HALO®

innovated by

advanced materials technology

Cannabinoid Separation Using HALO® LPH-C18

Conner McHale
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Advanced Materials Technology

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Presentation Outline

- Advanced Materials Technology
    - Superficially Porous Particles (SPP)
    - Product Portfolio
- HALO® 90Å, LPH-C18
- Cannabis Potency Testing Method Development
- HALO® 1.5 mm ID
- Technical Resources

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

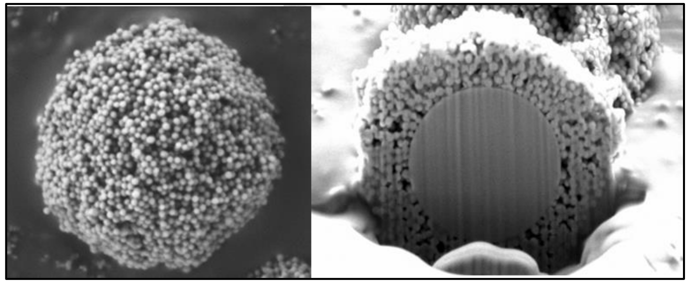
Facility

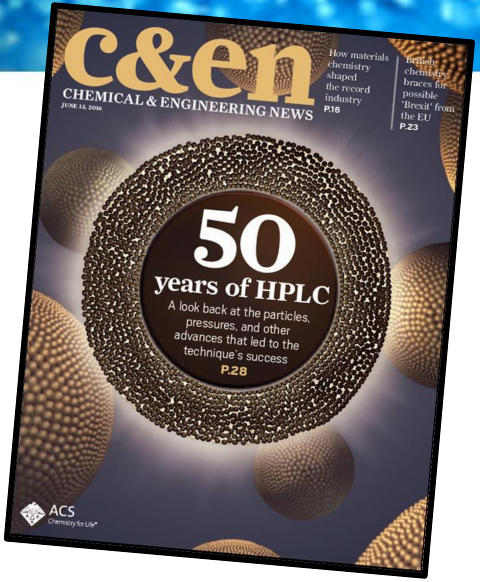
- Fully equipped state of the art laboratories
- All operations handled in Wilmington, DE
  - R&D, Applications, QA/QC, Manufacturing, Sales and Marketing

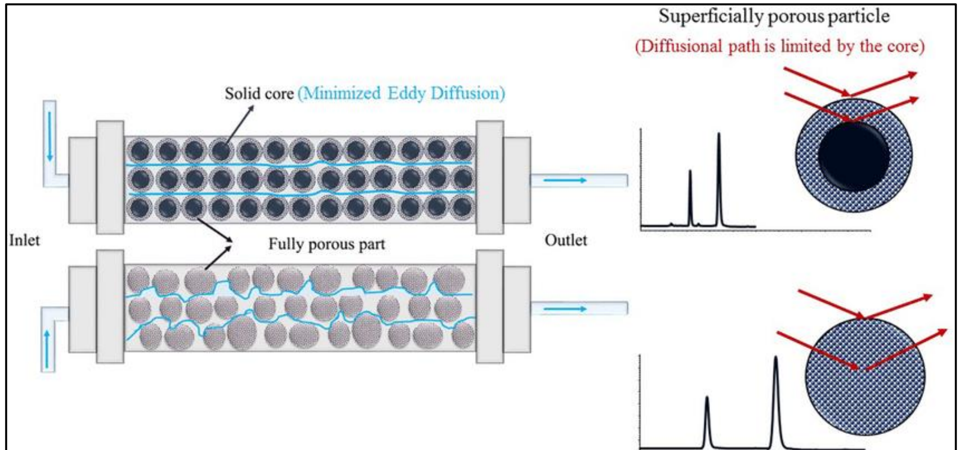
AMT is a company of innovators and continues to grow and deliver enabling materials to market. Our incredible team is our greatest resource.

Superficially Porous Particle Technology (SPP)

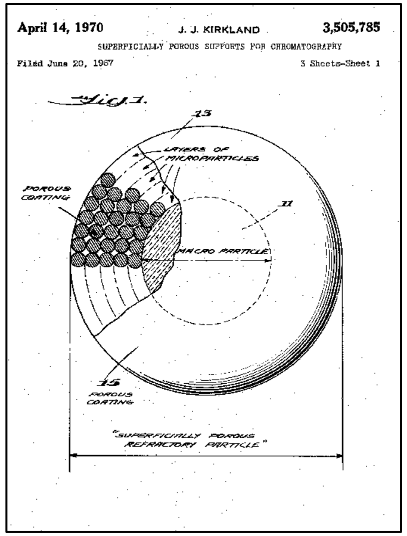
-High Purity Silica Particles (2, 2.7, 3.4, 5 µm)
-Bonded Phase Shell Fused to Solid Core
-Shell Consists of Different Pore Sizes
(90, 160, 400, 1000Å)

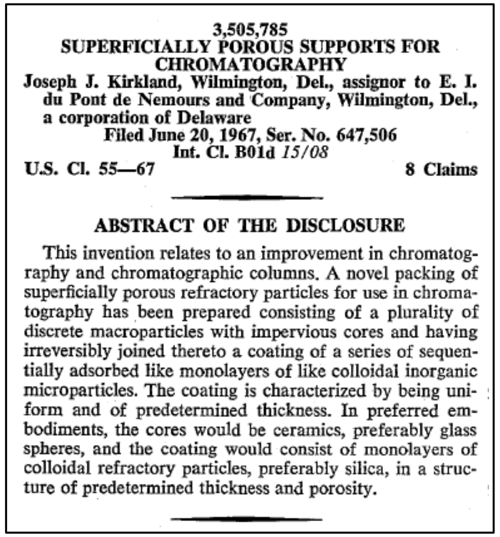






The History of the Core–Shell Particles and Applications in Active Pharmaceutical Ingredients Via Liquid Chromatography - Scientific Figure on ResearchGate.



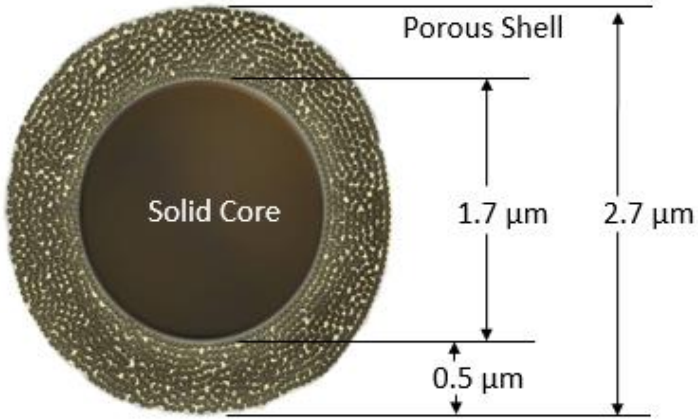


3,505,785
SUPERFICIALLY POROUS SUPPORTS FOR CHROMATOGRAPHY
Joseph J. Kirkland, Wilmington, Del., assignor to E. I. du Pont de Nemours and Company, Wilmington, Del., a corporation of Delaware
Filed June 20, 1967, Ser. No. 647,506
Int. Cl. B01d 15/08
U.S. Cl. 55—67 8 Claims

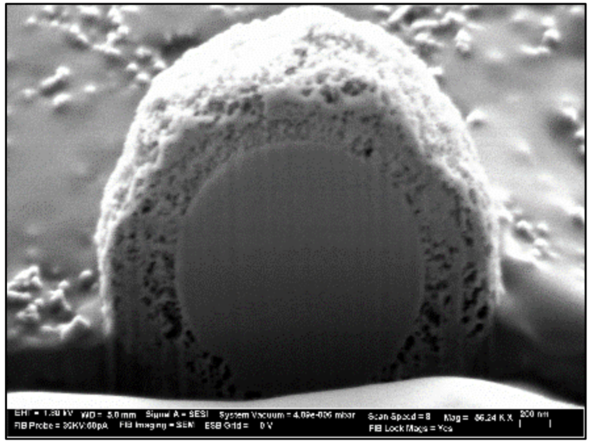
ABSTRACT OF THE DISCLOSURE

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.

Shell with pores

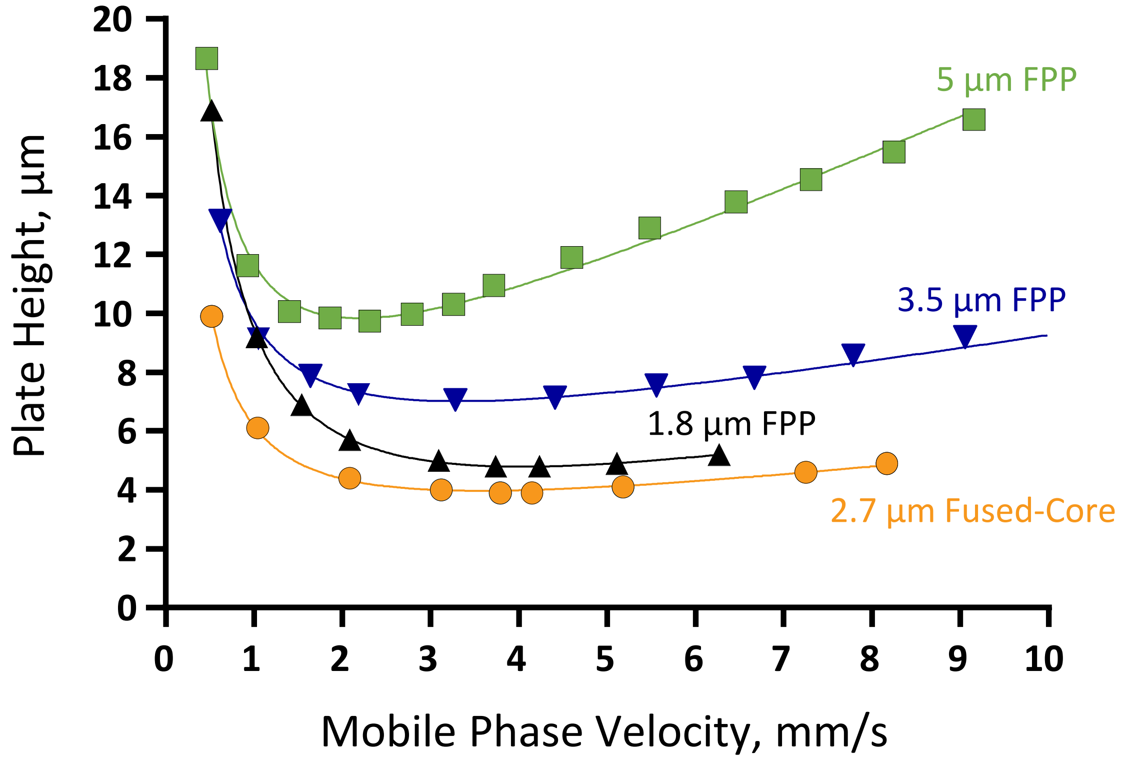


SEM Particle Cross- section

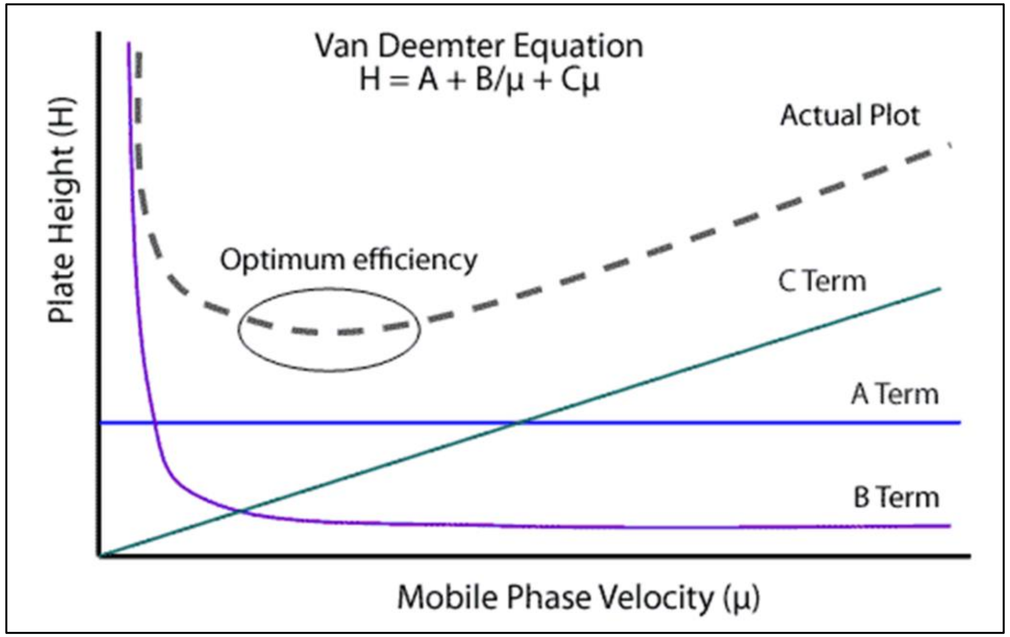


How SPP Benefits Separations?

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

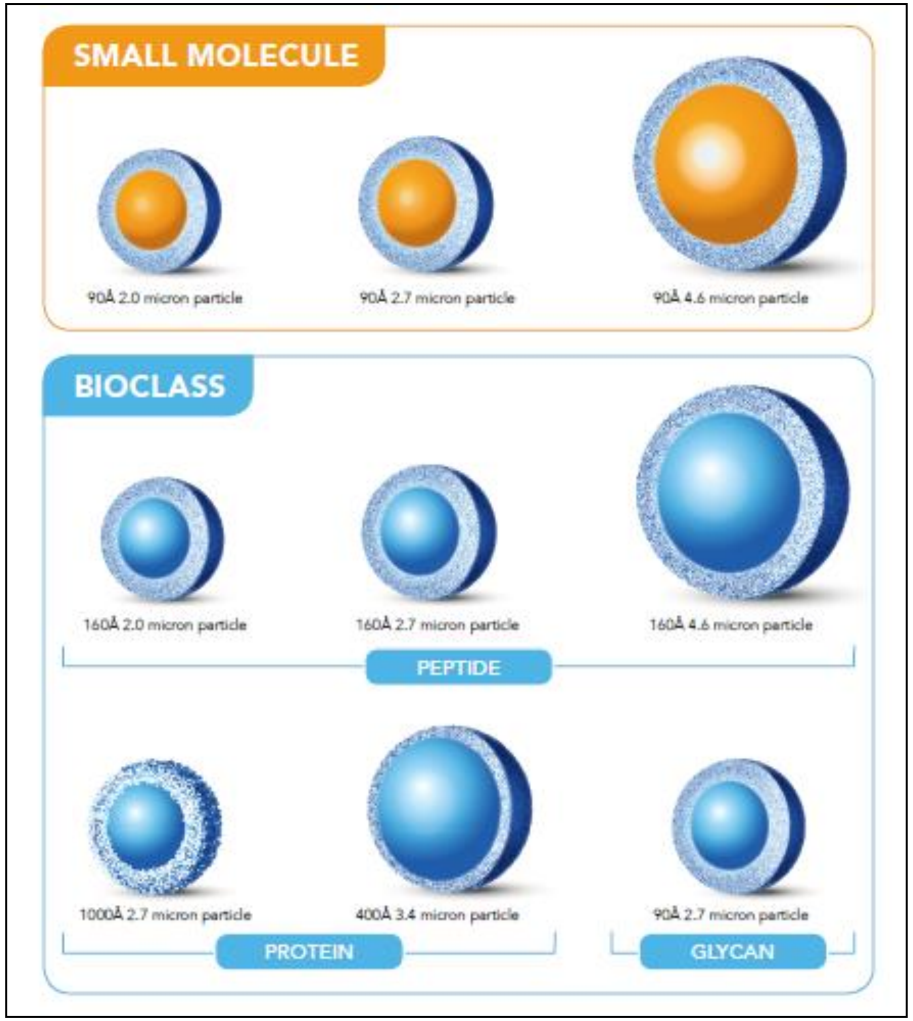
A = eddy diffusion term (particle size and how well bed was packed) **30 - 40% smaller**

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

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

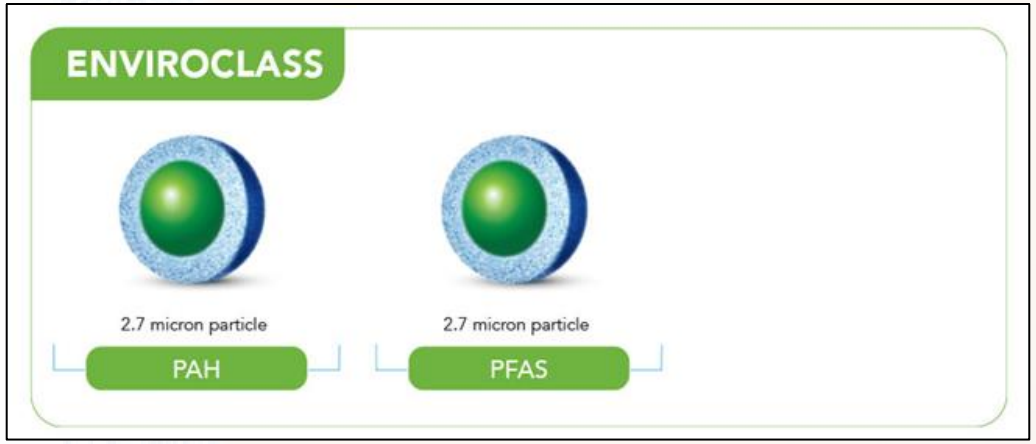
µ = mobile phase linear velocity (L/t_0)

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
- Many different column dimensions from capillary to semi-prep.



HALO 90 Å, 2.7 µm LPH-C18

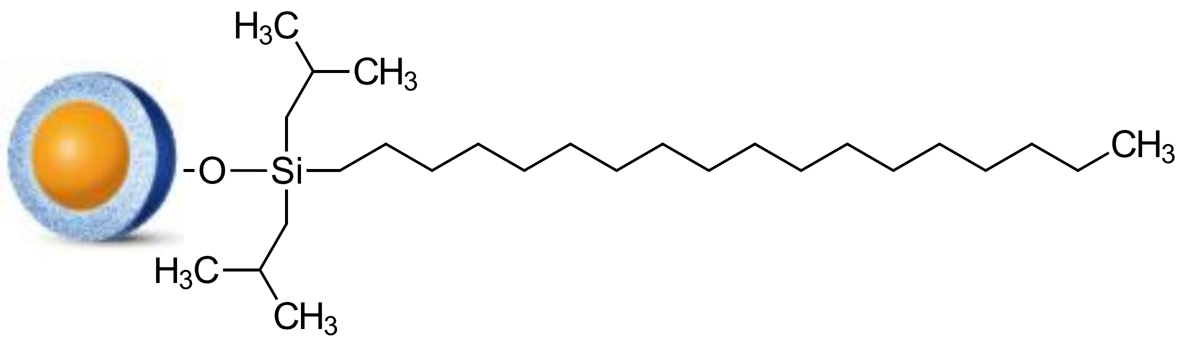
Introducing a low pH compatible, 90 Å, superficially porous particle C18 phase useful for any chromatographer running under low pH conditions. The sterically protected ligand reduced acidic hydrolysis which enables low pH mobile phases to be used without sacrificing column performance over time.

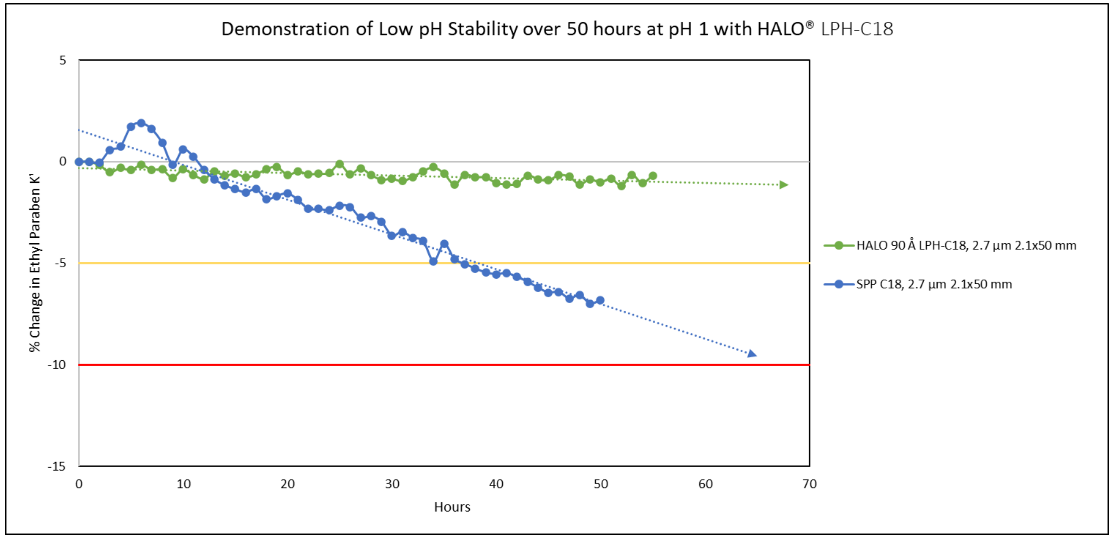
SPECIFICATIONS

Ligand: diisobutyloctadecylsilane
Particle Size: 2 µm, 2.7 µm
Pore Size: 90 Å

USP Designation: L1
Carbon Load: 6.5%
Surface Area:
2 µm: 120 m2/g
2.7 µm: 135 m2/g

Endcapped: No
Low pH Limit /Max T: 1/90 °C
High pH Limit/Max T: 8/40 °C





Pertinent for Cannabis because we prefer low pH to suppress acidic cannabinoids and improve peak shape

Cannabinoid Testing

A California marijuana company is being sued over the potency of its joints

By Zoe Sottile and Claudia Dominguez, CNN

Published 1:39 PM EDT, Wed October 26, 2022

More potent pot tends to be priced higher in California

Market share and price of legal marijuana by listed level of THC, 2020

THC LEVEL		SHARE OF TRANSACTIONS	RETAIL PRICE/GRAM
Very Low	<7%	0.3%	$8.40
Low	7-14	4.0	5.31
Medium	14-21	39.8	7.37
High	21-28	44.7	11.06
Very High	>=28	11.1	12.89

Cured flower products only.

SOURCE: FLOWHUB

JOURNAL OF NATURAL PRODUCTS
Article
Cite This: J. Nat. Prod. 2019, 82, 3047−3055
pubs.acs.org/jnp

Pharmacokinetics of Phytocannabinoid Acids and Anticonvulsant Effect of Cannabidiolic Acid in a Mouse Model of Dravet Syndrome

Lyndsey L. Anderson,[†,‡] Ivan K. Low,[†] Samuel D. Banister,[†,§] Iain S. McGregor,[†,⊥] and Jonathon C. Arnold[*,†,‡]

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[‡]Discipline of Pharmacology, Faculty of Medicine and Health, The University of Sydney, Sydney, New South Wales 2006, Australia
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[⊥]School of Psychology, Faculty of Science, The University of Sydney, Sydney, New South Wales 2006, Australia

Journal of Natural Products
Article

Table 3. Physiochemical Properties of Phytocannabinoid Acids

phytocannabinoid	molecular weight	pK_a	log P	TPSA (Å)	rotatable bonds	CNS MPO score
CBCA	358.4	2.87	6.91	66.76	8	3.79
CBDA	358.5	2.91	6.63	77.76	7	3.59
CBDVA	330.4	2.91	5.94	77.76	5	4.04
CBGA	360.5	2.92	7.35	77.76	10	3.23
CBGVA	332.4	2.92	6.46	77.76	8	3.68
THCA	358.4	2.89	6.25	66.76	5	4.12

Standardizing Cannabis Lab Testing Nationally

NCLC recommends that the following cannabinoids be included in a standardized potency panel for all products:

- Δ9-THC
- Δ9-THCA
- Δ8-THC
- CBD
- CBDA
- CBN
- CBNA
- CBG
- CBGA

Further, the group recommends that any cannabinoid on the label should be required to be on the cannabinoid testing panel for all products, such cannabinoids to include, but not be limited to, the following:

- CBDV
- CBDVA
- THCV
- THCVA
- CBL
- CBLA
- CBC
- CBCA
- Δ10-THC
- Δ9,11-THC
- THCO acetate
- HHC

Buffer	pKa	Operating pH range
Phosphate, pk1	2.1	1.1-3.1
pK2	7.2	6.2-8.2
pK3	12.3	11.3-13.3 (not advisable)
Citrate, pK1	3.1	2.1-4.1
pK2	4.7	3.7-5.7
pK3	5.4	4.4-6.4
Formate	3.8	2.8-4.8
Acetate	4.8	3.8-5.8
Tris (hydroxymethyl)- aminomethane	8.3	7.3-9.3 (use at 8.0 or below)

Selection of formic acid/ ammonium formate: MS friendly and acidic cannabinoid pKa values are ~3

Journal of Natural Products

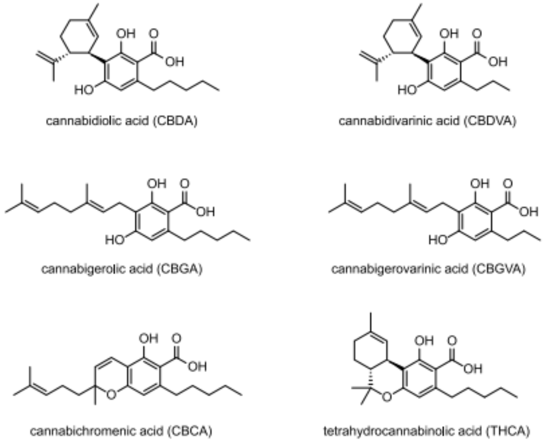
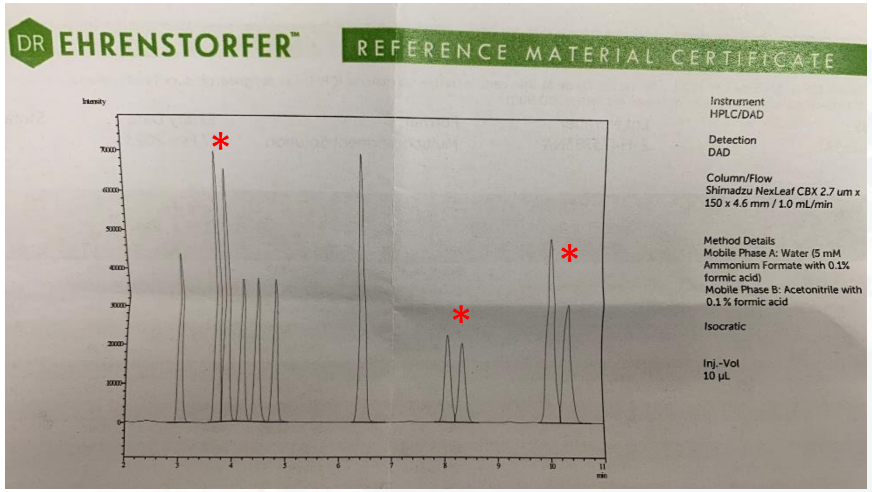
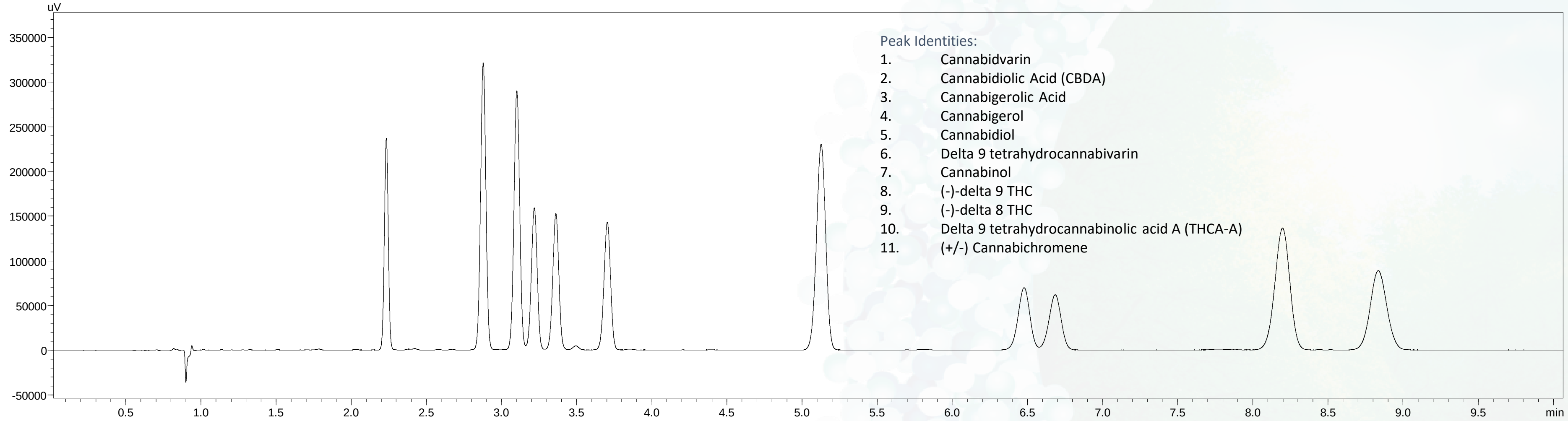


Figure 1. Chemical structures of phytocannabinoid acids used in this study.

Testing Conditions:
Mobile Phase: A: 5mM Ammonium Formate, 0.1% Formic Acid
B: Acetonitrile, 0.1% Formic Acid
Isocratic Separation: 75% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 228 nm
Injection: 1 µl
Temperature: 30ºC
Flow Rate: 1.5 mL/min.
Back Pressure: 345 bar
Sample: LGC DRE-A50000255AL
Sample Solvent: 75/25 ACN/ Water
Column: HALO 90Å LPH-C18, 2.7µm, 4.6 x 150mm





Testing Conditions:

Mobile Phase: A: 5mM Ammonium Formate, 0.1% Formic Acid
B: Acetonitrile, 0.1% Formic Acid

Isocratic Separation: 75% B

Instrument: Shimadzu Nexera X2

Wavelength: PDA, 228 nm

Injection: 1 µl

Temperature: 30°C

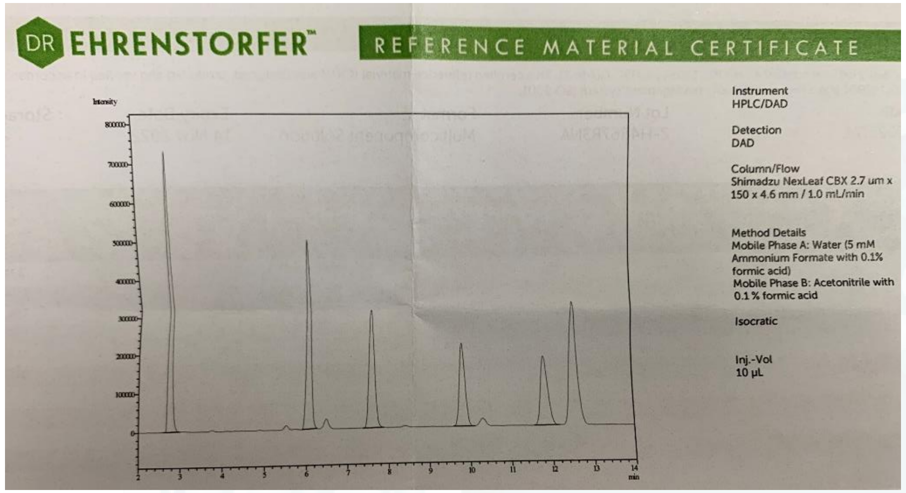
Flow Rate: 1.5 mL/min.

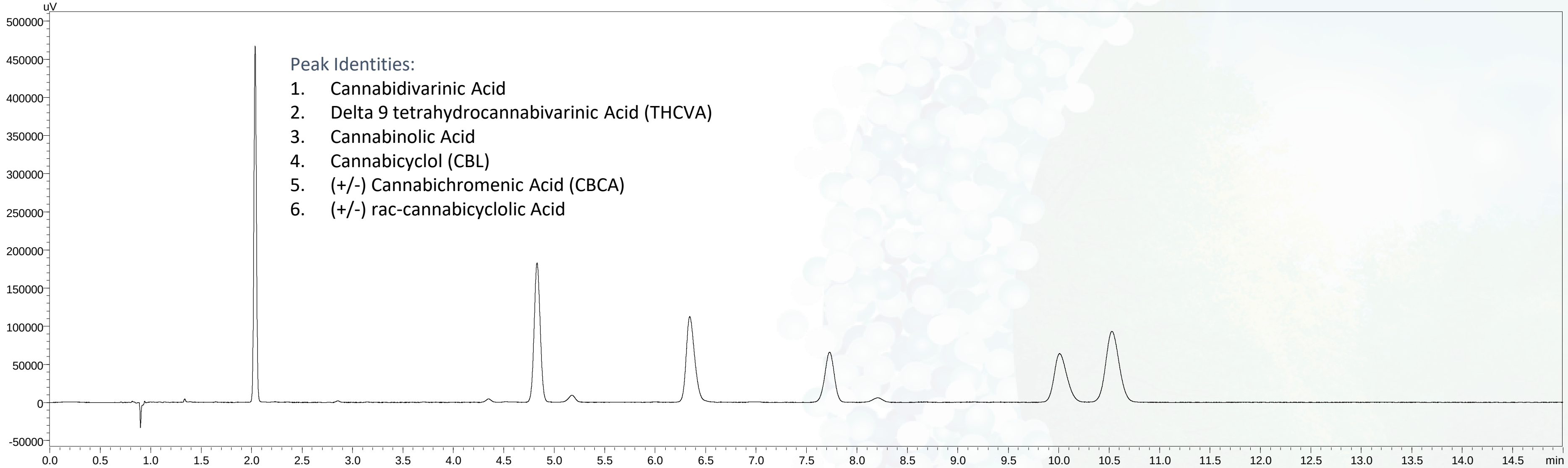
Back Pressure: 345 bar

Sample: LGC DRE-A50000257AL

Sample Solvent: 75/25 ACN/ Water

Column: HALO 90Å LPH-C18, 2.7µm, 4.6 x 150mm





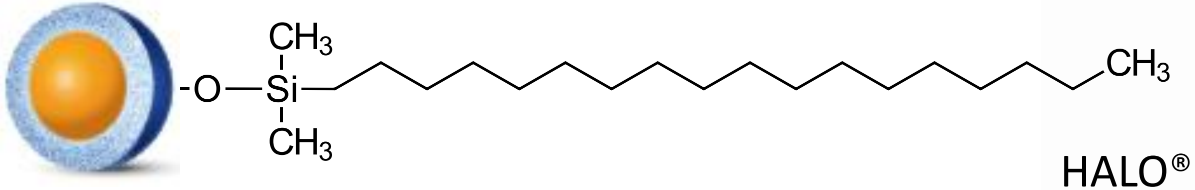
Peak Identities:

1. Cannabidivarinic Acid
2. Delta 9 tetrahydrocannabivarinic Acid (THCVA)
3. Cannabinolic Acid
4. Cannabicyclol (CBL)
5. (+/-) Cannabichromenic Acid (CBCA)
6. (+/-) rac-cannabicyclolic Acid

Another C18 Column?

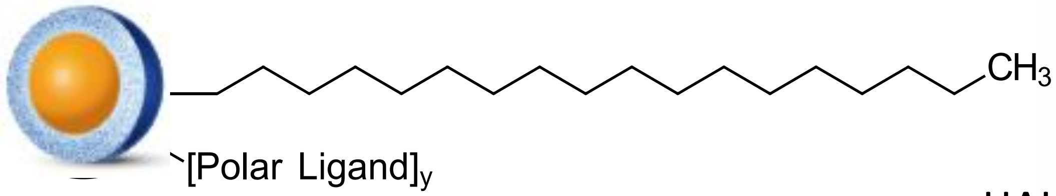
HALO®

Features and Benefits



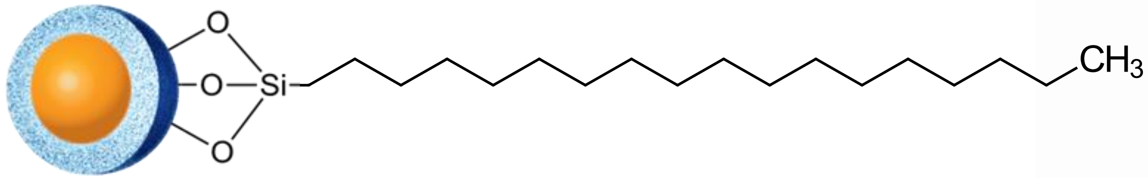
HALO® C18

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



HALO® AQ-C18

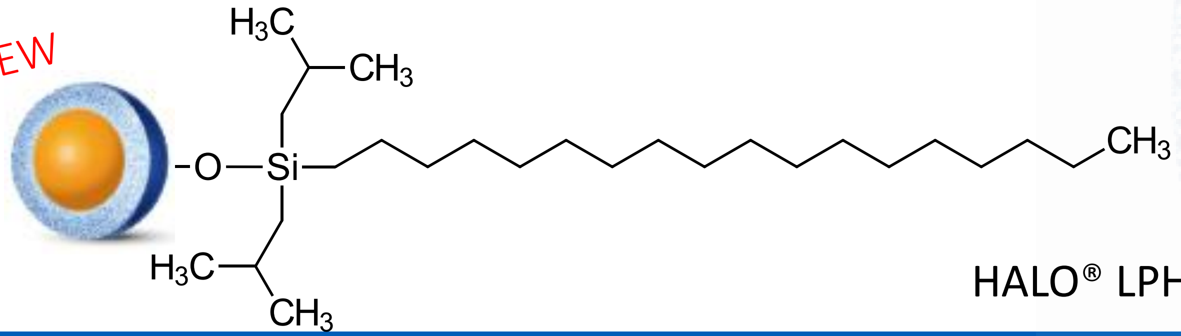
- Resistant to dewetting, making it 100% aqueous mobile phase compatible
- Enhanced retention and selectivity for polar molecules



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 employing mobile phases with very low pH and elevated temperature for acidic and neutral compounds.

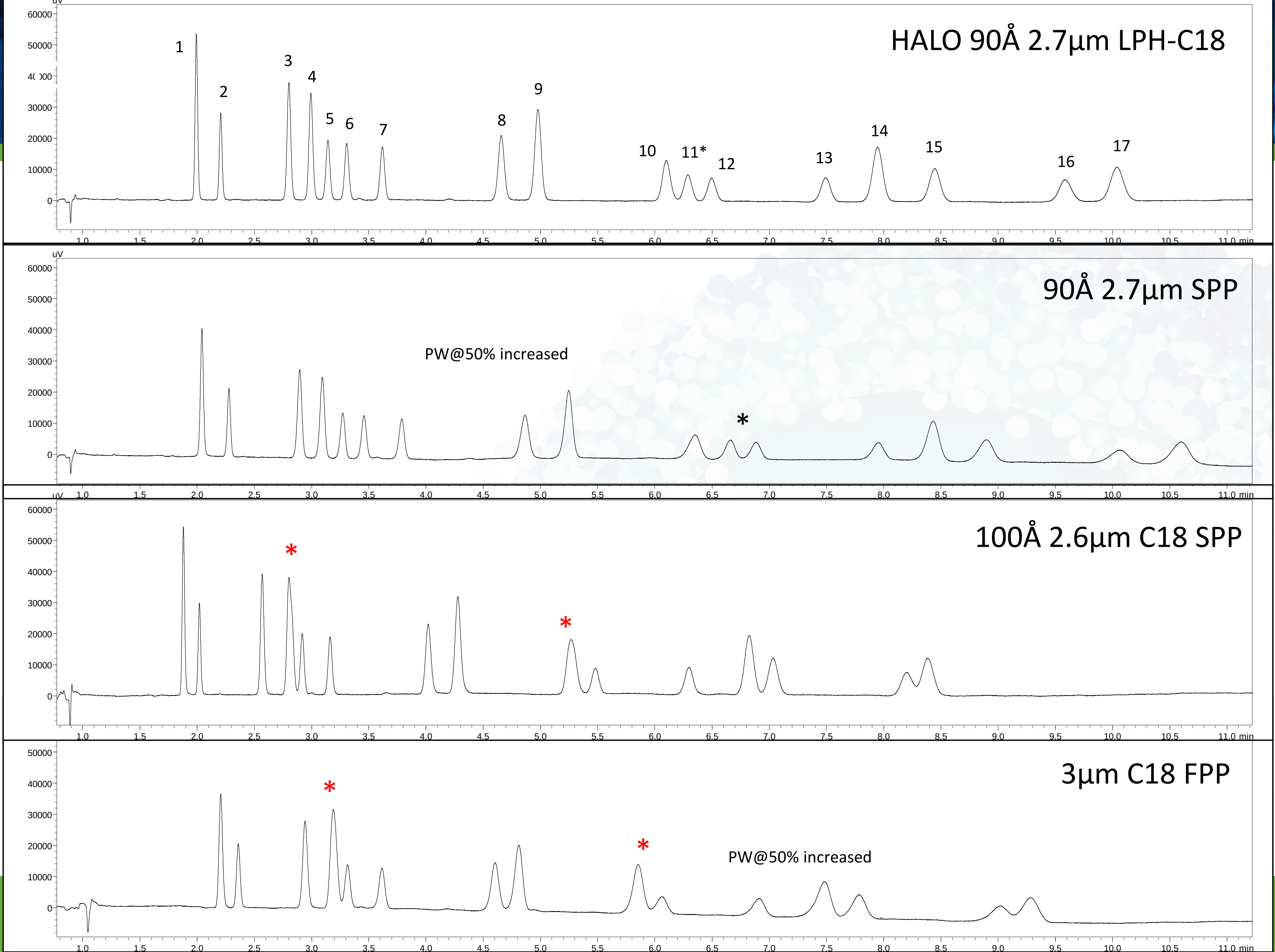
Cannabinoid HALO Phase Screening

HALO vs. Others

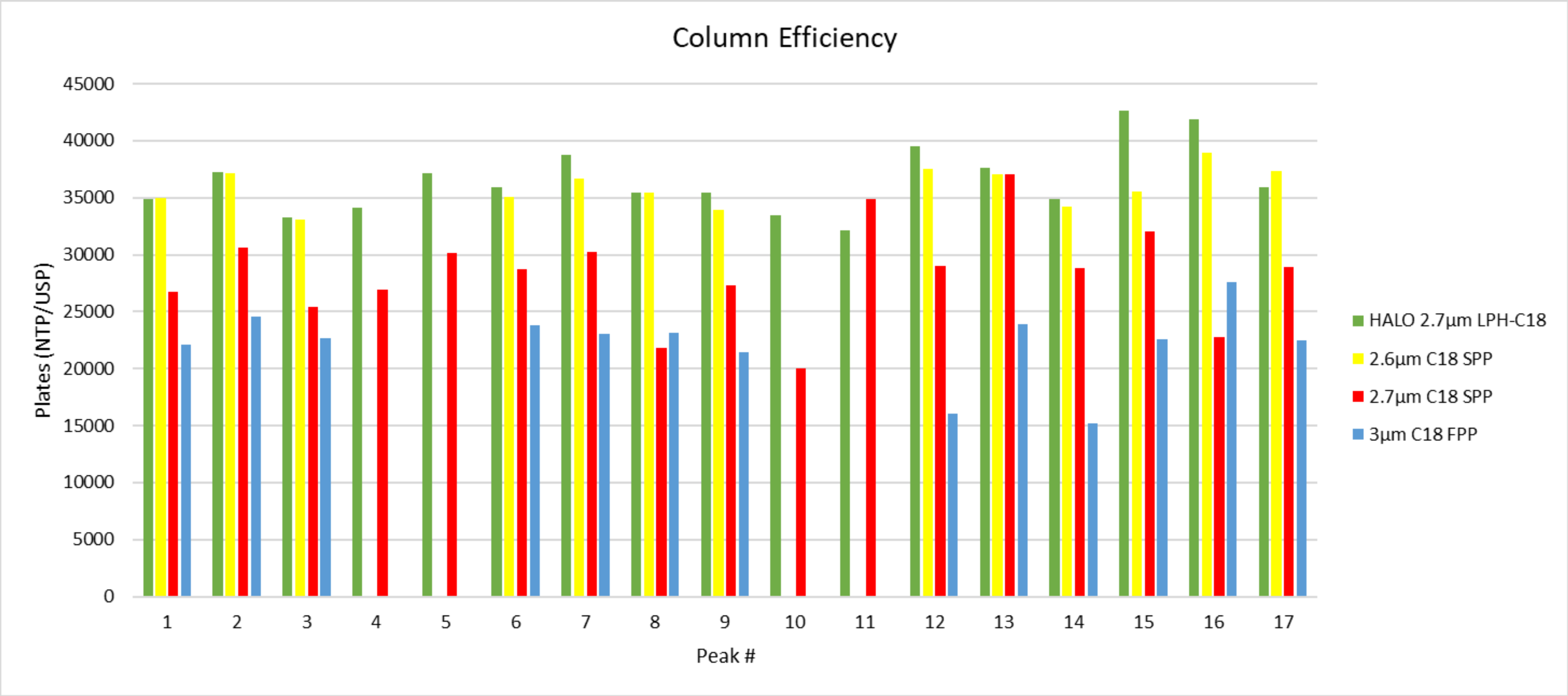
Testing Conditions:
Mobile Phase: A: 5mM Ammonium Formate, 0.1% Formic Acid
B: Acetonitrile, 0.1% Formic Acid
Isocratic Separation: 75% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 228 nm
Injection: 1 µl
Temperature: 30°C
Flow Rate: 1.5 mL/min.
Sample Solvent: 75/25 ACN/ Water, 0.1% FA
Column Dimension: 4.6 x 150mm

PEAK IDENTITIES

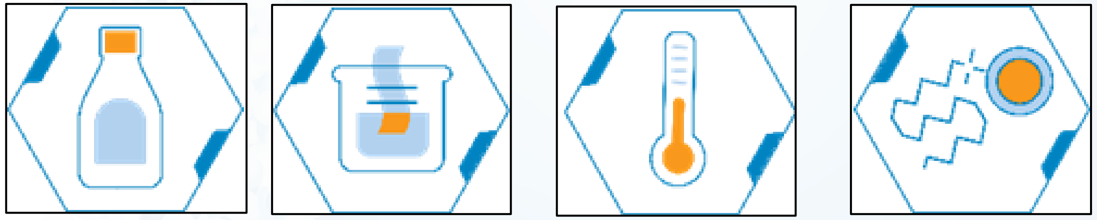
1. Cannabidivarinic acid (CBDVA)
2. Cannabidivarin (CBDV)
3. Cannabidiolic acid (CBDA)
4. Cannabigerolic acid (CBGA)
5. Cannabigerol (CBG)
6. Cannabidiol (CBD)
7. Tetrahydrocannabivarin (THCV)
8. Tetrahydrocannabivarinic acid (THCVA)
9. Cannabinol (CBN)
10. Cannabinolic acid (CBNA)
11. delta 9- Tetrahydrocannabinol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



Column Efficiency: SPP vs. FPP



Method Development



Acidic Modifiers

Journal of Natural Products Article

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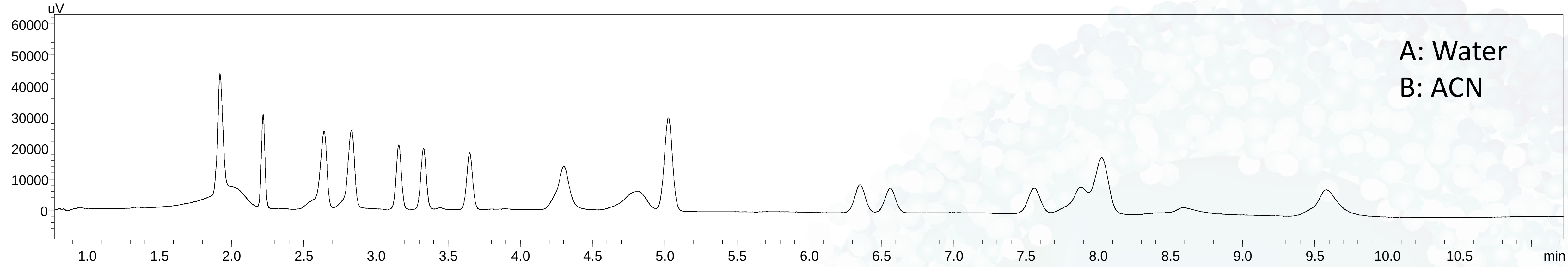
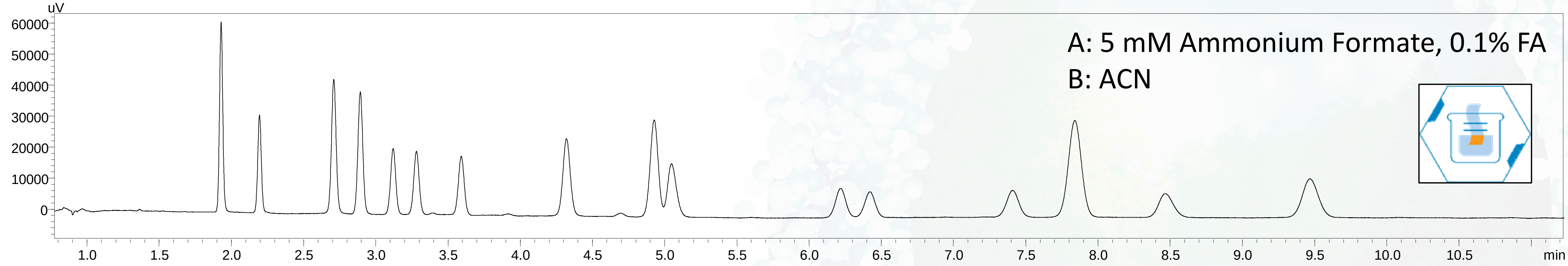
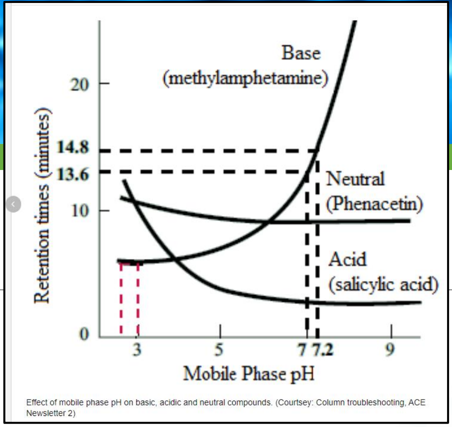
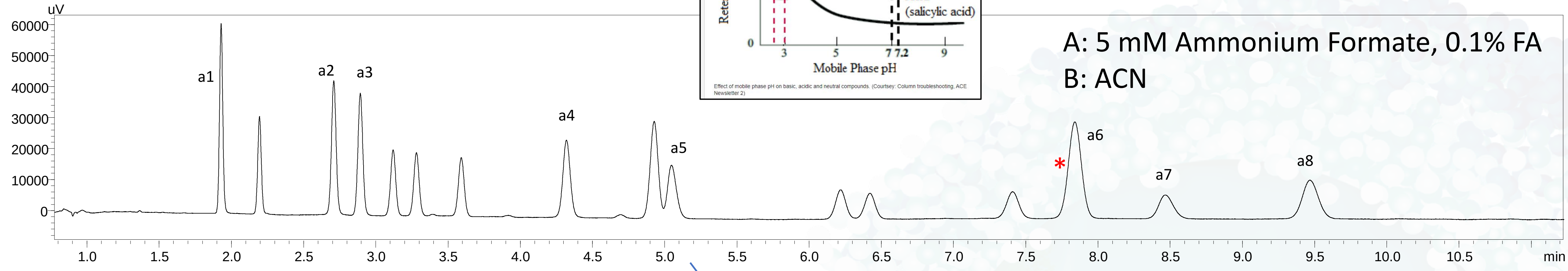
THCA	358.4	2.89	6.25	66.76	5	4.12



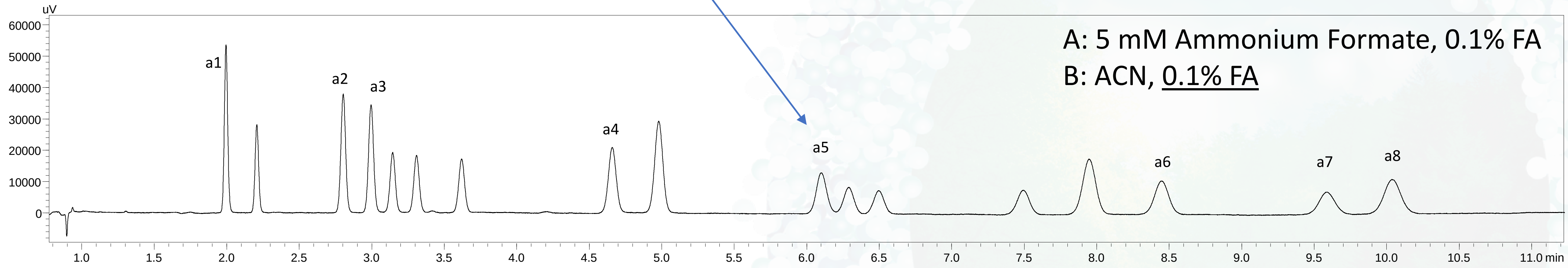


pKa



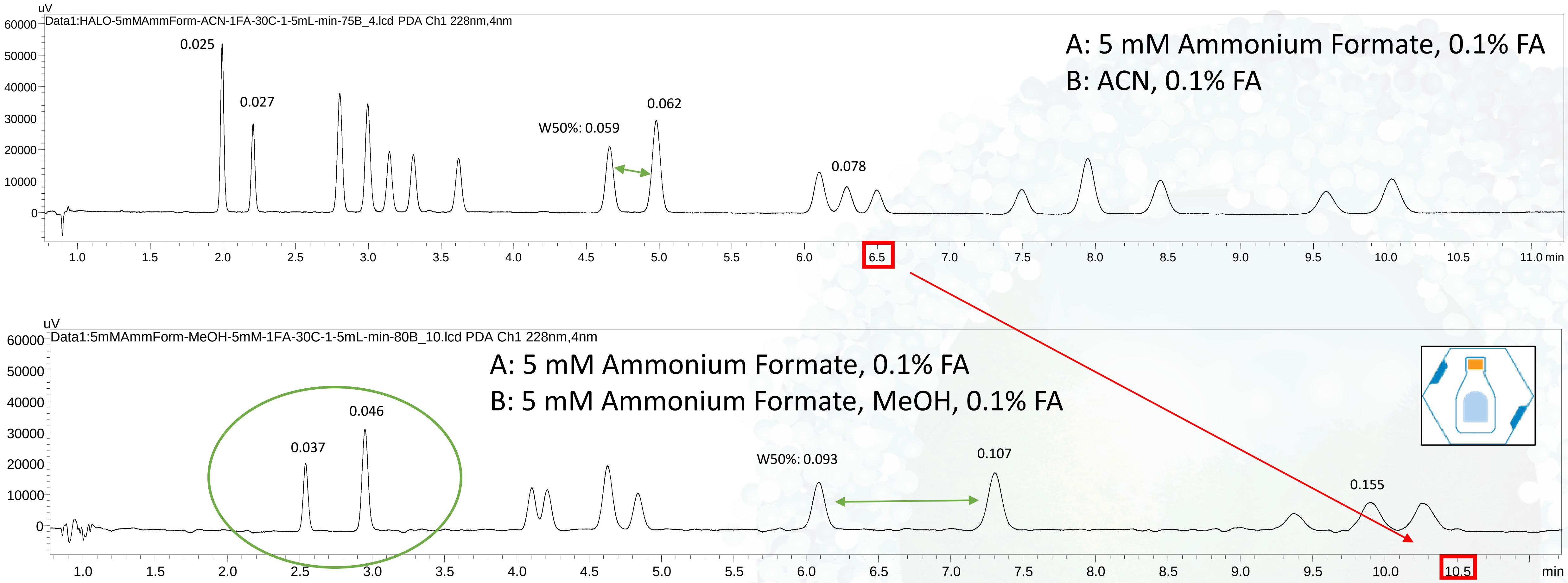


A: 5 mM Ammonium Formate, 0.1% FA
B: ACN



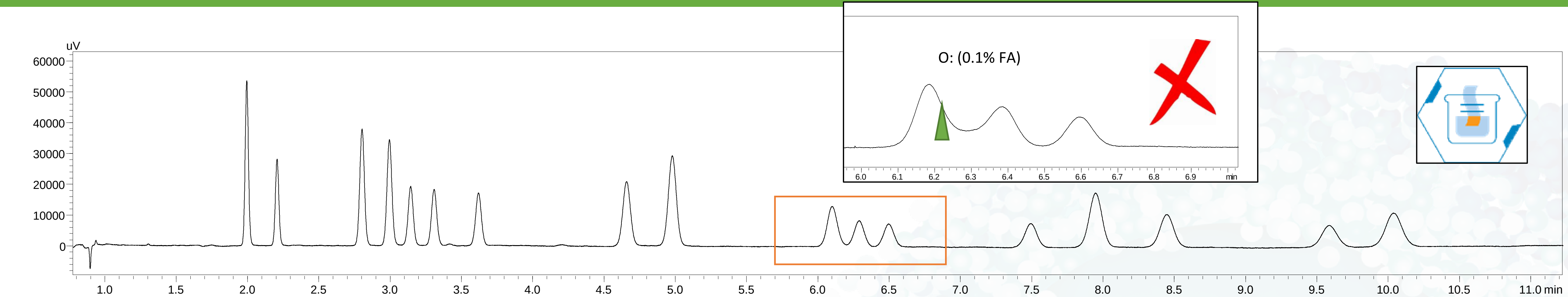
A: 5 mM Ammonium Formate, 0.1% FA
B: ACN, <u>0.1% FA</u>

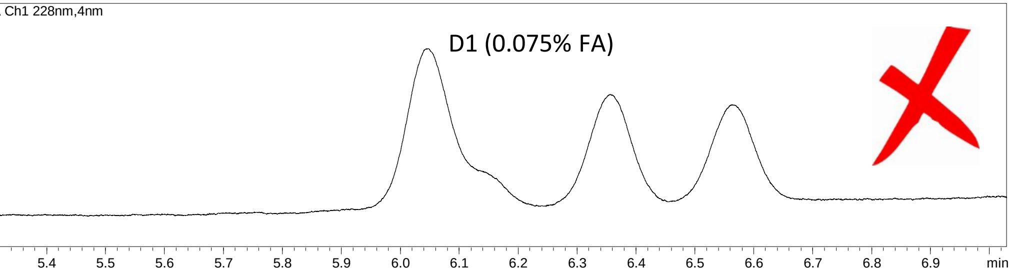
Don't Forget About Methanol!

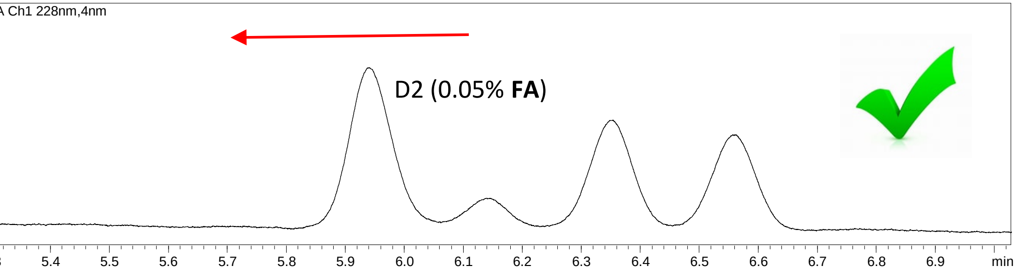


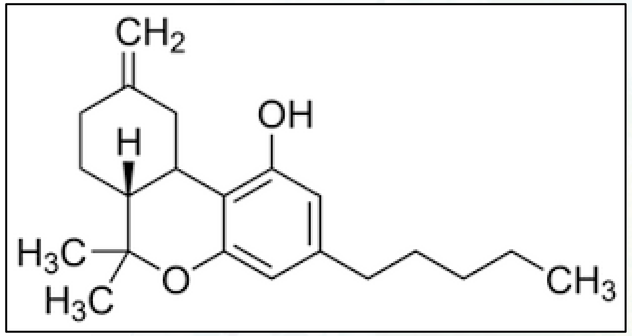
-some resolution benefits, changes in selectivity, however, wider peak widths (lower sensitivity) and coelutions using methanol

More than 100 Cannabinoids: Exo-THC Analysis

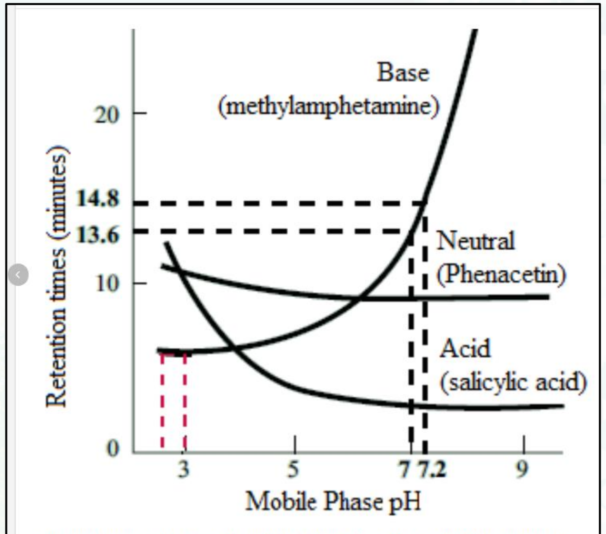








Exo-THC is also known as Delta-9,11-THC. And while this isomer is a known byproduct formed during the synthesis of THC (dronabinol), it can also be formed by many of these post-extraction processes used to "clean up" the extracts.

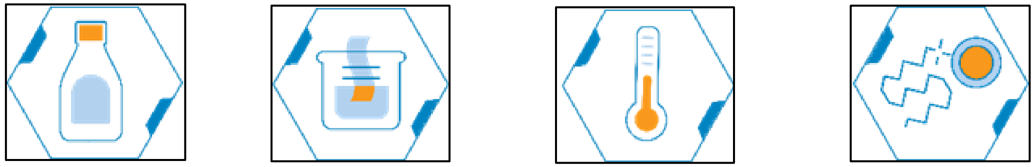


Effect of mobile phase pH on basic, acidic and neutral compounds. (Courtsey: Column troubleshooting, ACE Newsletter 2)

Mix of 18 Cannabinoids: HALO 90Å LPH-C18

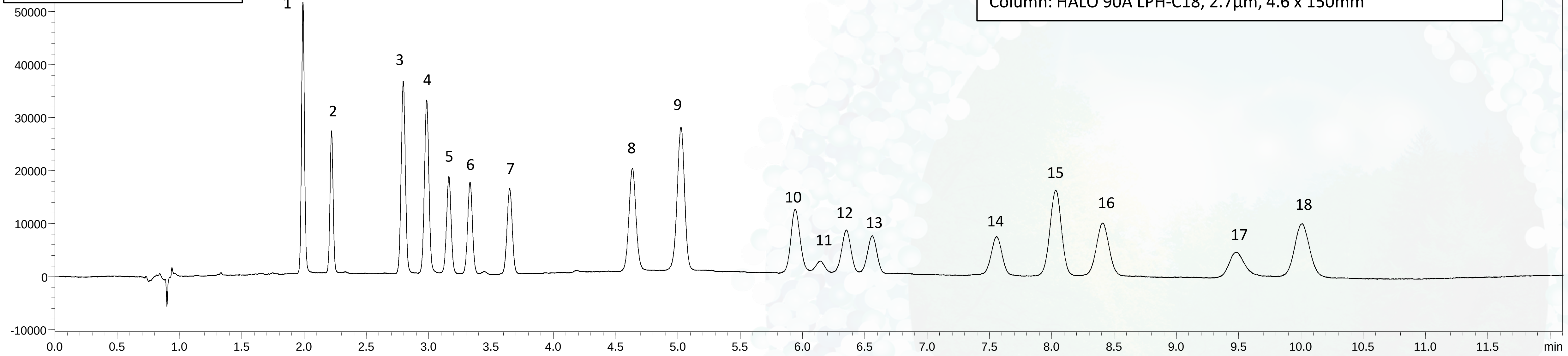
PEAK IDENTITIES:

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. 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)



Testing Conditions:

Mobile Phase: A: 5mM Ammonium Formate, 0.1% Formic Acid
B: Acetonitrile, 0.05% Formic Acid
Isocratic Separation: 75% B
Instrument: Shimadzu Nexera X2
Wavelength: PDA, 228 nm
Injection: 0.2 µl
Temperature: 30°C
Flow Rate: 1.5 mL/min.
Back Pressure: 345 bar
Sample Solvent: 75/25 ACN/ Water, 0.1% FA
Column: HALO 90Å LPH-C18, 2.7µm, 4.6 x 150mm



LC-MS: HALO 90Å 2µm LPH-C18

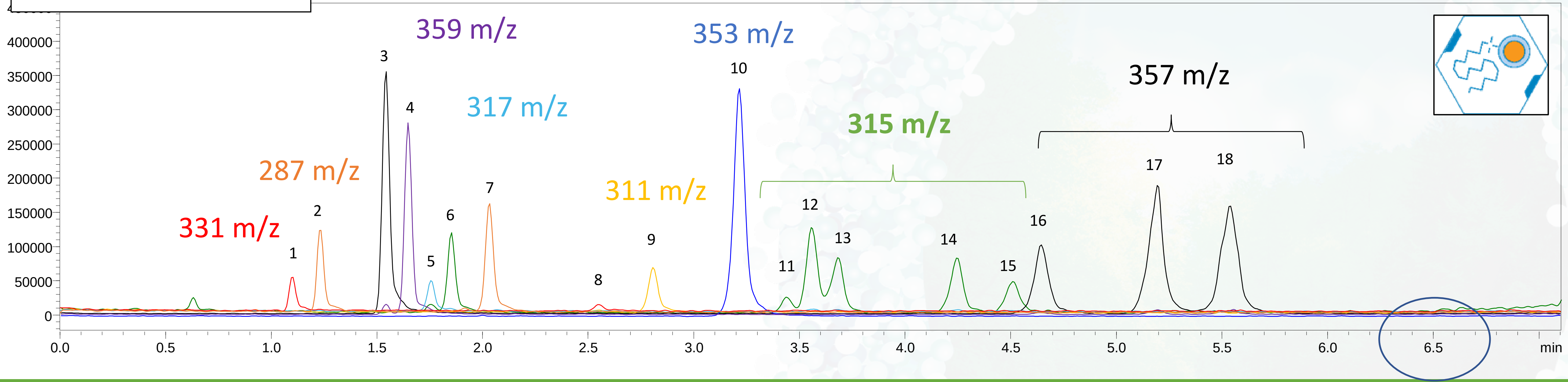
HALO®

PEAK IDENTITIES

1. Cannabidivarinic acid (CBDVA, (+) 331 m/z)
2. Cannabidivarin (CBDV, (+) 287 m/z)
3. Cannabidiolic acid (CBDA, (-) 357 m/z)
4. Cannabigerolic acid (CBGA, (-) 359 m/z)
5. Cannabigerol (CBG, (+) 317 m/z)
6. Cannabidiol (CBD, (+) 315 m/z)
7. Tetrahydrocannabivarin (THCV, (+) 287 m/z)
8. Tetrahydrocannabivarinic acid (THCVA, (+) 331 m/z)
9. Cannabinol (CBN, (+) 311 m/z)
10. Cannabinolic acid (CBNA, (-) 353 m/z)
11. Exo-tetrahydrocannabinol (EXO-THC, (+) 315 m/z)
12. delta 9- Tetrahydrocannabinol (D9-THC, (+)315 m/z)
13. delta 8- Tetrahydrocannabinol (D8-THC, (+) 315 m/z)
14. Cannabicycol (CBL, (+) 315 m/z)
15. Cannabichromene (CBC, (+) 315 m/z)
16. Tetrahydrocannabinolic acid A (THCA-A, (-) 357 m/z)
17. Cannabichromenic acid (CBCA, (-) 357 m/z)
18. Cannabicyclolic acid (CBLA, (-) 357 m/z)

Testing Conditions:

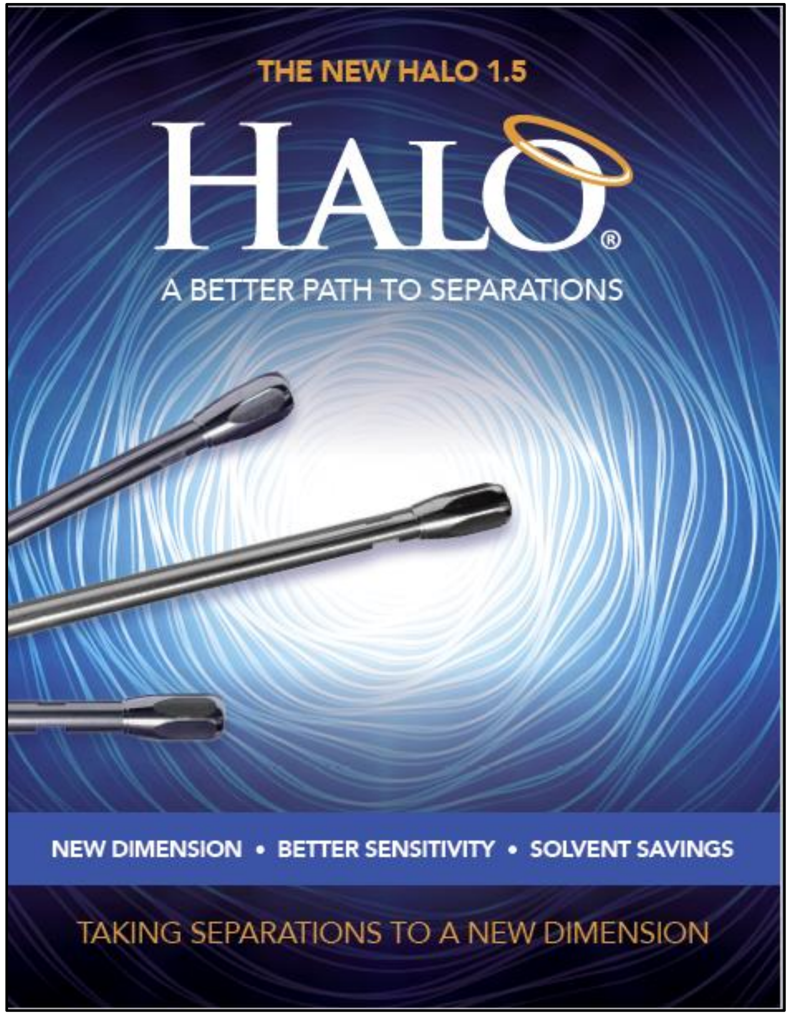
Mobile Phase: A: 5mM Ammonium Formate, 0.1% Formic Acid
B: Acetonitrile, 0.05% Formic Acid
Isocratic Separation: 75% B
Instrument: Shimadzu LC-MS 8040/ Nexera X2
Wavelength: PDA, 228 nm
Injection: 0.2 µl
Temperature: 30°C
Flow Rate: 0.4 mL/min.
Back Pressure: 275 bar
Sample Solvent: 75/25 ACN/ Water, 0.1% FA
Column: HALO 90Å LPH-C18, 2µm, 2.1 x 100mm



A NEW DIMENSION IN SEPARATIONS

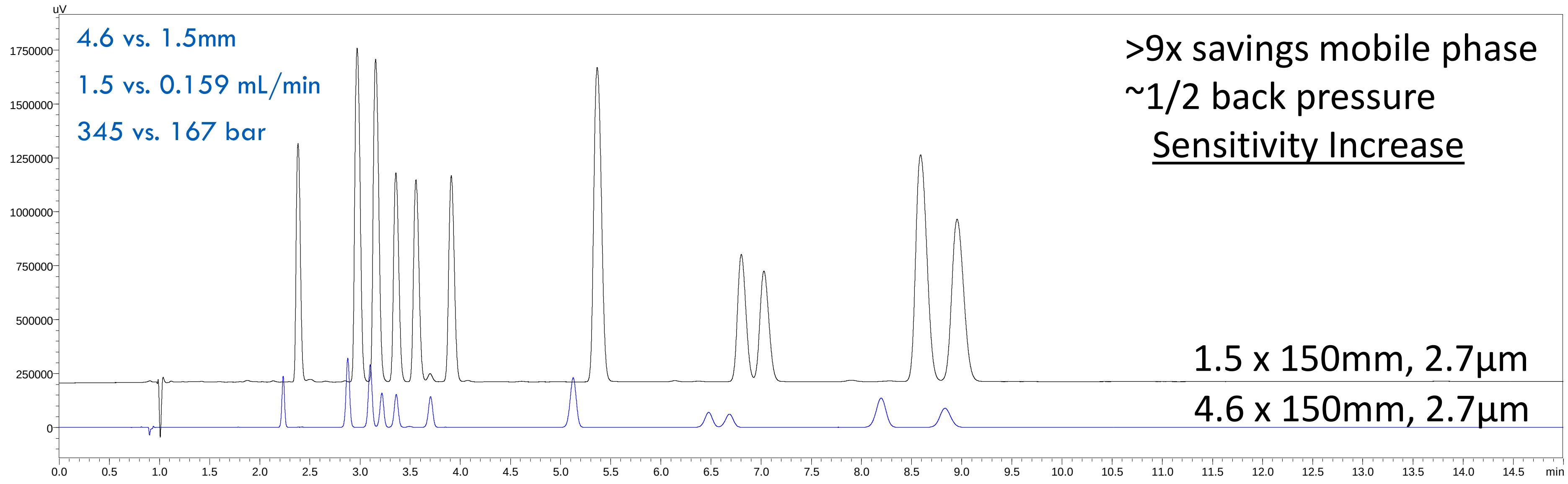
HALO®

MORE PERFORMANCE FROM UHPLC AND LCMS SYSTEMS

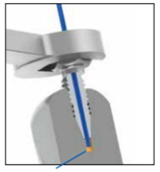


- More sensitivity from conventional UHPLC systems
- Higher ionization efficiencies from LCMS systems
- Reduced solvent consumption compared to 2.1 mm id columns (and greater)
- Easy to implement microflow solution

HALO® 1.5 mm ID: R&D/ MS Apps.



MarvelXACT™ VS. CONVENTIONAL CONED FITTINGS

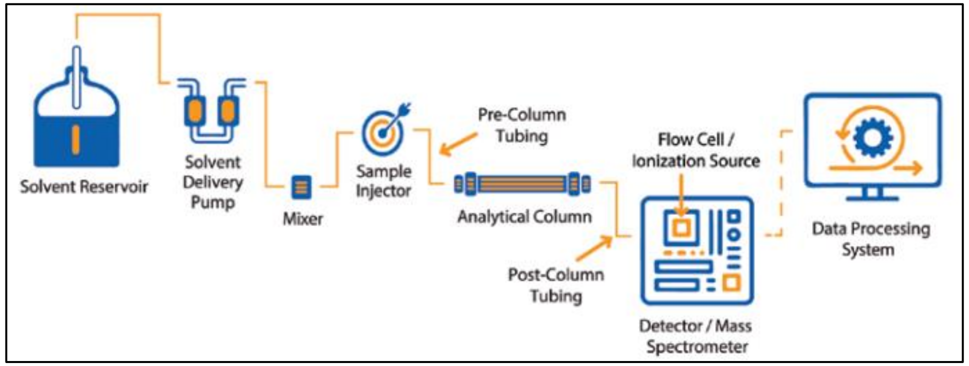


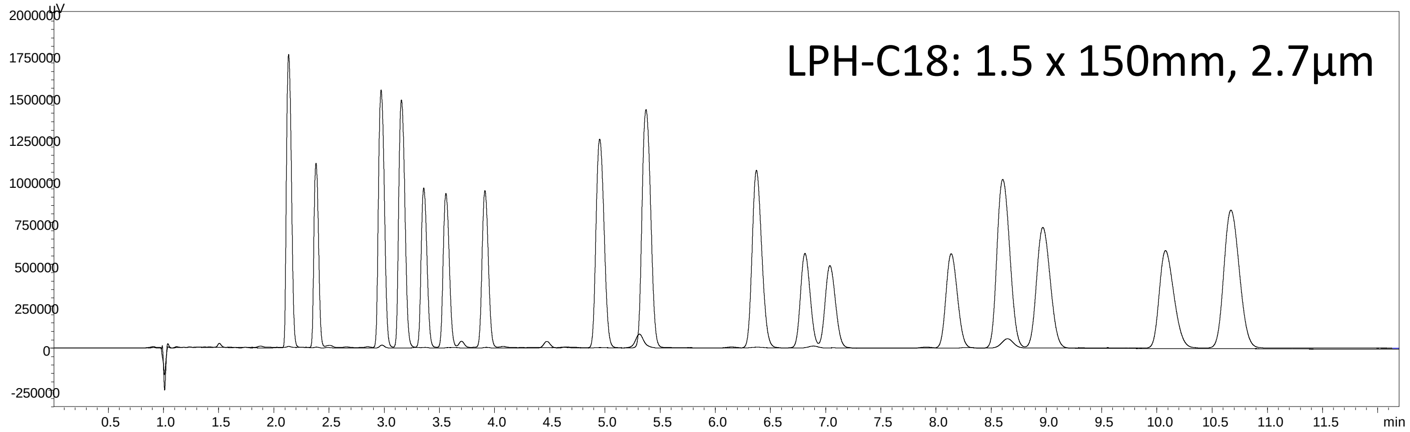
EXTRA INTERNAL VOLUME

Conventional coned fittings require a ferrule in conjunction with a fitting for proper sealing. They depend on tools, to improve sealing performance, which significantly increases probability of extra internal volume and poor chromatography results. The mechanical tightening increases wear leading to higher replacement costs.

ZERO DEAD VOLUME

MarvelXACT™ fittings do not depend on ferrules. They seal with hand tightening at the bottom of the port, which significantly reduces required torque and enables many more connects and disconnects reducing wear and increasing product life. An enhanced proprietary tip design also ensures zero dead volume (ZDV) and better chromatography results.





Website, LinkedIn, YouTube, Facebook

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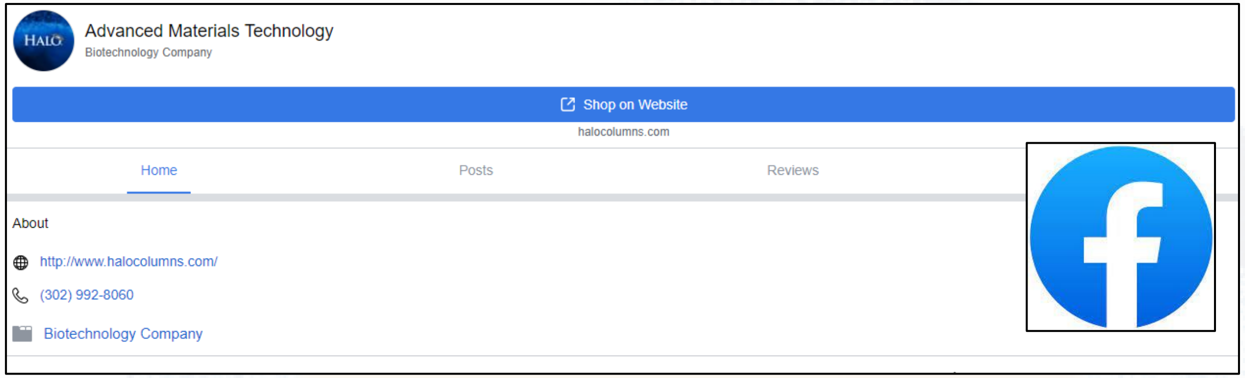
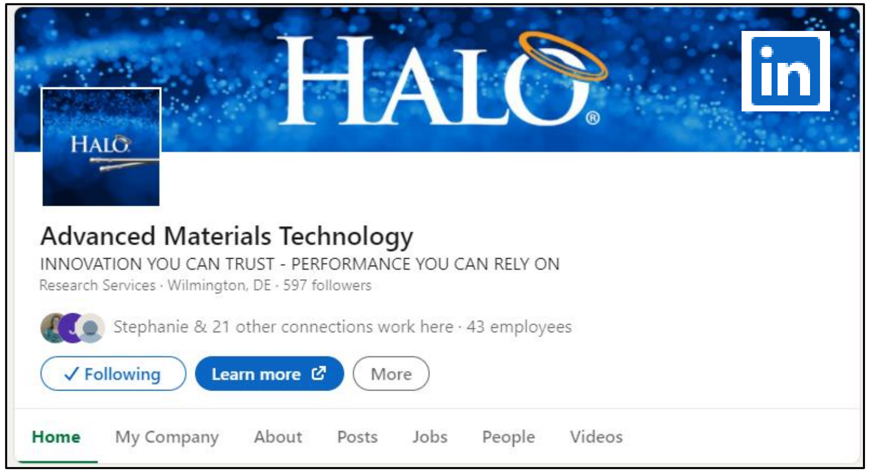
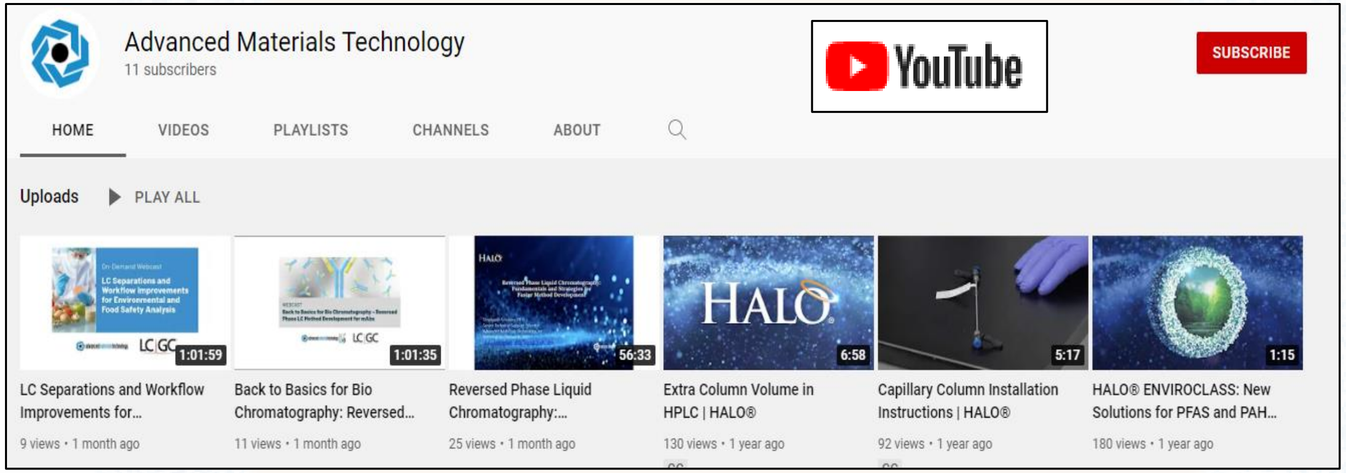
[HALO® HPLC Columns for Chromatography Separation | LC Columns (halocolumns.com)](https://halocolumns.com)



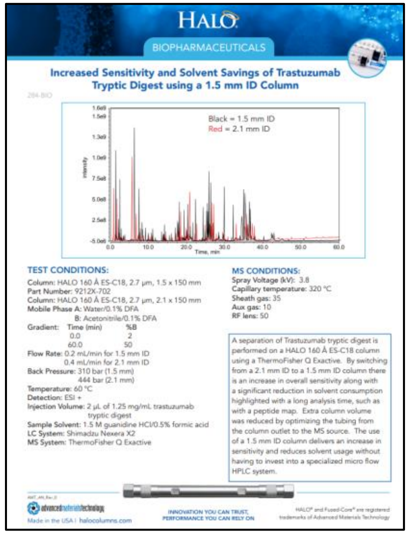


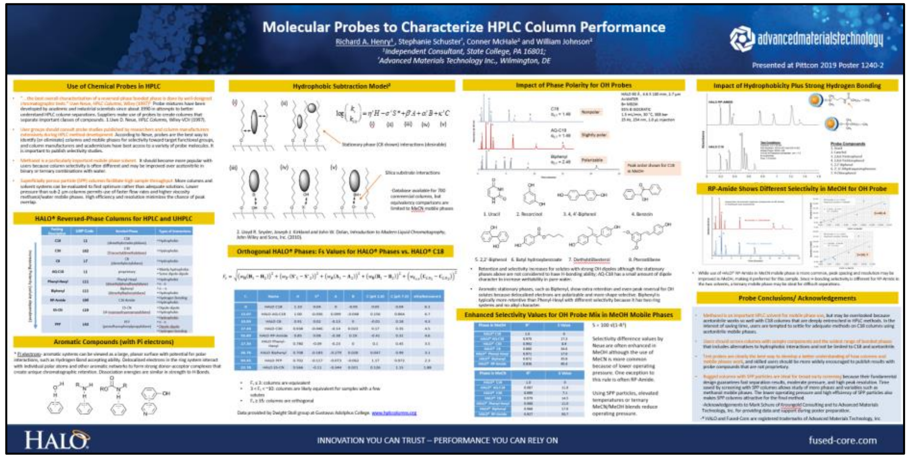


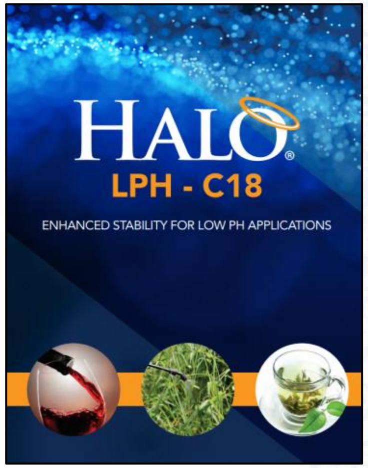
Technical Resources

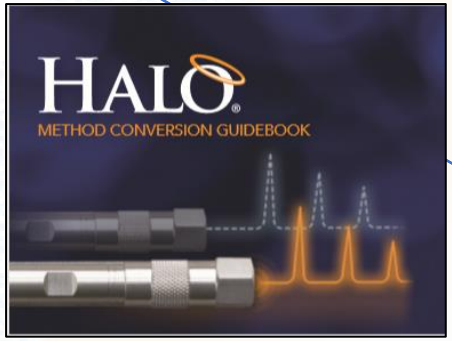
[HALO® HPLC Columns for Chromatography Separation | LC Columns (halocolumns.com)]

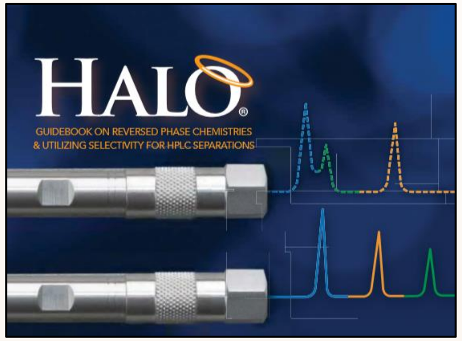
Application Notes | Conference Papers | Product Literature | Technical Documents | Videos









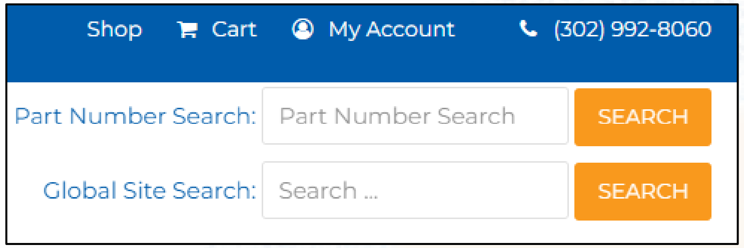


Webinar: Reversed Phase Liquid Chromatography: Fundamentals and Strategies for Faster Method Development

Questions?

Sales, Technical and Marketing Materials:

- www.halocolumns.com



Technical Support:

- support@advanced-materials-tech.com

Sales Questions/Sales Orders:

- sales@advanced-materials-tech.com

Special thanks to LGC for supplying standards!

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