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FUSED-SILICA NARROW-BORE MICROPARTICLE-PACKED-COLUMN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Narrow-bore fused-silica liquid chromatography columns with I.D. $\leqslant$ 500 μm and packed with $\leqslant$ 10-μm particles in lengths from 10 cm to a few meters can be efficiently prepared. A 1-m fused-silica column with 110,000 theoretical plates can be packed with 3-μm C_{18} bonded-phase particles. Narrow-bore microparticle-packed columns can be used in conjunction with a constant-pressure piston pump, a splitter injector and an on-column UV absorbance detector. A 45 cm $\times$ 300 μm I.D. column packed with 3-μm C_{18} bonded-phase particles allows baseline separation of an Environmental Protection Agency priority pollutant mixture containing sixteen polynuclear aromatic hydrocarbons. The use of the effect of solvent modifier(s) in the sample solution to improve solute resolution, peak detection, and column selectivity and to reduce analysis time of the narrow-bore microparticle-packed-column high-performance liquid chromatography is proposed and demonstrated. The practical importance of the solvent effect in narrow-bore packed-column high-performance liquid chromatography is also discussed.

INTRODUCTION

Recent advances in high-performance liquid chromatography (HPLC) column technology center on open tubular columns[1–9] and microparticle-short-packed columns[7–10]. The interest in open tubular columns is due to the following reasons: (1) a 10 to 10^2 fold saving in solvent consumption compared with conventional packed-column HPLC using columns of 1–5 mm I.D.; (2) higher resolution can be achieved using long microbore columns; (3) minimum sample requirements provide the potential for trace analysis in cases of limited available sample volume, such as in the analysis of biological fluids; and (4) the low flow-rates should facilitate direct interfacing to mass spectrometers and flame-based chromatographic detectors[10]. The advantages of directly interfacing with flame-based detectors are particularly important due to the fact that these detectors offer greater sensitivity and selectivity (elemental specificity) than do conventional LC detectors. As discussed previously[11], with the use of open tubular columns of I.D. less than 30 μm, the resolving power, speed of analysis and column peak capacity can be equal to or better than that achieved with

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the state-of-the-art 5-μm-particle-packed columns having a reduced plate height of 2 and a reduced velocity of 5 in a given analysis time. However, the stringent requirements imposed by microbore open tubular columns on the stability of the hydraulic pumps at low flow-rates, nanoliter sample volume injection, submillimeter connector tubing and subnanoliter detector flow cells present substantial impediments to the full use of available column performance[11]. In addition, columns with I.D. less than 30 μm are difficult to prepare and have limited partition ratios (*e.g.*, k less than 3), sample capacities (*e.g.*, less than 1 μg), column selectivity and ranges of applicable sample mass and concentration.

Compared with conventional 25–30 cm × 4–5 mm I.D. LC columns, microparticle-short-packed columns (10 cm × 1–5 mm I.D.) packed with 3–10-μm particles offer some savings in solvent consumption, a decreased analysis time and enhanced detection of solutes. However, the application of microparticle-*short*-packed columns is limited to samples containing not more than several dozen components, due to a limited column-peak capacity and theoretical plate number. In addition, the direct interfacing of such microparticle-packed columns to a mass spectrometer or a flame-based chromatographic detector for solute detection and characterization cannot be obtained at the flow-rates used (1–10 ml/min).

It is the purpose of this paper to propose and to examine the use of columns with I.D. less than 500 μm and packed with ⩽10-μm particles in lengths from 10 cm to a few meters. *Narrow-bore microparticle-packed columns**, as suggested here, are normally operated at the same linear velocity as conventional packed columns, 0.5–2 mm/sec, dependent upon the particle size used. Because of the small I.D., the volumetric flow-rates are between 0.1 and 10 μl/min. This low mobile-phase flow-rate allows easy, direct interfacing of the column to a mass spectrometer[12,13] and/or a flame-based chromatographic detector[11] so that more sensitive and specific LC detection can be obtained. The proposed narrow-bore microparticle-packed column LC provides both the advantages of open tubular column and short microparticle-packed column LC in terms of solvent-saving, fast analysis, and minimum sample size requirement. In addition, the packed bed in the narrow bore column is stable due to the difficulty of rearrangement and resettling of the tightly packed particles in a very small cross-sectional area. The use of long columns (0.5–2 m) provides the high resolving power and peak capacity required for complex sample analysis.

In this report, the chromatographic performance and practical aspects of narrow-bore microparticle-packed columns of I.D. ranging from 57 to 376 μm and packed with 3-, 5- and 10-μm C_{18} bonded reversed-phase particles are evaluated in conjunction with a constant-pressure piston pump, a splitter injector[1] and an on-column** UV absorbance detector[14].

EXPERIMENTAL

A Varian Model 8500 high-pressure piston pump was used in the constant-pressure mode. Flow-rates as low as 0.1 μl/min were obtained without any modification of the pump. A 0.5-μl Valco (Houston, TX, U.S.A.) internal sample loop valve was used for sample injection. The splitting of the injected sample was achieved by

* Patent pending.

** U.S. patent application S.N. 223,445.

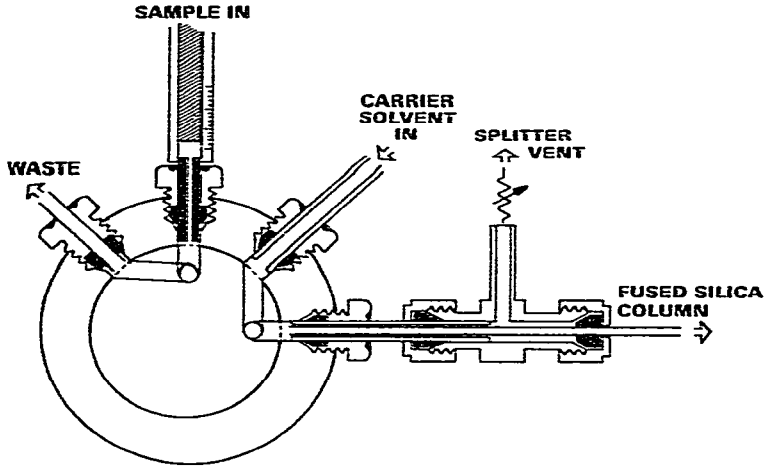

Fig. 1. The modified Valco 0.5-μl internal loop sampling valve for split injection in narrow-bore packed column HPLC.

utilizing the inlet of the narrow-bore microparticle-packed column as a split point, which is located inside the housing of the Valco valve as shown in Fig. 1. Fused-silica tubing (courtesy of Mr. Ernest Dawes, Scientific Glass Engineering) with I.D. ranging from 57 to 376 μm and with lengths up to 2 m was packed with 3-, 5- and 10-μm C_{18} bonded-phase particles using a slurry-packing technique. The reversed-phase octadecylsiloxane was chemically bonded onto the microparticulate silica before packing into the microbore columns. The mobile phase used in the study was 70:30 acetonitrile–water under isocratic conditions.

A Jasco UVIDEC-III UV spectrometer (Jasco, Tokyo, Japan) with a time constant of 0.5 sec in conjunction with on-column flow cell detection[14] was used as shown in Fig. 2.

Experimental data collected in real time using a conventional chromatographic data system were processed using a chromatographic column evaluation routine[16] in which chromatographic performance parameters such as peak width, retention time, partition ratio, plate height, peak area, peak height, statistic moments, peak skewness and peak asymmetry are calculated.

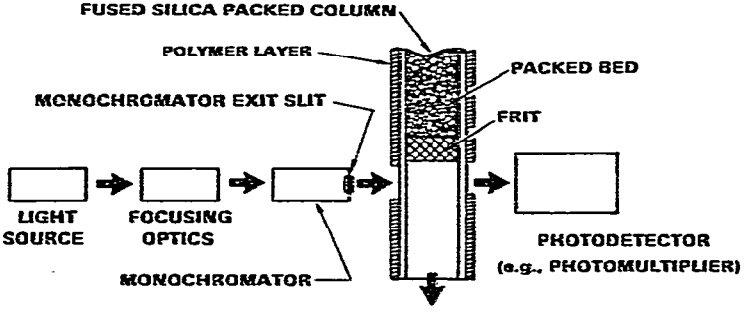

Fig. 2. Schematic diagram for on-column detection using narrow-bore fused-silica microparticle-packed column.

RESULTS AND DISCUSSION

Fused-silica columns were used as both the separation column and as the flow cell for on-column UV absorbance detection. The use of the column end as an on-column absorbance flow cell eliminates the need for column-to-detector connections and provides a sub-nanoliter detector flow cell which contributes no significant band broadening to the eluted peaks. The use of thin-wall flexible fused-silica tubing as the LC column material provides the following advantages:(1) good mechanical strength, flexibility and, thus, ease of handling; (2) inlet pressure ranges up to 800 atm may be applied and, thus, long columns can be prepared and used; (3) a smooth inner surface minimizes solute zone-spreading due to wall effects; (4) good chemical inertness and low metal content minimize chemical and physical interactions between solute molecules and the column walls; (5) good optical transparency allows visual observation of the column packed bed as well as on-column absorbance detection in the UV region; (6) small outside diameters of the fused silica columns allow easy interfacing into injector and detector with minimum extra-column effects; (7) no connectors or interface tubing are required if split injection and on-column detection are used; and (8) columns can be coiled and thus the design of a column oven is simplified.

In addition, the temperature effect[15] as normally observed due to the temperature gradient across the column cross-sectional plane and along the column axial direction is minimized for fused-silica narrow-bore microparticle-packed columns. The narrow column diameter, the thin fused-silica wall, and the low volumetric flow-rates insure that heat generated by friction at high operating pressures can be rapidly equilibrated with the ambient or column oven temperature. The absence of a significant thermal effect allows the use of microparticles ($\leqslant$ 10 μm) and long column lengths at high operating pressures for high column efficiency and resolving power.

Instrumental extra-column zone-spreading effects

Extra-column zone-spreading effects arise, in general, from three major sources: injectors, connectors and detectors. Because of the small elution volumes (small peak widths) and flow-rates in narrow-bore microparticle-packed column LC, the design requirements for a narrow-bore microparticle-packed column liquid chromatograph are quite stringent. In this work, no column-to-injector and detector[14] interface tubings were used. Split injection utilizes the narrow-bore packed-column inlet as a splitter such that a fraction of sample is split to the column and the remaining fraction is purged to waste. Injector dead-volume effects are minimized at high injector flow-rates and split ratios.

On-column detection[14] uses the column end as a detector flow cell for optical detection. The extra-column zone-spreading contribution to the percentage loss in peak resolution, $\%\Delta R_s$, can be expressed as

$$\%\Delta R_s = \left(1 - \left[1 + \frac{H'L'}{HL}\right]^{-1/2}\right) \times 100\% \quad (1)$$

where H' and H are the height equivalent to a theoretical plate for the flow cell and the column, respectively, and L' and L are the entrance/exit slit length for the detector flow cell and the column length, respectively. For a column of circular cross-section, as an example, H' can be calculated according to eqn. 2:

$$H' = \frac{2\pi r^2 D_m}{F} + \frac{F}{24\pi D_m} \quad (2)$$

where r, F, and D_m are the column radius, the mobile-phase volumetric flow-rate, and the solute–mobile phase binary molecular diffusion coefficient.

Assuming a 1 m × 200 μm I.D. narrow-bore packed column of 5-μm C_{18} bonded-phase particles, a reduced plate height, h, of between 2 and 8 can be obtained at a flow-rate of 1 μl/min. The percentage loss of resolution, $\%\Delta R_s$, against reduced plate height, h, using on-column detection with a 0.3-cm entrance/exit slit optical cell height and a 200-μm light path-length is calculated and given in Fig. 3.

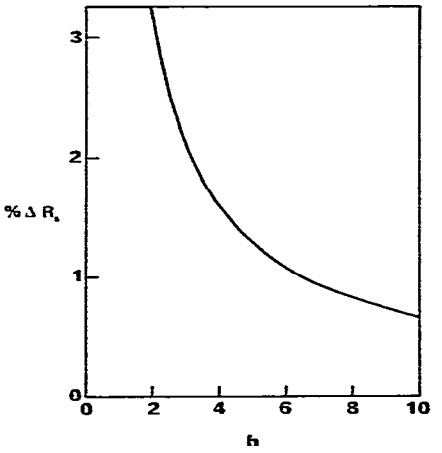

Fig. 3. Theoretically calculated percentage loss in peak resolution using on-column detection for a 5-μm particle 1 m × 200 μm I.D. packed column. A 300 × 200 μm I.D. on-column detector flow cell was assumed.

Fig. 3 indicates that a 300 × 200 μm I.D. cylindrical on-column flow cell contributes *ca.* 3% to the loss of peak resolution obtained by a 1 m × 200 μm I.D. narrow-bore microparticle-packed column having a reduced plate height of 2 and particle diameter of 5 μm. The loss in peak resolution is greatly reduced for columns of lower efficiency. For example, the same on-column detector contributes less than 1% loss to the peak resolution if the column has a reduced plate height of greater than 6.5. Experimentally observed reduced plate heights for the narrow-bore microparticle-packed columns reported here ranged from 3 to 6, and so the on-column detector contribution to the loss of peak resolution was *ca.* 1–2%.

The injector could become a major source of extra-column zone-spreading if the internal volume of the injector cannot be effectively swept by the mobile solvent. Effective sweeping of the internal volume of the injector can be achieved either with high split ratios or with high mobile-solvent flow-rates.

Fig. 4 shows a comparison of the plate heights, H, *vs.* partition ratios, k', for polynuclear aromatic hydrocarbon (PAH) components eluted from a 30 cm × 376 μm I.D. narrow-bore column, packed with 5-μm C_{18} bonded-phase silica gel particles. Direct injection was obtained by using a Valco loop valve with a 0.5-μl internal

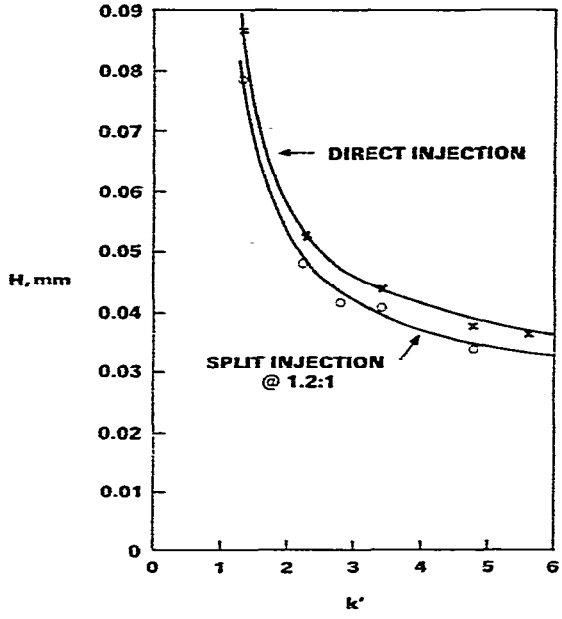

Fig. 4. A comparison of injector dead-volume effect on zone-spreading for PAH samples: benzene, $k' = 1.26$; toluene, $k' = 2.25$; naphthalene, $k' = 2.75$; fluorene, $k' = 3.4$; phenanthrene, $k' = 4.8$; pyrene, $k' = 5.7$. Column mobile solvent (70:30 acetonitrile–water) and splitter vent flow-rates were 9.24 and 10.9 μl/min, respectively. A 30 cm × 376 μm I.D. column packed with 5-μm particles was used.

loop volume, where the injector mobile flow-rate equals the column flow-rate at 9.24 μl/min. Split injection was obtained by splitting the injector flow-rate of 20.3 μl/min at 1.2 to 1, and it allowed a 9.24 μl/min mobile-phase flow-rate in the column. Plate heights obtained by using direct injection were nearly 10% higher than that obtained by using split injection.

At low column mobile-phase flow-rates, the injector dead-volume contribution to solute zone-spreading could be substantial if a long residence time in the injector were allowed for the sample. Fig. 5 shows the injector dead-volume effect on the measured plate heights for the same sample and conditions as that for Fig. 4, but the column flow-rate is 3.5 μl/min. The plate heights obtained by using direct injection ranged from 25 to 75% higher than those obtained by using a 2.1 to 1 split injection. A conventional direct injector (loop valve) is obviously not satisfactory if narrow-bore microparticle-packed columns are to be operated at their optimum efficiencies. A 2.1 to 1 splitter has a significantly reduced injector dead-volume effect at low mobile-solvent flow-rates. However, the negative slopes of H *vs.* k' on Figs. 4 and 5 indicate that a significant degree of solute zone-spreading is the result of the injector dead-volume effect. High split ratios (*e.g.*, $\geqslant 10$ to 1) are desirable if no zone-spreading contribution from the injector is allowed and if low column mobile-phase flow-rates are used.

Column efficiency versus column inner diameters

Column efficiency in terms of plate height is compared in Fig. 6 for columns

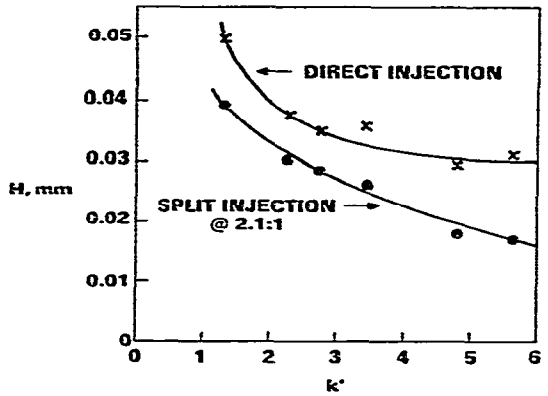

Fig. 5. The effect of injector dead volume on the measured column efficiency in terms of plate height, H. Column and mobile solvent used were the same as for Fig. 4. Column mobile phase and splitter vent flow-rates were 3.52 and 7.27 μl/min, respectively.

with 130-, 210- and 310-μm I.D. columns packed with the same 5-μm C_{18} bonded-phase particles under similar slurry-packing conditions. All three columns were 1 m in length. Three of five columns of each I.D. size were studied.

The performance of the 210-μm column at low flow-rates is superior to that obtained from both 130- and 310-μm columns. A reduced plate height of 4.4 was measured for the 210-μm column and a reduced plate height of 5.5 was obtained for the 130- and 310-μm columns. The reduced plate heights reported here, however, were two to three times higher than the commonly assumed optimum value of 2. The column packing procedure thus requires further optimization. Even so, long columns can be used and good reproducibility in column efficiency was obtainable, under the same conditions.

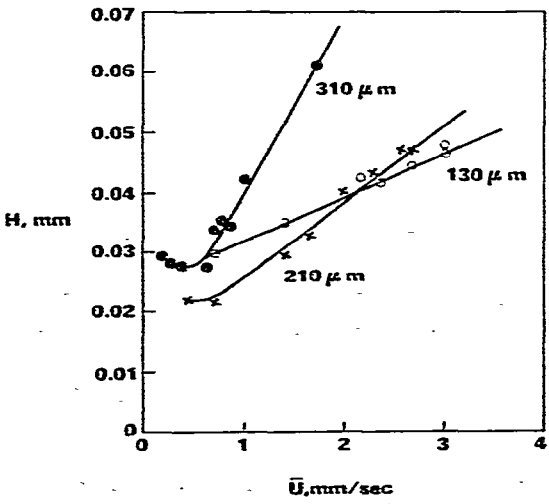

Fig. 6. Performance of 5-μm MicroPak MCH-5 reversed-phase packing in narrow-bore packed columns. Test compound was pyrene at $k' = 5.7$.

Fig. 6 also shows that the slope of H *vs.* $\bar{u}$ for the 310-μm column is the highest among the three studied here. This could indicate a non-uniform packed bed due to the non-optimized packing technique used here. Columns of 130 μm I.D. had the lowest slope of H *vs.* $\bar{u}$. This indicates that the smaller column I.D., the smaller the flow-rate effects. The short narrow-bore microparticle-packed column of 130 μm I.D. would thus be the best among the three for operating at high flow-rates for fast analysis.

Resolving power of microbore packed columns

The resolving power of conventional packed columns is limited by the total available column theoretical plate number. Conventional 5-μm particle packed columns with a length of 30 cm can generate 15,000 to 25,000 plates. Recently developed 3-μm particle packed columns, available in 10–15 cm lengths, generate 10,000 to 20,000 plates. Complex sample analysis with baseline resolution of most components can be difficult using conventional packed columns limited to less than 25,000 plates.

The van Deemter plot for 1 m × 330 μm I.D. columns packed with spherical 3 μm C_{18} bonded silica particles is shown in Fig. 7. (RoSil, courtesy of Dr. M. Verzele, State University of Ghent, Belgium). A minimum plate height of 9.7 μm corresponding to a reduced plate height of 3.2 was obtained. The total column plate number exceeded 110,000 for the flow-rate range studied. The flow-rate did not significantly affect plate height in the range 0.5–1.5 mm/sec.

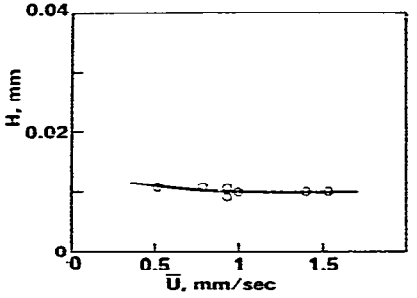

Fig. 7. Performance of a 3-μm C_{18} bonded-phase particle 330 μm I.D. packed column. Test compound was pyrene.

Application of the high plate count available in such a long, narrow-bore packed column is demonstrated in the isocratic separation of an Environmental Protection Agency (EPA) priority pollutant mixture of sixteen PAH components, shown in Fig. 8. Baseline resolution of the PAH mixture was obtained using a 50,000-plate, 45 cm × 330 μm I.D. column packed with the 3-μm C_{18} material (RoSil) discussed in the preceding paragraph. The mobile phase was 70:30 acetonitrile–water and the flow-rate was 3.7 μl/min ($\bar{u}$ = 1.2 mm/sec). The high plate count of the long microcolumn was necessary to achieve baseline resolution of the PAH pair benzo[*a*]anthracene/chrysene (peaks 9, 10).

Solvent usage in the PAH separation was 600 μl for the 180-min separation. This represents a solvent use reduction of 65 fold relative to a conventional 15 cm × 4.6 mm I.D. column packed with 3-μm C_{18} material, generating only 20,000 plates.

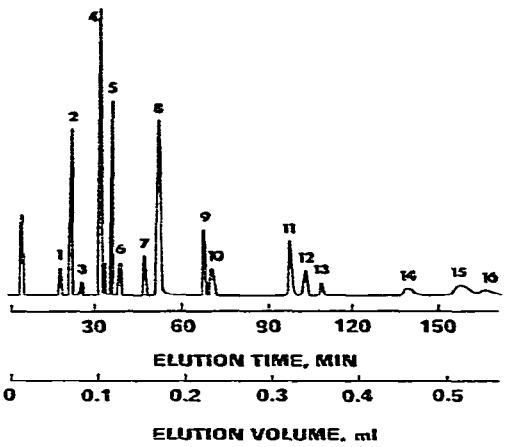

Fig. 8. Separation of sixteen-component PAH mixture on a 50,000-plate, 45 cm × 330 μm I.D., 3-μm C_{18} packed column. Peak identifications are: 1, naphthalene; 2, acenaphthalene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benzo[*a*]anthracene; 10, chrysene; 11, benzo[*b*]fluoranthene; 12, benzo[*k*]fluoranthene; 13, benzo[*a*]pyrene; 14, dibenzo[*a,h*]anthracene; 15, benzo[*ghi*]perylene; 16, indeno[1,2,3-*cd*]pyrene.

Solvent effect in narrow-bore column HPLC

In column LC, the use of solvent and solvent modifier(s) to improve column selectivity and resolution of sample components and to reduce the analysis time of well-retained components are practised either by premixing the solvent and its modifier(s) in isocratic operations or by step or linear solvent programming in programmed elution chromatography. To date, there have been few reports[17,18] on the use of solvent or solvent modifier(s) in the sample solution for the improvement of solute resolution, column selectivity and reduction in analysis time. In general, because of the large column cross-sectional area and mobile-solvent flow-rates used in conventional column (4–6 mm I.D.) HPLC, a large volume of solvent modifier(s) in the sample would be necessary in order to exert an appreciable solvent effect. This large volume of solvent modifier(s) in the sample solution may precipitate the sample components and disturb the equilibrium of the phase system at the head of the column. In practice, the same solvent as the mobile phase is used to dissolve the sample solutes and to prevent such problems. For trace analysis, on-column trace enrichment utilizing weak solvent for trace-component preconcentration at the inlet of the column has been reported[19]. However, its application has been limited to enhancement of sample detectivity, and the use of step or linear solvent programming was necessary.

Narrow-bore packed column HPLC as proposed here utilizes columns of I.D. less than 500 μm, and packed with ≤ 10 μm particles in lengths from 10 cm to a few meters. Because of the narrow bore of the columns, low mobile-solvent flow-rates in the range 0.1–10 μl/min are used. This low flow-rate and the limited surface area of the narrow-bore column packed bed allow easy and effective modification of the mobile-solvent system by the injection of a small amount of solvent modifier(s) into the column. The solvent modifier(s), in practice, can be injected as one of the solvent components in the sample solution or as a sample into the column at the time the modification of mobile solvent system is desirable.

An example of this technique is shown in Fig. 9, where pure methanol at a flow-rate of 3.1 μl/min was used as the mobile solvent for the elution of a sample mixture containing *ca.* 10–50 ng each of benzenethiol, hexanethiol, octanethiol and dodecanethiol in methanol solution, or in methanol plus a modifier (methylene chloride or water) from a C_{18} chemical-bonded reversed-phase on 5-μm-silica-gel-particle, 50 cm × 250 μm I.D. narrow-bore packed column. The mobile-solvent hold-up volume measured for the column was 14.8 μl. Sample sizes injected were 0.6 μl for all experiments.

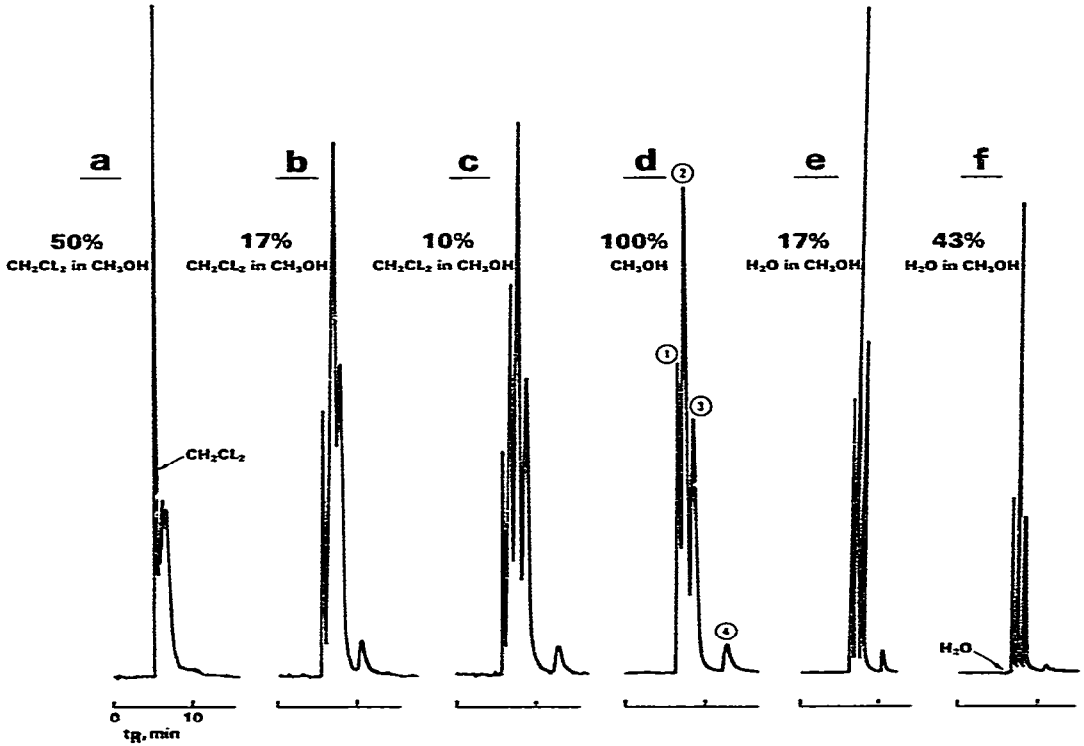

Fig. 9. Solvent effect in narrow-bore column HPLC. Solutes are 1, benzenethiol; 2, hexanethiol; 3, octanethiol and 4, dodecanethiol. Solvent modifier peaks are identified in Figs. 9a and 9f. Solvent modifiers and their concentrations are shown. The sample volume was 0.6 μl for all experiments. Methanol was used as mobile solvent. A C_{18} chemical-bonded reversed-phase 5-μm silica-gel particle packed column (50 cm × 250 μm I.D.) was used.

The example demonstrated in Fig. 9 shows the effects of a strong and a weak solvent modifier on both the retention and resolution of the thiol components. First, with the use of a strong solvent modifier such as methylene chloride in the sample solution, the solubility of the solvents into the mobile solvent is increased. The effects of the strong solvent modifier are thus a reduction in the analysis time and the loss of resolution for those components eluted right after the solvent modifier(s). The effectiveness of the strong solvent modifier on the loss of resolution and the reduction in the analysis time depends on the amount of the solvent modifier injected into the column. As shown in Fig. 9c, a 10% methylene chloride (modifier) in methanol

sample does not have a significant effect on either solute retention times or resolutions from that obtained (Fig. 9d) using pure methanol as solvent for both mobile phase and sample solution. However, as shown in Fig. 9b, a 17% methylene chloride in methanol sample solution was observed to reduce the retention times of the thiol compounds by more than 20% and to reduce the resolution of benzenethiol, hexanethiol and octanethiol. Near complete loss of resolution of thiols as shown in Fig. 9a was observed when a 50% methylene chloride methanol sample solution was injected.

In the case of the use of a weak solvent modifier (*e.g.*, water) in the sample solution, an on-column focusing of the injected solute zone is obtained due to reduced solubility of sample solutes in the mobile solvent. This on-column focusing of the initial solute sample zone results in a significant improvement in peak resolution and allows a large sample volume to be injected into the narrow-bore packed column with minimum injector-dead-volume and sample-volume effects on the column efficiency. Fig. 9c shows the resolution and peak shape of the thiol compounds obtained under the same conditions as that for Figs. 9a–d, except that the injected 0.6-μl sample mixture contains 17% water in methanol. A dramatic improvement in solute resolution, a reduction in solute peak width and an enhancement in solute sample detection due to a narrower peak width and less mobile solvent dilution were demonstrated.

A comparison of Figs. 9d and 9e in terms of the thiol retention times suggests that 0.1 μl (*i.e.*, 17% of 0.6-μl sample injected) of water injected into a 50 cm × 250 μm I.D., 5-μm particle packed column at a mobile solvent (methanol) flow-rate of 3.1 μl/min is just sufficient to allow solute initial zone focusing at the inlet of the column. There is no significant effect on the retention of those thiols eluted near the solvent modifier (water in this case) peak.

By increasing the amount of solvent modifier injected into the column, the mobile-solvent system in the front portion of the column is modified and the solubility of the solute compounds in the mobile solvent can be adjusted to achieve higher capacity ratio (k), selectivity, and resolving power (R) for the solutes eluted right after solvent modifier. Fig. 9f shows a baseline resolution of benzenethiol, hexanethiol and octanethiol obtained by increasing the water concentration in the sample mixture to 43%. The amount of water injected into the column in this case is 0.26 μl, or 1.7% of the column free volume. This relatively high volume of water injected into the column provides not only the on-column focusing of this initial solute zone but also the effective modification of the mobile-solvent system during the time that solutes remain in solution in water–methanol mixed solvents. As a result, better resolution and near 10% increases in retention times for those thiols eluted near the water peak were observed. It should also be pointed out that the use of weak solvent modifier in the injected sample will affect the peak shape (due to on-column focusing) but not the retention time of those well-retained sample components because the time spent by those well-retained solutes in solution in the solvent-solvent modifier mobile-phase system is short relative to their retention times. The total analysis time for solutes with partition ratio higher than 2 is normally not affected by the utilization of the weak solvent modifier for improving solute resolution, column selectivity, peak width and detection. The solvent effect demonstrated above in narrow-bore-column HPLC could also be applied to conventional packed column HPLC by scaling up the amount of solvent modifier needed according to flow-rate and column cross-sectional area ratio to be injected to achieve the solvent effect.

The practical importance of the suggested solvent effect in narrow-bore-column HPLC are: (1) the injection of a strong solvent modifier at the time of sample injection or at any desirable time reduces the analysis time of a well-retained sample without using a multicomponent solvent programming system, and thus, a single-pump hydraulic system could be utilized in narrow-bore-column HPLC; (2) the use of strong solvent modifier allows better peak detection due to less dilution of solute zones; (3) the injection of a weak solvent modifier at the time of sample injection or at any desirable time enhances peak resolution, and thus difficult and complex sample separation can be obtained; (4) the use of a weak solvent modifier allows on-column focusing and thus improves solute peak width and detection; (5) the use of a weak solvent modifier will not significantly increase the analysis time of well-retained compounds; and (6) a single mobile solvent or mixture of solvents in the pumping system minimizes solvent waste, exchange solvent difficulties, and cross-contamination of the narrow-bore-column HPLC system.

CONCLUSIONS

High-efficiency fused-silica narrow-bore columns packed with $\leqslant 10$ μm particles in lengths of up to a few meters can be prepared. The long length narrow-bore packed columns allow extremely high resolving power and peak capacity for the analysis of complex samples. A 1-m column with 110,000 theoretical plates was prepared and used in conjunction with a constant-pressure piston pump, a splitter injector and an on-column UV detector.

The use of fused-silica tubing for column material allows on-column optical detection and eliminates the column-to-detector connector and interface tubing. Optimum performance of the narrow-bore microparticle-packed columns can, therefore, be obtained.

Narrow-bore packed columns with 3- and 5-μm particles have minimum plate heights of *ca.* 10 and 20 μm, respectively. Further study in the optimization of column packing techniques may allow more efficient columns to be prepared. Narrow-bore columns packed with 3-μm spherical C_{18} bonded-phase particles achieved over 100,000 theoretical plates for a 1-m column. A 0.45 m $\times$ 330 μm I.D. 3-μm particle packed column allowed baseline separation of an EPA priority pollutant mixture containing sixteen PAH components.

The use of solvent effect in narrow-bore packed column HPLC, by the injection of a solvent modifier at the time of the modification of mobile-solvent system, is desirable in that it allows a single-pump hydraulic system to be utilized in narrow-bore microparticle-packed column HPLC for enhancing speed of analysis, solute detection and solute resolution.

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REFERENCES

1 F. J. Yang, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 3 (1980) 589.
2 K. Hibi, D. Ishii, I. Fujishima, T. Takeuchi and T. Nakanishi, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 21.
3 T. Tsuda, K. Hibi, T. Nakanishi, T. Takeuchi and D. Ishii, *J. Chromatogr.*, 158 (1978) 227.
4 K. Hibi, T. Tsuda, T. Takeuchi, T. Nakanishi and D. Ishii, *J. Chromatogr.*, 175 (1979) 105.
5 D. Ishii, T. Tsuda and T. Takeuchi, *J. Chromatogr.*, 185 (1979) 73.
6 T. Tsuda and G. Nakagawa, *J. Chromatogr.*, 199 (1980) 249.
7 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 169 (1979) 51.
8 R. P. W. Scott and P. Kucera, *J. Chromatogr.*, 185 (1979) 27.
9 R. P. W. Scott, P. Kucera and M. Munroe, *J. Chromatogr.*, 186 (1979) 475.
10 V. L. McGuffin and M. Novotny, *Anal. Chem.*, 53 (1981) 946.
11 F. J. Yang, in R. E. Kaiser (Editor), *Proceedings of the Fourth International Symposium on Capillary Chromatography*, Institute of Chromatography, Bad Durkheim, G.F.R., 1981, pp. 139–175.
12 S. Tsuge, Y. Hirata and T. Takeuchi, *Anal. Chem.*, 51 (1981) 946.
13 D. Ishii and T. Takeuchi, *J. Chromatogr. Sci.*, 18 (1980) 462.
14 F. J. Yang, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1981) 83.
15 I. Halasz, R. Endele and J. Asshauer, *J. Chromatogr.*, 112 (1975) 37.
16 F. J. Yang, A. C. Brown III and S. P. Cram, *Amer. Lab.*, August, 1978.
17 N. A. Parris, *Instrumental Liquid Chromatography*, Elsevier, New York, 1976, pp. 230–232.
18 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley, New York, 1979, 2nd ed., p. 298.
19 J. N. Little and G. J. Fallick, *J. Chromatogr.*, 112 (1975) 389.