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HIGH OVEN TEMPERATURE ON-COLUMN INJECTION IN CAPILLARY GAS CHROMATOGRAPHY

I. SAMPLE INTRODUCTION

K. GROB, Jr.* and T. LÄUBLI
Kantonales Labor, P.O. Box, CH-8030 Zürich (Switzerland)
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SUMMARY

High oven temperature on-column injection, *i.e.*, on-column injection at column temperatures well above the boiling point of the solvent, is highly attractive for rapid analysis at elevated temperatures. Two concepts are described, involving the use of a temporarily cooled column inlet at least 10 cm long such as is achieved by the extended secondary cooling (Carlo Erba), the movable on-column injector (J & W) and the oven-independently thermostated column inlet (Varian). According to the first concept, a relatively small sample volume (*ca.* 0.5 μl, depending on the length of the temporarily cooled inlet) is injected, the liquid coating the wall of the cooled inlet. The second concept accepts larger sample volumes and uses the cooled inlet as a buffer zone in order to prevent violent solvent evaporation from rejecting sample liquid into the injector. Movements of the sample material within the column inlet are described, from which technical requirements are derived.

INTRODUCTION

The basic concept of on-column injection in capillary gas chromatography (GC) is extremely simple, as the sample is introduced directly into the column inlet, circumventing all the steps involved in extra-column vaporization which are known to present problems. On-column injection is accordingly reliable for quantitative analysis. The basic logic is again simple: losses of sample material (also related to discrimination and high standard deviations) due to injection are ruled out, provided that no sample material returns behind the injection point or is lost backwards out of the column.

A key problem determining the working rules of the technique is related to the volume of the injection zone: the volume in the column inlet is far smaller than the volume of vapour created by evaporation of the sample solvent. As a result, rapid sample evaporation creates an increased pressure in the column inlet, which pushes sample material backwards out of the column. Therefore, it is necessary to provide sufficient time for sample evaporation, allowing the carrier gas to remove the gen-

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erated vapour continuously. This is achieved either by injection at low column temperatures (conventional on-column injection) or by slow introduction of the sample liquid from a cool zone into the heated zone (the kind of techniques to be considered in this paper).

Solvent evaporation at low column temperature

The conventional working rules for on-column injection[1] require the column temperature during injection and solvent evaporation to be below the boiling point of the sample (solvent) at the carrier gas inlet pressure in order to restrict the sample vapour pressure to below the carrier gas inlet pressure. This maximum injection temperature is therefore slightly above the boiling point of the solvent under standard conditions, depending on the inlet pressure applied[2].

On-column injection at low column temperatures proved to be highly reliable: it is reliable for quantitative analysis as it rules out rejection of sample material, but it also ensures that solvent effects optimally reconcentrate the initial bands of volatile solutes. There is no thermal defocusing, the temperature of the column inlet (and its increase) requires no special attention and the technique remains the same whether 0.2-or 200-μl volumes of sample liquid are injected[3]. Hence it contributes to the simplicity of on-column injection.

On the other hand, on-column injection at column temperatures below the boiling point of the solvent often renders analyses awkward. First, the required cooling of the oven is time consuming. If, for example, triglycerides are analysed as a solution in *n*-hexane, the column must be cooled below 70–75°C for injection and solvent evaporation. Subsequently, the oven is heated to above 300°C again for elution of the triglycerides. In addition to being awkward, the cooling step also causes problems concerning the stability of the baseline and reproducibility of absolute retention times. It is therefore understandable that there is much interest in high-temperature on-column injection, even if this means leaving the safe ground of classical conditions and if certain drawbacks must be accepted.

Approaches to high-temperature on-column injection

In 1978 Grob[4] suggested that sample rejection due to violent sample evaporation could be overcome by injecting slowly; assuming instant evaporation of the sample solvent, only such an amount of volatile material should be introduced per unit time that its vapour could be immediately discharged through the column by the carrier gas. However, using conventional on-column injectors, this concurrent solvent evaporation sometimes caused severe losses of high-boiling solute material[1]. Part of these losses were due to sample evaporation inside the tip of the syringe needle reaching into the oven-thermostated, hot column inlet. Another part was caused by sample evaporation on the outer wall of the needle; slow injection causes sample liquid to be drawn backwards into the narrow space between the needle and the column wall by capillary forces. As solvent evaporation proceeds from the rear to the front (the carrier gas picks up vapour from the rear), solvent evaporation occurred primarily between the needle and the column wall, depositing high-boiling solute material on the outer wall of the syringe needle. Some aspects of slow injection at high oven temperatures could be substantially improved by cooling the injection

zone during sample introduction by secondary cooling[5]. Nevertheless, slow injection was abandoned because its reliability was unsatisfactory for the high demands in on-column injection, particularly when using volatile solvents.

At Varian, Yang[6] and Hinshaw and Yang[7] constructed an on-column injector equipped with a device thermostating an inlet section of the column about 12 cm long independently of the oven. This inlet can be kept at low temperature while the oven temperature is far above the boiling point of the solvent. This inlet section can only retain about 0.5 μl of liquid; if larger sample volumes are injected, some liquid flows into the hot column. However, the cool inlet section serves as a buffer, preventing sample material from being rejected as far as into the injector.

The investigations reported in this paper were carried out using a movable on-column injector (J & W, Rancho Cordova, CA, U.S.A.). However, the results and conclusions are expected to be independent of whether the inlet was cooled by the device of Varian (Palo Alto, CA, U.S.A.), by pulling the column inlet out of the GC oven or by using an extended secondary cooling (Carlo Erba, Milan, Italy).

CONCEPTS OF ON-COLUMN INJECTION INTO TEMPORARILY COOLED COLUMN INLETS

Small sample volumes coating cooled column inlet

According to the first of two existing concepts, the sample liquid is coated as a film on the internal wall of the temporarily cooled column inlet. The sample volume injected is kept small enough to prevent sample liquid from flowing into the oven-thermostated column. Solvent evaporation is carried out at a low temperature, and the inlet section is heated to or above the oven temperature only after the solvent has completely evaporated. This technique avoids the generation of large volumes of vapour through rapid heating.

Geeraert *et al.*[8] applied this technique, using a home-made movable on-column injector, to the analysis of triglycerides. Sample volumes between 0.2 of 0.3 μl were injected into the column inlet kept in the atmosphere above the GC oven.

Cool buffer zone preventing rejection from column

The second concept tolerates larger sample volumes and accepts that sample liquid flows out of the cooled inlet into the entrance of the hot, oven-thermostated column. However, liquids cannot really penetrate into a column section at a temperature that far exceeds the boiling point of the sample as the sample liquid is stopped by its own vapour pressure. Therefore, the front of the sample plug remains in the zone where the column temperature changes from below to above the boiling point of the solvent, and solvent evaporation takes place there. After completion of solvent evaporation, the column inlet is heated, allowing the solute material to start the chromatographic separation process.

EXPERIMENTAL

Apparatus

Experiments were carried out using a movable on-column injector from J & W. This injector is fixed to a telescopic system, allowing the injector (and the column inlet) to be moved up and down by 133 mm. The system was mounted on an old gas

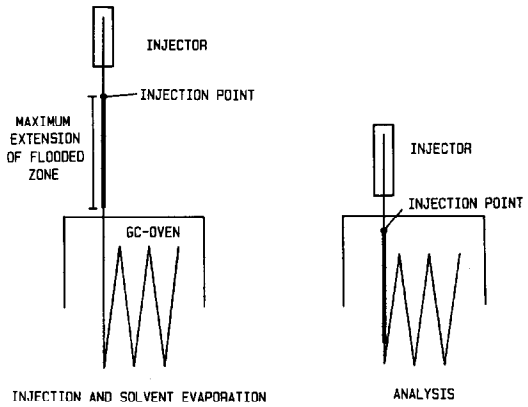

Fig. 1. Working principles of the moving on-column injector used for high-temperature on-column injection. Injection is carried out with the injector in the up position, pulling some 10 cm of the column inlet out of the oven-thermostated zone. The sample floods this cool inlet. After completion of solvent evaporation the injector is moved into the down position, introducing the solute-coated inlet into the oven. The injection point must be positioned such that it is clearly oven-thermostated when the injector is in the down position.

chromatograph (Carlo Erba Model 2150), leaving a gap of 15 mm between the roof of the oven and the base plate of the injector, stuffed with insulation material. This, however, does not fully preclude that a column section just above the base plate of the injector may be warmed by heat irradiated from the mounting bolt or by hot air from the oven blown along the column inlet through the mounting bolt.

Glass capillary columns (8–10 m × 0.31 mm I.D.) coated with PS 255 (a methylsilicone) of 0.4 μm film thickness were used. These columns were equipped with fused-silica inlets of 1–1.5 m × 0.32 mm I.D., either deactivated but uncoated (retention gap) or coated with stationary phase of the same film thickness as the separation column. Connections were prepared by fusion[9].

Description of the system

During chromatographic runs the movable on-column injector is in down position (Fig. 1). In this position the injection point must be located within the GC oven (preferably 10–15 mm below the possibly cooler oven roof) in order to ensure that all sample material deposited on the wall of the column inlet is oven-thermostated during the chromatographic run. This requires adjustment of the length of the syringe needle to the given installation (17 cm in our case).

Movable on-column injectors work as conventional on-column injectors if left permanently in the down position. This is suitable for injections carried out at column temperatures at or below the boiling point of the solvent.

Moving the injector into the up position, a length of 133 mm of the column inlet is pulled out of the GC oven and cooled to a temperature between 25 and 40°C, depending on the oven temperature and the distance from the base plate. However, the useful length of the cooled column inlet section is less than 133 mm; it only spans from the base plate of the injector to the injection point (Fig. 1). In our case it was 100 mm.

Movement of the sample liquid in the column inlet

Movement of the sample liquid in the column inlet was observed visually, either using a fused-silica capillary inlet from which the polyimide coating had been burnt off, or by replacing the top part of the column inlet by a glass capillary with a strongly etched internal surface (giving it a whitish aspect when dry and becoming transparent when coated with liquid).

Movement of solute material was also determined visually, injecting solutions containing 0.1–1% of perylene (a polynuclear aromatic hydrocarbon eluting from standard columns at *ca.* 250°C) and observing the fluorescence of the latter under UV light (366 nm).

RESULTS AND DISCUSSION

Behaviour of the sample liquid in the column inlet

In the first instance the sample liquid injected into the column inlet kept below the boiling point of the solvent behaves as previously described in the context of band broadening in space[10]: the injected sample liquid forms a plug closing the bore of the column. The latter is pushed into the column by the carrier gas, coating the column wall behind itself with a thick film of sample liquid until the plug is exhausted. Mostly, this primary flow of liquid, lasting for a fraction of a second, is followed by a secondary flow along the column wall, which tends to reduce the thick sample layer to a mechanically stable film.

The length of the column inlet flooded by the sample liquid depends on a number of parameters, but as a first approximation it may be assumed that liquids wetting the column (stationary phase) surface flood 20–25 cm of the column inlet per microlitre of injected liquid, 25–30 cm if the inlet is uncoated (retention gap)[11].

In on-column injection into temporarily cooled column inlets, three cases can be distinguished according to the injected sample volume.

(a) According to the first concept described above, with the sample liquid coating the cooled inlet, the length of the flooded zone must not exceed that of the cooled inlet. If the useful length of the cooled inlet is 10 cm, the maximum sample volume is 0.4–0.5 μl if the inlet is coated and 0.3–0.4 μl if it is uncoated.

(b) If slightly larger sample volumes are injected (second concept), some sample liquid flows into the entrance of the oven-thermostated column by the secondary flow of liquid along the column wall. However, rapid solvent evaporation prevents the liquid from entering a column section at a temperature considerably exceeding the boiling point of the solvent. Solvent vapour replaces most of the carrier gas passing through the column, with the consequence that the carrier gas flow in the inlet is at least strongly reduced. This in turn reduces the flow of sample liquid into the hot part of the column. This partly self-regulating system causes the solvent evaporation at the entrance of the oven-thermostated column to be fairly mild. If some liquid does flow too far into the hot column (delayed evaporation), followed by rapid evaporation, the vapour pressure created is discharged by a flow of vapour backwards into the cooled inlet. Such returning vapour does not move far owing to rapid recondensation.

(c) On further increasing the sample volume (under typical conditions and assuming a useful length of the cool inlet of 10 cm, to a volume exceeding about 0.8

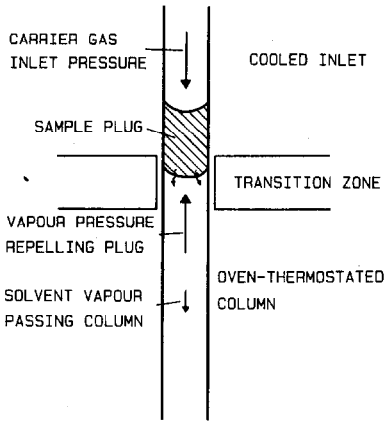

Fig. 2. Solvent evaporation from a stationary sample plug, positioned such that the front of the plug is located at a point where the temperature corresponds to the boiling point of the solvent at the inlet pressure. The plug is kept stationary as the solvent vapour pressure equals the carrier gas inlet pressure.

μl), the sample plug (primary flow) reaches the hot column section and a more violent mechanism becomes active. In contrast to case (b), the column bore is closed by the liquid, preventing easy discharge of excessive sample vapour towards the cooler rear. There is some "bumping", considered in more detail below.

Rejection of sample liquid

Theoretically, a plug of liquid pushed into a hot column should be stopped and driven back to a position such that its front is at a temperature causing the sample (solvent) vapour pressure to be equal to the carrier gas inlet pressure. This should result in a stationary plug as shown in Fig. 2, from the front of which the solvent would evaporate. However, as described in a previous paper[12], in reality the sample plug does not find a stable position in a process as quiet as that described above; owing to delayed evaporation the sample plug enters the hot column much further than it is supposed to (the smooth surface of a stationary phase film efficiently hinders the onset of evaporation). Once evaporation is initiated, evaporation is violent, and the high sample vapour pressure created at the evaporation sites (often the sample plug is split) throws the sample plug(s) back into the cool column inlet. The plugs move a considerable distance until they come to a stop, *i.e.*, until the vapour in front of them has recondensed. A fraction of a second later, the carrier gas inlet pressure exceeds the solvent vapour pressure and drives the (often recombined) plugs back towards the heated zone, and the whole process starts again.

Typical "stopping distances", *i.e.*, distances covered within the cool inlet until the plug of liquid comes to a stop, were found to range between 6 and 11 cm. Liquids that do not wet the surface of the column inlet are driven further back (more than 15 cm) because liquids glide more easily on the unwetted surface but possibly also because recondensation is hindered. The latter observation is of particular importance if on-column injection at elevated column temperatures is used to avoid band broadening in space by samples that do not wet the stationary phase[13].

Effect of prematurely heating the inlet

A delicate point when using temporarily cooled column inlets for high-temperature on-column injection concerns the selection of the moment when the inlet is heated (by lowering the injector into the down position, switching off the secondary cooling or initiating heating of the independently thermostated inlet section). Most producers of temporarily cooled inlets recommend heating this inlet immediately after completion of injection. Corresponding guidelines have been elaborated for slow injection where cooling of the inlet serves for preventing evaporation inside the syringe needle[5]. However, our case is fundamentally different and guidelines must not be transferred blindely.

The sample liquid is coated on the wall of the cooled inlet in order to provide the solvent with the time required for gentle evaporation and transport through the column. Similarly, the concept of considering the cool inlet as a buffer for retaining rejected sample liquid requires that cooling is maintained up to completion of solvent evaporation. Premature heating creates an excessive volume of vapour and a back-flow of the latter. In other words, the benefits of the cooling are nullified.

Experimental observations confirmed this view in general, although presenting a more complex picture. Movements of the liquid were followed visually, injecting volatile solvents into a column at 200°C and lowering the injector into the down position immediately after injection.

On injecting volumes up to 0.3 μl no returning liquid could be observed above the base plate of the injector. Injections of larger volumes, however, created a hump of liquid *ca.* 2–5 mm above the point where the column inlet left the heated zone (mounting bolt in the injector base plate). This standing wave of liquid remained there until solvent evaporation was almost completed. If the injector was lowered slowly, the hump of liquid moved backwards at a corresponding speed, remaining stationary relative to the mounting bolt.

Visual observation of the solvent does not allow one to distinguish the extent to which the solvent returned towards the injector as liquid or as vapour recondensing in the cooler part. This differentiation, however, is crucial, as return of liquid in the liquid phase carries all dissolved solute material along, whereas evaporation and recondensation leaves higher boiling material at the evaporation site.

Movements of the solute material were studied using perylene as a marker. Injections of various volumes and using different solvents were carried out at an oven temperature of 250°C (as required for the isothermal analysis of perylene), again lowering the injector immediately after the injection. Observations were not uniform; in many instances the fluorescence of perylene became clearly visible within the hump of back-flowing liquid. In other instances, however, the hump of returned liquid did not show noticeable fluorescence. However, even in these instances some perylene was located in the transition zone of the inlet located between the oven-thermostated and the cooled sections, *i.e.*, the part situated in the mouting bolt. The extent to which perylene was carried backwards behind the injection point appeared to depend on many factors, among which the speed of lowering the injector was important. A large proportion of perylene returned when the plug of injected liquid did not disappear before the injector was lowered because the vapour pressure created at the front of the plug drove the latter backwards behind the injection point. Considerable back-flow in the liquid phase is also obtained if the stream of back-flowing vapour

drives waves of liquid along the column wall into the rear of the inlet.

In practice, it is not important how much solute material is flushed behind the injection point. As long as such a return cannot be reliably ruled out, the inlet can only be heated when (nearly?) all of the solvent has been evaporated. Currently used guidelines must be changed accordingly.

Sample plug returning to needle tip

The mechanism under certain conditions causing the sample plug to return to the tip of the still inserted syringe needle and its consequences for analyses (discrimination, memory effects) have been described previously[14]. The carrier gas ahead of the freshly injected sample plug acts as a spring; it is compressed by the injected sample liquid (which enters the column at a flow-rate far exceeding that of the carrier gas). The compressed gas slows the movement of the sample plug and, under certain conditions, pushes it back again towards the tip of the still inserted syringe needle (a process that takes place during a fraction of a second). If the returned sample plug touches the needle tip, some liquid is pulled by capillary forces backwards between the needle and the column wall. When the syringe needle is withdrawn, this liquid is pulled backwards further up the column neck, often up to the column entrance. The column neck thus becomes coated with sample liquid, the excess of sample liquid flowing back into the oven-thermostated column. The sample film contains about 30 nl of liquid per centimetre of column length (0.3 mm I.D.). The amount of sample material deposited on the wall of the neck between the column entrance and the injection point depends on the length of the latter, but can easily reach 100–300 nl.

The sample material pulled backwards into the permanently cooled column neck kept inside the injector behaves as sample material, reaching there owing to premature heating of the temporarily cooled inlet section or excessively violent rejection of liquid at the beginning of the heated column (see above). The effects observed in the chromatograms depend on the volatility of the solutes and the retention power in the column inlet, in particular on whether a retention gap is used as a column inlet.

Solutes of high volatility in the cool column inlet return rapidly to the oven-thermostated column and are chromatographed normally together with the bulk of the solute material, forming perfectly shaped peaks representing the total amount of injected solute material. On the other hand, solute material of low volatility remains in the column neck during the whole run. Again, perfectly sharp peaks are obtained, but of insufficient area (discrimination at the high-temperature end of chromatograms!). The lost material is likely to create memory effects; it is washed back into the oven-thermostated column by a following injection bringing liquid into the column neck[14].

Solute material of intermediate volatility returns into the oven-thermostated column with a delay. If this delay is small, the affected solute material is eluted as the tail of a peak. A greater delay causes the tail to become broader, or invisible if the solute material merely lifts the baseline to a marginal extent. Resulting peaks may appear to be of perfect shape. However, the peak areas are insufficient. In contrast to solute materials of even lower volatility, no memory effects are observed as the deposit is removed before the subsequent injection.

Fig. 3a shows an isothermal chromatogram (160°C) of solutes of intermediate

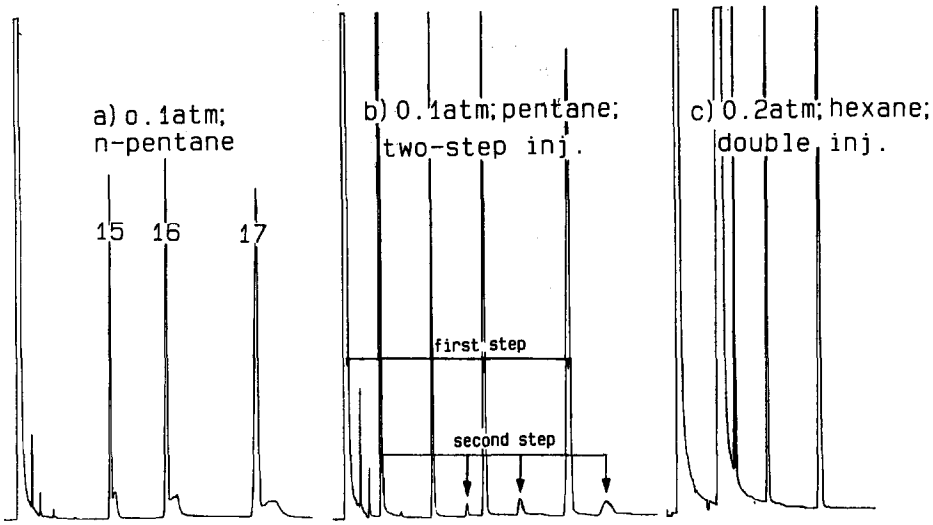

Fig. 3. Certain conditions cause the injected sample liquid to be pulled backwards between the walls of the column and the syringe needle. As the needle is withdrawn, this liquid is pulled further backwards into the column neck. The effects on chromatography depend on volatility of the solutes. Isothermal runs at 160°C; 0.5-μl injections of C_{15}–C_{17} *n*-alkanes (15–17) in *n*-pentane (a,b) and *n*-hexane (c). (a) Normal injection under conditions causing some sample liquid to be pulled into the column neck. Solutes return from there with a delay, forming the broad shoulders eluted after the main peaks. (b) After a normal injection the injector was moved into the up position again and a 5-cm section of column inlet was pulled out of the injector. Lowering the injector caused thermal desorption of solute material from the formerly cooled inlet, resulting in the small peaks eluted after the main peaks with the same distance as the "air" peak of the second "injection" is away from the solvent peak. (c) Normal injection under conditions that do not cause a return of sample liquid behind the injection point, followed by an injection of pure solvent (*n*-hexane) into the column neck to rinse possible deposits of solute material into the oven-thermostated column.

volatility (C_{15}–C_{17} *n*-alkanes) obtained by injection of 0.5 μl of an *n*-pentane solution on to an 8 m × 0.31 mm I.D. capillary column coated with PS-255 up to the column entrance (no retention gap). Injection was carried out with the injector in the up position, and the injector was lowered (the column inlet heated) 10 s after injection. Both the high volatility of the solvent and the relatively low carrier gas flow-rate (about 2 ml/min of H_2) contributed to the fact that the plug of injected sample liquid returned to the tip of the syringe needle. Broad shoulders are observed in the chromatogram, eluted just after the sharp peaks and representing material that entered the oven-thermostated column with a delay due to slow release from the permanently cooled rear part of the column inlet behind the injection point. According to the peak-area ratios, about 30% of the sample was withdrawn into the column neck, *i.e.*, about 0.15 μl of sample liquid, coating a column section about 5 cm long. Return of solute material into the cool column neck was confirmed by a modified injection procedure in which injection was carried out as in (a), lowering the injector 10 s after injection, but the solutes were allowed only 10 s to leave the temporarily cooled inlet by lifting the injector into the up position again after 10 s. In this way the warm inlet section behind the injection point was cooled to ambient temperature also. Then a 5-cm section of the column inlet was pulled out of the injector, accordingly shifting the injection point further into the oven. Lowering the injector into the down position

caused a formerly permanently cool rear column inlet section to become oven-thermostated and desorption of the solute material withdrawn into it.

Two points can be observed in Fig. 3b. First, the broad shoulders observed in chromatogram (a) are absent, which is the result of the rear column inlet having been cooled to a temperature that does not cause desorption of the solutes at a rate that produces a visible deflection of the recorder pen. Second, small, slightly broadened peaks are observed at a distance from their parent peaks corresponding to the time difference between lowering the injector for the first and the second times. These small peaks represent solute material from the cool rear of the inlet, which has only been heated by the second "injection". The areas of these secondary peaks differ from those of the shoulders in chromatogram (a), first because the amount of withdrawn solute material is poorly reproduced, and second because some solute material slowly passed on to the oven-thermostated column without a noticeable rise in the baseline before lowering the injector the second time.

The same evidence as obtained by the above experiment was achieved by subsequent injection of pure solvent into the column head instead of pulling the inlet partially out of the injector. The sample was injected, the inlet heated and cooled again (as in the above experiment), followed by injection of a 1-μl volume of pure *n*-hexane into the column neck just below the column entrance. This solvent rinses possible solute material from the permanently cooled rear part of the inlet into the oven-thermostated column. Such rinsing is highly efficient if an uncoated inlet (retention gap) is used. Rinsing solute material from a coated surface, however, is incomplete.

Fig. 3c shows the result of a double injection carried out under conditions that prevent the return of the sample plug to the tip of the syringe needle; *n*-hexane was used instead of the more volatile *n*-pentane and the carrier gas inlet pressure was doubled. The chromatogram shows the two solvent peaks but no secondary peaks of the solutes, confirming that no solute material was withdrawn far behind the injection point.

Return of the sample liquid to the needle tip was found to be a severe problem, first because such a return occurred frequently, and second because of the length of the column neck that was coated with a large volume of sample liquid. Hence, the effects on quantitative analysis are not negligible.

Frequently, sample liquid was not only pulled backwards up to the column entrance, but out of the column into the needle guide zone and the rotating valve of the injector. As the restriction above the rotating valve consists of a glass tube, the liquid adhering to the needle tip and being smeared within this top section of the injector could easily be observed. Of course, such losses and the resulting contamination of the injector (memory effects) cannot be tolerated.

CONCLUSIONS

It should be emphasized that comments on the movable on-column injector refer to high-temperature on-column injection and not to the use of the injector for conventional on-column injection at lower column temperatures. In fact, the injector was not designed for high-temperature on-column injection, and the latter technique should be considered as an additional possibility offered by injectors that allow tem-

porarily cooling of a column inlet section such as the movable on-column injector.

This paper has dealt only with the process of sample introduction, where several sources of problems, mostly resulting in general or selective losses of solute material, were identified. Part II will deal with possible problems caused by deformed initial solute bands (resulting in deformed peaks or losses in peak area).

On the one hand, high-temperature on-column injection is very convenient for many applications, and on the other, the quantitative results obtained were clearly less reliable than those produced by conventional on-column injection. Sometimes absolute peak areas varied within wide ranges, indicating non-reproducible losses of sample material. In some rare instances memory effects were observed. Finally, the precision and accuracy of the relative peak areas often did not correspond to those obtained by conventional on-column injection. Nevertheless, results were obtained that could not have been produced by vaporizing injection.

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