

PERFORMANCE OF CHEMICALLY-FABRICATED FRITS IN PACKED CAPILLARY LC COLUMNS

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INTRODUCTION

Capillary liquid chromatography has been explored as a technique for the future developments in the fields of proteomics and medical analysis, as well as has become commonly used by scientists in drug discovery studies. There is a number of advantages this technique offer over conventional liquid chromatography, such as increased sensitivity (by reducing the internal diameter) and the smaller sample size (down to a few femtomoles), enabling the interfacing the LC system directly with mass spectrometers. One of the challenges to achieve good separation (that becomes important especially when extremely small flow rates are used) is to minimize any void volumes that might be present within the system.

The optimized ProteCol design (Figure 1) that is perfect for LC-MS applications is extremely robust, offering chromatography with very good peak shapes and excellent efficiencies. This integral design incorporates mechanical frits that are used to retain packing material within the column body. Highly permeable, chemically-fabricated frits (CFFs) might be used to achieve uniform flows, eliminate the risk of unnecessary void volumes and the associated band broadening effects as well as avoiding potential non-specific interactions on a metal surface which can cause a loss of sample and/or reducing chromatographic performance.

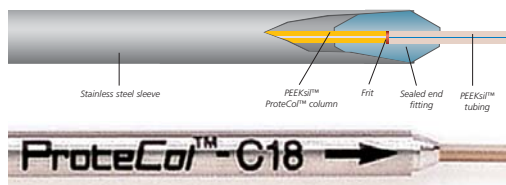


Figure 1. The ProteCol™ Capillary Column (cross section and photo) showing the integrated fused silica lined PEEK™ (PEEKsil™) tubing column design.

MANUFACTURE OF CAPILLARY COLUMNS WITH CFFS

One approach to creating frits in-situ involves the following steps:

STEP 1

Capillary Column Slurry Packing

Capillary LC columns are slurry packed using the standard packing procedure. The packing conditions (slurry and packing solvents, packing pressures, packing time and time for depressurising) are optimized according to the packing material.

STEP 2

Chemical Frit Fabrication

A number of approaches can be utilized in preparation of chemical frits. In this case, unique metal oxide chemistries appeared to be the most suitable to achieve the optimum chromatographic column performance (Figure 2).

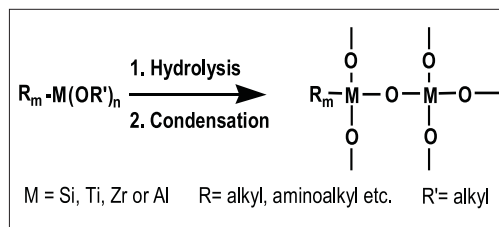


Figure 2. A general chemical approach to CFFs.

A diluted frit solution is applied to the ends of a packed column, followed by the appropriate treatment of the column ends during which the polymerization of the system proceeds to completion.

STEP 3

Frit and Column Performance Evaluation

MECHANICAL STABILITY

The requirements for pressure limits for CFFs depend on the fabrication methods, i.e., the order of the column packing and the frit fabrication. If the CFFs are formed first, it is important that they withstand the packing pressures. Otherwise, normal running pressures (much lower compared to packing pressures) become the maximum pressure requirement.

To establish the maximum pressures for CFFs, a capillary LC column with a CFF (prepared as described in Figure 2) was connected to the packing apparatus with pressures increased to 8500psi over extended period of time. CFFs did not fail at any stage throughout this experiment, and no material leakage was observed.

It was established that the increased frit solution concentration or the repetitive solution application did not lead to an increased mechanical strength. The frit length was measured as 0.7-0.9mm.

CHROMATOGRAPHIC PERFORMANCE

The performance of a column with CFF is shown in Figure 3. Excellent peak symmetries (1.14) and efficiencies 112,000N/m with no peak broadening were achieved. Column backpressures were noted to be half of those when a mechanical frit is used, indicating a presence of a homogeneous, highly permeable CFF.

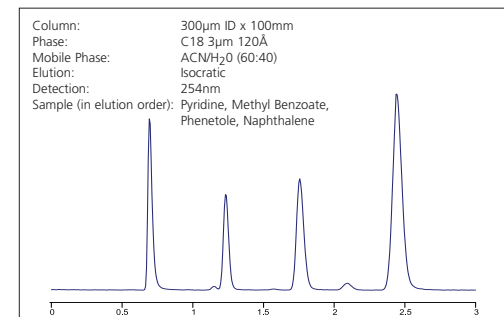


Figure 3. Standard test mix on a capillary LC column with CFFs.

The retention time stability data is in Table 1. It appeared that over a number of injections (20) the %RSD was quite low (0.8%) (Note: the %RSD requirement according to USP is less <1% over 6 injections).

Table 1. Retention Time Stability.

Injection no.	Retention Time	Column:	150µm ID x 100mm
1	13.58	Phase:	C18 3µm 300Å
5	13.55	Mobile Phase:	ACN/H ₂ O (60:40)
10	13.33	Analyte:	Naphthalene
15	13.24	Elution:	Isocratic
20	13.30	Detection:	254nm
Average (of 20)	13.36		
Std Deviation	0.110		
% RSD	0.82		

CONCLUSIONS

The above described experimental technology enables manufacture of capillary columns with chemically-fabricated frits that effectively retain packing materials and are able to withstand high packing pressures. The CFFs show good permeability, performing well in the pressure driven flow mode, with columns exhibiting lower backpressures (compared to those with mechanical frits). The %RSD on the retention time appeared to be within the range acceptable in industries where the stringent specifications are in place.

