



Enhanced Sensitivity for Peptide and Protein Applications Using the 1.5mm ID Column

Peter Pellegrinelli Stephanie Schuster, Conner McHale, Ben Libert Advanced Materials Technology, Inc.

Introducing the 1.5mm ID Column









"I want more **sensitivity** with my conventional UHPLC system..."

"I need an **easy to use** microflow solution..."

"How can I get **more performance** from my LC and LCMS systems?"

"We have a goal to reduce solvent consumption..."

What does the 1.5 I.D. Deliver?

- ✓ More Sensitivity from conventional UHPLC Systems
- ✓ Higher Ionization Efficiencies from LCMS systems
- Reduced Solvent Consumption compared to 2.1 mm and larger ID columns
- Easy to Implement microflow solution on uHPLC instruments
 Sturdy Design eliminates possible bed disturbance during shipment or use

Benefits of smaller I.D.'s

- Reduced solvent usage
 - Up to 9x less solvent used compared to a 4.6mm I.D.
 - 4x less solvent used compared to a 3.0mm I.D.
 - 2x less solvent used compared to a 2.1mm I.D.
- Increased LOD response (peak heights)
- Increased LOQ (area counts)
- Reduction in usage for valuable samples
 - Increased sensitivity leads to similar response with less sample



Signal increases as I.D. decreases with identical sample load on column.



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Sources of Extracolumn (EC) Dispersion



Advantages of the 1.5mm I.D.

- Why stop at the 1.5mm ID instead of going lower
 - Too much efficiency is lost from ECV
 - Peak widths increased therefore sensitivity is lost
- The 1.5 maintains efficiency
 - The 2.1 is efficient but at the cost of signal
 - The 1.0 has more signal but is less efficient and requires flows that may exceed specification of uHPLC instrument
 - The 1.5 bridges the gap between analytical and capillary LC Instruments and allows excellent separation data to be generated at flows of 200 uL/min which are within typical uHPLC specs.



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



Bio Examples on the 1.5mm ID







HALO 160 Å ES-C18



Ligand: DIISOBUTYLOCTADECYLSILANE USP Designation: L1 ^{H₃} Available Particle Sizes: 2, 2.7, 5 μm Pore Size: 160 Å

HALO 1000 Å DIPHENYL



Ligand: DIPHENYLMETHYL USP Designation: L11 Available Particle Sizes: 2.7 μm Pore Size: 1000 Å

Increased MS Ionization of Intact Trastuzumab



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% npropanol/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 RE lens: 50

Increased MS Ionization of Reduced Trastuzumab



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

Increased Solvent Savings on a Peptide Map



halocolumns.com

Increased Response in UV of Peptides and Small Proteins



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

Full Scan of an intact mAB via LC/MS

2µL inj. trastuzumab IdeS digest (2µg)



- The 1.0mm ID column has an increase in TIC but with a significant increase in peak width
- The 1.5 TIC 2.7 fold increase in area ratio over the 2.1.
- The 1.5 demonstrates benefits over the 2.1 without the required investment into a specialized system.

Conclusion

- The Benefits of the 1.5mm I.D. HPLC column
 - Increased response in all separations that employ a concentration detector
 - Further gains in sensitivity from Increased ionization efficiencies in LCMS separations
 - UHPLC may be used; does not require a capillary flow LC system
 - Easy to install on a standard UHPLC system
 - Sample usage can be reduced
 - Reduced solvent usage compared to higher ID's
- Some uHPLC instruments have more EC dispersion than others
 - Pre/post column tubing and flow cell contribute to dispersion
 - Band broadening can be managed

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





• More options coming soon!

More Info on the 1.5mm ID

- Poster presented 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. <u>https://doi.org/10.1016/j.chroma.2022.463207</u>.
 - 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. <u>https://doi.org/10.1016/j.chroma.2021.462258</u>.
- Talks from HPLC 2022
 - <u>New 1.5mm UHPLC Columns Enable Robust Separations with Increased Sensitivity and Solvent Savings</u>
 - Enhanced Sensitivity for Intact Monoclonal Antibody Analysis via LCMS using a Novel UHPLC Column Design
- The all new 1.5mm I.D.
 - <u>https://halocolumns.com/introducing-the-new-halo-1-5/</u>

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Questions

support@advanced-materials-tech.com

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