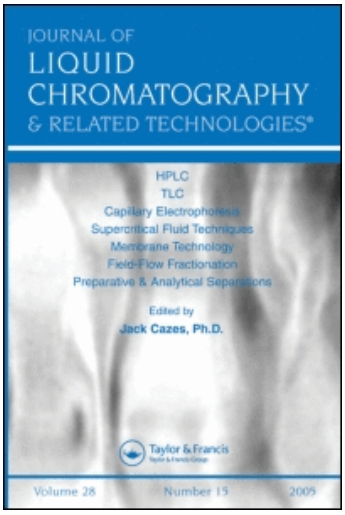

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PREPARATION OF HIGH EFFICIENCY COLUMNS FOR HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

This method employs a specially constructed slurry reservoir, which features an internal taper and a quick-disconnect pump fitting. A major innovation is the use of a three-solvent system to maintain a slurry of packing material as a "slug" within the reservoir. A measured amount of carbon tetrachloride is poured into the empty reservoir-column assembly. The column packing material is introduced as an isopropanol slurry and the reservoir is filled with isooctane. The assembly is then connected to a Haskel DSTV 122C pump, and the column is packed with isooctane at 10,000 psi pressure.

This procedure provides HPLC columns with efficiencies of 40,000 plates per meter for 10-micron silica, 30,000 plates per meter for 10-micron C_{18}, and 70,000 plates per meter for 5-micron C_{18} packing materials. We have used this method for preparation of over 35 HPLC columns during the past two years.

INTRODUCTION

The general procedure and list of required apparatus for the production of HPLC columns have been previously described (1,2).

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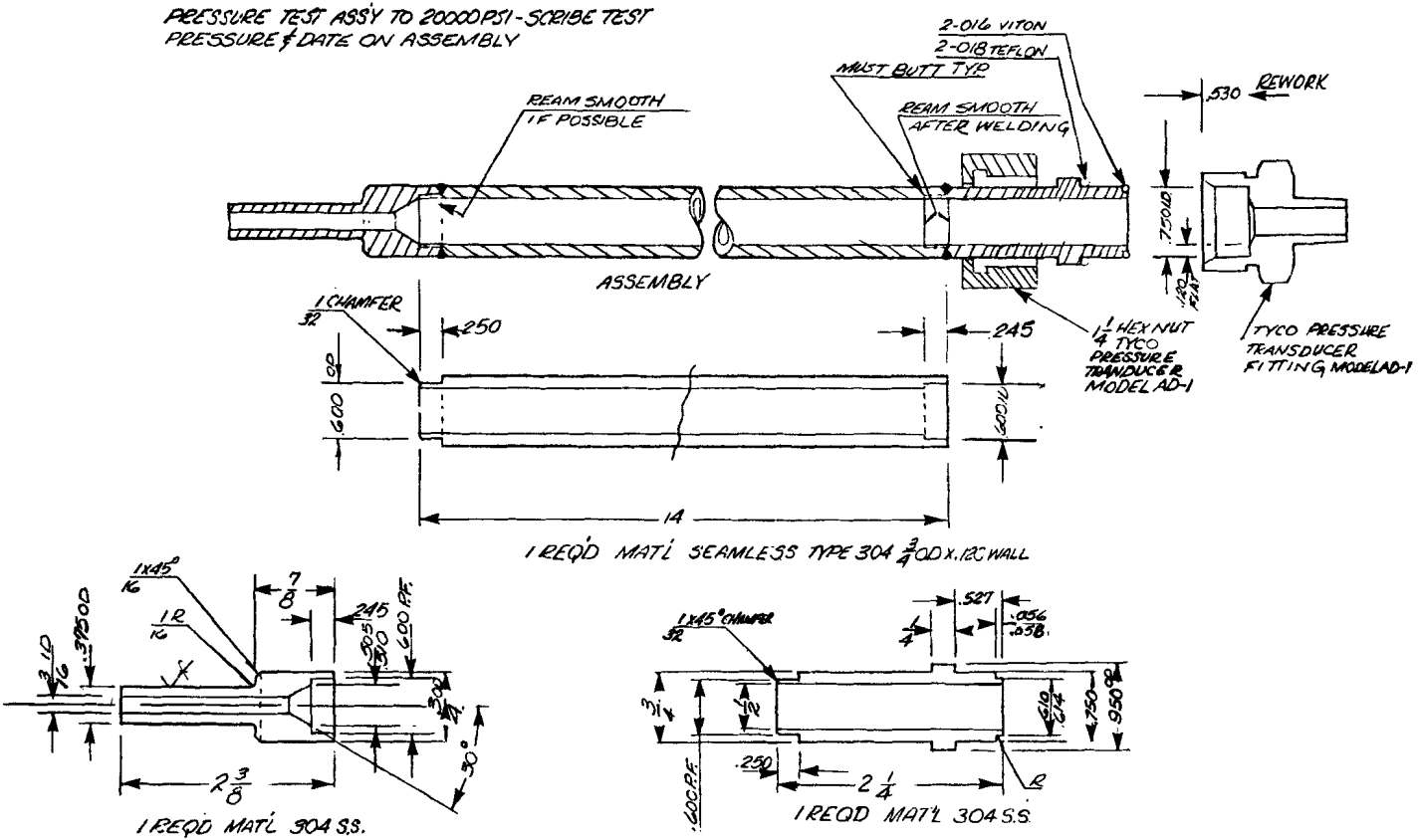

FIGURE 1.

Many papers have appeared in the literature, each adding to the general technique. Examples are the use of a ball valve by Kikta (3) to allow for a rapid connection to the pressurizing solvent, the use of a hand-tightened slurry vessel by Berendsen (4) to allow rapid attachment, and the recommendation by Uden (5) that the slurry reservoir should have an internal taper to prevent turbulent flow. Since a slurry reservoir that had an internal taper and that could be attached to the pumping system by means of a hand-tightened fitting was not commercially available, a device meeting these requirements was designed and constructed (Figure 1).

Initial attempts at column packing utilized the generally recommended techniques of filling the column with isopropanol, adding a slurry of packing in isopropanol, pressurizing, and packing with isooctane. These produced usable columns, but they were rather low in plate count (10,000 to 12,000 plates/meter). On the basis that less intermixing in the slurry reservoir would occur if a denser solvent was used in the column, carbon tetrachloride was selected to fill the column. This produced columns with at least twice the plate count as previously obtained. An attempt was made to produce almost immiscible layers by using methanol as the slurry solvent. Unfortunately, methanol is an ineffective slurry solvent, and this system produced very poor columns. Apparently, the function of the solvent in producing a stable slurry is more important than its immiscibility with regard to the other solvents in the system.

Pouring isopropanol slowly down the side of a cylinder on top of a layer of carbon tetrachloride and then carefully adding a layer of isooctane produces a three-layer system. This concept has been used to maintain the slurry of the packing as a "slug" between the carbon tetrachloride contained in the column and the isooctane in the upper portion of the slurry reservoir. This system produced the best columns.

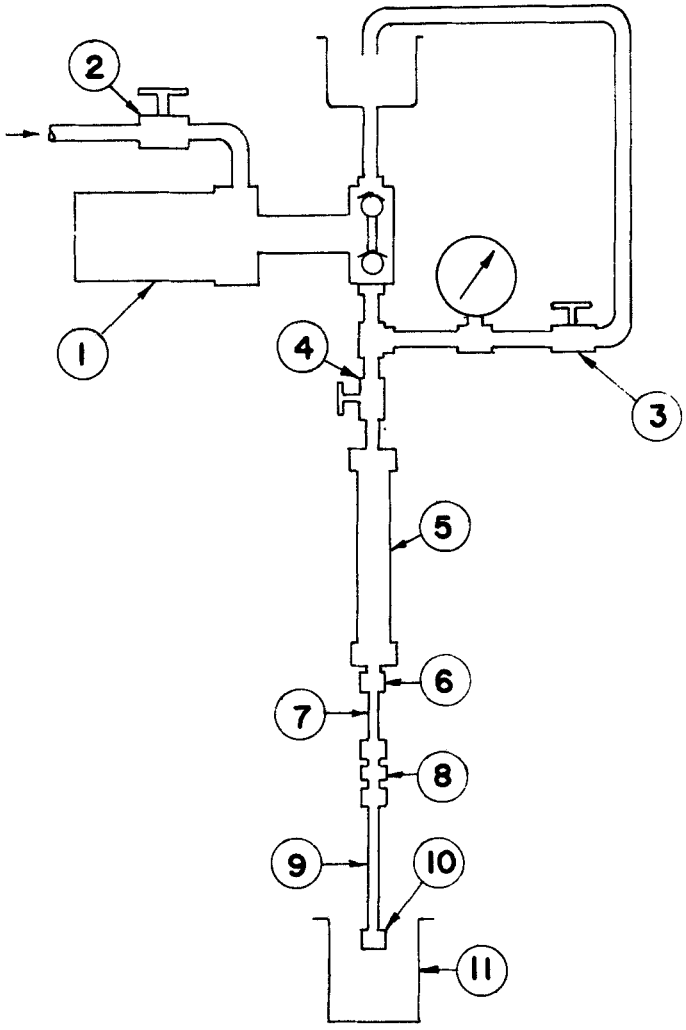

FIGURE 2

1. Pump, Haskel DSTV 122C.
2. Main Valve--located between the high flow regulator and the pump.
3. Bypass Valve--to allow pumped solvent to recycle into the solvent reservoir.

   Note: All of the above plus an air-line filter, pressure gauge, and connecting tubing are available as Assembly No. 29426-122 from Haskel Engineering and Supply Co., Burbank, California.

APPARATUS

See Figure 2.

MATERIALS

1. Carbon Tetrachloride, ACS Reagent.
2. Isopropyl Alcohol (Propanol-2), HPLC grade.
3. Isooctane (2,2,4-trimethylpentane), HPLC grade.
4. Spherisorb S10W (10-micron spherical silica)
   Spherisorb S5 ODS (5-micron spherical silica with octadecyl reverse phase coating)
   Spherisorb S10ODS (10-micron spherical silica with octadecyl reverse phase coating)
   Manufactured by Phase Separations, Ltd. and available through Alltech Associates.

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4. Column Shutoff Valve - Whitey SS-4PDF4. (Whitey Co., Cleveland, Ohio).
   Although this valve is nominally rated at 6000 psi, we have been using this type with no problems since the valve is only used to release pressure.
5. Slurry Reservoir (Figure 1)--304SS. - 3/4" O.D. x 1/2" I.D. x 17" L welded to 3/8" O.D. x 3/16" I.D. x 1-1/2" L with an internal taper.
   Note: Similar reservoirs are now commercially available. These are manufactured by Scientific Systems Inc. and are distributed by Applied Science.
6. Reducing Union - Stainless Steel, 3/8" x 1/4" (Swagelock).
7. Pre-Column - 1/4" O.D. x 4.6 mm I.D. x 5 cm L., Stainless Steel, "Li-Chroma" (Alltech Assoc.)
8. Union - Stainless Steel, 1/4" x 1/4" bored through (Swagelock).
9. HPLC column - 1/4" x 4.6 mm I.D. x 25 cm L, Stainless Steel, "Li-Chroma" (Alltech Assoc.).
10. End Fitting - Parker 4-1 Z2HBZ-2-SS C-1/4" column end fitting, inverted nut, with 2-micron internal frit (Forberg Scientific, Oak Park, MI.).
11. Effluent Reservoir--one-gallon metal can.

INITIAL SETUP

The Haskel pumping system was modified by replacing the original column shutoff valve (manufactured by Dragon) with the Whitey valve. This was originally done due to repeated failures of the Dragon valve. Later, however, we suspected that the "straight-through" design of the Whitey valve may contribute to increased efficiency of the columns produced. The pump should be rigidly secured to allow ample space below to attach the reservoir and column. This will require at least 3-1/2 feet below the outlet of the column shutoff valve. A 1/2" air line supplying at least 85 psi is required to drive the pump at 10,000 psi output. The slurry reservoir should be thoroughly cleaned of cutting oils, welding scale, and metal chips by scrubbing all internal surfaces with a paste of aluminum oxide and green soap, rinsing with distilled water and acetone, and blow drying with nitrogen.

PROCEDURE

The solvent tank is filled with isooctane with the main valve and column shutoff valve closed. After opening the bypass valve slightly, the air supply is turned on and the regulator is adjusted to approximately 20 psi. The main valve is then slowly opened, and the pump is allowed to cycle slowly until the lines are primed. The bypass valve is slowly closed, and the regulator is adjusted until the gauge indicates 10,000 psi. The main valve is then closed, and the bypass valve is slowly opened to relieve the pressure and then closed again.

The end fitting (with 2-micron frit) is seated onto the outlet of an empty 4.6 mm I.D. x 1/4" O.D. x 25 cm column. The column is attached by the 1/4" bored-through union to the outlet of the slurry reservoir, and this assembly is set aside. The inlet end of this column should butt snugly against the pre-column, inside of the 1/4" - 1/4" union.

The required amount of packing (2.9g of S10W or 3.5g of S5 ODS or S10 ODS) is slurried in 25 ml of isopropyl alcohol in

a 50-ml Erlenmeyer flask. This slurry is sonicated under vacuum for one minute. A 25-ml aliquot of carbon tetrachloride is poured into the top of the column-reservoir assembly, followed by the freshly sonicated slurry. This slurry should be carefully poured down the side of the reservoir, taking care not to leave any residue on the top fitting. The reservoir is then filled by carefully running isooctane down the side of the reservoir until filled. The reservoir is then quickly attached to the pumping system by means of the Tyco Pressure Transducer fittings (model AD-1) on the top of the reservoir and the outlet of the column shutoff valve. This fitting will withstand 10,000 psi with only hand tightening. The column shutoff valve is opened, and the valve and adjacent tubing are tapped to release any bubbles. The column shutoff is then closed, the bypass valve is opened, and the pump is turned on and cycled slowly to purge any air from the lines. After a few cycles, the bypass valve is slowly closed, the regulator is turned up until the gauge registers 10,000 psi, and the main valve is opened fully. This column shutoff valve is opened suddenly with one motion, and the pump is allowed to cycle at full output. Since the initial surge will be somewhat violent, a large (1 gallon) container should be provided under the column outlet to contain the spray. This will quickly subside to a fine stream. After 100 ml of this fine stream has been collected, the main valve for the pump is completely turned off. The system is allowed to sit until the gauge registers 0 psi. The bypass valve is then opened, and the column is carefully removed from the column-reservoir assembly. There should be a small amount of packing in the short pre-column tube. The packing at the column inlet is carefully leveled and an inlet end fitting (with 2-micron frit) is attached.

The column may now be equilibrated and checked for quality. The equilibration for the silica columns is performed by pumping acetone, methylene chloride, and heptane at 3.5 ml/min for 30 minutes each, in succession. The equilibration for the C_{18}

reverse-phase columns is performed in a similar manner using methylene chloride, methanol, and 60:40 acetonitrile:water at 3.5 ml/min for 20 minutes each.

The quality of the column is determined by a plate count measurement. The conditions used for the silica columns are as follows: mobile phase, heptane; flow, 0.5 ml/min; detection at 254 nm, 0.1A full scale; 10-mv recorder at 2 in/min; 20 μl injection (Rheodyne Model 7120) of a solution of benzene in heptane (approximately 0.01% by volume - enough to produce a 70-80% deflection on the recorder). The conditions used for the C_{18} reverse-phase columns are as follows: mobile phase, 60:40 acetonitrile:water; flow, 0.5 ml/min; detection of 254 nm, 0.1A full scale; 10 mv recorder at 0.5 in/min; 10-ul injection of a solution of acenaphthene in the mobile phase (approximately 0.01% weight to volume - enough to produce a 70-80% deflection on the recorder). Equipment used was a Model 6000A pump and Model 440 UV detector (Water Assoc.).

CALCULATION (Figure 3)

The horizontal distance from the point of injection to the to the apex of the peak (t_R) is measured. The width of the peak at half peak height (W) is also measured. The plate count (N) is then calculated:

$$N = 5.54\left(\frac{t_R}{W}\right)^2$$

The symmetry of the peak can be determined by calculating the "asymmetry factor". A vertical line, normal to the baseline, is dropped through the apex of the peak. A point is marked on this line at a distance from the baseline that is 10% of the peak height. A line is drawn through this point, parallel to the baseline, that intercepts both sides of the peak. The distance along this line from the vertical line to the right-hand peak intercept (Y) is measured. The distance to the left-hand inter-

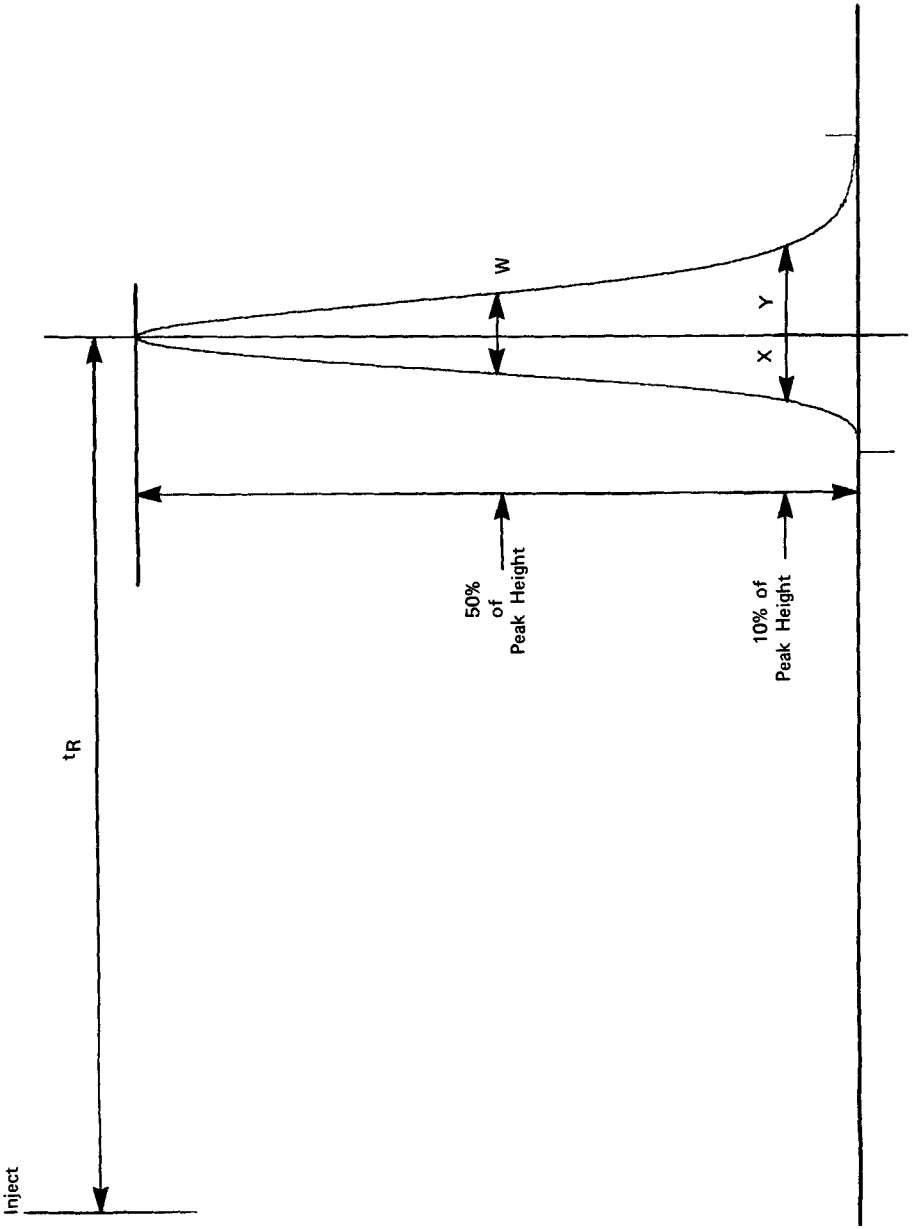

FIGURE 3

cept (X) is similarly measured. The "asymmetry factor" at 10% peak height is then calculated

$$As(10\%) = Y/X$$

Results of these calculations on columns prepared by this technique are as follows:

Col.No.	Packing	N/Meter	As (10%)
12	S1ØW	43,064	-
13	"	41,124	-
15	S1ØODS	24,388	-
16	"	21,284	-
19	"	25,059	-
20	"	25,035	-
21	S5ODS	74,843	-
22	"	73,506	-
24	S1ØODS	23,111	-
25	"	30,337	-
26	"	23,396	-
27	"	38,820	-
35	S5ODS	66,004	0.75
37	S1ØODS	44,413	-
38	"	41,093	-
39A	"	34,147	1.30
40	"	50,580	1.12
41	"	43,946	1.25
42	"	24,945	1.35
43	"	39,903	1.08
44	"	37,968	1.14
45	"	23,926	1.08
46	"	28,512	1.39
47	"	27,033	1.49
48	"	26,541	1.61
49	"	22,784	1.50
50	"	29,075	1.25
51	"	27,381	1.46
59	S5W	33,532	1.16
60	"	29,300	1.32
61	"	26,718	0.98
62	S1ØW	38,779	1.41
63	S1ØW	41,616	1.14
64	S5W	48,116	1.28
65	"	34,045	1.38

There is reason to suspect that the S5W columns gave lower plate counts than expected due to adsorbed water on the stock

packing. The recommendation of Majors (6) on heating the silica gel to 200°C prior to use as an HPLC packing was not followed in preparing the silica columns. Also, HPLC grade heptane was used as the mobile phase in determining the plate count for the silica columns. Since no special precautions (such as drying over molecular sieve, etc.) were followed, the number listed may not represent the ultimate plate count for a column.

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I wish to acknowledge the efforts of T. J. Kona in the design and construction of the slurry reservoir.

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