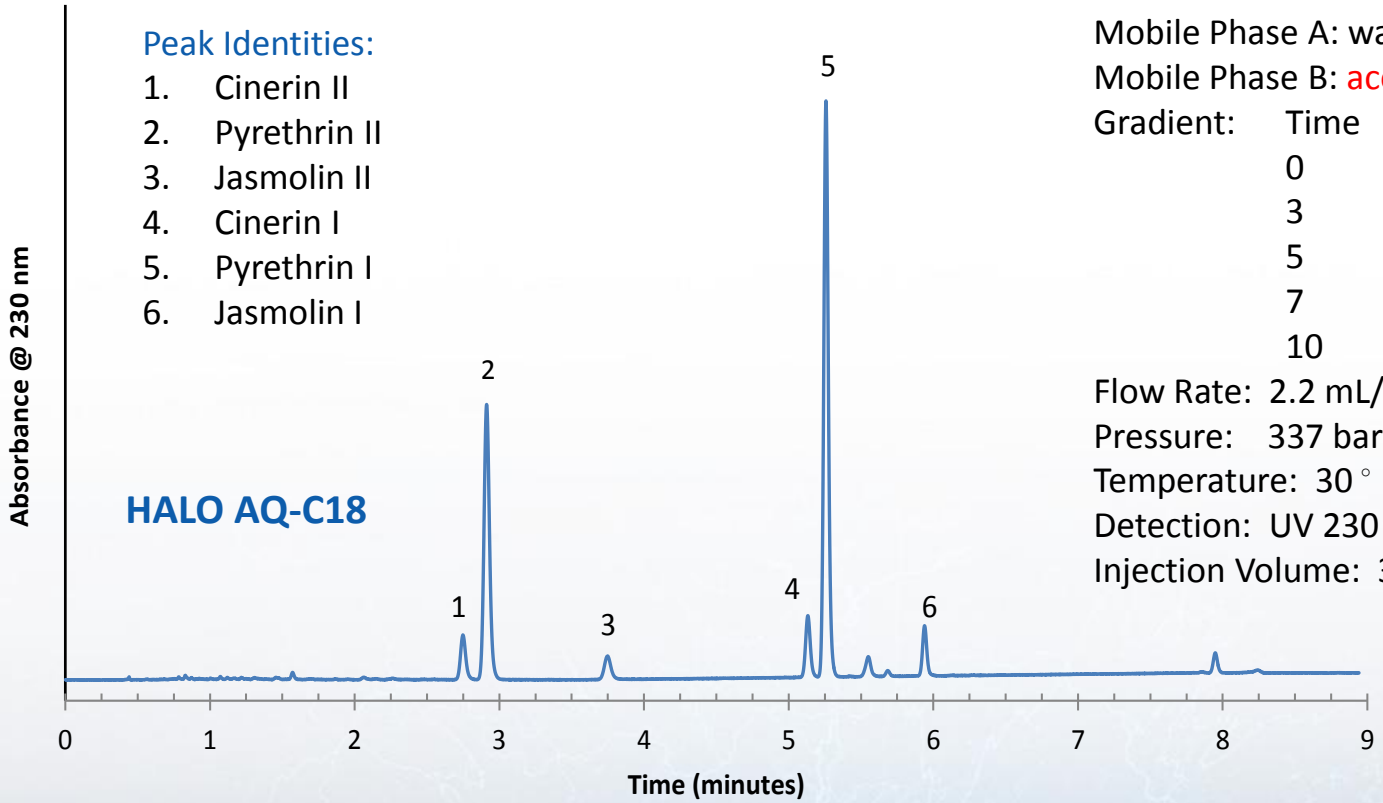
2.6 µm SPP Aqueous Compatible Phase
- **Less retention than HALO AQ-C18**
- **Peak 1 closer to solvent front**

Organic Acids



TEST CONDITIONS:
Column: HALO 90Å, AQ-C18, 2.7 µm, 4.6 x 250mm
Isocratic: 20mM Potassium Phosphate buffer pH: 2.7
Flow Rate: 1.0 mL/min
Pressure: 307 bar
Temperature: 40 °C
Detection: UV 214 nm, PDA
Injection Volume: 20 µL
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

Pyrethrins

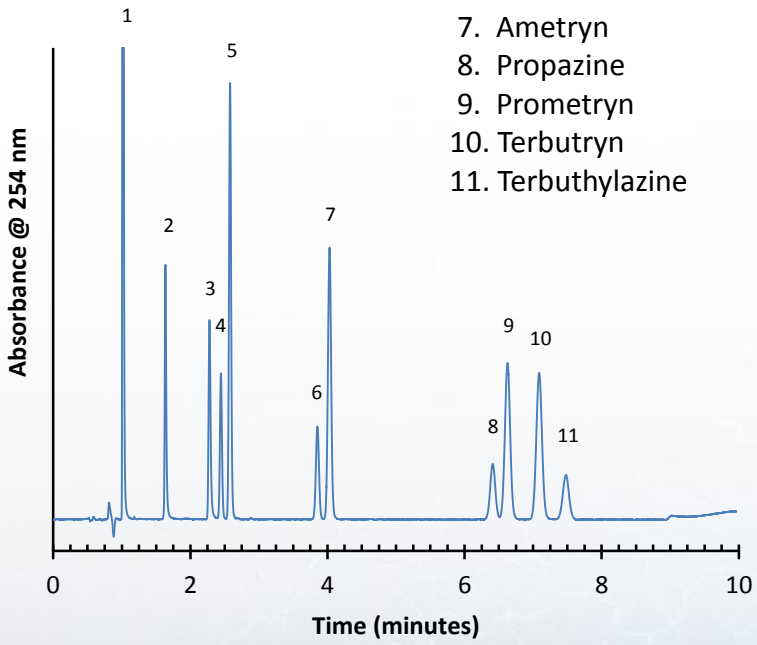


Column: HALO 90Å, AQ-C18, 2.7 µm, 4.6 x 150 mm
Mobile Phase A: water
Mobile Phase B: acetonitrile

Gradient:	Time	%B
	0	67
	3	67
	5	80
	7	90
	10	90

Flow Rate: 2.2 mL/min
Pressure: 337 bar
Temperature: 30 ° C
Detection: UV 230 nm, PDA
Injection Volume: 3 µL

Triazine Pesticides



Peak Identities:

1. Acetone (solvent)
2. Atraton
3. Prometon
4. Simazine
5. Simetryn
6. Atrazine
7. Ametryn
8. Propazine
9. Prometryn
10. Terbutryn
11. Terbuthylazine

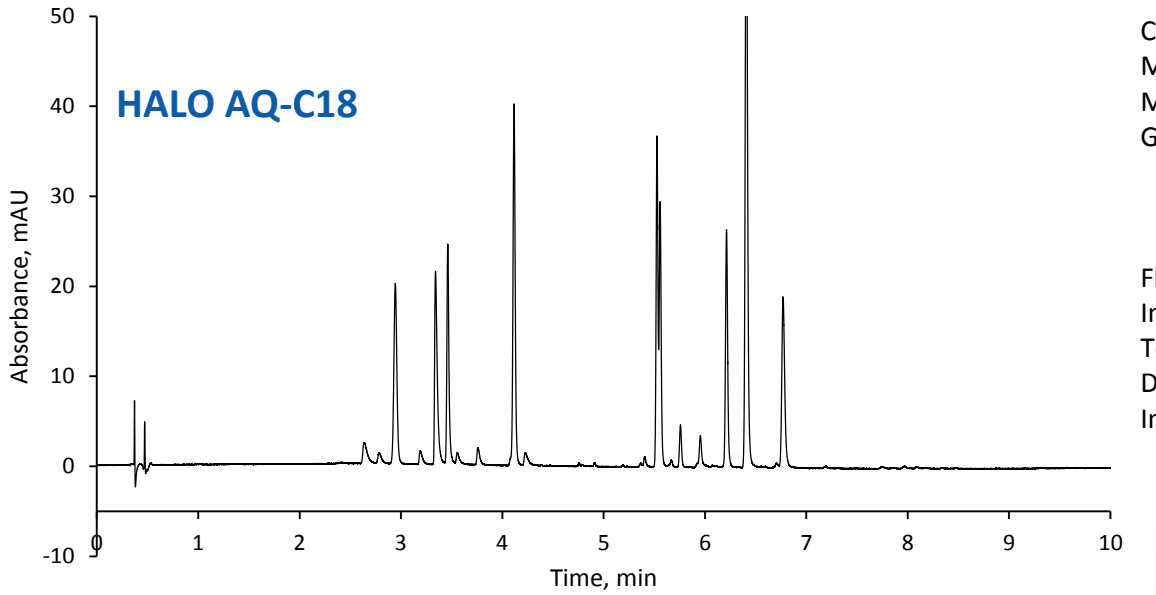
TEST CONDITIONS:

Column: HALO AQ-C18, 2.7 µm, 4.6 x 150 mm
Mobile Phase:
A= 0.02 M sodium phosphate buffer, pH=3.0
B= Acetonitrile
Mobile phase::

Time	%B
0.0	40
8.0	40
10.0	75

Flow Rate: 1.6 mL/min.
Pressure: 310 bar at start
Temperature: 35°C
Detection: UV 254 nm, VWD
Injection Volume: 2.0 µL
Sample Solvent: 25/75: acetone/acetonitrile
Response Time: 0.02 sec.
Data rate: 25 Hz.
Flow Cell: 2.5 µL semi-micro
LC System: Shimadzu Prominence UFLC XR

Complex Drug Mix with Acetonitrile Gradient



Column: HALO 90Å, AQ-C18, 2.7 µm, 3.0 x 50 mm
Mobile Phase A: 10 mM ammonium acetate, pH 5.75
Mobile Phase B: acetonitrile

Gradient:	Time	%B
	0	2
	1	2
	11	90

Flow Rate: 0.6 mL/min
Initial Pressure: 115 bar
Temperature: 30 ° C
Detection: UV 254 nm, PDA
Injection Volume: 2 µL

Sample Contains:

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

Summary

- HALO AQ-C18 is a stable, reproducible bonded phase that is compatible with 100% aqueous mobile phases as well as combinations with organic modifiers such as MeOH, MeCN, and others.
- A proprietary polar component has been added to the C18 stationary phase to increase the aqueous wettability of the surface and impart reproducible, unique selectivity to the stationary phase.
- When slightly different resolution is needed for polar analytes, HALO AQ-C18 should be evaluated under both MeCN and MeOH conditions.

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