# Determination of Fluridone Residues in Meat, Milk, Eggs, and Crops by High-Performance Liquid Chromatography or Gas Chromatography

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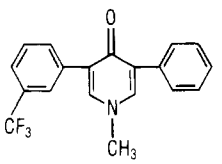
A residue method is described for the determination of the aquatic herbicide fluridone in meat, milk, eggs, and crops. Fluridone is extracted from meat, eggs, or crops with methanol, and the herbicide is extracted from milk with disposable C18 cartridges. Extracts are purified by liquid partitioning and/or alumina column chromatography. The purified extracts are concentrated and then measured by high-performance liquid chromatography with UV detection at 313 nm. The residue method has a detection limit of approximately 0.05 ppm for all sample types. Recoveries fortified with 0.05 and 0.10 ppm of fluridone have averaged 86 ± 10% for meat, 80 ± 4% for milk, 100 ± 8% for eggs, and 90 ± 12% for crops. The derivatization of fluridone with phosphorus tribromide for determination by gas chromatography with electron capture detection can be utilized as an alternative or confirmatory procedure.

Fluridone, 1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4(1*H*)-pyridinone (I), is an aquatic herbicide that results in the management of troublesome vascular aquatic



I

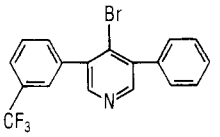
weeds at low application rates (Parka et al., 1978; McCowen et al., 1979; Grant et al., 1979; Rivera et al., 1979; Arnold, 1979; Sanders et al., 1979). The bioconcentration and field dissipation of fluridone and its degradation products in aquatic environments have been reported previously (West et al., 1979, 1983; Muir et al., 1980; West and Parka, 1981; Muir and Grift, 1982). The physical and chemical properties, metabolic and environmental transformations, and methods of analysis of fluridone have been reviewed (West, 1984).

Since animals may drink water treated with fluridone and crops may be irrigated with water containing the herbicide, analytical methods are needed for the enforcement of residue tolerances in the raw agricultural commodities. Prior to the development of residue methods, however, it was necessary to determine the nature of the residue in edible crops and animal products.

Uptake and metabolism studies with representative crops that were furrow or sprinkler irrigated with water containing radiolabeled fluridone indicated that the parent compound was the primary residue and that no major metabolites had formed (Berard and Rainey, 1981). Also, an investigation of the uptake, translocation, and metabolism of radiolabeled fluridone in corn, soybean, and cotton plants indicated that the parent compound was not metabolized by these crops (Berard et al., 1978). Animal metabolism studies have indicated that no major identifiable residues occurred in the meat, milk, or eggs of animals consuming water containing radiolabeled fluridone (Berard and Rainey, 1981).

Thus, on the basis of results of the radiolabeled studies, residue methods have been developed for the parent compound in meat, milk, eggs, and crops. Previous residue methods have been published utilizing gas chromatography with electron capture detection (GC-ECD) of fluridone in fish (West, 1978; West and Burger, 1980) and in plants and crops (West, 1978). A method utilizing high-performance liquid chromatography (HPLC) with UV detection of fluridone in fish and crayfish has also been developed (West and Day, 1986). The HPLC method involved an acidic hydrolysis step to release conjugated residues of fluridone and its hydroxylated metabolite, followed by cleanup on a disposable Florisil cartridge prior to separation of the two compounds on a reversed-phase C18 column.

The GC methods require a derivatization of fluridone with phosphorus tribromide to form a brominated derivative (II) suitable for electron capture detection. In this



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paper, methods are described for the determination of underivatized fluridone in milk, animal tissues, and crops by HPLC. The HPLC methods are advantageous in that they are more rapid and avoid the use of harzardous derivatizing reagents.

## EXPERIMENTAL SECTION

**Apparatus, Chemicals, and Reagents.** All solvents for HPLC and GC assays were HPLC and pesticide grade, respectively. 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. Sep-Pak C18 cartridges were used in conjunction with a Sep-Pak cartridge rack (Waters Associates, Inc.).

The gas chromatograph was a Hewlett-Packard Model 402 equipped with an electron capture detector. The column was a 180 cm × 0.4 cm (i.d.) borosilicate glass tube containing 3% OV-101 on 80/100-mesh Chromosorb W-HP. The oven, detector, and injection block were operated at 195, 275, and 230 °C, respectively.

The HPLC system consisted of a Waters Model 6000A solvent delivery system operated at a flow rate of 1.0 mL/min, a Waters Model 440 absorbance detector (fixed wavelength 313 nm) operated at 0.02 AUFS, a Waters Model 710A intelligent sample processor (100-μL injec-

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tion), a Houston Instruments Omni Scribe strip chart recorder operated at a chart speed of 0.167 cm/min, and a μBondapak C18 column with a Co-Pell ODS guard column (Whatman, Inc.). The mobile phase was methanol–water (70:30) at a flow rate of 1.0 mL/min.

**Residue Analysis.** (*A*) *Milk.* Two disposable C18 cartridges were joined together by means of a short piece of Tygon tubing (2 cm × 0.6 cm (i.d.)). The joined cartridges were then attached to the cartridge rack, which was adjusted to the discard (A) setting. The cartridge reservoirs were attached to the top of the joined cartridges, and the cartridges were rinsed with 20 mL of methanol, followed by 20 mL of water. (The vacuum was adjusted to result in the formation of discrete drops of liquid eluting from the cartridges.)

The milk sample (20 mL) was added to the reservoir and was passed through the cartridges, followed by the addition of 20 mL of water to elute residual milk droplets. After the vacuum had pulled essentially all of the residual water droplets through the cartridges, the cartridge rack was adjusted to the eluate collection (B) setting. Fluridone was eluted from the cartridges into the cartridge rack collection tubes with 8 mL of methanol. The methanol eluate was quantitatively transferred to a 125-mL evaporating flask, and the solvent was evaporated with use of a Rinco rotary vacuum evaporator and a 35–45 °C water bath. The residue was dissolved in 5 mL of hexane–dichloromethane (70:30, v/v), and the samples were purified by the alumina column procedure described for the analysis of meat, egg, and crop samples in section B.

(*B*) *Meat, Eggs, and Crops.* A 25-g sample of finely ground meat or crop sample, or a 25-g sample of blended eggs (yolks plus whites), was weighed into a 0.5-L jar. Methanol was added to result in a total extraction volume of 100 mL with allowance for the moisture content of the sample. (For lightweight, bulky crop samples such as grass or straw, a total of 200 mL was used.) The sample was shaken on a gyratory shaker at 250 rpm for at least 30 min. The extract was filtered through a funnel containing folded filter paper into a graduated cylinder. A 20-mL aliquot (40 mL for lightweight, bulky samples) was transferred to a 250–mL separatory funnel containing an equal volume of aqueous 5% NaCl solution. Hexane (40 mL) was added, and the separatory funnel was shaken vigorously for at least 20 s. The phases were allowed to separate, and the aqueous (lower) phase was drained into a beaker. The hexane (upper) phase was discarded, and the aqueous phase was returned to the separatory funnel. The partitioning step was repeated with a second 40-mL aliquot of hexane, which was discarded. Fluridone was then extracted from the aqueous phase by partitioning with three 40-mL aliquots of dichloromethane. After each partitioning, the dichloromethane (lower) phase was separated and drained through a funnel containing sodium sulfate into a 250-mL evaporating flask. The sodium sulfate was then rinsed with 15–20 mL of dichloromethane. The combined dichloromethane extract was evaporated to dryness with use of a rotary vacuum evaporator and a 35–45 °C water bath, and the residue was dissolved in 5 mL of hexane–dichloromethane (70:30, v/v) for purification by alumina column chromatography.

Prior to use, each new batch of alumina was standardized to determine the exact elution pattern of fluridone. The alumina was standardized by charging the column with 10 μg of fluridone and collecting 5 10-mL fractions of hexane–dichloromethane (70:30), followed by 12 10-mL fractions of dichloromethane. The eluate fractions were evaporated, dissolved in methanol–water (70:30), and injected into the HPLC to determine the elution pattern.

The alumina column chromatography purification was accomplished by wet-packing a glass column (250 mm × 14 mm (i.d.)) with 10 mL (9.6 g) of 4.0% water-deactivated alumina in a total of at least 40 mL of hexane–dichloromethane (70:30). The eluant was drained into a beaker. A 1-cm layer of sodium sulfate was added on top of the alumina, and the column was rinsed with 10–15 mL of hexane–dichloromethane (70:30). The sample extract was added to the column in 5 mL of hexane–dichloromethane (70:30). The flask was rinsed with two 5-mL aliquots of hexane–dichloromethane (70:30), which were separately added to the column and drained. The column was rinsed with an additional 25 mL of hexane–dichloromethane, followed by 20 mL of dichloromethane. All of the eluate collected thus far was discarded. Fluridone was then eluted from the column with 50 mL of dichloromethane into a 125-mL evaporating flask. The eluant was evapoarted to dryness with use of a rotary vacuum evaporator and a 35–45 °C water bath. The residue was dissolved in 4.0 mL of mobile phase (methanol–water, 70:30) for analysis for HPLC using the apparatus conditions listed previously.

## RESULTS AND DISCUSSION

**Method Validation and Analytical Recovery Efficiencies.** The analytical method was validated by determining the recovery efficiency for untreated control samples fortified with 0.05–0.1 ppm of fluridone. Recovery efficiencies were determined for whole milk, eggs (yolks plus whites), representative meat tissues, and representative crops. Meat tissues included in the validation study were beef liver, chicken lean, and ground pork sausage containing appreciable fat. Two representative crops from each of 13 different agricultural commodity groupings were analyzed (Table I). The recoveries are summarized in Tables I and II. Overall, the recovery averaged 80 ± 4% for milk ($n$ = 11), 100 ± 8% for eggs ($n$ = 12), 86 ± 10% for meat ($n$ = 35), and 90 ± 12% for crops ($n$ = 66).

A few of the crops listed in Table I (i.e., corn and soybean forage plants, alfalfa, orchardgrass, and grapefruit) produced interfering peaks on the HPLC chromatogram. However, it was possible to analyze these crops by a previously published method (West, 1978), which utilized gas chromatography with electron capture detection of a brominated fluridone derivative (II). The overall recovery averaged 69 ± 15% ($n$ = 10) for the gas chromatographic method.

The detection limit for the HPLC method (based upon a peak height response of at least 3 times base-line noise) was approximately 0.05 ppm for all of the different sample types. Representative chromatograms are contained in Figures 1–5.

**Storage Stability.** Since crop samples may be stored for varying periods of time prior to assay, a storage stability study was conducted with 19 different crop samples. Control samples fortified with 0.1 ppm of fluridone were stored in a freezer for 3–76 days before assay. The results, which are summarized in Table III, indicated that the recovery of fluridone from stored samples averaged 95 ± 14% of theory. Thus, instability of fluridone during freezer storage of crop samples was not a problem within the time frame studied.

A storage stability study was not conducted for meat, milk, or eggs because none of these sample types have been analyzed in our laboratory. Radiolabeled metabolism studies with [$^{14}$C]fluridone have demonstrated that meat, milk, and eggs from animals dosed with fluridone did not

**Table I. Recovery of Fluridone from Crops Representative of 13 Different Commodity Groupings**

| commodity gp | crop | method | % recovery 0.05 μg/g | % recovery 0.1 μg/g |
|---|---|---|---|---|
| citrus crops | grapefruit | GC | | 60 |
| | | | | 105 |
| | orange | HPLC | | 98 |
| | | | | 98 |
| cucurbits | cucumber | HPLC | | 81 |
| | | | | 88 |
| | squash | HPLC | | 100 |
| | | | | 94 |
| forage grasses | orchardgrass | GC | | 76 |
| | | | | 63 |
| | corn plant | GC | | 58 |
| | | | | 55 |
| forage legumes | alfalfa | GC | | 76 |
| | | | | 63 |
| | soybean plant | GC | | 65 |
| | | | | 64 |
| fruiting vegetables | tomato | HPLC | | 86 |
| | | | | 100 |
| | green pepper | HPLC | | 83 |
| | | | | 89 |
| grain crops | corn (grain) | HPLC | | 93 |
| | | | | 76 |
| | wheat (grain) | HPLC | 97 | 97 |
| | | | 102 | 110 |
| | | | 97 | 89 |
| | | | | 83 |
| | | | | 83 |
| | wheat (straw) | HPLC | | 72 |
| | | | | 72 |
| leafy vegetables | cabbage | HPLC | | 95 |
| | | | | 72 |
| | lettuce | HPLC | | 100 |
| | | | | 100 |
| nut crops | almond (meat) | HPLC | | 90 |
| | | | | 102 |
| | almond (shell) | HPLC | | 115 |
| | | | | 106 |
| | walnut (meat) | HPLC | | 99 |
| | | | | 96 |
| | walnut (shell) | HPLC | | 99 |
| | | | | 78 |
| pome fruits | apple | HPLC | | 92 |
| | | | | 92 |
| | pear | HPLC | | 103 |
| | | | | 97 |
| root crops | carrot | HPLC | | 83 |
| | | | | 57 |
| | potato | HPLC | 101 | 93 |
| | | | 93 | 72 |
| | | | 110 | 72 |
| | | | | 83 |
| | | | | 89 |
| seed/pod vegetables | snapbean | HPLC | | 103 |
| | | | | 96 |
| | soybean | HPLC | | 88 |
| | | | | 76 |
| small fruits | strawberry | HPLC | | 57 |
| | | | | 68 |
| | grape | HPLC | 80 | 88 |
| | | | 112 | 86 |
| | | | 96 | 92 |
| | | | | 79 |
| | | | | 97 |
| stone fruits | plum | HPLC | | 73 |
| | | | | 85 |
| | peach | HPLC | | 96 |
| | | | | 89 |

contain residues of the herbicide. Thus, the residue method for these commodities was developed for tolerance enforcement only.

**Critical Factors and Method Ruggedness.** To determine the ruggedness of the residue method, several factors were investigated to determine their effects upon analyte recovery. The potential loss of fluridone during Rinco evaporating steps was studied as a function of

**Table II. Recovery of Fluridone from Milk, Eggs, and Representative Meat Tissues**

| sample type | ppm fortified | N | % recovery range | % recovery av | % recovery SD |
|---|---|---|---|---|---|
| whole milk | 0.05 | 5 | 74–86 | 80 | 5 |
| | 0.1 | 6 | 75–85 | 79 | 4 |
| eggs | 0.05 | 6 | 92–100 | 95 | 4 |
| | 0.1 | 6 | 97–116 | 105 | 8 |
| beef liver | 0.05 | 6 | 88–104 | 95 | 7 |
| | 0.1 | 6 | 89–98 | 92 | 4 |
| pork sausage | 0.05 | 6 | 71–94 | 84 | 8 |
| | 0.1 | 6 | 71–90 | 82 | 11 |
| chicken lean | 0.05 | 6 | 73–95 | 80 | 9 |
| | 0.1 | 6 | 59–89 | 80 | 11 |

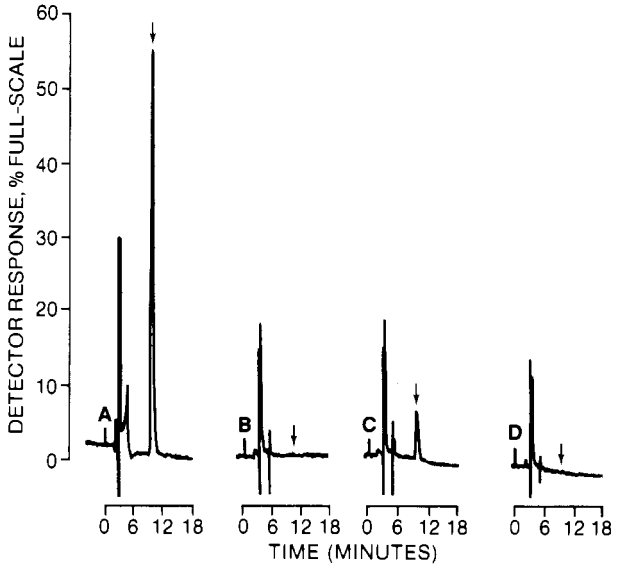


**Figure 1.** High-performance liquid chromatograms demonstrating the determination of fluridone in walnuts (letters indicate injection time and arrows indicate retention time of fluridone): (A) fluridone standard, 100 ng; (B) untreated control walnuts containing no detectable fluridone residue; (C) control walnuts fortified with 0.1 ppm of fluridone, equivalent to a 96% recovery; (D) walnut meat from a tree irrigated with water treated with fluridone, containing no detectable residue.

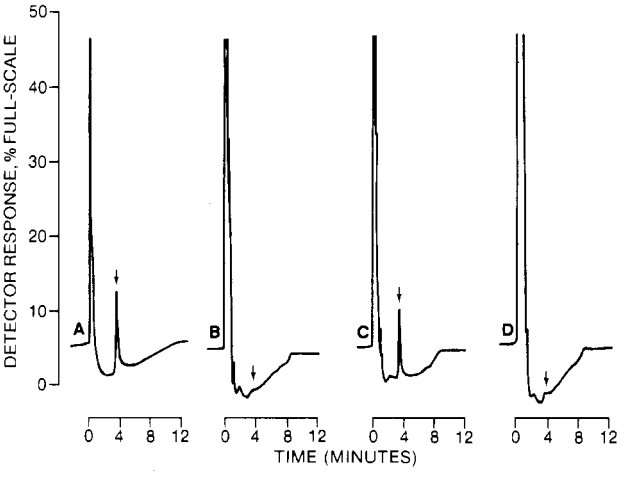


**Figure 2.** Gas chromatograms demonstrating the determination of fluridone as its brominated derivative (II) in grapefruit (letters indicate injection time and arrows indicate retention time of the derivative): (A) brominated standard, 0.19 ng; (B) untreated control grapefruit containing no detectable fluridone residue; (C) control grapefruit fortified with 0.10 ppm of fluridone, equivalent to a 105% recovery; (D) grapefruit from a tree irrigated with water treated with fluridone, containing no detectable residue.

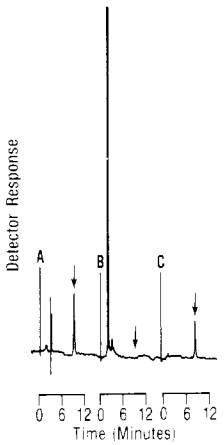


**Figure 3.** High-performance liquid chromatograms demonstrating the determination of fluridone in whole milk: (A) fluridone standard, 25 ng; (B) control milk containing no detectable fluridone residue; (C) control milk fortified with 0.05 ppm of fluridone, equivalent to a 77% recovery.

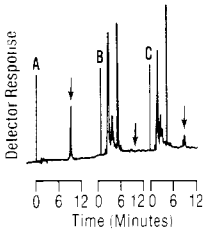


**Figure 4.** High-performance liquid chromatograms demonstrating the determination of fluridone in eggs: (A) fluridone standard, 25 ng; (B) control eggs containing no detectable fluridone residue; (C) control eggs fortified with 0.05 ppm of fluridone, equivalent to a 92% recovery.

**Table III. Storage Stability Data for Fluridone in Frozen Crop Samples Fortified with 0.1 ppm of Fluridone**

| crop | days in storage | % recovery |
|---|---|---|
| wheat grain | 7 | 96 |
| potato | 42 | 85 |
| carrot | 43 | 105 |
| green pepper | 36 | 104 |
| tomato | 14 | 85 |
| cabbage | 34 | 118 |
| zucchini squash | 44 | 77 |
| cucumber | 43 | 118 |
| grape | 76 | 86 |
| plum | 75 | 100 |
| walnut meat | 54 | 78 |
| walnut shells | 54 | 112 |
| almond meat | 38 | 53 |
| almond shells | 38 | 75 |
| grape | 76 | 103 |
| orange | 42 | 111 |
| peach | 3 | 104 |
| apple | 6 | 100 |
| pear | 20 | 97 |

evaporation time and temperature. No loss of the compound was observed when samples were left on the Rinco for up to 5 min after solvent evaporation at temperatures as high as 45 °C.

The stability of fluridone in the final assay solution (methanol–water) was investigated. The results suggested that the compound is stable when the final solution is stored for at least 6 days at room temperature or for at least 19 days under refrigeration. Stability for longer periods of time was not investigated.

The linear detector response occurred over a range of 5–200 ng of fluridone injected. The injection of 5 ng of fluridone produced a response equivalent to approximately 3 times base-line noise.

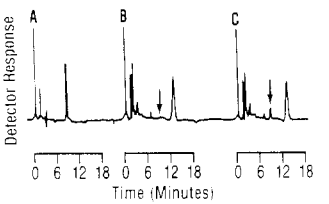


**Figure 5.** High-performance liquid chromatograms demonstrating the determination of fluridone in ground pork sausage: (A) fluridone standard, 25 ng; (B) control sausage containing no detectable fluridone residue; (C) control sausage fortified with 0.05 ppm fluridone, equivalent to an 86% recovery.

**Registry No.** Fluridone, 59756-60-4.

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