INCREASED SENSITIVITY AND SOLVENT SAVINGS WITH NOVEL 1.5 MM ID STAINLESS STEEL UHPLC COLUMNS

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MAC-MOD
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Outline

- Desire to move to smaller ID columns
- Benefits of smaller IDs with novel 1.5 mm ID UHPLC Columns
- Q & A

The Move to Smaller ID Columns

- HPLC columns were originally 4.6 mm ID operated at 1 mL/min+
- 3.0 mm ID columns introduced as a means to save solvent
  - 47% solvent savings going from a 4.6 x 100 mm @ 1.5 mL/min to a 3.0 x 100 mm @ 0.8 mL/min
- Short columns with 2.1 mm ID columns introduced for use with UHPLC and for interfacing to mass spectrometers

- Impact of Smaller ID Columns
  - Effects of viscous friction are diminished
  - Signal intensity is increased when same sample concentration used
  - Less solvent consumed = reduced consumable & waste disposal costs

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Fundamental Effects in Transitioning to Smaller Internal Column Diameters

Viscous Friction

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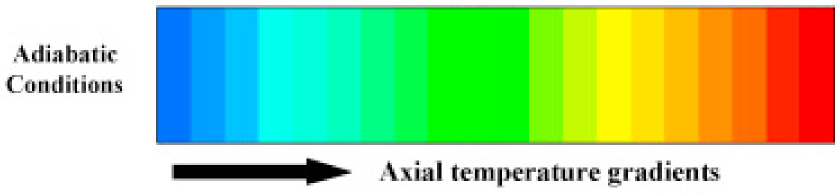
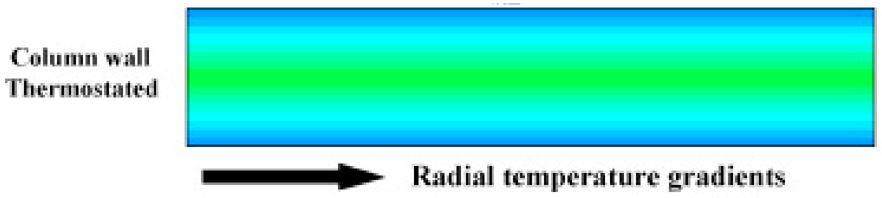
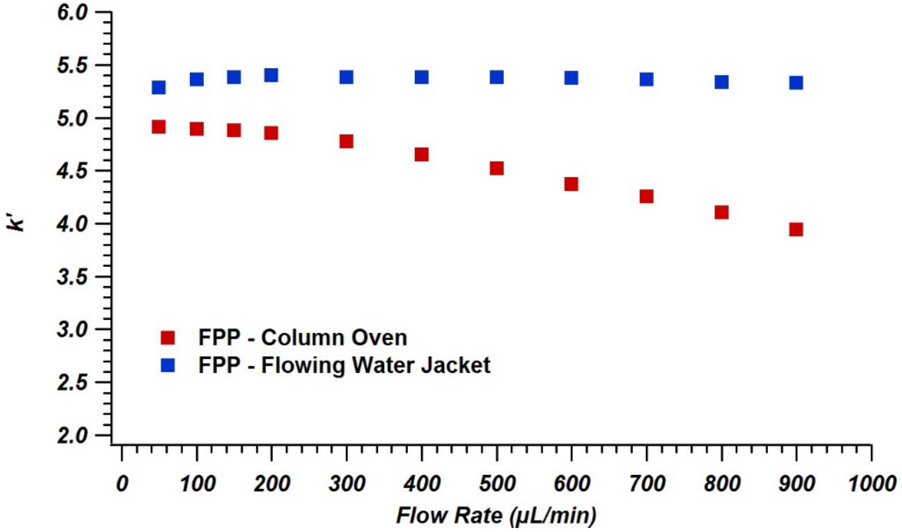
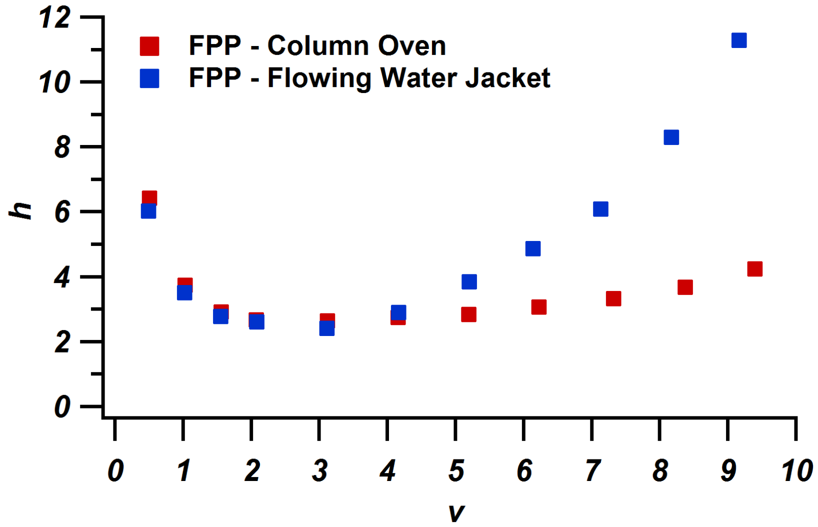
Formation of Thermal Gradients Due to Viscous Friction

$$Power = F\Delta P$$









Axial Gradients:

Less reproducible elution times

Radial Gradients:

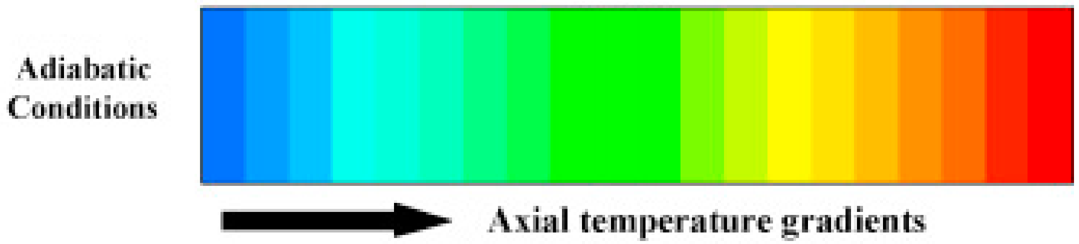
Lower efficiencies

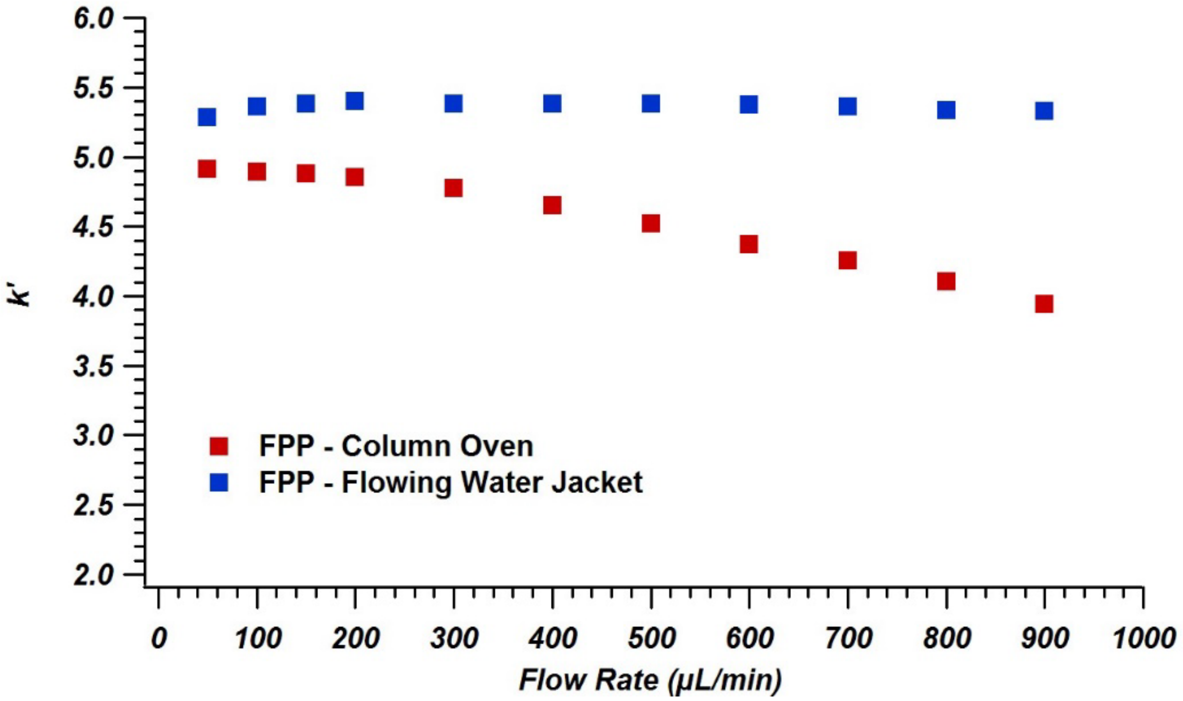
(≈ 4 W/m and up)

Gritti, F., Guiochon, G. *J. Chrom. A* **2009**, *1216*, 1353-1362.

Grinias, J. P., et. al. *J. Chrom. A* **2014**, *1371*, 261-264.

Formation of Axial Thermal Gradients Due to Viscous Friction





Axial Thermal Gradients are maximized in (quasi)-adiabatic environments:

Insulated columns or still-air oven conditions (no convective movement from oven fan)

Higher Efficiency, but higher Δt_r across flow rate range

Gritti, F., Guiochon, G. *J. Chrom. A* **2009**, *1216*, 1353-1362.
Grinias, J. P., et. al. *J. Chrom. A* **2014**, *1371*, 261-264.

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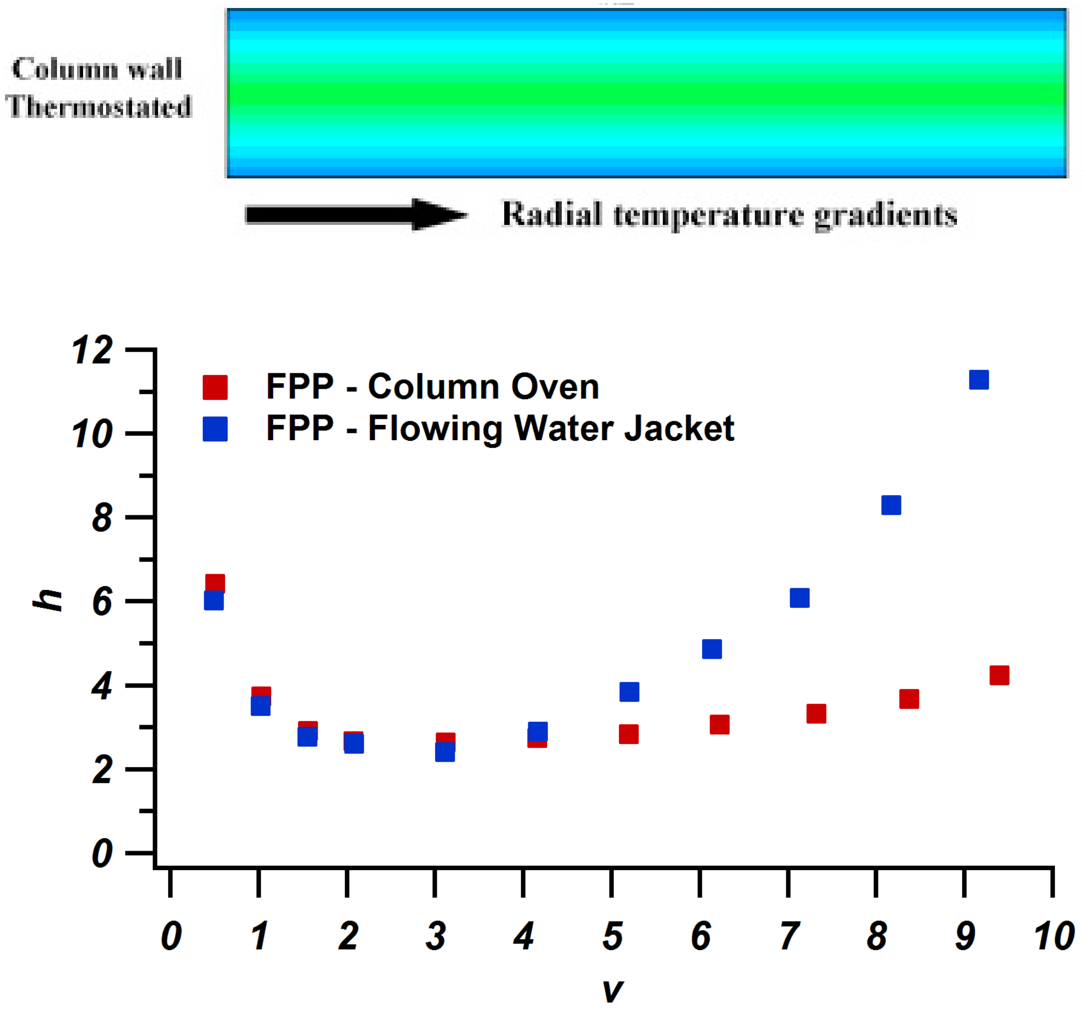
Formation of Radial Thermal Gradients Due to Viscous Friction



Radial Thermal Gradients are maximized in (quasi)-isothermal environments:

Air/water-jacketed columns or forced-air oven conditions (convective movement from oven fan)

Lower Δt_r across flow rate range, but lower efficiency

Gritti, F., Guiochon, G. *J. Chrom. A* **2009**, *1216*, 1353-1362.
Grinias, J. P., et. al. *J. Chrom. A* **2014**, *1371*, 261-264.

Considerations for Reducing Viscous Friction: Smaller Internal Column Diameter

$$Power = F\Delta P$$

Column inner diameter	Flow rate	Pressure	Power
4.6 mm	2.0 ml min^{-1}	7000 bar	24.0 W
2.0 mm	380 µl min^{-1}	7000 bar	4.5 W
1.0 mm	95 µl min^{-1}	7000 bar	1.1 W
500 µm	24 µl min^{-1}	7000 bar	280 mW
250 µm	5.9 µl min^{-1}	7000 bar	71 mW
100 µm	940 nl min^{-1}	7000 bar	11 mW
50 µm	240 nl min^{-1}	7000 bar	2.9 mW

[a]Power equals heat divided by time, which equals flow rate times pressure drop. Assume 1.0-µm particles packed in a 50-cm-long column.

[b]24 W equals 344 calories min^{-1} or 172 calories ml^{-1}.

Enough power to boil mobile phase before it exits the column!

Jorgenson, J. W. Annu. Rev. Anal. Chem. 2010 3:129

Fundamental Effects in Transitioning to Smaller Internal Column Diameters

Peak Signal

Internal Column Diameter and Concentration-Sensitive Detection

- **Most LC detectors are concentration-sensitive**
  - **LOD is improved when LC delivers highly concentrated sample**
    - **Minimize dilution in mobile phase**
  - **Flow rate optimum scales with ratio of square of radius of column**

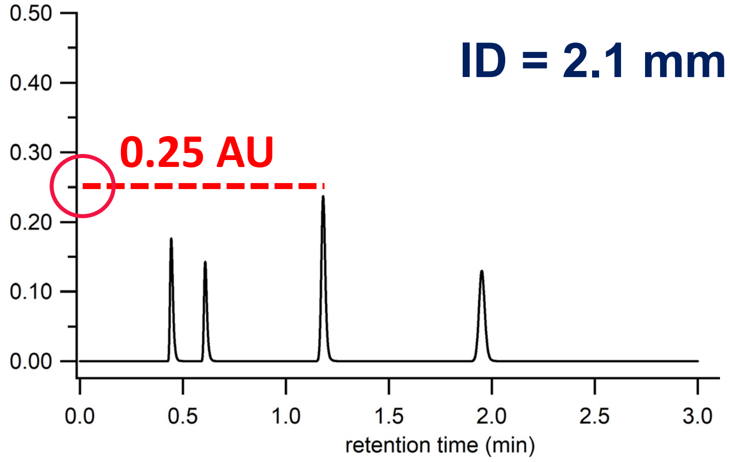
Example: 4.6 mm to 1.0 mm i.d. column

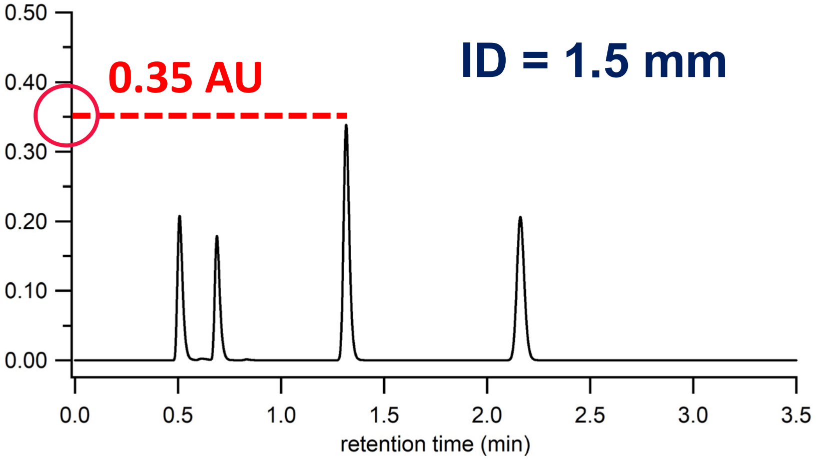
1 mL/min to around 50 µL/min

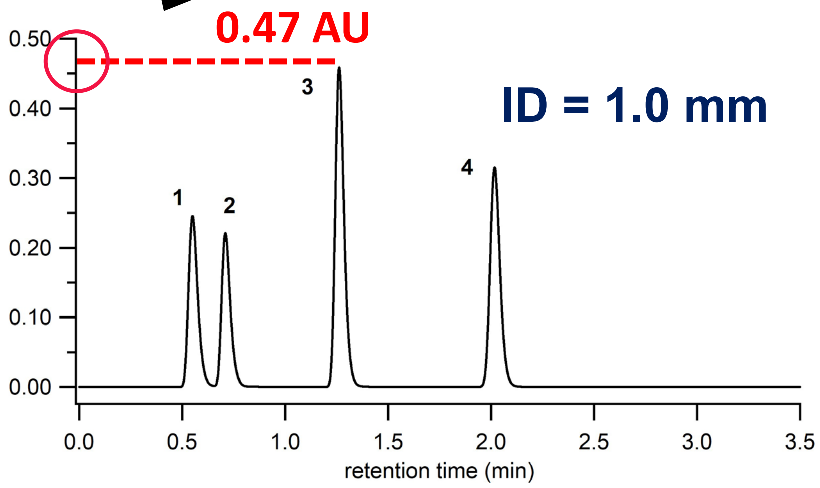
(1000µL/min)/(50µL/min) = 20-fold enhancement

****This calculation based on <u>identical</u> sample load (same sample concentration and injection volume)***

Comparison of Absorbance Signal with Varying Column Diameter







Signal increases as i.d. decreases with identical sample load on column.

Fundamental Effects in Transitioning to Smaller Internal Column Diameters

Extra-Column Effects

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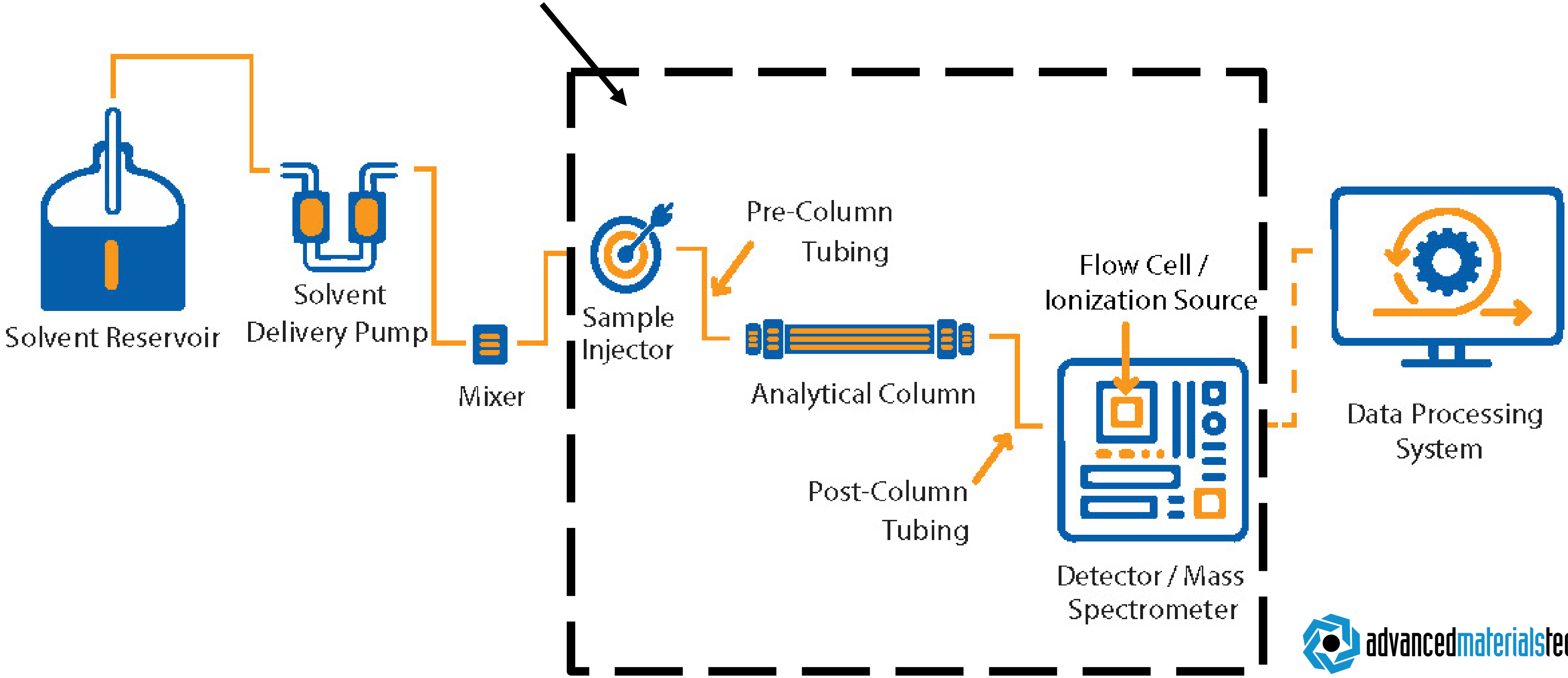
Sources of Extra-Column Band Broadening

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



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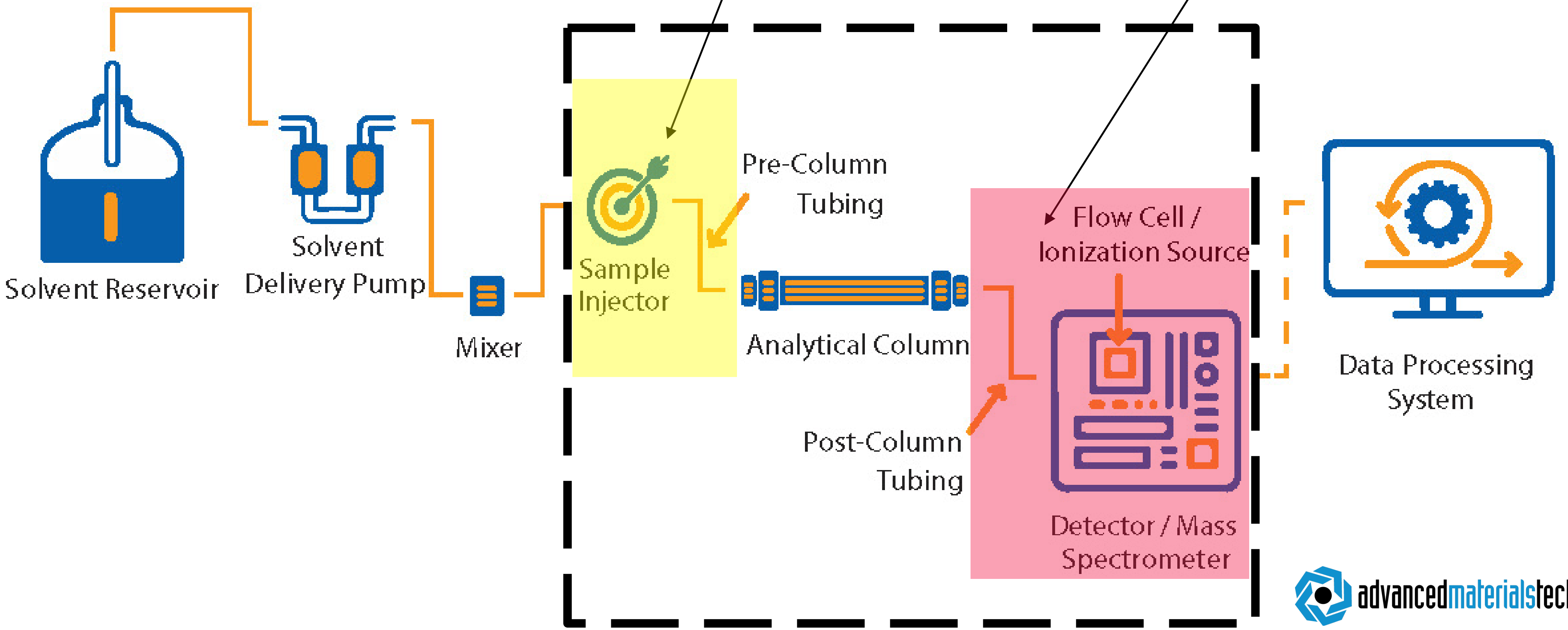
Sources of Extra-Column Band Broadening

Yellow = pre-column
Red = post-column

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



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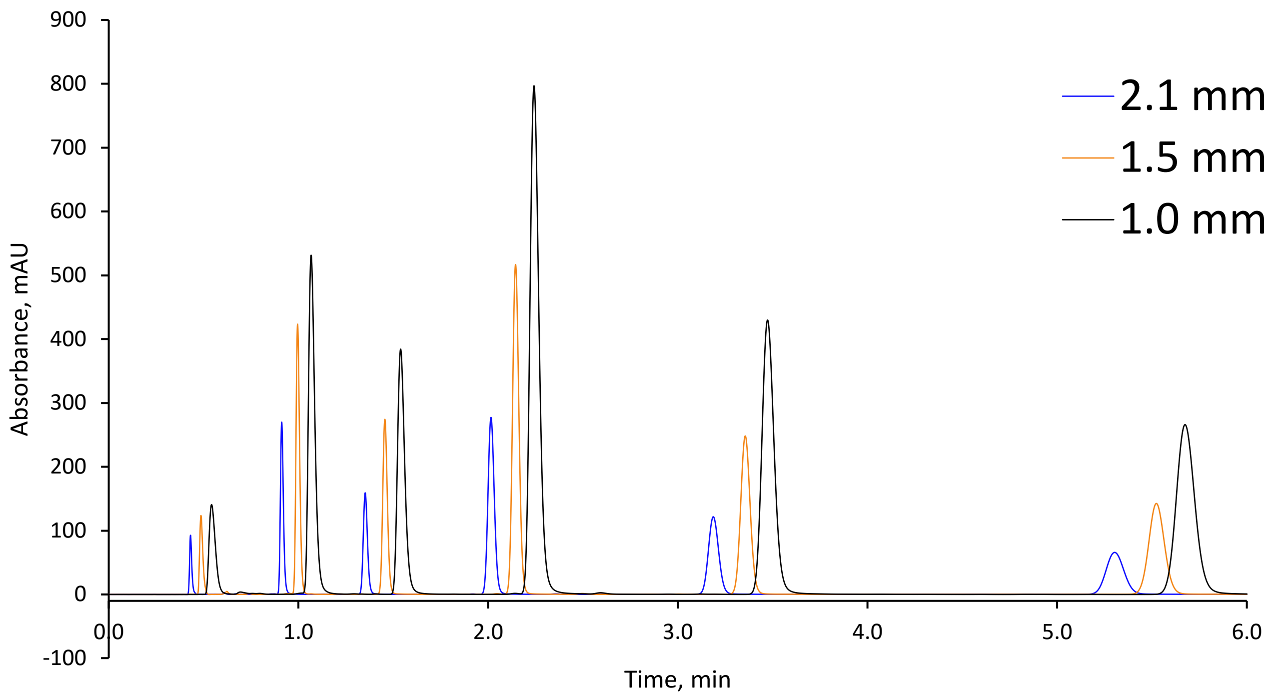
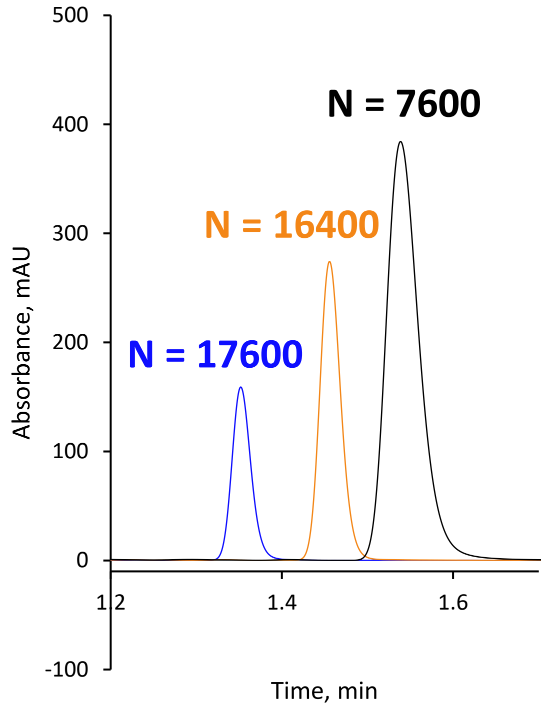
Pros & Cons in Shifting from 2.1 mm i.d. to 1.0 mm i.d.





In move from 2.1 mm i.d. to 1.0 mm i.d., signal increases and viscous friction decreases, but there is a significant loss in efficiency primarily due to extracolumn effects. 1.5 mm i.d. columns can provide a compromise between these effects.

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Summary

- Smaller ID columns are less subject to the effects of viscous heating

- Smaller ID 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

A NEW DIMENSION IN SEPARATIONS

What benefit does it offer?

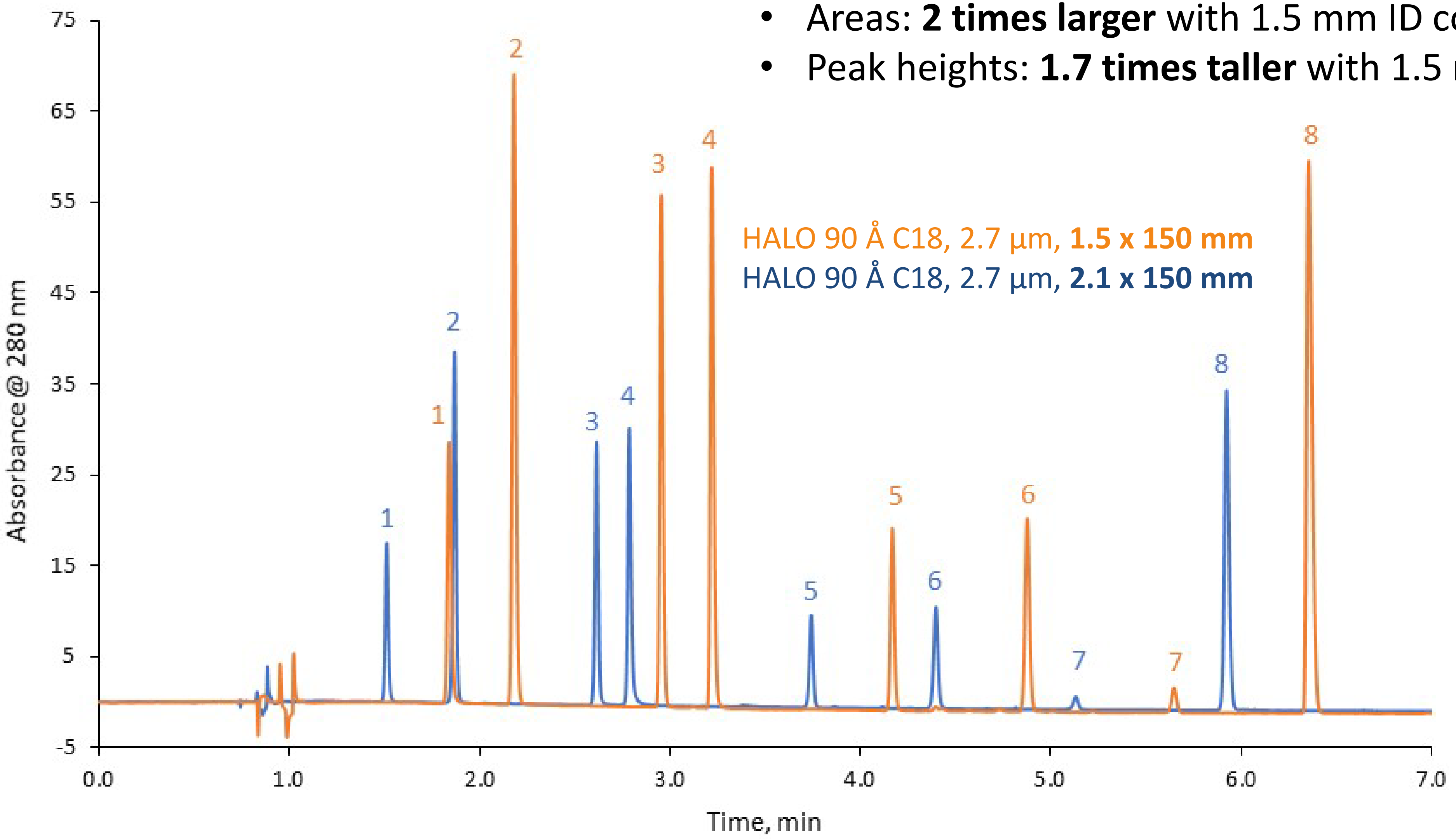
MORE PERFORMANCE FROM UHPLC AND LCMS SYSTEMS

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

More sensitivity from conventional UHPLC systems

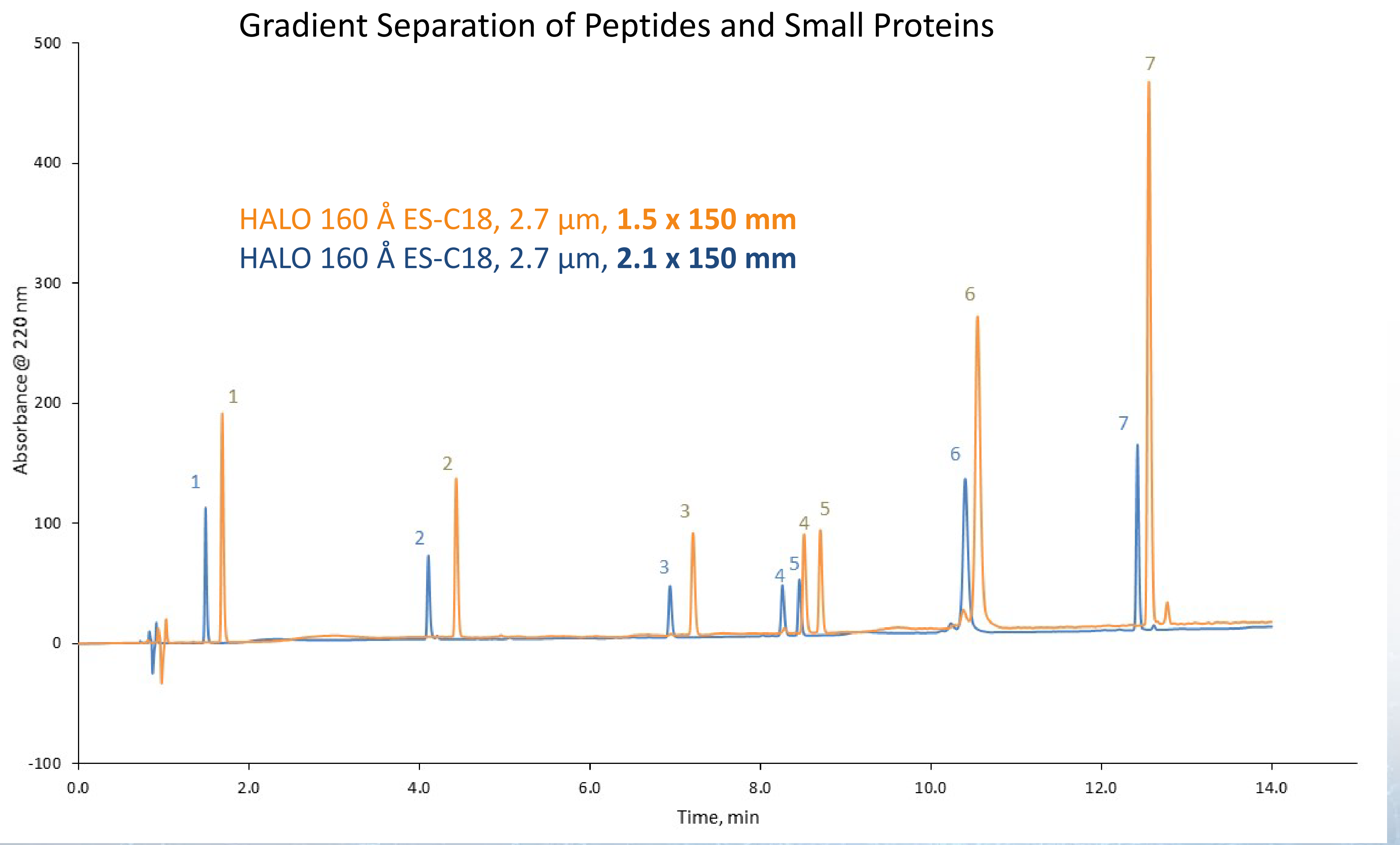
Comparison of Gradient Separation of OTC Cough and Cold Medicines

- Areas: **2 times larger** with 1.5 mm ID column
- Peak heights: **1.7 times taller** with 1.5 mm ID column



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

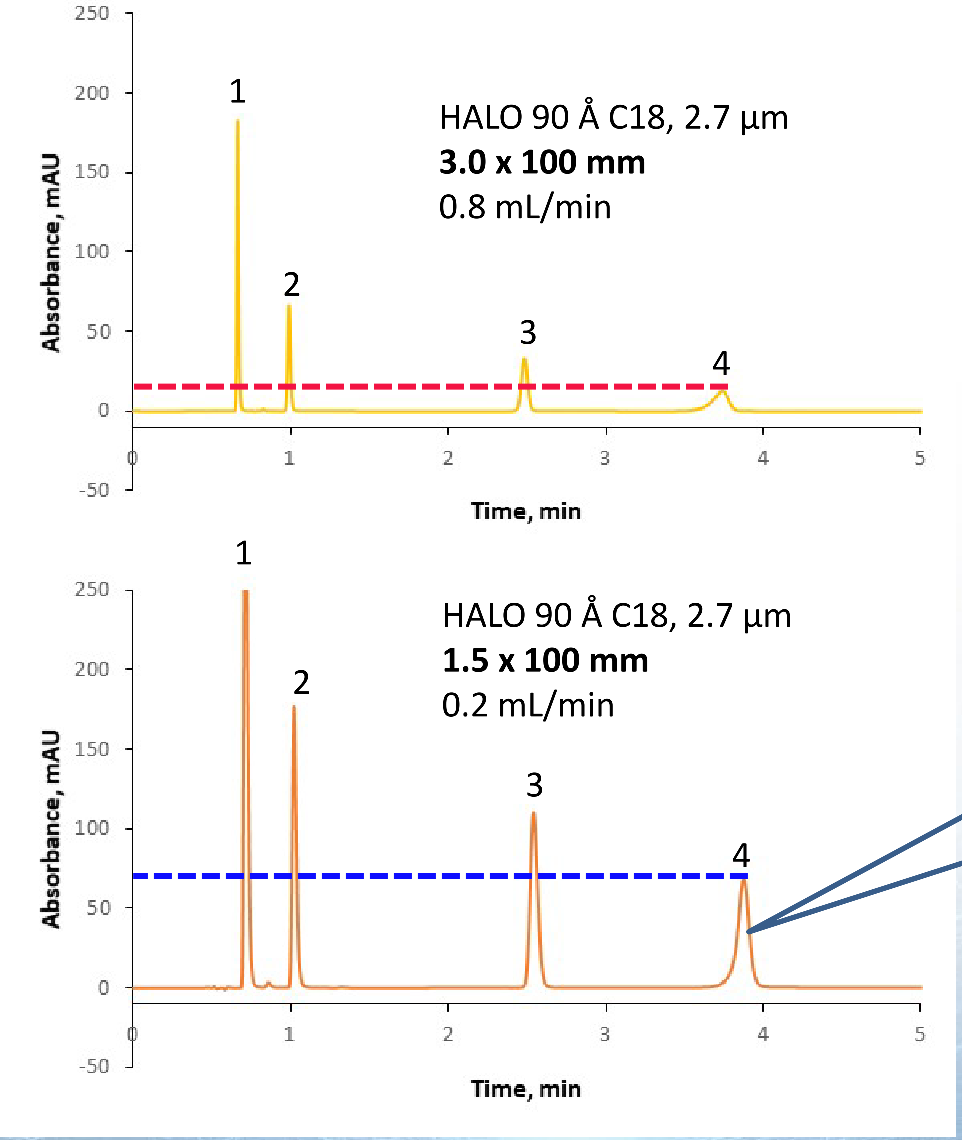
More sensitivity from conventional UHPLC systems



1. Gly-Tyr
2. Val-Tyr-Val
3. Methionine Enkephalin
4. Angiotensin II
5. Leucine Enkephalin
6. RNase A
7. Insulin

Enhanced Impurity Identification

Isocratic Separation Comparing 1.5 mm to 3.0 mm ID column

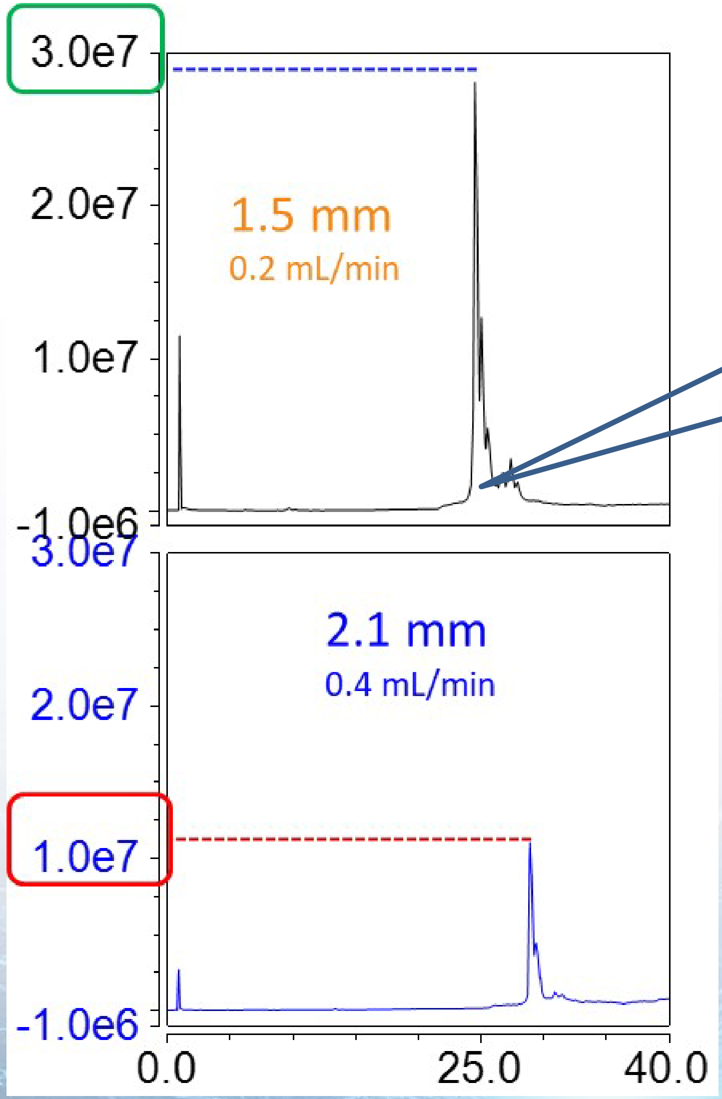


1. Acetaminophen
2. Caffeine
3. Aspirin
4. Salicylic Acid

Both area and peak height are increased!

Higher ionization efficiencies from LCMS systems - 1

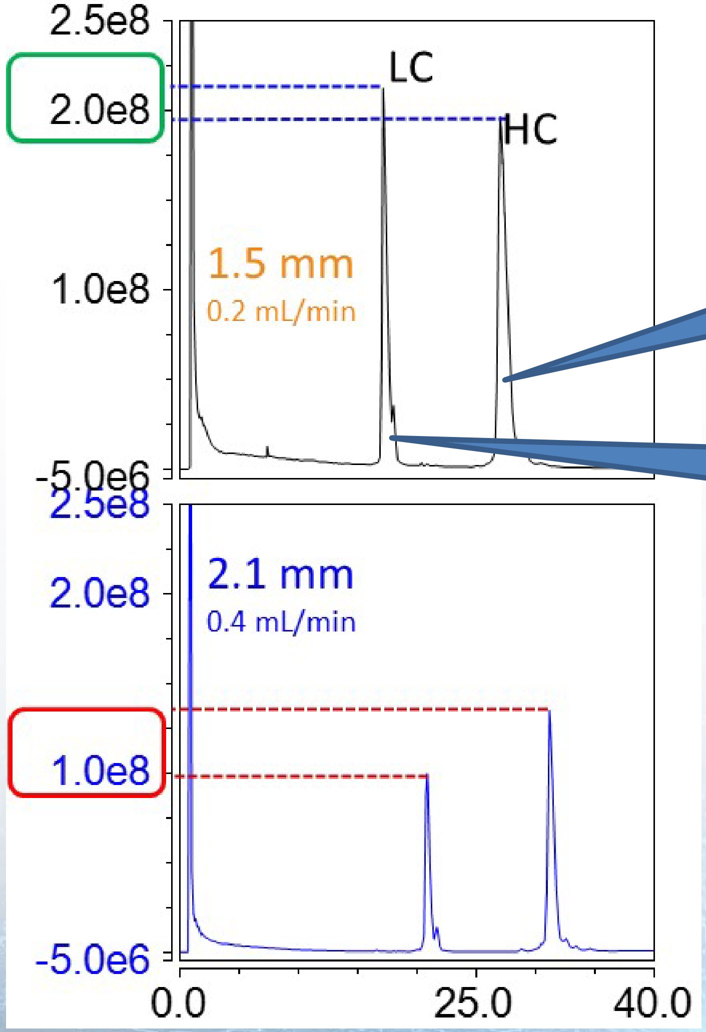
Intact Trastuzumab using HALO 1000 Å Diphenyl under Gradient Conditions



Area is 2.9x larger and peak height is increased

Higher ionization efficiencies from LCMS systems - 2

Reduced and Alkylated Trastuzumab
using HALO 1000 Å Diphenyl

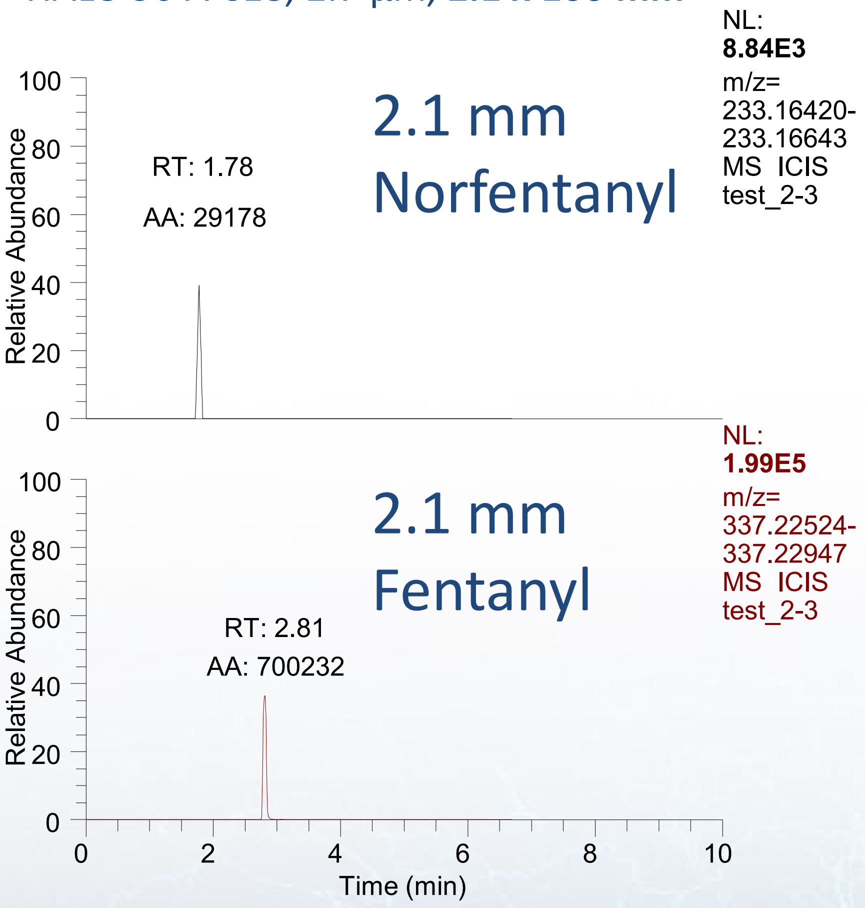


Heavy Chain (HC)
Area is 2.3x larger

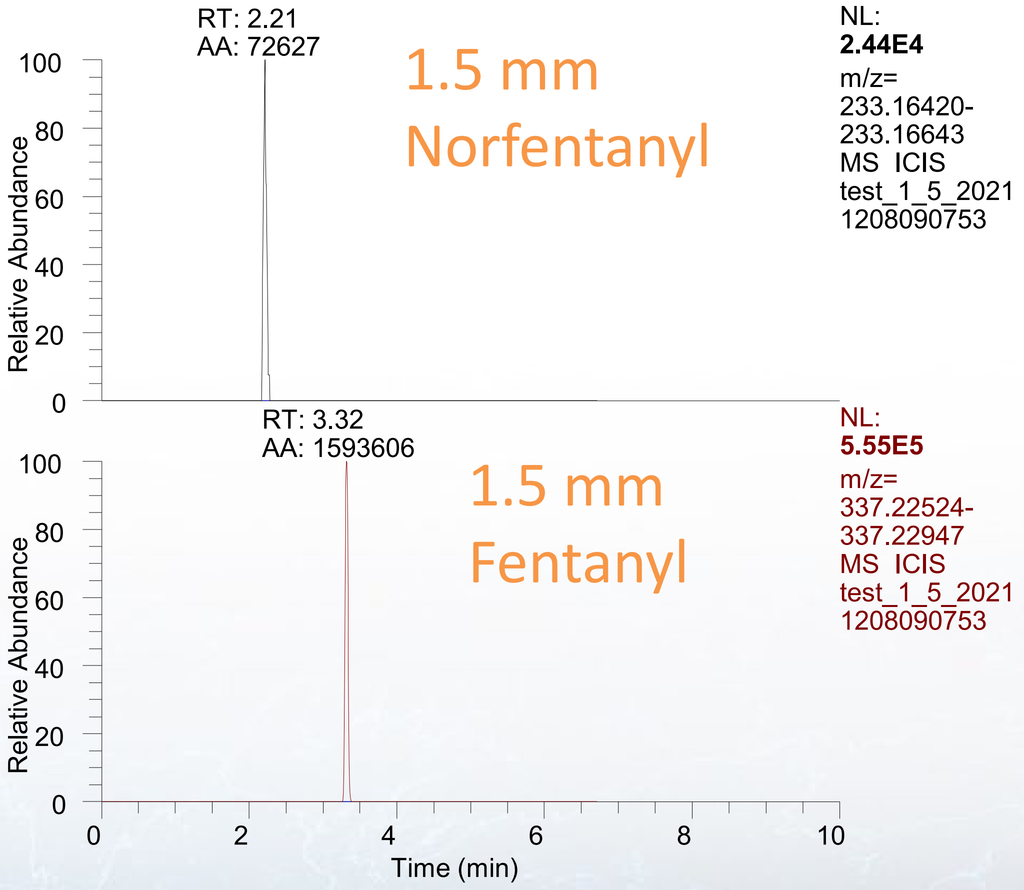
Light Chain (LC)
Area is 2.7x larger

Higher ionization efficiencies from LCMS systems - 3

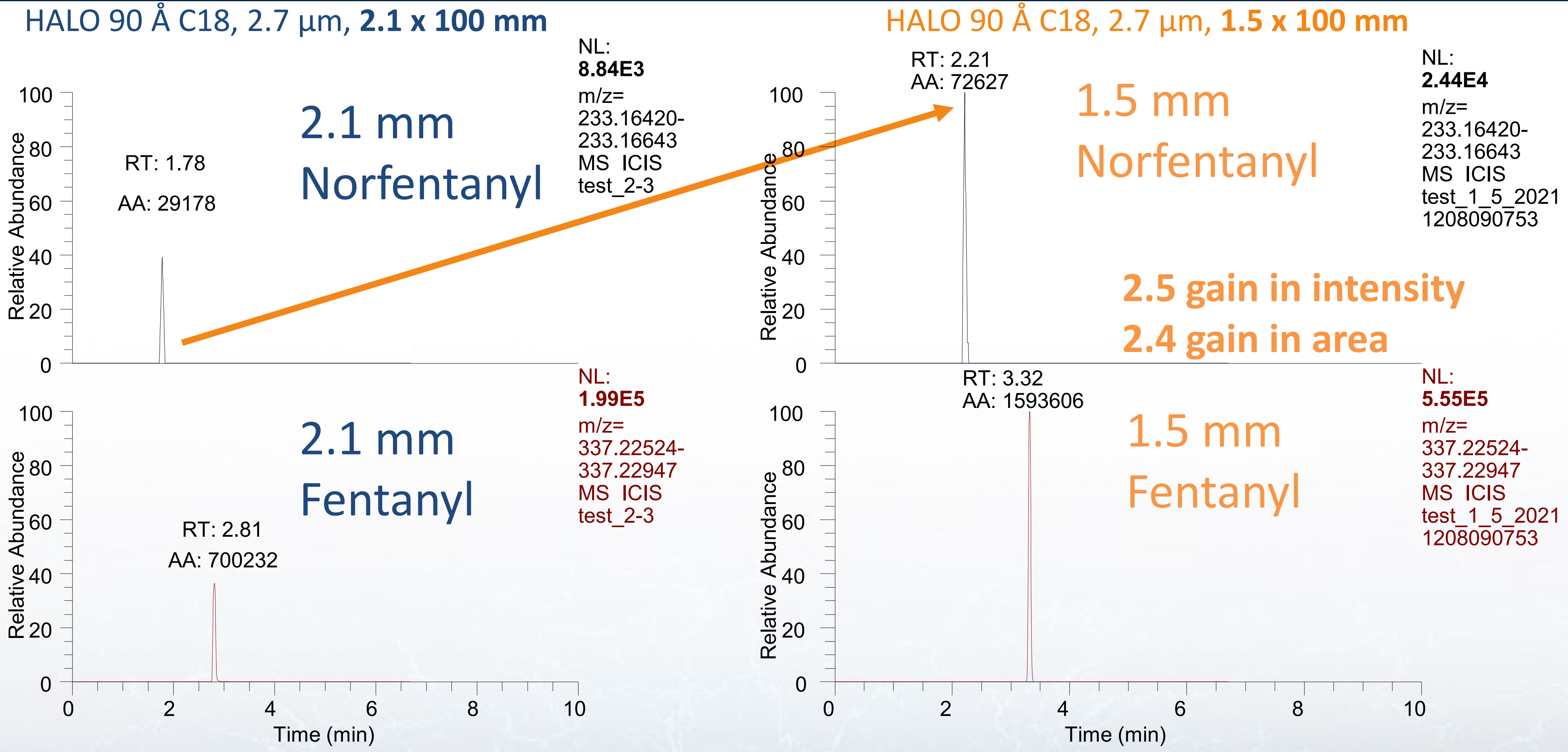
HALO 90 Å C18, 2.7 µm, **2.1 x 100 mm**



HALO 90 Å C18, 2.7 µm, **1.5 x 100 mm**



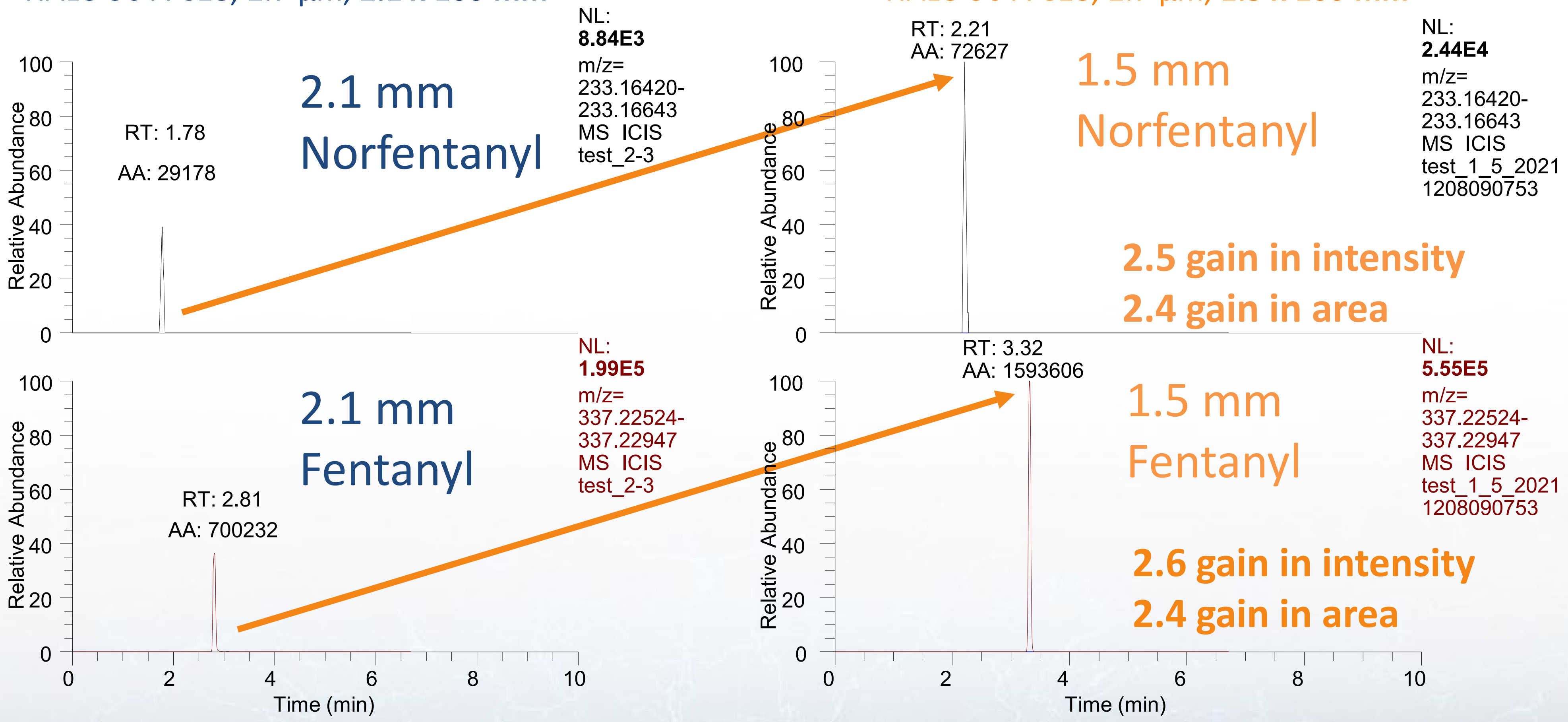
Higher ionization efficiencies from LCMS systems - 3



Higher ionization efficiencies from LCMS systems - 3

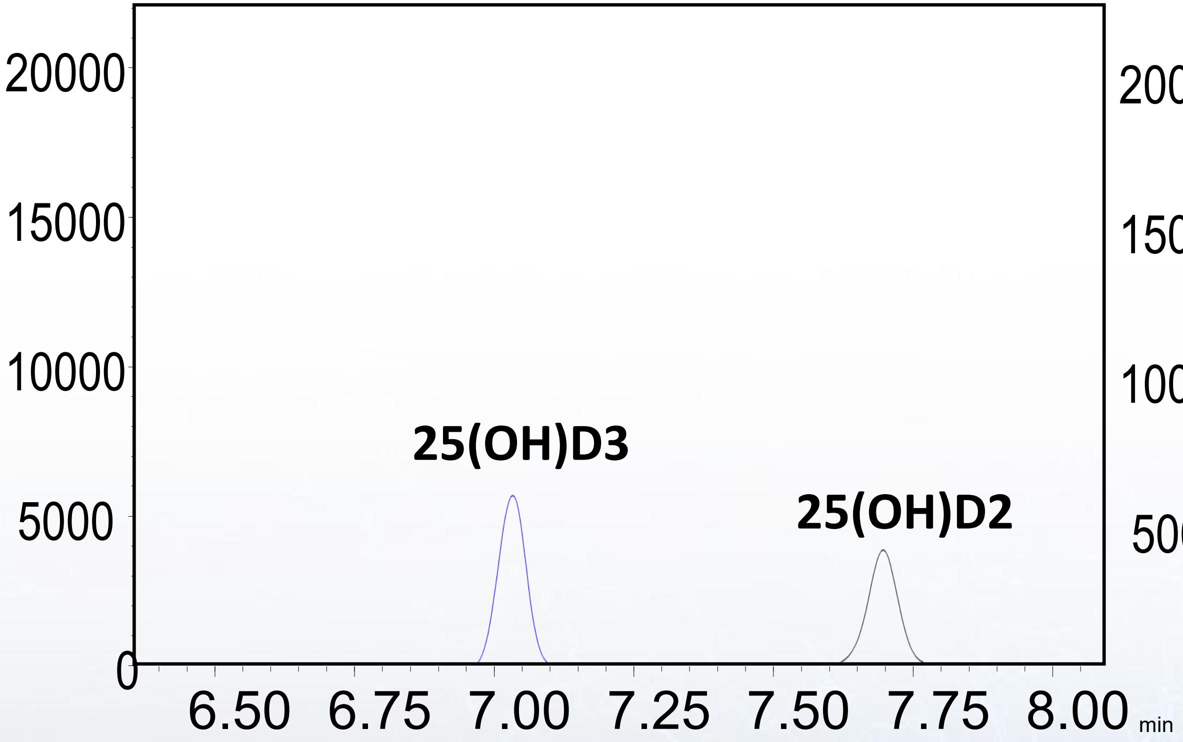
HALO 90 Å C18, 2.7 µm, **2.1 x 100 mm**

HALO 90 Å C18, 2.7 µm, **1.5 x 100 mm**

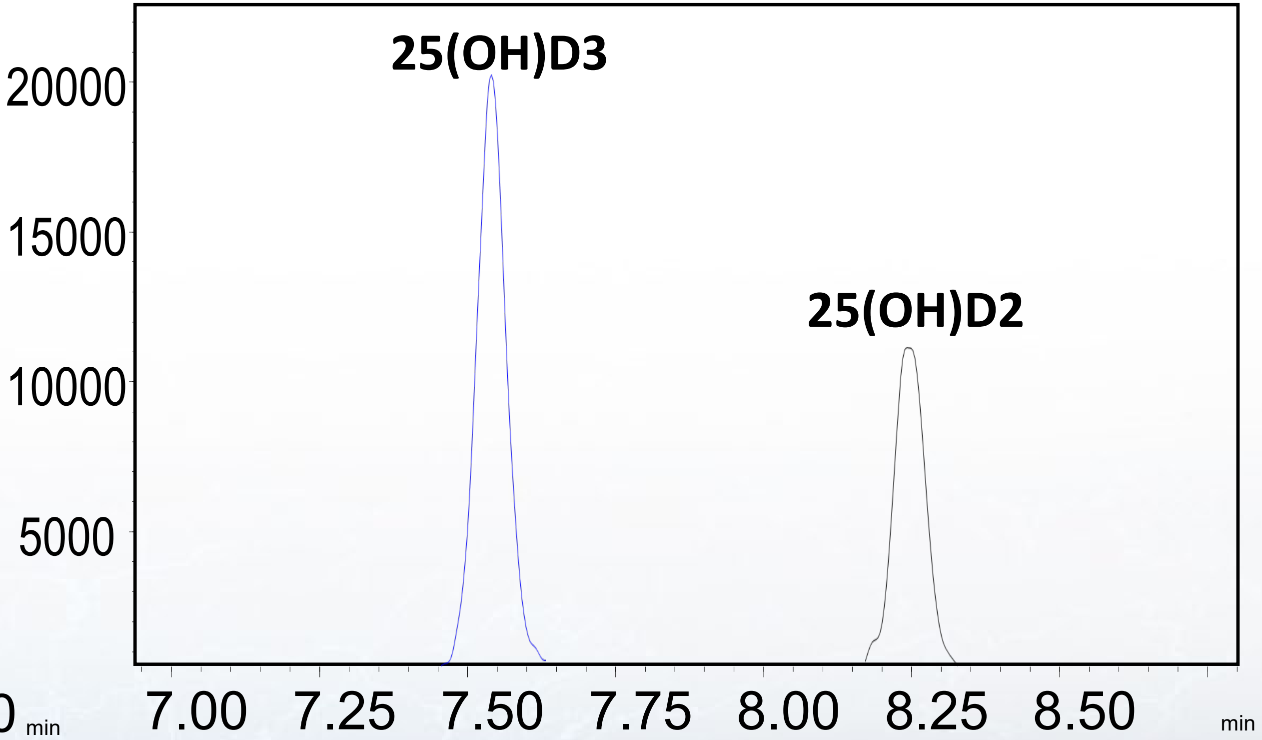


Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

HALO 90 Å C18, 2.7 µm, **2.1 x 100 mm**



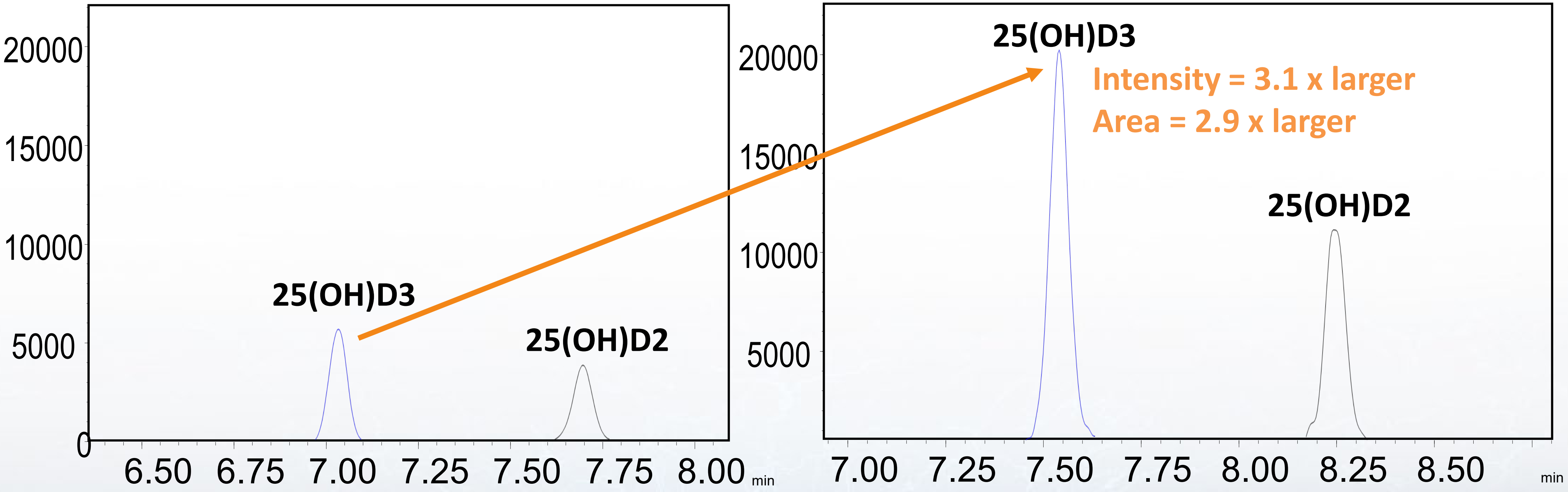
HALO 90 Å C18, 2.7 µm, **1.5 x 100 mm**



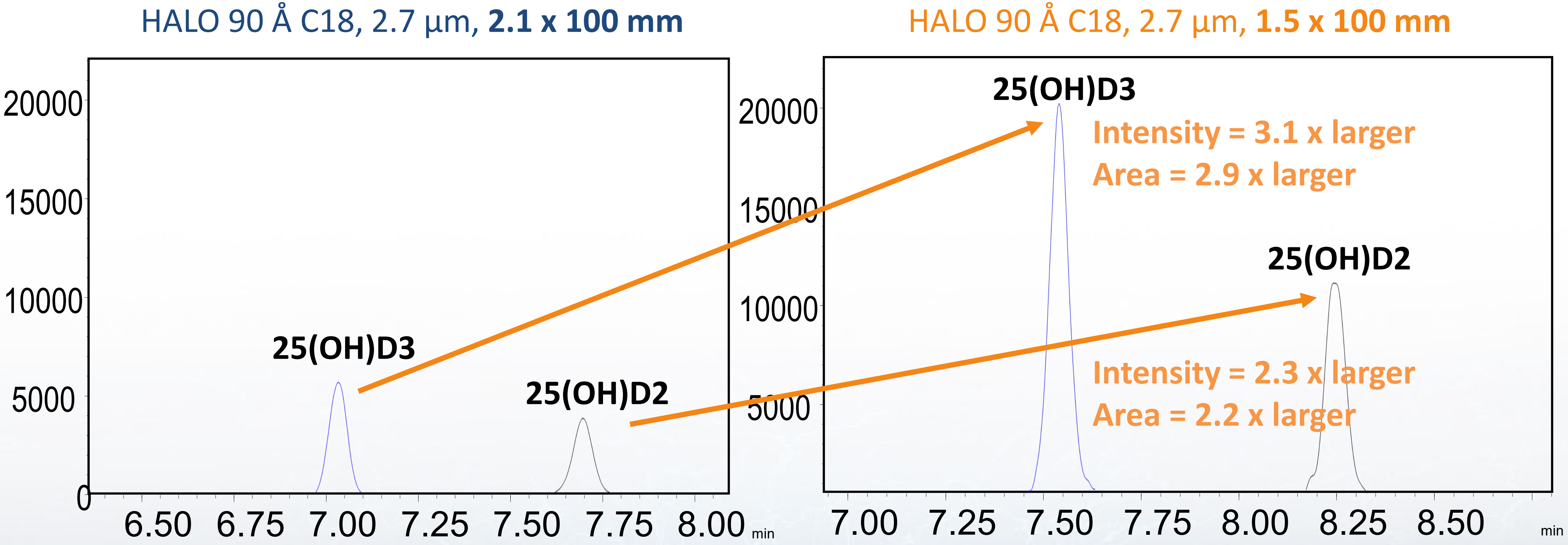
Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

HALO 90 Å C18, 2.7 µm, **2.1 x 100 mm**

HALO 90 Å C18, 2.7 µm, **1.5 x 100 mm**

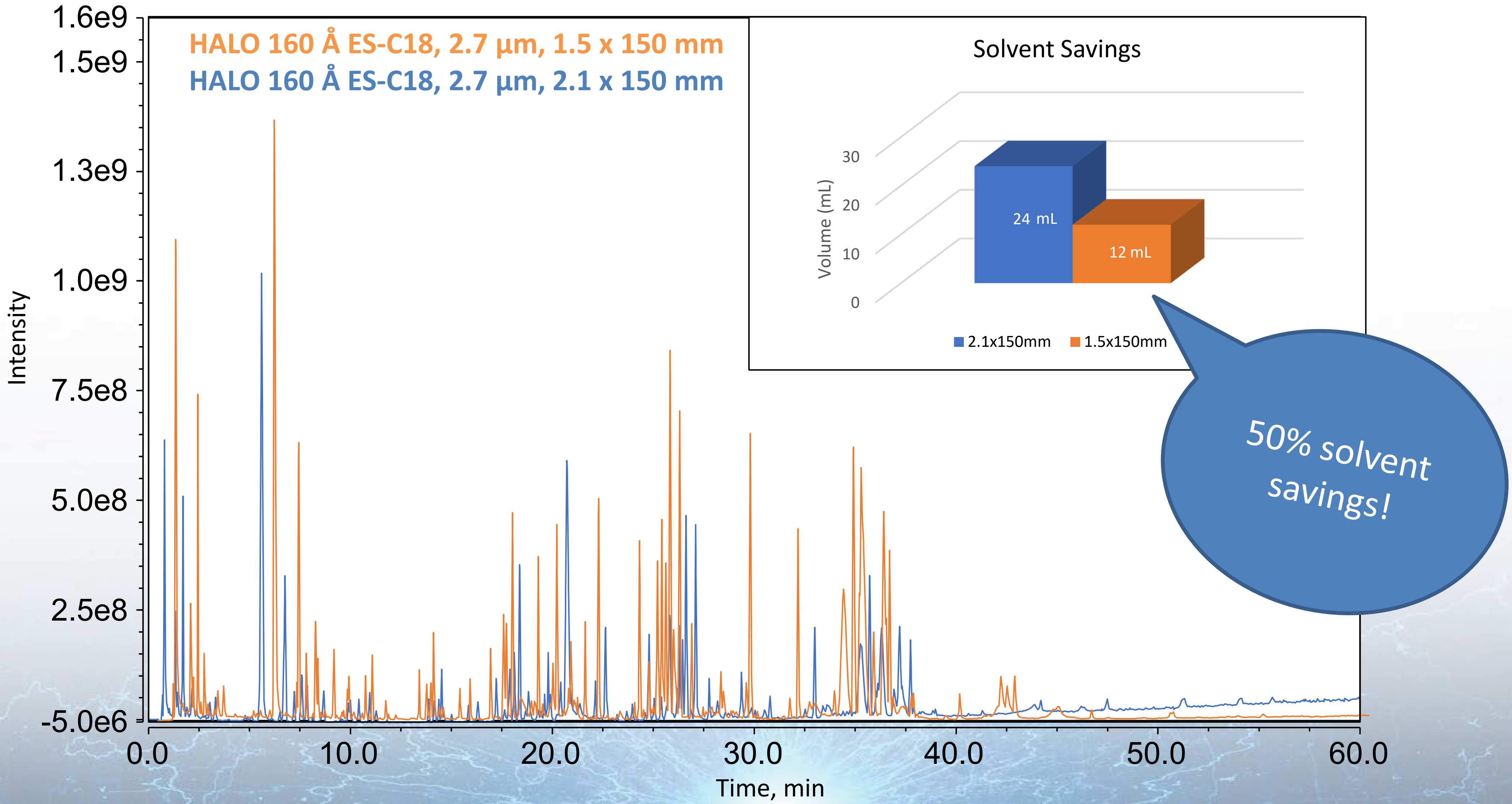


Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

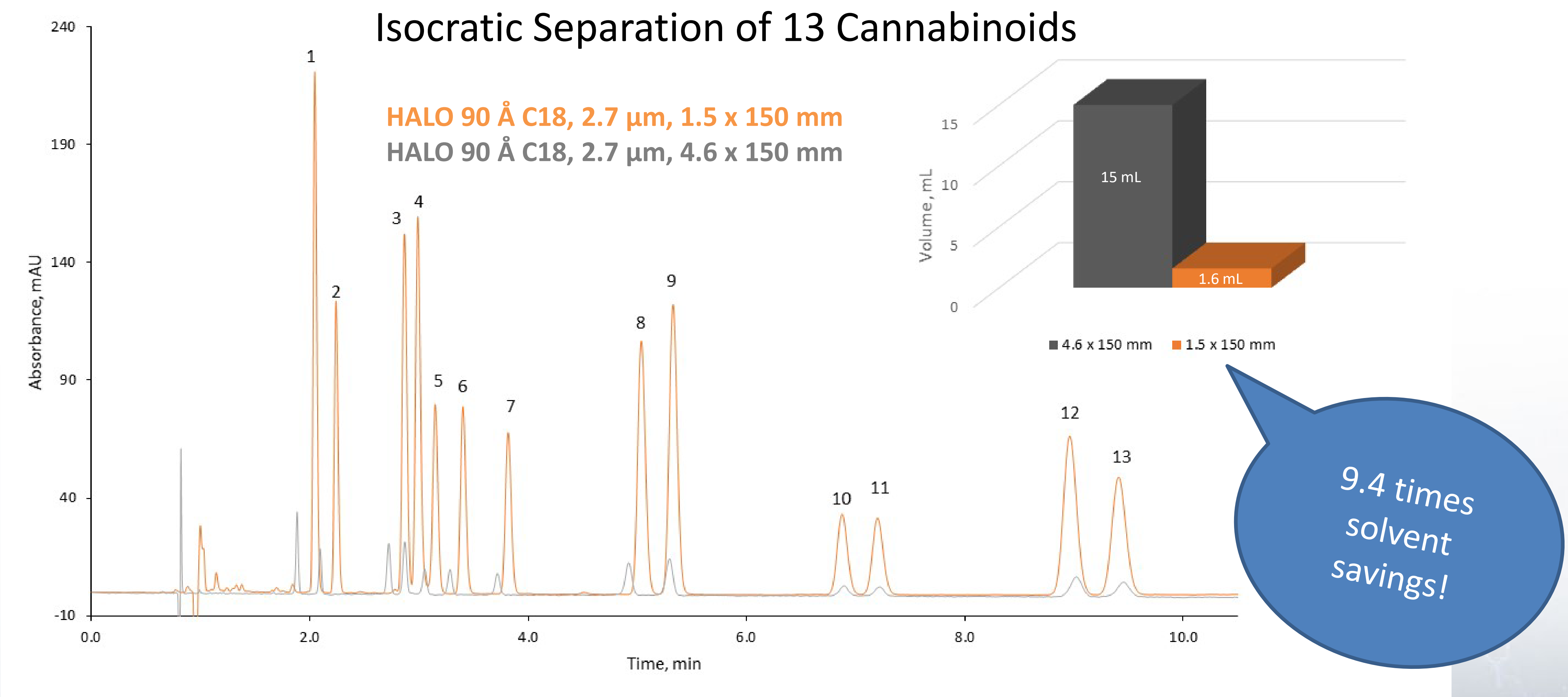


Reduced solvent consumption compared to 2.1 mm id columns

Peptide Map of Trastuzumab under Gradient Conditions



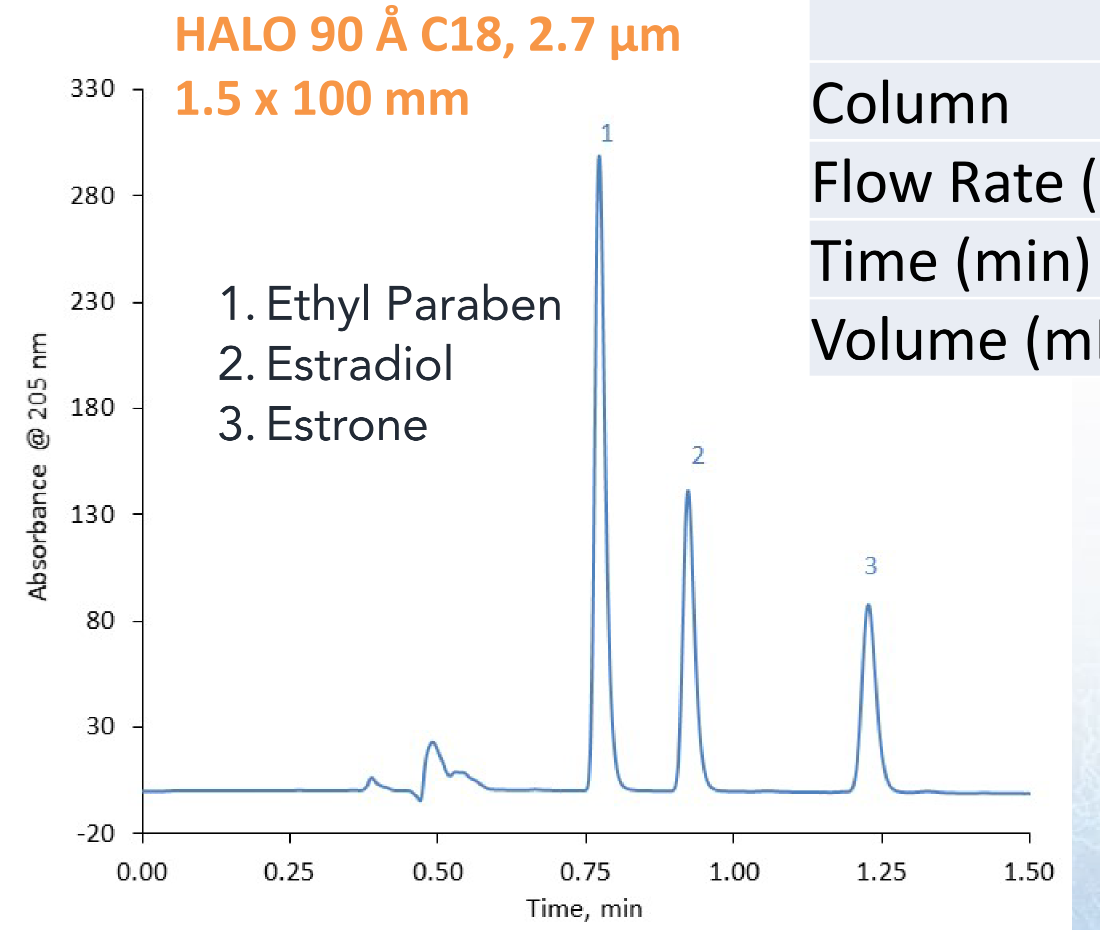
Reduced solvent consumption compared to 4.6 mm id columns



Peak identities (in order) are CBDVA, CBDV, CBDA, CBGA, CBG, CBD, THCV, THCVA, CBN, Δ9-THC, Δ8-THC, CBC, and THCA.

Reduced solvent consumption when modernizing methods

USP Method for Estradiol

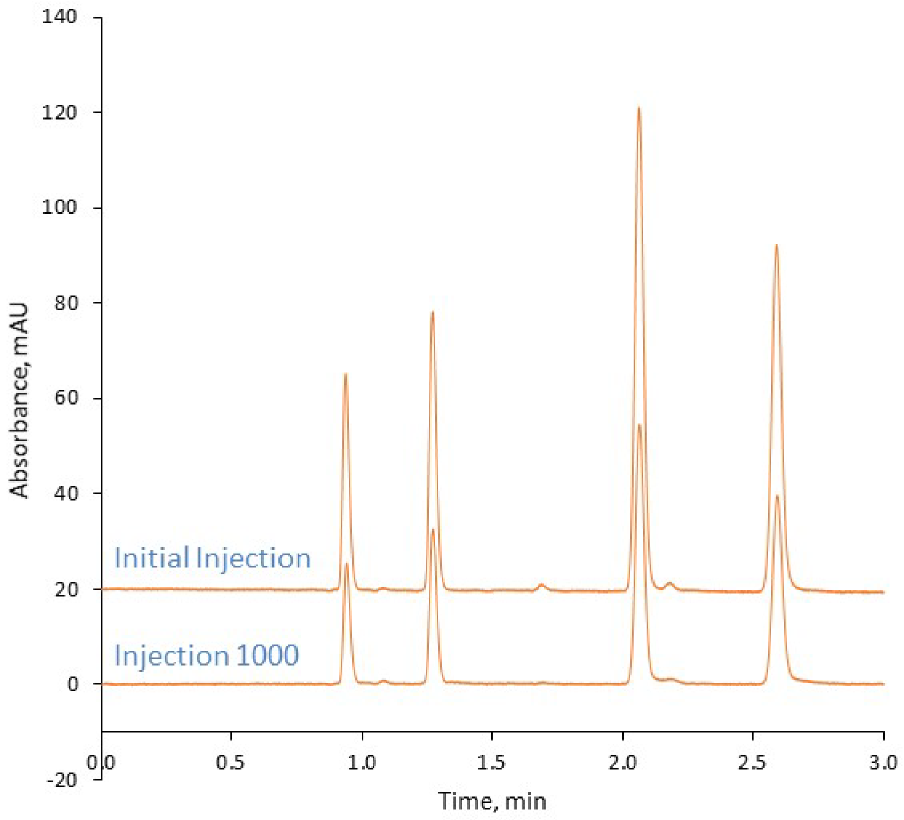


	Original Method	Modified Method
Column	3.9 x 300 mm	1.5 x 100 mm
Flow Rate (mL/min)	1	0.2
Time (min)	8	1.5
Volume (mL)	8	0.3

96% solvent savings!

1.5 mm ID Stability – Example 1

- HALO 1000 Å Diphenyl in 1.5 mm ID column hardware was tested at 600 bar for 1000 injections.
- No loss in efficiency or retention was observed over the course of the experiment.



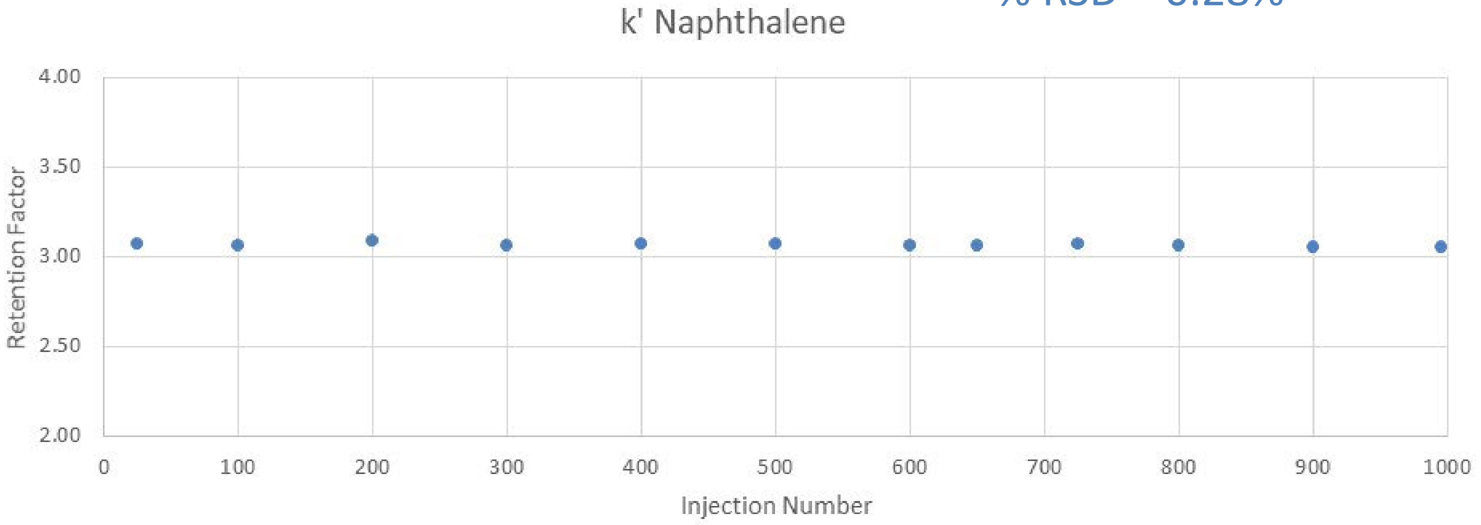
TEST CONDITIONS:

Column: HALO 1000 Å Diphenyl, 2.7 µm, 1.5 x 150 mm
Mobile Phase A: Water
B: Acetonitrile
Isocratic: 25 %B
Flow Rate: 0.4 mL/min
Back Pressure: 600 bar
Temperature: 30 °C
Detection: 254 nm, PDA
Injection Volume: 0.2 µL
Sample Solvent: 60/40 ACN/ Water
Data Rate: 200 Hz
Response Time: 0.005 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

1.5 mm ID Stability – Example 2

- A HALO 90 Å C18 1.5 mm ID column was run for 1000 injections
- Retention factor of naphthalene was stable across all of the injections

% RSD = 0.28%



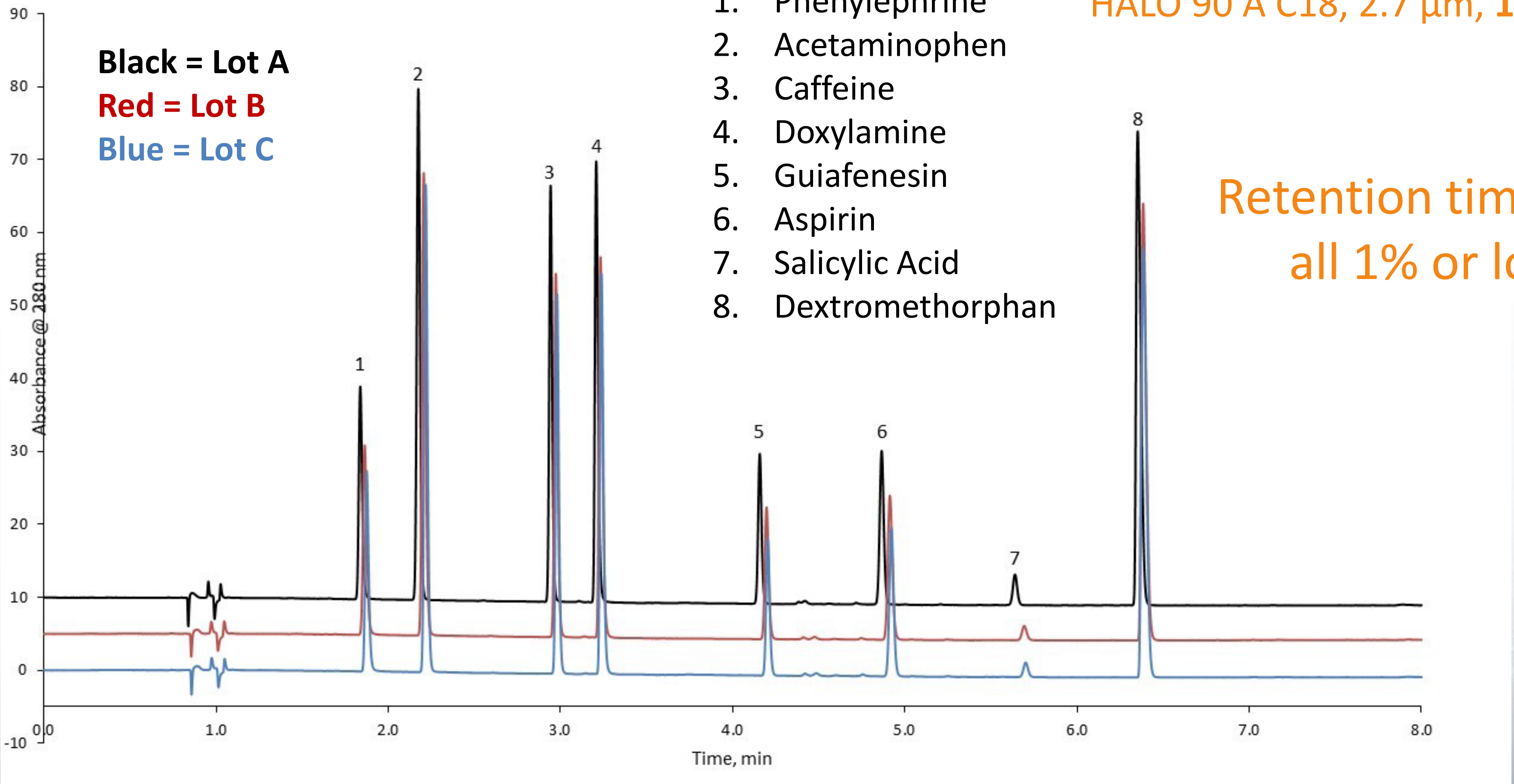
TEST CONDITIONS:

Column: HALO 90 Å C18, 2.7 µm, 1.5 x 150 mm
Mobile Phase A: Water
B: Acetonitrile
Isocratic: 60 %B
Flow Rate: 0.6 mL/min
Back Pressure: ~600 bar
Temperature: 30 °C
Detection: 254 nm, PDA

Injection Volume: 0.2 µL
Sample Solvent: 60/40 ACN/ Water
Data Rate: 200 Hz
Response Time: 0.005 sec.
Flow Cell: 1 µL
LC System: Shimadzu Nexera X2

HALO® 1.5 mm ID Column Hardware Reproducibility

Excellent reproducibility from 3 different lots of column hardware



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

HALO 90 Å C18, 2.7 µm, **1.5 x 150 mm**

Retention time %RSDs all 1% or lower!

Easy to implement microflow solution

Column looks and feels like a 2.1 mm...

Successful implementation requires system optimization via:

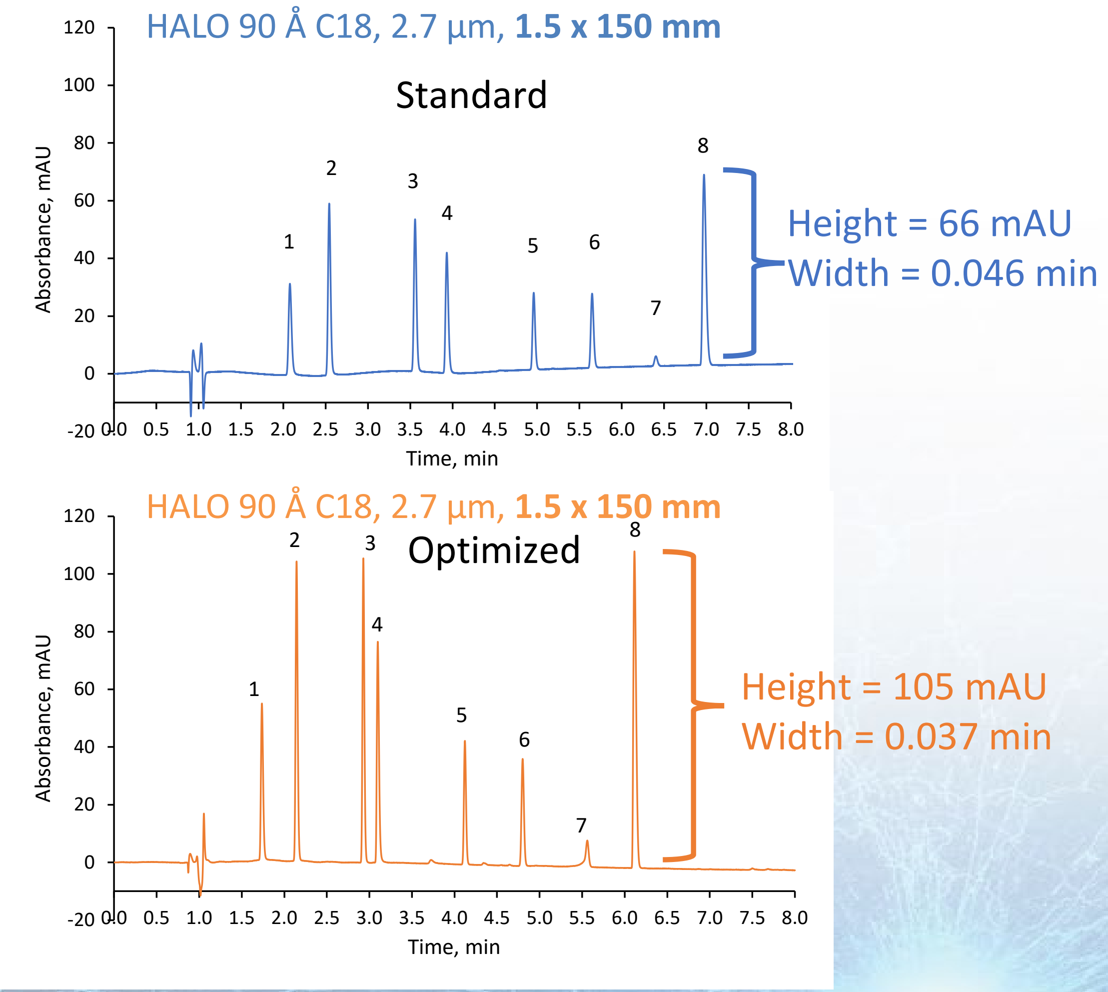
- Hardware
- Accessories

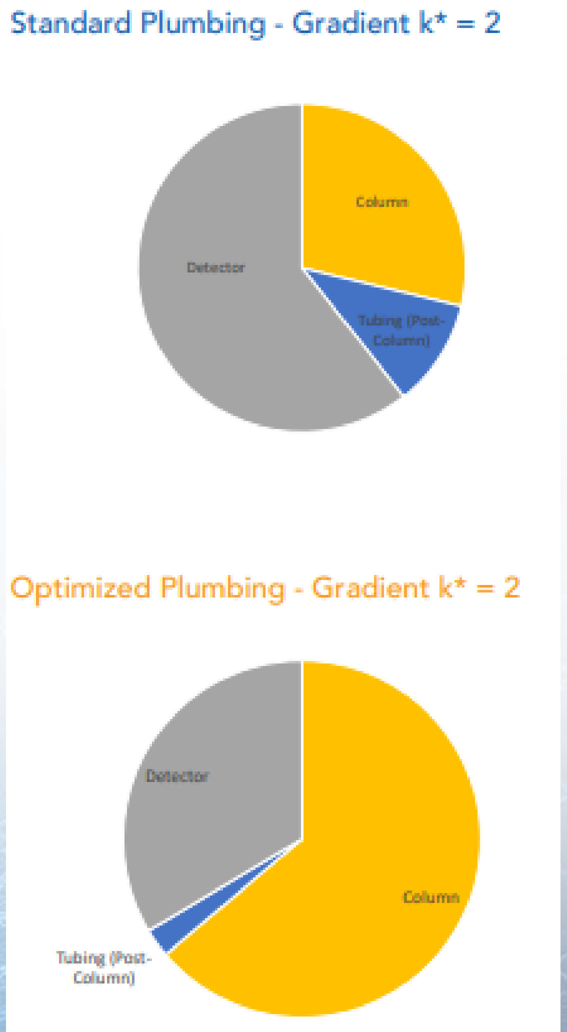
Success using HALO® 1.5 mm ID

- System
- Connectors
- Method Transfer to 1.5 mm

Variance Charts – Gradient

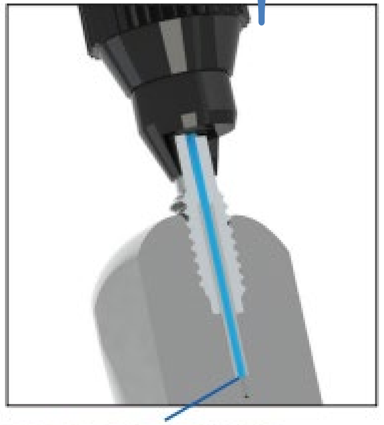
Where Has My Efficiency Gone? Impacts of Extracolumn Peak Broadening on Performance
4 part series in LCGC North America from Dwight R. Stoll, Thomas Lauer, & Ken Broeckhoven
http://www.multidlc.org/dispersion_calculator



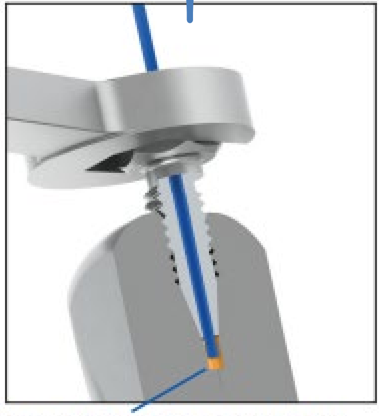


AMT MarvelXACT™ Connectors

- 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

MarvelXACT and MarvelX are registered trademarks of IDEX.

How to transfer a method to a 1.5 mm ID column?

- Scale flow rate

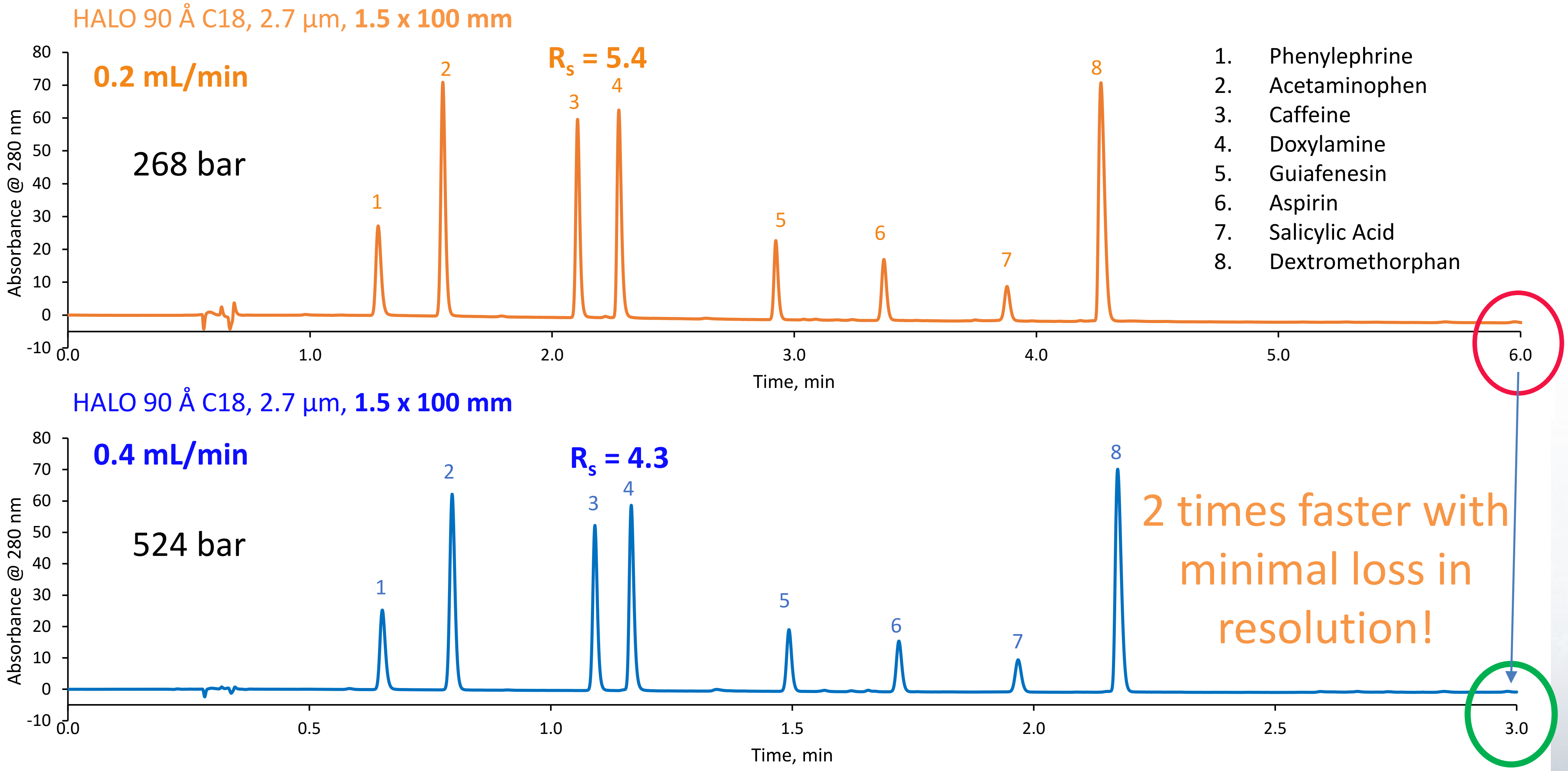
$$F_2 = F_1 \times \frac{(\pi R_2)^2}{(\pi R_1)^2} = F_1 \times \frac{(R_2)^2}{(R_1)^2} = F_1 \times \frac{(D_2)^2}{(D_1)^2}$$

F_2 = scaled flow rate
F_1 = original flow rate
D_2 = column ID being transferred to
D_1 = original column ID

	COLUMN IDS				
	4.6	3.0	2.1	1.5	1.0
FLOW RATES (mL/min)	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

Benefits of Fused-Core® in a 1.5 mm



HALO® 1.5 mm ID Columns

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

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

Summary

- ✓ More sensitivity from conventional UHPLC systems
- ✓ Higher ionization efficiencies from LCMS systems
- ✓ Reduced solvent consumption compared to 2.1 mm id columns
- ✓ Easy to implement microflow solution with existing systems
- ✓ Made by a trusted manufacturer of Fused-Core® columns in a 9001 ISO-certified facility
- ✓ Available in chemistries for small molecule, peptide, and protein separations

Q&A

technical@mac-mod.com

Photo by Camylla Battani on Unsplash