Journal of Chromatography, 198 (1980) 389–396
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,042

PEAK IDENTIFICATION IN CAPILLARY GAS CHROMATOGRAPHY BY SIMULTANEOUS FLAME IONIZATION DETECTION AND ^{14}C-DETECTION

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(First received April 23rd, 1980; revised manuscript received June 19th, 1980)

SUMMARY

The simultaneous peak identification by radiocarbon and flame ionization detection in the effluents from capillary gas chromatography is reported. A constant portion of the effluent is split off, the organic matter converted into CO_2 and the $^{14}CO_2$ monitored using an anthracene flow cell. Adsorption and condensation in the detection system ("memory" effects) are thereby avoided and identical conditions can be used for a variety of compounds. The system proved to be suitable for the analysis of pesticide metabolites with a specific activity down to 37 kBq/mg, the limit of detection being 7 Bq.

INTRODUCTION

Gas chromatography–mass spectrometry (GC–MS) is a reliable and effective method for evaluation of the structure of metabolites[1–6]. Biological samples containing metabolites are complex mixtures of components. Open-tubular capillary columns are capable of separating the individual fractions by GC, and the pure components are then introduced into the mass spectrometer for structure analysis. However, the GC fraction consisting of the unknown metabolites is usually not precisely defined, and therefore a set of fractions must be analyzed by MS. The amount of data produced accumulates and the selection of reliable structural information becomes increasingly difficult.

In metabolism studies using radiolabelled compounds it is therefore desirable to detect the ^{14}C-labelled metabolite fractions during GC separation, as only these fractions need to be analyzed in MS. Simultaneous detection of radioactivity and mass in GC has been reported by several authors[7–10], but not in capillary gas chromatography. Therefore, we have developed capillary GC with simultaneous and continuous detection of mass and radioactivity.

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EXPERIMENTAL

Principle of the method

A schematic view of the apparatus is presented in Fig. 1. The effluent of the glass capillary column was split at a ratio of 9:1. The minor portion was introduced into the flame ionization detector (FID), the major one combusted to CO_2 and measured continuously in a radioactivity flow monitor using an anthracene cell.

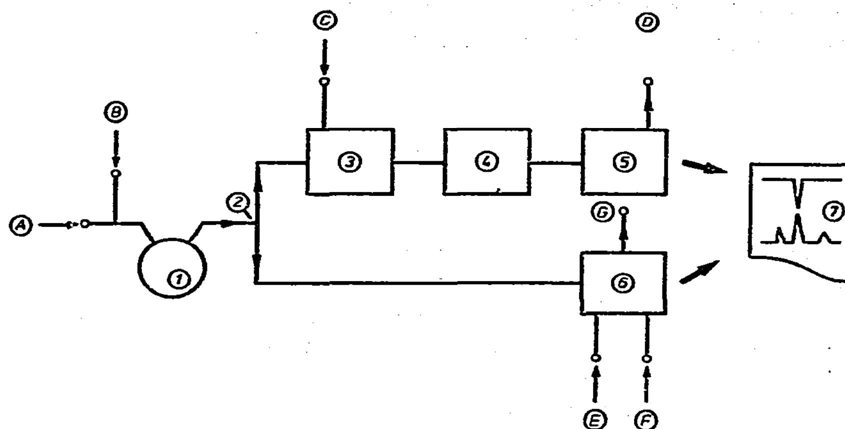

Fig. 1. Schematic description of gas flow and apparatus used for ^{14}C-detection in capillary GC. A = Carrier gas; B = injection; C = oxygen; D = outlet pump; E = air; F = hydrogen; G = exhaust. 1 = Capillary column; 2 = outlet splitter; 3 = combustion furnace; 4 = ^{14}C-flow cell; 5 = pump; 6 = FID; 7 = recorder.

Column oven and splitter

A Model 2151 Fractovap (Carlo Erba, Milan, Italy) was used, equipped with an injection system according to Grob and Grob[11]. Capillary glass columns (10–50 m × 0.3 mm I.D.) were purchased from Jaeggi (Trogen, Switzerland). Helium served as carrier gas (0.5–1.0 bar, 1–5 ml/min) and as purge gas for the injector port (17 ml/min). The columns were usually operated between 50°C and 250°C with various temperature programs. The volumes injected were 0.5–3 µl. The outlet splitter was a modification of the Neuner-Jehle splitter[12] as illustrated in Fig. 2. The splitting ratio was predetermined by the inner diameter of the outlet platinum–iridium capillaries. A ratio of 9:1 was found suitable for our purposes. One tube of the splitter was connected directly with the FID, the other one with the combustion furnace. The splitter was regularly cleaned according to the methods described by Christiansen[6].

Combustion furnace

The combustion furnace is shown in Fig. 3. The splitter effluents (1–3.5 ml/min) were transferred through a heated transfer line (300°C) into a quartz tube (inner volume 200 µl) which contained the combustion catalyst (cobaltous oxide). Oxygen gas was fed at 1.5 ml/min into the tube which was maintained at 650°C by a heating coil (platinum) powered by a variable transformer. The combustion tube is open to the

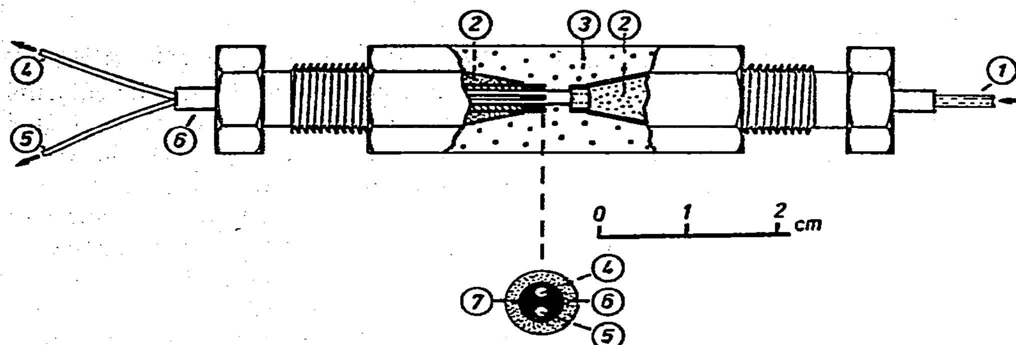

Fig. 2. Design of the outlet splitter. 1 = Glass capillary; 2 = 1/16-in. Swagelock GC ferrules; 3 = Swagelock GC union; 4, 5 = Pt–Ir capillary tube; 6 = 1/16-in. steel capillary tube; 7 = seal of silver solder. Below: exploded view of vertical cross-section of te splitter.

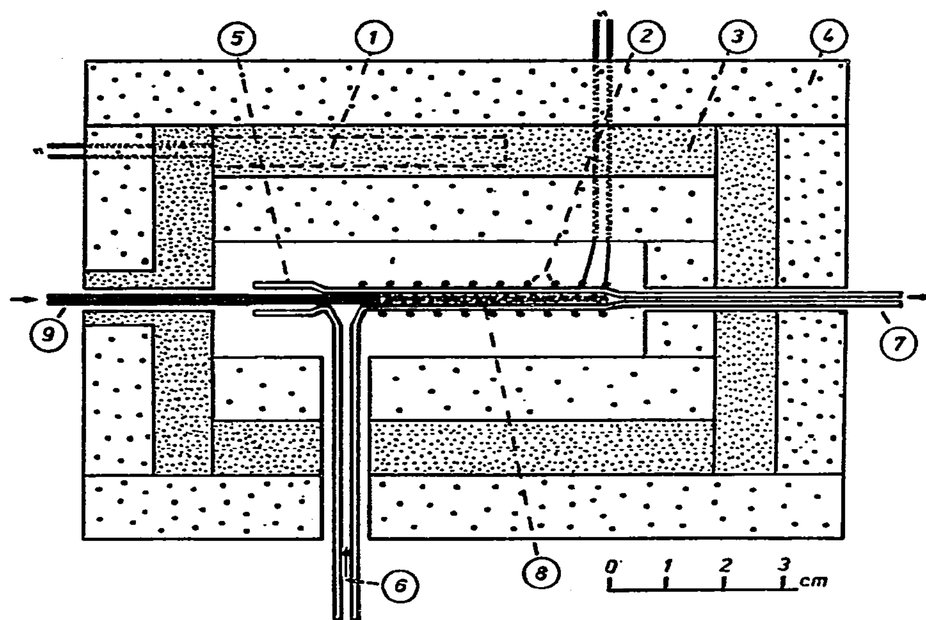

Fig. 3. Design of combustion furnace. 1 = Heating cartridge; 2 = heating coil; 3 = aluminium block; 4 = marinite housing; 5 = quartz combustion tube; 6 = oxygen inlet; 7 = transfer tube to flow cell; 8 = oxidation catalyst; 9 = inlet capillary.

atmosphere along the inlet capillary, which itself impinges directly on the combustion catalyst, back pressure onto the splitter thereby being avoided.

The aluminium block (3) heated by the cartridge (1) is maintained at a minimum temperature of 200°C. Condensation in the transfer capillary and in the combustion tube is thereby avoided.

Radioactivity flow monitor

^{14}C-Monitoring was carried out by a BF 5026 flow monitor (Dr. Berthold, Wildbad, G.F.R.) equipped with an anthracene cell (total volume 7 ml). The effluents from the combustion furnace (2.5–5 ml/min) were transferred to the cell by PTFE tubing (1/16 in. O.D.). A suction pump, Type AL No. 17 KL (W. Wirth, Basel, Switzerland), connected to the flow cell furnished a negative pressure (suction rate, 12–14 ml/min). The BF 5026 was usually operated with a sensitivity of 1000 cpm and a time constant of 2 sec. The lower and the upper thresholds of the analyzer channel A were 250 and 850 respectively. Using the time constant of 2 sec, the background of the operating system was characterized by a standard deviation of 0.37 Bq.

The anthracene (Fluka, Buchs, Switzerland) was recrystallized from toluene–ethyl acetate (8:2) and washed with ethyl acetate, then with 5% aqueous formic acid.

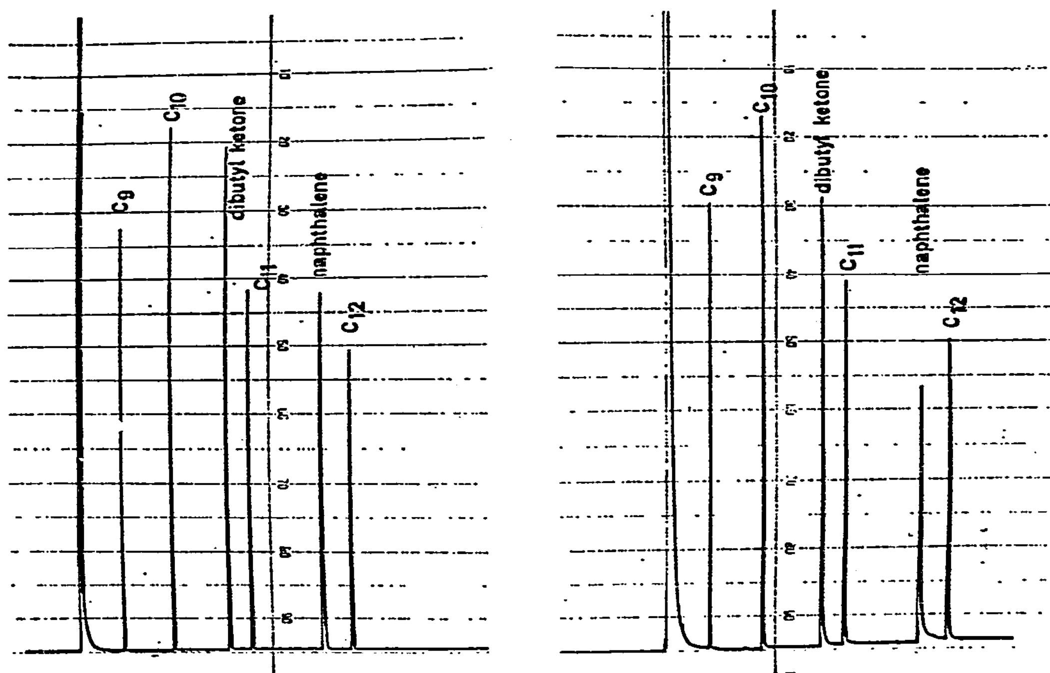

Fig. 4. Separation of a test mixture without (left) and with (right) effluent splitter. Order of elution: nonane, decane, dibutyl ketone, undecane, naphthalene and dodecane.

RESULTS

Influence of the effluent splitter on the GC resolution

As demonstrated in Fig. 4, the separation of the test mixture is slightly affected by the effluent splitter. The strong tailing of the naphthalene peak is probably caused by Carbowax remaining in the effluent splitter after its deactivation.

Reproducibility and sensitivity of the ^{14}C-detection

[^{14}C]2,6-Dimethylaniline (32 Bq) was injected repeatedly onto the capillary

column and detected with a peak height eight times the "noise" of the detector (defined as 4 × SD background, *i.e.* 1.5 Bq). Under these experimental conditions the ^{14}C-peak height of four consecutive injections was calculated to be 12 ± 0.4 Bq (mean ± SD, $n = 4$).

Application

GC analyses of typical samples are presented in Figs. 5–7.

DISCUSSION

Simultaneous detection of ^{14}C-radioactivity and mass (FID) in capillary GC was successfully performed largely due to the following features:

(1) An outlet splitter with a dead volume of approximately 10 µl.

(2) A combustion tube with a dead volume less than 100 µl in a total volume of 200 µl.

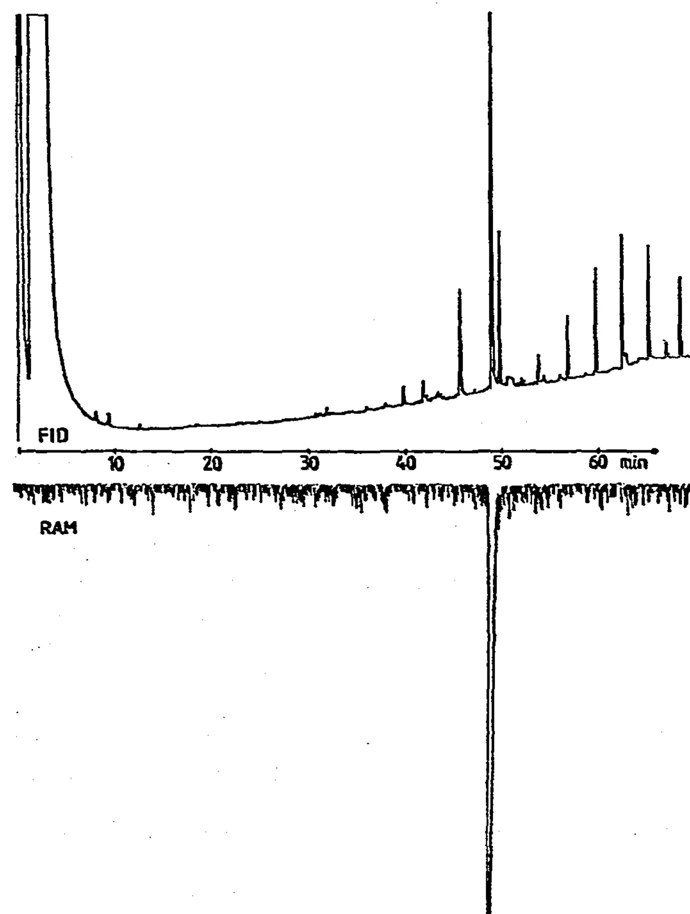

Fig. 5. Analysis of a non-polar metabolite of an agrochemical isolated from potato leaf tissue and partially purified. 167 Bq (1 µl) of radioactive material (specific activity, 800 kBq/mg) were analyzed. Column, 25 m × 0.25 mm SE 54; temperature programmed from 60°C to 250°C at 3°/min.

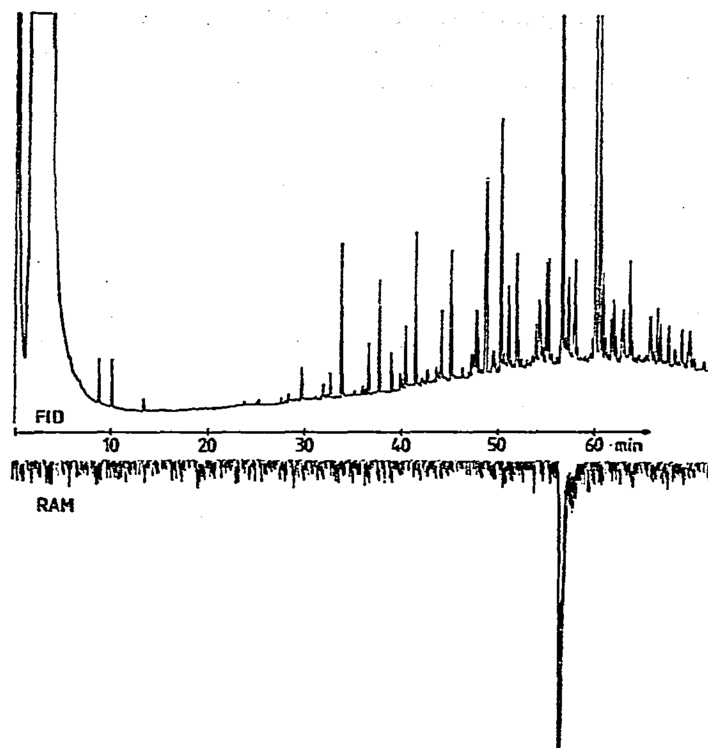

Fig. 6. Analysis of a polar metabolite of an agrochemical isolated as in Fig. 5. After methylation using diazomethane, 67 Bq (1 μl) of the radioactive material (specific activity, 318 k Bq/mg) were analyzed. Column as in Fig. 5.

Since organic materials in the GC effluents are not oxidized completely in this small volume, addition of oxygen is necessary. The catalyst would be contaminated and its activity soon exhausted without the supply of oxygen.

(3) Pressure waves generated by combustion in the tube are prevented from being tranferred back into the GC system (splitter and column) by the fact that the combustion tube is open to the atmosphere and by the presence of the suction pump.

(4) Oxidation of total organic material to CO_2 proved to have the following advantages: no contamination of the ^{14}C-detection system by organic matter, and hence no "memory" effects; no ^{14}C-recovery problems caused by the individual organic compounds; and the ^{14}C-detection must be optimized for only one compound namely CO_2. Furthermore, combustion to $^{14}CO_2$ allowed the use of an anthracene cell, which represents an improvement over glass and liquid scintillators with regard to counting efficiency and handling.

Under practical conditions the peak resolution R of the ^{14}C-detection system

$$R = \Delta t / w$$

where t = retention times, w = peak width at half peak height, was about one-half that of the FID detection. 6.7 Bq of injected radioactivity resulted in a peak height

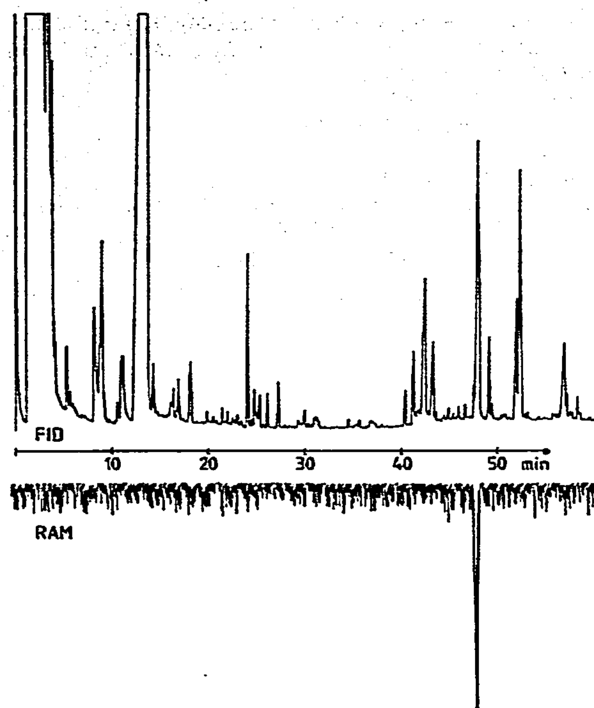

Fig. 7. Analysis of a polar metabolite of an agrochemical isolated from grape leaf tissue and partially purified. After ethylation using diazoethane, 34 Bq (1 μl) of the radioactive material (specific activity, 185 kBq/mg) were analyzed. Column as in Fig. 5, but programmed from 50°C to 250°C at 3°/min.

above background equivalent to six times the standard deviation of the background. Thus, 0.2 μg of a metabolite with a specific activity of k Bq/mg were detected during capillary gas chromatography. This amount was considered to represent the "limit of detection" of our system.

In conclusion, it was possible to measure continuously the ^{14}C-radioactivity in capillary GC effluents simultaneously with mass detection. Thus the presence of a ^{14}C-labelled metabolite in a given GC fraction could be verified immediately, and the MS data directly related to it.

ACKNOWLEDGEMENT

The technical assistance of Mrs. M. Fuchs (this Division) is acknowledged. We thank Mr. H. Wegmüller (this Division) for the preparation of Figs. 1–3 and Mrs. E. Schlatter for typing the manuscript. Thanks are also due to Mr. W. Blum and Dr. W. Richter (Central Function Research, Ciba-Geigy Ltd., Basel) for their helpful cooperation.

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