Investigating the effect of column ID on sensitivity when using Micro LC columns

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Overview

Micro flow application requires the use of columns and traps with a smaller inner diameter (ID) that allow lower flow rates than traditional analytical methods while still maintaining the desired linear velocity. Through the use of lower flow rates, the ionization efficiency in LC-MS is increased providing greater sensitivity when providing greater sensitivity where the amount of available sample is limited.

In this application note, we investigate the ability to scale separations developed on traditional analytical columns (2.1 mm ID) to micro flow rate scale columns (0.3 and 0.5 mm ID) to take advantage of the sensitivity gains through miniaturization without the need for new method development and optimization. The comparison was generated using a representative sample of 20 stable-isotope-labelled (SIL) peptides under typical reversed-phase mobile phase conditions using a SCIEX® 5500 QTRAP MS/MS detector. This application demonstrates the effect on sensitivity and selectivity when column ID is reduced into a micro LC format.

Micro LC Conditions for All Examples

Column: Kinetex® 2.6 µm XB-C18
Dimension: 50 x 2.1 mm
50 x 0.5 mm
50 x 0.3 mm
Part No. 00B-4496-AN
00B-4496-AF
00B-4496-AC
Mobile Phase: A: Water with 0.1 % Formic Acid
B: Acetonitrile with 0.1 % Formic Acid
Gradient:

Time (min)	% B
0	3
10	40
12	80
14	80
15	3
20	3

Flow Rate: See Figures for Adjusted Flow Rate for Maintained Linear Velocity
Temperature: Ambient (25 °C)
Detector: MS/MS SCIEX® QTRAP® 5500
Injector Temp.: 4 °C
Column Temp.: 25 °C
Injection Volume: See Figures for Adjusted Flow Rate for Scaled Injection Volume in respect to column ID
Sample: 20 stable-isotope-labeled (SIL) Peptide mix

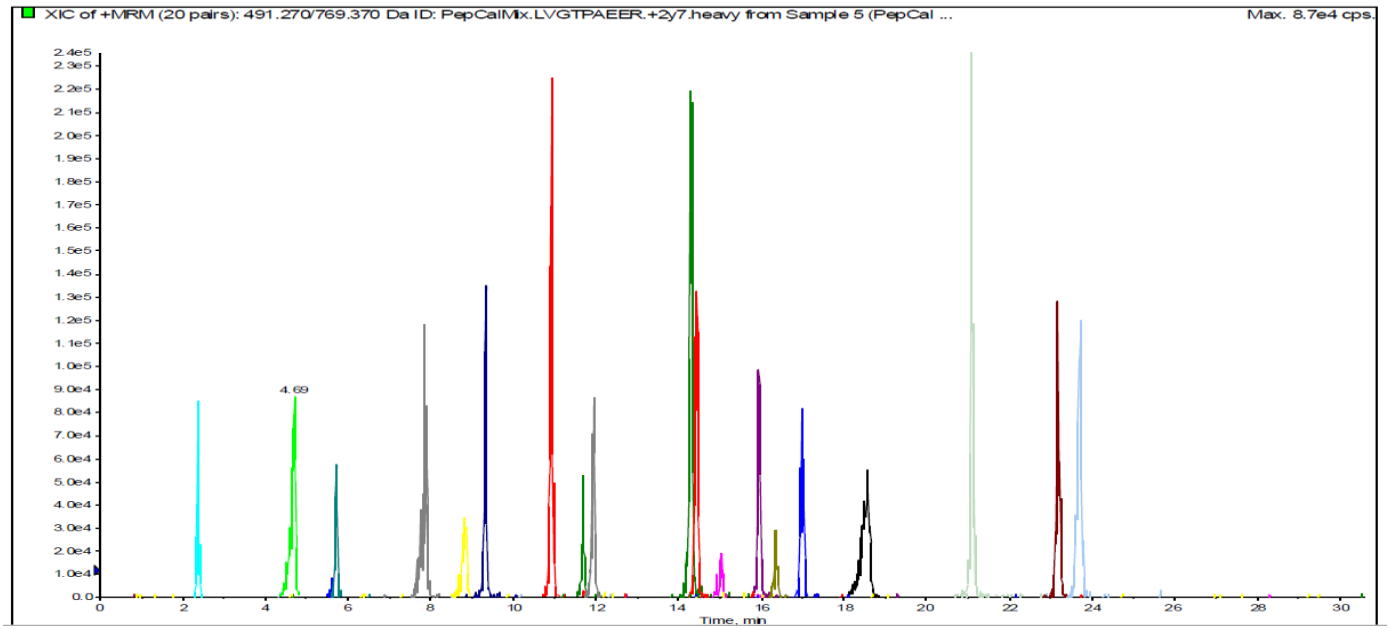
Precursor Mz	Product Mz	Peptide Sequence
485.25	856.41	IGNEQGVSR
491.27	769.37	LVGTPAEER
408.55	593.35	AETSELHTSLK
473.26	555.32	GAYVEVTAK
583.31	753.41	AVGANPEQLTR
519.8	422.29	LDSTSIPVAK
593.8	729.38	SAEGLDASASLR
739.36	999.51	YDSINNTEVSGIR
533.32	711.41	AGLIVAEGVTK
657.34	724.37	YIELAPGVDNSK
768.9	725.39	ALENDIGVPSDATVK
636.35	759.44	VGNEIQYVALR
677.86	649.41	DGTFAVDGPGVIAK
758.91	957.51	SPYVITGPGVVEYK
549.29	721.39	GFTAYYIPR
964.98	596.34	TVESLFPEEAETPGSAVR
613.35	878.51	VFTPLEVDVAK
540.27	796.35	LGLDFDSFR
883.47	713.41	AVYFYAPQIPLYANK
569.83	711.42	SGGLLWQLVR

Results and Discussion

To simulate this, we injected increasingly smaller injection volumes on the analytical scale column and the micro scale columns and compared the peak height response at the various injection amounts. The same peptide mixture as the previous example was used for this investigation for consistency of comparison. At a 20 µL injection (200 fmol on column) good sensitivity was achieved on the analytical scale column, however, when the mass injected was decreased to 10 fmol the detection of the peptides above signal-to-noise was challenging because it was below the limits of detection configuration (**Figure. 1**).

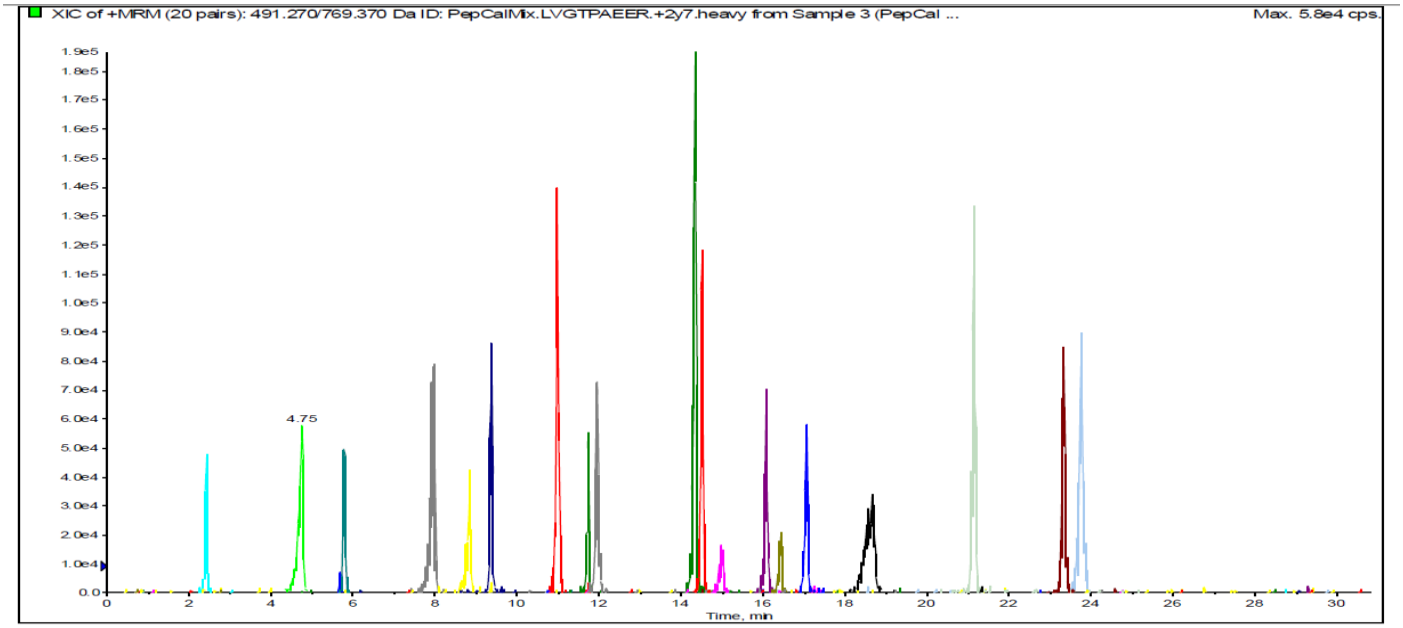
Figure 1. Comparison of mass load using the Kinetex XB-C18 2.6 µm 50 x 2.1 mm run at 500 µL/min

Fig. 1. A: Injection Volume: 200 fmol



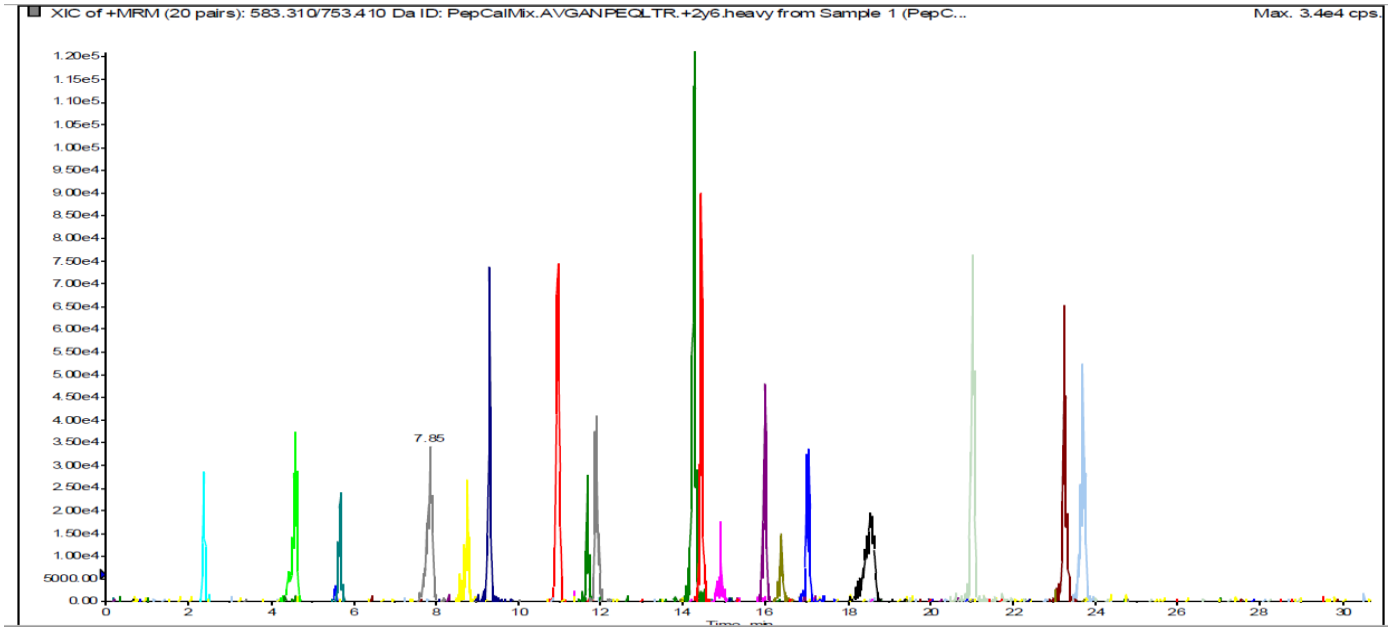
App ID 25890

Fig. 1. B: Injection Volume: 150 fmol



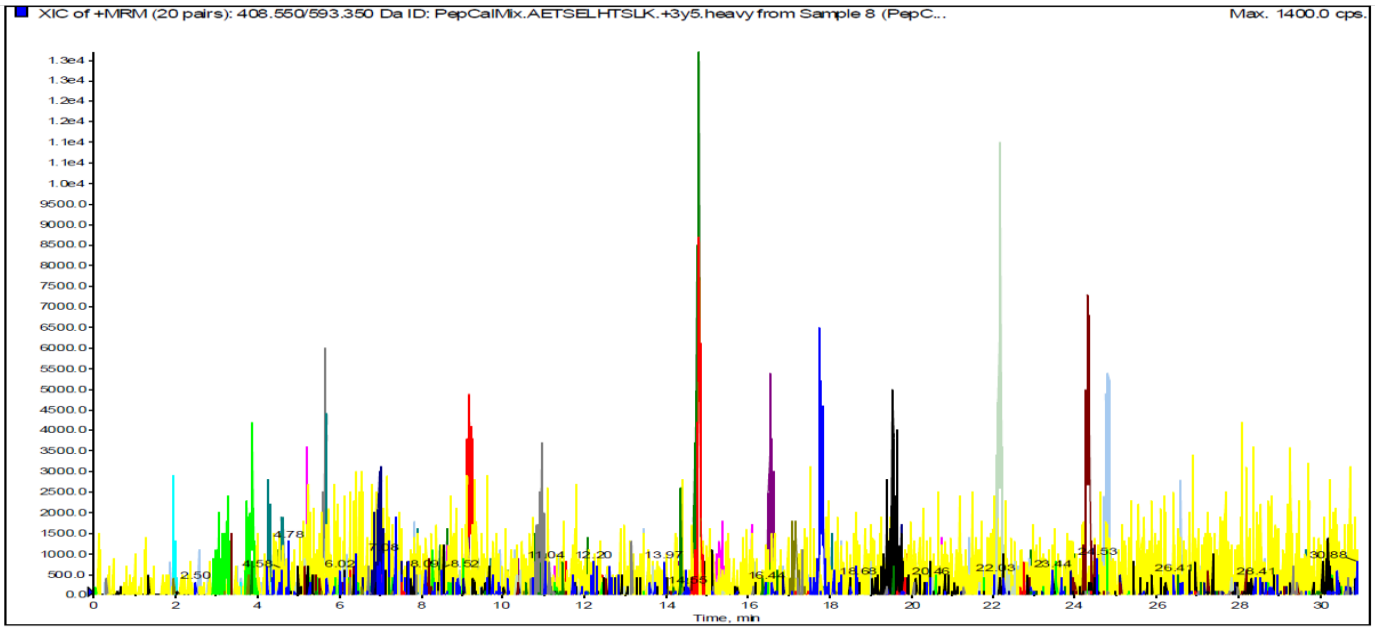
App ID 25889

Fig. 1. C: Injection Volume: 100 fmol



App ID 25888

Fig. 1. D: Injection Volume: 10 fmol



App ID 25887

When comparing the selectivity of both the 0.3 mm and 0.5 mm ID columns (**Figure 2**) no change in selectivity was observed despite a narrower column ID Flow rate was adjusted to maintain constant linear velocity and the gradient profile kept the same.

The 0.3 mm column offered an improvement in sensitivity over the 0.5 mm column as is to be expected however as we can clearly see from the two chromatograms no changes in elution order or retention time were observed despite the increase in column ID

Figure 2. **C**omparison of a 1 µL injection on a 0.3 mm and 0.5 mm ID Kinetex XB-C18 core-shell 50 mm column

Fig. 2. A: Kinetex 2.6 µm XB-C18 50 x 0.5 mm, Injection Volume 1 µl, Flow Rate 30 µl/min

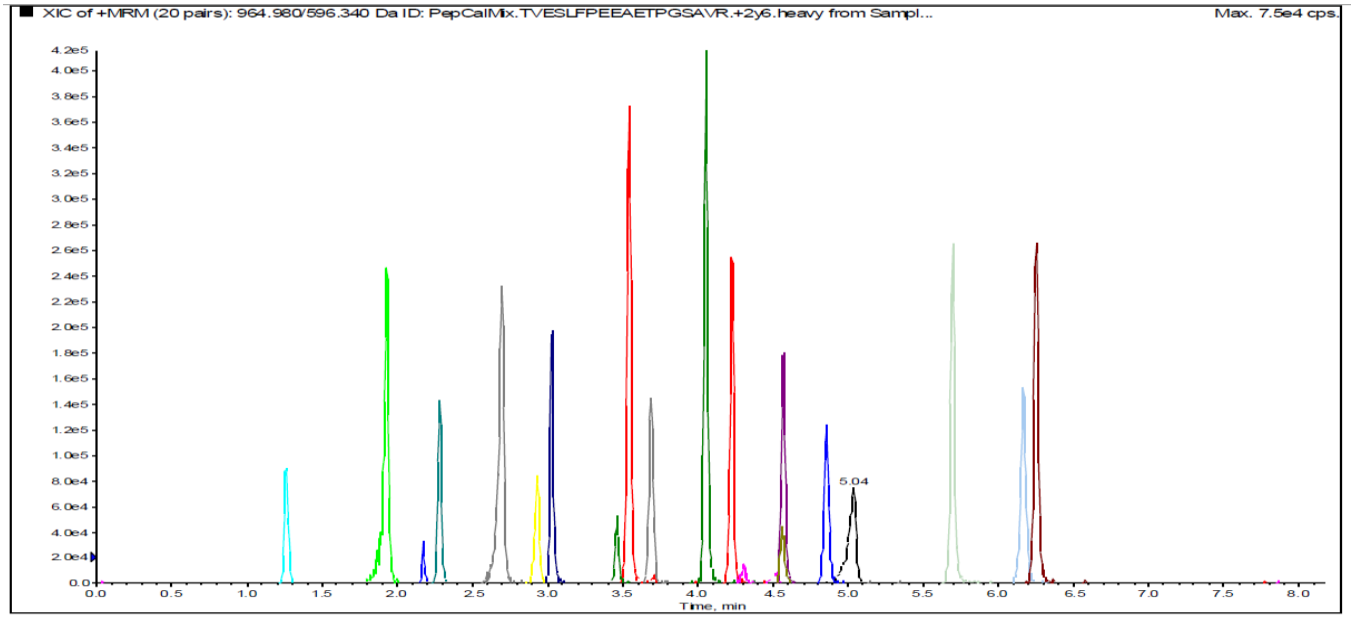


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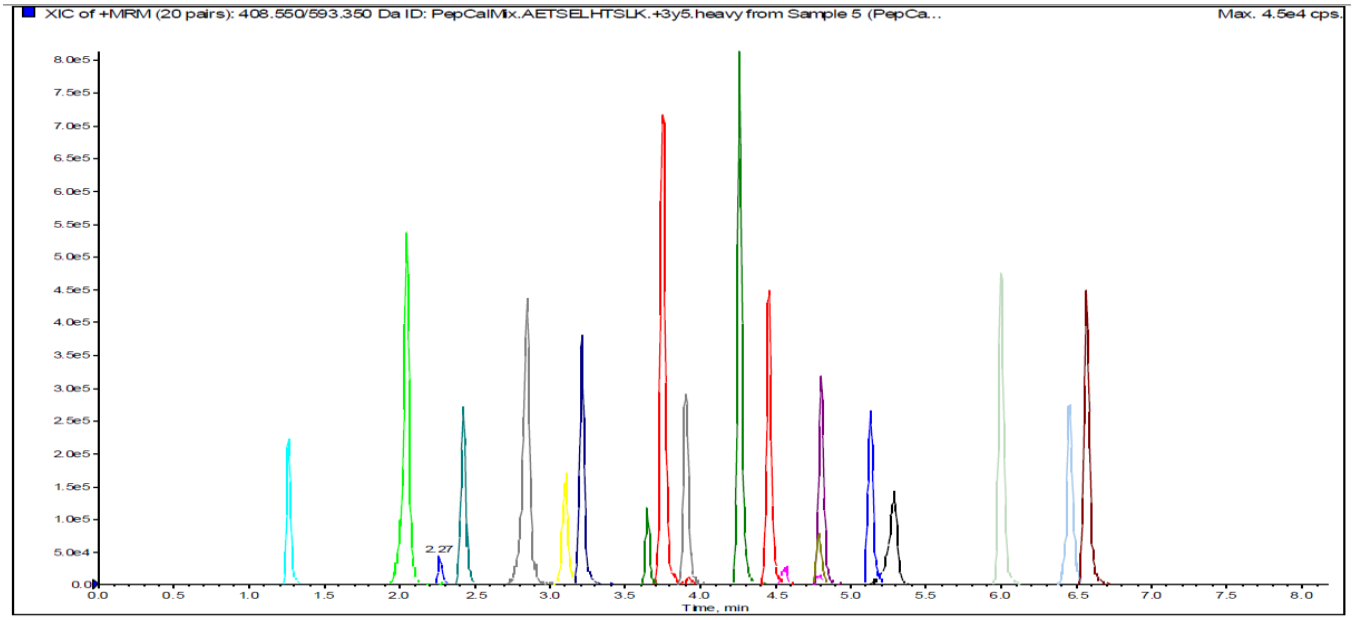
Fig. 2. B: Kinetex 2.6 µm C18 50 x 0.3 mm, Injection Volume 1 µl, Flow Rate 10 µl/min



App ID 25921

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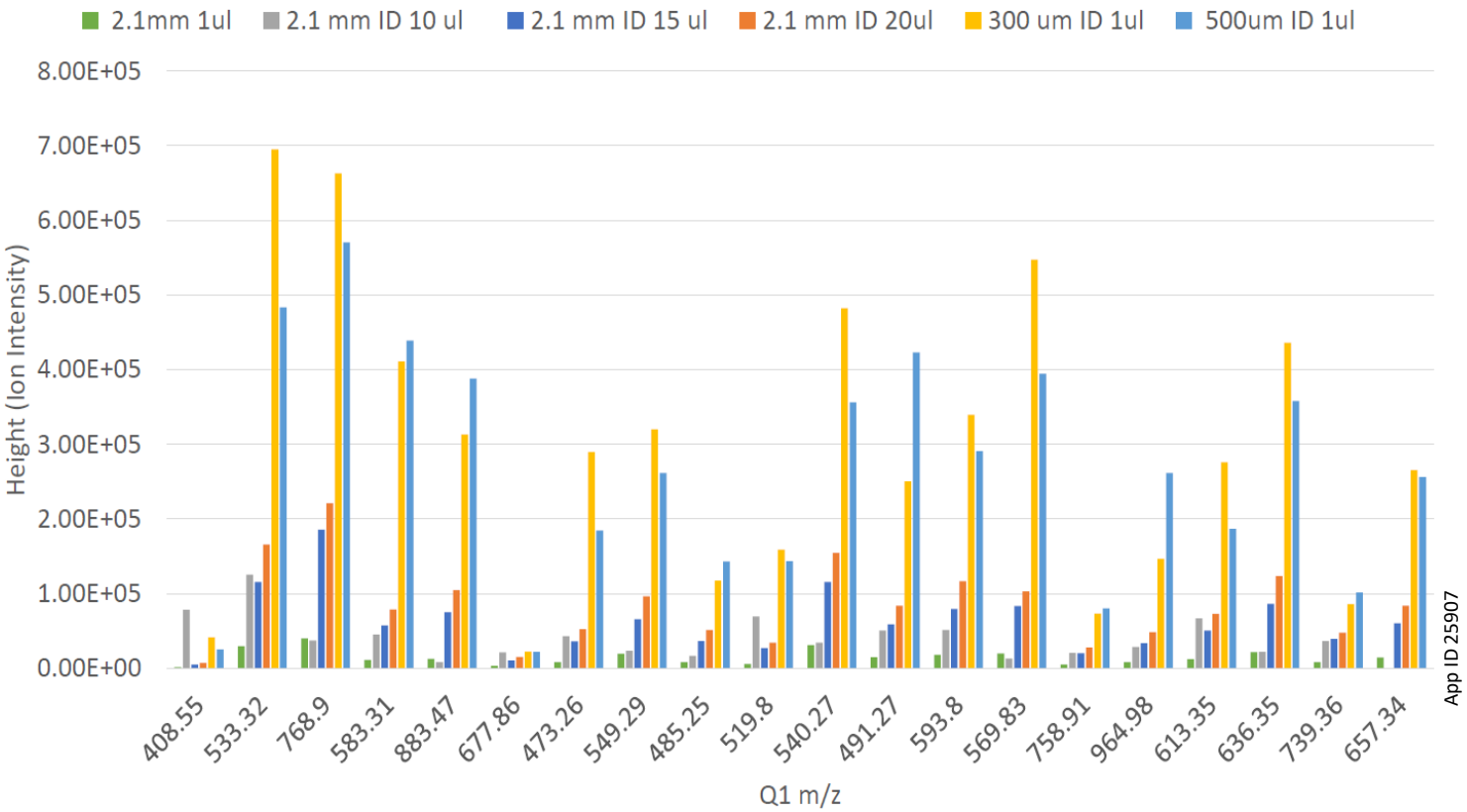
In applications where there is limited sample available, the ability to inject samples as small as 1 µL with no detriment to detection offers a significant advantage and has been found to be especially important for peptide quantitation. When the column ID is reduced the flow rate can be also be reduced to maintaining the same linear velocity and separation but increases the ionization efficiency and thus enhancing MS sensitivity.

Throughout this application, the column length and gradient profile was maintained, and we observed no difference in selectivity across the different column ID's demonstrating the scalability of the analytical format (2.1 mm) and micro LC format (0.3-0.5 mm) depending on the requirements of the analysis.

Figure 2. Column Scalability Comparison of height (Ion Intensity) for a 10 fmol/µl PepCalMix



Conclusion

In conclusion, when available sample is limited or the analyte concentration is low, scaling your column ID to a micro LC format can significantly help to improve the sensitivity of your method. In this example, we saw that by using a 0.3 mm ID column with the same gradient slope as a 2.1 mm analytical column, we gained improved sensitivity at a mass load of 10 fmol whilst maintaining the same selectivity. In this application, we have demonstrated that there is a significant improvement in sensitivity when using either a 0.3 mm or 0.5 mm ID column which offers chromatographers an opportunity to improve sensitivity without altering selectivity. An additional concern when smaller column ID's are used is the potential effect of extra-column volume stemming from the injector, tubing or detector which can potentially lead to a loss of separation efficiency known as band broadening; when we evaluated these narrower column ID's under comparable conditions to the 2.1 mm columns the was no detriment to peak width which could be attributed to band broadening.

Need a different column size or sample preparation format?

No problem! We have a majority of our available dimensions up on www.phenomenex.com, but if you can't find what you need right away, our super helpful Technical Specialists can guide you to the solution via our online chat portal www.phenomenex.com/ChatNow.

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