



Superficially Porous Particles with Polar C18 Selectivity

Richard A. Henry, Technical Consultant,
983 Greenbriar Drive, State College, PA 16801
(hyperLC@comcast.net)

Stephanie A. Schuster, Chuping Luo, Conner W. McHale, Robert E. Moran,
William L. Johnson

Advanced Materials Technology, Inc.
3521 Silverside Road, Suite 1-K, Quillen Bldg.
Wilmington, DE 19810

Overview

- HALO AQ-C18

- Objectives

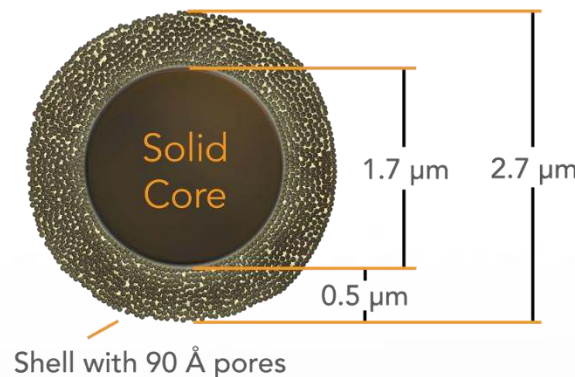
- C18 stability and ease of use
 - C18 reproducibility
 - Extended use to 100% aqueous and low organic mobile phases
 - Alternate selectivity for polar molecules vs pure C18
 - Become equally useful in both methanol and acetonitrile mobile phases

- Applications

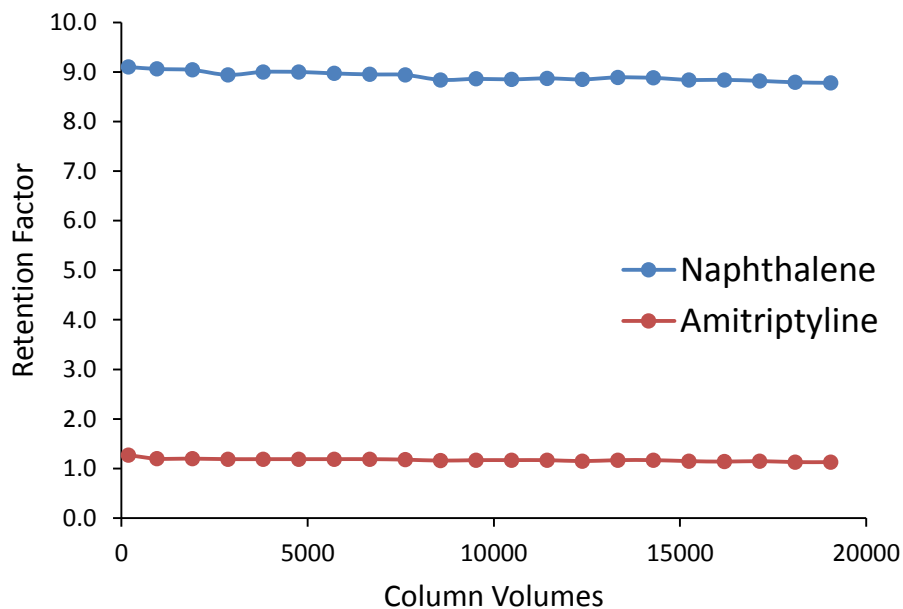
- Brain Neurotransmitters
 - Nucleic Acid Bases
 - Cannabinoids
 - Pyrethrins
 - Organic Acids
 - Drugs and Metabolites

HALO AQ-C18

- Particle type:
 - Type B superficially porous silica
 - 2.7 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
 - Phase chemistry: C18 with novel addition of dipole character (proprietary treatment to increase versatility)



HALO AQ-C18 Stability

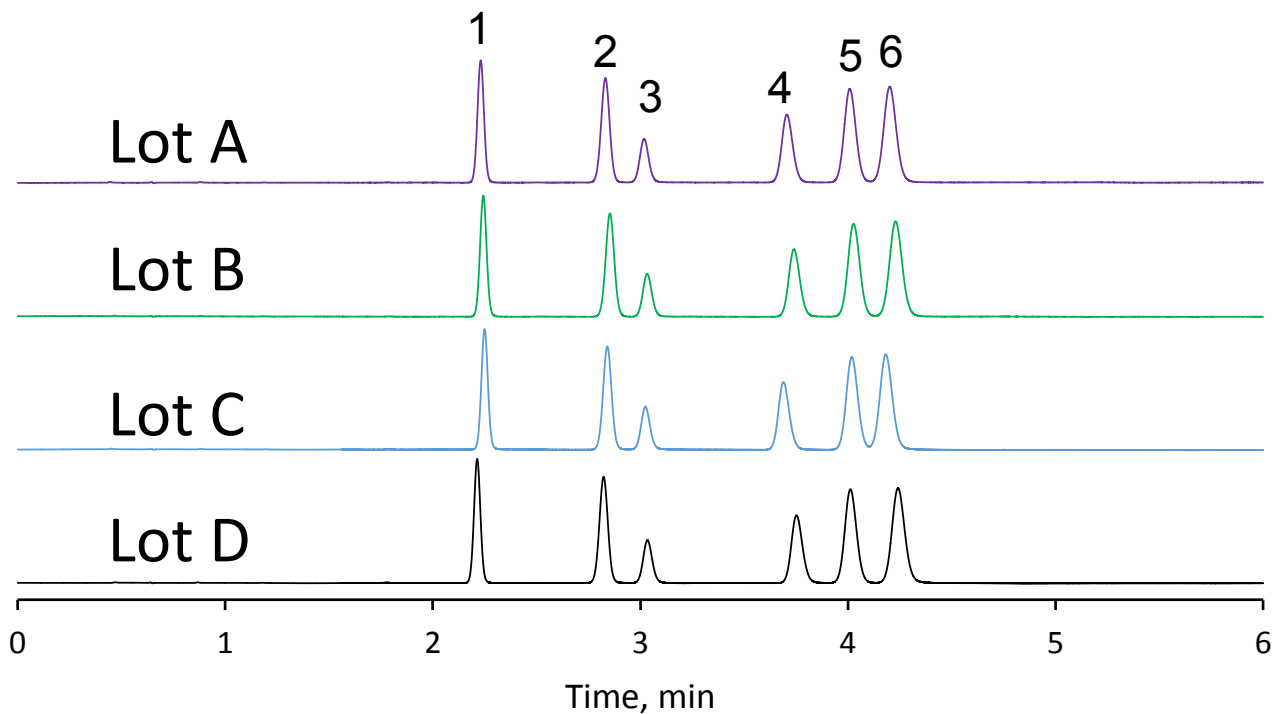


Minimal retention loss observed after ~ 19,000 column volumes

Column: 2.1 x 50 mm
HALO 90Å AQ-C18, 2.7 µm
Part Number: 92812-422
Mobile Phase A: 50 mM KCl/HCl, pH 2
Mobile Phase B: acetonitrile
Isocratic: 60/40 A/B

Flow Rate: 1.0 mL/min
Pressure: 235 bar
Temperature: 60 °C
Detection: 254 nm
Injection: 0.5 µL
Sample: uracil, amitriptyline, naphthalene

HALO AQ-C18: Lot-to-Lot Reproducibility



Sample:

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

Columns: 4.6 x 100 mm
HALO 90Å AQ-C18, 2.7 µm

Part Number: 92814-622

Isocratic: 50/50 water/methanol

Flow Rate: 1.3 mL/min

Temperature: 35 °C

Detection: 254 nm

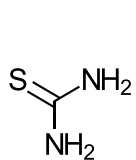
Injection: 0.5 µL

- Retention factor %RSDs < 1.5%
- Alpha values %RSDs < 1%

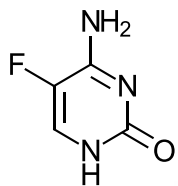
HALO AQ-C18: RESISTS DEWETTING

pH 2 Dewetting results: Nucleic Acid Bases

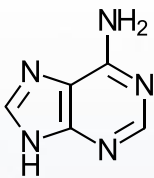
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



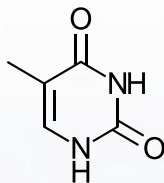
Thiourea



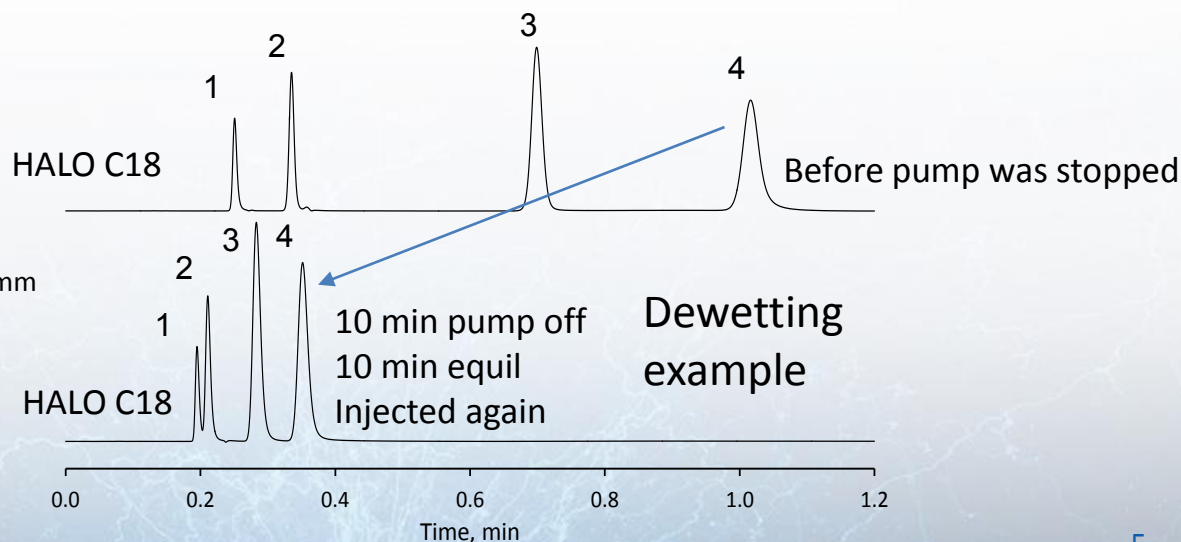
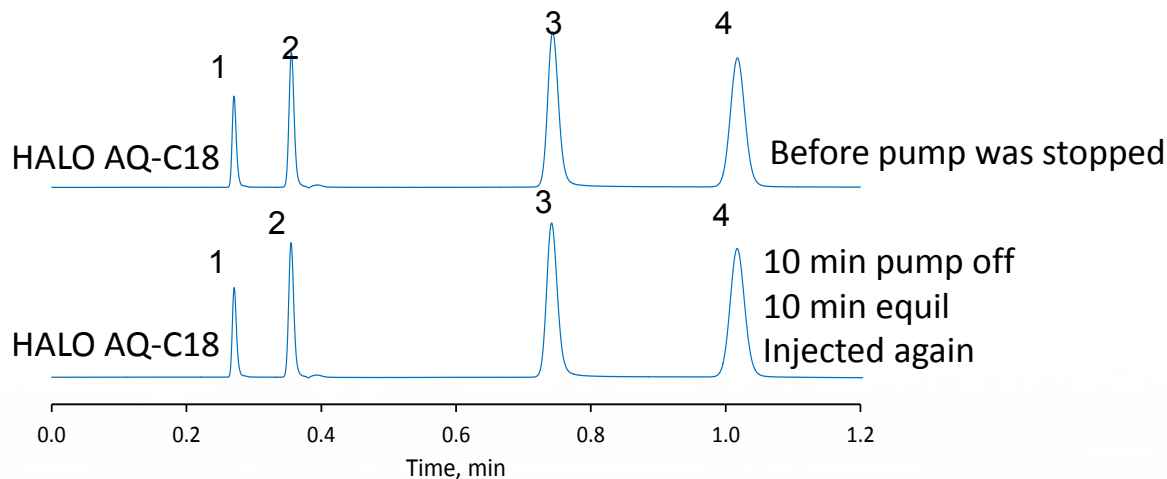
5-Fluorocytosine



Adenine



Thymine



Columns: HALO 90Å, AQ-C18, 2.7 μ m, 4.6 x 50 mm
 HALO 90Å, C18, 2.7 μ m, 4.6 x 50 mm

Isocratic: Water/0.1% TFA

Flow Rate: 2.0 mL/min

Pressure: 290 bar

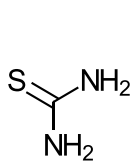
Temperature: 30 °C

Detection: UV 254 nm, PDA

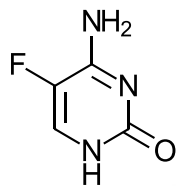
Injection Volume: 0.5 μ L

pH 7 Dewetting results: Nucleic Acid Bases

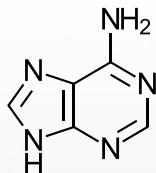
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



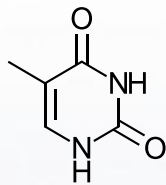
Thiourea



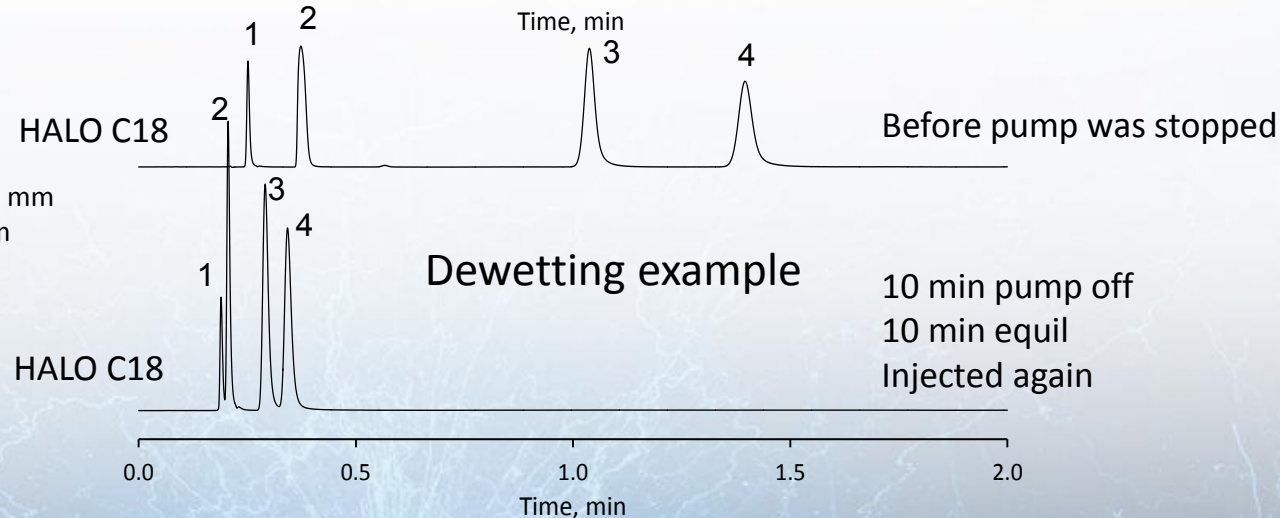
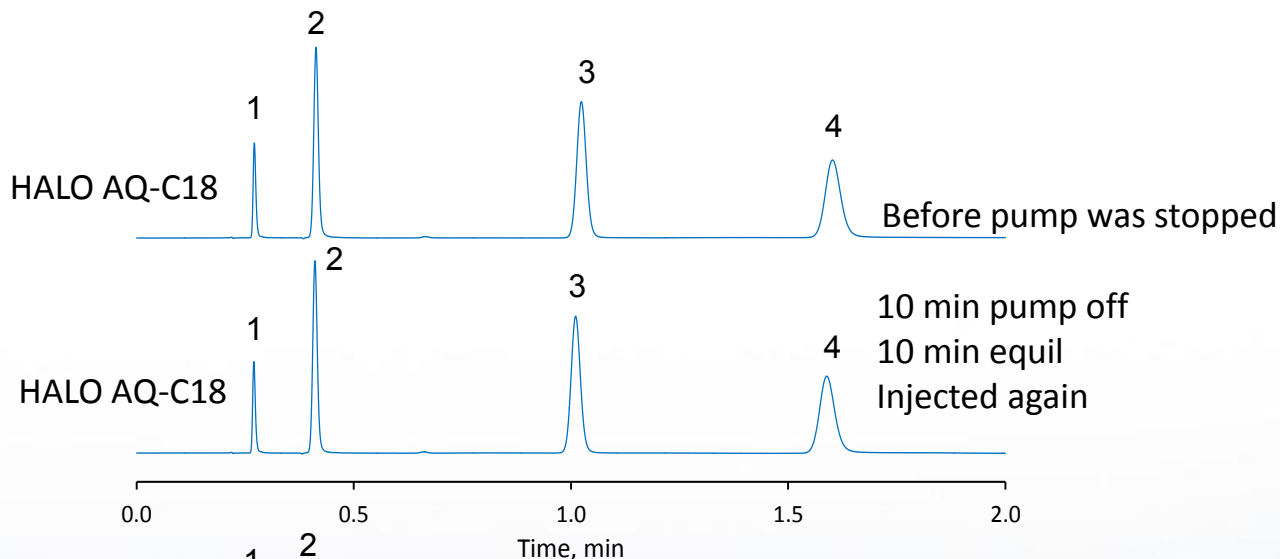
5-Fluorocytosine



Adenine



Thymine



Columns: HALO 90Å, AQ-C18, 2.7 μ m, 4.6 x 50 mm

HALO 90Å, C18, 2.7 μ m, 4.6 x 50 mm

Isocratic: 20 mM Phosphate buffer, pH 7

Flow Rate: 2.0 mL/min

Pressure: 290 bar

Temperature: 30 °C

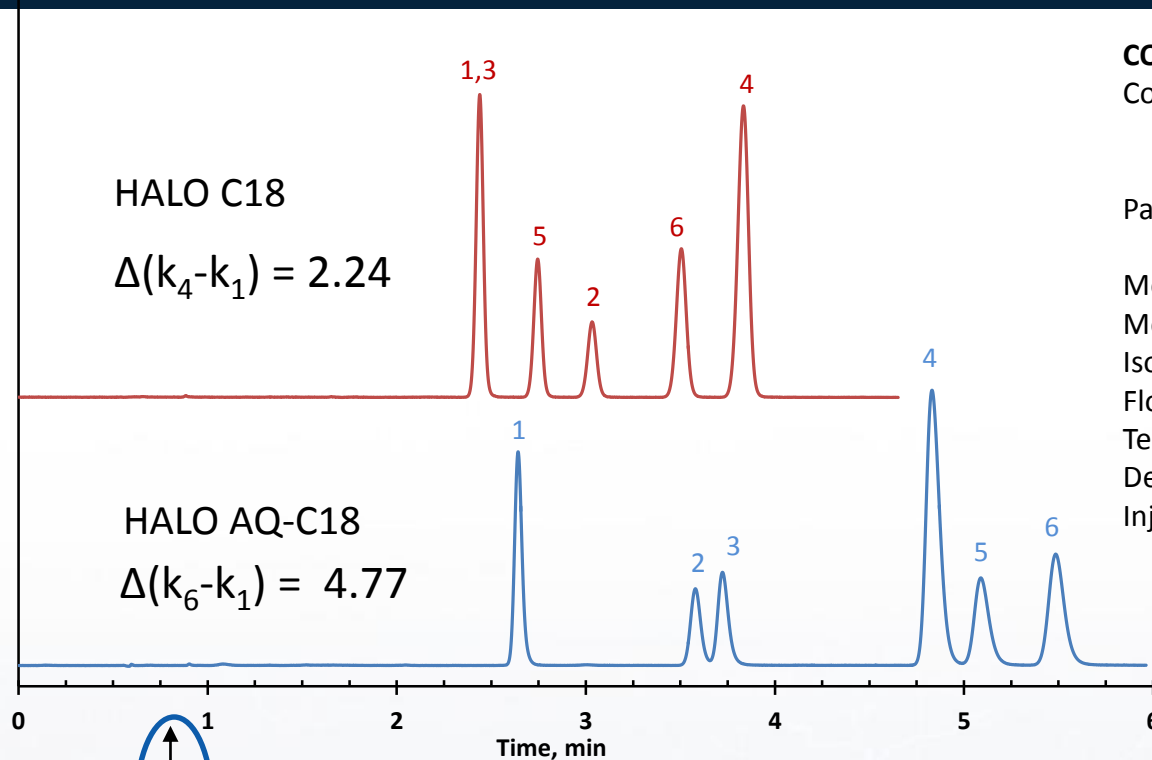
Detection: UV 254 nm, PDA

Injection Volume: 0.5 μ L

HALO AQ-C18: ENHANCED POLAR SELECTIVITY

Enhanced Selectivity Window for Polar Groups

Absorbance @ 254 nm



CONDITIONS:

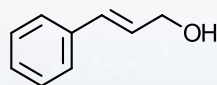
Columns: 4.6 x 100 mm
 HALO 90Å C18, 2.7 μ m (top)
 HALO 90Å AQ-C18, 2.7 μ m (bottom)

Part Numbers: 92814-602
 92814-622

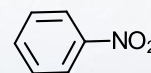
Mobile Phase A: water
 Mobile Phase B: **methanol**

Isocratic: 48/52 A/B instrument mixed
 Flow Rate: 1.4 mL/min
 Temperature: 30 °C
 Detection: 254 nm
 Injection: 0.5 μ L

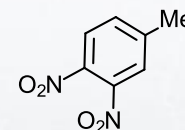
t_0



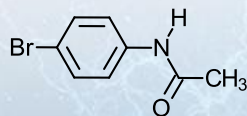
1. Cinnamyl alcohol



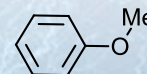
3. Nitrobenzene



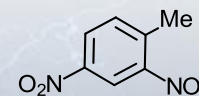
5. 3,4-Dinitrotoluene



2. 4-Bromoacetanilide

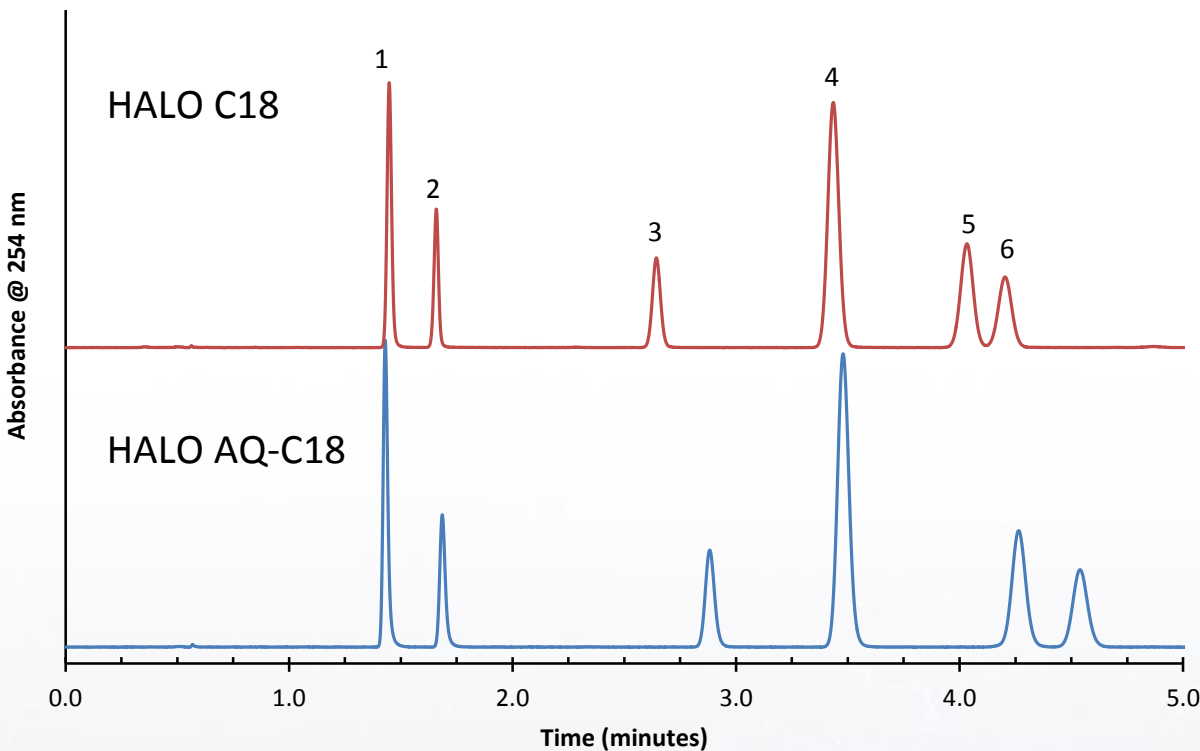


4. Anisole



6. 2,4-Dinitrotoluene

Comparison of HALO AQ-C18 vs. HALO C18 using Acetonitrile



CONDITIONS:

Columns: 4.6 x 100 mm
HALO 90Å C18, 2.7 μm (top)
HALO 90Å AQ-C18, 2.7 μm (bottom)

Part Numbers: 92814-602
92814-622

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

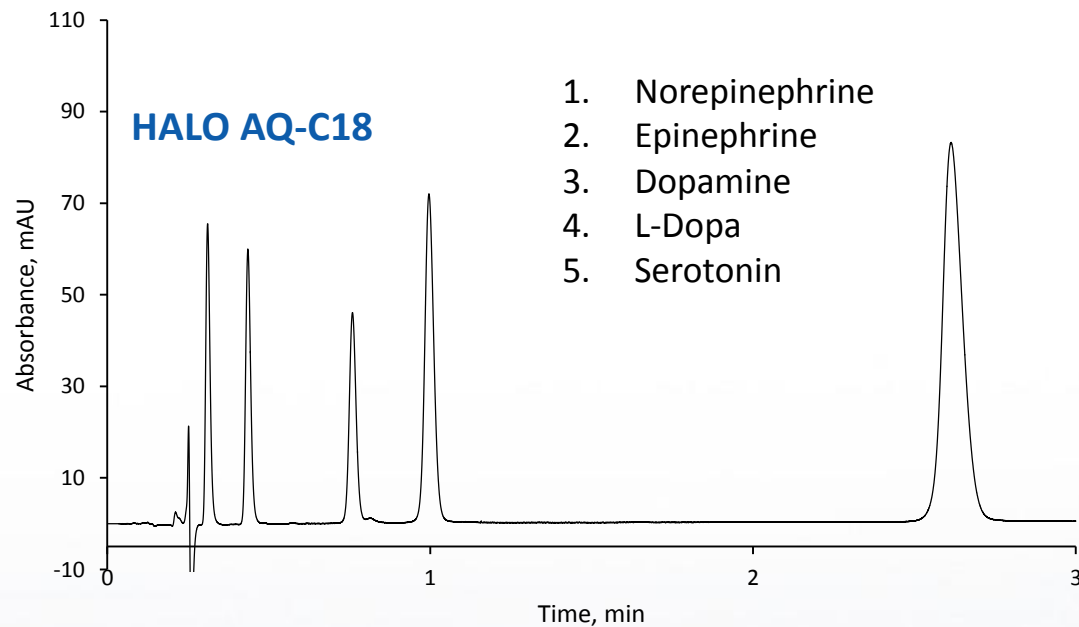
PEAK IDENTITIES

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

HALO AQ-C18: APPLICATIONS

Brain Neurotransmitters

Column: HALO 90Å, AQ-C18, 2.7 μm , 2.1 x 50 mm
Isocratic: **Water/0.1% TFA**
Flow Rate: 0.5 mL/min
Pressure: 185 bar
Temperature: 30 ° C
Detection: UV 254 nm, PDA
Injection Volume: 1 μL



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**

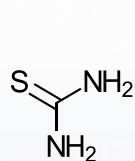
Flow Rate: 2.0 mL/min

Pressure: 290 bar

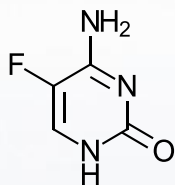
Temperature: 30 ° C

Detection: UV 254 nm, PDA

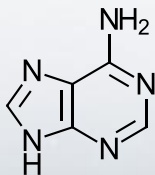
Injection Volume: 0.5 µL



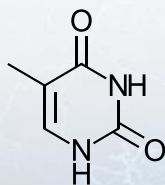
Thiourea



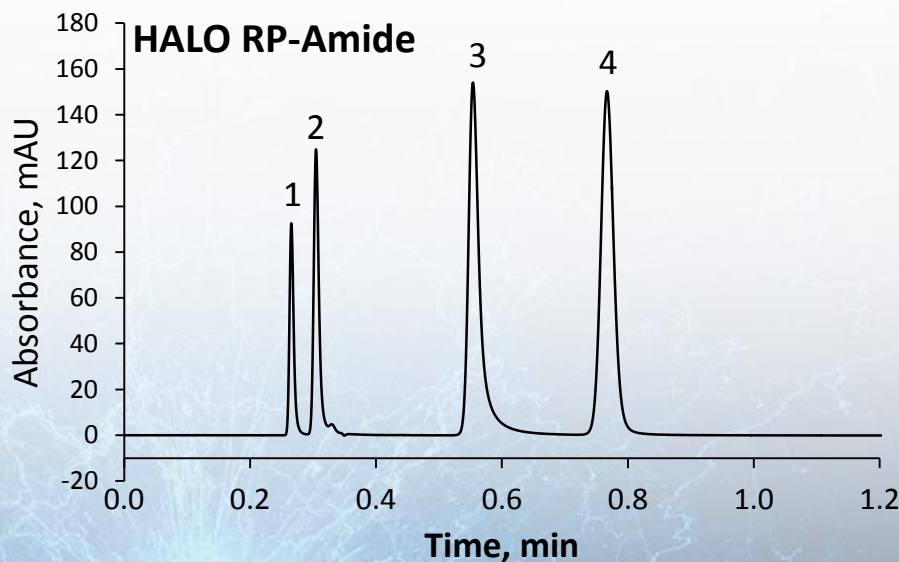
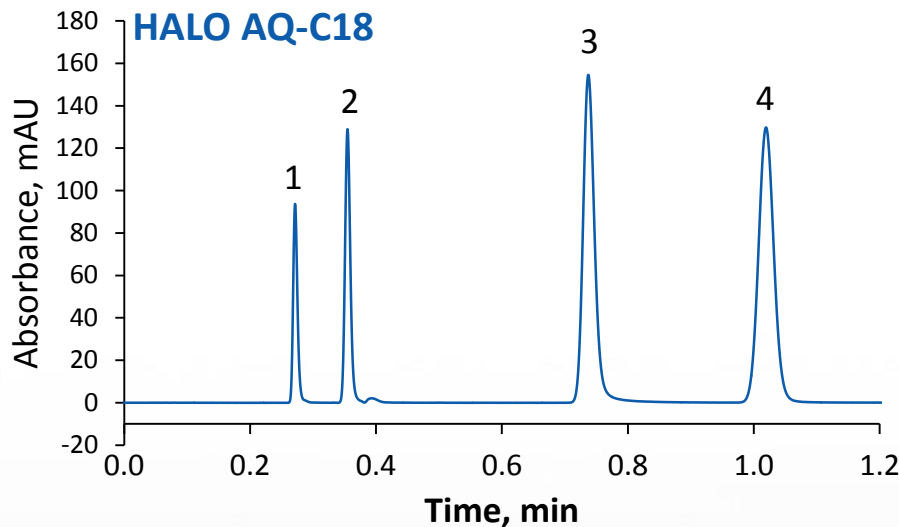
5-Fluorocytosine



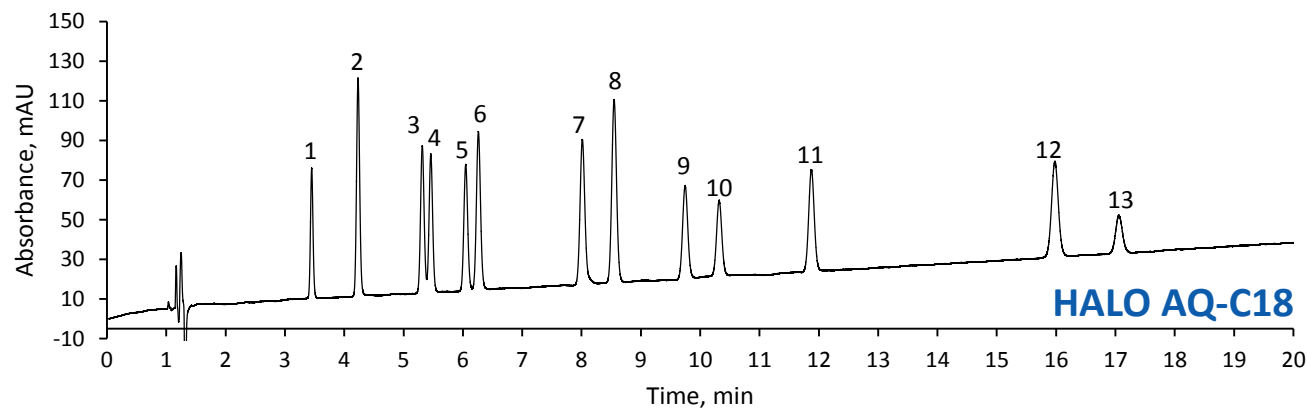
Adenine



Thymine

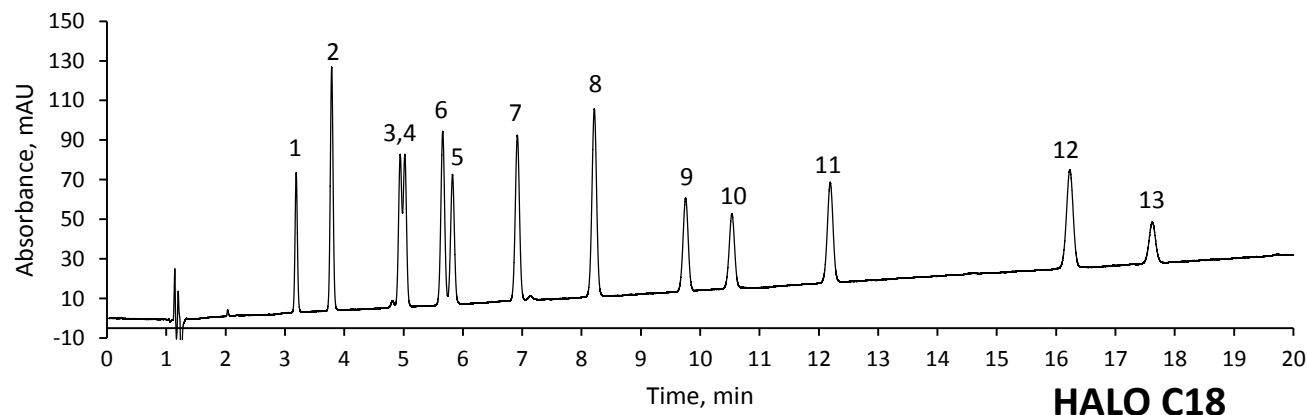


Cannabinoids: Methanol Mobile Phase Conditions



PEAK IDENTITIES

1. CBDVA
2. CBDV
3. CBDA
4. CBGA
5. CBD
6. CBG
7. THCV
8. CBN
9. Δ^9 -THC
10. Δ^8 -THC
11. CBC
12. THCA
13. CBCA



Columns: HALO 90Å, AQ-C18, 2.7 μ m, 3 x 150 mm

HALO 90Å, C18, 2.7 μ m, 3 x 150 mm

Mobile Phase A: Water/0.1% formic acid

Mobile Phase B: **Methanol/0.085% formic acid**

Gradient: 80-100% B in 38 min

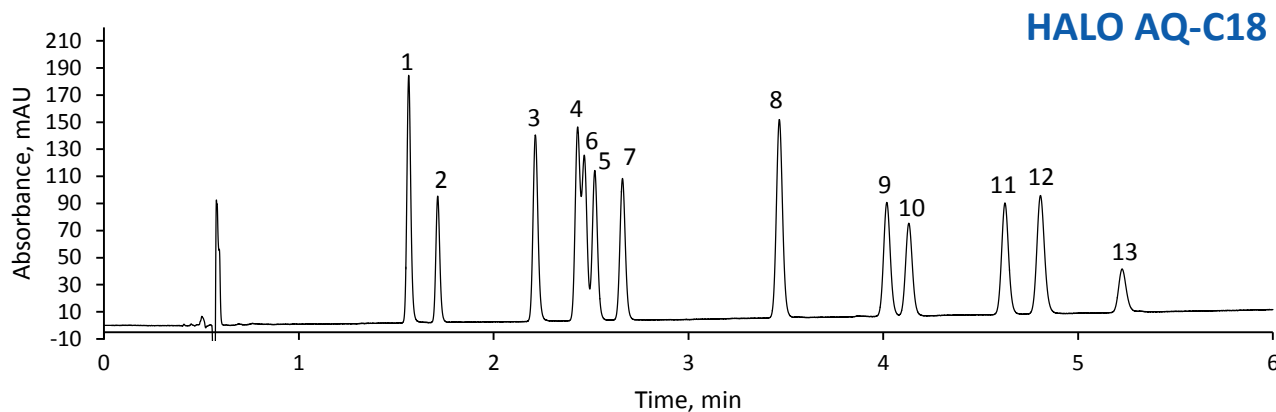
Flow Rate: 0.5 mL/min

Temperature: 32 °C

Detection: UV 220 nm, PDA

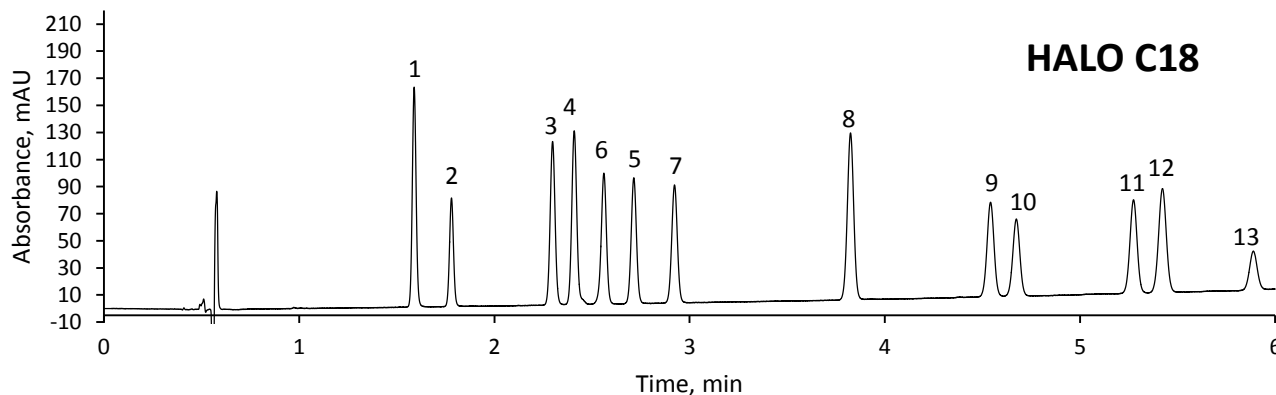
Injection Volume: 0.6 μ L

Cannabinoids: Acetonitrile Mobile Phase Conditions



PEAK IDENTITIES

1. CBDVA
2. CBDV
3. CBDA
4. CBGA
5. CBD
6. CBG
7. THCV
8. CBN
9. Δ^9 -THC
10. Δ^8 -THC
11. CBC
12. THCA
13. CBCA



Columns: HALO 90Å, AQ-C18, 2.7 μ m, 3 x 150 mm

HALO 90Å, C18, 2.7 μ m, 3 x 150 mm

Mobile Phase A: Water/0.1% formic acid

Mobile Phase B: **Acetonitrile/0.085% formic acid**

Gradient: 70-88% B in 6 min

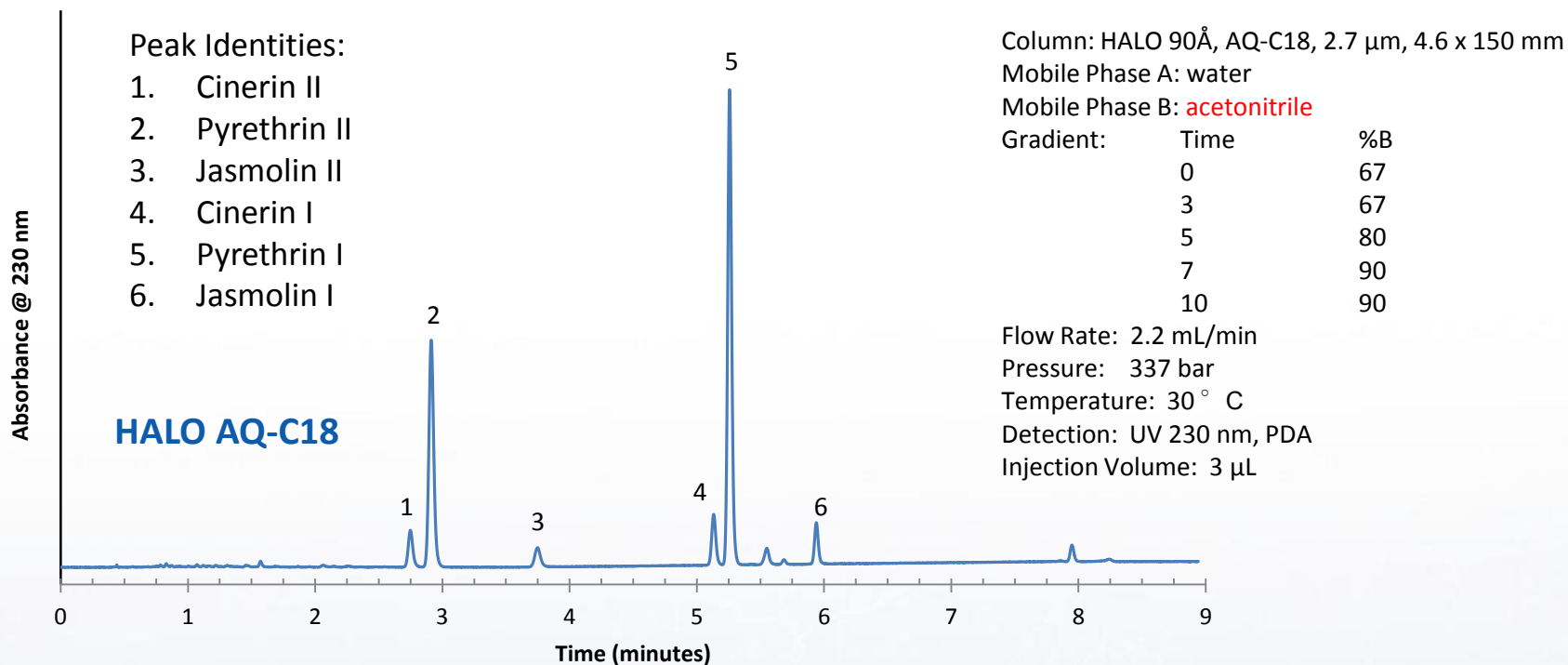
Flow Rate: 1.0 mL/min

Temperature: 30 °C

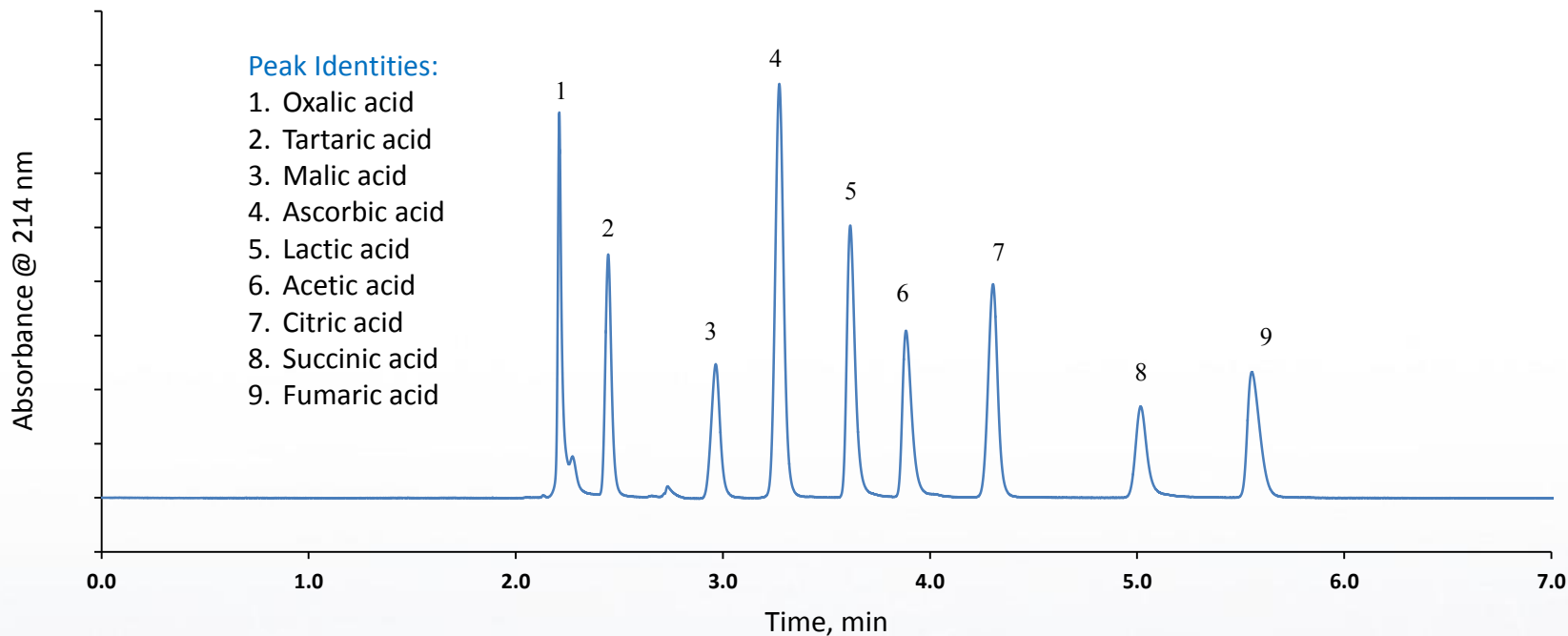
Detection: UV 220 nm, PDA

Injection Volume: 0.6 μ L

Pyrethrins



Organic Acids



TEST CONDITIONS:

Column: HALO 90Å, AQ-C18, 2.7 μ m, 4.6 x 250mm

Part Number: 92814-922

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 μ L

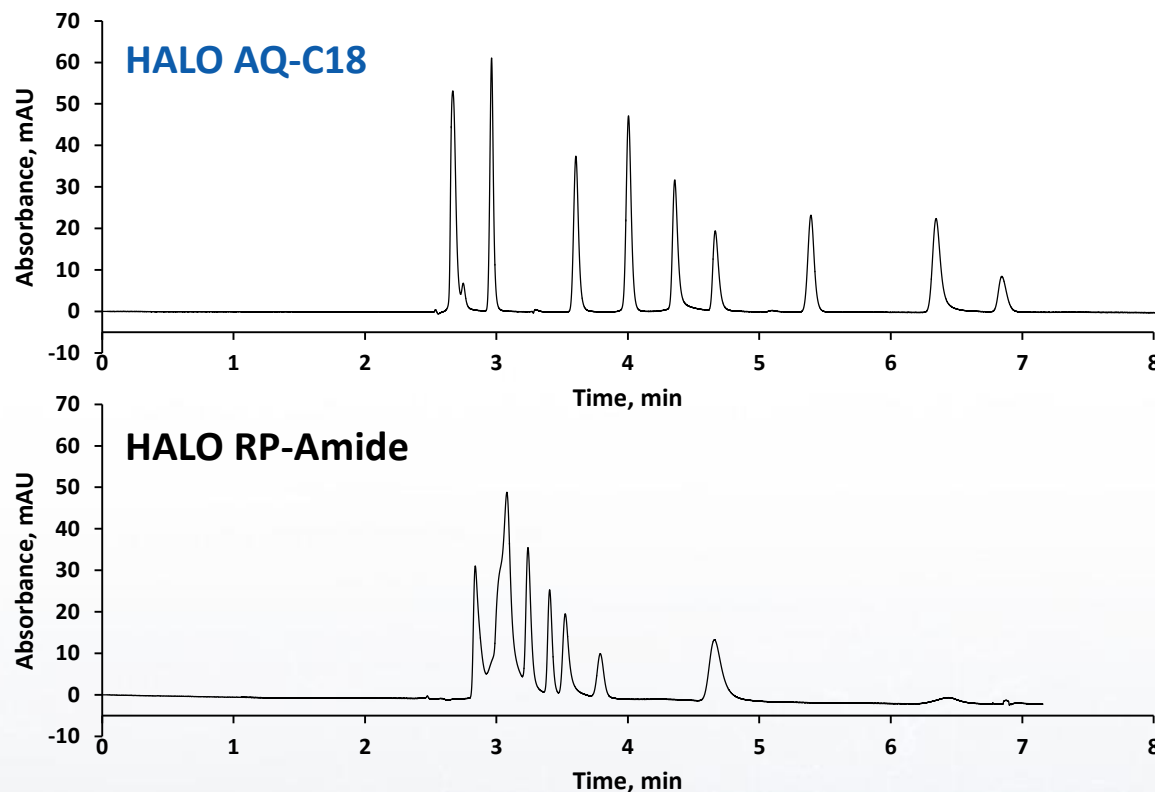
Data Rate: 100 Hz

Response Time: 0.025 sec

Flow Cell: 1 μ L

LC System: Shimadzu Nexera X2

Organic Acids: AQ-C18 compared to RP-Amide



TEST CONDITIONS:

Columns: HALO 90Å, AQ-C18, 2.7 μm , 2 each 4.6 x 150mm

HALO 90Å, C18, 2.7 μm , 2 each 4.6 x 150mm

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

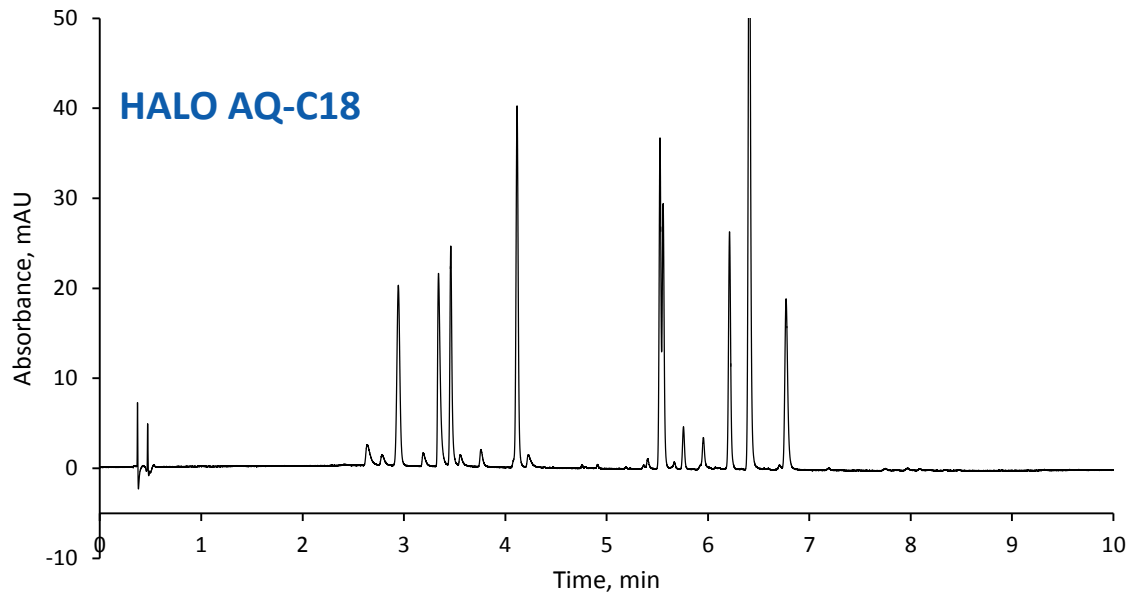
Flow Cell: 1 μL

LC System: Shimadzu Nexera X2

Sample Contains:

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

Complex Drug Mix with Acetonitrile Gradient



Column: HALO 90Å, AQ-C18, 2.7 μ m, 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: mL/min

Initial Pressure: 115 bar

Temperature: 30 ° C

Detection: UV 254 nm, PDA

Injection Volume: 2 μ L

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

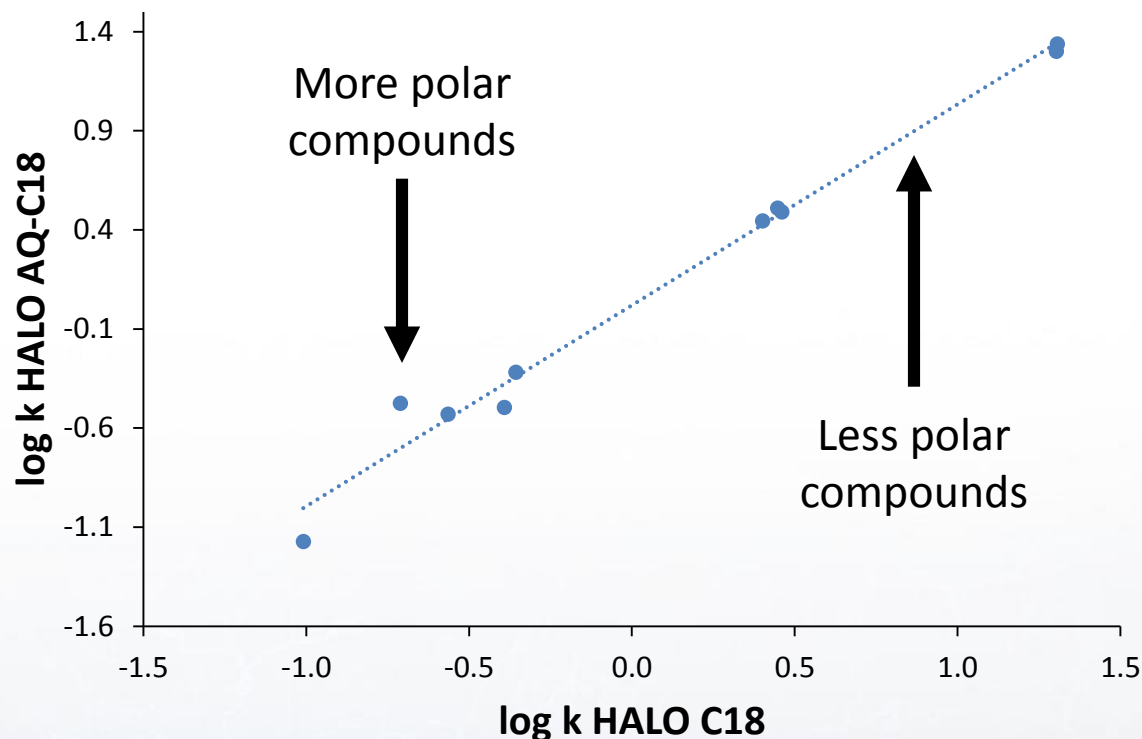
Kappa-Kappa Selectivity Plot

Columns: HALO 90Å, AQ-C18, 2.7 μm , 4.6 x 100 mm
HALO 90Å, C18, 2.7 μm , 4.6 x 100 mm
Isocratic: 60/40 20 mM Phosphate buffer, pH 7/ACN
Flow Rate: 1.0 mL/min
Temperature: 30 ° C
Detection: UV 254 nm, PDA

Peak Identities:

(more polar to less polar)

1. Benzoic Acid
2. Procainamide
3. Caffeine
4. Tolbutamide
5. 3,4-Dihydroxybenzonitrile
6. Acetophenone
7. Benzonitrile
8. Dimethylphthalate
9. Valerophenone
10. Naphthalene



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 to impart unique selectivity to the phase.
- When more resolution is desired for polar analytes, HALO AQ-C18 should be evaluated under both MeCN and MeOH conditions.

Acknowledgements

- Joe DeStefano, Tim Langlois, and Barry Boyes
- Ben Libert and Justin Godinho
- Matt Jackson and Brian Wagner

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Cannabinoid Structures

