FENTHION

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DETERMINATIVE METHOD

A. INTRODUCTION

1. Theory

A weighed portion of tissue is ground in a mixture of acetone and chloroform and the aqueous phase is discarded. The organic phase is evaporated to dryness and the residue partitioned between petroleum ether and acetonitrile. The acetonitrile layer is evaporated to dryness and the residue is treated with an oxidation mixture and extracted with hydrochloric acid. The oxygen analog, sulfone, is extracted from the aqueous phase in chloroform and evaporated to dryness. The residue is dissolved in acetone and is quantitatively analyzed by means of a gas-liquid chromatograph using a KCl thermionic detector.

2. Applicability

This procedure is applicable to the determination of fenthion (0,0-Dimethyl 0-[4-(methylthio)-m-tolyl] phosphorothioate) and its metabolites in animal tissue.

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B. EQUIPMENT

1. Apparatus	a. Assorted laboratory glassware. b. Blenders: Waring or equivalent, equipped with one-quart jars. c. Centrifuge: Damon/IEC model BE-SO, or equivalent. d. Food chopper: Hobart or equivalent.
2. Instrumentation	a. Gas chromatograph: equipped with a detector, or equivalent. b. Rotary vacuum evaporator: all glass.

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C. REAGENTS AND SOLUTIONS

Reagent and Solution List

a. Acetone: Reagent, ACS, redistilled.

b. Acetonitrile: Technical, redistilled.

c. Chloroform: Reagent, ACS, redistilled.

d. m-Chloroperbenzoic acid, F.M.C. Corp., Villa Park, IL.

e. m-Chloroperbenzoic acid reagent: 10% (w/v) in isopropyl ether. Observe warnings on container label. Prepare only the amount needed.

f. Hydrochloric acid: 2.0N.

g. Hyflo Super-Cel: Johns-Manville.

h. Isopropyl ether: Reagent, ACS, Fisher Cat. #E-141.

i. Petroleum ether: Redistilled.

j. Sodium hydroxide: Analytical reagent, 0.5N.

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D. STANDARDS

1. Source

EPA
Director
Environmental Monitoring System Laboratory
Office of Research & Development
Cincinnati, OH 45268
513-569-7610 (use modem)

2. Preparation of Standards

Fenthion standard solution (5 μg/mL in acetone).

a. Weigh 0.05 g fenthion standard into a clean 100 mL volumetric flask. Make to volume with reagent acetone and shake to mix.

b. Transfer 1 mL of this solution to a clean 100 mL volumetric flask. Make to volume with reagent acetone and shake to mix. This flask contains 5 μg/mL of fenthion.

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E. EXTRACTION PROCEDURE

1. Sample Preparation

a. Grind the entire sample in a food chopper in the presence of an equal amount of dry ice.

b. Place the sample material in frozen storage overnight to allow the dry ice to sublime.

2. Sample Extraction—Fat Tissue

a. Weigh 25 g of the chopped sample into a blender jar.

b. Add 200 mL petroleum ether and blend for three minutes at high speed.

c. Filter, with suction, through a Whatman #42 filter paper covered with a ⅛-inch layer of Hyflo Super-Cel.

d. Transfer the filtrate to a 500 mL separatory funnel.

NOTE: If the filtrate becomes cloudy, warm the solution before the transfer is made.

e. Return the filter cake and filter paper to the blender jar.

f. Add 200 mL acetonitrile and blend for two minutes at high speed.

g. Filter, with suction, through a Whatman #42 filter paper into the same filter flask.

h. Transfer the filtrate to the separatory funnel containing the petroleum ether extract.

i. Shake the separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower phase into a second 500 mL separatory funnel containing 100 mL of Skellysolve B.

j. Shake the second separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower phase into a 1,000 mL round-bottom flask.

k. Repeat the extractions in steps i and j, using 200 mL portions of acetonitrile.

l. Evaporate the combined acetonitrile extracts (600 mL) to dryness on the evaporator at 40° C. Continue with step E.4.

3. Sample Extraction—Other Tissues

a. Weigh 50 g of the chopped sample into a blender jar.

b. Add 15 g of Hyflo Super-Cel and 200 mL acetone and blend for three minutes at high speed.

c. Filter, with suction, through Whatman #42 filter paper.

d. Transfer the filtrate to a 1,000 mL separatory funnel.

e. Return the filter cake and filter paper to the blender jar.

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E. EXTRACTION PROCEDURE (Continued)

f. Add 200 mL chloroform and blend at high speed for three minutes.

g. Filter, with suction, through a Whatman #42 filter paper covered with a ⅛-inch layer of Hyflo Super-Cel into the same flask.

h. Rinse the blender jar with 100 mL chloroform and add the rinsings to the dry filter cake.

i. Transfer the filtrate to the separatory funnel containing the acetone extract.

j. Shake the separatory funnel for 20 seconds, allow the layers to separate, and draw off the lower phase through a 32 cm Whatman #12 fluted filter paper into a 1,000 mL round-bottom flask.

k. Evaporate the sample to dryness on the rotary evaporator at 40° C.

l. Transfer the sample residue to a 500 mL separatory funnel with 200 mL petroleum ether.

m. Rinse the flask with 200 mL acetonitrile and add the rinsings to the separatory funnel containing the petroleum ether solution.

n. Shake the separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower phase into a second 500 mL separatory funnel containing 100 mL petroleum ether.

o. Shake the second separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower phase into a 100 mL round-bottom flask.

p. Repeat the extractions in steps n and o with two 100 mL portions of acetonitrile.

q. Evaporate the combined acetonitrile extracts (400 mL) to dryness on the evaporator at 40° C.

4. Oxidation—All Samples

Start an appropriate fenthion standard.

a. Dissolve the residue from the acetonitrile evaporation in 10 mL of the m-chloroperbenzoic acid reagent.

b. Allow the sample to stand at room temperature for 30 minutes with occasional swirling.

NOTE: Do not allow the sample to remain in contact with the concentrated oxidant for more than 45 minutes.

c. Add 10 mL isopropyl ether and transfer the sample to a 125 mL centrifuge separatory funnel.

d. Shake the separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower phase into a second 125 mL centrifuge separatory funnel containing 20 mL isopropyl ether.

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E. EXTRACTION PROCEDURE (Continued)

e. Add 80 mL of 2.0N hydrochloric acid to the first separatory funnel and shake for 30 seconds.

f. Shake the second separatory funnel for 30 seconds, if necessary; then centrifuge the sample for 5 minutes at 800 rpm.

g. Draw off the lower phase into a 500 mL separatory funnel.

h. Draw off the lower phase of the first 125 mL separatory funnel into the second 125 mL separatory funnel and repeat steps f and g.

i. Add 200 mL chloroform to the 500 mL separatory funnel containing the aqueous extracts (160 mL).

j. Shake the separatory funnel for 30 seconds, allow the layers to separate, and draw off the lower phase into a second 500 mL separatory funnel.

k. Repeat step j with 100 mL chloroform.

l. Add 100 mL of 0.5N sodium hydroxide to the combined chloroform extracts and shake the separatory funnel for 30 seconds.

m. Allow the layers to separate, and draw off the lower phase through a 32 cm Whatman #12 fluted filter paper into a 500 mL round-bottom flask.

n. Evaporate the sample to dryness on the evaporator at 40° C, removing any traces of chloroform with a gentle stream of air.

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F. ANALYTICAL QUANTITATION

1. Instrumental Settings and Conditions

Chromatographic conditions.

a. Column: 16 inch × 3 mm i.d. borosilicate glass column packed with 10% D.C. 200 and 0.2% QF-1, or equivalent, on 80-100 mesh Gas Chrom Q.

b. Carrier gas: Nitrogen, 35 mL/min, 40 psi.

c. Hydrogen flow: 35 mL/min, 12 psi.

d. Air flow: 425 mL/min, 30 psi.

e. Temperature:

i. Column—210° C.

ii. Injection port—225° C.

iii. Detector—240° C.

f. Recorder chart speed: ½-inch per minute.

g. Electrometer range: 100.

h. Attenuation: 1.

2. General Operation

a. Dissolve the residue from oxidation in 2 mL acetone.

b. Using a microliter syringe, inject 4 μL of the sample or standard solution into the gas chromatograph.

c. Identify the fenthion peak by its retention time and measure the area produced on the recorder strip chart with a polar planimeter. At the operating conditions employed, the retention time for the fenthion oxygen analog sulfone is 3.5 minutes.

3. Reference

Anderson, R. J., et. al., *Journal of Agriculture and Food Chemistry*. 14, 6, 619 (1961).

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G. CALCULATIONS

Procedure

Calculation of the ppm of fenthion in a sample is done by use of the following equation in which response for an unknown is compared to the response for an amount of standard fenthion carried through the procedure from the oxidation step. In the case of a 50 g sample, 5 μg of standard fenthion is used, which corresponds to 0.1 ppm.

$$\text{ppm} = \frac{\frac{\text{Area (Sample)}}{\text{Area (Standard)}} \times \frac{\text{Attenuation (Sample)}}{\text{Attenuation (Standard)}} \times \frac{\text{Std. inj.}}{\text{Sample wt. in g}}}{\text{Dilution Factor}}$$

When using the aliquots and dilutions described in the above procedure, the equation simplifies to:

$$\text{ppm} = \frac{\text{Area (Sample)}}{\text{Area (Standard)}} \times \frac{\text{Attenuation (Sample)}}{\text{Attenuation (Standard)}} \times 0.1$$

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H. HAZARD ANALYSIS

1. Method Title	Determination of Fenthion Residues in Animal Tissues		
2. Required Protective Equipment	Safety glasses, plastic gloves, lab coat.		
3. Procedure Steps		*Hazards*	*Recommended Safe Procedures*
	C. Reagents		
	Acetone Acetonitrile Chloroform m-Chloroperbenzoic acid	These reagents are very flammable and corrosive, and the vapors are extremely irritating to the skin, eyes, and respiratory system.	These solvents should only be used in an efficient fume hood, away from any electrical heating devices.
	D. Extraction Procedure		
	Extraction of fat tissue Extraction of other tissues	The relatively large volumes of reagents listed above plus petroleum ether present an increased risk. The concentration of these solvents also poses a danger (rotary evaporation).	It is important that these steps be performed in the fume hood.
4. Disposal Procedures	Organic solvents	See above	Segregate chlorinated from nonchlorinated solvents to the extent possible. Hold in designated storage cans until disposed of by the contractor or in-house specialist.