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Improved Method for the Determination of Fenamiphos and Its Sulfoxide and Sulfone Using Capillary Gas Chromatography and Thermionic Detection

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Methodology is presented for the determination of the nematicide fenamiphos (Nemacur) and its two major oxidative metabolites, fenamiphos sulfoxide and fenamiphos sulfone, in turfgrass and soil. Special consideration was given to improve analysis time, sample cleanup, and analytical resolution as compared to published methodology. Fenamiphos and its sulfone were separated from plant waxes and the sulfoxide metabolite on a Florisil cleanup column. Greater cleanup of the fenamiphos and sulfone fraction was obtained with a charcoal–cellulose column. Residue levels were quantified using capillary gas chromatogrpahy and nitrogen–phosphorus specific thermionic detection. Recoveries of the three chemicals from samples fortified from 0.005 to 1.0 ppm ranged from 80.1% to 96.3% depending on the analyte and sample matrix.

INTRODUCTION

Fenamiphos (Nemacur, ethyl 3-methyl-4-(methylthio)-phenyl (1-methylethyl)phosphoramidate) is a potent nematicide that exhibits some systemic insecticidal character (Mobay Chemical, 1982). The chemical is registered on a wide variety of crops and ornamentals including pineapple, grapes, and turfgrass. In plants and soils fenamiphos degrades readily by oxidation at the thioether linkage to form fenamiphos sulfoxide and fenamiphos sulfone (Waggoner, 1972; Waggoner and Khasawinah, 1974).

In preparation for a fenamiphos turfgrass study requiring monitoring of the parent compound and its sulfoxide and sulfone, published analytical methods were reviewed. Unsuitable for this study were methods that determined parent compound only (Sagredos and Eckert, 1977) or that determined parent and metabolites as the sulfone oxidation product following treatment with potassium permanganate (Thornton, 1971). Two published methods (Bowman, 1972; Brown, 1981) were attempted, but difficulties were encountered in the areas of sample cleanup and analyte recovery. These problems were overcome with a method that also reduces analysis time compared to published methods and takes advantage of the power and speed of capillary gas chromatography.

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EXPERIMENTAL SECTION

Apparatus. Extractions were performed on a Tekmar Ultra Turrax tissumizer. A Hewlett-Packard HP-5710A gas chromatograph was used equipped with split injection and Hewlett-Packard Model HP-18789A dual nitrogen–phosphorus specific detectors. The capillary column used was a J&W Scientific DB-5 (0.251 mm i.d. × 25 m, d_f = 0.25 μm). A Hewlett-Packard HP-3380A integrator was used for gas chromatography peak quantitations.

Reagents. Florisil (Floridin Co.; PR Grade, 60/100 mesh), anhydrous Na_2SO_4, and Whatman CF11 cellulose powder were used as received. Nuchar C-190 (charcoal) was acid washed (Brown, 1975), and a 2:5 mixture (w/w) of nuchar–cellulose powder (Brown, 1981) was prepared and mixed by tumbling overnight. All solvents were pesticide residue grade or equivalent. The analytical standard of fenamiphos (98%) was obtained from Chem Service, Inc. West Chester, PA. The analytical standards of fenamiphos sulfoxide (99%) and fenamiphos sulfone (99%) were supplied by Mobay Chemical Corp., Kansas City, MO.

Sample Preparation. Loamy sand soil from Lindcove, CA, was seived through a 6-mesh screen. The soil contained 11.0% moisture and was stored in airtight plastic bags. Turfgrass was sampled from a golf course putting green on the San Francisco peninsula using a reel-type

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lawn mower set at $^5/_{16}$ in. Clippings were approximately 0.5 cm in length and contained 72.0% moisture. The turfgrass samples were placed in plastic bags, immediately frozen with dry ice, and then kept at −18 °C. Twenty-gram aliquots of soil and turfgrass were placed in 250-mL Erlenmeyer flasks for the recovery studies.

Sample Fortification. All stock solutions were made from ethyl acetate. Soil and turfgrass aliquots were fortified by syringe with appropriate volumes of 10 or 100 ng/μL stock solutions. The solvent was allowed to evaporate for 10 min in a fume hood under a gentle stream of air. Soils were then brought to 50% moisture (w/w) and allowed to sit for 10 min prior to extraction.

Sample Extraction. A 180-mL portion of ethyl acetate–acetone (4/1, v/v) was added to the soil or turfgrass sample. The sample was extracted for 2 min on the tissumizer operating at full speed. The mixture was filtered through a Buchner funnel containing Whatman #1 filter paper into a 500-mL vacuum flask. The filter cake and filter paper were returned to the 250-mL Erlenmeyer flask along with 100 mL of fresh extracting solvent. The mixture was extracted for 2 min on the tissumizer at full speed and filtered as before, the filtrates being combined. The extraction flask and filter cake were rinsed with a fresh 50-mL aliquot of extracting solvent, and the rinsate was combined with the previously obtained filtrates. The extracted sample was transfered to a 500-mL round-bottom flask through a bed of anhydrous Na_2SO_4 to remove any particulates and some water. Approximately 10 g (1 teaspoon) of anhydrous Na_2SO_4 was added to the turfgrass samples. A 50-μL portion of a 1% OV-101 in ethyl acetate was added to the sample as a keeper and the sample concentrated just to dryness in vacuo on a 35 °C rotary evaporator equipped with a dry-ice-filled cold finger. A 10-mL portion of benzene was added and swirled to dissolve the sample in preparation for the cleanup step. *Note*: A variety of health hazards are associated with the use of benzene. All work using benzene sould be performed in a properly functioning fume hood, and solvent-resistant gloves should be worn to minimize exposure.

Column Cleanup. Two glass columns (10 mm i.d. × 300 mm, 75-mL reservoir) were plugged at the bottom with wads of glass wool. To one column was added 1 cm of anhydrous Na_2SO_4, 3 g of Florisil, and a cap of 1 cm of anhydrous Na_2SO_4. In the second column was sandwiched 1 g of the nuchar–cellulose (2:5 w/w) mixture between two 1-cm layers of anhydrous Na_2SO_4. In a fume hood, both columns were rinsed with benzene and a receiving vessel placed under the Florisil column. The sample was applied to the Florisil column, and the round-bottom flask was immediately rinsed with 10 mL of 2.5% acetone in benzene (v/v). The sample extract was allowed to reach the top of the Na_2SO_4, and the rinse was applied to the column. The rinse step was repeated with a second 10-mL aliquot at 2.5% acetone in benzene. The second rinse was allowed to pass through the Florisil column and all eluate generated to this point discarded. The Florisil column was positioned above the nuchar column to allow it to drip into the nuchar column. A 200-mL round-bottom flask was placed under the nuchar column, and fenamiphos and fenamiphos sulfone were eluted from the Florisil column with 40 mL of 25.0% acetone in benzene. The Florisil column was placed over a 100-mL round-bottom flask and fenamiphos sulfoxide eluted with 45 mL of 80.0% acetone in benzene. When the Florisil column eluate that contained fenapmiphos and its sulfone had passed through the nuchar column, 20 mL of 25.0% acetone in benzene was added to the nuchar column. The chromatographic process through the nuchar column can be expedited by applying 5–10 psi of air pressure to the top of the column without the loss of effective sample cleanup. To each of the two fractions generated in the cleanup step was added 50 μL of 1% OV-101 in ethyl acetate as a keeper, and the two fractions were concentrated in vacuo on the 35 °C rotary evaporator. For gas chromatographic analysis, the fernamiphos and sulfone fraction was brought up to an appropriate volume in a 5-mL test tube with ethyl acetate. The fenamiphos sulfoxide fraction was brought to volume in a 5-mL test tube with acetone for gas chromatographic analysis.

Table I. Data for the Recovery of Fenamiphos and Its Sulfoxide and Sulfone from Soil and Turfgrass

fortific level, ppm	% rec ± SD (n = 3)		
	fenamiphos	fenamiphos sulfoxide	fenamiphos sulfone
	Soil		
1.0	88.0 ± 1.7	80.1 ± 6.4	97.0 ± 4.6
0.1	88.5 ± 8.1	83.3 ± 5.8	94.0 ± 6.1
0.005	94.0 ± 3.5	86.4 ± 3.7	95.3 ± 3.5
	Turfgrass		
1.0	91.3 ± 3.5	84.3 ± 2.9	91.9 ± 3.8
0.1	87.2 ± 5.1	87.1 ± 5.6	96.3 ± 2.5
0.01	85.5 ± 3.1	83.6 ± 6.0[a]	93.8 ± 4.2

[a] Recovery value for fortification at 0.05 ppm.

Gas Chromatographic Analysis. Standards of fenamiphos and fenamiphos sulfone were combined in ethyl acetate for use in quantitation by gas chromatography. Fenamiphos sulfoxide standards were made separately in ethyl acetate. Samples fortified at 0.005 and 0.01 ppm were brought to 0.5 and 1.0 mL, respectively, for GC analysis, and these samples were compared to 0.2 ng/μL standards. Other fortification levels were brought to volumes appropriate for comparison to 1 ng/μL standards.

Injection into the gas chromatograph were made by using the cold-needle solvent flush technique with a split ratio of 1:20 and an inlet temperature of 250 °C. The DB-5 fused silica capillary column was operated at 235 °C with hydrogen as the carrier gas with a linear velocity of 80 cm/s. The detector end of the capillary column was fed to the top of the thermionic detector jet. The detector was operated at 300 °C and had gas flows of 30 mL/min nitrogen makeup, 1.4 mL/min hydrogen, and 50 mL/min air. Quantitation was performed by integration of peak area and comparison to external standards. Retention times for the analyses under these conditions were 1.90 min for fenamiphos, 3.74 min for fenamiphos sulfoxide, and 3.83 min for fenamiphos sulfone.

RESULTS AND DISCUSSION

The average recoveries of fenamiphos and its two major thio oxidation products from soil are shown in Table I. The soil moisture content was brought to 50% (w/w) to promote complete extraction of field-incurred residues (Brown, 1981). Figures 1a and 2a show gas chromatograms of fenamiphos and metabolite standards at levels of 0.4 ng injected. Figure 1 chromatograms are of fractions containing fenamiphos and its sulfone while Figure 2 chromatograms show fenamiphos sulfoxide. Recoveries of fenamiphos averaged 90.2% from soils fortified at 0.005–1.0 ppm on a wet weight basis. Fenamiphos sulfoxide and fenamiphos had average recoveries of 83.3% and 95.4%, respectively, from soils fortified at levels of 0.005 to 1.0 ppm. Figures 1b and 2b contain gas chromatograms from untreated soil. Figures 1c and 2c are for soil fortified at 0.005 ppm. The presence of turfgrass coextractives in

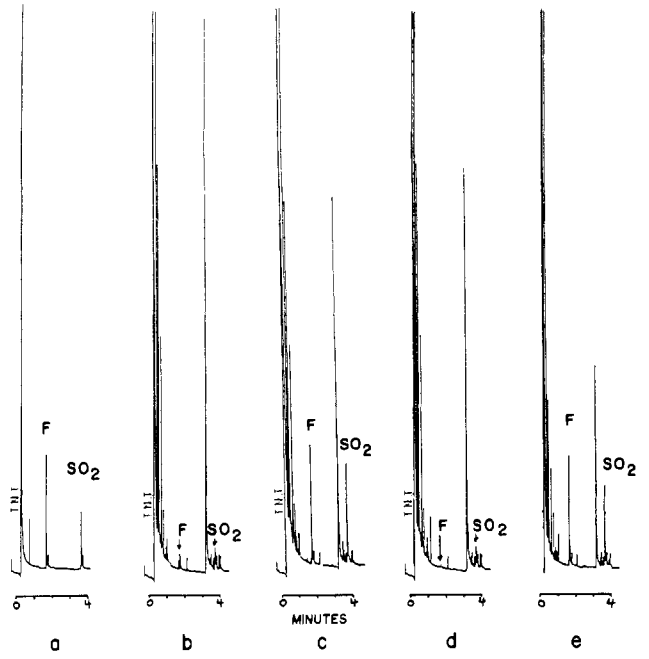

Figure 1. Fenamiphos and fenamiphos sulfone analysis: (a) 0.4 ng each of standards of fenamiphos and fenamiphos sulfone; (b) the fenamiphos and sulfone fraction from untreated soil; (c) fortified at 0.005 ppm (injections are equivalent to 80 mg of soil); (d) and (e) fenamiphos and sulfone fractions from untreated turfgrass and from turfgrass fortified at 0.01 ppm, respectively (injections equivalent to 40 mg of turfgrass). Fenamiphos is F, and fenamiphos sulfone is SO_2.

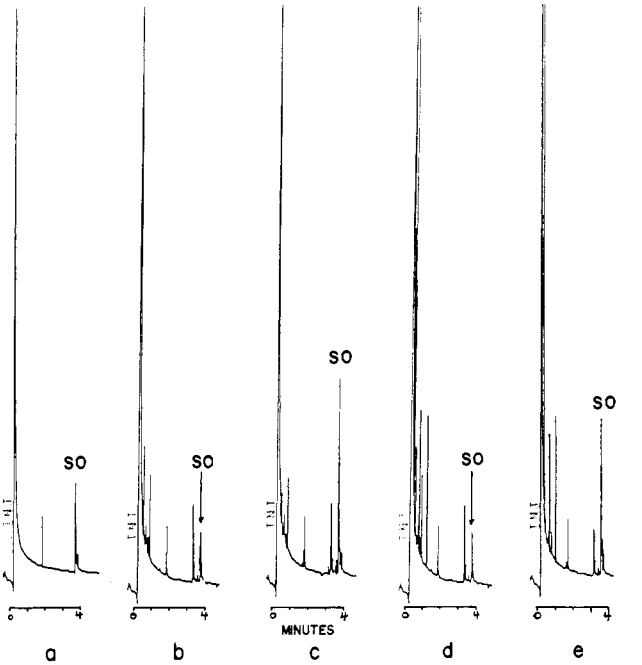

Figure 2. Fenamiphos sulfoxide analysis: (a) chromatogram of 0.4 ng of fenamiphos sulfoxide standard. Other chromatograms show fenamiphos sulfoxide fractions from soil that was untreated (b) or fortified at 0.005 ppm (c). The injections are equivalent to 80 mg of soil. Fenamiphos sulfoxide fractions from untreated turfgrass and from turfgrass fortified at 0.05 ppm are shown in (d) and (e), respectively (injections are equivalent to 8 mg of turfgrass). Fenamiphos sulfoxide is SO.

the samples following cleanup increased the minimum method sensitivity of fenamiphos and its metabolites by 2–10-fold. Fenamiphos and fenamiphos sulfone had average recoveries of 88.0% and 94.0.0%, respectively, from turfgrass samples fortified at 0.01–1.0 ppm. Fenamiphos sulfoxide was recovered with an average efficiency of 85.0% from samples fortified at 0.05–1.0 ppm. Figures 1d and 2d show gas chromatograms of an untreated turfgrass sample, and Figures 1e and 2e represent a sample fortified at 0.05 ppm.

Sample cleanup was the focus of most of the problems encountered in this laboratory with published fenamiphos procedures. Previous procedures used a silica gel column with either hexane–acetone or benzene–acetone solvent mixtures to achieve cleanup and analyte separation (Bowman, 1972; Brown, 1981). In both procedures, the analytes were separated into a fraction containing fenamiphos and its sulfone and a second fraction containing fenamiphos sulfoxide. This was done since adequate separation of the sulfoxide from the sulfone was difficult to obtain by gas chromatography. Isolating the sulfoxide from the other two chemicals of interest provided an additional benefit in allowing the further cleanup of the fenamiphos and fenamiphos sulfone fraction by nuchar, a means that would irreversibly bind the sulfoxide.

The first cleanup procedure attempted in this laboratory utilized activated silica and hexane–acetone as the eluting solvents. This solvent pair was chosen to avoid the hazards associated with the use of benzene. A problem occurred with the hexane–acetone solvent pair, when large amounts of coextracted turfgrass waxes were not removed from the sample. These waxes precipitated from solution upon freezing and, when present in the sample, caused severe overload and contamination of the gas chromatograph. Efforts to remove the plant waxes from the sample by filtering the frozen sample or rinsing the silica gel column with various concentrations of hexane–acetone met with failure. Insufficient separation of fenamiphos from the plant waxes was always observed. Other solvent systems using hexane and various polar solvents did not yield a useful combination.

Improved turfgrass sample cleanup was provided on silica gel using benzene–acetone as the eluting solvent pair. This solvent pair was able to separate fenamiphos from plant waxes, but breakdown of fenamiphos was observed. In order to remove plant waxes from the sample, the activated silica gel column was rinsed with 10–20 mL of 2.5% acetone in benzene. The waxes eluted before fenamiphos, but 5–20 of the applied fenamiphos degraded to its sulfoxide in agreement with a previous publication (Bowman, 1972). The breakdown of fenamiphos on activated silica gel was also seen with toluene–acetone mixtures to approximately the same extent as benzene–acetone mixtures. Breakdown was only observed if a rinse step prior to fenamiphos elution was used and if one of the elution solvents was aromatic. When the moisture content of the silica gel was increased to 15% (w/w), no breakdown of fenamphos was observed. However, the cleanup obtained using silica gel with 15% water was inadequate. Substances of sample origin coeluted from the GC column with the sulfone and the sulfoxide making quantitation impossible.

The use of activated Florisil and nuchar (charcoal) as adsorbants and benzene–acetone as the eluting solvent pair provided the best observed sample cleanup. Plant waxes were separated from the analytes on the Florisil column. The Florisil column, like a silica gel column, effects the separation of fenamiphos and fenamiphos sulfone from fenamiphos sulfoxide. The fraction containing the parent compound and the sulfone metabolite were subjected to further cleanup on a nuchar–cellulose column. No breakdown of fenamiphos was observed at any step of the cleanup procedure.

Other cleanup procedures for the fenamiphos sulfoxide fraction were also investigated by column chromatography

and absorbants such as nuchar, neutral alumina, and SepPack C-18 cartridges. Although a number of solvent systems were tried with each polar adsorbant, fenamiphos sulfoxide did not elute from the carbon or alumina columns, even when methanol was used. Reversed-phase cleanup using C-18 cartridges was prone to adsorbant overload by turfgrass plant coextractives.

Injections into the gas chromatography were made at a very low split ratio of 1:20. This provided good sensitivity without column overload. The low split ratio was made possible by the use of hydrogen as the carrier gas. Since hydrogen operates at twice the linear velocity of helium carrier gas, the gas flow through the injector is twice as great for the same split ratio. This means that if helium is the carrier gas and a split ratio of 1:40 is required for adequate flushing of the injector, than a split ratio of 1:20 could be used if hydrogen was the carrier, resulting in detection limits being reduced in half simply by changing carrier gases. Although glass wool in split injector inserts is not generally recommended for optimum injection reproducability and sample descrimination (Grob, 1981), in the field of pesticide residue analysis, glass or quartz wool in the injector can be highly desirable. Column contamination can be significantly reduced since the glass wool plug provides a surface where most nonvolatile materials can condense prior to entering the capillary column. If the samples for GC analysis are somewhat dirty, as most environmental samples are, the glass wool can save many centimeters of capillary column that would otherwise be rendered useless. Multiple injections of soil and turfgrass samples caused the glass wool to become contaminated, and it was replaced several times without loss of column performance. With glass wool in the injector insert, the reproducability of repetitive 2-μL manual injections was $\pm 5\%$ in this study, which is acceptable for most applications.

Fenamiphos sulfoxide and, to a lesser degree, fenamiphos sulfone are relatively difficult to chromatograph on the gas chromatograph unless the system is very inert. Poorly silanized injector inserts, activated columns, or improper placement of the column in the detector can lead to extremely low response and badly tailing peaks.

Separation of fenamiphos sulfoxide and fenamiphos sulfone on a 25-m DB-5 (5% phenyl methylsilicone) capillary column and a 25-m DB-1 (100% methylsilicone) was attempted. In each case, base-line resolution is nearly obtained for a new column but is insufficient for quantitative purposes. An older column that has lost some efficiency does not come close to delivering useable separations. Base-line separation of the sulfoxide from the sulfone should be readily attained on a 50–60-m DB-1 or DB-5 capillary column, but at the expense of long analysis times. Since these columns were not available in the laboratory, the hypothesis was not tested. A 15-m DB-225 (50% (cyanopropyl)phenyl dimethylsilicone) capillary column gave excellent separation of the fenamiphos metabolites, but analysis times were very long (35 min) at the 220 °C upper temperature limit of the column. A 15-m DB-1701 (14% (cyanopropyl)phenyl dimethylsilicone) capillary column operating at 250 °C provided good separation of all three chemicals with an analysis time of 5 min. Unfortunately, this column was not available for routine use and was not used for this study.

Previously published methods used flame photometric detection in the phosphorus mode to quantitate fenamiphos residues. In this study a nitrogen–phorphorus specific thermionic detector was used without any unusual sample-related interferences or operation problems. The gas chromatograph could detect 100 pg of fenamiphos injected, which is about 6 pg to the detector. Detection of fenamiphos sulfoxide and fenamiphos sulfone was about 10 pg each to the detector.

The ease and speed of analytical procedure are of great importance to the analyst, especially when a large number of samples is involved. Three areas of this procedure were improved over previous procedures to minimize analysis time. The first area was in the sample extraction step. The use of a tissumizer to extract field-treated samples was found to give similar results as compared to a 4-h Soxhlet extraction. Sample extraction using the tissumizer requires about 10 min/sample as compared to approximately 5 h/sample required to perform Soxhlet extractions. The second area that was modified in the method was in the choice of extracting solvent. The extracting solvent was chosen to eliminate a separatory funnel type extraction and provide high solubility to a wide range of polar and nonpolar solutes. The solvent forms an azeotrope with a relatively high percentage of water (about 3% water at 150 mm). Since approximately 300 mL of extracting solvent is used per sample, up to 9 mL of water could be removed from a 20-g sample without an aqueous/organic solvent partition step. Samples with higher moisture contents can be accommodated by adding 10 g of Na_2SO_4 to the sample prior to concentrating the extraction solvent. Water-soluble coextractives from the samples that would be removed in an aqueous/organic solvent partition did not interfere with the analysis. Separatory funnel extractions are relatively slow and usually result in a sample extract requiring a concentration step prior to the next manipulation. If the only reason for the extraction step was to remove water or change solvent polarities, a solvent that forms an azeotrope with water can save time. The gas chromatograph was the third area where analysis time was reduced. The capillary column gave more resolving power and also reduced analysis time compared to a packed column. Using hydrogen as the carrier gas is also a time saver, cutting the GC analysis time in half as compared to using helium.

Registry No. Fenamiphos, 22224-92-6; fenamiphos sulfoxide, 31972-43-7; fenamiphos sulfone, 31972-44-8.

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