Journal of Chromatography, 203 (1981) 193–205
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,356

BENEFITS OF A SPECIAL COOLING SYSTEM TO IMPROVE PRECISION AND ACCURACY IN NON-VAPORIZING ON-COLUMN INJECTION PROCEDURES

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SUMMARY

The use of a special cooling system to cool the first part of the capillary column during rapid, non-vaporizing on-column injection eliminates or at least drastically reduces the consequences of the sample back-returning process. This process produces sample loss, discrimination and injector contamination, not only at oven temperatures around or above the boiling point of the solvent, but also at lower temperatures. The results obtained show that the cooling system is successful in keeping the sample inside the capillary within a reasonably wide range of operating conditions, thereby improving the precision and accuracy of the chromatographic data.

INTRODUCTION

During the last few years the development of capillary column technology has led to columns of better quality. The implication of this is that quantitation is now a valid aim. The critical aspects are moving from the field of column technology to the realm of instrumentation.

It is recognized today that the principal area of instrumentation that can give rise to difficulties is the sample injection system. This often determines the precision and accuracy of the results and represents the main source of error. Apart from maintaining the efficiency of the capillary, the injection system must ensure the transfer of the sample from the syringe to the column in a non-discriminating, non-destructive and non-contaminating manner.

Vaporizing injection systems, which are commonly used in capillary gas chromatography, can change the sample composition during its passage from the syringe to the column. Thermal and catalytic decomposition of sample components inside the vaporization chamber and the discriminating processes which take place in the syringe needle[1] and the vaporization chamber contribute to the change in sample composition. These changes are especially important when the samples are thermolabile or contain

0021-9673/81/0000–0000/$02.50

components with a wide range of volatilities. These phenomena are linked with the sample vaporization process and the operating conditions required for vaporization.

Good injector design and the optimization of operating parameters can reduce the influence of these effects but generally cannot eliminate them. Quantitative analysis therefore requires, in these instances, the use of more than one internal standard and a careful analysis of sample behaviour during its transfer to the capillary.

The simplest method of avoiding these unwanted effects is to introduce the liquid samples directly into the capillary column without vaporization. This idea, used many years ago for sample introduction into packed column and suggested by Desty[2] in 1965 for sample injection into the capillary column, has only recently been resurrected by Schomburg *et al.*[3] and Grob and Grob[4,5].

PROCESSES WHICH CAN PRODUCE SAMPLE LOSS, DISCRIMINATION OF SAMPLE COMPONENTS AND INJECTOR CONTAMINATION DURING ON-COLUMN SAMPLING PROCEDURES

The main advantage of this non-vaporizing on-column injection technique is the non-discriminating, non-destructive and non-contaminating injection of the sample into the capillary column. However, this is only true if processes that cause sample loss, discrimination and injector contamination are eliminated or reduced to such an extent that their consequences have a negligible effect.

All problems result from the difficulties encountered in the complete and irreversible transfer of the sample from the syringe to the capillary (Table I).

If the sample is injected slowly the undesirable processes occur in the syringe needle[6] or on its outer surface[7]. As shown recently by Grob and Neukom[7] and supported by our own results, under normal operating conditions the rapid injection technique must be used to avoid the drawbacks produced by the part of the sample which remains on the external wall of the needle. On the other hand, the rapid injection technique can produce a back-return of part of the sample already in the column and as a consequence produce sample loss, discrimination of sample components and injector contamination.

Sample back-return from the column during rapid on-column injection and procedures used to eliminate its effects

Grob and Neukom[7] suggested that the back-return of sample explains the sample losses observed when the sample is injected quickly at oven temperatures above the boiling point of the solvent. We have found that this process is active even at oven temperatures below the boiling point of the solvent. This difference is probably due to the fact that with rapid injection at oven temperatures around the boiling point of the solvent the sample loss mechanism produces less effects on relatively volatile alkanes, which are partly recovered by the carrier gas, than on heavier compounds.

Therefore, by using samples containing alkanes with a wide range of volatilities we have been able to establish the effect of the back-returning process even at lower oven temperatures.

Moreover, we have found that by using a special cooling system[8] it is possible to avoid or considerably reduce the effects of the sample back-returning process

TABLE I

UNDESIRABLE PROCESSES PRODUCING SAMPLE LOSS, DISCRIMINATION AND INJECTOR CONTAMINATION IN NON-VAPORIZING ON-COLUMN INJECTION SYSTEMS

Type of process	*Mechanism*	*Effects*	*Influence of injector design and operating conditions*
Incomplete and discriminative sample transfer from the syringe to the column	Partial evaporation of the sample out of the syringe needle. This process happens if during the injection part of the syringe needle is heated by a hot environment. The heavy compounds remain partly in the needle while the solvent and the volatiles enter more than expected[6]	Discrimination of sample compounds	Strongly influenced by the injector design and especially by the possibility of dissipating the heat received from any hot environment. The process is especially critical if the sample is injected slowly at temperatures around or above the boiling point of the solvent. The "secondary cooling" avoids it by cooling the bottom part of the needle, down to the injection point, during the injection
	The liquid sample is only partially transferred inside the column. The other part remains on the syringe needle tip and external wall and is partly removed when the syringe is withdrawn. The removed part is concentrated in heavy compounds, the volatiles being partly recovered by the carrier[7]	Sample loss; discrimination of sample compounds; injector contamination	Relatively dependent on the injector design owing to the recovery of volatiles by the carrier gas and the part of the sample which is wiped off in the injector when the syringe is removed. Critical only in slow injections. Strongly dependent on operating conditions: sample size, syringe type, column, I.D., oven temperature, carrier gas flow-rate. "Secondary cooling" does not help. Rapid injection avoids it
Back-ejection of part of the sample from the capillary column inlet	During the rapid injection part of the sample from the rear part of the jet is back-expelled owing to the shock wave created by the rapid evaporation of the front part of the jet. The back-returned vapour and droplets are re-condensed and re-trapped on the external wall of the syringe needle and on the internal wall of the injector[7] (this work)	Sample loss; discrimination of sample compounds; injector contamination	Influenced by the injector design owing especially to the possibility of controlling the back-return of the sample by the effects of the "secondary cooling" system (see also the above-mentioned remarks). Important especially in rapid injection even at temperatures below or around the solvent boiling point and moderate amounts of sample. Critical at higher oven temperatures and larger sample sizes. The "secondary cooling" avoids it (within a reasonable range of conditions) by cooling the first part of the column which acts as a trap.

within a relatively wide range of oven temperatures and sample volumes. The purpose of this extremely simple cooling system[8], called "secondary cooling", is to cool the initial part of the capillary column during the injection and to isolate it from the hot environment (Fig. 1).

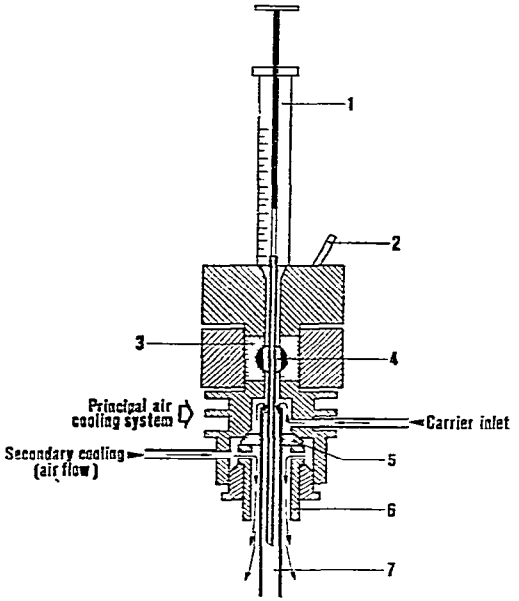

Fig. 1. Modified Grob-type on-column injector, including the secondary cooling system. 1 = Microsyringe; 2 = valve lever; 3 = valve seal; 4 = stainless-steel rotating valve; 5 = column seal; 6 = cooling jacket; 7 = capillary column.

In a previous paper[6] we showed the efficiency of this system in avoiding discrimination of sample components in the syringe needle. In this paper we try to demonstrate the additional benefits of the secondary cooling system in avoiding or considerably reducing the consequences of the sample back-returning process. The stream of air (or another coolant) flowing along the column creates an "intermediate" zone where the temperature varies between that of the injector body, cooled to a value close to room temperature by the "principal cooling system", and that of the "hot" part of the column, entirely controlled by the oven.

According to the model proposed by Grob and Neukom[7], when the sample is injected quickly, small droplets are formed and the jet is ejected far into a zone under the oven control (Fig. 2a). Owing to their large total surface the droplets are quickly evaporated. Rapid evaporation of the sample, representing the front of the jet, creates a pressure wave which produces back-ejection of part of the droplets and vapour representing the rear part of the jet (Fig. 2b). This part of the sample will be re-condensed and re-trapped inside the column, on the outer wall of the syringe needle or inside the injector body, or will eventually pass through the injector to the atmosphere. This depends on the nature and the amount of sample, the design of the injector and the operating conditions.

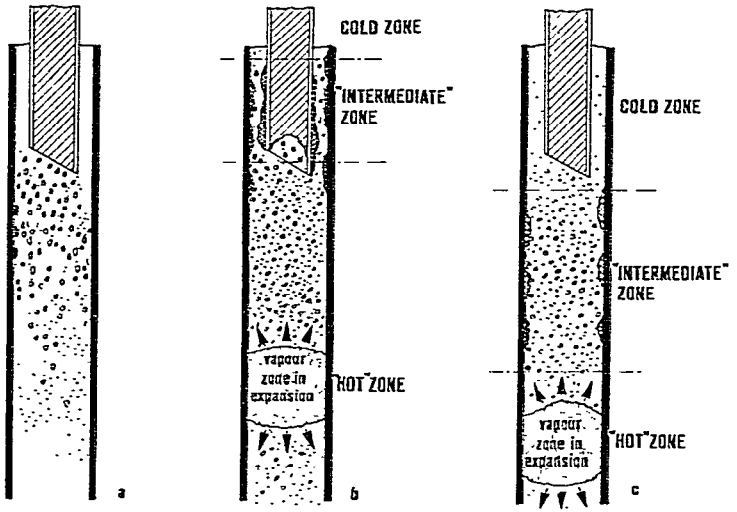

Fig. 2. The back-return of part of the sample during the rapid on-column injection according to the model proposed by Grob and Neukom[7] and the simple model proposed by us to explain the retention of the sample inside the column by the secondary cooling system (not to scale). (a) The jet of sample entering the column during the rapid injection. (b) Without secondary cooling, at oven temperatures around or moderately above the boiling point of the solvent, the rapid evaporation of the front part of the jet produces back-ejection of the rear part. The back-returned droplets and vapour are re-trapped and re-condensed in the "intermediate" zone placed around and above the injection point. (c) Under the same operating conditions but using secondary cooling the "intermediate" zone is shifted and produces "thermal focusing" of the reverse sample flow inside the capillary column. Air cooling can also displace the "rapid evaporation zone" down to the column, attenuating the magnitude of the pressure wave.

At temperatures around or slightly higher than the boiling point of the solvent, without secondary cooling, the sample is re-condensed and re-trapped in the injector area and therefore lost mainly as a liquid when the syringe needle is removed[7]. The heavy components are therefore lost more than the volatiles, the latter being partially recovered by the carrier gas. At higher oven temperatures, a large amount of sample is lost as vapours and small droplets, passing through the injector to the atmosphere. The discrimination is reversed because the volatile components are now preferentially vented.

The back-returned amount of sample depends on many factors and it is almost impossible to quantify it. It is therefore obvious that in order to obtain precise and accurate quantitative results the back-returned sample must be kept inside the column. A possible solution is to reduce the rate of evaporation speed by injecting the sample at a low enough temperature. However, this solution is limited by the fact that the oven temperature must be kept relatively far below the boiling point of the solvent to obtain good precision and accuracy. Moreover, the temperature must be decreased when a large sample volume is injected. This increases the risk of destroying the column by the formation of a liquid plug transported by the carrier through a long tract before being completely evaporated, thus washing off and accumulating the stationary phase. Therefore, injection at low temperatures is generally limited to small sample sizes. The injection at low temperatures has other practical drawbacks.

A relative large number of samples, which for reasons connected with the sample preparation procedures, are normally dissolved in relatively volatile solvents, have to be analysed at relatively high oven temperatures. Much time is therefore spent in cooling the oven to a convenient temperature. If, in addition, the on-column injector is designed in such a way that it continuously accumulates heat from the oven or other sources and is unable to dissipate it, the operator has to wait for a long time until the injection system can be used[9].

The second solution, which has a considerable number of advantages, is temporary cooling of the first tract of the column during injection, using the "secondary cooling" system. The results show that in this mode the sample is kept entirely inside the column for a reasonable wide range of oven temperatures around the boiling point of the solvent and a relatively large range of sample sizes.

In addition to the data presented in the previous paper[6] and here, to support our conclusions other data were obtained in different laboratories using the same type of on-column injector and the secondary cooling system. They all led to the same conclusions. For example, Verzele *et al.*[10] recently reported extremely precise and accurate quantitative data on the piperine content of pepper and pepper extracts by injecting solutions in methylene chloride at 100°C, *i.e.*, 60°C above the boiling point of the solvent.

The effect of secondary cooling can be explained by assuming that the back-returning flow of sample is thermally focused inside the column, in the "intermediate" zone now situated below the injection point (Fig. 2c). Moreover, the cooling of the initial part of the column can probably displace the rapid evaporation zone down into the column, attenuating the effects of the pressure wave.

The recovery of the part of the sample re-condensed and re-trapped in the "intermediate" area of the column is realized as soon as the air stream is switched off. This is due to the special design of the system[6] and to the low thermal mass of the capillary, which can reach the oven temperature in a few seconds. At temperatures considerably higher than the boiling point of the solvent secondary cooling with an air stream is not powerful enough to prevent sample loss.

By using a more effective coolant than air, the permissible oven temperature range can be extended (these aspects will form the subject of a future paper).

EXPERIMENTAL

In order to illustrate the performance of secondary cooling and to justify the mechanism proposed, we used different test mixtures of *n*-alkanes, ranging from *n*-nonane to *n*-tetracontane, dissolved in equal weights in different solvents. The solvents used were *n*-hexane (b.p. 69°C) and acetone (b.p. 56°C). Concentrations between 10 and 20 ng/μl were used for *n*-hexane solutions and around 10 ng/μl for acetone solutions. *n*-Alkanes were used, of course, because of their practically equal response factors for the flame-ionization detector. The experiments were performed on a Fractovap Model 4160 gas chromatograph (Carlo Erba, Milan, Italy) fitted, as standard, with the on-column injector shown in Fig. 1. Glass capillary columns of 0.32 mm I.D. were used. Hydrogen was used as the carrier gas at a flow-rate of 3 ml/min during the injection. The peak areas were measured with Spectra-Physics Model 4100 integrators.

The exceptionally high precision of the peak areas obtained by the cold on-column injection allowed us to use them directly as a measure of the amount of components really transferred to the column. In fact, when secondary cooling is used, the relative standard deviations of peak areas are between 1 and 2%, whereas without cooling the values range from 2.5 to 4%. Our results are supported by those obtained in other laboratories[7,10,11].

Owing to the use of the absolute peak areas, special attention was paid to the constancy of the detector operating parameters. We analysed variations of the peak areas with sample size and component volatility using different injection techniques and different oven temperatures during the injection. Slow and rapid injections, with and without secondary air cooling, were tested. In all instances we used a Hamilton Model 701 SN microsyringe with a Model GA 32 needle (0.23 mm O.D., 0.1 mm I.D.) with a length of 75 mm.

The values reported were calculated on the basis of a minimum of twelve replicate injections for each set of parameters.

RESULTS AND DISCUSSION

Variation of peak area with sample size

According to the mechanism discussed above, the back-ejected part of the sample must increase with increase in sample volume and oven temperature. Therefore, it was expected that air cooling would be efficient even at relatively high oven temperatures provided that the amount of sample is not too large. The results shown in Fig. 3 support this statement.

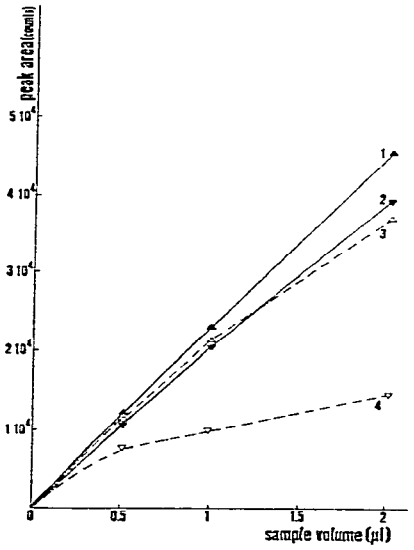

Fig. 3. Variation of *n*-tetradecane peak area with sample size. Rapid injection of an *n*-hexane solution containing about 10 ng/µl at two oven temperatures: 65°C with (1) and without (2) cooling, and 100°C with (3) and without (4) cooling.

They also show that the differences between peak areas obtained with and without cooling are significant even at temperatures below the boiling point of the solvent and especially for large sample sizes. These differences become very considerable at higher oven temperatures.

We observed that at higher oven temperatures slow injections gave better results than rapid injections. This is explained by the fact that under these conditions, the back-ejection of the sample becomes so important that slow injection improves the results even if part of the sample remains on the external wall of the needle. Secondary cooling is again essential in order to prevent discrimination of the sample components in the syringe needle[6].

According to our supposition, the air cooling system must be efficient enough if the oven temperature is not too high. The results shown in Fig. 4 demonstrate that for temperatures around the boiling point of the solvent the air cooling ensures complete recovery of the tested sample components, regardless of their volatility, even if relatively large amounts of sample are injected.

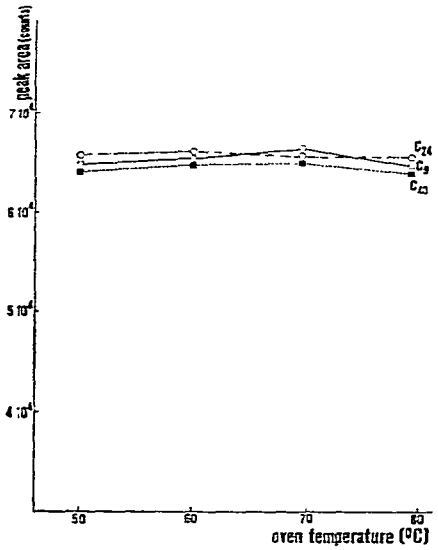

Fig. 4. Variation of *n*-nonane (□), *n*-tetracosane (○) and *n*-tetracontane (■) peak areas with oven temperature. Rapid injection with secondary cooling of 2 μl of *n*-hexane solution containing about 20 ng/μl of each alkane.

Discrimination of sample components produced by different injection techniques

To clarify further and demonstrate the influence of the cooling system, we measured the variation of the *n*-alkane peak areas with the carbon number using different injection techniques and oven temperatures. Discrimination curves, similar to those used by Grob and Neukom[1], were obtained for each injection technique (rapid or slow, with or without cooling) and set of operating conditions. The relative positions of these curves and the changes induced by variation of the oven temperature gave useful information about the sample loss mechanism.

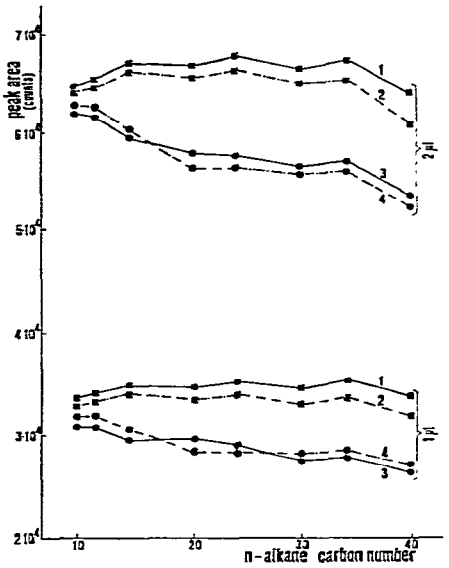

Fig. 5. Discrimination curves of *n*-alkanes (peak area *versus* carbon atom number) for different amounts of sample injected at 55°C using different injection techniques. Test mixture in *n*-hexane (b.p. 69°C) containing about 20 ng/μl of each component. The upper curves correspond to 2 μl and the lower curves to 1 μl of injected sample. (1) Rapid, cooling; (2) rapid, no cooling; (3) slow, cooling; (4) slow, no cooling.

We shall first consider the discrimination curves obtained by injecting 1 and 2 μl of *n*-alkane test mixture in *n*-hexane at 55°C, which is an oven temperature considerably lower than the boiling point of the solvent (Fig. 5).

The curves show that even at this low oven temperature the back-returning process is still active and therefore the secondary cooling improves the accuracy of the results. However, the sample loss and discrimination are not too high if the sample size is not too large. With smaller sample sizes the main effects of the random back-returning process consist in a decrease in the precision of the peak area. The relative standard deviations of peak areas obtained without cooling are twice those found when cooling is used (Table II).

TABLE II

IMPROVEMENT OF PRECISION OF GAS CHROMATOGRAPHIC DATA BY USING THE COLD ON-COLUMN INJECTION TECHNIQUE

Oven temperature: 55°C.

Solvent	*Amount of sample (μl)*	*Sampling conditions*	*Relative standard deviation of peak area (%)*							
			C_9	C_{11}	C_{14}	C_{20}	C_{24}	C_{30}	C_{34}	C_{40}
n-Hexane	1 μl	Cooling	1.6	1.6	1.6	1.3	1.5	1.5	1.5	1.6
		No cooling	2.8	3.5	3.3	2.9	2.9	2.7	2.5	2.6
	2 μl	Cooling	1.3	1.3	1.3	1.6	1.8	1.8	1.9	1.7
		No cooling	3.0	3.1	3.1	2.8	2.7	2.7	2.8	2.8
Acetone	1 μl	Cooling	2.0	1.4	1.5	1.4	1.4	1.3	2.2	—
		No cooling	4.1	2.7	3.0	2.7	2.7	3.2	4.3	—

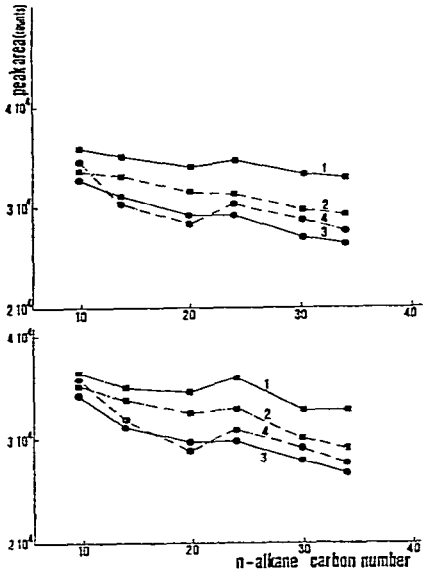

Fig. 6. Discrimination curves of *n*-alkanes injected at 65°C (lower curves) and 70°C (upper curves) using different injection techniques; 1 μl of test mixture in *n*-hexane (b.p. 69°C) containing about 20 ng/μl of each component. (1) Rapid, cooling; (2) rapid, no cooling; (3) slow, cooling; (4) rapid, no cooling.

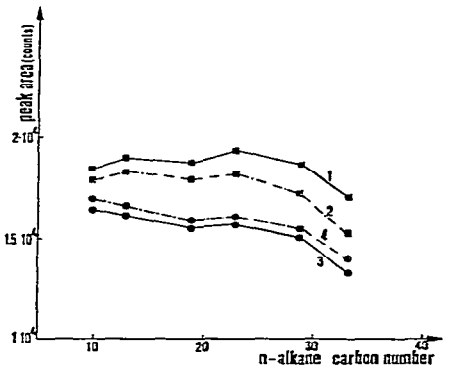

Fig. 7. Discrimination curves for *n*-alkanes dissolved in acetone and injected at 55°C using different injection techniques; 1 μl of test mixture in acetone (b.p. 56°C) containing about 10 ng/μl of each component. (1) Rapid, cooling; (2) rapid, no cooling; (3) slow, cooling; (4) slow, no cooling.

By increasing the oven temperature to a value close to the boiling point of the solvent, the pattern of the discrimination curves changes (Fig. 6). Whereas the curve corresponding to rapid injection with cooling remains almost unchanged, that characterizing rapid injection without cooling becomes gradually inclined and shifted down to the area specific to slow injections. Sample loss and discrimination increase, the heavy components being lost more than the volatilities. On changing the solvent from *n*-hexane to acetone we found the same behaviour (Fig. 7 and Table II).

The results obtained support the hypothesis advanced by Grob and Neukom[7]. At oven temperatures around or moderately higher than the boiling point of the solvent, the back-ejected part of the sample is transported mainly out of the system by the same mechanism as that producing sample loss in slow injections. They also support our hypothesis explaining the beneficial influence of the cooling system.

Results obtained with a specially modified on-column injector having a duckbill rubber valve instead of the usual stainless-steel rotating valve

In order to show that the back-returning sample process and the solution adopted to keep the sample inside the column are not peculiar to the design of the on-column injector used, we performed some experiments with a specially modified on-column injector, in which the stainless-steel rotating valve was replaced with a silicone-rubber duckbill Vernay valve (Fig. 8).

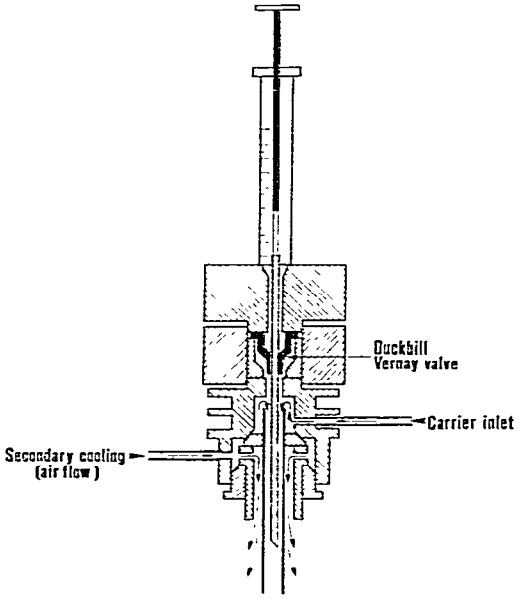

Fig. 8. Modified on-column injector with a duckbill rubber valve instead of the normal stainless-steel rotating valve.

The duckbill rubber valve must in principle ensure relative tightness of the system during the injection. In fact, we checked different types of valves (Vernay Models VA 3143, 3272 and 3197) made of Buna N and silicone-rubber and, despite the fact that all of them were initially leak free, they experienced continuous leaks after a few injections.

We tested both injectors under the same operating conditions by injecting rapidly 2 μl of an *n*-alkane test mixture in *n*-hexane at 70°C (Fig. 9). The curves obtained with the cooling system are more or less the same, whereas those obtained without cooling show a larger sample loss in the modified on-column injector with a rubber duckbill valve. We were unable to find a simple explanation of this result.

However, these data and the relative standard deviations of the peak areas listed in Table III demonstrate that the back-return of the sample during the rapid injection is not peculiar to the structure of the injector but represents a process linked

TABLE III

IMPROVEMENT OF PRECISION OF GAS CHROMATOGRAPHIC DATA BY USING THE COLD ON-COLUMN INJECTION TECHNIQUE

On-column injector with duckbill Vernay valve instead of stainless-steel rotating valve. Oven temperature: 70°C.

Solvent	*Amount of sample*	*Sampling conditions*	*Relative standard deviation of peak area (%)*							
			C_9	C_{11}	C_{14}	C_{20}	C_{24}	C_{30}	C_{34}	C_{40}
n-Hexane	2 μl	Cooling	2.0	2.0	1.6	1.4	1.3	1.7	1.7	1.9
		No cooling	2.9	2.9	3.0	3.5	3.5	3.8	4.0	4.0

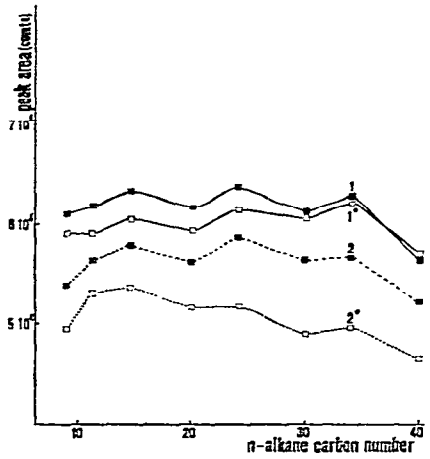

Fig. 9. Discrimination curves for *n*-alkanes obtained by rapid injection with (1 and 1*) and without (2 and 2*) cooling using a normal on-column injector (curves 1 and 2) and the specially modified on-column injector with rubber duckbill valve (curves 1* and 2*).

with this type of sampling. The data also show that the use of a secondary cooling system represents a general solution for avoiding or considerably reducing the effects of this undesirable process.

CONCLUSIONS

(1) The results support the model proposed by Grob and Neukom[7] to explain sample loss and discrimination of sample components during the slow on-column injection. As suggested, at oven temperatures around the boiling point of the solvent rapid injection must be used instead of slow injection.

(2) The results also support the model proposed by the same authors to explain the sample loss in rapid on-column injection through the back-ejection of the sample from the capillary. This process is not peculiar to a specific type of injector but is characteristic of this injection technique.

(3) The sample back-returning process is active not only at oven temperatures higher than the boiling point of the solvent but even at temperatures around or below it. It therefore affects the precision and accuracy of gas chromatographic data even at relatively low oven temperatures. These unwanted effects are magnified when the amount of sample and the oven temperature increase.

(4) The use of drastically reduced oven temperatures during the injection in order to avoid the back-return of the sample has important limitations. For example, the sample size is limited by the risk of destroying the column and the analysis time is prolonged owing to the additional time needed to reach the imposed operating conditions.

(5) The secondary cooling system can be used to avoid or considerably reduce the effects of the sample back-returning process. The secondary cooling is efficient for a relative large range of operating conditions and sample sizes.

As illustrated by our results and by those obtained in other laboratories, the secondary cooling system permits relatively large amounts of sample to be injected at temperatures higher than the boiling point of the solvent, giving extremely precise and accurate chromatographic data. This is true even for complex samples containing components with a wide range of volatilities.

The results demonstrate that the use of the secondary cooling system increases significantly the precision and accuracy of the data even at oven temperatures considerably lower than the boiling point of the solvent.

ACKNOWLEDGEMENTS

We are indebted to Messrs. A. Sironi and T. Jackson for reading the manuscript and to Mrs. O. Sestagalli for secretarial aid.

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