Some Separation Characteristics of an OV-101/OV-210 Column for Organochlorinated Pesticides with Particular Reference to the Separation of Photoendrin and Endrin.

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When determining the sensitivity of methods for the analysis of organochlorine pesticides at the ng/l (part per trillion) level, it is important to obtain and maintain optimum conditions on the gas chromatograph. Having taken into account the design of the instrument, septum conditions, quality of the carrier gas, gas filter efficiency, gas line, rotameter condition and so on, the single most important factor is column efficiency. Columns of low bleed rate and high stability to withstand continuous daily usage are highly desirable. The column has been rightly referred to as the heart of the gas chromatograph. This communication reports preliminary findings on a mixed OV-silicone column, which has several characteristics complimenting those of a DC-11/QF-1 column used in previous work[1]. One of the most notable features of this OV-silicone column is its ability to differentiate photoendrin and endrin. This report discusses the separation of these two pesticides and other characteristics of an OV-101/OV-210 column.

Materials and Method

Reagents

Pesticide grade solvents without further purification were used for the synthesis of photoendrin and for the preparation of standard solutions.

OV-101 and OV-210 silicone oils were obtained commercially.

Instrument

A Tracor MicroTek Model 220 equipped with dual ^{63}Ni electron-capture detectors supplying 10 mc and two 6' x 1/4" OD U-shaped glass columns packed with the following was used:

A. Column I-was packed with 4% DC-11 and 6% QF-1 on Chromosorb W, 60-80 mesh, AW, DMCS treated and conditioned for 1 week at 240°C before use.

B. Column II-packed with 3.6% OV-101 and 5.5% OV-210 on Chromosorb W, 80-100 mesh, AW, DMCS treated and conditioned at 250°C for 78 hours before use.

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Operating parameters were:
column temperature - 200°C for both columns;
detector temperature - for column I, 260°C, for column II 265°C;
temperature of injection block (with glass insert) - both columns 210°C;
nitrogen carrier gas - column I at 75 ml/min
column II at 80 ml/min;
and recorder speed - two Honeywell Electronik 194 at 1"/5 min.

Infrared spectra were run on a Perkin-Elmer Model 457 instrument utilizing Nujol, Flourolope and KBr disc techniques.

Melting points were determined on a Mettler FPI automatic melting point apparatus.

A Rayonet photochemical reactor model RPR-208 equipped with ultra violet (u.v.) lights of 2537°A was used for the syntheses of photoendrin.

Nuclear Magnetic Resonance spectra were kindly run by Dr. W.J. ApSimon and Dr. J. Buccini of Carleton University, Ottawa.

Synthesis of Photoendrin

5 gm of purified endrin was dissolved in 1.5 l. of pesticide grade hexane and irradiated by u.v. lights with pure nitrogen bubbling gently through the solution during reaction. At the end of 78 hrs. the reaction mixture was filtered and the crystals washed with ether. The ether and supernatant hexane solution were combined and concentrated. The resulting precipitate was filtered out of the mixed solvent, washed with ether and then combined with the original crystals, obtained above. The combined solids (3.8 gms) were recrystallized six times from chloroform to obtain white crystals (2.2 gm) m.p. 232.3°C and had an IR spectrum identical to the literature data[2]. NMR analysis (in $CDCl_3$) confirmed the structure as reported in literature[2]. Since no conclusion can be drawn from the published NMR spectrum due to the high noise to signal ratio, a better solvent (deuterated dimethyl sulfoxide) and decoupling technique were used to determine the chlorine position.

Preparation of OV-Silicone Column

The following procedure is slightly modified from that of Mendoza et al[3].

0.8 gm of OV-101 and 1.2 gm of OV-210 were weighed individually in a suitable glass weighing boat. Each was then transferred to a 500 ml round bottom flask fitted with a reflux condenser, using warmed (65-70°C) pesticide grade ethyl acetate. Approximately 200 ml was used to ensure that the 20 gm of solid support to be added later would be completely covered. The contents of the flask were heated with a heating mantle whose temperature was adjusted by a variable transformer. The variac setting was such

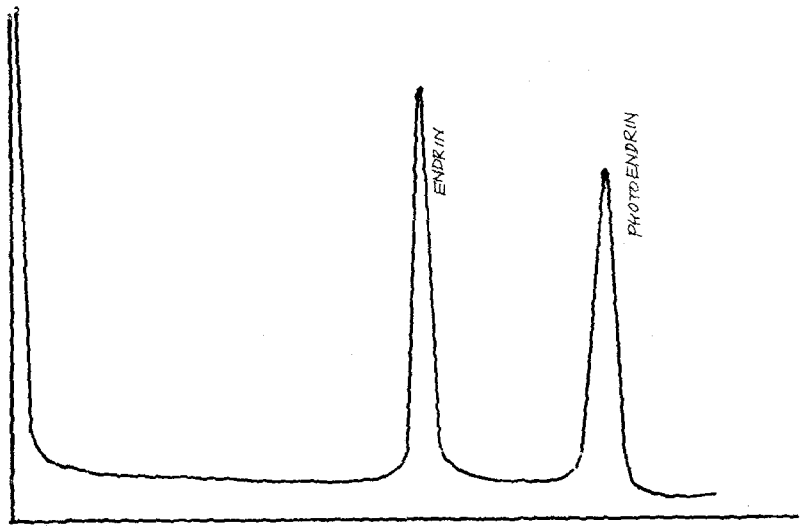

Fig. 1. Separation of endrin and photoendrin on a OV-101/ OV-210 column.

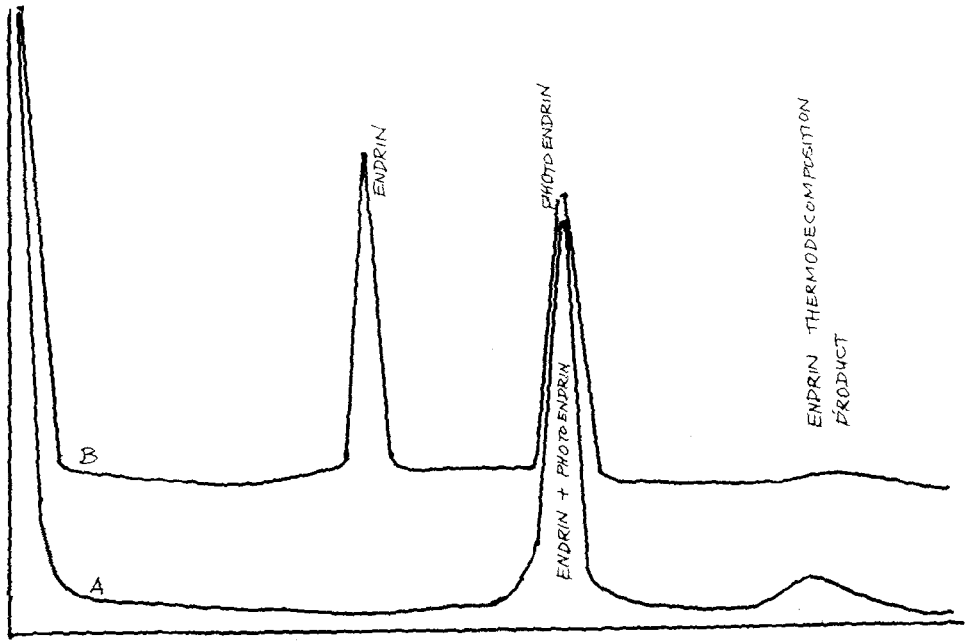

Fig. 2. Chromatograms showing a mixture of endrin and photoendrin on some DC-11/QF-1 columns, before preconditioning (A) and after (B) preconditioning for endrin.

that light refluxing occured (setting at approx. 20). After heating with periodic swirling until the polymers were completely dissolved, 20 gm of Chromosorb W, AW, DMCS treated, 80/100 mesh, was carefully added to the flask which was gently swirled. The flask and contents were then heated at a reduced variac setting of approximately 15 and again gently swirled periodically for another 2 hrs. At the completion, the flask was placed on a Buchi Rotavapor and with gentle rotation and slight vacuum the solvent was evaporated using a warm water (40°C) bath until the excess solvent was removed. Rotation was then stopped and residual solvent removed by allowing a gentle stream of air to pass over the column material for approximately 1/2 hour. The flask was then placed in an oven at 75°C overnight. The free flowing column material that remained next day was transferred to an evaporating dish and dried further at 250°C for 4 hours. The column material was then cooled in a dessicator and stored in a suitable air tight container.

Results & Discussion

Before discussing the characteristics of the OV-silicone column, the synthesis of photoendrin merits some comments. When the procedure of Zabik et al[2] was repeated, difficulties in obtaining pure photoendrin were encountered, particularly when more than 10 gm of endrin in 1 litre of hexane was irradiated. This was due to the simultaneous formation of endrin ketone, which may be attributed to the u.v. reaction on endrin particularly in the solid state; however it is felt that the increasing acidity of the hexane solution during reaction may be the major contributing factor in its formation. Consequently it was observed that nitrogen bubbled gently through the reaction mixture during irradiation greatly reduced the formation of endrin ketone, probably because of the expulsion of acidic gas during reaction. This bubbling of nitrogen facilitated the purification of photoendrin by reducing the ratio of endrin ketone produced. Since both the ketone and photoendrin have similar elution patterns on florisil columns[4,5] and rf values on TLC plates, it is only by tedious fractional recrystallization from $CHCl_3$ that purified photoendrin was obtained.

Figure 1 illustrates the elution pattern of endrin and photoendrin on an OV-silicone column. It has been observed, as mentioned by Zabik et al[2] that endrin and photoendrin have the same or similar retention times on several different GLC columns. We also found that some newly packed DC-11/QF-1 columns did not resolve these two compounds without extensive preconditioning for endrin (Fig 2A). This is due to the fact that endrin was decomposed extensively on this column and only the decomposition products appeared on the chromatogram. The major decomposition product had a similar retention time as that of photoendrin (Fig 2A). After repeated injections of endrin at ug/ul level (with detector disconnected), decomposition of endrin on the column was drastically reduced so that endrin and photoendrin can be resolved into two well separated peaks (Fig 2B). The extent of

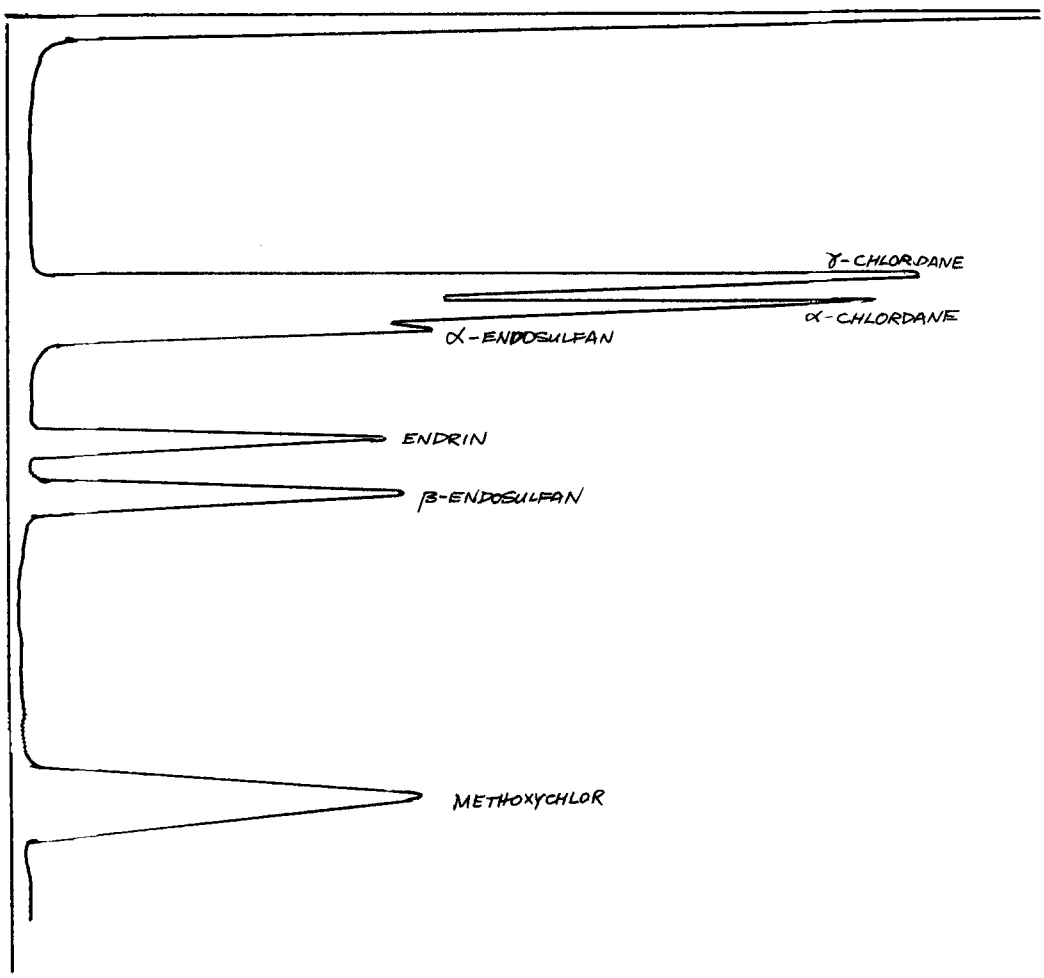

Fig 3. A typical chromatogram from an OV-101/OV-210 column.

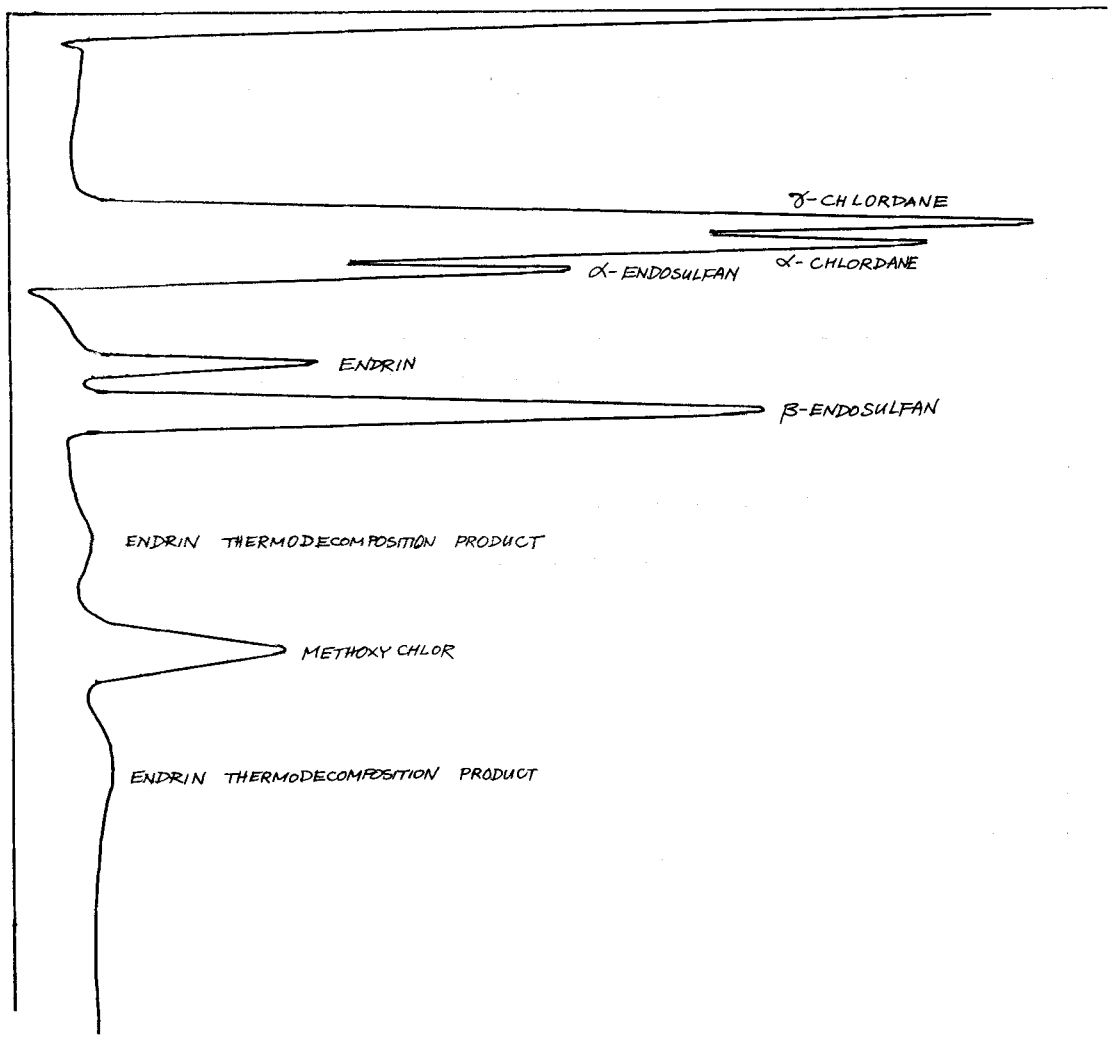

Fig 4. A chromatogram from a DC-11/QF-1 column after preconditioning for endrin.

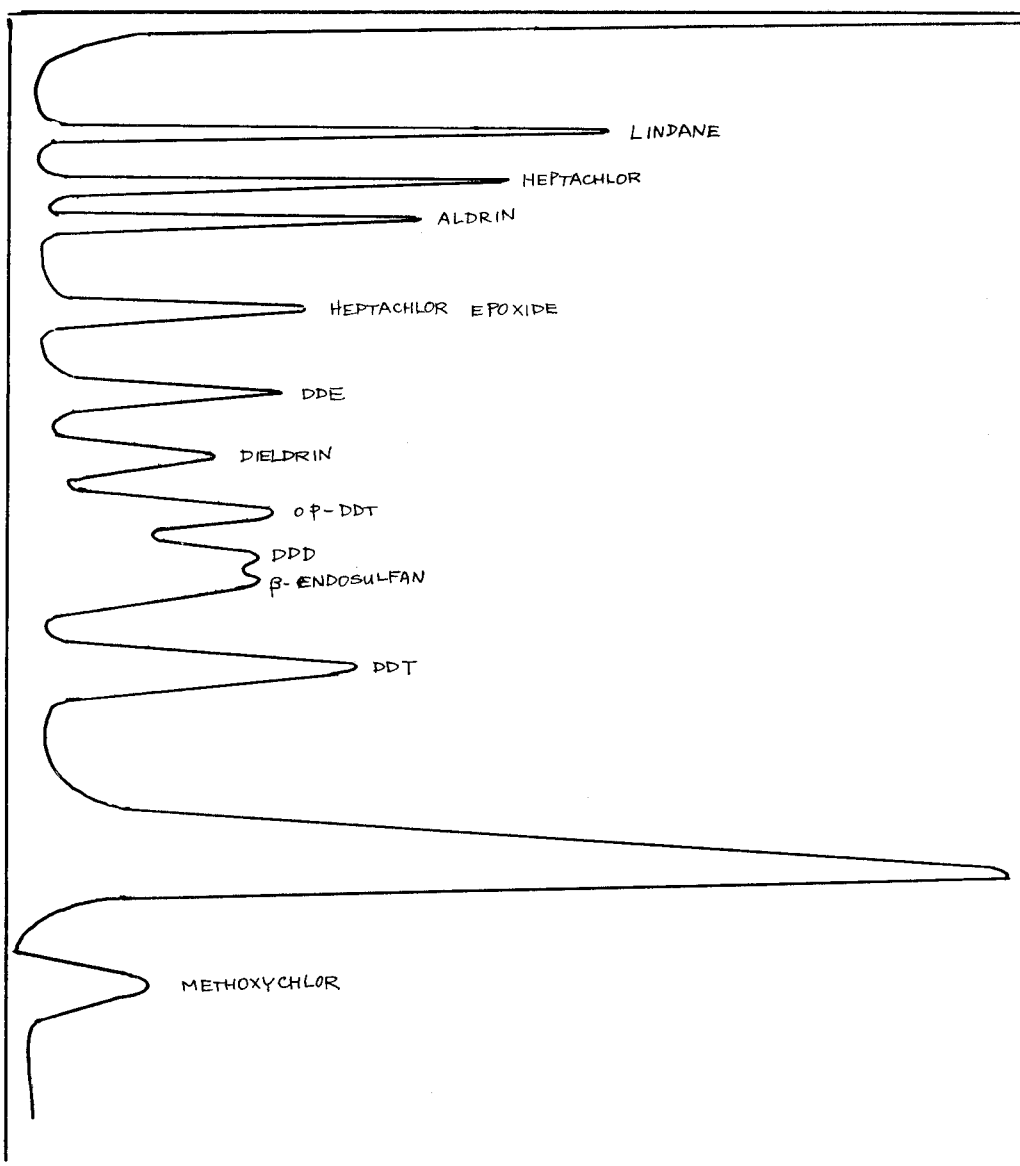

Fig 5. Gas chromatographic elution pattern of some pesticides on an OV-101/OV-210 column.

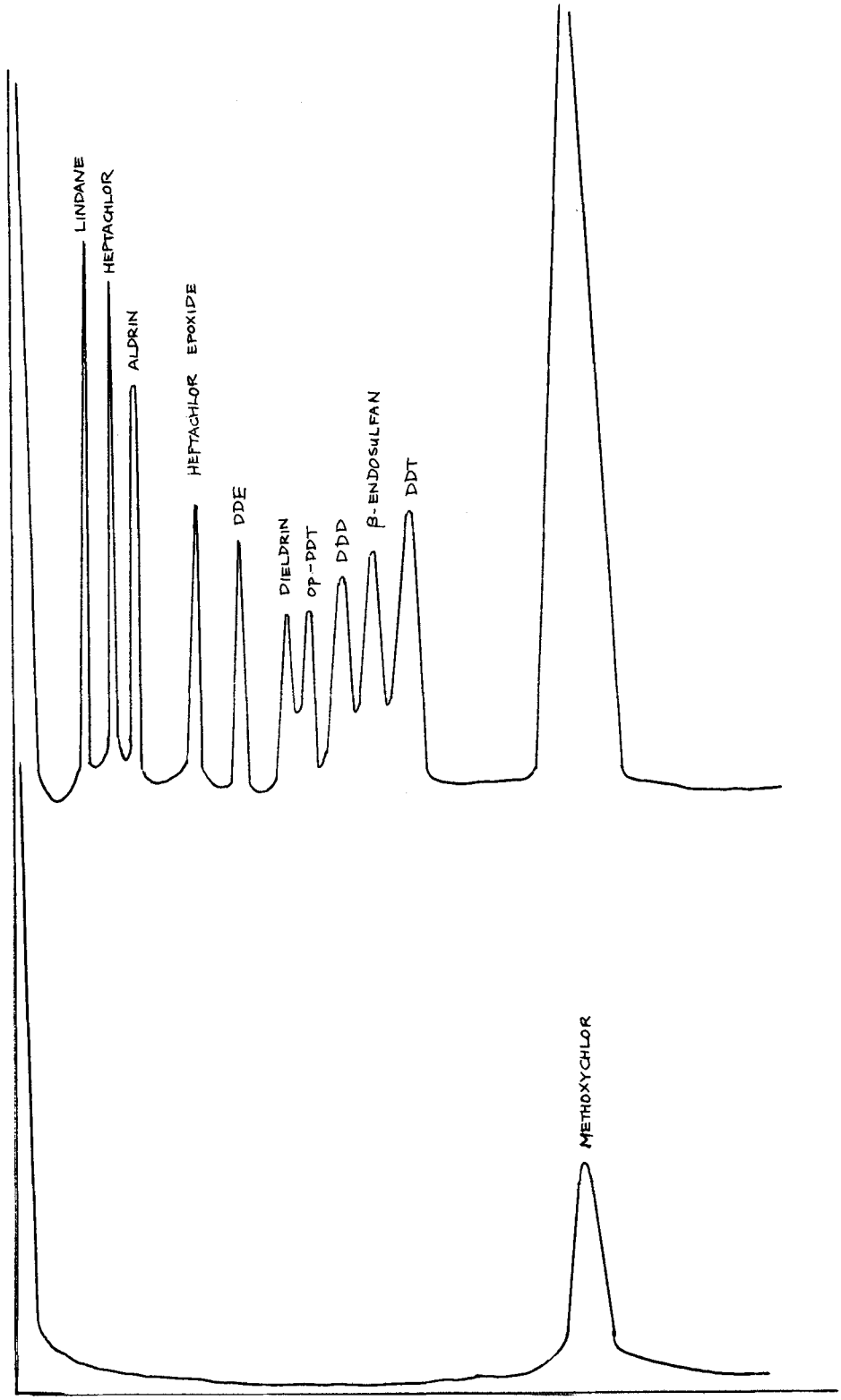

Fig 6. Gas Chromatographic elution pattern of some pesticides on a DC-11/QF-1 column.

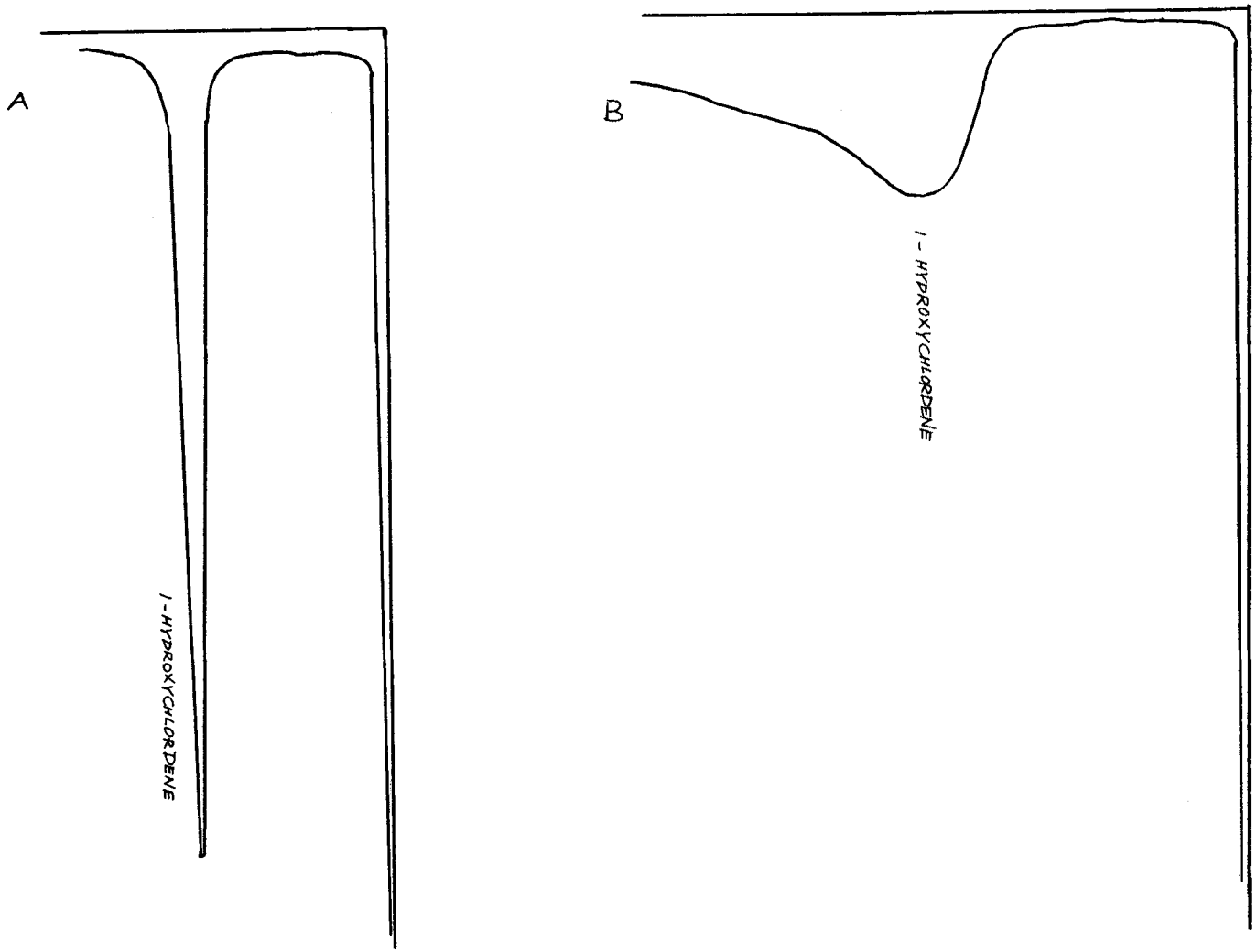

Fig 7. Behaviour of 1-hydroxychlordene on an OV-101/OV-210 (A) and a DC-11/QF-1 column (B). Both columns are without preconditioning for this compound.

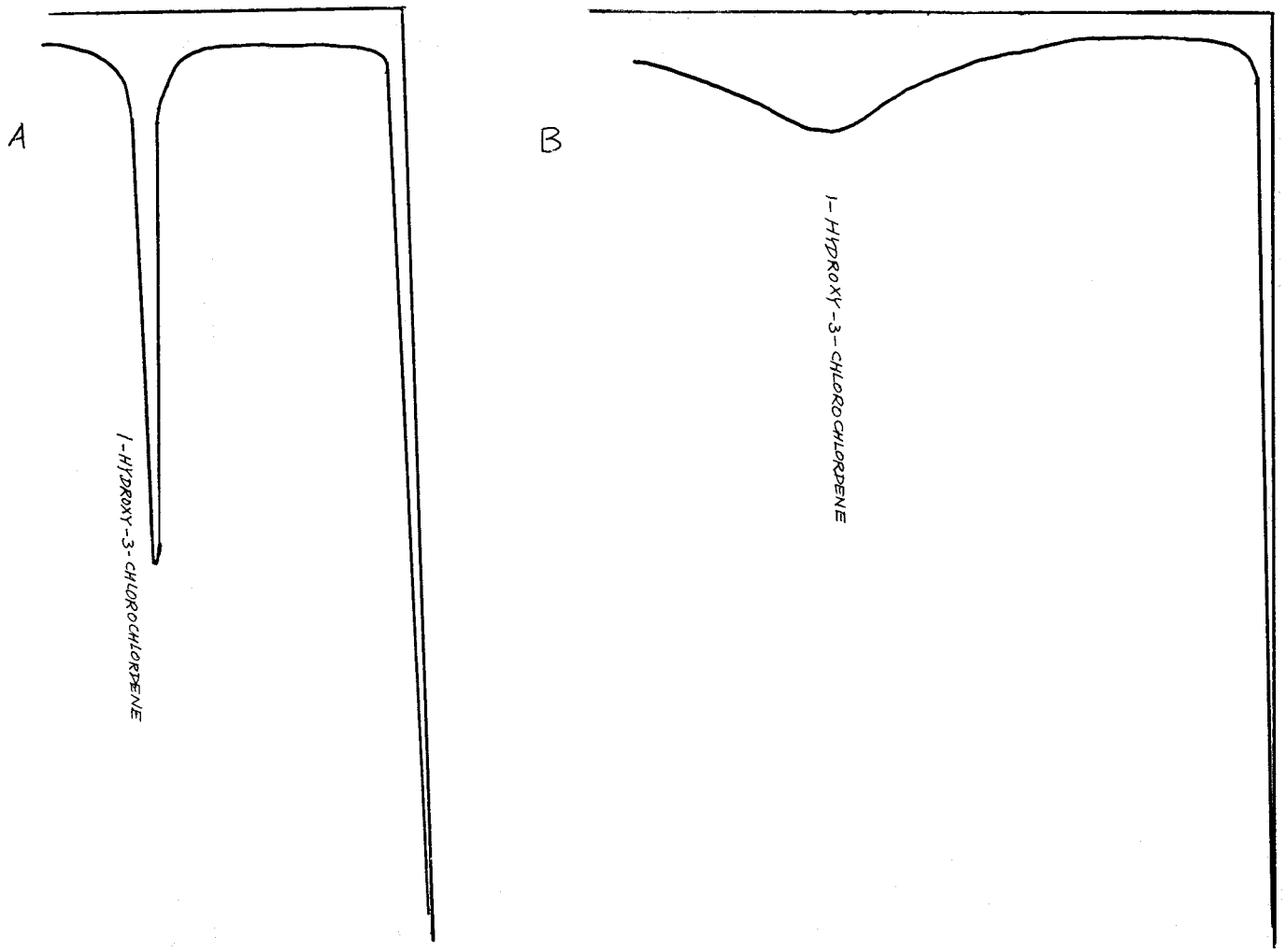

Fig. 8. Behaviour of 1-hydroxy-3-chlorochlordene on an OV-101/OV-210 column (A) and a DC-11/QF-1 column (B). Both columns are without preconditioning for this compound.

preconditioning depends on different batches of column packing material prepared. It must be emphasized that such extensive decomposition was not always observed for all batches of DC-11/QF-1 column packing materials prepared. In contrast, such decomposition pattern of endrin was not observed with all the batches of OV-101/OV-210 column packing material which we have so far prepared. In fact, all the OV-silicone columns we used did not thermally isomerize endrin (without preconditioning for endrin) to such an extent as do many other columns such as the DC-11/QF-1 column and SE-30 column. At normal injection concentration used (100 pg to 1000 pg) the chromatogram of endrin displayed one single sharp peak (Fig 1 and 3). Therefore, the detection limit for endrin would be lowered by using an OV-101/OV-210 column.

This OV-silicone column not only provides a means to separate these two compounds but it still retains good separation properties for the routine analysis of other organochlorinated pesticides. Fig 3 and 5 display the elution pattern of some common organochlorinated pesticides from this column. Inclusion of chromatographs from a DC-11/QF-1 column (Fig. 4 and 6) are for comparison. Although the present OV-silicone column separated endrin and photoendrin without preconditioning for endrin, it did not give as good a separation of α -endosulfan and cis-chlordane (Fig. 3) as did the DC-11/QF-1 column as shown in Fig. 4. Figure 5 illustrates the separation of dieldrin and o,p'-DDT and the resulting doublet for p,p'-DDD and β -endosulfan on the OV-silicone column. Contrastly, this situation was reversed for the DC-11/QF-1 column (Fig. 6). The unlabeled large peak is an unidentified co-extracted compound from some distilled water with polyethylene and tygon connections. This peak caused interference with p,p'-methoxychlor on the DC-11/QF-1 column (Fig. 6), whereas it was resolved on the OV-silicone column (Fig. 5).

One of the most desireable characteristics of the mixed OV-silicone column is its apparent increased sensitivity to 1-hydroxychlordene and 1-hydroxy-3-chlorochlordene as illustrated in Figures 7 and 8. In contrast, the DC-11/QF-1 column required repeated injections of these alcohols before it began to approach this sensitivity, and even then it was only temporary. There are several major reasons for detecting 1-hydroxychlordene at low levels in a water sample. Firstly, heptachlor is known to hydrolyze to 1-hydroxychlordene in water[6] at a very rapid rate; in fact this laboratory has observed that when 1 l. of water is spiked with 10 to 100 ng of heptachlor, complete hydrolysis occurs in less than 1 week at room temperature. Further, 50% conversion occurs in the first 2 or 3 days. It is therefore desireable to have a sensitive and quick screening method for 1-hydroxychlordene for water quality studies. Secondly, in many cases, the subsequent silylation or acetylation step in the $AgCO_3$/aqEtOH[7] or potasium tert-butoxide/tert-butanol[8] procedure for the confirmation of heptachlor and heptachlor epoxide can be eliminated due to the enhanced sensitivity in

detecting these alcohols using the OV-101/OV-210 column.

This OV-silicone column gave sharp peaks to polar compounds such as hydroxyl compounds: dicofol, cis-[9] and trans-aldrin diols and so on. It appears that this column is ideal for oxygen analogs of organophosphates. This aspect is under investigation and findings will be reported at a later date.

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