Implementing 1.5 mm inner diameter columns into LC-MS bottom-up proteomic workflows

H. Ritchie[1], S. Jadeja[2], D. K. Naplekov[2], J. Lenčo[2]
[1]*Advanced Materials Technology Inc., Wilmington, DE;*
[2] Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Králové, Charles University, Hradec Králové, Czech Republic

HPLC 2023
Duesseldorf, Germany

INTRODUCTION

- With an increased sensitivity of the mass spectrometers, microbore and narrow bore columns are gaining popularity for LC-MS bottom-up proteomic applications where robustness and high sample throughput are preferred over absolute sensitivity.
- In 2018, Lenčo et al. demonstrated that 1.0 mm microbore columns could replace typical nanoflow columns in many proteomic applications with only a 5-fold greater peptide sample needed. [1] Since then, analytical columns with a 1.0 mm inner diameter have been considered a default choice for microflow LC-MS-based proteomic analyses.
- However, columns with an inner diameter of 1.0 mm can inherently not provide the separation performance typically seen for 2.1 mm columns because they suffer from significant trans-column eddy dispersion and are sensitive to extra-column peak broadening.
- Both effects are significantly reduced in the recently introduced column with an untypical inner diameter of 1.5 mm.
- The columns with an inner diameter of 1.5 mm potentially represent a reasonable balance between the sensitivity of methods relying on 1.0 mm inner diameter columns and the chromatographic performance of 2.1 mm columns. [2,3]

AIM OF THE STUDY

In our study, we sought to evaluate the potential of 1.5 mm inner diameter columns for high-flow LC-MS-based bottom-up proteomics.

EXPERIMENTAL

Samples
1) Mixture of 11 iRT peptides with defined retentivity on C18-based stationary phase
2) Tryptic digest of therapeutic monoclonal antibody trastuzumab (Herceptin, Roche)
3) Tryptic digest of a live vaccine strain of a bacterium *F. tularensis*
4) Tryptic digest of Jurkat human cell line

Instrumentation
All analyses were performed using Vanquish Horizon UHPLC instrument coupled to Q Exactive HF-X orbitrap-based mass spectrometer (both from Thermo Fisher Scientific). To minimize the post-column band dispersion, 50 µm outlet capillaries and 75 µm spray needle were used.

Components of the mobile phase
Component A: 0.1% formic acid in water

Component B1: 0.1% formic acid in 80% acetonitrile
Component B2: 0.1% formic acid in 100% acetonitrile (for complex samples)

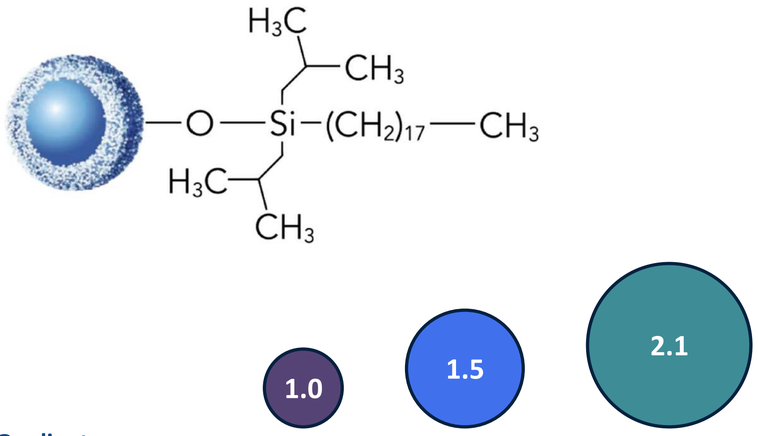
Columns and flow rates

1.0 × 150 mm HALO 160 Å ES-C18, 2.7 µm operated at a flow rate of 51 µL/min

1.5 × 150 mm HALO 160 Å ES-C18, 2.7 µm operated at a flow rate of 115 µL/min

2.1 × 150 mm HALO 160 Å ES-C18, 2.7 µm operated at a flow rate of 225 µL/min

All columns were operated at 55 °C.



Gradients

Sample	
1) 11 iRT peptides	2.5% to 52.5% of comp. B1 in 12 min
2) Tryptic digest of trastuzumab	2.0% to 50.0% of comp. B1 in 22 min
3) *F. tularensis* LVS tryptic digest	2.0% to 50.0% of comp. B2 in 30 min
4) Jurkat cells tryptic digest	2.0% to 40.0% of comp. B2 in 15 min 2.0% to 40.0% of comp. B2 in 30 min 2.0% to 40.0% of comp. B2 in 60 min

ESI settings (auto-default)

Parameter	51 µL/min	115 µL/min	225 µL/min
Sheath gas flow rate	30	37	46
Auxiliary gas flow rate	10	10	10
Sweep gas flow rate	1	1	2
Spray voltage (kV)	3.5	3.5	3.5
Capillary temp. (°C)	250	250	252
Auxiliary gas temp. (°C)	151	230	403
Depth of the ESI needle	halfway between B-C		

MS settings

Parameter	
MS1 range	350-1500 *m/z*
MS1 resolution	60,000
Intensity threshold for MS2	1×10^5
Isolation window for MS2	1.8 *m/z*
Normalized collision energy	27
MS2 resolution	15,000

Data analysis
Software:
- Byonic (Protein Metrics)

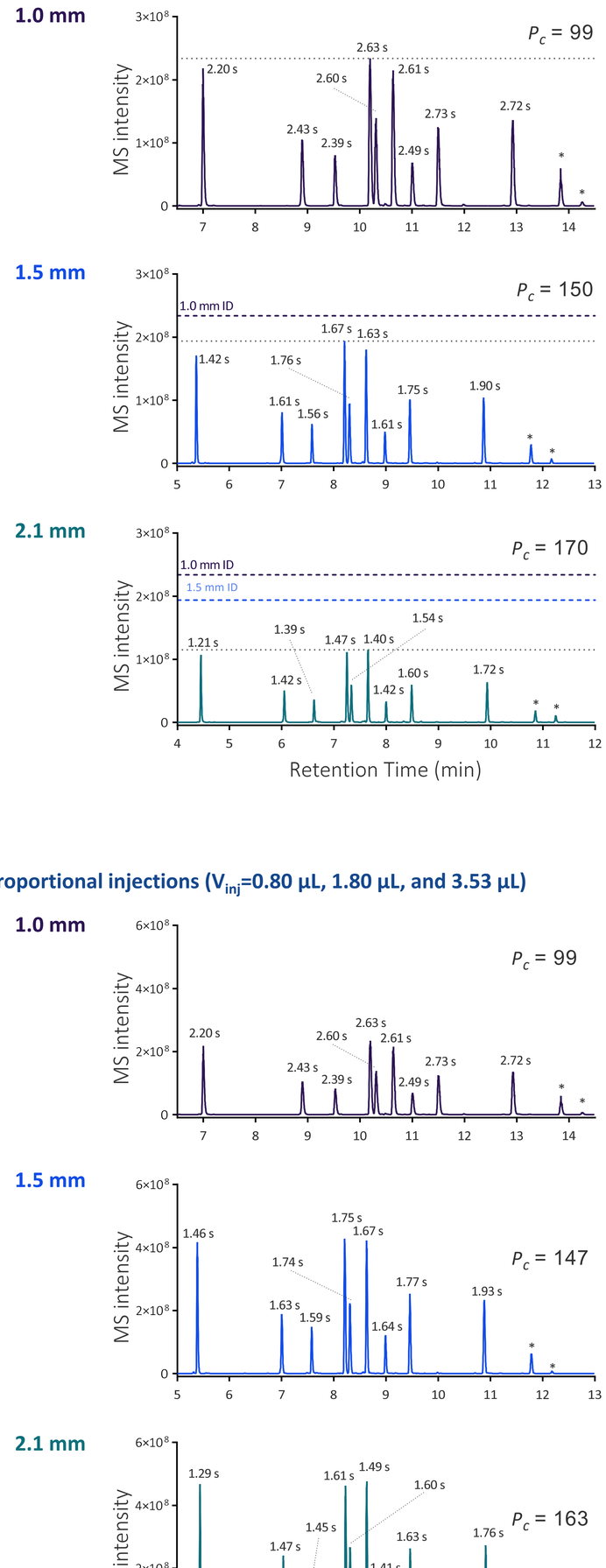
Tolerances used for spectra identification and LC-MS peaks extraction:
- MS1 7 ppm and MS2 17 ppm

Considered modifications and cleavage specificity:
- Carbamidomethyl or thiomethylation @ Cys
- Deamidated @ Asn
- Glu->pyro-Glu or Gln->pyro-Glu @ NTerm
- Oxidation @ Met
- Acetyl @ Protein NTerm (only for Jurkat cell proteins)
- Semitryptic cleavage

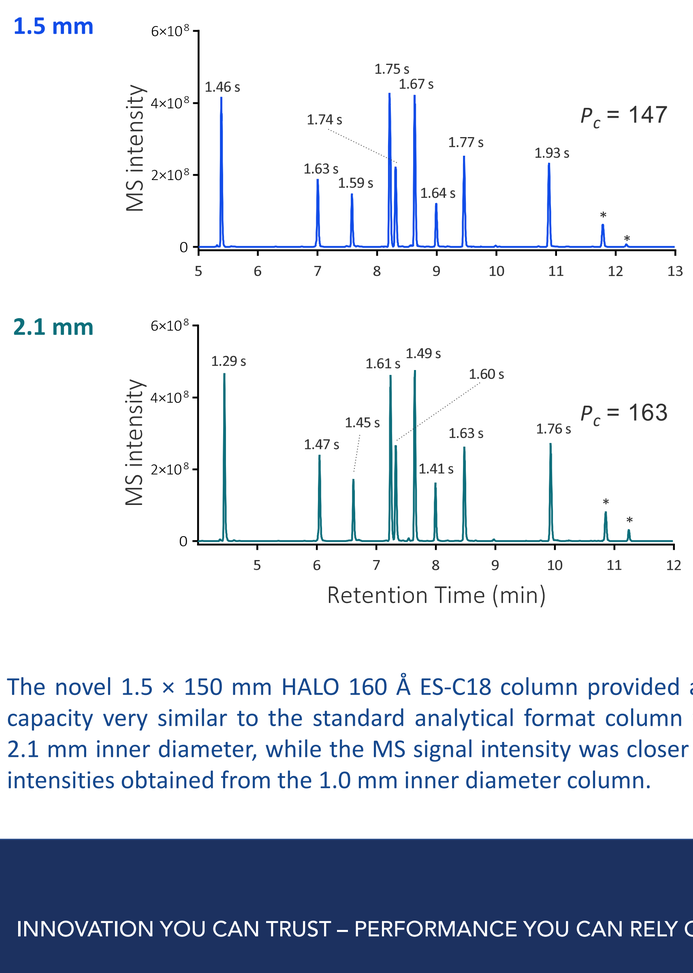
RESULTS

1) LC-MS analysis of simple peptide mixture

Constant injections (V_{inj}=0.80 µL)

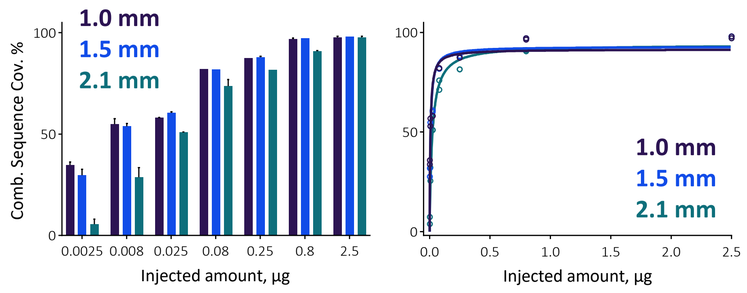


Proportional injections (V_{inj}=0.80 µL, 1.80 µL, and 3.53 µL)



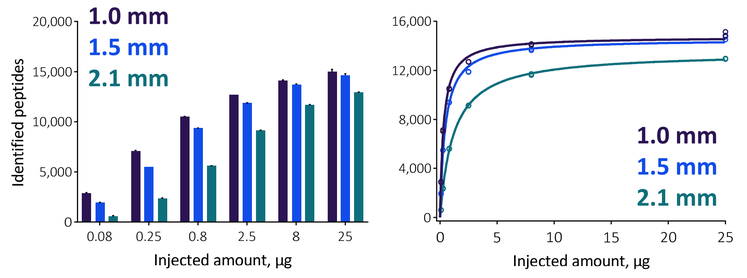
- The novel 1.5 × 150 mm HALO 160 Å ES-C18 column provided a peak capacity very similar to the standard analytical format column with a 2.1 mm inner diameter, while the MS signal intensity was closer to the intensities obtained from the 1.0 mm inner diameter column.

2) Peptide mapping of trastuzumab digest



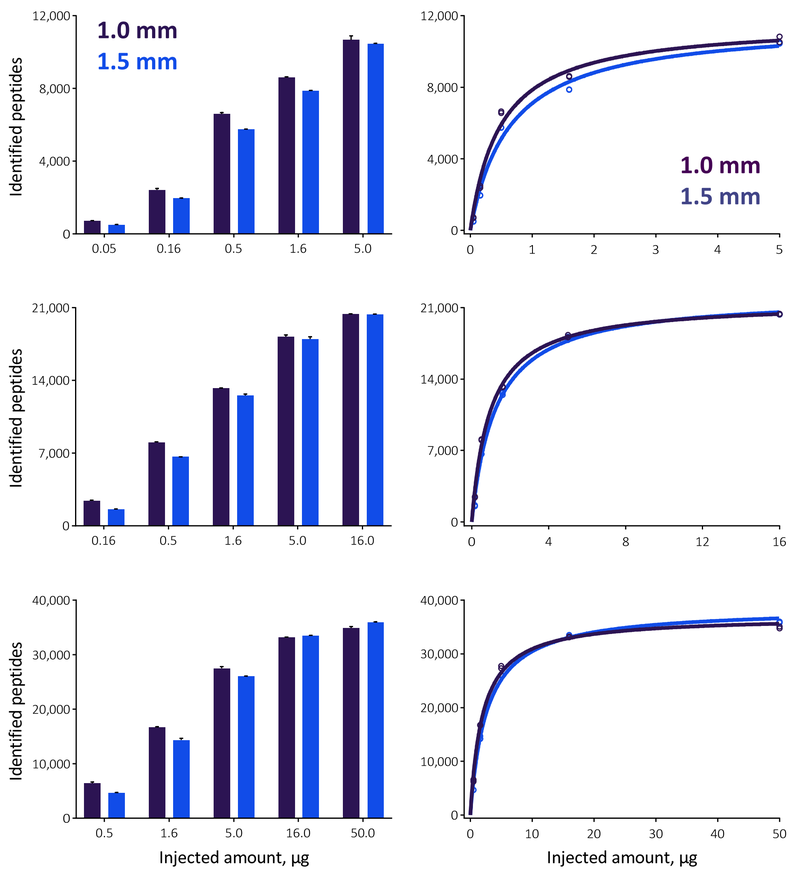
- The novel 1.5 × 150 mm HALO 160 Å ES-C18 column provided almost identical and often slightly better sequence coverage of trastuzumab than the 1.0 × 150 column.
- Both lower inner diameter columns provided 100% sequence coverage from injecting 0.8 µg of the trastuzumab digest.
- Compared to the 2.1 × 150 mm column, the novel 1.5 mm column can save 50% of the solvent and around 70% of the sample to provide the same results with only 0.65 min extra time.

3) Proteomic analysis of tryptic digest of *F. tularensis*



- At higher sample loads, the novel 1.5 × 150 mm HALO 160 Å ES-C18 column provided a very similar number of identified peptides from the medium complex sample as the 1.0 × 150 column. The 2.1 × 150 column provided fewer identified peptides.

4) Proteomic analysis of tryptic digest of Jurkat cells



- At higher sample loads, the novel 1.5 × 150 mm HALO 160 Å ES-C18 column provided a number of identified peptides from the most complex sample involved in the study similar or, in longer gradients, even slightly better than the 1.0 × 150 column.

CONCLUSIONS

- The obtained results demonstrated that the better chromatographic performance of 1.5 mm inner diameter columns could balance the sensitivity of 1.0 mm columns in bottom-up LC-MS proteomics.
- The 1.5 mm inner diameter columns should be of particular interest to researchers seeking a highly robust and high-throughput column format.

REFERENCES

[1] J. Lenčo et al., Conventional-Flow Liquid Chromatography–Mass Spectrometry for Exploratory Bottom-Up Proteomic Analyses. Anal. Chem. 2018, 90, 5381.
[2] S. Fekete et al., Using 1.5 mm internal diameter columns for optimal compatibility with current liquid chromatographic systems. J Chromatogr A 2021, 1650, 462258.
[3] B. P. Libert et al., Implementing 1.5 mm internal diameter columns into analytical workflows. J Chromatogr A 2022, 1676, 463207.

ACKNOWLEDGMENT

S. Jadeja, D. K. Naplekov, and J. Lenčo from the Faculty of Pharmacy in Hradec Králové gratefully acknowledge the financial support of the Project of the Czech Science Foundation (GAČR No. 22-21620S) and the SVV Project No. 260 662.