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PRACTICAL NARROW-BORE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY SYSTEM USING COMMERCIAL EQUIPMENT AND 1-mm BORE COLUMNS PACKED IN THE LABORATORY

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SUMMARY

The construction of a robust narrow-bore high-performance liquid chromatograph is described which involves a commercial pump, injector and detector and columns (300 × 1 mm I.D.) prepared in the laboratory. The columns were constructed from glass-lined stainless-steel tubing with unique zero-dead volume end assemblies. Full details of the column packing procedure are given which uses a conventional column packing pump at pressures less than 10 000 p.s.i. Results are presented from a study to establish the most appropriate slurry liquids and slurry concentrations. Both silica and ODS-silica columns have been prepared and their performance is discussed with reference to their suitability for routine analysis.

INTRODUCTION

Most high-performance liquid chromatography (HPLC) is currently conducted with columns of 4–5 mm internal diameter but the last five years has seen considerable interest in the utilisation of columns with reduced internal diameters. This is demonstrated by a large number of research papers (and several extensive reviews[1–6]) concerning this topic. Three different types of column have emerged, (*viz.* narrow-bore packed, packed microcapillaries and open-tubular) but are often referred to collectively as "microbore" columns which can be very confusing. At the present time only narrow-bore packed columns appear to be suitable for routine work and these are essentially conventional HPLC columns with reduced internal diameters containing packing materials of 3–10 μm.

Despite the vast literature concerning the development of narrow-bore columns few analytical laboratories are currently using them for routine applications despite numerous claims of their advantages over conventional columns. This can be attributed to several factors. Firstly, until relatively recently little commercial equipment specifically designed for narrow-bore work was available and few application laboratories have been prepared to make the extensive modifications to existing hardware as demonstrated in the literature. Secondly, commercial narrow-bore columns have been relatively expensive while few published papers give sufficient experimental

details to facilitate the packing of such columns in the laboratory. Finally, some confusion remains concerning the merits of narrow-bore columns in routine analysis and many laboratories await further clarification before making the considerable investment necessary to convert existing systems. With these considerations in mind the practicality of using narrow-bore HPLC columns for routine work has been examined and the present paper describes the construction of 1 mm bore columns, gives details of our packing procedure and demonstrates the performance of the columns using readily available commercial equipment requiring minimal modification. A second paper[7] will examine the potential advantages of these columns over conventional HPLC columns with particular reference to the types of problem encountered in forensic drug analysis.

It is now well established that the packing of small particles (3–10 μm) into conventional HPLC columns requires pumping a slurry of the material into the empty column at a relatively high pressure. Nevertheless numerous variations on this basic theme have been published as has been indicated in recent reviews[8,9]. The many variables include the nature of the slurry liquid and the pressurising solvent, the concentration of the slurry, the method of dispersal (*e.g.* ultrasonic vibration), the use of constant pressure or constant flow pumps, the packing pressure, the shape and size of the slurry reservoir, and the use of procedures to prevent sedimentation (*e.g.* upward packing, slurry stirring). In addition, several criteria are used to evaluate the success of a column packing method and these include the column plate count, the permeability and stability of the packed bed, the production of symmetrical peaks and the speed and reproducibility of the procedure. Practical experience has demonstrated that good columns can be produced using a wide range of different procedures but attempts to optimise the many variables and identify the best methods have met with limited success. The results of such studies have often been contradictory and few general guidelines have emerged. After many such experiments Verzele *et al.*[10] have even concluded that every packing material, including different commercial brands of the same type, requires an individualised packing procedure to produce columns of optimum performance.

It is generally agreed that narrow-bore HPLC columns are more difficult to pack than conventional columns. Scott and Kucera[1,11,12] have advocated the use of very high packing pressures (25 000 p.s.i.) and have recommended low viscosity balanced density slurries for silica packings and acetonitrile for octadecyl-silica (ODS-silica). Balanced density slurries have also been used by Hermansson[13], Bowermaster and McNair[14], Apffel *et al.*[15] and Menet *et al.*[16] for a wide range of different packing materials. The last publication also recommends the use of very high packing pressures with dilute slurries and stresses the importance of a cone shaped connector between the slurry reservoir and the empty column. Vadukul and Loscombe[17,18] have packed narrow-bore columns using low viscosity slurry solvents and a stirred reservoir. Meyer and Hartwick[19] have also used a low viscosity slurry solvent, isopropanol, for packing 1 mm bore columns and describe systematic studies to optimise the procedure. The above publications all involved the packing of stainless-steel (SS) columns of around 1 mm I.D. but recent work by Takeuchi and Ishii[20], Yang[21], Hirata and Jinno[22] and Novotny *et al.*[23] has involved the use of glass or fused-silica tubing (0.1–0.5 mm I.D.) for column fabrication. These columns have been packed at lower pressures (typically $< 10\,000$ p.s.i.) with low viscosity slurry solvents.

The operation of narrow-bore HPLC columns requires the use of miniaturized injectors and detector flow-cells in order to avoid extra-column band broadening and thus realise the true performance of the columns. The emphasis of the present paper is on the feasibility of setting up reliable narrow-bore HPLC systems for routine analysis and hence the selection of the hardware (pump, injector, UV detector) has been based on its commercial availability, deliberately avoiding the need for extensive modifications in the laboratory. The valve injector selected had loop sizes ranging from 0.5 to 5 μl while the UV detector had a flow-cell of 0.3 μl being the smallest available in any UV detector on the British market at the time of commencing this work[24]. The use of 1 mm I.D. columns in this study was influenced by the dimensions of these components since an excessive loss in performance might be expected with columns of smaller internal diameters[25]. In addition, such columns are usually operated at flow-rates between 10 and 100 μl/min and a dual-piston analytical pump was modified by the manufacturer to deliver these flow-rates. Adhering to the general philosophy of using equipment which is readily available all column packing has been conducted with a commercial packing pump capable of pressures up to about 10 000 p.s.i.

EXPERIMENTAL

Materials

Methanol and hexane were HPLC grade obtained from Rathburn Chemicals (Walkerburn, U.K.). Acetone, ethanediol, nitrobenzene, pentane, phenol and toluene were analytical grades from BDH (Poole, U.K.). Anisole, *p*-cresol, 2,5-dimethylphenol, 3,5-dinitrobenzonitrile, 3,5-dinitrobenzyl chloride and 2,6-dinitrotoluene were obtained from Aldrich (Gillingham, U.K.). All other chemicals were general purpose grades from BDH. Water was distilled in glass in the laboratory.

Three HPLC packing materials were obtained from Shandon Southern Products (Runcorn, U.K.), *viz.* Hypersil-silica with mean particle diameters of 4.13 and 5.73 μm respectively and Hypersil-ODS with a mean particle diameter of 5.49 μm.

Column construction

Columns were constructed from glass-lined SS tubing (30 cm × 1/8 in. O.D. × 1 mm I.D.) obtained from Scientific Glass Engineering (Milton Keynes, U.K., Part No. 2737). Each end of the column was terminated in the same way with a 1/4-in. O.D. tube brazed onto the glass-lined tubing and a conventional zero-dead volume end-fitting (Fig. 1).

A length of SS rod (1/4 in. O.D.) was cut on a lathe (3.5 cm) and drilled along its length (No. 30 British Standard bit) such that the glass-lined tubing gave a close fit when inserted into the hole. The metal surfaces to be brazed were first cleaned with fine emery-paper and then immersed in 50% sulphuric acid for 15 min. After rinsing with distilled water, the glass-lined tube was inserted into the outer tube taking care to align the ends of the tubes. The assembly was clamped in a vertical position with the ends pointing down. A short length of a silver brazing alloy (melting point 620–630°C) was wrapped around the glass-lined tubing and allowed to rest on the top of the outer tube. The region of the joint was then covered with a thick paste of Tenacity Flux No. 5 in water. Heat was carefully applied to the outer tube using

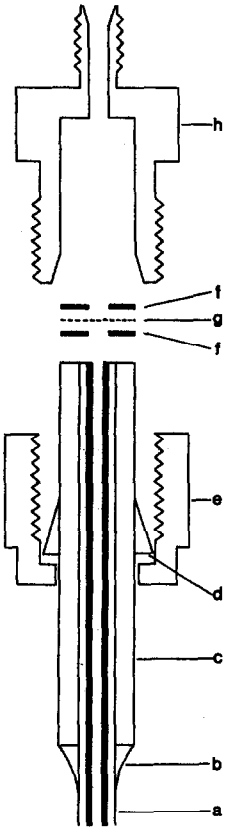

Fig. 1. Construction of narrow-bore column end assemblies. a = Glass-lined SS tubing (30 cm × 1/8 in. O.D. × 1 mm I.D.); b = silver brazing; c = SS rod (3.5 cm × 1/4 in. O.D.) drilled along its length to accommodate the glass-lined tubing; d = SS ferrule (1/4 in.); e = SS nut (1/4 in.); f = PTFE washer (0.005 in. thick); g = SS mesh (1/4 in.); h = drilled out zero-dead volume end-fitting (1/4 in.).

a fine gas-air torch until the brazing alloy was seen to melt when the source of heat was quickly removed to prevent any damage to the glass-lining of the inner tube. After allowing the joint to cool slowly to room temperature the excess flux was removed.

The end-fitting used was a Parker-Hanifin drilled out SS 1/4–1/16 in. reducing union (HETP, Macclesfield, U.K., Part No. 200) and was held in position by a 1/4-in. SS compression ferrule. The packing material was held in position by a 1/4-in. SS mesh disc (HETP, Part No. 206) which was sandwiched between two 1/4-in. washers made from PTFE sheet (0.005 in.) having 1-mm diameter holes cut in the centres.

Column packing

The columns were packed with conventional HPLC column packing equipment (Shandon Southern Products) based on a pneumatic amplifier pump and capable of pressures up to about 10 000 p.s.i. The apparatus included a 3-way switching

valve such that the liquid being pumped could be changed without interrupting the flow. The outlet from the pump was connected to a coil of SS tubing (1/16 in. O.D. × 1.09 mm I.D.) from Phase Separations (Queensferry, U.K., Part No. T1009) which acted as a slurry reservoir (Fig. 2). Three such reservoirs were constructed with tubing lengths of 150, 375 and 1000 cm having volumes of *ca.* 1.4, 3.5 and 9.4 ml respectively. The connection between the pump and the slurry reservoir involved a drilled out 1/16-in. union with a side arm (HETP, Part No. 387) and a straight through 2-way stop valve (Scientific Glass Engineering, Part No. SSI-15MW-A200). The outlet side of this valve was terminated with a luer syringe connector (HETP, Part No. 437). This arrangement allowed the slurry reservoir to be loaded rapidly from a luer syringe without the need to disconnect any fittings.

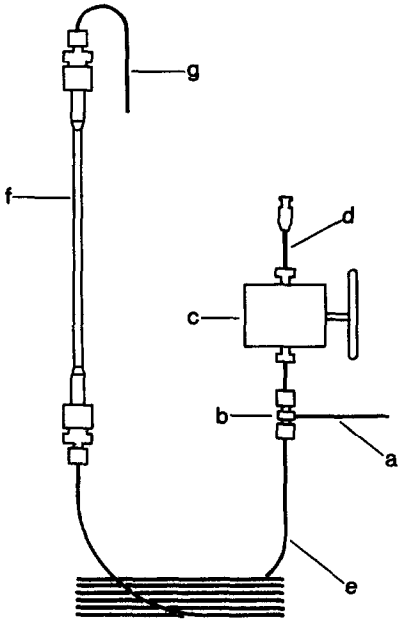

Fig. 2. Narrow-bore HPLC column packing apparatus. a = SS inlet tube (1/16 in.) from the HPLC packing pump; b = drilled out union (1/16 in.) with a side-arm brazed into position; c = straight through stop valve; d = luer syringe connector; e = slurry reservoir constructed from SS tubing (1/16 in. O.D. × 1.09 mm I.D.); f = empty narrow-bore column; g = PTFE outlet tube (1/16 in. O.D. × 0.8 mm I.D.).

The outlet end of the slurry reservoir was connected to the empty column using a 1/16-in. SS nut and ferrule. The outlet end of the column was assembled as shown in Fig. 1 while the mesh and PTFE washers were removed from the inlet end. Columns were packed in an upward direction with a PTFE tube (1/16 in. O.D. × 0.8 mm I.D.) connected to the column outlet such that the waste solvent could be safely collected. This was particularly important when handling the toxic halogenated slurry solvents.

Various slurry liquids were tested during the course of this study including methanol, methanol–ethanediol (1:1, v/v), aqueous ammonium hydroxide (0.001 *M*), iodomethane–methanol (9:1, w/w) and tetrabromoethane–methanol (4:1, w/w). The

last two mixtures, containing halogenated solvents, tended to discolour on storage; this was effectively removed by passing the liquids through disposable cartridges (*ca.* 23 × 9 mm I.D.) packed with silica (Sep-Pak silica) obtained from Waters Assoc. (Northwich, U.K.) immediately before use.

The packing procedure now used routinely for both silica and ODS-silica is described below. The equipment was set up as shown in Fig. 2 with the 1.4-ml slurry reservoir in position and the liquid reservoirs of the packing pump containing hexane, methanol and 50% aqueous methanol. After priming the pump with hexane and setting the pressure to 8500–9000 p.s.i. against a stop valve the pump was switched off and the pressure allowed to subside. The slurry reservoir filling valve was opened and a compressed air line applied to the luer fitting such that solvent was pushed out of the waste line leaving the slurry reservoir and column completely empty. The glass barrel of a gas-tight syringe (V. A. Howe, London, U.K., Part No. 1001TLL, 1 ml) was then attached to the luer fitting.

Packing material (190 mg) was weighed into a graduated glass test tube (5 ml) with a stopper and the slurry liquid [tetrabromoethane–methanol, 4:1 (w/w), 1 ml] was added. The tube was shaken vigorously to disperse the packing material and then placed in an ultrasonic bath for 15 min. The slurry was decanted into the syringe barrel using a pasteur pipette and pushed into the slurry reservoir using the PTFE tipped plunger although this was not always necessary as the slurry often moved into the coil without assistance. The slurry reservoir filling valve was then closed and the packing pump switched on. Hexane was pumped for about 15 min and then methanol for a further 15 min when the packing was considered to be complete. With ODS-silica packing materials the column was further conditioned with 50% aqueous methanol. Towards the end of the packing procedure the column was inverted by gently bending the tubing of the slurry reservoir and then the pump was switched off. When the pressure had subsided the column was disconnected and the SS mesh with PTFE washers placed in position.

Column testing

Chromatography was performed with a Waters M6000A pump modified by the manufacturer to slow down the speed of the electric motor, with an internal switch used to select a reduction in flow-rate of × 10 or × 100. Samples were introduced with a Rheodyne 7410 microbore injection valve fitted with a loop filler port (Model 70-11). A range of internal sample loops (0.5, 1, 2 and 5 μl) were used with this valve. The eluate was monitored with a Jasco Uvidec-100-III UV spectrophotometer (Lea Scientific, Milton Keynes, U.K.). Chromatograms were recorded with a Linear Model 500 chart recorder having a pen response of <0.5 s full scale.

The UV detector was fitted with a micro-cell cassette available from the manufacturer which contained a small flow-cell (0.3 μl) consisting of a length of quartz tubing (0.6 mm O.D., 0.3 mm I.D.). The construction is shown in Fig. 3 along with the details of the connections from the end of the narrow-bore column used to minimise the dead-volume. A further reduction in dead-volume was achieved by each column having its own unique inlet connection consisting of a short length of SS tubing (5 cm × 1/16 in. O.D. × 0.006 in. I.D.) held into the end-fitting by a SS ferrule such that the tube outlet touched the mesh disc. This approach eliminated problems arising from variations in the drilling of each end-fitting.

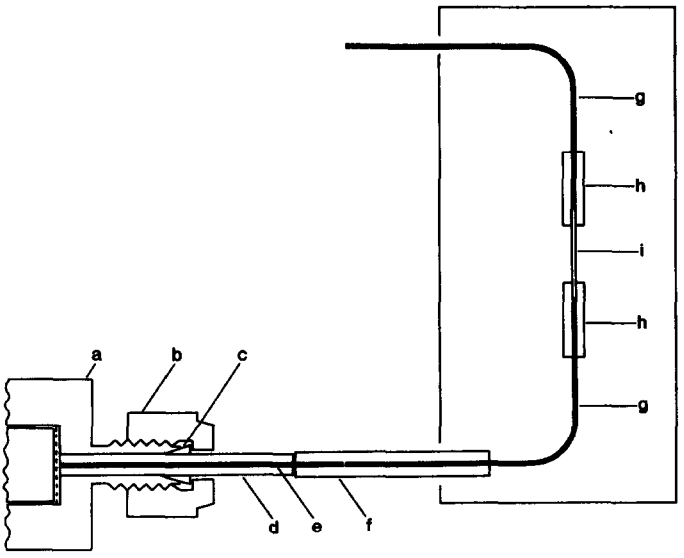

Fig. 3. Connections between the column outlet and the UV detector flow-cell. a = Narrow-bore end-fitting; b = SS nut (1/16 in.); c = PTFE ferrule (1/16 in.); d = PTFE tubing (3.5 cm × 1/16 in. O.D. × 0.023 in. I.D.); e = SS tubing (4 cm × 0.7 mm O.D. × 0.1 mm I.D.); f = PTFE tubing (3 cm × 2 mm O.D. × 0.5 mm I.D.); g = SS tubing (6 cm × 0.6 mm O.D. × 0.3 mm I.D. with two 0.15-mm wires inserted into the bore to reduce dead volume); h = PTFE tubing (1 cm × 1/16 in. O.D. × 0.5 mm I.D.); i = quartz tubing (1.25 cm × 0.6 mm O.D. × 0.3 mm I.D.).

The column testing procedures were essentially those recommended by Bristow and Knox[26]. An eluent of hexane–methanol (99.5:0.5, v/v) was used for silica columns and methanol–water (60:40, v/v) for ODS-silica columns. Detection wavelengths of 235 and 270 nm were used for the normal-phase and reversed-phase systems respectively. The injection valve was fitted with a 0.5-μl loop for column testing while the performance of the pump was checked at regular intervals by connecting an appropriate graduated syringe with no plunger to the outlet from the detector and timing the progress of the liquid along the scale.

The test mixture for silica columns was prepared by dissolving acetophenone (30 mg), 1,3-dinitrobenzene (15 mg), 3,5-dinitrobenzonitrile (20 mg), 3,5-dinitrobenzyl chloride (20 mg), 2,6-dinitrotoluene (20 mg) and nitrobenzene (40 mg) in methanol (1 ml), adding pentane (50 ml) and then making up to a volume of 200 ml with hexane. Toluene (1.5 mg per ml) was also added to this solution for some test procedures.

The test solution for ODS-silica columns was prepared by dissolving acetone (716 mg), phenol (15 mg), *p*-cresol (29 mg), 2,5-dimethylphenol (29 mg), anisole (24 mg) and phenetole (35 mg) in methanol–water (80:20, v/v) (50 ml).

The measurements taken from the test chromatograms were used to calculate the conventional parameters used to assess the performance of packed HPLC columns. These included plate number (N), height equivalent of a theoretical plate (HETP), reduced plate height (h), separation impedance (E), flow resistance parameter (φ) and total column porosity (ε_{tot}). Calculations followed published proce-

dures[26] using peak widths at half height to estimate column efficiencies. They were carried out using an interactive computer program written in BASIC for the Prime 550 computer by the author.

RESULTS AND DISCUSSION

The selection of glass-lined SS tubing for the column construction was based on published data which suggests that columns with smooth interior walls give higher plate counts when packed under identical conditions[20,27,28]. The performance of narrow-bore columns fabricated from glass or fused-silica tubing (0.1–0.5 mm)[20–23] has most closely approached the theoretical limits which can be achieved with conventional columns. However, the flexibility of such tubing is a practical disadvantage for routine handling which is overcome by the use of rigid glass-lined tubing.

The end-fittings of conventional SS HPLC columns are usually held in position by compression ferrules on the column tubing. Initial experiments with the glass-lined tubing (1 mm I.D.) using SS one-piece ferrules to attach 1/8-in. zero-dead volume end-fittings cracked the glass-lining. Similar results were obtained using two-piece SS or brass ferrules. The method which was finally adopted is shown in Fig. 1 and involved silver brazing a short length of 1/4-in. diameter SS tubing onto the end of the glass-lined tubing and then using a 1/4-in. zero-dead volume end-fitting with a SS ferrule. The performance of columns constructed in this way was greatly improved by the inclusion of PTFE washers each side of the mesh disc to minimise any dead volume associated with the drilling of the end-fitting leaving a slightly conical surface at the bottom of the 1/4-in. hole.

Preliminary experiments using a conventional slurry reservoir (*ca.* 30 ml, 8 mm I.D.) to pack silica (5.73 μm) into the 1-mm bore columns (300 mm) gave poor efficiencies while the packing procedure took several hours. The adoption of a slurry reservoir of comparable internal diameter to the column (Fig. 2) gave columns of satisfactory performance with the total procedure taking less than one hour. Using this apparatus a series of experiments was conducted to examine the effect of different slurry liquids on the column packing procedure and the results are presented in Table I. All packing procedures were identical except for the selection of the appropriate slurry liquid and the solvent used to push the slurry into the column. Two columns were used in this study and each packing procedure was tested on both columns. The slurry liquids were selected to be representative of the different approaches to slurry packing which have been reported in the literature, *viz.* low viscosity[29–33], high viscosity[27,34], aqueous ammonia[35] and balanced density[36–39] slurries. The plate counts for six compounds in the silica test mixture measured at a flow-rate of 80 μl/min indicated that the balanced density slurries of iodomethane–methanol and tetrabromoethane–methanol gave the best results. The results did not demonstrate any clear advantages between the two balanced density slurry liquids.

The influence of slurry concentration was investigated by packing a single column with the same weight of silica dispersed in different volumes of tetrabromoethane–methanol. In each experiment the slurry reservoir was selected to have a volume just large enough to accommodate the slurry such that the column started to pack as soon as the packing pump was switched on with no time delay. The results are shown in Table II and clearly indicate that concentrated slurries are

TABLE I

EFFECT OF DIFFERENT SLURRY LIQUIDS ON COLUMN PACKING

Columns: A and B both 300 × 1 mm I.D. Slurry preparation: Hypersil-silica (5.73 μm), 175 mg dispersed in 3 ml of the slurry liquid. Packing pressure: 8500 p.s.i. Column testing procedure involved an eluent of hexane–methanol (99.5:0.5, v/v) at a flow-rate of 80 μl/min. Compounds 1–6 are nitrobenzene, acetophenone, 2,6-dinitrotoluene, 1,3-dinitrobenzene, 2,6-dinitrobenzylchloride and 2,6-dinitrobenzonitrile respectively with capacity factors (k') ranging from 0.44 to 5.92.

Packing procedure		*Column*	*Plate counts**					
Slurry liquid	*Pressurising solvent*		*1*	*2*	*3*	*4*	*5*	*6*
Methanol	Methanol	A	1932	1844	1799	1824	1683	1634
		B	2435	2446	2585	2620	2422	2560
Methanol–ethanediol (1:1, v/v)	Methanol	A	2043	1940	1929	2079	1755	1632
		B	2214	2262	2212	2436	2287	1206
Ammonium hydroxide (0.001 *M*)	Methanol	A	2527	2670	2550	2788	2649	2867
		B	1583	1504	1470	1590	1480	1472
Iodomethane–methanol (9:1, w/w)	Hexane	A	9239	8716	10 743	11 745	11 732	11 849
		B	8293	8453	10 084	11 841	11 811	12 890
Tetrabromoethane–methanol (4:1, w/w)	Hexane	A	9204	10 334	10 633	12 411	12 140	12 823
		B	8197	9239	9204	10 991	10 870	10 642

* Mean of 3 determinations.

preferred. This has the additional advantage of speeding up the packing procedure since less time is required to pump the relatively viscous slurry liquid through the column. Balanced density slurry packing procedures for conventional HPLC columns have become rather unpopular in recent years and this can be attributed to the toxicity and high cost of the halogenated solvents used in the preparation of the slurry liquids. The small slurry volumes used for packing narrow-bore columns in this study overcome these objections both from an economic viewpoint and by the ease with which such small quantities can be safely contained thus reducing exposure risks.

TABLE II

EFFECT OF SLURRY CONCENTRATION ON COLUMN PACKING

Column: 300 × 1 mm I.D. Slurry liquid: tetrabromoethane–methanol (4:1, w/w). Packing material: Hypersil-silica (5.73 μm). Slurry concentration: 170 mg of packing material dispersed in the appropriate volume of slurry liquid. Packing pressure: 8500 p.s.i. Column testing procedure involved an eluent of hexane–methanol (99.5:0.5, v/v) at a flow-rate of 80 μl/min.

Slurry volume (ml)	*Slurry reservoir volume (ml)*	*Plate count for 2,6-dinitrotoluene*
1	1.4	14 294
3	3.5	12 502
9	9.4	12 036

The present slurry packing procedures have now been used for over 18 months and have been applied to a wide range of different packing materials. The procedures have proved to be very reproducible and a series of identical columns packed with the same batch of Hypersil-silica gave plate counts with a coefficient of variation of 6.7% (n = 7).

The procedure now adopted routinely for packing narrow-bore columns involves a slurry liquid of tetrabromoethane–methanol (4:1, w/w) with a slurry volume of 1 ml. This procedure has been applied to materials of different particle sizes and Fig. 4 shows the results of experiments to examine the relationship between the column efficiency and the eluent flow-rate for a test compound (2,6-dinitrotoluene, k' = 1.22) on typical columns packed with 4.13 and 5.73 μm silica. Fig. 4A gives a plot of plate count (N) against flow-rate for the two materials with both columns giving 14 500–15 000 plates at optimum velocity. Thus, no improvement in plate count was achieved by using the material of smaller particle size. Fig. 4B shows the same data plotted as reduced plate height (h) against reduced velocity (v). These are dimensionless parameters which allow comparisons to be made with the theoretical expectations for packed columns[26]. The 4.13-μm material gave minimum reduced

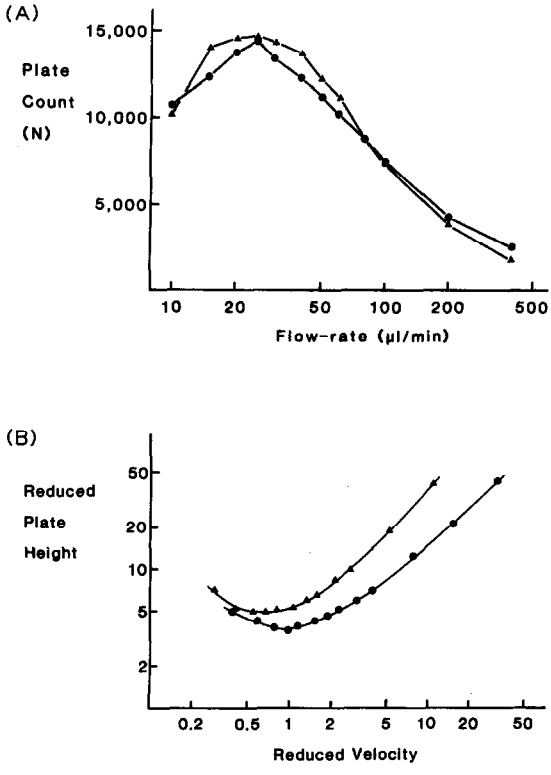

Fig. 4. The performance of silica narrow-bore HPLC columns. (A) Plots of plate count against flow-rate; (B) plots of reduced plate height against reduced velocity. Eluent: hexane–methanol (99.5:0.5, v/v). Detection: 235 nm. Test compound: 2,6-dinitrotoluene (k' = 1.22). Injection volume: 0.5 μl. Packing materials: Hypersil-silica (mean particle size: ● = 5.73 μm, ▲ = 4.13 μm).

plate heights of about 5 while the 5.73-μm material gave values of about 3.5. It is clear that the smaller particle size material is less successfully packed using the present packing procedure.

Although the optimum performance of the narrow-bore columns packed with 5.73-μm silica (h = *ca.* 3.5) falls short of that which can be achieved with well packed conventional columns (h = 2–3) the results compare favourably with previous publications involving the packing of 1 mm bore columns[12–19] where the procedures used have often been less convenient involving high pressures (>20 000 p.s.i.) or long packing times. The packing procedure has also been applied to ODS-silica (5.49 μm) using the same slurry liquid and Fig. 5 shows a plot of reduced plate height against reduced velocity for *p*-cresol (k' = 1.03) on a typical column. As for silica columns typical reduced plate heights at optimum velocity were *ca.* 3.5. It is interesting to note that Scott and Kucera[11] found that different slurry liquids were required for packing silica and ODS-silica into 1 mm bore columns.

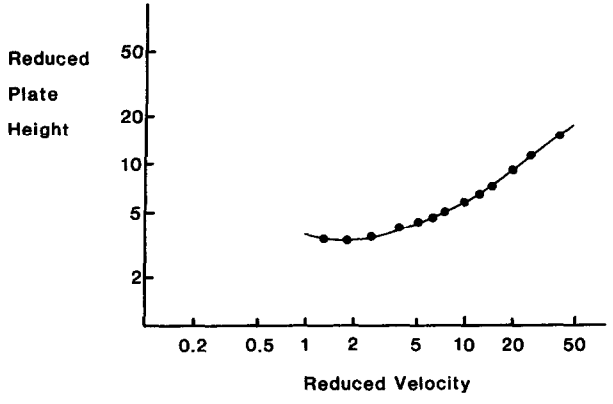

Fig. 5. The performance of an ODS-silica narrow-bore HPLC column. Eluent: methanol–water (60:40, v/v). Detection: 270 nm. Test compound: *p*-cresol (k' = 1.03). Injection volume: 0.5 μl. Column: Hypersil-ODS (5.49 μm).

The column testing procedures adopted were essentially those recommended by Bristow and Knox[26]. The test mixture for ODS-silica columns was identical to that described, *viz.* acetone, phenol, *p*-cresol, 2,5-dimethylphenol, anisole and phenetole. However, the mixture used for testing silica columns was modified from that recommended, replacing 1,3,5-trinitrobenzene which is difficult to obtain, with three new compounds (1,3-dinitrobenzene, 3,5-dinitrobenzylchloride and 3,5-dinitrobenzonitrile) which extend the retention range to k' = 5.9. Fig. 6 shows chromatograms obtained for the test mixture on a 5.73-μm silica column at two different flow-rates. The slower flow-rate of 25 μl/min is close to the minimum in the reduced plate height against reduced velocity curve (Fig. 4B) and although demonstrating optimum efficiencies the analysis times are rather long. The second chromatogram at a flow-rate of 80 μl/min, which is equivalent to a flow-rate of 2 ml/min with a conventional (5 mm) column, indicates that a satisfactory performance can be achieved on these columns with reasonable analysis times. Fig. 7 shows a chromatogram obtained with

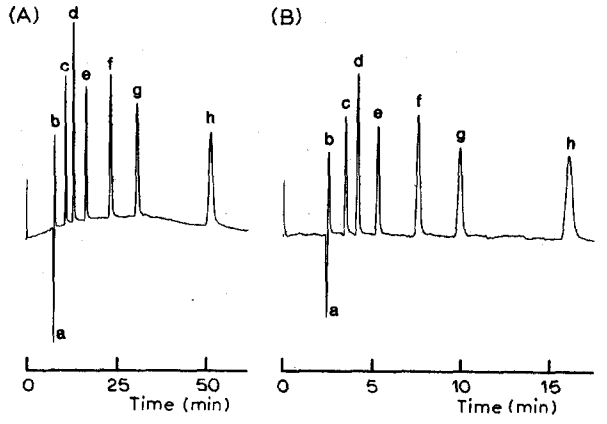

Fig. 6. Chromatograms of a test mixture on a silica narrow-bore column. Flow-rate: (A) 25 μl/min; (B) 80 μl/min (with operating pressures of 120 and 390 p.s.i. respectively). Column: Hypersil-silica, 5.73 μm (300 × 1 mm I.D.). Eluent: hexane–methanol (99.5:0.5, v/v). Detection: 235 nm. Injection volume: 0.5 μl. Peaks: a = pentane; b = toluene; c = nitrobenzene; d = acetophenone; e = 2,6-dinitrotoluene; f = 1,3-dinitrobenzene; g = 2,6-dinitrobenzylchloride; h = 2,6-dinitrobenzonitrile. The test compounds (a–h) have capacity factors (k') of 0, 0.05, 0.44, 0.72, 1.22, 2.13, 3.13 and 5.92 respectively.

the test mixture on an ODS-silica column. The peaks in all chromatograms show good peak shapes and it is clear that the column efficiencies are very satisfactory for routine analysis. Furthermore the operating pressures at typical working flow-rates were comparable to those which would be expected with conventional columns at identical linear flow-rates.

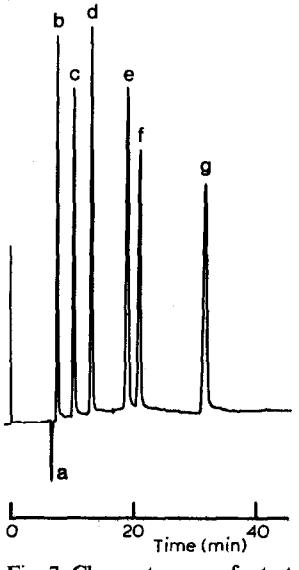

Fig. 7. Chromatogram of a test mixture on an ODS-silica narrow-bore column. Column: Hypersil-ODS, 5.49 μm (300 × 1 mm I.D.). Eluent: methanol–water (60:40, v/v). Flow-rate: 25 μl/min. Pressure: 850 p.s.i. Detection: 270 nm. Injection volume: 0.5 μl. Peaks: a = methanol–water disturbance; b = acetone; c = phenol; d = *p*-cresol; e = 2,5-dimethylphenol; f = anisole; g = phenetole. The test compounds (a–g) have capacity factors (k') of 0, 0.17, 0.60, 1.03, 1.90, 2.20 and 3.82 respectively.

Further comparison of the narrow-bore columns with conventional columns has been made by the calculation of flow resistance parameters (φ). This is a dimensionless parameter which measures the resistance to eluent flow allowing for the column length, the eluent viscosity and the particle size of the packing material. A value of about 500 is typical for conventional slurry packed HPLC columns while the present narrow-bore columns (both silica and ODS-silica) have all shown similar values (500 ± 100). This must indicate that the density of the packed bed in the narrow-bore columns is similar to that in the larger bore columns and this was confirmed by measurements of the total porosity (ε_{tot}). This parameter represents the fraction of the column bed volume which is occupied by the mobile phase and shows typical values of 0.7–0.8 for conventional columns. The narrow-bore columns showed ε_{tot} values of *ca.* 0.72 for ODS-silica (5.49 μm) and *ca.* 0.83 for silica (5.73 μm).

The separation impedance (E) is generally regarded as one of the best parameters to describe the overall performance of packed HPLC columns[26]. It is dimensionless and measures performance in terms of a high plate count, low elution time and the economical use of pressure; a decrease in E represents an improved performance. It is simply related to the flow resistance parameter and the reduced plate height by $E = h^2\varphi$ and thus an optimum value of *ca.* 2000 is expected for conventional columns with $h = 2$ and $\varphi = 500$. The present narrow-bore columns packed with 5.73-μm silica and 5.49-μm ODS-silica gave E values of 5500–6000 at optimum flow-rates. As the flow resistance parameter for the narrow-bore columns was measured to be similar to that in conventional columns it is clearly the non-optimal efficiency (high h value) which gives the high E value.

The application of the present narrow-bore columns to practical analytical problems requires some guidelines concerning the influence of sample injection on column performance and experiments have been conducted to examine both injection volume and mass loading. A reversed-phase HPLC system was chosen for these studies involving a Hypersil-ODS column (300 × 1 mm I.D.) and a buffered aqueous methanolic eluent [methanol–0.1 *M* sodium dihydrogenphosphate (50:50, v/v)] with injections of the drug phenacetin dissolved in the eluent. Phenacetin has a capacity factor of *ca.* 1.6 on this HPLC system. In the first series of experiments the same weight of drug (2 μg) was injected onto the column using the four different loops available for the injection valve. In each case the sample loop was completely filled with a solution of phenacetin at the appropriate concentration. An eluent flow-rate of 50 μl/min was used with detection at 220 nm. The results clearly demonstrated that there was little difference in column efficiency between the 0.5-, 1- and 2-μl loops (N = 6305, 6378 and 6347 respectively) while the 5-μl loop led to a fall in the observed column performance (N = 5637). Although a limiting injection volume of 2 μl is very small compared to conventional HPLC columns and may require samples to be further concentrated before analysis, the selection of an injection solvent with a lower elution strength should enable the injection volume to be further increased while maintaining the column performance[40].

The critical mass loading of a column has been suggested as the mass of sample which causes a 30% deterioration in the column efficiency observed with small loads[41]. Fig. 8 shows a plot of plate count against the weight of phenacetin injected in a 2-μl sample loop and using the above definition it can be seen that the maximum loading is around 10 μg for the present reversed-phase HPLC system.

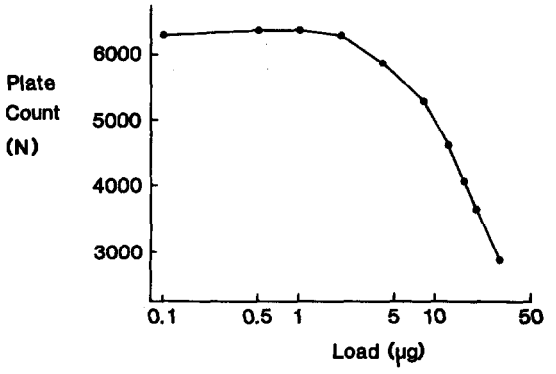

Fig. 8. The influence of mass loading on the observed column performance. Column: Hypersil-ODS, 5.49 μm (300 × 1 mm I.D.). Eluent: methanol–aqueous sodium dihydrogen phosphate (0.1 *M*) (50:50, v/v). Flow-rate: 50 μl/min. Compound injected: phenacetin dissolved in the eluent. Injection volume: 2 μl.

CONCLUSION

The present work has demonstrated a practical narrow-bore HPLC system which uses commercial equipment and columns (1-mm bore) packed in the laboratory. The columns are constructed from glass-lined SS tubing being terminated by unique end assemblies. Full details of a packing procedure are described which has proved to be both easy and reliable for both silica and ODS-silica. The packing procedure has been used in the authors laboratory for over 18 months and has consistently given columns with satisfactory performance with a very low failure rate. Furthermore the operation of these columns with the commercial hardware described has proved to be as straightforward as using conventional HPLC systems.

The performance of the columns is similar to that which has already been achieved by other workers with 1-mm bore columns but the simplicity of the packing procedure using a conventional HPLC column packing pump at < 10 000 p.s.i. is a considerable advantage. The failure to achieve reduced plate heights with the present 1-mm bore columns as good as those obtained with conventional columns may have several causes, but it is most probably the simple consequence of the narrow-bore columns being less well packed. Nevertheless, such factors as the influence of the column wall on the chromatography, extra column band broadening from the valve injector, detector flow-cell and column inlet/outlet fittings, and slow response times from the detector and chart recorder may all contribute to the overall band broadening observed in the chromatogram during a performance test. Undoubtedly, improvements could be attempted in many of these areas. There is clearly much scope for further optimisation of the packing procedure and the use of special home-made equipment may help to overcome some of the other problems as has already been demonstrated in the literature. However, it was not the intention of the present paper to strive for the maximum column performance but to describe a practical narrow-bore system with a performance satisfactory for routine analysis which can easily be set up in any laboratory.

It is hoped that the demonstration of such a practical narrow-bore system will

encourage analysts to consider the use of these columns for applications in their own fields. Data from routine analysis are urgently required by which the various claims and counter claims concerning the advantages of narrow-bore columns can be tested and the analyst can be guided in the purchase of new HPLC instrumentation with regard to the necessity for "microbore" capability. With this intention, a second paper[7] will consider the potential advantages of 1-mm bore HPLC columns with reference to routine applications in the field of forensic drug analysis.

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