APPLICATIONS

Going from Small to Tiny: Increasing Sensitivity by Scaling Down from Micro to Nano Flow LC

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Introduction

A powerful tool in discovery proteomics that is used to increase the effectiveness of the MS efficiency is to miniaturize the LC separation. When moving from discovery to routine analysis, it is beneficial to move to micro LC for improved robustness and reliability of results. However, this transition is not always straight forward and can be time consuming. Having scalable LC columns and traps available in both micro and nano formats makes the transition from discovery to routine analysis much faster and easier.

Chromatographic separations are scaled down by decreasing the column inner diameter (ID) while maintaining linear velocity. This results in a decreased flow rate, increased analyte concentration and increased sensitivity. As opposed to analytical flow, micro and nano flow deal with much smaller column IDs and flow rates (**Table 1**). While Micro flow offers excellent sensitivity and sample throughput, Nano flow can be an excellent option when sample amount is limited (**Table 1**), and high sensitivity is needed in order to achieve low limits of detection and quantitation. In this technical note we explore column miniaturization effects attained by reducing column ID from 300 µm to 75 µm and its effects on sensitivity.

Results and Discussion

20 different peptides at the same concentration were analyzed on both the micro and nano column (**Table 2**). By decreasing column ID from micro to nano, there was a 10-fold increase in sensitivity across all peptides monitored in this method (**Figure 1**). There was also an increase in peak area (**Figure 2**) and ion intensity (**Figure 3**) across all peptides that were analyzed when scaling down from a micro to a nano column. This is summarized in **Table 3** as an average 7.8 fold increase when moving from a micro LC column to a nano LC column. There was also an increase in the signal-to-noise (S/N) ratio as the column ID was decreased, further suggesting an increase in sensitivity of detection in the nano LC column versus the micro LC column.

Table 1

Column Type	Column Internal Diameter (ID)	Typical Flow Rates	Typical Sample Load
Nano LC	50-75 µm	200-500 nL/min	100-200 ng
Micro LC	150-500 µm	1-50 µL/min	1-10 µg
Analytical	1-8 mm	0.02-5mL/min	0.010- 1.5 mg

LC Conditions

Column: Kinetex® 2.6 µm XB-C18
bioZen™ 2.6 µm Peptide XB-C18
Dimensions: 150 x 0.3 mm
150 x 0.075 mm
Part No.: 00F-4496-AC
00F-4768-AW-21
Pressure (bar): 200 bar
Mobile Phase: A: 0.1 % Formic Acid in Water
B: 0.1 % Formic Acid in Acetonitrile
Gradient:

Time (min)	% B
0	3
30	40
35	70
40	80
41	3
45	3

Flow Rate: Kinetex: 4 µL/min
bioZen: 250 nL/min
Temperature: 25 °C
LC System: NanoLC™ 425 (SCIEX®)
Detection: MS/MS
Detector: 6500 QTRAP® (SCIEX)
Injection Volume: 1 µL
Sample: See Table 2

MS Conditions

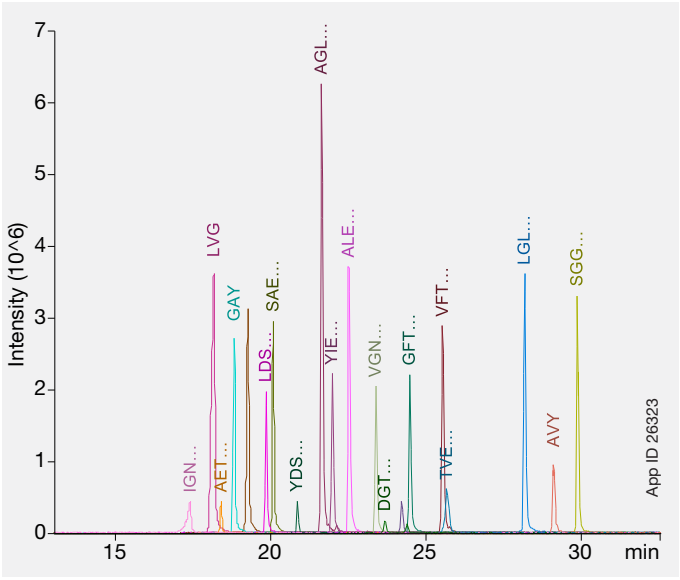
CUR: 30
IS: 2500
GS1: 35
GS2: 0
IHT: 150
CAD: Low
EP: 10
DP: 80
IHT: 150
CXP: 15

Table 2.

ID	Q1	Q3	Parameter	Value
PepCalMix.AETSELHTSLK.+3y5.heavy	408.55	593.35	CE	24.3
PepCalMix.AGLIVAEGVTK.+2y7.heavy	533.32	711.41	CE	26.5
PepCalMix.ALENDIGVPSDATVK.+2y7.heavy	768.9	725.39	CE	35.6
PepCalMix.AVGANPEQLTR.+2y6.heavy	583.31	753.41	CE	31.5
PepCalMix.AVYFYAPQIPLYANK.+2y6.heavy	883.47	713.41	CE	47.9
PepCalMix.DGTFAVDGPGVIAK.+2y7.heavy	677.86	649.41	CE	33.6
PepCalMix.GAYVEVTAK.+2y5.heavy	473.26	555.32	CE	23.9
PepCalMix.GFTAYYIPR.+2y5.heavy	549.29	721.39	CE	26.9
PepCalMix.IGNEQGVSR.+2y8.heavy	485.25	856.41	CE	26.7
PepCalMix.LDSTSIPVAK.+2y4.heavy	519.8	422.29	CE	34
PepCalMix.LGLDFDSFR.+2y6.heavy	540.27	796.35	CE	26.7
PepCalMix.LVGTPAEER.+2y7.heavy	491.27	769.37	CE	23.4
PepCalMix.SAEGLDASASLR.+2y7.heavy	593.8	729.38	CE	30
PepCalMix.SGGLLWQLVR.+2y5.heavy	569.83	711.42	CE	29.6
PepCalMix.SPYVITGPGVVEYK.+2y9.heavy	758.91	957.51	CE	37.8
PepCalMix.TVESLFPEEAETPGSAVR.+2y6.heavy	964.98	596.34	CE	42.4
PepCalMix.VFTPLEVDVAK.+2y8.heavy	613.35	878.51	CE	26.3
PepCalMix.VGNEIQYVALR.+2y6.heavy	636.35	759.44	CE	33.5
PepCalMix.YDSINNTEVSGIR.+2y9.heavy	739.36	999.51	CE	40.3
PepCalMix.YIELAPGVDNSK.+2y7.heavy	657.34	724.37	CE	29

Figure 1.
Ion chromatogram comparison of the MRM traces using bioZen™ column with nano flow (left) and Kinetex® column with micro flow (right). The MRM chromatogram for the 20 peptides shows an increase in sensitivity of about 10-fold.

bioZen Nano Column



Kinetex Micro Column

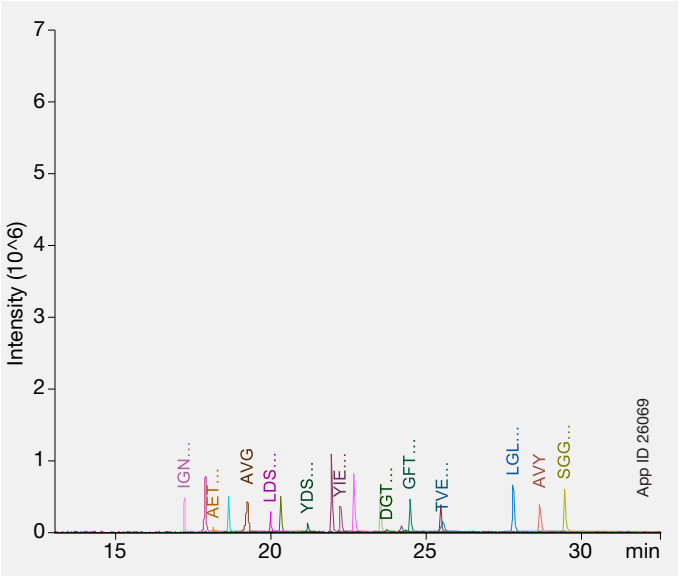


Figure 2.
Area increase when scaling down from the Kinetex column with micro flow (gray) to the bioZen column with nano flow (blue) column for each of the 20 peptides.

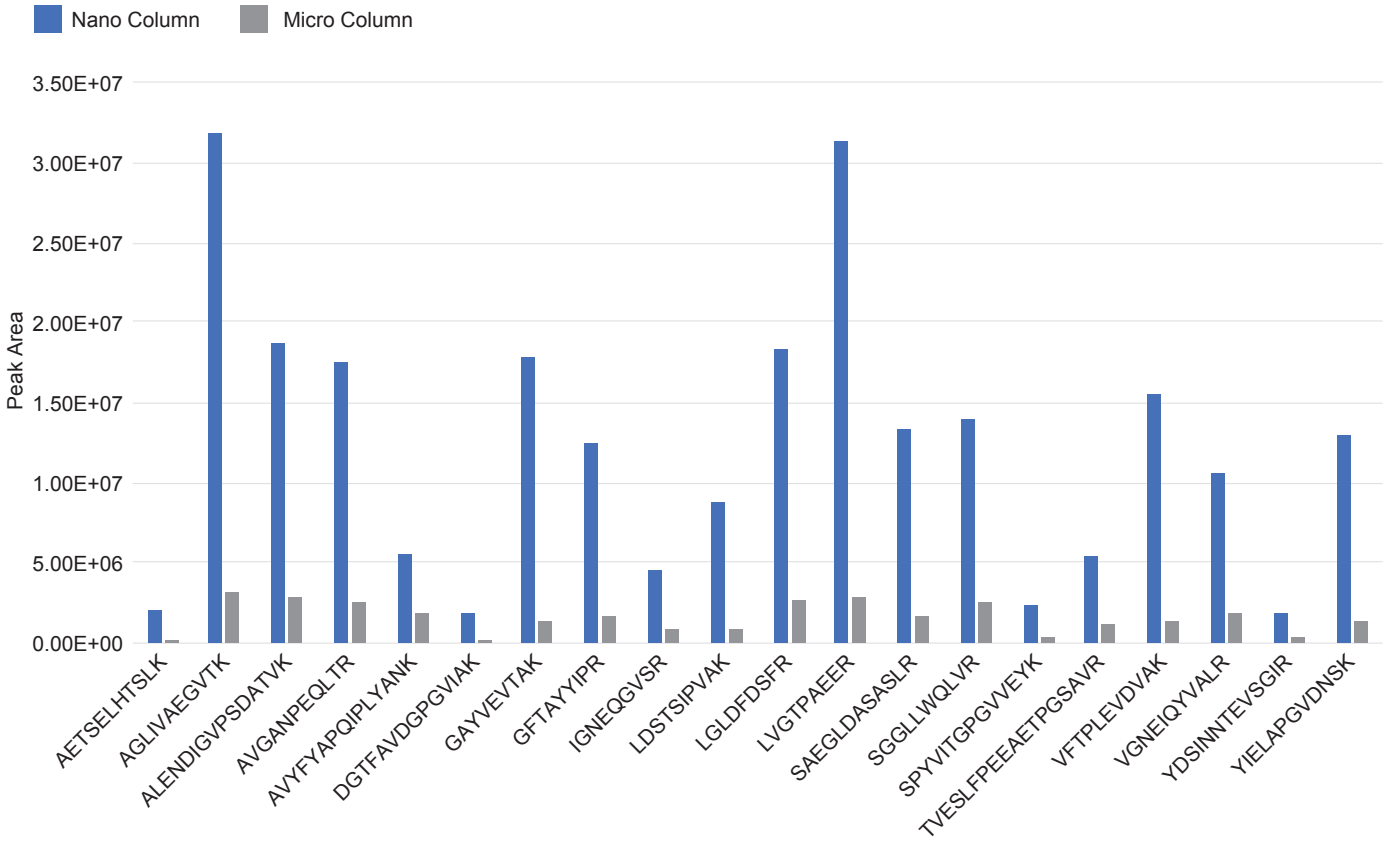


Figure 3.
Ion intensity increase when scaling down from the Kinetex® column with micro flow (gray) to the bioZen™ column with nano flow (blue) for each of the 20 peptides.

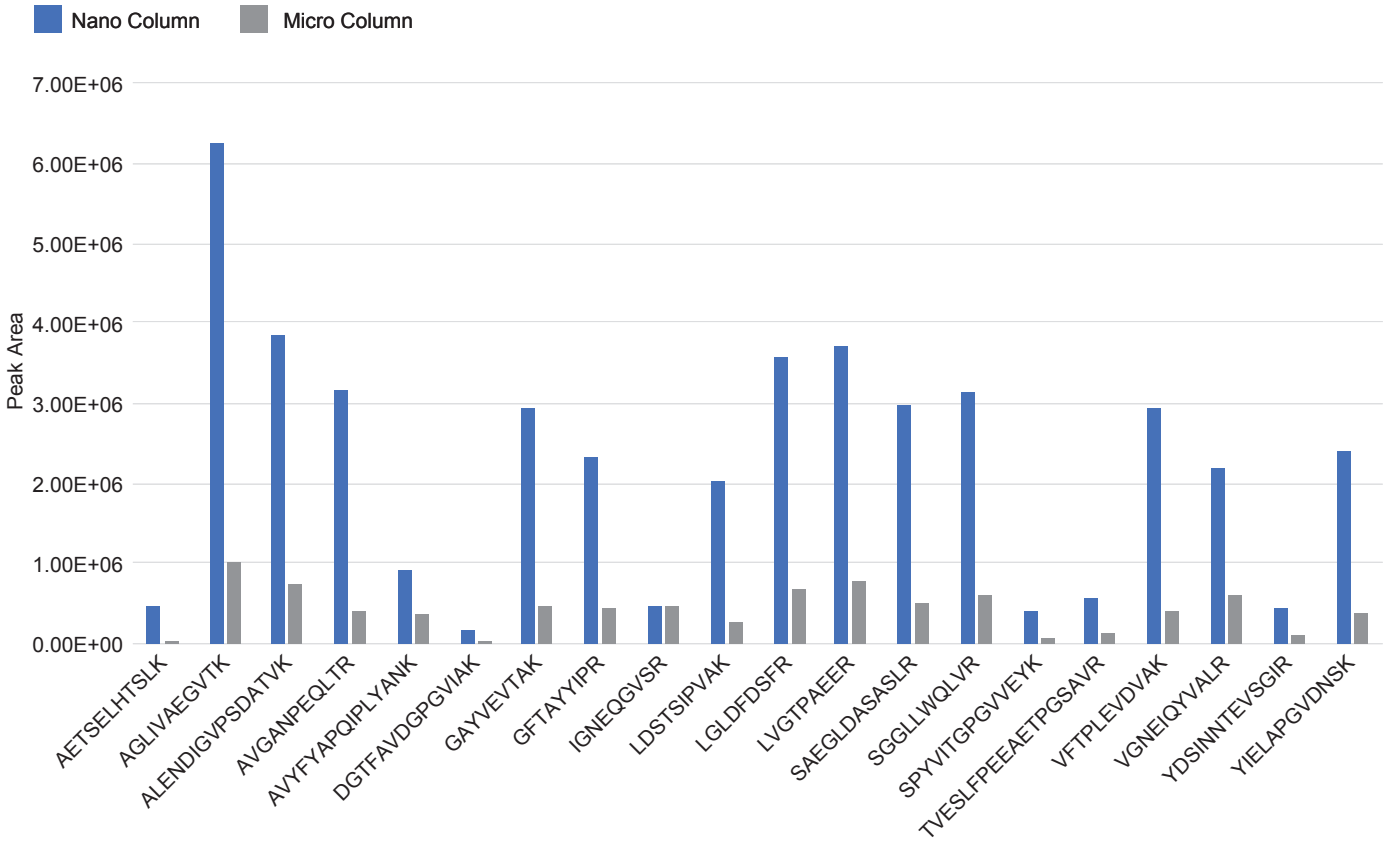


Table 3.
Increase in peak area, peak height, and S/N when moving from the 0.3 mm ID Kinetex micro column to the 0.075 mm ID bioZen nano column for each of the isotopically labeled peptides.

Peptide	Increase in Relative Peak Area	Increase in Relative Peak Height	Increase in Relative S/N
PEPCALMIX.AETSELHTSLK.+3Y5.HEAVY	13	9	9
PEPCALMIX.AGLIVAEGVTK.+2Y7.HEAVY	10	6	3
PEPCALMIX.ALENDIGVPSDATVK.+2Y7.HEAVY	7	5	4
PEPCALMIX.AVGANPEQLTR.+2Y6.HEAVY	7	8	6
PEPCALMIX.AVYFYAPQIPLYANK.+2Y6.HEAVY	3	2	2
PEPCALMIX.DGTFAVDGPGVIAK.+2Y7.HEAVY	7	7	7
PEPCALMIX.GAYVEVTAK.+2Y5.HEAVY	14	6	6
PEPCALMIX.GFTAYYIPR.+2Y5.HEAVY	7	5	5
PEPCALMIX.IGNEQGVSR.+2Y8.HEAVY	5	1	1
PEPCALMIX.LDSTSIPVAK.+2Y4.HEAVY	10	8	6
PEPCALMIX.LGLDFDSFR.+2Y6.HEAVY	7	6	3
PEPCALMIX.LVGTPAEER.+2Y7.HEAVY	11	5	3
PEPCALMIX.SAEGLDASASLR.+2Y7.HEAVY	8	6	6
PEPCALMIX.SGGLLWQLVR.+2Y5.HEAVY	6	6	6
PEPCALMIX.SPYVITGPGVVEYK.+2Y9.HEAVY	6	5	5
PepCalMix.TVESLFPEEAETPGSAVR.+2y6.heavy	4	4	4
PEPCALMIX.VFTPLEVDVAK.+2Y8.HEAVY	22	8	7
PEPCALMIX.VGNEIQYVALR.+2Y6.HEAVY	6	4	4
PEPCALMIX.YDSINNTEVSGIR.+2Y9.HEAVY	5	3	3
PEPCALMIX.YIELAPGVDNSK.+2Y7.HEAVY	9	7	7

Conclusion

Limited sample size can cause low sensitivity and detectability on normal analytical LC columns and traps. Scaling down the column ID increases the robustness of the ion intensity that is detected. Micro LC columns are one way to do this. However, decreasing the column ID to a nano LC column, provides even better results for small sample volumes. We tested this concept by comparing a Kinetex micro LC column to a bioZen nano LC column and as the data presented here suggests, nano LC columns offer much higher sensitivity, greater peak area, and increased ion intensity when sample volume is a limiting factor. However, by maintaining the same linear velocity and gradient slope, the selectivity of the separation is maintained and so no method optimization is needed when moving from nano to micro or vice versa.

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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-4605-AW-21

bioZen Nano LC Columns with Fused Silica Inlet/Outlet Fittings

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-4601-AW-11

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

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

Kinetex® 2.6µm Micro LC Columns (mm)

Phases	50 x 0.30	100 x 0.30	150 x 0.30	50 x 0.50	100 x 0.50	150 x 0.50
XB-C18	00B-4496-AC	00D-4496-AC	00F-4496-AC	00B-4496-AF	00D-4496-AF	00F-4496-AF

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