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Column Chromatographic Method for Cleaning Up Extracts from Biological Material and Simultaneous Separation of PCBs and DDE

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Gas chromatographic determination of organochlorine insecticides in biological material is often complicated because of the simultaneous presence of polychlorinated biphenyls (PCBs). A separation is therefore advantageous. Several column chromatographic procedures are described in the literature (REYNOLD 1969, HOLDEN and MARSDEN 1969, ARMOUR and BURKE 1970, SNYDER and REINERT 1971, BERG et al.1972, ERNEY 1974).

In order to establish such a column chromatographic separation procedure at our department, we used as a base those methods which had been reported to be successful in separation of PCBs and DDE. Our duplication efforts did not prove to be very satisfactory. For one reason the fine grained silica gel types used gave slow elution and therefore the large quantities of eluting agent necessary led to a prolonged eluting time. We therefore chose a coarser grained silica gel. In addition to a separation procedure, we also wanted a simultaneous clean-up of extracts of biological material. Such a combination column is described in this report.

MATERIALS AND METHODS

Reagents

a) Adsorbents: Silica gel, Woelm 0.063-0.2 mm or 0.05-0.2 mm for column chromatography (M.Woelm,Eschwege, W.Germany). Kiesel gel 60 for column chromatography, grain size 0.063-0.125 mm (Art.9386,Merck), Silicic acid, 100 mesh, AR (Mallinckrodt No. 2847).

These reagents were Soxhlet extracted with n-hexane for 4 hr and activated at 130°C for at least 24 hr. Thereafter they were transferred to a flask with a tightly fitting glass stopper and placed in a desiccator. After cooling, known quantities of water were added in some cases and the flask, shaken thoroughly for an hour, was then stored in a desiccator.

Aluminum oxide 90 active neutral, for column chromatography (Art.1077 Merck). It was Soxhlet extracted with n-hexane 4 hr and activated at 800°C for at least 8 hr. After cooling, the aluminum oxide was deactivated with 5% distilled water and thereafter

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treated in the same way as the silica gel.

b) Solvents: n-hexane Art.9688 Merck, petroleum ether boiling point 40-60°C, Art.909 Merck, diethyl ether supplied by A/S Den norske Eterfabrikk. The solvents were redistilled in glass and tested before use by concentrating 50 fold before injection into the gas chromatograph.

c) Standards: p,p'-DDE, p,p'-DDD, p,p'-DDT and lindane, purchased from Analytical Standards, Sweden. PCBs, Clophen A50, from Farbenfabriken Bayer Aktiengesellschaft Leverkusen.

Apparatus

a) Gas chromatograph: Varian 1700 with electroncapture detector(^{3}H). Columns: 150 cm x 2 mm ID glass columns, one packed with 10% QF-1 and one with 4% SF-96 on Chromosorb W, 100-120 mesh. Operating conditions: Nitrogen 30 ml/min, injector column and detector temperatures were 190°, 180°, and 200°C, respectively.

b) Clean-up/separation column: Glass column with inner diameter of 0.8 cm, with teflon stopcock and a 50 ml reservoir at the top.

Experimental procedure

A hexane-washed cotton wool plug was first placed into the glass column, which was then filled with hexane and packed with 2 to 6 g of the described silica gel types. Up to 1 ml of a standard solution of Clophen A 50 (4000 ng/ml), lindane (50 ng/ml), DDE (200 ng/ml), DDD (500 ng/ml), and DDT (800 ng/ml) were introduced into the column. Elution was carried out either with hexane or petroleum ether, and separation was obtained by after a predetermined volume adding 10% diethyl ether. Elution rate was regulated to about 1.5 ml/min. The course of elution as regards the individual components was determined by fractionating the eluate and analysing each fraction by gas chromatography. Thereafter a combination column was made which in addition to the most suitable silica gel type was packed with 2 g of aluminum oxide at the top. An elution trial was carried out with a sample from the same standard mixture. The same components were thereafter added to uncontaminated cod liver oil and trial solutions with 20-60 mg of this cod liver oil were introduced into the column in order to simultaneously test separation and clean-up capacity. Finally, samples of extracts of biological material were introduced into the column. Evaporation of small volumes of solvent was carried out over a water bath at 40°C, and in a stream of dry air, whilst large volumes were evaporated using a rotary evaporator and a round flask to which was welded a 5 cm long glass tube with a diameter of 0.5 cm and marked with divisions at 0.5 and 1 ml.

RESULTS AND DISCUSSION

Separation trials were carried out using the three described silica gel types with the standard mixture. With 3-10 g kiesel gel 60 or silicic acid slow elution and unsatisfactory separation resulted, especially of PCBs and DDE which were the most difficult to separate. By using 4 g silica gel with a grain size of 0.063-0.2 mm or 0.05-0.2 mm, it was possible to separate PCBs and DDE. The separation efficiency of this column was dependent to a marked extent on the degree of activation of the silica gel. Deactivation with 0-0.5% water gave satisfactory separation, but already with 1.5% water added to the silica gel, PCBs and DDE were eluted partially together as shown in Fig.1. Hexane and petroleum ether were found to be equally effective as eluting agents. To simplify matters, only hexane is mentioned in the text, henceforward.

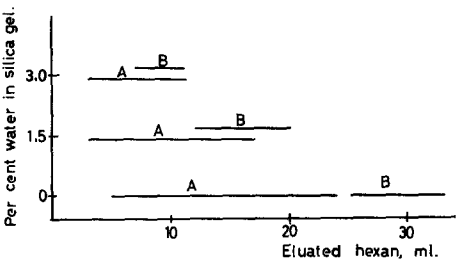

Fig.1. Effect of silica gel water content on elution and separation of Clophen A 50 (A) and p,p'-DDE (B). 1-5 ml fractions of hexane eluate analyzed.

In order to achieve a simultaneous separation and clean-up of extracts from biological material, the column was packed with silica gel and aluminum oxcide as mentioned previously. This did not change the elution process as far as the mentioned components were concerned. PCBs (Clophen A 50) was eluted with around 24 ml hexane, whilst DDE as the first of the remaining components began to come around 25 ml eluted hexane (Fig.2). Every new batch should be checked for separation ability, as small changes in eluating volumes have been observed between batches.

The aluminum oxide/silica gel column was tested with samples of biological material to investigate repeatability and recovery. Fourteen samples of 20 mg of old uncontaminated cod liver oil were fortified with the standard solution and introduced into the column. The elution volume for PCBs (Clophen A 50) did not exceed 24 ml hexane, called fraction A, whilst DDT, DDD, DDE and lindane were thereafter eluted with 20 ml hexane containing 10% diethyl ether, fraction B. The

results of the recovery experiments are shown in Table I.

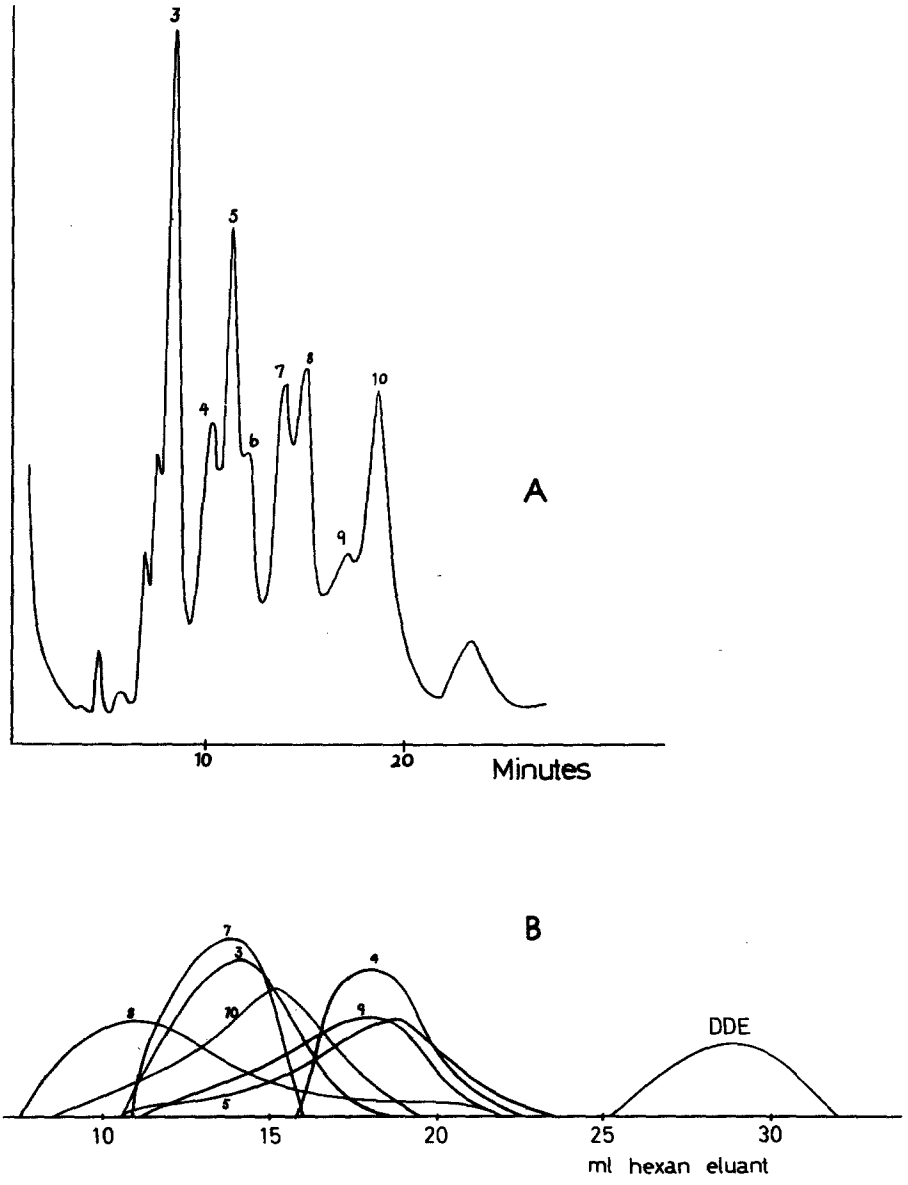

Fig.2. Gas chromatogram of Clophen A 50 (A), and hexane elution pattern of Clophen A 50 and p,p'-DDE (B) from a silica gel-aluminum oxide column.

The separation efficiency of the column proved to be dependent on the concentration of the organochlorines in the sample. For example, good separation was achieved with 1 µg/ml PCBs and 0.5 µg/ml DDE, whilst a ten-fold increase in concentration led to 4-5% of the total amount of PCBs being eluted together with 5-6% of the total DDE. In cases where biological material has residues of organochlorines exceeding these concentration levels, regard should be given to this by, for example, diluting the lipid extract of the sample in order to achieve optimal conditions for separation.

TABLE I

Recovery experiments of PCBs, lindane, DDT and metabolites in fortified cod liver oil samples, by use of deactivated aluminum oxide and silica gel column chromatography.

Fraction	Test substance	Concentration of test substance, µg/ml	Percentage recovery of 14 parallels	
			Mean	Range
A hexane	PCB	0.8 - 8	99	(89 - 112)
B 20 ml hexane and 10% diethyl ether	Lindane	0.01 - 0.1	93	(87 - 112)
	DDE	0.04 - 0.4	93	(80 - 104)
	DDD	0.1 - 1.0	97	(87 - 114)
	DDT	1.6 - 16	97	(83 - 116)

The amount of fat introduced into the column was varied from 20 to 60 mg without this having any demonstrable effect on the separation of PCBs and DDE.

The method was used in connection with the analysis of contaminated seabird egg material and cod liver oil. 20 mg of extracted fat dissolved in hexane was introduced into the column. A better separation of PCBs and DDE was found with this material than in the described experiments in which pure standards were used. Fig.3 shows a gas chromatogram of a seabird egg with 88 ppm PCBs and 33 ppm DDE calculated on fat basis before and after separation. This seems to indicate that the degree of chlorination of PCBs influences the column's separation characteristics. This supposition is supported by MASUMOTO (1975) who, using a column packed with silicic acid, found that of the analysed PCB compounds, only the most chlorinated, Arochlor 1260, was satisfactorily separated from DDE.

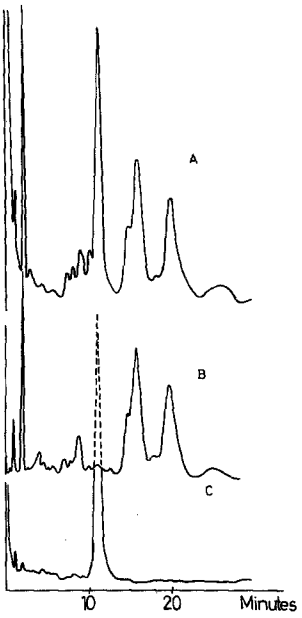

Fig.3. Chromatograms of a seabird egg extract containing residues of PCBs and p,p'-DDE before and after separation on silica gel-aluminum oxide column. A: the extract treated with sulfuric acid, B: hexane eluate and C: hexane with 10% diethyl ether eluate from the column.

The method described for cleaning up extracts of biological material and simultaneous separations of PCBs and DDE is relatively rapid and requires comparatively small amounts of chemicals. The silica gel seemed to be stable during prolonged storage in a desiccator, in that it was used up to one month after activation without any differences in its characteristics being observed.

If the method's premises regarding the amount of fat and concentration of organochlorines are fulfilled, the method should be very suitable for use in connection with gas chromatographic analysis of biological material with regard to organochlorine insecticides and PCBs.

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