Journal of Chromatography, 217 (1981) 91–98
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,902

APPLICATION OF THE SPLIT–SPLITLESS INJECTOR TO ENVIRONMENTAL ANALYSIS

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SUMMARY

In environmental analysis the concentrations of the compounds under investigation are often at the detection limit of the analytical methods available. In gas chromatography highly sensitive detectors such as the electron-capture or the nitrogen–phosphorus detector are used, but in capillary gas chromatography there is the severe disadvantage that only minute amounts of the isolated material reach the detector. These problems can be overcome by use of the split–splitless injection technique. Compounds with boiling points higher than approximately 250°C are quantitatively trapped in the glass insert of the injector and, by rapid heating, transferred completely to the capillary column, resulting in a 10- to 500-fold increase in the overall sensitivity. The solvent used is vented off prior to the chromatographic step so that the column is protected from washing-out effects. Various standard solutions and real samples originating from rain water and foods containing organochlorine or organophosphorus compounds were analysed by the split–splitless mode in comparison with the conventional split technique. It was demonstrated that comparable performance with essentially higher sensitivity is achieved with the former mode.

INTRODUCTION

There is an increasing demand for the efficient analysis of substances such as pesticides, fertilizers, plasticizers or polychlorinated biphenyls (PCBs) in environmental and human samples. Often the concentrations of the compounds to be monitored are at the detection limit of the analytical methods available. Today, gas chromatography (GC) is used mostly because of its high specificity, sensitivity and resolution. High performance is achieved by the application of glass capillary columns and element-specific detectors such as the electron-capture (ECD) and the nitrogen–phosphorus detector (NPD). However, there is the severe disadvantage that only a minute fraction of the isolated material reaches the detector as a consequence of the small volumes injected and due to splitting; frequently only one thousandth or less of the sample is measured.

Recently we described a cold injection system for the application of very large

0021-9673/81/0000–0000/$02.50

amounts of isolated material to glass capillary columns[1,2]. The use of this split–splitless technique leads to an increase in the overall sensitivity of a GC method by a factor of 10–500 or more, as shown in the analysis of clinically interesting compounds such as homovanillic acid[3].

We describe here the application of this technique to environmental problems, using as an example organochlorine and organophosphorus compounds in food and water samples.

EXPERIMENTAL

Reagents and materials

All solvents were of analytical-reagent grade. Standard materials were obtained from E. Merck (Darmstadt, G.F.R.) and Analabs (North Haven, CT, U.S.A.).

Gas chromatography

A Model 3700 gas chromatograph (Varian, Palo Alto, CA, U.S.A.) equipped with a split–splitless injector was used as described previously[1,2].

Organochlorine pesticides and polychlorinated biphenyls. The starting temperature of the injector was 30°C, injection rate 1–2 $\mu l \cdot sec^{-1}$ and vaporization temperature 185°C. A 25-m SE-30 glass capillary column (Bruker-Franzen Analytik, Bremen, G.F.R.) was used with nitrogen as the carrier gas (flow-rate 1 $ml \cdot min^{-1}$), trapping temperature 120°C, temperature programme 160–260°C at 5°C $\cdot min^{-1}$. A ^{63}Ni constant-current, pulse-modulated ECD was used, with nitrogen as make-up gas (flow-rate 30 $ml \cdot min^{-1}$), temperature 330°C.

Organophosphorus pesticides. The starting temperature and injection rate were as above and the vaporization temperature was 240°C. The column conditions were as above, except for a trapping temperature of 120°C and temperature programming from 180 tot 260°C at of 8°C $\cdot min^{-1}$. A NPD was used with helium as make-up gas (flow-rate 30 $ml \cdot min^{-1}$), a hydrogen flow-rate of 5 $ml \cdot min^{-1}$, an air flow-rate of 175 $ml \cdot min^{-1}$, a bias voltage of 4 V and a temperature of 300°C.

Using the split mode for comparison, the following conditions were used: splitting ratio, 1:10; injector temperature, 185°C for organochlorine compounds and 240°C for organophosphorus compounds; other conditions as above.

Combined gas chromatography–mass spectrometry

For positive identification of the compounds indicated in the figures, mass spectrometry was performed with a MAT 311 A double-focusing mass spectrometer combined with a Model 1400 gas chromatograph (Finnigan-MAT, Bremen, G.F.R.). The gas chromatograph was equipped with the same split–splitless injection system. The glass capillary column was coupled to the ion source of the mass spectrometer with an open split-type all-glass connection according to Henneberg *et al.*[4].

Sample preparation

PCBs and organochlorine pesticides from food samples (rabbit, venison and cheese) were isolated according to ref. 5. After triturating the specimen with sodium sulphate the compounds of interest were extracted with light petroleum (b.p. 40–60°C), pre-purified on a silica gel column loaded with 30% of water and eluted with light petroleum again.

Water samples were extracted with *n*-hexane in a micro-separator and used without further purification[6].

The phosphorus esters were isolated from apples according to ref. 7. After homogenizing in the presence of acetone, parathion was extracted with chloroform. The chloroform phase was evaporated to dryness and the residue dissolved in acetone.

RESULTS AND DISCUSSION

Reproducibility

The repeated injection ($n = 8$) of 10 μl of a standard solution containing eleven organochlorine pesticides and polychlorinated biphenyls corresponding to 1 ng of each on to the column resulted in good reproducibility (Table I). With the exception of the most volatile compounds investigated the peak areas calculated without and with internal standardization (aldrin as reference substance) showed coefficients of variation from 2.3 to 3.9 % and from 0.6 to 1.9 %, respectively. These results are better than those obtained recently by Onuska and Thomson[8] with the split technique.

Neither retention times nor peak areas are influenced by the solvents used[2].

TABLE I

REPRODUCIBILITY OF PEAK AREAS ($n = 8$)

Compound	*Coefficient of variation (%)*	
	Without internal standardization	*With internal standardization*
2,6-Dichlorobiphenyl	7.0	4.9
Hexachlorobenzene	5.2	3.2
γ-Hexachlorocyclohexane	2.3	0.6
2,3,6-Trichlorobiphenyl	3.5	1.3
2,2′,4,5′-Tetrachlorobiphenyl	3.3	1.1
Aldrin	2.4	–
2,2′,3,4,5-Pentachlorobiphenyl	3.4	1.2
DDE	2.6	0.9
p,p′-DDD	3.0	1.4
o,p′-DDT	3.0	0.9
p,p′-DDT	3.9	1.9

Sensitivity

As an example of the increase in overall sensitivity, 1 μl of 2-chlorobiphenyl in acetone corresponding to 10 ng was injected. With respect to the different attenuations in the split–splitless and split mode, the same peak heights were obtained (Fig. 1). In addition, the chromatogram demonstrates the quantitative removal of the solvent in this cold injection technique.

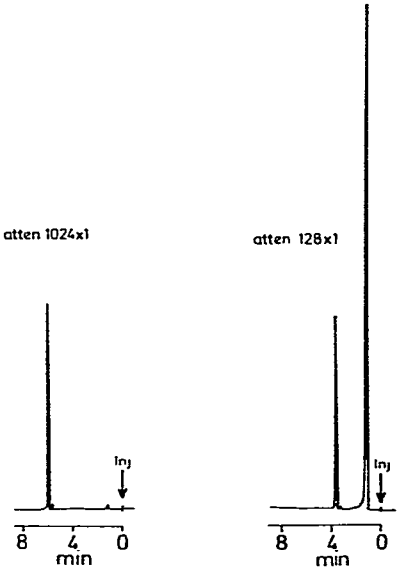

Fig. 1. Glass capillary gas chromatogram of 2-chlorobiphenyl; volumes injected 1 μl each, corresponding to 10 ng. Left, split–splitless mode; right, split mode (split 1:10).

Limitations

2-Chlorobiphenyl (b.p. 274°C) can still be transferred to the column quantitatively by the injection technique described. For compounds with lower boiling points, losses of material during the evaporation step are observed using a temperature of approximately 30°C. This agrees well with our previous results with saturated hydrocarbons[2].

Application to organochlorine compounds

Eleven organochlorinated compounds were chromatographed on an SE-30 glass capillary column by the split–splitless and split injection techniques. As can be seen from the chromatograms, the column efficiency is not affected by the different injection modes (Fig. 2).

A technical preparation of different isomers of penta- to heptachlorobiphenyls (Clophen A 60) was also separated by both techniques. The chromatograms (Fig. 3) demonstrate especially impressively that split–splitless injection does not influence the high resolution of capillary GC.

Samples of biological interest (rain water, cheese and fatty tissue from deer and rabbit) were analysed. The volumes injected were approximately the same. A comparison between the two injection techniques showed the superior sensitivity of the cold split–splitless mode (Figs. 4–7).

The high separation efficiency of glass capillary columns remains unchanged even if biological samples are applied with a large excess of matrix.

Despite the high loading of the injector insert with crude material, up to 20 injections can be performed without changing the glass insert.

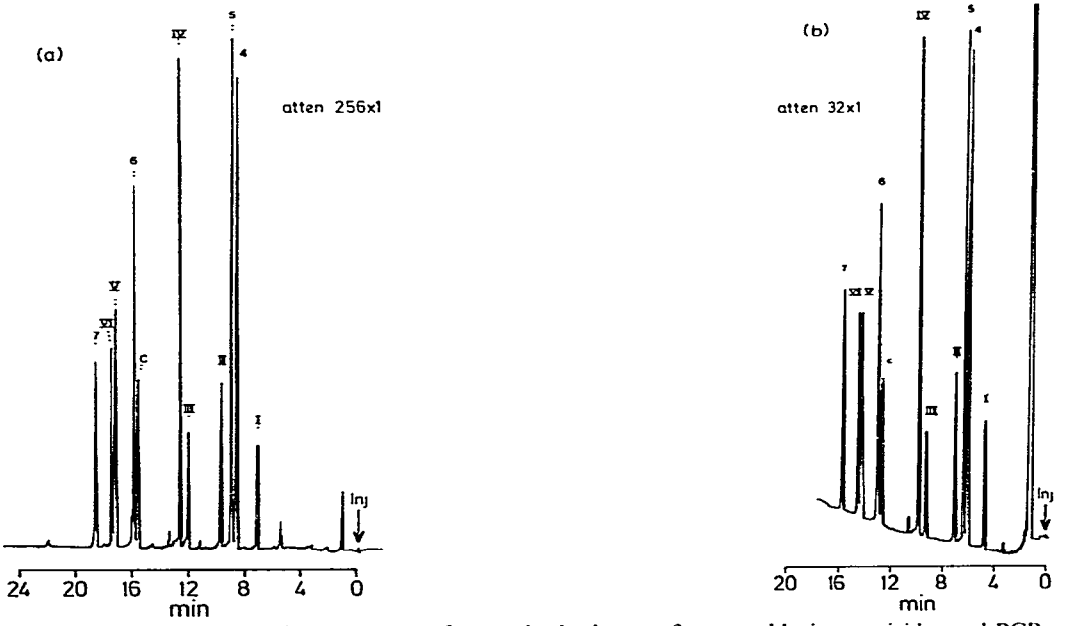

Fig. 2. Glass capillary gas chromatogram of a standard mixture of organochlorine pesticides and PCBs; volume injected 1 μl, corresponding to 1 ng of each component. 4 = Hexachlorobenzene; 5 = γ-hexachlorocyclohexane; 6 = *p,p'*-DDE; 7 = *p,p'*-DDT; I = 2,6-dichlorobiphenyl; II = 2,3,6-trichlorobiphenyl; III = 2,2',4,5'-tetrachlorobiphenyl; IV = aldrin; V = *p,p'*-DDD; VI = *o,p'*-DDT; c = 2,2',3',4,5-pentachlorobiphenyl. (a) Split–splitless mode; (b) split mode (split 1:10).

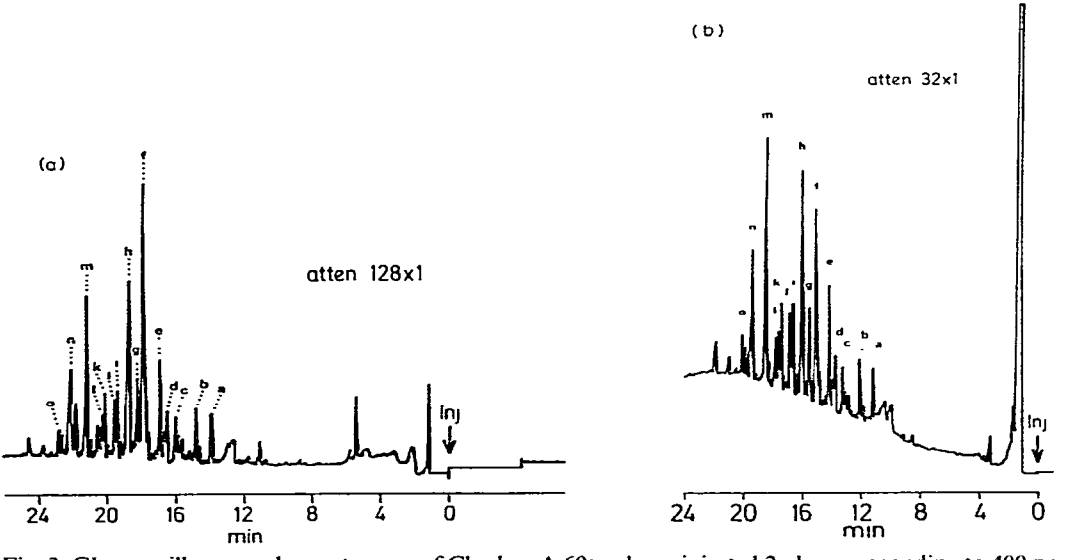

Fig. 3. Glass capillary gas chromatogram of Clophen A 60; volume injected 2 μl, corresponding to 400 pg (a) or 40 pg (b) on-column. a–c = Pentachlorobiphenyls; d–h = hexachlorobiphenyls; i–o = heptachlorobiphenyls. (a) Split–splitless mode; (b) split mode (split 1:5).

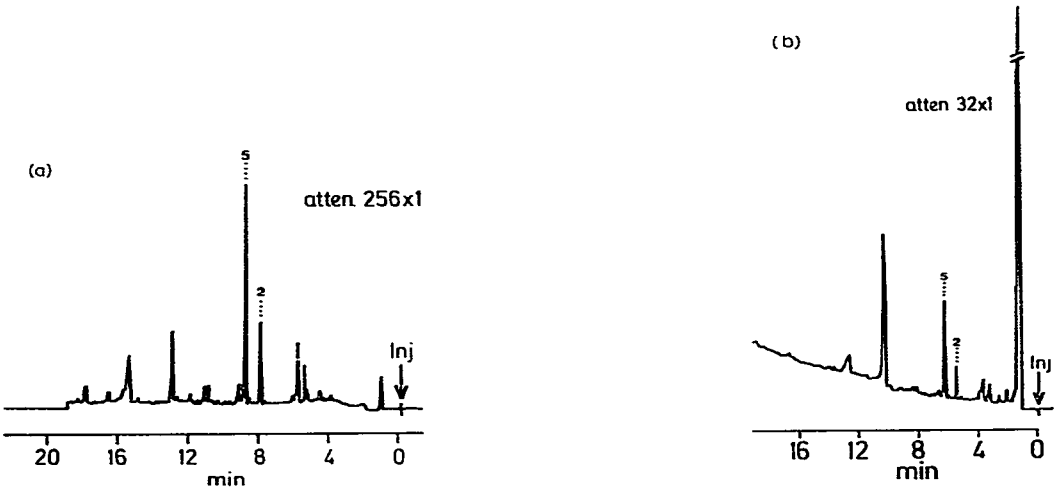

Fig. 4. Glass capillary gas chromatogram of an *n*-hexane extract from a 2-l rain water sample from Schauinsland; volume injected 1 μl. 2 = α- and 5 = γ-hexachlorocyclohexane (lindane). (a) Split–splitless mode; (b) split mode (split 1:10).

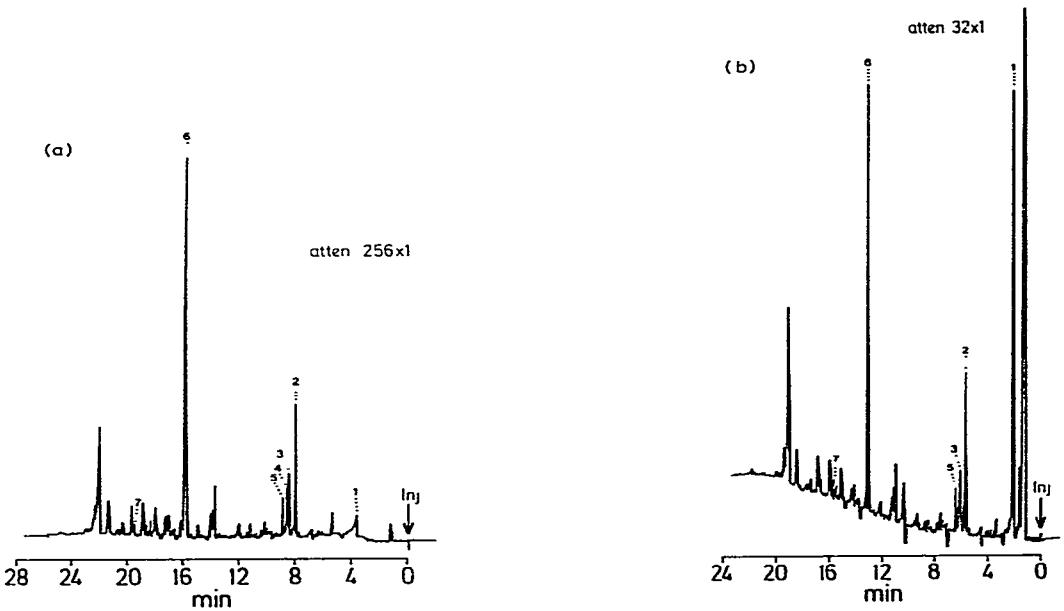

Fig. 5. Glass capillary gas chromatogram of a pentane extract of Bulgarian ewe cheese. Volumes injected: (a) 3 μl; (b) 2 μl. 1 = Perchlorobutadiene; 2 = α-, 3 = β- and 5 = γ-hexachlorocyclohexane; 4 = hexachlorobenzene; 6 = *p,p'*-DDE; 7 = *p,p'*-DDT. (a) Split–splitless mode; (b) split mode (split 1:10).

Application to organophosphorus compounds

Even with more polar compounds, no significant adsorption takes place in the injector, as could be shown for the dimethylthiophosphinic derivative of homovanillic acid methyl ester[3]. This could also be demonstrated for determination of the thiophosphorous ester parathion, which gave identical results under split–splitless and split

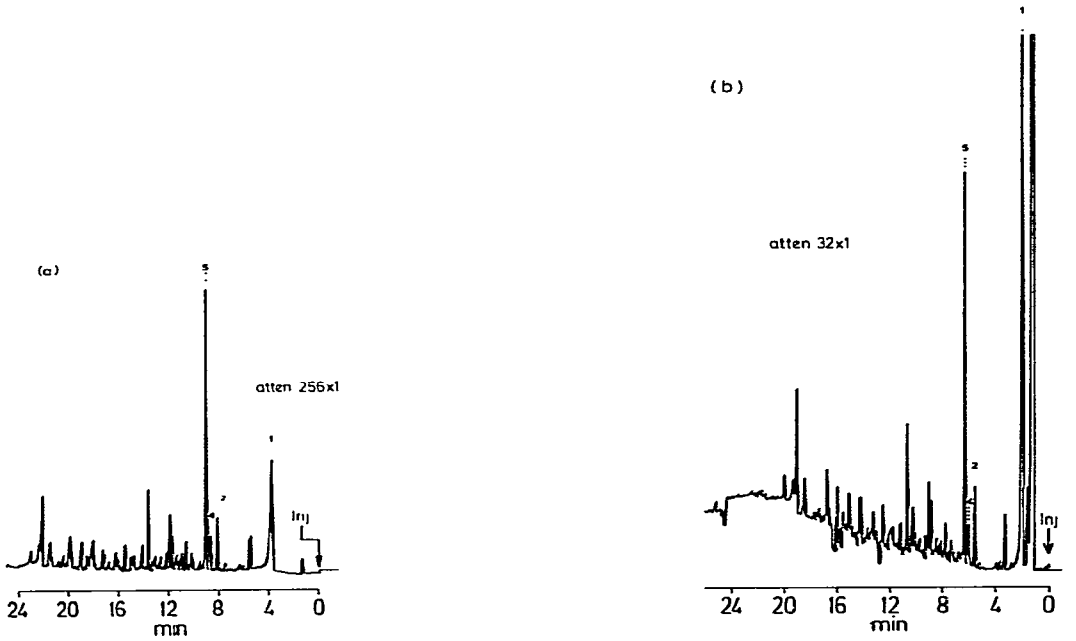

Fig. 6. Glass capillary gas chromatogram of a pentane extract of fatty tissue of venison. Volumes injected: (a) 3 μl; (b) 2 μl. 1 = Perchlorobutadiene; 2 = α- and 5 = γ-hexachlorocyclohexane; 4 = hexachlorobenzene. (a) Split–splitless mode; (b) split mode (split 1:10).

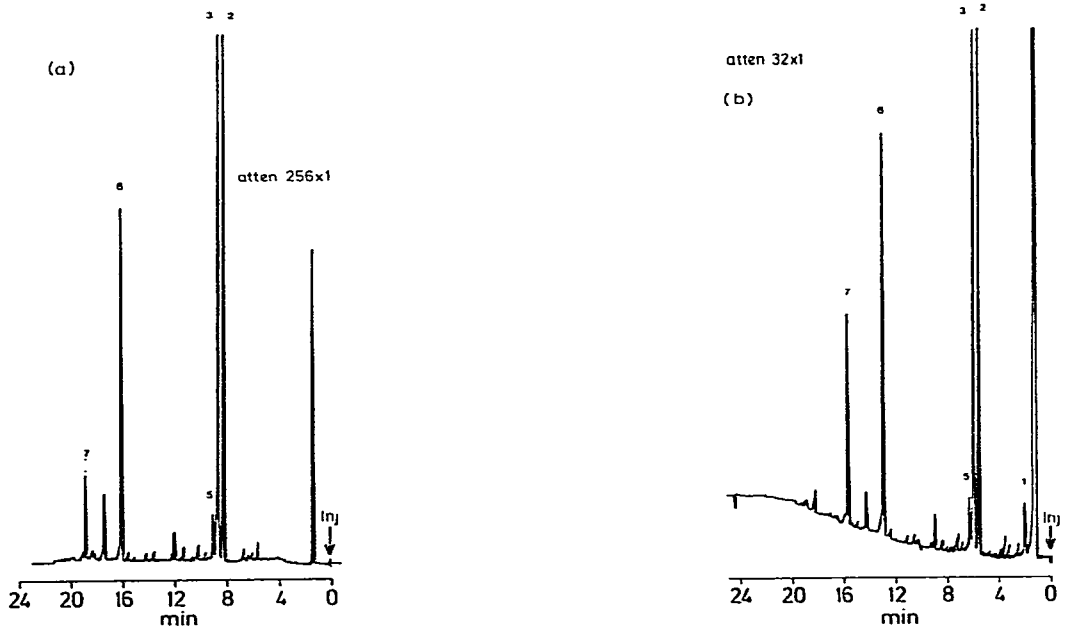

Fig. 7. Glass capillary gas chromatogram of a pentane extract of fatty tissue of house rabbit. Volumes injected: (a) 3 μl; (b) 2 μl. 1 = Perchlorobutadiene, 2 = α-, 3 = β- and 5 = γ-hexachlorocyclohexane; 6 = *p,p'*-DDE; 7 = *p,p'*-DDT. (a) Split–splitless mode; (b) split mode (split 1:10).

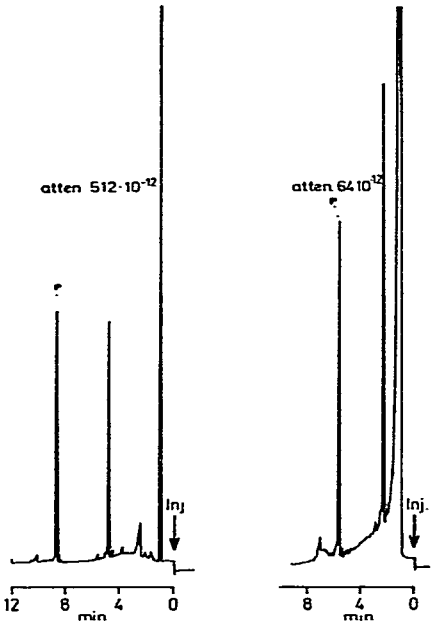

Fig. 8. Glass capillary gas chromatogram of an acetone extract of apples. Volume injected 1 μl. P = Parathion. Left, split–splitless mode; right, split mode (split 1:10).

conditions. The matrix in a very crude extract of apples did not influence the performance of the GC system (Fig. 8).

In many instances less extreme requirements are set on the analytical procedure because large amounts of material to be examined are available. Frequently, however, the amount of material to be analysed is limited and therefore the sensitivity of the technique normally used is not sufficient. In addition, a decrease in the amount of sample needed leads to a reduction in expenditure of time and labour during the clean-up procedure.

ACKNOWLEDGEMENTS

We are greatly indebted to Dr. L. Weil (Institut für Wasserchemie und Chemische Balneologie der Techn. Universität München, G.F.R.), Mrs. A. Ruschenburg (Landesuntersuchungsamt für das Gesundheitswesen Südbayern, München, G.F.R.) and Dr. E. Möllhoff (Bayer AG, Leverkusen, G.F.R.) for providing the samples.

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