

US EPA ARCHIVE DOCUMENT

Agricultural Analytical Chemistry  
Lilly Research Laboratories  
Division of Eli Lilly and Company  
Greenfield, Indiana 46140

DETERMINATION OF FLURIDONE<sup>1</sup> RESIDUE IN MEAT, MILK,  
OR EGGS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

AM-AA-CA-R060-AA-755

Principle

Fluridone is extracted from milk onto SEP-PAK C<sub>18</sub> cartridges, and the herbicide is eluted from the cartridges with methanol. Fluridone is extracted from meat or eggs by shaking with methanol. The extracts are purified by liquid-liquid partitioning and alumina column chromatography. The purified extracts are concentrated and fluridone is measured by high-performance liquid chromatography with a U.V. detector operated at a fixed wavelength of 313 nm.

Chemicals and Reagents

1. Solvents

- a. Methanol, reagent grade
- b. Hexane, reagent-grade, redistilled
- c. Dichloromethane, reagent grade, redistilled
- d. Methanol, HPLC grade
- e. Water, HPLC grade

2. Solutions

- a. HPLC mobile phase--methanol (HPLC grade):water (HPLC grade), 70:30 (V/V), filtered and degassed.
- b. Hexane:dichloromethane (70:30, V/V)
- c. Sodium chloride, aqueous (50/o, W/V)

3. Sodium sulfate, anhydrous, methanol washed

4. Alumina, Alcoa F-20, deactivated with 40/o water (V/W), see Section H.

Equipment

1. Rotary vacuum evaporator (Rinco or equivalent) with water bath heated to approximately 35-45°C

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<sup>1</sup> 1-methyl-3-phenyl-5-[3-trifluoromethyl)phenyl]-4-(1H)-pyridinone (coded EL-171).

2. Magnetic stirrer and stirring bar
3. Gyrotory shaker (New Brunswick Model G33 or equivalent)
4. Chromatography columns--250 mm x 14 mm i.d., equipped with stopcock and a 250 ml reservoir
5. SEP-PAK C<sub>18</sub> cartridges (Waters Associates) and a SEP-PAK extraction apparatus (Figure 1)
6. Syringe, glass, with Luer tip
7. A high pressure liquid chromatograph consisting of the following components (or equivalent models):

Waters Model 6000A solvent delivery system

Waters Model 440 Absorbance Detector (fixed wavelength, 313 nm)

Waters Model 710A Intelligent Sample Processor

Houston Instruments Omni Scribe Strip Chart Recorder, 1-10mV

### Procedure

#### A. Preparation of Standard Solutions

1. Standard Solution A (fluridone, 1.0 mg/ml)--dissolve 100 mg of fluridone analytical standard in methanol (HPLC grade) in a 100-ml volumetric flask and dilute to volume.
2. Standard Solution B (fluridone, 10.0 mcg/ml)--transfer a 1.0 ml aliquot of Standard Solution A to a 100-ml volumetric flask and dilute to volume with methanol:water (70:30).
3. Standard Solution C (fluridone, 2.5 mcg/ml)--transfer a 10.0 ml aliquot of Standard Solution B to a 40-ml volumetric flask and dilute to volume with methanol:water (70:30).
4. Standard Solution D (fluridone, 0.25 mcg/ml)--transfer a 5.0-ml aliquot of Standard Solution C to a 50-ml volumetric flask and dilute to volume with methanol:water (70:30).
5. Standard Solution E (fluridone, 10.0 mcg/ml)--transfer 0.5 ml of Standard Solution A to a 50-ml volumetric flask and dilute to volume with hexane:dichloromethane (70:30).

#### B. Fortification of Recovery Samples

With each set of samples, prepare recovery samples in duplicate by fortifying two 25-g aliquots of a control meat or egg sample with 0.5 ml of Standard Solution C. Fortify 20 ml of control milk with 0.4 ml of Standard Solution C. If insufficient control sample is available, prepare duplicate system recoveries by fortifying two system blanks (100 ml of methanol) with 0.5 ml of Standard Solution C. Also analyze an untreated control (if available) and a system blank with each set of samples.

## C. Sample Extraction

## 1. Milk

- a. Using a short piece of Tygon tubing approximately 2.0 cm in length and 0.6 cm i.d., join the short end of a SEP-PAK C<sub>18</sub> cartridge to the long end of a second cartridge. Then attach the long end of the joined cartridges to the glass tubing leading to a graduated cylinder (Figure 1) and attach the short end to the three-way T-shaped stopcock on the extraction apparatus.
- b. Using vacuum, rinse the SEP-PAKs by pulling 20 ml of HPLC-grade methanol through the cartridges, followed by 20 ml of deionized water. Discard the rinses.
- c. Add 20 ml of the milk sample to a graduated cylinder. Using vacuum, pull the milk through the cartridges and then rinse the cartridges with 20 ml of deionized water. After the vacuum has pulled nearly all of the residual water droplets through the cartridges, detach the SEP-PAKs from the apparatus and discard the eluates.
- d. Attach the long end of one of the cartridges to the Luer tip of a dry glass syringe and elute fluridone by pumping 10 ml of HPLC-grade methanol through the cartridge. Collect the eluate in a 125-ml evaporating flask. Repeat with the second cartridge, combining the two methanol eluates in the same flask.
- e. Evaporate the combined eluates to dryness using a rotary vacuum evaporator and a 35-45°C water bath.
- f. Dissolve the residue in 5 ml of hexane:dichloromethane (70:30). Proceed to the alumina column purification procedure in Section E.

## 2. Meat or Eggs

- a. Weigh 25 g of ground meat sample or 25 g of blended egg sample into a pint mason jar. Add methanol (reagent-grade) to result in a total extraction volume of 100 ml with allowance for the moisture content of the sample. Shake the sample for 30 minutes at 250 rpm on a gyratory shaker.
- b. Transfer a 20-ml aliquot of the methanol extract to a graduated cylinder by pouring through a funnel containing folded filter paper.

## D. Liquid-Liquid Partition

1. Transfer the aliquot from step C-2 to a separatory funnel containing 20 ml of 50/o sodium chloride solution.

2. Rinse the graduated cylinder with 40 ml of hexane and transfer the rinse to the separatory funnel.
3. Shake the separatory funnel for approximately 20 seconds. Allow the phases to separate and drain the aqueous (lower) phase into a beaker. Discard the hexane (upper) phase and return the aqueous phase to the separatory funnel.
4. Extract the aqueous phase three times by shaking with three 40-ml aliquots of dichloromethane. Allow the phases to separate and drain the dichloromethane (lower) phase through a funnel containing sodium sulfate into a 250-ml evaporating flask. After the third extraction, rinse the sodium sulfate with 15-20 ml of dichloromethane.
5. Evaporate the dichloromethane just to dryness with a rotary vacuum evaporator and a 35-45°C water bath. Dissolve the residue in 5 ml of hexane:dichloromethane (70:30).

#### E. Alumina Column Purification

1. Prepare a column with standardized, deactivated alumina according to the procedure in Section H.
2. Add the sample extract from step D-5 to the column and drain to the top of the sodium sulfate. Discard the eluate.
3. Rinse the flask with 5 ml of hexane:dichloromethane (70:30) and add the rinse to the column. Drain to the top of the sodium sulfate and repeat with a second 5-ml rinse.
4. Wash the column with an additional 25 ml of hexane:dichloromethane (70:30) and discard the eluate.
5. Wash the column with 20 ml of dichloromethane and discard the eluate.
6. Add an additional 50 ml of dichloromethane and collect the eluate in a clean 125 ml evaporating flask.

NOTE: See Section H for standardizing the elution volume to be collected.

7. Evaporate the dichloromethane just to dryness with a rotary vacuum evaporator and a 35-45°C water bath.
8. Dissolve the residue in 4.0 ml of methanol:water (70:30). If the solution contains suspended particulate matter, filter the solution into an HPLC sample vial.

#### F. HPLC Measurement

1. Measure the HPLC peak height response for fluridone using the instrumentation listed in the EQUIPMENT section and the following analytical parameters.

NOTE: The parameters listed below may be modified as needed to compensate for daily variations in instrument performance. The parameters used for the analysis should be recorded.

Column-- $\mu$ Bondapak C<sub>18</sub> or Lichrosorb RP-18 with a Co-Pell ODS guard column

Mobile phase--methanol:water (70:30)

Flow rate--1.0 ml/min

Injection volume--100 microliters

Attenuation--0.02 AUFS

Chart speed--0.167 cm/min

Direct Standard--Standard Solution D

2. During the sample analysis, periodically determine the HPLC peak height for Standard Solution D. Use the average peak height for calculating the results in Section G.

## G. Calculations

1. Percent Recovery =

$$\frac{\text{PH}_{\text{rec}}}{\text{PH}_{\text{std}}} \times C \times V \times \text{AF} \times 100\% / \text{mcg fortified}$$

where: PH<sub>rec</sub> = peak height (cm) of recovery sample

PH<sub>std</sub> = average peak height (cm) of standard

C = concentration (mcg/ml) of standard

V = final volume (ml), including dilutions

AF = aliquot factor (AF = 1 for milk, and AF = 5 for meat or eggs)

2. Residue (ppm) =

$$\frac{\text{PH}_{\text{sa}}}{\text{PH}_{\text{std}}} \times C \times V \times \text{AF} \times 100\% / \text{W} \times \% \text{ Recovery}$$

where: PH<sub>sa</sub> = peak height (cm) of sample

W = weight (g) or volume (ml) of sample extracted

## H. Deactivation and Standardization of Alumina

NOTE: Each batch of alumina must be deactivated and standardized prior to initial use.

## 1. Deactivation of Alumina

- a. Determine the loss on drying of the alumina as received by heating at 110°C for at least 4 hours. Add sufficient deionized water to result in a total moisture content of 4.00/o (v/w).
- b. Tumble the alumina in a closed container for at least one hour.

## 2. Packing of Alumina Column

- a. Place a plug of glass wool in the bottom of a chromatography column. With the stopcock closed, add 15 ml of hexane:dichloromethane (70:30) followed by 10 ml (9.6 g) of alumina and 15 ml of hexane:dichloromethane (70:30).
- b. Push a plastic rod through the column packing, open the stopcock, and thoroughly stir the packing as it begins to settle. Remove the rod and rinse the column reservoir with 5 ml of hexane:dichloromethane (70:30).
- c. Add approximately 1 cm of sodium sulfate, rinse the reservoir with 5 ml of hexane:dichloromethane (70:30), and drain the solvent to the top of the sodium sulfate.

## 3. Standardization of Alumina

- a. Add 1.0 ml of Standard Solution E to the column, followed by 4 ml of hexane:dichloromethane (70:30) and drain the eluant to the top of the column.
- b. Add 5 ml of hexane:dichloromethane (70:30) and drain to the top of the column.
- c. Wash the column with 30 ml of hexane:dichloromethane (70:30) and discard the eluate.
- d. Elute the column with 120 ml of dichloromethane in 10 ml fractions and collect each fraction in separate 125 ml evaporating flasks.
- e. Evaporate the fractions just to dryness and dissolve the residues in 5.0 ml of methanol:water (70:30).
- f. Analyze each fraction by HPLC using the parameters listed in Section F. Compare the peak height response of fluridone in the column fractions with that of Standard Solution D to determine

the elution pattern of fluridone, and adjust the volume of dichloromethane to be collected in Step E-6 accordingly.

DISCUSSION

The residue method has a limit of detection (three times background) of approximately 0.05 ppm. Recoveries obtained by fortifying control samples with 0.05 ppm and 0.10 ppm of fluridone are summarized in Table I. Chromatograms demonstrating the recovery of fluridone from milk, eggs, beef liver, ground pork, and chicken lean are contained in Figures 2 through 6.

S. D. West  
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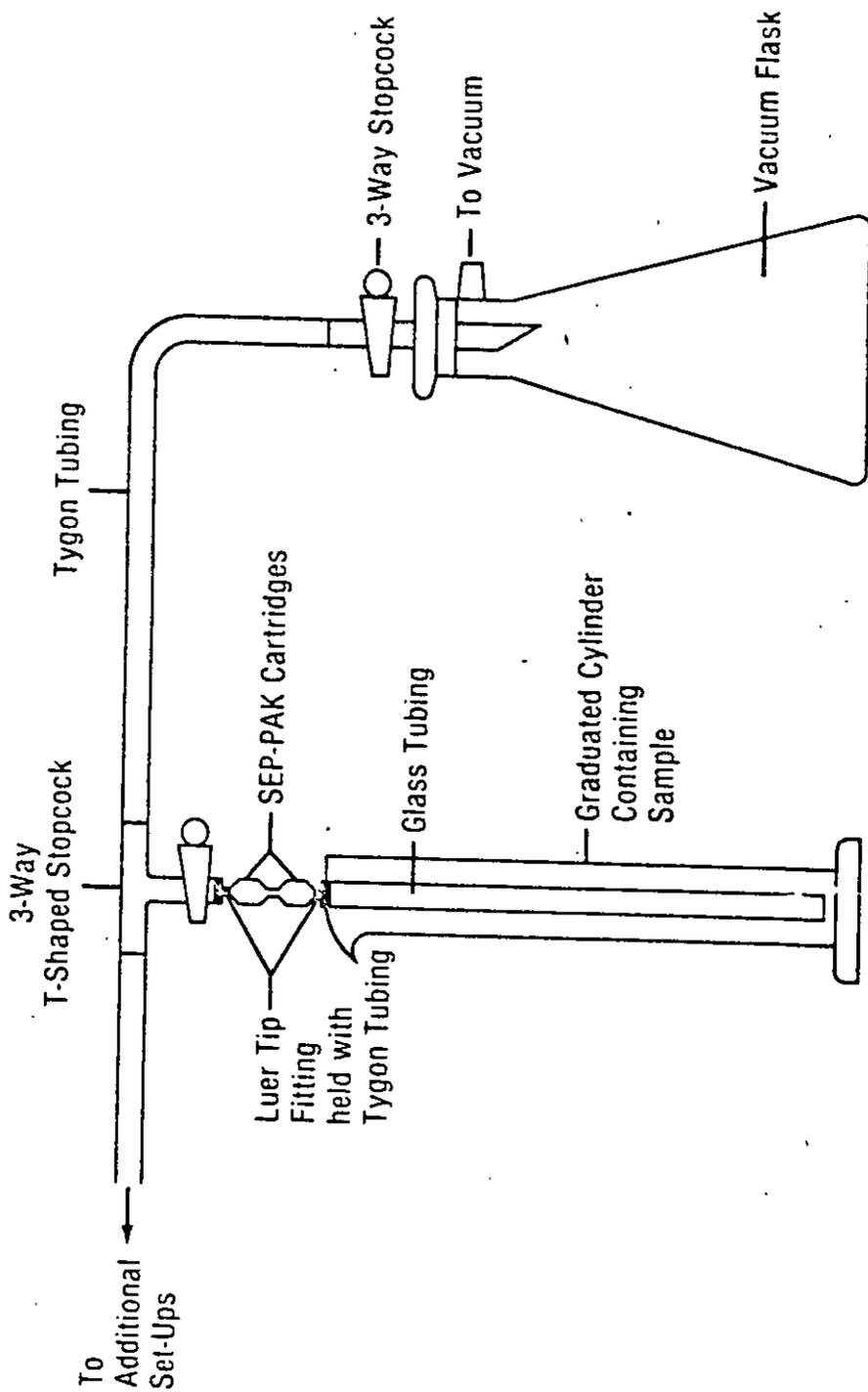
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TABLE I  
FLURIDONE RECOVERIES

<u>Sample Type</u>	<u>Fortification Level (ppm)</u>	<u>N</u>	<u>Percent Recovery (<math>\bar{X} \pm s.d.</math>)</u>
Milk	0.05	5	79 $\pm$ 5
	0.10	6	79 $\pm$ 4
Eggs	0.05	6	95 $\pm$ 4
	0.10	6	105 $\pm$ 8
Beef Liver	0.05	6	95 $\pm$ 7
	0.10	6	92 $\pm$ 4
Ground Pork	0.05	6	84 $\pm$ 8
	0.10	5	82 $\pm$ 11
Chicken Lean	0.05	6	80 $\pm$ 9
	0.10	6	80 $\pm$ 11

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Figure 1  
SEP-PAK Extraction Apparatus



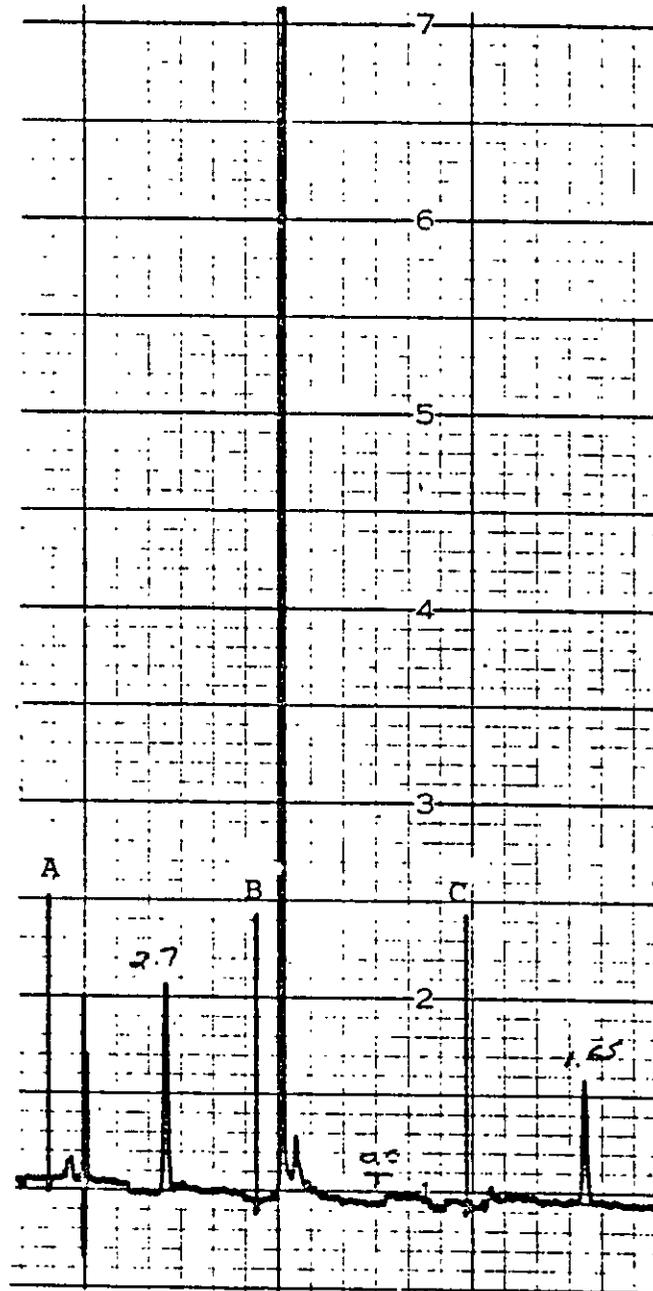


Figure 2. Recovery of fluridone from milk:  
(A) fluridone standard, 2.5 ng;  
(B) control milk containing no  
detectable fluridone; (C) control  
milk fortified with 0.05 ppm of  
fluridone, equivalent to a 77.3%  
recovery.

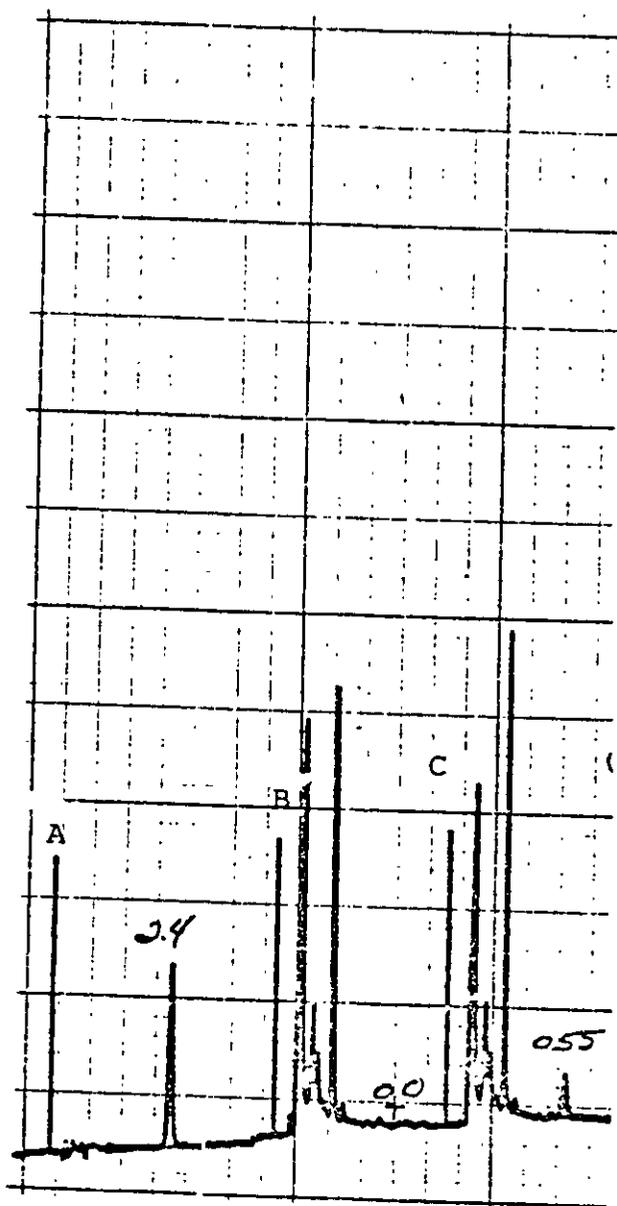


Figure 3. Recovery of fluridone from eggs:  
(A) fluridone standard, 25 ng;  
(B) control eggs containing no detectable fluridone; (C) control eggs fortified with 0.05 ppm of fluridone, equivalent to 91.7% recovery.

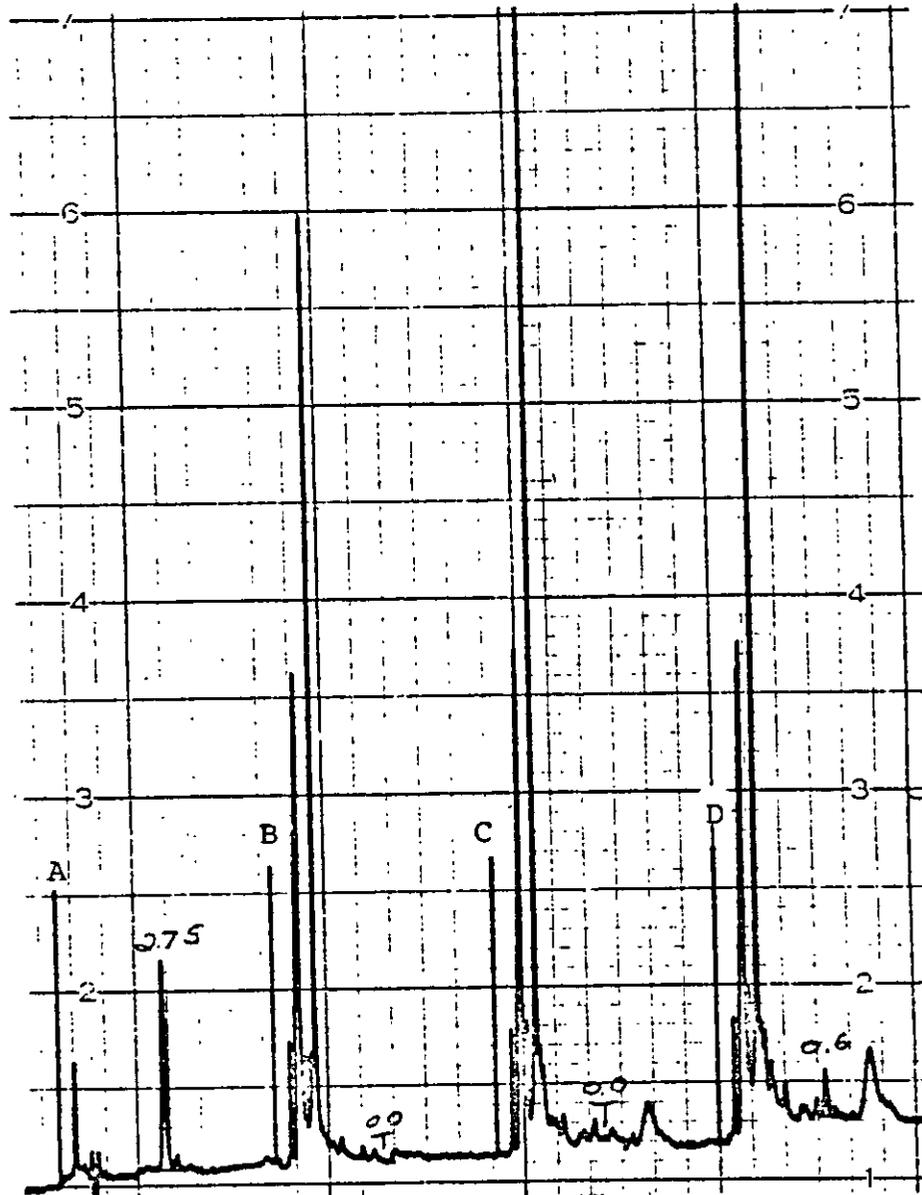


Figure 4. Recovery of fluridone from beef liver:  
(A) fluridone standard, 25 ng;  
(B) system (reagent) blank; (C) control  
liver containing no detectable fluridone;  
(D) control liver fortified with 0.05  
ppm of fluridone, equivalent to an 88.9%  
recovery.

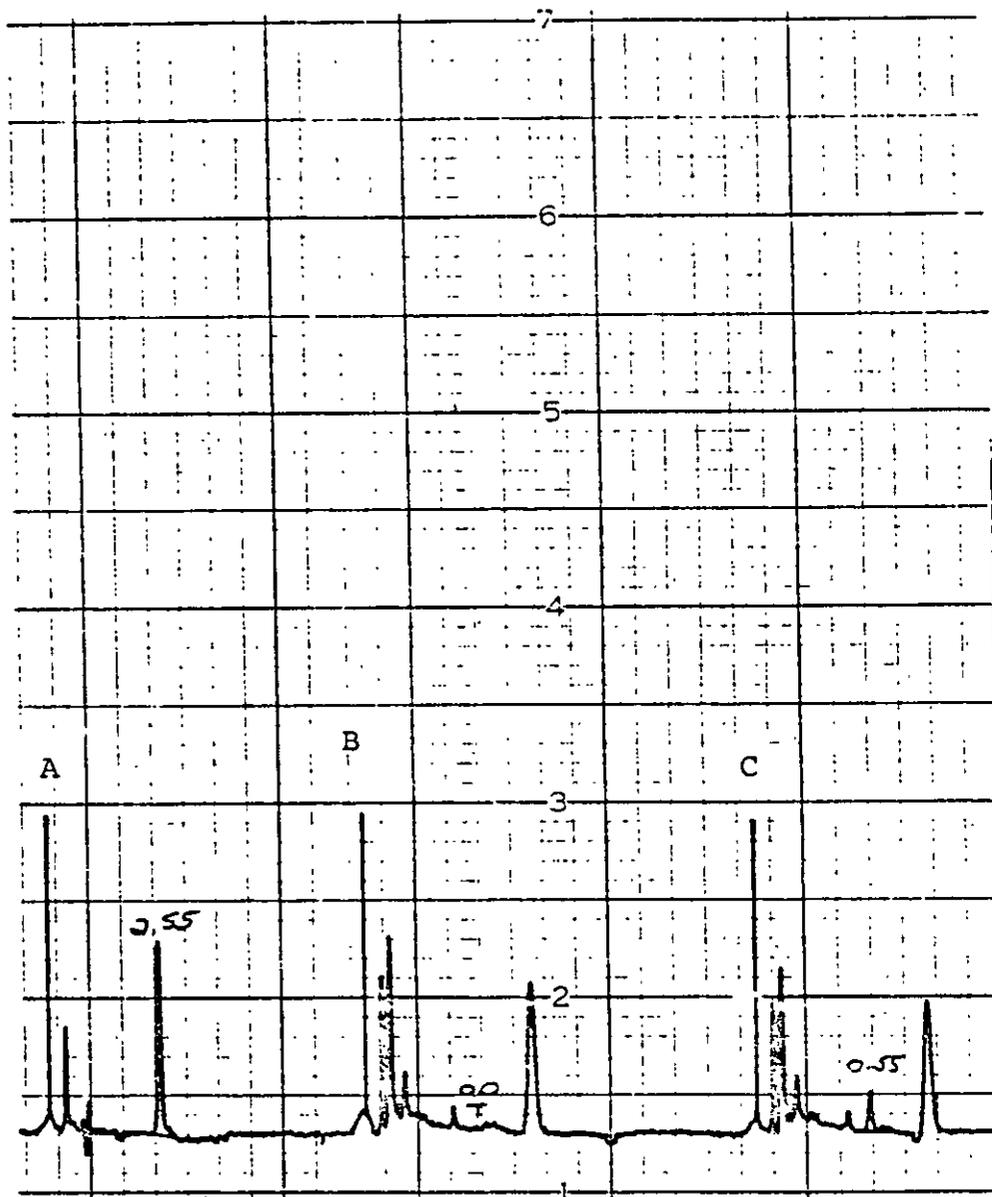


Figure 5. Recovery of fluridone from ground pork:  
(A) fluridone standard, 25 ng; (B) control  
pork containing no detectable fluridone;  
(C) control pork fortified with 0.05 ppm  
of fluridone, equivalent to an 86.3% recovery.

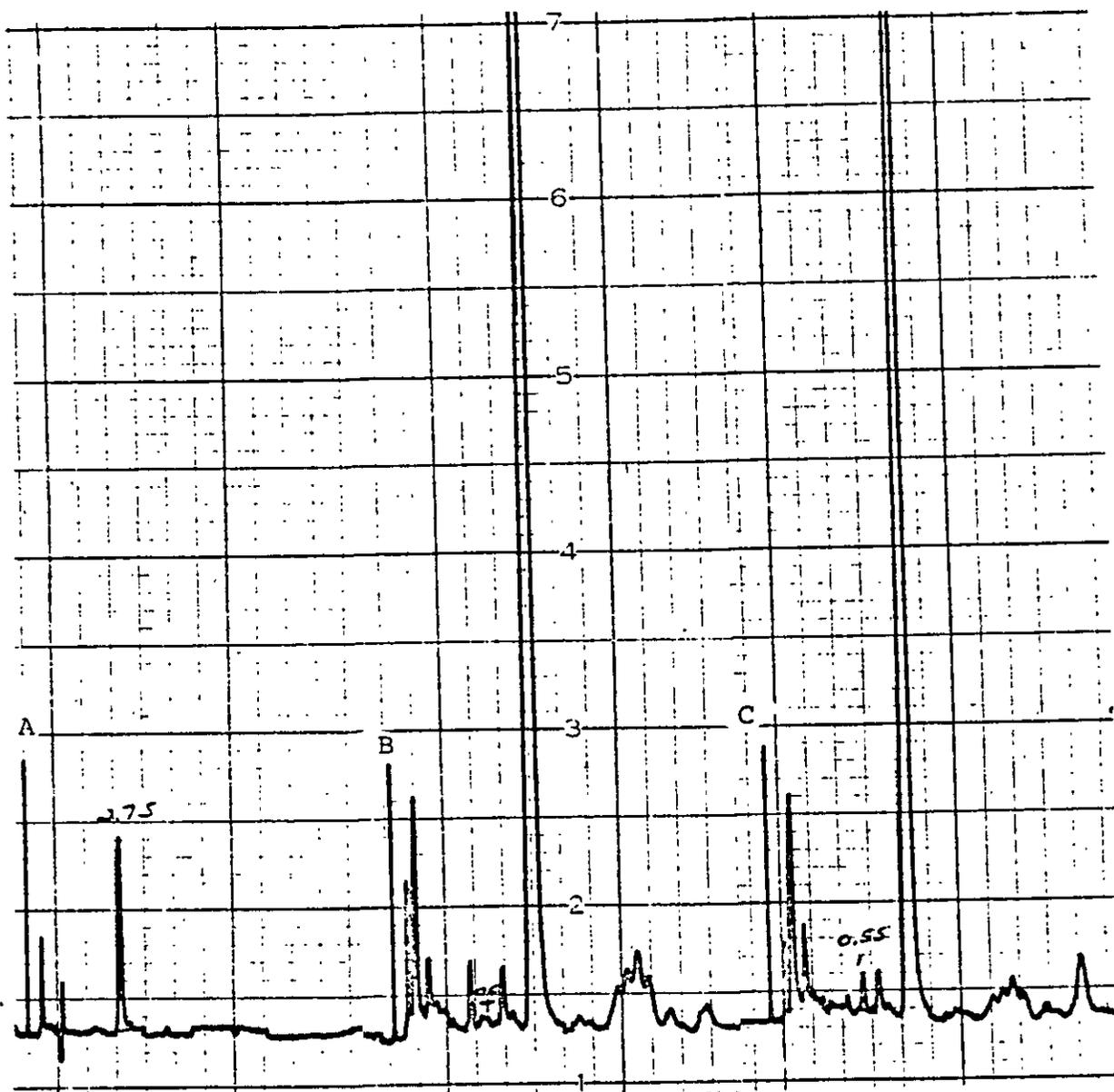


Figure 6. Recovery of fluridone from chicken lean:  
(A) fluridone standard, 25 ng; (B) control  
chicken containing no detectable fluridone;  
(C) control chicken fortified with 0.05 ppm  
of fluridone, equivalent to an 80.0%  
recovery.