

Photodegradation of Sulfa Drugs by Fluorescent Light

Thin layer chromatographic and liquid chromatographic procedures were used to show that sulfonamides containing a heterocyclic amine moiety and free N^1 acidic hydrogen will photodegrade under fluorescent light in model systems containing riboflavin. The photodegradation product was characteristic of the drug. In-depth studies on sulfamethazine showed that the drug also photodegraded in the presence of lumichrome and flavin mononucleotide; the rate of photodegradation depended on the photosensitizer and its concentration. Crude polar liver extracts sensitized the photodegradation of sulfamethazine, but to a degree less than expected on the basis of reported riboflavin content of livers. It is recommended that procedures for quantitating sulfa drugs and their metabolites be performed in subdued lighting and/or that amber or low actinic vessels be used to prevent losses due to photochemical reactions.

In the course of studies on the development of procedures for sulfa drugs and their metabolites in swine and chicken livers (1, 2), unexplained losses of the drugs and/or metabolites occasionally occurred during the isolation procedure. Preliminary investigations into the source of these decreased values led to the observation that sulfa drugs are susceptible to photochemical reactions by fluorescent light in the presence of polar extracts of liver tissues. Since previous studies have demonstrated that vitamin B₂ (riboflavin) catalyzes photochemical reactions in a variety of biological materials (3, 4) and liver contains relatively large amounts of this vitamin (5), studies on the susceptibility of sulfa drugs to photochemical reactions by fluorescent light in model systems were initiated. This communication reports the results of studies on a num-

ber of sulfa drugs and alerts the analyst to a potential problem in analyzing for these drugs in tissue samples.

Experimental

Irradiation

Aqueous methanol solutions of sulfa drugs or their derivatives, 1 mL in 60 × 17 mm screw-cap flint glass vials, were exposed to fluorescent light (250 ft-candles) for 5–60 min at ambient temperature in the presence of riboflavin (J. T. Baker Chemical Co., Phillipsburg, NJ 08865), flavin mononucleotide (FMN) sodium salt (Sigma Chemical Co., St. Louis, MO 63178), or lumichrome (Pfaltz and Bauer, Stamford, CT 06902). The light source consisted of two 15 watt cool white fluorescent bulbs (Westinghouse F15T8/cw) positioned 15.25 cm above the sample vials which were placed in a horizontal position.

Thin Layer Chromatography (TLC) Studies

Approximately 75–100 µg drug and 35 µg riboflavin in 1 mL 50% aqueous methanol was exposed to fluorescent light for 60 min. Following exposure, 1–2 µL samples were subjected to TLC on 2.5 × 10.0 cm glass plates with 250 µm layer of silica gel G (Analtech, Newark, DE 19711). Plates were developed to 0.5 cm initially, dried in a hot air oven at 50°C, and redeveloped to 5 cm. Developing solvents: (a) chloroform–ethyl acetate–methanol (5 + 5 + 1), and (b) chloroform–methanol (1 + 1) or upper layer of butanol–acetic acid–water (4 + 1 + 5). Sulfa drugs, N^1 -methyl derivatives, and photodegradation products containing a primary aromatic amine were visualized with the Bratton-Marshall (B-M) spray reagent previously described (6). N^4 -Acetyl com-

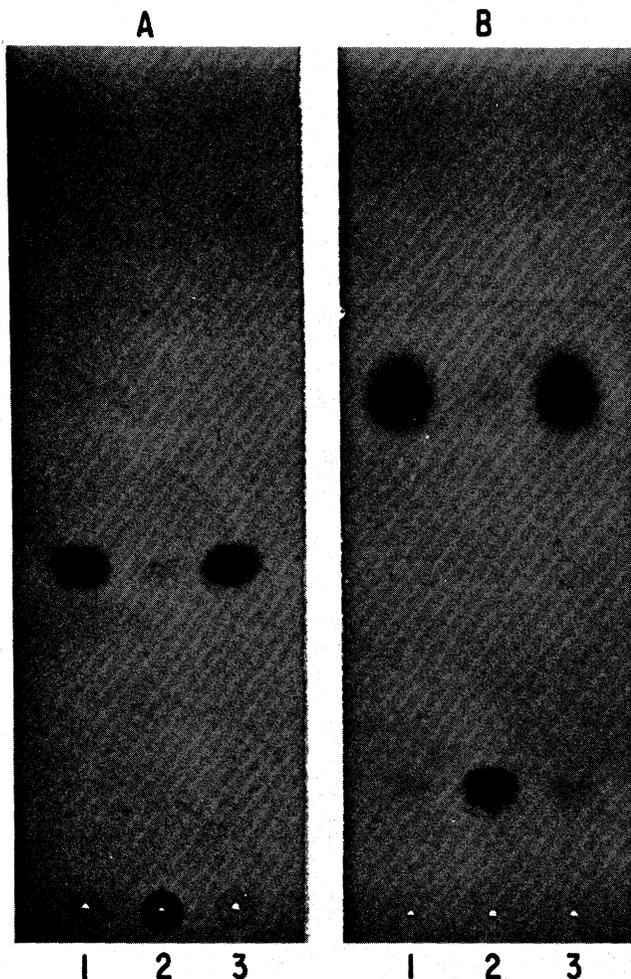


Figure 1. TLC chromatograms of 1, sulfamethazine + riboflavin control; 2, sulfamethazine + riboflavin exposed to 250 ft-candles fluorescent light for 1 h; and 3, sulfamethazine exposed to 250 ft-candles fluorescent light for 1 h. Solvent systems: A, chloroform-ethyl acetate-methanol (5 + 5 + 1); B, upper layer *N*-butanol-acetic acid-water (4 + 1 + 5).

pounds were visualized with the *N*-chlorination procedure of Schwartz and Sherman (7). *N*⁴-Glucopyranosylsulfamethazine and its photodegradation product were visualized with the B-M spray reagent following acid hydrolysis (1). Photodegradation was considered positive when the color intensity of the parent compound obviously decreased and/or an additional spot(s) appeared within 1 h of light exposure.

Liquid Chromatography (LC) Studies

A solution of 3.54 μg sulfamethazine and 3.6 μg riboflavin or 2.3 μg lumichrome in 1 mL methanol-water (30 + 70) was exposed to fluorescent light as described above; 3.54 μg sulfamethazine in 1 mL aqueous methanol solution served as a control. At predetermined intervals, 50 μL samples were subjected to LC. Sulfamethazine remaining in the exposed solutions was determined by comparing peak heights at the appropriate retention time. The LC system consisted of an Altex Model 100 A pump (Altex Scientific, Inc., Berkeley, CA 94710) connected to a Schoeffel Model SF 770 Spectroflow variable wavelength detector operated at 254 nm; Altex Model 210 sampling valve with a 50 μL loop; 25 cm \times 4.6 mm id column of 5 μm Alltech C₁₈ (Alltech Associates, Deerfield, IL 60015); mobile phase methanol-water (30 + 70); flow rate 1 mL/min. Retention times: sulfamethazine 8.8 min; riboflavin 15.0 min; lumichrome 55.8 min.

Polar Liver Extracts

An aliquot of 5 g frozen, ground swine or chicken liver was shaken 45 min (Ika-Vibrax VXR shaker, Tekmar Co., Cincinnati, OH 45222) with 20 mL water, methanol, or acetone in 50 mL polypropylene screw-cap centrifuge tube. The samples were centrifuged at 3000 rpm for 5 min. The aqueous supernate was recovered and filtered through a plug of glass wool. The methanol and acetone supernates were recovered and evaporated to near dryness at 50°C under a stream of nitrogen. The residues were taken up in 15 mL water and filtered. The aqueous suspensions were passed through a 5 cm column of Duolite ES-863 resin (Diamond Shamrock Corp., Cleveland, OH 44114) prepared as previously described (1). Preliminary studies showed that riboflavin and FMN were readily removed from aqueous solutions by the resin. The columns were washed with 15 mL water and the flavins eluted with 5 mL methanol. The effluents were extracted with 5 mL hexane and evaporated to near dryness. One mL of 50% aqueous methanol containing 54 μg *S*-methazine was added and the samples were exposed to fluorescent light for 1 h. Following exposure, 2 μL samples were subjected to TLC.

Results and Discussion

The role of riboflavin in the photodegradation of sulfa drugs by fluorescent light in model systems is demonstrated by the TLC results in Figure 1, in which there is a concurrent loss of sulfamethazine and appearance of a polar B-M positive compound. The appearance of a singular sulfamethazine photodegradation product is further illustrated by the LC tracings in Figure 2. As determined by TLC, as little as 0.1 μg riboflavin/mL solution was found to sensitize the photochemical reaction and the rate of photodegradation of sulfamethazine was demonstrated to be a function of the riboflavin concentration and light intensity. The degradation of sulfamethazine in the presence of 3.6 μg riboflavin/mL solution was a first-order type reaction, where $K(\text{s}^{-1}) = 1.86 \times 10^{-3}$. An equimolar concentration of lumichrome, shown by LC to be the major photodegradation product of riboflavin in these studies, also catalyzed sulfamethazine photodegradation but at a slower rate [$K(\text{s}^{-1}) = 4.72 \times 10^{-4}$]. Significantly, lumichrome is not further degraded in the process.

A compilation of the results on a variety of sulfa drugs and drug derivatives in aqueous methanol solutions subjected to fluorescent light for 1 h in the presence of riboflavin is as follows:

Compounds Photodegraded

Sulfamethazine	Sulfaquinoxaline
Sulfamerazine	Sulfaethoxy pyridazine
Sulfadiazine	Sulfabromomethazine
Sulfapyridine	<i>N</i> ⁴ -Acetylsulfamethazine
Sulfathiazole	<i>N</i> ⁴ -Acetylsulfamerazine
Sulfadimethoxine	<i>N</i> ⁴ -Glucopyranosylsulfamethazine

Compounds Not Photodegraded

Sulfaguanidine	Sulfanitran
Sulfanilamide	<i>N</i> ¹ -Methylsulfamethazine
Sulfabenz	<i>N</i> ¹ -Methylsulfamerazine

With the exception of sulfathiazole, which photodegraded to several compounds, all photodegraded drugs resulted in one product, characteristic of the drug. All sulfa drugs and derivatives gave negative results in the absence of riboflavin, and degradation stopped on removal of the light source. An analysis of these results suggests that the sulfonamide must contain a heterocyclic amine moiety and free *N*¹ acid hydro-

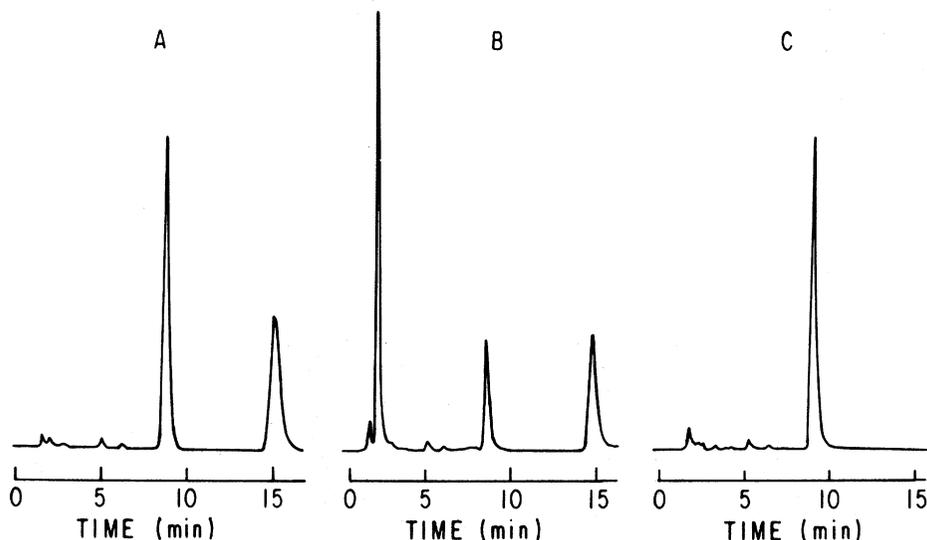


Figure 2. LC chromatograms of A, sulfamethazine + riboflavin control; B, sulfamethazine + riboflavin exposed to 250 ft-candles of fluorescent light for 15 min; and C, sulfamethazine exposed to 250 ft-candles of fluorescent light for 15 min. Retention times: photodegradation product, 2.3 min; sulfamethazine, 8.8 min; and riboflavin, 15 min.

gen on the $-SO_2NH-$ linkage to undergo photodegradation. The results are unaffected by substituents on the N^4 amino group.

TLC of polar liver extracts, spiked with sulfamethazine and exposed to fluorescent light for 1 h, showed the presence of the same photodegradation product as observed in model systems of sulfamethazine. The extent of the photodegradation was not as extensive as expected on the basis of reported riboflavin levels of swine and chicken livers (5). Spiking the tissue extracts with an additional 7.2 μg riboflavin increased the amount of photodegradation but not to the same extent observed in model systems, indicating the relatively crude extracts contained compounds that inhibited or competed with the photodegradation of sulfamethazine. Riboflavin appeared to be photodegraded more readily to lumichrome in the liver extracts which may explain, in part, the slower rate of photodegradation compared with the model system. A further explanation in chicken livers may lie in the observations of Reyes et al. (8) that 85% of riboflavin in chicken liver is present as FMN, which was shown by TLC of model systems to sensitize the photodegradation at a rate comparable to lumichrome.

In conclusion, the results reported herein indicate that it is necessary for the analyst to take precautionary measures to

protect liver extracts in which riboflavin, FMN, or lumichrome and sulfa drugs and/or their metabolites are present to prevent losses due to photodegradation as the result of fluorescent light. Protecting such solutions from direct natural light is self-evident. It is recommended that analyses be conducted in subdued lighting and/or that solutions be maintained in protective amber or low-actinic vessels.

REFERENCES

- (1) Parks, O. W. (1984) *J. Assoc. Off. Anal. Chem.* **67**, 566-569
- (2) Parks, O. W., & Fiddler, W. (1983) 97th Annual International Meeting Assoc. Off. Anal. Chem., Oct. 3-6, Washington, DC
- (3) Penzer, G. R., & Radda, G. K. (1967) *Chem. Soc. Quart. Rev.* **21**, 43-65
- (4) Sattar, A., & deMan, J. M. (1975) *Crit. Rev. Food Sci. Nutr.* **7**, 13-37
- (5) *Composition of Foods* (1963) U.S. Department of Agriculture Handbook No. 8, Government Printing Office, Washington, DC
- (6) Parks, O. W. (1982) *J. Assoc. Off. Anal. Chem.* **65**, 632-634
- (7) Schwartz, D. P., & Sherman, J. T. (1982) *J. Chromatogr.* **240**, 206-208
- (8) Reyes, E. S. P., Norris, K. M., Taylor, C., & Potts, D. (1983) 97th Annual International Meeting Assoc. Off. Anal. Chem., Oct. 3-6, Washington, DC