Journal of Chromatography, 178 (1979) 387–392

CHROM. 12,127

EVALUATION OF INJECTION TECHNIQUES FOR TRIGLYCERIDES IN CAPILLARY GAS CHROMATOGRAPHY

K. GROB, Jr.
Kantonales Laboratorium, P.O. Box, 8030 Zurich (Switzerland)
(Received June 21st, 1979)

SUMMARY

Precision and accuracy of vaporization, as well as cold on-column injections, were studied using triglycerides as a sample of very high boiling point. Cold on-column injection gave by far the most reproducible results (with standard deviations of 1 to 3%) normalized on internal standard. A weak discrimination of the larger triglycerides was assumed to be due to losses in the chromatographic column. Using a classical vaporizing injector, *ca.* 20% of the triglycerides were lost because of insufficient elution out of the syringe needle. Such losses explain virtually all the systematic errors occurring during splitless injections. However, discrimination especially of the higher boiling triglycerides, as observed with split sampling, was consistently higher, pointing to an additional, very inconsistent mechanism producing changes in the original composition of the part of the sample which enters the column. Standard deviations of data obtained with splitless injections were 9–13%, and 15–30% for split sampling.

INTRODUCTION

Triglycerides are probably the highest boiling compounds at present analyzed routinely by gas chromatography (GC)[1,2]. Up to now these compounds have been separated almost exclusively on packed columns. Little has been published on the use of glass capillary columns to analyze triglycerides. Schomburg *et al.*[3] reported some chromatograms using the polar Poly S 179 as stationary phase. Schulte[4] used nonpolar phases on "$BaCO_3$"-type columns. But according to his and our experience, these columns had fairly short lifetimes. We found that persilanized support surfaces[5] coated with apolar phases are most suitable for routine analyses[6]. Such columns could be used for several hundred runs (up to 355°) without significant changes in their performance, such as separation efficiency, retention (film homogeneity and amount of stationary phase) and adsorption (1-octanol still eluted to 80% of its theoretical peak height after such use). Fig. 1 shows a chromatogram of the triglycerides of rape seed oil with a large proportion of erucic acid, ranging from the group 54 containing the tri-18's (usually the end of a triglyceride chromatogram) up to the group 64 with molecular weights around 1030 daltons. The chromatogram

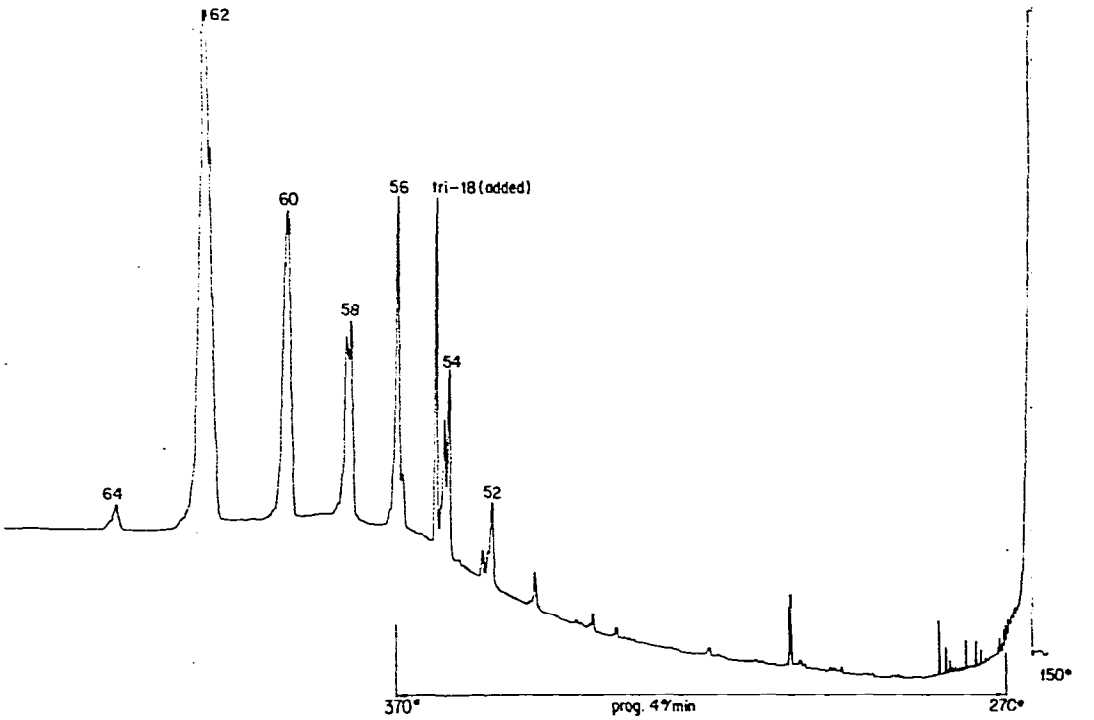

Fig. 1. Chromatogram of triglycerides of rape seed oil with a high content of erucic acid, ranging in molecular weight to above 1000 daltons. The triglycerides are grouped according to their total number of carbon atoms in the fatty acid chains. Separation within these groups is poor due to the complexity of isomers.

was obtained after about 20 runs up to 370°, during which the column properties were not noticeably altered.

Another problem associated with capillary GC (to some degree also with packed column GC) is the quantitative aspect of triglyceride analysis, largely due to poor sampling techniques. Triglycerides are clearly out of the range for which the classical vaporizing injectors have been conceived. Their vapour pressures are too low to allow a reasonable evaporation in the injector. Thus elution out of the syringe needle is likely to be insufficient. Evaporation from a solid surface, as during a "moving needle" injection, does not look like being a viable method either. The smallest amount of dirt in the injector would be expected to retain triglycerides, and insufficiently heated parts near the septum would have a stronger effect on this type of sample than on virtually any other. The fact that cold on-column injection[7–9,*] does not require an evaporation step for the sample to enter the column, and that the sample is injected by a mechanical movement of a liquid rather than by an evaporation out of the syringe needle, makes this technique clearly the method of choice for triglycerides. Nevertheless the opportunity to study the limitations of the

* Having the sample leaving the syringe needle as a liquid is as important as depositing it inside the column. Thus "cold on-column injection", or just "cold injection", may be more informative than "on-column injection".

vaporizing injector with these extremely high boiling substances promoted us to run some quantitative experiments.

EXPERIMENTAL AND RESULTS

Cold on-column injection

Our test mixture contained equal amounts of *n*-pentadecane (*n*-C_{15}) and the triglycerides tri-10, -12, -14, -16, -18 and tri-18:1, dissolved in hexane. This sample was separated on a SE 52 column (10 m × 0.3 mm), film thickness 0.12 μm, with persilanized support[5], at 100° to elute *n*-C_{15} and a temperature program of 5°/min from 240 to 350° for the triglycerides (0.8 atm H_2 as carrier gas).

Cold injections (using an injector modified according to ref. 9) gave results with by far the lowest standard deviation: for peak areas normalized on *n*-C_{15} as internal standard, the standard deviations were between 1.7 and 3.2%; for triglyceride peak areas normalized on tri-10, the S.D.s were between 0.8 and 1.3% (18 injections). However, as shown in Fig. 2, the triglyceride peak areas were only *ca.* 80% of that of the alkane *n*-C_{15} (present in the same amount). The triglyceride response on the flame ionization detector (FID), as estimated by the CH-content, is *ca.* 83% of that of an alkane for tri-10 and 89% for tri-18. The differences between the expected and the observed peak areas seem to be significant and indicate some discrimination of the larger molecules, increasing from *ca.* 3% for tri-10 to nearly 10% for tri-18. We do not believe that these losses occur during the injection. The fact that the first injection onto a column and (to a lesser degree) the first injection in the morning showed higher discrimination than the following ones points to the

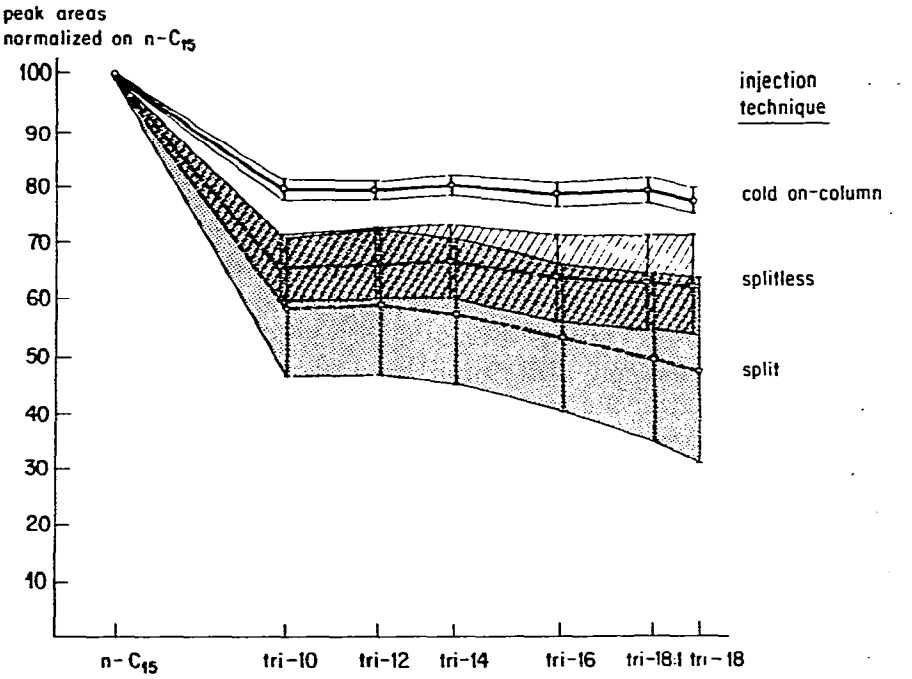

Fig. 2. Peak areas and their standard deviations for triglycerides normalized on the alkane *n*-C_{15} as obtained with different injection techniques (peak areas not corrected by the response factors on FID).

possibility that some losses occur in the column. Experiments with two other (similar) columns showed slightly larger triglyceride peak areas (hence somewhat lower adsorption or degradation of the triglycerides).

Splitless injection

For the vaporizing injection, using a syringe to introduce the sample, we were interested in differentiating between discrimination occurring in the injector and the losses due to insufficient elution out of the syringe needle. The latter were determined on the basis of the n-C_{15} peak area, because this alkane was previously found to leave the needle satisfactorily, its boiling point being some 150° below the injector temperature (400°)[10]. Conditions: syringe, 10 μl, with a needle of length 3 in. and a dead volume of 1 μl; injector, Carlo Erba (Milan, Italy), as described[11], usually equipped with a glass liner (80 mm × 4 mm I.D.) which was carefully kept clean during these experiments.

As previously shown for alkanes[10], the "hot needle" injection technique gave the lowest discrimination and the highest reproducibility of the results also for the triglycerides: the empty needle is preheated in the injector before the sample is rapidly introduced into it. The "hot needle" technique proved to be considerably superior to the "solvent flush" method. The solvent plug behind the sample did not noticeably improve the elution of the triglycerides, since the pure solvent does little or no washing of the very hot walls of the syringe needle (rather the solvent glides over a cushion of vapour, even when the injection is done as quickly as possible, thus minimizing the warming up of the needle). All the following data refer to the "hot needle" technique.

Discrimination occurring with vaporizing injection (split or splitless) was calculated on the basis of peak areas obtained by cold injections.

The insufficient elution out of the syringe needle accounted for nearly all the discrimination of the triglycerides observed with splitless injection. The losses amounted to 14–20% of the peak area of n-C_{15}. Reinjections of the material left in the syringe needle as described[10] have shown that some 7% of the n-C_{15} (corresponding approximately to the proportion of whole solution observed near the plunger after the injection) and 20–28% of the triglycerides remained in the needle. This result with 1 μl sample volumes (needle content) was not changed basically when 2 μl of the sample were introduced. As shown in Fig. 2, all triglycerides were affected by these losses by a similar proportion. Thus, as soon as a triglyceride is used as internal standard, quantitations suffer relatively little from these losses (great errors occur only if the compounds contained in the sample range from those able to leave the needle as a vapour to those expelled exclusively as droplets). However, standard deviations of results with splitless injections (fifteen runs) were some four to six times higher than of those obtained by cold injection (9–13%). This standard deviation was not significantly reduced when the peak areas were normalized on tri-10 instead of n-C_{15}.

Split injections

Split injections gave inconsistent results, depending on factors we did not understand. However, the discrimination of the triglycerides over n-C_{15} was constantly higher than for splitless injections. The proportion of triglycerides remaining

in the syringe needle was found to be some 25–50% higher than for the splitless sampling (the proportion of n-C_{15} left in the needle being approximately the same). This probably reflects an insufficiently preheated carrier gas at the relatively high gas flow-rates through the injector during the split injection (for these experiments the split flow was 150 ml/min, using a split ratio of *ca.* 1:50). For about a third of the runs, these losses in the syringe needle explained the observed discrimination fairly well, while for the others there must have been an additional, very inconsistent mechanism leading to an increased discrimination mainly of the larger triglycerides. Consecutive determinations usually gave standard deviations between 10 and 18%. However, the next mornings' data showed (with the same consistency) different mean values. Nine sets of results were obtained on the same instrument and the same column, but on different days during nearly 2 months. For the total of 54 runs, the standard deviation was between 21 and 35% if the triglyceride peak areas were normalized on n-C_{15}, and between 15 and 24% when tri-10 was used as internal standard.

It is of interest that the use of 1.5 in. syringe needles (providing a distance of 5 cm between the tip of the needle and the column entry) gave results with somewhat lower discrimination than injections with 3 in. needles (1 cm distance). Standard deviations of the fourteen runs with the shorter needle were some 4% lower. As the proportion of sample material left in the syringe needle was about the same in each case, we conclude that the length of the syringe needle influences the discrimination mechanism occurring in the injector. This finding seemed to indicate that the non-homogeneity of the sample vapour at the split point could be the cause of the extra discrimination and the high variance of the results. However, the introduction of glass wool into the liner of the injector, as suggested for example by Schomburg *et al.*[12] had a clearly negative effect: it increased the discrimination as well as the standard deviation. Part of these increased losses are probably due to condensation or degradation of the triglycerides on the glass wool surface as was demonstrated by the fact that splitless sampling into such injectors caused the heavier triglycerides to disappear almost completely.

The extra discrimination which seemed to be fairly specific for split injections (and which was not due to selective elution out of the syringe needle) affected the various triglycerides differently. In strongly discriminated runs some 20% of the tri-10 eluted from the syringe needle disappeared, whereas the corresponding proportion for the tri-18 was *ca.* 50%. Hence this kind of error can hardly be matched with an appropriate choice of an internal standard.

DISCUSSION

We were not really surprised that cold (on-column) injection gave much better results than the conventional vaporizing sampling since cold injection deposits the sample in its original liquid form into the capillary inlet and shortens the sampling process drastically, thus eliminating a large number of potential changes in the sample composition. However, we were surprised indeed that the sample discrimination in the vaporizing injector was hardly significant in the case of splitless injections. When a triglyceride is used as internal standard (eliminating errors due to the syringe needle) the mean values seem to be fairly accurate. However, split in-

jections were found to be inappropriate for the analyses of triglycerides, since accuracy and precision were poor.

If triglycerides were injected without a solvent, one or a few droplets would leave the syringe, usually falling to the bottom of the injector, and virtually no triglyceride material enters the column. There is only a low probability that one of these droplets will hit the head of the column. For such samples the (volatile) solvent is of great importance, not only as a diluent, but also to promote sufficient evaporation. The solvent thus serves as a propellent, producing an aerosol by the spray effect. We assume then that most of the triglyceride sample leaves the syringe as very small droplets and enters the capillary as a fog rather than as a real vapour.

We find it difficult to explain the high standard deviation resulting from split or splitless injections. For splitless sampling it would not have been surprising if triglyceride material had condensed on the surfaces in the injector, especially the cold spots toward the septum (measured to be at 150° when the injector was set at 400°), possibly also on dirt or septum particles which have fallen into the glass liner. As the transfer time (splitless period) was 40–50 sec there was plenty of time for such condensation. Condensed materials are not likely to re-evaporate rapidly and should therefore have exhibited large losses. However, instead of such rather reproducible losses, only a random variance was observed.

It is not easy to rationalize the results of the split sampling. The large number of small particles in an aerosol would be expected to be split in a reproducible manner (according to laws of statistics) which would not exclude systematic discrimination of parts of the sample due to the different diffusivity of the particles and vaporized molecules at the split point[13] or due to an uneven split ratio for different sample components reaching the split point at different times (caused by the fluctuation of the split ratio during the injection[14]). However, these processes cannot easily explain the experimental data.

REFERENCES

1 A. Kuksis, in G. V. Marinetti (Editor), *Lipid Chromatographic Analyses*, Vol. L, Marcel Dekker, New York, Basle, 2nd ed., 1976, p. 215.
2 W. Eckert, *Fette, Seifen, Anstrichm.*, 79 (1977) 360.
3 G. Schomburg, R. Dielmann, H. Husmann and F. Weeke, *J. Chromatogr.*, 122 (1976) 55.
4 E. Schulte, *12th International Symposium on Chromatography, Baden-Baden, 1978.*
5 K. Grob, G. Grob and K. Grob, Jr., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 31.
6 K. Grob, H. P. Neukom and R. Battaglia, *J. Amer. Oil Chem. Soc.*, in press.
7 K. Grob and K. Grob, Jr., *J. Chromatogr.*, 151 (1978) 311.
8 K. Grob, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 263.
9 M. Galli, S. Trestianu and K. Grob, Jr., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 366.
10 K. Grob, Jr. and H. P. Neukom, *J. High Resolut. Chromatogr. Chromatogr.Commun.*, 2 (1979) 15.
11 K. Grob and K. Grob, Jr., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 57.
12 G. Schomburg, H. Behlan, R. Dielmann, F. Weeke and H. Husmann, *J. Chromatogr.*, 142 (1977) 87.
13 H. Bruderreck, W. Schneider and I. Halasz, *J. Gas Chromatogr.*, 5 (1967) 217.
14 K. Grob and H. P. Neukom, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, in press.