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AUTOMATIC INJECTION IN HIGH-RESOLUTION GAS CHROMATOGRAPHY: A PROGRAMMED TEMPERATURE VAPORIZER AS A GENERAL PURPOSE INJECTION SYSTEM

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SUMMARY

A new method of splitless sampling for capillary column gas chromatography is presented, providing a total sample injection without discrimination effects and without substance loss due to the thermal decomposition, adsorption or condensation. The method is based on a programmed temperature vaporizer and a capillary pre-column allowing the introduction of the sample at low temperature by means of a regular syringe. After sample injection, the capillary pre-column is quickly (15 sec) heated to the vaporizing temperature and then the split valves are opened for a fast purge of the injection area. The new system allows the use of an automatic liquid sampler.

INTRODUCTION

In high-resolution gas chromatography (GC) no other parameter has received more attention than the sample injection. It is very difficult to obtain highly efficient separations and highly reproducible, accurate and representative quantitative results on very small samples or on very dilute solutions. The criteria for a high-performance capillary sampling system have been described[1].

Several different types of sampling and injection techniques have been developed, however none could be considered as "universal". It is recognized that the stream splitting technique, which is as well established as capillary GC, is the easiest injection system, but it generally gives less accurate quantitative results.

The Grob splitless technique[2] has a much broader application but suffers from severe limitations in the case of high-molecular-weight substances. Also, temperature sensitive substances may be destroyed or damaged inside the vaporizer. The cold "on-column" Grob injection system[3] is the newest sampling system. It has some very important features and is considered today as the most interesting tool for sample introduction into a capillary column. However, even after some small improvements[4], this system also has several operational drawbacks (1–3) and performance limitations (4–6):

(1) The first part of the column needs to be straightened for several centimetres without restriction of the inside diameter. The inside of the cut end must be conically

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widened by means of a diamond pen. This represents a very difficult task for unskilled workers.

(2) Special syringes with ultrathin needles must be employed. Even with the narrowest needle, capillary columns having I.D. less than 0.3 mm cannot be used. Columns with I.D. greater than 0.35 mm are also excluded.

(3) The present design does not allow automation of injections. In practice, full automatic sampling is still not possible.

(4) Non-volatile residues, always present in biological or environmental extracts, solidify and accumulate at the injection point, making an absorbing layer after a few injections.

(5) There is always a danger of losing part of the sample by capillary movement back along the syringe needle.

(6) The reproducibility of retention time is insufficient, at least for the first components eluted, for use of modern computerized techniques of peak identification and reporting.

In order to overcome these limitations, we set out to develop a new injection system. The basic requirements of such a system are:

(1) To avoid high temperatures upon introduction of the syringe needle and when the sample is injected. (This is the primary source of component discrimination, thermal effects on labile components, non-reproducibility of sample amount entering the column and sample recovery.)

(2) To avoid the use of the column as an intrinsic element of the sampling system. (This is the source of absorption phenomena, is the cause of the difficulty in preparation of the column inlet and the reason why small I.D. columns cannot be used.)

(3) To allow different sampling modes without modifications of the sampling system and without removing the capillary column.

(4) To allow the use of a fully automatic liquid sampler.

The criteria have been met by the development of a programmed temperature vaporizer* (PTV) in which is inserted a capillary pre-column having an I.D. large enough to accept a standard syringe needle. In other words, the sample injection is carried out in a similar manner to the Grob splitless technique, but with the following differences:

(a) during injection, the vaporizer is kept cold and quickly heated when the syringe needle is removed

(b) the insert is a glass tube of suitable I.D.

EXPERIMENTAL

The PTV injector

A cross-sectional view of the Dani PTV injector with the capillary pre-column installed is shown in Fig. 1. The capillary pre-column is a straight glass tube (55 × 2 mm O.D.). The inside diameter, chosen to meet the requirements of different injections modes, may be varied from 0.5 to 1.5 mm. The tube of smaller diameter is

* Patent pending.

widened into a cone at the inlet to facilitate the introduction of the syringe needle; the other end is ground flat. The glass tube is kept firmly in place by a graphite seal just below the carrier gas inlet. This seal prevents the carrier gas from circulating around the capillary pre-column and assures a flow through the capillary pre-column even when it is packed tightly with silanized glass or quartz wool. The capillary pre-column is easily installed and removed from the top of the injector.

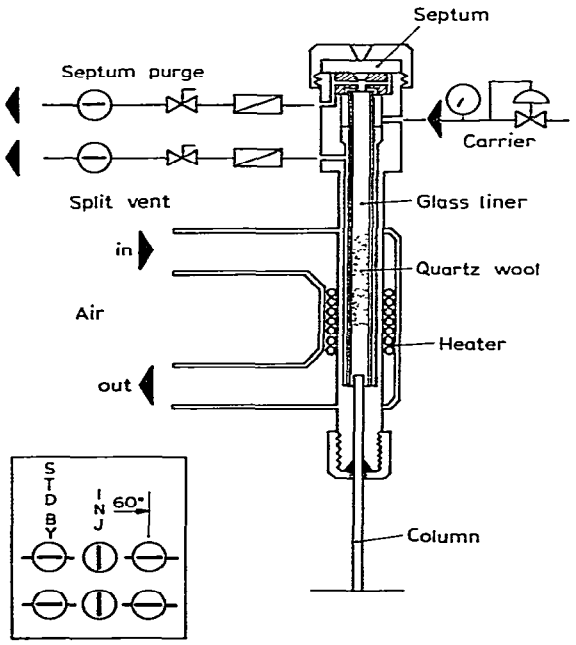


Fig. 1. Cross-sectional view of the Dani PTV.

The inlet port consists of a soft rubber septum. This part of the injector is always cold and its temperature is never higher than 50°C even when high-molecular-weight substances require a high vaporization temperature. The temperature controller surrounding the sample injector provides operations according to the temperature profile shown on Fig. 2. The exceptional performance is obtained by means of a flow of compressed air and a very efficient heater.

The glass capillary column penetrates a few millimetres into the vaporizer. It is straightened for several centimetres, cut and the edges flattened. Fused silica columns can be mounted without any modification. The PTV injector accepts capillary columns of practically any I.D.; the maximum O.D. is 1.2 mm.

Two vent lines are provided: one for the septum purge on the top of the injector, preventing septum contamination from reaching the pre-column; the second, on the bottom, is used in the split sampling mode and for a quick purge of the pre-column in the splitless mode. Both lines include a needle valve for adjusting the optimum purge and split flow-rate. An automatic shut-off valve is mounted on the lower split line.

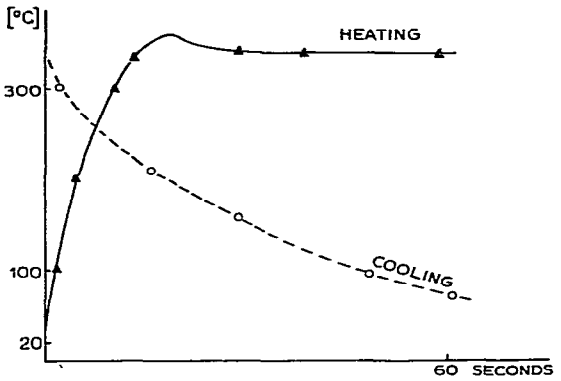


Fig. 2. Temperature profile as a function of time during the temperature increase and during the cooling step.

A regular Hamilton syringe is used for injection with the needle 50 mm long, 0.45 mm nominal O.D., 5–10 μl capacity or its equivalent. The sample injection sequence is as follows: the shut-off valve of the lower splitter is closed; the sample is injected via a regular syringe; the vaporizer temperature is increased to the optimum preset limit; after 30–90 sec, the shut-off valve is opened; the oven temperature program is started; when the oven is cooled, the vaporizer is cooled to the injection temperature.

GC apparatus

All experiments were conducted on a Dani 3800 PTV gas chromatograph equipped with flame ionization (FID), electron-capture (ECD) and nitrogen–phosphorus (NPD) detectors, separately or in combination. The main features of this gas chromatograph have already been described[5]. It has facilities for setting the initial and final PTV temperatures, the upper temperature delay and for timing the split valve open-close functions.

The autosampler

Automatic injections were performed with the Dani ALS 3840 autosampler. This consists of three basic units, for sample storage, injection and control. The sample storage unit is a segmented carousel made up of five racks capable of holding up to 50 samples in screw-capped septum sealed vials. The racks are removable and replaceable without interrupting the analysis sequence. The injection unit consists of an electropneumatic system and of an arm in which a regular syringe is firmly mounted. During this operation, the syringe is automatically positioned over the sample vials, the washing solvent cuvette, the waste line or the GC injection port. The syringe is also moved up and down in order to dip the needle in the sample or in the washing solvent and for the final injection. The syringe piston is operated by means of the same pneumatic servomotor. The control unit provides the facilities for actuating the injection program.

In designing this autosampler, particular care has been devoted to meeting all the requirements of a capillary column sampling system. The needle is introduced into the vaporizer according to the technique suggested by Grob and Newcome[6]:

before entering the vaporizer, the sample is withdrawn from the needle, the empty needle enters the vaporizer and the sample is pushed out after a few seconds. The syringe is washed just after the injection and then moved to the stand-by position, some distance from the injection port, which is already clean. Clean-up is performed with pure solvent, up to nine times: each time the solvent is discarded. Before sampling, the syringe is washed again with the sample, also up to nine times (each time the sample is discarded), and finally the piston is moved up and down (four times) with the needle dipped in the sample to eliminate air bubbles. In the stand-by position, the injection port area is free and normal injections can be carried out.

The computer integrator and recording system

Shimadzu CR1-A and Dani Astra 1200 data systems were used for all the measurements. Readings from combinations of detectors were recorded on a dual pen Omniscribe recorder.

The capillary columns

Two columns were used: (a) glass, 20 m × 0.35 mm I.D. × 0.95 mm O.D., persilanized and coated with OV-1 silicon gum, thickness 0.05 μm; (b) fused silica, 10 m × 0.25 mm I.D. × 0.35 mm O.D., deactivated and coated with OV-1 silicon gum, thickness 0.1 μm. The glass capillary column fitted end-to-end with the pre-column. The fused-silica column penetrated 5 mm into the pre-column.

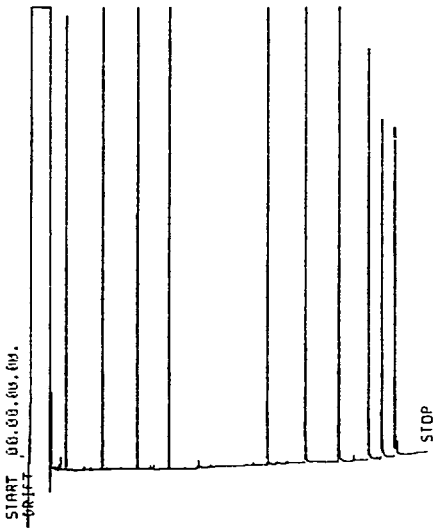


Fig. 3. Full automatic sampling with PTV injection of a C_{10}–C_{40} *n*-alkanes mixture nominally containing 25 ng/μl of each homologue diluted in *n*-hexane. Column: fused silica, 10 m × 0.2 mm I.D. 0.1 μm OV-1. Temperature: PTV, 70–310°C; column, 60–320°C (10°C/min). Carrier: hydrogen, 0.6 bar.

TABLE I

RETENTION TIME (t_R) AND NORMALIZED PEAK AREA REPRODUCIBILITY WITH PTV TOTAL INJECTION MODE ON A GLASS CAPILLARY COLUMN

Samples injected: 10 × 0.5 µl, 20 × 1 µl, 10 × 2 µl containing 25 ng (nominal) of each alkane. Automatic sampling. For conditions see Fig. 3.

n-Alkanes	*0.5 µl*				*1 µl*				*2 µl*			
	t_R		*Peak area*		t_R		*Peak area*		t_R		*Peak area*	
	$\bar{x}$ *(min)*	*S.D. (%)*	$\bar{x}$	*S.D. (%)*	$\bar{x}$ *(min)*	*S.D. (%)*	$\bar{x}$	*S.D. (%)*	$\bar{x}$ *(min)*	*S.D. (%)*	$\bar{x}$	*S.D. (%)*
C_{10}	2.17	0.95	7.3	6	2.16	0.95	7.2	5	2.19	0.95	7.5	4
C_{12}	5.52	0.36	10.2	1.5	5.5	0.36	9.9	1.1	5.53	0.36	10.0	0.9
C_{14}	8.48	0.12	10.4	1.2	8.46	0.12	10.4	0.95	8.49	0.12	10.3	0.8
C_{16}	10.98	0.09	10.7	1.3	10.95	0.09	10.8	1.2	10.99	0.09	10.7	1
C_{24}	18.68	0.05	10.8	1.3	18.65	0.05	10.8	1.3	18.69	0.05	10.7	1
C_{28}	21.68	0.05	10.6	1.4	21.64	0.05	11.7	1.2	21.68	0.05	10.6	1
C_{32}	24.29	0.04	11.5	1.5	24.26	0.04	10.4	1.5	24.3	0.04	11.4	1.1
C_{36}	26.52	0.04	9.6	2	26.5	0.04	9.6	1.8	26.53	0.04	9.6	1.6
C_{38}	27.65	0.04	9.7	2.2	27.64	0.04	9.6	2	27.67	0.04	9.7	1.8
C_{40}	28.67	0.03	9.2	3	28.65	0.03	9.2	2.5	28.68	0.03	9.4	1.9

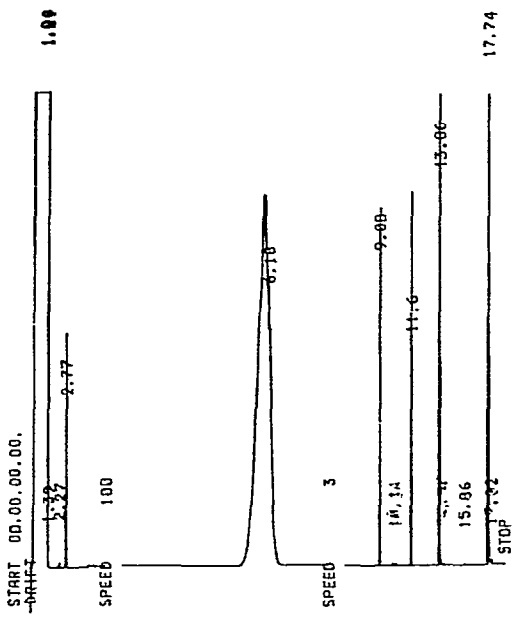


Fig. 4. Manual sampling with PTV injection of 0.5 µl of a C_{10}–C_{22} *n*-alkanes mixture nominally containing 100 ng/µl of each homologue diluted in *n*-hexane. Column and conditions as in Fig. 3. Carrier: hydrogen, 0.4 bar. Chart speed increased to 100 mm/min during C_{12} alkane elution (t_R = 6.18 min).

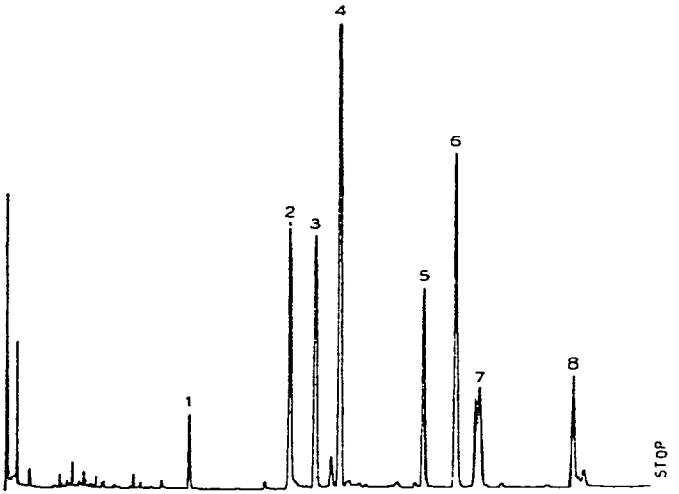


Fig. 5. Manual sampling with PTV injection of 0.5 μl pesticides (50 ppb of each dissolved in *n*-hexane). Column: fused silica, 10 m × 0.2 mm I.D., 0.1 μm OV-1. Temperature: PTV, 70–200°C; column, 60–130°C (max. speed), 130–200°C (5°C/min). Carrier: hydrogen, 0.4 bar. ECD pulse mode, constant current. Peaks: 1 = lindane; 2 = met-parathion; 3 = ronnel; 4 = aldrin; 5 = thiodan; 6 = dieldrin; 7 = endrin; 8 = DDT.

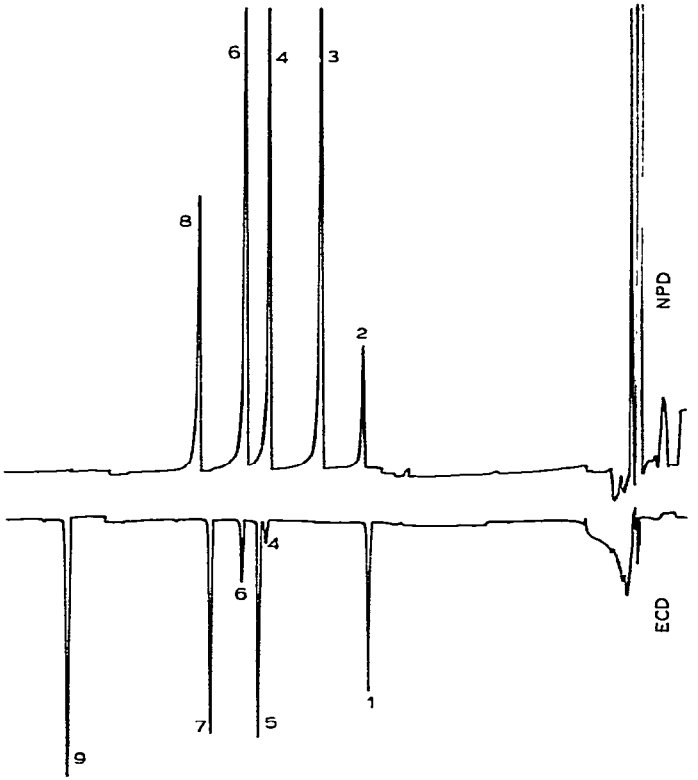


Fig. 6. Manual sampling with PTV injection of 1 μl nitrogen-containing phosphoretted and chlorinated pesticides in *n*-hexane. Column: glass, 25 m × 0.32 mm I.D., 0.2 m OV-1. Temperature: PTV, 70–180°C; column, 60–140°C (max. speed), 140–210°C (5°C/min). Detectors: ECD + NPD in series. Peaks: 1 = lindane; 2 = simazine; 3 = diazinon; 4 = met-parathion; 5 = heptachlor; 6 = ronnel; 7 = aldrin; 8 = fenthion; 9 = dieldrin.

RESULTS AND DISCUSSION

Fig. 3 shows the chromatogram of a C_{10}–C_{40} *n*-alkane mixture and Table I summarizes the average values from twenty injections performed with a fused-silica (0.25 mm I.D.) column.

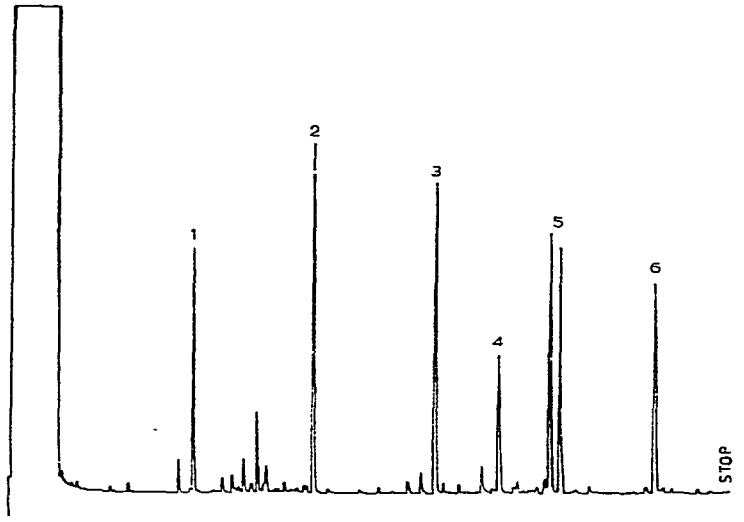
The following conclusions can be drawn:

(a) The reproducibility of the retention time, as expected from a fully automatic injection system, is more than enough for the identification of individual peaks.

(b) The quantitation of such a broad-molecular-weight mixture is also good, taking into account that the amount of each component in the sample is in the nanogram range.

(c) The sampling system is suitable for injections of 0.5 to 2 μl.

Fig. 4 shows a recording with the chart speed increased during the elution of a peak (t_R = 6.18 min). The perfect peak symmetry is a clear indication that no peak distortion occurs when the injection is made with the PTV.



C-R1A
SMPL # 00
FILE # 1
REPT # 1290
METHOD 241

#	NAME	TIME	CONC	MK	AREA
0		4.1	1.1366		1143
0		4.46	9.6695	V	9725
0		5.65	1.6263	V	1635
0		5.97	2.8923	V	2909
0		6.19	1.5187	V	1527
0		7.32	16.542	V	16638
0		10.24	16.3224		16417
0		11.33	1.2323	TV	1239
0		11.75	8.3448	V	8393
0		12.93	4.328	V	4353
0		12.98	11.2968	V	11362
0		13.2	11.1226	V	11187
0		15.43	3.348		3367
0		15.46	4.8898	V	4918
0		15.47	5.729	V	5762
	TOTAL		99.9999		100580

Fig. 7. Manual sampling with PTV injection of a mixture of polyaromatic hydrocarbons dissolved in xylene. Column: fused silica, 10 m × 0.2 mm I.D., 0.1 μm OV-1. Temperature: PTV, 140–310°C; column, 115–280°C (10°C/min). Peaks: 1 = anthracene; 2 = pyrene; 3 = benzanthracene; 4 = chrysene; 5 = benzopyrenes; 6 = benzoperylene.

Ultra-trace analysis

Fig. 5 shows a pesticides analysis performed with the ECD. Provided that the quartz wool and glass tube are properly deactivated by silanization, the pesticides can be detected at the ppb (10^9) level. Fig. 6 illustrates the analysis of chlorinated and phosphoretted pesticides obtained with the use of combined ECD–NPD.

Poorly volatile substances

Fig. 7 shows the chromatogram of a mixture of polyaromatic hydrocarbons. In this case the PTV temperature was increased to 350°C.

Split injection

The PTV injector could also be used for split mode injection in cases where a sample must be injected undiluted. Fig. 8 shows an example of this type of application. The only modifications are that the capillary pre-column has a larger I.D. (1.2 mm) and is packed tightly with glass wool. All the advantages and disadvantages of the injection technique are retained.

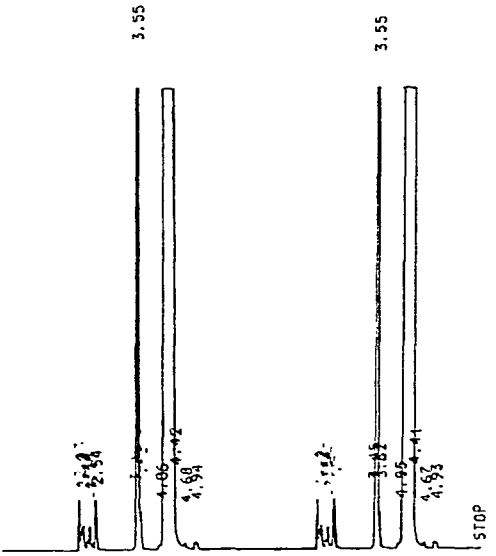


Fig. 8. Manual sampling with split injection mode of aromatic hydrocarbons (impurities in toluene). Column: glass, 25 m × 0.33 mm I.D., 0.15 μm Carbowax 20M. Temperature: PTV, 200 C. Split: 200 ml/min. Capillary pre-column: 1.2 mm I.D., packed with glass wool.

CONCLUSIONS

The PTV injector has proved to be a general purpose tool for introducing the sample without discrimination and losses into a high-resolution gas chromatograph equipped with capillary column. Its advantages over other total sample injection techniques can be summarized as follows.

The operational advantages include the use of standard syringes, and any type and diameter of capillary columns. The PTV does not require careful handling of the capillary column inlet edge, and the capillary pre-column is easily removable and

replaceable. A fully automatic liquid sampler can be used with a choice of injection modes (split, splitless, hot splitless). Improved performance is achieved due to reproducibility of retention time and to the lack of sample loss, capillary phenomena or adsorption inside the column.

Since the total sample injection and the vaporization step take place only after the syringe needle has been removed from the capillary pre-column, there are no discrimination effects.

REFERENCES

1 F. J. Yang, A. C. Brown, III and S. P. Cram, *J. Chromatogr.*, 158 (1978) 91.
2 K. Grob and G. Grob, *J. Chromatogr. Sci.*, 7 (1969) 584.
3 K. Grob and K. Grob, Jr., *J. Chromatogr.*, 151 (1978) 311.
4 M. Galli, S. Trestianu and K. Grob, Jr., *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 366.
5 DANI TB 3800/6800-HR, DANI, Monza, 1981.
6 K. Grob, Jr. and H. P. Newcome, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 563.
7 F. Poy, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 2 (1979) 243.