Determination of Residue Levels of the Herbicide Fluridone by Electron-Capture Gas Chromatography

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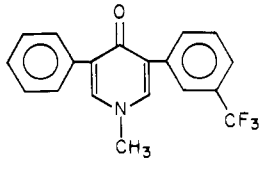
Analytical procedures are described for determining residues of the herbicide fluridone, 1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4(1*H*)-pyridinone, in soil, water, plant tissue, processed cottonseed oil, fish, zooplankton, and animal feed. Following suitable extraction and purification steps, a novel bromination derivative is prepared by reacting fluridone with phosphorus tribromide in the presence of pyridine. Measurement of the derivative is accomplished by gas chromatography with electron-capture detection. The method is capable of determining 0.5 ppb of fluridone in water and 10–30 ppb in other substrates.

Fluridone, 1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone, is a new broad spectrum preemergence herbicide currently being developed by Elanco Products Co. (a Division of Eli Lilly and Co.). The chemical and physical properties, toxicological data, herbicidal properties, and field performance of fluridone have previously been described (Waldrep and Taylor, 1976, 1977; Webster et al., 1977). The absorption and translocation of fluridone in cotton, soybeans, corn, and rice has also been reported (Berard and Rainey, 1977).

The electron-capture detector and the element specific detectors commonly employed in gas chromatography exhibit insufficient response to fluridone for the determination of residues at the part per billion level. Consequently, it is necessary to derivatize fluridone to form a product with increased sensitivity to one of these detection systems.

In this paper a novel, pyridine-catalyzed reaction is described for converting microgram and submicrogram quantities of fluridone (I) to a brominated product (II)



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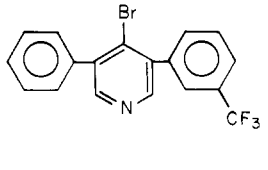

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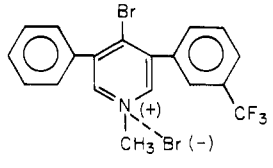

III

which is subsequently measured by electron-capture gas chromatography (EC-GC). In addition, procedures using this technique are described for determining fluridone residues in several types of environmental and agricultural samples.

EXPERIMENTAL SECTION

Apparatus, Chemicals, and Reagents. The analytical standard of fluridone was obtained from Lilly Research Laboratories (Greenfield, Ind.). All solvents were reagent grade. Hexane, benzene, and dichloromethane were redistilled in glass. Practical grade phosphorus tribromide (Matheson, Coleman, and Bell) and technical pyridine (Fisher Scientific) were used as received. Anhydrous sodium sulfate was washed with methanol and dried at 50 °C for 16 h. Alumina (Alcoa F-20) was dried at 110 °C for 16 h, deactivated with 4.0% water (v/w), and tumbled for 1 h in a closed container.

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Table I. Fortification and Recovery Levels of Fluridone in Various Samples

Sample	Fortification level, ppm	No. of fortifications	% recov range (Av)
Water	0.001	11	80-116 (93)
Soil	0.01-0.04	15	55-90 (76)
Aquatic plants	0.02	4	79-97 (88)
Rice grain	0.02-0.05	3	94-139 (110)
Rice hulls	0.02	1	68 (68)
Rice straw	0.05-0.50	6	71-113 (84)
Cottonseed	0.01-0.10	8	84-104 (88)
Cottonseed oil	0.05	1	83 (83)
Fish	0.02-0.04	10	65-121 (89)
Zooplankton	0.02-0.10	4	51-92 (75)
Animal feed	0.02-1.00	2	108-115 (112)

The gas chromatograph was a Hewlett-Packard Model 402 equipped with a ^{63}Ni electron-capture detector. The column was a 180 cm × 0.3 cm i.d. borosilicate glass tube containing 2% OV-17 on 80/100 mesh Chromosorb W-HP. The oven, detector, and injection block were operated at 195, 290, and 230 °C, respectively.

Extraction Procedures. Untreated control and fortified recovery samples were assayed in the same manner as experimental samples to determine background interference and recovery levels. Typical fortification levels and recoveries have been summarized in Table I.

Soil (25 g) was weighed into a pint Mason jar. A 50:50 mixture of methanol and 2 N sodium hydroxide (100 mL) was added and the fluid level was marked on the jar. The jar was covered with a watch glass and the sample was boiled in a boiling water bath. Methanol was added periodically to maintain the fluid level. After boiling for 1 h, the sample was cooled to room temperature and methanol was added to restore the original volume. A 10-mL aliquot of the supernatant liquid was transferred to a 125-mL separatory funnel containing 50 mL of 5% sodium chloride solution.

Finely ground plant tissue (25 g) or animal feed (10–25 g) was weighed into a pint Mason jar. Methanol was added to give a total extraction volume of 100 mL with allowance for moisture content of the sample. Bulky samples such as rice straw and hulls frequently required larger extraction volumes. The samples were blended for 10 min with an Omni mixer, or finely ground samples were shaken for 30

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min at 300 rpm on a platform shaker. An aliquot of known volume (10–30 mL) was transferred to a 250-mL separatory funnel containing 50 mL of 5% sodium chloride solution.

Processed cottonseed oil (2.0 g) was weighed into a 125-mL evaporating flask and dissolved in 30 mL of hexane. Methanol (30 mL) was then added to the flask, which was swirled to thoroughly mix the solution. The entire solution was transferred to a 250-mL separatory funnel containing 50 mL of 5% sodium chloride solution.

Sliced and chopped fish tissue (25 g) was weighed into a half-pint Mason jar. Methanol was added to give a total extraction volume of 100 mL with allowance for moisture content of the fish. The sample was blended for 10 min with a Waring blender, and a 10.0-mL aliquot was transferred to a 125-mL separatory funnel containing 50 mL of 5% sodium chloride solution.

Zooplankton (1–3 g) was weighed into a pint Mason jar. Methanol (20 mL) was added and the sample was shaken for 30 min at 300 rpm on a platform shaker. The entire sample was poured through folded filter paper into a 250-mL separatory funnel containing 50 mL of 5% sodium chloride solution. The Mason jar and the filter cake were rinsed twice with 5-mL portions of methanol.

Water (100 mL) was transferred to a 250-mL separatory funnel containing 30 mL of dichloromethane.

Liquid–Liquid Partition Procedure. Hexane (30 mL) was added to separatory funnels containing extracts of plant tissue, animal feed, fish, or zooplankton. (Extracts of cottonseed oil already contained 30 mL of hexane. Water and soil extracts did not require a hexane–water partition). The separatory funnels were shaken for 30 s, the phases were allowed to separate, and the aqueous layers were drained into a beaker. The hexane layers were discarded and the aqueous solutions were returned to the separatory funnels.

Cottonseed oil and rice straw frequently required an additional hexane wash. Following the second hexane wash, methanol was evaporated from the aqueous phase of cottonseed oil samples with a rotary vacuum evaporator and a 40 °C water bath. The removal of methanol at this stage increased recoveries for cottonseed oil samples only and was unnecessary for other types of samples.

Fluridone was extracted from the aqueous phase of all samples by shaking with two 30-mL portions of dichloromethane and draining the dichloromethane through a funnel containing sodium sulfate into a 125-mL evaporating flask. The sodium sulfate was washed with 10 mL of dichloromethane, and the sample was evaporated to dryness with a rotary vacuum evaporator and a 40 °C water bath.

Derivatization Procedure. Due to the hazardous nature of phosphorus tribromide, the derivatization procedure was performed in a hood. Contact with the reagent was avoided, and safety glasses were worn at all times.

Phosphorus tribromide (2 mL) was added to the flask and swirled briefly. One drop of pyridine was added and swirled. Boiling chips were added to the flask, and an air-cooled condensing tube (18.5 cm × 2.2 cm i.d.) was fitted into the neck of the flask. The flask was placed on a hot plate preheated to 150–170 °C. After 20 min the flask was removed from the hot plate and cooled at room temperature for 15 min. After the flask had cooled, 50 mL of cold 5% sodium hydroxide solution was poured through the top of the condensing tube to destroy the excess phosphorus tribromide. (Failure to cool the flask before adding the sodium hydroxide may result in a violent reaction. However, when the flask is properly cooled, the addition of the sodium hydroxide solution causes the reaction mixture to boil and fume mildly.) The flask was swirled, the condensing tube was removed, and the flask was cooled at room temperature for an additional 20 min.

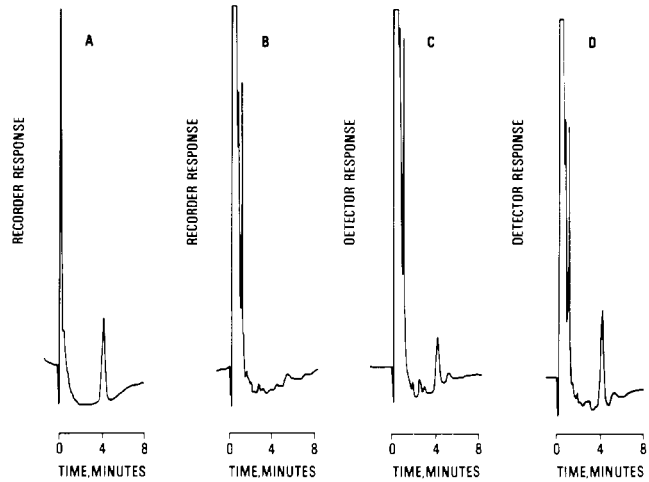

Figure 1. Gas–liquid chromatograms illustrating the determination of fluridone in soil as the brominated derivative II: (A) standard (II), equivalent to 0.19 ng of fluridone; (B) soil extract (untreated control); (C) soil extract (recovery, 0.02 ppm); (D) soil extract (experimental sample, 0.038 ppm).

The derivatization procedure was also performed for 100 μg of fluridone standard. The resulting brominated standard was used for preparing solutions for obtaining the standard response curve as described in the Gas Chromatography section.

Liquid–Liquid Partitioning Procedure. The reactant solution was transferred to a 125-mL separatory funnel. The flask was rinsed with 20 mL of dichloromethane, which was added to the separatory funnel. The resulting mixture was shaken for 30 s, and the dichloromethane layer was drained through a funnel containing sodium sulfate into a 125-mL evaporating flask. The extraction was repeated with a second 20 mL of dichloromethane, the sodium sulfate was rinsed with 10 mL of dichloromethane, and the combined extracts were evaporated to dryness with a rotary vaccum evaporator and a 40 °C water bath. The residue was dissolved in 5 mL of hexane.

Alumina Column Chromatography. A glass chromatography column (250 mm × 14 mm i.d.) containing a 250-mL reservoir was packed with 13 mL (11.4 g) of standardized, deactivated alumina, and topped with a 1.5-cm layer of sodium sulfate. The column was washed with 20 mL of hexane which was discarded. The sample was placed on the column with two 5-mL portions of hexane. Each portion was drained to the top of the sodium sulfate before additional portions were added to the column. An additional 50 mL of hexane was added and the eluent was discarded. The column was then eluted with 30 mL of benzene. The first 10 mL was discarded and the remaining 20 mL was collected in a 125-mL evaporating flask. The sample was evaporated to dryness with a rotary vacuum evaporator and a 40 °C water bath. The residues from experimental samples were dissolved in 1.0–3.0 mL of benzene for analysis by EC-GC. The 100 μg of standard brominated fluridone was dissolved in 100 mL of benzene for preparation of solutions for determining the standard response curve as described in the Gas Chromatography section.

Gas Chromatography. Solutions for obtaining the standard response curve were prepared from aliquots of the brominated fluridone standard (II) which was prepared as described above. Aliquots were diluted with benzene

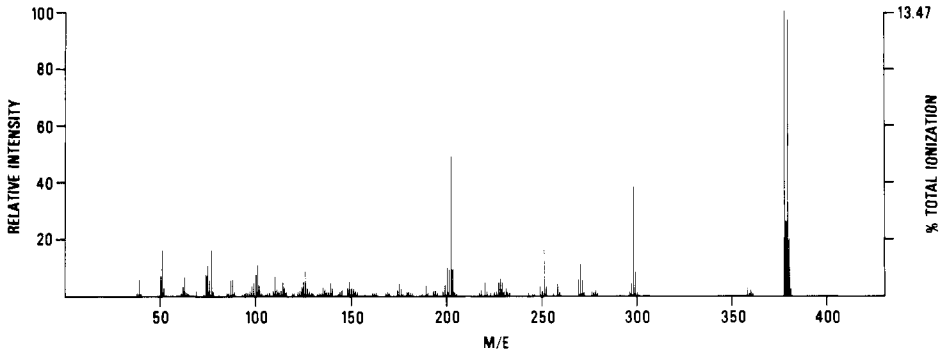

Figure 2. Normalized mass spectrum of the fluridone brominated derivative II.

to yield standard solutions of II equivalent to 0.01–0.05 μg/mL of fluridone. The injection of 0.04–0.20 ng of II resulted in a linear response curve with peak heights normally ranging from about 1–7 cm. Fluridone residues in experimental samples and fortified recoveries were determined by comparison with the standard response curve. Representative chromatograms are shown in Figure 1.

RESULTS AND DISCUSSION

The methods presented in this paper have been successfully applied to the determination of fluridone residues in soil, water, plant tissue, animal feed, fish, zooplankton, and processed cottonseed oil. Untreated control samples fortified with fluridone were carried through the assay procedure to determine levels of recovery, and the results are summarized in Table I. The methods have been capable of determining 0.5 part per billion (ppb) of fluridone in water and 10 ppb in soil, animal feed, cottonseed oil, and many types of plant tissue. The test sensitivity varied from 20–30 ppb for bulky plant samples such as rice hulls and straw.

Nonextractable residues of [^{14}C]fluridone were sometimes encountered when soil aged for at least 30 days was extracted with acetonitrile. Soil binding occurred primarily in soils which were air dried prior to analysis. However, boiling with the sodium hydroxide–methanol mixture consistently extracted 90–100% of the aged radioactivity from soil.

Less hazardous solvents may be substituted for benzene, if desired. The brominated derivative may be eluted from the alumina column with redistilled 1-chlorobutane, and residues may be dissolved in redistilled hexane or toluene for injection into the gas chromatograph.

The derivatization reaction did not proceed consistently without the addition of pyridine or dimethylformamide. The catalytic action of these two reagents is probably related to their electron-donating characteristics. Based upon measurements by gas chromatography with flame ionization detection, milligram amounts of fluridone reacted quantitatively to yield a single derivative. Quantitative yields were also obtained with microgram and submicrogram amounts of fluridone. The latter observation was based upon a similarity of EC-GC detector responses to solutions of 0.1 μg/mL of II prepared by derivatizing 1.0 mg, 1.0 μg, and 0.1 μg of fluridone.

The structure of the resulting derivative was confirmed to be that of II by GC–MS. The normalized mass spectrum in Figure 2 indicates that the brominated derivative has a molecular weight of 377. The molecular ion is also the base peak. The M and M + 2 peaks of nearly equal intensity are characteristic of a single bromine, and the peak at m/e 298 is due to the loss of –Br from the molecular ion.

The derivatization of a stable ketone moiety with phosphorus tribromide to form an electron-capturing derivative for low-level detection appears to be a novel reaction, and the mechanism of the derivatization is uncertain. It is possible that the reaction proceeds via a quaternary ammonium salt (III) resulting from the replacement of the oxygen with a single bromine atom and the subsequent aromatization of the pyridinone ring. The intermediate would then decompose during heating to yield II and methyl bromide. This mechanism, however, is only hypothetical at this time.

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Received for review August 18, 1977. Accepted January 16, 1978.