

A NEW COLUMN DESIGN FOR CAPILLARY LC

Hans-Jürgen Wirth, Peter Dawes and Ern Dawes
SGE International Pty Ltd. 7 Argent Place, Ringwood, Victoria 3134, Australia.

ABSTRACT

The recent development of areas like medical trace analysis, biotechnology and proteomics has increased the demand for powerful and sensitive separation techniques and sample analysis. Even though the advantages of capillary columns in liquid chromatography were recognized early on it is only recently that instrument manufacturers are able to provide equipment able to handle the specific demands of capillary LC.

SGE has developed a range of capillary columns and accessories with a focus on reducing extra-column void volumes – one key aspect for successful capillary LC. Reduction of void volumes was always an issue in optimized liquid chromatography but with the extremely low flow rates used in nano-flow capillary LC the subject of void volumes becomes very important.

PERFORMANCE IMPROVEMENT IN CAPILLARY LC

- Column design
- Minimizing the extra-column void volumes
- Minimizing the number of connections
- Choice of connection tubing material

COLUMN DESIGN

- Column body and connection tubing made of fused silica lined PEEK™ – combination of inertness of fused silica with robustness of PEEK
- Column IDs: 75 to 300µm, suitable for nano-flow applications
- Column length: 50, 100 and 150mm
- Integrated connection tubing: 200mm inlet, 100mm outlet (25 or 50µm ID, 1/16" or 1/32" OD), it reduces the number of necessary connections and optimizes flow path
- Packing material: 3µm silica, 120 or 300Å pore size (C4, C18 and SCX). Small particles combined with large pore size ideal for high efficient peptide and protein analysis

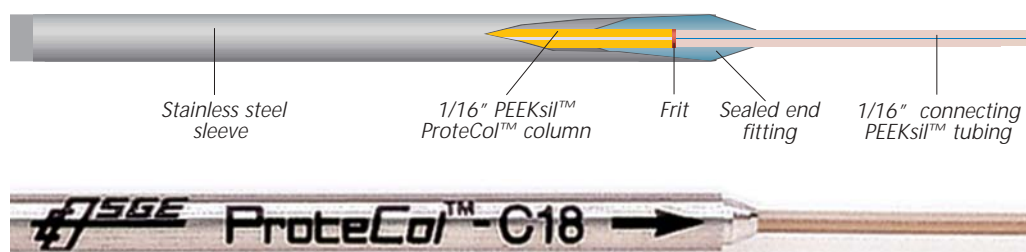


Figure 1. The ProteCol™ Capillary Column (cross section and photo) showing the integrated fused silica lined PEEK™ (PEEKsil™) tubing column design.

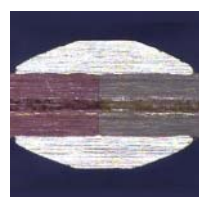
CONNECTION TUBING FOR ZERO-VOLUME CONNECTIONS

When connecting capillary tubing a number of factors are important to avoid sample dispersion.

- **Avoiding gaps at the butt connection**
Gaps will add to the void volume and sample trapped in gaps is slow to wash out causing peak tailing
- **Reducing number of connections**
Each connection will cause a slight disturbance in the flow path
- **Alignment of the bore**
Perfectly aligned bores allow continuous laminar flow thus minimizing sample dispersion



Tubing ends should be precisely square (left) to avoid volumes at the connection joint.



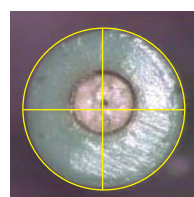
Square tubing ends allow for volume free butt connections.



Same requirements apply for column ends and filtering connectors.



Concentricity of bores in 1/32" PEEKsil tubing. SGE PEEKsil (left) and a competitor's tubing.



MINIMIZING EXTRA-COLUMN VOID VOLUMES

The connection tubing contributes to void volumes in two ways:

- Directly by its internal volume
- Indirectly by void volumes introduced at the joints

Column ID	Extra Particle Volume	Tubing ID	Tubing Volume	% of Total Mobile Phase Volume
4.6mm	1339µL	150µm	8.84µL	0.66%
300µm	5.7µL	50µm	0.98µL	14.7%
150µm	1.4µL	50µm	0.98µL	41.2%

Table 1. Contribution of connection tubing to dead volume (column length = 100mm; tubing length = 500mm)

The optimization of the connection volume becomes more and more important as the column ID is reduced.

Any additional volume has an adverse effect on the chromatographic performance. The influence is shown by extending a 50mm column (150µm ID, C18) with 50, 100, 200 and 400mm of 50µm ID tubing (98, 196, 393 and 785nL respectively)

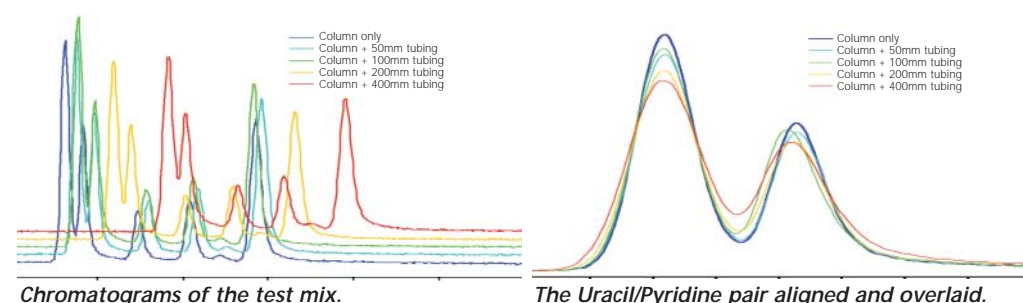


Figure 2. Chromatograms on a 50mm x 150µm ID column (3µm C18, 300Å), mobile phase: 60% Acetonitrile : 40% water, flow rate: 1.0µL/min, Sample (in elution order): Uracil, Pyridine, Methyl Benzoate, Phenetole, Naphthalene

NUMBER OF CONNECTIONS

With connection tubing of 50µm ID and smaller the concentricity of the bore and the quality of the tubing end will have an impact on the column performance.

In this example a 100mm x 300µm ID C18-column was extended by a total of 400mm tubing using 1 to 8 additional connections (1 x 400mm, 2 x 200mm to 8 x 50mm). The test mix was injected and the naphthalene peak was overlaid. An increase in peak broadening and peak tailing can be observed with increasing number of connections.

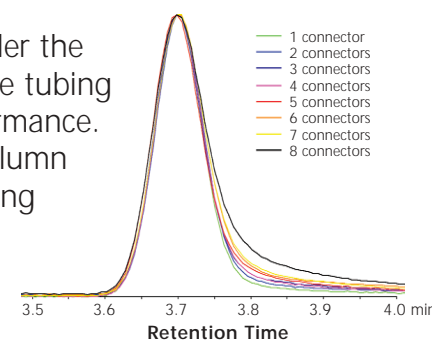


Figure 3. Influence of connections on peak shape.

COLUMN AND CONNECTION TUBING MATERIAL

Tubing material can have an effect on the peak shape due to nonspecific interaction and due to surface morphology (disturbing laminar flow through surface roughness). Three different tubing materials were compared (stainless steel, PEEK and PEEKsil™).

The sample mix was injection onto 1000mm of 100µm ID tubing of stainless steel, PEEK and fused silica lined PEEK (PEEKsil). The peak shape was investigated.

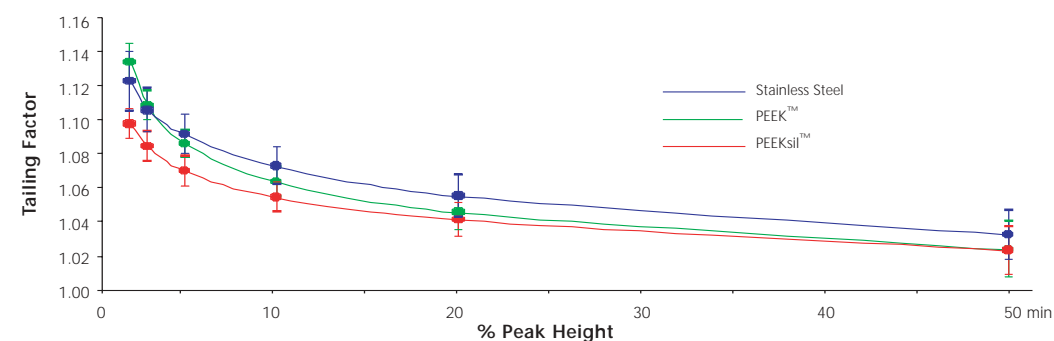


Figure 4. Comparison between PEEK™, PEEKsil™ and stainless steel tubing.

CONCLUSION

The importance of system optimization on the chromatographic performance in capillary LC has been demonstrated. There are a number of factors outside the column which can be directly addressed by the HPLC user. SGE provides an integrated system of high performance columns and accessories which make the system optimization easier.