HALO®

advancedmaterialstechnology

Opportunities for Developing Improved Separation Methods with Modern UHPLC Instruments

Peter Pellegrinelli

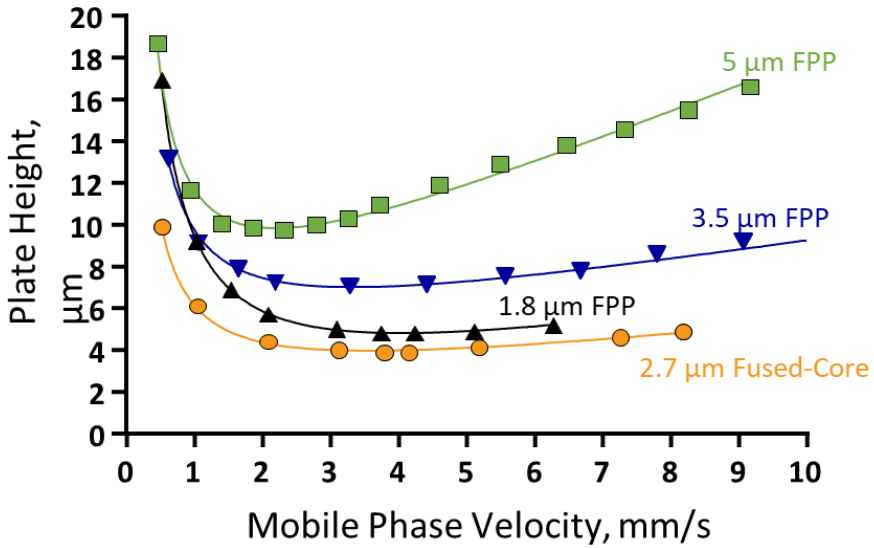
Stephanie Schuster, Conner McHale

Advanced Materials Technology, Inc.

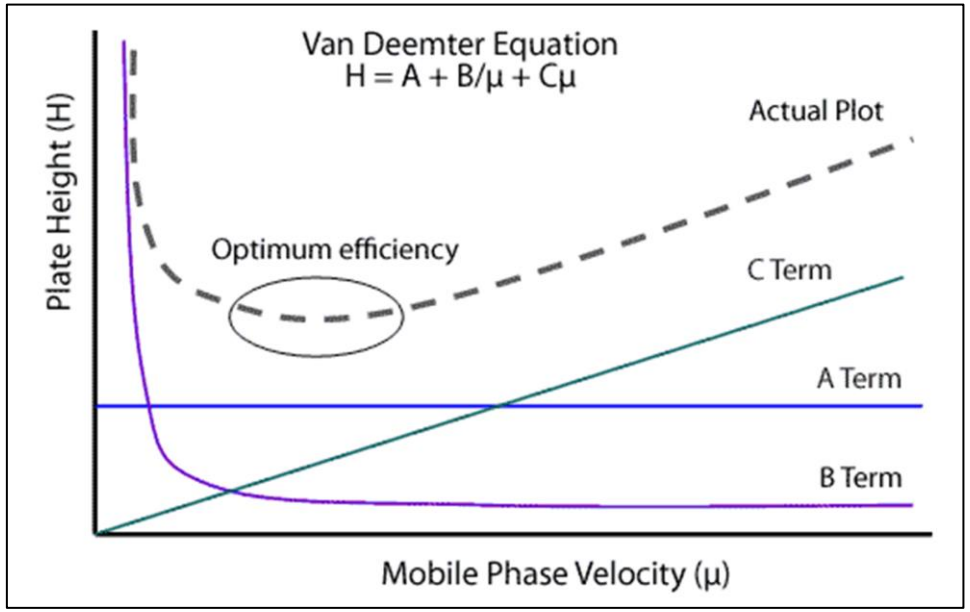
Improved Instrumentation

- HPLC and UHPLC systems have improved
  - Lower internal volumes
  - Higher back pressure capabilities
  - High flow rate accuracy and repeatability
  - Less carryover for each injection
- HPLC technology will continue to evolve
  - HPLC column technology must improve as well

Switching Particle Type
FPP vs SPP



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)

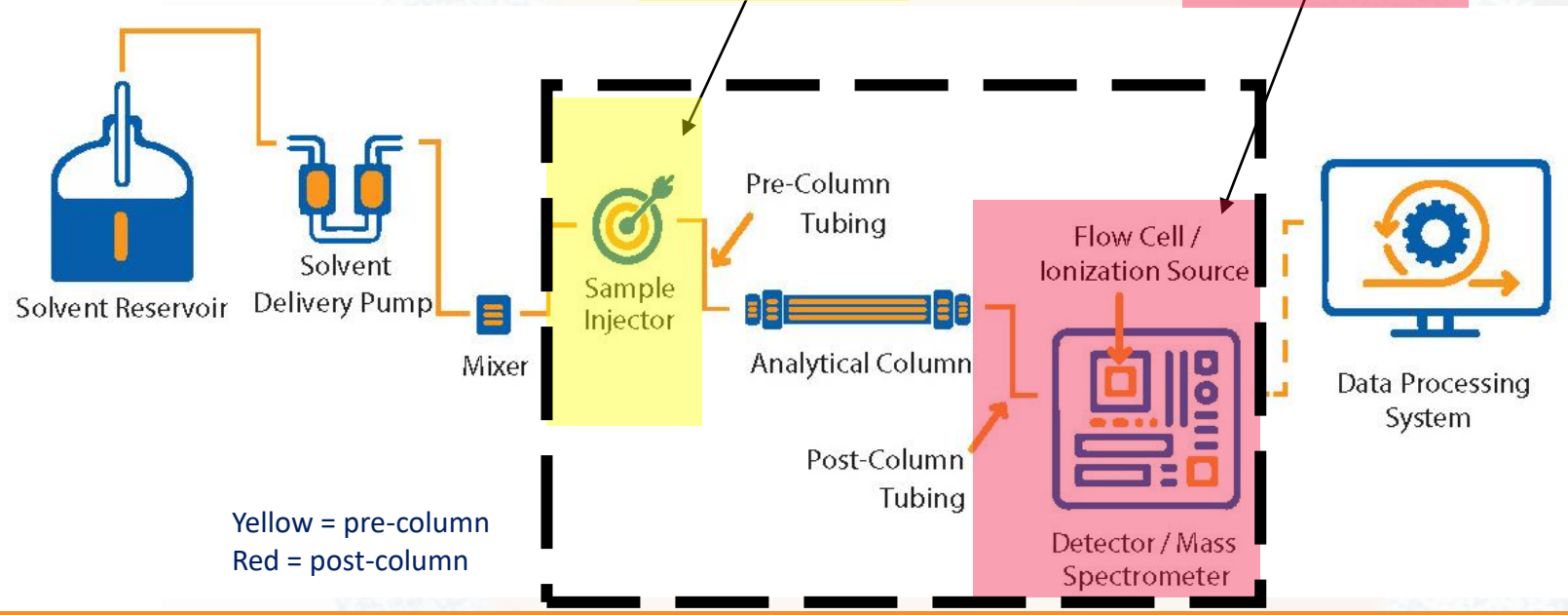
Reduced ID Columns & Particle Sizes

- ✓More Sensitivity from conventional UHPLC Systems
- ✓Higher Ionization Efficiencies from LCMS systems
- ✓Reduced Solvent Consumption compared to larger ID columns
- ✓Reduced back pressure
- ✓Easy to Implement microflow solution
- ✓Robust Hardware that requires less care

Getting the Most from a Column

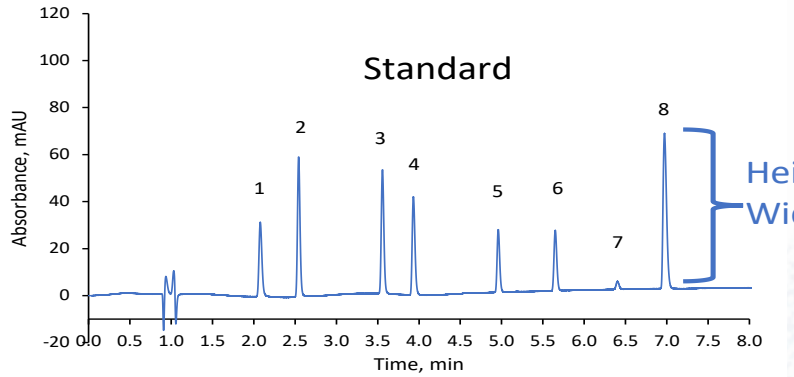
- Reduce any extracolumn dispersion
  - Pre-column tubing
  - Post-column tubing
  - Detector/Mass

$$\sigma^2_{V,tot} = \sigma^2_{V,pre-col} + \sigma^2_{V,col} + \sigma^2_{V,post-col}$$

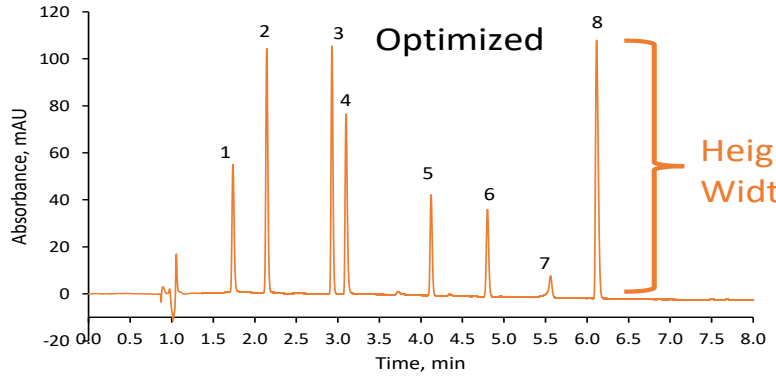


Getting the Most from a Column

- Minimize dispersion from detector and tubing



Height = 66 mAU
Width = 0.046 min



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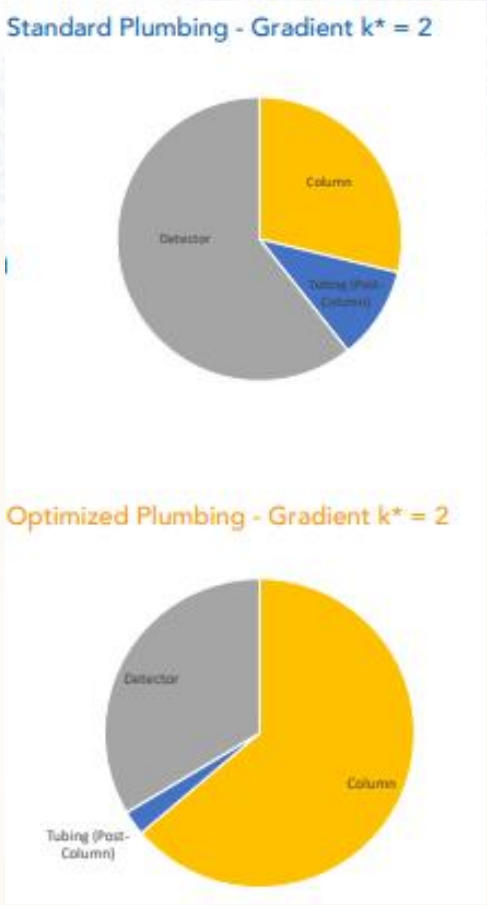
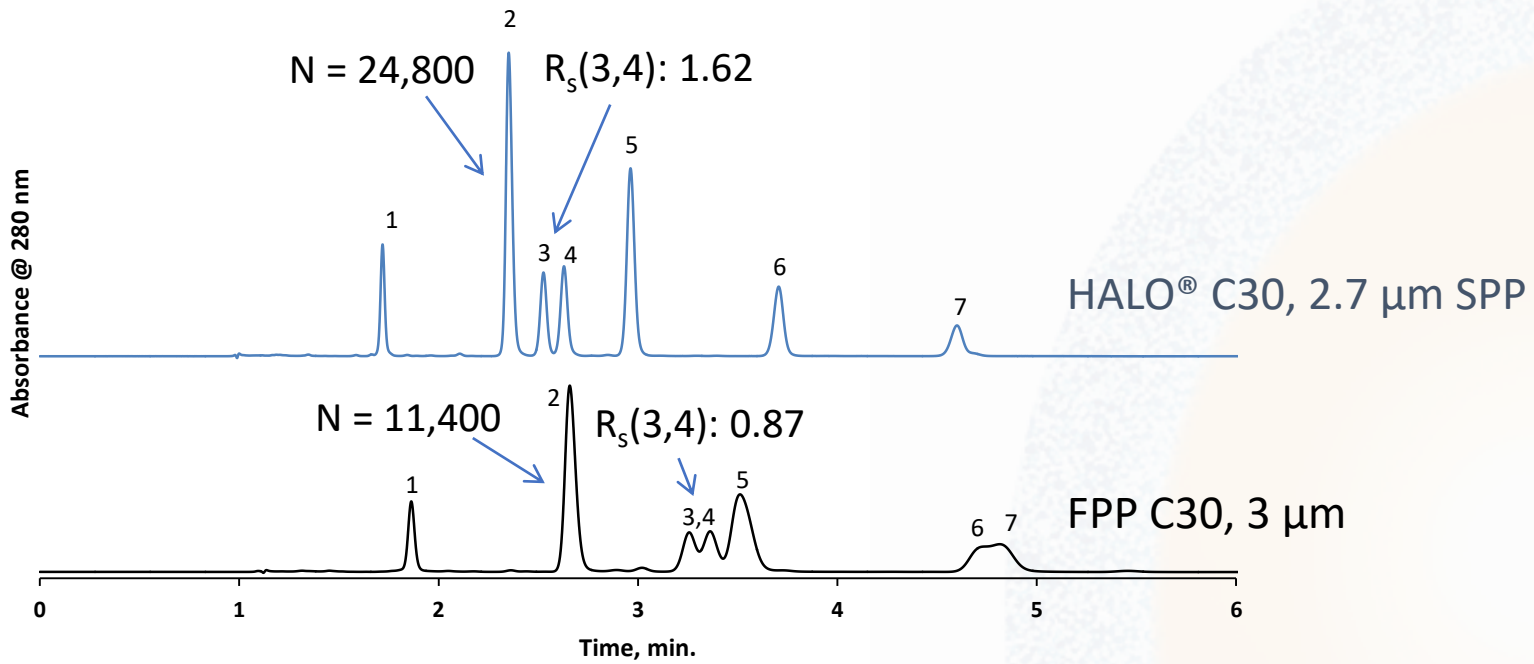


http://www.multidlc.org/dispersion_calculator

Increased Efficiency Demonstrated Using Fat Soluble Vitamins

Sharper peaks and increased resolution with the HALO® C30 column!

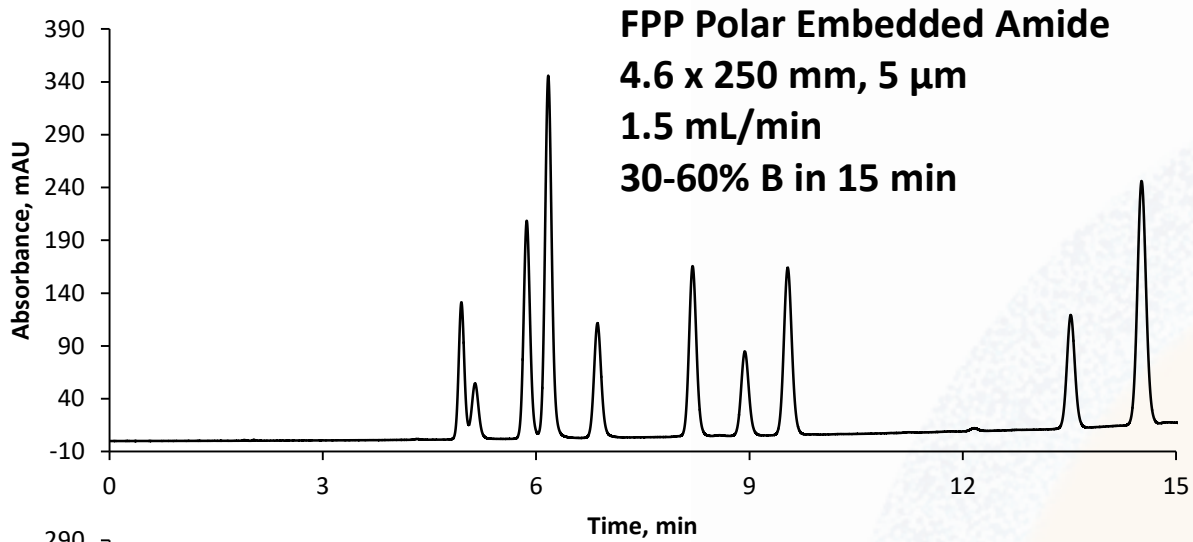


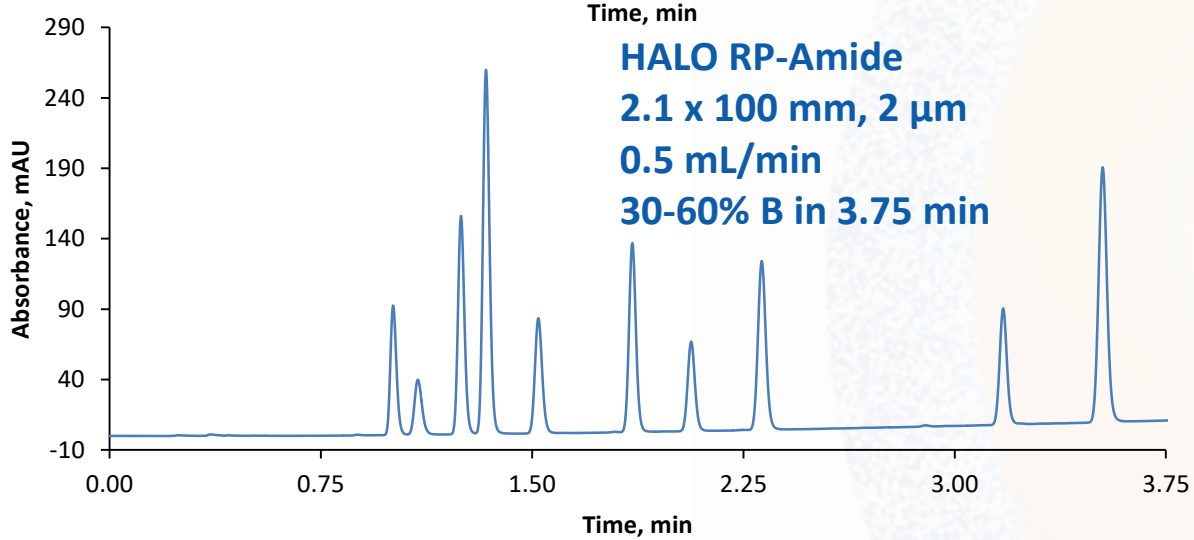
PEAK IDENTITIES:

1. Retinyl acetate (A)
2. Delta tocopherol (E)
3. Ergocalciferol (D2)
4. Cholecalciferol (D3)
5. Alpha tocopherol (E)
6. DL-alpha-tocopherol acetate (E)
7. 2,3-*trans*-phylloquinone (K)

Isocratic: 100% Methanol
Wavelength: 280nm
Injection: 2 µL
Temperature: 30 °C
Flow Rate: 1.5 mL/min
Columns: 4.6 x 150 mm

Increased Speed & Reduced Mobile Phase Consumption





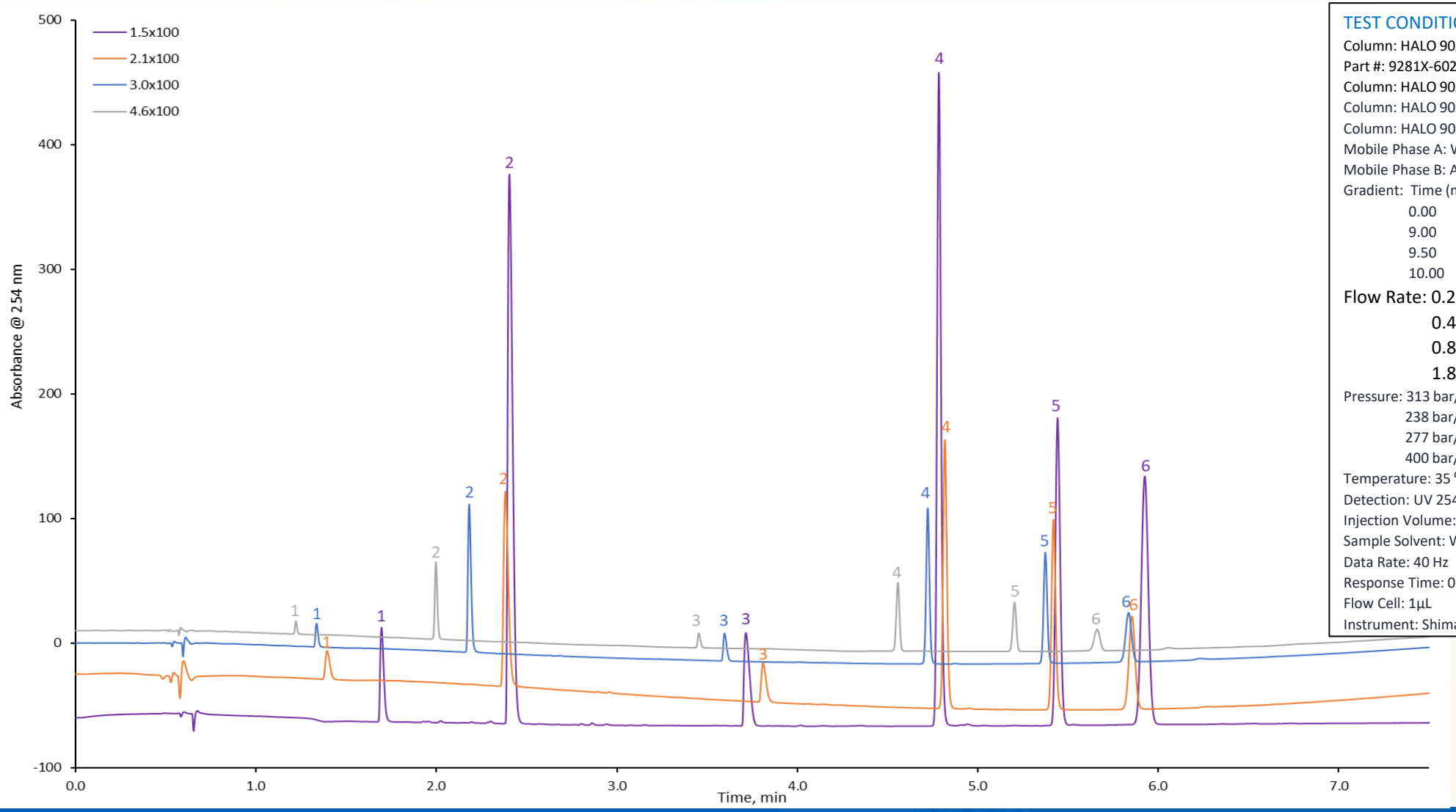
PEAK IDENTITIES:

1. homovanillic acid
2. caffeic acid
3. syringic acid
4. vanillic acid
5. chlorogenic acid
6. sinapic acid
7. ferulic acid
8. *p*-coumaric acid
9. *trans*-cinnamic acid
10. resveratrol

SPP method

- 4x faster
- 12x less solvent

Decreasing ID to Improve Sensitivity



TEST CONDITIONS

Column: HALO 90Å C18, 2.7 µm, 1.5 x100 mm
Part #: 9281X-602
Column: HALO 90Å C18, 2.7 µm, 2.1 x 100 mm
Column: HALO 90Å C18, 2.7 µm, 3.0 x 100 mm
Column: HALO 90Å C18, 2.7 µm, 4.6 x 100 mm
Mobile Phase A: Water/ 0.1% DFA
Mobile Phase B: ACN/ 0.1% DFA
Gradient:

Time (min)	%B
0.00	05
9.00	85
9.50	05
10.00	05

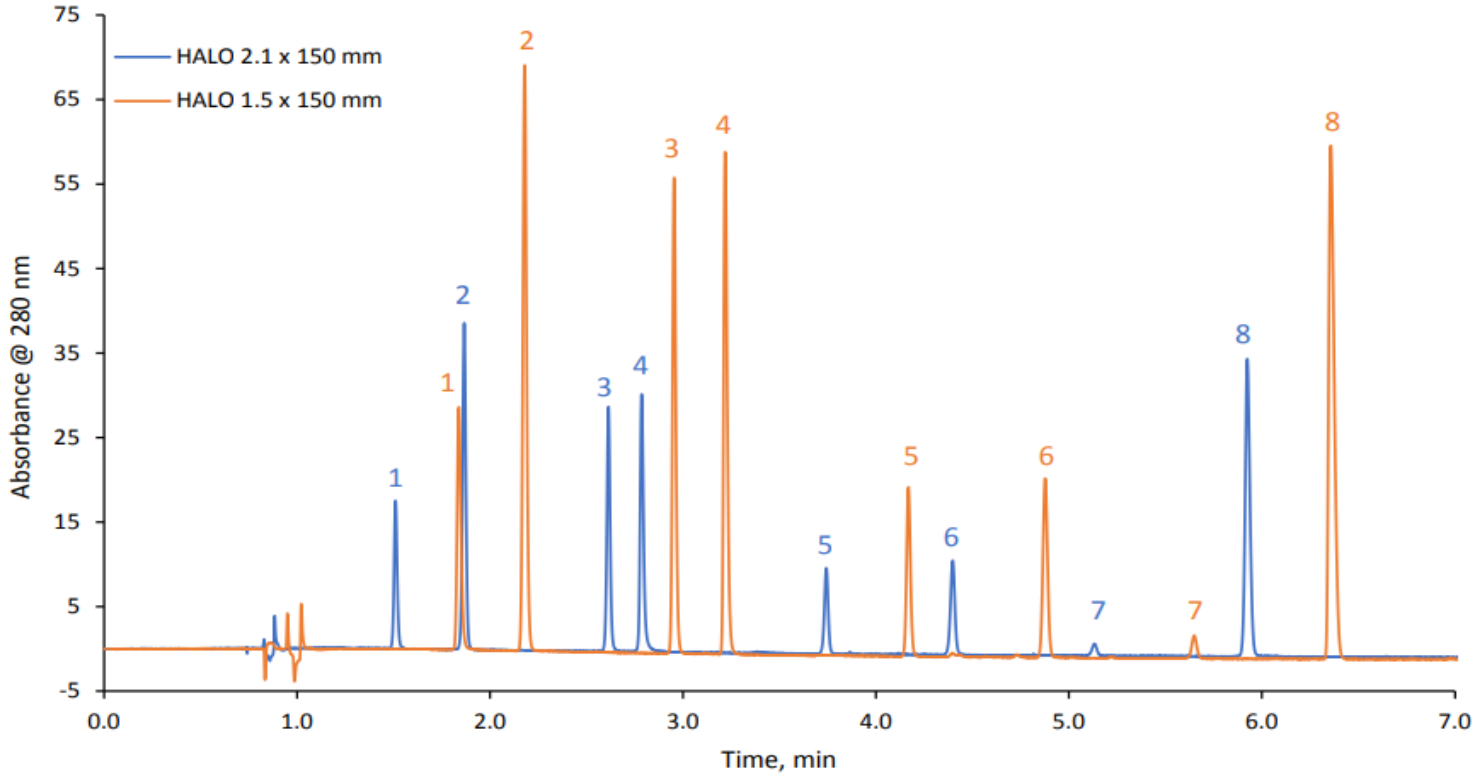
Flow Rate: 0.2 mL/min for 1.5mm
0.4 mL/min for 2.1mm
0.8 mL/min for 3.0mm
1.88 mL/min for 4.6mm
Pressure: 313 bar/1.5mm
238 bar/2.1mm
277 bar/3.0mm
400 bar/4.6mm
Temperature: 35 °C
Detection: UV 254 nm, PDA
Injection Volume: 1.0 µL
Sample Solvent: Water/ACN 50/50
Data Rate: 40 Hz
Response Time: 0.100 sec.
Flow Cell: 1µL
Instrument: Shimadzu Nexera X2

PEAK IDENTITIES

1. Atenolol
2. Pindolol
3. Propranolol
4. Indoprofen
5. Naproxen
6. Warfarin

Increased Sensitivity of OTC Medicines

With extracolumn dispersion minimized, the 1.5 mm ID column shows taller peaks compared to 2.1 mm ID column providing greatest benefit for minor components.

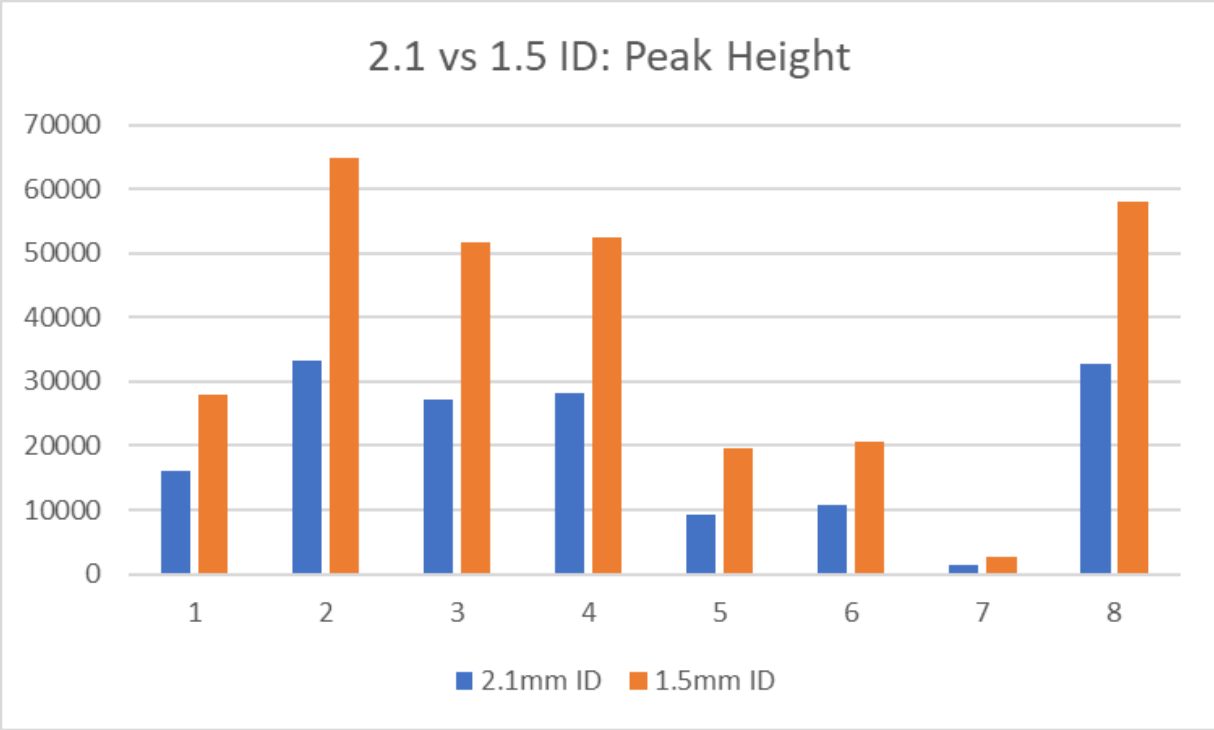


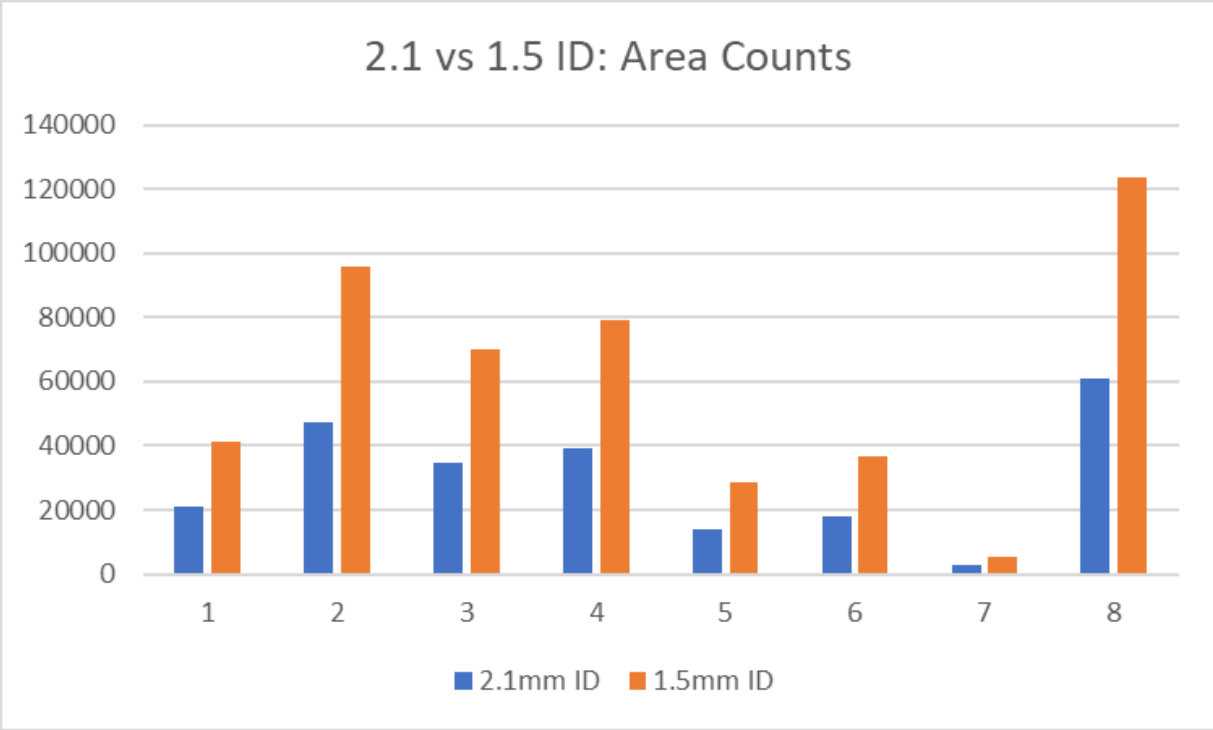
PEAK #	COMPOUND
1	Phenylephrine
2	Acetaminophen
3	Caffeine
4	Doxylamine
5	Guiafenesin
6	Aspirin
7	Salicylic Acid
8	Dextromethorphan

TEST CONDITIONS:

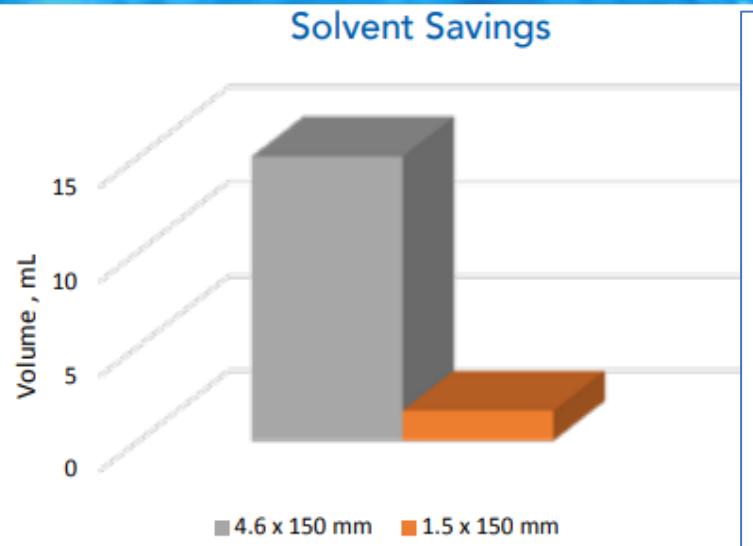
Mobile Phase A: Water/0.15% TFA
Mobile Phase B: ACN/0.1% TFA
Gradient: 5-50 %B in 8 min
Flow Rate: 0.2 mL/min for 1.5 mm
0.4 mL/min for 2.1 mm
Pressure: 425 bar/1.5 mm
470 bar/2.1 mm
Temperature: 35 °C
Injection Volume: 0.5 µL
Detection: UV 280 nm, PDA
Instrument: Shimadzu Nexera X2

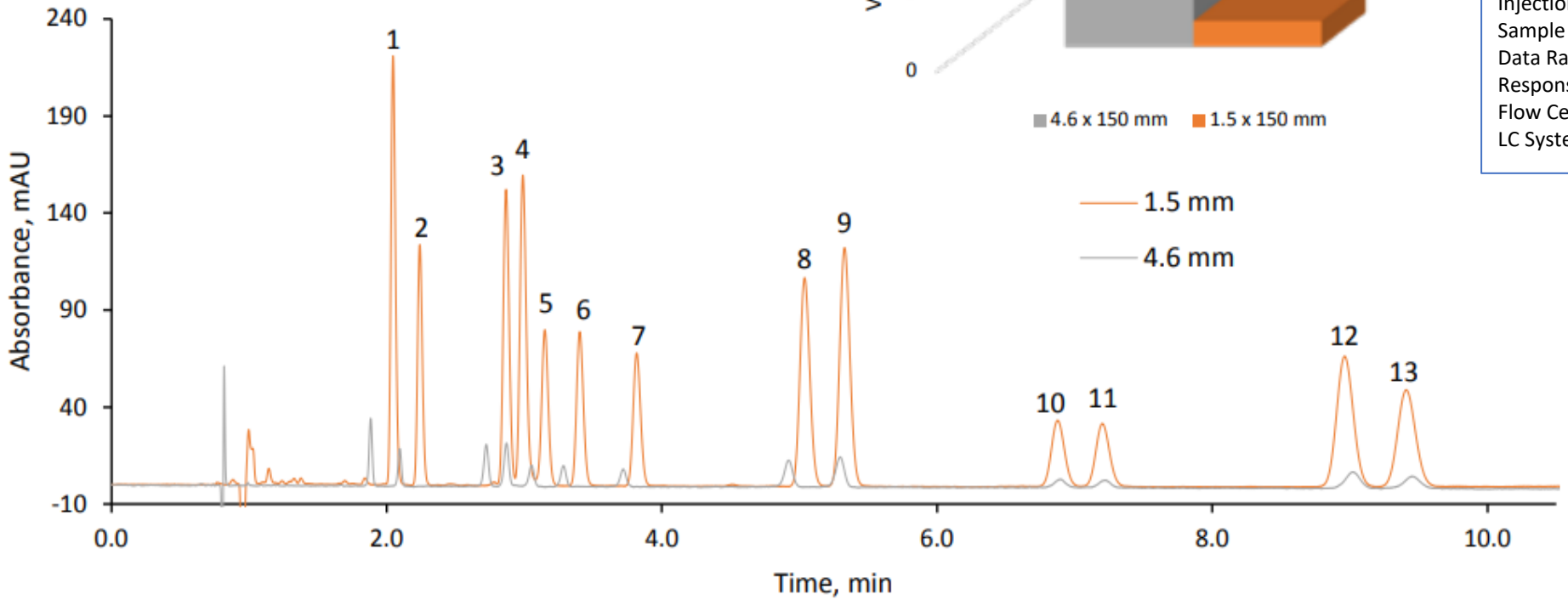
2.1 vs 1.5mm ID
OTC Data





Solvent Savings from a 4.6 to 1.5 ID

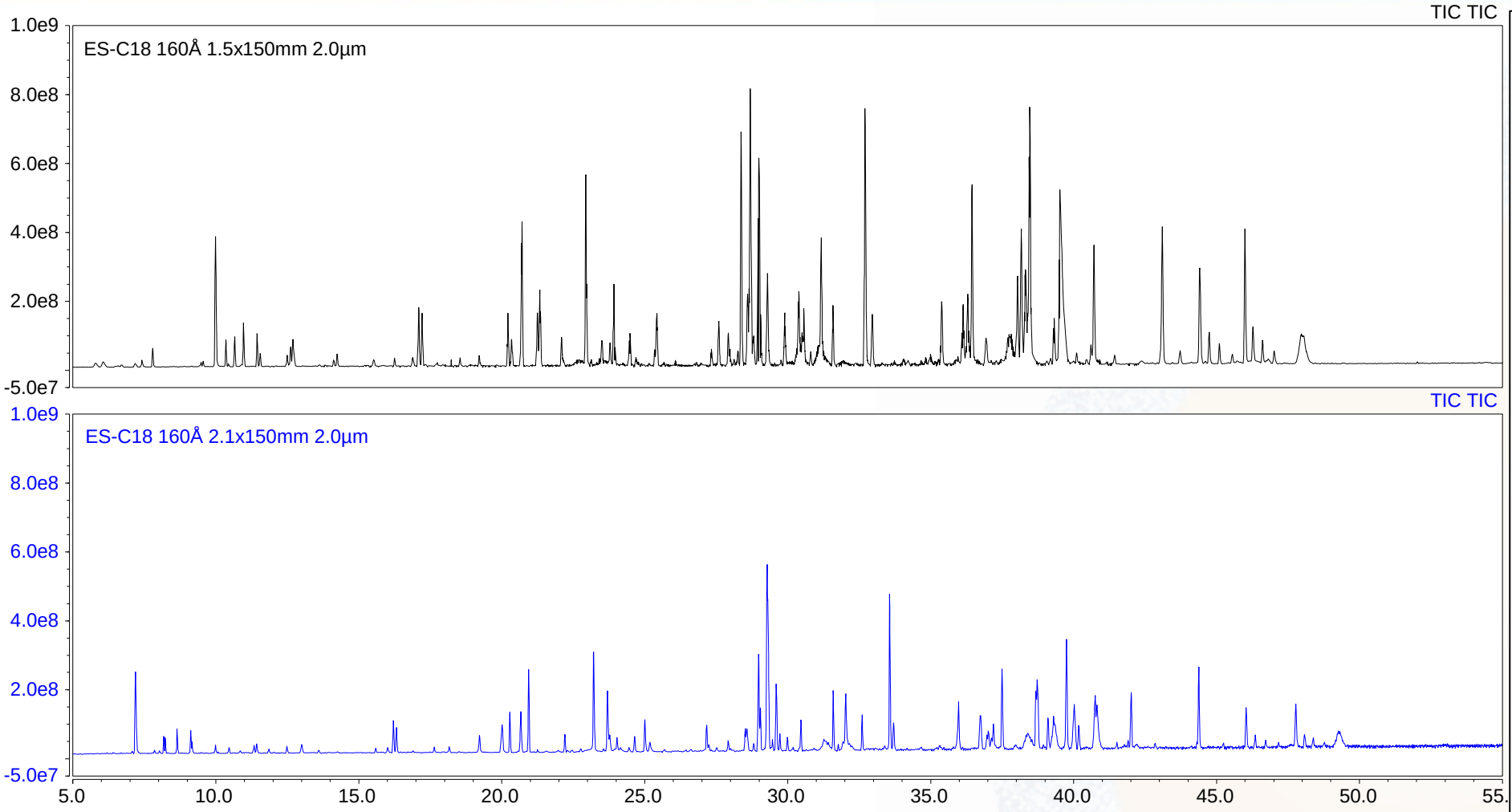




TEST CONDITIONS:
Column: HALO 90Å C18, 2.7 µm, 1.5 x 150mm
Part Number: 9281X-702
Column: HALO 90Å C18, 2.7 µm, 4.6 x 150mm
Mobile Phase A: Water/ 0.1% Formic Acid
Mobile Phase B: Acetonitrile/ 0.1% Formic Acid
Isocratic: 75% B
Flow Rate: 0.159 mL/min (1.5x150)
Flow Rate: 1.5 mL/min (4.6x150)
Temperature: 30°C
Detection: UV 228 nm, PDA
Injection Volume: 0.5 µL
Sample Solvent: 75/25 ACN/ Water
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

PEAK IDENTITIES:
1. CBDVA
2. CBDV
3. CBDA
4. CBGA
5. CBG
6. CBD
7. THCV
8. THCVA
9. CBN
10. 9-THC
11. 8-THC
12. CBC
13. THCA

Increased MS Response of Peptides



TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.0 µm, 1.5 x 150 mm
Column: HALO 160 Å ES-C18 , 2.0 µm, 2.1 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

Flow Rate: 0.2 mL/min for 1.5mm
0.4 mL/min for 2.1mm
Pressure: 372 – Bar 1.5
670 – Bar 2.1
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

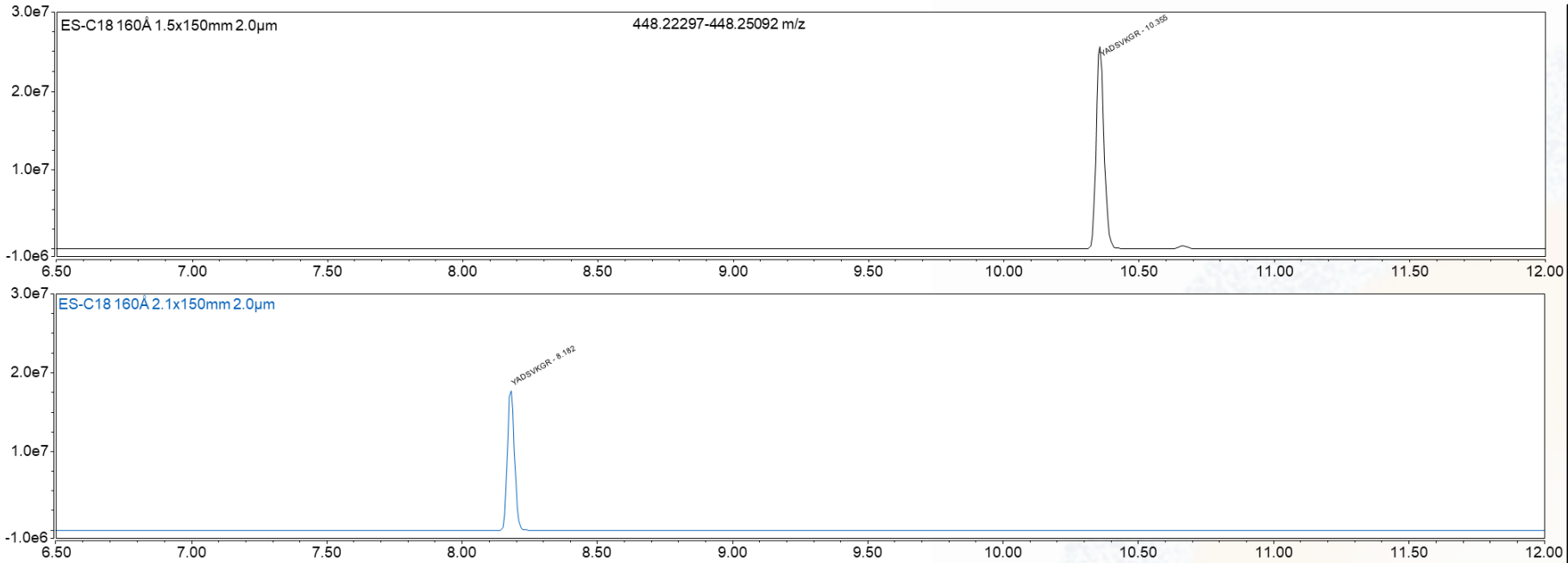
Tubing Optimization:

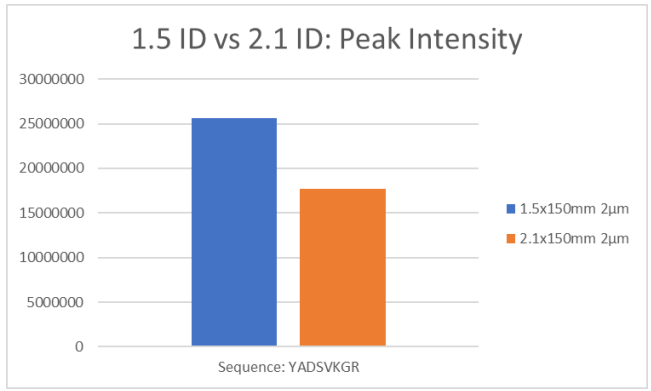
50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

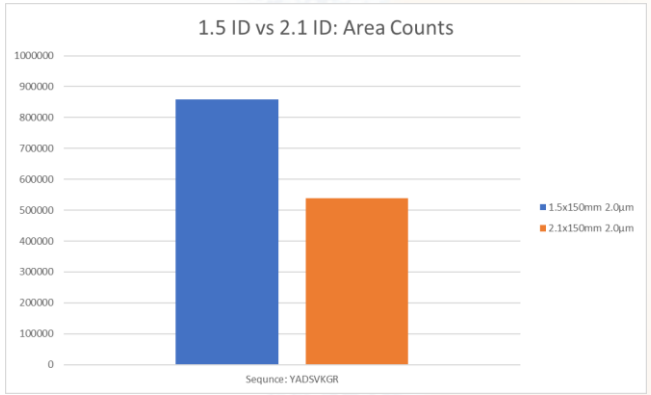
MS CONDITIONS:

System: ThermoFisher Q Exactive
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

Increased MS Response of Peptides







TEST CONDITIONS:

Column: HALO 160 Å ES-C18 , 2.0 µm, 1.5 x 150 mm
Column: HALO 160 Å ES-C18 , 2.0 µm, 2.1 x 150 mm
Mobile Phase A: Water, 0.1% DFA
Mobile Phase B: Acetonitrile, 0.1% DFA
Gradient:

Time	%B
0.5	2
60.5	50
61.0	70
65.0	70
65.5	2
70.0	Stop

Flow Rate: 0.2 mL/min for 1.5mm
0.4 mL/min for 2.1mm
Pressure: 372 – Bar 1.5
670 – Bar 2.1
Temperature: 60 °C
Injection Volume: 1 µL
Sample: 1mg/mL Trastuzumab tryptic digest
Sample Solvent: 1.5M Guanidine HCl/0.5% Formic Acid/~50mM Tris pH 7.8
LC System: Shimadzu Nexera X2

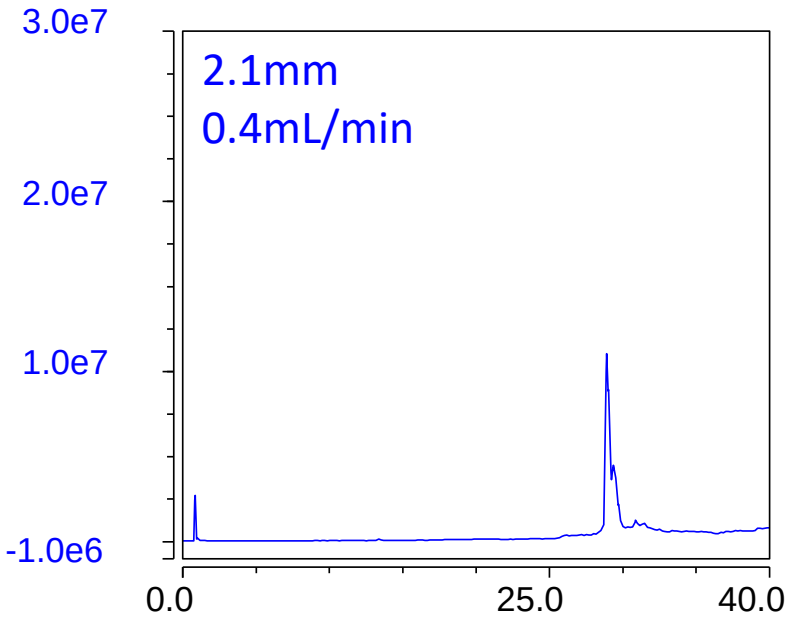
Tubing Optimization:

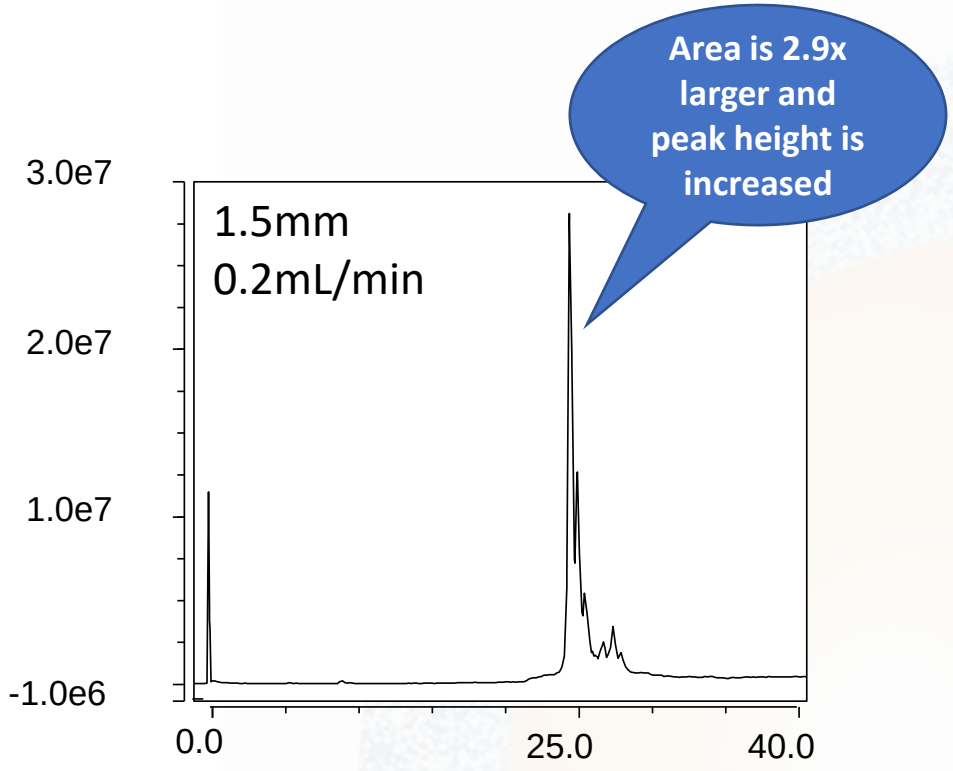
50µm x 600mm Column to Diverter Valve
50µm x 350mm Diverter Valve to Ground
50µm x 100mm Ground to Source

MS CONDITIONS:

System: ThermoFisher Q Exactive
Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

Intact Trastuzumab on a HALO 1000 Å Diphenyl





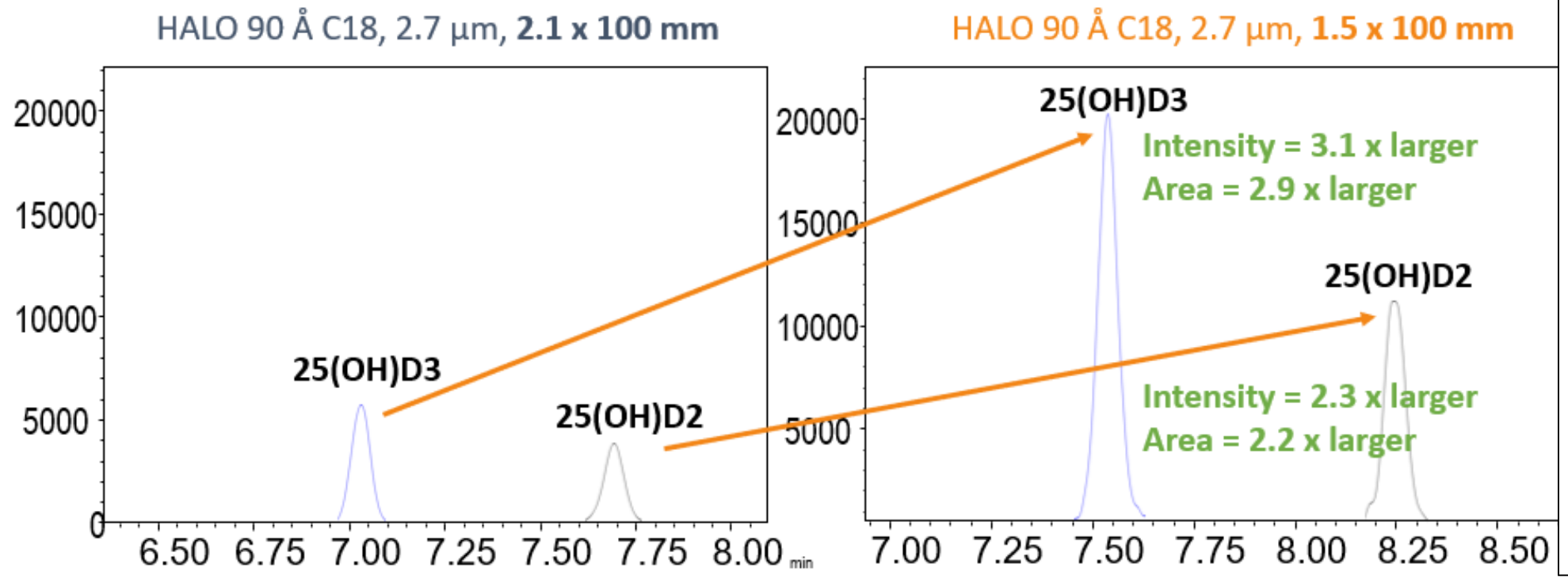
TEST CONDITIONS

Column: HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm
Part Number: 9212X-702
Column: HALO 1000 Å Diphenyl, 2.7 µm, 2.1 x 150 mm
Part Number: 92712-726
Mobile Phase A: Water/0.1% DFA
B: 50% Acetonitrile/50% n-propanol/0.1% DFA
Gradient: 27-36 %B in 40 min
Flow Rate: 0.2 mL/min for 1.5 mm ID
0.4 mL/min for 2.1 mm ID
Back Pressure: 252 bar (1.5 mm)
272 bar (2.1 mm)
Temperature: 60 °C
Detection: PDA, 220 nm
Injection Volume: 3 µL of 1.0 mg/mL of Trastuzumab
Sample Solvent: 100 mM ammonium bicarbonate
LC System: Shimadzu Nexera X2
MS System: ThermoFisher Q Exactive

MS CONDITIONS

Spray Voltage (kV): 3.8
Capillary temperature: 320 °C
Sheath gas: 35
Aux gas: 10
RF lens: 50

Increased Response of Vitamin D Metabolites



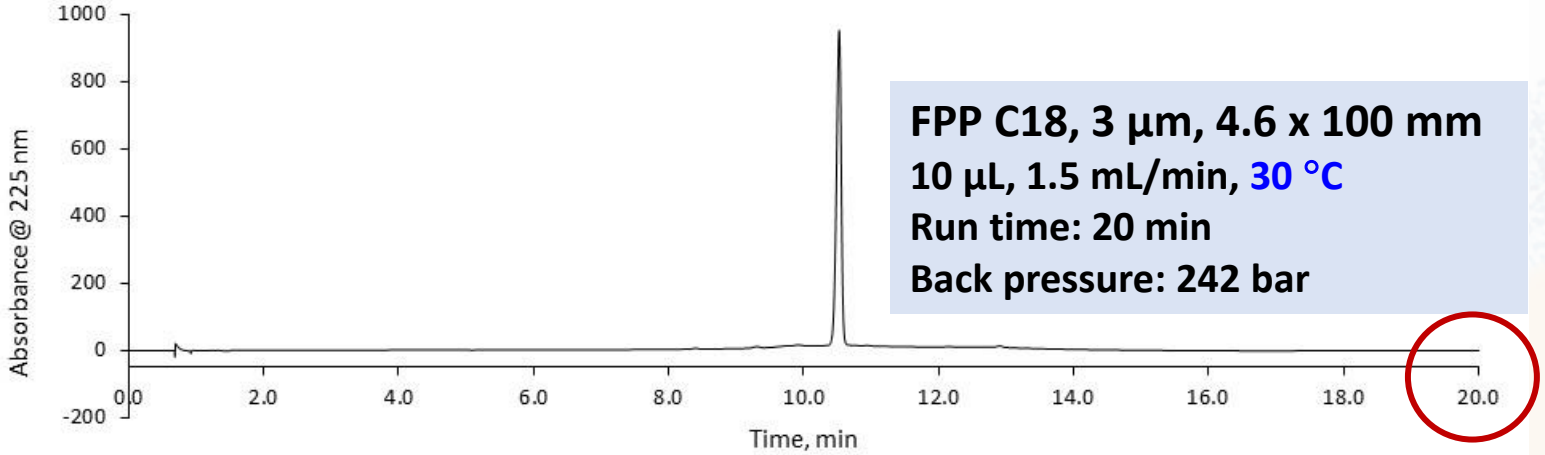
TEST CONDITIONS:
Column: HALO 90 Å C18, 2.7µm, 2.1x100 mm
Column: HALO 90 Å C18, 2.7µm, 1.5x100 mm
Part Number: 9281X-602
Tubing (Column to Source): AMT MarvelXACT™ 50µm x 600mm
Part Number: PS7050600
Mobile Phase A: Water, 0.1 % Formic Acid
Mobile Phase B: ACN, 0.1 % Formic Acid
Flow Rate: 0.4 mL/min for 2.1 mm
0.2 mL/min for 1.5 mm
Pressure: 370 bar for 2.1 mm
229 bar for 1.5 mm
Temperature: 40 °C
Injection Volume: 1.0 µL @ 500 ppb
Sample Solvent: 50/50/ ACN/H_2O
Detection: +ESI/APCI MS/MS
LC System: Shimadzu Nexera X2
ESI LCMS system: Shimadzu LCMS-8040
Gradient:

Time	%B
0.00	50
10.80	92.5
11.48	95
12.75	100
13.75	100
14.25	50
20.00	End

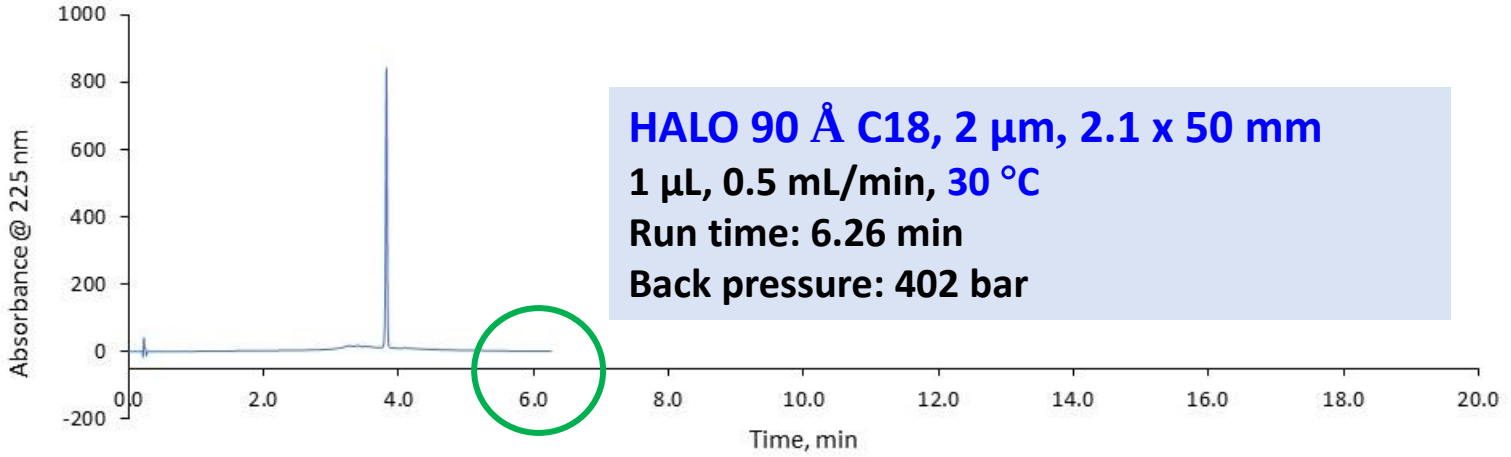
MS Source Conditions: ESI +/APCI
Spray Voltage: 4.5 kV
Nebulizing gas: 2 L/min
Drying gas: 15 L/min
DL temp: 250 °C
Heat Block: 300 °C

PEAK IDENTITIES
1. 25-Hydroxyvitamin D3 (25(OH)D3) m/z - 383.4
2. 25-Hydroxyvitamin D2 (25(OH)D2) m/z - 395.3

USP Monograph for Itraconazole – Modified



FPP C18, 3 µm, 4.6 x 100 mm
10 µL, 1.5 mL/min, 30 °C
Run time: 20 min
Back pressure: 242 bar



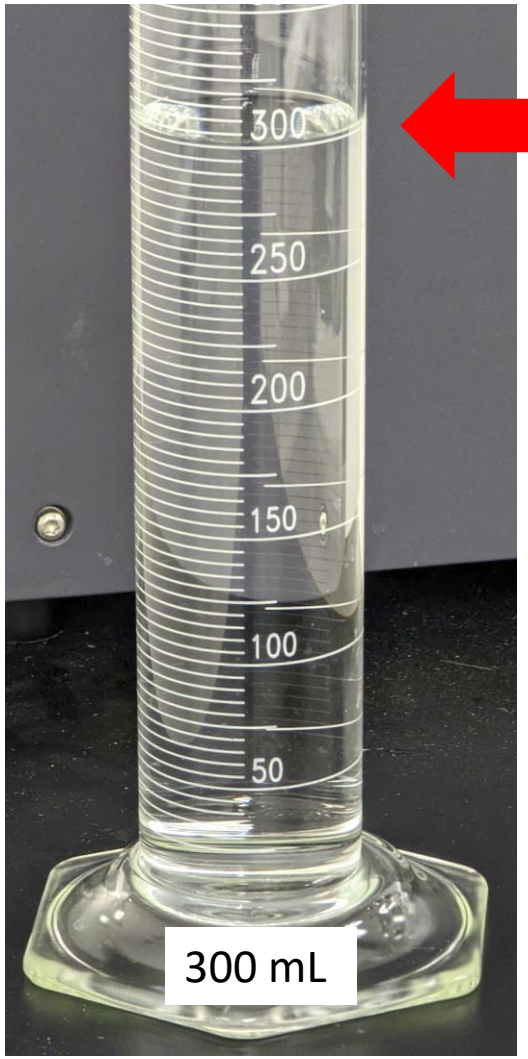
HALO 90 Å C18, 2 µm, 2.1 x 50 mm
1 µL, 0.5 mL/min, 30 °C
Run time: 6.26 min
Back pressure: 402 bar

Method was moved to a shorter SPP column with smaller i.d. and smaller particle size

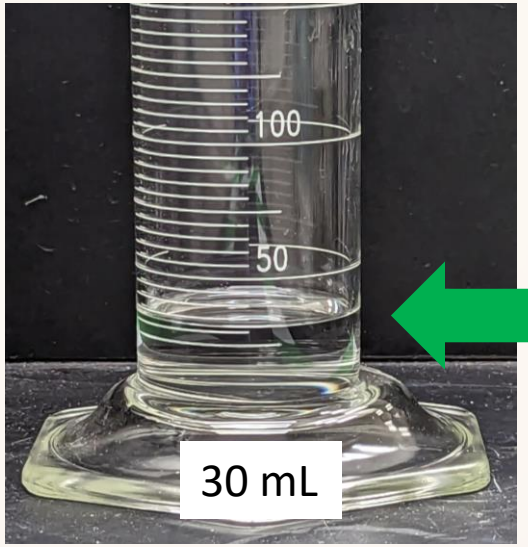
Total run time is > 3 times faster with the HALO® column!

USP Monograph for Itraconazole: Mobile Phase Reduction

For 10 injections:



compared to



10X reduction in mobile phase used!

Summary

- Optimization through column technology can be simple during method development
  - Switching particle type or size while keeping phase chemistry
  - Decreasing column length/ID
  - Changes to the method
- New column tech comes with multiple perks
  - Increased sensitivity
  - Faster run times
  - Increased solvent savings
  - Less sample injected

Acknowledgements

- AMT
  - Stephanie Schuster
  - Conner McHale
  - Stephanie Rosenberg
- Richard Henry

Questions

advancedmaterialstechnology

halocolumns.com

3521 Silverside Road, Suite 1-K
Quillen Building
Wilmington, DE 19810

(302) 992-8060