LPH-C18: A C18 Column Alternative

Advanced Materials Technology
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advancedmaterialstechnology

- Founded in 2005 by Tim Langlois and Joe DeStefano
- First company to commercially manufacture sub 3 µm superficially porous particles – *Fused-Core®*
- Personnel
  - >50 employees

- Facility
  - Fully equipped state of the art laboratories
  - All operations handled in Wilmington, DE
    - R&D, Applications, QA/QC, Manufacturing, Sales and Marketing

International Organization for Standardization ISO 9001

ANAB ANSI National Accreditation Board ACCREDITED ISO/IEC 17065 PRODUCT CERTIFICATION BODY

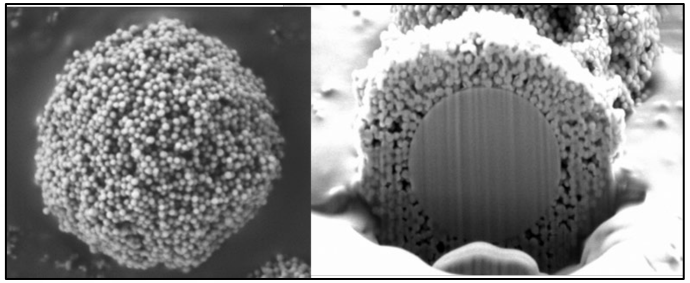
ASR American Systems Registrar

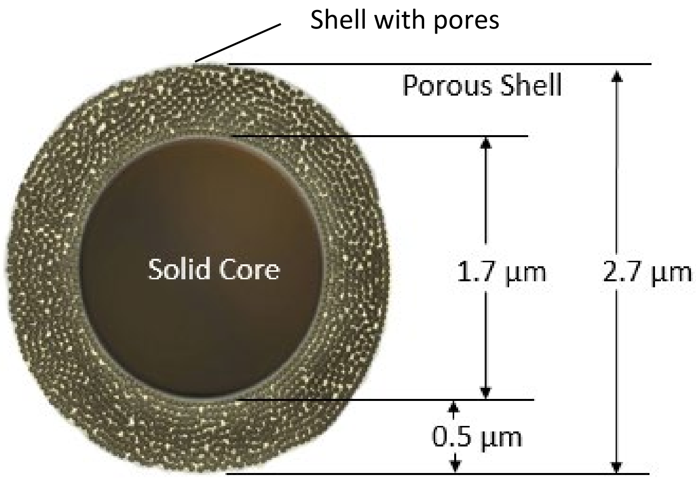
Outline

- Superficially Porous Particle (SPP) Technology
- How SPP benefits separations?
- AMT particles and stationary phases
- HALO® LPH-C18
    - Stability
    - Lot-to-lot
    - Applications

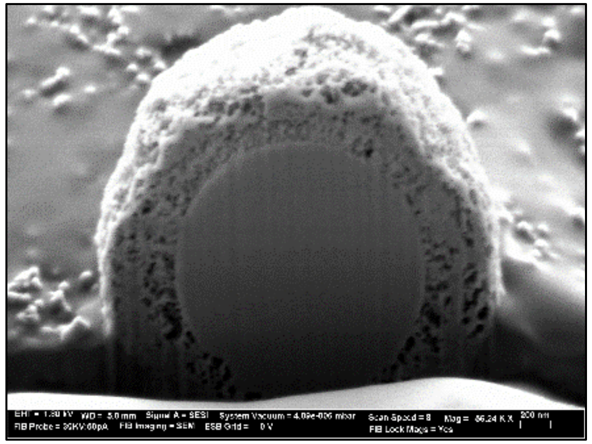
Superficially Porous Particle (SPP) Technology

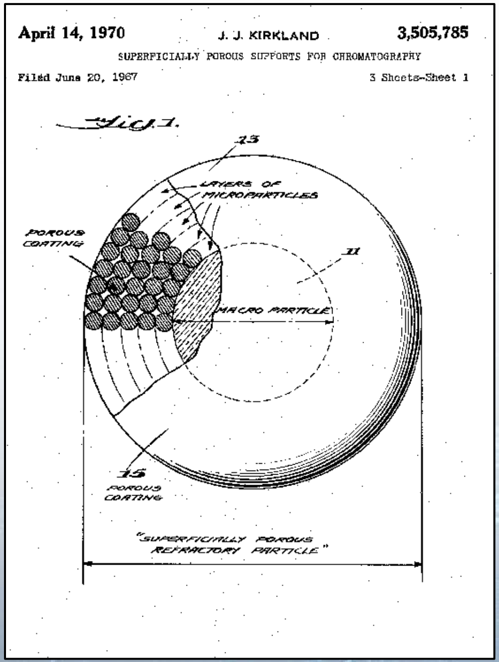
-High Purity Silica Particles (2, 2.7, 3.4, 5 µm)
-Porous Shell Fused to Solid Core
-Porosity of the shell (can be 90, 160, 400, or 1000 Å)





SEM Particle Cross- section



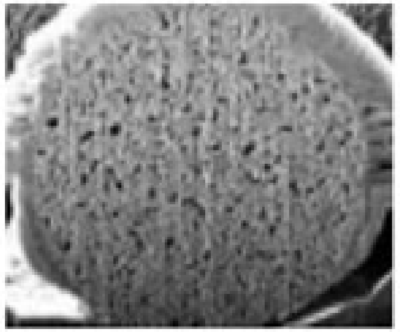


3,505,785
SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY
Joseph J. Kirkland, Wilmington, Del., assignor to E. I. du Pont de Nemours and Company, Wilmington, Del., a corporation of Delaware
Filed June 20, 1967, Ser. No. 647,506
Int. Cl. B01d 15/08
U.S. Cl. 55—67 8 Claims

ABSTRACT OF THE DISCLOSURE

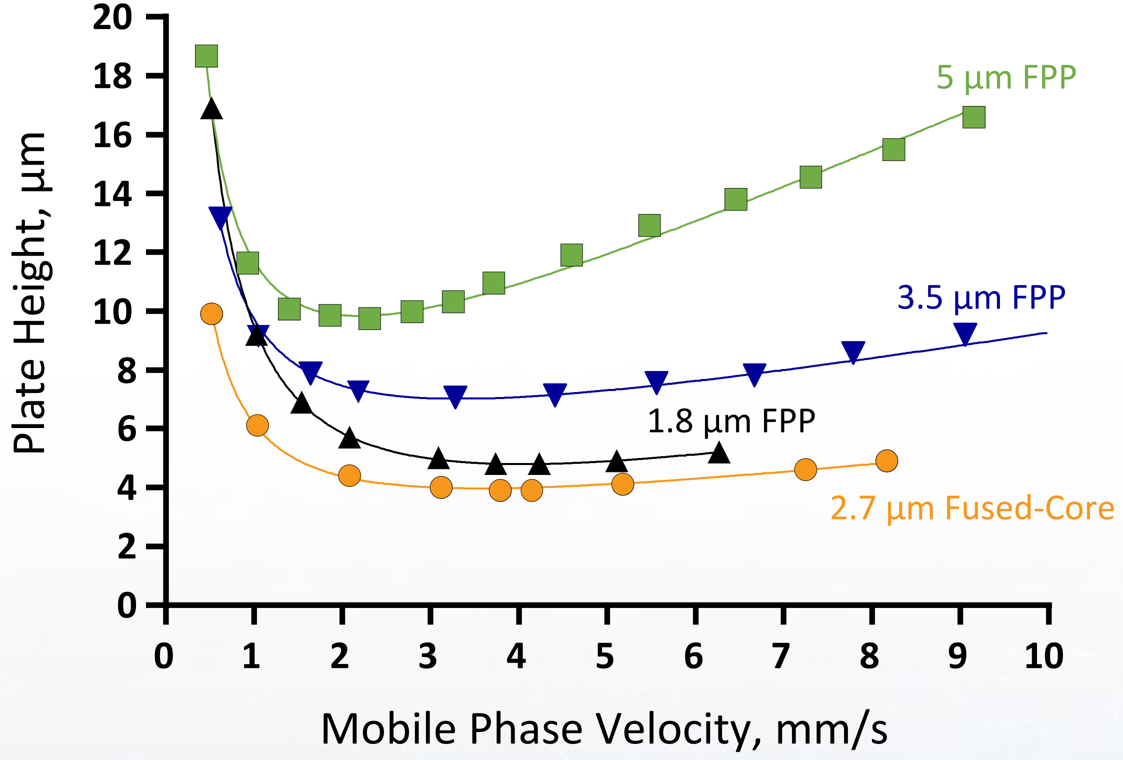
This invention relates to an improvement in chromatography and chromatographic columns. A novel packing of superficially porous refractory particles for use in chromatography has been prepared consisting of a plurality of discrete macroparticles with impervious cores and having irreversibly joined thereto a coating of a series of sequentially adsorbed like monolayers of like colloidal inorganic microparticles. The coating is characterized by being uniform and of predetermined thickness. In preferred embodiments, the cores would be ceramics, preferably glass spheres, and the coating would consist of monolayers of colloidal refractory particles, preferably silica, in a structure of predetermined thickness and porosity.

Fully Porous Particle (FPP)

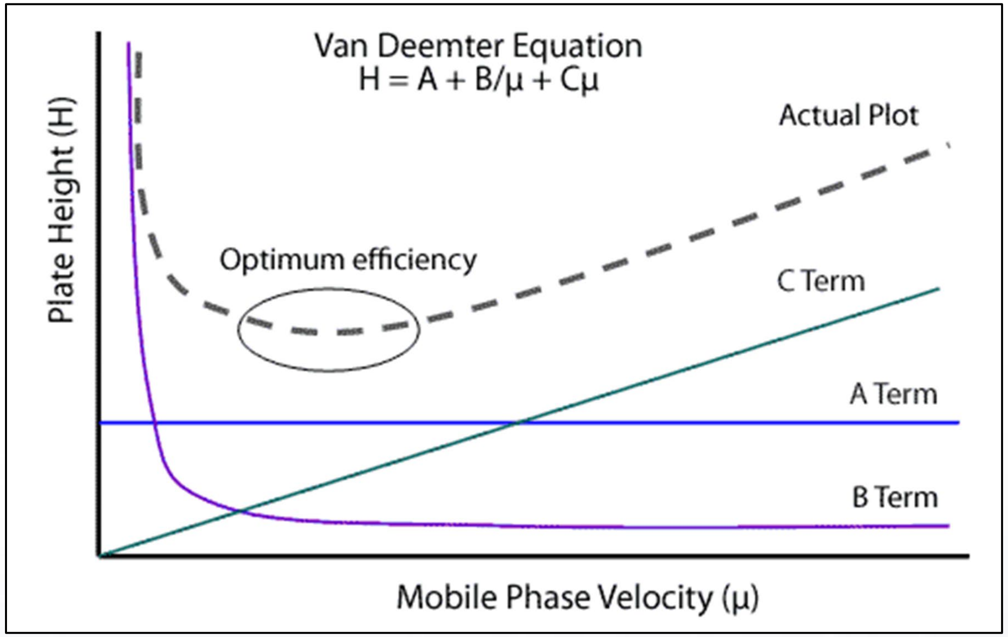


How SPP Benefits Separations?

Speed and Efficiency



J.J. DeStefano, T.J. Langlois, & J.J. Kirkland, ***J. Chromatogr. Sci.***, 2008, 46(3), 254-260



$$H = A + \frac{B}{\mu} + C\mu$$

Effect of Particle Size and Type

Columns: 4.6 x 50 mm
5 µm FPP C18
3.5 µm FPP C18
1.8 µm FPP C18
2.7 µm HALO C18

Solute: naphthalene
Mobile phase: 60% ACN/40% water
Temperature:24 °C

van Deemter Equation

H = height equivalent to theoretical plate

A = eddy diffusion term (particle size and how well bed was packed) **30 - 40% smaller**

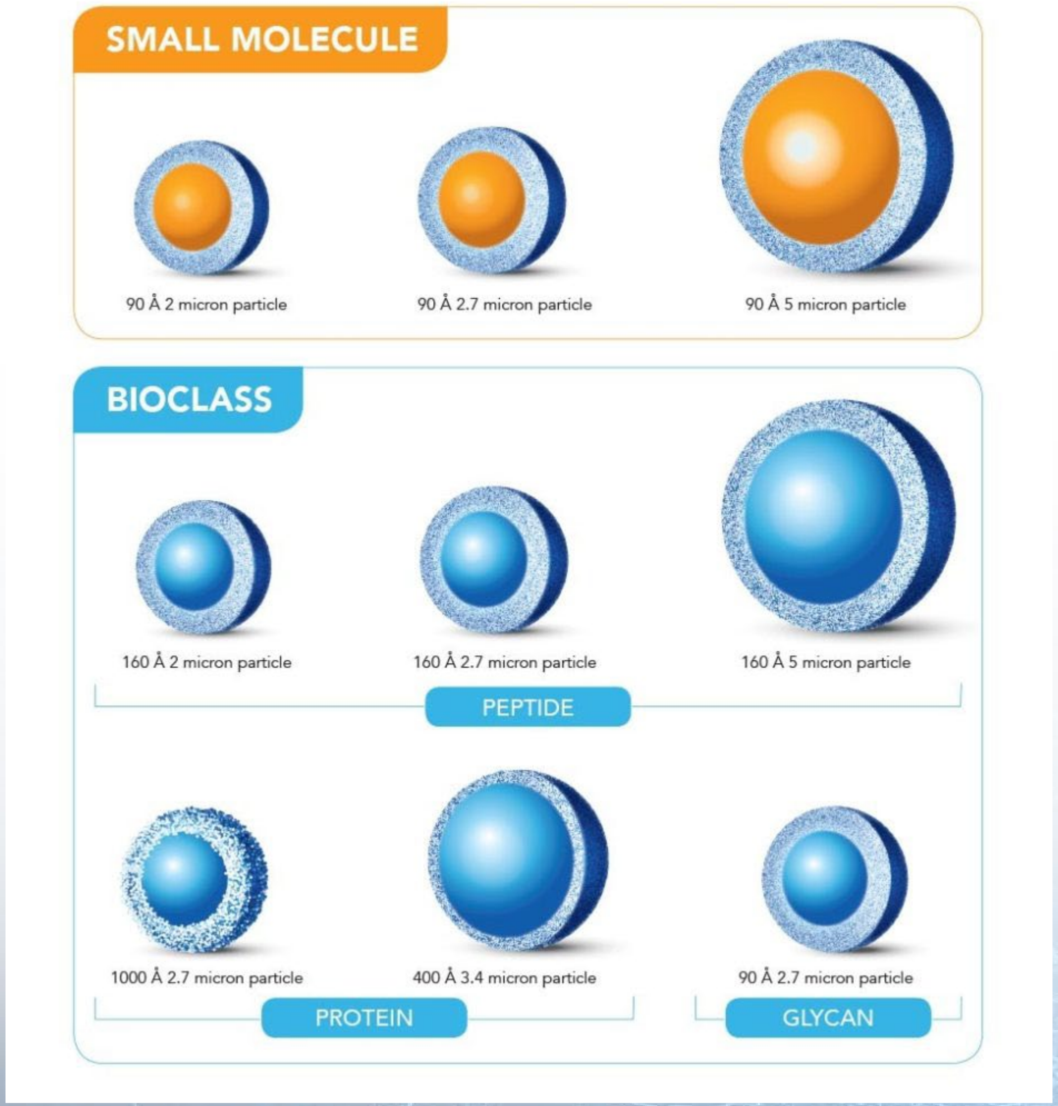
B = longitudinal diffusion term **25 - 30% smaller**

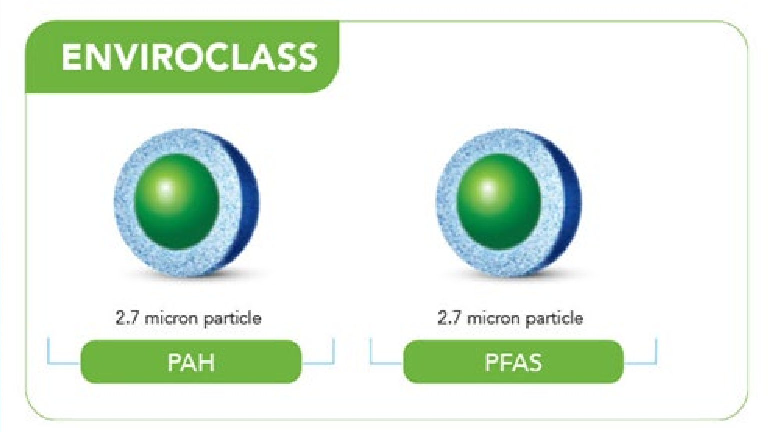
C = resistance to mass transfer term (kinetics of the analyte b/w mobile phase and stationary phase)

µ = mobile phase linear velocity (L/t_0)

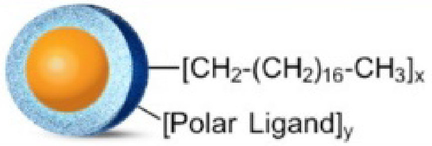
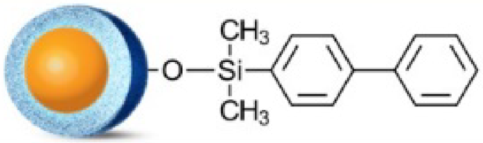
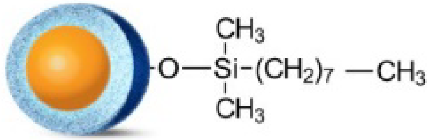
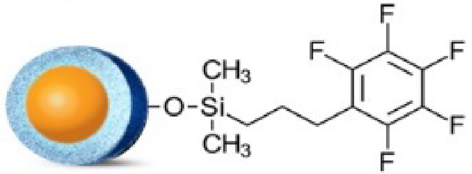
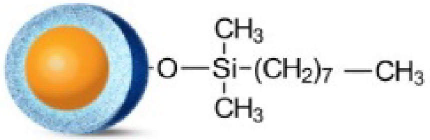
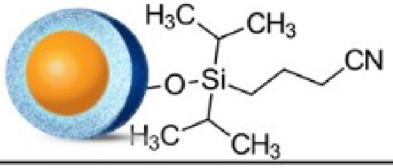
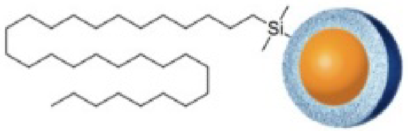
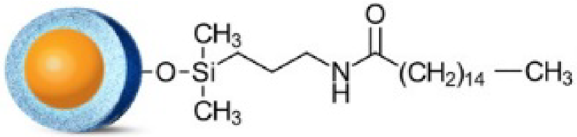
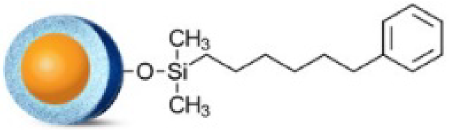
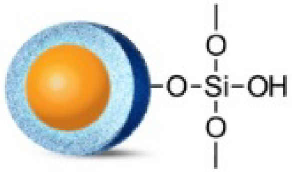
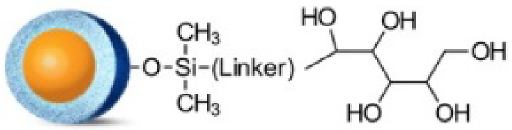
AMT Product Portfolio

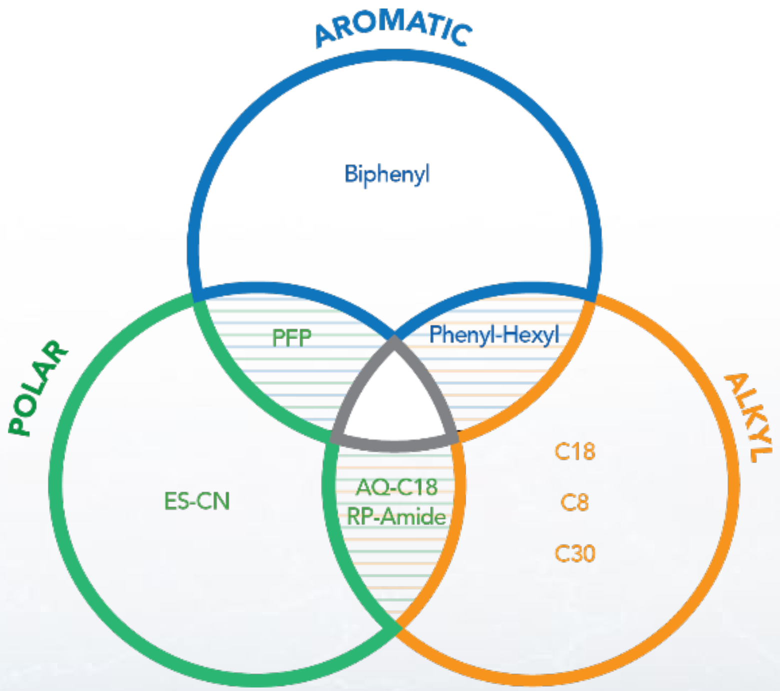
- Portfolio of Products
  - Varying particle morphologies to meet separation needs (particle size, core size, shell thickness, pore size)
  - Various chemistries for selectivity of analytes across small molecule to large molecule including reversed phase and HILIC options
  - Many different column dimensions from capillary to semi-prep





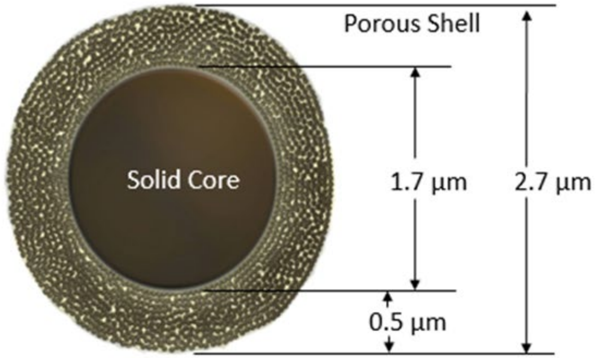
HALO®: Small Molecule Phases

AQ-C18		Biphenyl	
C8		PFP	
C18		ES-CN ES = extra stable	
C30		RP-Amide	
Phenyl-Hexyl		HILIC	
		Penta-HILIC	

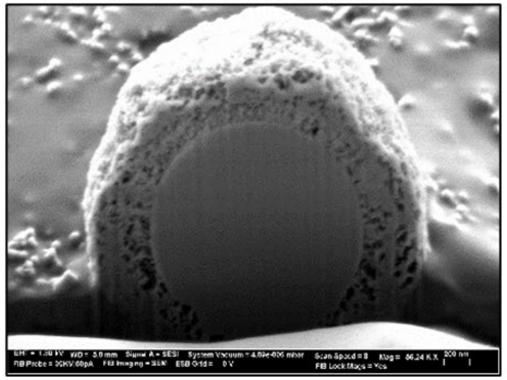


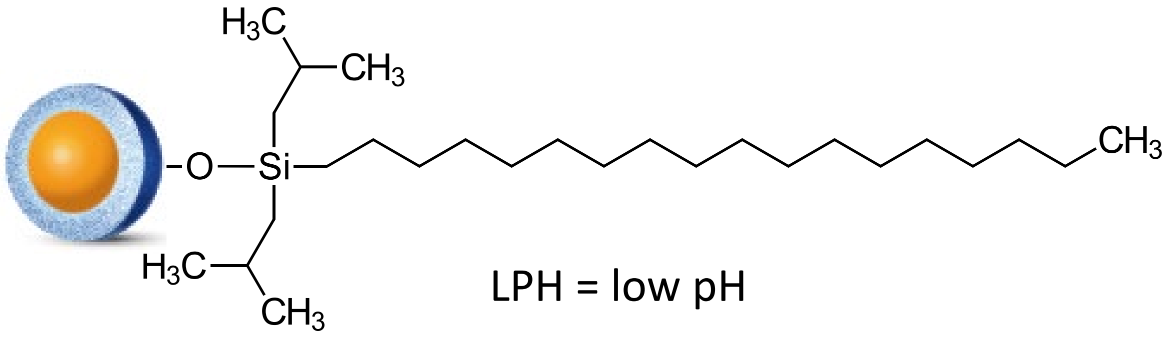
HALO 90 Å LPH-C18

Shell with pores



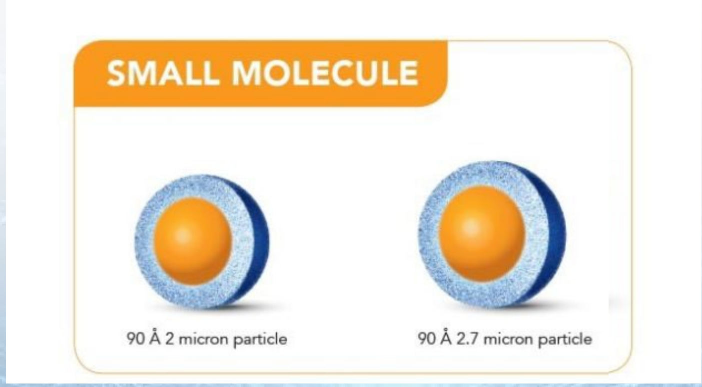
SEM Particle Cross- section



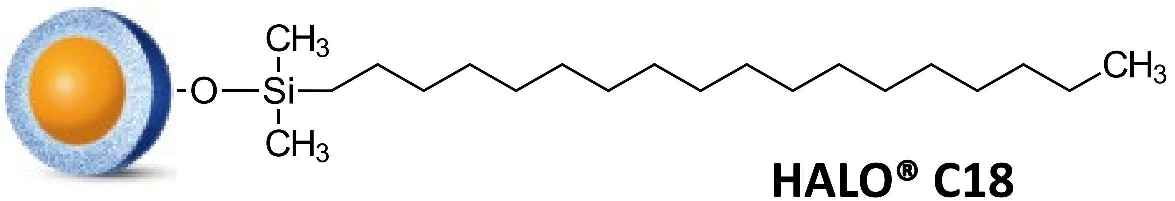


LPH = low pH

Bonded Phase	USP Designation	Particle Size (µm)	Carbon Load (%)	Surface Area (m²/g)	Low pH/ T Limit	High pH/ T Limit	Endcapped
LPH-C18	L1	2	6.5	120	1/ 90°C	8/40°C	No
		2.7	6.5	135			



Another C18 Column?

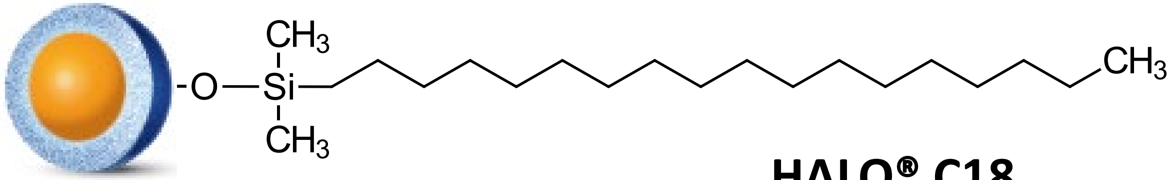


HALO® C18

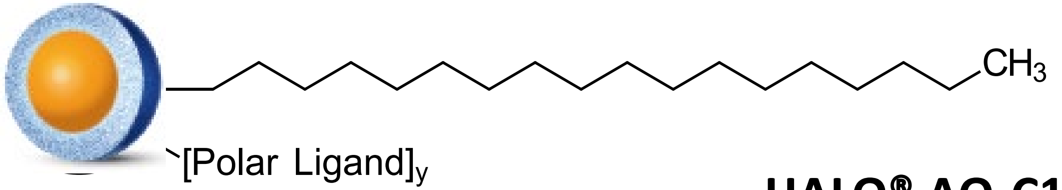
Features and Benefits

- The standard for retaining and separating a broad range of analyte polarities

Another C18 Column?



HALO® C18

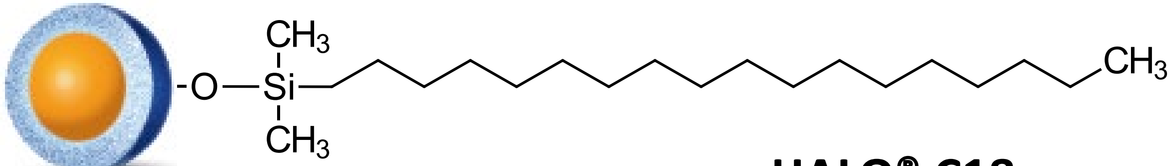


HALO® AQ-C18

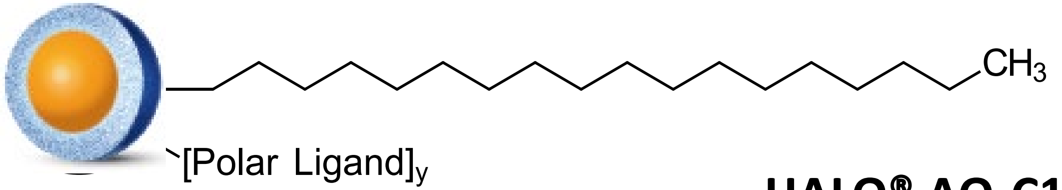
Features and Benefits

- The standard for retaining and separating a broad range of analyte polarities
- Resistant to dewetting, making it 100% aqueous mobile phase compatible
- Enhanced retention and selectivity for polar molecules

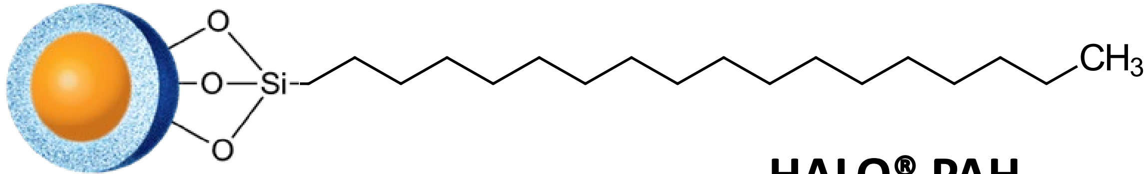
Another C18 Column?



HALO® C18



HALO® AQ-C18



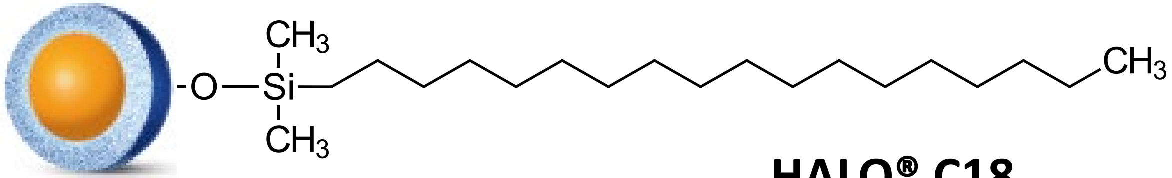
HALO® PAH

Features and Benefits

- The standard for retaining and separating a broad range of analyte polarities
- Resistant to dewetting, making it 100% aqueous mobile phase compatible
- Enhanced retention and selectivity for polar molecules
- Optimized for polycyclic aromatic hydrocarbons (PAH)
- Polymeric bonding that promotes shape selectivity

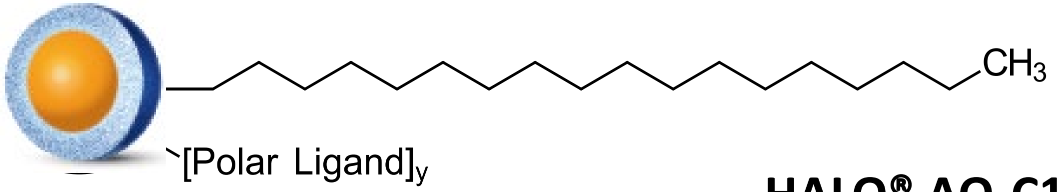
Another C18 Column?

Features and Benefits



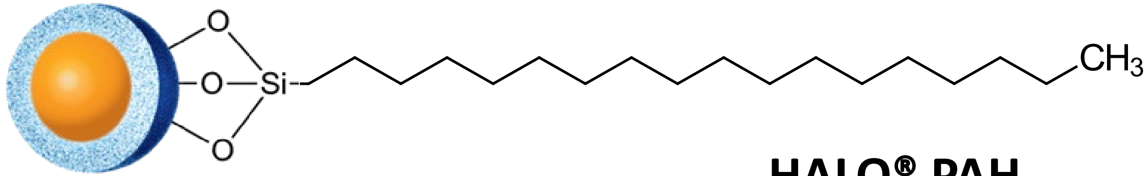
HALO® C18

- The standard for retaining and separating a broad range of analyte polarities



HALO® AQ-C18

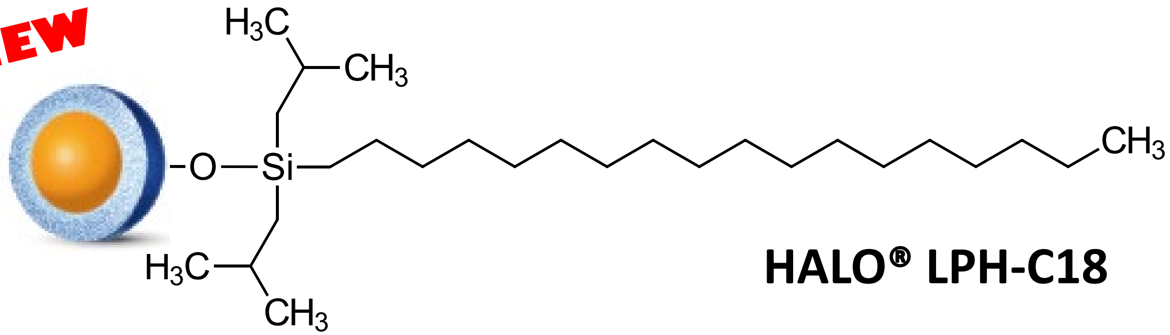
- Resistant to dewetting, making it 100% aqueous mobile phase compatible
- Enhanced retention and selectivity for polar molecules



HALO® PAH

- Optimized for polycyclic aromatic hydrocarbons (PAH)
- polymeric bonding that promotes shape selectivity

NEW

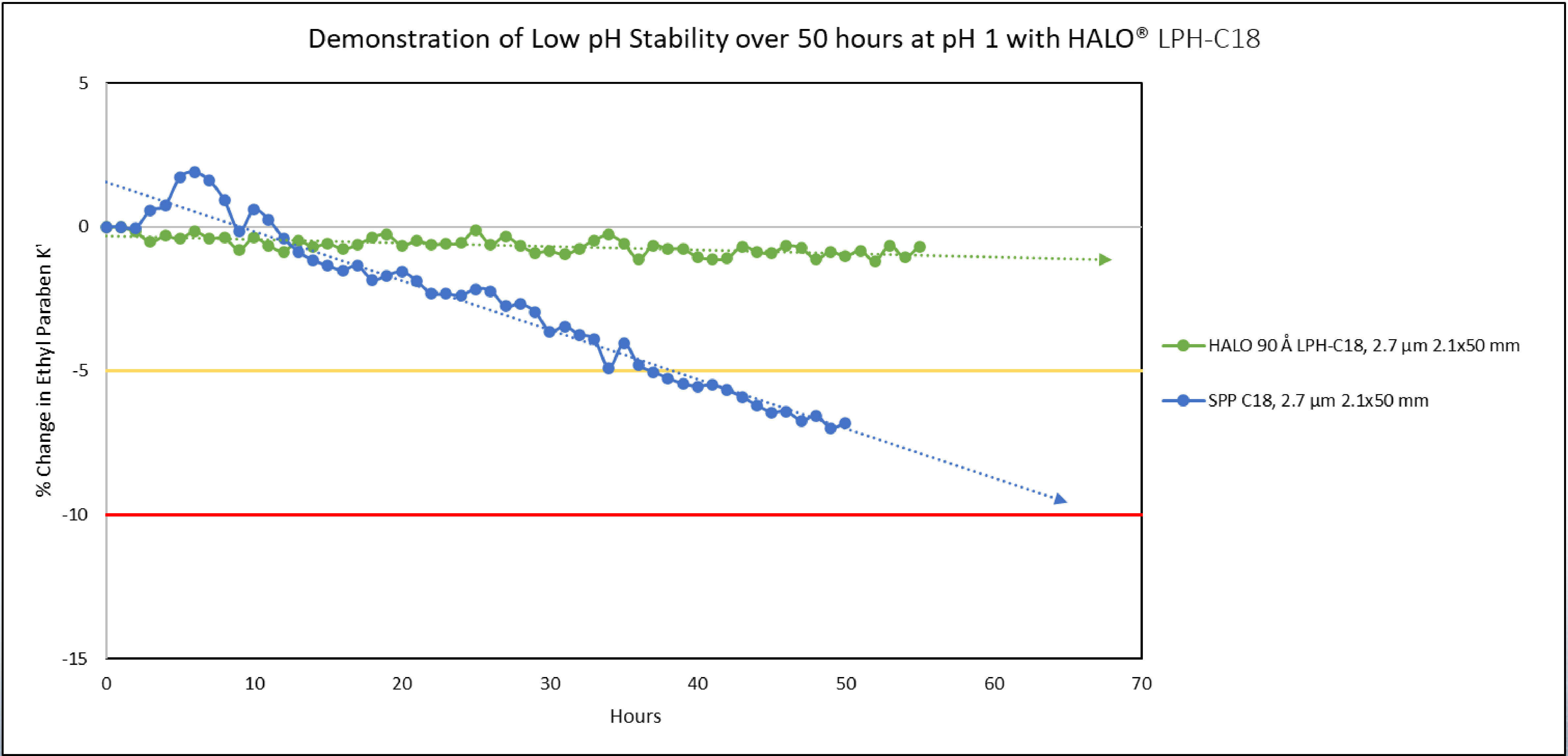


HALO® LPH-C18

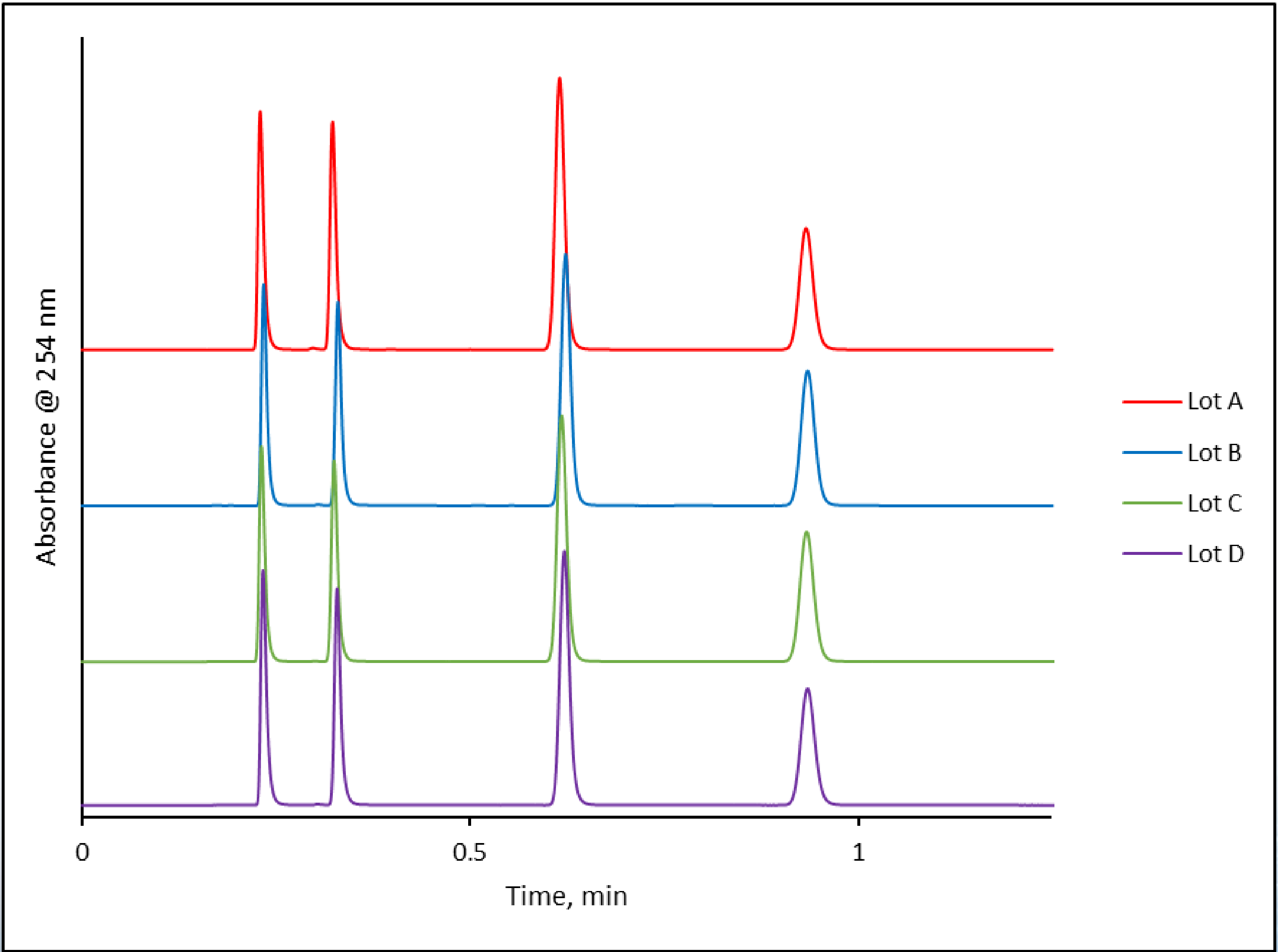
- Optimized for small molecule reversed-phase HPLC separations using low pH mobile phases and elevated temperature for acidic and neutral compounds

Use 160Å ES-C18 for larger molecules/peptides

LPH-C18: Stability: pH 1, 60°C

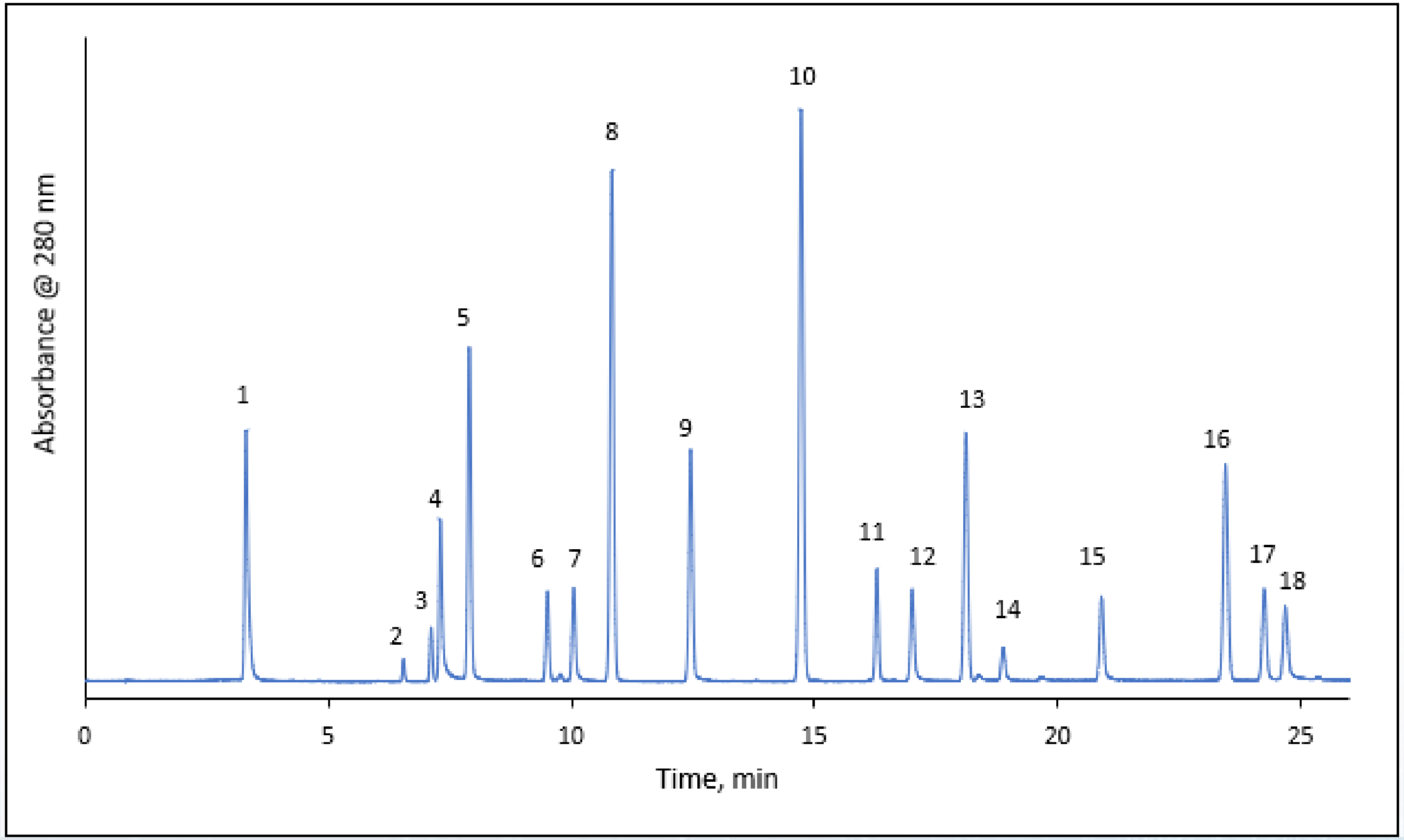


LPH-C18: Lot-to-Lot



TEST CONDITIONS
Column: HALO 90 Å LPH-C18, 2.7 µm 4.6 x 50 mm
Part Number: 92824-416
Mobile Phase A: Water
B: Acetonitrile
Isocratic: 60% B
Flow Rate: 1.8 mL/min
Temperature: 30 °C
Detection: 254 nm
Injection Volume: 2.0 µL
Peak Identities: uracil, phenol, 1-chloro-4-nitrobenzene, naphthalene (in elution order)
Sample Solvent: 60/40 ACN/ Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

LC-UV of Polyphenols



TEST CONDITIONS

Column: HALO 90 Å LPH-C18, 2.7 µm 2.1x100 mm
Part Number: 92822-616
Mobile Phase A: Water/ 0.1% Formic Acid
Mobile Phase B: Acetonitrile/ 0.1% Formic Acid
Gradient:

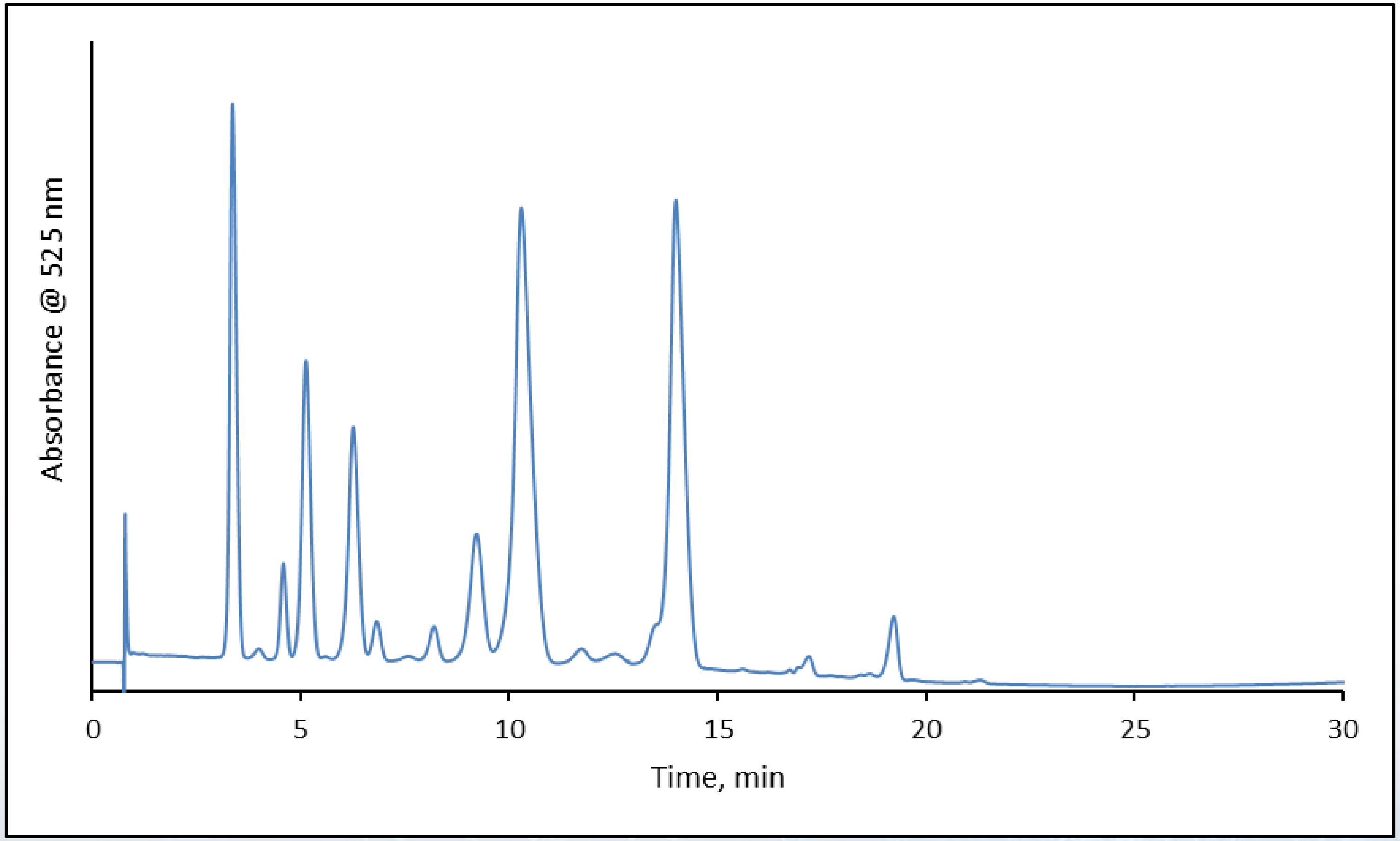
Time (min)	%B
0.0	0
3.5	8
7.1	10
25.0	30
26.0	40
27.0	100
29.0	100
30.0	0
35.0	0

Flow Rate: 0.3 mL/min
Pressure: 159 bar
Temperature: 30 °C
Detection: UV 280 nm, PDA
Injection Volume: 0.7 µL
Sample Solvent: Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

1. Gallic Acid
2. Epigallocatechin
3. Chlorogenic Acid
4. Catechin
5. Caffeic Acid
6. Epicatechin
7. Epigallocatechin Gallate
8. p-Coumaric Acid
9. Ferulic Acid
10. o-Coumaric Acid
11. Quercitrin
12. Myricetin
13. Resveratrol
14. Morin
15. Quercetin
16. Naringenin
17. Apigenin
18. Kaempferol

LC-UV of Anthocyanins Extracted from Blueberries



TEST CONDITIONS

Column: HALO 90 Å LPH-C18, 2.7 µm 2.1x100 mm
Part Number: 92822-616
Mobile Phase A: Water/ 3% Phosphoric Acid (pH: 1.4)
Mobile Phase B: Methanol
Gradient:

Time	%B
0.0	23
10.8	26
29.8	60

Flow Rate: 0.27 mL/min
Pressure: 144 bar
Temperature: 30°C
Detection: UV 525 nm, PDA
Injection Volume: 4.5 µL
Sample Solvent: Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

LC-MS Pesticide Screening of Barley

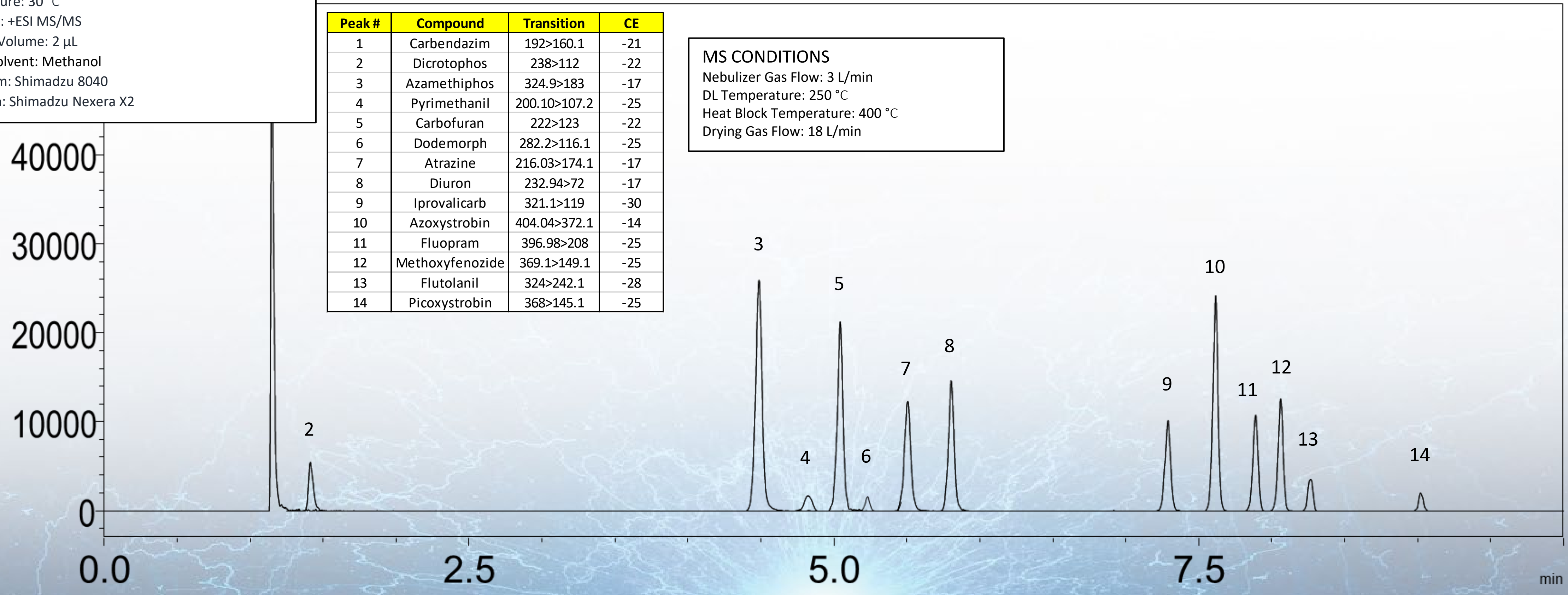
TEST CONDITIONS
Column: HALO 90 Å LPH-C18 2 µm, 2.1x100 mm
Part Number: 91822-616
Mobile Phase A: Water, 0.1% Formic Acid
Mobile Phase B: Acetonitrile, 0.1% Formic Acid
Gradient:

Time	%B
0.0	30
1.0	30
12.0	100
16.0	100

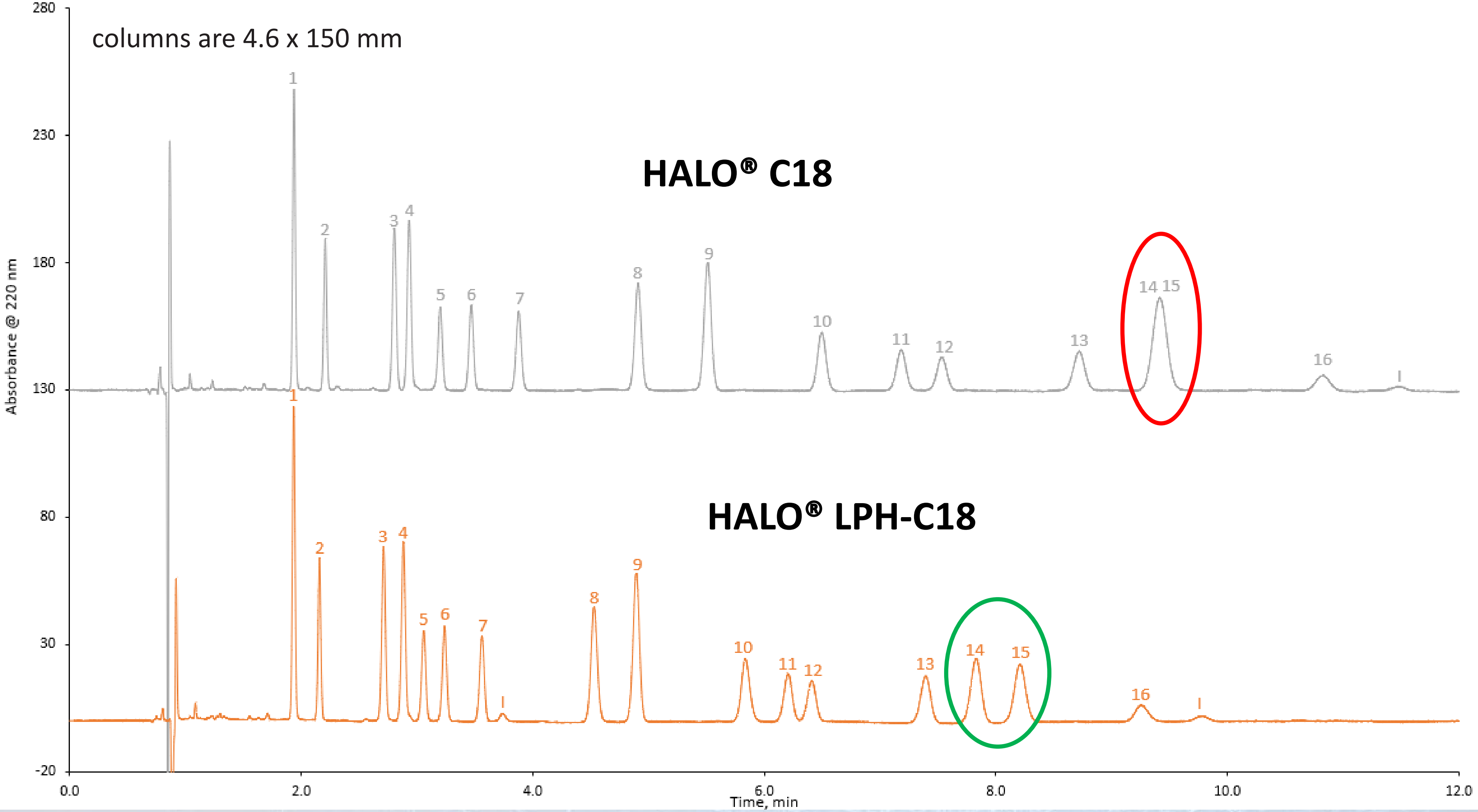
Flow Rate: 0.2 mL/min
Pressure: 235 bar
Temperature: 30 °C
Detection: +ESI MS/MS
Injection Volume: 2 µL
Sample Solvent: Methanol
MS System: Shimadzu 8040
LC System: Shimadzu Nexera X2

Peak #	Compound	Transition	CE
1	Carbendazim	192>160.1	-21
2	Dicrotophos	238>112	-22
3	Azamethiphos	324.9>183	-17
4	Pyrimethanil	200.10>107.2	-25
5	Carbofuran	222>123	-22
6	Dodemorph	282.2>116.1	-25
7	Atrazine	216.03>174.1	-17
8	Diuron	232.94>72	-17
9	Iprovalicarb	321.1>119	-30
10	Azoxystrobin	404.04>372.1	-14
11	Fluopram	396.98>208	-25
12	Methoxyfenozide	369.1>149.1	-25
13	Flutolanil	324>242.1	-28
14	Picoxystrobin	368>145.1	-25

MS CONDITIONS
Nebulizer Gas Flow: 3 L/min
DL Temperature: 250 °C
Heat Block Temperature: 400 °C
Drying Gas Flow: 18 L/min



16 Cannabinoid Separation: HALO® C18 compared to HALO ® LPH-C18

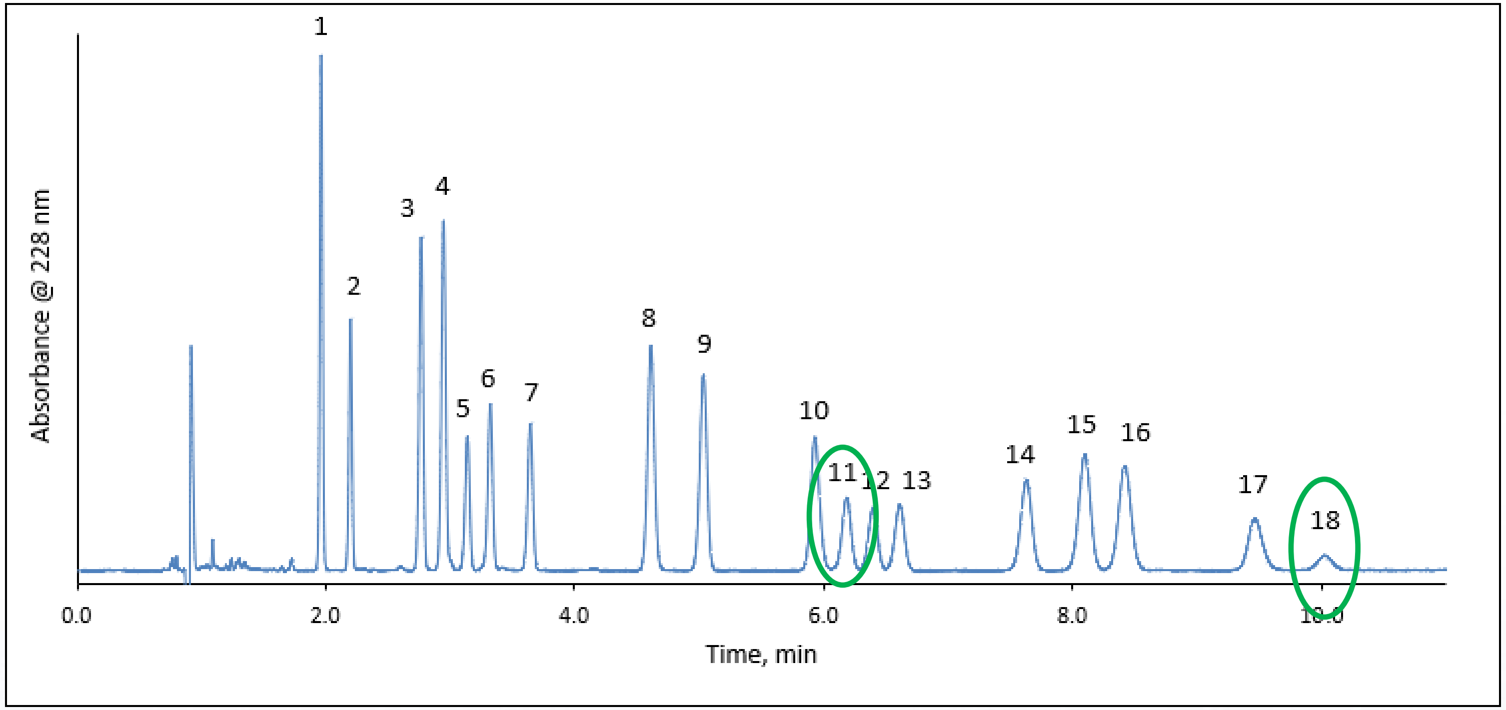


Peak Identities:

1. Cannabidivarinic acid (CBDVA)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Tetrahydrocannabivarinic acid (THCVA)
9. Cannabinol (CBN)
10. Cannabinolic acid (CBNA)
11. Δ9-Tetrahydrocannabinol (Δ9-THC)
12. Δ8-Tetrahydrocannabinol (Δ8-THC)
13. Cannabicyclol (CBL)
14. Cannabichromene (CBC)
15. Tetrahydrocannabinolic acid A (THCA-A)
16. Cannabichromenic acid (CBCA)

Mobile Phase A: 5mM Ammonium Formate in Water + 0.1% Formic Acid (pH = 3)
Mobile Phase B: Acetonitrile + 0.1% Formic Acid
Isocratic: 25/75 A/B
Flow Rate: 1.5 mL/min

Fast Separation of 18 Cannabinoids



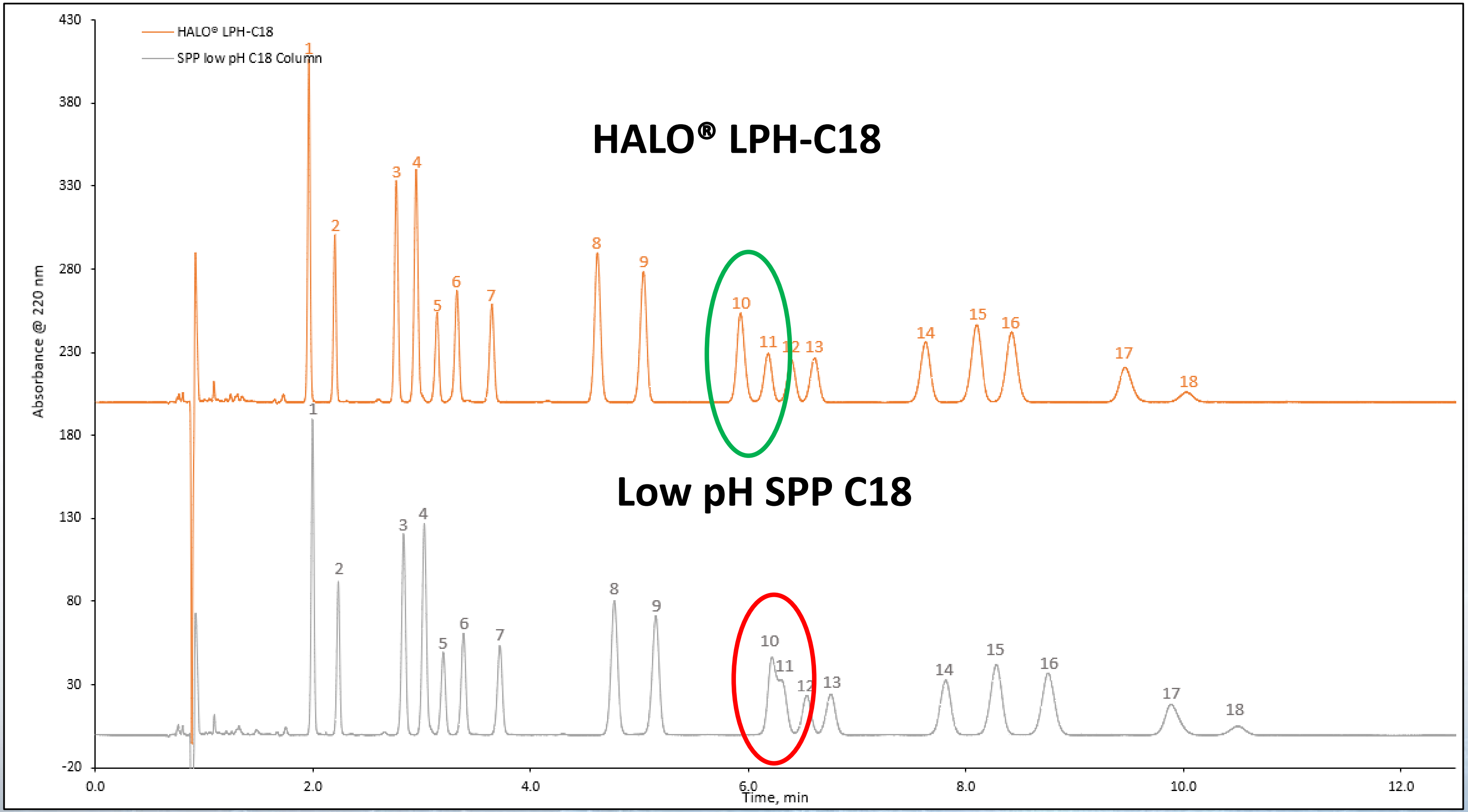
TEST CONDITIONS

Column: HALO 90 Å LPH-C18, 2.7 µm, 4.6 x 150mm
Part Number: 92824-716
Mobile Phase A: 5 mM Ammonium Formate, 0.1% Formic Acid
B: Acetonitrile, 0.1% Formic Acid
Isocratic: 75% B
Flow Rate: 1.5 mL/min
Pressure: 232 bar
Temperature: 30°C
Detection: PDA, UV: 228 nm
Injection Volume: 3 µL
Sample Solvent: 75/25 MeOH/ Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µl
LC System: Shimadzu Nexera X2

PEAK IDENTITIES

1. Cannabidivarinic acid (CBDVA)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Tetrahydrocannabivarinic acid (THCVA)
9. Cannabinol (CBN)
10. Cannabinolic acid (CBNA)
11. Exo-tetrahydrocannabinol (EXO-THC)
12. delta 9- Tetrahydrocannabinol (D9-THC)
13. delta 8- Tetrahydrocannabinol (D8-THC)
14. Cannabicycol (CBL)
15. Cannabichromene (CBC)
16. Tetrahydrocannabinolic acid A (THCA-A)
17. Cannabichromenic acid (CBCA)
18. Cannabicyclolic acid (CBLA)

18 Cannabinoid Separation: HALO LPH-C18 compared to low pH SPP C18



PEAK IDENTITIES

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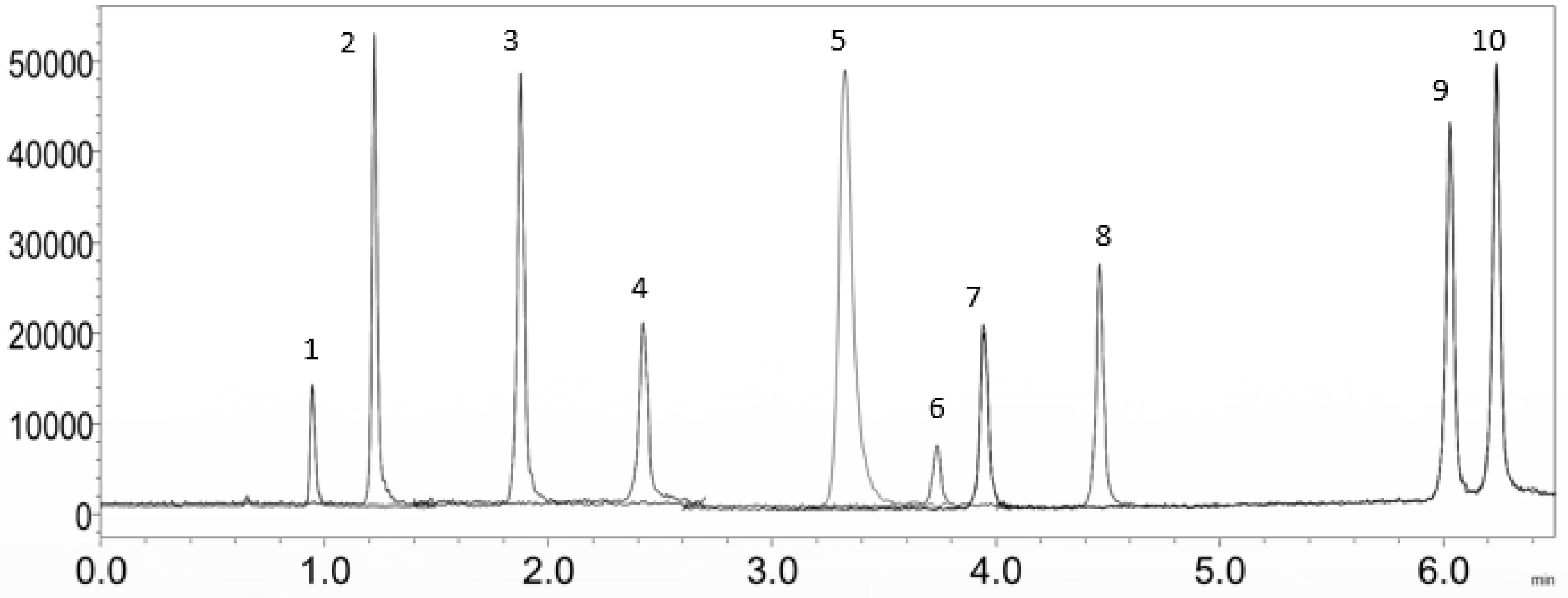
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Tetrahydrocannabivarinic acid (THCVA)
9. Cannabinol (CBN)
10. Cannabinolic acid (CBNA)
11. Exo-tetrahydrocannabinol (EXO-THC)
12. delta 9- Tetrahydrocannabinol (D9-THC)
13. delta 8- Tetrahydrocannabinol (D8-THC)
14. Cannabicycol (CBL)
15. Cannabichromene (CBC)
16. Tetrahydrocannabinolic acid A (THCA-A)
17. Cannabichromenic acid (CBCA)
18. Cannabicyclolic acid (CBLA)

LC-MS of Catechins and Caffeine in Tea



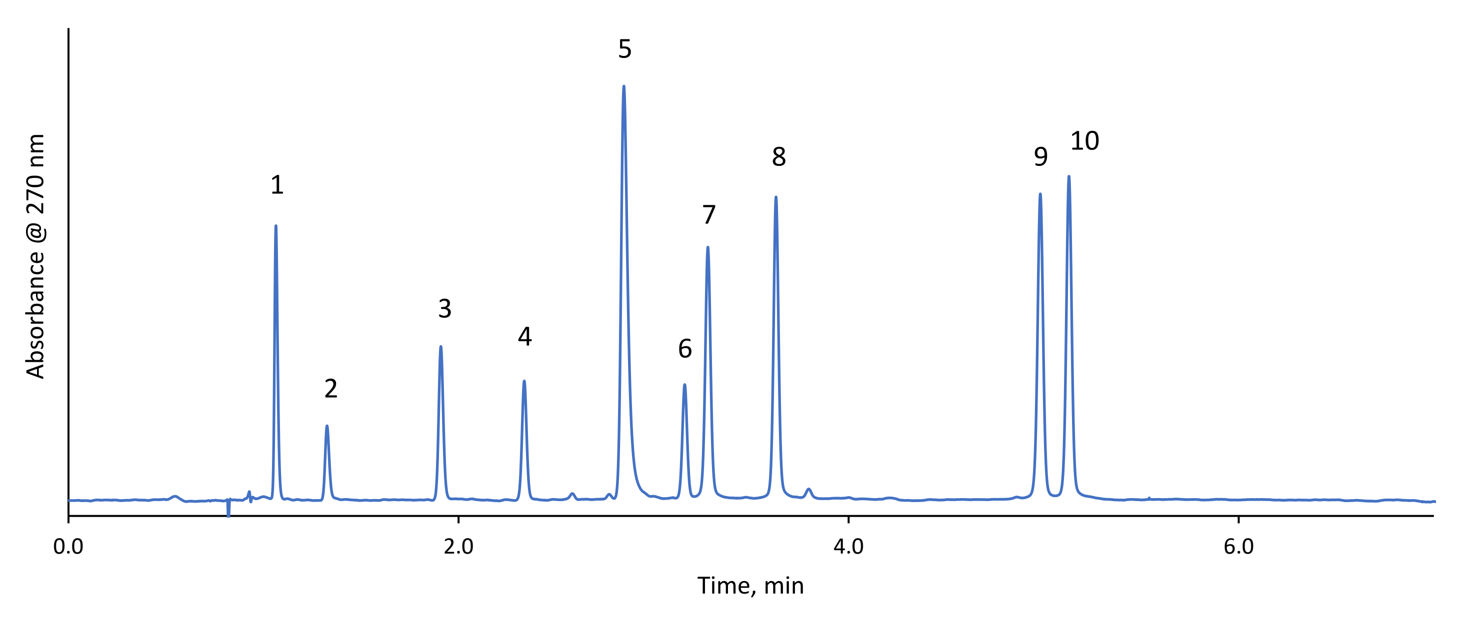
Peak #	Compound	M/Z
1	Gallic Acid	169
2	Gallocatechin	305
3	Epigallocatechin	305
4	Catechin	289
5	Caffeine	195
6	Epicatechin	289
7	Epigallocatechin Gallate	457
8	Gallocatechin Gallate	457
9	Epicatechin Gallate	441
10	Catechin Gallate	441

TEST CONDITIONS
Column: HALO 90 Å LPH-C18 2 µm, 2.1x100 mm
Part Number: 91822-616
Mobile Phase A: Water, 0.2% Formic Acid (pH 2.45)
Mobile Phase B: Acetonitrile, 0.2% Formic Acid

Gradient:	Time	%B
	0.0	10
	1.0	10
	6.0	21
	7.0	21

Flow Rate: 0.3 mL/min
Pressure: 438 bar
Temperature: 40 °C
Detection: +/- ESI MS/MS
Injection Volume: 2 µL
Sample Solvent: Water
MS System: Shimadzu 8040
LC System: Shimadzu Nexera X2

LC-UV of Catechins and Caffeine in Tea



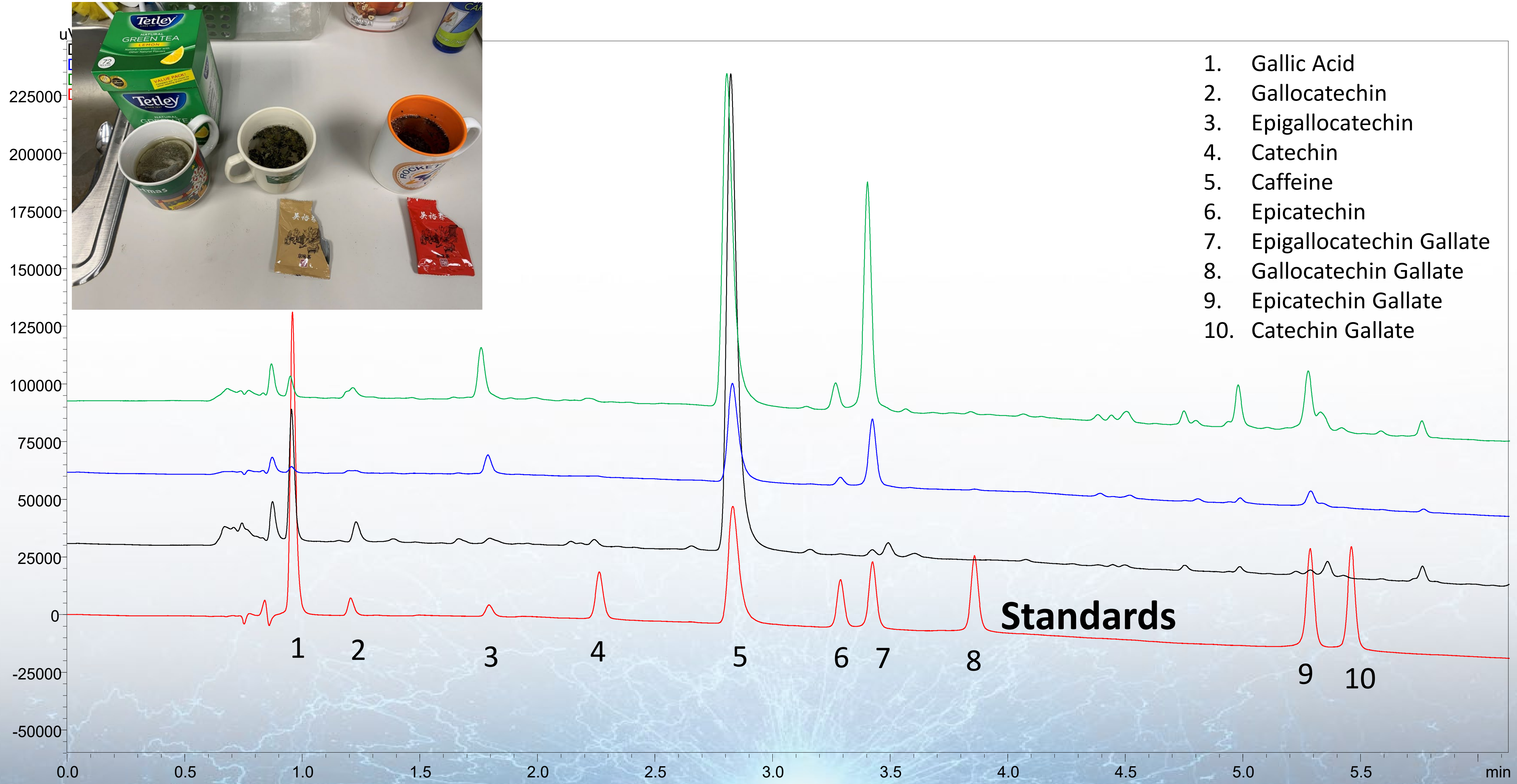
TEST CONDITIONS
Column: HALO 90 Å LPH-C18 2.7 µm, 4.6x150 mm
Part Number: 92824-716
Mobile Phase A: Water, 0.2% Formic Acid (pH: 2.45)
Mobile Phase B: Acetonitrile, 0.2% Formic Acid

Gradient:	Time	%B
	0.0	10
	0.5	10
	6.0	23
	7.0	23

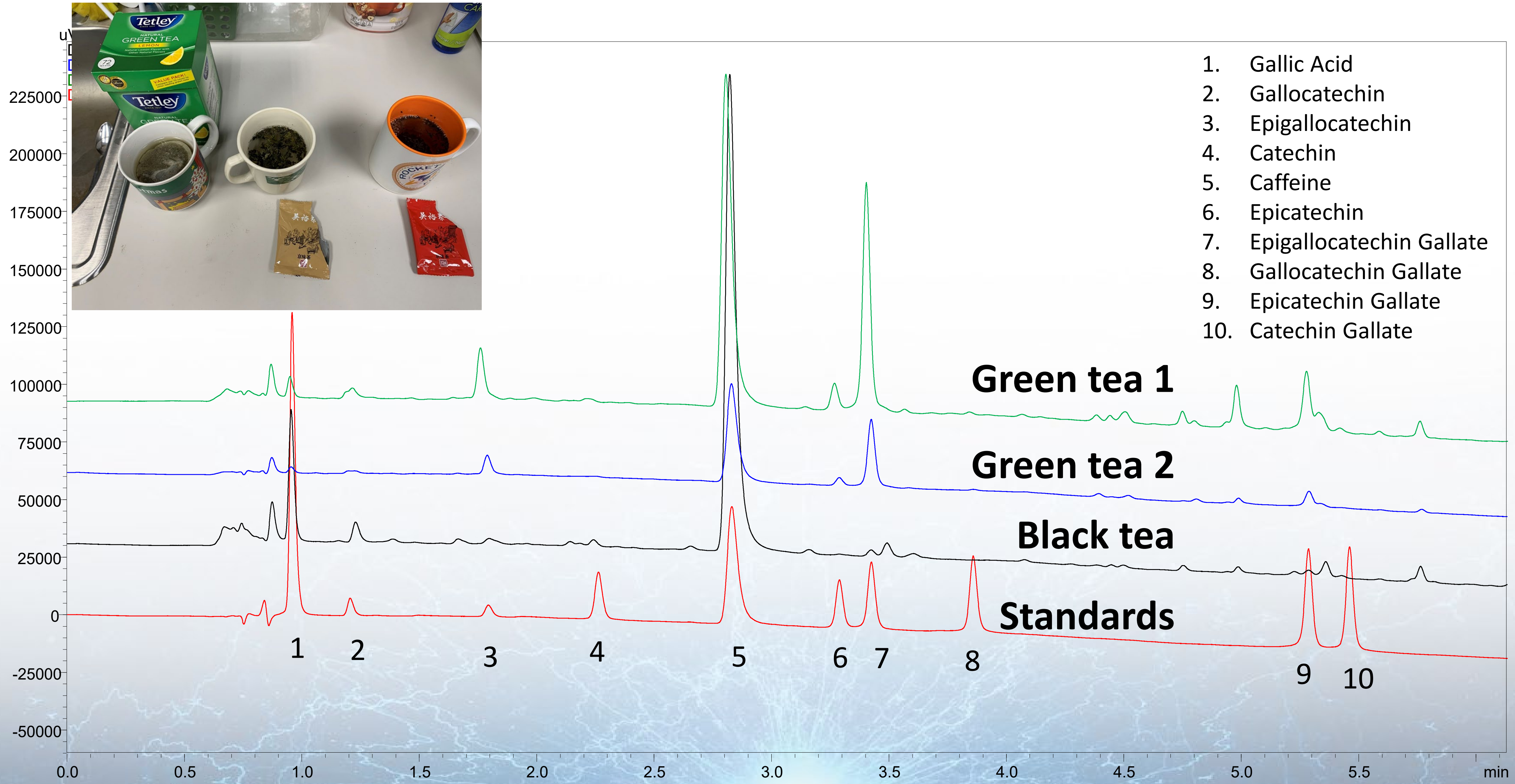
Flow Rate: 1.8 mL/min
Pressure: 395 bar
Temperature: 40 °C
Detection: PDA, UV 270 nm
Injection Volume: 5 µL
Sample Solvent: 90/10 Water/ Acetonitrile
LC System: Shimadzu Nexera X2

Peak #	Compound	M/Z
1	Gallic Acid	169
2	Gallocatechin	305
3	Epigallocatechin	305
4	Catechin	289
5	Caffeine	195
6	Epicatechin	289
7	Epigallocatechin Gallate	457
8	Gallocatechin Gallate	457
9	Epicatechin Gallate	441
10	Catechin Gallate	441

Catechins and Caffeine in Tea: Comparison of 3 Teas



Catechins and Caffeine in Tea: Comparison of 3 Teas



HALO 90 Å LPH-C18 Summary

- Improved stability with low pH mobile phases
  - Low pH/Temperature Limit: 1/90 °C
- Highly reproducible bonded phase coverage
- USP classification: L1
- Available Particle Sizes: 2 µm, 2.7 µm
  - New Column I.D.: 1.5 mm I.D. for 2.7 µm particle size

Questions?

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