IMPLEMENTING 1.5 MM INTERNAL DIAMETER COLUMNS INTO ANALYTICAL WORKFLOWS

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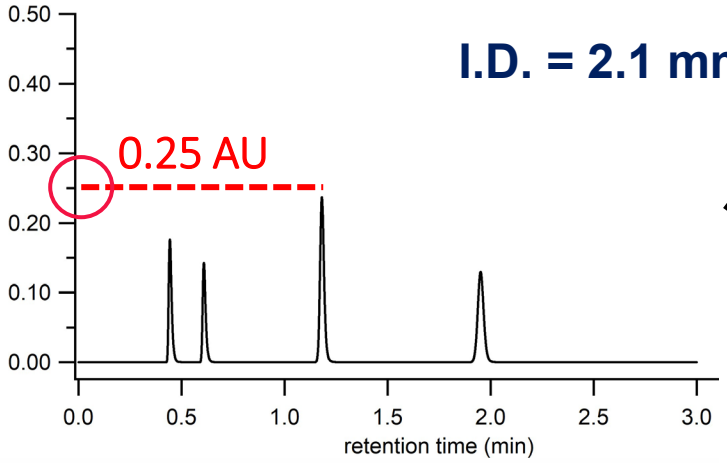
Outline

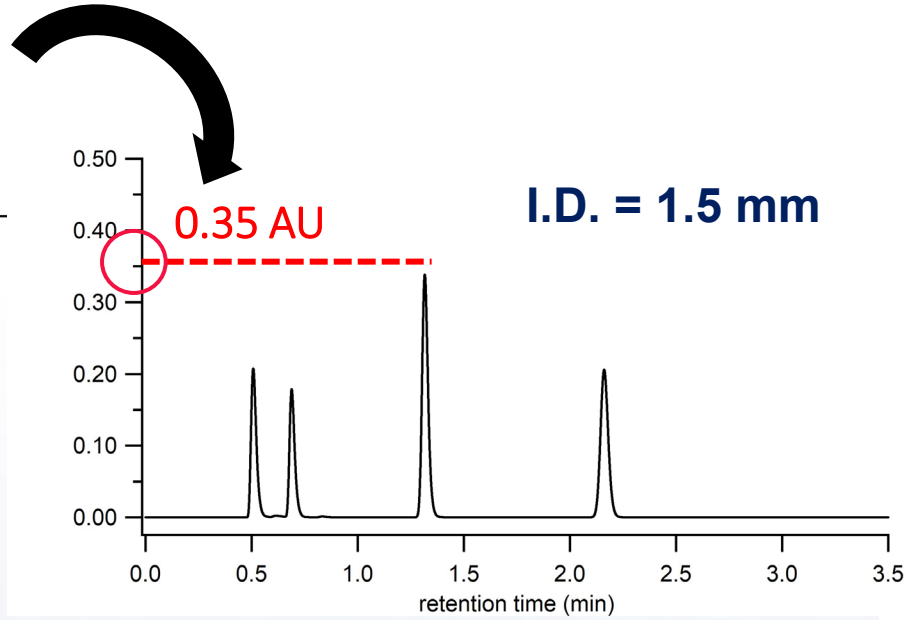
- **Comparing the 1.5 mm internal diameter (i.d.) column performance to 2.1 mm and 1.0 mm i.d. columns:**
  - Small molecule absorbance detection (UV)

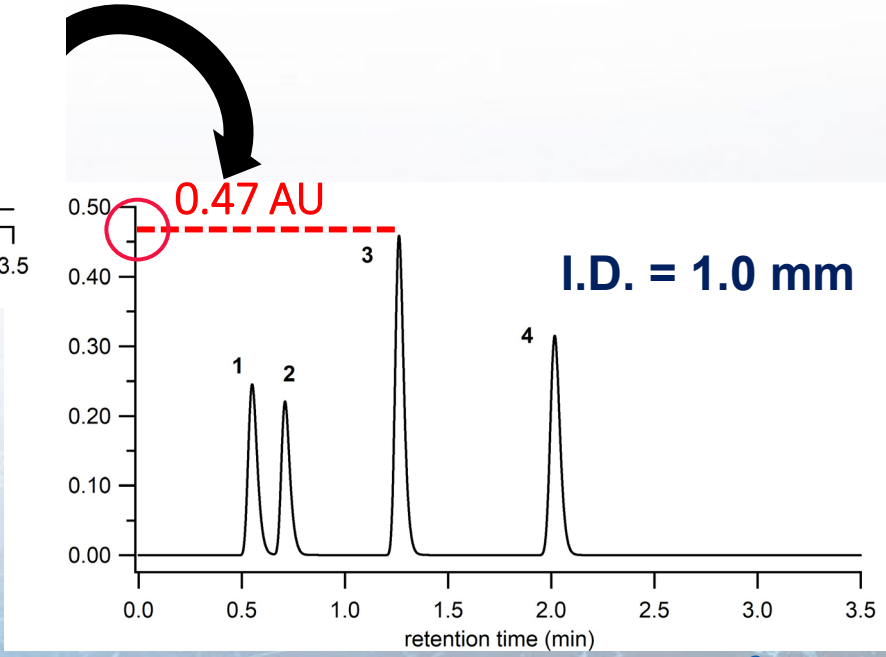
- **Measure differences in LCMS mAb analysis between the 2.1 mm, 1.5 mm, and 1.0 mm i.d. columns:**
  - mAb analysis → intact, subunit, peptide
  - Difluoroacetic acid (DFA) mobile phase modifier used to improve peak shape

- **Pros and Cons of switching from a 1.5 mm i.d. to a 2.1 mm or 1.0 mm i.d.**
  - Experimental limitations? e.g. sample concentration.. analysis time.. Solvent consumption..

Comparison of Absorbance Signal with Varying Column Diameter







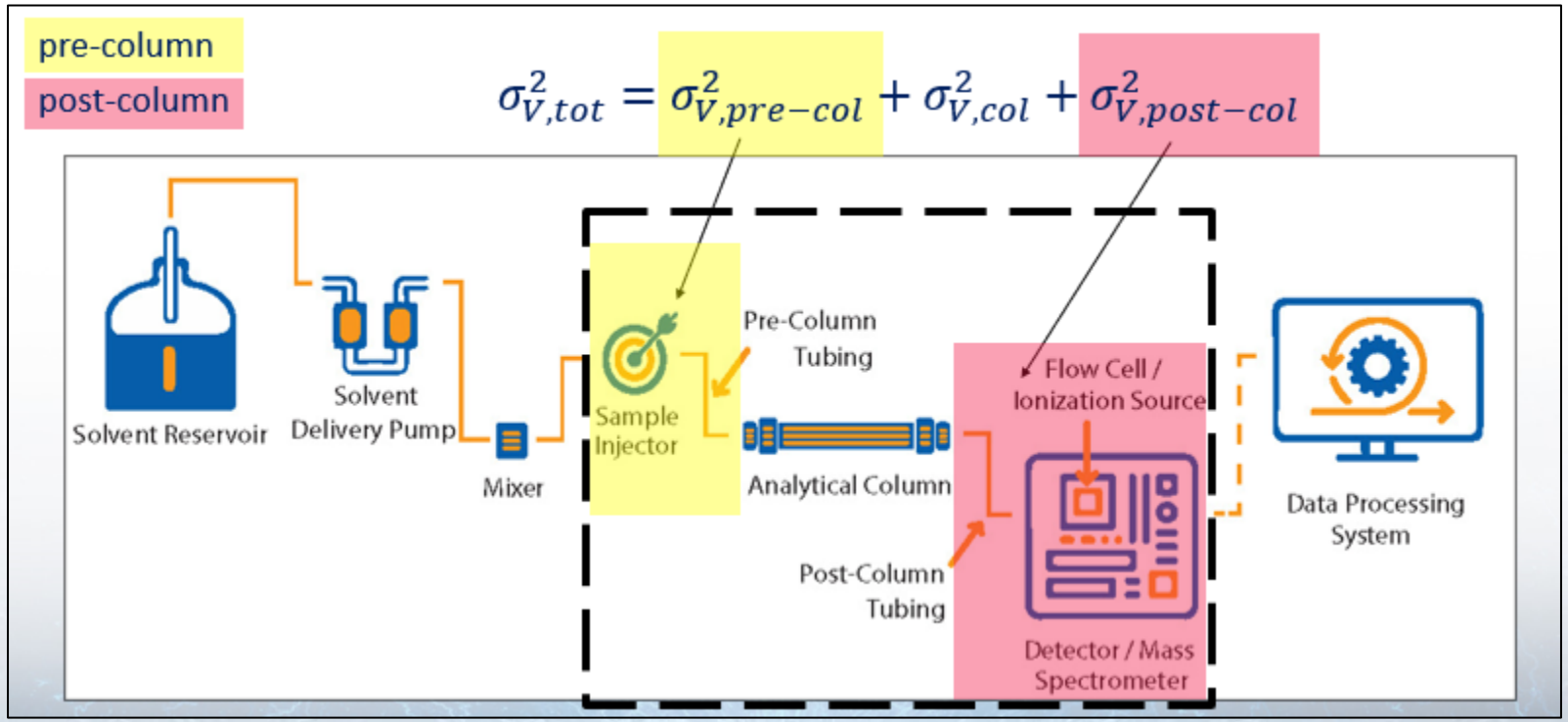
Signal increases as column i.d. decreases with identical sample load on column.

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**
- **2.1 mm i.d. (& shorter columns) introduced for use with UHPLC and for interfacing to mass spectrometers**

The Move to Smaller I.D. Columns

- **Signal intensity is increased when <u>same</u> sample concentration used**
- **Impact of** Extra Column Dispersion **must be considered**



Internal Column Diameter and Concentration-Sensitive Detection

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

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

Godinho & Grinias, HPLC 2018 Capillary LC Short Course

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F_2 = scaled flow rate
F_1 = original flow rate
D_2 = column i.d. being transferred to
D_1 = original column i.d.

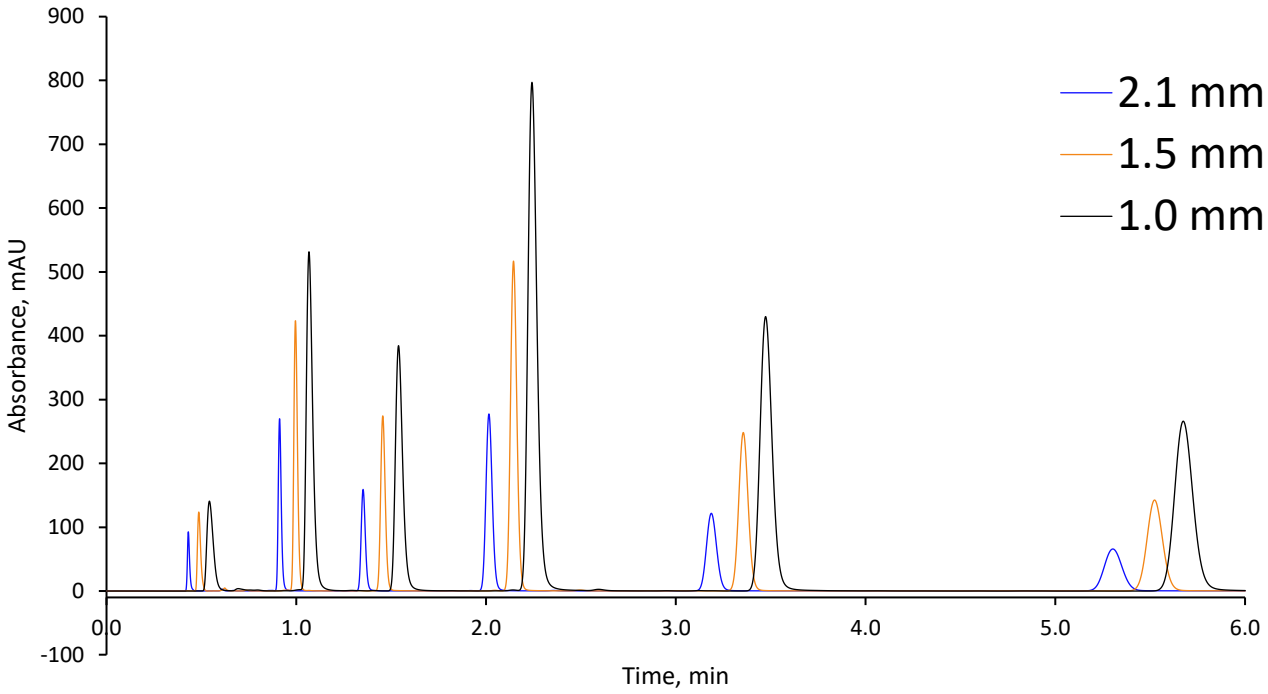
	COLUMN IDS				
	4.6	3.0	2.1	1.5	1.0
FLOW RATES (mL/min)	0.96	0.41	0.20	0.10	0.045
	1.44	0.61	0.30	0.15	0.068
	1.92	0.82	0.40	0.20	0.091
	2.40	1.02	0.50	0.26	0.113
	2.88	1.22	0.60	0.31	0.136

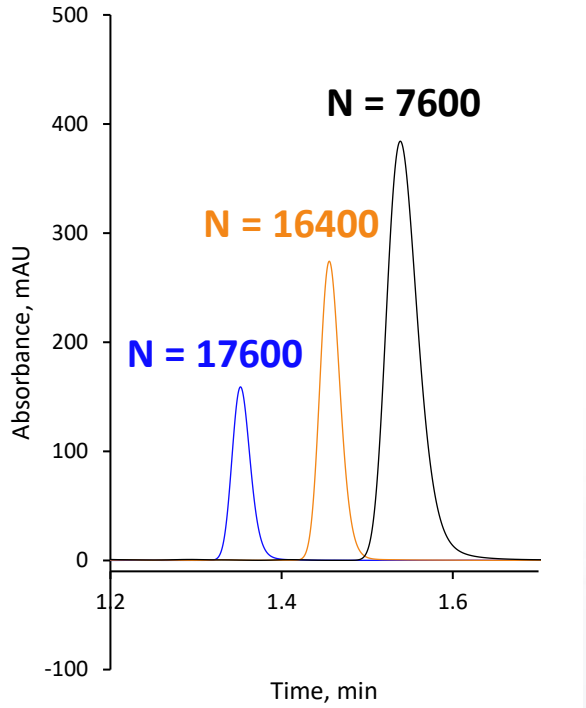
- **$ Consumables**
- **$ Waste disposal**

***Gradient method**: add injection time delay to account for dwell volume.

****Injection volume**: scale injection to maintain signal or keep same injection volume for increased signal.

Pros & Cons: Shifting from 2.1 mm I.D. to 1.0 mm I.D.

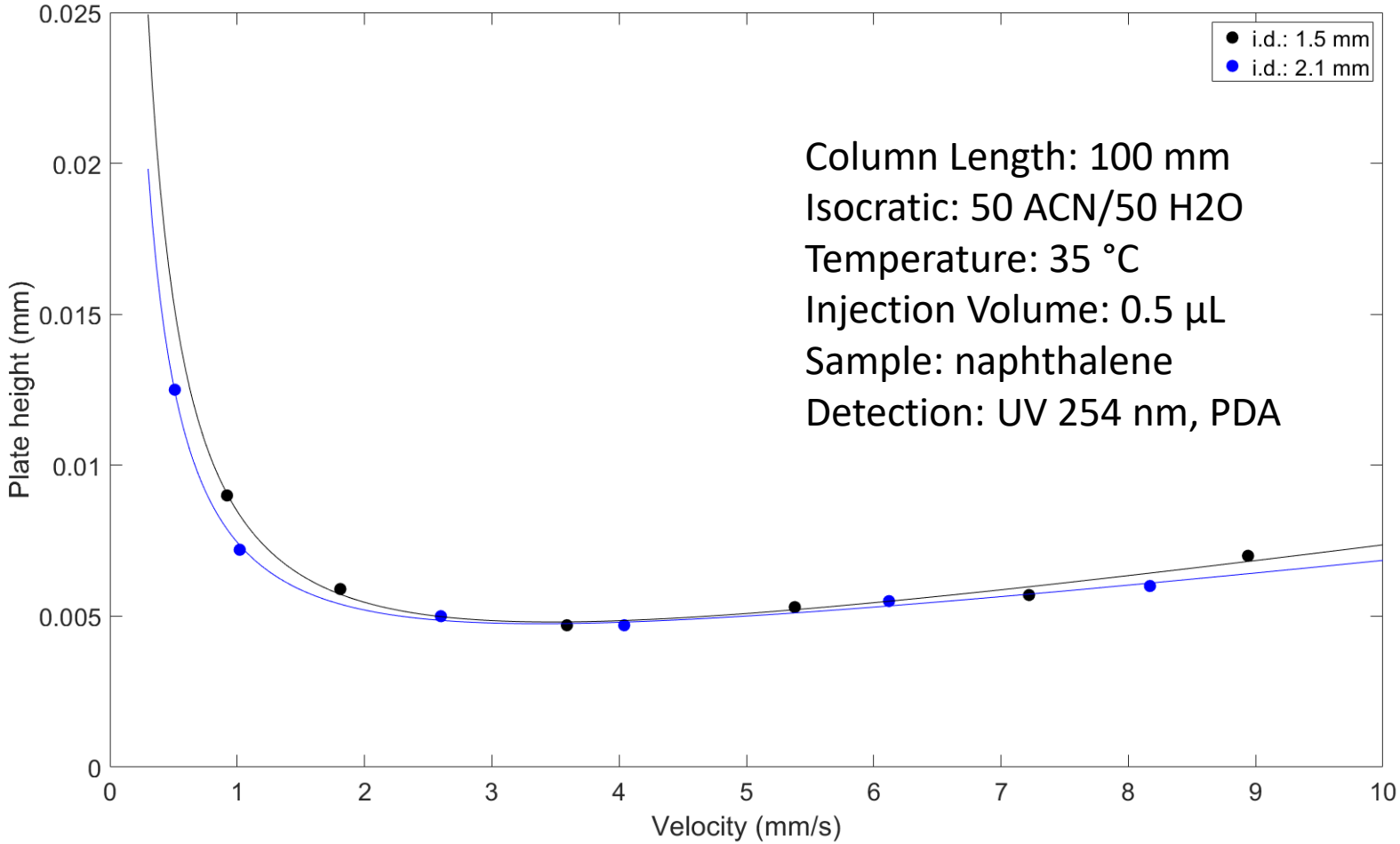




In move from 2.1 mm I.D. to 1.0 mm I.D., signal increases, but there is a *significant loss in efficiency primarily due to extracolumn effects.*

1.5 mm I.D. columns can provide a compromise between these effects.

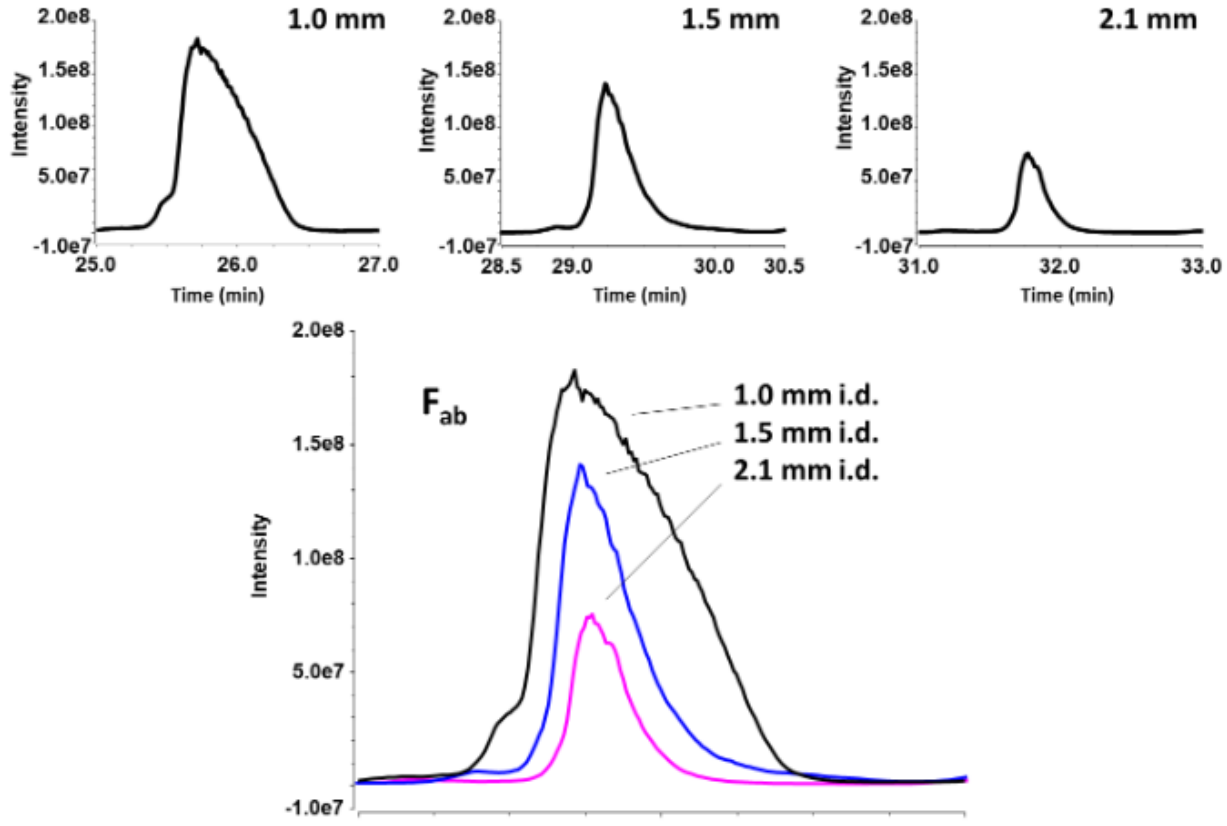
van Deemter Comparison: 1.5 mm to 2.1 mm



Why does 1.5 mm matter for biopharma separations?

**Total Ion Current, Full Scan [800 – 4000m/z], 3pt. MA

2µL inj. trastuzumab IdeS digest (2µg)



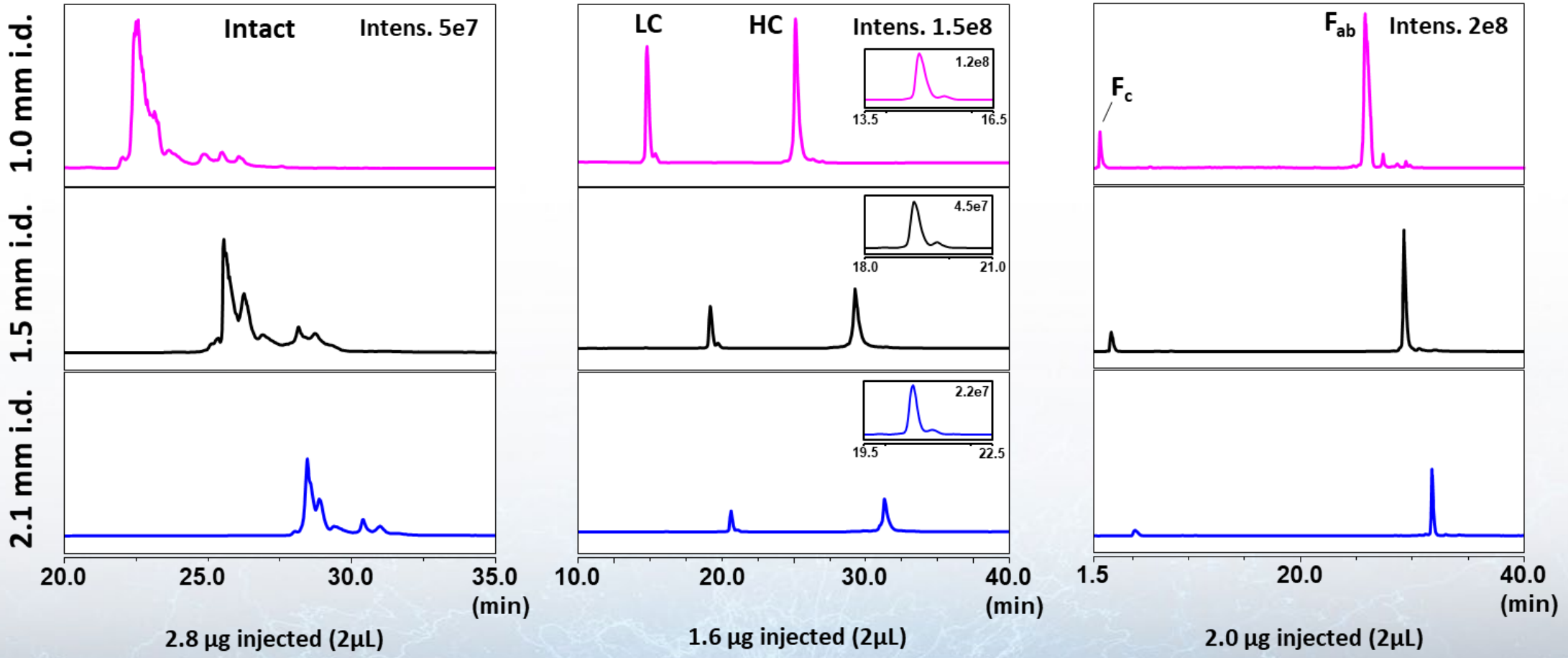
mAb characterization by LC/MS:

- 1.0 mm column obtained an increase in TIC; significant increase in PW50%
- 2.1 mm i.d. vs 1.5 mm i.d.: obtained a 2.7-fold increase in TIC Area Ratio; TIC Area Ratio = TIC $Area_{1.5mm}$/TIC $Area_{2.1mm}$
- With 1.5 mm i.d., can we demonstrate the immediate benefits observed without instrument tuning simply by reducing column internal diameter to 1.5 mm?

**Differences in ESI at different flow rates plays role in TIC intensity

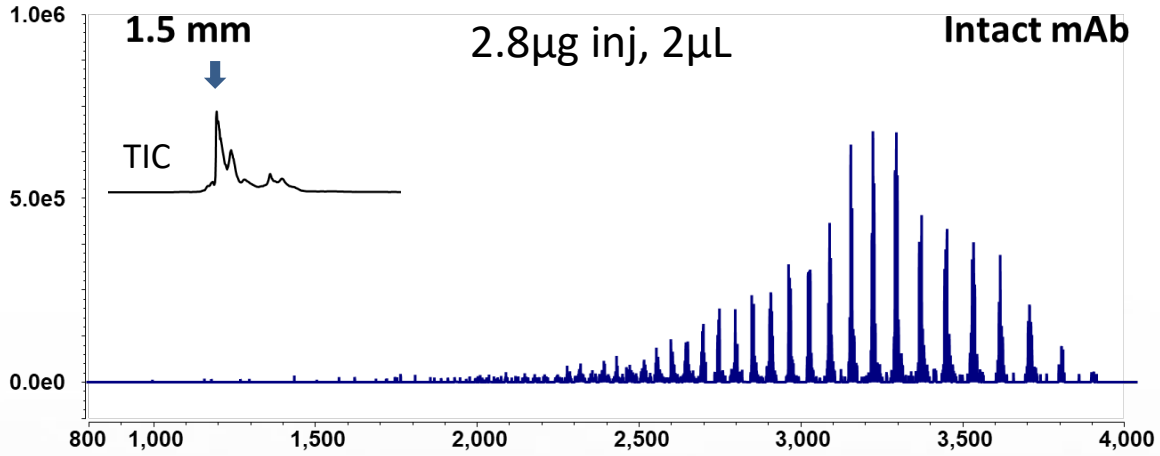
Trastuzumab LC/MS: 1000Å Diphenyl 150mm 2.7µm

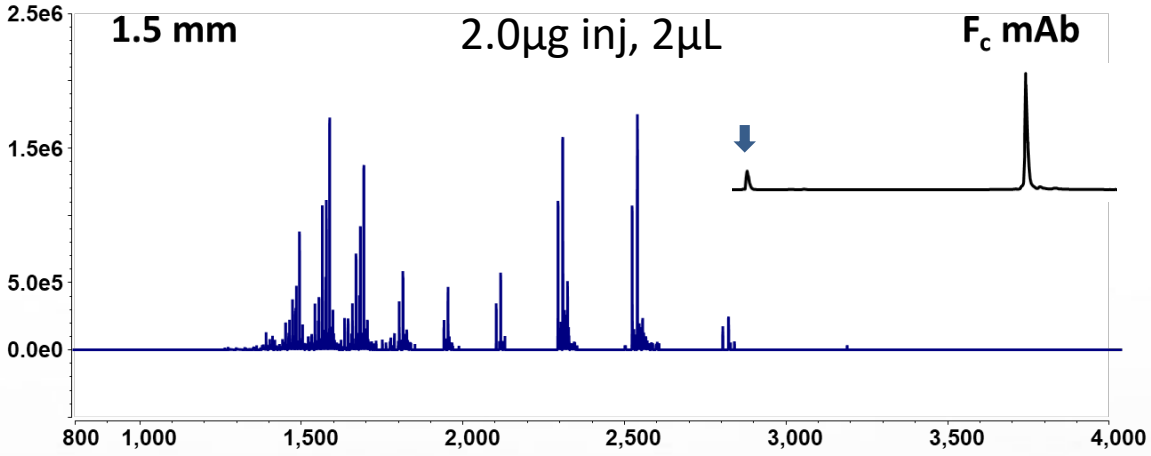
Total Ion Current, Full Scan [800 – 4000m/z], 3pt. MA

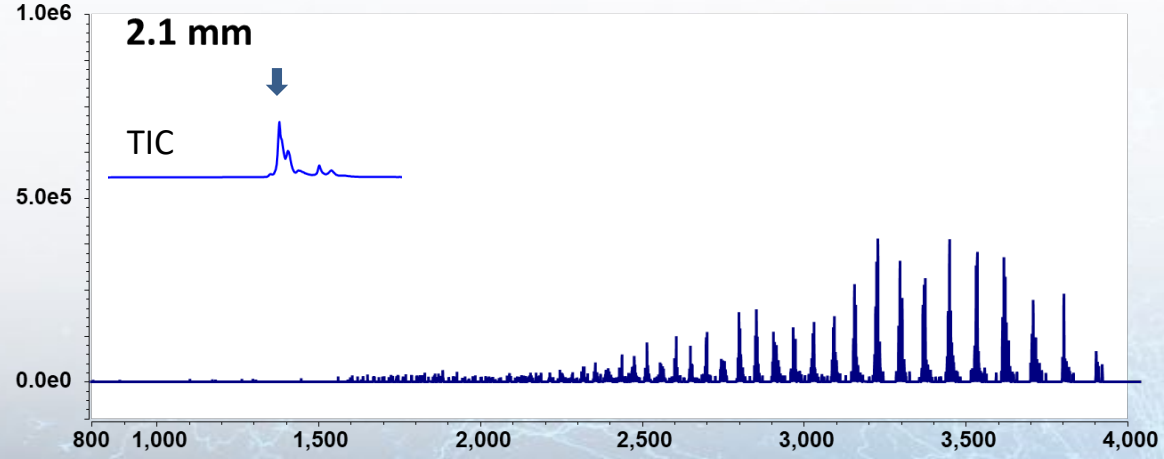


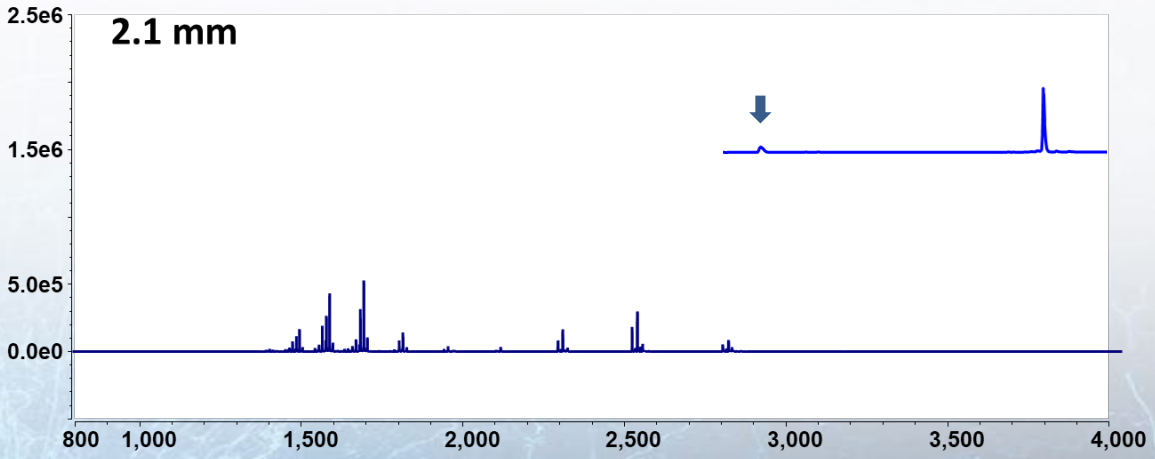
Adapted from Fig. 2 B.P. Libert, J.M. Godinho, S.W. Foster, J.P. Grinias, B.E. Boyes, Implementing 1.5 mm internal diameter columns into analytical workflows, J. Chromatogr. A, 1676 (2022) 463207

Comparison of MS charge state envelopes

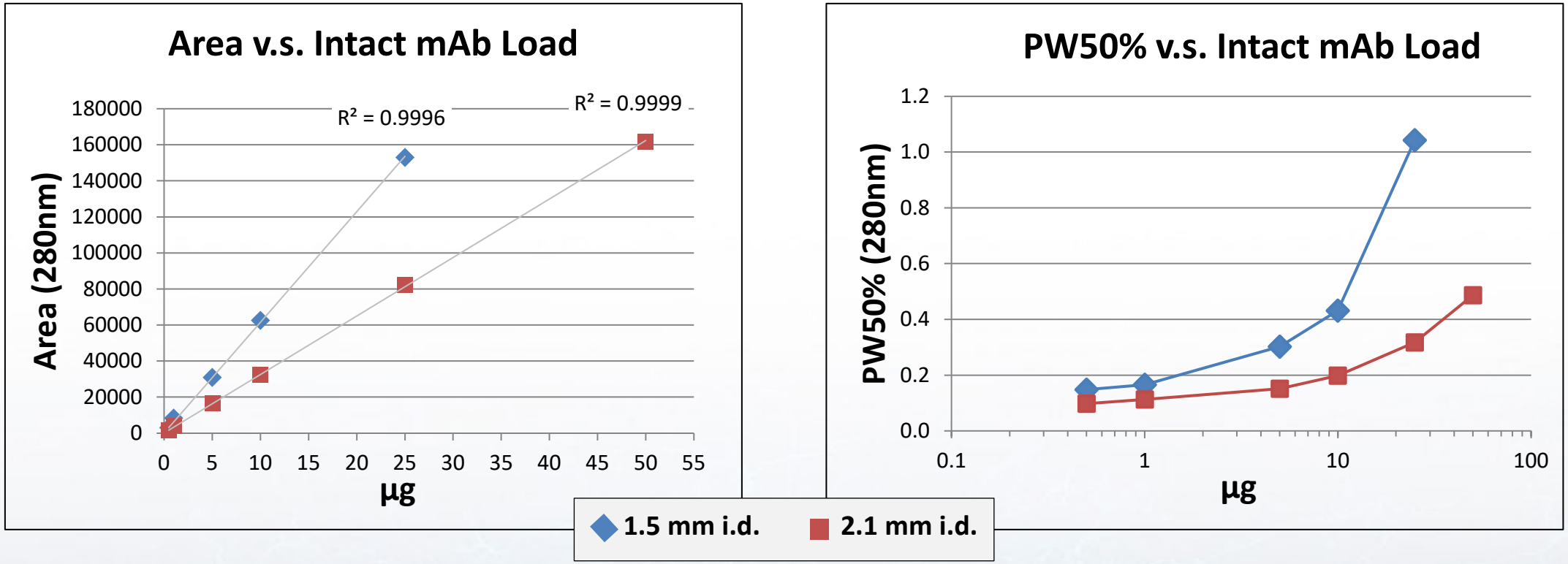








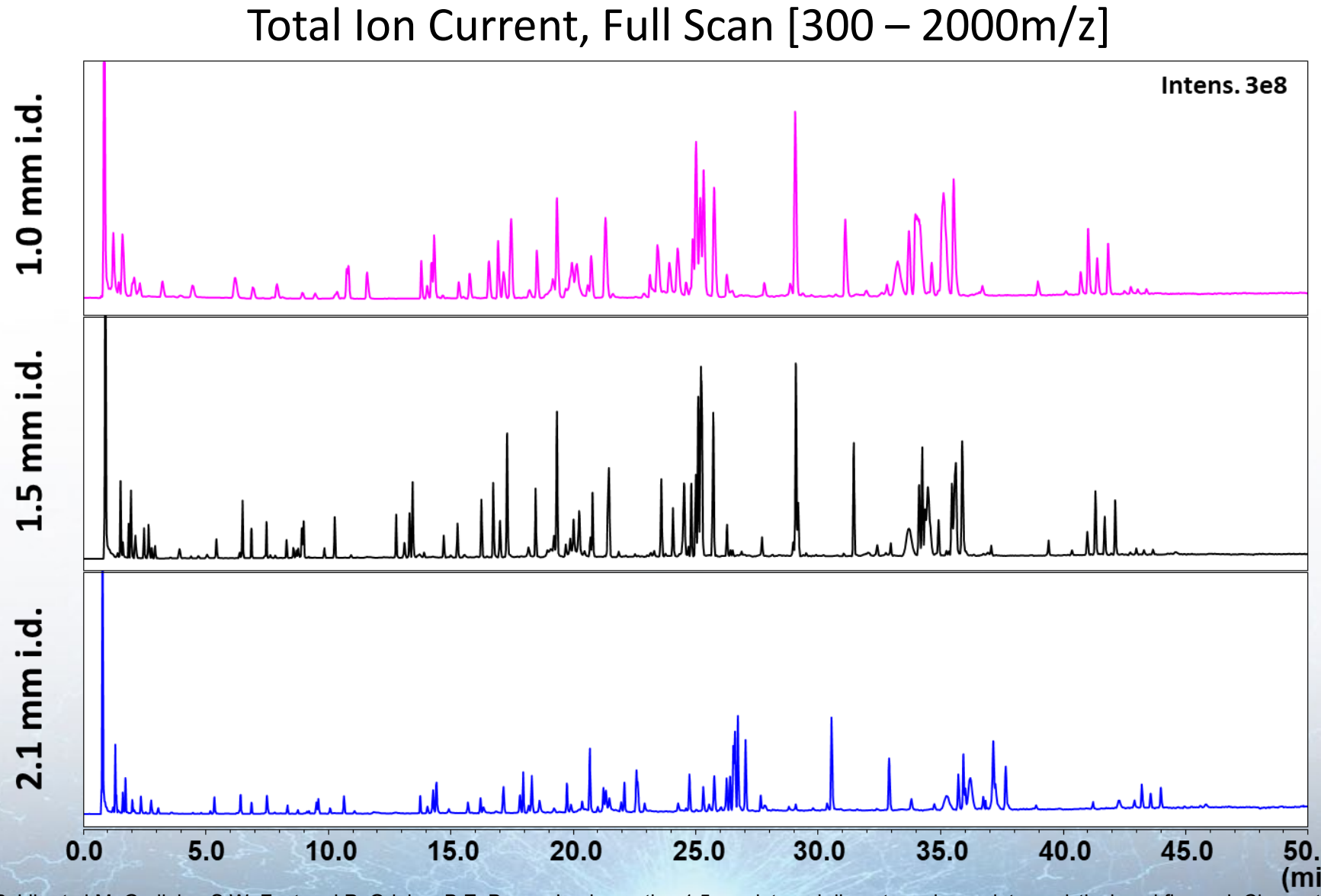
Intact mAb Load Tolerance: 1000Å Diphenyl 2.7µm



- **1.5 x 150mm trastuzumab linear range 0.5 - 25µg; 2.1 x 150mm trastuzumab linear range 0.5 - 50µg**
- **Non-linear isotherm observed at high mass load**

B. Libert, Presented at ASMS 2021 Poster WP 198

mAb tryptic digest on 160Å ES-C18 150mm 2.7µm



0.3µg digest on-column

2-50%B in 60 min, 60°C

<u>Flow: column i.d.</u>
0.1mL/min: 1.0mm
0.2mL/min: 1.5mm
0.4mL/min: 2.1mm

ES-C18 150mm
2.7µm 160Å

(A) 0.1%DFA H2O
(B) 0.1%DFA ACN
(*Gradient Delay*)

Adapted from Fig. 3 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

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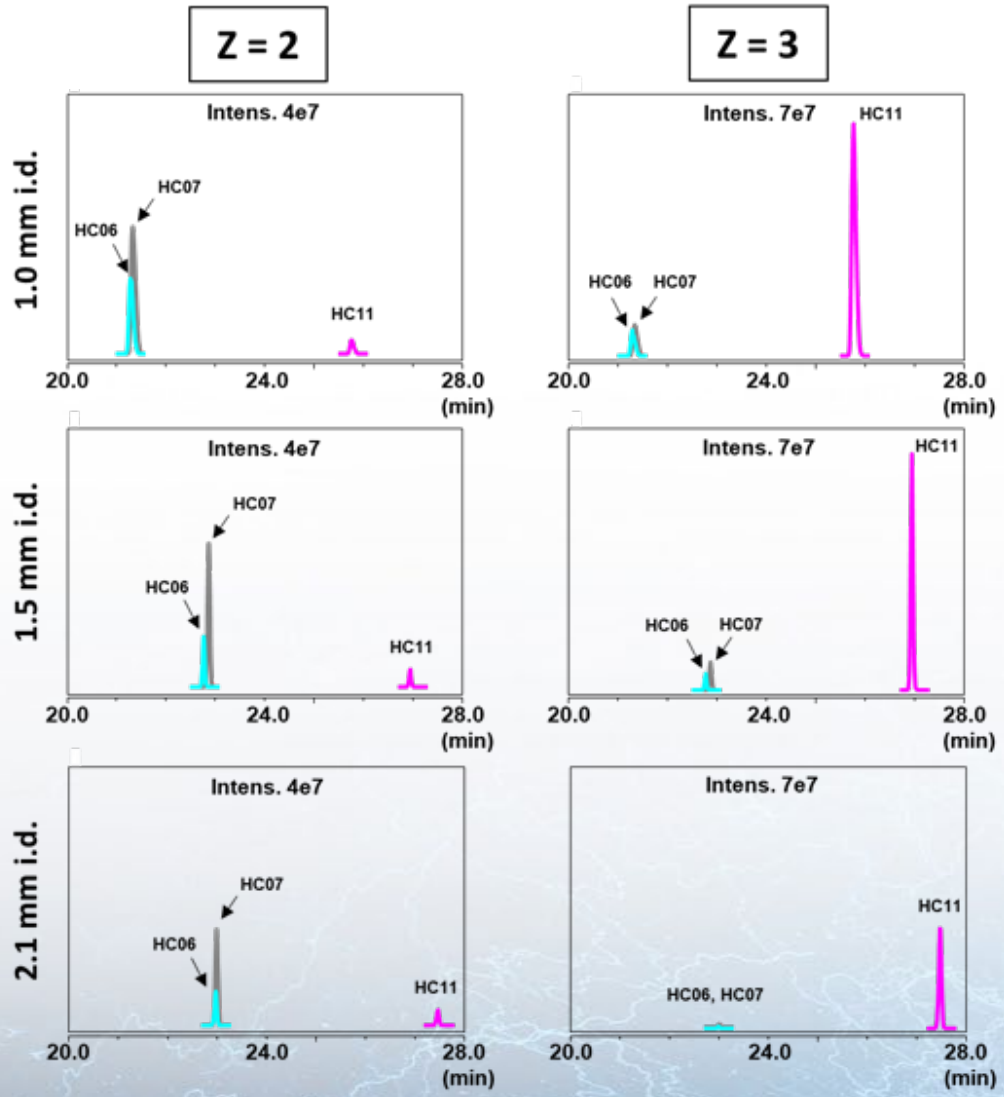
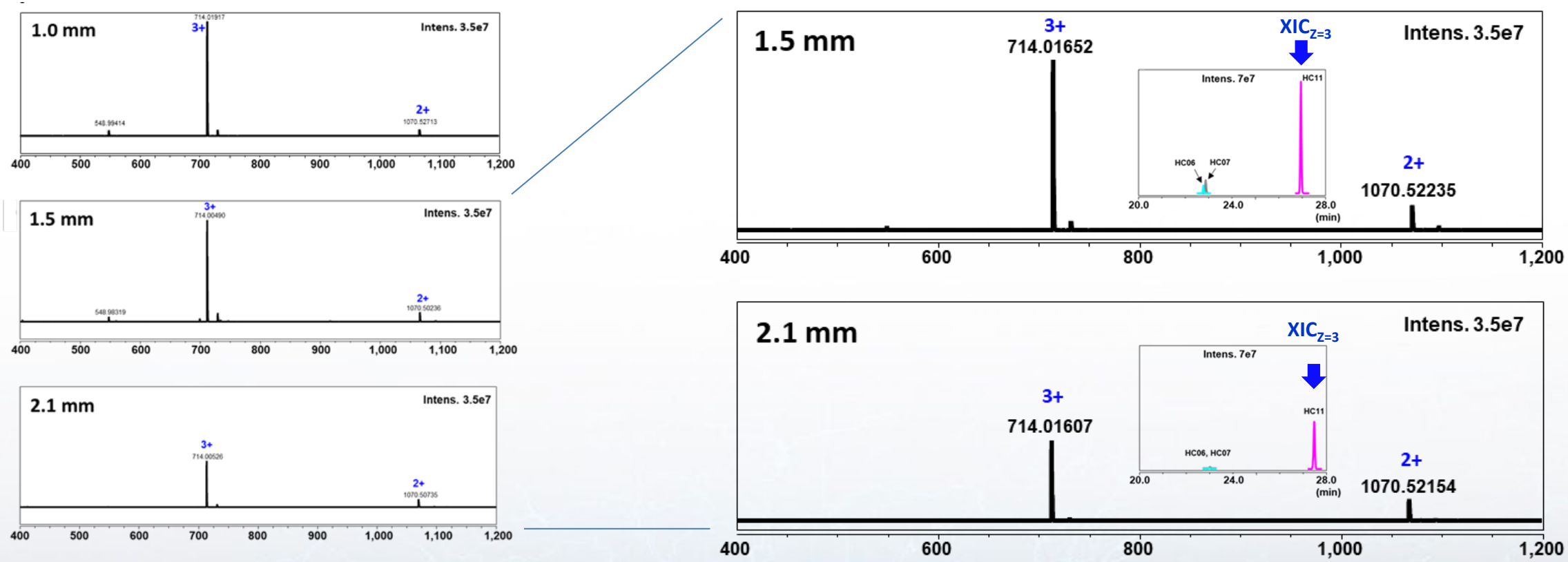


Extracted Ion Current (XIC):
Peptides HC06, HC07, and HC11

- **1.5 mm obtained XIC tryptic peptide species with increased relative m/z intensity at higher charge state occupancies (vs 2.1mm)**
- **In general, the 1.5 mm i.d. column obtained a 1.7-fold increase in XIC area counts (vs 2.1mm)**
- **Differences in ESI at different flow rates play role in XIC intensity**

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

Charge Envelope Comparison of Heavy Chain mAb 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

Summary

2-fold increase in UV area counts for selection of small molecule standards

- By reducing column i.d. from 2.1 mm to 1.5 mm

2-fold or greater increase in MS (TIC/XIC) area counts; moving 2.1 to 1.5 mm i.d.:

✓ Intact mAb, light & heavy chain, IdeS F_c / F_{ab}, tryptic peptides

Solvent consumption decreased by ½ (1.5 mm i.d.) compared to 2.1 mm i.d.

- *Simplicity of implementation with existing HPLC systems*
- More optimization with 1.0 mm i.d. columns for high performance mAb analysis (UV or LCMS)
- *Increased sample loading on 1.5 mm vs 1.0 mm; increased UV/MS signal 1.5 mm vs 2.1 mm (equal load)*

HALO® SPP chemistries currently available to 1.5 mm i.d. users:

✓ **1000 Å C4, Diphenyl**

✓ **160 Å ES-C18**

✓ **90 Å C18, Low pH-C18**

Acknowledgements

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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.