Journal of Chromatography, 208 (1981) 209–216
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,560

PREPARATION OF WALL-COATED OPEN TUBULAR GLASS (PYREX) CAPILLARY COLUMNS WITH POLAR STATIONARY PHASES, USING SUPEROX™-4 AS A SURFACE PRETREATING AND DEACTIVATING AGENT

R. F ARRENDALE*, R. F SEVERSON and O T CHORTYK
Tobacco and Health Laboratory, Science and Education Administration/Agricultural Research, U S. Department of Agriculture, P.O. Box 5677, Athens, GA 30613 (U S.A)
(Received November 17th, 1980)

SUMMARY

Wall-coated open tubular (WCOT) glass (Pyrex) capillary columns were prepared with three different polar stationary phases (Carbowax 20M, Superox™-4, and SP-1000) using Superox-4 as a surface pretreating and deactivating agent. Methods for the laboratory preparation and practical application of these columns are given. Columns prepared by this technique were efficient, thermally stable, and low in surface activity. The Superox-4 pretreatment and deactivation technique produced high quality polar WCOT columns, and no surface roughening procedure was required. The technique offered a substantial decrease in column preparation time. In addition, columns prepared by this technique were comparable to those from commercial suppliers or those produced by conventional preparation techniques.

INTRODUCTION

In a recent article from this laboratory, we introduced the technique of using Superox™-4 as a surface pretreating and deactivating agent for the preparation of wall-coated open tubular (WCOT) glass capillary columns[1]. Columns prepared by this procedure compared favorably with columns prepared by a conventional procedure that included a surface roughening step[2,3]. The Superox-4 procedure eliminated the need for a surface roughening step even for preparing columns with polar liquid phases.

The unique characteristics of Superox-4 are responsible for the success of the technique. This polyethylene glycol (mol.wt. $4 \cdot 10^6$) is thermally stable (even at 300°C in the absence of oxygen), coats smoothly and evenly on bare glass surfaces, and resists droplet formation even during heating[4,5].

Our preparation procedure involves the application of a non-extractable layer of Superox-4 to the glass surface, and is similar to Cronin's[6] adaptation of the method of Aue *et al.*[7] for deactivating solid surfaces. This non-extractable layer of polar polymer gives the glass surface a polar character. Superox-4 on the treated glass

surface interacts intermolecularly with the polar liquid phase and thus stabilizes it on the glass surface. Also, the Superox-4 layer efficiently deactivates the glass surface by covering a large portion of the chemically reactive sites.

In this paper, we report the use of the Superox-4 procedure to prepare WCOT columns with three polar liquid phases (Carbowax 20M, Superox-4, and SP-1000). The columns so prepared were evaluated for efficiency, stability and activity. Separations achieved with these columns are given and discussed.

EXPERIMENTAL*

In Fig. 1, our procedure for preparing WCOT glass capillary columns with Carbowax 20M, Superox-4 and SP-1000 by the Superox-4 pretreatment and deactivation procedure is set out.

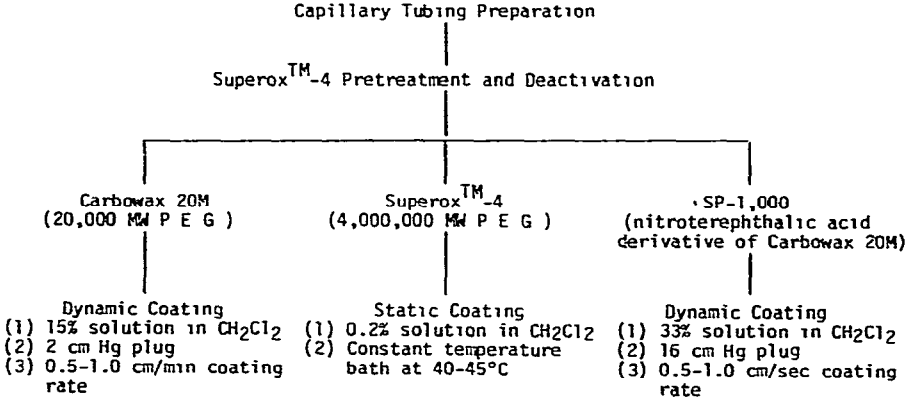

Fig 1. Scheme used for preparing WCOT glass capillary columns with polar stationary phases. PEG = Polyethylene glycol; MW = molecular weight.

Materials

Superox-4 was obtained from Alltech (Arlington Heights, IL, U.S.A.); Carbowax 20M from Analabs (North Haven, CT, U.S.A.); and SP-1000 from Supelco (Bellefonte, PA, U.S.A.). All solvents were Burdick and Jackson (Muskegon, MI, U.S.A.) "distilled-in-glass" grade and were redistilled in glass before use.

Capillary tubing preparation

Freshly drawn Pyrex glass capillary tubing was rinsed sequentially with concentrated hydrochloric acid, acetone, and methylene chloride; it was then dried under nitrogen[1].

Superox-4 pretreatment and deactivation

The clean, dry capillary tubing was rinsed with a dilute solution of Superox-4 in methylene chloride. Previously, 0.1–0.5% solutions were used[1], but we have since

* Reference to a company or product name does not imply approval or recommendation by the U.S Department of Agriculture

found that a 0.2% solution is best for columns of I.D. 0.25 mm or less, and that more concentrated solutions may be used with columns of larger bore. A plug of Superox-4 solution, occupying 10–20% of the capillary tubing length, was pushed through the column at a rate of 2–3 cm/min. Faster rates tended to leave excessive amounts of solution on the tubing wall, thus causing smaller plugs of solution to trail behind the main plug. Formation of these small plugs appeared to have little effect on the quality of the columns produced, provided that the integrity of the main plug was maintained throughout the rinsing step. After the tubing had dried (nitrogen flow), the ends were sealed with a flame from a small propane torch. During the sealing process, oxygen in the air was excluded by a positive flow of high-purity nitrogen maintained through the tubing. We tested the first seal by immersing the end in a beaker of water and increasing the nitrogen pressure. The other end of the capillary tubing was then broken off near the connection to the nitrogen source and sealed with the propane torch. Several attempts were usually necessary to achieve an adequate seal due to the rapid escape of nitrogen from the column. Each seal was checked by the water-immersion method. Finally, the sealed capillary tubing was heated in an oven at 300°C for 1 h. Best results were obtained when we repeated this procedure twice

Liquid phase coating

Carbowax 20M and SP-1000 were coated on the tubing by the mercury-plug dynamic-coating technique of Schomburg and Husman[8]. Solutions (15%, w/v, for Carbowax 20M and 33%, w/v, for SP-1000) were prepared in methylene chloride. About 10% of the capillary tubing length was filled with coating solution, followed by a high-purity mercury plug of appropriate length under vacuum. Columns were then coated by pushing the mercury plug and coating solution through the column under nitrogen pressure at 0.5–1.0 cm/min for Carbowax 20M and 0.5–1.0 cm/sec for SP-1000.

Superox-4 was coated onto the tubing by the static coating technique developed by Bouche and Verzele[9]. The capillary tubing was filled with the coating solution supplied from a reservoir under nitrogen pressure (0.2%, see Fig. 1). Next, the open end of the capillary tubing was submerged in a water-soluble glue, with the coating solution still flowing from the end. The other end of the tubing was then connected to a vacuum source, and the glue was drawn into the column a distance of about 10 cm. The vacuum was disconnected, and the glue plug was allowed to harden for 24 h. After the plug had hardened, the capillary tubing was placed in a constant-temperature water bath (40°C). The column end containing the glue plug was straightened before filling so that the plug could easily be kept dry. After the capillary tubing had equilibrated, the open end was connected to a vacuum pump, and the solvent was slowly evaporated, so that the liquid phase was deposited on the tubing surface as a thin film.

Column conditioning and evaluation

After the WCOT columns had been coated, they were dried under nitrogen flow. Next, the column ends were straightened to fit the configuration of the injection port and detector of the gas chromatograph; a low flow of air was maintained through the columns while the ends were being straightened with a propane torch. After straightening, the ends were rinsed with methylene chloride, re-coated with a 0.2

mg/ml solution of Superox-4 in methylene chloride, and dried for 45 min under nitrogen flow. After installation in the chromatograph, each column was temperature-programmed (2°/min) from 40° to 150°C, maintained at the final temperature for 1 h, then evaluated for activity and total number of effective plates. Column activity was evaluated from the appearance of the chromatogram for a polarity-standard mixture. The number of effective theoretical plates was calculated based on the hexadecane peak, with a capacity ratio (k') of 5. If these results were satisfactory, each column was conditioned to the maximum operating temperature before analytical use.

RESULTS AND DISCUSSION

According to conventional preparation techniques, the preparation of WCOT columns with polar liquid phases relies upon two basic principles of film stabilization[2,10,11]; Grob[11] defined these two principles as chemical and geometric. Chemical film stabilization is due to intermolecular attraction between functional groups on the glass surface and the functional groups of the stationary liquid phase. Geometric film stabilization involves reducing the contact angle between the liquid phase and the glass surface[2]. When this is done, the intermolecular attractive forces between like molecules of the stationary liquid phase are reduced, and adjacent pools of stationary liquid phase are held in the cavities of the roughened surface by capillary action[12]. Most conventional techniques for preparing polar WCOT columns emphasize the geometric means of stabilizing the liquid phase film by including surface-roughening procedures[2,3,13–16]. Our method of using Superox-4 as a surface-pretreating (surface modification) and deactivating agent emphasizes the chemical means of liquid phase film stabilization over the geometric method. Superox-4, like Carbowax 20M[1–3], deactivates solid surfaces and also evenly and smoothly coats them, so that surface roughening is not necessary. All three polar liquid phases chosen for this have the capacity to interact chemically with the Superox-4 surface-bonded layer. Carbowax 20M and Superox-4 have functional groups identical to those of the Superox-4 layer. SP-1000 (a nitroterephthalic acid derivative of Carbowax 20M) also has a polyethylene glycol moiety in its structure and is therefore capable of interacting chemically with the Superox-4 surface-bonded layer. These chemical interactions are similar to those that occur between like molecules of the respective liquid phases, and result in the stabilization of the polar stationary liquid phase films on the deactivated glass surface.

In our first publication on the Superox-4 method of WCOT column preparation[1], we showed that Carbowax 20M columns prepared by the dynamic-coating method had high efficiency and low activity. We have used Carbowax 20M columns heated to 250°C under programming conditions and at 230°C under isothermal conditions with little change in column characteristics. Thus, the thermal stability of columns with this stationary liquid phase appears to be good. As with all liquid phases that contain a polyethylene glycol moiety, oxygen must be removed from carrier gases if column stability and neutrality are to be maintained[2,10].

Verzele and co-workers[4,5] investigated Superox-4 as a liquid phase and found it to possess several unique properties, many of which arise from its gum-like behavior. Our results with Superox-4 as a liquid phase are in agreement with their findings.

Fig. 2 shows an activity-standard chromatogram obtained with a 15-m Superox-4 WCOT column. Good surface activity was indicated by the excellent peak shapes for all components in the polarity standard. The peak heights of 2,4-dimethylaniline and 2,6-dimethylphenol are indicative of their relative amounts in the mixture and, thus, of low acid–base surface activity. Superox-4 WCOT columns showed good thermal stability at 250°C under constant use. Thermal stability at higher temperatures was demonstrated under programmed conditions. Verzele and co-workers obtained similar results, with use of various preparation techniques[4,5]. Superox-4 columns can be used to separate a variety of constituents Fig. 3 shows a chromatogram of the methyl esters of a mixture of fatty acids separated with the Superox-4 column; note the separation of the C_{18} fatty acid methyl ester isomers. We selected Superox-4 columns for the quantitative determination of many tobacco and tobacco smoke constituents, including alkaloids[17], aliphatic hydrocarbons, fatty acid methyl esters and duvatrienediols.

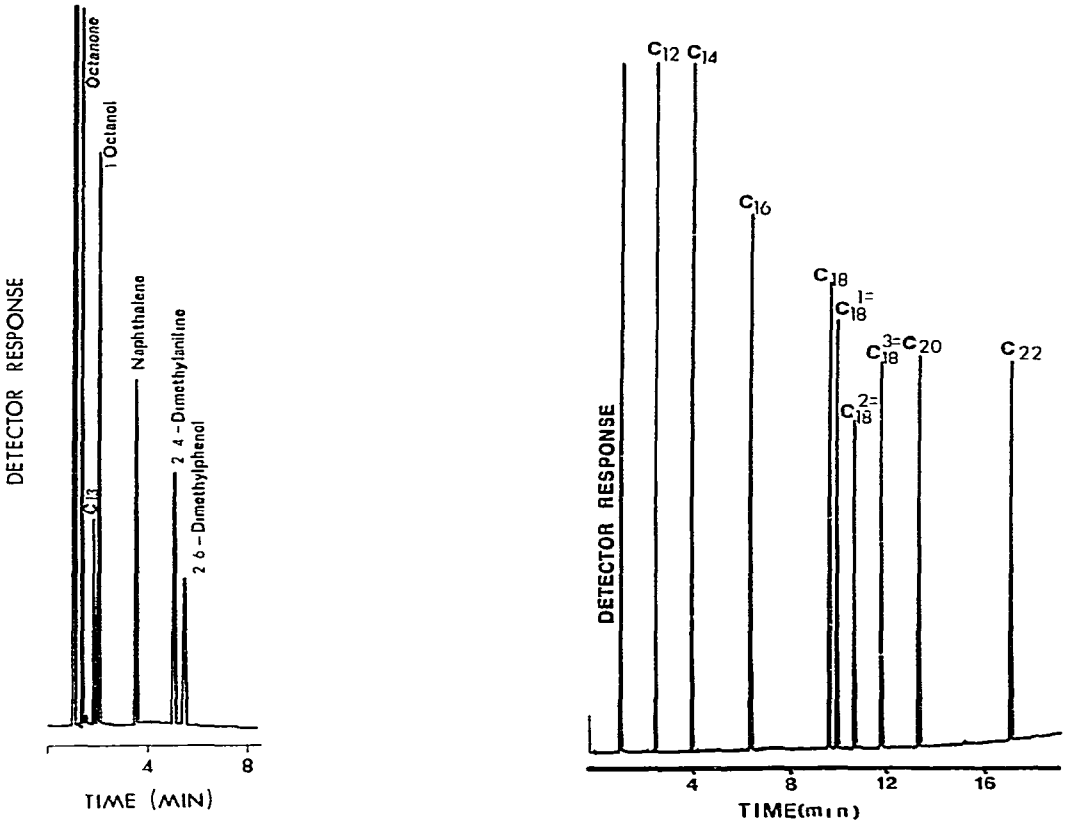

Fig 2 Activity-standard chromatogram (FID) obtained using a Superox-4 WCOT column prepared by the Superox-4 pretreatment and deactivation procedure Conditions. 15 m × 0 25 mm I D ; 130°C; 28 cm/sec helium flow; split injection mode

Fig 3. Fatty acid methyl ester mixture chromatogram (FID) obtained using a Superox-4 column Conditions· 26 m × 0 25 mm I D ; temperature-programmed from 160 to 250°C at 4°C/min; 28 cm/sec helium flow; split injection mode

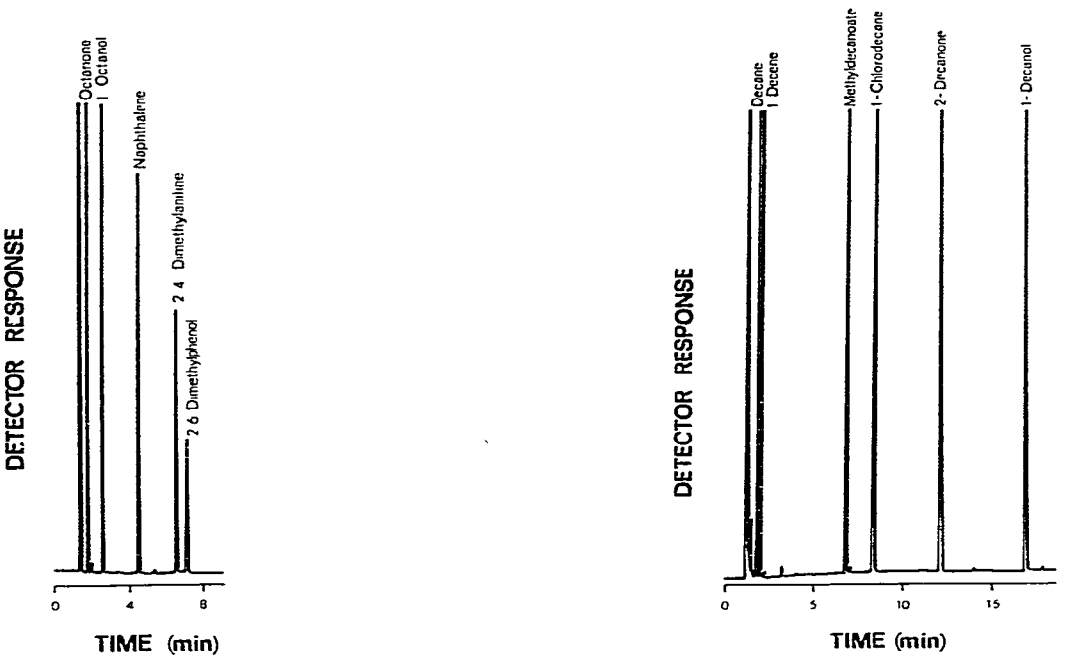

Fig 4. Activity-standard chromatogram (FID) obtained using an SP-1000 WCOT column prepared by the Superox™-4 pretreatment and deactivation procedure Conditions 24 m × 0.25 mm I D.; 150°C; 27 cm/sec helium flow; split injection mode

Fig 5 Decane derivatives separated on an SP-1000 WCOT column (FID) Conditions· 24 m × 0.25 mm I.D., temperature programmed from 70 to 150°C at 4°C/min; 28 cm/sec helium flow; split injection mode

Fig. 4 shows the analysis of our polarity-standard mixture with a 24-m SP-1000 column. The peak shapes for all components are very good, and there is no evidence of peak-height reduction due to adsorption of the sample components. Fig. 5 shows the separation of a standard mixture containing a series of decane derivatives on an SP-1000 WCOT column prepared by the Superox-4 procedure; note the discrimination between the different functional groups by this polar column. SP-1000 columns are often used in the analysis of flavor- and aroma-producing compounds from a variety of sources. Fig. 6 is a chromatogram of a flue-cured-tobacco flavoring mixture obtained with an SP-1000 column. Good separation and excellent peak shape were obtained for this mixture, which included such flavor components as vanillin, piperonal, and *p*-anisaldehyde.

The column characteristics of an SP-1000 column were monitored over several weeks of constant use so that column stability could be evaluated (Fig. 7). When the activity-standard chromatogram was obtained with a freshly coated SP-1000 column that had been conditioned for 1 h at 150°C (Fig. 7A), the baseline was irregular and the 2,4-dimethylaniline peak was low. The adsorption of 2,4-dimethylaniline was probably due to excess acid left in the SP-1000 liquid phase during the preparation of this nitroterephthalic acid derivative of Carbowax 20M. The column was then completely conditioned at 230°C and then used for several days to analyze flavor- and

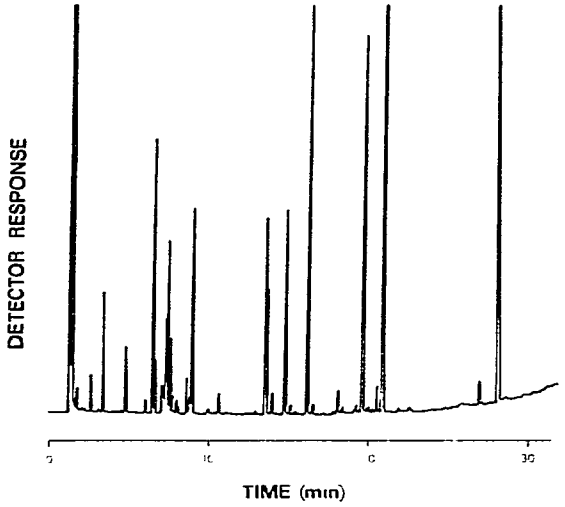

Fig 6 Flue-cured-tobacco flavoring mixture separated using an SP-1000 WCOT column (FID) Conditions. 24 m × 0 25 mm I.D ; temperature-programmed from 100 to 230°C at 4°C/min; 28 cm/sec helium flow; split injection mode

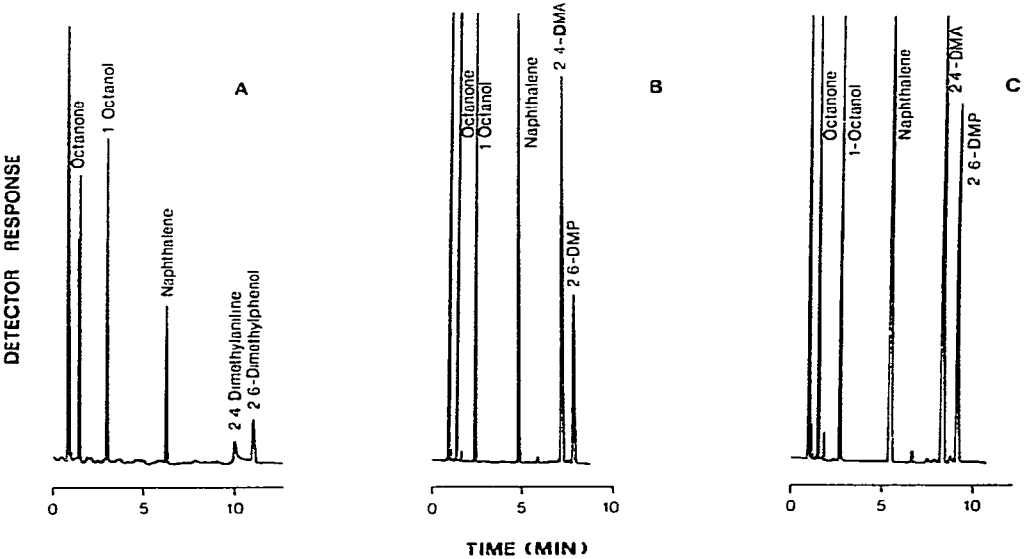

Fig 7. Activity-standard chromatogram (FID) obtained using an SP-1000 WCOT column: Chromatogram A obtained after initially conditioning the column to 150°C for 1 h; chromatogram B obtained after completely conditioning the column at 230°C; chromatogram C obtained after 30 analyses using the splitless injection mode Conditions· 24 m × 0 25 mm I D ; 150°C; 28 cm/sec helium flow; split injection mode

aroma-producing components. Commercial suppliers recommend that their SP-1000 columns coated on glass be heated only to 220°C. We even heated this column to 240°C for short periods of time. Fig. 7B shows the analysis of the standard-activity mixture after this period of use. The column retained its activity toward the com-

ponents of the standard mixture and indicated good column stability. Fig. 7C shows the separation of the polarity-standard mixture separated with the same SP-1000 column after weeks of use, having been used to analyze more than 50 samples. Most of the analyses were performed with the chromatograph in the splitless (Grob) injection mode[18]. The column retained its high efficiency and low activity after 2 months of use, thus indicating its excellent stability.

The SP-1000 and Superox-4 columns were not as efficient as the Carbowax 20M columns because their liquid phase films were thicker. However, the number of effective plates for the SP-1000 and Superox-4 columns was high (2000 to 2500 per metre, based on the hexadecane peak at a k' value of 5). It has been shown that acid–base effects are reduced and that thermal stability increases with thicker liquid phase films[19].

In conclusion, polar WCOT columns prepared by the Superox-4 pretreatment and deactivation procedure were efficient, thermally stable, and possessed low surface activities. The characteristics of these columns were comparable to those of commercially prepared columns as described by the suppliers, or those of columns prepared by conventional techniques that included surface roughening steps.

REFERENCES

1 R. F. Arrendale, L B Smith and L B. Rogers, *J High Resolut Chromatogr. Chromatogr. Commun*, 3 (1980) 115.
2 K. Grob and G Grob, *J. Chromatogr*, 125 (1976) 471.
3 K. Grob, G. Grob and K. Grob, Jr., *Chromatographia*, 10 (1977) 181
4 M. Verzele and P. Sandra, *J Chromatogr*, 158 (1978) 111
5 P. Sandra, M Verzele, M. Verstappe and J. Verzele, *J High Resolut. Chromatogr. Chromatogr Commun*, 2 (1979) 288
6 D A Cronin, *J Chromatogr*, 97 (1974) 263
7 W. A Aue, C. R. Hastings and S. Kapila, *J. Chromatogr*, 77 (1973) 299.
8 G Schomburg and H. Husman, *Chromatographia*, 8 (1975) 517
9 J. Bouche and M. Verzele. *J Gas Chromatogr*, 6 (1968) 501
10 M. Verzele, *J. High Resolut. Chromatogr. Chromatogr. Commun*, 2 (1979) 647.
11 K. Grob, *J. High Resolut Chromatogr. Chromatogr Commun*, 2 (1979) 599
12 J. C. Giddings, *Anal. Chem*, 34 (1962) 458
13 K Tesarik and M. Novotny, in H G Stuppe (Editor), *Gas Chromatography 1968*, Akademie-Verlag, Berlin, 1968, p 575
14 G. Alexander and G A F M Rutten, *Chromatographia*, 6 (1973) 231.
15 J D. Schieke, N. R. Comins and V. Pretorius, *J. Chromatogr.*, 112 (1975) 97.
16 H. T. Badings, J. J. G van der Pol and J. G. Wassink, *J High Resolut. Chromatogr. Chromatogr Commun*, 2 (1979) 297.
17 R F. Severson, K. L. McDuffie, R F. Arrendale, G. R. Gwynn, J. F. Chaplin and A. W. Johnson, *J. Chromatogr*, 211 (1981) in press
18 K. Grob and K Grob, Jr, *J. Chromatogr*, 94 (1973) 53
19 K Grob, Jr. and K. Grob, *Chromatographia*, 10 (1977) 250.