AN EVALUATION OF VENTED PROGRAMMED TEMPERATURE PRECOLUMNS IN GAS–LIQUID CHROMATOGRAPHY

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One of the principal problems encountered in the analysis of high molecular weight compounds by gas chromatography is satisfactory introduction of the sample onto the column. This was pointed out by RENSHAW AND BIRAN[1] who developed a relatively simple apparatus and technique whereby a dilute solution of methyl esters could be accurately measured into a "spoon", the solvent evaporated, and the "spoon" introduced into the heated portion of the chromatograph. The technique as described worked satisfactorily for C_{12} and higher fatty acid methyl esters. KIRKLAND[2] also developed a similar method in which the solvent was "evaporated" within the chromatograph as opposed to the external evaporation technique of RENSHAW AND BIRAN. In KIRKLAND'S method, the solution was injected into a flash vaporizer, carried into a cold column where condensation of high boiling components occurred, and the solvent vented to the atmosphere by means of a valve arrangement preceding a second (partition) column. After closing the vent valve, a traveling furnace moved the high boiling constituents through the first column and onto the second column where partition and subsequent separation occurred. A third method of sample introduction is possible with packed columns by utilizing temperature programming of the partition column. The solution can be vaporized, carried onto a "cold" partition column where the high boiling constituents are immobilized in the liquid phase while the solvent passes through the column. This method is the least satisfactory of the three because extreme solvent tailing generally occurs, particularly with low liquid phase loadings on the column.

When using packed columns and large solution injections, argon ionization detectors become overloaded with resulting electrode arcing while hydrogen flame detectors are often extinguished by the sudden pressure surge due to flash vaporization of the solvent. In addition, the solvent often removes liquid phase from the front of the column (exposing the support) and carries part of the column liquid phase into the detector with a subsequent unstable increase in background current and decreased sensitivity. Packed columns can handle large volumes of solvent, however, and this is undoubtedly one of the principal reasons they are preferred over the more efficient Golay columns which normally require a stream splitter and, effectively, more concentrated solutions for equivalent sensitivity.

KIRKLAND'S method and the temperature programming method both normally utilize heated injection ports which often result in sample decomposition, particu-

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larly with drugs, pesticides, and organo-metallics. Because of this, direct on-column injection techniques are again becoming popular, particularly in preparative separations[3].

Recently we have investigated a concept similar to that suggested by KIRKLAND. Basically the method consists of injecting a relatively large volume of solution into a short precolumn initially at low temperature and venting off the solvent at a point between this short column and the partition column (see Fig. 1). The vent is then

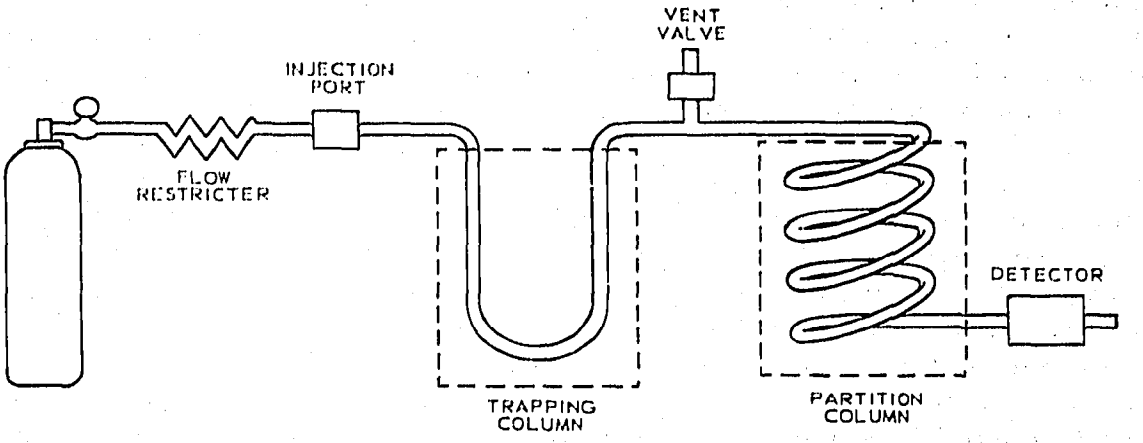

Fig. 1. Schematic of the precolumn-vent system.

closed and the precolumn temperature is programmed rapidly to transfer the higher boiling components onto the partition column. In this way, under optimum conditions, only traces of the solvent are introduced onto the partition column so that neither tailing nor detector overloading occurs. (This basic concept has recently been reported by KARMEN, WALKER AND BOWMAN[4] who eliminated the partition column to simply analyze for total lipids after venting off the low boiling solvent.)

EXPERIMENTAL

The first precolumn investigated was a $^1/_8$ in. O.D., $^1/_{16}$ in. I.D. by 8 in. stainless steel tube packed with uncoated $^{100}/_{120}$ mesh silicon carbide. This was used in series with a $^1/_{16}$ in. I.D. by 5 ft. partition column packed with 5 % SE 30 Silicone gum rubber (General Electric) on $^{100}/_{170}$ mesh Anakrom ABS (Analytical Engineering Laboratories). The partition column and an all-metal vent valve were maintained at 70°. The precolumn was wrapped with asbestos-covered nichrome heating wire. Flow rate was 24 ml/min at the detector and 30 ml/min at the vent outlet.

The second precolumn investigated was an uncoated $^1/_{16}$ in. O.D., 0.020 in. I.D. by 4 ft. length of stainless steel capillary tubing. The first 1.5 in. of the capillary was expanded to an internal diameter of approximately 0.035 in. to allow for insertion of Hamilton syringe needles. A modified Swagelok fitting was used with a Burrell silicone septum seal for needle insertion. This capillary precolumn was used in series with a 35 ft., 0.010-in. I.D. capillary column internally coated with SE 30 silicone rubber. The vent valve separating the precolumn from the partition column consisted of a 6 in. length of $^1/_{16}$ in. O.D. capillary tubing which led to a specially constructed, neoprene-sealed on-off toggle valve of minimum dead volume. The Golay column was maintained at 60°. The precolumn was electrically isolated at one

end to become a self-heating resistance element when connected to a 6 V transformer through an on-off switch. The flow rate was 12 ml/min at the detector and 25 ml/min at the vent outlet.

A conventional Beckman hydrogen flame ionization detector and electrometer amplifier were used in both cases for effluent detection.

In order to briefly investigate the possibility of using this system for high speed, high temperature chromatography, a special hydrogen flame unit was constructed. This consisted of a 1.5 in. diameter brass base around which 20 ft. of $^1/_{16}$ in. O.D., 0.020-in. I.D. stainless steel tubing was wrapped. The detector was machined into this base as were holes for air and hydrogen lines, a cartridge heater, a "Y" connector to join a capillary precolumn, vent line, and partition column, and a thermocouple. Four feet of $^1/_{16}$ in. O.D., 0.020-in. I.D. stainless steel tubing was used for the precolumn and a 2 in. piece of $^1/_{16}$ in. O.D., 0.020-in. I.D. tubing was used for the vent. All connections were designed to eliminate dead space and were silver-soldered. A 10 V transformer was used to heat the precolumn by isolation of one end to become a self-heating resistance element in the same manner as described above. The temperature rise was measured by attaching a thermocouple to the precolumn; at 10 V the precolumn heated from 25° to 250° in 15 sec with a helium flow of 40 ml/min (optimum flow rate for this system) passing through the precolumn. The partition column was coated in place with Versamid 900 (General Mills) and was maintained at 200°.

In all cases flow was limited by the use of a capillary flow restrictor and two-stage pressure regulator preceding the gas chromatographic systems. The flow difference between the vent and the detector with the first two systems was due to the flow resistance of the partition columns. In the third, high-speed system, flow rate was controlled almost entirely by the resistance of the capillary flow restrictor preceding the system so that the same flow rate was measured at the vent as at the detector. The flow resistance of the partition columns made possible the use of simple on-off valves for the vent. Helium was used in each case as the carrier gas.

RESULTS AND PERFORMANCE

To determine the maximum amount of solvent (diethyl ether) which could be injected into the precolumn without resulting in sample carry-over through the precolumn and out the vent, a series of dilutions were prepared of fatty acid methyl esters which differed in solute concentration by factors of 2. Increasing volumes of solution were then injected into the systems such that the sample weight remained constant. The determination of ester loss was then made in the case of the packed column by comparing the response for each ester injection with that of reference injections without venting. With the Golay column, this method of comparison was not possible because direct injection of the solvent into the column was not feasible. However, the responses for the first four dilutions using 2, 4, 8, and 16 μl injections were identical and as a result, it was assumed that no loss occurred.

It was found that with the $^1/_{16}$ in. I.D. by 8 in. precolumn packed with silicon carbide that up to 200 μl of solution could be injected without any loss of C_5 and C_6 fatty acid methyl esters when using a vent time of 1 min at a vent temperature of 25°. Larger injections resulted in some carry-over of the esters through the vent. The

1-min vent time was optimum for these relatively volatile esters with vent times shorter than 30 sec resulting in solvent being carried onto the partition column while vent times longer than 3 min resulted in some loss of these esters. It was estimated that more than 95 % of the solvent is vented within the first 10 sec. With the capillary precolumn using C_8 and C_9 fatty acid methyl esters as the chromatographic samples, up to 32 μl of solution could be injected using a 10-sec vent time (optimum) before a significant amount of solvent was carried onto the partition column. By using a longer vent time (up to 3 min) as much as 64 μl could be injected, but when larger solution volumes were used, serious nonreproducible losses of the esters through the vent occurred. The 32-μl to 64-μl injections used with the capillary injection system represented approximately $^1/_4$ to $^1/_3$ the internal volume of the tubing. Fig. 2 shows a typical chromatogram of the C_8 and C_9 fatty acid methyl esters chromatographed using this system.

In using the Golay system designed for high speed chromatography, it was found that after establishment of optimum flow rates, vent time, and precolumn heating

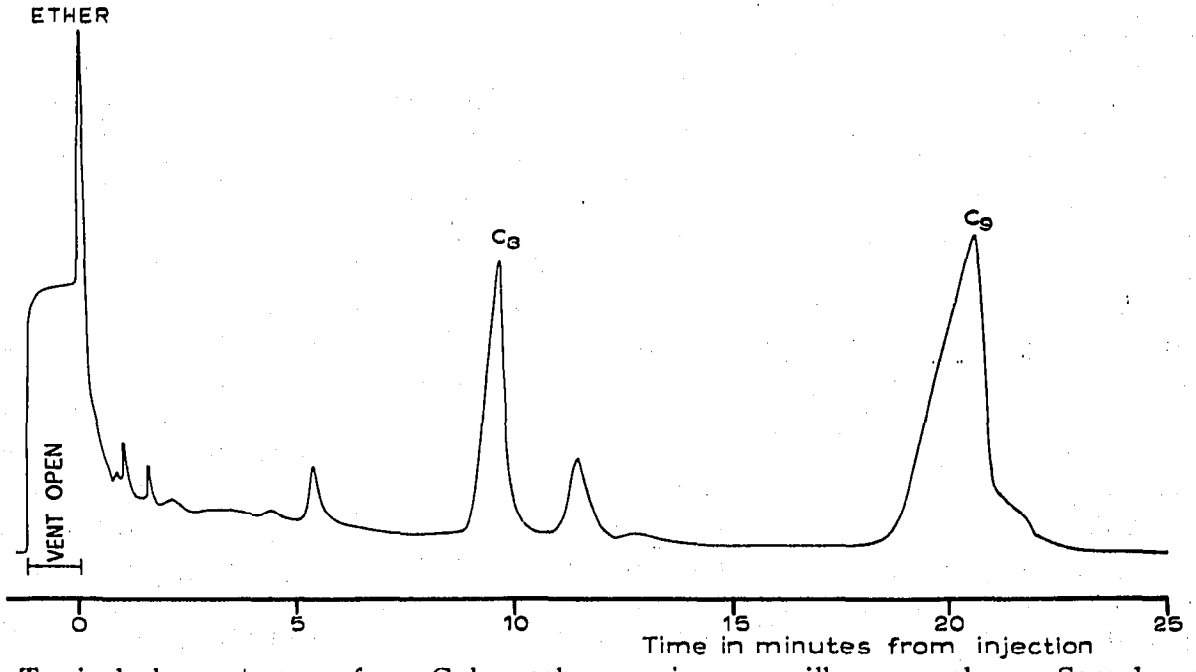

Fig. 2. Typical chromatogram from Golay column using a capillary precolumn. Sample: 20 μl injection containing 1.2×10^{-6} g methyl octanoate (b.p. 193°) and $2.5 \cdot 10^{-6}$ g methyl nonanoate (b.p. 215°). Conditions described in text.

time, it was possible to reproducibly separate the NIH Metabolism Study Section Standard Mixture "C" of C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18} and C_{20} fatty acid methyl esters in 30 sec with a total analysis time of 1 min. The first four components were not completely separated, but were identifiable (see Fig. 3).

APPLICATION TO PROGRAMMED TEMPERATURE AND ISOTHERMAL COMMERCIAL CHROMATOGRAPHS

On the basis of the work discussed above, two commercial gas chromatographs were modified to enable the use of this system. In the first modified chromatograph

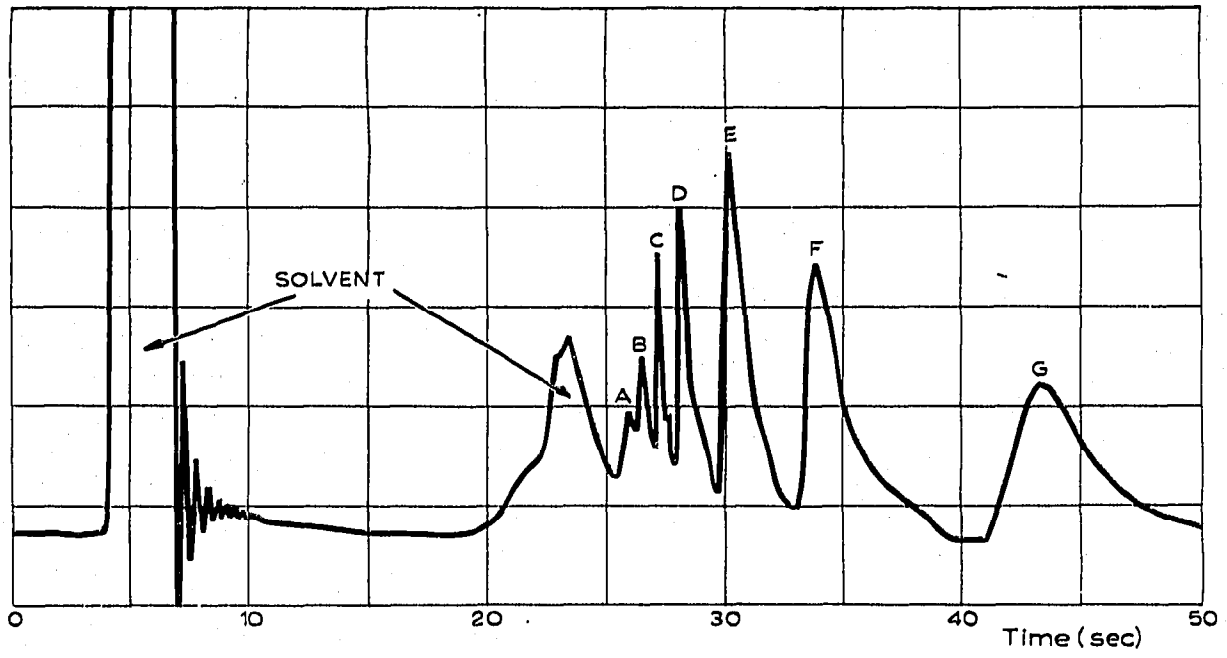

Fig. 3. Oscilloscope tracing of high speed methyl ester chromatography. Initial solvent peak is due to short vent time (13 sec). The second solvent peak is residual solvent held by condensed methyl esters. Sample size: 2 μl of 0.05 % ester mixture described in text.

(Beckman Thermotrac), the injection port was replaced with a 6-in. long precolumn filled with silicon carbide. The vent was placed in the feedthrough connector which joins the precolumn with the partition column. It was found that condensation of higher molecular weight esters occurred in this connector which was thermally attached to a heat sink (the oven wall). Subsequent slow vaporization of the esters from the connector as the column oven temperature was raised resulted in broad, unsymmetrical peaks as shown in Fig. 4. This was initially eliminated by maintaining the connector at the same temperature as the detector, however, it was found that when certain drugs and pesticides were chromatographed, decomposition occurred at this joint just as decomposition had occurred in conventional flash vaporizers. Consequently, a "floating" connection (one not physically or thermally connected to the oven wall) was used. In this case chromatograms exhibiting good symmetrical

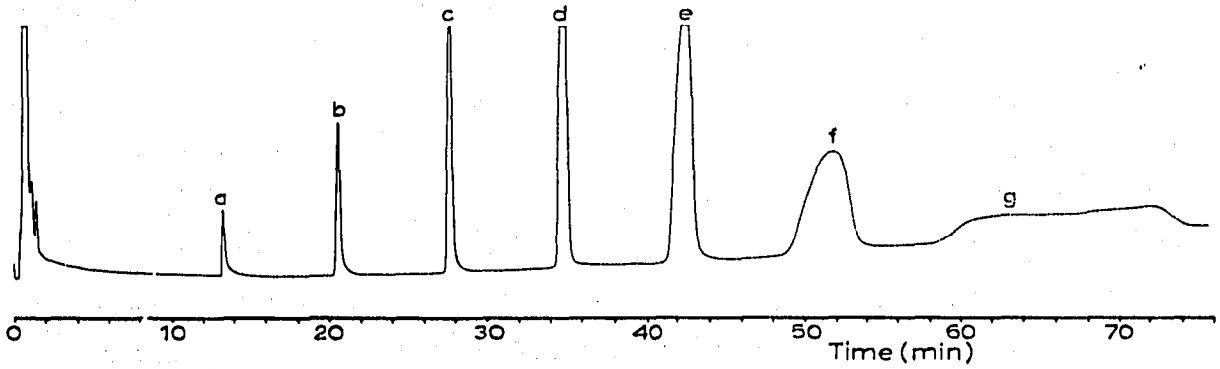

Fig. 4. Programmed temperature precolumn, programmed temperature partition column operation showing effect of cold junction between columns. Sample size: 100 μl of 0.05 % ester mixture described in text.

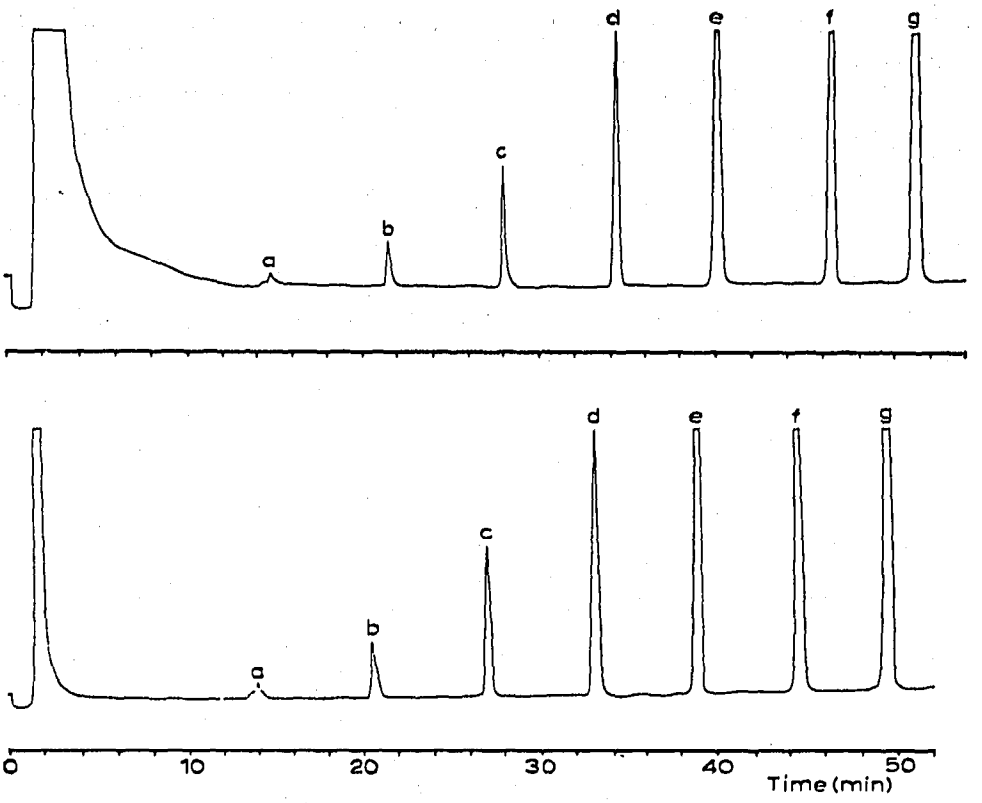

Fig. 5. Comparison of programmed temperature precolumn, programmed temperature partition column operation. Top: without solvent venting. Bottom: with solvent venting (10 sec vent). Sample same as in Fig. 4.

peaks were obtainable and no sample decomposition occurred. Fig. 5 illustrates chromatograms obtained through the use of this system; (a) with solvent venting and (b) without solvent venting for methyl ester analysis. No loss of C_8 and higher esters occurs with venting and much larger sample injections can be made without venting when the solvent is injected onto a cold precolumn than when injected into a hot vaporizer. In regard to this second factor, we had previously found that a 10-μl injection of diethyl ether into a flash vaporizer invariably resulted in extinction of the hydrogen flame. By comparison, since installation of this system, the flame has never been extinguished by injections of ether up to 100 μl in volume when using the same column and detector and the same carrier, hydrogen, and air flow rates.

Fig. 6 compares the isothermal partition column operation of a Barber Coleman model 61-c incorporating this system (a) with the precolumn maintained at 250°, (b) with venting and precolumn programming, (c) without venting but with programmed precolumn temperature. Effluent detection was by the Lovelock diode argon ionization detector. The improvement of chromatograms obtained with this system over conventional flash vaporization systems is readily apparent. Somewhat analogous to hydrogen flame detection, larger volumes of solvent can be injected without venting before electrode arcing occurs.

Although we have found no case where it is more desirable to use a hot (isothermal) flash vaporizer, an autotransformer does provide a means for isothermal operation and was used on the Barber Coleman to provide a means by which the effect of varying the heating rate of the precolumn could be investigated. The rate of precolumn

temperature rise is important, but is not critical within limits. If the rate is too slow, unsymmetrical peaks with shoulders occur for each component. We have found that 100-W cartridge heaters, operated at 115 V provide adequate heating rates when using carrier flow rates of less than 100 ml/min provided a low mass heat transfer block such as aluminum is used to provide even heat transfer from the cartridge heaters to the precolumn. We normally use flow rates of about 20 to 40 ml/min through $^1/_{16}$ in. I.D. columns and at these flow rates, no problems have been encountered.

With this system "instantaneous" injections are not required and indeed, are not desirable. Better results are obtained, particularly with solvent venting, if the

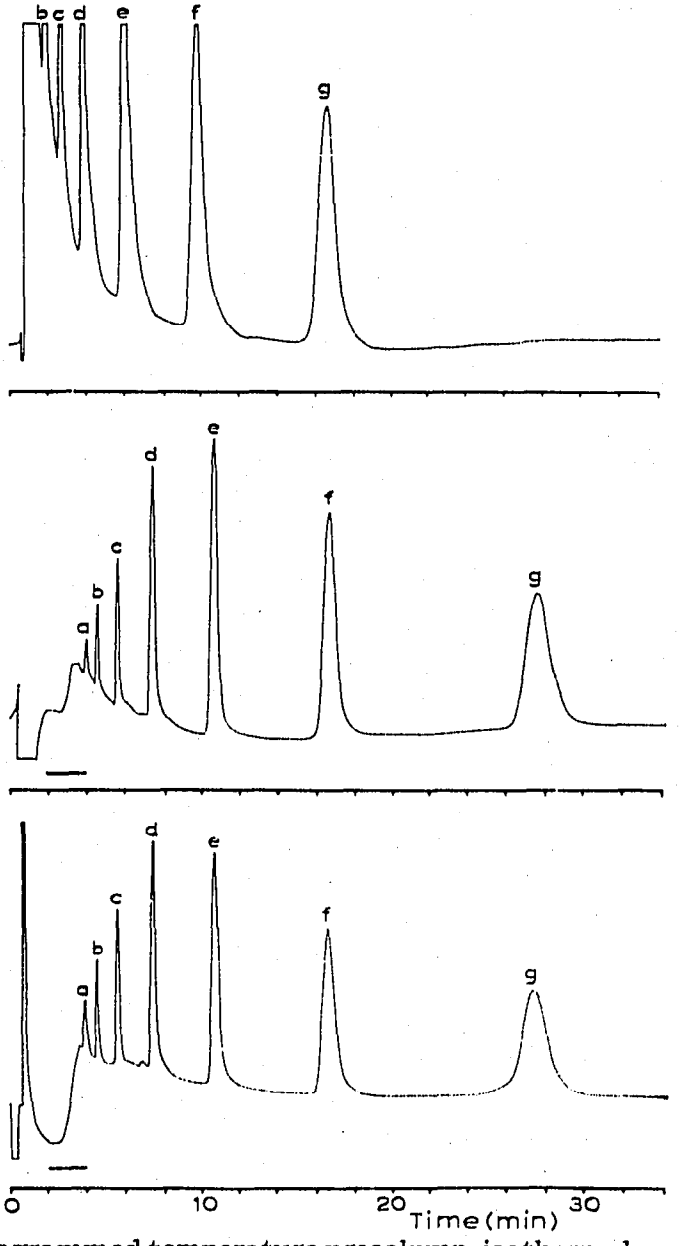

Fig. 6. Comparison of programmed temperature precolumn, isothermal partition column operation. Top: 5 μl of 0.05 % ester solution injected onto hot precolumn without venting. Middle: 3 μl of 0.05 % ester solution injected onto cold precolumn without venting followed by programmed precolumn. Bottom: 3 μl of 0.05 % ester solution injected onto cold precolumn with 10 sec vent followed by programmed precolumn.

transfer of solution from the syringe to the precolumn is allowed to proceed over several seconds. With high boiling components it is even possible to make several repeat injections of dilute solutions before programming the precolumn, thereby effecting several-fold concentration of the sample.

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SUMMARY

The use of vented, programmed temperature precolumns in gas chromatography is described. The use of these precolumns facilitates the analysis of thermally unstable compounds, enables high speed chromatography of high molecular weight compounds, and aids in the analysis of trace amounts of high molecular weight materials in large volumes of low boiling solvents, particularly when using isothermal partition column operation.

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