Journal of Chromatography, 186 (1979) 197–205

CHROM. 12,194

CAPILLARY GAS CHROMATOGRAPHIC INJECTION SYSTEM FOR LARGE SAMPLE VOLUMES

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SUMMARY

The injection system for capillary gas chromatography described can solve problems encountered in the trace analysis of high-boiling compounds. It increases the overall sensitivity, because the whole sample can be used for the gas chromatographic separation and detection. Up to 250 μl can be injected with an injection rate of about 1–10 μl/sec. The retention times are not affected by the volume injected or the nature of the solvents used. The linearity of the response is not influenced by the sample volume. The reproducibility (coefficient of variation) of the peak areas is less than 1%. The handling of the injector is simple and the glass insert can be changed in less than 1 min.

INTRODUCTION

Recently we described a new injection system for glass capillary gas chromatography[1]. In 1964 a similar concept applicable to packed and Golay columns was published by Abel[2]. Our injection can solve the problems encountered in the trace analysis of high-boiling compounds. In the usual sample splitting mode only a small part of the minute amounts of the isolated material is analysed while the major part of the sample is wasted. In the past, therefore, several approaches which would overcome these difficulties were recommended. One of the most common methods is the Grob-type splitless injection technique, in which dilute solutions are injected directly[3–5]. In another method the sample is concentrated by removing the solvent before it is placed in the injector[6–9].

Because all of these approaches have some drawbacks in the trace analysis of naturally occurring high-boiling compounds, we developed an injector system which can easily be handled and operated with a high degree of precision and accuracy.

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EXPERIMENTAL

Injection system (Fig.1)

The split–splitless injector described here was designed for a Varian 3700 gas chromatograph. It can also be used in a Varian 1440 model.

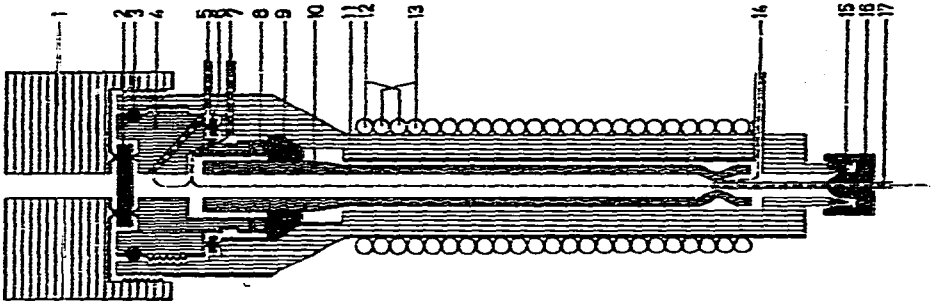

Fig. 1. Cross-sectional view (schematic) of the split–splitless injector. 1 = Injector cap; 2 = septum; 3 = silicone O-ring; 4 = insert holder; 5 = septum cleaning; 6 = aluminium washer; 7 = carrier gas; 8 = back ferrule (1/4 in.); 9 = graphite ferrule (1/4 in.); 10 = glass insert; 11 = injector body (stainless steel); 12 = Thermocoax heater; 13 = carrier gas; 14 = split exit; 15 = graphite ferrule (1/16 in.); 16 = nut (1/16 in.); 17 = glass capillary column.

The original injection port and the electronic heating control were replaced by our injection system. The thin-walled stainless-steel tubing (11) (Fig. 1) has a low thermal capacity and can be heated very rapidly by a Thermocoax heater (12) (TUT, Philips Industrie, Hamburg, G.F.R.). For pre-heating the carrier gas line (7 ÷ 13) is wrapped around the body next to the heater coil (12). The borosilicate glass insert (10) is filled with a pre-washed glass-wool plug and is constricted at the lower end in order to obtain an optimal gas stream at the split point. It can be changed very rapidly (within 1 min) by unscrewing the insert-holder (4) and pulling out the glass tube (10) after removing the cap (1) with the septum (2). Mounting is accomplished in the reverse order. Splitting (14) was controlled by a vent-off valve. The septum purge (5) is adjusted by a needle valve. The insert (10) is sealed by a 1/4-in. graphite ferrule (9) and a stainless-steel back ferrule (8). The insert holder (4) is sealed by an O-ring (silicone rubber) (3) and an aluminium washer (6). The vented vapours of the column split should be sucked off by an open-coupled exhaust line to prevent inhalation by the operator.

The columns used were 25-m SE-30 open-tubular glass capillary columns (17) (I.D. 0.3 mm) with nitrogen as the carrier gas.

Principle of operation

Prior to the introduction of the sample, the injector is cooled to the appropriate temperature by a stream of air on the outside of the injector body. This starting temperature has to be somewhat higher than the boiling point of the solvent used. Within approximately 40 sec up to 250 μl of the sample are injected slowly with the split fully opened. The splitting ratio is 1:1000. The solvent is evaporated and blown by the carrier gas through the vent. The low-volatility

sample constituents remain on the glass-wool plug. After a few seconds the split valve is closed and the interior of the injector is heated immediately to the volatilization temperature of the sample. The column temperature is maintained at its initial temperature during this step. The sample is trapped on the column. After a few minutes the programme is run and the separation performed as usual. Cooling of the injector can be started as soon as 5 min after the introduction of the sample.

RESULTS AND DISCUSSION

Heating characteristics

The Thermocoax heaters have the following advantages over conventional heating wire: they can be wrapped round the stainless-steel tubing in order to achieve maximum heat transfer, and the cold end construction technique enables very large amounts of heat to be transferred without connection problems.

Utilizing these features, we were able to heat the interior of the glass insert of the injector within a few seconds (Fig. 2). For the TUT heater the upper limit was 50 V, corresponding to 15 A. In practice, 20–30 V were sufficient. Applying this voltage a rise in temperature from 60° to 340° occurs within approximately 60 sec. The heat transfer from the heater coil to the interior depends largely on the thermal resistance of the glass insert.

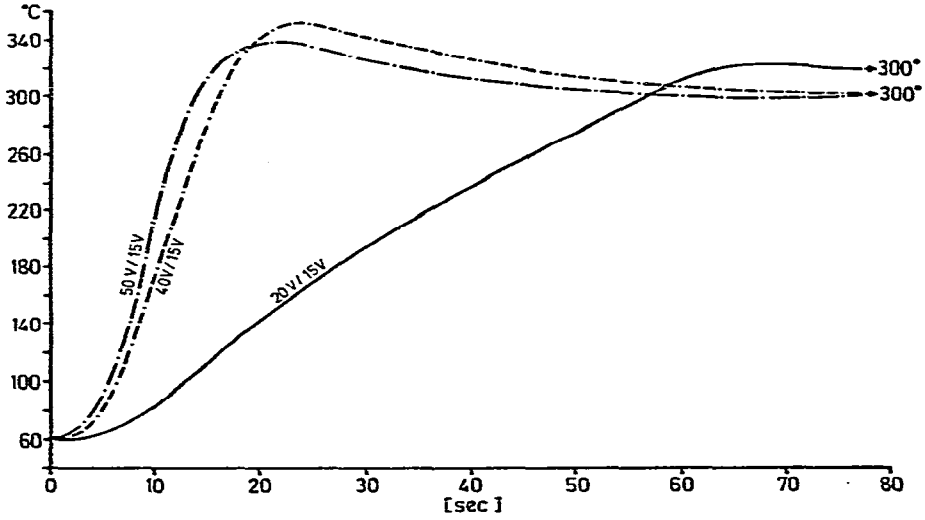

Fig. 2. Heating characteristics of the Thermocoax heater. The first value represents the rapid heating voltage, the second the constant heating voltage (shown on the curve). The temperatures of the interior of the glass insert are given.

Qualitative and quantitative evaluation of the injection system

The experiments were performed with test mixtures consisting of a homologous series of even-numbered hydrocarbons (n-C_{18} to n-C_{32}), as proposed by Schomburg *et al.*[10], and dimethylthiophosphinic esters of aromatic hydroxy acids[11].

TABLE I

EFFECT OF THE HEATING RATE (VOLTAGE APPLIED) ON RETENTION TIMES
The volume injected was 20 μl.

Hydrocarbon	*B.p.*$_{760\,mm}$ (°C)	*Retention time (sec)*		
		15 V	*20 V*	*40 V*
n-C_{18}	316	638	635	630
n-C_{20}	343	794	792	789
n-C_{22}	369	928	925	925
n-C_{24}	391	1048	1047	1045
n-C_{26}	412	1159	1158	1155
n-C_{28}	432	1262	1262	1257
n-C_{30}	450	1358	1356	1353
n-C_{32}	467	1448	1445	1443

Influence of the heating rate on retention times and peak width

The data were obtained from injections at three different heating voltages (15, 20 and 40 V). From Table I it can be seen that the alteration of the heating rate in this range produces no noticeable variation of retention times, the coefficient of variation (c.v.) being less than 0.5%.

The peak width, however, increased distinctly when the voltage was lowered from 20 to 15 V (Table II).

Effects of solvents used on retention times and peak areas

The hydrocarbons were dissolved in solvents of different polarity with a wide range of boiling points (33–126°), resulting in a concentration of 12.5 ng/μl. A sample volume of 20 μl of each solution was slowly injected (within 20 sec) and the split closed after 30 sec. The injector was flushed after 50 sec from 60° to 300° and the hydrocarbons were trapped on the glass capillary column at 140° for 6 min. Then the temperature programme, from 140° to 315° at 8°/min, was started.

TABLE II

EFFECT OF THE HEATING RATE (VOLTAGE APPLIED) ON PEAK WIDTH
The volume injected was 20 μl.

Hydrocarbon	*B.p.*$_{760\,mm}$ (°C)	*Peak width (sec)*		
		15 V	*20 V*	*40 V*
n-C_{18}	316	3.3	2.3	2.3
n-C_{20}	343	2.3	2.1	2.2
n-C_{22}	369	2.3	2.1	2.1
n-C_{24}	391	2.4	2.1	2.1
n-C_{26}	412	2.4	2.0	2.0
n-C_{28}	432	2.3	2.2	2.2
n-C_{30}	450	2.4	2.3	2.2
n-C_{32}	467	2.5	2.3	2.2
$\bar{x}$		2.49	2.18	2.16

In contrast to the findings with the conventional Grob-type splitless sampling technique[12], no influence of the solvents on the retention times was observed (Table III). This was expected because the solvent is vented and cannot participate in the separation step.

TABLE III

EFFECTS OF THE SOLVENTS USED ON RETENTION TIMES

The volume injected was 20 μl.

Solvent	*B.p.*$_{760\,mm}$ (°C)	*Retention time (sec)*							
		n-C_{18}	*n*-C_{20}	*n*-C_{22}	*n*-C_{24}	*n*-C_{26}	*n*-C_{28}	*n*-C_{30}	*n*-C_{32}
n-Pentane	36	641	798	932	1053	1164	1266	1362	1452
n-Hexane	68	641	798	934	1055	1166	1268	1364	1454
n-Octane	126	639	798	933	1055	1165	1268	1363	1453
Isooctane	116	642	799	934	1055	1166	1269	1364	1454
Diethyl ether	35	643	799	934	1055	1166	1269	1365	1455
Dioxane	100	640	797	932	1052	1163	1266	1361	1451
Tetrahydrofuran	65	641	799	933	1055	1165	1268	1363	1453
Acetone	56	641	798	934	1055	1166	1268	1364	1454
Ethyl acetate	77	641	799	934	1055	1166	1268	1364	1454
Dichloromethane	40	642	799	934	1055	1165	1268	1364	1453
Carbon tetrachloride	77	641	798	934	1055	1166	1268	1364	1454
Carbon disulphide	46	640	797	932	1053	1164	1267	1362	1452
Toluene	110	641	798	934	1054	1165	1268	1363	1453
$\bar{x}$		641.0	798.2	933.4	1054.4	1165.2	1267.8	1363.3	1453.2
s		1.0	0.73	0.87	1.04	0.99	0.93	1.12	1.09
C.v. (%)		0.16	0.09	0.09	0.1	0.08	0.07	0.08	0.07

The peak areas also show no deviations (Table IV). Merely the areas of the *n*-C_{18} peaks vary somewhat more, probably owing to the loss of this hydrocarbon with the highest vapour pressure.

Reproducibility of retention times and peak areas

High-performance capillary systems should meet the following criteria with respect to reproducibility: c.v. of the retention times less than 0.1% and c.v. of the normalized peak areas less than 1.0% (ref. 12). From Tables III and IV the reproducibility can also be derived. Despite the adverse conditions experienced with different types of solvents, we were able to meet both of these stringent requirements with the injection system described, with the exception of the c.v. of the peak area of *n*-C_{18}.

Effects of sample volume on the linearity of the response

A 1-μl volume of five concentrations of dimethylthiophosphinic ester of homovanillic acid methyl ester (1–100 ng/μl) were injected together with the internal standard (25 ng/μl of the dimethylthiophosphinic ester of 3-hydroxyphenylacetic acid methyl ester). The correlation coefficient of this calibration graph was 0.9997 (Fig. 3).

TABLE IV

EFFECTS OF THE SOLVENT USED ON RELATIVE AND TOTAL PEAK AREAS

The volume injected was 20 μl.

Solvent	*B.p.*$_{760\,mm}$ (°C)	*Relative peak area (%)*								*Total peak area (arbitrary units)*	*C.v.** (%)
		n-C_{18}	*n*-C_{20}	*n*-C_{22}	*n*-C_{24}	*n*-C_{26}	*n*-C_{28}	*n*-C_{30}	*n*-C_{32}		
n-Pentane	36	10.8	12.6	12.8	12.7	12.9	12.9	12.5	12.8	1231	1.2
n-Hexane	68	10.8	12.8	12.9	12.7	12.9	12.8	12.4	12.7	1225	1.3
n-Octane	126	10.2	12.6	12.1	13.0	13.0	12.9	12.5	12.7	1253	2.6
Isooctane	116	11.2	12.7	12.7	12.6	12.8	13.0	12.4	12.6	1263	1.5
Diethyl ether	35	10.5	12.8	12.9	12.8	12.9	12.9	12.4	12.8	1273	1.4
Dioxane	100	11.5	12.8	12.8	12.6	12.8	12.7	12.3	12.5	1268	1.5
Tetrahydrofuran	65	11.4	12.9	12.7	12.6	12.8	12.8	12.3	12.5	1248	1.6
Acetone	56	10.8	13.0	13.1	12.7	12.7	12.9	12.3	12.5	1284	2.2
Ethyl acetate	77	10.5	12.8	12.9	12.8	12.9	12.9	12.4	12.8	1240	1.4
Dichloromethane	40	11.7	12.7	12.6	12.6	12.7	12.9	12.3	12.5	1260	1.5
Carbon tetrachloride	77	10.3	12.7	12.9	12.8	13.0	13.0	12.5	12.8	1295	1.4
Carbon disulphide	46	9.7	12.6	12.9	12.9	13.1	13.1	12.7	13.0	1250	1.5
Toluene	110	10.9	12.8	12.8	12.7	12.8	12.8	12.5	12.7	1273	0.9
$\bar{x}$		10.79	12.75	12.78	12.73	12.85	12.89	12.42	12.68	1258.7	
s		0.56	0.12	0.24	0.13	0.13	0.10	0.12	0.16	20.3	
C.v. (%)		5.2	0.90	1.90	1.0	1.0	0.81	0.93	1.2	1.6	

* Calculated without *n*-C_{18}.

In another experiment these solutions were diluted 20-fold and 20 μl of each were injected. This corresponds to the same amount of solutes on the column as described in the previous paragraph. The correlation coefficient of the latter was 0.9999 (Fig. 3). This means that no solute discrimination occurs with these rather polar and easily adsorbed compounds.

Similar results were obtained with hydrocarbons. Gas chromatograms from a 1-μl (Fig. 4A) and a 20-μl (Fig. 4B) injection of the original and the 1:20 diluted solution of even-numbered alkanes, respectively, were identical.

Advantages of the split–splitless injection technique

This solvent-free chromatography has real advantages over the common splitless injection techniques.

Solid-state injection systems. Quantitative transfer of up to a 20-μl sample volume is possible with the pre-column technique[6]. The concentration step is performed, however, outside the gas chromatograph. The type of packing of the pre-column is critical, and only chemically bonded stationary phases are adequate for these purposes.

The moving needle system[7,8] allows the application of sample volumes only up to 5 μl. The Curie-point evaporation injector[9] is very expensive because a high-frequency generator is necessary in addition to the sophisticated injector construction. Coating of the ferromagnetic wire with the sample is performed outside the injector and seems to be troublesome.

Grob-type injection system. The performance of the splitless sampling technique of Grob and co-workers[3–5] depends on a large number of experimental variables[10].

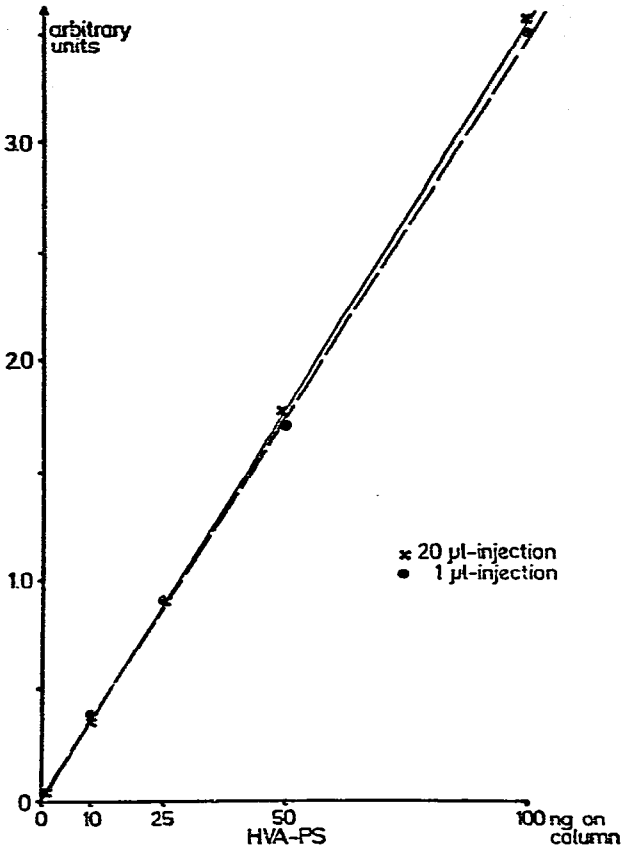

Fig. 3. Effects of sample volume on the linearity of the response.

This method is particularly suitable for low-boiling substances which are eluted near the tail of the solvent peak. A disadvantage of this technique is the high loading of the column with large amounts of solvents.

Disadvantages of the split–splitless injection technique

As in solid-state injection systems, a sufficient difference between the boiling points of the solutes and solvents is a prerequisite. Therefore, this technique can be applied only to higher boiling compounds.

A disadvantage of all large-volume sampling methods is the rapid contamination of the insert[12]. In some instances the glass insert has to be changed after each analysis. This factor has been considered in the physical construction of our device. The replacement of the insert is quick and simple.

CONCLUSIONS

In our opinion, the following features of this new sampling technique significantly increase the overall sensitivity in the gas chromatographic trace analysis of high-

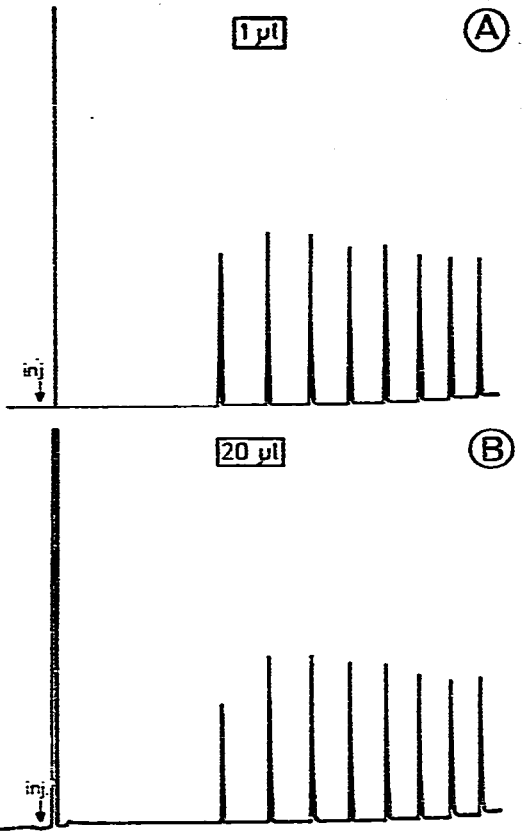

Fig. 4. Gas chromatograms from (A) a 1-µl and (B) a 20-µl injection of a series of even-numbered hydrocarbons (n-C_{18} to n-C_{32}) dissolved in n-hexane. The concentration of solution B was a 1:20 dilution of solution A. The peak areas correspond to 250 ng of each hydrocarbon. Injector: starting temperature 60°, vaporization temperature 320°. Column, 25 m × 0.3 mm I.D. SE-30; carrier gas, nitrogen at a flow-rate of 1.5 ml/min; temperature programme, heating from 140° (6 min) to 315° at 8°/min; flame-ionization detector; temperature, 290°.

boiling compounds without complicating the handling:

(a) sample sizes between 1 and 250 µl may be injected;

(b) the normally used split technique also can be performed;

(c) rate of sample injection *ca.* 1–10 µl/sec;

(d) injector purge time *ca.* 30 sec;

(e) no effects of volume injected on the retention times (c.v. less than 0.1%) or the peak width;

(f) no effect of solvents used on the retention times (c.v. less than 0.1%);

(g) no effect of sample volume on the linearity of the response;

(h) high reproducibility of total peak area (c.v. 1.6%) and normalized peak areas (c.v. less than 1.0%).

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