Table II. Standard Error Distribution

$S_{y \cdot x}$	% of standard curves with $S_{y \cdot x} \leq$ desired value: in muscle[a]	in liver[b]
0.005	35.4	9.8
0.010	71.1	49.5
0.015	93.2	73.4
0.020	100.0	87.8
0.025		96.7
0.030		100.0

[a] 384 determinations. [b] 364 determinations.

GC-MS, there will be a 95% probability of detecting all violative samples screened by establishing the confirmation threshold at 0.11–$2S_{y\cdot x}$ ppm for sulfamethazine. The data in Table II demonstrate that in most cases $S_{y\cdot x}$ is small enough to set the limit at 0.07 ppm or greater, which should minimize the number of nonviolative samples carried on to the confirmation step. Examples of how the threshold varies depending on $S_{y\cdot x}$ are shown in Figure 4.

The use of fluorescence in situ scanning in conjunction with an internal standard and preadsorbent TLC permits the use of rapid cleanup procedures in quantitative residue screening. The extension of this technique to other sulfonamides of interest as well as the adaptability of the rapid cleanup to GC-MS analysis is currently under investigation.

LITERATURE CITED

Bevill, R. F.; Schemske, K. M.; Luther, H. G.; Dzierzak, E. A.; Limpuka, M.; Felt, D. R. *J. Agric. Food Chem.* **1978**, *26*, 1201–1203.

Code of Federal Regulations, 1977, 21. Food Additive Regulation 558.128; New Animal Drug Regulations 546.110 (e), 546.113 (b), 520.2260, 566.670.

Goodspeed, D. P.; Simpson, R. M.; Ashworth, R. B.; Shafer, J. W.; Cook, H. R. *J. Assoc. Off. Anal. Chem.* **1978**, *61*, 1050–1053.

Halpaap, H.; Krebs, K. F. *J. Chromatogr.* **1977**, *142*, 823–853.

Imai, K.; Bohlen, P.; Stern, S.; Undefriemd, S. *Arch. Biochem. Biophys.* **1974**, *161*, 161–163.

Sigel, C. W.; Woolley, J. L.; Nichol, C. A. *J. Pharm. Sci.* **1975**, *64*, 973–976.

Suhre, F. B.; Simpson, R. M.; Shafer, J. W., U.S. Department of Agriculture, Beltsville, MD, unpublished data, 1980.

Tishler, F.; Sutter, J. L.; Bathish, J. N.; Hagman, H. E. *J. Agric. Food Chem.* **1968**, *16*, 50–53.

Touchstone, J. C.; Sherma, J.; Dobbins, M. F.; Hansen, G. R. *J. Chromatogr.* **1976**, *124*, 111–114.

Received for review July 18, 1980. Accepted January 28, 1981.

High-Pressure Liquid Chromatographic Determination of the Herbicide Fluridone in Cottonseed

Sheldon D. West

A method is described for determining fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl]-4-(1*H*)-pyridinone] in cottonseed at levels as low as 0.05 ppm. Fluridone is extracted from cottonseed with methanol. Purification of the sample extracts is accomplished by aqueous–hexane and aqueous–dichloromethane partitioning, followed by alumina column chromatography. The purified extracts are concentrated and then measured by reverse-phase high-pressure liquid chromatography on μBondapak C_{18} by utilizing methanol–water (65:35) as the mobile phase. Detection is accomplished with a fixed-wavelength UV detector at 254 nm. Recoveries averaged 84.3% for untreated cottonseed fortified with 0.05–0.20 ppm of fluridone. The method is evaluated by analyzing cottonseed samples from fields treated at rates of 0.3 and 0.8 lb/acre fluridone 335 days after a preplant soil incorporation of the herbicide.

Fluridone [1-methyl-3-phenyl-5-[3-(trifluoromethyl)-phenyl]-4-(1*H*)-pyridinone] has exhibited broad spectrum preemergence herbicidal activity for weed control in cotton (Waldrep and Taylor, 1976). Consequently, a method is needed for determining residues of the herbicide in cottonseed. A previously published method (West, 1978) involved the derivatization of fluridone with phosphorus tribromide for measurement by gas chromatography with electron capture detection at a test sensitivity of 0.01 ppm. In this paper, a method is described for determining fluridone in cottonseed by high-pressure liquid chromatography (LC) with UV detection at 254 nm. The high-pressure LC procedure eliminates the need to derivatize fluridone, thereby reducing sample analysis time and improving analytical precision.

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

EXPERIMENTAL SECTION

Apparatus, Chemicals, and Reagents. High-pressure LC grade water (J. T. Baker) and high-pressure LC grade methanol (Waters Associates) were used for the high-pressure LC mobile phase. Hexane was pesticide grade, distilled in glass. Dichloromethane (reagent grade) was redistilled, and reagent-grade methanol was used as received. Anhydrous sodium sulfate was washed with methanol and dried at 50 °C for 16 h. Neutral Alumina F-20 (Alcoa) was dried at 110 °C for 16 h, deactivated with 4.0% water (v/w), and tumbled for 1 h in a closed container.

The liquid chromatographic system consisted of a Waters Model 6000A solvent delivery system, a Waters Model 440 absorbance detector (fixed wavelength, 254 nm) operated at 0.02 AUFS, a Waters Model 710A Intelligent sample processor (200-μL injection), a Houston Instru-

0021-8561/81/1429-0624$01.25/0 © 1981 American Chemical Society

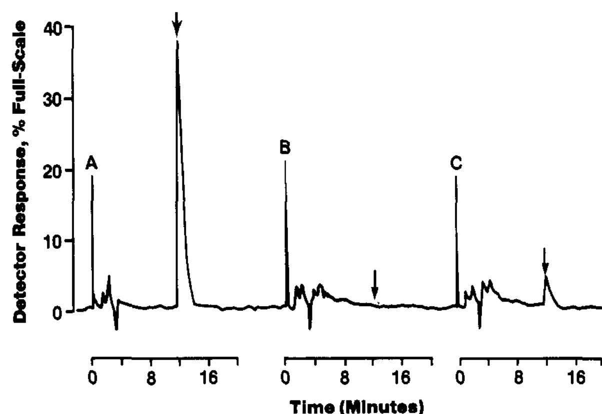

Figure 1. High-pressure liquid chromatograms demonstrating recovery of fluridone from cottonseed (letters indicate injection time and arrows indicate retention time of fluridone): (A) fluridone standard (200 ng); (B) untreated cottonseed; (C) untreated cottonseed fortified with 0.10 ppm of fluridone (equivalent to an 89% recovery).

ments Omni Scribe strip chart recorder, and a μBondapak C_{18} column (3.9 mm i.d. × 30 cm) with a Co-Pell ODS guard column (Whatman, Inc.). The mobile phase was methanol–water, 65:35, and its flow rate was adjusted to 1 mL/min. Under these conditions, the retention time for fluridone was ~12 min. Quantitation was accomplished by comparison of sample peak heights with a 1.0 μg/mL direct standard. The injection volume was 200 μL.

Additional equipment included a gyratory shaker (New Brunswick Model 33), glass chromatography columns (250 × 14 mm i.d.) equipped with stopcocks and 250-mL reservoirs, and rotary vacuum evaporators.

Extraction Procedure. A 25-g aliquot of delinted and finely ground cottonseed was weighed into a 1-pt Mason jar. Methanol (100 mL) was added, and the sample was shaken for 30 min at 300 rpm on a gyratory shaker. The methanol extract was poured through folded filter paper into a graduated cylinder until 20 mL of extract was collected.

Liquid–Liquid Partition Procedure. The 20-mL aliquot was transferred to a separatory funnel containing 20 mL of 5% aqueous sodium chloride solution. The aqueous phase was extracted 3 times with 40-mL portions of hexane, and the hexane washes were discarded. Fluridone was then extracted from the aqueous phase with three 20-mL portions of dichloromethane, which were combined and dried by draining through a funnel containing sodium sulfate into a 125-mL evaporating flask. The combined extract was evaporated to dryness with a rotary vacuum evaporator and a 40 °C water bath.

Alumina Column Chromatography. A chromatograhic column (25 × 1.4 cm i.d.) was prepared by wet packing 10 mL (9.8 g) of 4.0% water-deactivated alumina with hexane–dichloromethane (70:30) in a glass column and topping the column with a 1-cm layer anhydrous sodium sulfate. (Prior to initial use, each batch of alumina was standardized to determine the elution pattern of fluridone.) The evaporated sample extract was added to the column in three 5-mL rinses of hexane–dichloromethane (70:30). The column was washed with an additional 25 mL of hexane–dichloromethane (70:30), followed by 20 mL of dichloromethane. The eluate to this point was discarded. Fluridone was then eluted from the column with 50 mL of dichloromethane, and the eluate was collected in a 125-mL evaporating flask. The solvent was evaporated to dryness with a rotary vacuum evaporator and a 40 °C water bath. The residue was dissolved in 4.0 mL of methanol–water (65:35) for measurement by high-pressure LC as described above.

RESULTS AND DISCUSSION

Chromatograms demonstrating the determination of fluridone in cottonseed are contained in Figure 1. The method as described is capable of determining fluridone at levels as low as 0.05 ppm. The precision of the method was evaluated by analyzing untreated cottonseed fortified with 0.05, 0.10, and 0.20 ppm of fluridone, and the recoveries averaged 81.6 ± 7.0%, 89.8 ± 10.7%, and 80.0 ± 10.1%, respectively. For comparison, recoveries of fluridone at the 0.05-ppm level using the derivatization procedure for gas chromatography (West, 1978) averaged 90.2 ± 19.7%. Elimination of the derivatization steps also reduced sample preparation time by ~2–3 h.

The residue method was further evaluated by analyzing cottonseed (Acala SJ2 variety) from fields in Fresno, CA, treated with 0.3–0.8 lb of fluridone/acre. The herbicide was applied as a preplant soil incorporation, and the cottonseed was harvested 335 days after application. Fluridone was not detected in any of the cottonseed samples. These results, obtained under actual field use conditions, agree with those of Berard et al. (1978), who attributed the absence of [^{14}C]fluridone in cottonseed to limited uptake and translocation of the herbicide by the plant.

LITERATURE CITED

Berard, D. F.; Rainey, D. P.; Lin, C. C. *Weed Sci.* **1978**, *26*, 252.
Waldrep, T. W.; Taylor, H. M. *J. Agric. Food. Chem.* **1976**, *24*, 1250.
West, S. D. *J. Agric. Food Chem.* **1978**, *26*, 644.

Received for review August 11, 1980. Revised January 23, 1981. Accepted February 20, 1981.