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ANALYSIS FOR POLYCHLORINATED BIPHENYLS BY DUAL CAPILLARY COLUMN GAS CHROMATOGRAPHY

by

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MANAGEMENT PERSPECTIVE

A method has been developed whereby the various PCB congeners found in Aroclors can be identified and quantified on two GC capillary columns simultaneously. Also, the usual organo-chlorine target compounds can be quantitated and identified in the same chromatogram. With the present system, 125 different compounds can be identified and quantitated. The agreement between this methodology and existing methodology has been found to be very good. In addition, only one analysis is necessary using the new methodology rather than the two presently required with the attendant saving of analysis time. Using computer programs developed with the method, a time saving of half an hour per sample by the analyst is anticipated.

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PERSPECTIVE-GESTION

On a mis au point une méthode qui permet d'identifier et de doser simultanément les divers congénères de BPC se trouvant dans les Aroclors au moyen de deux colonnes capillaires de CG. Ce chromatogramme permet aussi d'identifier et de doser les organochlorés habituellement recherchés. Dans sa forme actuelle, ce système permet d'identifier et de doser 125 composés. On a par ailleurs constaté une très bonne concordance entre cette méthode et celle qui s'utilise actuellement. En outre, cette dernière nécessite deux analyses, tandis qu'avec la nouvelle méthode on n'en fait qu'une, ce qui fait gagner du temps. En utilisant les programmes informatiques mis au point pour la nouvelle méthode, on devrait réduire la durée de l'analyse d'une demi-heure par échantillon.

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ABSTRACT

A dual column, gas chromatographic, congener-specific methodology has been developed that permits determination of polychlorinated-biphenyl (PCB) concentrations in environmental water and sediment samples. The linearity of the detector responses were checked over a range of 1000 at the sub ppb range of total PCB in the dual column mode, with less than 10^{-12} g of each congener impinging on the detector. The chromatographic method was checked to ensure that there was minimal interference by the organochlorines to the PCB analysis and vice-versa. PCB standards were analyzed and they yielded values within 10% of the expected amounts. Extracts from sediments were analyzed and the organo-chlorine pesticide (OC) values were compared with, and agreed with, values obtained using standard methods. The PCB values obtained from the standard and presented methods agreed within a factor of two. Use of the method provides results that are at least comparable with present methodology and requires less time.

Key Words: PCB, Congeners, Quantification, Capillary, Dual Column

RÉSUMÉ

On a mis au point une méthode d'analyse par chromatographie en phase gazeuse sur colonnes jumelées spécifique aux composés congénères qui permet de doser les biphényles polychlorés (BPC) des échantillons d'eau et de sédiments prélevés en milieu naturel. On a vérifié la linéarité de la détection en colonnes jumelées dans une plage de 1 000 à des concentrations totales de BPC inférieures à 1 p.p. 10^9, avec moins de 10^{-12} g de chacun des congénères agissant sur le détecteur. On s'est aussi assuré que l'interférence mutuelle des organochlorés et des BPC était minime dans l'analyse par chromatographie. L'analyse de BPC étalons a donné des valeurs se trouvant dans une marge de 10 % des valeurs escomptées. On a analysé des extraits de sédiments et constaté que les concentrations de pesticides organochlorés (OC) mesurées concordaient avec les valeurs obtenues par les méthodes standard. Dans le cas des BPC, les valeurs obtenues par la méthode standard et les concentrations mesurées par la méthode présentée ici concordaient à l'intérieur d'une plage d'un facteur 2. La nouvelle méthode donne des résultats au moins comparables à ceux qu'on obtient avec la méthode actuelle et prend moins de temps.

Mots-clés : BPC, congénères, dosage, capillaire, colonnes jumelées

1.0 INTRODUCTION

The synthesis of all 209 PCB congeners and their subsequent characterization by capillary column gas chromatography (1) has made possible the analysis of PCB mixtures (2,3,) and environmental samples (4) for individual PCB congeners. This has been achieved by using single capillary column gas chromatography. Previously, PCB analysis was accomplished by using single packed column gas chromatography (5). The analysis using packed columns involves summing certain peaks which contain many unresolved congeners. This type of calculation best provides total PCB concentrations. Using capillary columns, the individual congeners can be separated, their individual concentrations can be summed and then the results reported by congener or the degree of substitution about the biphenyl structure.

This method works well for standards and clean environmental samples. For samples from the aquatic media, other compounds in the sample may be eluted with the particular PCB despite the effort expended for the cleanup steps (e.g., 6). When there is a coelutant, two errors can be introduced in the analysis. First, if a target compound is present, there is an enhanced value for that compound. Second, if the target compound is not present, but a peak does occur at the appropriate retention time, the compound causing the peak may be erroneously identified as the target compound. Also, negative peaks may occur in the chromatogram caused by a compound which gives rise to this behaviour not being removed during the cleanup step or because of some contamination of the detector. If a negative peak occurs near a target peak, such as a PCB congener, there will be an enhanced value for that target compound, as the baseline is shifted to a lower value.

Chemists analyzing samples collected from the aquatic milieu use two GC methods (4,6) to analyze the first fraction of the cleanup step (6), often expending considerable time analyzing the PCB chromatogram. To effectively utilize the capabilities of a computer and state-of-the-art GC technology, a method was developed that yields more

reliable analysis of PCBs than is currently used. Also, other target compounds could be analyzed concurrently. Using dual column gas chromatography in the split/splitless mode, and interfacing with a mini-computer, the reported methodology was developed. The use of the second column permits a comparison of the retention times of the PCBs and other target compounds as to presence of a particular compound as well as providing a check on the concentrations for each compound. With the abundance of information from such an analysis, specially tailored computer programs were created which can match the congeners present and their concentrations, thereby reducing the work of the analyst.

2.0 METHOD AND MATERIALS

The gas chromatograph was an HP 5890 equipped with dual EC detectors and was controlled by the Laboratory Automated System (LAS) software of the Real Time Executive (RET-A) operating system of an HP 1000 computer. Each injection of 1µL per column was performed by an HP7235A automatic sampler, and the injector was operated in the split/splitless mode. All samples were injected in the splitless mode with the purge activated after 0.75 min. Preliminary integration was performed on two HP-3392A integrators, one for the output from each column-detector combination. The EC detectors contained Ni^{63} foil operated at 300°C and had Ar/Me (95/5%) makeup gas mixed with the normal H_2 carrier gas. The column head pressure was 17 psi. An initial temperature of 80°C was maintained for 2 minutes, then the temperature was increased by 10°C/min to 140°C, then the rate reduced to 2°C/min until the temperature reached 255°C at which time the run was complete. The capillary columns had liquid phase DB-1, XE-52, or DB-5 and were supplied either by Hiresco (Mississauga, Ontario) or J.&W. through their distributors. The columns were 30 m in length with internal diameters of 0.25 mm. The liquid film thickness was 0.25 µm for each column. For all analysis presented here, guard columns were positioned before the working columns.

The organochlorines, including aldrin, heptachlor, hexachlorobenzene, octachlorostyrene, p,p'-DDE, o,p'-DDT, p,p'-DDT, photomirex, mirex, di's-, tri's-, 1,2,3,4-tetre-, penta, and hexa-chlorobenzenes were obtained from the National Depository of the National Bureau of Standards (Washington, D.C.). Also the Aroclor mixtures of 1221, 1016, 1254, and 1262 were obtained from this source. The solutions, either individually or in mixtures were made up to appropriate concentrations in isooctane. A calibration mixture was prepared by combining 1000 parts of Aroclor 1221, 500 parts of Aroclor 1016, 350 parts of Aroclor 1254 and 300 parts of Aroclor 1262 according to Onuska et al. (2).

During a series of runs, an isooctane blank was injected after every second sample. For preliminary investigations, at least ten aliquots were injected. The results were downloaded from the computer and appropriate programs written on the HP 1000 would permit calculation of statistical data or determination of the PCB concentration by congener distribution or the degree of substitution.

The first step in calculating the concentration of the PCBs in the samples was to determine if the particular congener appears on the chromatographic traces of each chromatogram. Several congeners may elute as doublets on one column but as two peaks on the other. In this instance, if the doublet peak was present and two peaks identified as corresponding to the congeners in the doublet on the other column, both congeners would be considered present. If only one of the congeners was in the chromatogram of the second column, it would be considered as being present, and the doublet on the first column in fact being the trace of a single compound. It was found more convenient for one column to be used for the quantitation and the second to be used for the confirmation. Once it was established that the particular congener was present in both chromatographic traces, the concentration from each detector was compared. If the difference in the concentration for a particular congener on one column exceeded that on another by more than a factor of five, the lower value was to be used.

3.0 RESULTS AND DISCUSSION

Before attempting to establish a method that can quantitate the PCB congeners and other target organochlorine compounds, good chromatography as denoted by symmetrical peak shape and resolution between closely eluting compounds had to be obtained for the PCB mixture on both columns. When this was attained, the results had to be reproducible, which is a function of the injection and the detector response as well as the chromatography. Preliminary work was carried out using six Aroclor 1262 solutions encompassing the range of 5.0 to 0.04×10^{-9} total PCB, and chromatographed on an XE-52 capillary column. Analyzing the response for the 27 major congeners, coefficients of variance (CV) of less than 10% were calculated for each congener at concentration, and the CV was below 5% for three-quarters of the 159 mean values calculated. When the means for each congener as a function of concentrates was treated by linear regression analysis, correlation coefficients greater than 0.998 were calculated. The detector response was deemed linear over the concentration range studied which encompasses those concentrations found in environmental samples (e.g. 4). The XE-52 column was then replaced with a DB-1 liquid phase column. Once the results obtained from this column were considered acceptable for the PCBs, organochlorines and chlorobenzenes, the DB-1 and DB-5 columns were installed together in a dual column mode, ensuring equal amounts of the injection reached each detector (7). The OC mixture was then chromatographed together with the PCB standard. There were some minor overlaps, but these were easily corrected by comparing the results from the two columns. An example is that of aldrin and congener 29 which coelute on the DB-1 column, a simple cross reference to aldrin and congener 29 on the other column, first for the presence or absence, and second for the concentration will give an indication that both are present, or if there is only one, as well as the relative concentrations.

The standard PCB solution containing 2150 pg/μL, using the dual mode, yielded results that were reproducible. The CV was less than

5% for peaks whose retention time was less than 35 min and CVs of less than 10% for the remainder of the peaks. The DB-1 results were slightly more reproducible than those from the DB-5 column, and the influence of the guard columns was to increase the CV by about 2% for each peak whose retention time was greater than 20 min. Typical chromatograms are shown in Fig. 1. A solution containing the congeners in the four Aroclors used to make the standard was serially diluted and the resulting solutions chromatographed, with each being injected ten times. The concentrations ranged from 2250 pg to 30 pg total PCB impinging on the detector. .The plots of response versus concentration were linear with correlation coefficients above 0.98 for the majority of the congeners. A standard solution of the organochlorines expected to elute with the PCBs in the cleanup was next injected. These too were shown to give a linear response on the detector over a concentration range of 30 pg to 2250 pg total PCB, the range where the detector response would be expected for environmental samples containing compounds at the trace level. The response factors were then entered into two calibration tables, one for each column and detector combination. Then standards were analyzed. Listed in Table 1 are the total PCB concentrations of various standards and Aroclors, as well as the anticipated values. Each total is the sum of the congeners identified on both columns. The agreement between measured and expected values is good.

Then environmental sediment and water samples were investigated. In Table 2 the results are listed for a standard sediment extract, a spiking solution containing PCB and OCs, and the standard sediment extract with the spike added before the cleanup step. Considering the DB-1 column results only, 73 pb of total PCB were found in the reference sediment and 214.6 ppb were determined in the spike. The spiking solution contained 200 pg/μL. By calculation, the spiked sediment contained 306.4 ppb of total PCB compared to an anticipated 288.4 ppb. The results arose from 52 peaks being identified as PCB congeners in the spike and 59 peaks identified as PCB congeners in the spiked sediment. Also included in Table 2 are the results derived from

the standard method of analysis. The spiked sediment and the untreated sediment have lower total PCB values than calculated by the present method, but the values for the spiking solution agree. This portion of Table 2 indicates that the congener methodology can analyze samples at least as well as the methodology presently used, but it also provides a more comprehensive understanding of exactly which PCB congeners are present. The method requires summing of the concentrations of each contributing congener, including any error derived fom calculating the concentration. The more congeners present, the greater the possible error. However, the values presented in Tables 1 and the first part of Table 2 indicate that the total concentrations generated by this method are in general agreement with the anticipated values.

These samples also contained organochlorines which coelute with the PCBs during the cleanup stage. These are also listed in Table 2. The values derived from the spiking solution (column 5) agree with the expected values (column 7) and are closer to the values in column 7 than those obtained using the methodology developed for OC quantitation (column 6). The values for the di- and tri-chlorobenzenes in the sediment and spiked sediment indicate that these compounds are present but the quantitation is poor. These compounds elute near the beginning of the chromatogram, just after the solvent peak and in the area where small impurities from the silica gel cleanup may interfere with the analysis. Values for later eluting organochlorines listed in Table 3 do agree with the expected values.

A number of extracts of samples were then analyzed using the methodology presented here as well as by the standard methods. Table 3(a) and 3(b) list the results for four samples, three representative sediment samples and one representative water sample. Also included are the results of analysis conducted by qualified laboratory staff on the same samples using standard techniques. The organochlorines are listed in Table 3(a). Each entry denotes the particular compound being detected on both columns and is the average of the two calculated values, unless one value is five times greater than the other, in which

case the lower value is entered. In the table, this value is underlined to emphasize this manipulation. The values listed in this part of Table 4 show that the results for the two methods agree within the same order of magnitude, but with slightly more compounds being found using this methodology. Analyzing QC standards with both methods showed a better performance of the congener specific method.

Table 3(b) lists the PCB concentrations, as totals and these are listed as amounts per degree of substitution as well as the number of contributing congeners. The results for both methods agree reasonably well. Both are of the same order of magnitude, and follow the same trend. The congeners present in the Aroclors were used to standardize the calibration table and therefore would be expected to present acceptable results.

Prior to quantifying, this congener specific method requires that each compound be identified on both columns. Then the concentrations calculated from the response of each detector is checked to ensure that a coeluting contaminant is not interfering. If one of the responses is too high, the analyst can substitute the lower result obtained from the other column. This procedure provides confirmation both as to the presence of a particular compound as well as its concentration. Although the prime objective was to produce a method that quantitates PCBs in environmental samples, other target compunds are also identified and quantitated. The results presented here show that the new method is at least as accurate as the standard method and can accurately produce PCB results based on the contribution of individual congeners. This reduces the number of analyses that need to be done, which results in a real saving of equipment time. Also by using a computer to collate the results of the dual capillary column analysis, a further saving of time is achieved.

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TABLE 1

ANALYSIS OF PCB STANDARDS

Standard (Aroclor)	Expected Concentration (pg/μL)	Found Concentration (pg/μL)
1016	41.9	37.6
1262	50.0	47.2
1242	200.0	187.4
1254	200.0	182.2
1260	200.0	201.3
1221+1016+	2150.0	2252 (OV-1)
1254+1262		2001 (DB-5)
1242+1254	200.0	187.0
1260		

TABLE 2

PCB AND ORGANOCHLORINE ANALYSIS OF REFERENCE SEDIMENT

Compound	Spiked Sediment (a)	Spiked Sediment (b)	Sediment (a)	Sediment (b)	Spike (a)	Spike (b)	Expected
	(1)	(2)	(3)	(4)	(5)	(6)	(7)
	(ng/g)		(ng/g)		(ng/g)		(ng/g)
PCB	306.4	227	73.8	41.4	214.6	213	200
No. congeners	59		33		52		
1,3-DiClB[1]	23.9	24.2	6.6	n.d.	48.3	40.1	50
1,4-DiClB	39.3	26.2	19.8	6.1	52.1	40.9	50
1,2-DiClB[1]	36.8	27.0	30.4	0.8	53.5	41.1	50
1,3,5-TriClB[2]	5.4	3.9	2.4	n.d.	6.5	5.3	5
1,2,4-TriClB	10.3	8.8	10.7	8.7	6.6	6.0	5
1,2,3-TriClB	26.6	5.4	20.6	2.2	6.0	6.3	5
1,2,3-4-TetB[3]	17.2	12.4	12.8	19.0	18.3	7.8	5
PentaClB[4]	8.7	10.4	4.0	4.4	6.6	5.2	5
HCB[5]	13.0	10.0	3.7	2.6	9.7	4.4	5
Heptachlor	6.9	7.5	n.d.	n.d.	7.5	8.8	5
Aldrin	10.9	6.0	n.d.	n.d.	8.3	9.4	5
p,p'-DDT	22.3	38.3	n.d.	n.d.	23.4	57.9	15
p,p'-DDE	18.0	21.2	3.1	3.2	15.9	26.0	10
o,p'-DDT	17.2	21.2	n.d.	n.d.	17.9	35.1	15
Mirex	10.2	13.3	0.5	n.d.	11.3	21.3	10

(a) results obtained using new methodology

(b) results obtained using standard methodology; n.d. = not detectable

[1] = Dichlorobenzene; [2] = Trichlorobenzene; [3] = Tetrachlorobenzene

[4] = Pentachlorobenzene; [5] = Hexachlorobenzene

TABLE 3(a)

COMPARISON BETWEEN RESULTS DERIVED FROM PRESENTED METHODOLOGY AND STANDARD METHOD

Compound	1 (a)	(b)	2 (a)	(b)	3 (a)	(b)	4 (a)	(b)
	(ng/g)		(ng/g)		(ng/g)		(ng/g)	
1,3-DiClB[1]	n.d.	n.d.	5.7	n.d.	3.7	n.d.	n.d.	n.d.
1,4-DiClB	11.2	6.9	14.9	6.1	7.8	n.d.	11.8	n.d.
1,2-DiClB[1]	4.6	2.4	12.5	0.8	n.d.	n.d.	81.0	67.8
1,3,5-TriClB[2]	1.5	0.7	3.0	n.d.	n.d.	n.d.	0.9	n.d.
1,2,4-TriClB	3.0	3.2	9.2	8.7	n.d.	n.d.	1.7	1.6
1,2,3-TriClB	16.9	n.d.	20.6	2.2	11.2	n.d.	1.0	n.d.
HexClCycPeDi[3]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.24	n.d.
1,2,3-4-Tetr[4]	4.2	5.4	14.6	19.0	0.2	n.d.	0.6	0.5
PentaClB[4]	5.9	5.2	5.0	4.4	n.d.	n.d.	0.7	0.6
HCB[5]	34.2	22.9	4.4	2.6	n.d.	n.d.	2.7	2.0
OctaClStyre[6]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	1.7	n.d.
p,p'-DDT	n.d.	n.d.	n.d.	n.d.	2.0	n.d.	2.1	n.d.
p,p'-DDE	n.d.	n.d.	2.9	3.2	5.6	6.6	4.8	4.7
Photomirex	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	0.5	n.d.
Mirex	0.6	n.d.	0.4	n.d.	n.d.	n.d.	1.9	2.1

(a) results obtained using new methodology

(b) results obtained using standard methodology; n.d. = not detectable

[1] = Dichlorobenzene; [2] = Trichlorobenzene; [3] = Tetrachlorobenzene

[4] = Pentachlorobenzene; [5] = Hexachlorobenzene

TABLE 3(b)

POLYCHLORINATED BIPHENYLS
Number of Contributing Congeners and Concentrations

	1		2		3		4	
	(a) (ng/g)	(b)	(a) (ng/g)	(b)	(a) (ng/g)	(b)	(a) (ng/g)	(b)
Total PCB	532.7	444.5	73.8	41.4	24.4	17.7	33.9	63.9
Mono-Subst.								
# Contrib.	0		0		0		0	
Conc.								
Di-Subst.								
# Contrib.	1		0		0		0	
Conc.	1.0							
Tri-Subst.								
# Contrib.	6		4		1		0	
Conc.	91.7		12.4		6.8			
Tetra-Subst.	10		9		5		4	
# Contrib.	205.0		24.0		6.1		12.0	
Penta-Subst.								
# Contrib.	13		9		6		7	
Conc.	194.5		29.3		8.6		19.0	
Hexa-Subst.								
# Contrib.	8		5		3		2	
Conc.	34.8		5.0		2.3		2.9	
Hepta-Subst.								
# Contrib.	5		3		2		0	
Conc.	4.3		2.0		0.5			
Octa-Subst.								
# Contrib.	2		0		0		0	
Conc.	1.4							
Nona-Sutst.								
# Contrib.	0		0		0		0	
Conc.								

(a) results obtained using new methodology
(b) results obtained using standard methodology;

LIST OF FIGURES

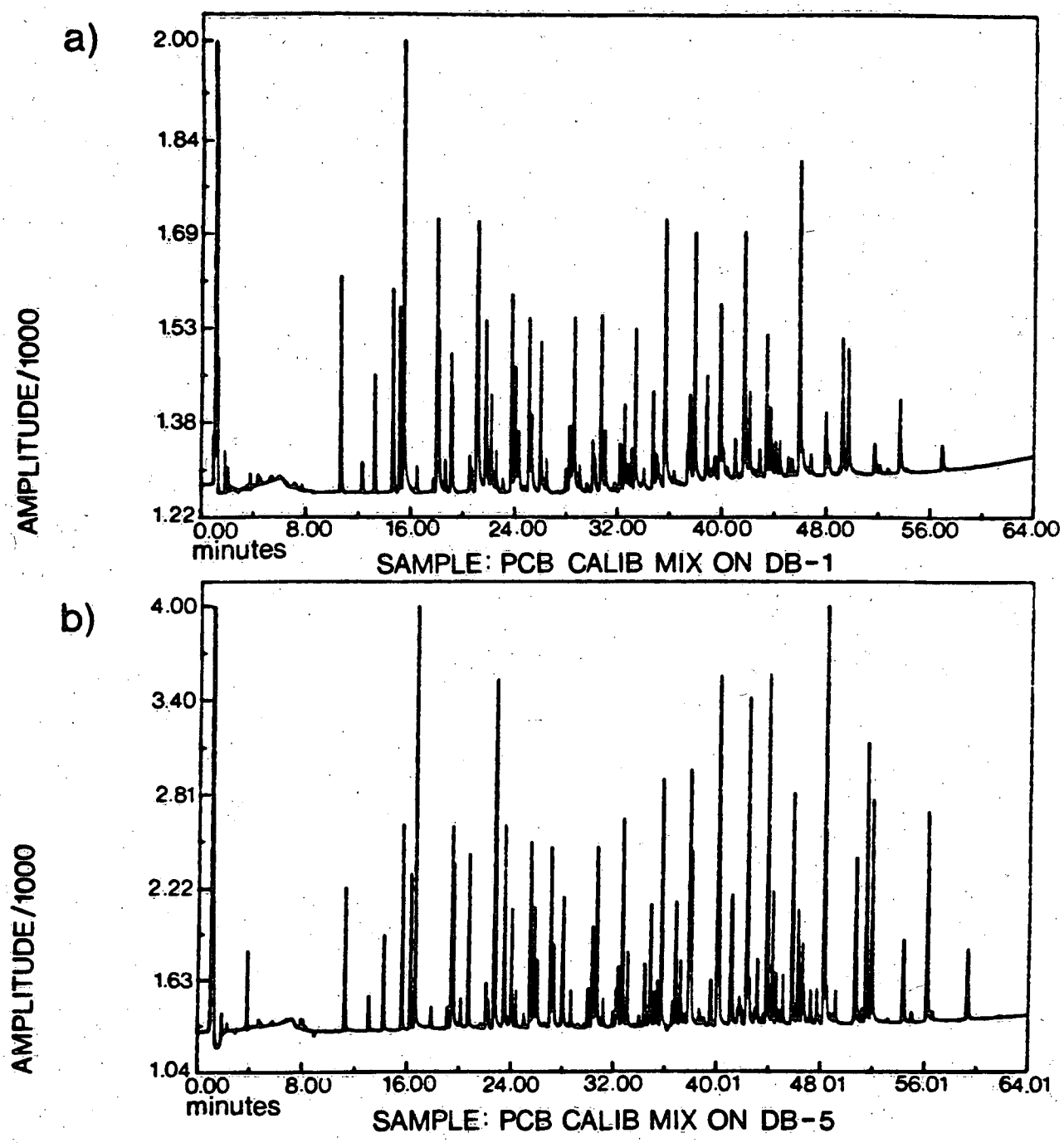

FIGURE 1. Dual Column Chromatograms of Calibration Mixture