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METHOD FOR THE SEPARATION OF ORGANOCHLORINE RESIDUES BEFORE GAS–LIQUID CHROMATOGRAPHIC ANALYSIS

DAVID E. WELLS and SAMUEL J. JOHNSTONE
Freshwater Fisheries Laboratory, Pitlochry (Great Britain)
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SUMMARY

The adsorption characteristics of alumina and silica for column chromatography have been assessed to develop a method, using a single solvent, for the separation of seventeen organochlorine residues into four eluates prior to gas–liquid chromatographic analysis. The effects on the adsorbents of thermal activation and subsequent deactivation with water, variation of column size and choice of eluents have been critically examined. The lipid capacity of the alumina and the effect of co-extracted materials from animal tissue upon the elution profile of the organochlorines have been determined.

INTRODUCTION

A number of methods have been described[1–5] using adsorption chromatography for the preparation of extracts of chlorinated hydrocarbon residues from environmental samples for gas–liquid chromatographic (GLC) analysis with electron capture detection (ECD). Morley[6] reviewed a number of high surface energy adsorbents used for the clean-up of pesticide residues and Moats[1] compared silicic acid, alumina, decolourizing carbon and Florisil for the clean-up of butter fats. He found little success with the first three and required a 5% deactivation with water on Florisil to elute dieldrin with methylene chloride. Florisil has been widely used, but due to the variability observed between batches[7] some workers have preferred to use alumina, which has a higher adsorption coefficient, particularly for lipids. Law and Goerlitz[3] found that Florisil did not clean-up river water extracts as well as alumina and Leoni[4] used a silicic acid column, deactivated to 5% with water to clean-up similar types of aqueous extracts.

The method used in this laboratory, to date, has been based on the twin column clean-up and separation techniques developed by Holden and Marsden[2] and has been extended to analyse organochlorine residues in rain, river and sea water, plankton and algae, as well as fish tissue. The method employs 2 g alumina columns (deactivated with 5% water) and *n*-hexane as the eluent; the alumina retaining the co-extracted lipid material and many of the unwanted contaminants. The constituent organochlorines are then separated into two groups using a 2-g silica column (deactivated

with 5% water). The first group, which includes polychlorinated biphenyls (PCBs) and *p,p'*-DDE, is eluted with *n*-hexane, followed by 10% diethyl ether in *n*-hexane to remove *p,p'*-DDT and the more polar organochlorines.

Although this technique has been satisfactory for much of the routine analysis of organochlorine residues in fish tissue, a number of disadvantages have become apparent during the analysis of other types of sample. Many samples (*e.g.*, rain, river waters and sediments), even after concentration of the solvent extract, contain concentrations of organochlorines at or near the minimum detection limit of the instrumentation. The analysis, therefore, demands a stable baseline, free from background interference derived from the solvents, adsorbents and contaminated glassware. To a large extent this was obtained by scrupulous cleaning, but it was often difficult to remove the background interference from the 10% diethyl ether–hexane eluate. Solvents were only used when their GLC chromatograms were free of interfering peaks after a one hundred-fold concentration by evaporation, but impurities were often extracted from the silica using this solvent mixture, even after the silicic acid had been fired at 600° prior to deactivation. These impurities were particularly obtrusive in the determination of hexachlorocyclohexane (HCH) isomers, which appear early in the GLC chromatogram, and their elimination was necessary to avoid misinterpretation of the results.

Some commercial DDT formulations contain up to 20–30% of the *o,p'* isomer which may therefore subsequently appear in natural samples. To quantify the DDT isomers in these samples a complete separation from PCBs into another fraction was required. The technique of Holden and Marsden[2] divides the *o,p'*-DDT approximately 1:1 between the two silica eluates, part being included with the PCB fraction, while the remainder is included in the second eluate with *p,p'*-DDT, *p,p*-DDD and dieldrin. Ideally the *o,p'*-DDT also requires isolation from *p,p'*-DDD and dieldrin, as this separation would not only simplify the total DDT analysis but also avoid the possibility of confusion with other residues, *e.g.*, endrin, chlordane.

The technique of using alumina and silica micro-columns has been developed to enable seventeen organochlorine residues to be separated into four eluates using a single solvent, *n*-hexane. The compounds have been separated in such a manner that all residues in each eluate (with the exception of *p,p'*-DDE and the PCBs) can be completely resolved on a single GLC column. The reliability and reproducibility of the adsorbents chosen have been critically examined for use with standard solutions and extracts from natural samples of varying origins and organic content.

EXPERIMENTAL

Borosilicate glass micro-columns 450 × 6 mm I.D. were used, with a taper at the lower end and a solvent reservoir at the top. Hexane-washed cotton wool balls were used to plug the tapered end and support the column packing. Alumina powder, initially BDH (Poole, Great Britain) No. 27076 and subsequently Reeve Angel (Clifton, N.J., U.S.A.) type A11-O-col, was activated at 800° for 4 h, cooled, and deactivated to the desired level with hexane-washed distilled water. The Reeve Angel A11-O-col alumina (Reeve Angel, Clifton, N.J., U.S.A.) was sieved to remove the fines and agglomerates and the particle size band 64–125 μm was retained (85% of the total material). The silica Merck No. 7734 70-325 (ASTM) (Merck, Darmstadt, G.F.R.)

was activated at 600° for 4 h, cooled to 150°, placed in a vacuum desiccator and cooled to room temperature under reduced pressure. Each complete batch of the activated material (400 g for alumina and 200 g for silica) was deactivated to the required level immediately by shaking with distilled water. Both deactivated materials were stored in stoppered flasks. Sodium sulphate (AR), used as a drying agent, was heated to 200° for 4 h, cooled and stored in a glass-stoppered bottle. High purity glass-distilled *n*-hexane and acetone (Rathburn, Walkerburn, Great Britain) were used for rinsing the glassware, and as solvents. A 100-ml aliquot of *n*-hexane from each batch was concentrated to 1 ml and examined for any background interference.

The adsorption columns were freshly prepared for each sample. Each empty column was rinsed with acetone followed by hexane and allowed to drain and air-dry. The column packing was measured out by volume using a calibrated tube, poured into the column and packed by tapping the sides of the column. A small charge (200 mg) of anhydrous sodium sulphate was placed at the column head to ensure complete dryness of the sample before passing to the adsorbent. The sample or standard, contained in 1 ml of *n*-hexane, was pipetted on column and allowed to drain into the column before the sample rinsings and the hexane charge were added to the reservoir. The appropriate eluate volumes were collected in graduated tubes and evaporated to 1 ml, either for analysis by GLC or for further separation. The final eluates were injected into a Varian 1400 single column chromatograph fitted with a tritium ECD instrument. The GLC glass column was 1525 × 2 mm I.D., containing 4% SE-30 + 6% OV-210 on Chromosorb W HP (80–100 mesh). Nitrogen was used as a carrier gas at a flow-rate of 30–35 ml/min. The column and injector temperatures were 200° and the detector temperature was 220°. Identification was based on relative retention times using dieldrin as a reference, and samples were quantified by comparing the peak heights with those from standard pesticide solutions. These standards were injected at similar times to the sample to minimise errors in sensitivity caused by fluctuations of the instrument.

RESULTS

Adsorbent activation

An investigation of the effect of thermal treatment on the purity and the final activity of both alumina and silica used as adsorption column packing confirmed that a high temperature firing at 600–800°, as outlined previously, was necessary to minimise the trace impurities. The high purity obtained by thermal treatment outweighed the inherent loss of activity, particularly of the alumina, due to firing.

Column size

The diameter and length of the micro-columns were varied to improve the chromatographic efficiency of the packing. However the wider columns (8 and 10 mm) gave much poorer resolution while longer columns of narrower bore (4–5 mm) increased the residence time by restricting the solvent flow, without any significant gain in separation. The 6 mm I.D. column was therefore chosen for the remaining experiments.

Silica columns

The original 2-g columns of silica, deactivated with 5% water did not completely separate *o,p'*-DDT from the PCBs and *p,p'*-DDE, but this was improved by increasing the column size from 2 to 2.5 g and by continuing to elute with *n*-hexane until all the *o,p'*-DDT and *p,p'*-DDT were removed. However, it was still necessary to remove the more polar species from the column with 10% diethyl ether in *n*-hexane.

The optimum activity of the silica column for the *o,p'*-DDT–*p,p'*-DDE separation was found by varying the water content from 0 to 7% and monitoring the effect upon the elution pattern (Table I). The results show that the best separation was obtained when 3% water was added to the silica. Further additions of water to 5% and 7% speeded up the elution, but the resolution of the column tended to decrease. The active silica, being strongly hygroscopic, readily altered its activity upon exposure to moist air and was found unsuitable for use, particularly after long-term storage. The silica deactivated with 3% water offered the best resolution and this was chosen for inclusion in the present scheme. Although there is an overlap of *p,p'*-DDE (62%) and *o,p'*-DDT (17%) at the 6-ml elution volume, the separation was completed by changing the collection tube at a more appropriate point druing the elution, *e.g.* at 5.5 ml, giving less than 8% of *o,p'*-DDT in the first eluate.

TABLE I

EFFECT OF WATER CONTENT ON THE SEPARATION CHARACTERISTICS OF SILICA

Silica (2.5 g; Merck No. 7734) activated at 600° for 4 h; eluent, *n*-hexane; column, 6 mm I.D. glass.

Elution (% water)	*Pesticide*	*Recovery (%)*				
		Volume (ml)				
		1–4	*5*	*6*	*7*	*8–13*
0	*p,p'*-DDE	—	3	14	45	38
	o,p'-DDT	—	—	—	2	98
	p,p'-DDT	—	—	—	—	100
2	*p,p'*-DDE	—	52	41	7	—
	o,p'-DDT	—	—	30	26	44
	p,p'-DDT	—	—	—	5	95
3	*p,p'*-DDE	—	38	62	—	—
	o,p'-DDT	—	—	17	25	58
	p,p'-DDT	—	—	—	1	99
5	*p,p'*-DDE	50	50	—	—	—
	o,p'-DDT	—	43	34	20	3
	p,p'-DDT	—	—	10	51	39
7	*p,p'*-DDE	55	43	2	—	—
	o,p'-DDT	—	21	57	22	—
	p,p'-DDT	—	—	8	48	44

The reproducibility of this separation was strongly dependent upon the standardization of the activation and subsequent deactivation of the adsorbent. Each batch, initially 100 g, remained stable throughout its shelf-life (4–5 weeks) giving reproducible chromatographic results. However, it was found that the elution volume

and the degree of separation varied from batch to batch and occasionally, using the previous method of Holden and Marsden[2], a 2-g silica column, deactivated to 5% with water, did not successfully separate *p,p'*-DDE and *p,p'*-DDT. Prior to this study the silica (500 g) was activated and stored in a vacuum desiccator, and working quantities (100 g) were subsequently deactivated and tested when required. Consequently the moisture content of the active adsorbent was dependent upon the number of times it was removed from the desiccator and exposed to the atmosphere, and the effectiveness of the vacuum seal on the desiccator. It was found that the failure of the deactivated material in separating the pesticide residues was related to the age of the active silica. A comparison of the separation obtained from freshly activated silica and active silica, three months old, both deactivated with 3% water, is given in Table II. When this older active silica had been freshly made and tested it had given acceptable results for the separation of *p,p'*-DDE and *o,p'*-DDT. However after three months had elapsed it gave poor resolution, indicating the limited shelf-life of the active material. Reliable and reproducible results were obtained from silica if the material was deactivated immediately after cooling from 600°. Each batch once deactivated was tested and the chromatographic characteristics were found to remain constant throughout the lifetime of the batch.

TABLE II

EFFECT OF AGE OF ACTIVE SILICA ON THE CHROMATOGRAPHIC PROPERTIES AFTER DEACTIVATION

Silica (2.5 g; Merck No. 7734) deactivated with 3% water; eluent, *n*-hexane; column, 6 mm I.D. glass. A, column prepared from activated silica, 3 months old; B, column prepared from activated silica fired the same day.

Elution volume (ml)	*Recovery (%)*					
	A			*B*		
	p,p'-DDE	*o,p'-DDT*	*p,p'-DDT*	*p,p'-DDE*	*o,p'-DDT*	*p,p'-DDT*
1–5	82	3	—	73	4	—
5–13	18	97	100	27	96	100
1–5.5	83	25	—	96	6	—
5.5–13	17	75	100	4	94	100
1–6	86	36	—	97	35	—
6–13	14	64	100	3	65	100

Although the silica columns were able to separate the early eluting compounds by using *n*-hexane, it was still necessary to add a more polar solvent to remove *p,p'*-DDD, dieldrin and the HCH isomers. The purity of the mixed solvent after eluting from the silica column was frequently checked and found unacceptable due to extracted impurities from the silica. Prewashing the packed columns had previously been investigated, but the process was both time-consuming and tended to give erratic results. Two other alternatives were considered, namely a different solvent of similar polarity (dielectric constant) or removal of the more polar compounds before the silica

column stage. The latter appeared to be a more promising alternative, utilizing the alumina column to effect the separation.

Alumina columns

Most extracts from environmental samples require the removal of unwanted co-extracted materials before being introduced into a GLC or gas chromatography–mass spectrometry (GC–MS) system, and alumina has been successfully used for this purpose. Although it is more absorbent than silica, the alumina is less polar at similar water-content levels, and only requires *n*-hexane as a solvent to elute the organochlorine residues of current interest from the column. By careful manipulation of the column parameters a division at a suitable point during the elution was considered possible, to separate the more polar compounds which have a larger retention volume on the silica column. The most useful division of the alumina column eluate falls between *p,p'*-DDT and *p,p'*-DDD. The PCBs and *p,p'*-DDE are eluted prior to the *p,p'*-DDT and dieldrin is removed long after *p,p'*-DDD.

The original 2 g alumina, deactivated to 5% with water, used for clean-up did not achieve this, but the results from work by Holden and Marsen[2] gave every indication that it could be done by changing the column conditions. The resolution was much improved by decreasing the water content of the alumina from 5 to 3% and by increasing the column loading from 2 to 3 g. However, using the BDH aluminium hydroxide as a starting material, the elution time to remove dieldrin from the column was increased from 3 to 5 h. This extension, although not an analytical problem, did offset the advantages gained in column performance for a routine method.

Another grade of alumina (Reeve Angel alumina A11-O-col) gave a superior performance with a reduced elution time. This material was sieved and the particle size band 64–125 μm retained. The elution time for dieldrin using a 3-g column was reduced from 5 to 1.2 h, which was a 50% reduction in time over the original columns.

TABLE III

SEPARATION OF ORGANOCHLORINES ON ALUMINA

3 g Al_2O_3 (A11-O-col) 64–125 μm activated at 800° for 6 h and deactivated with 3% water.

Elution volume (ml)	*Recovery (%)*							
	p,p'-DDE	*o,p'-DDT*	*p,p'-DDT*	*p,p'-DDD*	*α-HCH*	*γ-HCH*	*Dieldrin*	*β-HCH*
1								
2	74	44						
3	26	56	46					
4			54					
5					9			
6					54			
7–12				100	37	100		
13–17								
18–22							82	
23–27							18	
28–32								
33–37								
38–42								66
43–47								34

TABLE IV

ELUTION PATTERN OF SEVENTEEN ORGANOCHLORINE RESIDUES ON ALUMINA AND SILICA COLUMNS

3 g Al_2O_3 (A11-O-col) containing 4% water; 2.5 g SiO_2 (Merck No. 7734) containing 3% water. Eluent, *n*-hexane; columns, 6 mm I.D. glass.

Elution volume (ml)	*HCB*	*PCB (1254)*	*Aldrin*	*Hepta-chlor*	*p,p'-DDE*	*o,p'-DDT*	*p,p'-DDT*	*α-HCH*	*γ-HCH*	*γ-Chlor-dane*	*p,p'-DDD*	*Endo-sulfan I*	*Hepta-chlor epoxide*	*Endrin*	*Dieldrin*	*p,p'-DCBP*	*β-HCH*
*Silica**																	
1–3	100																
4		30	9														
5		54	48		32												
6		15	37	14	57												
7			6	60	12	59											
8–14				26		41	100										
Alumina																	
5								9		18							
6								56		57							
7								30	4	23	26						
8								5	28	2	49						
9									56		23	3					
10									12		2	33					
11												38					
12												22	2	4			
13												4	16	21			
14													32	34			
15													33	29			
16–17													17	12	3		
18–19															39		
20–21															52		
22–23															6		
24–25																	
26–30																3	53
31–35																92	47
36–40																5	
41–45																	

* First 4 ml from alumina column chromatographed on silica (see Fig. 1).

The separation between *p,p'*-DDT and *p,p'*-DDD was increased from 0.5 to 3–4 ml in elution volume and *α*- and *β*-HCH were also isolated from the early eluting peaks. However a considerable increase in the elution volume (over 50 ml) was necessary to remove *β*-HCH from the column (Table III). Reduction of the activity of the Reeve Angel alumina by increasing the water content from 3 to 4% decreased the total elution volume without a loss in resolution between *p,p'*-DDT and *p,p'*-DDD. This gave an extremely useful separation of a number of organochlorine residues when used in conjunction with the silica columns, as described earlier (Table IV).

With the modified alumina column the collection tubes were exchanged after the elution of *p,p'*-DDD. This fulfilled a dual role by isolating *α*- and *γ*-HCH from *β*-HCH in particular, and by separating the later eluting compounds into two groups to assist in the quantitative analysis.

Lipid content of extracts

The elution profile and recovery of organochlorine residues on alumina has been observed to alter with increasing lipid content of the sample[5], and it was necessary to determine the effect of other co-extracted materials upon the performance of the columns which had been developed using standard solutions. A series of solutions were made containing a fixed concentration of organochlorines with a range of concentrations of cod liver oil. One milliliter of each soluiton was chromatographed on a 3-g Reeve Angel alumina column, deactivated to 4% with water, and separated using

TABLE V

EFFECT OF LIPID CONTENT ON THE RECOVERY OF ORGANOCHLORINES FROM ALUMINA

3 g Al_2O_3 (A11-O-col) 64–125 *μ*m activated at 800° for 6 h and deactivated with 4% water. Eluent, *n*-hexane; column, 6 mm I.D. glass.

Lipid content (mg)	*Elution volume (ml)*	*Recovery (%)*							
		p,p'-DDE	*o,p'-DDT*	*p,p'-DDT*	*p,p'-DDD*	*α-HCH*	*γ-HCH*	*Dieldrin*	*β-HCH*
0	1–4	112	100	108					
	5–12				110	104	90		
	13–35							98	86
21	1–4	100	94	101					
	5–12				109	104	99		
	13–35							104	87
38	1–4	104	110	112					
	5–12				109	106	105	28	
	13–35							82	87
61	1–4	104	98	104					
	5–12				104	96	94	87	
	13–35							24	103
89	1–4	92	—	112	*	21	—		
	5–12		—		77	**	—	73	**
	13–35		—						38
178	1–4	84	—	103	*	81			
	5–12		—			**		37	**
	13–35		—						16

* *p,p'*-DDD found in 1–4 ml masked by PCBs present in the oil.

** *γ*-HCH and *β*-HCH overlapped in the same eluate.

the elution columes found most suitable for the lipid-free standards. The background values obtained for the organochlorines in the original oil were subtracted to obtain the percentage recovery values of the added compounds (Table V). At no or low lipid content the separations and recoveries are quantitative and reproducible, except for β-HCH which tends to be retained on the column. As the lipid content is increased the elution profile is gradually compressed, decreasing the elution volume of the more polar materials. This compression did not initially affect the early sections of the profile, but with further additions of fat (*ca.* 90 mg) *p,p'*-DDD and γ-HCH began to emerge along with *p,p'*-DDE and *p,p'*-DDT. At this loading and at higher lipid values the recoveries of the residues became variable and unreliable. These variations in column performance with lipid content do not preclude the use of the method for the analysis of animal tissue extracts provided that the weight of lipid transferred to the column is controlled, the maximum acceptable lipid loading being 60 mg for a 3-g column. This lipid limit was easily maintained by analysing for the extractable residues prior to the organochlorine residue analysis. For an extract of high lipid, but suspected low organochlorine content a small 1-g alumina column was used prior to the main 3-g separation column. The Reeve Angel alumina has a lipid-holding capacity of approximately 50 mg/g. This was determined by loading a series of 1-g alumina columns with a range of weights of lipid and measuring the amount eluted by 40 ml of hexane (Table VI).

TABLE VI

LIPID HOLDING CAPACITY OF ALUMINA

1 g Al_2O_3 (A11-O-col) 64–125 μm activated at 800° for 6 h and deactivated with 4% water. Eluent, 40 ml *n*-hexane.

Lipid	*Weight of lipid (mg)*			
On column	52.5	101.6	145.1	213.0
Recovered	3.7	51.6	102.7	165.8
Retained	48.8	50.0	42.7	47.2

This 1-g column could therefore be used to reduce the fat content in the sample prior to separation on the main 3-g alumina column. A volume of 15 ml of *n*-hexane was required to elute a sample of β-HCH from a 1-g alumina column, deactivated to 4% with water.

The partial elution of dieldrin in the second eluate caused by the presence of more than 20 mg lipid was avoided by dividing the eluting *n*-hexane at 10 ml. This was found suitable for non-lipid samples as well as tissue extracts. The standards used for testing each batch of deactivated alumina were in hexane solution, but standardization can be improved by the addition of 50 mg/ml lipid.

The separation procedure

The final form of the separation procedure is given in Fig. 1. This flow diagram outlines the complete method and lists the constituent compounds in each eluate. The chromatogram obtained from the separation of the listed organochlorines (Table IV) is given in Fig. 2a–d and can be compared to the traces obtained by the separation with silica alone, Fig. 2e. The compounds which are currently of particular interest in

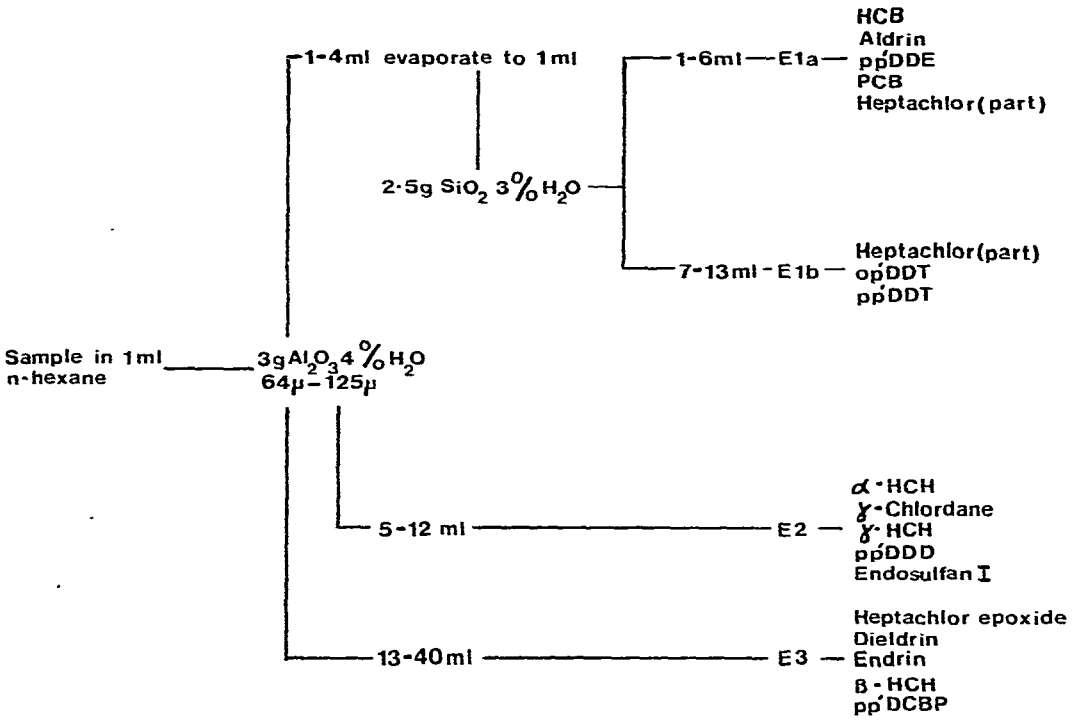

Fig. 1. Flow diagram for the separation of seventeen organochlorine residues using alumina and silica microcolumns and *n*-hexane as the eluent.

environmental samples are clearly separated into four eluates. Heptachlor which is rarely detected in United Kingdom samples, is split between two eluates, but should it occur its presence would not be masked in GLC analysis by other organochlorines included here.

DISCUSSION

This new technique (Fig. 1), incorporating the dry packed alumina and silica columns, has significantly shortened the time required for analysis and improved the identification and quantification of organochlorine residues in natural samples. The time spent on clean-up and separation has been decreased by 50%, giving an overall processing time of approximately 4 h prior to GLC analysis. While the later eluates (5–40 ml) are developing on the alumina column the first alumina eluate can be separated on silica. Both alumina and silica can be reliably deactivated from freshly activated materials in quantities of up to 500 g and will retain their activity for at least three months.

The preparation of the columns and the measurement of the elution volumes were found to be quite critical, particularly at the silica column stage. Although the silica performance was acceptably reproducible, the amount of *p,p'*-DDE which was found in the second (E1b) eluate varied from 2–10%, and the level of *o,p'*-DDT found in the first elutae was occasionally as much as 8% but generally less than 5%. This

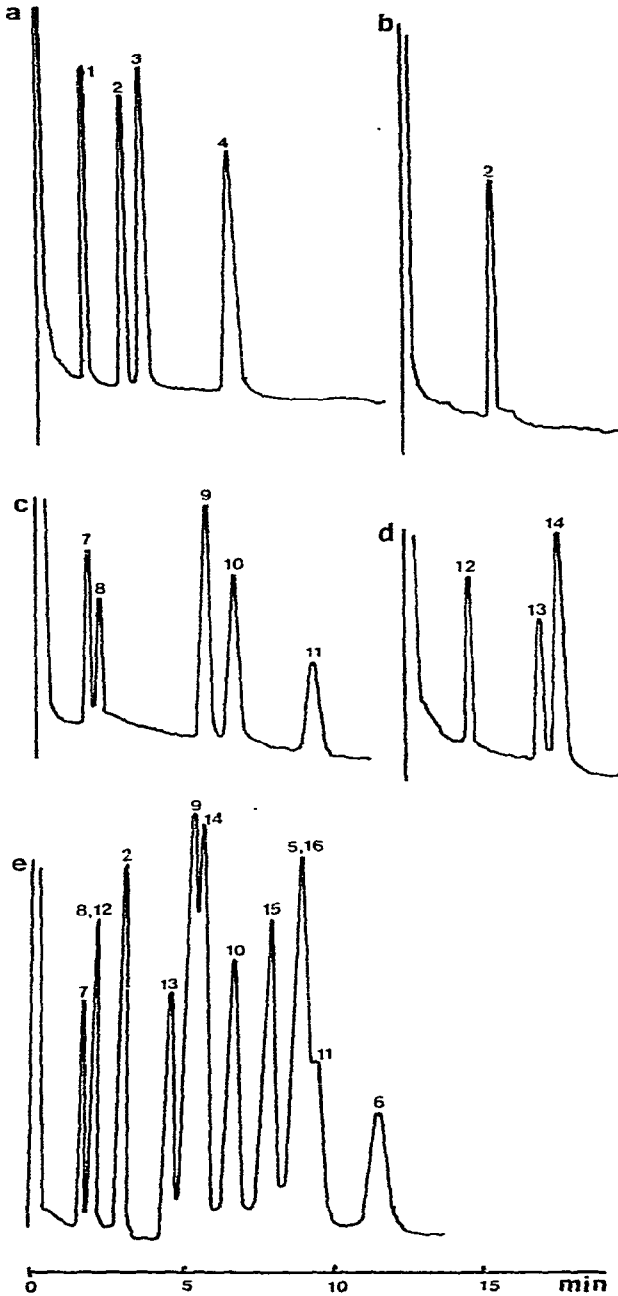

Fig. 2. Chromatograms obtained from (a–d) the eluates using the current method, and (e) the method of Holden and Marsden[2]. Column, 4% SE-30 + 6% OV-210 on Chromosorb W HP; temperature, 200°, nitrogen carrier gas, 35 ml/min. (a) Eluate 1a; 1 = HCB, 2 = heptachlor, 3 = aldrin, 4 = *p,p'*-DDE. (b) Eluate 1b; 2 = heptachlor, 5 = *o,p'*-DDT, 6 = *p,p'*-DDT. (c) Eluate 2; 7 = α-HCH, 8 = γ-HCH, 9 = γ-chlordane, 10 = endosulfan I, 11 = *p,p'*-DDD. (d) Eluate 3; 12 = β-HCH, 13 = *p,p'*-DCBP, 14 = heptachlor epoxide, 15 = dieldrin, 16 = endrin. (e) Eluate 2 (Holden and Marsden[2]) which incorporates eluates 1b, 2 and 3 of the new method.

variation reflected the difficulty in packing a column with identical chromatographic characteristics and in measuring small volumes of volatile solvent accurately.

The identification and quantification of *o,p'*-DDT and HCH isomers have been simplified by altering the characteristics of the silica, and by removing the more polar compounds at the alumina stage. This avoids the use of 10% diethyl ether in *n*-hexane on the silica columns. The separation of the HCH isomers into two eluates (E2 for α- and γ-HCH, E3 for β-HCH) on alumina also assists in their identification.

The four eluates obtained in the preparation of each sample increase the overall chromatographic time, but this is not without justification. As well as improving the separation of the more common organochloriens present it reduces the possibility of confusion with unknown substances. The method has been successfully applied to the analysis of a considerable variety of environmental samples. The analysis of animal tissue extracts is only limited by the amount of lipid which can be applied to the alumina columns, but reproducible results can be obtained if this does not exceed 60 mg.

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