Superficially Porous Particles with Polar C18 Selectivity

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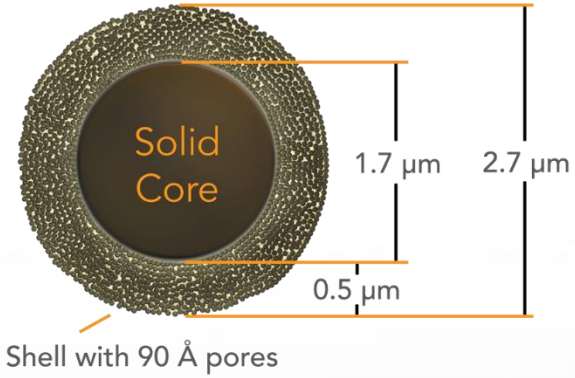
Overview

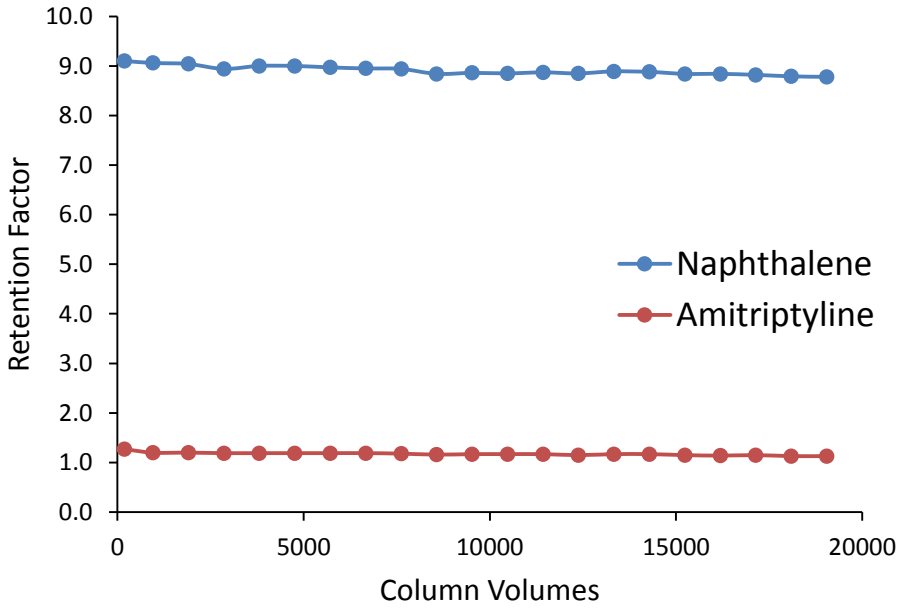
- HALO AQ-C18
  - Objectives
    - C18 stability and ease of use
    - C18 reproducibility
    - Extended use to 100% aqueous and low organic mobile phases
    - Alternate selectivity for polar molecules vs pure C18
      - Become equally useful in both methanol and acetonitrile mobile phases
  - Applications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

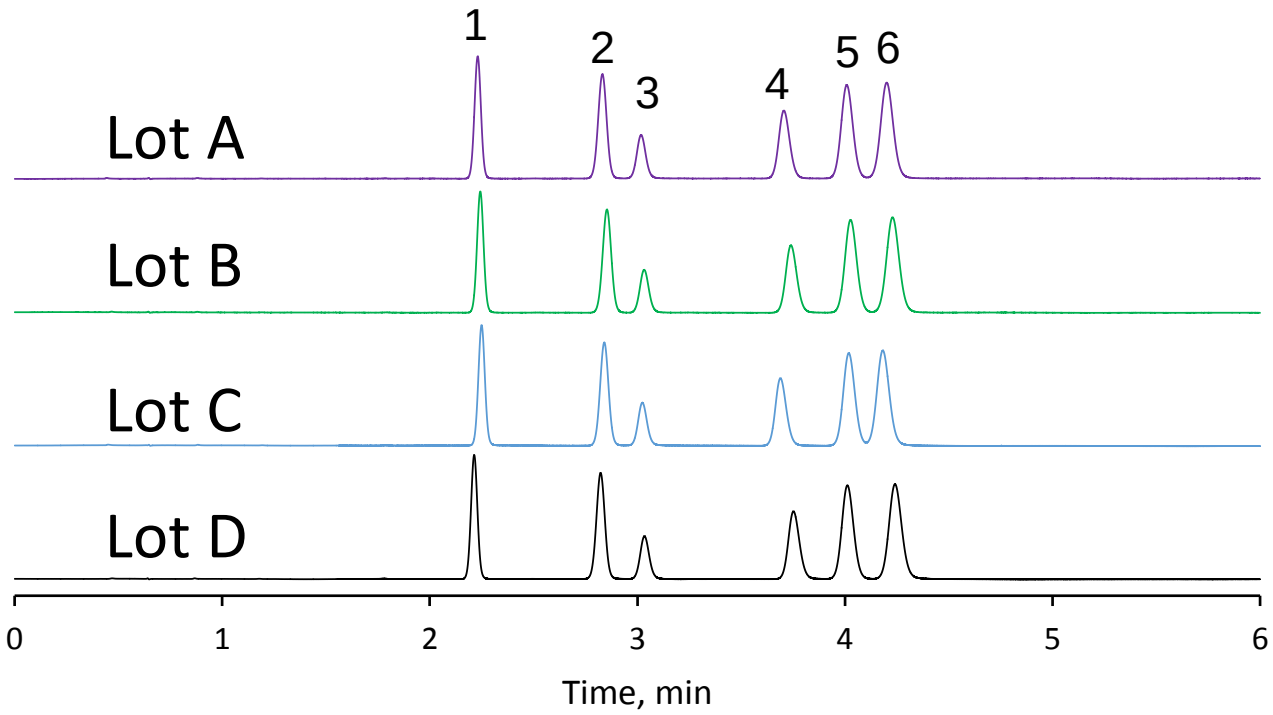


Minimal retention loss observed after ~ 19,000 column volumes

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

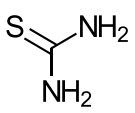
Columns:	4.6 x 100 mm HALO 90Å AQ-C18, 2.7 µm
Part Number:	92814-622
Isocratic:	50/50 water/methanol
Flow Rate:	1.3 mL/min
Temperature:	35 °C
Detection:	254 nm
Injection:	0.5 µL

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

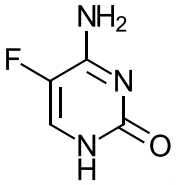
HALO AQ-C18: RESISTS DEWETTING

pH 2 Dewetting results: Nucleic Acid Bases

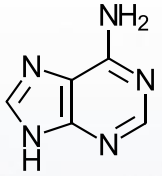
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



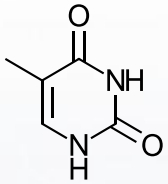
Thiourea



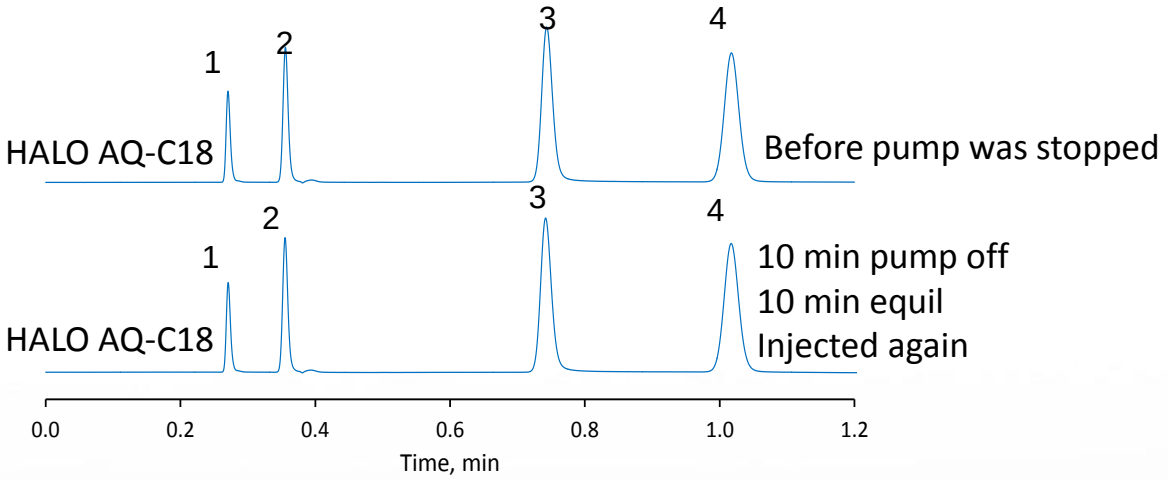
5-Fluorocytosine

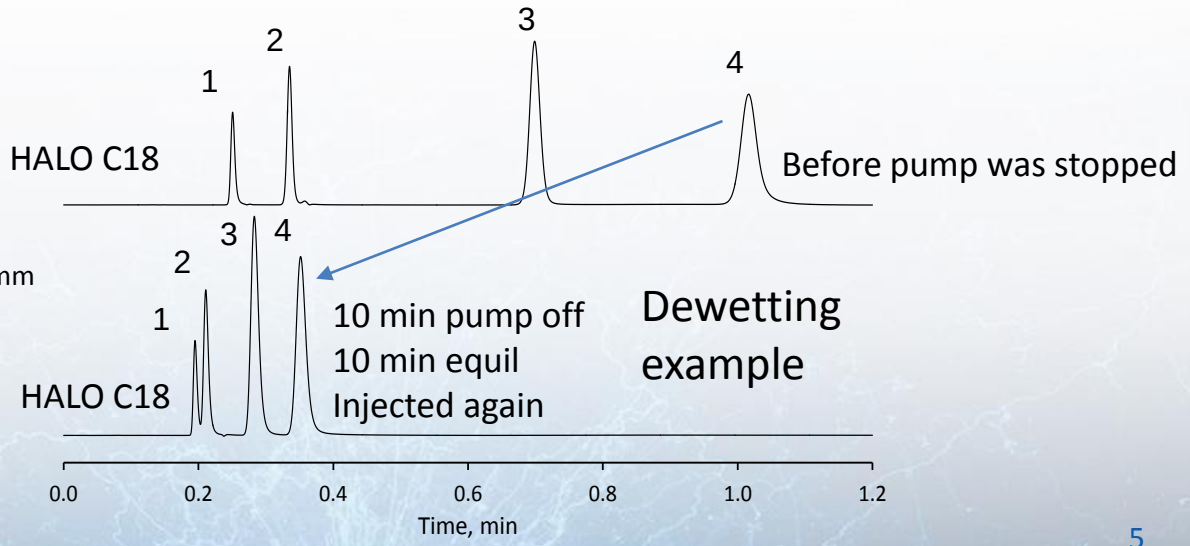


Adenine



Thymine





Columns: HALO 90Å, AQ-C18, 2.7 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

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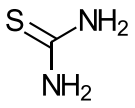
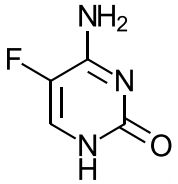
pH 7 Dewetting results: Nucleic Acid Bases

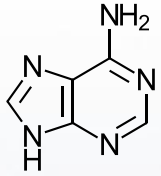
1. Thiourea
2. 5-Fluorocytosine
3. Adenine
4. Thymine



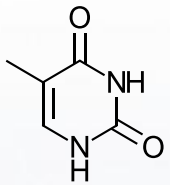
Thiourea



5-Fluorocytosine

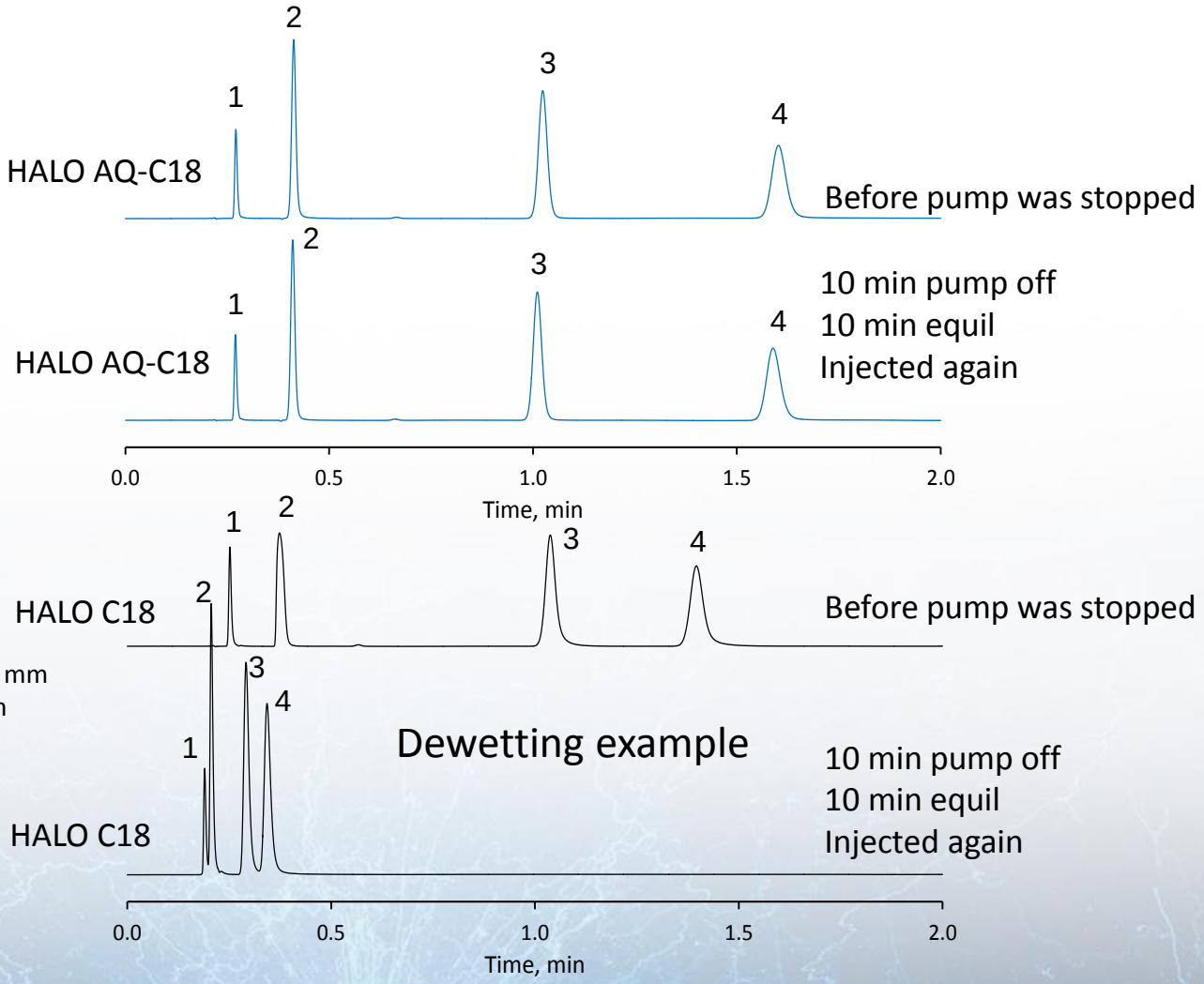


Adenine



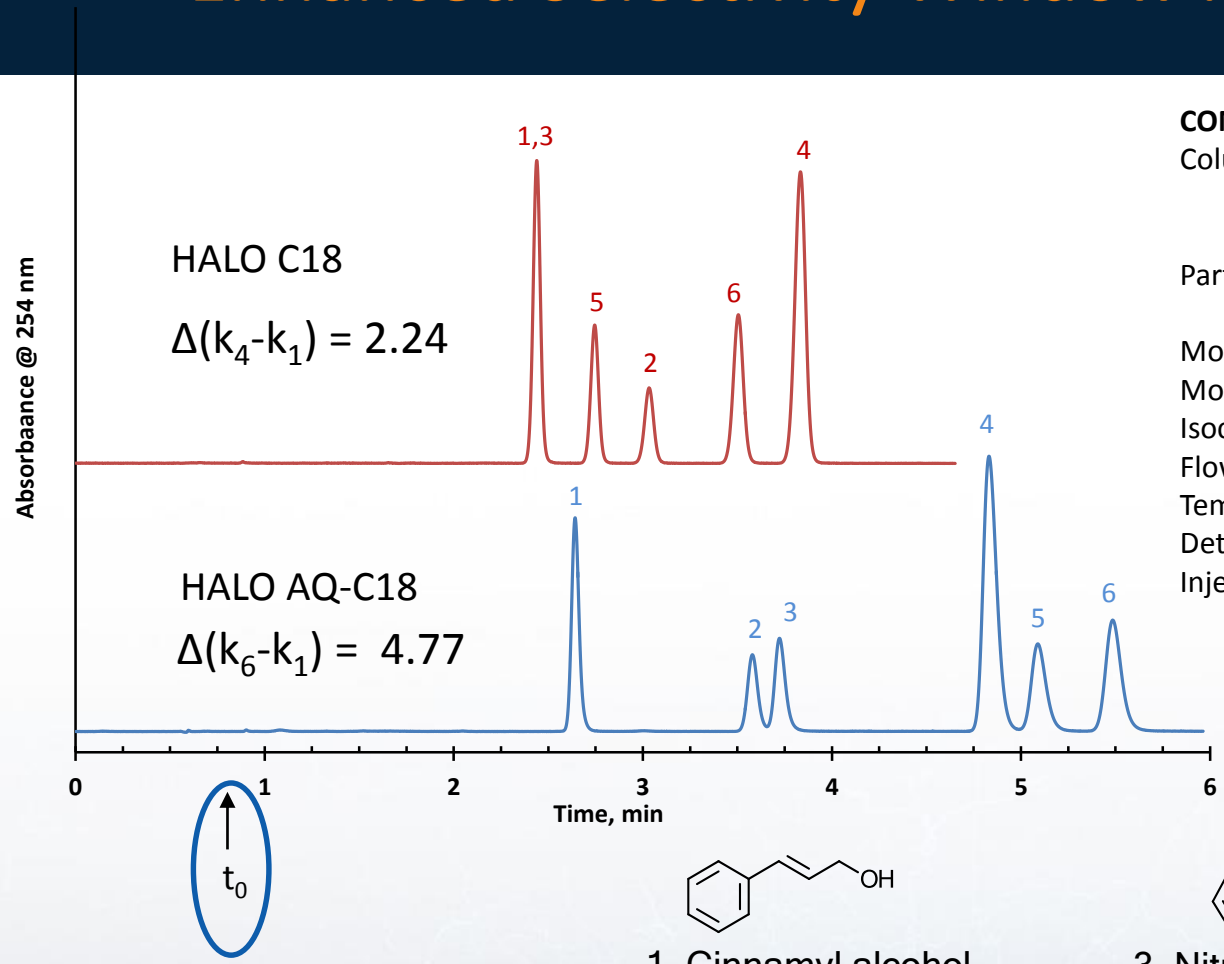
Thymine

Columns: HALO 90Å, AQ-C18, 2.7 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: ENHANCED POLAR SELECTIVITY

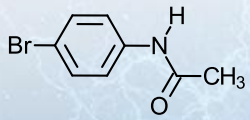
Enhanced Selectivity Window for Polar Groups



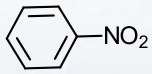
CONDITIONS:

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

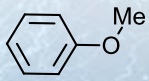
1. Cinnamyl alcohol



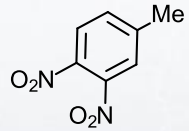
2. 4-Bromoacetanilide



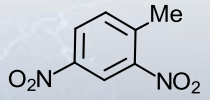
3. Nitrobenzene



4 . Anisole

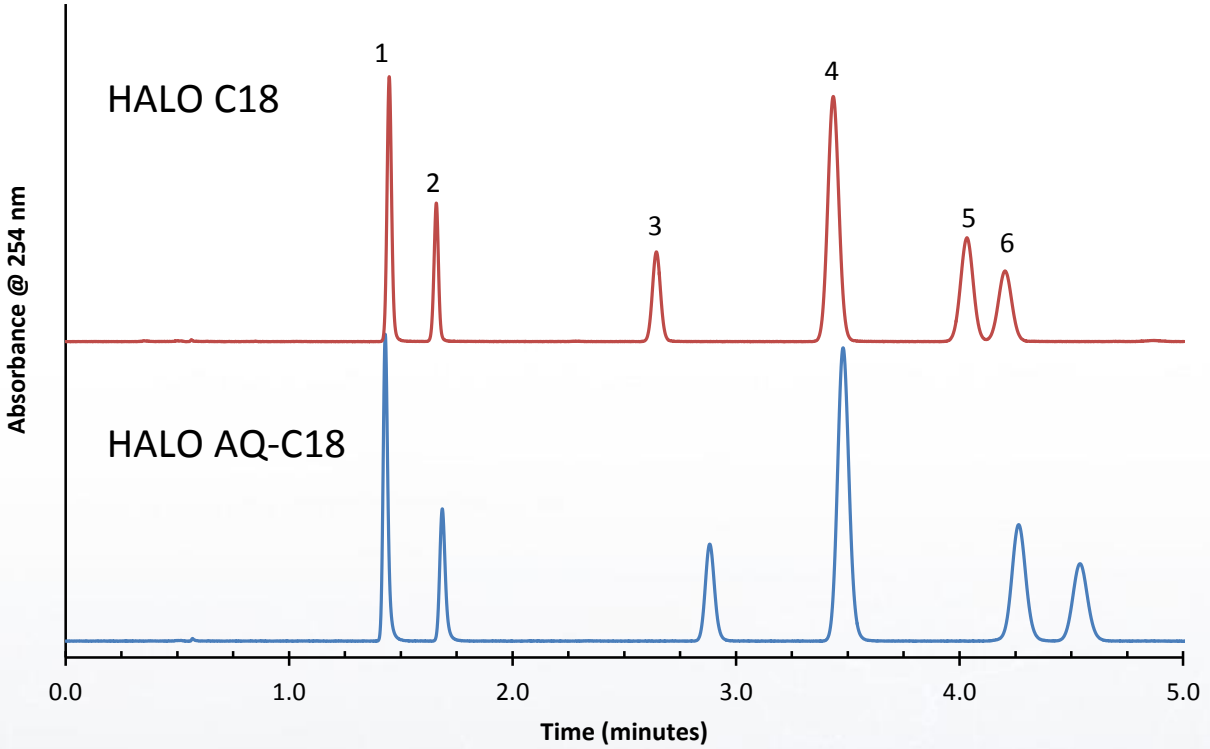


5. 3,4-Dinitrotoluene



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

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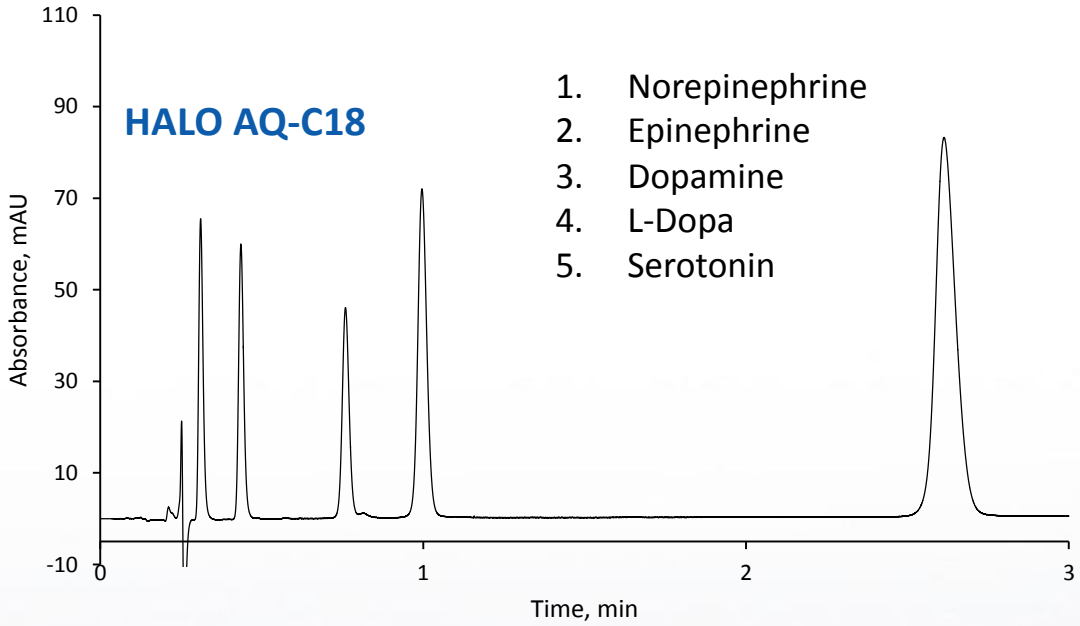
HALO AQ-C18: APPLICATIONS

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



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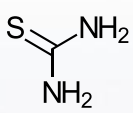
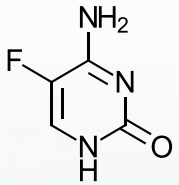
Nucleic Acid Bases

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

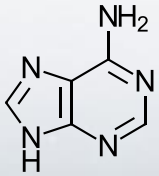
Columns: HALO 90Å, AQ-C18, 2.7 µm, 4.6 x 50 mm
HALO 90Å, RP-Amide, 2.7 µm, 4.6 x 50 mm
Isocratic: Water/0.1% TFA
Flow Rate: 2.0 mL/min
Pressure: 290 bar
Temperature: 30 ° C
Detection: UV 254 nm, PDA
Injection Volume: 0.5 µL



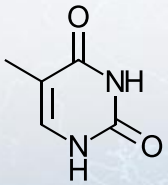
Thiourea



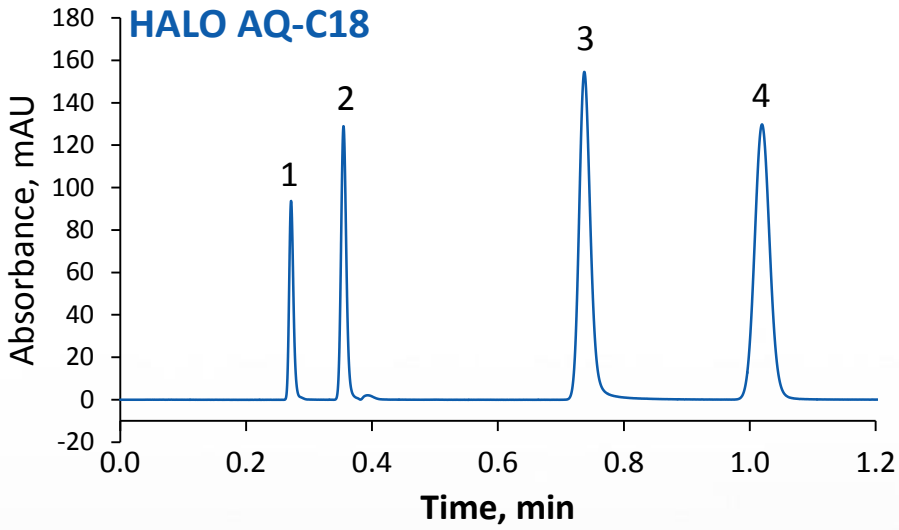
5-Fluorocytosine

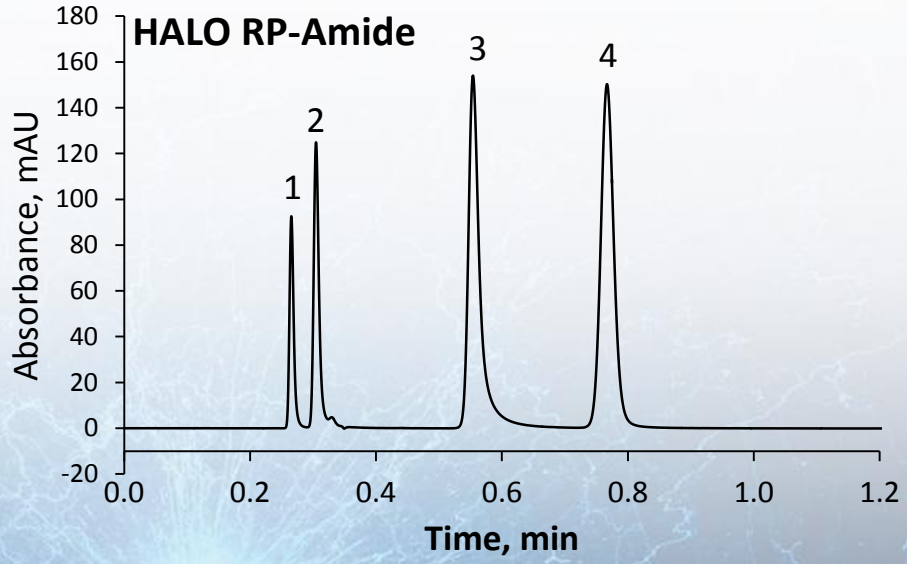


Adenine



Thymine





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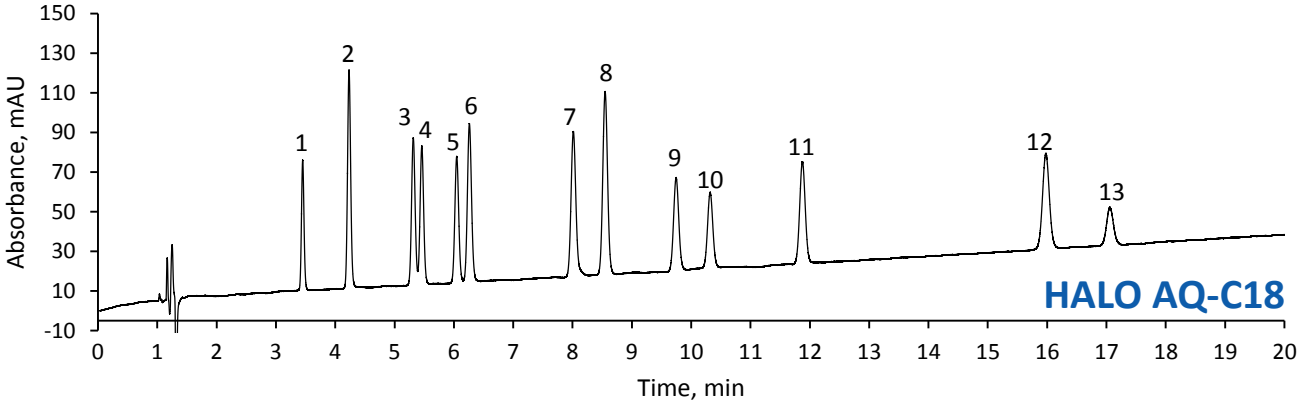
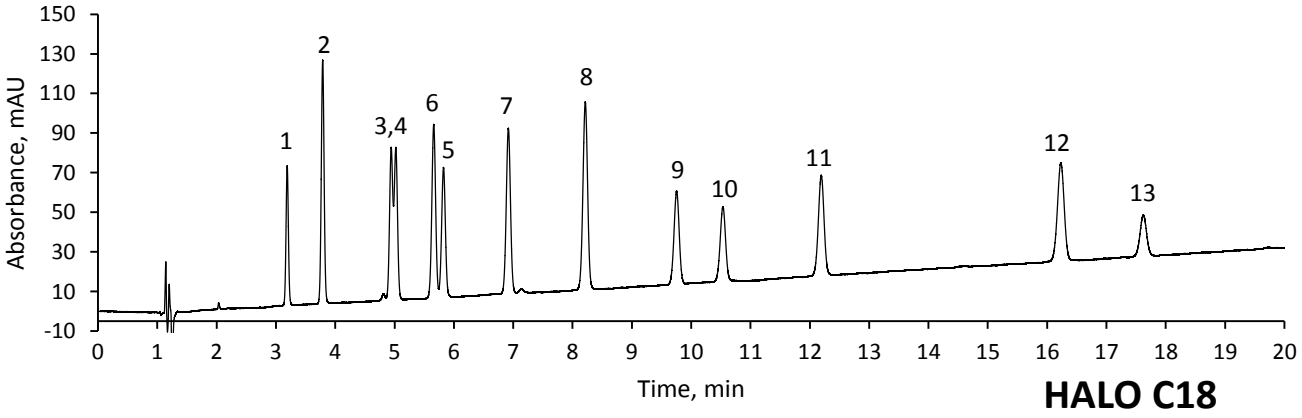
Cannabinoids: Methanol Mobile Phase Conditions





PEAK IDENTITIES

1. CBDVA
2. CBDV
3. CBDA
4. CBGA
5. CBD
6. CBG
7. THCV
8. CBN
9. Δ9-THC
10. Δ8-THC
11. CBC
12. THCA
13. CBCA

Columns: HALO 90Å, AQ-C18, 2.7 µm, 3 x 150 mm
HALO 90Å, C18, 2.7 µm, 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 µL

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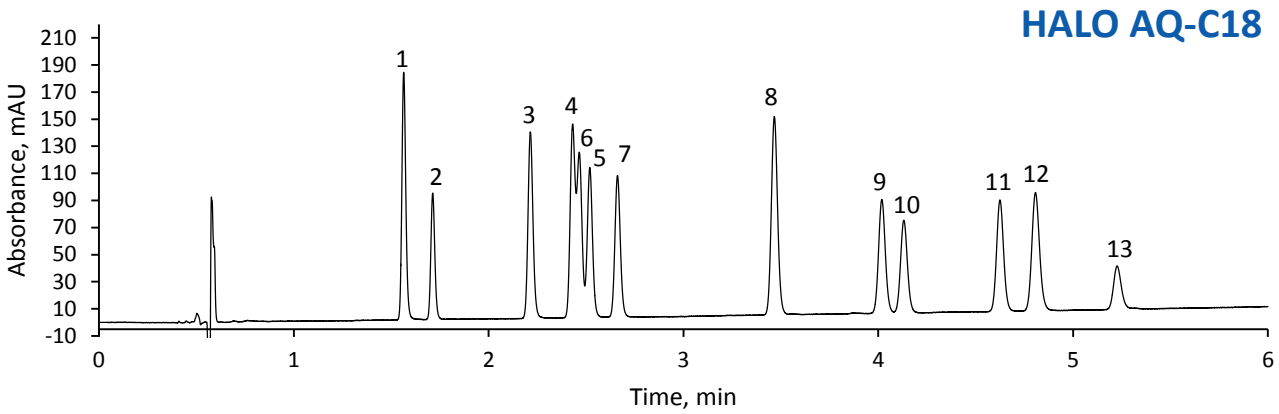
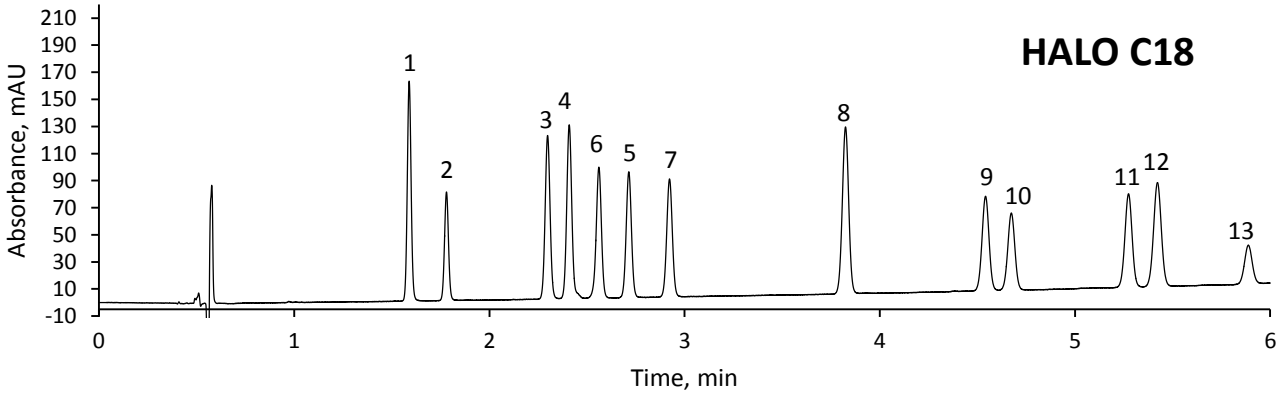
Cannabinoids: Acetonitrile Mobile Phase Conditions





PEAK IDENTITIES

1. CBDVA
2. CBDV
3. CBDA
4. CBGA
5. CBD
6. CBG
7. THCV
8. CBN
9. Δ9-THC
10. Δ8-THC
11. CBC
12. THCA
13. CBCA

Columns: HALO 90Å, AQ-C18, 2.7 µm, 3 x 150 mm
HALO 90Å, C18, 2.7 µm, 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 µL

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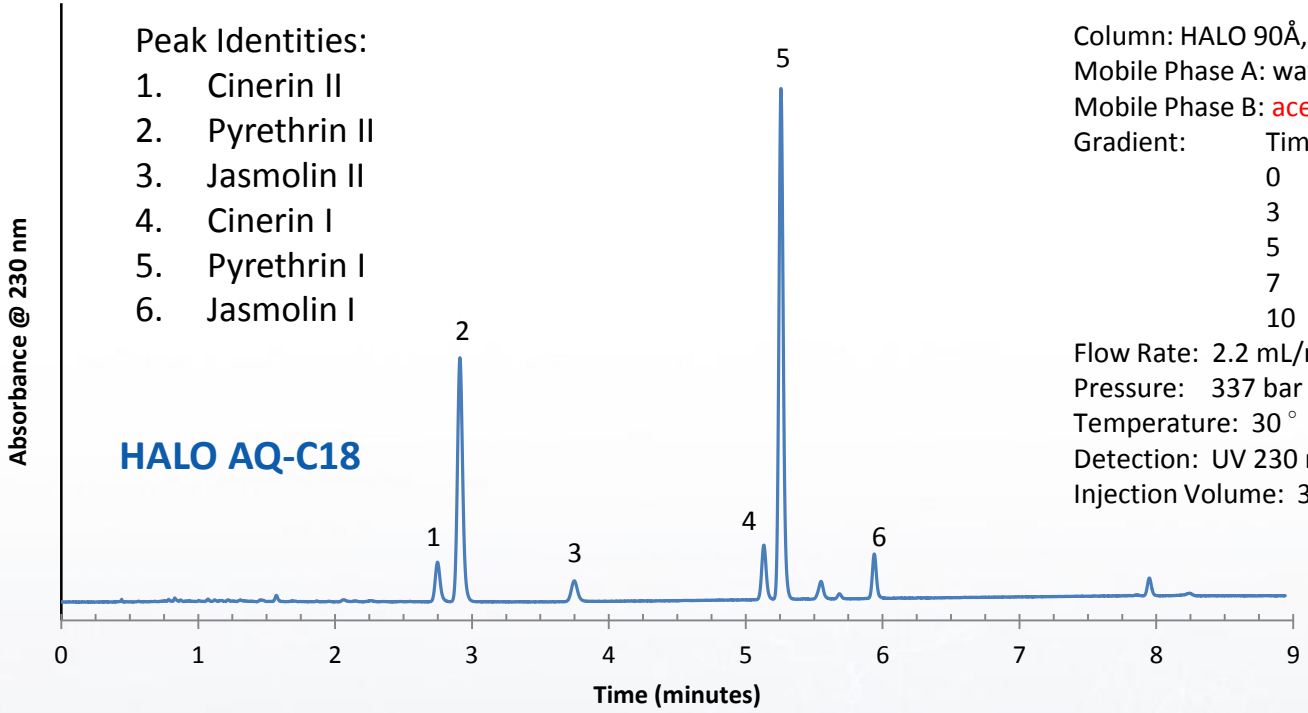
Pyrethrins



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

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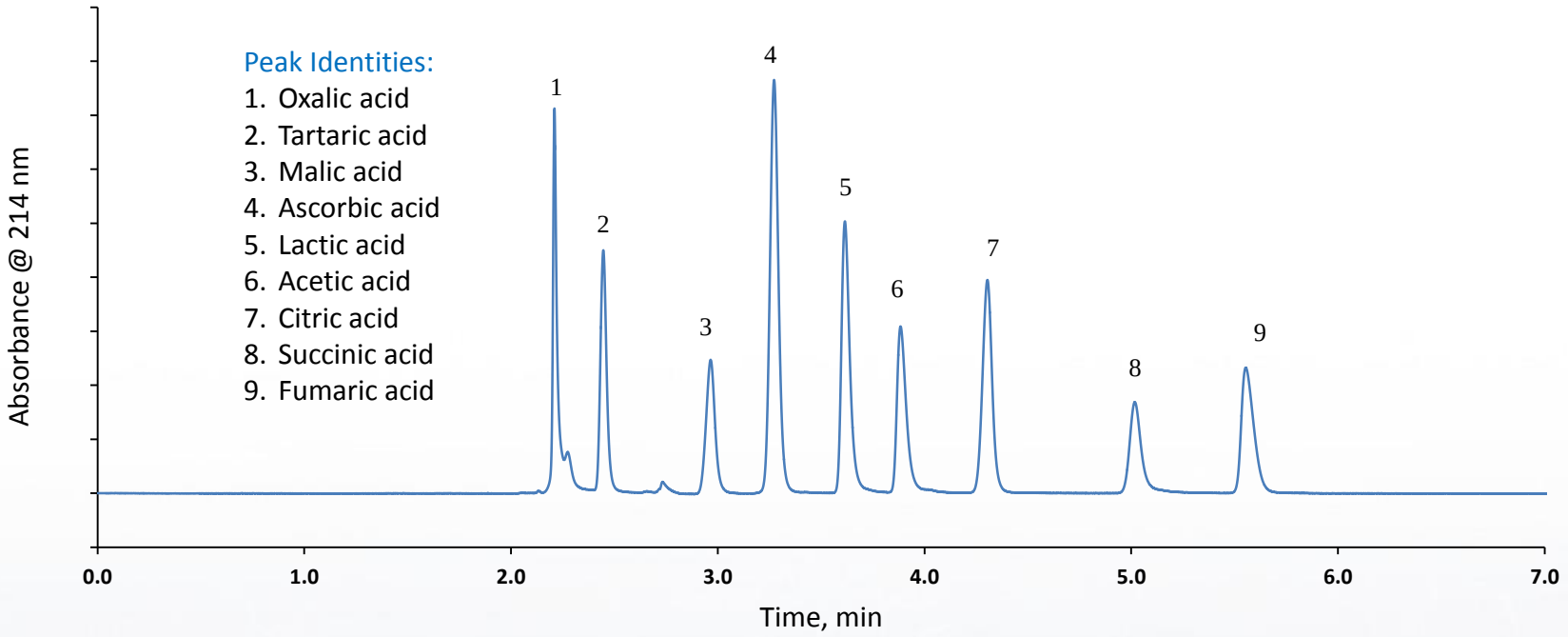
Organic Acids



TEST CONDITIONS:
Column: HALO 90Å, AQ-C18, 2.7 µm, 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 µL
Data Rate: 100 Hz
Response Time: 0.025 sec
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

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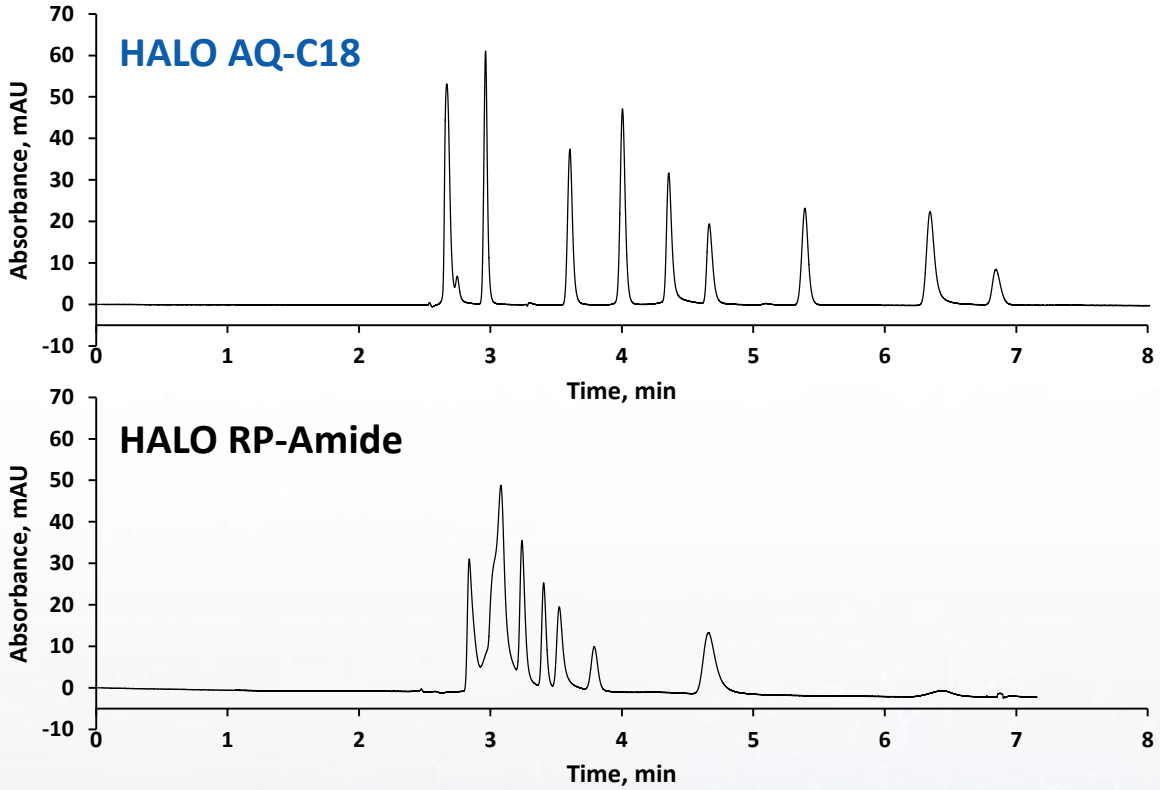
Organic Acids: AQ-C18 compared to RP-Amide



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

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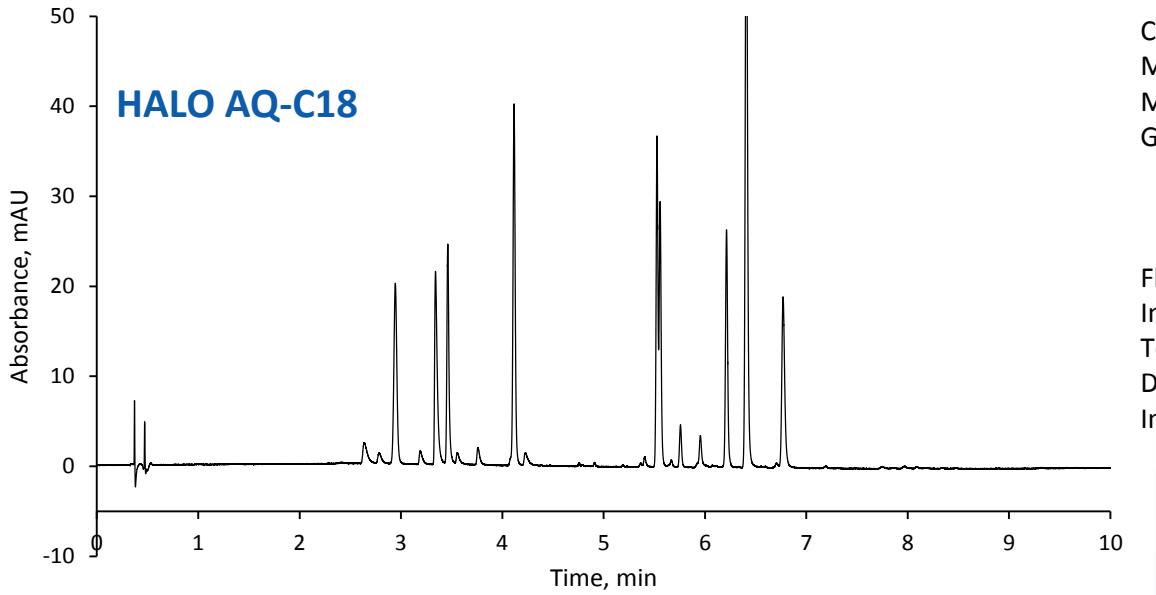
Complex Drug Mix with Acetonitrile Gradient



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: 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

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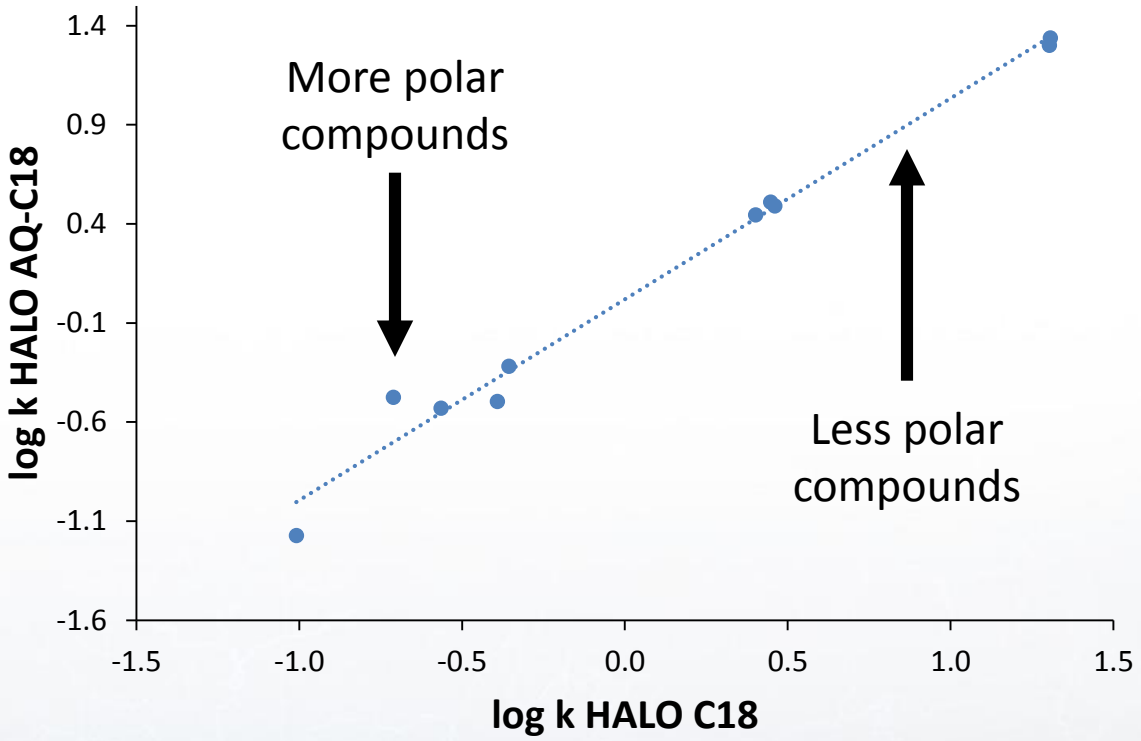
Kappa-Kappa Selectivity Plot

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. Dimethylphathalate
9. Valerophenone
10. Naphthalene



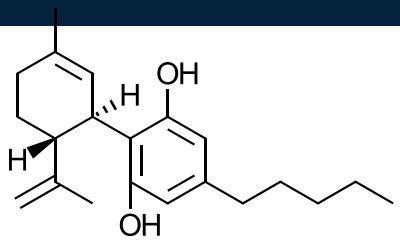
Summary

- 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.

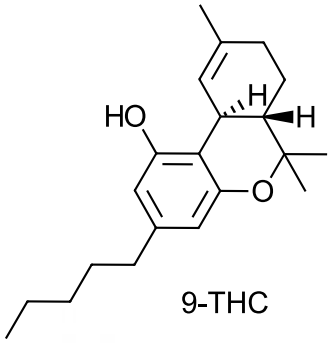
Acknowledgements

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

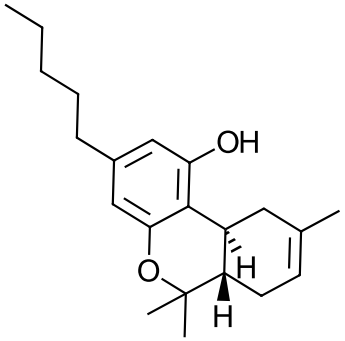
Cannabinoid Structures



CBD



9-THC



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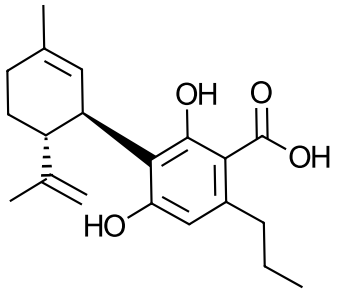
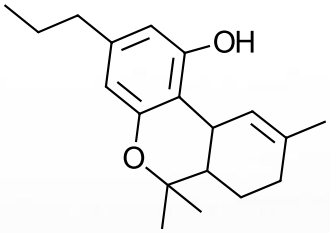


CBDVA



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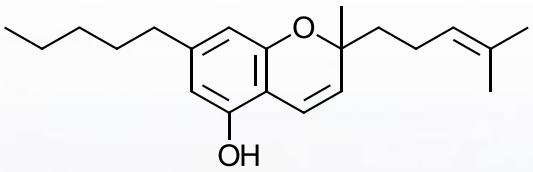

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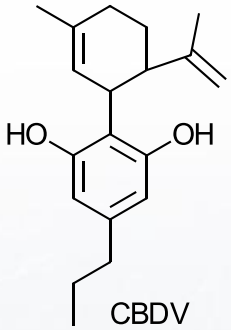

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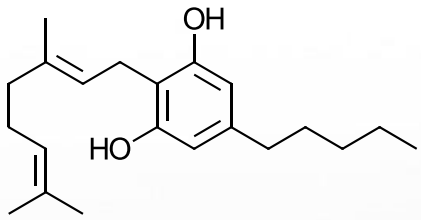

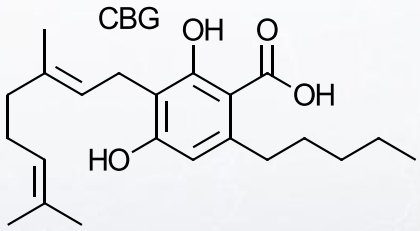

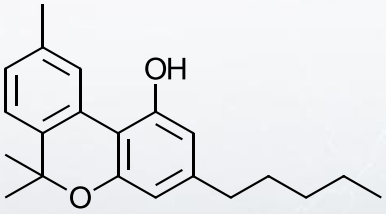

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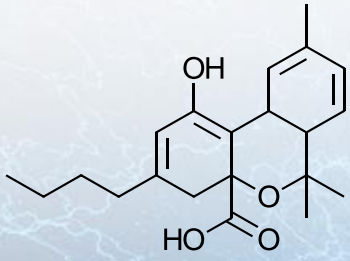

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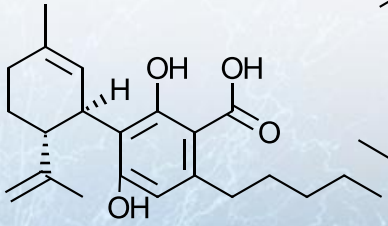
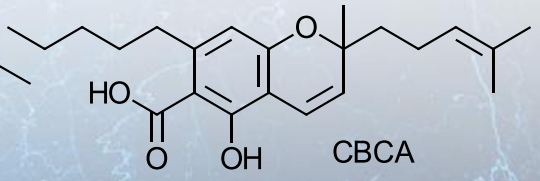


CBDA

CBGA



CBCA

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