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A Simple and Convenient Way to Pack Capillary LC Columns

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Abstract

Packing your own columns for capillary and nano LC is very common in the research community but has a number of disadvantages over commercially packed columns. In a lab it is harder to get the columns reproducible due the variation in bed height, the manufacture of the frits and the fact that fused silica capillaries are fragile. Packing your own columns also require a pressurised packing bomb and a high pressure gas supply.

We have developed a column packing kit where the fused silica capillaries are replaced by PEEKsil®, a PEEK coated fused silica capillary which is much more robust than fused silica tubing. Columns with the desired ID and length are butt connected to connection capillaries with integrated low-volume screens and slurry packed using any HPLC pump as the packing solvent delivery system. The resulting columns have a precise bed height, are more robust than fused silica columns and can be reversed for column cleaning purposes. Independent test have also shown them to be equal to or superior in performance to commercially available columns.

The MyCapLC™ column

The column consists of a column body from 1/16 " OD PEEKsil and column inlet and outlet capillary from 1/32 " OD PEEKsil. The column and the connection capillaries are joined by a PEEK double-ended ferrule which also contains a 0.5 µm stainless steel woven screen as a frit. The thickness of the screen is about 30 µm keeping the void volume to a minimum. The design also allows the ID of the connection capillaries to be smaller than the column ID.
Column IDs range from 75 µm to 530 µm and column lengths are typically 50, 100 or 150 mm. While the standard is 1/32 " OD PEEKsil for the connection tubing any combination of connection tubing (1/16 ", 1/32 " or 360 µm) can be used with the same technique.

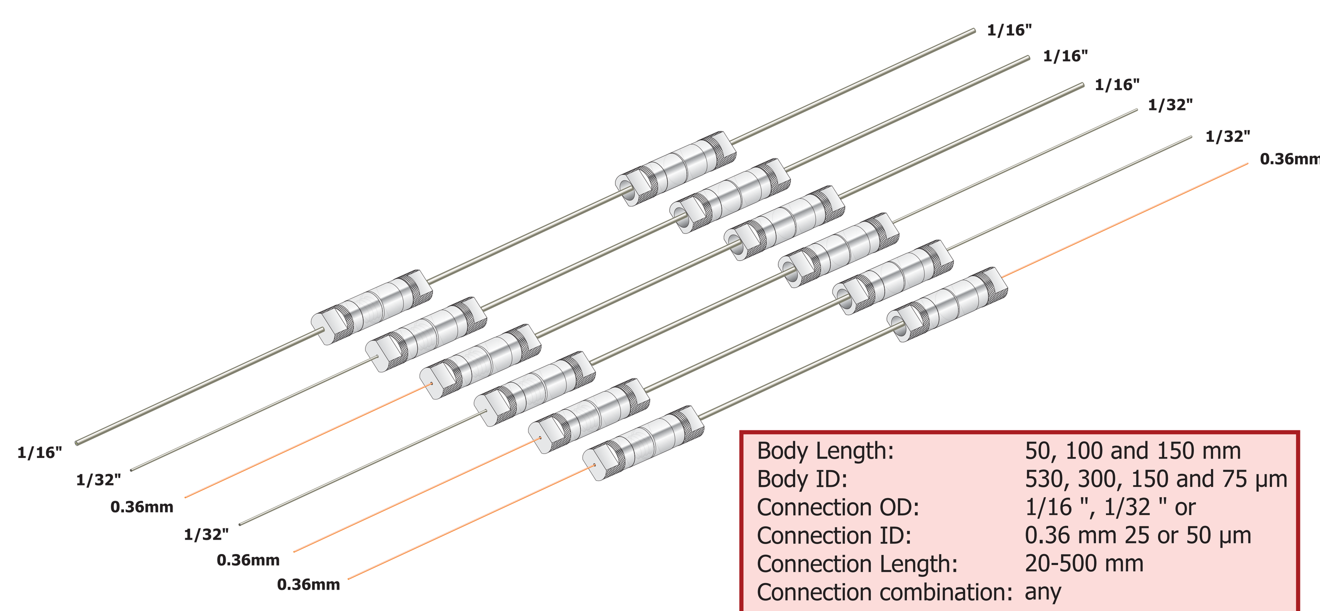


Figure 2: Possible combinations of capillaries for MyCapLC columns.

The packing process

The outlet part of the column is assembled first and attached to the slurry reservoir (175 µL capacity). The stationary phase is suspended in 175 µL of slurry solvent. For reversed phase materials a 1:1 mixture of chloroform and cyclo-hexanol will form a stable slurry but good results can also be achieved with chloroform alone. Table 1 shows the required amounts of silica to pack a column.

Column ID [µm]	Column Length [mm] 50	100	150
75	0.14	0.28	0.42
150	0.6	1.2	1.8
300	2.3	4.6	6.9
530	7	14	21

Table 1: Required amounts of silica in mg.

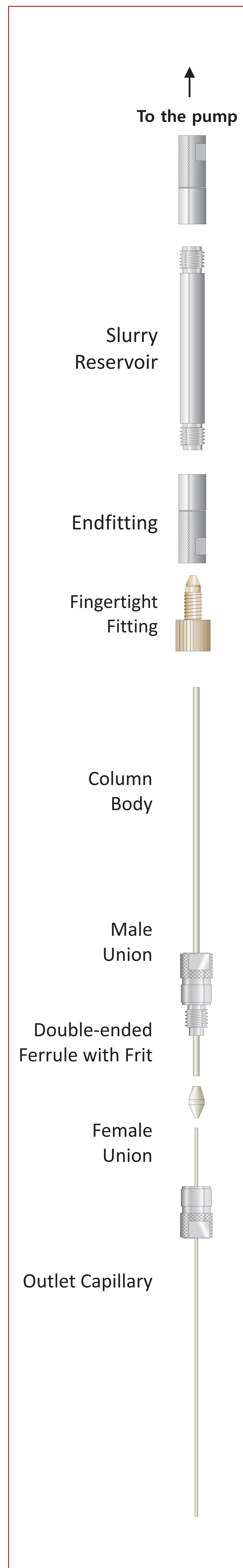
The slurry is filled into the reservoir with a pasteur pipette or a syringe and the closed reservoir is attached to the outlet of an HPLC system. Packing proceeds under constant flow with 50-80% acetonitrile or methanol as the packing solvent. The required flow rate is dependent on the ID of the column and a guideline of recommended flow rates is shown in table 2:

Column ID [µm]	Column Length [mm] 50	100	150
75	0.6	0.6	0.5
150	2.5	2.5	2.0
300	10	10	8
530	30	30	24

Table 2: Recommended flow rates in µL/min to pack MyCapLC columns.

During the packing process the pressure will increase as the bed height increases. Once the content of the slurry reservoir has be pumped through the column the pressure will change due to the change in viscosity between the slurry and the packing solvent. When the column is finished, it is removed from the reservoir and the inlet capillary is attached.

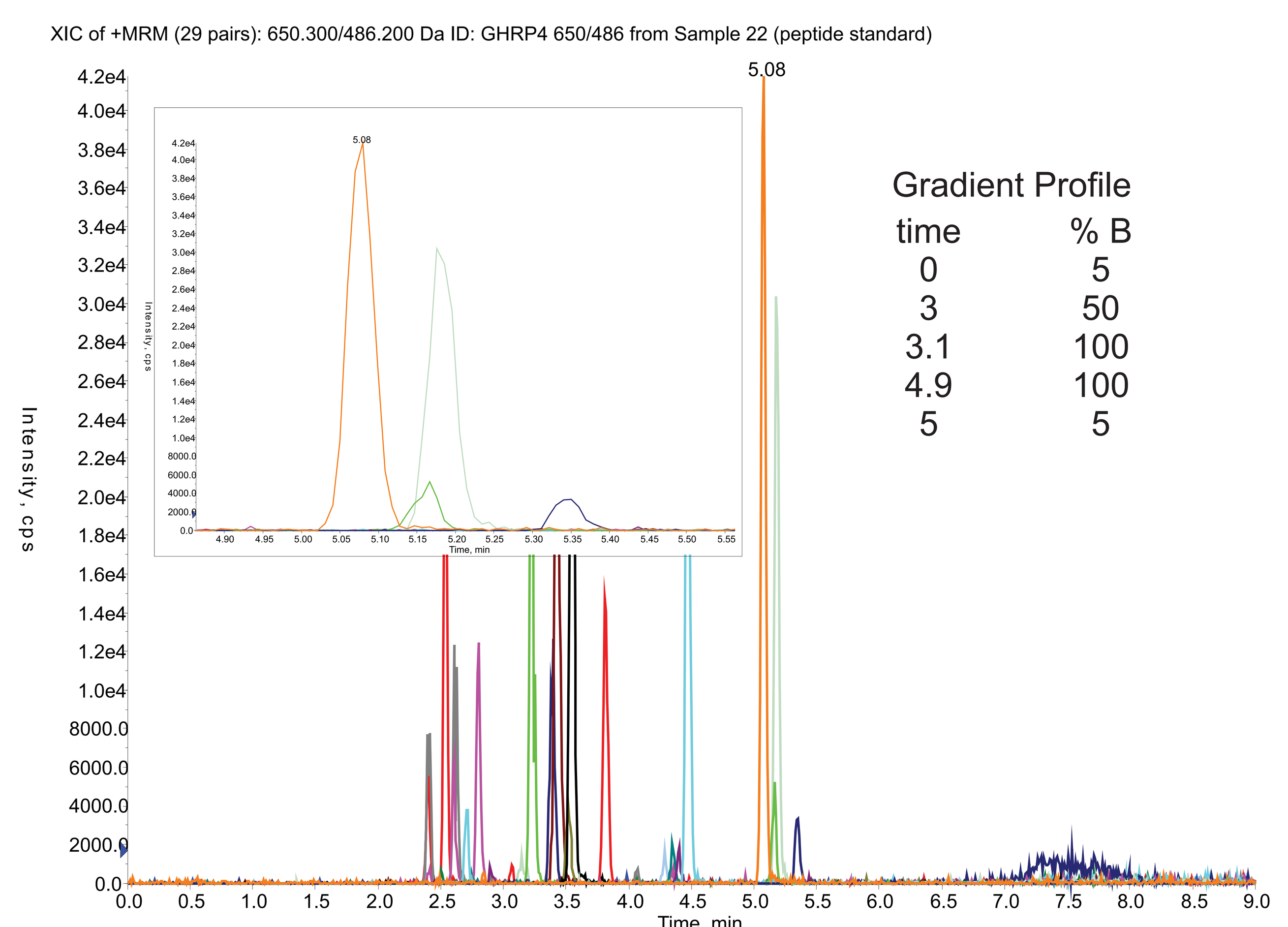
Packing assembly



MyCapLC column performance

The first set of data was produced by R. Steel from the Racing Analytical Services Laboratory (RASL) in Flemington Vic. A sample of peptide standards were separated on a in-house packed 100 mm x 0.53 mm ID column:

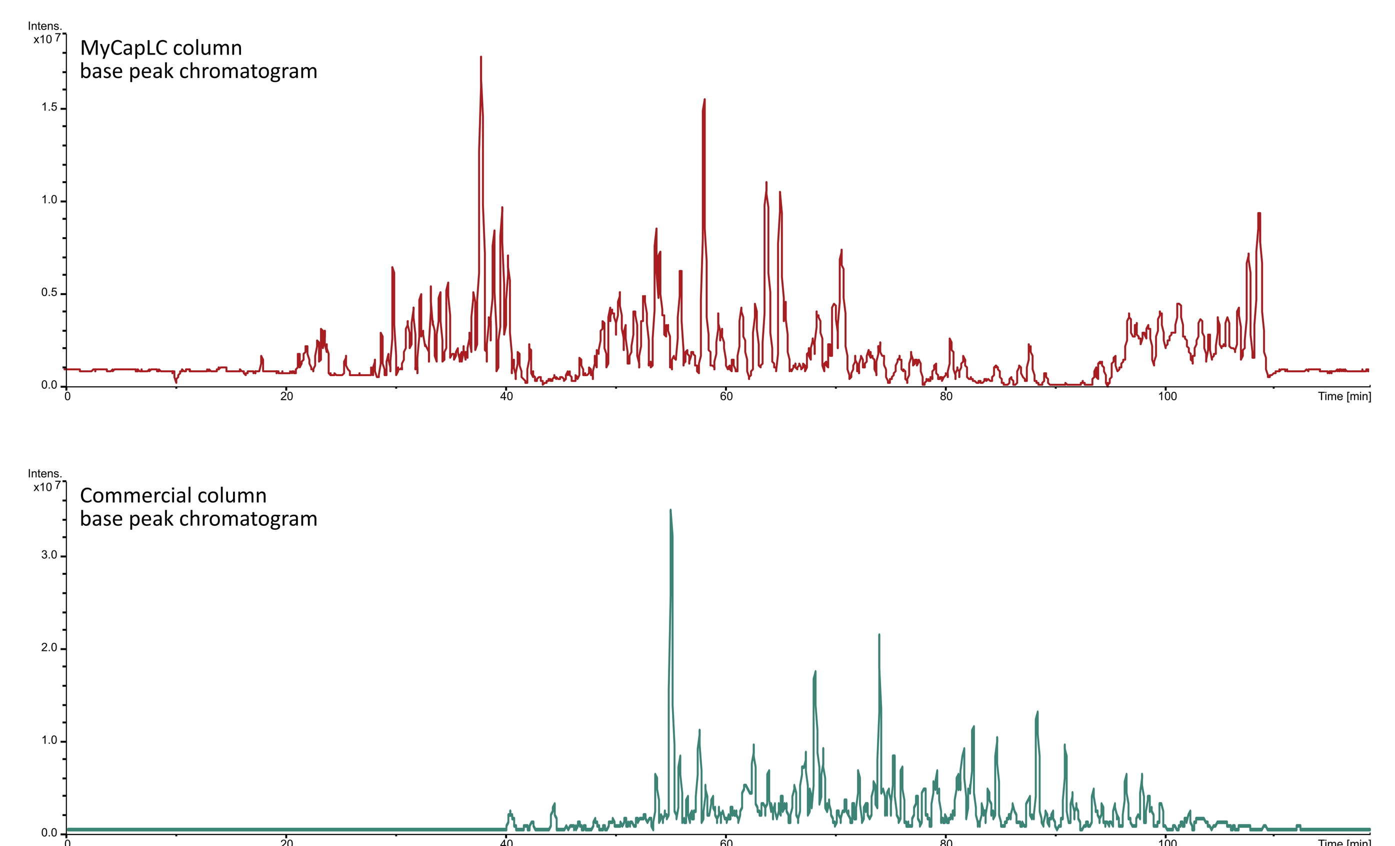
Column: MyCapLC C18, 3 µm 200 Å, 150 x 0.53 mm
Mobile phase A: 0.1% formic acid in water
Mobile phase B: 0.1% formic acid in acetonitrile
Flow rate: 20 µL/min



The peak width of the peak at 5.08 minutes at half peak hight was 2.4 sec!

The second example is the analysis of a zebra fish brain homogenate performed by Georgia Arentz from the Adelaide Proteomics Centre. A MyCapLC column with 300 µm ID and 100 mm length was compared to a commercially available column with the same dimensions and under the same conditions.

Nano-LC−ESI-MS/MS was performed on an Ultimate 3000 RSLC system (Thermo-Fisher Scientific) coupled to an Impact II™ QTOF mass spectrometer (Bruker Daltonics) via an Advance CaptiveSpray source (Bruker Daltonics). Peptide samples were pre-concentrated onto a C18 trapping column (Acclaim PepMap100 C18 75 µm × 20 mm, Thermo-Fisher Scientific) at a flow rate of 5 µL / min in 2% ACN 0.1% TFA for 10 minutes. Peptide separation was performed using the columns supplied by Trajan at a flow rate of 0.9 µL/ min using a linear gradient from 5 to 45% B (A: 5% ACN 0.1% FA, B: 80% ACN 0.1% FA) over 80 minutes, followed by a 15 minute wash with 90% B, and an 15 minute equilibration with 95% A.



It was possible to identify 167 proteins on the MyCapLC column compared to 137 proteins identified with the commercial column.

Conclusions

We have developed a kit which allows the analysts to pack their own capillary columns without the need for specialized equipment. The resulting columns perform as well or better then the pre-packed columns available commercially and are fraction of the cost. The unique design of the column hardware makes these columns more reproducible and more robust than self-packed fused silica columns.

Acknowledgements

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