Journal of Chromatography, 151 (1978) 311–320

CHROM. 10,607

ON-COLUMN INJECTION ON TO GLASS CAPILLARY COLUMNS

K. GROB
GC-Laboratory, ETH Zürich, EAWAG, 8600 Dübendorf (Switzerland)
and
K. GROB, Jr.
Inst. f. allg. Botanik/Pflanzenphysiologie, ETH Zürich, Sonneggstrasse 5, 8092 Zürich (Switzerland)
(Received September 5th, 1977)

SUMMARY

Sample introduction on to capillary columns includes evaporation of the sample as an important step. Except for volatile substances, this evaporation step entails deleterious effects and is the primary cause of the limited precision and accuracy of quantitative analyses performed with capillary columns. A second factor that limits the potential of capillary columns is the injection septum. A solution to the problems seems to be on-column injection by means of an injector functioning without a septum. There are important arguments in favour of maintaining the classical syringe for injection, and such an injector is described and some examples of applications are given.

PROBLEMS WITH INJECTION ON TO CAPILLARY COLUMNS

At present, sample introduction on to capillary columns is carried out routinely according to a few well known methods. The results seem to be satisfactory and there have been no signs of widespread concern. A more detailed study of the situation reveals, however, that fundamental problems exist.

The problems were recognized even in the early days of capillary column gas chromatography (GC). As far as we know, the first practical attempts to improve the injection technique were made in 1962 by Zlatkis and Walker[1], who reported on-column injection on to wide-bore steel capillary columns. In 1965, Desty[2] presented the most thorough study of the problem. He stated that probably the only solution to the problem of properly introducing high-boiling or thermally labile substances would be to "inject the very small amount required directly on to the column where it can dissolve in the stationary phase in the top few plates and thus be subjected to the lowest possible temperatures —that of the column itself"; this statement is still valid, but nevertheless it seems to have been overlooked. Even in chapters on sample introduction, several textbooks written after 1965 do not refer to Desty's ideas.

Considerably later, Verzele *et al.*[3] and Badings *et al.*[4] reported on-column injection on to glass capillary columns, and Verzele *et al.* indicated some doubtful

aspects of conventional injection, especially with respect to quantitative work. Both groups, however, recommended on-column injection for wide-bore (I.D. 0.6 and 0.9 mm, respectively) capillaries only.

Schomburg *et al.*[5] were the first to apply on-column injection with regular-size (I.D. *ca.* 0.3 mm) glass capillary columns and to describe equipment for the practical use of the method. We have been working in the same field for a long period and we present here our experiences and results.

Problems with conventional injection

Sample introduction on to 0.2–0.4 mm wide capillary columns has always been carried out via an evaporation step, regardless of whether stream splitting, splitless injection, the falling needle or a pre-column was applied. This step is not based on necessity. The reason why the evaporation step nevertheless has been generally accepted for almost 20 years may derive partly from tradition. GC was originally developed for the analysis of gases, so that a gasification step as the start of an analytical run would seem to be justified. This approach was automatically conveyed into today's conditions, under which most injected samples are, after vaporization, fully or predominantly condensed again on a short section of the column inlet. The initial evaporation is, therefore, no longer directly related to the chromatographic process, but is simply part of a sample introduction procedure (a poor one).

The obvious drawbacks of the evaporation step are the following.

(1) The sample is efficiently diluted with carrier gas, while in the subsequent step every care and a great deal of sophistication are taken to concentrate it again to yield a narrow starting band.

(2) Thermally labile sample components may be altered, especially when flash evaporation has to be applied.

(3) It is difficult to reproduce an exact amount of sample actually reaching the column.

(4) Increasing quantitative discrimination occurs with sample components showing an increasing tendency to be adsorbed.

(5) With increasing injector temperature, an increasing part of the sample is evaporated from the metal surface of the hot syringe needle. Under these conditions, "all-glass" injectors in fact hardly exist. In summary, the problems with quantitative work on capillary columns, (as Verzele *et al.*[3] express it: ". . . reproducibility is excellent, but the results are wrong"), are caused by injection via evaporation.

Injection has been the major, if not the only, respect in which packed columns have been superior to capillary columns. On-column injection completely eliminates all of the above drawbacks, thus favouring the analysis of heat-sensitive material and exact quantitative work.

Role and effects of the septum

While gas chromatographs may have very different designs, almost all of them carry a septum on top of the injection port. Obviously it is the convenience of this component that has led to its general acceptance. Nevertheless, injection through an elastic septum is another basically poor technique, the replacement of which will probably be an important task in the future, at least in capillary column GC.

Septum flushing devices, which have become common in injection ports, over-

come two weaknesses of the septum: firstly, adsorption and subsequent release of vaporized sample causing tailing or artifacts, and secondly constant release of volatile plastizisers causing ghost peaks and baseline drift in temperature programmed runs. Unfortunately, the latter effect is not completely eliminated even by a perfectly working flushing system, because the syringe needle, when passing through the septum, is covered with trace amounts of organic contaminants which are transferred into the vaporizer. The corresponding residual septum effect may become troublesome in analyses run with high sensitivity and especially in high-temperature work.

A third effect of the septum cannot be controlled by a flushing device. Almost every passage of the syringe needle cuts a minute rubber particle out of the septum and, during injection, the liquid or vapour stream leaving the needle flushes this particle into the injector cavity. It is obvious that plastic deposits accumulating in the injector in this way are undesirable, hence the emphasis on an injector design that permits easy and frequent cleaning. In our experience, the transfer of septum particles is less dependent on the shape of the needle tip than is commonly believed.

These facts lead to the almost inevitable conclusion that the septum is an undesirable component, particularly in capillary column GC as the importance of septum effects increases with decreasing amounts of sample material introduced. It is surprising, therefore, that there have been few attempts to improve the injection step. In the near future, a new impetus for such attempts may result from attempts to achieve direct sample introduction into capillary columns. The transfer of septum particles into the inlet section of a capillary column entails, of course, much more negative effects than transfer into an open injector cavity. Under the conditions of on-column injection, therefore, the septum is not an undesirable but rather a prohibited component.

REALIZATION OF ON-COLUMN INJECTION

Principles governing on-column injection

On-column injection works perfectly provided that a number of requirements regarding temperature and flow conditions are fulfilled, as follows.

Temperature conditions. It is the most elementary principle of the method that the sample be evaporated from the column wall to start chromatographic migration. Thus, the evaporation point (Fig. 1) is identical with the injection point. The sample has to arrive at this point without premature evaporation, which would cause loss of sample and contamination of the equipment. From this, the following basic requirements, regarding the design of an injector, are easily deduced. Firstly, the injector assembly has to be cooled, *e.g.*, to a maximum temperature of 35° if solvents such as pentane and diethyl ether are to be used. Secondly, the injection point has to be sufficiently distant from the oven wall to be fully within the temperature control of the oven, but not too distant to avoid warming up the injection needle with consequent evaporation from the needle. The optimal distance greatly depends on the design of the oven; an average distance is 10 mm.

Pressure and flow conditions. Here again there are two fundamental requirements. Firstly, there must be the possibility of controlling, over a wide range, the rate at which the liquid sample leaves the injection needle at the injection point. This necessity is best explained by means of a typical application. If we assume that 2 μl of

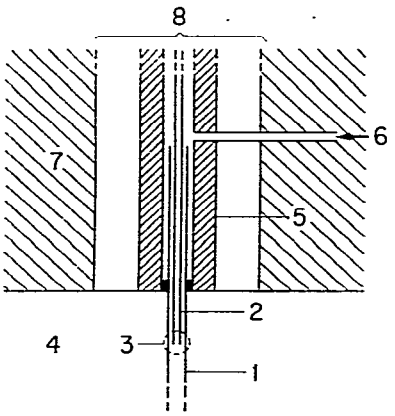

Fig. 1. Schematic diagram of an on-column injector. 1, Capillary column; 2, injection needle; 3, injection point; 4, column oven; 5, injector body; 6, carrier gas inlet; 7, oven insulation; 8, cooled volume.

moderately to high-boiling sample material dissolved in pentane have to be injected at an oven temperature of 100°, then rapid injection would lead to explosive evaporation with a sudden pressure increase. A large part of the sample would immediately be flushed back into the cool injector, where the sample would be deposited, with the exception only of its most volatile components. If the same injection is carried out at a controlled rate, *e.g.*, within 3 sec, no significant increase in pressure is caused. The gently produced pentane vapour mixes with the carrier gas while the material of interest is cold-trapped at the injection point, forming an ideally short starting band for the subsequent analysis. With equipment that does not permit control of the injection rate, only very small samples can be injected. A rapid 2-μl injection at an oven temperature reduced to 40° to prevent sudden evaporation with pressure shock would result in a liquid plug migrating through some length of the capillary column before shortening and finally disappearing. Obviously this alternative is again undesirable.

The second requirement concerns the design and the manipulation of the component carrying the injection needle. It must be designed such that the needle releases no sample material within the injector, *i.e.*, in the cold part of the capillary column. Part of the released material might then be flushed by the carrier gas into the heated column. The remainder, if sufficiently volatile, would vaporize slowly or would not vaporize at all but would be rinsed away by the next injection.

A further requirement is so fundamental that it might seem too trivial to be mentioned. It means the possibility of selecting and determining the injected sample size. In the context of on-column injection, however, this requirement is far from trivial.

Producing short starting bands. The simplest and most direct means of obtaining a very short sample plug at the column inlet is to inject small samples very rapidly. In many applications, however, large samples have to be introduced. Thus the question arises of how, under the conditions of on-column injection, large samples can be concentrated to short starting zones. The answer is that the same two principles

used with conventional injection apply, namely cold trapping and the solvent effect. Cold trapping, as shown in the above example, is even more efficient with on-column injection. Because of much higher solvent concentrations, the solvent effect requires some additional care. A more detailed discussion of the application technique will be given in a forthcoming paper.

On-column injection with a syringe

One of several possible components for carrying the injection needle is a syringe, and throughout our work we have been trying to keep the syringe as an injection tool for the following reasons. The syringe is the simplest and safest means and, at the same time, fulfils three basic requirements, namely sample volume determination, injection rate control and full control of sample release (no premature sample loss). In addition, the syringe is an extremely convenient tool; it is easily cleaned, sample contamination is easily avoided and it does not consume more sample than is needed for one run. It is well known (see, *e.g.*, the study by Grant and Clarke[6]) that severe quantitative errors can be produced by a syringe. Detailed study shows, however, that the main source of trouble is partial sample vaporization out of the hot syringe needle, whereby part of the sample is fractionated in a poorly reproducible way. In contrast, when used for on-column injection with a cold injector, the syringe works under its most ideal conditions.

The outer diameter of the syringe needle should be at least 0.05 mm less than the inner diameter of the column. A needle that fits too tightly into the column over a length of several centimetres will almost plug the column and the flow conditions for the carrier gas will therefore be considerably altered during the injection period. Recommendable and commercially available syringes for on-column injection into 0.3-mm and wider columns are, *e.g.*, the Hamilton No. 75 or No. 701 with a 32-gauge needle (O.D. 0.23 mm), and with an optional needle length that has to be adapted to the gas chromatograph in use, according to the outlines given in the previous section.

The great problem with the use of syringes is that we have to work without an injection septum and it is therefore difficult to stabilize the pressure of the carrier gas at the column inlet during injection. One means of effecting this stabilization is to use a pressure lock system, but this unfortunately precludes practical work with the usual syringes because of their impractical length and the inconstant shape and diameter of the glass barrel. Schomburg *et al.*[5] used the pressure lock principle and consequently sacrificed the important advantages of the syringe.

*Design and functioning of an on-column injector**

Our solution to the problem of constant-pressure injection without a septum may be considered as a quasi-pressure lock system which, compared with a real pressure lock, is not completely leak-free during the injection period. The design of our injector (Fig. 2) is best explained by discussing the steps involved in the operation of injection. Before starting an injection, the stop valve is in its normal, *i.e.*, closed position. The syringe (we use a Hamilton No. 75 with an 80-mm long 32-gauge needle) is filled without taking account of the volume of the needle as it is not emptied during

* The device has become available from Brechbueler AG, 8902 Urdorf, Switzerland, representative of Carlo Erba Strumentazione S.p.A.

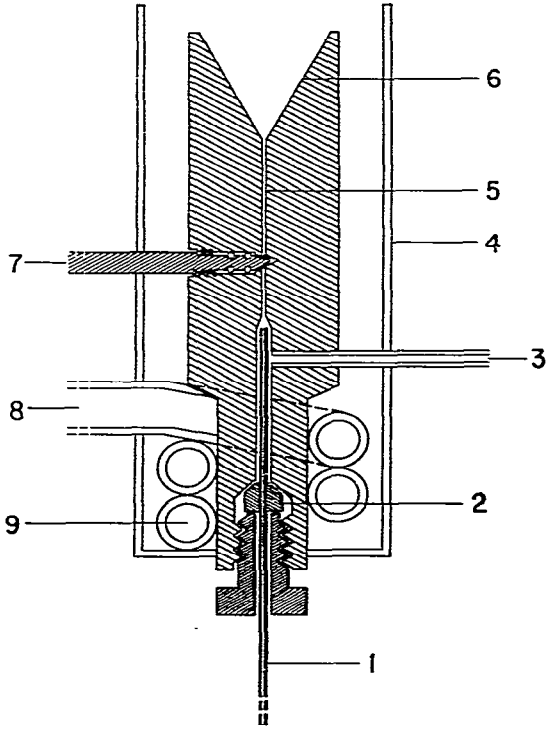

Fig. 2. Construction of an on-column injector. 1, Glass capillary column; 2, graphite fitting; 3, carrier gas inlet; 4, steel beaker; 5, 0.3-mm channel; 6, conical aperture; 7, stop valve; 8, coiled copper tubing, cold air in; 9, cold air out. The assembly is mounted in the oven insulation so that the column fitting is accessible from the column oven.

injection. The conical aperture on top of the injector guides the needle into the 0.3-mm channel. The introduction of the needle close to the stop valve, a position which is indicated by an external mark, means that the channel is almost completely blocked. Therefore, upon opening the valve, the gas flow through the channel is so low that no measurable pressure drop results at the column inlet. The syringe is now pushed down and the needle is guided into the capillary column. The conical aperture of the injector leads the glass barrel into its final position. At the same time, the tip of the needle reaches the injection point (Fig. 1), provided that the needle has the appropriate length. Now the syringe plunger is depressed rapidly in the case of a 0.1–0.2-μl injection, or at a controlled rate when a larger volume is injected. After injection, the syringe is moved back to the level with the tip just above the stop valve. The valve is then closed and the syringe is withdrawn.

A steel beaker keeps the injector isolated from the hot insulating material of the oven top. Cold air is conducted around the lower part of the injector body through copper tubing. After leaving the tube at the bottom of the beaker, the air flushes the free space between the injector and the beaker. An important further detail is that a relatively wide-bore (I.D. 1 mm) tube is used for the carrier gas supply, otherwise even the small leakage through the injector channel might cause a measurable pressure drop in the injector.

Before mounting the capillary column, the cut (not flamed) end is internally

widened conically by means of a diamond pen and the outer edges are smoothened with a file. During this operation, a high flow-rate of gas enters the column at the opposite end so as to prevent glass dust from entering the column.

COMPARISON OF ON-COLUMN WITH CONVENTIONAL INJECTION

Quantitative analysis of adsorption-sensitive material

The work reported in this section has been performed by the organic-analytical group of the Swiss Federal Institute for Water Resources and Water Pollution Control (EAWAG) and has been described in part elsewhere[7].

The sample used for checking the reproducibility (precision) of repeated runs was a sediment extract dissolved in methylene chloride containing individual polynuclear aromatic hydrocarbons (PAHs) at concentrations of *ca.* 1 ng/μl. Thus, at least 1 μl, without splitting, had to be introduced into the column in order to obtain sufficient signals from the flame-ionization detector. To check the accuracy, a standard PAH solution of similar concentration was prepared. From the integrated peak areas, response factors for the individual PAHs were calculated with respect to the internal standard (1-chlorotetradecane).

With the use of the same glass capillary column (20 m $\times$ 0.3 mm I.D., SE-52), the following injection procedures were compared. For on-column injection, 2 μl of the methylene chloride solution were slowly injected within 8 sec on to the column at an oven temperature of 80°. For conventional injection without splitting, 2 μl of the same solution were injected rapidly into the closed injection port at 270° while the column was maintained at room temperature. After 30 sec, the injector was flushed with carrier gas. Rapid injection was applied, although slow injection would have been desirable in the conventional procedure to reduce diffusion of sample vapour towards metallic parts of the injector. During slow injection, however, the syringe needle becomes so hot that the sample is completely evaporated inside the needle, *i.e.*, on a metallic surface. On the one hand, this results in partial evaporation for only certain PAHs; while on the other hand, some substances undergo breakdown reactions. For the same reason, injector temperatures above 270° did not improve the evaporation step.

As Table I shows, on-column injection yields standard deviations that are more than three times lower from four repeated runs. It is interesting to compare the standard deviations for minor (*e.g.*, benzo[*a*]pyrene) with that of major (*e.g.*, perylene) components. While conventional injection produces a much higher relative

TABLE I

COMPARISON OF RESULTS OF ON-COLUMN AND CONVENTIONAL INJECTION

PAH	*Relative standard deviation (%)*		*Response factor*	
	On-column	*Conventional*	*On-column*	*Conventional*
Phenanthrene	3.8	17	0.62	0.74
Fluoranthene	3.4	16	0.62	0.78
Pyrene	4.2	12	0.63	0.79
Benzo[*a*]pyrene	5.7	22	0.66	1.03
Perylene	5.3	10	0.69	1.02
Coronene	3.2	—	0.70	—

standard deviation for the minor component, the corresponding figure obtained from on-column injection is only slightly increased. Considering the absolute level of the standard deviations, it should be remembered that trace components present in a very complex mixture have been analysed.

The information obtained from standard deviations is in good agreement with that from response factors. While after on-column injection the response factors increase rather slowly with increasing molecular weight (which we attribute to residual adsorption effects by the column), a much more pronounced increase after conventional injection is observed. This increase, indicating a strong discrimination of substances with decreasing volatility, must be caused by adsorption processes that occur in the injector cavity as the same column was used for both types of injection. It should be added that every care was taken to minimize adsorption by using an ideal vaporizer geometry that yields the minimal contact of the sample with metal surfaces, and by carefully avoiding adsorbing impurities.

Both comparisons confirmed our experience that important advantages are gained when the sample evaporation is omitted as a step in the injection procedure.

Analysis of heat-sensitive substances

As an example we chose mustard oil from radishes, which is currently under investigation in our laboratory. The structure of the main components is shown in Fig. 3. Quantitative analysis of the substances is especially difficult, for three reasons.

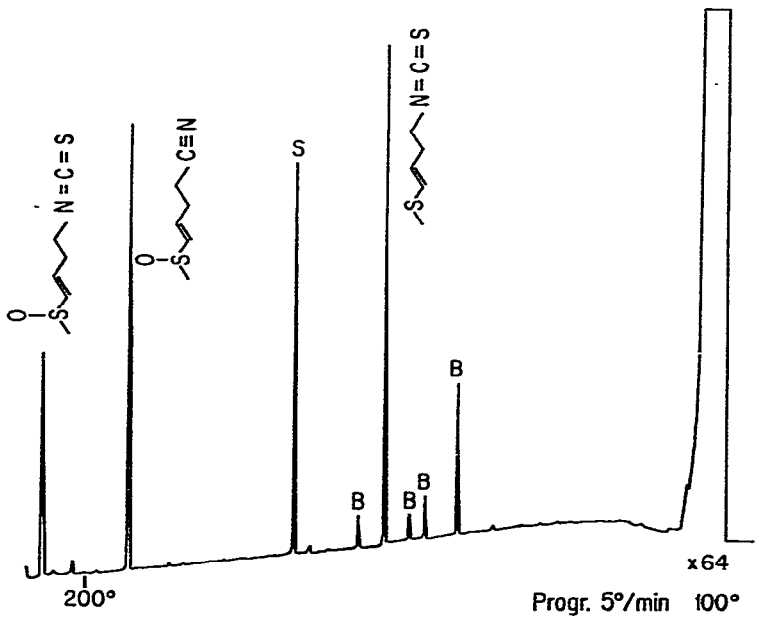

Fig. 3. Analysis of mustard oil. Column, 15 m × 0.3 mm I.D., 0.08-μm Pluronic 64 on acidic support. Conventional injection: 1.0 μl of methylene chloride solution, splitless, column at room temperature, rapid heating to 100°, programmed at 5°/min to 200°. Detector: NPD, N-mode. Main mustard oil components are indicated by their formulae. B, Breakdown products; S, internal standard (1-cyanopentadecane, 5 ng/μl). After on-column injection, no breakdown products are observed.

Firstly, they are so reactive that, in order to avoid losses during clean-up procedures, we preferred to analyse the raw methylene chloride extract from radishes. Secondly, the substances are extremely sensitive to adsorption, and thirdly they can be analysed on acidic columns only.

According to our experience, quantitative analysis of low concentrations of mustard oil components on capillary columns is hardly feasible with conventional injection. Regular manipulation of the syringe produces severe discrimination (of the order of 50% for the last component) of the heavier substances owing to adsorption on the syringe needle. This problem is effectively overcome by means of a modified manipulation. The sample is completely drawn back into the glass barrel. After introducing the syringe into the vaporizer, the injection is delayed for 5–10 sec, during which period the empty needle is fully warmed up before the sample is moved into it. This manipulation, however, produces breakdown reactions caused by contact between the sensitive substances and the hot metal surface. The use of nickel instead of stainless steel as the needle material did not improve the results, while with platinum needles even increased breakdown effects were observed. The chromatogram in Fig. 3 represents the best compromise between losses by adsorption and losses by breakdown reactions. Important amounts of breakdown products are still eluted while, on the other hand, relative standard deviations of repeated runs, mainly due to adsorption, are still of the order of 20–30%. The results are not improved when a plug of pure solvent is injected after the sample.

After on-column injection no breakdown products are observed. At the same time, there is no longer any evidence for adsorption effects during injection. Thus, on-column injection is the only means of analysing reasonably sensitive substances such as mustard oil components.

Comparison over a wide volatility range

Finally, we wished to check on-column injection not for extreme sample characteristics (volatility, instability) but with the aim of comparing the new technique with the common ones under the usual conditions, *i.e.*, with chemically and thermally stable samples with volatilities ranging from moderately high to moderately low. We

TABLE II

COMPARISON OF RESULTS FOR ANALYSIS OF FATTY ACID METHYL ESTERS

Analyses performed at Kantonales Laboratorium, Zürich; we thank Dr. E. Romann for permission to publish the results.

Number of carbon atoms in fatty acid	*Relative standard deviation (%)*		
	On-column (0.25–1.0 µl, 1:100,000, injection rate 0.2–5.0 sec)	*Splitless (1 µl, 1:100,000)*	*With splitting (1 µl, 1:4000–1:10,000, splitting ratio 1:2–1:150)*
8	1.6	1.2	1.8
14	1.3	2.4	0.7
16	1.7	3.4	2.9
18	1.6	5.8	3.8
20	1.1	5.1	3.4
22	1.2	6.1	3.5

selected a series of fatty acid methyl esters in which the ester of lauric acid (C_{12}) was chosen as an internal standard. With every injection technique, eight analyses were run. The relative standard deviations are given in Table II.

Before comparing the results, the following details should be taken into account. For the eight injections with splitting as well as for the on-column injections relatively wide variations of the injection parameters (see Table II) were applied as we consider such variations to be realistic, *e.g.*, when the same analysis is run by a different operator. Further, one must realize that for splitless injection, in comparison with injection with splitting, a 10–20-fold more dilute solution was used. Thus, splitless injection is effected under distinctly more difficult conditions. It is important to note that the same difficult conditions were also present with on-column injection.

The most obvious comparison is that between splitless and on-column injection, as both were run under similar conditions. It is not surprising that the deviations for splitless injection are approximately three times greater, part of the sample remaining in the closed vaporizer for up to 30 sec with the corresponding opportunity for intense diffusion. The diffusion becomes increasingly troublesome with decreasing volatility, which is clearly illustrated by the increasing deviations with increasing molecular weight. It seems more surprising that injection with splitting, despite the higher concentrations used, gives almost double the standard deviations obtained with on-column injection. A further important fact is that only on-column injection shows no dependence of the standard deviations on volatility.

ACKNOWLEDGEMENTS

This work was generously sponsored by F. J. Burrus & Cie, Boncourt. We are indebted to Dr. Stuart Wakeham for reading the manuscript.

REFERENCES

1 A. Zlatkis and J. Q. Walker, *J. Gas Chromatogr.*, 1 (1963) 9.
2 D. H. Desty, *Advan. Chromatogr.*, 1 (1965) 218.
3 M. Verzele, M. Verstappe, P. Sandra, E. van Luchene and A. Vuye, *J. Chromatogr. Sci.*, 10 (1972) 668.
4 H. T. Badings, J. J. G. van der Pool and J. G. Wassink, *Chromatographia*, 8 (1975) 442.
5 G. Schomburg, R. Dielmann, H. Husmann and F. Weeke, *Chromatographia*, 10 (1977) 383.
6 D. W. Grant and A. Clarke, *J. Chromatogr.*, 97 (1974) 115.
7 W. Giger and C. Schaffner, *Anal. Chem.*, in press.