A Rapid Micromethod of Sample Cleanup for Gas Chromatographic Analysis of Insecticidal Residues in Plant, Animal, Soil, and Surface and Ground Water Extracts[1]

by A. M. KADOUM[2]
Department of Entomology
Kansas State University, Manhattan, Kansas

In the application of gas chromatography with electron capture detection for insecticide residue analysis, one of the most difficult problems is the quantitative separation of the insecticide from interfering biological substances.

Many of the cleanup methods described in the literature do not efficiently remove the interfering substances from extracts for gas chromatography (1, 3, 4, 5, 12). Adsorption column chromatography using Florisil is probably the most widely accepted method. However, for satisfactory cleanup of plant and animal extracts using Florisil, some further cleanup is necessary prior to chromatography (9). Moreover, variable recoveries resulting from substantial retention of some insecticides on various adsorbents have been reported (7, 8, 11).

The objective was to develop a rapid cleanup method with reproducible high recovery of insecticides for the extracts of

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[1]Contribution No. 924, Department of Entomology, Kansas Agricultural Experiment Station, Manhattan, Kansas, U. S. A. Research supported in part by Regional Research Projects NCM-37 and NC-85, and Kansas projects 687 and 481. The assistance of Mr. Larry Cox, Research Assistant, is greatly appreciated.

[2]Assistant Professor of Entomology.

Bulletin of Environmental Contamination & Toxicology,
Vol. 2, No. 5, 1967, published by Springer-Verlag New York Inc.

a wide variety of samples encountered in insecticide residue determinations. To achieve this objective a silica gel (60-200 mesh) microcolumn was discovered to separate the most common chlorinated and phosphate insecticides from moderate amounts of fats, waxes and pigments.

Materials and Methods

Reagents and Equipment. Stock solutions were prepared in hexane (1 ug/ul) from analytical grade aldrin, dieldrin, heptachlor, heptachlor epoxide, endrin, parathion, diazinon and malathion. These insecticides were furnished by various insecticide manufacturers.

Standard solutions (1 ug/ml) for fortification were prepared by pipetting 100 ul of the stock solution into a 100 ml volumetric flask, which was diluted to volume with hexane.

Eluting solvents were hexane, 2%, 10%, 40%, 60%, and 70% (v/v) solutions of benzene in hexane, benzene, and 8% (v/v) ethyl acetate in benzene.

All solvents were nanograde (Mallinckrodt Chemical, St. Louis, Mo.) unless otherwise specified.

Gas chromatographs included: (1) Aerograph model 600-C with Aerograph programer model #328 Hydrogen flame ionization detector with phosphorus kit; and (2) RSCO model #600 series equipped with electron-capture detector.

Disposable pipets, 5 3/4 in. in length and tip opening approximately 1 mm and top opening 5 mm inside diameter were used as microcolumns.

Activated silica gel, high purity grade 950 (60-200 mesh) (Fisher Scientific Company, St. Louis, Mo.) was used as column adsorbent.

Special wooden holders were constructed to hold the disposable pipet-type microcolumn during chromatography (Fig. 1). Two wooden boards 10 1/2 in. long, 4 in. wide and 3/16 in. thick were mounted parallel and horizontally 1 1/4 in. apart, on two wooden supports. Forty holes were drilled to accommodate the microcolumns. Each hole was 9/32 in. and 7/32 in. in diameter in the upper and lower board, respectively.

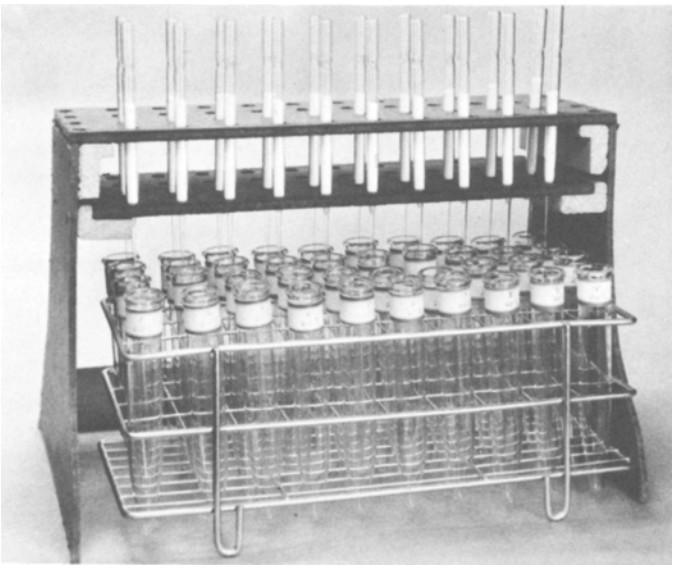

Figure 1. Wooden holder demonstrating method of elution and illustrating 20 disposable pipet microcolumns, 40 centrifuge tubes used as receivers for the eluate.

Column Chromatography. A silica gel chromatographic microcolumn was prepared by packing a plug of glass wool loosely into a disposable pipet about 1 1/2 in. from the tip, and then adding one gram of silica gel. The microcolumn was tapped to obtain good packing and then washed with 5 ml hexane. Extracts of grains, plant tissue, soil, ground water and animal tissue were prepared by standard techniques (2). Prior to column chromatography, solvent extracts were evaporated to dryness and the residue was redissolved in one ml of n-hexane. For partial deactivation of silica gel, one ml standard solution or the concentrated extract in hexane used for charging the microcolumn was saturated with 5 ul of distilled water and was transferred quantitatively to the chromatographic microcolumn. It was then allowed to percolate through the microcolumn at a rate of one ml per one to two minutes. The walls of the chromatographic microcolumn were rinsed with small portions of hexane. When the solvent reached the top of the silica gel, elution with the desired eluting solvent was commenced.

A series of solvent mixtures with varying polarities were used for elution. The eluate was collected in a 15 ml graduated centrifuge tube as indicated in Figure 1. When the desired volume was collected and the eluting solvent had reached the upper surface of the silica gel, the receiver (centrifuge tube) was changed. Elution was continued with the next higher polarity eluting mixture and the percolate from the microcolumn was

collected. Using 35-40°C water bath, the eluate obtained was concentrated to one ml volume by a stream of nitrogen.

Recovery experiments at the 0.1 and 1 ppm level were conducted on the chlorinated hydrocarbons and organophosphorus insecticides, respectively.

Results and Discussion

The column chromatography on silica gel reported herein provides an effective sample cleanup method for the separation of insecticide residues from pigments, fats and/or waxes which cause instrumentation inferference and give erratic results.

A number of solvent systems were studied for the selective elution of insecticides from silica gel. The eluting solvent mixtures used (Table I) were the most satisfactory of several solvent systems studied and appear to be the key to successful separation of chlorinated and organophosphorus insecticides from interfering materials on silica gel.

During the experimental testing of this procedure, significant and consistent losses of the insecticides were noted. Saturation of the concentrated hexane extract used for charging the microcolumn with 5 ul of distilled water per ml prior to chromatography, increased the average recovery of all tested insecticides from varying percentage recoveries to a total recovery. This indicates the necessity for saturation of insecticide concentrate extract with distilled water. The purified grade 950 silica gel (60-200 mesh) was used as received without any further activation.

TABLE I

Selective elution of insecticides from microcolumn packed with 1 gm silica gel

Insecticide	Solvent system	Distribution in eluate, % recovery from GLC. Total volume of eluate (ml)		
		4 ml	6 ml	8 ml
Aldrin	Hexane	65 ± 8[a]	100 ± 0	100 ± 0
Heptachlor	2% (v/v) benzene in hexane	60 ± 5	94 ± 3	100 ± 0
Heptachlor epoxide	40% (v/v) benzene in hexane	97 ± 1	100 ± 0	100 ± 0
Dieldrin	60% (v/v) benzene in hexane	91 ± 7	100 ± 0	100 ± 0
Endrin	70% (v/v) benzene in hexane	93 ± 5	100 ± 0	100 ± 0
Parathion	Benzene	84 ± 6	95 ± 2	100 ± 0
Diazinon	8% (v/v) ethyl-acetate in benzene	39 ± 5	94 ± 2	100 ± 0
Malathion	8% (v/v) ethyl-acetate in benzene	98 ± 1	100 ± 0	100 ± 0

(a) Recovery is the average of 4 replicates to nearest percent.

Many lots of silica gel were tested and gave essentially identical results. Recoveries of standard compounds were determined for each insecticide separately. Standard recoveries were calculated from the peak area of a known quantity of insecticide in hexane added to the appropriate substrate which had been precleaned by the procedure described herein, prior to the addition of insecticide. The recovery data for tested insecticides were expressed in terms of percent recovery of insecticide.

The eluate was collected in three volumes to indicate the approximate volume required to elute the various insecticides. Recoveries of different insecticides were approximately 100% in 6-8 ml of eluate (Table I). Differences between lots of the same type were not troublesome with the eluting solvents used.

Application of this method was used for extracts of plants, grains, fish, mice, soil and large amounts of ground and surface water prepared and extracted by standard techniques. Insecticides were added to equivalent portions of controls of the various extracts. All insecticides tested in the recovery experiments, using different extracts, were completely eluted in 6 ml of eluate, indicating total recovery.

The cleanup of the extracts was satisfactory for gas chromatographic detection using 10 gm of soil, plants and grains; 2-3 gm of animal tissue; and 20 liters of ground and surface water.

Success was attained in making separations and determinations of insecticides at 0.001 ppm in plants, grain, and soil; 0.004 ppm in animal tissue; and 0.001 ppb in water extracts.

A minimum background was encountered in the chromatogram by discarding the 10% (v/v) benzene in hexane eluate before eluting the heptachlor epoxide, dieldrin, endrin, parathion, diazinon and malathion.

It has been observed that Florisil appears to bind some insecticides. Recoveries may be low, and in our experience, high electron-capture background may be encountered. Moreover, Florisil does not effectively separate insecticides from the waxy materials present in plant extracts and considerable amounts of fats in animal extracts (9). Silica gel, on the other hand, removes waxes, pigments and fats. This makes the technique particularly useful for rapid chromatographic cleanup of different types of extracts. This technique has shortened the time for sample cleanup prior to gas chromatographic analysis.

The use of n-hexane-acetonitrile and petroleum ether-acetonitrile methods for cleanup of sample extracts were not sufficient (6, 10). Small amounts of interfering substances were still not completely removed. These residual biological materials can be eliminated by chromatography on silica gel, which could be used as a one-step cleanup method or in combination with partitioning methods of cleanup.

This cleanup technique was sufficient for gas chromatographic analysis with electron capture detector, and hydrogen flame ionization detector with the phosphorous kit, for chlorinated hydrocarbons and organophosphorus insecticides, respectively. It

can be used with samples of sufficient size, using silica gel macrocolumn chromatography.

The characteristics of silica gel and the factors that influence the elution of different insecticide residues from the adsorbent are currently under investigation.

Although intended primarily for the determination of tested insecticides, this procedure should apply equally well for the analysis of other insecticides.

Summary

A simple microcolumn chromatographic cleanup method for chlorinated and organophosphorus insecticides has been developed in which a high purity grade 950 of activated silica gel (60-200 mesh) is used. Insecticides are selectively eluted from the adsorbent by different solvent mixtures varying in polarity. Procedures are described for cleanup of residues in plants, grains, animal tissues, soil, and large amounts of ground and surface water.

Cleanup is satisfactory for gas chromatographic analysis. All tested insecticides were recovered quantitatively.

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