

Liquid Chromatographic Determination of Incurred Nitrofurazone Residues in Chicken Tissues

OWEN W. PARKS

U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, Philadelphia, PA 19118

LEON F. KUBENA

U.S. Department of Agriculture, Agricultural Research Service, Veterinary Toxicology and Entomology Laboratory, College Station, TX 77841

One-day-old chicks were raised to maturity on a diet fortified with 0.0055% nitrofurazone. Analyses of tissue extracts by a liquid chromatography/electrochemical detection method revealed that the distribution of residues between liver, breast, and thigh muscle differed significantly from that previously reported in birds that were dosed with the drug before sacrifice. Differences between ground and unground tissues were also observed, suggesting that residues are not distributed evenly throughout the same tissue.

Nitrofurazone (5-nitro-2-furaldehydesemicarbazone), when fed continuously, is effective in preventing coccidiosis in chickens (1). However, information is lacking on residues of the drug in the tissues of treated birds, as a result of the rapid metabolism of the nitrofurazone and previous absence of sensitive methods of detection. Recently, we reported a liquid chromatographic (LC) method capable of detecting nitrofurazone residues in fortified chicken tissues at the low ppb level (2). This method was applied to the tissues of birds that had been placed on a diet fortified with 0.0055% nitrofurazone as 1-day-old chicks and continued on the diet to maturity. This communication reports the levels of the drug found in the liver, breast, and thigh muscle, as well as other observations.

METHOD

Reagents and Materials

(a) *Solvents, tissue homogenizer, centrifuge, sand, neutral alumina.*—Same as in Reference 2.

(b) *Drugs.*—Nitrofurazone (Norwich-Eaton Pharmaceuticals, Norwich, NY 13815); Amifur™ medicated premix containing 50 g nitrofurazone/lb (SmithKline Animal Health Products, West Chester, PA 19380).

(c) *Liquid chromatography/electrochemical detection.*—LC-5000 precision pump (Isco Inc., Lincoln, NE 68505) connected to Model LC-4B amperometric detector (Bioanalytical Systems, Inc., West Lafayette, IN 47905); glassy carbon electrode -0.8 V vs Ag/AgCl, 5–10 μ A full scale. Altex Model 210A sampling valve with 50 μ L loop. Recorder: Fisher Recordall Series 5000 at 10 mV full scale; chart speed 1 cm/min. Column: 25 cm \times 4.6 mm id 5 μ m Supelcosil LC-18 (Supelco, Inc., Bellefonte, PA 16823). Mobile phase: pH 6.0 phosphate buffer (0.05M monobasic potassium phosphate solution containing 0.001M EDTA adjusted to pH 6.0 with 1N NaOH)—methanol (57.5 + 42.5) purged with helium. Elute samples isocratically at 1.0 mL/min.

Feeding Trials

One-day-old male broiler chicks were placed on a commercial-type starter-grower diet fortified with 0.0055% nitrofurazone. At 42 days of age, 9 birds were sacrificed. An additional 9 medicated birds were removed to a control feed for 2 days and then sacrificed. Immediately after sacrifice, livers were removed, placed in a plastic bag, and frozen in liquid nitrogen to limit postmortem metabolism of the drug. Breast and thigh muscle were removed as quickly as possible and frozen to less than -50°C . All tissues were maintained at less than -50°C to -20°C before analyses. Tissues removed from 9 birds raised for 42 days on a nonmedicated feed served as controls.

Tissue Preparation

Frozen tissues from 2 birds were partially thawed, cubed, and blended together in a chilled (4°C) Waring blender. The blended tissues were quickly packed in plastic bags and refrozen in dry ice. The tissues of a single bird were left unground and frozen.

Determination and Quantitation

Analyses were conducted as previously described (2). Daily standard solutions containing ca 2.0, 5.0, 10.0, 84.0, and 168.0 ng/2 mL pH 6.0 phosphate buffer—methanol (1 + 1) were prepared from stock solutions of nitrofurazone in dimethylformamide. Average detector response factor (*R.F.*) was determined by relating concentration to measured peak

Table 1. Concentration of nitrofurazone in tissues of zero birds

Bird No.	Concn, ppb		
	Liver	Thigh	Breast
1024	146.2 ± 3.8	2.22 ± 0.11	2.64 ^a
1028			
1033	120.1 ± 8.0	2.20 ± 0.22	1.39 ^a
1040			
1047	87.4 ± 13.2	1.17 ± 0.00	0.69 ^b
1053			
1058	147.8 ± 5.27	2.30 ± 0.05	2.72 ± 0.14
1065			
1067 ^c	63.1 ± 14.5	9.11 ± 4.91	5.36 ± 2.31

^a Single determination.

^b Detected in 1 sample only.

^c Unground tissues.

heights (ng/mm). Concentration of nitrofurazone in incurred tissue was determined by the following formula:

$$\text{Concentration, ppb} = \frac{R.F. (\text{ng/mm}) \times \text{peak height (mm)}}{1.875 \text{ g tissue} \times 0.75}$$

where 0.75 represents percent recovery from fortified tissues (2). Duplicate samples were analyzed on different days.

Results and Discussion

Table 1 summarizes the results of analyses of tissue samples from birds sacrificed while on the nitrofurazone-fortified feed (zero time birds). With the exception of Liver 1047-1053, duplicate determinations were generally in good agreement among the blended samples. Concentrations determined in duplicate unground tissues (Bird 1067) varied considerably, suggesting that the residues were not uniformly distributed throughout the tissue. Furthermore, the ratios of total residues in the breast and thigh muscle to liver tissue in the unground samples were 7-8 times as great as the ratios determined in the ground tissues. The reason for this is unclear but may be a reflection of the uneven distribution of residues in tissues, differences between birds, or poor extraction of residues from unground liver tissues. Nitrofurazone was not detected in the tissues of birds removed from the medicated feed 2 days before sacrifice (minimum level of detection, 0.5 ppb).

In addition to the parent drug (*R*, 5.1 min), a small peak, presumably a metabolite, was present in all chromatograms of extracts of incurred liver tissues (Figure 1). The peak had the same retention time (4.0 min) as that previously observed in studies on incurred furazolidone tissues (3). This small peak could be produced in control liver tissues fortified with 200 ppb nitrofurazone by incubating 30 min at 37°C. Stability of the unidentified metabolite for further incubation and/or frozen

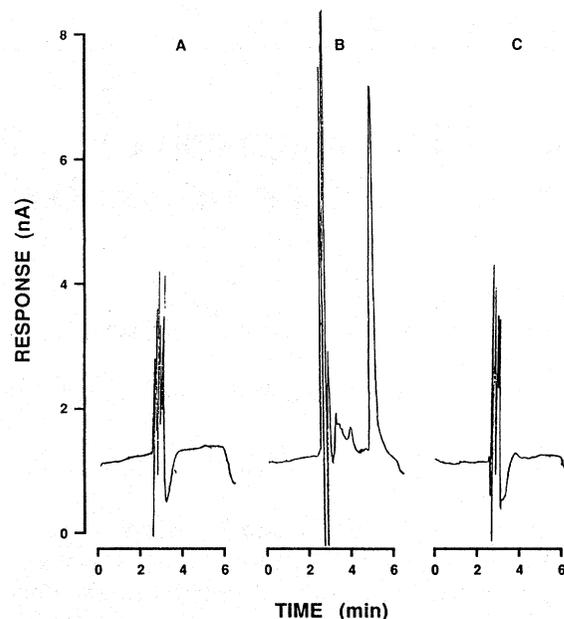


Figure 1. Chromatograms of liver extracts of (A) birds maintained on nonmedicated control feed, (B) birds fed nitrofurazone-fortified feed continuously until sacrifice, and (C) birds fed nitrofurazone-fortified feed continuously, then moved to control feed 2 days before sacrifice. Electrochemical detection: potential -0.8 V; attenuation 10 nA full scale.

storage was similar to that observed in the furazolidone studies (3).

The distribution of residues in the tissues in this study differs significantly from that reported by Sugden et al. (4). The latter observed average concentrations (870 ppb) 15 times as great in muscle tissue as in liver tissue 8 h after birds were dosed with 50 mg nitrofurazone. The distribution approached equality (6 ppb) after 24 h. The birds in this study consumed, on the average, approximately 8 mg nitrofurazone (150 g feed) in the 24 h period before sacrifice. These observations suggest that differences occur in drug uptake and/or metabolism between dosing and normal feeding practices.

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