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APPLICATIONS

bioZen™ 2.6 µm Nano Peptide XB-C18 Column Core-shell Technology Results in Improved Peptide Identifications in HeLa Cell Lysates in Comparison to Competitor Brands With and Without a Nano-Trap Column

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Introduction

Miniaturized LC-MS is employed in situations where the amount of sample for analysis is very small.[1] Some common application areas where miniaturized LC-MS and specifically nano LC-MS are employed are Omics applications including proteomics, metabolomics, lipidomics, and foodomics.[1,3,6-7] Moving to smaller ID (internal diameter) columns while maintaining the same mass injected onto the column improves the ionization efficiency since there is less sample dilution due to the chromatographic process.[1-5] The increased ionization efficiency leads to improved sensitivity.

Another interesting area of research in LC optimization over the last 10 years has been the use of core-shell particles. In general, these particles consist of a solid non-porous core surrounded by a porous silica layer containing the chromatographic stationary phase. While there have been many different explanations for the chromatographic behavior of these particles, it is generally observed and reported that by going from a fully porous particle to a core-shell particle there is a substantial increase in chromatographic efficiency, leading to narrower peaks and higher peak capacities.[8-11] While there has been some variability in the reported performance benefit between different size core-shell and fully porous particles, this has been generally shown to be a function of the performance and availability of different instrumentation.[12-13] Most of the investigations into core-shell particle performance has been limited to analytical scale columns. The ability to utilize the performance benefits of core-shell based nano columns, using commercially available LC-MS equipment, has received little attention.[1-5, 8-11]

In this application we investigate the impact of sub-2 µm fully porous particles and core-shell particles in nano LC-MS with commercially available instrumentation and the effect they have on separation performance as well as protein and peptide identifications in proteomics.

LC Conditions

Column: bioZen Nano 2.6 µm Peptide XB-C18
Waters® nanoEase™ 5 µm M/Z Symmetry C18
Thermo Fisher Acclaim™ PepMap™ 100 3 µm nanoViper™ C18
Dimensions: 150 x 0.075 mm
150 x 0.075 mm
150 x 0.075 mm
Part No.: 00F-4768-AW-11 (bioZen)
Trap: bioZen RP1
Waters nanoEase 1.7 µm M/Z Peptide BEH C18
Thermo Fisher Acclaim PepMap 100 nanoViper
Dimensions: 10 x 0.075 mm
20 x 0.180 mm
20 x 0.1 mm
Part No.: 05N-4252-W (bioZen)
Pressure: 250 bar
Mobile Phase: A: 98 % water, 2 % Acetonitrile, 0.2 % Formic Acid
B: 80 % Acetonitrile, 20 % water, 0.2 % Formic Acid
Gradient:

Time (min)	% B
0	2
7.5	6
90	25
120	40
121	98
136	98

Flow Rate: 350 nL/min
Temperature: 25 °C
LC System: Thermo Fisher EASY-nLC™EASY-nLC™ 1200
Detection: nanoESI
Detector: Orbitrap™ HF
Injection Volume: 2 µL

Results and Discussion

We investigated nano LC-MS separations done in both direct inject and trap and elute modes and compared those to not only a traditionally fully porous 3 µm column but also a fully porous sub-2 µm column. **Figure 1** shows chromatograms obtained from these three columns with a mixture of 20 isotopically labeled peptides. Plots of the average peak width and tailing factor that were obtained in the separations are shown in **Figure 2**. Moving from a fully porous 3 µm column to a fully porous sub-2 µm column results in a 53 % decrease in the average peak width. The backpressure, however, needed to run the separation with the sub-2 µm column was 5200 psi whereas it was 1800 psi with the 3 µm column. Backpressure increases with the square of the particle size so we would expect an increase of 3.1x for the sub-2 µm column which is very close to what was experimentally observed. When we performed the same separation using a 2.6 µm core-shell column we observed peak widths identical to what was obtained on the fully porous sub-2 µm column. The separation on the core-shell particle also required a backpressure of 2000 psi, which is significantly lower than that of the sub-2 µm column.

To investigate the effect that the narrow peak widths of both the core-shell and sub-2 µm column have on peptide and protein identifications, these columns were used to separate and analyze the HeLa S3 quality control standard. In **Figure 3**, the number of protein and peptide identifications that were obtained on a fully porous 3 µm, fully porous sub-2 µm and core-shell 2.6 um column are shown. There was a substantial increase in the number of peptide (12 %) and protein (10 %) identifications observed by moving from the fully porous 3 µm column to either the sub-2 µm fully porous or core-shell 2.6 µm column in direct inject mode.

We also investigated the effect of sub-2 µm fully porous and 2.6 µm core-shell particles in trap and elute mode and their impact on the number of protein and peptide identifications. Since the fully porous columns used for this comparison were manufactured by different vendors, the traps that we used in conjunction with those columns were the ones that were recommended by their respective vendors. In addition to having different particles and stationary phases in the traps, they also had different internal physical dimensions, and therefore volumes. It is important to note this as the experiments were performed in forward elute mode where such differences can play a role in overall performance. In **Figure 4**, the number of protein and peptide identifications that were obtained on a fully porous 3 µm, sub-2 µm and 2.6 µm core-shell column in trap and elute mode are shown. There was a substantial difference between the number of peptide (10 %) and protein (7 %) identifications that were obtained when using the fully porous sub-2 µm column and trap system versus the core-shell column and trap system. This can be explained at least in part by the aforementioned differences in trap architecture. The trap for the fully porous sub-2 µm column was 20 x 0.180 mm whereas the trap for the core-shell based column was 10 x 0.075 mm. **Table 1** shows the average peak widths for the fully porous 3 µm, sub-2 µm, and 2.6 µm core-shell column, both in direct inject and trap and elute mode. We can see that for the fully porous sub-2 µm column and trap system there is an increase in the average peak width from 9 seconds to 12 seconds by the addition of the large volume trap, substantially lowering the peak capacity in trap and elute mode.

Figure 1.
Nano LC-MS/MS chromatograms obtained from a mixture of 20 isotopically labeled peptides injected on 150 x 0.075 mm columns packed with 2.6 µm core-shell XB-C18, Thermo Fisher Acclaim™ PepMap™ nanoViper™ 3 µm fully porous C18, and Waters® NanoEase™ M/Z Peptide BEH 1.7 µm fully porous C18 particles.

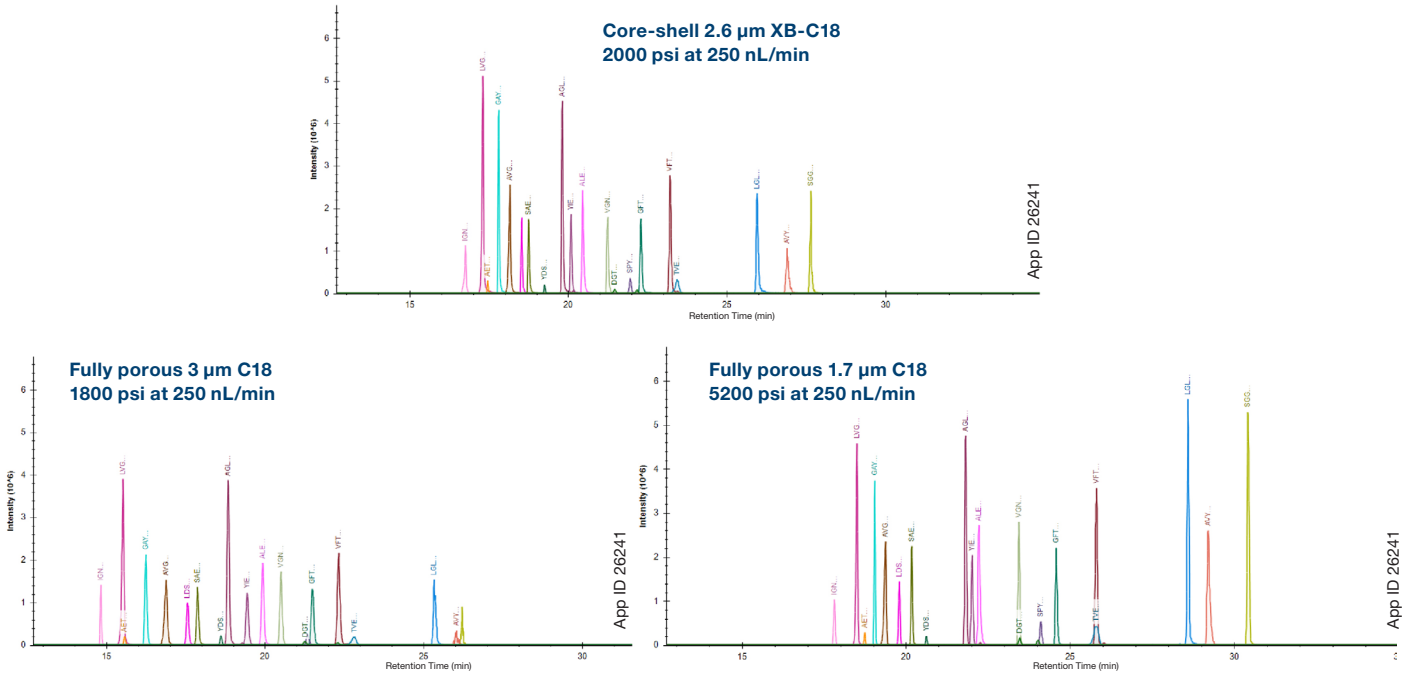


Figure 2.
Peak widths and tailing factors obtained from a mixture of 20 isotopically labeled peptides injected on 150 x 0.075 mm columns packed with 2.6 µm core-shell XB-C18, Thermo Fisher Acclaim™ PepMap™ nanoViper™ 3 µm fully porous C18, and Waters® NanoEase™ M/Z Peptide BEH 1.7 µm fully porous C18 particles, respectively

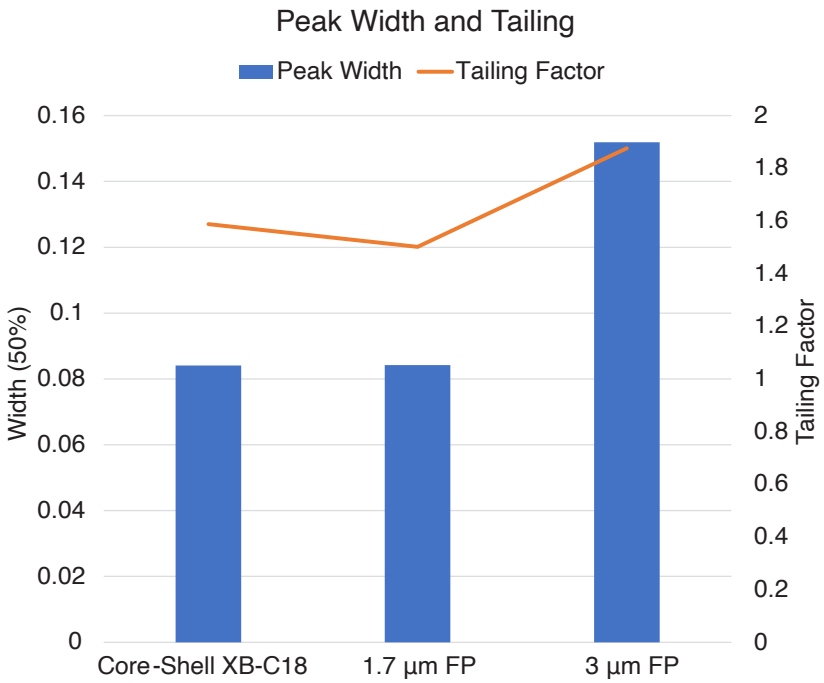
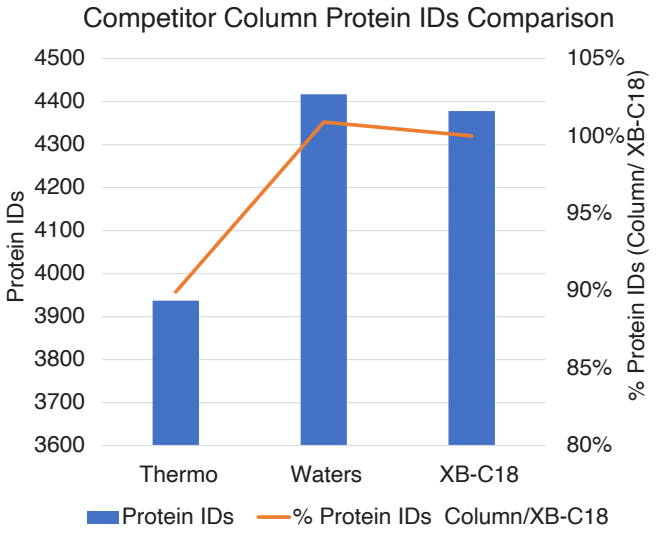


Figure 3.
Number of proteins and peptides that were identified on a nano LC-MS analysis of a digested HeLa sample using a Thermo Fisher Acclaim PepMap nanoViper C18, Waters NanoEase M/Z Peptide BEH C18, and bioZen™ 2.6 µm XB-C18, in direct inject mode.



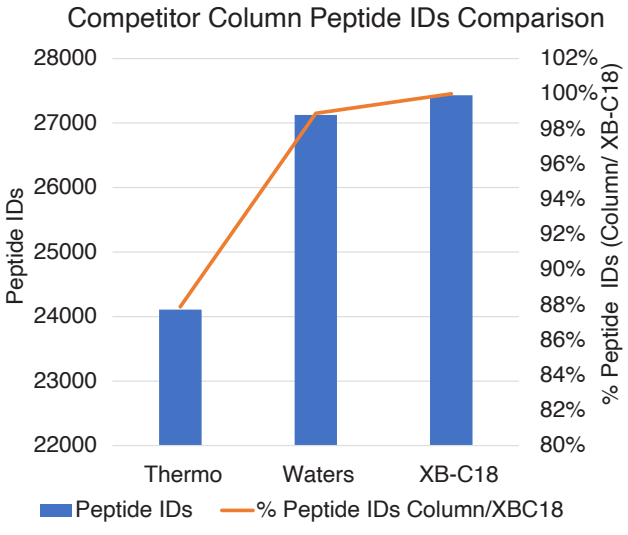
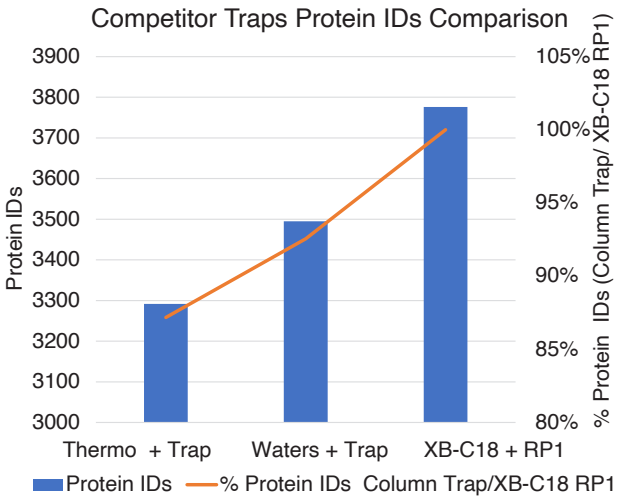


Figure 4.
Number of proteins and peptides that were identified on a nano LC-MS analysis of a digested HeLa sample using a Thermo Fisher Acclaim™ PepMap™ nanoViper™ C18, Waters® NanoEase™ M/Z Peptide BEH C18, and bioZen™ 2.6 µm XB-C18, in trap and elute mode with Thermo Fisher Acclaim PepMap nanoViper, Waters nanoEase M/Z Symmetry C18, and Nano Trap RP-1 (General RP) traps, respectively.



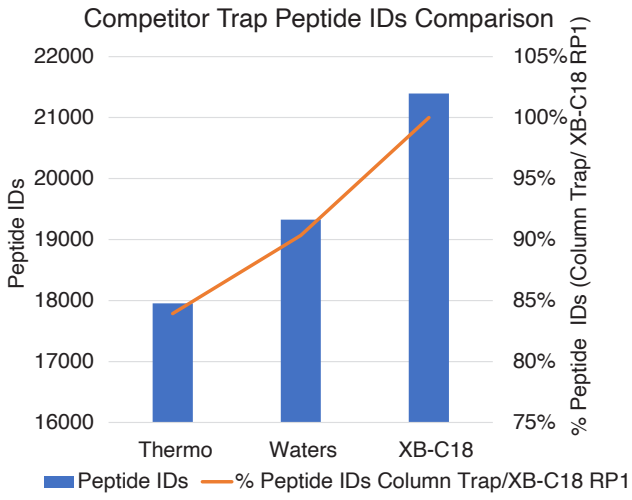


Table 1.
Average peak width and peak capacity for nano column and trap combination with the theoretical loss of peak capacity due to peak broadening and gradient window reduction as well as the observed reduction in protein identifications.

Column	Trap	Average Peak Width (s)	Peak Capacity	Theoretical % Identifications	Observed % Identifications
bioZen 2.6 µm XB-C18 150 x 0.075 mm	None	10	720	—	—
bioZen 2.6 µm XB-C18 150 x 0.075 mm	RP1 10 x 0.075 mm	11	545	76	78
Waters NanoEase M/Z Peptide BEH C18 130 Å, 1.7 µm, 75 µm x 150 mm	None	9	800	—	—
	Waters nanoEase M/Z Symmetry C18 100 Å,5 µm, 180 µm x 20 mm	13	508	63	80
Thermo Acclaim PepMap 100 75 µm x 15 cm, nanoViper C18, 3 µm, 100 Å	None	13	554	—	—
	Thermo Acclaim PepMap 100 µm x 2 cm nanoViper	13	508	92	85

Conclusions

The miniaturization of LC columns is a powerful tool to increase MS sensitivity. By combining nano LC column technology with highly efficient column packing materials such as core-shell particles, a laboratory can increase the benefits through narrower chromatographic peaks. The narrower peaks lead to higher sensitivity as well as increases in the number of protein and peptide identifications by as much as 10 %. As with analytical scale LC separations the choice of column chemistry can greatly affect the separation. The core-shell particle technology also provides the added benefit of producing higher chromatographic efficiency at significantly lower pressures than sub-2 µm fully porous particles. When moving from direct inject to trap and elute, it is important to take into account the additional gradient delay volume that is added and adjust the gradient and the method appropriately as to not lose peak capacity.

References

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Ordering Information

bioZen™ Nano LC Columns with Integrated SecurityLINK™ Fingertight Fitting

Phases	150 x 0.075 mm	250 x 0.075 mm	500 x 0.075 mm
bioZen 3 µm Peptide PS-C18	00F-4771-AW-21	00G-4771-AW-21	–
bioZen 2.6 µm Peptide XB-C18	00F-4768-AW-21	00G-4768-AW-21	–
bioZen 3 µm Polar C18	00F-4782-AW-21	00G-4782-AW-21	–
bioZen 5 µm Peptide XB-C18	–	–	00J-4792-AW-21

bioZen Nano LC Columns with Open Fused-Silica Inlet Fitting

Phases	150 x 0.075 mm	250 x 0.075 mm	500 x 0.075 mm
bioZen 3 µm Peptide PS-C18	00F-4771-AW-11	00G-4771-AW-11	–
bioZen 2.6 µm Peptide XB-C18	00F-4768-AW-11	00G-4768-AW-11	–
bioZen 3 µm Polar C18	00F-4782-AW-11	00G-4782-AW-11	–
bioZen 5 µm Peptide XB-C18	–	–	00J-4792-AW-11

bioZen Trap Columns

Nano Trap Columns		
Phases	**10 x 0.075 mm**	**Unit**
RP1 (General RP)	05N-4252-AW	3/pk
RP2 (Aqueous Stable RP)	05N-4754-AW	3/pk

PEEKLok™ Trap Fittings

Trap Fittings		
Part No.	**Description**	**Unit**
AQ0-7602	PEEKLok fittings with 6-40 thread for 1/32" OD tubing (2 x fittings, 6 x ferrules and 1 x tightening tool)	ea
AQ0-7603	PEEKLok fittings with 6-32 thread for 1/32" OD tubing (2 x fittings, 6 x ferrules and 1 x tightening tool)	ea
AQ0-7600	PEEKLok fittings with 10-32 thread for 1/32"OD tubing with low profile hex head (2 x fittings, 6 x ferrules and 1 x wrench)	ea

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