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Note

Separation of organochlorine residues by adsorption chromatography prior to capillary gas chromatography

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The earlier methods developed by Holden and Marsden[1] and Wells and Johnstone[2] for the clean-up and separation of organochlorine residues (OCs) in tissue, sediment and water extracts using twin columns of alumina and silica have been further exploited to include a wider range of contaminants. Initially this sample preparation scheme was used to remove the lipid and co-extracts which interfered with the analysis by gas chromatography (GC)–electron capture detection (ECD). Although much of this clean-up is now possible using gel permeation chromatography (GPC)[3,4] the separation scheme, as outlined in this paper, can serve as group-separation low-resolution chromatography to clarify and aid the final resolution and identification by capillary GC with ECD and/or mass spectrometry. The scheme and the list of determinands are by no means exhaustive, but it has now been expanded to include a wider range of important groups of contaminants which are determined by a number of workers using our original methods, and this paper should serve as an update.

The elution scheme on alumina and silica columns separates some major isomer mixtures, *e.g.*, polychlorinated biphenyls (PCBs) and toxaphene from each other and from other groups *e.g.*, DDT, "Drins", chlordanes, the neutral OCs, some basic and acidic compounds (*e.g.*, chlorophenols and chlorosulphonamido diphenyl ethers)[5].

EXPERIMENTAL

Apparatus

The alumina and silica columns were 200 × 6 mm I.D. borosilicate glass columns with a taper at the lower end and a solvent reservoir at the top.

Reagents

n-Hexane, glass-distilled grade was obtained from Rathburn (Walkerburn, U.K.). Solutions of diethyl ether (1% and 30%) in *n*-hexane, and of acetic acid (15%) in *n*-hexane were prepared.

Basic alumina was obtained from BDH (Merck 1097) it was heated to 200°C for 2 h and deactivated to 4% (v/w) water (Brockmann activity II-III) with hexane-washed distilled water, and then stored in a stoppered bottle. Acidic alumina

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was prepared from the basic alumina by washing it with 1 *M* hydrochloric acid, filtering the slurry, drying at 200°C for 4 h, cooling and deactivating to 4% water with hexane-washed distilled water.

Merck 7734 silica was used, activated at 600°C for 4 h; deactivated to 3% water with hexane-washed distilled water; stored in a stoppered bottle. Sodium sulphate, anhydrous, granular, analytical grade, was dried at 200°C for 4 h. Cotton wool was rolled into 5-mm balls, washed in hexane and air dried.

Organochlorine pesticides were obtained from the U.S. Environmental Protection Agency, Research Triangle Park, NC, U.S.A. PCB isomers were obtained from Chromatography Services (UK). Chloroanilides and chlorosulphonamides were obtained by purification of industrial mixtures[5,6].

All other compounds were obtained from Aldrich (UK).

RESULTS AND DISCUSSION

All glassware used was cleaned overnight in Haemo-sol at the recommended strength at 60°C. It was rinsed with distilled water and dried in a hot-air dryer. The glassware was rinsed with acetone and hexane before use. The continuous assessment of the blanks during analysis has indicated that this is a very satisfactory method of cleaning.

The adsorption columns were freshly prepared for each sample. Each empty column was rinsed with acetone followed by *n*-hexane and allowed to drain and air dry. They were then plugged at the tapered lower end with a cotton-wool ball which was transfered to the column with tweezers and tamped in position with a clean glass rod. The alumina columns were filled with acidic alumina (2 g), measured by volume using a calibrated cup, and settled by tapping the column side. This was followed by basic alumina (1 g) and sodium sulphate (200 mg). The silica columns were prepared in a similar manner with silica (3 g) and sodium sulphate (200 mg).

The types of sample which have been cleaned-up and separated on these columns are extracts of water, sewage sludge, final effluent, sediment, fish and sea mammal tissue. Before the sample extract could be prepared by this scheme it was necessary to determine the lipid/wax content of the extract by evaporation of a known volume and determining the residue by weight, and to limit the weight of the extract to 50 mg per column. This limit prevented the column from being overloaded with fat and reducing the resolution and adsorption capacity of the alumina[2]. If a larger amount of sample was required for analysis it was possible to remove the bulk of waxes/lipids using either GPC[3,4] or by using the mixed alumina column without any group separation. Overloading the column with excessive amounts of fat produces a "concertina" effect[2] on the elution pattern of the determinands. In this situation the separation on the alumina columns would need to be repeated, once the level of fat was below 50 mg/ml, to obtain the necessary degree of separation reported here.

The sample was pipetted on to the head of the alumina column and allowed to soak into the packing. Sample rinsings were added to obtain a quantitative transfer. When this had travelled into the column the solvent reservoir was filled with *n*-hexane (45 ml). The anhydrous sodium sulphate was present to remove any remaining moisture from the sample and preserve the activity of the column.

Any compounds with a labile proton, such as phenol, sulphonamides and acids

were adsorbed on to the basic alumina in the top third of the column, while the base-neutrals were eluted. Each eluate was collected in a glass tube of the appropriate size according to the scheme shown in Fig. 1. The contents of each eluate determined so far is given in Table I. The volumes declared in this scheme serve only as an indication of the amount of solvent required for each eluate. The definitive values were always determined for each batch of adsorbent using a calibration mixture and the calibration was valid for each batch provided that the adsorbent was kept in a screw-top container. The basic and acidic alumina were prepared in 250- and 500-g lots, which was sufficient for *ca.* 250 samples. The test mixture contains α-HCH (hexachlorocyclohexane), γ-HCH, 4,4'-DDE, 4,4'-DDT. 4,4'-DDD at 0.2 mg/l and dieldrin at 0.1 mg/l. For calibration this solution was applied to the alumina column and the eluate was collected every ml for 7 ml and then each ml between 11 and 14 ml. The separation volume was selected where 4,4'-DDE and 4,4'-DDT (eluate 1) are split from α-HCH, γ-HCH and 4,4'-DDD (eluate 2), and from dieldrin (eluate 3). The other separations on alumina were not so critial and occurred at the volumes specified. The reproducibility of the separation from batch to batch has been found to be within 1–1.5 ml.

The first eluate (0–4 ml) contained all of the chlorobenzenes although only the tetra to hexchlorobenzene is determined in this scheme; the more volatile components

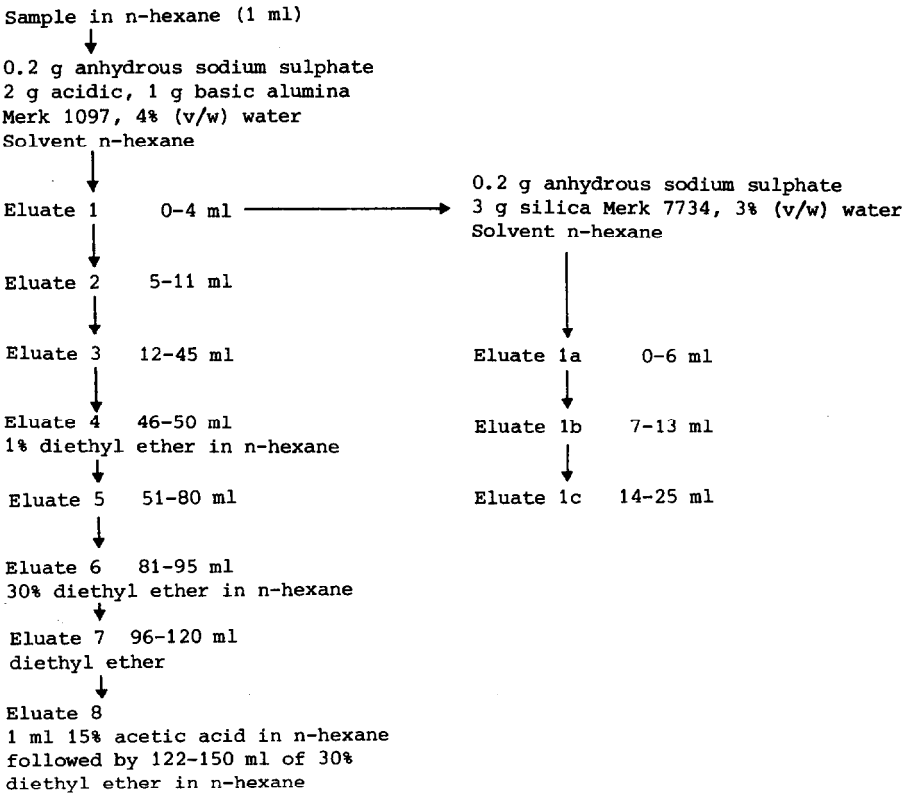

Fig. 1. Elution scheme.

TABLE I

COMPONENTS OF THE ELUATES

Eluate 1a	*Eluate 1b*	*Eluate 1c*
Chlorobenzenes including hexachlorobenzene	Heptachlor (50%)	Hexachlorophene
Polychlorinated biphenyls	α-Chlordene	Toxaphene
Heptachlor (50%)	γ-Chlordene	
Aldrin	2,4'-DDE (75%)	
2,4'-DDE (25%)	2,4'-DDT	
4,4'-DDE	4,4'-DDT	
Mirex		
Eluate 2	*Eluate 3*	
α-HCH	Heptachlor epoxide	
γ-HCH	Dieldrin	
α-Chlordane	Endrin	
γ-Chlordane	β-HCH	
Oxychlordane	4,4'-Dichlorobenzophenone	
Endosulfan I and II	Chloroaminodiphenyl ethers	
trans-Nonachlor	Pentachloroaniline	
2,4'-DDD	Tetrachloroaniline	
4,4'-DDD		
Eluate 5	*Eluate 6*	*Eluate 8*
cis-Permethrin	Trichloroaniline	Phenol
trans-Permethrin	Dichloroaniline	Chlorophenols
	Chlorotrifluoromethylaniline	Chlorosulphonamides

being more amenable to head space analysis. The PCBs are a major group in this eluate and are accompanied by the polychlorinated naphthalenes (PCNs) and polychlorinated terphenyls (PCTs); the latter two groups exist in very low concentrations in natural samples and tend to be dominated by the PCBs. This eluate also contained most of the DDT group (2,4'-DDD and 4,4'-DDD excepted), heptachlor, aldrin, chlordene, hexachlorophene and toxaphene. Such a complex eluate required further separation prior to analysis, even by capillary GC, and this was achieved using the silica column (see below).

The second eluate (5–11 ml) contained the α- and γ-isomers of HCH and chlordane, oxychlordane, endosulphan I and II, *trans*-nonachlor, 2,4'-DDD, and 4,4'-DDD. This is a relatively straightforward eluate and is usually not complicated by other co-extracted compounds in most environmental samples.

The third eluate (12–45 ml) contained heptachlor epoxide, dieldrin and endrin, β-HCH, 4,4'-dichlorobenzophenone, polychloroaminodiphenylethers (PADs), pentachloroaniline (PCA), and tetrachloroaniline (TCA). The inclusion of the PADs in this eluate, the permethrin in eluate 5, chlorotrifluoromethylaniline in eluate 6 and the chlorosulphonamido diphenyl ethers in eluate 8 was as a result of the development of a comprehensive method for the separation of the active ingredients of the main mothproofing agents used in the woollen textile industry in Scotland[6–8].

Eluate 4 (46–50 ml) was increased in polarity by the addition of 1% diethyl ether. This eluate contained no determinands in the current scheme, but was separated from the next fraction because some unknown interfering compounds, particu-

larly from sewage sludge extracts, co-eluted with permethrin in the following fraction: eluate 5 (51–80 ml). The larger volume (30 ml) and small increase in polarity from pure hexane (eluate 3) were required to remove the permethrin and separate it from the more polar moieties.

Eluate 6 (81–95 ml) used 30% diethyl ether in *n*-hexane to remove the trichloroaniline, dichloroaniline and chlorotrifluoromethylaniline from the alumina column. Separation of this particular group can only be successfully achieved if most of the less polar waxes and lipids have previously been removed from the extract using GPC.

The seventh eluate (96–120 ml) was 100% diethyl ether which purged the column of much of the remaining coextractants. This was discarded.

The basic alumina at the head of the column was acidified with 15% glacial acetic acid in *n*-hexane (1 ml). This volume was allowed to remain in contact with the basic alumina for 2–3 min to release all of the acidic components which had been adsorbed onto this upper section of column[5]. Finally, eluate 8 (121–150 ml), 30% diethyl ether in *n*-hexane, was collected. This contained all of the acidic fraction including the phenols, chlorophenols and chlorosulphonamides.

The first fraction from the alumina column was concentrated to 1 ml in a stream of dry air and pipetted onto the silica column. When this had travelled into the column bed the sample tube rinsings in *n*-hexane were added to the column and the reservoir charged with 25 ml of *n*-hexane. The elution scheme for the silica column is given in Fig. 1. As with the alumina column, the volumes of each eluate depended on the precise activity of the particular batch of silica. Each batch was prepared in 500-g lots and tested with a solution containing 4,4′-DDE (0.05 mg/l), 2,4′-DDT and 4,4′-DDT (0.1 mg/l). The correct volume for the eluate 1a was set between the elution of 4,4′-DDE and 2,4′-DDT; with the former being in eluate 1a and the latter in eluate 1b. The second split was not as critical and was set at 13 ml for the separation of the compounds listed in this scheme.

The eluate 1a now contained all of the chlorobenzenes, PCBs, aldrin, mirex and 4,4′-DDE. A small fraction of the 2,4′-DDE was split into this group along with 50% of the heptachlor. In any separation scheme, with this complexity, it is difficult to obtain all of the components and/or groups in discrete fractions and in this case two of the less abundant OCs were split between eluates 1a and 1b. (N.B. This refers to samples taken some distance from any local source of these pollutants.)

Eluate 1b contained the remaining (50%) heptachlor, α- and γ-chlordane, 2,4′-DDE (75%), 2,4′-DDT and 4,4′-DDT. These were effectively separated from the toxaphene fraction (>90%–95%) which was removed in the following eluate 1c, along with hexachlorophene. Such a clear separation between the PCBs, DDT group and the toxaphene, with a single solvent has greatly improved the identification specificity of these ubiquitous environmental pollutants. Like Tai *et al.*[9], we found that the activity and grade of silica required careful control, to obtain a good separation of PCBs and toxaphene. However, unlike them, we have used a dry packing technique and have only used *n*-hexane as an eluent.

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