Simultaneous Clean Up of Fish Fat Containing Low Levels of Residues and Separation of PCB from Chlorinated Pesticides by Thin-layer Chromatography

by MARJA LIISA HATTULA

Department of Chemistry
University of Jyväskylä
Jyväskylä, Finland

PCB, polychlorinated biphenyls, have been widely reported and they have been found to be a great environmental impact [NELSON et al. (1972)]. The contamination of different ecosystems has aroused the attention of ecologists and pesticide analysts and according to RISEBROUGH (1969) the contamination of marine ecosystems with persistent compounds which are nonpolar and therefore water-insoluble both makes questionable the use of the sea as a source of human food and causes hazards to wildlife especially to birds. The contamination of water ecosystems has resulted in monitoring programs in several countries [DUKE et al. (1970), GIAM et al. (1972), OTTERLIND et al. (1971), PORTMANN (1970), ZITCO (1971)].

In this work a method is described which was developed during a monitoring program of the fish of Finland containing low levels of residues, PCB > 0.05 ppm in average and total DDT (DDT + DDE + DDD) > 0.01 ppm in the wet weight of the tissue.

TLC has been used mainly for clean up before GLC separation [BAGLEY et al. (1970), FEHRINGER and WESTFALL (1971), LICHTENSTEIN et al. (1969), MULHERN et al. (1971), DE VOS and PEET (1971), WESTÖÖ and NORÉN (1970)] but also for the final determination [BUSH and FA-CHUN (1973)] . In most cases the clean up of the fat extract has been made by liquid-liquid partition chromatography or by column chromatography. If, however, the fat contents of the sample is small and the level of residues is low, losses may occur during the process and evaporation of great volumes of solvents makes the process inconvenient.

The purpose of the present work was to develope a method in which small amounts of fat containing low levels of residues could be treated with a minimum amount of equipment and solvents for clean up and separation before the final analysis by GLC. The method has been tested for 300 specimens of fish as a supplementary method for sulfuric acid clean up [AHLING and JENSEN (1970), BJERK and HOLT (1971), JENSEN et al. (1972)] in a program consisting the analyses of 1000 specimens of the fish of Finland.

MATERIALS AND METHODS

PCB-standards

Clophen® A 50 and 60 were kindly provided by Bayer Company, Leverkusen, Germany. Stock solutions of 0.1 % were prepared in re-distilled hexane and solutions of different concentrations were diluted therefrom.

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Adsorbent

Kieselgel G nach Stahl für Dünnsichtchromatographie (Merck).

Pesticide standards

p,p'DDE, p,p'DDT, p,p'DDD, o,p'DDT, aldrin, lindane, dieldrin and endrin were obtained from the Analytical standards, Unilab Research Corporation, Berkeley, California, USA. The solutions were made as PCB solutions.

Developing solvents

Dichloromethane, p.a., redistilled (Merck, Darmstadt, Germany) n-heptane, redistilled over KOH (May and Baker LTD, Dagenham, England).

Spray reagent

0.5 % diphenylamine solution according to ADAMOVIĆ (1968).

Eluents

Diethylether, p.a. (Merck) and Cyclohexane, p.a. redistilled (Merck).

Instrumentation for TLC

Desaga standard model applicator, mounting board, rying rack and chromatographic tank 10x22x23 cm (Desaga, Heidelberg, Germany). Light: Philips U.V. 30 w 57413/40 M6. Spotting pipets from 5 to 100 µl, H.E. Pedersen, Danmark. Plates of window glass 20x20 cm.

Chromic acid solution

According to WESTÖÖ and NORÉN (1970).

Gas chromatographic equipment

Varian Model 600-D Aerograph with ^{3}H EC-detector, glass columns 1.5 m, inside diameter 1.5 mm, injector temperature 225°C, column and detector 190°C and 200°C, respectively. The column fillings used were a) 4 % SF-96 on Chromosorb W (HMDS) 100-120 mesh and b) a mixture filling containing 65 parts of 8 % QF-1 and 35 parts of 4 % SF-96 on Chromosorb W 100-120 mesh.

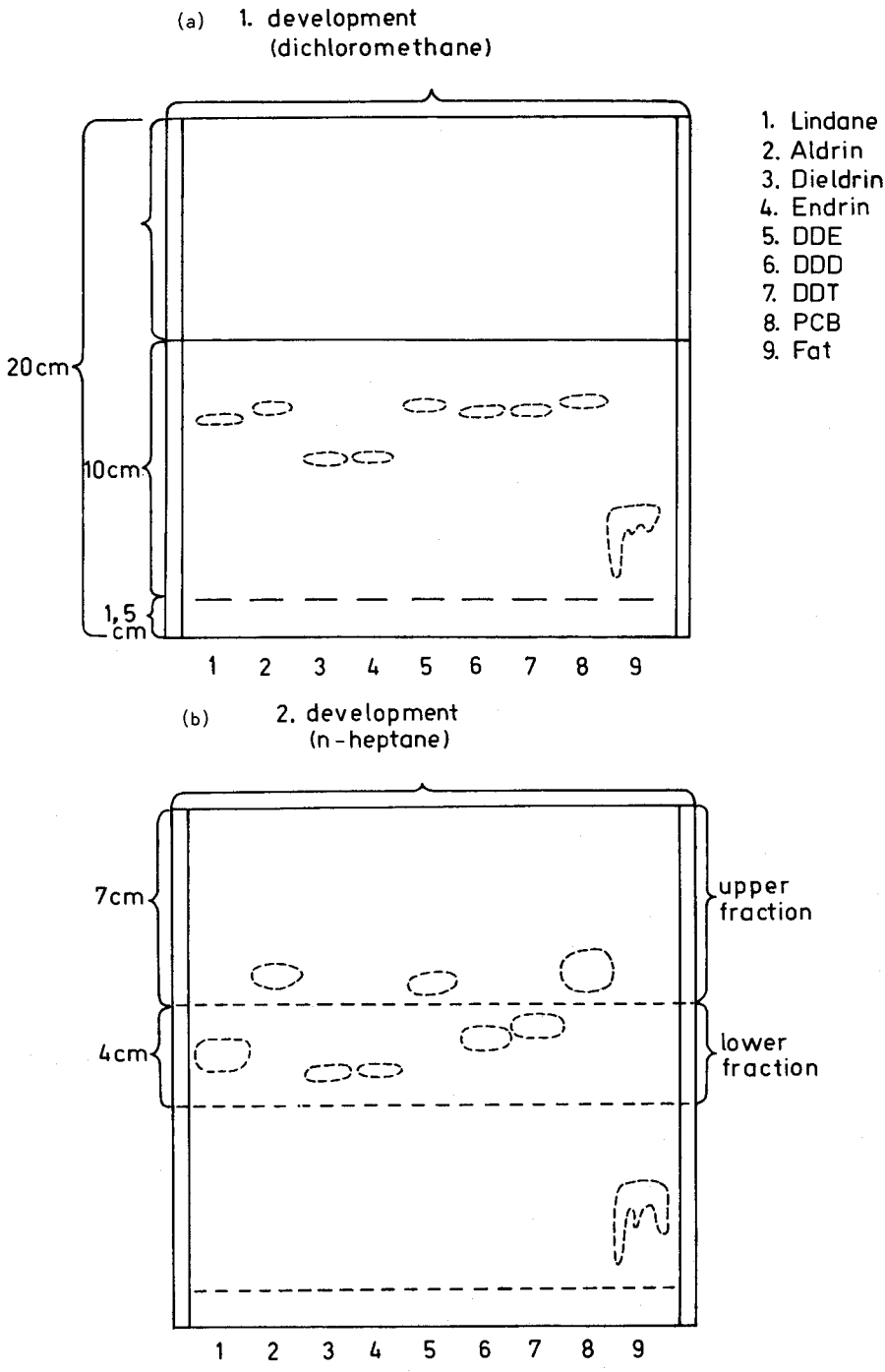
TLC PROCEDURE AND RESULTS

The plates were prepared by mixing the adsorbent 1:1 (w/w) with distilled water and the thin-layer thickness of 1 mm was chosen for final analyses. The plates were activated for two hours at 120°C before use.

A line was drawn by pencil 0.5 cm from the side edges and 1 cm from the upper edge to show the end of the solvent front. The spotting was made 1.5 cm from the lower edge and 100 µl of the standard solution (10 µg/ml) was applied per spot. The fish fat was diluted in n-hexane and the maximum of 15 mg was applied per spot.

The plate was first developed in 100 ml dichloromethane to the halfway point, which was marked in advance (figure 1a) and then dried and moved to another tank containing 100 ml n-heptane. The method was standardized at room temperature (20-22°C) so that the first run took 12 minutes and the second one 37 minutes. The dry plate was sprayed with diphenylamine solution and kept under UV-light for 3 minutes. Compounds were coloured as follows:

Figure 1. PCB and chlorinated pesticides on TLC in a two stage process



lindane bright violet, PCB light violet, DDE, DDD and DDT grey, aldrin light green, dieldrin and endrin bright green and the fat yellowish. The R_f values are presented in table 1.

TABLE 1

R_f values of fish fat, chlorinated pesticides and PCB in a two stage process

Compound	R_f
fat	0.23
dieldrin	0.41
endrin	0.41
lindane	0.54
p,p'DDD	0.54
p,p'DDT	0.55
o,p'DDT	0.55
aldrin	0.63
p,p'DDE	0.67
PCB	0.67

On the basis of the R_f values two groups could be separated of which the upper one consisted of PCB, aldrin and p,p'DDE and the lower one p,p'DDT, o,p'DDT and p,p'DDD, dieldrin and endrin. The fat remained lowest (figure 1b).

The method was then used in the quantitative analysis as follows. A plate was devided by vertical lines into four sections three of which were used for samples and one for the standards. The maximum fat loading was 15 mg per section which was applied as a thin line as well as the standards. The development process was completed as above but during the spraying the sample part was covered with aluminium foil.

After spraying the two fractions were marked according to visible standard lines (only PCB, p,p'DDE and dieldrin were used in routine analyses) and the fractions were scraped with a spatula into glass columns (10x1 cm) with a pipette end. The columns were stoppered with acid and hexane-washed glass wool. The adsorbent was tapped to make a tight column free from air bubbles and the residues were eluted into volumetric tubes by the mixture of 20 % diethylether in cyclohexane. 1 ml the eluent was sufficient for 90-100 % recoveries of PCB but not for most of the pesticides and 2 ml was the final volume taken. The compounds were then separated by GLC, the upper fraction was chromatographed in the mixture column and the lower fraction in SF-96 column. The calculation of PCB was carried out by using the method of HOLDEN (1970) and the heights of 9 peaks of the total 13 were included in the calculations. For determining p,p'DDE the method of WESTÖÖ and NORÉN (1970) was modified as follows: After chromatographing the upper fraction the solution was transferred into a chilled glass stoppered tube. Chromic acid solution was added 1:1 (v/v) and the mixture was shaken until the brownish colour was turned green. The acid layer was carefully moved by suction and the solvent layer was washed twice with 2 ml of cold distilled

water and dried with anhydrous sodium sulphate. The sample was chromatographed again and p,p'DDE was calculated as described by WESTÖÖ and NORÉN (1970).

DISCUSSION

The method was used as a supplementary method for sulfuric acid clean up in which PCB and DDT -type compounds remain unaffected but the epoxide structures are destroyed. For routine analyses the H_2SO_4-treatment was sufficient for determining PCB because for calculations the measuring of 9 noninterferring peaks of the total 13 was considered to be sufficient. The TLC method was used mainly for checking the levels of dieldrin, endrin, and the DDT-type compounds. A good separation could be obtained by using the mixture column for the upper fraction and the SF-96 column for the lower fraction.

The clean up method was then compared with two other methods used simultaneously as a clean up method e.g. sulfuric acid treatment and the column chromatographic clean up (Al_2O_3-column) described by HOLDEN and MARSDEN (1969). A great amount of fish fat was extracted by Soxhlet and made 1 % solution in n-hexane. The homogenous solution was then analyzed by using the clean up method as the only changing parameter. The residues observed in the fat were aldrin, p,p'DDE, p,p'DDT and PCB; dieldrin, endrin and o,p'DDT are known to be very rare in the wildlife of Finland SILTANEN et al. (1971) .

The results are presented in table 2.

TABLE 2

The yield of PCB and pesticides from honogenous fish fat after different clean up methods

	ng residue/ml purified solution			
	aldrin	p,p'DDE	p,p'DDT	PCB
H_2SO_4 treatment	4.9	19	20.2	39
s_d	0.5	3	1.2	3
Al_2O_3 -column	4.1	18.7	20	39
s_d	0.4	0.7	2	2
TLC	4.8	14.4	20	41
s_d	0.3	1.3	2	3

The results show only slight difference except for DDE which may result from an incomplete extraction from the adsorbent with the remainder in the lower fraction not being noticed.

In routine analyses, however, the components of the upper fraction were determined after sulfuric acid treatment and only the lower fraction was scraped and analysed quantitatively which made the procedure rapid.

One of the drawbacks of the method described was the uneven quality of the adsorbent. In some batches peaks having the same retention time with lindane or aldrin could be observed. Washing the adsorbent with hot water or solvents or washing the plates before use changed, however, the surface qualities too much for reproducible results and the batch had to be replaced. The method described has been applied later in cases when the fat yield has been low as in the residue analyses of plankton, bottom invertebrates and water plants and if the amount of the fat is limited, less than 10 mg, the TLC method is the only method applied for clean up.

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