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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF POLYCHLORINATED BIPHENYLS

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SUMMARY

High-performance liquid chromatography on a reversed-phase, microparticle column (μBondapak C_{18}) employing gradients of water and acetonitrile as the mobile phase has been used to resolve the commercial mixtures of polychlorinated biphenyls (PCBs), Aroclor 1221, 1016 and 1254. Individual PCBs (49) have been chromatographed under similar conditions and used as standards upon which the tentative identification and quantitation of some of the major components of the Aroclors have been based.

INTRODUCTION

The widespread introduction of mixtures of polychlorinated biphenyls (PCBs) into the environment[1] and the subsequent persistence of the more highly chlorinated constituents in animals[2] and man[3] has potentiated numerous investigations into an analytical method for the determination of PCBs. The large number of different PCBs possible (209 + biphenyl), the lack of synthetic samples of all of the PCBs, the presence of other halogenated pesticides as impurities and the low levels of the PCBs in many biological samples combine to hinder development of such an analytical procedure.

Gas chromatographic methods are most frequently utilized for PCB analysis, principally because of the sensitivity of the electron-capture detector (ECD) for chlorinated compounds. Methods have been published on gas chromatographic analysis using single[4] or multiple[5] packed columns and glass capillary columns[6]. The ECD responses of the individual PCBs show great variation and, in the absence of synthetic samples of particular PCBs, quantitation is usually based on predicted responses extrapolated from available PCBs. In many instances the resolution of the components is not sufficient for accurate analysis.

In view of the failure of gas chromatographic methods to yield completely satisfactory analyses of PCBs, the use of high-performance liquid chromatography

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(HPLC) presents an alternative procedure worthy of investigation. Only one group has reported on the use of HPLC or high-speed liquid chromatography (HSLC) to separate and quantitate the individual PCB components in commercial mixtures of PCBs[7,8]. A silica gel column which elutes the higher chlorinated PCBs initially was utilized in the normal phase. This system produced a reasonable separation of the lower chlorinated PCBs present predominantly in the commercial mixture Aroclor 1221, but was less efficient in separating the more highly chlorinated PCBs present in Aroclors 1254 and 1260.

The desirability of having an improved analytical procedure for PCBs prompted us to investigate the potential of reversed-phase HPLC. We report here on the analyses of three commercial mixtures of PCBs, Aroclor 1221, 1016 and 1254, and of 49 commercially synthesized individual PCBs.

EXPERIMENTAL

Aroclor 1254, 1016 and 1222 were obtained from Monsanto (St. Louis, Mo., U.S.A.) and were used without further purification or fractionation. Individual PCBs were purchased from Analabs (North Haven, Conn., U.S.A.) or RFR Corp. (Hope, R.I., U.S.A.) and were used as purchased. HPLC analysis of the individual PCBs indicated that the greatest level of impurity was 5% based only on integrated peak areas on the chromatograms. For most of the individual PCBs only a single peak was detected by the HPLC system utilized. The acetonitrile used for eluting the HPLC columns was glass distilled (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.). Water was deionized, glass distilled and filtered through a 0.22-μm membrane (Millipore, Bedford, Mass., U.S.A.) prior to chromatographic use.

A Waters Assoc. Model 244 liquid chromatograph equipped with a Hewlett-Packard Model 3385A automation system (recording integrator) was utilized. All solutions were monitored at 254 nm. The column (30 cm $\times$ 4 mm I.D.) was packed with reversed-phase, microparticle silica (μBondapak C_{18}; Waters Assoc., Milford, Mass., U.S.A.). The solvents used were I, water–acetonitrile (9:1), and II, acetonitrile–water (9:1). All individual PCBs and Aroclors were loaded on to the column in tetrahydrofuran solutions at concentrations of 5 mg/ml. With individual PCBs 1-μl injections were routinely used, and for Aroclor solutions 5-μl injections were used. The elution conditions for the various Aroclor mixtures were varied so as to obtain the best resolution of components with acetonitrile and water as eluents. For Aroclor 1221 elution was initiated with a solvent mixture of 60% of solvent II and 40% of solvent I and the solvent composition was altered in a non-linear gradient (gradient No. 7, Waters Assoc. Model 660 solvent programmer) over a period of 30 min to achieve 100% of solvent II. Elution was continued for a further 6 min. Solvent flow-rates were 2 ml/min throughout. For Aroclor 1016 a solvent mixture of 55% of solvent II and 45% of solvent I (*i.e.*, 54% of acetonitrile) was used isocratically. For Aroclor 1254 the same conditions were used as for Aroclor 1221 but the period of the gradient change was 40 min. A mixture of Aroclor 1254, 1221 and 1016 in the ratio of 1:1:1 (w/w/w) was chromatographed using the conditions developed for Aroclor 1254. The individual PCBs were chromatographed using conditions corresponding to that Aroclor mixture in which their isomeric series was most prominent. The nomenclature used for the individual PCBs is based on that of Brinkman and co-workers[7,8].

Quantitation of all of the Aroclor components that could be so analysed was performed by comparison of integrated peak areas using responses calibrated from weighed samples of individual PCBs.

RESULTS AND DISCUSSION

The absolute retention times and UV detector absorbance responses at 254 nm of 49 individual PCBs are presented in Table I. Detector responses are reported relative to that of biphenyl (arbitrarily set at 1.0). A number of relationships can be derived from the data in Table I and used for predicting the approximate chromatographic behavior of those individual PCBs not yet available synthetically. (i) In general, increasing the chlorine content of PCBs is a major factor in increasing the retention time. Consequently, isomeric groups tend to be eluted at similar times. (ii) Chloro substituents on PCBs in the 2- or 6- (*ortho*) positions decrease retention times relative to PCBs without such substituents, and this effect is enhanced when *o*-chlorosubstituents are on opposite phenyl rings (*e.g.*, the retention times of 2,2′-dichloro- and 2,6-dichlorobiphenyl are 10.24 and 11.09 min, respectively). The effect of two or more *o*-chloro substituents on a PCB is to decrease its retention time to correspond more closely with those of the isomeric series having one lower chlorine number (*e.g.*, 2,2′-dichloro- and 2,6-dichlorobiphenyl have lower retention times than 4-chlorobiphenyl and 2,3,6,2′,3′,6′-hexachlorobiphenyl has a retention time lower than those of some of the pentachlorobiphenyls tested). (iii) A chloro substituent on the 4- (*para*) position increases retention times relative to effects of 3- or 5- (*meta*) substituents (*e.g.*, the retention time of 2,4-dichlorobiphenyl at 14.31 min exceeds those of 2,3-dichloro- and 2,5-dichlorobiphenyl at 12.71 and 13.81 min, respectively). (iv) The UV detector responses were in agreement with previously reported spectral studies[9] and UV detector responses[7,8]. Thus, within an isomeric series of PCBs, the UV detector absorbance responses decrease with increasing retention time, although there are a number of exceptions. (v) 2- or 6-Chloro substituents diminish the responses of PCBs relative to those with no substituents in the *ortho* position, and this effect is again enhanced when the *ortho* substituents are on opposite phenyl rings [*e.g.*, the response of 2,2′-dichlorobiphenyl (0.047) is less than that of 2,6-dichlorobiphenyl (0.075), which in turn is markedly less than that of 2,3-dichlorobiphenyl (0.278) or 2,4-dichlorobiphenyl (0.508)]. (vi) A chloro substituent in the 4-position enhances the response of a PCB relative to that with a 3- or 5-substituent [*e.g.*, compare the responses of 2,4′-dichlorobiphenyl (0.468) with those of 2,3-dichloro-(0.278) and 2,5-dichlorobiphenyl (0.295)].

The HPLC column used in these studies separates on the basis of the relative extents of hydrophobic affinities. It can thus be concluded that *o*-chloro substituents, which force the two phenyl rings of biphenyl out of coplanarity, tend to decrease the hydrophobicity while *p*-chloro substituents increase the hydrophobicity of PCBs relative to those with the same numbers of chloro substituents but in different positions.

The detector responses of the PCBs are based on the κ absorbance band, which has been attributed to the conjugated biphenyl system[9]. *o*-Chloro substituents cause a hypsochromic shift of this band together with a decrease in extinction[9], which results in a diminished response at 254 nm. It is possible that the relatively high responses observed for the nona- and decachlorinated biphenyls (Table I) are a

TABLE I

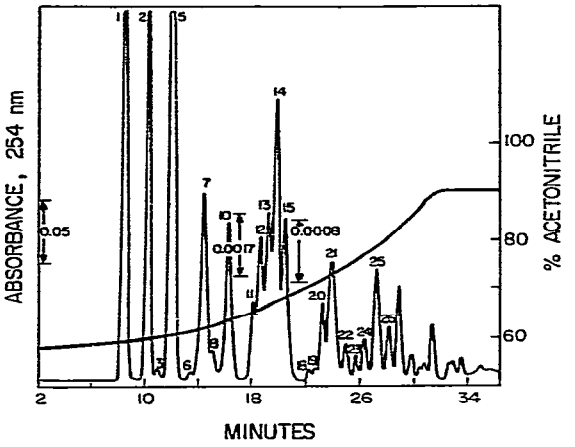
HPLC RETENTION TIMES AND UV SPECTRAL RESPONSES FOR INDIVIDUAL PCBs

UV detector at 254 nm wavelength.

PCB	*Retention time (min)*	*Spectral response (relative to biphenyl = 1000)*	*Analysis conditions*
Biphenyl	7.62	1.000	Aroclor 1221 assay
2-	9.39	0.255	
3-	9.91	0.725	
4-	11.12	0.917	
2,2′-	10.24	0.047	
2,6-	11.09	0.075	
2,3-	12.71	0.278	
2,4′-	13.57	0.465	
2,5-	13.81	0.295	
2,4-	14.31	0.508	
3,3′-	14.80	0.562	
3,4-	15.11	0.917	
4,4′-	15.43	1.087	
3,5-	16.63	0.685	
2,5,2′-	23.59	0.046	Aroclor 1016 assay
2,3,6-	23.82	0.042	
2,4,6-	28.67	0.093	
2,3,4-	30.05	0.394	
3,4,2′-	31.08	0.327	
2,5,3′-	32.12	0.190	
2,5,4′-	33.61	0.506	
2,4,5-	34.02	0.309	
2,4,4′-	36.31	0.674	
2,6,2′,6′-	14.61	0.007	Aroclor 1254 assay
2,3,2′,3′-	18.41	0.173	
2,3,2′,5′-	19.80	0.034	
2,5,2′,5′-	21.10	0.037	
3,4,3′,4′-	21.15	0.086	
2,3,5,6-	21.64	0.092	
2,4,2′,5′-	22.31	0.093	
2,4,2′,4′-	22.80	0.156	
2,3,4,5-	24.95	0.352	
2,5,3′,4′-	24.96	0.364	
2,4,3′,4′-	25.80	0.564	
2,4,5,2′,3′-	24.91	0.088	
2,3,6,2′,5′-	25.48	0.063	
2,3,4,2′,5′-	26.09	0.088	
2,3,4,5,6-	27.21	0.176	
2,4,5,2′,5′-	27.42	0.089	
2,3,6,2′,3′,6′-	25.81	0.007	
2,3,5,6,2′,5′-	28.64	0.019	
2,3,4,2′,3′,4′-	30.83	0.163	
2,4,6,2′,4′,6′-	31.44	0.047	
2,3,4,2′,4′,5′-	31.85	0.245	
2,4,5,2′,4′,5′-	32.88	0.146	
2,3,4,5,6,2′,5′-	34.10	0.096	
2,3,5,6,2′,3′,5′,6′-	37.36	0.024	
2,3,4,5,6,2′,3′,4′,5′-	41.96	0.204	
2,3,4,5,6,2′,3′,4′,5′,6′-	45.12	0.140	

consequence of the enhanced extinction of the "main band"[9] of these compounds. Although the "main bands" for these PCBs are centered at 216 nm, their absorbances tail well past 254 nm[9].

The chromatograms of Aroclor 1221, 1016 and 1254 and a 1:1:1 (w/w/w) mixture of the three Aroclors are depicted in Figs. 1–4. Some of the major constituents of the Aroclors have been tentatively assigned and quantitated, based on comparisons of retention times and responses with those of the available standards (Table I) together with previously reported results[4–8,10]. The sequence of numbering of the



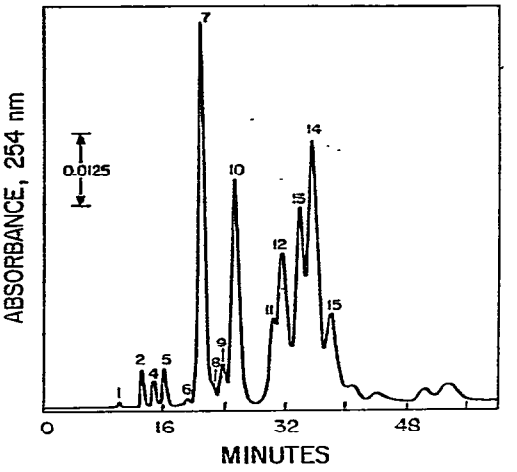


Fig. 1. HPLC separation of Aroclor 1221 (5 mg/ml in tetrahydrofuran) on a μBondapak C_{18} column monitored at 254 nm. Initial conditions, 40% water–acetonitrile (9:1) and 60% water–acetonitrile (1:9); final conditions, 100% water–acetonitrile (1:9); gradient period, 30 min; flow-rate, 2 ml/min; injection volume, 5 μl; amount injected, 25 μg.

Fig. 2. HPLC separation of Aroclor 1016 (5 mg/ml in tetrahydrofuran) on a μBondapak C_{18} column monitored at 254 nm. Conditions, 45% water–acetonitrile (9:1) and 55% water–acetonitrile (1:9); flow-rate, 2 ml/min; injection volume, 5 μl; amount injected, 25 μg.

peaks includes all peaks observed in all the chromatograms and thus some numbers may be absent from a particular chromatogram. Final identification of the components represented by the major peaks can only be made using mass spectrometry and nuclear magnetic resonance spectrometry, and studies are continuing in our laboratories along these lines.

For Aroclor 1221 (21% by weight of chlorine), 31 components were distinguishable chromatographically (Fig. 1). The seven major components have been tentatively identified and quantitated (Table II). 3-Chlorobiphenyl is not completely resolved from an excess of 2-chlorobiphenyl and a trace amount of the former may be present and thus increase our reported value for the latter, which is slightly higher than previously reported values. It is apparent that the patterns of minor components of Aroclor 1221 closely resemble those of the major components of Aroclor 1016 and 1254 (compare Fig. 1 with Figs. 2 and 3).

For Aroclor 1016 (41% by weight of chlorine), 18 components were distinguishable chromatographically (Fig. 2). The resolution of the components was inferior

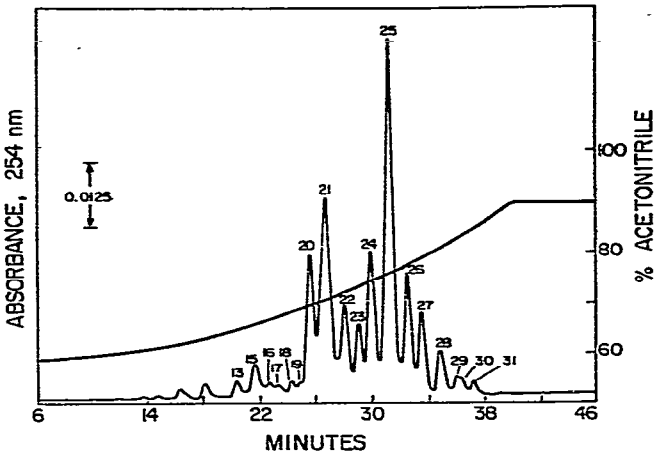


Fig. 3. HPLC separation of Aroclor 1254 (5 mg/ml in tetrahydrofuran) on a μBondapak C_{18} column monitored at 254 nm. Initial conditions, 40% water–acetonitrile (1:9) and 60% water–acetonitrile (1:9); final conditions, 100% water–acetonitrile (1:9); gradient time, 40 min; flow-rate, 2 ml/min; injection volume, 5 μl; amount injected, 25 μg.

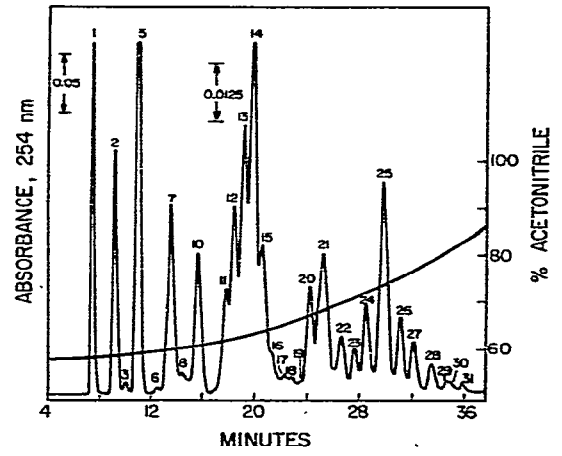


Fig. 4. HPLC separation of Aroclor 1221–Aroclor 1016–Aroclor 1254 (1:1:1, w/w/w) (1.67 mg/ml of each in tetrahydrofuran) on a μBondapak C_{18} column monitored at 254 nm. Initial conditions, 40% water–acetonitrile (9:1) and 60% water–acetonitrile (1:9); final conditions, 100% water–acetonitrile (1:9); gradient time, 40 min; flow-rate, 2 ml/min; injection volume, 10 μl; amount injected, 50 μg total.

to that obtained with Aroclor 1221 and 1254. The peaks up to and including No. 10 have been tentatively identified and together make up approximately 30% by weight of the sample (Table III). The retention time of peak 13 corresponds with that of 2,3,2′,5′-tetrachlorobiphenyl but, based on the known response of this compound, the peak would represent greater than 100% by weight of the sample. It must therefore be assumed that some other PCB co-chromatographs with 2,3,2′,5′-tetrachlorobiphenyl. Peaks 11, 12, 13, 14 and 15, which together represent approximately 70% by

TABLE II
COMPOSITION OF AROCLOR 1221

Peak No.	*PCB*	*Content (wt.-%)*			
		This study	*Ref. 5**	*Ref. 7***	*Ref. 10*
1	Biphenyl	12.6	15.85	13	12.7
2	2-	38.2	32.14	33	28.4
3	2,2'-	5.8	4.81	7.5	9.2
5	4-	21.7	19.07	17	18.7
7	2,4'-	13.4	10.17	15	13.6
8	2,4-	1.6	2.72	1.5	3.5
10	4,4'-	4.7	3.65	5	6.2

* Gas chromatographic method using six columns.
** HPLC method.

weight of Aroclor 1254, have retention times representative of trichlorobiphenyls (but excluding those with high *o*-chloro substitution) and highly *ortho* substituted tetrachlorobiphenyls. The latter, however, are unlikely to be solely represented by any of these peaks for the same reason as discussed previously for excluding 2,3,2',5'-tetrachlorobiphenyl.

TABLE III
COMPOSITION OF AROCLOR 1016

Peak No.	*PCB*	*Content (wt.-%)*
1	Biphenyl	0.03
2	2-	1.1
5	4-	0.4
7	2,4'-	12.0
9	2,5,2'-	12.7
10	4,4'-	3.4

For Aroclor 1254 (54% by weight of chlorine), 22 components were distinguishable chromatographically (Fig. 3). Those components which have been tentatively identified and quantitated are shown in Table IV. The major peak (based on integrated areas) is No. 25 and probably represents a pentachloro- or hexachlorobiphenyl which has less than two *ortho* substituents. Higher numbers of *ortho* substituents would have responses that would preclude the possibility of a peak of such magnitude.

Although the analysis of PCBs by HPLC using a UV detector at 254 nm has been shown to be a viable method, producing separation of some components that are superior to those of gas chromatographic methods, the relative lack of sensitivity presents a problem. In the present study, 25 μg of the Aroclors were used for each analysis. This could be reduced to 3 μg with the HPLC used in this study without markedly reducing the accuracy of the analysis. The HPLC system permits a much greater volume of sample to be loaded per analysis than is possible with gas chromatographic systems and this, together with the use of higher concentrations of sample

TABLE IV
COMPOSITION OF AROCLOR 1254

Peak No.	*PCB*	*Content (wt.-%)*
13	2,3,2′,5′-	5.3
15	2,5,2′,5′-	10.3
20	2,5,3′,4′-	3.3
22	2,4,5,2′,5′-	11.7
26	2,3,4,2′,4′,5′-	4.9
27	2,4,5,2′,4′,5′-	5.3

solutions, would to a large extent overcome the relatively diminished sensitivity of the HPLC method.

Apart from the potential to analyse environmental samples of PCBs, the HPLC method provides a simple means for purifying individual PCBs, which can then be used for investigations where trace impurities could significantly alter results. The removal of dibenzofuran impurities from PCB samples to be used in induction studies would be particularly significant. The limited nature of the UV detector could also be used to advantage by effectively eliminating the interference of other non-UV-absorbing xenobiotics in environmental samples during PCB analysis. The use of variable-wavelength detectors should facilitate this. Two examples of such xenobiotics are the insecticides mirex and chlordane.

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