CHROM. 4936

A SIMPLE QUANTITATIVE METHOD FOR TRAPPING AND TRANSFER OF LOW CONCENTRATION GAS CHROMATOGRAPHIC FRACTIONS SUITABLE FOR USE WITH SMALL DIAMETER GLASS COLUMN SYSTEMS

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(Received July 13th, 1970)

SUMMARY

A technique suitable for the quantitative trapping and transfer of fractions from glass porous layer open tubular columns is described. Fractions are collected in short U-traps of the cooled columns and may be rapidly and efficiently transferred to other glass columns for additional separation and identification by combined gas chromatography–mass spectrometry. High and low boiling compounds down to levels of 20 ng or lower can be handled with ease. A procedure for selectively reducing large interfering peaks is discussed. The method is especially useful for dealing with labile compounds, since there is minimal contact of the sample with hot metal surfaces at all stages.

INTRODUCTION

The analysis of complex mixtures by gas chromatography (GC) frequently necessitates the trapping out of fractions from a column either for subsequent analysis on a column of different polarity or for examination by some other analytical technique. Such complexity is encountered in the area of food flavour chemistry, where combined gas chromatography–mass spectrometry (MS) is, at the present time, the most widely used and versatile technique for studying the multi-component mixtures of volatile compounds isolated from various foodstuffs, usually in amounts at the p.p.m. levels. In most flavour essences only some of the regions of a complex chromatogram contain peaks (or groups of peaks) of olfactory significance and often these are present in relatively low concentrations.

One problem in analysing such essences is the following. Since high efficiency wall-coated or porous layer open tubular (PLOT) columns are normally used in the analysis of such mixtures, it is frequently not possible to inject enough sample onto the column to permit MS analysis of these low concentration components without impairing the separating power of the column by overloading.

Another problem which often arises in this type of analysis is caused by the presence of some components in such high relative concentrations that the mass

spectrum of a minor compound eluted on its tail is obscured. A high residual background from the large peak can make it very difficult to decipher a weak superimposed spectrum. This difficulty may sometimes, though not always, be overcome by analysing the mixture on a number of columns of different polarity.

One method of solving the first of the above problems is to inject a large sample onto a packed column and to trap out the desired region for rechromatography on a high efficiency column. If it is desired to trap out a very narrow region and minimise interference from large peaks surrounding the region of interest, repeated charges of sample may be applied to the capillary or PLOT column, the required fractions being concentrated in an appropriate trap and then rerun. Minor components of interest, which follow a large interfering peak, may be obtained relatively free of the latter by trapping out repeatedly fractions on the tail of the large component.

In recent years several authors[1–5] have described a variety of techniques for the collection and transfer of small amounts of samples eluted from packed and, to a somewhat lesser extent, from capillary columns[6]. When dealing with small diameter column systems there are two main requirements which a trapping and transfer technique should fulfil:

(a) Minimal loss of sample should take place during both the collection or concentration step and in the transfer stage.

(b) The trapped sample should be presented to the column in a sharp narrow band, so that the total available efficiency of the column is used.

This is particularly important where a minor component is trapped on the tail of a major peak.

In this paper, a technique is described for the trapping and transfer of fractions between glass PLOT columns which satisfies both of the above requirements. Recoveries are quantitative for compounds covering a wide boiling range at levels down to 20 ng or even lower. The methods permit the concentration of small peaks from a PLOT column, as well as the selective bypassing of large interfering peaks during the course of a run.

EXPERIMENTAL

Apparatus

In the work discussed here glass PLOT columns were prepared by a method recently described[7]. The fraction collecting traps were made from short straight lengths of the same PLOT tubing as follows. A piece of tubing, about 135 mm long, was heated in the middle over a micro bunsen flame and bent into the shape of a narrow U. Each open end was then turned down on the flame to give two short lengths (10 mm) at angles of 90° to the rest of the tube.

A Pye Model 104 gas chromatograph, equipped with a heated flame detector and a heated injection head, was used. A small volume injection system which fitted directly to the standard compression coupling of the Pye injection head, was made from a length of glass capillary tubing (6.25 mm O.D. × 1.05 mm I.D.) in the following manner. A length of capillary tube was carefully heated in a gas–oxygen flame and drawn out to give a narrow portion of approximately 1 mm diameter. The tube was then cut off to a length of 65 mm above the narrow portion so that when connected to

the injection port the narrow portion extended just below the bottom of the heated injection zone into the oven.

In order to make connections between the injection system and the column and between the column and a trap, short lengths (20 mm) of thin-wall PTFE tubing (bore 0.75 mm) were used in conjunction with 20-mm long hypodermic needles (21 gauge) from which the syringe adaptor fittings were cut off. The pieces of PTFE tubing were fitted carefully over the narrowed end of the injection system and over both ends of the column and any trap after softening the tubing over a very low bunsen flame. On cooling a tight leak-proof seal formed between the glass and PTFE tubing. A tiny spot of 'Araldite' resin was applied around each joint to reinforce it for operation at up to 220°. The cut-off ends of 21-gauge hypodermic needles were then inserted through approximately half the length of the connecting tube at one end of both the column and trap. A little 'Araldite' was placed arount the junction of the PTFE and the needle. Connection of the column to the injection system was then achieved by simply pushing the needle into the PTFE connection on the latter.

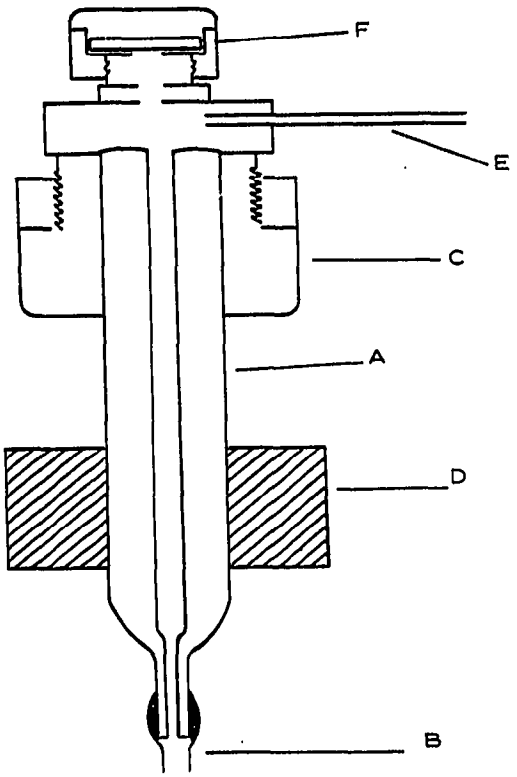


Fig. 1. Glass injection system. (A) Glass capillary tube (6.25 mm O.D. × 1.05 mm I.D.); (B) PTFE connection to column; (C) compression nut; (D) injection heater; (E) carrier gas inlet; (F) septum.

This method of connecting glass PLOT column systems possesses low dead volume and has provided a leak-proof injection system when tested with carrier gas pressures of 1.2 kg/cm^2 and column operating temperatures up to 220°. The injection system and column connection method are shown in Fig. 1.

The column, which was positioned in the oven by suspending it from a length of thin wire attached to a hook fixed to the roof of the oven, was connected to the FID and trap via a splitter system constructed from a three-way 1.55-mm O.D.

stainless steel coupling*. Two hypodermic needles, each 20 mm long, were silver soldered to two short lengths of stainless steel capillary tubing (10 mm × 1.55 mm O.D.) and were used to connect the PLOT column and trap to the coupling. The connection between the latter and the detector was made by silver soldering a 100-mm length of narrow stainless steel capillary tubing (0.5 mm O.D. × 0.3 mm I.D.) to a 10-mm length of 1.55-mm diameter tube. The narrow capillary was inserted through the line leading from the base of the detector into the oven, effectively reducing the dead volume of this portion. The split ratio between the FID and trap may be set to any required value by restricting the narrow capillary tube with suitable lengths of thin stainless steel wire. A split ratio of 9:1 in favour of the trap was used in the experiments described here.

A piece of thin-wall PTFE tubing, approximately 60 cm long, was connected to

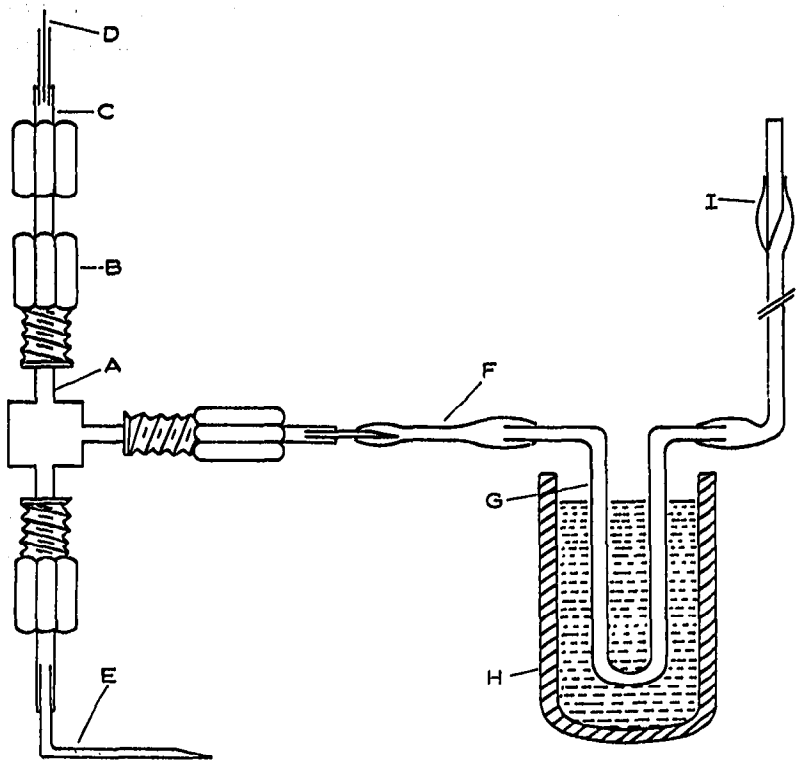


Fig. 2. Fraction collector and splitter arrangement. (A) 1.55 mm O.D. three-way connector; (B) connecting nut; (C) connection to FID; (D) restricting wire; (E) hypodermic connection to column; (F) PTFE connecting tube; (G) PLOT trap; (H) liquid nitrogen; (I) external blanking hypodermic connection.

the exit of the glass trap and led through a small hole bored through the roof of the chromatographic oven. This enabled the effluent from the column to be directed as desired during the course of a run to the FID by simply inserting a blanked-off hypodermic needle into the PTFE tubing outside the oven, thereby stopping the flow of carrier gas through the trap. The arrangement of the splitter and the trap is shown in Fig. 2.

* Simplifix Couplings Ltd., Maidenhead, Berks.

Trapping and transfer of fractions

Trapping of fractions is carried out by immersing the PLOT U-tube in a small Dewar flask of 30 ml capacity, which is filled with liquid nitrogen. If a number of peaks are to be collected at different times during a run with the splitter *in situ*, the U-tube is normally held in the coolant from the start of the run. When just one peak or group of peaks is to be trapped during a run, the splitter arrangement may be dispensed with and the trap connected directly between the column and the FID. In this case the trap is cooled just before the peak of interest emerges. To obviate the need to open the oven for insertion of the coolant under the trap, it is convenient to enclose the Dewar flask in a small wire cage, which can be raised or lowered within the oven by means of a length of thin copper wire protruding through a small hole in the roof of the oven. The wire is held in position by means of a small clamp fixed on the roof of the oven.

After collection of a fraction, the trap, still immersed in the liquid nitrogen, is removed from the oven and plugs of blanked off PTFE tubing are placed over each end. It may be stored indefinitely under these conditions without loss or contamination. For rechromatography of the trapped sample the column is disconnected from the injection block and the cooled trap inserted between the latter and the column. If the splitter is in position, the open end is blocked with a plug of blanked off PTFE tubing. The newly made connections are carefully checked for leaks. As soon as the oven attains the required starting temperature, the flask of coolant is removed by lowering from under the trap and the run allowed to proceed.

Contact of the sample with metal surfaces is kept to a minimum in the system described above, occurring only within the splitter and the hypodermic connectors, which possess a very low combined dead volume.

Trapping experiments were carried out with two different PLOT columns, a 12-m length coated with a 1% w/v solution of Carbowax 20M solution in methylene chloride and a 25-m length coated with a 0.2% w/v solution. The trap itself was also coated with a 0.2% solution of the same phase.

RESULTS AND DISCUSSION

The effectiveness of the trapping system was tested for a range of carrier gas flow rates from 2 to 20 ml/min using the 12-m column and diethyl ether as the test substance. Replicate charges (0.5 ml) of a vapour sample containing 0.2% v/v of diethyl ether in air were injected for each flow rate with the traps either cooled or uncooled. The chromatograph attenuation was increased by a factor of 100 when the trap was cooled. Comparison of peak heights showed the trapping system to be 100% efficient at 2–15 ml/min and 99.8% at 20 ml/min.

The quantitative aspect of the combined sample trapping and transferring process was investigated with a mixture of compounds having a range of polarities, consisting of a 0.5% v/v solution of undecane, *p*-xylene, *n*-pentanol and 2-octanone in diethyl ether. With the trap inserted directly between the 12-m column and the detector 0.2-μl charges of sample were injected onto the column at a temperature of 115° and a flow rate of 5 ml/min of helium. The ratio of each peak area relative to undecane was determined. The injection was repeated, but this time the trap was cooled immediately after elution of the undecane peak and the three components collected. The trapped fraction was then rechromatographed by the procedure de-

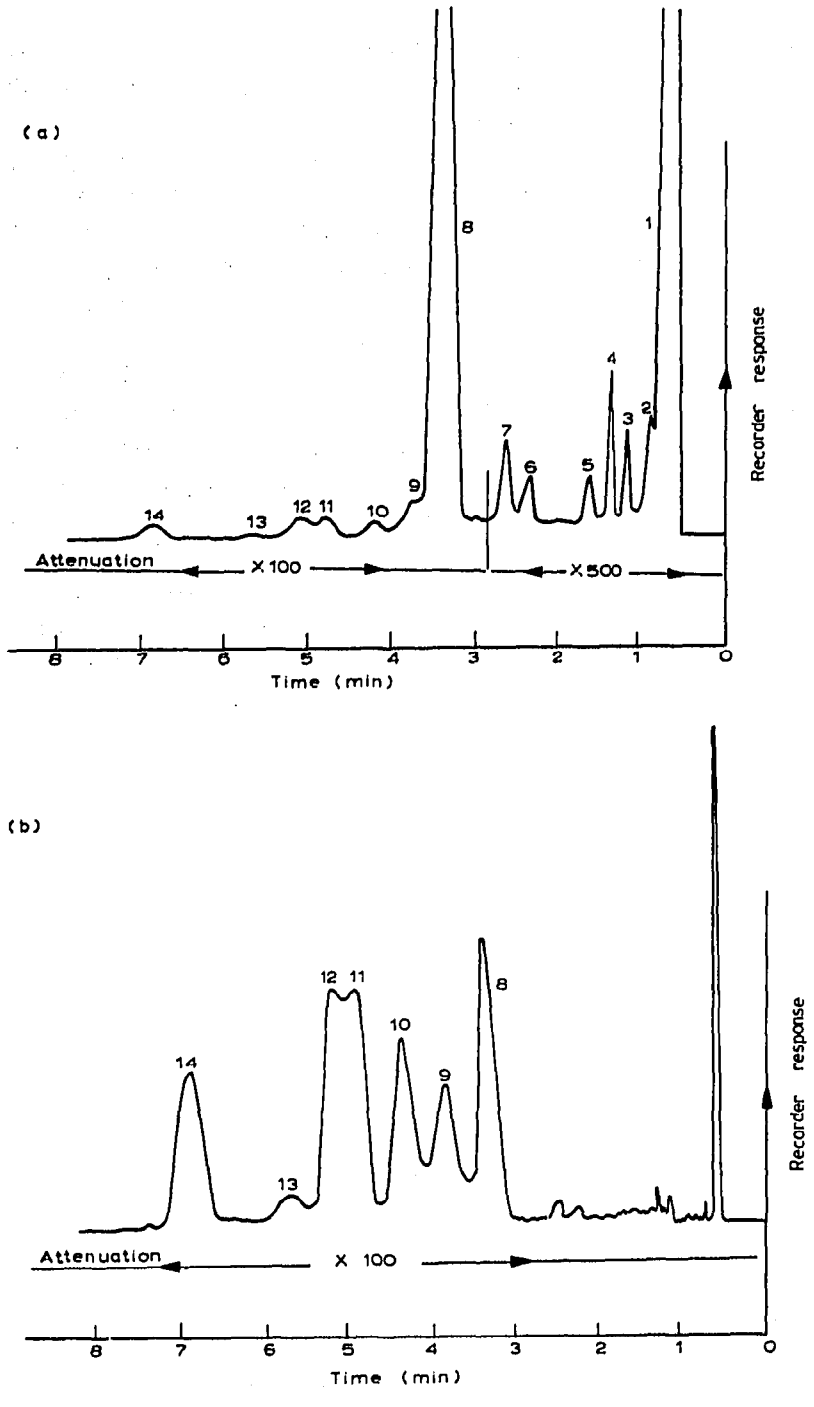


Fig. 3. Chromatograms of (a) a 0.5-μl injection of a synthetic mixture and (b) a rerun of a trapped fraction from a 5-μl injection showing a concentration of minor peaks. Column, 12 m × 0.5 mm PLOT, coated with 1% w/v Carbowax 20M; temperature 115°; flow rate, 6 ml/min helium.

scribed above and the peak area ratios relative to the undecane peak again determined. There was excellent agreement between the ratios obtained for both the trapped and untrapped runs in replicate experiments, giving overall trapping and transfer efficiencies in the range 97–100% for each of the three components. It has been possible

to obtain almost quantitative recoveries from the trapping and transfer of quantities as low as 20 ng of *n*-pentanol.

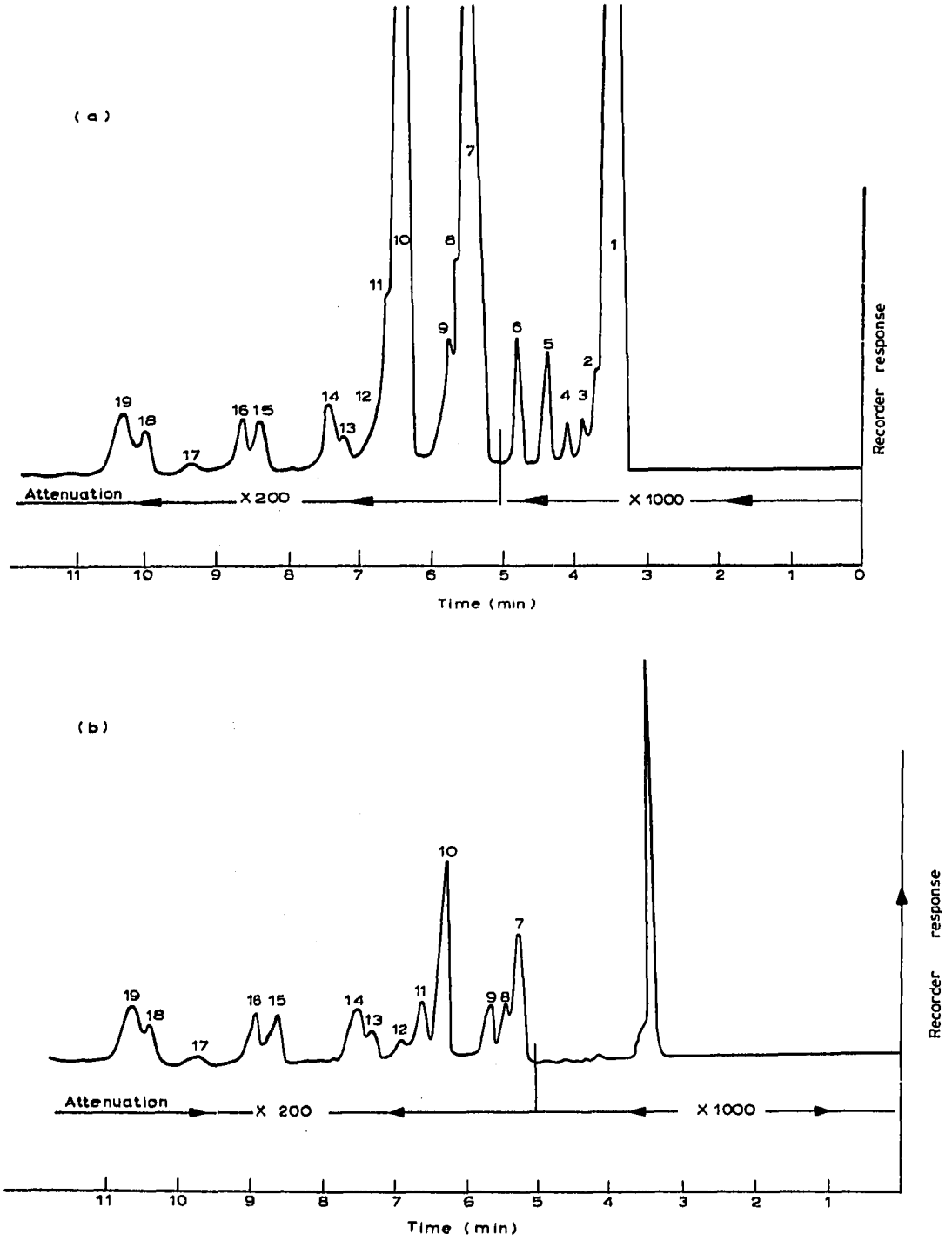
An example of concentrating an area of low concentration following a large peak is shown in chromatograms (a) and (b) in Fig. 3. Chromatogram (a) represents a 0.5-μl injection of a synthetic mixture of aliphatic alcohols, ketones and hydrocarbons in ether, corresponding to a weight of 50 μg for 2-octanone (peak 8) and between 0.2–1 μg for dodecane (9), *n*-hexanol (10), 4-octanol (11), 3-octanol (12) and *n*-heptanol (14). The column was then overloaded by injecting 5 μl of the same mixture. The coolant was applied to the trap just as the trailing edge of peak 8 came back on scale on the recorder chart, and the subsequent small peaks were collected. Chromatogram (b) shows the rerun of the trapped fraction under the same conditions. Peak 9 is seen to be much enhanced relative to peak 8 and the recoveries of the other minor components are clearly excellent, peak heights being enhanced by virtually the expected ratio of 10.

Fig. 4 represents a more complex situation, where two minor components, ethyl hexanoate (8) and *o*-ethylbenzene (9), eluted on the tail of a major component, *n*-pentanol (7), are followed by another major component, 2-octanone (10), on the tail of which occurs another minor component, dodecane (11). Fig. 4a shows the chromatography of a 0.2-μl sample injected with the splitter in position, but with the entire column effluent directed to the detector. With the trap cooled a second 0.2-μl sample was injected. The column effluent was initially directed to the detector by blocking off the flow through the splitter in the manner already described. As soon as the trailing edge of the major peak 7 came back on scale the effluent was directed through the trap to collect peaks 8 and 9. Just before the large peak 10 appeared, the trap was again blanked off until the latter appeared on scale and then the remaining peaks on the chromatogram were collected in the trap. These include *n*-hexanol (14), 4-octanol (15), 3-octanol (16), tridecane (18) and *n*-heptanol (19). Chromatogram 4b represents a rerun of the trapped components on the same column. The results of trapping and transferring can be seen to have caused no loss even in the resolution of incompletely separated peaks and the interference of the large peaks 7 and 10 with the minor components on their respective trailing edges has been markedly reduced. Fig. 4c shows the rerun of a fraction from a 1-μl sample of the same mixture trapped out as described above and indicates how small peaks can be concentrated from different regions of a chromatogram during the course of a run.

In the experiments described above vaporisation of the trapped fractions has been effectively achieved by simply allowing the trap to heat to oven temperature on removal of the coolant. Adequate vaporisation for compounds boiling up to around 300° is obtainable without difficulty. A sample containing 2 μg each of the *n*-paraffin series tridecane to heptadecane was injected on the 20-m column at 80° and programmed at 8°/min. From a second sample the five compounds were collected in the trap and then rechromatographed under the same conditions. The width at half height for each peak was identical for both the trapped and untrapped runs. If lower initial oven temperatures, *e.g.* 40–50°, are employed in the temperature-programmed analysis of wide boiling range mixtures it may be useful to assist vaporisation of the trapped sample by placing a beaker of hot water under the trap after removal of the coolant.

It may be necessary when dealing with mixtures of wide boiling range to trap out fractions at various times in the duration of a run. Consequently, the length of the cooled portion of the PLOT trap becomes increasingly less as the liquid nitrogen in

the Dewar evaporates and some loss of previously trapped lower-boiling compounds might be expected each time the carrier gas is bypassed through the trap to collect a fraction. In a relevant experiment 0.2 μl of diethyl ether were injected onto the 12-m column with the trap connected to the detector and immersed in a full flask of liquid nitrogen. The chromatograph was programmed from 70° at 3°/min with the attenuation set at × 50. No signal was registered on the recorder until the temperature reached



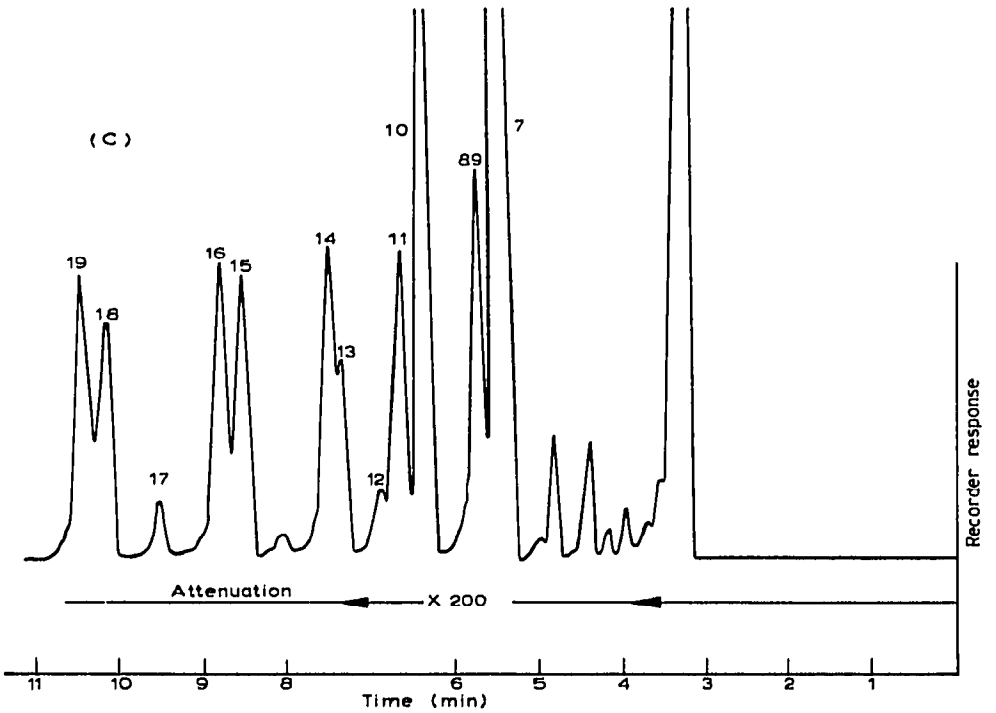


Fig. 4. Chromatograms of (a) a 0.2-μl synthetic mixture run without splitting; (b) a rerun of a trapped fraction from a 0.2-μl sample after selective reduction of peaks 7 and 10; (c) a rerun of a trapped fraction from a 1-μl sample showing a concentration of minor components. Column, 25 m × 0.5 mm PLOT, coated with 0.2% w/v Carbowax 20M; temperature, initially 80° (3 min), then programmed at 3°/min; flow rate, 2 ml/min helium.

195°, when the ether peak broke through in a sharp band, the trap having run completely dry.

The techniques presented in this paper have been developed particularly for use in the study of the complex mixtures represented by food volatiles which may contain labile compounds at the higher-boiling end. The minimal contact of the sample with large hot metal surfaces at all stages in collection and subsequent handling of trapped fractions is a particular advantage of the method and should make it applicable to a wide range of other problems involving labile compounds in trace amounts.

ACKNOWLEDGEMENT

Financial support from the Science Research Council for this work is gratefully acknowledged.

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