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EXPERIMENTS WITH COLD ON-COLUMN INJECTION

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SUMMARY

Several parameters influence the peak distortion and peak splitting that can occur with cold on-column injection in capillary gas chromatography. Differences for non-polar and polar stationary phases, when non-polar and polar solvents are injected, illustrate this. A possible explanation of the phenomena is given.

INTRODUCTION

The problems encountered with cold on-column injection when large samples (>2 μl) of non-polar solvents are injected or even when small samples (<1 μl) of polar solvents such as methanol are injected on non-polar polysiloxane columns are well known[1–5]. Peak distortion and peak splitting then make qualitative and quantitative analysis impossible. To avoid this "peak broadening-in-space" effect, a retention gap, *i.e.*, a capillary inlet free of stationary phase has been proposed[2,3]. This solves most of the problems encountered in the routine practice of capillary gas chromatography (GC) with cold on-column injection. More particularly, a retention gap is needed when polar solvents such as methanol are injected into non-polar columns. In the course of experiments with normal and immobilized polyethylene glycol (Superox 20M) films, we observed that neither non-polar nor polar solvents produced peak distortion. Therefore, peak distortion effects of cold on-column injection are not as general as are currently believed. We therefore decided to study this phenomenon. This paper describes observations on the injection of apolar and polar solvents into columns with non-polar stationary phases of the polydimethylsiloxane type and into columns with polar phases of the polyethylene glycol type.

EXPERIMENTAL

Observations on non-polar columns of the polydimethylsiloxane type

A 1-μl volume of a solution of the usual polarity mixture in *n*-hexane or dichloromethane injected on-column into 20 m $\times$ 0.3 mm I.D. columns coated with

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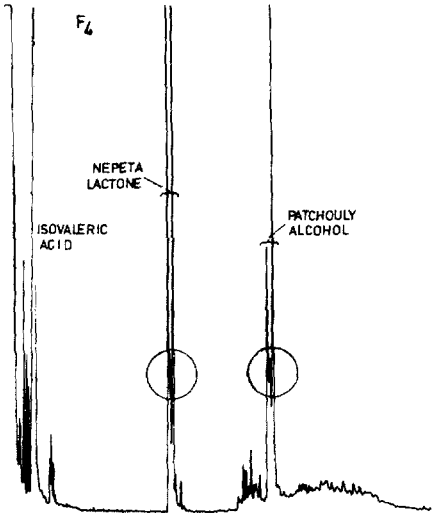


Fig. 1. Chromatogram of fraction of the essential oil of *Valeriana celtica* L. Solvent: methanol; 1 μl injected on-column.

OV-1, SE-52 or SE-54 (immobilized or not) does not produce noticeable peak distortion. When the same amount of a methanol solution is injected, the compounds are eluted as split-distorted peaks. The degree of distortion increases with increasing retention, *e.g.*, 2,3-butanediol shows a perfect peak shape whereas the esters are broadened and split.

Until recently, we could avoid this problem mostly by selecting an appropriate solvent compatible with on-column injection. When utilizing the direct introduction of 1–2 μl of methanol solutions from a high-performance liquid chromatographic (HPLC) analysis, peak splitting was very troublesome. The analysis of a fraction collected from a step-elution HPLC analysis of the essential oil of *Valeriana celtica* L. with *n*-hexane and methanol illustrates the problem. The fraction of the polar compounds was contained 1 ml of *n*-hexane and 6 ml of methanol in a two-phase system. Both phases were injected on-column (1 μl) onto a 20 m × 0.3 mm I.D. HTS-OV-1 glass capillary column with a film thickness of 0.3 μm. Straightening of the column was performed under nitrogen.

Fig. 1 shows the chromatogram of the methanol layer and Fig. 2 that of the *n*-hexane layer. It is obvious that methanol causes peak splitting but *n*-hexane does not. Connecting a retention gap of 1 m × 0.3 mm of high-temperature silylated and uncoated fused-silica tubing to the main column resulted, for methanol injections, in the same peak shapes as obtained in Fig. 2. When the experiments were repeated on a 20 m × 0.3 mm I.D. immobilized SE-54 fused-silica column without a retention gap, there was less peak splitting than with the glass capillary column (see Fig. 1).

The reason for this was not clear until we examined the first 10 cm of the glass column. During straightening, the column inlet was overheated, creating irregularities on the surface. This roughening thus caused more peak distortion than that obtained on the smooth fused-silica surface. This effect was emphasized by overheating the inlet section (15 cm) of a water- and hydrochloric acid–glass column (see Fig. 3).

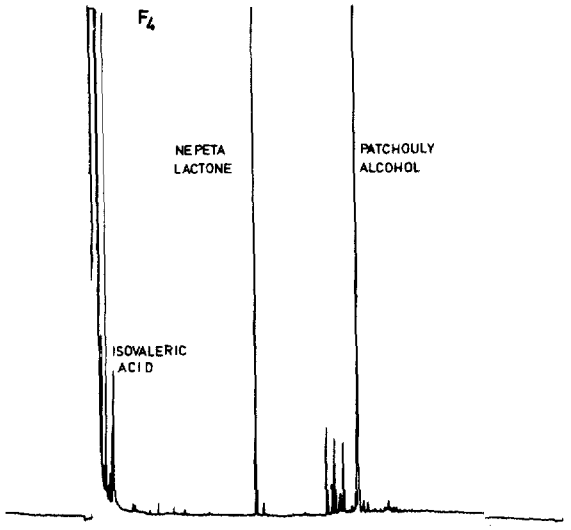


Fig. 2. Chromatogram of fraction of the essential oil of *Valeriana celtica* L. Solvent: *n*-hexane; 1 μl injected on-column.

Hydrocarbons, even dissolved in *n*-hexane, now showed severe splitting, comparable to the peak splitting in Fig. 1, even when only 1-μl samples were injected.

Probably, owing to the very intense roughening, the flooding zone for *n*-hexane was not homogenous. Solvent droplet formation occurred in the inlet zone, comparable to the methanol droplet formation on non-polar polydimethylsiloxane films. When polar solvents are injected into non-polar columns, the use of a retention gap is therefore necessary.

Large sample sizes of non-polar solvents on non-polar columns also cause peak broadening and even peak splitting. These phenomena have been discussed by Grob[1–3], Knauss *et al.*[5], Trestianu[6], Munari *et al.*[7] and Wang *et al.*[8]. When large sample sizes are injected, a retention gap of 1 m is no longer sufficient and, therefore, it must be adapted to the problem at hand. The approach proposed by Wang *et al.*[8] for avoiding peak broadening by, *e.g.*, injecting large sample sizes above the boiling point of the solvent, drastically improves the peak shape of the compounds, but

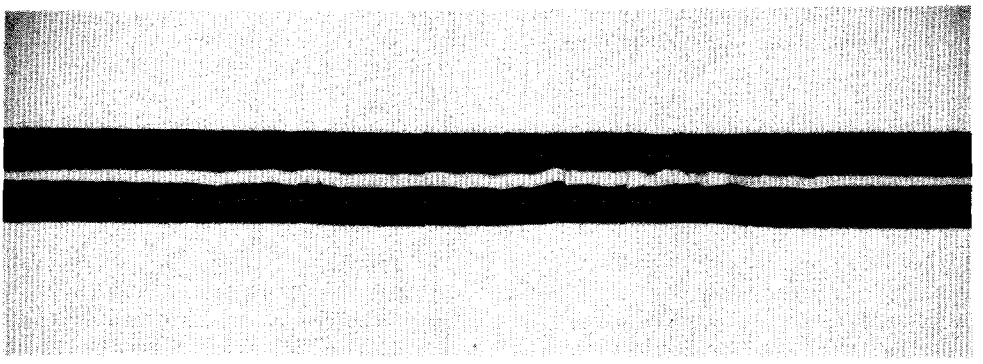


Fig. 3. Overheated column inlet.

compared with cold on-column injection discrimination also drastically increases. Injections at high temperature lead to a short flooding zone, as vaporization occurs immediately.

Construction and connection of a retention gap

The simplest way to obtain a retention gap is to wash out the column over the length needed (50–60 cm per μl injected). Washing must be carried out after coating and before conditioning or immobilization. This technique can only be applied by the limited number of chromatographers who are making their own columns. Commercial columns are mostly pre-conditioned and, at present, cross-linked or immobilized, so that the stationary phase film can no longer be removed by washing. The proposal of Grob[3] that manufacturers should produce columns with a retention gap of 3 m is therefore sound. However, it is doubtful that this will be realized in the near future. The connection of a pre-column retention gap to the separation column is a more realistic approach. This offers the advantage that when the column inlet, *i.e.*, the retention gap, is contaminated, replacement of the retention gap is feasible. Because of the problems in straightening glass columns, a retention gap must be made from fused-silica tubing. In our hands, the best and easiest way to connect the retention gap to the capillary column is by means of a polyimide seal[9]. The response we received from chromatographers applying this technique was very positive, although some experience is needed to master this technique. The methods applied for glass–fused-silica and fused-silica–fused-silica connections are detailed below.

Glass–fused-silica connections

The glass capillary inlet (0.3 mm I.D.) is conically enlarged with a diamond drill or a heated tungsten point to produce a housing for the fused-silica retention gap. The capillary column and retention gap are placed in a device installed in the GC oven (CE 4160) and connected to an arm of the capillary holder (Fig. 4). The device consists of a T-piece with the perpendicular arms on top. The upper arm bears a fitting with a 0.8-mm bore. The fused-silica retention gap, connected on one side to the on-column injector, is introduced in the fitting. Both columns are coupled and the fused-silica tubing is fixed by means of a GC septum in the fitting nut.

A small drop of polyimide prepolymer (PI-2550; DuPont) is slightly heated on the top of a spatula, and a thin layer is applied to the connection point. Polymerization is carried out at 110°C for 20 min. Two or three additional layers of polyimide are applied in the same manner.

A slow flow of carrier gas can be introduced into the column during polymerization, although this is not necessary for non-polar coatings.

Fused-silica–fused-silica connections

A small polyimide cylinder (8 × 3 mm) is drilled with a 0.45-mm bore for 0.32-mm columns and with a 0.35-mm bore for 0.25-mm columns (see Fig. 5). The fused-silica columns just fit into the bores. Four millimetres of a fused-silica retention gap are introduced into the cylinder. A small drop of polyimide is applied to the connection and the device is heated at 110°C for 20 min. The main fused-silica capillary is then introduced into the cylinder at the other side and the procedure is repeated. In this way, a zero-dead-volume connection is obtained. We have not ex-

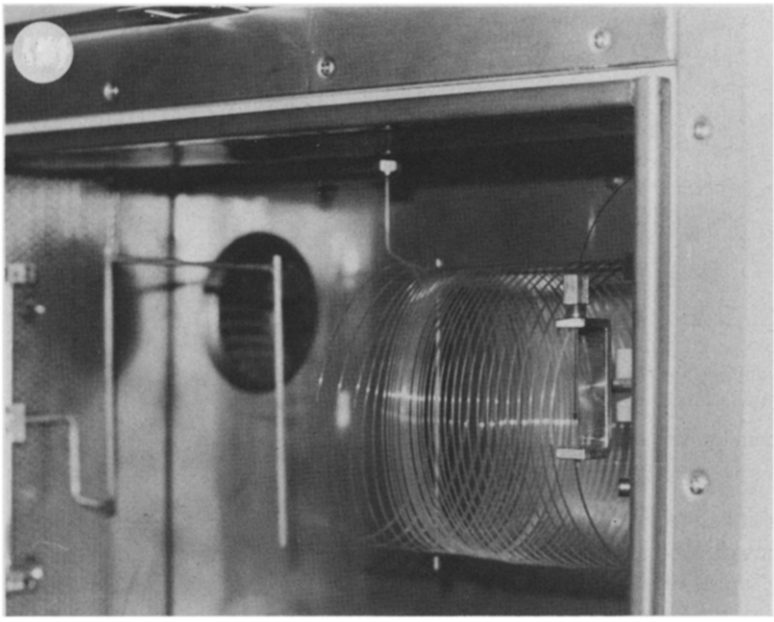


Fig. 4. Device for glass–fused-silica connections.

perienced any effect of the polyimide on the chromatographic behaviour of the main column.

Observations with polar columns of the PEG type

Chromatography of 1 μl of *n*-hexane and methanol solutions of the polarity mixture on Superox 20M capillary columns did not show peak distortion. This is not decisive because on injecting methanol, the stationary phase could have been stripped over a distance of several decimetres, creating a retention gap in the inlet section. Therefore, further experiments were carried out on immobilized Superox 20M films. Several sample sizes of *n*-hexane and methanol solutions of *n*-tetradecane, nonanol-1 and the methyl esters of decanoic, undecanoic and dodecanoic acid were injected into a 20 m × 0.3 mm I.D. glass capillary column, coated with 0.12 μm of immobilized Superox 20M[10]. For both methanol and *n*-hexane, injection peak splitting phenomena were absent. Figs. 6–8 show the influence of the sample size on the "Trennzahl" (*TZ*) values calculated for the methyl esters of undecanoic acid and dodecanoic acid. The parameters modified were the column temperature during injection and the injection rate (fast and slow injection). The *TZ* value for split injection of 1 μl of a methanol solution of the two esters was 19.5. The *TZ* values for on-column injections of 1 μl methanol and *n*-hexane were approximately 5% lower. The *TZ* values rapidly

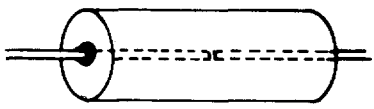
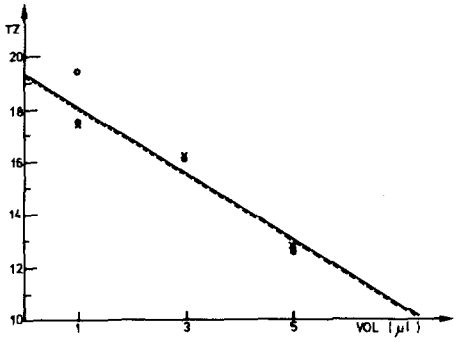


Fig. 5. Fused-silica–fused-silica connection.



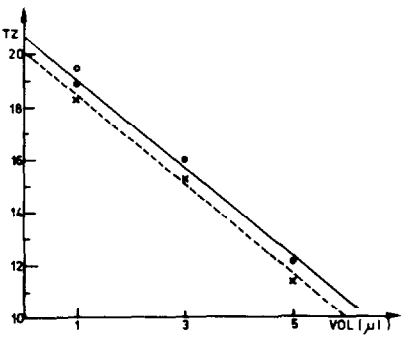


Fig. 6. *TZ versus* amount injected for slow injection: 1 µl, 2 sec; 3 µl, 8 sec; 5 µl, 15 sec. Column (Superox 20M CL) at room temperature. ○, Split; ×, on-column methanol, ●, on-column *n*-hexane.

Fig. 7. *TZ versus* amount injected for fast injection (1 sec). Column (Superox 20M CL) at room temperature.

decreased with increasing sample sizes (the increase of the flooding zone accounts for this), but the slopes of the curves for methanol or *n*-hexane solutions were equal. In general, lower *TZ* values were found for methanol solutions. As observed visually, the length of the flooding zone for methanol injections was 1.5–2 times the length of the flooding zone for *n*-hexane solutions, and this can explain the small decrease.

The same experiments were repeated with a fused-silica column, coated with 0.35 µm of immobilized Superox 20M. The decrease in *TZ* value on injecting 1 and 5 µl of methanol solutions was only 5 *TZ* units, compared with the loss of 8 *TZ* units on the thin-film column. By fixation of methanol on the thick layer of the polar stationary phase, the flooding zone is probably shortened, resulting in less broadening on injection of large samples.

Also, the analysis of essential oil fractions dissolved in *n*-hexane or methanol did not show any peak distortion or peak splitting. The chromatographic pattern was exactly the same. There is only a small decrease in efficiency compared with split injection on injecting 1-µl amounts on-column. This decrease in resolution is compensated for by the important advantages of on-column injection, *e.g.*, low discrimination, excellent quantitative reproducibility and minimal thermal degradation of labile compounds.

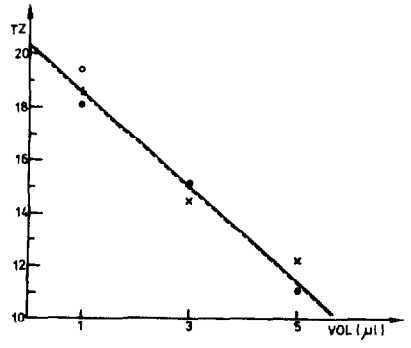


Fig. 8. *TZ versus* amount injected for fast injection. Column (Superox 20M CL) at 65°C.

During the investigations with polar columns, we have never observed peak splitting, as occurs on non-polar columns. On non-polar columns injections of 1 μl of methanol solutions resulted in halving of the TZ value compared with n-hexane solutions.

Therefore, we must conclude that the nature of the stationary phase is an important parameter in on-column injection phenomena.

Possible explanation of the phenomena

Peak deformations can have many causes, most of which are well understood. Tailing can be due to dead volumes in the chromatographic systems. Leading and severe tailing are caused by the incompatibility or polarity difference of the analytes and the stationary phase. Double peaks and shoulders can arise through wall defects or double injections. The distortion and splitting phenomena that can occur after cold on-column injection are, however, a recent development. The explanations so far advanced in the literature do not appear very convincing to us. Our ideas on the subject are as follows. A liquid plug, injected into a capillary column, is immediately pushed forward in liquid form by the carrier gas and at the same time starts to evaporate. Evaporation is determined by the amount injected, boiling point of the solvent, column temperature and heat of vaporization of the solvent. The last parameter is much higher for polar than non-polar solvents. Polar solvents therefore vaporise much more slowly. After cold on-column injection of large samples in a transparant glass capillary column, liquid can actually be seen to travel through several column coils before the liquid becomes invisible. The liquid partly vaporises, but is also taken up by the stationary film, which therefore becomes temporarily much thicker. This liquid travels through the column as in normal chromatography by evaporation and recondensation.

From the above reasoning we draw the conclusion that with cold on-column injection the mixture is deposited over a longer column section than with other injection techniques. This causes some band broadening, more for a polar solvent such as methanol than for a non-polar solvent such as n-hexane. The flooded column length is indeed longer after methanol than after n-hexane injection. However, this band broadening has nothing to do with peak distortion or peak splitting.

On injection, a liquid plug is introduced into the column. This plug is forced open by the carrier gas. If this situation prevails for the duration of the chromatographic experiment there is no peak distortion or splitting. However, the slightest uneveness in the column wall (bend, wall irregularity, speck of dust, etc.) can give rise to the temporary appearance of a solvent lens or column obstruction. If this phenomenon occurs outside an advancing chromatographic band, a normal peak will result; if it occurs in a band, a split peak or severe band broadening will occur. If several such obstructions appear under a band, multiple peaks can result. For discrete, virtually normal chromatographic peaks to appear, the obstruction should last only a very short time. This, then is, the peak splitting phenomenon. If the obstruction lasts for some time, or if it is not an off–on phenomenon but rather a more lasting occurrence, severe peak broadening will follow. It is essential that the temporary obstruction or lens formation occurs while the band is passing and ceases to exist before the band has passed completely, otherwise no splitting can occur. Splitting or the appearance of two peaks for a single compound can only be explained

by a phenomenon that occurs in the band and retards that portion of the band which has not yet passed the disturbance in the column. This explanation agrees with the observation that distortion–splitting is favoured by straightening a piece of the column. Such straightening must introduce wall irregularities. The above discussion also agrees with the fact that peak distortion–splitting is favoured by larger sample volumes and by polar solvents, which evaporate more slowly. That polar solvents do not lead to peak distortion–splitting on polar phases can also be understood by the better compatibility of the two substances. This leads to slower evaporation of the solvent from the stationary phase and less chance of lens formation.

Another conclusion is that the inner diameter of the columns is an important parameter, smaller diameters leading more easily to disturbances. However, peak distortion and splitting are a very complex subject and more experiments need to be carried out for a complete understanding of this phenomenon.

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