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"TEMPERATURE-PROGRAMMED" GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION*

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SUMMARY

A flow-controlled, temperature-programmed precolumn, a vent valve, and a pressure-controlled, isothermal analytical column were combined to achieve high-sensitivity electron capture gas chromatography. The separations of chlorinated hydrocarbons conducted with this system demonstrate its potential for introducing temperature programming, solvent venting and peak cutting abilities to the common gas–liquid chromatographic analysis by electron capture.

INTRODUCTION

Since the inception of gas chromatography (GC), analysts have strived to achieve theretofore impossible separations. One of the paths of these investigations has been the serial linking of chromatographic columns and instruments[1–8]. Some investigators have found increased resolution when columns filled with the same chromatographic packing but differing in diameter have been so linked[9–11].

Eventually analysts became interested not only in the achievement of new separations, but also in the reduction of analysis time and in the protection of at least part of the chromatographic column system and the detector from late eluting, undesirable components of the sample. The technique which came to be known as backflushing fulfilled these requirements successfully[12–16].

Trace analysts, who, in their analyses, must inject large volumes of very dilute solutions, and analytical biochemists, who frequently rely on derivatization of biological extracts, realized the value of the ability to vent the solvent and/or derivatization solution to the atmosphere before the detector became swamped[2,17–20].

Precolumns have been an integral part of the scientific endeavor toward simpler, easier, and more effective analyses[21–34]. Various lengths of chromatographic packings have been placed before the analytical column for various reasons: to extend the life of the analytical column[23]; to serve as reaction loops or reaction precolumns in the selective retardation or subtraction of certain functional groups or classes of compounds[24–27]; to serve as introductory systems for capillary gas chromato-

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graphs[28,29,32,35]; to vent the solvent and protect the following chromatographic system (*i.e.* column and detector); to reduce analysis time, and to adapt instruments for backflushing[30–34,36].

Crossley[31] utilized a precolumn system which was constructed so as to backflush the precolumn, thus protecting the following system from late eluting, undesirable material while at the same time reducing analysis time. Kirsten and Mattsson[32] achieved the same results with a precolumn which could be plugged into a gas chromatograph equipped with an electron capture detector (ECD). Evrard designed an elegant precolumn system for GC of biological extracts[33], utilizing a temperature-programmed precolumn which was equipped for solvent venting, and later adapted it for septumless injection[34]. Croll[36] modified an ECD-equipped gas chromatograph with a precolumn system which allowed the precolumn to be backflushed with negligible baseline upset by valve switching.

The primary goal of this investigation was to devise a GC system utilizing an ECD which could be used with some type of temperature programming at high sensitivity. This problem is known to be difficult to solve[37–39]. At the same time, the secondary goals were to incorporate into our system some of the advantages of the previously discussed approaches, *i.e.* solvent and/or derivatization reagent vent, reduction of analysis time, and protection of the chromatographic column and detector (especially the radioactive foil) from late eluting, undesirable materials.

EXPERIMENTAL

The carrier gas flow system

A Barber-Colman Model 5000 gas chromatograph equipped with a 300-mCi tritium source ECD was slightly modified in this study. The system devised is shown in Fig. 1. The prepurified nitrogen carrier gas supply (1) was maintained at 60 p.s.i. throughout the study by a two-stage stainless-steel regulator (Matheson 3104) and was purified with Linde 5A molecular sieve. Just after the filter, the carrier gas stream was split into two streams "a" and "b" which passed through rotameters (2). Stream "a" was *flow*-controlled (3) (Brooks No. 8744 differential flow controller) and purified (4) with a 22-in. length of 1/4-in. O.D. copper tubing packed with 30–60 mesh activated charcoal, before it entered the precolumn (5). Stream "b" was *pressure*-controlled (6) (Brooks No. 8601 pressure regulator) before it passed into the back of the isothermal column in the gas chromatograph. A 0.5-l stainless-steel ballast tank (7) (Whitey

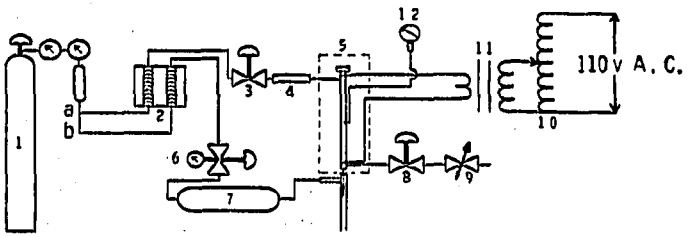

Fig. 1. The carrier gas flow system and the electrical connections. 1 = Carrier gas supply; 2 = rotameters; 3 = flow controller; 4 = charcoal filter; 5 = precolumn; 6 = pressure regulator; 7 = ballast tank; 8 = needle valve; 9 = on–off valve; 10 = autotransformer; 11 = stepdown transformer; 12 = thermocouple readont.

DOT 3E 1800 10M71) was placed between the pressure regulator and the instrument. Stream "a" was carried in 1/8-in. O.D. copper tubing while stream "b" was carried in 1/4-in. O.D. copper tubing. For solvent venting, a flow controller (8) (needle valve type) and a Whitey on–off valve (9) were used.

All flow lines were scrupulously cleaned with acetone, benzene, and pesticide quality hexane; even then the charcoal filter (4) was found to be necessary. Since the system was designed for solvent venting and since it was felt that the pressure controller (6) may not adequately cope with sudden, large demands of gas, the ballast tank (7) was added. In addition, stream "b" flowed exclusively in 1/4-in. O.D. copper tubing in an attempt to remove any flow restrictions between the pressure regulator and the analytical column.

The precolumn

The precolumn and the connections to the analytical column in the instrument are shown in Fig. 2. The precolumn was made from a 12-in. segment of a 3/8-in. O.D. stainless-steel tube. A small piece of 3/8-in. O.D. stainless-steel rod was silver-soldered to the bottom end, was bored out as shown, and a 1/16-in. O.D. stainless-steel tube was silver-soldered in the axis of the precolumn to transfer the precolumn effluent to the gas chromatograph, while a 1/8-in. O.D. stainless-steel tube was silver-soldered perpendicular to the axis of the precolumn to serve as the vent line. At the top end another length of 1/8-in. O.D. tubing was silver-soldered perpendicular to the axis of the precolumn to serve as the carrier gas inlet. A threaded ring was added at the very top to accommodate a 5/16-in. Swagelock stainless-steel nut, a washer, and a septum.

The connection to the analytical column was made at the normal injection port of the instrument. A 1/4- to 1/16-in. Swagelock reducer was bored out so as to accommodate the 1/16-in. O.D. delivery tube from the precolumn. The delivery tube then just penetrated the top of the analytical column packing. PTFE ferrules were used on the analytical column. A 1/4-in. copper tee was added to the normal carrier gas entry port on the analytical column so that it could be used as a supplementary injection port.

The precolumn itself was a 1/4-in. O.D. glass tube which was positioned inside the stainless-steel tube assembly. The glass tube was centered by two O-rings made from blue silicone rubber septum material and a thin band of PTFE. The glass tube was just long enough to fit snugly between the upper septum and a washer made from blue silicone rubber septum material. This configuration insured that all the carrier gas which passed into the stainless-steel tube assembly passed through the precolumn. A small hole (2 mm diameter) was cut in the glass column approximately 5 mm above the side-arm carrier for entrance of the partially warmed carrier gas.

The electrical connections

The precolumn assembly was heated by passing current through it. As shown in Fig. 1, an autotransformer (10) provided power to a 1.10 to 0.8-V stepdown transformer (11) which was connected to the ends of the precolumn assembly by 10-gauge copper wire. A thermocouple (12) provided temperature readout. Due to the high temperatures reached, silver-solder was used in making all electrical connections. The entire assembly was insulated with glass wool and fiberglass wrap 3-in. thick on all sides. The temperature was controlled by varying the autotransformer setting.

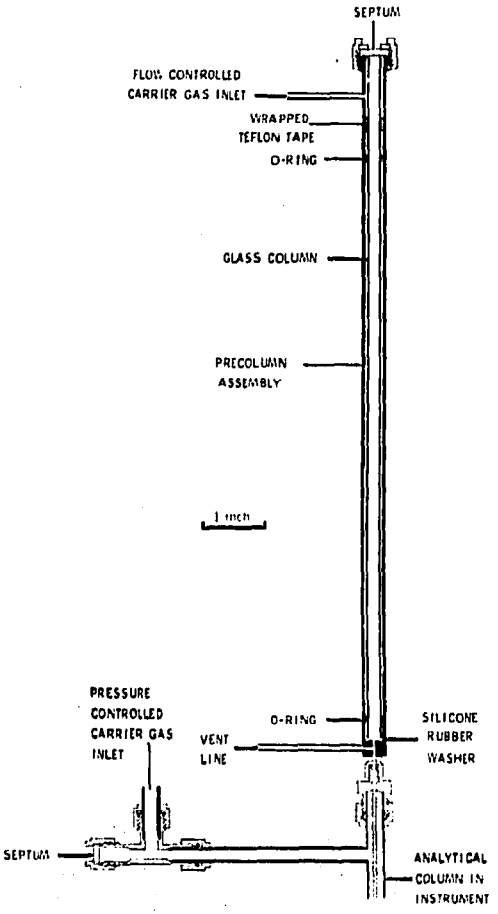

Fig. 2. Precolumn device and the connections to the analytical column.

The columns

The analytical column was a 6 ft. × 1/4 in. O.D. Pyrex glass U-tube designed to fit into the instrument. Both the precolumn and the analytical column were packed with a common mixed phase which was chosen since it did not retard the model mixtures of chlorinated hydrocarbons unduly, but still provided adequate separation. The precolumn flow was 45 ml/min and the total flow at the detector was 85 ml/min.

RESULTS AND DISCUSSION

It is generally accepted that, in most laboratories, the baseline stability of ECDs is usually critically dependent on the flow of carrier gas reaching the detector. Recently an interesting theoretical work[40] was published which claims that the standing current is independent of the flow-rate under certain rigorous conditions. The true relationship between the two under commonly-found analytical conditions is complex and, to some degree, still uncertain. The idea for this system was to

temperature program the precolumn while operating the analytical column in the instrument isothermally. Since the flow delivered to the detector by even a differential flow controller decreases as the back-pressure in the intermediary column increases, the baseline would be expected to change, and it normally does. It was felt that if the carrier gas pressure at the head of the isothermal column was held constant, then the flow to the detector should remain constant, thus ensuring a stable baseline. The flow reaching the detector then is dependent only on the pressure at the column head and is independent of the precolumn flow. Therefore, such a system should be capable of temperature programming and solvent venting. Upon solvent venting, the entire precolumn effluent, plus some carrier gas from the analytical column, is vented to the atmosphere; the gas supply to the analytical column head must be variable between limits wide enough to take up the slack of the lost precolumn effluent and still maintain constant pressure. The system described was designed on this basis.

The primary goal of this investigation was to devise a gas chromatographic system utilizing an ECD which could be temperature programmed at high sensitivity. Fig. 3a shows isothermal chromatography of 110 pg each of designated chlorinated hydrocarbons on the analytical column only (injection through the supplementary injection port). Figs. 3b and 3c show the chromatograms obtained when the precolumn

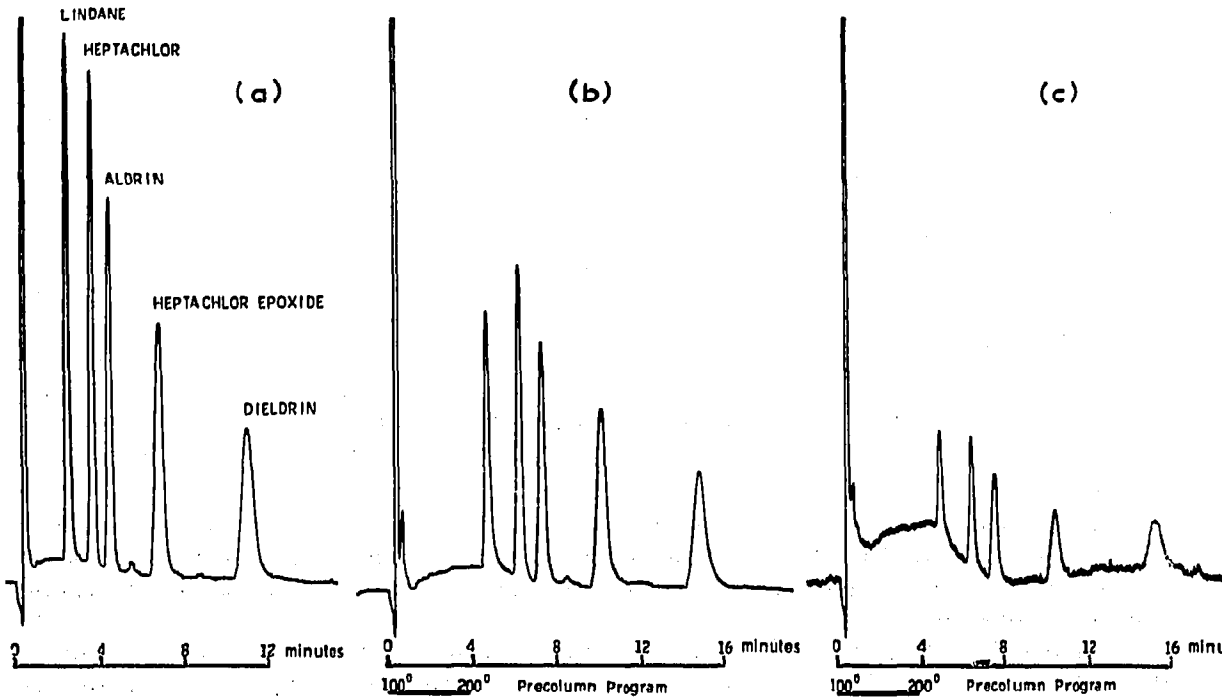

Fig. 3. (a) Chlorinated hydrocarbons (110 pg each) injected into supplementary injection port; attenuation, × 300; analytical column, 150° isothermal; flows: precolumn, 45 ml/min, total flow to detector, 85 ml/min; pressure at head of analytical column, 19 p.s.i.; detector, 215°; precolumn, 200° isothermal. (b) Chlorinated hydrocarbons (100 pg each) injected into precolumn; same conditions; precolumn programmed 100–200° at 27°/min. (c) Chlorinated hydrocarbons (10 pg each) injected into precolumn; same conditions except attenuation × 100; precolumn programmed 100–200° at 27°/min.

TABLE I

EFFECT OF THE PRECOLUMN TEMPERATURE PROGRAM ON RESOLUTION

iso = isothermal; progr. = programmed.

Column conditions		*Heptachlor epoxide–dieldrin resolution*
Analytical column	*Precolumn*	
150° iso	Bypassed	4.86
150° iso	27°/min progr.	5.04
150° iso	20°/min progr.	4.94
150° iso	12°/min progr.	4.69
150° iso	190° iso	4.95
150° iso	150° iso	3.37

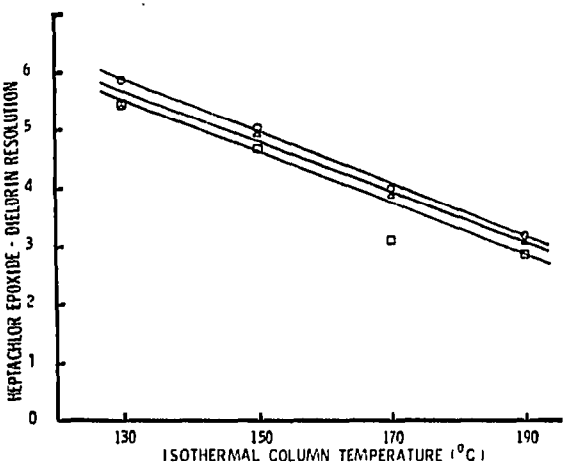

Fig. 4. Effects of isothermal and temperature-programmed precolumn on resolution. Precolumn temperature program rates were: 27°/min (○—○), 20°/min (△—△) and 12°/min (□—□).

was temperature programmed from 100 to 200°. In Fig. 3b each peak corresponds to 100 pg, while in Fig. 3c each peak corresponds to only 10 pg. Note in Table I that the resolution of the temperature-programmed run is slightly better than that of the isothermal run.

The effect of the precolumn temperature-program rate is fairly small. Evidently the resolution obtained on the chromatograms is a function not only of the two columns and the temperature-program rate, but also of other certain factors. Fig. 4 shows the combined effects of isothermal column temperature and precolumn temperature-program rates. There is no doubt that the fastest program rate gave the best resolution, yet it was puzzling that when injections were made onto a hot (190°) precolumn, the resolution was intermediate between the isothermal run and the temperature-programmed run (Table I). Note also that as the program rate decreased, the resolu-

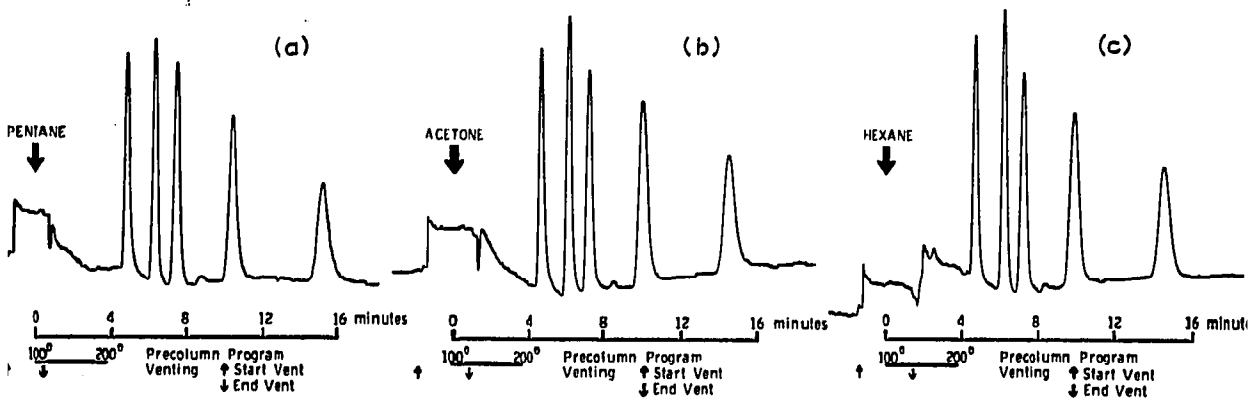

Fig. 5. (a) Chlorinated hydrocarbons (100 pg each) injected into precolumn; attenuation, × 300; analytical column, 150° isothermal; flows: precolumn, 45 ml/min; total pressure at head of analytical column, 18 p.s.i.; flow to detector, 85 ml/min; detector, 215°; precolumn programmed, 100–200° at 27°/min; 2 μl of pentane vented at a rate of 50 ml/min carrier gas. (b) Chlorinated hydrocarbons (100 pg each) injected into precolumn; same conditions; 2 μl of acetone vented at a rate of 50 ml/min. (c) Chlorinated hydrocarbons (100 pg each) injected into precolumn; same conditions; 100 μl of hexane vented at a rate of 100 ml/min.

tion decreased, so that even at 12°/min, the resolution was lower than isothermal chromatography at 150° on the analytical column alone. One possible explanation for the decreased resolution at low program rates is that the chromatographic zone may be broadened as it traverses the distance between precolumn and analytical column. The broadening could be a function of the geometry of the union between the columns or of mixing which occurs during addition of the supplementary flow, or of the prevelant temperature gradients. No further attempt was made to explain these phenomena. Nevertheless, the precolumn system described can be "temperature programmed" at high sensitivity with negligible baseline drift.

Figs. 5a, 5b, and 5c show solvent vents of 2 μl of pentane, 2 μl of acetone, and 100 μl of hexane, respectively. The system was not capable of venting methylene chloride or chloroform satisfactorily.

Fig. 6 shows peak cutting capabilities. In Fig. 6b note how the analysis time is shortened when late-eluting peaks are vented. In Figs. 6c and 6d note that it was not possible to completely cut out heptachlor epoxide. Presumably the precolumn was too short to completely resolve all the peaks thus making some cuts difficult. No further attempt was made to define or remedy the situation.

Fig. 7 indicates how complex mixtures eluting at the beginning of a chromatogram could be resolved. Note that in the case of complex mixtures a temperature program can better characterize early-eluting peaks, while late-eluting peaks (which are not shown here) would not be unduly retained. Fig. 7a shows isothermal chromatography on the analytical column at 205°. Fig. 7b shows a precolumn temperature program.

The primary goal of this investigation, *i.e.* demonstrating that a temperature-programmed precolumn could be used with electron capture detection, was achieved. The secondary goals were at least partially realized in the successful solvent vent and peak cutting. The system should be applicable to routine analyses and especially so to trace analyses and analyses of crude biological extracts, since the solvents may be

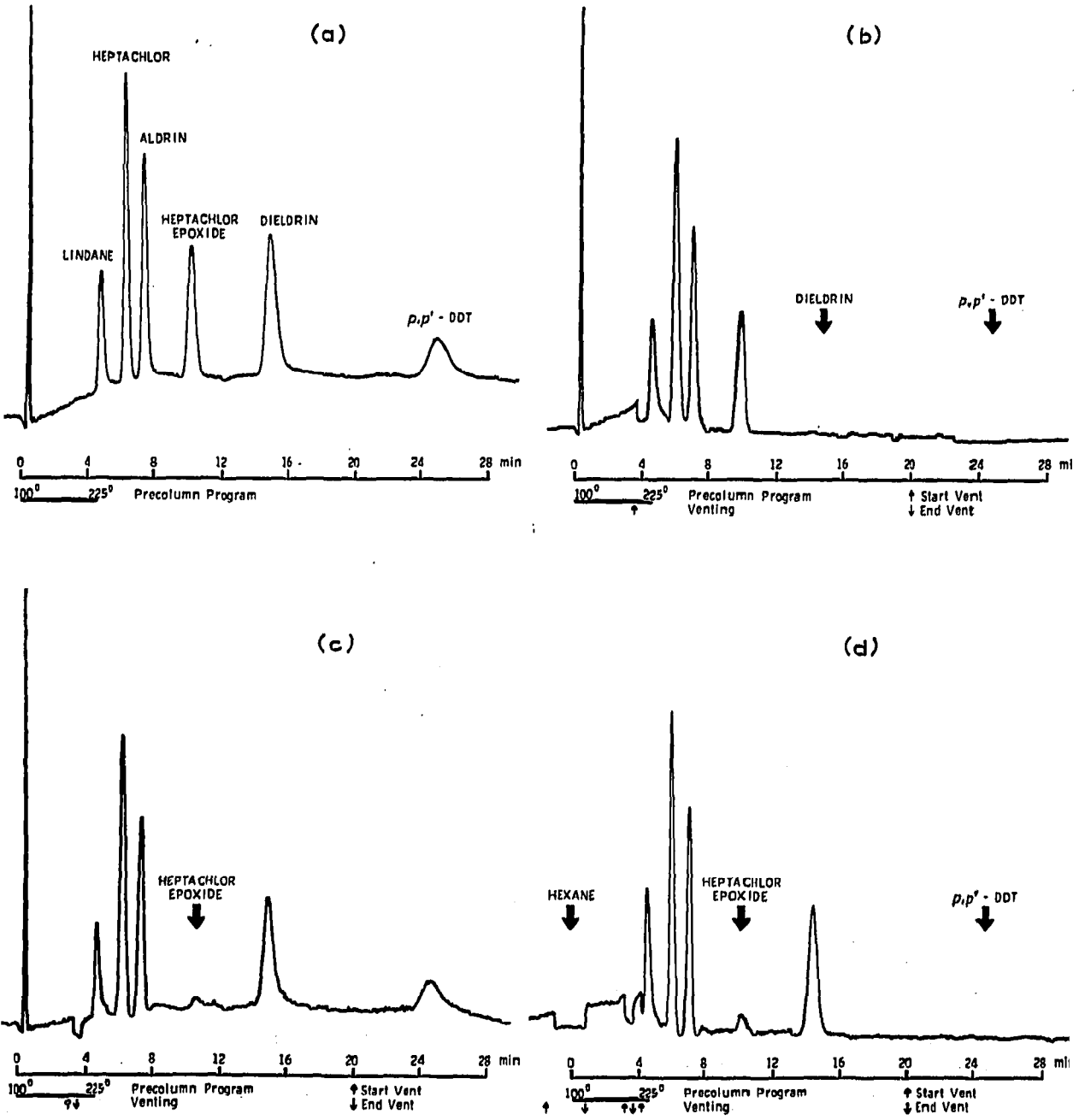

Fig. 6. (a) Chlorinated hydrocarbon mixture injected into precolumn; precolumn temperature programmed. (b)–(d) Injections of model mixture showing potential for peak 'cutting'.

vented and the contaminated precolumn can be changed easily and quickly. Further work in the serial linking of two gas chromatographs —*i.e.* a temperature-programmed and an isothermal one— would be most interesting.

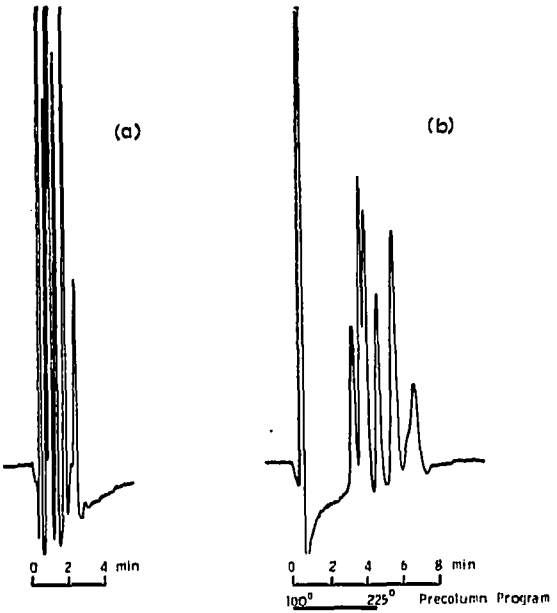

Fig. 7. (a) Test mixture injected into supplementary injection port; attenuation, × 300; analytical column, 205°; flows: precolumn, 45 ml/min, pressure at head of analytical column, 18 p.s.i.; total flow to detector, 70 ml/min; detector, 215°; precolumn, 200° isothermal. (b) The same mixture injected into precolumn; same conditions; precolumn programmed 100–225° at 27°/min.

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