Separation of Three Chlorodibenzo-p-dioxins from Some Polychlorinated Biphenyls by Chromatography on Aluminum Oxide Column

By MILDRED L. PORTER and JERRY A. BURKE, (Industrial Chemicals Residue Branch, Division of Chemical Technology, Food and Drug Administration, Washington, D. C. 20204)

Abstract

The separation of 2,3-dichlorodibenzo-p-dioxin, 2,3,7-trichlorodibenzo-p-dioxin and 2,3,7,8-tetrachlorodibenzo-p-dioxin from polychlorinated biphenyls by column chromatography on aluminum oxide is described. Polychlorinated biphenyls were first eluted with 1% methylene chloride/hexane and the chlorodioxins were then eluted with 20% methylene chloride/hexane. Recoveries of both polychlorinated biphenyls and chlorodioxins approximated 100%.

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Residues of polychlorinated biphenyls (PCB) are found in fish, bird and animal specimens from different parts of the world (1-2). Westöö reported the presence of PCB with high frequency in refined fats and oils and foods of animal origin (3). These compounds are extracted and isolated along with pesticides and are detected by gas chromatographic (GLC) systems normally employed in analyses for chlorinated pesticides.

Chlorodibenzo-p-dioxins (chlorodioxins) may be found in chlorophenols (4) which are widely used in agriculture and industry. Chlorodioxins have been associated with the "chick edema factor" in by-product fat (4). Some pesticide formulations have been found to contain certain chlorodioxins (5). The 2,3,7,8-tetrachlorodibenzo-p-dioxin (tetra) has an extremely high toxicity (6). For this reason, analytical methodology for residues of the chlorodioxins, especially the 2,3,7,8-tetrachloro isomer, must be capable of detecting very low residue concentrations.

In tests to determine behavior of tetra in multi pesticide residue methodology (7), we found a similarity of behavior of tetra and PCB (Aroclors®). Both were eluted from Florisil together and along with DDT and

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its analogs. The GLC retention time of tetra on DC-200 and mixed QF-1/DC-200 liquid phases was in the retention time range of the multicomponent PCB. GLC determination of tetra in the presence of PCB (9) did not appear feasible. Tetra was not resolved from p,p' -DDT on either liquid phase. Both tetra and PCB were unchanged by reflux with alcoholic KOH; p,p' -DDT was dehydrochlorinated to p,p' -DDE. Tetra and PCB were eluted together from a silicic acid column (7) with petroleum ether eluant. The silicic acid column is used for the separation of some PCB from DDT and analogs. In our experiments tetra and PCB were eluted together from the silicic acid column (9) with petroleum ether eluant.

The similarity in behavior of tetra and PCB in the various analytical procedures and the frequent occurrence of PCB as a residue indicates a possibility of interference by PCB in analysis for residues of tetra and perhaps other chlorodioxins.

Thin layer chromatography on aluminum oxide with n-heptane mobile solvent (7) resolved PCB (Aroclor® 1254) from tetra. Migration relative to p,p' -TDE was: tetra, 2.12; Aroclor® 1254, 2.54. This suggested column chromatography on aluminum oxide as a means of separating PCB, to permit quantitation of tetra by GLC.

Experimental

A number of experiments were conducted with different lengths of the aluminum oxide column and with different proportions and volumes of hexane/methylene chloride eluants to determine optimum conditions for separating tetra and PCB (Aroclor® 1254). Further investigation of separation and recoveries included 2,3-dichloro and 2,3,7-trichlorodibenzo-p-dioxins and Aroclors® 1260 and 1262. One ml of tetra (Standard a)

plus one ml of PCB mixture (Standard c) or one ml of chlorodioxin mixture (Standard b) plus one ml of the PCB mixture were used in these experiments.

Standards

(a) 2,3,7,8-tetrachlorodibenzo-p-dioxin in hexane.--2.12 µg/ml.

(b) Dioxin mixture in hexane.--2,3-dichlorodibenzo-p-dioxin; 1.59 µg/ml; 2,3,7-trichlorodibenzo-p-dioxin, 1.28 µg/ml; 2,3,7,8-tetrachlorodibenzo-p-dioxin, 1.23 µg/ml.

(c) PCB mixture in hexane.--Aroclor® 1254, 0.16 µg/ml; Aroclor® 1260, 0.16 µg/ml; Aroclor® 1262. 0.16 µg/ml.

Reagents

(a) Aluminum oxide.--Merck, "suitable for chromatographic adsorption", heated overnite in covered beaker in 130°C oven and allowed to cool in desiccator.

(b) Eluant A.--1% methylene chloride/hexane; dilute 1 ml methylene chloride to 100 ml with hexane.

(c) Eluant B.--20% methylene chloride/hexane; dilute 20 ml methylene chloride with hexane. Allow mixture to reach room temperature and adjust volume to 100 ml with hexane.

Apparatus

(a) Disposable pipets.--100 mm x 4 mm i.d.

(b) Micro evaporative concentrator.--No. K569250 with 10 ml tube (K570050), Kontes Glass Co., Vineland, New Jersey, 08360.

(c) Gas chromatograph.--equipped with electron capture detector and 6' x 4 mm i.d. glass column packed with 3% OV-101 on 80/100 mesh Chromsorb WHP.

Operating conditions: N_2 carrier flow, 120 ml/min; column and detector temperature, 200°C; injection temperature 225°C. Concentric design electron capture detector operated at d c voltage to produce 1/2 full scale recorder deflection when full scale deflection is 1×10^{-9} amperes.

Separation

1. The following paragraph Safety Precautions should be added following Apparatus: Chlorodioxins were not weighed in the author's laboratory. All work was done with standard solutions (see "Standards") furnished by another FDA laboratory. Safety glasses and disposable plastic gloves were worn. Bench tops were covered with disposable adsorbent mats. The effluent from the electron capture GLC detector was passed into a trap containing corn oil. Sample concentrations were carried out in the hood. Gloves, towels, mats, pipets, containers of solution, and aluminum oxide columns were placed in plastic bags at the end of each experiment. Sealed bags were picked up weekly for incineration.

the chromatographic tube were rinsed and the ... of 6 ml Eluant A (1% methylene chloride/hexane). Eluate A was concentrated to a suitable volume, using a micro evaporative concentrator and analyzed by electron capture GLC. Recovery of PCB approximated 100%. An additional 1 ml of Eluant A was passed through the column and the eluate analyzed separately. No chlorodioxins or PCB were present.

The receiver was changed and the column eluted with 6 ml Eluant B (20% methylene chloride/hexane). Eluate B was concentrated and analyzed in the same manner as Eluate A. Recovery of the three chlorodioxin isomers approximated 100%. An additional 1 ml of Eluant B was passed through the column and the eluate analyzed separately. No chlorodioxins were present.

Results and Discussion

The separation of PCB (mixture of Aroclors® 1254, 1260, 1262) from chlorodioxins (mixture of 2,7-di, 2,3,7-tri, and 2,3,7,8-tetrachlorodibenzo-p-dioxin) on a column of aluminum oxide was complete. The PCB were first

eluted with 6 ml 1% methylene chloride/hexane and the chlorodioxins were then eluted with 6 ml 20% methylene chloride/hexane. Recoveries of both PCB and chlorodioxins approximated 100%. No difference in separation or recovery was observed with acid, basic, or neutral aluminum oxide from three manufacturers.

Retention times of the chlorodioxins relative to dieldrin at the GLC conditions described under apparatus were: 2,7-dichloro, 0.93; 2,3,7-dichloro, 1.67; 2,3,7,8-tetrachloro, 3.0. Approximate quantities of the chlorodioxins required for one half scale recorder deflection at the GLC conditions described under apparatus were: 2,7-dichloro, 40 ng; 2,3,7-dichloro, 20 ng; 2,3,7,8-tetrachloro, 18 ng. Response tended to improved (smaller quantity for 1/2 scale recorder deflection) with use of the GLC column.

Summary

Separation of PCB from chlorodioxins was achieved by column chromatography on aluminum oxide. This technique should be useful in analyzing samples containing PCB for residues of the chlorodioxins.

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