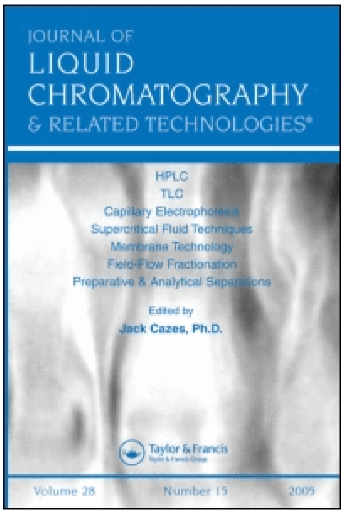

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P. A. Hyldburg[a]; C. M. Sparacino[a]; J. W. Hines[a]; C. D. Keller[a]
[a] Analytical and Chemical Sciences Research Triangle Institute, North Carolina

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DEMONSTRATION OF THE FEASIBILITY OF RADIALLY COMPRESSED MICROBORE HPLC COLUMNS

P. A. Hyldburg, C. M. Sparacino, J. W. Hines, and C. D. Keller

Analytical and Chemical Sciences
Research Triangle Institute
P. O. Box 12194
Research Triangle Park, North Carolina 27709

ABSTRACT

This work involved the development of radially compressed, microbore high performance liquid chromatography (HPLC) columns. The design of the overall system and the column are described, and the problems associated with the design features are reported. Variables examined during the course of this work included the column material, column length, packing method, flow rate, radial compression pressure, and internal column pressure. Efficiencies (expressed as plates/meter) are shown for various combinations of those variables and are compared to those obtained using a commercial, steel microbore column.

INTRODUCTION

The complexity of smoke condensates has strained the ability of high resolution capillary gas chromatography to provide a separation of the hundreds of volatile components that they contain. Further, the lack of a similar high resolution technique for the non-volatile components (e.g., high resolution HPLC) has severely hampered attempts to identify and quantitate hazardous constituents of smoke condensates. The current work has attempted to address this problem by development of high resolution HPLC technology based upon radially compressed, microbore columns.

The principle of radial compression relies simply upon the ability of soft, polymeric column walls to effectively eliminate "wall effects" when compressed inward by sufficient external pressure (1). Thus, radial compression should provide a means of obtaining the maximum possible efficiency of which the particular column is capable. There are currently available conventional sized (4-5 mm ID) HPLC columns which employ radial compression technology (2). It was the purpose of this work to apply this technology to microbore HPLC columns in the expectation of obtaining high resolution, coupled with the special properties (e.g., high mass sensitivity, compatibility with direct injection LC/MS, etc.) demonstrated for steel walled microbore columns, along with the advantages of long flexible columns.

INSTRUMENTATION

HPLC System

The HPLC system used for the evaluation of radially compressed microbore columns consisted of a model M590 dual-piston pump (Waters Assoc.) capable of providing flow rates down to 1 µL/min at pressures as great as 6000 psi. The pump was capable of operating in either the constant flow or constant pressure mode. Injections were made using a Model 3XL valve-loop injector (SSI) with a 0.2 µL loop volume. The detector was a Model 440 fixed-wavelength UV (Waters Assoc.) at 254 nm and was fitted with a specially modified, low-volume cell (1.2 µL). Columns were connected to the injector and detector by 5 cm lengths of 1/16" OD x 0.004-0.005" ID stainless steel tubing. In addition, the normal inlet-outlet configuration of the detector cell was reversed to accommodate the low dead volume requirements of micro-bore columns.

Radial Compression System

A diagram of the system used for radial compression of micro-bore columns is shown in Figure 1. Pressure was provided by a pneumatic pump, Model MCP-71 (Haskel), with a pressure maximum of 8,800 psi. Propylene glycol was used as the hydraulic fluid. The compression reservoir itself was fabricated from 1" ID stainless steel tube capped with 1" column end fittings (Whatman) drilled to 1/16" ID. The reservoir was connected to the pneumatic pump through a tee with a Model SS-4PDM4-F4 high pressure ball valve (Whitey) to release hydraulic pressure. The arrangement shown in Figure 1 allowed the entire column along with its end fittings to be contained inside of the compression reservoir. Only the 1/16" X 0.005" ID transfer tubing passed outside of the reservoir (through the 1" - 1/16" end fillings) to the injector and detector. The design of the microbore column end fittings is discussed in the sections below.

COLUMN PREPARATION AND EVALUATION

Throughout this research, the most difficult problem was design of microbore column end fittings that would 1) hold the column and the interior packing material in place, 2) withstand internal pressures approaching the maximum for the column material (i.e., *ca*. 1000 psi for PTFE, *ca* 3000 psi for polyethylene used later), and 3) withstand the externally applied radial pressure (>2000 psi). The end fitting shown in Figure 2 was developed to meet these criteria and was used on the initial PTFE columns discussed below.

Dry-Packed PTFE Columns

Initial flexible-wall, microbore columns were prepared from commercially available PTFE tubing with 1.5 mm ID (1/16") and 3.2

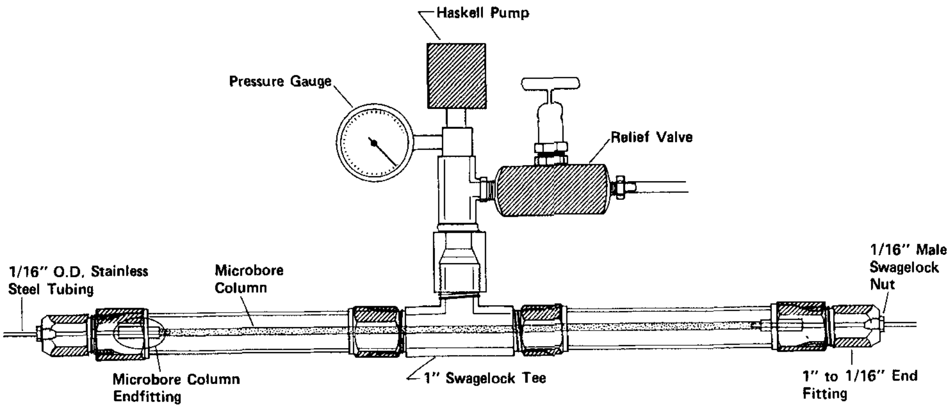

Figure 1. Schematic Diagram of Compression System

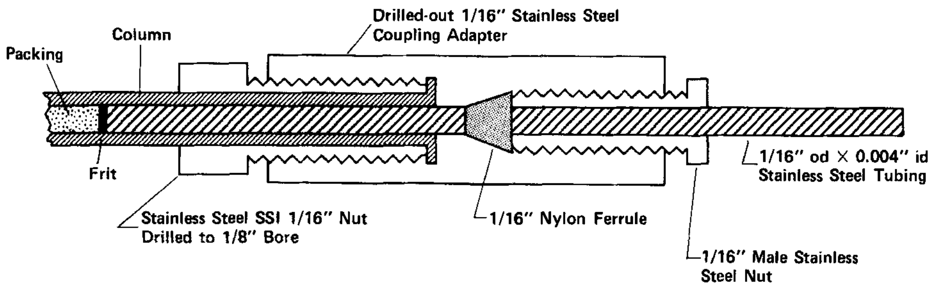

Figure 2. Schematic of Column End Fitting Design Utilizing Flared Column Ends

mm OD (1/8"). This size was chosen to facilitate use with readily available 1.5 mm diameter SS frits to contain the packing material and with standard 1/16" and 1/8" HPLC fittings. To further simplify initial column development and to test the feasibility of the end fitting design and the radial compression concept, the first columns were dry-packed with Zorbax ODS 8-9 micron (DuPont) reverse phase column packing.

Dry-packing of a 250 mm length of PTFE tubing was performed in much the same manner as for packed GC columns. A 2 µm porosity, 1/16" (1.5 mm) diameter SS frit was placed in the outlet end of the column and held in place within the fitting by the 1/16" male, flare-fit nut. The outlet end was then attached to an aspirator vacuum and the packing material slowly introduced into the inlet end. When the column was full, the inlet end was terminated with a similar fitting containing a frit (2 µm porosity X 1/16" diameter). The final column length was about 210 mm.

Performance of the dry-packed column is summarized in Table 1 (Column #1). Without radial compression, the column generated only 7,700 plates/meter using methyl anisate as the test solute (k' = 7.6) and eluting with 40% acetonitrile/water. However, the application of radial compression (2000 psi) increased the performance by 44% (11,100 plates/meter). As expected, radial compression also increased the internal pressure from 520 to 650 psi at the 100 µL/min flow rate. Reduction of the flow rate to 30 µL/min produced a further increase in efficiency to 20,000 plates/meter. The latter value was comparable to previous slurry-packed PTFE columns (3-9).

<u>Slurry-Packed PTFE Columns</u>

It is well established that the most efficient method of packing microparticulate HPLC columns is slurry-packing under high solvent velocity. The problem of containing the high internal column pressures generated by the high solvent velocity is severe with columns made of PTFE tubing. The initial approach to packing PTFE columns was simply to keep the internal column pressure just below the bursting limit of the PTFE tube. However, in practice this limit was found to vary from <u>ca</u> 300-1000 psi depending upon the uniformity of the tubing and the end fitting design. Packing material was suspended in about 5 mL of isopropanol/CCl_4 (1:13) as the slurry solvent.

Table 1. Summary of Results for Radially Compressed Micro-bore Columns

Column Number	Packing Material	Column Material	Length (cm)	Packing Method	Flow Rate (μL/min)	Radial Compression (psi)	Internal (psi)	Plates per meter
1	Zorbax C8 (8-9 μm)	PTFE	21	Dry	100	0	520	7,700
					100	2000	650	11,100
					30	2000	170	20,000
2	Spherisorb C8 (10 μm)	PTFE	24	Slurry	100	0	490	3,200
					100	2000	745	7,700
3	Spherisorb C8 (10 μm)	Polyethylene	21-25	Slurry	100	0	400	4,350
					100	2000	300	9,100
4	Spherisorb C8 (10 μm)	Polyethylene	24	Slurry/Radial Compression	100	400	300	2,900
					100	3000	550	7,700
5	Spherisorb C8 (10 μm)	Polyethylene	23	Slurry/Radial Compression	100	1000	690	3,100
					100	3000	630	4,760
					10	3000	68	10,300
6	Partisil C8 (10 μm)	Glass-lined Stainless Steel	25	Commercial	100	-	745	20,000
					25	-	130	31,250

Tubing Dimensions: PTFE = 1.5mm ID x 3.2mm OD
Polyethylene = 1.6mm ID x 3.2mm OD
Glass-lined Stainless Steel = 1.0mm ID x 3.2mm OD

Test Component: Methyl Anisate

Attempts to produce PTFE columns using this slurry-packing technique failed due to 1) disintegration of the flared PTFE tube under pressure at the terminal fittings (inlet or outlet), 2) splitting of weak points in the nonuniform tubing wall, 3) leakage of the terminal fittings, and 4) nonuniform deposition of the microparticulate during the packing process. Major factors in these failures appeared to be the cold flow characteristics of the PTFE and the difficulty of controlling the packing pressure in the small diameter column. A single column, packed with Spherisorb C8 (10 µm), was obtained in these attempts. The performance of this column is summarized in Table 1 (Column #2) and was uniformly poor (i.e., <8000 plates/meter with 2000 psi radial compression). Note, however, that the application of radial compression increased column performance over 140% versus the non-radially compressed mode. The lifetime of the column was short due to the PTFE cold flow characteristics, which produced drastic changes in the column length and diameter under radial compression and internal flow. This behavior also resulted in rapid deterioration of the terminal fittings with loss of column packing and clogging of connection lines. The use of PTFE as a column material was therefore abandoned in favor of a more stable polymer.

Slurry-Packed Polyethylene Columns

The only known polymers sufficiently impervious to most HPLC solvents (besides PTFE) are high density polyethylene or polypropylene. However, since a commercial source of tubing with an ID in the range 0.8-1.5 mm could not be located, polyethylene tubing of size 1.6 mm ID x 3.2 mm OD was extruded in-house. Although the tubing was shown to survive internal pressures up to 1000 psi, the initial attempts to pack this tubing as described earlier produced leaks in the terminal fittings.

Changes were made to the terminal fitting design in order to improve their reliability. First, the flange at the end of the

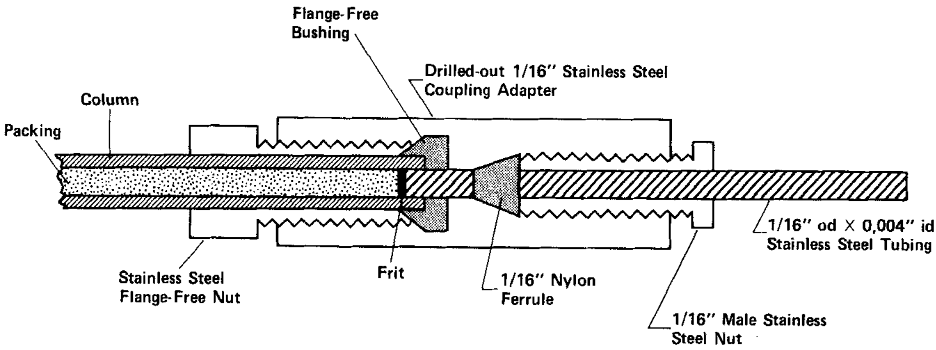

Figure 3. Schematic of Column End Fitting Design Utilizing Flange-Free Bushings

polyethylene tube was replaced with a Kel-F Flange-Free® bushing (Alltech/Applied Science), which acted more like a conventional ferrule. Small burrs on the inside of the bushing prevented the polymer tube from slipping out. Second, the frit was placed just inside the end of the column to apply additional pressure on it via the bushing. This design is shown in Figure 3.

A polyethylene column was slurry-packed at a maximum pressure limit of 1000 psi using the procedure described for PTFE columns above. The performance of the column was evaluated using methyl anisate both with and without radial compression (2000 psi). The performance is summarized (Column #3) in Table 1. Although the overall performance was improved over that obtained with slurry-packed PTFE columns, it was still poor (9100 plates/meter with radial compression). Radial compression, however, provided a 110% increase in performance over the uncompressed mode of operation.

Application of radial compression during packing appeared to provide a means of both compressing the column tightly around the frits (to prevent leakage of packing material) and adding additional support for the column wall against much higher internal packing pressures (>1000 psi). A preliminary evaluation of the radial compression limits for empty polyethylene tubing showed that it would typically withstand up to 2000 psi externally applied pressure. Once filled with packing, the column should withstand much greater external pressures.

Several attempts were made to slurry-pack polyethylene columns at constant internal pressure (4000 psi) using simultaneously applied radial compression. The performance of two columns successfully packed by this procedure are summarized in Table 1 as Column #4 and Column #5, respectively. These column evaluations were performed with at least minimal radial compression in order to prevent leakage of packing material around the outlet frit. In this case, an increase in overall efficiency of >50% was obtained by increasing radial compression from 1000 psi to 3000 psi (Column #5).

Comparison of Radially Compressed with Steel Microbore Columns

For purposes of comparison, a commercial microbore HPLC column was evaluated along with the radially compressed, microbore column prepared above (Column #5). The commercial column contained Partisil C8 (10 μm particles) and was made of 1.0 mm ID x 3.2 mm OD glass-lined SS with a length of 250 mm. Van Deemter plots (HETP *versus* linear velocity) were generated for both columns over a flow rate range of 1-200 μL/min. Column #5 was radially-compressed at 3000 psi. The results are summarized in Figure 4. To examine the effect of radial compression, a Van Deemter plot was also generated for Column #5 under 1100 psi radial compression.

As expected, the commercial microbore column gave approximately three times the efficiency of the radially-compressed column and deteriorated little in performance over the entire flow range. The performance of the radially-compressed column is interesting in that performance appears to be unaffected by flow after an initial loss as the flow increased from the optimum (10

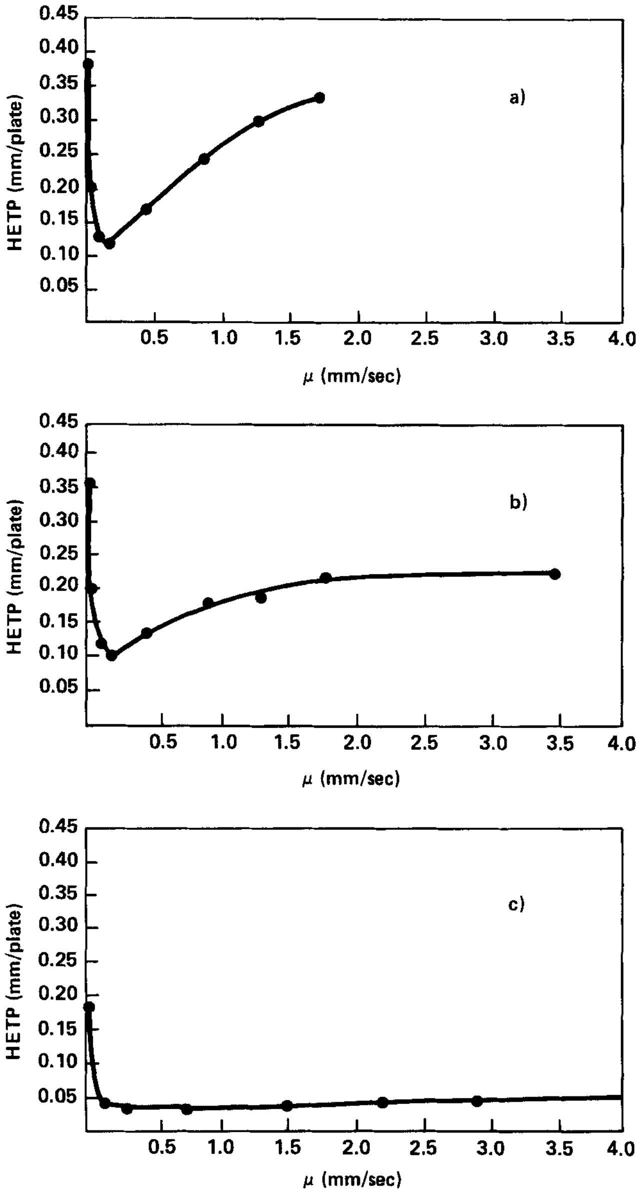

Figure 4. Van Deemter Plot of (a) Commercial, Steel Microbore Column (1 mm ID); (b) Radially Compressed Column Compressed at 3100 psi, (c) Radially Compressed Column Compressed at 1100 psi.

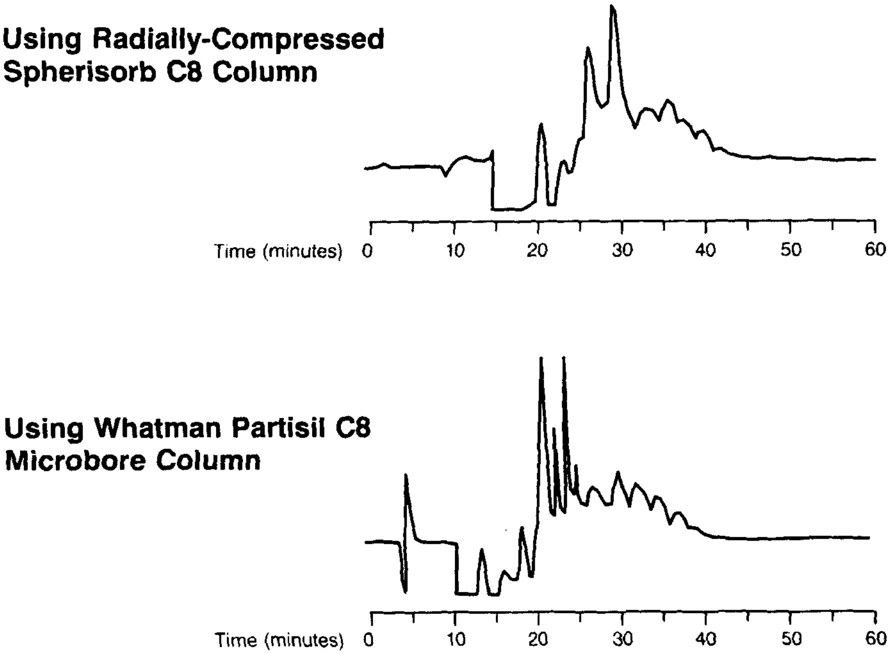

Figure 5. HPLC/UV Chromatograms of Marijuana Smoke Condensate Extract.

µL/min). This immunity to increased flow disappears completely when the radial compression is reduced to 1100 psi (from 3000 psi). The comparative separation of a complex basic fraction of marijuana smoke condensate on the commercial and radially compressed, microbore columns is shown in Figure 5.

CONCLUSIONS

The primary result of this study was confirmation of the increase in column performance (up to 150%) afforded by radial compression of the microbore column wall. Major difficulties were encountered in the engineering of terminal column fittings that could withstand both internal and external pressures in the range 2000-5000 psi. Improperly designed terminal fittings produced 1) leakage of radial compression fluid into the column, 2) leakage of mobile phase out of the column, 3) leakage of packing material around column frits, or 4) disintegration of the column material at the terminal fitting. Obtaining the optimum performance of which microbore columns should be capable (i.e., >100,000 plates/meter) via radially-compression will also require better initial packing of the column bed. Thus, higher slurry-packing pressures are desirable and should be obtained with improvements in the terminal fittings.

In addition to improvements in the packing procedure, the affect of a number of variables on the performance of radially-compressed microbore columns should be examined

- column ID (0.8-1.5 mm)
- column wall thickness (0.5-1.25 mm)
- column length (20-200 cm)
- type of stationary phase (bonded silica, silica, polymer beads)
- particle shape (spherical, irregular)
- particle size (3-10 micron)
- slurry solvent (constant density, high viscosity)
- flow rate (1-200 µL/min)
- injection volume
- detector volume and type
- radial compression pressure
- column temperature

All of these variables provide areas for further evaluation and development of radially-compressed microbore columns.

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