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ANALYSIS OF POLYCHLORINATED BIPHENYLS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND CAPILLARY GAS–LIQUID CHROMATOGRAPHY

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SUMMARY

Seventy fractions were obtained by vacuum distillation of a PCB mixture containing 42% of chlorine. Four of these fractions were chosen, the composition of which covered the whole of the observed region, and analysed by means of high-performance liquid chromatography (HPLC) on silica gel using *n*-pentane as the mobile phase and capillary gas–liquid chromatography (GLC) using OV-101 and Carbowax 20M as stationary phases at 200 °C. The chosen distillation fractions were further prepared by HPLC, each yielding 10–14 samples. Individual PCB standards and all samples were analysed by HPLC and capillary GLC. These procedures permitted the identification of those compounds which are eluted simultaneously under the conditions used in either HPLC or capillary GLC alone.

INTRODUCTION

The production of polychlorinated biphenyls (PCBs), their industrial use and their environmental occurrence are most often monitored by gas chromatography, alone or combined with mass spectrometry (GC–MS)[1]. Recently the high-performance liquid chromatographic (HPLC) analysis of PCBs was described[2,3]. Many pairs of chlorinated biphenyls are eluted simultaneously under given conditions[4,5], as we found during an analysis of Aroclor 1242 by capillary gas–liquid chromatography (GLC) and mass spectrometry. It is clear that neither HPLC nor capillary GLC alone permits the separation and identification of all components of PCB mixtures.

It was the aim of the present work to establish the problems that can be encountered during the analysis of PCBs by capillary GLC and HPLC, and the advantages that can be obtained by a combination of both methods. The preparative use of HPLC and the high resolving power of capillary GLC are considered.

EXPERIMENTAL

Liquid chromatography

A Varian 8500 liquid chromatograph with a syringe pump connected with a CDS 111 (Varian, Palo Alto, Calif., U.S.A.) chromatographic data system was used. Sample injection was performed by the stop-flow technique with 5- and 10-μl syringes (Hamilton, Reno, Nev., U.S.A.). A Variscan UV spectrophotometer (Varian) with a variable wavelength was used as a detector. The volume of the flow-through cells was 8 μl and the path length was 1 cm. The detector was set at 205 and 254 nm. The signal was monitored by a dual-channel strip-chart recorder at two sensitivity values (200 and 50 mV). The columns used were packed with 5-μm spherical silica gel (Pragosil SI 5), manufactured in cooperation with the Institute of Nuclear Research, Řež, and the Prague Institute of Chemical Technology, Czechoslovakia. All columns were prepared in the Laboratory of Synthetic Fuels, Prague, and packed by the slurry technique[6]. The following columns were used.

Column A. This was a stainless-steel column (10 cm × 2 mm I.D.). For an *n*-pentane flow-rate of 20 ml/h the column efficiency for naphthalene (capacity ratio = 3.1) was 1800 theoretical plates.

Column B. This was a glass column (25 cm × 2.7 mm I.D.) with stainless-steel fittings. For an *n*-pentane flow-rate of 50 ml/h the column efficiency for naphthalene (capacity ratio = 5.8) was 6000 theoretical plates.

Column C. This was a stainless-steel column (25 cm × 4.7 mm I.D.). For an *n*-pentane flow-rate of 100 ml/h the column efficiency for naphthalene (capacity ratio = 5.4) was 15000 theoretical plates.

Column D. This was a stainless-steel column (25 cm × 8.0 mm I.D.). For an *n*-pentane flow-rate of 400 ml/h the column efficiency for benzene (capacity ratio = 1.6) was 8000 theoretical plates.

Preparative liquid chromatography

Volumes of 7 μl of liquid fractions (fractions Nos. 10, 37, 52) and 10 μl of a saturated isooctane solution of fraction No. 66 were injected into the preparative column (column D) with an *n*-pentane flow rate of 400 ml/h. Fractions were collected in test-tubes situated directly after the detector output. The volume between the detector and the test-tube was formed by an output capillary (10 cm × 0.2 mm I.D.). *n*-Pentane was spontaneously evaporated from the fractions received.

Total solids were dissolved in 1 ml of isooctane and analysed by liquid chromatography on an analytical column (column C) and by GLC on capillary columns (columns E and F).

Gas chromatography

A Fractovap Model 2300 gas chromatograph (Erba Science, Milan, Italy) was used, with a flame-ionization detector and an all-glass splitter, which enabled capillary columns to be used. Nitrogen was used as the carrier gas. Capillary columns were prepared as described earlier[5].

Column E. The column was etched with HCl for 24 h at 620 °K. The OV-101 stationary phase was coated on the column walls by the dynamic method.

Column F. The column was etched with HCl for 24 h at 620 °K. The Carbowax 20M stationary phase was coated on the column walls by the dynamic method.

The operating conditions for the analysis of PCBs by GLC are given in Table I.

TABLE I

OPERATING CONDITIONS FOR ANALYSIS OF PCBs BY GAS CHROMATOGRAPHY

Parameter	*Column*	
	E	*F*
Length (m)	60	50
I.D. (mm)	0.25	0.25
Liquid phase	OV 101	Carbowax 20M
Column temperature (°C)	200	200
Injector port temperature (°C)	275	275
Nitrogen flow-rate (ml/min)	0.3–0.6	0.3–0.5
Nitrogen pressure (atm)	1.5	1.0
Splitting ratio	1:150	1:150

Samples

A mixture of PCBs containing 42% of chlorine was distilled into 70 fractions by fractional vacuum distillation in an all-glass apparatus. All fractions were analysed by GLC on column E. The fractions chosen for PCB analysis (HPLC and HPLC–GLC combination) were Nos. 10, 37, 52 and 66.

The following standard chlorinated biphenyls were used for quantitative analysis: 3-, 2,2'-, 2,3'-, 2,5-, 2,4'-, 2,6-, 4,4'-, 2,4-, 3,5-, 3,3'-, 3,4'-, 2,5,2'-, 3,5,3'-, 2,5,4'-2,4,4'-, 3,4,2'-, 2,6,2',6'-, 2,4,2',4'-, 2,5,2',5'-, 2,5,3',4'-, 2,4,2',5'-, 2,3,2',3'-,2,3,2',5'-, 3,4,3',4'- and 2,3,4,2',5'- (Analabs, North Haven, Conn., U.S.A.).

RESULTS AND DISCUSSION

PCB mixtures containing 42% of chlorine are rather complicated. Capillary GLC analysis on both a polar and a non-polar stationary phase permits the resolution of approximately 50 compounds (Figs. 1 and 2). As the number of theoretically possible monochloro-, dichloro-, trichloro- and tetrachlorobiphenyls is 81, and in a PCB mixture containing 42% of chlorine there are also pentachloro derivatives[1,4], we considered that capillary GLC alone would be unable to resolve all components in the mixture.

Distillation fractions 10, 37, 52 and 66 (see Fig. 1), which contained all components of the starting mixture, were chosen for further experiments. For an orientation HPLC analysis of distillation fractions, the short column A was used. A better separation of the mixture was achieved on the glass column (column B), the efficiency of which is similar to that of commonly used commercial columns. The best resolution was obtained on a column with a larger inner diameter (column C). However, analyses on this column are more time consuming, and the consumption of the mobile phase is greater. The separation of fraction No. 52 on columns A, B and C is illustrated in Fig. 3.

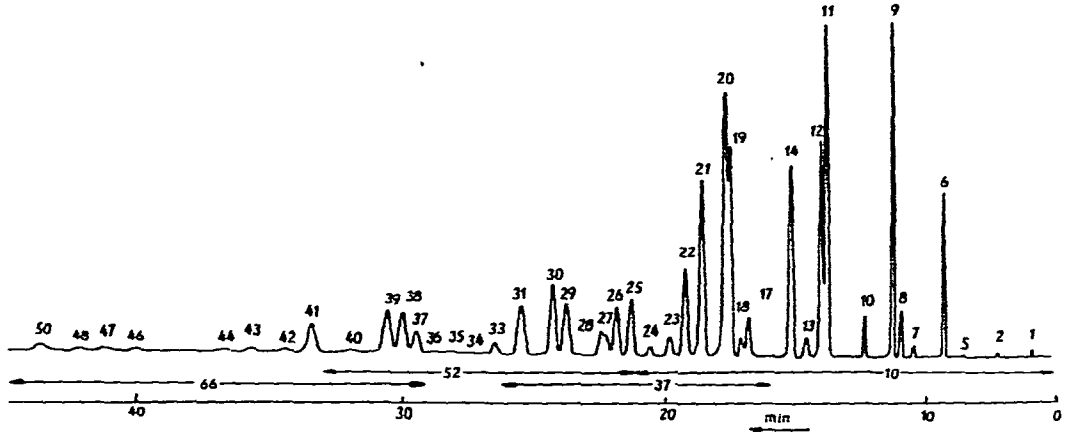

Fig. 1. Separation of a PCB mixture by GLC on a glass capillary column coated with OV-101 at 200 °C (column E). Numbering of peaks as in Tables II and III. The regions of distillation fractions 10, 37, 52 and 66 are designated with arrows.

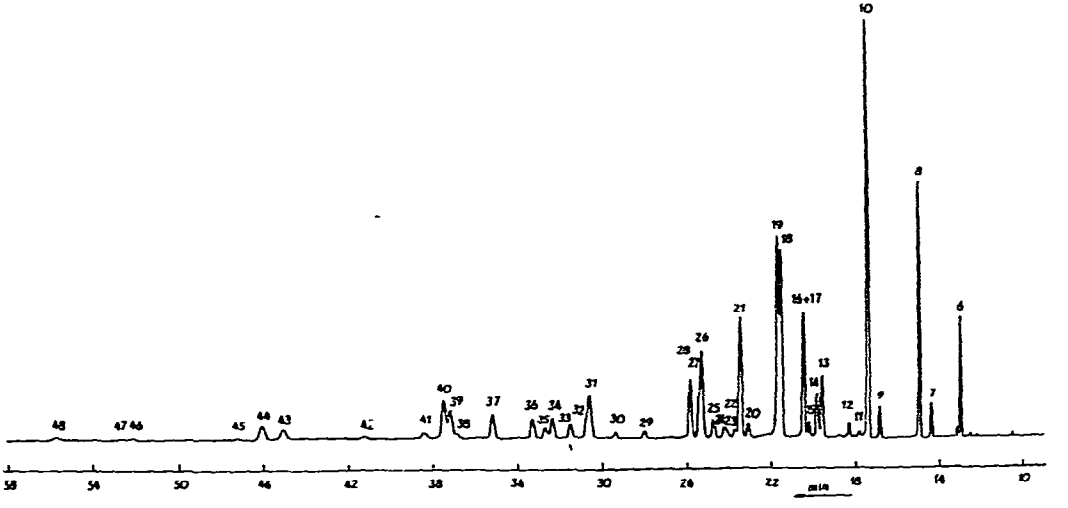

Fig. 2. Separation of PCB mixture by GLC on a glass capillary column coated with Carbowax 20M at 200 °C (column F). Numbering of peaks as in Tables II and III.

A comparison of diagrams[2] obtained by detection at 205 and 254 nm contributed to the qualitative analysis of mixture, and chromatograms of fraction No. 70 at both wavelengths are compared in Fig. 4. *n*-Pentane was used as the mobile phase; when preparing spectrally pure *n*-pentane we did not experience the difficulties that are usually encountered when purifying *n*-hexane.

HPLC analysis of distillation fractions 10, 37, 52 and 66 with *n*-pentane as the mobile phase on column C at 205 nm is shown in Fig. 5. It is apparent that with increasing number of chlorine atoms in the biphenyl molecule, the analysis time decreases, which is in agreement with published data[2]. Fig. 6 illustrates the analysis of

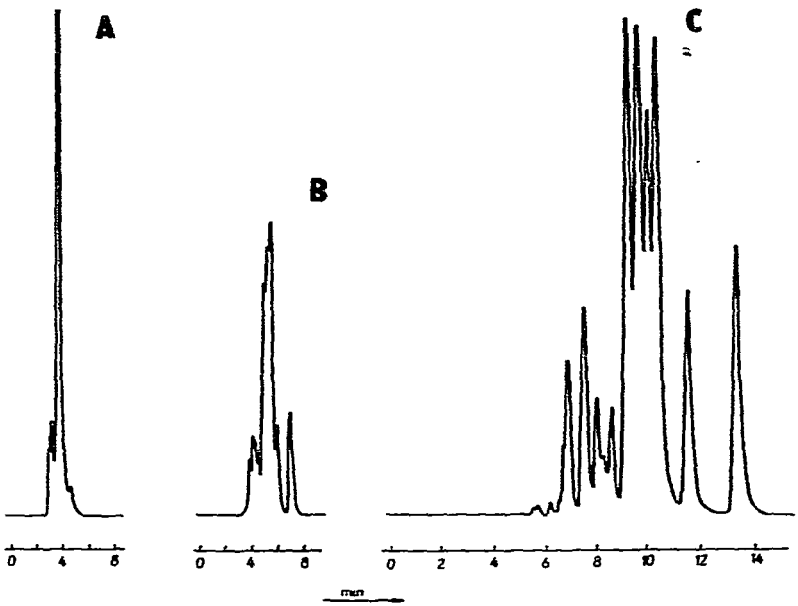

Fig. 3. Separation of fraction 52 by HPLC on columns of various efficiency with *n*-pentane as mobile phase. A, Column A, flow-rate 20 ml/h; B, column B, flow-rate 40 ml/h; C, column C, flow-rate 100 ml/h.

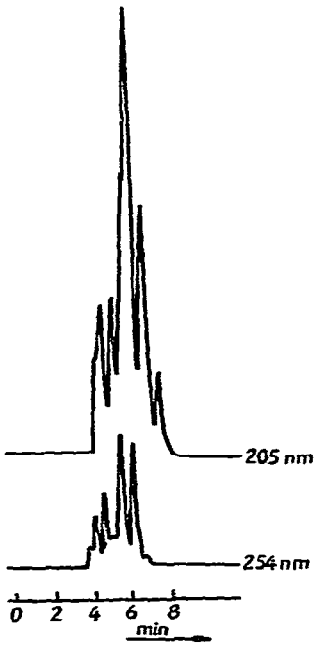

Fig. 4. Comparison of separation of fraction 70 by HPLC on column B with an *n*-pentane flow-rate of 40 ml/h, detected at two wavelengths.

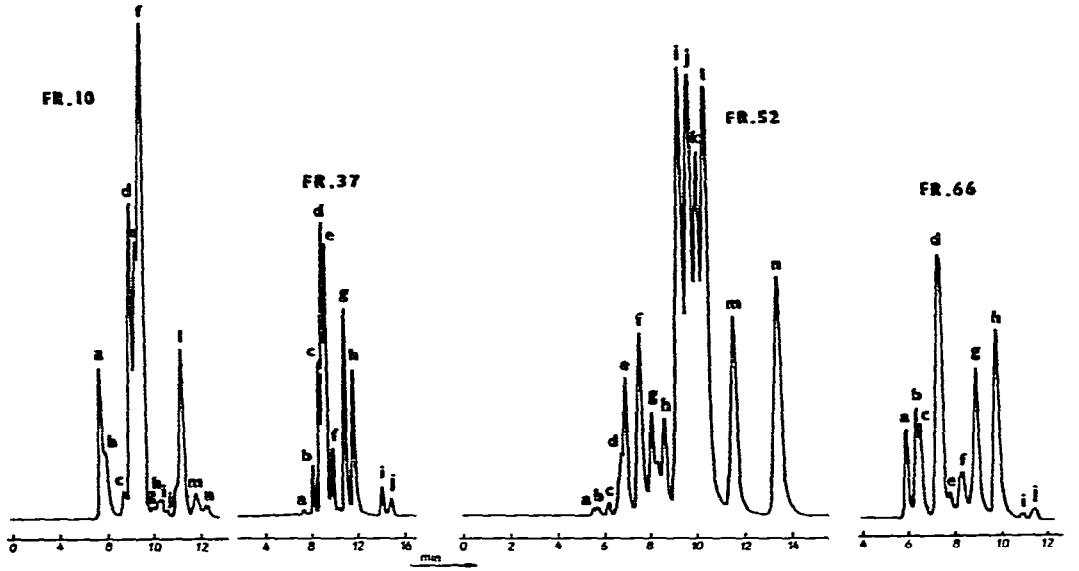

Fig. 5. Separation of fractions 10, 37, 52 and 66 by HPLC on column C with *n*-pentane at a flow-rate of 100 ml/h. Numbering of peaks as in Tables II and III.

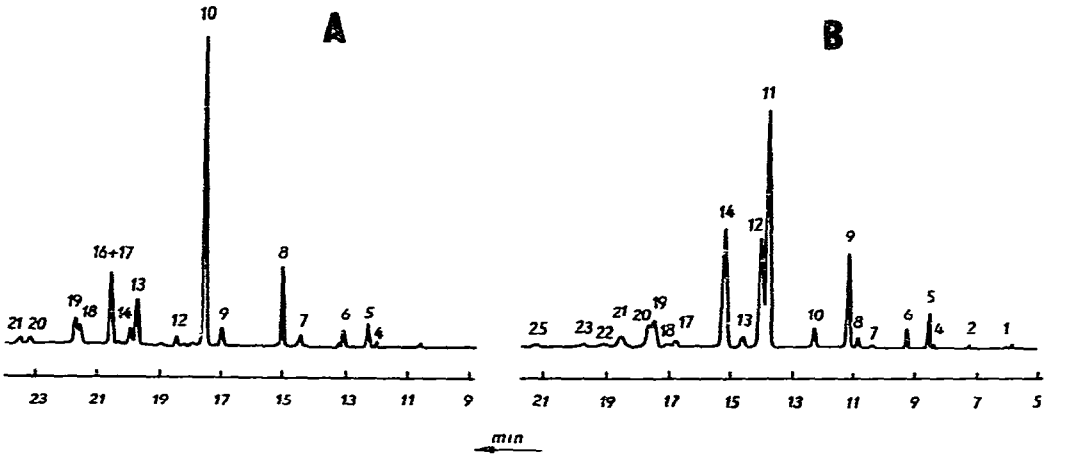

Fig. 6. Separation of fraction 10 by capillary GLC on Carbowax 20M (A) and OV-101 (B) at 200 °C. Numbering of peaks as in Tables II and III.

distillation fraction 10 by capillary GLC on Carbowax 20M and OV-101 stationary phases. Although efficient capillary columns were used, overlapping of the peaks of several PCBs on both stationary phases occurred.

By means of liquid chromatographic preparation on column D, 14 samples were obtained from fraction 10, 11 samples from fraction 37, 13 samples from fraction 52 and 11 samples from fraction 66. The sample preparation and HPLC analysis on column C for fraction 37 is shown in Fig. 7. The volume of samples prepared was 4 ml. After evaporation of *n*-pentane and dissolution of the sample in isooctane, the

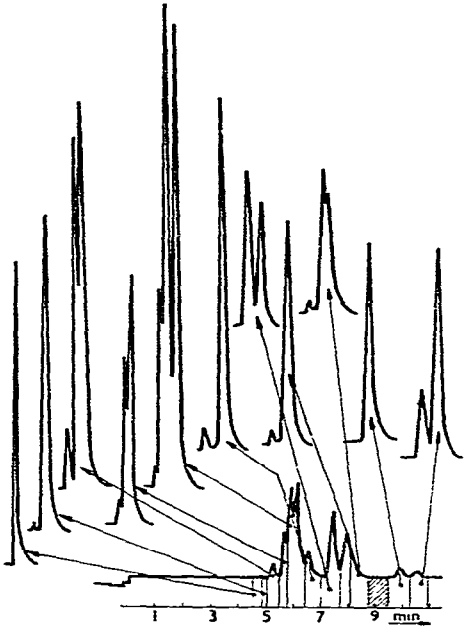

Fig. 7. Preparative LC of fraction 37 and HPLC analyses of all samples. Preparative: column D, *n*-pentane flow-rate 400 ml/h. Analytical: column C, *n*-pentane flow-rate 100 ml/h.

amount of the sample was sufficient for approximately 100 analyses by both liquid and gas chromatography. The feed was relatively large from the point of view of the amount of silica gel in the column, leading to a decrease in preparative column efficiency. The column was operating near its linear capacity limit, and it can therefore be reasonably postulated that for a lower feed or a larger column packing mass (*i.e.*, using a larger column) a better separation of the compounds would be achieved. On the other hand, under given conditions narrower fractions can be obtained, *i.e.*, smaller amounts of purer compounds, sufficient for further analyses. In addition to HPLC, all samples were also analysed by capillary GLC on both stationary phases.

Analyses of some samples obtained by preparation of distillation fraction 10 are shown in Fig. 8. The assignment of the peaks in Fig. 8 corresponds to that in Figs. 5 and 6. The optimal preparation was the isolation of a compound with a relatively high purity (*e.g.*, 2,3,2′-trichlorobiphenyl, Fig. 8d). Fig. 8e serves as an example of the separate of compounds with only a slight difference in adsorption properties, but with different volatilities. A difficult separation of compounds both by HPLC and GC is shown in Fig. 8a. An interesting case, when the relative volatilities of components are nearly identical and the compounds differ in their adsorption properties, is shown in Figs. 8b and 8d; two peaks in HPLC (d and l) correspond to peak No. 14 on the non-polar stationary phase.

PCB structures determined for fraction 10 are assigned to individual peaks from Figs. 5, 6 and 10 in Table II. With an increasing number of chlorine atoms the analysis becomes more difficult. Fig. 9 gives results from three samples obtained by preparation of fraction 37. Table III summarizes PCB structures assigned to individual

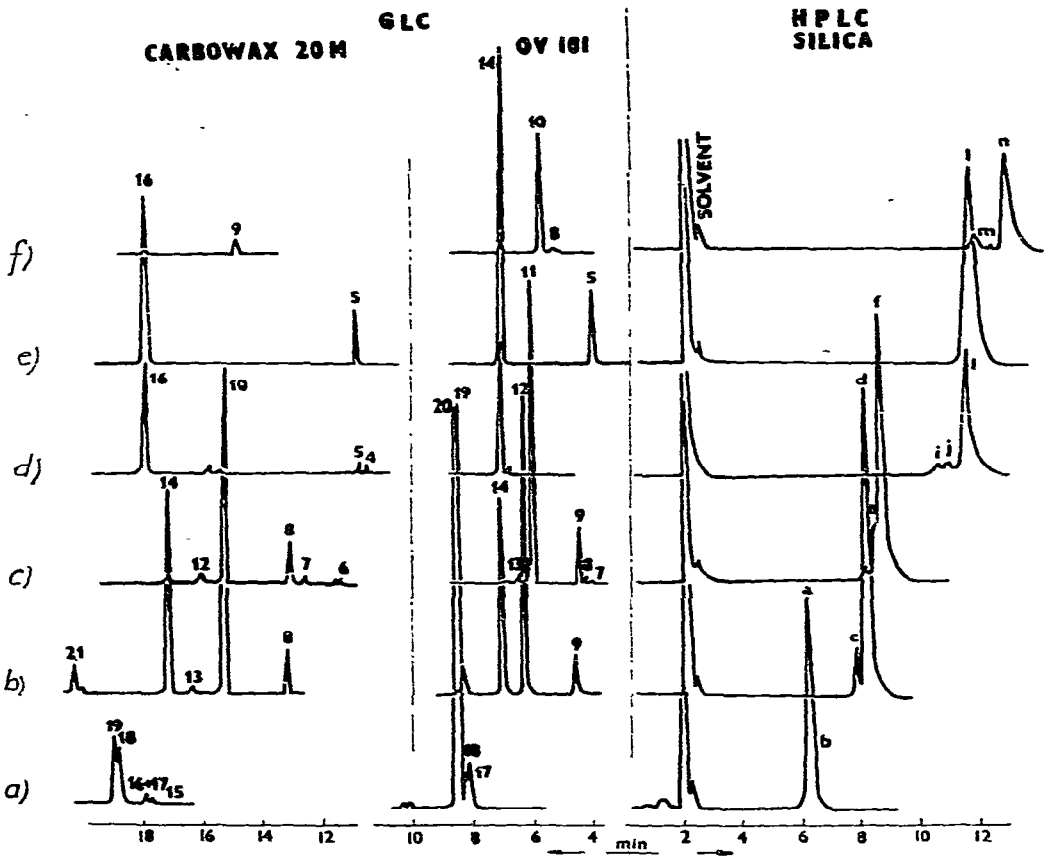

Fig. 8. HPLC and capillary GLC analyses of some samples obtained by preparative LC of fraction 10. HPLC, column C, *n*-pentane flow-rate 100 ml/h. Capillary GLC, see Table I.

TABLE II

IDENTIFICATION OF INDIVIDUAL PCBs IN FRACTION 10 USING A COMBINATION OF HPLC AND GLC

GLC peak No.		*HPLC peak No.*	*Structure*
OV-101	*Carbowax 20M*		
4	3		3-
5	5	l	4-
6	6		2,2′-
7	7		2,4-
8		l	2,3′-
9	8	e	2,4′-
10		m	2.6,2′-
11	10	f	2,5,2′-
12	14	e	4,4′-
12	10	d	2,4,2′-
14	16 or 17	l	2,3,2′-
14	13		
17	16 or 17	b	2,5,3′-
18	15	a	2,4,3′-
19	19	b	2,5,4′-
20	18	a	2,4,4′-
21	21	c	3,4,2′-

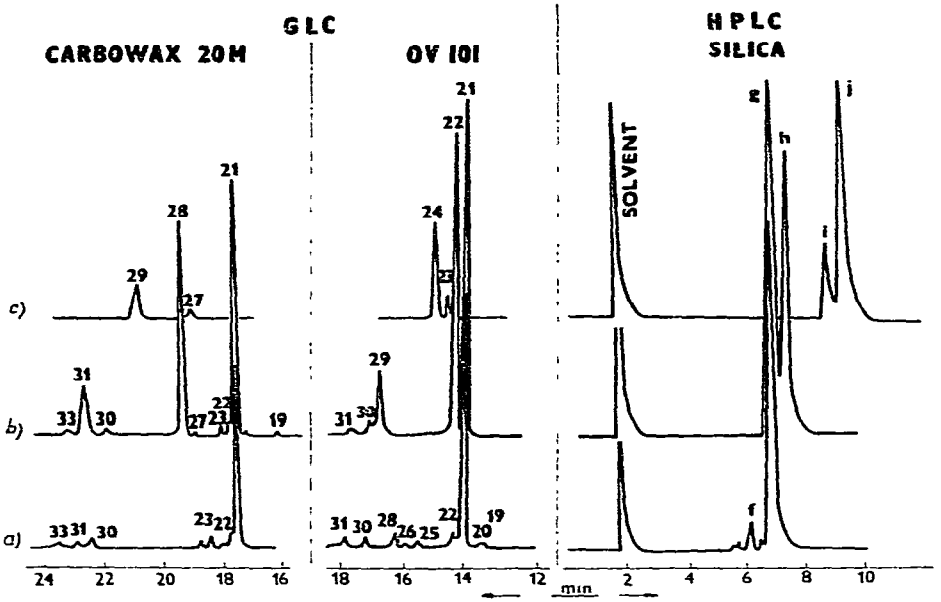

Fig. 9. HPLC and capillary GLC analyses of some samples obtained by preparative LC of fraction 37. HPLC, column C, *n*-pentane flow-rate 100 ml/h. Capillary GLC, see Table I.

peaks in fraction 37 (Figs. 1, 2, 5 and 9). Fraction 52 is composed mostly of tetrachloro derivatives, and it is therefore difficult to exploit for the identification of components in liquid chromatography the dependence of the number of chlorine atoms and the elution time[2,3].

For illustration, chromatograms of some samples obtained by preparation of fraction 52 are shown in Fig. 10. An unambiguous assignment of 2,5,2′,5′-, 2,3,2′,5′- and 2,3,2′,4′-tetrachlorobiphenyls was attained in this fraction. Similar identification

TABLE III

IDENTIFICATION OF THE MAIN INDIVIDUAL PCBs IN FRACTION 37 USING A COMBINATION OF HPLC AND GLC

GLC peak No.		*HPLC peak No.*	*Structure*
OV-101	*Carbowax 20M*		
19	19	e	2,5,4′-
20	18	d	2,4,4′-
21	26, 21	b, g	3,4,2′- + ?
22	28	h	
23	27	i	
24	29	j	
25	26	l	2,5,2′,5′-
26	26	l	2,4,2′,5′-
27	26		2,4,2′,4′-

problems occur for fraction 66 which, in addition to tetrachloro derivatives, also contains some pentachloro derivatives.

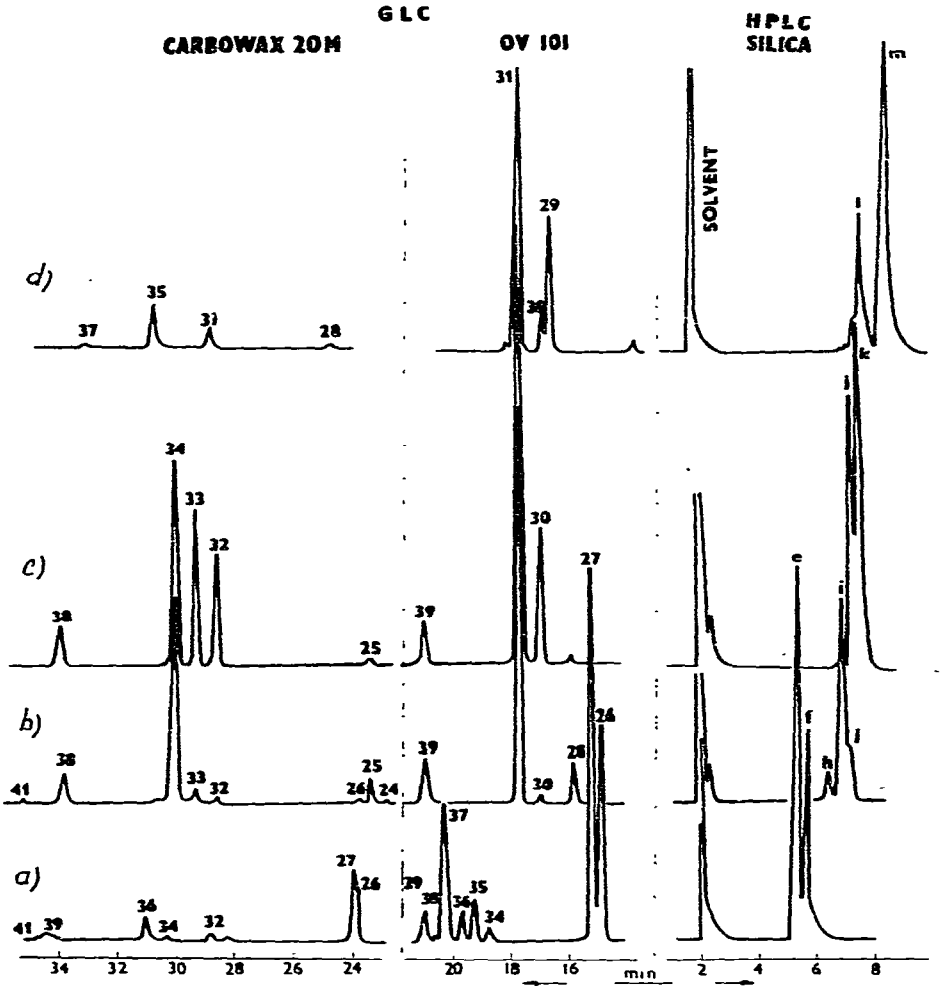

Fig. 10. HPLC and capillary GLC analyses of some samples obtained by preparative LC of fraction 52. HPLC, column C, *n*-pentane flow rate 100 ml/h. Capillary GLC, see Table I.

Chromatograms of two samples prepared from fraction 66 are shown in Fig. 11. Two PCB components that are well resolved by liquid chromatography yield only one peak on non-polar OV-101 stationary phase. We found by combined GC–MS[7] that it is a 1:3 mixture of penta- and tetrachloro derivatives. Our current work is concerned with problems in the analysis of tetra- and pentachloro derivatives.

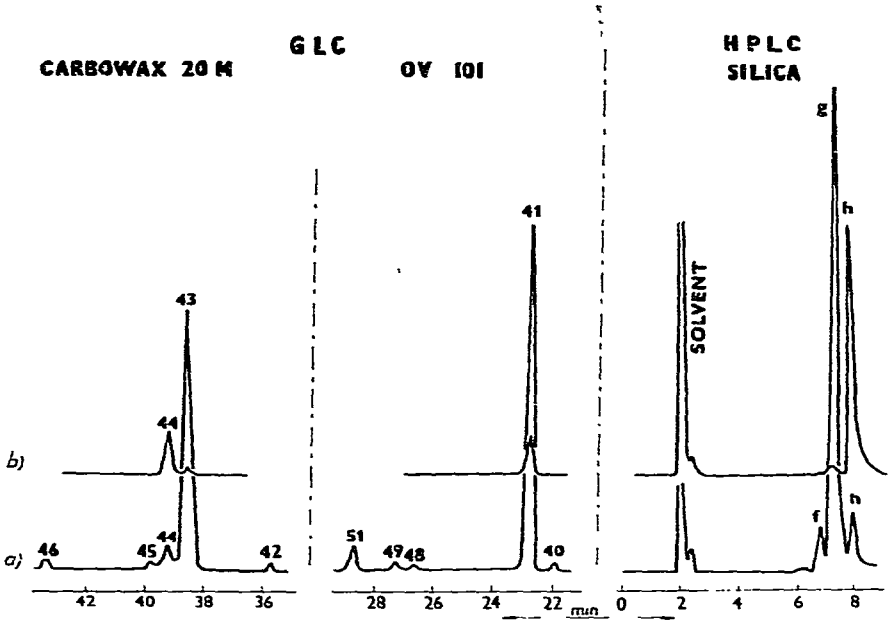

Fig. 11. HPLC and capillary GLC analyses of some samples obtained by preparative LC of fraction 66. HPLC, column C, *n*-pentane flow-rate 100 ml/h. Capillary GLC, see Table I.

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