*Journal of Chromatography*, 189 (1980) 109–117
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 12,444

# FACTORS AFFECTING THE ACCURACY AND PRECISION OF COLD ON-COLUMN INJECTIONS IN CAPILLARY GAS CHROMATOGRAPHY

K. GROB, Jr. and H. P. NEUKOM
*Kantonales Laboratorium, P.O. Box, 8030 Zürich (Switzerland)*
(Received September 10th, 1979)

## SUMMARY

The conditions used in cold on-column injection were studied in order to optimize them. The transfer of the liquid sample from the syringe needle to the internal wall of the column is a critical factor. It has to occur by mechanical motion, and not by evaporation. Non-discriminated runs were obtained on rapidly pushing the plunger. The use of 0.5-$\mu$l syringes was unsatisfactory because of the insufficient speed of the liquid at the needle tip. On the other hand, the rapid injection of large volumes of liquid may produce too much sample vapour, which is then partly lost by back-flow through the column entrance. Such losses are avoided when the column temperature is not higher than the boiling point of the solvent. Temperatures far below the boiling point are unsatisfactory because of possible phase stripping in the front coils of the capillary. Thus, rapid injection of volumes between 0.25 and 8 $\mu$l at column temperatures around or, for small sample sizes, also below the boiling point of the solvent are recommended. These conditions assure an ideal solvent effect independent of the sample volume.

## INTRODUCTION

Recent practice has shown that the term "on-column" can be misinterpreted. An essential feature of the originally reported technique[1–3] is the avoidance of evaporation before the sample reaches the internal wall of the column. Modified methods have been described[4] in which evaporation is still an essential step. Therefore, in the interests of unambiguous terminology, we feel obliged to call our technique "cold on-column" injection.

Increasing evidence has shown that sampling with classical vaporizing injectors suffers from severe problems, causing results with high standard deviations. Calibration runs are needed in order to adapt the quantitative information to the true values. It is not likely that vaporizing injectors, even after further development, will produce highly precise and accurate results. Nevertheless, they are useful in a broad range of applications, especially for qualitative analysis and for crude and contaminated samples.

Cold injection of liquid samples has the advantage of avoiding thermal degrada-

tion, and most of the processes creating discrimination as typical of vaporizing sampling[5], *i.e.* the problem that the composition of the sample entering the column is not the same as for the sample injected. However, one problem that still has to be considered carefully is the transfer of the sample from the exit of the syringe to the internal surface of the column. In vaporizing injectors this is done by a combination of mechanical shift and evaporation. The sample volume injected then is the volume read on the barrel plus an extra amount evaporated (incompletely) out of the syringe needle or, with 1-$\mu$l syringes, out of the liquid remaining between the tungsten plunger and the steel tubing of the syringe needle. Other injectors avoiding sampling by syringes may overcome some of these problems, but they still suffer from insufficient transfer of the sample owing to fractionated evaporation.

With cold injection, similar problems have to be considered. The mechanical transfer of liquid from one device to another is never complete. The inevitable relative error increases with decreasing volume (for instance, of pipettes), whereby at least reproducible, instead of complete, transfer is attempted. Similar, but greater, problems arise with the transfer of a fraction of a microlitre. In gaschromatography (GC) there is the additional problem that volatile components of non-transferred material may partly evaporate. This means that high-boiling compounds are transferred mechanically (as desired), while unknown additional amounts of volatiles are sampled upon evaporation. There is no easy technique to avoid this problem. Rinsing with pure solvent appears attractive but is difficult to achieve under the conditions of cold injection.

We tested the extent of losses through insufficient sample transfer in cold on-column injection, and optimized the technique to avoid discrimination and to produce maximum precision and accuracy. Also, as a consequence, a number of related technical problems had to be studied.

## TRANSFER OF LIQUID SAMPLES

The basic problem is easily comprehended. "Injection" of, *e.g.*, 0.05 $\mu$l of a liquid into the open air with a 0.5-$\mu$l syringe (SGE, Melbourne, Australia) produces a minute droplet attached to the needle tip, where it undergoes fractionated evaporation. When the same is done inside a capillary tube, a fraction of the liquid is transferred to the wall of the tube. However, the transferred fraction is far from being reproducible, as it depends strongly on factors such as the exact position of the needle tip with respect to the wall of the tube and the wettability of the wall with respect to the particular liquid. Larger volumes of liquid will form droplets large enough to be detached from the needle tip, with the possible exception of a relatively small remainder. The material remaining at the needle tip partly evaporates in the stream of carrier gas, but only releasing volatiles. The high-boiling components are lost as they are pulled out of the injector when the syringe is removed.

A further problem is caused by the fraction of the sample that is pulled up between the syringe needle and the column wall (in spite of the carrier gas flow). As the needle is removed, part of this liquid is wiped off in the column end and another part is pulled off on the needle. Thus, uncontrolled contamination and loss of sample occur, discriminating against heavier substances.

Using a 5- or 10-$\mu$l syringe (Hamilton, Bonaduz, Switzerland, or SGE, Mel-

bourne, Australia), both problems are overcome by rapidly pushing the plunger. Because of its high velocity, of the order of several metres per second, the sample is blown off the needle as a stream of small droplets. Virtually no sample remains at the needle orifice, and no sample is pulled up between the needle and the column.

Table I shows results obtained with a test mixture containing equal amounts of $C_7$–$C_{28}$ alkanes. Rapid injection completely prevented discrimination, which was severe with slow injection. With *n*-hexane instead of *n*-pentane as the solvent, and with the column still at room temperature, evaporation of the liquid from the needle is slowed down, and discrimination is reduced to about half the extent observed with *n*-pentane. This might suggest that solvents with boiling points far above the column temperature should be selected. However, with sample size above 0.5 μl, phase stripping may occur.

TABLE I

DEPENDANCE OF DISCRIMINATION ON THE INJECTION SPEED

Peak areas of alkanes normalized on *n*-heptane in a mixture containing equal amounts of components. Injections of 1 μl with a 10-μl syringe, transferring the sample during different periods of time. Column temperature, 30°; (hydrogen) flow-rate, 2.5 ml/min; solvents, *n*-pentane and *n*-hexane. Only rapid injections produce satisfactory, *i.e.*, virtually complete, transfer of the liquid sample from the tip of the syringe needle to the column.

| *Alkane* | *Rapid (n-pentane)* | *Slow (n-pentane)* | | | | *Slow (n-hexane)* | |
|---|---|---|---|---|---|---|---|
| | | *1 sec* | *2.5 sec* | *5 sec* | *10 sec* | *2 sec* | *5 sec* |
| $C_{12}$ | 1.0042 | 1.0001 | 0.9623 | 0.9848 | 0.9641 | 1.0020 | 0.9915 |
| $C_{20}$ | 1.0093 | 0.9637 | 0.8421 | 0.6857 | 0.5189 | 0.9553 | 0.8714 |
| $C_{28}$ | 1.0043 | 0.9586 | 0.7453 | 0.5158 | 0.3596 | 0.8937 | 0.7603 |

SAMPLE VOLUME

Vaporizing injection by the syringe restricts the practical volume of the sample to between 1 and 3 μl; 1 μl is the needle volume of an average syringe, while 3 μl produces a maximum vapour volume with respect to the free volume of most injectors (we do not recommend the use of 1-μl syringes for reasons explained elsewhere[5]).

With cold on-column injection, the range of practical sample volumes is much larger. The upper limit is controlled by the risk of damaging the column, and the lower limit by unsatisfactory sample transfer between the syringe needle and the column wall. Large amounts of solvent do not cause phase stripping, provided that the column temperature is near to or above the boiling point of the sample. However, with cold on-column injection, the choice of column temperature is very limited, and temperature selection becomes more critical the larger is the sample volume. Excessive temperatures, leading to too rapid evaporation of the sample in the column, may create a back-flow due to a pressure increase near the injection point. Temperatures below the boiling point of the sample promote phase stripping. An 8-μl volume of *n*-pentane injected at a column temperature of 30° produces one or a few liquid plugs in the capillary with a total length of the order of 10 cm. While travelling into the column, the liquid disappears only after passing a column length of about 1–2 m (depending on the carrier gas inlet pressure). Such plugs essentially block

the carrier gas flow, thus preventing rapid evaporation. Therefore, either a sufficiently long column section should be uncoated, or a higher column temperature has to be selected. With an optimum column temperature, and/or with an uncoated column inlet section, sample volumes of the order of 10 $\mu$l can reasonably be injected.

Table II shows peak areas of three alkanes, normalized on an internal standard, obtained with sample volumes from 0.1 to 8 $\mu$l (mean values of 4–8 runs). Injections were rapid, using a 5- or 10-$\mu$l syringe. The column temperature was 30° and the solvent was *n*-pentane. Results from large sample sizes do not show significant discrimination. The standard deviation was 0.3–1.5%.

TABLE II

DEPENDENCE OF ACCURACY ON SAMPLE SIZE

| *Alkane* | *Sample volume (µl)* | | | | | | |
|---|---|---|---|---|---|---|---|
| | *0.1* | *0.2* | *0.5* | *1.0* | *2* | *4* | *8* |
| $C_{12}$ | 1.0423 | 1.0373 | 1.0013 | 0.9976 | 1.0000 | 1.0027 | 1.0127 |
| $C_{20}$ | 0.9721 | 0.9786 | 0.9988 | 0.9833 | 1.0054 | 1.0003 | 1.0125 |
| $C_{28}$ | 0.9509 | 0.9639 | 1.0004 | 0.9932 | 1.0016 | 0.9988 | 1.0094 |

Small sample sizes do not create problems with phase stripping. Thus, the column temperature may be far below the boiling point of the solvent. The lower limit of the sample volume is given by the sample transfer from the needle tip to the column wall, which causes discrmination of high-boiling compounds. For injecting small sample sizes, the SGE 0.5-$\mu$l syringe may seem to be ideal. However, injections with this syringe usually did not give accurate results. Sample volumes of 0.2 $\mu$l yielded discriminated and non-discriminated runs, while 0.05 $\mu$l always produced discrimination of high-boiling substances. Injections of the smallest practical volumes with 5- or 10-$\mu$l syringes gave more accurate results.

Table III compares 0.5- and 5-$\mu$l syringes, using *n*-hexane as the solvent at a column temperature of 30°. For sample sizes of 0.2 $\mu$l and less, high-boiling components were discriminated with both syringes, but even better results were obtained with the larger syringe. The values in Table II for 0.1- and 0.2-$\mu$l samples were

TABLE III

COMPARISON OF 0.5-$\mu$l (SGE) AND 5-$\mu$l SYRINGES (SGE OR HAMILTON) WITH RESPECT TO DISCRIMINATION

Peak areas normalized on *n*-heptane as internal standard; mean values of 3–5 runs. In addition to the higher discrimination obtained using the small syringe, standard deviations were considerably higher (of the order of 6%, compared with 1.5% for 0.2-$\mu$l injections with the 5-$\mu$l syringe). Solvent, *n*-hexane; column temperature during the injection, 30°. Injections were made as rapidly as possible.

| *Alkane* | *0.5-µl syringe* | | | *5-µl syringe* | | |
|---|---|---|---|---|---|---|
| | *0.05 µl* | *0.1 µl* | *0.2 µl* | *0.1 µl* | *0.2 µl* | *0.5 µl* |
| $C_{12}$ | 0.9347 | 0.9468 | 0.9604 | 0.9905 | 0.9881 | 1.0075 |
| $C_{20}$ | 0.8678 | 0.8920 | 0.9951 | 0.9865 | 0.9977 | 1.0071 |
| $C_{28}$ | 0.7306 | 0.8719 | 0.9890 | 0.9644 | 0.9956 | 1.0082 |

obtained with the 5-$\mu$l syringe, but using *n*-pentane instead of *n*-hexane as the solvent at 30°. The results were typically discriminated in both senses: the high-boiling substances owing to insufficient sample transfer, and the volatiles, including the internal standard *n*-heptane, owing to loss out of the needle before the injection was made. Thus, these runs showed maximum peak areas around the $C_{10}$ alkane. This was no longer observed with *n*-hexane as the solvent.

We consider that the higher accuracy of the results obtained with the larger syringes (for the same sample volumes) is due to the different speeds of the liquid leaving the syringe needle. On pushing the plunger at the same speed into a 5- and 0.5-$\mu$l syringe, the liquid moves almost 10 times faster in the needle of the larger syringe. Based on these observations, we conclude that the minimum practical sample volume is given by the volume that can be injected, with reasonable accuracy, with a 5- or 10-$\mu$l syringe. This volume is of the order of 0.3 $\mu$l.

## COLUMN TEMPERATURE DURING INJECTION

In a column with a temperature far above the boiling point of the solvent, the sample evaporates so rapidly that the vapour cannot be transported by the carrier gas flow. At the usual gas flows of 2–3 ml/min (hydrogen), the vapour from 0.2 $\mu$l of *n*-pentane is transported per second. If more vapour is generated, a zone of increased pressure is built up, which causes extra flows in both directions. The centre of this flow may be about 10 cm away from the needle tip with rapid injection. This is the distance that the droplets leaving the needle may travel before they hit the column wall. At relatively high column temperatures, the first portion of vapour creates a pressure barrier, which pushes the next portion backwards towards the syringe needle and the column inlet, where some material may be lost. In Fig. 1 such losses are determined for three alkanes as solvents at different temperatures.

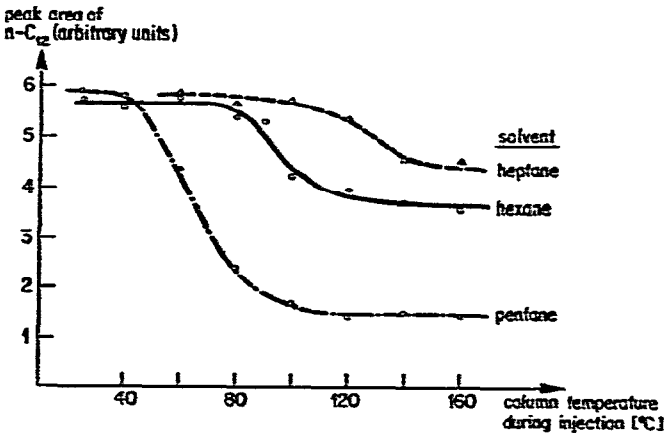


Fig. 1. Sample losses at too high injection temperatures: peak area of *n*-dodecane in three different solvents, keeping the capillary at different temperatures during the injections; temperature increase for the elution of *n*-dodecane after 1 min. Injection volume, 1 $\mu$l; carrier gas flow-rate, 2.5 ml/min; column, 20 m × 0.30 mm I.D. coated with 0.6-$\mu$m SE-52. Areas are reduced when the evaporation of the sample in the capillary inlet is too rapid. A pressure increase causes a back-flow of sample vapour. Vapours leave the capillary by the entry and are vented through the open injector valve.

At column temperatures above 100°, about 75% of the sample was lost when the solvent was *n*-pentane. With *n*-hexane and *n*-heptane much smaller proportions of the sample left the column (even at higher temperatures). This may be explained by a cold trapping effect exerted by the uppermost column section in the cooled injector, where the less volatile solvents condense and prevent sample loss.

A considerable amount of sample may condense on the outer wall of the syringe needle. When the syringe was withdrawn immediately after the injection, the losses were of the order of 20% (see Table IV with experimental conditions). When the needle remained in the injector for a further 5 sec, the losses more than doubled. This observation was explained by assuming that some time was needed in order to build up an efficient back-flow.

TABLE IV

EXPERIMENTAL PEAK AREAS FOR *n*-DODECANE IN *n*-HEXANE (1:50,000) INJECTED INTO A COLUMN KEPT AT 140°

Sample volume, 1 μl; carrier gas flow-rate, 2.5 ml/min. Areas expressed as a percentage of the area obtained at column temperatures below 70°. Variable: period of time the syringe needle was kept in the capillary after (rapid) injection and before taking it out.

| *Time (sec)* | *Peak area (%)* |
|---|---|
| 0 | 83 |
| 3 | 81 |
| 5 | 60 |
| 10 | 57 |

Moderately volatile materials, if they have strayed into the cooled column inlet, return from there with a delay, creating strongly tailing peaks. High-boiling materials such as triglycerides remain there until they are washed down by another injection. With relatively high-boiling solvents, such as *n*-nonane, a similar observation was made for the solvent itself. As long as the column temperature was near the boiling point, the solvent peak was wellshaped. At higher temperatures it started to broaden and tail, especially when the syringe was withdrawn with a delay.

For cold on-column injection the upper temperature limit of the column should be near the boiling point of the solvent. It is influenced by a variety of factors such as carrier gas flow-rate (determining the amount of vapour transported per unit time), the sample volume, the evaporation energy of the solvent (strongly cooling the column locally), and a number of poorly defined variables such as the length of column wetted by the liquid sample and the position of the needle tip in the column.

Fig. 2 shows the peak areas of *n*-dodecane dissolved in *n*-pentane, injected at 80° with various carrier gas flow-rates (sample volume 1 μl). Even with high flow-rates the losses were of the order of 20%, increasing to 80% at low flow-rates.

The critical upper column temperature was almost independent of the sample volume. Fig. 3 shows the results obtained with a solution of *n*-nonane in acetone. Below a certain temperature no losses were detected, regardless of the sample size. Above this critical temperature the losses increased sharply, then being larger for the large injections. This allows one to conclude that the upper column temperature limit is practically independent of the sample volume.

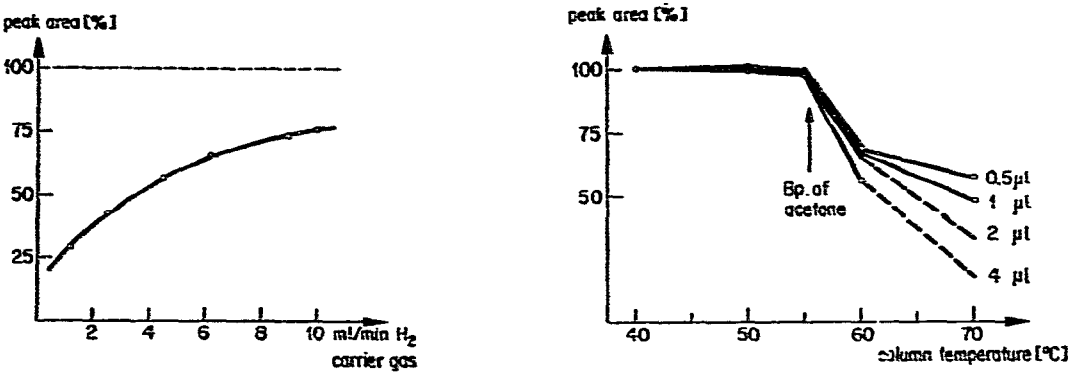


Fig. 2. Dependence of sample vapour losses on the carrier gas flow-rate for injections at too high column temperatures (causing back-flow of sample vapour): peak area of *n*-nonane in *n*-pentane (1:50,000) injected at 80°. Sample size, 1 µl. Peak areas as a percentage of that obtained at a column temperature of 30°. Sample losses strongly decrease with increasing carrier gas flow-rate, but they cannot be avoided totally.

Fig. 3. Critical upper column temperature for rapid cold on-column sampling of solutions in acetone: peak area of *n*-nonane dissolved in acetone (1:50,000). Injections of various amounts at different column temperatures; carrier gas (hydrogen) flow-rate, 2.5 ml/min. The mean peak area of 1-µl injections at 40° was set at 100%. It can be concluded that such solutions should not be injected at column temperatures above the boiling point of acetone (56°), because otherwise too rapid evaporation of the sample causes sample losses. Further, the critical upper column temperature is fairly independent of the sample volume (but not the losses above this temperature).

The upper limit of the column temperature that does not produce losses due to back-flow was not found to bear a simple relationship to the boiling point of the solvent. Data for *n*-pentane to *n*-heptane suggested a limit of 7° above the boiling point. For chloroform, losses became detectable at 70°, indicating a limit of 5° above the boiling point. Acetone (Fig. 3) should not be injected at any column temperature above the boiling point (all values determined at a carrier gas flow-rate of 2.5 ml/min). Thus the boiling point of the solvent seems to be a reasonable upper temperature limit for cold on-column injection.

It is tempting to speculate that the simplest way of preventing losses due to back-flow is to inject small sample volumes. The situation was examined with an *n*-pentane solution injected at 80° (Fig. 4). At a carrier gas flow-rate of 2.5 ml/min the maximum tolerable volume is about 0.1 µl, an amount which was indicated above to be too small to be transferred accurately, thus causing discrimination of high-boiling components. A 0.1-µl volume corresponds to *ca.* 20 µl of vapour (filling about 15 cm of capillary column). As small amounts cannot be "shot" deeply into the column, it is not surprising that losses occur readily.

It was surprising that discrimination in runs with high proportions of lost sample material (injections at exceedingly high column temperatures) was low (Table V). Volatiles (as *n*-heptane) were lost by higher proportions than *n*-octane or *n*-dodecane. Another typical feature of injections at column temperatures above the critical limit are high standard deviations for values normalized on an internal standard as well as for absolute peak areas.

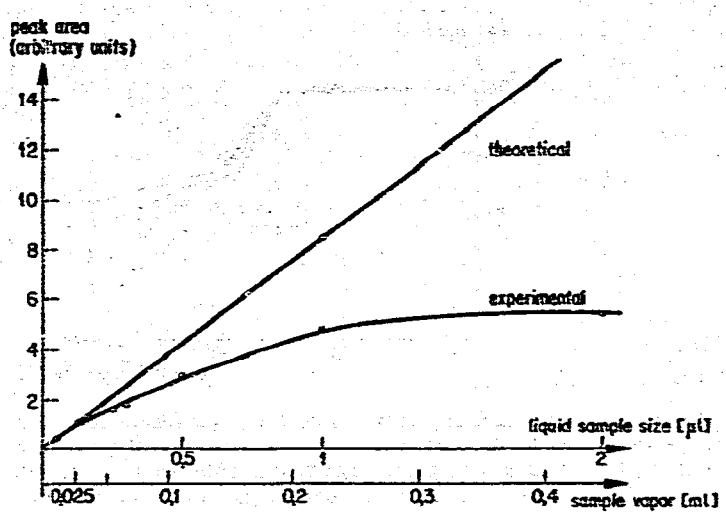


Fig. 4. Dependence of sample losses on the sample volume with exceedingly high column temperature during the injection: peak area of *n*-nonane in *n*-pentane, injected at 80°. Carrier gas flow-rate, 2.5 ml/min. Losses would only be neglectable for sample sizes below 0.1 µl, an amount which cannot be satisfactorily transferred from the syringe needle to the column.

TABLE V

ACCURACY AND PRECISION OF RESULTS OBTAINED BY INJECTIONS INTO A CAPILLARY KEPT NEAR THE BOILING POINT OF THE SOLVENT (*n*-PENTANE) AND FAR ABOVE IT

At a column temperature of 70°, 50–60% of the sample was lost by back-flow. Nevertheless, discrimination was relatively weak, but standard deviations were strongly increased (10 and 6 runs).

| *Alkane* | *30°* | | *70°* | |
|---|---|---|---|---|
| | *Peak area* | *Relative standard deviation (%)* | *Peak area* | *Relative standard deviation (%)* |
| $C_8$ | 0.9982 | 0.55 | 1.0590 | 3.7 |
| $C_{12}$ | 1.0038 | 0.38 | 1.0809 | 6.5 |
| $C_{16}$ | 0.9936 | 0.70 | 1.0980 | 7.8 |
| $C_{20}$ | 1.0008 | 0.45 | 1.0654 | 7.2 |
| $C_{24}$ | 0.9936 | 0.60 | 1.0295 | 7.6 |
| $C_{28}$ | 1.0071 | 0.44 | 1.0352 | 8.6 |

## CONCLUSIONS

These studies show that the conditions for most accurate cold on-column injections with syringes have to be selected more carefully than had been thought before. The concept presented here was strongly influenced by the new finding that only rapid injection provides virtually complete transfer of liquid sample on to the column. A number of aspects concerning cold injection had to be adapted accordingly. The method of slow injection to avoid a zone of excessive gas pressure (thus avoiding back-flow), as recommended earlier[3], had to be replaced. A new way had to

be found of eliminating problems caused by back-flow. The solution to the problem is an optimized column temperature during injection. The temperature is selected to permit rapid injection of any sample volume, yielding sufficiently slow evaporation to avoid back-flow, while being sufficiently high not to produce phase stripping. This temperature is found to be close to the boiling point of the solvent. It is constant for a wide range of sample volumes and shows little dependence on carrier gas flow-rate.

These conditions dictated by the injection technique have to be matched with those of chromatography. The main problem is solved automatically, because according previous work[3], injection on to a column the temperature of which is close to the boiling point of the solvent represents the ideal situation for the solvent effect, *i.e.*, perfect re-concentration of vapour clouds of any volume, without the risk of phase stripping (note that this coincidence of optimum conditions holds only for cold on-column injection; for vaporizing splitless injection a lower column temperature is required).

The sample size was found to be limited by a minimum to ensure sufficient transfer of the liquid sample from the syringe to the column wall (at least 0.2 $\mu$l), and a maximum which can still be injected rapidly. Very large volumes[6] are probably out of the recommended range as they have to be injected slowly.

The test mixture used for this study contained relatively volatile compounds ($C_7$–$C_{28}$). It was selected because discrimination in cold injection affects low-boiling to moderately volatile material more than high-boiling substances. Losses of high-boiling substances, when injected incorrectly, are relatively constant over a broad range of substances. Thus, reasonably accurate results are obtained with a suitably selected internal standard.

## REFERENCES

1 G. Schomburg, R. Dielmann, H. Husmann and F. Weeke, *Chromatographia*, 10 (1977) 383.
2 K. Grob and K. Grob, Jr., *J. Chromatogr.*, 151 (1978) 311.
3 K. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 263.
4 H. Kern and B. Brander, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 312.
5 K. Grob, Jr. and H. P. Neukom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 15.
6 J. Roeraade, *3rd International Symposium on Glass Capillary Chromatography, Hindelang, 1979.*