

CHROM. 17 489

METHOD FOR TESTING RETENTION GAPS AND DATA ON THE GAS CHROMATOGRAPHIC RETENTION POWER OF DIFFERENTLY DEACTIVATED CAPILLARY TUBES

K. GROB, Jr.* and H. P. NEUKOM

Kantonales Labor, P.-O. Box, 8030 Zürich (Switzerland)

(Received December 18th, 1984)

SUMMARY

A testing procedure is described that allows the rapid determination of (a) the retention power in an uncoated pre-column used as a retention gap and (b) adsorption effects in such capillary tubes, and which is also of interest for checking for contamination of the retention gap by non-volatile sample by-products. Data on the retention power of uncoated capillaries deactivated by silylation and using Carbowax are presented, providing some interesting information on the deactivation procedures commonly used for column preparation. In particular, it was found that silylation forming trimethylsilyl or dimethylphenylsilyl groups yields very different surface properties.

INTRODUCTION

A retention gap is an uncoated column inlet¹ used to reduce the effects of non-volatile sample by-products on the chromatographic performance of the column² and to reconcentrate bands broadened in space³. The length of the retention gap varies between about 0.2 and 50 m, depending on the length of the zone flooded by the sample liquid^{4,5}.

An optimal retention gap is characterized by the following:

(a) A low retention power in order to provide a maximal reconcentration effect⁶. The reduction in the band length when a solute passes from the retention gap into the coated separation column is approximately equal to the ratio of the retention powers in these two column parts. Hence the reconcentration effect is most important if a thick-film column is used together with a retention gap of minimal retention power⁷.

(b) The wall of the retention gap should be inert in order to prevent adsorption of polar solutes and to minimize their retention.

(c) The retention gap must be wetted by the sample liquid because otherwise the liquid runs too far into the column. Wettability is a particularly severe problem for retention gaps deactivated by silylation with hexamethyldisilazane (HMDS) or similar reagents that form trimethylsilyl groups⁴.

A standardized test for retention gaps is desirable for two purposes: first, for the development of an optimized preparation procedure for pre-columns to serve as retention gaps, and second, for the testing of used retention gaps to assess their state, in particular to check for contamination by non-volatile sample by-products, which increase both the retention power and adsorptivity in the retention gap.

Testing of retention gaps has much in common with testing of uncoated capillary columns used to control the quality of the surface deactivation before coating. Such a method was described by Schomburg *et al.*⁸ in 1977 and involved the use of a polar (Carbowax) pre-column in order to elute separated, sharp solute bands into the piece of capillary tube to be tested (of length about 2 m). *n*-Butanol was used as a test component, introduced by split injection as a solution in *n*-hexane. Peak shapes were determined qualitatively to assess the adsorptivity. Grob and Grob⁹ refined the selection of the test components and stressed the need for quantitative determination of the peak sizes in order to detect irreversible adsorption.

Uncoated capillary tubes were tested for the determination of adsorptivity, whereas the test of retention gaps concentrates primarily on the measurement of retention powers. We recently determined the "depth of retention gaps" by two methods, one of them again involving the use of a coated pre-column. Retention times of some solutes were determined with and without the retention gap attached to this column. The other method involved the determination of the residual band width after creating a known band broadening in space, *i.e.*, an assessment of the retention power via a measured reconcentration factor⁶.

The results of this investigation allowed the following conclusions to be drawn: (1) roughening (etching) of the internal column walls creates an increased retention power compared with smooth surfaces owing to the large surface area; (2) Carbowax-deactivated capillary tubes show 2–4 times stronger retention than silylated tubes; (3) silylated fused-silica tubes exhibit only half the retention power of untreated fused-silica tubes even when an alkane is used as a test solute.

The above methods were developed for short pieces of capillary tubing to be tested. However, as the retention power in the uncoated pre-column is critical primarily for long pieces of tubing, a much simpler method can be applied that is suitable for a rapid routine test.

This paper describes the proposed testing procedure and lists further results concerning the evaluation of preparation procedures for retention gaps. In the context of the latter subject a recent paper by Rutten *et al.*¹⁰, dealing with the high-temperature silylation of silica material obtained after leaching of glass or fused-silica surfaces, is of interest. Their study provided some new information about the still poorly understood subject of column deactivation, where up to now pragmatic evaluations of the effects on column quality have prevailed. The silylation products were studied, using fumed silica and NMR spectroscopy. The authors concluded that the originally formed trimethylsilyl or phenyldimethylsilyl groups [using hexamethyldisilazane (HMDS) or 1,2-diphenyl-1,1,3,3-tetramethyldisilazane (DPTMDS) as silylation reagents, respectively] became dimethylsilyl groups at elevated temperatures with the silicon bonded to two oxygen atoms, losing a methyl or the phenyl group. On HMDS-treated surfaces this transformation occurred at 350°C and on DPTMDS-deactivated surfaces at 250°C. According to these results, silylation at 400°C (commonly used for the preparation of apolar glass capillary columns¹¹)

should result in the same surface characteristics whether silylation is carried out with HMDS or DPTMDS. On the other hand, the authors also found that the formation of the double bonds to the silica structure occurred only above 460°C for the HMDS- and above 400°C for the DPMDS-treated material if the silica was thoroughly dried before silylation. It was interesting to compare these results, inevitably obtained under somewhat artificial conditions, with those obtained by the retention gap test discussed below. Previously we found that the wettabilities of the HMDS- and DPTMDS-treated surfaces are very different⁴. Here we report that the retention powers of the two types of surfaces are also very different. This may be interpreted as proof that the phenyl groups are not eliminated during the silylation procedure, as was predicted to occur by Rutten *et al.*; however, the proof is not rigorous.

The retention power determined by the retention gap test is expressed in terms of the "apparent film thickness" of an apolar stationary phase (OV-1). This unit is suitable for expressing a characteristic of the wall surface (the phase ratio β is also dependent on the column diameter). However, the main advantage is the easy comparison with the retention power of the coated separation column (at least as long as the latter is coated with an apolar stationary phase).

EXPERIMENTAL

Determination of the retention power

The capillary tube to be tested is installed between a split injector and a flame-ionisation detector. The oven temperature is set at 70°C and the carrier gas flow-rate at a few ml/min; the split flow-rate is adjusted to about 50 ml/min. The dead time of this system is determined by injection of 1 μ l of methane (or another fuel gas) using a stop-watch. Then 5–200 μ l of headspace gas, taken directly from the bottles of the test components, are injected and the retention times of the maxima of the peaks measured with the stop-watch. Test solutes of different types of compounds (*n*-alkanes, methyl esters, 1-alkanols and aromatics) are selected to give retention times exceeding the dead time of the column by 10–200%. The determined retention times divided by (dead time – 1) results in adjusted partition ratios, k' , used to calculate apparent film thicknesses.

The calculation of apparent film thicknesses presupposes a calibration of the adjusted partition ratios, k' , using columns coated with a known thickness of OV-1. This calibration was carried out using four columns of length 8–20 m, coated with OV-1 of 0.080–0.3 μ m film thickness, again injecting the test solutes as headspace at an oven temperature of 70°C. Mean k' values, extrapolated to a film thickness of 1 nm, are listed in Table I. The k' value determined for a solute on a retention gap is divided by the corresponding value in Table I to give an apparent film thickness in nanometres.

As an example, the injection of 10 μ l of *n*-tetradecane headspace on to a 50 m \times 0.30 mm I.D. glass capillary deactivated with DPTMDS gave a peak with a retention time of 154 sec (Fig. 2). The dead time of this capillary was 70 sec, hence $k' = 1.2$. The calibrated value for a 1 nm film thickness in Table I is 0.28. Hence the apparent film thickness in this tube was 4.3 nm.

TABLE I

ADJUSTED PARTITION RATIOS, k' , AT 70°C ON AN APOLAR STATIONARY PHASE (OV-1) EXTRAPOLATED TO A FILM THICKNESS OF 1 nm

Compound	k'	Compound	k'
<i>n</i> -Tridecane	0.13	Methyl octanoate	0.032
<i>n</i> -Tetradecane	0.28	Methyl decanoate	0.14
<i>n</i> -Pentadecane	0.58	Methyl dodecanoate	0.66
<i>n</i> -Hexadecane	1.17	1-Octanol	0.022
1-Ethyl naphthalene	0.20	1-Nonanol	0.047
		1-Decanol	0.11
		1-Undecanol	0.21

Determination of adsorptivity

Adsorptivity in the retention gap is determined qualitatively by examination of the peak shapes. Detection of irreversible adsorption, *e.g.*, by acid-base interactions, is not possible in this way.

Retention gaps are easily overloaded because of the absence of a film of liquid phase. Overloading results in a peak with a rapid up-slope and a slower return to the baseline (Fig. 1), and hence in peak asymmetry, being the mirror image of the overloaded peak eluted from a coated column. This distortion resembles that due to adsorption effects. However, the peak shape approaches a triangle rather than showing a broad tail. The shift in retention times due to overloading is not important.

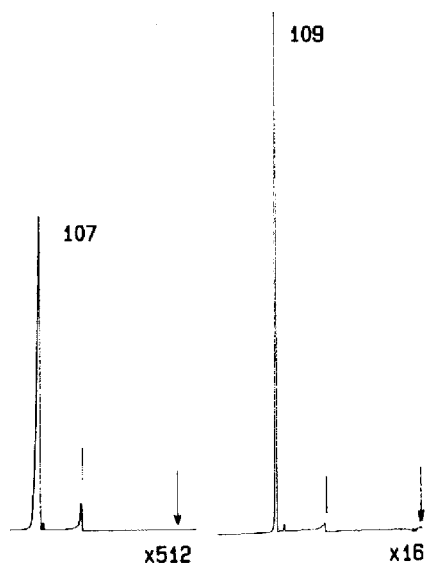


Fig. 1. Overloading effect on uncoated capillary tubes. 50 m \times 0.32 mm I.D. glass capillary deactivated with DPTMDS; *n*-tridecane as test solute. Note the different attenuations. Retention times indicated in seconds. The peak deformation by overloading does not correspond to that observed using coated columns and may be misinterpreted as adsorptivity.

RESULTS

Adsorptivity

Fig. 2 shows the test results obtained from two freshly prepared glass retention gaps (both $50\text{ m} \times 0.30\text{ mm I.D.}$), one silylated with HMDS and the other with DPTMDS. The differences in retention powers (k' values) are discussed below. The peak shapes are typical of retention gaps of this type: there is some overloading, despite the low attenuation ($\times 8$), and also some adsorption.

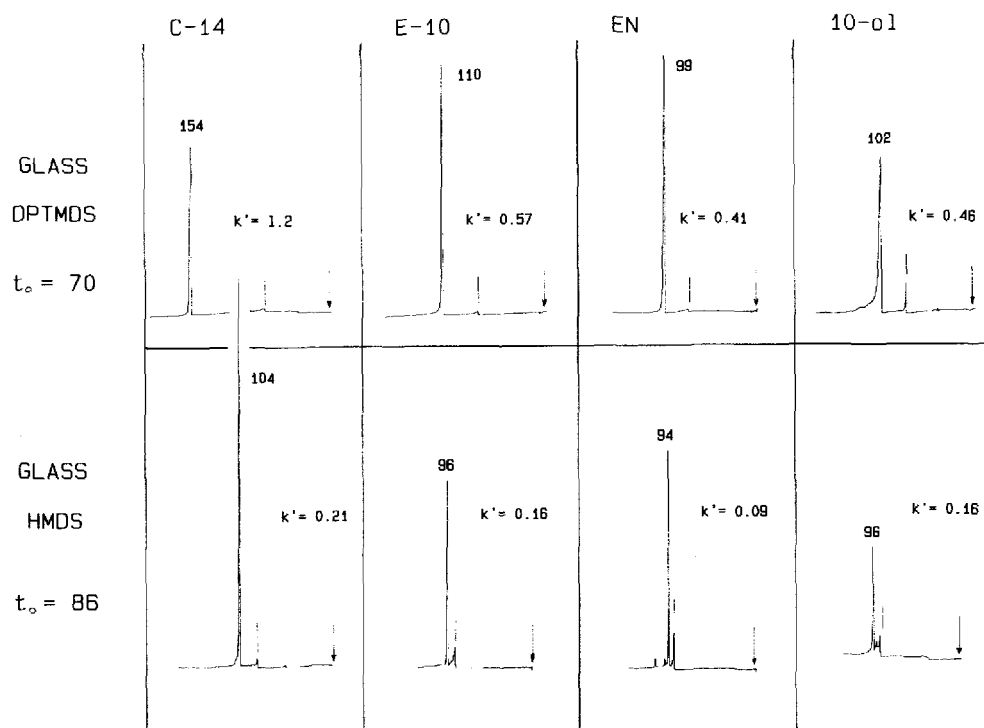


Fig. 2. Test results for two freshly prepared $50\text{ m} \times 0.30\text{ mm I.D.}$ glass capillaries deactivated with DPTMDS and HMDS. Test solutes: *n*-tetradecane (C-14), methyl decanoate (E-10), ethylnaphthalene (EN) and 1-decanol (10-ol). Retention times indicated in seconds.

Fig. 3 shows the test results obtained from a $50\text{ m} \times 0.32\text{ mm I.D.}$ fused-silica retention gap silylated with DPTMDS after extended use for coupled high-performance liquid chromatographic-gas chromatographic (HPLC-GC) applications. It was tested because peaks eluted from the separation column had started to tail, indicating additional band broadening and insufficient reconcentration by the retention gap. The test showed strong adsorptivity and a considerably increased retention power (determined by retention times measured for the top of the peaks). After rinsing with methanol and dichloromethane, the test results improved (Fig. 3) but were still far from the original excellent values. Parts of the retention gap are still in use for less demanding applications (2-5 m pieces, where the reconcentration power is less critical).

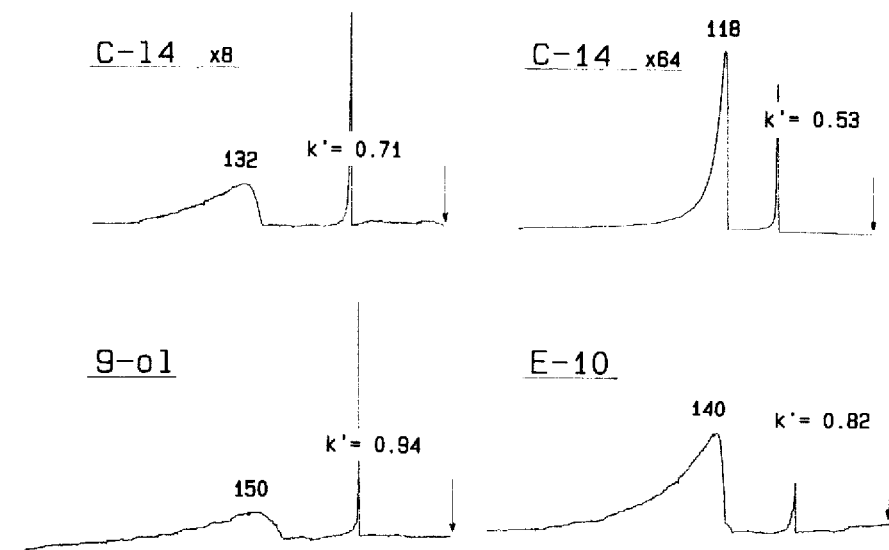


Fig. 3. Test of a 50 m \times 0.32 mm I.D. fused-silica retention gap deactivated with DPTMDS after intensive use and rinsing with solvent. Strong adsorption is due to contamination with non-volatile and non-extractable material. Note the dependence of adsorption on the amount of *n*-tetradecane injected.

It is interesting that the *n*-tetradecane peak was at least as strongly distorted as the methyl decanoate peak and nearly as much as that of 1-nonanol (all tests with attenuation \times 8). This suggests that a large proportion of the adsorptivity is not due to polar adsorptive sites but inhomogeneously distributed retaining dirt. On the other hand, the dependence of peak deformation on the amount of test solute injected still matches that of classical adsorption effects (see the peak of *n*-tetradecane at attenuation \times 64).

Retention power of various types of retention gaps

Table II lists the most interesting types of retention gaps and the retention powers determined by the described test. It allows the following conclusions to be

TABLE II

RETENTION POWERS (APPARENT FILM THICKNESSES) OF DIFFERENT TYPES OF RETENTION GAPS

Test solutes: *n*-C₁₄, *n*-tetradecane; EN, 1-ethylnaphthalene; E₁₀, methyl decanoate; 9-ol, 1-nonanol.

Column	<i>n</i> -C ₁₄	EN	E ₁₀	9-ol
Fused silica:				
HMDS	0.3	0.3	0.2	1.3
DPTMDS	1.1	0.5	1.8	2.2
Glass:				
Carbowax	4.1	7.6	7.2	27
HMDS	0.8	0.4	0.7	1.5
DPTMDS	3.1	1.4	2.3	3.1

drawn: (a) silylated fused-silica tubes show lower retention powers than analogously treated glass tubes, probably owing to the more intensive leaching of the glass prior to the silylation; (b) capillary tubes silylated with HMDS exhibit substantially lower retention powers than those deactivated by DPTMDS; and (c) the Carbowax-deactivated capillary (leached, dried and deactivated) has a strong retention power, particularly for polar solutes.

Influence of leaching temperature

Despite its higher retention power compared with fused silica, the glass pre-column is of great interest for use as a retention gap because of its very low cost. Samples containing considerable amounts of non-volatile by-products tend to "fill in" the retention gap, and often these dirt deposits cannot be removed by rinsing with solvent. An additional interest in the investigation of an optimized preparation of the glass retention gaps was the insight obtained into the deactivation procedures used for the preparation of coated columns.

Glass capillaries are leached with 20% hydrochloric acid at elevated temperature overnight¹¹. This leaching step extracts salts and opens up the silica structure. The depth of the leached layer depends on the leaching temperature. The resulting gel layer is stabilized by drying at 300–450°C under a gas flow.

The effect of the leaching temperature on the retention characteristics of the silylated glass tube is shown in Table III. The results for several members of a class of compound are given to indicate the deviations observed in practice. The results for the HMDS-deactivated tubes suggest that the increase in the leaching temperature from 120 to 140°C substantially decreases the polarity of the surface, effectively reducing the retention power for polar solutes, but hardly influencing that of apolar solutes. A leaching temperature of 140°C appears to be sufficient to remove the adsorptive material from the glass surface. The results obtained for the DPTMDS-treated surface show the same tendency. However, a leaching temperature of 160°C is clearly superior to one of 140°C. As the leaching temperature also affects the retention power for alkanes, effects other than adsorptivity appear to be involved.

TABLE III

APPARENT FILM THICKNESSES (nm) OF GLASS CAPILLARIES (0.30 mm I.D.) LEACHED AT VARIOUS TEMPERATURES AND DEACTIVATED BY SILYLATION AT 400°C

n-C₁₄ = *n*-tetradecane; *n*-C₁₅ = *n*-pentadecane; EN = 1-ethylnaphthalene; E₈ = methyl octanoate; E₁₀ = methyl decanoate; E₁₂ = methyl dodecanoate; 9-ol = 1-nonanol; 10-ol = 1-decanol.

Silylating reagent	Leaching temperature (°C)	<i>n</i> -C ₁₄	<i>n</i> -C ₁₅	EN	E ₈	E ₁₀	E ₁₂	9-ol	10-ol
HMDS	120	0.8	0.8	2.5	3.8	2.2	1.6	4.8	3.8
	140	0.6	0.5	0.5		0.8	0.5	2.2	1.3
	160	0.8		0.4	0.7	0.8	0.6	1.5	1.1
DPTMDS	120	6.5		2.4		8.0	4.5	4.8	5.4
	140		2.0		3.6	2.8	5.0	4.4	
	160	3.4	3.3	1.4		2.3	1.8	3.1	3.4

TABLE IV

APPARENT FILM THICKNESSES (nm) OF GLASS CAPILLARIES (50 m × 0.30 mm I.D.) LEACHED AT 140°C AND SILYLATED WITH HMDS or DPTMDS AT 300 AND 400°C

n-C₁₄ = *n*-tetradecane; EN = 1-ethylnaphthalene; E₁₂ = methyl dodecanoate; 10-ol = 1-decanol.

Silylating reagent	Silylation temperature (°C)	<i>n</i> -C ₁₄	EN	E ₁₂	10-ol
HMDS	300	0.6	0.3	0.4	0.8
	400	0.6	0.5	0.5	1.3
DPTMDS	300	5.1	1.9	2.8	4.1
	400	4.7	2.0	2.8	4.4

Silylation temperature

For silylation the capillaries were flushed with nitrogen, flame sealed and kept at 300 or 400°C for 14 h¹¹. As shown in Table IV, there is no important change in terms of retention characteristics whether the columns were silylated at 300 or 400°C. In particular, there is no support for the hypothesis that phenyl groups would have been eliminated if the DPTMDS-treated capillaries were heated at 400°C. The retention power for 1-ethylnaphthalene (which would be expected to be most sensitively affected by phenyl groups) remained virtually constant. Further, the two differently silylated columns remained as different whether silylated at 300 or 400°C.

Carbowax-deactivated capillaries

Sections of 8 m of a Duran glass capillary, leached at 160°C and dried overnight at 300°C, were flushed with solutions of Carbowax 1000 in dichloromethane and heated at 280°C for 1 h under a flow of hydrogen. They were again flushed with dichloromethane to remove extractable Carbowax, and heated at 250°C before testing.

Table V shows the influence of the Carbowax concentration on the retention characteristics of the deactivated capillaries. The results obtained with a length of capillary that had been treated in the same way but with no Carbowax in the flushing solution are not given. From this capillary only *n*-nonane (!) eluted as a broad, strong-

TABLE V

APPARENT FILM THICKNESSES (nm) IN CARBOWAX-DEACTIVATED GLASS CAPILLARIES AS A FUNCTION OF THE CARBOWAX CONCENTRATION IN THE DEACTIVATION SOLUTION

n-C₁₄ = *n*-tetradecane; EN = 1-ethylnaphthalene; E₁₀ = methyl decanoate; 9-ol = nonanol.

Carbowax concentration (%)	<i>n</i> -C ₁₄	EN	E ₁₀	9-ol
0.01	10	12	25	210
0.1	4.8	6.5	7.0	24
1.0	4.1	7.6	7.2	27
10	4.2	8.2	7.2	26

ly distorted peak. The treatment with 0.01% Carbowax solution still resulted in a strongly retaining capillary, not saturating the most active sites. On the other hand, deactivation solutions containing 0.1–10% of Carbowax 1000 produced columns with identical properties, which indicates that the 0.1% solution introduced a sufficient amount of Carbowax to saturate the active sites, and that any additional amount neither has a deactivation effect nor is fixed to the surface to remain in the capillary after flushing with solvent.

CONCLUSIONS

Complex systems call for methods for testing the performance of its parts, and the pre-columns used as retention gaps are no exception. Retention gaps must be tested in order to assess their quality when they are new, and also to facilitate the identification of the source of the problem if, *e.g.*, peaks are broadened or distorted.

The retention powers given are of help in selecting an appropriately deactivated pre-column for a particular application: (a) the DPTMDS-deactivated retention gap has excellent properties concerning wettability and thermostability and its retention power is reasonably low; (b) the HMDS-treated capillaries show substantially lower retention powers than all the others tested, but they are wetted only by alkanes and ethers as solvents. If the retention gap is used exclusively for solutions in these solvents, this is the best choice; and (c) Carbowax deactivation is simple and rapid to carry out in the laboratory and such pre-columns are cheap and attractive if dirty samples cause a high consumption of pre-column material. On the other hand, Carbowax-deactivated pre-columns are not optimally inert, have a relatively poor thermal stability and are not suitable if a high reconcentration factor is needed (if the flooded zone and the pre-column exceed a few metres in length).

The surface characteristics determined by the retention gap test give interesting information on the surface properties obtained by the deactivation procedures and on the procedures themselves. The data show that the Carbowax concentration in the deactivation solution is not critical, as all concentrations between 0.1 and 10% give the same results. The choice of the silylation reagent is more important than often thought. Surfaces treated with HMDS and DPTMDS behave differently, and the difference does not diminish if a higher silylation temperature (400°C) is used.

ACKNOWLEDGEMENTS

The glass retention gaps were prepared by G. and K. Grob and the fused-silica retention gaps by M. Galli (MEGA, Milan).

REFERENCES

- 1 K. Grob, Jr., *J. Chromatogr.*, 237 (1982) 15.
- 2 K. Grob, Jr., *J. Chromatogr.*, 287 (1984) 1.
- 3 K. Grob, Jr., *J. Chromatogr.*, 213 (1981) 3.
- 4 K. Grob, Jr., H. P. Neukom and M.-L. Riekkola, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 319.
- 5 K. Grob, Jr. and B. Schilling, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 531.
- 6 K. Grob, Jr. and K. Grob, *J. Chromatogr.*, 270 (1983) 17.

- 7 K. Grob, Jr., *J. Chromatogr.*, 328 (1985) in press.
- 8 G. Schomburg, H. Husmann and F. Weeke, *Chromatographia*, 10 (1977) 580.
- 9 K. Grob and G. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 302.
- 10 G. Rutten, A. van de Ven, J. de Haan, L. van de Ven and J. Rijks, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 607.
- 11 K. Grob, G. Grob, W. Blum and W. Walther, *J. Chromatogr.*, 244 (1982) 197.