



EXPLORING THE IMPROVEMENTS ENABLED BY 1.5 MM ID UHPLC SPP COLUMNS

Stephanie A. Schuster

Peter Pellegrinelli, Conner McHale, Benjamin Libert

Advanced Materials Technology, Inc.



EAS 2022

November 15, 2022

What benefits does 1.5 mm offer?

MORE PERFORMANCE FROM UHPLC AND LCMS SYSTEMS

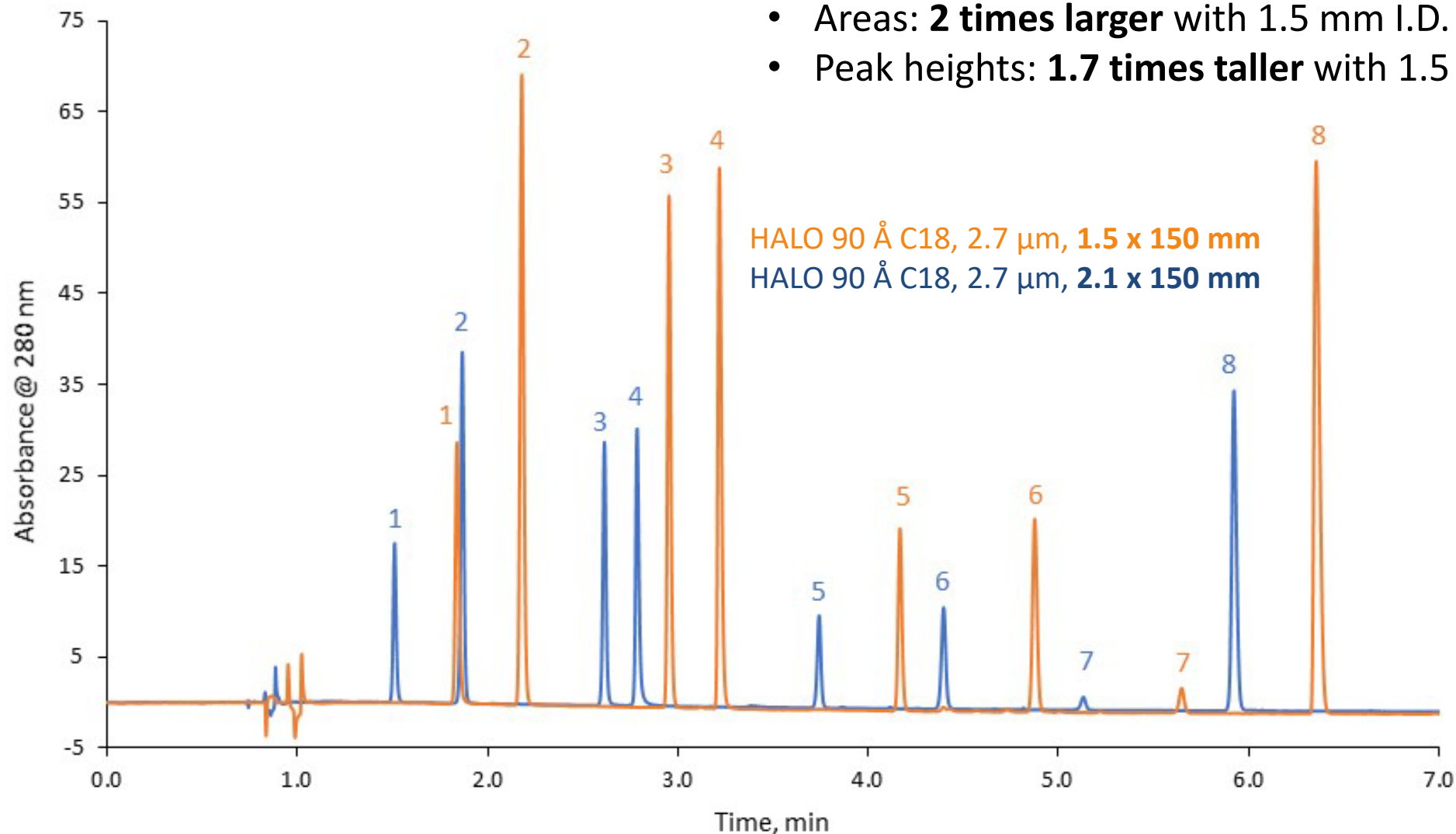
- ✓ **More sensitivity** from conventional UHPLC systems*
- ✓ **Higher ionization efficiencies** from LCMS systems
- ✓ **Reduced solvent consumption** compared to 2.1 mm I.D. columns (and greater)
- ✓ **Easy to implement** microflow solution with existing systems

* UV and other concentration sensitive detectors

More sensitivity from conventional UHPLC systems

Comparison of Gradient UV Separation of OTC Cough and Cold Medicines

- Areas: **2 times larger** with 1.5 mm I.D. column
- Peak heights: **1.7 times taller** with 1.5 mm I.D. column



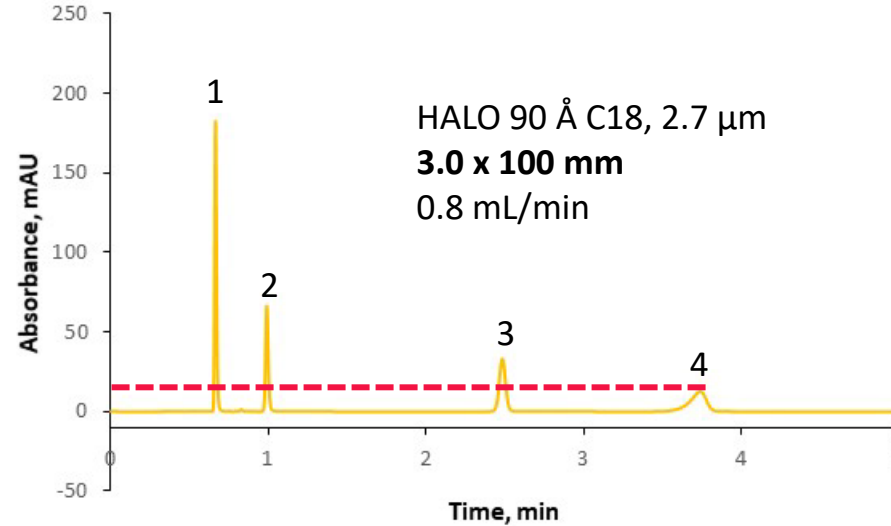
1. Phenylephrine
2. Acetaminophen
3. Caffeine
4. Doxylamine
5. Guaifenesin
6. Aspirin
7. Salicylic Acid
8. Dextromethorphan

Same sample
concentration and
injection volume used
for both column I.D.s

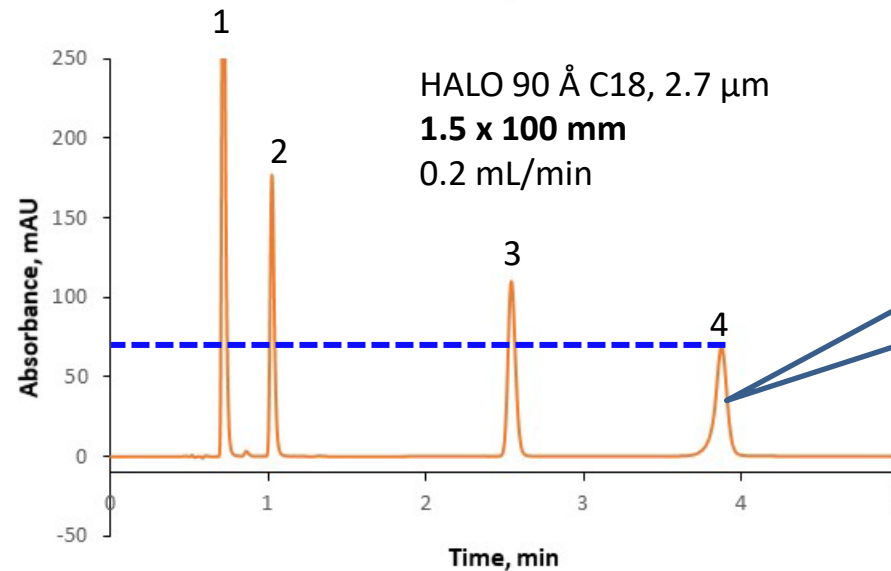
Enhanced Impurity Identification

Isocratic Separation Comparing 1.5 mm to 3.0 mm I.D. column

Isocratic: 70/30 water/methanol/1.5%
glacial acetic acid
Flow Rate: 0.2 mL/min for 1.5 mm
0.8 mL/min for 3.0 mm
Pressure: 426 bar/1.5 mm
622 bar/3.0 mm
Temperature: 35 °C
Injection Volume: 0.5 µL
Detection: UV 280 nm, PDA



1. Acetaminophen
2. Caffeine
3. Aspirin
4. Salicylic Acid

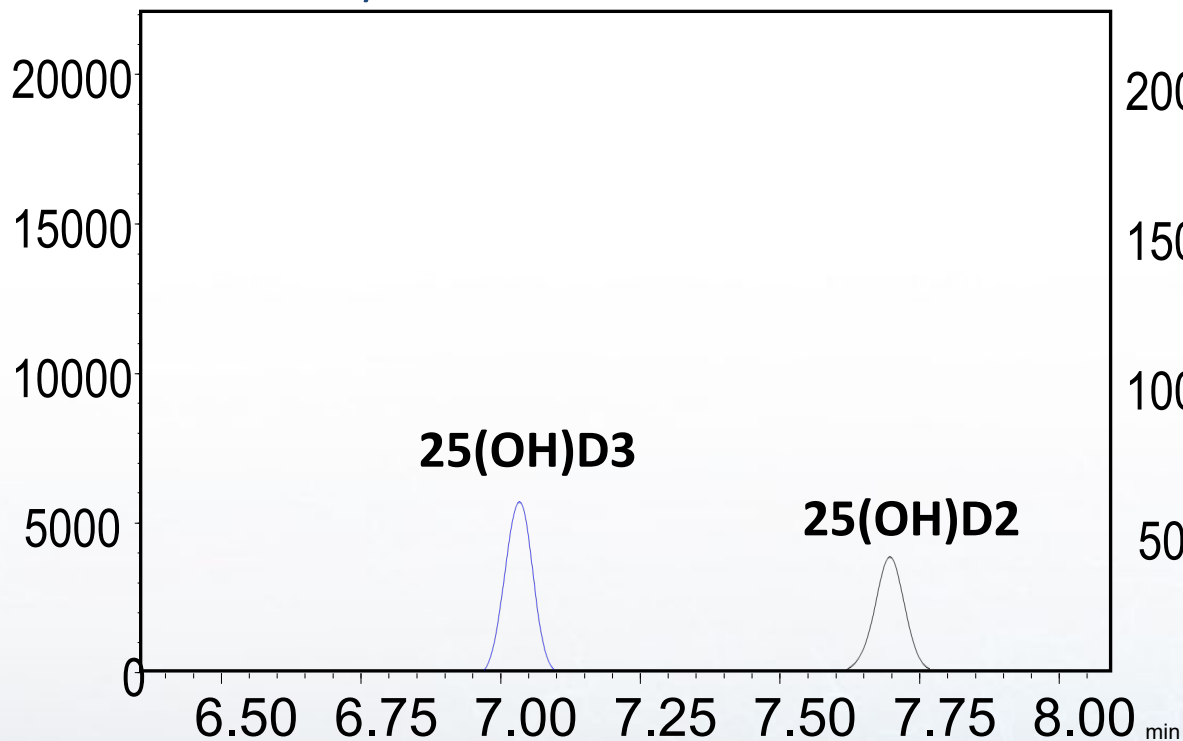


Both area and peak height are increased!



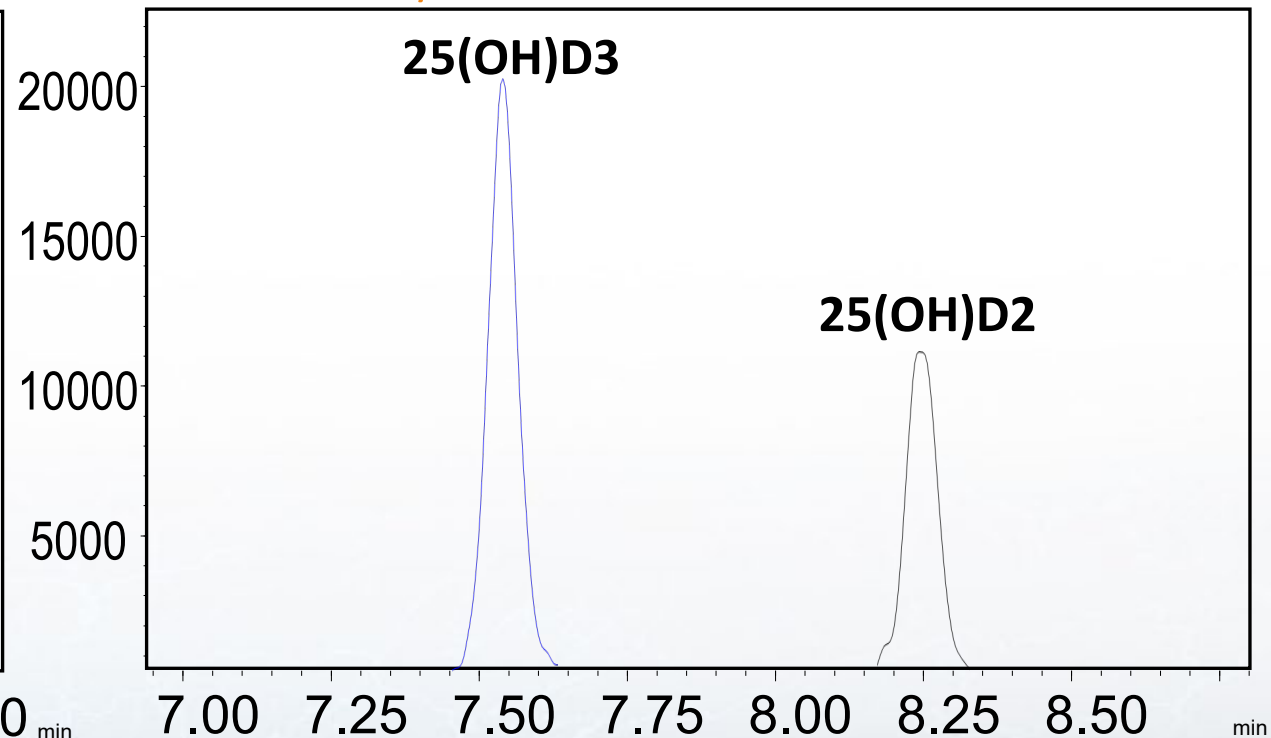
Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

HALO 90 Å C18, 2.7 μm , 2.1 x 100 mm
0.4 mL/min



Gradient: 50-92.5% ACN/0.1% formic acid in 10.8 min
Injection Volume: 1.0 μL @ 500 ppb

HALO 90 Å C18, 2.7 μm , 1.5 x 100 mm
0.2 mL/min

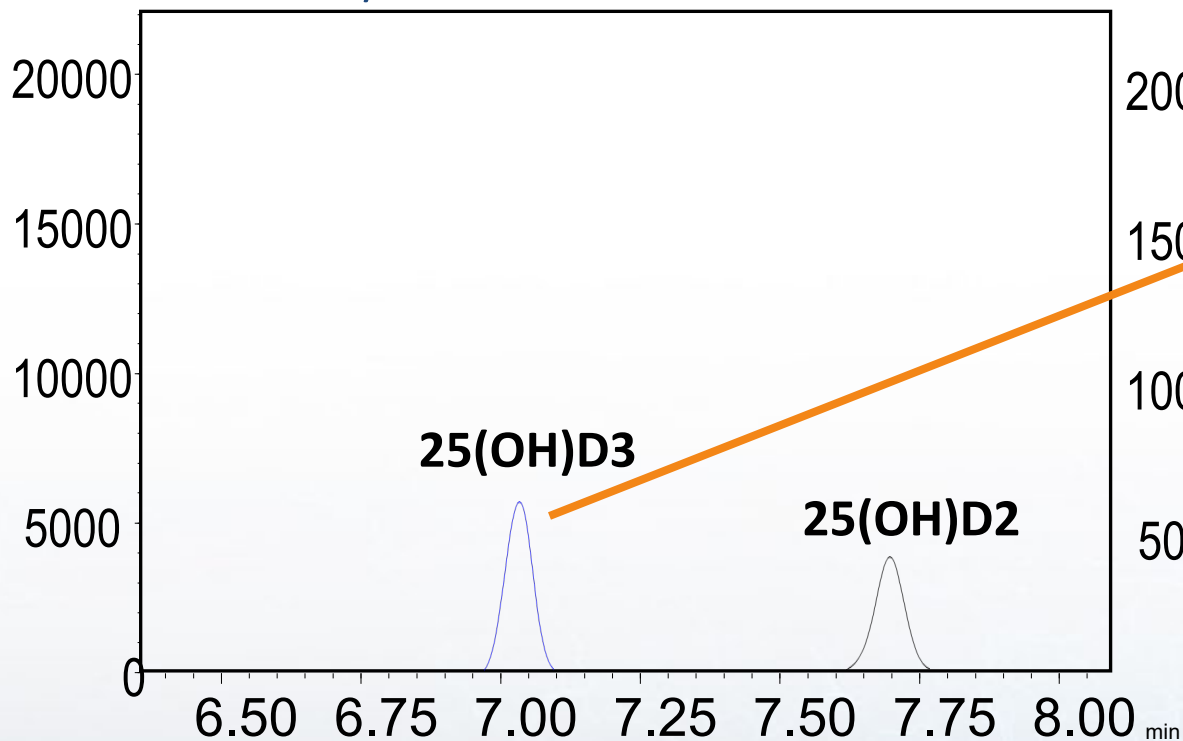


Temperature: 40 °C
Instrument: Shimadzu LCMS-8040



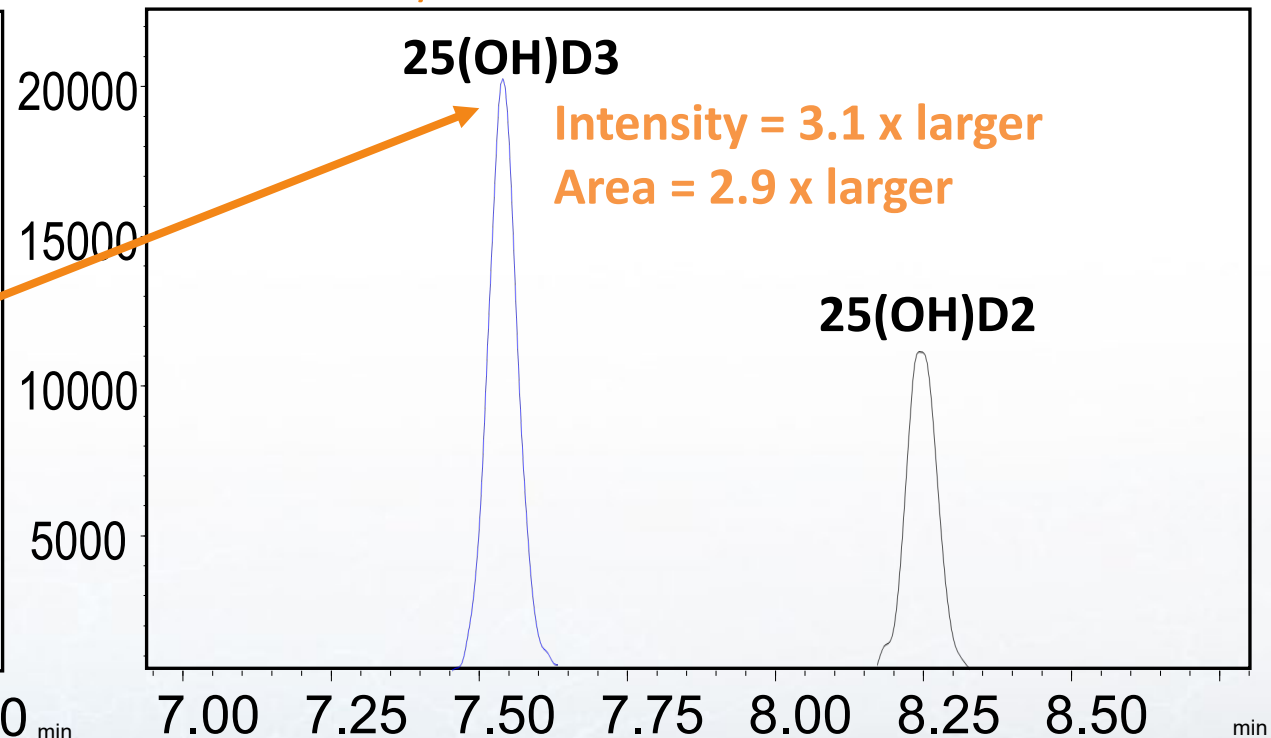
Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

HALO 90 Å C18, 2.7 μm , 2.1 x 100 mm
0.4 mL/min



Gradient: 50-92.5% ACN/0.1% formic acid in 10.8 min
Injection Volume: 1.0 μL @ 500 ppb

HALO 90 Å C18, 2.7 μm , 1.5 x 100 mm
0.2 mL/min

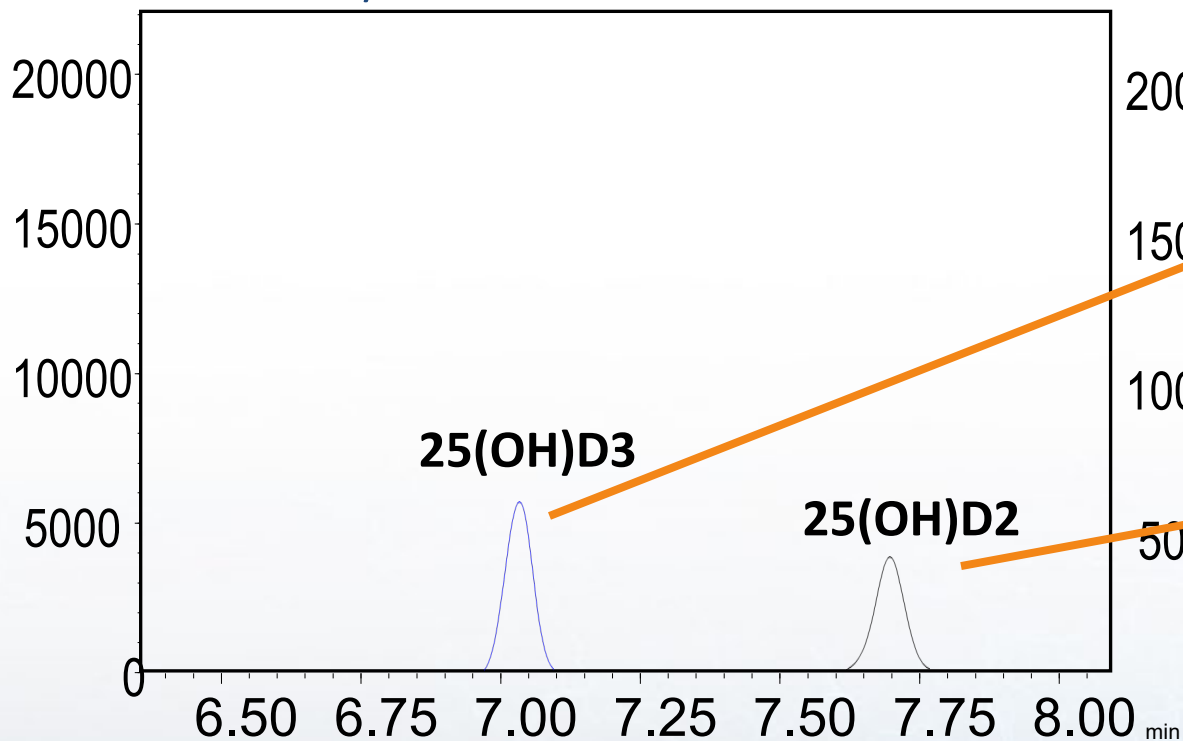


Temperature: 40 °C
Instrument: Shimadzu LCMS-8040



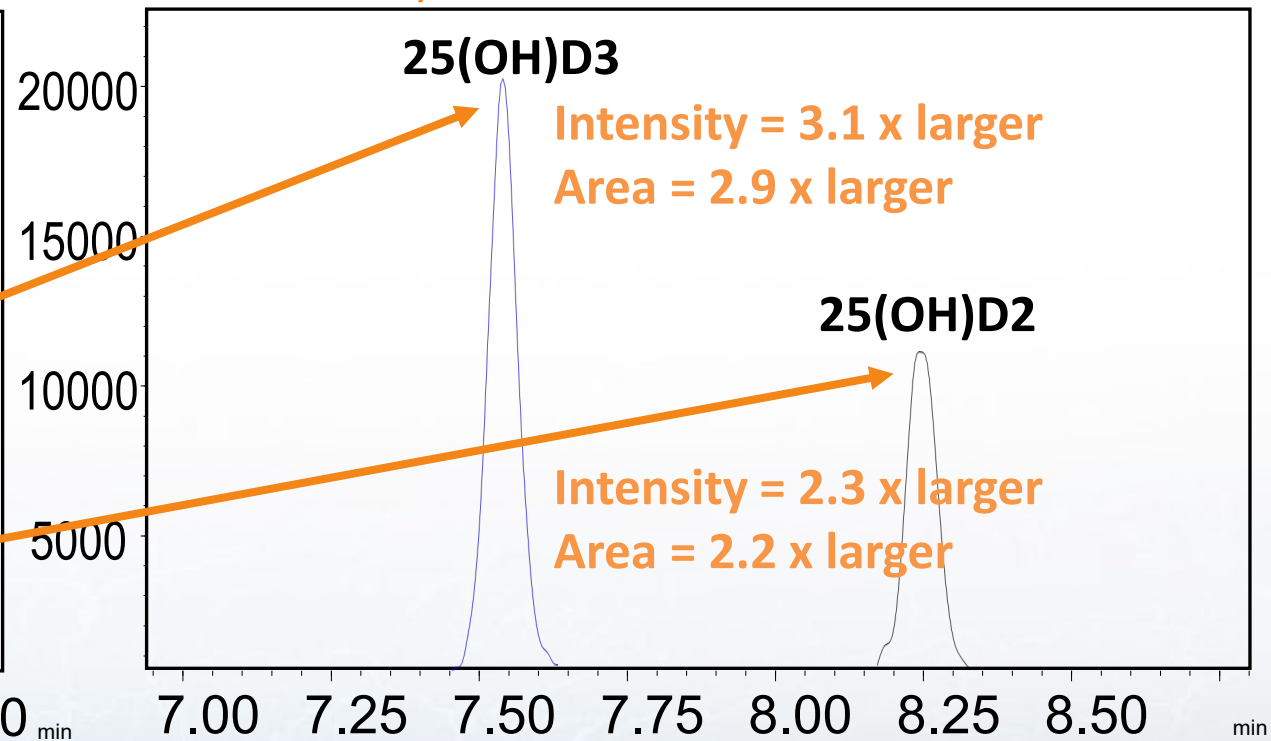
Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

HALO 90 Å C18, 2.7 μm , 2.1 x 100 mm
0.4 mL/min



Gradient: 50-92.5% ACN/0.1% formic acid in 10.8 min
Injection Volume: 1.0 μL @ 500 ppb

HALO 90 Å C18, 2.7 μm , 1.5 x 100 mm
0.2 mL/min



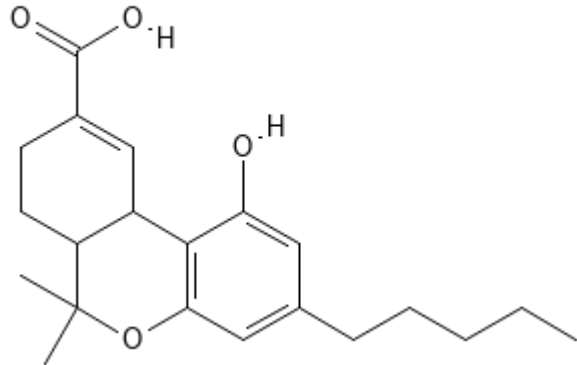
Temperature: 40 °C
Instrument: Shimadzu LCMS-8040



Increased sensitivity with LCMS systems:

(±)-11-nor-9-Carboxy- Δ 9-THC and (±)-11-nor-9-Carboxy- Δ 8-THC

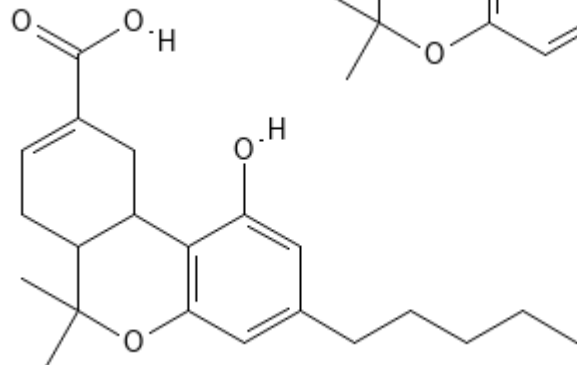
(±)-11-nor-9-Carboxy- Δ 9-THC



HALO 90 Å C18, 2.7 μ m, 1.5 x 100 mm

Δ 8

Δ 9



(±)-11-nor-9-Carboxy- Δ 8-THC

HALO 90 Å C18, 2.7 μ m, 2.1 x 100 mm

Δ 8

Δ 9

— 2.1 mm

— 1.5 mm

Chromatographic data for the 1 μ g/mL standard on both columns. Chromatograms are to scale.



PennState

Data courtesy of Paola Roldán-Arroyo and Dr. William Campbell
The Pennsylvania State University Forensics Program

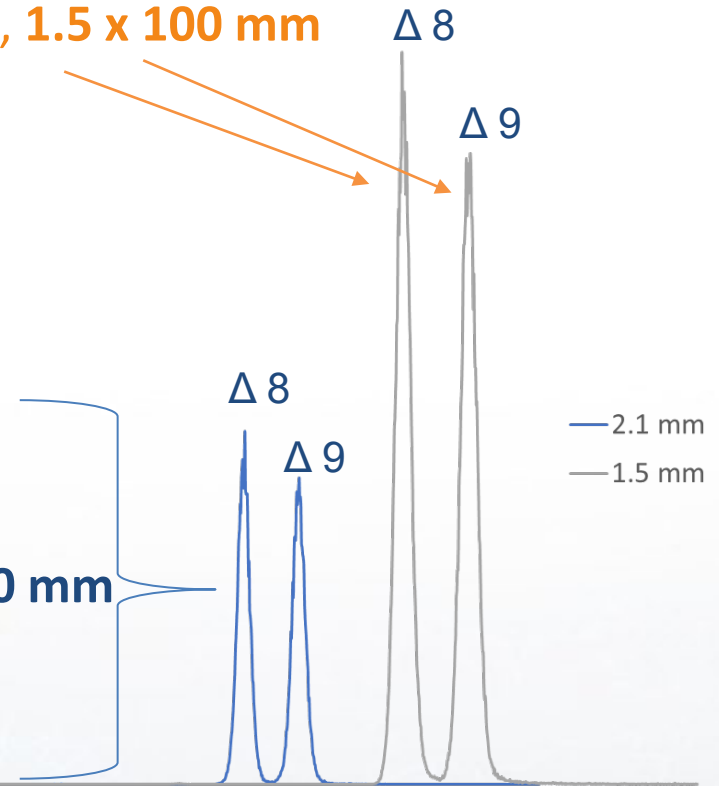


Increased sensitivity with LCMS systems: (±)-11-nor-9-Carboxy-Δ9-THC and (±)-11-nor-9-Carboxy-Δ8-THC

HALO 90 Å C18, 2.7 μm, 1.5 x 100 mm

Analyte	R ²	LOD μg/mL	LOQ μg/mL	Column ID
Delta 8 Carboxy	0.9986	0.0180	0.0545	2.1 mm
Delta 8 Carboxy	0.9997	0.0086	0.0259	1.5 mm
Delta 9 Carboxy	0.9981	0.0204	0.0618	2.1 mm
Delta 9 Carboxy	0.9997	0.0081	0.0245	1.5 mm

HALO 90 Å C18, 2.7 μm, 2.1 x 100 mm



Chromatographic data for the 1 μg/mL standard on both columns. Chromatograms are to scale.



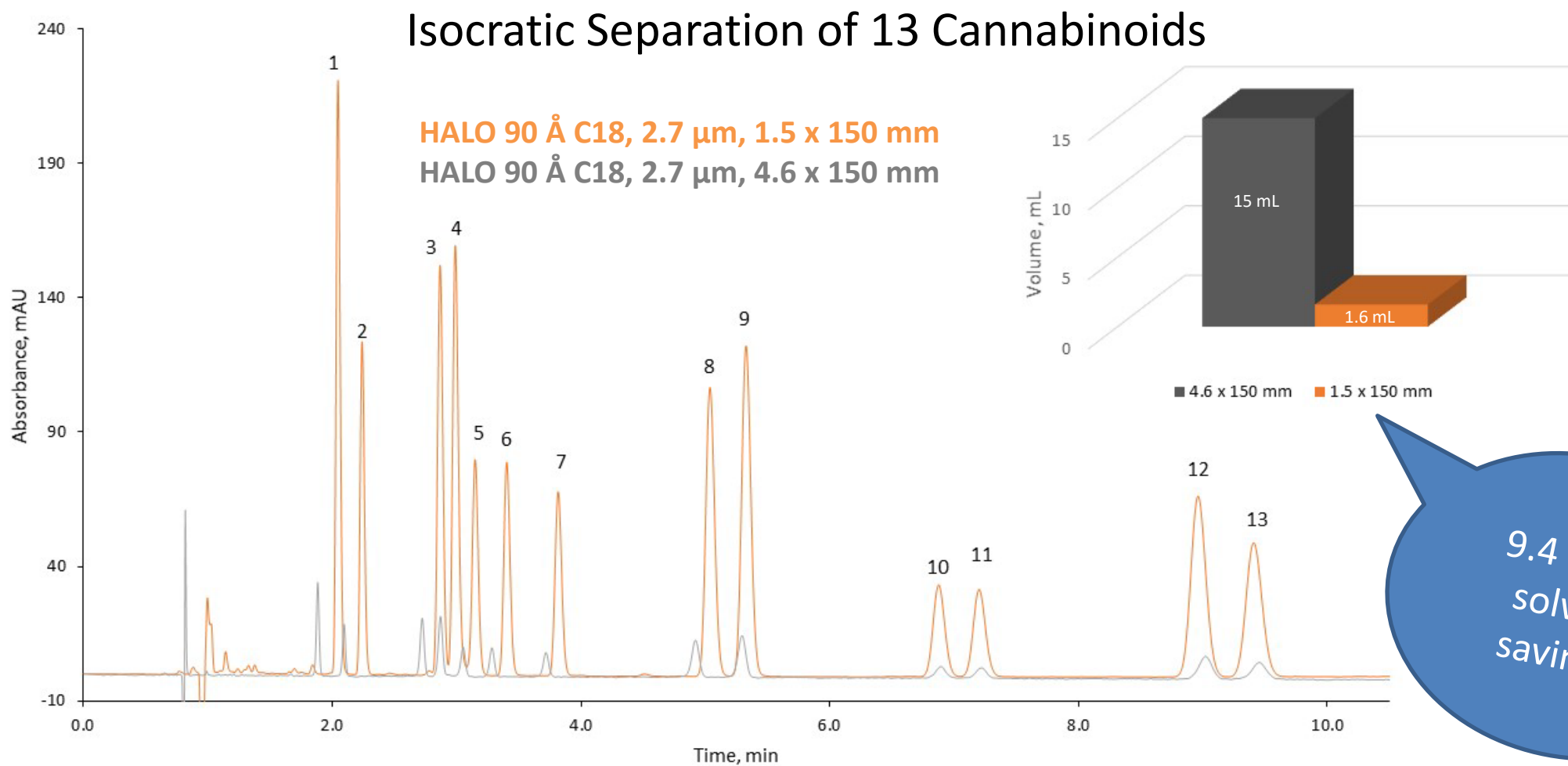
Data courtesy of Paola Roldán-Arroyo and Dr. William Campbell
The Pennsylvania State University Forensics Program

Sensitivity Effects with LCMS systems:

Benzodiazepines Example

Drug	HALO 1.5 mm Column		HALO 2.1 mm Column		Ratio of 2.1/1.5	
	LOD (µg/mL)	LOQ (µg/mL)	LOD (µg/mL)	LOQ (µg/mL)	LOD	LOQ
7-aminoflunitrazepam	0.010	0.031	0.016	0.048	1.56	1.56
oxazepam	0.009	0.026	0.023	0.069	2.66	2.66
chlordiazepoxide	0.013	0.039	0.028	0.084	2.15	2.15
alprazolam	0.009	0.026	0.018	0.055	2.09	2.09
flunitrazepam	0.005	0.015	0.014	0.043	2.81	2.81
nitrazepam	0.012	0.036	0.020	0.061	1.70	1.70
7-aminoclonazepam	0.017	0.052	0.019	0.057	1.09	1.09
temazepam	0.012	0.036	0.014	0.043	1.21	1.21
clonazepam	0.004	0.012	0.010	0.031	2.62	2.62
α-hydroxyalprazolam	0.008	0.024	0.018	0.054	2.20	2.20
nordiazepam	0.015	0.047	0.030	0.091	1.95	1.95
diazepam	0.007	0.021	0.011	0.032	1.53	1.53
N-desmethyflunitrazepam	0.011	0.035	0.012	0.037	1.06	1.06
bromazepam	0.010	0.031	0.017	0.053	1.68	1.68
lorazepam	0.014	0.042	0.016	0.048	1.15	1.15

Reduced solvent consumption compared to 4.6 mm I.D. columns



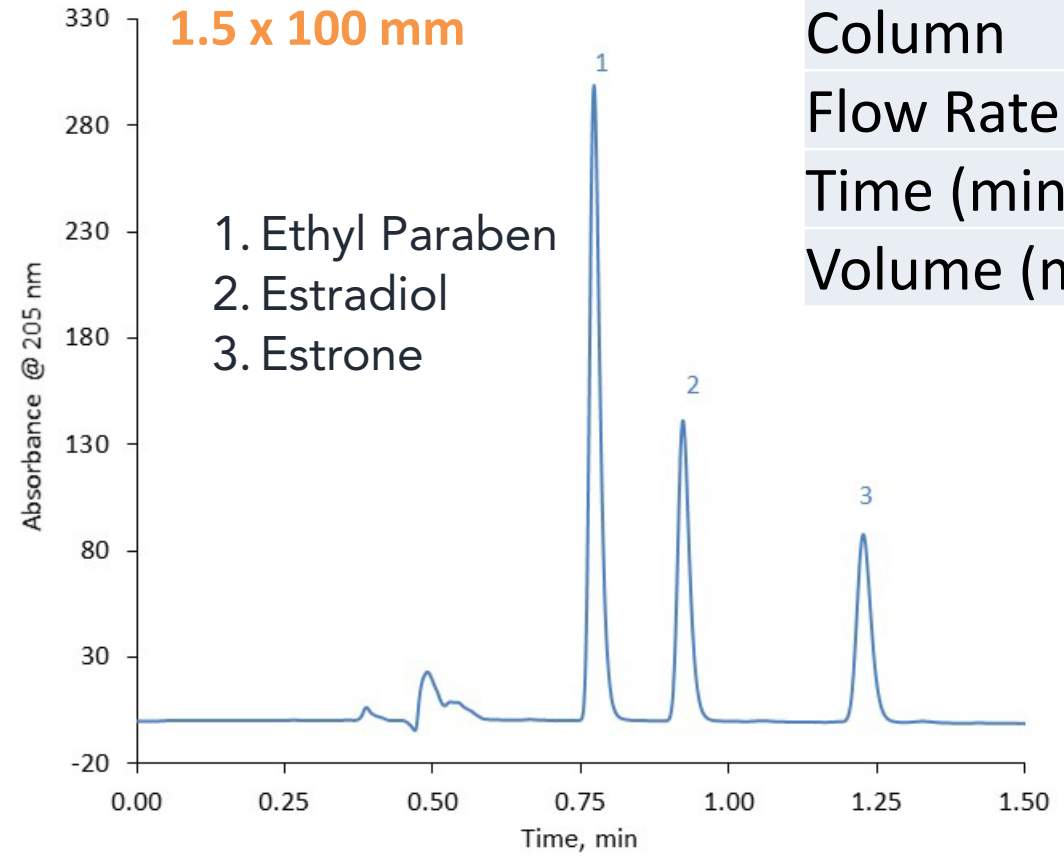
Peak identities (in order) are CBDVA, CBDV, CBDA, CBGA, CBG, CBD, THCV, THCVA, CBN, Δ^9 -THC, Δ^8 -THC, CBC, and THCA.



Reduced solvent consumption when modernizing methods

USP Method for Estradiol

HALO 90 Å C18, 2.7 µm
1.5 x 100 mm

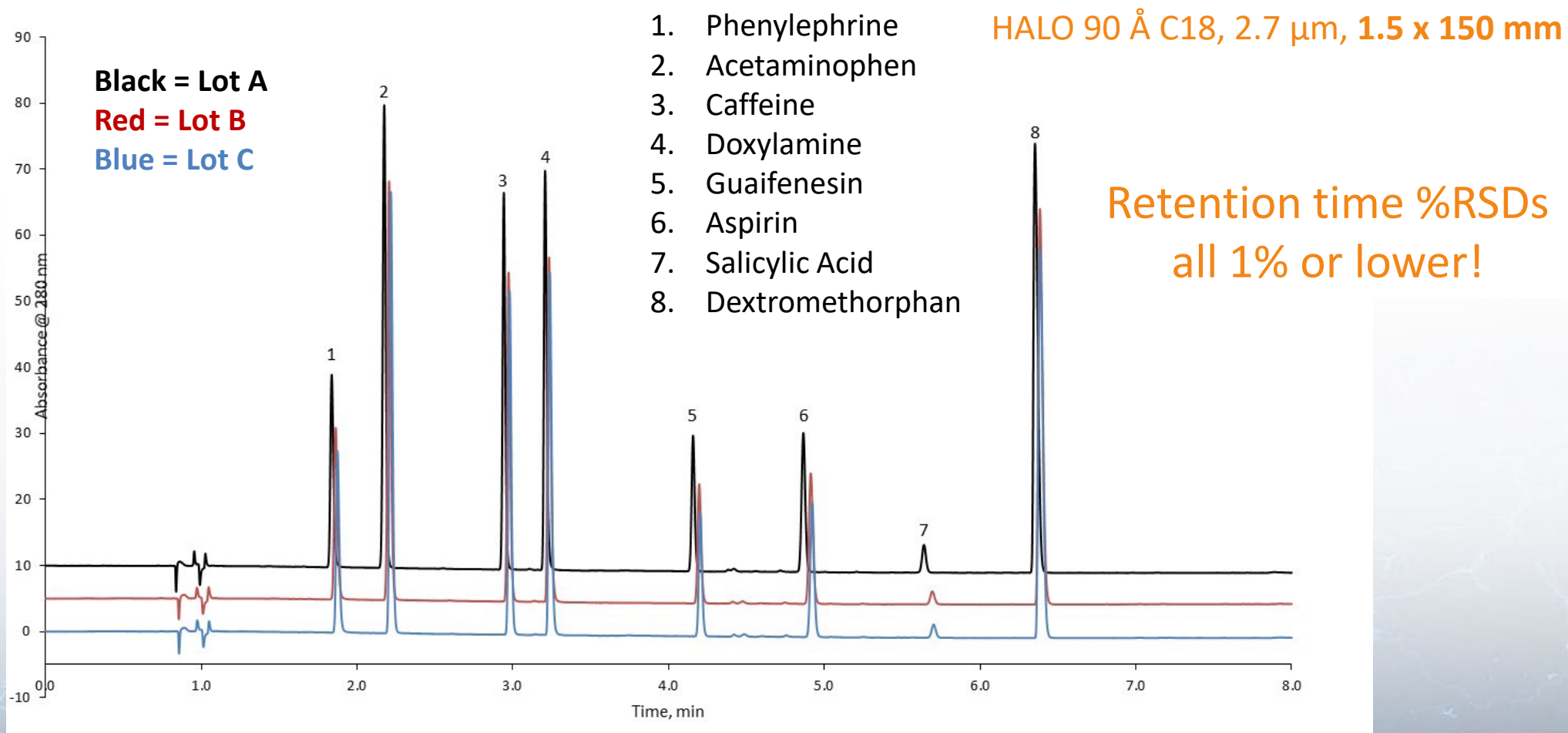


	Original Method	Modified Method
Column	3.9 x 300 mm	1.5 x 100 mm
Flow Rate (mL/min)	1	0.2
Time (min)	8	1.5
Volume (mL)	8	0.3

96% solvent savings!

HALO[®] 1.5 mm I.D. Column Hardware Reproducibility

Excellent reproducibility from 3 different lots of column hardware





Easy to implement microflow solution

Column looks and feels like a 2.1 mm...



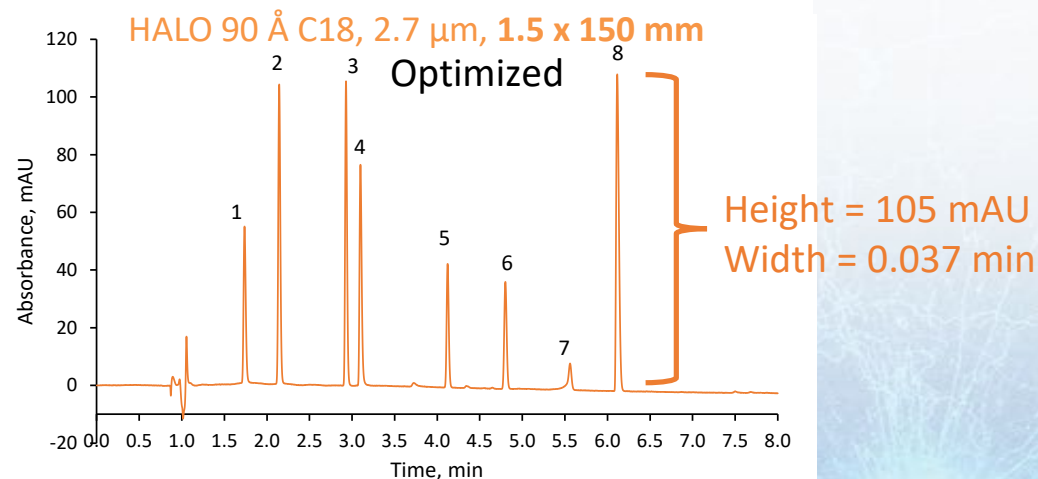
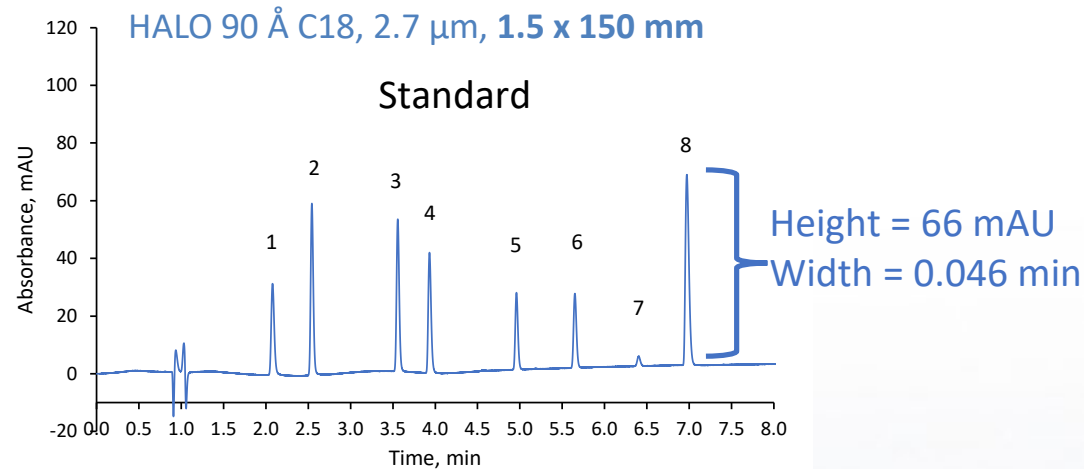
Not possible to bend/cause bed disturbance

Success using HALO[®] 1.5 mm I.D.

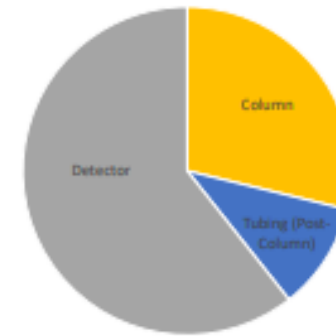
- System
- Connectors
- Method Transfer to 1.5 mm

How do I Estimate Extracolumn Dispersion?

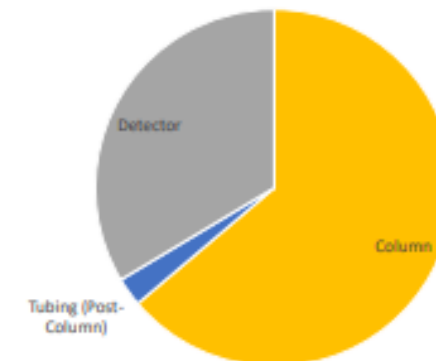
Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance
4 part series in LCGC North America from Dwight R. Stoll, Thomas Lauer, & Ken Broeckhoven
http://www.multilc.org/dispersion_calculator



Standard Plumbing - Gradient $k^* = 2$

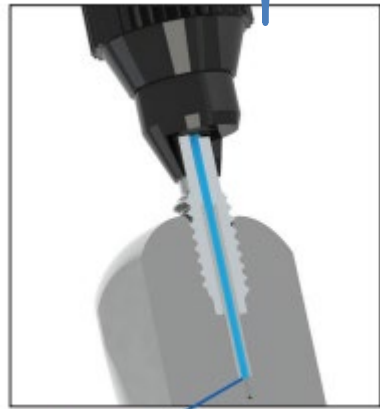


Optimized Plumbing - Gradient $k^* = 2$

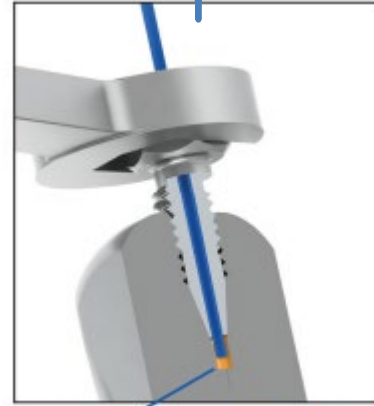


AMT MarvelXACT™ Connectors

- Difference between MarvelXACT™ and ferrule fitting



ZERO DEAD VOLUME



EXTRA INTERNAL VOLUME

- PEEKsil™ and PEEK-Lined Stainless Steel options
- Volume included with dimension for easy selection

Material
PEEKsil™
Dimension
75µm x 600mm, 2650 nl

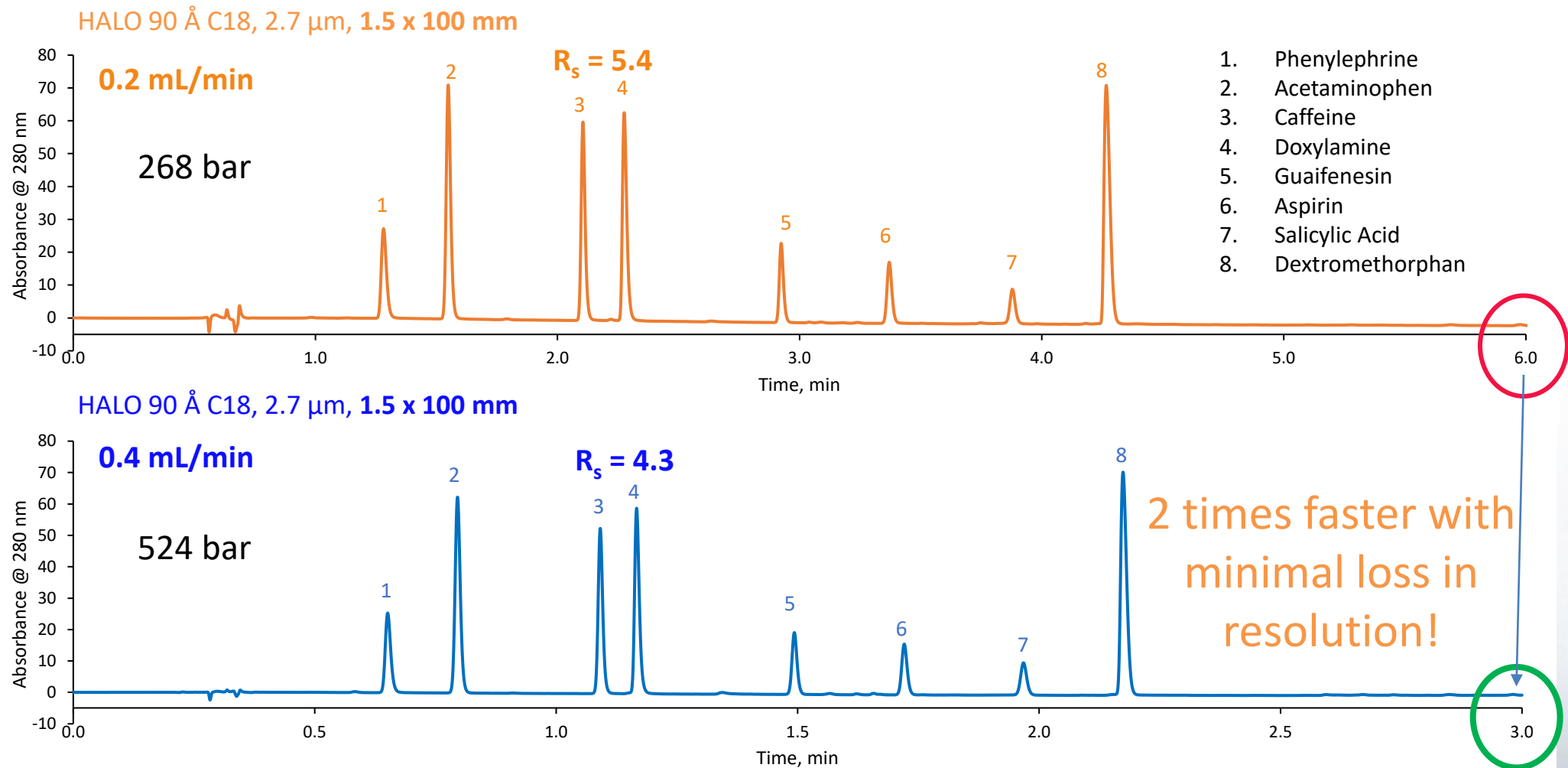
How to transfer a method to a 1.5 mm I.D. column?

- Scale flow rate

FLOW RATES (mL/min)	COLUMN IDS				
	4.6	3.0	2.1	1.5	1.0
	0.96	0.41	0.20	0.10	0.045
	1.44	0.61	0.30	0.15	0.068
	1.92	0.82	0.40	0.20	0.091
	2.40	1.02	0.50	0.26	0.113
	2.88	1.22	0.60	0.31	0.136

- If gradient method, add injection time delay to account for dwell volume
- Scale injection volume to maintain signal or keep same injection volume for increased signal as you reduce the column I.D.

Benefits of Fused-Core[®] in a 1.5 mm



Gradient separation where the flow rate has been doubled and gradient time cut in half

HALO® 1.5 mm I.D. Columns

Chemistries & pore sizes available for small molecules, peptides, and proteins

- HALO 90 Å C18
- HALO 90 Å LPH-C18
- HALO 160 Å ES-C18
- HALO 1000 Å C4
- HALO 1000 Å Diphenyl



Wed. 9:30 AM Liquid Chromatography Applications for Better Separations

HALO® 1.5 mm I.D. Columns

Chemistries & pore sizes available for small molecules, peptides, and proteins

- HALO 90 Å C18
- HALO 90 Å LPH-C18
- HALO 160 Å ES-C18
- HALO 1000 Å C4
- HALO 1000 Å Diphenyl



Wed. 2:00 PM Proteomics & Metabolomics: Challenges and Recent Developments
Enhanced Sensitivity for Peptide and Protein Applications Using the 1.5mm ID Column, Peter Pellegrinelli

HALO[®] 1.5 mm I.D. Columns

Chemistries & pore sizes available for small molecules, peptides, and proteins

- HALO 90 Å C18
- HALO 90 Å LPH-C18
- HALO 160 Å ES-C18
- HALO 1000 Å C4
- HALO 1000 Å Diphenyl



More products to be added soon!

Summary

- ✓ 1.5 mm I.D. columns offer benefits of increased signal and reduced solvent consumption
 - ✓ To realize the benefit of increased signal, the impact of extra-column effects of the UHPLC and/or MS system being used must be minimized
 - ✓ Optimization of the MS source parameters may be necessary
- ✓ Easy to implement microflow solution with existing systems
 - ✓ 100 $\mu\text{L}/\text{min}$ – 500 $\mu\text{L}/\text{min}$ & pumps that are able to deliver gradients
- ✓ Made by a trusted manufacturer of Fused-Core[®] columns in a 9001 ISO-certified facility

Acknowledgements

- AMT
- Jim Grinias, Rowan University
- Justin Godinho, GSK
- William Campbell, The Pennsylvania State University
- Thomas Waeghe

Where do I Find More Info on 1.5 mm ID columns?

- Journal Articles

- B.P. Libert, J.M. Godinho, S.W. Foster, J.P. Grinias, B.E. Boyes, Implementing 1.5 mm internal diameter columns into analytical workflows, J. Chromatogr. A. 1676 (2022) 463207.
<https://doi.org/10.1016/j.chroma.2022.463207>.
- S. Fekete, A. Murisier, G.L. Losacco, J. Lawhorn, J.M. Godinho, H. Ritchie, B.E. Boyes, D. Guillarme, Using 1.5 mm internal diameter columns for optimal compatibility with current liquid chromatographic systems, J. Chromatogr. A. 1650 (2021) 462258.
<https://doi.org/10.1016/j.chroma.2021.462258>.

Questions

support@advanced-materials-tech.com



Photo by [Camylla Battani](#) on [Unsplash](#)