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Note

Simultaneous clean up and fractionation of organochlorine compounds by adsorption chromatography

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The determination of organochlorine pesticides and polychlorinated biphenyls (PCBs) in biological materials by high-resolution gas chromatography with electron-capture detection (HRGC–ECD) is complicated by the presence of lipids and co-eluting compounds. In order to overcome several of these problems, Holden and Marsden[1] developed a method based on adsorption chromatography, including a clean up step and a separation step. Several workers[2–4] have improved this method and recently Wells *et al.*[5] presented an elution scheme for a wide range of organochlorine compounds.

A serious drawback of the method mentioned above is that it is time consuming. At least two separate adsorption chromatography columns have to be used, together with intermediate steps, *e.g.*, concentration, drying, etc., leading to an increased risk of contamination. Kveseth and Brevik[6] introduced the idea of simultaneous clean up and separation of biological samples. Using a single column, they succeeded in cleaning up egg and fish oil samples and separated the organochlorine contents into two groups, *viz.*, one containing total PCBs, and the other containing lindane, total DDT, total DDE and total DDD. No information was obtained about the individual components, however.

When large numbers of samples have to be analyzed, as is the case in environmental studies, much time may be saved by a reduction in the sample pretreatment. HRGC–ECD [or HRGC–mass spectrometry (MS)] enables the analyst to identify and quantify individual PCB congeners and pesticides and has become a commonly accepted method of analysis for organochlorine compounds in the environment. It makes high demands upon the quality of the extract to be injected. This paper describes the performance of a single adsorption chromatography column with two adsorbents for a simultaneous clean up and fractionation of biological tissues prior to HRGC–ECD analysis of the individual PCB congeners and 19 organochlorine pesticides.

EXPERIMENTAL

Apparatus

The adsorption column comprised 200 mm × 5 mm I.D. borosilicate glass

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with an eluent reservoir of 50 ml at the top and a glass frit at the lower end.

Capillary gas chromatography was carried out on a Packard Becker 430 gas chromatograph equipped with a nickel-63 electron-capture detector. Fused-silica (25 m × 0.22 mm I.D.) CP-Sil 5 CB or CP-Sil 8 CB columns (Chrompack, Middelburg, The Netherlands) were used. Temperatures injector (splitless), 260°C; detector, 305°C; oven, 60°C (2 min isothermal) to 295°C at a rate of 4°C/min. Helium was used as the carrier gas and the detector was purged with nitrogen.

All glassware was heated overnight at 250°C and rinsed with acetone, ethanol and *n*-hexane before use.

Reagents

n-Hexane, high-performance liquid chromatography (HPLC) grade, was obtained from Baker (Deventer, The Netherlands) and refluxed and distilled over metallic sodium. Diethyl ether, analytical grade, obtained from Baker, was purified over aluminium oxide and distilled before use; a 10% (v/v) solution in *n*-hexane was prepared for each elution.

Aluminium oxide, Woelm B super I (Woelm, Eschwege, F.R.G.) was activated at 200°C for at least 12 h and subsequently deactivated with 5% (w/w) doubly distilled water. Silica gel (Kieselgel 60, 70–230 mesh; Merck, Darmstadt, F.R.G.) was activated at 140°C for at least 12 h and subsequently deactivated with 0.5% (w/w) doubly distilled water.

Sodium sulphate, anhydrous (Baker, analytical grade), was used as drying agent.

The PCB congeners were a gift from L. G. M. Th. Tuinstra (Rikilt, Wageningen, The Netherlands) or were obtained from Ultra Scientific (Hope, RI, U.S.A.); organochlorine pesticides were gifts from U. A. Th. Brinkman (Free University, Amsterdam, The Netherlands) and P. Frintrop (RIZA, Lelystad, The Netherlands).

RESULTS AND DISCUSSION

The adsorption columns were freshly prepared for each elution. Clean columns were dry packed with 4.0 g deactivated silica gel, 0.5 g sodium sulphate, 2.0 g deactivated aluminium oxide and 0.5 g sodium sulphate (silica gel layer at the bottom). A vibrator was used to obtain a proper packing. The column was pre-eluted with *n*-hexane. A 1-ml volume of a sample extract or an analytical standard solution was applied on top of the column and allowed to soak into the packing. Next, the reservoir was filled with 35 ml of *n*-hexane. The eluate was collected in fractions (1–5) of 10, 10, 5, 5 and 5 ml, respectively. Subsequently the reservoir was filled with 35 ml of the diethyl ether–hexane mixture and another five fractions (6–10) of 5, 5, 10, 10 and 5 ml were collected. Each fraction was concentrated to 1 ml in a stream of nitrogen and analyzed by HRGC–ECD. In this manner an elution profile was obtained for each compound.

In order to reduce the analysis time, the ten fractions had to be reduced to a lower number. Combination of fractions 1–5 (I) and 6–10 (II), respectively, yielded two main fractions, the former (I) containing all PCBs, polychlorobenzenes, hexachlorobutadiene, aldrin, mirex and part of the total p,p'-DDE and heptachlor present in the extract, the latter (II) containing the remainder of the pesticides, as is seen

from the right-hand side of Table I. If required, a further separation of fraction I is possible into a subfraction Ia containing apolar pesticides and Ib containing PCBs. To that end, fractions 1 and 2 from the original elution should be combined to give Ia, and fractions 3–5 to give Ib. The described conditions for HRGC–ECD, however, allow for a sufficient separation of these compounds in one GC experiment.

Recoveries were calculated from the chromatograms of the standard solution before and after use of the adsorption column. The results are given in Table I. Most of the components show recoveries within the 80–120% range. For a few compounds however lower recoveries are observed. The low recoveries for hexachlorocyclohexanes (HCHs) are ascribed to evaporation losses during the analytical procedure. The use of an alternative injector, *viz.*, an on-column injector, may improve the results. As to endosulfan, the low recoveries for the α isomer result from the dynamic equilibrium existing between the α and β isomers. The aluminium oxide of the adsorption

TABLE I

RECOVERIES (%) OF ORGANOCHLORINE COMPOUNDS IN FRACTIONS COLLECTED FROM THE ADSORPTION COLUMN

Conditions as in the Experimental section. HCH = Hexachlorocyclohexane; QCB = pentachlorobenzene; HCB = hexachlorobenzene; HCBu = hexachlorobutadiene; CB = chlorobiphenyl.

Compound	*Fraction No.*												*Total*
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*I*	*II*	
α-HCH								68				68	68
γ-HCH								69				69	69
QCB	48	43									91		91
HCB	89	22	12								123		123
HCBu		100									100		100
Mirex		89									89		89
Heptachlor					81	29					81	29	110
Heptachlorepoxide								82				82	82
α-Endosulfan								48				48	48
β-Endosulfan											0	0	0
Aldrin			49	32							81		81
Dieldrin								10	72	6		88	88
Endrin								88				88	88
o,p'-DDE								79				79	79
o,p'-DDD								87				87	87
o,p'-DDT								92				92	92
p,p'-DDE					10	60	39				10	99	109
p,p'-DDD								83				83	83
p,p'-DDT								84				84	84
2,4,4'-Tri-CB				49	55						104		104
2,2',5,5'-Tetra-CB				43	66						109		109
2,2',4,5,5'-Penta-CB			19	70							89		89
2,3,4,5,6-Penta-CB			23	62							85		85
2,2',3,4,4',5-Hexa-CB			11	63	8						82		82
2,2',4,4',5,5'-Hexa-CB			72	13							85		85
2,2',3,4,4',5,5'-Hepta-CB			80	7							87		87

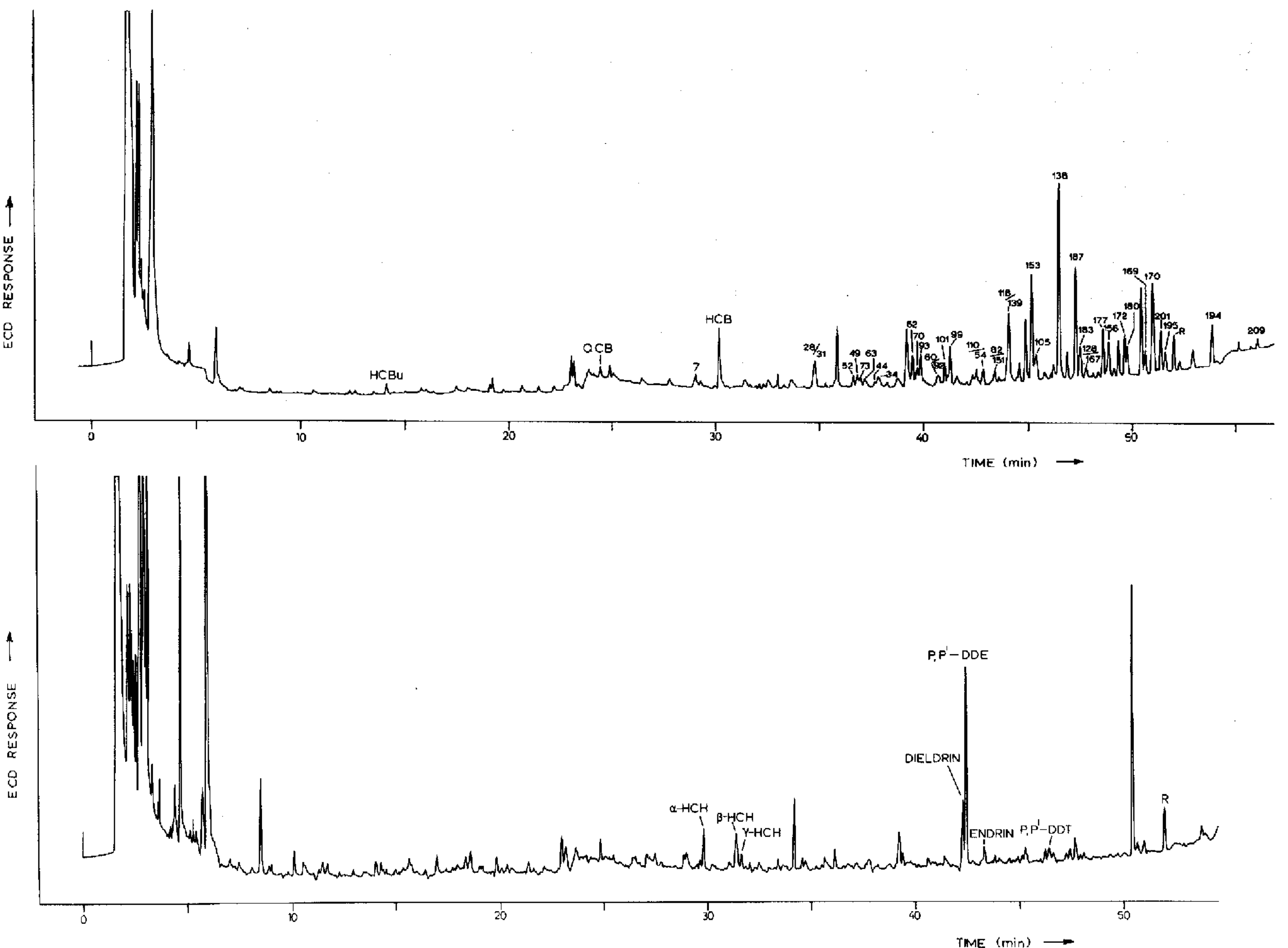

Fig. 1. HRGC–ECD chromatograms of fraction I (top) and fraction II (bottom) of an extract of adipose tissue from a juvenile dunlin (*Calidris alpina alpina*) captured in the Dutch Wadden Sea. The extract was purified and fractionated on the column, as described in the Experimental section. In the top chromatogram the IUPAC numbering of the PCB compounds is given.

column strongly retains the β isomer, resulting in a zero recovery for the latter, because of the slow establishment of the new equilibrium.

As is seen from Table I, the more polar PCB congeners, *i.e.*, the less chlorinated ones, tend to elute later. Thus a dichlorobiphenyl may be eluted in fraction II. However, upon testing a technical PCB mixture containing dichlorobiphenyls, it appeared that all the PCB congeners were found in fraction I.

The clean up efficiency was tested by means of samples of biological tissues and sediments. Extracts of bird liver, bird adipose tissue, invertebrates and fish oils were examined and found to be efficiently purified and fractionated[7]. An example is given in Fig. 1. The amount of adsorbents used in the adsorption column allows for a maximum application of about 60 mg of lipids of animal origin. For sediments, usually an additional step to remove elemental sulphur is required. Prior to the adsorption chromatography, we employed a procedure described by Jensen *et al.*[8]. Again a good result was observed[7].

The present procedure was used in an EC interlaboratory test on the analysis of individual PCB congeners in fish oils[9]. Results in good agreement with other participants (coefficients of variation ranging from 11 to 24% at the 0.04–0.3 mg/kg level per congener, 14 laboratories) were obtained. The described procedure therefore is a useful improvement upon existing methods. It enables a considerable reduction in both time and the amounts of solvents required for pretreatment of environmental samples, and is adapted to the current demands of high resolution organochlorine compound analysis.

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