Journal of Chromatography, 279 (1983) 49–57
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 126

DEACTIVATION AND COATING OF NON-POLAR 50-μm I.D. CAPILLARY COLUMNS

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SUMMARY

The deactivation and coating of 50-μm I.D. capillary columns was studied, using borosilicate and fused-silica columns with over 10^5 theoretical plates. A device is described for the convenient introduction of fluids by means of nitrogen pressure up to 80 bar. The device permits fluid switching while the working pressure is maintained. Established procedures for leaching and polysiloxane degradation, which are normally used with 0.25-mm I.D. capillaries gave low coating efficiencies when applied to the 50-μm I.D. columns studied. Modified procedures are described.

For borosilicate columns an acceptable level of deactivation has been achieved. A slight residual activity towards alcohols and amines could not be prevented. Excellent deactivation was observed for the fused-silica columns, even when only 15 pg of polar test compounds where injected. Chromatograms demonstrating the separation of terpenes and of underivatized drugs are given.

INTRODUCTION

High-resolution gas chromatography has become the method of choice for the analysis of the vaporizable fraction of samples of very complex composition. Capillary columns with plate numbers exceeding 10^5 are widely used for this purpose, normally giving analysis times between 0.5 and 2 h. These long analysis times can be drastically shortened, without loss of resolution, by reducing the dimensions of the capillary column. Schutjes *et al.*[1,2] recently investigated 8 m × 50 μm I.D. columns and observed that the analysis time decreases in proportion to the column inner diameter. In this paper, deactivation methods for borosilicate and fused-silica 50-μm I.D. columns are presented. Chromatograms obtained from polar samples are discussed.

EXPERIMENTAL

Borosilicate columns (Duran 50; Schott, Wertheim, F.R.G.) of 50 μm I.D. were drawn with a laboratory-made precision drawing machine. Fused-silica tubing of 50 μm I.D. was obtained from SGE (Melbourne, Australia). *n*-Pentane, 40% hy-

0021-9673/83/$03.00

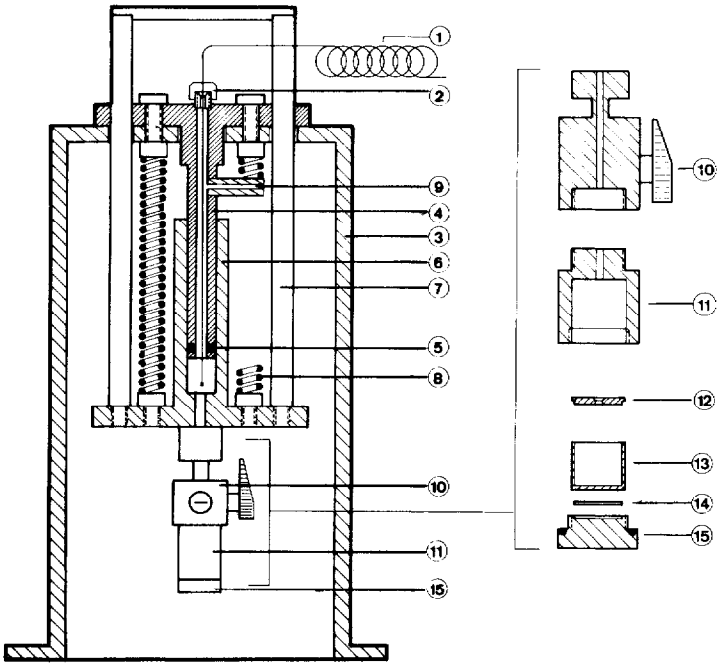

Fig. 1. Schematic drawing of the fluid introduction device (not on scale; lever not shown). 1 = Capillary column; 2 = PTFE ferrule; 3 = stand; 4 = upper compartment; 5 = Viton O-ring; 6 = lower compartment; 7 = frame; 8 = spring; 9 = pressurizing gas supply; 10 = ball valve; 11 = housing; 12 = anti-diffusion cap; 13 = vessel; 14 = PTFE washer; 15 = nut.

drofluoric acid (both of analytical-reagent grade), 30% hydrochloric acid and 65% nitric acid (both of Suprapur grade) were obtained from Merck (Darmstadt, F.R.G.). OV-1 was obtained from Ohio Valley Corp. (Marietta, OH, U.S.A.). Hexamethyldisilazane (HMDS) and diphenyltetramethyldisilazane (DPTMDS) were obtained from Fluka (Buchs, Switzerland).

For convenient filling of 50-μm I.D. capillary columns with liquids, elevated pressures are required, as the permeability of a capillary tube decreases with the square of its inner diameter. A fluid introduction device was therefore built, which employs nitrogen as the pressurizing gas, up to 80 bar, and permits fluid switching while the working pressure is maintained. A schematic drawing is shown in Fig. 1. The device incorporates two hollow cilindrical sections with matched diameters. The upper compartment (4) can be moved inside the lower part (6) similar to a plunger moving inside the barrel of a syringe. A Viton O-ring (5) provides a simple, but very effective, gas-tight seal. The column is connected with a PTFE ferrule to the upper compartment, which is firmly clamped to a stand. The lower compartment is provided with a ball valve and a housing for the vessel containing the liquid, and is attached to a frame, which can be moved with a lever. To minimize diffusion losses, the vessel is covered with a PTFE cap, having a 2-mm hole for the column. The outside diameter of the vessel is smaller than the inner diameter of the housing, so no pressure drop can build up across the vessel wall during operation. There is no risk of spillage when the working pressure is suddenly changed. PTFE vessels are employed when working with corrosive acids.

At installation, the column end is placed about 2 mm above the ball valve, the frame being held in the lower position. The device is then pressurised with nitrogen, using a Tescom (MN, U.S.A.) Model 44-1123-24 high-pressure regulator. By moving the frame, either liquid from the vessel or nitrogen gas can be introduced into the column. After the ball valve is closed, the vessel housing can be depressurized and the vessel can be changed. Meanwhile the column remains at the working pressure and is purged with nitrogen.

The chromatograms were obtained with a Carlo Erba (Milan, Italy) Fractovap 2900 gas chromatograph, equipped with a flame-ionization detector. A Tescom high-pressure regulator was installed in the carrier gas line. An SP4100 (Spectra-Physics, Santa Clara, CA, U.S.A.) computing integrator was used for data handling.

RESULTS

In our initial studies[1,2] we employed 50-μm I.D. columns, which where not deactivated. The columns were rinsed with organic solvents and then coated. With this simple procedure, few difficulties were met. A coating efficiency surpassing 75% was normally obtained with the static coating technique, employing water-glass of low viscosity for column end sealing[3]. This sealing method resulted in no failures, provided that the column front was closed with a septum before the water-glass was drawn in.

Dynamic film deposition with a solution of 15% (v/v) OV-1 in pentane, which was passed through the column at a rate of 2–3 cm/min, generally gave coating efficiencies between 55 and 70%, thus being inferior to static coating. Surprisingly, the film thickness obtained with the dynamic method was observed to decrease by a factor of 5 when the OV-1 solution was followed by a mercury plug.

Borosilicate columns

The deactivation of 50-μm I.D. borosilicate columns was initially carried out by procedures known to give good results when applied to conventional 0.25-mm I.D. capillary columns. These methods involved leaching of the column with a 20% hydrochloric acid solution for 12 h at 180°C, flushing with distilled water and dehydration at 250°C. Either persilylation[4,5] or polysiloxane degradation (PSD)[6,7] was then employed as the final deactivation step. After these treatments, the 50-μm I.D. columns unfortunately could no longer be coated with reasonable efficiency. Dynamic film deposition gave coating efficiencies of only 20–35% and the column even became blocked when the static method was tried. When viewed under a scanning electron microscope, the dynamically deposited film displayed a finely crinkled structure. On employing milder leaching conditions, the stationary film regained its usual smooth appearance, but coating still failed. Further investigations led to the conclusion that the deactivation step must be carried out in the presence of a solvent, thus preventing the build-up of unwanted solid deposits on the column wall. Employing this modification, well deactivated non-polar columns with a coating efficiency of about 80% were obtained. Column end straightening was carried out with a gently heated electrical device.

The deactivated borosilicate columns were examined by the quality test of Grob *et al.*[8]. To ensure comparable test conditions, the temperature programming

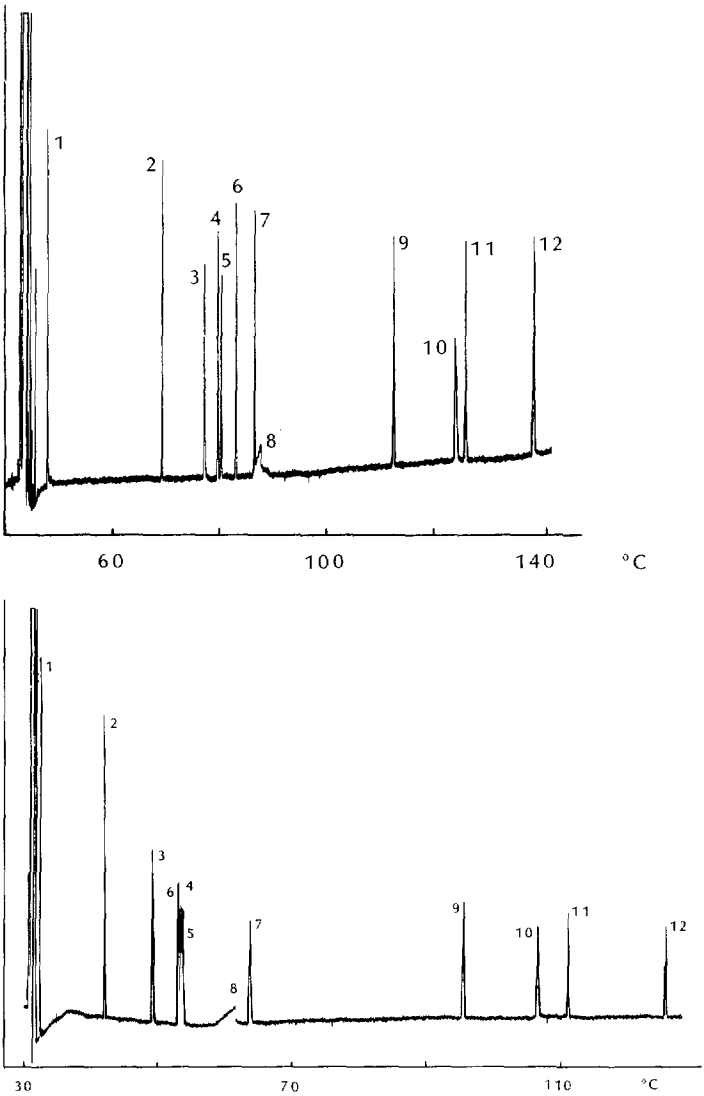

Fig. 2. Chromatograms of the Grob mixture, obtained with helium, on a 7.5 m × 50 μm I.D. persilylated borosilicate OV-1 column, programmed from 40 to 150°C at 3.5°C/min (upper chromatogram) and on a 6 m × 50 μm I.D. PSD-deactivated fused-silica SE-54 column, programmed from 30 to 140°C at 4°C/min (lower chromatogram). Sensitivity: 10^{-12} A f.s. Peaks: 1 = 2,3-butanediol; 2 = *n*-decane; 3 = 1-octanol; 4 = 2,6-dimethylphenol (DMP); 5 = nonanal; 6 = *n*-undecane; 7 = 2,6-dimethylaniline (DMA); 8 = 2-ethylhexanoic acid; 9 = methyl decanoate; 10 = dicyclohexylamine; 11 = methyl undecanoate; 12 = methyl dodecanoate.

rate for the 50-μm I.D. columns was made five times larger than the rate prescribed for columns of 0.25 mm I.D., in accordance with ref. 2. A representative test result is shown in Fig. 2 (top). This chromatogram was obtained on a column leached with 15% hydrochloric acid for 16 h at 150°C, flushed with 1 *M* hydrochloric acid, distilled water and methanol, dehydrated for 1 h at 180°C under a flow of nitrogen and then

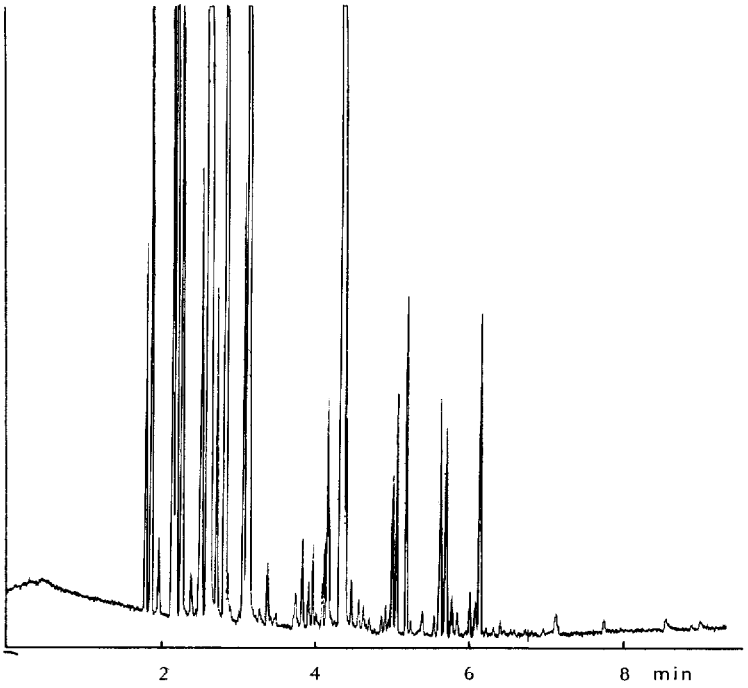

Fig. 3. Chromatogram of bergamot oil on a persilylated 8 m × 50 μm I.D. borosilicate column, coated with OV-1. Oven temperature programmed from 60 to 170°C at 20°C/min, then to 240°C at 35°C/min. Carrier gas: helium. Inlet pressure: 10 bar.

filled with HMDS–DPTMDS–pentane (1:1:2). The column was heated at 400°C for 6 h with flame-sealed ends, then flushed with pentane and methanol, dried and statically coated. The Grob test revealed a slightly diminished response towards DMP and nonanal. Dicyclohexylamine is quantitatively eluted. However, this peak is broadened, and thus lowered, by a mechanism that is not yet understood. Octanol clearly tails. Even stronger tailing and partial adsorption were observed when 1-aminodecane was injected. This tailing for octanol and aminodecane was found with each of four borosilicate columns studied, including two columns that were deactivated by polysiloxane degradation. The tailing is presumably due to insufficient leaching.

Deactivated borosilicate columns have been applied successfully to the analysis of essential oils, underivatized and methoxime-trimethylsilyl (MO-TMS) derivatized steroids and alkylphenols. The separation of bergamot oil on an 8 m × 50 μm I.D. OV-1 column is shown in Fig. 3. Most of the compounds are monoterpenes. All of the compounds with a retention time below 3 min are known to have the same molecular weight of 136.

A crude sample containing alkylphenols, supplied by Dr. T. Tóth (Eötvös Lorand University, Budapest, Hungary), was analysed in triplicate at 100°C on a persilylated 7.5 m × 50 μm I.D. borosilicate column and on a 25 m × 0.25 mm I.D., PSD-deactivated soft glass column. Both columns were coated with OV-1 (see Table I). The Kováts retention indices obtained with the two columns differ by at most 0.6

TABLE I

KOVÁTS INDICES (*I*) AND RELATIVE PEAK AREAS OBTAINED AT 100°C FOR A MIXTURE OF UNDERIVATIZED ALKYLPHENOLS ON TWO COLUMNS

Columns: 7.5 × 50 μm I.D. persilylated borosilicate and 25 m × 0.25 mm I.D. PSD-deactivated soft glass columns, both coated with OV-1.

*Compound**	*7.5 m × 50 μm I.D. column*		*25 m × 0.25 mm I.D. column*	
	I	*Rel. peak area*	*I*	*Rel. peak area*
Phenol	948.1	–	948.5	–
2-Methylphenol	1025.6	–	1025.4	–
3-Methylphenol	1045.9	–	1045.4	–
3,5-Dimethylphenol	1139.7	28.6	1139.3	27.9
2,3-Dimethylphenol	1148.3	1.0	1148.5	1.0
3,4-Dimethylphenol	1163.0	1.6	1163.1	1.7
3-Ethylphenol	1177.3	1.4	1177.0	1.6
8	1214.5	20.7	1214.2	20.0
9	1229.8	9.3	1229.8	9.1
10	1248.7	16.1	1248.4	15.9
11	1281.1	6.0	1281.7	6.6
12	1321.6	12.0	1321.5	12.6
13	1333.2	3.3	1333.5	3.7

* The numbered compounds are ethyldimethylphenol or ethyltrimethylphenol isomers.

units, the average deviation being less than 0.1 unit. The quantitative data obtained compare well. Thus, excellent agreement is observed.

Fused-silica columns

Fused-silica columns of 50 μm I.D. were first rinsed with an aqueous solution containing 2% hydrofluoric acid and 2% nitric acid for 30 min and then for a short time with 2% hydrochloric acid, as recommended by Onushka[9]. The columns then were flushed with distilled water, dehydrated for 3 h at 280°C under a flow of nitrogen and then filled with a solution of 0.6% (v/v) OV-1 in pentane. The columns were flame-sealed at one end, leaving the other end open, and were enclosed in an aluminium box, which was continuously purged with nitrogen. The box was heated to 420°C at 12°C/min and then maintained at this temperature for 1 h. After they had cooled, the columns were rinsed with pentane and statically coated.

When the flush with distilled water was omitted from the above procedure, a white, gel-like substance was seen to be pushed out of the column as the OV-1 solution was introduced. Sometimes the column even became blocked. Slightly active columns were obtained when polysiloxane degradation was carried out below 400°C. The column properties then also changed unpredictably during the conditioning step.

Fused-silica columns with very good inertness have been prepared by the procedure described above. The chromatogram obtained on a 6 m × 50 μm I.D. fused-silica SE-54 column for the Grob mixture (Fig. 2, bottom) shows some distinct differences when compared with the Grob test of the borosilicate OV-1 column (Fig. 2, top). Owing to the slight difference in polarity between the two phases, *n*-undecane and DMP as well as 2-ethylhexanoic acid and DMA, are eluted in a different order.

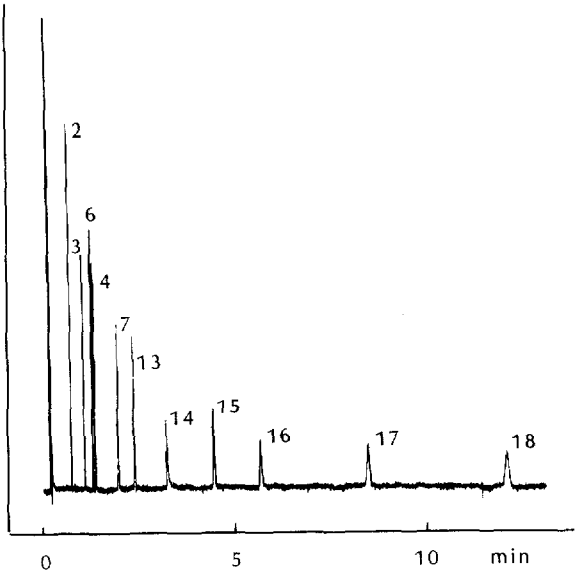

Fig. 4. Chromatogram of polarity test mixture, analysed at 100°C on a 6 m × 50 μm I.D. PSD-deactivated fused-silica column coated with SE-54. Amount of sample introduced into the column: 15 pg per compound. Carrier gas: helium. Peaks: 2, 3, 4, 6 and 7 as in Fig. 2: 13 = *n*-dodecane; 14 = 1-aminodecane; 15 = *n*-tridecane; 16 = nicotine; 17 = *n*-tetradecane; 18 = acenaphthene.

On SE-54, *n*-undecane, DMP and nonanal severely overlap, which hampers the quantitative evaluation of the test. The inertness of the fused-silica column appears to be superior to the deactivation of the borosilicate surface. The fused-silica column shows better peak heights for octanol and dicyclohexylamine, while octanol is not affected by tailing.

The deactivation of the fused-silica column was studied in more detail by using a polarity test mixture, containing equal amounts (by weight) of each test compound (Fig. 4). Only 15 pg of each compound were introduced into the 50 μm I.D. column, which is 20–100 times less than the amounts normally used for testing 0.25-mm I.D. capillary columns. Well separated, symmetrical peaks were observed for all substances, except 1-aminodecane, which showed moderate tailing. In contrast to the Grob mixture, the polarity mixture was analysed under isothermal conditions. As a consequence, the deactivation of the column cannot be judged easily from the peak heights shown in Fig. 4. However, the peak areas reveal a quantitative recovery for all compounds, except 1-aminodecane. The response factors calculated for octanol, DMA and DMP, based on *n*-undecane as the reference compound, are in very close agreement with values published by Schomburg[10]. The DMA/DMP peak-area ratio was measured to be 1.04, indicating that the deactivated surface is nearly neutral.

The deactivated fused-silica columns are suitable for a wide range of applications, such as the analysis of underivatized drugs. For instance, paracetamol, which is very easily adsorbed, is eluted with only minor tailing from such columns (Fig. 5).

Cross-linked phases

Immobilization of the stationary phase with organic peroxides restored some

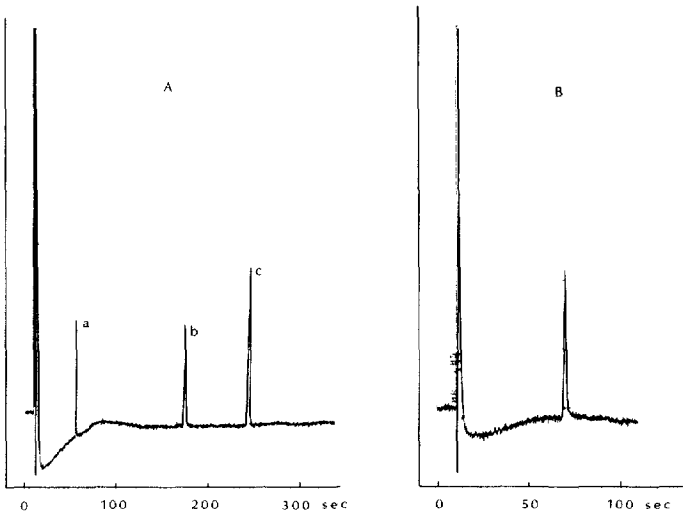

Fig. 5. Chromatograms of underivatized drugs. (A) Separation of (a) caffeine, (b) phenacetin and (c) cocaine at 90°C; (B) paracetamol at 180°C. Column as in Fig. 4.

activity to the deactivated 50-μm I.D. columns. Immobilization, however, also permitted the direct introduction of liquid samples. For this purpose, a special glass liner of 0.4 mm bore was installed in the cold injection port of the gas chromatograph. An amount of 1.5 μl of the liquid sample was introduced into the liner with a syringe. A small fraction of the liquid plug then entered the column, while the remaining part moved towards the splitter. A chromatogram thus obtained is presented in Fig. 6, showing the separation of 1-ppm amounts of some test compounds dissolved in hexane.

DISCUSSION

Well deactivated non-polar 50 μm I.D. columns with a good coating efficiency

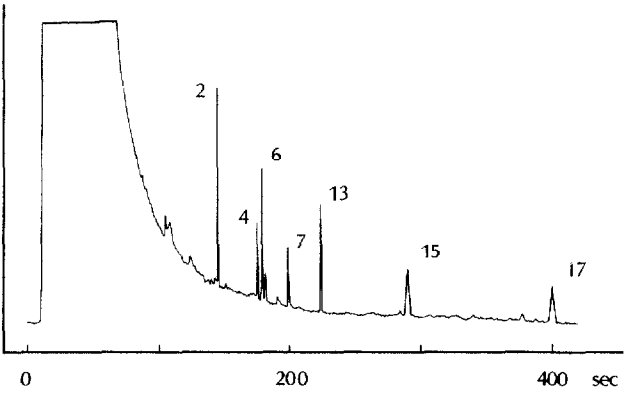

Fig. 6. Separation of 1-ppm amounts of test compounds, dissolved in hexane, on a 6 m × 50 μm I.D. fused silica column coated with immobilized OV-1, by use of a liquid-splitting "on-column" injector. Injector temperature, 55°C. Oven temperature, maintained at 60°C for 75 sec, then programmed at 30°C/min to 120°C. Carrier gas: helium. Inlet pressure: 8 bar. Peaks as in Figs. 2 and 4.

can be prepared by relatively simple techniques. The columns can be operated with conventional modern gas chromatographs, the replacement of the carrier gas pressure regulator being the major modification required. Large savings in time may be realized, in particular in the analysis of complex mixtures requiring capillary columns of high plate number. Columns coated with immobilized phases are expected to become important tools in supercritical fluid chromatography. In view of the above, we strongly advocate that very-narrow-bore columns with inner diameters between 30 and 100 μm be made widely available to all chromatographers in the near future.

ACKNOWLEDGEMENTS

We thank Mr. E. Dawes, Scientific Glass Engineering, Melbourne, Australia, for the generous gift of the 50-μm I.D. fused-silica capillary tubing and Mr. H. van Leuken, Eindhoven University of Technology, for expert technical assistance.

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