II was one of the two major factors governing chemical distribution and loss from the system, the other being soil type.

Relatively little of the amount applied for any of the chemicals, except dieldrin (Figure 1), reached the animals. Even though Table II shows high values for certain invertebrates (e.g., crickets), the concentration relative to the application rate remains low. Dieldrin affected vole survival as is evident from the observed mortalities and associated high concentrations of dieldrin materials in the bodies and brains of the voles.

Conclusions. The following conclusions are based on the data from these two experiments. (1) The comparable results for the methylparathion experiments in both studies indicate that the TMC is suitable for examining organophosphates and that reproducible results are obtainable. (2) The increased airflow used in experiment II substantially altered the mass balance and residue distribution of methylparathion within the TMC ecosystem. (3) Soil type affected mass balance and residue distribution within the TMC. (4) The presence of bound residues of the organophosphates suggested that, within the TMC under certain conditions (e.g., low airflow), they may be characterized as fairly persistent.

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Determination of Sulfamethazine in Swine Tissues by Quantitative Thin-Layer Chromatography

Michael H. Thomas,* Karen E. Soroka, Randy M. Simpson, and Robert L. Epstein

A thin-layer chromatography method suitable for rapid screening for the presence of sulfamethazine residues in swine tissue is presented. Tissue is extracted with ethyl acetate, back-extracted into methylene chloride, and concentrated. Detection is by optical in situ scanning of the fluorescamine derivative. At 0.1 ppm a relative standard deviation of less than 6% is achieved.

Sulfamethazine is widely used for the treatment of bacterial infections in swine where, like other sulfonamides, it acts as a competitive inhibitor of *p*-aminobenzoic acid in the biosynthesis of folic acid in the invading organism. Current regulations (Code of Federal Regulations, 1977) allow a tolerance of 0.1 ppm in uncooked edible tissues. USDA Food Safety and Quality Service (FSQS) laboratories presently screen tissues qualitatively by a TLC–GLC procedure (Goodspeed et al., 1978). Quantitation and confirmation of the drug is accomplished by a spectrophotometric method (Tishler et al., 1968). Recently, a GC–MS method (Suhre et al., 1980) has been developed as a more sensitive and specific replacement for the Tishler procedure. For minimization of analysis time and the number of samples requiring GC–MS confirmation, an accurate, precise, but rapid screening procedure is required. Quantitative thin-layer chromatography was selected because of its inherent ability to analyze many samples simultaneously while maintaining sensitivity and selectivity through fluorescence detection. This technique has previously been applied to sulfadiazine in tissue (Sigel et al., 1975) and to sulfamethazine in plasma (Bevill et al., 1978). In neither case was the confidence interval nor long-term reproducibility of the method reported. These parameters take on critical importance when an attempt is made to establish a quantitative limit below which no further confirmation will be made. A level of precision much higher than is normally associated with chromatographic residue procedures (especially thin layer) has been achieved through the combined use of an internal standard

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and preadsorbent TLC plates. Furthermore, preadsorbent TLC plates tolerate the application of cruder extracts, allowing simpler sample preparation. Application of relatively crude tissue extracts to conventional silica gel typically causes severe tailing and poor quantitation. Properly utilized, preadsorbent or preconcentrating zones minimize factors like occlusion that cause poor quantitative reproducibility on conventional plates.

EXPERIMENTAL SECTION

Apparatus. An Aminco-Bowman (Silver Spring, MD) spectrophotofluorometer with a TLC scanning attachment was used for all quantitation. Excitation and emission wavelengths were 410 and 500 nm, respectively. Fluorescence was measured via transmission. Plates were scanned at 0.77 mm/s, the slowest available scan speed.

Reagents. Precoated 20 × 20 cm TLC plates containing a preadsorbent spotting area (LK6D, Whatman, Inc., Clifton, NJ) were used without activation or predevelopment.

All solvents except *tert*-butanol were distilled in glass (Burdick & Jackson Laboratories, Inc., Muskegon, MI). The *tert*-butanol was reagent grade (Fisher Scientific Co., Springfield, NJ).

Fluram (fluorescamine) was obtained from Pierce Chemical Co., Rockford, IL. The derivatizing solution was prepared by dissolving ~30 mg of fluorescamine in 250 mL of acetone. This was replaced after treating eight to nine plates.

Sulfamethazine was obtained from Pfaltz and Bauer, Stamford, CT, and sulfapyridine was obtained from Sigma Chemical Co., St. Louis, MO. Fortification standards of each drug were prepared in methanol (1.0 μg/mL).

Extraction and Sample Preparation. A 2.5-g sample of liver or muscle was weighed into a 50-mL polypropylene centrifuge tube and fortified with 0.1 ppm of sulfapyridine. After 15–30 min was allowed for adsorption of the drug, 25 mL of ethyl acetate was added, and the contents were macerated for 30 s by using a Tekmar SDT tissumizer (Cinncinati, OH). The tube was then capped and mechanically shaken for 10 min. After centrifugation (5 min at 2500 rpm), the ethyl acetate was transferred to a clean 50-mL polypropylene centrifuge tube, the tissue discarded, and 10 mL of 1 N hydrochloric acid added to the tube containing the ethyl acetate extract. The tube was again mechanically shaken for 5 min and the layers were completely separated by centrifugation (5 min at 2500 rpm). The organic phase was aspirated and discarded, and the pH of the aqueous phase adjusted to 6.5 by addition of 1 mL of 10 N NaOH and a final adjustment was made with 0.1 N NaOH or 0.1 N HCl. The aqueous phase was then extracted with 10 mL of methylene chloride by mechanically shaking the tube for 5 min. The layers were completely separated by centrifugation as before. If emulsions formed, these were minimized by centrifugation at 3500 rpm for 10 min. The aqueous phase was aspirated and discarded. The contents of the tube were evaporated to dryness under a stream of nitrogen at 40 °C by using an N-Evap (Organomation, Northborough, MA). During the evaporation, the walls of the tube were periodically rinsed with additional methylene chloride. The residue was reconstituted in 100 μL of methanol. The procedure is summarized in Figure 1.

Thin-Layer Chromatography. A 20-μL portion of sample was applied to the preadsorbent layer via a microliter syringe, taking care to apply the sample in one smooth continuous stroke to maximize uniformity of concentration across the band (Halpaap and Krebs, 1977). The plate was developed in a saturated tank containing

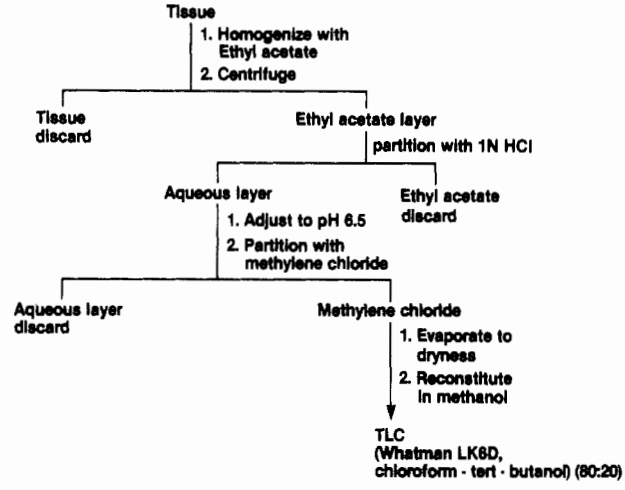

Figure 1. Schematic of the procedure.

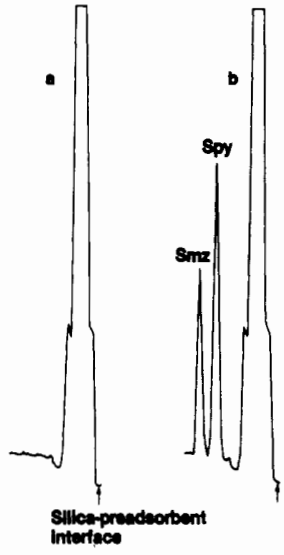

Figure 2. Scan of thin-layer chromatogram of (a) control muscle and (b) control muscle fortified with 0.1 ppm of sulfamethazine (Smz) and 0.1 ppm of sulfapyridine (Spy). Conditions are described under Experimental Section.

chloroform–*tert*-butanol (80:20) and then dried in an oven at 100 °C for 1 min. For liver samples chromatography was improved by preceding the above step by a short development (~1.5 cm) in methanol and drying in an oven at 100 °C for 30 s. The fluorescamine derivatization was accomplished by quickly dipping the dried plate in the fluorescamine solution. Derivatization was complete after air drying for 15–30 min. No need was found to further stabilize the derivatives as reported elsewhere (Imai et al., 1974; Touchstone et al., 1976) as they were stable for at least 2–3 h.

Quantitation. To compensate for any matrix effects, we constructed the standard curve of the ratio of peak heights of sulfamethazine to sulfapyridine vs. sulfamethazine concentration using fortified tissue rather than standard solutions. The chromatograms of control tissue and fortified tissue are shown in Figure 2.

RESULTS AND DISCUSSION

Optical in situ quantitation of the fluorescamine derivative is an extremely sensitive and selective technique for the determination of sulfamethazine residues in tissue. Less than 1 ng of the sulfonamide can be detected, an amount equivalent to 0.002 ppm in tissue. During the development of the method one control liver sample con-

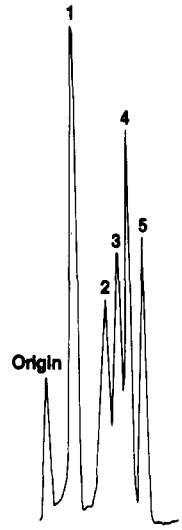

Figure 3. Scan of thin-layer chromatogram after fluorescamine derivatization of the five common sulfonamides used for veterinary purposes. $R_f \times 100$: (1) sulfathiazole, -18.5; (2) sulfamethazine, -37.0; (3) sulfaquinoxaline, -38.6; (4) sulfadimethoxine, -44.6; (5) sulfabromomethazine, -50.8.

Table I. Accuracy and Reproducibility of Sulfamethazine Concentration As Determined by TLC

tissue	ppm added	ppm found (n = 20)	mean within-day COV[a] (6 days)	day-to-day COV	$\frac{\text{ppm found}}{\text{ppm added}} \times 100$
muscle	0.05	0.050	4.65	7.65	100
muscle	0.10	0.101	3.86	5.56	101
muscle	0.20	0.204	3.51	3.35	102
liver	0.05	0.048	8.23	6.98	95.8
liver	0.10	0.101	5.87	3.63	101
liver	0.20	0.203	6.48	2.83	102

[a] COV = coefficient of variation.

tained a band at the R_f of sulfamethazine equivalent to 0.005–0.015 ppm by multiple determinations. When analyzed by GC–MS, the sample was found to contain 0.012 ppm of sulfamethazine.

The method is linear over the range 0.05–0.5 ppm, which is more than adequate for screening purposes. If desired, the upper limit can be increased by diluting the ethyl acetate extracts of samples containing greater than 0.5 ppm of sulfamethazine.

The selectivity of fluorescence detection allows the use of considerably less complex extraction procedures than would be required for gas chromatographic assays with electron capture detection or HPLC with UV absorption detection. The thin-layer chromatogram for control tissue is completely free of interfering peaks, and it should be possible to readily extend the method to other sulfonamides. With the chloroform–*tert*-butanol solvent system, the five most commonly used verterinary sulfonamides can be resolved (Figure 3). In this laboratory one analyst can assay 24 samples/day inclusive of fortified control samples.

The absolute recovery of sulfamethazine and sulfapyridine were found to be 48.8% and 43.2%, respectively, by the external standard. Although the recovery of sulfapyridine is slightly lower than that of sulfamethazine, the important consideration is that run-to-run variation in the recovery of the two compounds is of the same magnitude and sign because of the similarity in structure and pH profiles. Hence, the internal standard corrects

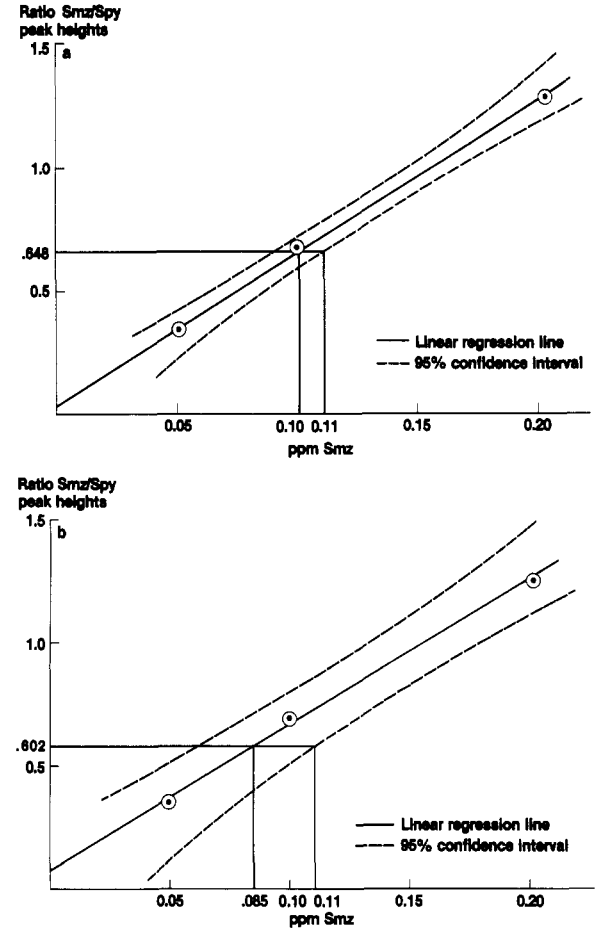

Figure 4. (a) Actual standard curve in muscle; $S_{y \cdot x}$ equal to 0.005 ppm. In this study 35.4% of all standard curves had $S_{y \cdot x} \leq 0.005$. As shown, the confirmation threshold would be 0.10 ppm to detect all 0.11-ppm residues by using the 95% confidence interval. (b) Actual standard curve in muscle; $S_{y \cdot x}$ equal to 0.01 ppm. The data in Table II indicate that 71.1% of all standard curves had $S_{y \cdot x} \leq 0.01$ ppm. To detect all 0.11-ppm sulfamethazine residues with 95% confidence requires a threshold of 0.085 ppm.

both for recovery and recovery variability.

The use of a chemically similar internal standard enables very high levels of accuracy and precision to be obtained (Table I). These characteristics are maintained on a day-to-day as well as on a within-day basis. The average daily variability when expressed as the percent relative standard deviation at the tolerance level in tissue (0.1 ppm) was 5.6% in muscle and 3.6% in liver.

For examination of the long-term day-to-day performance of the method, the following experiment was performed. Four replicates at three concentration levels were run daily in fortified swine tissue. This was done in both liver and muscle. Each day therefore produced $(4)^3$ or 64 possible three-point standard curves. In this way it was possible to generate several hundred "uses" of the method in a short period of time.

An examination of the standard curves generated in both tissues allows an estimation of the performance of the procedure as a quantitative screen. The correlation coefficient (r) is very good, especially in muscle where it is typically greater than 0.998. More useful is the standard error of estimate ($S_{y \cdot x}$) of the regression line which can be used to establish a screen limit or confirmation threshold on a statistical rather than empirical basis. For a particular confidence interval, as $S_{y \cdot x}$ becomes smaller, the screen limit rises and approaches the tolerance level. For example, if all residues above 0.11 ppm are to be confirmed by

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 0.11–2$S_{y \cdot x}$ 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.

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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.

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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-

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