NEW 1.5 MM UHPLC COLUMNS ENABLE ROBUST SEPARATIONS WITH INCREASED SENSITIVITY AND SOLVENT SAVINGS

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Advanced Materials Technology, Inc.

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Outline

- Desire to move to smaller I.D. columns
- Benefits of smaller I.D.s with novel 1.5 mm I.D. UHPLC Columns
- Summary

The Move to Smaller I.D. Columns

- HPLC columns were originally 4.6 mm I.D. operated at 1 mL/min+
- 3.0 mm I.D. 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 I.D. introduced for use with UHPLC and for interfacing to mass spectrometers

Impact of Smaller I.D. Columns

- Signal intensity is increased when same sample concentration used

- Impact of Extra column dispersion must be considered

- Less solvent consumed = reduced consumable & waste disposal costs

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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

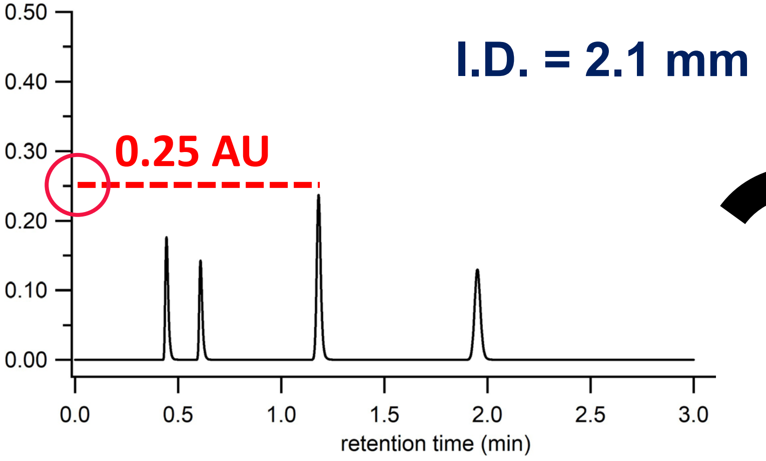
Example: 4.6 mm to 1.0 mm I.D. column

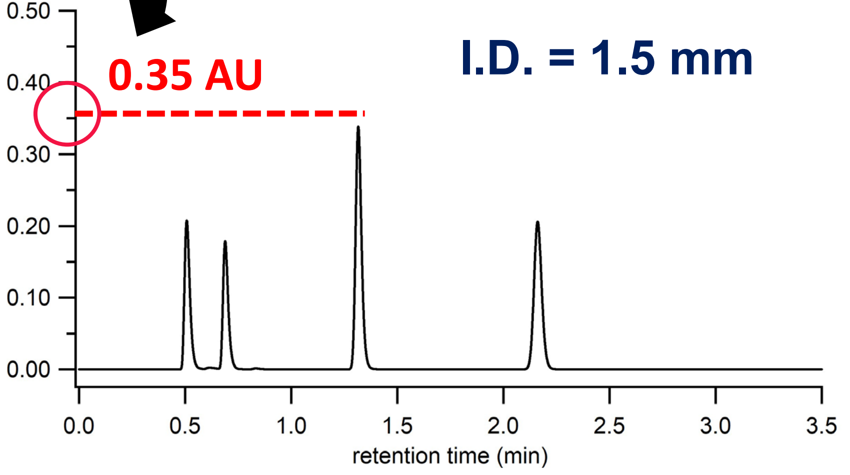
1 mL/min to around 50 µL/min

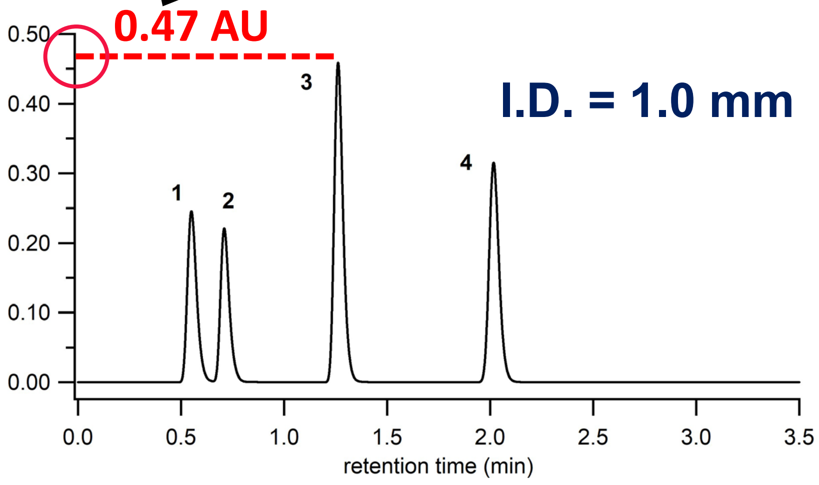
(1000µL/min)/(50µL/min) = 20-fold enhancement

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.

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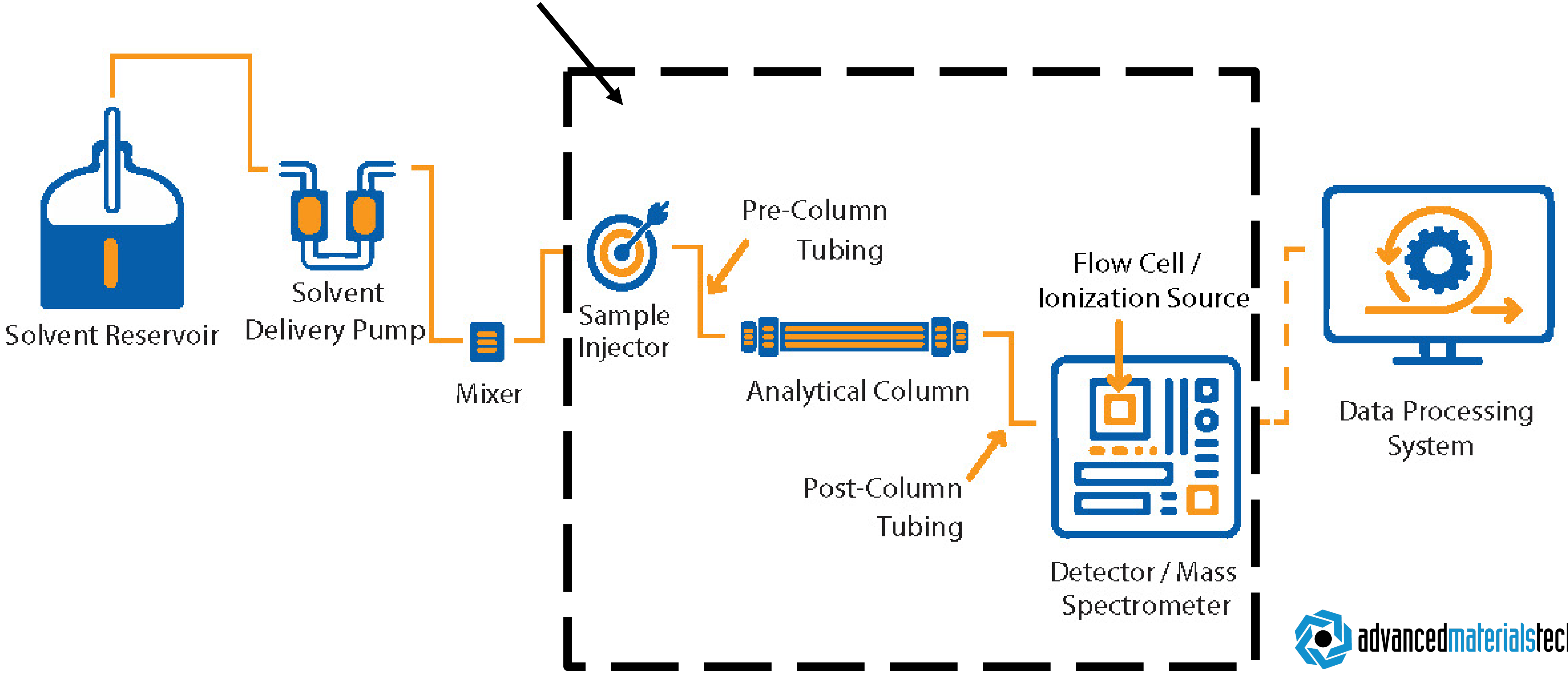
Total Band Broadening/Dispersion

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



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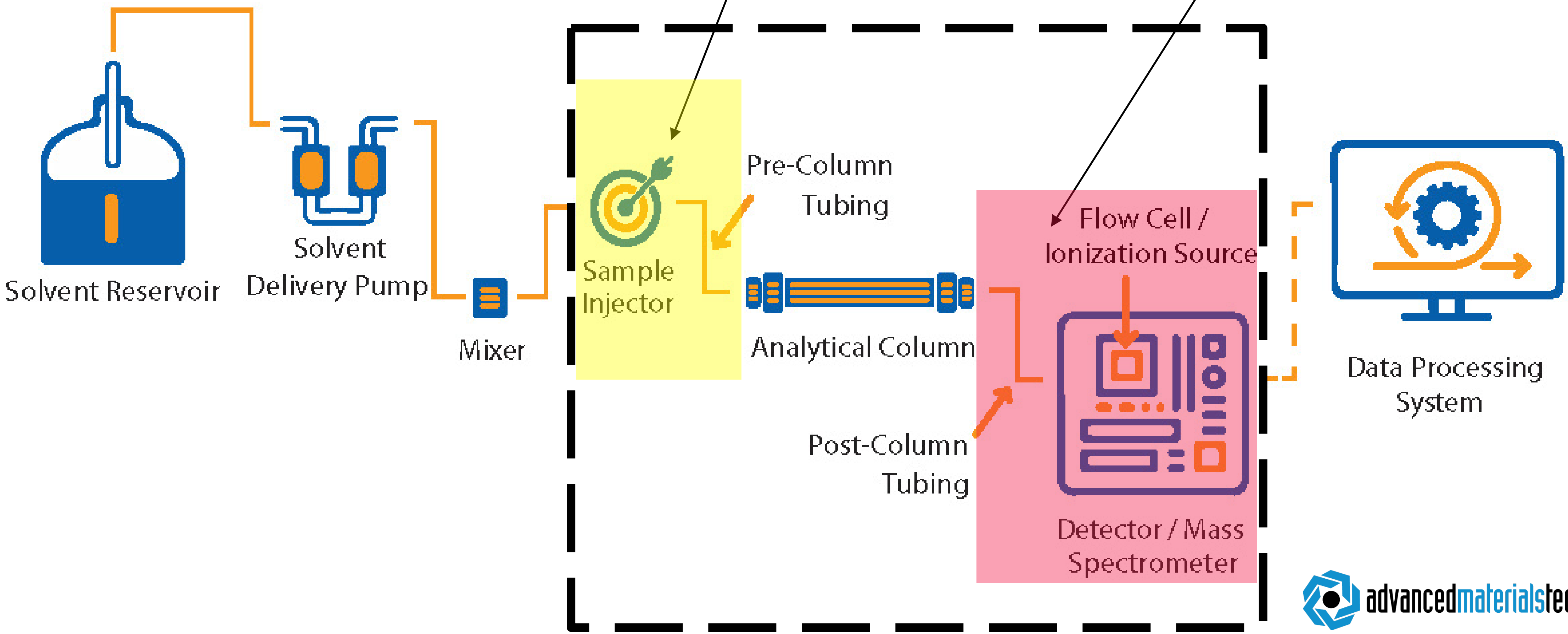
Sources of Extra-Column Band Broadening

Yellow = pre-column
Red = post-column

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



Relationship Between Extracolumn Dispersion and Extracolumn Volume

- $ECD = \left(\frac{ECV}{4}\right)^2$
- 2 μL^2 of ECD = 5.66 µL of ECV
- **ECD is measured in μL^2 while ECV is measured in µL**
- **Much easier to visualize ECV, but more useful to know ECD since this value is an additive portion of the total dispersion in the system, which includes the column:**

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

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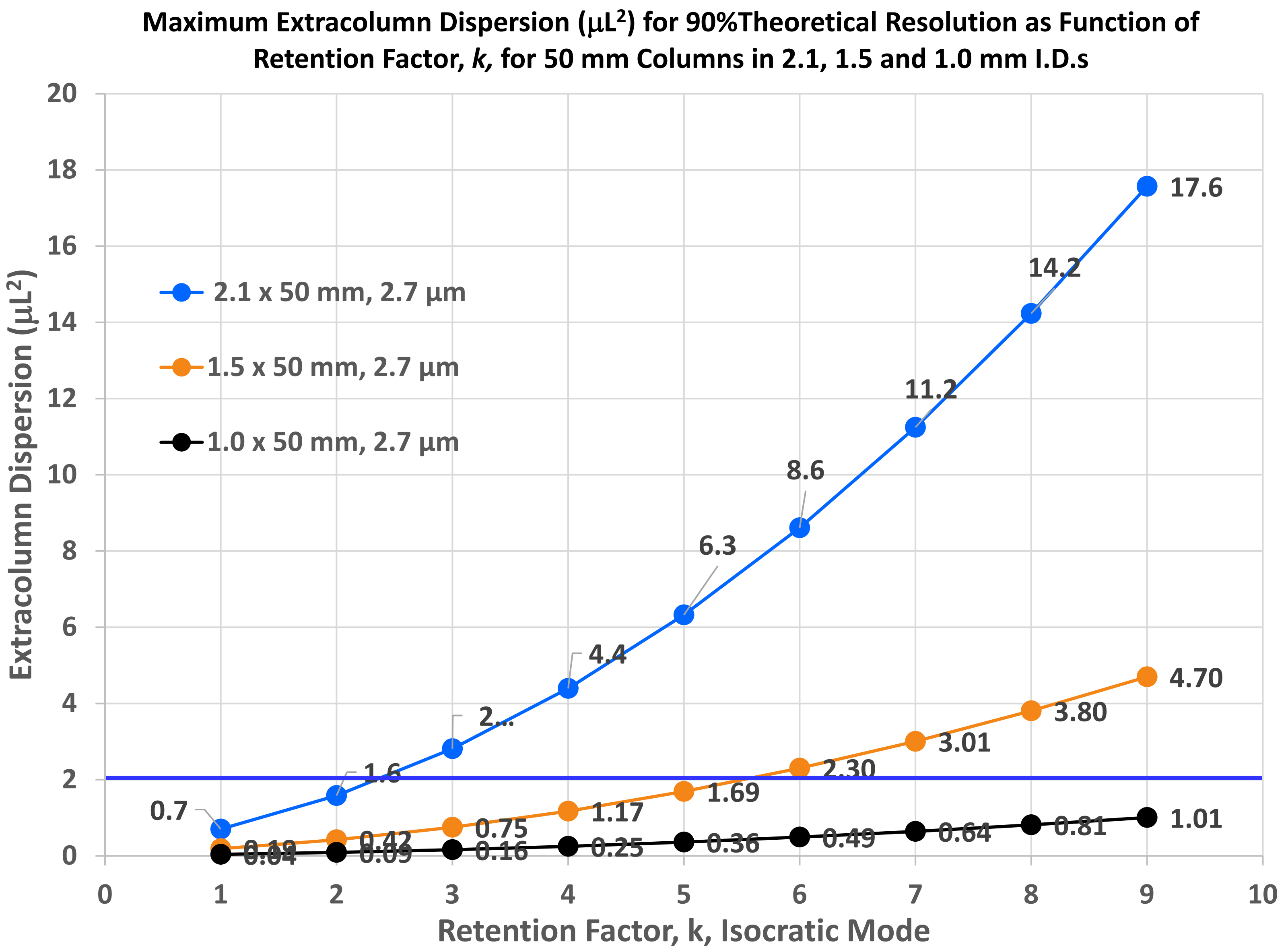
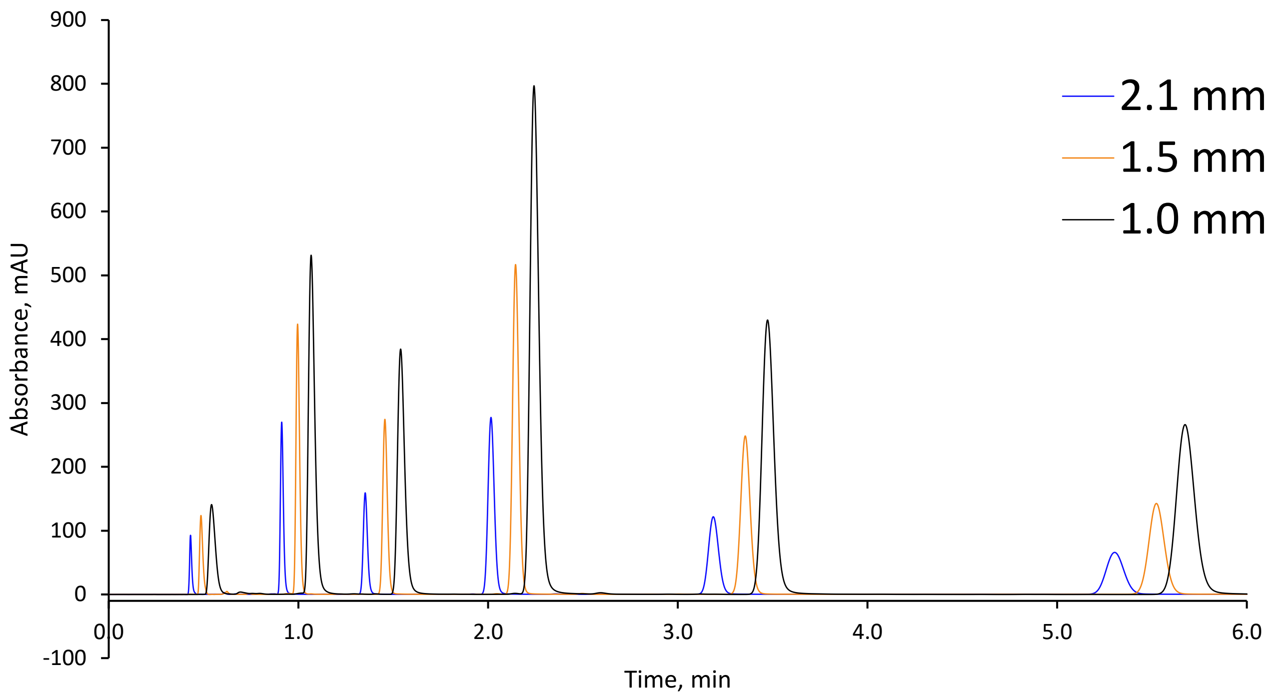
Q: How much extracolumn dispersion (ECD) is too much?

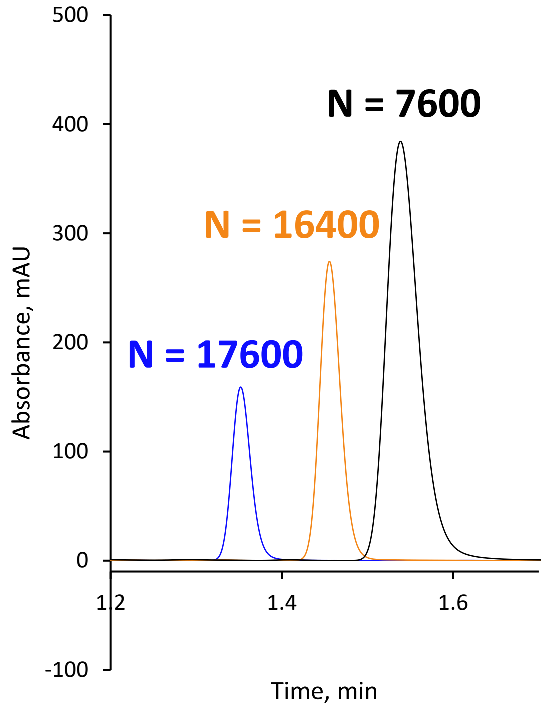


A: For isocratic separations, it depends on the column I.D. and retention factor

- As the column I.D. is reduced, the impact of ECD increases
- As k increases, the maximum amount of dispersion for 90% resolution increases
- For optimized Nexera in our lab, we estimated the dispersion to be 2 μL^2 so need k = 6 for 90% of resolution

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, 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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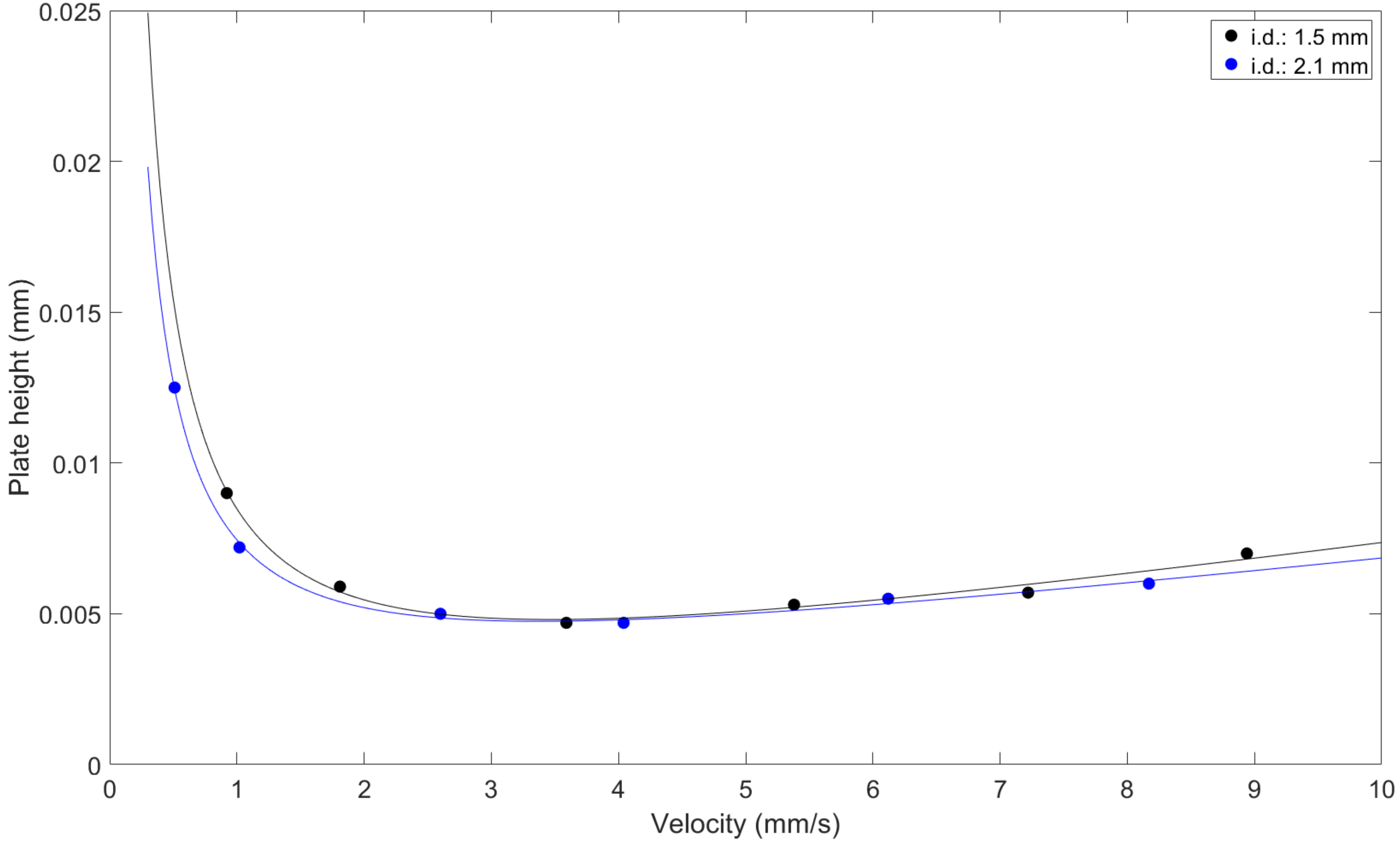
van Deemter Comparison: 1.5 mm to 2.1 mm



Column ID: as indicated
Column Length: 100 mm
Isocratic: 50/50 water/acetonitrile
Temperature: 35 °C
Injection Volume: 0.5 µL
Sample: naphthalene
Detection: UV 254 nm, PDA

Summary

- Smaller 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

A NEW DIMENSION IN SEPARATIONS

What benefits does 1.5 mm offer?

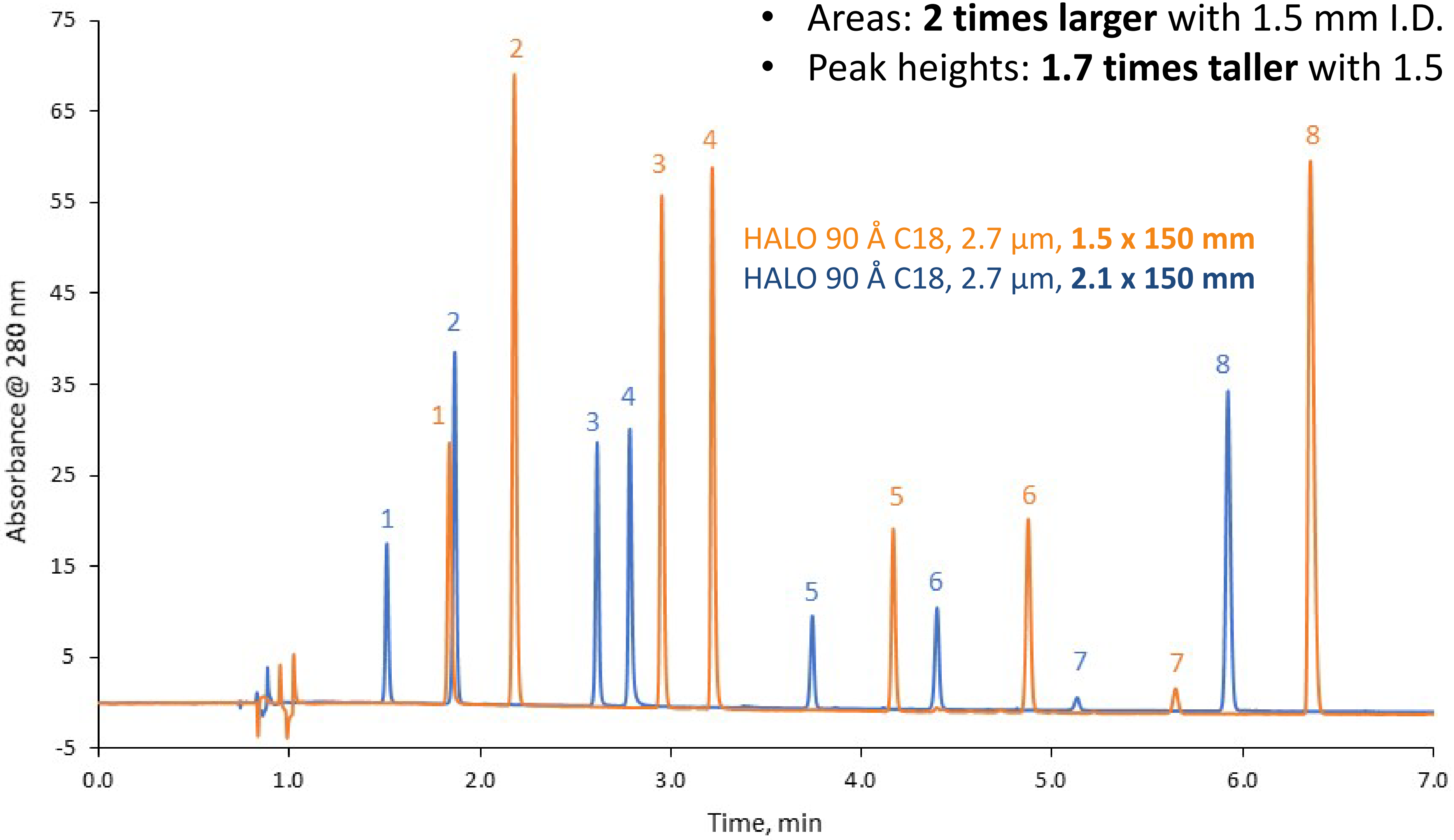
MORE PERFORMANCE FROM UHPLC AND LCMS SYSTEMS

- ✓ 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

More sensitivity from conventional UHPLC systems

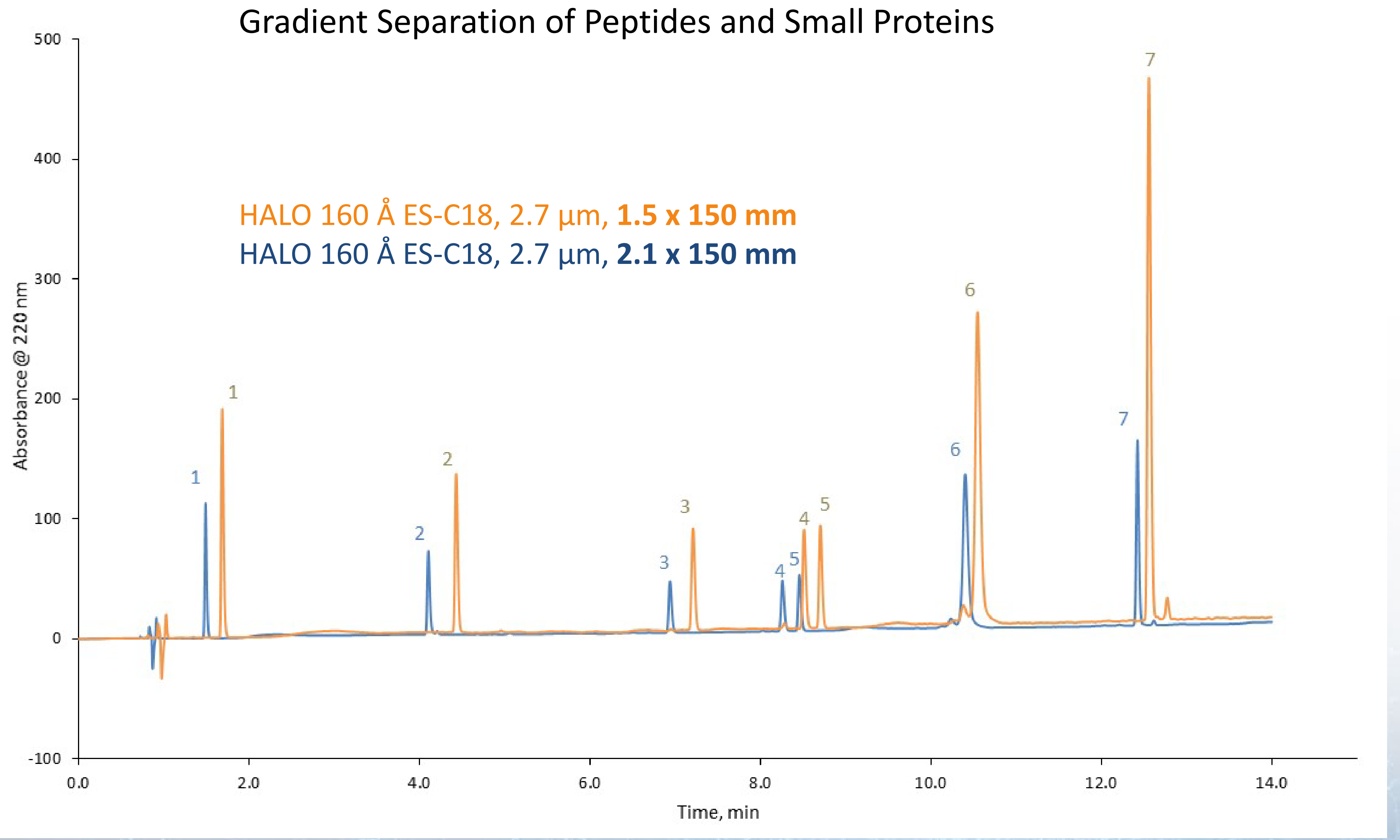
Comparison of Gradient Separation of OTC Cough and Cold Medicines

- 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

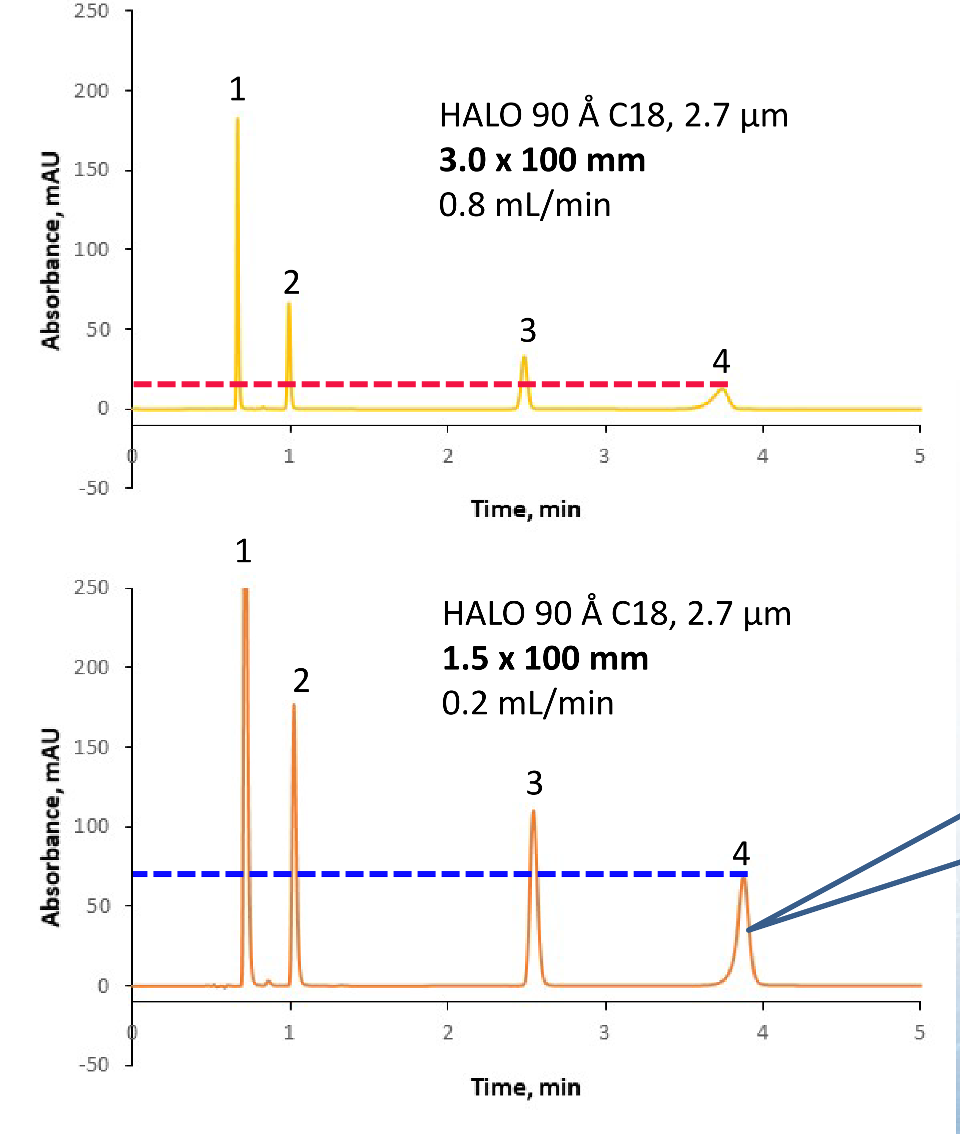
More sensitivity from conventional UHPLC systems



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 I.D. column



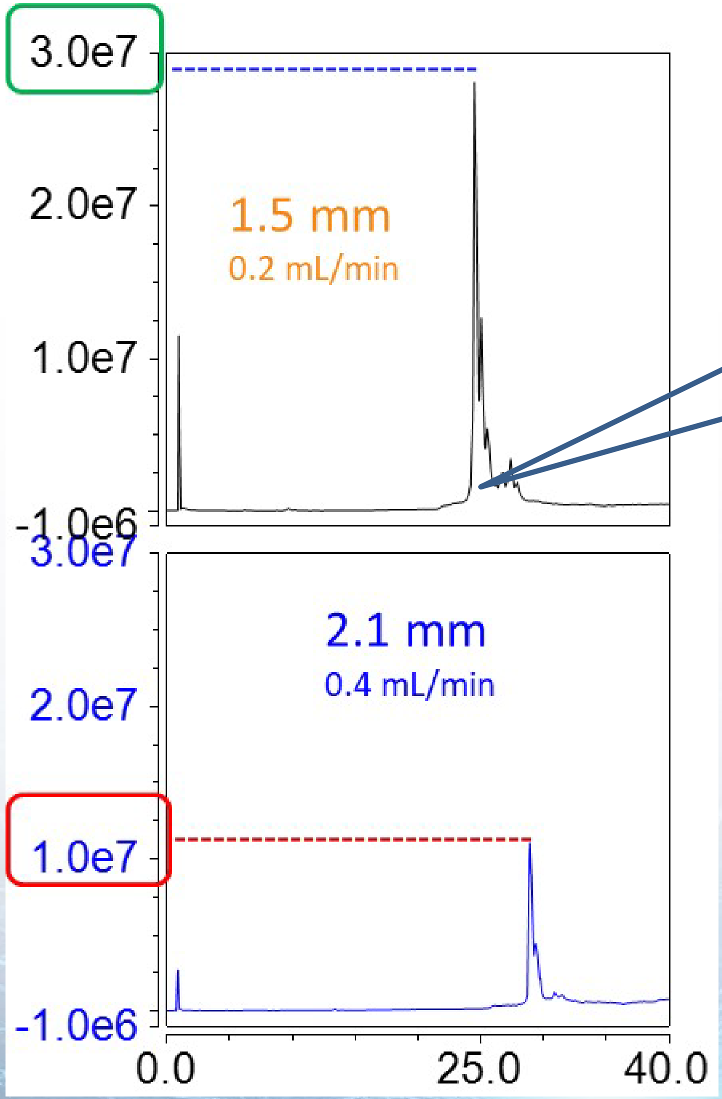
1. Acetaminophen
2. Caffeine
3. Aspirin
4. Salicylic Acid

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

Both area and peak height are increased!

Higher ionization efficiencies from LCMS systems - 1

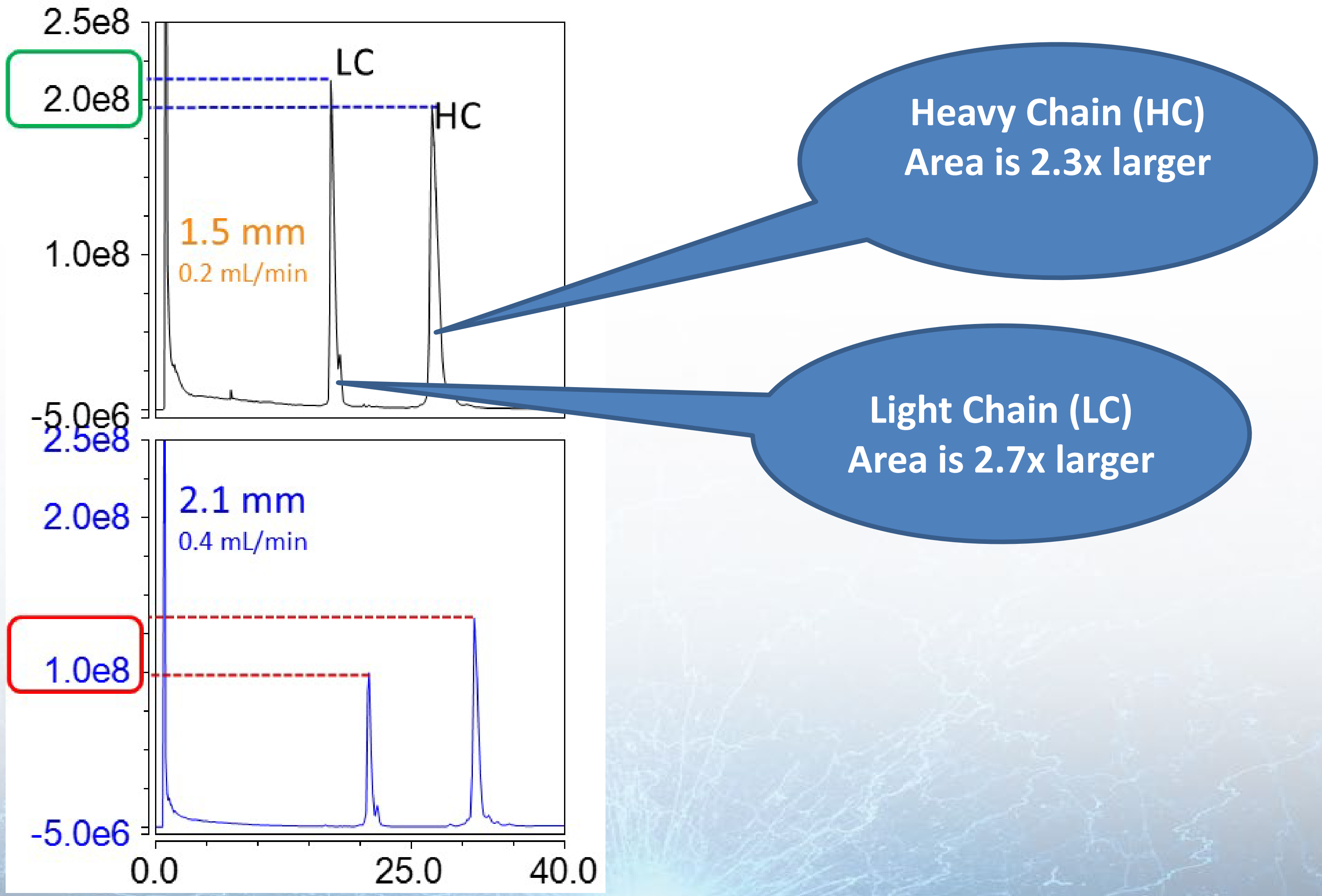
Intact Trastuzumab using HALO 1000 Å Diphenyl under Gradient Conditions



Area is 2.9x larger and peak height is increased

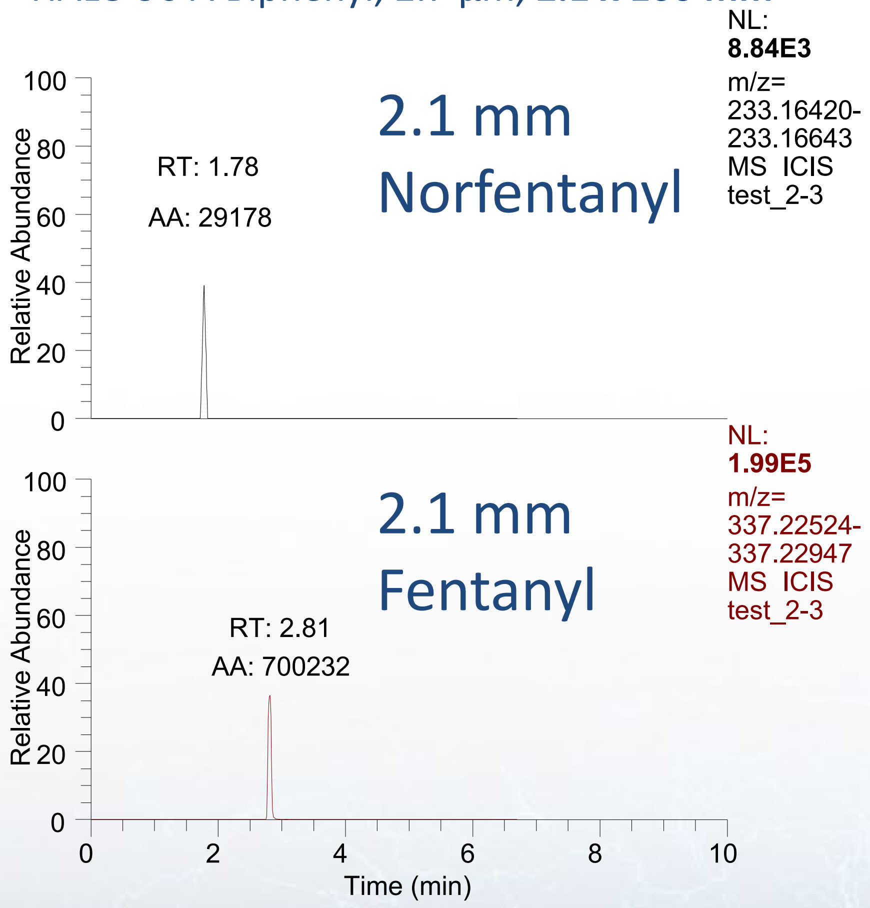
Higher ionization efficiencies from LCMS systems - 2

Reduced and Alkylated Trastuzumab
using HALO 1000 Å Diphenyl

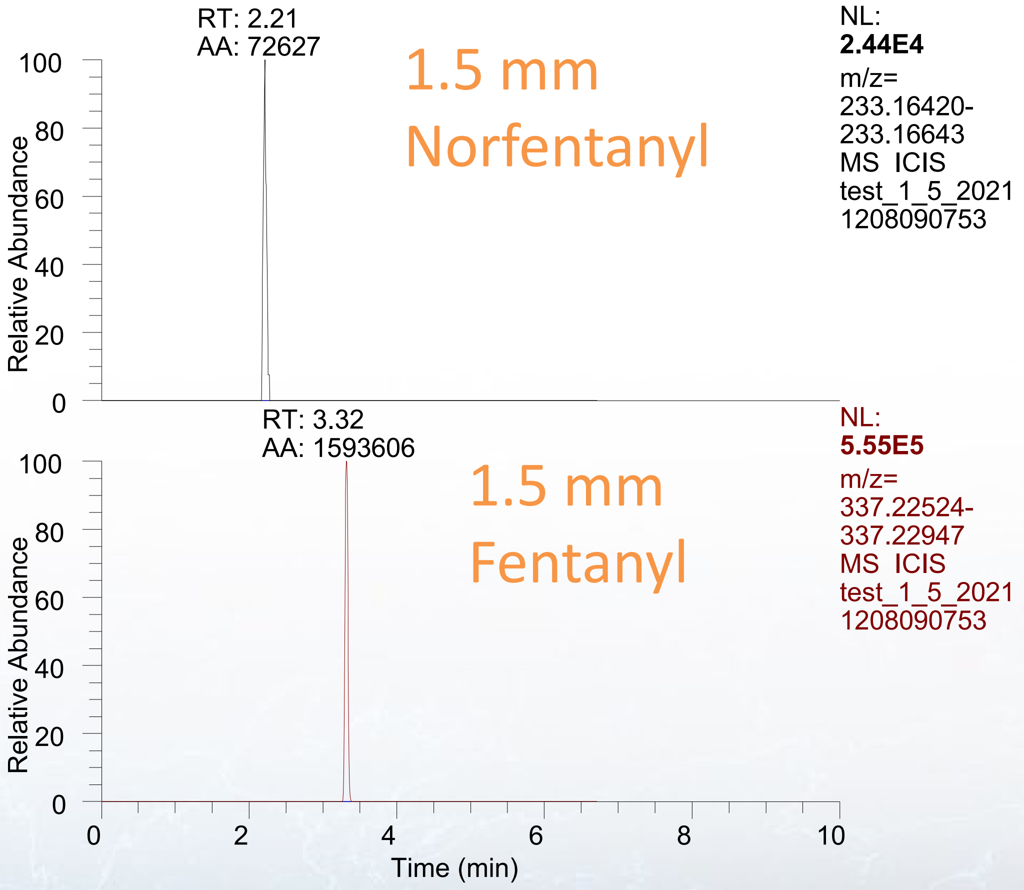


Higher ionization efficiencies from LCMS systems - 3

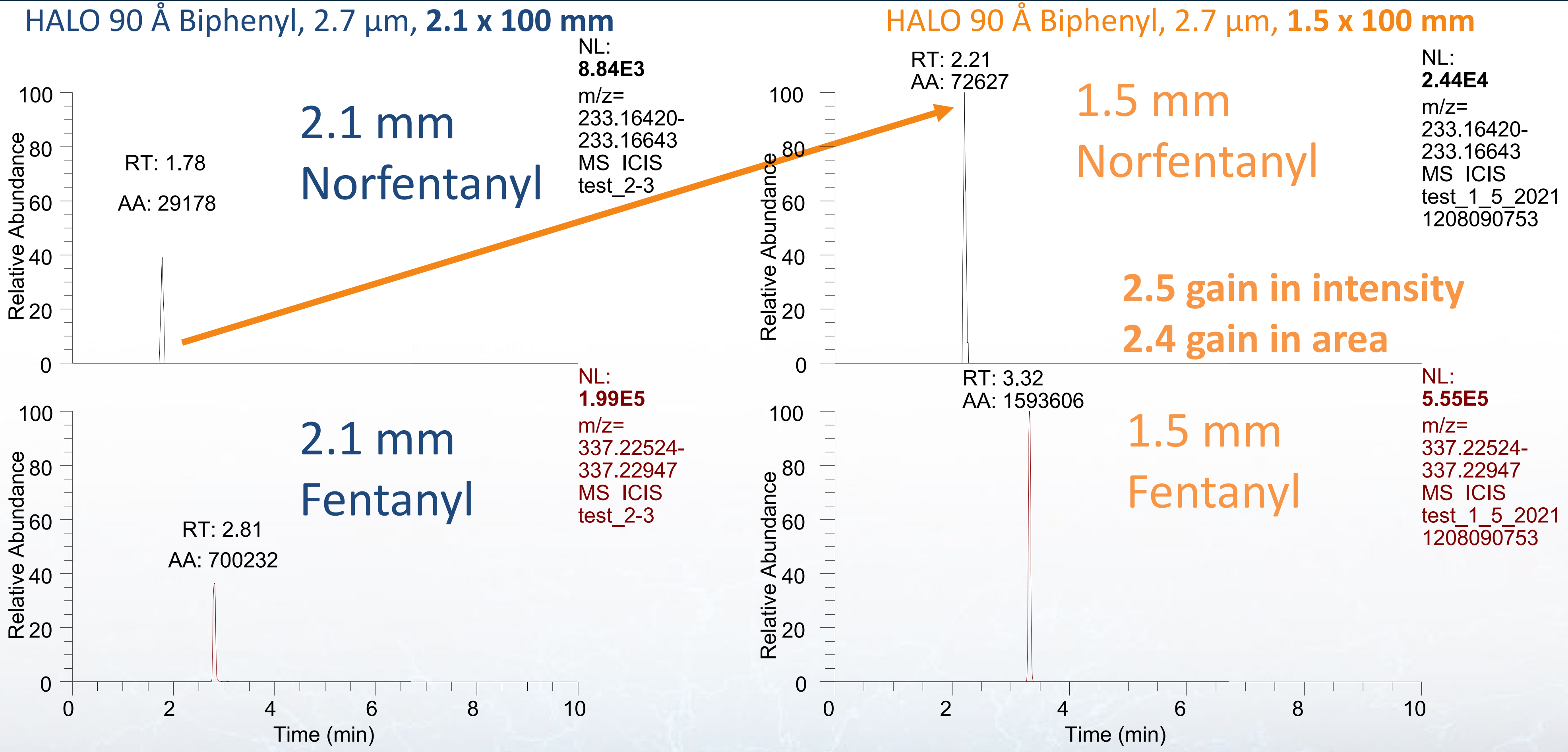
HALO 90 Å Biphenyl, 2.7 µm, **2.1 x 100 mm**



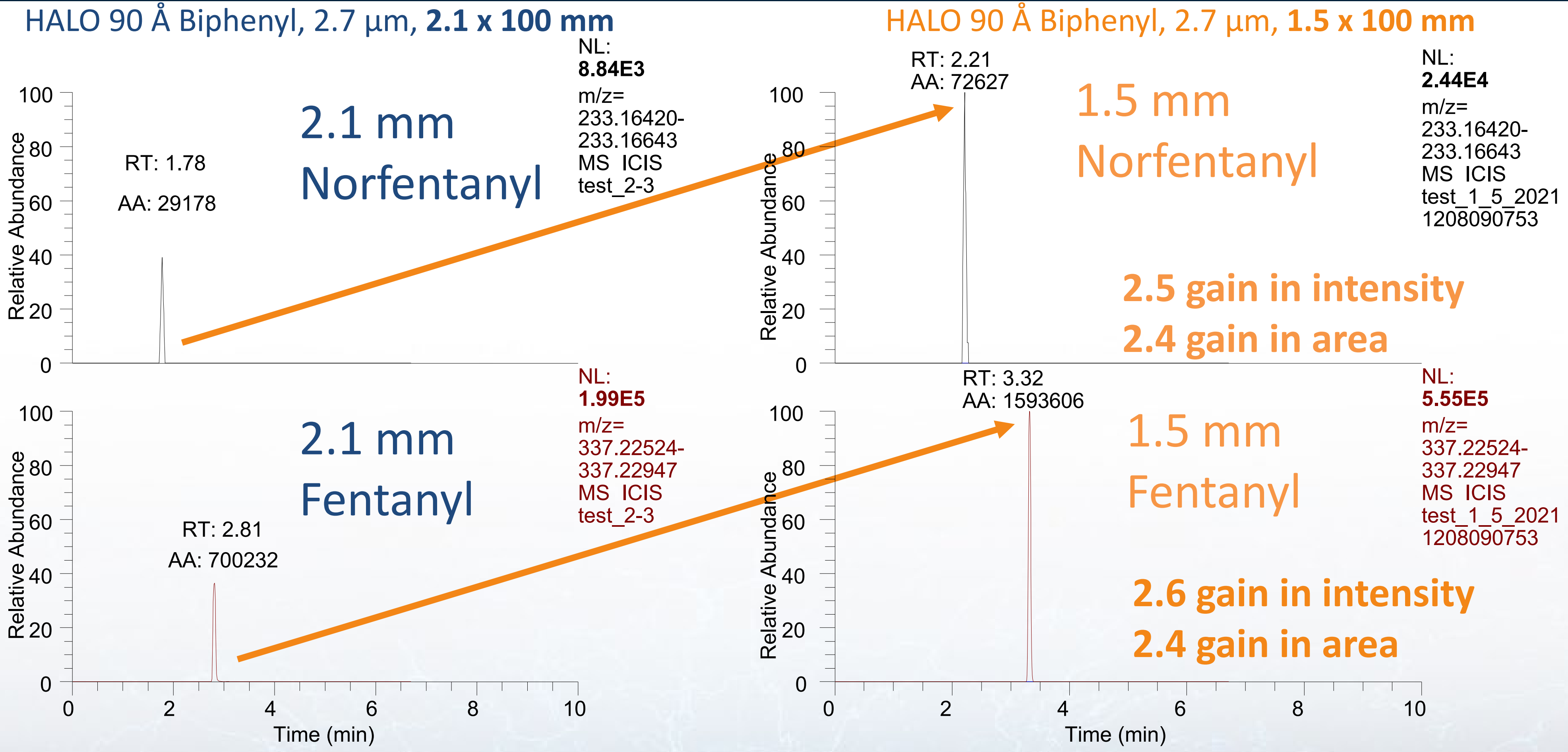
HALO 90 Å Biphenyl, 2.7 µm, **1.5 x 100 mm**



Higher ionization efficiencies from LCMS systems - 3

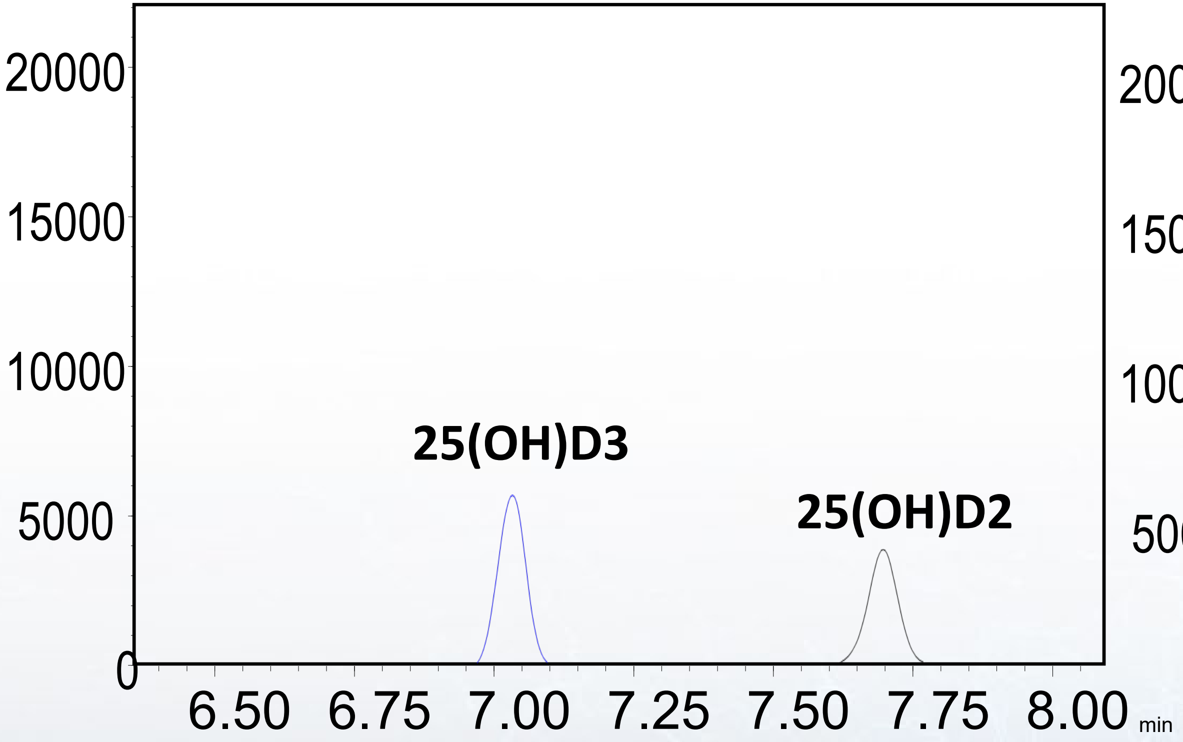


Higher ionization efficiencies from LCMS systems - 3

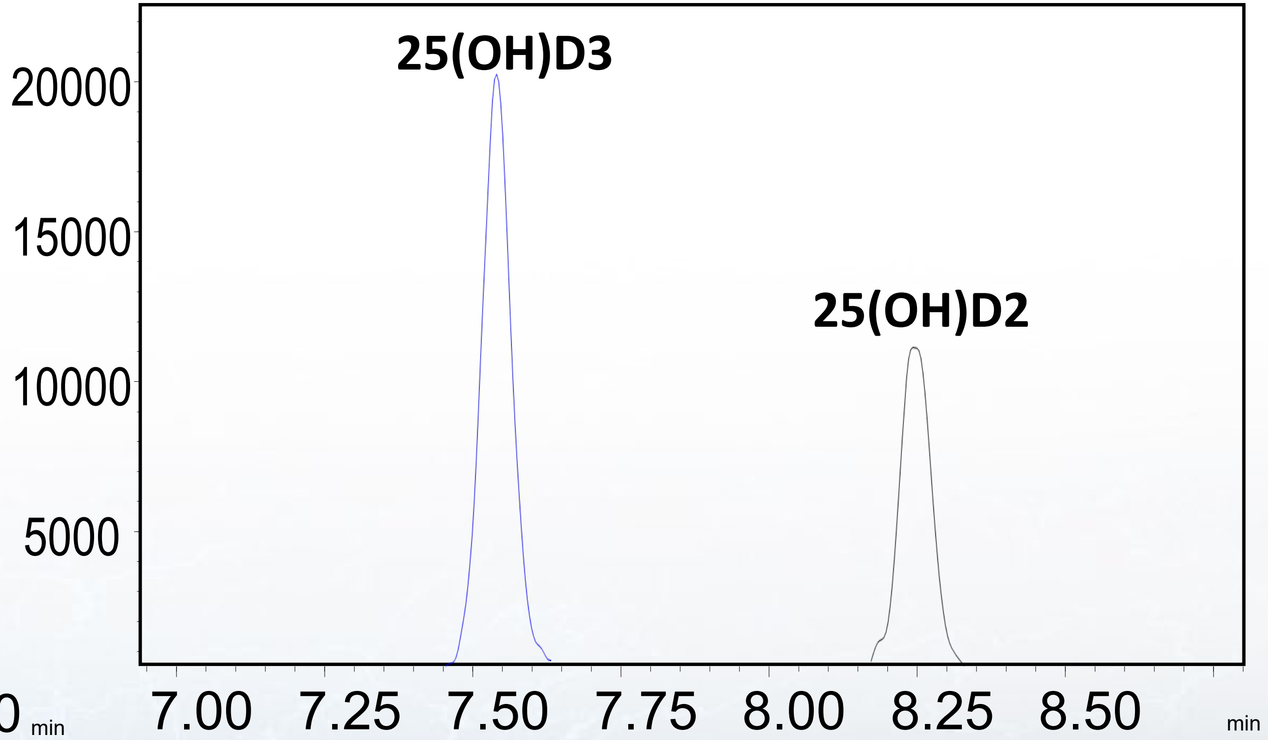


Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

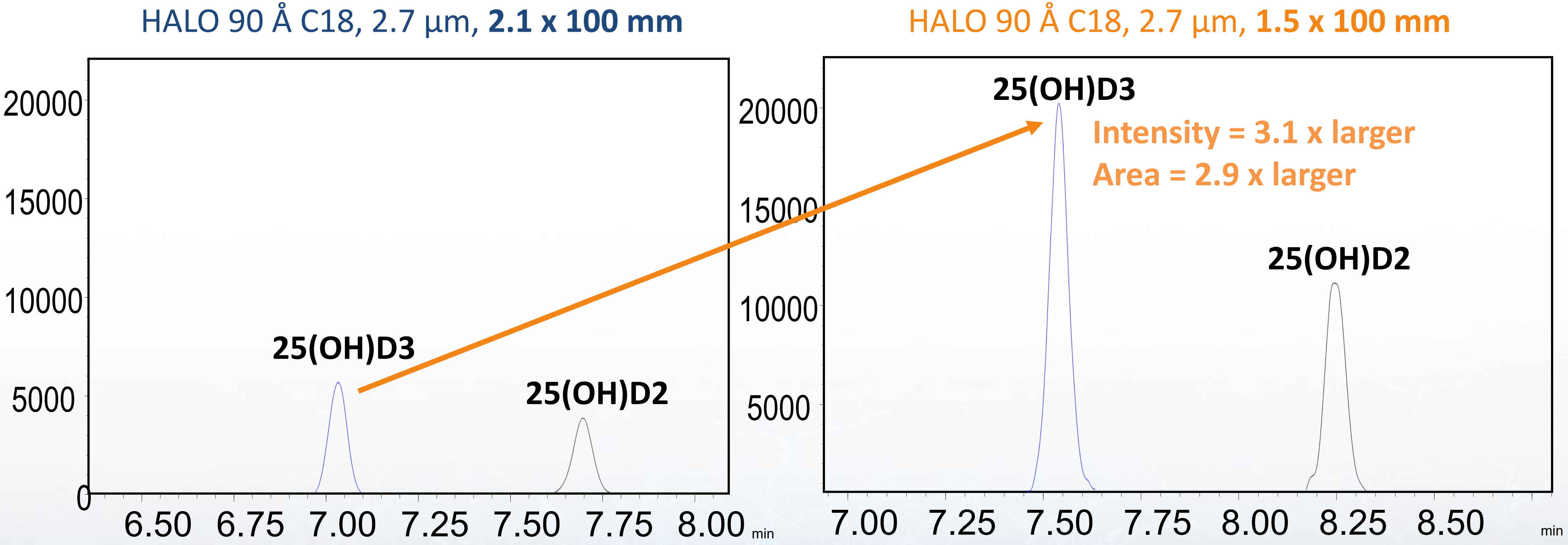
HALO 90 Å C18, 2.7 µm, **2.1 x 100 mm**



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



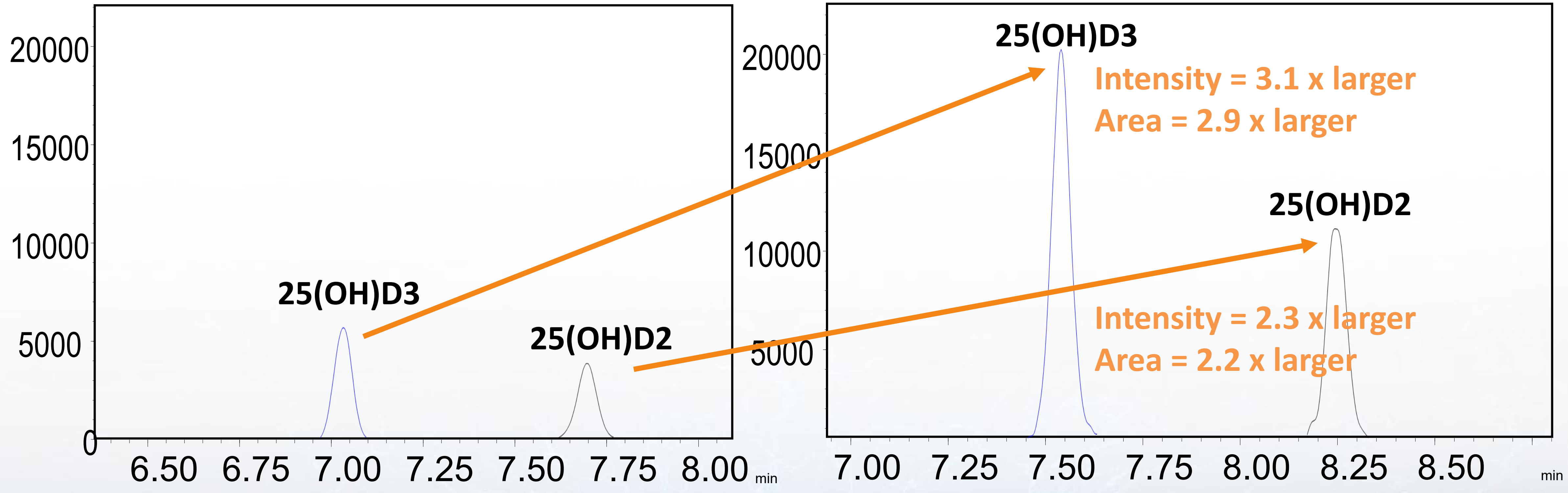
Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites



Higher ionization efficiencies from LCMS systems – Vitamin D Metabolites

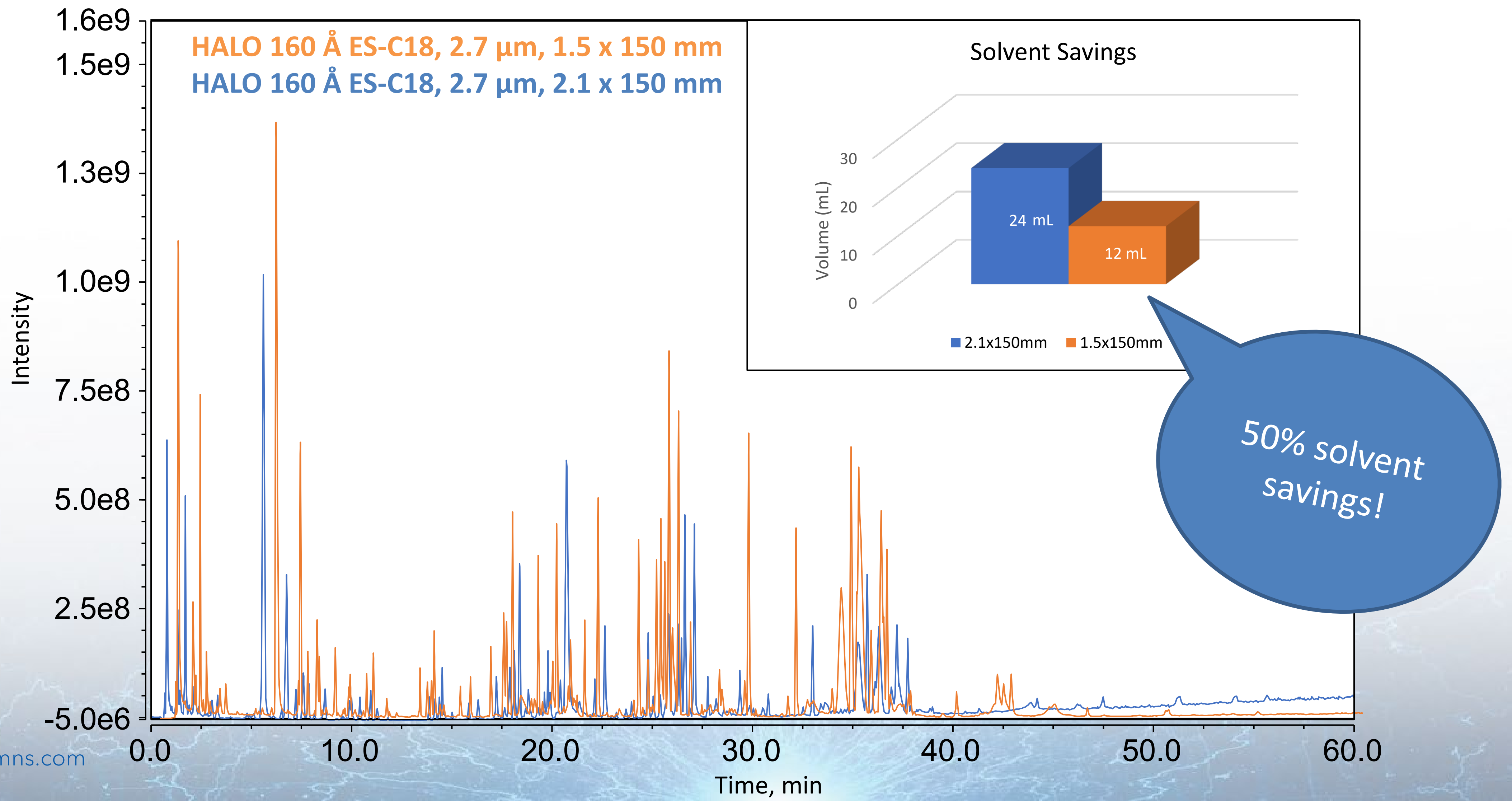
HALO 90 Å C18, 2.7 µm, **2.1 x 100 mm**

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

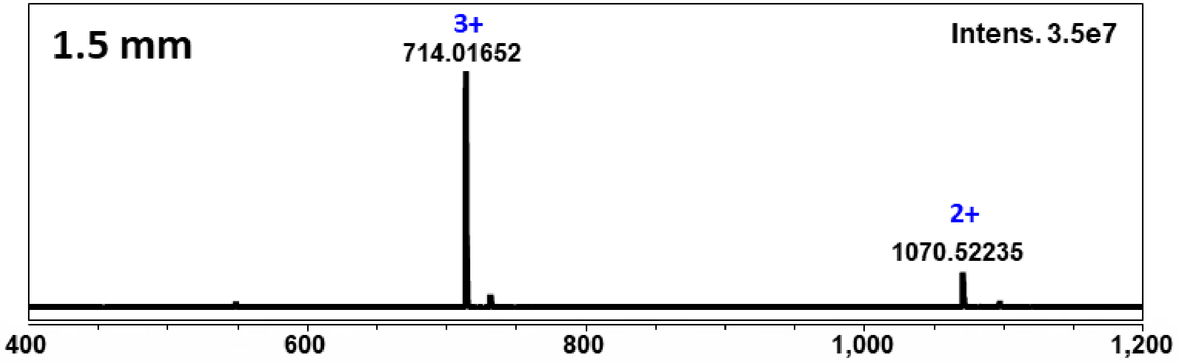


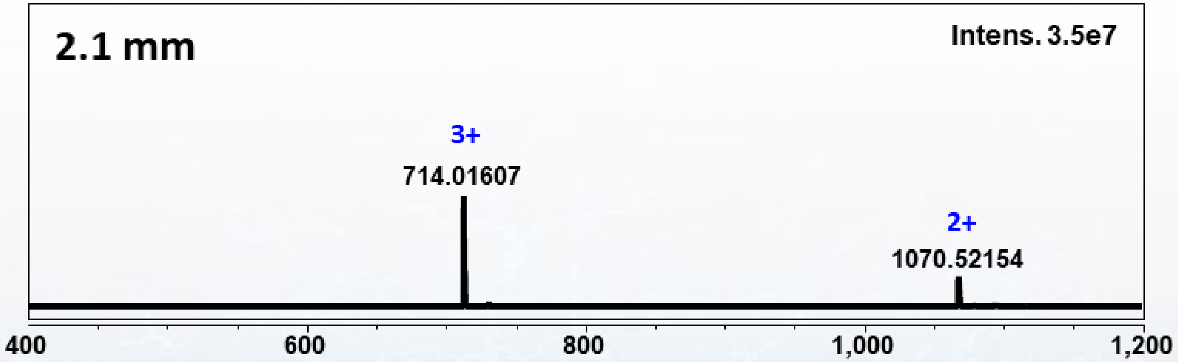
Reduced solvent consumption compared to 2.1 mm I.D. columns

Peptide Map of Trastuzumab under Gradient Conditions



Charge Envelope Comparison of Heavy Chain Peptide HC11



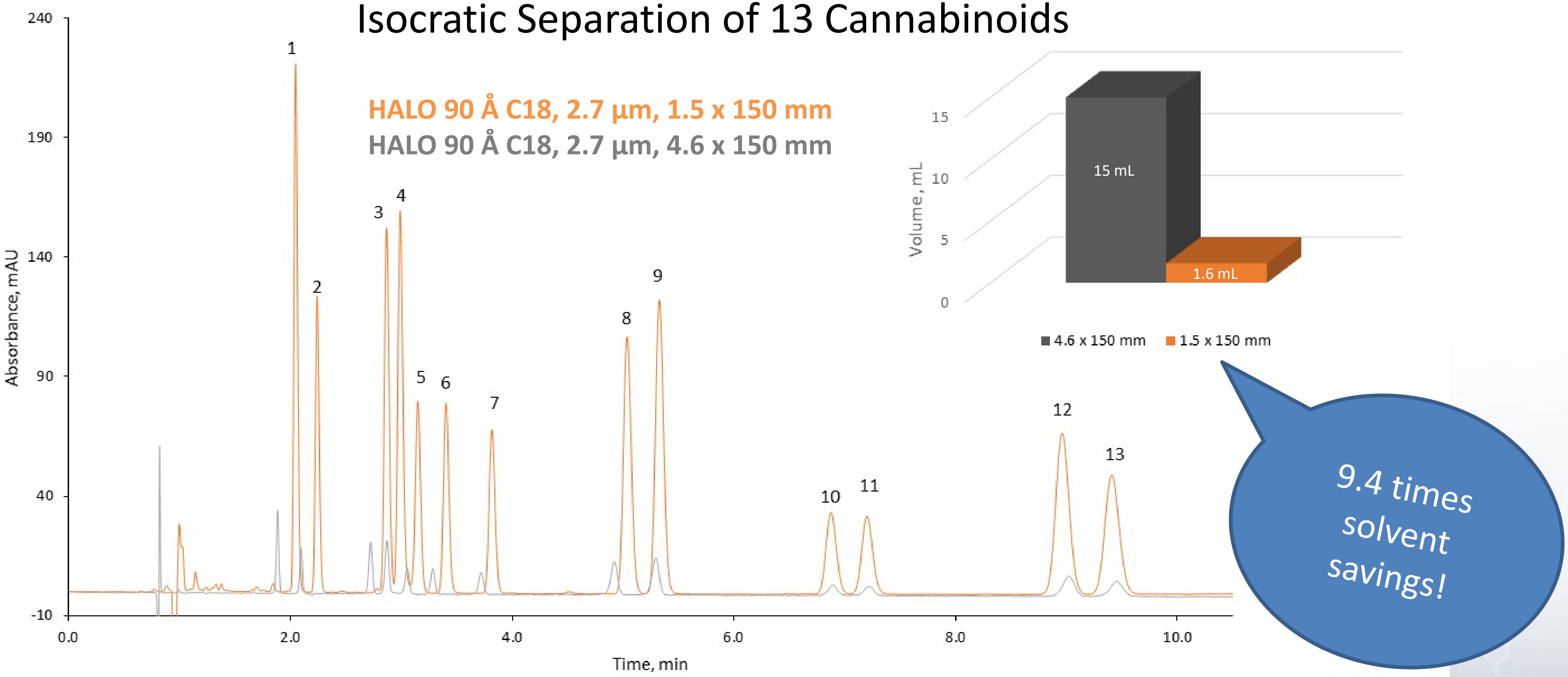


0.3µg trastuzumab tryptic digest, 2-50%B in 60 min 60°C ES-C18 150mm 2.7µm 160A; (A) 0.1%DFA H2O (B) 0.1%DFA ACN (*Gradient Delay*)

Adapted from Fig. 4 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

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

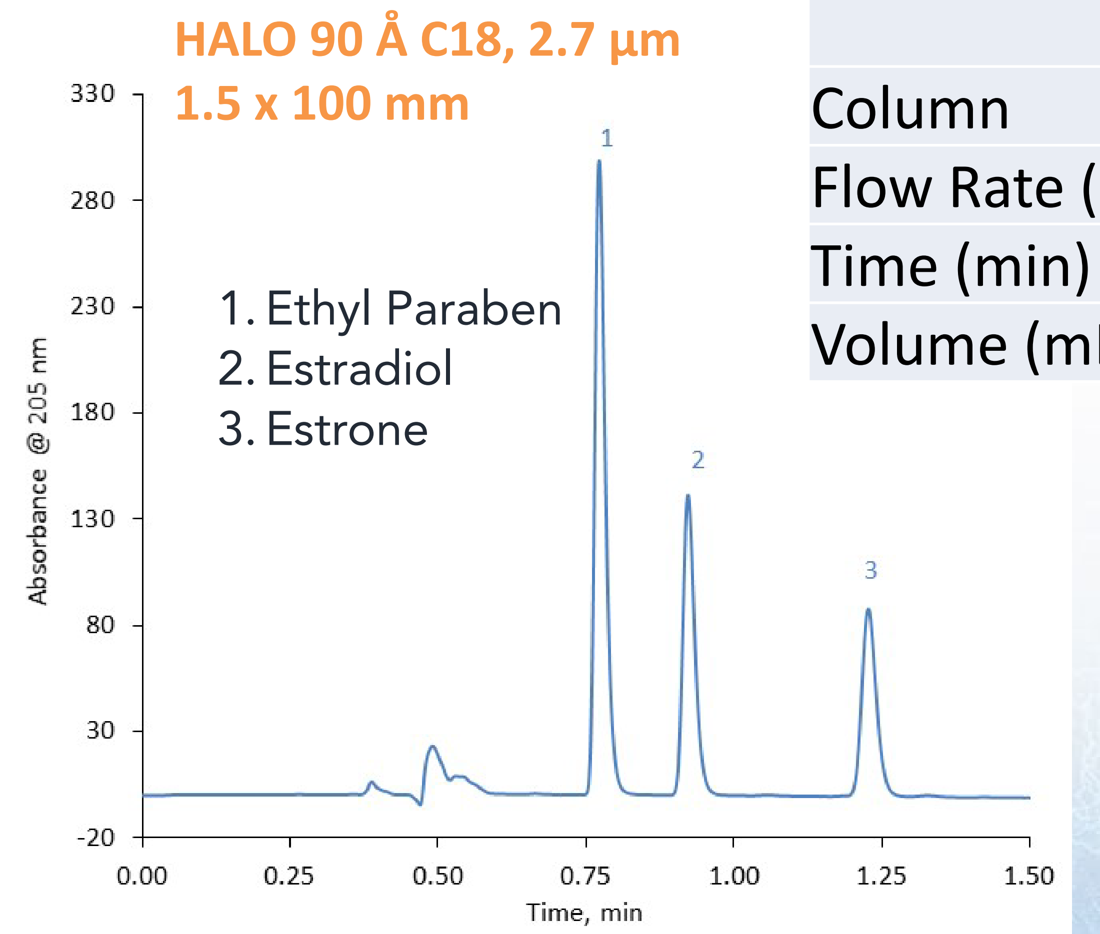
Isocratic Separation of 13 Cannabinoids



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

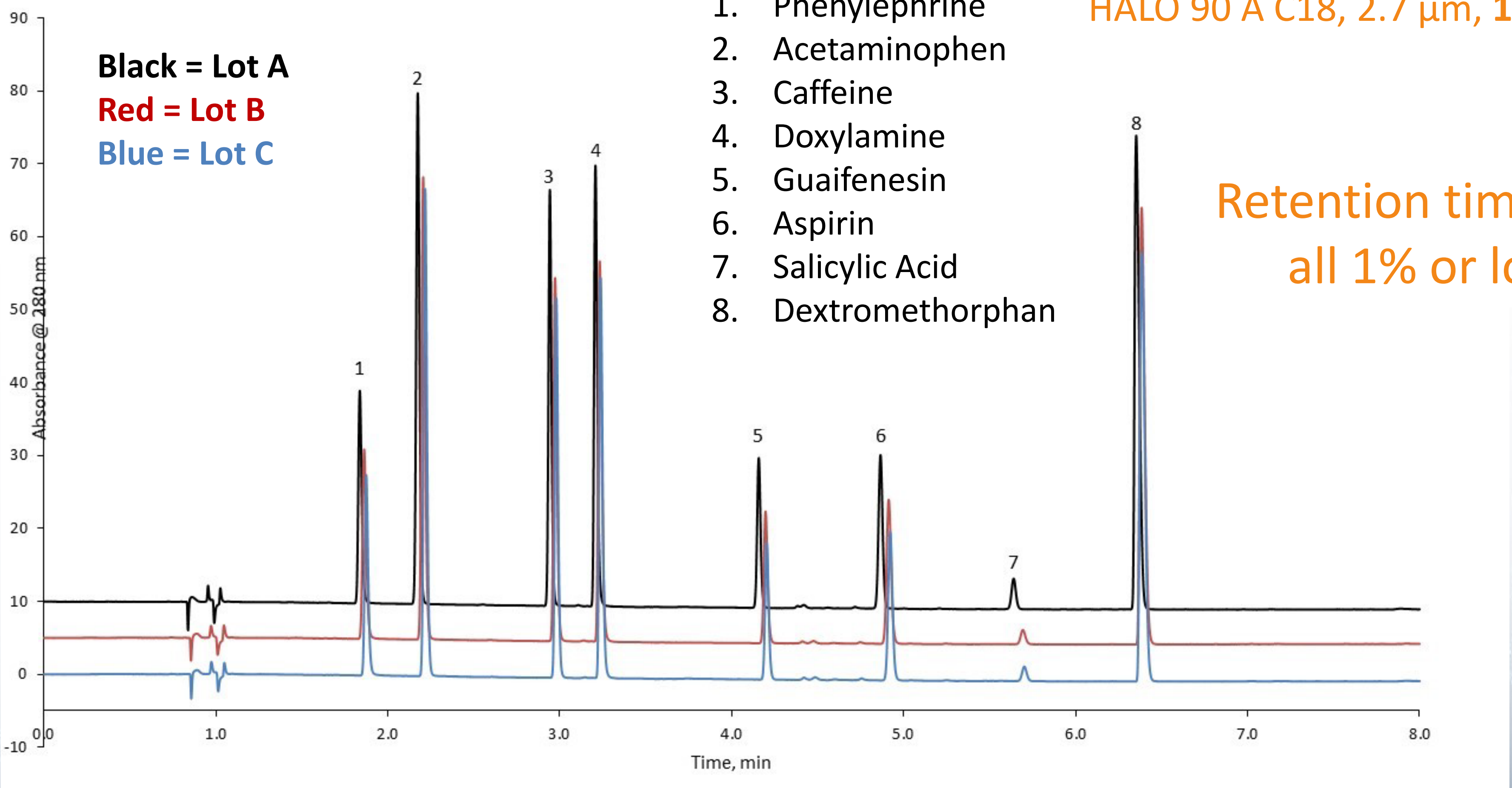


	Original Method	Modified Method
Column	3.9 x 300 mm	1.5 x 100 mm
Flow Rate (mL/min)	1	0.2
Time (min)	8	1.5
Volume (mL)	8	0.3

96% solvent savings!

HALO® 1.5 mm I.D. Column Hardware Reproducibility

Excellent reproducibility from 3 different lots of column hardware



1. Phenylephrine
2. Acetaminophen
3. Caffeine
4. Doxylamine
5. Guaifenesin
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

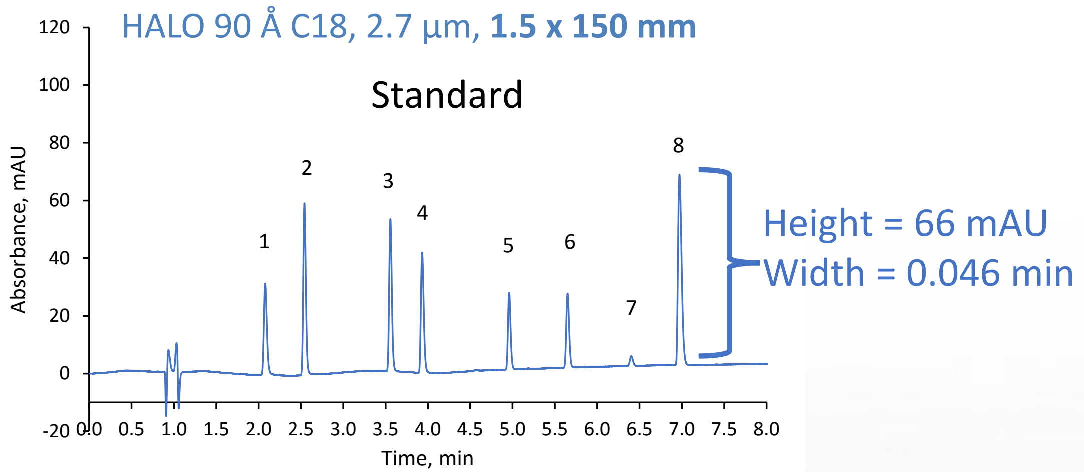
Column looks and feels like a 2.1 mm…

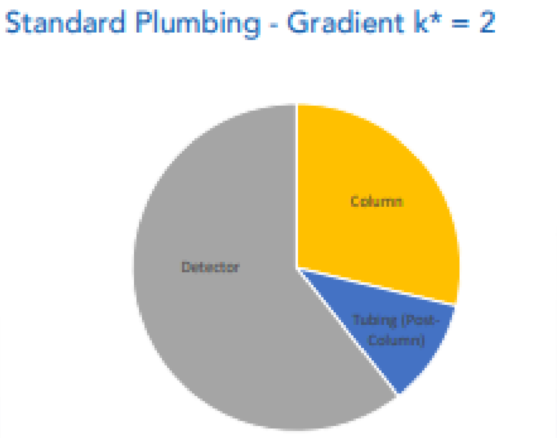
Success using HALO® 1.5 mm I.D.

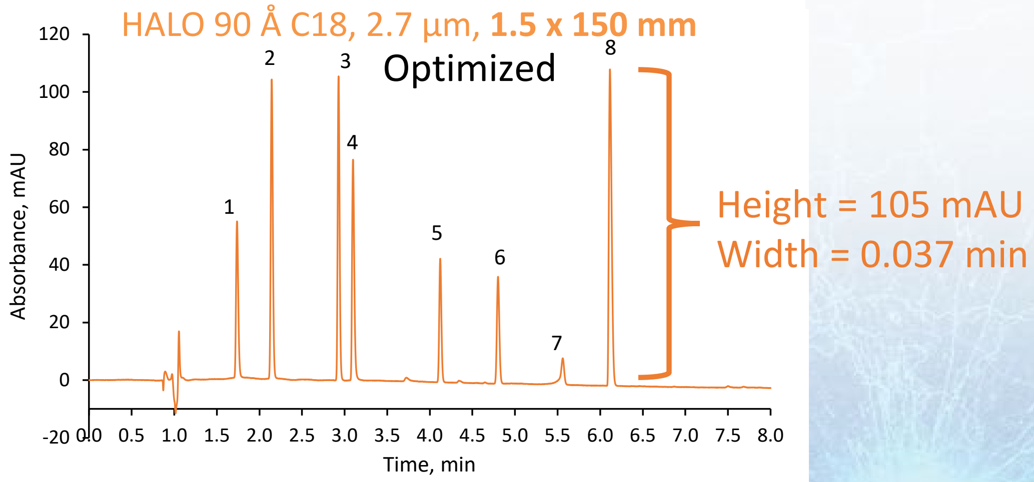
- System
- Connectors
- Method Transfer to 1.5 mm

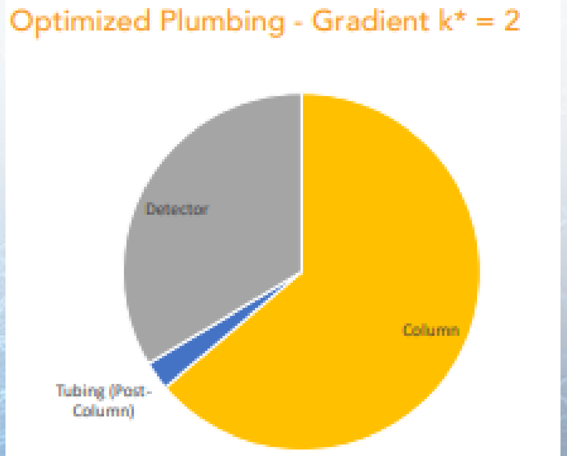
How do I Estimate Extracolumn Dispersion?

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.multidlc.org/dispersion_calculator



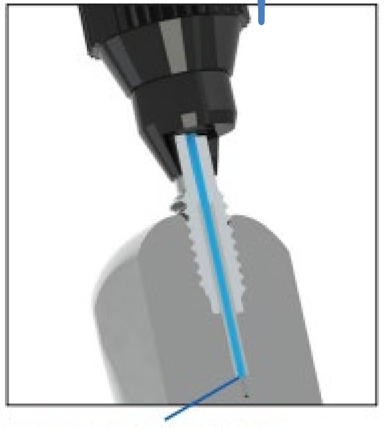


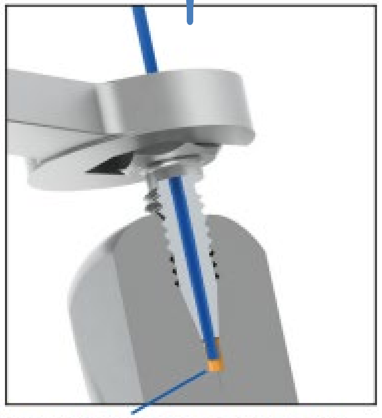




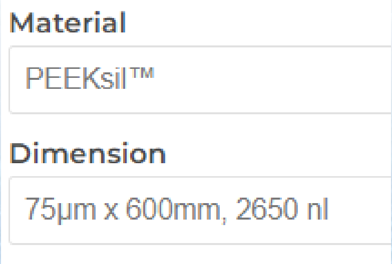
AMT MarvelXACT™ Connectors

- Difference between MarvelXACT™ and ferrule fitting





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



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

- Scale flow rate

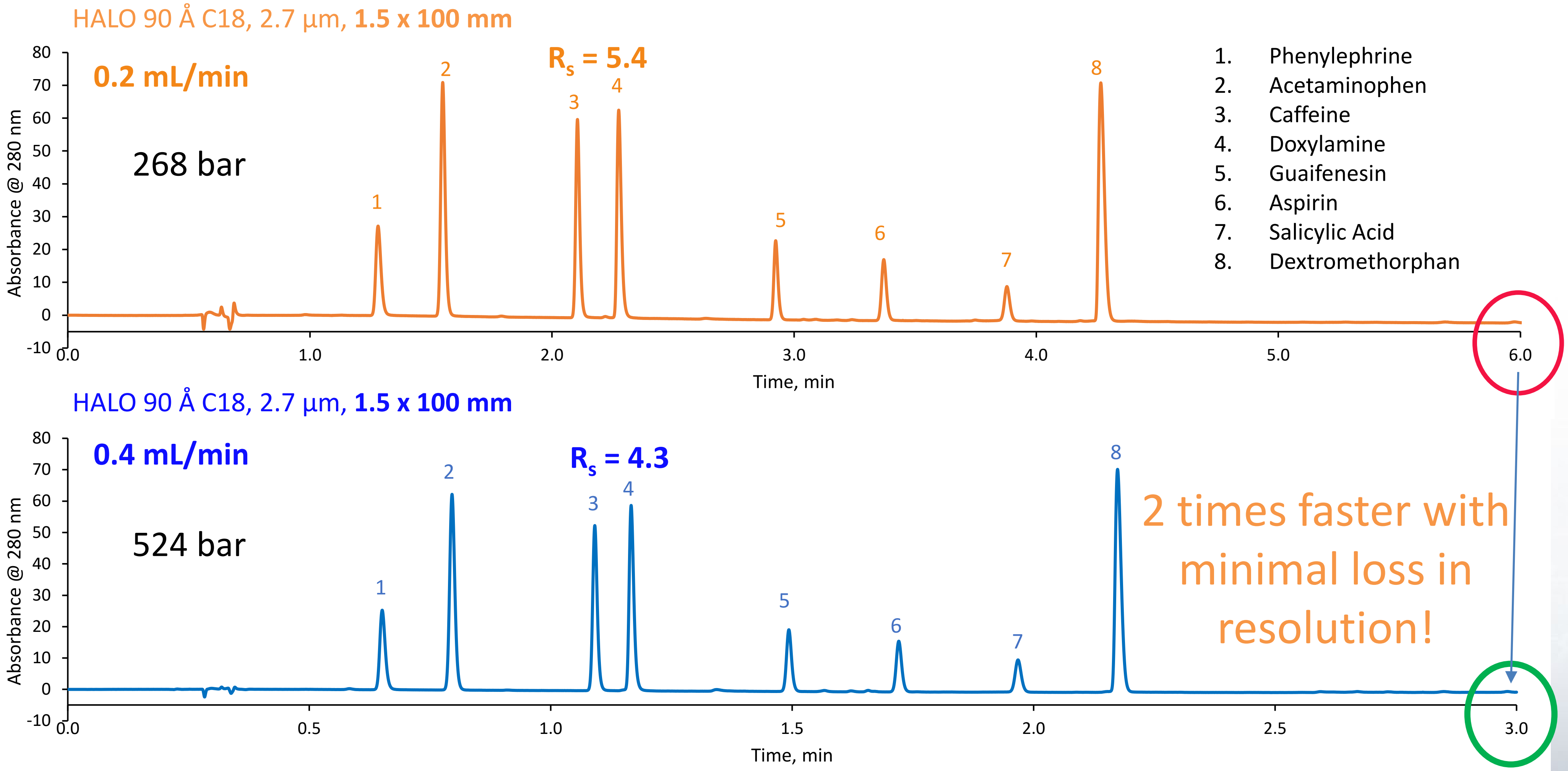
$$F_2 = F_1 \times \frac{(\pi R_2)^2}{(\pi R_1)^2} = F_1 \times \frac{(R_2)^2}{(R_1)^2} = F_1 \times \frac{(D_2)^2}{(D_1)^2}$$

F_2 = scaled flow rate
F_1 = original flow rate
D_2 = column I.D. being transferred to
D_1 = original column I.D.

	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 I.D. 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 I.D. 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

HALO® and Fused-Core® are registered trademarks of Advanced Materials Technology

Acknowledgements

- AMT
- Jim Grinias, Rowan University
- Justin Godinho, GSK
- Mark Schure, Kroungold Analytical Inc.
- Thomas Waeghe

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

- Poster – presented today at HPLC 2022
  - P-T-719 Optimizing Older UHPLC Systems for use with New, Smaller ID Columns Merlin Bicking, ACCTA, Inc., Saint Paul, MN, USA; Richard Henry, Consultant, Sanibel, FL, USA
- 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

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Photo by Camylla Battani on Unsplash