

# Stability of Sulfaquinoxaline, Sulfadimethoxine, and Their $N^4$ -Acetyl Derivatives in Chicken Tissues During Frozen Storage

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**The  $N^4$ -acetyl derivatives of sulfaquinoxaline and sulfadimethoxine were stable in fortified chicken liver and thigh muscle tissues during frozen storage for 1 year at  $-20$  and  $-70^\circ\text{C}$ . In contrast, the parent compounds depleted approximately 35% in liver tissues at  $-20^\circ\text{C}$ . The transformation of the depleted sulfa drugs to their  $N^4$ -glucopyranosyl derivatives was negligible, suggesting that products other than glucosides resulted during the storage period.**

In previous studies, sulfamethazine (1) and the monoamino metabolites of zoalene (2) depleted in incurred and fortified tissues, respectively, during storage at  $-20^\circ\text{C}$ . In both earlier studies, evidence was presented that the depletion of residues in liver tissue was, at least partially, the result of transformation to their  $N^4$ -glucopyranosyl derivatives. This study was conducted to determine if the depletion of sulfa drug residues was limited to those containing the free primary arylamino functional group or also included their  $N^4$ -acetyl metabolites.

## Experimental

### Reagents and Apparatus

(a) *Solvents*.—Ethyl acetate (EtOAc) and methanol (MeOH) (American Burdick and Jackson Laboratories, Inc., Muskegon, MI), chloroform National Formulary ( $\text{CHCl}_3$ ) (J. T. Baker, Inc., Phillipsburg, NJ),  $N,N$ -dimethylformamide (DMF) (Fisher Scientific Co., Fair Lawn, NJ).

(b) *Parent compounds*.—Sulfadimethoxine (Sigma Chemical Co., St. Louis, MO), sulfaquinoxaline (Pfaltz and Bauer, Inc., Waterbury, CT).

(c)  *$N^4$ -Acetylsulfadimethoxine and  $N^4$ -acetylsulfaquinoxaline*.—Sulfa drug (50 mg) was dissolved in 2.5 mL glacial acetic acid, and 2.5 mL acetic anhydride was added. The mixture was heated ( $60^\circ\text{C}$  bath) for 10 min, 6 mL  $\text{H}_2\text{O}$  was added, and the solution was kept overnight at  $4^\circ\text{C}$ . The precipitated

$N^4$ -acetyl derivatives were recovered and recrystallized from ethanol-water and dried under reduced pressure at  $60^\circ\text{C}$ .

(d)  *$N^4$ -Glucopyranosylsulfadimethoxine and  $N^4$ -glucopyranosylsulfaquinoxaline*.—Prepared according to the method of Paulson et al. (3) and purified as previously described (2) for  $N^4$ -glucopyranosyl derivatives of 3-amino-5-nitrotoluamide and 5-amino-3-nitrotoluamide.

(e) *Shaker*.—Tekmar VXR power unit with VX8 shaker head (Thomas Scientific, Swedesboro, NJ).

(f) *Centrifuge*.—Refrigerated IEC Centra-7R, rotor No. 822A (International Equipment Co., Division of Damon Corp., Needleham Heights, MA).

(g) *Neutral alumina*.—Brockman activity I, 80–200 mesh (Fisher Scientific Co.). Add 650 mg neutral alumina to disposable filter column (Fisher Scientific Co., Cat. No. 11-387-50). Pack firmly by gently tapping top of column. Add 0.25 cm of sand. Wash column with two 1 mL portions of  $\text{CHCl}_3$  before use for isolation of parent drugs and  $N^4$ -acetyl derivatives.

(h) *Liquid chromatography*.—Column, 25 cm  $\times$  4.6 mm id, 5  $\mu\text{m}$  thickness, Supelcosil LC-18 (Supelco, Inc., Supelco Park, Bellefonte, PA). Mobile phase, pH 6.0 phosphate buffer (0.05M monobasic potassium phosphate containing 0.001M EDTA adjusted to pH 6.0 with 1N NaOH)—MeOH (6.5 + 3.5) purged with helium. Chromatograph analytes isocratically at 0.9 mL/min. Instrumentation, LC-5000 precision pump (Isco Inc., Lincoln, NE). Detectors, Model 1000S diode array detector (Applied Biosystems, Inc., Foster City, CA) at 265 nm, 0.01 absorbance units full scale (AUFS), with 20  $\mu\text{L}$  loop for sulfadimethoxine and derivatives; Model LC-4B amperometric detector (Bioanalytical Systems, Inc., West Lafayette, IN) with glassy carbon electrode set at  $-1.0$  V vs Ag—AgCl electrode, 100 nA full scale, 100  $\mu\text{L}$  loop for sulfaquinoxaline and derivatives. Recorder, Fisher Recordall series 5000 recorder at 10 mV full scale and chart speed at 0.5 cm/min.

### Preparation of Fortified Samples

The same procedure as previously described (2) was followed, except that 2.5 g tissue was fortified with 4.0 ppm sulfadimethoxine and  $N^4$ -acetylsulfadimethoxine from stock MeOH solutions (2  $\mu\text{g}/\mu\text{L}$ ) or 4.00 ppm sulfaquinoxaline and  $N^4$ -acetylsulfaquinoxaline from DMF stock solutions (2  $\mu\text{g}/\mu\text{L}$ ). After 1 year of storage, all samples were held at  $-70^\circ\text{C}$  until analyzed. Stored control tissue samples were forti-

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fied with 4.00 ppm of drugs just before analysis of the stored, fortified tissues.

### Analytical Procedures

(a) *Isolation of sulfadimethoxine, N<sup>4</sup>-acetylsulfadimethoxine, sulfaquinoxaline, and N<sup>4</sup>-acetylsulfaquinoxaline.*—Add 10 mL CHCl<sub>3</sub>-EtOAc (7.5 + 2.5) to fortified tissue in 50 mL screw-capped polypropylene centrifuge tube. Shake for 20 min at medium setting. Centrifuge for 10 min at 3500 rpm. Recover organic extract (do not remove any aqueous phase). Filter through plug of glass wool in 4 mL disposable Pasteur pipet. Collect filtrate. Return extracted tissue in centrifuge tube to -20°C storage. Pass 2.5 mL organic extract through column containing 650 mg Al<sub>2</sub>O<sub>3</sub>. Wash column with 1.5 mL CHCl<sub>3</sub> in 0.5 mL increments. Dry column under reduced pressure. Elute column with mobile phase and collect first 4 mL effluent. After mixing of the effluent, add 2 mL of the effluent to 9 mL screw-capped vial and inject onto LC column (see reference 2 for injection procedure for electrochemical detection).

(b) *Isolation of N<sup>4</sup>-glucopyranosylsulfaquinoxaline.*—For isolation from liver tissue, add 8.25 mL 10% aqueous DMF to previously extracted tissue from previous procedure. Shake for 20 min at medium setting. Centrifuge at 3500 rpm for 10 min. Recover aqueous phase and filter through plug of glass wool. Pass 2.5 mL extract through 3 mL (500 mg) C<sub>18</sub> Baker solid-phase extraction (SPE) column (J.T. Baker, Inc.). Rinse sides of column with 0.5 mL H<sub>2</sub>O. Wash C<sub>18</sub> column with an additional 2 mL H<sub>2</sub>O. Remove excess H<sub>2</sub>O under reduced pressure. Wash C<sub>18</sub> column with 1 mL 10% aqueous MeOH. Remove excess under reduced pressure. Elute column with mobile phase and collect first 2 mL effluent. Inject 100 µL onto LC column. Detect electrochemically. For isolation from thigh tissue, add 8.25 mL H<sub>2</sub>O to extracted tissue. Proceed as for liver tissue except do not wash C<sub>18</sub> SPE column with 10% MeOH.

(c) *Isolation of N<sup>4</sup>-glucopyranosylsulfadimethoxine.*—Add 18.25 mL MeOH to CHCl<sub>3</sub>-EtOAc extracted tissues from previous procedure. Shake for 20 min at medium setting. Centrifuge for 10 min at 3500 rpm. Recover extract and filter through plug of glass wool. Pass 15 mL extract through 3.25 cm neutral alumina prepared as previously described (2). Wash column with 5 mL MeOH in increments of 1.0, 1.0, 1.0, and 2.0 mL. Dry column under reduced pressure. Elute column with mobile phase and collect first 2 mL effluent. Inject 20 µL onto LC column.

### Results and Discussion

Table 1 summarizes the average concentrations of sulfadimethoxine, N<sup>4</sup>-acetylsulfadimethoxine, sulfaquinoxaline, and N<sup>4</sup>-acetylsulfaquinoxaline in tissues fortified with 4.00 ppm after 1 year of storage at -20 and -70°C. The N<sup>4</sup>-acetyl derivatives were essentially stable in both liver and muscle tissue during the storage period.

As expected, the parent sulfa drugs depleted (approximately 35%) in liver tissue during storage at -20°C. However, unlike in previous studies on sulfamethazine (1) and the monoamino metabolite of zoalene (2), their transformation to N<sup>4</sup>-glucopyranosyl derivatives could not be confirmed. Small peaks at the retention times of the glucosides were observed in liver samples stored at -20°C, which were absent in stored controls, but these peaks accounted for less than 10% of the depletion of the drugs. Acid hydrolysis studies, as used in the zoalene metabolite studies, were ineffective in confirming the presence of glucosides. Under the assumption that depletion of any N<sup>4</sup>-glucosides themselves may have occurred after the additional storage period at -70°C before analyses (up to 6 months), liver tissues were fortified with 4.0 ppm of the N<sup>4</sup>-glucopyranosyl derivatives of sulfadimethoxine and sulfaquinoxaline and stored for 5 to 6 months at -70°C. No significant depletion of the glucosides was observed after the storage period. Future studies will be directed toward determining the nature of the depletion of drugs in this study and those of the monoamino metabolites of zoalene not accounted for in the previous study. In this respect, it is noteworthy that Sheth et al. (4) reported the formation of 1-(N<sup>4</sup>-sulfathiazole)-1-deoxy-D-fructose, a non-acid-hydrolyzable amadori compound, in model systems of sulfathiazole and fructose. It was obvious from visual observations of the stored liver tissues in this study that a dark brown discoloration of the tissues occurred at -20 but not at -70°C.

The results of this study demonstrate that, unlike the parent compounds, the N<sup>4</sup>-acetyl derivatives of sulfaquinoxaline and sulfadimethoxine do not become depleted in liver and tissues during storage at -20°C. Furthermore, a comparison of these results with those previously reported (1, 2) indicates that although depletion of structurally similar drug residues (containing a free primary arylamino functional group) occurs in liver tissue, the extent of depletion and the kind of end products vary among drugs. The extent of depletion, however, may be the result of differences between incurred residues versus fortified

**Table 1. Concentration<sup>a</sup> of sulfa drugs and derivatives in tissues fortified with sulfaquinoxaline, N<sup>4</sup>-acetylsulfaquinoxaline, sulfadimethoxine, and N<sup>4</sup>-acetylsulfadimethoxine at 4.0 ppm after 1 year of frozen storage**

Tissue	Temp., °C	Concentration ± SD, ppm			
		Sulfaquinoxaline	N <sup>4</sup> -Acetylsulfaquinoxaline	Sulfadimethoxine	N <sup>4</sup> -Acetylsulfadimethoxine
Liver	-20	2.49 ± 0.16	3.77 ± 0.07	2.69 ± 0.09	3.79 ± 0.15
Liver	-70	3.76 ± 0.12	3.87 ± 0.02	3.81 ± 0.14	4.00 ± 0.03
Thigh	-20	3.75 ± 0.25	4.12 ± 0.07	3.67 ± 0.13	3.92 ± 0.11
Thigh	-70	4.11 ± 0.14	4.13 ± 0.05	4.00 ± 0.14	4.08 ± 0.14

<sup>a</sup> Average of 4 fortified tissues; SD, standard deviation.