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"BAND BROADENING IN SPACE" AND THE "RETENTION GAP" IN CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

Peaks may be broadened, distorted or even split if the sample components are spread out in the column inlet by large amounts of condensed solvent. This "band broadening in space" occurs during splitless injections with the solvent effect and cold on-column sampling. The features of the band broadening in space are summarized. Peak distortion is often found to be less pronounced than would be expected from the length of the flooded column section. It is assumed that this is due to partial transport of the solvent within the flooded zone by the vapour phase. Minor temperature gradients in the column inlet may greatly influence the peak distortion.

The "retention gap" is proposed as a method of eliminating peak distortion caused by band broadening in space. The retention in the column inlet (the approximate length of the flooded zone) is reduced to accelerate the migration of the spread sample components and to reconcentrate them at the beginning of the regular film of the stationary phase. The peak broadening is reduced by a factor corresponding to the reduction of the film thickness in the retention gap zone compared with the remainder of the column. For the routine analysis of free sterols it is shown that the retention gap completely eliminates the peak distortion and, at the same time, greatly reduces problems caused by by-products of the sample which disturb or degrade the stationary phase in the flooded zone of the column inlet.

INTRODUCTION

In a previous paper[1] we described the phenomenon of "band broadening in space". We also investigated its origin when it occurs associated with splitless injections with the solvent effect or cold on-column sampling. These two injection techniques create large amounts of condensed solvent in the column inlet and the thick layer of liquid is not stable. The rapid stream of carrier gas drives the liquid further into the column, flooding 20–60 cm of the column inlet. When the liquid is spread out as a thin, stable film, the expansion stops and the solvent evaporates. In splitless injection a considerable proportion and in cold on-column injection all of the sample material is dissolved in the flooding liquid. Hence it becomes spread out into the column as far as the flowing liquid reaches. The resulting bands are sufficiently broad

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to cause severe peak broadening at least for short to medium sized columns. Commonly peaks are not only broadened, but are also distorted or split into two maxima, which is due to the uneven distribution of the sample material over the flooded section of the column. We used the expression "band broadening in space" because the parameter that is common to the bands of all sample components is the geographical distribution in the column. This distinguishes it from the "band broadening in time" where the bands of the different components do not have the same local extension within the column. The latter bands expand by gas chromatographic migration during a fixed period of time, resulting in a geographically more extended band for a rapidly migrating substance than for a highly retained substance.

Typical features of the band broadening in space are as follows:

(1) Peaks eluting up to 40°C above the column temperature during the injection are not distorted. Distortion increases with increasing elution temperature for the range of compounds chromatographed within the following *ca.* 50°C and is the most drastic for solutes eluted at elevated column temperatures.

(2) For splitless injections only the peak is distorted which is related to the proportion of sample having reached the flooded zone. Various amounts of the sample components (depending on their volatility) are trapped in the warm, bottom part of the vaporizing injector and may elute as a separated, undistroted peak.

(3) The pattern of the peak distortion (the shape of the peak or its splitting) is very similar for all components of a sample. Above the critical temperature where band broadening in space becomes active, all peaks are affected.

(4) The broadening effect on the peaks as measured, *e.g.*, in millimetres on the chart paper is proportional to the retention of the peak. In isothermal runs the distortion of the peaks increases with retention; in temperature programming the peaks are more drastically affected if slow programming rates are applied.

(5) The effects of the band broadening in space are the more pronounced the shorter is the column, because the distortion of the peaks and the reduction of the separation efficiency are directly related to the proportion of the flooded column compared with the total length of the capillary.

(6) The distortion increases with increasing sample volume because the larger amount of liquid extends the flooded zone. In cold on-column sampling peaks are commonly broadened only when the sample volume is below about 0.8 μl. They are usually split for sample sizes exceeding 1 μl.

The decrease in the separation efficiency due to the band broadening in space may be massive. For a cold on-column injection of 1 μl of sample into a 15-m column, the separation efficiency may be reduced by a factor of about two in terms of resolution (determined, *e.g.*, as separation number, TZ) or by a factor of four in terms of theoretical plates.

OCCURRENCE OF BROADENING IN SPACE

Peak broadening is often less drastic than expected on the basis of the length of the capillary inlet section, which becomes wet during the injection. In splitless injection this may be due to the fact that the proportion of the sample components which are trapped in the bottom of the injector is so dominating that the broadened prepeak of the material spread in the flooded zone is hardly visible, which is a dangerous source of error for quantitative analyses.

In cold on-column sampling no peak broadening may be observed during a series of chromatograms, whereas under apparently identical conditions strongly distorted peaks may result another time. According to our experience, band broadening in space is seldom visible to its full extent if the solvent is pentane or hexane. On the other hand, it is always evident if the solvent is more polar such as acetone, diethyl ether or an alcohol. The fact that the sample components are often spread out less far than to the front of the zone with condensed solvent leads us to conclude that the transport of the solvent occurred at least partially through the vapour phase. If the liquid expands by evaporation and recondensation the sample components are not carried along, and hence their bands are not broadened. A minor proportion of transport through the vapour phase is sufficient to block the expansion of the sample components.

The proportion of the liquid transferred through the liquid and the vapour phase seems to depend on the evaporation energy of a solvent. Further, it depends on minor details of the conditions, which is also the reason for the often poor reproducibility of the extent and the pattern of the peak distortion. We noted that minor temperature gradients in the inlet section of the column are very important. The distortion was changed by turning the column into another position in the gas chromatographic (GC) oven, as illustrated in Fig. 1. The same mixture of *n*-alkanes

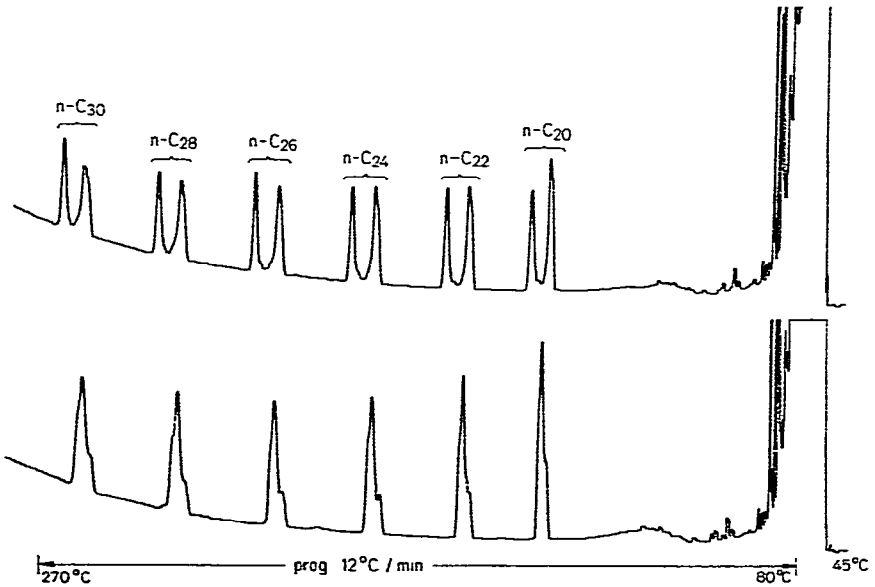

Fig. 1. Comparison of the distortion pattern of peaks in two chromatograms run under identical conditions except for the position of the column inlet in the GC oven. Volumes of 2 μl of the even-carbon C_{20}–C_{30} *n*-alkanes diluted 1:50,000 in acetone; cold on-column injection at 45°C into a 5 m × 0.30 mm I.D. glass capillary coated with 0.6 μm of OV-73 with a fully coated inlet section; temperature programme, 10°C/min from 80 to 270°C. For the upper chromatogram the column turned from the injector towards the centre, and for the lower chromatogram towards the corner of the GC oven. The distortion pattern shows that the alkanes were distributed differently within the flooded zone. The materials were located at the ends of the flooded zone in the upper chromatogram and remained together in the lower chromatogram. We assume that these differences are due to different proportions of solvent transported through the vapour phase.

dissolved in acetone was injected twice. All analytical conditions were kept constant except that the column was turned by about 60° about the straightened inlet section. Instead of going from the injector towards a corner, the column turned towards the centre of the oven. The distribution of the sample material within the flooded zone changed completely. In one instance the material was split into two portions located near the ends of the flooded zone. In the other position of the column the materials remained together, thus producing a single, although distorted, peak.

We assume that the phenomenon of band broadening in space may not only be observed as a result of the flooding solvent as described up to now. We expect that certain types of peak tailing behave in the same way and that some undesirable solvent effects producing distorted peaks might be better understood when relating them to this kind of band broadening. However, these subjects need to be explored further.

THE RETENTION GAP

Band broadening in space cannot be corrected by the conventional reconcentration techniques used to eliminate the effects of the band broadening in time, *i.e.*, cold trapping and the solvent effect[2]. Band broadening in space is in fact even an undesirable side-effect of the solvent effect. The reconcentration of the bands resulting from band broadening in time requires a temporary increase in the retention in the column inlet. However, GC retention does not hinder the sample spreading out in the liquid phase.

The concept of the reconcentration of the bands broadened in space calls for the opposite: instead of an increase in the retention it requires a retention gap, *i.e.*, an inlet section of the column with a negligible retention compared with the separating major part of the column. The retention gap accelerates the migration of the sample components and allows them to be reconcentrated on the beginning of the regular film of the stationary phase. The length of the retention gap zone has to include the complete flooded zone. Hence about 60 cm should be sufficient for sample volumes up to 1.5 μl.

The mechanism of reconcentration by the retention gap may be described differently for temperature-programmed and isothermal runs. Both explanations have their benefits. They might appear to be different just because of the simplifications involved.

Assuming that a wash of the column inlet extracted 90% of the stationary phase, the sample components migrate through this section of the capillary at a temperature decreased by about 50°C. In temperature-programmed runs the components move at relatively low temperature through the column inlet as far as to the beginning of the regular coating of the stationary phase, where they are focused by the increasing retention. As they have to "wait" there, the front and rear ends of the broadened band nearly meet each other. The components wait until the column temperature is sufficiently increased to allow their chromatography.

In isothermal GC a reduced retention in the column inlet increases the speed of the migration of the sample components. The acceleration is approximately proportional to the reduction of the film thickness of the stationary phase (phase ratio). Thus a 90% extraction of the stationary phase accelerates chromatography by a factor of

ten. As a consequence, the sample arrives at the beginning of the regular film within a time shortened by a factor of ten, *i.e.*, the original width of the band is reduced by 90%. Alternatively, looking at the front and rear ends of the band, the front of the band migrates on the regular coating by a tenth of the speed of the rear end until the latter also reaches the regular film. Thus the rear end is able to reduce its delay to the front by 90%.

The retention gap does not completely eliminate the effects of the band broadening in space because "retention gap" never means "zero retention". However, as in most instances it is easy to reduce the retention in the column inlet by a large factor, the peak broadening rapidly becomes negligible.

Fig. 2 and Table I show some experimental data on the depth of the retention gap required to render peak distortion negligible. We used a capillary with an extremely thick coating (2 μm of OV-73) and varied the retention of its inlet section. First, the column was 7 m long and coated throughout the inlet (end-section straightened electrically under nitrogen[3]). Then 1 m of the column was replaced with 1-m pieces of other columns with a known, lower film thickness, reducing the film thickness stepwise by factors of two. The stationary phase of all columns was immobilized[4] to prevent phase stripping. The effects on peak broadening were determined by cold on-column injections of 1.5 μl of the even-carbon C_{20}–C_{30} *n*-alkanes diluted 1:50,000 in acetone. The chromatogram on the bottom of Fig. 2 shows the full effect of the band broadening in space as obtained by the column inlet coated with the 2 μm film. The peaks are split and broadened by a factor exceeding four. The chromatogram next to the bottom was obtained from the 6-m column with a 2-μm coating attached to a 1-m inlet section with a 1-μm film (using shrinkable PTFE tubing). Peak splitting and broadening were strongly reduced but were still severe. The next chromatogram was obtained from the configuration with an inlet section of a quarter of the film thickness of the main column. Peak splitting disappeared, but peaks were still noticeably distorted and broadened. The top chromatogram was made on a column attached to a 1-m persilanized but uncoated inlet section. In this chromatogram the peaks were no longer distorted and their width corresponded to a chromatogram made by a split injection.

Table I lists the peak widths at half-height obtained from chromatograms as shown in Fig. 2 (although the widths at half-heigth have to be considered as rough approximations when peaks are distorted or even split). The broadening effect due to band broadening in space was calculated by subtraction of the peak width of the capillary with the uncoated pre-column from the peak widths of the columns with coated inlets. The data show that the broadening effect is halved when the thickness of the coating of the pre-column is halved. This is in agreement with the mechanism of the retention gap as described above; the sample components are accelerated by the same proportion as the film thickness (phase ratio) of the inlet section is reduced. If the two maxima of the split peak reach the beginning of the stationary phase in half the time, the distance between the two maxima is also halved.

The data in Table I are based on moderately extreme conditions; the column was short (6 m) and the sample volume was fairly large (1.5 μl), using acetone, which provides the virtually complete realization of the broadening effect. Under these still realistic conditions a decrease in the film thickness in the retention gap area by a factor of ten still gave considerable peak broadening (a decrease in separation ef-

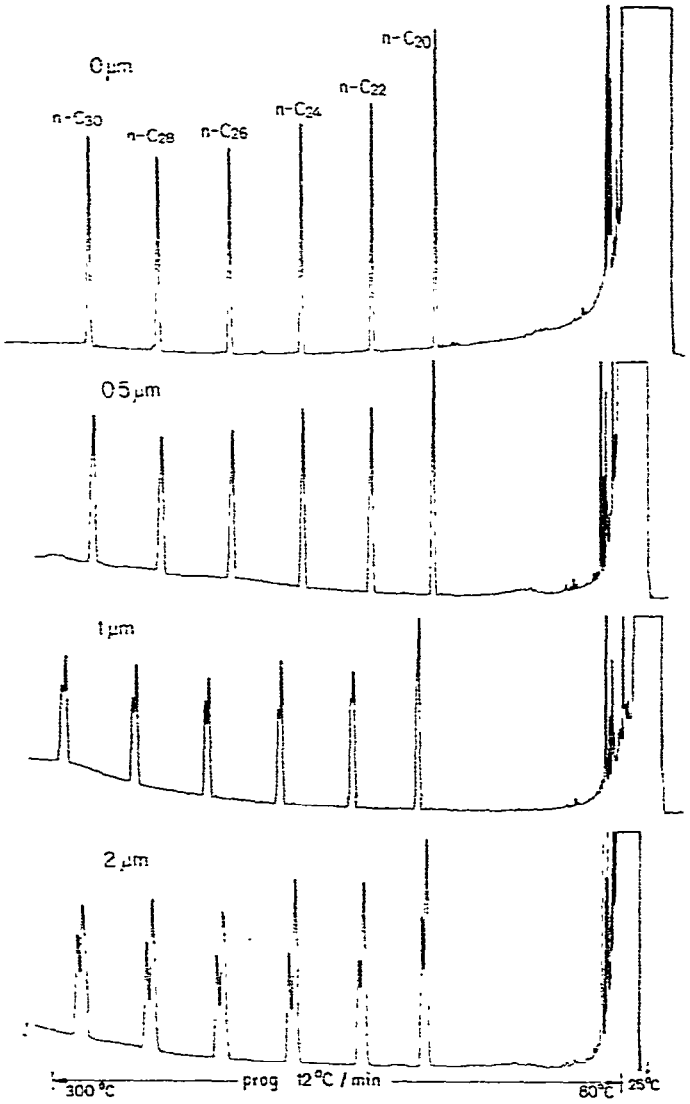

Fig. 2. Effect of the film thickness in the column inlet on the peak distortion by the band broadening in space. The bottom chromatogram was made on a 7-m column coated with 2 µm of OV-73. For the chromatograms above, 1 m of the column inlet was replaced with 1 m of column coated with only 1 and 0.5 µm of the same stationary phase. For the top chromatogram the column inlet consisted of persilanized but uncoated tubing. Volumes of 1.5 µl of the indicated *n*-alkanes, diluted 1:50,000 in acetone, were injected with an open oven door by cold on-column injection; 0.1 atm of hydrogen as carrier gas; temperature programme 12°C/min from 80 to 300°C, started 20 sec after the injection. The broadening effect is halved for each reduction of the film thickness in the column inlet by a factor of two. The top chromatogram with the uncoated pre-column gave the same peak width as a split injection. Peak widths and broadening effects, listed in Table I, indicate that a retention gap to render peak distortion negligible requires a reduction of the film thickness in the retention gap zone by a factor of 50–100. Under conditions creating less distortion by the band broadening in space, a smaller reduction is sufficient.

TABLE I

PEAK WIDTHS AT HALF-HEIGHT AND BROADENING EFFECTS FOR C_{22}, C_{26} and C_{30} *n*-ALKANES WHEN 2 μl OF THEIR ACETONE SOLUTION WERE INJECTED INTO A 6-m COLUMN COATED WITH 2 μm OF OV-73 WITH ATTACHED 1-m INLET SECTIONS COATED WITH VARIOUS FILM THICKNESSES

Film thickness of inlet (μm)	*Peak width (mm)*			*Broadening effect (mm)*		
	n-C_{22}	*n*-C_{26}	*n*-C_{30}	*n*-C_{22}	*n*-C_{26}	*n*-C_{30}
2	4.8	5.0	5.2	3.2	3.8	4.0
1	2.4	2.8	3.2	1.4	1.6	2.0
0.5	1.8	2.2	2.3	0.8	1.0	1.1
0.25	1.3	1.7	1.8	0.3	0.5	0.6
0.08	1.1	1.2	1.3	0.1	0	0.1
0	1.0	1.2	1.2	–	–	–

ficiency in terms of separation number, TZ, of about 40%). A retention gap to reduce the peak broadening to a negligible level required a reduction in the retention by a factor of 50–100. However, for longer columns and smaller sample volumes a reduction in the retention by a factor of 10 is sufficient.

The model considering the mechanism of the retention gap in isothermal runs is useful as it allows correct predictions. However, if the chromatogram is run isothermally at the column temperature during the injection, band broadening in space does not occur. The conditions creating broadened and distorted peaks always involve steps which resemble temperature programming. For an analysis above the boiling point of the solvent, the column temperature during sampling must be decreased. As soon as the sampling process is finished, the column temperature is rapidly increased to the temperature of the analysis. Thus even isothermal analyses include in fact a (controlled or uncontrolled) step of temperature programming.

It may be objected that an uncoated section of the column behaves as a dead volume because it allows diffusion processes to broaden the bands, but it does not contribute to the separation. However, this holds true in only a single case: if the column is used isothermally at the temperature during the injection and if the sample is introduced by a split injection. Even in this instance, the broadening is small because the residence time of the sample in this column section is short. As the components are virtually not retained, the residence time is just a fraction of the dead time of the column which is equal to the proportion of the retention gap on the total length of the column. For a retention gap of 1-m length in a 10-m column and for a component eluted with a capacity factor $k = 5$, the peak is estimated to be broadened by 2%. Under the usual working conditions it is even less. As soon as temperature programming is involved, the re-concentration on the beginning of the regular film of the stationary phase, eliminates the broadening effect. In splitless or cold on-column injection the broadening effect is reduced by the solvent effect if working isothermally or again is completely eliminated as soon as a substantial temperature increase after the injection is involved.

Fig. 3 gives an example of a routine analysis where the retention gap is a prerequisite for reasonable chromatography. Sterols, *e.g.*, from fats, margarines or

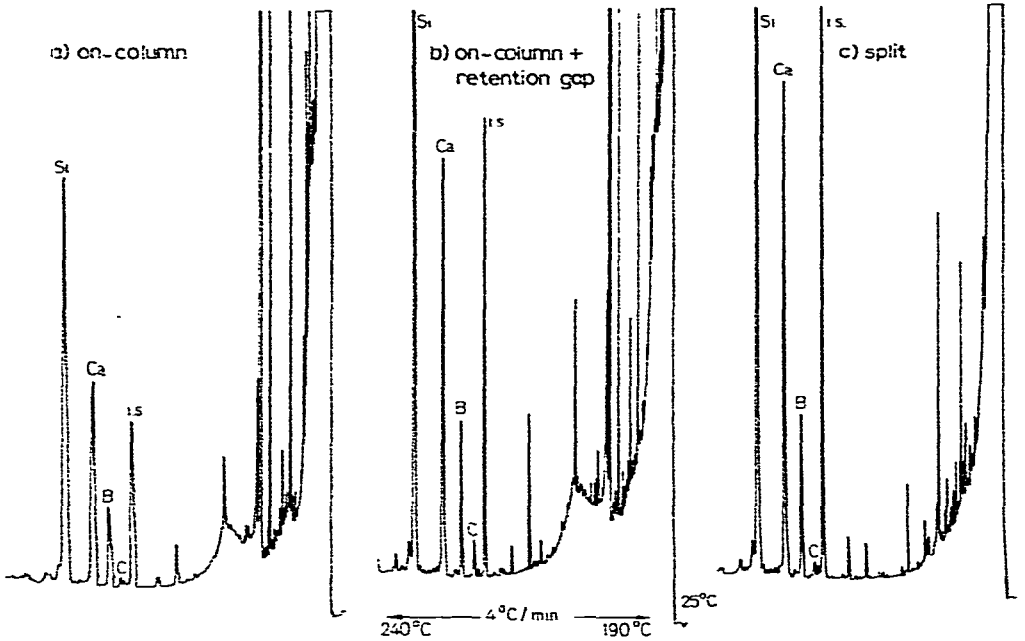

Fig. 3. Analysis of free sterols (from rapeseed oil) as an application of the retention gap. (a) Cold on-column injection of 0.7 μl of an ethereal solution at 25°C; 15 m × 0.32 mm I.D. glass capillary, coated with 0.15 μm of OV-73 throughout the column; 0.3 atm of hydrogen as carrier gas; temperature programme 4°C/min from 190 to 240°C. Peaks: i.s. = internal standard (*n*-triacontane); C = cholesterol; B = brassicasterol; Ca = campesterol; Si = sitosterol. All peaks are distorted by the band broadening in space. (b) As (a) but a 60-cm length of a persilanized, uncoated capillary was linked to the column inlet to serve as a retention gap. Peak widths are now the same as in (c) for a vaporizing split injection. Cold on-column sampling is preferable to split sampling because of the accuracy of the results.

similar foodstuffs, are easily analysed in their underivatized form on a moderately well deactivated capillary column. For several applications an accuracy of the results in the range 2–5% is required. The large amount of the material available would allow a vaporizing split injection, the most rapid and most convenient injection technique for this application. However, unless the conditions are well optimized and quantitation is based on carefully designed calibration procedures, the required accuracy is not guaranteed. The sterols enter the column by a smaller proportion than the internal standard (an *n*-alkane), and this proportion is difficult to reproduce. For split injection the use of the alkali-stable coprostanol as an internal standard is preferable, but it still gives high deviations. Accuracy is no problem when using cold on-column sampling. However, as chromatogram A in Fig. 3 shows, the band broadening in space requires attention. This chromatogram was obtained by an injection of 0.7 μl of an ethereal solution of the unsaponifiable material of rapeseed oil on a 15 m × 0.30 mm I.D. glass capillary coated with 0.15 μm of OV-73. Thus conditions did not favour band broadening in space as the sample volume was relatively small and for many purposes a third of the column length would have done the separation just as well.

The use of a 60-cm long persilanized and uncoated pre-column (connected by shrinkable PTFE tubing) solves two problems with this analysis simultaneously. It

serves as a retention gap, completely eliminating the peak broadening effects as shown by the comparison of chromatograms B and C, representing cold on-column injection with a retention gap and vaporizing split injection, respectively. Second, the empty pre-column greatly reduces the problems with the by-products extracted from the strongly alkaline aqueous solution. In routine practice we neither wash the ethereal solution intensively to remove the alkali, nor do we dry it. In particular, basic salts rapidly destroy the silicone phases. As alkaline salts are not volatile, they are deposited exclusively in the flooded inlet section on the column. The absence of stationary phase in this section of the column prevents the resulting bleeding and peak tailing due to accumulated degradation products.

CONCLUSIONS

Band broadening in space hinders analyses of certain injection techniques as long as it is not recognized and understood. However, the introduction of a retention gap by keeping the column inlet free from stationary phase is so easy that band broadening in space cannot be considered a problem.

We have recommended the use of capillary inlets free from stationary phase for many years, although for another reason. Most problems with capillary columns during their use are due to deficiencies in the inlet sections. The replacement of the inlet section, either by breaking it off or by replacing a pre-column, renovates most used columns[5,6]. However, the absence of stationary phase in the inlet section avoids these problems to a large extent. Hence the use of columns with an empty inlet section now has another advantage due to the retention gap.

The retention gap may be introduced in many ways, but in to our experience most of them have limitations. These technical aspects will be discussed in a forthcoming paper.

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