Method to Determine Fenthion and Five Oxidative Metabolites in Fat

A method was modified for determining fenthion (*O,O*-dimethyl *O*-[3-methyl-4-(methylthio)phenyl]-phosphorothioate) and five oxidative metabolites in fat. With this method, fenthion, its sulfoxide, its sulfone, and the oxygen analogue, and its sulfone were analyzed by gas chromatography with the flame photometric detector and a 3% OV-1 column. The oxygen analogue sulfoxide was analyzed by liquid chromatography with a μ Bondapak C 18 column. Recoveries from samples of fat fortified with the compounds were in the range of 81–100%.

Fenthion (*O,O*-dimethyl *O*-[3-methyl-4-(methylthio)-phenyl] phosphorothioate) is an organophosphorus insecticide that is used to control a variety of insects including the larval stages of the common cattle grub (*Hypoderma lineatum*). For control of this parasite the insecticide is applied either as a "pour-on" or "spot-on" formulation to the backs of the cattle. Fenthion (P=S,S) has several metabolites including five oxidative metabolites. Since the oxidative metabolites are more likely to be found in fat than the products of hydrolysis, only those metabolites were studied. The oxidative metabolites include the sulfoxide (P=S,SO) and the sulfone (P=S,-SO_2) of fenthion, the oxygen analogue (P=O,S) and its sulfoxide (P=O,SO) and sulfone (P=O,SO_2). Since the lipophilic properties of fenthion are well known (Möllhoff, 1971), one would expect greater residues of these compounds in fat than in muscle tissue of treated cattle. Methods for the extraction, separation, and quantitation of fenthion and metabolites from a variety of biological samples are available in Anderson et al. (1966), Bowman and Beroza (1968, 1969), and Suffet et al. (1967). Bowman and Beroza (1968) were the only ones to develop a method by which all six compounds could be analyzed individually by gas chromatography. Their method was developed for use with the substrates—corn, grass, and milk. We attempted to use their method for the extraction, cleanup, and quantitation of these six compounds in fat and found that it had to be modified to satisfy our needs.

The following is a description of the modified method we used to extract, separate, and analyze samples of fat for residues of fenthion and five oxidative metabolites.

EXPERIMENTAL SECTION

All chemicals were reagent grade or better. Isopropyl alcohol (chromatoquality reagent) obtained from Matheson, Coleman and Bell was used unchanged. All other solvents used—acetone, acetonitrile, hexane, and benzene—were redistilled in glass. The silica gel (60–200 mesh Baker analyzed reagent obtained from J. T. Baker) was used without drying but the anhydrous, reagent grade sodium sulfate was dried 3 h at 600 °C prior to use. The gas chromatograph, Micro Tek Model 220, was equipped with a flame photometric detector and a 4 mm i.d. × 1.22 m borosilicate glass column packed with Chromosorb 750 (80–100 mesh) coated with 3% OV-1. Carrier gas was prepurified nitrogen at a flow rate of 94 mL/min. The column was operated at 190 °C, injector at 225 °C, and detector at 180 °C in the phosphorus mode. A Polytron Homogenizer with a PT-20-ST generator was used to homogenize the fat samples in the extraction solvent. The liquid chromatograph, Waters Model 204ALC/GPC, was equipped with a Model 400 absorbance detector, a 254-nm wavelength filter, and a 4 mm i.d. × 30 cm μ Bondapak C 18 column. The solvent mixture was acetonitrile–H_2O (45:55) delivered at a flow rate of 1.2 mL/min. Analytical standards of fenthion and metabolites used in this study were supplied by Mobay Chemical Corp., Kansas City, Mo.

Extraction. Samples of fat (2 g) were weighed into 30-mL beakers. A 10-mL portion of boiling hexane was added, and the sample was homogenized with the Polytron Homogenizer at medium speed for approximately 1 min. The fat, dissolved in hexane, was filtered through 10 cm of anhydrous sodium sulfate in 13 mm i.d. × 20 cm glass column into a 250-mL separatory funnel. Another 10-mL portion of boiling hexane was added to the beaker, and the remaining tissue was blended and filtered as before. This step was then repeated. Then 30 mL of boiling hexane was used to rinse the generator shaft and the beaker and to wash any remaining fat or pesticide from the drying column into the separatory funnel. Twenty-five milliliters of acetonitrile saturated with hexane was added to the separatory funnel, and the funnel was shaken moderately for 1 min. After 5 min for phase separation, the acetonitrile layer was drained into a 125-mL flat-bottom boiling flask. This partitioning step was repeated twice, and all three portions of acetonitrile were combined in the boiling flask. Boiling chips were added to the combined acetonitrile extracts, a three-ball Snyder column was attached to the flask, and the acetonitrile was evaporated to ca. 5 mL on a hot plate. Twenty milliliters of hexane was added to the flask after partial cooling, and the solution was again evaporated to a low volume. (Hexane and acetonitrile form an azeotropic mixture.) The addition of hexane followed by evaporation was repeated twice or until all traces of acetonitrile were removed. The sample was quantitatively transferred to a 15-mL centrifuge tube and concentrated to ca. 1 mL with a Kontes tube heater and a slow stream of dry prepurified nitrogen.

Cleanup. The clean up column, as described by Bowman and Beroza (1968) (10 mm i.d. × 30 cm), was prepared by adding, in order, a glass wool plug, 2 g of anhydrous sodium sulfate, 4 g of silica gel, and 2 g of anhydrous sodium sulfate. The column was prewashed with 20 mL of 0.5% isopropyl alcohol in benzene. The prewash solvent was allowed to drain through the column until the liquid level reached ca. 2.5 cm above the packing. The liquid was held at this level for 30 min. Immediately after draining the solvent to the top of the column, the hexane solution from the extraction step containing the compounds of interest was quantitatively transferred to the column. The compounds were eluted from the column by solvent mixtures. Information about the elution of the various compounds from the chromatographic column is given in Table I. All three fractions were collected into 50-mL centrifuge tubes and were concentrated with a modified Kontes tube heater and a slow steam of dry prepurified nitrogen.

Quantitation. To achieve optimum sensitivity, the gas chromatographic column (3% OV-1) had to be sensitized by repeated injections of the appropriate standards (200 ng/μL) prior to the analysis of an unknown sample. The sensitization was accomplished faster by injecting standards contained in fat extracts. The gas chromatograph was used to analyze the compounds in fractions 1

Table I. Solvent Mixtures Used to Elute Compounds from the Chromatographic Column

eluting solvent	parts used: isopropyl alcohol	acetone	benzene	compounds eluted
1	0.5	0.5	99.0	P=S,S; P=S,SO_2
2	0.5	15.0	85.0	P=O,S; P=O,SO_2; P=S,SO
3	0.5	99.5	0	P=O,SO

Table II. Percent Recovery of Fenthion and Metabolites from Fortified Samples of Fat

compd	retention time, min	percent recov.[a]	range of fortification, μg/g[b]	eluting fraction
P=S,S	1.42	97.5	0.006-5.0	1
P=S,SO_2	3.42	99.3	0.05-1.0	1
P=O,S	1.25	90.4	0.013-0.25	2
P=O,SO_2	2.75	83.4	0.25-0.5	2
P=S,SO	3.42	99.8	0.05-0.5	2
P=O,SO	2.88[c]	81.8	0.1-0.5	3

[a] Average of two or more fortified samples. [b] The lower figure was the lowest level that could be accurately quantitated. [c] This metabolite was quantitated by liquid chromatography. (Retention volume was 3.46 mL). All other compounds were quantitated by gas chromatography.

and 2, i.e., P=S,S, P=S,SO_2, P=O,S, P-O,SO_2, and P=S,SO. After concentration, the residues were taken up in 0.5 to 10 mL of benzene. Usually 2-μL injections were used for the gas chromatographic analysis, but these varied with sample volume and concentration. Quantitation was achieved by comparing peak heights of unknowns to those from standard solutions. However, standards of P=S,SO were prepared in the fraction 2 eluate of control fat because of the improved response of the detector to this compound when the fat extract was present.

The liquid chromatograph was used to analyze the residue of fenthion oxygen analogue sulfoxide (P=O,SO) in fraction 3. Fraction 3 was transferred to a 5-mL centrifuge tube with three successive hexane rinses and was evaporated to near dryness with the Kontes tube heater. The sample was carefully taken to dryness with a dry nitrogen stream without heat to remove all traces of the eluting solvents. The residue was then taken up in 0.2 mL of acetonitrile–H_2O (45:55) by mixing with a vortex mixer. Injection volume was 5 μL. The flow rate was 1.2 mL/min, and the retention time was 2.88 min.

RESULTS AND DISCUSSION

Known amounts of standard solutions of fenthion and oxidative metabolites individually and together were added to preweighed 2-g samples of fat. These samples were held at room temperature for 10 min after fortification to allow the solvent to evaporate prior to extraction. Recoveries averaged 92.0% and ranged from about 81.8% for P=O,SO to 99.8% for P=S,SO. These results, along with the range of fortification, the levels that could be accurately quantitated, and the retention of time of each compound in the gas or liquid chromatograph are shown in Table II. Other fortified samples were held at room temperature for various time intervals up to 1 h prior to extraction. No differences in percent recovery could be found due to the length of time between fortification and extraction.

The metabolite, fenthion oxygen analogue sulfoxide (P=O,SO), can be analyzed by gas chromatography by the conditions reported here but the liquid chromatograph is much more sensitive (ca. 5×). Also, the OV-1 column used in the GC analysis is very difficult to sensitize to this compound.

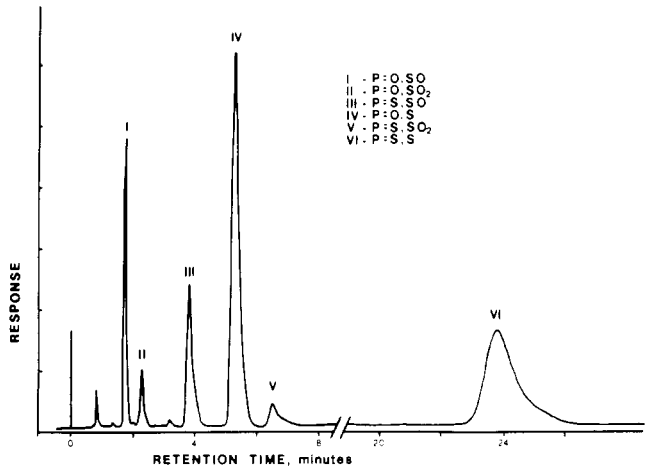

Figure 1. Liquid chromatogram of the separation of fenthion and oxidative metabolites obtained with LC equipped with μ Bondapak C 18 column, UV detector at 254 nm, and using an acetonitrile–water (45:55) solvent system at 2 mL/min.

Liquid chromatography was used as another means to separate and identify the parent compound and oxidative metabolites. The liquid chromatograph was used to separate all six compounds of interest on a μ Bondapak C 18 column with a solvent system consisting of acetonitrile–water (45:55). The chromatogram shown in Figure 1 was obtained from a standard mixture of the six compounds and a flow rate of 2 mL/min. The absolute retention times using the LC conditions mentioned above were: 1.73, 2.28, 3.82, 5.28, 6.50, and 23.74 min for P=O,SO, P=O,SO_2, P=S,SO, P=O,S, P=S,SO_2, and P=S,S, respectively.

LITERATURE CITED

Anderson, R. J., Thornton, J. S., Anderson, C. A., Katague, D. B., *J. Agric. Food Chem.* **14**, 619 (1966).
Bowman, M. C., Beroza, M. J., *J. Agric. Food Chem.* **16**, 399 (1968).
Bowman, M. C., Beroza, M., *J. Assoc. Off. Agric. Chem.* **52**, 1231 (1969).
Möllhoff, E., *Pestic. Sci.* **2**, 179 (1971).
Suffet, I. H., Faust, S. D., Carey, W. F., *Environ. Sci. Technol.* **1**, 639 (1967).

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