*Journal of Chromatography*, 279 (1983) 431–438
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 121

# MICROBORE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC COLUMNS: SPEED, EFFICIENCY, SENSITIVITY AND TEMPERATURE PROGRAMMING

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## SUMMARY

Microbore columns (1 mm I.D.) were compared with conventional 4.6 mm I.D. columns with respect to speed, efficiency and sensitivity. If the column lengths, particle diameter, packing efficiency, mobile phase and linear velocity are the same, most chromatographic properties such as speed (analysis time), efficiency, pressure drop and sensitivity are also the same. The major differences are the column volumes, the void volumes and the difference in volumetric flow-rates for equal linear flow velocities. The low flow-rates of microbore columns (30–200 $\mu$l/min), however, do save on solvent consumption and do make for easier and more efficient direct interfacing to other instruments. Temperature programming of microbore columns produces faster analyses for large $k'$ values, increased sensitivity (due primarily to sharper peak shape) and increases the range of compounds that can be handled in an isocratic mode.

## INTRODUCTION

Microbore and capillary high-performance liquid chromatographic (HPLC) columns are generating a great deal of interest, mostly owing to the dramatic impact of capillary gas chromatographic (GC) columns; unfortunately, owing to major differences in the mobile phases of GC and HPLC, no major improvement in HPLC columns is forseen in the near future. Microbore columns (commonly defined as packed columns with inner diameters ranging from 0.5 to 1.0 mm) have at various times been claimed to exhibit greater sensitivity[1], greater resolution[2], faster analyses[3,4] and the capability of generating large numbers of theoretical plates when coupled together[5].

Capillary HPLC (really open-tubular columns) have already demonstrated a large number of theoretical plates and very good resolution of complex sample mixtures[6,7]; however, these types of columns require very long analysis times and special instrumentation, which is not commercially available. The low sample capacity of capillary columns demands extremely sensitive and very low-volume detectors[8], which are also currently unavailable. It does not appear that capillary HPLC columns will be a useful product in the next few years. Considerable research efforts will be required to make them workable in most chromatographic laboratories.

Microbore columns, however, have been commercially available for the past 2 years. There are at least five column suppliers in Europe and the U.S.A. today, and

0021-9673/83/$03.00 

there are several commercial instruments on the market with microbore capability. Microbore columns are being used and will be used more in the future. This paper summarizes our experience of the last 2 years with microbore columns.

## EXPERIMENTAL

### *Microbore columns*

Microbore columns were made with specially prepared 1-mm I.D. stainless-steel tubing, the inside surface of which had been polished to a mirror finish. In our experience, this is a critical step. Takenchi and Ishii[9] have compared various column tubing materials and concluded that a smooth internal surface is essential for high column efficiencies. A slurry, carbon tetrachloride–methanol (95:5), was packed into the columns under an increasing pressure from 1000 to 10,000 p.s.i. (70 to 700 bar) at 3000 p.s.i./min (200 bar/min) by means of a Haskel DST-122 air driven fluid pump (Haskel Engineering and Supply, Burbank, CA, U.S.A.). It was felt that increasing the pressure during packing would result in a more uniform packing density. Column efficiencies served to verify this hypothesis. The columns were flushed with 25 ml of methanol before being removed from the pump. The results reported here were all obtained on 10, 7 and 5 $\mu$m RP-18 materials, but silica gel (Si-60), amino, cyano and diol packings have also been successfully prepared in our laboratory.

### *Instrumentation*

A MACS microbore system (EM Science, Gibbstown, NJ, U.S.A.) was used for most of this study. It includes a 0.5-$\mu$l injector (MACS 500), a low-volume delivery pump (MACS 100) and a variable-wavelength UV detector with 0.5-$\mu$l detector cells (MACS 700).

Voltage for heating the columns was provided by a Powerstat Type 116 variable transformer (Superior Electric, Bristol, CT, U.S.A.), stepped down to 0–6.3 V by an F-16X filament transformer (Triad-Utrad, Huntington, IN, U.S.A.). The column temperature was monitored with an AD590J two-terminal IC temperature transducer (Analog Devices, Norwood, MA, U.S.A.).

The solvents used were HPLC-grade methanol, acetonitrile and water (Burdick and Jackson, Muskegon, MI, U.S.A.). Samples were obtained from Aldrich Chemical (Milwaukee, WI, U.S.A.) and used as received.

## RESULTS AND DISCUSSION

Most conclusions drawn about microbore columns seem to be made under non-optimal conditions for one of the columns. Microbore columns are merely smaller versions of standard HPLC columns and the same principles and theories apply. If the column variables, such as length, particle diameter, type of sorbent, mobile phase, temperature and linear velocity are held constant, the important chromatographic parameters, such as retention time, void volume, capacity factor, efficiency (plate number, $N$, or reduced plate height, $h$), pressure drop and selectivity ($\alpha$) will be the same. Sensitivity may be higher or lower, depending on the sample mass injected and the detector cell volume, as will be discussed shortly. Table I summarizes the differences in the two column types.

TABLE I

DIFFERENCES BETWEEN CONVENTIONAL AND MICROBORE COLUMNS.

| *Column* | *I.D. (mm)* | $V_o(\mu l)$ | *Volume (μl) (25 cm length)* | *Flow-rate* |
|---|---|---|---|---|
| Conventional | 4.4 | 3300 | 4200 | 1 ml/min |
| Microbore | 1.0 | 160 | 200 | 50 μl/min |

* For equal linear velocity.

*Microbore column characteristics*

The primary difference is the smaller inner diameter and, therefore, the smaller column volume; this means a smaller volumetric flow-rate (typically 30–200μl/min). This smaller flow-rate makes microbore columns attractive for directly coupled chromatographic systems, such as LC–LC, LC–GC, LC–mass spectrometry, LC–Fourier transform infrared spectroscopy, and LC–nuclear magnetic resonance spectroscopy. It also means considerable savings in the volume of mobile phase used.

As a direct result of the lower flow-rate, peaks will be eluted in a smaller volume of mobile phase (see Table I). Table II lists the peak width, expressed as $\sigma$, in microlitres for a series of capacity factors ($k'$); $\sigma$ was calculated from the equation $\sigma = [(k'+1)V_0]/\sqrt{N}$, where $V_0$ is the void volume. Table II also shows the percentage increase in $\sigma$ due to different detector cell volumes. These values were calculated by statistical moment analysis of an integrated Gaussian function. By adjusting the integration width (therefore the cell volume) and sequencing across the peak, synthetic peaks were generated, the $\sigma$ of which was determined as the square root of the second statistical moment. These calculations ignore the peak width contribution caused by parabolic flow profiles and mixing in a detector cell, focusing only on the contribution due to the static cell volume. These calculations show that for small $k'$ values it is necessary to use a smaller cell volume in order to minimize the band broadening. For $k'$ values greater than 5, a normal detector cell volume (*i.e.* 8 μl) would show only a small decrease in resolution.

The small peak volumes of microbore columns may demand the injection of smaller than normal sample volumes. Microbore columns can show excessive band spreading if too large a sample loop is used. This is seen as a loss in resolution. Fig. 1 shows this effect dramatically. The first chromatogram (A) shows a good chromato-

TABLE II

Numerical analysis of contribution of detector volume to observed peak width

Column: 25 cm × 1 mm I.D.; 10μm, $N$=12,500; $V_o$=157 μl.

| $k_1$ | *Peak width, σ(μl)* | *Contribution ( %) Detector volume (μl) 0.5* | | |
|---|---|---|---|---|
| *8.0* | | | | |
| 1 | 2.81 | 0.27 | 6.93 | 67.99 |
| 2 | 4.21 | 0.13 | 3.26 | 30.38 |
| 5 | 8.43 | 0.05 | 1.25 | 7.67 |
| 10 | 15.45 | 0.03 | 0.6 | 2.32 |

gram on a 50-cm microbore column, when 0.5 μl of sample in a strong solvent (pure methanol) was injected. The mobile phase was methanol–water (80:20) in all chromatograms shown. The second chromatogram (B) is for the same sample, but 5.0 μl were injected. The sample was diluted 10-fold, so that the same sample mass was injected. Obviously, this sample volume is excessive, and a dramatic loss in resolution is observed. The third chromatogram (C) illustrates a solution to this problem. The same sample volume and mass as in B were injected in a solvent weaker than the mobile phase [methanol–water(50:50)]. The weak solvent allows the sample to be strongly sorbed at the column inlet in a narrow band. The stronger mobile phase coming in behind the weak sample solvent then solubilizes the sample and generates a chromatogram comparable to A. In fact, the plate count is slightly higher. This use of a weaker sample solvent to minimize band spreading applies to all column diameters[10,11]. The effect of excessive sample volume is merely seen more frequently with microbore columns.

The small volumes of microbore columns also demand low sample volumes and low detector volumes for very fast analysis (short columns) and very high resolution. Commercial instruments, optimized for larger bore columns, are incapable of providing the high performance level of microbore columns in these instances. Microbore columns can be used in these instruments, but there will be a sacrifice in chromatographic results (see Table II).

*Microbore chromatographic characteristics*

With the above-mentioned column characteristics in mind, it is possible to evaluate microbore columns with respect to the common chromatographic objectives of speed, efficiency and sensitivity.

*Speed.* Analysis time is primarily dependent on column length and particle diameter. Shorter columns produce faster analyses, but unless the particle size is also reduced, a shorter column will produce a lower resolution. For equal lengths, equal particle diameters and flow-rates generating the required number of plates per second, microbore columns are as fast as regular columns. One advantage of microbore columns is that the small column volumes make fast analyses at very fast flow-rates more acceptable in terms of solvent consumption. Fig. 2 illustrates this point. The fast analysis at 10 times the optimal flow-rate generates a good chromatogram in less than 2.5 min,

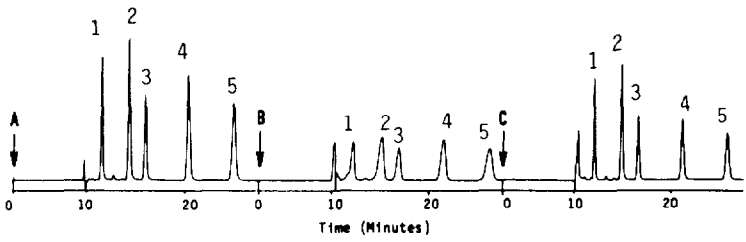


Fig. 1. Excessive sample solvent effect in microbore columns: (A) normal injection volume; (B) excessive sample volume; (C) use of weak sample solvent to overcome the sample volume effects. Column, 50 cm × 1 mm I.D., RP-18, 10 μm; sample, (a) phenol, (b) ethylphenol, (c) anisole, (d) toluene, (e) ethylbenzene; detection, UV (254 nm); Mobile phase methanol–water (80:20), in all instances.

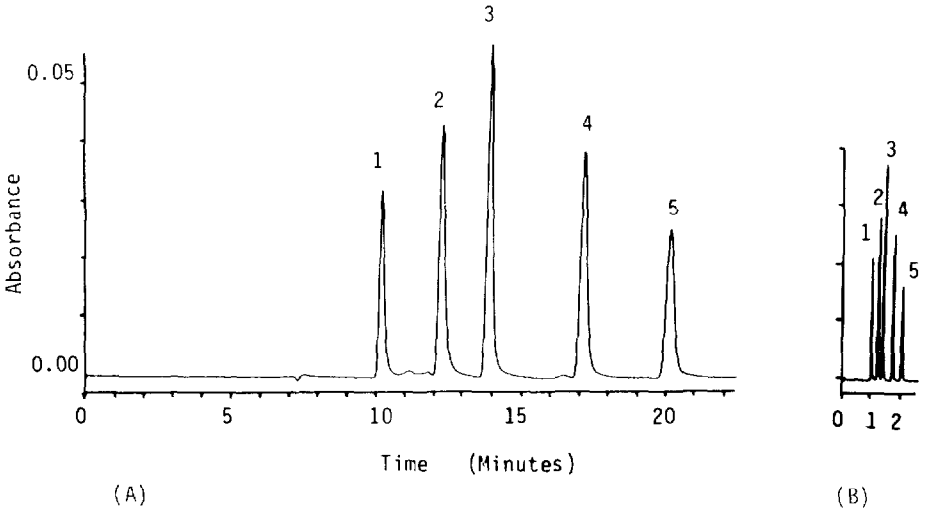


Fig. 2. Illustration of a high-speed analysis with microbore columns: 50-cm microbore column, 10 μm RP-18(A) 30 μl/min, 400 p.s.i.; (B) 300 μl/min, 4000 p.s.i. Same sample as in Fig. 1.

consuming only 300 μl/min of solvent. For the same analysis time, a conventional-bore column would use 6ml/min of solvent.

An important consideration in high-speed analysis is that very short microbore columns (10 cm or shorter) produce such narrow band widths that the band broadening due to extra-column effects becomes critical. This is not as much of a problem with a short and wide packed columns (*e.g.*, 100 x 6 mm).

*Efficiency.* Column efficiency is determined by many factors, including column length, particle diameter, mobile phase, temperature, flow-rate, sample mass and volume and the technique for packing the column. Our studies have shown that 4.6 and 1 mm I.D. columns produce reduced plate heights of around 2. Fig. 3 shows a separation of 16 PTH amino acids on a 50 cm x 1 mm I.D. column, packed with 5 μm particles of RP-18, which generated 45,000 plates ($h$=2.2) on peak 4 (PTH-threonine). A step gradient was used.

*Sensitivity.* Several publications have claimed greater sensitivity for microbore systems, but these comparisons have not been totally valid. If the sample mass is decreased 20-fold in accordance with a 20-fold smaller sorbent bed, there will be a 20-fold loss in sensitivity. However, if the volumetric flow-rate is also reduced 20-fold (same linear velocity), there is a 20-fold increase in sensitivity, because the sample is contained in one twentieth of the mobile phase. This means equal sensitivity for both columns with the same detectors. If high-speed or high-resolution analyses are required, it may be necessary to reduce the detecor cell volume (see earlier discussion). If this reduced detector volume means a reduced cell path length, say from 10 to 2 mm, there would be a five-fold loss in sensitivity for microbore systems. If, however, the cell path length is the same, and the same sample mass is placed on both columns, there can be a 10–20-fold increase in sensitivity for microbore systems.

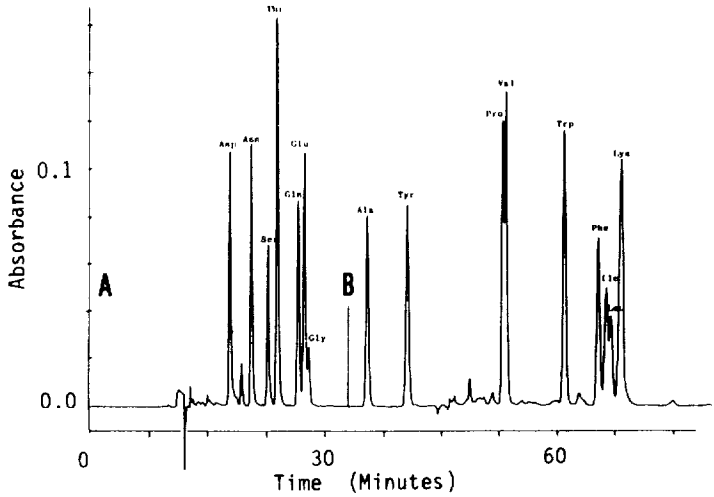


Fig. 3. E. High-resolution analysis of PTH-amino acids. Column, 50 cm × 1 mm I.D., 5 μm, RP-18; mobile phase, step gradient, (A) methanol-acetonitrile–7.5 mmol sodium acetate (20:3:77)(pH–4), (B) methanol–acetonitrile–12 mmol sodium acetate (2:3:5)(pH–4); injection volume, 25 μl.

*Temperature-programmed microbore HPLC*

Poppe *et al.*[12] suggested that one way to reduce the debilitating effects of radial thermal gradients created by viscous mobile phases at high velocities was to reduce the column diameter. If radial temperature gradients were present in narrow-bore columns, they would quickly level out across the narrow column bed. The introduction of temperature-programmed microbore HPLC in our laboratory owes its success to this

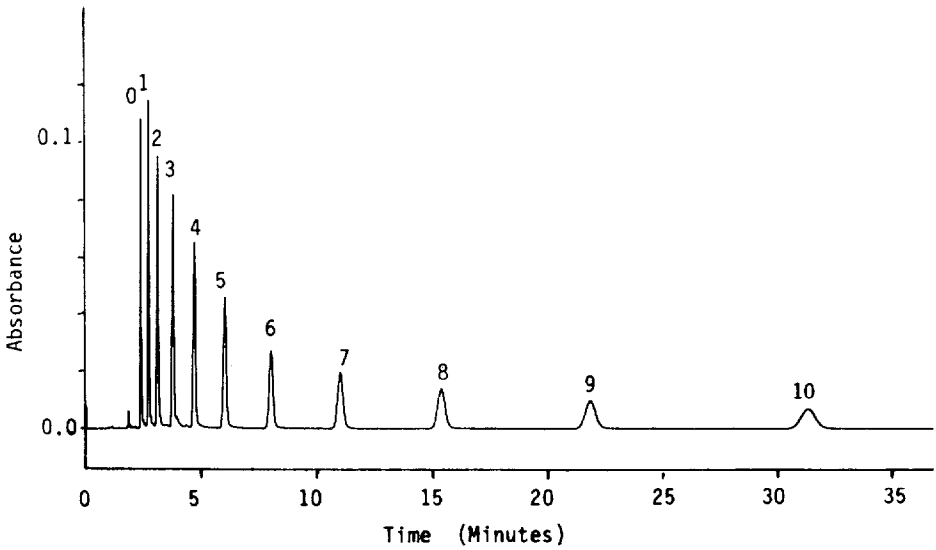


Fig. 4. Isothermal separation of *n*-alkylbenzenes at 25°C. Column, 50 cm × 1 mm I.D., 7 μm RP-18; mobile phase, acetonitrile–water (80:20) at 250 μl/min; pressure, 3000 p.s.i.; detection, UV (210 mm). Numbers above peaks refer to the number of carbon atoms in the alkyl side-chain.

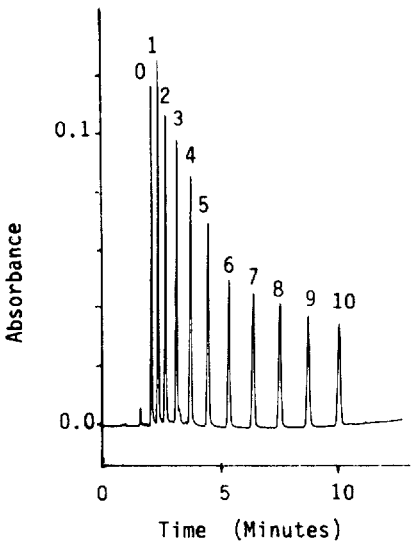


Fig. 5. Temperature-programmed separation of alkylbenzenes. Temperature increased from 25 to 100°C at 7.5°C/min; final pressure, 1500 p.s.i. All other conditions as Fig. 4.

fast equilibration time. Fig. 4 shows the isothermal separation at 25°C of a series of alkylbenzenes; Fig. 5 shows the temperature-programmed separation of the same sample.

The simplest method of heating the column is to pass an electrical current through the stainless-steel column tubing. The column then becomes its own heater. At 3 V r.m.s. the column dissipates enough power to heat the solvent at the column outlet to 100°C. During temperature programming, the column must be thermally insulated if a linear increase in power is to provide a linear increase in column temperature. The thermal mass (mass × specific heat) of the stainless-steel tubing is roughly equal to that of the sorbent and solvent, resulting in good thermal matching. Electrical heating results in a linear increase in temperature from the inlet to the outlet. By using ovens or circulating baths, a more constant temperature profile from the inlet to the outlet can be obtained.

The following advantages of temperature programming over isothermal HPLC are observed when a programme from 25 to 100°C is used: (1) a 75 % reduction in retention times for late-eluted peaks; (2) a four-fold increase in sensitivity for late-eluted peaks due to both lower retention volumes and higher column efficiencies, caused by the increase in mass transfer in both the mobile and stationary phases; and (3) a 50 % reduction in pressure drop. This lower pressure requirement could be used for flow programming at constant pressure. Research is underway to extend the understanding of the processes involved and of the effects on chromatographic performance under temperature-programmed conditions.

## CONCLUSION

The main chromatographic performance factors exhibited by microbore columns

in comparison with conventional columns, may be summarized as follows: Speed: determined primarily by column length, flow-rate and pasticle diameter; should be equivalent for equal parameters. Efficiency: determined primarily by length, particle diameter, flow-rate, sample size and column packing techniques; should be equivalent for equal parameters. Sensitivity: determined by sample mass, volumetric flow-rate and detector cell path length; should be equivalent for equal parameters; may be less for microbore columns if a small-volume detector cell is required for high resolution or very high speed. Temperature programming: can be used to reduce analysis time, increase sensitivity and extend the scope of the column.

ACKNOWLEDGEMENTS

This work was supported by NASA grants NAG-1-246 and NAG-1-343.

CHROMF121A - 12-10-83 14-13-18

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